

INTRODUCTION TO BIOTECHNOLOGY LABORATORY MANUAL



Donna Barron
Hudson Valley Community College

Introduction to Biotechnology Laboratory Manual

This text is disseminated via the Open Education Resource (OER) LibreTexts Project (<https://LibreTexts.org>) and like the thousands of other texts available within this powerful platform, it is freely available for reading, printing, and "consuming."

The LibreTexts mission is to bring together students, faculty, and scholars in a collaborative effort to provide an accessible, and comprehensive platform that empowers our community to develop, curate, adapt, and adopt openly licensed resources and technologies; through these efforts we can reduce the financial burden born from traditional educational resource costs, ensuring education is more accessible for students and communities worldwide.

Most, but not all, pages in the library have licenses that may allow individuals to make changes, save, and print this book. Carefully consult the applicable license(s) before pursuing such effects. Instructors can adopt existing LibreTexts texts or Remix them to quickly build course-specific resources to meet the needs of their students. Unlike traditional textbooks, LibreTexts' web based origins allow powerful integration of advanced features and new technologies to support learning.



LibreTexts is the adaptable, user-friendly non-profit open education resource platform that educators trust for creating, customizing, and sharing accessible, interactive textbooks, adaptive homework, and ancillary materials. We collaborate with individuals and organizations to champion open education initiatives, support institutional publishing programs, drive curriculum development projects, and more.

The LibreTexts libraries are Powered by [NICE CXone Expert](#) and was supported by the Department of Education Open Textbook Pilot Project, the California Education Learning Lab, the UC Davis Office of the Provost, the UC Davis Library, the California State University Affordable Learning Solutions Program, and Merlot. This material is based upon work supported by the National Science Foundation under Grant No. 1246120, 1525057, and 1413739.

Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation nor the US Department of Education.

Have questions or comments? For information about adoptions or adaptations contact info@LibreTexts.org or visit our main website at <https://LibreTexts.org>.

This text was compiled on 05/01/2026

TABLE OF CONTENTS

Licensing

1: Lab Safety and Lab Notebook

- 1.1: Student Learning Outcomes
- 1.2: Introduction
- 1.3: PART I- Setting Up a Legal Scientific Notebook
- 1.4: PART II- Lab Safety
- 1.5: Study Questions
- 1.6: PUZZLE- Lab Safety and Lab Notebooks

2: Metrics and Measurements

- 2.1: Student Learning Outcomes
- 2.2: PART I- Metrics
- 2.3: Part II- Measuring Using The Metric System
- 2.4: Study Questions

3: Micropipetting

- 3.1: Student Learning Outcomes
- 3.2: Introduction To Micropipetting
- 3.3: Part I- Choosing And Setting The Micropipette
- 3.4: Part II- Pipetting Practice
- 3.5: Study Questions

4: pH and Buffers

- 4.1: Student Learning Outcomes
- 4.2: Introduction
- 4.3: Prelab Reading Assignment- Chemistry Review
- 4.4: Activity 1- Measuring pH
- 4.5: Activity 2- Preparation of an Acetate Buffer
- 4.6: Activity 3- Effect of Adding Acid and Base to Acetate Buffer
- 4.7: Study Questions

5: Paternity and Gel Electrophoresis

- 5.1: Student Learning Outcomes
- 5.2: Part I- Agarose Gel Electrophoresis
- 5.3: Part II- Paternity Case- Who is the Father of My Kittens?
- 5.4: Study Questions

6: Restriction Digest and Gel Electrophoresis (New)

- 6.1: Student Learning Outcomes
- 6.2: Introduction
- 6.3: Part I- Restriction Digest
- 6.4: Part II- Casting Agarose Gel

- 6.5: Part III- Practice Pipetting
- 6.6: Part IV- Gel Electrophoresis
- 6.7: Study Questions

7: Transformation

- 7.1: Student Learning Outcomes
- 7.2: Introduction
- 7.3: Transforming Bacteria with Plasmids
- 7.4: Study Questions

8: Column Chromatography (Rev)

- 8.1: Student Learning Outcomes
- 8.2: Introduction
- 8.3: Pre Lab Activity- Think Pair Share-Discuss What You Know
- 8.4: Part I- Lysis of Bacterial Cells
- 8.5: Part II- Separating the Green Fluorescent Protein with Column Chromatography
- 8.6: Study Questions

9: Serial Dilutions and Standard Curve with a Microplate

- 9.1: Student Learning Outcomes
- 9.2: Part I- Serial Dilutions
- 9.3: Part II- Standard Curves
- 9.4: Study Questions

10: Good Manufacturing Practices (GMP) with New Table

- 10.1: Student Learning Outcomes
- 10.2: Part I- Review Terms and Background Information
- 10.3: Part II- Train and Prepare for a Production Run
- 10.4: Part III- Production and Documentation
- 10.5: Study Questions

[Index](#)

[Glossary](#)

[Detailed Licensing](#)

[Detailed Licensing](#)

Licensing

A detailed breakdown of this resource's licensing can be found in [Back Matter/Detailed Licensing](#).

CHAPTER OVERVIEW

1: Lab Safety and Lab Notebook

- 1.1: Student Learning Outcomes
- 1.2: Introduction
- 1.3: PART I- Setting Up a Legal Scientific Notebook
- 1.4: PART II- Lab Safety
- 1.5: Study Questions
- 1.6: PUZZLE- Lab Safety and Lab Notebooks

This page titled [1: Lab Safety and Lab Notebook](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by Donna Barron.

1.1: Student Learning Outcomes

Goals

- Understand the importance of a well-kept notebook
- Learn how to enter information and maintain a legal scientific notebook
- Familiarize students with basic safety rules
- Allow students to become familiar with the safety equipment in the laboratory



Student Learning Outcomes

- Be able to interpret data on safety diamond.
- Record information into a notebook in a legally acceptable format.
- Format a table of contents.
- Make appropriate corrections to their notebook.
- Engage in good laboratory practices.
- Know how to properly use PPE.
- Know the location and proper use of emergency equipment.
- Recognize the meaning of common laboratory safety signs.

This page titled [1.1: Student Learning Outcomes](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

1.2: Introduction

Regardless if you work at a biotechnology company or in an academic research lab, keeping a lab notebook is a requirement. The Food and Drug Administration's (FDA) handbook states, "if it isn't written down, it wasn't done." The lab notebook is considered a legal document and can be used in court to settle patent disputes or to report a specialist's finding in paternity suits or criminal cases. Often times, the lab notebook is used as a starting point for other scientists who work in the lab. Lab notebooks are always maintained for the following reasons:

- To record the steps an individual has carried out and to document their observations
- To establish ownership in case of a patent dispute or other legal issues
- To establish guidelines used to evaluate the process in which a product was made and to evaluate the product itself
- To follow the production of a product through the manufacturing process
- To create a contract between a company and consumers and/or between a company and regulatory agencies
- To demonstrate a procedure was done correctly
- To develop, follow, and evaluate standard operating procedures (SOP)

Without documentation, even quality work is worthless. In the event of an experiment failing, the documentation allows for a scientist to review their protocol and make adjustments for future experiments. As stated above, in industry, lab notebooks are legal documents. They are used to determine product quality, patent rights, and liability. Notebooks are always treated as if they will be used in court because the consequences of not doing so could be devastating to a company.

This page titled [1.2: Introduction](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

1.3: PART I- Setting Up a Legal Scientific Notebook

A notebook should contain what equipment and materials were used and what steps were followed to show that the equipment and materials were validated before use. In case of an audit by government regulatory agencies, a company must be able to produce documentation that proves that they are following **Good Manufacturing Practices (GMPs)**. If they find that a notebook was missing information or was not legible, then the company may be fined or held liable for damages in a product lawsuit. The importance of a well-kept notebook cannot be emphasized enough.

Guidelines For Setting Up A Legal Scientific Notebook

1. Obtain a **bound notebook**, such as a composition book. Spiral notebooks are not acceptable.
- *For the purpose of this course, pages suitable for a lab notebook are included in the appendix. Your instructor may direct you to print a specific number of pages and bind them with staples along the long left edge to serve as a low-cost substitute for an official bound notebook.
2. Use only blue or black pen to make entries. **NO PENCIL ALLOWED.**
3. Label the outside of your notebook with the information:
4. Make pages 1-4 the Table of Contents. Write "Table of Contents" at the top of each of these pages. Record the title of each lab or entry and the page number for each.
5. Each page in the notebook must be numbered. Number each page of the notebook in the upper corner with the first page of the notebook being "page 1." The back of the first page will be "page 2" and so on.
6. Record the date of each entry at the top of each page.
7. Sign and date the bottom of each page.
8. For each experiment (or activity) that you perform, you should include the purpose for the experiment, the materials and methods you use, any data tables, figures or graphs properly labelled with a title, and a conclusion.
9. Do not erase errors. Just draw a single line through an erroneous entry, then add your initials. Enter the correct entry nearby.
10. Never leave blank spaces. Draw a diagonal line through all open or blank space.
11. If an entry continues on a different page, include "go to page ____" on the bottom right hand of the page to let the reader know where the rest of the information is located.
12. Graphs and other small sheets of paper can be pasted into the notebook using a glue stick.
13. Always include enough details for someone else to successfully duplicate the work you have recorded.
14. Label all figures, tables and calculations. Figures should be labeled on the **bottom left** and tables are labeled on the **top left**.
15. Never remove pages from your notebook.

ACTIVITY: Analysis of Sample Lab Notebook Entries

1. Using blue or black pen complete steps A-E of the "guidelines for setting up a legal scientific notebook."
2. Enter a title in your lab notebook page (and in Table of Contents) as "Analysis of Sample Lab Notebook Entries."
3. Label the top of page 5 Lab A: Analysis of Sample Lab Notebook Entries.
4. Each student should obtain a copy of the "Sample student notebook entries."
5. Review each of the three samples by yourself. You may choose to use the notebook scoring rubric provided in the appendix of this manual. Once you have reviewed the entries you can discuss your opinions with your group members. Make a list of what the student did well and what the student could have improved upon.
6. **Draw 3 tables into your notebook, one for each student entry you will be evaluating.**

Table 1.1: Review of Sample Lab Notebook: Entry #1 Score: ____ / 10 pt

What did student do well?	Improvements Needed?

7. If you were grading these lab entries, what categories/criteria would you use to assign points? If you were the grader, what overall score (out of 10 points) would you assign the samples?

This page titled [1.3: PART I- Setting Up a Legal Scientific Notebook](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

1.4: PART II- Lab Safety

Introduction

Laboratory safety involves all the measures taken by the laboratory worker, laboratory owner, institution and regulatory agencies to eliminate potential harm to human health and well-being. Although steps are taken to reduce risks in the workplace, safety is a matter of **personal responsibility**. A biotechnology lab may have several safety hazards that must be known and understood by all students or employees working in the lab. It is the responsibility of each person in the lab to know and follow basic laboratory safety rules, to understand how to safely operate equipment, understand the hazards of materials they are working with and to work to reduce potential risks. In the event of an accident, it is critical to know the location and use of emergency equipment. Having this knowledge should help to prevent accidents and minimize damage that might occur in the event of an accident.

A. Laboratory Safety Equipment

Draw Table 1.2 in your lab notebook. Fill in the location of all of these lab safety items:

Telephone and Campus Emergency Number, Fire Alarm, Fire Extinguisher, Eye Wash, Shower, Glass Waste Container, Biohazard Waste Container, Lab Coats, Goggles, Gloves, Disinfectant, First Aid Kit, Broom / Dust Pan, Emergency Class Evacuation site

Table 1.2: Laboratory Safety Equipment and Location

Safety Equipment	Location
Telephone and Campus Emergency Number	
Fire Alarm	

Laboratory Safety Guidelines and Contract

General Rules

- No guests are allowed in the lab.
- Know emergency procedures, use and location of emergency equipment (emergency exits, fire extinguishers, fire blanket, eye wash station, first aid kit, and broken glass container).
- In case of fire, evacuate the room and assemble outside the building.
- Report all accidents, no matter how insignificant they appear, to a laboratory supervisor
- Be aware of your surroundings and potential dangers created by others.

Personal Protection

- Do not smoke, eat, drink, chew gum, or apply cosmetics in a laboratory.
- Wear protective clothing such as long pants, closed-toe shoes, a lab coat, and goggles.
- Tie long hair up or behind the shoulders. Do not wear long, dangling jewelry or scarfs.
- Dispose of gloves in the laboratory trash. Do not wear lab coats and gloves into public areas. You will need to dispose of gloves and take off your lab coat before leaving the lab.
- Cover cuts or scrapes with a sterile, waterproof bandage before entering a lab.
- Wash skin immediately and thoroughly if contaminated by chemicals or microorganisms.
- Wash your hands regularly, with soap and water, especially after working with bacteria.
- If you are pregnant or ill, please let your lab instructor know immediately.
- Let your lab instructor know before leaving the classroom.

Handling Chemicals

- Keep all containers capped with the appropriate lid. Clearly label items produced in the lab.
- If a chemical is splashed into the eyes or skin, flush for 15 minutes.
- Clean up spills and broken glass immediately. Use broom and dustpan to pick up broken glass.
- Keep chemicals away from direct heat or sunlight. Keep containers of alcohol, acetone, and other flammable liquids away from flames.

- Read labels carefully. Be aware of hazardous chemicals and precautions for safe use.
- Follow instructions about proper disposal of lab reagents.

Handling Equipment

- Keep your work area clean and clutter-free.
- Be aware of your potential impact on others.
- Notify lab supervisor of malfunctioning equipment.
- If you do not know how to use an instrument or equipment, then do not touch it.
- Do not use laboratory equipment without first receiving instruction in its use.
- Keep balances clean and dry, always use weigh paper/boats.
- Never leave heat sources unattended. Be careful when using hot plates or burners. Note that there is often no visible flame, glow or sign that objects are hot.

I have read the lab safety guidelines, found all lab safety equipment, and understand the procedure about emergency class evacuation. I will conduct myself in a safe and conscientious manner and take proper care in the use of all lab equipment.

Signature _____ Date _____

B. Lab Safety Video

1. While watching the lab safety video, take notes on as many safety hazards as you notice.
2. When the video is over, discuss the hazards you observed with your group and what can be done to work safely using good laboratory practices.
3. Make Table 1.3 in your notebook and organize your observations.

Table 1.3: Laboratory Hazards and Corrections

Laboratory Hazard Observed	Correction/Good Laboratory Practices (GLP)

C. NFPA Activity

NFPA Ratings (National Fire Protection Association)

A quick assessment of a chemical's health hazards that is usually available on its container is a rating by the National Fire Protection Association (NFPA). A color-coded diamond shape lists numbers rating a hazard as:

 NFPA Rating Explanation Guide 					
RATING NUMBER	HEALTH HAZARD	FLAMMABILITY HAZARD	INSTABILITY HAZARD	RATING SYMBOL	SPECIAL HAZARD
4	Can be lethal	Will vaporize and readily burn at normal temperatures	May explode at normal temperatures and pressures	ALK	Alkaline
3	Can cause serious or permanent injury	Can be ignited under almost all ambient temperatures	May explode at high temperature or shock	ACID	Acidic
2	Can cause temporary incapacitation or residual injury	Must be heated or high ambient temperature to burn	Violent chemical change at high temperatures or pressures	COR	Corrosive
1	Can cause significant irritation	Must be preheated before ignition can occur	Normally stable. High temperatures make unstable	OX	Oxidizing
0	No hazard	Will not burn	Stable		Radioactive
					Reacts violently or explosively with water
					Reacts violently or explosively with water and oxidizing

This chart for reference only - For complete specifications consult the NFPA 704 Standard
NFPA Chart 1: www.compliancestore.com

Figure 1.1. NFPA Ratings

Procedure

1. Walk around the room and locate two items that display the NFPA safety diamond.
2. Draw each diamond in your lab notebook and clearly label the reagent that it is describing.
3. Read the diamond and comment on the characteristic and hazards of the two items located.

Chemical Hazards and Waste Disposal

Many chemicals can NOT be poured down the sink. Labs must be aware of the university, state and federal guidelines. Be sure you know which chemicals are hazardous, require special storage or must be placed into marked waste jugs, kept in fume hoods until sent to the proper hazardous waste disposal. Know which materials are considered biohazards and have to be autoclaved.

Medical and Biohazardous Waste Treatment and Disposal Chart, U. California

TYPE OF WASTE	DEFINITION	REQUIRED TREATMENT
Medical Waste	Human blood or materials of biological origin with known or suspected infectious components	Waste is placed in red biohazard bag; minimum autoclave temperature of 121°C (250°F) for 30 minutes at 15 psi.
Liquid Medical Waste	Tissue culture media of human blood or infectious or potentially infectious materials	Decontaminate using 10% bleach solution for a 30-minute contact time. Dispose down the drain with water.
Sharps Waste	Anything that is contaminated with medical or biohazardous waste and that can pierce skin, e.g., hypodermic needles, syringes, broken pipettes, glass, razor blades, scalpels, etc.	Sharps containers.
Biohazardous Waste	Human and animal tissue culture Culture dishes or devices used to transfer, inoculate, or mix cultures; recombinant DNA, transgenic organisms, plant materials, animal blood, and animal cell lines	Waste is placed in red biohazard bag; minimum autoclave temperature of 121°C (250°F) for 30 minutes at 15 psi. Autoclave tape must be placed on the bag. Biohazardous waste must be disposed of within 7 days of being generated if stored above 0°C.
Liquid Biohazardous Waste	Non-infectious tissue culture	Same as liquid medical waste above.

D. Safety Data Sheets (SDS)

A Safety Data Sheet (previously called Material Safety Data Sheet) for each chemical is required by the Occupational Safety and Health Administration (OSHA). Sections 1-8 contain information about the identification, hazards, composition, safe handling practices, and emergency control measures. Sections 9 through 11 and 16 contain technical and scientific information, such as physical and chemical properties, stability and reactivity, toxicology, and exposure control.

Draw table 1.3 in your lab notebook and answer the following.

Using the SDS for sodium hydroxide provided to your group, determine what should be done in the event that the reagent:

- got on your skin
- splashed into your eyes
- was inhaled

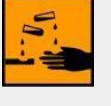
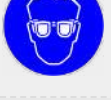
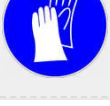
Table 1.3: Safety Procedures for Sodium Hydroxide (NaOH)

Adverse Event	Response to Event
NaOH in eyes	
NaOH on skin	
NaOH inhaled	

E. Science Laboratory Safety Signs

Write the meaning of each symbol.

Table 1.4

This page titled [1.4: PART II- Lab Safety](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

1.5: Study Questions

1. Review lab safety signs and know their meanings.
 2. What are the types of PPE required in a laboratory?
 3. How should you dispose of glass waste?
 4. How should you dispose of biohazard waste?
 5. What should you do if a chemical gets in eyes?
 6. Given a laboratory hazard, be able to describe the correction of GLP needed in the lab.
 7. What do the 4 diamonds in the NFPA represent?
 8. Why must a scientist keep a lab notebook?
 9. In industry, a lab notebook is a legal document. What can it be used to determine?
 10. What is GMP?
 11. What are the Do's and Don'ts of keeping a legal notebook?
-

This page titled [1.5: Study Questions](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

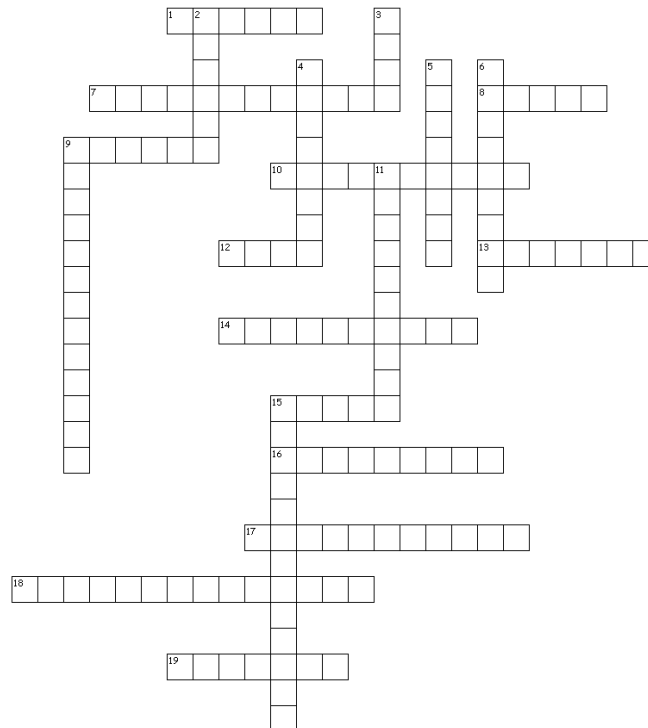
1.6: PUZZLE- Lab Safety and Lab Notebooks

Terms:

Alkaline, Biohazardous, Broom, Contents, Corrosive, Diamond, Flammability, Four, Glass, Health, Instability, Laboratory, Legal, Manufacturing, OSHA, Oxidizing, PPE, Sharps, Special

Across	Down
1. broken test tubes and beakers should be placed in the ____ waste container	2. the lab notebook is considered a ____ document and can be used in court
7. hazard that the yellow color on the NFPA sign indicates	3. abbreviation for Personal Protective Equipment
8. governmental agency that regulates chemical safety data sheets	4. shape of sign that lists the chemical ratings from the National Fire Protection Association
9. broken test tubes/beakers should be swept with a ____ and dust pan	5. hazard that the white color on the NFPA sign indicates
10. COR on the NFPA sign	6. table found at the front of a scientific lab notebook
12. instrument used to write in a scientific lab notebook	9. type of waste containing recombinant DNA and tissue culture dishes
13. used razors, scalpels and needles should be placed in the ____ waste container	11. OX on the NFPA sign
14. SOP stands for Standard ____ Procedures	15. hazard that the red color on the NFPA sign indicates
15. rating number for the most dangerous hazards in the NFPA sign	
16. ALK on the NFPA sign	
17. GLP stands for Good ____ Practices	
18. GMP stands for Good ____ Practices	
19. hazard that the blue color on the NFPA sign indicates	

Puzzle: Lab Safety and Lab Notebooks



This page titled [1.6: PUZZLE- Lab Safety and Lab Notebooks](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

CHAPTER OVERVIEW

2: Metrics and Measurements

[2.1: Student Learning Outcomes](#)

[2.2: PART I- Metrics](#)

[2.3: Part II- Measuring Using The Metric System](#)

[2.4: Study Questions](#)

This page titled [2: Metrics and Measurements](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

2.1: Student Learning Outcomes

Goals

- Review the metric system
- Learn to convert between metric units.
- Use various instruments found in the biotechnology lab
- Measure mass and volume with precision and accuracy.
- Pipet with precision and accuracy
- Learn how to use a micropipette to measure very small volumes.



