



Personalized Medicine: Diagnosing and Targeting HER2 and ER+ Breast Cancer MiniLab

Student Guide

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Based on original activities developed by

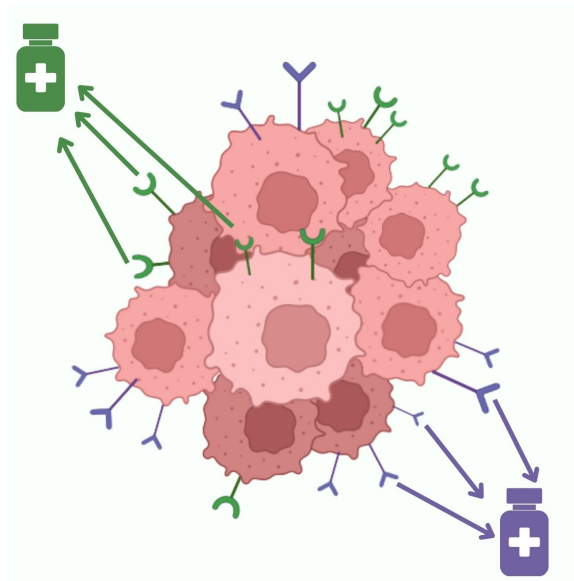


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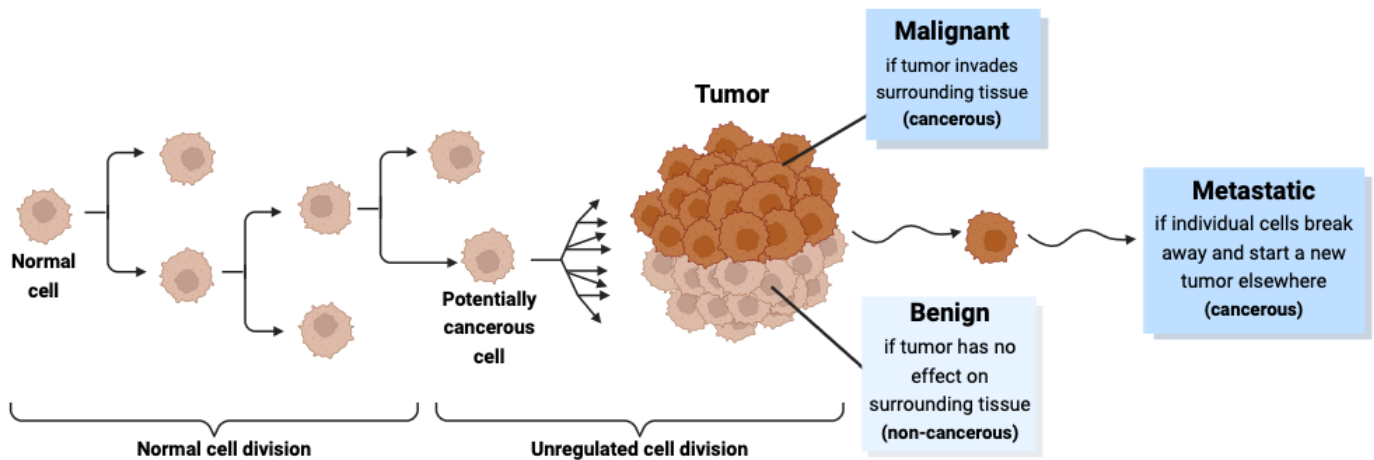
Laboratory Safety

1. Wear lab coats, gloves, and eye protection as required by district protocol.
2. Use caution with all electrical equipment such as PCR machines and electrophoresis units.
3. Heating and pouring molten agarose is a splash hazard. Use caution when handling hot liquids. Wear eye protection and gloves to prevent burns.
4. Wash your hands thoroughly after handling biological materials and chemicals.

What is Cancer?

In this MiniLab, you'll explore how normal and cancer cells work, learn about types of breast cancer, and discover how doctors diagnose and treat certain breast cancers using targeted therapies.

Cancer is a disease where cells grow and divide out of control. Normally, the body controls cell growth and damaged cells die off. But cancer cells can avoid controlled cell death and continue dividing, allowing them to continue to grow when they should be destroyed. This can lead to tumors, which are masses of extra cells. Tumors can be **benign** (not cancerous) or **malignant** (cancerous). Malignant tumors can spread to other parts of the body, which is called **metastasis**.



Created in [BioRender.com](https://www.biorender.com) 

Quick treatment after diagnosis is important, but finding the right treatment can take time since cancer is complex and different for each person.

Treatment of Cancer

Doctors use different treatments to help people fight cancer. The main goal is to stop cancer cells from growing or to remove the tumor. Common treatments include surgery, radiation, chemotherapy, immunotherapy, hormone therapy, stem cell transplants, and targeted therapy.

