

BARBER LAB SEQUENCING PROTOCOL (ABI 377)

Gel Preparation

1. Wash glass plates using 1% Alconox (VWR 21835-032) and a rubber glove. DO NOT let the bottoms of the plates (laser reading area) touch anything hard.
2. Wash Alconox off with warm tap water, then rinse plates well with DD water. While rinsing, rub read area of plate with gloved hands. Rinse with top of plates facing downward so that rinse water moves away from reading area. Place plates in drying rack tops down so that the laser reading area is not scratched by the rack. Allow to air dry completely.
3. Make up acrylamide solution if there is not enough in the refrigerator (25 mL).

In a 250mL beaker add the following:

1	Magnetic stir bar
36g	Urea (2 vials)
10mL	Long Ranger Gel Solution (Cat. No. 50611)
20mL	5x TBE buffer
42mL	ddH ₂ O

4. Place solution on magnetic stir plate for 10-15 minutes until all urea has gone into solution
5. Filter acrylamide solution using Whatmann glass microfiber filters. Store in refrigerator.
6. Place non-eared plate onto Otter Casting Rack. Be sure that plate serial numbers are on bottom. Plate should be dry, clean, and free of lint or other debris
7. Lightly wet spacers with ddH₂O and place them along the sides of the plate. Wetting spacers facilitates the sliding of the top plate as well as cohesion of spacers to bottom plate. Water should not drip onto main plate surface.
8. Place eared plate, ears first, slightly overlapping the bottom of the non-eared plate. Plates should overlap by about 1 inch. Be sure that serial number of top plate is facing up.
9. Measure out 25 mL of acrylamide solution in a graduated cylinder. Transfer to a beaker and add 125 μ L of freshly prepared APS (0.05g in 500 μ L ddH₂O —this solution should not be more than 1 week old, Fisher Cat. No. A682-500) and 17 μ L of TEMED (ICN Cat. No. 195516). Mix well by swirling, but take care not to introduce air bubbles into the solution.

10. Gently pour a small puddle of the activated acrylamide near the edge of the overlapping plates. Surface tension will draw acrylamide between the plates. As gel spreads fully across plates, gently push top plate towards the top of the bottom plate. Continue to pour acrylamide as needed. Watch the bottom of the plates closely to ensure that no bubbles are introduced. Pushing plates too quickly will introduce bubbles. Pushing too slowly will result in excessive loss of acrylamide from dripping around the spacers. If bubbles form at bottom edge, push top plate back to release bubbles, then continue.
11. Once gel has been completely poured, pour a small bead of acrylamide at the top (leading edge) of the gel. Then take the casting comb, place one corner on the bead of acrylamide and gently lower the comb onto the plate. Angling the comb will prevent bubbles from being trapped beneath the comb, which will affect the leading edge of the gel once clamped. Once comb is fully on plate, gently edge one corner in between the two plates. Continue until the comb is fully in place.
12. Clamp plates together. Place 3 combs across the top of the gel and 4 clamps on opposite sides of the gel at the same time. Clamps should be placed on the areas where the comb and spacers sit.
13. Allow polymerization to continue for at least 2 hours. 30 minutes into polymerization, place a moist paper towel near the bottom of the plate to reduce desiccation.

Sequencing Set Up

Before starting a sequencing run be sure that the computer is not connected to another computer, that filesharing is turned off, and that AppleTalk is inactive.

1. Begin by washing the sequencing plates. Remove clamps, and rinse excess acrylamide in the sink. Use a small amount of 1% alconox to clean the laser reading area. Remove casting comb and rinse ALL acrylamide out of the well. Check carefully looking into a light source, as the acrylamide debris may be easily overlooked. Rinse plates first with warm tap water, then rinse well with DD water. Again, the plates should be oriented bottom up so that rinse water moves away from the reading area. Turn plate on it's side and wick excess water from the well using a kimwipe.
2. Allow plates to airdry or use the compressed air bottles to speed things up. Make sure the air bottle is not inverted or tilted or it will spray the propellant all over the plate! **It is critical that NOTHING ever touch the laser reading area.**
3. While plates continue to dry, make up 1500 mL 1x TBE buffer by diluting 300 mL of 5x buffer in 1200 mL water. TBE should be fresh and free of precipitate. For optimal results, this TBE should be the same stock used to make the gel.

