



College of the Canyons: Introduction to Biotechnology

Gel Electrophoresis and DNA Analysis: Post Lab

1. What functional group is responsible for DNA's negative charge? Draw and name it.
Phosphate: PO_4^{-3}

2. If you were to use a 4% agarose gel instead of a .8% agarose gel, what effects would this make on the electrophoresis gel and why? Give three examples, ideas.

The fragments would move less in 4% gel as increasing % means more "mesh" (agarose) which slows DNA. Tighter bands @ 4%, the tighter mesh also compresses the bands making them "crisper". The voltage would have to be increased to achieve the same amount of separation (or increase the run time). The gel will also be a bit more difficult to photograph as the concentrated agarose obscures the DNA (sort of like looking through fog)...

3. Name / describe three roles of the buffer solution in DNA gel electrophoresis
 - a) Keeps pH stable,
 - b) preserves DNA,
 - c) conducts electricity to allow current to flow
 - d) deactivates degrading enzymes by removing key ions that these enzymes require.

4. Explain why "ladder DNA" was run with your gel. Name / describe three ideas relating to this idea.

Standard with known amount/mass (create standard curve), helps to check if cyber green will stain DNA, determine mass or amount of DNA for unknown samples by comparing with the standard (AKA Ladder DNA).

5. What possible errors could occur if loading dye was not added to your sample before the loading of the wells? Name two issues / problems.

Little (if any) DNA would get into gel as loading dye helps it to sink. Also, hard to visually follow DNA as it migrates through gel, so may stop the gel at the wrong time.

6. When graphing your distance migrated results, what form should the graph take (axis, units, etc) so that the results can be interpolated easily?

Semi log with distance migrated on linear axis (mm units) and mass on log axis (base pair units).

7. Explain how Ethidium Bromide allows us to visualize the DNA fragments. What are two features ANY DNA dye should have to be effective?

The dye must be able to a) bind with DNA very specifically, and B) be highly visible under some sort of lighting treatment. So..the Et-Br (and cybersafe for that matter) binds with the DNA and can absorb UV light and re-emit a visible light (in the orange spectrum for Et-Br and in the green spectrum for cybersafe).

8. Describe how an endonuclease can be used to determine if a piece of DNA is linear or circular. Hint: review lab on gel electrophoresis. Remember, cut a loop once, get one piece of DNA, cut a linear piece once and get two pieces of DNA.