GENETICS LABORATORY MANUAL

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All Figures were designed and created in BioRender.

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LABORATORY SAFETY PROTOCOLS

You are expected to read the appropriate sections of this manual before coming to lab. You are also expected to follow instructions provided in each exercise. Inform your instructor if you do not understand a procedure.

1. Notify your instructor if you are pregnant, allergic to any chemicals (such as latex), or have another medical condition that requires precautionary measures in the laboratory.
2. Place book bags, large purses, etc., under your lab bench or where instructed. The only things on the lab bench should be what you need for lab that day.
3. Do not eat, drink, handle contact lenses, or apply cosmetics in the lab.
4. Confine long hair, loose clothing, and dangling jewelry.
5. Wear close-toed shoes and be aware that some chemicals can stain clothing.
6. Cover cuts, scrapes or other wounds with a bandage.
7. Assume all chemical and reagents are poisonous and act accordingly.
8. Never pipet chemicals by mouth. Use pipettes, or other devices as directed, to measure and transfer chemicals.
9. Do not pour chemicals, or other materials, back into "stock bottles" unless told to do so.
10. Put the right cap back on reagent bottles.
11. Dispose of reagents, or equipment, as instructed.
12. Keep all chemicals away from edge of lab bench to avoid spills.
13. Wash skin immediately and thoroughly if contaminated by chemicals or microorganisms.
14. Report all spills, no matter how minor, to your instructor immediately.
15. Do not leave heat sources unattended.
16. Use appropriate apparatus when handling hot glassware.
17. Never point a test tube that is being heated in the direction of someone else.
18. Report accidents immediately. Do not attempt to clean up glassware that is dropped and shatters.
19. Other broken glassware, and glass slides, should be disposed of in "Sharps" containers.
20. Be particularly careful; when handling scalpels, razor blades, scissors, etc.
21. Know the location of the fire extinguisher, eye wash station, first aid kit, glass disposal boxes and clean-up materials for spills.
22. Wipe off your bench at the end of lab. Wash, dry and replace all the materials and equipment you used.

Molecular Lab Skills

In this activity, you will learn basic techniques used in molecular biology laboratories, such as micropipetting, centrifugation, and agarose gel preparation and loading. Mastering these techniques is critical for successful experimentation, so please read the entire activity BEFORE beginning.

Micropipettes

Micropipettes are used to accurately measure and transfer small volumes (microliters/µL to milliliters/mL) of liquid and are one of the most widely used instruments in any molecular laboratory. There are two types of micropipettes: variable and fixed. As the name implies, fixed micropipettes are designed to transfer a "fixed" amount of liquid. This type of micropipette cannot be adjusted to withdraw more or less volume. More commonly, variable micropipettes pipettes are used, as these can be adjusted to withdraw a specific volume **within a range**.

As shown in Figure 1, different sized micropipettes can accommodate different volumes (or ranges of volumes), and the volume that any micropipette can accommodate is usually indicated on the top (or occasionally the side). As a general "rule of thumb", always select the SMALLEST size pipet that will handle the volume you wish to transfer. Accuracy decreases as you use unnecessarily large pipets for small volumes.

Figure 1: Variable micropipettes can accommodate different volumes. For example, the P200 micropipette can accommodate and deliver any volume between 20-200 µl. If a volume greater than 200 µl or smaller than 20 µl is required, the P1000 or P20 must be used, respectively. **NEVER exceed the upper or lower limits of these pipettes!**

Micropipette	Maximum volume	Optimal range for use
P20	20 µL	2 - 20 µL
P200	200 µL	20 - 200 µL
P1000	1000 µL	200 - 1000 µL

Your instructor will help you familiarize yourself with the basic parts of the micropipette (listed below and shown in Figure 2) before use.

- **Plunger** button (operating button) – to aspirate and dispense the solution
- **Tip ejector** button – to discard tips after use
- **Volume adjustment** knob –to adjust the volume (in variable micropipette)
- **Volume indicator**/readout – displays the set volume

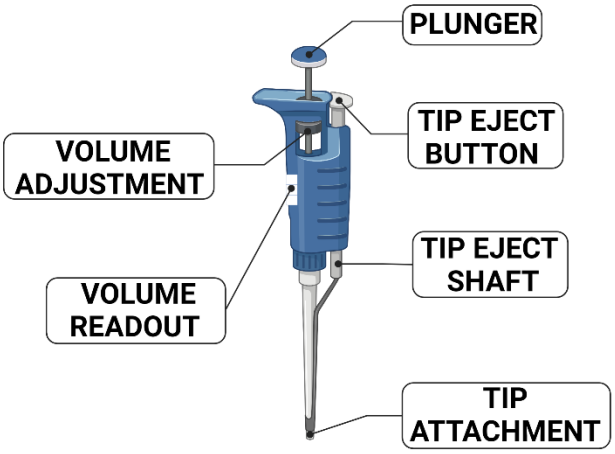
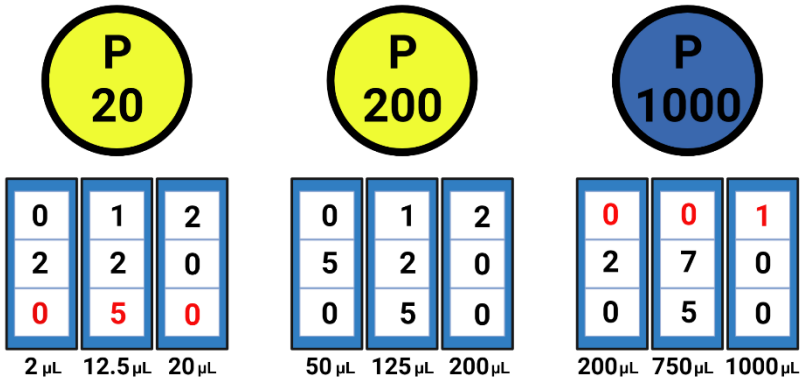


Figure 2: Micropipette parts

The volume that a micropipette is calibrated/set to will be displayed in the volume indicator. The volume is adjusted by turning the volume adjustment knob either clockwise or counterclockwise. Notice that the number display may contain one or more decimal values (Figure 3). It is ***critical*** that you understand how to read the volume indicator so that you transfer the correct volumes! If you are uncertain about how to interpret the volumes, or how to set the correct volume, ask your instructor for help.

Figure 3: Volume calibration examples. The P1000 reads **milli**liters, while the other micropipettors read **micro**liters. The red and black numbers are separated by a decimal.



Micropipette tips are necessary to transfer liquids. Like micropipettes, plastic micropipette tips come in different sizes, and it is important to use the correct tip for the volume you want to transfer. Using an incorrect tip poses a risk of bringing liquid into the shaft of the micropipette, which can ***damage it***. Always check the box of tips to ensure you are using the correct size for the micropipette being used. If you are unsure, ask your instructor.

Gather your supplies and read through all steps of the activity before you begin.

Materials: micropipettes & tips, loading dye, water, 1.5 mL centrifuge tubes, 0.5 mL (PCR) tubes, electronic balance

Lab Procedure

1. Select the P200 and adjust the volume to 155 µL.
2. Select the correct box of tips to accommodate the volume listed above.
3. Place a tip on the micropipette by opening the cover of the tip box and inserting the micropipette shaft into a tip and push down firmly until it is seated. Remove the micropipette with the attached tip and **be sure to close the tip box to keep the tips as sterile as possible**. **NOTE: Do not allow the pipet tip to touch any object (including your gloves, clothes, hair, skin, bench).**

4. Draw fluid (water or loading dye, as instructed) into the micropipette tip:
    a. The plunger will stop at two different positions when it is depressed. Push the plunger down slowly to the point of **first** resistance: this is the load volume.
    b. While holding the plunger at the load volume set point, put the tip into the solution so that it is immersed just enough to cover the end
    c. **Slowly** allow the plunger to go all the way back up, drawing fluid into the tip. ***Make sure the tip is submerged in the fluid at all times to prevent air from being brought into the tip.***

5. Transfer the fluid to an empty microfuge tube:
    a. Place the tip all the way to the bottom of the tube you are transferring into.
    b. **Slowly** push the plunger down to the first 'stop' to expel the contents of the tip.
    c. **Do not** allow the plunger to go back to the 'up' position when you are done expelling the liquid. Continue to hold your thumb at the first 'stop'. If necessary, you can continue pushing past the first 'stop' to expel any remaining fluids in the tip.
    d. Remove the tip from the fluid in the tube, then allow the plunger to go back to the 'up' position with your thumb.
    e. Dispose of tip in appropriate container. Note: **Never** use the same tip to transfer different fluids!!!!

6. Select the P1000 and set to 653 µL
7. Repeat steps 2-5
8. Select the P20 and set to 6 µL
9. Repeat steps 2-5

A simple way to check the calibration of your micropipette is using the fact that 1 mL of dH_2O has a mass of 1g. Pipet a range of volumes spanning the pipette's useable range and weigh them with an electronic balance. Pipets having greater than 5 % error should be recalibrated.

The microcentrifuge

Centrifugation of samples is important for many biological applications, such as separating DNA/RNA or proteins from solution, microfiltration of aqueous samples or simply to collect the last drops of liquid into the bottom of the tube. A microcentrifuge, also called a microfuge, is used to spin small (2 mL or less) liquid samples at high speeds (generally tens of thousands X g-force). Because the rotor spins at such speeds, it is **extremely important** to **counterbalance** a tube (or tubes) with another tube (or tubes) of similar contents. ***Failure to counterbalance tubes properly can result in damage to the rotor and/or cause serious injury!***

In most cases, the best way to counterbalance is to use a tube filled to the same level as your sample tube with tap water. Tubes should be placed directly across from one another, as shown in Figure 4.

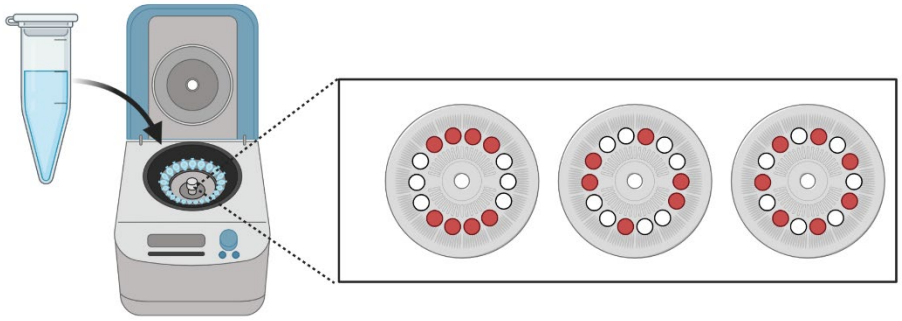


Figure 4: Proper microcentrifuge counterbalance. Red circles indicate tubes. In each example, the weight of the tubes is evenly distributed (counterbalanced) in the rotor.

Gather your supplies and read through all steps of the activity before you begin.

Materials: micropipettes & tips, water, 1.5 mL centrifuge tubes, 0.5 mL (PCR) tubes, microcentrifuge

Lab Procedure

1. Fill a microcentrifuge tube with 250 µL of water.

2. Place in the microfuge.

3. Balance it with another tube of the same size and containing the same amount of liquid. Place them exactly opposite each other in the rotor. Close the lid.

4. Turn on the microfuge and allow it to spin for 2 - 3 seconds before stopping.

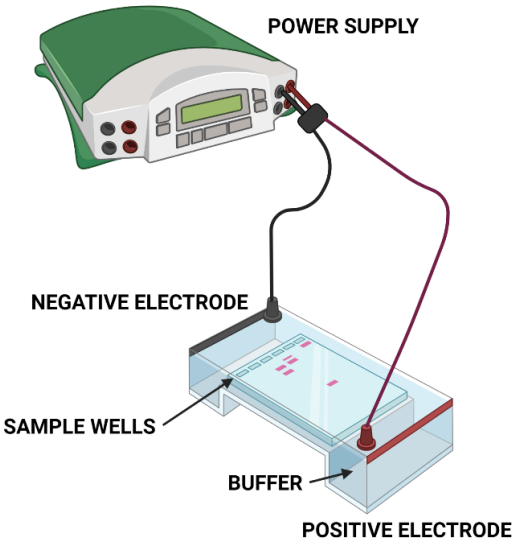
5. Fill a third tube with the same amount of water. Arrange all 3 tubes in the rotor, making sure they are counterbalanced. Repeat step 4.

Sometimes it may be necessary to use centrifuge tubes that are smaller than the standard 1.5 mL tube, such as 0.5 mL (PCR) tubes. Adaptors can be inserted into the rotor holes to accommodate these smaller tubes. Ask your instructor if you will practice centrifuging smaller tubes.

Preparation and Loading of Agarose Gels

Gel electrophoresis is another commonly used technique that separates molecules (such as DNA) based on size and charge. When an electric current is applied across the gel, negatively charged molecules move toward a positively charged electrode (Figure 5). Smaller molecules move more quickly through the gel than larger molecules, resulting in the fragments being arranged in order of size.

Figure 5: Gel electrophoresis sorts molecules based on charge and size.



The gel consists of a permeable matrix, like a sieve, through which molecules can travel. Agarose gels are commonly used to separate fragments of DNA. The concentration of agarose used to make the gel depends on the size of the DNA fragments: higher agarose concentrations equate to a denser matrix, best suited for smaller DNA fragments, while larger molecules require a lower concentration of agarose.

To make a gel, agarose powder is mixed with an electrophoresis buffer (typically TAE or TBE) and heated until all of the agarose powder has melted. The mixture is then poured into a gel tray and a "comb" is placed at one end to make wells for the samples. Once the gel has solidified, it is placed into an electrophoresis chamber, the comb is removed and electrophoresis buffer is poured into the tank until the surface of the gel is covered (Figure 5). Samples are then added to the wells. In most cases, a dye is added to the samples to increase viscosity (to prevent the sample from floating out of the well) and visibility (to help the researcher when loading samples).

Agarose gels are prepared using a w/v percentage of agarose in a buffer solution. For example, a 1% gel contains 1 g agarose in 100 ml 1X TAE. For a 1.5% gel, 1.5 g is added to 100 ml 1X TAE. Generally, 50mL is adequate to prepare a single gel.

The buffer used in electrophoresis conducts the electric current. The type of buffer used depends on the approximate size of the DNA fragments in the sample. Depending on the laboratory, you may be provided a concentrated buffer solution (e.g., 10X, 50X.) that you will need to dilute. Always read the label of the buffer solution before diluting. *Failure to dilute the TAE/TBE will result in very slow migration of the samples.*

In the next part of this laboratory exercise, you will prepare a 0.5% agarose gel and practice loading samples into the gel.

Gather your supplies and read through all steps of the activity before you begin.

Materials: micropipettes & tips, agarose, concentrated TAE buffer (50X, 10X, etc.), electronic balance, microwave oven and/or hot plates, weigh boat/tray, parafilm, general glassware (flasks, graduated cylinders), gel electrophoresis chamber & power supply, gel tray & comb, oven mitt or hot pad, tape or dam for gel tray

Lab Procedure

1. Seal both ends of a gel-casting tray with tape, dams, or any other method appropriate for the gel tray you are using. Be sure to seal it well to prevent leakage. Place a comb n the gel tray (there are grooves on the gel tray for the comb to fit into). Set aside.

2. Calculate the amount of agarose needed for a 0.5% agarose gel (50 mL volume). **Check your calculations with your instructor.**

3. Using a weigh boat, measure the appropriate amount of agarose and transfer to a 125 mL Erlenmeyer flask.

4. Using a graduated cylinder, measure 50 mL of 1X TAE electrophoresis buffer and add this to the flask containing the agarose.

5. Cover the flask with parafilm or plastic wrap. Swirl to mix.

6. Dissolve the agarose by heating the solution in a microwave (*If necessary, a hot plate can be used to dissolve the agarose: ask your instructor). Begin by heating for 30 seconds. Remove and swirl flask contents. Heat the solution again for 15 seconds. Watch the flask carefully and stop the microwave **BEFORE** the solution begins to boil violently and upward out of the flask.

7. Remove the flask from microwave. ***Caution: flask will be hot!*** Use oven mitt or hot pad provided. Observe the solution - no solid particles should be visible and the solution should be transparent. It may be necessary to heat the flask one or two more times in 15 second intervals.

8. Allow the solution to cool. To determine if the melted solution is cool enough to pour, touch the bottom of the flask to inside of your wrist: when it is hot (just uncomfortable, but not yet not “warm”) to touch the solution is ready to pour.

9. Pour the liquid agarose solution into the prepared gel tray.

10. Allow the gel to solidify (about 20 - 30 minutes).

11. Once the gel is semi-translucent and firm, remove the tape and comb.

12. Carefully transfer the gel tray to an electrophoresis chamber. Make sure that the wells are near the **black** electrode (cathode) end of the chamber.

13. Add enough electrophoresis buffer (about 50 mL) to cover the gel.

14. Obtain a tube of practice loading dye and pipette 20 µL into the wells of your gel:

a. Draw 20 µL into a micropipette tip.
b. Carefully guide the micropipette tip into the well of the gel, but not so far that it is touching the agarose at the bottom. *Be careful to avoid puncturing the well.*
c. **Slowly** depress the plunger, letting the contents settle to the bottom as you pipette as you push. ***Do not push the plunger all the way down, as this will introduce air bubbles into the wells and lead to the spilling of the well's contents!***
d. Repeat for all wells.

15. Place the lid on top of the electrophoresis chamber, making sure that the black and red ends are correctly oriented. The black lead will be attached at the end of the gel near the samples. The red lead will be attached at the other end.

16. Connect the chamber to the power supply.

17. Select the voltage and time and start the electrophoresis (time and voltage will vary according to your instructor).

Cell Cycle Mutations

Cancer is uncontrolled cell division. When cells are healthy, they grow and divide in a regulated manner by responding to levels of **regulatory proteins**. When levels of these regulatory proteins are disrupted (or when the proteins don't function) cells can continue to divide, producing daughter cells that are also unregulated. Eventually, this forms a cluster of cells called a tumor. *Note: some cancer cells (blood, lymph) circulate rather than forming solid tumors.

Genes provide the instructions for all cellular proteins. Mutations in some genes will lead to cancer because the corresponding proteins aren't produced properly, or don't function properly, enabling cells to reproduce too quickly or pass genetic surveillance systems without the appropriate signal.

