Bio 208 Lab 14 – PCR Prelab Name Erika Neugebauer

NOTE: This part of the lab is **DUE** when you arrive at the **BEGINNING OF THE LAB PERIOD**. IF THIS IS NOT COMPLETED BEFORE THE LAB PERIOD, 5 POINTS WILL BE DEDUCTED FROM THE LAB TOTAL GRADE (20 pts).

Part 1 – A copy of the **Edvotek 330- The Molecular Biology of DNA Polymerase Chain Reaction Student Handout** is included with this email. You do not have to print out the entire the Edvotek handout (only the experimental procedure, **Edvotek 330- PCR Experimental Procedure**) which is also attached as a separate document. All handouts are on Blackboard. You will need to read the Edvotek handout in order to answer questions in this prelab and be prepared to complete the lab. Part of the prelab assignment is to do the following:

______1. Bring **Edvotek 330- PCR Experimental Procedure** to lab (see Part 3) _____2. Bring safety goggles to lab

Part 2 – PCR is explained in pp. 5-7 of The **Edvotek 330 PCR Handout** and **Section 17.3 The Polymerase Chain Reaction Is a Powerful Technique for Copying DNA, p. 346+** in your **Essentials of Genetics** textbook. Answer the questions below.

1. IN PLAIN WORDS explain the purpose of PCR (if you copy from Edvotek, you will get no points).

The purpose of PCR is multiply a small amount of DNA to a substantial example, which can then be used for different tests.

2. Figure 17-8, p. 348 in your textbook, and Figure 1, p. 7 in the Edvotek handout diagram the PCR process. PCR involves three steps which are repeated many times (the number of repeats are called cycles). For each of the three steps listed below, explain IN PLAIN WORDS:
i.. Explain what happens in the step. ii. Explain how it is accomplished (if you simply copy from Edvotek, you will get no points).

Step 1 – Denaturation alters the structure creating single strands of DNA. This is done by heating the DNA to 95°C.

Step 2 – Annealing occurs when lowering the temperature (54°C), which causes primers to join or pair up with each single strand template.

Step 3 – Increasing the temperature to 72°C causes extension to happen, which is when polymerase copies the template to make the DNA double stranded again.

3. Page 3 in the Edvotek handout lists the following ingredients that are necessary to carry out PCR for our lab. Explain the role for each of the items listed below (if you simply copy from Edvotek, you will get no points).

dNTP mixture (might have to read p. 5 if you can't figure it out) –
This mixture together with the primer mix and helps the annealing process of joining primers to the single strand.

Taq DNA Polymerase – This polymerase is very heat resistant and copies the DNA single strand template in step 3.

Primer mix – This is mixed with the dNTP mixture. These primers line up with the ends of the DNA strands in step 2.

DNA template – This is where the primers join to create + amplify more double stranded DNA.

4. Kary Mullis who developed PCR in the 1980s was later awarded the Nobel prize for his achievement. Some think the key to his success was the use of the *Taq* DNA Polymerase. Explain **where** *Taq* DNA Polymerase comes from **AND why** its discovery was so important to PCR.

Taq DNA come from Thermus aquaticus bacterium which are extremely heat resistant since they live in hot springs. This was important because PCR has some high temperatures that polymerase needs to survive through & not get denatured.

5. PCR is actually a conceptually simple idea. In three steps, a DNA strand is copied once resulting in two strands of DNA. The three steps are repeated with the two strands each being copied resulting in 4 strands. Repeat them again and you will have 8 strands. These three steps make up one cycle. After three cycles, we ended up with 8 strands ($2^3 = 8$ where 3 is the number of cycles). In this lab we will carry out PCR for 15 cycles and 30 cycles. Using the formula, $2^n = N_n$ where n is the number of cycles and N = number of DNA copies, calculate the number of copies we will make in the lab for 15 cycles (N_{15}) and 30 cycles (N_{30}). NOTE: we actually will end up with many more copies because we will start with more than one original copy.

N_{15} = 32,768 N_{30} = 1,073,741,824

Part 3 – We will be following the procedure outlined in **Module I –Option A** (pp.10-11) and **Module II**, p. 15, and p. 33 (staining) in **Edvotek 330- PCR Experimental Procedure** handout. Underline (or highlight) key steps and/or write notes and questions in the margins. **Be prepared to show this to the lab instructor at the beginning of Thursday's lab.**

Bio 208 Lab 14 – PCR Analysis **Name** Josi Zarcia.

DUE: Wednesday, December 10

Overview of Lab. PCR is used to greatly increase the number of copies of DNA fragments from a few copies to billions in a matter of an hour or two. Basically, PCR mimics replication in a non-cellular environment by providing all the components necessary for replication and inducing the process through moderating the temperature of the DNA environment. Three changes in temperature over a 2-3 minute period will create one replication event, resulting in a doubling of the DNA. If the temperature changes are repeated again, the amount of DNA will be doubled again. Figure 17-8, p. 348 in your textbook, and Figure 1, p. 7 in the Edvotek handout illustrate this diagrammatically. Each series of temperature changes is referred to as a cycle. Each cycle doubles the amount of DNA that was available at the beginning of that cycle. Assuming that you would start with one double strand of DNA, the number of copies of DNA generated through PCR would be equal to 2^n where n = number the cycles. For example, if you started with a single double strand of DNA and ran PCR for 5 cycles, you would end up with $2^5 = 32$ copies of DNA when you were done. In reality, 32 copies of DNA is not very much DNA to a genetics researcher. Therefore, they carry out PCR for many more cycles than 5. In this lab we conduct PCR on the same DNA strands for 15 cycles and for 30 cycles. Our analysis will be fairly straightforward. We will compare three different samples of the same DNA: 1. part of the original sample that did not undergo PCR: 2. part of the original sample that went through 15 PCR cycles; and 3. part of the original sample that went through 30 PCR cycles. We will test the effectiveness of PCR by conducting electrophoresis on the samples. The DNA strands are the same length, therefore they should travel the same distance in the electrophoresis. However, if there is more DNA, the band will take up more stain and be easier to see. The lab will be carried out by five teams (2-3 people on a team). We will follow the protocol as outlined in the Edvotek handout with specific adjustments outlined below.

Everyone in the lab will be required to wear goggles and gloves

Materials.

Each team should identify the following items needed which will be provided on a tray:

- Tubes with PCR reaction pellets
- three 0.5 ml tubes for electrophoresis samples
- one 0.2 ml tubes for PCR sample
- small plastic tray (for electrophoresis staining)
- small plastic tray (for electrophoresis destaining)
- forceps
- Tube C (Standard DNA markers)
- Tube D (Enzyme grade ultrapure water)
- Tube E (DNA template)
- Permanent marker
- Electrophoresis apparatus
- Electrophoresis buffer solution
- Ethidium Bromide staining instructions handout
- FlashBlue Staining instructions handout
- Tube B (Primer Mix)
- InstaStain Ethidium Bromide/FlashBlue Stain
- 10X gel loading solution

The following items will be shared by all teams:

- Microcentrifuge
- DNA visualization light (regular)
- DNA visualization light (UV)
- PCR Intsrument

Part 1 – PCR

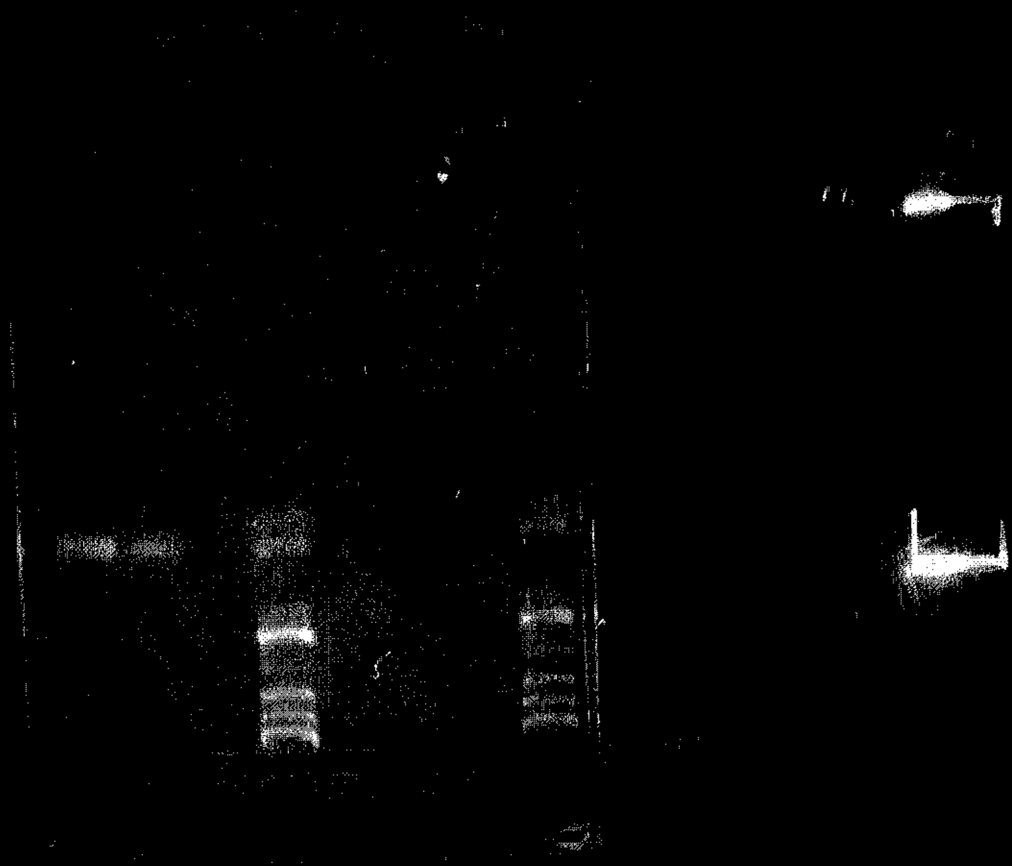
1. The Experiment Overview (p. 8) gives a brief summary of what we will be doing. Note: We will not do Part III – Size Determination of the PCR Amplified DNA Fragment
2. Follow steps 1-15 on pages 10-11 for conducting your PCR. Note the following steps
   - STEP 3 – We will be using 0.2 µl sized tubes designed for Concordia's PCR instrument.
   - STEP 7 – Concordia's PCR instrument has a heated lid (therefore, wax beads not needed)
   - STEP 8 – The PCR has been pre-programmed to match the specifications listed. You will be shown this on the PCR machine.
   - STEP 10 – We did not have to add wax (see STEP 7 above)
   - STEP 12 – we are not storing our samples overnight (unless we are way behind in time)

