

DNA Sequencing Electrophoresis: Lots of Detail, but a very employable skill.

Prepare Electrophoresis Chamber

Clean Plates

- DI water, soap and nylon scrubby
- If gloves are used, wash off residues
- Final DI water rinse, Et-OH rinse (identify insides of plates)
- Sigma coat (to avoid gel sticking), ethanol rinse and dry

Assemble Plates

- Teflon Plates (0.4 mm) along long sides of plate
- Tape around perimeter
- Clamps over spacers (NOT GLASS) to secure

Pour Gel

- PAGE Gel. Make all reagents fresh and BEWARE as polyacrylamide is toxic!
- Need Acrylamide (monomer) bis acrylamide (cross linker) and fresh ammonia persulfate (catalyst)
- Gel takes about 20 minutes to set, so work quickly
- Add solution to one corner with syringe and capillary action will pull in
- Add comb (ensure no bubbles)
- Add some extra acrylamide to top of gel to retard evaporation

Buffer: Side bar: as demonstrated in video, single buffer was used. Can use multiple buffers to create gradients, and pH gradients will affect migration of charged DNA molecules (can also make a gradient out of PAGE % [] as well)

Note that the gel is stratified in 3 tiers. Solution 2 at bottom, 1&2 in middle, and 1 on top.

Why not just 1 on top of 2.. to avoid abrupt transition.

Assemble Apparatus

Insert gel into apparatus with small plates facing towards back.

Clamp into position and fill top reservoir. Rinse comb with buffer to dislodge bubble (bubbles will decrease polymerization) Is this step logical in sequence?

Pre-electrophoresis

- Black plug at top (as DNA moves away from negative)
- Set watts (Why? Voltage and amperage are both a function of watts)
- Run briefly to warm gel and buffer (to permit more stable running)

Review of sequencing reactions (use this time to edit your notes)

Add Samples

Loading dye has 4 functions: increase density, diagnostic (will show if gel is erratic) and shows if there are leaks between teeth, allows for visualization of fragment progress.

2 Dyes used:

- Bromophenol blue: Corresponds to 26 BP fragment
- Xylene-cyanol: Corresponds to 106 BP fragment

Electrophoresis

Sequential addition of samples will allow for more of gel to be read.

First loaded samples will show largest BP fragments, and later samples will show smaller DNA fragments.

Interview “pearls of wisdom,” Clamps and sharks tooth combs have made for better gel runs.

Disassemble Apparatus

Power off, then disconnect

Drain top reservoir

Remove plates, Remove top plate and blot gel to paper.

Dry with dryer or 10 % acetic acid and oven.

Autoradiography

To X-ray... FYI

Results: Note: Gel as lots of idiosyncrasies. Do not bother to memorize, as current gel devices can read with lasers.