There are 2 types of genetic mutations: **Acquired mutations** are the most common cause of cancer. They result from damage to genes during a person's life. For example, this could be a breast cell or a colon cell, which then goes on to divide many times and forms a tumor. **Germline mutations** are less common. A germline mutation occurs in a sperm cell or egg cell and can pass from generation to generation. Cancer caused by germline mutations is called inherited cancer.

A single mutation will likely not cause cancer. Usually, cancer occurs from multiple mutations over a lifetime. That is why cancer occurs more often in older people. They have had more opportunities for mutations to build up.

What are surveillance systems? Throughout the cell cycle, there are several checkpoints that regulate progression from one phase to the next. Progression through these checkpoints is largely determined by **cyclin-dependent kinases** (CDKs), enzymes that are activated by binding to proteins called **cyclins.** When CDKs are activated (by binding to cyclins) they trigger signaling cascades that lead to progression through the cell cycle (i.e., the cell moves forward to the next phase). These checkpoints assess things such as DNA damage, spindle formation, etc., and trigger **apoptosis** (programmed cell death) if damage cannot be repaired. These checkpoints are important because if a damaged cell continues to divide, it can lead to cancer.

Why isn't it easy to track cancer? Since cancer is due to several mutations in several genes, it is hard to see which path it will take. Several mechanisms contribute to a cell completing its cycle, including **proto-oncogenes**, **tumor suppressor genes**, and **DNA repair genes**.

Proto-oncogene: These genes signal the cell cycle to move forward, like a green light signal. A mutated proto-oncogene is called an **oncogene:** this gene will allow the cell to divide without the proper signal.

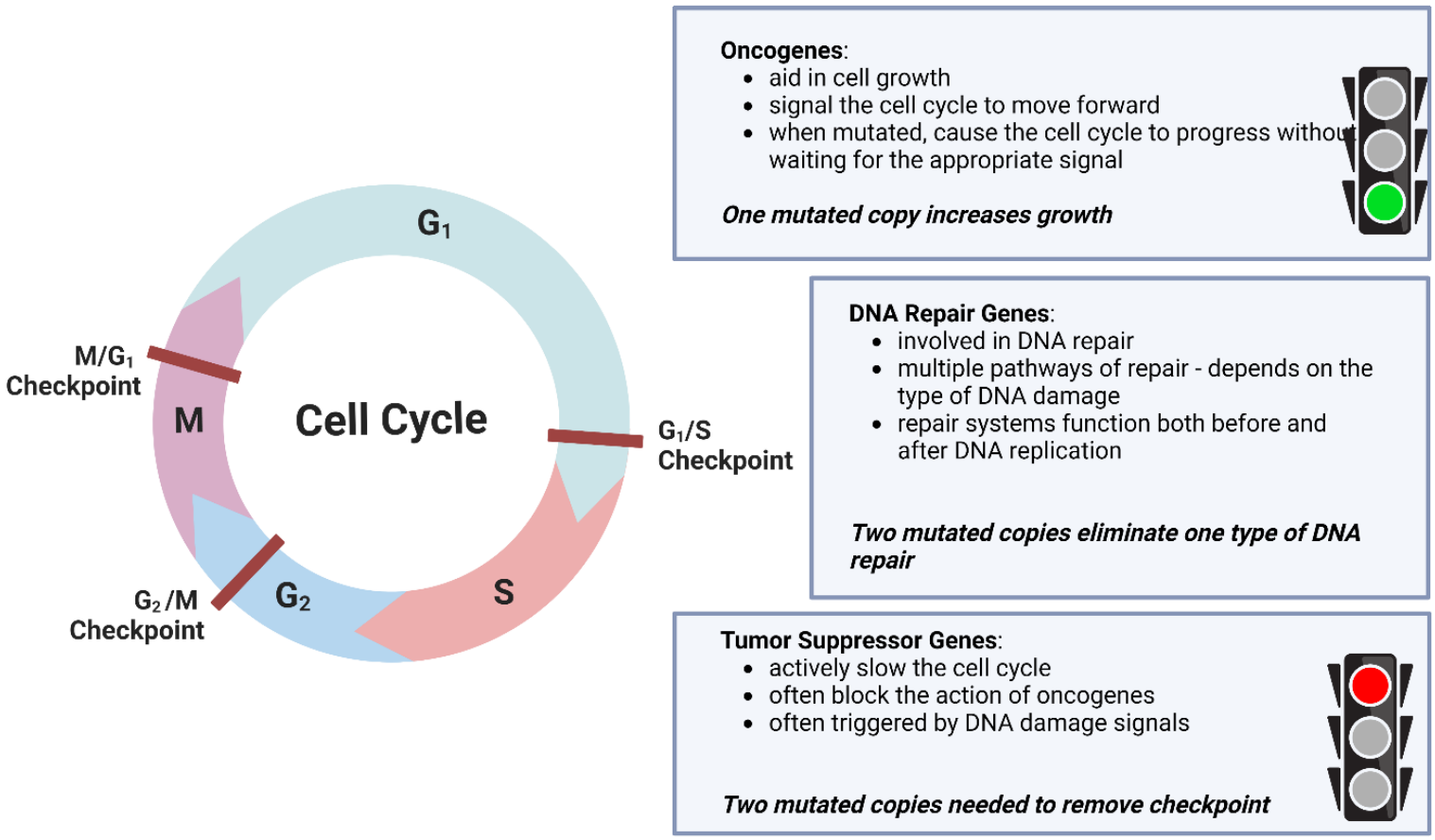
Tumor Suppressor Genes: These genes are activated when the cell is damaged. They trigger apoptosis

DNA Repair Genes: These genes work before and after DNA replication occurs in the cycle to repair damage to DNA. There are multiple pathways for DNA Repair Genes, so if one is damaged, another can become active.

What happens when there is a mutation in one of these "surveillance genes"? Can it be fixed or counteracted by another gene? For example, if we get a mutation in a tumor suppressor gene, can it be fixed via a DNA Repair Gene? What if we also get a mutation in a DNA repair gene? These are some of the different pathways that cancer can take, and it can be hard for researchers to predict where the next mutation might occur.

<u>Can you model multiple cell divisions to identify mutation patterns?</u> In this activity, you will learn about the multiple pathways that lead to cancer by simulating the cell cycle: the class will represent a population of cells, and you will represent a single cell preparing to undergo cell division. By rolling dice, you may (or may not) acquire mutations that alter the cell cycle. If you acquire a mutation, you will determine what regulatory mechanisms are damaged and then describe the impact of that damage on the rate of cell division. You may also find that you have a germline mutation, which may (or may not) impact the cell cycle.

Figure 1: The cell cycle. Note the checkpoints and affected processes.



Gather your supplies and read through all steps of the activity before you begin.

Materials: tubes filled with red, white, and yellow beads, timer, dice, mutation cards

Lab Procedure

1. Set a timer for 15 minutes

2. Start the timer.

3. Draw a bead from your tube, then decide what kind of mutation your cell has:
   - A **white** bead = no de novo mutations. **Wait 1 minute before drawing another bead.**
   - A **yellow** bead = mutations that do not affect the rate of cell division. Roll the die to determine the type of mutation from the mutation card. **Wait 1 minute before drawing another bead.**
   - A **red** bead = mutations that speed the rate of cell division. Roll the die to determine the type of mutation from the mutation card. This mutation reduces cell cycle time. **Wait 30 seconds before drawing another bead.**

4. **While you wait** (as described above), fill in **Table 1** according to the mutation you acquired (in the appropriate "Round of Cell Divisions" row):

   - A **white** bead - Place a tally mark in the row under the "No mutation" column
   - A **yellow** bead - Place a tally mark in the corresponding column
   - A **red** bead –Place a tally mark in the corresponding column

5. Continue play until the first player/group completes Table 1 (or according to the time set by your instructor).

6. Tally the total number of cell divisions, the total number of mutations, and the total number of each type of mutation. Record the totals.

7. Examine **Figure 1** to determine how the cell cycle is affected by your mutations. Consider the number of mutations needed to disable each checkpoint:
   - **Oncogenes**—1 mutated copy removes a checkpoint and increases the rate of division.
   - **Tumor suppressor genes**—2 mutated copies are needed to remove a checkpoint.
   - **DNA repair genes**—2 mutated copies eliminate 1 type of DNA repair.

8. Draw a large X over any disabled checkpoint.

9. Discuss your results with the class and compare the total number of cell divisions and mutations acquired by different groups. Identify which cells "became cancerous" and compare the mutation patterns of those cancerous cells.

Table 1: Mutations Chart

DICE ROLL →		1	2	3	4	5	6	
ROUND OF CELL DIVISION		Oncogene mutation acting at G1/S checkpoint	Oncogene mutation acting at G2/M checkpoint	Tumor suppressor mutation acting at G1/S checkpoint	Tumor suppressor mutation acting at G2/M checkpoint	DNA repair gene damage	DNA mutation but no impact	No mutation
	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
	9							
	10							
	11							
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	23							
	24							
	25							
	26							
	27							
	28							
	29							
	30							
	TOTALS							

Total Rounds of Cell Division Completed:	
Total Number of Mutations:	

CONCLUSIONS

1. Based on the class results, (each student/student group representing a cell), describe how a tumor might result from this group of cells.

2. Explain the impact of the following mutations on the cell cycle:

- Oncogene mutation
- DNA repair gene damage
- Tumor Suppressor Gene Mutation

3. Why might it be helpful for cancer researchers to understand the different types of mutations that influence the cell cycle?

4. Is there one mutation you think that researchers should watch more closely than another? Why?

**This exercise was adapted from the Hudson Alpha Institute for Biotechnology "Collecting Cancer Causing Changes" classroom resource.*

Chi Square Corn Analysis

Introduction: This laboratory investigates a dihybrid cross as shown in an ear of corn. There are four grain phenotypes in the ear of genetic corn shown below: **Purple & Smooth (A), Purple & Shrunken (B), Yellow & Smooth (C) and Yellow & Shrunken (D).**

These four grain phenotypes are produced by the following two pairs of heterozygous genes (P & p and S & s) located on two pairs of homologous chromosomes (each gene on a separate chromosome):

Dominant Genes	**Recessive Genes**
P = Purple	**p = Yellow**
S = Smooth	**s = Shrunken**

Table 1 shows a dihybrid cross between two heterozygous parents (PpSs X PpSs). The gametes of each parent are shown along the top and left sides of the table. This cross produced the ear of genetic corn shown at the beginning of this activity. The table shows four different phenotypes with the following phenotypic ratios: 9 Purple & Smooth, 3 Purple & Shrunken, 3Yellow & Smooth, and 1yellow and shrunken. There are nine different genotypes in the table: PPSS (1), PPSs (2), PpSS (2), PpSs (4), PPss (1), Ppss (2), ppSS (1), ppSs (2) and ppss (1).

Table 1: Dihybrid cross with heterozygous parents

Gametes	PS	Ps	pS	ps
PS	PPSS	PPSs	PpSS	PpSs
Ps	PPSs	PPss	PpSs	Ppss
pS	PpSS	PpSs	ppSS	ppSs
Ps	PpSs	Ppss	ppSs	ppss

Your instructor will distribute corn (or pictures) for you to examine in this activity. Once you receive your sample(s), compare to the picture above to be sure that you understand the different phenotypes.

Gather your supplies and read through all steps of the activity before you begin.

Materials: segregating ears of corn (or pictures), hybrid cross cards

Lab Procedure

1. Count the number of each kernel in your ear of corn and record your results below.

	Number Counted
Purple & smooth	
Purple & shrunken	
Yellow & smooth	
Yellow & shrunken	
TOTAL	

2. Did you obtain a 9:3:3:1 ratio? To determine if the deviations from your observed data are due to chance alone or if the data is significantly different, you need to use a **chi square test**. The table below will help you make the calculations.

	Observed Number	**Expected Number**	**[observed – expected]^2 / expected**
Purple & smooth		Total x 9/16 =	
Purple & shrunken		Total x 3/16 =	
Yellow & smooth		Total x 3/16 =	
Yellow & shrunken		Total x 1/16 =	
TOTAL		**CHI SQUARE VALUE ========> (add the numbers from the rows above)**	

3. Using the table below, determine if your chi square value is a good fit with your data. The degrees of freedom (df) is the number of possible phenotypes minus 1. In your case, 4 - 1 = 3. Find the number in that row that is closest to your chi square value. **Circle that number**.

Good Fit Between Ear & Data							Poor Fit	
df	.90	.70	.60	.50	.20	.10	.05	.01
1	.02	.15	.31	.46	1.64	2.71	3.85	6.64
2	.21	.71	1.05	1.39	3.22	4.60	5.99	9.21
3	.58	1.42	1.85	2.37	4.64	6.25	7.82	11.34
4	1.06	2.20	2.78	3.36	5.99	7.78	9.49	13.28

4. Explain what it means to have a "good fit" or a "poor fit". Does your chi square analysis of real corn data support the hypothesis that the parental generation was PpSs x PpSs?

Obtain a **HYBRID CROSS CARD**, A or B, that shows a cross between two homozygous parents. List the genotypes for the P generation:

Parent 1 _____

Parent 2 _____

1. Which allele is dominant or recessive? How do you know?

2. Does the card show a monohybrid or dihybrid cross? How do you know?

3. List the genotype(s) of the F1 generation shown on your card.

4. Show the results of crossing two F1 individuals to obtain an F2. List the phenotypic ratios.

Working in groups, perform a cross using A-1 and B-1 as the P generation.

1. Will you treat this as a monohybrid or dihybrid cross? Explain.

2. List the genotypes and phenotypes for the P and F1 of this cross.

3. Show the results of crossing two F1 individuals to obtain an F2. List the phenotypic ratios.

Chi Square Practice

1. A large ear of corn has a total of 433 grains, including 271 Purple & starchy, 73 Purple & sweet, 63 Yellow & starchy, and 26 Yellow & sweet. Your Tentative Hypothesis: This ear of corn was produced by a dihybrid cross (PpSs x PpSs) involving two pairs of heterozygous genes resulting in a theoretical (expected) ratio of 9:3:3:1. Objective: Test your hypothesis using chi square and probability values.

2. In a certain reptile, eyes can be either black or yellow. Two black eyed lizards are crossed, and the result is 72 black eyed lizards, and 28 yellow-eyed lizards. Your Tentative Hypothesis: The black eyed parents were Bb x Bb. Objective: Test your hypothesis using chi square analysis. In this set, because only two values (traits) are examined, the degrees of freedom (df) is 1. SHOW ALL WORK!

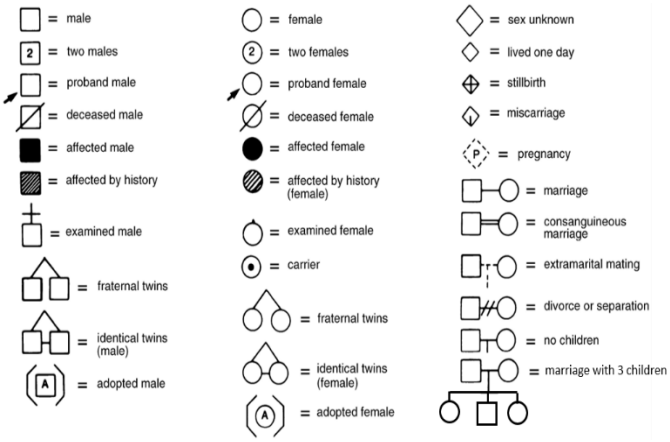
3. A sample of mice (all from the same parents) shows 58 Black hair, black eyes | 16 Black hair, red eyes | 19 White hair, black eyes 7 | White hair, red eyes Your tentative hypothesis: (what are the parents?) Objective: Use a chi square analysis to support your hypothesis.

Detecting Inherited Forms of Cancer

For this lab, assume you are a genetic counselor. It is your job to advise patients of their risk for inherited disorders based upon family history or clinical findings and to explain the nature of the disorder and risks to other family members. In **part 1** of the activity, you will be working with an individual who has a family history of colon cancer that may be caused by an ***autosomal dominant disorder*** known as hereditary nonpolyposis colorectal cancer, or HNPCC.

For **part 2** of the activity, you will assume the role of a laboratory technician to complete and analyze a DNA-based diagnostic test identifying which members of this family have inherited the cancer-causing mutation.

What is genetic testing? Genetic testing is any type of medical test that identifies changes in chromosomes, genes, or proteins. Genetic testing often identifies specific changes that may lead to or predispose an individual to a particular condition. If a patient decides to go forward with genetic testing, a DNA sample is obtained and sent to a laboratory to extract the patient's DNA and examine the genes associated with the disease.



Obtaining a **pedigree** (**Figure 1**) is often the first step in determining who may benefit from a genetic test. Pedigrees show relationships between family members as well as inheritance patterns for a trait of interest, and help identify at-risk individuals. Not every individual in the family will be a candidate for testing and not every candidate for testing will choose to be tested.

Figure 1: A pedigree is a diagram showing family history and relationships. Individuals affected with the trait/disease of interest are indicated by shaded symbols. Males are represented by squares, females with circles, and a mating relationship indicated with a line connecting the two. A ***proband*** is an individual who typically serves as the starting point for a genetic study within a family.

This lab activity focuses on **HNPCC** (hereditary nonpolyposis colorectal cancer), a type of colorectal cancer. HNPCC, also known as Lynch syndrome, results in polyps (abnormal growths) in the colon that ultimately may become cancerous. Pedigree studies have shown that HNPCC is inherited in an autosomal dominant fashion.

There are four identified genes (MLH1, MSH2, MSH6, and PMS2) that can cause HNPCC when mutated. These genes are known as caretaker genes-they scan the genome to identify and help repair DNA mutations. As with nearly all human genes, two copies of each are present, one inherited maternally and the other, paternally. HNPCC occurs when both copies of a specific caretaker gene are mutated. In most cases, only one copy of the mutation is inherited from a parent, leaving every cell with only a single working copy. If a cell in the colon acquires a mutation in the working copy, the cell loses some of its ability to detect and repair DNA damage. Eventually, the buildup of this additional genetic damage leads to colorectal cancer.

Inheriting a mutation in one of these HNPCC genes does not guarantee the formation of cancer, but studies suggest that individuals who inherit one HNPCC mutation have a dramatically increased risk (80%) of developing colorectal cancer compared to those who don't (5%). The symptoms of colon cancer are often not observed until late in the cancer's development, so early detection is critical. Genetic screening can help identify at-risk individuals.