Student Learning Outcomes

- Convert between metric units for mass, volume and size
- Use a gram balance to obtain the mass of an object
- Make accurate and precise measurements with a graduated cylinder and serological pipette
- Calculate percent error for a given measurement
- Read, set, and operate a micropipette.
- Determine which pipette should be used to measure a specific volume.
- Determine how accurately you can measure with each micropipette.

This page titled [2.1: Student Learning Outcomes](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

2.2: PART I- Metrics

Introduction To Metrics

Working in a biotechnology lab requires knowledge of the metric system. The metric system uses standardized units of measurement for length, mass, and volume, ensuring measurements are reproducible and easily made. Appropriate instruments are used to make these measurements. For example, balances measure mass in grams and graduated cylinders measure volume in milliliters.

The metric system has base measurements. The meter is used to measure distances; the liter measures volume; and the gram measures mass. A measurement must always consist of a number and a unit, for example, 2 m, conveys the length is twice that of the base unit of length, the meter. The abbreviations are permitted when expressing measurements. The metric system allows for easy conversion between units as everything is base 10. This means you will either multiply or divide by ten as you convert from one unit to another. For example, one decameter is 10 times larger than a meter. Therefore, you need 10 meters to equal a decameter. A kilometer is 1000 times larger than a meter. Therefore, you need 1000 m to equal one kilometer.

Table 2.1: Base Units of Measure

Length:	meter (m)
Mass:	gram (g)
Volume:	Liter (L)
Time:	second (s)
Temperature:	Celsius (C)

Table 2.2: Metric Prefixes

Kilo- (k)	1,000	10^3
Hecto- (h)	100	10^2
Deca- (da)	10	10^1
One	1	10^0
Deci- (d)	0.1 (= 1/10)	10^{-1}
Centi- (c)	0.01 (=1/100)	10^{-2}
Milli- (m)	0.001 (=1/1,000)	10^{-3}
Micro- (μ)	0.000001 (=1/1,000,000)	10^{-6}

Steps for Converting Metric Units

1. Memorize the Table 1 and know how to use metric prefixes. You can use the helpful **pneumonic** below.

<u>K</u> ing	<u>H</u> enry	<u>D</u> oes	<u>U</u> sually	<u>D</u> rink	<u>C</u> hocolate	<u>M</u> ilk	
Kilo-	Hecto-	Deca-	Base units	Deci-	Centi-	Milli-	Micro
k	h	da	m, L, g	d	c	m	μ

2. When you are converting a smaller unit to a larger unit, you move the decimal point to the left the appropriate number of steps. Keep in mind each time you move the decimal point you are dividing by 10.
3. When you are converting from a larger unit to a smaller, you will move the decimal point to the right. This means each time you move the decimal point you are multiplying by 10.
4. Write down the number you are converting for example (100 cm). Then right in the decimal point. It is always right after the ones place to the right of the number.

100 P 100.

5. If you want to convert 100 cm to meters (m) you would now look at your chart and determine how many “steps” you have to move the decimal to the right or left. From centimeter to meter you have to take 2 steps to the left. That means you must move your decimal 2 places to the left.

100. cm P 1.00 meters

Metric Conversion Practice

Using the steps above, complete the following problems in your lab notebook.

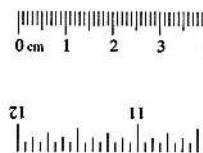
1. 50mm P ____ cm
2. 50cm P ____ km
3. 700mL P ____ L
4. 20m P ____ μm
5. 3dm P ____ m
6. 15kg P ____ cg
7. 55L P ____ mL
8. 52mg P ____ μg

This page titled [2.2: PART I- Metrics](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

2.3: Part II- Measuring Using The Metric System

A. Taking Linear Measurements With A Ruler

Linear measurements in science are in metric units. The basic unit is the METER (m). The rulers you will be using today are CENTIMETER (cm) rulers. There are 100 cm in a meter. If you look at the ruler, you will see 10 hatch marks between each centimeter marking. Each hatch mark represents a MILLIMETER (mm). There are 10 millimeters in a centimeter.



Materials

- 5-6 washers of various sizes
- Centimeter ruler

Procedure

1. Obtain 5 **washers** from your instructor.
2. Order the washers on a piece of paper from the **smallest** diameter to the **largest**, labeling them #1-5.
3. Using a centimeter ruler, record the **diameter** of each washer in centimeters. See Figure 2 for an image on how to measure the diameter.
4. Record your results in Table 1.
5. Convert all your washer diameter measurements to millimeters and meters. RECORD in Table 1.
6. Keep your washers in order as you will be using them later.

Results

Draw the following table in your lab notebook including the title of the table.

Table 2.1: Diameter Measurements of Washers with Unit Conversions

WASHER #	DIAMETER OF WASHER (cm)	DIAMETER OF WASHER (m)	DIAMETER OF WASHER (mm)
1			
2			
3			
4			
5			

B. Taking Mass Measurements With An Electronic Balance

Weight measurements in science are also in metric units. The basic unit is the gram (g). The electronic balances you will be using today are gram balances. The model you will be using will accurately measure to 0.01 gram. There are 1000 grams in a kilogram. One of the most common units used is the milligram (mg). There are 1000 milligrams in a gram. If you need a VERY small amount of something, you measure it in micrograms (μg). There are 1,000,000 μg in a gram. Some conversions are indicated below:

$$1000\text{g} = 1\text{kg}$$

$$1\text{g} = 1000\text{mg}$$

$$1\text{g} = 1,000,000\mu\text{g} (10^6 \mu\text{g})$$

$$1\text{mg} = 1000\mu\text{g}$$

Materials

- 5 washers of various sizes that were previously measured.
- Gram balance

Procedure

1. Press the on button and wait for the balance to display zeros on screen.
2. If the screen doesn't display zeros, press the "zero" or "tare" button.
3. Once the machine displays zeros (0.00g), place your washer on the center of the platform.
4. Wait for the scale to achieve a stable reading (numbers are not fluctuating).
5. Record your mass in grams in Table 2 for each washer starting from smallest to largest.

Results

1. Draw the following table in your lab manual including the title.
2. Record your results in grams (g) and then convert those masses to kg and mgs.

Table 2.2: Weight Measurements of Washers with Unit Conversions

WASHER #	WEIGHT OF WASHER (g)	WEIGHT OF WASHER (kg)	WEIGHT OF WASHER (mg)
1			
2			
3			
4			
5			

C. Volumetric Measurements

The metric unit for volume is the LITER (L). There are 1000 milliliters (mL) in one liter. Another common unit in volume is the microliter (μL). There are 10^6 μL in one liter and 1000 μL in one milliliter. Some common conversions are shown below:

$$1\text{L} = 1000\text{mL}$$

$$1\text{L} = 1,000,000\mu\text{L} \text{ (10}^6\mu\text{L)}$$

$$1\text{ml} = 1000\mu\text{L}$$

You will need to become familiar with the different types of instrumentation and glassware that you will be using throughout this semester. Today, we will focus on glassware and devices that measure larger volumes of liquid. You will also determine when a particular device is appropriate to use based on the volume that you are dispensing. The types of measuring devices are very different if you want to measure and dispense a liter vs. a milliliter!

Graduated Cylinder

You will use this to dispense large volumes that are more than 10 mL. You will be using various size graduated cylinders, ranging from 20mL – 2000 mL (2L), in this class (Figure 3A).

Serological Pipet

These pipettes accurately dispense volumes of 1mL to 10mL and can be used for volumes up to 50 mL. You will be using mostly 5mL and 10mL serological pipettes in this class.

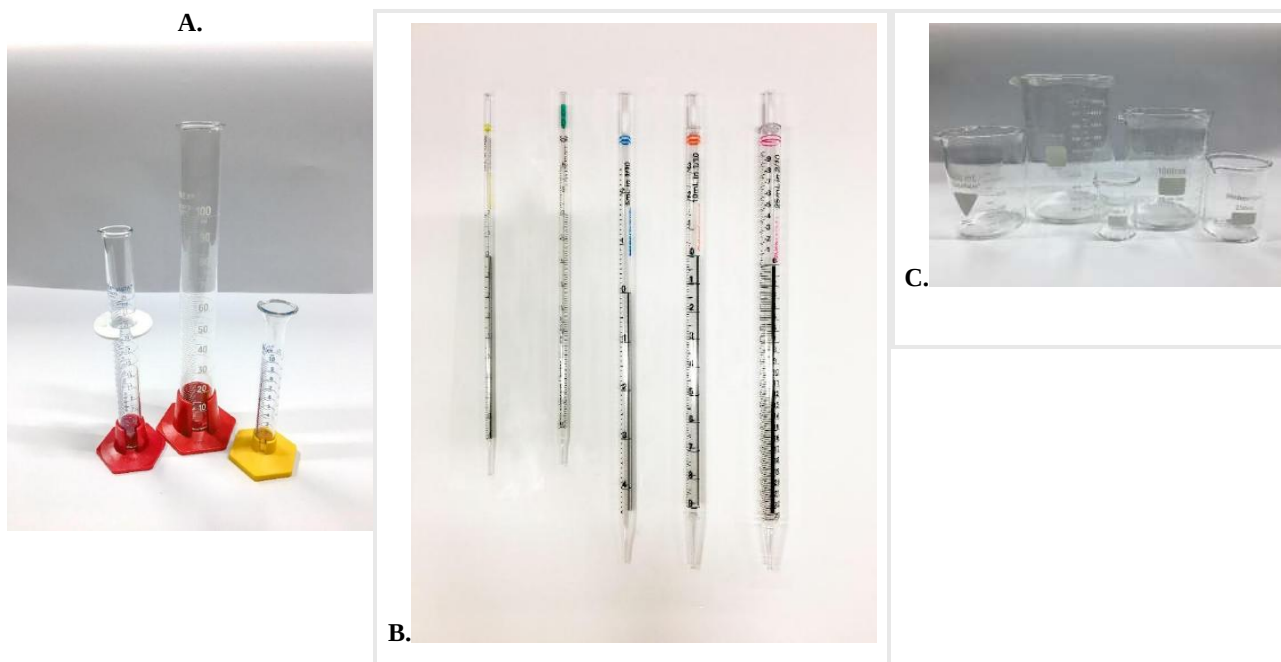


Figure 2.5. Instrumentation and glassware used to measure large volumes. Figure 2.5.A illustrates several sizes of graduated cylinders. Figure 2.4.B illustrates various sizes of serological pipettes. Figure 2.4.C illustrates various sizes of beakers.

Materials

- 1 - 50ml beaker
- Squir bottle with diH₂O
- 1 - 50 ml graduated cylinder
- 1 - gram scale
- 1 - 5ml serological pipette
- 1 pipette pump or electronic pipet aide

Procedure

Graduated Cylinder Measurements

1. Draw a table 3 in your lab manual as shown on the following page.
2. Obtain a 50 mL beaker. Weigh and record the weight in grams on Table 3 under “Weight of container”. This is the container you will use to weigh your water. It is not what you will use to measure in this experiment.
3. The **theoretical** amount of water you will be measuring using a graduated cylinder is 43 mL. This has been recorded in Table 3.
4. Using a squirt bottle, squirt 43 mL of water into a graduated cylinder. Be sure to read from the bottom of the meniscus.
5. Pour the 43 mL from the graduated cylinder into the weighed beaker.
6. Weigh the beaker with the water and record on Table 3 under “weight of container and water.”
7. Determine the weight of the water and record as “weight of water only”.
8. Convert this weight to mL. Water has a density of 1g/mL. Because water has a density of 1g/mL, then the number g=mLs (50ml=50g) Record this number as “actual volume dispensed”.
9. Determine the % error for each of your measurements as follows:

$$\% \text{ Error} = \left| \frac{\text{Theoretical amount of water} - \text{Actual volume dispensed}}{\text{Theoretical amount of water}} \right| \times 100$$

10. If you are not within an error range of +/- 5% then try again.

Serological Pipet

1. Dry the 50ml beaker you used previously. You already weighed this container in the previous exercise. Write the weight of the container you previously attained.
2. Obtain a 5 mL serological pipet and a pipet pump. Put on the pipet pump. DO NOT SHOVE THE PIPET way up into the pump. Use the dial to draw 3.7mL of deionized water into the pipet from a 50 mL graduated cylinder.
3. Dispense the water into the weighed beaker dialing in the opposite direction. To get the last bit out of the pipet, quickly dial one way then the other.
4. Dispense the 3.7 mL into the weighed beaker and determine its mass (g). Record this value in your table as “weight of container and water.”
5. Determine the weight of the water and record. If you accurately dispensed 3.7 mL, the weight difference should be very close to 3.7g.
6. Convert the weight to mL and record.
7. Determine the % error as described in the previous section and record on Table 1-3.
8. If you are not with in an error range of +/- 5% then try again

Results

Table 2.3: Measuring Volumes of Water and Determining the Accuracy of the Measurements

Device used	Theoretical volume dispensed (mL)	Weight of container (g)	Weight of container and water (g)	Weight of water only (g)	Actual volume dispensed (mL)	% Error
50mL graduated cylinder- try #1	43					
50mL graduated cylinder- try #2 (if needed)	43					
5mL serological pipet- try #1	3.7					
5mL serological pipet- try #2 (if needed)	3.7					

D. Accuracy And Precision

When making measurements, both precision and accuracy are extremely important. **Precision** of a measurement refers to the **closeness of repeated measurements or the reproducibility** of the measurement (how small the range is), while **accuracy** describes how close the measurement is to the true value or expected value.

Precision can be affected by the measuring instrument. If a student uses a thermometer calibrated to the nearest degree to measure the temperature of a beaker of water and a second student uses a thermometer that is calibrated to the nearest ten degrees for the same exercise, the reproducibility or precision of measurements will be greater for the student using the thermometer calibrated to the nearest degree. The thermometer with the greatest number of calibrations (smallest increments) is the most precise, and thus the reproducibility is greater.

Accuracy, however, is dependent upon both the **precision of the measuring instrument** and the **technical skills** of the individual taking the measurement. You will perform an experiment to determine which instrument is most accurate and precise for measuring 10ml of liquid. Once you have obtained your group data, we will use Google Docs to create a spreadsheet with the class data. You will use the class data set to determine which instrument was most accurate and most precise.

To calculate the “**mean**” or “**average**”, you will add all the measurements and then divide by the number of measurements. For example, if three groups weighed a washer and the measurements for weight were 9 grams, 11 grams, and 10 grams, to calculate the mean you would add 9+10+11 and then divide by 3 (because there were 3 measurements) and you would get a mean of 10

grams. To calculate the **range**, you would take the smallest measurement and subtract that from the largest measurement. In the last example, the range would be 11-9, which would give you a range of 2. As a class we will fill in the following tables and you will include them in your lab notebook (making sure to give EACH table a title!!).

Materials

- 1 - 50ml beaker
- Squirt bottle with diH₂O
- 1 - 25 ml graduated cylinder
- 1 - gram scale
- 1 - 10ml serological pipette
- 1 pipette pump or electronic pipet aide

Procedure

1. Obtain a 50 mL beaker. Place the beaker on the scale and hit “tare.” This will cancel out the weight of the beaker.
2. Obtain a second 50 mL beaker and use it to measure 10 mL of deionized water. Pour the measured 10 mL of water into the 50 mL beaker on the scale. Record the weight in grams under the number “1” in table 1-5 (which means that this is your first attempt to measure 10 mL with the beaker), which is equivalent to the mL of water (because remember that water has a density of 1 g/mL).
3. Dispose of the water in the beaker that is on the scale. Dry the beaker between measurements. Repeat steps 1-3 for a total of 4 trials using a beaker to measure 10 mL water.
4. Obtain a 25 mL graduated cylinder and measure 10 mL of deionized water. Pour the measured 10 mL of water into the 50 mL beaker on the scale. Record the weight in grams under the number “1” (which means that this is your first attempt to measure 10 mL with the graduated cylinder), which is **equivalent** to the mL of water (because remember that water has a density of 1 g/mL).
5. Dispose of the water in the beaker that is on the scale. Repeat steps 4-5 for a total of 4 trials using a graduated cylinder to measure 10 mL water.
6. Obtain a 10 mL serological pipet and measure 10 mL of deionized water. Pour the measured 10 mL of water into the 50 mL beaker on the scale. Record the weight in grams under the number “1” (which means that this is your first attempt to measure 10 mL with the serological pipet), which is equivalent to the mL of water (because remember that water has a density of 1 g/mL).
7. Dispose of the water in the beaker that is on the scale. Repeat steps 6-7 for a total of 4 trials using a serological pipet to measure 10 mL water.
8. When finished, dump out all the water and place your used glassware on the cart in the front of the room.
9. Determine the average and the range for your data for each measuring device and record in table 1-5.
10. Record your averages for each measuring device in Table 2.1-2.6 for the class data.
11. Record your ranges for each measuring device in Table 2.1-2.7 for the class data.

Results

Table 2.4

Measuring Device Used to Measure 10 mL	Actual Volume Dispensed for 4 trials (mL)					
	1	2	3	4	Average	Range
beaker						
Graduated cylinder						
Serological pipet						

Table 2.5

Measuring Device Used to Measure 10 mL	Class Average Actual Volume Dispensed for 4 trials (mL)				
	A	B	C	D	Class Average
Beaker					
Graduated cylinder					
Serological pipet					

Table 2.6

Measuring Device Used to Measure 10 mL	Class Range of Actual Volume Dispensed for 4 trials (mL)				
	A	B	C	D	Average Class Range
Beaker					
Graduated cylinder					
Serological pipet					

Conclusion

Use your data and the questions below to write your conclusion in your lab notebook

1. For the volume measurements, which glassware used to measure 10 mL was the most precise?
2. Which measuring device was the least precise? Explain in detail why you think that particular device is the most precise.
3. For the glassware used to measure 10 mL, which instrument was the most accurate?
4. Was the instrument that was most precise the same one that is the most accurate?
5. Why or why not (in your answer be sure to include which measuring device was the most precise and which one was the most accurate)?
6. What can contribute to a larger % error when making a measurement? If you had to repeat a measurement in Table 3, be sure to indicate why you think your % error was outside of the acceptable range.

This page titled [2.3: Part II- Measuring Using The Metric System](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

2.4: Study Questions

1. Convert the following:
 - a. 345 mL = _____ μL
 - b. 0.34 dag = _____ kg
 - c. 5.2 km = _____ mm
 2. Which equipment would you use to measure 10 mL, 5 mL, 1 mL?
 3. Be able to calculate your percent error.
-

This page titled [2.4: Study Questions](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

CHAPTER OVERVIEW

3: Micropipetting

- [3.1: Student Learning Outcomes](#)
- [3.2: Introduction To Micropipetting](#)
- [3.3: Part I- Choosing And Setting The Micropipette](#)
- [3.4: Part II- Pipetting Practice](#)
- [3.5: Study Questions](#)

This page titled [3: Micropipetting](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

3.1: Student Learning Outcomes

Goals

- Use various instruments found in the biotechnology lab
- Measure volume with precision and accuracy
- Pipet with precision and accuracy
- Learn how to use a micropipette to measure very small volumes



Student Learning Outcomes

- Make accurate and precise measurements with micropipettes and serological pipettes.
- Calculate percent error for a given measurement.
- Read, set, and operate a micropipette.
- Determine which pipette should be used to measure a specific volume.
- Determine how accurately you can measure with each micropipette.

This page titled [3.1: Student Learning Outcomes](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

3.2: Introduction To Micropipetting

The ability to measure very small amounts, **microliters (μl)**, of liquid chemicals or reagents is a fundamental skill needed in the biotechnology or research lab. Scientists use a device called a **micropipette** to measure these very small volumes with **accuracy**. This activity introduces the technique of micropipetting. Remember, as with all fine motor skills, this new skill will require practice and determination. Be sure to operate the micropipette slowly and carefully.

This page titled [3.2: Introduction To Micropipetting](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

3.3: Part I- Choosing And Setting The Micropipette

There are several sizes of micropipettes used in the biotechnology lab. Today, you will be using the P-1000, P-200, and P-20. The P-1000 measures volumes between 100-1000 μL , the P-200 measures volumes between 20-200 μL , and the P-20 measures volumes in the 2-20 μL range. It is important to always pick the correct micropipette for the volume to be measured.

Looking at Figure 3.1, you can see that each micropipette has a similar but different display window. For the P1000, the red number indicates the thousands place, followed by the hundreds, tens, and the ones displayed as small vertical lines. Each line represents 2 μL . The P-200 is read differently. The display from the top down reads, hundreds, tens, ones, and the vertical lines are considered 0.2 μL . Finally, the P-20 can be read from the top down tens, ones, and the red tenths.

