

# Membrane Comb<sup>1</sup> Protocols

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## Resuspending and Loading Cycle Sequencing Product onto Membrane Combs

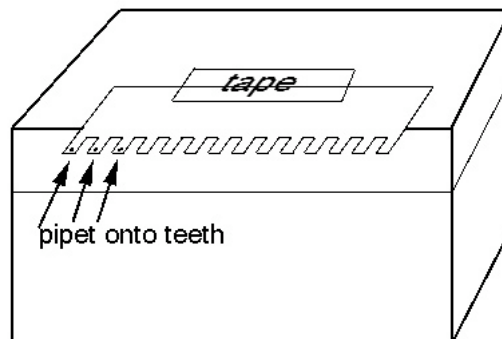
After you have tried your usual cycle sequencing products, you can determine your own standard conditions for resuspending and loading. **For a start, resuspend in 3 ul and load 0.5 ul per tooth.**

Here's why:

Loading too much can be a problem, since you can't stagger lanes to make tracking easier, and adjacent lanes will overlap if they are too bright. So (depending on just how bright your cycle sequencing reactions usually are), you may want to resuspend in 3 ul (medium-bright signal) or just 2 ul (low-medium low signal) and then consistently load just 0.5 ul onto the comb for the first try. This leaves plenty left over, so that if some lanes are too dim, you can reload w/ 1.0 ul on another gel, or (if you leave a space on either side to preclude overlap problems) up to about 1.2 ul. If you normally have super-bright lanes, resuspend in 4 ul/load 0.5 ul per tooth.

Because the combs are so efficient at getting cycle-sequencing products onto the gel without leakage or loss to the buffer chamber, you can "load" less than usual and still have nice bright signal. Background seems much reduced as well, so even fairly dim signal (i.e. almost invisible until you crank the contrast WAY up) may still produce very useable sequence.

"Loading" a comb involves pipetting cycle sequencing product directly onto the tooth. Although the comb supplier will sell you a fancy loading tray, you could (alternatively) tape the comb onto the top of a plastic tip box, with the teeth facing you and projecting off the edge of the box. If you are going to routinely use 96 tooth combs, buying the loading tray from the GelCompany (U.S.\$180) may be worth it – it has little wells that you pipet your samples into, then just "dip" the teeth of the comb into the wells to load them all at once. For precise loading volumes, use a 2 ul pipet. Rinse the pipet tip several times between samples in a little beaker of distilled water, and you won't have to change tips between samples...



Because you can't stagger lanes to facilitate tracking, insert a space between every 10 teeth or so, and/or stagger samples sequenced with different primers, to make adjacent lanes distinct and easy to track.

## Which Combs to Order

The GelCompany sells a variety of comb types, some with one tooth spacing for "GeneScan" and others for "Sequencing". Some are called "laminated" and some are "unlaminated" (although the difference is not readily apparent). In order to re-use combs, order the "CAM" types CAM36, CAM48, CAM 64 and CAM96 for 36, 48, 64 and 96 lanes, respectively. (For some reason they all have four extra teeth – avoid using the outer two teeth on each side – unless centered perfectly they may be outside your data collection window!.)

## Casting the Gel

Always use standard plates, NOT the ground-down "96-lane" front plate, even for 96 lane membrane combs. Cast the gel as usual using the back of a 0.2 mm thick 36 – 64 lane comb, to form a groove at the top of the gel. Clean the gel groove as usual after polymerization – flush out the sticky acrylamide blobs from the gel groove while cleaning the outside of your plates prior to the pre-run. Groove flushing and fit of the plastic casting comb are not nearly the issues they are when trying to load the old-fashioned way, between the teeth of a nasty leaky plastic comb...

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<sup>1</sup> from The Gel Company, see <http://www.gelcompany.com> for prices and ordering info., or by phone at 415-247-8760 Tell them Frank sent you.

### **Pre-run Conditions**

Create a special PRE-RUN MODULE (open "Collection", then open an existing module by clicking on the icon next to the RUN selector window, modify, then "save as") with 0 volts, 0 watts, 0 amps. This way you can do the pre-run with buffer in only the bottom chamber (to keep it moist and prevent Red Rain – remember Red Rain??) to heat up the gel without even installing the upper buffer chamber. You should mount the front heating plate, just don't mount and fill the upper chamber until AFTER you have inserted the comb.

### **Inserting the Comb**

After your PRE-RUN has heated the gel to near 50°, CANCEL the PRE-RUN, then fill the gel groove with 20% Ficoll (w/syringe and needle) – it will only take about 5 ml to fill the gel groove and displace any water that may be remaining in it. Replace any remaining Ficoll in the syringe back into the working stock bottle (its relatively expensive and time-consuming to prepare – see directions below). Insert the pre-loaded comb: dip into the Ficoll in the groove to moisten the teeth; tilt the top of the comb toward you, then push back till the teeth contact the back plate; carefully slide the teeth down the back plate and into the groove until they touch the bottom of the gel groove. NOW you can carefully fill the upper buffer chamber with buffer, making sure not to rinse the Ficoll out of the gel groove. Start the RUN. PAUSE after 1.0-1.5 minutes (yes, that's one to one and a half MINUTES) and then remove the comb (set it aside to clean and re-use if it isn't already worn and tattered). Flush the gel groove (use the same syringe and needle used to load Ficoll) with buffer several times to displace ALL the Ficoll. RESUME, and complete the run. (NOTE: if you try to run in your sample with the 0 volt 0 amp PRE-RUN module it will NOT work. This is the voice of unfortunate experience talking.)

### **Re-Using Membrane Combs**

To clean the membrane comb for re-use (36- and 48-tooth combs can be reused 4+ times, 64 and 96-tooth combs can be reused 1-2 times): rinse in a distilled water stream to flush away the Ficoll. Dry on a tip box w/ teeth extended over edge (i.e. loading position as in above figure), in a drying oven for a few minutes or in a safe, protected drawer for a few hours. Replace the dry comb back in its protective sleeve till next use.

### **Preparing ~20% Ficoll Loading Solution**

Weigh out 15-20 g Ficoll 400 and add to ~80-85 g of water, add magnetic stir bar. Heat to 50° on a stir plate, it will take an hour or so to go into solution. Add a dollop (1-2 g or so) Amberlite (MB-150 or equivalent) mixed bed ion exchange resin. Continue stirring for 30 mins-1 hour more. Filter through a sterile 0.45 um (or so) filter, decant 20-40 ml into a sterile bottle as a working stock but KEEP THIS IN THE FRIDGE except when you are filling your gel groove. Ficoll kept at room temperature gets re-ionized (or something) and causes strange lane-jags and compressions near the beginning of the run.