CEREAL KILLER: DIAGNOSING OYSTER DISEASES USING PCR

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Grade Level
High School

Subject Area
Biology

VA SEA is a collaborative project between the Chesapeake Bay National Estuarine Research Reserve, the Virginia Institute of Marine Science's Marine Advisory Program, and Virginia Sea Grant. The VA SEA project is made possible through funding from the National Estuarine Research Reserve System Science Collaborative, which supports collaborative research that addresses coastal management problems important to the reserves. The Science Collaborative is funded by the National Oceanic and Atmospheric Administration and managed by the University of Michigan Water Center.

Title: Cereal Killer: Diagnosing Oyster Diseases Using PCR

Focus: Understand the basis of polymerase chain reaction (PCR) by constructing a model of the technique using cereal and pipe cleaners.

Grade Level: High School Biology

VA Science Standards:

BIO.1 The student will demonstrate an understanding of scientific and engineering practices by

- asking questions and defining problems
- interpreting, analyzing, and evaluating data
- developing and using models
- obtaining, evaluating, and communicating information

BIO.5 The student will investigate and understand that there are common mechanisms for inheritance. Key ideas include

- DNA has structure and is the foundation for protein synthesis

Learning Objectives:

- ✓ Students will make observations about dying oysters and discuss possible reasons for oyster mortality
- ✓ Students will compare plots and interpret different data types to argue which variable(s) are most important
- ✓ Students will build models of DNA to understand the mechanisms of polymerase chain reactions (PCR)
- ✓ Students will use their PCR models to determine which disease likely caused oyster mortality

Total length of time required for the lesson:

90-120 minutes total class time; Advance preparation of lab materials – 1-2 hour(s), Lab setup – 5 minutes, Introduction – 10 minutes, Activity #1 –20 minutes, Lecture – 15 minutes, Activity #2 – 30-60 min, Discussion – 5 minutes, Breakdown and clean-up – 5 minutes.

Key words, vocabulary:

- Parasite: an organism that lives in or on another species and benefits and harms that species
- DNA: (deoxyribonucleic acid) molecules that carry genetic codes that are used for the development and functioning of an organism

- Primer: short, single-stranded DNA sequences that match specific sequences in a target sequence, used in PCR
- PCR: (polymerase chain reaction) laboratory technique used to make millions of copies of a specific DNA sequence
- Nucleotide: basic building blocks of DNA (A, G, T, C)
- Pathology: the study and diagnosis of disease
- Electrophoresis: the movement of charged particles in a fluid or gel from the influence of an electric field.
- Chesapeake Bay: a large estuary located on the East Coast of the US, where oysters live
- Keystone species: a species on which other species in an ecosystem largely depend, such that if it were removed the ecosystem would change drastically.

Background information:

In the Chesapeake Bay, oysters play important ecological and economical roles. Not only do they support a $30-million-dollar aquaculture industry[1], but oysters are keystone species that provide habitat, food, and protection for a wide variety of species in the bay. Additionally, oysters help filter nutrients and algae out of the water, thereby increasing water quality. Unfortunately, today oyster populations in the Chesapeake Bay only make up a fraction of historic numbers, largely due to overharvest, pollution, and disease. When mass mortalities occur, scientists are often asked to disentangle the long list of possible reasons for these mortality events, which can include a number of different disease-causing parasites. Since the late 1950's, oysters have dealt with a number of disease pressures, that continue to threaten wild and aquacultured oysters today. Testing for these diseases in aquaculture farms has become common practice in efforts to reduce the spread and learn more about these parasites. One widely used technique for diagnosing disease is polymerase chain reaction (PCR), where millions of copies of a targeted DNA sequence from a specific parasite are made, if they are present in the sample. In this lesson, students will be presented with a crime-scene-like case study of a farm that has experienced a massive mortality of oysters. Students will interpret a variety of graphs and figures of environmental data to determine that a disease likely caused the event. Then the students will be presented with three major "suspect" oyster parasites – MSX, Dermo, and Bonamia – and model a PCR using cereal to determine which parasite was the killer.

Materials & Supplies:

- Froot Loops or off-brand fruit loops with at least four colors of ringed cereal (ex: fruit spins, tootie fruities)
- pipe cleaners
- permanent marker
- Computer and projector for accompanying PowerPoint
- Paper and pencils
- Lab Prep Manual

Classroom Set up:

Students should work in groups of four-five students, with a table and enough space to construct either 2 or 8 (depending on time constraints) double-stranded DNA molecules using a bowl of dry cereal and pipe cleaners. Set up however best for this.