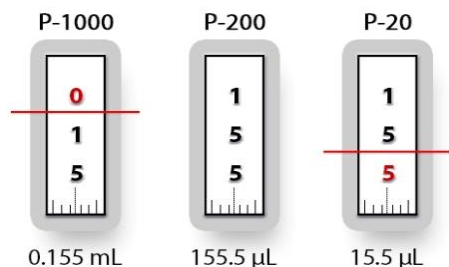


Figure 3.1: Reading a micropipette

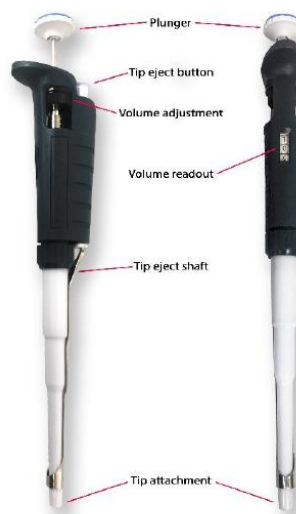


Figure 3.2: Parts of a micropipette

A. Choosing Your Micropipette

For each amount listed below, indicate the correct micropipette needed to measure the volume accurately then set the pipette to the indicated amount and show your partner.

1. 567 μL : _____ Partner observation: _____
2. 160 μL : _____ Partner observation: _____
3. 700 μL : _____ Partner observation: _____
4. 25 μL : _____ Partner observation: _____
5. 15 μL : _____ Partner observation: _____

B. Setting Your Micropipette

Materials

- P-20 micropipette
- P-20 tips
- Waste container
- Tube of red dye in tube rack
- Laminated sheet for pipetting

Procedure

1. Each student will load 5, 10, 15, and 20 μL of red dye onto the laminated sheet.
2. Locate the p-20 and set the dial to 5 μL .

3. Hold the micropipette in your dominant hand, and gently but securely place the end of the micropipette into the proper size tip. Once the tip is on, be careful **not to touch the tip on anything!** If your tip touches the bench, lab coat etc. eject the tip into the waste container and get a new clean pipet tip.
4. With your other hand, open the cap of the tube of red dye and bring the tube of red dye to eye level,
5. Push the micropipette plunger down to **the first stop and hold your thumb in this position.**
6. Place the pipet tip into the red dye solution.
7. Gently release your thumb from the plunger to draw fluid into the tip.
8. Confirm that the tip has liquid and that no bubbles are present within the tip.
9. Close the tube of red dye and place back in tube rack.
10. Gently touch the tip to the center of the circle labeled 5ul and slowly push all the way down (to second stop) on the plunger to dispense the liquid.
11. Repeat this process for the remaining volumes.
12. Be sure to watch your groupmates to provide feedback and help with their technique.

Results

Take a picture or draw a picture of your spots and include this in your lab notebook as Figure 1. Make sure the figure has a title.

Conclusion

1. Observe if your spots were similar in size to your groupmates.
2. Which volume had the most variability?
3. **What could have contributed to your spot being too large or small?**

This page titled [3.3: Part I- Choosing And Setting The Micropipette](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

3.4: Part II- Pipetting Practice

A. Microplate Art

Materials

- p20 pipette (1)
- p200 micropipette (1)
- P-20/P-200 tips
- Microplate art set (design cards, colored dyes, and 96-well microplate) (1)
- Analytical or electronic balance

Procedure

1. Obtain a 96-well microplate, a design card, and tubes of colored dyes.
2. Write the Microplate Art Design number in your lab notebook.
3. Using the gram balance, obtain the weight of your 96 well microplate and record in your notebook.
4. Using the p200 micropipette with tip, dispense 50 μ l of dye into the wells written on the design card.
5. Once you have finished pipetting, weigh your completed microplate, and record in your lab notebook.

Results

1. Be sure to record your weight in grams of your microplate pre/post pipetting in your lab notebook.
2. Using these values, calculate your percent error of the microplate you just created. Include the calculation in your lab notebook.
3. Take a picture of your microplate design and include this in your lab notebook.

Conclusion

1. Was your percent error below +/- 5%? If your percent error was above this range, elaborate on the potential causes.
2. Did your pattern look correct? How could you avoid errors in the future?

B. Micropipette Practice Matrix

Materials

- p20 pipette (1)
- p200 micropipette (1)
- 1.5ml microfuge tubes (3)
- Permanent marker
- Analytical or electronic balance

Procedure

1. Label three microfuge tubes: 1, 2, 3,
2. Weigh each tube before placing any liquid inside.
3. Draw table 3.1 in your lab notebook and use it to record your data.

Results

Table 3.1: Calculating Accuracy for Micropipetting

Tube #	Weight of tube (g)	Weight of tube + dye (g)	Theoretical weight of dye	Actual weight of dye	% Error
1					
2					
3					

4. Deliver the volumes indicated in Table 3.2 into each of the 3 labeled tubes.

Table 3.2: Volumes to be Pipetted into each Tube

Tube #	Micropipette	Red Dye (μL)	Blue Dye (μL)	Green Dye (μL)
1	P1000	210	435	332
2	P200	110	153	67
3	P20	15	17	10

5. Weigh each tube after pipetting.
6. Determine the theoretical weight of the dye using the information about the weight of a mL of the dye solution at room temperature provided by your instructor.
7. Determine the % error for each tube.

Conclusion

Based on your data comment on the following in your lab notebook:

1. Which micropipette gave the most precise measurement?
2. Which micropipette gave the most accurate measurement?
3. **What may have contributed to higher percent errors?**

This page titled [3.4: Part II- Pipetting Practice](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

3.5: Study Questions

1. Convert the following:
 - a. 345 mL = _____ μ l
 - b. 0.54 mL = _____ μ l
 - c. 5.2 L = _____ mL
 2. Which micropipette would you choose to measure 550 μ l? 17 μ l? 167 μ l?
 3. Make 3 suggestions that other biotechnologists can use to improve micropipetting accuracy.
 4. Assuming that the density of water is 1 gram per milliliter, how much should 550 μ L of water weigh?
 - a. 17 μ L of water?
 - b. 167 μ L of water?
 5. What is the formula to calculate percent error?
 6. What is the maximum volume you can set for each micropipette (P-1000, P-200, P-20)?
-

This page titled [3.5: Study Questions](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

CHAPTER OVERVIEW

4: pH and Buffers

- [4.1: Student Learning Outcomes](#)
- [4.2: Introduction](#)
- [4.3: Prelab Reading Assignment- Chemistry Review](#)
- [4.4: Activity 1- Measuring pH](#)
- [4.5: Activity 2- Preparation of an Acetate Buffer](#)
- [4.6: Activity 3- Effect of Adding Acid and Base to Acetate Buffer](#)
- [4.7: Study Questions](#)

This page titled [4: pH and Buffers](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

4.1: Student Learning Outcomes

Goals

- Accurately measure the pH of solutions using pH indicator strips and a pH meter
- Create buffer solutions and test the effects of adding acid and base to each



Student Learning Outcomes

- Understand the pH scale
- Know how to correctly use pH indicator strips and a pH meter
- Understand the function and composition of a buffer
- Relate pH values and the role of buffers to cellular function and living systems

This page titled [4.1: Student Learning Outcomes](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

4.2: Introduction

The pH of solutions is an important characteristic. Cells must maintain a constant pH so that the enzymes and processes taking place inside the cells will continue as needed. Chemical and enzymatic reactions are typically dependent on a specific pH range. Thus, it is important to understand pH and be able to determine the pH of various solutions.



Figure 7.1: The pH meter

The pH scale is a familiar concept for students who study science. The pH value of a solution reflects the relative concentration of hydrogen ions (H^+) or protons to the concentration of hydroxide ions (OH^-) in a solution. Solutions with a pH value less than 7 are acidic and those with a value greater than 7 are basic, or alkaline. The value 7 is neutral meaning the amount of H^+ in a solution is equal to the amount of OH^- in a solution. Pure water H_2O , which can dissociate naturally into H^+ and OH^- ions, would have a value of 7.

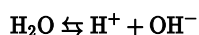


Table 7.2: The pH Scale

[H ⁺] in mol/L	pH	[OH ⁻] in mol/L	pH Classification	[H ⁺] in mol/L	pH	[OH ⁻] in mol/L	pH Classification
1.0	0	10 ⁻¹⁴	Acidic	10 ⁻⁸	8	10 ⁻⁶	Basic
0.1	1	10 ⁻¹³		10 ⁻⁹	9	10 ⁻⁵	
0.01	2	10 ⁻¹²		10 ⁻¹⁰	10	10 ⁻⁴	
0.001	3	10 ⁻¹¹		10 ⁻¹¹	11	0.001	
10 ⁻⁴	4	10 ⁻¹⁰		10 ⁻¹²	12	0.01	
10 ⁻⁵	5	10 ⁻⁹		10 ⁻¹³	13	0.1	
10 ⁻⁶	6	10 ⁻⁸		10 ⁻¹⁴	14	1.0	
10 ⁻⁷	7	10 ⁻⁷	Neutral				

This page titled [4.2: Introduction](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

4.3: Prelab Reading Assignment- Chemistry Review

In a chemical equation, variables that are surrounded by brackets “[” and “]” are expressions of concentration, or the specific amount of a molecule in a given volume of solution. For example, if you see “[H⁺]” in an equation, this is read as “the concentration of hydrogen ion”.

The concentration of a solution is often expressed in units of moles per liter (mol/L). Just as one “dozen” represents a quantity of 12 items, one “mole” represents a quantity of approximately 6.022 X 10²³ items.

one dozen molecules = 12 molecules

one mole of molecules = 602,200,000,000,000,000,000,000 molecules!

Note

“n” is used in equations to indicate a quantity measured in moles. For example if you see “nAcid” in an equation, this is read as “moles of acid”.

The term “Molarity” indicates that a solution’s concentration is in units of moles per liter. A one molar solution (1 M) contains one mole of solute within each liter of that solution. Reagents used in the laboratory will often be labeled with their concentrations expressed in terms of molarity.

The relative concentration of H⁺ or OH⁻ may change very dramatically in solutions, so a logarithmic scale (called pH) instead of a linear scale is used to express concentration. These equations can be used to calculate the pH based on hydrogen ion concentration or vice versa:

$$\text{pH} = -\log [\text{H}^+] \quad \text{and} \quad [\text{H}^+] = 10^{-\text{pH}}$$

Buffers

A buffer is a mixture of a weak acid (HA) and its salt (e.g., NaA), and is sometimes referred to as a **conjugate acid-base pair**. As mentioned above, buffers have a major role in stabilizing the pH of living systems. Vertebrate organisms maintain the pH of blood using a buffer composed of a mixture of carbonic acid (H₂CO₃) and sodium bicarbonate (Na⁺HCO₃⁻). The weak acid in this buffer is carbonic acid and the salt is sodium bicarbonate. When dissolved in water, sodium bicarbonate disassociates completely into sodium ions (Na⁺) and bicarbonate ions (HCO₃⁻). The H₂CO₃ is the conjugate acid of HCO₃⁻ and the HCO₃⁻ is the conjugate base of H₂CO₃. Together, this conjugate acid-base pair functions as the bicarbonate buffer system.

Buffer systems are also of particular importance to experimental cell biology.

The pH of a buffer solution may be calculated as follows:

$$\text{pH} = \text{pK}_a + \log \frac{n_A}{n_{HA}}$$

Where pK_a = dissociation constant of the acid, n_A = initial number of moles of salt in the buffer, and n_{HA} = initial number of moles of acid in the buffer. If you know these values, it is possible to accurately calculate the pH of a buffer system before you create it!

The pK_a of acetic acid (used in today’s experiment) is 4.75

$$n_A = \text{volume of conjugate base (mL)} \times \frac{11}{1000\text{ml}} \times \text{concentration of conjugate base (mol/L)}$$

$$n_{HA} = \text{volume of conjugate acid (mL)} \times \frac{11}{1000\text{ml}} \times \text{concentration of conjugate acid (mol/L)}$$

Use of pH Indicator Strips

The pH of a solution can be roughly approximated using strips of paper treated with color changing indicator reagents. The strips are dipped into the solution to be tested for several seconds and then removed. The color of the indicator strip is then compared to

a reference chart, often printed on the side of the strip's container. The reference color on the chart that most closely matches the color of the reacted strip will have a pH value printed below it and that will be the approximate pH. One advantage to using pH indicator strips is that they are relatively inexpensive, easy to use, and are adequate for determining pH where an error of ± 1 pH unit is acceptable. A more accurate method of determining pH is to use a calibrated pH meter, which can determine the exact pH to one or more decimal places depending on the quality of the device.

Use of a pH Meter

The pH meter is essentially a voltmeter designed to measure the potential difference between a reference electrode and a sensory electrode. The reference electrode will usually contain a solution of AgCl_2 . The external solution (or liquid to be tested) contains varying concentrations of protons. The instrument expresses the potential difference between these solutions either as a millivolt difference or translates it into a pH reading. Obtaining accurate readings with a pH meter depends on effective calibration, and the degree of static charge, and the temperature, as well as other factors. Particularly important to the proper use of a pH meter is accurate standardization, as glass electrodes must be carefully calibrated using buffers of known pH. Generally it is desirable to calibrate the meter with a standard buffer of a pH as close as possible to the pH of the solution to be measured. The problem of static charge buildup on the electrode can be eliminated by blotting (not rubbing) the surface of the electrode dry when transferring from one solution to another.

Calibrate the pH meter for pH 4, 7, and 10 before taking measurements. If calibrated properly, your pH meter should produce measurements with an accuracy of ± 0.06 pH units. Always test your meter after calibration using the standard buffers and recalibrate the meter if necessary before proceeding.

Your instructor will demonstrate the proper calibration, care, and use of the meter. Be sure to take good notes!

This page titled [4.3: Prelab Reading Assignment- Chemistry Review](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

4.4: Activity 1- Measuring pH

Materials

Per group of 4:

- 1 Set of 4 unknown solutions (in 30 mL tubes with screw top lids)
- 1 container of pH indicator strips and color reference chart
- 1 pair of forceps
- 1 pH meter (calibrated – See instructor for directions)

Procedure

1. Obtain a set of unknown solutions from instructor.
2. Measure the pH of each solution using the pH indicator strips first. Hold the strips with the forceps. ***Use a new strip for each solution!***
3. Record your data in Table 1.
4. Measure the pH of each solution using the pH meter. ***Be sure to rinse the tip of the probe with DI water before putting the probe into each sample! (Ask the instructor for instructions if you are not sure how to properly calibrate and use the pH meter).***
5. Record your data in Table 7.1.

Table 7.1. Measured pH values of Known Test Solutions

Unknown Solution	pH value measured using pH indicator strips:	pH value measured using pH meter:	Expected pH value: (Ask Instructor)
A			
B			
C			
D			

Data Analysis

- How do your pH indicator strip values compare to your pH meter values?
- Check your measured pH values with those of the other teams. Are your values similar?
- Check with your instructor to see what the actual pH values should be. How accurate were you?

This page titled [4.4: Activity 1- Measuring pH](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

4.5: Activity 2- Preparation of an Acetate Buffer

Materials

Per Class:

- 1 bottle stock solution of 0.1 M acetic acid (CH_3COOH)
- 1 bottle stock solution of 0.1 M sodium acetate ($\text{Na}^+\text{CH}_3\text{COO}^-$)

Per Group of 4:

- 6 clean 30 mL plastic tubes
- 2 clean 5 mL serological pipettes
- 2 pipette pumps (10 mL capacity)
- 1 Sharpie Marker

Procedure

1. Using a sharpie marker, label the two 30 mL tubes - one as “Acetic Acid” and the other “Sodium Acetate”. Fill each tube up with the correct stock solution.
2. Using a sharpie marker, label each of the two 5 mL pipettes - one as “AA” and the other as “SA”. To avoid contamination, DO NOT dip pipettes into stock solution bottles and ONLY use the designated pipette to transfer either acetic acid or sodium acetate from your group’s labeled tubes.
3. **Using a sharpie marker, label a clean 30 mL tube as “Buffer 1”, another as “Buffer 2”, the third as “Buffer 3”, and the fourth as “H₂O”. Each student in your group will take one tube. If there are only 3 students, one of you can also take the “H₂O” tube. Write your names into the first column of table 2 next to the tube(s) you will be working with.**
4. Create the acetate buffers using your marked serological pipettes and the specified volumes of acetic acid and sodium acetate in Table 2. **Be sure to accurately pipet the volumes indicated to get good results! Review proper pipetting technique with your instructor if necessary.**

(For the “H₂O” tube, simply fill the tube about a third full with pure deionized water)

5. Close the lids and gently shake each tube for about 20 seconds or more to mix the contents.
6. Measure the pH of each solution with the pH meter using proper technique and enter your measurements in table 2.

This page titled [4.5: Activity 2- Preparation of an Acetate Buffer](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

4.6: Activity 3- Effect of Adding Acid and Base to Acetate Buffer

Materials

Per Group of 4:

- **Everything From Activity (2 above)**
- 30 mL dropper bottle of 0.1 M HCl (Hydrochloric Acid)
- 30 mL dropper bottle of 0.1 M NaOH (Sodium Hydroxide)

Procedure

1. Add a single drop of HCl to each of your team's 4 tubes. Close the lids and gently shake the tubes to thoroughly mix the contents.
2. Measure the pH of each solution and enter the pH values in table 3.
3. Continue adding drops of HCl according to the table, measuring pH, and recording values.
4. When you have completed Table 3, you will now start adding drops of NaOH (base) to your tubes according to table 4.
5. Be sure to shake the tubes to mix the contents thoroughly before measuring pH and entering the values in Table 4.
6. Look at your results and compare the pH changes in your 4 tubes. What do you notice about the pH changes when you compare them?
7. Compare your pH values to those of the other teams. Ask your instructor for the expected values.

Table 8.2. Experimental Acetate Buffers Mixing Chart

Student	Tube	Volume of Acetic Acid (mL)	Volume of Sodium Acetate (mL)	Measured pH	Expected pH (Ask Instructor)
	Buffer 1	5.0	5.0		
	Buffer 2	7.0	3.0		
	Buffer 3	3.0	7.0		
	H ₂ O	None	None		

Table 8.3. Effect of Adding 0.1 M HCl (acid) to Acetate Buffers and Water

Tube	pH after a total of ____ drops of acid (HCl) added		
1 drop	2 drops	3 drops	
Buffer 1			
Buffer 2			
Buffer 3			
H ₂ O			

Table 8.4. Effect of Adding 0.1 M NaOH (base) to Acetate Buffers and Water

Tube	pH after a total of ____ drops of base (NaOH) added		
1 drop	2 drops	3 drops	
Buffer 1			
Buffer 2			
Buffer 3			
H ₂ O			

This page titled [4.6: Activity 3- Effect of Adding Acid and Base to Acetate Buffer](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

4.7: Study Questions

1. What range of pH values indicates that a solution is acidic? Basic?
 2. In general, how does the relative concentration of hydrogen ions [H^+] compare to that of hydroxide ions [OH^-] in a neutral, acidic, and basic solution?
 3. Based on your observations, how would you describe what a buffer does?
 4. What factors determine the accuracy of a reading with a pH meter?
 5. What is the pH of a solution that has a hydrogen ion concentration of $2.46 \times 10^{-5} \text{ M}$?
 6. What is the expected pH of a buffer made from 25.7 mL of 2.0 M Acetic acid and 0.0492 L of 0.90-M Sodium acetate?
-

This page titled [4.7: Study Questions](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

CHAPTER OVERVIEW

5: Paternity and Gel Electrophoresis

5.1: Student Learning Outcomes

5.2: Part I- Agarose Gel Electrophoresis

5.3: Part II- Paternity Case- Who is the Father of My Kittens?

5.4: Study Questions

This page titled [5: Paternity and Gel Electrophoresis](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

5.1: Student Learning Outcomes

Goals

- Run agarose gel electrophoresis on samples
- Learn how to analyze DNA fingerprints



Student Learning Outcomes

- Separate molecules by electrophoresis
- Analyze bands resulting from gel electrophoresis
- Determine the paternity of offspring using DNA fingerprint analysis

This page titled [5.1: Student Learning Outcomes](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

5.2: Part I- Agarose Gel Electrophoresis

Introduction

Gel electrophoresis is a very common and useful technique for separating DNA, RNA, and protein molecules on the basis of molecular size and charge. Agarose is a polysaccharide, found in seaweed, that forms a gel matrix (meshwork of various-sized holes). When an electric current is passed through the buffer and the gel, the molecules in the sample move toward the electrode with the opposite charge. DNA, RNA and protein molecules typically have a negative overall charge and will move toward the positive electrode. Smaller molecules can move through the matrix of the gel faster than larger molecules.

DNA fingerprinting uses band patterns that result from specific treatment of DNA samples to identify the relationship of a sample to reference samples. In this lab, you will run a simulation of a DNA fingerprinting activity in order to become familiar with this process. You will load samples on a gel and separate the bands in each sample to create specific patterns using gel electrophoresis.

Preparing Agarose Gels

There are various running buffers that can be used to separate DNA. A few of the commonly used buffers are called TAE (Tris Acetate EDTA), TBE (Tris Borate EDTA), and SB (Sodium Borate). Always use the same buffer to make the agarose gel and run the electrophoresis.

Materials

- Agarose powder
- Spatula
- Weigh boat
- 20x stock
- Graduated cylinders
- PipetAid
- Serological pipet
- Flask with vented cap

Equipment

- Balance
- Microwave

Procedure

A. Prepare 500 ml of 1X Sodium Borate buffer from a 20X stock solution.

1. Measure 25 mL 20X Sodium Borate buffer stock in a 25 mL graduated cylinder and pour into a 500 mL container.
2. Measure 475 mL DI-water in a 500 mL graduated cylinder and pour into the same container.
3. Cap tightly and mix.

B. Prepare 50 ml 0.8% (w/v) agarose in 1X Sodium Borate buffer to pour four Mini One gels.

Note

Note that agarose (and gelatin) are two substances that are prepared in a special way, as they can only dissolve in boiling liquids. So, we NEVER put agarose (and gelatin) powder into graduated cylinders. Instead we pour the powder into the final container (Erlenmeyer flask).