- **Surgery** removes the tumor from the body. It works best when the cancer hasn't spread.
- **Radiation therapy** uses high-energy waves (like X-rays) to damage cancer cell DNA, making it hard for them to grow. It can shrink tumors before surgery or destroy leftover cancer cells after.
- **Chemotherapy** uses strong drugs to kill fast-growing cells like cancer. For example, the drug Taxol blocks cell division, which kills cancer cells. Chemotherapy can shrink tumors, slow cancer, or treat cancer that has spread.

In this MiniLab, you'll learn more about targeted therapies for HER2-positive and hormone receptor-positive breast cancers.

HER2 and Breast Cancer

HER2 (**H**uman **E**pidermal Growth Factor **R**eceptor **2**) is a protein found on cell surfaces that helps control normal cell growth and division. The **HER2 gene** makes this protein, and most cells have two copies of the gene.

In **HER2-positive breast cancer**, the HER2 gene is **amplified**, meaning the cell has many extra copies, sometimes more than 20. This causes the cell to make too many HER2 proteins on the surface of the cell, which send constant "grow" signals, even without external instructions. As a result, the cancer cells divide quickly and form aggressive tumors that grow faster, spread more easily, and are more likely to come back after treatment.

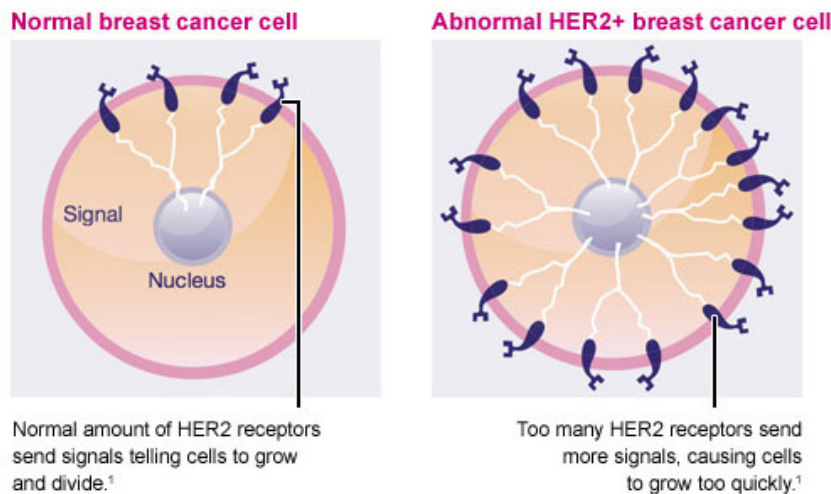


Image credit: https://www.aboutcancer.com/herceptin_0211.htm

Pre-Lab Question

3. **Describe** the relationship between HER2 amplification and breast cancer.

4. Knowing that mutations in DNA repair mechanisms lead to copying errors during replication, a failure at which checkpoint could have happened to cause the number of HER2 genes to be copied excessively? **Explain** your answer.

Detection of HER2 Gene Amplification

There are three diagnostic tests for HER2 amplification, which are Immunohistochemistry (IHC), Fluorescence In Situ Hybridization (FISH), and qPCR.

- **IHC** uses staining to measure HER2 protein levels. A score of **0** means HER2-negative, while **3+** means HER2-positive. It's quick, widely available, and gives a visual result, but scores of **1+ or 2+** can be unclear.
- **FISH** uses fluorescent probes to compare the number of HER2 gene copies to a control gene on chromosome 17. A ratio above **2.0** means HER2-positive. FISH is very accurate, especially when IHC results are uncertain.
- **qPCR** is a newer test that measures HER2 gene copies directly. If qPCR shows a normal number of HER2 genes, the sample is **HER2-negative**. If there are many extra copies, it is **HER2-positive**, meaning the cancer is likely driven by HER2 gene amplification and could respond to HER2-targeted treatments.

Pre-Lab Question

5. **Describe** the three methods for detecting HER2 amplification.

Treatment of HER2+ Breast Cancer

Because HER2 is an important driver of **HER2-positive breast cancer**, it is a major target for treatment. **Trastuzumab (Herceptin)** is a monoclonal antibody that attaches to the HER2 receptor proteins on cancer cells. This blocks HER2's signals to grow and divide, slowing or shrinking the tumor.

Trastuzumab also helps the immune system attack cancer. Once it binds to HER2 protein, it marks the cancer cells so immune cells can find and destroy them—a process called **ADCC** (antibody-dependent cellular cytotoxicity).

Finally, Trastuzumab can also block growth signals inside the cancer cell. It is often given with chemotherapy or other HER2 drugs like **Pertuzumab**, which targets a different part of HER2 for an even stronger effect.

Pre-Lab Question

6. **Describe** the three modes of action of Trastuzumab.

Hormone Receptor-Positive Breast Cancer

Some breast cancers grow in response to hormones like **estrogen** or **progesterone**. Estrogen is a chemical messenger that helps control the menstrual cycle, supports reproductive health, and plays a key role in developing female body traits.

Cells have **estrogen receptors**—proteins that act like locks opened by estrogen, the “key.” When estrogen binds to these receptors, it sends signals that make the cell grow and divide.