Teacher Preparation:

Prepare lesson activity by constructing starting DNA strands (two pipe cleaners with 20 froot loops on each pipe cleaner) and 3-9 primer sets for each group (each primer set is composed of two pipe cleaners with 5 froot loops on each). See attached Lab Prep Manual for specific instructions.

Each group should have:

Two complimentary DNA strands

One set each of primer sets #1, #2, and #3

Six additional sets of primer set #1 **OR** a colored copy of *Products of 3 Rounds of PCR* handout to save time (once they identify the correct primer)

Oyster Murder Part I: the crime scene worksheet* (can be distributed to each student, if resources available. Pages 1 & 2 can be laminated for reuse)

Oyster Murder Part II: who dunnit? worksheet* (can be distributed to each student, if resources available. Page 1 can be laminated for reuse)

*the last page(s) of each worksheet are not designed to be reusable; print in black and white.

Procedure:

1. **Engagement**

Begin the PowerPoint, *Oyster Mass Murder* (slides 1-4)

- See slides for specific notes with suggested dialog and discussion
- Set the crime scene on slide 1 by explaining what an oyster is and where it lives, and how it has been murdered.
- Focus on slide 2 which provides a side-by-side visual of a healthy and dying oyster.
- Ask students:
    - If you had to eat one of these oysters, which one would you eat? Why?
    - What differences do you notice between the oyster on the left and the oyster on the right?

2. **Exploration**

- Ask students to brainstorm in pairs 2-3 possible reasons why the oysters may be dying (5 mins), and then ask a few pairs to share out one from their list.
    - Some possible reasons could include: pollution (chemical, plastic, eutrophication), disease, starvation, temperature/environmental stress, predation

- Further explore the role of oysters in our ecosystems and human society by presenting slide 3. If students are not familiar with this animal and its role as a keystone species, view the optional 1:00 video linked at the bottom of the slide for more information.
- Split the class into groups of four-five students and hand out the "Oyster Murder Part 1: The Crime Scene" worksheet. Give them 20 minutes to fill out that worksheet, before coming together as a class to decide the likely reason for the oyster mortality on the farm.

3. Explanation

Continue the PowerPoint, *Oyster Mass Murder,* from slide 5 until slide 11. (15 min)

4. Elaboration

- Distribute initial primer sets (#1, #2, #3), DNA strands, and a bowl of fruit loops to each group.
- Students will first need to determine which primer set matches their DNA strand following the instructions on "Oyster Murder Part 2: Who Dunnit?" **Slide 12 can be projected as a visual instruction manual** to help students understand the activity.
- Once students have finished their first round of PCR by creating two double-stranded DNA molecules with the correct primer and fruit loops, distribute the rest of the correct primer sets OR the "Products of 3 Rounds of PCR" handout to each group. The former requires students to construct an additional 12 single strands of DNA which could add a substantial amount of time to the lesson, and lesson prep time as teachers will have to construct 6 more primer sets for each group. Consider making this a competition between groups to see which group can build their correct DNA strands fastest. To save on time (and resources), teachers can opt to print out the "Products of 3 Rounds of PCR" worksheet for each group to analyze after they determine the correct PCR primer.
- Students should complete two more rounds of PCR, with a final count of 8 double stranded DNA molecules, OR use the "Products of 3 Rounds of PCR" worksheet to answer questions on the "Who Dunnit?" worksheet.

5. Evaluation

- Have students work through the questions at the end of the worksheet, and review the answers as a class to make sure all groups have correctly modeled three rounds of PCR and understand how the technique is used for disease diagnostics.
- Briefly present slide 13 and 14, with suggested dialogue in the notes of the PowerPoint.

Assessment

Students will be assessed based on their performance on the interpreting graphs worksheet, PCR activity, and follow up worksheet questions.

