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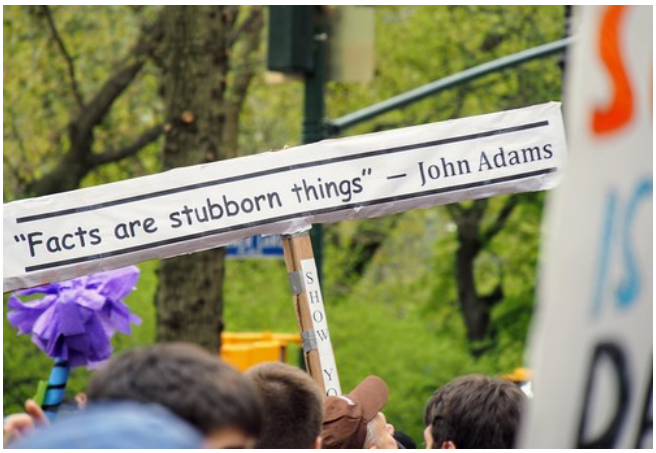
2.1.1.5: REPORTING ON SCIENCE

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2.1.1.1: INTRODUCTION

THE SCIENTIFIC METHOD

Scientists use a methodology for systematically investigating natural phenomena. This method uses existing information or observations to acquire new information or validate previous knowledge. These knowledge types come from empirical (experiential) or measured information. Empirical and measured data (or knowledge) are referred to as **observations**. While **empirical** data comes from experiences, science has developed into a mode of inquiry using **experimentation**. Experimental science uses the pre-existing base of knowledge to ask a testable question called a **hypothesis**. As a youngster, we're incorrectly taught that a hypothesis is ~~*an educated guess*~~. Formulating previous observations and measurements into a cohesive line of inquiry requires no guessing. People often have "theories" on something, when they actually have hypotheses based on their observations and assumptions.



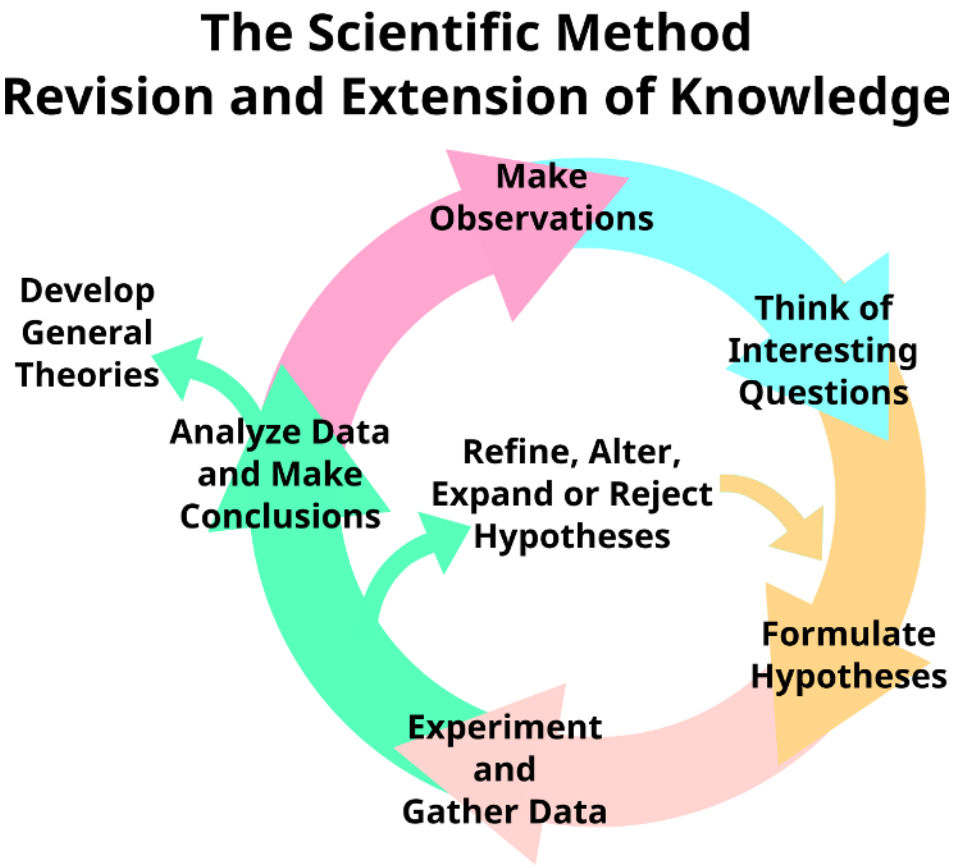


EXPERIMENTAL SCIENCE

Hypothesis testing is the means by which experimental science is conducted. Experimental science is designed to enhance the understanding of a problem and removing biases from the interpretation. The goal of hypothesis testing is to try every way possible to disqualify the validity of the hypothesis. By doing so, the experimenter removes any biases in the experimental design. If the experimenter is unable to invalidate the hypothesis, the hypothesis becomes more valid and better able to act as a predictor of phenomena.

Experiments utilize **controls**. In a controlled experiment, there is a positive and negative control. These controls act as references in the experiment. A **positive control** is an experimental condition where the expected outcome that is tested will be produced. This control is necessary to assess the validity of a test or treatment. There can be multiple instances used as a positive control to examine the sensitivity of the experiment. A **negative control** is an experimental condition where the expected outcome is known not to occur. This type of control sometimes comes in the form of a sham or mock treatment such as giving someone a sugar pill (a **placebo**).

Through the use of experimental science and hypothesis testing, an increased refinement of existing knowledge can aid in designing new hypotheses. Hypothesis testing is re-iterative. That is to say, we use new knowledge to continue to enhance our understanding of the universe.



The scientific method is a reiterative process based on testing and revising knowledge. (CC-BY-NC-SA Jeremy Seto)

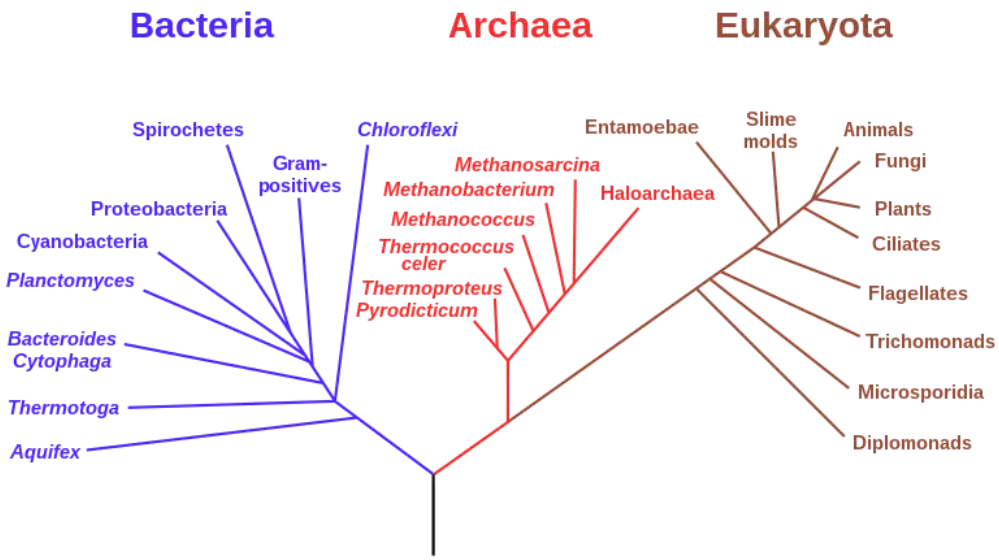
THEORIES

A **scientific theory** comes from repeated substantiation of multiple tested hypotheses. That is to say, confirmed hypotheses, observations, and experiments permit scientists to formulate a cohesive idea that integrates multiple substantiated pieces of evidence. As with hypotheses, theories are designed to be predictive and falsifiable. In the common language, we often hear the word theory to mean a conjecture, and as already discussed, conjectures based on evidence can be formulated into testable hypotheses.

When a theory is accepted by a predominant population of the specialists, it is referred to as a **scientific principle**. An example of a scientific principle is the ***theory of evolution by natural selection***. Numerous tested hypotheses have been confirmed that lead to the understanding of natural selection as a method of evolution. This theory allows scientists to understand the underlying relatedness of all living things on the planet. Additionally, it unifies the disparate fields of Biology that can utilize the theory in a predictive manner. It is therefore also referred to as a **unifying principle** of Biology.

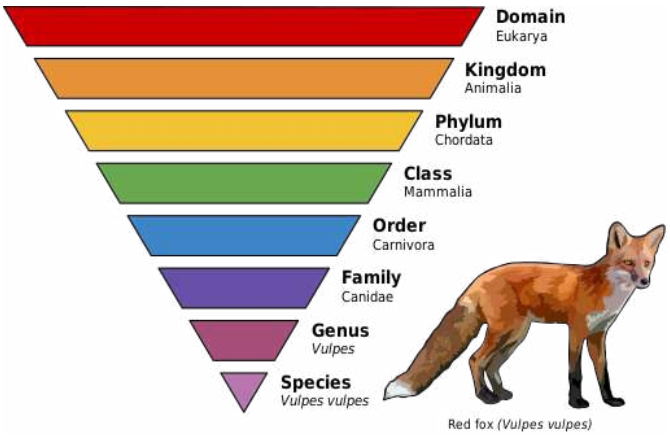
CLASSIFICATION OF LIFE

All living things on Earth share a relationship. The rules that govern life processes can be generalized across all **organisms** (living things), as well as non-living biological entities (viruses). The relatedness of organisms is often visualized as a **phylogenetic tree**. This tree is a hierarchical classification system that groups organisms together based on common features and used these similarities to name them. This is referred to as **taxonomy** with the broadest category is called a **domain**.



The phylogenetic tree of life describing the inter-relatedness of all living things on Earth.

Three domains exist, **Archaea**, **Bacteria,** and **Eukarya**. Archaea and Bacteria are also grouped together as **Prokaryotes** (*pro*– before; *karya*– nucleus). Eukarya (*eu*– true; *karya*– nucleus), or eukaryotes, is a group of organisms that have nuclei. The second most inclusive or broad category is **Kingdom**. Humans are in the Kingdom of animals. The third most inclusive or broad category is **Phylum**. Humans are in the phylum called Chordata. Each level of organization can be further subdivided and you may be more familiar with the subphylum called Vertebrata. Within this division, humans fall in the **class** of mammals. Amongst the mammals, humans are in the **order** of primates. Humans are categorized into a narrower group of organisms in the **family** of great apes or hominids. Within this family, humans fall into the **genus** of *Homo*. Biologists use a method of identifying specific organisms called binomial nomenclature. Binomial nomenclature uses the most specific groupings of taxonomy (genus and **species**) as a two-part name. While humans are of the species sapiens, the species name of humans using binomial nomenclature is *Homo sapiens*.



Taxonomic ranking of the red fox illustrating the inclusiveness of Domain and the exclusiveness of the Species level of categorization.

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2.1.1.2: MICROSCOPY

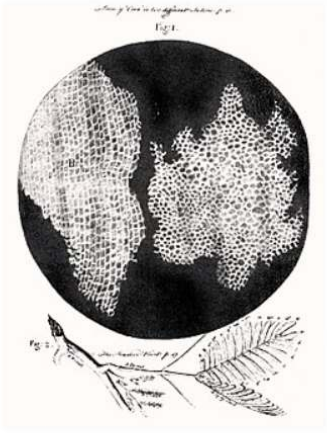
THE LIGHT MICROSCOPE

HOOKE'S CELL

In 1665, Robert Hooke published *Micrographia*, a book that illustrated highly magnified items that included insects and plants. This book spurred on interest in the sciences to examine the microscopic world using lenses but is also notable for Hooke's observations of cork where he used the word "cell" in a biological sense for the first time.



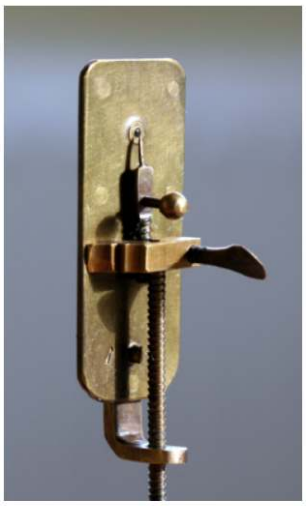
Illustration of Hooke's microscope



The empty chambers of cork inspired Hooke to coin the term "Cell"

THE FATHER OF MICROBIOLOGY: VAN LEEUWENHOEK

The Dutch tradesman Antonie van Leeuwenhoek used high power magnifying lenses to examine the parts of insects and to examine the quality of fabric in his drapery business. He began to experiment with pulling glass to generate lenses and developed a simple microscope to observe samples. Using a simple single lens with a specimen mounted on a point, he was able to identify the first microscopic "animalcules" (*little animals*) that will be later known as protozoa (*original animals*).



van Leeuwenhoek's microscope

Though van Leeuwenhoek's apparatus was simple, the magnifying power of his lenses and his curiosity enabled him to perform great scientific observations on the microscopic world. He was ridiculed for fabricating his observations of protists at first. Ever the scientist, van Leeuwenhoek examined samples of his own diarrhea to discover *Giardia intestinalis*. While he did not make the connection of the causative nature of this microorganism, he described the details of the way this organism could propel itself through the medium in great detail.



Modern micrograph of Giardia intestinalis (Kingdom Protista)

MODERN COMPOUND MICROSCOPE

Unlike van Leeuwenhoek's single-lens microscope, we now combine the magnifying power of multiple lenses in what is called a compound microscope.



1. Ocular lens or eyepiece
2. Nose Piece/ Lens Carousel
3. Objective lens
4. Course Focus Knob
5. Fine Focus Knob
6. Stage
7. Lamp
8. Condenser
9. Stage control

USING THE LIGHT MICROSCOPE



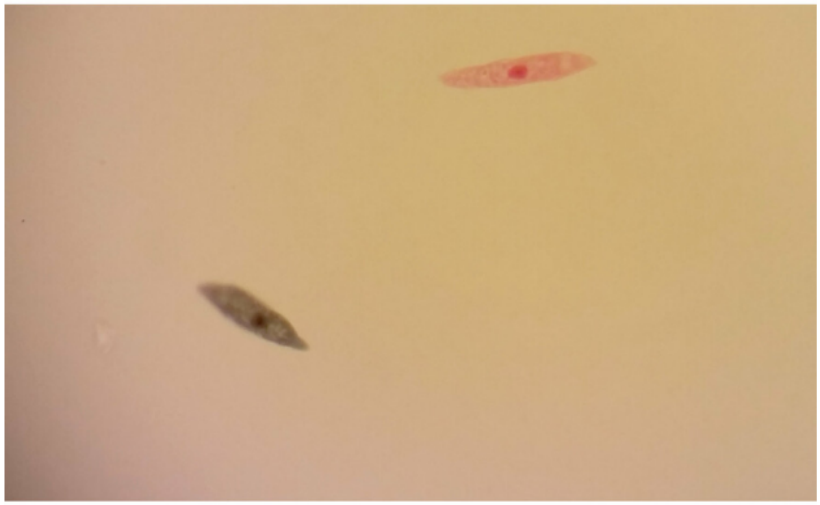


Daphnia magna, the freshwater flea is a small crustacean (scanning magnification)



Low magnification of Daphnia magna

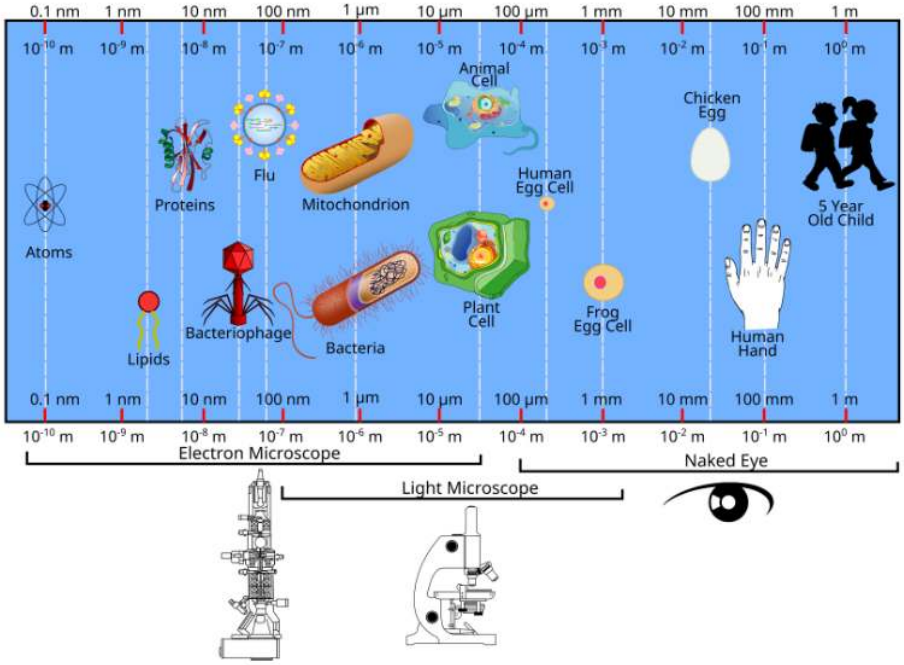






Protozoa. Top: Amoeba Center: Euglena Bottom: Paramecium

SCALE



Credit: Jeremy Seto and derived from works by IgniX, Gringer, Jiver, Ninjatacoshell, Ali Zifan (CC-BY-SA)

MAGNIFICATION

Magnification is the process of enlarging the appearance of an object. We calculate the magnification of an object by indicating the fold change in size. So if something appears to be double the size of the real item, then it is obviously magnified 2X. Because there is a magnification by the eye-piece (**ocular lens**), as well as the **objective lenses**, our final magnification of an item is the product of those two lenses.

The lowest magnification objective lens (usually 4X or 5X) is referred to as a **scanning lens**. There is also usually a low power lens at 10X and a higher magnification lens at 40X. There may be a higher magnification lens at 100X but these usually require oil to function properly and are often reserved for microbiology labs.

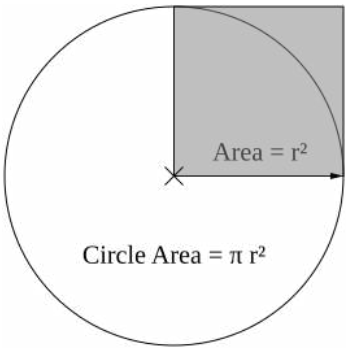
- What is the power of the ocular lens?
- We can calculate that as:
  $\text{Magnification}_{\text{total}} = \text{Magnification}_{\text{objective}} \text{ X } \text{Magnification}_{\text{ocular}}$
- With this in mind, fill in the following table:

	Objective Magnification	Ocular Magnification	Total Magnification
Scanning			
Low Power			
High Power			
Oil Immersion	100X		

FIELD OF VIEW (FOV)

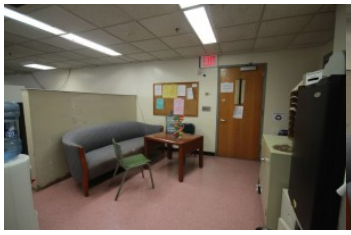
In a microscope, we ordinarily observe things within a circular space (or field) as defined by the lenses. We refer to this observable area as the **field of view (FOV)**. Understanding the size of the FOV is important because actual sizes of the object can be calculated using the Magnification of the lenses.

FOV can be described as the area of a circle:



$Area = \pi r^2$

What are the effects of magnification on FOV?



1) Lowest Magnification



2) Low Magnification



3) High Magnification



4) Highest Magnification

In image 1, we can see a model of DNA on a table with a water bottle and a large area of the room. Image 2 displays less of the room in the background but the DNA model is larger in appearance because the magnification is greater. In image 3, we no longer see evidence of a door and the DNA model is much larger than before. In image 4, we no longer see the table the model and water bottle rest upon. While the

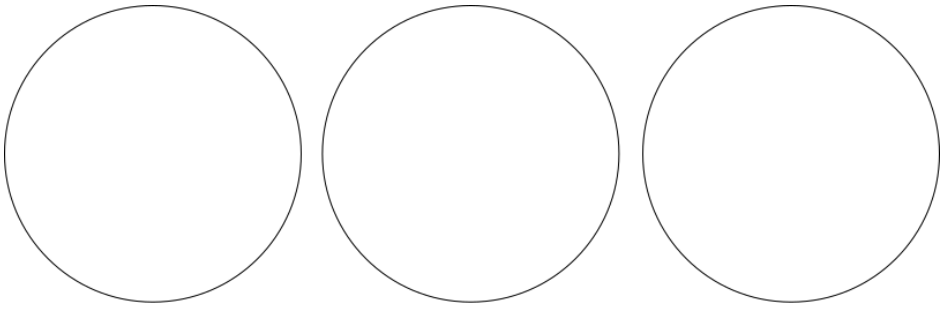
last image is largest, we see less of the surrounding objects. We have higher magnification at the cost of the field of view. FOV is inversely related to the magnification level.

FIELD OF VIEW CALCULATION

1. Examine a ruler under scanning magnification
   - Measure the diameter in mm.
   - Diameter= _____
   - Radius= _____
   - Calculate the field of view at this magnification= _____
2. Examine a ruler under low magnification (10x)
   - Measure the diameter in mm.
   - Diameter= _____
   - Radius= _____
   - Calculate the field of view at this magnification= _____
3. What is the relationship in the between the magnification and field of view? _____
4. What is the proportion of change in the field of view when doubling the magnification? _____

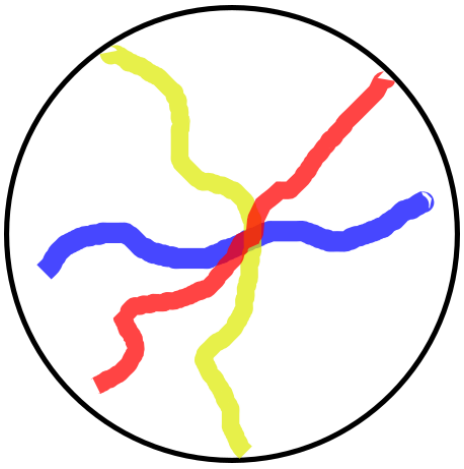
THE LETTER "E"

1. Orient a slide with the Letter "e" so that it is read as an "e" without magnification
2. Draw the "e" at scanning, low and high magnification



DEPTH OF FIELD

1. Examine the slide of colored threads under scanning power so the cross-point of the threads is at the center of the field.



2. Raise the magnification to the low power objective

- What do we notice about the threads and the focus?
- How can we explain this observation with respect to the threads?

- Close the diaphragm so allow a pinpoint of light through the slide. What effect does this have on the image?

We notice that when we observe 3 overlapping threads of different color under a microscope, we can focus on one thread at a time. Similarly, when we zoom in a great deal on the DNA model below, we notice that the print on the water bottle is not sharp.

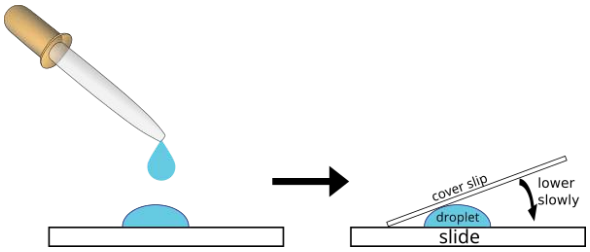


Highest Magnification with shallow depth of field. Notice how the label on the water bottle is blurry while the lettering on the DNA model is sharp.

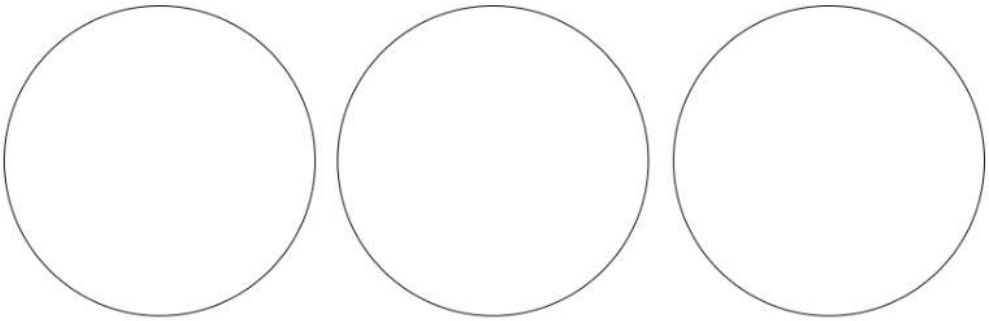
We know that the water bottle is behind the DNA molecule. Under the microscope, the threads of differing color are also stacked on top of each other. We recognize that they are on different planes because they are three dimensional. Each thread has depth and does not occupy the same exact space. If we focus on the print of the water bottle on the image above, we would no longer see the lettering on the DNA molecule sharply. We refer to this concept as **Depth of Field (DOF)**. Under the microscope, at low magnification, we can make out fewer finer details. However, most items appear on the same plane in this case and or comparably sharp. But as we increase the magnification and see finer details, the distances between the various planes in view become more apparent. We can see a similar phenomenon at low magnification of the DNA model. At the low magnification, we may not be able to read the print on the water bottle, but the bottle and DNA molecule are of a similar distance from our view that the small difference in apparent depth is not as noticeable. We can still draw on other visual cues to know that the bottle is behind the model, but the sharpness of both items are equivalent.

EXAMINING CELLS

1. Choose a prepared slide of a Protist (*Euglena*, *Amoeba*, *Paramecium*)
2. Prepare a wet mount of a drop of pond water and place a coverslip over the drop.
3. Swab the inside of your cheek
    1. Roll the swab across a slide
    2. Drop some methylene blue onto the slide
    3. Place a coverslip over the drop
    4. Visualize and draw your cheek cells



4. Document your observations by drawing the cells and by using your phone to snap an image.



REAL BIOLOGICAL EXAMPLES

We can see the concepts of FOV and DOF in the following pictures.



In an even more extreme close-up (higher magnification), we would have difficulty focusing on both the eyes and beak since there is depth and distance between those features.

HOW DO WE USE MICROSCOPES

In our lab, we look at some pond water. What do we see? Why is this significant? How does the microscope help us study these items? What is the utility of the concepts of magnification, FOV, and DOF when we use microscopes to study biological samples?



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2.1.1.3: UNITS OF MEASURE

THE METRIC SYSTEM

The **metric system** is an internationally agreed-upon measurement system based on decimals or powers of 10. Scientists use a refined version called the **International System of Units** (abbreviated **SI**). In biology, you will often find a need to describe measurements of length, volume, mass, time, temperature or amount of substance.

INTERNATIONAL SYSTEM OF UNITS

BASIC SI UNITS		
MEASURE	**SI UNIT**	**SYMBOL**
length	meter	**m**
mass	kilogram	**kg**
time	second	**s**
temperature	Kelvin *(Celsius is used in Biology)*	**K** *(°C)*
quantity	mole	**mol**
current	Ampere	**A**
luminosity	candela	**cd**

METRIC UNITS

- length: meter (**m**)
- volume: liter (**L**)
- mass: gram (**g**)
- time: second (**s**)
- temperature: Celsius (**°C**)
  - Kelvin (**K**) is a unit of thermodynamic temperature and is the SI unit. The Kelvin scale in the same as the Celsius or centigrade scale but offset by 273.16
  - Biology uses Celsius predominantly because of the range in which organisms live.
- amount of substance: mole (**mol**)
  - A mole is a number representing 6.022×10^{23} of something
  - Just as a pair of shoes equals 2 shoes, a mole of shoes is 6.022×10^{23} shoes
  - Just as a dozen eggs equals 12 eggs, a mole of eggs is 6.022×10^{23} eggs

METRIC PREFIXES IN EVERYDAY USE			
PREFIX	**SYMBOL**	**SCIENTIFIC NOTATION**	**FACTOR**
tera	T	10^{12}	1 000 000 000 000
giga	G	10^{9}	1 000 000 000
mega	M	10^{6}	1 000 000
kilo	k	10^{3}	1 000
hecto	h	10^{2}	100
deca	da	10^{1}	10
BASE UNIT	(none)	10^{0}	1
deci	d	10^{-1}	0.1
centi	c	10^{-2}	0.01
milli	m	10^{-3}	0.001
micro	μ	10^{-6}	0.000 001
nano	n	10^{-9}	0.000 000 001
pico	p	10^{-12}	0.000 000 000 001

STRATEGY FOR CONVERSIONS

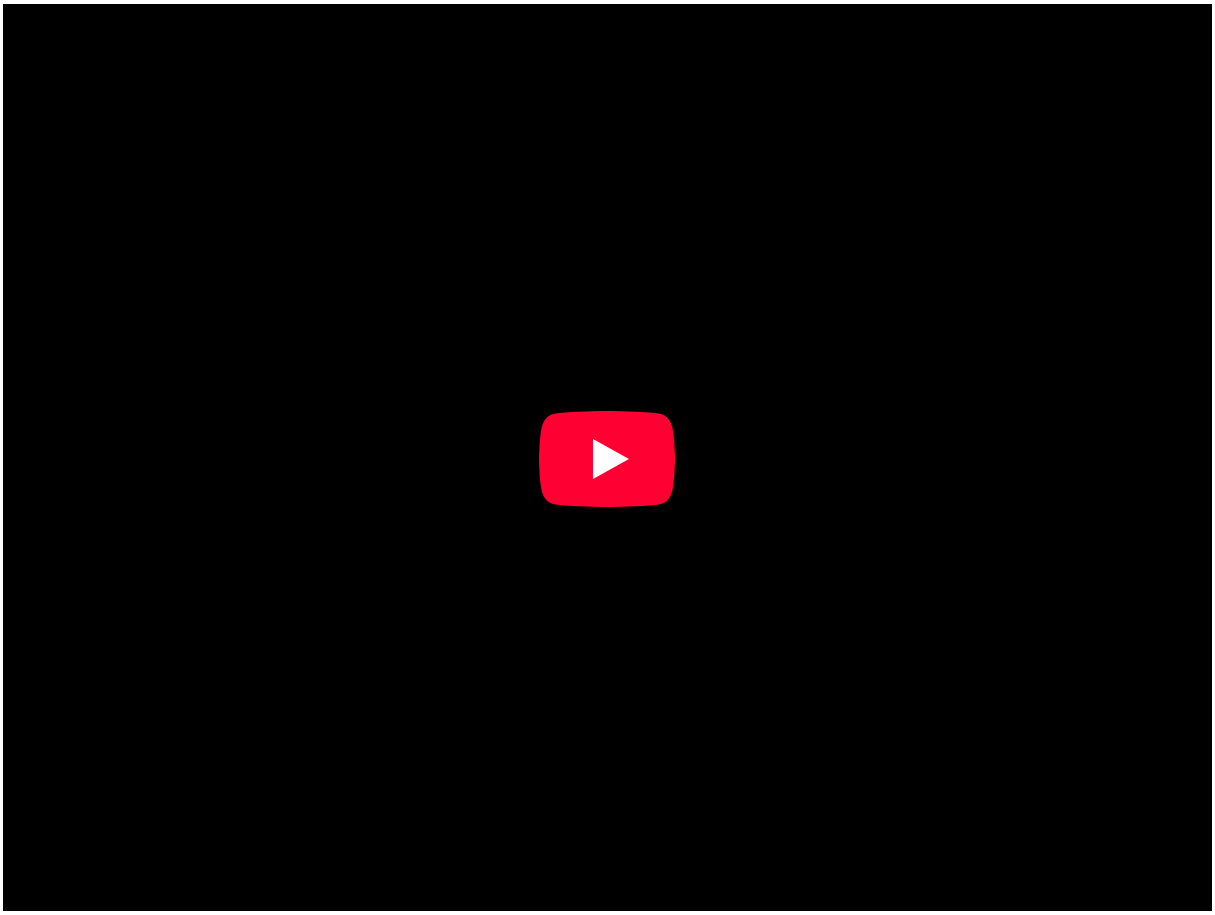
1. What unit is being asked for?
   - 500ml = ____L → liters
2. What unit are you starting from?
   - 500ml = ____L → milliliters
3. Which unit is larger? By how much is that unit larger?
   - Liters are the larger unit. Liters are 1,000X (10^3) greater than milliliters.
4. Which direction are we moving?
   - Since we are moving to a larger unit, our value will be smaller. In this case, the value is smaller by 1,000X
   - In other words, the value is 1/1000 or 0.001 the value.
   - So what is the answer?

FACTORING OUT

Using the idea of factors of ten, you can assess the difference between the two units and cancel out the original unit algebraically to reach the desired final unit.

- 500ml=_____L
  - $1ml = \frac{1}{1000}L$ or, $\frac{1L}{1000ml}$
    - which states 1000 milliliter in every 1 liter
  - $500ml \times \frac{1L}{1000ml} = \frac{500L}{1000} = 0.5L$
    - pay attention to the units and how we've canceled out the ml in the numerator of 500ml and in the denominator in the conversion of 1L in 1000ml

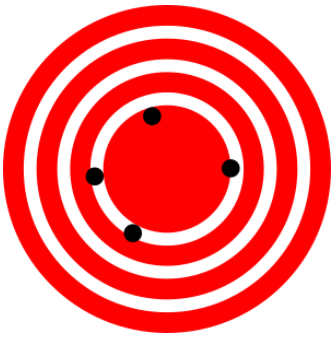
ADDITIONAL RESOURCES





ACCURACY AND PRECISION

Accuracy refers to how closely a measured value agrees with the correct or target value.
Precision refers to how closely individual measurements agree with each other and reflects the repeatability in those measurements.



This illustrates accuracy. Measurements are on target.



This illustrates precision. Measurements are very close to each other and repeatable.



This illustrates Accuracy AND Precision. Each measurement is on target and also highly repeatable.

Instruments have a finite amount of accuracy and it is important to report measurements within that level of accuracy. **Significant figures**, report the number of digits that are known to some degree of confidence with the measuring device. With the increased sensitivity of the equipment, the number of significant figures increases.

PIPETTING BASICS

TYPES OF MICROPIPETTORS

Pipettors are made by many different manufacturers and thus all do not look the same. Learning to correctly use one type of pipettor will provide you the knowledge to use others as they share the same method of distributing small volumes. This lab will illustrate the Rainin Pipetman® micropipettors.

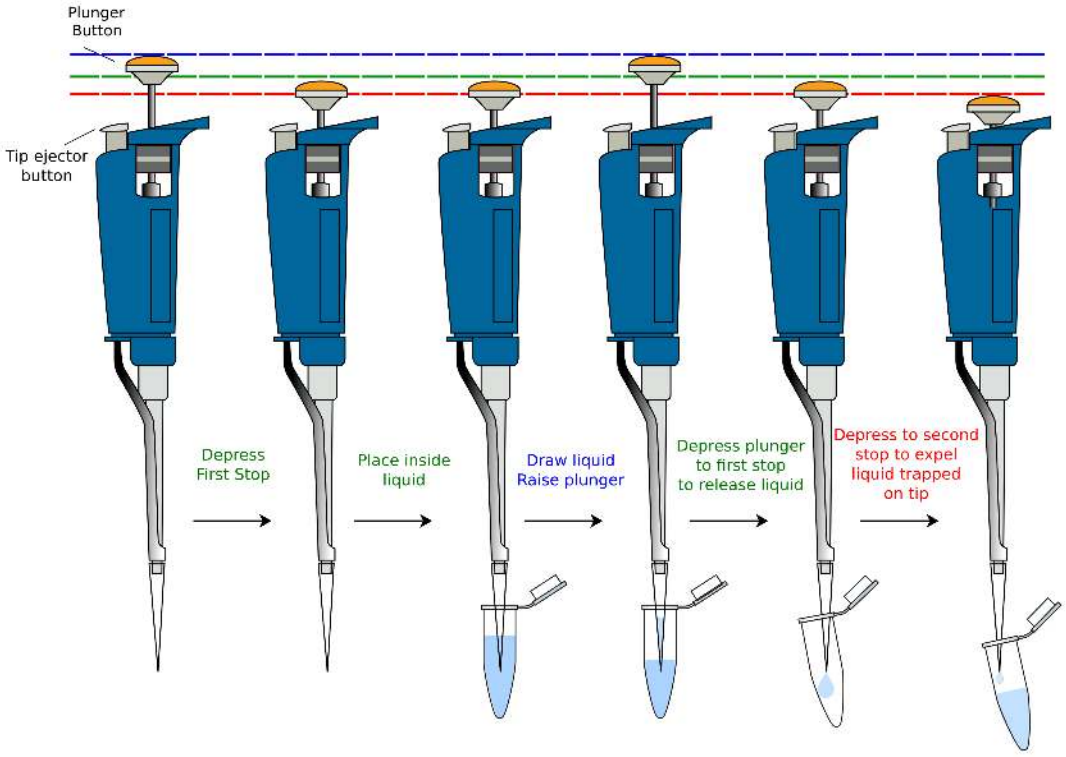
The top of the plunger shows the pipettor size for the Pipetman models. Each pipettor has its own volume range and it is **CRITICAL** to use a pipettor only in its proper volume range. The "P-number" represents the maximum volume in µL that the pipettor can measure. Pipettors are more accurate in the *upper* part of their range. 20 µL should be measured with a P20 rather than with a P200. The four pipettor sizes (P10, P20, P200, P1000) used in our lab will measure from 1 µL – 1000 µL as shown below.

CORRECTLY ADJUSTING THE PIPETTORS

	P10	P20	P200	P1000
Volume Range (µL)	1-10	2-20	20-200 50-200 *	200-1000
	tens 0 ones 4 tenths 7 4.7µl	tens 1 ones 7 tenths 8 17.8µl	hundreds 1 tens 5 ones 4 154µl	thousands 0 hundreds 8 tens 6 860µl

Gilson Pipetman pipetting ranges chart. Note that the P200 officially has a range from 50-200µl

TUTORIAL ON PROPER USAGE



Pipetting sequence. Ensure the plunger is depressed outside of the liquid to displace air and avoid blowing bubbles into solution. Carefully draw the plunger up slowly and follow the liquid to avoid drawing air. Depress the plunger in destination tube to the first stop. Depress to the second stop if fluid remains in the tip to expel full volume.

https://www.youtube.com/watch?v=uEy_NGDfo_8

RULES FOR USE OF THE MICROPIPETTORS

These are precision instruments which can easily be damaged. Treat them with respect and care. They are essential for your success in this course and shared amongst numerous students.

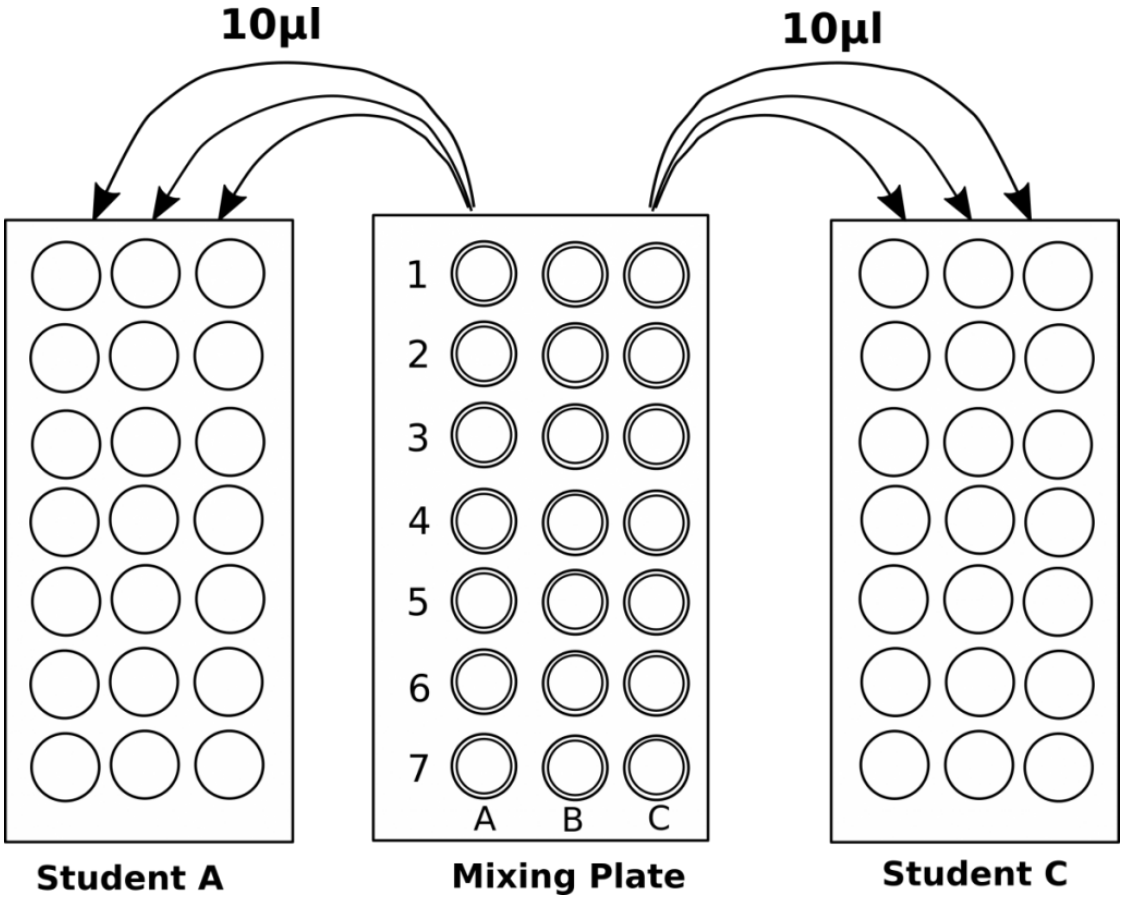
1. Never measure higher or lower than the range of the pipettor allows.
   - An exception to this rule is the P200 labeled 50-200 µl.
   - While we have P100 pipettors for this range, they appear too similar to P20 that they are often confused.
   - Originally, P200 were labeled 20-200 µl and we know that the lower range is less precise on these.
2. Never turn the volume adjuster above or below this range indicated on the pipettor or you risk breaking the instrument.

3. Never allow liquid to get into the micropipettor.
    - This causes contamination.
    - This weakens the seal on the o-rings and can damage them.
4. Never use the micropipettor without a tip.
5. Never invert or lay down the micropipettor with liquid in the tip.
    - Liquids will roll into the piston this way.
6. Never let the plunger snap back when filling or ejecting liquid.
7. Never immerse the barrel in fluid.
    - This causes contamination.
8. Never set the micropipettor on the edge of the bench; this may result in the micropipettor falling or being knocked onto the floor.

EXERCISE: PIPETTING PRACTICE

1. Prepare seven dye mixtures as illustrated in the table below.
    1. One student mix samples in column A and a second mixes in column C.
    2. Column B is left empty and used if one student makes a mistake.
2. Each dye mixture prepared in the first well to reach a total volume of 45 µl.
3. Pipet 10 µl in triplicate from each well of the mixing plate into the center of the appropriate circles on the target card.

Well #	Buffer (µl)	Red (µl)	Blue (µl)	Yellow (µl)	Glycerol (µl)	Alcohol (µl)	Total (µl)
1	40	5	-	-	-	-	**45**
2	15	-	-	10	10	10	**45**
3	20	5	10	10	-	-	**45**
4	30	-	15	-	-	-	**45**
5	22	13	-	-	-	10	**45**
6	25	-	10	-	10	-	**45**
7	20	6	-	6	13	-	**45**



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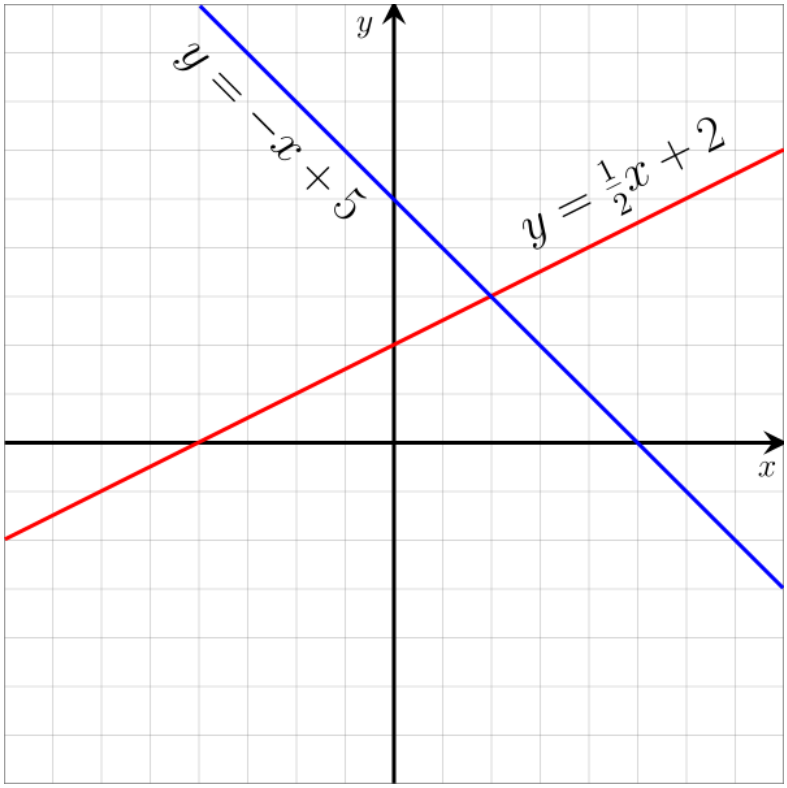
- **1.3: Units of Measure** by Bio-OER is licensed CC BY-NC-SA 4.0.

2.1.1.4: QUANTITATIVE SKILLS

VARIABLES

Experimental science looks at cause and effect types of relationships. Controlled experiments vary one of the factors or traits to observe the effect on another factor or trait. These factors are called **variables**. A **dependent variable** is something that is observed and expected to change as a result of modifying another factor in the experiment. That is to say, the outcome *depends* on another factor. Another name for dependent variable is **responding variable**. The **independent variable** is the factor or condition that is changing or being changed by the experimenter. Sometimes waiting is the condition that is changing, making the independent variable: time. Since we change the independent variable, it is also called the **manipulated variable**.

GRAPHING A LINE



A graph displaying 2 lines and their equations

A line can be described mathematically by the equation:

$$y = mx + b$$

This is referred to as the **slope-intercept form**. The 2 variables **y** and **x** refer to coordinates along each axis. The term **m** refers to the change in the y-values over the change in the x-values. This is referred to as the **slope of the line**. The term **b**, the **y-intercept**, is the y-value where the line crosses the y-axis.

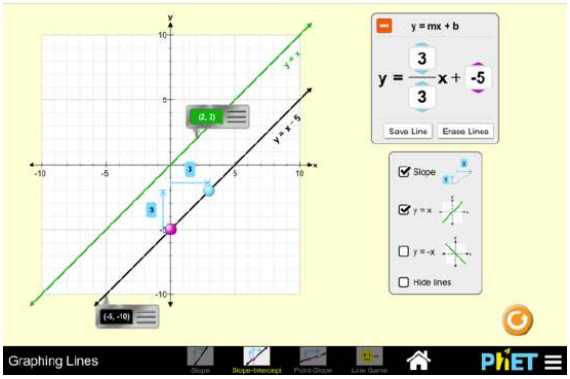
$$m = \frac{y_2 - y_1}{x_2 - x_1} \text{ OR } m = \frac{\Delta y}{\Delta x}$$

This is how the slope of a line (m) is determined.

The slope of the line indicates the relationship between the two variables, x and y. The equation of the line $y = mx + b$ indicates to us that "y" occurs as a function of changes to "x". Sometimes this is represented by the equation $f(x) = mx + b$. Since "y" depends on "x", "y is the dependent variable and "x" is the independent variable. As "x" changes, how does "y" change in response? This is what the slope reveals to us.

For more review, visit the following link.

SLOPE ACTIVITY



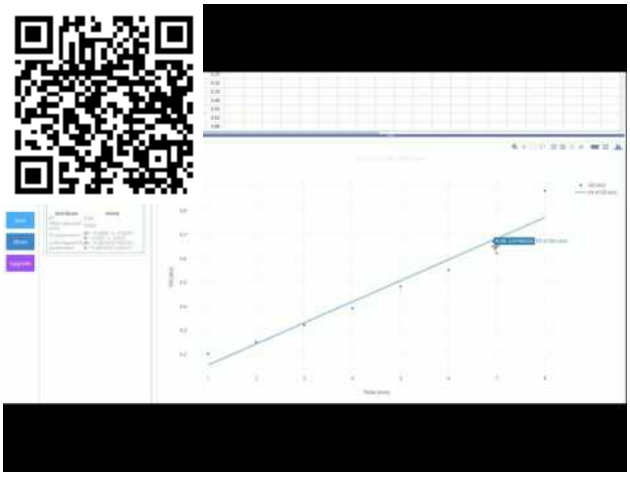
Click here to run the simulation.

DATA PLOTTING ACTIVITY

1. Use the following data from the USDA to plot a **scatterplot** and generate a trend-line.
2. Use the info from the table below or download (a text file that can be opened in notepad/textedit)
3. Follow a tutorial on how to graph a scatterplot with line of best fit (trend-line)
4. Or use this tutorial in plot.ly
    - Show the equation of the trend-line on the graph
    - Ensure that “lbs. Mozzarella” is the y-axis
    - Ensure that “Year” is the x-axis
    - Remember not to use a line graph

Year	lbs. Mozzarella
2000	9.33
2001	9.70
2002	9.66
2003	9.65
2004	9.94
2005	10.19
2006	10.52
2007	11.02
2008	10.57
2009	10.63





WHAT DO WE LEARN?

1. What does this scatterplot tell us about the relationship between consumption of mozzarella in relationship to years?
2. How would this graph influence the way you invest in a mozzarella cheese company? Can you predict anything about the future of cheese consumption?
3. What does the slope of this line indicate to you?
    a. Use mathematics to illustrate this point.
    b. The slope has a unit related to “lbs.” and “year”, what is this unit?

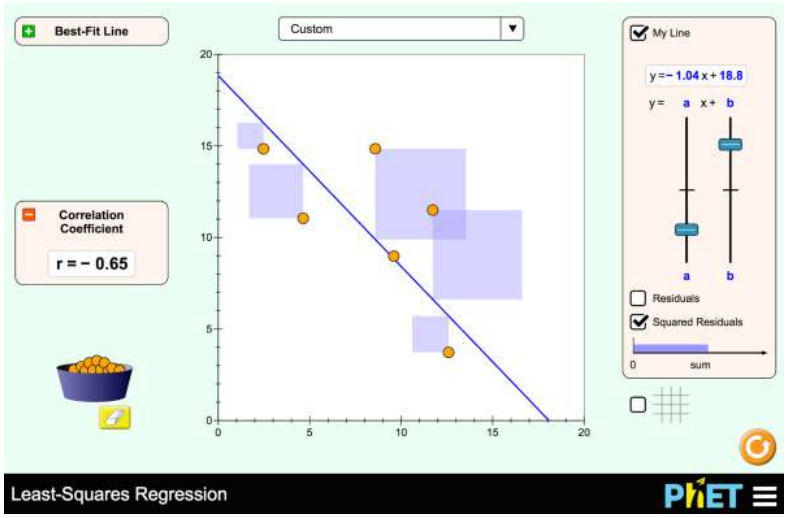
CREATING A LINE OF BEST FIT

Not all points collected will fall on a straight line. A **Line of Best Fit** or a **Trendline** approximates the average of those points through a mathematical process called the **least-squares** method. While one could “eyeball” this line, the least-squares method uses the data to minimize the distance from all those points to the line to have an averaging effect.

1. Create a column of data for “x” values, “y” values, x^2 and xy
2. At the bottom of these columns, sum the data. Σx, Σy, Σx^2, Σxy
3. Calculate the slope from these values
    - $m = \frac{N(\Sigma xy) - \Sigma x \Sigma y}{N(\Sigma x^2) - (\Sigma x)^2}$ (where N=number of entries in a column)
4. Calculate the y-intercept from these values

- $b = \frac{N(\Sigma y) - m(\Sigma x)}{N}$

5. Which provides use the function $f(x) = mx + b$



Click here to run the simulation.

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2.1.1.5: REPORTING ON SCIENCE

LAB REPORTS

TITLE

A description of the main idea or question of the lab. This can also highlight a key finding or question.

ABSTRACT

A brief summary of the main question, methods, and findings. This is usually the last thing written but the first thing presented in order to grab the attention of the reader.

A rough breakdown of an abstract would contain about:

- 3 sentences worth of introduction with the key question
- 2 sentences of major methodology
- 3-6 sentences of the major results and conclusions drawn from them

Lengths will vary, but using this framework, you will not deviate too far from having a reader lose interest.

INTRODUCTION

Introduce the background that is relevant for forming the hypotheses being tested. What were the previous observations or prior knowledge used to come to these ideas? State the actual hypotheses to be tested and how it will further the understanding of the issue.

MATERIALS AND METHODS

This section is a little like a cooking recipe. The main steps taken should be summarized as standard prose in a manner that anyone could follow and repeat. This is written in the past tense and 3rd person. Do not write in the first person as *you* have nothing to do with the experiments. Explain “What was done with which reagents?”

RESULTS

This section is descriptive of what was observed. Figures and tables serve as a summary of the results to illustrate the data. They also serve as guides to outline the text of the section. Slowly describe each figure or table. Expand these points into sentences and paragraphs. Present the data as fully as possible, including stuff that at the moment does not quite make sense. This is written in the past tense and 3rd person. Conclusions are not provided in this section as they are made from analyzing the information and synthesizing the results.

DISCUSSIONS

Discussions are the conclusions made by analyzing the results. At this time, you will be able to re-emphasize the original hypotheses made in the introduction. Indicate whether or not the hypotheses were demonstrated sufficiently. If this is not the case, offer alternatives and interpretations. Can you improve or modify your hypotheses? Explain how multiple lines of evidence corroborate each other and help to further the understanding of the problem.

REFERENCES

Prior knowledge requires demonstration of strength or validity. The references should be from primary sources and should illustrate the point of the statement. The section is presented in numerous formats as a bibliography, but citations are inserted near the text where knowledge or statements are displayed. Use a reference Manager to insert citations and format the bibliography. An excellent free reference manager with a plug-in to common word processors is Zotero.

ADDITIONAL RESOURCES

- MIT OCW

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SECTION OVERVIEW

2.1.2: CHEMISTRY

2.1.2.1: INTRODUCTION

2.1.2.2: WATER

2.1.2.3: BIOLOGICALLY IMPORTANT MACROMOLECULES

2.1.2.4: CHROMATOGRAPHY

2.1.2.5: PH

2.1.2.6: PH (ACTIVITY)

2.1.2.7: CARBOHYDRATES

2.1.2.8: LIPIDS

2.1.2.9: PROTEINS

2.1.2.10: NUCLEIC ACIDS

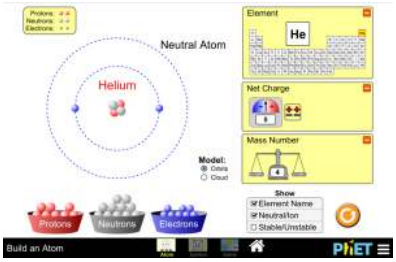
2.1.2.11: BIOLOGICAL MOLECULES (CONCEPT)

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2.1.2.1: INTRODUCTION

CHEMISTRY

Self-Guided activity: http://phet.colorado.edu/files/activities/3807/BAA-Student-Handout.doc



Follow the self-guided activity for a review of basic chemistry before continuing to the organic chemistry section.

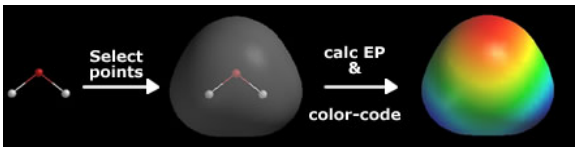
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2.1.2.2: WATER

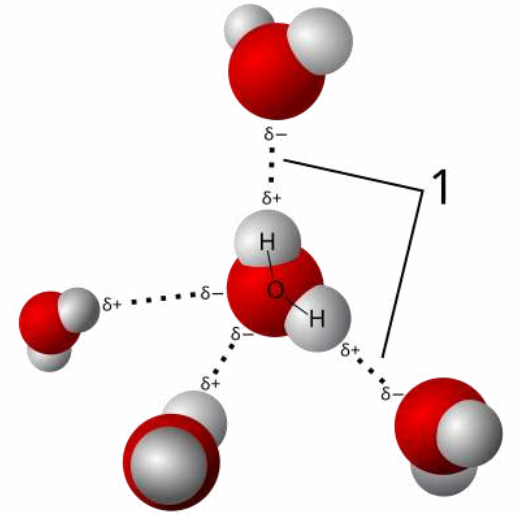
POLAR COVALENT BONDS

H_2O is a polar covalent molecule. The Bonds between the H atoms and the O atom arise from sharing electrons. These shared electrons form to satisfy the **octet rule**. However, oxygen is a "selfish" sharer. This **electronegative** aspect of oxygen means that the electrons of the H_2O molecule preferentially associate near the oxygen atom, creating partial charges. We indicate this by placing a $\boldsymbol{\delta^-}$near the O and $\boldsymbol{\delta^+}$'s near the H atoms. These partial charges make the H_2O **polar**.



The electron cloud around a water molecule lingers around the oxygen molecule to render it partially negative. Red illustrates the partial negative end of the molecule while blue indicates the partial positive.

Because of this polarity, H_2O molecules arrange in a highly structured way. Use the following **simulation to explore polarity of molecules**.



These weak associations that arise from the polar: polar attractions are referred to as **Hydrogen Bonds (H-bonds)**. While independently weak, the summation of all the H-bonds is very strong. These associations give rise to the special properties of water: **surface tension**, **cohesion**, **adhesion**, and **high specific heat capacity**.

- **Hydrogen Bond Simulation**

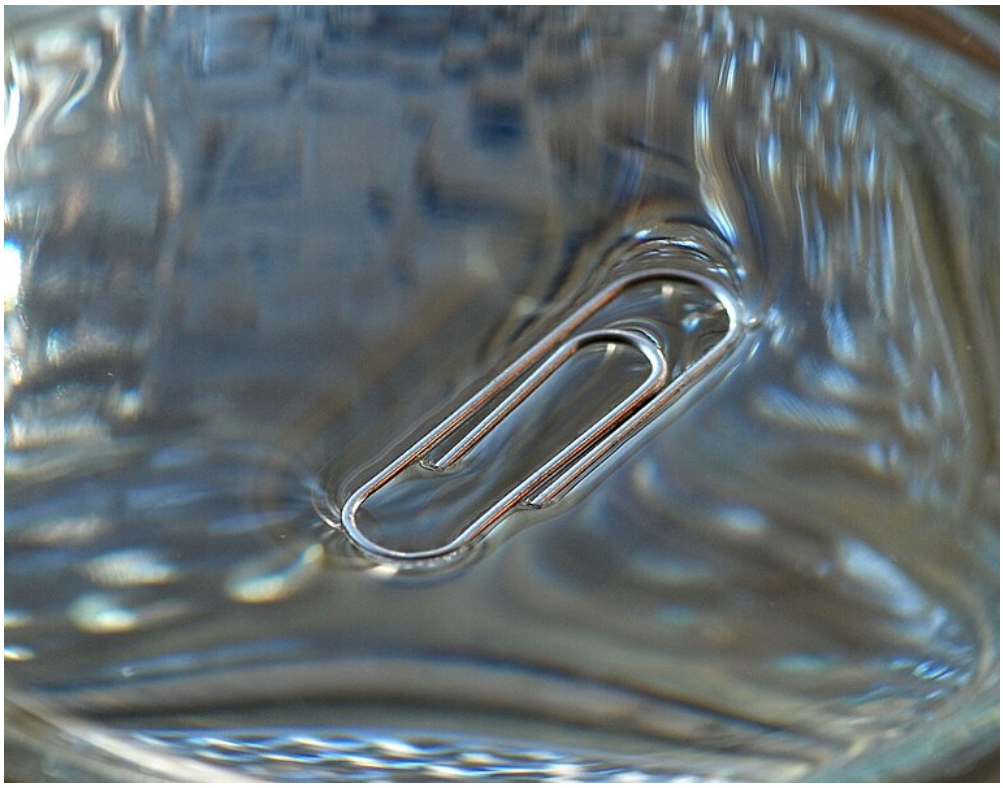
Polar materials mix with polar materials. Things that can dissolve in water are also polar and referred to as being **hydrophilic** (*hydro* = water, *philic* = liking). Non-polar substances do not interact or mix with polar solvents and are referred to as **hydrophobic** (*hydro* = water, *phobic* = hating). Since carbon and hydrogen share electrons equally, organic compounds are non-polar. Oil is a hydrocarbon that does not mix well with water or vinegar. Vinegar, however, is a polar compound that interacts with water. Detergents are called **amphiphilic** (*amphi* = both; *philic* = liking) because they have portions that are non-polar and portions that are very polar. Detergents can, therefore, dissolve in hydrocarbons and water. Water alone cannot effectively remove oil from your skin but a detergent can dissolve the oil and carry it away in water.

File:Sodium laurylsulfonate V.1.svg

The common detergent in soap – Sodium Lauryl Sulfate

SURFACE TENSION

Surface tension presents as an invisible film that encompasses the surface of water. The attractive forces arising from the intermolecular **cohesion** holds the surface of water together.



This paperclip would sink if it broke through the surface of the water.



This water strider is not on top of the water because it is light. It has not broken through the surface of the water and is, therefore, on top of the water.

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SOLUTIONS

Water is an excellent solvent of other polar compounds. Table salt (NaCl) ionizes readily in water. The δ^- O associate around Na^+ while the δ^+ H associate with the Cl^-. If NaCl is dissolved in H_2O, what do you think happens to the intermolecular interactions between water molecules? What do you think would happen to the H-bonds? Would you expect there to be a difference in the surface tension? How do you think this explains the difference of boiling or freezing?

RUNNING ON WATER



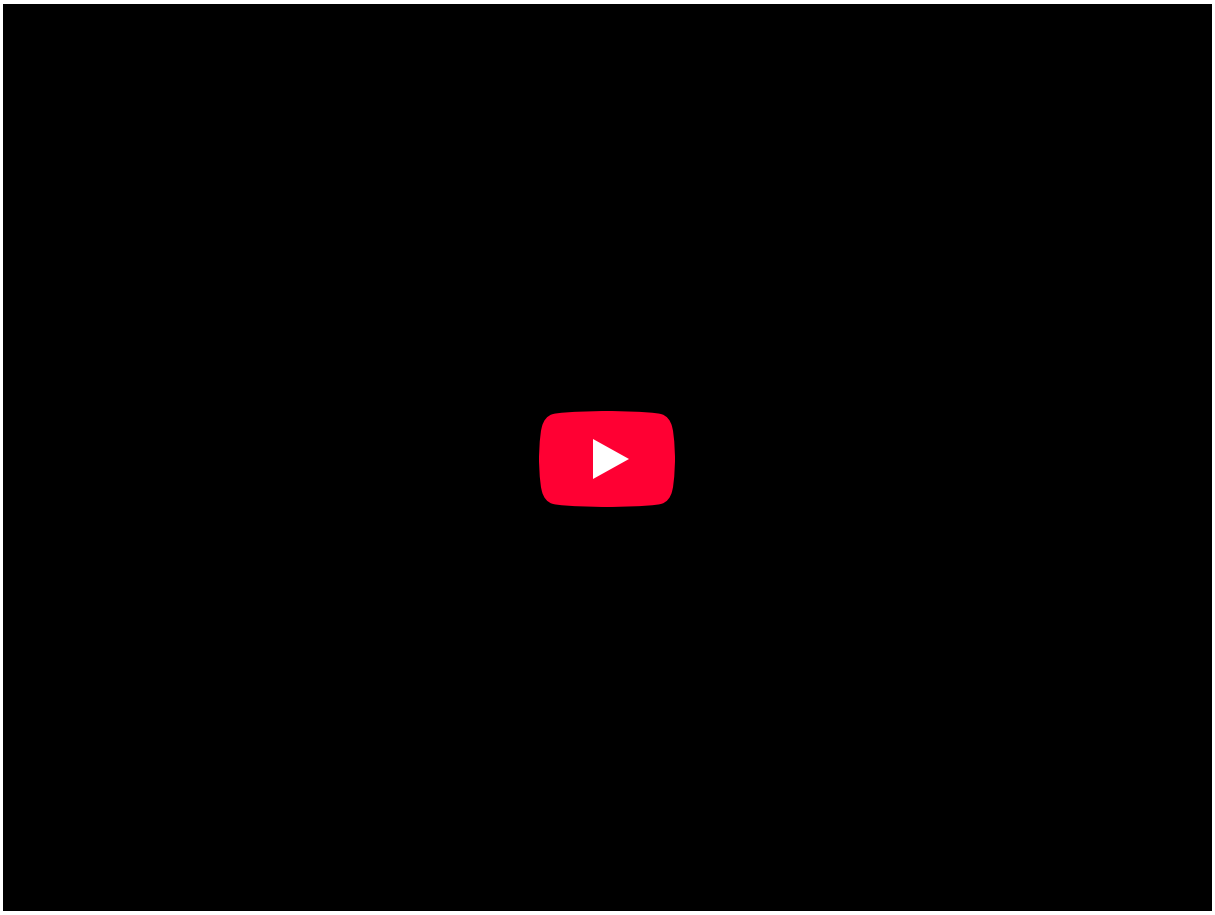
Basiliscus plumifrons (green basilisk)



Basiliscus basiliscus (common basilisk)

Lizards of the genus *Basiliscus* have the nickname “Jesus Christ lizard” for their very special adaptation regarding water.

In the face of danger, these lizards run on their hind legs across the water to escape predation. Their hind legs have long toes that help in increasing surface area to distribute their weight so they can propel themselves on the surface of the water. They do not sink because the surface tension of the water is not broken by the large surface covered by their feet. After about 4.5m, they lose sufficient momentum to propel themselves on the water surface and break through. H-bonds enable this adaptation.





Video from National Geographic

WHAT IF?

The namesake of these lizards walked on water but was also able to turn water into wine. Wine is a solution of ethanol (11%) in water. Ethanol has a polar end and a non-polar end.

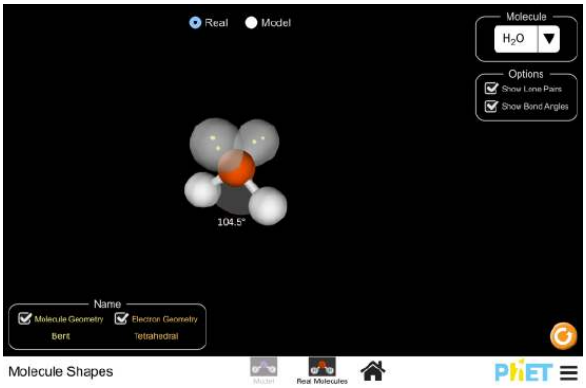
- How successful would the basilisk run on wine?
- Why?
- How could you test this without necessarily using a lizard?

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2.1.2.3: BIOLOGICALLY IMPORTANT MACROMOLECULES

UNDERSTANDING CHEMISTRY IN 3D



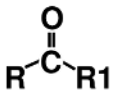
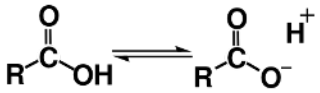
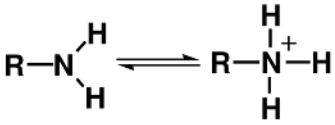
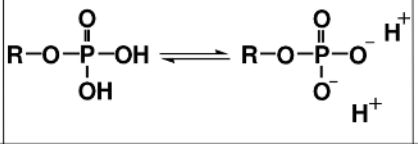
1. Click on the image above to launch the simulation.
2. Choose “Real Molecules”.
3. Click on “Show Bond Angles”.
   - You may also deselect “Show Lone Pairs”.
4. Drag the atoms to understand the 3-D shape.
5. Choose CH_4 from the drop-down menu of molecules.
   - CH_4 is methane, the simplest organic molecule.
   - Notice the restraints on the physical space occupied by the atoms as you drag the atoms around.
   - Remember these bond angles and constraints when we explore organic chemistry.
   - Toggling between “Model” and “Real” will illustrate if the position of the atoms are influenced by other factors we don’t see.
6. Choose NH_4 from the drop-down menu.
   - NH_4 is ammonia, a polar solvent.
   - NH_4 is **NOT** an organic molecule.
   - Toggle the lone pairs to see how these electrons play a role in the positioning of atoms.
7. Choose CO_2 from the drop-down.
   - Explore this non-organic molecule to visualize the effects of double bonds on geometry.

ORGANIC CHEMISTRY

Living things are composed of **organic molecules** primarily made up of the elements carbon and hydrogen. Molecules of hydrogen and carbon (referred to as **hydrocarbons**) have the property of being **nonpolar**. Yet 70- 90% of cells are composed of water (a **polar** compound). Polar substances mix with other polar substances. Likewise, non-polar substances interact with other non-polar compounds. Polar and non-polar compounds are immiscible (unable to mix).

SO HOW DO CELLS KEEP FROM FALLING APART IN A LIQUID ENVIRONMENT?

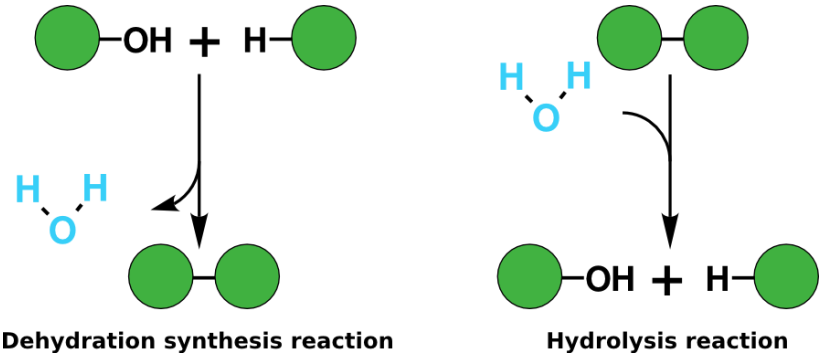
Functional groups are clusters of atoms in a group that impart a new “function” to the compound they are attached to. Hydrocarbons in cells have functional groups attached to them that permit them to interact with the water environment of the cell. These functional groups also define the type of molecule it is based on the characteristics of those groups.

Group Name	Structure	Compound Name	Property
Hydroxyl	R–OH	alcohol	polar
Carbonyl		Aldehyde (R1=H) Ketone (R1=C)	polar
Carboxyl		acid	polar acidic
Amino		amine	polar basic
Sulfhydryl	R–SH	thiol	polar
Phosphate			polar acidic

Functional Groups. *Functional groups commonly found in organic compounds. R is a placeholder chemical that can be anything (like a hydrocarbon chain). The functional groups in this table are those that add polar or charged properties to the hydrocarbon chains. Bi-directional arrows indicate that those functional groups dynamically ionize and reach an equilibrium in solution.*

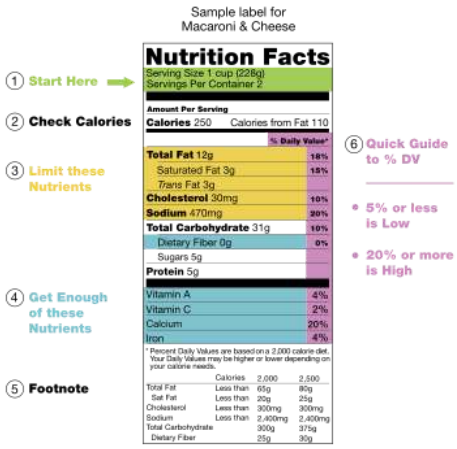
HOW ARE MACROMOLECULES ASSEMBLED?

The common organic compounds of living organisms are **carbohydrates**, **proteins**, **lipids**, and **nucleic acids**. Each of these are macromolecules or **polymers** made of smaller subunits called **monomers**. The bonds between these subunits are formed by a process called **dehydration synthesis**. This process requires energy; a molecule of water is removed (dehydration) and a covalent bond is formed between the subunits. Because a new water molecule is formed, this is also referred to as *condensation*. The opposite where water and energy are used to break apart polymers into simpler monomers is called **hydrolysis** (***hydro***– water, ***lysis***– to break or split).



Polymer formation. Dehydration reactions join monomers. Hydrolysis splits polymers.

WHERE DO WE FIND MACROMOLECULES?



A nutrition label illustrates the breakdown of chemical components of Macaroni and Cheese. This is not limited to the macromolecules discussed here. Items like Iron and Sodium are ions that are important for the function of the cell.

ADDITIONAL RESOURCES

- Carbohydrates http://www.visionlearning.com/en/library/Biology/2/Carbohydrates/61
- Lipids http://www.visionlearning.com/en/library/Biology/2/Lipids/207/reading
- Fats and Proteins http://www.visionlearning.com/en/library/Biology/2/Fats-and-Proteins/62

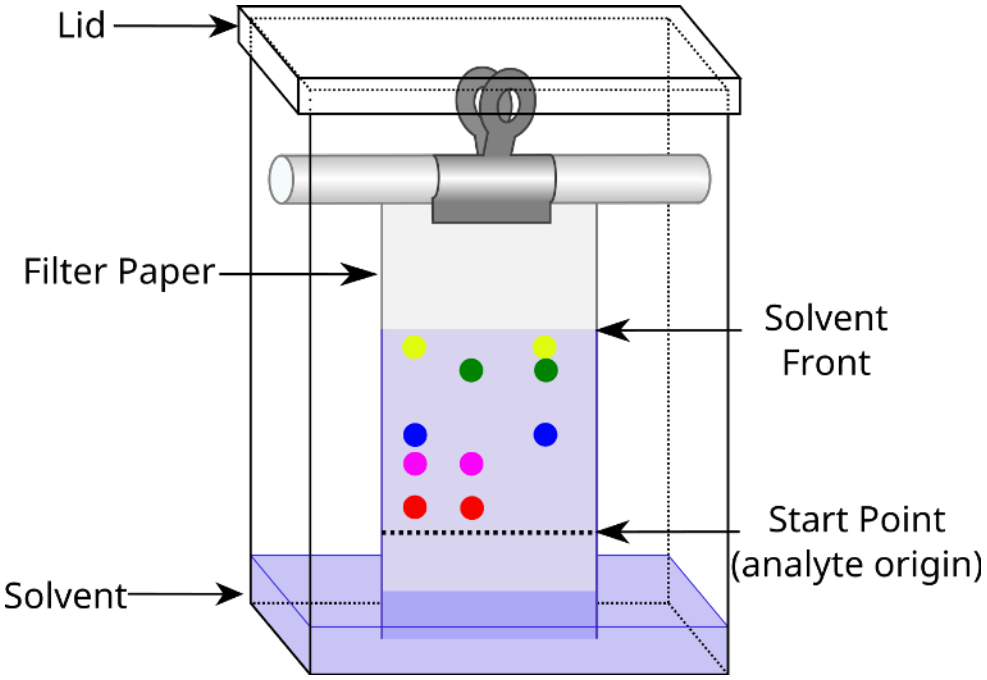
VOCABULARY

- Carbohydrates
- Reducing sugar
- Polymer
- Monomer
- Monosaccharide
- Disaccharide
- Polysaccharide
- Dehydration synthesis
- Hydrolysis
- Reduction
- Aldehyde
- Ketone
- Lipid

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2.1.2.4: CHROMATOGRAPHY



Credit: Theresa Knott [CC BY-SA 3.0 or GFDL]

Chromatography is a collective term for a set of analytical techniques used to separate mixtures. *Chroma* means color and *graph* means to write or draw. Paper chromatography is an analytical technique used to separate mixtures of chemicals (sometimes colored pigments) using a partitioning method. The paper in this method is called the **stationary phase** because it does not move and serves as a substrate or surface for the separation. **Analytes** (substances being analyzed) are separated from each other based on a **differential affinity** to a solvent. The solvent dissolves and carries the analytes along the matrix of the stationary phase. Since the solvent moves through a wicking action, it is called the **mobile phase**.

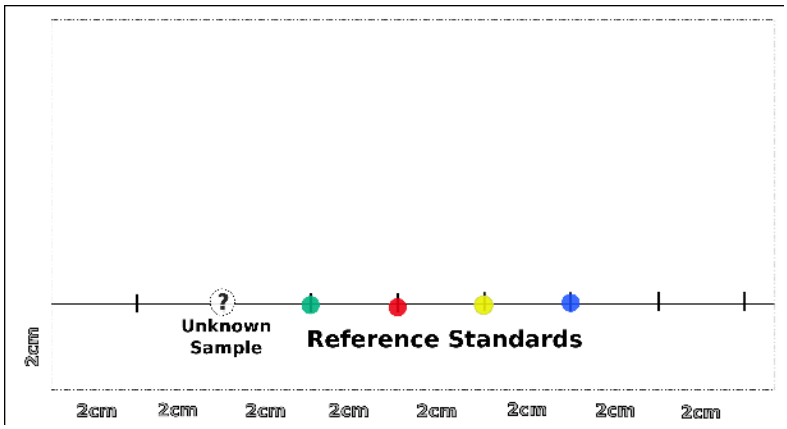
The distance that the analyte migrates along the paper related to the total distance that the solvent or mobile phase moves is called the **Retention Factor** or $\mathbf{R_F}$.

$$R_F = \frac{D_A}{D_S} = \frac{migrationdistanceofanalyte}{migrationdistanceofsolvent}$$

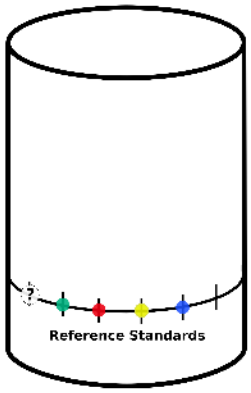
ARE THE FOOD COLORINGS USED IN COLORED CANDY THE SAME AS THE FD&C APPROVED CHEMICALS?

How many colored spots do you expect to see for each reference standard?

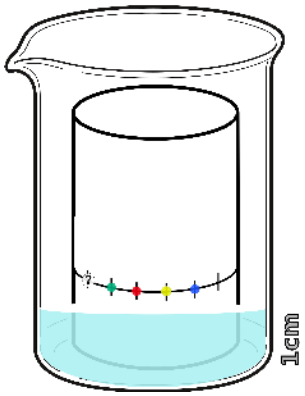
1. Obtain a 25 cm square piece of chromatography paper that will fit into the beaker that will serve as the chromatography chamber.
2. Draw a pencil line across the lower end of the chromatography paper about 2 cm from the bottom.
3. Draw additional vertical tick marks along this line every 2 cm.
4. Place colored candy in a flask with 2 ml ethanol until the color dissolves into the solution.
5. Using an applicator, create a very small spot on a tick mark and allow to dry.
6. Repeat application on the spot to make a very small and dark spot.
7. Continue to spot reference standards along other tick marks. These reference standards are food coloring.



8. Place approximately 1 cm of mobile phase solution (a very polar saltwater solution) into the beaker.
9. Roll the filter paper into a cylinder and fix with staples.



10. Place cylinder into the beaker and cover for 20 minutes **or** until the mobile phase reaches 2 cm from the top of the paper.



11. Mark the final distance of the mobile phase and dry the filter.
12. Measure the distance of each spot from the starting point.
    1. Each spot is a separate analyte.
    2. Some spots separate into multiple analytes.
    3. Measure **EACH** one.
13. Measure the distance from the starting point to the final point that the solvent reached.
14. Calculate R_f values and tabulate results.

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TABLE OF REFERENCE STANDARDS

Reference Standard	Distance (cm)	R_F
Green	D_1=	R_{F1}=
	D_2=	R_{F2}=
	D_3=	R_{F3}=
Red	D_1=	R_{F1}=
	D_2=	R_{F2}=
	D_3=	R_{F3}=
Yellow	D_1=	R_{F1}=
	D_2=	R_{F2}=
	D_3=	R_{F3}=
Blue	D_1=	R_{F1}=
	D_2=	R_{F2}=
	D_3=	R_{F3}=

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TABLE OF UNKNOWNS

Unknown	Distance (cm)	R_F
Brown Candy	D_1=	R_{F1}=
	D_2=	R_{F2}=
	D_3=	R_{F3}=
Blue Candy	D_1=	R_{F1}=
	D_2=	R_{F2}=
	D_3=	R_{F3}=
Red Candy	D_1=	R_{F1}=
	D_2=	R_{F2}=
	D_3=	R_{F3}=
Green Candy	D_1=	R_{F1}=
	D_2=	R_{F2}=
	D_3=	R_{F3}=
Orange Candy	D_1=	R_{F1}=
	D_2=	R_{F2}=
	D_3=	R_{F3}=
Yellow Candy	D_1=	R_{F1}=
	D_2=	R_{F2}=
	D_3=	R_{F3}=

REFLECT

1. Compare and average the R_F values of each analyte across the entire class.
2. Did you predict the number of spots that would appear from each analyte (reference or candy)?
3. Assuming all the dye molecules are of the same mass, what influenced the migration patterns of each spot?
4. Were the colors used in the candy the same as the references?
5. What does it mean if the candy color didn't match anything from the food colors from the cake decorating set used as references?

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2.1.2.5: PH

PH

ACIDS AND BASES

We can call any compound that adds H^+ ions (a free proton) into solution an **acid**. Along with this, we would expect that any compound that would decrease the concentration of free H^+ of a solution as a **base**. **pH** is the *power of* H^+ of a solution. We define this *power* as a **molar concentration** of H^+ in solution. This concentration invariably ends up being a relatively small number (though great in absolute numbers) and is expressed as a decimal number. Because the range of the concentrations is so great, we express these numbers as logarithmic numbers to avoid writing many 0's after the decimal and to facilitate communicating the concentration. Since these numbers are so (relatively) small, we use the negative logarithm to describe this concentration.

Mathematically defined, $pH = -\log_{10}[H^+]$

The pH scale ranges so that anything below pH 7 is acidic and anything above pH 7 is alkaline. So a smaller number is more acidic. But didn't we just state that something acidic contains more H^+ ions? Remember, because we are dealing with a negative Logarithm, this means the concentration is higher.

LOGARITHMIC SCALES

If we have a quantity that is 10^2, we know that translates into 100. Just as if we have a quantity of 10^4, we know that translates into 10000. Just as it becomes inconvenient to keep writing all those 0's, it's really impractical to write many many 0's after a decimal. It's really hard to talk about too! So we likewise will express numbers like 0.0001 as 10^{-4}. A logarithm is the reverse function of an exponent. Therefore:

- $\log_{10}(0.0001) = -4$
- $\log_{10}(10^{-4}) = -4$

So how do we define a solution that is pH 2? Well, we already decided that this solution is below pH 7 — making it an acid. But what does this mean in terms of H^+ ion concentration?
Let's work this out algebraically:

- $pH = -\log_{10}[H^+]$ Let's bring the (-) over to the other side
- $-pH = \log_{10}[H^+]$ Now let's reverse the Log → base 10
- $10^{-pH} = [H^+]$ Plug in the pH → molar concentration of $[H^+]$

As we can now see, a solution of pH 2 is acidic because the molar concentration of $[H^+]$ is 10^{-2}mole/L or 0.01M

DISSOCIATION OF IONS: THAT NUMBER IS SMALL!

It's not a small number. Remember that a mole is 6.022 X 10^{23}. That's a very large number! Think about it! A solution of pH 4 is acidic, but if we plug in the formula, we realize that this is equal to 0.0001M H^+ – less than pH 2 at 0.01M!

But let's compare it to the $[H^+]$ content of H_2O. Now I'm going to sound crazier! Water can be thought of as being in an equilibrium where some of the molecules are ionizing and deionizing. We can express this in 2 ways:

- $H_2O \leftrightharpoons H^+ + OH^-$
- $2H_2O \leftrightharpoons H_3O^+ + OH^-$

So at any given point, a liter of H_2O at neutral pH (7) has 10^{-7} moles of H^+ ions. Incidentally, it also has 10^{-7} moles of OH^- in solution. The second expression indicates the formation of a hydronium ion (H_3O^+) instead of a free proton in solution. So something that is pH 2 is a stronger acid than pH 4, right? Nope. That just indicates the amount of free protons in solution. It is more acidic but acid strength means something else. When we talk about **strong acids**, it means that it is more likely to donate a proton to the solution because it is more likely to ionize. Let's look at the following:

- $HA \leftrightharpoons H^+(aq) + A^-(aq)$ Where HA is an acid dissociating in solution

If this dissociation is very high, then we say that it is a strong acid. Similarly, a compound like NaOH readily dissociates completely in solution and provides OH^- ions that can readily remove H^+ from the solution—a strong base! We speak of dissociation in terms of rates and we express this as the acid dissociation constant, $\mathbf{K_a}$. This is calculated using the concentrations of $[H^+]$ (proton), $[A^-]$ (conjugate base) and [HA] (non-dissociated) at equilibrium:

- $K_a = \frac{[H^+][A^-]}{[HA]}$

Just like the orders of magnitude we have when discussing pH, the rates of dissociation are more conveniently communicated on a logarithmic scale.

- $pK_a = -\log_{10} K_a$

Think about it this way, if the concentration of the dissociated ions is very high, the numerator in the rate is very high → K_a is great. In other words, at equilibrium, the dissociation reaction looks more unidirectional than bi-directional as the compound is readily ionized:

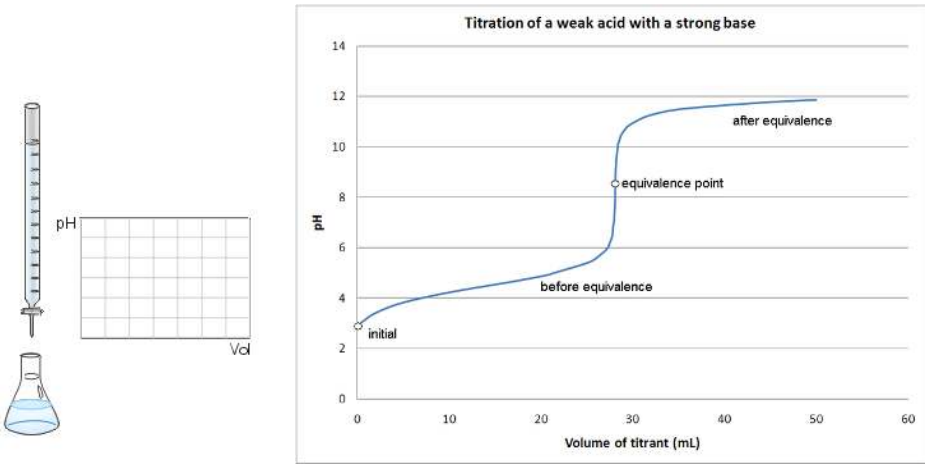
- $HA \rightarrow H^+(aq) + A^-(aq)$

On this scale, we refer to anything with a pK_a < -2 as a strong acid since it will readily dissociate in solution. This form of the dissociation constant is extremely useful in estimating the pH of **buffered solutions** and for finding the equilibrium pH of the acid-base reaction (between the proton and the conjugate base). We can estimate the pH by utilizing the Henderson-Hasselbalch Equation:

- $pH = pK_a + \log_{10}\left(\frac{[A^-]}{[HA]}\right)$

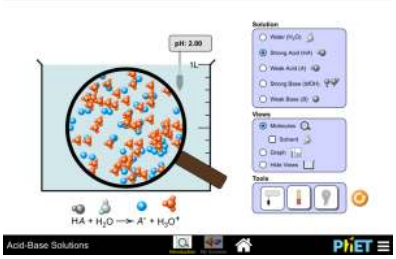
BUFFERED SOLUTIONS

A **buffer** is something that resists change. A **buffered solution** is one that consists of a weak acid or weak base that will control the pH of a solution. Imagine a buffer to be a reservoir of available H+ or OH- ions. If a buffered solution is pH 2, adding a basic solution to it will not cause a drastic change to the pH because the reservoir of H+ will continuously neutralize the base. Eventually, this store or reservoir of H+ will be depleted. When this happens, the pH will suddenly change. The range in which acid or base is added without a significant change in pH is called the buffered zone or the **buffering capacity**. When this store of H+ or buffering capacity is expended, we have reached the **equivalence point** that describes the point at which the base has completely neutralized the weak acid.



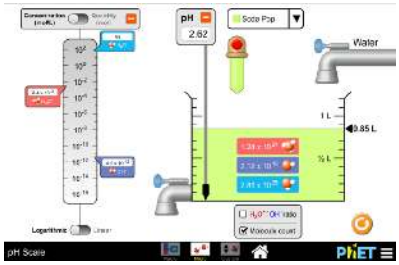
Titration of an acid by a base The solution has a good buffering capacity between pH 3 and pH 5

ACIDS AND BASES SIMULATION



Click here to run the simulation on Acids and Bases

PH SIMULATION



Click here to run the simulation on pH scales

Use the table below to indicate whether an item is an acid or a base and what you predict the pH to be. We can determine these in class through measuring.

Solution	Acid or Base	Predicted pH	Actual pH
Coffee			
Cola			
Distilled H_2O			
Detergent			
Bleach			
Apple Juice			
Antacid Solution			

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2.1.2.6: PH (ACTIVITY)

INQUIRE!

- What is the mechanism of action for antacids?
- Do they all antacids have the same efficacy?

EXPLORE: DETERMINE THE PH OF COMMON SOLUTIONS

1. Use the table below to indicate whether an item is an acid or a base and what you predict the pH to be. We can determine these in class through measuring.

Solution	Acid or Base	Predicted pH	Actual pH
Coffee			
Cola			
Distilled H_2O			
Detergent			
Bleach			
Apple Juice			
Antacid Solution			

2. Using the pH meter, measure the pH and validate your predictions.

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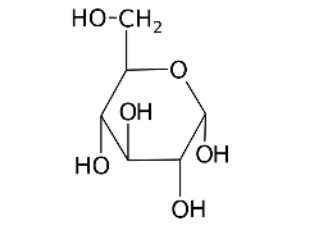
2.1.2.7: CARBOHYDRATES

INTRODUCTION: CARBOHYDRATES

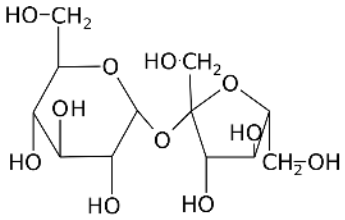
Carbohydrates serve 2 major functions: energy and structure. As energy, they can be simple for fast utilization or complex for storage. Simple sugars are monomers called **monosaccharides**. These are readily taken into cells and used immediately for energy. The most important monosaccharide is glucose ($C_6H_{12}O_6$), since it is the preferred energy source for cells. The conversion of this chemical into cellular energy can be described by the equation below:

$$C_6H_{12}O_6\ (s) + 6\ O_2\ (g) \rightarrow 6\ CO_2\ (g) + 6\ H_2O\ (l) + \text{energy}$$

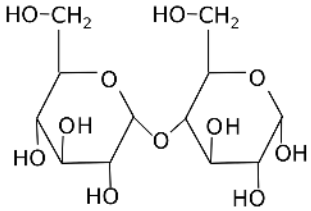
Long polymers of carbohydrates are called **polysaccharides** and are not readily taken into cells for use as energy. These are used often for energy storage. Examples of energy storage molecules are amylose, or **starch,** (plants) and **glycogen** (animals). Some polysaccharides are so long and complex that they are used for structures like **cellulose** in the cell walls of plants. Cellulose is very large and practically indigestible, making it unsuitable as a readily available energy source for cells.



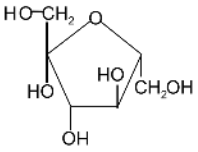
Glucose, a monosaccharide



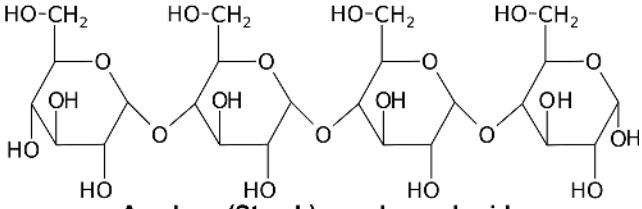
Sucrose, a disaccharide



Maltose, a disaccharide



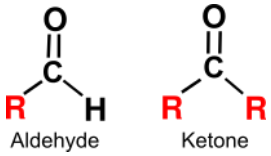
Fructose, a monosaccharide



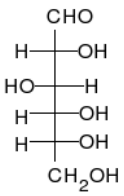
Amylose (Starch), a polysaccharide

Carbohydrates: *Carbohydrates are composed of sugar units referred to as -saccharides.*

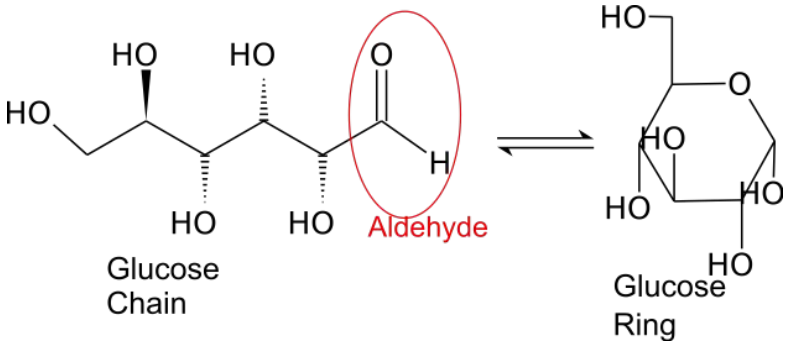
Many monosaccharides such as glucose and fructose are **reducing sugars,** meaning that they possess free **aldehyde** or **ketone** groups that reduce weak oxidizing agents such as the copper in Benedict's reagent. The double bond in the carbonyl group is a source of electrons that can be donated to something else. That is to say, those electrons can be "*lost*" by the sugar and "*gained*" by another chemical. **Benedict's reagent** contains cupric (copper) ion complexed with citrate in alkaline solution. Benedict's test identifies reducing sugars based on their ability to reduce the cupric (Cu^{2+}) ions to cuprous oxide (Cu^{+}) at basic (high) pH. Cuprous oxide is green to reddish-orange. Roughly speaking, **reduction** is a type of chemical reaction that is paired with **oxidation**. In oxidation/reduction reactions (**RedOx**), some chemical loses electrons (oxidized) to another chemical that gains them (reduced). We remember whether a compound is reduced or gained by using the pneumonic: LEO goes GER or **L**oss of **E**lectrons is **O**xidation & **G**ain of **E**lectrons is **R**eduction.



Monosaccharides contain a carbonyl group. The carbonyl is a source of electrons (the double bond on the oxygen). These electrons can be donated (or lost and oxidized) to reduce another compound (that gains those electrons).



Glucose is the preferred carbohydrate of cells. In solution, it can change from a linear chain to a ring.



Monosaccharides are capable of **isomerizing**. This means they alternate in structure from a linear chain to a ring form in solution. In the chain form, the aldehyde is free to donate (lose) electrons to reduce another compound. When monosaccharides undergo dehydration synthesis to form polymers, they can no longer isomerize into chains with free aldehydes and are unable to act as reducing sugars. Green color indicates a small amount of reducing sugars, and reddish-orange color indicates an abundance of reducing sugars. Non-reducing sugars produce no change in color (i.e., the solution remains blue).

Oxidized Benedict's reagent (Cu^{2+}) + Reducing sugar (R-C=O)
(blue)

Heat
High pH

Reduced Benedict's reagent (Cu^{+}) + Oxidized sugar (R-COOH)
(green to reddish orange)

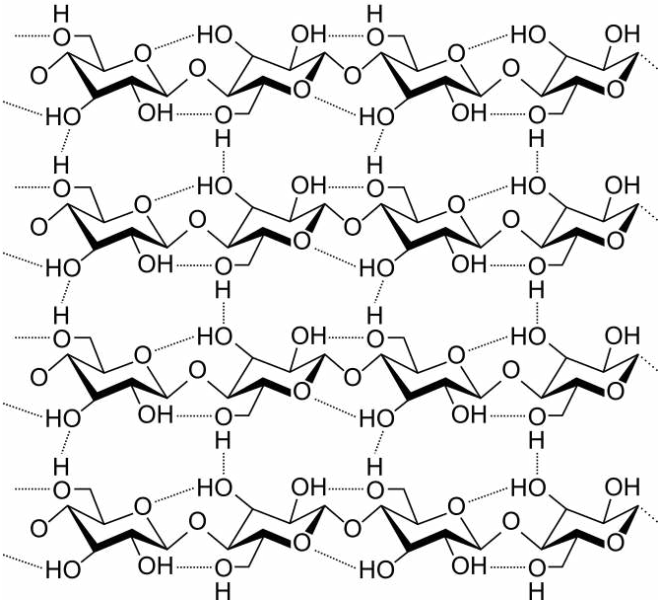
Note: Cu^{2+} has fewer electrons than Cu^{+}.

When monosaccharides undergo dehydration synthesis to form polymers, they can no longer isomerize into chains with free aldehydes and are unable to act as reducing sugars. Green color indicates a small amount of reducing sugars and reddish-orange color indicates an abundance of reducing sugars. Non-reducing sugars produce no change in color (i.e., the solution remains blue).

STRUCTURAL CARBOHYDRATES

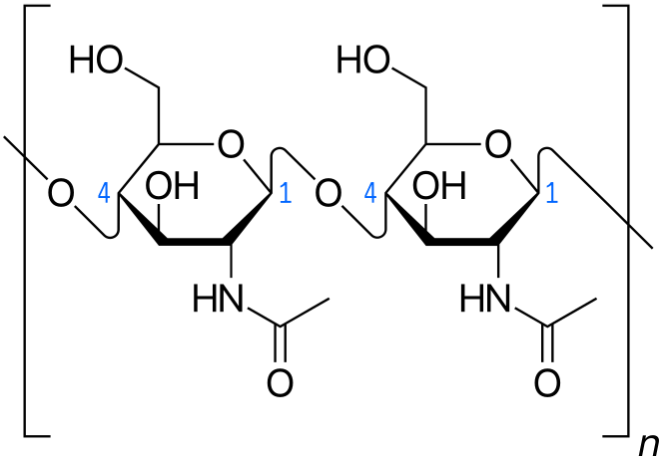
In food, more complex carbohydrates are derived from larger polysaccharides. These larger carbohydrates are fairly insoluble in water. Dietary fiber is the name given to indigestible materials in food most often derived from the complex carbohydrates from vegetable material. Some of this material serves the plants as a structural component of the cells and is completely insoluble. **Cellulose** is the major structural carbohydrate found in plant cell walls. Similarly, animals and fungi have structural carbohydrates that are composed of the indigestible compound called **chitin**. We will not be testing for these items.

Cellulose (plant structural)



Cellulose is a complex carbohydrate of glucose molecules. It is the major structural component of plant cell walls. It' structural durability is enhanced by intramolecular hydrogen bonds.

Chitin (animal and fungi)



Chitin is a structural carbohydrate found in animal shells or fungi cell walls. The polymer contains amide groups that differentiate it from other carbohydrates composed of glucose.



A cicada molting from its shell made of chitin.

TEST YOUR KNOWLEDGE

- http://www.visionlearning.com/en/library/Biology/2/Carbohydrates/61/quiz

DETECTION OF CARBOHYDRATES (ACTIVITY)

MATERIALS:

- potato juice
- apple juice
- urine sample 1
- urine sample 2
- reducing sugar solution
- starch solution
- Benedict's Reagent
- Sucrose solution
- glucose solutions
- distilled water
- hot plates
- beakers of water
- test tubes
- test tube rack

STOP AND THINK

- Use your senses and previous observations/experiences about the qualities of the experimentals.
- Formulate some hypotheses about the carbohydrate content of the experimentals or unknowns.
  - Identify if the sample is experimental or control before making a hypothesis.

QUESTION: ARE THERE SIMPLE REDUCING SUGARS IN MY JUICE? ARE THERE SIMPLE REDUCING SUGARS IN MY URINE?

Diabetes mellitus is a disease that refers to the inability of the cells to take in glucose. The word diabetes refers to urination and mellitus refers to sweetness. Since the cells of diabetics cannot remove glucose from the blood, there is an excess of glucose circulating that is eliminated in the urine. The traditional method of diagnosing someone with diabetes mellitus was to taste the sweetness of the patient's urine. Let's use Benedict's test for the detection process instead of the unhygienic alternative.

Make a hypothesis and ask what we would predict from a Benedict's test if testing a urine sample of someone with diabetes mellitus.

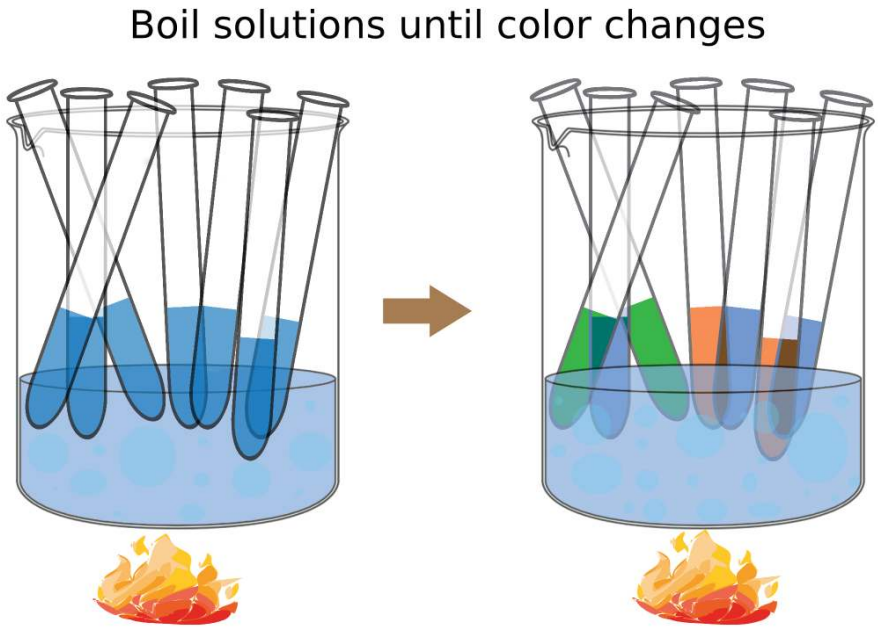
BENEDICT'S TEST FOR REDUCING SUGARS

1. Obtain 9 test-tubes and number them 1-9.

2. Add to each tube the materials to be tested. Your instructor may ask you to test some additional materials. If so, include additional numbered test tubes.
3. Indicate in the table whether the sample you are testing is positive control, a negative control, or an experimental.
4. Before you begin the heating of the samples, predict the color change (if any) for each sample. (use the sample type to aid in your prediction)
5. Add 40 drops (or 2 ml) Benedict's solution to each tube.
6. Place all of the tubes in a boiling water bath for 3 min or until a noticeable color change and observe colors during this time.
7. After 3 minutes, remove the tubes from the water bath and let them cool to room temperature. Record the color of their contents in the Table.

Table:Solution And Color Reactions For Benedict's Test For Reducing Sugars

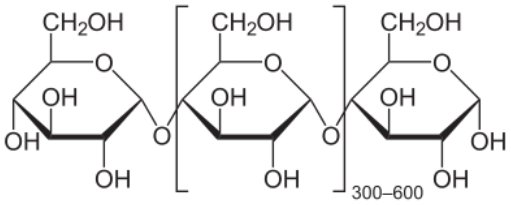
Tube	Solution	Type of Sample (unknown, + control, - control)	Hypothesized Color	Benedict's Test Color
1	10 drops apple juice			
2	10 drops potato juice			
3	10 drops sucrose solution			
4	10 drops glucose solution			
5	10 drops distilled water	- **Control**	**Blue**	
6	10 drops reducing sugar	+ **Control**	**Red**	
7	10 drops starch solution			
8	10 drops Urine sample 1			
9	10 drops Urine sample 2			



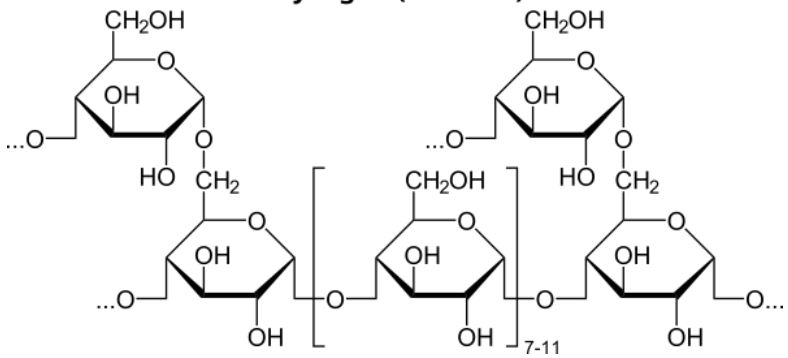
IODINE TEST FOR STARCH

Carbohydrates that are used for energy storage are not reducing sugars since they are polymers that lack free aldehydes. Plant cells store energy in the form of starches like amylose or pectin. Since these molecules are larger than monosaccharides or disaccharides, they are not sweet to the taste and are not very soluble in water. **Iodine** (iodine-potassium iodide, I_2KI) staining distinguishes starch from monosaccharides, disaccharides, and other polysaccharides. The basis for this test is that starch is a coiled polymer of glucose — iodine interacts with these coiled molecules and becomes bluish-black. Iodine does not react with other carbohydrates that are not coiled and remains yellowish brown. Therefore, a bluish-black color is a positive test for starch, and a yellowish-brown color (i.e., no color change) is a negative test for starch. Notably, glycogen, a common energy storage polysaccharide in animals, has a slightly different structure than does starch and produces only an intermediate color reaction.