1. Measure 50 mL 1X buffer with a 50 mL graduated cylinder and pour into an Erlenmeyer flask.
2. Measure 0.4 g of agarose powder and pour into the same flask. This will look like an opaque slurry.
3. Use a vented cap if possible (or no cap). Place the flask in a microwave oven and turn on (for 1 minute) at high setting. Keep a close watch - do not allow the liquid to boil over!
4. Watch for bubbling of the liquid. As soon as the liquid starts to bubble, stop the microwave, use mitts or silicone "Hot Hands" to swirl the flask 2-3 times.

5. Then replace and turn on the microwave again for the second boil.
6. As soon as the liquid starts to bubble, stop the microwave, use mitts or “Hot Hands” to hold the flask to the ceiling light. Look carefully for any specks or crystals in the liquid. When there are no more specks visible in the clear solution, then the agarose has completely melted.
7. *Place the flask on the table and allow to cool to 60°C. When you are first able to hold the flask with bare hands, then the agarose is cool enough to pour into trays. Do not pour agarose too hot, as the casting gels will warp and crackle. Do not cool agarose too long, as the gel will not polymerize evenly.*
8. *Prepare the casting trays and practice loading gel samples while you wait.*

Casting Agarose Gels (Mini One Embitec Gel System)

1. Place two clear acrylic casting trays into the white casting stand.
2. Insert the 9-well comb into the proper slot of the casting stand.
3. It is best to use a Pipet-Aid and 25 mL Serological Pipet to measure and transfer 12.5 mL of melted agarose solution into each casting tray. Or pour agarose solution until 1/3 up the comb.
4. Quickly move or pop air bubbles with a pipet tip or gel comb. Insert the gel comb into the proper slot.
5. Do not move or bump the gel tray until the gel has solidified in about 15 minutes.
6. Store extra agarose solution in flask, covered with parafilm or cap, at 4°C for later use.

Practice Loading Gel Samples

Loading gel samples is a skill that takes practice to learn! The actual agarose gel that you will be using for electrophoresis is very delicate and can easily be punctured. The practice gel cannot be punctured, but provides the same size wells to dispense your samples. As DNA is clear, usually a colored loading dye is added to the DNA samples. High-density glycerol is also added to the loading dye, so that the DNA samples will fall to the bottom of the well quickly.

Lab Tip

When loading samples into the gel wells, only push the micropipette plunger to the first stop, do NOT push to the second stop. Be sure to keep your thumb pressed down on the plunger, until the micropipette is completely out of the buffer tank.

1. Obtain a practice urethane gel.
2. Cover the gel with deionized water. If there are bubbles in the wells, use a plastic transfer pipet to remove the bubbles.
3. Use a pipet tip on a P20 micropipette and set dial to 10.0 uL.
4. Push and hold micropipette plunger to the first stop.
5. Pick up 10 uL of the practice red dye and slowly release your thumb.
6. Hold the micropipette vertically (see diagram) over the practice gel, such the tip is below the water level and just above the well. There is no need for tip to enter into the well, as the heavy glycerol will pull the sample down into the bottom of the well.

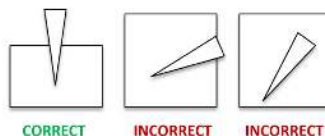


Diagram of disposable tip over wells

7. Push the plunger to the FIRST STOP only and keep your thumb there. (Optional: try pushing to the second stop to see what happens.)
8. Move your entire arm up, so that the micropipette is out of the water, then allow your thumb to come off the plunger. (Optional: try releasing your thumb while the pipette is still in the water to see what happens).
9. Practice loading 10.0 µL of the red dye into three or more wells of the practice gel.
10. You should be able to see the colored sample drop down to the bottom of the wells.

 Lab Tip

If done correctly, all of the sample will stay in the well. Check if there is any colored dye floating away from the top of the well, which means that the sample may contaminate another well. Check if there is any dye leaking out from the bottom of well, which means that you punctured the well and the sample may not enter the gel.

This page titled [5.2: Part I- Agarose Gel Electrophoresis](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

5.3: Part II- Paternity Case- Who is the Father of My Kittens?

Mary has a white cat named “Honey” who was lost for two days about three months ago. She now has four kittens (photo 1) and Mary wants to know if the two neighboring cats, “Tom” or “Butch,” could be the father of each kitten. To analyze their DNA fingerprint, Mary has collected hair follicles from each adult cat and kitten, extracted DNA, and amplified DNA using the polymerase chain reaction.

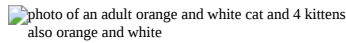


Figure 11.2. Honey (left) and her four kittens (in order): Cream, Molasses, Ginger, Sugar

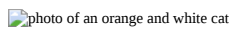
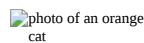
 

Figure 11.3. Potential fathers: Tom (left) and Butch (right)

Hypothesis

Using the photo, complete table 1 with your prediction of the father of each kitten.

Table 11.1. Hypothesis on Paternity of Kittens

KITTEN	POTENTIAL FATHER	REASONING
Cream		
Molasses		
Ginger		
Sugar		

Materials

Reagents

- Pre-cast 0.8% agarose gel
- Seven samples in microfuge tubes. One for each feline
- 135-150 mL 1X sodium borate running buffer (enough to submerge the agarose gel)

Equipment and Supplies

- Electrophoresis system such as MiniOne or other brand with the gel chamber and power supply
- P-20 micropipettes and appropriate tips
- Cell phone or camera to photograph the gel to document results

Procedure

Note

The following is completed when the electrophoresis chamber has been prepared with an 0.8% agarose gel and 1x Sodium Borate buffer in the chamber. Do not forget to document your procedure appropriately in your laboratory notebook.

1. Obtain the “DNA samples” – there are seven microfuge tubes labeled P- V.
2. Using a P-20 micropipettor and a pipet tip, measure 10µL from Tube P and transfer into the first well of the agarose gel. Be sure to follow the gel loading order noted in Column 1 – *Well*.
3. Using a new tip for each sample, transfer 10µL of each sample into new wells of the gel.
4. Be sure to keep track of your sample loading, if you do not follow the table below. If there were any problems with the loading (punctured gel, not enough sample), be sure to write in the NOTE column.

Loading notes: Include any deviations or notes in your laboratory notebook

Table 11.2.

Well	Tube	DNA Sample 10µL	Notes for loading
1	P	Tom (male)	
2	Q	Cream (kitten)	
3	R	Molasses (kitten)	
4	S	Honey (female)	
5	T	Ginger (kitten)	
6	U	Sugar (kitten)	
7	V	Butch (male)	

6. If using a MiniOne electrophoresis system, run the gel for 15 minutes, until color bands separate. If using another electrophoresis system, run the gel at 135V until the dye front is.
7. For best viewing of the results, pick up the casting tray (with gel) out of the buffer tank, slide the gel onto a white laminated paper, label the samples (and your team name) and take a photo.
8. **Because DNA samples will diffuse through the agarose gels, you should always record results quickly once the electrophoresis has been turned off.**

Analysis

1. Use colored pencils to record the band patterns (color the appropriate blocks) in the Data Table below.

Table 11.3. Colored “DNA” Bands Separated by Agarose Gel Electrophoresis

Band	Tom Male	Cream	Molasses	Honey Female	Ginger	Sugar	Butch Male
Blue #1							
Blue #2							
Pink #1							
Purple #1							
Yellow #1							
Yellow #2							

- Carefully consider each band of all four kitten samples and determine whether the band matches Tom, Honey or Butch. For the kitten samples (columns QRTU) in Data Table 3, write (within the colored blocks) who matches that band -- Tom, Honey, or Butch.
- Draw your conclusions based on the "DNA evidence".
- Fill in the 2nd and 3rd column in table 4 below. Compare your hypothesis in table 1 where you guessed the father for each kitten based on appearances to your conclusion regarding the father based on the DNA evidence. Was your hypothesis correct for each kitten?
- What is the specific evidence that justifies your conclusion determining each kitten's father? Fill your responses to these questions in the table below.

Table 11.4. Comparison of Hypothesis and Conclusion backed by Experimental Evidence

KITTEN	FATHER based on visual	FATHER based on DNA	Evidence
Cream			
Molasses			
Ginger			
Sugar			

This page titled [5.3: Part II- Paternity Case- Who is the Father of My Kittens?](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

5.4: Study Questions

1. During gel electrophoresis, DNA will migrate toward which electrode?
 2. If you had DNA molecules that were small, medium, long, and extra long, which would be closest to the bottom of the gel after electrophoresis?
 3. What do you know about the pattern of bands that result from an offspring as they relate to the mother and father?
 4. If you ran DNA fingerprint analysis but the band pattern for the offspring was not matching either parent, what would you conclude?
-

This page titled [5.4: Study Questions](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

CHAPTER OVERVIEW

6: Restriction Digest and Gel Electrophoresis (New)

[6.1: Student Learning Outcomes](#)

[6.2: Introduction](#)

[6.3: Part I- Restriction Digest](#)

[6.4: Part II- Casting Agarose Gel](#)

[6.5: Part III- Practice Pipetting](#)

[6.6: Part IV- Gel Electrophoresis](#)

[6.7: Study Questions](#)

This page titled [6: Restriction Digest and Gel Electrophoresis \(New\)](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

6.1: Student Learning Outcomes

Goals

- Understand the function of restriction enzymes
- Conduct analysis of DNA fragments by gel electrophoresis



Student Learning Outcomes

- Read a plasmid map to determine restriction sites and fragment sizes
- Determine if restriction enzyme recognition sequences are palindromes
- Predict the sizes of DNA fragments formed after a restriction digest
- Compare gel electrophoresis bands to determine DNA sizes

This page titled [6.1: Student Learning Outcomes](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

6.2: Introduction

Recombinant DNA technology is possible due to several tools useful for manipulating DNA molecules and transforming cells -- including plasmids, restriction enzymes and DNA ligase. This lab introduces you to plasmids and restriction enzymes, as well as the lab technique of gel electrophoresis. Later lab experiments will introduce you to the other tools of biotechnology.

Restriction enzymes (also called **restriction endonucleases**) are proteins made by many bacterial species, to defend against viral infections. Each restriction enzyme moves along a DNA molecule until it finds a specific **recognition sequence** in the DNA. The enzyme cuts the double-stranded DNA, resulting in DNA fragments. Over 3000 restriction enzymes that recognize short (4-8 bp) palindromic sequences have been discovered.

Figure 1 shows the recognition sequence for restriction enzyme Hind III. Notice that the recognition sequence is a **palindrome**, and reads the same going forwards and backwards. The Hind III enzyme makes a **staggered** cut of the DNA, and produces fragments that have single stranded areas called “sticky ends”.

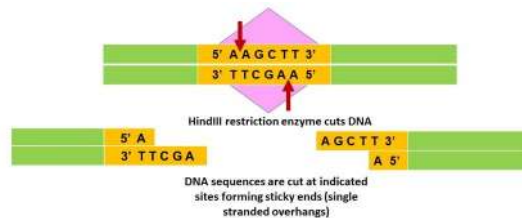


Figure 1: Recognition sequence for HindIII and the resulting cut made to DNA.

Figure 2 shows the recognition sequence of two other restriction enzymes Sca I and Pst I. Enzyme Pst I makes a staggered cut of the DNA at its recognition sequence. But restriction enzyme Sca I makes a **blunt** cut at its recognition sequence to generate DNA fragments with no sticky ends.

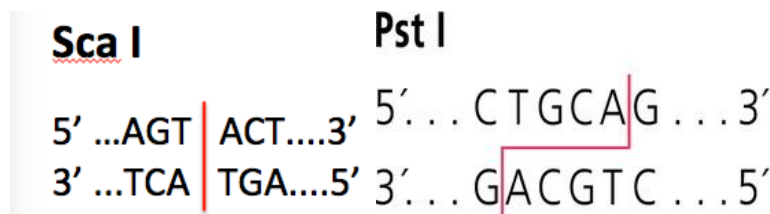


Figure 2: Recognition sequence of two other restriction enzymes Sca I and Pst I

Bacterial cells have all of their genes (genome) in a single circular chromosome. But bacterial cells can also carry non-essential pieces of DNA called **plasmids**. A plasmid is a small circular DNA that is able to replicate itself, and can carry a few genes from cell to cell. Scientists are able to design recombinant plasmids to carry specific genes into a target host cell.

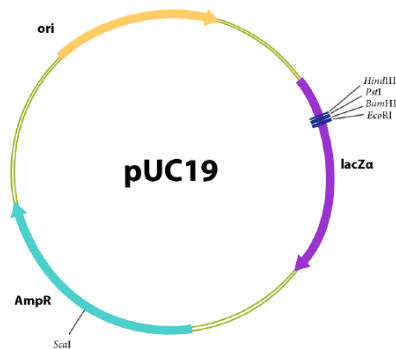


Figure 12.3: pUC19 plasmid map

The genetic map of a plasmid “pUC19” is shown in Figure 3. The total size of the plasmid is 2686 bp. There is a Pst I recognition site at position 439, Hind III recognition site at position 447, and Sca I recognition site at 2179. If one restriction enzyme is used to cut pUC19 plasmid, what would be produced?

Determine what DNA fragments are produced when two restriction enzymes are used to cut pUC19 plasmid DNA.

Table 12.1 Predicted DNA Fragments from Restriction Digest of pUC19 Plasmid

Cut with Restriction enzymes	Sca I and Pst I	Sca I and Hind III	Pst I and Hind III
Resulting DNA fragment sizes			

This page titled [6.2: Introduction](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

6.3: Part I- Restriction Digest

Agar is a polysaccharide derived from red algae. The agar powder is first dissolved in a boiling liquid, and then cooled to form a gelatinous solid matrix. As microbes cannot digest agar, this material is used commonly in laboratories to hold the nutrients that bacteria need.

Materials

- Clean microfuge tubes
- Permanent marker
- P-20 micropipette
- Box of disposable pipette tips
- Waste container for used tips and microfuge tubes
- Microfuge tube rack
- Microfuge tubes containing:
 - pUC19 plasmid DNA
 - pUS2 plasmid DNA
- Pst 1 Restriction enzyme
- Sca 1 Restriction enzyme
- Restriction buffer
- Deionized water

Equipment

- Microcentrifuge
- 37°C water bath (or dry bath or incubator)

Method

1. Use a Sharpie to label the top and side of 3 clean microfuge tubes A B C and your group name.
2. Follow the reagent table below and dispense the proper amounts of reagents to the labeled tubes.
3. Use new tips for different reagents. Add reagents to the solution at bottom of tube. Always check that your pipet tip is empty after dispensing the reagent.

Tube	DNA	Pst 1 RE	Sca 1 RE	Restriction Buffer	Water
A	4 uL pUS2	2 uL		4 uL	
B	4 uL pUC19	2 uL	2 uL	2 uL	
C	4 uL pUC19	2 uL		4 uL	
D	4 uL pUC19				6 uL

3. Cap tubes tightly Place two tubes directly across from each other in the microcentrifuge.
4. Spin for five seconds to pool the reagents at the bottom of each tube.
5. Place tubes into a floating rack in the 37°C water bath for at least one hour (but no more than two hours.)
6. After the incubation is complete, you will analyze the contents by gel electrophoresis in Part IV.

This page titled [6.3: Part I- Restriction Digest](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

6.4: Part II- Casting Agarose Gel

Agarose is a complex carbohydrate found in seaweed. If agarose is dissolved in a boiling liquid and then cooled, the solution converts into a solid gel matrix. The agarose solution will be poured into a casting tray to form the desired gel shape. A gel comb has teeth that is used to form the “wells” or holes for loading the samples. You will be prepare and cast a 1% agarose gel with electrophoresis buffer.

Materials

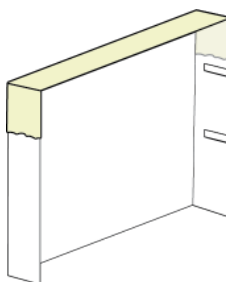
- Agarose powder
- Weigh boat
- Spatula
- Masking tape
- 100 Graduated cylinder
- 250 mL Erlenmeyer flask
- Electrophoresis Gel Casting tray
- Gel comb
- Deionized or distilled water
- 1X Electrophoresis buffer
- Heat-resistant silicone mitts or tongs
- Electronic or analytical balance
- Microwave

Method

Note

If you have concentrated electrophoresis buffer stock, you must dilute the stock to 1X working concentration before preparing agarose solutions or running gel electrophoresis.

- For 20X stock, combine 25 mL 20X stock with 475 mL deionized water to make 500 mL 1X buffer.
- For 50X stock, combine 10 mL 50X stock with 490 mL deionized water to make 500 mL 1X buffer.



1. Using the graduated cylinder, measure 100 mL of the 1X electrophoresis buffer.
2. Using an electronic scale, measure 1.0 g of agarose powder.
3. Pour some of the measured buffer into an 250 mL Erlenmeyer flask. Pour in the measured agarose powder. Pour some of the measured buffer into the agarose weigh boat, and pour into flask. Repeat until all agarose has been transferred to flask. Pour rest of buffer into flask.
4. Cover the opening of the flask with plastic wrap. Use a pipette tip to poke a small hole in the plastic wrap.
5. Place the covered flask in a microwave and heat on high. When you see bubbles form in the solution, pause the microwave, use oven mitts to gently swirl the flask a few times.
6. Continue microwaving the flask until the liquid starts to bubble again. Using oven mitts, hold the flask to the light and swirl the solution. Look carefully to check that there are no specks or swirls of agarose suspended in the liquid. If liquid is clear, then the agarose is dissolved. Wait five minutes for the agarose to cool.

 Note

Instructor will announce if you have a casting stand system and do not need to tape each tray.

7. Prepare the acrylic electrophoresis gel trays by taping the two open ends of each tray. Be sure to press tape firmly along the entire edge of the tray with your fingernail. If using masking tape, you can see a difference in the tape translucence.
8. Place a comb in each tray before adding the agarose solution.
9. When the agarose solution has cooled to the point that you can safely touch the bottom of the flask (~60°C; about five minutes), pour agarose solution into each casting tray, so that the solution covers about 2 mm of each comb.

 Note

Each Mini One gel requires 12.5 mL agarose solution, each casting tray holds two gels = 25 mL total.

10. Once the gels solidify (which will take around 30 minutes), pull the comb out of each gel. Pull it straight out without wiggling it back and forth; this will minimize damage to the front wall of the well.

This page titled [6.4: Part II- Casting Agarose Gel](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

6.5: Part III- Practice Pipetting

While you are waiting for the restriction digest to incubate, you can practice loading samples into a practice gel. As agarose gels are very easy to puncture through, it is important to have good technique for loading the samples. As the gel wells are small, only push the micropipette to the **FIRST** stop to dispense the sample. Purified DNA looks like water, so a colored dye is added to ensure that you can see the sample loading into the well. Glycerol, a viscous liquid, is added to the loading dye to ensure that the DNA sample will sink to the bottom of the well.



Materials

- P-20 micropipette
- Box of disposable pipette tips
- Waste container for used tips
- Tube of colored dye/glycerol
- Agarose or polyurethane practice gel
- Agarose powder
- Weigh boat
- Spatula
- Masking tape

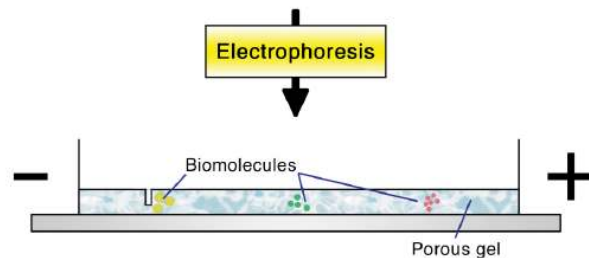
Method

1. Watch the video or instructor demonstration.
2. Submerge the practice gel with water or buffer.
3. Practice loading 5 uL and 10 uL colored dye into several wells of a practice gel. Do not change tips for this practice.
4. Place your elbow on the table to steady your pipette hand. If needed, also use your other hand to support your pipette hand.

This page titled [6.5: Part III- Practice Pipetting](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

6.6: Part IV- Gel Electrophoresis

Gel electrophoresis is a technique to use electrical current to separate a mixture of molecules such as DNA, RNA, and proteins. The electrophoresis buffer contains ions to conduct electric current. As DNA molecules are negatively charged, they will migrate towards the positive electrode (red). The solidified agarose gel matrix will have pores of various sizes (similar to a sponge), so the size, shape and charge of the molecules can affect the rate of travel through the agarose gel. Smaller molecules move faster than the larger molecules.



DNA can be visualized with various dyes. Scientists typically use ethidium bromide (either inside the agarose gel or as post-stain after the gel run). As ethidium bromide is mutagenic, we will not be using that in our class. Instead, we will use gel green stain, which is compatible with the blue LED transilluminators (eg. MiniOne). The alternative stain is gel red, which works with the uV transilluminators.

Materials

- Agarose powder
- Weigh boat
- Spatula
- Masking tape
- 100 Graduated cylinder
- 250 mL Erlenmeyer flask
- Electrophoresis Gel Casting tray
- Gel comb
- Deionized or distilled water
- 1X Electrophoresis buffer
- Heat-resistant silicone mitts or tongs
- Electronic or analytical balance
- Microwave

Method

Setting Up the DNA Samples

1. Find your tubes from the restriction digest (Part 1).
2. Add 2 uL of Gel green Loading dye into each of the sample tubes. Pipet up and down twice to mix the liquid.
3. Place tubes in a balanced configuration in a MicroCentrifuge and spin for five seconds.

Setting Up the Electrophoresis System

1. Watch videos and follow instructions for placing the gel tray into the electrophoresis buffer tank.
2. Fill the buffer tank with 1X Electrophoresis buffer, ensuring that the entire gel is completely submerged. You want about 1 mm liquid layer above the gel, but not too much buffer as that can build up resistance.
3. Check that the gel is oriented with sample wells closest to the negative electrode (black). Check that the power cord can reach easily. Check that the gel box will not need to be moved for 30 minutes.

