Array Post-Processing Protocol

DeRisi Lab, UC San Francisco, June 2001

Post processing can be broken down into three distinct steps: re-hydration, blocking, and denaturing. Since the DNA solution dries to the outer edge of the spot during the printing process, the goal of re-hydration is to allow the DNA a chance to more evenly distribute across the surface of the spot. This will help eliminate the donut-shaped appearance of the spots and will also increase the amount of total DNA bound after processing. Poly-l-Lysine arrays require that exposed amines be blocked prior to hybridization to prevent binding of labeled material. The blocking process is a race between excess DNA molecules leaving spots to bind near-by exposed lysine, and the succinic anhydride reacting and capping the amines. For this reason, this particular step in the process has been historically troublesome for first-time users to master. The last step consists of boiling and fixing the arrays. The boiling step is meant to denature the double stranded DNA molecules and thus enhance hybridization availability.

Both re-hydration and boiling may be omitted, but at the cost of overall array sensitivity. Note that for oligo-based chips, the boiling step is not needed at all since all molecules are already single stranded.

Materials

Metal slide racks (Shandon Lipshaw)
Re-hydration trays (Sigma H6644)
Centrifuge capable of spinning slide racks
Succinic anhydride (Aldrich 23,969-0)
1-methyl-2-pyrrolidinone (Aldrich 32,863-4)
1M Sodium Borate Solution, pH 8.0. (Prepare using Boric Acid (Fisher A73-500), pH'd with NaOH)
Diamond-tipped glass etching pen (VWR 52865-005)
Optional: StrataLinker for UV cross linking

Protocol

1. Choose 20-30 slides to be processed. Add enough distilled water to a large 4L beaker such that a slide rack will be completely submerged when placed inside. Place the beaker on a heating plate to boil.

2. Handle all slides with **powder-free gloves**. Determine correct orientation of slide. With the etching pen, **lightly** mark the boundaries of the array on **back** side of the slide. After processing, the arrays will not be visible.

3. Pour 100 ml 0.5x SSC into hydration tray and warm on slide warmer. Set the warmer to 42C. Note that if you may wish to omit the rehydration steps and proceed directly to step 6 if the spots on your slides are extremely tightly packed (less than 20 microns of space between).

Rehydration will slightly expand the size of the spots which can lead to spots actually touching.

4. Set slide array side down and observe spots until full hydration is achieved. Hydration times will vary depending on printing, but will usually be between 1-5 minutes. Under-hydration results in less DNA bound within a spot, and over-hydration will cause spots to run together.

5. Upon reaching full hydration, immediately snap dry slide by flipping it quickly onto a heating block (pre-heated to 140 ˚C). Do this in one smooth motion, with one hand, pinching the array at one end and flipping it over as you move it to the hot plate. Make sure you have got it array-side is up! The array should dry within 1-2 seconds. Leave the slide on the block for an additional second. Remove the slide and place into a metal slide rack.

6. Measure 335 ml of 1-methyl-2-pyrrolidinone into a clean, dry 500mL beaker. Dissolve 5.5g of succinic anhydride using a stirbar. Note that the stock bottle of solid succinic anhydride should be stored under desiccation and vacuum. Do not use if exposed to moisture!

7. **IMMEDIATELY** after succinic anhydride dissolves, mix in 15 ml of 1M sodium borate **pH 8.0.** Quickly pour the buffered blocking solution into a clean, dry glass slide dish. Plunge the slides rapidly into blocking solution and vigorously shake, keeping the tops of the slides under the level of solution. After 30 seoconds of plunge-mixing, put a lid on the glass box, and let shake gently on a rotator for 15 minutes.

8. Reduce the heat on the boiling water so that temp is approx. 95°C. Drain excess blocking solution off slides for approximately 5 seconds and transfer slide rack to the boiling water such that the rack is completely submerged. Gently “swish” the rack back and forth under the water for a few seconds. Incubate for 60 seconds. If you choose not to boil, then we suggest using distilled water at room temperature instead. Part of the purpose of this step is to remove the organic solvent.

9. **Quickly** transfer the rack to a glass dish of 95% EtOH and plunge to mix. Make sure the EtOH is crystal-clear. Do not use if it appears to have particulates or if it appears cloudy. Place the slide rack on a micro-titer plate carrier and spin in a benchtop centrifuge (such as a Beckman GS-6 or Allegra) for 1 minute at 550 rpm.

10. After spinning, the slides should be clean and dry. Remove the slides from the rack and store in a plastic (not wood!) microscope slide box. Arrays may be used immediately.

Protocol Variations and Tips

• If the methyl pyrrolidinone appears yellowish, do not use.
• Do no use succinic anhydride that has been exposed to moisture.
• If you observe streaks of DNA, or “comet tails,” the initial plunge-mix of arrays into the succinic anhydride solution was too slow.
• Not all lysine slides are equal. Several people have noted that slides can contain sub-regions where the lysine coating appears to be “weaker.” This is manifested during the hybridization where the region once occupied by spotted DNA is now able to bind fluorescent hybridization probe. This is presumably due to spotted DNA leaving the surface during the boiling step, since this step represents the harshest treatment. The resulting array scans appear to have regions where all the spots are one bright uniform color. Two protocol variations seem to mitigate this effect. The first is to simply omit the boiling step which comes at the cost of some sensitivity loss. The second is to block the slides in the succinic anhyride solution for 5 minutes, transfer to 95 ˚C water for 60 seconds, and then plunge back into the succinct anhydride solution for another 10 minutes. After this, plunge into 95% EtOH to remove organic solvent. The rationale being that “loose” DNA will leave the slide during the boiling. The exposed amines will be blocked by the second succinic anhydride incubation.
• UV crosslinking appears to enhance binding of long-oligo DNA to lysine slides. However, crosslinking seems to have little or no effect on binding of PCR product, as measured by hybridization intensity. If you choose to cross-link, we suggest an energy of 550mJ.