Name:_____ Class: _____ Date: _____

Polymerase Chain Reaction (PCR) Introduction

Introduction:

When a DNA sample is collected from an organism or from a crime scene, there is often very little DNA in the collected sample. If there are only a few desired DNA fragments in a sample placed in a gel, there will not be enough DNA to form a band on the gel. Scientists need millions of copies of the sequence they want to study. Scientists can use Polymerase Chain Reaction (PCR for short) to **amplify a specific sequence**, or make many, many copies of the particular fragment they are interested in.

To do a PCR reaction, the scientist must know the sequence of the ends of the DNA fragment he/she is interested in. The scientists designs small pieces of RNA called **primers** that can base-pair to the ends of the desired fragment. These RNA primers mark the ends of the segment the scientist wants to study and tell the reaction what DNA fragment to copy. In the diagram below, the double line represents the DNA strand, arrows represent the RNA primers, and the small vertical lines represent base-pairs between the primer and the DNA strand. The scientist also adds water, spare nucleotides, and an enzyme that copies DNA.

Initial Sample (only a few copies in scientist's sample)

Inside the tubes in the PCR machine called a thermal cycler, multiple complex steps take place over the course of a few hours to copy the sequence many times.

PCR product:

Millions of copies of the desired fragment between and including the sequences that base-paired with the RNA primers in the tube

Remember:

Often the initial sample has other DNA that the scientist doesn't want to study. By performing the PCR reaction, the scientist makes copies of the DNA fragment that he/she does want to study. The DNA that the scientist doesn't want to study **is still in the product tube**, but only in small numbers. The desired fragment will outnumber the undesired DNA by millions to 1.

Once the scientist has a tube containing millions of copies of the DNA (s)he wants to study, the DNA can be isolated on a DNA gel using a process called DNA gel electrophoresis.

Name:_____ Class: _____ Date: _____

Polymerase Chain Reaction (PCR) Questions

Answer the following questions in complete sentences.

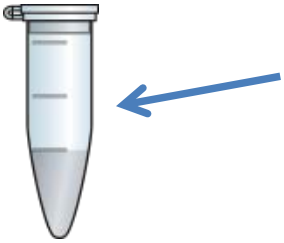
1. In what situation do scientists need to use the PCR reaction?

_____

_____

_____

2. Make a list of the chemicals and reactants go into the tube before it goes in the PCR machine.



_____

_____

_____

_____

3. What is the PCR machine called? _____

4. What is the purpose of the RNA primers in the reaction?

_____

_____

5. How can scientists see what is in their reaction tube after the PCR reaction?

_____

_____

6. Why are the spare nucleotides needed in the reaction?

_____

_____

7. What would you expect would happen if the scientist forgot to put the DNA copying enzyme in the tube before putting the tube in the machine?

_____

_____

Name:_____ Class: _____ Date: _____

Restriction Enzyme Analysis: Introduction

Introduction:
Restriction enzymes are special enzymes harvested from unique bacteria that scientists use to cut DNA. Restriction enzymes are like little "molecular scissors"! They cut DNA but only at specific sequences. Examine the DNA sequence below and find the sequence GAATTC in the top DNA strand. Circle it and the bases below the sequence.

Uncut DNA:

TGATCGTGGAATTCGATGATCGATGCTAGCTGAA
ACTAGCACCTTAAGCTACTAGCTACGATCGACTT

Notice that the sequence below the GAATTC sequence (CTTAAG) is identical, just backwards! A lot of restriction enzymes recognize sequences called "palindromic sequences".

The restriction enzyme cuts the sequence into two pieces:

G | AATTC
CTTAA | G

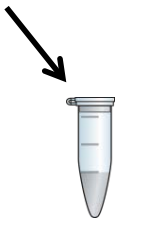
If the sequence above was cut, the results would look like this:

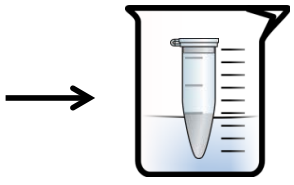
Piece 1:
TGATCGTGG
ACTAGCACCTTAA

Piece 2:
AATTCGATGATCGATGCTAGCTGAA
GCTACTAGCTACGATCGACTT

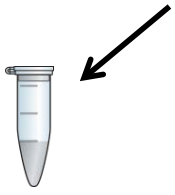
The procedure for performing Restriction Enzyme Analysis is shown below:

Uncut DNA + Restriction Enzyme





Cut DNA with smaller fragments, cut at specific sites



The scientist places the tube in a beaker/hot water bath at the enzyme's ideal temperature.