Part 2 – Electrophoresis

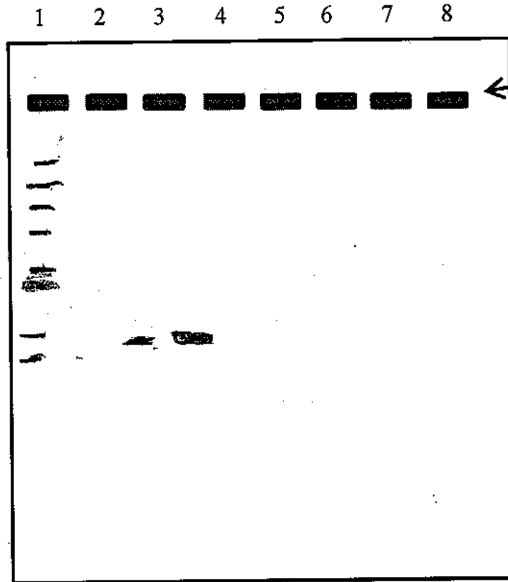
1. Gels were prepared ahead of time for this lab.
2. Since each team only needs 4 lanes in a gel, two teams will share a gel with one team using lanes 1-4 and the other using lanes 5-8.
3. Follow steps 1-8 on page 15 for carrying out electrophoresis.

Part 3 – Staining your electrophoresis gels – two options

Option 1 – Instastain Ethidium Bromide Cards - Follow the procedure for staining and destaining the gel as outlined in steps 1-5, Appendix F, page 33.



Part 4 – PCR results. Write out the source of DNA that was in each lane. Mark the approximate positions for the DNA fragment bands in your electrophoresis gel as well as the other team's on the diagram below. Attempt to recreate the approximate intensity of the band (estimating band intensity is the purpose for carrying out electrophoresis in this lab). The best way to show the intensity of the band would be to photograph the gel. If you photograph your gel and hand in a printed copy, you will receive extra credit. You may also want to describe the band intensities to the side of the diagram.



Wells

Team 1 TS + EN

Lane 1: Standard DNA fragments

Lane 2: Control rxn sample, 0 cycles

Lane 3: Reaction sample after 15 cycles

Lane 4: Reaction sample after 30 cycles

Team 2 ______

~~Lane 5: Standard DNA fragments~~

~~Lane 6:~~ ______

~~Lane 7:~~ ______

~~Lane 8:~~ ______

1. Lanes 1 and 5 should have 9 bands which are standard DNA fragments. Their sizes in base pairs and in order from largest to smallest are: 23130, 9416, 6557, 4361, 3000, 2322, 2027, 725 and 570. To the left of the figure, **label the standard bands with their appropriate sizes**. The standard bands in both lanes should be identical, therefore, one label for both will be sufficient. If one of the 9 bands does not appear in either lane, you may have to compare you membrane to others and try and determine which band(s) did not show.

2. Discuss your results. **First,** tell whether you were able to see the effectiveness of PCR as a means of *amplifying* DNA? **Then,** explain why there were differences seen (or expected) in the three lanes (lanes 2-4 and lanes 6-8). In other words, what was different about the DNA samples put in each lane. Yes, we were able to see the effectiveness because the thicker band in lane 4 tells us that there are many more DNA strands copied. No bands showed in lane 2 because no replication occurred. Lane 3 was smaller than lane 4 because it was not in the PCR as long, + not replicated as much. Lane 4 was in the PCR for twice as long.

Part 5 – Post Lab Assignment. Use Google Scholar (if you do not know about Google Scholar, you should) or some other internet tool and find a research article in which PCR was used. Report the following information from the article below.

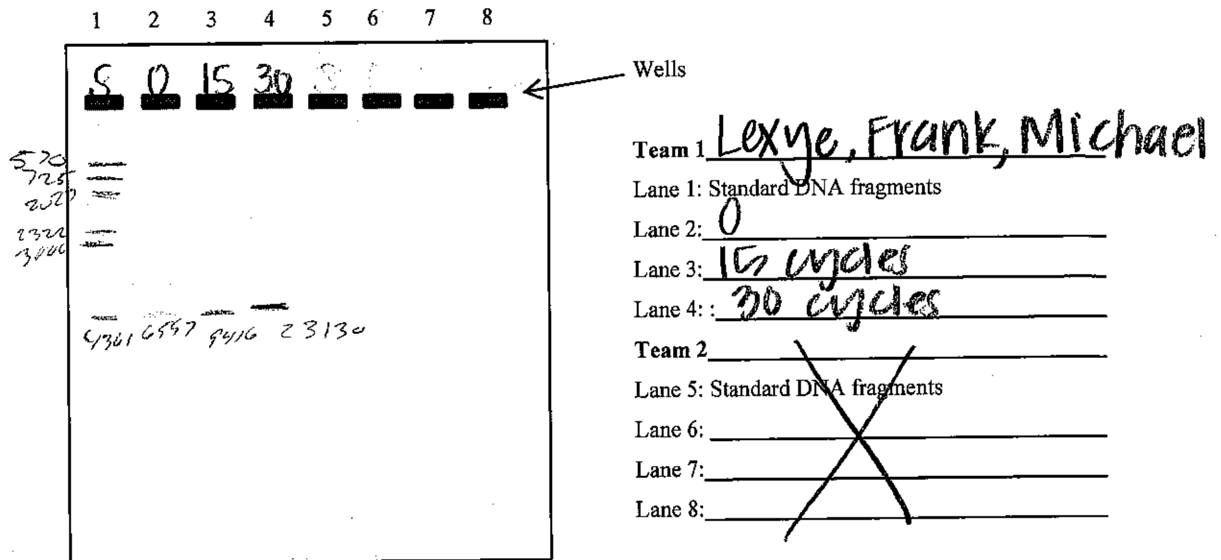
1. Cite the article using proper citation (author year method). Two examples are below:
   Welinder, C. and L. Ekblad. 2011. Coomassie Staining as Loading Control in Western Blot Analysis. J. Proteome Res 10(3): 1416–1419.
   Smith, T. E., P. R. Olsen, and J. E. Ward. 2002. Fragmentation in an Appalachian Forest. Ecology 89:918-925.
   Chetverina, Alexander B., Helena V. Chetverina, Timur R. Samatov, and Victor I. Ugarov. 2002. Molecular Colony Diagnostics: Detection and Quantitation of Viral Nucleic Acids by In-Gel PCR. BioTechniques 33: 150-156.

2. Explain the purpose/goal of the research and why PCR was used in the research.
   They used PCR to amplify HIV-1 RNA and HBV DNA to detect a low quantity of viral molecules in huge sample of DNA or RNA. They found that HIV-1 RNA was amplified with no interference.

3. Go to Methods section of the paper and find the following information for how the PCR was carried out:
   i. The number of PCR cycles carried out (we performed 15 and 30 cycles on our samples): 43 cycles
   ii. The temperatures and times used for each step of the cycle (ours was (94 C for 1 m; 45° C for 1 m; 72° C for 1 m):
   3 cycles: (94°C at 15s; annealing 45; 72°C at 60s)
   40 cycles: (94°C at 6s; annealing 45; 72°C at 60s)

*did not give temperature for annealing

Part 4 – PCR results. Write out the source of DNA that was in each lane. Mark the approximate positions for the DNA fragment bands in your electrophoresis gel as well as the other team's on the diagram below. Attempt to recreate the approximate intensity of the band (estimating band intensity is the purpose for carrying out electrophoresis in this lab). The best way to show the intensity of the band would be to photograph the gel. If you photograph your gel and hand in a printed copy, you will receive extra credit. You may also want to describe the band intensities to the side of the diagram.



1. Lanes 1 and 5 should have 9 bands which are standard DNA fragments. Their sizes in base pairs and in order from largest to smallest are: 23130, 9416, 6557, 4361, 3000, 2322, 2027, 725 and 570. To the left of the figure, **label the standard bands with their appropriate sizes.** The standard bands in both lanes should be identical, therefore, one label for both will be sufficient. If one of the 9 bands does not appear in either lane, you may have to compare you membrane to others and try and determine which band(s) did not show.

2. Discuss your results. **First**, tell whether you were able to see the effectiveness of PCR as a means of *amplifying* DNA? **Then,** explain why there were differences seen (or expected) in the three lanes (lanes 2-4 and lanes 6-8). In other words, what was different about the DNA samples put in each lane.

   We were able to see the effectiveness because more DNA was cut the longer it was left in allowing a more distinct band.

Part 5 – Post Lab Assignment. Use Google Scholar (if you do not know about Google Scholar, you should) or some other internet tool and find a research article in which PCR was used. Report the following information from the article below.