Amylose or Starch (Plants)



Glycogen (Animals)



Plants store carbohydrates as a simple repeating polymer of glucose called starch. Amylose is a type of starch. Animal cells store glucose into a storage polymer called glycogen which is slightly more complicated than amylose.

ACTIVITY: IODINE TEST FOR STARCH

1. Obtain 7 test-tubes and number them 1-7.
2. Hypothesis Testing: Indicate in the table if the sample is experimental or control. Predict your expected color changes for each sample.
3. Add to each tube the materials to be tested as indicated in the table below. Your instructor may ask you to test some additional materials. If so, include additional numbered test tubes.
4. Add 10 drops of iodine to each tube. This test does **NOT** require boiling.
5. Record the color of the tubes' contents in the table below.

Table: Solution And Color Reactions For Iodine Test For Starch

Tube	Solution	Type of Sample (unknown, + control, - control)	Hypothesized Color	Iodine Test Color
1	20 drops apple juice			
2	20 drops potato juice			
3	20 drops sucrose solution			
4	20 drops glucose solution			
5	20 drops distilled H_2O	- **Control**	**Yellow**	
6	20 drops reducing sugar			
7	20 drops starch solution	+ **Control**	**Black-Purple**	

QUESTIONS FOR REFLECTION

1. In the Benedict's test, which of the solutions is a positive control? Which is a negative control?
2. Which is a reducing sugar, sucrose or glucose?
3. Which contains more reducing sugars, potato juice or onion juice?
4. Is there a difference between the storage of sugars in onions and potatoes?
5. Which patient sample likely comes from a diabetic patient and how do we know this?
6. In the Iodine test, which of the solutions is a positive control? Which is a negative control?

7. Which is more positive for the iodine test: onion juice or potato juice?
8. What can you infer about the storage of carbohydrates in onions? In potatoes?
9. Describe the half-reaction $\mathbf{Cu^{2+} \rightarrow Cu^{+}}$ as oxidation or reduction.
10. Describe the half-reaction $\mathbf{Cu^{+} \rightarrow Cu}$ as oxidation or reduction.

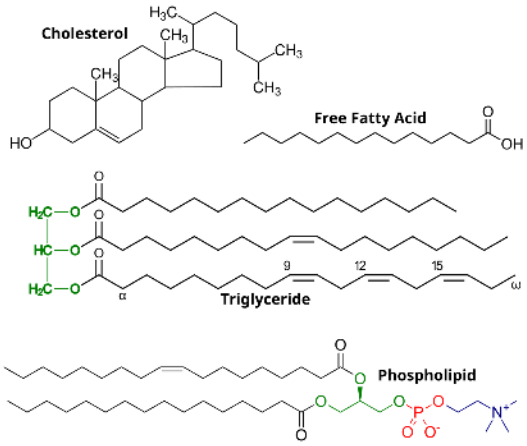
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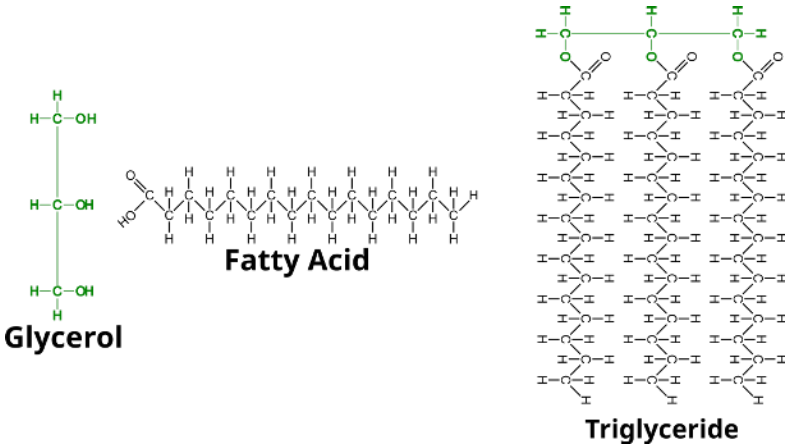
2.1.2.8: LIPIDS

STRUCTURE OF LIPIDS

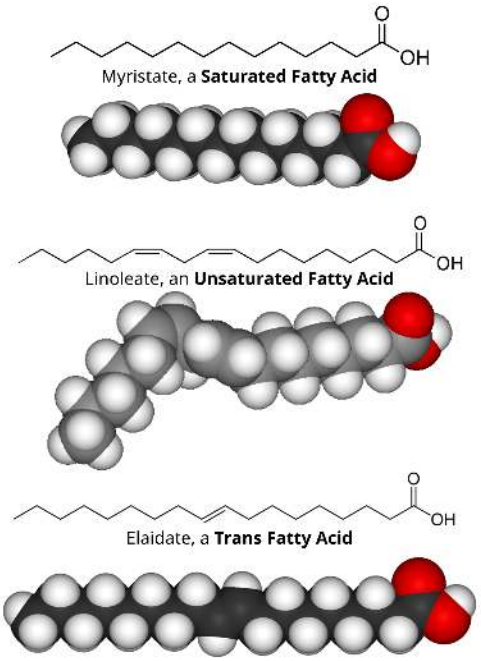
Lipids are the class of macromolecules that mostly serve as long-term energy storage. Additionally, they serve as signaling molecules, water sealant, structure, and insulation. Lipids are insoluble in polar solvents such as water and are soluble in nonpolar solvents such as ether and acetone.



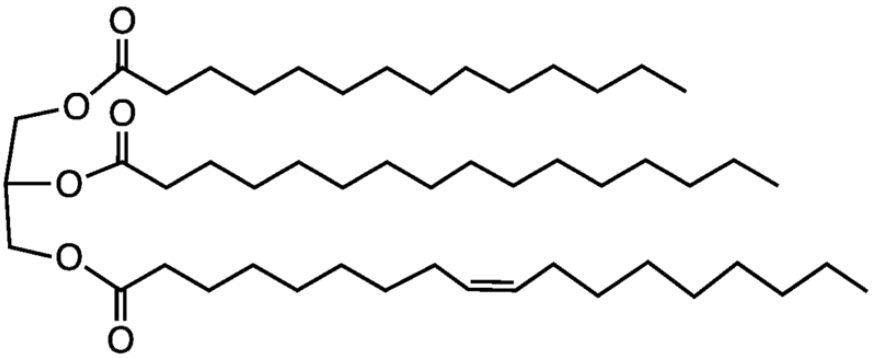
Fats or triglycerides are made of glycerol and three fatty acid chains. They form through 3 dehydration synthesis reactions between a hydroxyl of the glycerol and the carboxyl group of the fatty acid.



SATURATED VERSUS UNSATURATED FATS



- A saturated fatty acid. The molecule takes up little space in three dimensions. Many molecules can stack upon each other. Saturated fats are solid at room temperature.
- A polyunsaturated fatty acid. A kink from the double bond increases the amount of three-dimensional space that the molecule fills. Unsaturated fats tend to be liquid at room temperature.
- A trans-fatty acid. Despite an unsaturated bond, the molecule fills as much space as a saturated fatty acid and is solid at room temperature. Trans fats usually arise from artificial saturation techniques.



Butterfat is almost completely saturated. Notice how molecules can stack very closely.



Because butterfat can stack together very closely, it is dense and found as a solid at room temperature. Credit: Steve Karg (CC BY 2.5)

TESTING FOR LIPIDS

Tests for lipids are based on a lipid's ability to selectively absorb pigments in fat-soluble dyes such as Oil Red O or Sudan IV.

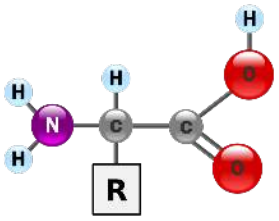
Tube	Solution	Control (+/-) or Experimental	Reaction Description
1	1 ml vegetable oil + water		
2	1 ml vegetable oil + Oil Red O		
3	1 ml water + Oil Red O		
4	1 ml Lipid Solution + Oil Red O		

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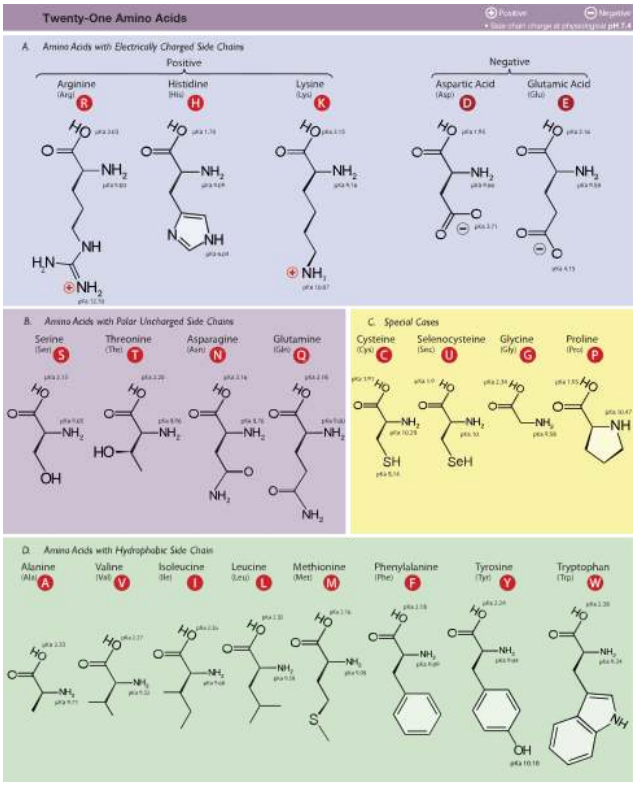
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2.1.2.9: PROTEINS

PROTEINS ARE POLYMERS OF AMINO ACIDS

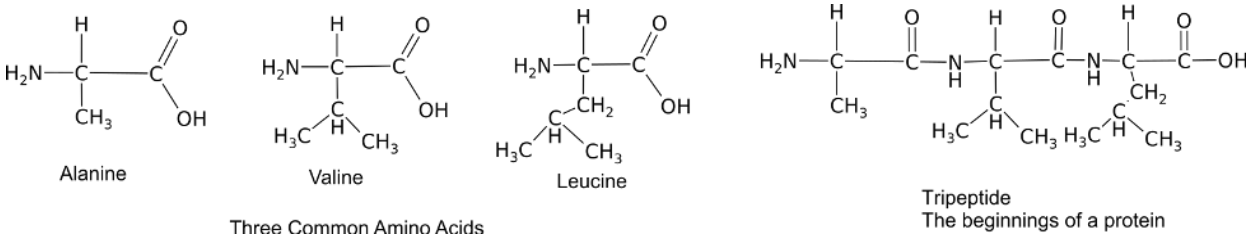


Proteins provide much of the structural and functional capacity of cells. Proteins are composed of monomers called amino acids. **Amino Acids** are hydrocarbons that have an **amino group** (-NH_2) and an acidic **carboxyl group** (-COOH). The R group represents a hydrocarbon chain with a modification that alters the properties of the amino acid. 20 universal amino acids are used to construct proteins. The variation in functional groups along the amino acid chain gives rise to the functional diversity of proteins.



20 amino acids and their properties. A 21st amino acid on this table represents the non-universally found selenocysteine.

Monomers bond together through a dehydration synthesis reaction between adjacent amino and carboxyl groups to yield a **peptide bond**.



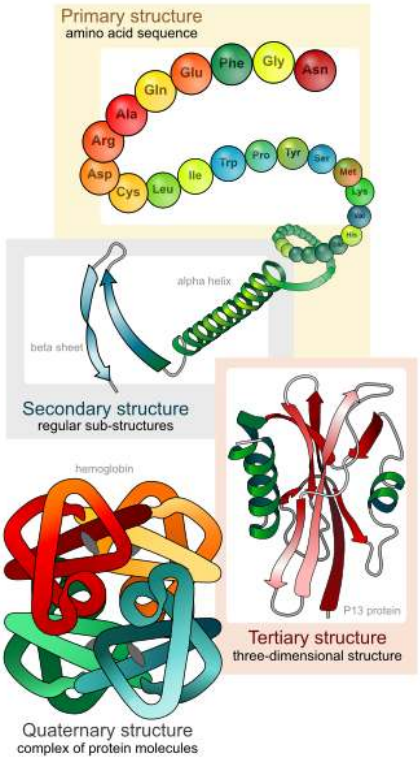
Three amino acids bound into a tripeptide.

HOW AMINO ACIDS INTERACT WITH EACH OTHER AND THE ENVIRONMENT

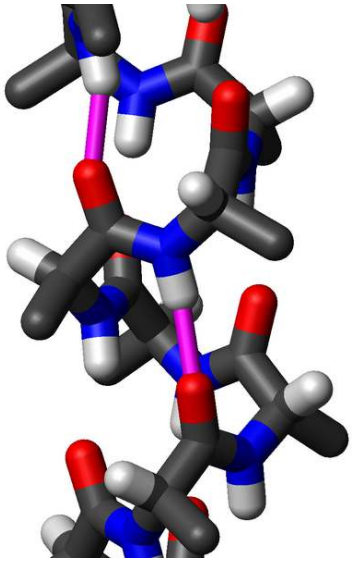
Use the following simulation to test how a polypeptide chain with fold based on the type of solution it is in and the composition of the amino acids.

- Protein Folding Simulation (CC BY 4.0 Concord Consortium)

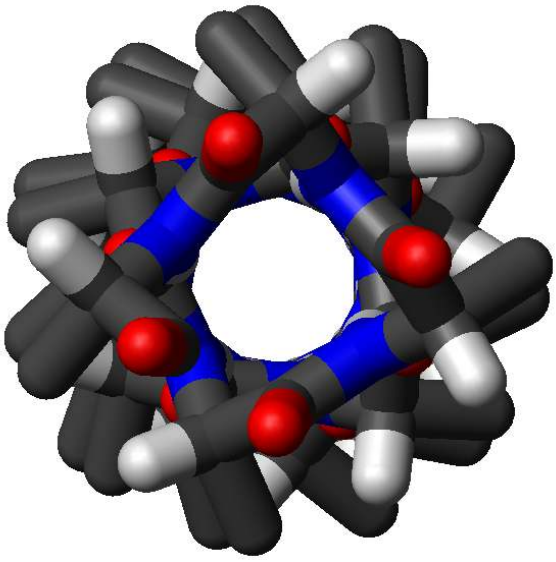
LEVELS OF STRUCTURE



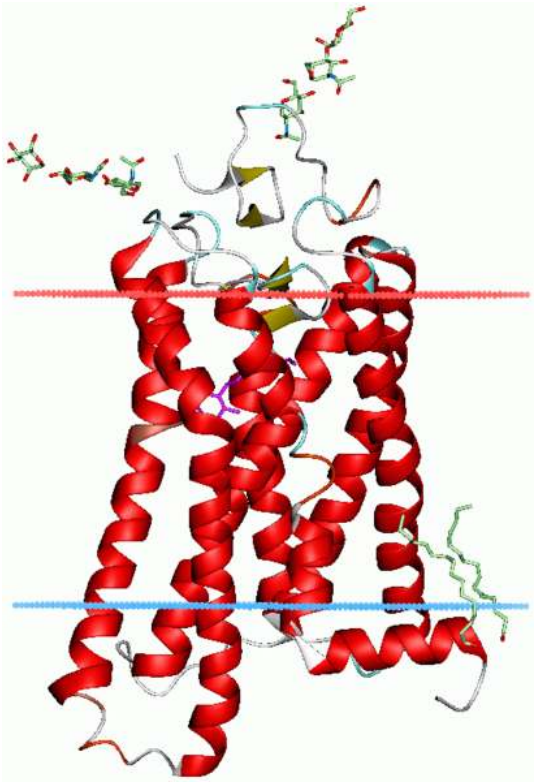
- **Primary Structure (1°)**: The sequence of amino acids read from the Amino or N-terminal end of the molecule to the Carboxyl or C-terminal end
  - Tyr-Cys-Arg-Phe-Leu-Val-….
- **Secondary Structure (2°)**: local three-dimensional structures that form from interactions of amino acids, like hydrogen bonding
  - **Alpha Helix** – coils occurring from the H-bonds between N-H and C=O groups along the backbone of the protein



Side view of α-helix illustrating H-bonds in magenta between carboxyl oxygen (red) and amine nitrogen (blue)

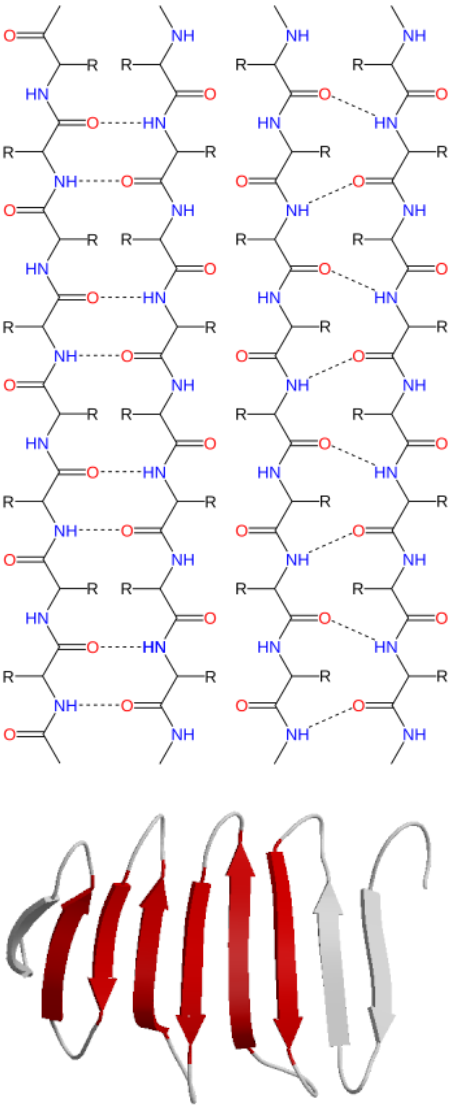


Top-down view of an α-helix



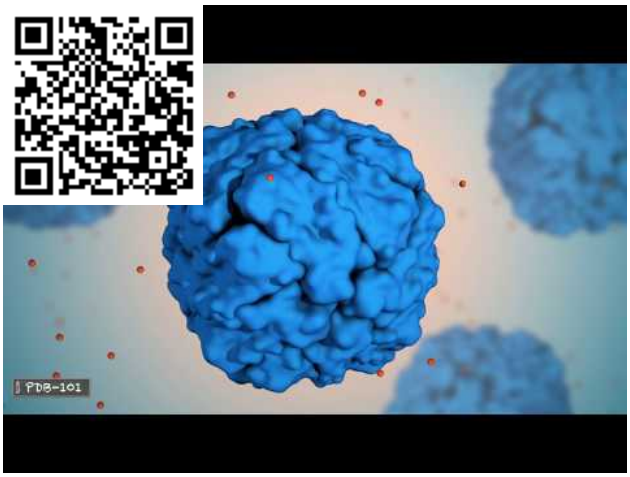
Side view of ribbon diagram of α-helices traversing a membrane.

- **Beta Sheets** – laterally connected strands or sheets of amino acids occurring from the H-bonds between N-H and C=O groups along the backbone of the protein

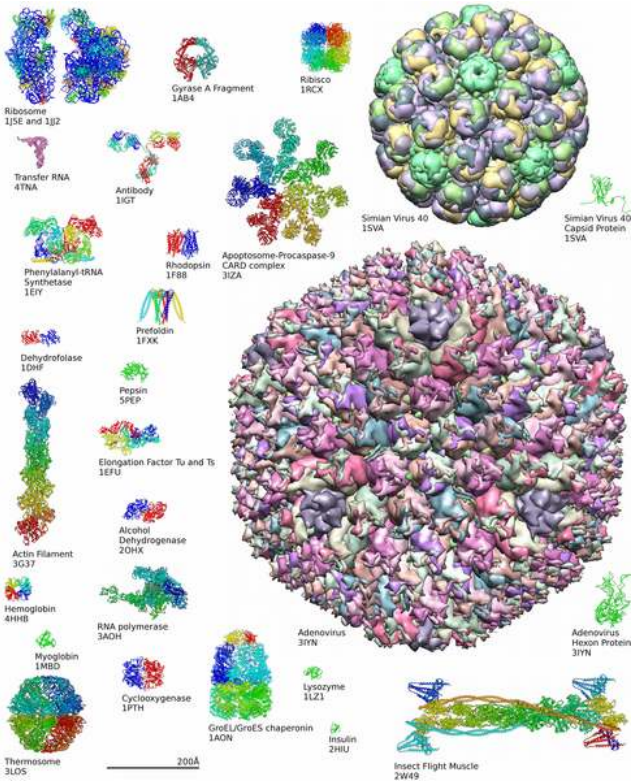


Ribbon diagram of β-sheets

- **Tertiary Structure (3°)**: overall 3-D structure of the peptide chain
- **Quaternary Structure(4°)**: multimeric protein structure from assembling multiple peptide subunits



DIVERSITY OF PROTEINS

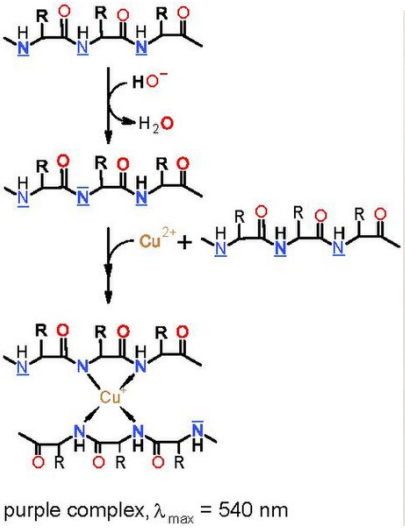


Learn more about the complexity of protein structures at the Protein Data Bank.

PROTEIN DETECTION (ACTIVITY)

PROTEIN DETECTION THEORY:

Proteins can be detected through the use of the **Biuret test**. Specifically, peptide bonds (C-N bonds) in proteins complex with Cu^{2+} in Biuret reagent and produce a violet color. A Cu^{2+} must complex with four to six peptide bonds to produce a color; therefore, free amino acids do not positively react. Long polypeptides (proteins) have many peptide bonds and produce a positive reaction to the reagent. **Biuret reagent** is an alkaline solution of 1% $CuSO_4$, copper sulfate. The violet color is a positive test for the presence of protein, and the intensity of the color is proportional to the number of peptide bonds in the solution.





BIURET TEST

1. Examine the table below. Indicate if the sample is a negative control, positive control or an experimental.
2. Predict the color change of the solution.
   - Formulate a hypothesis about the components of the experimentals.

3. Obtain 6 test tubes and number them 1-6.
4. Add the materials listed in the table.
5. Add 3 drops of Biuret reagent (1.0% $CuSO_4$ with NaOH) to each tube and mix.
6. Record the color of the tubes' contents in Table.

Tube	Solution	Control (+/-) or Experimental	Predicted Color Change	Actual Color Change
1	2 ml urine sample 1			
2	2 ml urine sample 2			
3	2 ml egg albumin			
4	2 ml amino acid solution			
5	2 ml distilled water			
6	2 ml protein solution			

CONCLUSIONS ABOUT THE URINE SAMPLES

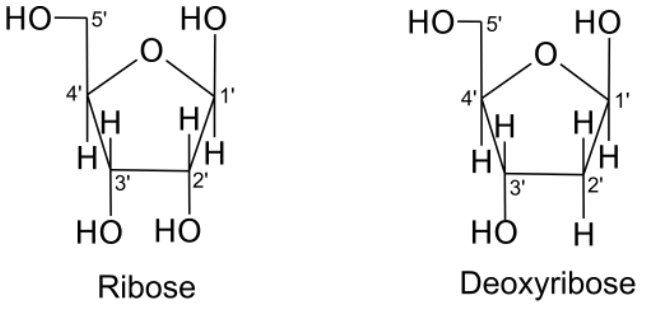
Based on the results of the Benedict's test and the Biuret test, can we make any conclusions?

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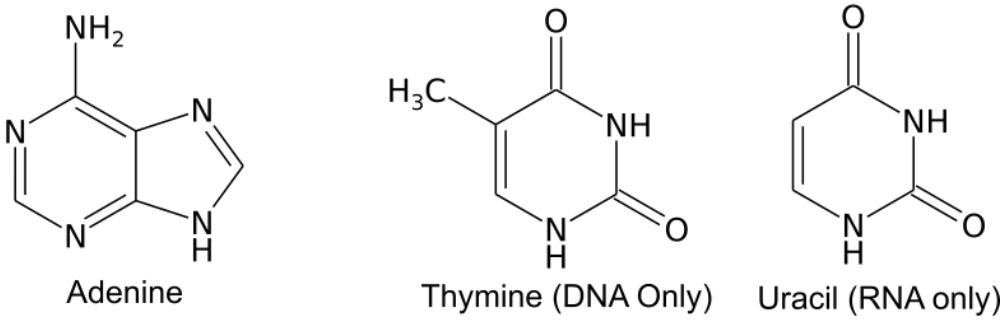
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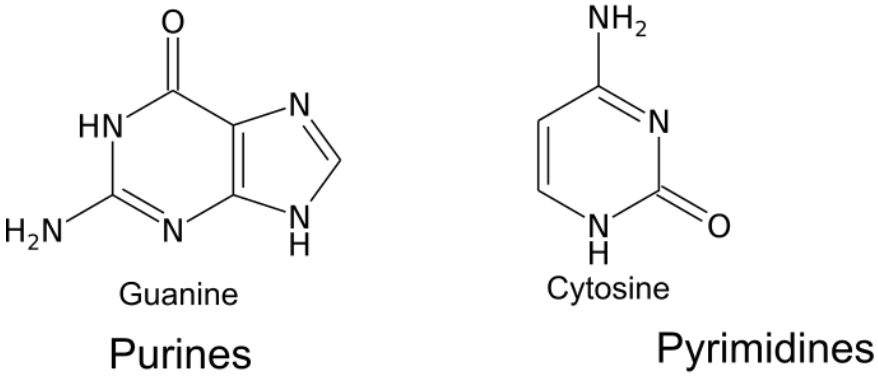
2.1.2.10: NUCLEIC ACIDS

NUCLEIC ACIDS



The Two Sugar Subunits Of Nucleic Acids

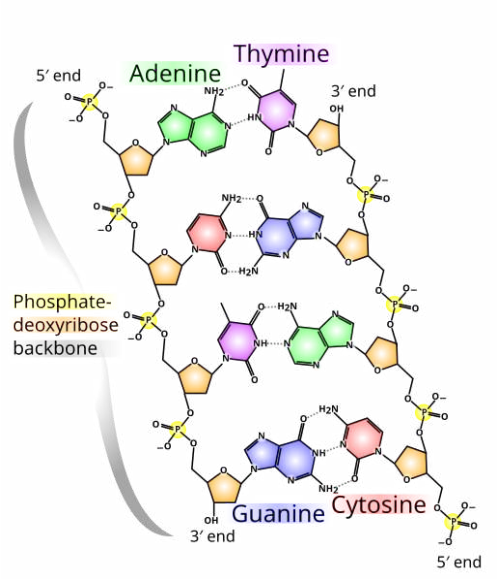




The Five Nitrogenous Bases of Nucleic Acids

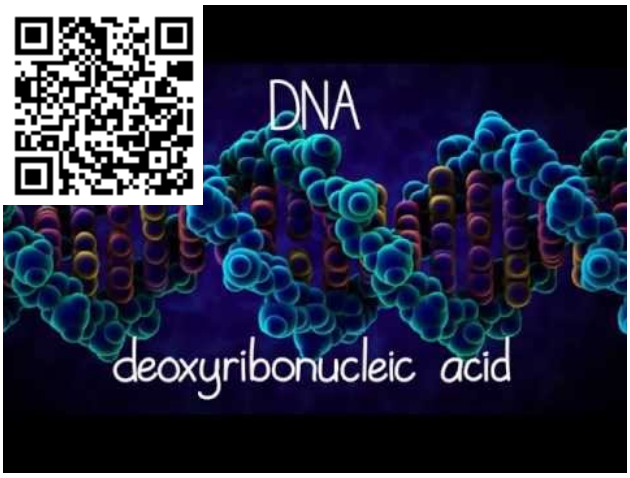
Nucleic acids are composed of linked nucleotides. DNA includes the sugar, deoxyribose, combined with phosphate groups and combinations of thymine, cytosine, guanine, and adenine. RNA includes the sugar, ribose with phosphate groups and combinations of uracil, cytosine, guanine, and adenine.

DNA and RNA are nucleic acids and make up the genetic instructions of an organism. Their monomers are called **nucleotides**, which are made up of individual subunits. Nucleotides consist of a 5-Carbon sugar (a pentose), a charged phosphate and a **nitrogenous base** (Adenine, Guanine, Thymine, Cytosine or Uracil). Each carbon of the pentose has a position designation from 1 through 5. One major difference between DNA and RNA is that DNA contains deoxyribose and RNA contains ribose. The discriminating feature between these pentoses is at the 2′ position where a hydroxyl group in ribose is substituted with a hydrogen.

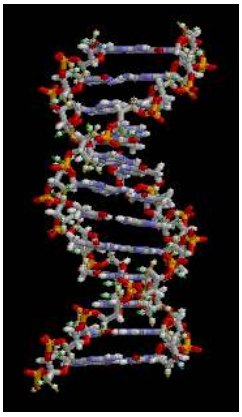


DNA has a double-helical structure. Two antiparallel strands are bound by hydrogen bonds.

The following video illustrates the structure and properties of DNA.



DNA is a double helical molecule. Two antiparallel strands are bound together by hydrogen bonds. Adenine forms 2 H-bonds with Thymine. Guanine forms 3 H-bonds with Cytosine. This AT & GC matching is referred to as **complementarity**. While the nitrogenous bases are found on the interior of the double helix (like rungs on a ladder), the repeating backbone of pentose sugar and phosphate form the backbone of the molecule. Notice that phosphate has a negative charge. This makes DNA and RNA, overall negatively charged.



There are 10 bases for every complete turn in the double helix of DNA.

NUCLEIC ACIDS: DNA EXTRACTION AND DISCHE'S DIPHENYLAMINE TEST (ACTIVITY)

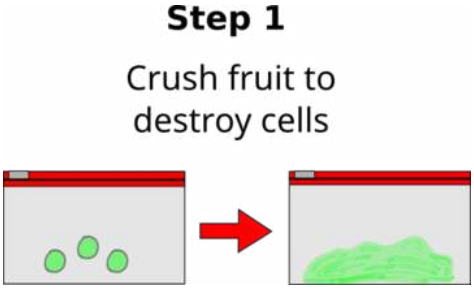
PRELAB QUESTIONS

1. What are fruits?
    a. Where do they come from?
    b. What are they made of?
2. Use phylogeny to classify plants (DKPCOFGS)
3. Where is DNA located within the fruits? Where is it located in you?
4. Why would you want to extract DNA from an organism?
5. What class of molecule is DNA?

EXTRACTION OF DNA FROM FRUIT

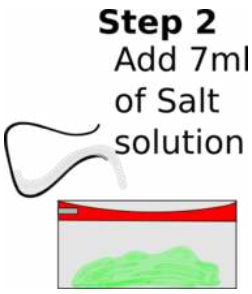
single panel instructions can be found at https://github.com/jeremyseto/bio-oer/blob/master/figures/chemistry/DNA/fruitdnaisolation.svg

1. Mash about 10g or 3cm of over-ripe banana OR 3 grapes OR 1 strawberry in zip-top bag.



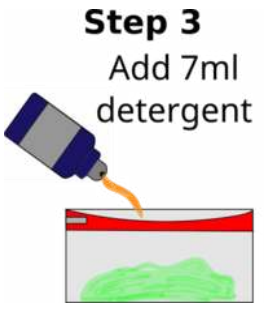
- Over-ripe banana is best since the cell walls are already decomposing
- Physical mashing continues to break up the cell walls

2. Add 7ml of salt solution.



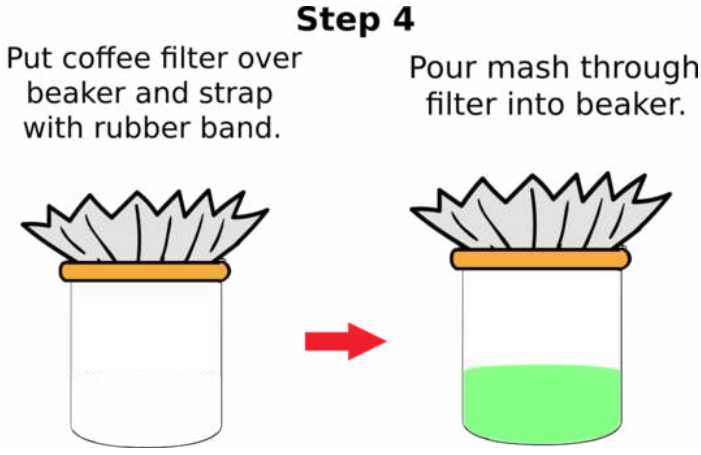
- The salt solution helps the DNA to aggregate (clump together).

3. Add 7ml of liquid detergent and mix.



- Dissolves the lipids in the cell and nuclear membranes
- Releases DNA into the salt solution

4. Place a coffee filter over a cup or beaker and fasten with an elastic band.

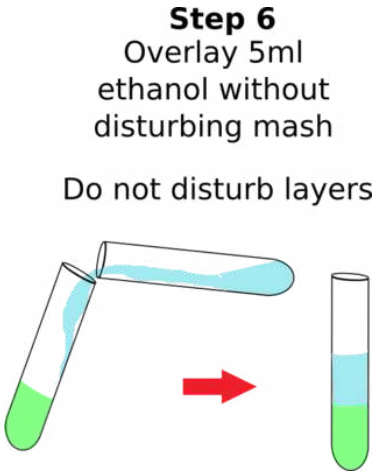


- Pour mash through the filter into a beaker

5. Pour about 5ml of filtrate into a test tube.

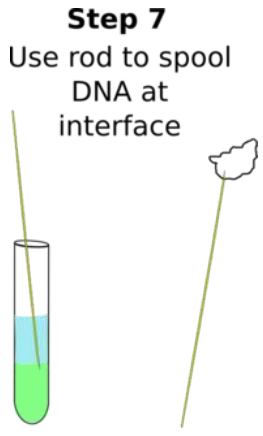


6. Slowly pour an **EQUAL** volume of cold ethanol down the side of the tube to form a layer on top of the fruit fluid.



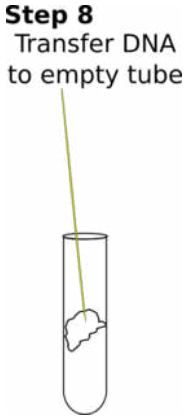
- Carefully run the alcohol down the side to form a separate layer on top of the fruit solution.
- Do not mix the alcohol and banana solution.
- Ice-cold 100% ethanol works best.

7. Spool the DNA: use a plastic loop or glass rod to gently swirl at the interface of the two solutions.



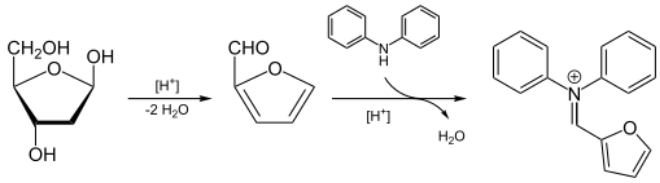
- The interface is where the two solutions meet.
- DNA is not soluble in alcohol.
- Bubbles may form around a wooly substance (this is the DNA).

8. Transfer the DNA.



DISCHE DIPHENYLAMINE TEST FOR DNA

DNA can be identified chemically with the **Dische diphenylamine test**. Acidic conditions convert deoxyribose to a molecule that binds with diphenylamine to form a blue complex. The intensity of the blue color is proportional to the concentration of DNA. The Dische's Test will detect the deoxyribose of DNA and will not interact with the ribose in RNA. The amount of blue corresponds to the amount of DNA in solution.



The diphenylamine compound of the Dische's test interacts with the deoxyribose of DNA to yield a blue coloration.

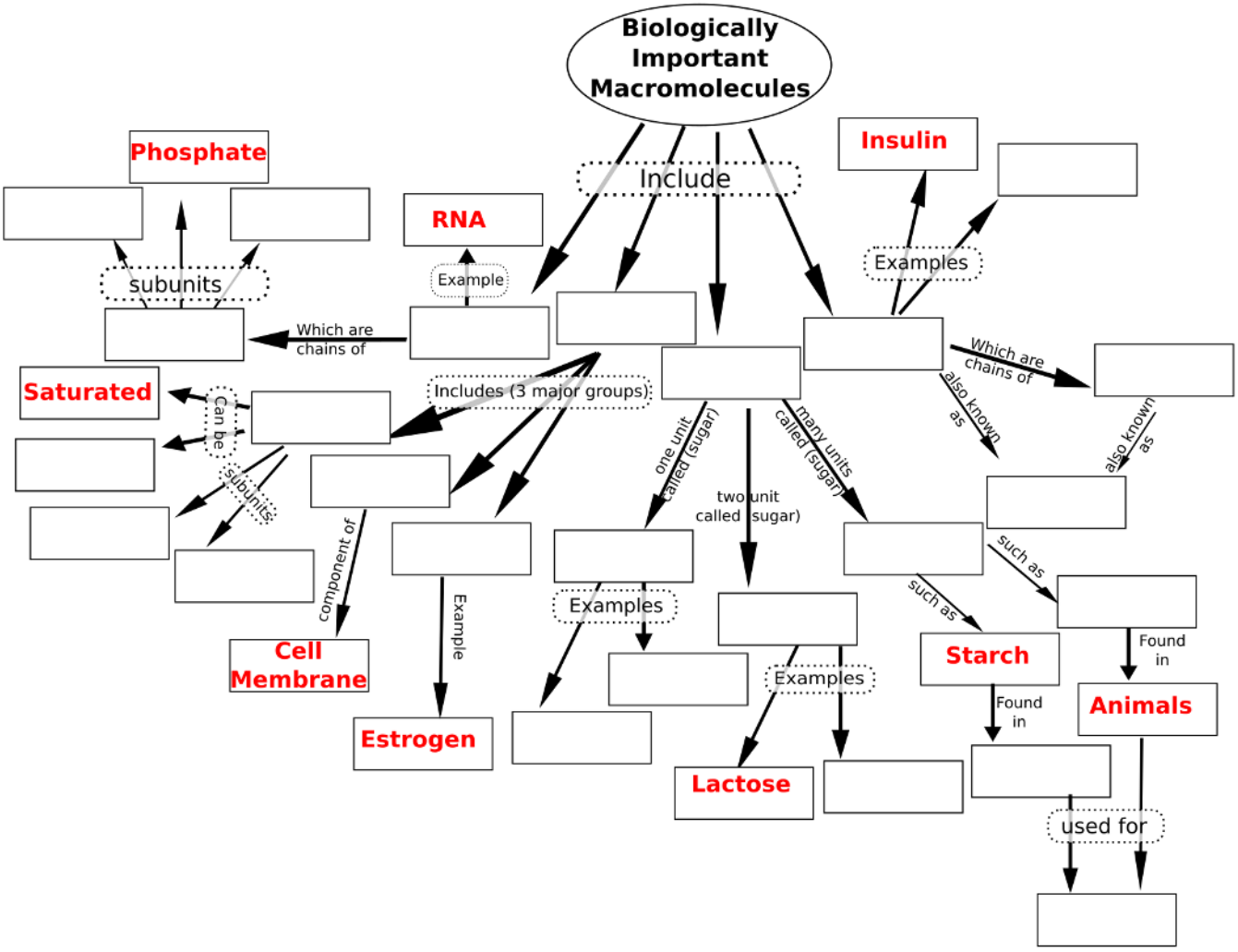
1. Obtain 3 test tubes and number them 1-3.
2. Suspend the spooled DNA in 3 ml of distilled water. MIX.
3. Add to tubes:
    1. 2 ml of DNA solution
    2. 1 ml of DNA solution with 1 ml H_2O
    3. 2 ml of H_2O
4. Add 2 ml of the Dische's diphenylamine reagent to each tube and mix thoroughly.
5. Place in a boiling water bath for 10 minutes.
6. Evaluate your results. A clear tube indicates no nucleic acids. A blue color indicates the presence of DNA. A greenish color indicates the presence of RNA.

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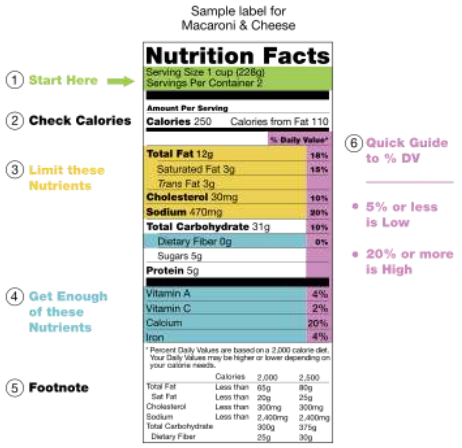
2.1.2.11: BIOLOGICAL MOLECULES (CONCEPT)

MACROMOLECULES CONCEPT MAP



Download the PDF.

SUMMARIZING MACROMOLECULE DETECTION:



A nutrition label illustrates the breakdown of chemical components of Macaroni and Cheese. This is not limited to the macromolecules discussed here. Items like Iron and Sodium are ions that are important for the function of the cell.

Fill in the table below to illustrate how molecules in the above nutrition table were detected. What would you use as a Positive control? What would be the outcome of that Positive Control (color)? What about negative controls? Using the nutrition label above or a similar one, indicate what the test result would be if using the individual tests and indicate if that molecule is absent or present. Remember, if the sugars (simple reducing sugars) and the dietary fiber don't add up to the total carbohydrates, the remainder is starch.

Table 1. Determination of Biological Macromolecules in **Some Food Item**

Test Name	Test Purpose	Positive Control		Negative Control		Food Item	
		Control substance	Color Result	Control substance	Color Result	Color Result	Present or Absent
Benedict's							
Iodine							
Biuret							
Oil Red O							

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SECTION OVERVIEW

2.1.3: QUANTITATIVE DETERMINATIONS

2.1.3.1: INTRODUCTION

2.1.3.2: ENZYMES

2.1.3.3: EXPLORING BEER'S LAW (VIRTUAL)

2.1.3.4: QUANTITATIVE DETECTION OF PROTEIN (ACTIVITY)

2.1.3.5: QUANTITATIVE DETECTION OF PROTEINS (SPECTROVIS PLUS)

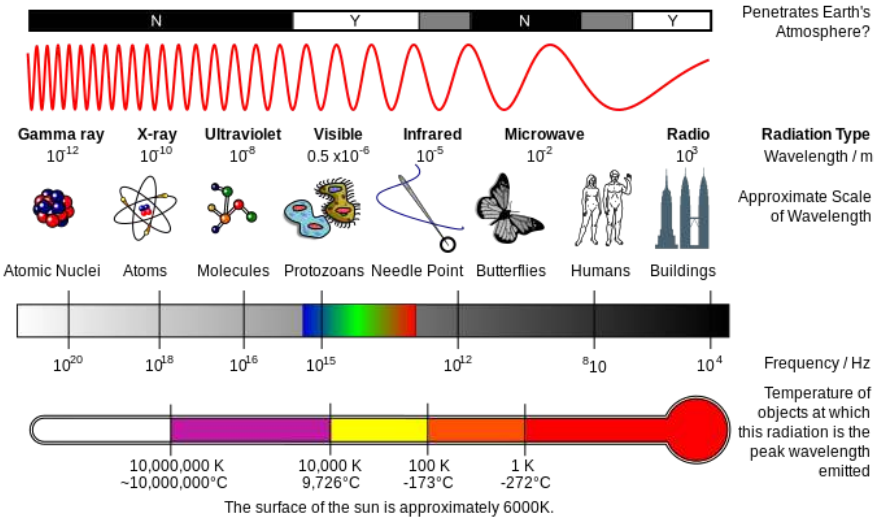
2.1.3.6: ENZYME KINETICS (ACTIVITY)

2.1.3.7: ENZYME KINETICS WITH SPECTROVIS

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2.1.3.1: INTRODUCTION

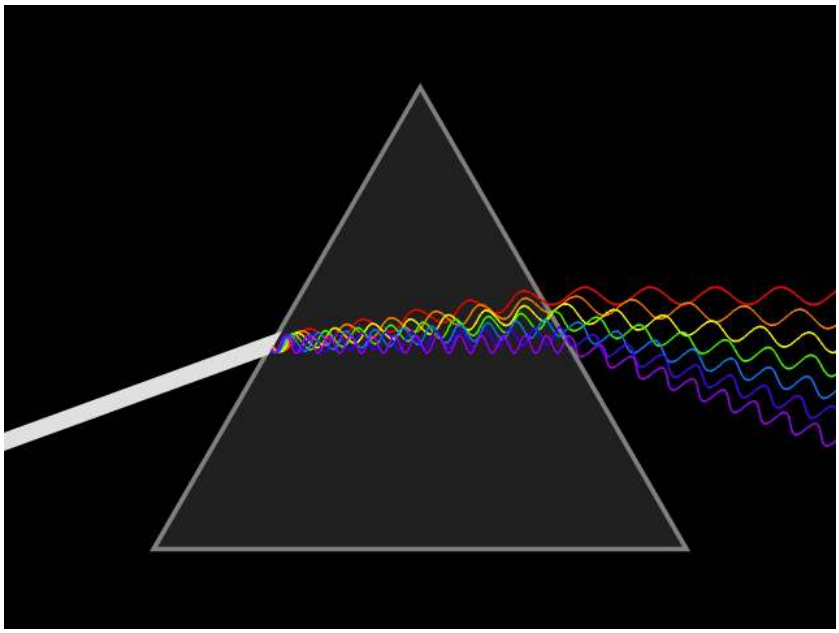
PROPERTIES OF LIGHT



Credit: Inductiveload, NASA (CC-BY-SA 3.0)

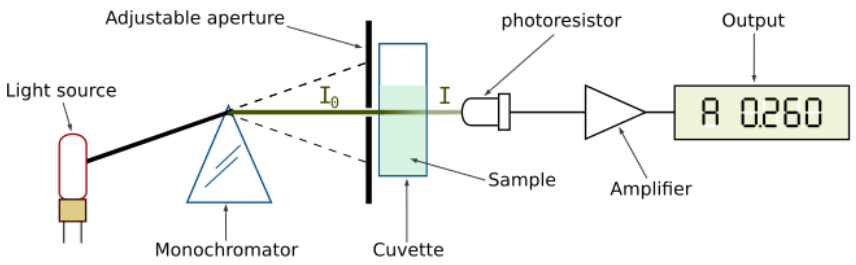
Light is a type of energy that travels as a wave-particle. The **wavelength** of light is the distances between peaks in the waves as light travels. Wavelengths are measured in nanometers (nm) and different wavelengths of light represent differing colors. White light is a mixture of the visible light **spectrum**. Light of long wavelengths (infra-red) and very short wavelengths (ultraviolet) are invisible to humans but can be observed by other organisms. As the wavelength decreases, the energy of the light is increased.

Diffraction of light through a prism exposes the components wavelengths of light.



SPECTROPHOTOMETRY

Spectrophotometers (***spectro***-image/color; ***photo***-*light*; ***meter***-measure) are used for chemical analysis of solutions based on properties of absorption or transmission.



Schematic of a spectrophotometer. The monochromator is a prism that splits the light. A single wavelength of light is focused through the aperture to pass through the solution in the cuvette. GYassineMrabetTalk [CC-BY-SA].

Transmittance refers to the amount of light that passes through the solution.

$$T = \frac{I}{I_0}$$

The transmittance of a light source through a cuvette. The intensity of light, $\mathbf{I_0}$, decreases as it passes through the solution. The light detected by the sensor, **I**, reflects the transmittance of the solution.

If the light is being absorbed by chemicals in the solution, this results in a lower transmission. **Absorbance** is therefore inversely related to transmittance as expressed by the equation:

$$A = -\log_{10} T$$

Follow the virtual demonstration at http://www.virtual-labs.leeds.ac.uk/pres/spectrophotometry/ (CC-BY-NC-SA) for a more in-depth explanation of spectrophotometry.

BEER'S LAW

Beer's Law is a relationship between the concentration or amount of a dissolved substance in a solution that is reducing the amount of transmitted light due to the absorption of the radiant energy. **Lambert's Law** states that the reduction of transmittance was related to the length of the path of light. As the light path increases through a substance, there is a reduction in transmittance. Collectively, these ideas are referred to as **Beer-Lambert Law**, but most observers will control the path length and simply refer to it as Beer's Law.

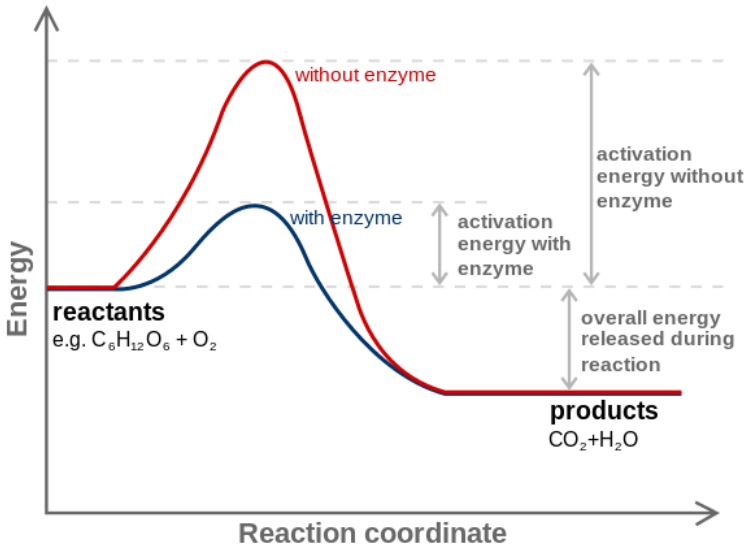
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2.1.3.2: ENZYMES

ENERGY AND CATALYSTS

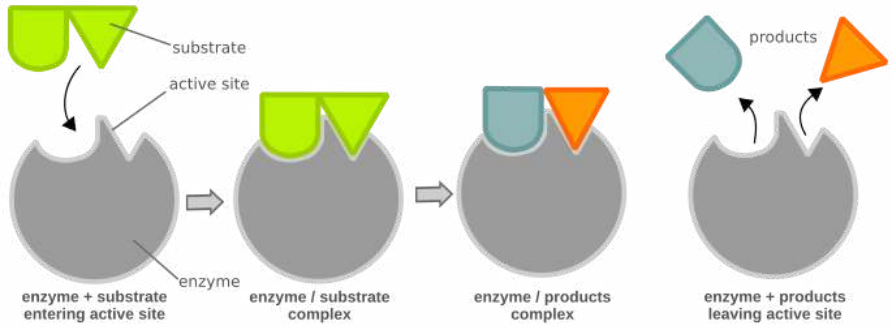
In biological systems, **energy** is roughly defined as the capacity to do work. Molecules are held together by electrons. Breaking and building these bonds requires an input of energy. The energy needed to initiate such reactions is referred to as **activation energy** (E_A). Sometimes the necessary energy to initiate a reaction is so large that it greatly limits the likelihood of the reaction ever occurring. **Catalysts** are chemicals that take part in facilitating reactions by reducing the energy of activation. If the activation energy is reduced, the likelihood of a reaction occurring is greatly enhanced. In cells, the catalysts are often made of proteins and called **enzymes**.



*Reaction coordinate of an **exothermic** reaction with and without an enzyme. The enzyme reduced the E_A to facilitate the likelihood that the reaction occurs. This **catabolic** reaction breaks complex things down, thus increasing entropy and releasing energy into the system.*

ENZYMES

Reactants in enzymatic reactions are called **substrates**. They have an imperfect fit to a binding domain of the enzyme called the **active site**. Substrate binding to this active site induces a change in the shape of the protein that coordinates the substrate into a transition state that will reduce the amount of E_A required for the reaction to go to completion. The **induced fit** of the protein also aids in coordinating other cofactors or **coenzymes** that will aid in the reaction.

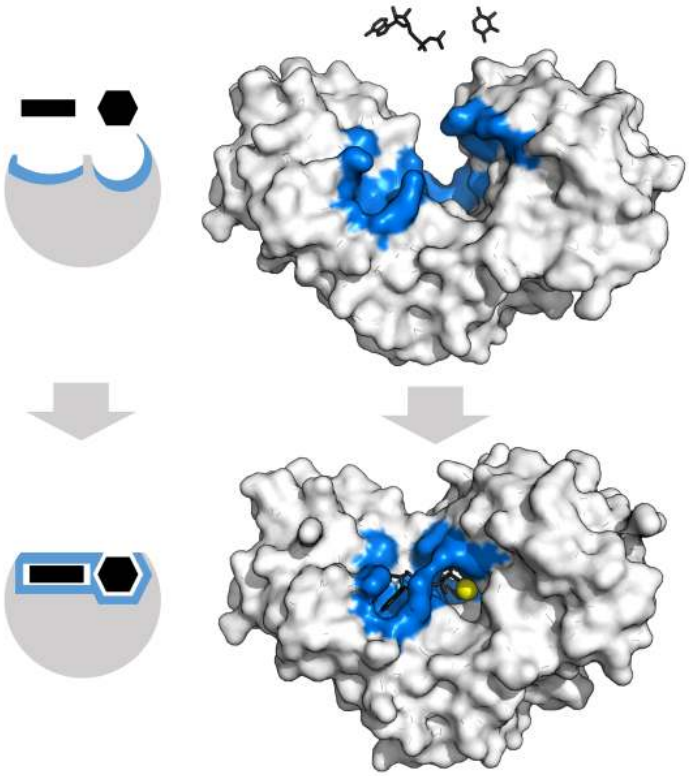


***Induced fit** model of enzymes and substrates. The active site of the protein is an imperfect match for the substrate. Intermolecular interactions between the enzyme and substrate induce a new fit that facilitates the formation of a **transition state** and results in the catalysis of the reaction.*

The reaction follows the standard flow where the Enzyme (E) and the Substrate (S) interact to form an Enzyme-Substrate Complex (ES). The ES then dissociates into Enzyme and the resultant Product (P).

$$E + S \Rightarrow ES \Rightarrow E + P$$

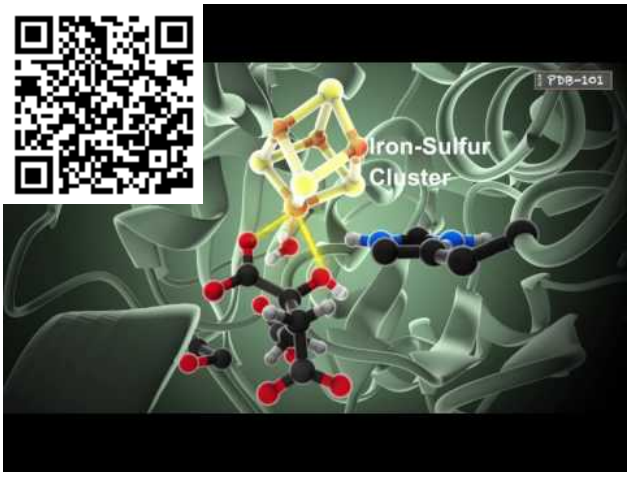
The induced fit of the enzyme-substrate complex coordinates the transition state to facilitate the reaction. This induced fit occurs through non-covalent means that result in a tugging on the molecules (an application of energy) while molecules are coaxed into the reactions.



Hexokinase enzyme interacts with an ATP and a hexose. These interactions alter slightly the structure of the enzyme (induced fit). This pulling on the enzyme and the substrates aids in catalyzing the reaction through coordinating the molecules, sometimes with the aid of cofactors and coenzymes. The yellow sphere represents the cofactor Mg^{2+}.

Coenzymes can be covalently linked to amino acid side chains of the enzyme and are also referred to as **prosthetic groups**. While prosthetic groups are organic in nature, they may also involve the coordination of metal ions, like the heme group which binds to iron. These prosthetic groups enhance the repertoire of the amino acids to provide additional functioning to the entire protein. Early coenzymes were described as being vital to normal functioning and were characterized as organic molecules with amine groups. Because of this coincidence, they were referred to as **vitamins** (for *vital amines*) though not all vitamins have amine groups. The trace metal ions that work with these groups are also required and represent the minerals on food items.





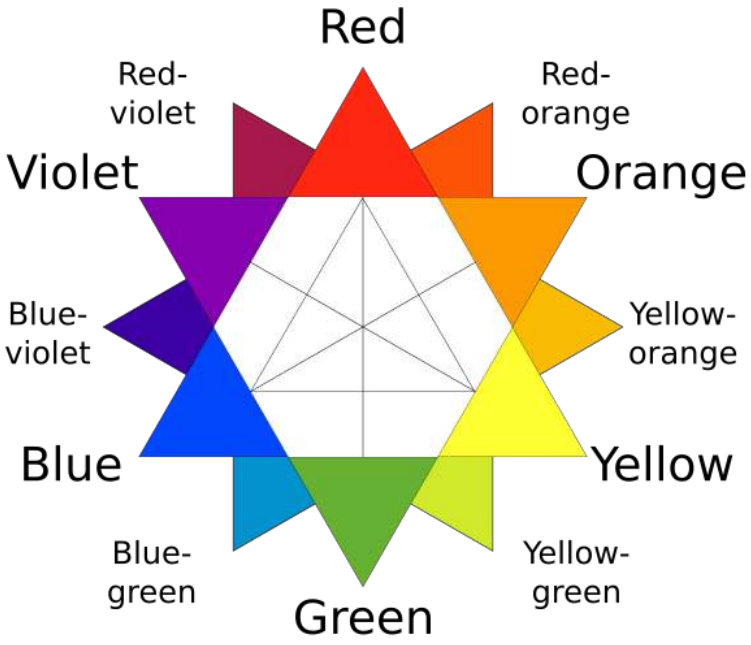
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2.1.3.3: EXPLORING BEER'S LAW (VIRTUAL)

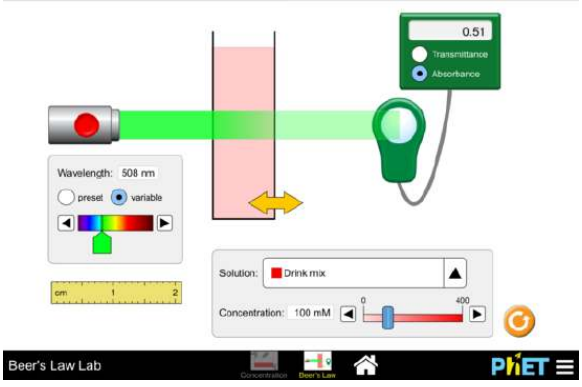
Use the link below to launch a simulation where you can alter the properties involved in spectrophotometry and examine the Beer-Lambert Law. Increase and decrease the concentration slider in the simulation:

1. What happens to the contents in the cuvette?
2. How does this change the Transmittance and Absorbance readings?
3. Click “variable” and use the slider. What happens to the readings when the Wavelength of the laser is a similar color as the solution in the cuvette?
4. Consult the color star below and find the color wavelength that is opposite of the color of the solution. Set the laser to this color using “variable” and the slider.



1. What is the effect on Transmittance and Absorbance with this color?

2. Using the previous observations (using variable wavelength slider), how would you use the relationship on Transmittance/Absorbance to best measure the concentration of a solution?



Click on image above to begin Beer’s Law Simulation

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2.1.3.4: QUANTITATIVE DETECTION OF PROTEIN (ACTIVITY)

EXPERIMENTAL BACKGROUND

Bovine Serum Albumin (BSA) is a protein that circulates in the blood of cows. Purified BSA can be used with Biuret solution in serial dilutions to generate a **Standard Curve**. The standard curve will illustrate the relationship between concentration (the **dependent variable**) and absorbance at 540 nm (the **independent variable**). We can then use this curve to estimate the concentration of unknown samples.

1. On a graph, do you remember which axis is the dependent and which is the independent variable?
2. In the table below, can you identify which samples are the **negative controls** and which are the **positive controls**?
3. What is the prediction of the absorbance or color intensity of the different tubes?

DILUTE BSA STANDARDS

1. Label 9 tubes 1-9.
2. Combine the components of the table below to generate the appropriate concentration of solutions.

	Blank	BSA Standard Dilution						Unknowns	
	A 0 mg/ml	B 1 mg/ml	C 2 mg/ml	D 4 mg/ml	E 6 mg/ml	F 8 mg/ml	G 10 mg/ml	H ? mg/ml	I ? mg/ml
ml BSA	0	0.1	0.2	0.4	0.6	0.8	1	---	---
ml H2O	1	0.9	0.8	0.6	0.4	0.2	0	---	---
ml unknown	---	---	---	---	---	---	---	1	1
ml Biuret	4	4	4	4	4	4	4	4	4

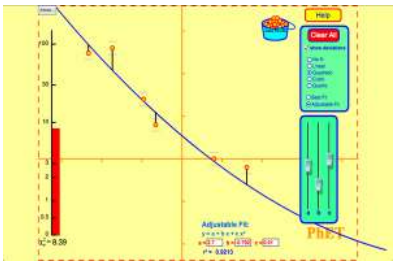
3. Place tube 1 (**blank**) into a cuvette and measure the absorbance (**A**) in the spectrophotometer at 540 nm.
4. Calibrate the spectrophotometer to read 0 at $\mathbf{A_{540nm}}$.
5. Sequentially read each sample at $\mathbf{A_{540nm}}$ and record values in the table below.

Tube #	BSA (mg/ml)	A_{540nm}
A	**0**	
B	**1**	
C	**2**	
D	**4**	
E	**6**	
F	**8**	
G	**10**	
Do NOT plot the values below. Use the plot to estimate the concentration of the samples.		
H		
I		

6. Plot each BSA dilution in a spreadsheet program like Excel as a scatterplot.
7. Generate best-fit line for these standards with the equation of the line.
8. Use the equation of the line to estimate the concentration of the unknown sample.

CURVE FITTING

Run the simulation below to understand how you can use the standard dilution series to estimate your sample concentrations.

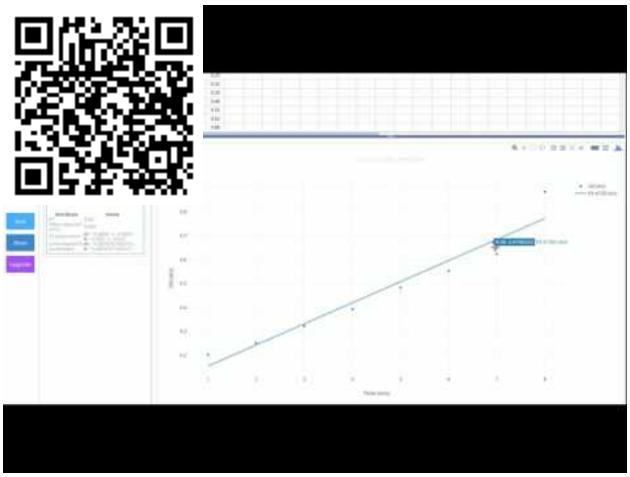


Click on the image above to begin simulation on curve fitting.

SCATTERPLOT TUTORIAL

Use the tutorial below and watch at 1.25X to plot your own data.





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2.1.3.5: QUANTITATIVE DETECTION OF PROTEINS (SPECTROVIS PLUS)

EXPERIMENTAL BACKGROUND

Bovine Serum Albumin (BSA) is a protein that circulates in the blood of cows. Purified BSA can be used with Biuret solution in serial dilutions to generate a **Standard Curve**. The standard curve will illustrate the relationship between concentration (the **dependent variable**) and absorbance at 540 nm (the **independent variable**). We can then use this curve to estimate the concentration of unknown samples.

1. On a graph, do you remember which axis is the dependent and which is the independent variable?
2. In the table below, can you identify which samples are the **negative controls** and which are the **positive controls**?
3. What is the prediction of the absorbance or color intensity of the different tubes?

DILUTE BSA STANDARDS

1. Label 9 tubes 1-9
2. Combine the components of the table below to generate the appropriate concentration of solutions

	Blank	BSA Standard Dilution						Unknowns	
	A 0 mg/ml	B 1 mg/ml	C 2 mg/ml	D 4 mg/ml	E 6 mg/ml	F 8 mg/ml	G 10 mg/ml	H ? mg/ml	I ? mg/ml
ml BSA	0	0.1	0.2	0.4	0.6	0.8	1	---	---
ml H2O	1	0.9	0.8	0.6	0.4	0.2	0	---	---
ml unknown	---	---	---	---	---	---	---	1	1
ml Biuret	4	4	4	4	4	4	4	4	4

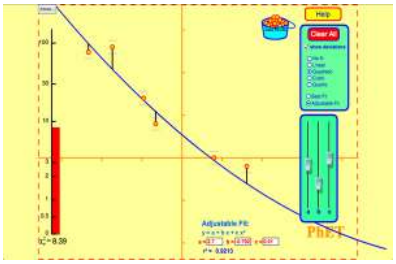
3. The instructor will begin to set-up the units for distribution.
    1. Connect SpectroVis Plus to LabQuest2.
    2. Power on.
    3. Select the LabQuest App.
    4. On the “Meter” tab, tap on **Mode.**
        1. Change the mode to “**Events with Entry**“.
        2. Enter the Name: Concentration.
        3. Enter Units: mg/ml.
        4. Select “OK”.
        5. If a message appears about saving run, choose Discard.
        6. Tap on the red area showing Absorbance to trigger menu items
            1. Select “**Change Wavelength**“.
            2. Enter 540.
        7. Tap on the red area showing Absorbance to trigger menu items.
            1. Select “Calibrate”.
            2. Select “Warm Up”.
            3. Select Finish Calibration.
4. Place tube 1 (**0 mg/ml**) into a cuvette for measuring the absorbance (**A**) in the SpectroVis Plus.
    - Tap on the file cabinet icon to store this data.
5. Sequentially read each sample at the stored wavelength ($\mathbf{A_{540nm}}$)and record values in the table below.

Tube #	BSA (mg/ml)	A_{540nm}
A	0	
B	1	
C	2	
D	4	
E	6	
F	8	
G	10	
Do NOT plot the values below. Use the plot to estimate the concentration of the samples.		
H		
I		

1. Plot each BSA dilution in plot.ly as a scatterplot (Log-on using Facebook/Google/Twitter credentials for free).
2. Generate best-fit line for these standards with the equation of the line.
3. Use the equation of the line to estimate the concentration of the unknown sample.

CURVE FITTING

Run the simulation below to understand how you can use the standard dilution series to estimate your sample concentrations.



Click on the image above to begin simulation on curve fitting.

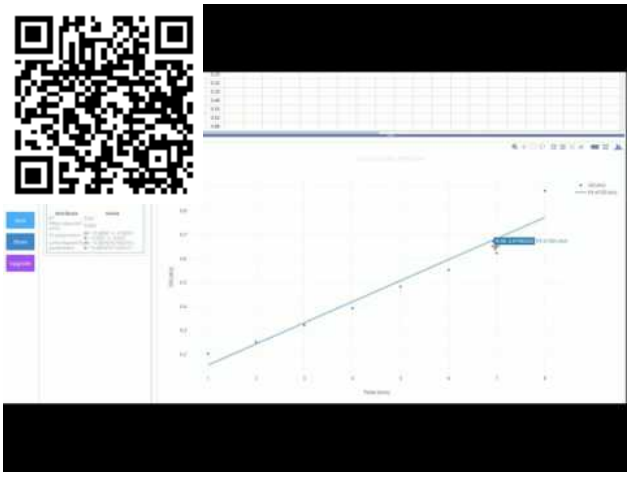
LABQUEST2 AND SPECTROVIS TUTORIAL



SCATTERPLOT TUTORIAL

You can watch this tutorial at 1.25X and pause when needed.



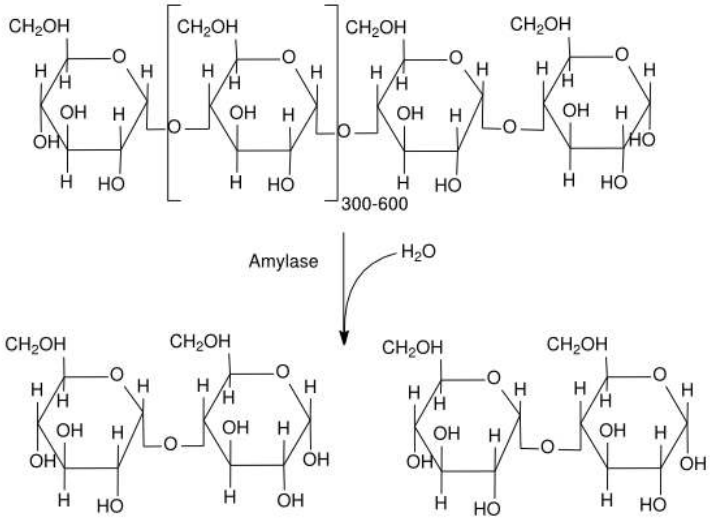


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2.1.3.6: ENZYME KINETICS (ACTIVITY)

THE ENZYME

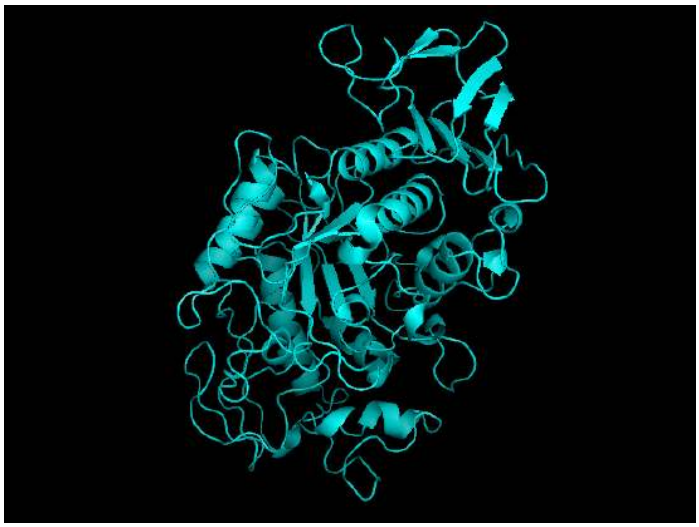


Amylase is an enzyme that breaks down amylose (starch) into glucose molecules.

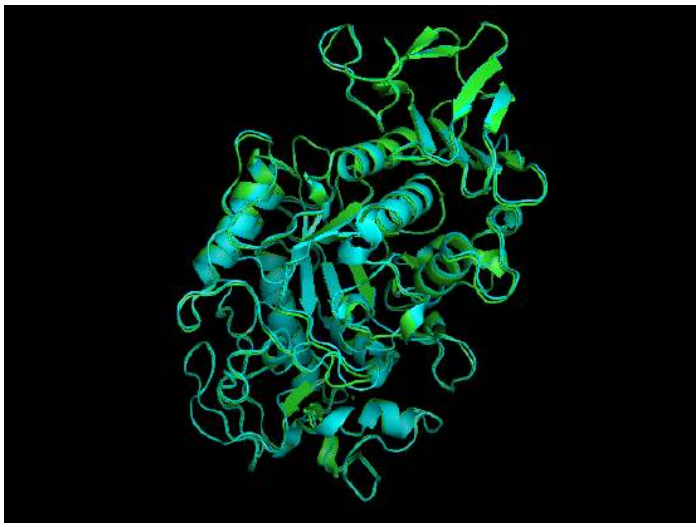
1. What test can be used to indicate the presence of Starch?
2. What parameters would influence the ability of the enzyme to facilitate the rate of the reaction?
3. What is the role of an enzyme in a chemical reaction and what is it made of?
4. What test can be used to indicate the presence of glucose?



Salivary amylase is produced in the mouth, where digestion begins.



Pancreatic amylase is produced in the pancreas and is supplied to the duodenum of the small intestines.



*Overlay of salivary (**green**) and pancreatic (**teal**) amylase molecules.*

EFFECT OF TEMPERATURE ON ENZYME ACTIVITY

1. Add 5 ml of H_2O to a tube (this is the BLANK).
2. Add 5 ml of starch (substrate) to 3 separate tubes.
    - One on ice (0°C), one on the bench (25°C), and one in a 40°C water bath
3. Add 2 drops of iodine to each tube and mix: Blank, 0°C, 25°C, and 40°C.
4. Read the Blank in the spectrophotometer and calibrate it to 100% transmittance at 560nm.
5. Read each tube in the spectrophotometer. This is time 0 minute.
6. Add 35 drops of amylase solution to each tube simultaneously. Mix and ensure incubation is occurring at the correct temperature.
7. At 2 minute intervals, quickly read **ALL** tubes in the spectrophotometer and immediately return the tubes to the proper temperature.
8. Continue reading the samples every 2 minutes until you reach 22 minutes on the table below.

Time (min)	0ºC % Trans	25ºC % Trans	40ºC % Trans
0			
2			
4			
6			
8			
10			
12			
14			
16			
18			
20			
22			

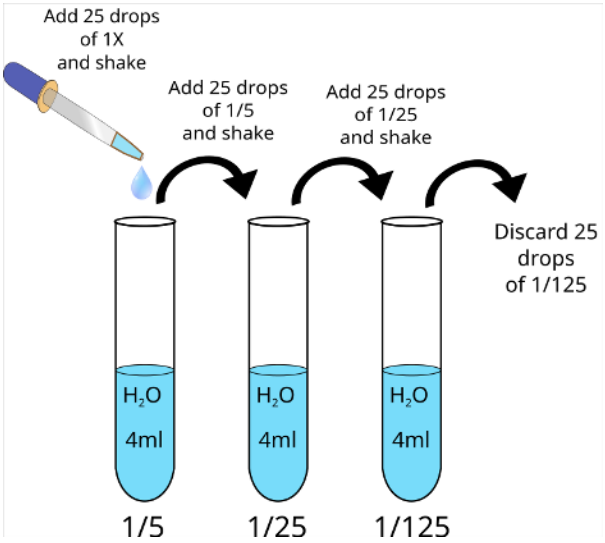
EFFECT OF PH ON ENZYME ACTIVITY

1. Add 5 ml of water to an empty tube (this is the BLANK)
2. To 3 separate tubes, add 2.5 ml of buffer pH 3, pH 5 or pH 7
    1. Add 2.5 ml of starch (substrate) to each of these tubes (excluding BLANK)
3. Add 2 drops of iodine to each tube and mix: Blank, pH 3, pH 5, pH 7
4. Read the Blank in the spectrophotometer and calibrate it to 100% transmittance at 560nm
5. Read each tube in the spectrophotometer. This is time 0 min.
6. Add 35 drops of amylase solution to each tube simultaneously, mix to homogeneity.
7. At 2 minute intervals, quickly read **ALL** tubes in the spectrophotometer.
8. Continue reading the samples every 2 minutes until you reach 22 minutes on the table below.

Time (min)	pH 3 % Trans	pH 5 % Trans	pH 7 % Trans
0			
2			
4			
6			
8			
10			
12			
14			
16			
18			
20			
22			

EFFECT OF ENZYME CONCENTRATION ON ENZYME ACTIVITY

1. Add 5 ml of water to an empty tube (this is the BLANK)
2. Add 5 ml of pH 7 buffer to 3 separate tubes.
3. Follow the dilution scheme below:



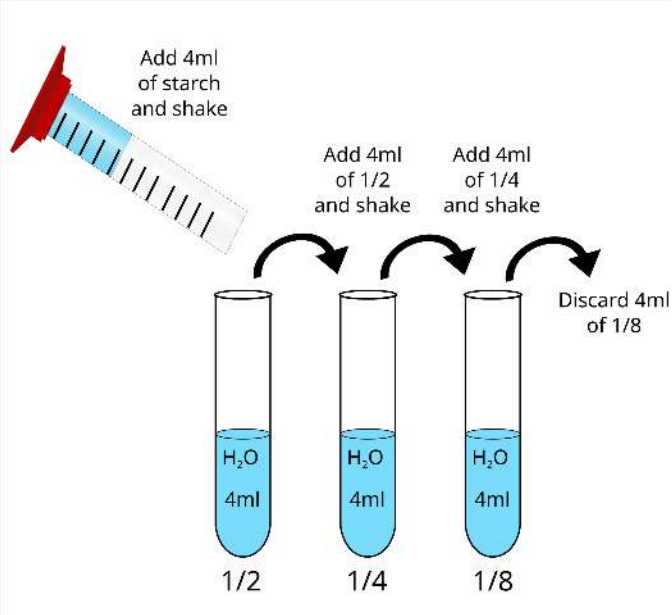
Dilution scheme for amylase (enzyme)

4. In 4 separate tubes, ADD 4 ml of starch solution.
   - Label them 1x, 1/5x, 1/25x, 1/125x
5. Add 2 drops of iodine to each starch tube and the Blank.
6. Read the Blank in the spectrophotometer and calibrate it to 100% transmittance at 560nm.
7. Read each tube in the spectrophotometer. This is time 0 minute.
8. Add 35 drops of diluted amylase solutions to the appropriately labeled tubes simultaneously and mix.
   - Each tube receives a different Amylase dilution.
9. At 2 minute intervals, quickly read **ALL** tubes in the spectrophotometer.
10. Continue reading the samples every 2 minutes until you reach 22 minutes on the table below.

Time (min)	1 X % Trans	1/5 X % Trans	1/25 X % Trans	1/125 X % Trans
0				
2				
4				
6				
8				
10				
12				
14				
16				
18				
20				
22				

EFFECT OF SUBSTRATE CONCENTRATION ON ENZYME ACTIVITY

1. Add 5 ml of water to an empty tube (this is the BLANK)
2. Add 5 ml of pH 7 buffer to 3 separate tubes
3. Follow the dilution scheme below:



4. In 4 cuvettes, ADD 4 ml of the diluted starch solution.
    - Label them 1x, 1/2x, 1/4x, 1/8x.
5. Add 2 drops of iodine to each starch tube and the Blank.
6. Read the Blank in the spectrophotometer and calibrate it to 100% transmittance at 560nm.
7. Read each tube in the spectrophotometer. This is time 0 minute.
8. Add 35 drops of amylase solution to each tube simultaneously and mix.
9. At 2 minute intervals, quickly read **ALL** tubes in the spectrophotometer.
10. Continue reading the samples every 2 minutes until you reach 22 minutes on the table below.

Time (min)	1 X % Trans	1/2 X % Trans	1/4 X % Trans	1/8 X % Trans
0				
2				
4				
6				
8				
10				
12				
14				
16				
18				
20				
22				

PLOT THE RESULTS

1. Using a plot.ly, plot the data on the same chart.
2. Calculate the line of best fit of each dataset.
3. The slope represents the activity of the enzyme in each condition. What is the unit of this activity?

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2.1.3.7: ENZYME KINETICS WITH SPECTROVIS

ENZYME KINETICS OF TURNIP PEROXIDASE

Hydrogen peroxide (H_2O_2) is a strong oxidizing agent that can damage cells and is formed as a by-product of oxygen consumption. Fortunately, aerobic cells contain peroxidases that break down peroxide into water and oxygen. This enzyme reduces hydrogen peroxide into H_2O by oxidizing an organic compound (AH_2 -> A).

$$H_2O_2 + AH_2 \xrightarrow{\text{peroxidase}} 2H_2O + A$$

This exercise uses turnip extract as a source of peroxidase. This turnip extract requires a source of electrons (a reducing agent) in order for the reaction to occur. In this case, a colorless organic compound called **guaiacol** is used. Guaiacol is oxidized in the process of converting the peroxide and becomes brown. The enzymatic activity can then be traced using a spectrophotometer to measure the amount of brown being formed.

SET-UP AND CALIBRATE LABQUEST WITH SPECTROVIS PLUS

1. Set-up the cuvette to serve as a Blank.
    1. Add 10 drops 0.02% hydrogen peroxide.
    2. Add 5 drops 0.2% guaiacol.
    3. Add 20 drops of pH 7 buffer.
    4. Add 10 drops extraction buffered.
2. Connect a LabQuest2 to a SpectroVis plus and start the data collection program.
3. Calibrate the SpectroVis.
    1. Change Mode.
        1. Select “Time-Based” from the dropdown menu and press OK when done.

    - Rate: 0.5 samples/s
    - Interval: 2 s/sample
    - Duration: 200 s

2. Press the Play button (green arrow).

1. Perform Warm-up for 90 seconds.

1. Choose "Finish Calibration".

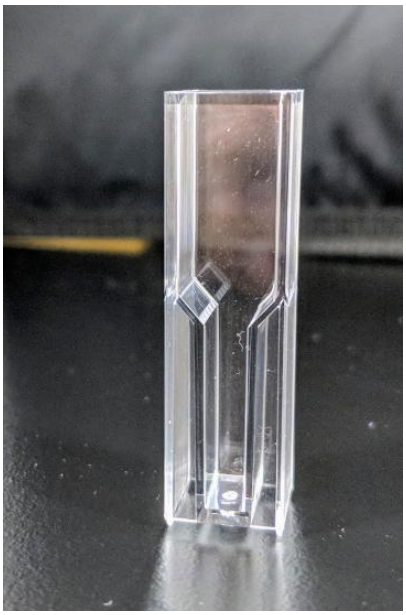
2. Press "OK".

2. Press the "Stop" button (Red Square).

4. Select “Meter Icon” (top left most of the display).
    1. Press the large red area showing the current absorbance reading.
    2. Choose “Change Wavelength”.
    3. Set Wavelength to 500nm.

EFFECT OF PH ON PEROXIDASE ACTIVITY

1. Set-up the cuvette for pH 3.
    1. Add 10 drops 0.02% hydrogen peroxide.
    2. Add 5 drops 0.2% guaiacol.
    3. Add 20 drops of pH 3 buffer.
    4. Add 10 drops turnip extraction last.
    5. Quickly invert the cuvette and place the cuvette into the Spectrovis Plus.
    6. Press play button to begin recording data (choose “discard data” if prompted).
        1. After 200s, remove SpectroVis from USB.
        2. Insert a USB flash drive and wait 1 minute.
        3. Press File → Export → choose USB icon and rename the file and add “.csv” to the end of the file name.
        4. Press OK.
2. Sequentially repeat the experiment exchanging the pH buffer with pH 5, 7, 10.



EFFECT OF TEMPERATURE ON PEROXIDASE ACTIVITY

1. Set-up the cuvette for 0ºC
    1. Add 10 drops 0.02% hydrogen peroxide (incubated at 0ºC for 10 minutes).
    2. Add 5 drops 0.2% guaiacol.
    3. Add 20 drops of extraction buffer at 0ºC.
    4. Add 10 drops turnip extraction (incubated at 0ºC for 10 minutes) last.
    5. Quickly invert the cuvette and place the cuvette into the Spectrovis Plus.
    6. Press play button to begin recording data (choose “discard data” if prompted).
        1. After 200s, remove SpectroVis from USB.
        2. Insert a USB flash drive and wait 1 minute.
        3. Press File → Export → choose USB icon and rename the file and add “.csv” to the end of the file name.
        4. Press OK.
2. Sequentially repeat the experiment exchanging the buffers stored at 20ºC, 40ºC, 60ºC.

EFFECT OF SUBSTRATE CONCENTRATION ON PEROXIDASE ACTIVITY

1. Set-up the cuvette for 1X substrate.
    1. Add 10 drops 0.02% hydrogen peroxide.
    2. Add 5 drops 0.2% guaiacol.
    3. Add 20 drops of extraction buffer.
    4. Add 10 drops turnip extraction last.
    5. Quickly invert the cuvette and place the cuvette into the Spectrovis Plus.
    6. Press play button to begin recording data (choose “discard data” if prompted).
        1. After 200s, remove SpectroVis from USB.
        2. Insert a USB flash drive and wait 1 minute.
        3. Press File → Export → choose USB icon and rename the file and add “.csv” to the end of the file name.
        4. Press OK.
2. Sequentially repeat the experiment with different amounts of buffer and peroxide:
    1. Repeat the experiment with 0.2X substrate and export data to a USB drive.
    2. Repeat the experiment with 2X substrate and export data to a USB drive.
    3. Repeat the experiment with 3X substrate and export data to a USB drive.

	0.2X substrate	1X substrate	2X substrate	3X substrate
Drops Peroxide	2	10	20	30
Drops Guaiacol	5	5	5	5
Drops Extraction Buffer	28	20	10	0
Drops Extract	10	10	10	10

EFFECT OF ENZYME CONCENTRATION ON PEROXIDASE ACTIVITY

1. Set-up the cuvette for 1X enzyme.
    1. Add 10 drops 0.02% hydrogen peroxide.
    2. Add 5 drops 0.2% guaiacol.
    3. Add 20 drops of extraction buffer.
    4. Add 10 drops turnip extraction last.
    5. Quickly invert the cuvette and place the cuvette into the Spectrovis Plus.
    6. Press play button to begin recording data (choose “discard data” if prompted).
        1. After 200s, remove SpectroVis from USB.
        2. Insert a USB flash drive and wait 1 minute.
        3. Press File → Export → choose USB icon and rename the file and add “.csv” to the end of the file name.
        4. Press OK.
2. Sequentially repeat the experiment with different amounts of buffer and extract:
    1. Perform the experiment on 1X enzyme and export data to a USB drive.
    2. Repeat the experiment with 0.2X enzyme and export data to a USB drive.
    3. Repeat the experiment with 2X enzyme and export data to a USB drive.
    4. Repeat the experiment with 3X enzyme and export data to a USB drive.

	0.2X enzyme	1X enzyme	2X enzyme	3X enzyme
Drops Peroxide	10	10	10	10
Drops Guaiacol	5	5	5	5
Drops Extraction Buffer	28	20	10	0
Drops Extract	2	10	20	30

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SECTION OVERVIEW

2.1.4: OSMOSIS AND DIFFUSION

2.1.4.1: INTRODUCTION

2.1.4.2: WHY ARE CELLS SMALL? (ACTIVITY)

2.1.4.3: DO LARGER THINGS DIFFUSE FASTER? (ACTIVITY)

2.1.4.4: GUMMY BEARS- TONICITY (ACTIVITY)

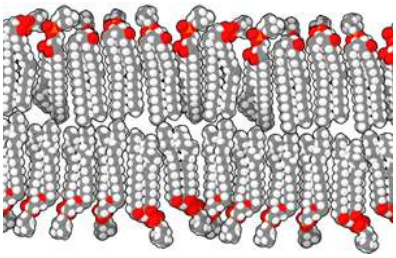
2.1.4.5: OBSERVING OSMOSIS (ACTIVITY)

2.1.4.6: AGAR CUBES (PREPARATION)

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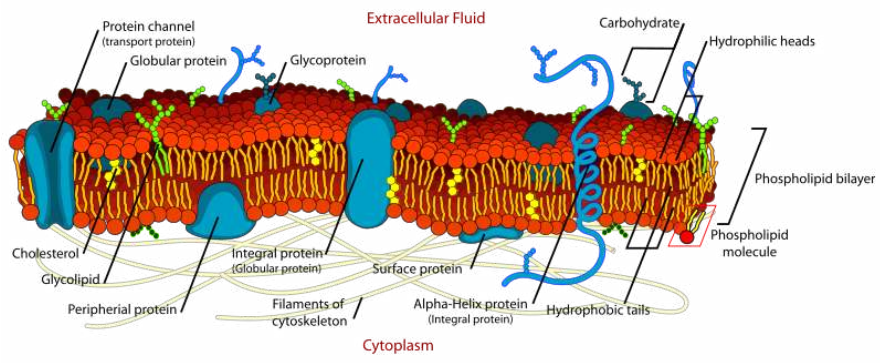
2.1.4.1: INTRODUCTION

UNDERSTANDING MEMBRANES



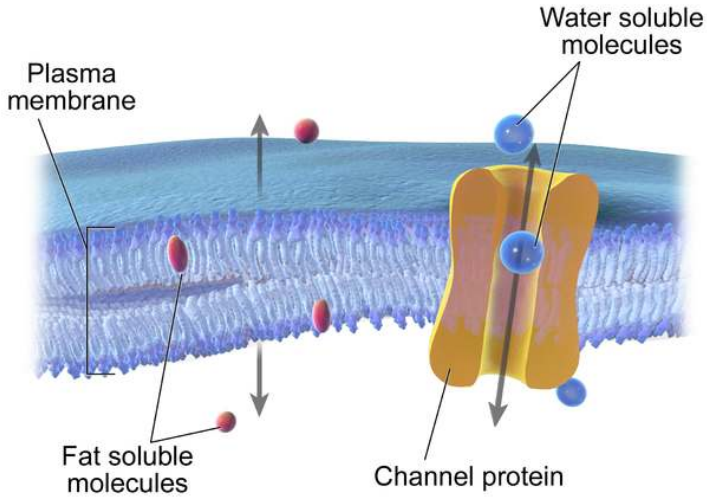
The cell membrane is the barrier that separates the cytoplasm from the external world. The cell membrane consists primarily of phospholipids in a bilayer. Phospholipids are amphipathic with a polar head (phosphate group) and a hydrophobic tail (2 hydrocarbon chains). Due to the chemical properties of the heads being attracted to water and the tails having a desire to avoid water, phospholipids self-assemble into micelles. Cell membranes form from a phospholipid bilayer where the lipid tails interact with each other and the phosphate heads face the external water environment or the internal cytoplasm of the cell.

The cell membrane does not solely consist of phospholipids but also have proteins and cholesterol inserted into the bilayer. As the image of the bilayer above indicates, the molecules are constantly moving and flow in a lateral motion. Cholesterol modulates the fluidity of this motion. Proteins associated with the membrane may sit on either side (**peripheral proteins**) of the membrane or pass through both layers of the membrane (**transmembrane proteins**). The model that describes the components of the cellular membrane is referred to as the **Fluid Mosaic Model**. This model states that the cell membrane is a mosaic of 1) Phospholipids 2) Proteins 3) Cholesterol that moves about in a side to side motion.



The fluid mosaic of phospholipids, proteins, and cholesterol that create the selective barrier between the interior and the exterior of the cell.

Small uncharged molecules pass through the double layer of phospholipids. Polar, charged or large molecules have great difficulty passing through the membrane and require the aid of transmembrane proteins. An example of a transmembrane protein that facilitates the movement of a polar substance is aquaporin, which permits the free movement of water.



Diffusion Across the Plasma Membrane

DIFFUSION

Diffusion is the net movement of a substance from high concentration to low concentration. This difference in concentration is referred to as a **concentration gradient**. This movement does not require any external energy but uses the free energy intrinsic to the system.

- Temperature Effects on Diffusion
  - Temperature/Diffusion Simulation
- Molecular Mass Effects on Diffusion
  - Size/Mass/Diffusion Simulation



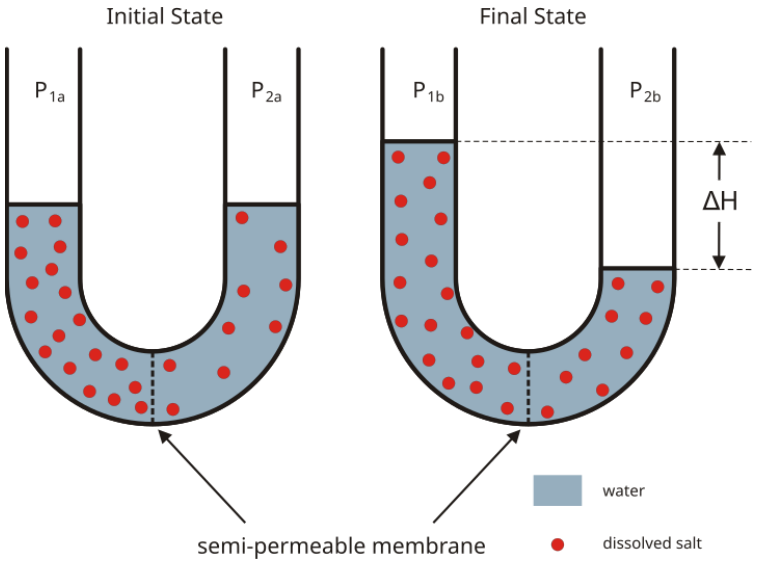
Diffusion

Concentrated dye diffuses along the concentration gradient until reaching equilibrium (no net movement).

OSMOSIS

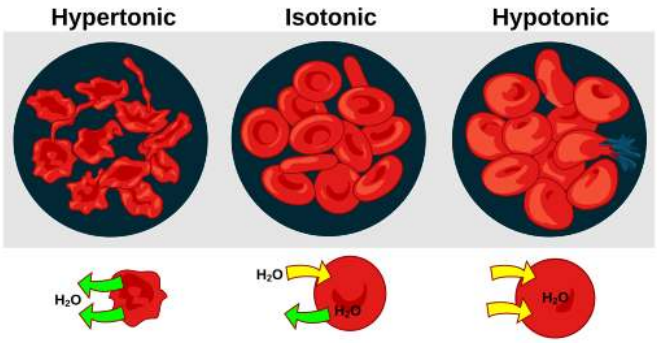
Osmosis is a special case of diffusion. Instead of observing the net change in solute, osmosis follows the net movement of a solvent across a **semipermeable membrane**. Since a semi-permeable membrane permits specific things to pass through, some solutes are partitioned.

- Simulation of diffusion through a semipermeable membrane (CC-BY 4.0 Concord Consortium)

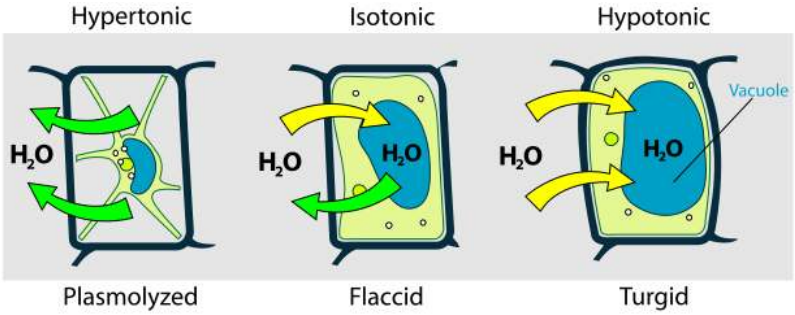


A semi-permeable membrane allows the solvent to pass but not this red salt molecule. The water moves along the concentration gradient (of water) . This movement of water causes an osmotic pressure.

A cell lacking a cell wall is affected greatly by the tonicity of the environment. In a **hypertonic** solution where the concentration of dissolved solute is high, water will be drawn out of the cell. In a **hypotonic** solution where the concentration of dissolved solute is lower than the interior of the cell, the cell will be under great **osmotic pressure** from the environmental water moving in and can rupture.



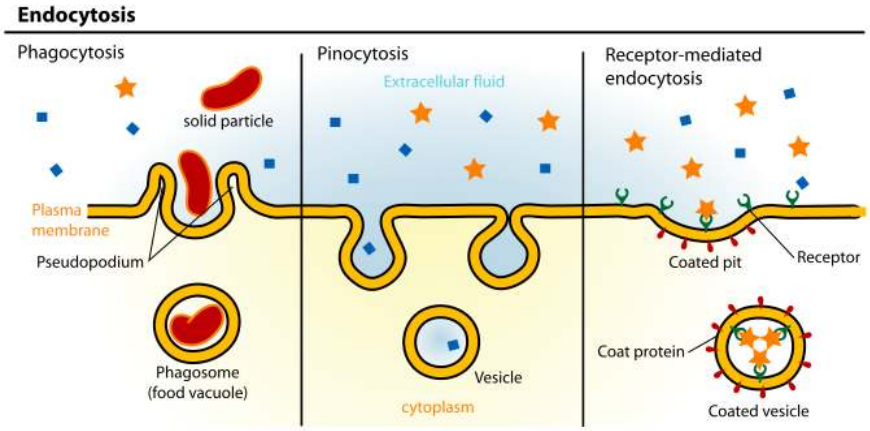
Plants have rigid cell walls composed of cellulose. These cell walls permit for maintenance of cellular integrity when the external environment is **hypotonic** (less dissolved substances). In this situation, the water moves into the cell. Without the cell wall, the cell would burst open from the excessive water pressure entering the cell. This state of swelling is referred to as turgid, resulting from **turgor pressure**.

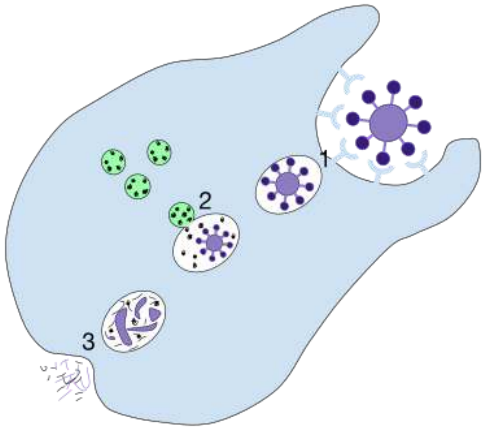


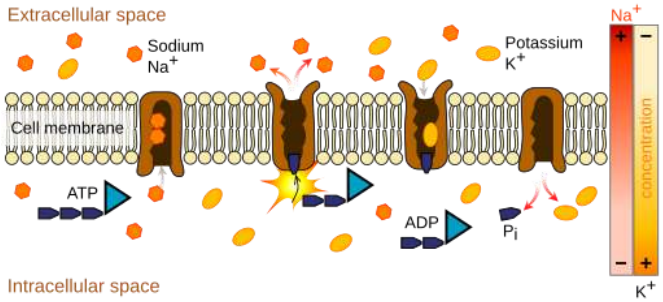
Cell walls of a plant retain the shape of the cell despite the state of external tonicity.

When the exterior environment is **hypertonic** (greater amount of dissolved substances), the reverse condition occurs whereby the cellular fluid exiting the cell reduces the size of the cytoplasm. This condition is referred to as **plasmolysis**.

ACTIVE TRANSPORT MECHANISMS







FURTHER READING

- http://www.visionlearning.com/en/library/Biology/2/Membranes-I/198/reading
- http://www.visionlearning.com/en/library/Biology/2/Membranes-II/204
- http://www.biologycorner.com/bio1/notes_diffusion.html (CC-BY-NC)

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2.1.4.2: WHY ARE CELLS SMALL? (ACTIVITY)

INTRODUCTION

1. Take 3 blocks of agar of different size (1cm, 2cm, 3cm) → these are our cell models.
2. Measure the length, width, and height of each cube using a ruler.
3. Calculate the area of each face of the cubes and add all the areas together for a single cube.

- A cube has 6 faces → the total surface area is the same as the area of one side multiplied by 6.

4. Calculate the volume of each cube.
5. Report the surface area-to-volume in the table below.

DATA TABLE: CALCULATING SURFACE AREA-TO-VOLUME RATIO

Cell Model (Cube)	Length	Width	Height	Total Surface Area	Volume of cell	Surface Area: Volume
1						
2						
3						

STOP AND THINK

- Which cube has the greatest surface area:volume ratio?
- Which cube has the smallest surface area:volume ratio?
- **Hypothesize**: In an osmosis or diffusion experiment, which cube size would have the greatest diffusion rate?

PROCEDURES

1. Each group will acquire three agar cubes: A 3cm cube, a 2cm cube, and a 1cm cube. **CUT AS ACCURATELY AS POSSIBLE**. *(This may be already completed for you.)*
2. Place cubes into a beaker and submerge with 200 ml NaOH.
3. Let the cubes soak for approximately 10 minutes.
4. Periodically, gently stir the solution, or turn the cubes over.
5. After 10 minutes, remove the NaOH solution.
6. Blot the cubes with a paper towel.
7. Promptly cut each cube in half and measure the depth to which the pink color has penetrated. Sketch each block's cross-section.
8. Record the volume that has remained white in color.
9. Do the following calculations for each cube and complete the following data table:

DATA TABLE: CALCULATION OF DIFFUSION AREA-TO-VOLUME

Cube Size	Cube volume (cm^3) (V_{total})	Volume white (cm^3) (V_{white})	Sketch of each Cube	Volume of the diffused cube ($V_{total} - V_{white}$) = ($V_{diffused}$)	Percent Diffusion ($V_{diffused}/V_{total}$)	Surface Area: Volume (from the previous table)
1 cm.						
2 cm.						
3 cm.						

CONCLUDE

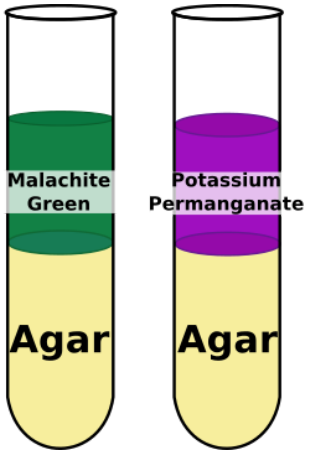
1. Which cube had the greatest percentage of diffusion?
2. Did this meet your expectations with your hypothesis?
3. If you designed a large cell, would it be a large sphere or something long and flat?

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2.1.4.3: DO LARGER THINGS DIFFUSE FASTER? (ACTIVITY)

INTRODUCTION



Agar is a gelatinous substance derived from a structural carbohydrate found in seaweed. It is often used in cooking as a vegetarian alternative to gelatin and can be used as a thickener. Microbiologists pour plates of agar containing nutrients in order to isolate and grow bacteria and other microbes. As with gelatin, the long fibery nature of this structural carbohydrate permits it to be melted and tangled together in a mesh-like network where the spaces between molecules are filled with solution. Altering the amount of fluid solution will change the pores between fibers. More fluid will create a looser gel that has larger spaces between molecules. Reducing the fluid solution volume will conversely create a stiffer gel with smaller spaces between fibers.

1. Take 2 tubes of agar and a solution of Malachite green (365 g/mole) and a solution of Potassium permanganate (164 g/mole).
2. Mark the top of the agar on the outside of the tube (the starting point).
3. Add 10 drops of malachite green to one tube and 10 drops of Potassium permanganate to the other.
4. Take note of the time.
5. At 20 minute intervals, measure the distance from the top that the agar has moved. Do this for at least 1 hour.
6. Plot the data and compare the trends. Describe the rate of diffusion for each.

STOP AND THINK

- **Hypothesize** which solution will move faster through the agar and provide a reason.

DIFFUSION SPEED OF DYE MOLECULES		
DYE	**MOLECULAR WEIGHT**	**HYPOTHESIS** (FAST OR SLOW DIFFUSION)
Malachite Green	365 g/mole	
Potassium Permanganate	164 g/mole	

CONCLUDE

- Which solution actually moved faster?
  _____
- Did this meet your expectations?
  _____
- Propose a reason why a certain dye moved faster._____
- **Review through simulation**

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2.1.4.4: GUMMY BEARS- TONICITY (ACTIVITY)

INTRODUCTION

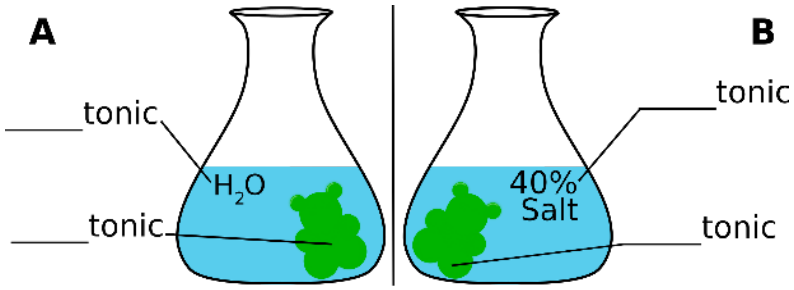
We're all familiar with gelatin (like the Jello brand). Gummy candies are made of gelatin. Gelatin is a protein that exists as long fibers. When gelatin is dissolved in a liquid and cooled, the gelatin fibers tangle together in a mesh-like network. The space in between the gelatin molecules is filled with the fluid it was dissolved in. Gummy candies are considerably more firm than the gelatin molds we have as desserts because they contain a lot less fluid. Nonetheless, gummy candies are filled with a sugary solution with coloring. Like a cell, a gummy candy placed in solution will be affected by the properties of osmosis when submerged in different solutions.

STOP AND THINK

- Is distilled water hypertonic, hypotonic, or isotonic compared to the sugar solution inside a gummy candy?
- Based on your answer, hypothesize if a gummy candy submerged in distilled water or 40% salt solution will swell faster? Label the diagram below with your hypothesis.

PROCEDURES

1. Obtain 2 gummy bears and place them in 2 different small flasks.
2. Drown 1 bear in distilled water and drown the other in 40% salt solution.
3. At the end of the lab session, remove the bears from the solution and document the size difference with your mobile phone.



