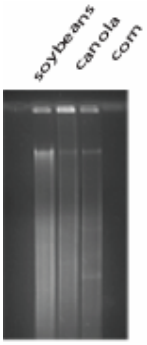
Appendix B. Phillips *et al.*
PCR Exercise

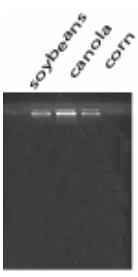
Carlos and Emily, two novice scientists, were asked to test a local farmer's crops for the presence of the *bar* gene, which confers resistance to Roundup™, a commercially available herbicide that the farmer would like to use in her field. However they are having trouble with the lab procedures. Please help them figure out why their experiments do not work.

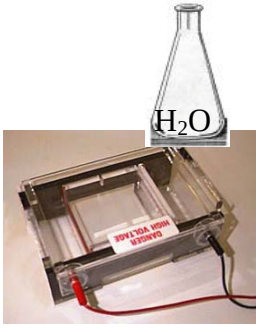
First, Carlos isolated genomic DNA from several crops. He decided to separate the total genomic DNA out on a gel after restriction enzyme digest and see which crops contained the *bar* gene. Observe the gel to the right and explain to Carlos why his gel is inconclusive. Why is PCR a more appropriate technique for testing for the presence of the *bar* gene?



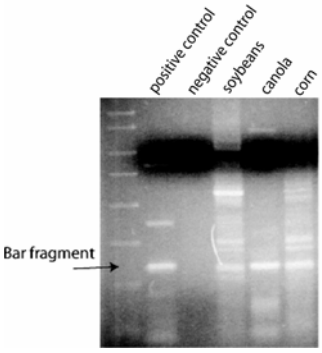
2. Now that they have decided to use PCR to test for the presence of the resistance gene, Emily has designed primers to amplify a 300-bp fragment of the *bar* gene and is ready to set up the reactions. However, she does not have a protocol for PCR. Please list the reagents Emily should use in her reactions (amounts not necessary) and explain the function of each reagent. Why are two primers necessary for amplification?

3. When they were ready to run the PCR samples on an agarose gel, Carlos loaded the gel, added water to the gel tank and connected the electricity. Afterwards, Emily examined Carlos'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 Carlos decided to try the experiment one last time. They added all the right components to the reaction, but added 10 μl of DNA instead of 1 μl. They finished the reaction anxious to see the results. The gel to the left depicts their results. Explain why they have lots of higher molecular weight bands other than the band corresponding to the *bar* gene. (Consider the first few cycles of PCR.)



5. What can Carlos and Emily conclude about the farmer's crops?