In **estrogen receptor-positive (ER+)** breast cancer, the cancer cells have too many estrogen receptors. This means estrogen strongly fuels their growth. The more estrogen these cancer cells are exposed to, the faster they grow and spread.

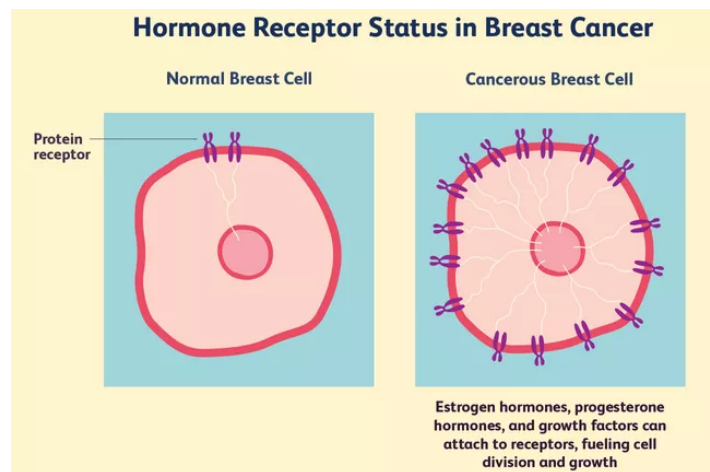


Image credit: <https://www.verywellhealth.com/hormone-receptor-status-and-diagnosis-430106> / Gary Ferster

In ER+ breast cancer cells, estrogen binds to its receptor and activates genes inside the cancer cell that promote growth and division. As a result, ER+ breast cancers tend to be fueled by the presence of estrogen in the body. The more estrogen the cancer cells are exposed to, the more they grow and spread.

Pre-Lab Question

8. **Explain** the difference between estrogen receptors on normal breast cells and breast cancer cells that results in ER+ breast cancer.

Detection of Hormone Receptor-Positive Breast Cancer

To detect **hormone receptor-positive** breast cancer, doctors take a **biopsy**, which removes a small sample of the tumor. This can be done with a needle (fine needle aspiration or core needle biopsy) or surgery. The sample is sent to a lab, where a pathologist looks at the cells under a microscope. Cancer cells often appear irregular and disorganized compared to normal cells.

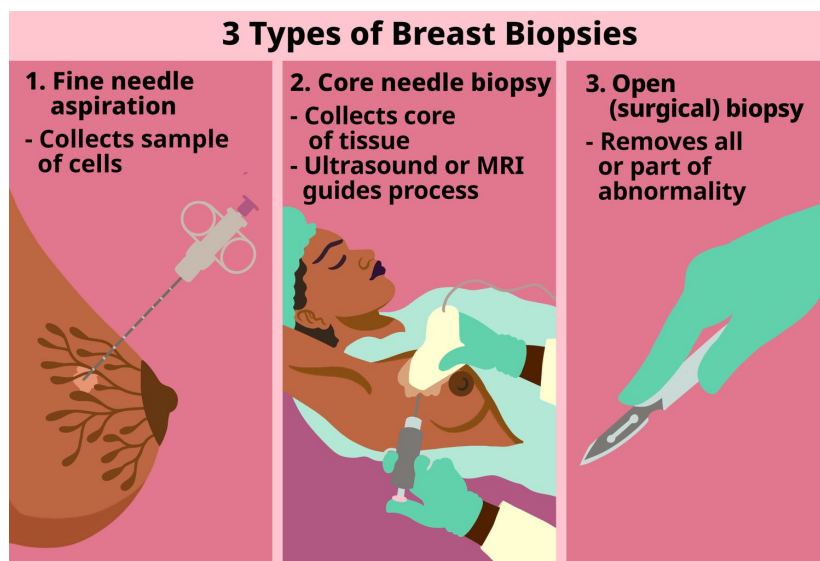


Image credit: <https://www.verywellhealth.com/breast-biopsy-7966144>

Next, the tissue is tested for **estrogen (ER)** and **progesterone (PR)** receptors using tests like **immunohistochemistry (IHC)** or **immunofluorescence (IF)**.

- **IHC** stains cells with special antibodies that attach to ER or PR, which result in a brown or red color. If at least 1% of cells have these receptors, the cancer is hormone receptor-positive. (American Cancer Society. (n.d.)
- **IF** uses fluorescent dyes to highlight different cell parts, so researchers can see where they are in the cell and how they might affect cancer growth. Under a fluorescence microscope, various stains let researchers see cell structure, actin filaments, and where GPER is positioned, helping study its role in cancer growth and hormone response. The following stains are used in an IF test:
 - **Hoechst stain** – stains the **nucleus** blue, showing its size and shape.
 - **Phalloidin + GFP** – stains **F-actin** green, highlighting actin filaments in the cell.
 - **Alexa Fluor 594–tagged antibody** – stains **GPER**, a cell membrane estrogen receptor, red, showing its location and distribution.