Hypothesized swelling of the bear based on tonicity

Condition	Tonicity Inside Bear Relative to the Solution	Tonicity Outside relative to the Bear	Hypothesis about swelling
A			
B			

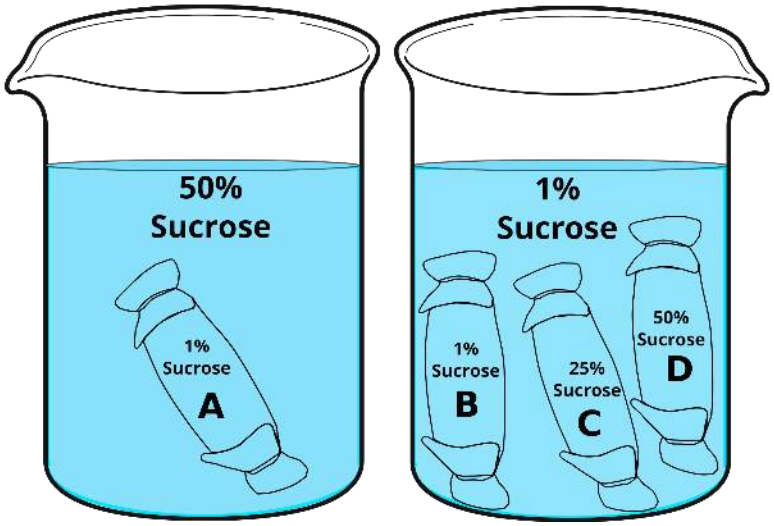
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2.1.4.5: OBSERVING OSMOSIS (ACTIVITY)

OBSERVE OSMOSIS ALONG A FREE ENERGY GRADIENT

1. Obtain four pieces of water-soaked dialysis tubing 15 cm long and eight pieces of string. Seal one end of each tube by tying it into a knot.
2. Open the other end of the tube by rolling it between your thumb and finger.
    1. Write A, B, C, D on 4 pieces of paper.
    2. Insert the labels into individual bags.
3. Fill the bags with the contents shown in the figure below with 10 ml of solution.
    A. **Bag A** 10 ml 1% Sucrose
    B. **Bag B** 10 ml 1% Sucrose
    C. **Bag C** 10 ml 25% Sucrose
    D. **Bag D** 10 ml 50% Sucrose
4. For each bag, loosely fold the open end and press on the sides to push the fluid up slightly and remove most of the air bubbles. Tie the folded ends securely, rinse the bags, and check for leaks.
5. Blot excess water from the outside of the bags and weigh each bag to the nearest 0.1 gram.
6. Record the weights in **Data Table 1: Weight of Dialysis Bags**.
7. Place bags B, C, and D in a beaker or large bowl filled with 1% sucrose. Record the time.
8. Place bag A in an empty beaker and fill the beaker with just enough 50% sucrose to cover the bag. Record the time.
9. Remove the bags from the beakers at 10-minute intervals for the next hour, blot them dry, and weigh them to the nearest 0.1 g. Handle the bags delicately to avoid leaks, and quickly return the bags to their respective containers. For each 10-minute interval record the total weight of each bag and its contents in **Data Table 1**. Then calculate and record in **Data table 2: Change in Weight of Dialysis Bags** the change in weight from the initial weight.



STOP AND THINK

- Define the tonicity of the solution inside the bag relative to the outside
- Based on your definitions, **hypothesize** the direction the solution will move (in or out of the bag) and fill in the table below

HYPOTHESIZED MOVEMENT OF SOLUTION BASED ON TONICITY

Bag	Tonicity Inside Bag Relative to the Solution	Tonicity of Outside Solution relative to the Bag	Hypothesized solution movement (in, out, none)
A			
B			
C			
D			

Data Table 1: Weight of Dialysis Bags

Bag	0 Min	10 Min	20 Min	30 Min	40 Min	50 Min	60 Min
A							
B							
C							
D							

Data Table 2: Net Weight Change of Dialysis Bags

Bag	0 Min (WT_0-WT_0)	10 Min (WT_{10}-WT_0)	20 Min (WT_{20}-WT_0)	30 Min (WT_{30}-WT_0)	40 Min (WT_{40}-WT_0)	50 Min (WT_{50}-WT_0)	60 Min (WT_{60}-WT_0)
A	**0**						
B	**0**						
C	**0**						
D	**0**						

PRESENT YOUR DATA

- Plot your data using only the Change in Weight. (subtract the Initial Weight at 0 minutes from the Total Weight at each time point)
  - Using a computer, create a scatter plot of the data from Table 2 and calculate the equation of the line
- Change in Weight = Total $Weight_{(current\_time)}$ – Initial $Weight_{(time\_0)}$

CONCLUDE

- Did your results match your hypotheses? _____
- What do the slopes of the lines generated from plotting Change Weight indicate to you? _____
- Can you analyze and articulate in words what has occurred with respect to these slopes? _____ _____ _____ _____ _____ _____

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2.1.4.6: AGAR CUBES (PREPARATION)

PROCEDURES

1. Prepare a 2% solution of agar.
   - Mix 20 g of agar with 1 L of distilled water.
2. Heat almost to a boil. Stir frequently until the solution is clear.
3. Remove from heat and add 10 mL of 1% phenolphthalein solution.
   - 1 g phenolphthalein in 100 mL 95% ethanol (If pink, titrate with HCl until clear.)
4. Pour agar into a shallow tray to a depth of 3 cm and allow it to set (overnight).

Silicone Ice-cube trays can be used. It is difficult to find them in the appropriate metric measurements. 3 cm cubes are approximately 1.2 inches and there are molds for this.

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SECTION OVERVIEW

2.1.5: CELLULAR RESPIRATION

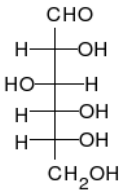
2.1.5.1: INTRODUCTION

2.1.5.2: FERMENTATION

2.1.5.3: CELL RESPIRATION (CONCEPT)

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2.1.5.1: INTRODUCTION



Glucose is the preferred carbohydrate of cells. In solution, it can change from a linear chain to a ring.

Energy is stored in the bonds of the carbohydrates. Breaking these bonds releases that energy. Crushing sugar crystals creates tiny electrical fields that give off invisible ultraviolet light. The wintergreen chemical (methyl salicylate) gets excited by these excited electrons and fluoresces in a visible blue wavelength. This phenomenon is called triboluminescence.

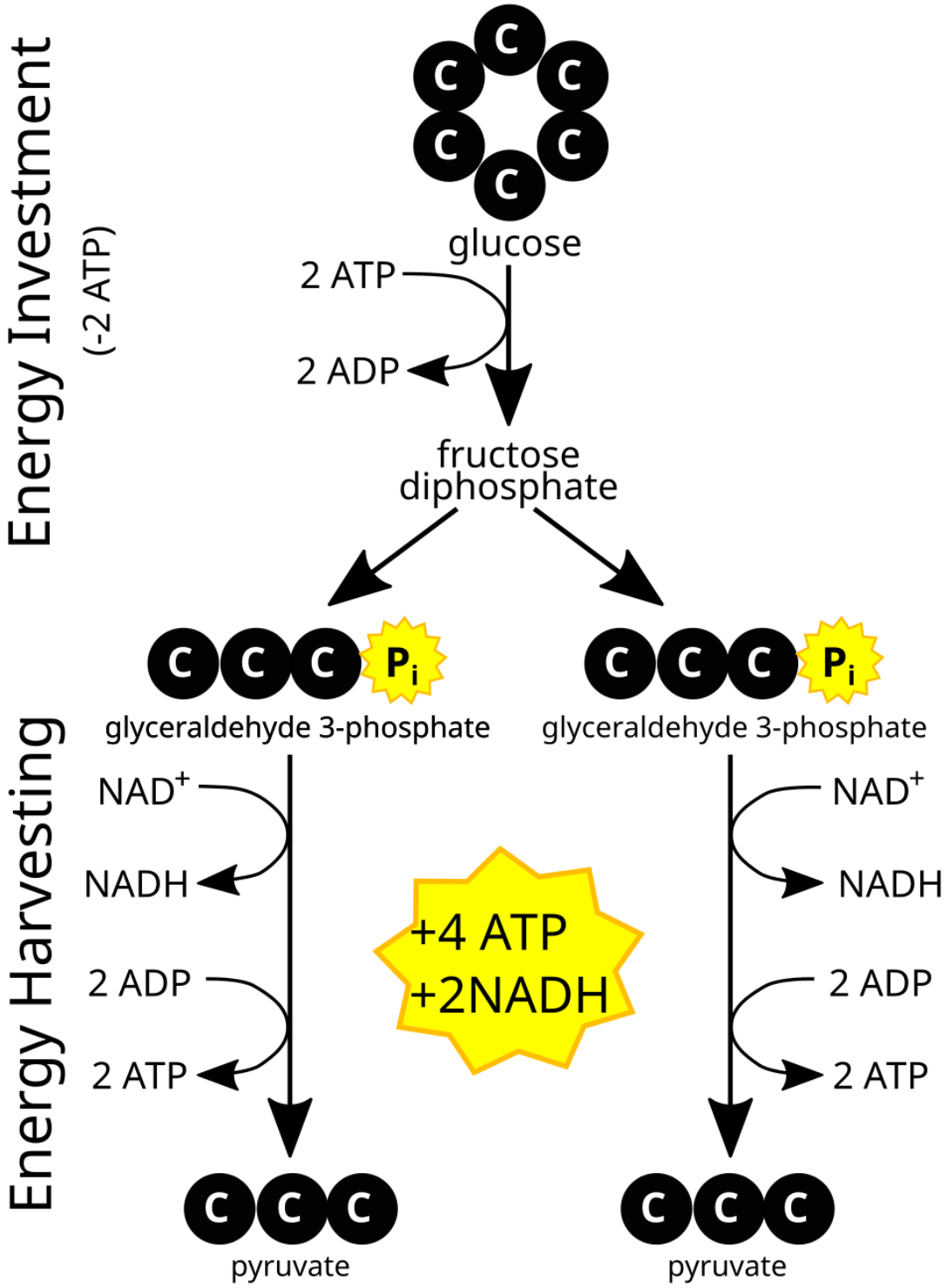




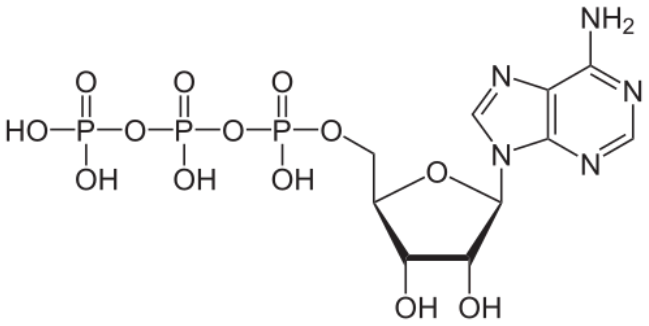
GLYCOLYSIS

Glucose is the preferred carbohydrate of cells. **Glycolysis** (***glyco*** – sugar; ***lysis*** – splitting) is a universal process of all cells that occurs in the cytosol whereby the glucose (a 6-carbon sugar) is split into two pyruvates (a 3-carbon molecule) molecules to generate ATP and reduced NADH. **ATP** (adenosine triphosphate) is the energy currency of the cell that stores chemical energy in 3 high energy phosphate bonds.

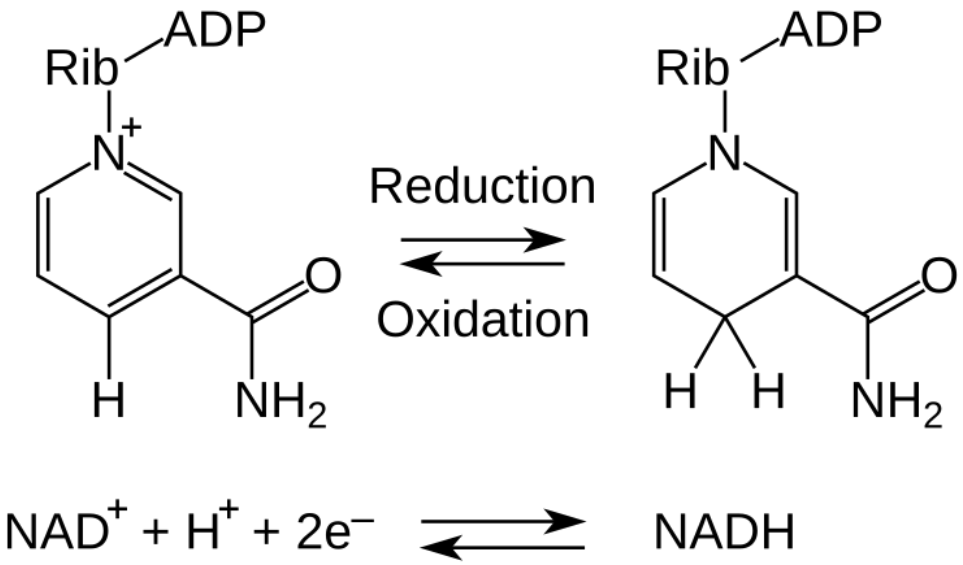
NADH (reduced nicotinamide adenine dinucleotide) is a high energy electron carrier that acts as a coenzyme in reactions and as a rechargeable battery of sorts. The uncharged state that is not carrying high energy electrons is called **NAD^+**.



Glycolysis is the splitting of glucose into 2 pyruvate molecules to generate 2 NADH and 2ATP molecules.



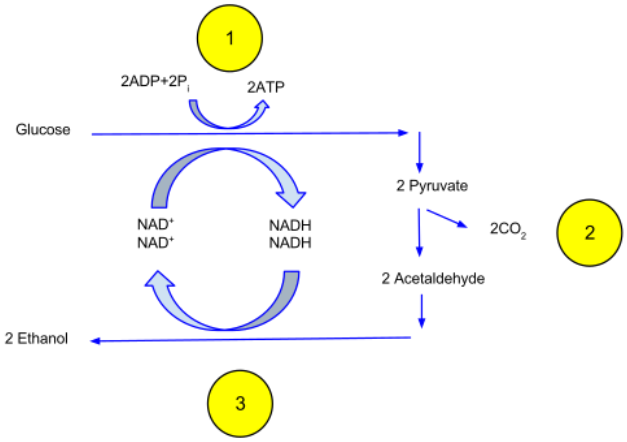
ATP contains 3 high energy phosphates and acts as cellular energy currency.



NADH is the reduced form of NAD+. The High energy electrons associated with the reduced form come with a Hydrogen atom.

FERMENTATION

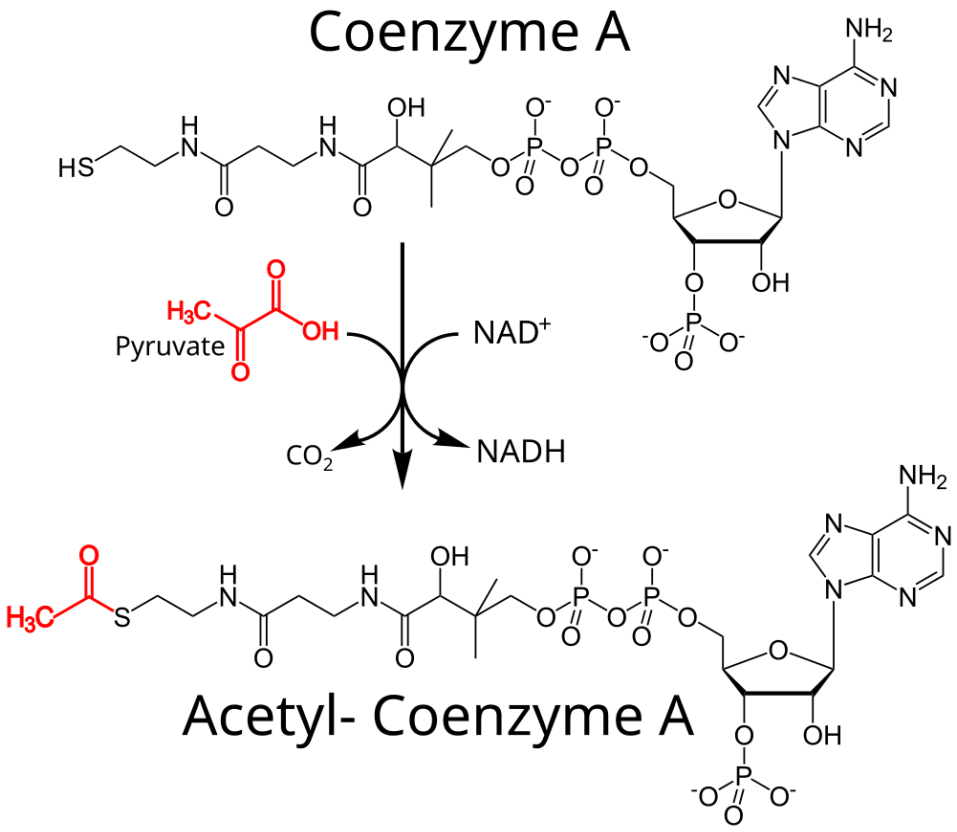
In the absence of oxygen, cells may decide to utilize the pyruvate from glycolysis to rapidly generate additional ATP molecules in a process called fermentation. **Fermentation** is the anaerobic process of reducing pyruvate to generate ATP. This process uses the NADH generated from glycolysis as the reducing agents. Fermentation is a familiar process that occurs in yeast to generate ethanol. In other organisms, like humans, fermentation results in the production of lactic acid. Both lactic acid and ethanol are toxic, but this aids the cells in generating ATP when energy is required rapidly. Fermentation also generates CO_2 as a waste molecule as pyruvate is broken down into a 2-carbon compound.



Fermentation in yeast generates ATP in the absence of oxygen but yields little ATP at the cost of the reduced NADH. Credit: Davidcarmack (CC-BY-SA)

THE PREPARATORY REACTION

In the presence of O_2, aerobic organisms will use a reaction of pyruvate decarboxylation in the cytosol. This reaction generates a molecule of **Acetyl-CoA** from the Coenzyme A which can enter the mitochondria.

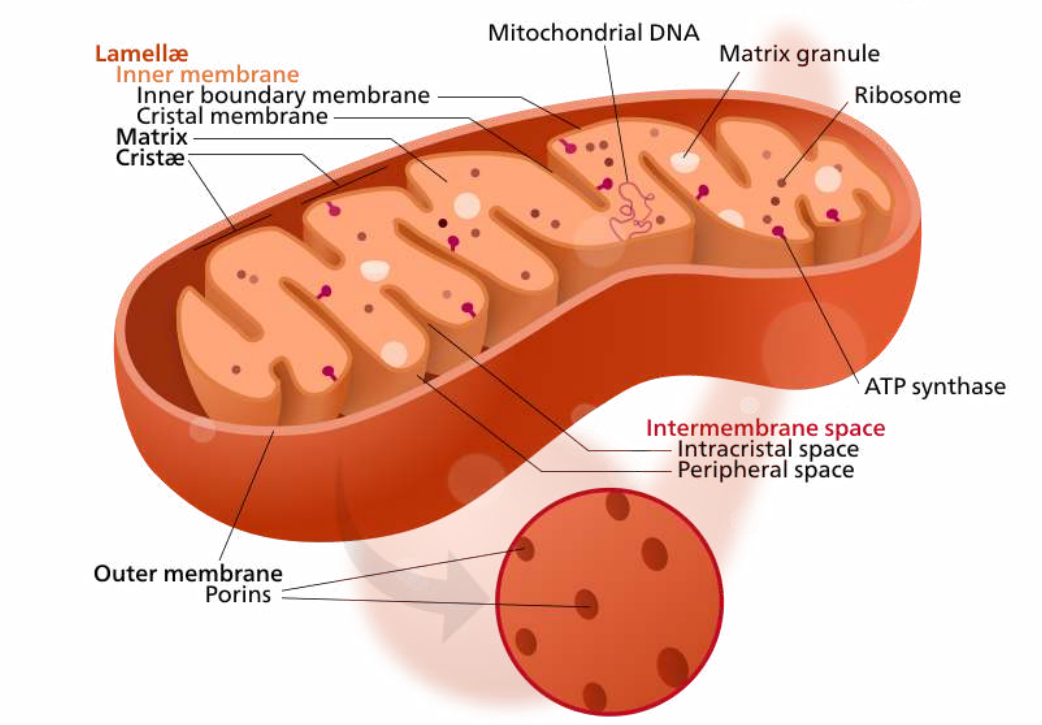


Coenzyme A (CoA) is charged with an Acetyl group (2 carbon compound) to generate Acetyl-CoA and a CO_2.

When there is an excess of carbohydrates, the Acetyl-CoA is used as a starting point for long-term energy storage in lipid synthesis.

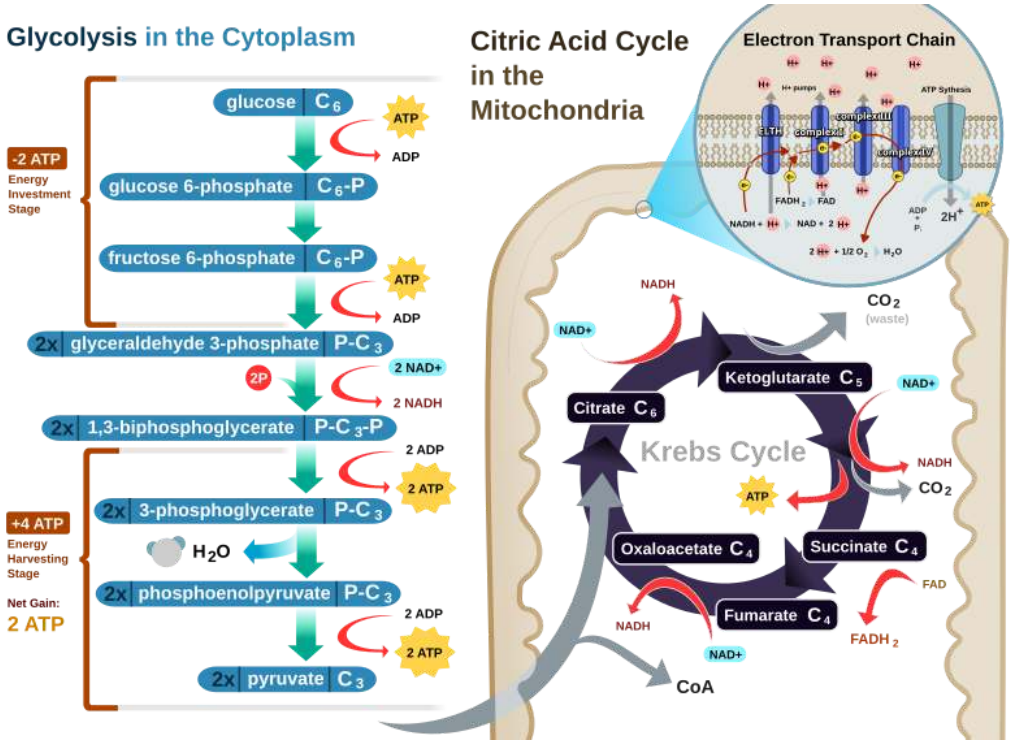
MITOCHONDRIA

Mitochondria are the power station of eukaryotic cells. They are derived from a process described by the **endosymbiotic theory** whereby aerobic prokaryotes were engulfed by a proto-eukaryote. In this mutualistic arrangement, the prokaryote detoxified the deadly O_2 gas in the environment and used it to fully break down glucose to yield many ATP molecules. Evidence for this theory comes from the independent replication of the mitochondria, the bacterial-like mitochondrial DNA, the bacterial-like mitochondrial ribosomes, the bacterial lipids found in the inner membrane and the eukaryotic nature of the outer membrane. Mitochondria are genomically similar to bacteria of the order Rickettsiales. Some bacteria of this order are still free-living and some are intracellular pathogens.



Credit: Kelvinsong (CC-BY-SA 3.0)

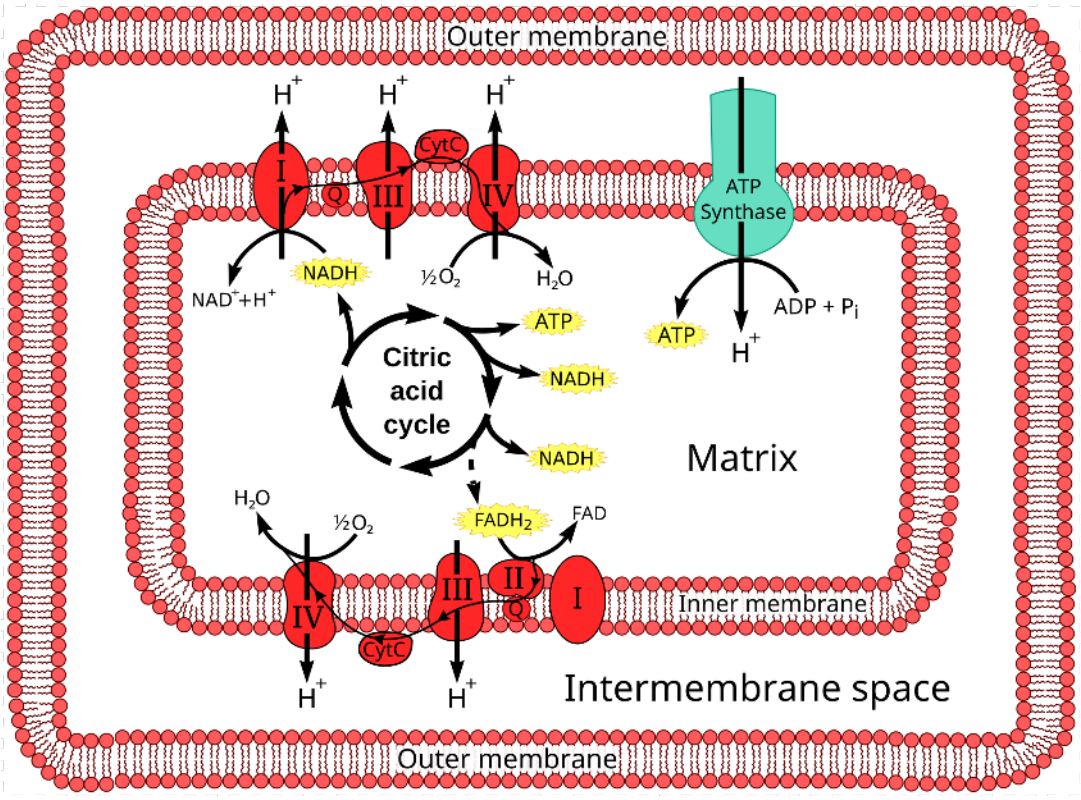
AEROBIC RESPIRATION



Cellular Respiration. Left side is glycolysis (anaerobic). The Right side is what occurs in the presence of oxygen in eukaryotes. The aerobic reactions occur inside the mitochondria after being fed Acetyl-CoA molecules from the cytoplasmic preparatory reaction. Credit: RegisFrey (CC-BY-SA 3.0)

Acetyl-CoA enters the mitochondrial matrix where it is used in the **Krebs Cycle** (aka Tricarboxylic acid cycle (TCA), aka Citric acid cycle). For each pyruvate, there are 2 turns of the cycle where additional NADH and another high energy electron carrier **$FADH_2$** (flavin adenine dinucleotide) are generated. The electrons stored by NADH and $FADH_2$ are transferred to proteins called **cytochromes** that have

metal centers for conducting these electrons. In the process of moving these electrons, the cytochromes in this **Electron Transport Chains** (ETC) power the movement of protons into the intermembrane space. The terminus of these electrons is an O_2 molecule that is reduced into 1/2 H_2O molecules. This apparent movement of water molecules from the chemical synthesis is termed **chemiosmosis**. A channel in the membrane called **ATP synthase** acts as a gateway for the H^+ back into the matrix, but use this motion to convert ADP into ATP.



*Closeup of the **Electron Transport Chain** (ETC) that takes place on the inner membrane of mitochondria. This is where oxygen is utilized as the final electron acceptor. Reduction of 1/2 O_2 results in the generation of a water molecule (**chemiosmosis**). Credit: Jeremy Seto (CC-BY-NC-SA 3.0)*

METABOLIC POOL

The catabolic pathways involved in the glycolysis and the Krebs cycle constitute the **metabolic pool** that supplies building blocks for other anabolic reactions in the cell. An excess of carbohydrates can result in an accumulation of Acetyl-CoA molecules. If there is a great excess of Acetyl-CoA, the acetyl groups can be committed to fatty acid synthesis for long-term energy storage. Glycolytic products can also be the starting point for amino acid synthesis. 3-phosphoglycerate can be used to synthesize glycine, cysteine and serine. Pyruvate can be used to generate alanine, valine, and leucine. Oxaloacetate from the Krebs cycle can be used as a starting point for aspartate, lysine, asparagine, methionine, threonine, and isoleucine. Glutamate and glutamine are synthesized from α-ketoglutarate formed during the Krebs cycle. While most of the 20 amino acids can be synthesized *de novo*, there are 9 **essential amino acids** in humans that can not be synthesized in sufficient quantity and therefore must be gained from the diet. These essential amino acids include histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine.

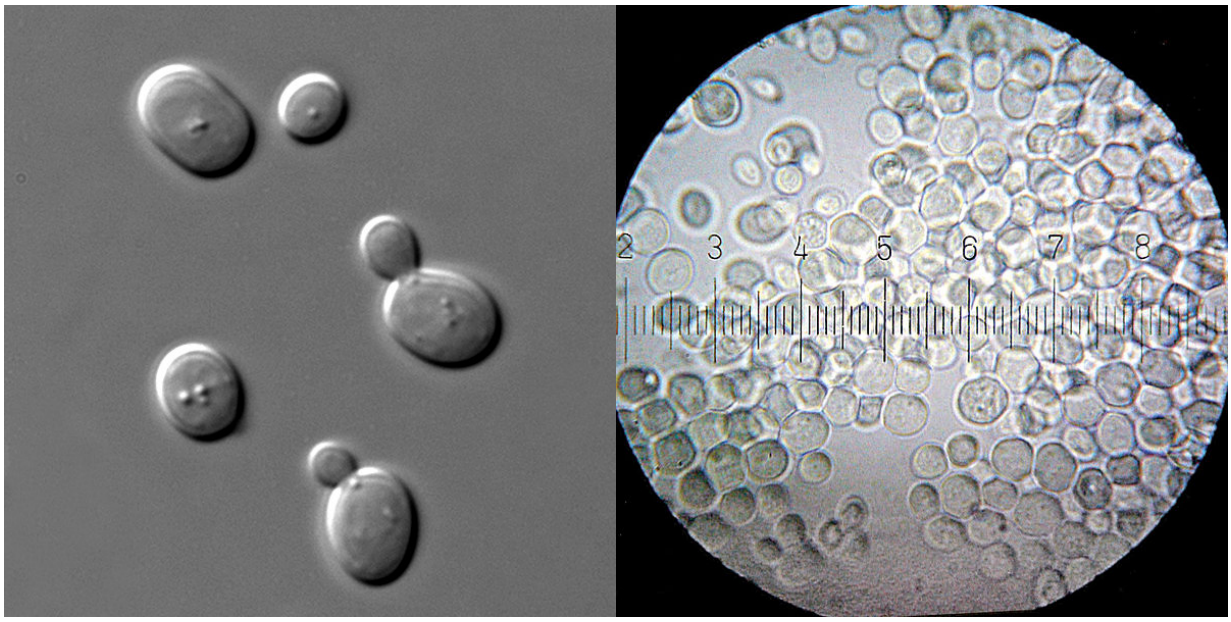
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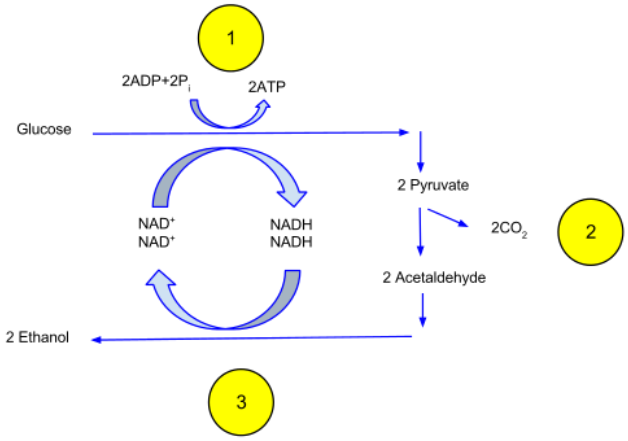
2.1.5.2: FERMENTATION

INTRODUCTION

Yeasts are single-celled fungi. The species called *Saccharomyces cerevisiae* is commonly called Baker's or Brewer's yeast. Like other eukaryotes with mitochondria, yeast can use oxygen to generate ATP in the process of **oxidative phosphorylation**. These yeast are **facultative aerobes** which means they can also switch to an anaerobic mechanism of ATP production called fermentation.

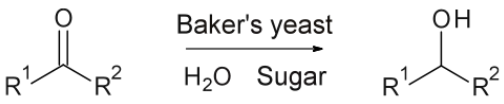


In all organisms, the process of glycolysis occurs anaerobically in the cytoplasm to produce two pyruvate molecules from a single glucose. This process produces 2 new ATP molecules and reduced nicotinamide adenine dinucleotide (NADH).



In ethanol fermentation. One glucose molecule breaks down into two pyruvates via glycolysis (1). The energy from these exothermic reactions is used to bind inorganic phosphates to ADP and convert NAD+ to NADH. The two pyruvates are then broken down into two Acetaldehyde and give off two CO2 as a waste product (2). The two Acetaldehydes are then reduced to two ethanol, and NADH is oxidized back into NAD+ (3).

Fermentation is an anaerobic process that occurs in the cytoplasm and quickly generates an additional ATP through the reduction of pyruvate. NADH is the source of electrons in this process that is oxidized to NAD+. Many organisms will ferment to generate lactic acid and CO_2 from the pyruvate in order to generate ATP. Yeast fermentation produces **ethanol**.



FERMENTATION SET-UP

1. Mix the solutions in the table below in a fermentation tube.
2. Eliminate the air bubble in the sealed end of the fermentation tube.
3. Place rubber stopper into the open end of the fermentation tube and place at the appropriate temperature for an hour.
4. Predict the amount of CO_2 generated in the last column of the table using +, – or +++.
    - After an hour, measure the headspace created by the bubbles and compare with your predictions.

TUBE	YEAST	SUGAR	ADDITIVE	TEMP.	CO_2 generation
1	10 ml yeast	None	20 ml H_2O	25°C	
2	10 ml yeast	10 ml glucose	10 ml H_2O	25°C	
3	10 ml yeast	10 ml glucose	10 ml H_2O	37°C	
4	10 ml yeast	10 ml lactose	10 ml H_2O	37°C	
5	10 ml yeast	10 ml sucrose	10 ml H_2O	37°C	
6	10 ml yeast	10 ml maltose	10 ml H_2O	37°C	
7	10 ml yeast	10 ml glucose	10 ml 0.1M $MgSO_4$	37°C	
8	10 ml yeast	10 ml glucose	10 ml 0.1 M NaF	37°C	
9	10 ml yeast	10 ml lactose	10 ml H_2O + LactAid pill	37°C	

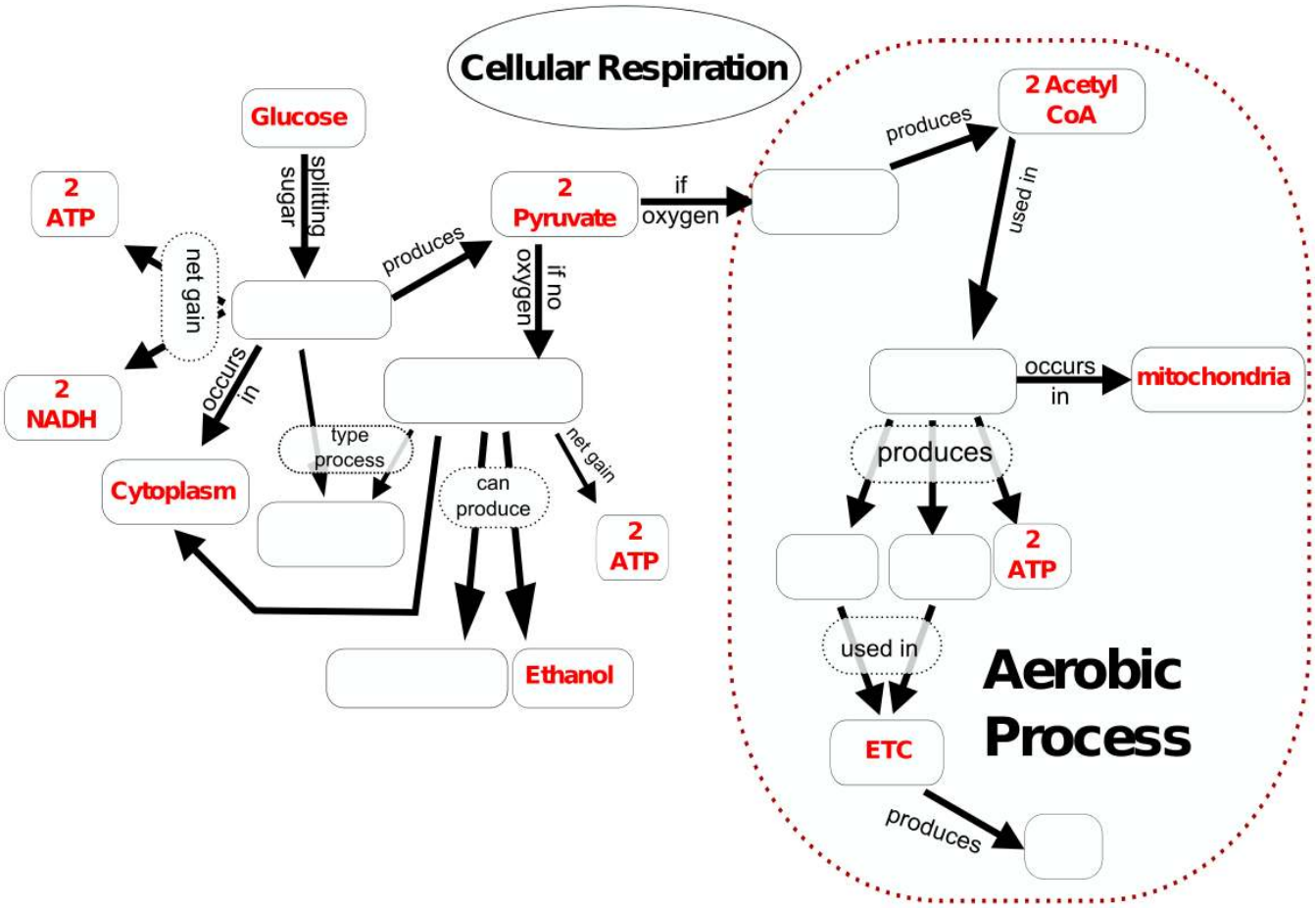
QUESTIONS TO DIRECT HYPOTHESIS FORMATION

1. What is the preferred energy source of all cells?
2. What types of sugars are being used in each tube (monosaccharide, disaccharide, etc)?
3. What effect should temperature have on the fermentation reactions?
4. What does CO_2 indicate in these tubes?
5. What do you think the additives do? What effect will they have?

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2.1.5.3: CELL RESPIRATION (CONCEPT)



Cellular Respiration concept map.

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SECTION OVERVIEW

2.1.6: PHOTOSYNTHESIS

2.1.6.1: INTRODUCTION

2.1.6.2: PHOTOSYNTHETIC PIGMENTS

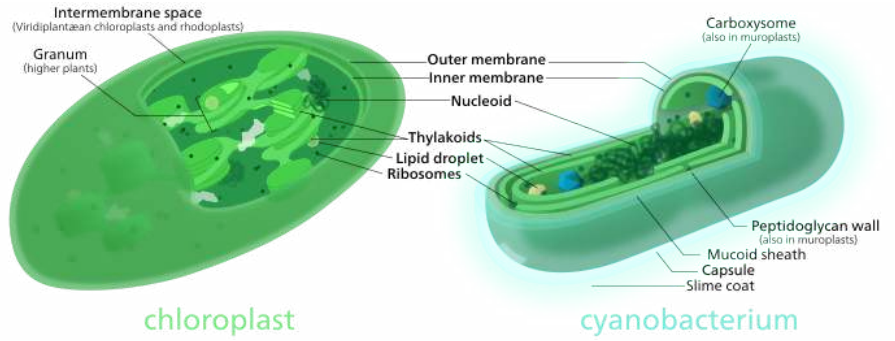
2.1.6.3: ABSORBANCE SPECTRA OF PHOTOSYNTHETIC PIGMENTS

2.1.6.4: PHOTOSYNTHESIS (CONCEPT)

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2.1.6.1: INTRODUCTION

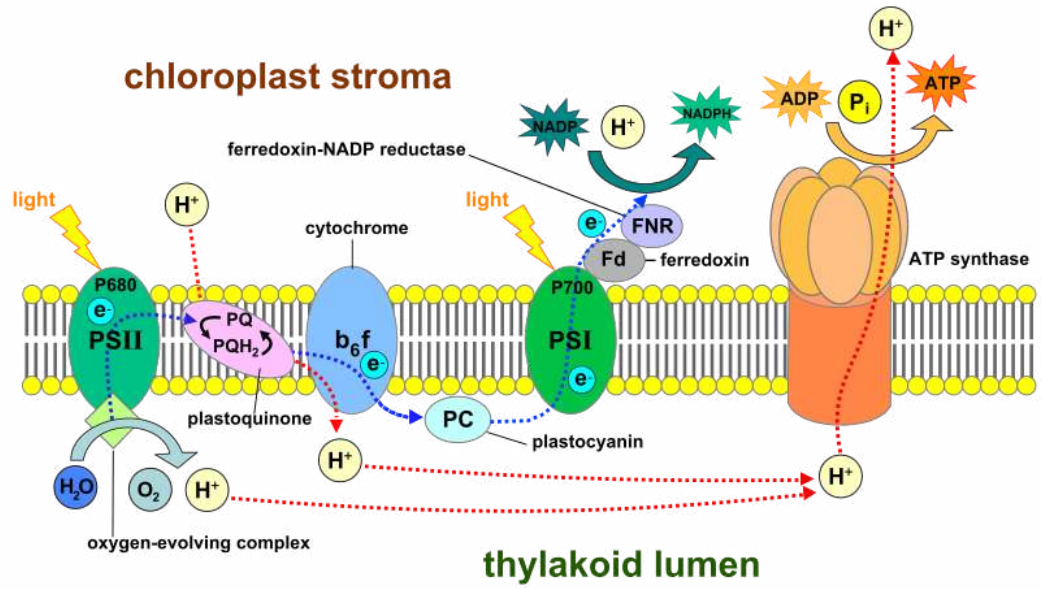
CHLOROPLASTS



Credit: elvinsong [CC-BY-SA 3.0]

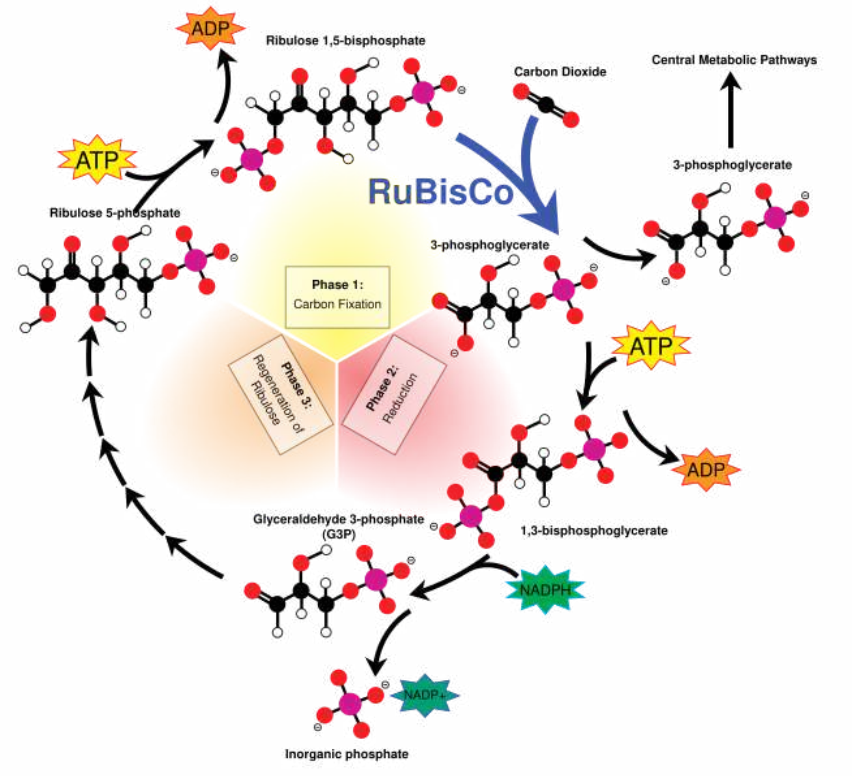
Chloroplasts arose through a second endosymbiotic event in plants and various protists. These light-harvesting organelles share a similarity in structure and genome to photoautotrophic cyanobacteria.

LIGHT-HARVESTING



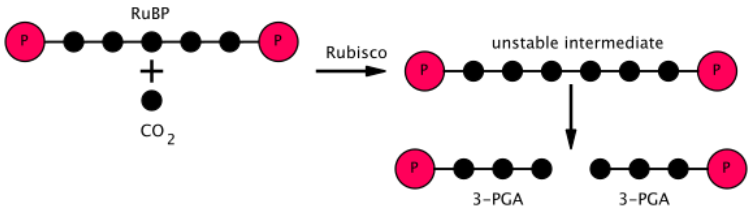
The thylakoid membranes of chloroplasts and cyanobacteria provide additional surface area for energy capture of light to occur. The light-dependent reactions in chloroplasts utilize two protein complexes referred to as **Photosystem I** (PSI) and **Photosystem II** (PSII) located on the thylakoid membranes. At the center of each photosystem complexes are **photopigments** optimized to absorb specific wavelengths of light. When light is absorbed in a photosystem, an electron is excited and transferred to the electron transport chain. In PSII, the electron is regenerated by splitting of two water molecules into $4H^+ + 4e^- + O_2$. As the electrons move through the ETC, protons are pumped into the thylakoid space. The ETC leads to the reduction of a high energy electron carrier $NADP^+$ to **NADPH**. Since this pathway uses consumes water in a chemical reaction, the apparent loss of water in the thylakoid space is referred to as chemiosmosis.

PSI is also known as the cyclic pathway since the excited electron runs through a closed circuit of the ETC to regenerate the lost electron. This closed circuit also generates a proton gradient through powering of a proton pump but does not lead to the reduction of NADPH. As with the ETC-powered proton pump in mitochondria, the proton gradient is used to power ATP-synthase in producing ATP molecules.

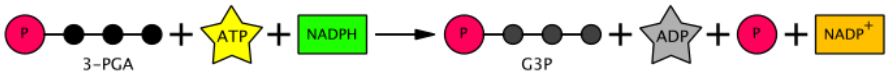


Credit: Mike Jones [CC-BY-SA 3.0]

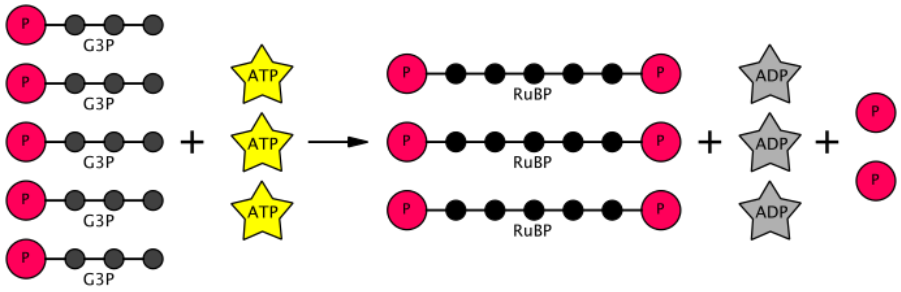
The light-independent reactions are also known as the dark reactions or **Calvin Cycle** and utilize the ATP and NADPH from the light-dependent reactions to fix gaseous CO_2 into carbohydrate backbones. Photosynthesis is often simplified into $6CO_2 + 6H_2O$ + light –> $C_6H_{12}O_6 + 6O_2$. However, the true product is 3-phosphoglycerate that can be used to generate longer carbohydrates like glucose. The starting point of **carbon fixation** is the carbohydrate Ribulose 1,5-bisphosphate. The enzyme Ribulose Bisphosphate Carboxylase (**RuBisCO**) captures a CO_2 molecule onto Ribulose 1,5-bisphosphate to generate 2 molecules of 3-phosphoglycerate which can enter the process of **gluconeogenesis** to generate glucose. ATP from the light reactions can then facilitate the conversion of 3-phosphoglycerate to 1,3 bisphosphoglycerate which can be reduced by NADPH to glyceraldehyde-3-phosphate (G3P). G3P can then be used to regenerate Ribulose 1,5-bisphosphate.



1: Carbon fixation by RuBisCO

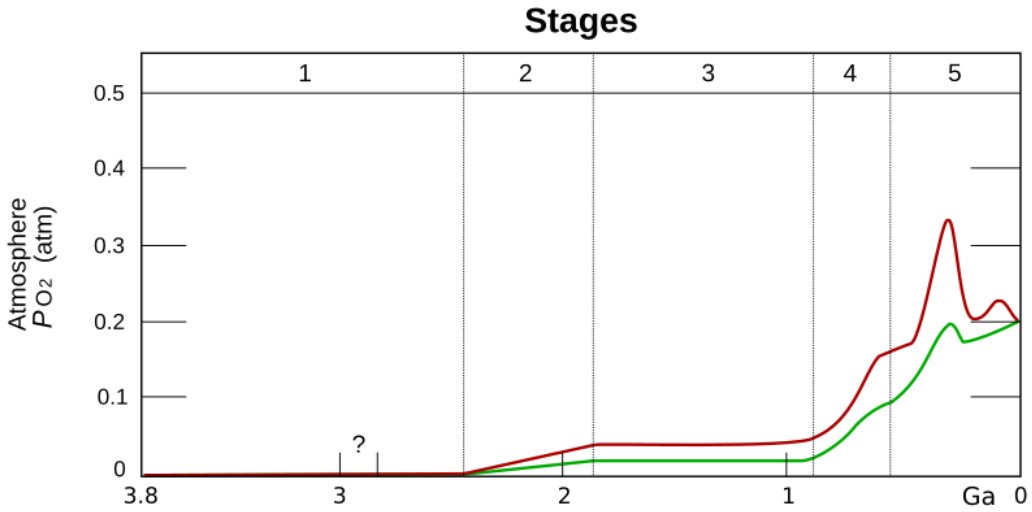


2: Reduction by NADPH



3: Ribulose, 5-bisphosphate regeneration

THE GREAT OXYGENATION EVENT



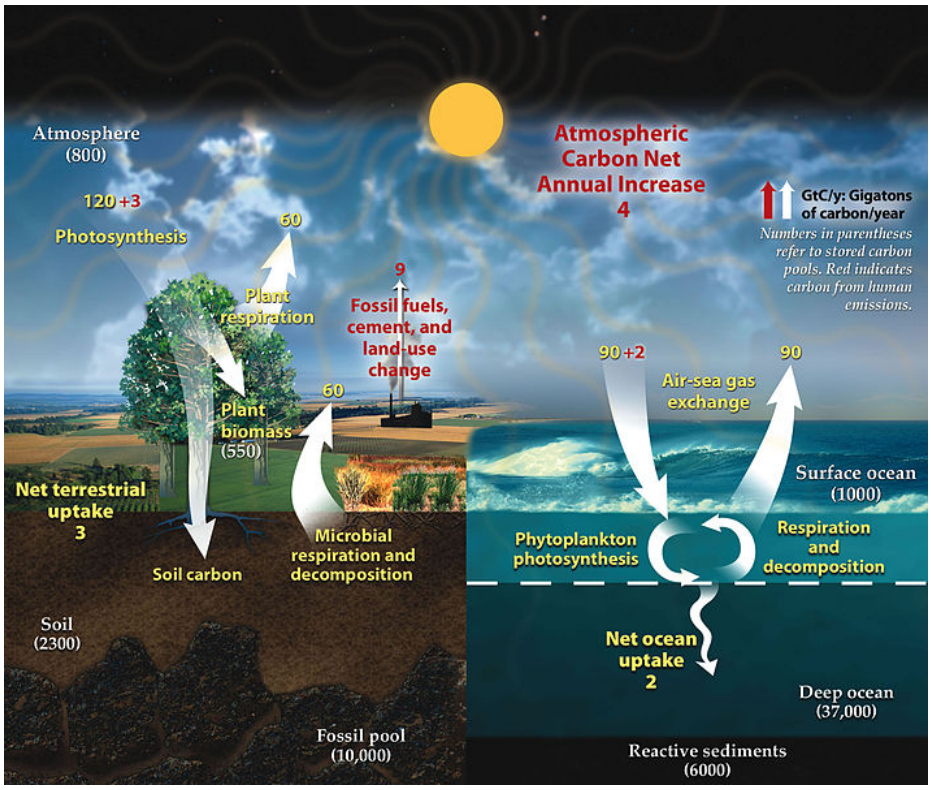
Two estimates of the evolution of atmospheric O_2. The upper red and lower green lines represent the range of the estimates. Stage 1 (3.85–2.45 Ga) represents the primordial reducing atmosphere. Stage 2 (2.45–1.85 Ga) coincides with the emergence of oceanic cyanobacteria where O_2 was being absorbed by the oceans and sediment. O_2 escaped the oceans during Stage 3 (1.85–0.85 Ga). O_2sinks filled in Stage 4 (0.85–0.54 Ga) and Stage 5 (0.54 Ga–present) leading to atmospheric accumulation.



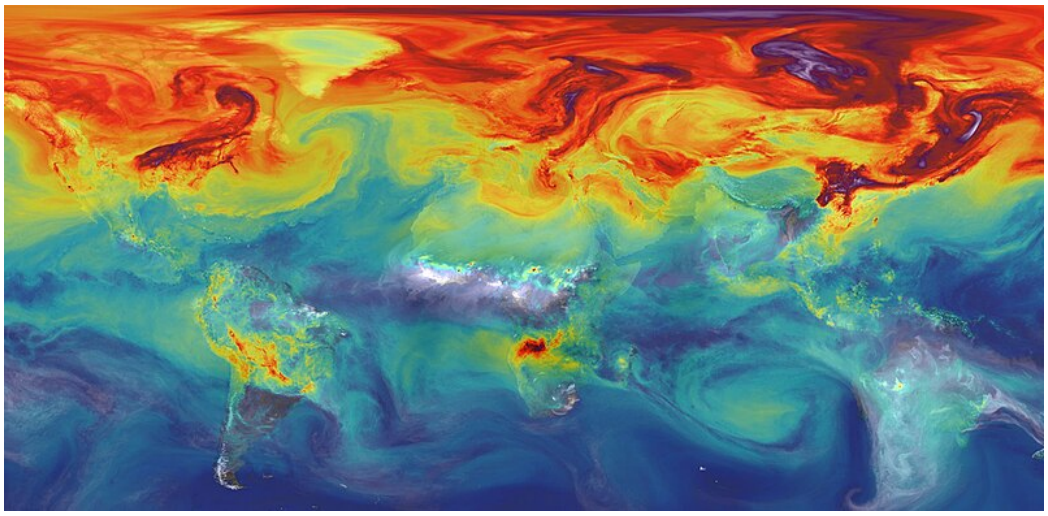
Banded iron formations in 2.1 billion-year-old rock illustrate the oxidation of dissolved oceanic iron that precipitated in response to accumulating O_2 concentrations.







The Carbon Cycle illustrates carbon sequestration and release between various carbon sinks.



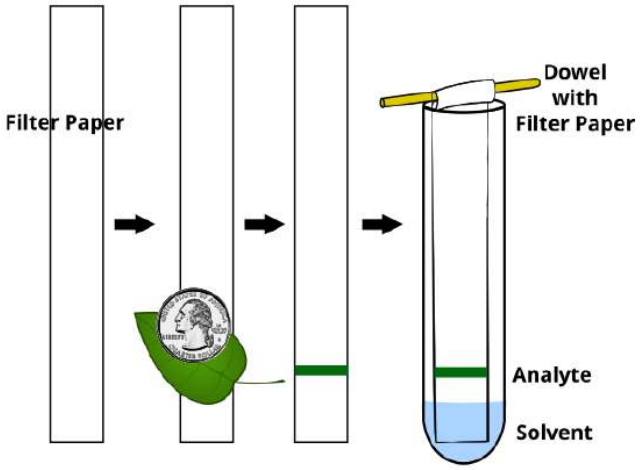
Projection of atmospheric CO_2 accumulation without reduction of fossil fuel reduction by NASA.

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2.1.6.2: PHOTOSYNTHETIC PIGMENTS

EXTRACT AND SEPARATE THE PIGMENTS



1. Lay a strip of filter paper on the bench.
2. About 2 cm from the bottom of the strip, place a fresh spinach leaf and rub a coin across the leaf to transfer pigment to the strip.
3. The instructor will be provided with a spoonful of *Spirulina* powder that has been soaked in 10ml acetone overnight.
    - On a separate strip, the instructor will apply the Spirulina extract approximately 2cm from the bottom of the strip.
4. Suspend the strips by a dowel or paper clip in a tube with about 3ml chromatography solution *(2 isooctane: 1 acetone: 1 diethyl ether*).
5. Develop the strips until the solvent reaches about 2 cm from the top.

CHROMATOGRAPHY ANALYSIS

1. How many different pigments separate from the spinach extract? From the spirulina?
2. Are all pigments represented between the two extracts?
3. The mobile phase is non-polar. What are the properties of each pigment?
4. Measure the R_f of each pigment.

Pigment Color	Distance (cm)	R_F
	D_A= D_S=	
	D_A= D_S=	
	D_A= D_S=	
	D_A= D_S=	

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2.1.6.3: ABSORBANCE SPECTRA OF PHOTOSYNTHETIC PIGMENTS

PRELAB EXERCISE

1. Fill the *Color* field in the table below
2. Use plot.ly to create a **line** graph with the 3 samples below (A, B, C)
   - Plot *% Reflectance* on the Y-axis and *Wavelength (nm)* on the X-axis

		% Reflectance		
Color	**nm**	**A**	**B**	**C**
	400	68	92	78
	425	40	71	77
	450	90	38	51
	475	97	49	57
	500	100	92	45
	525	100	100	66
	550	96	97	100
	575	98	96	100
	600	96	98	100
	625	97	80	100
	650	79	71	100
	675	56	96	100
	700	88	100	100

STOP AND THINK: REFLECTANCE

A sign of plant health is viewed through the near infra-red. While we cannot see this spectrum of light with our eyes, we can use other sensors to detect this light. Compare the images of the Black & White with the Infra-red image. What differences can you see in the 2 images that will help you understand how this is a useful measure of plant health? How do you think this corresponds to the table above?



The English Garden

Reflectance Slider shows an overlay of the next images.



The English Garden (black & white)



The English Garden (near infra-red)

Visible light wavelengths (between 400nm-700nm) are strongly absorbed by the pigments in leaves (Chlorophylls, Xanthophylls, and Carotenoids). These pigments utilize the energy of these wavelengths to take part in the light reactions. The cellular structure of leaves does not absorb wavelengths longer than these wavelengths (>700nm in the infra-red range). By comparing the amount of visible light to the amount of near infra-red lights that are reflected, one can gauge the relative health of leaves, forests, or jungles. This is the rough description of the Normalized Differential Vegetation Index (NDVI) that scientists use in conjunction with satellite imagery to assess the health of vegetation.

THE ROLE OF LIGHT IN CARBOHYDRATE SYNTHESIS

1. Pick a leaf from a geranium exposed to light and one kept in the dark for 48 hours.
    - Keep the stem on the leaf grown in the light.
    - Remove the stem from the leaf grown in the dark.
2. Hydrolyze the cell walls of the geranium leaves by boiling in a water bath for 5 minutes or until it looks like over-cooked vegetables).
3. ***"****Bleach****"*** the leaves by removing the pigments. Place the leaves in hot alcohol for 7 minutes or until they turn white.
    1. Save this green solution for Absorbance Spectrum exercise.
4. Remove the leaves and place it in a petri dish.
5. Add iodine to the dish. If starch is present, the leaf will turn a deep bluish-black color.
6. Photograph the leaf with your phone to document the effects of light on carbohydrate storage.

MEASURING THE ABSORBANCE

1. Connect the Spectrovis to the LabQuest2.
2. Turn on the Labquest2 units.
3. Choose the Labquest app.
4. Select the icon that looks like X|Y.

5. Press the green Play button on the bottom left.
6. Press OK to calibrate.
7. Let the machine calibrate for 90 seconds.
8. Choose “Finish calibration”.
9. Insert the Geranium pigment from the bleaching reaction.
    1. Do NOT use Acetone in these plastic cuvettes since it will frost over the plastic.
10. Press the Red Stop button.
11. Students should record the absorbance values at every 10 nm from 380nm-700nm.
12. The professor will prepare *Spirulina* extract diluted in ethanol in a cuvette and obtain the continuous absorbance spectrum.
13. Plot Relative Absorbance against *wavelength* using a line graph and compare the absorption spectrum of the extracts.
    1. Relative Absorbance sets the maximum value in each dataset as a denominator.
    2. Every value is divided by this maximum value.

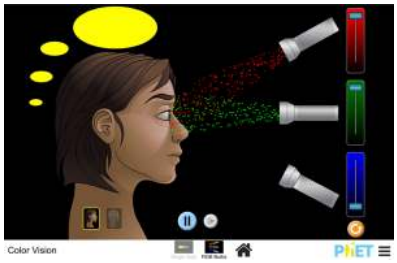
This page titled 2.1.6.3: Absorbance Spectra of Photosynthetic Pigments is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Bio-OER.

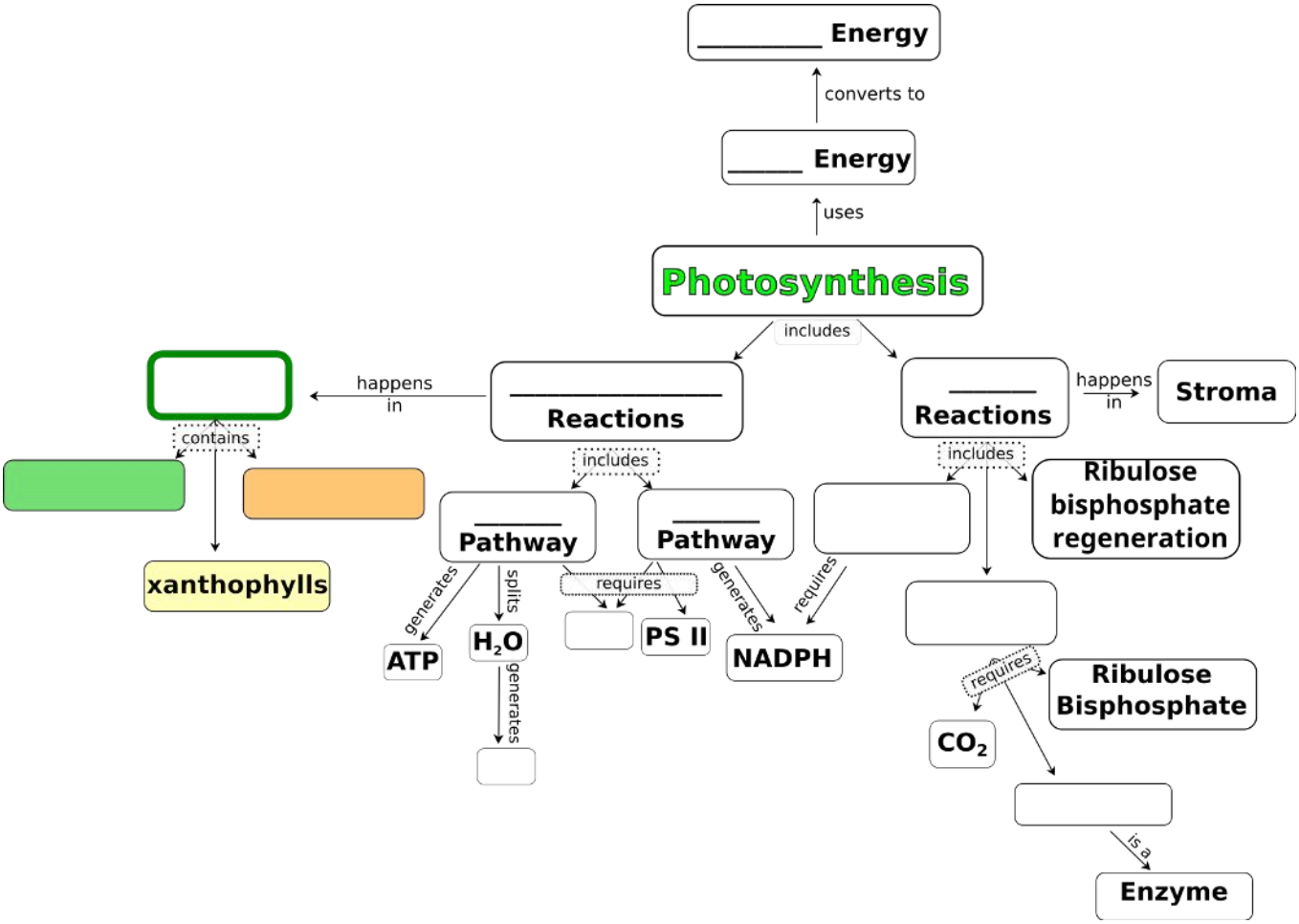
- **6.3: Absorbance Spectra of Photosynthetic Pigments** by Bio-OER is licensed CC BY-NC-SA 4.0.

2.1.6.4: PHOTOSYNTHESIS (CONCEPT)

EXPLORING LIGHT

Run the simulation below to understand how white light, specific wavelengths of light, and filtered light work.





Concept map for Photosynthesis. Download PDF

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SECTION OVERVIEW

2.1.7: CELL DIVISION

2.1.7.1: INTRODUCTION

2.1.7.2: MITOSIS (ACTIVITY)

2.1.7.3: MODELING MITOSIS AND MEIOSIS (ACTIVITY)

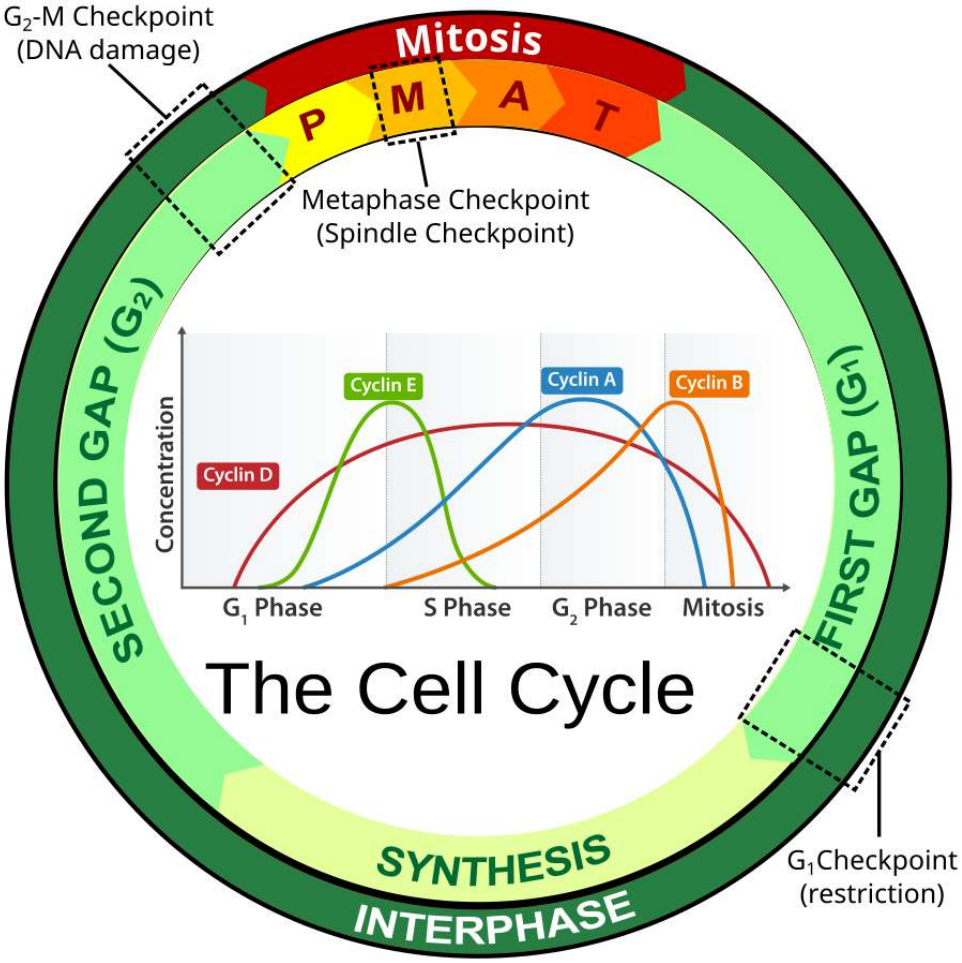
2.1.7.4: CHROMOSOMES AND KARYOTYPES

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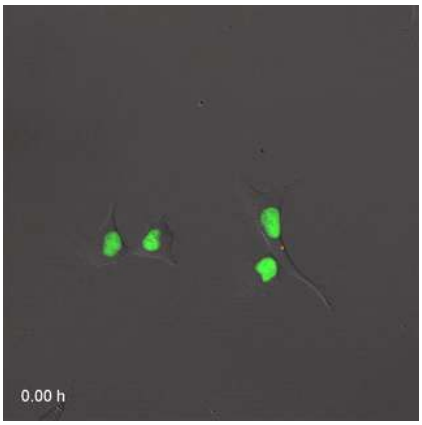
2.1.7.1: INTRODUCTION

INTRODUCTION: THE CELL CYCLE AND MITOSIS

The cell cycle refers to a series of events that describe the metabolic processes of growth and replication of cells. The bulk of the cell cycle is spent in the "living phase", known as **interphase**. Interphase is further broken down into 3 distinct phases: G_1 (Gap 1), S (Synthesis) and G_2 (Gap 2). **G_1** is the phase of growth when the cell is accumulating resources to live and grow. After attaining a certain size and having amassed enough raw materials, a checkpoint is reached where the cell uses biochemical markers to decide if the next phase should be entered. If the cell is in an environment with enough nutrients in the environment, enough space and having reached the appropriate size, the cell will enter the S phase. **S phase** is when metabolism is shifted towards the replication (or synthesis) of the genetic material. During S phase, the amount of DNA in the nucleus is doubled and copied exactly in preparation to divide. The chromosomes at the end of G_1 consist of a single **chromatid**. At the end of S phase, each chromosome consists of two identical **sister chromatids** joined at the **centromere**. When the DNA synthesis is complete, the cell continues on to the second growth phase called **G_2**. Another checkpoint takes place at the end of G_2 to ensure the fidelity of the replicated DNA and to re-establish the success of the cell's capacity to divide in the environment. If conditions are favorable, the cell continues on to mitosis.

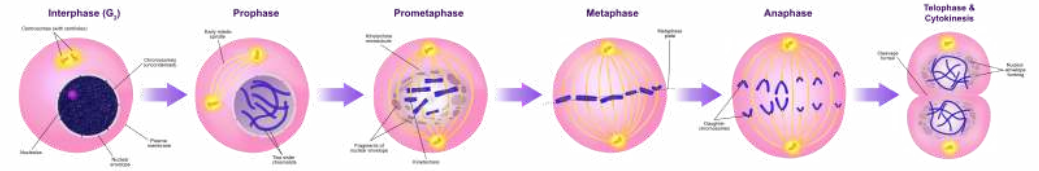


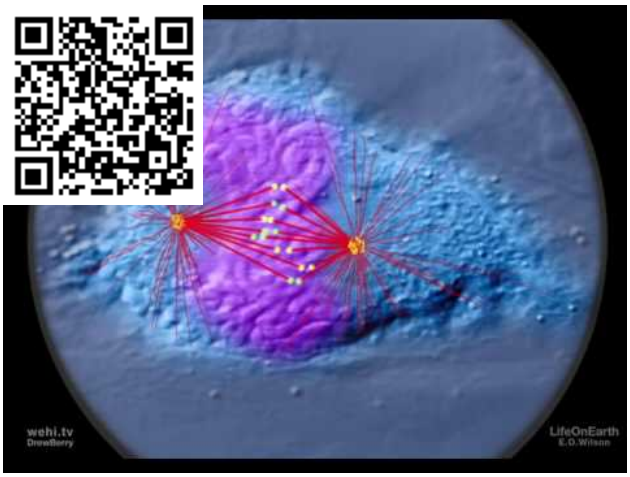
Eukaryotic cell cycle is governed by the expression of cyclin proteins along with their activity. Credit: Jeremy Seto (CC-BY-SA)

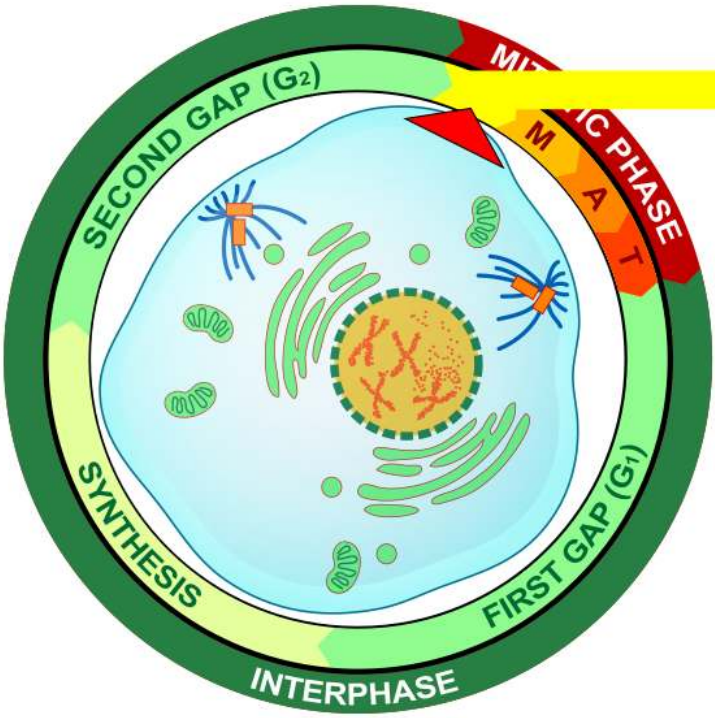


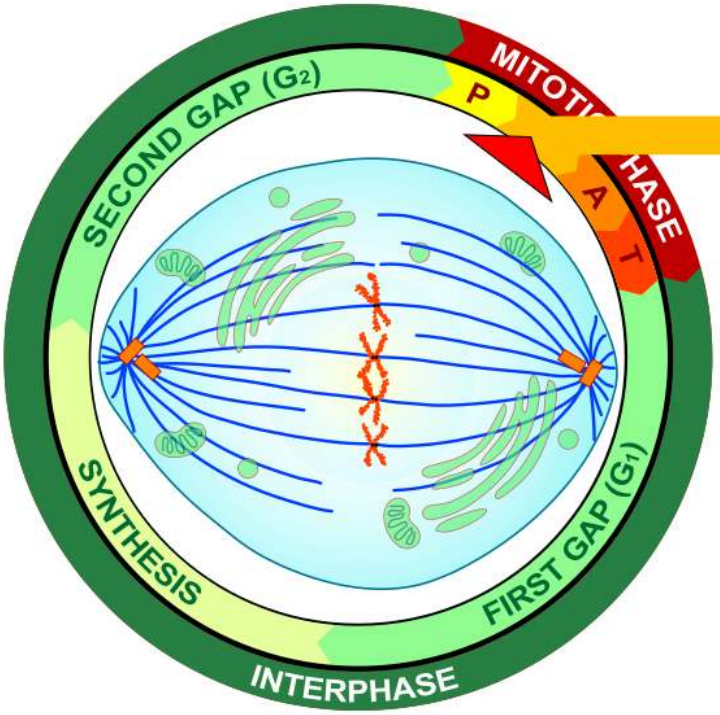
Mammalian cells in culture going through the Cell Cycle. Green marker proteins expressed during the G1 phase. Red marker proteins are expressed during S/G2/M. During the G1 to S transition, fluorescence disappears as the marker proteins also transition in expression.

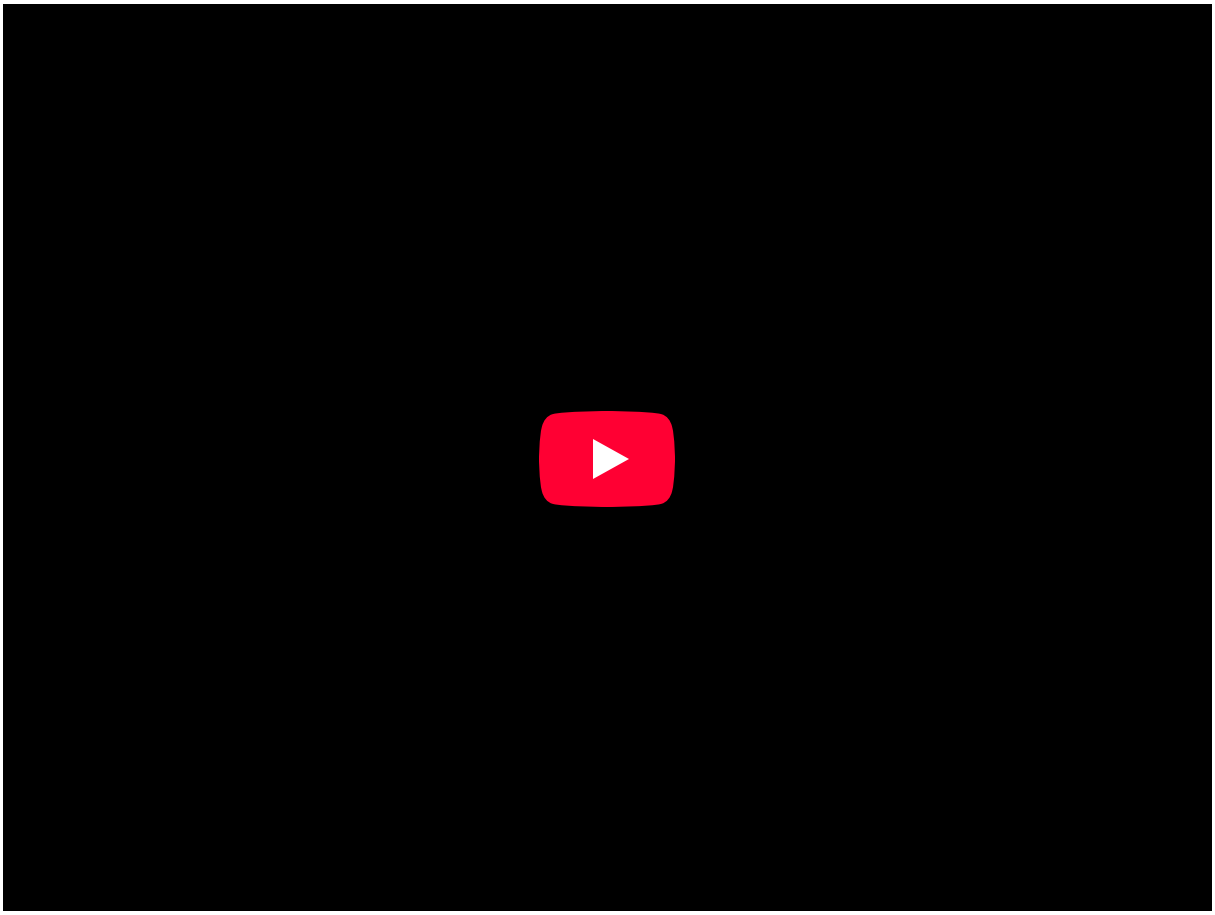
Mitosis is the process of nuclear division used in conjunction with cytokinesis to produce 2 identical daughter cells. **Cytokinesis** is the actual separation of these two cells enclosed in their own cellular membranes. Unicellular organisms utilize this process of division in order to reproduce asexually. Prokaryotic organisms lack a nucleus, therefore they undergo a different process called binary fission. Multicellular eukaryotes undergo mitosis for repairing tissue and for growth. The process of mitosis is only a short period of the lifespan of cells. Mitosis is traditionally divided into four stages: **prophase**, **metaphase**, **anaphase,** and **telophase**. The actual events of mitosis are not discreet but occur in a continuous sequence—separation of mitosis into four stages is merely convenient for our discussion and organization. During these stages, important cellular structures are synthesized and perform the mechanics of mitosis. For example, in animal cells, two microtubule-organizing centers called **centrioles** replicate. The pairs of centrioles move apart and form an axis of proteinaceous microtubules between them called **spindle fibers.** These spindle fibers act as motors that pull at the centromeres of chromosomes and separate the sister chromatids into newly recognized chromosomes. The spindles also push against each other to stretch the cell in preparation of forming two new nuclei and separate cells. In animal cells, a contractile ring of actin fibers cinches together around the midline of the cell to coordinate cytokinesis. This cinching of the cell membrane creates a structure called the **cleavage furrow**. Eventually, the cinching of the membrane completely separates into two daughter cells. Plant cells require the production of new cell wall material between daughter cells. Instead of a cleavage furrow, the two cells are separated by a series of vesicles derived from the Golgi. These vesicles fuse together along the midline and simultaneously secrete cellulose into the space between the two cells. This series of vesicles is called the **cell plate**.

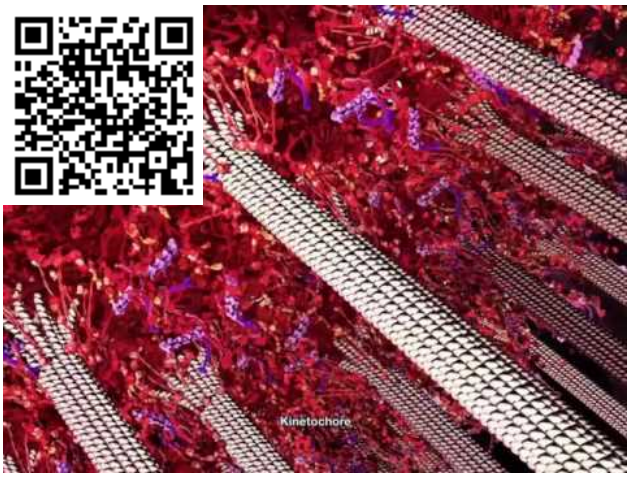



wehi.tv
LifeOnEarth
E.O.Wilson

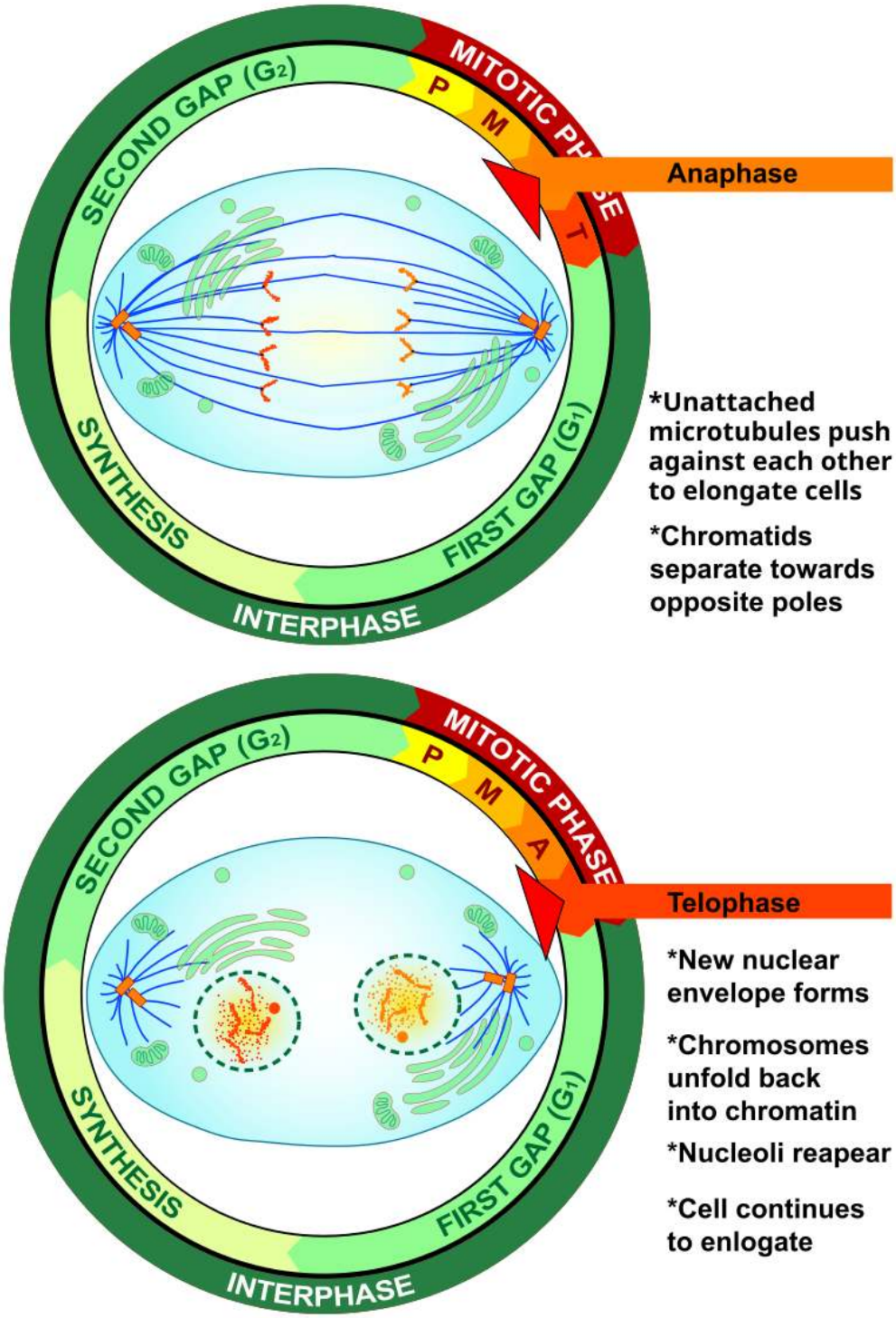

SECOND GAP (G2)
SYNTHESIS
FIRST GAP (G1)
INTERPHASE
M
A
T
Prophase
*Nucleolus disappears
*Chromatin condenses into chromosomes
*Separation of centrosomes
*Formation of the mitotic spindle


SECOND GAP (G2)
SYNTHESIS
FIRST GAP (G1)
INTERPHASE
P
A
T
Metaphase
*Chromosome align in the metaphase plate

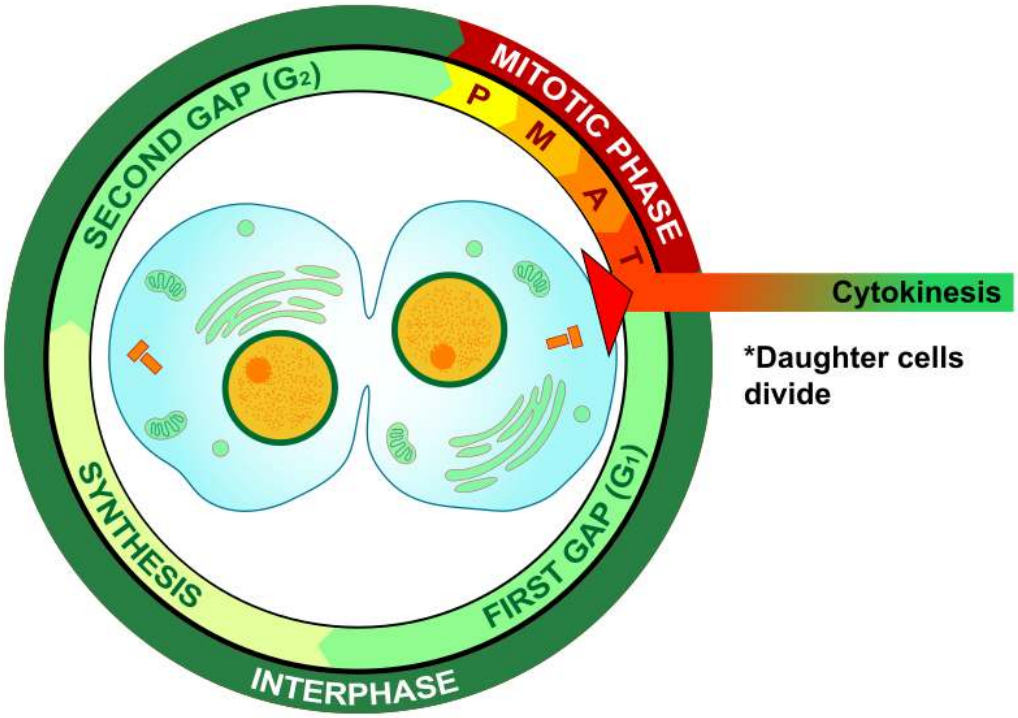




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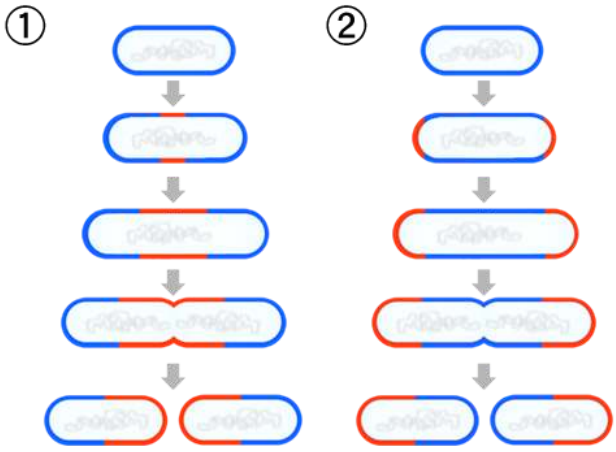


MITOSIS REVIEW

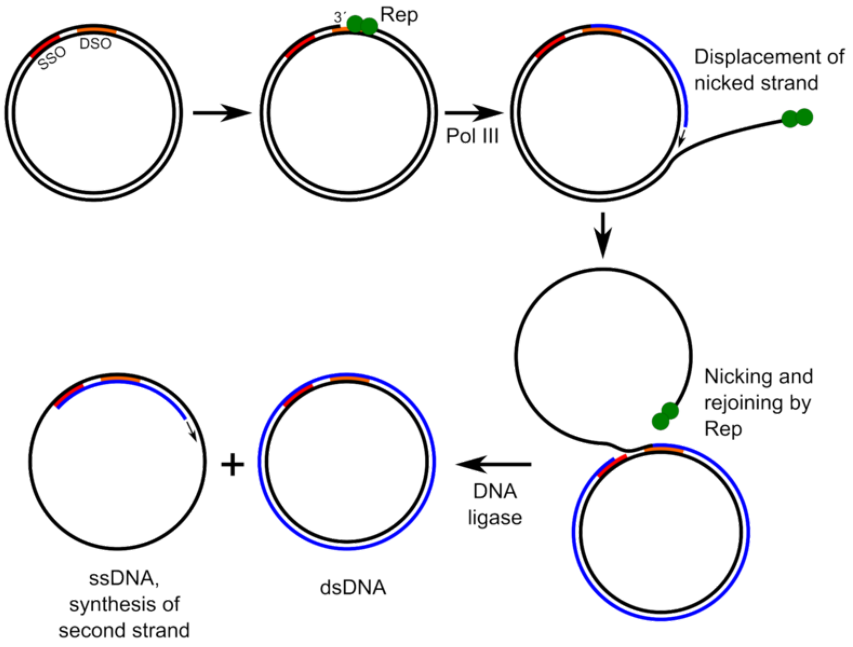
- Khan Academy Review
- Mitosis Flash Card Review
- Mitosis Sequence Review

BINARY FISSION

Mitosis refers to nuclear division, which happens to coincide with cytokinesis. Prokaryotes do not have nuclei, therefore they do not undergo a process of mitosis. Instead, prokaryotes undergo the process of binary fission. Growth of new membranes during the cellular growth and expansion in a prokaryote occurs by 2 mechanisms **1)** along the center of the cell or **2)** apically.



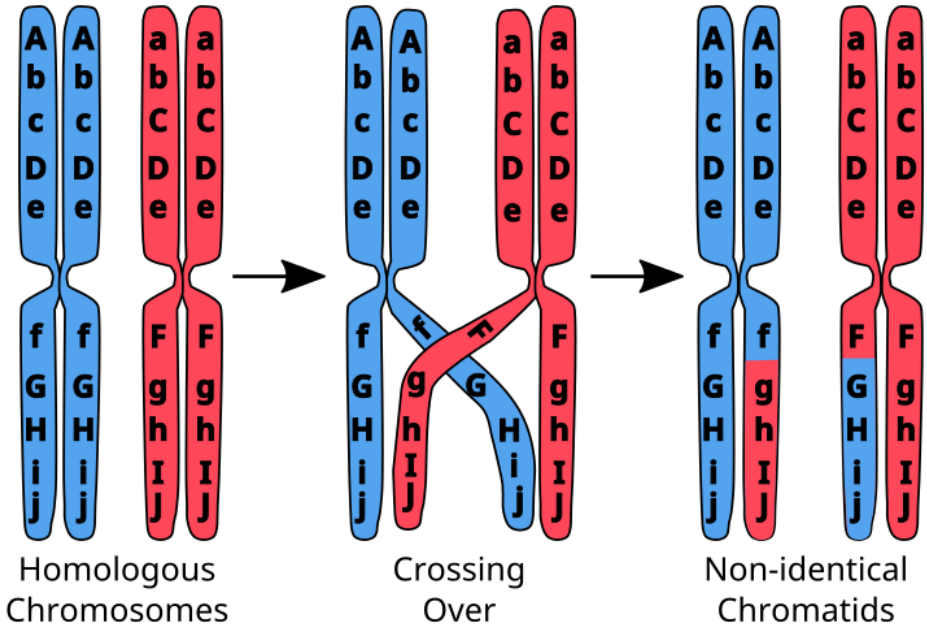
The chromosome of prokaryotes is organized in a circular form. A mechanism called the **rolling circle model** describes the replication of the chromosome that accompanies binary fission. This is also the mechanism utilized by extrachromosomal DNA called plasmids.



INTRODUCTION: MEIOSIS

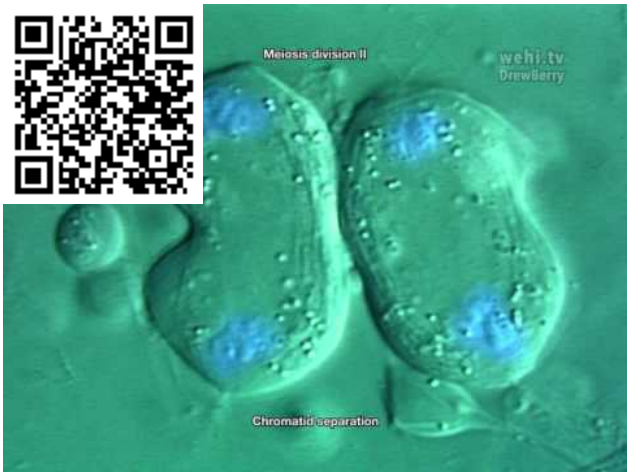
Meiosis is a process of nuclear division that reduces the number of chromosomes in the resulting cells by half. Thus, meiosis is sometimes called “reductional division.” For many organisms, the resulting cells become specialized “sex cells” or **gametes**. In organisms that reproduce sexually, chromosomes are typically **diploid** (**2N**) or occur as double sets (**homologous pairs**) in each nucleus. Each homolog of a pair has the same sites or loci for the same genes. You might recognize that you have one set of chromosomes from your mother and the remaining set from your father. Meiosis reduces the number of chromosomes to a **haploid** (**1N**) or single set. This reduction is significant because a cell with a haploid number of chromosomes can fuse with another haploid cell during sexual reproduction and restore the original, diploid number of chromosomes to the new individual. In addition to reducing the number of chromosomes, meiosis shuffles the genetic material so that each resulting cell carries a new and unique set of genes in a process of **independent assortment**.

As in mitosis, meiosis is preceded by replication of each chromosome to form two chromatids attached at a centromere. However, reduction of the chromosome number and production of new genetic combinations result from two events that don’t occur in mitosis. First, meiosis includes two rounds of chromosome separation. Chromosomes are replicated before the first round, but not before the second round. Thus, the genetic material is replicated once and divided twice. This produces half the original number of chromosomes.

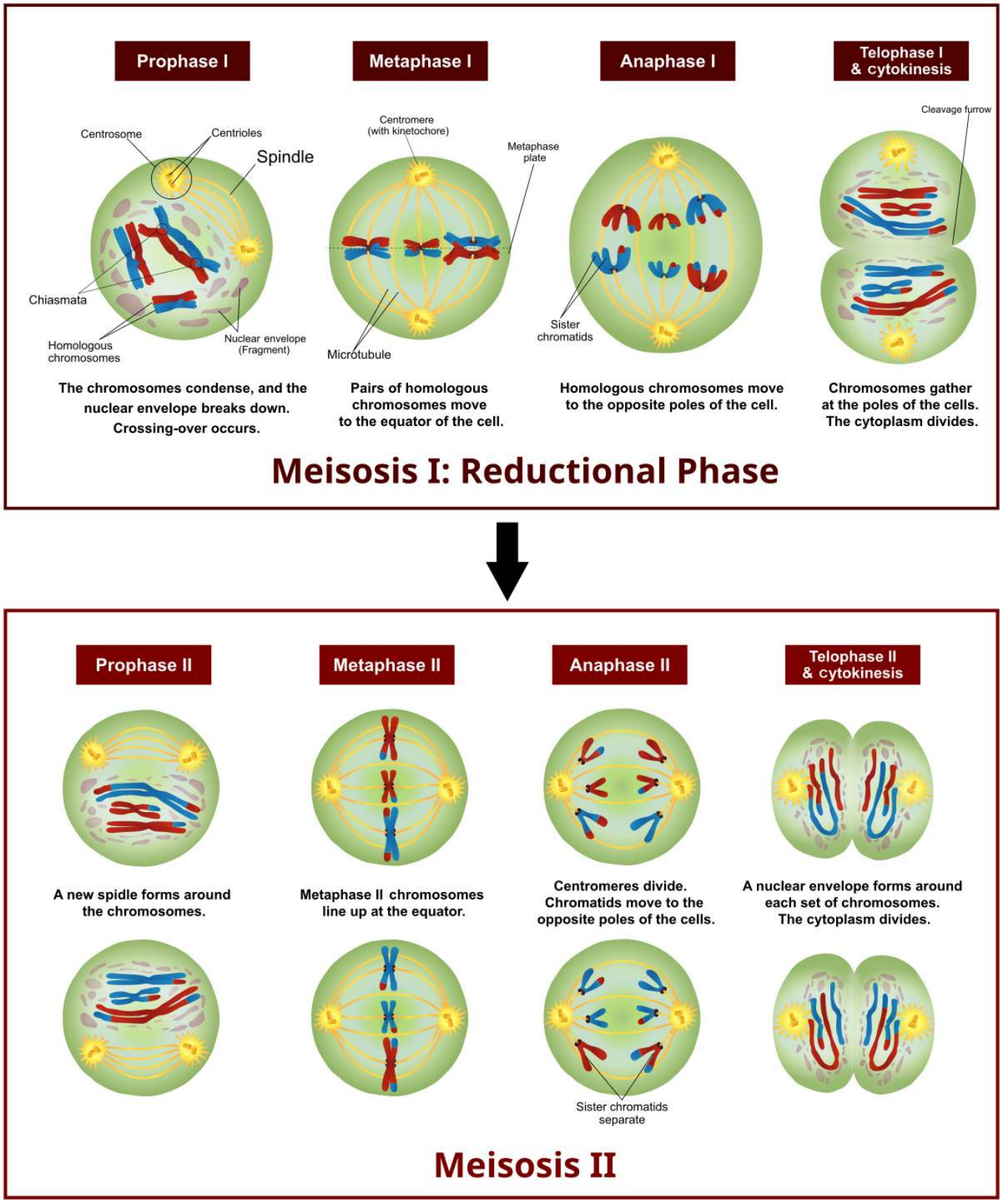


Crossing over between chromatids of homologous chromosomes increases genetic diversity during meiosis I. Synapsis occurs during prophase I as the homologous chromosomes begin to pair up. Credit: Jeremy Seto (CC-BY-NC-SA)

Second, during an early stage of meiosis each chromosome (comprised of two chromatids) pairs along its length with its homolog. This pairing of homologous chromosomes results in a physical touching called **synapsis**, during which the four chromatids (a tetrad) exchange various segments of genetic material. This exchange of genetic material is called **crossing-over** and produces new genetic combinations. During crossing-over there is no gain or loss of genetic material. But afterward, each chromatid of the chromosomes contains different segments (alleles) that is exchanged with other chromatids.



STAGES AND EVENTS OF MEIOSIS



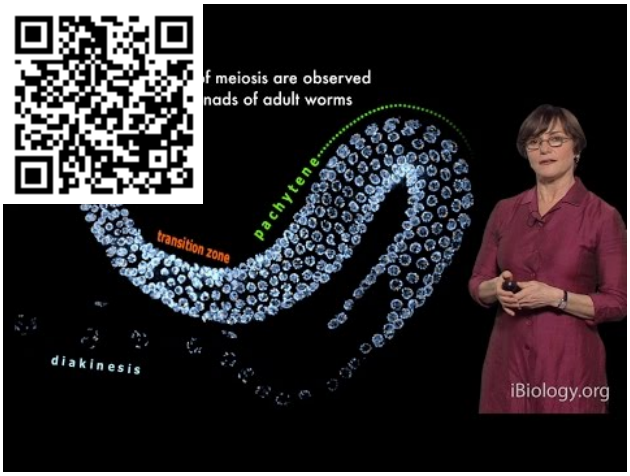
Stages of Meiosis. Credit: ***Ali Zifan*** *(CC-BY 4.0)*

Although meiosis is a continuous process, we can study it more easily by dividing it into stages just as we did for mitosis. Indeed, meiosis and mitosis are similar, and their corresponding stages of prophase, metaphase, anaphase, and telophase have much in common. However, meiosis is longer than mitosis because meiosis involves two nuclear divisions instead of one. These two divisions are called Meiosis I and

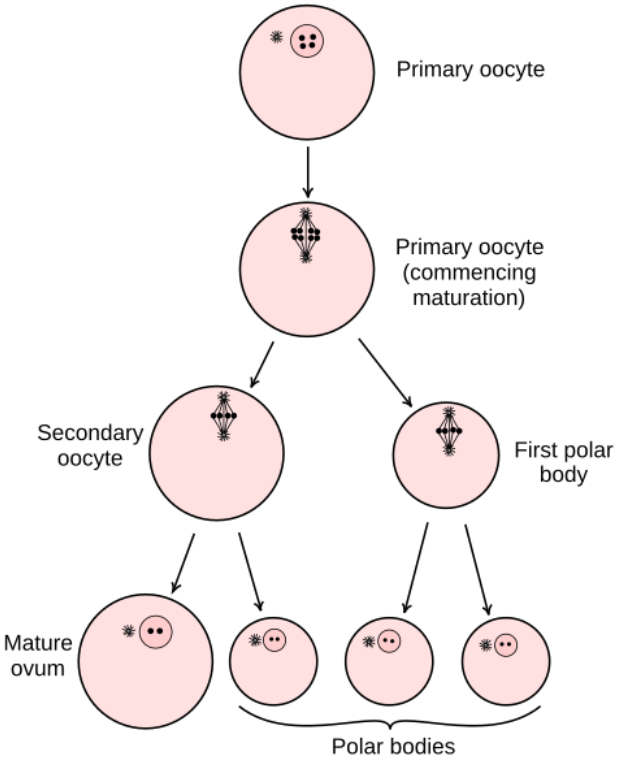
Meiosis II. The chromosome number is reduced (**reductional division**) during Meiosis I, and chromatids comprising each chromosome are separated in Meiosis II. Each division involves the events of prophase, metaphase, anaphase, and telophase.