After 30-60 minutes, the scientist heats up the tube to denature or "disable" the enzyme to stop the reaction.

The scientist can use a micropipette to take the new DNA fragments in solution and place them in a gel for Gel Electrophoresis Analysis.

Name:_____ Class: _____ Date: _____

Restriction Enzyme Analysis: Questions

1. Why do scientists use restriction enzymes?

_____

_____

2. What would scientists use to see their newly cut fragments of DNA?

_____

_____

3. Look at the sequences below. If you add a restriction enzyme(that cuts at GAATTC) to the uncut DNA, how many DNA fragments will result? Circle the fragments.

Uncut DNA 1:

TGATCGTGGAATTCGATGATCGAATTCGCTAGCTGAATTCAAAAAA
ACTAGCACCTTAAGCTACTAGCTTAAGCGATCGACTTAAGTTTTTT

Number of fragments: _____ Length of fragments: _____ basepairs (ignore ends with unpaired bases)

Uncut DNA 2:

TGATCGTGGACTTCGATGATCGAATTCGCTAGCTGAATTCAAAAAA
ACTAGCACCTGAAGCTACTAGCTTAAGCGATCGACTTAAGTTTTTT

Number of fragments: _____ Length of fragments: _____ basepairs (ignore ends with unpaired bases)

Uncut DNA 3:

TGATCGTGGACTTCGATGATCGAATTCGCTAGCTGCATTCAAAAAA
ACTAGCACCTGAAGCTACTAGCTTAAGCGATCGACGTAAGTTTTTT

Number of fragments: _____ Length of fragments: _____ basepairs (ignore ends with unpaired bases)

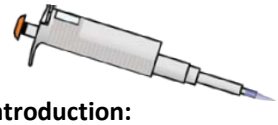
4. You are given a new sample of DNA that matches one of the uncut samples above (1, 2, or 3). How could you use restriction enzyme analysis to match your unknown sample to one of the known samples (1, 2, or 3)? Explain below.

_____

_____

_____

Name:_____ Class: _____ Date: _____



Gel Electrophoresis Introduction

Introduction:

Gel Electrophoresis is a lab procedure scientists use to separate pieces of DNA by **length**.

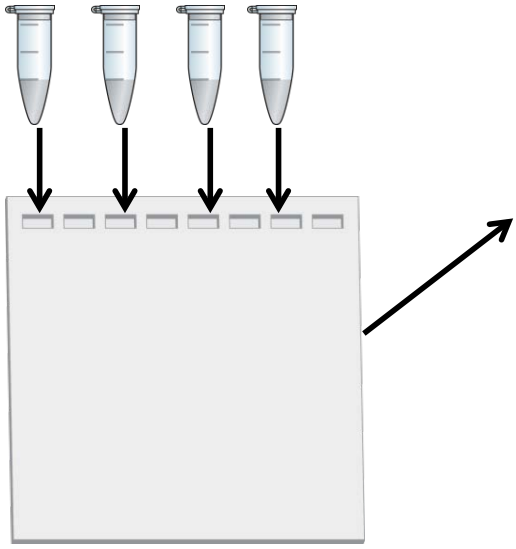
How it works:

1. A scientist uses a micropipette (a hand-held tool that acts like a precise mini-syringe) to place samples of DNA inside wells in a gel square made of agarose, a thick substance that looks similar to really thick, translucent Jello. The gel also contains a chemical called ethidium bromide. Ethidium bromide sticks to the DNA and causes the DNA to glow under UV light.
2. The gel is placed in a liquid solution called "buffer" inside a gel electrophoresis chamber. The buffer helps electricity flow through the gel.
3. An electric current is applied to the solution inside the gel electrophoresis chamber by hooking the box up to a power supply.
4. DNA is negatively charged, so it is attracted to the positive electrode of the gel electrophoresis box and starts to move through the gel towards the positive electrode.
5. Small DNA fragments move faster through the thick "mesh" or "matrix" of the agarose gel. Larger DNA fragments travel slower.
6. After some time, the gel is removed from the box. The DNA fragments (stained with ethidium bromide) form bands on the gel that can be viewed by shining a UV light through the gel.