Family Pedigree

Use the information below and the rules of pedigree construction (Figure 1) **to draw a family pedigree** for HNPCC. Be sure to include the names and ages of all family members, as well as the age of diagnosis if a patient has or has had the disease. Also indicate the age of death if the patient has died. Use "dx" to indicate the age of diagnosis, "d" to indicate the age of death, and "yo" for years old.

Your patient Bob is 45 years old and in good health. His wife Jane is 42 and also in good health. Bob and Jane have a 19 year old son Steven and a 21 year old daughter Claire. Bob explains that his sister Susan (age 50) was diagnosed with colon cancer at age 42. His brother Marshall is 47 and was recently diagnosed with colon cancer. Bob's last sibling (Sara) is 38.

Bob's parents both died young: his father Robert was killed in the war at age 35, and his mother Elizabeth was killed in a car accident at age 40. Elizabeth has a brother Stan (age 70) who was diagnosed at age 50 with colon cancer. Elizabeth also had three sisters and two brothers, who remain living. Bob's father (Robert) had three brothers, who are d eased, and three sisters, all of whom are still living. Bob's paternal grandparents both died in their 60s, causes unknown. Bob's maternal grandmother died at age 42 from ovarian cancer and his maternal grandfather died at age 85.

Bob's wife Jane has two bothers: one has two sons and one has a daughter. Jane also has a sister who has three daughters. Jane's parents are living. Her mother Roberta is 67, and her father Herschel is 70. Roberta has three living sisters, two living brothers, and a brother who died in his 70s from a heart attack. Roberta's parents (Jane's maternal grandparents) are deceased. The grandfather died at age 65 from a heart attack, and the grandmother at 75 with breast cancer.

Herschel has two deceased sisters and a 78 year old brother, Warren. Warren was diagnosed four years ago with colon cancer. Herschel's parents are deceased: his father died at age 80 and his mother at 75. Both Bob and Jane are African American. There is no other known cancer on either side of the family.

After constructing your pedigree, answer the following questions.

1. Describe Bob's risk for colon cancer. Describe Jane's risk.

2. As Bob's genetic counselor, what issues might you choose to discuss with Bob?

3. Is there anyone in either family you would like to have more information for? Explain.

4. What is the relationship between cell cycle regulation and cancer? What makes a cancerous cell different from a normal cell?

Genetic Testing

On the basis of the information determined about the family, genetic testing was offered to Marshall, Bob's brother who was recently diagnosed with cancer. This test examined the DNA sequence of all four genes known to be associated with HNPCC. A single nucleotide change was found in the MSH2 gene. Clinical studies have shown that this mutation is associated with HNPCC. As a result, genetic testing has been recommended for those in the family who wish to participate.

You are the laboratory technician responsible for completing the genetic testing for these family members. You will be given tubes of prepared DNA, each taken from a blood sample collected from a different family member who agreed to be tested. The DNA has been amplified by PCR (polymerase chain reaction) to produce millions of copies of the specific region of DNA that contains the mutation identified in Marshall. Enough DNA is created by this process that it can be loaded onto an agarose gel and visualized by gel electrophoresis.

Previous research has shown that the single-nucleotide change found in Marshall's DNA creates a sequence recognized by a restriction enzyme, a type of protein that cuts DNA at specific sequences. *If the mutation is present, mixing the amplified DNA fragment with the restriction enzyme will cut the DNA into two smaller pieces of differing size*. If the mutation is not present, the restriction enzyme will not recognize the DNA sequence and the amplified fragment will remain intact.

As a lab technician, you will separate and visualize the DNA fragments from each family member by gel electrophoresis. Upon viewing the finished gel, you will analyze the fragments of DNA that represent the area around the possible mutation and record the results for each family member.

Gather your supplies and read through all steps of the activity before you begin.

Materials: microcentrifuge, micropipettes & tips, electrophoresis chamber, power supply, gel tray & comb, tape or other method of sealing gel tray, DNA samples: family members, controls (Normal DNA, Tumor DNA, Heterozygote DNA), 1 kb DNA ladder, DNA stain & staining tray, 1.2% agarose gel, 1x TBE buffer

Lab Procedure

1. Prepare a 1.2% agarose gel using 1X TBE buffer and pour into gel tray.

2. When the agarose has set, unseal the ends of the gel tray and place the tray in the electrophoresis chamber so that the comb is at the negative (black, cathode) end.

3. Use 1x TBE buffer to fill the electrophoresis chamber to the level that just covers the entire gel.

4. Remove the comb by gently pulling it straight up. Take care not to rip the wells.

5. Make sure that the sample wells left by the comb are completely submerged. If "dimples" are observed around the wells, slowly add buffer until the dimples disappear.

6. Pipet 20 µL of each DNA sample into a separate well of the gel as indicated below. ***Remember to use a fresh pipet tip for each sample.***

Lane 1 - 1-kb DNA ladder

Lane 2 - Control (Normal Allele) DNA

Lane 3 - Control (Tumor) DNA

Lane 4 - Control (Heterozygote) DNA

Lane 5 - Stan, Bob's Uncle (age 70), with previous colon cancer diagnosis

Lane 6 - Susan, Bob's sister (age 50), with colon cancer

Lane 7 - Marshall, Bob's brother (age 47), with known HNPCC mutation

Lane 8 - Sara, Bob's sister (age 38)

Lane 9 - Bob

Lane 10 - Warren, Jane's uncle (age 78), with previous colon cancer diagnosis

Lane 11 – Jane

Lane 12 - Steven, Bob's son

Lane 13 - Claire, Bob's daughter

* If students are splitting a set of samples, then two gels should be loaded as follows:

Gel 1

Lane 1 - 1-kb DNA ladder

Lane 2 - Control (Normal Allele) DNA

Lane 3 - Control (Tumor) DNA

Lane 4 - Control (Heterozygote) DNA

Lane 5 - Stan, Bob's Uncle (age 70), with previous colon cancer diagnosis

Lane 6 - Susan, Bob's sister (age 50), with colon cancer

Lane 7 - Marshall, Bob's brother (age 47), with known HNPCC mutation

Gel 2

Lane 1 - 1-kb DNA ladder

Lane 2 - Control (Normal Allele) DNA

Lane 3 - Sara, Bob's sister (age 38)

Lane 4 - Bob

Lane 5 - Warren, Jane's uncle (age 78}, with previous colon cancer diagnosis

Lane 6 - Jane

Lane 7 - Steven, Bob's son

Lane 8 - Claire, Bob's daughter

7. After all samples have been loaded, connect the electrophoresis equipment to the power supply and run the gel until the loading dye is approximately 1cm from the end of the gel (125V for 45 minutes).

8. Stain & rinse your gel according to your instructor.

After gel electrophoresis, determine the position of DNA fragments by comparing them with the DNA ladder. Next, **draw a representation of your gel,** marking and labeling each lane and appropriate sizes of DNA bands. Once you have drawn your gel, determine which family member(s) have inherited the HNPCC mutation and use this data to ***complete the pedigree you created in part 1 of this activity*.**

Discussion Questions

1. Does having the mutation mean that an individual will definitely develop cancer? Why or why not?

2. According to the genetic test results, what is the risk that Bob's daughter Claire will pass the HNPCC mutation along to her children?

3. Assume that Bob did not want to know his mutation status and declined to be tested; however, Bob's daughter Claire very much wants to be tested.

- Under these circumstances, and assuming that Jane is negative for the mutation, a positive test on Claire will reveal Bob's mutation status as well. Who has the right to determine whether Bob's daughter should be tested? Does Claire's age make a difference? If she is tested and found to be positive, how should she discuss the results with Bob?

4. Who outside the family has a right to know this genetic information? Explain.

**This exercise was adapted from the Hudson Alpha Institute for Biotechnology "HNPCC: Detecting Inherited Forms of Cancer" classroom resource.*

Southern Blots: Detecting Sickle Cell Anemia

Southern blotting is a laboratory technique used to analyze an organism's total DNA, also known as its genome, in order to identify a specific sequence of interest. The technique, developed in 1975 by Professor Sir Edwin Southern, can be used for the diagnosis and/or study of genetic diseases, forensic applications, paternity testing and more.

Southern blots identify restriction fragment length polymorphisms (**RFLPs**) in DNA samples. An RFLP is the result of a restriction site that is present in the genomes of some members of a population, but not others (due to an alteration in the nucleotide sequence). RFLPs are identified using a probe that spans the polymorphic region, so the presence or absence of the polymorphic restriction site is determined by the number and sizes of the DNA fragments that are detected.

During a Southern blot, DNA from a biological sample (such as blood or tissue) is digested with a restriction enzyme, and the resulting DNA fragments are separated by gel electrophoresis. The DNA fragments are transferred from the gel onto a solid membrane, which is then incubated with a DNA probe labeled with a radioactive, fluorescent or chemical tag. The tag allows any DNA fragments containing complementary sequences to the DNA probe to be visualized. Southern blots are a very accurate diagnostic tool, since the probes used are specific for a known DNA sequence. The steps involved in a Southern Blot are detailed below.

Table 1: Southern Blotting Overview	
Digest	DNA is cleaved by restriction enzymes, which recognize and cut DNA at specific sequences. The size of the resulting DNA fragments can be predicted based on the enzymes used.
Separate	Digested DNA is separated according to size using gel electrophoresis.
Depurination & Denature	The gel is placed in an acid solution to separate the sugar phosphate backbone (depurination), then placed in a base solution to separate the two DNA strands (denaturation).
Transfer	DNA is transferred from the gel to a nitrocellulose membrane.
Fix	DNA is secured to the membrane (using heat, vacuum, UV or combination)
Hybridize	Membrane is incubated with a labeled probe SPECIFIC for the sequence of interest
Visualize	Florescent probes use UV light. Radiolabeled probes are visualized by autoradiograph (x-ray film). Chromogenic detection utilizes enzymes and color-changing solutions.

Sickle Cell Disease (SCD) results from a single base mutation in the gene that codes for the hemoglobin protein, causing the blood cells to become stiff & deformed ("sickle" shaped). The symptoms of SCD range from tiredness to heart attack to death. Detection of SCD is challenging because of the range of symptoms. Often, blood samples are viewed under a microscope to diagnose SCD, or hemoglobin levels are measured to diagnose the disease. For newborn babies however, the detection of SCD is further complicated due to the production of fetal hemoglobin, which could mask the symptoms of the disease. Thus, for new parents, a more accurate test is needed. Today, you will perform a Southern Blot to test DNA samples from a family (mother, father and child) concerned about SCD.

Gather your supplies and read through all steps of the activity before you begin.

Materials: 0.8% agarose gel, electrophoresis buffer (1X TAE), electrophoresis chamber & power source, gel tray & comb, DNA samples (+/- and heterozygous controls, mother, father, child), nylon membranes, filter paper, paper towels, 200 mL of DEPURINATION SOLUTION: 0.25N HCl, 400 mL of DENATURATION SOLUTION: 0.5M NaOH / 0.6M NaCl, detection/hybridization solution, **incubator set to 80°C**, forceps, spatula, ink pen, plastic trays, microwave oven or hot plate, electronic balance, laboratory glassware (flasks, graduated cylinders), plastic wrap, tape, scissors, latex gloves – NOT POWDERED GLOVES! (If necessary, rinse and dry)

In this part of the activity, you will be given DNA samples from a family (mother, father and child) that have been digested with a restriction enzyme that recognizes (and cuts) a sequence within the hemoglobin gene, mutations in the recognition (restriction) site will result in different sized DNA fragments compared to normal DNA samples. You will separate the DNA fragments using gel electrophoresis, then immediately depurinate/denature the DNA in the gel and transfer it to a nylon membrane.

Lab Procedure Part I: Electrophoresis

1. Prepare 0.8% agarose gel using 1X electrophoresis buffer (TAE).

2. Place the gel (still on the tray) into the electrophoresis chamber, cover with electrophoresis buffer.

3. Load 20 µl of each DNA sample into the appropriate well:

Lane 1: Normal sample DNA

Lane 2: Heterozygous DNA

Lane 3: Disease sample DNA

Lane 4: Mother's DNA sample

Lane 5: Child's DNA sample

Lane 6: Father's DNA sample

4. Run gel for **30 minutes at 125V.**

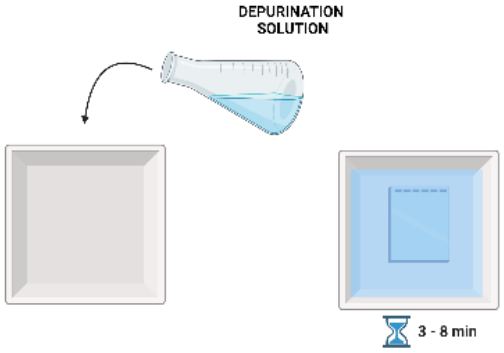
5. After electrophoresis is complete, remove the gel and casting tray from the electrophoresis chamber and proceed **IMMEDIATELY** to the next step.

DEPURINATION (20 minutes) - ***The depurination step should not last too long, since very short fragments attach less firmly to the membrane.*** **READ THE PROTOCOL BEFORE CONTINUING!!!**

1. Add 200 mL **of DEPURINATION SOLUTION (0.25 N HCl)** to a plastic tray.

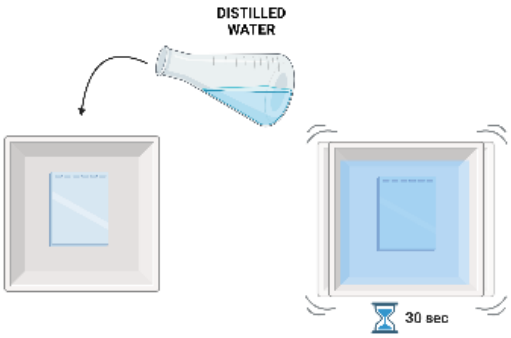
2. Transfer gel to the tray, making sure that it is covered by the solution.

3. Incubate at room temperature for 3-8 minutes. ***STOP incubation once the blue dye in your gel turns to a green/yellow color! *The color change is due to bromophenol blue in the samples, which is a pH indicator: the change from blue to yellow, indicates that the pH has decreased, and the DNA has effectively been depurinated ("nicked").***



4. **Immediately** pour depurination solution into a waste container.

5. **Rinse**: Add distilled water to the tray and gently shake for 30 seconds. Discard water in the appropriate waste container, then **REPEAT 2 times.**



<u>DENATURATION (35-40 minutes)</u>

1. Add 200 ml of **DENATURATION SOLUTION (0.5 M NaOH/6 M NaCl)** to the tray

2. **Incubate** at room temperature for 15 minutes. ***The density of the solution will cause the gel to float, so check often and shake to keep it immersed!***

3. Discard the denaturation solution in the appropriate waste container.

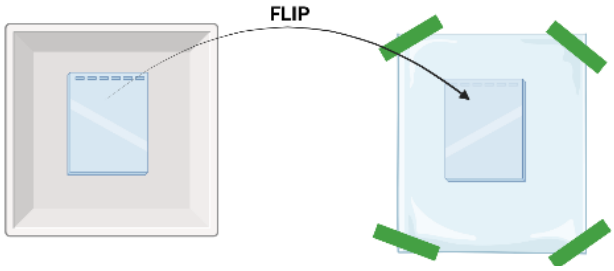
4.vREPEAT with NEW denaturation solution, incubating for 15 minutes.

5. **DO NOT discard the solution**. **Read ahead** so that you understand what to do at the end of this 15-minute incubation.

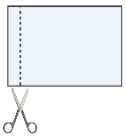
SOUTHERN BLOT TRANSFER (10-15 minutes) - ***Oils from your hands can interfere with the transfer, so WEAR GLOVES and use 2 CLEAN FORCEPS for the following steps***

1. PREPARE your lab bench by spreading out a small sheet of plastic wrap, TAPING the edges to keep it flat.

2. Place your gel **well side down** onto the plastic wrap so that the exposed (top side) of the gel is smooth.

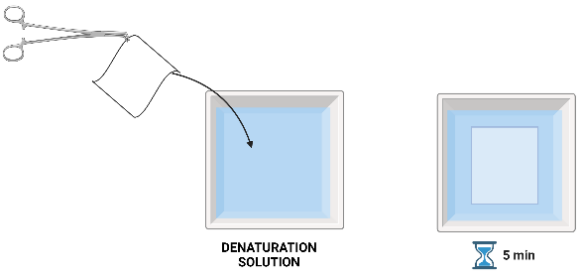


3. Using forceps and scissors, **carefully** trim the nylon membrane and filter paper to the size of the gel.



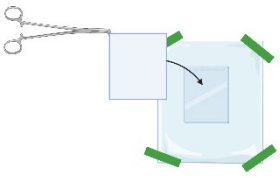
4. SLIDE the membrane out of the blue cover and TRANSFER to the denaturation solution from the last step.

For best results, ADD the membrane to the solution in a U shape so that the middle comes in contact with the solution first! The solution can then slowly spread out to both edges.



5. SOAK the membrane for 5 minutes.

6. Using forceps, REMOVE the membrane from the tray and PLACE on top of your gel.



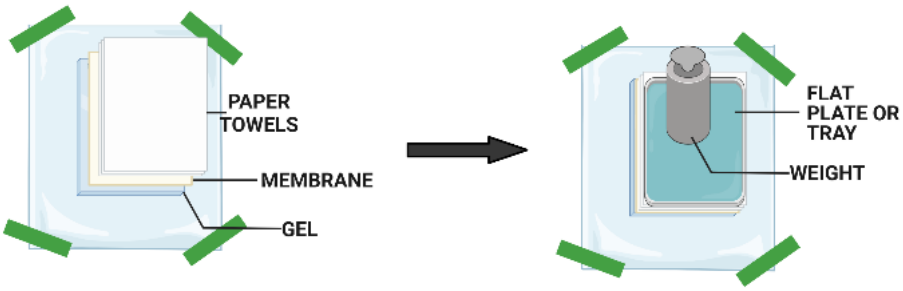
7. Place the filter paper on top of the nylon membrane.

8. REMOVE any air bubbles from the top of the gel, membrane, and filter paper using a large pipette tip or similar cylindrical object.