4. Open Collection module of Sequencing Analysis. Click "new". Chose "create new sample sheet". Create a sample sheet for your sequences.
5. Place dried plates into plate sequencer mounting rack. Notches on the non-eared plate should fit snugly against the pins on the plate mounting rack. Lock plate into rack. No force should be required to lock in plate. If plate does not lock in easily, check alignment of plates in rack. Misaligned plates result in poor sequences.
6. Put lower buffer chamber into place and plug into 377. Add 1X TBE bugger to lower buffer chamber. Fill to sharpie mark. Place plate and rack into 377. Again, this should be achieved easily. Plates should align with pins and mounting clamps. No force should be required to lock in rack. If rack does not lock in easily, check alignment of rack and try again.
7. In the collection module of Sequencing Analysis, choose "new". Choose "new sequence run". Close door of 377. Choose "Plate check E" and run "Plate Check". After 1-2 minutes scan lines should appear. These should be flat, and without any significant peaks. If the machine does not appear to be responding quit the program and restart.
8. Set prerun module to ficoll. Place the heating plate into position. Connect the grounding wire and two coolant hoses.
9. Close door and start pre-run to preheat gel. When temperature hits 50C, gel is ready to load. While gel is preheating, resuspend pelleted sequencing reactions and load samples onto membrane comb.

RESUSPEND PELLETT FOR ELECTROPHORESIS

1. Resuspend pellet in 3 μ L of a 1:10 blue 377 dye/formamide solution. Make sure your invisible pellet is resuspended by pumping your pipetman. How do you know? You don't.
2. Following initial resuspension, vortex strip tubes for 10-15 seconds. To be sure all the reaction has settled in the bottom of the tube, either shake down strip tubes or centrifuge briefly.
3. Heat these reactions to 95°C for 3 minutes and then plunge them in ice to dissociate the DNA strands and keep them dissociated. (Program 95 on thermocycler).
4. Load 1 μ l of sample onto tooth of comb. Forward and reverse reactions for each sample should be run next to one another to facilitate easy analysis.
5. Allow samples to dry on comb for at least 20 minutes.

Loading Gel

1. Stop prerun. Add sample sheet. Adjust Run Module to 36E-1200. Time should be 7 hours. Filter should be dRhod, BDset{any primer}.
2. Start run, press pause and open the 377 door. Using a pipetmen fill well with ~400 μ l of 20% Ficoll Solution (Gel Company Cat. No. DAL25; stored in fridge). Be very careful not to get ficoll on back of plate, as it is very sticky. Add a thin line of ficoll along the top edge of well.
3. Load membrane comb into well.
4. Working quickly, but carefully, attach upper buffer tray. Fill with buffer. Restart run for 1 minute. Stop run. Remove comb. Rinse ficoll solution from well using syringe. Be sure no ficoll remains. Cover upper buffer chamber. Close door. Restart run. Check status and log windows to make sure everything is working properly. Check scan lines and gel image to ensure that machine is collecting data.
5. Rinse membrane comb and allow to dry.

Gel Take Down

1. Open door of 377. Drain buffer from both upper and lower buffer chambers
2. Unplug heating plate ground wire and cooling tubes. Remove heating plate. You must remove heating plate prior to the upper chamber, or the heating plate will stick to the glass plates.
3. Remove upper buffer chamber.
4. Remove gel in mounting rack. Be sure that plates are still secured in mounting rack.
5. Remove lower buffer chamber. Wipe excess buffer/condensation from 377.
6. Rinse upper and lower buffer chamber and upper buffer chamber cover.
7. Remove plates from mounting rack. Rinse mounting rack. Pay special attention to lower portion where it sits in buffer.
8. Using a plastic wedge, separate glass plates. Stick wedge underneath ear of plate. DO NOT pry with wedge, simply insert fully. Plates should separate easily.

9. Rinse plates thoroughly of buffer and acrylamide. Wash with 1% alconox. Rinse well in DD water and place in drying rack top down. Remember, it may be you that uses these plates next. Be sure they are clean and ready to use again.
10. Use a paper towel to remove gel from second plate. By placing towel flat against gel, gel will stick to towel and remove easily. DO NOT wipe towel across plates, this will cause scratching. Rinse, wash with 1% alconox, then rinse again and place in drying rack upside down.
11. Look around the room and make sure room is clean and tidy for the next person to use the facility.
12. Copy your run folder onto the current month's sequence folder on DUDE then move run folder into your own directory if you wish to save it.

****Run Folders will be burned onto a CDROM at the end of every month****