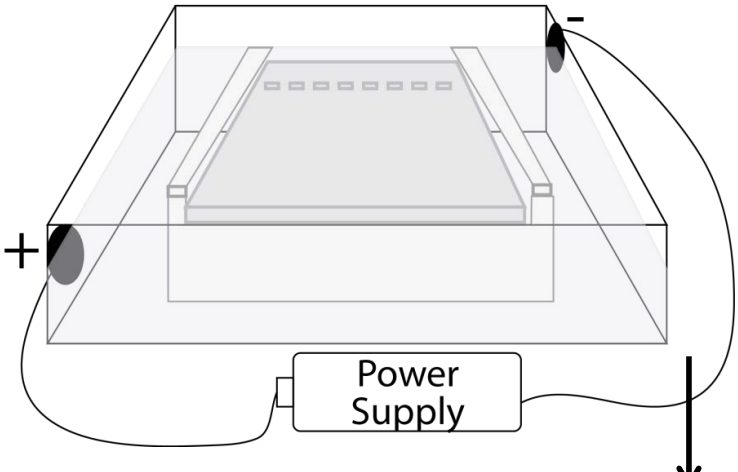
In the diagram below, 10, 100, 500, 1000 identifies the length of the DNA fragments in each tube in base pairs.

Wells

1	3	5	7
10	100	500	1000 and 100



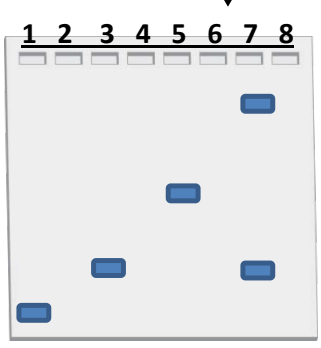
Gel Electrophoresis Chamber for Approximately 30 Minutes



Note that lane 1 (containing the smallest length DNA fragment) traveled the furthest on the gel towards the positive electrode. And a fragment in lane 7 traveled the least.

See in Lane 7 that two different size DNA fragments in the sample are separated on the gel. You know the fragment that traveled farther is the same length (100 bp) as the fragment in lane 3, because it traveled the same distance.

Also, notice that no DNA solution was added to wells 2, 4, 6, or 8 and there is nothing in those lanes.



Under UV Light

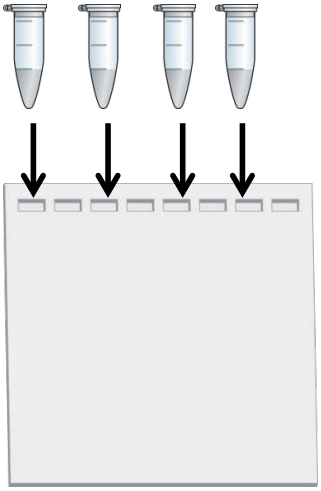
Name:_____ Class: _____ Date: _____

Gel Electrophoresis is useful in identifying the size of an unknown DNA fragment. Scientists compare unknown DNA samples to a control sample called **a marker sample or a "ladder"**. The ladder sample contains several known lengths of DNA.

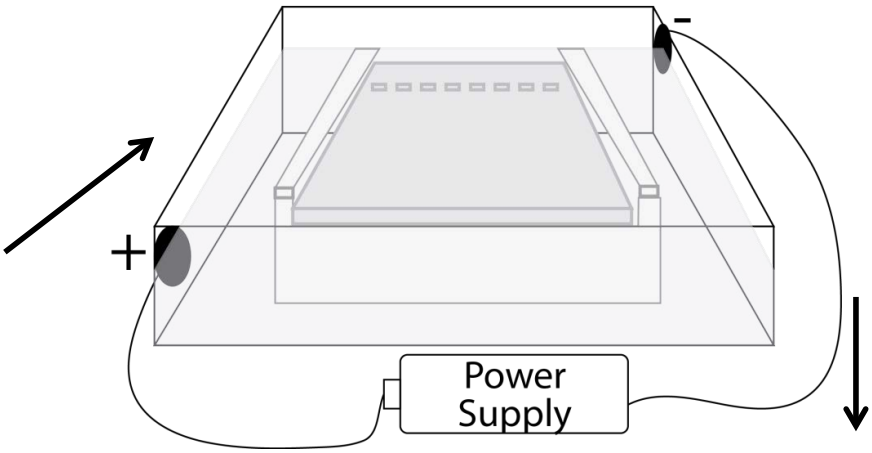
In the example below, the ladder contains known fragments of DNA with lengths 100, 300, 500, 800, 1000, and 2000 base pairs long.

