Appendix D: Pre-lab Assignments and Gel Electrophoresis Worksheet

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PCR Pre-Lab

Please use your carbonless lab notebook to write out your answers.

1) What two techniques will we use to detect the presence of a deletion in the genome of *C. elegans* mutants?

2) What is a primer? What is its specific function in this lab?

3) What is special about the DNA polymerase used in our PCR reaction?

4) How does nested PCR differ from regular PCR?

5) Why are we using nested PCR?

6) What will we use as the template for the external nested PCR reaction? for the internal PCR reaction?

7) How will we visualize our PCR products?

8) On what basis does agarose gel electrophoresis separate pieces of DNA? How can we use it to tell us the size of specific pieces of DNA?

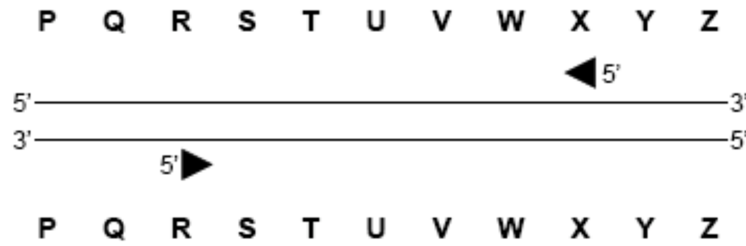
9) What will be used as a positive control for the nested PCR? What will it tell us?

10) What will be used as a negative control? What will it tell us?

11) List three pieces of information that can be learned about a *C. elegans* gene on Worbase (www.wormbase.org).

12) You have an amino acid sequence and are interested in determining its potential protein domains, which resource (website) would you use?

13) A thorough understanding of the process of PCR will help in the analysis of your results. The figure below represents a segment of double-stranded DNA with 100 base pair segments denoted by each letter. The primers are indicated by the arrows for both the top and bottom DNA strands. In the following questions, a "copy" of DNA refers to a double-stranded piece of DNA. Consulting the following web site will help: http://www.dnalc.org/ddnalc/resources/pcr.html



A) Draw the first three cycles of PCR indicating the products labeled with letter designations, and directionality (5'→ 3'). For each cycle, indicate how many copies of target double stranded-DNA and "intermediate DNA" (the DNA includes target DNA region plus a bit of the flanking DNA and/or the original DNA strand) you would have after each cycle assuming you began with one double strand DNA template.

Please answer the following questions assuming there have been four cycles of PCR.

B) What is the total number of target DNA copies and the total number of intermediate DNA copies assuming you began with one double strand DNA template? Note: Target DNA copies contain only the target DNA on both strands.

C) What is the ratio of intermediate to target DNA copies in the fourth cycle? How would this ratio change after twenty cycles? How does this ratio affect what you see in the DNA gel?

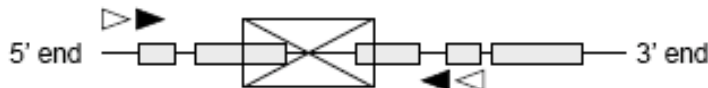
14) Predict the band patterns we could expect to obtain for the following samples and sketch them on the gel below.

lane a) A DNA ladder with the following fragment sizes: 500 bp, 1 kB, 1.5 kB, 2 kB, 3 kB, 4 kB, 5 kB. Note that 1 kB = 1000 bp.

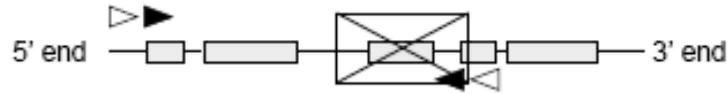
lane b) Internal reaction from a nested PCR reaction performed on a gene from wild-type *C. elegans* using the primers shown (internal primers are 3 kB apart).

