

Array Post-processing Protocol

Adapted from DeRisi, Schoolnik, and Somero Lab protocols (developed by Andy Gracey), DeRisi microarray course notes, and personal experience.

Goal: After the arrays have been printed with DNA spots, this protocol is used to bind the DNA more tightly to the slide, remove excess DNA that hasn't been bound, and block any free lysines on the poly-lysine slide coating (those free lysines are "sticky" and if not blocked they could non-specifically bind labeled probe during an array hybridization.) Since slides age more quickly after they've been post-processed, it makes sense to just post-process a small batch at a time, as they are needed.

Required Equipment *most of it in my bench cupboards*

slide rack(s)	piece of cardboard
a 1L and a 50ml Erlenmeyer flask	centrifuge with adapter for slide rack
2 3L beakers	diamond scribe etcher (in my bench drawer)
50ml Erlenmeyer	powder-free gloves
rotator-table	UV-crosslinker (Stratagene above Steve's bench)
humid chamber (Sigma H6644)	
heating block	
dust-free board for cross-linking (e.g.	

Required Chemicals

20X SSC, 10% SDS
1M Sodium Borate, filtered (make from Boric acid, adjust pH with NaOH): Boric Acid's FW = 61.83
1-Methyl-2-pyrrolidinone (anhydrous, 99.5%, FW=99.13) - Sigma M6762-1L - **do not use if it appears yellowish**
Succinic Anhydride, 6g (99+%, FW=100.07, a moisture sensitive irritant) - Aldrich 239690-50G - **do not use if it has been exposed to moisture. Keep in a dessicator, sealed with parafilm.**
95% Ethanol (**do not make from 100%, it is made differently**) - **do not use if it is cloudy or has particulates**

Before you start: *prep work can take up to ~ an hour*

**** always rinse all glassware, etc. with Milli-Q water before use to remove dust****

**** always use powder-free gloves when working with arrays!****

1) take Methyl-pyrrolidinone out of fridge and put in hood to come to room temperature.

2) make up *fresh* Na-Borate (*do this first so it has time to cool before pHing*)

you'll need 25.71 mls, so make e.g. 40mls and then discard excess:

in a 50ml Erlenmeyer, with a small stir bar:

35mls Milli-Q H₂O

2.473g Boric Acid

mix on high, with heat, until dissolved

cool to room temp

adjust to pH = 8.0 with 10N NaOH (takes >20 drops, so start there.

Do NOT work back with HCl if you go past. Start fresh with new solution.)

check volume - add Milli-Q H₂O up to 40mls

filter through 0.2um syringe-filter, into a 50ml Falcon tube

- 3) wipe down bench area with EtOH
- 4) heat up heat block on bench to at least 90°C
- 5) place in the chem. hood:
 - stirring plate for preparation of blocking solution
 - shaker, with secondary containment tray taped down
- 6) make up pre-wash solution: 1X SSC/ 0.1% SDS
for 700mls (to use for 1 rack of slides, in a 3L beaker):
 - 658mls Milli-Q H₂O
 - 35mls of 20X SSC
 - 7mls of 10% SDSmix briefly with stir bar, remove stir bar, and cover beaker.

Step 1. Rehydrate the slides

This step recovers the spot's circularity, and decreases "donuting" of the spots.

- a) wipe down the bench with 70% EtOH and kimwipe (*not paper towel*) to remove dust
- b) put warm tap water into humid chamber, and place chamber next to heat block
- c) Rehydrate slides by inverting (array side down toward steamy water) them over warm water in a slide-staining chamber – **don't use much water or it can splash up on slides**. Watch until the spots become glistening and juicy, **3-10 minutes**. (Under-hydration causes too little DNA to stay adhered to the spot during the subsequent washing, and over-hydration causes the spots to be blebby. AG says 2-3" is usually sufficient, the DeRisi protocol says 1-10".)
Be careful not to allow the water to touch the array.
- d) Immediately flip them (array side up!) onto a heating block (inverted, about 90°C). Watch the steam evaporate. When the array spots dry in a rapid wave-like pattern, remove them from the heating block. This takes about **5 seconds**. Do 1 slide at a time.