4. Draw and label in your notebook how the samples will be loaded in the gel. Check whether you will be sharing the gel with another group.
5. Using a new tip for each sample, load the DNA samples carefully into the gel wells.
6. When all the samples have been loaded, close the cover over the electrophoresis box. [Note: Gel green is especially sensitive to light, so do not leave the Mini One light on during the electrophoresis].
7. Connect the electrical leads to the power supply. Connect both leads to the same channel, with cathode (–) to cathode (black to black) and anode (+) to anode (red to red). [Note: Mini One system must have orange hood in place to turn on].
8. Turn on the power supply and set the voltage to 130–135 V. [Note: Mini One system do not have adjustable voltage].
9. After turning on the power on the gel boxes, look for bubbles forming on the negative electrode (to show electric current) and that dyes are moving toward the correct direction. If running the wrong way, wait until dyes are inside the agarose gel, then turn the gel 180° and restart the run..
10. Do not allow the loading dye to run off the gel. Be sure to turn off the power switch and unplug the electrodes from the power supply. Do this by grasping the electrode at the plastic plug, NOT the cord.
11. Carefully remove the cover from the gel box and pick up the gel tray. Slide the gel onto plastic wrap on top of the appropriate transilluminator. Take a photo.
12. Compare the sizes of the DNA ladder to the pUC19 fragments.
 - i. The pPSU1 cut with Pst1 has fragments of 4100, 2000, 1000, 900, 800, 700, and 500.
 - ii. The pPSU2 cut with Pst I have sizes of 4100, 1500, 600, 500, 400, 300, 200, 100, 50.
13. What sizes are the pUC19 DNA fragments?

Results

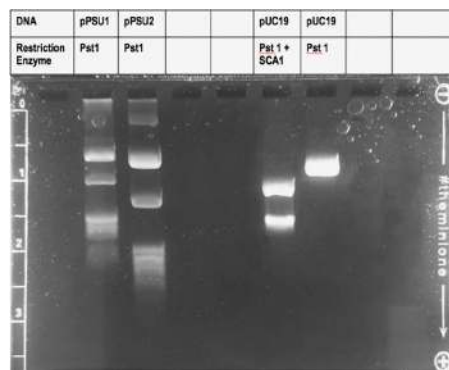


Figure 7: Restriction digests after electrophoresis

This page titled [6.6: Part IV- Gel Electrophoresis](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

6.7: Study Questions

1. What role do restriction enzymes have in nature?
 2. What is a palindrome? How does that relate to restriction enzymes?
 3. Why do molecular biology research labs always have microwaves?
 4. Why should you not ever eat in a molecular biology research lab?
 5. How do you dispense samples into an agarose gel?
-

This page titled [6.7: Study Questions](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

CHAPTER OVERVIEW

7: Transformation

[7.1: Student Learning Outcomes](#)

[7.2: Introduction](#)

[7.3: Transforming Bacteria with Plasmids](#)

[7.4: Study Questions](#)

This page titled [7: Transformation](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

7.1: Student Learning Outcomes

Goals

- Describe the role of transformation in the gene cloning process
- Explain the purpose of each control in the transformation experiment
- Explain how the information encoded in a gene is expressed as a *trait*



Student Learning Outcomes

- Carryout a transformation
- Predict the growth results for both negative control, plasmid containing reaction on both antibiotic-containing and nutrient agar only media
- Develop a reasoning, if predicted growth results don't match actual growth results

This page titled [7.1: Student Learning Outcomes](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

7.2: Introduction

A plasmid is an ideal vector for carrying DNA sequences from one organism to another. Plasmids used in genetic engineering are equipped with (1) a *promoter* that enables gene **transcription**, (2) a sequence for the initiation of DNA replication (*ori* site), and (3) an antibiotic resistance gene.

Transforming Bacteria with Recombinant Plasmid

Inserting a gene into a plasmid vector is an important first step in the gene cloning process. However, if the ultimate goal is to produce a large amount of a particular protein, the plasmid must replicate to make sure that there are many copies of the gene and the gene of interest must be *expressed*. Both activities can only occur inside a cell. Therefore, in this lab we will put a **recombinant** plasmid into *E. coli* bacteria through a process that is called *transformation*, because it changes the DNA content of the bacteria.

The plasmid will be taken up by bacteria where it replicates, and its genes will be expressed using the bacterial cellular machinery. If a gene of interest has been inserted into the plasmid vector, the bacteria produces the product encoded by that gene.

In this exercise, you will carry out the transformation of *E. coli* bacteria using a recombinant plasmid that contains a gene that produces colored proteins.

Bacteria Transformation

Once a recombinant plasmid is made that contains a gene of interest, such as insulin, the plasmid can enter bacterial cells by a process called transformation. **Figure 13.1** illustrates transformation. The uptake of DNA from the environment of a bacterial cell occurs with a very low efficiency in nature. *E. coli* bacteria have complex plasma membranes that separate the external environment from the internal environment of the cell and carefully regulate which substances can enter and exit the cell. In addition, the cell wall is negatively charged and repels negatively charged DNA molecules.

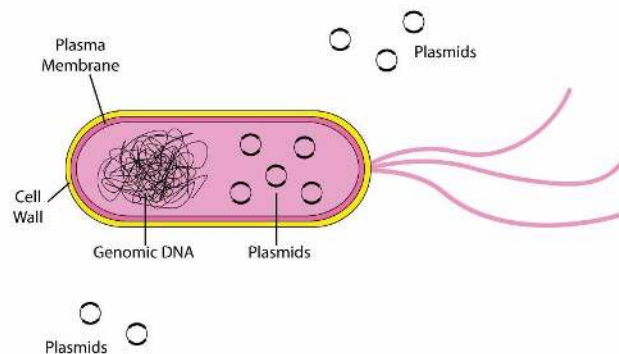


Figure 13.1: Bacterial transformation

In order to increase the efficiency of DNA uptake, bacteria are treated in two ways. First, the *E. coli* bacteria are placed in a solution that contains positive calcium ions, which neutralize the negative charge on the cells' outer membranes, enabling DNA molecules to cross the plasma membranes and enter the cell. Next, the bacteria are subjected to a heat shock, a sudden increase in temperature, which causes the pressure outside the cell to increase. This pressure difference enables the plasmid DNA to enter the bacterial cell from the outside.

Cells treated with calcium and heat are considered *competent* to take up DNA more efficiently, but even with this treatment only about 1 in 10,000 bacterial cells takes up a plasmid in its environment. So how can the bacteria that have taken up the recombinant plasmid be identified? Recall that an important component of a recombinant plasmid is a gene for antibiotic resistance. If you place bacterial cells in the presence of the **antibiotic**, only those cells that have the recombinant plasmid will grow.

From Plasmid DNA to Protein

Once a recombinant plasmid has entered the bacterial cell, DNA polymerase initiates replication at the *ori* site, and the plasmid replicates using the bacterial DNA replication enzymes. These multiple copies of plasmids can now produce the protein of interest, such as insulin, in large quantities. Usually to get the bacteria to make the protein of interest, it must be induced by adding a chemical which will encourage transcription of the gene. In this process, the information encoded in the human DNA is transferred from DNA to protein using the transcription and translation machinery of the cell (see **Figure 13.2**). The protein may alter the observable traits of the organism.

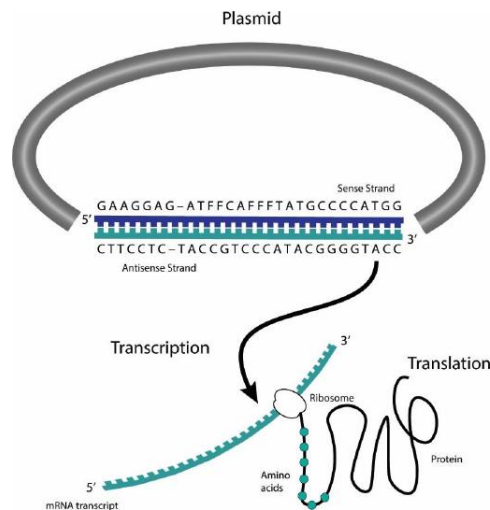


Figure 13.2: Gene expression from a plasmid in the bacterial cell

Genetic engineering is only possible because genes from different organisms can be expressed in bacteria. On Earth, all life is related, and the way that information is encoded in DNA is universal. As you may already know, proteins are made up of smaller subunits called **amino acids**, and a sequence of three **nucleotides** in DNA code for a single amino acid. These three-nucleotide sequences are called **codons**. For example, the codon TTG codes for the amino acid tryptophan, whereas the codon AAG codes for the amino acid lysine. In many cases, more than one codon can encode the same amino acid. For example, AAA is also a codon for lysine. In addition, there are informational codons, such as the *start codon* (ATG) and the *stop codon* (TTA), which show where in the DNA sequence the code for the protein begins and ends.

This page titled [7.2: Introduction](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

7.3: Transforming Bacteria with Plasmids

In this laboratory you will transform *E. coli* bacteria with plasmids. Using *E. coli* bacteria that have been pretreated with calcium chloride, you will divide the bacteria into two groups: a control group to which no **plasmid** is added, and a treatment group to which you add the plasmids. After heat-shocking both groups of cells, you will grow them under several different conditions:

- The control group is grown on nutrient agar (a *medium* that supports bacterial growth).
- The control group is grown in the presence of nutrient agar and the antibiotic ampicillin.
- The treatment group is grown in the presence of nutrient agar.
- The treatment group is grown in the presence of nutrient agar and the antibiotic ampicillin.
- The treatment group is grown in the presence of nutrient agar, ampicillin, and an inducer (such as IPTG).

By examining the growth of bacteria under these conditions, you can verify that your procedure worked, and you can identify the bacteria transformed with the plasmid that you added to the bacteria. How will you know if you are successful? In the examples we have recommended, the recombinant bacteria will have a new and highly visible trait: It will now produce colored protein, which makes the cells themselves colored!

The relevant components of this plasmid are the gene for the colored protein, the inducible promoter, and the ampicillin resistance gene (*ampR*). The *ampR* gene confers resistance to the antibiotic ampicillin. (Biotechnologists call these genes *selectable markers* because only bacteria having the gene will survive in the presence of an antibiotic.) If the inducer is present in the bacteria, the promoter will be “turned on” so RNA polymerase can transcribe the gene of interest. This will allow protein to be produced.

Prelab Questions

Discuss the following questions with your group and be prepared to share your answers with the class.

- Ampicillin is an antibiotic that kills bacterial cells by disrupting the formation of cell walls. However, the plasmid has the ampicillin resistance gene, which produces a protein that breaks down ampicillin. What is the purpose of growing bacteria that have been transformed in the presence of ampicillin?
- What will happen when bacterial cells that contain the plasmid are not given the chemical inducer?
- In the lab, you will add samples of the control group (no plasmid) P⁻ and the treatment group (with plasmid) P⁺ to plates that contain various combinations of nutrient agar (NA), ampicillin (AMP), and the chemical inducer (IND).

What are your predictions for the growth you would expect for each combination? Fill in **Table 13.1** by indicating whether you predict growth would occur or not.

- Read through the Procedures below and outline the steps, using words and a flowchart in your lab notebook.

Table 13.1 Predictions

Transformation of <i>E. coli</i>		
Medium	No plasmid control	Treatment with plasmid
Nutrient Agar		
Nutrient Agar + Ampicillin		
Nutrient Agar + Ampicillin + Inducer		

Materials

Reagents

- A rack with the following:
 - Microfuge tube of plasmid
 - Microfuge tube of Nutrient Broth (NB)
 - Microfuge tube of 100 µL of chilled competent *E. coli* cells (CC)
 - (CC tube must be kept on ice at all times)

- 3 Petri plates with agar:
 - 1 of NA/amp
 - 1 of NA/amp/ind

Equipment and Supplies

- Styrofoam cup of crushed ice (fill cup first with ice before taking CC tube.)
- 2 1.5-mL microfuge tubes
- Permanent marker
- Disposable gloves
- P-20 micropipette
- P-200 micropipette
- Tip box of disposable pipette tips
- Pack of cell spreaders (will be shared among groups)
- 42°C water bath with floating microfuge tube rack (will be shared among all groups)
- Timer or clock (will be shared among all groups)
- Tape (will be shared among all groups)
- 37°C incubator (will be shared among all groups)
- Biohazard bag for materials that come into contact with *E. coli* cells (will be shared among groups)
- Waste container (will be shared among groups)

Safety

- All appropriate safety precautions and attire required for a science laboratory should be used. Please refer to your instructions.
- Use caution when handling *E. coli* bacteria and use *aseptic technique*. Aseptic technique is a set of procedures that ensure protection of the lab worker and protection of a bacterial sample, which is necessary for the experiment to be successful. Specifically:
 - Do not touch anything that has been or will be in contact with *E. coli* bacteria. Students handling equipment that comes into contact with bacteria should wear gloves.
 - Try to avoid spills or contamination of surfaces with anything that has been in contact with *E. coli* bacteria. Immediately inform your teacher if a spill or contamination occurs.
 - When you have finished using microfuge tubes, pipette tips, and cell spreaders, place them immediately into the biohazard bag or waste container, as directed by your teacher.
 - When directed to do so, place your Petri plates back into the original sleeves and in the biohazard bag.
 - Wash your hands well with soap after completing the lab.

Procedure

1. Check your rack to make sure that you have the reagents listed.
2. Obtain a CC tube from the ice-filled container, placing it in a Styrofoam cup of ice. **The competent cells in this lab must be kept cold—be sure to pick up microfuge tubes by the upper rim to avoid warming the cells.**
3. Label two clean microfuge tubes “P–” and “P+.”

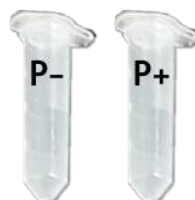


Figure 13.3. Labeled microfuge tubes

4. Place the P– and P+ tubes in the Styrofoam cup of ice with the CC tube. **Bacterial transformation requires sterile techniques. It is essential that these directions be followed precisely.**
5. Using the larger P-200 micropipette, add the competent cells from the CC tube to the P– and P+ tubes:
 - a. Set the P-200 micropipette to 50 µL.

- b. Very carefully, re-suspend the bacterial cells in the CC tube by gently pumping the pipette two times in the solution.
- c. Add 50 μL of CC to each of the empty chilled tubes (P– and P+), holding each tube at its rim to keep it cold, and return each tube quickly to the ice. **To avoid contamination, be sure to use a new micropipette tip for each addition.**
6. Using the P-20 pipette, add plasmid to the tube labeled “P+”:
 - a. Set the P-20 micropipette to 10.0 μL .
 - b. Hold the chilled P+ tube by the upper rim and add 10.0 μL of LIG. Mix the solutions by pumping the pipette two times in the liquids, and return the P+ tube to the ice.
7. Keep the P– and P+ tubes on ice for 15 minutes.
8. While the cells are on ice, prepare your three agar Petri plates—one plate each of NA, NA/amp, and NA/amp/ind:
 - a. Label the bottom of each plate (the part that contains the agar) with your group number and class period. Write small and on the edge of the plate.
 - b. With the plates closed, draw a line on the NA plate and the NA/amp plate that divides each plate in the middle. Label half of each plate “P–” and the other half “P+.” Label the NA/amp/ind plate “P+.” The plates will be arranged as follows:



Figure 13.4. Labeled plates

9. Following the 15-minute **incubation** on ice, carry the P– and P+ tubes (in the cup of ice) to the 42°C water bath. Place the two tubes in the floating microfuge tube rack in the water bath for **EXACTLY 45 SECONDS**.
10. After the 45-second heat shock, immediately place the tubes back on ice and leave them there for at least a minute.
11. Using the large P-200 micropipette, add NB to the P– and P+ tubes:



Figure 13.5 How to distribute the no plasmid control treatment onto media

- a. Set the P-200 micropipette to 150 μL .
- b. Add 150 μL of NB to the P– tube. Cap the tube and gently flick two or three times to mix.
- c. Add 150 μL of NB to the P+ tube. Cap the tube and gently flick it two or three times to mix.
12. If time permits, allow the cells in the P– and P+ tubes to incubate at room temperature for 15 minutes.
13. Add cells from the P– tube onto your NA and NA/amp plates:
 - a. Set the P-200 micropipette to 50 μL .
 - b. Gently pump the pipette two or three times in the P– tube to suspend the cells, and load 50 μL of the P– cells.
 - c. Open the lid of the NA plate, like a “clamshell,” and add 50 μL of cells from the P– tube to the section marked “P–.” Close the lid.
 - d. Again, gently pump the pipette two or three times in the P– tube to suspend the cells, and load 50 μL of the P– cells.
 - e. Open the lid of the NA/amp plate, like a clamshell, and add 50 μL of cells from the P– tube to the section marked “P–.” Close the lid.
14. Spread the cells from the P– tube on your NA and NA/amp plates:

- a. Open the package of sterile cell spreaders at the end closest to the spreader handles. Remove only one spreader, and close the package to keep the others sterile.
 - b. Open the lid to the NA plate, like a clamshell, and spread the cells evenly across the entire P⁻ side of the plate by gently moving the spreader across the agar surface. (Keep the cells on the P⁻ side of the plate.) Close the lid.
 - c. Carefully spread the P⁻ cells on the NA/amp plate, using the same spreader and technique. **Hold the spreader by the handle and do not allow the bent end to touch any surface, as this will contaminate the spreader. Place the used spreader in the biohazard bag.**
15. Add cells from the P⁺ tube to your NA, NA/amp, and NA/amp/ind plates:



Figure 13.6. How to distribute the plasmid treatment onto media

- a. Make sure that the P-200 micropipette is set to 50 μ L.
 - b. Gently pump the pipette two or three times in the P⁺ tube to suspend the cells, and load 50 μ L of the P⁺ cells.
 - c. Open the lid of the NA plate, like a clamshell, and add 50.0 μ L of cells from the P⁺ tube to the section marked “P⁺.” Close the lid.
 - d. Again, gently pump the pipette two or three times in the P⁺ tube to suspend the cells, and load 50 μ L of the P⁺ cells.
 - e. Open the lid of the NA/amp plate, like a clamshell, and add 50.0 μ L of cells from the P⁺ tube to the section marked “P⁺.” Close the lid.
 - f. Set the P-200 micropipette to 100 μ L, gently pump the pipette two or three times in the P⁺ tube, and load 100 μ L of the P⁺ cells.
 - g. Open the lid of the NA/amp/ind plate, like a clamshell, and add 100.0 μ L of P⁺ cells to various areas across the surface—not just a single spot. Close the lid.
16. Spread the cells from the P⁺ tube on your NA, NA/amp, and NA/amp/ind plates:

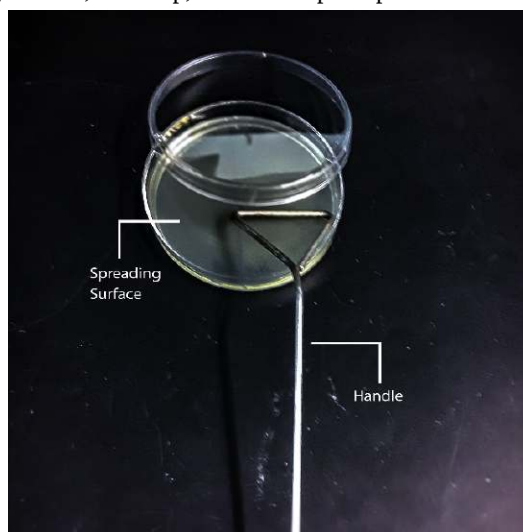


Figure 13.7. Spreading the *E.coli* onto the medium surface

- a. Open the package of sterile cell spreaders at the end closest to the spreader handles. Remove only one spreader and close the package to keep the others sterile.
- b. Open the lid to the NA plate, like a clamshell, and evenly spread the cells on the P⁺ side of the plate (and only on this side) by gently moving the spreader across the agar surface. Close the lid.

- c. Carefully spread the P⁺ cells on the NA/amp plate using the same spreader and technique.
- d. Carefully spread the P⁺ cells on the NA/amp/ind plate using the same spreader. Then gently rotate the plate beneath the P⁺ spreader so that the cells can be spread over the entire surface of this plate. Close the lid. **Hold the spreader by the handle and do not allow the bent end to touch any surface, as this will contaminate the spreader. Place the used spreader in the biohazard bag.**
17. Allow all three plates to sit right side up for five minutes. Using provided tape, tape all three plates together and label tape with your group number and class period.
18. Place the plates in the 37°C incubator upside down to prevent **condensation** from dripping onto the cells.
19. Place all microfuge tubes, pipette tips, and cell spreaders in the biohazard bag.
20. Incubate the plates for 24–36 hours at 37°C.
21. Examine the plates and in your notebook record the amount of growth on each half.

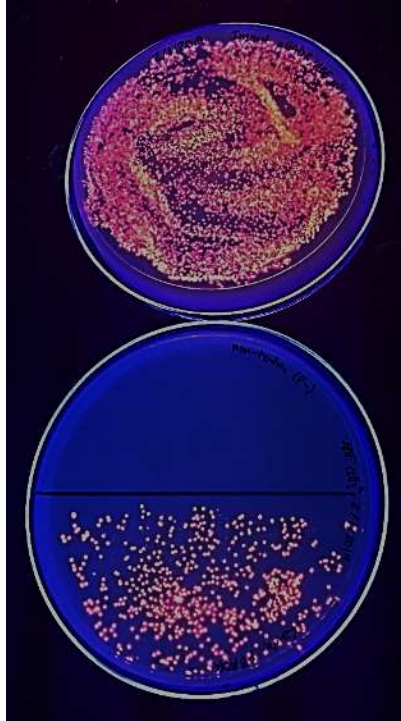


Figure 13.8. *E. coli* transformed with a plasmid with a gene encoding fluorescent protein growing on NA+Amp+IND

22. Discard the Petri plates in the biohazard bag when directed to do so.

Analysis



Table 13.9. Transformed *E. coli* producing colored molecules NA+AMP+IND

1. Look at the results of your transformation. Fill out **Table 13.2** with observations on whether you see growth or not on the different media.
2. Do your actual results match your predicted results? If not, what differences do you see and what are some explanation for these differences?