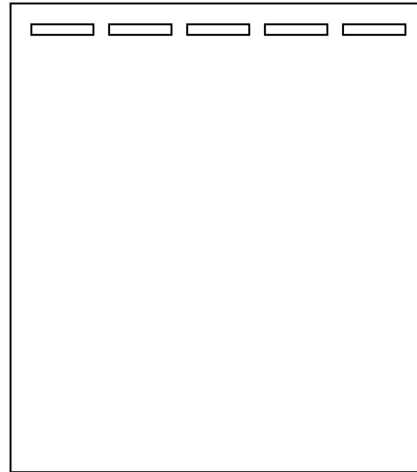
lane c) Internal reaction from a nested PCR reaction performed on a gene from *C. elegans*, using the same primers, with a 500 bp deletion in the region shown.

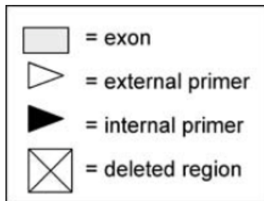
lane d) Internal reaction from a nested PCR reaction performed on a gene from *C. elegans* with a 1 kB deletion in the region shown.



lane e) Internal reaction from a nested PCR reaction PCR reaction performed on a gene from *C. elegans* with a 1 kB deletion in the region shown.







15) In lab, you will also be using nested PCR and gel electrophoresis to amplify and visualize DNA from your feeding RNAi worms. Assuming that you use the same primers and external/internal PCR procedure as in question 14, which lane (b, c, d, or e) on the gel above would best describe the DNA band you would see from your RNAi worms?

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PCR Pre-Lab (KEY)

Please use your carbonless lab notebook to write out your answers.

1) What two techniques will we use to detect the presence of a deletion in the genome of *C. elegans* mutants?
Nested PCR and agarose gel electrophoresis.

2) What is a primer? What is its specific function in this lab?
Primers are short, single-stranded DNA pieces that are complementary to the ends of a DNA sequence that we are interested in amplifying. The primers are used to "direct" the DNA polymerase to make copies of DNA.

3) What is special about the DNA polymerase used in our PCR reaction?
The polymerase is isolated from bacteria (*Thermus aquaticus*) that live in hot springs. The bacteria are adapted to high temperatures, so the Taq polymerase does not denature during the PCR process even though temperatures reach 100°C.

4) How does nested PCR differ from regular PCR?
Nested PCR involves performing two separate PCR reactions.

5) Why are we using nested PCR?
Nested PCR helps to promote greater amplification and specificity when working with large complex pools of DNA, like genomic DNA.

6) What will we use as the template for the external nested PCR reaction? for the internal PCR reaction?
Template for external: genomic DNA from *C. elegans*. Template for internal: the external PCR reaction.

7) How will we visualize our PCR products?
Through agarose gel electrophoresis.

8) On what basis does agarose gel electrophoresis separate pieces of DNA? How can we use it to tell us the size of specific pieces of DNA?
Agarose gel electrophoresis separates DNA pieces on the basis of charge, size, and shape (configuration). To find the size of our unknown DNA sequences, we compare their migration distances with those of molecular weight standards, or markers.

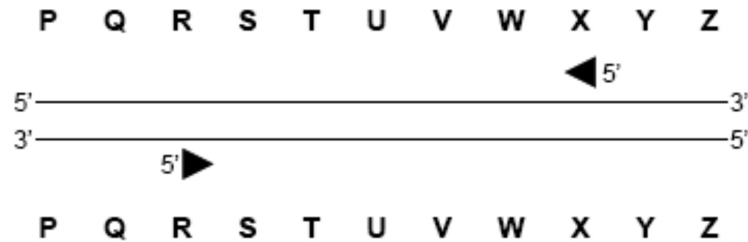
9) What will be used as a positive control for the nested PCR? What will it tell us?
Genomic DNA from wild-type *C. elegans* will serve as a positive control. We know that wild-type *C. elegans* should not have a deletion in the gene. PCR should yield a nice band on our agarose gel of an expected molecular weight. This will let us know that our current materials and techniques are sound.