9. PLACE a 5·6 cm stack of paper towels on top of the filter paper.

10. PLACE an empty tray or flat plate on top of the paper towels.

11. PLACE a 500g weight (500 ml beaker w/water) on top of the tray.



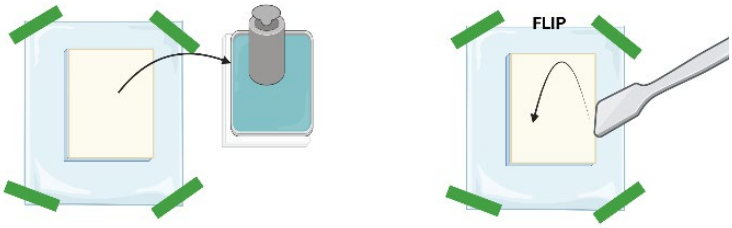
12. **Do not disturb for 24 - 48 hours to allow the transfer.** The DNA will not be efficiently transferred if the blot short-circuits of capillary flow. If leaving for over 24 hours, use parafilm strips to seal the edges of the gel.

PART 2: In this step, the DNA will be fixed to the nylon membrane and incubated with a solution containing a labeled probe that is complimentary to a sequence in the HEMOGLOBIN gene.

SOUTHERN BLOT FIXATION (35-45 minutes)

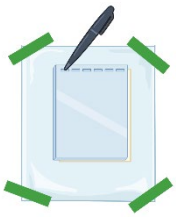
1. Remove the weight and items on top of the filter paper (weight, tray & paper towels), but leave the filter paper.

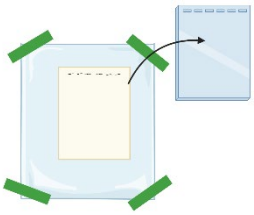
2. FLIP the remaining stack (filter paper-membrane-gel) so that the gel is facing up. This can be done with a spatula, or with forceps and rinsed gloves.



3. Use a **pen** to DRAW through the sample wells on the gel and mark their position on the nylon membrane.

4. Carefully REMOVE and DISCARD the gel with forceps or a spatula.





5. VERIFY that the transfer has occurred by the presence of the blue tracking dye on the membrane. Using a **pen**, gently LABEL this side of the membrane with your group ID/initials (**this is the side that the DNA is on so keep the label small).**

6. **FIXATION**: PLACE the membrane between two sheets of filter paper or paper towels and INCUBATE at **80°C for 30 minutes.**

OPTIONAL STOPPING POINT: Once you have performed the fixation step then the membrane can be stored until you are ready to continue. Store the membrane between the two sheets of filter paper, at room temperature, and away from moisture.

DETECTION & ANALYSIS (30-45 minutes)

1. ADD 60 ml of hybridization solution (Blue-Blot DNA Stain) to a sealable bag or tray.

2. Place your membrane (from previous) in the bag or tray. ***If you are using a tray, keep the DNA side facing up.***

3. SOAK the membrane for 5 -15 minutes making sure that the membrane stays covered by the solution.

4. WASH the membrane by adding 200 mL of distilled water, gently shaking the bag or tray, then pour out the water and REPEAT until the DNA bands are clearly visible against a light blue background.

5. REMOVE the membrane from the water and AIR DRY. Once dry, membranes can be stored in your laboratory notebook.

Discussion Questions

1. Examine your banding pattern. How many bands do you observe in each column?

2. Compare the banding pattern created by the Mother, Child, & Father's DNA to the controls.
    a. List the genotype of each sample (which samples are homozygous dominant, heterozygous, or homozygous recessive).

    b. Do any of the family members have the disease?

    c. If the father and mother have another child, what is the chance that the child will have the disease?

3. Describe the steps involved in a Southern Blot, explaining the significance of each.

*This exercise was adapted from EDVO-KIT activity #315, www.edvotek.com

Cytogenetics and Karyotyping

In this activity, you will take on the role of a ***cytogeneticist*** working in a hospital. A case study will be given to you for review. You will then construct a ***karyotype*** for your patient and attempt to make a diagnosis.

Introduction

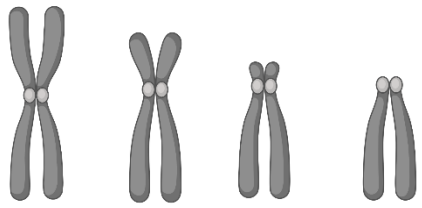
A cytogeneticist is a geneticist who specializes in the study of chromosomal structure and function. In order to think like a cytogeneticist, here are a few things you will need to know.

DNA in a healthy cell is never "naked" but is tightly wrapped around proteins and is referred to as ***chromatin***. In this state, the DNA/protein complex appears as long thin strands that cannot be distinguished from one another. During cell division, however, DNA is further condensed into visible ***chromosomes***.

The DNA of all living organisms is organized into chromosomes. Most human cells contain 46 chromosomes: a maternal set of 23 (contributed by the mother's egg) and a paternal set of 23 (contributed by the father's sperm). Thus, humans have 23 pairs of ***homologous*** or paired chromosomes. Within each set of chromosomes there is one ***sex chromosome*** (XX or XY) and 22 other chromosomes, called ***autosomes***.

Chromosomes vary in size and shape. ***Centromere*** location is also a feature used to distinguish one chromosome from another. ***Metacentric*** chromosomes have arms of roughly equal lengths. The arms of ***submetacentric*** chromosomes are unequal: the shorter arm is called the ***p arm***, the longer are is called the ***q arm***. ***Acrocentric*** chromosomes have a centromere that is even closer to one end of the chromosome, making their p arms even shorter in relation to their q arms. ***Telocentric*** chromosomes (not normally present in humans) have the centromere positioned in the region called a telomere (**Figure 1).**

Figure 1: Chromosome structure: centromere position



A ***karyotype*** is an image of an individual's chromosomes, usually obtained by Giemsa staining (G-banding). ***Heterochromatic*** regions of DNA are highly condensed regions of the chromosome. These regions contain an abundance of adenine and thymine (A-T rich) and stain more darkly. Less condensed (and transcriptionally active) ***Euchromatin*** is rich with guanine and cytosine nucleotides and incorporates less Giemsa stain, thus appearing as lighter bands (G-bands).

The reverse of G-banding is obtained in R-banding. Since A-T rich regions are more susceptible to denaturation than C-G regions, the resulting band pattern is the reverse of G-banding: A-T regions are lighter, while C-G regions are darker.

Because there is a unique pattern of light and dark bands for each chromosome, this technique can be used to uniquely identify each chromosome, as well as chromosomal abnormalities. Chromosomes are typically prepared for karyotyping with the sister chromatids so closely aligned that they appear as a single structure (in other words, they look like an "I" rather than an "X") (**Figure 2**).

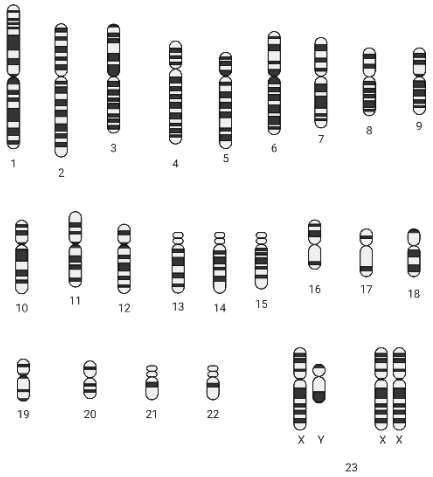


Figure 2: Human karyotype showing banding patterns

What types of samples are often obtained for karyotyping? Cells may be obtained from various sources for karyotype analysis, including blood/skin/other tissue, chorionic villi or amniotic fluid. Chorionic villus sampling (CVS) is performed during a pregnancy. This procedure involves removing part of the placenta so the cells can be analyzed. It can be performed at 10-12 weeks of gestation and carries a 1-2% risk of miscarriage. Amniocentesis (the process of withdrawing amniotic fluid surrounding the fetus) can be conducted between 15-20 weeks of gestation and carries a 1% or less risk of miscarriage (https://www.hopkinsmedicine.org).

What are the benefits of karyotyping? The results of a karyotype can provide answers or a diagnosis prenatally, to help parents make important decisions such as planning for the care needed at birth, or make alternative reproductive decisions. In postnatal cases, the results can be used to diagnose syndromes associated with chromosomal abnormalities.

Tests are available for pregnant females to screen for chromosomal disorders. These screening tests measure proteins in the blood of pregnant women to identify cases that should be more extensively tested (via amniocentesis to obtain fetal cells, for example). Maternal screening tests are not entirely accurate, and abnormal pregnancies can remain undetected by the maternal blood screen. Additionally, women who are carrying a normal fetus may have an abnormal screening test and then deal with the stress of deciding whether or not to undergo amniocentesis.

Gather your supplies and read through all steps of the activity before you begin.

Materials: cytogenetics report, chromosome board

Lab Procedure

1. You will receive a Chromosome board containing a case study and set of patient chromosomes (reusable decals). Each case study has a Case ID and a unique color. The color of the patient chromosomes matches the color that is printed around the case study section of the chromosome board. Confirm that the colors of the chromosomes and the board match.

2. Select a chromosome decal from the "cryostorage" area of the board and sketch it on your Cytogenetics Report. Fill in the appropriate noting the centromere, telomere, and p and q arms. Note the centromere position and identify the chromosome as metacentric, submetacentric, or acrocentric.

3. Read the case study for your patient (listed on the chromosome board).

4. On the Cytogenetics Report, record patient information, including name, case ID, reason for referral, patient age, and source of cells.

5. Next, **construct a karyotype** by identifying the homolog for each chromosome listed on your board and placing it on the board in the proper position.

6. Once the karyotype is complete, analyze it for chromosomal anomalies (number and/or structure).

7. Record chromosome number, gender, and chromosomal findings on the Cytogenetics Report.

8. Determine the suggested diagnosis. ***you may need to do some research to properly diagnose your patient**

9. Complete the Cytogenetics Report on your patient to include **patient diagnosis**, **chromosomal findings** and **notes for a caregiver** (if applicable).

Cytogenetics Report

Select a chromosome from the cryostorage area. Sketch the chromosome, labeling the p arm, q arm, centromere, and telomere.

Chromosome type (circle the correct option)	metacentric	submetacentric	acrocentric

Patient Name:	Case Study ID	Age

Why is the patient being referred for karyotyping?	Source of Cells for Karyotyping: Blood _____ Amniocytes _____ Chorionic Villi _____ Other (specify) _____
Total Number of Chromosomes Observed:	Gender
Chromosomal Findings: _____no observable chromosomal abnormalities _____monosomy (chromosome #____) _____trisomy (chromosome #____) (arm____) _____deletion (chromosome #____) (arm____) _____insertion (chromosome #____) (arm____) _____inversion (chromosome #____) (arm____) _____translocation (chromosome #s____ and ____)	**Patient Diagnosis:**

Notes for patient's caregiver with additional implications of the diagnosis, including life expectancy, complications, available treatments, and support group information.

Summary Questions:

1. Explain how a karyotype is prepared.

2. What are the limitations to cytogenetics, with regard to diagnosing genetic diseases/disorders?

3. Why is cytogenetics/karyotyping important?

Forensic Science and PCR

DNA fingerprinting is a technique used to determine the genotype of a DNA sample and compare it to other DNA "profiles". For example, crime scenes often contain biological evidence (such as blood, semen, hairs, saliva, bones, pieces of skin) from which DNA can be extracted. If the DNA profile obtained from evidence discovered at the scene of a crime matches the DNA profile of a suspect, this person is included as a potentially guilty person; if the two DNA profiles do not match, the individual is excluded from the suspect pool. This technique is used around the world for investigations of missing persons, mass disasters, human rights violations, and paternity.

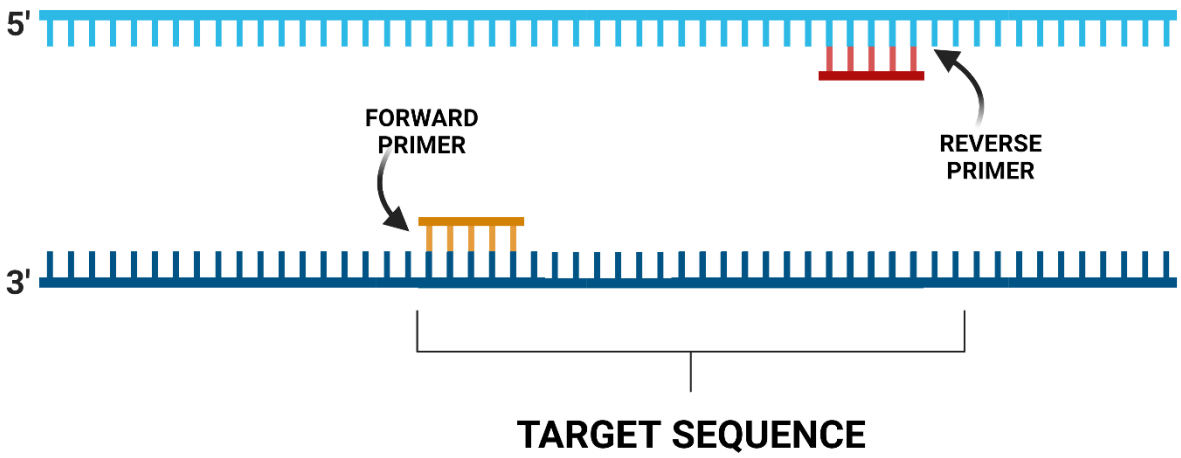
What is PCR? PCR (polymerase chain reaction) produces large amounts of a specific piece of DNA from trace amounts of starting material (template). The template can be any form of double-stranded DNA. A researcher can take trace amounts of DNA from a drop of blood, a single hair follicle, or a cheek cell and use PCR to generate millions of copies of a desired DNA fragment. In theory, only a single template strand is needed to generate millions of new DNA molecules. Prior to PCR, it would have been impossible to do forensic or genetic studies with this small amount of DNA. Thus, the ability to **amplify** the precise sequence of DNA that a researcher wishes to study or manipulate is the true power of PCR.

PCR is such a powerful tool is its simplicity and specificity. The specificity of PCR is its ability to target and amplify one specific segment of DNA a few hundred base pairs in length out of a complete genome of over 3 billion base pairs. In addition, all that is required for PCR is at least one DNA template strand, DNA polymerase, two DNA primers, and the four nucleotide building block subunits of DNA (A, G, T, and C) otherwise known as **deoxynucleotide triphosphates** (**dNTPs**) (adenine, guanine, thymine, cytosine). These nucleotides are provided in a reaction buffer called a **master mix**. The master mix contains **dNTP**s, a special buffer to maintain optimum pH, salts, and $MgCl_2$. Salts and magnesium ions (also known as cofactors) are needed for the DNA polymerase to perform optimally.

PCR amplification is DNA replication in a test tube. The portion of the DNA you want to make copies of is called the target sequence. The sample of DNA obtained at a crime scene and the suspect's DNA samples contain the target sequence. The 3 basic steps of PCR are listed below.

> **Denaturation**: Before new DNA synthesis can begin the double stranded DNA template must be unwound and separated into single strands. In cells this is carried out by a family of enzymes. In PCR, heat is used to "melt apart" (denature) the double stranded DNA template.
>
> **Annealing**: Before a target region of DNA can be amplified, one must determine short sequences of DNA upstream (at the 5' end) and downstream (at the 3' end) of the target loci region of interest. These areas are then used to make short pieces of DNA, called ***primers*** or oligonucleotides, which are complementary to regions upstream and downstream of the target loci region. Primers serve as start and stop points for amplifying the target region of the DNA to be copied.
>
> **Extension**: Primers are needed because the DNA polymerase requires an already existing nucleotide chain to bind and add nucleotides to one at a time. Once the polymerase locates and binds to template DNA and the primer, it initiates the addition of nucleotides and synthesizes new copies of the double stranded template DNA by adding nucleotides onto the primer and extending it. Therefore, primers provide a starting point for the DNA polymerase.

Figure 1: PCR Overview



These 3 steps – denaturation, annealing, and extension together make up one PCR cycle. A complete PCR reaction involves many repetitions of a single PCR cycle. In this experiment, your PCR reactions will cycle 35 times.

The enzyme used in PCR is a special DNA polymerase called ***Taq polymerase.*** This enzyme was isolated from a thermophilic bacterium, *Thermus aquaticus (Taq*), which lives in high-temperature steam vents such as those found in Yellowstone National Park. Taq polymerase is well-suited for PCR because it is able to withstand the temperature of 95 °C which is required for DNA strand separation without denaturing.

Two template strands are created from the original template after each complete cycle of the strand synthesis reaction – denaturation, annealing, and extension. It is called the polymerase chain reaction because exponential growth of the number of template molecules occurs after each cycle is complete, i.e., the number of DNA copies doubles at each cycle. Therefore, after 35 cycles there will be 2^{35} times more copies than at the beginning. After 35 cycles, the DNA of interest has been amplified sufficiently to be visualized using gel electrophoresis and DNA stains. This allows researchers to determine the presence or absence of the desired PCR products.

The DNA sequences used in DNA fingerprinting are non-coding regions that contain **Short Tandem Repeats (STRs)**. STRs are short DNA sequences that are repeated in a tandem fashion. For example, TH01 is a TCAT sequence repeated within the human genome as shown in Figure 2 below:

…CGG**TCATTCATTCATTCATTCATTCATTCAT**TCAT…

Figure 2: Two sample genotypes – each individual has two alleles		
		# REPEATS
SUSPECT 1	TCATTCATTCATTCATTCAT	5
SUSPECT 1	TCATTCATTCAT	3
SUSPECT 2	TCATTCATTCATTCATTCATTCATTCATTCATTCAT	10
SUSPECT 2	TCATTCATTCATTCATTCATTCAT	6

For the TH01 STR locus, there are many alternate alleles that differ from each other by the number of [TCAT] repeats present in the sequence. More than 20 different alleles of TH01 have been found in people worldwide, but each individual has only two of these: one inherited from each parent. As shown in the figure, suspect 1 suspect 2 have different alleles.

Using primers specific to the DNA sequences on either side of the [TCAT] STR, billions of copies of each of the two original TH01 alleles in any one person's DNA type can be amplified using PCR. To visualize the results, PCR products can be "sorted" using **gel electrophoresis.** Following gel electrophoresis (which separates the PCR products according to size), the pattern of bands is compared to an Allele Ladder to identify the alleles present in the original samples.

As shown in figure 3, PCR has been performed on DNA from 2 suspects using primers specific for the TH01 locus.