3. How many colored colonies were present on your NA/AMP/IND plate?

Table 13.2 Observations

Transformation of <i>E. coli</i>		
Medium	No plasmid control	Treatment with plasmid
Nutrient Agar		
Nutrient Agar + Ampicillin		
Nutrient Agar + Ampicillin + Inducer		

This page titled [7.3: Transforming Bacteria with Plasmids](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

7.4: Study Questions

1. Why did the colored colonies only appear on the NA/amp/ind plate and not the NA/amp plate?
 2. Recombinant plasmids are engineered so that they can replicate in the cell independently of the chromosome replication. Why is it important to have multiple copies of a recombinant plasmid within a cell?
 3. How is the information encoded in the inserted gene expressed as a trait? Be sure to use what you have previously learned about gene expression and the relationship between DNA, RNA, protein, and traits.
 4. Why is it possible for bacteria to make human protein, such as insulin, or a sea anemone protein, such as the red fluorescent protein?
-

This page titled [7.4: Study Questions](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

CHAPTER OVERVIEW

8: Column Chromatography (Rev)

[8.1: Student Learning Outcomes](#)

[8.2: Introduction](#)

[8.3: Pre Lab Activity- Think Pair Share-Discuss What You Know](#)

[8.4: Part I- Lysis of Bacterial Cells](#)

[8.5: Part II- Separating the Green Fluorescent Protein with Column Chromatography](#)

[8.6: Study Questions](#)

This page titled [8: Column Chromatography \(Rev\)](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by Donna Barron.

8.1: Student Learning Outcomes

Goals

- Explain the conditions for bacterial growth and relate it to the goal of collecting protein
- Explain what is meant by protein folding
- Describe the relationship between a protein's *conformation* (three-dimensional shape) and function of the protein
- Use column chromatography to separate proteins



Student Learning Outcomes

- Lyse bacterial cells to release protein
- Purify GFP via hydrophobic interaction column
- Identify the location of GFP throughout the purification process

This page titled [8.1: Student Learning Outcomes](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

8.2: Introduction

Proteins are biological molecules made of chains of amino acids that take on a three-dimensional shape called a **conformation**. A specific conformation is achieved by folding of the amino acid chain in a way that the protein can perform a specific job or function for the cell. If the protein does not fold in the specific way, it cannot do the job it otherwise would. Proteins can be purified and used as therapeutic agents (drugs) to treat patients with specific conditions. Because purifying specific human proteins can be difficult or impossible from human or animal tissues, we can employ cells such as bacteria like *E. coli* to do the job. These cells are genetically engineered to make the proteins of interest in large amounts that are more easily purified.

An example of a therapeutic protein is insulin, used to treat diabetes. This was the first genetically engineered protein to be introduced as a therapeutic agent. The gene for human insulin was engineered into a plasmid and this recombinant plasmid was introduced into *E. coli*. The bacterial cells made insulin which was purified to be used for treating diabetes.

Similarly, green fluorescent protein (gfp) can be produced and purified. Prior to this lab, the *gfp* gene was introduced by the process of genetic engineering into a recombinant plasmid capable of allowing *E. coli* to produce protein from the genetic instructions. Producing protein from a gene is termed **expression**. This recombinant plasmid was introduced into *E. coli* using the process of transformation. This laboratory uses the process of obtaining purified green fluorescent protein to model how therapeutic proteins are purified for human treatments.

You will be provided a culture of bacteria that have been grown and induced (stimulated) to produce gfp. You will treat the bacteria with a solution that will lyse (break open) the cells to release the protein. Then you will employ the process of column chromatography, the focus of this laboratory. Column chromatography is a method of allowing a solution to flow over a substance that will selectively bind and separate the components of the solution. In this case, tiny beads are packed into a tube-like column and the solution obtained through bacterial lysis is allowed to flow over the beads. As you pass other solutions over the column, you will be able to collect some of the solution flowing through the beads which will contain a much more concentrated and pure gfp.

Growing Bacterial Cells

The pattern of growth for bacterial cells is well understood. In the laboratory setting, the goal is to grow cells and have them produce the protein we are interested in purifying. When using optimal conditions to grow *E. coli*, four phases of growth will occur (see Figure 14.1).

1. During *lag phase* no cell division occurs. Cells are preparing to divide by making new enzymes and proteins as well as copies of their DNA. Cells enlarge.

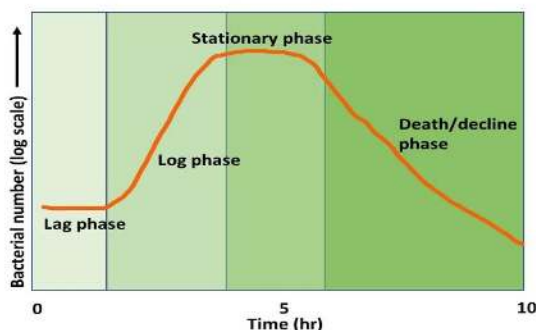


Figure 8.2.1: Copy and Paste Caption here. (Copyright; author via source)

2. In the *log phase*, a doubling of the population is occurring. This is termed logarithmic growth. Cells undergo binary fission (cells divide in half) approximately every 20 minutes for *E. coli* under optimal conditions. Other types of bacteria, and *E. coli* under less than ideal conditions, will divide at different rates.
3. The *stationary phase* occurs after the log phase when there is no overall change in the population size. During this period, cell division is equivalent to cell death and happens as resources such as nutrients and oxygen are depleted and waste products are building up in the environment.

During the decline or death phase of a bacterial culture, cells are dying faster than they replicate. The cell population is decreasing. This occurs as waste builds further and the food supply is exhausted.

This page titled [8.2: Introduction](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

8.3: Pre Lab Activity- Think Pair Share-Discuss What You Know

Prior to the lab period you should read the material, take some notes on your thoughts on the following questions (Think). In lab, you will be asked to discuss with your partner your thoughts and hear theirs (Pair). Take additional notes on new insights during this small discussion. Finally, you may be asked to discuss your ideas with the class (Share). It is not expected that you know all the answers before the class discussion but by the end of the activity, you should have the questions answered completely.

1. What is the term for bacterial reproduction? Describe this process.
2. What roles do proteins play within cells?
3. What happens to protein function if a protein loses, or never correctly achieves, the prescribed conformation?
4. How does the order of amino acids relate to protein conformation and thus protein function?
5. Since we can control when to “turn on” or express our gene, when is the best time to do so? To help you decide ask yourself:
 - i. Would producing protein take energy?
 - ii. Would a greater number of cells produce more of your protein of interest than fewer cells?
 - iii. Can proteins degrade if left too long?

As you read through the procedures for bacterial lysis and column chromatography, create a flow chart using words and pictures to describe the process. Compare your flow charts with each other prior to beginning lab. Note any differences and attempt to resolve which flow chart(s) has/have more accurate information. Adjust your own flow chart as necessary.

6. Compare your flow charts with each other prior to beginning lab. Note any differences and attempt to resolve which flow chart(s) has/have more accurate information. Adjust your own flow chart as necessary.

Protein Purification

Transformed bacteria can multiply in culture and produce the protein of interest. If this protein is to be used therapeutically, it will need to be purified. This means that other cellular components, including other proteins, must be separated from your protein. Column chromatography is a common method to separate proteins.

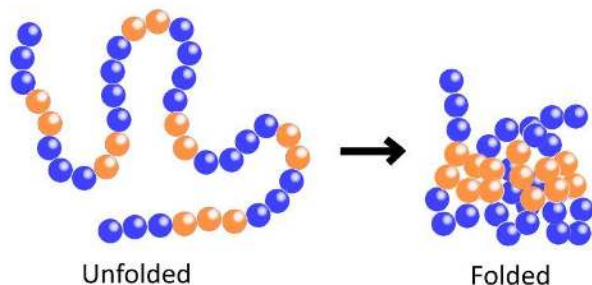


Figure 14.2. Protein

Proteins are made of amino acids. Individual amino acids have different properties such as hydrophobicity (water-hating) or hydrophilicity (liking water), ionic charge, or the ability to form weak or strong bonds with other amino acids. When a protein is first made in a cell, it is a long chain of amino acids in an order determined by the gene. The order of the amino acids in a protein determines how the chain will fold to produce a three dimensional protein conformation (See Figure 14.2). This specific conformation will have different amino acids interacting with each other in specific ways. Amino acids facing the environment in a folded protein can interact with other molecules. Specific groups of amino acids near each other can form binding sites to interact with other specific molecules. Overall, these relationships determine protein structure and thus protein function. Imagine proteins involved in enzymatic reactions, as channels in membranes, in transporting other molecules, or for binding DNA. These proteins all have very specific binding interactions determined by amino acids in specific locations in a folded protein.

Question: If individual amino acids are swapped or deleted in an amino acid chain, do you imagine this would affect the function of a protein?

The rules for protein folding are not perfectly understood and is an area of active scientific investigation. However, a few basic rules have been discovered. Factors that cause proteins to fold in specific ways include:

1. Weak bonds will form between amino acids with a negative and a positive charge.
2. Strong (covalent) bonds will form between sulfur-containing amino acids. These are called disulfide bridges.
3. Hydrophilic amino acids locate to the outer surfaces of proteins because they interact with the cell environment, which is mostly water. Hydrophobic amino acids hide on the inside of proteins or embed within cell membranes to avoid contact with the water in the environment.

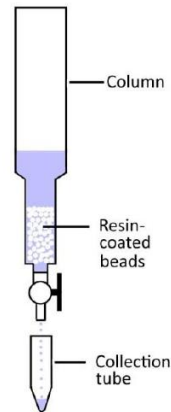


Figure 14.3. An image of a column with resin beads and a collection tube

Depending on the content of amino acids in a specific protein, overall it will take on a hydrophobic or hydrophilic character. Column chromatography can separate hydrophilic and hydrophobic proteins from the rest of the cell contents. Small beads coated with a material called a resin are packed into a column. The resin attracts proteins which will bind to the resin as other cell contents flow past. For hydrophobic proteins to stick, they must be treated to expose the typically buried hydrophobic amino acids. Buffer solutions can be used to cause proteins to denature (unfold) and expose the amino acids that will be attracted to the resin.

Different buffers are passed over the column in an order determined to best separate the proteins of interest from the rest of the cell contents. Figure 14.4 shows three solutions used to separate green fluorescent protein from the rest of the cell. The binding buffer denatures proteins so that the hydrophobic amino acids stick to the resin. The wash buffer removes loosely adherent proteins and material from the column leaving the more strongly attached protein of interest. Finally, the elution buffer, which has a low buffer concentration, causes the protein to begin to refold to hide the hydrophobic amino acids which releases the protein from the resin coated beads in the column. The portion or *fraction* of fluid exiting the column that contains your protein can be captured in a container and saved. DOES SIZE PLAY A ROLE? DO WE SAY ANYTHING ABOUT IT?

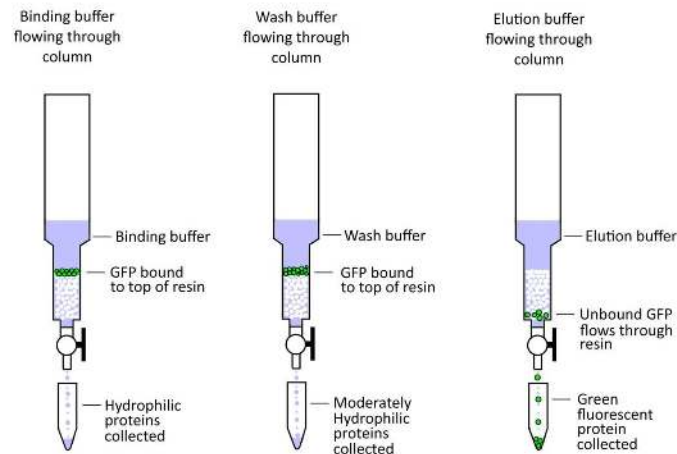


Figure 14.4. Separation of green fluorescent protein by hydrophobicity using column chromatography

Question: Do you believe that all types of protein would use the same types of resin-coated beads and the same types of buffers to become purified? Explain your answer.

This page titled [8.3: Pre Lab Activity- Think Pair Share-Discuss What You Know](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

8.4: Part I- Lysis of Bacterial Cells

Previously, bacteria were transformed with a recombinant plasmid capable of expressing *gfp* when cells were induced. The reason the cells can be induced to produce protein is that within the plasmid, in front of the gene for *gfp*, there is a special sequence of DNA that will respond if a chemical is placed in the media. This chemical is called an inducer and signals that the gene should be “turned on” and messenger RNA should be transcribed from the DNA instructions and the protein should be produced. The plasmid also contains the selectable ampicillin resistance gene to ensure that the cells growing in your culture are cells that contain your plasmid.

Prior to this lab period, cells were grown in the presence of ampicillin until late in log phase. The cells in culture divide and each cell contains many copies of the plasmid. At late log phase, the chemical inducer was added to the medium to turn on the *gfp* gene and the cells were allowed to continue to grow and produce *gfp*.

First, you will collect your cells and break them open. This process is called cell lysis. After the cells are lysed, you will use column chromatography to purify *gfp*.

Materials

Reagents (changed slightly)

- Microfuge tube rack with the following tubes:
 - LB/amp/ind culture of *E. coli* cells (EC)
 - elution buffer (EB)
 - lysis buffer (LyB)
- Additional 1,000 μ L (1 mL) of the LB/amp/ind culture of *E. coli* cells (obtain from your instructor in step 6)

Equipment and Supplies (changed slightly)

- P-200 micropipette
- Tip box of disposable pipette tips
- Permanent marker
- Microcentrifuge (shared with class)
- Vortex mixer (shared with class)
- Long wave UV light
- Liquid waste container
- Sharps container
- Biohazard bag for materials that come into contact with *E. coli* cells (shared with class)

Safety Reminder

Appropriate safety precautions should be used at all times. These will be reviewed by your instructor and can be found in the beginning of your laboratory manual which you should refer to before you begin this procedure. Aseptic technique is required when handling *E. coli* and materials that have come in contact with the bacterial culture. Remember that aseptic technique are the procedures used to protect your culture and samples from contamination but also protect you.

1. Disinfect your work area and wash your hands before beginning an experiment.
2. Never touch anything that has come in contact with the *E. coli*. This includes pipettes, spreaders, and the interior of tubes. Pipet tips should never touch anything except the material to be transferred. Spreaders and pipettes should only be handled from the end that will not touch bacteria.
3. When handling petri dishes, only open the lid enough to work with the agar surface and then close the lid immediately. This will avoid contamination, such as fungal spores from the air, landing on your agar plate.
4. If something becomes accidentally contaminated, speak to your instructor to inquire if a replacement is appropriate and available.
5. Avoid spills. If one occurs, notify your instructor immediately for help in cleaning it appropriately.

6. Contaminated waste such as used microfuge tubes and cell spreaders will be placed in the biohazard bag. Pipet tips will be placed in a sharps container.
7. Only when directed to do so will you dispose of your used petri dishes in the biohazardous waste.
8. Be sure to clean your work area and wash your hands before exiting the lab.

Procedures

1. Check your rack to make sure that you have the reagents listed for *Part I*.
2. Examine the EC tube with a long wave UV light and record its color in your notebook.
3. Before you can lyse the cells, you will need to separate the cells from the growth *medium*. To do this, spin the EC tube in the microcentrifuge for five minutes.
4. Carefully remove the EC tube from the microcentrifuge to avoid disturbing the solid pellet that contains the bacterial cells.
5. Set the P-200 micropipette to 200 μ L, place a new tip on the micropipette, and carefully remove the supernatant (liquid) from the EC tube without disturbing the cell pellet. (You can dispense the supernatant into the liquid waste container.)
6. Bring the EC tube to your teacher, who will add 1,000 μ L (1 mL) of the LB/ amp/ind culture of *E. coli* to your EC tube.
7. Repeat steps 3–5 (spin the tube for 5 minutes and remove the liquid). What color is the supernatant? The pellet? What are the contents of each?
8. Use the micropipette to remove as much of the liquid as you can without touching the cell pellet.
9. Using the P-200 pipette, add 150 μ L of elution buffer (EB) to the cell pellet in the EC tube.
10. Close the cap of the EC tube tightly and resuspend the cells using a vortex mixer. If you do not have a vortex mixer you can repeatedly drag the tube vigorously across the surface of the microfuge tube rack. Examine the EC tube carefully. If there are visible clumps of cells, continue vortexing or dragging the tube to resuspend the cells.
11. Using the P-200 pipette, add 150 μ L of Lysis buffer (LyB) to the EC tube. Close the cap of the EC tube tightly and mix the contents using the vortex mixer or by dragging the tube vigorously across the surface of the microfuge tube rack several times.
12. Label the EC tube with your group number and class period and give it to your instructor. Your teacher will incubate the cells at room temperature overnight.
13. Place all microfuge tubes and pipette tips in the biohazard bag.

This page titled [8.4: Part I- Lysis of Bacterial Cells](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

8.5: Part II- Separating the Green Fluorescent Protein with Column Chromatography

Materials

Reagents

- Microfuge tube rack with the microfuge tube of lysed cells (EC, from Part I of this lab)
- Containers with the following:
 - Binding buffer (BB) = 4.0 M Ammonium sulfate solution
 - Wash buffer (WB) = 1.3 M Ammonium sulfate solution
 - Elution buffer (EB) = 10 mM Tris, 1 mM EDTA, pH 8.0 solution
- 20% Ethanol solution (For cleaning and storing resin in columns at the end of lab)

Equipment and Supplies

- 2 1.5-mL microfuge tubes
- Liquid waste container
- P-1,000 micropipette
- Tip box of disposable pipette tips
- Chromatography column
- Microcentrifuge (will be shared among all groups)
- Waste container (will be shared among groups)

Procedure

1. Assign tasks in your group. Have one person make sure that your materials are ready (steps 2–3), another person set up the chromatography column (steps 4–5), and another person spin the lysed cells and remove the supernatant (step 6–7).
2. Check to make sure that you have all the reagents listed.
3. Label two clean microfuge tubes “SUPER” and “GFP.”
4. Set up your chromatography column as directed by your instructor, being careful not to dislodge the stopcock attached to the lower portion of the tube. **Do not allow the column to run dry.**
5. Prepare the column:
 - Set the liquid waste collection container under the stopcock.
 - Carefully open the column by turning the stopcock valve, and allow the liquid to drain into the waste collection container.
 - Close the valve once there is about 1–2 mm of liquid left above the resin bed.
 - Using the P-1000 micropipette and a new tip, add 1,000 μL (1 mL) of binding buffer (BB) gently down the side of the chromatography column.
 - Open the column by turning the stopcock valve, and allow the liquid to drain into the waste collection container.
 - Close the valve once there is about 1–2 mm of liquid left above the resin bed.
 - Make sure that the liquid is no longer draining from the column into the waste container.
6. Spin the EC tube in the microcentrifuge for five minutes to create a pellet of the cell debris.
7. Examine the tube. You should see a supernatant and a solid pellet. Use the long wave UV light to examine your tube. What color is the supernatant? The pellet? What are the contents of each?
8. Using the P-1,000 pipette, carefully remove 200 μL of EC supernatant without disturbing the cell debris pellet, and dispense the supernatant into the SUPER tube. If you dislodge the debris pellet, you will have to centrifuge the tube again.
9. Using a new tip, add 200 μL of binding buffer (BB) to the SUPER tube. Mix by gently pumping the solution in and out.
10. Using the same tip but changing the volume, add 400 μL of the SUPER tube mixture to the chromatography column. Carefully dispense the solution down the side of the column to avoid disturbing the surface of the resin bed.
11. Open the valve and allow the solution in the column to drain into the waste collection container. Close the valve once there is about 1–2 mm of liquid left above the resin bed.
12. Examine the column and locate the green fluorescent protein. Is it spread throughout the resin bed, or does it appear to be restricted to a single band? Record your observations in your notebook.

13. Using a new tip, add 1,000 μL (1 mL) of wash buffer (WB) gently down the side of the chromatography column.
14. Open the valve and allow the solution in the column to drain into the waste collection container. Close the valve once there is about 1–2 mm of liquid left above the resin bed.
15. Examine the column with the long wave UV lamp and locate the green fluorescent protein. Has the location of the green fluorescent protein changed in the resin bed?
16. Using a new tip, add 1,000 μL of elution buffer (EB) twice (2 mL total), gently, down the side of the chromatography column.
17. Set the GFP tube under the stopcock. Open the valve and allow the part of the eluate that is fluorescing under UV light to drain into the GFP tube. Close the valve and cap the tube when done.
18. Set the waste collection container back under the stopcock. Open the valve and allow the rest of the eluate to drain into the waste container. Close the valve once there is about 1–2 mm of liquid left above the resin bed.
19. Using a new tip, add 1,000 μL of 20% ethanol three times (3 mL total) gently, down the side of the chromatography column.
20. This time, open the valve and allow the solution in the column to drain into the waste collection container until there is about 1 cm of liquid left above the resin bed then close the valve securely.
21. Now place the caps back on the column tightly to prepare it for storage.
22. Pour the contents of the waste collection container down the sink drain.

Data Analysis

Compare your GFP tube with GFP tubes from other groups. Is there a difference in intensity of color from sample to sample under UV? Record your observations in your notebook.