1. Cite the article using proper citation (author year method). Two examples are below:
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   Garibyan, Lilit. 2013. Journal of Investigative Dermatology. 133, e6. doi: 10.1038

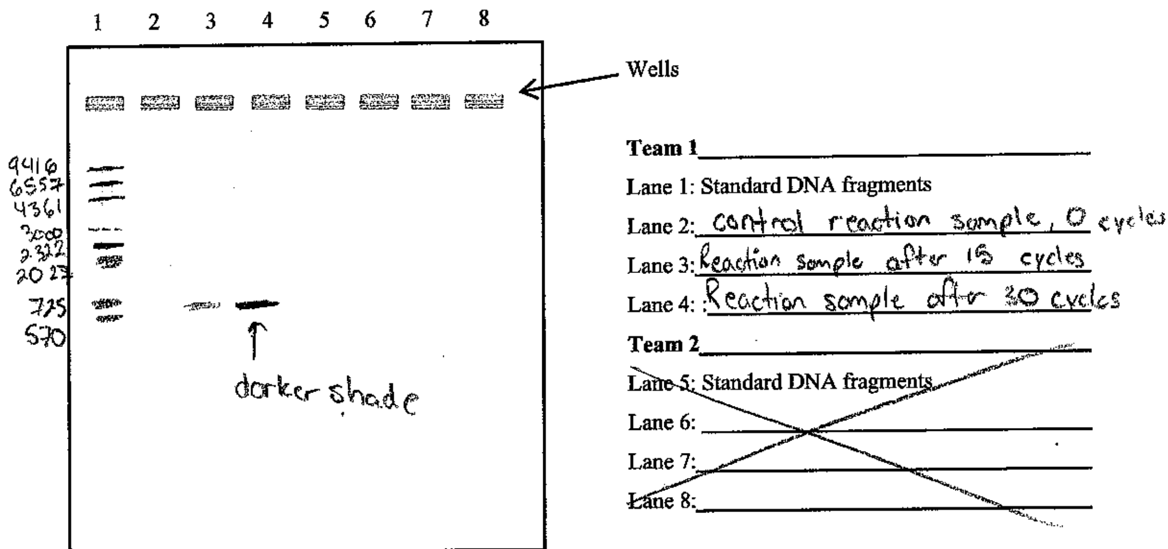
2. Explain the purpose/goal of the research and why PCR was used in the research.

   PCR is a sensative technique that allows rapid amplification of a specific segment of DNA.

3. Go to Methods section of the paper and find the following information for how the PCR was carried out:
   i. The number of PCR cycles carried out (we performed 15 and 30 cycles on our samples): 40 cycles
   ii. The temperatures and times used for each step of the cycle (ours was (94 C for 1 m; 45° C for 1 m; 72° C for 1 m):
   2 min: 95°C, 10 sec: 94°C, 1 min: 50-68°C,

Part 4 – PCR results. Write out the source of DNA that was in each lane. Mark the approximate positions for the DNA fragment bands in your electrophoresis gel as well as the other team's on the diagram below. Attempt to recreate the approximate intensity of the band (estimating band intensity is the purpose for carrying out electrophoresis in this lab). The best way to show the intensity of the band would be to photograph the gel. If you photograph your gel and hand in a printed copy, you will receive extra credit. You may also want to describe the band intensities to the side of the diagram.

* 23130 not seen



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2. Discuss your results. **First**, tell whether you were able to see the effectiveness of PCR as a means of *amplifying* DNA? **Then,** explain why there were differences seen (or expected) in the three lanes (lanes 2-4 and lanes 6-8). In other words, what was different about the DNA samples put in each lane.

Our sample did not show the effects of PCR due to not enough initial DNA. However, other classmates PCR worked. Lane 2 there was nothing because it didn't go through the PCR cycle. Lane 3 had a light shaded line because it went through 15 cycles. Lane 4 was darker because it went through 30 cycles meaning more DNA.

Part 5 – Post Lab Assignment. Use Google Scholar (if you do not know about Google Scholar, you should) or some other internet tool and find a research article in which PCR was used. Report the following information from the article below.

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   Ochman, H., Gerber, A.S. and D. L. Hartl. 1988. Genetic Applications of an Inverse Polymerase Chain Reaction. Genetics 120:621-623.

2. Explain the purpose/goal of the research and why PCR was used in the research.

   These researchers were interested in the DNA flanking the known DNA sequence. By using PCR but inverting the DNA primers they are able to amplify the flanking DNA. The research was to hopefully identify possible sites for insertion of transposable elements. Also to look at DNA sequences left behind after partial excision

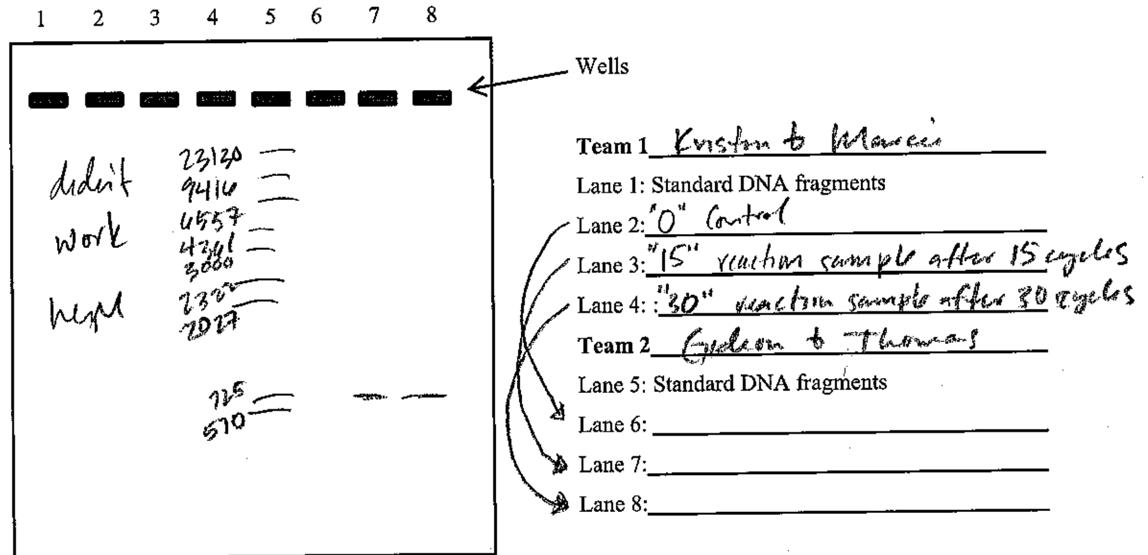
3. Go to Methods section of the paper and find the following information for how the PCR was carried out:

   i. The number of PCR cycles carried out (we performed 15 and 30 cycles on our samples): 30 cycles

   ii. The temperatures and times used for each step of the cycle (ours was (94 C for 1 m; 45° C for 1 m; 72° C for 1 m):

   30 cycles at 94° for 1.5 min, 48° 1.0 min, 70° 4.0 min

Part 4 – PCR results. Write out the source of DNA that was in each lane. Mark the approximate positions for the DNA fragment bands in your electrophoresis gel as well as the other team's on the diagram below. Attempt to recreate the approximate intensity of the band (estimating band intensity is the purpose for carrying out electrophoresis in this lab). The best way to show the intensity of the band would be to photograph the gel. If you photograph your gel and hand in a printed copy, you will receive extra credit. You may also want to describe the band intensities to the side of the diagram.



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2. Discuss your results. **First**, tell whether you were able to see the effectiveness of PCR as a means of *amplifying* DNA? **Then,** explain why there were differences seen (or expected) in the three lanes (lanes 2-4 and lanes 6-8). In other words, what was different about the DNA samples put in each lane.

   Yes we were able to see that it amplified the DNA because they showed as dark bands, while the DNA not amplified didn't show. The DNA not amplified (lane 6) didn't show because while there is DNA present, it is a very small amount so no band showed up.

Part 5 – Post Lab Assignment. Use Google Scholar (if you do not know about Google Scholar, you should) or some other internet tool and find a research article in which PCR was used. Report the following information from the article below.

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   K. Edwards, C. Johnstone and C. Thompson. 1991. A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acids Research, Vol. 19, No. 6: 1349

2. Explain the purpose/goal of the research and why PCR was used in the research.

   PCR is usually used for mammal DNA, in this research they developed a way to prepare the plant DNA so they could use PCR. The research experiment found out that ~~the~~ it is possible to extract within 15 minutes without using any hazardous organic solvents.

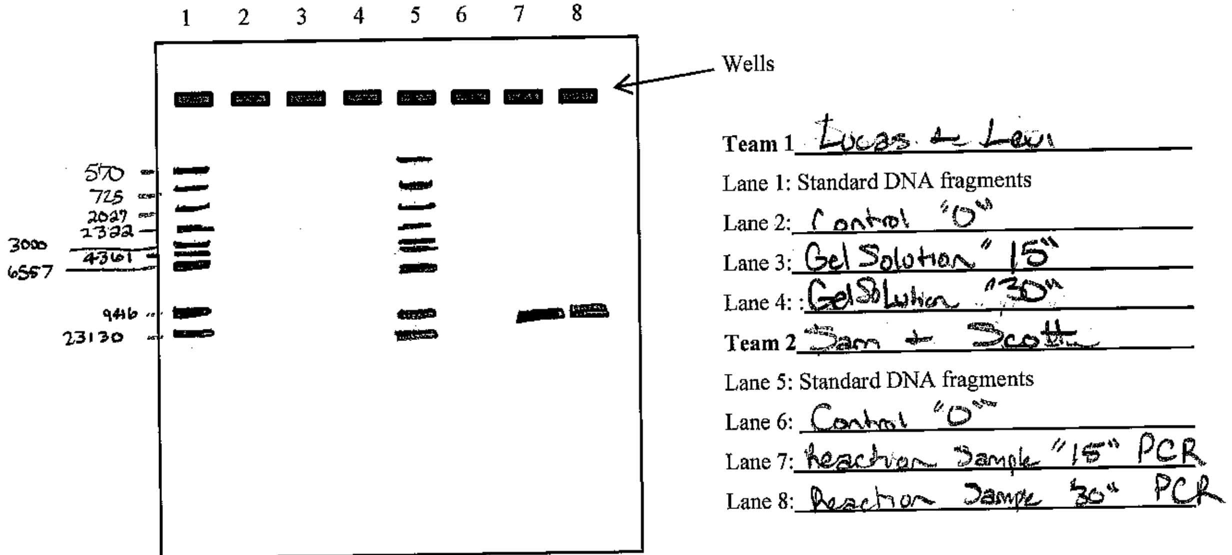
3. Go to Methods section of the paper and find the following information for how the PCR was carried out:

   i. The number of PCR cycles carried out (we performed 15 and 30 cycles on our samples): 35 cycles

   ii. The temperatures and times used for each step of the cycle (ours was (94 C for 1 m; 45° C for 1 m; 72° C for 1 m):

   95 °C for 45 seconds
   66 °C for 30 seconds
   73 °C for 2.5 minutes

Part 4 – PCR results. Write out the source of DNA that was in each lane. Mark the approximate positions for the DNA fragment bands in your electrophoresis gel as well as the other team's on the diagram below. Attempt to recreate the approximate intensity of the band (estimating band intensity is the purpose for carrying out electrophoresis in this lab). The best way to show the intensity of the band would be to photograph the gel. If you photograph your gel and hand in a printed copy, you will receive extra credit. You may also want to describe the band intensities to the side of the diagram.