Wells/Lanes #

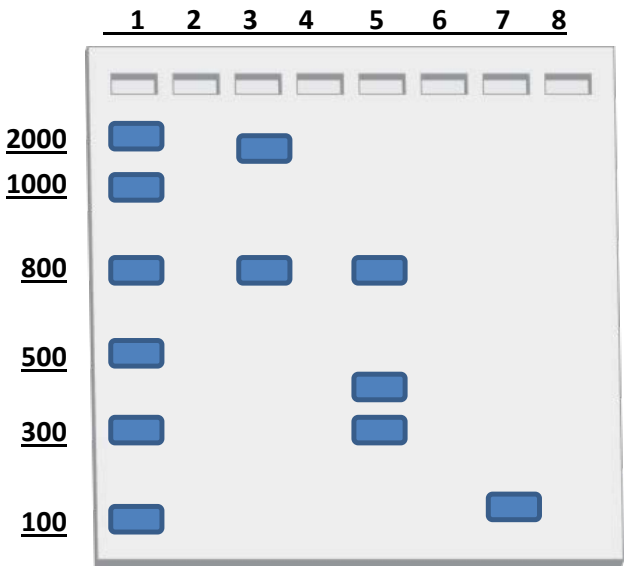
1	3	5	7
Ladder	?	?	?



Gel Electrophoresis Chamber for Approximately 30 Minutes



Under UV Light



Because we know the ladder's standard fragment size, we can use this to estimate the unknown fragments in lanes 3, 5, and 7.

In Lane 3, there are at least 2 different fragments: one has a length of between 1000 and 2000 bp. One has a length close to 800 bp.

In Lane 5, there are at least 3 different DNA fragments. One is about 800 bp, one is between 300 and 500 (estimate 400), and one is about 300 bp.

In Lane 7, there is at least 1 fragment that is close to 100 base pairs in length.

It is important to note what DNA gel electrophoresis cannot do. When reading a DNA gel's bands, you cannot know if a **sample has 2 or more different fragments of the same length.** In the gel above, lane 7 might have 1, 2, 3, 4, or many different DNA fragments with different sequences of very similar length (around 100 bp). There is no way to distinguish between different DNA fragments with the same length using just DNA gel electrophoresis.

Name:_____ Class: _____ Date: _____

Gel Electrophoresis Questions #1

Answer the following in complete sentences.