This page titled [8.5: Part II- Separating the Green Fluorescent Protein with Column Chromatography](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

8.6: Study Questions

1. What characteristics of amino acids are important for protein conformation?
 2. How is protein conformation related to protein function?
 3. Did you notice if your lysed cell solution looked more or less bright than the fraction you collected from the column? If you observed a difference, why might that be the case?
 4. Why are you able to use the column to separate your green fluorescent protein from the other cellular components?
 5. For this procedure, what could be adjusted to increase purity of your protein in the sample?
-

This page titled [8.6: Study Questions](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

CHAPTER OVERVIEW

9: Serial Dilutions and Standard Curve with a Microplate

[9.1: Student Learning Outcomes](#)

[9.2: Part I- Serial Dilutions](#)

[9.3: Part II- Standard Curves](#)

[9.4: Study Questions](#)

This page titled [9: Serial Dilutions and Standard Curve with a Microplate](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

9.1: Student Learning Outcomes

Goals

- Perform several serial dilutions
- Use spectrophotometry to measure the absorbance of solutions



Student Learning Outcomes

- Create a series of solutions of decreasing concentrations via serial dilutions.
- Measure the absorbance of solutions with a microplate reader.
- Generate standard curves in Excel.
- Evaluate the quality of standard curves by their R² value.

This page titled [9.1: Student Learning Outcomes](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

9.2: Part I- Serial Dilutions

Introduction

A **Serial dilution** is a series of dilutions made sequentially, using the same dilution factor for each step. The dilution factor is the initial volume divided by the final solution volume. For example, if you take 1 part of a sample and add 9 parts of water (solvent), then you have made a 1:10 dilution; this has a dilution factor of 10. These serial dilutions are often used to determine the approximate concentration of an enzyme (or molecule) to be quantified in an assay. Serial dilutions allow for small aliquots to be diluted instead of wasting large quantities of materials, are cost-effective, and are easy to prepare.

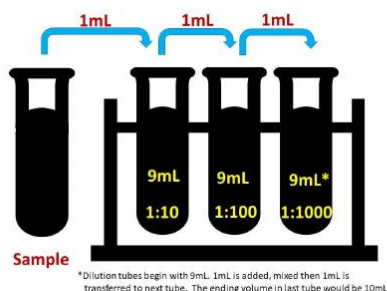


Figure 18.1: Dilution Series

Figure 18.1 shows a ten-fold serial dilution, which can also be called a 1:10 dilution, or a series with dilution factor of 10. To determine the concentration at each step of the series, you divide the previous concentration by the dilution factor.

Diagram of 1:2 Serial Dilutions

In your notebook, draw a diagram showing the serial dilutions for the 6KMnO_4 solutions you are preparing. In the diagram, indicate the volume being withdrawn from the concentrated solution, the volume of water added, the concentration of the new solution, and the total volume.

Practice Calculations

Problem 1. Assume the original sample used in Figure 1 contained 400 g/L of Reagent X.

- Then the first 1:10 dilution tube would have a concentration of $400/10 =$ _____
- Then the second 1:10 dilution would have a concentration of _____

Problem 2. Assume the original sample used in Figure is considered 100% concentration.

- Then the first 1:10 dilution tube would have a _____ % concentration.
- The second 1:10 dilution tube would have a _____ % concentration.

Problem 3. To make a serial dilution with a dilution factor of 5, you would need to add 1 part of the reagent plus _____ parts of water to make a total of 5 parts. This five-fold serial dilution would have concentrations of 100%, _____% in first diluted tube, _____% in second diluted tube, _____% in third diluted tube.

Problem 4. Suppose the third diluted tube of a two-fold serial dilution has a concentration of 300 g/L.

- That means that the second diluted tube has a concentration of _____
- The first diluted tube has a concentration of _____
- The original tube has a concentration of _____
- What formula could you use to calculate the concentration of the original tube from the problem statement?

Activity: Making Serial Dilutions

Materials

Reagents

- Blue food dye

- DI H₂O
- 96 well microplate (dry)

Supplies

- P200 Micropipette
- Box of P200 Pipet tips

Note

Use one pipet tip for each serial dilution.

Procedure

Preparing Two-Fold Serial Dilution (Dilution Factor of Two)

1. Obtain a clean, dry 96 well microplate, always touching the edges only. Use a dry clean paper towel to wipe off any fingerprints on the bottom of the plate.
2. Hold plate up to the light and check that there are no dirty spots on the three rows that you will use.
3. (Optional) You can scan the plate with no liquid, to find out the baseline absorbance of the plastic.
4. Pipet 100 uL of DI-water into the first 5 wells of row A (A1-A5).
5. Pipet 100 uL of the original blue dye into the first well (A1). Carefully pipet up and down twice to mix.
6. You do not need to change the pipet tip. But make sure that you released all liquid into the first well.
7. Transfer 100 uL of the mixture into the next well (A2). Mix carefully and release all liquid.
8. Transfer 100 uL of the mixture into the next well (A3). Mix carefully and release all liquid.
9. Transfer 100 uL of the mixture into the next well (A4). Mix carefully and release all liquid.
10. Transfer 100 uL of the mixture into the next well (A5). Mix carefully and release all liquid.
11. Transfer 100 uL of the mixture into the next well (A6). Take a photo of the wells on top of a white paper.

Preparing Four-Fold Serial Dilution (Dilution Factor of Four)

1. Using a new pipet tip, pipet 150 uL of DI-water into the first 5 wells of row B (B1-B5).
2. You do not need to change the pipet tip. Pipet 50 uL of the original blue dye into the first well (B1). Carefully pipet up and down twice to mix. Then make sure that you released all liquid into the first well.
3. Transfer 50 uL of the mixture into the next well (B2). Mix carefully and release all liquid.
4. Transfer 50 uL of the mixture into the next well (B3). Mix carefully and release all liquid.
5. Transfer 50 uL of the mixture into the next well (B4). Mix carefully and release all liquid.
6. Transfer 50 uL of the mixture into the next well (B5). Mix carefully and release all liquid.
7. Transfer 50 uL of the mixture into the next well (B6). This ensures that all wells B1-B5 have the same volume.
8. Take a photo of the wells on top of a white paper.

Preparing Five-Fold Serial Dilution (Dilution Factor of Five)

1. Using a new pipet tip, pipet 160 uL of DI-water into the first 5 wells of row C (C1-C5).
2. You do not need to change the pipet tip. Pipet 40 uL of the original blue dye into the first well (C1). Carefully pipet up and down twice to mix. Then make sure that you released all liquid into the first well.
3. Transfer 40 uL of the mixture into the next well (C2). Mix carefully and release all liquid.
4. Transfer 40 uL of the mixture into the next well (C3). Mix carefully and release all liquid.
5. Transfer 40 uL of the mixture into the next well (C4). Mix carefully and release all liquid.
6. Transfer 40 uL of the mixture into the next well (C5). Mix carefully and release all liquid.
7. Transfer 40 uL of the mixture into the next well (C6). This ensures that all wells C1-C5 have the same volume.
8. Take a photo of the wells on top of a white paper.

Activity: Measuring Absorbance

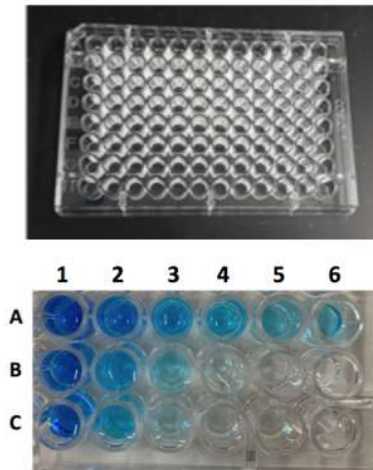


Figure 18.2 Microplate and Serial Dilutions

A microplate reader is a spectrophotometric instrument that can measure the absorbance of 96 different samples at one time. Does that save time compared to working with individual cuvettes and a spectrophotometer? We will use a microplate with 96 wells, so that you can perform all of your serial dilutions onto one plate and scan the entire plate with the microplate reader once. The microplate has rows marked A-H and columns marked #1-12. Using blue dye, you will make a 1:2 serial dilution on row A, make a 1:4 serial dilution on row B, and a 1:5 serial dilution on row C.

Materials

Equipment

- Microplate Reader and cables
- Laptop computer with program installed to run microplate reader

Procedure

1. Attach the cable from laptop computer to microplate reader.
2. Power “ON” the laptop computer and the microplate reader.
3. Open the computer program to run the microplate reader.
4. Push the button to open the microplate reader and expose the microplate loading platform.
5. Place your microplate securely into the holder area, ensuring that well A1 is at the top left corner.
6. Push the button to close the microplate reader.
7. On the computer program, start “read new plate” using wavelength 595 nm (for blue dye).
8. With the computer mouse, highlight the cells corresponding to the microplate wells that you used. Take a photo of the computer screen and note the Absorbance data in the tables below.
9. Calculate the dye concentration for each well, by dividing the dilution factor for each step of the serial dilution. The original dye is 100% concentration.

Data Table 18.1.

Absorbance Measurements of Two-Fold Serial Dilution with Microplate Reader					
Well	A1	A2	A3	A4	A5
Absorbance @ 595 nm					
Dye Concentration					

Data Table 18.2.

Absorbance Measurements of Four-Fold Serial Dilution with Microplate Reader					
Well	A1	A2	A3	A4	A5
Absorbance @ 595 nm					
Dye Concentration					

Data Table 18.3.

Absorbance Measurements of Five-Fold Serial Dilution with Microplate Reader					
Well	A1	A2	A3	A4	A5
Absorbance @ 595 nm					
Dye Concentration					

This page titled [9.2: Part I- Serial Dilutions](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

9.3: Part II- Standard Curves

Introduction

Standard curves (also known as calibration curves) show the relationship between two quantities. The standard curves are most often used to determine the concentration of “unknown” samples by comparing them to reference samples with “known” concentrations. Later in the course, we will use standard curves to measure amounts of extracted protein and to determine the size of DNA molecules. In today’s lab, you made three serial dilutions and should be able to calculate the concentrations for each dilution. Using Excel, you will prepare standard curves for each serial dilution and determine if your standard curve is accurate. Then you will determine the concentration of unknown samples, using your standard curves.

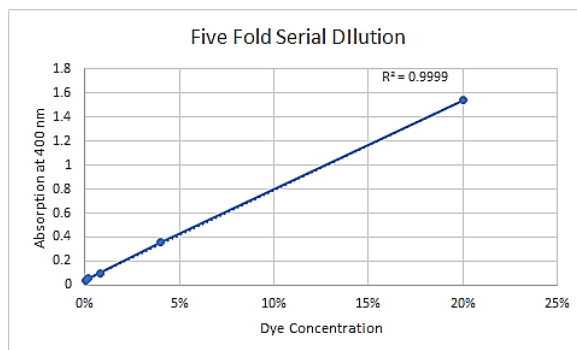


Figure 18.3. Standard Curve of Five Fold Serial Dilution

The R-squared value (R^2) is the correlation coefficient or the square of the correlation. For the standard curve, this value measures how strong the linear relationship is between the reagent concentration (X-axis) and the absorbance value (Y-axis). If the R^2 value = 1, then that shows a perfect positive relationship. Since your standard curves are generated from the serial dilutions you pipetted, the R^2 values can also show how accurate your pipetting skills are.

Activity: Making a Standard Curve for Each Serial Dilution

1. Enter the data into Excel.
2. Select the data values with your mouse. On the Insert tab, click on the Scatter icon and select Scatter with Straight Lines and Markers from its drop-down menu to generate the standard curve.
3. Be sure to add graph title and labels for X and Y axes.
4. To add a trendline to the graph, right-click on the standard curve line in the chart to display a pop-up menu of plot-related actions. Choose Add Trendline from this menu.
5. Select “display equation on chart” and “display R-squared value on chart”. Ideally, the R^2 value should be greater than 0.99.
6. Print the standard curves and add to your notebook.

Activity: Determining the Concentration of “Unknown” Samples

1. Your instructor will have several unknown samples.
2. Determine the absorbance values of each sample.
3. On your standard curve, use the graph equation to solve for the corresponding concentration of these samples. Or estimate from the line graph.

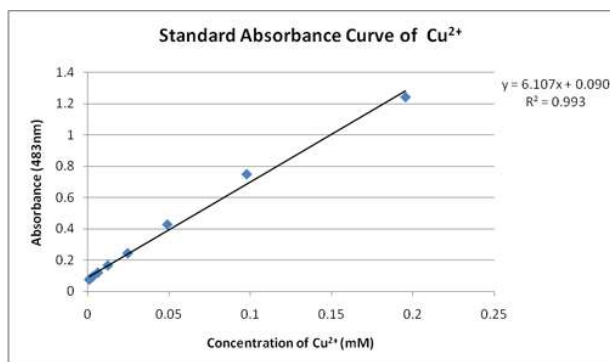
Data Table 18.4.

Unknown Sample	Absorbance	Concentration Compared to Serial Dilution Graphs		
		2-fold	4-fold	5-fold

This page titled [9.3: Part II- Standard Curves](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

9.4: Study Questions

1. Using a serial dilution, describe how you would prepare 10 mL of 1.0%, 0.1% and 0.01% solutions of **NaOH**. The stock solution is 10% **NaOH**. Draw diagrams as part of your descriptions/protocols.
2. Using the depicted standard curve, calculate the concentration of an unknown solution if its absorbance value is 0.55.



3. Evaluate the quality of the standard curve (see diagram) by using the R^2 value.

This page titled [9.4: Study Questions](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

CHAPTER OVERVIEW

10: Good Manufacturing Practices (GMP) with New Table

10.1: Student Learning Outcomes

10.2: Part I- Review Terms and Background Information

10.3: Part II- Train and Prepare for a Production Run

10.4: Part III- Production and Documentation

10.5: Study Questions

This page titled [10: Good Manufacturing Practices \(GMP\) with New Table](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

10.1: Student Learning Outcomes

Goals

- Make a batch of GMP popcorn within a specified timeframe (1.5 hours)
- Use Standard Operating Procedures to accomplish the manufacturing task
- Understand the complexity of the GMP process
- Understand Quality Control and Quality Assurance
- Understand the need for team members to complete individual jobs appropriately to accomplish goals

Learning Objectives

- Understand the role of Standard Operating Procedures (SOPs) and Batch/Lot Records
- Employ the principles of a quality systems approach to manufacturing
- Learn the difference between Quality Control and Quality Assurance QC/QA
- Practice teamwork

This page titled [10.1: Student Learning Outcomes](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

10.2: Part I- Review Terms and Background Information

Introduction

In everyday speech, quality is a relative attribute like beauty. However, in the Life Sciences or any other manufacturing-related industry quality means **compliance to specifications**. **Quality systems** are comprised of the policies, processes, and procedures for maintaining a product with specifications.

This lab exercise will utilize the manufacturing of a popcorn snack as an example where **good manufacturing practices (GMPs)** can be followed. The exercise used the components of a Quality System. Students are the employees with tasks to accomplish within the defined QA/QC parameters.

Quality Management Systems (QMS)

Quality is the business of the entire company.



Figure 20.1. Relationship between the Quality System, Quality Assurance, and Quality Control

Generally, companies build their quality systems by first making a commitment by management to design and deliver a ‘quality’ product. The **term quality management system** refers to the organizational resources, processes and procedures to implement quality management. In the case of pharmaceuticals, diagnostics and medical devices, these requirements mean the product is EXACTLY what was tested in clinical trials.

Quality product management is usually implemented through two departments; **quality assurance (QA)** and **quality control (QC)**.

The QA department plans activities and develops the processes that guarantee the accuracy and precision of outputs. QC performs the actual tests during the process and on the final product to ensure that specifications are met. Quality Assurance is process oriented and focuses on defect prevention, while quality control is product oriented and focuses on defect identification. QC refers to a measuring process, or to check a result and provide assurance that all activities are performing within predetermined limits.

Often, QMS systems are explained in this simple statement.

“Say what you do, do what you say, document It.”

SAY WHAT YOU DO = SOPs

Standard Operating Procedures (SOPs) and documentation are essential components of a **QA program**. The management team is responsible for ensuring adherence to the QA plan and SOPs. Essential to this process is documentation of the proposed quality system and processes used by the company.

Perhaps the most important form of documentation is SOPs. SOPs are validated methods that are at the technical core of the product or service. To determine whether methods are fit for their intended purpose, the selected methods must have established accuracy, precision, calibration and limits of detection and quantification. All methods must be fully validated for accuracy and precision before a company starts to sell its product or service.

DO WHAT YOU SAY = Follow the Process (SOPs)

Quality systems are implemented in many sectors of the life sciences industry including manufacturing, a testing laboratory, clinical trials or even Research and Development. In all cases the SOPs must be followed. In the example of a manufactured product, all manufacturing employees follow the defined process and document that they have done so. The QC department provides the process checks and balances. For example, they manage the acceptance and release of raw materials, in-process checks and testing of the final product.

DOCUMENT IT = Evidence of Quality

If you didn't write it down = you didn't do it!

No documentation = no **PROOF** you did it!

No documentation = **BAD** product and lost money for your company

Current Good Manufacturing Practices (cGMPs)

The general principles that all QMS have in common are:

1. Quality, safety, and effectiveness must be designed and built into the product, not tested or inspected into the product **Say what you do**
2. Each step in the manufacturing process must be documented and controlled to ensure the finished product meets design and regulatory specifications **Do what you say**
3. Process documentation provides evidence of compliance with cGMPs **Document it**

Statistical Process Control

Another aspect of a Quality program uses **statistical process control (SPC)**, which utilizes statistical methods to evaluate variability in procedures. In the design of the product, multiple data sets are obtained to establish accuracy and precision criteria. These criteria are documented in the **Master Record**. QC testing procedures ensure SPC through instrument calibration and validation, in process testing, final product testing, raw material testing etc. They use process control charts to monitor the process and procedures in order to manage variability.

The purpose of the Quality systems is to ensure quality. Since quality is defined as compliance to specifications, there must be set in place a 'statistical process control (SPC) to ensure this compliance. The SPC requires understanding of both the accuracy and precision required in the product.

Accuracy is the 'right answer' or 'bulls-eye. Accuracy is not always known but when it is, it is part of the process. **Precision** is reproducibility. Whatever the process or procedure, the quality system is there to ensure reproducibility.

Corrective and Preventative Actions (CAPA)

The purpose of the CAPA system is to serve as a feedback loop to identify and investigate all quality problems. CAPA policies are central to quality system. Part of the QC testing that takes place on every lot of product, is to assure compliance to specifications. If the specifications are not met, it is important to determine what is the root cause of the problem. Until the root cause is used to identify the cause of problems with accuracy and precision, product quality cannot be assured.

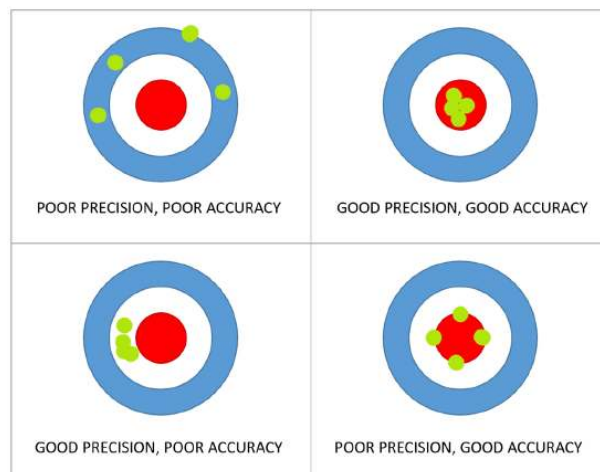


Figure 20.2. Accuracy and Precision

This page titled [10.2: Part I- Review Terms and Background Information](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

10.3: Part II- Train and Prepare for a Production Run

Table 20.1. Quality Assurance Versus Quality Control

Element	Quality Assurance (QA)	Quality Control (QC)
Definition	the activities focused on the processes for preventing mistakes and producing products free of defects	a set of activities concerned with monitoring and verifying resulting products meet the defined standards and specifications for quality
Purpose	Verification: Answer the question "Am I building the product correctly"? Prove the system meets all specified requirements at a particular stage.	Validation: Answer the question "Am I building the right product"? Ensure that the product meets customer expectation.
Type of process	Proactive	Reactive
Goal	Plan to prevent problems	Identify defects in the finished product Identify and correct source of product defects
Tools	Statistical Process Controls: Control Charts Run Charts	Statistical Quality Controls: Random Acceptance Sampling Range Charts Histograms Means Standard Deviation
Responsibility	Everyone	Quality Control Group

Lab Activity Flow Chart

Scenario: Your company, AWESOME SNACKS CO. (ASC), needs you to produce popcorn for their *Summer Snack Mix*. Your job will be to take the raw material and produce the popcorn that goes to the Snack Mixing Department

1. Employee Overview

- Supervisor (teacher) – Welcomes Employees (students) to Awesome Snack Company.
- Overview of QMS and why it's important to the company.

2. Group Formation

- Divide employees up into Quality Assurance Teams (5 employees each team)

3. Training

- Supervisor gives training form (see below, in QA packet) and packets to team

ASC TRAINING
—COMPLETE BEFORE STARTING PRODUCTION—

- Team Assigns Jobs to Members

- Material Control Tech.
- Quality Control Tech.
- Manufacturing Tech. I
- Manufacturing Tech II
- Quality Assurance Tech.

- QA Tech Reads SOP Standards 11-001 to team

- D. QA Tech distributes packets to each team member
- E. Team completes ASC Training Document.
- F. The completed ASC Training Document must be turned in to the supervisor (instructor) to receive material to start the production run.

4. Production

- A. Each technician performs their role
- B. Document everything on company forms
 - i. Put the following calculation from ASC Form 004 into your laboratory notebook:
 - a. Percent weight loss for each of the three bags.
 - b. Are you accepting or rejecting this batch? Why?
 - c. Statistical Process Control: Mean, Standard Deviation, and Coefficient of Variation
 - d. Post Production Product Specifications: Release or Reject
- C. Once everything is completed, QA will turn forms into Supervisor

5. Corrective Action Plan

- A. Class discussion of Statistical Process Control
- B. Corrective and Preventative Action plan is constructed.