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2. Discuss your results. **First**, tell whether you were able to see the effectiveness of PCR as a means of *amplifying* DNA? **Then**, explain why there were differences seen (or expected) in the three lanes (lanes 2-4 and lanes 6-8). In other words, what was different about the DNA samples put in each lane.
By means of amplifying the DNA, with no presence of bands in the 6th lane, the bands do become bolder as you move from lane 7 to 8 which would make sense do to more PCR time. The presence of no bands in 2-4 result from having the 10μL of Gel Loading Solution verse the DNA Template for Amplification in lanes 6-8. The absence in lane 6 results from no amplification of PCR to have time to reproduce more DNA strands because it was part of our control.

Part 5 – Post Lab Assignment. Use Google Scholar (if you do not know about Google Scholar, you should) or some other internet tool and find a research article in which PCR was used. Report the following information from the article below.

1. Cite the article using proper citation (author year method). Two examples are below:
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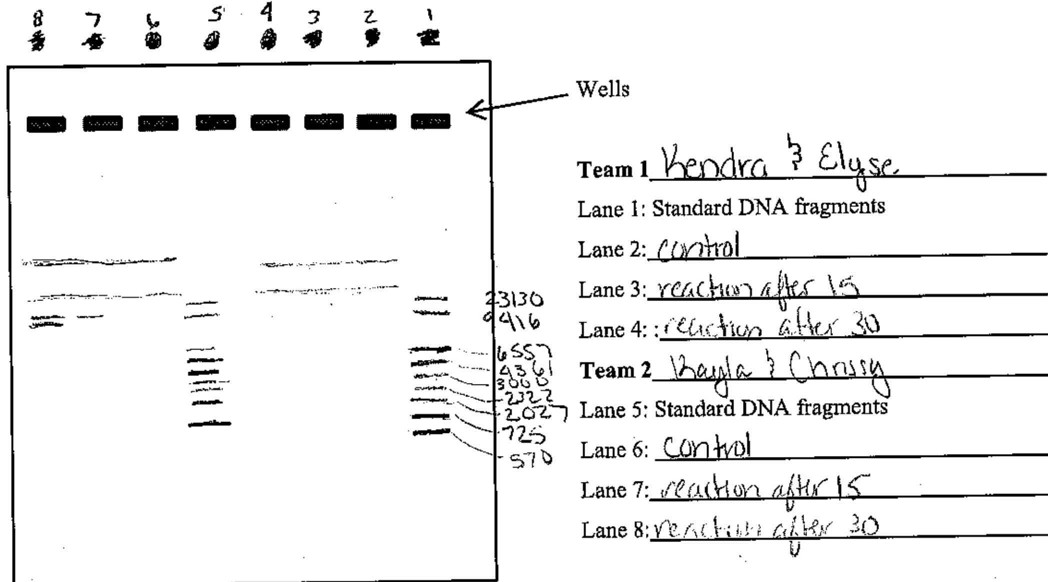
   Analytical Biochemistry, Volume 285, Issue 2, Page 194-204 Thomas D. Schmittgen, Brian A. Zakrajsek, Alan G. Mills, Vladimir Gorn, Michael J Singer, Michele W Reed.

2. Explain the purpose/goal of the research and why PCR was used in the research.
PCR was used for a serum-inducible transcription in Mouse fibroblasts (NIH 3T3). Both methods of end point and real time PCR methods to produce the DNA reproduction. Real time PCR had 4- to 5- log dynamic range of amplification to the end point of 1- log. From this they were able to arrange the first order of mRNA decay plots and also enhancing the precise and greater dynamic range of PCR amplification.

3. Go to Methods section of the paper and find the following information for how the PCR was carried out:
   i. The number of PCR cycles carried out (we performed 15 and 30 cycles on our samples): 30 + 55 cycles
   ii. The temperatures and times used for each step of the cycle (ours was (94 C for 1 m; 45° C for 1 m; 72° C for 1 m):
   37°C - 10min → 90°C - 5 min → 42°C - 45 min → 90° - 3 min → 26°C - 10min → 42°C - 45min → 75°C - 10min

Part 4 – PCR results. Write out the source of DNA that was in each lane. Mark the approximate positions for the DNA fragment bands in your electrophoresis gel as well as the other team's on the diagram below. Attempt to recreate the approximate intensity of the band (estimating band intensity is the purpose for carrying out electrophoresis in this lab). The best way to show the intensity of the band would be to photograph the gel. If you photograph your gel and hand in a printed copy, you will receive extra credit. You may also want to describe the band intensities to the side of the diagram.



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2. Discuss your results. **First**, tell whether you were able to see the effectiveness of PCR as a means of *amplifying* DNA? **Then,** explain why there were differences seen (or expected) in the three lanes (lanes 2-4 and lanes 6-8). In other words, what was different about the DNA samples put in each lane.

   The PCR effectiveness was seen after 30 cycles, not noticed alot after 15 cycles. Each cycle the DNA goes through the DNA is doubled. This means, it multiplies exponentially. The more DNA fragments in the sample the greater the amplification and easier the bands are to see.

Part 5 – Post Lab Assignment. Use Google Scholar (if you do not know about Google Scholar, you should) or some other internet tool and find a research article in which PCR was used. Report the following information from the article below.

1. Cite the article using proper citation (author year method). Two examples are below:
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   K. Edwards, C. Johnstone and C. Thompson. 1991. A simple and rapid method for the preparation of plant genomic DNA for PCR analysis

2. Explain the purpose/goal of the research and why PCR was used in the research.

   To develope a method for extracting a small amount of plant genomic DNA quickly, that can be used in PCR analysis. Plant tissues have presented difficulties in extracting nucleic acids. The PCR allows hundreds of samples to be processed within a day

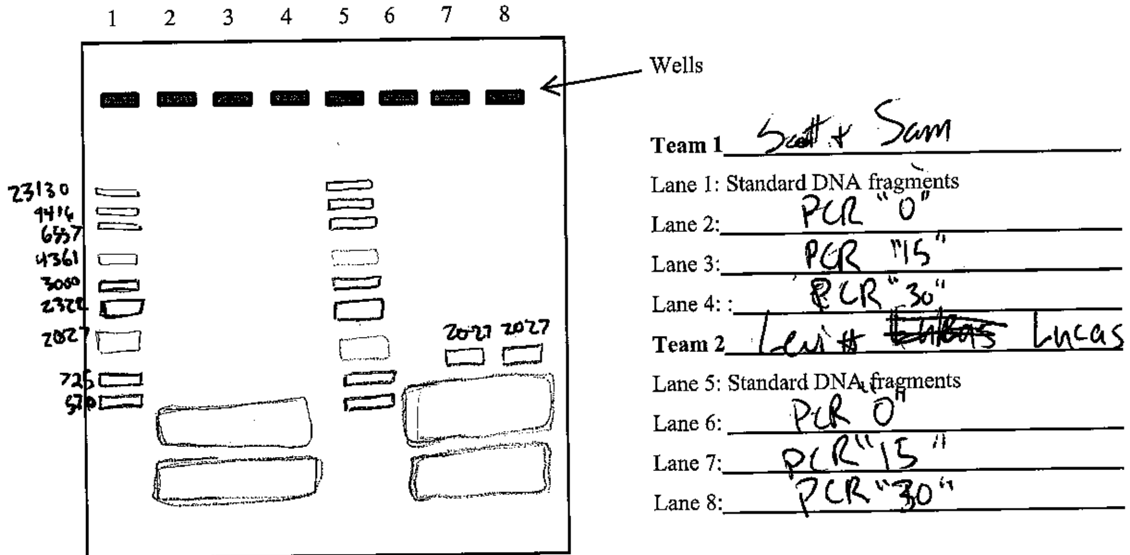
3. Go to Methods section of the paper and find the following information for how the PCR was carried out:

   i. The number of PCR cycles carried out (we performed 15 and 30 cycles on our samples): 35 cycles

   ii. The temperatures and times used for each step of the cycle (ours was (94 C for 1 m; 45° C for 1 m; 72° C for 1 m):

   95°C for 45sec., 66°C for 30sec., 72°C for 2.5min.

Part 4 – PCR results. Write out the source of DNA that was in each lane. Mark the approximate positions for the DNA fragment bands in your electrophoresis gel as well as the other team's on the diagram below. Attempt to recreate the approximate intensity of the band (estimating band intensity is the purpose for carrying out electrophoresis in this lab). The best way to show the intensity of the band would be to photograph the gel. If you photograph your gel and hand in a printed copy, you will receive extra credit. You may also want to describe the band intensities to the side of the diagram.