ADVANCED VIDEO OVERVIEW OF MEIOSIS

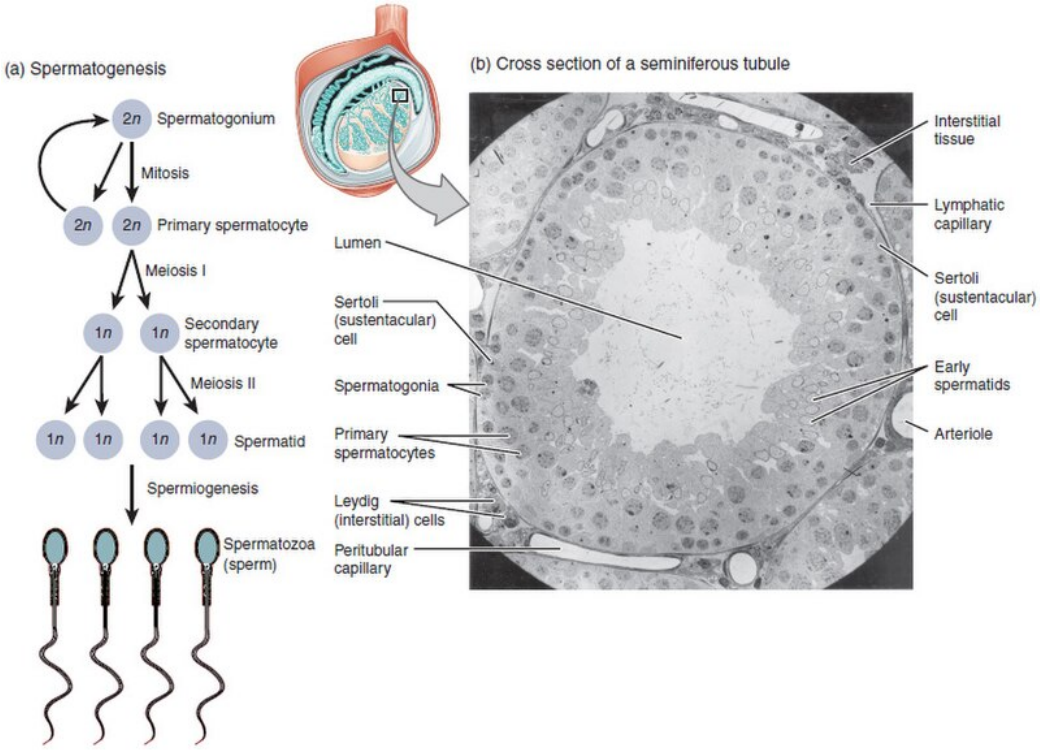




OOGENESIS



SPERMATOGENESIS

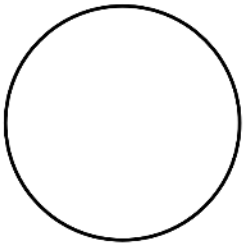


Comparing Mitosis and Meiosis

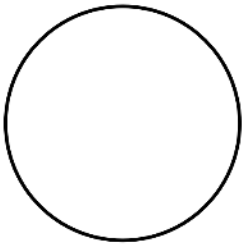
Using your pop bead knowledge, illustrate the phases below.
Note the ploidy where indicated (_N=_)

2N=4

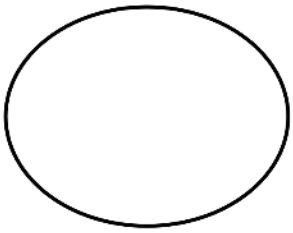
Prophase



Metaphase

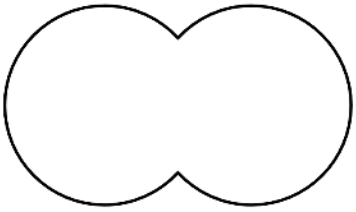


Anaphase



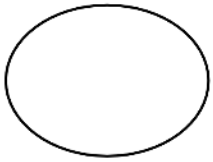
Telophase

_N=_

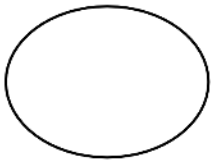


2N=4

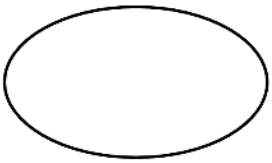
Prophase I



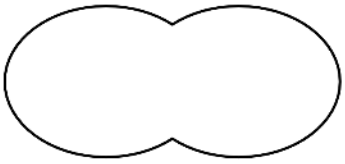
Metaphase I



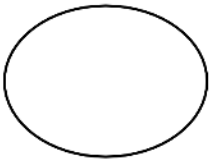
Anaphase I



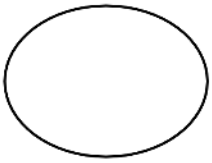
Telophase I

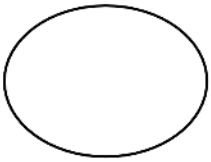


_N=_

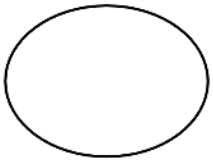


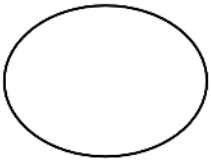
Prophase II



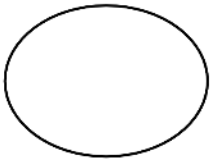


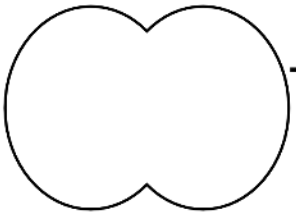
Metaphase II





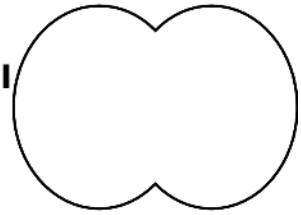
Anaphase II





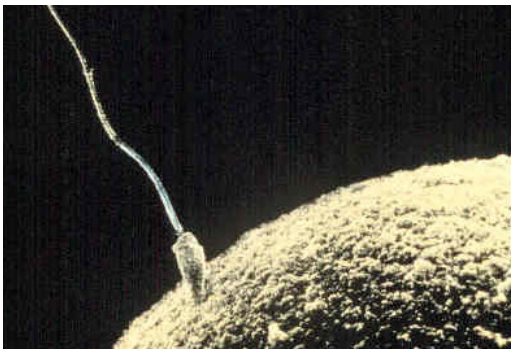
Telophase II

_N=_



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FERTILIZATION



Summary of Cell Division

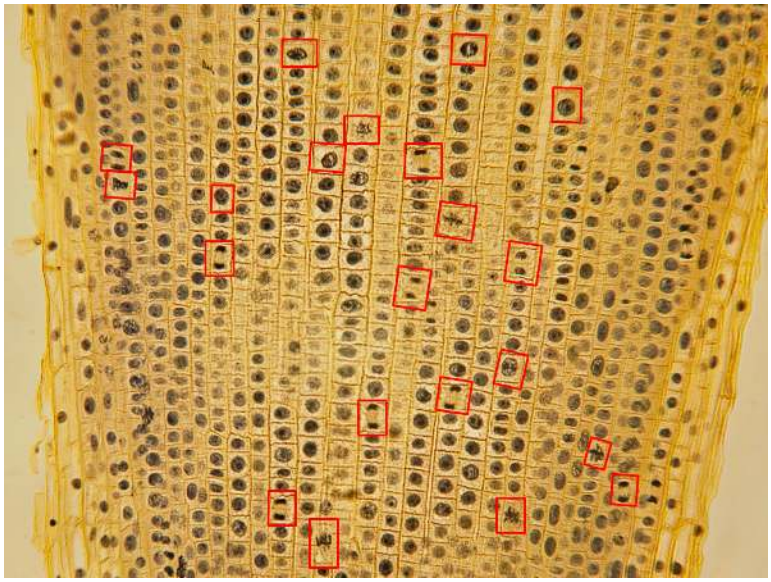
	Mitosis	Meiosis
Number of Cells at the Start		
Number of Cells at the End		
Number of Cell Divisions		
Chromosome Number (N)	Start:_____ End:_____	Start:_____ End:_____
Number of Chromosomes in the Cell at the Start of the Process (Human Cell)		
Number of Chromosomes in the Cell at the End of the Process (Human Cell).		
Daughter Cells (N or 2N)		
Daughter Cell Genetics (Identical or Non-identical)		
Purpose of Division		

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2.1.7.2: MITOSIS (ACTIVITY)

IDENTIFYING MITOTIC PHASES



Identifying phases of mitosis in the onion root tip. Click on the image to view a larger image.

The original figure without red boxes.

ACTIVITY: ESTIMATING THE TIME SPENT IN THE PHASES OF MITOSIS

Cells in your body reproduce at different rates. Skin cells reproduce frequently (about once per day); liver cells reproduce rarely (about once per year). Some specialized cells like nerve and muscle cells almost never reproduce and are in a special stage called G_0. The whole process of mitosis, prophase to telophase, takes approximately 90 min. In plants, an area of rapid growth is the tips of roots. This exercise uses onion root tips to illustrate the amount of time spent in each phase of mitosis.

1. Work as a team to look at onion root tips under the microscope. This area of the root is undergoing rapid cell reproduction.
    - If time is short, use the slides below
        - slide 1
        - slide 2
        - slide 3
        - slide 4
        - slide 5
        - slide 6
        - slide 7
2. Identify the phases of the cell cycle for 20 randomly chosen cells. Record this information in the table.
3. Trade results with 3 other people.
4. In an onion root tip, the entire cell cycle takes about 12 hours or 720 minutes
5. Calculate the percentage of time spent in each phase by counting the total number of cells in each phase (total in interphase, in prophase, etc.) and dividing each by the total number of cells you counted.
6. Multiply the percentage of time in each phase by the total time of the cell cycle (720 minutes) and this gives you an estimate of the time spent in each phase.

NUMBER OF CELLS IN EACH PHASE

	Interphase	Prophase	Metaphase	Anaphase	Telophase	Total
You (25)						
Partner 1 (25)						
Partner 2 (25)						
Partner 3 (25)						
Totals						

ESTIMATE OF TIME SPENT IN EACH PHASE OF THE CELL CYCLE.

	Interphase	Prophase	Metaphase	Anaphase	Telophase	Total
% of cells in each phase						100%
Estimated Time						720 minutes

TEST YOURSELF AT HOME

Use the following resource bio.rutgers.edu/~gb101/lab2_mitosis/section1_frames.html to test yourself and practice without a microscope.

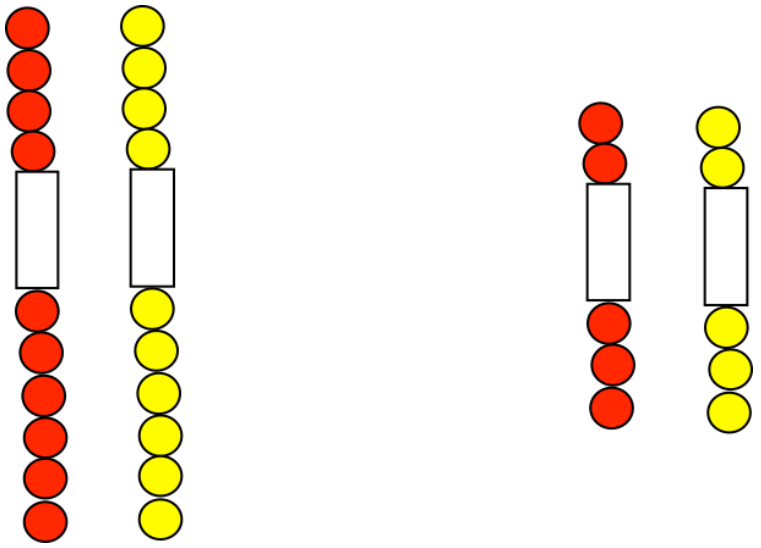
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2.1.7.3: MODELING MITOSIS AND MEIOSIS (ACTIVITY)

MITOSIS SIMULATION WITH POP-IT BEADS

Pop-beads are small, colored beads that can be joined together to simulate chromosome strands. We will use the pop-it beads to simulate the process that chromosomes undergo during cell division. Imagine that the beads represent long stretches of DNA that comprises the genetic instructions for the cell. Start with a cell with a chromosome number of 4 (4 chromosomes, or 2 homologous pairs). We will use red to identify the chromosome from the mother and yellow for the chromosome from the father.



Pop-it Bead Chromosomes (2N=4)

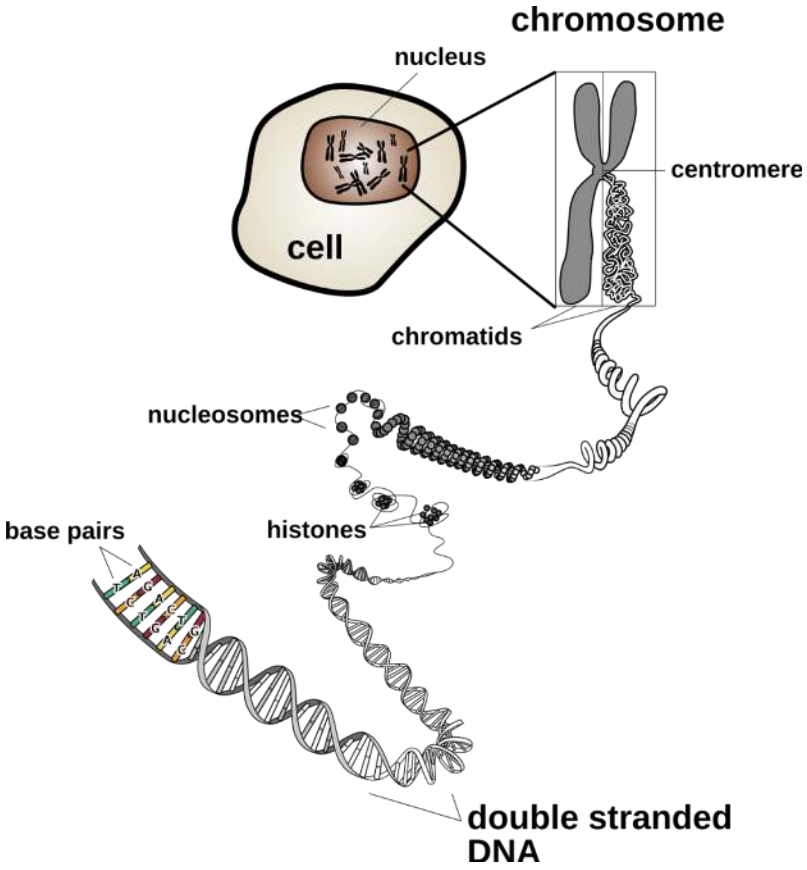
1. Make 4 chromosomes: two long chromosomes (one red and one yellow) and two short chromosomes (one red and one yellow). The two long chromosomes should each have the same number of beads, as should the two short chromosomes. The two long chromosomes are one homologous pair; the two short chromosomes are the second homologous pair.
2. *Simulate S phase*: Replicate your chromosomes by making an identical set of pop-it bead chromosomes (you should have a total of eight pop-it bead strands; four long and four short). Attach the identical replicas (chromatids) by their magnetic centromeres.
3. *Simulate Mitosis*: Move the “chromosomes” through each of the four stages of mitosis. Draw and label the pop- bead chromosomes for ONE of the phases on a separate sheet. It is not necessary to draw each individual bead.
4. Draw a large circle on the paper to represent the cell and the nucleus with a pencil.
5. Place your chromosomes in this and walk through each stage of Meiosis.
6. Erase the outlines as you go along and re-draw the boundaries of nuclei.
7. Document each stage with your cell phone:
    1. Prophase
    2. Metaphase
    3. Anaphase
    4. Telophase

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2.1.7.4: CHROMOSOMES AND KARYOTYPES

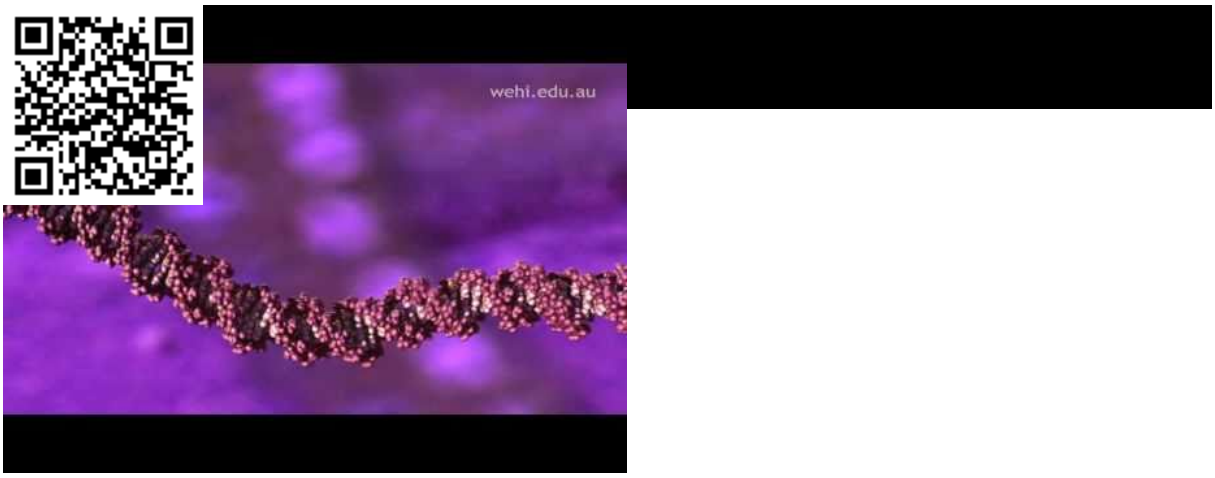
CHROMOSOMAL FEATURES



Chromosomes are made of double-stranded DNA molecules wound about histones and condensed into the familiar X-shape. Under regular functioning, these chromosomes are decondensed in the nucleus and not recognizable.

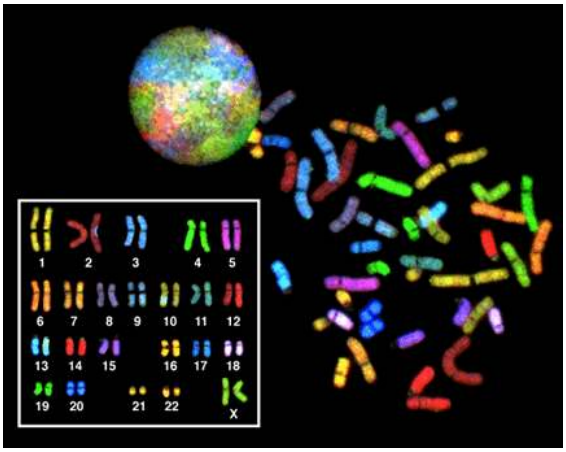
Chromosomes in Interphase are not visible individually. In preparation for nuclear division (mitosis or meiosis), they begin to organize tighter and condense in preparation for movement to subsequent daughter nuclei. The animation below illustrates the process of histone packaging and the molecular visualization of DNA replication. **Histones** are proteins that aid in the packaging of the chromosomes into organized coils that give rise to the recognizable chromosomes during metaphase.





Large-scale genomic rearrangements result in genetic abnormalities. Biologists utilize a technique called a **chromosome spread** followed by a **karyotype** or **karyogram**. To make a chromosome spread, one blocks the progression of mitosis at metaphase where chromosomes are condensed into the structures we are familiar with. A karyotype analysis is an arrangement of the chromosome spread into the homologous pairs of chromosomes.

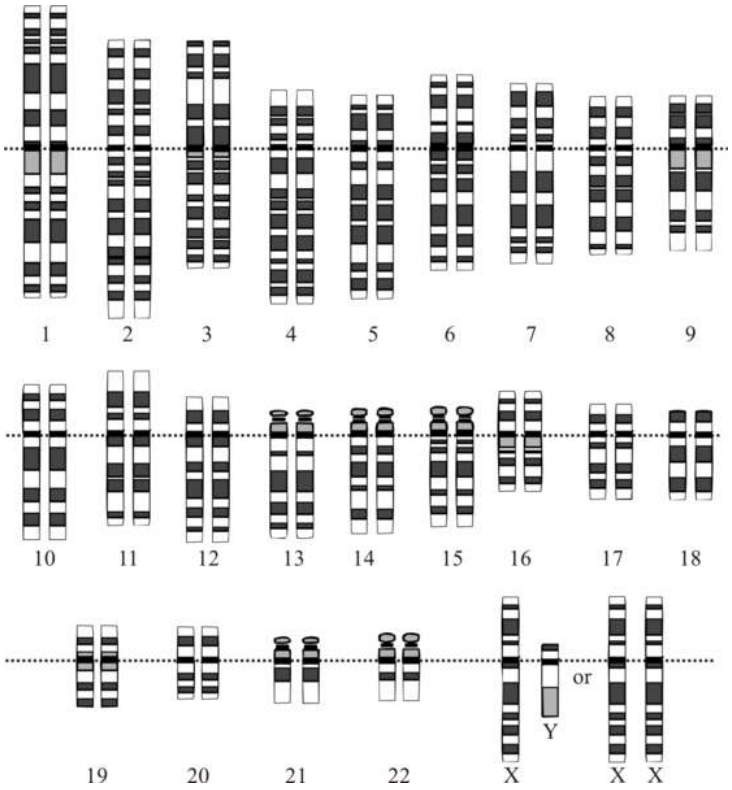
Events associated with the improper separation of chromosomes during metaphase results in an alteration of chromosome number in the subsequent generation of cells. Using the Pop-beads, we can understand better how the timing of these events will lead to differences in the karyotype.



A "spectral" karyotype of a female nucleus. Each homologous pair is "painted" to differentiate them.

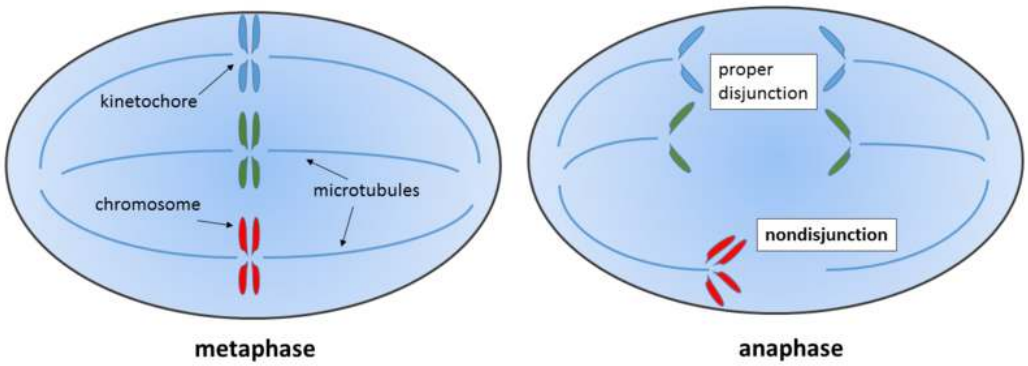
ABNORMAL KARYOTYPES

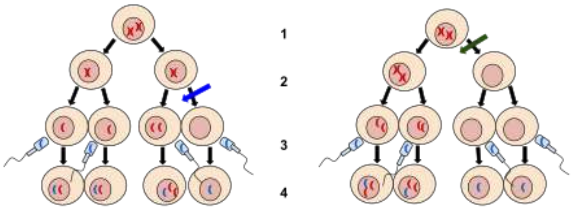
Down's Syndrome is a common genetic abnormality referred to as Trisomy 21. Instead of having the complement of 46 chromosomes of 22 homologous pairs plus 2 sex chromosomes, there are 47 chromosomes consisting of an additional Chromosome 21.



Standard Human Karyotype with 46 chromosomes. Both XX and XY are also shown here.

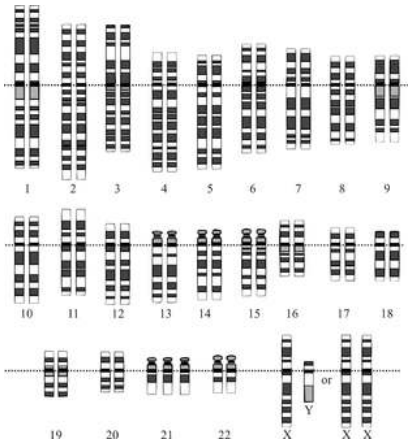
The appearance of extra or missing chromosomes arises during meiosis in an event called **nondisjunction**. After fertilization, a zygote with an improper chromosome complement occurs.





From left to right, the ploidy of the resultant zygotes: 2N, 2N, 2N+1, 2N-1; 2N+1, 2N+1, 2N-1, 2N-1

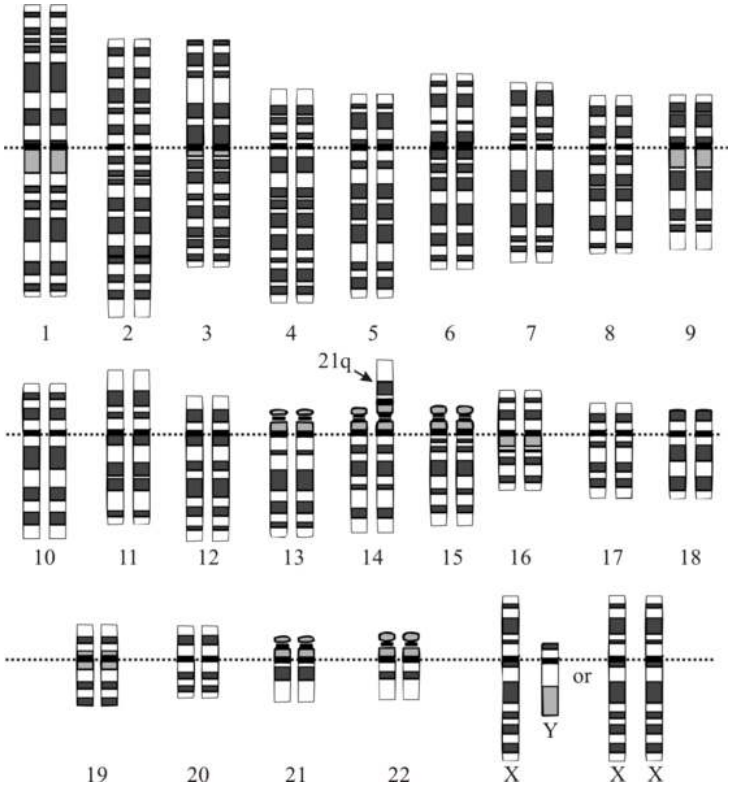
Nondisjunction can occur during Meiosis I or Meiosis II to yield **aneuploid** states of 2N+1 or 2N-1.



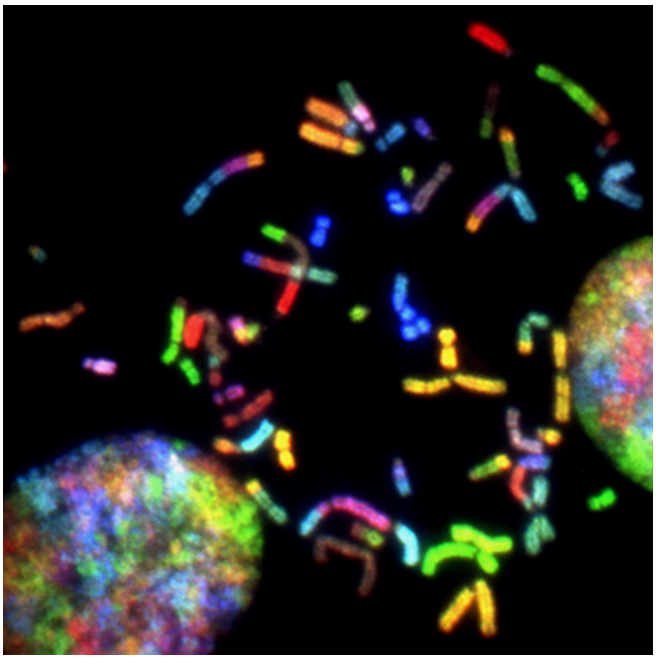
Down's syndrome karyotype showing the traditional trisomy 21.

TRANSLOCATION

Translocation is the movement of a piece or a whole chromosome onto another chromosome. The acrocentric nature of Chr 21 and it's small size makes it prone to an event called **Robertsonian Translocation** whereby two acrocentric chromosomes fuse.

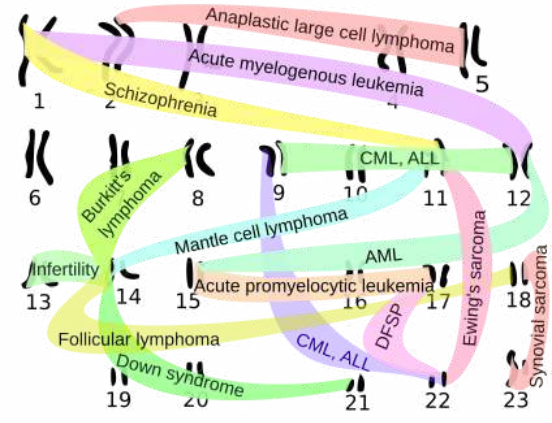


Down's Syndrome from a 14:21 translocation.



A spectral karyogram of a brain cancer (glioblastoma) illustrating multiple translocation problems and chromosome instability. Credit: Thomas Reid, NCI (CC-BY-NC)

Abnormalities in mitosis also occur and can result in diseases from translocations.



POLYPLOIDY

Some organisms and cells have entire sets of chromosomes additional to the standard 2N diploid. Cells that have extra sets in the formula of 3N are called **triploid**. If they are 4N, they are called **tetraploid**. This is different than the case of Down's syndrome, which has a chromosome complement of 2N+1. Any time there are abnormal numbers of chromosomes, cells are referred to as **aneuploid**. A special case of aneuploid occurs from having entire sets more of chromosomes—**polyploid**. Plants are especially robust in the regard of polyploidy and often have different species arise in such a way. Some plants become sterile in the case of polyploidy and will not produce seeds properly.



Wild bananas or plantains (Musa acuminata or Musa balbisiana) are deemed inedible because of their large seeds.

Have you ever seen a banana with these large seeds? The answer is most likely “No!” since these are not regarded as being edible. However, due to selective breeding practices, most edible plantains and bananas are hybrids of the two species *Musa acuminata* or *Musa balbisiana* that are 3N or 4N. In this case, the fruits are sterile and the seeds don’t develop. Other seedless fruits are also developed this way and require propagation through clonal means.

ADVANCED VIEW OF CHROMOSOME SEGREGATION





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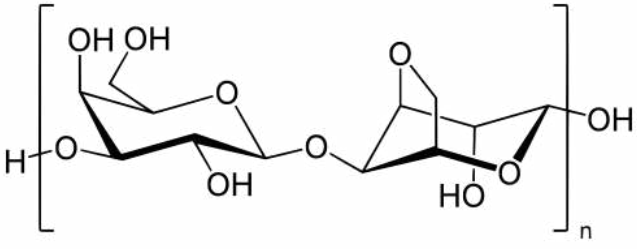
SECTION OVERVIEW

2.1.8: ANALYZING DNA

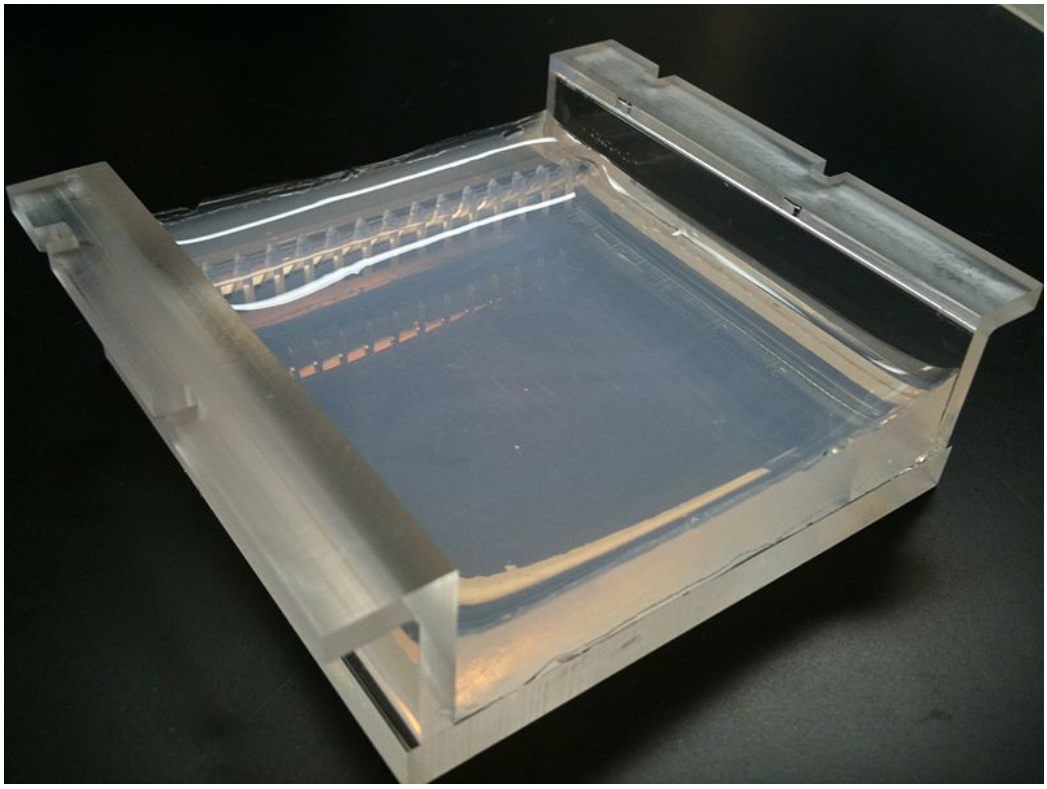
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2.1.8.1: INTRODUCTION

AGAROSE GEL ELECTROPHORESIS

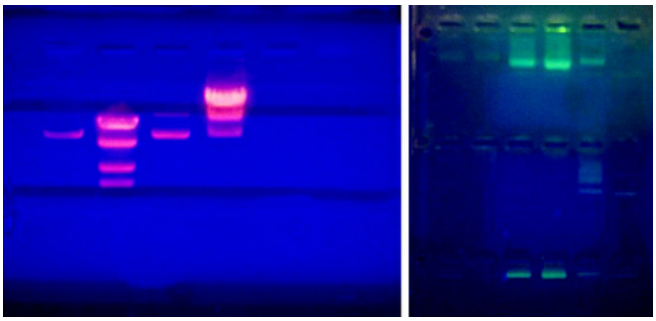


Agarose is a linear carbohydrate polymer purified from the cell walls of certain species of algae. Agar is a combination of the crude extract that contains agarose and the smaller polysaccharide agaropectin. When dissolved and melted in liquid, agarose strands become tangled together to form a netting that holds the fluid in a gel. Reduction of the fluid creates a higher percentage of the gel that is firmer and contains smaller pores within the netting.



Placing a **comb** within the melted agarose creates spaces that allow for the insertion of samples when the gel is solidified. Molecules can traverse through the pores as they are drawn by electrical currents. Charged compounds will migrate towards the electrode of opposite charge but migration rate will be influenced by the size of the molecules. Smaller compounds can easily traverse through the webbing while larger items are retarded by the pore size. Follow this simulation to get a better idea of how we use **Agarose Gel Electrophoresis** in molecular biology to study DNA fragments.

DNA molecules are not readily visible when **resolved** (separated) on an agarose gel. In order to visualize the molecules, a DNA dye must be administered to the gel. In research labs, a **DNA intercalating agent** called Ethidium Bromide is added to the molten gel and will bind to the DNA of the samples when run. Ethidium Bromide can then be visualized on a UV box that will fluoresce the compound and reveal bands where DNA is accumulated. Since Ethidium Bromide is known as a carcinogen, teaching labs will use a safer DNA intercalating agent known as Sybr Green. This can be visualized in a similar fashion but will fluoresce a green color instead.

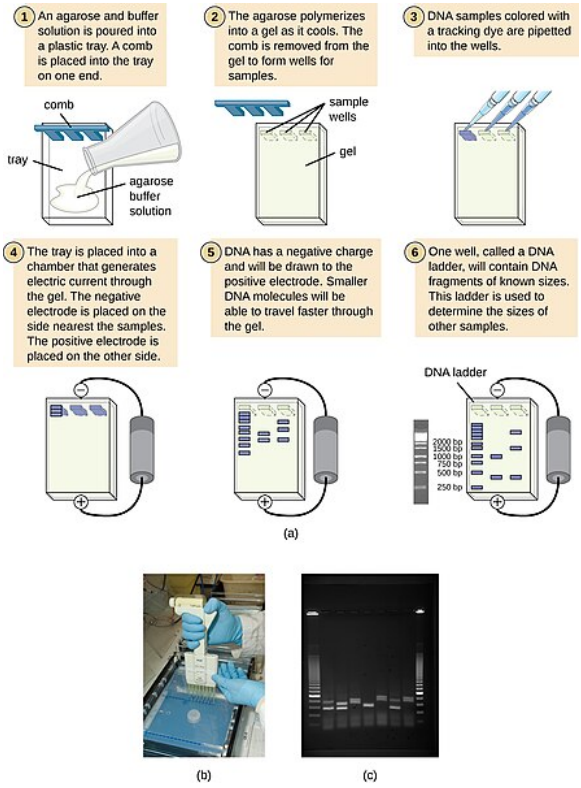


Agarose gels visualized on a UV transilluminator. Left shows a gel with Ethidium bromide. Right shows a gel with Sybr Green.

Agarose gels are made of and bathed in a buffered solution, usually of Tris-Borate-EDTA (**TBE**) or Tris-Acetate-EDTA (**TAE**). Regardless of the buffered solution, the buffer provides necessary electrolytes for the current to pass through and maintain the pH of the solution.

DNA samples are prepared in a buffer similar to the solution that it will be run in to ensure that the phosphate backbone of the DNA remains deprotonated and moves to the positive electrode. Additionally, **glycerol** or another compound is added to this buffer in order for the solution to sink into the wells without spreading out. A dye is often included in this loading buffer in order to visualize the loading in the wells and to track the relative progression of gel.

AGAROSE GEL SET-UP



Click here to watch the "Assembling the Rig & Loading/Running The Gel" video:

EXTERNAL RESOURCES

- Gel Electrophoresis Simulation
- The Structure of DNA http://www.nature.com/scitable/topicpage/discovery-of-dna-structure-and-function-watson-397
- http://learn.genetics.utah.edu/content/science/forensics/

ELECTROPHORESIS OF DYES (ACTIVITY)

1. Prepare a 1% agarose gel by adding 60ml Tris-Borate-EDTA buffer (TBE) to 0.6g agarose in an Erlenmeyer flask
2. Place the flask in a microwave or on the heat until agarose is melted.

- Stop periodically and swirl solution and do not permit to boil over.

3. Assemble the casting tray by blocking the ends with tape or plastic gaskets.
4. Place the comb into the center of the casting tray.
5. You may place the casting trays inside a refrigerator and pour the solution into the tray.
6. Wait until the gel is solidified.
7. Carefully separate the gaskets from the tray.
8. Remove the comb and place the casting tray into an electrophoresis chamber.
9. Cover the gel with TBE buffer.
10. Using a micropipettor, load 40-50µl dye samples sequentially into the wells.
11. Cover the electrophoresis chamber with the lid and ensure good contact between electrodes.
    - It is conventional that the **POSITIVE** side of the tank is nearest to you.
    - With the **POSITIVE** side nearest to you, load the samples from left to right.
12. Set the power supply to 100-120V and press the Run button (you should see bubbles at each electrode) and allow to run for at least 40 minutes.
13. After 40 minutes, stop the current and remove the gel in the casting tray.
14. Place tray on a white background and document your gel.

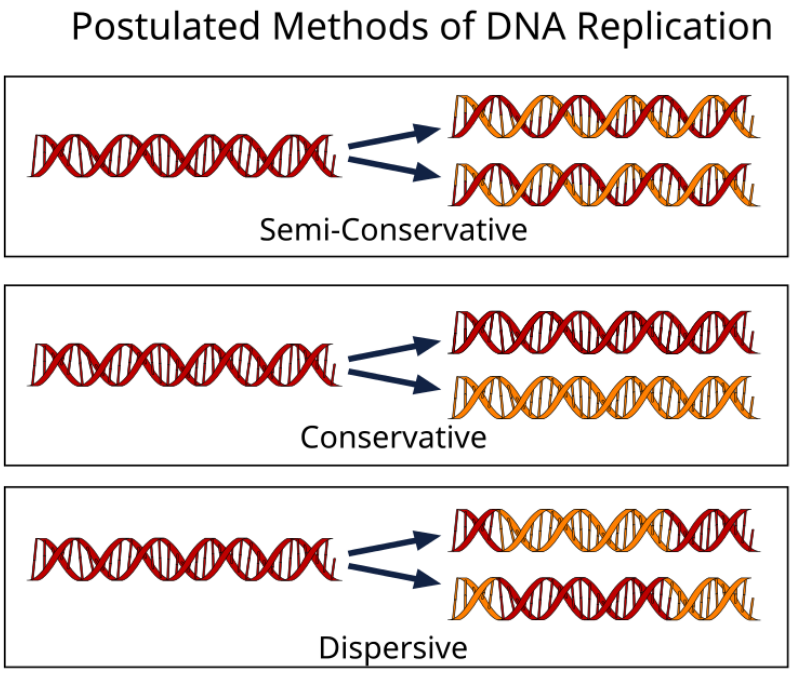
ACTIVITY FOLLOW-UP

1. What colors were the dyes originally before loading into the wells?
2. How many separate bands of dye are in each well following the run?
3. What does it mean that there are multiple bands in a lane? What does it mean that there is only one band in a lane?
4. What does the length of migration illustrate to us about the properties of the dye molecules?
5. In which direction did the dye molecules migrate? What does the direction of migration indicate about the analytes?
6. Are there lanes where there are multiple bands of the SAME color?

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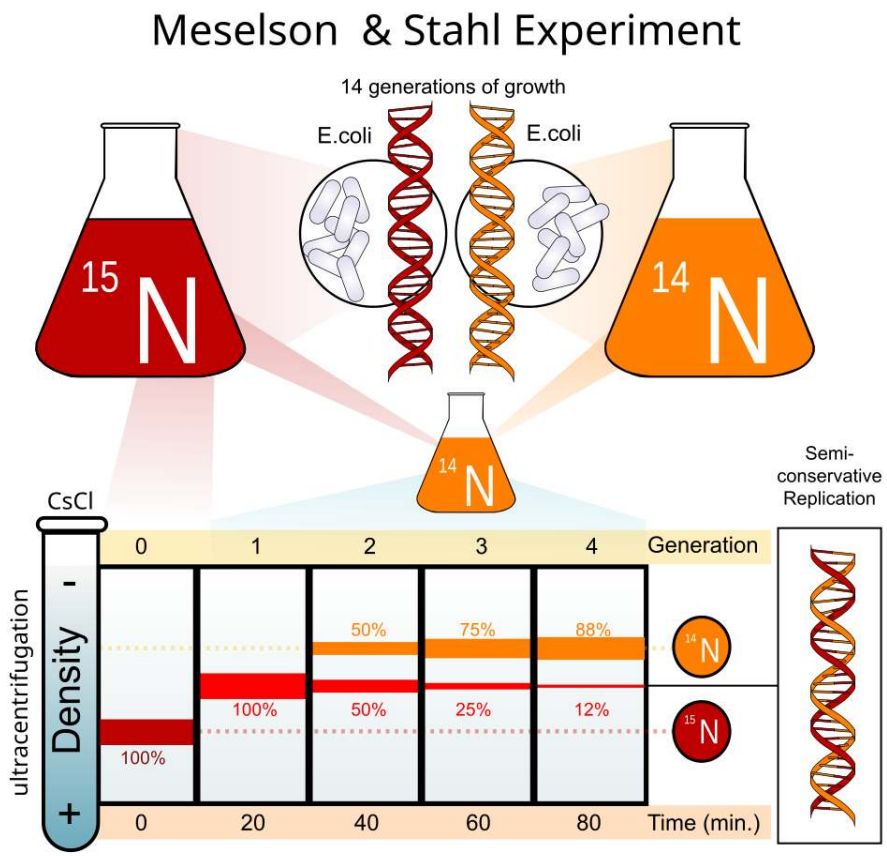
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2.1.8.2: DNA REPLICATION



Francis Crick proposed 3 models for how DNA might replicate. Credit: Jeremy Seto (CC-BY 3.0)

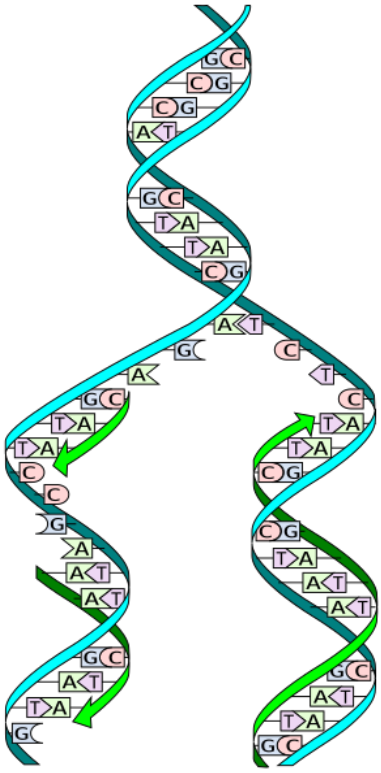
MESELSON & STAHL EXPERIMENT

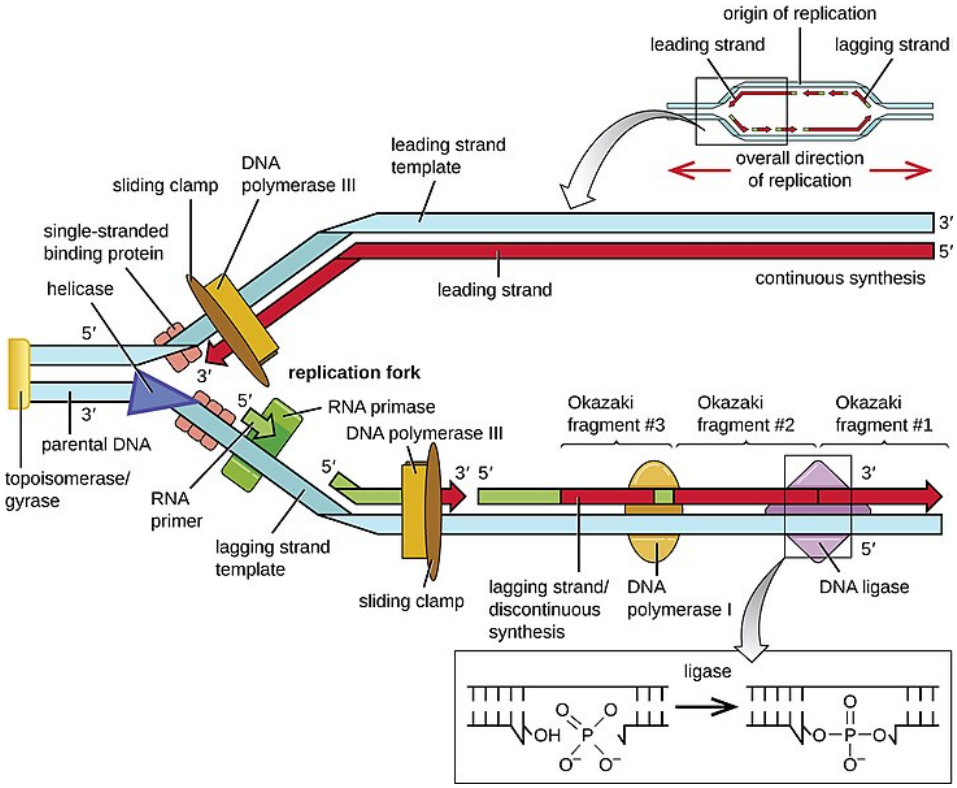


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Meselson & Stahl revealed semi-conservative replication as the method through use of radiolabeling DNA in bacteria. Credit: Jeremy Seto (CC0)

REPLICATION MACHINERY





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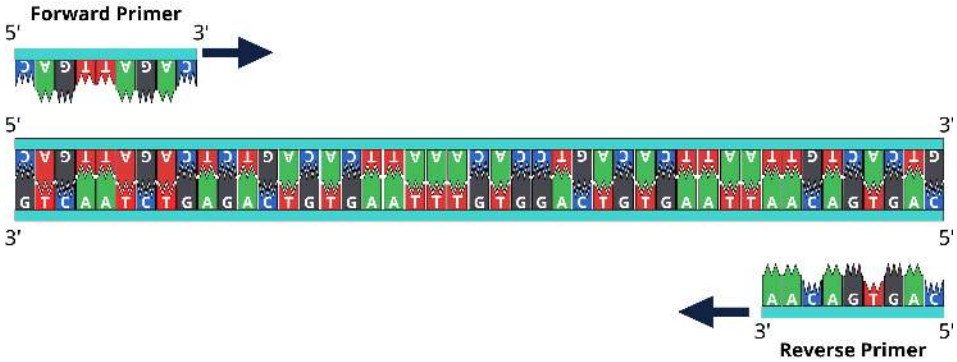


POLYMERASE CHAIN REACTION (PCR)

The **Polymerase Chain Reaction** (**PCR**) is a method of rapidly amplifying or copying a region of DNA in a tube. As the name implies, the technique uses a thermostable **DNA Polymerase** enzyme to mimic in a tube what happens within a cell during DNA replication. The chain reaction permits us to rapidly copy DNA from very minute source material in an exponential way. This technique is used in forensic science, genetic testing and cloning of rare genes. Because of the exponential copying process, a stray cell left behind can provide enough genetic material to make billions of copies of this DNA. The process of PCR can be observed in an animation found at Cold Spring Harbor Laboratory's DNA Learning Center website (http://www.dnalc.org/resources/3d/19-polymerase-chain-reaction.html).

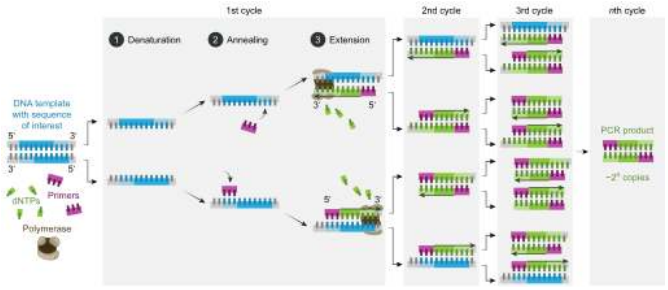






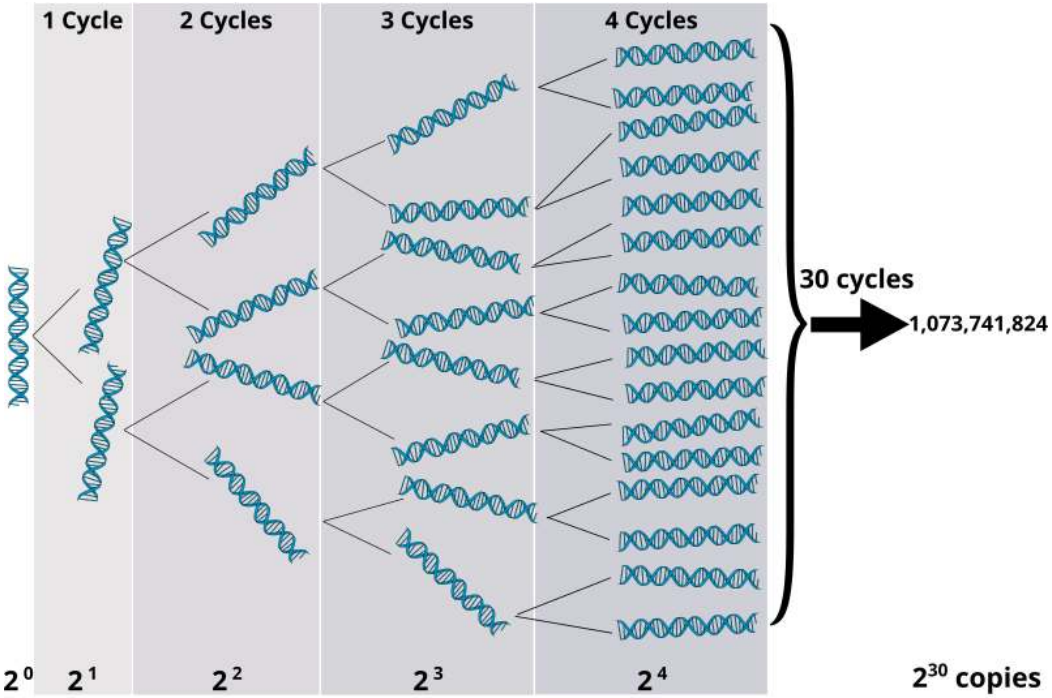
Primers are reverse complementary to one of the 2 strands of DNA. They flank the area of interest and become incorporated into the replicated product. Credit: Jeremy Seto (CC-BY 3.0)

As with any DNA replication process, one needs to start off with a **template**. The template is the source material that is meant for duplication. In this process, scientists are not interested in copying the entirety of the genome, just a small segment of interest. DNA polymerases require primers to begin the polymerization process. **Primers** are designed as small oligonucleotide segments that flank the area of interest. These are short strands of DNA that reverse complement to the DNA area of interest so that the DNA polymerase has a starting point and is guided only to the DNA segment of interest. These primers tend to be about 18-24 bases long.



Polymerase chain reactionHowever, a double-stranded DNA molecule is already base-paired together into a double helix so our primers can not interact. The first step of PCR is to separate the double-stranded DNA molecule by **denaturing** the H-bonds using high heat (95°C). The primer concentrations are much higher than the original template. The next step of PCR is called **annealing**. During this step, the temperature is reduced to a temperature of about 55°C. This temperature is still hot by our standards but is necessary to enhance the stringency of the correct base pairing of the primers to their targets on the template. The DNA Polymerase used in this process is derived from a bacteria that lives in very high temperatures and does not denature as other proteins would under such conditions (thermostable). The original enzyme was isolated from an organism called *Thermus aquaticus*, so we call the enzyme Taq polymerase or just **Taq** for short. This bacteria lives in hot springs where the temperatures are about 50°C but it thrives at a range between 50-80°C. The temperature is raised again to a higher temperature of 72°C for the polymerase to **extend** (also called **elongation**) or continue the polymerization step from the primer.

PCR is accomplished by cycling rapidly between these three steps: denature, anneal, and extension. The rate-limiting step is the extension which limits the length of DNA to be copied. If the original template is only a single copy, we would have 2 copies after the completion of a cycle. The subsequent cycle would have 4 copies, then 8, then 16, then 32, and so on. The doubling process is exponential so from 1 copy undergoing 30 cycles; we would have 2^{30} or 1,073,741,824 copies. This is over a billion copies in a few hours of time.



Exponential amplification by PCR. Credit: Jeremy Seto (CC-BY 3.0)

EXTERNAL RESOURCES

- Walkthrough of the steps in PCR
  - www.dnalc.org/view/15924-Making-many-copies-of-DNA.html

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- **8.2: DNA Replication** by Bio-OER is licensed CC BY-NC-SA 4.0.

2.1.8.3: VARIABLE NUMBER TANDEM REPEATS

POLYMORPHISMS

The difference in nucleotide sequences between humans lies between 0.1-0.4%. This means that people are greater than 99% similar. But when you look at your classmates around the room, you can see that that small difference amounts to quite a bit of variation within our species. The bulk of these differences aren't even within the coding sequences of genes but lie outside in regulatory regions that change the expression of those genes. Imagine if there were mutations to the coding sequences, this could be very deleterious to the well-being of the organism. We say that the coding sequences of genes that ultimately lead to proteins have **selective pressure** to remain the same. The areas outside of the coding sequences have a reduced and sometimes non-existent selection pressure. These areas are allowed to mutate in sequence and even expand or contract. Areas of changes or differences are called **polymorphic** (many forms). If you were to read a repetitive set of sequences and count the repetition, you' would make mistakes and lose count. Likewise, DNA polymerase will make errors or stutter in areas of repetitiveness and produce polymorphic regions.

TANDEM REPEATS

A type of polymorphism occurs due to these repeats expanding and contracting in non-coding regions. These regions are called variable number tandem repeats (**VNTRs**)or sometimes short tandem repeats (**STRs**). Any region or location on a chromosome is referred to as **locus** (loci for plural). Scientists use polymorphic loci that are known to contain VNTRs/STRs in order to differentiate people based on their DNA. This is often used in forensic science or in maternity/paternity cases. Any variation of a locus is referred to as an **allele**. In standard genetics, we often think of an allele as a variation of a gene that would result in a difference in a physical manifestation of that gene. In the case of STRs, these alleles are simply a difference in the number of repeats. This means the length of DNA within this locus is either longer or shorter and gives rise to many different alleles. VNTRs are referred to as minisatellites while STRs are called **microsatellites**.

CODIS

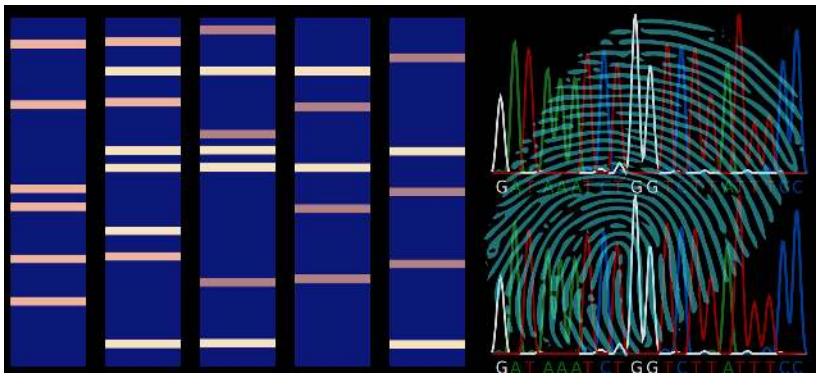


Figure **2.1.8.3.1**: DNA fingerprinting. Credit: Helixitta, The Photographer and Jeremy Seto (CC-BY-SA 3.0)

The FBI and local law enforcement agencies have developed a database called the Combined DNA Index System (**CoDIS**) that gathers data on a number of STRs. By establishing the number of repeats of a given locus, law enforcement officials can differentiate individuals based on the repeat length of these alleles. CoDIS uses a set of 20 loci that are tested together. As you would imagine, people are bound to have the same alleles of certain loci, especially if they were related. The use of 20 different loci makes it statistically improbable that 2 different people could be confused with each other. Think about this in terms of physical traits. As you increase the number of physical traits used to describe someone, you are less likely to confuse that person with someone else based on those combinations of traits. Using the CoDIS loci increases the stringency since there are many alleles for each locus. The twentieth locus in CoDIS (called AMEL) discriminates between male and female.

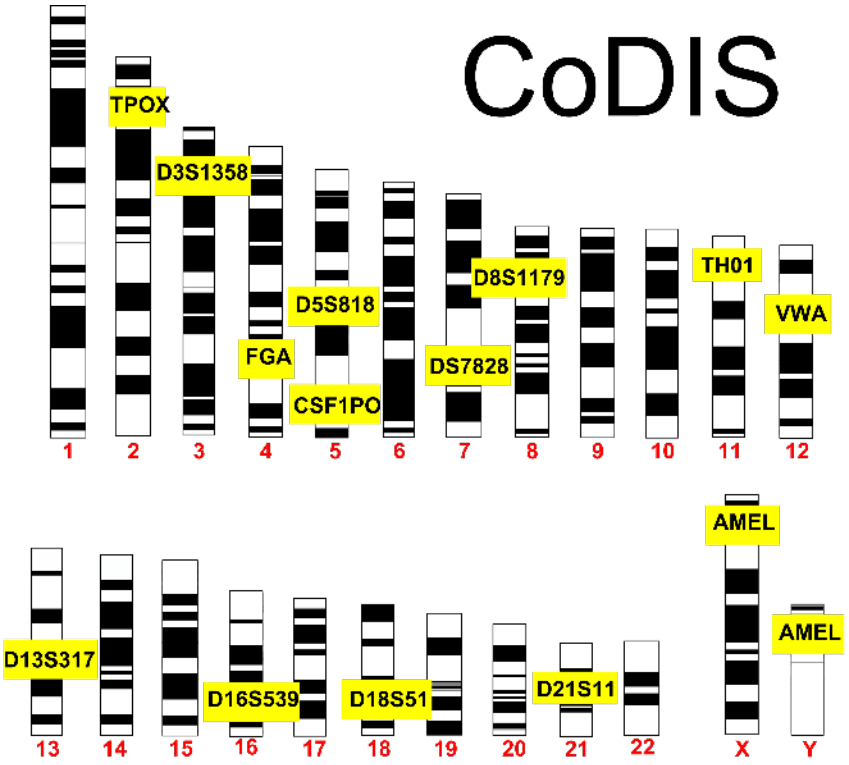


Figure 2.1.8.3.2: **CoDIS STRs:** The FBI utilizes 20 different loci to discriminate between people. AMEL discriminates by gender and is located on the X & Y. Credit: Jeremy Seto (CC-BY-NC-SA)

CRIME SCENE INVESTIGATION

This lab uses a CoDIS locus called TH01. TH01 is a locus on chromosome 11 that has a repeating sequence of TCAT. There are reported to be between 3-14 repeats in this locus. With the exception of X and Y in a male, all chromosomes have a homologous partner. Therefore, each individual will have 2 alleles for each CoDIS locus.

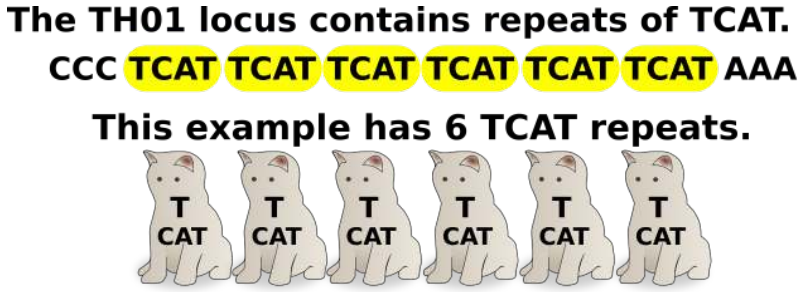


Figure 2.1.8.3.2: **TH01 STR:** Outside of the STR, there are flanking areas of known sequence. The primers that amplify TH01 in PCR recognize these flanking sequences to amplify the TCAT repeats. Credit: Jeremy Seto (CC-BY-NC-SA)

At a crime scene, criminals don't often leave massive amounts of tissue behind. Scant evidence in the form of a few cells found within bodily fluids or stray hairs can be enough to use as DNA evidence. DNA is extracted from these few cells and amplified by PCR using the specific primers that flank the STRs used in CoDIS.

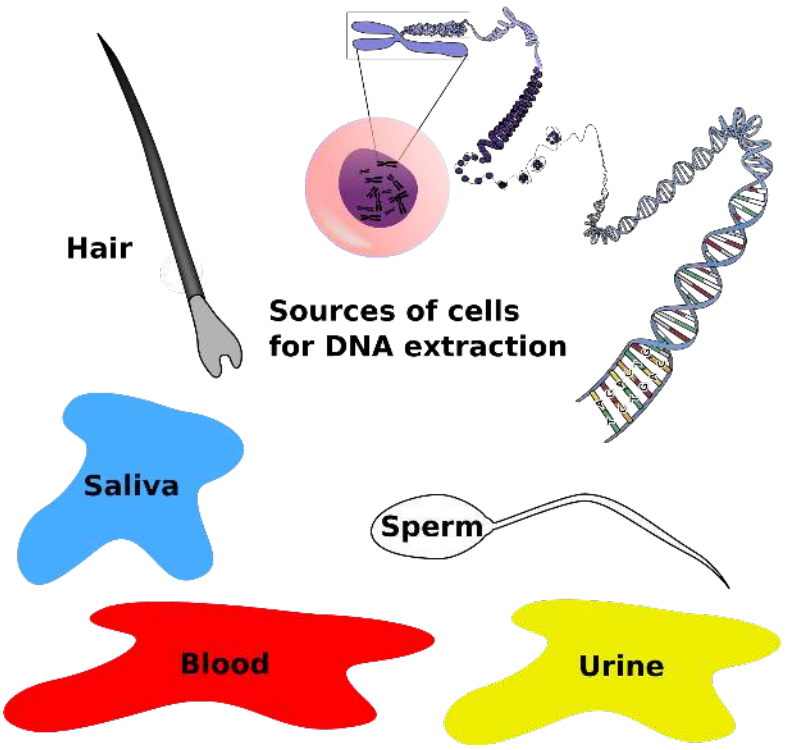
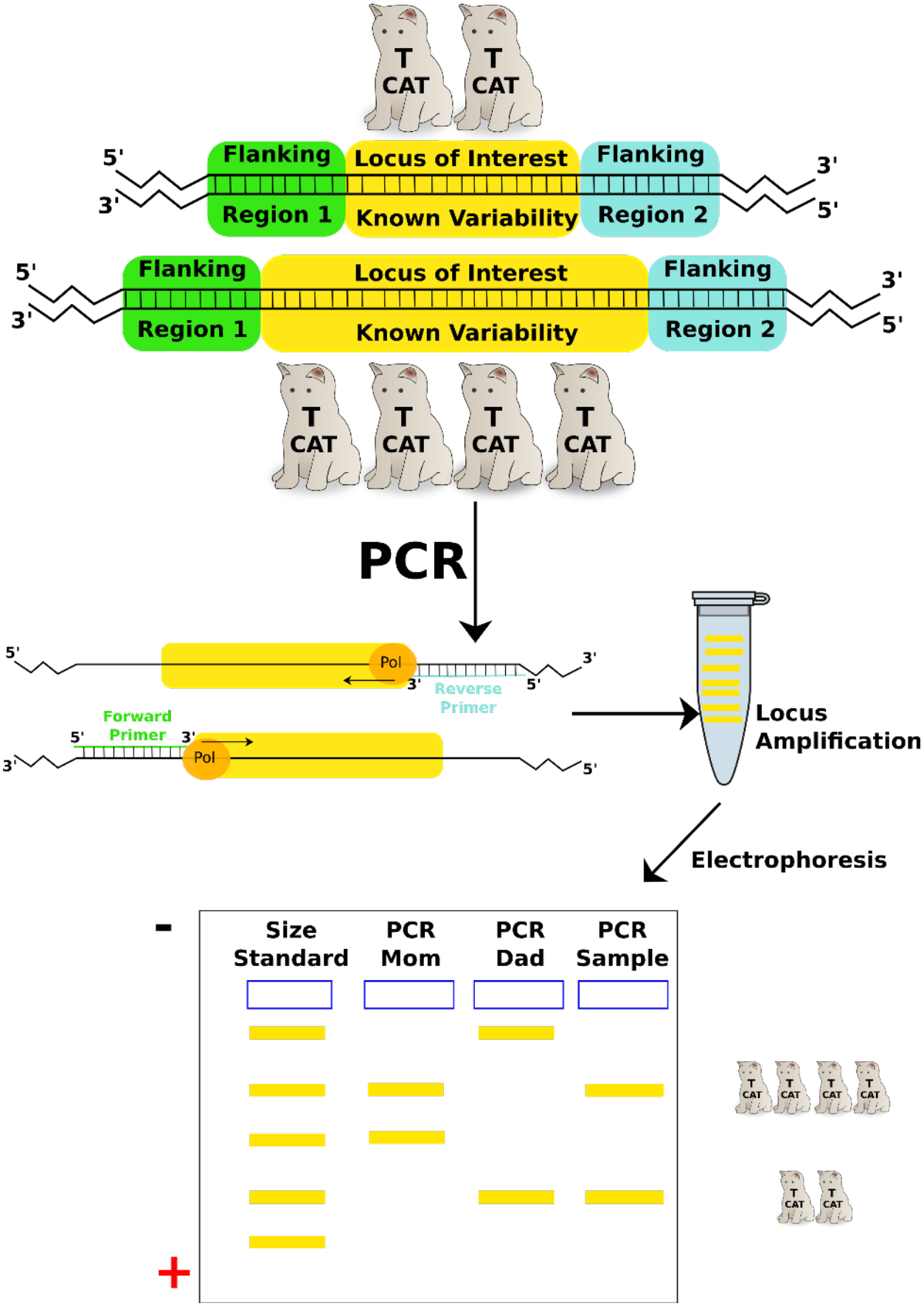


Figure **2.1.8.3.4**: **DNA evidence from a crime scene:** DNA can be extracted from cells found from various sources at a crime scene. PCR can amplify this small amount of DNA. Credit: Jeremy Seto (CC-BY-NC-SA)

Amplified DNA will be separated by gel electrophoresis and analyzed. Size reference standards and samples from the crime scene and the putative suspects would be analyzed together. In a paternity test, samples from the mother, the child, and the suspected father would be analyzed in the same manner. A simple cheek swab will supply enough cells for this test.



https://bio.libretexts.org/@go/page/114768

Figure **2.1.8.3.5**: **TH01 locus used in a Paternity/Maternity test:** Individual PCR reactions are run for each sample (mom, dad, and child). The TH01 primer pair specifically amplifies the locus. Each amplified sample is run on the same gel to resolve the different alleles of TH01 from each individual. From this test, the sample could be the offspring from these 2 parents but the use of more STRs would make it more definitive. Count the TCATs. Credit: Jeremy Seto (CC-BY-NC-SA)

EXTERNAL RESOURCES

- Flash animation walking through what an STR is: http://www.dnalc.org/view/15981-DNA-variations.html

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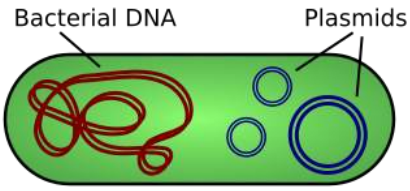
- **8.3: Variable Number Tandem Repeats** by Bio-OER is licensed CC BY-NC-SA 4.0.

2.1.8.4: PROPAGATING DNA IN BACTERIA

COMPLEMENTATION

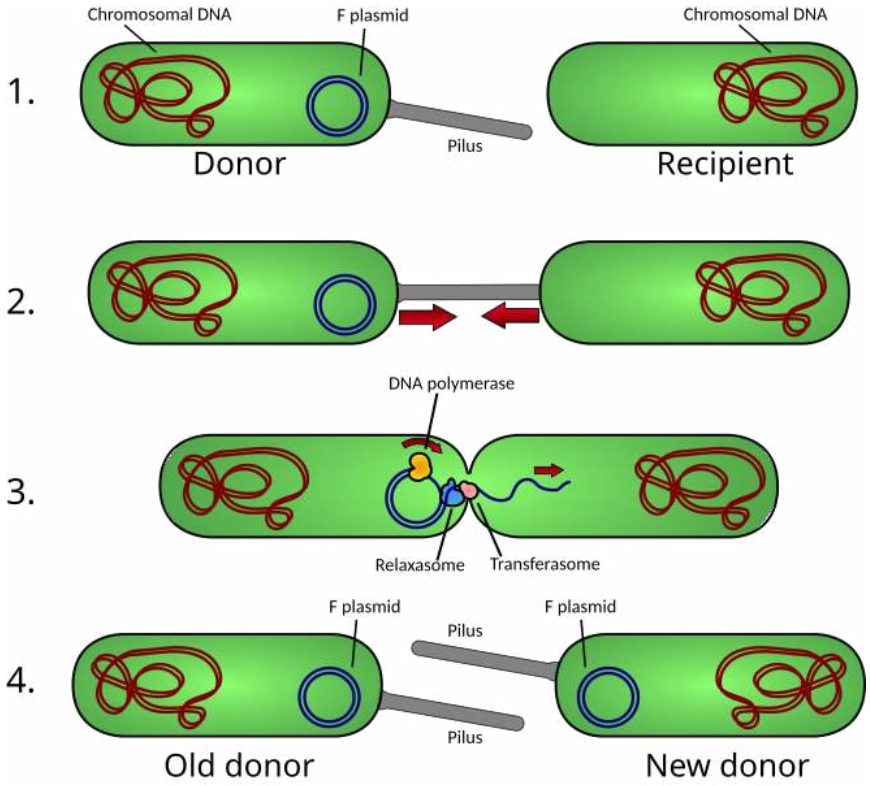
George Beadle and Edward Tatum first described the concept that each gene corresponded to an enzyme in a metabolic pathway by exposing the yeast *Neurospora crassa* to mutagenic conditions (Beadle & Tatum, 1941). Following these procedures, Joshua Lederberg continued these studies with Tatum where they generated two mutants strains in *Escherichia coli*. These bacteria were **auxotrophs**, unable to generate some basic nutrients necessary to sustain their growth. The two strains were described as met^{-} bio^{-} Thr^{+} Leu^{+} Thi^{+} (Strain A) and Met^{+} Bio^{+} thr^{-} leu^{-} thi^{-} (Strain B). Strain A can sufficiently synthesize the amino acids threonine, leucine, and the cofactor thiamine while deficient in producing the cofactor biotin and the amino acid methionine while the converse was true of Strain B. When either of these two strains was plated onto minimal media, no growth occurred. Supplementing minimal media with methionine and biotin permitted Strain A to grow as normal. When the two strains were mixed together and plated on minimal media, there was a growth of bacteria. The two strains were capable of complementing each other in some way as if a sexual exchange of genetic material had occurred (Lederberg & Tatum, 1946).

Bacteria are equipped with all the necessary capacities to replicate DNA. Common bacterial species have bee adapted for use in the lab to carry DNA and propagate it for uses in biotechnology. In addition to chromosomal DNA of the bacterial genome, bacteria also have extrachromosomal DNA called **plasmids**. These plasmids replicate independently of the bacterial chromosome and can occur in a high copy. These circular pieces of DNA are modified in labs to carry specific pieces of DNA so they can be studied or used for expression into proteins. Plasmids can naturally carry important traits, including antibiotic resistance. Plasmids are relatively small, ranging in size from 1000 bases to 1,000,000 bases long (1kb-1000kb).



*Bacterial DNA usually exists as a large **circular chromosome** (red). **Plasmids** are extrachromosomal and autonomously replicating pieces of DNA (blue).*

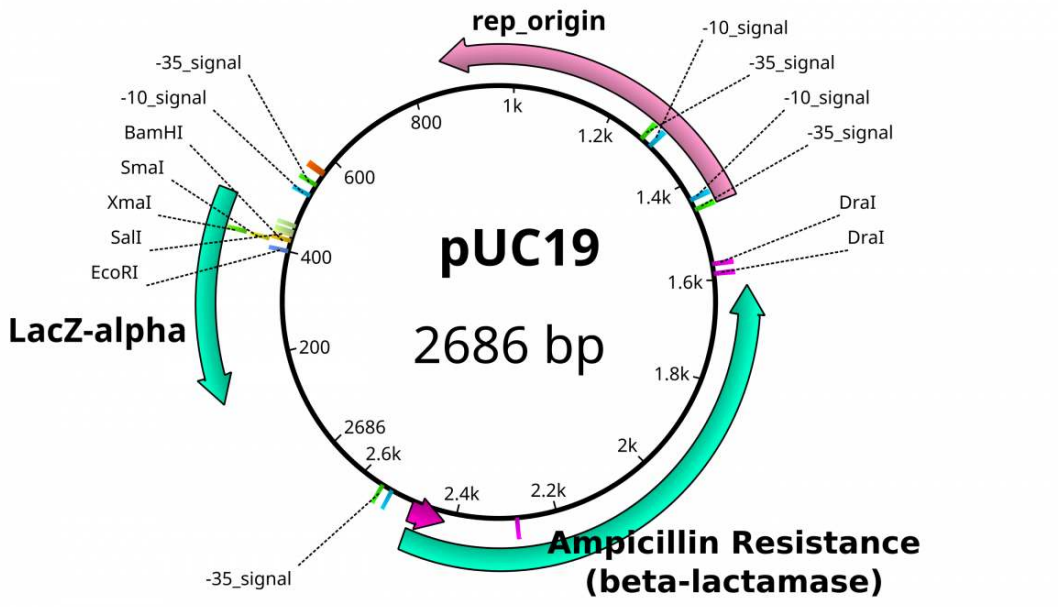
Through a process called **conjugation**, bacteria can “sexually” transfer genetic material to another by passing plasmids through a structure called a **conjugation pilus**.



Conjugation process between a plasmid bearing donor and a plasmid-less recipient. The donor creates a conjugation pilus to create a cytosolic bridge with the donor where the plasmid is replicated into the recipient through the rolling circle method of replication. The recipient then becomes competent to act as a donor.

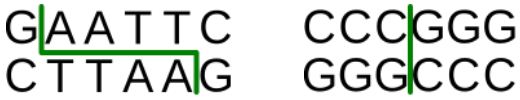
FEATURES OF PLASMIDS

Plasmids that are designed by Biologists to shuttle pieces of DNA for study are referred to as **vectors** because they *move* a piece of DNA.



These plasmid vectors have the same hallmarks as traditional plasmids with the capacity to replicate independently of the bacterial genome. The feature that allows these DNA's to replicate is called an **origin of replication** (ori) that is usually rich in A's and T's. However, these plasmid vectors have the additional properties that make them easy to work with and distinguishable from bacterial plasmids; a selection marker and a multiple cloning site. A **selection marker** usually comes in the form of a gene that encodes resistance to a specific antibiotic.

In the pictured plasmid, Ampicillin resistance granted by the β-lactamase gene. The **multiple cloning site** (**MCS**), also known as the polylinker, is the location in which the DNA of interest is incorporated into the vector. MCSs are defined by a set of unique sites where the DNA can be cut by **restriction endonucleases** (**RE**). As the name implies, restriction enzymes are "restricted" in their ability to cut or digest DNA. The restriction that is useful to biologists is usually **palindromic** DNA sequences. Palindromic sequences are the same sequence forwards and backward. Some examples of palindromes: RACE CAR, CIVIC, A MAN A PLAN A CANAL PANAMA. With respect to DNA, there are 2 strands that run antiparallel to each other. Therefore, the reverse complement of one strand is identical to the other.

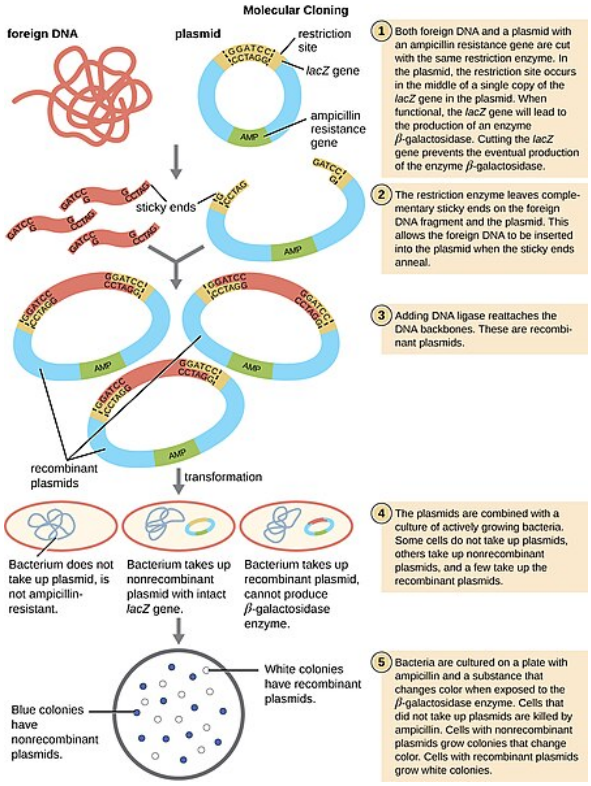


***EcoRI** generates sticky of cohesive ends **SmaI** generates blunt ends*

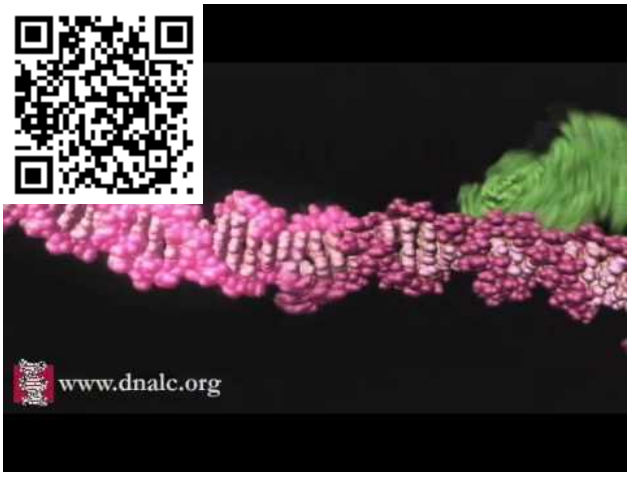
Restriction enzymes hydrolyze covalent phosphodiester bonds of the DNA to leave either "sticky/cohesive" ends or "blunt" ends. This distinction in cutting is important because an *EcoRI* sticky end can be used to match up a piece of DNA cut with the same enzyme in order to glue or ligate them back together. While endonucleases cut DNA, **ligases** join them back together. DNA digested with *EcoRI* can be ligated back together with another piece of DNA digested with *EcoRI*, but not to a piece digested with *SmaI*. Another blunt cutter is *EcoRV* with a recognition sequence of GAT | ATC.

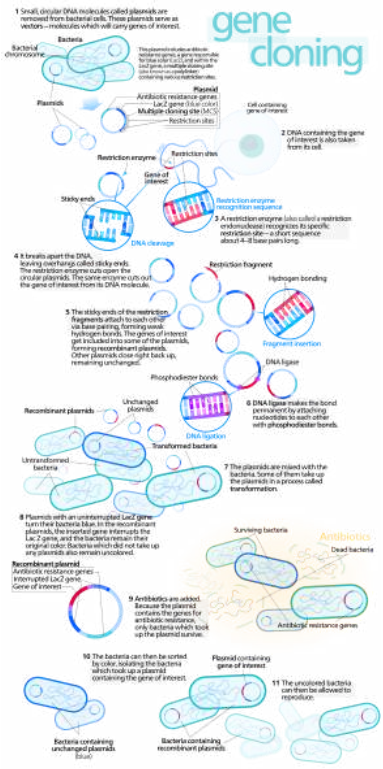
By "cutting and pasting" DNA into vectors, we can introduce foreign or exogenous DNA into bacteria. This type of DNA is now called **Recombinant DNA** and is the heart of biotechnology.

RECOMBINANT DNA TECHNOLOGY






www.dnalc.org



ADDITIONAL RESOURCES

1. http://www.dnalc.org/resources/3d/20-mechanism-of-recombination.html
2. http://www.dnalc.org/view/16705-Animation-34-Genes-can-be-moved-between-species-.html

QUESTIONS FOR THOUGHT

1. Why do you think that the origins of replication are made of A's and T's?
2. What is different about the types of bonds holding the double strands together versus phosphodiester bonds of the DNA backbone?
3. Can DNA be digested with *SmaI* be ligated to DNA digested with *EcoRV*?
4. If so, which enzyme will be able to digest this new DNA?

- *Beadle, G. W.; Tatum, E. L. (1941).* "Genetic Control of Biochemical Reactions in Neurospora". *Proceedings of the National Academy of Sciences*. **27** (11): 499–506. doi:10.1073/pnas.27.11.499. PMC 1078370. PMID 16588492
- *Lederberg J, Tatum EL (1946). "Gene recombination in E. coli". Nature. **158** (4016): 558. doi:10.1038/158558a0*

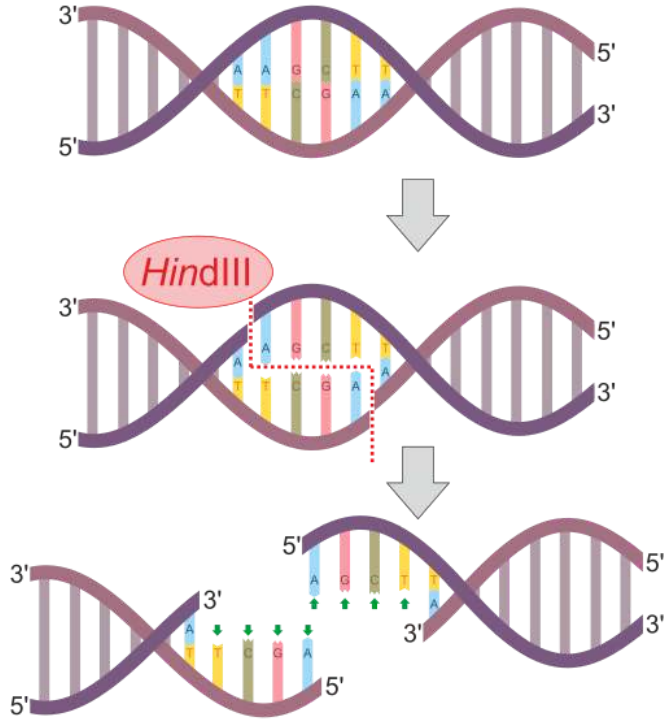
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- **8.4: Propagating DNA in Bacteria** by Bio-OER is licensed CC BY-NC-SA 4.0.

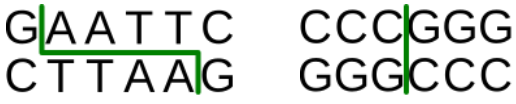
2.1.8.5: RESTRICTION ENZYMES

DNA can be cut by **restriction endonucleases** (**RE**). Endonucleases are enzymes that can hydrolyze the nucleic acid polymer by breaking the **phosphodiester** bond between the phosphate and the pentose on the nucleic acid backbone. This is a very strong covalent bond while the weaker hydrogen bonds maintain their interactions and double strandedness.

As the name implies, restriction endonucleases (or restriction enzymes) are "*restricted*" in their ability to cut or digest DNA. The restriction that is useful to biologists is usually **palindromic** DNA sequences. Palindromic sequences are the same sequence forwards and backwards. Some examples of palindromes: RACE CAR, CIVIC, A MAN A PLAN A CANAL PANAMA. With respect to DNA, there are 2 strands that run antiparallel to each other. Therefore, the reverse complement of one strand is identical to the other. Molecular biologists also tend to use these special molecular scissors that recognize palindromes of 6 or 8. By using 6-cutters or 8-cutters, the sequences occur throughout large stretches rarely, but often enough to be of utility.



Restriction enzymes hydrolyze covalent phosphodiester bonds of the DNA to leave either "sticky/cohesive" ends or "blunt" ends. This distinction in cutting is important because an *EcoRI* sticky end can be used to match up a piece of DNA cut with the same enzyme in order to glue or ligate them back together. While endonucleases cut DNA, **ligases** join them back together. DNA digested with *EcoRI* can be ligated back together with another piece of DNA digested with *EcoRI*, but not to a piece digested with *SmaI*. Another blunt cutter is *EcoRV* with a recognition sequence of GAT | ATC.



EcoRI *generates sticky of cohesive ends* ***SmaI*** *generates blunt ends*

RESTRICTION DIGESTIONS



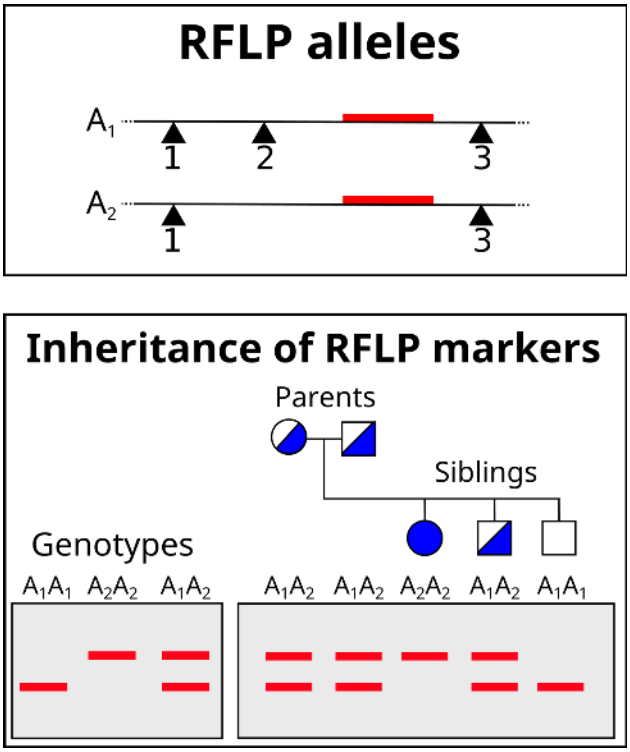


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2.1.8.6: DNA FINGERPRINTING (RFLP)

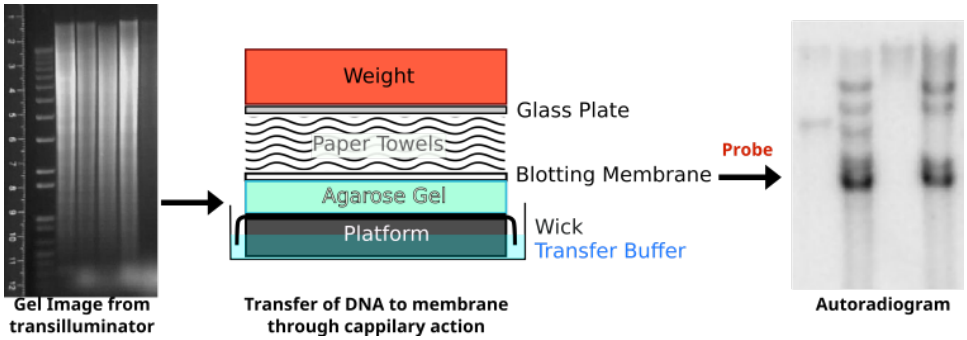
Restriction fragment length polymorphism (**RFLP**) is a technique that exploits variations in DNA sequences. DNA from differing sources will have variations or **polymorphisms** throughout the sequence. Using Restriction Enzymes, these differences in sequences may be teased out. However, if one were to take the entirety of the human genome and chop it up with a restriction enzyme, many indecipherable fragments would be made. In fact, the resulting agarose gel would simply show a large smear of DNA. RFLP analysis requires that a **probe** to a specific area of DNA be used to identify specific locations. Agarose gels would be transferred to a membrane or filter where they would be **hybridized** to these radioactive probes.



Homologous chromosomes with restriction sites noted by triangles. the rectangle sitting on the chromosomes correspond to a probe locus. Credit: Jeremy Seto (CC0)

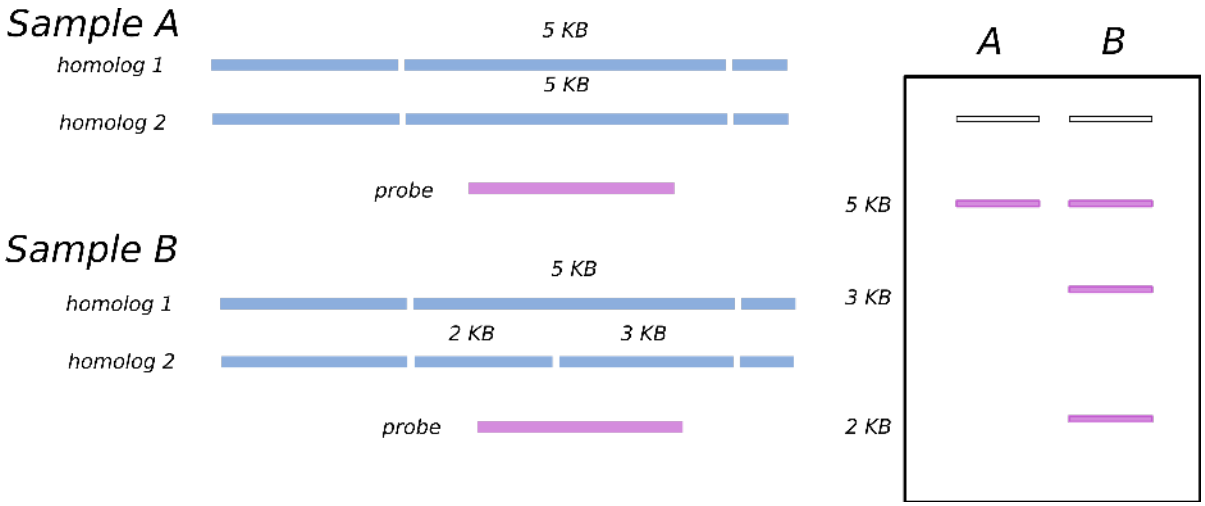
RFLP analysis was designed for forensic science to discriminate between people. Since people are 2N, they have pairs of homologous chromosomes with the same loci. However, these loci may contain different alleles. In this case, the phenotype for these alleles is the actual sequence that may or may not contain restriction sites. The presence or absence of a restriction site may arise from **single nucleotide polymorphisms** (**SNPs**) that reveal the natural variation between people.

The schematic below illustrates a comparison of restriction profiles between two sources. Note that the probe overlaps a restriction site in one of the alleles. This probe will be able to bind to both fragments given sufficient sequence overlap. Upon resolving on an agarose gel, genomic DNA that does not hybridize with the probe will obscure the locus of interest as a large smear. A filter is placed on top of the agarose and pressed against it to transfer the DNA in a process called **Southern Blotting**. Following a lengthy transfer, the filter is denatured to and incubated with the radioactive probe. To visualize this probe hybridization, a film is exposed to the filter and processed.



Following restriction digestion, the samples are resolved on an agarose gel. Digestion of genomic DNA will result in a large smear. Following transfer of the DNA onto a membrane through capillary action, the membrane is probed with radioactive probe DNA. The probe binds selectively to complementary sequences to reveal a series of distinct bands. An interactive demonstration of the first DNA fingerprinting.

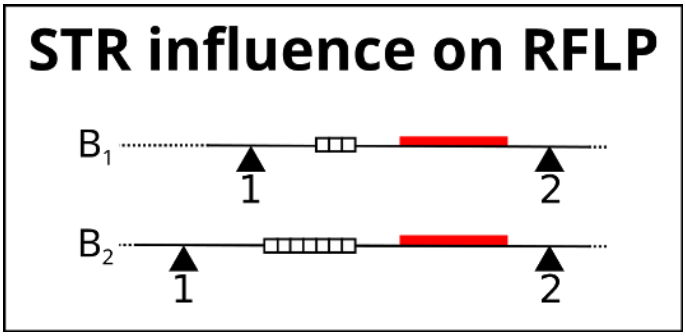
Credit: Oder Zeichner: abigail [or CC-BY-SA-3.0] /Autoradiogram



Sample A only reveals one band after processing because this person is homologous for the same allele. Sample B is heterozygous and reveals three bands.

Credit: Retama (CC-BY-SA 4.0)

RFLPs represent inheritable markers and can reveal relationships between different individuals. A pedigree can illustrate the relationship of the inherited alleles. The technique can be more informative if using multiple probes simultaneously for different loci or to use **multi-locus probes** that hybridize to multiple locations.



RFLPs may arise from differences in the STR/VNTR repeats between restriction sites. Credit: Jeremy Seto (CC0)

While RFLPs can arise from SNPs, they may also be caused by the expansion or contraction of repeated elements between restriction sites. These repeated elements of DNA are referred to as Variable Number Tandem Repeats (VNTR) and illustrate polymorphisms that normally occur in non-coding regions of the genome.

RELATED PAGES

- **Restriction Enzymes**
- **Analyzing DNA**
- **VNTR**
- **Genetics**

EXTERNAL RESOURCES

- Using Tandem Repeats for DNA Fingerprinting (Interview)
- The first DNA fingerprint (Flash Simulation)

DNA FINGERPRINTING (ACTIVITY)

1. The origin of the DNA samples for this exercise will be explained by the Instructor as numerous scenarios may be used (Edvotek Cat. #109).
2. Prepare a 1% agarose gel by adding 60ml Tris-Borate-EDTA buffer (TBE) to 0.6g agarose in an Erlenmeyer flask.
3. Place the flask in a microwave or on the heat until agarose is melted.
    - Stop periodically and swirl the solution. Do not permit to boil over.
4. Assemble the casting tray by blocking the ends with tape or plastic baskets.
5. Place the comb into the casting tray at the **NEGATIVE** end.
6. The instructor will add 6µl Sybr Safe to his/her own gel solution at this time.
7. You may place the casting trays inside a refrigerator and pour the solution into the tray.
8. Wait until the gel is solidified.
9. Carefully separate the gaskets from the tray ensuring not to tear apart the wells made by the comb.
10. Remove the comb and place the casting tray into an electrophoresis chamber.
11. Cover the gel with TBE buffer.
12. Using a micropipettor, load 40-50µl dye samples sequentially into the wells.
13. Cover the electrophoresis chamber with the lid and ensure good contact between electrodes.
    - It is conventional that the **POSITIVE** side of the tank is nearest to you.
    - With the **POSITIVE** side nearest to you, load the samples from left to right.
14. Set the power supply to 100-120V, press the Run button (you should see bubbles at each electrode), and allow to run for at least 40 minutes.
15. After 40 minutes, stop the current and remove the gel in the casting tray.
16. Slide the gels into the staining solution if they do not include Sybr Safe for visualization the subsequent meeting time.
17. The instructor will slide the gel onto a UV transilluminator behind a shield and show the results to the class.
    - Document the findings of the gel by photographing with your phone.
    - The instructor will discuss the results and ask for you to interpret the findings.

ACTIVITY FOLLOW-UP

1. Why are the samples loaded at the negative side of the gel?
2. What is the role of the dye in these samples? Should we be alarmed that the samples are all the same color?
3. What does it mean that there are multiple bands in a lane? What does it mean that there is only one band in a lane?
4. What can we conclude from the banding patterns in this forensics or paternity case? Is this sufficient data for these conclusions?

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2.1.8.7: CHEEK CELL DNA EXTRACTION

USING CYTOBRUSH

1. Use a sterile cytobrush and insert into mouth.
2. Brush the cytobrush on the inside of your cheek 25 times.
3. Swirl the cytobrush in 100 µl of Chelex suspension (10% w/v).
4. Place a centrifuge tube with Chelex and cell suspension on a 100 °C heat block for 10 minutes.
5. Centrifuge the tubes at maximum speed for 5 minutes.
6. DNA is in the supernatant. Avoid beads at the bottom.
7. Store DNA at -20 °C.

PCR WITH PCR BEADS

1. Add 22 µl of the primer mix (forward and reverse) to beads.
2. Ensure that the bead is dissolved.
3. Add 3 µl of DNA.

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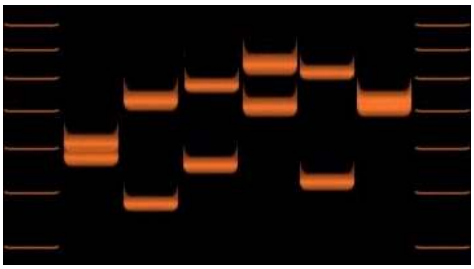
2.1.8.8: D1S80 VNTR (GENOTYPING)

The minisatellite marker D1S80 is located at 1p35-p36. This VNTR is 16 bases long. With a variation of alleles between 3-24 repeats, the locus displays enough diversity to aid in distinguishing between people. Although this is not a CoDIS marker, the use of multiple loci is required to definitively identify samples. The large repeat (16bp) permits the use of standard agarose gel electrophoresis to explore the diversity of this locus in our lab. PCR products range from 430bp to 814bp long.

- D1S80-for: 5′-GAAACTGGCCTCCAAACACTGCCCGCCG-3′
- D1S80-rev: 5′-GTCTTGTTGGAGATGCACGTGCCCCTTGC-3′

1. PCR the DNA samples extracted from cheek cells using the PCR Beads.
2. Pour 2% agarose into casting apparatus in the refrigerator.
    - 2 gels per class need to be made → 100ml of TBE with 2g agarose
    - Add 5µl SYBR safe solution into the molten agarose before casting.
    - Place 2 sets of combs into the gel → at one end and in the middle
3. Load the gel with a DNA ladder.
    - The sample is from the PCR.
4. Run the gel at 120V for 20 minutes.
5. Visualize on the UV transilluminator.

EXAMPLE RESULTS



1. How many alleles are visible in each lane?
2. Are the genotypes distinguishable between individuals?
3. Are any of the alleles common between individual samples?

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2.1.8.9: DNA MINIPREP BY ALKALINE LYSIS (ACTIVITY)

ALKALINE LYSIS

Once DNA is introduced and carried in bacteria, we would like to isolate the DNA again for further manipulation. In order to do so, bacteria containing the plasmid of interest is grown in a liquid culture of nutrient-rich broth made of yeast extract called Luria-Bertani Broth (**LB**). These cultured bacteria are grown until they are of a high concentration overnight. They are harvested through centrifugation and the broth is removed. The resulting pellet of bacteria is resuspended in a physiological buffer containing the chelator EDTA. A **chelator** is a chemical that removes divalent cations like Ca^{2+} or Mg^{2+} from solution. This is significant because divalent cations are necessary for DNA digesting enzymes to be active. By chelating the ions, the DNA we ultimately wish to purify will be safe from degradation.

After resuspension of the bacteria, an alkaline solution of 0.1N NaOH is mixed into the bacterial mix. This solution also contains an ionic detergent called sodium dodecyl sulfate (**SDS**) that aids in denaturing proteins and disrupting their interactions with the DNA. The mixture becomes viscous as the bacteria burst open and their contents leak into the solution. This basic solution is then neutralized with a potassium acetate buffer at pH5.5. As the solutions mix together, the pH approaches 7 and the potassium interacts with the SDS to cause the precipitation of the genomic chromosomal DNA and proteins. In order to separate the precipitate from the solution, the mixture is centrifuged at high speed to pellet the genomic DNA and protein. The **supernatant**, or solution, is transferred to a column containing a **silica membrane**. Under high salt conditions, DNA adheres to glass or silica. By passing the solution through this column, the plasmid DNA in the supernatant is trapped onto the silica membrane and removed from the solution. Additional washes are used to removed stray contaminants and remove the excessive salt. Plasmid DNA is finally removed from the column through **elution** by a low salt buffer. This low salt buffer is Tris pH 8 with EDTA (**TE**). Plasmid DNA can be stored stably in the TE buffer in the freezer for extended periods.

EXERCISE 1: PLASMID DNA MINI-PREP BY ALKALINE LYSIS

1. **Inoculate** 2 ml of rich medium (LB, YT, or Terrific Broth) containing the appropriate antibiotic with a single colony of transformed bacteria. Incubate the culture overnight at 37°C with vigorous shaking. (This is what you were provided)
    1. Each group should take 2 cultures
2. **Centrifuge** culture tubes directly at maximum for 5 minutes.
    1. If incapable of spinning in these tubes, transfer 1.5 ml of the culture into a microfuge tube (Eppendorf Tube).
    2. Centrifuge at maximum speed for 30 sec.
3. When centrifugation is complete, pour the broth solution into a container of bleach.
4. **Resuspend** the bacterial pellet in 250 µl of ice-cold P1 solution by vigorous shaking and transfer back into a microcentrifuge tube.

- P1 is a physiological solution of 50mM Tris at pH 8.
- P1 contains a Chelator called EDTA.
    - Chelators bind up excess divalent cations that are required for DNAse activity.

5. **Lyse**: Add 250µl of P2 solution to each bacterial suspension. Close the tube tightly, and mix the contents by inverting the tube gently five times. Do not vortex! Store the tube on ice.

- This is the lysis buffer containing the detergent Sodium Dodecyl Sulfate and NaOH.

6. **Neutralize:** Add 350 µl of ice-cold P3 solution. Close the tube and disperse lysis solution by inverting the tube several times. Store the tube on ice for 3-5 minutes.

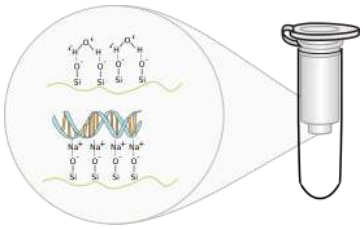
- This is the neutralization buffer containing Potassium Acetate.
- Neutralization restores pH to near 7 and also causes the precipitation of genomic DNA and proteins into a gloopy mess (snot-like).

7. Centrifuge the bacterial lysate at maximum speed for 5 minutes in a microfuge.

- Snot-like substances should be tightly packed into a pellet at the bottom of the tube after this step.
- The solution or supernatant contains the plasmid DNA.

8. **Column Purification of DNA**: Transfer the supernatant to a fresh tube with a silica-membrane column

- DNA likes to bind to glass under high salt conditions.
- The white membrane is made of glass fiber.



9. Centrifuge the supernatant through the column for 1 minute at maximum speed in a microfuge.

- The DNA will be bound to the membrane on the column (silica)

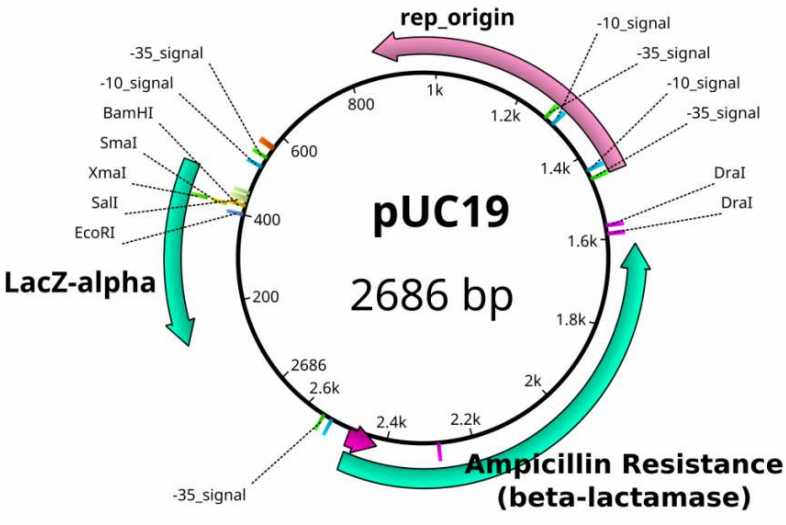
10. **Wash**: Discard flow-through and place column back into the waste tube. Wash column with 500 µl PE. Centrifuge the supernatant through the column for 1 minute at maximum in a microfuge.

