Agarose Gel Electrophoresis

Pouring a Standard 1% Agarose Gel:

1. Measure out 1g of agarose.

   Note: *Agarose gels are commonly used in concentrations of 0.7% to 2% depending on the size of bands needed to be separated. Simply adjust the amount of starting agarose to %g/100mL TAE (i.e. 2g/100mL will give you 2%).*

2. Pour agarose powder into microwavable flask along with 100mL of 1xTAE.

   Note: *The 7x7 tray takes ~40mL, the 15x10 tray ~90mL, the 15x15 tray takes 150mL.*

   Note: *TBE can be used instead of TAE, labs usually use one or the other, but there is very little difference between the two. TBE may be better for large fragments.*

3. Microwave for 1-3min (until the agarose is completely dissolved and there is a nice rolling boil).

   Note: *Caution HOT! Be careful stirring, eruptive boiling can occur.*

   Note: *It is a good idea to microwave for 30-45sec, stop and swirl, and then continue towards a boil. Keep an eye on it as the initial boil has a tendency to boil over.*

4. Let agarose solution cool down for 2min.

5. Add gel dye (we use gelRed (3uL/100mL - cheaper) or gelGreen (5uL/100mL - visible under blue light))

   Note: *Caution, although these dyes are supposed to be safer than EtBr, they are still potential mutagens. Always wear gloves when working with them.*

6. Clamp the gel tray in the gel-clamp, check it is level with the spirit-level, and pour the agarose into a gel tray with the well comb in place.

   Note: *Pour slowly to avoid bubbles which will disrupt the gel. Any bubbles can be pushed away from the well comb or towards the sides/edges of the gel with a pipette tip.*

7. Let it sit at room temperature for ~30 minutes (longer for low percentage agarose), until it has completely solidified.

   Note: *If you are in a hurry the gel can also be set more quickly if you place the gel tray at 4°C earlier so that it is already cold when the gel is poured into it.*

Loading Samples and Running an Agarose Gel:

1. Add loading buffer to each of your digest samples.

   Note: *Loading buffer serves two purposes: 1) it provides a visible dye that helps with gel loading and also allows you to gauge how far the gel has run; and 2) it contains a high % glycerol, so after adding it your sample is heavier than water and will settle to the bottom of the gel well, instead of diffusing in the buffer.*

2. Once solidified, place the agarose gel into the gel box (electrophoresis unit).
3. Fill gel box with 1xTAE (or TBE) until the gel is covered. Roughly (1mm over the gel.)
4. Carefully load a molecular weight ladder into the first lane of the gel.

   Note: *It helps to place a black card under the tray to provide contrast to the wells.*

5. Carefully load your samples into the additional wells of the gel.
6. Run the gel at 80-150V.

   Note: *A typical run time is about 1-1.5 hours, depending on the gel concentration and voltage.*

   Note: *Higher voltage runs the gel faster but heats the gel up more, reducing resolution. The larger the gel box, the higher the voltage you can run at.*

7. Turn OFF power, disconnect the electrodes from the power source, and then carefully remove the gel from the gel box.

   Note: *Don't leave the lid off the gel box. The water evaporates causing the buffer to become more concentrate. It also helps to prevent contamination.*

Tips and FAQ

- **How do you get better resolution of bands?**

  A few simple ways to increase the resolution (crispness) of your DNA bands include: a) running the gel at a lower voltage for a longer period of time; b) using a wider gel comb; or c) loading less DNA into well.

- **How do you get better separation of bands?**

  If you have similarly sized bands that are running too close together you can adjust the agarose percentage of the gel to get better separation. A higher percentage agarose gel will help resolve smaller bands from each other, and a lower percentage gel will help separate larger bands.

- **10% Rule:**

  For each sample you want to load on a gel, make 10% more volume than needed because several microliters can be lost in pipetting. For example, if you want to load 1.0µg in 10µL, make 1.1µg in 11µL.