### Preparing an acrylamide gel

1. Set up work area: White absorbent paper, spill catch tray, plate support (hunk of styrofoam).



2. New glass plates are treated by soaking in 2M sodium hydroxide for 1 hour (or more). One side of the shorter plate is treated with Rain‑X. Then, about every 10 runs, the long plate is cleaned again with 2N NaOH, and the short plate is treated with Rain-X.

3. In sink, wash the long glass plate with Alconox or liquid detergent and water, using just a gloved hand to rub. Rinse with tap distilled water. Dry the non-gel side with a paper towel, and set on styrofoam support. Dry the gel side with tissue paper (Kimwipes). Rinse with some 95% ethanol (pure grade from glass bottles) and some water, and dry with tissue paper (Kimwipes). Rinse again with just 95% ethanol, and dry again.

4. Repeat procedure with short plate.

5. Place spacers on long plate, and place short plate on top. Leave no gap between the thick spacer part and the top plate.



6. Place several binder clips on each side, centering the clip over the spacer.

7. Place a 3rd spacer at the bottom, and add more binder clips.

8. For a 60 ml gel, mix 12 ml 5× TBE and 13 ml dH2O. Place on hot plate, add 27 g urea and a stir bar, and warm the solution. Stir until dissolved.

9. Remove from heat, and bring to 51 ml volume with dH2O.

10. Filter through a 0.45 μm 25 mm diameter membrane filter using the 60 ml “Urea” syringe. Filter directly into the acrylamide squirt bottle.

1. Add 9 ml of 40% acrylamide stock. (Stock is 38 g acrylamide, and 2 g bis-acrylamide per each 100 ml)

**Final Gel Composition:**
5.7% Acrylamide
0.3% Bis-Acrylamide
7.49 M = 45% Urea
0.089 M Tris
0.089 M Boric Acid
0.002 M EDTA

12. When everything is ready, add 30 μl TEMED and 320 μl of 10% APS (to make, put 1 g APS in a 15 ml tube, bring to 10 ml with water). Swirl to mix, and **immediately** pour gel. (APS amount is increased gradually as it gets older)



13. If bubbles do get trapped, they can sometimes be pulled out with a bubble grabber. This is a hooked device made from thin plastic, e.g. old X-ray film.

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| 14. Insert combs about 4 mm. Cover with extra acrylamide solution.15. Add 2 binder clips, one over each half of comb. Tilt gel to 15° angle, and allow to polymerize for 1 to 1.5 hours. |  |

16. Pour excess acrylamide solution into marked waste bottle, and clean acrylamide squirt bottle immediately to prevent clogging.

17. Gel may be left overnight. Add moistened (filter paper) Kim-Wipes and cover with plastic film to prevent evaporation.

18. Remove combs and excess acrylamide. Wipe off any residue.

19. Close the drain valve on the gel apparatus.

20. Clamp gel in place in the gel apparatus. Tighten clamps firmly.

21. Mix 800 ml 1x TBE. (160 ml of 5× TBE, bring to 800 ml with water). Add buffer to top reservoir to just cover the short plate.

22. Use the 60 ml syringe to rinse out the well area. Pull up some buffer, and squirt it into the well area.

23. Pour remaining TBE into bottom reservoir. Fill from very right end to avoid bubbles between glass plates. If bubbles are caught, force out somehow with syringe and bent needle.

24. Pre-run at 60 watts for about 30 minutes.

25. Disconnect power cables. Clean out well area again with 60 ml syringe.

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| 26. Carefully remove combs so that teeth may not touch and dent the gel surface, but do not puncture top of the gel.27. Take samples. Samples are denatured for 3 minutes at 90°C (Program #90) and then kept on ice. |  |

28. Run for 2 hours at 60 watts.

### While gel is running

29. Get a piece of Whatman 3MM paper of 13 1/2 × 16 1/2 inches. Optional: cut off corner to line up with lower left side of gel.

### Disassembling the gel

30. Turn off power and remove cables.

31. Drain upper reservoir by opening drain valve.

32. Remove glass plates, blot off any surface liquid, and set on ice to cool, short plate on top. Let cool for 2 to 3 minutes.

33. Remove combs, and slide spacers out from the sides. Pry plates apart. Gel will hopefully stick only to the long (bottom) plate.

34. (Optional: Spray surface of gel with a little bit of water using a mister, to help the paper stick to the gel). Place the piece of Whatman 3MM paper on top of the gel. Smooth it with your fingers. Carefully peel the gel off, which will stick to the paper.

35. Place gel+paper on the drying machine, gel side up. Cover with plastic wrap, and dry under vacuum with heat set to 80°C for 50 minutes.

36. Dump liquid waste into appropriate radioactive waste bottle. If you fill the bottle, cap it, put a piece of tape on top with the current date, and put it in the cabinet under the sink in room 208

37. Wash plates, spacers, gel apparatus, etc.

38. When gel is dry, place gel side in contact with X-ray film for 36 to 72 hours in a room temperature cassette. This may be done by putting a sheet of X-ray film in the cassette (in the dark of course!), with the notched corner at the upper right. On top of this place the dried gel (gel side down) and close the cassette.