1. What tool is used to transfer small liquid solutions of DNA from tubes into the gel?

_____

2. What causes the DNA fragments to move within the gel?

_____

_____

3. Which DNA fragments move faster? Which DNA fragments move slower?

_____

_____

4. What causes the normally colorless DNA to glow under UV light?

_____

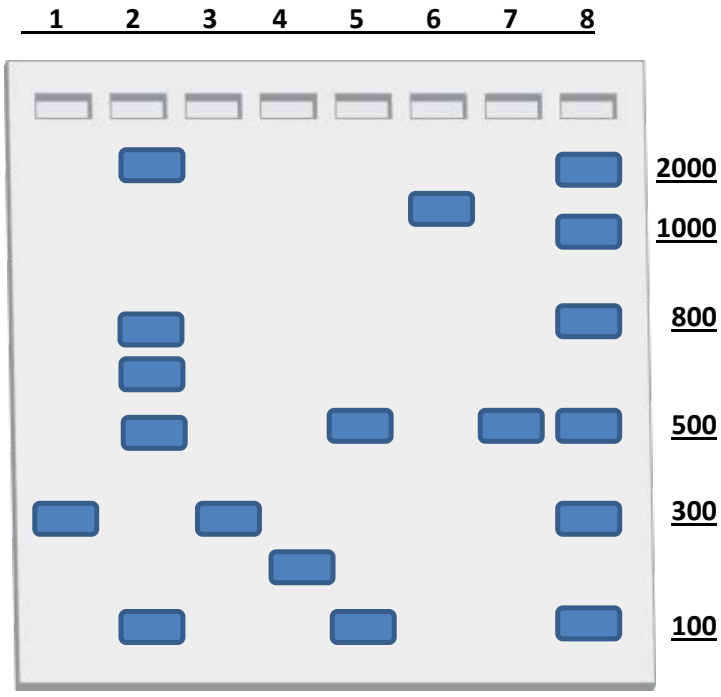
5. What is the purpose of using a marker or ladder in one lane on your gel?

_____

_____

6. In the table below, estimate the lengths of DNA fragments in each lane. Lane 8 has your marker and the lengths are written on the right.

Sample	DNA Fragment Sizes
1	
2	
3	
4	
5	
6	
7	



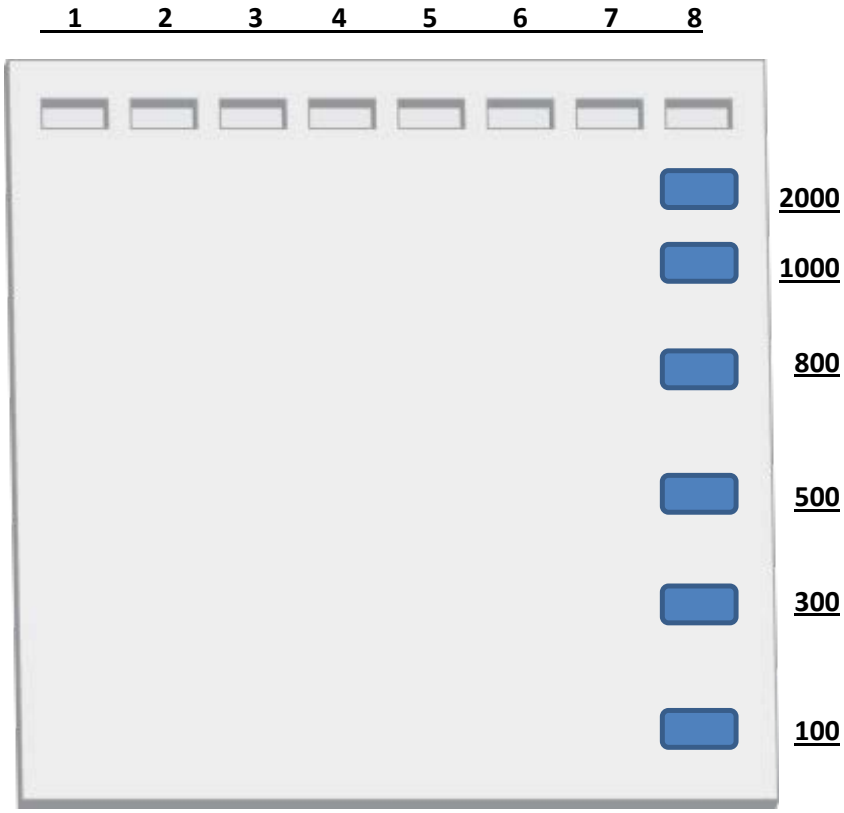
Name:_____ Class: _____ Date: _____

Gel Electrophoresis Questions #2

Answer the following in complete sentences.

7. In another experiment, you plan to run a gel on a set of samples. Use the table below with DNA fragment lengths and draw the bands on the gel. The marker bands are completed for you in lane 8.

Sample	DNA Fragment Sizes
1	300
2	1000, 200
3	100, 200, 300
4	700, 900, 1500
5	50
6	2000, 900
7	3000



8. What would you expect to happen if you left the gel accidentally in the gel electrophoresis chamber for too long?

_____

_____

9. A student makes a new gel using agarose, water, and buffer solution. The student loads their DNA samples in their wells and places the gel in the chamber for an appropriate length of time. When the student places the gel on the UV lightbox, no DNA bands show up at all. Even the marker lane is clear and has no bands! What mistake did the student make? Explain your answer.

