

SEED 2009

Day 4: Gel Electrophoresis

Learning Objective: In this lab exercise you will become familiar with the procedures for running gel electrophoresis

Background: Gel electrophoresis is a way in which nucleic acids of different lengths can be separated. DNA is a charged molecule; each nucleotide carries a negative charge. As a result, in the presence of an electric field, DNA will migrate toward the positive cathode (away from the negative anode). Because the gel is thick, the DNA does not go through it quickly; it gets slowed up in all of the tangles of the gel. The larger pieces of DNA get held up for longer periods of time than the shorter piece and therefore do not move as far in the gel. In this way, you are able to separate the DNA by different lengths.

The exercise: You will be running your PCR products on a gel along with the restriction digest of the vector from last week. You will observe the different length bands. If the appropriate bands are present, you will cut out the band for purification and recovery of the DNA next week.

Materials: 1% Agarose gel in 0.5X TBE buffer
TBE Running buffer
SybrSafe DNA Stain

Protocol

1. The gel has already been poured, cooled, and placed in the gel box with the proper amount of buffer. Observe the orientation of the gel in the gel box (note the red and black electrical connections and where the wells of the gel are situated).
2. On a piece of parafilm, make six 4 uL spots of 6X loading buffer. Make sure that they are far enough apart that they cannot mix!
3. Take 46 uL of each of your four PCR products and add them each to a different loading buffer spot. Label below the spots with an appropriate title.
4. Take 46 uL of the restriction digest from last week and add this to another spot. Label this spot 4A5 plasmid digest.
5. Take 46 uL of the undigested instructor 4A5 plasmid and add this to the final spot. Label this spot undigested 4A5 plasmid.

6. Mix the Samples by drawing the liquid in and out of the pipette several times.
7. Carefully pipette 10 uL of the instructor prepared DNA ladder into each of the wells on the sides of the gel. When pipetting into wells you must be very careful not to pierce the bottom of the gel and you must pipette slowly so that the liquid does not come out of the wells. NO BUBBLES. The loading buffer is more dense than the TBE running buffer so, if done gently, the sample will settle into the well.
8. **Very carefully** pipette the 50 uL of each sample with loading buffer into a different well of the gel. Again, be extremely careful as before! Make sure you record in your lab notebook which wells contain which samples!
9. When all of the wells are filled, place the cover on the gel box. Connect the cables (red and black) to the appropriate terminals on the power supply.
10. Set the power supply voltage to 125 V and start the gel running. Observe which direction the blue spots begin to move.
11. After the gel has run for 1 hour, you will see the blue color of the loading buffer has moved most of the way down the gel. Stop the gel running by turning off the power supply.
12. Look at the gel on the UV-light table. Make sure you wear the appropriate face shield to protect you from the ultraviolet light.
13. Observe the ladder pattern. Identify the length of the various bands. Record in your lab notebook.
14. Your PCR products should have a band that is 1000 bp in length. The digested vector should be slightly longer. If these bands are present, very carefully cut them out of the gel using a razor blade. Cut the smallest piece of gel possible which includes the bulk of the band. The slice should be very, very thin.
15. Place the gel slices into individual clean 1.5mL microfuge tubes and place them in the freezer until next week.