10) What will be used as a negative control? What will it tell us?
For the negative control we will prepare a sample with no DNA. This will let us know if any of the bands we see on our agarose gel are from DNA contamination in our stock solutions or from handling.

11) List three pieces of information that can be learned about a *C. elegans* gene on Worbase (www.wormbase.org).
Lots of possible answers, consult Wormbase.

12) You have an amino acid sequence and are interested in determining its potential protein domains, which resource (website) would you use?
pSMART.

13) A thorough understanding of the process of PCR will help in the analysis of your results. The figure below represents a segment of double-stranded DNA with 100 base pair segments denoted by each letter. The primers are indicated by the arrows for both the top and bottom DNA strands. In the following questions, a "copy" of DNA refers to a double-stranded piece of DNA. Consulting the following web site will help: http://www.dnalc.org/ddnalc/resources/pcr.html

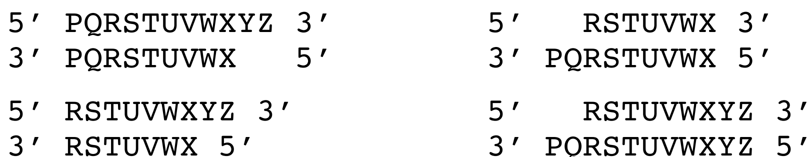


A) Draw the first three cycles of PCR indicating the products labeled with letter designations, and directionality (5'→ 3'). For each cycle, indicate how many copies of target double stranded-DNA and "intermediate DNA" (the DNA includes target DNA region plus a bit of the flanking DNA and/or the original DNA strand) you would have after each cycle assuming you began with one double strand DNA template.

Cycle #1: 2 copies intermediate DNA.

```
5′ PQRSTUVWXYZ 3′        5′   RSTUVWXYZ 3′
3′ PQRSTUVWX   5′        3′ PQRSTUVWXYZ 5′
```

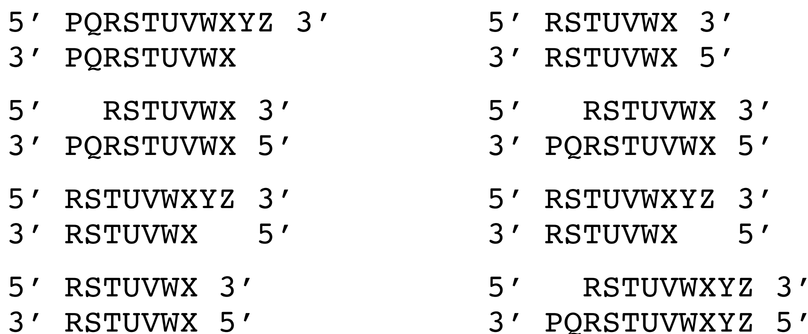
Cycle #2: 4 copies intermediate DNA.



```
5′ PQRSTUVWXYZ 3′        5′   RSTUVWX 3′
3′ PQRSTUVWX   5′        3′ PQRSTUVWX 5′

5′ RSTUVWXYZ 3′          5′   RSTUVWXYZ 3′
3′ RSTUVWX 5′            3′ PQRSTUVWXYZ 5′
```

Cycle #3: 6 copies intermediate DNA, 2 copies target DNA.



```
5′ PQRSTUVWXYZ 3′        5′ RSTUVWX 3′
3′ PQRSTUVWX             3′ RSTUVWX 5′

5′   RSTUVWX 3′          5′   RSTUVWX 3′
3′ PQRSTUVWX 5′          3′ PQRSTUVWX 5′

5′ RSTUVWXYZ 3′          5′ RSTUVWXYZ 3′
3′ RSTUVWX   5′          3′ RSTUVWX   5′

5′ RSTUVWX 3′            5′   RSTUVWXYZ 3′
3′ RSTUVWX 5′            3′ PQRSTUVWXYZ 5′
```

Please answer the following questions assuming there have been four cycles of PCR.