Team 1 Scott + Sam
Lane 1: Standard DNA fragments
Lane 2: PCR "0"
Lane 3: PCR "15"
Lane 4: : PCR "30"
Team 2 Levi + ~~Lukas~~ Lucas
Lane 5: Standard DNA fragments
Lane 6: PCR "0"
Lane 7: PCR "15"
Lane 8: PCR "30"

1. Lanes 1 and 5 should have 9 bands which are standard DNA fragments. Their sizes in base pairs and in order from largest to smallest are: 23130, 9416, 6557, 4361, 3000, 2322, 2027, 725 and 570. To the left of the figure, **label the standard bands with their appropriate sizes.** The standard bands in both lanes should be identical, therefore, one label for both will be sufficient. If one of the 9 bands does not appear in either lane, you may have to compare you membrane to others and try and determine which band(s) did not show.

2. Discuss your results. **First**, tell whether you were able to see the effectiveness of PCR as a means of *amplifying* DNA? **Then,** explain why there were differences seen (or expected) in the three lanes (lanes 2-4 and lanes 6-8). In other words, what was different about the DNA samples put in each lane.
Lane 8 seemed darker than lane 7 or 6. This is consistent with what we expected because its got exponentially more DNA in its lane. The more time it spends in the thermocycler the more copies of DNA and the thicker the line

Part 5 – Post Lab Assignment. Use Google Scholar (if you do not know about Google Scholar, you should) or some other internet tool and find a research article in which PCR was used. Report the following information from the article below.

1. Cite the article using proper citation (author year method). Two examples are below:
Welinder, C. and L. Ekblad. 2011. Coomassie Staining as Loading Control in Western Blot Analysis. J. Proteome Res 10(3): 1416–1419.
Smith, T. E., P. R. Olsen, and J. E. Ward. 2002. Fragmentation in an Appalachian Forest. Ecology 89:918-925.
Lo, D. and Hjelm, M. and Fidler, C. and Sargent, I. and Murphy, M. and Chamberlain, P. and Poon, P. and Redman, C. and Wainscoat, J. 1998. Prenatal Diagnoses of Fetal RhD Status by Molecular Analysis of Maternal Plasma. 339: 1734-1738

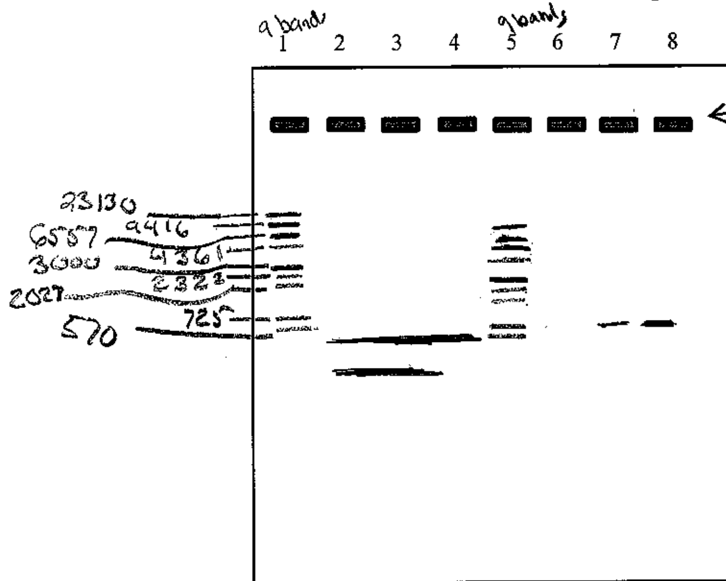
2. Explain the purpose/goal of the research and why PCR was used in the research.
They used PCR to do fetal blood testing to test for Rh isoimmunization. Rh isoimmunization is when the fetus' blood enters the mothers blood stream. When it does this, it creates antibodies that attack the fetus. PCR was used because it is the most effective way to test for this dangerous condition.

3. Go to Methods section of the paper and find the following information for how the PCR was carried out:

i. The number of PCR cycles carried out (we performed 15 and 30 cycles on our samples): 40 cycles

ii. The temperatures and times used for each step of the cycle (ours was (94 C for 1 m; 45° C for 1 m; 72° C for 1 m):
Denature for 10 min at 95°c and 40 cycles at 95°c for 15 sec and reannealing/extension for 1 min at 60°c

Part 4 – PCR results. Write out the source of DNA that was in each lane. Mark the approximate positions for the DNA fragment bands in your electrophoresis gel as well as the other team's on the diagram below. Attempt to recreate the approximate intensity of the band (estimating band intensity is the purpose for carrying out electrophoresis in this lab). The best way to show the intensity of the band would be to photograph the gel. If you photograph your gel and hand in a printed copy, you will receive extra credit. You may also want to describe the band intensities to the side of the diagram.



Wells

Team 1 Kendra; Elyse

Lane 1: Standard DNA fragments

Lane 2: 0 Control Reaction Sample, 0 cycle

Lane 3: 15 Reaction Sample after 15 cycles

Lane 4: 30 Reaction Sample after 30 cycles

Team 2 Kayla; Chrissy

Lane 5: Standard DNA fragments

Lane 6: 0 control reaction sample, 0 cycle

Lane 7: 15 Reaction sample after 15 cycles

Lane 8: 30 Reaction sample after 30 cycles.

1. Lanes 1 and 5 should have 9 bands which are standard DNA fragments. Their sizes in base pairs and in order from largest to smallest are: 23130, 9416, 6557, 4361, 3000, 2322, 2027, 725 and 570. To the left of the figure, **label the standard bands with their appropriate sizes.** The standard bands in both lanes should be identical, therefore, one label for both will be sufficient. If one of the 9 bands does not appear in either lane, you may have to compare you membrane to others and try and determine which band(s) did not show.

2. Discuss your results. **First,** tell whether you were able to see the effectiveness of PCR as a means of *amplifying* DNA? **Then,** explain why there were differences seen (or expected) in the three lanes (lanes 2-4 and lanes 6-8). In other words, what was different about the DNA samples put in each lane. Yes we were able to see the effectiveness of the the PCR amplifying the DNA because the control had zero cycles in the PCR and the band is not visible the band with 15 cycles is faint but visible and lane 8 which had 30 cycles in the PCR was the darkest band and most visible. The more cycles in the PCR the more amount of DNA produced

Part 5 – Post Lab Assignment. Use Google Scholar (if you do not know about Google Scholar, you should) or some other internet tool and find a research article in which PCR was used. Report the following information from the article below.

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Mariani, B. et al. 2014. Polymerase Chain Reaction Molecular Diagnostic Technology for Monitoring Chronic Osteomyelitis. Journal of Experimental Orthopaedics 1:9

2. Explain the purpose/goal of the research and why PCR was used in the research.

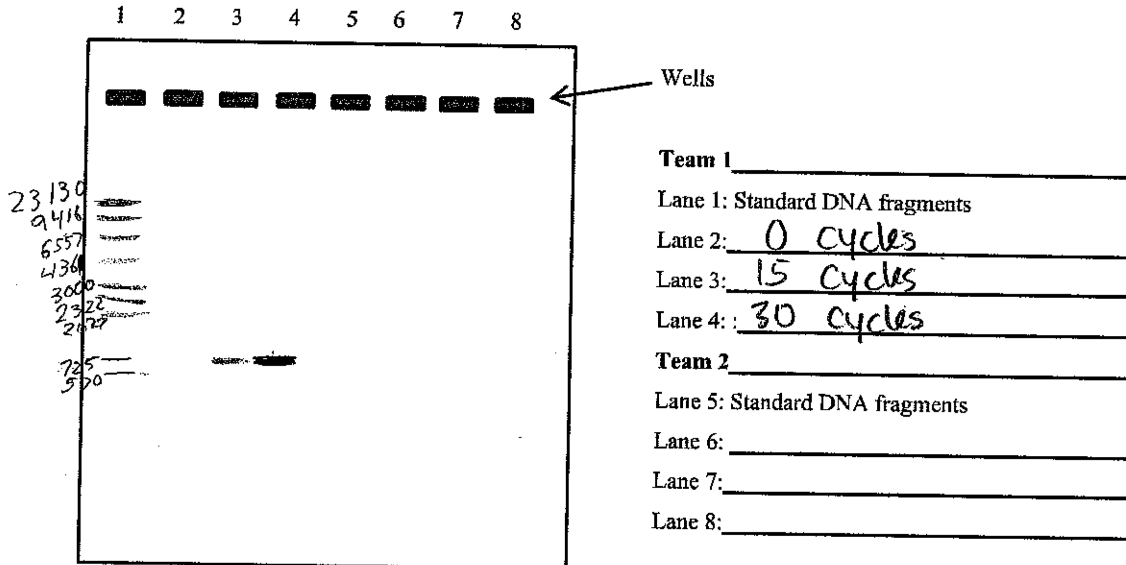
the goal is to determine the aplicabity of molecular diagnostic procedures for monitoring chronic osteomyelitis. PCR was useful for high-sensitivity detection and identification of bacteria associated with chronic osteomyelitis.

3. Go to Methods section of the paper and find the following information for how the PCR was carried out:
   i. The number of PCR cycles carried out (we performed 15 and 30 cycles on our samples): ① 30 ② 35 ③ 45
   ii. The temperatures and times used for each step of the cycle (ours was (94 C for 1 m; 45° C for 1 m; 72° C for 1 m):

① 94°C 1min, 55°C, 72°C
② 95°C 20 sec, 58°C 30, 72°C 45; 72° 5min, 4° ∞
③ 95°C 10 sec, 60°C 30sec

Bett Jam, Beth Holle, Kendell Shick

Part 4 – PCR results. Write out the source of DNA that was in each lane. Mark the approximate positions for the DNA fragment bands in your electrophoresis gel as well as the other team's on the diagram below. Attempt to recreate the approximate intensity of the band (estimating band intensity is the purpose for carrying out electrophoresis in this lab). The best way to show the intensity of the band would be to photograph the gel. If you photograph your gel and hand in a printed copy, you will receive extra credit. You may also want to describe the band intensities to the side of the diagram.