Activity: GMP Training

Materials

- 1 official SOP Standards for Employee Safety and Conduct: STANDARDS 11 -001 (see below)
- 1 official Awesome Snack Company (ASC) Documentation of Training Document (see below)
- 1 Set of the Standard Operating Procedures (SOPs) for Production:
 - MAT-11-001, MAN-11-001, MAN-11-002, MAN-11-003, QA-11-001, QC-11-001
- 1 set of production forms: ASC 001, ASC 002, ASC 003, ASC 004

Procedure

A. Form Groups, Assign Roles, Complete Training

1. The ideal group size is 5. Form groups and assign roles (jobs) to each member of Production Team. Jobs: Quality Assurance (QA), Quality Control (QC), Manufacturing Tech I, Manufacturing Tech II, Material Control (MC). Note: If the team is comprised of a group less than 5, 1 member must take multiple roles.
2. Follow the training instructions below. After training, the documentation of training document must be turned in to the supervisor (instructor) to receive material to start your production run.

AWESOME SNACK COMPANY			
Standard Operating Procedure (SOP)			
SOP: STANDARDS-11-001	TITLE: STANDARDS FOR EMPLOYEE SAFETY & CONDUCT		PAGE 1 OF 1
AREA: MAN/QUAL	REVISION: 4.3	EFFECTIVE DATE: 1/25/17	SUPERCEDES: 4.2.1
PREPARED BY: ASC TEAM			

Figure 20.3. The heading of SOP 11-001 from your packet

- A. **Quality Assurance (QA) Technician** will read SOP Standards-11-001 to the Team.
- B. **QA Technician** distributes signs, SOPs and ASC Forms to each team member
- C. Each team member reads SOP for his/her job to identify primary activity. (Ex. Weigh unpopped popcorn (UPP))
- D. **QA** fills out table 1 below. In the order presented, **QA** lists the activity and asks the name of who will do it.

ASC TRAINING
—COMPLETE BEFORE STARTING PRODUCTION—

PURPOSE

Every employee must be trained in conformance with GMP (Good Manufacturing Practices).

SCOPE

ALL ASC employees must be trained, acknowledge training, and receive sign-off from a supervisor.

ASC TRAINING

Not following the stated ASC guidelines may result in **written warning and/or termination** of employment

The ASC Guidelines include but are not limited to the following:

1. All hair that is shoulder length or longer must be tied back or contained in a hair net
2. Fingernails must be neatly trimmed. Fingernails should not protrude past the ends of the fingertips more than ¼ inch
3. Clothing and other personal belongings must be stored in designated areas
4. All jewelry is prohibited in all manufacturing areas with the exception of one smooth ring (no stones) on finger. This includes and is not limited to watches, multiple rings, ear, nose, tongue, eye, belly piercings or rings...etc. are not allowed
5. No items are allowed in pockets or affixed to clothing above the waist in the manufacturing area. The exception is for PPE (Personal Protective Equipment)
6. Electronic devices such as cell phone, iPods, blue tooth, radio, pager, MP3, handheld games, etc. These items may be stored in lockers. Employees may use these devices only in designated areas during breaks, lunch, before and/or after work
7. Areas must be left in a clean and organized manner at the end of each shift
8. No food (including gum, candy, nuts and/or similar snacks) and/or drinks can be stored or consumed in manufacturing areas
9. Every glass or plastic breakage in any manufacturing area must be reported to the Shift Supervisor or Safety Coordinator and a Quality Incident Report generated
10. Use the following format for dates: Day-Month-Year (ex. 05-Mar-2019)
11. When Initialing, use THREE initials, to include your middle initial

I, _____, certify that I have read and understand the *ASC Guidelines*.

Employee Full Name

Employee Signature

Supervisor Signature

Date

Date

Table 20.2: Assignment of Production Roles

ACTIVITY	WHO DOES THIS JOB? (NAME)	SOP/FORM
Inspect and/or Release, Reject UPP*		MAT-11-001 / ASC 002
Start Lot/Batch Record		QA-11-001 / ASC 001
Prepare and clean microwave/ balance		MAN-11-001, MAN-11-002, / ASC 003
Inspect Cleaning; OK production start		QC-11-001 / ASC 003
Weigh UPP		MAN-11-003 / ASC 004
Produce IPP*		MAN-11-003 / ASC 004
Place popcorn in Reject or Release		MAT-11-001 / ASC 002
Complete Lot/Batch Record		QA-11-001 / ASC 001
If necessary, initiate CAPA		

*UPP= unpopped popcorn; IPP= in process popcorn

- Print name and Initial on the OFFICIAL Documentation of Training document (looks like below) **after** training on Standards and SOPs is complete. Be sure to include your middle initial
- Once completed, Material Control turns in the document in to Supervisor (Instructor) to receive the starting material (UPP) and start production

Table 21.3: Documentation of Training

JOB TITLE	NAME (Print)	INITIALS (Include Middle Initial)
Material Control Tech 		
Quality Control Tech 		
Manufacturing Tech I 		
Manufacturing Tech II 		
Quality Assurance Tech 		

This is similar to the official document to be used and completed during the exercise.

This page titled [10.3: Part II- Train and Prepare for a Production Run](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

10.4: Part III- Production and Documentation

B. Perform Roles and Document Appropriately Using Company Forms

Procedure

1. Use official company SOP documents to perform the assigned roles in Table 1.
2. Document appropriately on the “official” Awesome Snack Company (ASC) forms as listed in Table 1

Results

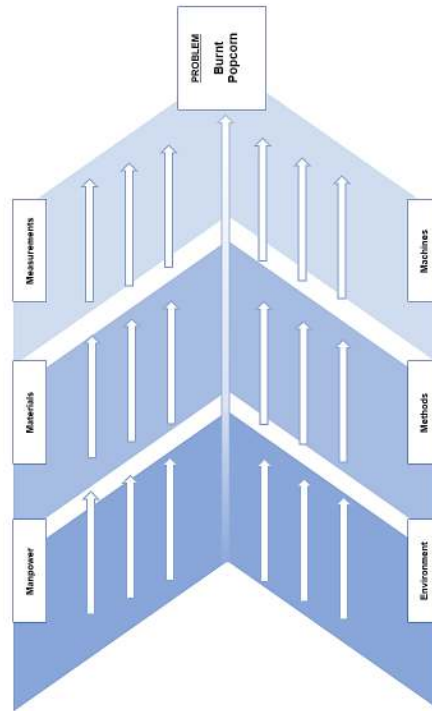
ASC form 004 (IPP production report) is to be completed by your team and turned in to your supervisor (instructor) along with ASC forms 001, 002, 003. Put the following calculations from this form into your laboratory notebook:

- a. Percent weight loss for each of the three bags. Are you **accepting** or **rejecting** this batch? Why?
- b. Statistical Process Control:
 - o Mean:
 - o Standard Deviation:
 - o Coefficient of Variation:
- c. Post Production Product Specifications: **Release** or **Reject**

This page titled [10.4: Part III- Production and Documentation](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

10.5: Study Questions

1. Did you need to implement CAPA? Explain the deviance that caused this procedural process to begin and explain the corrective and preventative process you implemented. This will be shared with the class during discussion.
2. The Ishigawa fishbone diagram (Figure 21.5, Page 21-10) is a tool used to determine the root cause of a problem. It enables a team to brainstorm and categorize all the potential problems that could be the cause of a problem so they can be systematically eliminated until the real cause or causes is/are identified.
3. With your team come up with all the potential reasons your popcorn may fail to meet quality specifications because it is burnt. Redraw the diagram, including the title, and fill it out in your laboratory notebook.



Attribution: *This lab was originally developed by the Southern CA Biotech Center @ Miramar College and is provided as an OER resource to support biotechnology education.*

This page titled [10.5: Study Questions](#) is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by [Donna Barron](#).

Detailed Licensing

Overview

Title: [Introduction to Biotechnology Laboratory Manual \(Barron\)](#)

Webpages: 73

Applicable Restrictions: Noncommercial

All licenses found:

- [CC BY-NC-SA 4.0](#): 98.6% (72 pages)
- [Undeclared](#): 1.4% (1 page)

By Page

- [Introduction to Biotechnology Laboratory Manual \(Barron\)](#) - [CC BY-NC-SA 4.0](#)
 - [Front Matter](#) - [CC BY-NC-SA 4.0](#)
 - [TitlePage](#) - [CC BY-NC-SA 4.0](#)
 - [InfoPage](#) - [CC BY-NC-SA 4.0](#)
 - [Table of Contents](#) - [Undeclared](#)
 - [Licensing](#) - [CC BY-NC-SA 4.0](#)
 - [1: Lab Safety and Lab Notebook](#) - [CC BY-NC-SA 4.0](#)
 - [1.1: Student Learning Outcomes](#) - [CC BY-NC-SA 4.0](#)
 - [1.2: Introduction](#) - [CC BY-NC-SA 4.0](#)
 - [1.3: PART I- Setting Up a Legal Scientific Notebook](#) - [CC BY-NC-SA 4.0](#)
 - [1.4: PART II- Lab Safety](#) - [CC BY-NC-SA 4.0](#)
 - [1.5: Study Questions](#) - [CC BY-NC-SA 4.0](#)
 - [1.6: PUZZLE- Lab Safety and Lab Notebooks](#) - [CC BY-NC-SA 4.0](#)
 - [2: Metrics and Measurements](#) - [CC BY-NC-SA 4.0](#)
 - [2.1: Student Learning Outcomes](#) - [CC BY-NC-SA 4.0](#)
 - [2.2: PART I- Metrics](#) - [CC BY-NC-SA 4.0](#)
 - [2.3: Part II- Measuring Using The Metric System](#) - [CC BY-NC-SA 4.0](#)
 - [2.4: Study Questions](#) - [CC BY-NC-SA 4.0](#)
 - [3: Micropipetting](#) - [CC BY-NC-SA 4.0](#)
 - [3.1: Student Learning Outcomes](#) - [CC BY-NC-SA 4.0](#)
 - [3.2: Introduction To Micropipetting](#) - [CC BY-NC-SA 4.0](#)
 - [3.3: Part I- Choosing And Setting The Micropipette](#) - [CC BY-NC-SA 4.0](#)
 - [3.4: Part II- Pipetting Practice](#) - [CC BY-NC-SA 4.0](#)
 - [3.5: Study Questions](#) - [CC BY-NC-SA 4.0](#)
 - [4: pH and Buffers](#) - [CC BY-NC-SA 4.0](#)
 - [4.1: Student Learning Outcomes](#) - [CC BY-NC-SA 4.0](#)
 - [4.2: Introduction](#) - [CC BY-NC-SA 4.0](#)
 - [4.3: Prelab Reading Assignment- Chemistry Review](#) - [CC BY-NC-SA 4.0](#)
 - [4.4: Activity 1- Measuring pH](#) - [CC BY-NC-SA 4.0](#)
 - [4.5: Activity 2- Preparation of an Acetate Buffer](#) - [CC BY-NC-SA 4.0](#)
 - [4.6: Activity 3- Effect of Adding Acid and Base to Acetate Buffer](#) - [CC BY-NC-SA 4.0](#)
 - [4.7: Study Questions](#) - [CC BY-NC-SA 4.0](#)
 - [5: Paternity and Gel Electrophoresis](#) - [CC BY-NC-SA 4.0](#)
 - [5.1: Student Learning Outcomes](#) - [CC BY-NC-SA 4.0](#)
 - [5.2: Part I- Agarose Gel Electrophoresis](#) - [CC BY-NC-SA 4.0](#)
 - [5.3: Part II- Paternity Case- Who is the Father of My Kittens?](#) - [CC BY-NC-SA 4.0](#)
 - [5.4: Study Questions](#) - [CC BY-NC-SA 4.0](#)
 - [6: Restriction Digest and Gel Electrophoresis \(New\)](#) - [CC BY-NC-SA 4.0](#)
 - [6.1: Student Learning Outcomes](#) - [CC BY-NC-SA 4.0](#)
 - [6.2: Introduction](#) - [CC BY-NC-SA 4.0](#)
 - [6.3: Part I- Restriction Digest](#) - [CC BY-NC-SA 4.0](#)
 - [6.4: Part II- Casting Agarose Gel](#) - [CC BY-NC-SA 4.0](#)
 - [6.5: Part III- Practice Pipetting](#) - [CC BY-NC-SA 4.0](#)
 - [6.6: Part IV- Gel Electrophoresis](#) - [CC BY-NC-SA 4.0](#)
 - [6.7: Study Questions](#) - [CC BY-NC-SA 4.0](#)
 - [7: Transformation](#) - [CC BY-NC-SA 4.0](#)
 - [7.1: Student Learning Outcomes](#) - [CC BY-NC-SA 4.0](#)
 - [7.2: Introduction](#) - [CC BY-NC-SA 4.0](#)
 - [7.3: Transforming Bacteria with Plasmids](#) - [CC BY-NC-SA 4.0](#)
 - [7.4: Study Questions](#) - [CC BY-NC-SA 4.0](#)
 - [8: Column Chromatography \(Rev\)](#) - [CC BY-NC-SA 4.0](#)
 - [8.1: Student Learning Outcomes](#) - [CC BY-NC-SA 4.0](#)
 - [8.2: Introduction](#) - [CC BY-NC-SA 4.0](#)
 - [8.3: Pre Lab Activity- Think Pair Share-Discuss What You Know](#) - [CC BY-NC-SA 4.0](#)

- 8.4: Part I- Lysis of Bacterial Cells - *CC BY-NC-SA 4.0*
- 8.5: Part II- Separating the Green Fluorescent Protein with Column Chromatography - *CC BY-NC-SA 4.0*
- 8.6: Study Questions - *CC BY-NC-SA 4.0*
- 9: Serial Dilutions and Standard Curve with a Microplate - *CC BY-NC-SA 4.0*
 - 9.1: Student Learning Outcomes - *CC BY-NC-SA 4.0*
 - 9.2: Part I- Serial Dilutions - *CC BY-NC-SA 4.0*
 - 9.3: Part II- Standard Curves - *CC BY-NC-SA 4.0*
 - 9.4: Study Questions - *CC BY-NC-SA 4.0*
- 10: Good Manufacturing Practices (GMP) with New Table - *CC BY-NC-SA 4.0*
 - 10.1: Student Learning Outcomes - *CC BY-NC-SA 4.0*
 - 10.2: Part I- Review Terms and Background Information - *CC BY-NC-SA 4.0*
 - 10.3: Part II- Train and Prepare for a Production Run - *CC BY-NC-SA 4.0*
 - 10.4: Part III- Production and Documentation - *CC BY-NC-SA 4.0*
 - 10.5: Study Questions - *CC BY-NC-SA 4.0*
- Back Matter - *CC BY-NC-SA 4.0*
 - Index - *CC BY-NC-SA 4.0*
 - Glossary - *CC BY-NC-SA 4.0*
 - Detailed Licensing - *CC BY-NC-SA 4.0*
 - Detailed Licensing - *CC BY-NC-SA 4.0*

Detailed Licensing

Overview

Title: [Introduction to Biotechnology Laboratory Manual \(Barron\)](#)

Webpages: 73

Applicable Restrictions: Noncommercial

All licenses found:

- [CC BY-NC-SA 4.0](#): 98.6% (72 pages)
- [Undeclared](#): 1.4% (1 page)

By Page

- [Introduction to Biotechnology Laboratory Manual \(Barron\)](#) - [CC BY-NC-SA 4.0](#)
 - [Front Matter](#) - [CC BY-NC-SA 4.0](#)
 - [TitlePage](#) - [CC BY-NC-SA 4.0](#)
 - [InfoPage](#) - [CC BY-NC-SA 4.0](#)
 - [Table of Contents](#) - [Undeclared](#)
 - [Licensing](#) - [CC BY-NC-SA 4.0](#)
 - [1: Lab Safety and Lab Notebook](#) - [CC BY-NC-SA 4.0](#)
 - [1.1: Student Learning Outcomes](#) - [CC BY-NC-SA 4.0](#)
 - [1.2: Introduction](#) - [CC BY-NC-SA 4.0](#)
 - [1.3: PART I- Setting Up a Legal Scientific Notebook](#) - [CC BY-NC-SA 4.0](#)
 - [1.4: PART II- Lab Safety](#) - [CC BY-NC-SA 4.0](#)
 - [1.5: Study Questions](#) - [CC BY-NC-SA 4.0](#)
 - [1.6: PUZZLE- Lab Safety and Lab Notebooks](#) - [CC BY-NC-SA 4.0](#)
 - [2: Metrics and Measurements](#) - [CC BY-NC-SA 4.0](#)
 - [2.1: Student Learning Outcomes](#) - [CC BY-NC-SA 4.0](#)
 - [2.2: PART I- Metrics](#) - [CC BY-NC-SA 4.0](#)
 - [2.3: Part II- Measuring Using The Metric System](#) - [CC BY-NC-SA 4.0](#)
 - [2.4: Study Questions](#) - [CC BY-NC-SA 4.0](#)
 - [3: Micropipetting](#) - [CC BY-NC-SA 4.0](#)
 - [3.1: Student Learning Outcomes](#) - [CC BY-NC-SA 4.0](#)
 - [3.2: Introduction To Micropipetting](#) - [CC BY-NC-SA 4.0](#)
 - [3.3: Part I- Choosing And Setting The Micropipette](#) - [CC BY-NC-SA 4.0](#)
 - [3.4: Part II- Pipetting Practice](#) - [CC BY-NC-SA 4.0](#)
 - [3.5: Study Questions](#) - [CC BY-NC-SA 4.0](#)
 - [4: pH and Buffers](#) - [CC BY-NC-SA 4.0](#)
 - [4.1: Student Learning Outcomes](#) - [CC BY-NC-SA 4.0](#)
 - [4.2: Introduction](#) - [CC BY-NC-SA 4.0](#)
 - [4.3: Prelab Reading Assignment- Chemistry Review](#) - [CC BY-NC-SA 4.0](#)
 - [4.4: Activity 1- Measuring pH](#) - [CC BY-NC-SA 4.0](#)
 - [4.5: Activity 2- Preparation of an Acetate Buffer](#) - [CC BY-NC-SA 4.0](#)
 - [4.6: Activity 3- Effect of Adding Acid and Base to Acetate Buffer](#) - [CC BY-NC-SA 4.0](#)
 - [4.7: Study Questions](#) - [CC BY-NC-SA 4.0](#)
 - [5: Paternity and Gel Electrophoresis](#) - [CC BY-NC-SA 4.0](#)
 - [5.1: Student Learning Outcomes](#) - [CC BY-NC-SA 4.0](#)
 - [5.2: Part I- Agarose Gel Electrophoresis](#) - [CC BY-NC-SA 4.0](#)
 - [5.3: Part II- Paternity Case- Who is the Father of My Kittens?](#) - [CC BY-NC-SA 4.0](#)
 - [5.4: Study Questions](#) - [CC BY-NC-SA 4.0](#)
 - [6: Restriction Digest and Gel Electrophoresis \(New\)](#) - [CC BY-NC-SA 4.0](#)
 - [6.1: Student Learning Outcomes](#) - [CC BY-NC-SA 4.0](#)
 - [6.2: Introduction](#) - [CC BY-NC-SA 4.0](#)
 - [6.3: Part I- Restriction Digest](#) - [CC BY-NC-SA 4.0](#)
 - [6.4: Part II- Casting Agarose Gel](#) - [CC BY-NC-SA 4.0](#)
 - [6.5: Part III- Practice Pipetting](#) - [CC BY-NC-SA 4.0](#)
 - [6.6: Part IV- Gel Electrophoresis](#) - [CC BY-NC-SA 4.0](#)
 - [6.7: Study Questions](#) - [CC BY-NC-SA 4.0](#)
 - [7: Transformation](#) - [CC BY-NC-SA 4.0](#)
 - [7.1: Student Learning Outcomes](#) - [CC BY-NC-SA 4.0](#)
 - [7.2: Introduction](#) - [CC BY-NC-SA 4.0](#)
 - [7.3: Transforming Bacteria with Plasmids](#) - [CC BY-NC-SA 4.0](#)
 - [7.4: Study Questions](#) - [CC BY-NC-SA 4.0](#)
 - [8: Column Chromatography \(Rev\)](#) - [CC BY-NC-SA 4.0](#)
 - [8.1: Student Learning Outcomes](#) - [CC BY-NC-SA 4.0](#)
 - [8.2: Introduction](#) - [CC BY-NC-SA 4.0](#)
 - [8.3: Pre Lab Activity- Think Pair Share-Discuss What You Know](#) - [CC BY-NC-SA 4.0](#)

- 8.4: Part I- Lysis of Bacterial Cells - *CC BY-NC-SA 4.0*
- 8.5: Part II- Separating the Green Fluorescent Protein with Column Chromatography - *CC BY-NC-SA 4.0*
- 8.6: Study Questions - *CC BY-NC-SA 4.0*
- 9: Serial Dilutions and Standard Curve with a Microplate - *CC BY-NC-SA 4.0*
 - 9.1: Student Learning Outcomes - *CC BY-NC-SA 4.0*
 - 9.2: Part I- Serial Dilutions - *CC BY-NC-SA 4.0*
 - 9.3: Part II- Standard Curves - *CC BY-NC-SA 4.0*
 - 9.4: Study Questions - *CC BY-NC-SA 4.0*
- 10: Good Manufacturing Practices (GMP) with New Table - *CC BY-NC-SA 4.0*
 - 10.1: Student Learning Outcomes - *CC BY-NC-SA 4.0*
 - 10.2: Part I- Review Terms and Background Information - *CC BY-NC-SA 4.0*
 - 10.3: Part II- Train and Prepare for a Production Run - *CC BY-NC-SA 4.0*
 - 10.4: Part III- Production and Documentation - *CC BY-NC-SA 4.0*
 - 10.5: Study Questions - *CC BY-NC-SA 4.0*
- Back Matter - *CC BY-NC-SA 4.0*
 - Index - *CC BY-NC-SA 4.0*
 - Glossary - *CC BY-NC-SA 4.0*
 - Detailed Licensing - *CC BY-NC-SA 4.0*
 - Detailed Licensing - *CC BY-NC-SA 4.0*