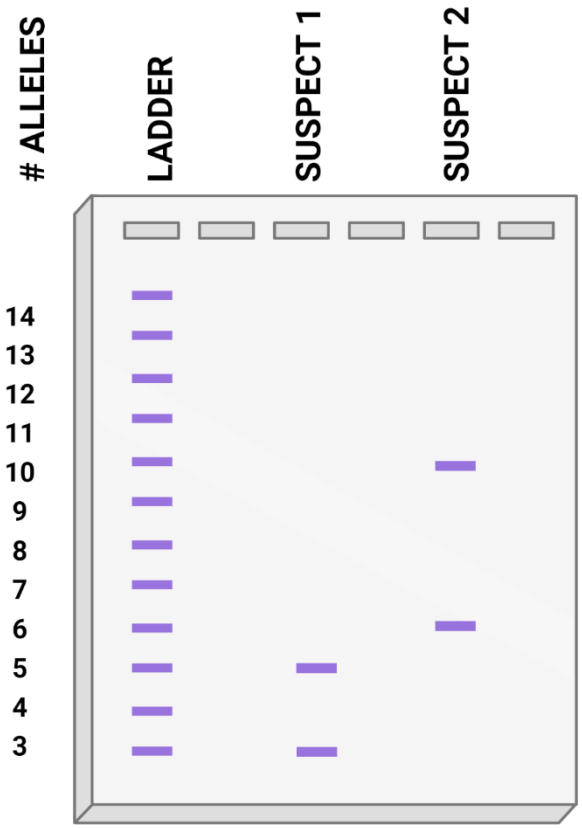


Figure 3: Example of gel electrophoresis results. DNA from two suspects was amplified using primers specific for "TH01" alleles.

You are about to conduct real world forensic DNA profiling. As a crime scene investigator, you will use the polymerase chain reaction (PCR) and agarose gel electrophoresis to analyze the DNA samples obtained from a hypothetical crime scene and four suspects. Your job is to identify the perpetrator.

Gather your supplies and read through all steps of the activity before you begin.

Materials: T100 Thermal Cycler, PCR tubes & adaptor tubes (5), marking pen, 2–20 µl adjustable micropipette & aerosol barrier tips, **ice bath** containing tubes of DNA (as below), DNA Samples: Crime Scene and 4 suspects (25µl of each sample indicated), Master Mix + primers (MMP, blue liquid, 120 µl)

*Your instructor will combine the Master Mix and Primers **(50MM:1P ratio)** and provide you with the "MMP"*

**** It is important to keep the master mix cold before use, so that the enzyme doesn't start to work before you add your DNA templates.**

***Keep tubes on ice throughout the procedure.**

Lab Procedure Day 1: PCR

1. Label PCR tubes CS, A, B, C, and D and include your group name/initials

2. Add 20 µl of corresponding DNA to each PCR tube.

3. Add 20 µl of the Master Mix + primers (**MMP**) to each PCR tube **(keep on ice)**

4. Mix the contents of your PCR tubes by gently pipetting up and down.

5. Once you've added MMP to a tube, **close the cap**. The solution in your PCR tubes should be blue. If it's not blue, talk to your instructor.

6. Place your capped PCR tubes in their adaptors on ice.

7. **When instructed to do so**, place your tubes in the thermal cycler.

8. Run PCR as follows: Denature at 94°C, 2:00; 35 cycles; 94°C/0:30; 52°C/0:30; 72°C/1:00; Repeat X34; Extension 10:00

Note: you will need 3% agarose gel for the next part of this activity. To make a single gel, add 1.5g agarose to 50 ml of 1XTAE buffer. Heat as instructed, then pour into gel tray.

Gather your supplies and read through all steps of the activity before you begin.

Materials: 3% agarose gel, PCR Samples from previous lab, 1X TAE buffer, Orange G loading dye, Crime Scene Investigator Allele Ladder (with loading dye), 2–20 µl adjustable pipet & aerosol barrier tips, gel electrophoresis chamber & power supply, DNA stain, gel staining tray

Lab Procedure Day 2: Electrophoresis

Today you will run an agarose gel to separate the PCR products from the previous lab.

1. Set up your gel electrophoresis chamber and cover your gel with 1X TAE Buffer.

2. Obtain your 5 PCR reactions from the previous lesson.

3. Add 10 µl Orange G loading dye to each PCR reaction tube and mix.

4. Load 20 µl of the allele ladder and 20 µl each sample into your gel.

***MAKE A NOTE OF THE ORDER IN WHICH YOU LOAD YOUR SAMPLES INTO THE WELLS!**

5. **Run gel at 100V for 30 minutes**.

6. When electrophoresis is complete, turn off the power and remove the lid from the gel box. Remove the gel tray and the gel from the gel box. Nudge the gel off the gel tray and carefully slide it into a container for staining.

There are two protocols for staining your gel. Your instructor will inform you which one you will use.

OPTION 1: OVERNIGHT STAINING (RECOMMENDED)

1. Add 120 ml of 1X Fast Blast DNA stain to your staining tray.

2. Let the gels stain for approximately 4–24 hours with gentle shaking for best results. No destaining is required.

3. The next day, pour off the stain into a waste beaker.

OPTION 2: QUICK STAINING (20 MINUTES)

This method will allow you to see bands quickly (within 15 minutes), but may require extensive de-staining to obtain optimal band-to-background intensity. Note: it is important to use **warm** tap water for the de-staining steps of this protocol.

1. Immerse your gel in 100x Fast Blast.

2. Stain the gel for 5 minutes with gentle agitation. Save the used stain for future use. Stain can be reused at least 7 times.

3. Transfer the gel into a tray and rinse with warm (40–55°) tap water for 10 seconds.

4. De-stain by washing three times in warm tap water for 5 minutes each, with gentle shaking for best results. You should be able to see bands after 10 minutes.

CONCLUSIONS

Once the gels have been stained, determine the alleles present in each sample and assign a DNA profile for each sample by comparing to the allele ladder. After drawing your gel (below) answer the questions at the end of this exercise.

DRAW YOUR GEL RESULTS BELOW:

1. Based on your results, can you determine who committed the crime? EXPLAIN.

2. What does PCR allow you to do with DNA?

3. What components do you need to perform PCR?

4. Why do you need each component?

5. Why do you need to perform PCR on DNA evidence from a crime scene?

6. What steps make up a PCR cycle, and what happens at each step?

7. Why do you need to perform PCR on DNA obtained from a Crime Scene?

8. What might you see if you ran a DNA sample extracted from evidence on a gel before PCR?

9. Why do forensic labs analyze non-coding DNA and not genes?

10. Why does DNA move through an agarose gel?

Restriction Digest of λ DNA

Learning Goals

In this lab, students will:

- Cut λ (lambda) DNA with three different restriction enzymes
- Make a standard curve using known DNA fragment sizes
- Use the standard curve to determine the size of DNA fragments in the unknowns
- Determine which enzyme was used in the three unknown reactions

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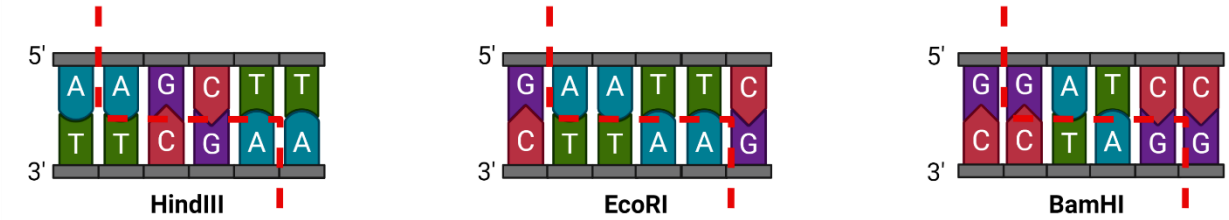
Introduction

Restriction endonucleases are enzymes that protect bacteria from viral infection. Bacteria have one circular chromosome that includes methyl groups that regulate protein-DNA interactions. Viral DNA does not have methyl groups; therefore, it is recognized as foreign and is cleaved by the enzyme. Restriction enzymes are named based on the source of the enzyme and a roman numeral (because each species of bacteria has several different restriction enzymes). The following table shows the restriction enzymes we will be working with in this lab.

Source	Enzyme	Pronunciation
H*aemophilus* ***in****fluenzae* Strain R**d**	HindIII	hen-dee-three
E*schericia* ***co****li* Strain **R**13	EcoRI	echo-are-one
B*acillus* ***am****yloliquefaciens* Strain **H**	BamHI	bam-aitch-one

Restriction enzymes cleave DNA at locations called restriction sites. Each enzyme has a unique site at which it cuts. The restriction sites for HindIII, EcoRI, and BamHI are shown below.

Answer question 1 before proceeding.



Lab Procedure: Week 1

All enzymes have an ideal temperature at which they function. All three of the restriction enzymes we are using today have an ideal temperature of 37°C. While that is the same as the average human body temperature, that is just a coincidence! After incubation at 37°C, you will move your tubes to 65°C to denature the enzymes for the next step.

Activity 1: Restriction Digests

Gather your supplies and read through all steps of the activity before you begin.

Per group - set of micropipettes, micropipette tips, plastic container for tip disposal, set of four 0.5 mL tubes (containing restriction enzymes), tube of λ DNA, tube float

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1. Label each tube with your Group #.

2. To each of the four tubes, add 20 µL of λ DNA, pipetting up and down to mix. Change tips between tubes.

3. Cap the tubes, place them in a tube float, and incubate at 37°C for at least 25 minutes (your instructor may have you move on to Activity 2 during this time).

4. After 20 minutes, move your tubes to the 65°C water bath for three minutes.

Clean-Up:

- Return the remainder of the λ DNA to your instructor.
- Empty used tips in the appropriate waste bin.

Answer questions 2 and 3 before proceeding.

Agarose gel electrophoresis is a technique used to separate macromolecules by size. DNA is negatively charged so the negative electrode will repel the DNA through the gel towards the positive electrode. The gel, on a molecular level, is like a sieve – larger fragments cannot travel as far as smaller fragments. Some very small fragments may even run out of the other end of the gel. In the next two activities, we will pour our gels, practice

loading, and resolve the products of our restriction digest on an agarose gel to separate the restriction fragments by their size.

Activity 2: Pouring the Gel and Practicing Loading

Gather your supplies and read through all steps of the activity before you begin.

Per group - set of micropipettes, micropipette tips, plastic container for tip disposal, gel casting tray, comb, tape, practice plate, methylene blue

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1. Seal the ends of the gel-casting tray with the tape provided and insert the comb at the farthest end of the gel casting tray.

3. Pour a tube of liquid agarose into the tray. Your instructor will inform you if you are to use the whole amount in the tube or if you need to make sure the agarose only covers about 1/3 of the comb teeth.

4. If there are any bubbles, use a pipette tip to move them to the end of the tray. DO NOT move or bump the tray while it solidifies.

5. While the gel is solidifying, retrieve a practice plate and flood it with water. This will mimic the environment you will experience when you load your gels (they will be in buffer).

6. Each student should load 20 µL of methylene blue into at least two separate wells. Continue practicing until you are comfortable with the procedure.

Clean-Up:

- Pour water from practice plate down the sink.
- Empty used tips in the appropriate waste bin.
- Return practice plates and methylene blue to the supply bench.

Answer questions 4 before proceeding.

Activity 3: Running the Gel

Gather your supplies and read through all steps of the activity before you begin.

Per group - set of micropipettes, micropipette tips, plastic container for tip disposal, electrophoresis chamber and lid, loading dye, SYBR™ Gold

1. Remove the tape from the gel tray and place it into the electrophoresis chamber with the wells closest to the negative electrode.

2. Fill the chamber with tris-borate-EDTA (TBE) buffer so that it is even on both sides and completely covers the gel.

3. Carefully remove the comb and ensure that the wells are full of buffer.

4. Add 2 µL of loading dye to each tube and mix by gently pipetting up and down. Change tips between tubes.

5. Add 2 µL of SYBR™ Gold to each tube and mix gently by pipetting up and down. Change tips between tubes.

Note: Loading dye weighs down the sample so that it stays in the well once loaded. SYBR™ gold makes the DNA UV reactive for documentation.

6. Carefully load 20 µL of each sample to a separate well, skipping a well before each sample (load samples in wells 2, 4, 6, and 8). Change tips between tubes. Be sure to note which well each sample is in.

7. Place the lid on the chamber and use tape to label it with your group number. Carefully move it to the supply bench, next to the power supplies. Your instructor will run the gels for 30 minutes at 130V and document your results for next week.

Clean-Up:

- Return the loading dye and SYBR™ Gold to your instructor.
- Empty used tips in the appropriate waste bin.
- Reset the bins and return them to the supply bench.

Answer questions 5 – 7 before proceeding.

Lab Procedure: Week 2

Restriction enzymes cut at specific DNA sequences and, since DNA is anti-parallel and bases always pair the same way, the enzyme will cut through both strands, resulting in restriction fragments. The more times the recognition sequence occurs in a piece of DNA, the more restriction fragments will be produced. The λ bacteriophage genome is a singular piece of DNA that is 48, 502 base pairs long. The recognition site for HindIII is 5' – AGCTT – 3' which occurs seven times in the λ genome. How many restriction fragments are expected?

Figure 1. Relative cut sites for HindIII in λ DNA. Cut sites are shown by the dotted lines. Fragments are numbered in red.

The fragment sizes are as follows: 1 – 23130; 2 – 2027; 3 – 2322; 4 – 564; 5 – 125; 6 – 9416; 7 – 6557; 8 – 4361. We will be using these known fragment sizes to make a standard curve based on the distance traveled in an agarose gel. The standard curve will then be used to determine the identity of the enzyme in the other restriction digests.

Activity 4: Making the standard curve

Gather your supplies and read through all steps of the activity before you begin.

Per student – print-out of results from Week 1, ruler, access to Excel

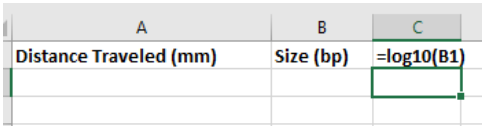
---

1. Rank the fragment sizes from Figure 1 from LARGEST to SMALLEST and record them in Table 1.

2. Measure the distance from the well to the center of the band for each restriction fragment in the HindIII digest. Record these results in Table 2.

3. In Excel, put the distance traveled in mm in one column and the corresponding size in another.

4. In the cell to the right of your largest band, type =log10( ) where the parentheses are the location of the size. Drag the corner of the box down to the bottom data point and it will fill in the logs for each band.



5. Highlight the column for distance traveled and the column with the log of the DNA size and insert the *first option* under scatter plot. The data points should NOT be connected.

6. Select your chart and click the + symbol on the right. Check the box next to "Show Trendline."

7. Right-click on the trendline and select "Format Trendline."

8. Click the box next to “Display equation on chart.” Record the equation here:

You will now use the standard curve generated in Activity 4 to determine the contents of the other three tubes. EcoRI cuts at the site 5’ – GAATTC – 3’ resulting in the following fragments: 1 – 21226; 2 – 4878; 3 – 5643; 4 – 7421; 5 – 5804; 6 – 3530. BamHI cuts at the site 5’ – GGATCC – 3’ resulting in the following fragments: 1 – 5505; 2 – 16841; 3 – 5626; 4 – 6527; 5 – 7233; 6 – 6770

Activity 5: Identification of unknowns.

1. Measure the distance traveled for each band in your unknowns and record in Table 2.

2. Use the slope of your standard curve to calculate the band sizes and record in Table 2.

3. Rank the band sizes from provided for EcoRI and BamHI in Table 3.

3. Compare your calculated band sizes to the provided band sizes to determine the contents of each tube.

Worksheet: Restriction Digest of λ DNA Week 1

Introduction:

1. What are restriction enzymes? Be specific regarding function and source.

Activity 1:

2. Why is it necessary to incubate the restriction digests at 37°C?

3. Why was it necessary to incubate the restriction digests at 65°C?

Activity 2:

4. Why did you add water to the gel before practicing loading?

Activity 3:

5. Why did you place the gel in the chamber with the wells closest to the black electrode?

6. What is the role of loading dye in this experiment? SYBR™ gold?

7. What is the purpose of gel electrophoresis in this experiment?

Data Summary: Restriction Digest of λ DNA Week 2

Table 1: _____

Band	1	2	3	4	5	6	7	8
Size								
Distance (mm)								

Table 2: _____

Band	Pink Tube		Yellow Tube		Blue Tube	
	Distance	Size	Distance	Size	Distance	Size
1						
2						
3						
4						
5						
6						

Table 3: _____

Tube	Green	Pink	Yellow	Blue
Contents				

*This lab activity was adapted from Dr. Sheryl Shanholtzer's and Virginia Michelich's adaptation of Carolina Biologicals

Investigating Chromosome 16 PV92 locus for *Alu* Insertion Polymorphism

Learning Goals

In this lab, students will:

- Describe the steps in a polymerase chain reaction (PCR)
- Explain what chemicals are needed in PCR and their roles, in addition to the template DNA
- Isolate, accurately pipette, and successfully amplify DNA fragments, followed by separating them by electrophoresis
- Use PCR to amplify a short nucleotide sequence from human chromosome 16
- Create a DNA fingerprint that shows the presence (+) or absence (-) of the *Alu* transposable element on the PV92 locus of chromosome 16

Introduction

Alu are short sequences of non-coding DNA (approximately 300 bp in length). The name "Alu" comes from the ability of these sequences to be cut using a restriction enzyme called *Alu1*. *Alu* elements are classified as SINES (short interspersed elements) and are thought to be derived from a protein export signaling gene. The human genome contains up to 1,000,000 *Alu* copies (10% of our genome).

Alu elements are an example of jumping genes (transposons). Transposable elements are pieces of genomic DNA that can copy themselves and be inserted into new areas of a chromosome. *Alu* elements are defective transposons because they lack an enzyme that enables them to copy their DNA. However, *Alu* elements can transpose if supplied with the proper enzyme from other transposons. Because several *Alu* element insertions are stable over time, they can be used for phylogenetic mapping.

In this lab, you will examine PV92 (a non-coding region) on Chromosome 16 for the presence of *Alu* insertion in your genome. Two alleles encode PV92, and these alleles are inherited via Mendelian genetics. The possible PV92 genotypes for the *Alu* insertion are + +, + -, or - - . These alleles can be separated using gel electrophoresis (see Figure 1 below).