B) What is the total number of target DNA copies and the total number of intermediate DNA copies assuming you began with one double strand DNA template? Note: Target DNA copies contain only the target DNA on both strands.

8 copies of intermediate DNA, 8 copies of target DNA.

C) What is the ratio of intermediate to target DNA copies in the fourth cycle? **(1:1)** How would this ratio change after twenty cycles? **(40:(2^{20} -40))** How does this ratio affect what you see in the DNA gel?

After 20 cycles the vast majority of DNA sequences that migrate through the gel will be target DNA copies, and so will converge in a prominent band.

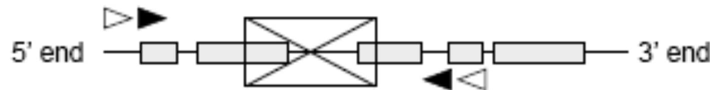
14) Predict the band patterns we could expect to obtain for the following samples and sketch them on the gel below.

lane a) A DNA ladder with the following fragment sizes: 500 bp, 1 kB, 1.5 kB, 2 kB, 3 kB, 4 kB, 5 kB. Note that 1 kB = 1000 bp.

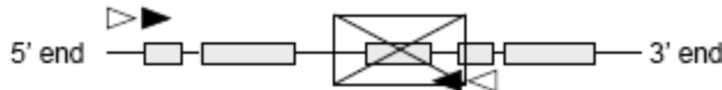
lane b) Internal reaction from a nested PCR reaction performed on a gene from wild-type *C. elegans* using the primers shown (internal primers are 3 kB apart).

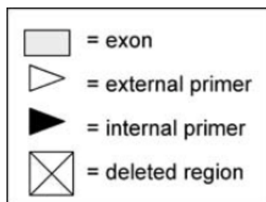
lane c) Internal reaction from a nested PCR reaction performed on a gene from *C. elegans*, using the same primers, with a 500 bp deletion <u>in the region shown</u>.

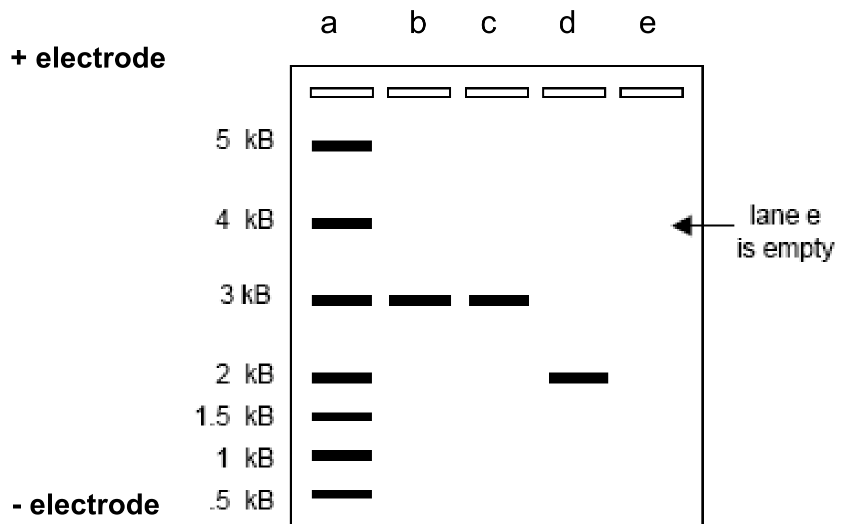
lane d) Internal reaction from a nested PCR reaction performed on a gene from *C. elegans* with a 1 kB deletion <u>in the region shown.</u>



lane e) Internal reaction from a nested PCR reaction PCR reaction performed on a gene from *C. elegans* with a 1 kB deletion <u>in the region shown</u>.







15) In lab, you will also be using nested PCR and gel electrophoresis to amplify and visualize DNA from your feeding RNAi worms. Assuming that you use the same primers and external/internal PCR procedure as in question 14, which lane (b, c, d, or e) on the gel above would best describe the DNA band you would see from your RNAi worms?