References

1. *Eastern Oysters*. Chesapeake Bay Foundation. (n.d.). Retrieved November 28, 2022, from https://www.cbf.org/about-the-bay/more-than-just-the-bay/chesapeake-wildlife/eastern-oysters/index.html

Acknowledgments

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Oyster Murder Part I: The Crime Scene

Introduction. On September 15, 2009, your friend who owns Selfish Shellfish Oyster Farm in James River, VA calls you in a panic. She tells you something terrible has happened – something has murdered more than half of her oysters, and many others are dying. Your friend begs you to help figure out what has caused this massive mortality on her aquaculture farm. You quickly phone all of your talented marine biologist co-workers, and as a group investigate the crime scene by compiling and collecting data about different environmental parameters that could potentially explain the murders.

Instructions. Analyze the data below and answer the following questions to narrow down your suspect list, before any more oysters get murdered!

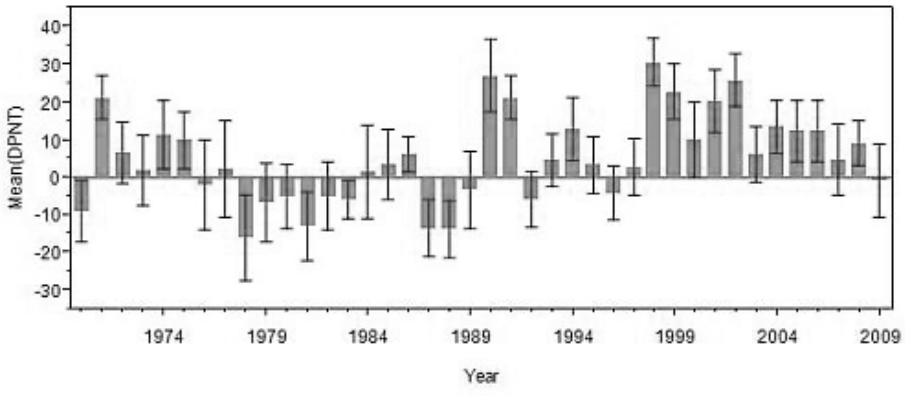


Figure 1. Mean annual departures from normal temperature. This graph shows how air temperature in Williamsburg, VA differs from the norm in a given year. Positive values indicate higher than normal temperatures, and negative values indicate lower than normal temperatures. Chart from:
https://www.vims.edu/ccrm/research/climate_change/data_sources/examples/environmental/index.php

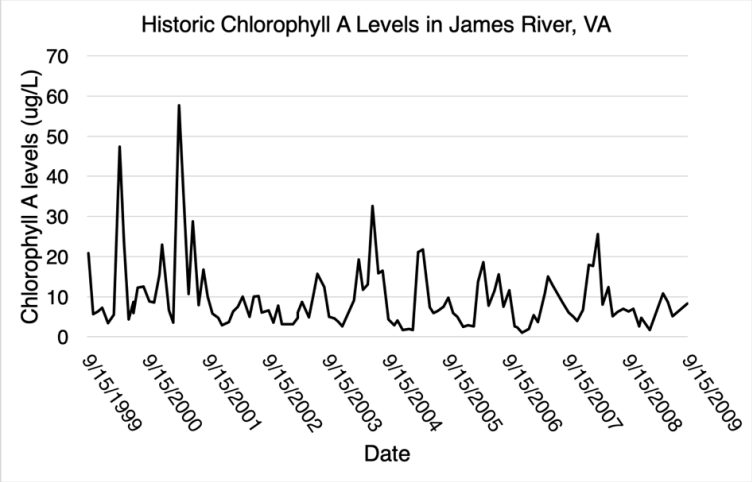


Figure 2. Historic Chlorophyll A levels in the James River. Chlorophyll A is the green pigment that algae (marine plants) use to absorb light, and can therefore be a good measure of water quality. Too much chlorophyll A means that the water may be polluted with nutrients from fertilizers and sewage, and too much algae is growing. Data from:
https://www.chesapeakebay.net/what/data