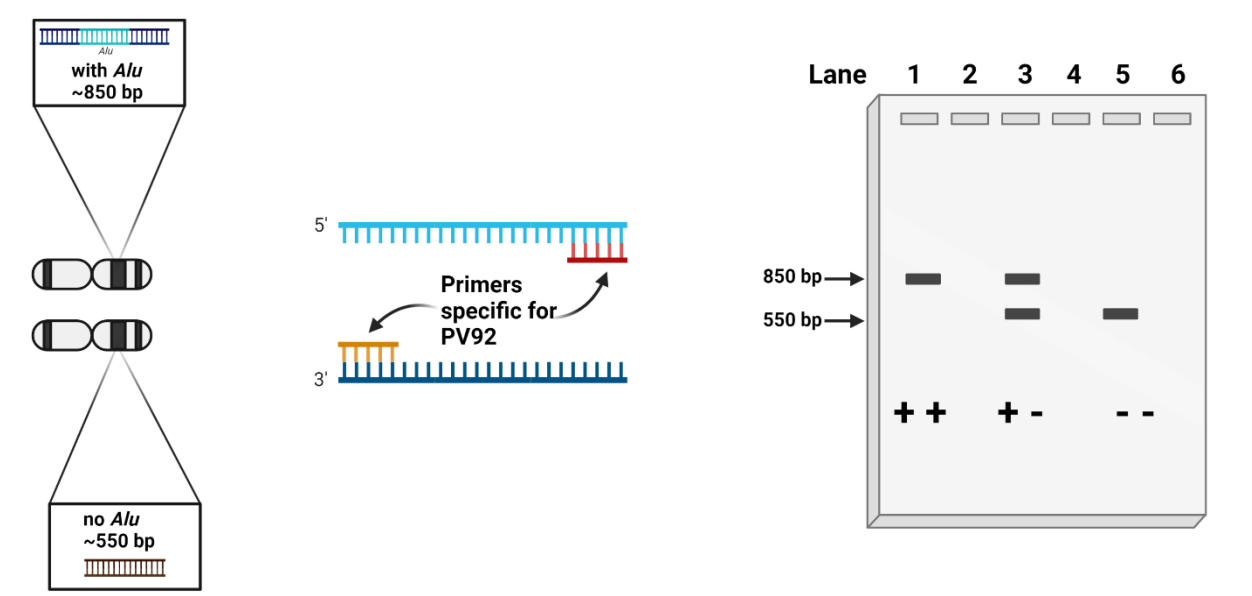


Figure 1: Illustration of a homologous pair of Chromosome 16, PCR primers, and possible results. The top chromosome contains the *Alu* insertion, while the bottom chromosome does not. Forward and reverse primers allow for PCR amplification of the PV92 locus. Following PCR and gel electrophoresis, + and – alleles can be visualized. The + allele is approximately 850 bp, and the – allele is approximately 550 bp in length. Genotypes (+ + in Lane 1, + - in Lane 3, and - - in Lane 5) can be visualized based on allelic size (see above).

NOTE:

This experiment will take place over two weeks. In week 1 we will isolate cheek cell DNA and amplify *Alu* fragments by PCR. In week 2, we will separate DNA fragments by gel electrophoresis.

Lab Procedure

In Activity 1, you will isolate DNA. The source of this template DNA is a sample of several thousand squamous cells obtained from your cheek. Cheek cells will be obtained using a saline mouthwash, concentrating them by centrifugation, and resuspending them in Chelex beads.

Here is a video on how to isolate cheeks cells for use in PCR:

Activity 1 (Week 1): Isolate Cheek Cell DNA

Gather your supplies and read through all activity steps before you begin.

Per Class: clinical centrifuge, dry-block incubator (set to 100 °C), ice bath, microfuge

Per group - set of micropipettes, micropipette tips, plastic container for tip disposal, permanent marker

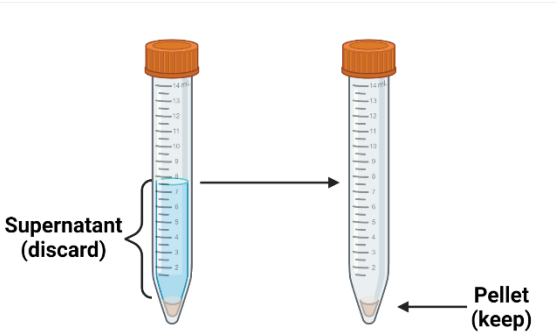
Per student - 1.5 mL centrifuge tube (2), 10 mL saline solution (0.85% NaCl), paper cup, locking-top centrifuge tube containing Chelex beads, disposable pipet

---

1. Place your initials on top of a 1.5 mL centrifuge tube
2. Place your initials on the 15 mL tube of saline solution (on the lid and side of the tube)
3. Pour the saline solution into your mouth and swish vigorously for 30 seconds
4. Spit saline solution into a paper cup
5. Carefully pour saline solution back into a 15 mL tube

   ⚠ NOTE: save the paper cup
6. Place your 15 mL tube into the clinical centrifuge with all your classmates' tubes
7. Spin tubes at full speed (with the brake on) for 10 min
8. Carefully pour off supernatant into a paper cup.

   ⚠ NOTE: you are keeping the cell pellet in the bottom of your 15 mL tube.



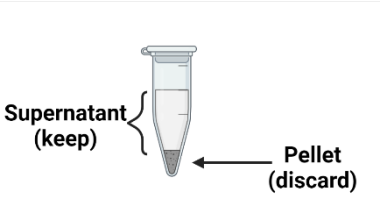
9. Use a sterile, disposable pipet to mix and resuspend Chelex beads in their 1.5 mL tube by pipetting several times.

   ⚠ NOTE: Make sure that Chelex beads do not resettle to the bottom of the 1.5 mL tube before next step.

   ⓘ Chelex beads bind metal ions that are released from cells. These metal ions can inhibit the PCR reaction, so using these beads ensures that are DNA sample is free from contaminants.
10. Transfer all resuspended Chelex beads to the 15 mL tube containing your cheek cell pellet.
11. Resuspend cheek cells fully in Chelex beads.
12. Transfer all cheek cell/Chelex bead suspension back to the 1.5 mL tube
13. Close 1.5 mL tube and lock lid by sliding the locking ball into the space in the lid
14. Incubate 1.5 mL tube in dry-block for 10 min
15. Place tube on ice for 3 -4 min

16. Pulse spin tube in a microfuge at full speed for 30 sec to pellet all debris

17. Transfer 200 µL of clear supernatant to a clean, labeled 1.5 mL tube



⚠ NOTE: This tube contains your DNA for Activity 2.

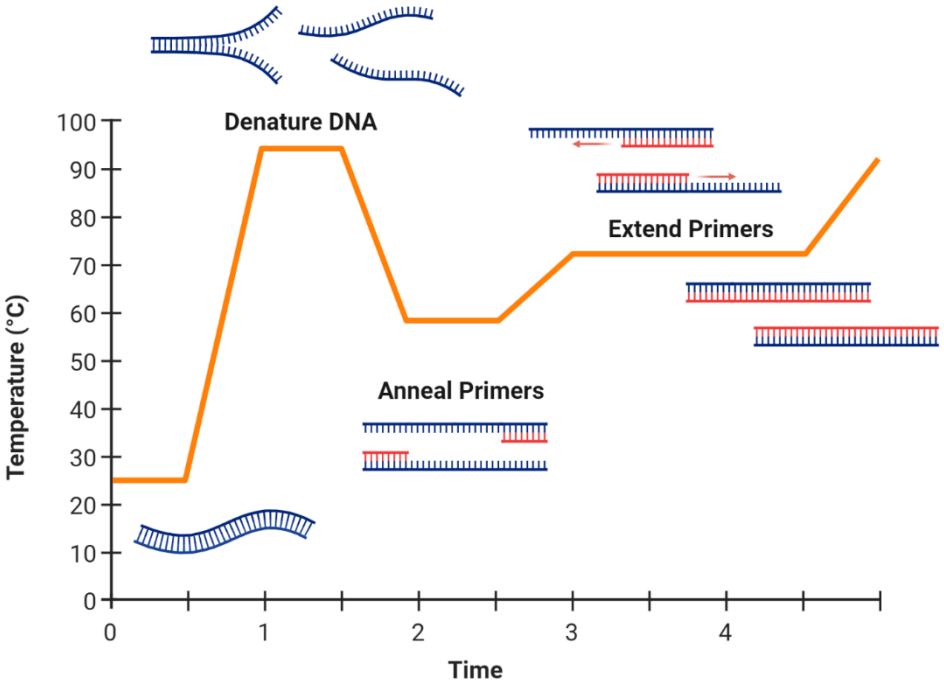
Clean-Up:

- ☐ Pour supernatant down the sink
- ☐ Throw away paper cup
- ☐ Dispose of 15 mL conical tube, pipette tips, 1.5 mL tube that contains cellular debris (step 16 tube)

In Activity 2, the clear supernatant obtained in Activity 1, which contains your chromosomal DNA, will be subjected to polymerase chain reaction (PCR). PCR is like a molecular copy machine, where a specific area of DNA is copied millions of times. This process uses repeated heating and cooling cycles to make these copies (see timing and temperatures below).

Your chromosomal DNA will be combined with all the necessary ingredients to make targeted copies: a buffered solution of heat-stable *Taq* polymerase, the four deoxynucleotide (dNTP) building blocks of DNA, oligonucleotide primers (for PV92 locus), and the cofactor magnesium chloride ($MgCl_2$) in a master mix.

This mixture will be placed into a DNA thermal cycler and taken through 30 cycles of:



- 30 sec @ 94 °C to denature the chromosomal DNA into single strands
- 30 sec @ 58 °C to allow for primers to bind to their complementary sequences on either side of the PV92 locus
- 30 sec @ 72 °C to allow *Taq* polymerase to extend the strand of DNA that begins with each primer

Primers used in Activity 2 bracket the PV92 locus on chromosome 16 and result in its selective amplification (copying). Taq polymerase only recognizes and binds to DNA with bound primer. Remember PV92 only has two alleles that indicate the presence (+) or absence (-) of the *Alu* transposable element at this location.

Here is the video link for Polymerase Chain Reaction:

Here is a video link for PCR amplification of cheek cell DNA:

Activity 2 (Week 1): Amplification of *Alu* Fragment by PCR

Gather your supplies and read through all activity steps before you begin.

Per Class: PV92 master mix (supplied by instructor), microfuge, ice bath, thermal cycler

Per group - set of micropipettes, micropipette tips, plastic container for tip disposal, permanent marker

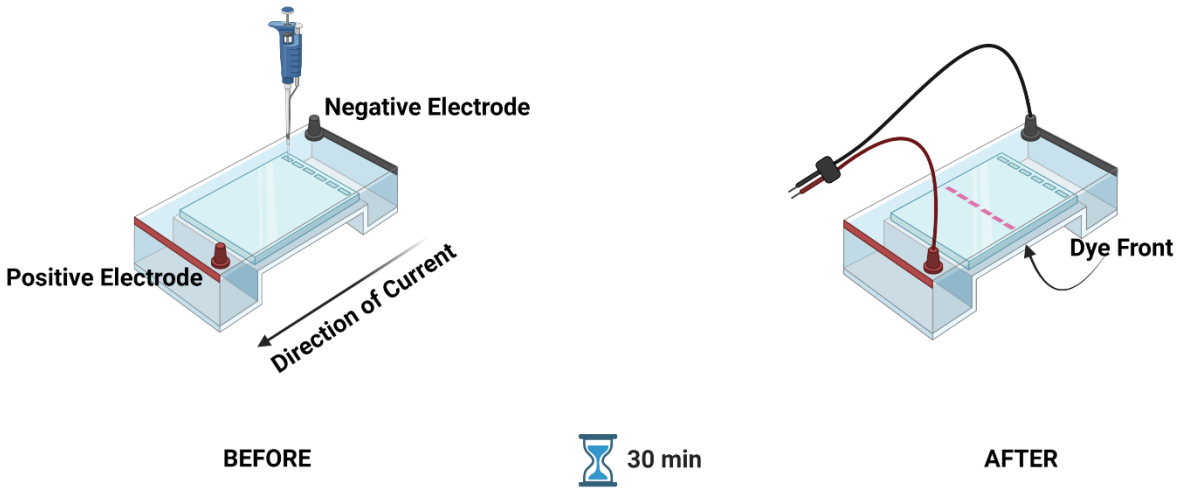
Per student – 200 µL of your chromosomal DNA, 0.5 mL PCR tube

1. Label your 0.5 mL PCR tube with your initials
2. Transfer 22.5 µL of PV92 master mix into your 0.5 mL tube
3. Add 2.5 µL of your DNA to the 0.5 mL tube
4. Close lid and flick tubes several times to mix
5. Pulse centrifuge 0.5 mL PCR to collect all contents at the bottom of the tube
6. Place 0.5 mL PCR tube into the thermal cycler
7. Instructor will start the thermal cycler once all tubes are placed inside. After the 30 cycles, tubes will be removed and stored in the freezer until next week.

Clean-Up:

- Dispose of the pipette tips into the appropriate waste bin
- Give the instructor your 1.5 mL tube that contains your chromosomal DNA. It will be frozen until next week.

In Activity 3, you will separate the amplified Alu fragments of DNA using gel electrophoresis. Gel electrophoresis uses an electrical current to pull negatively charged DNA through a semi-solid agarose gel toward a positively charged pole. DNA fragments migrate through the gel based on size and charge. The agarose gel acts like a colander/sieve such that small fragments move through the gel faster than larger fragments.



In this activity, you will load your amplified DNA products into a 1.5% agarose gel, along with a DNA ladder (size marker) and all products will be electrophoresed. In order to visualize your DNA (and the ladder), a dye must be added. Following the completion of gel electrophoresis, your DNA will be visualized and your Alu genotype will be determined.

Here is a video link that overviews gel electrophoresis of DNA fragments:

Activity 3 (Week 3): Separation of DNA fragments by gel electrophoresis

Gather your supplies and read through all steps of the activity before you begin.

Per Class: Gel rig electrical supply

Per group - set of micropipettes, micropipette tips, plastic container for tip disposal, permanent marker, masking tape, agarose gel casting tray, gel comb, 1.5% agarose solution (in 65 °C hot bath), electrophoresis gel rig, TBE, Sybr-Gold, DNA ladder

Per student – 0.5 mL PCR tube of amplified products

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1. Seal ends of casting tray with masking tape
2. Pour a tube (AMOUNT?) of 1.5% agarose into the sealed casting tray
3. Insert well comb into notches closest to the end of the casting tray
4. Pop and/or move any large bubbles that are close to well comb with a pipette tip
5. Allow gel to solidify for 10 min

⚠ NOTE: Do NOT bump or move casting tray while agarose is solidifying

6. Once gel has solidified, remove masking tape and gel comb
7. Place casting tray into gel rig
8. Fill gel rig to indicated “Max Fill” line with TBE

⚠ NOTE: The TBE will fill both side of the gel rig and will completely submerge the gel

9. Obtain you PCR tube from last week

ⓘ NOTE: The PCR tube contains your amplified chromosomal DNA for the PV92 locus

10. Add 2.5 µL of Sybr-Gold to your sample

11. Flick the tube to mix
12. Place your tube into a microfuge and pulse to collect all contents at bottom of the tube
13. Load 25 µL of your stained chromosomal DNA to one of the wells of your gel
14. Load 20 µL of DNA ladder to one lane of your gel
15. Run gel at 120 V for 25 mins
16. Remove gel with casting tray from gel rig
17. Bring your gel to your instructor at the UV transilluminator
18. Your instructor will help you to examine your gel as well as take a picture of your gel

⚠ NOTE: Do NOT look at the UV light without the shield in place.

Clean-Up:

- Dispose of your gel into the designated waste bin
- Dispose of your pipette tips, tubes
- Empty your gel rig of the TBE by pouring liquid down the sink and returning it to the supply counter

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Review Questions

What are *Alu* elements?

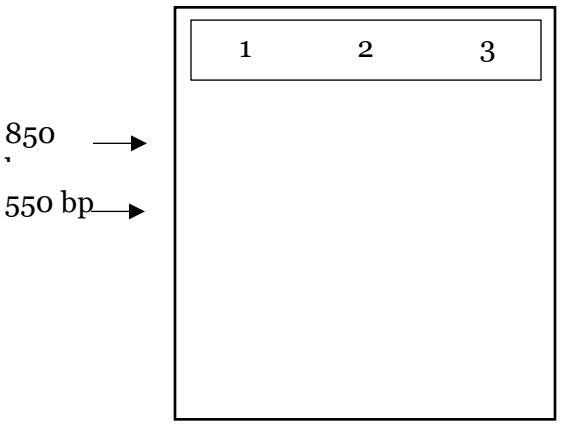
How are *Alu* elements used to trace human history?

What is PCR? What did we use it for?

What locus are we studying?

Describe what the following results mean AND sketch what they would look like (on the "gel"):

1. Double positive
2. Heterozygous
3. Double negative



Activity 1

1. Briefly describe the purpose of each step below:

Salt-water Swish

Chelex beads

Centrifugation

Heat incubation

Activity 2

1. Briefly describe the role of each component of the PCR Master Mix:

Taq polymerase

dNTPs

MgCl2

Primer

2. What is happening at each step of PCR?

94C

58C

72C

Post-lab

1. Which students in your group were homozygous at the pv92 locus and did nothave the *Alu* insertion?

2. Which students in your group were homozygous at the pv92 locus and have the *Alu* insertion?

3. Which students in your group were heterozygous at the pv92 locus and have only one *Alu* insertion?

4. Which samples had the most product from the PCR?

5. To which pole does the DNA move when current is applied?

6. Name the enzyme that catalyzes the addition of nucleotides to a single strand ofDNA:

7. During each cycle of replication, the number of strands increase by what amount?

8. What exactly is the role of the thermal cycler in the PCR process?

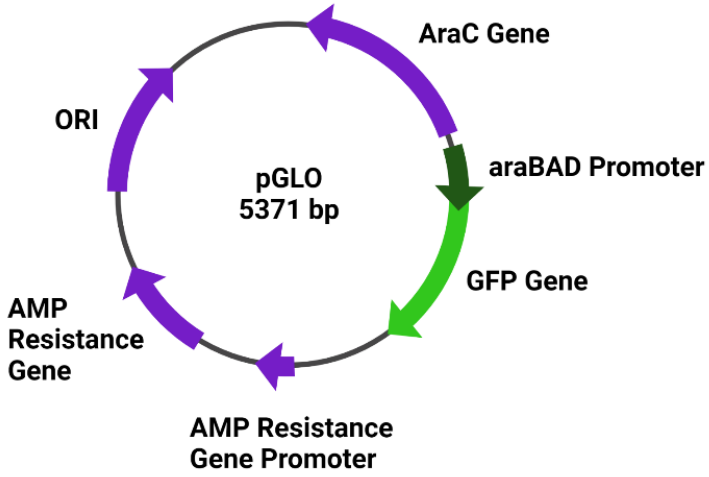
---

*This lab activity was adapted from Virginia Michelich's and Mike Stone's adaptation of Carolina Biologicals

pGLO Transformation

Genetic transformation, a process that involves the introduction and expression of foreign genes in a host organism. Genetic transformation is used in many areas of biotechnology. In agriculture, genes are introduced into crops to prevent spoiling or induce resistance to drought, among other things. Environmental scientists transform bacteria with genes enabling them to digest oil spills. There are also medical applications to this process. Some human diseases are treated using gene therapy, though the technology is still evolving.

