Gel electrophoresis

Introduction

Gel electrophoresis separates pieces of DNA by length.

Materials

- Agarose gel, 1 lane per sample, plus at least 1 lane for the ladder.
  - If you just poured the gel, make sure it has had **at least 30 minutes to set.**
  - If you have a large gel but only need a few lanes, cut out just a piece of it with a razor blade. Cut straight!
- Samples to run
- 6X NEB Purple Loading Dye
- Molecular weight standard (aka "ladder")
  - Commonly available ladders are **Hyperladder I** and **NEB 2-Log**.
- Gel box, lid, leads
- Electrophoresis power supply
- 1X TAE buffer, enough to fill the gel box.

Procedure

Prepare your samples

1. If your samples are frozen, thaw them completely, flick or vortex to mix, then pulse down in the microfuge.
2. If your samples are not already in loading dye, mix them **1:6** with **6X NEB Purple Loading Dye**

   Add 1 μl of loading dye for every 5 μl of sample.
   Pro tip: If you don't want to mix loading gel with your entire sample (PCR products, for example), cut off a strip of Parafilm; pipette 2-3 ul of sample onto the parafilm; add 1 ul loading dye; mix by pipetting and load directly.

Set up the gel box

3. Program the voltage on the power supply. For the small gel box, use **100V**; for the large gel box, use **150V.**
4. Program the time on the power supply. For a small analytical gel, set the timer for 30 minutes.

   For larger gels, start at 45 minutes, and then check regularly!
5. Attach the leads to the gel box cover. Make sure that the red (positive) lead is attached to the side of the box **farthest away from the wells in the gel.**

Remember, the DNA is negatively charged and will move toward the positive terminal. A useful neumonic for remembering this is **"Run to Red."**

6. If the gel box has not been used previously that day, empty it and rinse it out with DI water.

   The TAE buffer can be re-used, but if it's been sitting out for too long it evaporates and the salt concentration (and conductivity) changes. TAE is cheap; when in doubt, replace it.

7. Place the gel in the gel box.

8. Pour TAE into the gel box until it **just barely covers** the gel.

   The TAE's purpose is to conduct electricity; over-filling the gel box results in a larger conductive path, more current flow, and more heating (which can screw up your run.)

9. Check the wells to see that they are free of bubbles. If there are bubbles, blow them out by pipetting 100 μl of TAE from the gel box into the well.

Load your samples

10. Load your samples. For large combs, load **10 μl**; for small combs, load **5 μl.**

    Work quickly. The samples begin to diffuse in the buffer, leading to smeared bands.

11. Load the ladder in the last lane. Load 1/2 the volume of your samples: for large combs, load **5 μl,** for small combs, load **2.5 μl.**

12. Place the lid on the gel box. Make sure it is seated on the brass contacts.

Run the gel

13. Press the **Start** or **Run** button on the power supply.

14. Make sure the power supply doesn't complain about an open circuit. If it does, re-seat the gel box lid and press **Run** again.

15. Double-check that there are bubbles forming on the platinum wires at either end of the gel box.

16. Double-check that the red (positive) lead is on the side farthest from the wells. Remember, **Run to Red.**

17. Run until the **pink** band is 2/3 to 3/4 of the way down the gel. For small analytical gels, this should take 30 minutes. For larger gels, start at 45 minutes and check regularly!

Image the gel

18. If the power supply is still running, press the **Stop** button.

19. Lift the lid off of the gel box. **Lift the lid straight up. If you try to "hinge" it up, the lid will break.**

20. Transfer the gel to the GelDoc.

21. If it's not running, start QuantityOne from the toolbar.

    If necessary, click the top button on the toolbox to select the scanner.
    If necessary, reset the camera (as per instructions on the GelDoc computer.)

22. Press the **Epifluorescent Illumination** button on the GelDoc. Check the gel's position, zoom and focus.

    You want to be zoomed in so that the gel fills the field of view, and focussed so that the well edges are sharp.

23. Close the GelDoc door. Click **Auto Expose.**

    The **Auto Expose** functionality generally over-exposes my gels a bit. If your gel is over-exposed, remove 1/3 of the exposure time, type it into the **Exposure** box, then click **Manually Expose.**

24. In the **File** menu, select, **Export as JPEG...**. Save your gel to the **iGEM** folder on the desktop.

25. Open Benchling. Log in, and copy the gel to the **Description** page for the plasmid you're building.

    If there are multiple plasmids on the gel, save it to **each plasmid's Description.**

26. Discard the gel in the biowaste box. Wipe down the gel doc with a little water and a paper towel or Kimwipe.

Annotate the gel

27. **Immediately, before you forget what's where**, annotate the gel.

    List what is in each lane.
    Describe whether the pattern is what you expected or not. (You should have an *in silico* digestion to compare it to!)