How to make an agarose gel for electrophoresis

Agarose is expensive, so don't waste it. Don't make a huge gel if you don't have a lot of samples to run or if you don't need to run them that far.

Gels are described in terms of percents: 0.7%, 0.8%, 1%, and 1.2% are pretty common gel percentages. The percentage gel you run depends on a few things: what size fragment you're looking for, how good you need the separation of fragments to be, and whether or not you will be Gene Cleaning gel slices after you electrophorese them.

The percentage measurement is a weight/volume thing. For example, a 100% gel would be 100g agarose in 100mL TAE. (TAE is Tris-Acetate-EDTA; it's a buffer and we make gels with TAE and run them in TAE buffer.) You wouldn't make a 100% gel, though, that was just an example. More commonly, a 1% gel would be 1g agarose in 100mL TAE.

We have gel boxes and casting trays that vary in size. The volume of gel you will need to make will depend on the size of the casting tray. For the smallest gel trays, 30-40mL is a convenient volume. The wells of the gel are made by inserting a comb into the slots in the tray, and as the agarose hardens around the comb, wells are formed. The thicker you pour your gel, the deeper the wells will be.

To make a gel, first figure out what volume you want. You can pour water into the tray and when the wells look deep enough, you can record the volume and make your gel using that volume. Alternatively, you can make a set amount of gel and eyeball it as you pour it, saving the rest in a flask with Saran Wrap on the top (so the TAE doesn't evaporate out over time).

Then figure out what mass of agarose to use for the percentage gel you want. For example, if you are making a 30mL gel that you want to be 0.8%, the amount of agarose to use is 0.24g. Measure out the agarose using wax weighing paper. We keep some agarose in a scintillation vial near the balance; that way you don't need a spatula to measure it out. Put the agarose in an Erlenmeyer flask. Measure out the correct volume of TAE using a graduated cylinder. 1X TAE is in a jug thing near the door. Swirl the contents of the flask and cover the top with a paper towel.

Microwave your gel for however long it takes to melt completely. You don't want particulate matter in your gel. Be careful not to burn yourself, because it will be hot. Let it cool on the benchtop for five or so minutes (if you're in a big rush you can run water over the surface of the flask to cool it down).

Next add ethidium bromide -- this is a chemical that intercalates DNA and makes it visible under UV light. **EtBr is a potent mutagen, so make sure you don't get any on your fingers or on yourself, and don't spread it around the lab. You might want to wear gloves.** The amount of EtBr to add is as follows: of a 0.5mg/mL stock solution (that's what most of the stuff around the lab is), add 1/1000 to your gel. For example, if we go back to our 30mL gel, then you would add 30°L of EtBr.

The casting trays we have have this rubber stuff around the edges that makes a tight seal when you put them in the gel boxes sideways. They also have notches where you can insert a comb. The size comb to use depends on the width you want your wells to be. Swirl the flask immediately before pouring the gel into the tray to make sure it's mostly all at the same temperature; otherwise your gel will harden in a weird manner and will be ruined. It will take about 20 minutes for a small gel to harden enough to be used, longer for bigger gels. If you're in a big hurry, you can pour them in the cold room.

When you are finished pouring your gel, rinse out your Erlenmeyer really well with water before you put it in the wash tub, because whoever is doing the dishes will not want to get EtBr all over him/herself. Also, hardened agarose is hard to clean off of glassware.