POURING SEQUENCING GELS

1. Prepare acrylamide mix:
   -for large plate make 150 ml
   -make sure acrylamide is fresh i.e. < 2 months old

	150ml	500ml
Urea	72 g	240 g
40% acrylamide	22.5 ml	75 ml
5 x **TBE**	30 ml	100 ml
dH_2O	60.6 ml	202 ml

2. To water add urea and stir with low heat until dissolved (may take a while).

3. Wipe down glass plates with EtOH and pour about 500 µl Sigmacote onto top plate (plastic coated) in hood and let dry.

4. Clamp plates together with 0.4 mm spacers. Make sure bent end of spacer is near top and electrodes of clamps are on top. Place Whatman strip in bottom of casting tray.

5. Prepare fresh 25% ammonium persulfate (APS) ($250 mg ml^{-1}$).

6. Add acrylamide and TBE to dissolved urea.

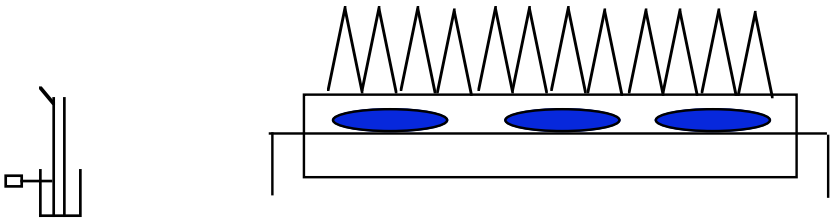
7. Let acrylamide mix, cool and filter sterilize through a Nalgene filter. Add 20 ml to a small beaker and add 40 µl TEMED and 40 µl 25% APS. Mix and quickly pour onto Whatman strip and seal plates into casting tray (set one corner in 1^{st} and follow down to other end to get good wicking). Screw in screws before it solidifies.

8. Get comb and syringe and make sure they're clean (0.4 mm). Stick Kimwipes into the buffer chamber to prevent spillage. Pour 60 ml into a larger beaker and add 60 µl TEMED and 60 µl APS to start polymerization.

9. With syringe suck up 50 ml and pour into corner keeping a small puddle at the tip of syringe. Keep flow constant and as the level approaches the top,

move syringe to center of the plates. Slowly lower down plates to rubber tubing.

10. Insert comb carefully, making sure not to bend the teeth, up to the bottom of the slots. Clamp plates together at top. Keep comb immersed in acrylamide and let dry overnight for best results. (Wet paper towels and place at the top and bottom of the gel. Wrap in Saran.)



10 x TBE (1L)
108 g Tris base
55 g boric acid
9.3 g $Na_2EDTA.H_20$

The pH should be 8.3, without adjustment. Autoclave.

RUNNING SAMPLES ON SEQUENCING GELS

1. Wash off excess acrylamide from top of the gel with water and remove comb and rinse top of gel with water.

2. Remove casting tray and place in gel holder. Fill upper chamber (between plates) to 1 cm of the top. Add 350-500 ml 1 x TBE buffer (no more than 500) into bottom chamber.

3. Flush the top of the gel with buffer using a syringe and a 18G needle. Insert the comb until it just enters the top of the gel. Flush the bottom out as well. Bubbles will cause samples to run to the edge.

4. Prerun the gel at 90W for 1 hr or until it reaches a temp of 50°C.

5. Place reactions in oven at 85°C. While samples are heating (2 min.) flush out wells to remove excess urea. Mark lanes: ..1b..1a..2b.

6. Load 2.5 to 3.0 μl reaction per lane.

7. If running short & longs runs, run 1st set for 3 hr until the top dye reaches the bottom.

8. Load second set and run till blue dye reaches the bottom (about 2.5 h).

9. Shut off power, blot bottom of gel with paper towels and carefully throw top buffer down drain.

10. With a thin spatula pry plates apart and place gel in **fixer** for 20'.

11. Cut two pieces of Whatman filter paper to fit film holder. Place two pieces of Whatman paper on top of gel, cut away extra gel and carefully lift gel from the plate. Place saran wrap on top of gel and dry with heat 1.5 to 2 hr.

12. Remove Saran and expose film overnight.

Gel fixer (1L)

10% methanol	100 ml

10% acetic acid	100 ml