Electrophoresis: Hints & Help

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Agarose Gel Electrophoresis Hints and Help

SUGGESTIONS AND REMINDERS

To maximize success with dye and DNA electrophoresis experiments and to avoid potential problems, several suggestions and reminders are listed below.

1. Use only distilled or deionized water to prepare buffers and gels. Do not use tap water.
2. To ensure that dye or DNA fragments are well resolved, make sure the gel formulation is correct and that electrophoresis is conducted for the recommended optimal amount of time.
3. Correctly dilute the concentrated buffer when preparing both the gel and electrophoresis (chamber) buffer. Remember that without buffer in the gel, there will be no dye or DNA mobility.
4. For optimal results, use fresh electrophoresis buffer prepared according to instructions.
5. Before performing the actual experiment, practice sample delivery techniques to avoid diluting the sample with buffer during gel loading.
6. For optimal electrophoretic separation, do not use voltages higher than 125 volts for agarose gel electrophoresis. Higher voltages can overheat and melt the gel.
7. To avoid loss of DNA fragments into the buffer, make sure the gel is properly oriented so the samples are electrophoresed from the negative electrode (cathode) towards the positive electrode (anode).

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8. DNA samples contain tracking dye, which moves through the gel ahead of most DNA (except extremely small fragments. Migration of the tracking dye will become clearly visible in the gel after approximately 10-15 minutes. (For dye electrophoresis, tracking dye is not necessary because the fragments are visible during electrophoretic separation.)

9. Do not move the apparatus after the samples have been loaded.
   - Moving the apparatus will dislodge the samples from the wells into the buffer and will compromise results.
   - If it is absolutely necessary to move the apparatus during electrophoresis, you may safely do so after the tracking dye has migrated at least 1 cm from the wells into the gel.

10. If DNA fragments or dye molecules are similar in size, fragments will migrate close to one another.
    - In general, longer electrophoretic runs will increase the separation between fragments of similar size.
    - Experiments which involve measurement of fragment molecular size or weight should be run at the recommended optimal time to ensure adequate separation.

11. To guard against gels that are missing small DNA fragments (small fragments move faster), remember that the tracking dye in the sample moves through the gel ahead of the smallest DNA fragments.
    - Terminate electrophoresis when the tracking dye has moved at least 3.5 to 4 centimeters from the wells and before it moves off the gel.
    - For convenience, the power source can be connected to a household automatic light timer to terminate the electrophoretic separation and avoid running samples off the end of the gel.

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12. Staining is not required for dye electrophoresis, but results must be analyzed upon completion of the electrophoretic separation. Because dye molecules are extremely small compared to DNA fragments, they will diffuse out of gel. Thus, gels involving the separation of dyes cannot be stored.

    Idea Suggestion: After electrophoresis, place the gel in a zip lock bag and place the gel on a computer scanner. Scan the gel and save the image for students.

13. For optimal results with DNA gels, stain the gel as soon as possible after electrophoresis. If DNA bands appear faint, repeat the procedure. Staining for a longer time will not harm the gel. Gels that have been re-stained with InstaStain® Methylene Blue may require longer destaining.

14. The figure at right represents a typical agarose gel which does not contain a DNA dye, such as methylene blue or ethidium bromide, during electrophoresis. The tracking dye is visible, but the DNA fragments will only be visible after staining.

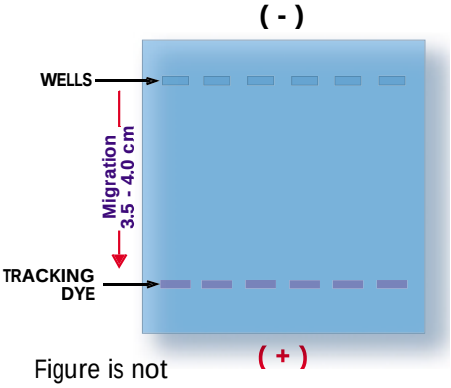

Figure is not drawn to scale.

If dyes are included in the agarose gel, the mobility of DNA bands can be distorted. If methylene blue is included in a gel, additional staining will be required. If ethidium bromide is added, an "ethidium bromide front" will result because the stain will move in the opposite direction (towards the negative electrode as opposed to the DNA which travels towards the positive electrode) and small bands (small DNA fragments) will not be stained.