- PE is a solution that helps to wash away the non-specifically bound substances

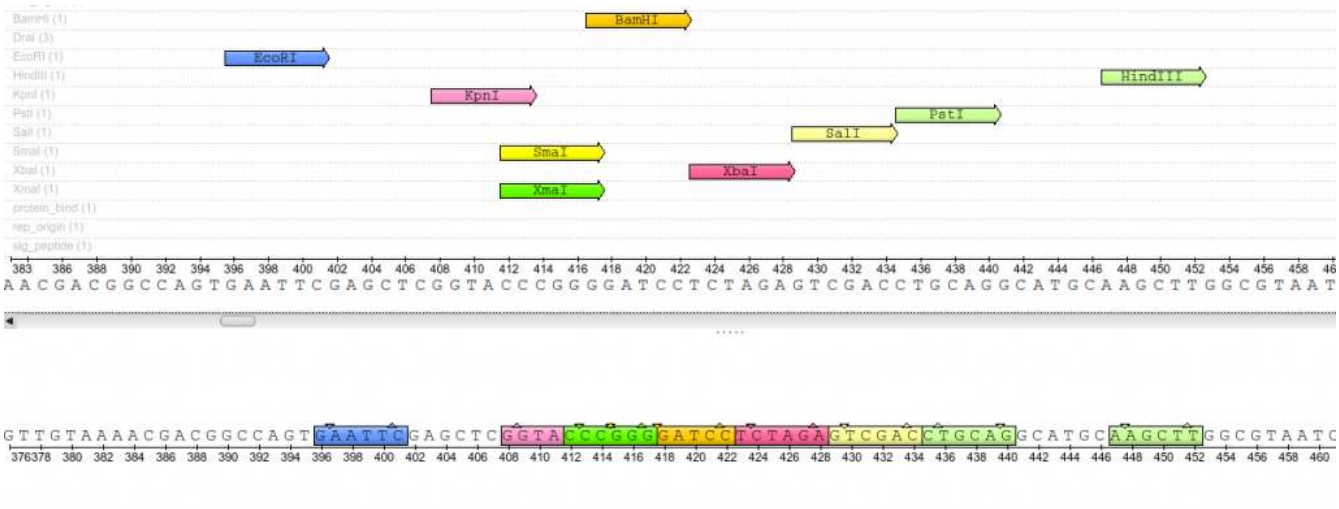
11. Discard flow-through and place column back into the waste tube. Wash Column with 700 µl PE. Centrifuge the supernatant through the column for 1 minute at maximum in a microfuge. Discard flow-through and repeat spin to dry column.
12. Place column in a fresh centrifuge tube and **Elute** the nucleic acids in 50 µl of TE (pH 8.0) by binding for 1 minute and spinning at maximum speed for 1 minute.

IDENTIFICATION OF PLASMID DNA

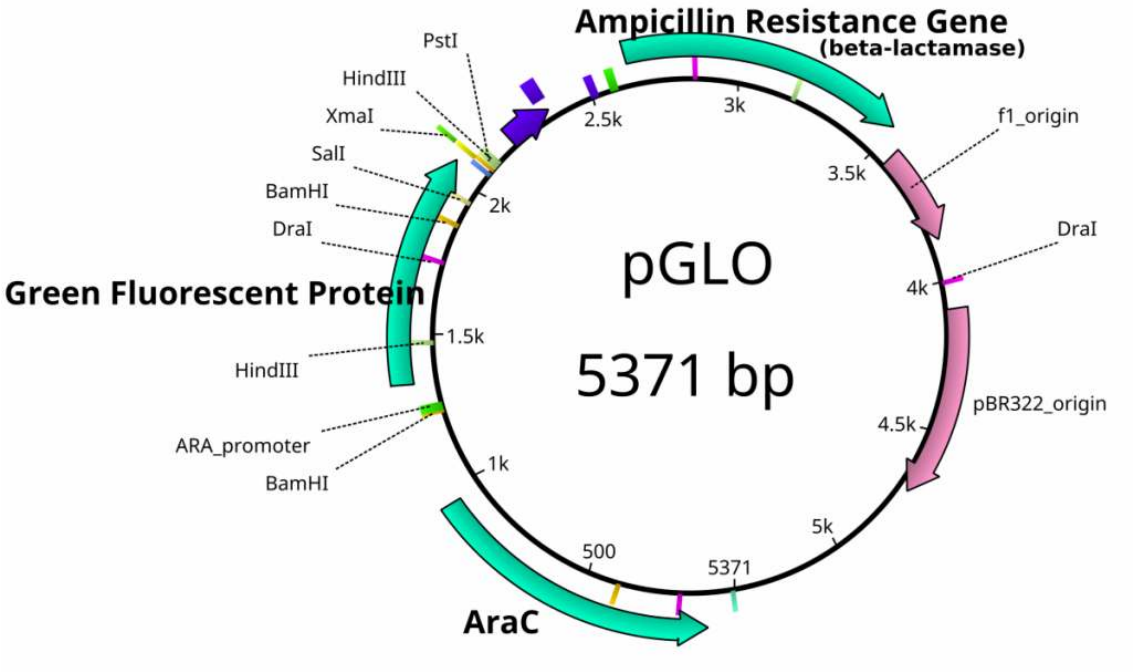
Once plasmids are isolated, they require identification. Plasmid vectors have known sequences and are mapped of their major features. Knowing the sequence of these pieces of DNA means knowing the locations of RE digestion sites. By using Res, digesting plasmids into known sizes aids in the verification of plasmid identity without the need to have the entire plasmid re-sequenced. A common plasmid is called pUC18 or pUC19. The “p” stands for plasmid, the “UC” stands for University of California (where it was designed) and 18 or 19 refer to the difference in the MCS. This plasmid is 2,686 base pairs or ~2.7kb (kilobase) long with a single *EcoRI* site in the MCS. Another plasmid of interest in learning Molecular Biology is called pGlo. This plasmid has a jellyfish gene in the MCS that codes for a protein that will fluoresce green when expressed under UV light. pGlo is 5.4kb long and contains a single *EcoRI* site. Once digested, by an enzyme, these plasmids can be identified based on size separation on an agarose gel. Usually, it is best to identify by using 2 different REs. Digestion is important before size comparisons since circular DNA migrates through agarose differently than linear DNA. Additionally, circular DNA can sometimes be “super-coiled” and lead to very rapid migration despite the size.



Feature map of pUC19 including the locations of some restriction enzymes.



Close up view of the pUC19 Multiple Cloning Site (MCS). These restriction sites appear only once throughout the entirety of the plasmid sequence.

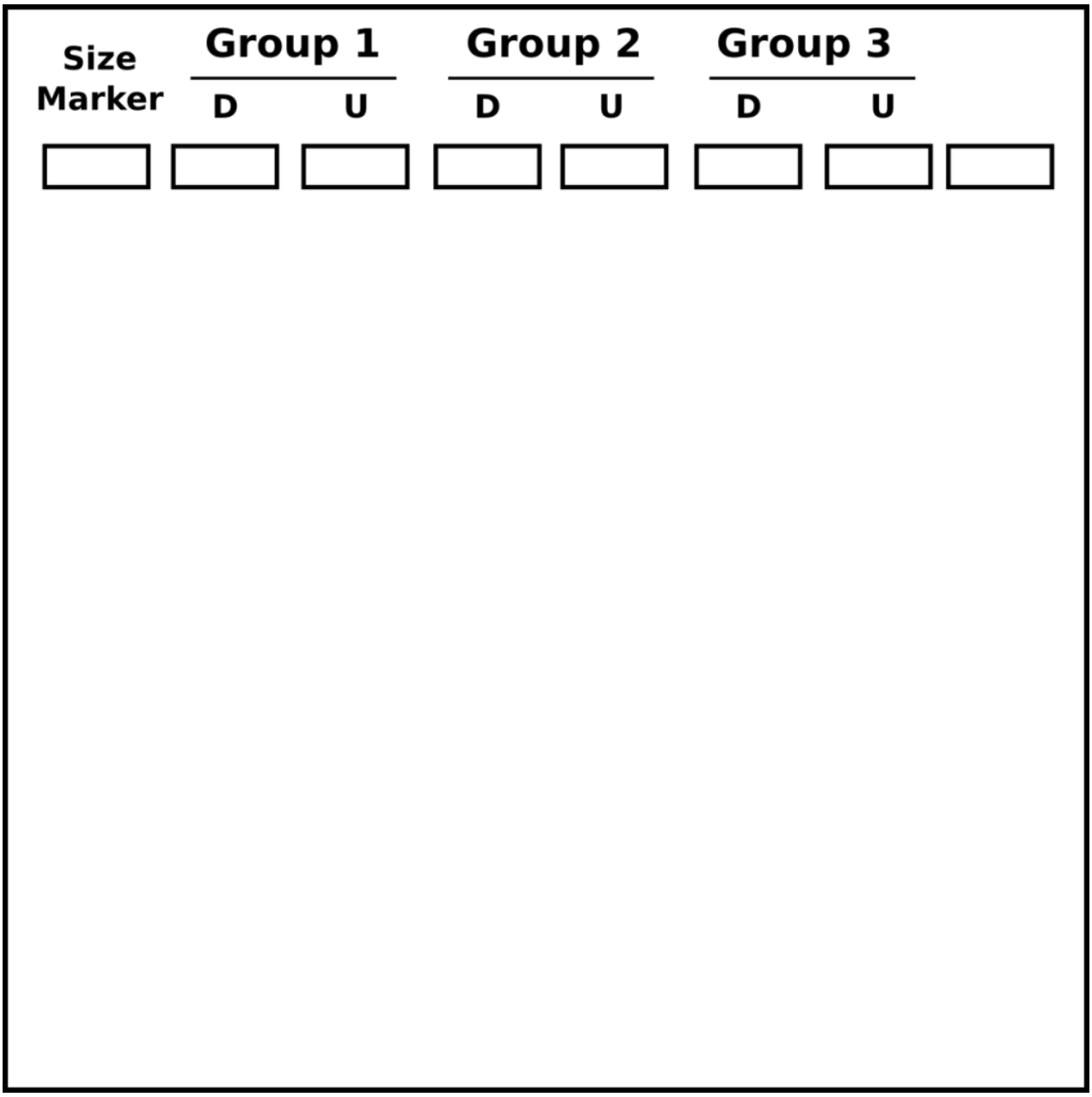


Plasmid feature map of pGlo. This is not a cloning vector, so an MCS does not exist.

EXERCISE 2: RESTRICTION DIGESTION IDENTIFICATION OF PLASMIDS

1. The class should prepare 2X 0.8% agarose gels by preparing 0.4g agarose in 50ml TBE buffer.
    1. Melt agarose solution by microwaving for 1 minute.
    2. Add 5µl Sybr Safe solution into the 100ml gel solution.
    3. Pour this solution into a casting tray inside the refrigerator.
    4. Insert a comb.
2. To a new tube add 2µl of plasmid DNA to 8µl of *Eco*RI fast digest mixture.
    1. 1µl Fast Digest Buffer
    2. 1µl Fast Digest *Eco*RI enzyme
    3. 6µl H_2O
3. Incubate at 37°C for 10 minutes.
4. Add 2µl of loading buffer to digestion mixture.

5. In a separate tube, combine 3µl plasmid DNA with 2µl loading buffer and 7µl of H_2O.
6. Load gel with an appropriate size ladder in the first lane, load the Digested plasmid in the next lanes, then the undigested plasmid.
    - 3 groups can load onto one gel
    - D=digested plasmid
    - U=undigested plasmid
7. Run the gel at 110V for 30 minutes and visualize on a UV transilluminator.
8. Document with your camera.



ADDITIONAL RESOURCES

- For help on this problem, please try out the *In silico* digestion activity.

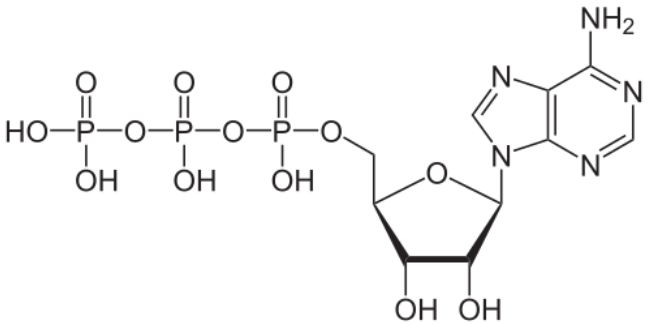
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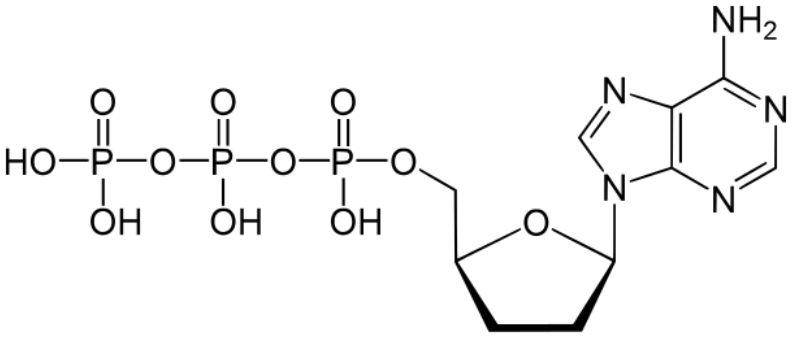
2.1.8.10: SANGER SEQUENCING OF DNA

RADIOACTIVE CHAIN TERMINATION

The polymerization of nucleic acids occurs in a 5′ → 3′ direction. The 5′ position has a phosphate group while the 3′ position of the hexose has a hydroxyl group. Polymerization depends on these 2 functional groups in order for a dehydration synthesis reaction to occur and extend the sugar-phosphate backbone of the nucleic acid. In the 1970s, Fred Sanger's group discovered a fundamentally new method of 'reading' the linear DNA sequence using special bases called **chain terminators** or **dideoxynucleotides**. The absence of a hydroxyl group at the 3′ position blocks the polymerization, resulting in termination. This method is still in use today and is called "Sanger dideoxynucleotide chain-termination method". This method originally used a radioactively labeled primer to initiate the sequencing reaction. Four reactions take place where each reaction is intentionally "poisoned" with a dideoxy chain terminator. For example, one reaction will have all 4 dNTPs (deoxynucleotide triphosphates) with the addition to a small amount of ddATP (dideoxyadenosine triphosphate). This reaction will result in a series of premature terminations of the polymerization specifically at different locations where an Adenine would be incorporated.

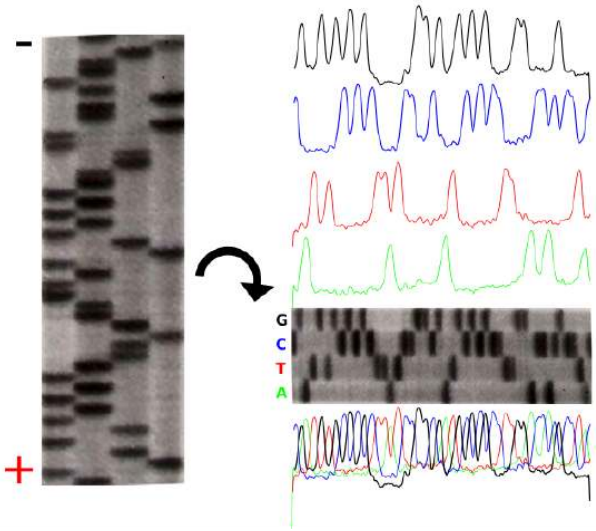


dATP is a natural monomer used in the polymerization of DNA. The 3'-OH is the attachment point of the next subsequent nucleotide.



The lack of a 3'-OH in this molecule of ddATP makes it a chain terminator that will prohibit the addition of another nucleotide to the DNA polymer.

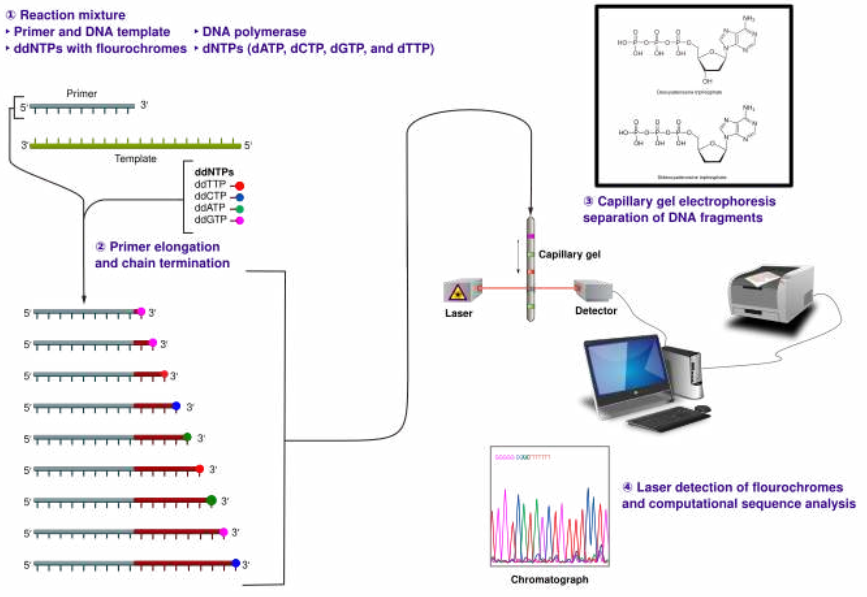
The product of these 4 separate sequencing reactions is run on a large polyacrylamide sequencing gels. The smallest fragments run through the gel the fastest and create a ladder-like pattern. This can be visualized through the use of an x-ray film that is sensitive to the radioactivity. Each lane of the gel corresponds to one of the four chain-terminating reactions. The bases are read sequentially from the bottom up and reveal the sequence of the DNA.



The sequencing gel can be manually scored. The profiles of each lane have been created using ImageJ to illustrate the banding pattern and subsequent sequence.

Credit: John Schmidt & Jeremy Seto (CC-BY-SA 3.0)

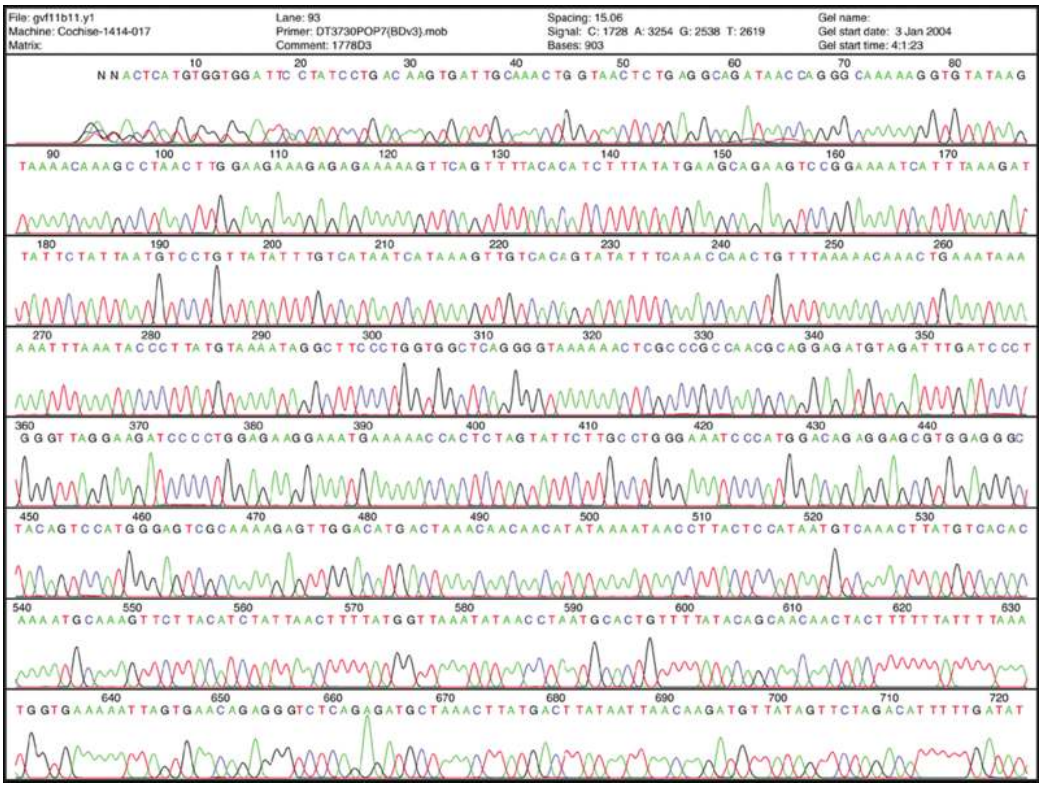
FLUORESCENT CHAIN TERMINATION AND CAPILLARY ELECTROPHORESIS



Credit: Estevezj (CC-BY-SA 3.0)

Radioactivity is dangerous and undesirable to work with so chain terminators with fluorescent tags were developed. This method synthesizes a series of DNA strands that are specifically fluorescent at the termination that is passed through a capillary electrophoresis system. As the fragments of DNA pass a laser and detector, the different fluorescent signal attributed to each ddNTP is identified and generates a chromatogram to represent the sequence. Fluorescent Chain Terminators are now used in reactions and run through a small capillary. The smallest fragments run through first and are detected to reveal a chromatogram.

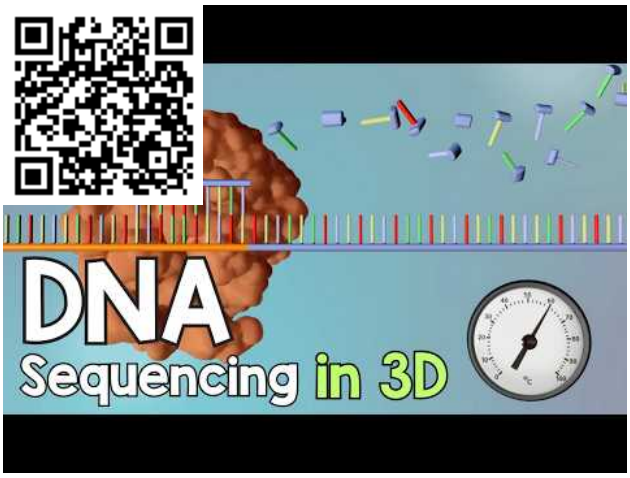
Fluorescent Chromatograms are used to score the nucleotide chain termination. The amplitude of each peak corresponds to the strength or certainty of the nucleotide call. Chromatogram files are usually provided alongside the sequence file with the extension ***.ab1** while the sequence files are provided as a text file in the **fasta** format. More about these files can be found here. The ab1 files are extremely important to analyze when there are ambiguity or sequencing errors. These ab1 files can also be used to ascribe a quality score on the base call.

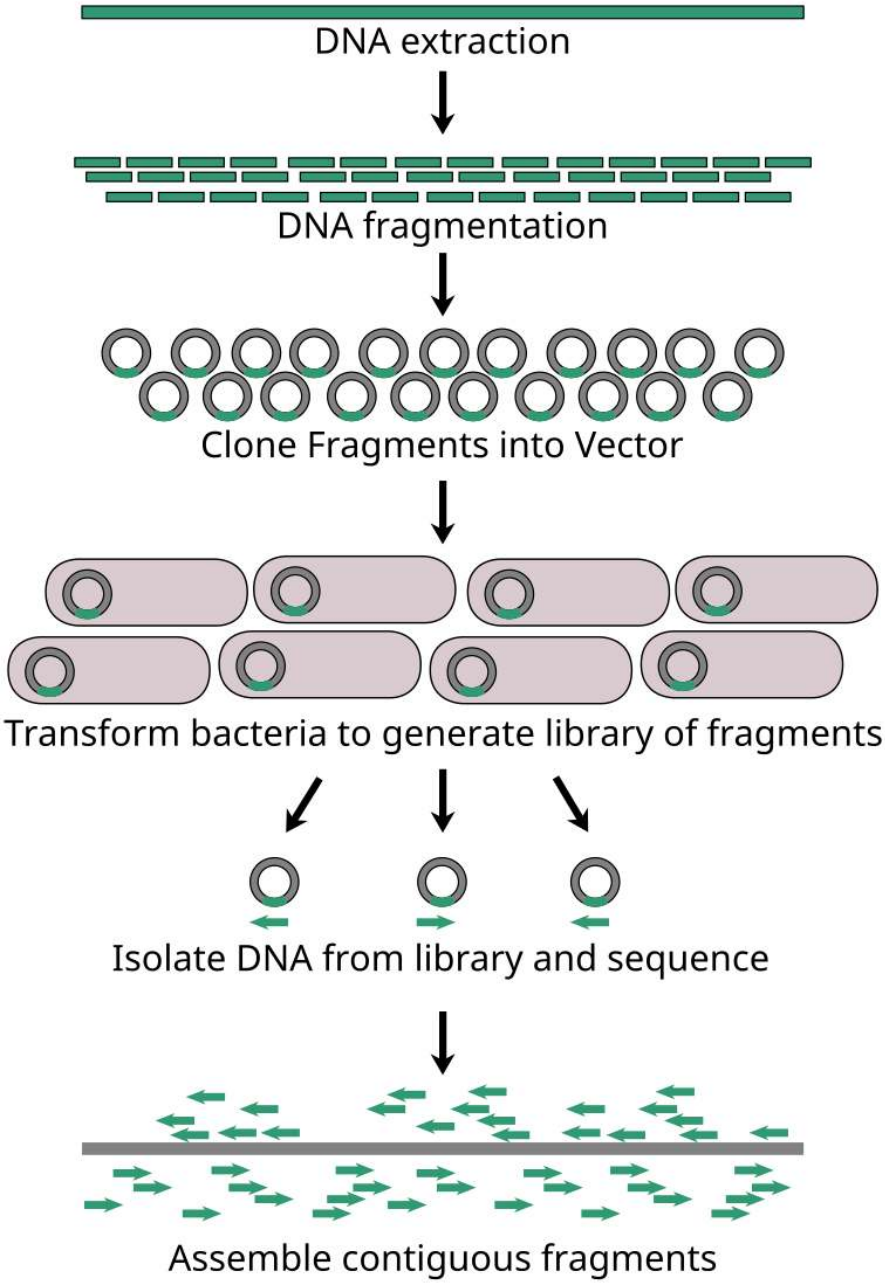


When there is too much ambiguity in the signal because of multiple peaks, you will often find an **N** in place of one of the 4 nucleotides (A, T, C, and G).

This video (source: www.yourgenome.org CC-BY) illustrates the mechanism of fluorescent chain termination and capillary electrophoresis.



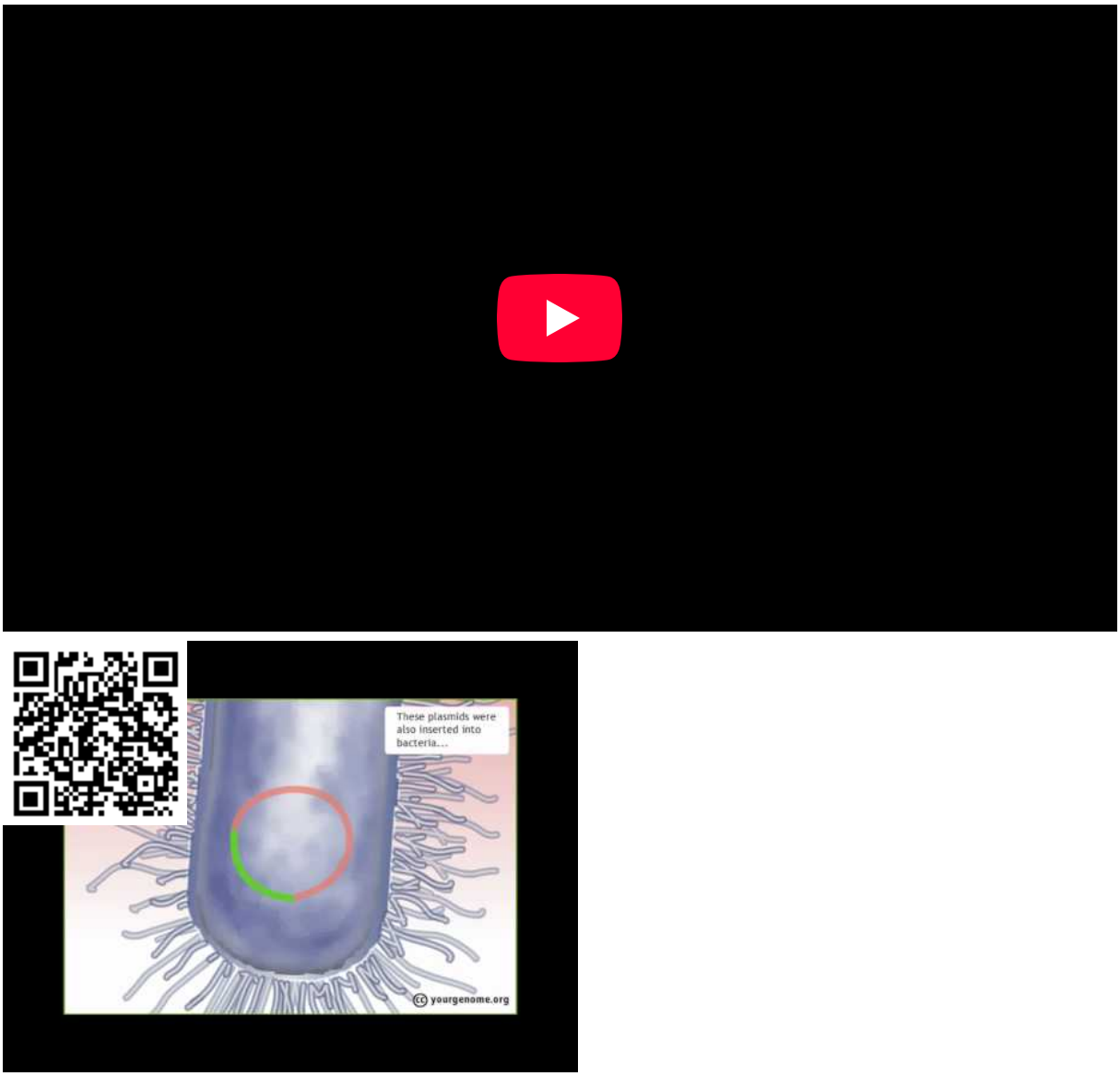

DNA
Sequencing in 3D



Credit: Jeremy Seto (CC-BY-NC-SA 3.0)

Traditional sequencing of genomes was a long and tedious process that cloned fragments of genomic DNA into plasmids to generate a genomic DNA library (**gDNA**). These plasmids were individually sequenced using Sanger sequencing methodology and computational was performed to identify overlapping pieces, like a jigsaw puzzle. This assembly would result in a draft scaffold.

The video below is taken from yourgenome.org (CC-BY) and illustrates the sequencing of the human genome through the shotgun sequencing approach.

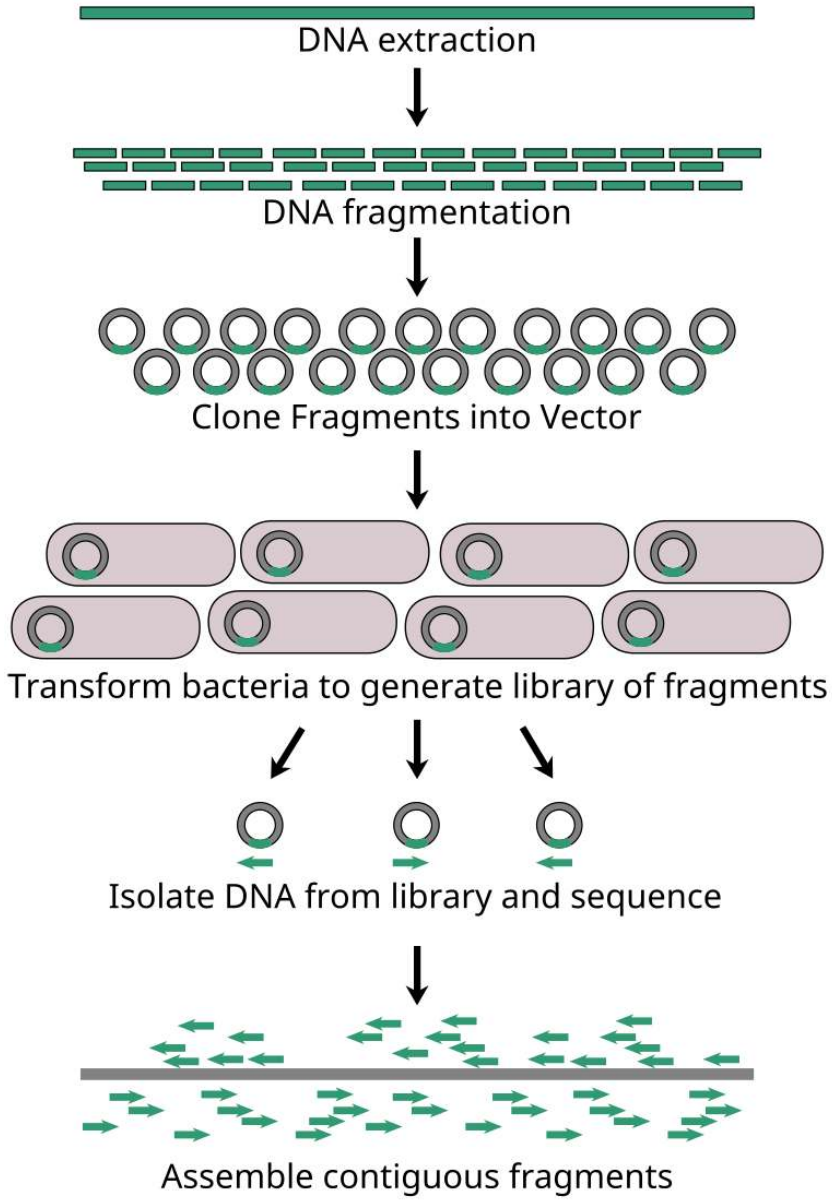


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2.1.8.11: NEXT GEN SEQUENCING

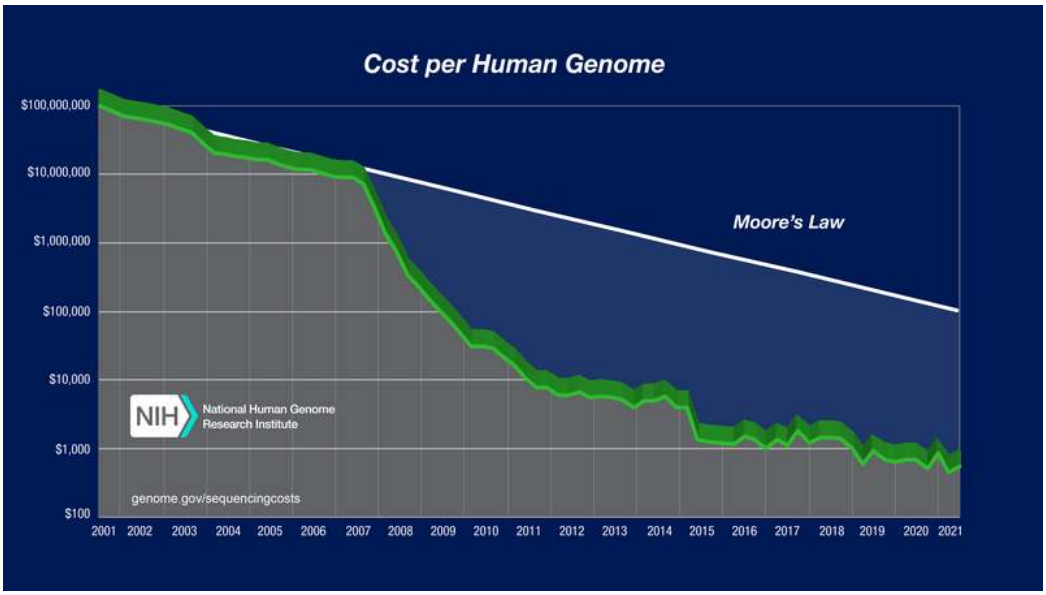
EXPANSION OF SEQUENCING TECHNOLOGY



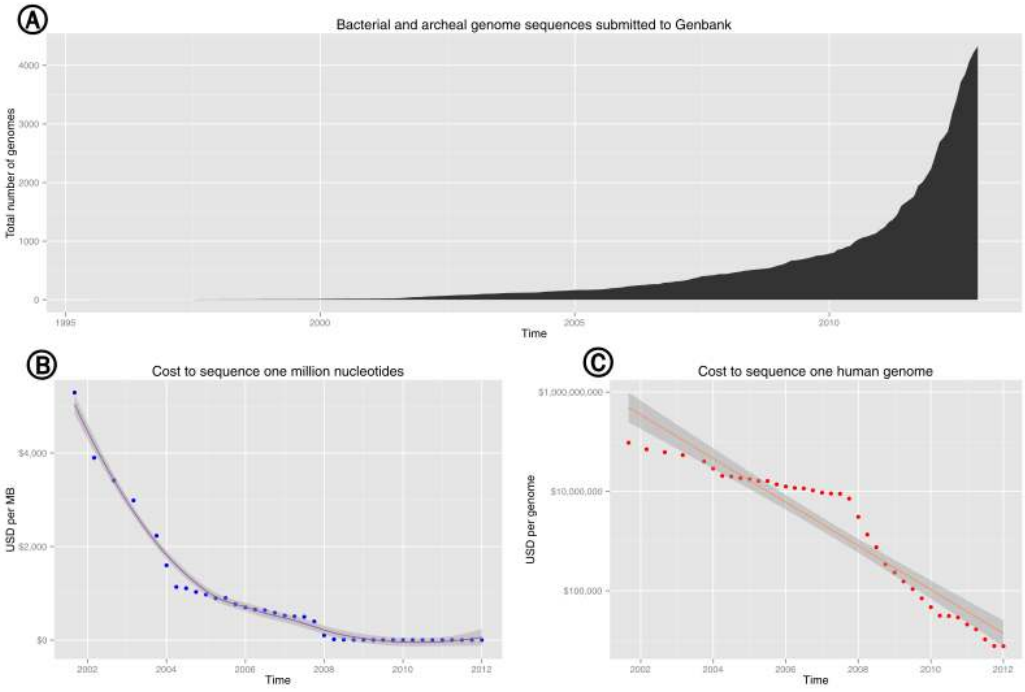
Credit: Jeremy Seto (CC-BY-NC-SA 3.0)

Traditional sequencing of genomes was a long and tedious process that cloned fragments of genomic DNA into plasmids to generate a genomic DNA library (**gDNA**). These plasmids were individually sequenced using Sanger sequencing methodology and computational was performed to identify overlapping pieces, like a jigsaw puzzle. This assembly would result in a draft scaffold.

As technology improved, the cost of sequencing genomes became less expensive. This technology outpaced Moore's Law, a semiconductor projection about the speed of computers as time progressed. A dramatic price decrease in the cost of genome sequencing occurred around 2008 due to technical advances.



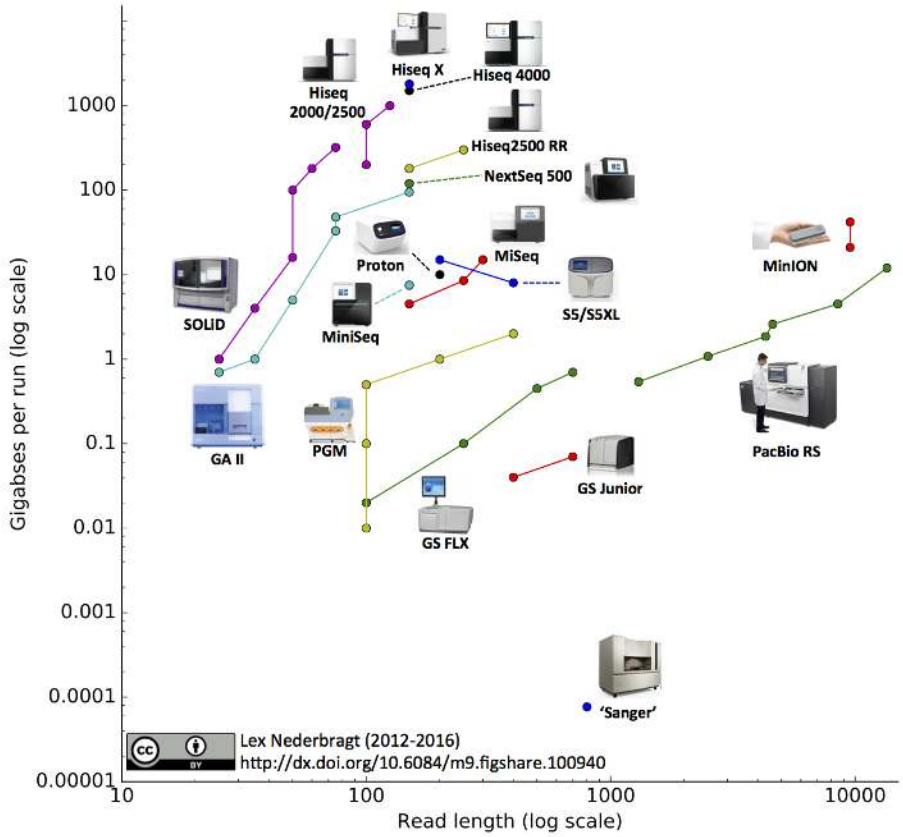
As the cost of genome sequencing decreased, a dramatic increase in genome deposition into Genbank was observed. These deposits reflected small genomes of bacteria and archaea.



Credit: Estevezj (CC-BY-SA 3.0)

The decrease in per nucleotide sequencing cost came from the parallelization of sequencing. Whereas Sanger Sequencing is capable of sequencing one stretch at a time, a parallel assembly of sequencing reactions has led to the high throughput sequencing often dubbed Next Generation Sequencing (**NGS**).

THE NEXT GENERATION OF SEQUENCING: HIGH-THROUGHPUT TECHNOLOGIES



Credit: Lex Nederbragt (CC-BY 3.0)

HIGH THROUGHPUT SEQUENCING APPLIED TO GENOME SEQUENCING (TEDED CC BY-NC-ND 4.0)

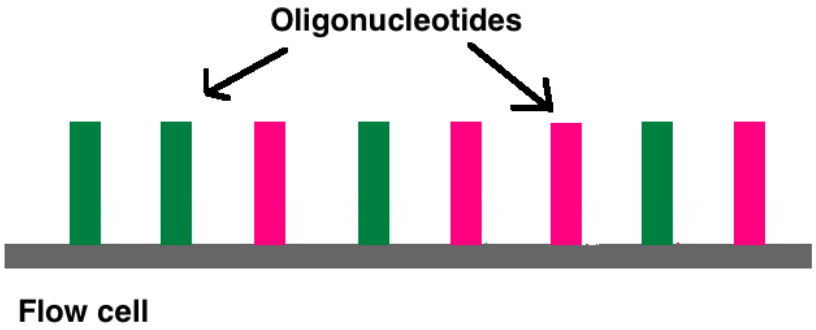




SHORT READ SEQUENCING BY SYNTHESIS

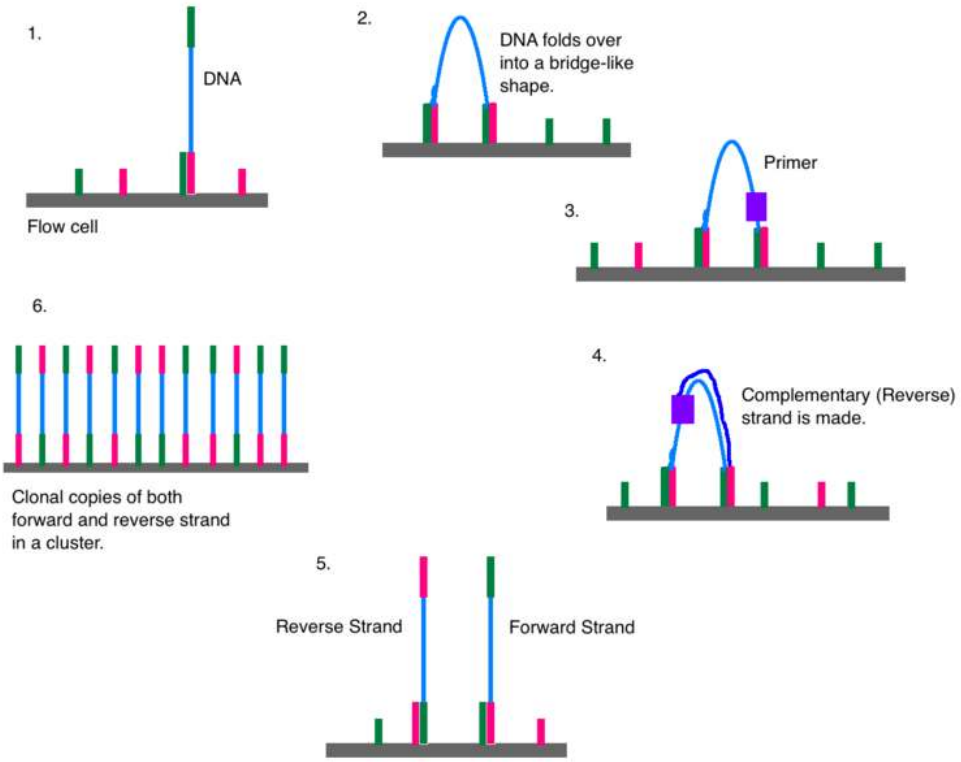
ILLUMINA

Illumina short-read sequencing uses flow cell technology where oligonucleotides complementary to adapter primers are physically seeded.



Flow cell surface with the adapter oligonucleotides. Credit: DMLapato (CC-BY-SA 4.0)

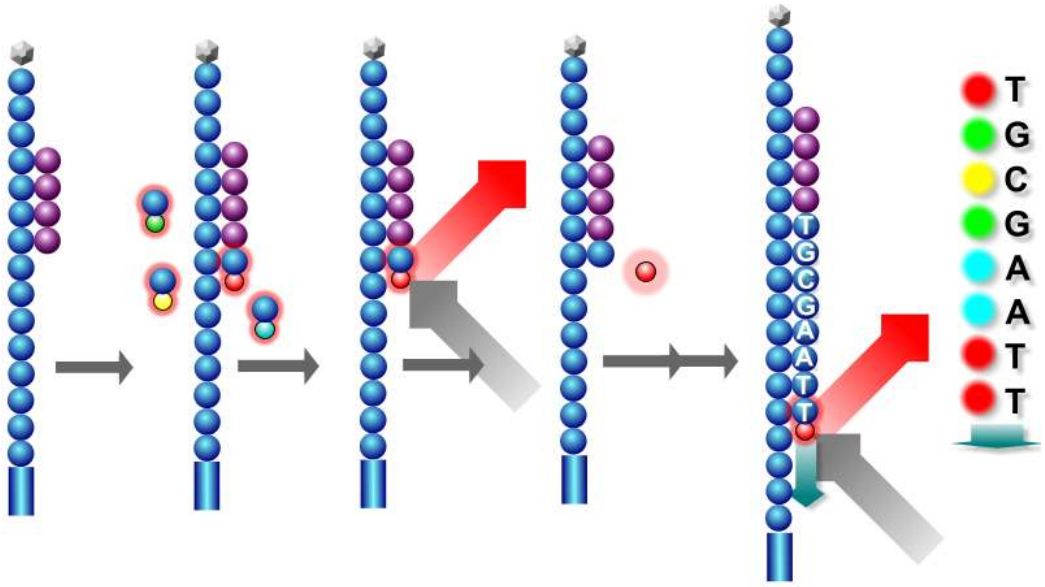
Fragmented DNA sequences are adapted with primers through ligation and hybridized to the flow cell. To increase the signal from sequencing, the short DNA sequences are amplified through a process called bridge amplification or cluster generation.



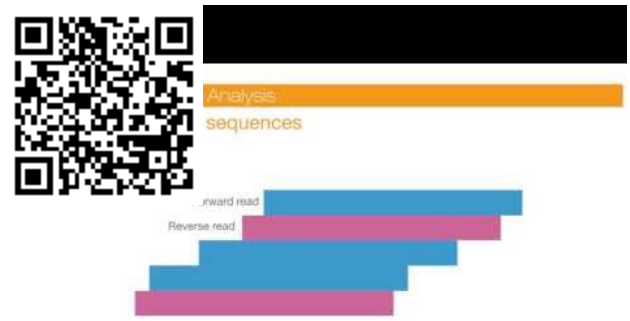
Cluster generation through bridge amplification. A low numb er of PCR cycles is used. Cluster generation aids in subsequent signal/noise determination.

Credit: DMLapato (CC-BY-SA 4.0)

The flow cell undergoes successive rounds of flooding with a fluorescent nucleotide, permitted to incorporate with a DNA polymerase and washed away. After each flood/wash cycle, fluorescent signals are measured to indicate the incorporation. Specific locations of fluorescence are tracked and consolidated to indicate the sequence at each registered point.



Each flow cycle introduces a fluorescent nucleotide for incorporation. Credit: Abizar Lakdawalla (CC-BY 3.0)



ION TORRENT

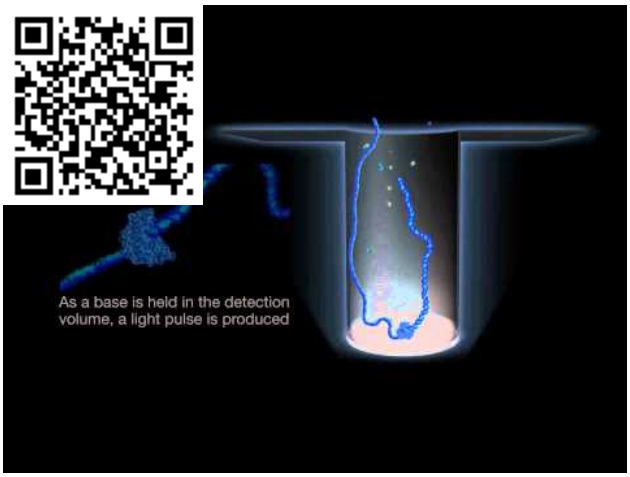
Fragmented DNA is ligated to adapter sequences and adhered onto microbeads. The beads are embedded into microwells on a semiconductor. Ion Torrent performs the sequencing reactions in an unbuffered solution since the semiconductor acts as a pH meter to identify nucleotide incorporation. Standard nucleotides are flooded onto the chip and incorporated. Because nucleotide incorporation creates a proton (H^+), a microenvironment of low pH is detected in the unbuffered solution.



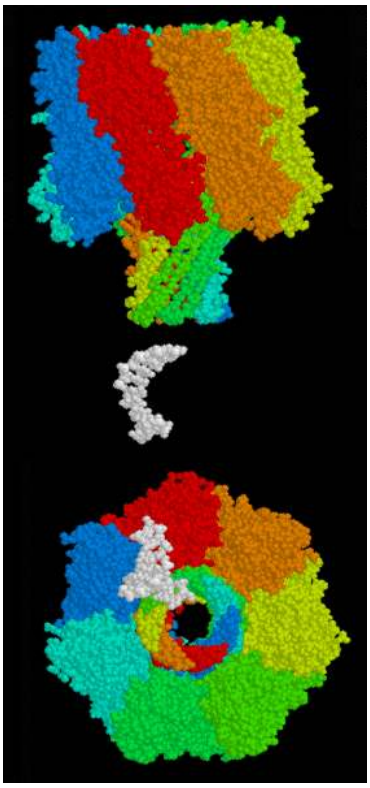
CULE REAL-TIME SEQUENCING

PAC BIO

Pac Bio uses nano wells with covalent-bonded DNA polymerase to sequence individual molecules of DNA. Fluorescent nucleotides are incorporated during synthesis reactions and real-time incorporation can be measured. Pac Bio sequencing has the advantage of sequencing fragments of 10-20kb, in stark contrast to the short-read methods.



OXFORD NANOPORE

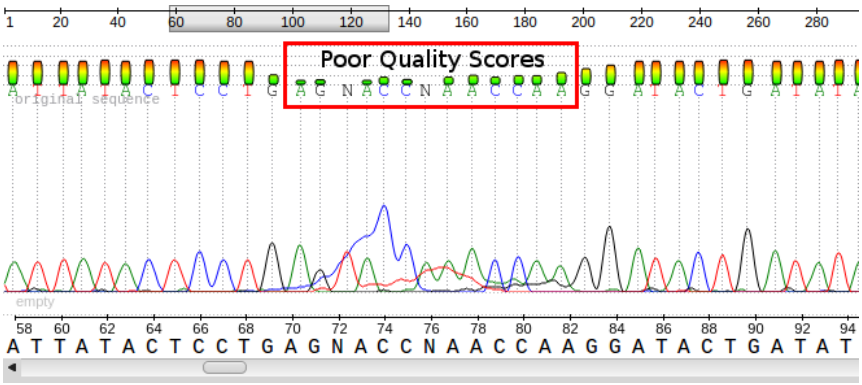


Credit: George Church (CC-BY 3.0)

Oxford Nanopore utilizes the protein alpha-hemolysin integrated onto a semiconductor chip. The pore size of the protein is the correct ssize for a single DNA molecule to fit through. A DNA Polymerase molecule is linked to the opening of the pore where the replicated DNA is fed through. As the DNA traverses the pore, the voltage changes are measured and mapped to the qualities of specific bases.

Link: youtu.be/BNz880V52rQ

SEQUENCE OUTPUT



A sample ab1 file displaying the base calls, the chromatograms and the quality scores for each base. Notice the poor quality in the red box and the corresponding peaks/bases.

The output file of the next-generation sequencing methods utilizes the **fastq** format. Like a fasta file, there is a header that describes the sequence. The first line is the header or title line which begins with '**@**' (remember that fasta begins with '>'). The second line is the actual raw sequence (once again similar to fasta). The third line has no meaning while the fourth line is filled with symbols as long as the sequence line. This last line is the quality score of the base call. As with the Sanger sequencing, there may be ambiguity with the base call of the sequence and the certainty is maintained in the quality score.

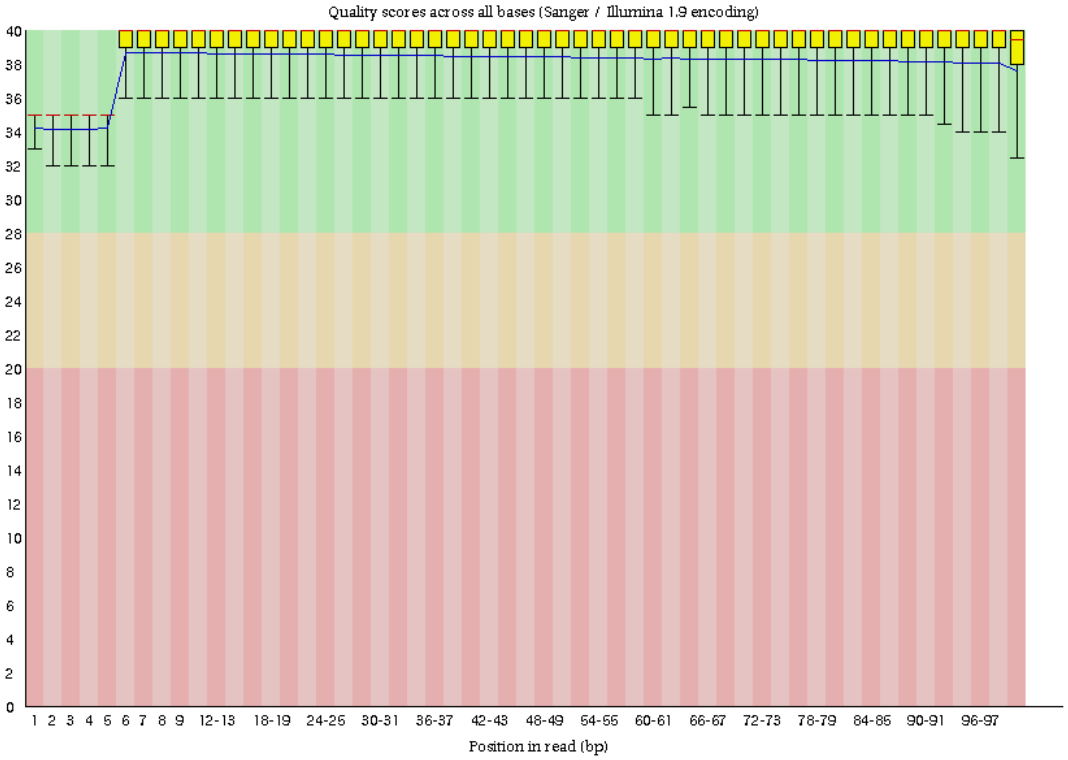
```
@GWZHISEQ02:207:H3VYHBCXX:1:1101:1465:1989 1:N:0:GTGGCC
AGAAGCCAAACGCATCATGGAAAAGCAGTTGCTCGAAGAACTCGAGCGACAGAGACAAGCTGAGCTTGCAGCACAAAAAGCCAGAGAGGAGGAAGAGAGAG
+
DBDDDIHIHHIIIHIHHHHIIIIIGHIIIIIIIIIIHIHHHEHHIIHIIIIIIHIHIIIIIHHIIIHHHIIIHIHIHIHIIHIHHH?@HHHIIHIHHHIGG
@GWZHISEQ02:207:H3VYHBCXX:1:1101:1715:1978 1:N:0:GTGGCC
NTACAGCCACGTGGAACCTGAGCACCATGAAGTCCCCCAAACCAGTGGGTTTTAAAGCAATCACCACAAAACTCCCTTTTTAGCTTTTGCTTGGGGTGGGG
+
#<DDDIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIHIIIHIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII
@GWZHISEQ02:207:H3VYHBCXX:1:1101:1695:1987 1:N:0:GTGGCC
GCCAAGCAAGCCTGCCCCTCAGCCCCCAGAGTGCACCCAATGACCCCCCTCCCTTGGGGTGACTCCTGATGAAGCACAACTCCCCACAGGGCCCCTAACCC
+
@BDADHIIIHIHIIIIHHIHIIHIIICHDHIIHIGEHIIHGFEHHHIIIIIIIIHHHHCHDIHHHIIHHIHIICCHFEHI?FGHIGIGGHGHHHHHIHHHH
@GWZHISEQ02:207:H3VYHBCXX:1:1101:1915:1948 1:N:0:GTGGCC
NAGTGTCTTTATTATTTATATTTTTTAAAAGCCAAACAATTCCAGCATACTCGTGATAACTTTGAGGATTAGATACATTTTCTTAACATTTTTTTCCCTTT
+
#<<<DGHIIIGIIIIII1GHIIIIIIHIIIHEHHIIHHIIIHGEHIHIIIIIGIIIIIIFHHEHIIIIGHIEEHIIIIIIIIIIHIHHHHHIIIIIGHIHII
@GWZHISEQ02:207:H3VYHBCXX:1:1101:2116:1940 1:N:0:GTGGCC
NGAGGGATAATCAGGCCGGACCTGAAGGGAGCGGAGCTGCAGCTACCGGAGTTGGATCGGTCATGGGACTTTGCAAGTGCCCCAAGAGGAAGGTGACGAAC
+
#<DBDIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIHIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII
```

Sample fastq file displaying 5 short-read sequences.

Phred scores were developed to assess the quality of the base calls arising from fluorescent Sanger sequencing during the Human Genome Project. The phred program scans the peaks of the chromatogram and scores based on the certainty or accuracy of the call. The scores are logarithmically based and scores greater than 20 represent greater than 99% accuracy of the base call.

Phred Quality Score	Probability of incorrect base call	Base call accuracy
10	1 in 10	90%
20	1 in 100	99%
30	1 in 1000	99.9%
40	1 in 10,000	99.99%
50	1 in 100,000	99.999%
60	1 in 1,000,000	99.9999%

Using the phred scores embedded in the last line of fastq files, poor quality reads can be removed. Using a program like FastQC permits the assessment of the reads and produces the graphical representation of quality.

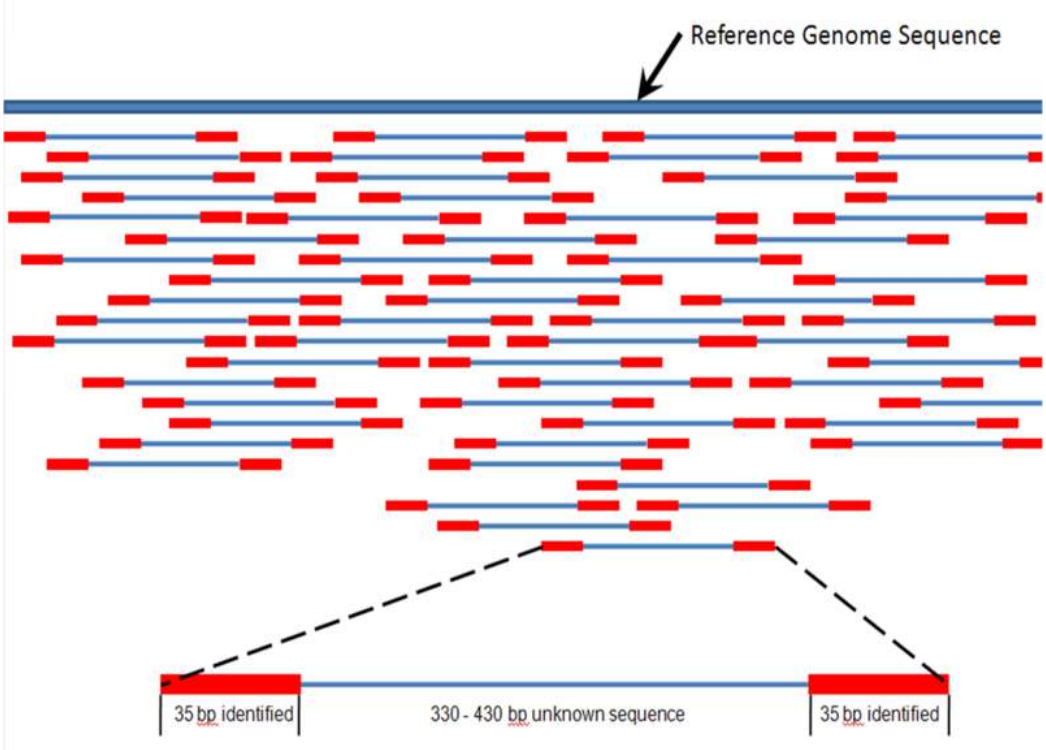


FastQC quality output illustrating the Phred score for each base call.

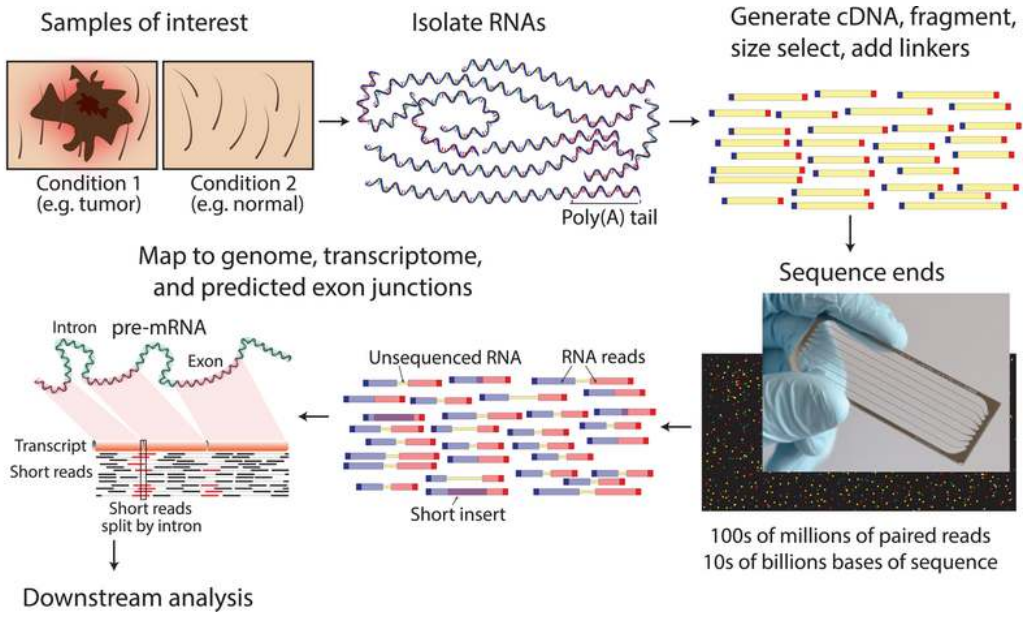
This short read sequence of about 100 nucleotides has all bases made at greater than 30, or > 99.9% accuracy.

ASSEMBLY AND ALIGNMENT

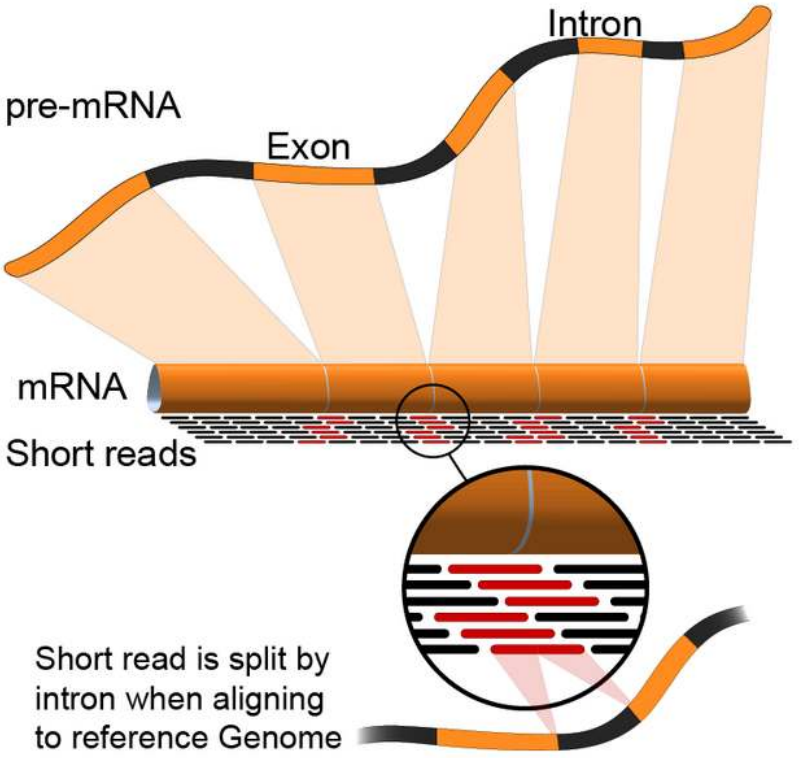
Sequences from short reads must be assembled into a usable sequence. To do so, a **reference genome** may aid in the assembly after adapter sequences are trimmed using automated methods. In the case that there is no reference genome, a related species may be used or a more computationally intensive process of ***de novo* assembly** must take place. With *de novo* assembly, it may be useful to have some long reads performed with PacBio to create scaffolds for generating the assembly into contiguous sequences, or **contigs**.



RNA-SEQ



Credit: Malachi Griffith, Jason R. Walker, Nicholas C. Spies, Benjamin J. Ainscough, Obi L. Griffith (CC-BY 4.0)
https://doi.org/10.1371/journal.pcbi.1004393

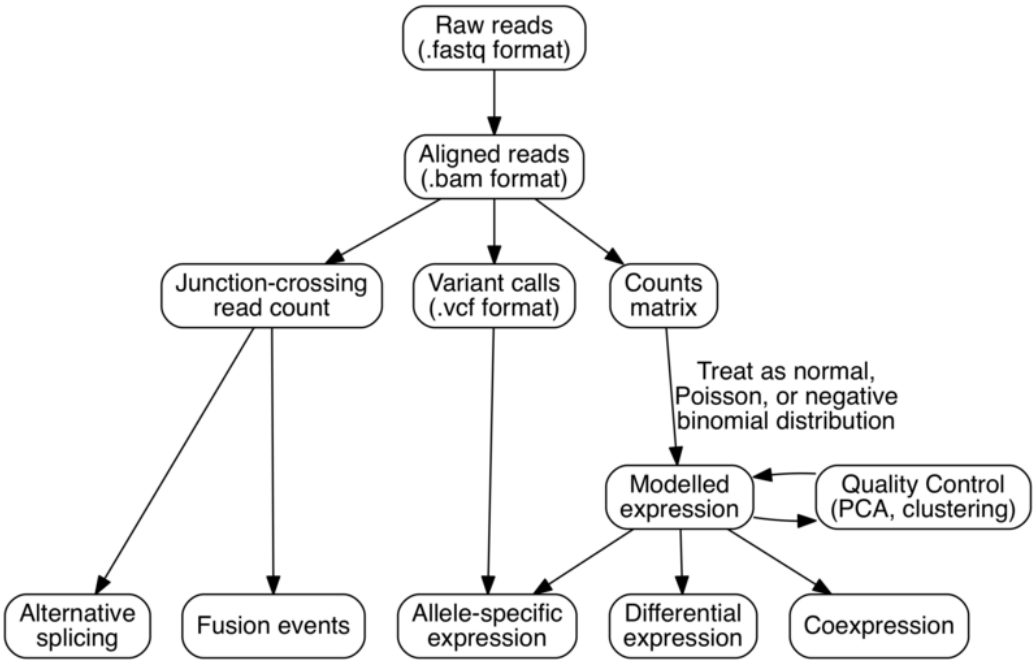


Credit: Rgocs (CC-BY)

RT-PCR and **RT-qPCR** can be used to measure the abundance of specific transcripts in a fairly low throughput way. Leveraging the concept of Reverse Transcription and coupling that to high-throughput sequencing technologies, transcripts can be sequenced and mapped to a genome to depict the quantity of transcripts as represented by the number of reads.

Given sufficient read coverage, novel splice isoforms can also be identified as different exon-exon junctions are identified.

The general workflow of RNA-Seq analysis follows:



Credit: Salubrious Toxin (CC-BY-SA 4.0)

ADVANCED VIDEO OF VARIANT CALLING FROM NGS TO DECIPHER A GENETIC SUSCEPTIBILITY



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SECTION OVERVIEW

2.1.9: GENETICS

2.1.9.1: INTRODUCTION

2.1.9.2: BITTER TASTE (ACTIVITY)

2.1.9.3: SEX-LINKED GENES

2.1.9.4: PROBABILITY AND CHI-SQUARE ANALYSIS

2.1.9.5: NON-MENDELIAN GENETICS

2.1.9.6: HARDY-WEINBERG AND POPULATION GENETICS

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2.1.9.1: INTRODUCTION

WRITING THE RULES OF HEREDITY:

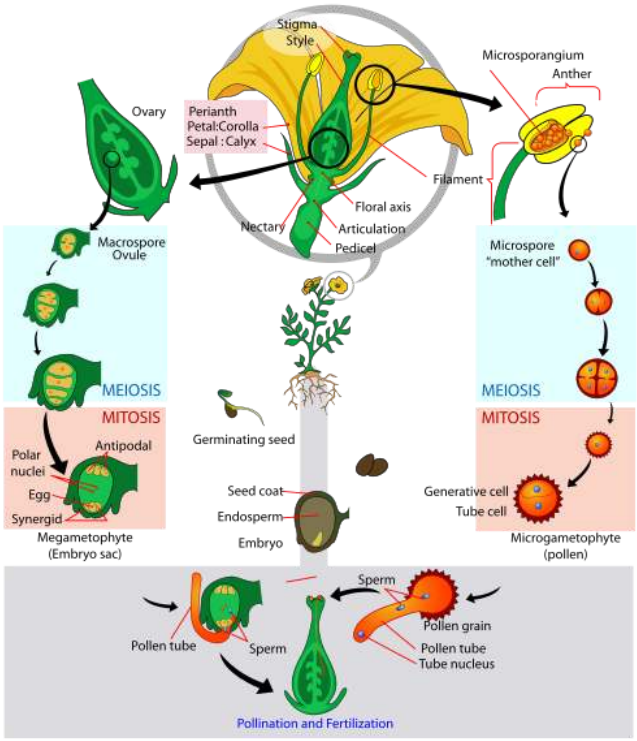


In the mid-1800s, an Augustinian friar named Gregor Mendel formalized quantitative observations on heredity in the pea plant. He undertook hybridization experiments that utilized purebred or ***true-breeding*** plants with specific qualities over many generations to observe the passage of these traits. Some of these physical traits included: seed shape, flower color, plant height, and pod shape.



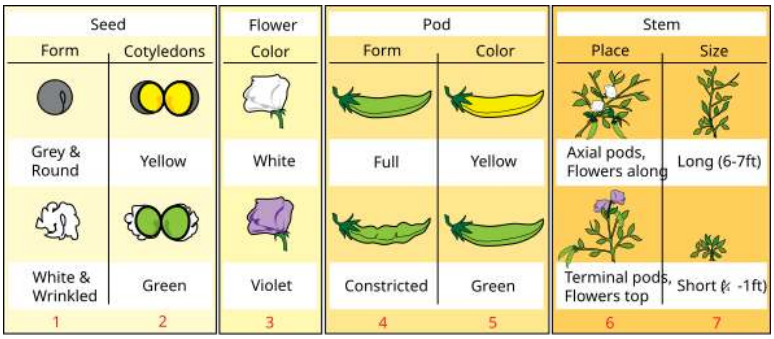
The pea plant (*Pisum sativum*) offered a great advantage of being able to control the fertilization process and having large quantities of offspring in a short period of time. In a simple experiment of tracking the passage of a single trait (**monohybrid cross**) like flower color through multiple generations, he was able to formulate rules of heredity. In this case, pea plants either produced white flowers or purple flowers for many generations (true-breeding purple flower or true breeding white flower). These true-breeding plants are referred to as the **Parental Generation** (**P**). By removing the male parts of the pea flower (anthers containing pollen), Mendel was able to control for self-pollination. The hybridization came from applying the pollen from one true-breeding plant to the female part (the pistil) of the opposite true-breeding plant. The subsequent offspring are referred to as the **First Filial Generation** (**F_1**). In the first generation, all the flowers are purple. Permitting self-pollination generates a **Second Filial Generation** (**F_2**). This generation sees the re-emergence of the white-flowered plants in an approximate ratio of 3 purple-flowered to 1 white-flowered plants.

Pea flowers



Male and female parts of flowers. Mendel removed the anthers containing pollen to prohibit self-pollination and selectively applied the pollen to stigmas in order to control the "hybridization".

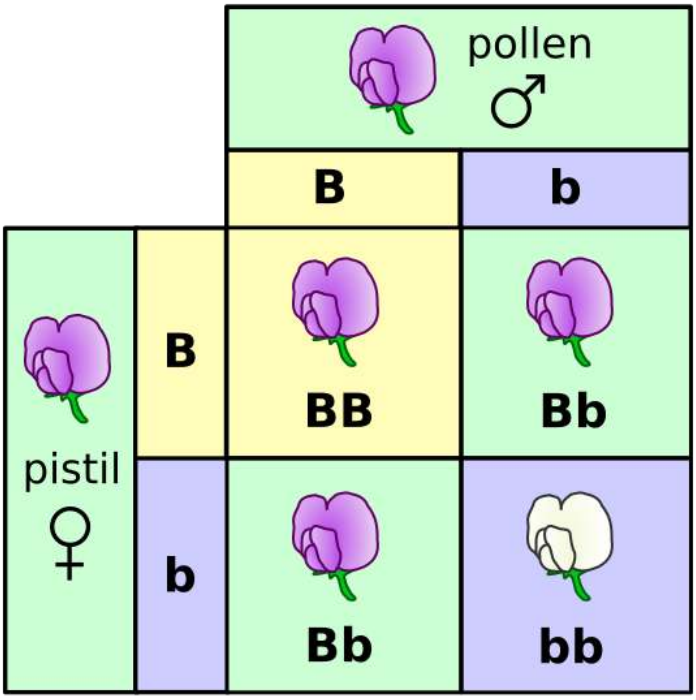
The loss of one variant on the trait in the F_1 plants with the re-emergence in the F_2prompted Mendel to propose that each individual contained 2 hereditary particles where each offspring would inherit 1 of these particles from each parent. Furthermore, the loss of one of the variants in the F_1 was explained by one variant masking the other, as he explained as being **dominant**. The re-emergence of the masked variation, or **recessive** trait, in the next generation was due to both particles being of the masked variety. We now refer to these hereditary particles as **genes** and the variants of the traits as **alleles**.



MENDEL'S RULES OF SEGREGATION AND DOMINANCE:

The observations and conclusions that Mendel made from the monohybrid cross identified that inheritance of a single trait could be described as passage of genes (particles) from parents to offspring. Each individual normally contained two particles and these particles would separate during the production of gametes. During sexual reproduction, each parent would contribute one of these particles to reconstitute offspring with 2 particles. In the modern language, we refer to the genetic make-up of the two "particles" (in this case, alleles) as the **genotype** and the physical manifestation of the traits as the **phenotype**. Therefore, Mendel's first rues of inheritance are as follows:

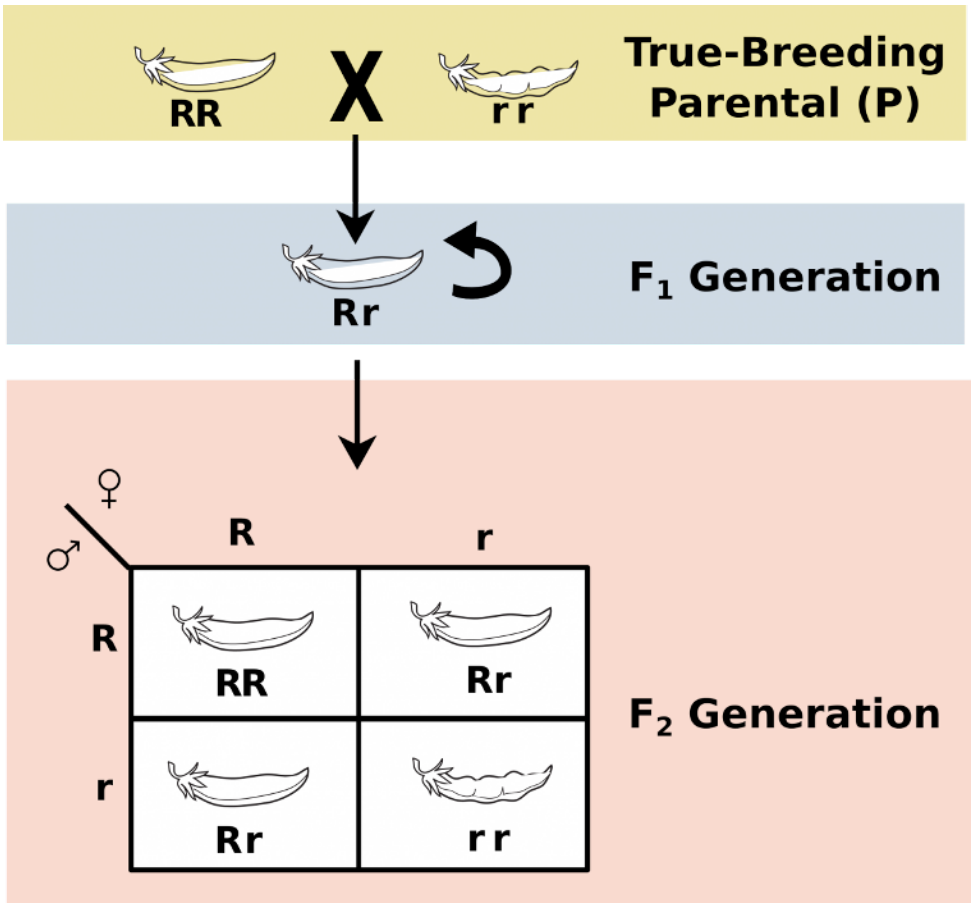
1. **Law of Segregation**
   - During gamete formation, the alleles for each gene segregate from each other so that each gamete carries only one allele for each gene
2. **Law of Dominance**
   - An organism with at least one dominant allele will have the phenotype of the dominant allele.
   - The recessive phenotype will only appear when the genotype contains 2 recessive alleles. This is referred to as **homozygous recessive**
   - The dominant phenotype will occur when the genotype contains either 2 dominant alleles (**homozygous dominant**) or one dominant and one recessive (**heterozygous**)



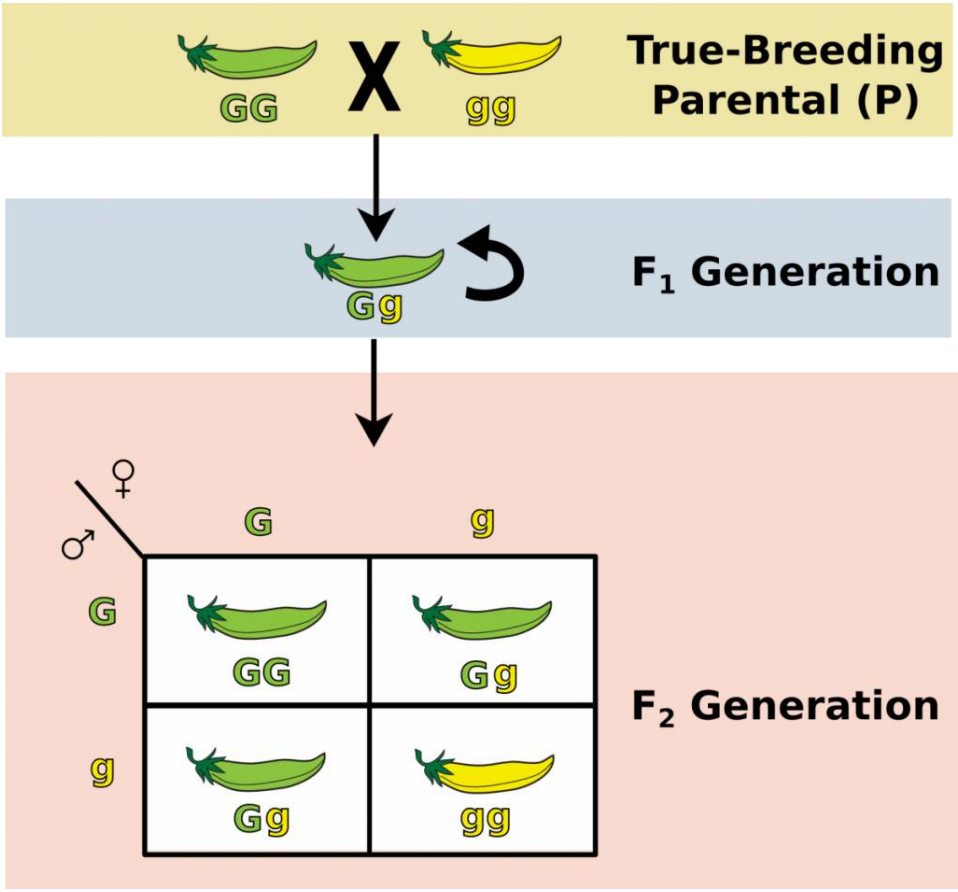
The F_1 cross (Punnett square) illustrating flower color inheritance in the F_2

Punnett Square is a tool devised to make predictions about the probability of traits observed in the offspring in the F_2 generation and illustrate the segregation during gamete formation.

THE SINGLE TRAIT CROSS (MONOHYBRID CROSS):



Monohybrid cross (one trait cross) observing the pod shape of peas.



Monohybrid cross (on trait cross) observing the pod color of peas.

CORN COLORATION IN AN F_2 POPULATION (ACTIVITY):



A corn cob contains hundreds of kernels. Each kernel is a seed that represents an individual organism. In the cob, we can easily see kernel color as a phenotype.

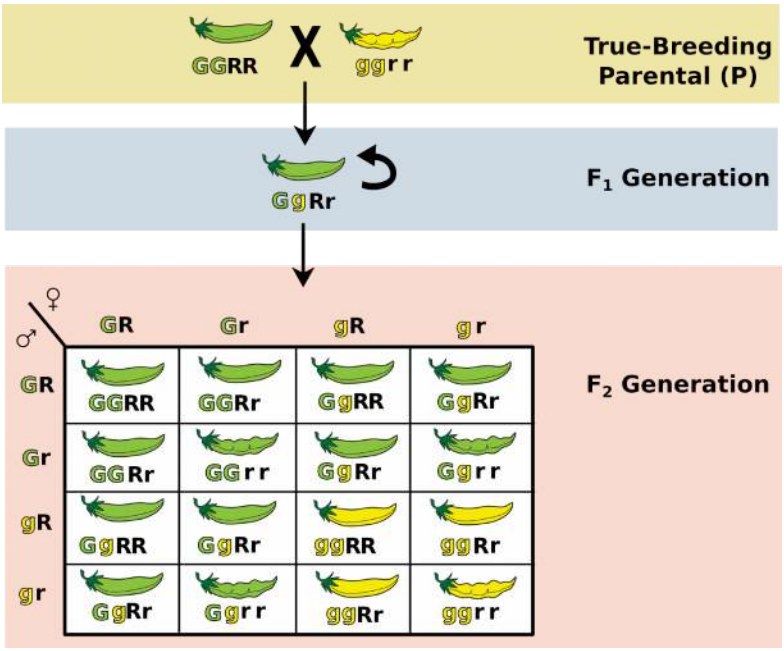
1. Retrieve an F_2 corn cob
2. Count a total of 100 kernels

1. Tally the number of Yellow Kernels within that 100 (in the dried state, anything yellow or honey-colored counts as yellow)
2. Tally the number of Purple Kernels within that 100 (in the dried state, purple colored kernels may appear brown)
3. Ignore any speckled kernels that may have yellow and purple within them

3. Compare numbers with the class as a whole
4. From the numbers:
    1. Is there a dominant color? _____
    2. Which is dominant, if there is? _____
    3. Create a Punnett square to illustrate the expected number of each color in a simple dominant:recessive paradigm.

THE TWO TRAIT CROSS (DIHYBRID CROSS):

Mendel continued his experimentation where he looked at two traits. These two trait crosses are called **dihybrid crosses**. While the monohybrid cross would yield 3:1 ratio of the phenotypes, the dihybrid crosses would yield 9:3:3:1 ratio of all the combinations of each phenotype.



MENDEL'S RULE OF INDEPENDENT ASSORTMENT:

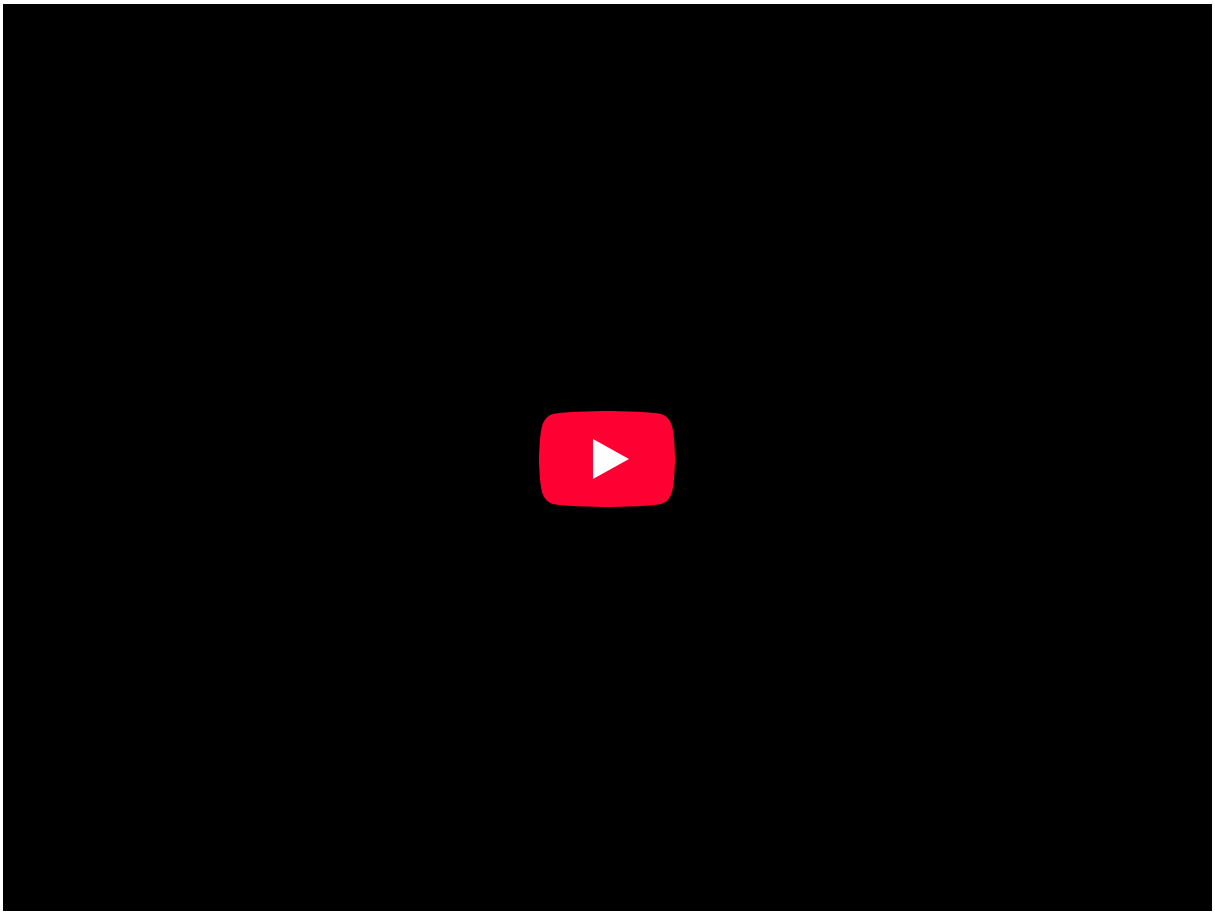
The dihybrid cross revealed another law of inheritance to Mendel. By observing the 9:3:3:1 ratio, Mendel concluded that traits were not tied to each other. That is to say, if a pea pod was yellow, it could still be either smooth or wrinkled in texture. This lack of linkage between genes yielding different characteristics was dubbed the **Law of Independent Assortment**. Genes for different traits can segregate independently during the formation of gametes.

KERNEL COLORATION AND TEXTURE IN AN F_2 POPULATION (ACTIVITY):

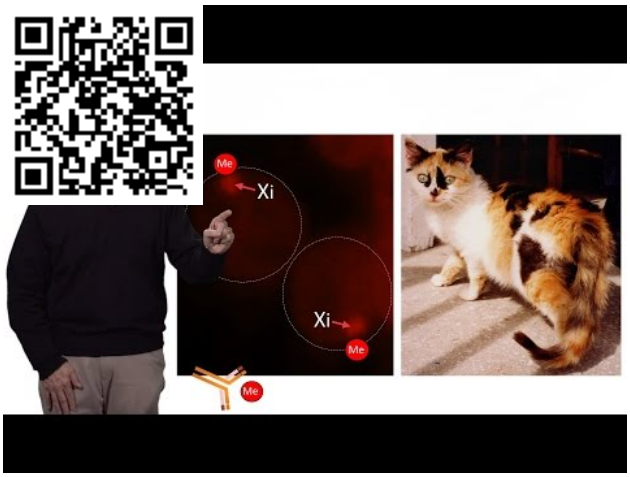


1. Retrieve a dihybrid F_2 corn cob
2. Count a total of 200 kernels
    1. Tally the number of Yellow Kernels that are rounded and smooth in texture
    2. Tally the number of Yellow Kernels that are shriveled and wrinkly in texture (honey-colored)
    3. Tally the number of Purple Kernels within that rounded and smooth in texture
    4. Tally the number of Purple Kernels within that are shriveled and wrinkly in texture
    5. Ignore any speckled kernels that may have yellow and purple within them
3. Compare numbers with the class as a whole
4. Each kernel constitutes an individual organism (a seed that can give rise to a whole new plant). From the numbers:
    1. Is there a dominant texture (smooth or shriveled)? _____
    2. Which is dominant, if there is? _____
    3. Is there a color that always pairs with a texture or do these characteristics *assort independently*? _____
    4. Create a Punnett square to illustrate the expected number of each color/texture combination in a simple dominant:recessive paradigm.

MODERN STUDY OF GENETICS:

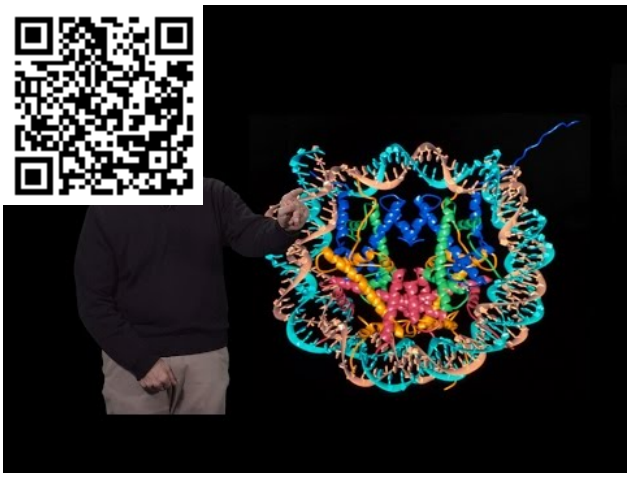


EPIGENETICS: BEYOND THE DNA



https://bio.libretexts.org/@go/page/114778

EPIGENETICS: ENVIRONMENT SHAPING EXPRESSION



ADDITIONAL RESOURCES:

- Mendelian Inheritance http://www.visionlearning.com/en/library/Biology/2/Mendel-and-Inheritance/129
- Independent Assortment http://www.visionlearning.com/en/library/Biology/2/Mendel-and-Independent-Assortment/145
- Genetics and Statistical Analysis http://www.nature.com/scitable/topicpage/genetics-and-statistical-analysis-34592
- Genetic Linkage http://www.nature.com/scitable/topicpage/discovery-and-types-of-genetic-linkage-500
- DNA as the Genetic Material http://www.nature.com/scitable/topicpage/discovery-of-dna-as-the-hereditary-material-340
- What is a gene? http://www.nature.com/scitable/topicpage/what-is-a-gene-colinearity-and-transcription-430

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2.1.9.2: BITTER TASTE (ACTIVITY)

GENETICS LEAVES A BAD TASTE IN MY MOUTH… OR NOT

Some of our personal preferences arise from the way we were brought up. Culture plays a role in our likes and dislikes. Likewise, our experiences play a role in how we respond to certain stimuli. Another major factor that plays a role in our preferences comes wired in our genome. The DNA in our cells is the instruction manual for who we are. We are programmed to seek out things of nutritive values in order to acquire raw materials like carbohydrates, proteins, and lipids. In our search for nutritive compounds, we have learned to avoid things that don't taste good. Bitter things have a tendency to be associated with toxic compounds in nature. When eating a food item for the first time, molecules hit our tongue and stimulate multiple sensations: sweet, sour, salty, savory, and bitter. Attributed to these multiple taste types are a diverse family of receptors that bind to the molecules that result in our perception of these sensations. Something bitter might make us learn to avoid this food item in the future.

One type of bitter receptor senses the presence of a chemical called phenylthiocarbamide (**PTC**). This chemical chemically resembles toxic compounds found in plants but is non-toxic. The ability to taste PTC comes from a gene called *TAS2R38*. This gene encodes a protein that on our tongues that communicates the bitterness of this chemical. There are two common alleles of this gene with at least five more uncommon variants. Within the two common forms, a **single nucleotide polymorphism** (**SNP**) is responsible for changing one amino acid in the receptor. It's this difference of one amino acid that results in the ability of the receptor to either respond or not respond to PTC. We inherit one copy of the gene from our father and one copy from our mother. How our parents' gametes formed and what alleles we received during the fertilization event determines how we respond to this chemical. Because we each have 2 copies of this gene, we can utilize simple Mendelian genetics to understand which allele is dominant or recessive.

1. Place a piece of "Control" paper on the tongue and indicate if there is a taste.
2. Place a piece of "PTC" paper on the tongue and indicate if there is a taste and the taste severity.
3. Fill out the table for the class to identify how many non-tasters, tasters, or super-tasters there are.
4. Indicate if you believe the trait is dominant or recessive (ability to taste or not taste).
5. Assign a descriptor allele for the dominant (a capital letter) or the recessive (a lowercase letter) and draw a Punnet square for the F_2 generation of 2 Heterozygous parents.
6. Compare the class tally of tasters and non-tasters in the class and discuss with your instructor if there is a clear dominance of this trait.

Table: PTC Tasting Tally

Phenotypes	Number	% Total
PTC Tasters (Dominant or Recessive)		
PTC Non-tasters (Dominant or Recessive)		
Total		

QUESTIONS:

1. How do you explain the presence of those who can't taste PTC, those who can taste it and those who really can't stand the taste of it?

2. This chemical is non-toxic and doesn't exist in nature. Do you think there is a **selective pressure** that confers an advantage to those who do taste it?

EXERCISE: CODING BITTERNESS:

Prior to this exercise, review the **Central Dogma**.

The full coding sequence of ***TAS2R38*** is 1,002 bases (334 amino acids) long. A segment of the gene is shown below where the SNP (in red) occurs. Variant 1 is the version of the gene that encodes for the ability to taste PTC. Variant 2 is the version of the gene that is unable to bind to PTC. This SNP mutation is called a **missense** mutation because it changes the amino acid. Some mutations cause the insertion of a premature stop codon. This **nonsense mutation** results in a truncated protein and can be disastrous to the function. We already know that the simple substitution of one nucleotide translates to a change in one amino acid and determines the ability to taste PTC. Imagine if a large group of amino acids from the protein was missing.

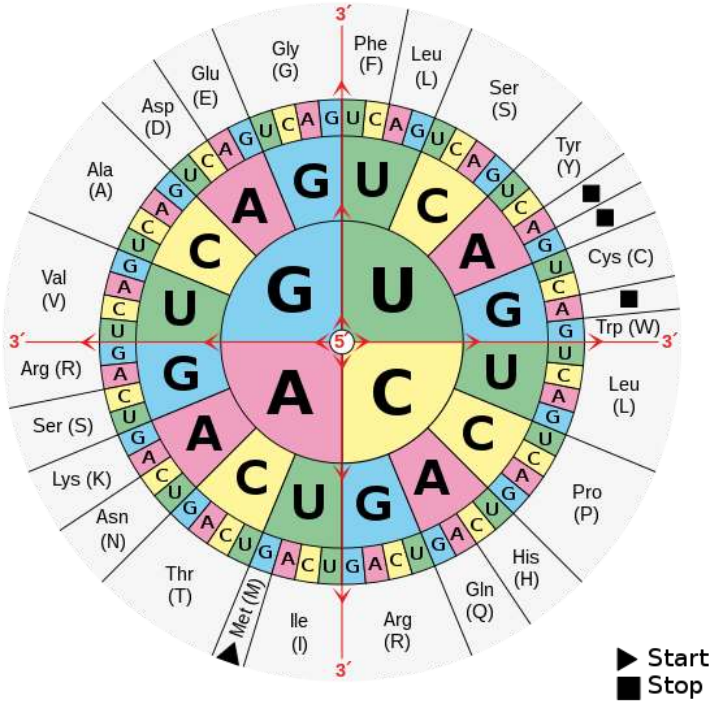
With the template strand ("Complement") information:

1. Write the sequence of the coding strand.
2. Write the sequence of the mRNA
3. Use the Genetic Code Chart to translate the amino acid sequence

Variant 1
Coding Strand : 5′-
Complement : 3′-TTC TCC GTC CGT GAC TCG-5′

mRNA : 5′-
Amino Acid :

Variant 2
Coding Strand : 5′-
Complement : 3′-TTC TCC GTC GGT GAC TCG-5′
mRNA : 5′-
Amino Acid :



PCR GENOTYPING THE TAS2R38 PTC RECEPTOR:

- 5’-CCTTCGTTTTCTTGGTGAATTTTTGGGATGTAGTGAAGAGGCGG-3’ (Forward Primer)
- 5′-AGGTTGGCTTGGTTTGCAATCATC-3′ (Reverse Primer)

1. PCR the DNA samples extracted from cheek cells using the PCR Beads.
2. Pour 2% agarose into casting apparatus in refrigerator.
   - 2 gels per class need to be made → 100ml of TBE with 2g agarose.
   - Add 5µl SYBR safe solution into the molten agarose before casting.
   - Place 2 sets of combs into the gel → at one end and in the middle.
3. Digest PCR product with *Hae* III.
   1. Remove 10µl of PCR product into a fresh tube.
   2. Add 1µl of *Hae*III enzyme into the tube.
   3. Incubate for 10 minutes at 37°C.
4. Load gel with DNA ladder, Digested and Undigested.
   - the undigested sample is from the original PCR.
5. Run gel at 120V for 20 minutes.
6. Visualize on the UV transilluminator.

SNP DETECTION:

The longer primer ends with the sequence “GG”. Both alleles at this locus will amplify equally well with this primer set, however, one allele will have the sequence “GGGC” and another “GGCC”. “GGCC” is the restriction site for the enzyme *Hae*III. The digestion of this

amplified DNA will be digestible for one allele and yield a DNA fragment the size of the large primer (44 bp) as well as the remainder of the amplicon. Because of this difference in digestion profile of the amplicon, we can identify the 2 alleles at this locus.

ANALYSIS QUESTIONS:

1. What is the size of the PCR product?
    - Perform an *in silico* PCR on the *Tas2R38* gene and identify the size of the amplicon.
2. The long primer is 44bp. If the amplicon of the allele digests, what are the sizes of fragments expected following *Hae*III?
3. Which allele is the one that can be identified through *Hae* III digestion?
    - Use the results of the PTC paper test.
4. Some lanes contain 3 bands instead of 1 or 2. Can you explain this?

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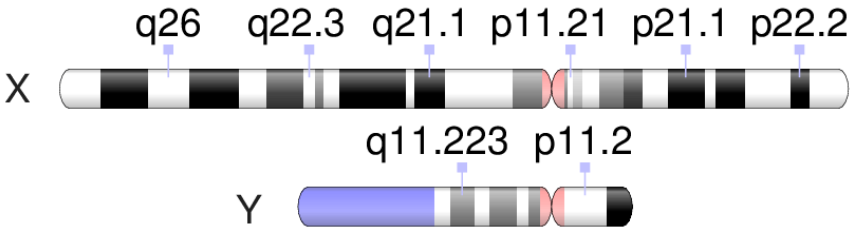
2.1.9.3: SEX-LINKED GENES

SEX CHROMOSOMES

For the most part, mammals have gender determined by the presence of the Y chromosome. This chromosome is gene-poor and a specific area called sex-determining region on Y (**SRY**) is responsible for the initiation of the male sex determination.

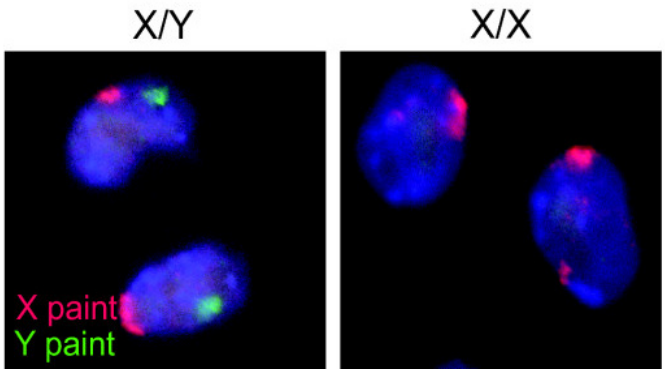


The X-chromosome is rich in genes while the Y-chromosome is a gene desert. The presence of an X-chromosome is absolutely necessary to produce a viable life form and the default gender of mammals is traditionally female.



Human X and Y chromosomes with G-Banding.

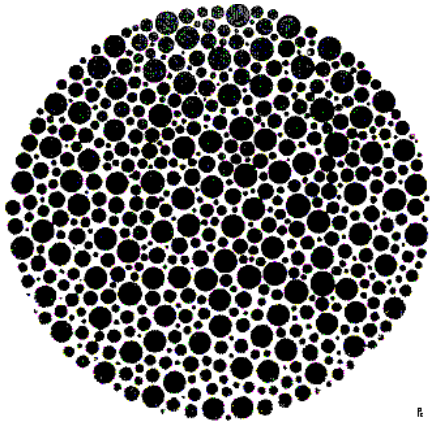
Chromosomal painting techniques can reveal the gender origin of mammalian cells. By using fluorescent marker sequences that can hybridize specifically to X or Y chromosomes through Fluorescence In Situ Hybridization (FISH), gender can be identified in cells.