1. Lanes 1 and 5 should have 9 bands which are standard DNA fragments. Their sizes in base pairs and in order from largest to smallest are: 23,130, 9416, 6557, 4361, 3000, 2322, 2027, 725 and 570. To the left of the figure, **label the standard bands with their appropriate sizes.** The standard bands in both lanes should be identical, therefore, one label for both will be sufficient. If one of the 9 bands does not appear in either lane, you may have to compare you membrane to others and try and determine which band(s) did not show.

2. Discuss your results. **First**, tell whether you were able to see the effectiveness of PCR as a means of *amplifying* DNA? **Then,** explain why there were differences seen (or expected) in the three lanes (lanes 2-4 and lanes 6-8). In other words, what was different about the DNA samples put in each lane.

Yes because the banding of lane 4 was more prominent than lane 3 which was even more prominent over lane 2. There was a difference because lane 4 was 30 cycles so there was N^{30} strands of DNA in lane 4. In lane 3 there was N^{15} strands used in the gel. In lane 2, only the original DNA (N) strands were used for electrophoresis.

Part 5 – Post Lab Assignment. Use Google Scholar (if you do not know about Google Scholar, you should) or some other internet tool and find a research article in which PCR was used. Report the following information from the article below.

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   Smith, T. E., P. R. Olsen, and J. E. Ward. 2002. Fragmentation in an Appalachian Forest. Ecology 89:918-925.

Machida, R. J., Knowlton, N., & Gilbert, J. A. (2012). PCR Primers for Metazoan Nuclear 18S and 28S Ribosomal DNA Sequences, Plos ONE, 7(9), 1-11.

2. Explain the purpose/goal of the research and why PCR was used in the research.

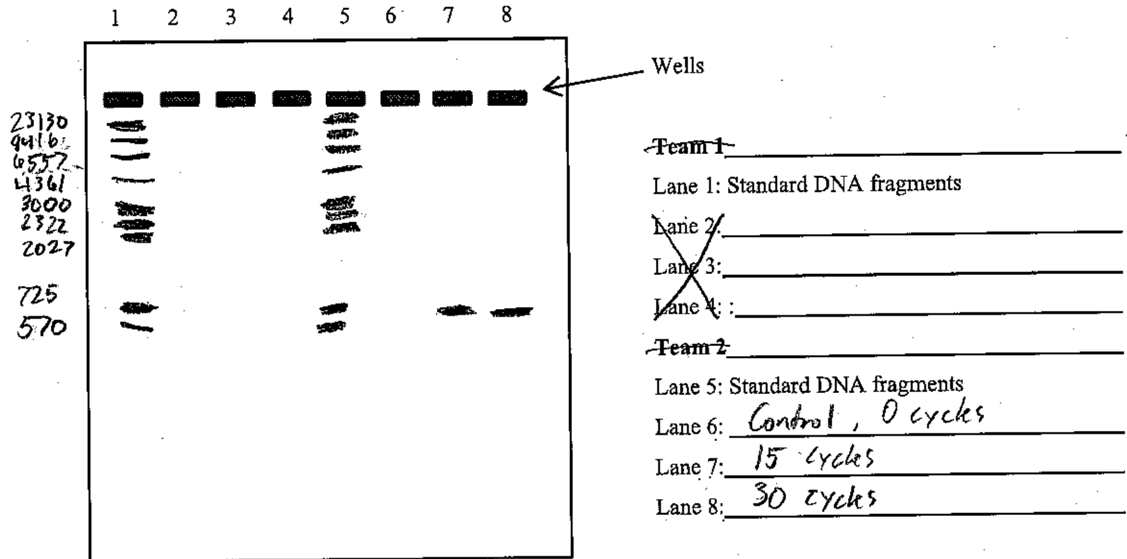
The goal of the research is to gather further understanding of animals, specifically those who live in marine life and how variability in genes compares between different phylums. The PCR was used to rapidly replicate allowing mutations to amplify.

3. Go to Methods section of the paper and find the following information for how the PCR was carried out:
   i. The number of PCR cycles carried out (we performed 15 and 30 cycles on our samples): 30 cycles

   ii. The temperatures and times used for each step of the cycle (ours was (94 C for 1 m; 45° C for 1 m; 72° C for 1 m):

95°C 10 sec
55°C 30 sec
72°C 60 sec

Part 4 – PCR results. Write out the source of DNA that was in each lane. Mark the approximate positions for the DNA fragment bands in your electrophoresis gel as well as the other team's on the diagram below. Attempt to recreate the approximate intensity of the band (estimating band intensity is the purpose for carrying out electrophoresis in this lab). The best way to show the intensity of the band would be to photograph the gel. If you photograph your gel and hand in a printed copy, you will receive extra credit. You may also want to describe the band intensities to the side of the diagram.



1. Lanes 1 and 5 should have 9 bands which are standard DNA fragments. Their sizes in base pairs and in order from largest to smallest are: 23130, 9416, 6557, 4361, 3000, 2322, 2027, 725 and 570. To the left of the figure, **label the standard bands with their appropriate sizes.** The standard bands in both lanes should be identical, therefore, one label for both will be sufficient. If one of the 9 bands does not appear in either lane, you may have to compare you membrane to others and try and determine which band(s) did not show.

2. Discuss your results. **First,** tell whether you were able to see the effectiveness of PCR as a means of *amplifying* DNA? **Then,** explain why there were differences seen (or expected) in the three lanes (lanes 2-4 and lanes 6-8). In other words, what was different about the DNA samples put in each lane.

Yes we can see the amplifying effects because the bands were brighter. Lane 1 + 5 was a standard DNA fragment so it showed 9 bands, Lane 6 was control so it didn't go through PCR and showed no bands. Lane 7 + 8 had a PCR pellet added to show the effects of the PCR. Lane 7 went through 15 cycles and Lane 8 went through 30 cycles so the Lane 8 band is brighter than the Lane 7 band.

Part 5 – Post Lab Assignment. Use Google Scholar (if you do not know about Google Scholar, you should) or some other internet tool and find a research article in which PCR was used. Report the following information from the article below.

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Welinder, C. and L. Ekblad. 2011. Coomassie Staining as Loading Control in Western Blot Analysis. J. Proteome Res 10(3): 1416–1419.

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Hokynar, Norja, Laitinen, Palomaki, Garbag-Chenon, Ranki, Hedman, Soderlund-Venermo, 2014. Detection and Differentiation of Parvovirus Variants by Commercial Quantitative Real-Time PCR Tests. American Society for Microbiology. Vol (52) Iss (12),

2. Explain the purpose/goal of the research and why PCR was used in the research.

The previous PCR tests attempting to detect Parvo Virus B19 genotypes could not identify each genotype independently. A different type of PCR test was originally needed for detecting each genotype. The new RealArt Parvo B19 LC PCR is capable of detecting all three genotypes on its own. This PCR was used to help amplify the already little genome present in the Parvo virus. This PCR amplifies all three Parvo B19 genomes and allows the results to be easily identified, read, compared and treated.

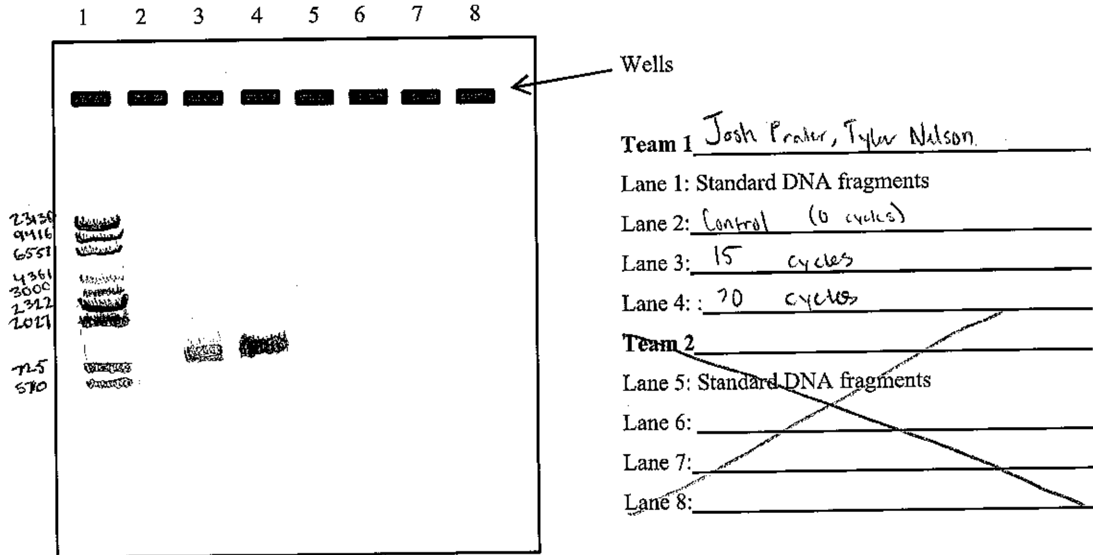
3. Go to Methods section of the paper and find the following information for how the PCR was carried out:

i. The number of PCR cycles carried out (we performed 15 and 30 cycles on our samples): 30 cycles

ii. The temperatures and times used for each step of the cycle (ours was (94 C for 1 m; 45° C for 1 m; 72° C for 1 m):

95°C for 15s, 40°C for 15s, 80°C for 1s

Part 4 – PCR results. Write out the source of DNA that was in each lane. Mark the approximate positions for the DNA fragment bands in your electrophoresis gel as well as the other team's on the diagram below. Attempt to recreate the approximate intensity of the band (estimating band intensity is the purpose for carrying out electrophoresis in this lab). The best way to show the intensity of the band would be to photograph the gel. If you photograph your gel and hand in a printed copy, you will receive extra credit. You may also want to describe the band intensities to the side of the diagram.