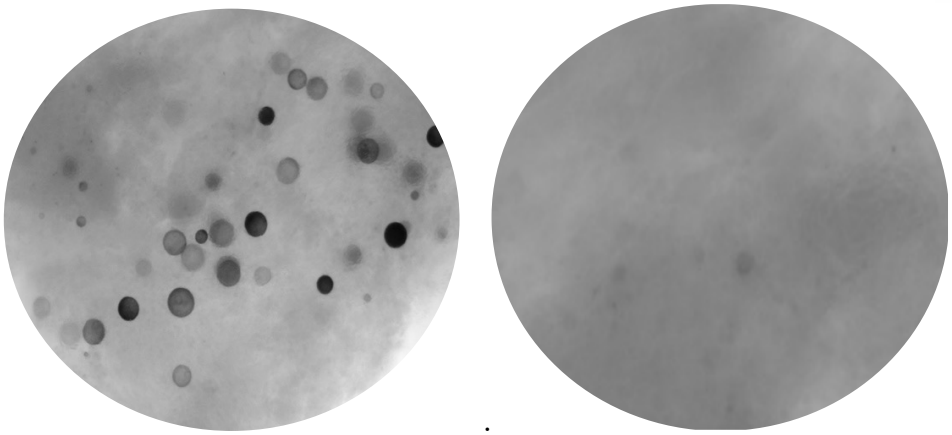


Figure 3. Disease cultures from dying and healthy oysters from Selfish Shellfish Oyster Farm. On the left is a microscopic image of a parasite culture created from a piece of tissue from a dying oyster, and on the right is a culture created from a tissue piece of a healthy oyster.
Image credit: Ryan Carnegie

Contaminant	Measured Levels	Action Levels
Mercury – heavy metal	0.0005 mg/L	0.002 mg/L
PFAS – chemical	57 ppt	70 ppt
Atrazine – pesticide	0.001 mg/L	0.003 mg/L
Microplastics	97 particles/L	1000 particles/L

Figure 4. Contaminant levels at Selfish Shellfish Oyster Farm on September 15, 2009. Measured levels indicate what you measured at the crime scene, while the action levels are high values that could potentially impact oysters and requires immediate action.

Name(s)_____

Date_____

1. Are the average temperatures higher, lower, or on par with normal temperatures in the year 2009? (Fig 1)

2. On average, has Williamsburg, VA been experiencing higher, lower, or normal temperatures from 1999-2009? (Fig 1)

3. What is Chlorophyll A and what is it a good indicator of? (Fig 2)

4. Describe the annual and decadal trend you see in chlorophyll A levels in the James River from 1999-2009. (Fig 2)

5. Estimate how many parasite cells (dark circles) you see in the dying oyster (left) and healthy oyster (right) in Figure 3.

6. Are any of the measured levels of contaminants listed in Figure 4 reaching levels that could potentially harm oysters?

7. Given all of the data compiled here, what do you think caused the oyster mortality event at Selfish Shellfish Oyster Farm?

Oyster Murder Part II: Who Dunnit?

Introduction Scientists in the Shellfish Pathology Lab at the Virginia Institute of Marine Science use different techniques to diagnose illness in oysters. One technique they use is called **Polymerase Chain Reaction (PCR).** To understand what a PCR is, we first need to understand the structure of DNA. **DNA** (which stands for deoxyribonucleic acid) are molecules that carry genetic codes that are used for the development and functioning of an organism. DNA sequences are made up of different combinations of just four **nucleotides** (A, G, T, C). Every living thing has a unique genetic code, so DNA is a very useful tool for identifying mystery organisms – or in our case identifying the oyster murderer! Much like detectives on criminal investigation TV shows, scientists collect DNA samples from dead oysters to figure out who the murderer is. They will then use their list of suspects to figure out which primers to use in their PCR reactions. **Primers** are short, single-stranded DNA sequences that match specific sequences of their suspects. If the suspect DNA is in their crime scene sample, that primer will bind to their sample DNA, and cause that DNA to amplify in massive amounts during the PCR. Scientists will then visualize their PCR products to see whether any DNA got amplified, which is the evidence they need to convict the murderer.

Instructions You have been given sample DNA extracted from a dying oyster and three sets of primers each of which is known to attach to a specific sequence of DNA from one of each murder suspect (MSX, Dermo, and *Bonamia*). Your group must determine which of the three primer sets attaches to your sample DNA.

1. **Denature:** Split your double stranded DNA sample into single strands, and arrange both strands from 3' → 5' (i.e. have the 3' end of each strand on the left, and the 5' end of each strand on the right).
2. Arrange all of your primer sets from 5' → 3' (5' ends on the left and 3' ends on the right). This is important because the 5' end will attach to the 3' end of your DNA sample, and then DNA replication occurs from 5'→ 3'.
3. **Annealing:** Figure out which primer set matches with your DNA sample given that:

   Green froot loops attach to **Blue** froot loops

   Orange froot loops attach to Yellow froot loops

Hint: Both primers in a set must attach to one of the two strands of DNA, and primers will not attach to the same single strand. Primers may not attach to the first nucleotide (froot loop) in your strand, they may attach somewhere in the middle of the DNA strand!

