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∅∅∅∅∅ **Gel Electrophoresis**

Gel electrophoresis functions as a method of separating DNA strands based on length. From this assessment we can glean a surprisingly varied amount of information-everything from determining if a PCR reaction proceeded correctly to finding mutations. Electrophoresis capitalizes on the negatively charged nature of DNA (or RNA) molecules as well their steric interactions with crosslinked polymers, which vary based on their size. Genetic material is set in a polymer gel made of *agarose* and pulled towards an electrode with an electric current; larger segments experience more interactions with the agarose polymer and hence move slower than larger ones. Once finished, "bands" are resolved on the material which can be compared by length; their actual length in basepairs can be roughly extrapolated from the gel with a logarithmic scale.

This protocol is involves the compound *ethidium bromide* which is an intercalating agent-that is, it can fit between the base pairs in a double helix; as a useful side effect, it can fluoresce when exposed to certain wavelengths of ultraviolet light and hence functions as an indespensible means of viewing the DNA. "Laboratory folklore" holds this compound as an extreme toxin, as it is capable of introducing frameshift mutations in genetic material *in vitro* by virtue of its shape and binding affinity. Tests on higher eukaryotes have the compound to be somewhat toxic; its toxicity, however, has been show to exist by the virtue of its *metabolites* and not the chemical itself.

It should hence be noted that while ethidium bromide is not the terrifying poison it has been made out to be, neither is it free of danger and should be handled with care.

Note that the "EtBr gel beaker" in the *materials list* will possess a "contaminated" label and can be found in theUSB3151 fume hood as of 5.28.2011. This fume hood will contain gel producing supplies.

Procedure I will produce the gel solution. Procedure *II* will cast the gel,*III* will prepare the loading dye and *IV* will load and run the final gel. Try to complete procedure *III* between *II* and *IV* while the gel is cooling.

Compounds
Agarose (powdered)
TAE or TBE buffer (1% dilution)
Ethidium bromide
Loading dye (sometimes referred to as "blue juice")
Your favorite DNA solution
DNA ladder/EtBr solution

Materials
Autopipettes (25mL)
PCR tube strip (one tube for each sample!)
EtBr gel beaker (*use only this beaker!-in fume hood!*)
Spatula
Weighing boat (2 required)
Gel holder
Gel cast
Pipeteman P20, tips

You will also need access to a
Autopipetter
High precision scale
Microwave (seriously)
Fume hood
Gel station
Ethidium bromide disposal container

External protocols
Using the gel viewer

Procedure I

1. Add **50mL TBE or TAE** buffer to the **EtBr gel beaker** with a **25mL autopipette**.
2. Carefully pour a minute amount of **powdered agarose** onto a **weighing boat**.

3. Slide back the glass panel covering the **high precision scale** and place a second **weighing boat** on the scale's pan. Tare the scale by pressing the (*on/zero*) button.

4. Gently scoop with a **spatula** (or pour) **700mg** powder from the first weighing boat into the second on the scale. Precision should be within ~±10 **mg.**

5. Take out the weighing boat with the 700mg powder from the scale and gently pour the full amount into the beaker of TBE buffer. Discard both boats and any excess powder. Swirl to mix.

6. Place the beaker in a **microwave.** Press the 30 seconds button to heat, then remove and swirl the solution when the time expires. **Repeat** until all agarose powder is dissolved or about 2 minutes of heating time have elapsed.

7. Set the beaker aside and let cool for ~4 minutes. The beaker should be warm, but not painful to touch or hold.

Please note that all disposable materials for procedures *II* and *IV* should be disposed of in the **ethidum bromide waste container.**

Procedure II

1. Add **10µL ethidium bromide** to the beaker with a **P20**. Swirl the beaker until the ethidium bromide is evenly dissolved.

2. Set the **gel holder** into a **cast**. Pour the contents of the beaker slowly into the cast.

3. Using a **P20** tip, pop bubbles in the solution-these will make the gel difficult to resolve. If a bubble will not pop, attempt to move it towards the edge of the cast.

4. Set the **comb** in the gel. Ensure the comb is level with the cast and holder. Seriously, don't forget this step.

5. Set the gel aside and wait 20-25 minutes to cool and solidify.

The next section will generate the loading solution that will load the gel. Make sure to have your DNA products on hand.

Procedure III

1. Tap the **loading dye** tube on the counter to force it to the bottom of the tube. Add **1.2µL**loading dye to each new **PCR tube** with a **P20**.

2. Add **4.8µL your favorite DNA solution** to each PCR tube with a **P20.** Mix by intaking and expelling the DNA/dye solution repeatedly with your pipette. Complete for each PCR sample.

Try not to use more volume than necessary above 6µL, as this will waste solution.

Procedure IV

1. Remove the now solidified gel from the cast by the holder. Remove the comb as well.

2. Place the gel in the **gel station**.

   ∝ *If the buffer has only been used once before you can skip the next step (3); the buffer needs replacement every two gels.*

3. Fill the tank in the gel station with **1% TBE or TAE buffer.** Empty any buffer currently in the tank.

4. Add **6µL DNA ladder** to the far left well of the gel with a **P20.**

5. Add **6µL 5:1 DNA/dye solution(s)** (from procedure *III*) to the other wells, taking note which well corresponds to which solution.

6. Place the **gel station's lid** on the top of the gel stations, making sure to press the electrical contact pins fully into their sockets. The polarity should be oriented such that the wells left by the comb are on the side of the **black** (negative) **electrode** so the DNA can migrate towards the **red** (positive)**electrode.**

7. Connect the gel station wires into the power supply, making sure to keep track of polarity. Set the gel station to **110V**. Make sure to turn on the power supply last; if you touch any part of the gel while 110V the current is active, you will die. Ensure bubbles are emanating from the electrodes in the gel station, as this indicates current flow through the TBE buffer. Wait **30-45 minutes.**

⇒ After the gel is complete, proceed to follow the protocol on *using the gel viewer.*