Agarose Gel Electrophoresis

DNA cutting review and showing of electrophoresis

Prepare agarose gel

Buffer preparation:
TAE (tris acetate) lower capacity.
TBE (tris borate) more costly with increased capacity (ability to conduct electrical current)
Agarose concentration as a function of resolution.

DNA sizes (Kb)	% agarose
30-1?	0.5% (does this make sense?)
12-0.8	0.4%
10-0.5	1.0%
7-0.4	1.2%
3-0.2	1.5%

Measure and boil
Agarose is weighed, buffer added, and heated, then cooled to 55 ^{o}C (only necessary if the tape will be burned.) Consider the following calulation and facts.
You have 10 X buffer and need 50 mls of 1.2 percent agarose.

So… 1.2 % is 1.2 grams per 100 mls. So 50 mls requires 0.6 grams. Add 0.6 grams agarose to 200 ml flask (flask should be at least 2X the volume of agarose to prevent boil over.) Add 5 mls of 10 X buffer (so that final volume will be 1X) and fill flask to 50 mls with DI water.

Pour liquid agarose: Avoid bubbles and ensure that the table is level. Note that different rigs have different idiosyncrasies.

FYI
Agarose is a a linear polysacharide (d-galactose-o(alpha) 3-6 anhydro –L galactose)
There are numerous types of agarose, each with specific features
Note that agar does not equal agarose (agar is impure and is used for culturing of microbes.)

Electrophoresis:
- General electrophoresis: 30-50 kb resolution
- Pulse field electrophoresis: 15 Mb (for genomic studies)
- Specialized agarose can resolve down to 50-20 bp.
- Also can permit reaction to take place in gel, and to easily remove the DNA.

Add samples

Loading buffer consists of:

- EDTA to chelate ions and stop enzymes
- bromophenol blue (to aid in loading and tracking) and equal apx. 300 bp fragment in migration rate.
- Xylenecyanol equals 4000 bp
- Sucrose and/or glycerol to increase sample density.

Place gel into the rig (gates are down, or tape is off) and add 1X TBE buffer until gel wells just disappear below fluid level.

Quantity of DNA is 200-500 ng maximum (to avoid smearing) and 1-5 ng is minimum for Et-Br visualization.

Add power: Samples are loaded near negative terminal (black by convention.) Connect all leads and then turn power on (as evidenced by bubble forming and amperage rising.)

Stain DNA: Et-Br standard information, and use UV to illuminate. Picture is taken with Polaroid camera with a Et-Br filter (appears orange)

Analyze: Standards are used to assess migration rate. Using semi-log/linear graph paper (bp/distance migrated, respectively.) Distance migrated can be measured, and using the graph the base pair size is interpolated (using graph directly.)