PREPARING AND RUNNING GENOMYX SEQUENCING APPARATUS
Adapted from BV's cookbook, commented by T.-C. He 1/15/2002

0. Prepare samples as per Cycle Sequencing or Sequenase protocols.

1. Clean plates with Windex--water--70% EtOH--100% EtOH using paper towels. If the plates are new, first apply "glass slick" to the notched plate by squirting about 5 ml (spread drops around; don't make a big puddle in the middle) onto the plate and slowly rubbing in with paper towels for about 2 minutes. Do not wipe off, instead rub in. Glass slick keeps the gel from sticking to the notched plates. Wash once with 100% ethanol.

2. Place two Styrofoam 50 ml conical racks on overlapping diapers, and rest the non-notched plate on the racks, labeled side facing the counter top. Wet the spacers (0.34 mm spacers for less resolution but ease of loading, 0.20 mm wedge spacers for longer reads) and place on sides of plate. Dry the spacers on the glass plate with paper towels.

3. Add APS (800 ul 10%) and TEMED (80 ul) to a 100ml of gel mix. Slowly pour and spread about 30ml of solution on the bottom part of the plate so that it forms a rectangular puddle. Be careful not to allow any bubbles. Place the notched plate vertically, with "ass-end" (this means the non-notched end) down, on top of the gel mix puddle, about one inch from the ass-end of the non-notched plate. GENTLY allow the non-notched plate to lie on top of the notched one, while keeping the ass-end of the notched plate in the puddle. This squeezes the puddle into a thin film that travels from the ass-end to the top-end of the plates. If you don't do it gently you'll get bubbles. Don't panic. Simply lift the top of the notched plate; the puddle gets unsqueezed and the bubbles burst spontaneously (or with your help). This method is much faster than the older one, particularly for thin or wedged gels, since the movement of the gel mix is driven by the weight of the notched plate, instead of (the much weaker) capillary forces. If at the end of the squeeze the mix didn't reach the top of the notched plate, ass-feed the gel mix into the interplate space to finish the job. The mix will be dragged by capillary action combined with your tapping, to avoid bubbles. When some of the gel is at the end, align plates. Place left thumb on spacer at bottom of the gel, and slowly slide the top plate to align it with the bottom plate. Pour a little gel mix into the top to finish off the job. Insert comb with teeth pointing up, and apply clips - two to each side and five to the top. NEVER PLACE CLIPS AT BOTTOM... WILL DISTORT GEL. Polymerize about 30-60 min.

4. Remove clips. Bring gel to sink, and rinse off excess acrylamide. DO NOT use spatulas or other sharp objects to remove the thin layer of acrylamide between the comb and the bevel of the notched plate; YOU'RE DAMAGING THE COMB. Simply remove the comb carefully and apply pressure towards the notched plate while you remove it and you'll take out the acrylamide. To make sure you don't have any acrylamide in the well space, you can drain all liquid from that space by gravity and

use paper towels to suck off the remaining liquid by capillary action. Any "liquid bubbles" remaining usually represent pieces of acrylamide left there. You can remove them with a comb - CAREFULLY, so as not to damage the teeth. Place upper buffer chamber on the top of the gel, tightening the top clips to snugness, BUT NO MORE. If you over tighten, expansion of the plates during a hot run will cause the ears of the notched plate to break off. The top buffer chamber never leaks! Don't worry!

5. Place gel in GENOMYX. Turn clips on either side to secure gel in the rig. Add buffer to bottom chamber, then shove in the gasket (this serves to decrease evaporation). Add buffer to the top chamber. Use bent syringe to clear out "soon-to-be" wells. Insert comb. Attach lead to upper chamber, and assemble top to upper chamber with swing-up attachments. Denature samples at 95 degrees for 30 seconds. Load. Attach top to upper buffer chamber with swing up fasteners, and attach lead. (Note: GENOMYX gels do not need to be pre-run).

6. Run gel - program **#12**. Input the appropriate length of run. Don't worry if you are not around... when the run ends, it begins a slow run to keep the bands from diffusing. After 8 hours, the bands will begin to diffuse. If you want to run the gel for more than 2.5 hours, it is a good idea to replace the top buffer at half-time.

7. Press stop. Remove the gel, still with the upper buffer chamber attached, to the sink. Remove the upper buffer chamber, allowing the buffer to run down the back of the gel. Now, go back to the machine, and run program **#60**. Press "pump," thus beginning to pump out the lower buffer chamber. Take the gel to counter, and place on diaper with the notched plate on top. Remove the notched plate by prying it up with a spatula FROM THE TOP END (otherwise you'll break the plates ears) - don't worry about destroying a few millimeters of the gel when you do this. Do not remove spacers at this point.

8. Place the plate with the gel attached back in the Genomyx machine, with the gel facing outside, and the top down. Close the front door of the Genomyx gently, leaving it partially ajar. Press dry. Allow drying for 10-15 minutes, until the urea is dry and crystallized. Shut off "dry," remove the gel from the machine and bring to the sink. (**Tip: if you completely close the door, it would actually take longer to dry the gel because of the limited airflow!**). Remove spacers. Place the gel (on the plate) on the rim of the sink in the following way: three corners of the plate are on the rim of the sink, and the fourth one is hanging in the air; one corner is on the distal rim (close to regular faucet), on top of a paper towel folded in three (as they come out of the dispenser) and once more; one corner is on the left rim, on top of a paper towel folded in three, and then twice; one corner is on the proximal rim; the paper towels are COMPLETELY under the plate, hanging into the sink. With this setting the plate is ALMOST horizontal, which allows the gel to withstand higher water flow. The low angle and the

paper towels ensure that the water flows into the sink. Since the sink's rim is tilted, it is a good idea to complement the set up with a paper towel on the distal left corner of the rim, extending from the wall into the sink; it will work as a capillary trap for water that gets out of the sink. Run both distilled AND TAP (hot is even better) water over the gel to wash off the urea; if you do it right, you can leave the plate there by itself. Continue running water for at least two minutes after all the urea crystals are gone. It is a good idea to rotate the plate 180 degrees (TURN OFF THE WATER TO DO THIS), so that all corners get a good wash. At the end of the wash, turn the tap water off first, and allow the distilled to run for another 30 seconds. If you do this right, you will need only one wash, which means that a well done wash and dry can take only about 30 minutes from stopping the gel to putting it on film! DO NOT TRY TO SAVE TIME ON THE WASHES! Drying takes at least 10 minutes (and the more urea you have the longer it might take); an extra minute of washing can save you a whole cycle of washing and drying! To decide whether you need to wash and dry again, check if the corners of the gel stick to your gloves when the gel is dry. If it sticks, even slightly, you need to wash again (sticking will get worse once the gel cools down).

9. Remove the glass plate/gel. In darkroom, place in a cassette gel side up, then the film, then another glass plate to hold the film in place. Replace the top of the cassette, fasten clips, and expose o/n. The fastest readable exposure obtained in this lab was 30 minutes (DAN's the champ), but overnight is standard. If you can't read after an overnight exposure, your reactions suck or the 33p is too old.

10. Once satisfactory autorad is obtained, wash off the gel by wetting it completely (DO NOT WALK AWAY AFTER PUTTING IT IN THE SINK WITH THE WATER RUNNING) and scraping it off with a razor blade. Dispose of waste to the radioactive waste container.