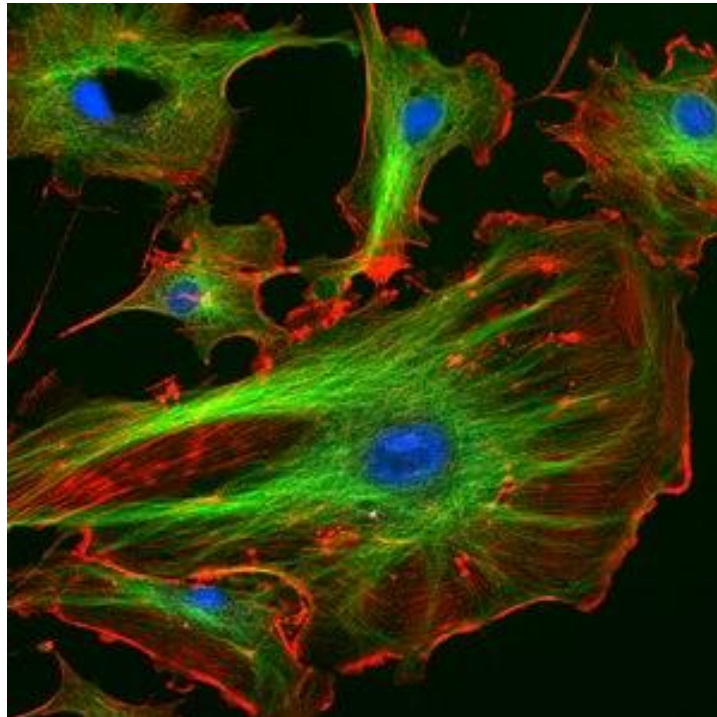


Image showing the blue staining of a cell nucleus using Hoechst stain, green staining of actin-GFP, and red staining of membrane proteins viewed under a fluorescent light microscope. Image credit: <https://en.wikipedia.org/wiki/Phalloidin>

Pre-Lab Question

9. When looking at an IF slide for a HER2 patient, which structure would give you an indication of the ER status?

| Stain | Color | Organelle/Part of Cell Stained | Purpose |
|--|-------|--------------------------------|---------|
| Hoechst | | | |
| Phalloidin and green fluorescent protein (GFP) | | | |
| Alexa Fluor 594 tagged GPER antibody | | | |

Treatment of of Hormone Receptor-Positive Breast Cancer

ER+ breast cancer grows in response to estrogen. Targeted treatments work by blocking estrogen's effect or lowering estrogen levels in the body.

- **Selective estrogen receptor modulators (SERM)** (like **Tamoxifen**) block estrogen receptors on breast cancer cells so estrogen can't make them grow. Tamoxifen blocks estrogen in breast tissue but can act like estrogen in other tissues, such as bones. It can be used by both pre- and postmenopausal women, and even in men.
- **Aromatase inhibitors** lower estrogen in postmenopausal women by blocking the enzyme that makes estrogen.
- **Selective estrogen receptor degraders (SERDs)** block estrogen receptors and also cause them to break down.
- **Luteinizing Hormone-Releasing Hormone agonists (LHRHs)** lower estrogen in premenopausal women by shutting down ovarian production.

These therapies help slow or stop the growth of ER+ breast cancer and can prevent it from coming back.

Pre-Lab Question

10. Compare and contrast the modes of action of the four main types of drugs used as targeted treatments for ER+ breast cancer.

| Drug | Mode of action |
|----------------------|----------------|
| SERMs | |
| Aromatase inhibitors | |
| SERDs | |
| LHRH agonists | |

Scenario

You are a medical lab technologist working with **12 breast cancer patients** (labeled A–L for privacy). Your job is to analyze their IF slides and HER2 status to find the best treatment for each one.

Biopsy samples were stained and viewed under a fluorescence microscope:

- **Hoechst** (blue) – stains the nucleus (DNA).
- **Phalloidin + GFP** (green) – stains actin filaments.
- **Red fluorescent antibody** – detects GPER, showing if the cancer is **estrogen receptor-positive (ER+)** or **negative (ER–)**.

DNA from each sample was tested for **HER2 gene amplification** using **PCR**. In this lab, PCR products are run on an **agarose gel** with GelGreen dye, which makes DNA glow under blue light.

Each sample has two bands:

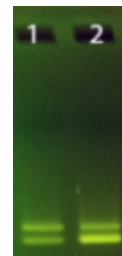
- **Reference gene** – always has 2 copies (164 bp), serves as internal control for brightness of 2 bands.
- **HER2 gene** – may have normal (2 copies) or extra copies (125 bp).

You compare the brightness of the HER2 band to the reference gene band from the same patient. If the HER2 band is much brighter, the HER2 gene is amplified, suggesting HER2-positive cancer. This gel method can show big differences in HER2 copy number, but it's less precise than FISH or qPCR.

Gel electrophoresis image, 162 bp reference gene and 125 bp HER2 gene.

Lane 1 - No amplification of HER2 gene (125 bp fragment)

Lane 2 - Amplification of HER2 gene (125 bp fragment)



12. **Explain** the purpose of the reference sample or internal control in this MiniLab?