In order to move genes from one organism to another, a vector is required. For bacterial transformations, ***plasmids*** are used as vectors. Plasmids are small circular pieces of DNA found in bacteria. Plasmid DNA usually contains genes for one or more traits that may be beneficial, such as antibiotic resistance. In nature, bacteria can transfer plasmids back and forth, enabling them to adapt to new environments. In this lab, you will transform *E. coli* with a gene that codes for Green Fluorescent Protein (GFP). The source of this gene is the bioluminescent jellyfish *Aequorea Victoria* (GFP causes this jellyfish glow in the dark). Following your transformation procedure, bacteria expressing the jellyfish gene will produce GFP, causing them to glow a brilliant green color under ultraviolet light.



The plasmid you will use, the ***pGLO plasmid***, has been genetically engineered to contain a gene for GFP, the ***bla gene*** which confers resistance to the antibiotic ***ampicillin***, and the ***araC gene***, a component of the ***arabinose*** operon. This special regulatory system allows us to control expression of GFP as follows: when the sugar arabinose is present, the ***araC*** gene product promotes the expression of GFP. If arabinose is absent from the growth media, the araC gene is not transcribed, so GFP is not expressed. Thus, the gene for GFP can be "switched on/off" in transformed cells by adding arabinose to the cells' nutrient medium.

Selection for cells that have been successfully transformed is accomplished by growth on ***ampillicin*** plates. Transformed cells will appear white (wild-type phenotype), whereas non-transformed cells should not grow. When cells are grown in nutrient medium containing ***arabinose*** as well, GFP expression is stimulated and the bacteria will glow green under UV light.

<u>Before beginning, design an experiment to test a transformation of *E.coli* with the pGLO plasmid.</u>

Gather your supplies and read through all steps of the activity before you begin.

Materials: *E. coli* starter plate (LB agar) ***bacteria must be actively growing**, transformation solution ($CaCl_2$, pH 6.1), rehydrated pGLO plasmid, poured agar plates (1 LB, 2 LB/amp, 1 LB/amp/ara), LB nutrient broth, foam microcentrifuge tube holder/float, container full of crushed ice (**not cubed**), 42°C water bath/thermometer, 37°C incubator, 2–20 µl adjustable volume micropipets/tips, microcentrifuge tubes, inoculation loops, sterile transfer pipets, UV Light

Transformation

The goal of genetic transformation is to change an organism's traits, also known as their phenotype. Before any change in the phenotype of an organism can be detected, a thorough examination of its natural (pre-transformation) phenotype must be made.

1. **Look at the colonies of *E. coli* on your starter plates**. List all observable traits or characteristics that can be described:

2. Label one microcentrifuge tube +pGLO and another -pGLO. **Include your initials/name.**

3. Label 4 LB plates/plate areas ON THE BOTTOM as follows (**include your initials/name)**:

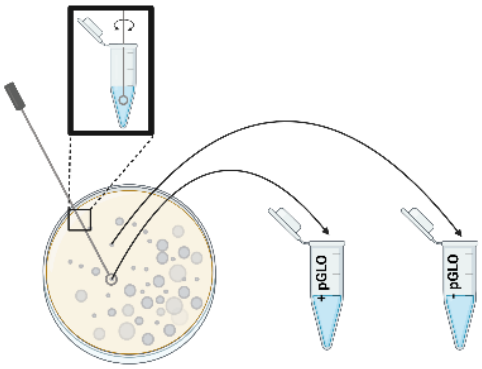
LB plate:	**- pGLO**
LB/amp plate:	**- pGLO**
LB/amp plate:	**+ pGLO**
LB/amp/ara plate:	**+ pGLO**

4. Using a **sterile transfer pipet**, add 250µl of transformation solution ($CaCl_2$) to each tube.

5. Place the tubes on ice.

6. Use a **sterile loop** to pick up 2–4 large colonies of bacteria from your starter plate. Select starter colonies that are "fat" (ie: 1–2 mm in diameter). It is important to take individual colonies (not a swab of bacteria from

the dense portion of the plate), since the bacteria must be actively growing to achieve high transformation efficiency. Choose only bacterial colonies that are uniformly circular with smooth edges.



7. Pick up the +pGLO tube and immerse the loop into the transformation solution at the bottom of the tube. Spin the loop between your index finger and thumb until the entire colony is dispersed in the transformation solution (with no floating chunks). Place the tube back in the tube rack in the ice. Using a new sterile loop, repeat for the -pGLO tube. Close both tubes. ***Examine the pGLO DNA solution with the UV lamp and note your observations.**

8. Add pGLO plasmid to the +pGLO tube:

a. Immerse a new sterile loop into the pGLO plasmid DNA stock tube. Withdraw a loopful (there should be a film of plasmid solution across the ring). Mix the loopful into the cell suspension of the +pGLO tube.
b. Optionally, pipet 10 µl of pGLO plasmid into the +pGLO tube & mix, then return to ice.

9. Incubate the tubes on ice for **10 min.** Make sure the tubes are all the way down and make contact with the ice.

- Why did you place the tubes on ice?
- Why do you need the $CaCl_2$ transformation solution?

After 10 minutes, you will perform a **heat shock.**

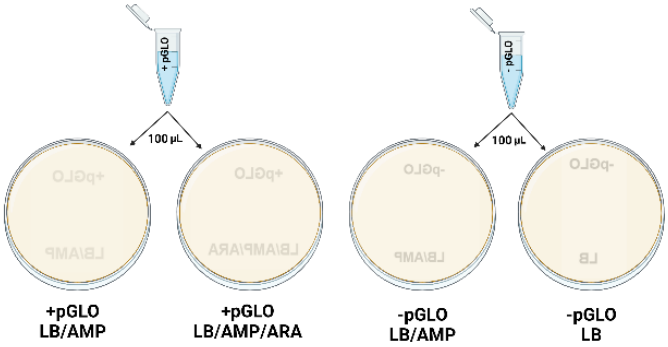
10. Using the foam rack as a holder, **quickly** transfer both the (+) pGLO and (-) pGLO tubes into the water bath, set at 42°C, for **exactly 50 sec**. Make sure to push the tubes all the way down so the bottom makes contact with the warm water. Check the temperature of the water bath with two thermometers to ensure accuracy.

11. After 50 seconds, **immediately** place both tubes back on ice.

12. Incubate tubes on ice for **2 min**.

13. Remove the tubes from the ice. Using a new **STERILE** pipet for **EACH TUBE**, add 250 µl of LB nutrient broth to the tubes.

14. Incubate the tubes for **10 min** at room temperature.



15. Gently flick the closed tubes with your finger to mix and resuspend the bacteria. Using a new sterile pipet for each tube, add 100 µl of the transformation (+pGLO) or control (-pGLO) suspensions to the appropriate agar plates as follows:

16. Use a new sterile loop for each plate. Spread the suspensions evenly around the surface of the LB nutrient agar by quickly skating the flat surface of a new sterile loop back and forth across the plate surface. DO NOT PRESS TOO DEEP INTO THE AGAR. Uncover one plate at a time and re-cover immediately after spreading the suspension of cells. This will minimize contamination.

17. Stack up your plates and tape them together. Put your group name and class period on the bottom of the stack and place the stack of plates upside down in the 37°C incubator until the next day (plates can be stored at RT or at 4°C if longer incubation period required).

- Why should you stack the plates upside down?

- On which of the plates do you expect to find bacteria most like the starter *E. coli* colonies you initially observed? Explain.

- If there are any genetically transformed bacterial cells, on which plate(s) would they most likely be located? Explain.

- What controls did you use in this experiment?

Analysis of results

Observe your plates under normal room lighting. Then turn out the lights and hold the ultraviolet light over the plates.

1. Carefully observe and draw what you see on each of the four plates.

2. Do you observe *E. coli* growing on the LB plate that does not contain ampicillin or arabinose? If so, explain.

3. From your results, can you tell if bacteria are ampicillin resistant by looking at them? Explain.

4. From the results that you obtained, how could you *prove* that the changes that occurred were due to the procedure that you performed?

5. An organism's traits are often caused by a combination of its genes and its environment. With regard to the green color observed in the genetically transformed bacteria: **Explain how environmental factors cause the genetically transformed bacteria to turn green.**

**This exercise was adapted from BIORAD kit #1660033EDU www.bio-rad.com*

Genetic Control of Serratia marcescens

Learning Goals

In this lab, students will:

- Define enzymes in terms of metabolic pathways: substrate, intermediate, end product
- Explain why end products in a metabolic pathway are not produced when an enzyme is dysfunctional
- Explain what potential products (if any) of a metabolic pathway will be made should an enzyme in that pathway be dysfunctional
- Differentiate between prototropic and auxotropic bacteria
- Explain the set-up for pair-wise feeding of auxotropic strains of *Serratia marcescens*
- Decipher the metabolic pathway of each of 3 auxotrophic strains of *Serratia marcescens* by observing result from pairwise feedings

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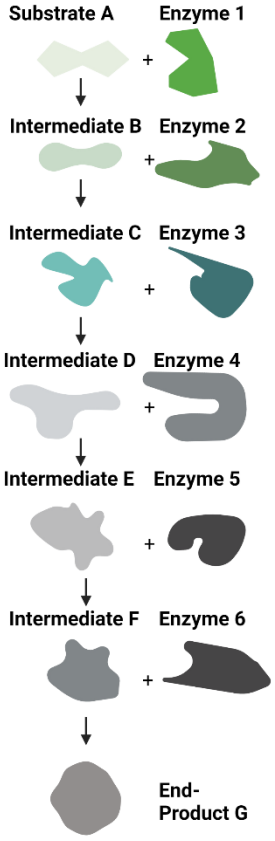
Introduction



Enzymes and metabolic pathways

Enzymes are proteins that function to speed up biochemical reactions (AKA: they are **catalysts**) within an organism that might not otherwise occur. The starting material that enzymes interact with are called **substrates**. Each biochemical reaction in the body requires a unique and specific enzyme, therefore each enzyme reacts with only one substrate. The resulting material that results from an enzymatic reaction is called the **end-product**.

When several enzymatic reactions occur to produce a single end-product, we refer to this as a **metabolic pathway**. For the end-product to be formed in a metabolic pathway, ALL enzymes in that pathway must be present and functional.

In this metabolic pathway, "A" is the substrate and "G" is the end-product. "B-F" are called **intermediates**, products that are made in the metabolic pathway that can react with a new enzyme, but they are not the end-product of the pathway. For "G" to be made, **ALL** enzymes must be **present AND functional**.

So, where do enzymes come from? Remember, enzymes are proteins that are encoded in the DNA. If the gene for the enzyme is non-functional, then that enzyme will not be made. Looking at the above metabolic pathway, if the gene that encodes Enzyme 3 is **non-functional**, then E3 will **not** be made. This means that the pathway will **stop** after Enzyme 2 (E2) acts on intermediate "B" to produce intermediate "C". Intermediate "C" will accumulate, but the pathway will not move any further. No end-product will be made. **NOTE**: if TOO much "C" accumulates within the cytoplasm of the cell (or a bacterium), it will be pumped out into the external environment.

But wait, there is more…. If all other enzymes in this pathway are present AND functional, it is possible to get end-product. How? Well, the cell (or bacterium in our case) can be "**FED**" an intermediate. To be "**FED**" means that a cell (or a bacterium in our case) picks up an intermediate from the external environment. If there is a concentration gradient, then it is possible for these intermediates to move into the cell from high concentration (outside cell) to low concentration (inside cell). Looking at the above metabolic pathway, if a cell (or a bacterium in our case) is "FED" intermediate "D" from the environment, then the rest of the intact (and functional) metabolic pathway will take intermediate "D" to end-product "G". **Experimental note**: We will be demonstrating this phenomenon with *Serratia marcescens*.

Serratia marcescens (S. marcescens)

In this lab, we will study how genes, which produce enzymes, control a specific biochemical pathway in bacteria called *Serratia marcescens*. This pathway leads to the production of a red pigment called **prodigioson**. We can see if this pigment is made by just observing the phenotype (the appearance) of *Serratia*.

S. marcescens is a rod-shaped, gram-negative bacteria that is found everywhere (water, soil, plants, humans). It is commonly found in the bathroom; that red/brown ring around your toilet bowl water line and around the drain of your bathtub or shower is thanks to *S. marcescens* and its **prodigioson** pathway. *S. marcescens* produces the **prodigioson** pigment when it is incubated at room temperature.

Prototrophic and Auxotrophic Bacteria

In this lab, we will study 3 strains of *S. marcescens* bacteria that are blocked in different parts of their **prodigioson** pathway due to a non-functional gene (which means the enzymes are not made). Strains of *S. marcescens* that lack functional genes (and thus enzymes) are not able to produce **prodigioson** are called **auxotrophs**. An auxotrophic organism is one that contains a mutation in one of the alleles that codes for an essential enzyme necessary for the end-product of the biochemical pathway.

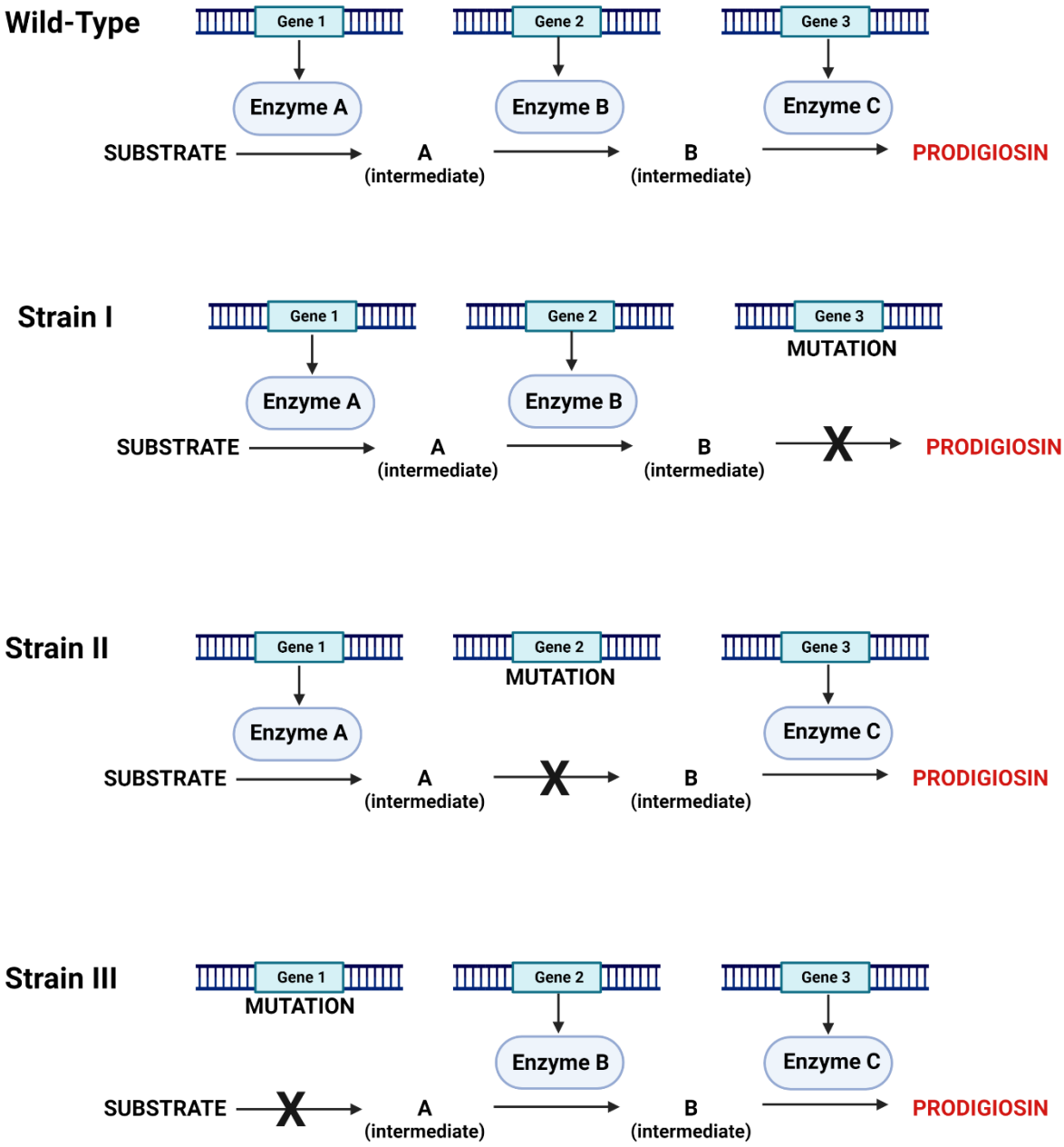
The WILDTYPE strain of *S. marcescens* has all genes (and thus enzymes) functional, so it is able to produce the **RED** pigment and is referred to as a **prototroph**. A prototrophic organism is one that is to produce the end-product of a biochemical pathway.

