Post Processing

Equipment	Chemicals
slide rack and dishes (Shandon Lipshaw #121)	1XSSC
500 ml beaker, 2 stir bars and shaker	1M Sodium Borate, filtered (make from Boris acid, adjust pH with NaOH)
hot plate and 4L beaker	1-Methyl-2-pyrrolidinone (anhydrous, 99.5%, FW=99.13)
humid chamber (Sigma H6644) and heating block	Succinic Anhydride (99+%, FW=100.07, a moisture sensitive irritant)
centrifuge with adapter for slide rack.	95% Ethanol

Before you start

a) heat up heat block to at least 90°C and invert the insert
b) place slide-staining chambers in front of the heating block.
c) fill slide staining chamber with SSC or water.
d) get a dust-free board needed for the cross-linking step.
e) place in the chem. hood:
   - large glass beaker with about 1 L boiling water, cover with foil as it is heated
   - stirring plate for preparation of blocking solution
   - shaker
f) for the preparation of the blocking solution, have a clean 500 ml beaker ready.
g) check if the following chemicals are available:
   - succinic anhydride (6.0 g)
   - 1-Methyl-2-pyrrolidinone (350 ml)
   - 15 ml 1 M Na-Borate, pH 8.0 (filtered)

1. Rehydrate the slides
   a) Rehydrate slides by inverting (array side down toward steamy water) them over warm water in a slide-staining chamber. Let the spots become glistening and juicy. *Be careful not to allow the water to touch the array.*

   b) 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.

2. UV cross link
   a) Place the slides, array side up, on a flat, dust-free board that fits into the UV cross-linker.

      Do not put them on a seran wrap surface since the slides stick to it.

   b) Irradiate with 600 uJoules UV light.

3. At this time, we place the slides in the slide rack and secure it with a strip of metal wire on top. Otherwise, there is a good chance that the slides will pop out of the rack during the vigorous washing in step 7.

   Skip prewash steps #4-#6 if the spots on the array are small. This step is to reduce pluming for arrays with an excess amount of dna.

   4. Gently wash slides with 1x SSC / 0.01%SDS for 30 sec
   5. Gently wash slides with 0.06x SSC for 30 sec.
   6. Dry slides in centrifuge at 600rpm 1-2 min.

7. Block free lysine (wear a lab coat when working with methyl pyrrolidinone)

   a) Add 6.0 g of succinic anhydride into 350 ml 1-Methyl-2-pyrrolidinone while stirring.

   b) As soon as the solids dissolve, quickly add 15 ml of the 1M Na-Borate pH 8, and pour the mixed solution into a slide washing tray (make sure the tray is small enough that 350ml of solution will completely cover the slides when they are placed inside).

   c) Quickly place the slides into the succinic anhydride solution and vigorously plunge for 60 seconds. Rotate at 60 rpm for 15 min.

      NOTE: The slides should be rapidly plunged into the organic acetic anhydride solution to quickly wash the unattached DNA away from the slides.
      Do not pour the solution over the slides.

   d) Remove the slide rack from the organic reaction mixture and place it immediately into the boiling water bath for 90 seconds.

   e) Transfer the slide rack to the 95% ethanol wash tray and carry it directly to the tabletop centrifuge.

   f) Spin dry the slides by centrifugation at 600 rpm for 2 min. Use a counter balance with the same number of slides in a rack.

   g) Carefully transfer the slides to a dry slide box for storage in a dessicator.