Step 2. UV cross link

This step helps the DNA stick to the poly-lysine.

- a) Place the slides, array side up, on a flat, dust-free board that fits into the UV cross-linker (I use a pre-cut piece of cardboard that I keep in my cupboard).
Do not put them on a seran wrap surface since the slides stick to it.
- b) Irradiate with 600 uJoules UV light – press the "**ENERGY**" button and then enter **600**, then press "**Start**". It will count down and beep when done. (Andy does his at 650 and does them in the metal rack, not laying them flat.)
- c) Before the next step, **etch the slides** with a diamond scribe (in the top middle drawer of my bench) to demarcate where the array is – after the pre-wash the spots will become invisible!

Step 3. Pre-Wash (a.k.a. the "shampoo method")

This step removes excess, unbound DNA to prevent "pluming" of the DNA out from the spots. Some protocols suggest skipping this step if the initial spots are small, however skipping this would then require an extra step later, which is not in this protocol.

- a) Place the slides in the slide rack and secure them with a strip of metal wire on top, or rubber bands. If rubber bands, then position as close to the rack edge as possible. (AG uses rubber bands.)
- b) rapidly plunge slides into 1x SSC / 0.1%SDS for 30 sec
- c) Gently wash slides with *lots* of Milli-Q water, swish rack back and forth (something like 5 consecutive gentle rinses of 30+ seconds each). Let sit in water briefly while preparing blocking solution. (Pre-wash protocols will vary on the details, but all have the same general principle. Some also spin slides dry before blocking, but AG doesn't, and drying doesn't appear necessary or beneficial.)

Step 4. Block free lysine

wear a lab coat when working with methyl pyrrolidinone

- a) In a 1L beaker, add **8.643g** of succinic anhydride into **526ml** 1-Methyl-2-pyrrolidinone while stirring with stir bar on stir plate.
- b) *As soon as* the solids dissolve, (though they may not dissolve completely, some protocols warn - mine always has dissolved), quickly add **23.57ml** of 1M Na-Borate pH 8, and pour the mixed solution into a 3L beaker.
- c) *Quickly* place the slides (in metal rack) into the succinic anhydride solution (**do not pour the solution over the slides**) and plunge up and down for 60 seconds. Rotate at 60 rpm for **1 hour if possible** - as little as 30 minutes probably OK. (AG's surface chemist friends say the process doesn't go to completion for about an hour, though some protocols call for as little as 15".) While blocking, set up 3L beaker with Milli-Q water on hotplate, in hood, and heat to boiling.
- d) Remove the slide rack from the organic reaction mixture and place it immediately into the boiling Milli-Q water bath (some protocols call for room temp, feel free to try this out and see which works better, just make a note of it! I haven't noticed a difference, actually) and wash thoroughly but gently by swishing rack back and forth for 90 seconds.
- e) Transfer the slide rack to a 3L beaker containing approximately 575mls of **95% ethanol (do not make from 100%)**, plunge slides to mix, and then carry directly in EtOH to the tabletop centrifuge.
- f) Spin dry the slides by centrifugation at **150 x g** for **2 min**. Use a counter balance with the same number (& orientation) of slides in a rack. (Balance slides are in the top right drawer of my bench).
- g) Carefully transfer the slides to a dry slide box for storage in a dessicator. Make sure the slide box is appropriately labeled.
- h) Collect methyl-pyrrolidinone solution as waste for periodic EHS pick-up.

Array Post-Processing Form:

Date: _____ Person: _____

Which slides being post-processed (printdate, series): _____

Total number = _____

Notes on solution making:

Notes on re-hydration:

Notes on pre-wash:

Time in to blocking solution: _____ Time out: _____

When out of blocking solution, into HOT or COLD water bath? (circle one)

Notes on blocking steps: