Blue-Gel electrophoresis – Pouring agarose gels (Preparatory activity)

Nucleic acid electrophoresis is a technique used to separate DNA or RNA fragments by size. DNA samples are pipetted into wells in a gel. The gel is placed in an electrophoresis unit. The entire gel is submerged in a buffer solution that carries an electric current. The electrophoresis unit has a positive pole (anode) and a negative pole (cathode). The electrophoresis instrument is turned on and the oppositely charged electric field causes the nucleic acid samples to migrate toward the positive anode because the sugar-phosphate backbone of the DNA is negatively charges. The larger DNA fragments migrate more slowly because they experience more resistance trying to move through the gel. Smaller fragments move more quickly to the anode and farther from the cathode. If the gel is run too long all the fragments will end up at the anode.

The nucleic acid to be separated can be prepared in several ways before separation by electrophoresis. In the case of large DNA molecules, the DNA is frequently cut into smaller fragments using DNA restriction endonucleases (or restriction enzymes). In other instances, such as PCR amplified samples, enzymes present in the sample that might affect the separation of the molecules are removed through various means before analysis. Once the nucleic acid is properly prepared, the samples of the nucleic acid solution are placed in the wells of the gel and a voltage is applied across the gel for a specified amount of time.

The DNA fragments of different lengths are visualized using a fluorescent dye specific for DNA, such as ethidium bromide. The gel shows bands corresponding to different nucleic acid molecules populations with different molecular weight. Fragment size is usually reported in "nucleotides", "base pairs" or "kb" (for thousands of base pairs) depending upon whether single- or double-stranded nucleic acid has been separated. Fragment size determination is typically done by comparison to commercially available DNA markers containing linear DNA fragments (a ladder) of known length.

The most commonly used gel for DNA electrophoresis is agarose (for relatively long DNA molecules) and polyacrylamide (for high resolution of short DNA molecules, for example in DNA sequencing). Gels are often run in a flat horizontal "slab" but sometimes in vertical slabs.

For short DNA segments such as 20 to 60 bp double stranded DNA, running them in Polyacrylamide gel (PAGE) will give better resolution.

DNA is negatively charged and will move to the positive pole of an electrophoresis setup.

1. Prepare a clean and dry agarose gel casting tray

place the small two side-walled clear casting tray inside one of the two white casting tray wells
 Place a comb at the top of the gel (8 lanes comb)

2. For each lab group, prepare a 2.4% agarose gel using electrophoresis buffer
 add 0.48 g of agarose to 20 ml of 1xTBE buffer

3. Heat the mixture using a microwave or hot plate … try about 30 seconds first, more if necessary
 Until agarose powder is dissolved and the solution becomes clear
 Use caution, as the mix tends to bubble over the top and is very hot

4. Let the agarose solution cool for about 2-3 min at room temperature.

 Swirl the flask intermittently

5. Add 2µL of GreenView Plus 10,000X per 20 mL blueGel mix.

6. Pour the cooled agarose solution into the gel-casting tray with comb

7. Allow gel to completely solidify (until firm to the touch) and remove the comb
 Typically, ~10 minutes for blueGel™ gels

8. Place the gel into the electrophoresis chamber and cover it with 20 mls of 1xTBE buffer

Gel electrophoresis – Running the gel

1. Make sure the gel is completely submerged in electrophoresis buffer
 Ensure that there are no air bubbles in the wells (shake the gel gently if bubbles need to be dislodged)

2. Load DNA samples onto the gel in the following sequence
 Lane 1: 10µL DNA ladder
 Lane 2: 15µL PCR product from **Test Food 1** (tube T1)
 Lane 3: 15µL PCR product from **Test Food 2** (tube T2)
 Lane 4: 15µL PCR product from **'*GMO Banana*'** (Tube G)
 Lane 5: 15µL PCR product from **'*non-GMO Banana*'** (Tube W)

Note: there is **no need to add gel loading dye to your samples**. The *miniPCR EZ PCR Master Mix* and *100 bp DNA Ladder* come premixed with loading dye, and ready to load on your gel!

3. Place the cover on the gel electrophoresis box

4. Press the on button.

5. Conduct electrophoresis for ~25 minutes, or until the colored dye has progressed to at least half the length of the gel
 Check that small bubbles are forming near the terminals in the box
 Longer electrophoresis times will result in better size resolution
 Lower voltages will result in longer electrophoresis times

Once electrophoresis is completed, turn the power off and turn the bulb on and look at your gel.
If you need to, you can turn off ambient lighting or use the portable