4. **Extension:** Add nucleotides (froot loops) according to the same matching scheme above (**Green-Blue**, Orange-Yellow) to the strands with the correct primers to complete your new DNA strands. *Remember:* DNA replication occurs from 5'→ 3', so only add froot loops to the 3' end of your primers until you reach the end of your DNA sample strand.
5. Once your group has identified the correct primer and completed the first round of PCR, your teacher will distribute six additional correct primer sets to your group. Complete 2 more rounds of PCR using these primers by following steps 1-4 twice. **ALTERNATIVELY,** your teacher will distribute the "Products of 3 rounds of PCR" handout.

Name(s)_____

Date_____

Complete the questions below after your group has completed 3 cycles of PCR with the correct primers or by using the "Products of 3 rounds of PCR" handout.

1. How many <u>double</u> stranded DNA molecules do you end up with at the end of 3 cycles of PCR?

2. Normal PCR's complete 25-35 cycles. How many <u>double</u> stranded DNA molecules would you have after 25 cycles? Hint: It is a lot of DNA! Ok to express your answer in terms of an exponent if you don't have a calculator.

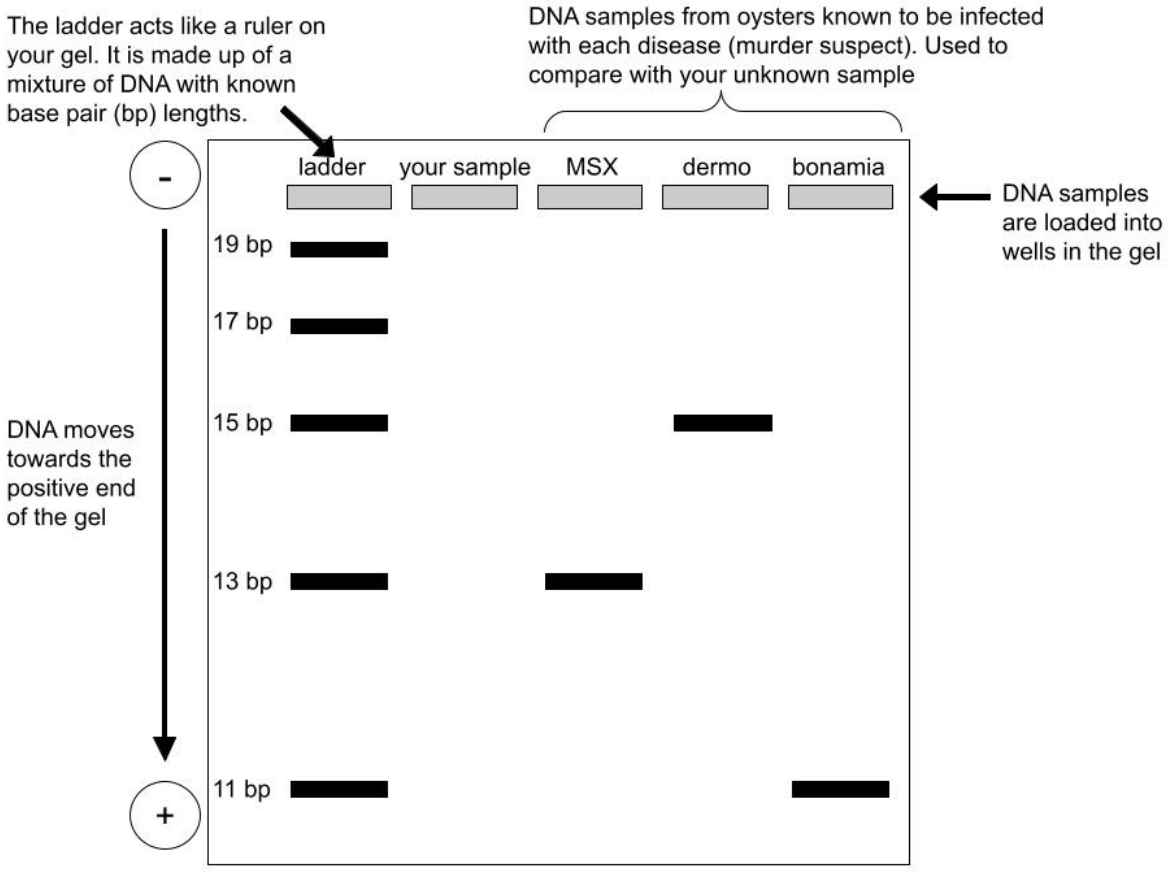
3. How many base pairs (froot loops long) are your shortest single stranded DNA molecules? Hint: include the froot loops in your primer.