The male cells have an X and a Y while the female cells have X and X combination. Credit: Janice Y Ahn, Jeannie T Lee [CC BY 2.0]

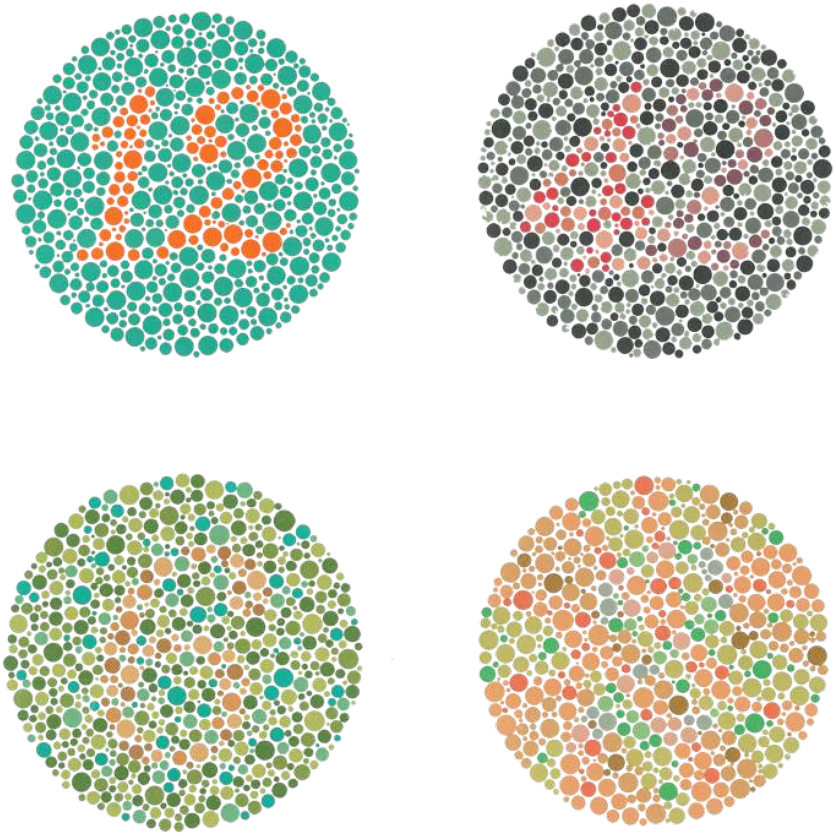
ISHIHARA TESTS (ACTIVITY)

The genes encoding photoreceptor proteins for the long wave-length (reds) and middle wavelengths (greens) reside on the X chromosome at Xq25. Since the Y-chromosome is not homologous, any mutation to either of these genes that render them non-functional results in an inability to perceive either of those colors. Men are more susceptible to the condition of red-green colorblindness since they are **hemizygous**. This means that there is no corresponding gene that could complement a deficient red or green photoreceptor gene.



Monochromatic representation of Ishihara test to a colorblind individual as it emerges to something visible to a color-sighted individual.

Dr. Shinobu Ishihara published his test for color perception in 1917 and this test is widely used to detect deficits in color perception. Below are examples of the Ishihara plates. Record the number that you perceive in each plate and discuss with the rest of the class.



Ishihara Test

1. As you go through the plates above, note the number that you see (if any).

2. The genes for the Red and Green receptors are on the X-chromosome, who are most affected by mutation? Create a Punnett Square to illustrate how this works.

3. Can women be color-blind for red/green?

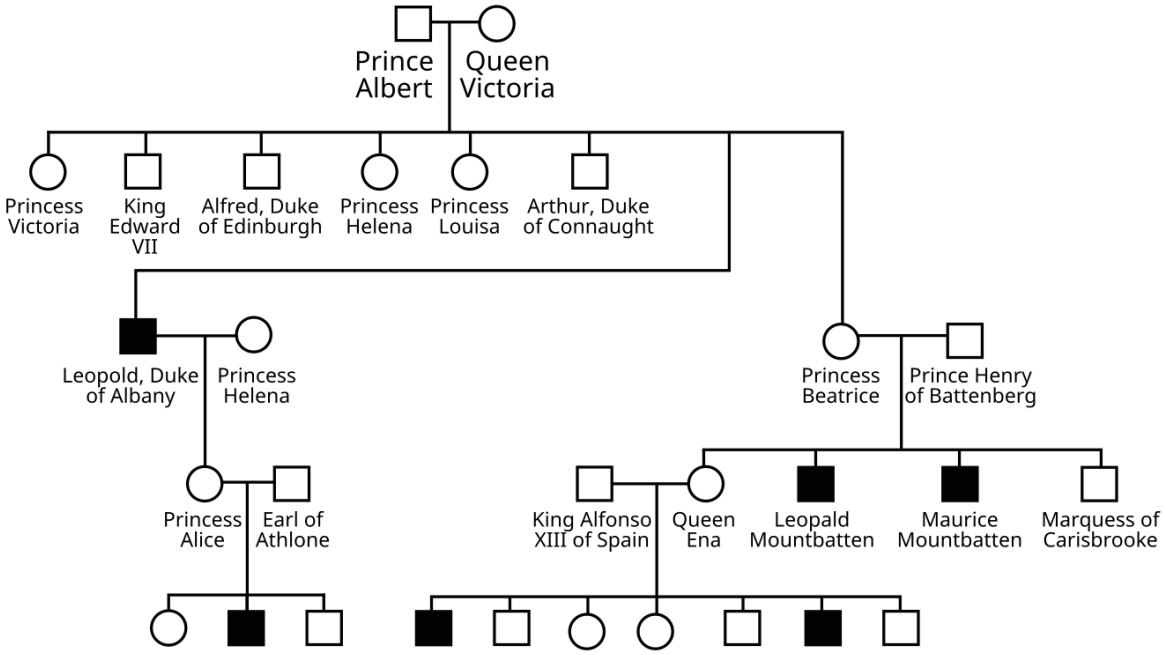
4. Humans have 3 color light receptors and have trichromatic vision. Some women are described as possibly having tetrachromatic vision (seeing 4 colors) and being able to discriminate colors invisible to the rest of us. Describe a mechanism for why this could happen. Why is there a possible gender bias?

SEQUENCE LINKS:

- Opsin 1 Long Wave sensitive gene (OPN1LW) – red photoreceptor (human)
- Opsin 1 Medium Wave sensitive gene (OPN1MW) – green photoreceptor (human)

THE CASE OF QUEEN VICTORIA

Hemophilia literally translates to *blood-loving*. This is a description of a series of disorders where an individual has an inability to clot blood after a cut. In modern times, clotting factors may be administered to an afflicted individual, but a prior treatment involved blood transfusions. A very famous family had a genetic predisposition to hemophilia and due to the proliferative nature of this family, we have some statistical power to verify predictions on the probabilities of passing the disease state. Below is a partial pedigree for Victoria, Queen of the United Kingdom of Great Britain and Ireland and Empress of India. The filled-in shapes represent individuals who suffered from hemophilia.



Credit: Jeremy Seto (CC-BY-SA)

QUESTIONS:

1. From the pedigree above, what can you say about this form of hemophilia with respect to dominance?

2. From this pedigree, can you comment on the probable chromosome where the deficiency occurs?

3. Assign genotypes for Prince Albert and Queen Victoria and perform a Punnett Square to illustrate if their offspring reflect your statements on dominance and chromosome location.

4. Albert and Victoria were 1st cousins. Do you believe this had anything to do with the propagation of this disease? What does your Punnett Square tell you?

5. Highlight the definitive carriers of the disease gene in the pedigree above.

ADDITIONAL RESOURCES:

- The full case study can be acquired at the National Center for Case Study Teaching in Science.
- Human Factor IX mRNA sequence

X-INACTIVATION

The mammalian X-chromosome contains significantly more genetic information than the Y-chromosome. This gene dosage is controlled for in females through a process called **X-inactivation** where one of the X-chromosomes is shut down and highly condensed into a **Barr body**. Inactivation of the X-chromosome occurs in a stochastic manner that results in females being cellular mosaics where a group of cells has

inactivated the paternal X-chromosome and other patches of cells have inactivated the maternal X-chromosome. The most striking example of **mosaicism** is the calico cat. A calico cat (tortoiseshell cat) is always a female. One of the genes that encodes coat color in cats resides on the X-chromosome and exist as either orange or black alleles. Due to the stochastic inactivation, the patterning of orange and black fur is a distinctive quality of calicos.



Credit: Howcheng [CC-BY-SA-3.0]

While the genetic information for the orange or black coat color exists in all cells, they are not equally expressed. This type of heritable trait in spite of the presence of the genetic material (DNA) is called **epigenetic** to imply that it is "above" (epi) genetics.

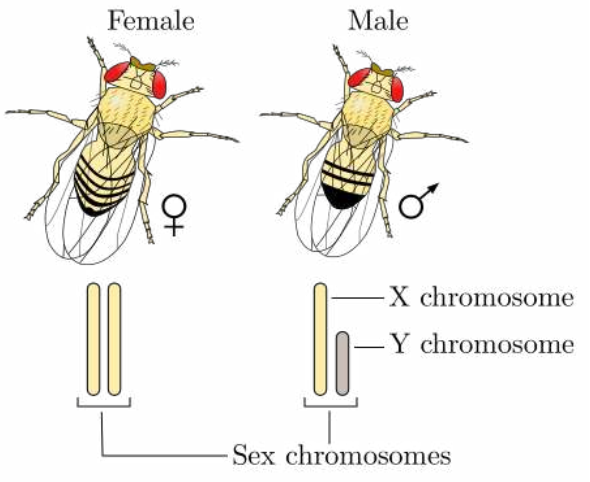
DROSOPHILA: THOMAS HUNT MORGAN

Around 1908, Thomas Hunt Morgan began to explore the genetics of what was to become a model organism, *Drosophila melanogaster* (Fruit fly). This small organism had a relatively short life cycle, great fecundity and was easily managed. From these flies that normally have red eye coloring, he and his students found white-eyed mutants. The lab noted that white-eyed flies were almost exclusively male. This gender imbalance led Morgan to believe that the trait was sex-linked.

File:EyeColors.jpg

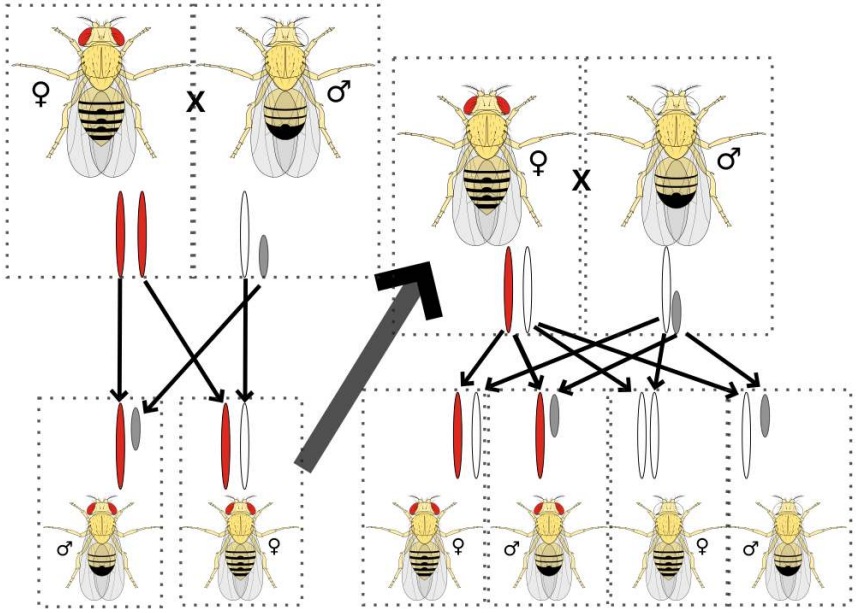
Eye colors (clockwise): brown, cinnabar, sepia, vermilion, white, wild. Also, the white-eyed fly has a yellow body, the sepia-eyed fly has a black body, and the brown-eyed fly has an ebony body. White-eyed flies have a gender imbalance and occur mostly in males.

In 1911, Morgan published a paper that described the inheritance patterns of 5 eye-colors in *Drosophila* (Morgan, 1911).



Drosophila follows a sex determination based on the ratio of X:A chromosomes and not by the presence of a Y as in mammals. A 1:1 ratio results in a female and a 1:2 ratio results in a male where the Y is ignored. The standard 2N = 8. Credit: GYassineMrabet [CC-BY-SA 4.0]

While DNA was not yet known as the source of genetic information, Morgan's studies revealed that the location of genes most likely resided on the chromosomes. By cataloging many mutations in the lab, he was able to construct a map of gene locations. His 1922 paper specifically stated that some traits were sex-linked and therefore residing on the sex chromosome. When performing crosses of white-eyed males to wild-type females, he continued to find white-eyed trait only in males. However, in the subsequent cross of females from that generation with white-eyed males, the presence of white-eyed males and females were revealed. This indicated that the white-eyed trait was recessive and resided on the X chromosome.



Analysis of the transmission of "White-Eyed" color in Drosophila. Credit: Jeremy Seto (CC-BY-SA)

Morgan received the Nobel Prize in Physiology or Medicine in 1933 for his inference of chromosomes being a physical mechanism for packaging genetic information in the cells.

- **Morgan** TH. THE ORIGIN OF FIVE MUTATIONS IN **EYE** COLOR IN **DROSOPHILA** AND THEIR MODES OF INHERITANCE. **Science**. 1911 Apr 7;33(849):534-7

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2.1.9.4: PROBABILITY AND CHI-SQUARE ANALYSIS

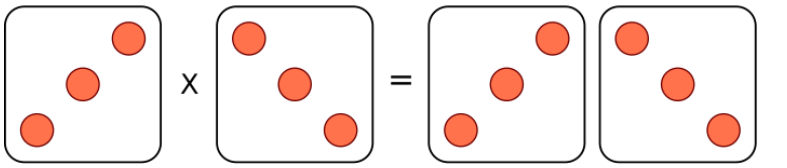
MENDEL'S OBSERVATIONS

Parental Cross	F_1 Phenotypes	F_2 Phenotypes	F_2 Ratios
Round x Wrinkled Seeds	All Round	5474 Round: 1850 Wrinkled	2.96:1
Yellow x Green Seeds	All Yellow	6022 Yellow: 2001 Green	3.01:1
Purple x White Flowers	All Purple	705 Purple: 224 White	3.15:1
Tall x Dwarf Plants	All Tall	787 Tall: 227 Dwarf	2.84:1

PROBABILITY: PAST PUNNETT SQUARES

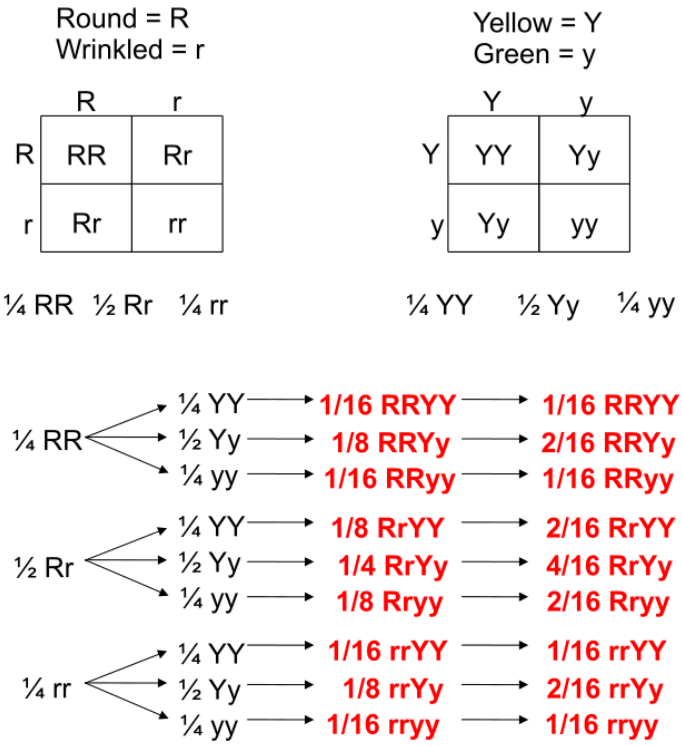
Punnett Squares are convenient for predicting the outcome of monohybrid or dihybrid crosses. The expectation of two heterozygous parents is 3:1 in a single trait cross or 9:3:3:1 in a two-trait cross. Performing a three or four trait cross becomes very messy. In these instances, it is better to follow the rules of probability. **Probability** is the chance that an event will occur expressed as a fraction or percentage. In the case of a monohybrid cross, 3:1 ratio means that there is a $\frac{3}{4}$ (0.75) chance of the dominant phenotype with a $\frac{1}{4}$ (0.25) chance of a recessive phenotype.

$$1/6 \text{ X } 1/6 = 1/36$$



A single die has a 1 in 6 chance of being a specific value. In this case, there is a $\frac{1}{6}$ probability of rolling a 3. It is understood that rolling a second die simultaneously is not influenced by the first and is therefore independent. This second die also has a $\frac{1}{6}$ chance of being a 3.

We can understand these rules of probability by applying them to the dihybrid cross and realizing we come to the same outcome as the 2 monohybrid Punnett Squares as with the single dihybrid Punnett Square.



This forked line method of calculating probability of offspring with various genotypes and phenotypes can be scaled and applied to more characteristics.

THE CHI-SQUARE TEST

The χ^2 statistic is used in genetics to illustrate if there are deviations from the expected outcomes of the alleles in a population. The general assumption of any statistical test is that there are no significant deviations between the measured results and the predicted ones. This lack of deviation is called the **null hypothesis** ($\mathbf{H_0}$). X^2 statistic uses a distribution table to compare results against at varying levels of probabilities or **critical values**. If the X^2 value is greater than the value at a specific probability, then the null hypothesis has been rejected and a significant deviation from predicted values was observed. Using Mendel's laws, we can count phenotypes after a cross to compare against those predicted by probabilities (or a Punnett Square).

Critical values for χ^2 test

p-val / DF	0.1	0.05	0.01	0.005
1	2.706	3.84	6.64	7.88
2	4.605	5.99	9.21	10.60
3	6.251	7.82	11.35	12.94
4	7.779	9.49	13.28	14.86
5	9.236	11.07	15.09	16.75
6	10.65	12.59	16.81	18.55
7	12.02	14.07	18.48	20.28
8	13.36	15.51	20.09	21.96
9	14.68	16.92	21.67	23.59
10	15.99	18.31	23.21	25.19

In order to use the table, one must determine the stringency of the test. The lower the p-value, the more stringent the statistics. **Degrees of Freedom** (**DF**) are also calculated to determine which value on the table to use. Degrees of Freedom is the number of classes or categories there are in the observations minus 1. DF=n-1

In the example of corn kernel color and texture, there are 4 classes: Purple & Smooth, Purple & Wrinkled, Yellow & Smooth, Yellow & Wrinkled. Therefore, DF = 4 – 1 = 3 and choosing $p < 0.05$ to be the threshold for significance (rejection of the null hypothesis), the X^2 must be greater than 7.82 in order to be significantly deviating from what is expected. With this dihybrid cross example, we expect a ratio of 9:3:3:1 in phenotypes where 1/16th of the population are recessive for both texture and color while $\frac{9}{16}$ of the population display both color and texture as the dominant. $\frac{3}{16}$ will be dominant for one phenotype while recessive for the other and the remaining $\frac{3}{16}$ will be the opposite combination.

With this in mind, we can predict or have expected outcomes using these ratios. Taking a total count of 200 events in a population, 9/16(200)=112.5 and so forth. Formally, the χ^2 value is generated by summing all combinations of:

$$\frac{(Observed - Expected)^2}{Expected} \tag{2.1.9.4.1}$$

CHI-SQUARE TEST: IS THIS COIN FAIR OR WEIGHTED? (ACTIVITY)

1. Everyone in the class should flip a coin 2x and record the result (assumes class is 24).
2. Fair coins are expected to land 50% heads and 50% tails.
   - 50% of 48 results should be 24.
   - 24 heads and 24 tails are already written in the “Expected” column.
3. As a class, compile the results in the “Observed” column (total of 48 coin flips).
4. In the last column, subtract the expected heads from the observed heads and square it, then divide by the number of expected heads.
5. In the last column, subtract the expected tails from the observed tails and square it, then divide by the number of expected tails.
6. Add the values together from the last column to generate the X^2 value.
7. Compare the value with the value at 0.05 with DF=1.
   - There are 2 classes or categories (head or tail), so DF = 2 – 1 = 1.
   - Were the coin flips fair (not significantly deviating from 50:50)?

Heads or Tails	Expected Number	Observed Number	$\frac{(\text{Observed-Expected})^2}{\text{Expected}}$
Heads	24		
Tails	24		
$\chi^2 = \sum \frac{(\text{Observed-Expected})^2}{\text{Expected}} \rightarrow$			

Let’s say that the coin tosses yielded 26 Heads and 22 Tails. Can we assume that the coin was unfair? If we toss a coin an odd number of times (eg. 51), then we would expect that the results would yield 25.5 (50%) Heads and 25.5 (50%) Tails. But this isn’t a possibility. This is when the X^2 test is important as it delineates whether 26:25 or 30:21 etc. are within the probability for a fair coin.

CHI-SQUARE TEST OF KERNEL COLORATION AND TEXTURE IN AN F_2 POPULATION (ACTIVITY)

1. From the counts, one can assume which phenotypes are dominant and recessive.
2. Fill in the “Observed” category with the appropriate counts.
3. Fill in the “Expected Ratio” with either 9/16, 3/16 or 1/16.
4. The total number of the counted event was 200, so multiply the “Expected Ratio” x 200 to generate the “Expected Number” fields.
5. Calculate the $\frac{(Observed-Expected)^2}{Expected}$ for each phenotype combination
6. Add all $\frac{(Observed-Expected)^2}{Expected}$ values together to generate the X^2 value and compare with the value on the table where DF=3.
7. Do we reject the Null Hypothesis or were the observed numbers as we expected as roughly 9:3:3:1?
   - What would it mean if the Null Hypothesis was rejected? Can you explain a case in which we have observed values that are significantly altered from what is expected?

Phenotype	Expected Ratio (9/16, 3/16,1/16)	Expected Number (Total # x Ratio)	Observed Number	$\frac{(\text{Observed-Expected})^2}{\text{Expected}}$
Purple & Smooth				
Purple & Wrinkly				
Yellow & Smooth				
Yellow & Wrinkly				
		$\chi^2 = \sum \frac{(\text{Observed-Expected})^2}{\text{Expected}} \rightarrow$		

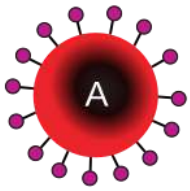
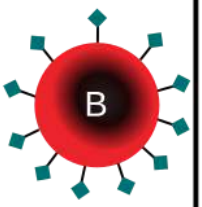
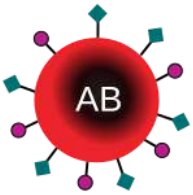
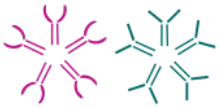
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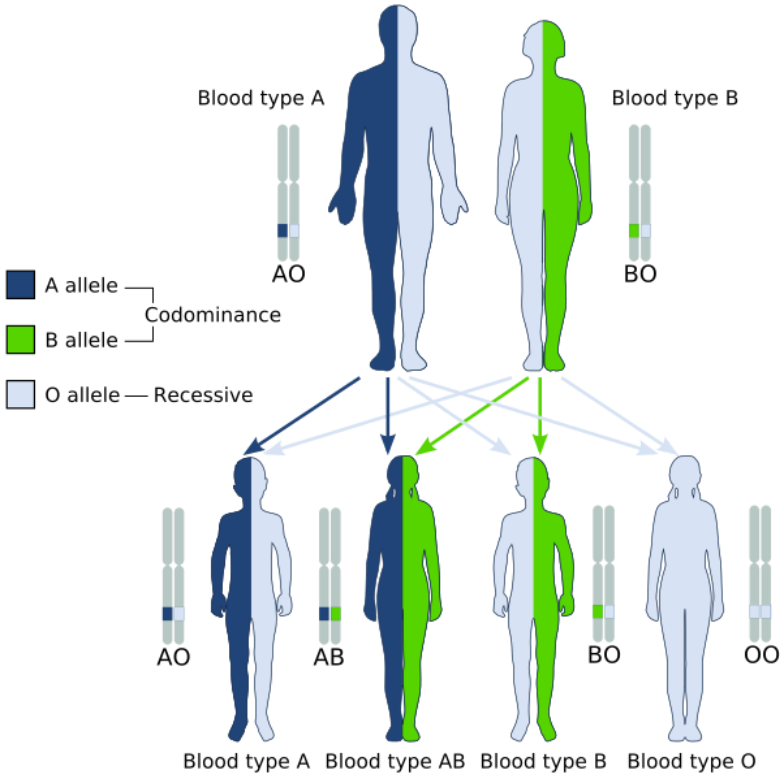
2.1.9.5: NON-MENDELIAN GENETICS

CO-DOMINANCE AND MULTIPLE ALLELES

Co-dominance is said to occur when there is an expression of two dominant alleles. The prototypical case for this is the human ABO blood grouping.

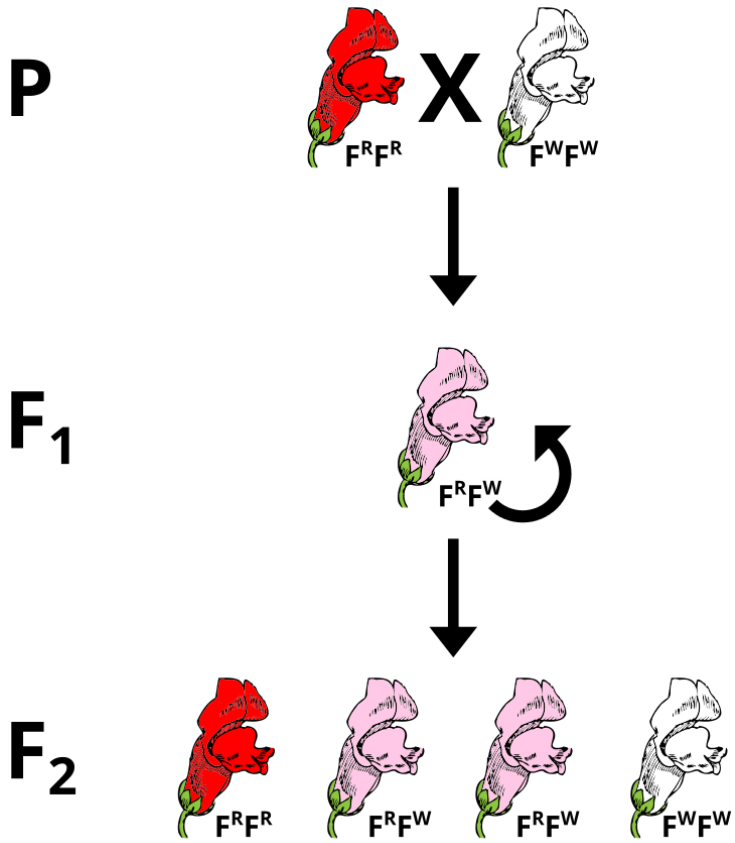
	Group A	Group B	Group AB	Group O
Red blood cell type				
Antibodies in plasma	 Anti-B	 Anti-A	None	 Anti-A and Anti-B
Antigens in red blood cell	A antigen	B antigen	 A and B antigens	None

Three alleles exist in the ABO system: A, B and O. This results in four blood types: A, B, O and the blended AB.



INCOMPLETE DOMINANCE

During Mendel's time, people believed in a concept of blending inheritance whereby offspring demonstrated intermediate phenotypes between those of the parental generation. This was refuted by Mendel's pea experiments that illustrated a Law of Dominance. Despite this, non-Mendelian inheritance can be observed in sex-linkage and co-dominance where the expected ratios of phenotypes are not observed clearly. **Incomplete dominance** superficially resembles the idea of blending inheritance, but can still be explained using Mendel's laws with modification. In this case, alleles do not exert full dominance and the offspring resemble a mixture of the two phenotypes.



Incomplete dominance in snapdragon flowers superficially appears like blending inheritance. Credit: Jeremy Seto (CC-BY-NC-SA)

The most obvious case of a two allele system that exhibits incomplete dominance is in the snapdragon flower. The alleles that give rise to flower coloration (Red or White) both express and the heterozygous genotype yields pink flowers. There are different ways to denote this. In this case, the superscripts of R or W refer to the red or white alleles, respectively. Since no clear dominance is in effect, using a shared letter to denote the common trait with the superscripts (or subscripts) permit for a clearer denotation of the ultimate genotype to phenotype translations.

PROBLEM: INCOMPLETE DOMINANCE

If pink flowers arose from blending inheritance, then subsequent crosses of pink flowers with either parental strain would continue to dilute the phenotype. Using a Punnet Square, perform a test cross between a heterozygous plant and a parental to predict the phenotypes of the offspring.

EPISTASIS AND MODIFIER GENES:



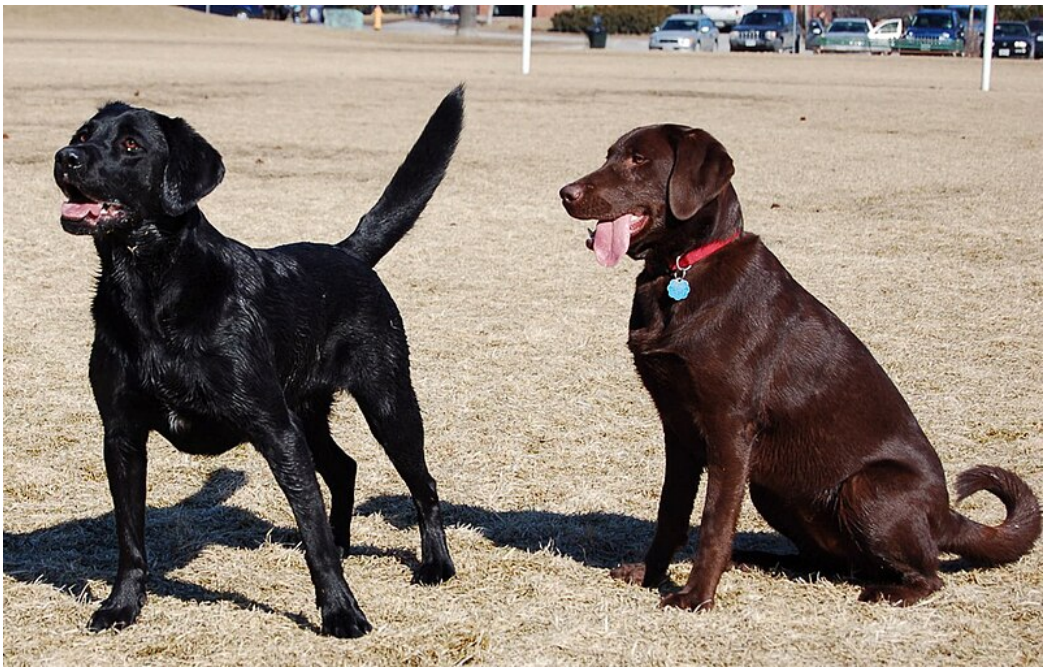
The interplay of multiple enzymes in a biochemical pathway will alter the phenotype. Some genes will modify the actions of another gene.
Credit: Jeremy Seto (CC0)

Genes do not exist in isolation and the gene products often interact in some way. **Epistasis** refers to the event where a gene at one locus is dependent on the expression of a gene at another genomic locus. Stated another way, one genetic locus acts as a modifier to another. This can be visualized easily in the case of labrador retriever coloration where three primary coat coloration schemes exist: black lab, chocolate lab, and yellow lab.



Chocolate lab (top), Black lab (middle), Yellow lab (bottom) coat colorations arise from the interaction of 2 gene loci, each with 2 alleles.
Credit: Erikeltic [CC-BY-SA 3.0]

Two genes are involved in the coloration of labradors. The first is a gene for a protein called TYRP1, which is localized to the melanosomes (pigment storing organelles). Three mutant alleles of this gene have been identified that reduce the function of the protein and yield lighter coloration. These three alleles can be noted as "***b***" while the functioning allele is called "***B***". A heterozygous (Bb) or a homozygous dominant individual will be black coated while a homozygous recessive (bb) individual will be brown.



Black lab (BB or Bb) and Chocolate lab (bb). Credit: dmealiffe[CC BY-SA 2.0]

The second gene is tied to the gene for Melanocortin 1 Receptor (MC1R) and influences if the eumelanin pigment is expressed in the fur. This gene has the alleles denoted "***E***" or "***e***". A yellow labrador will have a genotype of either *Bbee* or *bbee*.



Black lab (EE or Ee) and Yellow lab (ee) [CC0]

The interplay between these genes can be described by the following diagram:



Black lab (B_E_, Chocolate lab (bbE_), Yellow lab with dark skin where exposed (B _ee) and Yellow lab with light skin where exposed. Credit: Jeremy Seto (CC-BY-SA 3.0)

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2.1.9.6: HARDY-WEINBERG AND POPULATION GENETICS

HARDY-WEINBERG PRINCIPLE

The **Hardy-Weinberg principle** is a mathematical model used to describe the equilibrium of two alleles in a population in the absence of evolutionary forces. This model was derived independently by G.H. Hardy and Wilhelm Weinberg. It states that the allele and genotype frequencies across a population will remain constant across generations in the absence of evolutionary forces. This equilibrium makes several assumptions in order to be true:

1. An infinitely large population size
2. The organism involved is diploid.
3. The organism only reproduces sexually.
4. There are no overlapping generations.
5. Mating is random.
6. Allele frequencies equal in both genders
7. Absence of migration, mutation, or selection

As we can see, many items in the list above can not be controlled for but it allows for us to make a comparison in situations where expected evolutionary forces come into play (selection etc.).

HARDY-WEINBERG EQUILIBRIUM

The alleles in the equation are defined as the following:

- Genotype frequency is calculated by the following:

$$\text{genotype frequency} = \frac{\#\text{ individuals of given genotype}}{\text{total }\#\text{ individuals in population}} \tag{2.1.9.6.1}$$

- Allele frequency is calculated by the following:

$$\text{allele frequency} = \frac{\#\text{ of copies of an allele in a population}}{\text{total }\#\text{ of alleles in population}} \tag{2.1.9.6.2}$$

- In a two allele system with dominant/recessive, we designate the frequency of one as $\boldsymbol{p}$ and the other as $\boldsymbol{q}$ and standardize to:
  - $p =$ Dominant allele frequency
  - $q =$ recessive allele frequency
- Therefore the *total frequency of* ***all alleles*** in this system equal 100% (or 1)

$$p + q = 1 \tag{2.1.9.6.3}$$

- Likewise, the *total frequency of* ***all genotypes*** is expressed by the following quadratic where it also equals 1:

$$p^2 + 2pq + q^2 = 1 \tag{2.1.9.6.4}$$

  This equation is the Hardy-Weinberg theorem that states that there are no evolutionary forces at play that are altering the gene frequencies.

CALCULATING HARDY-WEINBERG EQUILIBRIUM (ACTIVITY)

This exercise refers to the PTC tasting exercise. One can test for selection for one allele within the population using this example. Though the class size is small, pooling results from multiple sections can enhance the exercise. Remember to surmise the dominant/recessive traits from the class counts.

Phenotype	Genotype	Genotype Frequency
Super Taster		
Taster		
Non-taster		

1. What is the recessive phenotype and how can we represent the genotype?

2. What is the dominant phenotype and how can we represent the genotypes?

3. What is the frequency of recessive genotype? (q^2)

4. What is the frequency of the recessive allele? (q)

5. What is the frequency of the dominant allele? (p=1-q)

6. Use Hardy-Weinberg to calculate the frequency of heterozygotes in the class. (2pq)

7. Use Hardy-Weinberg to calculate the frequency of homozygotes in the class. (p^2)

8. Using an aggregate of multiple sections, compare the local allelic and genotypic frequencies with what the Hardy-Weinberg would predict.

9. With this small number in mind, we can see that there are problems with the assumptions required for this principle. The instructor will perform the following simulation in class to illustrate the effects on multiple populations with the effects of selection and /or population limitations. A coefficient of fitness can be applied to illustrate a selective pressure against an allele.

- **Population Genetics Simulation of Alleles**

10. In the case of selective pressure, a **fitness coefficient** (***w***) can be introduced. A research article **http://www.jci.org/articles/view/64240** has shown that the Tas2R38 receptor aids in the immune response against *Pseudomonas*. Imagine a situation where there is an epidemic of antibiotic-resistant *Pseudomonas*. This would
show that the dominant allele will have a selective advantage.

- Modify the fitness coefficient in the Population Genetics Simulator and describe the effects this would have over many successive generations.

A CASE STUDY OF EVOLUTION: POPULATION GENETICS AT WORK



ADDITIONAL RESOURCES/EXPERIMENTS:

- Population Genetics Simulation of Alleles (Schaffer)
- Population Genetics Simulation of Alleles (Sheehy)
- *Lee RJ*, *Xiong G*, *Kofonow JM*, *Chen B*, *Lysenko A*, *Jiang P*, *Abraham V*, *Doghramji L*, *Adappa ND*, *Palmer JN*, *Kennedy DW*, *Beauchamp GK*, *Doulias PT*,*Ischiropoulos H*, *Kreindler JL*, *Reed DR*, *Cohen NA*. **T2R38 taste receptor polymorphisms underlie susceptibility to upper respiratory infection.** *J Clin Invest.* 2012;122(11):4145-4159. doi:10.1172/JCI64240.

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SECTION OVERVIEW

2.1.10: DNA AS THE GENETIC MATERIAL

2.1.10.1: INTRODUCTION

2.1.10.2: BACTERIAL TRANSFORMATION (ACTIVITY)

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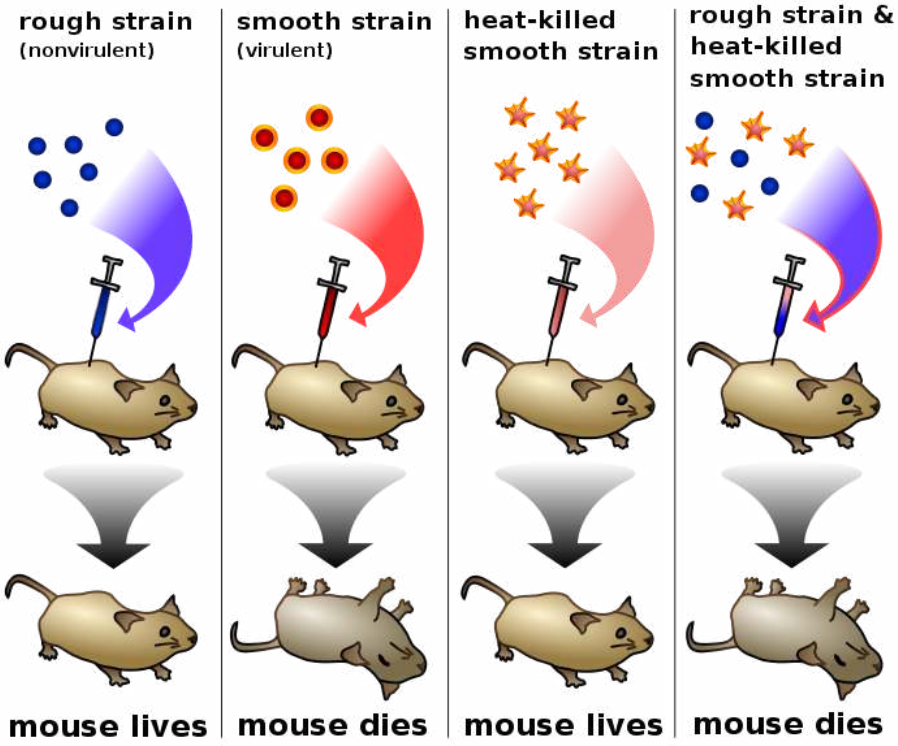
2.1.10.1: INTRODUCTION

HISTORY OF GENETIC TRANSFORMATION

Any uptake of genetic information from the external environment into cells that results in the expression of new traits is called **genetic transformation**. This process can occur naturally. Some bacteria are referred to as being “competent” to indicate that they are capable of taking DNA into the cell from the environment. This is referred to as **natural competence**. Bacteria are also capable of receiving DNA through the process of conjugation where plasmids from one bacteria are sent to another through the **conjugation pilus**. Other methods of introduction of foreign DNA include direct injection into the cytosol or through the use of viruses in a process called **transduction**. In eukaryotic cells, we refer to the introduction of DNA as **transfection**.

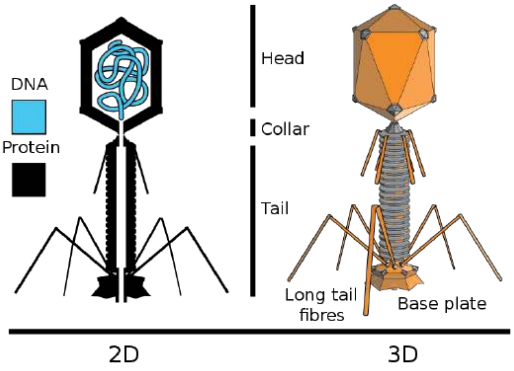
FREDERICK GRIFFITH AND THE TRANSFORMING AGENT

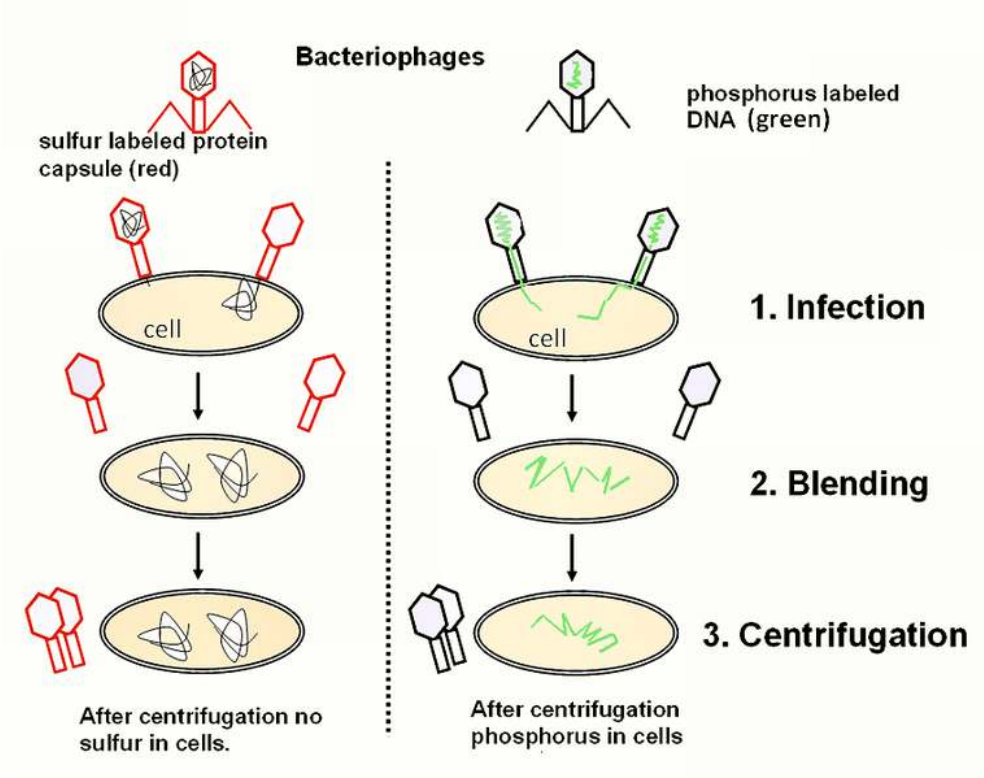
At the beginning of modern biology, the source of genetic material was not known to be DNA. In fact, many scientists thought DNA was too simple to perform this job. Scientists believed that proteins, with their 20 varied amino acids, were the carriers of genetic information. In an attempt to develop a vaccine for bacterial-induced pneumonia, Frederick Griffith was the first to describe the process of genetic transformation by accident in 1928. Griffith took a virulent strain of bacteria (smooth in appearance) that caused pneumonia and injected them into mice. This would result in the death of the mice. He also observed that injection of a rough bacteria did not cause any disease. After heat-killing the smooth bacteria, he discovered that living bacteria of the virulent strain was required for the disease to progress. Finally, he observed that injecting the heat-killed virulent bacteria with living bacteria of the non-virulent strain resulted in pneumonia and death in the mice. From this experiment, a **transforming agent** with the capacity to pass on a trait was found to be within the contents of those dead cells. But no one knew this agent to be DNA at that point.



HERSHEY AND CHASE

Hershey and Chase studied bacteriophage (phage=eater). Phages are bacterial viruses that infect bacteria and cause lysis of the cells. They have a very simple structure of a proteinaceous head/collar/tail and a DNA core. It was known that bacteria infected with phage were resistant to additional infection. In 1952 Hershey and Chase grew bacteriophage in conditions that would specifically label either the DNA or the protein with radioactivity. They subsequently took phage with radiolabeled DNA and infected bacteria. In parallel, they took phage with radiolabeled protein and infected another set of bacteria. After just enough time for infection, the bacterial cultures were placed into a blender to separate the bacteriophage from the bacteria.





Solutions were centrifuged to isolate bacteria from the phage. Bacteria were radioactive only when the phage grown in conditions to radiolabel DNA infected the bacteria to indicate that DNA might be the transforming agent.

QUESTIONS TO THINK ABOUT

1. What isotope would be used to label protein?
2. What isotope would be used to label just DNA?

AVERY, MCCARTY, AND MACCLEOD

In 1944, Oswald Avery, Colin MacCleod and Maclyn McCarty repeated Griffith's experiment. Instead of using heat-killed bacteria, these scientists isolated protein, carbohydrates, lipids and nucleic acids from the virulent strain and co-injected with the non-virulent bacteria. Carbohydrate extracts were ineffective at transforming bacteria. Protein extracts were incapable of causing transformation. Lipid injections were unable to result in virulence. Only nucleic acid samples treated with RNase were capable of transforming bacteria. When co-injecting with DNase, bacteria were not transformed. Along with Hershey-Chase, this definitively illustrated that DNA was the transforming agent capable of transferring genetic information.

MORE RESOURCES

- http://www.dnalc.org/view/16375-Animation-17-A-gene-is-made-of-DNA-.html

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2.1.10.2: BACTERIAL TRANSFORMATION (ACTIVITY)

BACTERIAL TRANSFORMATION

Escherichia coli are commensal gram-negative bacteria found in the guts of humans. They have the capacity to double every twenty minutes and make a favorable carrier of recombinant DNA. Plasmid DNA can be introduced into *E. coli* easily after making them competent. One method to achieve this is through chemical competence with heat shock. In this process, the bacteria are incubated in $CaCl_2$ solution on ice. The cold serves to slow down the molecular motion of the plasma membrane while the Ca^{2+} ions remove the charge-charge repulsion between the phospholipids and the negatively charged DNA seeking to gain entry into the cell. Cells are placed for a short period of time at 42°C to induce **heat shock**. This heat shock results in the cell taking up the DNA. This method is very low efficiency so many bacteria do not take in any DNA. Cells are allowed to recover from heat shock at 37°C in rich nutrient broth to allow for the production of the antibiotic resistance proteins encoded on the vector as a selection marker. Transformed cells are then spread across an agar plate containing the antibiotic which will then kill all non-transformed cells. Only the bacteria containing the vector with the antibiotic resistance gene will survive and replicate to form small colonies on the surface of the agar.

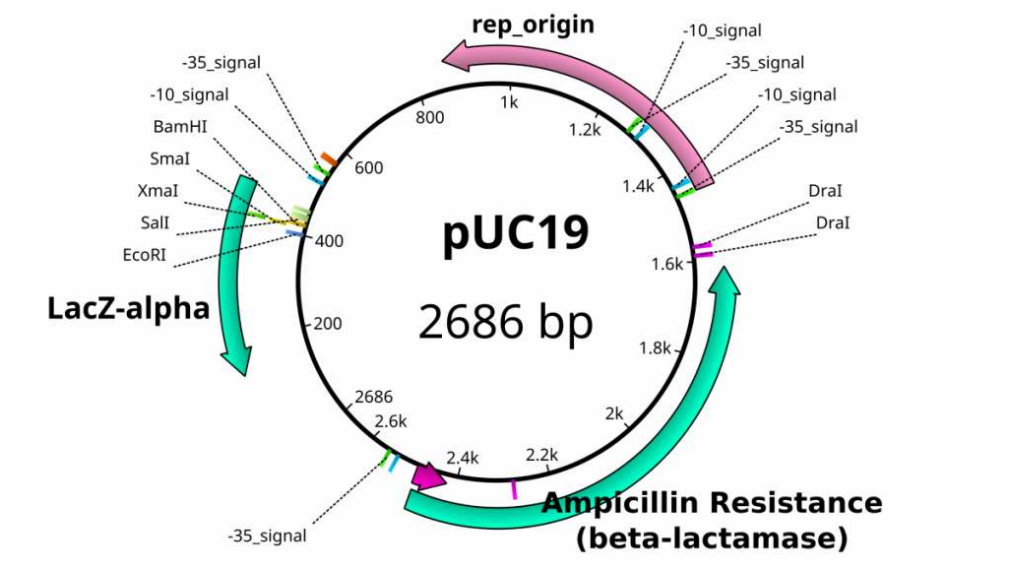
- http://www.dnalc.org/view/15918-Transformation.html
- http://www.dnalc.org/view/15916-DNA-...formation.html

EXERCISE: TRANSFORMATION OF BACTERIA WITH RE IDENTIFIED PLASMIDS

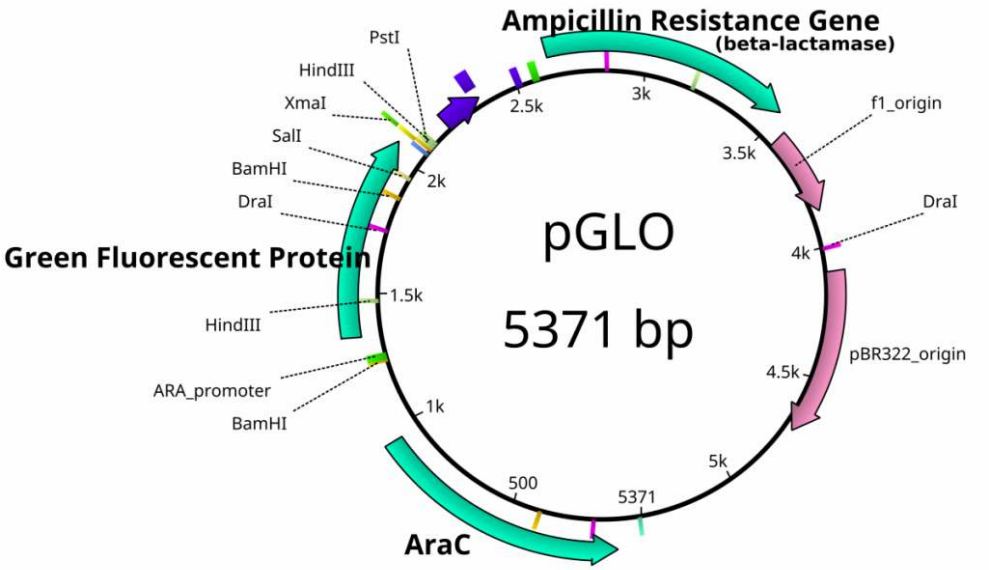
1. Each group retrieves the 2 mini-prepped plasmids from the previous week in the freezer and allow to thaw on ice.
2. Bring 2 agar plates to room temperature
    - 2 plates will contain antibiotic, X-Gal, and arabinose
3. For each plasmid, obtain 250µl of transformation buffer (50mM $CaCl_2$) in microfuge tubes and place on ice for 10 minutes.
4. Take an inoculating loop and remove a single colony of bacteria from a freshly streaked plate grown overnight.
5. Swirl bacteria in each tube containing transforming solution to distribute bacteria throughout the solution.
6. Pipette 5 µl of the plasmid into the tube and incubate on ice for 10 minutes.
7. During this incubation, flip the warmed plates and label them with your group names.
8. Place transformation tubes into 42°C heat block for 1 minute to heat shock the cells.
9. Add 500µl fresh SOC media (or LB) and incubate at 37°C for 15 minutes.
10. Pipette 150µl of transformation solution onto each plate and spread across the plate.
11. Turn plates agar side up and place them into 37°C incubator overnight. (your instructor will retrieve them and place them into the refrigerator).

HYPOTHESIZE: WHAT WILL I EXPECT OF MY TRANSFORMED CELLS?

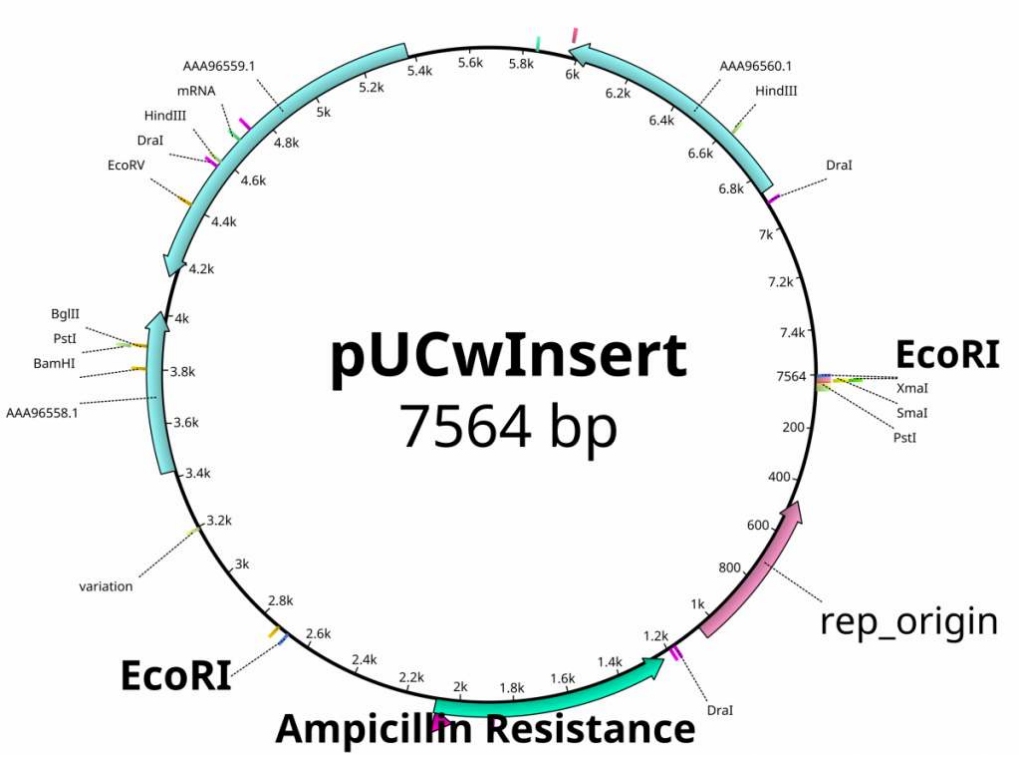
From the previous lab, we can identify our plasmids. The plasmids are either pGlo, pUC18/19 or pUC18/19 with a 6kb insert disrupting the LacZ gene. pGlo contains a gene that encodes the protein GFP that will fluoresce green under UV light and is 5.4kb. pUC is typically 2.7kb in size. LacZ is a gene encoding the protein β-Galactosidase, the enzyme that hydrolyzes lactose into the monosaccharides galactose and glucose. X-Gal is a chemical resembling lactose. However, upon hydrolysis, the molecule deposits a blue coloring into the cell.



pUC19 genbank file

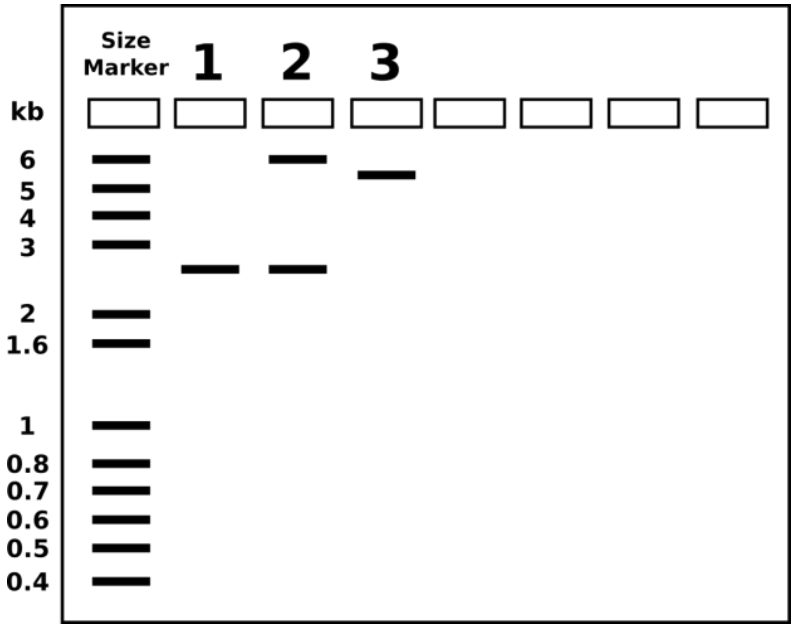


pGlo genbank file



pUC with insert file

1. If the previously mentioned plasmids were digested by *EcoRI*, label the lanes below with the appropriate plasmid (pGlo, pUC, and pUC-inserted)
2. Predict if your transformants will be green under UV, white in all conditions or blue.
3. For additional help on this problem, utilize the *In silico* digestion activity.



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SECTION OVERVIEW

2.1.11: GENE EXPRESSION

2.1.11.1: INTRODUCTION

2.1.11.2: PROKARYOTIC TRANSCRIPTION

2.1.11.3: RNA MINIPREP (ACTIVITY)

2.1.11.4: QUANTITATIVE NUCLEIC ACID MEASUREMENT

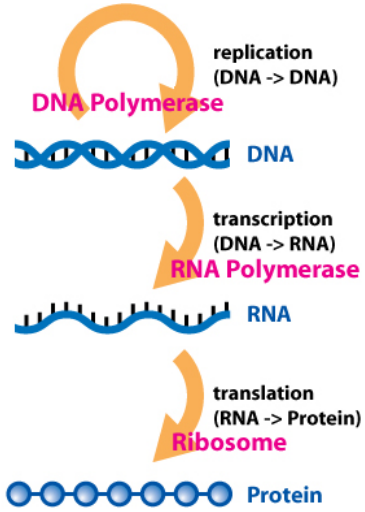
2.1.11.5: EUKARYOTIC TRANSCRIPTION

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2.1.11.1: INTRODUCTION

THE CENTRAL DOGMA

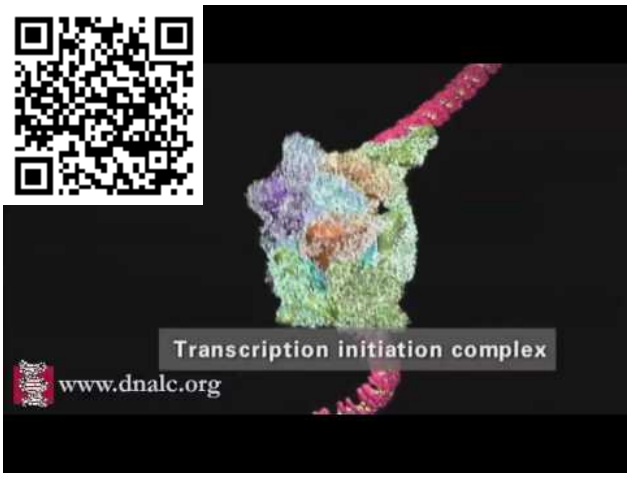
DNA was described as a molecule consisting of 2 anti-parallel strands in a double helix by Francis Crick and James Watson. The elegant model illustrated the intrinsic redundancy that made DNA a suitable data storage vessel for genetic information. Francis Crick later posited a notion of how this information went from storage to an actual program that runs cells. Crick first posited it as a "sequence hypothesis". This idea of information flow is called the **Central Dogma of Molecular Biology**. DNA stores the information that is expressed as an intermediate message of RNA. This RNA is then translated into amino acids to yield proteins.



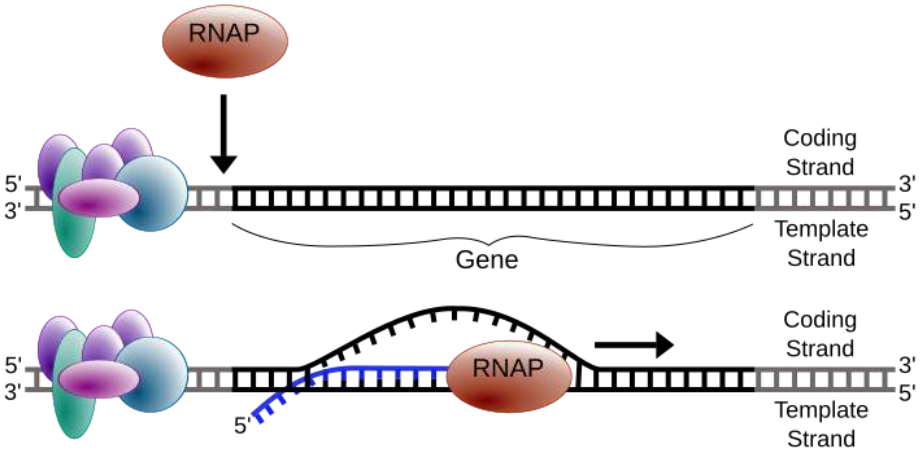
*The flow of information in cells. DNA serves as a template for copying itself (**replication**). DNA can also serve as a template for RNA (**transcription**). RNA is decoded into amino acids to generate proteins (**translation**). Credit Daniel Horspool (CC-BY-SA 3.0)*

TRANSCRIPTION

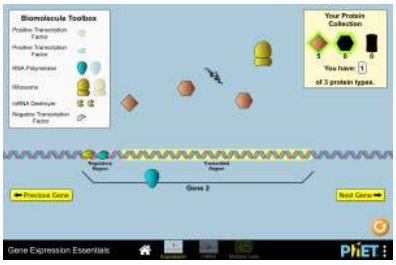


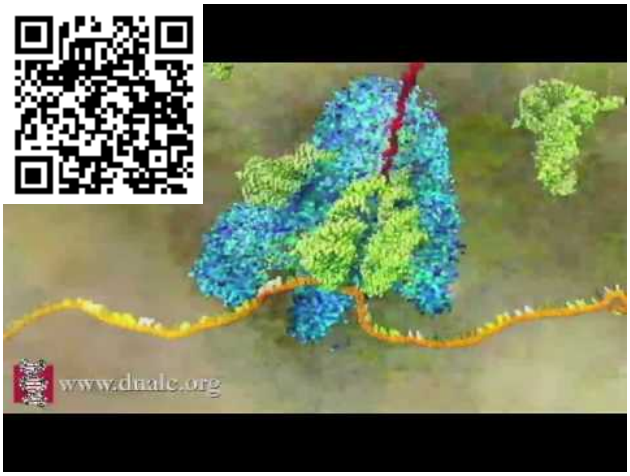


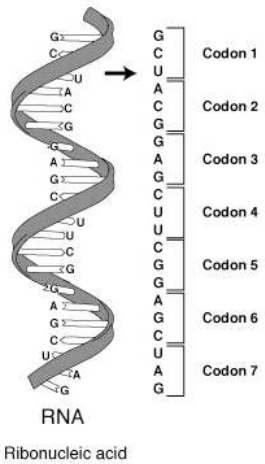
DNA is simply a storage vessel of genetic information. It sits in the nucleus and must be called upon through a process of **transcription** where an enzyme called **RNA Polymerase**"reads aloud" the stored information into a molecule called messenger RNA (**mRNA**). Since DNA is double-stranded in an **anti-parallel** fashion, we automatically know the sequence of the second strand by knowing the first. The mRNA is made through complementary base-pairing to the **template strand**, which is the reverse complement of the coding strand. The **coding strand** is the strand that reads identically in sequence to the mRNA with the exceptions of T's being replaced by U's.



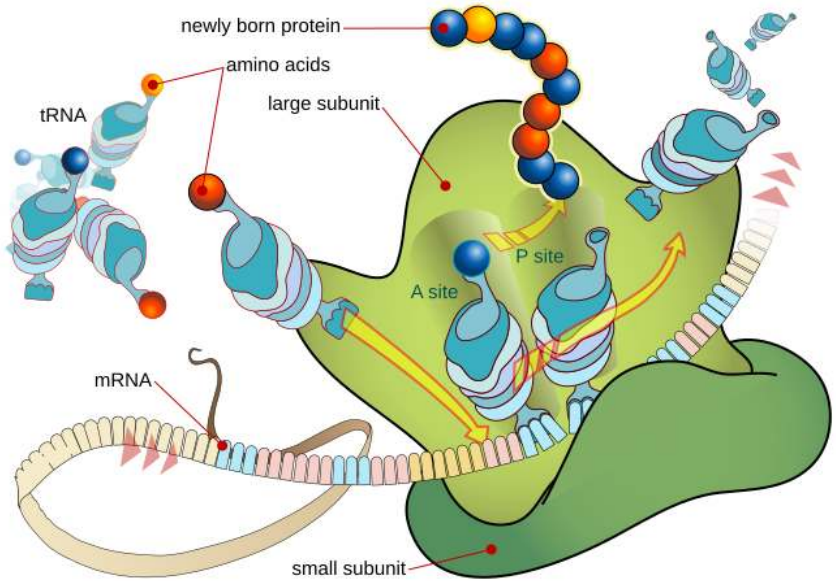
TRANSCRIPTION SIMULATION



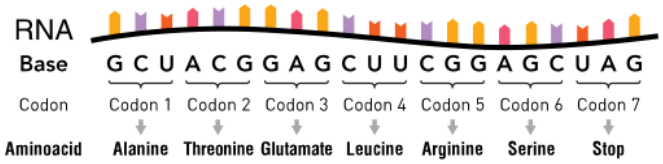


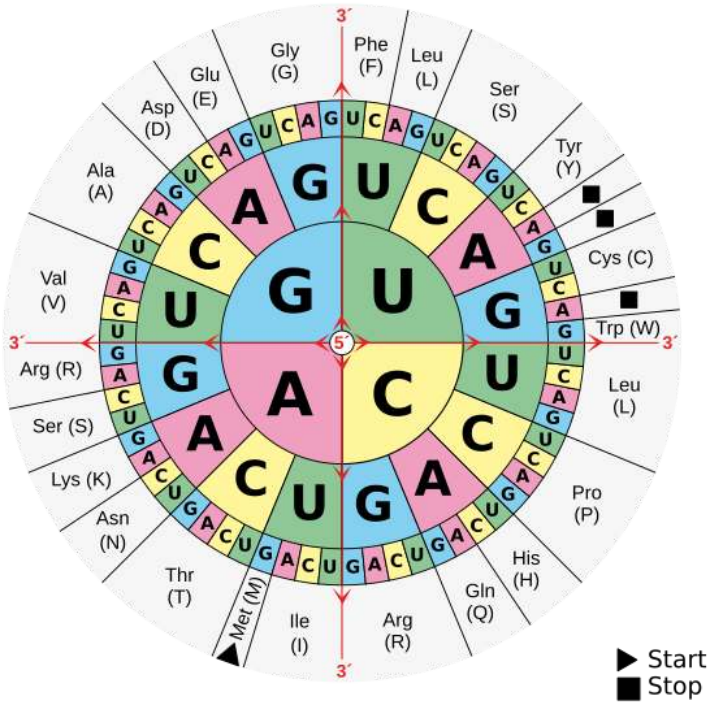


This coding strand is later decoded by the **ribosomes** with the help of transfer RNA's **tRNA**'s) that act as a decoder of the information and protein assembler in a process called **translation**. The ribosome scans along the mRNA and recognizes nucleotides in batches of 3 . These batches of 3 can be translated into an amino acid and are known as **codons**. Since there are 4 types of bases and they are read as groups of 3, there are 4^3 (or 64) combinations of these codons. However, there are only 20 amino acids used to build proteins. This indicates that there is room for redundancy. Three of these codons tell the ribosome to stop, like a period in a sentence. These are called **stop codons**. There is one special codon that performs double duty: ATG. The codon (ATG) that encodes the amino acid Methionine also acts as a **start codon** that tells the ribosome where to start reading from. Like nucleic acids, proteins have a polarity and are synthesized in an amino to carboxyl direction. We abbreviate this by terming the beginning of the protein sequence, N-terminal, and the ending of the sequence as the C-terminal.



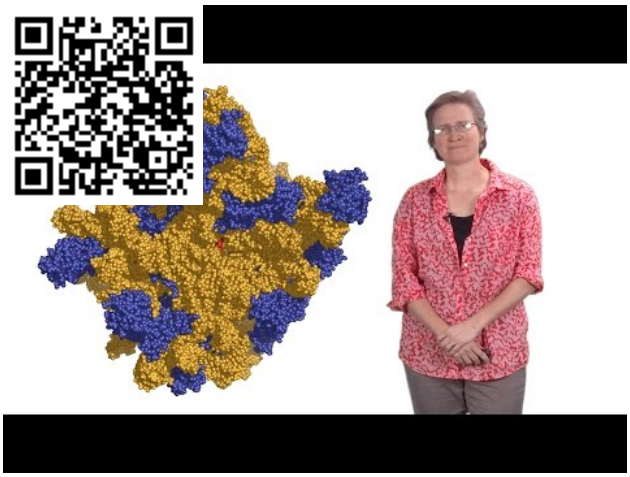
Ribosomes are large complexes of enzymes that coordinate the decoding of mRNA into amino acids to generate proteins.





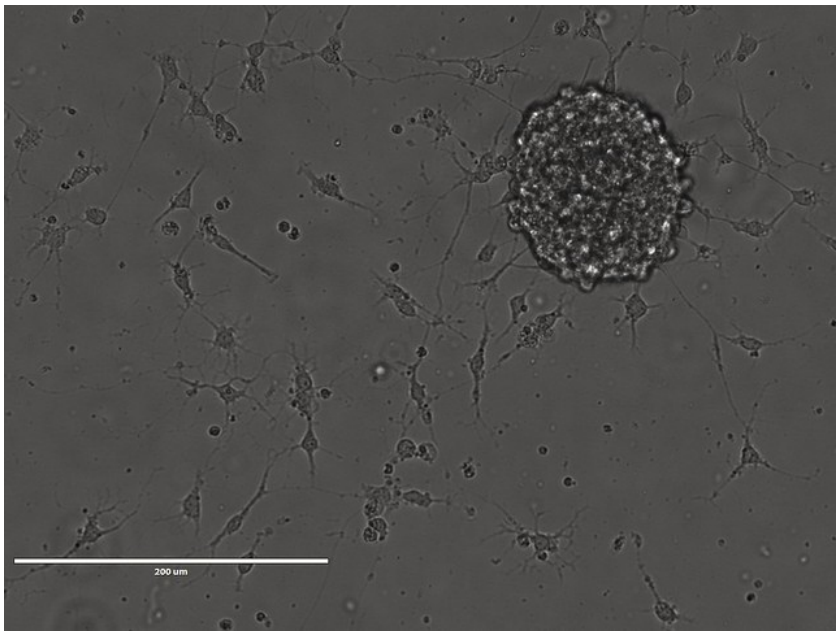
The standard genetic code.

ADVANCED VIDEO OF TRANSLATION



DECISIONS… DECISIONS…

What kinds of decisions are made for stem cells to differentiate into different cell types? What types of regulation occur during this process?



A cluster of neuronal progenitor cells (neurosphere) dissociates and differentiates into neurons.

VIDEO REVIEW

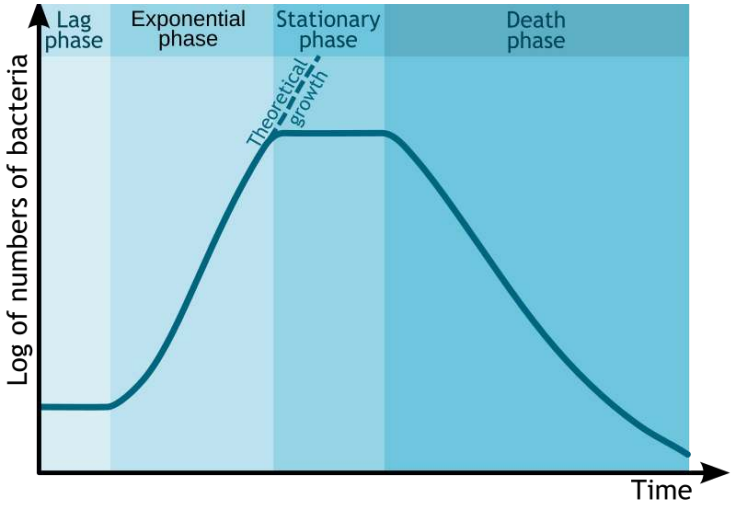
- Transcription Simulation (MIT License)
- **Transcription (CC-BY-NC-ND Cold Spring Harbor Lab – DNA Learning Center)**
  https://www.dnalc.org/content/c15/15510/transcription_basic.mp4
- **Translation (CC-BY-NC-ND Cold Spring Harbor Lab – DNA Learning Center)**
  https://www.dnalc.org/content/c15/15501/translation_basic.mp4

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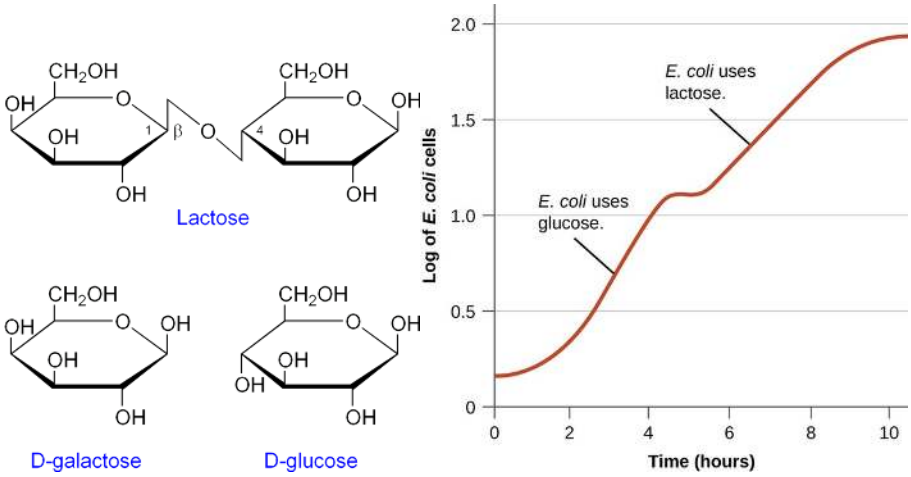
2.1.11.2: PROKARYOTIC TRANSCRIPTION

THE LACTOSE INTOLERANCE OF BACTERIA



The standard growth kinetics of E. coli are described by the curve. Credit: Michał Komorniczak (CC-BY-SA 3.0)

Glucose is the preferred energy source of cells. François Jacob and Jacques Monod sought to understand how bacteria made decisions to switch between different sugars as sources of energy. Jacob and Monod found that if glucose and lactose were presented as food for bacteria, there would be a **biphasic growth** pattern.

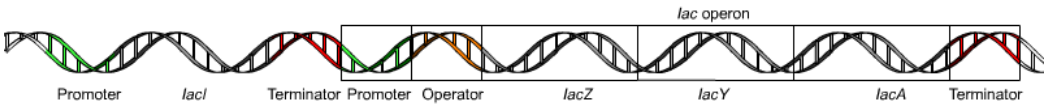


Credit: CNX OpenStax (CC-BY 4.0)

Jacob and Monod came to understand that the glucose would first be utilized (preferred source) and the lactose would be digested after the depletion of glucose. This occurred because under normal situations the bacteria would not have access to lactose and would waste energy by creating enzymes to digest it. The enzyme **β-galactosidase**, which is responsible for digesting lactose to the monomers galactose and glucose would only be induced under the conditions of low glucose and high lactose. Monod found that when lactose was the sole sugar, the expression of the enzyme β-galactosidase was induced and displayed a monophasic growth with a delay.

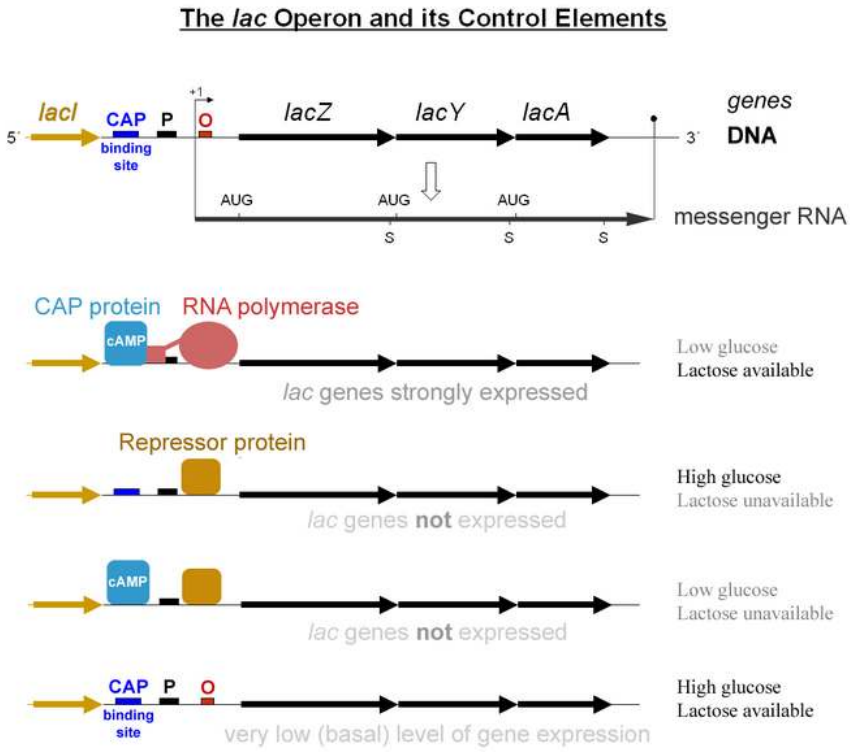
THE LAC OPERON

Jacob and Monod later found that the genes involved in utilizing lactose were clustered together in proximity under a coordinated control mechanism. This became known as the **Lac operon**.

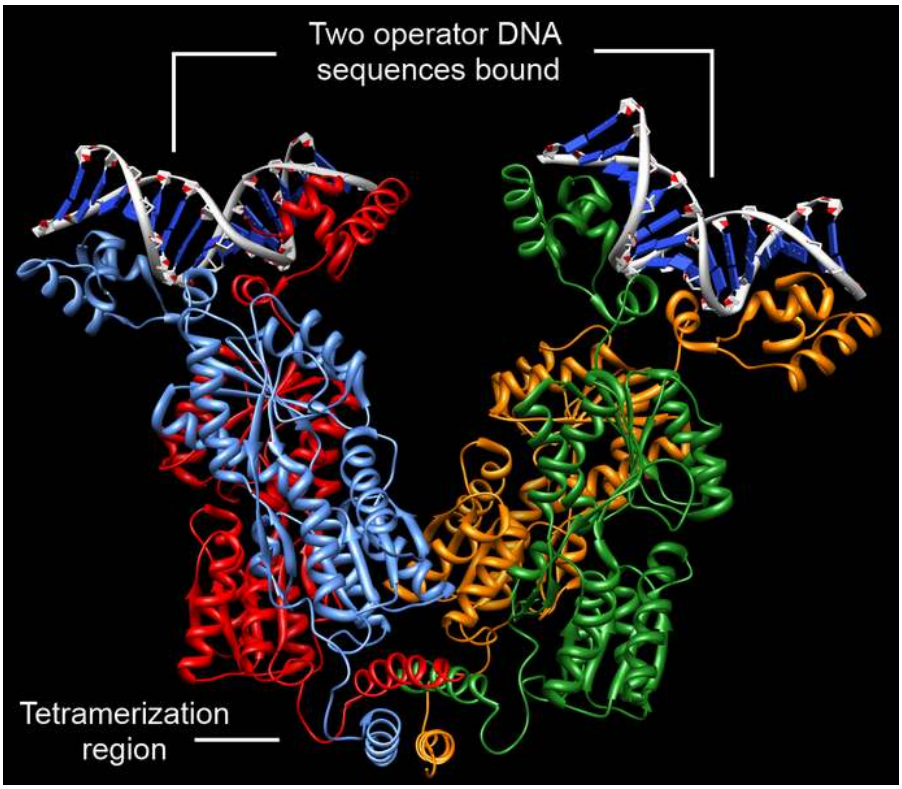


A schematic of the Lac Operon. LacZ, LacY, and LacA are transcribed as a single mRNA.

The usage of lactose as a source of energy is preferred by bacteria when glucose is not present. In the presence of abundant glucose, it would be a waste of energy and cellular resources to commit to synthesizing the mRNA and the protein for β-galactosidase. Unless lactose is present, a protein binds to a portion of the Lac promoter referred to as the **operator**. This **repressor** protein is encoded by another gene (***LacI***) outside of the gene cluster. Occasionally, the repressor unbinds to the operator and RNA Polymerase is permitted to transcribe the ***LacZ*** gene (β-galactosidase), ***LacY*** gene (permease), and ***LacA*** gene (acetylase). This "leakiness" of expression is important since the permease protein is needed on the surface of the cell to allow lactose into the cell if it is present in the environment. The presence of lactose causes the repressor to fall off the operator to grant RNA pol access to the DNA. When glucose is low, a protein called **CAP** (Catabolite Activated Protein) binds to the Lac promoter and works as a recruiter of RNA pol. The coordinated effects of CAP activation and Lac Repressor inactivation yield high transcription of the operon.

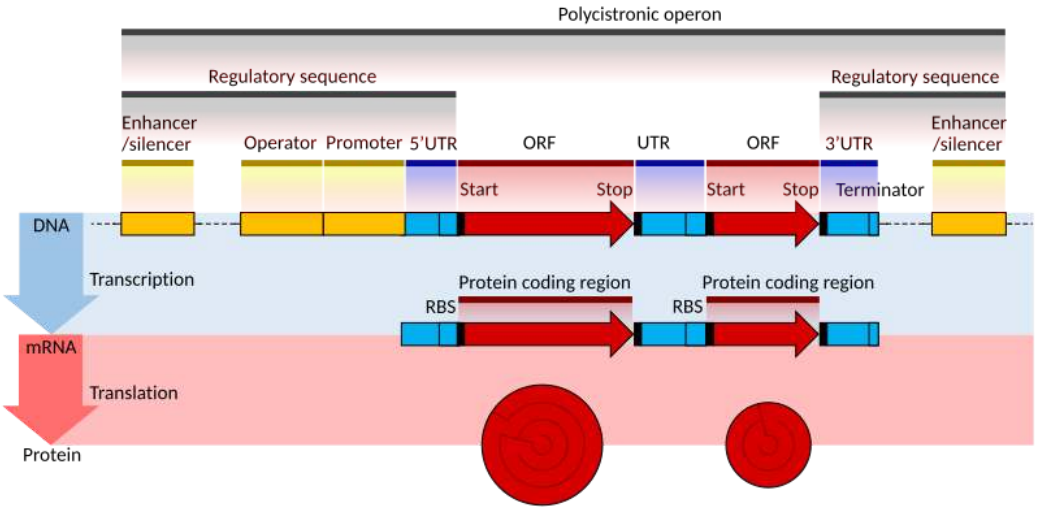


Credit: G3pro (CC-BY 2.0)



LacI bound to 2 DNA operator sequences.

Credit: SocratesJedi (CC-BY-SA 3.0)



LAC OPERON SIMULATION

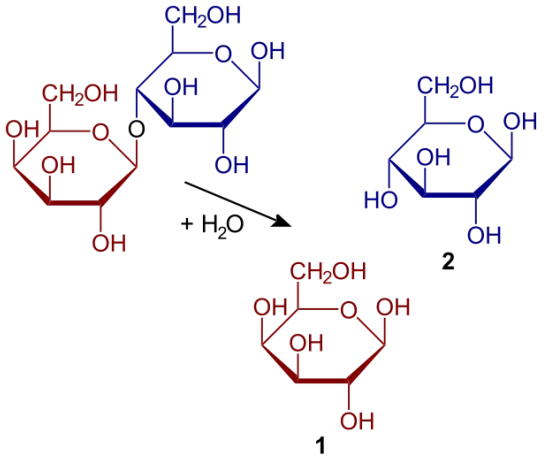
Launch the simulation below to explore the coordinated activation of the Lac Operon.

LACZ AS A REPORTER GENE

pUC19 contains LacZ DNA as a reporter gene to illustrate the presence of the functioning gene. Transcription of this gene is driven by the binding site for the RNA Polymerase subunit called **σ factor**. The σ factor binding site determines the directionality of the RNA polymerase since there is an option of transcribing in 2 directions. The standard σ factor binding site is often denoted as **-35 TTGACA…TATAAT -10** from the transcription initiation.

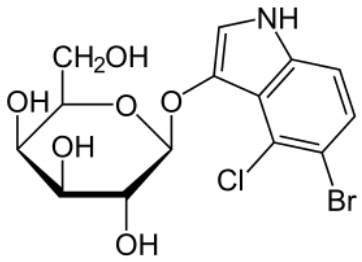
The multiple cloning site within the plasmid provides a convenient location to shuttle a foreign piece of DNA. When no foreign DNA is inserted to this space, the LacZ gene product β-galactosidase is functional. Disruption of the reading frame for this gene likewise disables

the functional product from being translated. By using chemical reporters, the integrity of this gene can be confirmed through enzymatic activity.

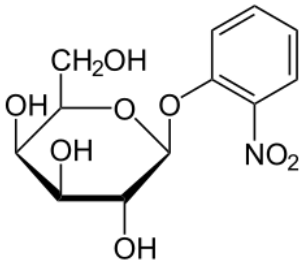


Hydrolysis of lactose to galactose and glucose

Two chemical reporters used to reveal the presence of a functioning LacZ are **X-Gal** (5-bromo-4-chloro-3-indolyl-β-D-galactoside5-bromo-4-chloro-3-indolyl-β-D-galactoside) and **ONPG** (orthonitrophenol).

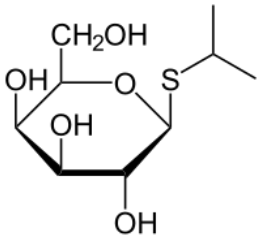


X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside) yields a blue color when cleaved by β-galactosidase



ONPG (orthonitrophenol) yields a yellow color upon cleavage by β-galactosidase

As in the case of the Lac operon, the *LacI* (repressor protein) will occupy the operator. This operator happens to be overlapping the -35 & -10 sequences. In order to fully activate these genes, the Lac repressor must be removed by binding to a lactose analog. In this case, the chemical **IPTG** (Isopropyl β-D-1-thiogalactopyranoside) is used since it is a non-cleavable analog that will perpetually bind to the Lac repressor.



IPTG

BLUE-WHITE SCREENING



Blue-White Screening reveals a phenotype of transformed bacteria based on the ability of X-Gal conversion by β-lactamase Credit: Stefan Walkowski (CC-BY-SA 4.0)

REFERENCES

- JACOB F, MONOD J. **Genetic regulatory mechanisms in the synthesis of proteins**. *J Mol Biol. 1961 Jun;3:318-56.*

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2.1.11.3: RNA MINIPREP (ACTIVITY)

EXERCISE: RNA MINIPREP

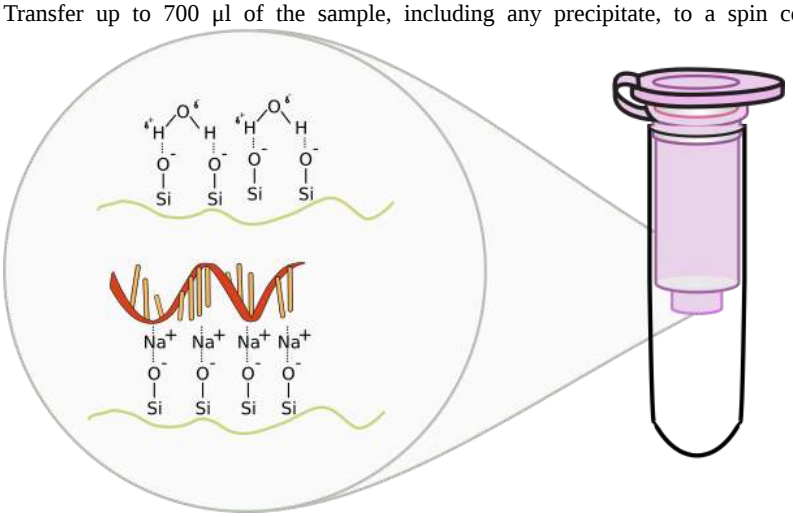
RNA purification occurs similar to DNA preparations. A silica-based column is used where DNA is excluded from binding based on size and through an additional DNA digestion step using the enzyme DNase I. RNA is extremely fragile and prone to degradation. Because of this, separate pipettes and plastics are usually used in labs to reduce the amount of exposure to environmental or experimental RNase. When handling RNA, be extremely careful of contaminating the buffers or samples. Always wear gloves as skin carries RNase enzymes. Refrain from talking as to not contaminate the area with RNase found in saliva.

RLT = RNA lysis buffer: contains guanidine, a harsh denaturant
RW1 = RNA wash buffer
RPE = Second RNA wash buffer with Ethanol
RDD = DNase digestion buffer

1. **Harvest** a maximum of 1 x 10^7 cells, as a cell pellet or by direct lysis in the vessel. Add the appropriate volume of Buffer RLT and vortex vigorously.

- If < 5 x 10^6 cells → 350 µl RLT (< 6cm plate)
- If ≤ 1 x 10^7 cells → 600 µl RLT (6-10cm plate)

2. Add 1 volume of 70% ethanol to the lysate, and mix well by pipetting. Do not centrifuge. Proceed immediately to the next step.
3. Transfer up to 700 µl of the sample, including any precipitate, to a spin column placed in a 2 ml collection tube (supplied).

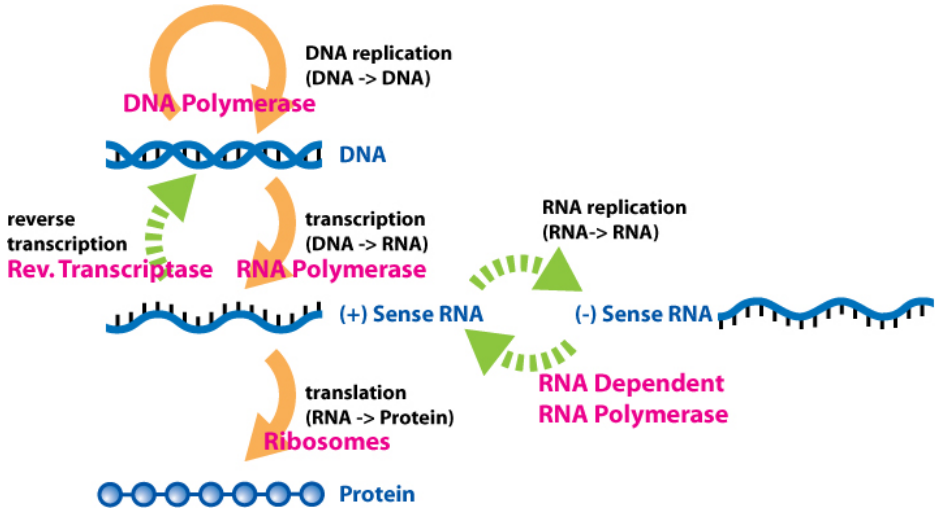


    1. Close the lid, and centrifuge for 15 s at ≥8000 x g.
    2. Discard the flow-through.
4. **Wash**: Add 350 µl Buffer RW1 to spin column, close lid, centrifuge for 15 s at ≥8000 x g (≥10,000 rpm). Discard flow-through.
5. Add 10 µl DNase I stock solution (see above) to 70 µl Buffer RDD. Mix by gently inverting the tube.
6. **Remove DNA** (optional): Add DNase I incubation mix (70 µl) directly to spin column membrane, and place on the benchtop (20–30°C) for 15 minutes.
7. **Wash**: Add 350 µl Buffer RW1 to spin column, close lid, centrifuge for 15 s at ≥8000 x g. Discard flow-through.
8. Add 700 µl Buffer RW1 to the spin column. Close the lid, and centrifuge for 15 s at ≥8000 x g. Discard the flow-through.
9. Add 500 µl Buffer RPE to the spin column. Close the lid, and centrifuge for 15 s at ≥8000 x g. Discard the flow-through.
10. Add 500 µl Buffer RPE to the spin column. Close the lid, and centrifuge for 2 min at ≥8000 x g.
11. Discard all flow-through and centrifuge at full speed for 1 min to dry the membrane.
12. Place the spin column in a new 1.5 ml collection tube. Add 30 µl RNase-free water directly to the spin column membrane.
13. Close the lid, and centrifuge for 1 min at ≥8000 x g to elute the RNA.
14. Add 30 µl RNase-free water directly to the spin column membrane. Close the lid, and centrifuge for 1 min at ≥8000 x g to elute the RNA.

REVERSE TRANSCRIPTION

The **Central Dogma** of Molecular Biology was proposed by Francis Crick, the co-describer of the double-stranded helical structure of DNA. This “dogma” was a statement to describe the flow of genetic information to show that DNA houses or stores data that is transcribed into RNA that is subsequently translated from nucleotides into amino acids through the machinery of the ribosomes. Since DNA is

relatively static in its ability to store genetic information, the expression of this stored data into the intermediate RNA or to the final protein product is of great significance. Imagine that the DNA in the nucleus of your cheek cells is identical to the DNA of the nucleus of cells in your liver. While the instructions are identical, these are clearly different cells that have a difference in the expression of proteins. Imagine a hard drive on a computer that stores information as 1's and 0's. These 1's and 0's do not have meaning until specific programs are called upon to act on this information. Likewise, different programs are called to use the instructions of your DNA to make a cheek cell different than a liver cell.



Credit: Daniel Horspool (CC-BY-SA 3.0)

In 1970, Howard Temin and David Baltimore independently isolated an enzyme from the Rous Sarcoma Virus and Murine Leukemia Virus, respectively. This enzyme was capable of violating the Central Dogma. The genomes of these viruses consist of RNA, not DNA. During the infection process, this enzyme is responsible for converting the RNA into DNA in a process called **reverse transcription**. This enzyme is logically called **reverse transcriptase** (RT). This discovery was rewarded with Nobel Prize in 1975. Later on, more viruses were discovered that were composed of RNA genomes that utilized this process, including HIV. Other enzymes within cells were also recognized to have reverse transcriptase activity, such as telomerase and retrotransposons. In molecular biology, these enzymes are used to convert mRNA into complementary copies of DNA called cDNA. The sum total of everything that is transcribed into RNA is referred to as the **transcriptome**. Synthesis of cDNA from any transcribed RNA can then be used for transcriptome analysis.

EXERCISE: REVERSE TRANSCRIPTION OF EUKARYOTIC MRNA

mRNA from eukaryotes are modified with 3′ polyadenylated tails. Oligo-dT primers can be used to prime the reverse transcription process of all mRNAs. All solutions should be kept on ice.

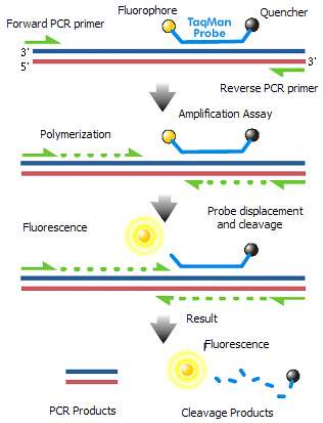
1. Determine the concentration of total RNA.
2. Adjust the concentration of RNA to 0.1mg/ml using Rnase free water.
3. Combine 10 ml RNA, 1 ml Oligo-dT (50µM), and 1 ml dNTP Mix (10 mM each).
4. Denature mixture at 65°C for 5 minutes and then place on ice.
5. Combine the following in a separate tube:
    1. 4µl Buffer 5X → contains all salts and pH buffer
    2. 2µl 0.1 M DTT → a reducing agent to mimic the cellular environment
    3. 1µl RNaseOUT (40U/µl) → an RnaseA inhibitor
    4. 1µl SuperScript III RT → the reverse transcriptase enzyme
6. After the denatured mixture has been sufficiently cooled, add 8µl enzyme mixture.
7. Incubate 45°C for 1 hour.
8. Deactivate enzyme by incubating 75°C for 10 minutes.
9. Store your cDNA in the freezer.

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2.1.11.4: QUANTITATIVE NUCLEIC ACID MEASUREMENT

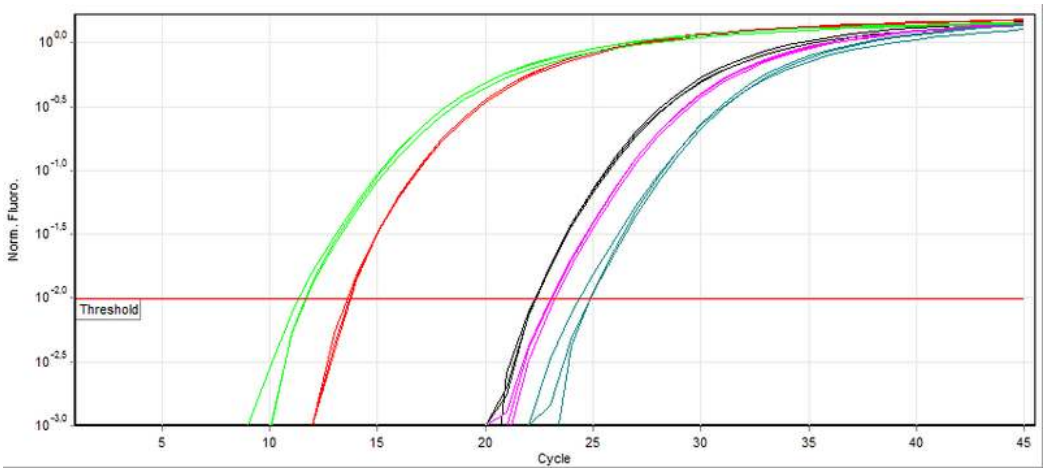
QUANTITATIVE PCR (QPCR)



Measurements can be made of individual genes of interest through PCR of those specific genes. A process known as Real-Time PCR or quantitative PCR (**qPCR**) is used to measure individual genes using fluorescence measurements. An intercalating agent that binds only to double-stranded DNA called **Sybr Green** is used in a qPCR machine that is measuring fluorescence after each cycle of PCR indirectly indicates the amount of amplified product. However, non-specific products of amplification may also be measured and not discriminated from the authentic amplicon.

An alternative to Sybr Green is exemplified by the TaqMan technology. With TaqMan, a third primer (**TaqMan probe**) is designed in the middle of the area to be amplified. This middle primer is designed with a hairpin self-complementarity so that the 5′ and 3′ ends are in close proximity. At one end, a **fluorescent reporter** is attached while the other terminus has a **quencher** that absorbs any fluorescence signal. Under normal circumstances, measurements of fluorescence will be very low. When PCR extension occurs, the Polymerase hydrolyzes this middle primer, thereby separating the quencher and reporter. The name TaqMan is a play on words since it is imagined that the polymerase is chewing up the probe like Pacman. With increased distance between quencher/reporter, the fluorescence signal from this probe can now be measured. This method is much more specific than Sybr Green. However, the use of specific probes increases the cost considerably.

THRESHOLD CYCLES (C_T)



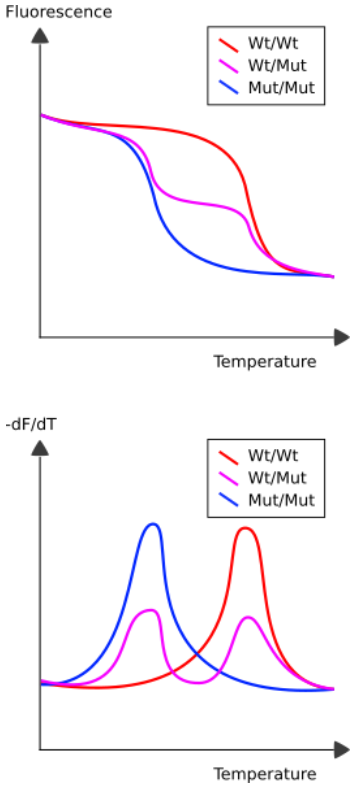
Credit: Zuzanna K. Filutowska (CC-BY-SA 3.0)

Fluorescence measurement early during the PCR process will be very low due to the small number of dsDNA molecules (Sybr Green) or most TaqMan primers being quenched. During this exponential DNA production, a threshold will be reached in which the fluorescence will linearly increase. A specific point where the fluorescence is clearly measurable is called the **Threshold Cycle** ($\mathbf{C_t}$) is used as a reference point to compare expression values.

Looking at the example of Sybr Green qPCR above, it can be observed that samples exponentially increasing at a lower cycle number (C_t) has a higher level of mRNA expression (towards the left) of that gene than samples with higher cycle number (towards the right). Notice that the fluorescence eventually plateaus and stops increasing. This is due to the depletion of raw materials for DNA production like dNTPs.

Since the PCR reactions theoretically represent a doubling of DNA after each cycle, the C_t values can be interpreted on a base 2 system. If there is a difference in C_t between two samples ($\mathbf{\Delta C_t}$) of 5 cycles, this corresponds to 2^5 or 32 fold difference. We can control for variations in the RNA preparation through comparing the fluorescence values of our gene of interest to a housekeeping gene like actin. The use of a house-keeping gene to normalize the initial input to the reactions and comparison between samples is referred to as **Relative Quantification**.

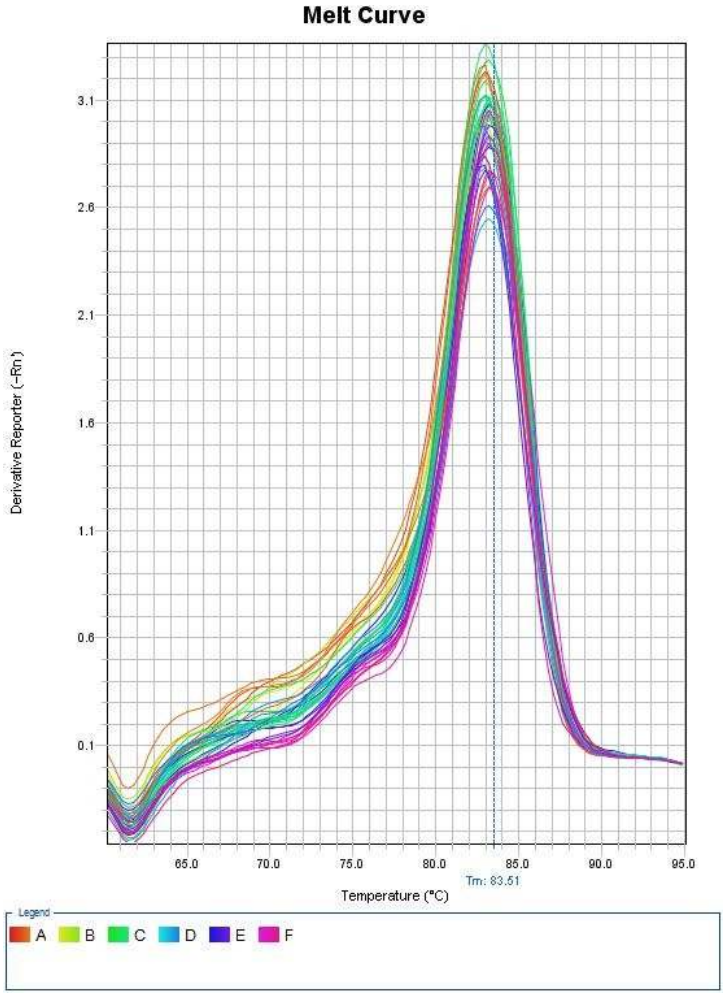
MELT CURVES FOR SYBR GREEN



The top panel illustrates the decrease in fluorescence as the temperature increases due to the dissociation of double-stranded DNA. The bottom panel illustrates the first derivative plot. Each peak in this example illustrates a different allele. The double peaks represent the presence of the 2 distinct alleles in the amplification products.

Credit: Seans Potato Business (CC-BY-SA 3.0)

When using Sybr Green, we need to ensure that the PCR is specific so that the fluorescence measurement truly reflect amplification of our gene of interest. At the end of each qPCR run (~40 cycles), a melt curve is performed. A **melting curve** (or dissociation curve) comes from constant measurements as the temperature is increased. As temperature increases, the DNA strands start to denature and fluorescence will begin to decrease. After complete separation of DNA strands, the fluorescence will again remain constant. The way this curve is viewed is through a derivative plot where the inflection in fluorescence reading is reported as the **melting temperature (Tm)**.



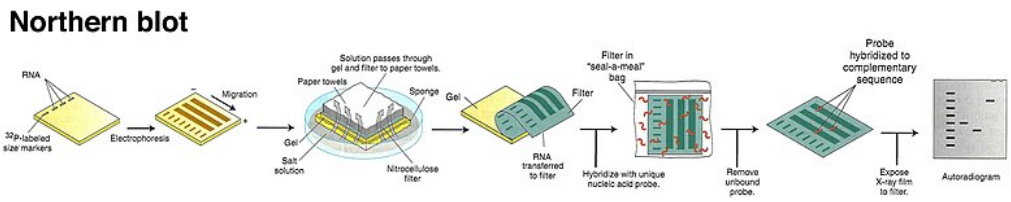
This melt curve illustrates each sample contains the same specific product with a melting temperature of 83.51°C.

Any peaks in this plot refer to a specific PCR product. If multiple peaks appear, the results will not be valid as they do not directly measure a single product.