1. Lanes 1 and 5 should have 9 bands which are standard DNA fragments. Their sizes in base pairs and in order from largest to smallest are: 23130, 9416, 6557, 4361, 3000, 2322, 2027, 725 and 570. To the left of the figure, **label the standard bands with their appropriate sizes.** The standard bands in both lanes should be identical, therefore, one label for both will be sufficient. If one of the 9 bands does not appear in either lane, you may have to compare you membrane to others and try and determine which band(s) did not show.

2. Discuss your results. **First**, tell whether you were able to see the effectiveness of PCR as a means of *amplifying* DNA? **Then,** explain why there were differences seen (or expected) in the three lanes (lanes 2-4 and lanes 6-8). In other words, what was different about the DNA samples put in each lane.

The effectiveness of Amplifying were obvious. In lane 2 there is no visible DNA, while a clear band shows in lane 3. Finally, a bright band shows in lane 4, meaning more DNA showed up with more cycles. This difference is due to the number of cycles shown with the DNA.

Part 5 – Post Lab Assignment. Use Google Scholar (if you do not know about Google Scholar, you should) or some other internet tool and find a research article in which PCR was used. Report the following information from the article below.

1. Cite the article using proper citation (author year method). Two examples are below:
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Serikawa, T., Kuramoto, T., Hilbert, P. and J.S Beckmann. 1992. Rat Gene Mapping Using PCR-Analyzed Microsatellites. pg 701-721.

2. Explain the purpose/goal of the research and why PCR was used in the research.

The purpose of the research was to construct a detailed rat genetic map to exploit the potential of the rat as an experimental animal. This detail is through microsatellites. These microsatellites create unique sequences, which then served as primer for the PCR. This way the PCR can replicate specific portions and genes of the rats DNA and fully build the genetic map.

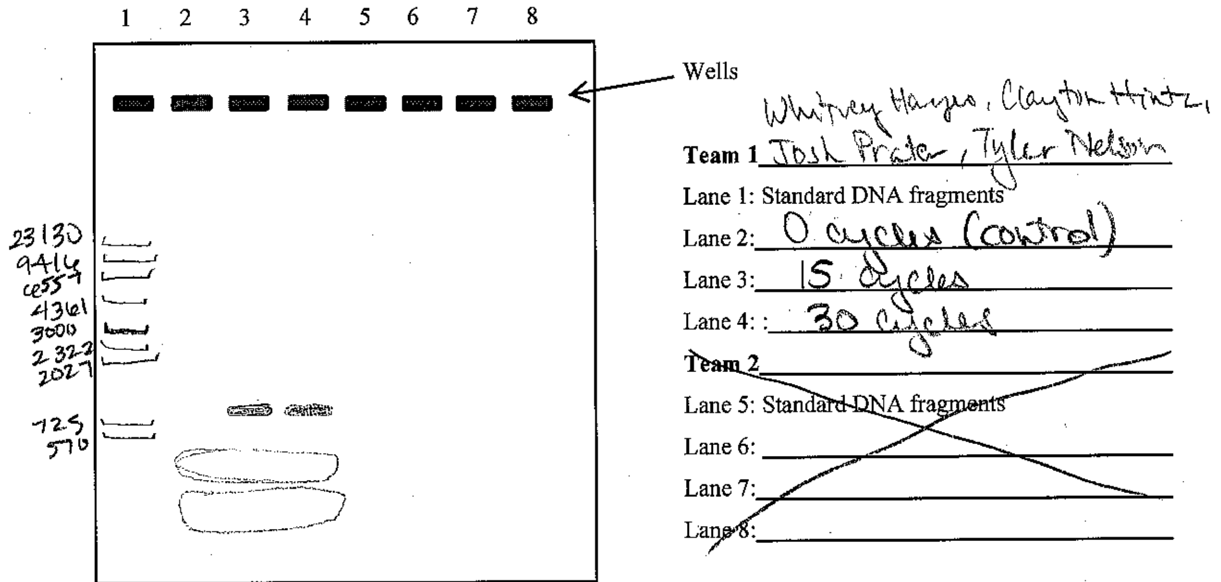
3. Go to Methods section of the paper and find the following information for how the PCR was carried out:

   i. The number of PCR cycles carried out (we performed 15 and 30 cycles on our samples): 35 cycles

   ii. The temperatures and times used for each step of the cycle (ours was (94 C for 1 m; 45° C for 1 m; 72° C for 1 m):

begun with 3 min at 94 at the beginning then ⟶ (1 min, 94°C)(1 min, ≈55°C)(30sec, 72°) Final 3min 72°

Part 4 – PCR results. Write out the source of DNA that was in each lane. Mark the approximate positions for the DNA fragment bands in your electrophoresis gel as well as the other team's on the diagram below. Attempt to recreate the approximate intensity of the band (estimating band intensity is the purpose for carrying out electrophoresis in this lab). The best way to show the intensity of the band would be to photograph the gel. If you photograph your gel and hand in a printed copy, you will receive extra credit. You may also want to describe the band intensities to the side of the diagram.



1. Lanes 1 and 5 should have 9 bands which are standard DNA fragments. Their sizes in base pairs and in order from largest to smallest are: 23130, 9416, 6557, 4361, 3000, 2322, 2027, 725 and 570. To the left of the figure, **label the standard bands with their appropriate sizes**. The standard bands in both lanes should be identical, therefore, one label for both will be sufficient. If one of the 9 bands does not appear in either lane, you may have to compare you membrane to others and try and determine which band(s) did not show.

2. Discuss your results. **First**, tell whether you were able to see the effectiveness of PCR as a means of *amplifying* DNA? **Then,** explain why there were differences seen (or expected) in the three lanes (lanes 2-4 and lanes 6-8). In other words, what was different about the DNA samples put in each lane.

   Our experiment showed obvious amplification of DNA. Lane 2 has no banding of DNA whereas Lane 3 has a clear band shown. Another clear band is apparent in Lane 4 meaning that more DNA was apparent after more cycles.

Part 5 – Post Lab Assignment. Use Google Scholar (if you do not know about Google Scholar, you should) or some other internet tool and find a research article in which PCR was used. Report the following information from the article below.

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2. Explain the purpose/goal of the research and why PCR was used in the research.

   The purpose of the research was to create a genetic map of the rat to show its DNA and show its potential as an experimental animal. The microsatellites in the experiment create unique sequences of DNA which were the primer for PCR. The PCR could then replicate some portions of genes to build the genetic map.

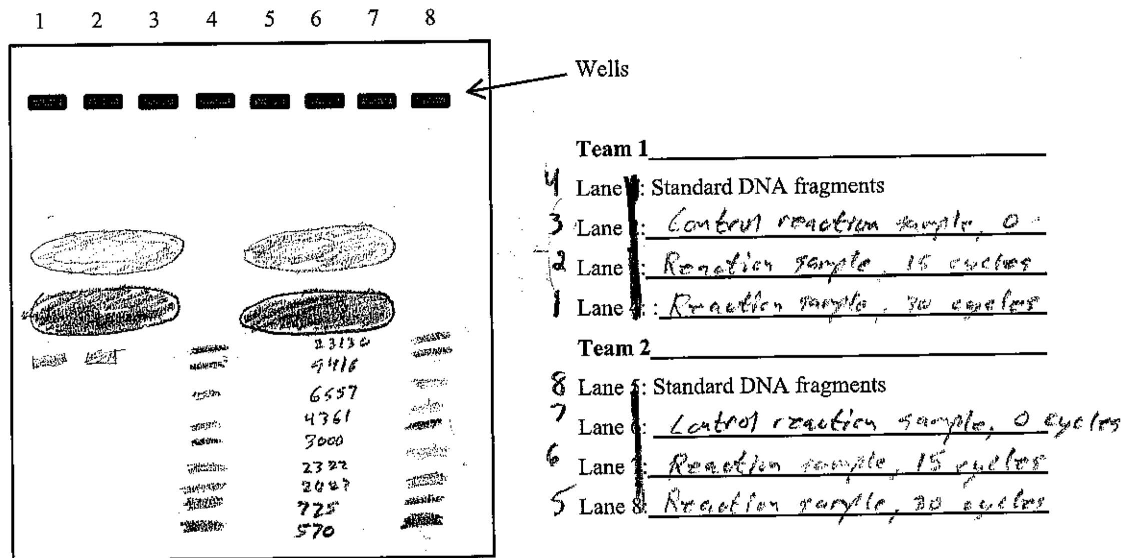
3. Go to Methods section of the paper and find the following information for how the PCR was carried out:

   i. The number of PCR cycles carried out (we performed 15 and 30 cycles on our samples): 45 cycles

   ii. The temperatures and times used for each step of the cycle (ours was (94 C for 1 m; 45° C for 1 m; 72° C for 1 m):

   1) 3min @ 94°C 2) 1 min @ 94°C 3) 1 min 55°C 4) 30 sec @ 72°C 5) 3min @ 72°C

Part 4 – PCR results. Write out the source of DNA that was in each lane. Mark the approximate positions for the DNA fragment bands in your electrophoresis gel as well as the other team's on the diagram below. Attempt to recreate the approximate intensity of the band (estimating band intensity is the purpose for carrying out electrophoresis in this lab). The best way to show the intensity of the band would be to photograph the gel. If you photograph your gel and hand in a printed copy, you will receive extra credit. You may also want to describe the band intensities to the side of the diagram.



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2. Discuss your results. **First**, tell whether you were able to see the effectiveness of PCR as a means of *amplifying* DNA? **Then,** explain why there were differences seen (or expected) in the three lanes (lanes 2-4 and lanes 6-8). In other words, what was different about the DNA samples put in each lane. In our gel we weren't able to determine a difference between the PCR and the control reaction sample. We examined other gels in the class and we able to determine differences. We were able to see two bands in the PCR because they were heated and cooled in the PCR machine.

Part 5 – Post Lab Assignment. Use Google Scholar (if you do not know about Google Scholar, you should) or some other internet tool and find a research article in which PCR was used. Report the following information from the article below.