4. How many of these shortest strands do you have compared to the longer strands?

The shortest strands are considered your target sequence, and will accumulate in large quantities after 25-35 cycles of PCR, while the other longer strands will not accumulate nearly as much because they include regions of DNA that aren't targeted by the primers.

When scientists perform a standard PCR, they don't know which primer (if any) have attached to their sample's DNA until they perform a gel electrophoresis. A gel electrophoresis is a method that separates mixtures of DNA according to their molecular sizes. A picture of one is shown below. An electric current is applied to a gel with loaded PCR products, and because DNA is negatively charged, the DNA begins to move to the positive end of the gel (opposites attract!). Smaller DNA fragments will move faster and further down the gel, while larger fragments will move slower and not get as far (think about if you were to push a shopping cart vs a truck down the road, which would you push faster and further?)

5. Below is a gel pre-loaded with a ladder (which is like a ruler with known base pair lengths) on the left, and bands for positive samples for the three murder suspects on the right. Draw the band associated with your target sequence (i.e. the shortest strand length) based on the PCR you performed under the well labeled "your sample".



6. According to your gel results, which disease is the oyster murderer?

7. What would you have seen on your gel if none of the primers were correct (i.e. none of the suspects had committed the murders)?

Products of 3 Rounds of PCR

Name(s)_____Instructor Key_____

Date_____

1. Are the average temperatures higher, lower, or on par with normal temperatures in the year 2009? (Fig 1)

Temperatures are normal (on par)

2. On average, has Williamsburg, VA been experiencing higher, lower, or normal temperatures from 1999-2009? (Fig 1)

Higher

3. What is Chlorophyll A and what is it a good indicator of? (Fig 2)

Chlorophyll A is the green pigment that algae (marine plants) use to absorb light, and can therefore be a good measure of water quality. Too much chlorophyll A means that the water may be polluted with nutrients from fertilizers and sewage, and too much algae is growing.

4. Describe the annual and decadal trend you see in chlorophyll A levels in the James River from 1999-2009. (Fig 2)

Annually, chlorophyll A oscillates from high values in the spring/summer time (plants grow in the spring/summer!) and drops in the winter time. Over decadal time scales, chlorophyll A has remained relatively stable, with the highest values reported in 1999-2001.

5. Estimate how many parasite cells (dark circles) you see in the dying oyster (left) and healthy oyster (right) in Figure 3.

25-35 cells in the dying oyster, 0 cells in the healthy oyster

6. Are any of the measured levels of contaminants listed in Figure 4 reaching levels that could potentially harm oysters?

No

7. Given all of the data compiled here, what do you think caused the oyster mortality event at Selfish Shellfish Oyster Farm? Explain why.

Disease. All other graphs/tables indicated very normal levels for the year 2009, with average temperature, normal Chlorophyll A levels, and low contaminant levels relative to action levels. The disease cultures though clearly showed that a sampled dying oyster had a disease, while a healthy oyster didn't, indicating that disease may be responsible for the mortality event.

Name(s)___Instructor Key_____

Date_____

Complete the questions below after your group has completed 3 cycles of PCR with the correct primers.

1. How many double stranded DNA molecules do you end up with at the end of 3 cycles of PCR?

   $2^3 = 8$

2. Normal PCR's complete 25-35 cycles. How many double stranded DNA molecules would you have after 25 cycles? Hint: It is a lot of DNA! Ok to express your answer in terms of an exponent if you don't have a calculator.

   $2^{25} = 33{,}554{,}432$

3. Count up how many nucleotides (froot loops) are on each of your DNA strands. How many base pairs (froot loops long) are your shortest single stranded DNA molecules? Hint: include the froot loops in your primer.