Lane "b" should most closely resemble the band you would see produced by DNA from feeding RNAi worms, because the RNAi process destroys your targeted gene's product (mRNA) but does not remove any genomic DNA.

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RNAi pre-lab

Please use your carbonless lab notebook to write out your answers.

1) What is the main objective of the RNAi lab exercise?

2) Briefly explain how the RNAi effect is achieved in one to two sentences.

3) One limitation of using RNAi for disease therapy is that you will possibly knockdown function of a different gene than intended - one with a similar sequence to the one you are targeting. From the NOVA video (http://www.pbs.org/wgbh/nova/sciencenow/3210/02.html), what are two other limitations?

4) Use one sentence to summarize the potential power of RNAi for research (described in the NOVA video):

5) Draw a basic picture of the flow of genetic information in the central dogma. Use the following terms in your drawing: *protein, gene, RNA*. Then indicate where dsRNA can cause an RNA interference (RNAi) effect on the flow of genetic information. You can use your textbook for help with this. Only use the space provided.

6) In our laboratory exercise, in which organism is the double stranded RNA made?

7) In our laboratory exercise, which organism will show the effect of the RNAi?

8) How does making dsRNA differ from normal transcription?

9) What would happen if a protein were translated from a strand of RNA transcribed from the non-template DNA strand (in other words, from a ssRNA strand complementary to an mRNA)?

10) When do you expect to see an effect of the feeding RNAi?

11) Do you expect to see the same phenotype with your feeding RNAi as you see with your deletion strain? Briefly explain your answer in the space provided.

12) There are several ingredients in the feeding RNAi plates and also in the bacterial cultures you will inoculate in lab. What function do the following chemicals have in the feeding RNAi?
    a. lactose
    b. tetracycline
    c. ampicillin

13) What will be used as a positive control for this lab? What is the expected phenotype? What will this tell us?

14) What should be used as negative controls? What is the expected phenotype? What will they tell us?

15) What are two ways bacteria can carry foreign genes?

16) Starting with a feeding clone draw out the molecular steps inside the bacterial cell that will take place to produce dsRNA. Include the lactose-responsive promoter, the T7 RNA polymerase, and the transcription process.

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RNAi pre-lab KEY

Please use your carbonless lab notebook to write out your answers.

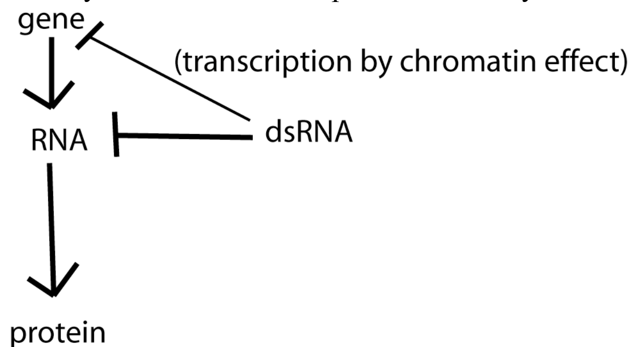
17) What is the main objective of the RNAi portion of this 4 week lab unit?
To knock down our gene of interest using feeding RNAi.

18) Briefly explain how the RNAi effect is achieved in one to two sentences.
Double stranded RNA causes matching mRNA to be degraded and can even stop the corresponding gene from being transcribed.

19) One limitation of using RNAi for disease therapy is that you will possibly knockdown function of a different gene than intended - one with a similar sequence to the one you are targeting. From the NOVA video (http://www.pbs.org/wgbh/nova/sciencenow/3210/02.html), what are two other limitations?
One: delivery to the right cells
Two: does the treatment last/length or effect

20) Use one sentence to summarize the potential power of RNAi for research (described in the NOVA video):
Using RNAi to turn off each gene of choice has the potential to tell you the function of the gene.

