

ABI 310 Clean-up and Set-up

By Alisa Ramakrishnan, Tuesday, July 06, 2004

- We use bad chemicals with this machine (polymer is a neurotoxin), so please always use gloves.
- Use only high quality ddH₂O for anything having to do with this machine, to avoid clogs in the capillary.*
- The block must be cleaned every 7-10 days, so estimate the amount of polymer you will need before filling the syringe (3-4uL per sample for a short capillary + amount for filling block). Any leftover polymer will not be able to be used, except for maybe priming the syringe later.
- Keep a sharp lookout for any white crystals anywhere on the block, syringe, etc (threads, holes, fittings, tubes, etc) and rinse them with water to get rid of them
- If you're going to do a run soon after you clean the machine, and you're familiar with the procedure, take the polymer and buffer you'll use and bring them to room temperature by letting them sit on the bench.
- If you're going to store the capillary for longer than a week, flush out the polymer in the capillary with water by running the 'seq fill capillary' module with water in the block and in the syringe instead of polymer (pg. 3-21)
- All page numbers refer to the ABI Prism 310 Genetic Analyzer User Guide for the 2004 PC Model

Preparation

1. Fill two 250mL beakers with ddH₂O (highest quality water)
2. Get tray of ABI310 cleaning supplies out
3. Put kim wipes on bench
4. Make sure you have two microcentrifuge tubes filled with water and capped with grey rubber septa for storing the capillary. Put them in a microcentrifuge tray.

Cleaning the Machine

1. Open ABI310 Collection Software
2. Take out any samples that are still in the machine and throw the tubes in the hazardous waste bin (open machine doors, push 'tray' button)

1-Capillary Storage (pg. 3-20)

Take out capillary and store it (shouldn't be exposed to air >30min)

1. Open capillary door
2. Take tape off capillary (be very careful not to break the capillary – it's fragile, and weakest at the capillary window)
3. Take capillary out of right-hand electrode hole (by samples)
4. Open laser door to release capillary
5. Loosen capillary fitting on block
6. Take capillary out of fitting
7. Put each end of the capillary in the microcentrifuge tubes prepared above

2-Buffer Vials and Waste Tube

1. Throw away the buffer and water in the vials on the autosampler
2. Take white lids off buffer vials
3. Remove grey septa and replace with new septa
4. Rinse everything with ddH₂O
5. Place on kim wipe to dry

3-Electrode (pg. 3-23)

1. Unscrew electrode (by autosampler)
2. Remove electrode and clean with water, dry with kim wipe
3. If it's bent, try to straighten it (pg. 3-26)
4. Replace in housing and screw back into machine

4-Syringe Soak (pg. 3-35)

Take out syringe

1. Under 'function' in the 'manual control' window, choose 'syringe home' and 'execute'
2. Unscrew syringe using the silver part at the base (do not unscrew by holding onto the top of the syringe, as that puts torque on the syringe barrel)
3. Squirt out extra polymer into a kim wipe (to be thrown into hazardous waste bucket)
4. Take syringe out and place in one of the water beakers
5. Lift plunger very slowly out of syringe barrel (while sucking up water)
 - a. Plunger is Teflon, but can be *easily damaged by microabrasions* as it is pulled in and out of the syringe barrel, so always move plunger slowly to extend its life (count 'one-thousand-one...up to five or more while removing). As is, with normal wear, we will probably have to replace it every year or more as it starts to leak.
6. Leave both syringe barrel and plunger in the beaker to soak

5-Block Cleaning (pg. 3-27)

1. Take off block
 - a. Take buffer reservoir off the block
 - i. Twist gently and pull the back end of the shot glass down to get it off the gasket
 - ii. Dispose of buffer in waste beaker
 - iii. Rinse out shot glass and place on the kim wipe to dry
 - b. Pull block directly towards you, and with little effort, it should be released from the rods holding it to the machine
 - c. Take out all four fittings by unscrewing slowly and gently (fittings are plastic, and we don't want to damage the threads at all)
 - d. Put fittings in the water beaker with the syringe to soak
2. Clean block
 - e. Attach extra luer fitting to one of the holes in the block
 - f. Fill 'wet' cleaning syringe with water from the 2nd beaker
 - g. Push water through the block (syringe attaches to luer fitting)
 - i. Put three syringes of water through each hole by blocking all but one of the holes with your fingers
 - ii. Move luer fitting to another hole and do the same
 - iii. Make sure the threads on the block are totally flooded with water to rinse out any polymer that might be lurking there
 - iv. Any polymer left in the block (tubes, threads, etc) will either turn to lactic acid or crystallize, and either option is not a good one
3. Dry block
 - a. Attach dry syringe to extra luer fitting and make sure water is out of the luer fitting
 - b. When refilling the dry syringe with air, make sure you're not sucking water into it by leaving it attached to a wet surface
 - c. Completely dry the block by forcing air through every hole/tube
 - d. Do your best – if there are little tiny bits of water that you just can't get out, it's ok
 - e. Leave on kim wipe to dry

6-Fittings Cleaning (pg. 3-27)

1. Use the 'wet' cleaning syringe to run water through each fitting
2. Make sure you rinse off the outside threads of the fittings as well
3. Use the 'dry' cleaning syringe to dry the fittings inside and out
4. Leave on kim wipe to dry

7-Syringe Cleaning (pg. 3-35)

1. Take off beige fitting on end of syringe barrel and leave it in the water beaker
2. Use squirt bottle or 'wet' syringe to clean threads and rinse syringe
3. Use 'wet' syringe to get water through the beige syringe fitting
4. Check O-rings on fitting and in syringe for any sign of decay – replace if necessary (we have extras in the 'sequencing supplies' drawer)
5. Rinse off plunger
6. Replace plunger in syringe barrel slowly (remember microabrasions, count slowly to five or more)
7. Replace fitting on end of syringe barrel
8. Leave on kim wipe to dry

8-Clean Machine (pg. 3-42)

1. Use a damp kim wipe to wipe down autosampler, windows, base, etc.
2. Wipe down anything you can – extra dirt can make the autosampler spark, or clog the capillary.