_____

_____

_____

_____

Name:_____ Class: _____ Date: _____


The Mysterious Death of Mr. Bawdee

Introduction:

You are a private investigator hired to investigate the murder of Mr. Bawdee. Unfortunately, the only evidence you have is DNA evidence collected from different items in the mansion he used to live in. He was killed following a dinner party at his mansion. Mr. Bawdee's body was found in the Dining Room. You obtain DNA samples from all of his party guests and from his body, currently in the morgue. The possible suspects include Professor Plum, Miss Scarlett, and Colonel Mustard. He was killed by a blunt force hit to the head and you also obtain DNA samples from several possible murder weapons. The possible weapons are the Lead Pipe (found in the Dining Room), the Candle Stick (found in the Hall), and the Wrench (found in the Kitchen).

DNA from Possible Weapons

Wrench

Candle Stick

Lead Pipe

DNA from Possible Suspects

Mr. Bawdee

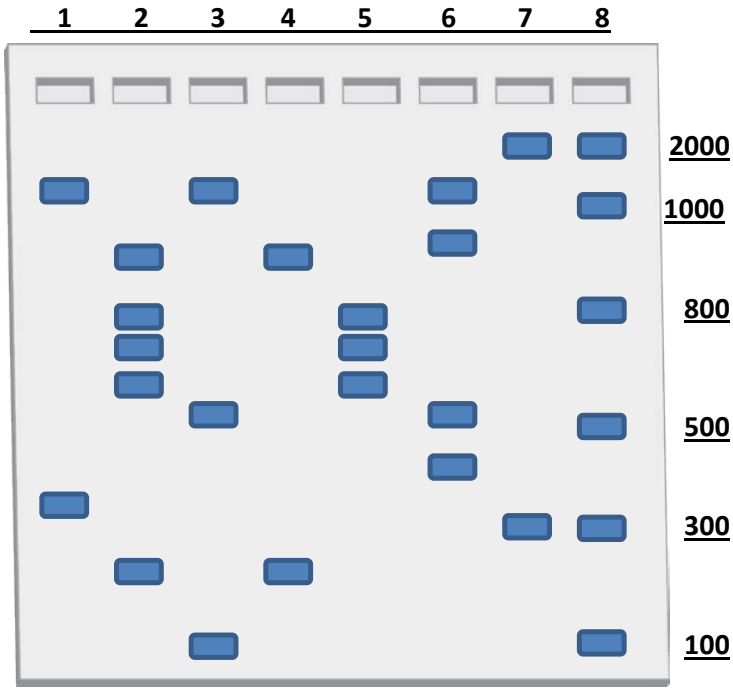
Prof. Plum

Miss Scarlett

Colonel Mustard

To examine your DNA samples, you use restriction enzymes to digest or "cut" the DNA into smaller fragments. In different DNA samples, restriction enzymes that cuts only at GAATTC sites will create different fragments. If two DNA samples match, the restriction enzyme should create the same fragments. You then run your samples on an agarose gel using the Gel Electrophoresis process. The gel's results are shown below. Lane 8 shows the ladder marker bands.

Sample	DNA Fragment Sizes (Estimated)
1 (Wrench)	
2 (Candle Stick)	
3 (Lead Pipe)	
4 (Mr. Bawdee)	
5 (Prof. Plum)	
6 (Miss Scarlett)	
7 (Colonel Mustard)	



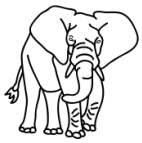
Mr. Bawdee was killed by _____ in the _____ with the _____.
(suspect) (place) (weapon)

Explain your answer:

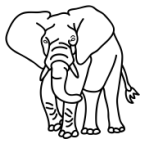
_____

_____

Name:_____ Class: _____ Date: _____



Who's the Daddy: Elephant Edition



Introduction:

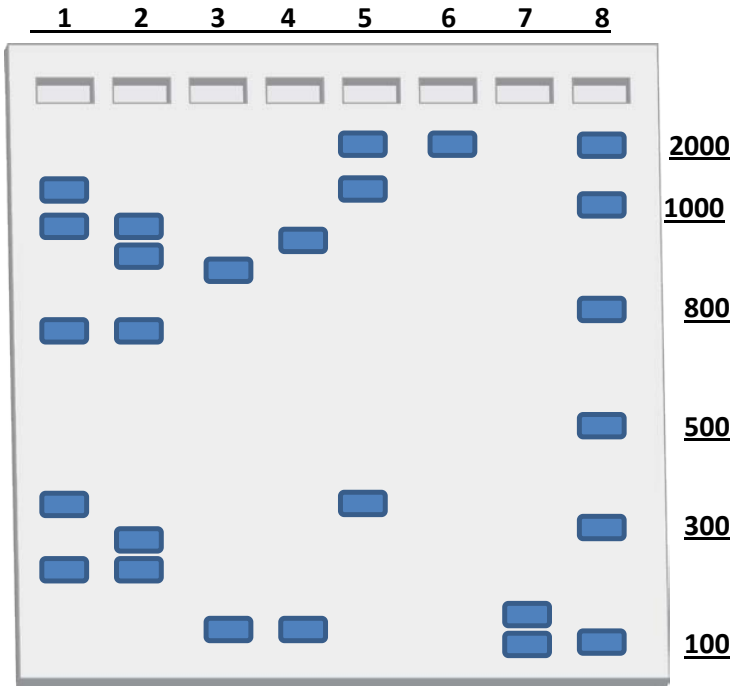
As an animal caretaker at one of the largest wildlife reserves in the world, it's your job to watch out for all your animals and keep watch for health problems. You discover that one of your female elephants (Ellie) is pregnant! After 21 long months, she gives birth to a healthy baby male elephant! In order to create a medical history file for your new baby elephant (Elmer), you take DNA samples from Ellie, Elmer, and 5 eligible bachelor males who may have sired the newest addition to the reserve.

DNA from Baby and Eligible Parent Elephants

Elmer **Ellie** **Edward** **Edmund** **Evan** **Eric** **Elijah**

To examine your DNA samples, you use restriction enzymes to digest or "cut" the DNA into smaller fragments. In different DNA samples, restriction enzymes that cut only at GAATTC sites will create different fragments. Baby **Elmer will share some fragments that match his mom Ellie. Any fragments that are present in Elmer that don't match Ellie's MUST match some of his father's DNA bands.** You then run your samples on an agarose gel using the Gel Electrophoresis process. The gel's results are shown below. Lane 8 shows the ladder marker bands.

Sample	DNA Fragment Sizes (Estimated)
1 (Elmer)	
2 (Ellie)	
3 (Edward)	
4 (Edmund)	
5 (Evan)	
6 (Eric)	
7 (Elijah)	



The father of baby Elmer is _____.

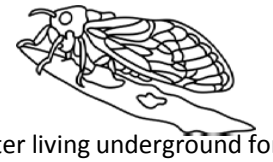
Explain your answer:

_____

_____

Name:_____ Class: _____ Date: _____

The Cicada Conundrum



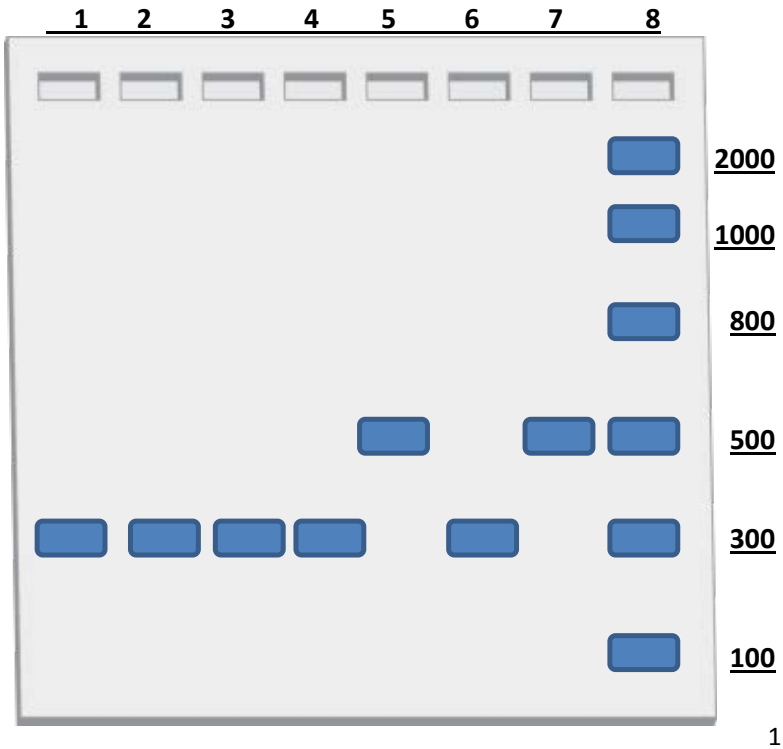
Introduction:
In North American summers, every year, there are cicada nymphs that emerge after living underground for a year or two. These nymphs, the immature form, crawl out of the ground, claw their way out of their old skin to form the adult form. The adults begin flying and singing very loudly, looking for mates. But the amazing thing about cicadas is that some species, that live in the same areas as the annual species, stay underground for 13 or 17 years! These longer-living species have synchronized broods, meaning that the cicadas of a particular species in a particular geographic area hatch all in the same few weeks in the same July and August. During these few short weeks, these cicadas mate with members of their own species, lay their eggs, and die, often covering people's yards with their bright orange and red speckled bodies. Annual and 17 year cicadas can occupy the same territory and it can be tough sometimes to identify which cicada is which. Adult seventeen year old cicadas usually have orange or reddish markings, while the adult annual cicadas often have green markings. But both species have translucent-brownish nymphs and it can be difficult to tell the difference during the nymph stage.

Your job: As an excited entomologist with a new PCR machine and a new gel electrophoresis chamber, you set out to study the nymphs underground in your back yard. Has the 17 year old cicada species taken root in your area? Do the annual cicadas outnumber the 17 year species underground? It's April, so all cicadas in your local ecosystem are still underground. You decide to dig for nymphs and to your surprise, you find 7 of them in one small area of your yard!

You perform some DNA extraction procedures and you collect their DNA in 7 small tubes.
You use PCR to isolate one particular segment of DNA from the cicada called *buzzer*. As far as scientists know, *buzzer* is a cicada specific DNA sequence, meaning so far scientists have only found this sequence in cicadas and not in other insects. You know from previous studies that when isolated using two RNA primers you have, *buzzer* is 300 base pairs long in the annual species of cicada. In the 17 year species, *buzzer* is 500 base pairs long. You run a DNA gel on your 7 PCR products from your 7 nymph DNA samples.

After PCR and Gel Electrophoresis - Gel Under UV Light

Lanes 1-7 have your nymph DNA samples. Lane 8 has a marker ladder with DNA fragments of standard sizes.



1. How many nymphs are annual cicadas? Which ones?

_____

_____

_____

2. How many nymphs are 17 year cicadas? Which ones?

_____

_____

_____

Name:_____ Class: _____ Date: _____

Your results seem promising, so you decide to do a larger scale study.

You spend a lot of time digging up your whole backyard, up to a few feet deep, to get more nymph samples. You find 300! You extract DNA, you run the PCR machine with your samples, and you run several DNA gels through the gel electrophoresis chamber. Here are your results.

Number of samples with 300 bp segment	Number of samples with 500 bp segment
120	170

3. What does this data suggest about the prevalence of the 17 year old cicada in your area? Would you expect them to outnumber the annual cicadas the next time they emerge, assuming that the number of annual cicadas stays about the same from year to year?

_____

_____

4. Notice that the number of your 300 bp samples and 500 bp samples add up to 290, not 300. Here is what happened to your other 10 samples.

9 samples have no bands show up on the gel in those lanes when the samples are run through the gel electrophoresis process.
1 sample had 1 band at 800 base pairs.

Write **two possible** explanations to explain why 9 of your samples have no bands on the gel. Explain your answer.

_____

_____

_____

_____

_____

_____

5. Write a possible explanation to explain why 1 of your samples have a strange 800 base pair band on the gel. Explain your answer.

_____

_____

_____

_____