Making Agarose Gels

Agarose is the most common medium used in gel electrophoresis. It is a polysaccharide polymer, which means it is made of long chains of carbohydrates. It is made mostly from a purified form of agar, which is made from seaweed. When heated, agarose is liquid, but it forms a gel when cool. As agarose solidifies, it forms a web of pores (MATRIX) that can be used to separate particles (like DNA) based on size. It can be thought of as a "molecular sieve."

Materials
weigh boats or foil trays
0.4g agarose powder
TAE Buffer 50x solution

Procedure

1. Using a weigh boat, measure 0.4 g agarose powder
2. Add BUFFER to fill the flask to the 50 ml line
3. Heat in microwave at 15 second intervals*, gently swirling between each heating time. The solution should be heated to boiling. At this stage, the solution will be clear and the agarose will be completely dissolved.

SAFETY NOTE: During heating, the agarose solution will become hotter than boiling water. Use hot mitts. Watch that it does not boil over and burn your hand as you remove the flask from the microwave. This can easily happen as the solution nears the boiling temperature.

4. Allow solution to cool to about 50-60 degrees Celsius. You won't need to measure this temperature, but can estimate it. The flask will be very warm to the touch, but you can hold it without getting burned.
5. Place the RUBBER DAMS on the edges of the CASTING TRAY and carefully pour the liquid agarose into the casting tray. Try to avoid creating bubbles.

Important note: If you pour agarose too hot, it can warp the casting tray. If you let it get too cold, it will solidify in your flask, making clean up more difficult.

Teacher tip: Rinse flasks out immediately so that liquid agarose doesn't gel in the flask. Then, clean up at the end will be much easier. DO NOT let students pour leftover agarose down the sink. If any is left, pour it in the trash. Since it is expensive, try not to have any left over.

6. Place the COMB in one of the notched areas on the casting tray. The position depends on the lab. Many labs will utilize samples that are negatively charged. These samples will be attracted to the positive (red) electrode. For these types of labs, be sure that the combs are placed on the side of the casting tray nearest the negative (black) electrode.
7. The gel will take 10-20 minutes to cool and will look cloudy when it is solidified. Gels can be made up to a week in advance if they are wrapped in plastic wrap or plastic bags and stored in the refrigerator.
8. When ready to use, carefully remove the comb and the rubber dams without tearing the wells.