21) Draw a <u>basic</u> picture of the flow of genetic information in the central dogma. Use the following terms in your drawing: *protein, gene, RNA*. Then indicate where dsRNA can cause an RNA interference (RNAi) effect on the flow of genetic information. You can use your textbook for help with this. Only use the space provided.



22) In our laboratory exercise, in which organism is the double stranded RNA made?
***E. coli* bacteria (that were specially engineered for this purpose).**

23) In our laboratory exercise, which organism will show the effect of the RNAi?
***C. elegans* worms.**

24) How does making dsRNA differ from normal transcription?
For making dsRNA, transcripts are made in both directions so that complementary strands are made. In normal transcription only the template strand is used to make ssRNA.

25) What would happen if a protein were translated from a strand of RNA transcribed from the non-template DNA strand (in other words, from a ssRNA strand complementary to an mRNA)?
A useless protein that would likely be short, because non-coding sequences often have early stop codons.

26) When do you expect to see an effect of the feeding RNAi?

We will look for the RNAi effect in the progeny of the worms that are fed the dsRNA. (timing is actually variable, depending on the gene and the strength of the effect, but the students may not know this)

27) Do you expect to see the same phenotype with your feeding RNAi as you see with your deletion strain? Briefly explain your answer in the space provided.
Possibly. RNAi can sometimes be weaker than gene deletions that cause strong loss of function. RNAi effects can sometimes be stronger, as well, because a genetic deletion could only result in a partial loss of function.

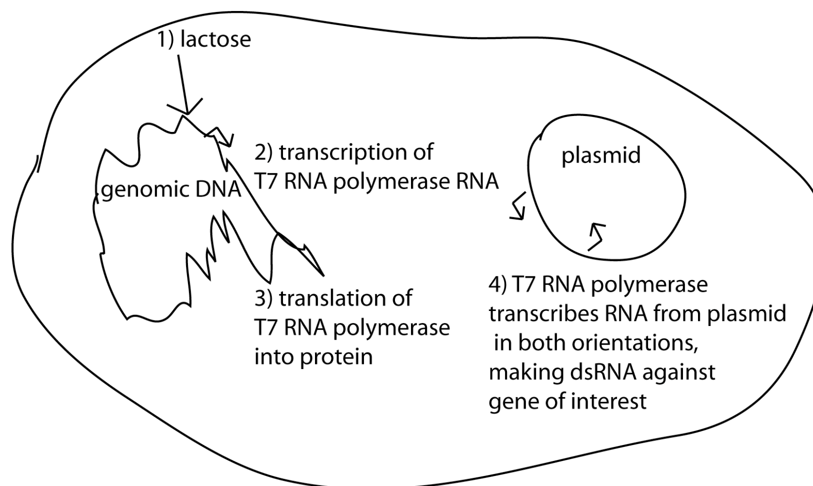
28) There are several ingredients in the feeding RNAi plates and also in the bacterial cultures you will inoculate in lab. What function do the following chemicals have in the feeding RNAi?
a. lactose
b. tetracycline
c. ampicillin
The lactose acts as a co-factor to activate transcription of T7 RNA polymerase.
Tetracycline is a selectable marker for integration of the T7 RNA polymerase gene into the *E. coli* genome.
Ampicillin is a selectable marker for the plasmid that contains DNA sequence matching our gene of interest.

29) What will be used as a positive control for this lab? What is the expected phenotype? What will this tell us?
Bacteria carrying a feeding vector containing sma-1 feeding RNAi clone. We expect this feeding clone to make the worms very small. It will tell us that the feeding plates are working.

30) What should be used as negative controls? What is the expected phenotype? What will they tell us?
One negative control is bacteria containing the empty feeding vector. The expected phenotype is >99% viability. This will tell us that the worms have been well cared for and that the feeding plates aren't toxic. This negative control will be compared to worms that have been taken through the same protocol except being placed on "normal" OP50 bacteria. We expect these worms will be normal. If they are healthier than the worms on the empty vector feeding plates that means that the lactose, tetracycline or ampicillin are affecting the health of the worms.