   15 base pair's

4. How many of these shortest single strands do you have compared to the longer strands?

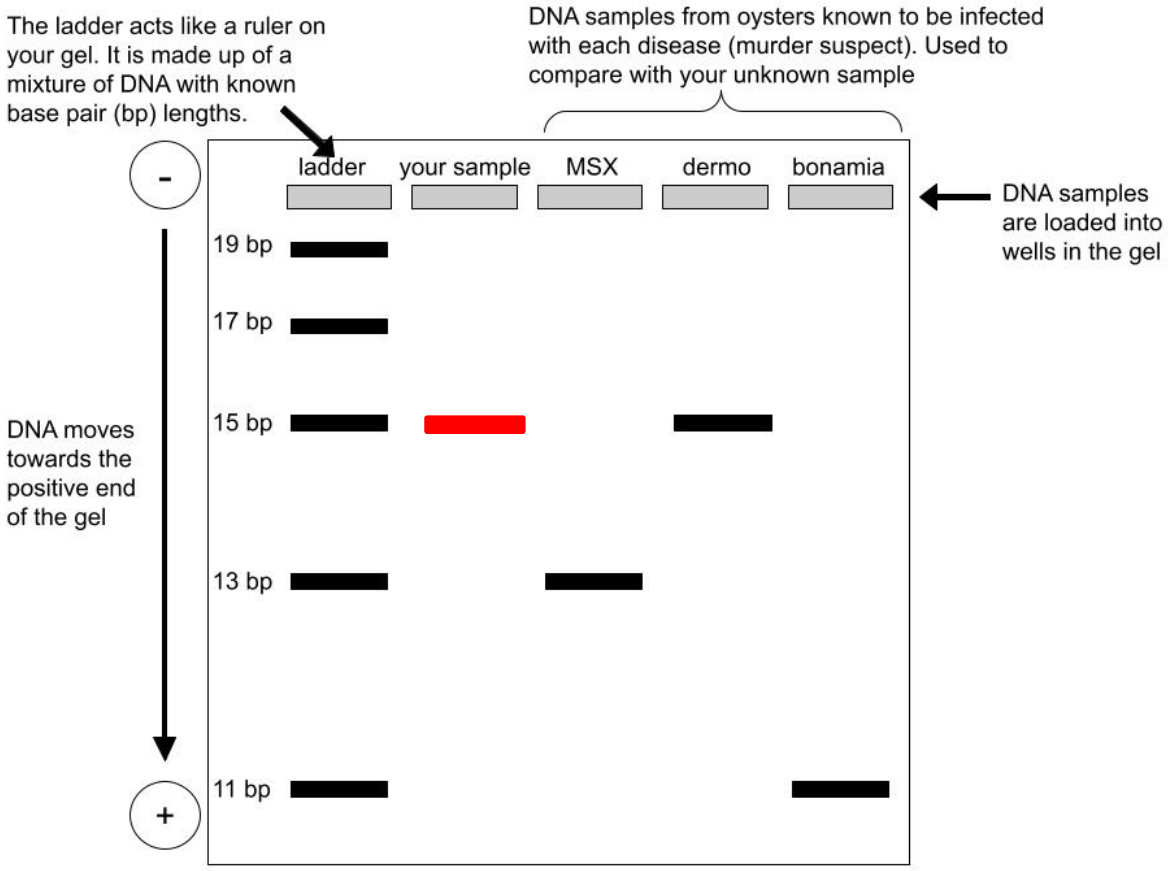
   11 strands that are 15 base pair's

   5 strands that are 20 base pair's

The shortest strands are considered your target sequence, and will accumulate in large quantities after 25-35 cycles of PCR, while the other longer strands will not accumulate nearly as much because they include regions of DNA that aren't targeted by the primers.

When scientists perform a standard PCR, they don't know which primer (if any) have attached to their sample's DNA until they perform a gel electrophoresis. A gel electrophoresis is a method that separates mixtures of DNA according to their molecular sizes. A picture of one is shown below. An electric current is applied to a gel with loaded PCR products, and because DNA is negatively charged, the DNA begins to move to the positive end of the gel (opposites attract!). Smaller DNA fragments will move faster and further down the gel, while larger fragments will move slower and not get as far (think about if you were to push a shopping cart vs a truck down the road, which would you push faster and further?)

5. Below is a gel pre-loaded with a ladder (which is like a ruler with known base pair lengths) on the left, and bands for positive samples for the three murder suspects on the right. Draw the band associated with your target sequence (i.e. the shortest strand length) based on the PCR you performed under the well labeled "your sample".