Your team's task is to run the PCR products from the 12 patients on an agarose gel, analyze the intensities of the DNA bands, and compare them to the reference bands. By examining the results, you will identify which patients have undergone HER2 gene amplification.

You will also examine the immunofluorescence images for each of the patients, determining whether each patient is ER+ or ER-. Based on this information, you will stratify the patients and recommend appropriate targeted therapies. Be sure to justify your treatment suggestions based on the molecular characteristics of each patient's cancer.

Part I: Electrophoresis

Materials

- 1 Minione® Casting System
- 1 MiniOne® Electrophoresis System
- 1 agarose GreenGel™ cup (2 %)
- 9 DNA sample aliquots
- 135 mL of running buffer
- 1 micropipette (2-20µL)
- 9 pipette tips

| Lane # | Sample Name | Volume |
|--------|---|--------|
| 1 | MiniOne® PCR Marker (750, 500, 250, 150, 50 bp) | 10 µL |
| 2 | Negative Control | 10 µL |
| 3 | Positive Control | 10 µL |
| 4 | Sample | 10 µL |
| 5 | Sample | 10 µL |
| 6 | Sample | 10 µL |
| 7 | Sample | 10 µL |
| 8 | Sample | 10 µL |
| 9 | Sample | 10 µL |

How to Cast a Gel

1. Place the MiniOne® Casting Stand on a level surface and place gel trays in the two cavities. For proper tray orientation place the tab edge of the tray on the left side. Insert the comb into the slots at the top of the casting stand with the 9-well side facing down.

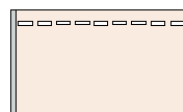
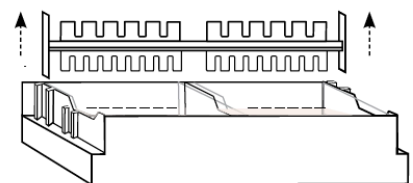
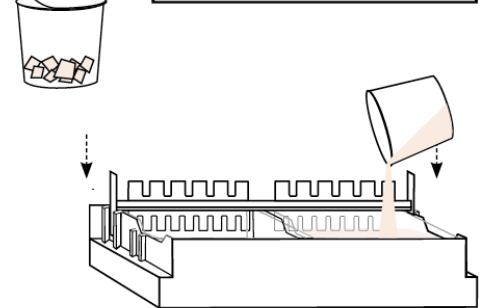
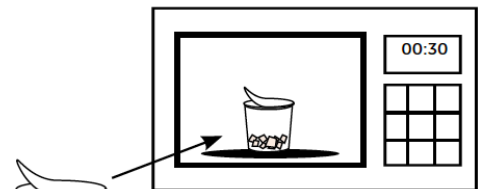
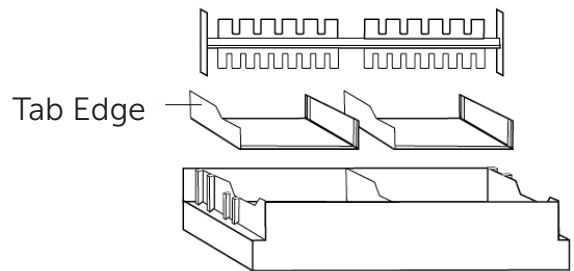
2. **Partially** peel the film off a GreenGel™ cup and microwave for 25-30 seconds. Allow to cool for 15 seconds. DO NOT microwave more than 5 gel cups at a time.

Safety requirement: Adult supervision required if students are handling gel cups!


3. One gel cup is for making one agarose gel! Slowly pour the hot agarose solution into a gel tray. Make sure there are no air bubbles in the agarose solution. Let the agarose gel solidify for 10 minutes or until opaque.

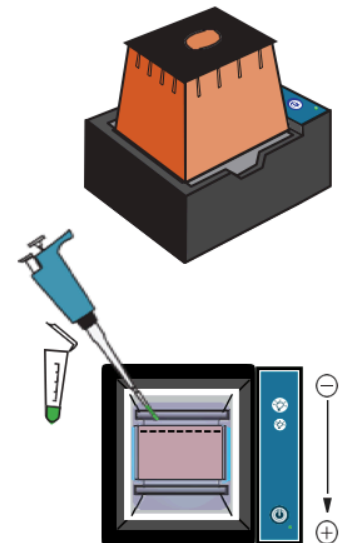
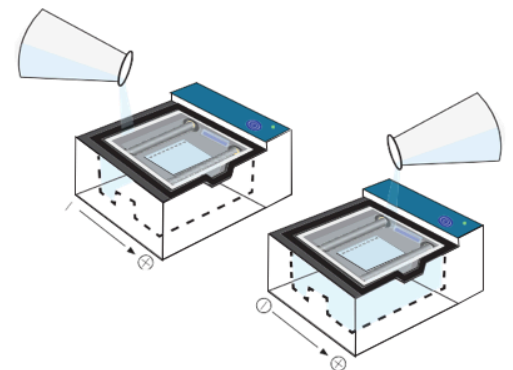
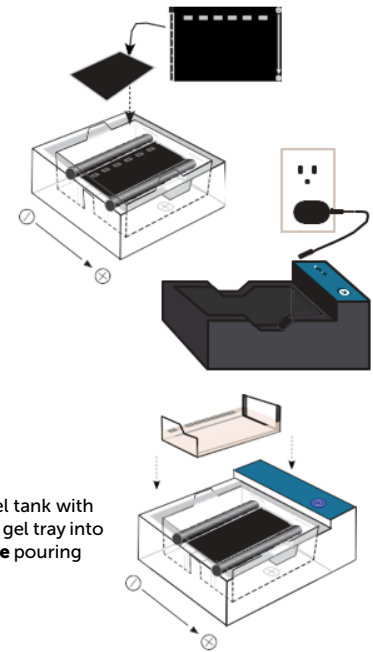
DO NOT disturb the gel until time is up.