31) What are two ways bacteria can carry foreign genes?
In a plasmid. Integrated into the genome.

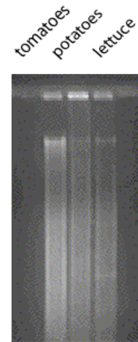
32) Starting with a feeding clone draw out the molecular steps inside the bacterial cell that will take place to produce dsRNA. Include the lactose-responsive promoter, the T7 RNA polymerase, and the transcription process.



PCR / Agarose Gel Electorphoresis Worksheet

Did you know that 60-70% of the food sold in US supermarkets contains at least small quantities of genetically modified products? Many fruits, vegetables, and grains have been genetically modified to promote increased yields, decreased pesticide/herbicide use, and increased nutritional content. This involves getting the plants to take up engineered DNA that confers these traits. Scott and Emily, two novice scientists, are doing an experiment to test if any of the plants in their garden have the *bar* gene, which confers resistance to Roundup™, a commercially available herbicide. They hypothesize that only the tomatoes have the resistance gene. However they are having trouble with the lab procedures. Please help them figure out why their experiments do not work.

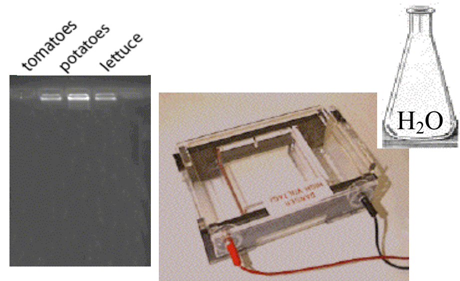
1. First, Scott and Emily isolated genomic DNA from several types of vegetables in the garden.

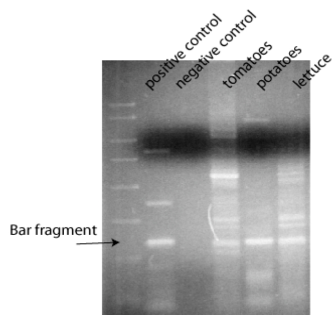


They decided to separate the total genomic DNA out on a gel and see which vegetables contained the *bar* gene. Observe the gel to the right and explain to Scott and Emily why the gel is inconclusive. Why is PCR a more appropriate technique for testing their hypothesis?

2. After this, Scott and Emily decide to use standard PCR to test for the presence of the resistance gene. They designed primers to amplify a 300-bp fragment of the *bar* gene and are ready to set up the reactions. However, they don't have a protocol for PCR. Please list the reagents they should use in the reactions (amounts not necessary) and explain the function of each reagent.

3. When they were ready to run the PCR samples on an agarose gel, Scott loaded the gel, added water to the gel tank and connected the electricity. Afterwards, Emily examined Scott's gel on a UV light box. Below is the gel she saw and she doesn't understand why the samples did not separate on the gel. Present a hypothesis to explain what happened and include your rationale.





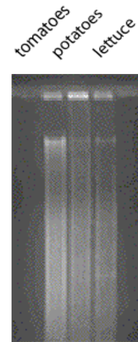
4. Finally, Emily and Scott decided to try the experiment one last time. They finished the reaction anxious to see the results. The gel to the left depicts what they saw. Explain why they have lots of bands other than the band corresponding to the *bar* gene. What could they try differently next time?

5. What can Emily and Scott conclude about their garden vegetables?

PCR / Agarose Gel Electorphoresis Worksheet

Did you know that 60-70% of the food sold in US supermarkets contains at least small quantities of genetically modified products? Many fruits, vegetables, and grains have been genetically modified to promote increased yields, decreased pesticide/herbicide use, and increased nutritional content. This involves getting the plants to take up engineered DNA that confers these traits. Scott and Emily, two novice scientists, are doing an experiment to test if any of the plants in their garden have the *bar* gene, which confers resistance to Roundup™, a commercially available herbicide. They hypothesize that only the tomatoes have the resistance gene. However they are having trouble with the lab procedures. Please help them figure out why their experiments do not work.

