

P. falciparum Oligo Array Post-Processing

(01/10/04)

Post processing steps: “Shampooing”, re-hydration, and blocking

Often the poly lysine coating on microarray slides becomes uneven with age and can lead to spurious background. We recommend (**OPTIONAL**) a simple “shampoo” procedure, which seems to consistently reduce the background even on NEW slides. Second, since the DNA solution dries to the outer edge of the spot during the printing process, a simple re-hydration step is used to allow the DNA a chance to more evenly distribute across the surface of the spot (**OPTIONAL**). This will help eliminate the donut-shaped appearance of the spots and will also increase the amount of total DNA bound after processing. Lastly, and most importantly, 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 free amines. For this reason, this particular step in the process has been historically troublesome for first-time users to master.

NOTE: Slides should be post-processed only when they are needed for immediate use (within the week).

Materials

Metal slide racks (Shandon Lipshaw)

Glass slide dishes

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), pHed with NaOH)

Diamond-tipped glass etching pen (VWR 52865-005)

StrataLinker for UV cross linking

Protocol

1. Choose the slides to be processed. Handle all slides with **powder-free gloves**. First, determine the correct orientation of slide. With the etching pen, **lightly** mark the boundaries of the array on the **backside** of the slide. After processing, the arrays will not be visible.

2. **Crosslink oligonucleotides** to enhance binding of long-oligo DNA to the poly lysine slides by exposing them to 60 mJ in Stratalinker **array side up**.
3. **“Shampoo” for pre-treatment of “old” microarrays:**
First, soak slides in 3x SSC + 0.2% SDS (pre-warmed to 65°) in a glass slide dish, 5 minutes. Transfer the slide rack to another glass slide dish containing distilled water (RT), 30 seconds. Rinse in 95% EtOH in yet another glass slide dish and then spin dry 5 minutes.

Rehydration: Note that **you may wish to omit the rehydration steps** and proceed directly to step 5 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. Pour 100 ml 0.5x SSC into hydration tray and turn on slide warmer to 42C. Turn on a heat block to max heat with the block inverted showing its smooth side. Set slide **array side down** in the hydration tray and observe spots until full hydration is achieved (spots glisten). Hydration times will vary depending on printing, but will usually be about 1 minute if you have utilized the “shampoo” method or 1-5 minutes if not. Under-hydration may result in less DNA bound within a spot, and over-hydration will cause spots to run together. Upon reaching full hydration, immediately snap-dry the slide by flipping it quickly (**array side up**) onto the heating block. 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. 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.

Blocking: The next two steps should be completed as quickly as possible:

5. Measure 335 ml of 1-methyl-2-pyrrolidinone into a clean, dry 500mL beaker. Add 5.5g of succinic anhydride and dissolve quickly using a stirbar. (Note that the stock bottle of crystalline succinic anhydride should be stored under desiccation and vacuum. **Do not use if exposed to moisture!**)
6. **IMMEDIATELY** after succinic anhydride dissolves, mix in 15 ml of 1M sodium borate, pH 8.0. Once stirred in, quickly pour the buffered blocking solution into a clean, dry glass slide dish. **Rapidly plunge** the slides (in slide rack) into blocking solution and **vigorously plunge up and down, keeping the tops of the slides under the level of solution**. After 30 seconds of plunge-mixing, put a lid on the glass box, and let shake gently on a rotator for 5 minutes.
7. Add enough distilled water (~1L) to a large 4L beaker such that a slide rack will be completely submerged when placed inside. After Step 6, drain excess blocking solution off the slides by lifting and tilting the rack for approximately 5 seconds and transfer the slide rack to the water (RT) such that the rack is completely

- submerged. Gently “swish” the rack back and forth under the water for a few seconds (circular wrist motion). Incubate for 60 seconds.
8. Remove the rack from the water and plunge back into the same buffered blocking solution used in step 6 above and again plunge-mix for 30 seconds, put a lid on the glass box, and let shake gently on a rotator for 5 minutes.
 9. Transfer the rack to a glass dish containing distilled water and plunge to mix. Then, transfer to a glass dish of 95% EtOH and plunge again. 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 5 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 not 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. The resulting array scans appear to have regions where all the spots are one bright uniform color. This effect seems to be eliminated by performing two blocking steps, as above. The rationale being that “loose” DNA will leave the slide during the water step. The newly exposed amines will be blocked by the second succinic anhydride incubation.