The 3 strains of *S. marcescens* that we will examine in this lab activity are auxotrophic strains. These auxotrophic strains are able to produce colored intermediates, but they are unable to produce the wildtype RED end product. We will set them up in feeding pairs to see if on strain can donate intermediates to another strain so that it can complete the pathway and produce prodigioson.

Lab Procedure

Activity 1 (Week 1): Examining metabolic pathways

In this activity, we will examine metabolic pathways and identify what genes are non-functional and thus which enzymes are non-functional in each pathway. We will examine what (if any) intermediate can be made. **NOTE:** Because these gene mutations do not provide the feedback loop necessary to turn off a response, intermediates accumulate. Because intermediates (if any) are being made in excess and they will be secreted by the bacteria into the external environment.



This figure shows schematics of the wildtype (prototrophic) and 3 non-functional (auxotrophic) **prodigioson** biochemical pathways. Examine each of these schematics and then watch the video about this figure. You should be able to answer questions under Activity 1 in the QUESTIONS portion of this lab exercise:

Activity 2 (Week 1): Set up of pairwise feedings

Now that we understand how auxotrophic pathways function, we will now set up feeding pairs to see if we can get any of these *S. marcescens* auxotrophic strains to complete the biochemical pathway and produce **prodigioson.** It is our hope that one of the auxotrophs in the feeding pair set-up will produce an intermediate in excess that can be picked up by the other auxotroph of the petri dish such that it can complete the pathway and make **prodigioson.**

Gather your supplies and read through all steps of the activity before you begin.

Per Class: Incubator (set to 25 °C)

Per group – 95% Ethanol (or other disinfectant), 2 Sharpie markers, striker, Bunsen burner, 2 loops, 6 petri dishes containing peptone-glycerol agar, auxotrophic strains of *S. marcescens* (933, X1120, and C11), wildtype strain of *S. marcescens*

Per student – Students will work as pairs to set up activity. Each group will require: 1 Sharpie, 1 loop, 3 petri dishes. Groups will share disinfectant, strains of bacteria, and Bunsen burner

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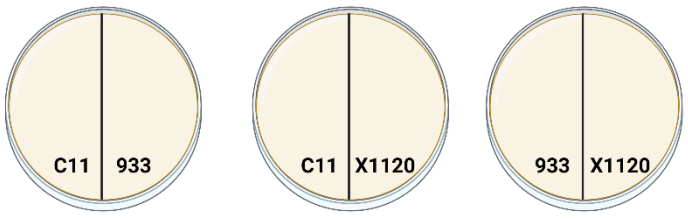
1. Disinfect workbench by spraying it down with disinfectant and wiping it down with paper towel

   ⚠ **NOTE:** Do NOT ignite Bunsen burner until disinfectant has completed dried on benchtop.

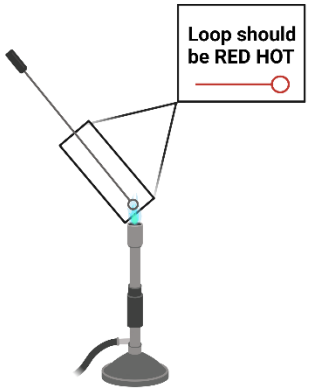
2. While waiting for disinfectant to dry, the label petri dishes on the bottom of plastic (not on lid side or on the actual agar!) as shown below for the following feeding pairs:



3. Connect Bunsen burner tubing to gas line on benchtop. Turn on gas.

4. Light Bunsen burner using striker

5. Sterilize loop by flaming and allow it to cool for approximately 30 sec



⚠ NOTE: Do NOT put the now sterile loop down on the benchtop.

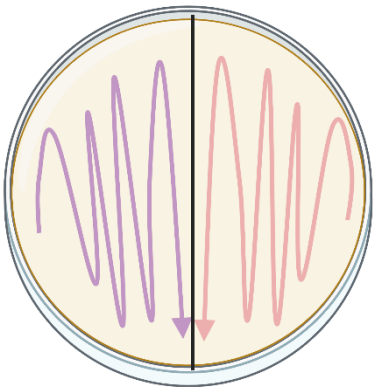
6. Open lid of auxotrophic bacteria strain that you will utilize first.

⚠ NOTE: Be sure to open lid under the "umbrella of sterility" of the Bunsen burner, otherwise there is a risk for contamination.

7. Using the sterile loop, gently scrape the auxotropic bacteria to obtain some on your loop.

⚠ NOTE: Be careful to not be too rough or you will break the agar that the bacteria is growing on. ALSO, you don't need much bacteria on your loop; meaning you DON'T need to see it on your loop to have enough!

8. Open lid of feeding pair petri dish close to flame (see Step 5 NOTE)



9. Transfer the auxotrophic bacteria to its appropriately labeled area on the feeding pair petri dish by making a "V" pattern as indicated in the figure to the right:

⚠ NOTE: Be sure to get the "V" as close to the line that is drawn in the middle of the plate as possible, but do NOT cross the line.

10. Replace lid on feeding pair petri dish
11. Sterilize the loop by flaming
12. Repeat steps 4-10 until all auxotrophic bacteria has been plated for the pairwise feeding experiment.
13. Turn all plates upside down (lid side down) and tape them together for your table. Label the tape with your table number
14. Place plates in incubator at 25 °C.
15. Answer questions under Activity 2 in the Questions portion of the laboratory exercise

Clean-Up:

1. Turn off gas and disconnect Bunsen burner. Return Bunsen burner to the group bin.
2. Return Sharpie markers, all stock plates of bacteria, loop, and striker to the group bin.

3. Discard any materials that have come in contact with bacteria (gloves, etc) to the designated autoclave bin (ask your instructor)

Activity 3 (Week 2): Read plates

This week we will determine which of the auxotrophic bacteria are able to complete the biochemical pathway and produce **prodigioson** through observation.

Gather your supplies and read through all steps of the activity before you begin.

Per Class:

Per group – Pairwise feeding plates set up the previous week, basket of stock plates (i.e.: wild-type *S. marcescens*, 3 auxotrophic strains of *S. marcescens*)

Per student – see "per group" materials

---

1. Retrieve your groups' plates
2. Examine your group's plates and compare to original stock cultures and wild-type *S. marcescens*
3. Answer the questions under Activity 3 in the Questions portion of the laboratory activity.

Clean-Up:

---

1. Return basket of stock plates to instructor
2. Discard gloves and pairwise feeding plates into designated autoclave bin

---

Lab Activity Questions:

Activity 1: Examining metabolic pathways

1. In regard to enzymatic pathways, what is an intermediate?

2. What is an auxotrophic bacteria?

3. For Strain 1:
    a. Which gene is non-functional?
    b. Can end-product be made?
    c. What CAN be made?

4. For Strain 2:
    a. Which gene is non-functional?
    b. Can end-product be made?
    c. What CAN be made?

5. For Strain 3:
    a. Which gene is non-functional?
    b. Can end-product be made?
    c. What CAN be made?

6. What is Strain 1 secreting?

    a. Strain 2?

    b. Strain 3?

Activity 2: Set up of pairwise feedings

7. Briefly describe how to set up a pairwise feeding experiment. Be specific and include ALL steps.

8. What was the purpose of the wild-type bacteria in this experiment?

9. What was the purpose of the reference plates in this experiment (stock plates used during set up and also in results basket)?

Activity 3 – Read plates

Complete the table below using results from the pairwise feeding experiment that your group set up:

Results Table:

Pairing	Strain	Color Before	Color After	Produced Prodigiosin?
C11 and 933	C11			
	933			
C11 and x1120	C11			
	x1120			
933 and x1120	933			
	x1120			

Use the results above and the diagram of the strains to determine the identity of each auxotroph (which strain are they?). Match the diagram to your data for C11, X1120, and 933 to find which strain these unknowns represent. Indicate below the identity of each strain:

- Strain I =
- Strain II =
- Strain III =

*This lab activity was adapted from Dr. Sheryl Shanholtzer's adaptation of an ABLE Workshop

Mapping Chromosomes with Crossing Over in *Sordaria*

Learning Goals

In this lab, students will

- Set up crosses of three strains of *Sordaria*
- Calculate the recombination frequency of each of the genes that control spore coat color
- Use recombination frequencies to construct a simple genetic map of the relative position of genes in relation to the centromere

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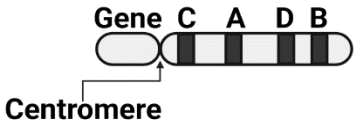
Introduction

Homologous chromosomes are the same size, have the same banding pattern when stained, and carry alleles for the same genes. In Meiosis I, crossing over adds genetic diversity by exchanging pieces of DNA between non-sister chromatids. Recombination frequency - how often genes are exchanged together - can be used to determine the location of a gene relative to the centromere. Recombination frequencies allow scientists to construct a genetic map where each percent represents one map unit. The maximum recombination frequency for any gene is 50% because if it is higher than that, they are being exchanged by chance. Let's look at a hypothetical example.

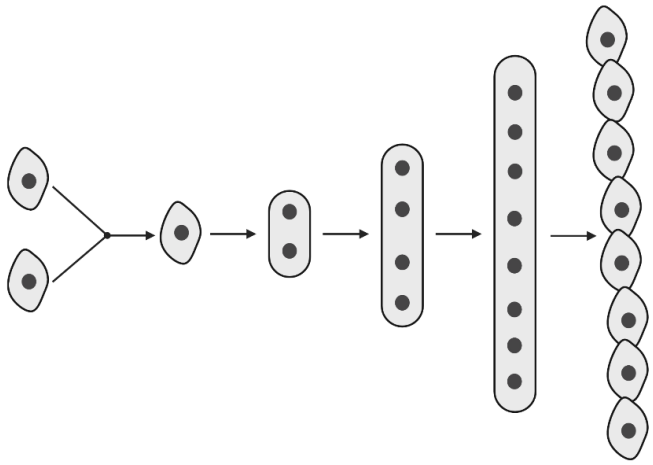
Gene	Recombination Frequency %	Genetic Map Positions are Relative
A	12.7	
B	37.9	
C	5.2	
D	21.4	

In this lab, we will be using three strains of the **haploid** fungus *Sordaria fimicola* to determine the relative locations of the two genes that control spore coat color. One gene controls production of the pigment melanin and the other controls deposition of the pigment in the cell wall (this is known as epistasis). The two genes controlling coat color each have two alleles, the wild type is indicated with a +. For a spore to be black, it must have the wild type allele for both genes. The table below shows the possible genotypes and phenotypes.

Genotype	$g^+ t^+$	$g^+ t$	$g\ t^+$	$g\ t$
Phenotype	black	gray	tan	colorless

Lab Procedure: Week 1

We will be setting up two cross-plates. One crossing black and tan and one crossing black and grey. The plates will be incubated at room temperature for a week. Over that time, the strains will sexually reproduce as shown in the figure to the right.



Two haploid spores will fuse to form a diploid zygote. The diploid zygote will undergo Meiosis I without cytokinesis resulting in a cell with two haploid nuclei. Meiosis II also occurs without cytokinesis, resulting in a cell with four haploid nuclei. Following Meiosis II, the haploid cell goes through mitosis and cytokinesis resulting in an asci with eight haploid spores.

Activity 1: Setting up the crosses

Gather your supplies and read through all steps of the activity before you begin.

Per group – two petri dishes with fungal growth agar, scalpel, 15 mL conical tube of ethanol, forceps, marker

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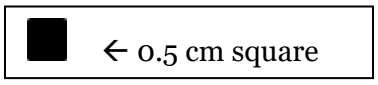
1. Using a marker, divide the dish into quadrants. Always label petri dishes on the bottom because the lid moves. Be sure to include your group # or some other way of identifying which samples are yours.

2. Label quadrants 1 and 3 **B** and the other two quadrants with **G** on one plate and **T** on the other.

3. Sterilize your scalpel by dipping the entire blade in the ethanol and laying it on a paper towel to dry.

4. To each plate, add a ~0.5 cm square of the appropriate strain with the agar facing up (fungus side touching the agar in the new plate). Gently press down on the squares to make sure they do not move. Sterilize scalpel between strains.

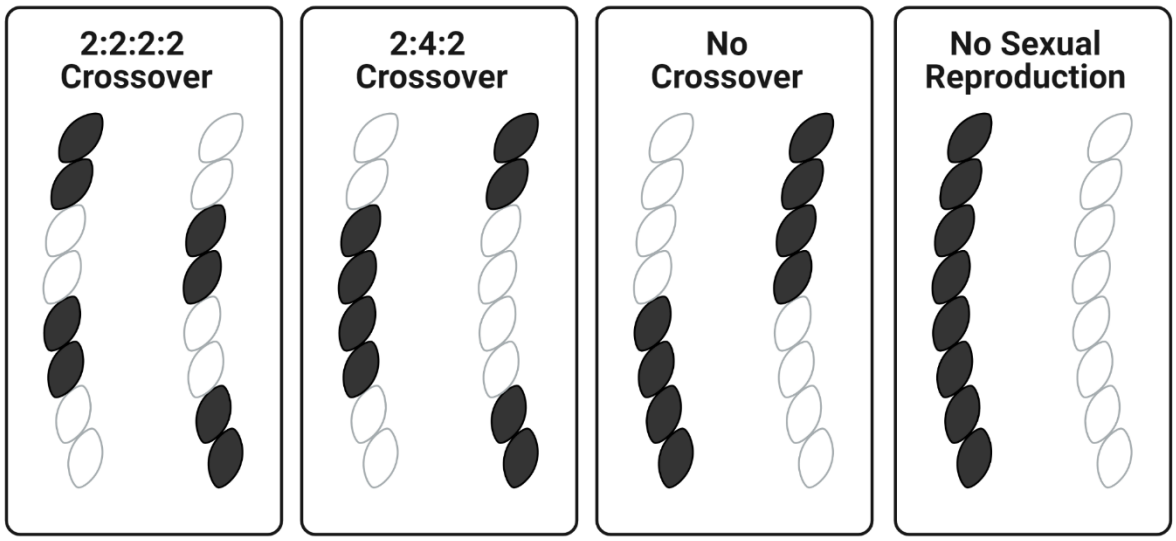


Clean-Up:

- ☐ Sterilize scalpel and return it to the bin
- ☐ Return stock plates to the supply bench

Answer question 4 before proceeding

In this activity, you will analyze practice data so that you are more comfortable when counting the result of the experiment next week. Crossover asci will either have a 2:2:2:2 or 2:4:2 color pattern. Non-crossover asci will have 4:4 patterns. Asci that are all the same color did not undergo sexual reproduction and are not counted. Because the eight spores in the asci are the result of mitosis, you must divide the recombination frequency by two to reflect that only half of the spores are produced in Meiosis II.



Activity 2: Practice Analysis

Gather your supplies and read through all steps of the activity before you begin.

Per student – card with illustration of ascospores, brain

Before you begin, discuss the matching question on the data summary with your group and make sure you know how you will analyze the practice data.

1. For each set of ascospores on your card, count how many asci have crossovers and how many do not. Do NOT mark on the cards with a sharpie.

2. Combine each group member's data and add it to Table 1. Gather data from other groups to complete the table.

3. Calculate the recombination frequency using this formula:

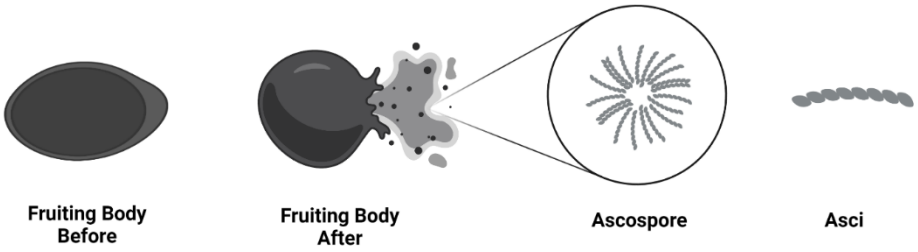
$$\text{Recombination Frequency} = \left[\left(\frac{\text{\# Crossover Asci}}{\text{\# Total Asci Counted}} \right) \div 2 \right] \times 100$$

Clean-Up:

- ☐ Make sure there are no marks on the simulated data cards
- ☐ Return cards to supply bin

Lab Procedure: Week 2

This week you will be collecting data to determine the recombination frequencies of the two genes controlling spore coat color. These numbers will allow you to make a simple genetic map of a chromosome found in *Sordaria fimicola*. Ascospores are inside the fruiting body of the fungus, you will need to rupture the fruiting body with enough pressure to release the ascospores but not so much pressure that you destroy the asci (which consists of eight spores). The fruiting bodies are the granular dark spots around the edge of the plate; the ones on the line between the strains are most likely to have sexual reproduction! It may take some practice to get the hang of scraping and making a wet mount so be patient!



Activity 3

Gather your supplies and read through all steps of the activity before you begin.

Per group – crosses from the previous week, two microscopes, microscope slides and cover slips, dropper bottle of diH_2O, dissecting needles, alcohol wipes

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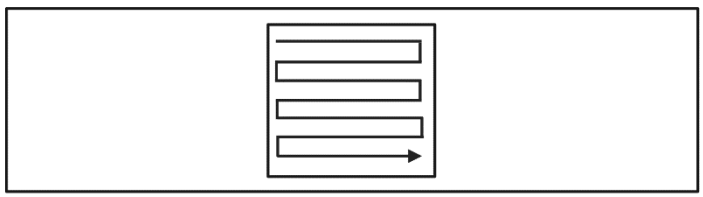
Tip: Tables should "divide and conquer" with half of the group counting the black and tan cross and the other counting the black and grey cross.

1. Place a drop of water in the center of a microscope slide. Carefully wipe the dissecting needle with the alcohol wipe and let it dry for about 30 seconds.

2. With the sterile dissecting needle, scrape ascospores from the plate without disturbing the agar. Add the ascospores to the water on the slide.

3. Add a cover slip and apply pressure (with your thumb or an eraser) to rupture the ascospores. If they are not ruptured enough, apply more pressure. If they are ruptured to the point that the asci have separated from one another, make a new wet mount.

4. Starting at the upper left corner of the cover slip, count the number of crossover and non-crossover asci. Systematically count all asci by moving from the top left corner to the bottom right corner as shown here.



5. Tally your results and gather data from other groups to complete your chart. Calculate the recombination frequency as in Activity 2 from last week.

6. Using the recombination frequencies of the grey and tan mutants, construct a genetic map.

Clean-Up:

- Place plates in the autoclave bin
- Place used microscope slides in the used slides bin
- Reset supply bin