1. First, Scott and Emily isolated genomic DNA from several types of vegetables in the garden.



They decided to separate the total genomic DNA out on a gel and see which vegetables contained the *bar* gene. Observe the gel to the right and explain to Scott and Emily why the gel is inconclusive. Why is PCR a more appropriate technique for testing their hypothesis?

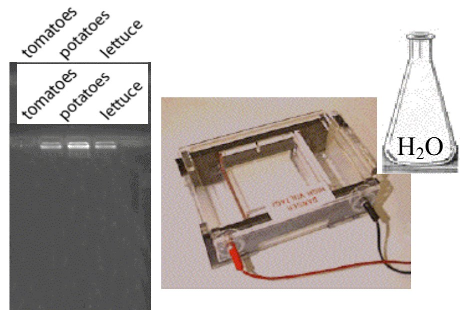
Dear Scott and Emily, by observing genomic DNA, you cannot determine the presence or absence of a specific gene. PCR allows for the amplification of a gene, if present, such that it can be detected on a gel.

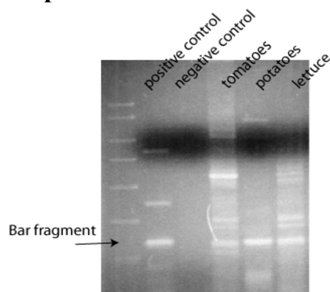
2. After this, Scott and Emily decide to use standard PCR to test for the presence of the resistance gene. They designed primers to amplify a 300-bp fragment of the *bar* gene and are ready to set up the reactions. However, they don't have a protocol for PCR. Please list the reagents they should use in the reactions (amounts not necessary) and explain the function of each reagent.

Genomic DNA – used as a template for DNA replication
Buffer – create appropriate reaction conditions such as salt concentration and pH
dNTPs – individual nucleotides to build the newly forming strand of DNA
Taq polymerase – the heat stable enzyme that carries out the replication process
Primers, forward and reverse – compliment and anneal to a specific site on the genomic DNA and directs the polymerase to this site so that replication can begin.

3. When they were ready to run the PCR samples on an agarose gel, Scott loaded the gel, added water to the gel tank and connected the electricity. Afterwards, Emily examined Scott's gel on a UV light box. Below is the gel she saw and she doesn't understand why the samples did not separate on the gel. Present a hypothesis to explain what happened and include your rationale.

Hypothesis: The gel did not run because water was added to the gel tank instead of running buffer. The buffer is essential because it contains salt, which allow for the conduction of a current across the gel. The current creates the charge differential that causes the negatively charged DNA to move toward the positive pole of the gel tank. This is what causes the separation of DNA.





4. Finally, Emily and Scott decided to try the experiment one last time. They finished the reaction anxious to see the results. The gel to the left depicts what they saw. Explain why they have lots of bands other than the band corresponding to the *bar* gene. What could they try differently next time?

The other bands may be due to the primers binding non-specifically to other similar sequences in the genomic DNA. This is a common problem when working with large complex mixtures of DNA. Next time they could try nested PCR, which involves performing two sets of PCR reactions. The first (external) PCR reaction amplifies the region of interest from genomic DNA and the second (internal) PCR reaction amplifies a smaller region using DNA from the external PCR reaction as template. Anything amplified non-specifically in the first round of PCR is unlikely to be amplified again in the second round. This should result in a decrease in the background bands on their gel.

5. What can Emily and Scott conclude about their garden vegetables?

All their vegetables contain the *bar* gene as determined by the presence of a band corresponding to the bar fragment in the positive control. All the vegetables should be resistant to Roundup.