4. Carefully remove comb when gel is ready. Remove gel tray with solidified gel from Casting Stand and wipe off any excess agarose from the bottom of the tray.




How to Load a Gel

1. Ensure the black viewing platform is in the gel tank. **Make sure the wells are aligned with the marks on the platform on the negative end.**
2. Plug the power supply into the wall and carefully insert the other end into the back of the MiniOne® Carriage.
3. Place the gel tank into the carriage so the carbon electrodes are touching the gold rivets and the tank sits level with the carriage.
4. Place the gel tray with the gel into the gel tank. The gel tank should not have any buffer in it when putting the gel tray with gel into it.
5. Turn the low intensity blue LED on by pressing the  button on the carriage.
6. Measure 135 mL of TAE running buffer and pour into **one side** of the gel tank. Watch the air push out between the gel tray and viewing platform. Once air has been removed from under the gel tray, pour remaining buffer into the **other side of the gel tank.**
7. Place photo hood on the carriage.
8. Press the power button which should now be a solid green light. If **green light is solid**, turn off the unit and proceed to loading gels.
9. Turn the low intensity blue light on by pressing the button on the carriage to help visualize the wells when loading.
10. Load 10 μL per well. Remember to change pipette tips for each sample. **Load your samples according to the order given in the sample chart.**

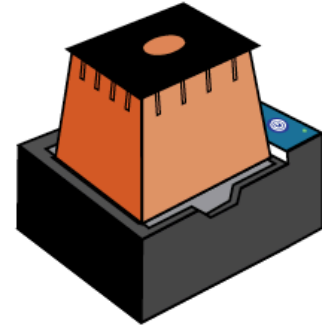


Run, Visualize and Capture Image


1. Once the gel is loaded, do not move it. Make sure the power supply is plugged in and place the photo hood on the carriage. Turn on the unit by pressing the  button. The green LED next to the button will turn on.

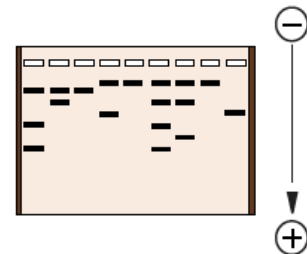
The green power LED will not turn on if:

- The tank is not properly placed inside the carriage.
- There is no buffer in the tank.
- The buffer is too diluted.
- The photo hood is not on the carriage.
- There is too little running buffer.
- The power supply is not plugged in. Check by turning on the blue LEDs.
- If the green power LED is blinking, please refer to the troubleshooting steps in the **MiniOne Electrophoresis Instruction Manual**



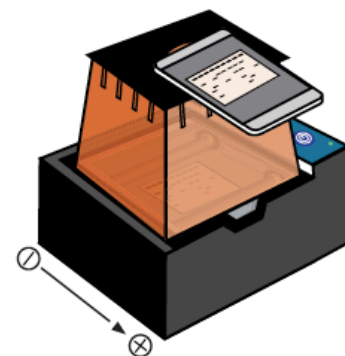
2. Have students periodically check the migration of the bands (~every five minutes).

3. Allow the gel to run **25 minutes** or until DNA separation is sufficient. Keep in mind small DNA samples run faster so it's important to periodically check where your bands are. After your run is complete, turn off the power by pressing the  button. Use the low intensity for viewing during the run. Light will weaken the fluorescent DNA signal.



4. Document your results.

Wipe off the condensation from the inside of the hood with a soft cloth if necessary, then place the hood back on the carriage. **Turn on** the high intensity light. Place your cell phone or camera directly on the photo hood to take a picture of the DNA. **DO NOT** zoom in as this will result in blurry pictures. (The photo hood is already at the optimal focal length for a smart device).