EXPRESSION MEASUREMENTS

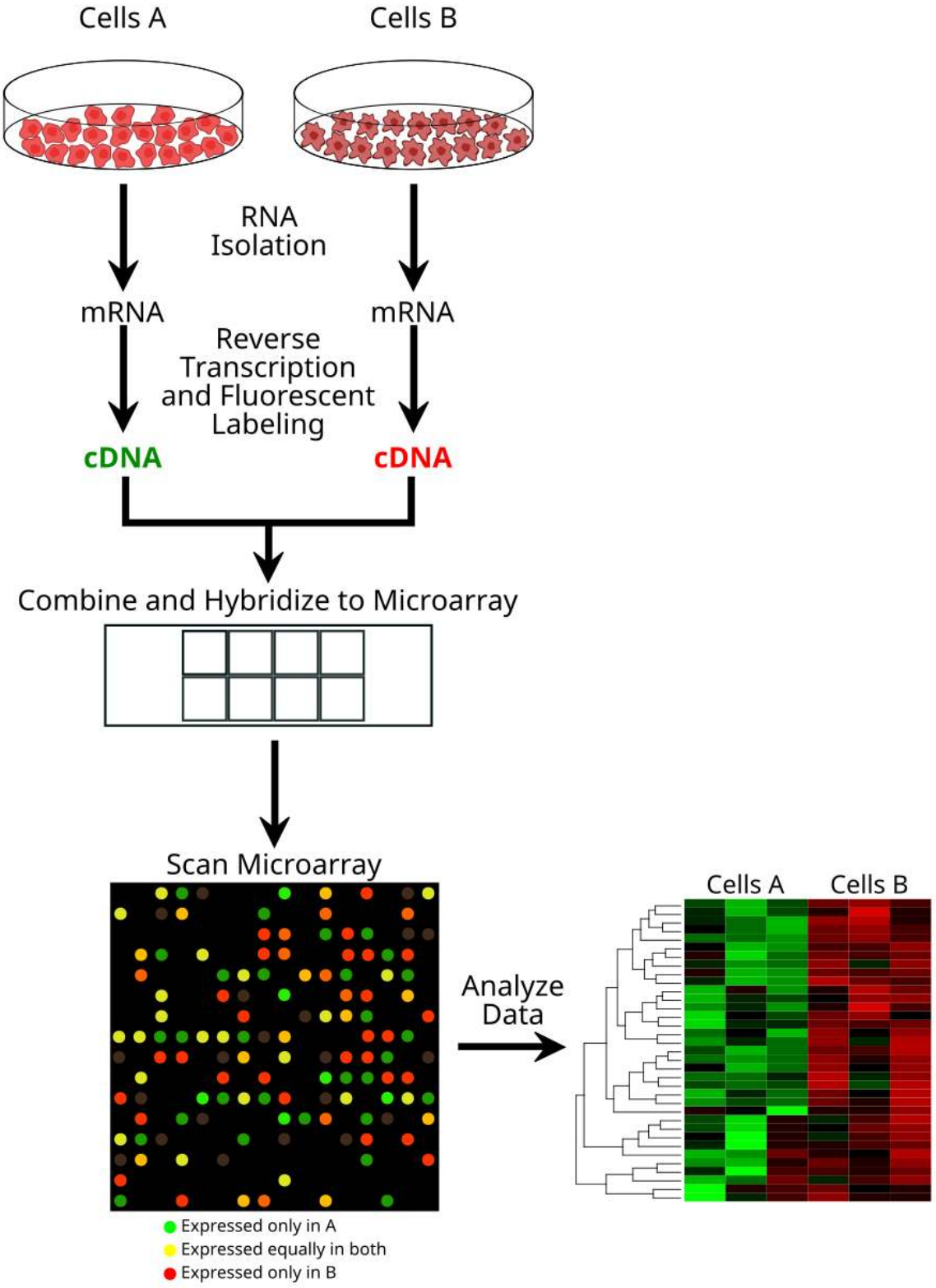
Differential gene expression refers to transcriptional programs activated by the cell under various conditions. “Differential” refers to a comparison of two or more states or timepoints. Using mRNA as an indirect measurement of protein, one can ascertain which proteins are linked to these different states. In eukaryotes, this can be assessed by enriching total RNA for polyA-containing mature mRNA. Through the use of oligo-(dT) containing resin, mRNA can be separated from non-protein encoding RNA. Likewise, performing a reverse-transcription using an oligo-(dT) primer will create a stable complementary DNA (cDNA) molecule that can be used with PCR. Using qPCR in this way is called **RT-PCR** or reverse-transcription polymerase chain reaction where specific primer pairs are used to amplify a small portion of a known gene.

HYBRIDIZATION-BASED METHODS AND MICROARRAYS:



Credit: FrozenMan (CC-BY-SA 4.0)

Prior to RT-PCR, the expression of individual genes was assessed through a hybridization-based approach. This method called for running RNA on an agarose gel and transferring the size-fractionated RNAs onto a membrane through a method called “blotting”. This transferred RNA was then hybridized to a radioactively labeled probe for a specific gene (corresponding to the reverse complementary sequence) and visualized by exposure to X-ray film in a process called **Northern Blotting**. The intensity of the band would be proportional to the amount of mRNA corresponding to the gene of interest. Re-probing with a housekeeping gene like actin would be used as a loading control to illustrate that a similar amount of total RNA was loaded into each well. Differences in sizes of the mRNA on the Northern Blot also revealed differences in splice variants of mature mRNA in the different states.



Credit: Jeremy Seto (CC-BY-NC-SA)

This technique was later adapted using non-radioactive methods. Using these non-radioactive methods, the reverse protocol was developed to measure multiple gene targets. By systematically immobilizing gene-specific probes onto a membrane or a microscope slide, an array of targets can be produced. In the simplest paradigm of having 2 states (control or experimental), cDNA from each sample can be used to generate fluorescent RNA that can hybridize to immobilized probes. Using 2 different fluorescent markers allows for the competitive

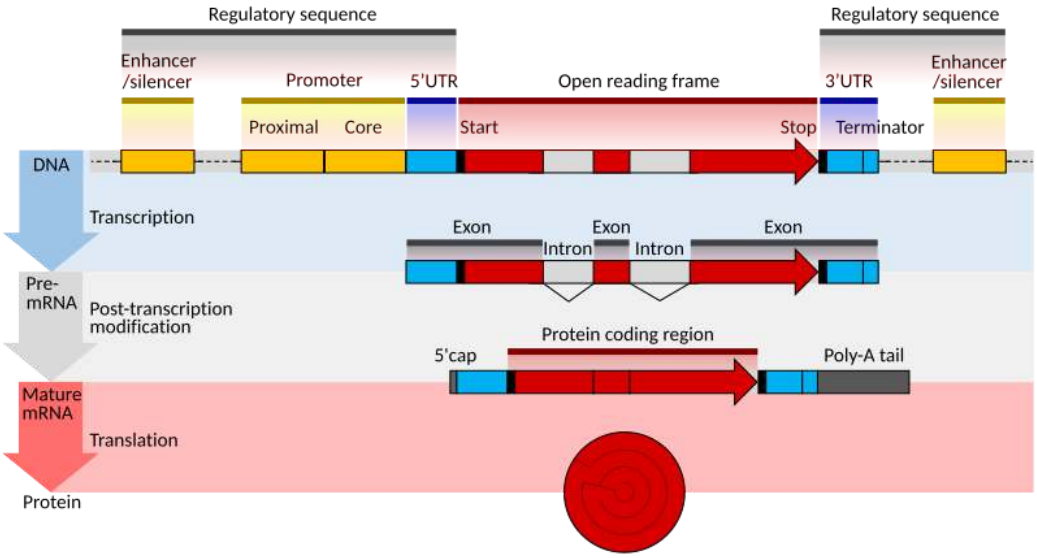
hybridization onto the array whereby the fluorescent signal in each channel can reveal the differential gene expression of the two states in a 2-color **microarray**.

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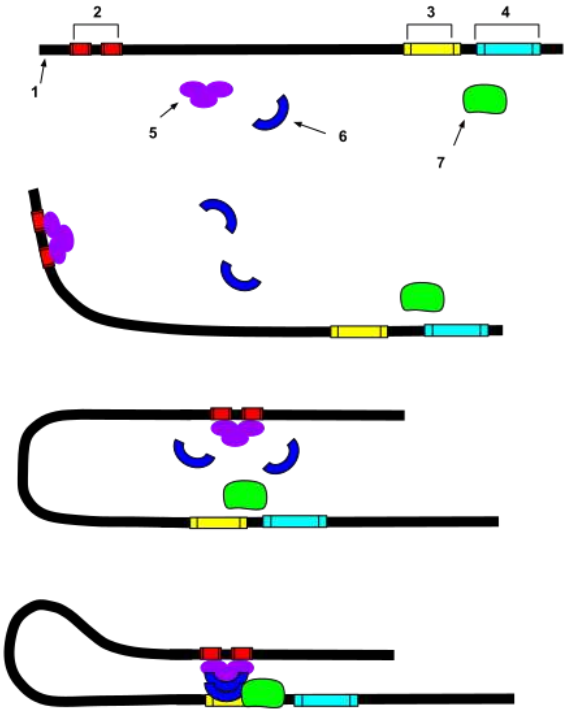
2.1.11.5: EUKARYOTIC TRANSCRIPTION

EUKARYOTIC GENE EXPRESSION



The Central Dogma in Eukaryotes. Genomic DNA of genes often contain introns that are spliced out when an RNA matures to an mRNA. This excision of introns can result in splice variants of the same gene with variants of the same protein. Credit: Thomas Shafee (CC-BY 4.0)

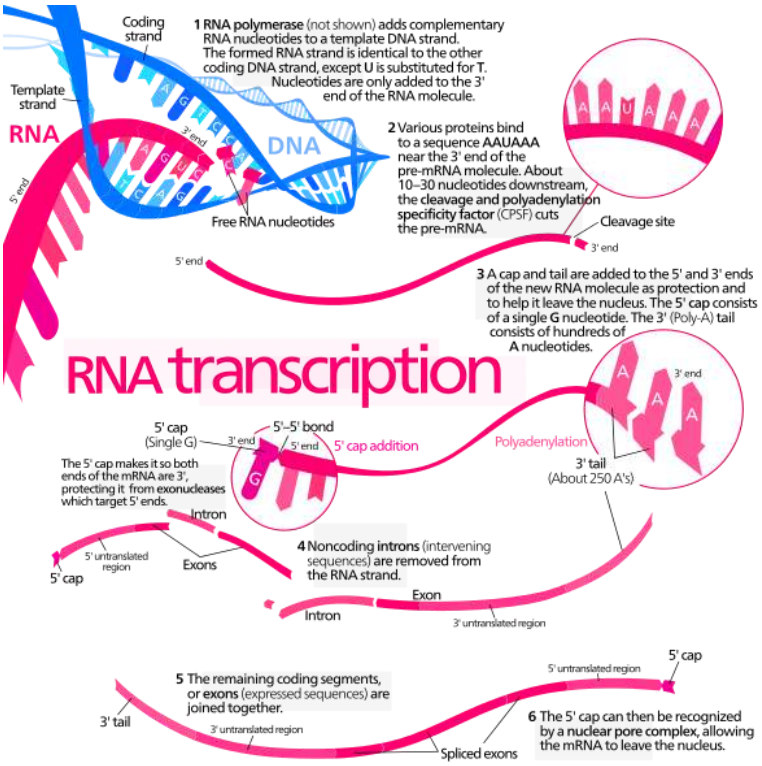
Unlike prokaryotic genes, the expression of genes in eukaryotic cells has complex systems of transcription factors that act on promoters to recruit RNA polymerases. Additionally, **enhancer elements** may reside many kilobases upstream of the promoter. These enhancers strengthen the transcription of the gene. In this case, **transcription activator proteins** or trans-activators augment the promoter activity.



1. DNA 2. Enhancer 3. Promoter 4. Gene 5. Transcription Activator Protein 6. Mediator Protein 7. RNA Polymerase

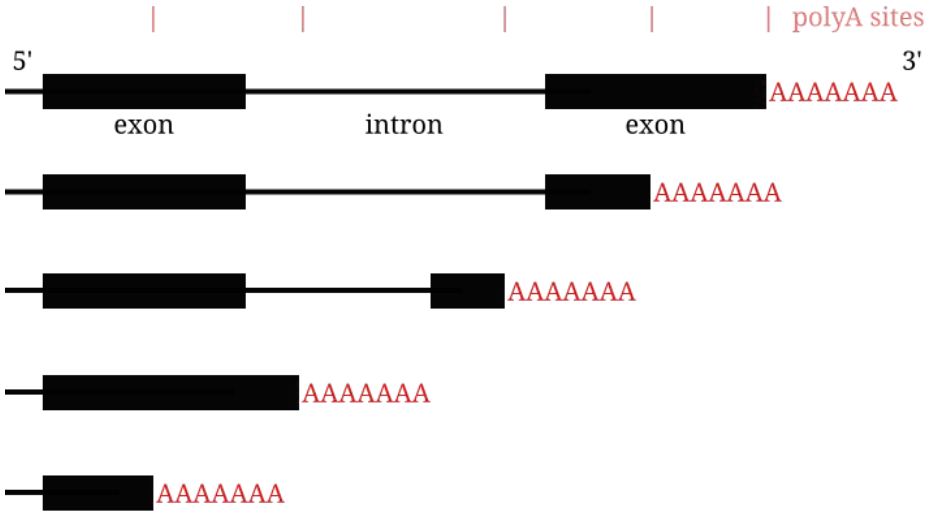
Credit: Jon Cheff (CC-BY-SA 4.0)

Mediator proteins (**coactivators**) form a multiprotein complex with the activators to recruit RNA polymerase to the promoter.



Credit: Kelvinsong (CC-BY-3.0)

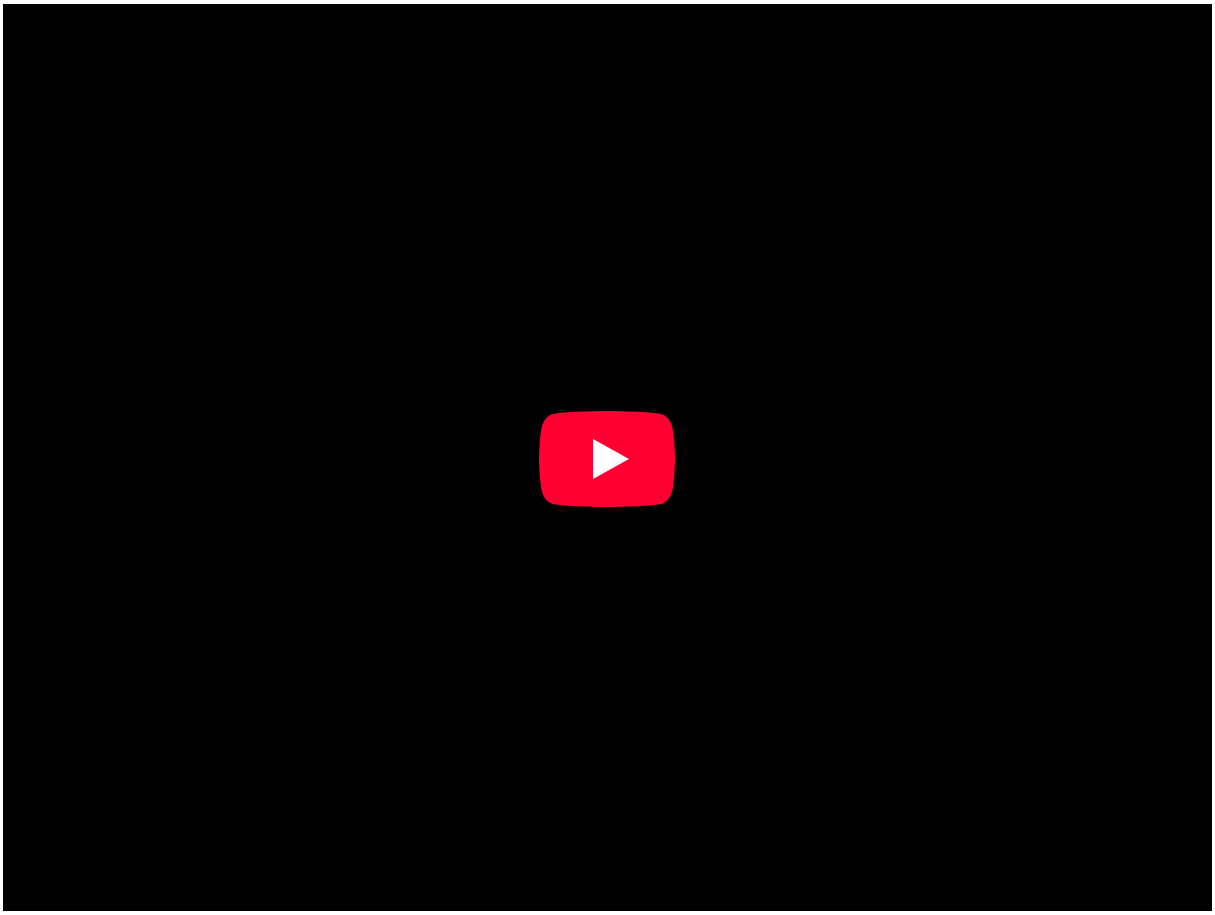
Eukaryotic genes may often contain **introns** (non-coding sequences) that are spliced out from the **exons** (coding sequences). This complexity permits for an increased variety of gene products. Mature eukaryotic mRNAs contain a 5′-methyl-Guanine followed by an untranslated leader sequence (**5′-UTR**), the coding sequences (**cds**), a 3′-untranslated region (**3′-UTR**) and a long stretch of Adenines (polyA tail).

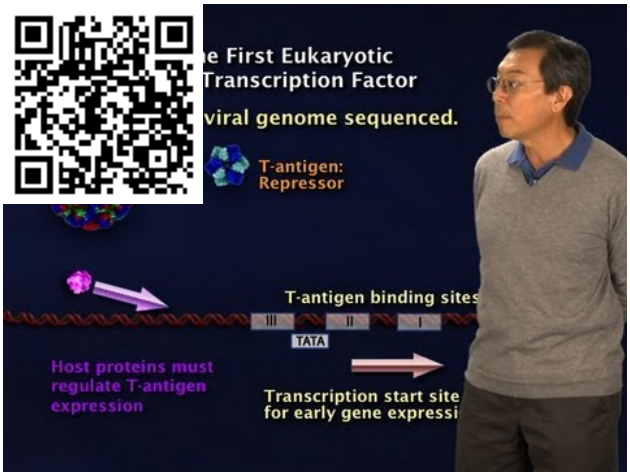


Expression is the most easily measured with RNA since nucleic acid manipulation is fairly simple with 4 different nucleotides. In eukaryotes, the messenger RNA (mRNA) intermediate that is transcribed from DNA contains a polyA tail that is used to separate these messages from other types of RNA that are abundant within cells (like ribosomal RNA). Through the use of an enzyme called **reverse transcriptase** (RT) and primers composed of deoxy-Thymidines (**oligo-dT** or dT_{18}), mRNA can be converted into a single strand of DNA that is complementary to the mRNA. This complimentary DNA is called **cDNA**. cDNA is very stable compared to the highly labile mRNA and is used for subsequent processing.

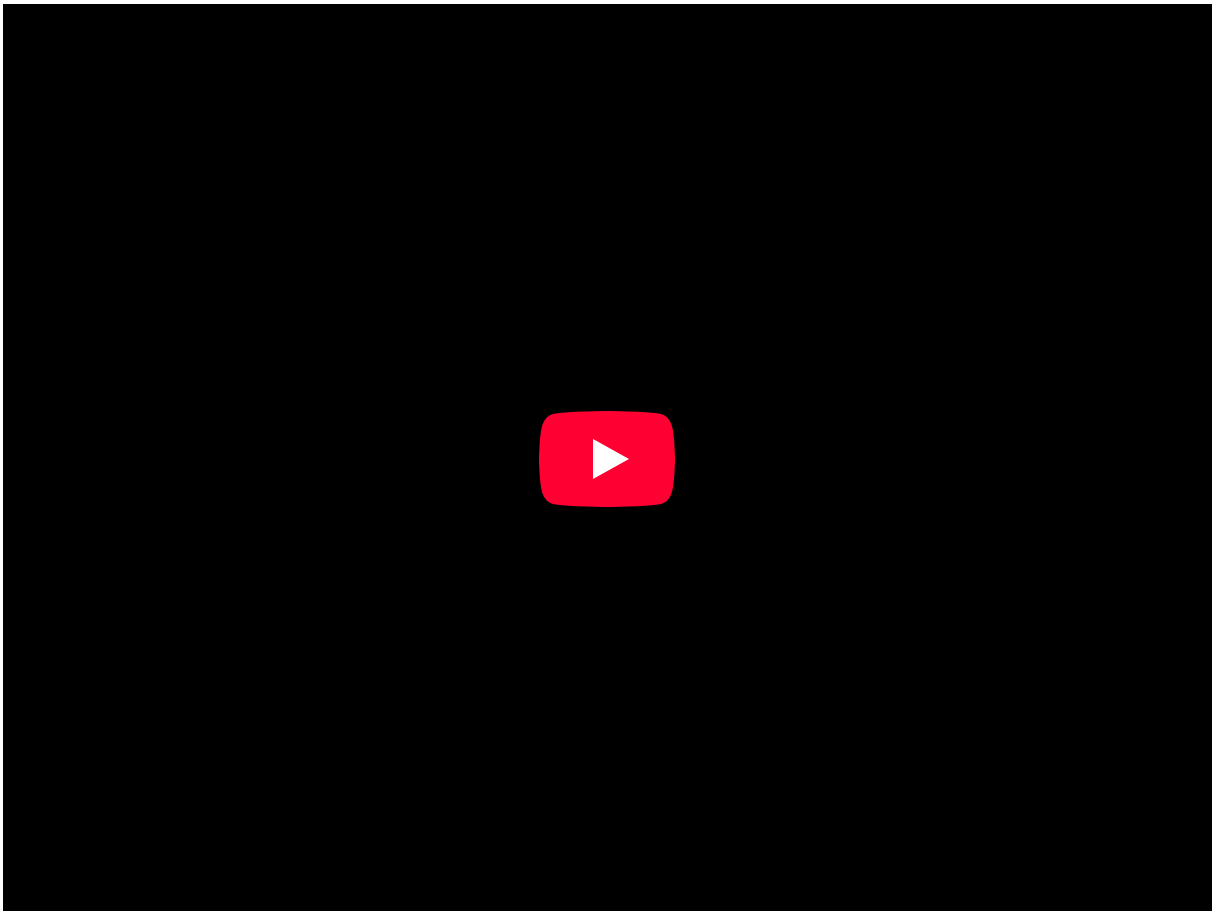
ADVANCED VIDEO OF EUKARYOTIC TRANSCRIPTION REGULATION

The first video describes the discovery of transcription factors that regulate the expression of eukaryotic genes.





The second video describes the complexity of gene expression that involves chromatin remodeling and enhancers. This video explores the roles and outcomes of differential gene expression.



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SECTION OVERVIEW

2.1.12: TRACING ORIGINS

2.1.12.1: INTRODUCTION

2.1.12.2: MATERNAL LINEAGE

2.1.12.3: MATERNAL LINEAGE (ACTIVITY)

2.1.12.4: ALU INSERTION (ACTIVITY)

2.1.12.5: TRANSPOSONS

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2.1.12.1: INTRODUCTION

BEING HUMAN



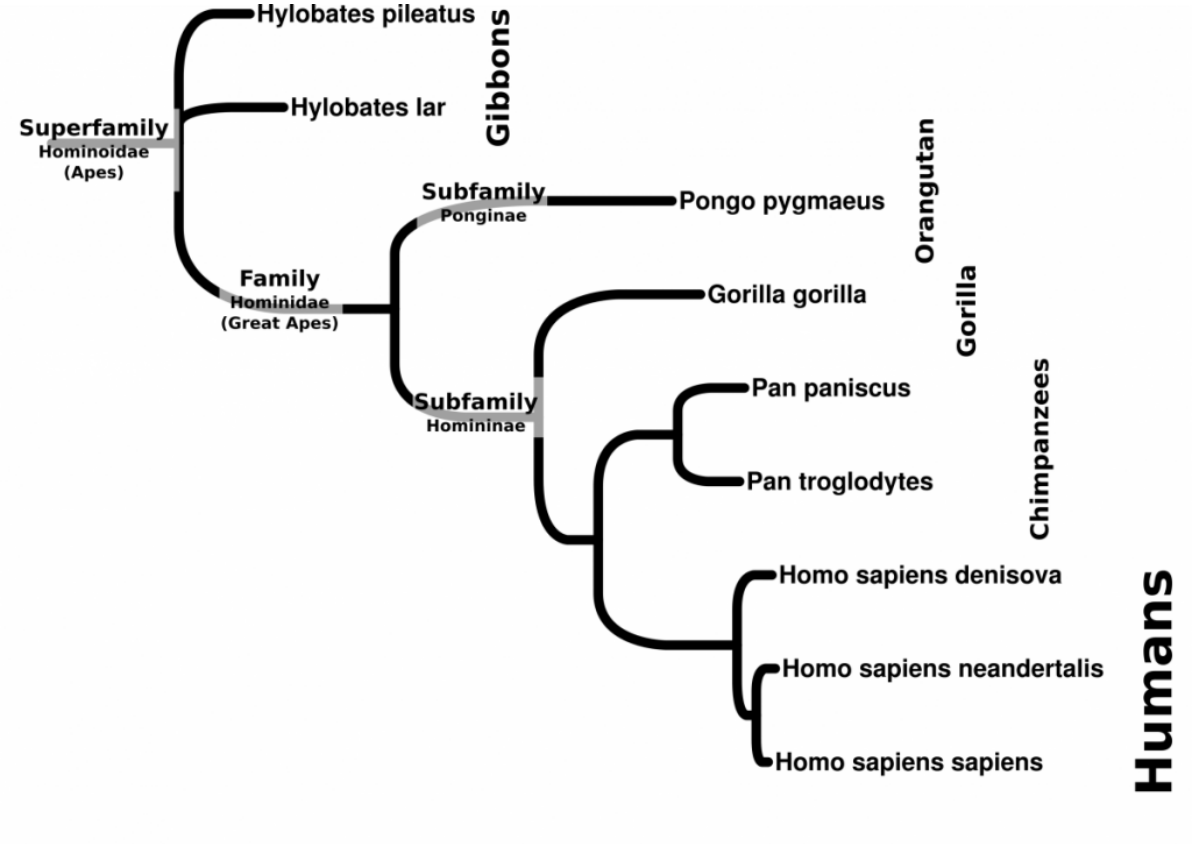
Drawings dating to approximately 30,000 years ago in the Chauvet Cave.

What constitutes being human? Many will point at cultural identity and leaving long-standing remnants of that culture. Such prehistorical artifacts like cave drawings and tools provide an anthropological framework for identifying what it is to be human, but the biological identity remains locked in the history of our DNA.



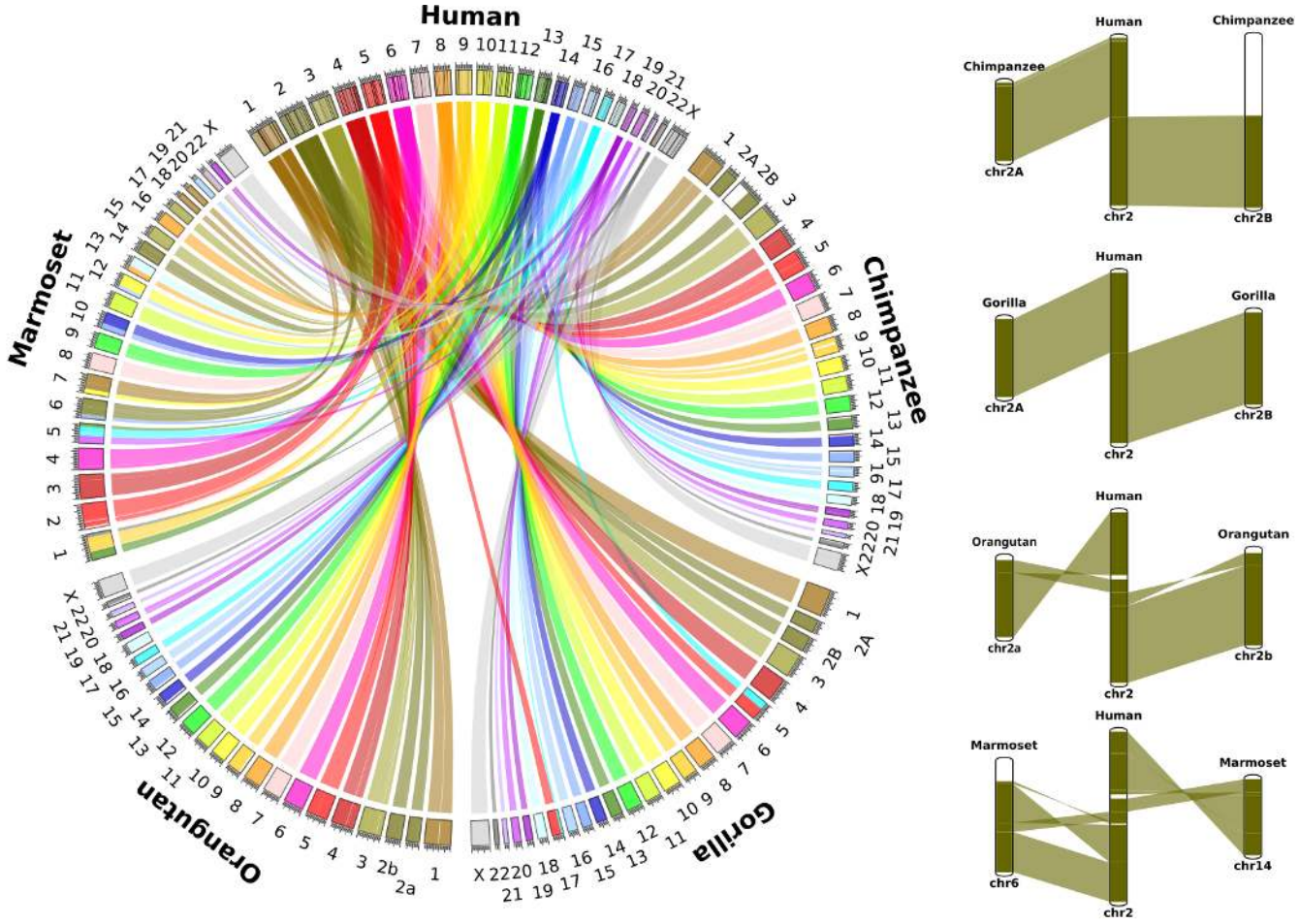
Spear points of the Clovis Culture in the Americas dating to approximately 13,000 years ago.

Credit: Bill Whittaker [CC-BY-SA 3.0]



Phylogenetic tree generated with Cytochrome Oxidase I (COI) genes.

Homo sapiens represent a branch of primates in the line of Great Apes. The family of Great Apes consists of four extant genera: *Homo*, *Pan*, *Gorilla*, *Pongo*. Karyotype analysis (Yunis *et al.*, 1982) reveals a shared genomic structure between the Great Apes. While humans have 46 chromosomes, the other Great Apes have 48. Molecular evidence at the DNA level indicates that Human Chromosome 2 is a fusion of 2 individual chromosomes. In the other Great Apes, these 2 Chromosomes are referred to as 2p and 2q to illustrate their synteny to the human counterpart.



Synteny map of Human, Chimpanzee, Gorilla, Orangutan, and Marmoset (non-ape primate). Mapping of chromosome 2a and 2b in the apes compared to 6 and 14 in the marmoset illustrates the relatedness of the chromosomal structure of the apes. Minor inversions are apparent in the orangutan chromosome.

Credit: Jeremy Seto [CC-BY-NC-SA]

Chimpanzees (*Pan*) are the closest living relatives to modern humans. It is commonly cited that less than 2% differences in their nucleotide sequences exist with humans (Chimpanzee Sequencing and Analysis Consortium, 2005). More recent findings in comparing the complement of genes (including duplication and gene loss events) now describe the difference in genomes at about 6% (Demuth JP, et al., 2006).



The Pan-Homo divergence. A display at the Cradle of Humankind illuminates the skulls of two extant Hominini with a series of model fossils from the Hominina subtribe of Australopithecina and Homo. Credit: Jeremy Seto [CC-BY-NC-SA] https://flic.kr/p/SmhHTd

THE GENUS *HOMO*



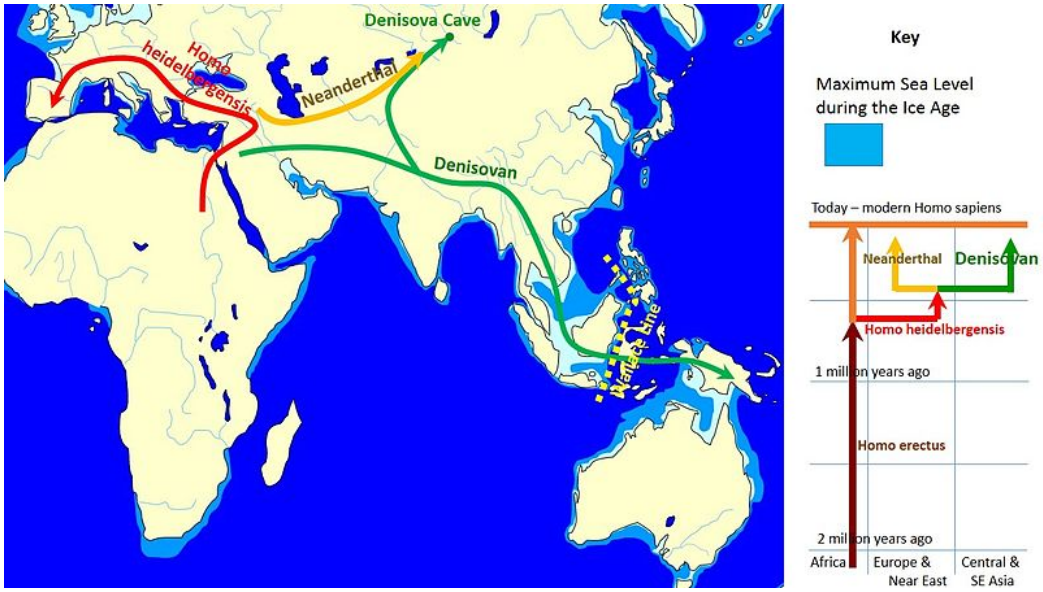
An underground lake at inside the Sterkfontein Cave system at the Cradle of Humankind (South Africa) Credit: Jeremy Seto [CC-BY-NC-SA] https://flic.kr/p/RczrEg

The rise of the human lineage is thought to arise in Africa. Fossils of *Austroloptihs* (southern apes) found in death traps, like those at the Cradle of Humankind, reveal a historical record of organisms inhabiting the landscape. The breaks in the ceiling of the caves provide opportunities for animals to fall inside these caves to their death. The limestone deposits of the caves serve as an environment for fossilization and mineralization of their remains. An abundance of fossilized hominids in these caves including *Australopithecus africanus*, *Australopithecus prometheus*, *Paranthropus boisei*, and the newly discovered *Homo naledi* continue to reveal the natural history of the genus *Homo* from 2.6 million to 200,000 years ago.



The entrance to the archaeological site at Sterkfontein, Cradle of Humankind (South Africa). Credit: Jeremy Seto [CC-BY-NC-SA] https://flic.kr/p/ULs2Sv

ANCIENT DNA OF HUMANS



In 2008, a piece of a finger bone and a molar from a Siberian Cave were found that differed slightly from that of modern humans and Neandertals. The cave, called Denisova Cave, maintains an average temperature of 0ºC year-round and the bones were suspected to contain viable soft tissue. An initial mitochondrial DNA analysis revealed that these hominids represented a distinct line of humans that overlapped with them in time (Krause *et al.*, 2010). Analysis of the full nuclear genome followed and indicated that interbreeding existed between these Denisovans, Neandertals and modern humans (Reich *et al.*, 2010). Furthermore, analysis of DNA from a 400,000-year-old femur in Spain revealed that these three lines diverged from the species *Homo heidelbergensis* with Denisovans closest in sequence similarity (Meyer *et al.*, 2016).

Between modern humans, markers found in the mtDNA can be used to trace the migrations and origins along the maternal line. Similarly, VNTRs found on the Y chromosome have revealed migration patterns along paternal lines within men. Other markers, like the insertion points of transposable elements, can be used to further describe the genetics and inheritance of modern humans while providing a snapshot into evolutionary history.

OTHER RESOURCES

- **Great Ape Mitochondrial Sequences**
- http://media.hhmi.org/biointeractive/click/Origins/01.html
- Yunis JJ, Prakash O. The origin of man: a chromosomal pictorial legacy. Science.1982 Mar 19;215(4539):1525-30.
- Chimpanzee Sequencing and Analysis Consortium. Initial sequence of the chimpanzee genome and comparison with the human genome. Nature. 2005 Sep 1;437(7055):69-87.
- Demuth JP, De Bie T, Stajich JE, Cristianini N, Hahn MW. **The evolution of mammalian gene families**. PLoS One. 2006 Dec 20;1:e85.
- Krause, Johannes; Fu, Qiaomei; Good, Jeffrey M.; Viola, Bence; Shunkov, Michael V.; Derevianko, Anatoli P. & Pääbo, Svante (2010), "The complete mitochondrial DNA genome of an unknown hominin from southern Siberia", Nature, **464** (7290): 894–897, doi:10.1038/nature08976, PMID 20336068
- Reich, David; Green, Richard E.; Kircher, Martin; Krause, Johannes; Patterson, Nick; Durand, Eric Y.; Viola, Bence; Briggs, Adrian W. & Stenzel, Udo (2010), "Genetic history of an archaic hominin group from Denisova Cave in Siberia", *Nature*, **468** (7327): 1053–1060, doi:10.1038/nature09710, PMID 21179161
- Meyer M, Arsuaga JL, de Filippo C, Nagel S, Aximu-Petri A, Nickel B, Martínez I, Gracia A, Bermúdez de Castro JM, Carbonell E, Viola B, Kelso J, Prüfer K, Pääbo S. **Nuclear DNA sequences from the Middle Pleistocene Sima de los Huesos hominins.** Nature. 2016 Mar 24;531(7595):504-7. doi: 10.1038/nature17405. PMID:26976447

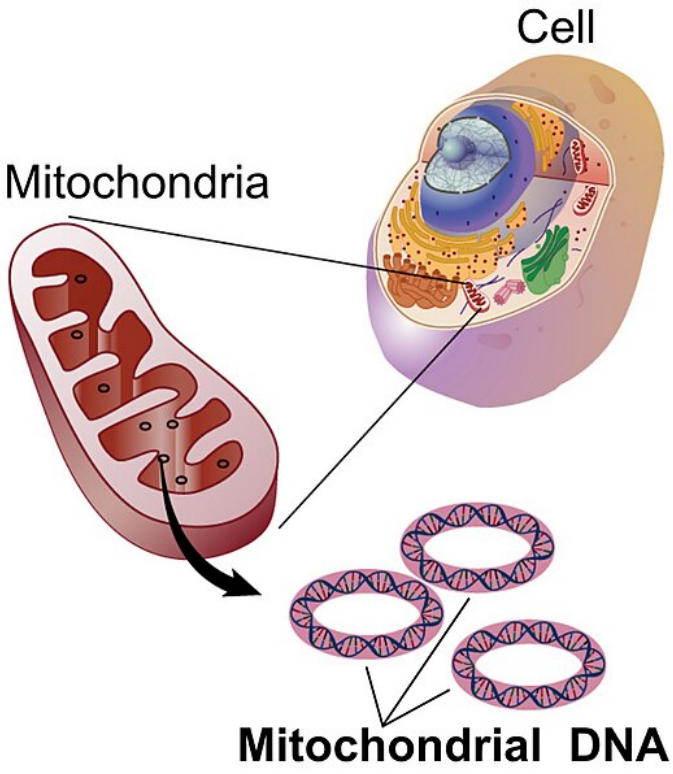
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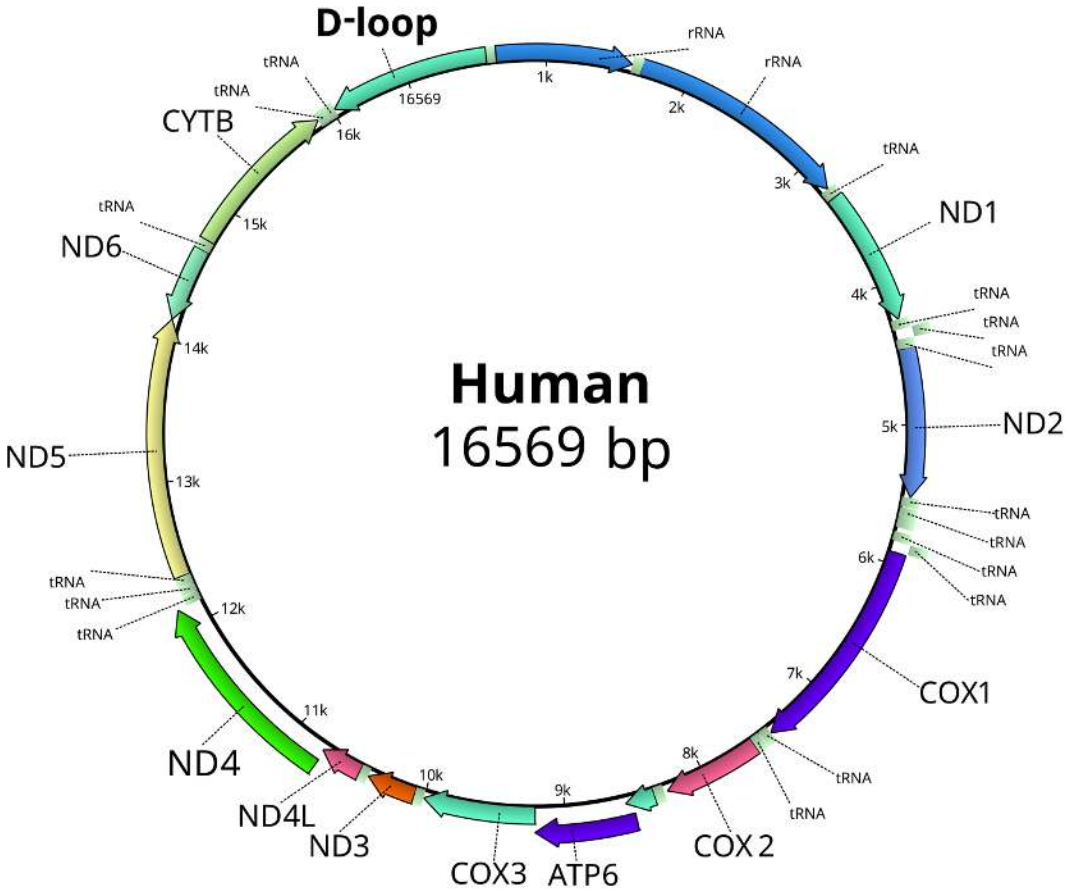
2.1.12.2: MATERNAL LINEAGE

MITOCHONDRIAL AND MATERNAL INHERITANCE

In addition to the 23 chromosomes inherited from mother and 23 chromosomes inherited from father, humans have an additional genome that is only inherited from the mother. This genome comes from the **endosymbiotic** organelle, the mitochondrion.



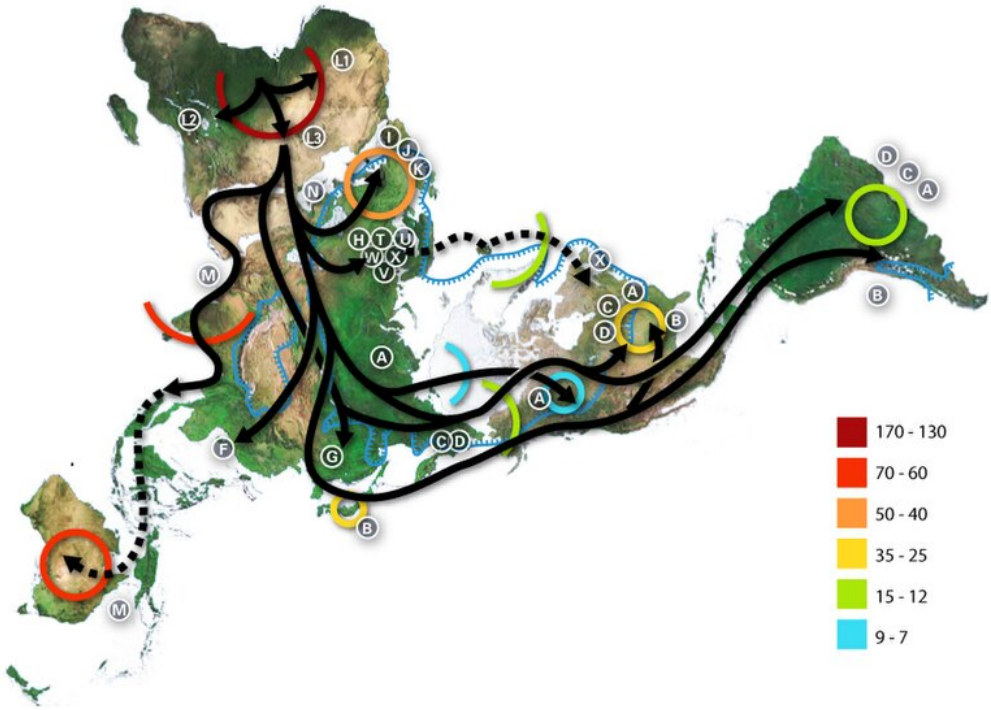
Mitochondria are thought to have arisen in the eukaryotic line when bacteria capable of detoxifying the deadly effects of atmospheric oxygen were engulfed by a eukaryote that did not proceed to consume it. Over the course of time, these formerly free-living bacteria became dependent on the eukaryotic cell environment while providing the benefit to the host cell of aerobic respiration. Hallmarks of this endosymbiotic event include the inner prokaryotic membrane surrounded by the outer eukaryotic membrane, the presence of prokaryotic ribosomes and most significantly, and the circular prokaryotic chromosome. Mitochondria still replicate independently of the host cell but can not survive outside of this cellular environment. Animal mitochondria have the simplest genomes of all mitochondrial genomes, ranging from 11-28kb. The human mitochondrial genome consists of 37 genes which are almost all devoted to processing ATP through oxidative phosphorylation.



Human mitochondrial genome

The human mitochondrial genome (genbank file) consists of 16,569 nucleotides (16.6kb). While most of this 16.6kb genome consists of protein-encoding genes, approximately 1.2kb non-coding DNA takes part in signals that control the expression of these genes and replication processes. It is the area of DNA where the double-strandedness is displaced and having the name **D-loop** (displacement loop). Mutations in this area generally have very little effect on the functioning of the mitochondria. Because of this reduced selection pressure on this area, this **control region** is also referred to as the **hypervariable region**. This hypervariable region actually has 10 times more SNPs than the nuclear genome. Due to this abundance of mutations, it is possible to track down the maternal line of an individual. Why just maternal? The human oocyte contains many mitochondria while sperm cells only contain mitochondria that power the flagellar motion. Upon fertilization, the flagellum and the associated mitochondria are lost, leaving the zygote with only maternal mitochondria.

The cluster of SNPs found in the mitochondrial control region are linked and are always inherited together. Because of the lack of paternal contribution, this linkage is referred to as a **haplotype**, or “half-type”. Tracking these polymorphic haplotypes, a family tree of humans was developed in the 1980s which concluded that humans arose from a metaphorical “Mitochondrial Eve” 200,000 years ago. As a metaphor to the Biblical Eve, this alludes to an origin but unlike the Biblical event, this does not mean that it was a single woman that gave rise to all of modern humanity. On the contrary, the metaphor merely indicates that a series of females; sisters and cousins, of this line gave rise to modern humans.



Migration map of mitochondrial haplogroups. Numbers represent 1000 years ago.

The use of mitochondria for this analysis provides great flexibility, especially from ancient sources. Unlike the nuclear genome which only has 2 copies of DNA per cell, the mitochondria are abundant in number and provide many copies of genome per cell. Ancient sources of DNA in fossils will most often have degradation of the DNA. The mitochondrial genome is just as likely to undergo degradation over time. However, the high copy number allows for gaps to be filled in easily. SNPs do not alter the overall size of the hypervariable region, therefore amplification by PCR can not resolve these differences based on agarose gel migration. However, **amplicons** (amplified copies) can be sent for sequencing whereby each nucleotide can be called out in succession and reveal the specific SNPs.

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2.1.12.3: MATERNAL LINEAGE (ACTIVITY)

THE PCR AMPLIFICATION OF THE MITOCHONDRIAL CONTROL REGION

There are 2 hypervariable regions within the control region of the mitochondria. This exercise amplifies just one of these. For more definitive results, both should be amplified and sequenced. This exercise will permit us to have a rough idea of the origins of our maternal line and we will be able to attribute ourselves to various tribes throughout the world. The human mitochondrial genome (genbank file).

Forward Primer 5'-TTAACTCCACCATTAGCACC-3'

Reverse Primer 5'-GAGGATGGTGGTCAAGGGAC-3'

1. PCR the previously extract DNA samples.

- Pour 2% agarose into casting apparatus in refrigerator.
- 2 gels per class need to be made → 100ml of TBE with 2g agarose
- Add 5µl SYBR safe solution into the molten agarose before casting.
- Place 2 sets of combs into the gel → at one end and in the middle.

2. Load the gel with DNA ladder and PCR.
3. Run the gel at 120V for 20 minutes.
4. Visualize on the UV transilluminator.
5. Document with a camera to verify amplification.
6. The instructor will submit viable reactions for sequencing.
7. **Analyze data during Bioinformatics Lab session.**
    1. Using NYCCT email address, register for an account at https://dnasubway.cyverse.org/.
    2. Retrieve reference mitochondrial sequences.
    3. Perform multiple sequence alignment using MUSCLE.
    4. Draw phylogenetic trees using PHYLIP and visualize using a FigTree.

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2.1.12.4: ALU INSERTION (ACTIVITY)

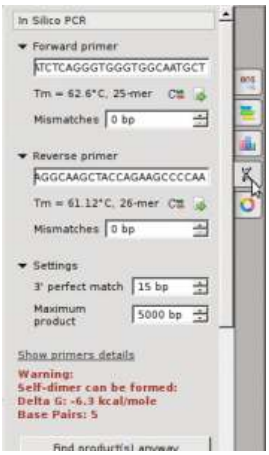
Alu's are unique SINEs that appear in the primate lineage and reveal the lineage and diversification of primates. While retrotransposons can disrupt gene (as in some cases of hemophilia), they often land outside of genes or within introns without effect. One example of a non-disruptive Alu element in humans is found in the location called **PV92** on chromosome 16. This element is of the youngest subfamily of Alu, called Ya5.

Since PV92 does not cause any deleterious effects, it can be used as a non-selected marker to illustrate lineage. Some people have an *Alu* element int his location while others do not. The presence or absence of this marker is viewed as an allele. This lab uses a primer that flanks the location of the *Alu* insertion that span 416 bp. If an *Alu* is present, the amplified DNA will be 300bp larger (the size of an *Alu*) at 731bp.

EXERCISE: IN SILICO PCR OF PV92

Forward primer: 5′ GGATCTCAGGGTGGGTGGCAATGCT 3′
Reverse primer: 5′ GAAAGGCAAGCTACCAGAAGCCCCAA 3′

1. Perform Virtual PCR **Informatics Exercise/Discussion.**
2. Visit BLAST: https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch
3. Paste both primers: GGATCTCAGGGTGGGTGGCAATGCT GAAAGGCAAGCTACCAGAAGCCCCAA
4. Choose "Somewhat Similar"
    1. Locate the locus of the product and the size.
5. Find the PCR fragments in Ugene
    1. Download the sample FASTA file: PV92 sample
    2. Open the file in Ugene and select option "As Separate Sequences in Viewer"
    3. Select the "In Silico PCR" button on the far right (double helix button) and insert the primers



4. A PCR product should be noted for one of the sequences after pressing "Find Products anyway"
5. Click on the second sequence in the viewer and Press "Find Products anyway"

EXERCISE: PCR GENOTYPE PV92 LOCUS

1. PCR the individual samples.
2. Pour 2% agarose into casting apparatus in the refrigerator.
    - 2 gels per class need to be made → 100ml of TBE with 2g agarose.
    - Add 5µl SYBR safe solution into the molten agarose before casting.
    - Place 2 sets of combs into the gel → at one end and in the middle.
3. Load DNA ladder and PCR samples.
4. Run gel at 120V for 30 minutes.
5. Visualize on UV transilluminator.
6. Score gels for the presence/absence of the alleles to determine genotype frequency in the class.

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2.1.12.5: TRANSPOSONS

TRANSPOSABLE ELEMENTS

Mobile genetic elements called **transposable elements** or **transposons** are located throughout the genome. These elements were first described in maize by Barbara McClintock at the Cold Spring Harbor Laboratory where she observed a disruption of coloring in corn kernels that did not follow simple Mendelian inheritance.





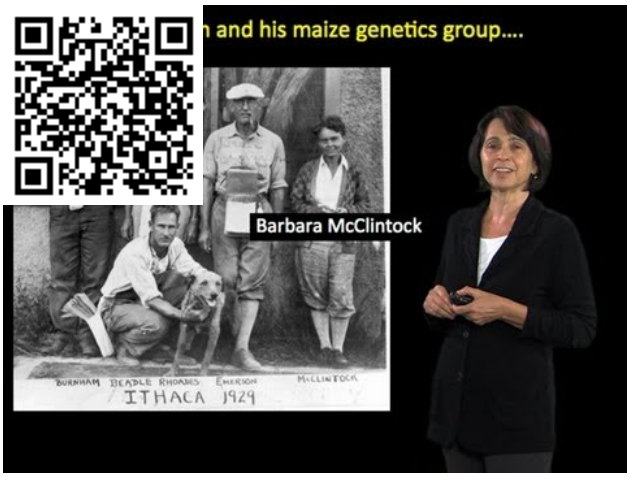
MCCLINTOCK'S CORN KERNELS



Each kernel represents a distinct new individual organism. Kernel color is described through simple Mendelian inheritance where purple is dominant over yellow. Dr. McClintock noticed that some kernels contained spots. She noticed that the coloration disruption could later reverse in subsequent generations. She described the phenomenon of this break in the Mendelian characteristics as a "genetic instability". Over time, she would come to realize that the spots in these kernels arose from the insertion of DNA into the area of genes that were involved in controlling kernel coloration.

Dr. McClintock's description of this phenomenon and the underlying mechanisms was extremely unpopular as it violated what was already known about the *fixity* of genetics. Though initially skeptical, biology has found that these "jumping genes" are found in every taxa including prokaryotes (where they are often associated with genes conferring antibiotic resistance) and she was later awarded the Nobel Prize. Approximately half of the human genome consists of transposons, making up the bulk of what was previously referred to as "junk DNA". **(See Animation: http://www.dnaftb.org/32/animation.html)**

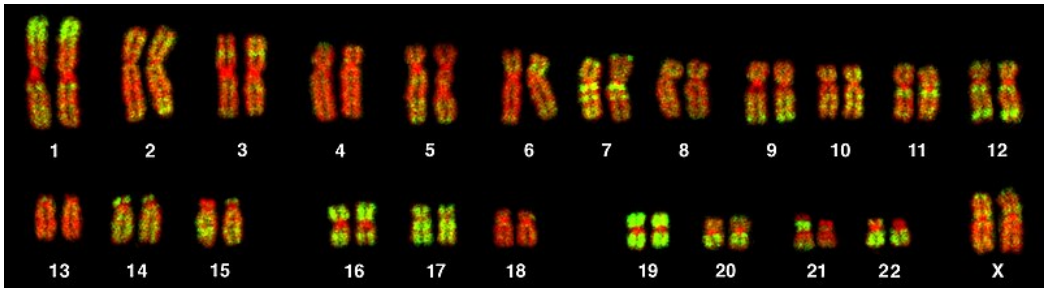
ADVANCED VIDEO OF GENE DISRUPTION BY JUMPING GENES



CLASSES OF TRANSPOSONS

Transposons can be autonomous or non-autonomous. **Autonomous transposons** encode their own **transposase** enzyme that facilitates the jumping of the gene while **non-autonomous transposons** require the transposase activity of another transposable element. Functional DNA transposons are autonomous and work through a "*cut and paste*" mechanism. DNA transposons are delineated by flanking terminal repeats that mark the location that the transposase excises the DNA. These DNA elements then re-integrate at a different location within the genome. The excision from DNA leaves marks of these flanking repeats that can be used to study the rate and level of DNA transposition events within a genome. The insertion of these transposons can affect the expression of nearby genes and can completely disrupt genes they land into as evidenced in the speckled corn kernels that McClintock described.

RNA transposons are called **retrotransposons** because they are transcribed into an mRNA and require a reverse transcription to integrate into the genome. The most common mobile element in the human genome is the Long Interspersed Nuclear Elements (**LINEs**) and the Short Interspersed Nuclear Elements (**SINEs**). These retrotransposons are most abundantly represented by the autonomous LINE1 (L1) and non-autonomous *Alu* elements, respectively. *Alu* elements rely on the expression of the L1 in order to be reverse-transcribed and integrated into the genome. These retrotransposons work in a "*copy and paste*" mechanism and are responsible for genomic expansion. As their classifications signify, LINEs are longer than SINEs. This is due to the presence of a second reading frame that encodes the transposase.

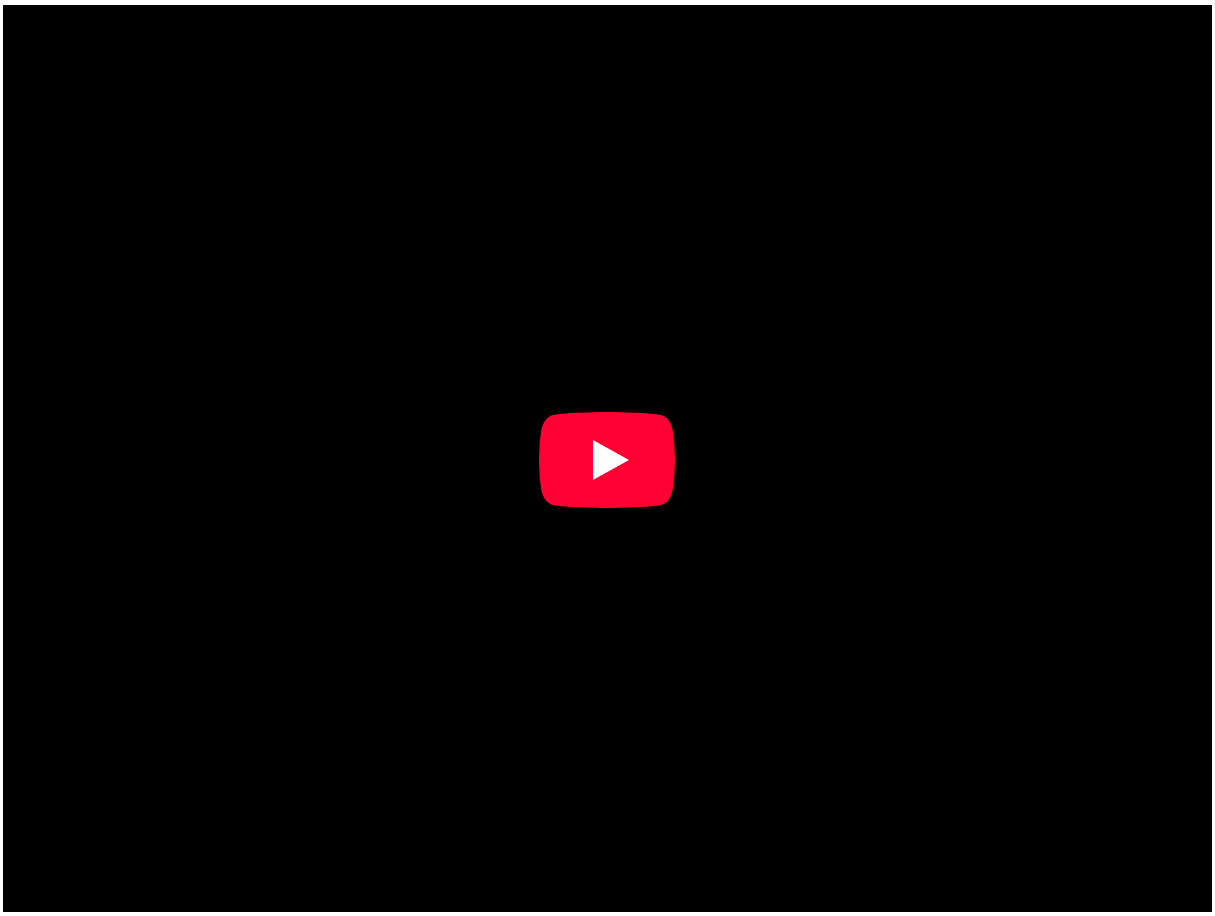


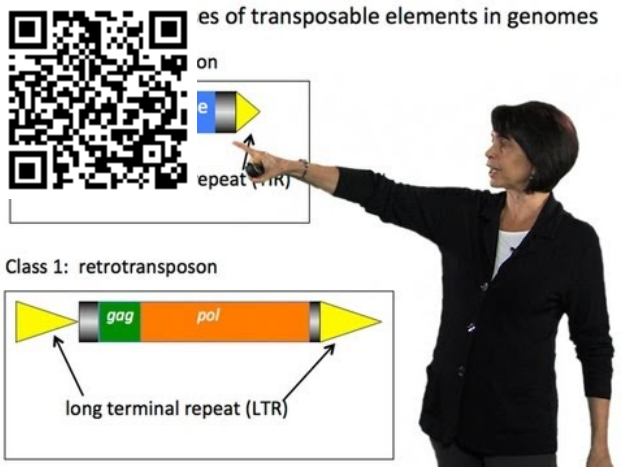
The Human Genome is littered with "junk" DNA. Green coloration corresponds to Alu sequences in this karyogram of the human genome.

Credit: Andreas Bolzer, Gregor Kreth, Irina Solovei, Daniela Koehler, Kaan Saracoglu, Christine Fauth, Stefan Müller, Roland Eils, Christoph Cremer, Michael R. Speicher, Thomas Cremer (CC-BY 2.5)

- Human L1 sequence (fasta)
- Human Ya5 Alu sequence (fasta)

ADVANCED VIDEO OF CLASSIFICATION OF TRANSPOSONS AND THE EVOLUTION OF GENOMES





FURTHER READING

- http://www.nature.com/scitable/topicpage/barbara-mcclintock-and-the-discovery-of-jumping-34083
- http://www.nature.com/scitable/topicpage/functions-and-utility-of-alu-jumping-genes-561

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SECTION OVERVIEW

2.1.13: DNA BARCODING

2.1.13.1: INTRODUCTION

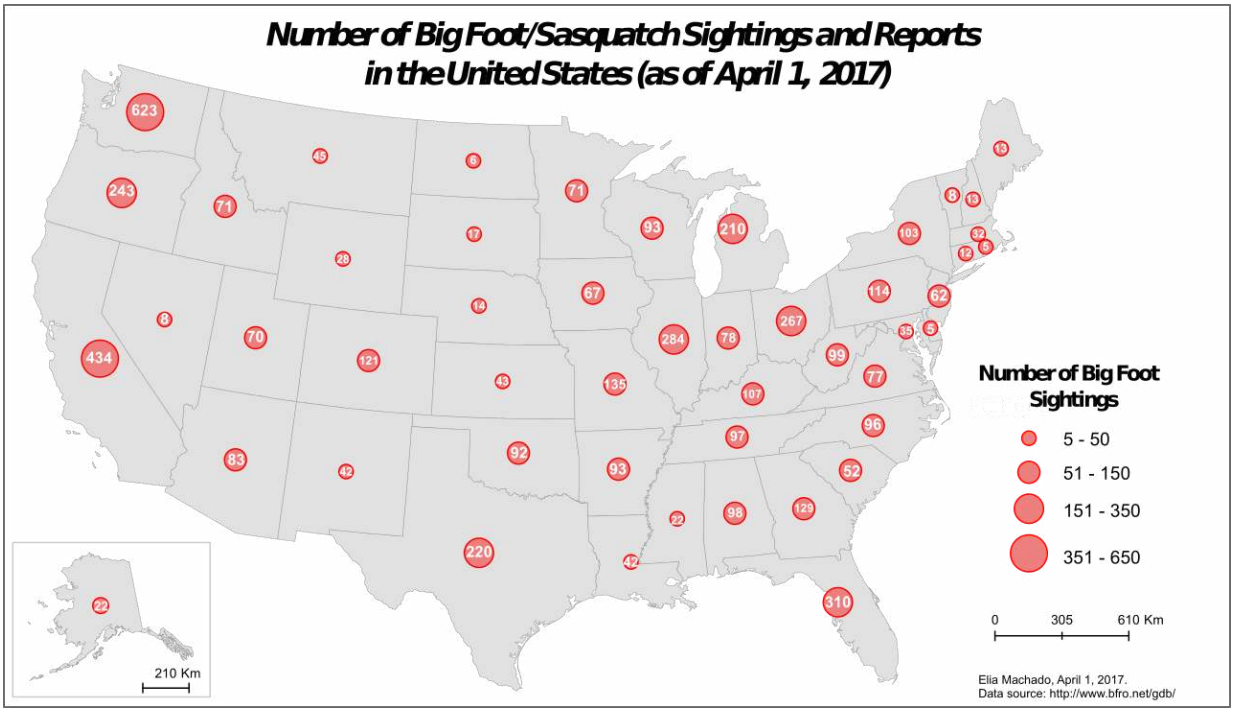
2.1.13.2: BARCODING (ACTIVITY)

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2.1.13.1: INTRODUCTION

CRYPTOZOOLOGY

Cryptozoology is a pseudoscience centered around the description of animals that have little or no evidence of existing. These mythical beasts include Bigfoot, Yeti, Sasquatch, jackalope, Loch Ness Monster, and chupacabra. Little evidence exists to illustrate their existence other than folklore.



Sightings of Bigfoot in North America. Credit: Elia Machado (CC-BY-NC-SA)

Sometimes, physical evidence is left behind like hair or feces. With DNA evidence, we can help to confirm the existence of these unknown creatures. Below features a table from Sykes et al. displaying results on supposed cryptic Apes (Bigfoot/Yeti) and what DNA evidence has revealed them to be.

ref. no.	location	attribution	GenBank sequence match	common name
25025	Ladakh, India	*yeti*	*U. maritimus*	polar bear
25191	Bhutan	*yeti/migyhur*	*U. maritimus*	polar bear
25092	Nepal	*yeti*	*Capricornis sumatraensis*	serow
25027	Russia	*almasty*	*U. arctos*	brown bear
25039	Russia	*almasty*	*Equus caballus*	horse
25040	Russia	*almasty*	*Bos taurus*	cow
25041	Russia	*almasty*	*Equus caballus*	horse
25073	Russia	*almasty*	*Equus caballus*	horse
25074	Russia	*almasty*	*U. americanus*	American black bear
25075	Russia	*almasty*	*P. lotor*	raccoon
25194	Russia	*almasty*	*U. arctos*	brown bear
25044	Sumatra	*orang pendek*	*Tapirus indicus*	Malaysian tapir
25035	AZ, USA	*bigfoot*	*P. lotor*	raccoon
25167	AZ, USA	*bigfoot*	*Ovis aries*	sheep
25104	CA, USA	*bigfoot*	*U. americanus*	American black bear
25106	CA, USA	*bigfoot*	*U. americanus*	American black bear
25081	MN, USA	*bigfoot*	*Erethizon dorsatum*	N. American porcupine
25082	MN, USA	*bigfoot*	*U. americanus*	American black bear
25202	OR, USA	*bigfoot*	*U. americanus*	American black bear
25212	OR, USA	*bigfoot*	*C. lupus/latrans/domesticus*	wolf/coyote/dog
25023	TX, USA	*bigfoot*	*Equus caballus*	horse
25072	TX, USA	*bigfoot*	*Homo sapiens*	human
25028	WA, USA	*bigfoot*	*U. americanus*	American black bear
25029	WA, USA	*bigfoot*	*C. lupus/latrans/domesticus*	wolf/coyote/dog
25030	WA, USA	*bigfoot*	*Bos taurus*	cow
25069	WA, USA	*bigfoot*	*Odocoileus virginianus/hemionus*	white-tailed/mule deer
25086	WA, USA	*bigfoot*	*Bos taurus*	cow
25093	WA, USA	*bigfoot*	*C. lupus/latrans/domesticus*	wolf/coyote/dog
25112	WA, USA	*bigfoot*	*Bos taurus*	cow
25113	WA, USA	*bigfoot*	*C. lupus/latrans/domesticus*	wolf/coyote/dog

Genetic analysis of hair samples attributed to yeti, bigfoot and other anomalous primates
Bryan C. Sykes, Rhettman A. Mullis, Christophe Hagenmuller, Terry W. Melton, Michel Sartori
Published 2 July 2014.DOI: 10.1098/rspb.2014.0161 CC-BY

Cryptozoological samples of hair believed to arise from legendary animals like Bigfoot, Sasquatch, Yeti, etc.

Table taken from http://rspb.royalsocietypublishing.org/content/281/1789/20140161 CC-BY

THE NEED FOR BARCODING

Taxonomy of living things was created by Carl von Linné, who formalized it by using a binomial classification system to differentiate organisms. Binomial nomenclature was used to describe a genus and a species name to each organism to provide an identity. These days, classifications of organisms is becoming increasingly important as a measurement of diversity in the face of habitat destruction and global climate change. There is no consensus on how many life forms exist on this planet, but the estimation of extinction rates is about 1 species per 100-1000 million species. Classification in Linné's day was mostly performed by morphological differences. This was carried on in fossils. However, morphology has many drawbacks, especially in sexually dimorphic species or species with multiple developmental morphologies.

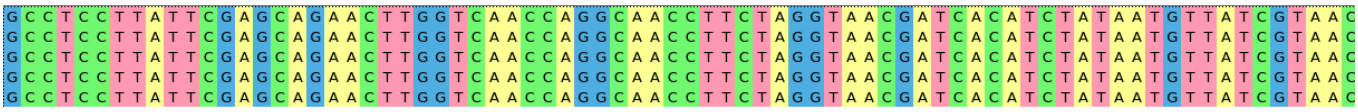


Larva (top) of the Green Lacewing and the adult (bottom)

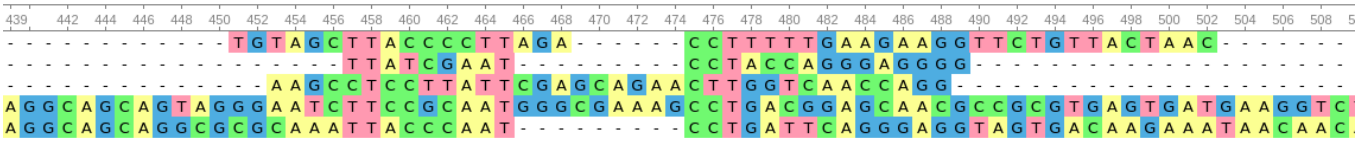
Molecular biology and DNA technologies have revolutionized the classification system of living things especially in providing the ability to match relatedness of these species. **DNA barcoding**, like the name implies, seeks to utilize DNA markers to differentially identify organisms. But what DNA markers should be used? What criteria do we use to develop barcodes? Discrimination, Universality, and Robustness are the criteria used to define the usefulness of barcodes.



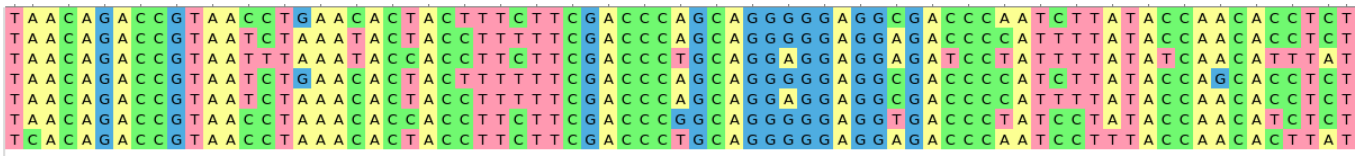
Since the goal of barcoding is to define specific organisms, discrimination is the *primary objective*. **Discrimination** refers to the difference of sequences that occur between species. However, science is easier when there is some universality in the locus used for discrimination. As it sounds, **universality** is an attempt to use the same locus in disparate genomes. While discrimination is about the uniqueness of sequences, universality seeks to use a single set of PCR primers that will be able to amplify that same distinct region with variable sequence similarity. If some region of DNA has absolutely no sequence deviation between species, this has great universality but poor discrimination. But if a sequence has very low sequence similarity, this is great for discrimination but has absolutely no universality and can not be amplified with the same set of primers. **Robustness** refers to the reliability of PCR amplification of a region. Some regions of DNA just don't amplify well or it is too difficult to design appropriate and unique primers for that locus.



A case where there is universality for designing primers, but not an area where discrimination can occur.



While discrimination of different organisms can occur in this situation, the lack of similarity in sequence would make it difficult to design primers. That is, the lack of universality in sequence would also make this PCR not robust.



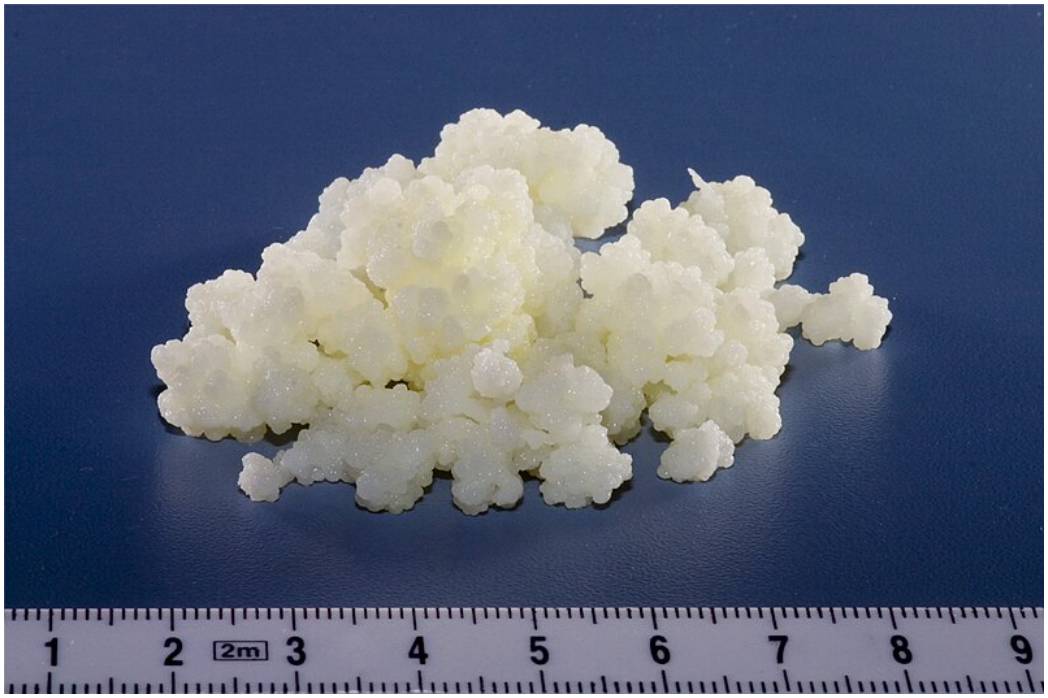
Enough variability in these sequences gives us the ability to discriminate between species. The high similarity provides us the universality required to design primers that may be robust enough to amplify by PCR.

Sometimes, species are so similar for one sequence that a second marker is required. Just as the standard UPC barcode has a series of vertical lines of different spacing and width, a 2-dimensional barcode adds that second dimension of information into a square of dots like in a QR code (Quick Response code). We can also utilize the second, the third, or the fourth set of loci that will aid in increased discrimination just as CoDIS utilizes multiple STR sites to define individual people. In animals, the most commonly used barcode is the mitochondrial gene, Cytochrome Oxidase I (**COI**). Since all animals have mitochondria and have this mitochondrial gene, it offers high universality. It is a robust locus that is easy to amplify and has high copy number with enough sequence deviation between species to discriminate between them.

Animal mitochondrial genomes vary from 16kb-22kb. However, plants, fungi, and protists have wildly different and larger mitochondrial genomes. For plants, we use a chloroplast gene, ribulose-bisphosphate carboxylase large subunit (**rbcL**) or maturase K (**matK**) (Hollingsworth et al. 2011). Prokaryotes are often discriminated by their **16s** rRNA gene while eukaryotes can be identified by **18s** rRNA. COI (a maternally transmitted gene) will not create a clear picture of species identity in the case of hybrid animals (mules, ligers, coydogs, etc.). Sometimes, closely related species are also indistinguishable by a single barcode, so the inclusion of 18s with COI may be necessary to define the identity of the species. Since it is so difficult to meet the three criteria (robustness, universality, and discrimination) for all species, having these multiple barcodes is important. Fungi prove to be difficult in identification by COI, so another marker called the internal transcribed spacer (**ITS**) is used to aid in their identification. We must also remember that not everything with chloroplasts are plants and, therefore, additional markers are used to identify protists.



Lichens are composite organisms composed of cyanobacteria or other algae with fungi. In this case, a single barcode would incorrectly identify the species.



Kefir granules represent colonies of mixed microbes that are used to generate kefir. Credit: A. Kniesel (CC-BY-SA 3.0)

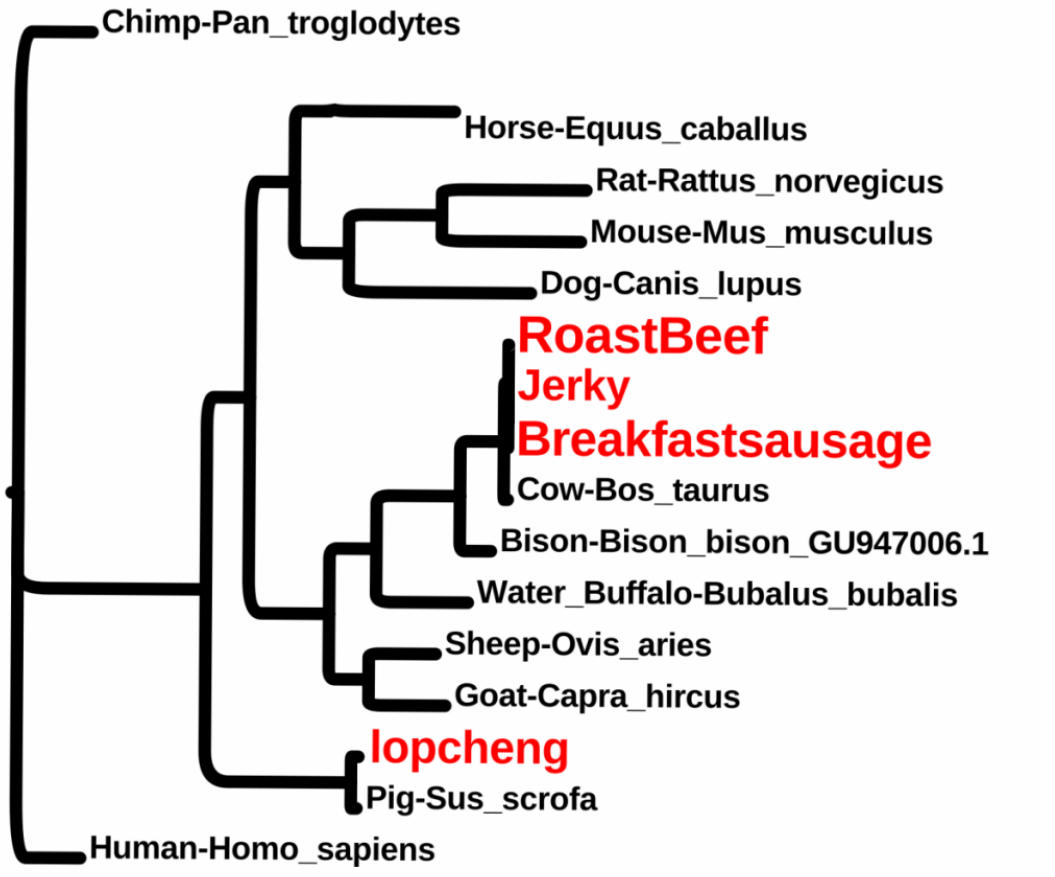


A symbiotic colony of bacteria and yeast is used to ferment kombucha. As the name implies, this is a complex composite colony of multiple species that contribute to the qualities of the kombucha. Credit: Lukas Chin (CC-BY-SA 4.0)

METABARCODING AND MICROBIOMES

CLASS RESULTS

Students wanted to check some food items. These included breakfast sausage from a Halal cart, “beef jerky” from the vending machine, roast beef from the cafeteria, and Chinese sausage (lopcheng).



For more class results, please visit https://openlab.citytech.cuny.edu/dna-barcodes/

FURTHER RESOURCES

- Reference Sequences
  - Gorilla COI: FASTA
  - Apple rbcL: FASTA
  - *Staphylococcus* 16S: FASTA
  - *Toxoplasma* 18S: FASTA
  - Porcinni mushroom ITS: FASTA
- Sykes BC, Mullis RA, Hagenmuller C, Melton TW, Sartori M. Genetic analysis of hair samples attributed to yeti, bigfoot and other anomalous primates. Proc Biol Sci. 2014 Aug 22;281(1789):20140161. doi: 10.1098/rspb.2014.0161.
- Hollingsworth PM, Graham SW, Little DP (2011) Choosing and Using a Plant DNA Barcode. PLOS ONE 6(5): e19254. https://doi.org/10.1371/journal.pone.0019254
- http://www.dnabarcoding101.org/

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2.1.13.2: BARCODING (ACTIVITY)

DNA BARCODING OF SAMPLES

1. Place sample in a clean 1.5 mL tube.
2. Add 100 µl of nuclear lysis solution to tube.
    - Twist a clean plastic pestle against the inner surface.
3. Add 500 µl more nuclear lysis solution to tube.
4. Incubate the tube in a water bath or heat block at 65°C for 5-15 minutes.
5. [Optional] Add 200 µl of protein precipitation solution to each tube incubate on ice for 5 minutes.
6. Centrifuge for 4 minutes at maximum speed to pellet protein and cell debris.
7. Transfer 600 µl of supernatant to a clean labeled tube.
8. Add 600 µl of isopropanol.
9. Centrifuge for 2 minutes at maximum speed to pellet the DNA.
10. Pour off the supernatant and add 600 µl of 70% ethanol to wash the pellet.
11. Centrifuge the tube for 2 minutes at maximum speed and carefully remove the solution.
12. Air-dry the pellet for 10 minutes and add 100 µl of the DNA rehydration solution (TE).
13. Incubate the DNA at 65°C for 5-10 minutes to dissolve.
14. Obtain a PCR tube containing Ready-To-Go PCR Bead. Label the tube with your identification number.
15. Use a micropipette with a fresh tip to add 23 µL of one of the following primer/loading dye mixes to each tube. Allow the beads to dissolve for 1 minute.
    - Plants: rbcL primers (rbcLaF / rbcLa rev)
    - Fish: COI primers (VF2_t1/ FishF2_t1/ FishR2_t1/ FR1d_t1)
    - Insects: (LepF1_t1/ LepR1_t1)
16. Add 2 µl of your DNA directly into the appropriate primer/loading dye mix.
17. Place tubes in a Thermal cycler.
18. Pour 2% agarose into casting apparatus in the refrigerator.
    1. 2 gels per class needed to be made → 100ml of TBE with 2g agarose
    2. Add 5 µl SYBR safe solution into the molten agarose before casting.
    3. Place 2 sets of combs into the gel → at one end and in the middle.
19. Load DNA ladder and PCR samples.
20. Run the gel at 120V for 30 minutes.
21. Visualize on the UV transilluminator.
22. Document with a camera.
23. Send amplicons of verified samples for sequencing.

- Plant rbcL gene
    - rbcLaf 5'- ATGTCACCACAAACAGAGACTAAAGC-3' (forward primer)
    - rbcLar 5'- GTAAAATCAAGTCCACCRCG-3' (reverse primer)
- Animal coi gene
    - lepF1 5'- ATTCAACCAATCATAAAGATATTGG -3' (forward primer)
    - lepR1 5'- TAAACTTCTGGATGTCCAAAAAATCA-3'(reverse primer)
    - vf1f 5'- TCTCAACCAACCACAAAGACATTGG-3' (forward primer)
    - vf1r 5'- TAGACTTCTGGGTGGCCAAAGAATCA-3' (reverse primer)

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SECTION OVERVIEW

2.1.14: GENETIC MODIFICATION

2.1.14.1: INTRODUCTION

2.1.14.2: GMO FOOD (ACTIVITY)

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2.1.14.1: INTRODUCTION

GENETIC MANIPULATION (SELECTION)

Genetic modification of organisms has been occurring through human manipulation since the beginning of agriculture. Humans selectively bred crops and livestock to propagate desirable traits in a process termed **artificial selection**. The original grass that gave rise to domesticated corn called teosinte hardly resembles what we think of when imagining modern maize.



Teosinte, the progenitor of maize. Corn came about due to selective breeding. Credit: John Doebley (CC-BY)

VARIATION: CROP DOMESTICATION

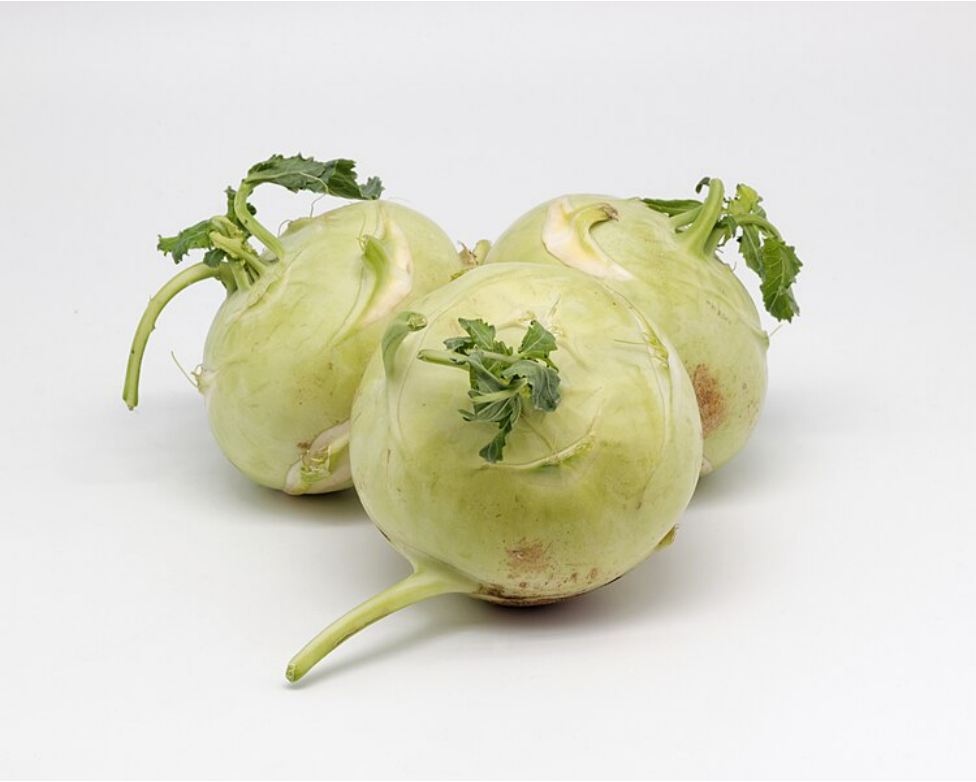
Selective breeding can yield a variety of features even within the same species. Below is a selection of vegetables of the species *Brassica oleracea* that have been developed into different varieties over the course of agricultural history.



Cabbage: Brassica oleracea var. capitata Credit: Forest & Kim Starr ***(CC-BY 3.0)***



Broccoli: Brassica oleracea var. italica Credit: Coyau (CC-BY-SA 3.0)



Kohlrabi: Brassica oleracea var. gongylodes Coyau (CC-BY-SA 3.0)



Romanesco: Brassica oleracea var. botrytis Credit: Richard Bartz (CC-BY-SA 2.5)

VARIATION: ANIMAL DOMESTICATION



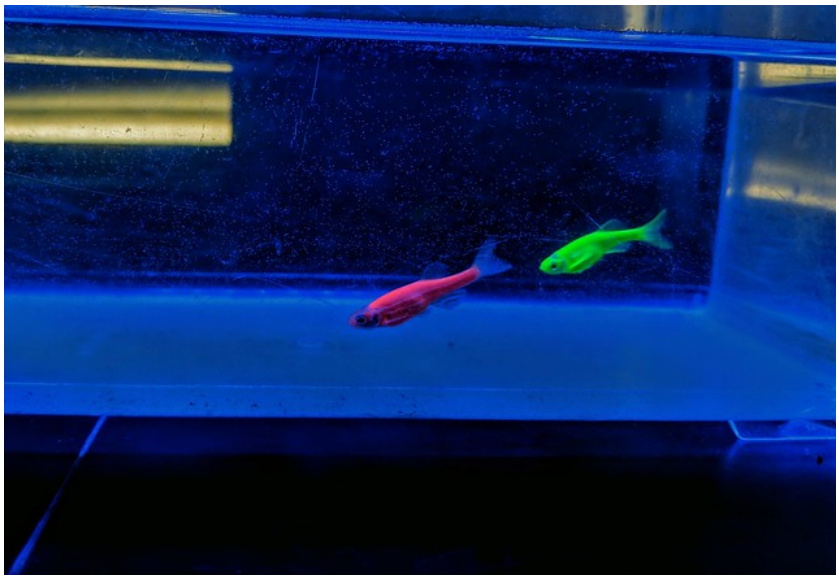
Credit: Mary Bloom, American Kennel Club (CC-BY-SA 4.0)



Companion animals like dogs underwent thousands of years of domestication and selection for traits that were desirable for different circumstances. A high degree of morphological diversity exists between dog breeds and their ancestral grey wolf progenitor.

GENETIC MANIPULATION (ENGINEERED)

Artificial selection takes multiple generations over a long period of time. With the advent of recombinant DNA and biotechnology, scientists can now genetically modify organisms through the introduction of foreign genes to provide desirable characteristics within one generation. This process does not require traits to naturally arise in a species.





GloFish are transgenic zebrafish (Danio rerio) expressing variants of GFP. Bottom features a wild-type fish. Credit: Azul (CC-BY)

GloFish® are novelty pets that have the insertion of various cnidarian fluorescent protein genes into the genome. These fish were released in the United States in 2003 and have subsequently been developed in red, orange, and blue varieties. Black tetras and tiger barbs are also now available.



Black tetra (Gymnocorymbus ternetzi) GloFish Credit: https://www.flickr.com/photos/fergy08/ (CC-BY 2.0)



Wild-type Black Tetra Credit: Fernandograu (CC-BY-SA 3.0)

GENETIC ENGINEERING IN PLANTS

With the advent of agribusiness, agriculture has become a profit-driven venture independent of food production. In this case, high production is paramount. Whereas traditional agriculture or artificial selection was slow and methodical, genetic modification in the context

of agribusiness is instantaneous through **genetic engineering**. The ***objective*** of genetic engineering is to transfer the DNA encoding a useful or favorable gene from an organism that carries that gene to one that does not. Simply inserting DNA into an organism does not result in expression. An appropriate promoter for the transgenic organism must be upstream of the gene of interest in order to drive transcription. In mammals, a strong promoter that will result in expression in every cell is the CMV promoter that is derived from cytomegalovirus. Likewise in plants, a strong promoter that works in every cell is derived from viral promoters like the **CaMV promoter** from cauliflower mosaic virus from the **35S gene** (a ribosomal RNA). (CaMV 35S sequence on NCBI)

Examples of useful traits include:

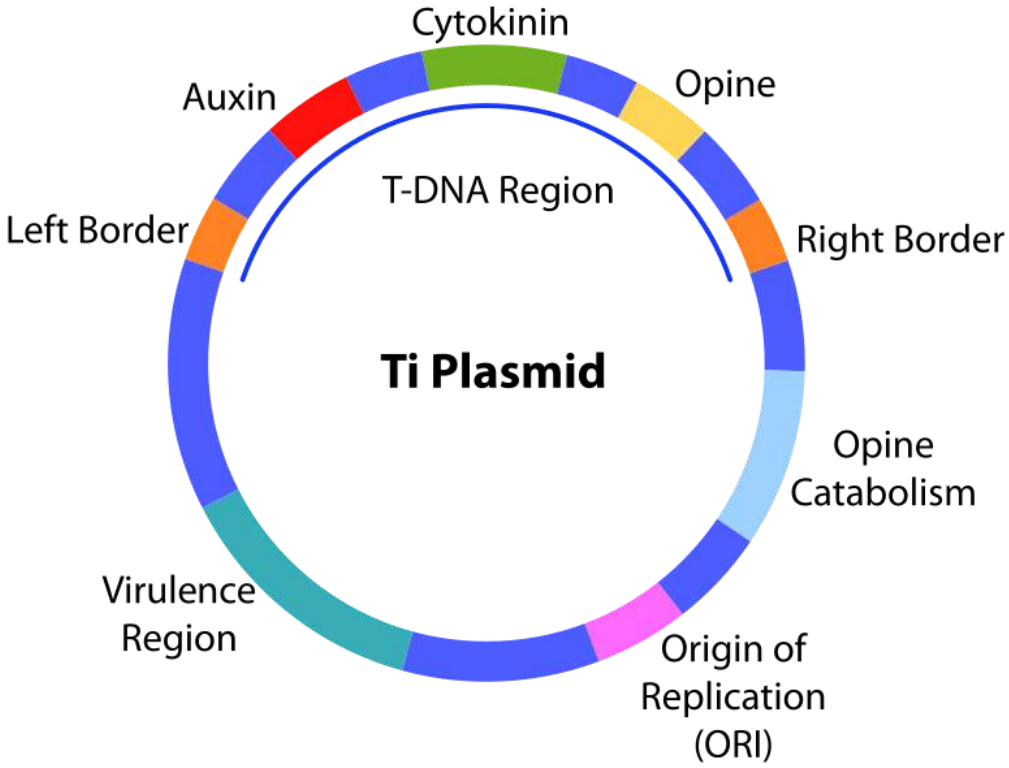
- Degrade herbicides
- Kill agricultural pests
- Synthesize critical nutrients
- To improve color and taste
- Resist damage during transit or prolonged storage.
- Increase in size
- Reduce time to market (more rapid growth or maturation)

Genetically Modified foods have become a hot topic of contention in recent times. These crops are generated through the infection of plant cells by a bacterium called ***Agrobacterium tumefaciens***. *Agrobacterium* is a gram-negative alphaproteobacterium of the family Rhizobiaceae which includes symbiotic nitrogen fixers found in legumes. Unlike those symbionts, *Agrobacterium* is a pathogenic soil bacterium known as a causative agent of crown galls (tumors).



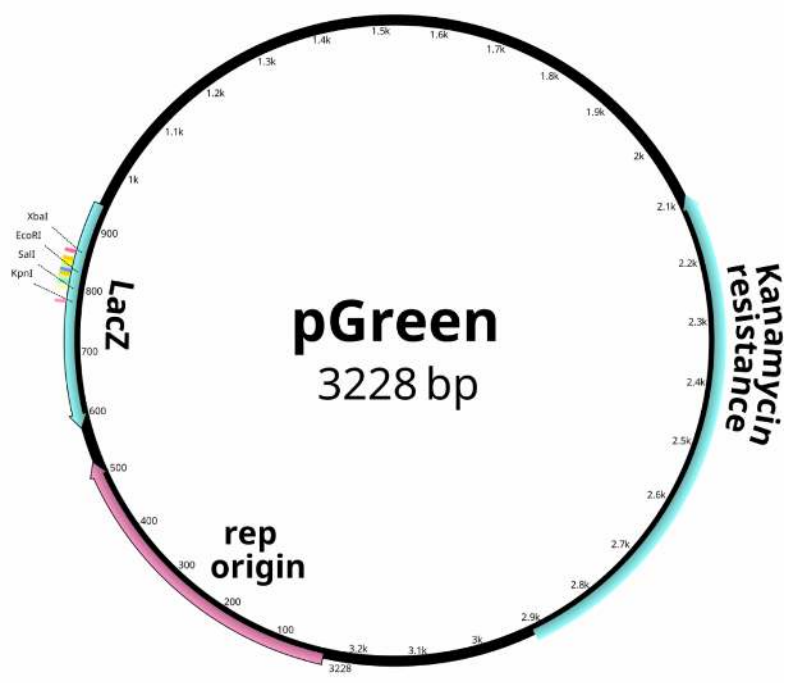
Crown gall on a Kalanchoë infected with Agrobacterium tumefaciens Credit: Bhai (CC-BY-SA 3.0)

The tumors are caused by the infection of plant cells by the bacterium and the subsequent insertion of the **T-DNA** ("Transfer DNA") that has a tumor inducing capability (**Ti**). Through the engineering of the T-DNA in a plasmid, selected genes can be delivered to plant cells through infection of transformed bacteria.

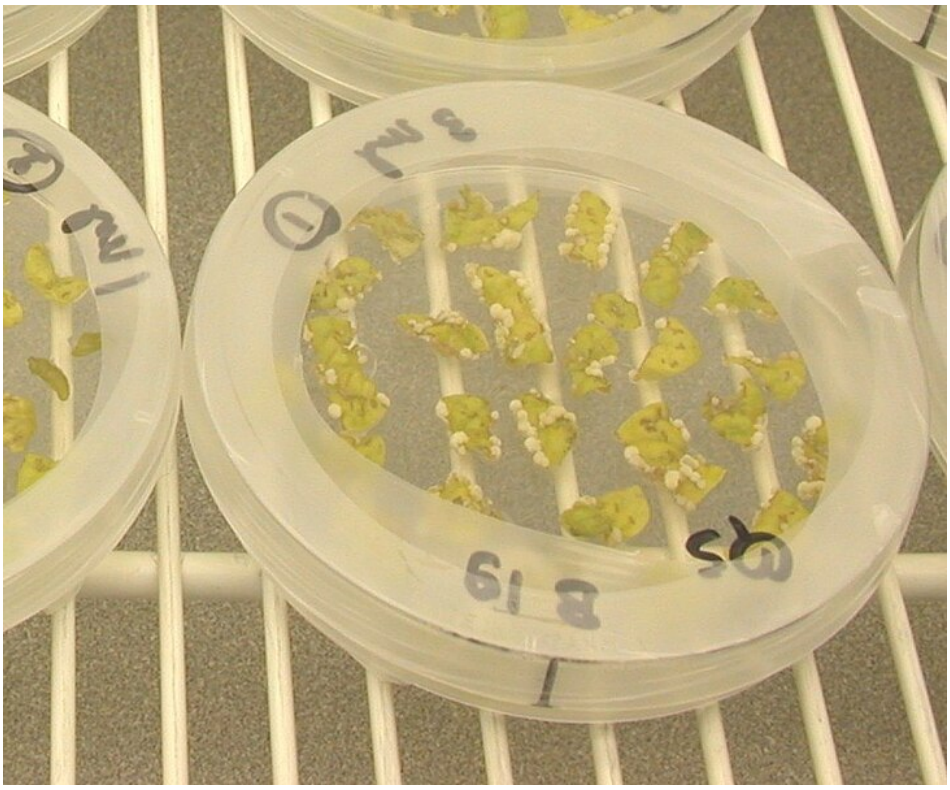


Ti plasmid has the T-DNA region replaced by the transgene. Ti is transformed into Agrobacterium which transduces the DNA into plant cells.

A modified Ti plasmid called **pGreen** was engineered to provide an MCS and selection marker for insertion of foreign genes of interest. In order for these genes to be expressed, they are driven by strong plant promoters like those from the CaMV 35S gene.

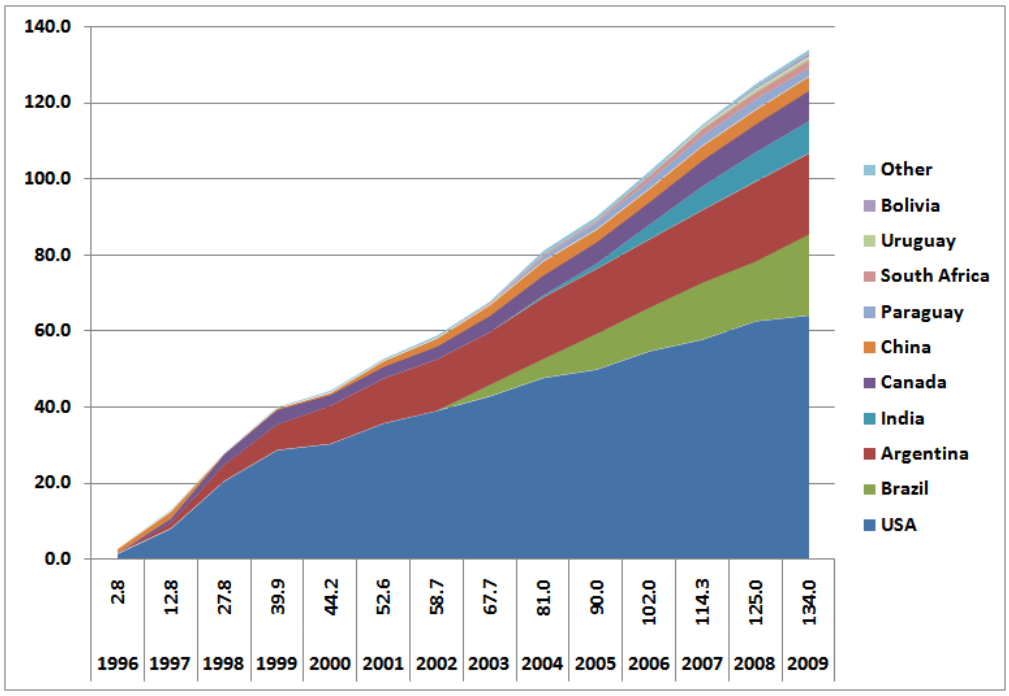


The plant to be engineered is cultured and infected with the transformed *Agrobacteria* that will then induce cysts that eventually root. The strong promoter of the CaMV 35S will constitutively express the gene in all cells of the plant.



Transformation of wild potato in culture using agrobacterium Credit: Seb951 (CC-BY-SA 3.0)

GROWTH OF GMO CROPS



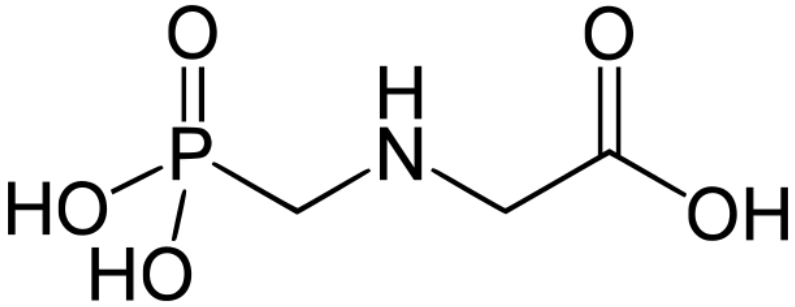
Land area used in millions of hectares.

DAMAGE RESISTANCE

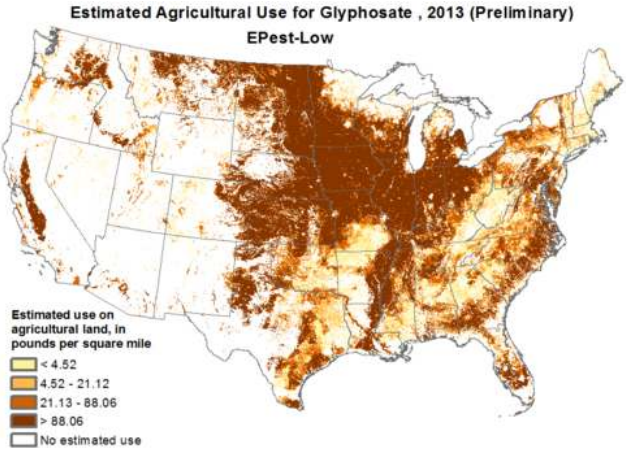
The first genetically modified crop FDA approved for sale was known as the Flavr Savr tomato. Tomatoes are prone to damage during shipping and are therefore picked before ripening. However, vine-ripened tomatoes have a richer flavor. Calgene developed the Flavr Savr and sold it to market between 1994-1997 in the United States. Flavr Savr was modified by inserting antisense into the genome that knocked-

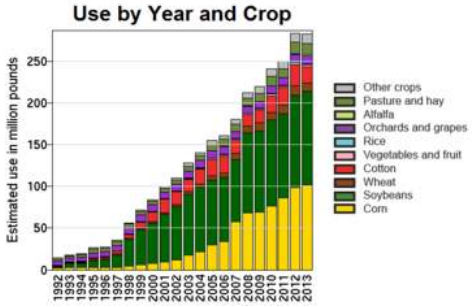
down the expression of polygalacturonase. Polygalacturonase degrades pectin in the cell walls of the fruit that results in softening, proclivity to damage, and eventual rotting.