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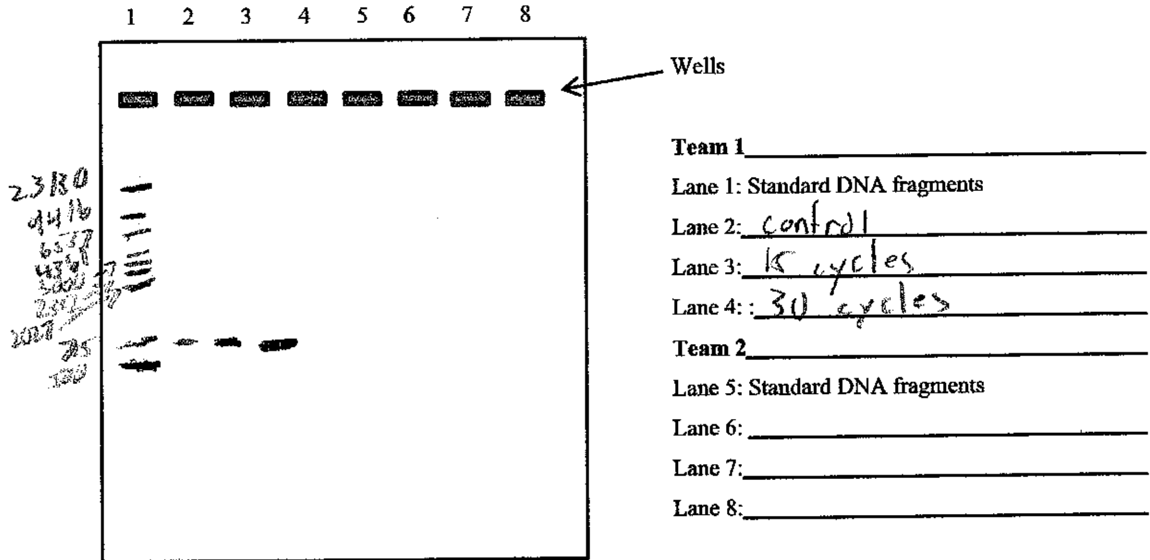
   Liu, Yao-Guang, and Yuanling Chen. 2007. High-efficiency thermal asymetric interlaced PCR for amplication of unknown flanking sequences

2. Explain the purpose/goal of the research and why PCR was used in the research.
   The goal is to study certain unknown genes in plants. To do this different PCR variations were used to amplify the unknown DNA fragments that are flanked by known DNA fragments

3. Go to Methods section of the paper and find the following information for how the PCR was carried out:
   i. The number of PCR cycles carried out (we performed 15 and 30 cycles on our samples): 16, 16, 12

   ii. The temperatures and times used for each step of the cycle (ours was (94 C for 1 m; 45° C for 1 m; 72° C for 1 m):
   Secondary tail-PCR – 94 °C for 0:30, 68 for 1:00, 72 for 3:00, 94 for 0:30, 68 for 1:00, 72 for 3:00, 94 for 0:30, 50 for 1:00, 72 for 3:00, 72 for 5:00, End

Part 4 – PCR results. Write out the source of DNA that was in each lane. Mark the approximate positions for the DNA fragment bands in your electrophoresis gel as well as the other team's on the diagram below. Attempt to recreate the approximate intensity of the band (estimating band intensity is the purpose for carrying out electrophoresis in this lab). The best way to show the intensity of the band would be to photograph the gel. If you photograph your gel and hand in a printed copy, you will receive extra credit. You may also want to describe the band intensities to the side of the diagram.



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2. Discuss your results. **First,** tell whether you were able to see the effectiveness of PCR as a means of *amplifying* DNA? **Then,** explain why there were differences seen (or expected) in the three lanes (lanes 2-4 and lanes 6-8). In other words, what was different about the DNA samples put in each lane.

   We were able to see the effectiveness. As the DNA went through more cycles, the DNA became thicker. This explains why the 30 cycles DNA was darkest.

Part 5 – Post Lab Assignment. Use Google Scholar (if you do not know about Google Scholar, you should) or some other internet tool and find a research article in which PCR was used. Report the following information from the article below.

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   Liu, Yao-Guang. Yuanling Chen. (2007) High-Efficiency Thermal Asymmetric Interlaced PCR for Amplification of Unknown Flanking Sequences. Short Technical Reports. 43(5). 649-653.

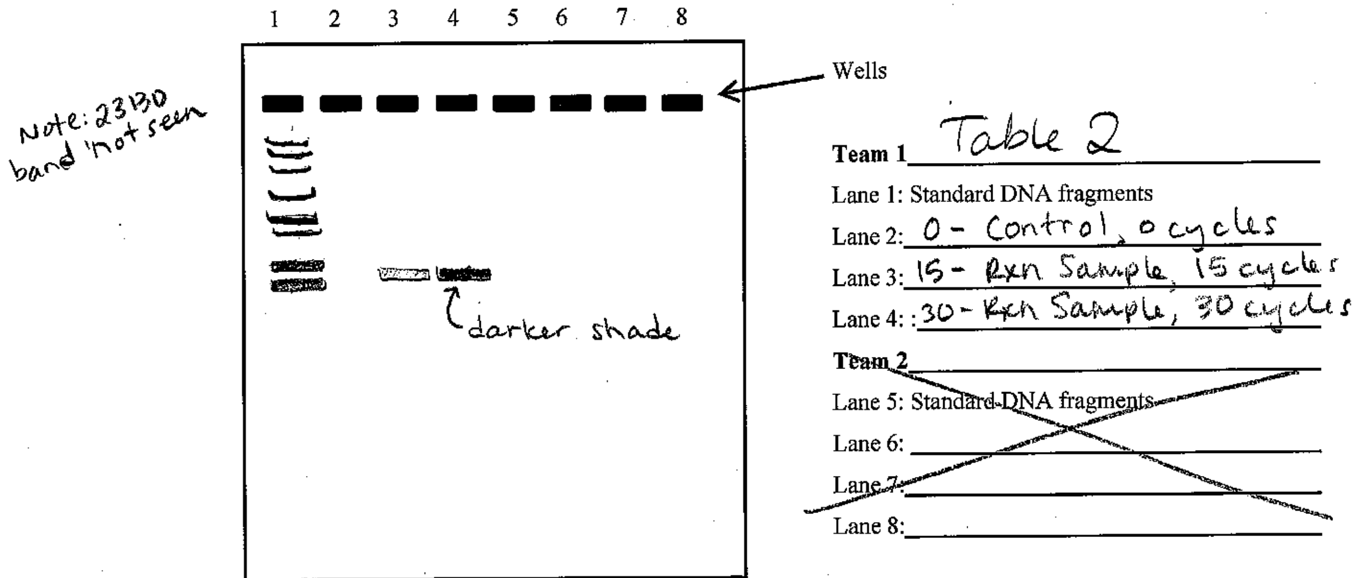
2. Explain the purpose/goal of the research and why PCR was used in the research.

   The goal was amplification of an unknown flanking sequence & to increase the success rate of TAIL-PCR. PCR is suitable for manipulating large number of samples,

3. Go to Methods section of the paper and find the following information for how the PCR was carried out:
   i. The number of PCR cycles carried out (we performed 15 and 30 cycles on our samples): 100 cycles

   ii. The temperatures and times used for each step of the cycle (ours was (94 C for 1 m; 45° C for 1 m; 72° C for 1 m):

Part 4 – PCR results. Write out the source of DNA that was in each lane. Mark the approximate positions for the DNA fragment bands in your electrophoresis gel as well as the other team's on the diagram below. Attempt to recreate the approximate intensity of the band (estimating band intensity is the purpose for carrying out electrophoresis in this lab). The best way to show the intensity of the band would be to photograph the gel. If you photograph your gel and hand in a printed copy, you will receive extra credit. You may also want to describe the band intensities to the side of the diagram.



1. Lanes 1 should have 9 bands which are standard DNA fragments. Their sizes in base pairs and in order from largest to smallest are: 23130, 9416, 6557, 4361, 3000, 2322, 2027, 725 and 570. To the left of the figure, **label the standard bands with their appropriate sizes.** The standard bands in both lanes should be identical, therefore, one label for both will be sufficient. If one of the 9 bands does not appear in either lane, you may have to compare you membrane to others and try and determine which band(s) did not show.

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Our sample did not show the effects, but class mate's did. This was probably due to not enough template DNA. The second lane didn't show anything because it was the control and didn't go through PCR. Lane third lane was a lighter shade then lane 4 because it only went through 15 cycles compared to 30 cycles of PCR.

Part 5 – Post Lab Assignment. Use Google Scholar (if you do not know about Google Scholar, you should) or some other internet tool and find a research article in which PCR was used. Report the following information from the article below.

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   Taberlet, P., Griffin, S., Goossens, B., Questiau, S., Manceau, V., Escaravage, N., Waits, L.P. & Bouvet, J. (1996). Reliable Genotyping of Samples with very Low DNA Quantities using PCR. Nucleic Acids Research, 24(16), 3189-3194. doi: 10.1093/nar/24.16.3189

2. Explain the purpose/goal of the research and why PCR was used in the research.

   This research was done to develop a technique to genotype at a microsatellite locus with only a few picograms of DNA. PCR allowed the small amount of DNA to be amplified so they could actually study the locus.

3. Go to Methods section of the paper and find the following information for how the PCR was carried out: ?

   i. The number of PCR cycles carried out (we performed 15 and 30 cycles on our samples): 57

   ii. The temperatures and times used for each step of the cycle (ours was (94 C for 1 m; 45° C for 1 m; 72° C for 1 m):

   20 cycles → 93°C for 30s, 55°C for 30s, 72°C for 1min 35 cycles → 93°C for 30s, 55°C for 30s, 72°C for 60s
   2 cycles → 93°C for 10s, 55°C for 30s, 72°C for 60s