5. Clean up. Follow teacher's instructions on disposal and clean up.

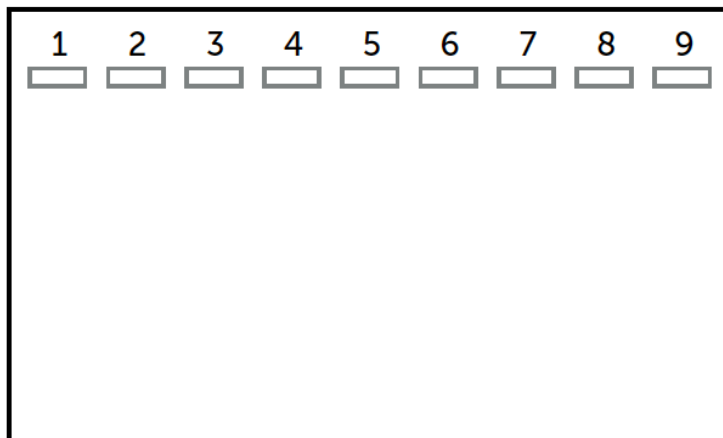
Clean Up

Note: All reagents in this lab can be disposed of as non-hazardous waste.

1. After collecting data and documenting results, remove the photo hood and unplug the power supply from the wall and from the back of the MiniOne® Carriage. Remove the clear running tank from the carriage and remove the gel and tray from the running tank.
2. Pour the used running buffer down the drain or into a waste beaker. Throw the gel away AND SAVE THE GEL TRAY. Rinse the clear plastic running tank, gel tray, comb, and casting system with DI or distilled water. Allow the tanks to fully air dry before storing.
3. Use a paper towel or Kimwipe™ to gently wipe the gold rivets in the carriage (where the electrodes connect) to ensure all moisture is removed. Wipe up any buffer that may have spilled into the black carriage. Follow any additional directions the instructor gives for clean up and storage.

Part II: Results

What does your gel look like? Record images of the gel in the gel below along with the resulting fragment sizes.



- Lane 1: _____
Lane 2: _____
Lane 3: _____
Lane 4: _____
Lane 5: _____
Lane 6: _____
Lane 7: _____
Lane 8: _____
Lane 9: _____

Post-Lab Questions

1. Based on the electrophoresis results, what is the HER2 status for your patients? Fill in the table below with the diagnosis
2. Based on the Immunofluorescence, what is the ER+ status of your patient? Fill in the table below with the diagnosis
3. Which treatment would be recommended for each of your patients and why? Complete the table below with your recommended course of treatment.

| Patient Sample | HER2 PCR | ER+ | Recommended Treatment |
|----------------|----------|-----|-----------------------|
| A | | | |
| B | | | |
| C | | | |
| D | | | |
| E | | | |
| F | | | |
| G | | | |
| H | | | |
| I | | | |
| J | | | |
| K | | | |
| L | | | |

Appendix A - What is Gel Electrophoresis?

Gel electrophoresis is a technique used in many areas of science to analyze the components of complex chemical mixtures. Mixtures of DNA, RNA, proteins, or dyes can be separated into their individual components based on molecular size and electrical charge using a separation matrix within an electric field.

The gel used in gel electrophoresis is a tangle of polymers forming a three-dimensional matrix with water-filled pores through which molecules migrate. A higher density of polymers creates smaller pores. Like the holes in a sieve or colander, the size of the pores has to be the appropriate size for the molecules being separated. Gels can be made from different substances depending on the application. One of the most commonly used and effective materials is agarose, a polymer extracted from seaweed. Agarose gels are formed (or cast) by pouring molten (melted) agarose into a tray where it solidifies into the desired shape as it cools. A comb is placed while the agarose is molten and then removed after it solidifies to create wells where the samples are loaded.

After the gel solidifies it is placed in an electrically conductive buffer between parallel positive ((+) anode) and negative ((-) cathode) electrodes

A voltage is applied between the electrodes, creating a uniform electric field within the gel. Molecules in the wells begin to move under the influence of the electric field: positively charged molecules migrate toward the (-) cathode and negatively charged molecules migrate toward the (+) anode.

The speed of a molecule's movement in an electric field is determined by the strength of its electric charge relative to its molecular weight. This is quantified as the charge to mass ratio. Speed of movement within a gel is also influenced by the size of the molecule relative to the pores in the gel. The polymers in the gel are like an obstacle course: smaller molecules maneuver easily through the pores, traveling faster and farther than large, bulky molecules. However, a large molecule can move faster through a gel than a smaller molecule when the strength of its charge relative to its mass is significantly higher. Shape can also affect how a molecule moves through the gel. Long spaghetti-like molecules will move slower than compact molecules, which slip easily through the pores. Molecules of the same size, shape, and charge will move together and form a distinct band. If multiple types or sizes of molecules are present in the sample, they will separate from each other and each will form a distinct band.

Appendix B - Polymerase Chain Reaction

Polymerase chain reaction (PCR) addresses two major challenges in molecular biology producing usable quantities of DNA for analysis and isolating a specific region of the genome. First, the DNA that scientists want to analyze is often collected in extremely small quantities (for example, a single drop of blood from a crime scene). To have usable quantities of DNA we must make many copies using the original DNA as a template. Second, among the whole genome we must find only the part we want to analyze, whether it is a gene that causes a disease or a sequence that can help identify a species. This is no small matter since the human genome is over 3 billion base pairs (bp) long and we are often interested in regions less than 300 bp long.