Setting up machine after cleaning

-Make sure polymer is at room temperature prior to loading it into the block/capillary

1-Fill Buffer Vials

1. Fill buffer vial with 1X sequencing buffer, cap, and place in slot #1 on autosampler
2. Fill 2nd vial with water, cap, and place in 2nd slot in autosampler
3. Fill 1.5mL lidless microcentrifuge tube with water, leave uncapped, and place in 3rd slot in autosampler (this is a waste vial)

2-Replace Block (pg. 3-29)

1. Make sure block is dry, including electrode part
2. Put beige fittings in block (see illustration on side of machine or pg. 3-12) by gently screwing them into the block so as not to hurt the threads. No need to really tighten them now.
3. With the buffer valve on your left, put the block back on the machine by pushing it in. Make sure the metal arm in the machine that opens and closes the buffer valve fits between the grooves on the valve mechanism on the block
4. Tighten all fittings except capillary fitting (finger tight- if you're really strong, don't really tighten them – it can crack the block. That's only if you're really strong, though. For me, I just tighten them as much as I comfortably can)
5. Fill buffer vial (shot glass) to line with buffer
6. Wet rim of vial with buffer
7. Place on block by pushing one side onto the gasket, then turning and tilting until the gasket is nicely seated in the vial

3-Replace capillary (pg. 3-15)

1. Remember, the capillary should be exposed to air no longer than 30min (unless filled with water prior to storage)
2. Insert the end by the laser window into the block capillary fitting (this is the end of the capillary with the dot)
3. This leaves one end of the capillary free while you're tightening the other end. With a long capillary, this can be a little awkward, but it eliminates possible torquing of the capillary and subsequent breaking at the capillary window.
4. Tighten capillary fitting so that the end of the capillary is directly under the t-junction of the polymer entry tube and the longest tube in the block
5. Place capillary by laser window, lining up the capillary window with the laser
6. Closer laser door (make sure the capillary window is still lined up correctly!)
7. Thread other end of capillary through the hole by the electrode on the right side of the machine
8. The end of the capillary should be within 1-3mm of the electrode. If not, bend the electrode slightly if possible
9. The end of the capillary should extend beyond the electrode about 0.5-1mm
10. Tape the capillary to the white heating board (for lack of a better term) to keep it from shifting

4-Calibrate Autosampler (pg. 3-4)

1. In "instrument" menu, choose "autosampler calibration"
2. Follow instructions, aligning capillary so the end almost almost almost touches the silver dots on the autosampler
3. Return capillary to buffer vial
 - a. Choose function 'autosampler to position' and enter '1' and execute
4. Raise autosampler to put capillary in buffer
 - a. Choose function 'autosampler up' and enter enough steps to put capillary in buffer

5-Fill syringe (pg. 3-37)

1. Check to make sure polymer in container has no bubbles or crystals.
 - a. Bubbles compress during the capillary filling and make it harder for the machine to fill the capillary. I sometimes leave small ones, though, by the syringe plunger, if I can't remove them.
 - b. Crystals will clog the capillary, so don't pick up any crystals into the syringe while filling, etc.
2. Slowly fill syringe about halfway with water, and push out the water again, *slowly*
3. Fill syringe very slowly to about 150uL with polymer (new or used)
 - a. This step is very important – if you fill it slowly, there should be no bubbles by the plunger. If there's a single small bubble, that's probably okay.
4. Push polymer out into kim wipe and dispose of kim wipe in hazardous waste
5. Now fill syringe with enough polymer to do your runs
 - a. For fragments on a short capillary, it takes about 4uL per run
 - b. For long capillaries, count on using about 7uL per run
 - c. Add 200uL more than you will need – this is for filling the block
6. Rinse off end of syringe with water, then dry with kim wipe
7. Make sure there are no bubbles in syringe – if there are, either wait for them to exit the top (if free-floating) or tap the syringe to try to dislodge bubbles on the plunger.

6-Fill Block (pg. 3-29)

1. Attach filled syringe to block by putting it through the white hole, then screwing it into the block (finger-tight will do)
2. Make sure (again) that fittings are tight
3. In the Manual Control window, choose function 'buffer valve close', then 'execute'
4. Loosen waste valve fitting (see diagram on side of machine)
5. Slowly push polymer into block and down waste valve fitting until it just starts to exit, then, while maintaining pressure on plunger, tighten the waste valve. This should fill that tube while leaving no air bubbles.
6. Now loosen the fitting on the upper right-hand side of the block. Push polymer through until it goes up through that tube, then tighten the fitting as above.
7. In the Manual Control window, choose function 'buffer valve open', then 'execute'
8. Push polymer through the rest of the block
9. Close buffer valve
10. Make sure there are no air bubbles in the block (or at least as few as possible)
11. Make sure the machine's syringe driver is toggled to the right, so it fits over the syringe plunger
12. In the Manual Control window, choose function 'syringe down', and lower the syringe driver until it touches the plunger

7-Fill Capillary (pg. 3-11)

1. Carefully note position of plunger
2. In addition to filling the capillary, this will detect any leaks. It should use about 4-7uL of polymer
3. Close all doors
4. In the Manual Control window, choose 'seq fill capillary' module, and start
5. Watch the plunger carefully, and if it moves more than 7-10uL, terminate the module and check for leaks.
6. This module takes about 10min

You're done!