

MICROARRAY FACILITY

Mount Sinai School of Medicine

Annenberg 19-80

Tel: (212) 241-8194

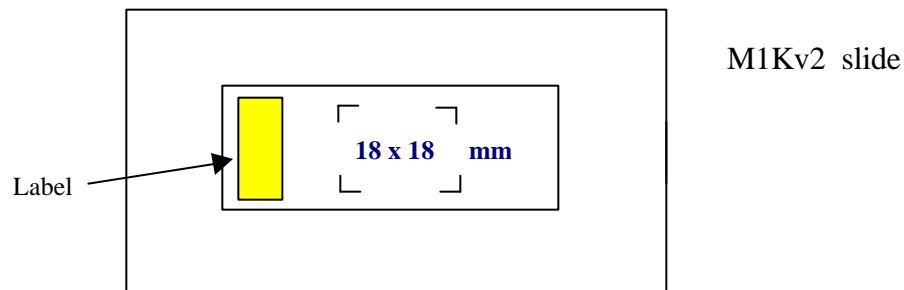
Fax: (212) 241-1627

Protocol #: D03

Preparation of Denatured Microarray Slide for hybridization

Note: a. Slides should be kept in a slide box all the time during storage of transportation.
b. Do not introduce any ink on the slide. It will generate fluorescent background.
c. In general, all arrayed slides are baked (will state otherwise). No further DNA cross-linking procedure is required. However, additional UV cross-linking procedure may increase the hybridization signal.

1. Before starting, place slide on a piece of paper (a 3" x 5" Post-It works fine) and draw an outline around it. Then, mark the area that the array is printed on (you can see the salt deposition). See the example below:



2. Arrayed DNA is vapor moistened (~2 second) over boiling water.
3. (optional) UV cross-link @ 300mJoule. Place the slide face up.
4. (optional) After UV cross-link, vapor moisten the array over boiling water again.
5. Heat snap slide on a hot plate (~2 second or it easily breaks).
6. Dissolve 1 gram of succinic anhydride (sigma) in 63 ml of n-methyl-pyrrolidinone, and stir until dissolved (prepare this solution fresh right before you start this protocol).
7. To this, add 7 ml of 0.2 M NaBorate, pH 8.0 (Boric Acid pH'd with NaOH). Mix well. The solution will turn yellow as time goes on. Do not use after it turns yellow.
8. Soak arrays in this solution for 15-30 min with gentle shaking (the solution should cover the entire array area).
*If you need to prepare more solution, scale up the component in proportion.
9. Rinse slide in 0.1% SDS for 30 seconds.
10. Rinse slide in filtered water for 1 min with one quick change of water.
11. Boil slide in 95° C water for 5 min.

12. Duck into ice-cold ethanol for 1 min.
13. Remove excess ethanol from slides by spinning the slide in a 50-ml conical tube at 1,500 rpm, RT for 5 min.
14. Slides are now ready for hybridization.