The History of PCR

As with many ideas in biotechnology, nature provides us with most of the tools we need. Every time a cell divides it makes two replicates of its entire genome with the help of specialized enzymes. The phenomenon of complementary base pairing should give you a hint as to how a specific region can be targeted. In the late 1970s Frederick Sanger developed a method for copying DNA in vitro that used short pieces of DNA, called primers to initiate replication by a DNA polymerase, similar to the RNA primers in your model of cellular DNA replication. An artificial primer used in the Sanger method has a sequence that allows it to bind at only one location in the target DNA sequence.

Using only one DNA primer, the Sanger method can only produce one copy of the target at a time. In 1983 Kary Mullis proposed a modification to this method where a second primer is used to initiate replication using the first copy as a template. Recall that DNA has an antiparallel structure where one strand runs in the opposite direction relative to the second strand. To use the first copy as a target for replication, the second primer that Mullis added needed to initiate replication in the opposite direction, thus one primer is called the forward primer and the other is called the reverse. With the original sequence and its copy serving as templates, two copies are produced instead of one. Further, these two copies can each be used as templates in a second round of copying, which produces four copies. After each round, or cycle, the number of copies has doubled. Over multiple cycles, billions of copies of the DNA sequence between the two primers are generated. This method is called Polymerase Chain Reaction (PCR) – *polymerase* because of the enzyme that is used to copy DNA and *chain reaction* because the products of one cycle serve as templates for the next round of copying. This rapid and efficient technology for generating usable quantities of DNA won Mullis the Nobel Prize in Chemistry. The first application of PCR was a test for sickle cell anemia.

How PCR Works

Instead of trying to recreate the cell's intricate biochemical machinery in a test tube, scientists rely on heat to control the various steps of the PCR reaction. For DNA to be copied the nucleotide bases must be exposed, which means double stranded DNA must be separated into single strands. Just as heat applied to an ice cube weakens the hydrogen bonds between water molecules and causes a phase transition to liquid water, heat applied to double-stranded DNA weakens hydrogen bonds between bases causing separation of the strands into single-stranded

DNA. As with ice, this is sometimes called melting, but is commonly referred to as denaturation. In a PCR cycle, denaturation is performed at 90-98°C for 5-30 seconds. This temperature is just below the boiling point of water.

Once the template DNA has been separated into single strands, the temperature is lowered to encourage primers to bind to their target sequences. Just as in the water analogy, lower temperature favors the formation of hydrogen bonds between molecules, in this case the primer and the template DNA. This step, called annealing, is typically between 45 and 65°C for 5-30 seconds. The ideal temperature and duration of the annealing step depends on the sequences of the primers being used. It must be low enough that hydrogen bonds are able to form between the primer and specific complementary sequence, but not so low that nonspecific, or random, binding between primer and template occurs.

DNA polymerase binds to the primer-template complex and begins to add nucleotides (dNTPs) onto the 3' end of the primer. This step, called extension, results in a new copy attached to the template as double-stranded DNA. The duration of the extension step depends on the length of the DNA segment being copied and can be anywhere from 5 seconds to 5 minutes.

At this point you may have noticed a problem – DNA polymerase, which the entire PCR process relies on, is a protein enzyme that needs a very specific three-dimensional structure to copy DNA. Before getting to the extension step, the entire PCR mixture, including the polymerase, has already been through the denaturation step where the reaction was heated almost to boiling. Anyone who has cracked an egg into a frying pan will know what high heat does to proteins!

In the early days of PCR, fresh polymerase was added to the reaction tube every cycle to replace the polymerase that had been destroyed by the denaturation step. Having to open the tube and add new enzyme for up to 30 cycles was expensive, inefficient, and increased the chances of contaminating the reaction. The innovation that would remove these obstacles came from an unusual source – Yellowstone National Park.

In the 1970s, scientists had isolated a species of bacteria called *Thermus aquaticus* from a hot spring in Yellowstone. *T. aquaticus* thrives at 75 - 80°C and can survive much higher temperatures. Its enzymes are similarly heat-tolerant. *E. coli*, the original source for the polymerase used in PCR, thrives at 37.5°C, the same temperature as the gut of mammals.

Since the biochemistry of DNA is similar across the tree of life, polymerase from *T. aquaticus* (called Taq Polymerase) can replace *E. coli* polymerase in PCR, with the modification that the extension step is performed at 70-75°C.

This innovation led to PCR becoming the ubiquitous, inexpensive, and efficient tool that it is today. Reactions can be set up once, sealed inside a tube, and placed in an automated thermal cycler. The thermal cycler (or PCR machine) is a specialized instrument that accurately and rapidly changes the temperature of the tube between denaturation, annealing, and extension. After 20-40 cycles of these three temperatures, billions of copies of the desired DNA product can be produced. The amplified DNA can be analyzed with gel electrophoresis at the end of the experiment or detected as they are being formed using more advanced equipment.

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