6. According to your gel results, which disease is the oyster murderer?

dermo

7. What would you have seen on your gel if none of the primers were correct (i.e. none of the suspects had committed the murders)?

You would see no band under the well for "your sample" as none of the DNA would have amplified.

Lab Prep Manual:

For each group of students, you will need to prepare 1 double stranded DNA molecule (two pipe cleaners with 20 froot loops on each pipe cleaner) and 3 sets of primers (6 pipe cleaners with 5 froot loops on each pipe cleaner). The froot loops must match the following color code for the activity to work:

G = green froot loop, Y= yellow, B= blue, O= orange

DNA molecule (5'→3'):

Strand 1: **GYYBOBOOGGBYYOGOYBBY**

Strand 2: **OGGOYBYOOGBBYYGYGOOB**

With a black sharpie marker write "5" on the first froot loop of the strand (green for strand #1, and orange for strand #2) and write "3" on the last froot loop of the strand (yellow for strand #1, and blue for strand #2). Write the numbers on the corresponding froot loop in three different spots, so students can easily identify the 5' and 3' ends. Coil the pipe cleaners with one strand going 5'→ 3' and the other going 3' → 5', to create a complimentary double stranded DNA strand.

Primer Set #1 (correct primer, 5'→3'):

Strand 1: **GYYBO**

Strand 2: **BYOOG**

Primer Set #2 (incorrect primer):

Strand 1: **BGBGB**

Strand 2: **OOOBG**

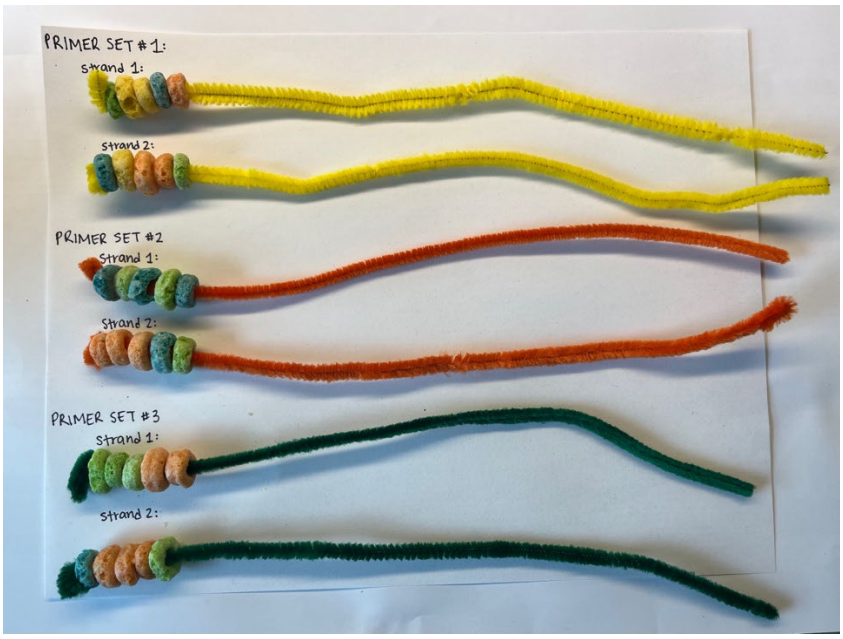
Primer Set #3 (incorrect primer):

Strand 1: **GGGOO**

Strand 2: **BOOOG**

Time and resource permitting, students should complete three rounds of PCR, using an additional six sets of primer set #1. Teachers will either distribute these six more sets (12 additional pipe cleaners for each group, with the froot loop color sequence from primer set #1 above), **OR** (to save time and resources) distribute the "Products of 3 rounds of PCR" handout **AFTER** the group has identified the correct primer set.

TIPS: Mark the 5’ and 3’ ends with a black sharpie. Once assembled, flip the second strand so its orientation is 3’→5’ and coil the two strands together to produce a double-stranded helix, to reinforce to students the structure of DNA.



TIPS: Use different colored pipe cleaners for each primer set for easy classroom set-up and clean-up. Fold over the 5’ end of the pipe cleaner (as pictured) so froot loops don’t slide off. You can label the first froot loop with a marker as the 5’ end or simply let students know that the folded/short end of the pipe cleaner is the 5’ end, so they add froot loops to the correct end of the primer sequence.