HERBICIDE RESISTANCE



Roundup is the trade-name of the herbicide **glyphosate** used in agriculture to control weed populations developed and patented by Monsanto. Glyphosate is absorbed through the foliage of plants and interferes with the enzymes that aid in the production of tyrosine, phenylalanine, and tryptophan. Plants and lower organisms generate aromatic amino acids through an enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase which is the target of this compound. A series of Roundup Ready crops were designed by the insertion of the *Agrobacterium* EPSP gene driven by the CaMV 35S promoter. This version of the gene is inherently resistant to glyphosate poisoning.





THE EMERGENCE OF SUPERWEEDS



Palmer amaranth (Amaranthus palmeri), commonly referred to as pigweed, is a pest species in cotton and soy fields that have become glyphosate-resistant.

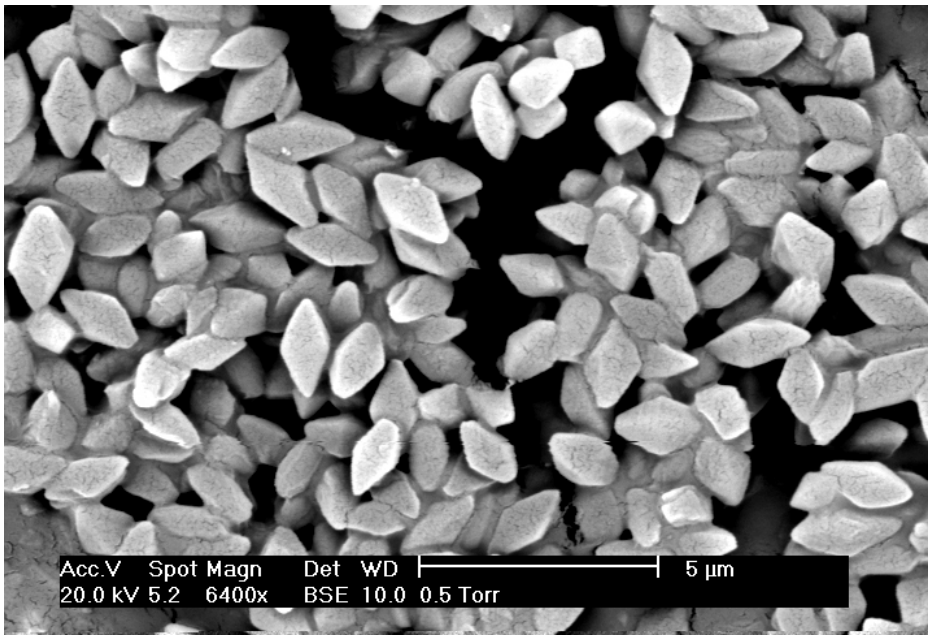
Credit: Pompilid (CC-BY-SA 3.0)

PEST RESISTANCE



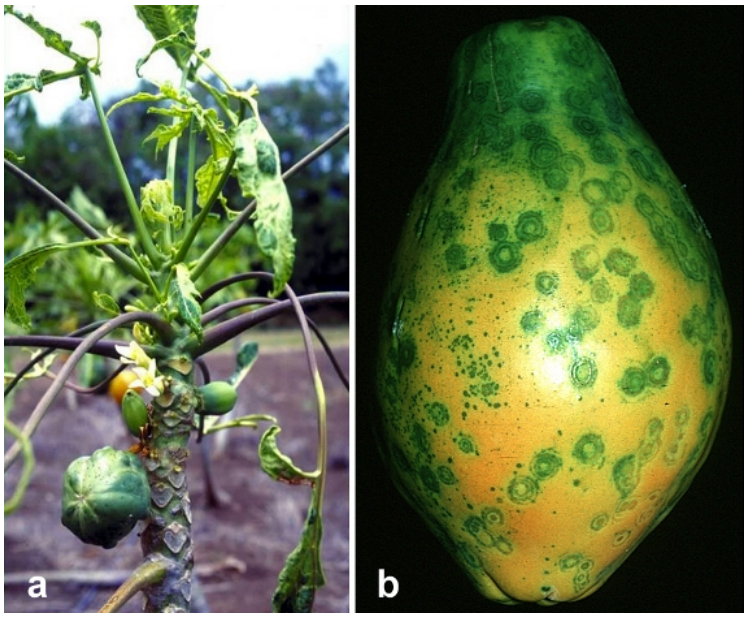
Credit: Cyndy Sims Parr (CC-BY-SA)

Crystals from the *Bacillus thuringiensis* (**Bt**), called ***Cry*** protein, are toxic to various insects: moths & butterflies, beetles, ants, wasps, flies & mosquitoes, bees, nematodes. The insertion of this gene into plants like corn (Bt-Corn) was designed to be resistant to pests.



DISEASE RESISTANCE

Papayas in the United States primarily come from Hawaii. A virus known as Papaya Ringspot Disease threatened the papaya crop in Hawaii. To combat this, papayas were genetically engineered to block viral entry into the papaya cells. Papayas purchased in Latin markets are most likely unmodified and usually come from Mexico where the ringspot disease is not yet a problem.



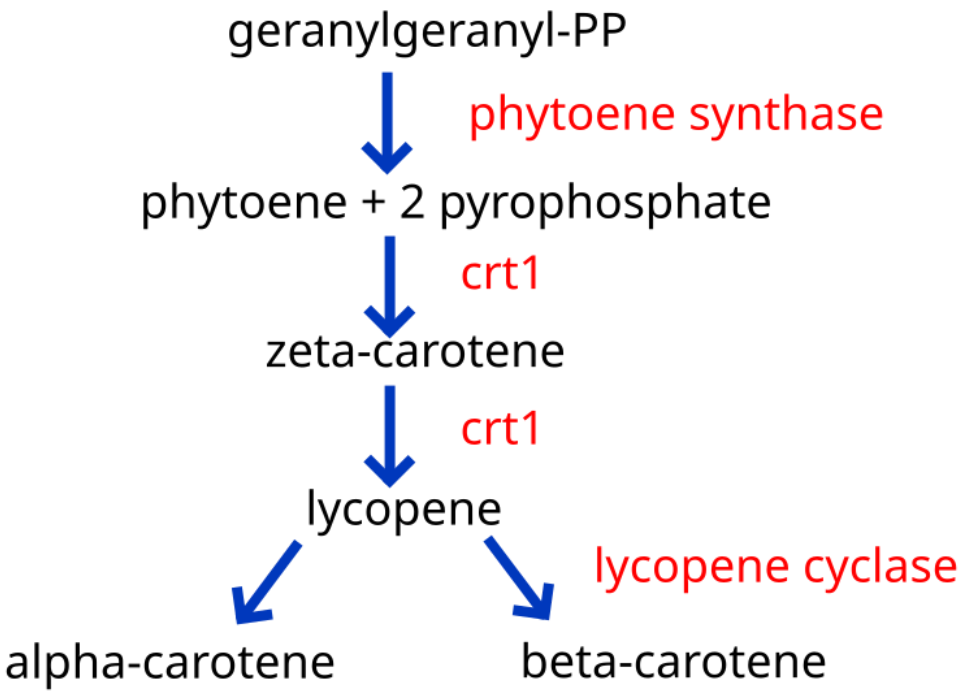
Plum Pox Virus is a threat to the genus *Prunus*. A genetically modified plum plant has been developed called C5. The cells in these plants silence the expression of plum pox coat protein if infected to block propagation of the virus.



An apricot infected with plum pox.



C5 resistant plums



Credit: en:user: Petaholmes, (CC-BY-SA 3.0)

Golden rice is a genetically engineered rice that is meant to address Vitamin A deficiency. It introduces enzyme genes from other species involved in the biosynthetic pathways for β-carotene production, a vitamin A precursor. It is estimated that millions of deaths and irreversible blindness occur in the third world each year due to Vitamin A deficiency and creating this rice was meant to address the problem.

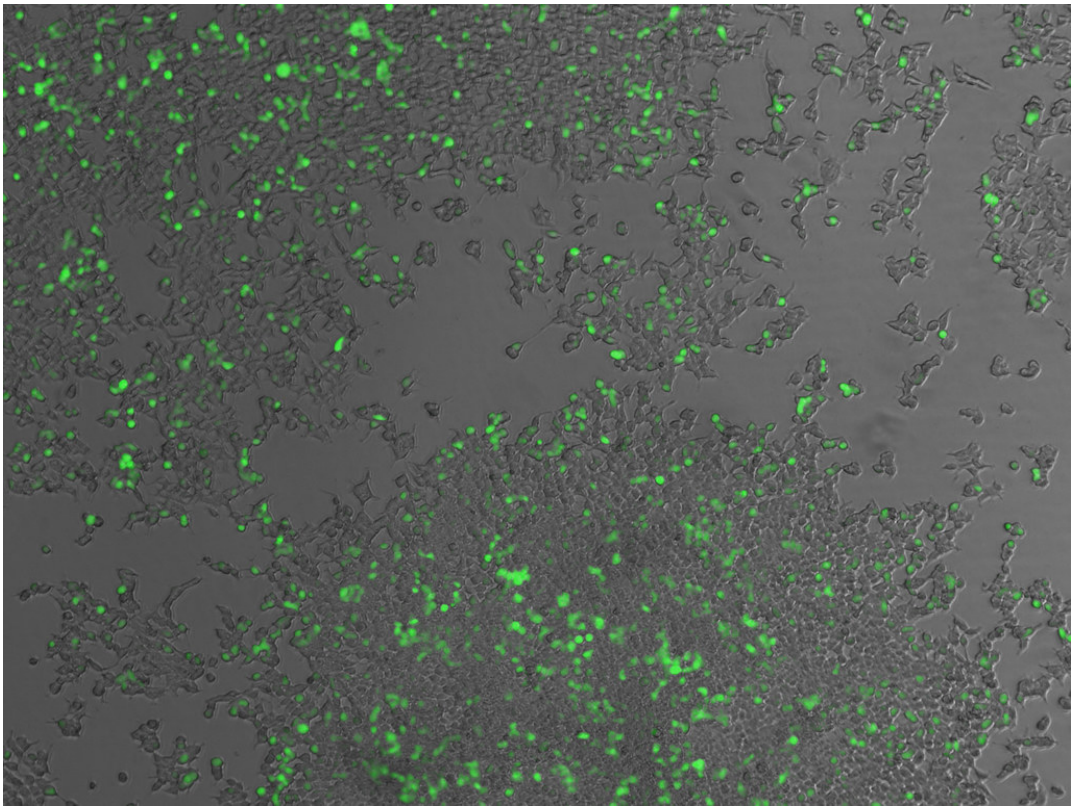
Rice is a staple in many cultures, therefore it is a good delivery system. Many controversies exist surrounding Golden Rice due to anti-GMO sentiment (from patenting systems), cultural sensitivities (white rice revered in certain cultures), and Vitamin A content/conversion doubts.



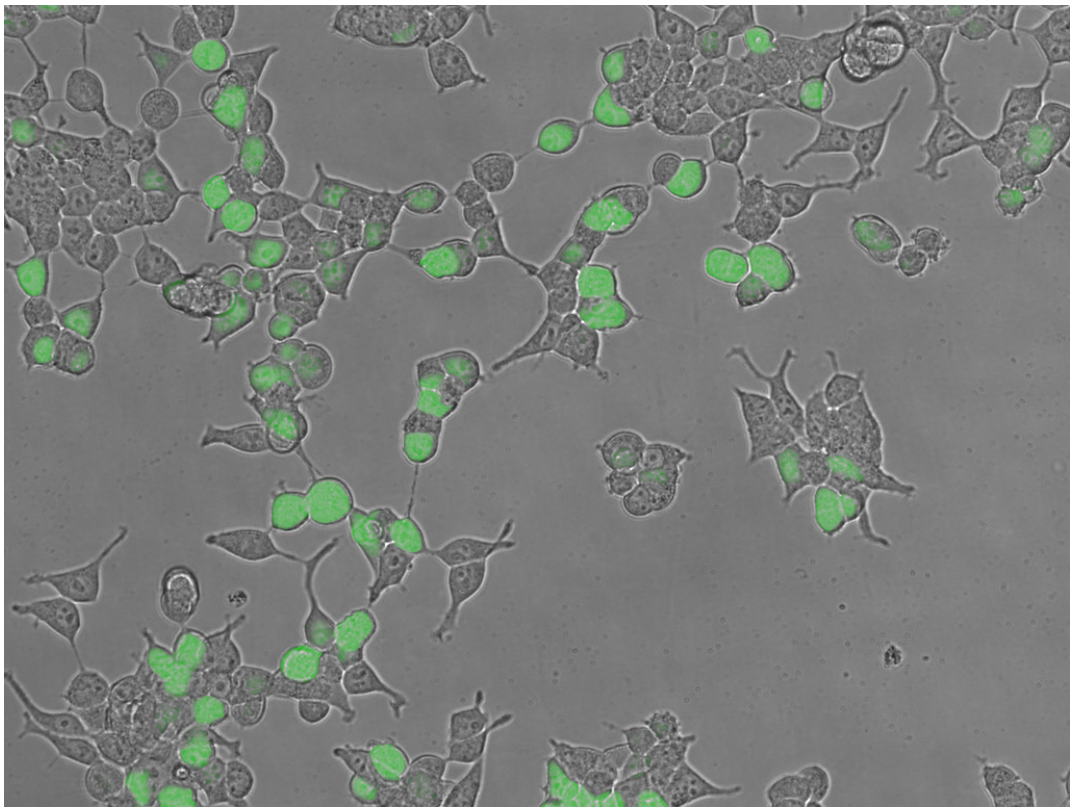
EXPLORE THE DEBATE

- Case studies reveal the complex truths behind GM crop myths.
- Case studies: A hard look at GM crops. Gilbert N. Nature. 2013 May 2;497(7447):24-6. doi: 10.1038/497024a

HETEROLOGOUS EXPRESSION IN TISSUE CULTURE

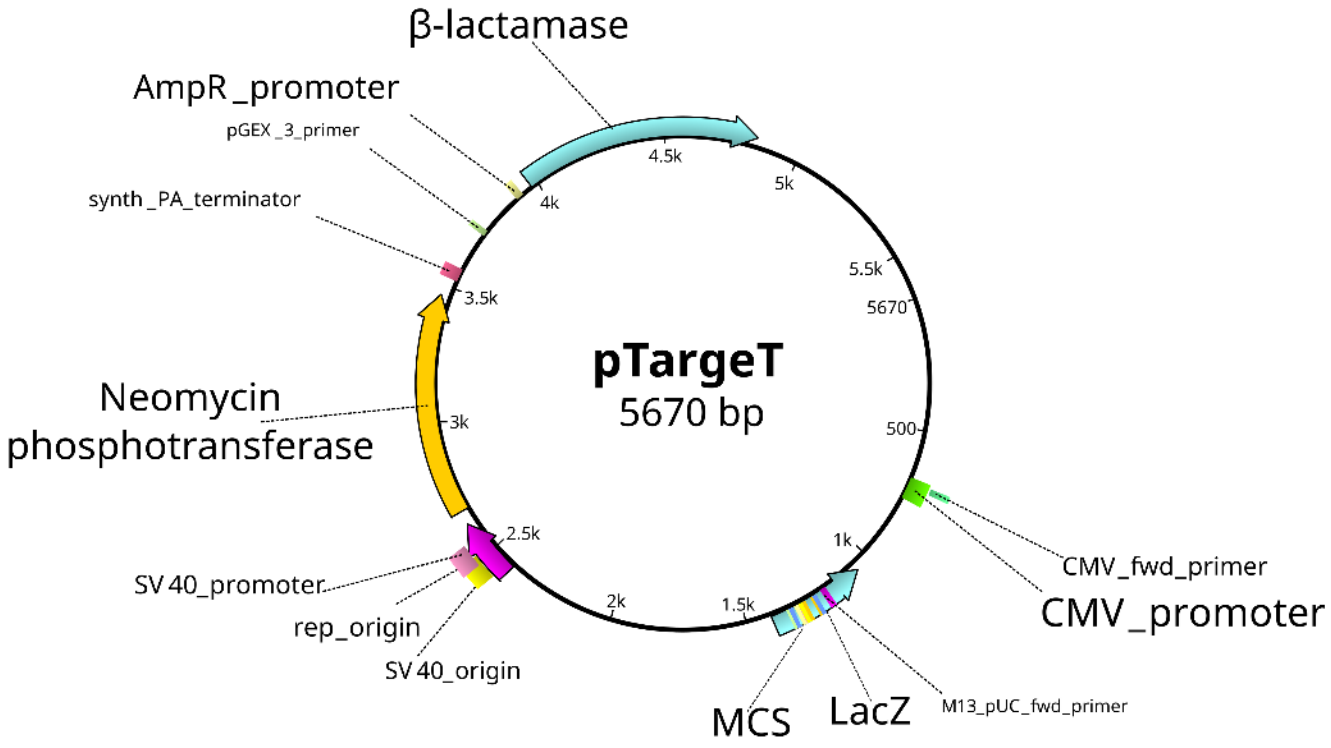


Human Embryonic Kidney cells (HEK293T) expressing the green fluorescent protein.



Higher magnification of HEK293T expressing the green fluorescent protein.

PLASMID STRUCTURE



pTarget mammalian expression vector. The MCS is inside the LacZ gene which permits for blue/white screening of the bacteria after cloning. The LacZ runs in the opposite orientation as the CMV promoter which will drive the gene transcription when inside a mammalian cell. SV40 origin servers as a plasmid replication origin if the cell line also expresses SV40 large T antigen, such as HEK293T cells.

Mammalian expression vectors contain the same hallmark features as bacterial plasmids: bacterial replication of origin and bacterial antibiotic resistance gene (β-lactamase or **Amp_R**). General bacterial plasmid features allow for the carrying and the propagation of the plasmid in a bacterial cell. Mammalian expression vectors additionally include a strong mammalian promoter (like **CMV** from the cytomegalovirus immediate early promoter) upstream of a multiple cloning site (**MCS**). Plasmids transfected into cells are transient in nature unless the DNA is selected for. The inclusion of a mammalian antibiotic resistance gene, like neomycin phosphotransferase (**Neo_R**), allows for the integration of the plasmid into the genome of the cell by using high concentrations of Neomycin or the analog G418.

LIPOFECTION

Cationic lipids can encapsulate plasmid DNA in liposomes. The cationic portions interact with the negatively charged plasma membrane to deliver the DNA into cells.

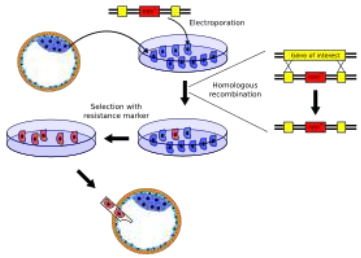
CALCIUM PHOSPHATE TRANSFECTION

Calcium chloride solution can be used to incubate with plasmid DNA. When this solution is mixed with a HEPES-buffered saline solution (HeBS) containing phosphate ions, the solution precipitates onto the surface of mammalian cells where they are taken up with the DNA.

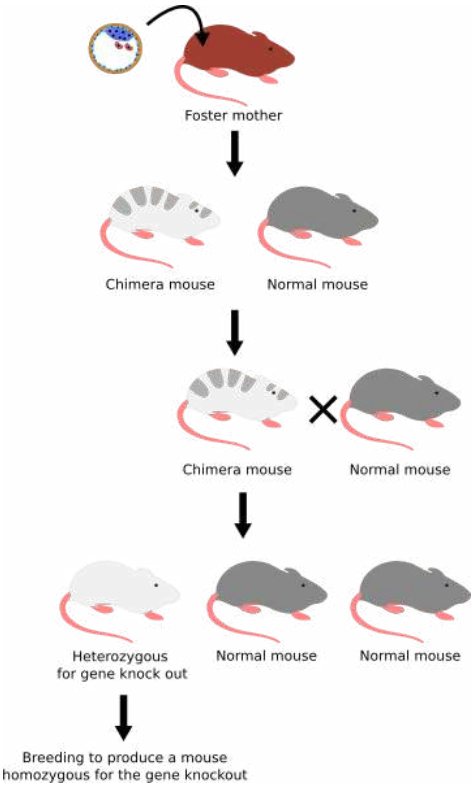
KNOCK-OUT & TRANSGENESIS

In the laboratory, model organisms are modified in order to understand the basic mechanisms of genes. The transformation of recombinant DNA into bacteria is an example of a genetic modification. Other model organisms, like mice, are used to study genes. Through recombinant DNA scientists can selectively ablate a gene, or create a **knock-out** (KO).

Embryonic stem (**ES**) cells are pluripotent cells with the capacity to differentiate into other cell types. Cultured ES cells can be transfected with plasmid DNA in order to genetically alter them. Linearized vectors containing a disrupted gene can homologously recombine with the native gene to replace it. Selection of cells with the disrupted gene by an antibiotic (like G418) enables the isolation and propagation of engineered ES cells.

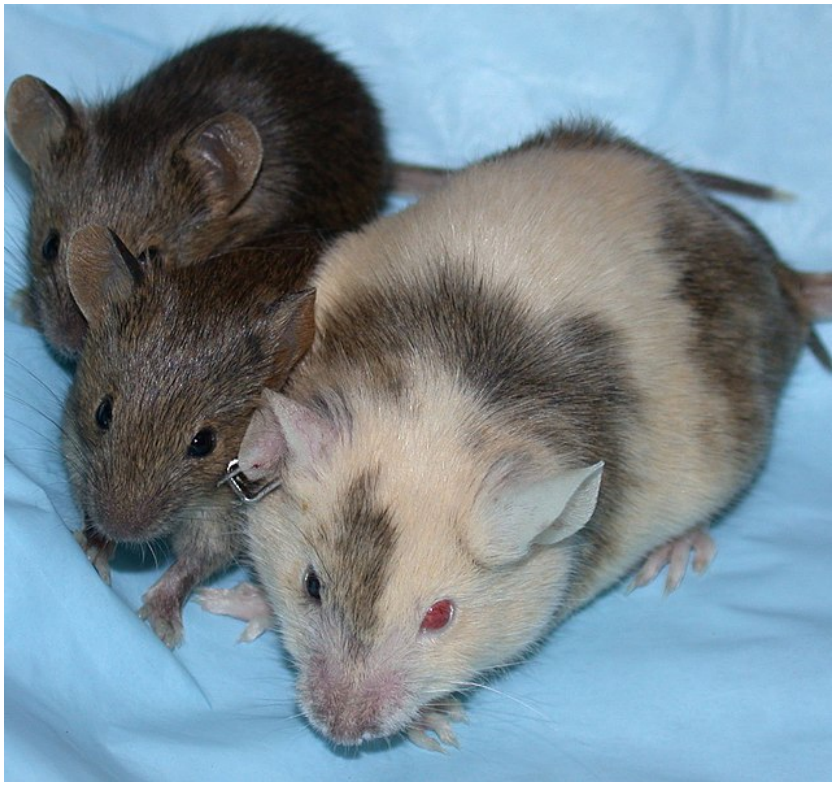


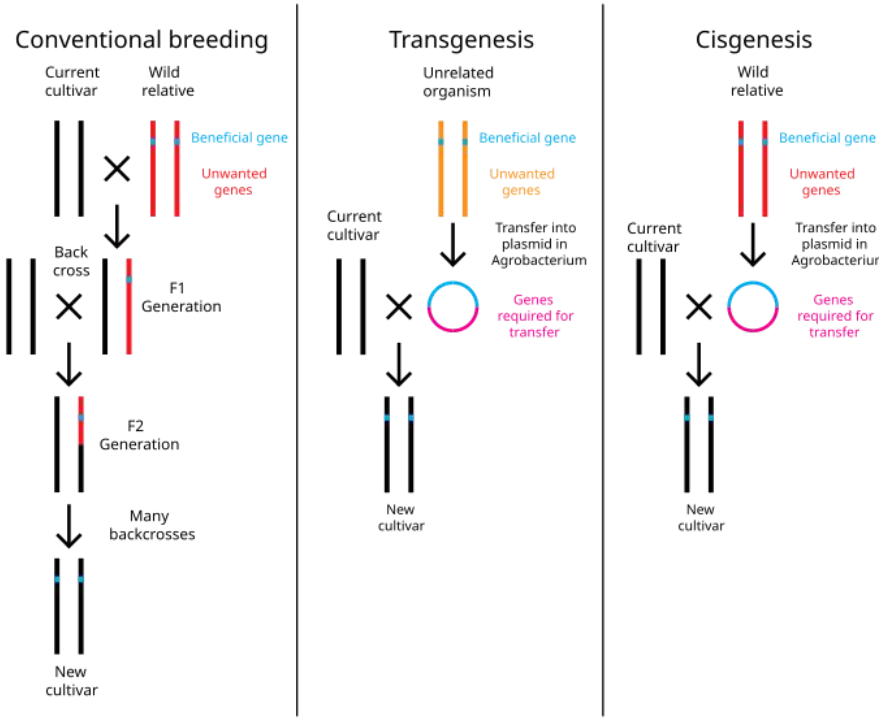
Credit: Kjaergaard (CC-BY- 3.0)



Credit: Kjaergaard (CC-BY- 3.0)

ES cells can be injected into mouse blastocysts and partially contribute to the subsequent mouse upon implantation into a mouse. These first mice are referred to as **chimeras** because they arise from mixtures of cells from 2 genetic sources. Germ-line transmission of the modified cells is desired and breeding of the chimera reveals heterozygous offspring of the engineered background. Full knock-out mice can be generated in the subsequent generation of breeding.



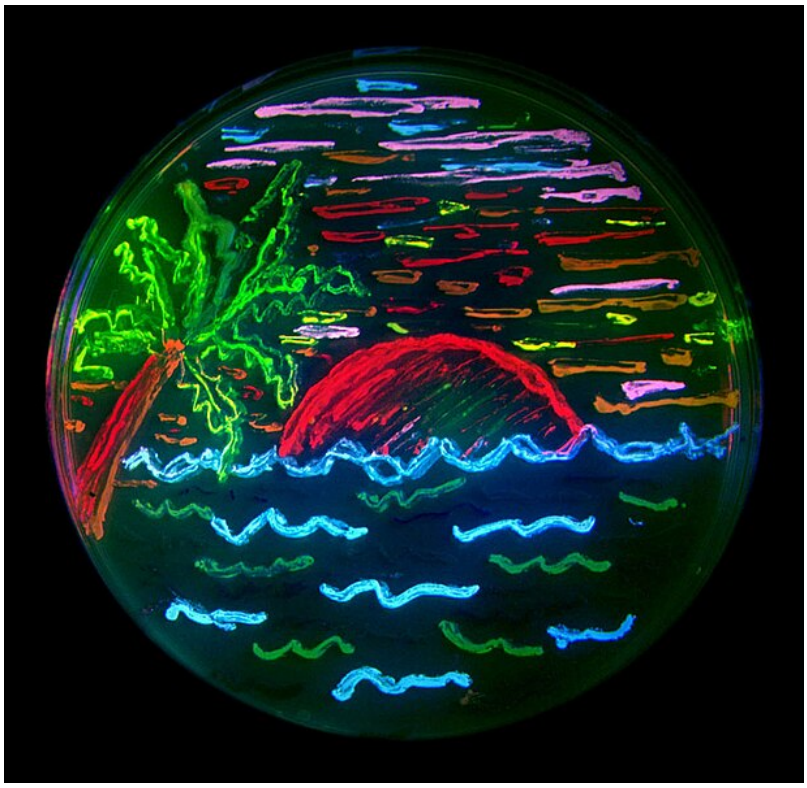


Credit: Smartse (CC-BY-SA 3.0)

Scientists can also overexpress or heterologously express foreign genes in what is termed **transgenic** organisms. As the name sounds, transgene refers to a gene from one place brought across into another.

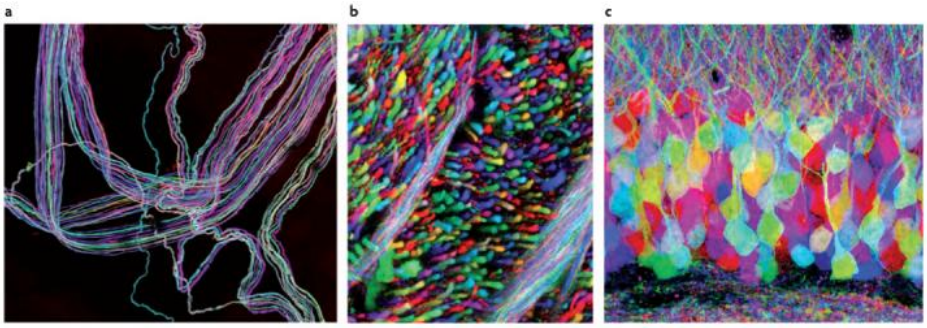
Transgenic and KO models permit scientists to study the roles of genes inside the organism and understand basic functioning.

Through mutagenesis, derivatives of the green fluorescent protein (GFP) have been produced to provide a palette of colors. Additionally, the subsequent discovery of similar genes from other cnidarian species have aided biotechnology by providing tracer molecules within developing organisms or within cells.

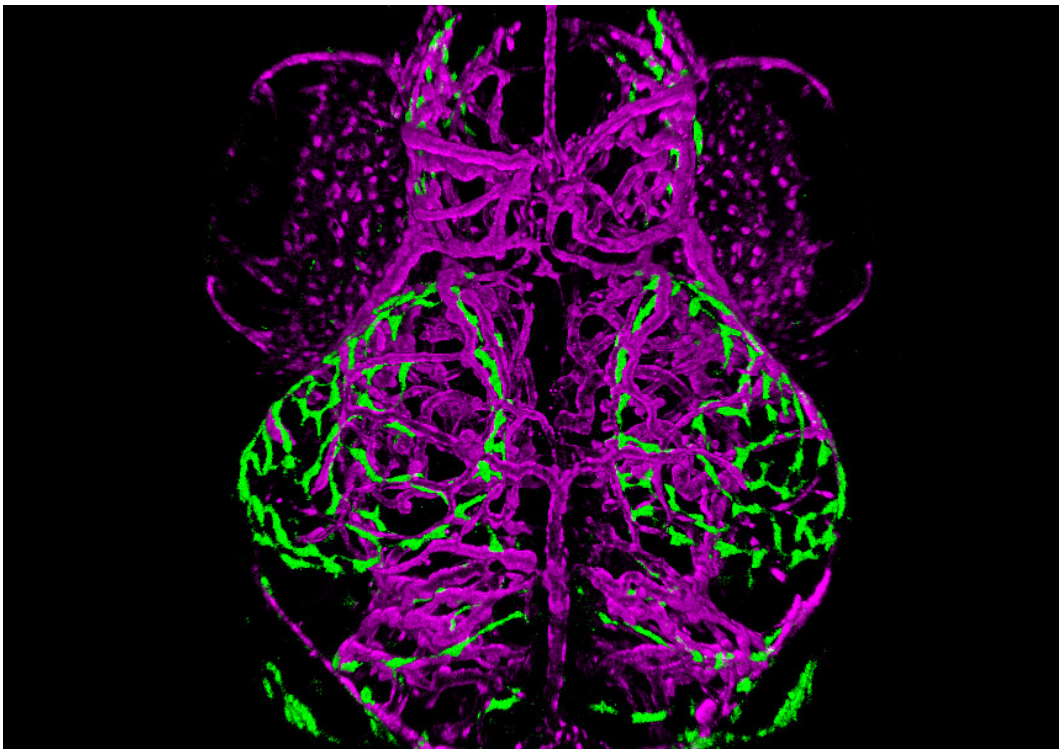


Bacteria expressing various GFP derivatives on agar from the lab of Nobel Laureate Roger Tsien.

While commercial organisms like GloFish are a novelty, directed insertion of GFP and the variants into the genome under different promoter systems allow scientists to understand the cell-specific functioning or contribution to the organism. An example of this can be found in developmental neurobiology where individual axons can be traced.



A "brainbow" is a system where a cassette of GFP variant genes are placed downstream of a neuronal promoter to permit the tracing of individual neurons and their axons in mice. Credit: Jeff W. Lichtman and Joshua R. Sanes (CC-BY 3.0)



Brain of a 10-day old double-transgenic zebrafish. Blood vessels are shown in magenta (Kdrl:mcherry) and a novel population of perivascular endothelial cells are shown in green (MRC1a:eGFP).

FURTHER READING

- CRISP-Cas9 https://www.neb.com/tools-and-resources/feature-articles/crispr-cas9-and-targeted-genome-editing-a-new-era-in-molecular-biology

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2.1.14.2: GMO FOOD (ACTIVITY)

PCR DETECTION OF GM FOOD

Briefly, genomic DNA will be isolated from food items derived from vegetation. Genetic modification will then be identified by PCR of the plant promoter used in genetic engineering, **CaMV 35S**. As a **positive control** for the appropriate extraction of DNA, PCR for **plant-specific tubulin** will be used.

1. Add 100 µL of lysis buffer to each tube containing the plant or food material.
2. Twist a clean plastic pestle against the inner surface of the 1.5-mL tube to forcefully grind the plant tissue or food product for 1 minute.
3. Add 900 µL of lysis buffer to each tube containing
4. Boil the samples for 5 minutes in a water bath
5. Spin for 2 minutes to pellet cell and food debris.
6. Transfer 350 µL of each supernatant to a fresh tube
7. Add 400 µL of isopropanol to each tube
8. Mix and leave at room temperature for 3 minutes.
9. Spin for 5 minutes.
10. Carefully pour off and discard the supernatant from each tube. Air-dry pellet.
11. Add 100 µL of TE buffer to each tube. 5 min at room temperature then keeps on ice.

PCR FOR 35S PROMOTER

1. Label one tube “35S FP” for food product.
2. Label one tube “35S WT” for wild-type soy: Negative control.
3. Label “35S RR” for Roundup Ready® soy plant: Positive control.
4. Different groups will only do one control.
5. Add **22.5 µL** of the 35S primer/loading dye mix to each tube containing PCR bead.
    - 5′-CCGACAGTGGTCCCAAAGATGGAC-3′ (Forward Primer)
    - 5′-ATATAGAGGAAGGGTCTTGCGAAGG-3′ (Reverse Primer)
6. Add **2.5 µL** of food product DNA to the reaction tube marked “35S FP.”
7. Add **2.5 µL** of wild-type or Roundup Ready® soybean DNA to the appropriate reaction tube marked “35S WT” or “35S RR.”

PCR FOR TUBULIN: (POSITIVE CONTROL FOR DNA QUALITY AND PCR CONDITIONS)

1. Label one tube “T FP” for food product.
2. Label one tube “T WT” for wild-type soy.
3. Label “T RR” for Roundup Ready® soy plant.
4. Different groups will only do one control.
5. Add **22.5 µL** of the Tubulin primer/loading dye mix to each tube containing PCR bead.
    - 5′-GGGATCCACTTCATGCTTTCGTCC-3′ (Forward Primer)
    - 5′-GGGAACCACATCACCACGGTACAT-3′ (Reverse Primer)
6. Add **2.5 µL** of food product DNA to the reaction tube marked “T FP.”
7. Add **2.5 µL** of wild-type or Roundup Ready® soybean DNA to the appropriate reaction tube marked “T WT” or “T RR.”

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SECTION OVERVIEW

2.1.15: PROTEIN PRODUCTION

2.1.15.1: INTRODUCTION

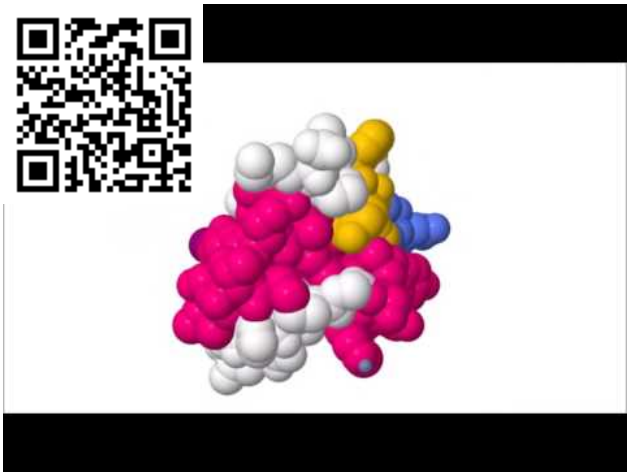
2.1.15.2: PROTEIN PURIFICATION (ACTIVITY)

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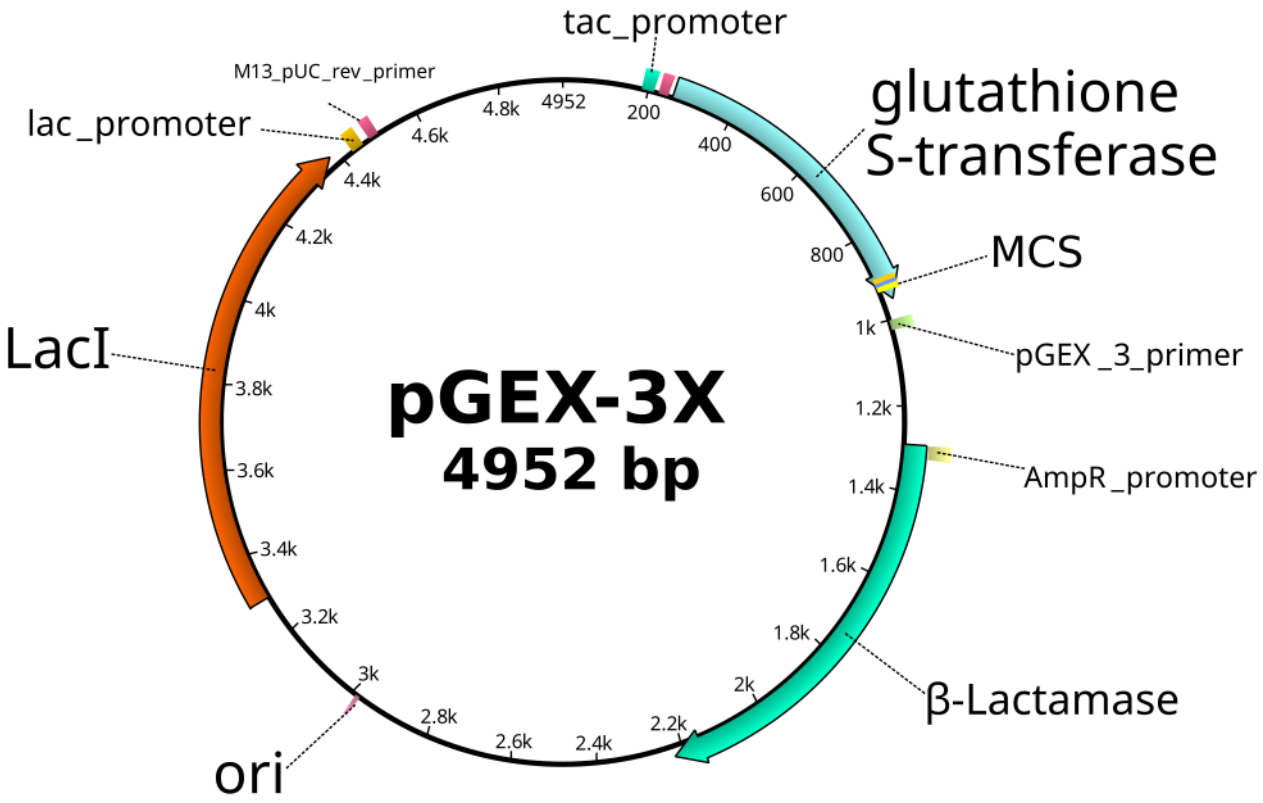
2.1.15.1: INTRODUCTION

PROTEIN EXPRESSION



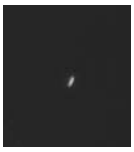


Recombinant DNA technology has many uses in basic scientific research to better understand the nature of living things. As a tool, recombinant DNA technology can be used to express proteins towards medical applications. Prior to biotechnology, type I diabetes (insulin-dependent) was treated by injection of insulin isolated from the pancreas of pigs. With the ability to express human proteins inside bacteria, yeast, and other cells, sacrificing pigs for porcine insulin is no longer necessary.



*Bacterial expression vector **pGEX-3X** contains the AmpR gene, the origin of replication, MCS downstream of the hybrid lac/trp promoter (tac) and the coding sequence for glutathione-S-transferase (GST). GST acts as a tag that is fused directly with the protein from the gene of interest and used to purify the protein with a glutathione resin.*

Bacteria or other cells can be engineered to express proteins through the process of cloning and transformation. Bacteria are advantageous because of their rapid life cycle and ease of growth. A bacterial expression vector contains the basic plasmid features: the origin of replication as well as antibiotic resistance gene. Often, an **affinity tag** will be used to aid in the purification of the protein. An example in the vector above shows the **GST** (glutathione-s-transferase) tag that can be purified with glutathione resin. Expression is only the first problem since bacteria are also synthesizing proteins that are required for the bacteria to grow and divide. Injecting these proteins in addition to insulin would cause an immune reaction that could be deadly. Therefore, it is required that overexpressed proteins be purified and isolated from other undesirable proteins.



Credit: Stewart EJ, Madden R, Paul G, Taddei F (CC-SA 3.0).

CRITERIA FOR CHOOSING AN EXPRESSION SYSTEM

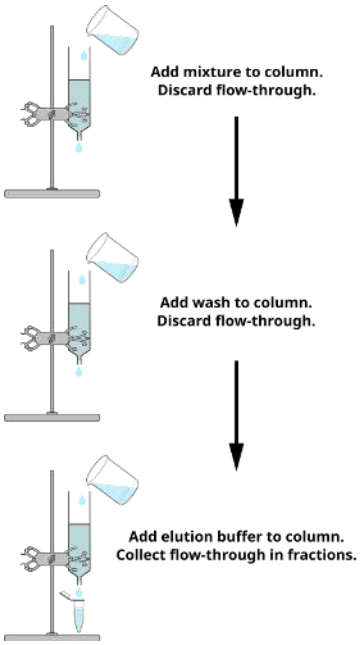
Desired characteristics	Bacteria	Yeast	Insect (baculovirus)	Mammalian cell culture (COS, CHO, HEK)
Cell growth	Rapid	Rapid	Slow	Slow
Complexity of growth medium	Minimum	Minimum	Complex	Complex
Cost of growth medium	Low	Low	High	High
Expression level	High	Low to high	Low to high	Low to moderate
Extracellular expression	Secretion to periplasm	Secretion to medium	Secretion to medium	Secretion to medium
Protein folding	Refolding usually required	Refolding may be required	Proper	Proper
Glycosylation	No	Yes	Some	Yes

Gene Expression Systems. Using nature for the art of expression (Fernandez, J.M. & Hoeffler, J.P., eds), Academic Press, San Diego, 1999.

Protein expression systems have inherent advantages and disadvantages. The table above summarizes the comparison of the various cellular systems of production (Fernandez & Hoeffler, 1999).

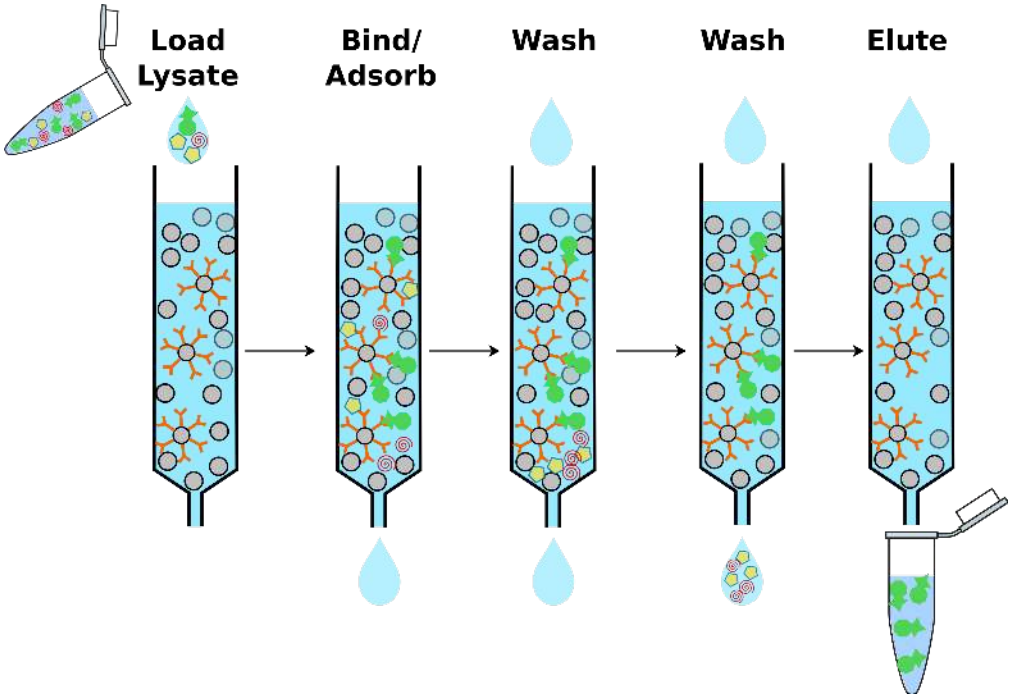
PURIFICATION

Different methods of isolation can be applied depending on the properties of the protein. **Ion exchange chromatography** is useful if the protein of interest has a specific charge that will interact with a resin packed with the opposite charge.





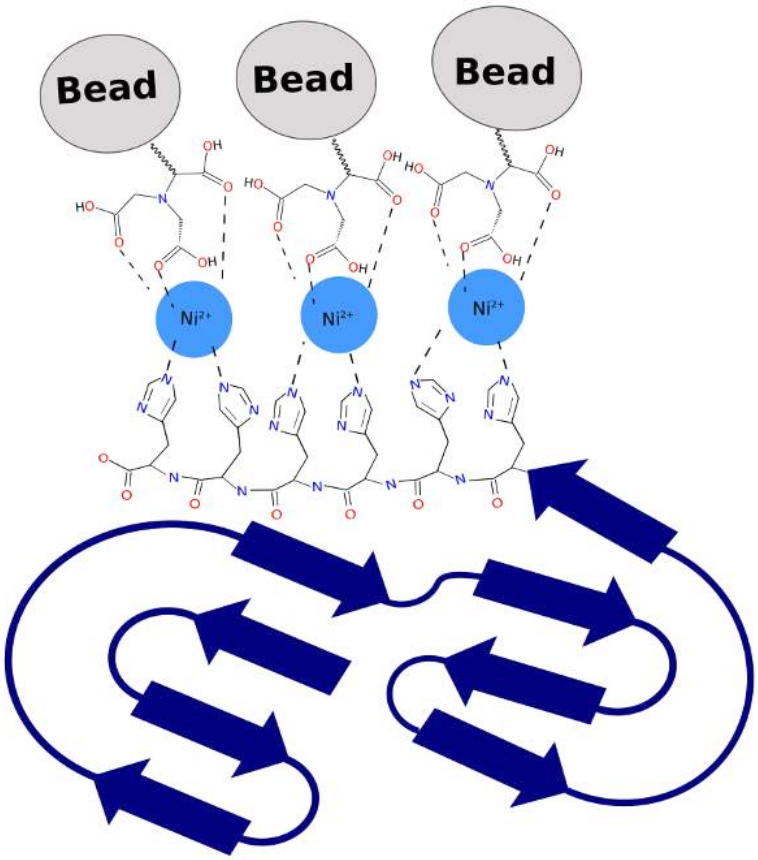
IMMUNOPRECIPITATION



Immunoprecipitation: Column is packed with Protein-A agarose which binds to antibodies. Cell lysates are then loaded onto the columns where they flow through and are allowed to interact with the antibody. Washes are performed to remove the non-specifically bound proteins. An elution buffer is used to disrupt the interaction of the antibody to the protein target.

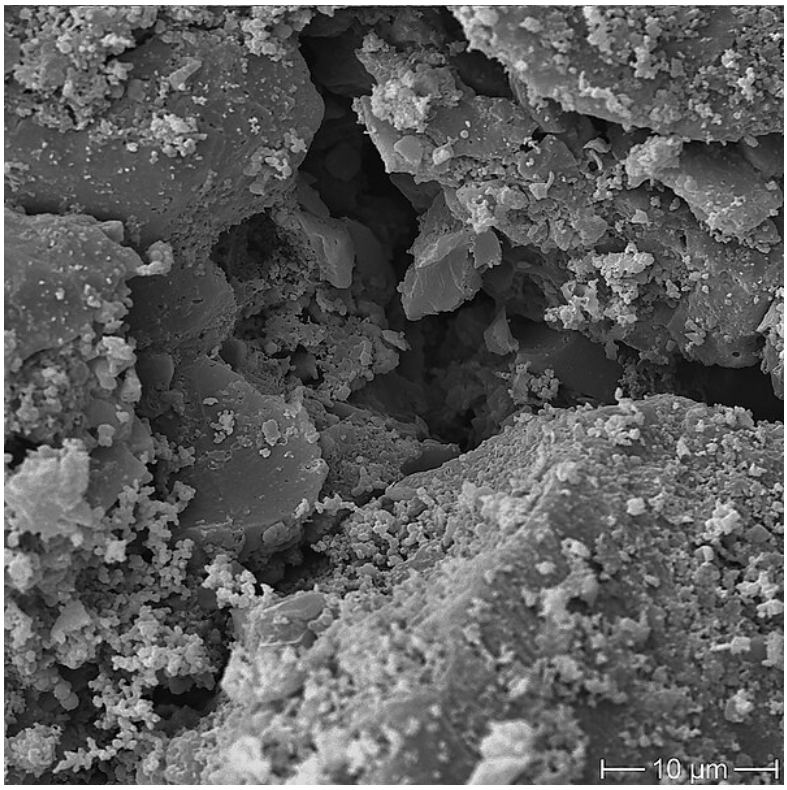
AFFINITY PURIFICATION

Affinity purification employs the use of specific antibodies that bind to the protein of interest very tightly to retain it on a column. With these techniques, the protein retained on the resin is washed numerous times to remove other proteins that are non-specifically sticking. A change in pH or ionic conditions then is used to disrupt the interaction with the resin and **elute** the proteins from the column. Proteins that are engineered to contain tags can be purified by antibodies specific to those tags. Also, the addition of 6 or more consecutive Histidine residues to the end of a protein makes them susceptible to purification with Nickel-NTA resin or Cobalt purification. In these cases, the 6XHis tag associates with these metal ions on the resin are selectively adhered.

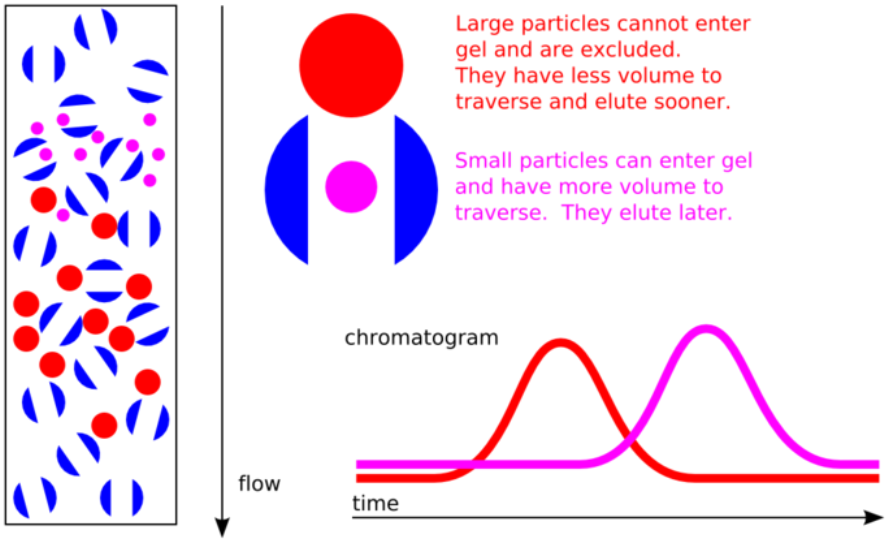


Nickel NTA resin coordinating the capture of a 6His tagged protein.

SIZE EXCLUSION



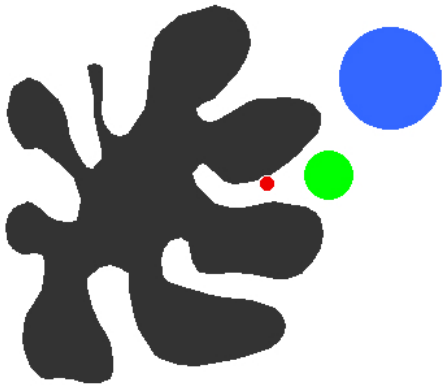
Credit: Mydriatic (CC-BY-SA 3.0)



Credit: Takometer(CC-BY 2.5)

Most of you are familiar with water purification filters. Before using these filters, you soak them in water and dark residue leaks out. This dark residue is activated charcoal. The activated charcoal has tiny microscopic pores that trap small items like ions and other particles. The primary goal of these filters is to remove metals and chlorine that are found in tap water. The porous nature of activated charcoal renders it useful for trapping molecules in water purification systems.

The process used to trap these small particles is called **size exclusion**. Unlike agarose gel electrophoresis where the smaller particles navigate through the matrix faster, size exclusion resins trap the smaller molecules.



The smaller the molecule, the longer they spend within the pores as they traverse through the matrix.

SIGNIFICANCE OF PURIFICATION



Credit: Hans Hillewaert (CC-BY)

All injectable drugs must be clean of endotoxins from bacteria. Purification of the protein of interest from bacterial lysates removes the dangerous pathogenic materials from that would otherwise activate host immune reactivity.

The horseshoe crab (*Limulus polyphemus*) performs a special function in the ecosystem by providing eggs for migratory birds to feed on. This organism also houses a special cell type in its hemolymph. The Limulus amoebocyte lysate (**LAL**) test is the most sensitive assay of detecting endotoxins from bacteria. Amoebocytes are collected from these organisms for use on testing batches of injectable drugs to ensure proper purification and safety.

REFERENCES

- Joseph M. Fernandez and James P. Hoeffler. Introduction: SO MANY POSSIBILITIES: HOW TO CHOOSE A SYSTEM TO ACHIEVE YOUR SPECIFIC GOAL, In Gene Expression Systems. Academic Press, San Diego. 1999, Pages 1-5, ISBN 9780122538407. http://dx.doi.org/10.1016/B978-012253840-7/50001-8.

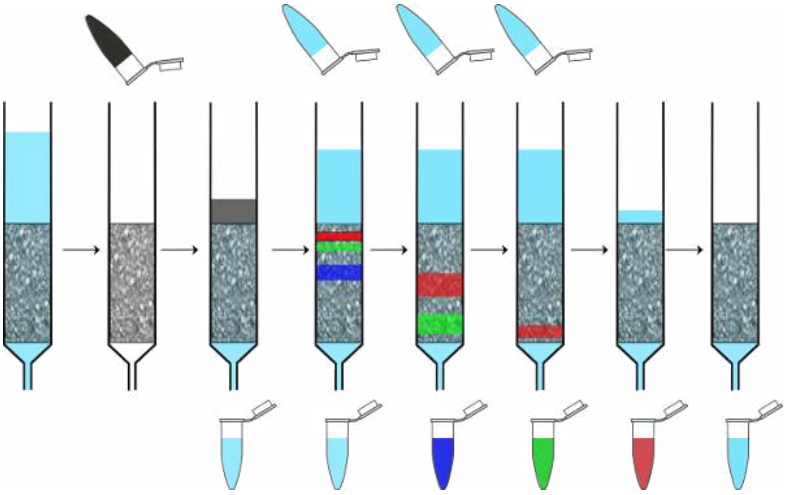
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2.1.15.2: PROTEIN PURIFICATION (ACTIVITY)

SIZE-EXCLUSION OF DYE MOLECULES

As a demonstration, the instructor may illustrate the concept of size exclusion on a set of mixed food coloring.

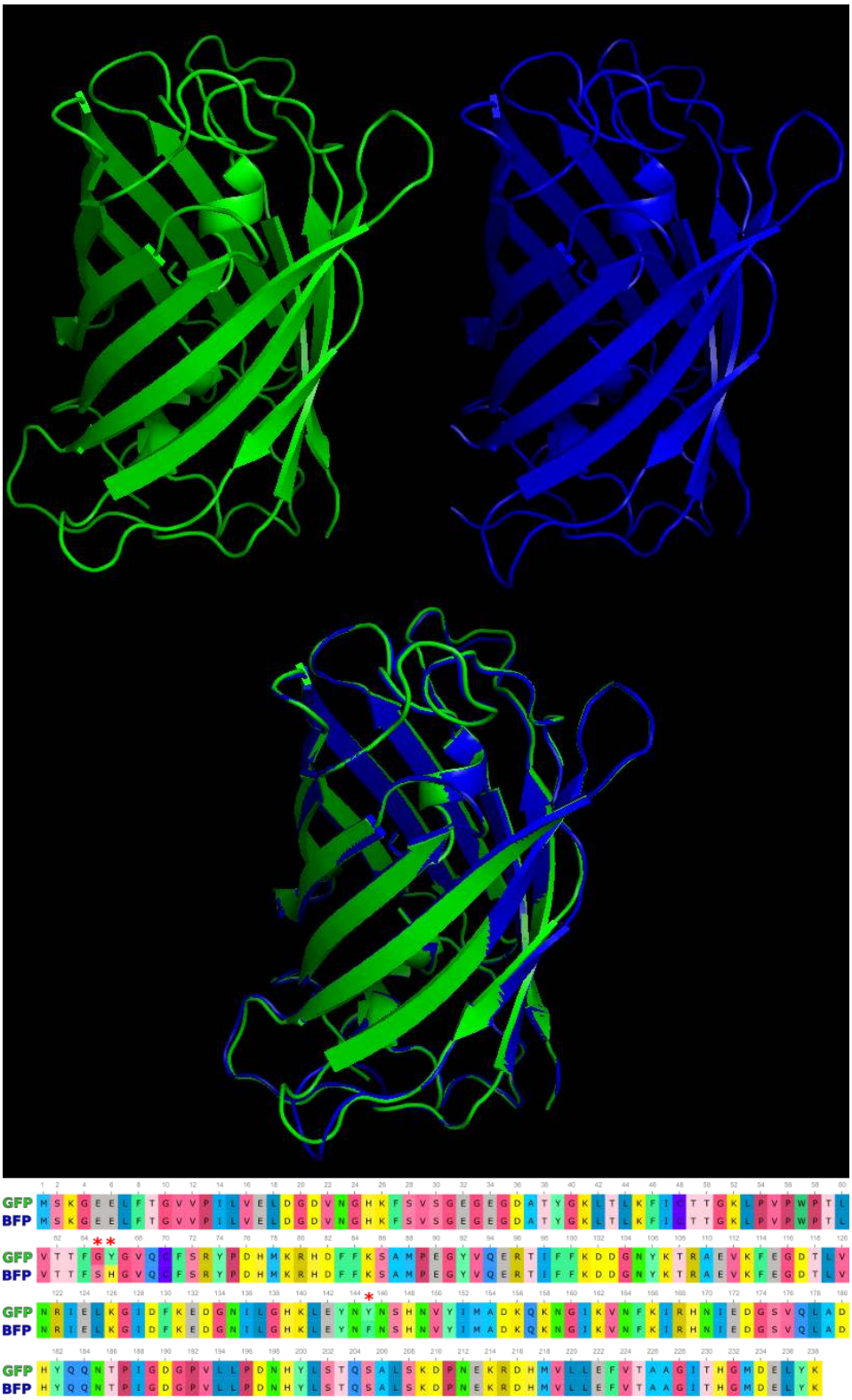


Size exclusion chromatography of food coloring.

1. Let the column empty over a beaker.
2. Carefully load 0.2 ml of food coloring mixture onto the column.
3. Place 10 tubes on a rack under the column.
4. Place a 1 ml buffer on the column and collect 0.5 ml fractions.
5. Continue to add buffer 1 ml at a time until all fractions have been collected.

SIZE-EXCLUSION OF PROTEINS

This exercise seeks to purify Green Fluorescent Protein (GFP) or Blue Fluorescent Protein (BFP) from the bacterial lysate. These proteins have a specific size of 238 amino acids and are 40,000 daltons (40kD). Based on their specific size, they will have a specific rate of migration through the size-exclusion resin. Remember that the bacterial lysate is full of additional proteins that are not your protein of interest that we are attempting to isolate.



3D model of GFP (Top Left), BFP (Top Right), structural alignment of GFP and BFP (Center) and the sequence alignment (Bottom) illustrating the 3 amino acid changes to produce the alternative protein. Red asterisks indicate the location of mutations.

Drops of fluid will be collected in fractions. The fractions containing the fluorescent proteins will be found only in specific fractions that will be visible under UV illumination.

1. Vertically mount the column on a ring stand. Make sure it is straight.

2. Slide the cap onto the spout at the bottom of the column.
3. Mix the slurry (molecular sieve) thoroughly by swirling or gently stirring.
4. Carefully pipet 2 ml of the mixed slurry into the column by letting it stream down the inside walls of the column.
5. Place an empty beaker under the column to collect wash buffer.
6. Remove the cap from the bottom of the column and allow the matrix to pack into the column.
7. Label eight microcentrifuge tubes #1-8.
8. Slowly load the column with 0.2ml of the GFP extract. Allow the extract to completely enter the column.
9. Add 1ml of the elution buffer on top of resin without disturbing the resin.
    - Add buffer slowly (several drops at a time) to avoid diluting the protein sample.
    - Using the graduated marks on the sides of the tubes, collect 0.5ml fractions in the labeled microcentrifuge tubes.
    - Continue to add 1ml buffer and collect fractions until all tubes are full.
10. Check all fractions by using long wave U.V. light to identify tubes that contain the fluorescent GFP or BFP proteins.
11. Further purification may be performed with a different resin with the few fractions containing the protein of interest.
12. Protein samples should be run on an acrylamide gel and stained against all proteins to check the purity of the sample or fluorescence measurements taken.

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SECTION OVERVIEW

2.1.16: BIOINFORMATICS

2.1.16.1: INTRODUCTION

2.1.16.2: SEQUENCE ANALYSIS

2.1.16.3: IN SILICO RESTRICTION

2.1.16.4: IN SILICO PCR

2.1.16.5: PRIMER-BLAST

2.1.16.6: MORPHOMETIC ANALYSIS

2.1.16.7: SEQUENCE ALIGNMENT AND TREE BUILDING

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2.1.16.1: INTRODUCTION

FASTA FORMAT

Biological sequences are passed to software in a standardized format referred to as **FASTA**. FASTA is a plain text format that can be read in any text editor (TextEdit, Notepad, VIM, TextWrangler, etc.). Nucleic acids (DNA and RNA) and Proteins are represented by single-letter nucleotides (A, T, C, G) or single letter amino acid (20 amino acids). FASTA sequences begin with a > character in the first line followed by some descriptive information about the sequence, like a sequence name. The next line consists of the sequence information. A FASTA file can contain multiple sequence entries all demarcated by a new line and a title line beginning with >.

EXAMPLE FASTA FILE

> Made-up nucleic acid sequence
ATATAGGGATTAGGATTAGAGGATAGAGGGGATTGCGCCG
> Another nucleic acid sequence in the same file
GGGTCGGGCTAGCGGAATCGGATTCGGCATTCGGATATTCGGATTCGGAT

FASTA files are plain text but usually have an extension indicating it as a sequence file: .fasta, .fa, .fna or even .txt

A list of single-letter codes for nucleic acids follows below:

Nucleic Acid Code	Meaning	Mnemonic
A	A	**A**denine
C	C	**C**ytosine
G	G	**G**uanine
T	T	**T**hymine
U	U	**U**racil
R	A or G	pu**R**ine
Y	C, T, or U	p**Y**rimidines
K	G, T, or U	bases which are **K**etones
M	A or C	bases with a**M**ino groups
S	C or G	**S**trong interaction
W	A, T, or U	**W**eak interaction
B	not A (i.e. C, G, T, or U)	**B** comes after A
D	not C (i.e. A, G, T, or U)	**D** comes after C
H	not G (i.e., A, C, T, or U)	**H** comes after G
V	neither T nor U (i.e. A, C, or G)	**V** comes after U
N	A, C, G, T, U	**N**ucleotide
X	masked	
–	Gap of indeterminate length	

GRAPHICAL SEQUENCE MANIPULATION

The exercises described here regarding bioinformatics will utilize a free and open-source software called Unipro UGENE.

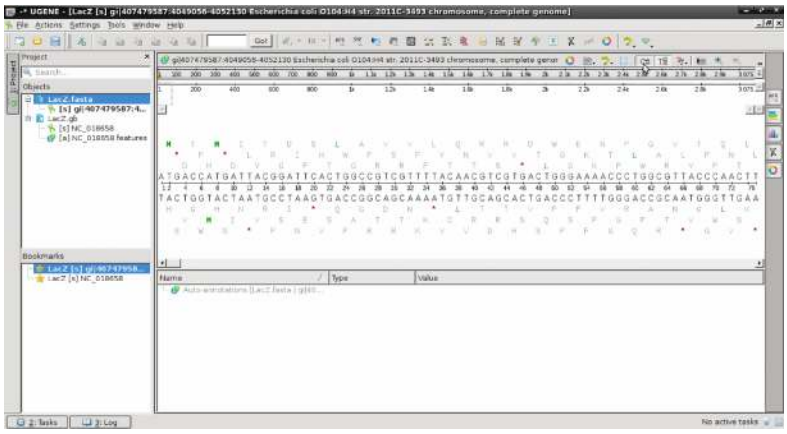
- Okonechnikov K, Golosova O, Fursov M, the UGENE team. **Unipro UGENE: a unified bioinformatics toolkit**.*Bioinformatics* 2012 28: 1166-1167. doi:10.1093/bioinformatics/bts09
- Golosova O, Henderson R, Vaskin Y, Gabrielian A, Grekhov G, Nagarajan V, Oler AJ, Quiñones M, Hurt D, Fursov M, Huyen Y.**Unipro UGENE NGS pipelines and components for variant calling, RNA-seq, and ChIP-seq data analyses**. *PeerJ 2014* 2:e644. doi:10.7717/peerj.644

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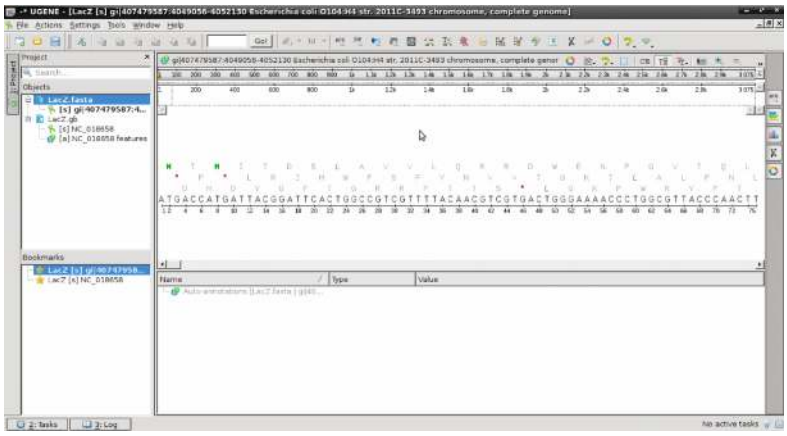
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2.1.16.2: SEQUENCE ANALYSIS

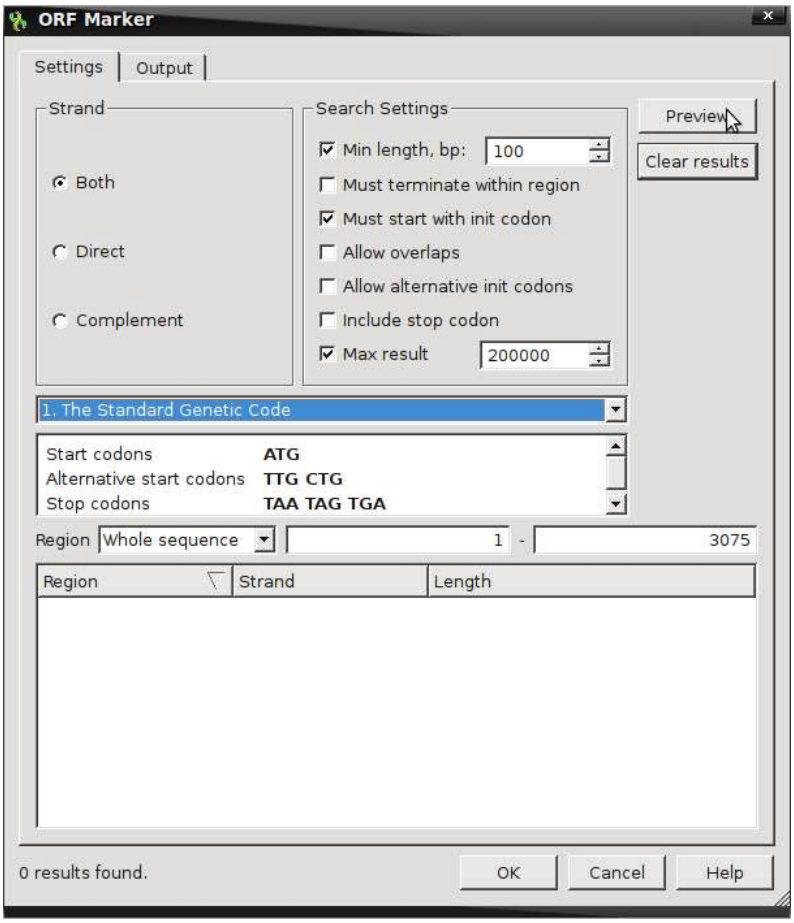
1. Download the file LacZ.gb and open in a text editor.
   - This is a **Genbank format** file that contains the sequence following the word '**ORIGIN**' and terminating with '//'.
   - Prior to the sequence is a batch of descriptive information including references, organism, and database cross-reference identifiers. While these don't mean much to you, the appropriate database within Genbank can be queried to reveal more information about the sequence.
2. Download the file LacZ.fasta and open in a text editor (NotePad).
   - Notice the simple structure of the **fasta** file beginning with the '>' and description of the sequence.
   - This is a DNA sequence. ***But DNA is usually double-stranded!*** We can assume the sequence of the second strand because it will be complementary to this one.
     - By convention: we know that this sequence is **5′ → 3′.**
   - This text contains a portion of the *E. coli* genome that includes a gene called LacZ.
   - This file does not contain any annotation to indicate where the gene sequence actually begins or ends.
3. Launch UGENE and open both files. They will appear on the left side "Objects" pane.
   - The default display automatically shows the reverse complement of the DNA strand and all 6 **Open Reading Frames** (ORFs).



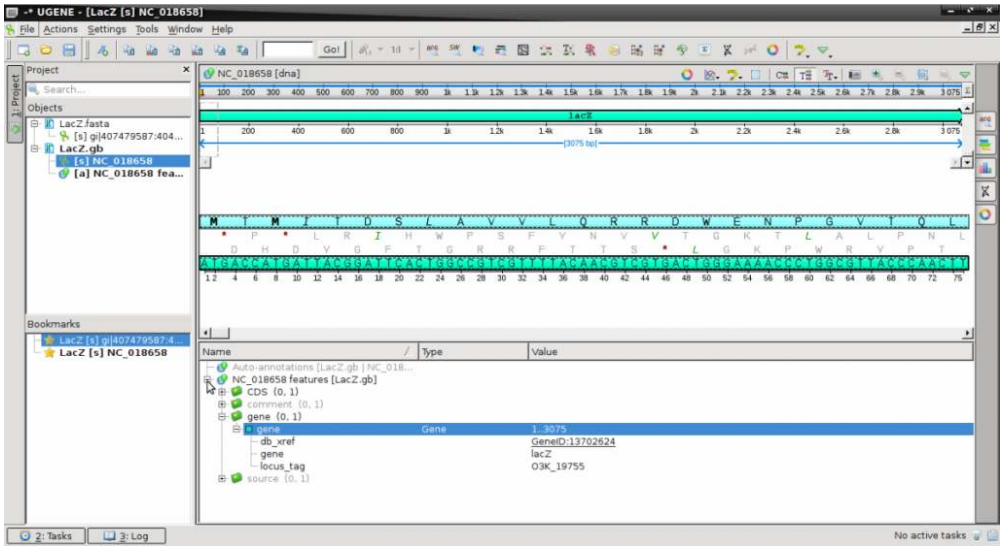
- To simplify the view, click on the '**C**' to remove the complementary strand (look at the cursor in the image).



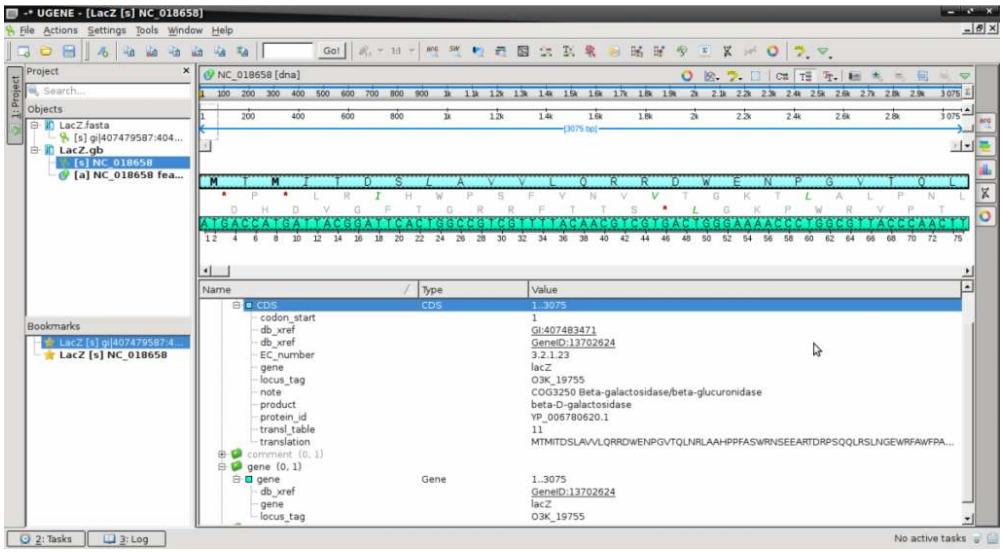
4. **Count the ORFs:**
   - Find ORFs by right-clicking on the sequence and select "**Analyze → Find ORFs**"
   - Default setting looks for ORFs on both strands with a minimum length of 100 nucleotides
   - The **Open Reading Frame** here is defined as something beginning with initiation or start codons from the Standard Genetic Code (**ATG**) and two additional alternative start codons (**TTG** & **CTG**) that is terminated by any one of the three standard stop codons (**TAA**, **TAG**, **TGA**)
   - Selecting Preview will provide the number of possible ORFs fitting these criteria.



5. Double click on the **LacZ.gb** in the **Objects** panel to activate the view.
    - This file now shows the same sequence with information about the DNA.



- Expand the various features in the Annotations pane at the bottom to explore the sequence features.



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2.1.16.3: IN SILICO RESTRICTION

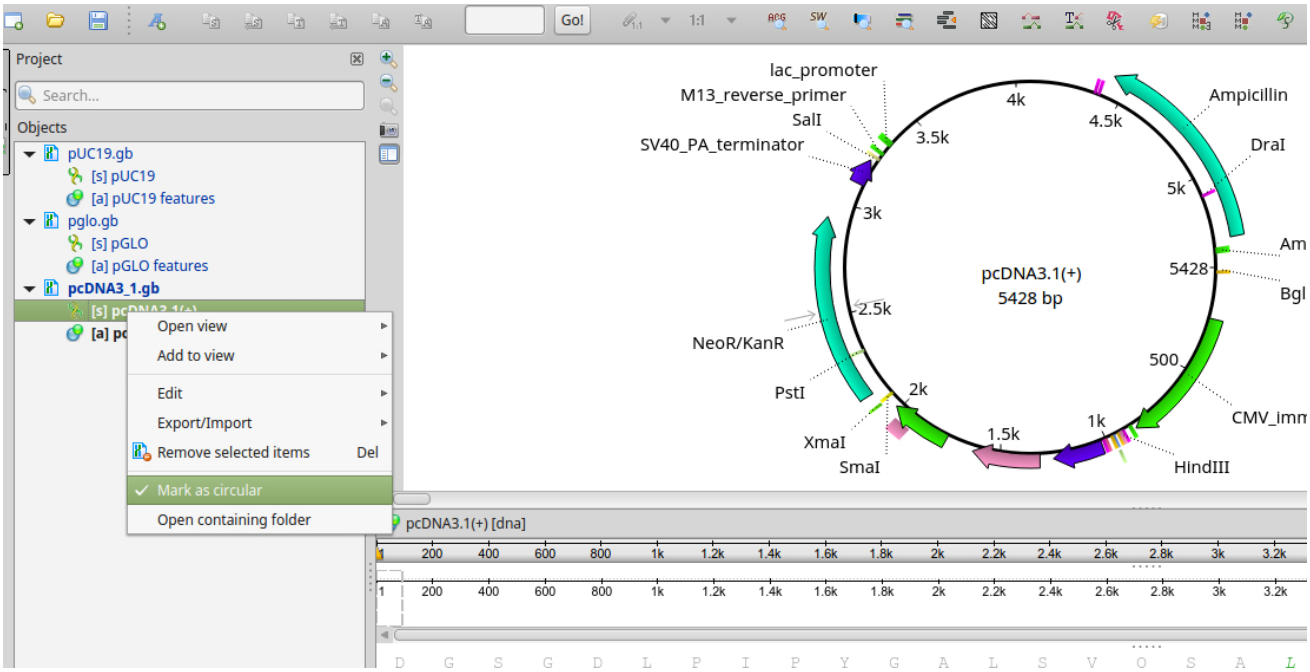
CONCEPT

Restriction enzymes act like molecular scissors. The ones we use in Molecular Biology are those that cut within known sequences that occur often enough, yet rare enough to cut our DNA into analyzable fragments. Molecular Biologists often use 6-cutters. This means that the site of digestion is “restricted” to a recognition sequence of 6 nucleotides. These nucleotides are usually palindromic as discussed before.

Imagine a linear piece of DNA as a piece of string. When cutting the string once, you result in 2 pieces. Now consider a plasmid. This was already discussed to be a circular piece of DNA. With a circle, there are no ends. cutting the plasmid once results in 1 piece of DNA as opposed to 2. Keep this in mind when digesting circular plasmids. In this case, nucleotide 1 is adjacent to and contiguous with the last nucleotide of the sequence.

IN SILICO DIGESTION

1. This activity is meant to supplement Identification of DNA (activity)
2. Launch UGENE and open the following files:
   - **pGlo.gb**
   - **pUC19.gb**
   - **pcDNA3.1.gb**
   - **pUC with Insert**
3. In the **Objects** menu, right-click on the sequences and select “**Mark as circular**”
   - The sequences will now be treated as circular DNA.
   - The first nucleotide and the last nucleotide become adjacent as a continuous sequence this way.



4. From the top menu, select **Actions → Analyze → Find restriction sites.**
   - This will load a set of default restriction enzymes
   - If none are loaded, select them individually (found in alphabetical order)
     - Choose: BamHI, BglII, ClaI, DraI, EcoRI, EcoRV, HindIII, KpnI, PstI, SalI, SmaI, XbaI, XmaI, NotI
5. **Actions → Cloning → Digest into fragments**
   - Choose your enzyme.
   - Try *Hind* III for each plasmid.
   - Fragments will be added to the circular view and annotations for these fragments will be added.

- You can use this information to calculate how many fragments come from enzymes based on how many times they cut and by the nucleotide coordinates found in the annotation of the fragment.

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2.1.16.4: IN SILICO PCR

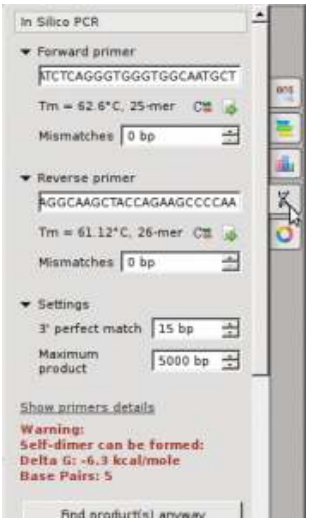
Using the primer sequences, one can determine the size and/or location of a PCR product. This can be done using BLAST or with a program like UGENE.

USING BLAST

1. Primers for the PV92 insertion.
   - Forward primer: 5′ GGATCTCAGGGTGGGTGGCAATGCT 3′
   - Reverse primer: 5′ GAAAGGCAAGCTACCAGAAGCCCCAA 3′
2. Visit BLAST: https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch.
3. Paste both primers:
   - GGATCTCAGGGTGGGTGGCAATGCTGAAAGGCAAGCTACCAGAAGCCCCAA
   - Remove the 5′ and 3′ numbers.
4. Choose "**Somewhat Similar**".
   - Locate the locus of the product and the size.

USING UGENE

1. Exercise using a human D-Loop Primers.
   - Forward Primer 5'-TTAACTCCACCATTAGCACC-3'
   - Reverse Primer 5'-GAGGATGGTGGTCAAGGGAC-3'
2. Download the sample Genbank file: Human Mitochondrial Genome.
3. Open the file in Ugene.
4. Select the "**In Silico PCR**" button on the far right (double helix button).
   - Insert forward and reverse primers in the appropriate spaces.



5. A PCR product should be noted for one of the sequences after pressing "**Find Products anyway**".

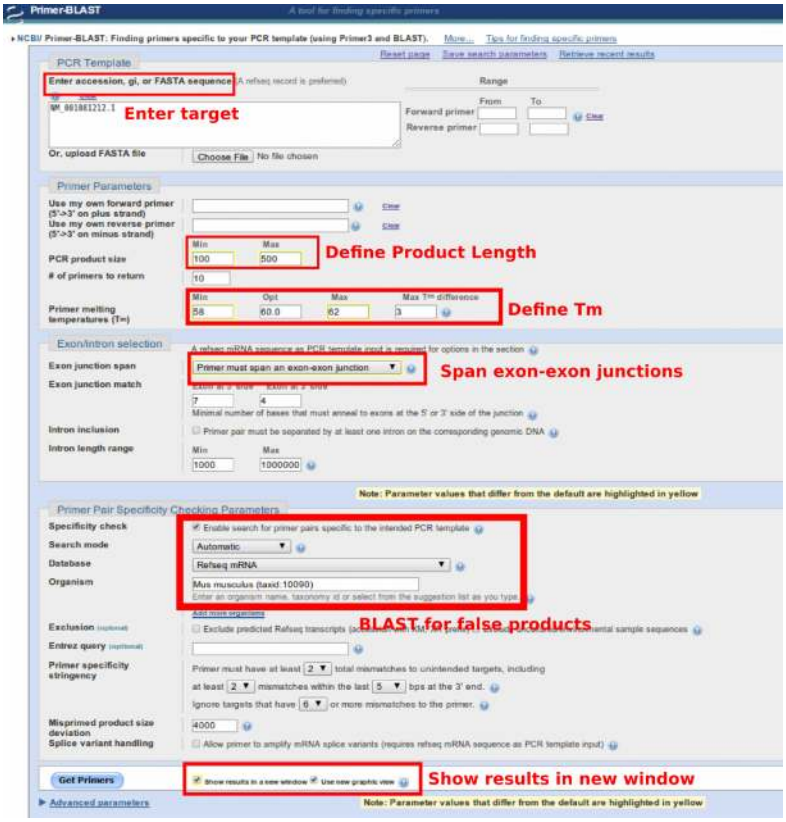
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2.1.16.5: PRIMER-BLAST

Primer-BLAST is a combination of a program called **Primer3** that aids in the design of primers with specific properties and BLAST. Primer-BLAST allows for the construction of primers for qPCR where the user can specify the melting temperature, reduce the amount of self-priming, and span exon-exon junctions in order to avoid amplification of contaminating genomic DNA. After the design of primers, each primer pair is sent into BLAST to identify if similar products within the genome of the model organism will also be primed and amplified. This process ensures that the primers designed fall within your design parameters and most likely only amplify your gene of interest.

1. Enter the sequence OR the NCBI accession number for the gene of interest.
2. Define the PCR product length.
    1. Limiting the product between 100-500 permits for good efficiency in qPCR.
    2. Longer products may not be efficiently replicated depending on your cycling protocol.
3. Define the desired melting temperature ($\mathbf{T_m}$) of the primers (the minimum, optimal, maximum, difference between the set).
    1. 60ºC is fairly high and will aid in the enhanced specificity of the primer with the target during amplification to avoid false priming.
    2. Try to have the T_m as close as possible so that they are annealing about equally.
4. Choose the option “Primer must span an exon-exon junction”.
    1. This aids in amplifying cDNA and not genomic DNA that may be contaminating.
    2. Do not select this if it a single exon gene as this will fail.
5. Select Refseq mRNA as the database to search against.
    1. **Refseq** provides sequences to naturally occurring sequences.
    2. Things like plasmid sequences or vector constructs do not show up in Refseq.
6. Select the organism you are BLASTing against.
    1. There are options for model organisms as well as cell lines.
    2. If you are using something like PC12 cells, you may use *Rattus norvegicus* or PC12 genome since that is also an option in the database.
7. Evaluate the location of the primers and the other parameters. We generally choose primers at the 3′ end of the RNA since RT reactions often have a 3′ bias in eukaryotes by using oligo-dT priming in the reverse transcription.



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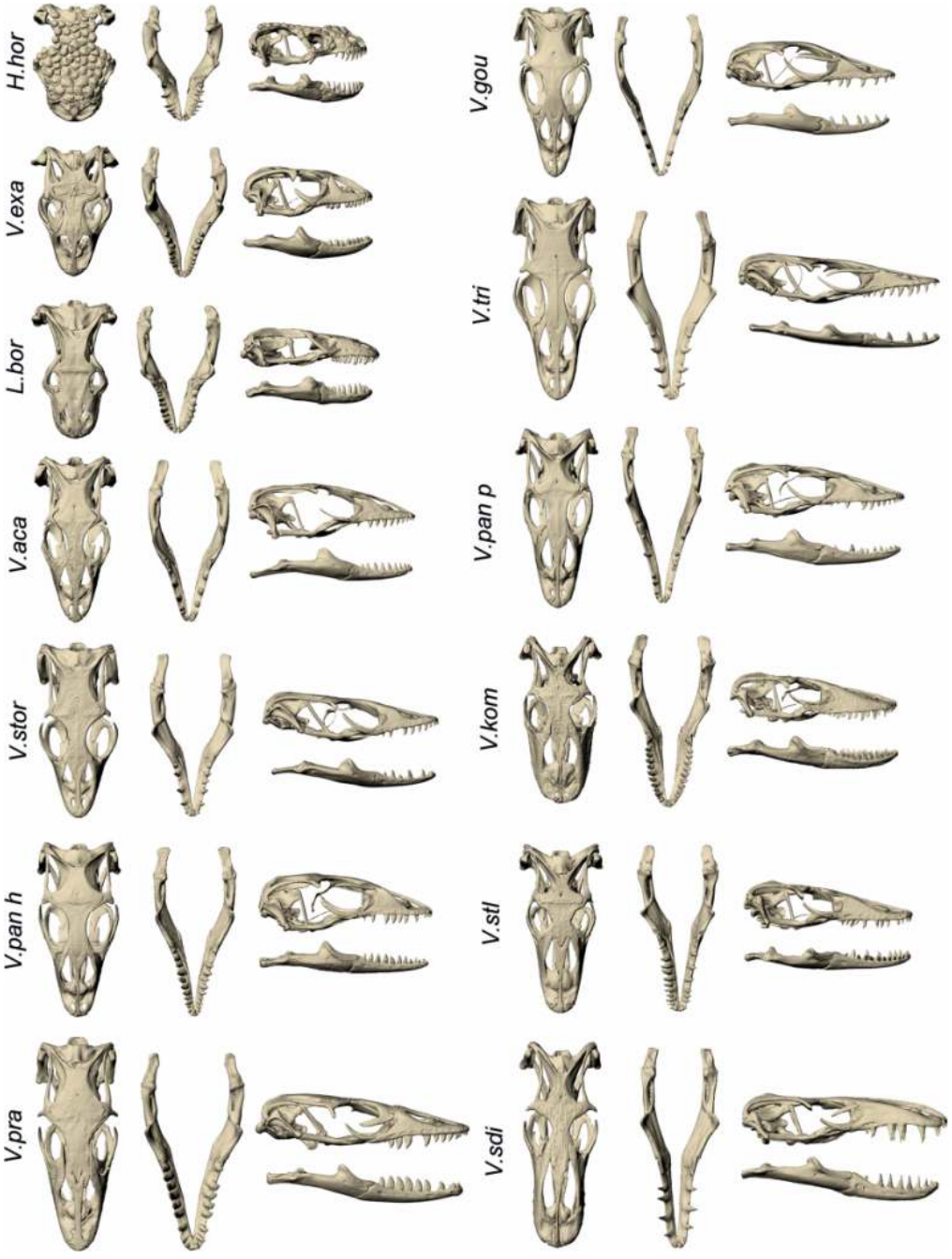
2.1.16.6: MORPHOMETIC ANALYSIS

MORPHOMETRICS AND PHYSICAL MARKERS

Morphometrics (*morpho*– shape; *metrics*– measurements) is the use of physical measurements to determine the relatedness of organisms. With extinct organisms that have died out long ago, DNA extraction proves to be difficult. Likewise, prior to DNA technologies to analyze species, **Linnean taxonomy** was ascribed to organisms based on similarities in features.

DESCRIBING SPECIES AND VARIATION OF MORPHOLOGIES

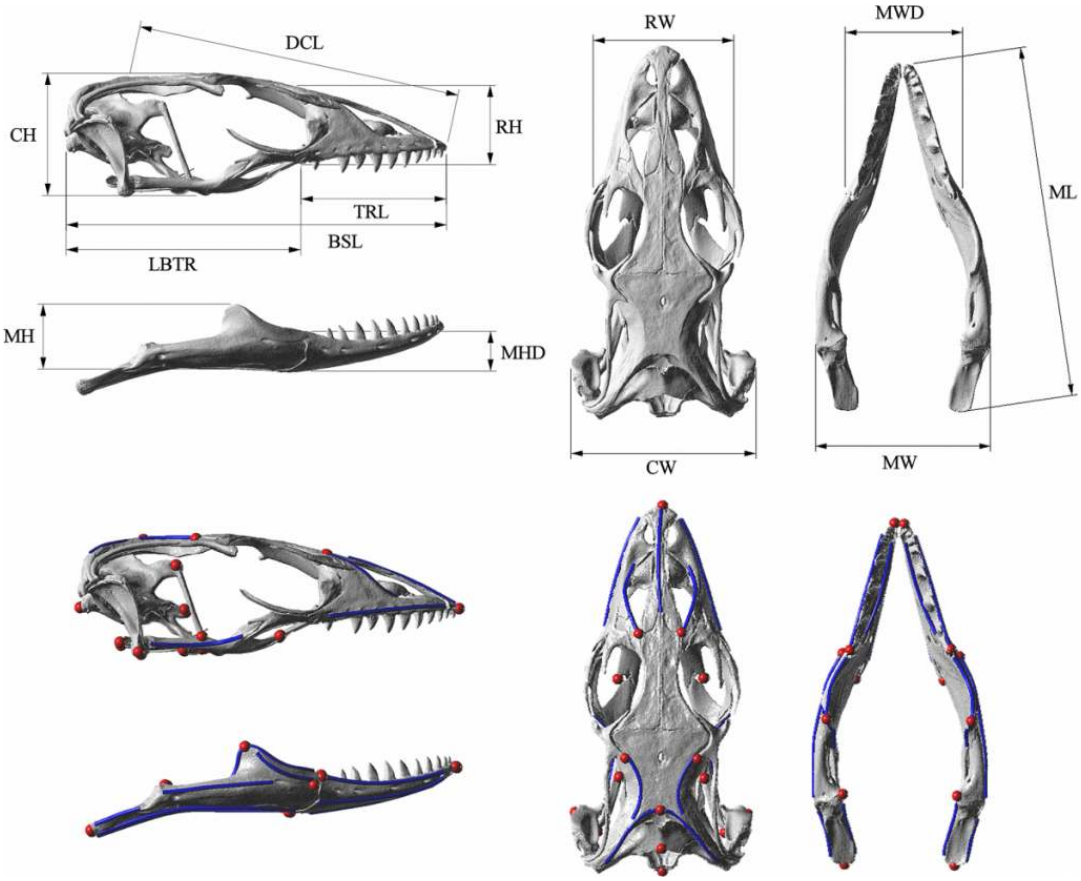
Below are images of skull landmarks of the lizard family Varanidae. This family includes monitor lizards and Komodo Dragons. As can be seen below, the general morphology of the skulls is similar enough that they all retain the same landmarks. The figure below also illustrates the diversity in these lizards that illustrate a large variation between species.



Skulls of the species involved in this analysis. McCurry et al. (2015) (CC-BY)

LANDMARKS STANDARDIZE MEASUREMENTS

Having a set of shared landmarks provides the opportunity to make systematic measurements of morphometric features.



Landmarks and measurement metrics for the morphometric analysis of skulls. McCurry et al. (2015) *(CC-BY)*

EUCLIDEAN DISTANCE TO MEASURE RELATEDNESS

Euclidean distance is a measurement derived from Pythagorean geometry that describes the shortest distance (d) between 2 points (A and B) as a straight line using triangulation. In a Cartesian space, the points can be defined:

$$A = (x_A, y_A)$$

and

$$B = (x_B, y_B)$$

Standard Pythagorean theorem can be expressed as:

$$x^2 + y^2 = d^2$$

To find the distance between the 2 points, we utilize algebra to calculate for d.

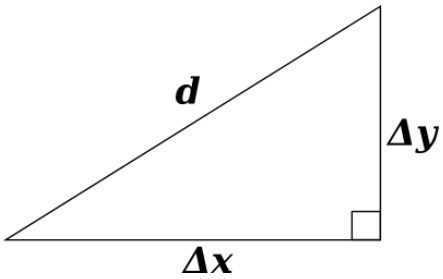
$$d = \sqrt{x^2 + y^2}$$

In this case, we expand to comparing the coordinates of the two points:

$$\Delta x = x_B - x_A$$

and

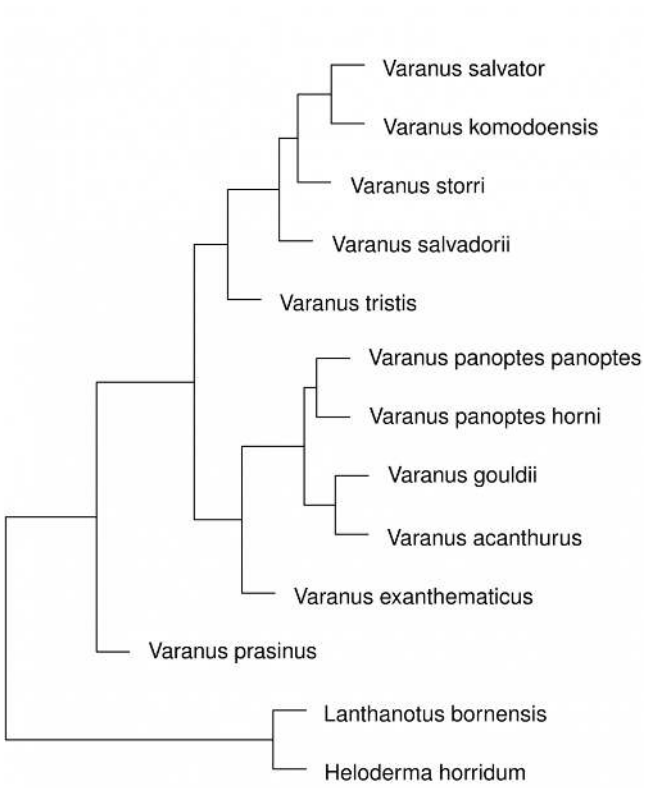
$$\Delta y = y_B - y_A$$



We can then expand this idea to include the differences in data points that describe the comparisons of multiple measurements.

$$d(\mathbf{X_i}, \mathbf{X_j}) = \sqrt{\sum_{k=1}^{p} (X_{ik} - X_{jk})^2}$$

CALCULATING DISTANCE WITH R



Varanoid Dendrogram from R

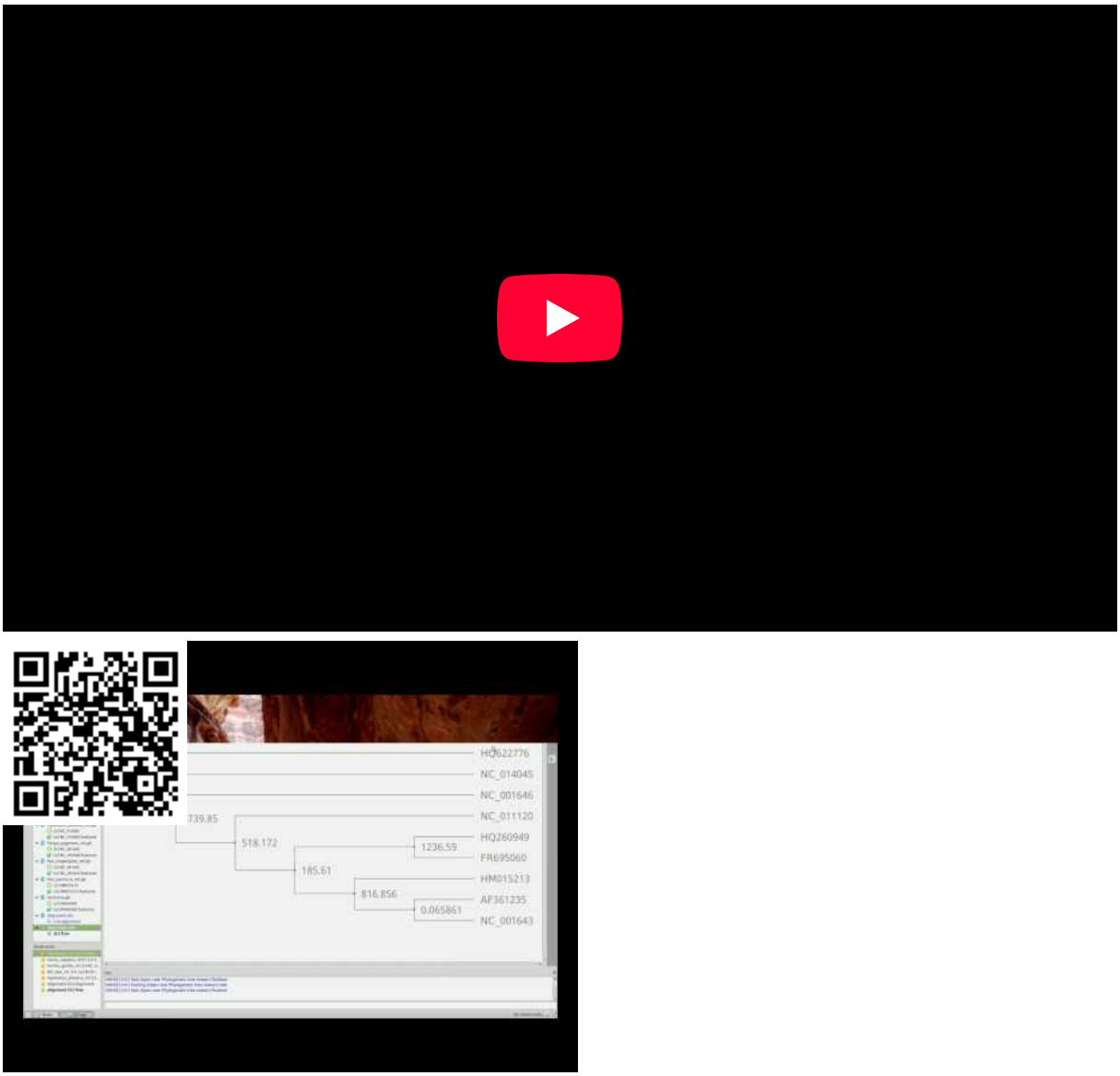
1. Download the **dataset** (McCurry *et al.* 2015) associated with this activity (a Comma Separated Value .csv file). This can be used in a spreadsheet or in a text editor. This data can be imported into R to determine the Euclidean distances of landmarks.
2. The following code in R will download the data set into a variable called “varanoid”, measure Euclidean distance and save a plot into a PDF file in a directory called “/tmp”.

```
## install curl for fetching from internet if it isn't
install.packages ( 'curl' )
## Load the curl library
library (curl)
## read the data of measurements and assign it to a variable 'varanoid'
varanoid = read.csv ( curl (
'https://raw.githubusercontent.com/jeremyseto/bio-oer/master/data/varanoid.csv' ))
## set the row names to the Species column
row.names (varanoid) = varanoid$Species
## remove the first column of the table to have purely numeric data
varanoid_truncated = (varanoid[,2:14])
## calculate distance using euclidean as the method
dist_measure = dist (varanoid_truncated, method= 'euclidean' )
## display dist_measure to look at the comparisons
dist_measure
varanoid_cluster = hclust (dist_measure)
## open PDF as a graphics device to save a file in the '/tmp' directory
pdf (file= '/tmp/varanoid_tree.pdf' )
plot (varanoid_cluster)
dev.off ()
## close the device to save the plot as pdf
```

DNA ANALYSIS

Before starting this activity, review **bioinformatics** and **sequence analysis**.

1. Search NCBI for mitochondrial sequences from the species involved in McCurry 2015. The data has been submitted by Ast (2001).
2. Find the sequences and identify/extract elements that are common to all.
3. Assemble the shared sequences in a text editor as a single FASTA file where each species is separated by a header (“>Species A”).
    - Notepad on Windows (but it’s better to download notepad++)
    - Textedit on Mac (but probably better to download TextWrangler)
    - Gedit on Linux
4. Save the file as “something.fasta”.
5. Perform a multiple sequence analysis using **UGENE.**
6. Generate a phylogenetic tree using UGENE. For this exercise, use Maximum Likelihood (PhyML) as the algorithm. File the tutorial below.
7. Compare the DNA with the morphometric analyses. What problems could we imagine arise if we rely solely on morphometry?



REFERENCES

- McCurry MR, Mahony M, Clausen PD, Quayle MR, Walmsley CW, Jessop TS, Wroe S, Richards H, McHenry CR. (2015) **The Relationship between Cranial Structure, Biomechanical Performance and Ecological Diversity in Varanoid Lizards.** *PLoS ONE* 10(6): e0130625. doi: 10.1371/journal.pone.0130625
- Ast, Jennifer C. (2001) **Mitochondrial DNA Evidence and Evolution in Varanoidea (Squamata)**. *Cladistics* 17(3): 211–26. http://www.sciencedirect.com/science/article/pii/S0748300701901690
- Fisher, R.A. (1936) **The use of multiple measurements in taxonomic problems.** *Annals of Eugenics*, 7: 179–188. doi:10.1111/j.1469-1809.1936.tb02137.x

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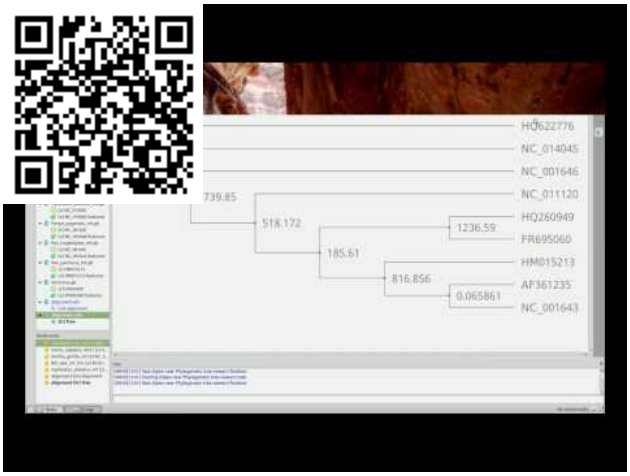
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2.1.16.7: SEQUENCE ALIGNMENT AND TREE BUILDING

UGENE

The following video illustrates the tree-building process using MUSCLE and PhyML in **UGENE**.





COMMAND LINE

The following requires:

- A UNIX-like environment like Linux or MacOS
- **MUSCLE** to perform a multiple sequence alignment
- **PhyML** to generate Maximum Likelihood
- **FigTree** to manipulate the tree

Download the example file **oranges**. In the download directory, perform the following:

```
unzip orange.zip
cd  orange
cat  ./*txt >> oranges.fasta  ## merges all files into a single fasta file
muscle -  in  oranges.fasta -phyiout oranges.phy
## -phyiout tells muscle to use the interleaved phylip format for output
phyml -i oranges.phy -m HKY85
## -m is for method and HKY85 is the default nucleotide method we used in UGENE
mv  oranges.phy_phyml_tree.txt oranges.nwk
## change the name of the output to reflect it is a nwk file
```

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