9-mer Hybridization Protocol

Visualization of printed DNA as a quality control check can be easily performed by hybridizing the array with a Cy-labeled random 9-mer sequence at room temperature.

Basic Protocol:

- use 150 pmol of Cy labeled 9-mer in a regular Hybridization mix
- hyb at Room Temperature 3-5 min.
- wash slide
- scan

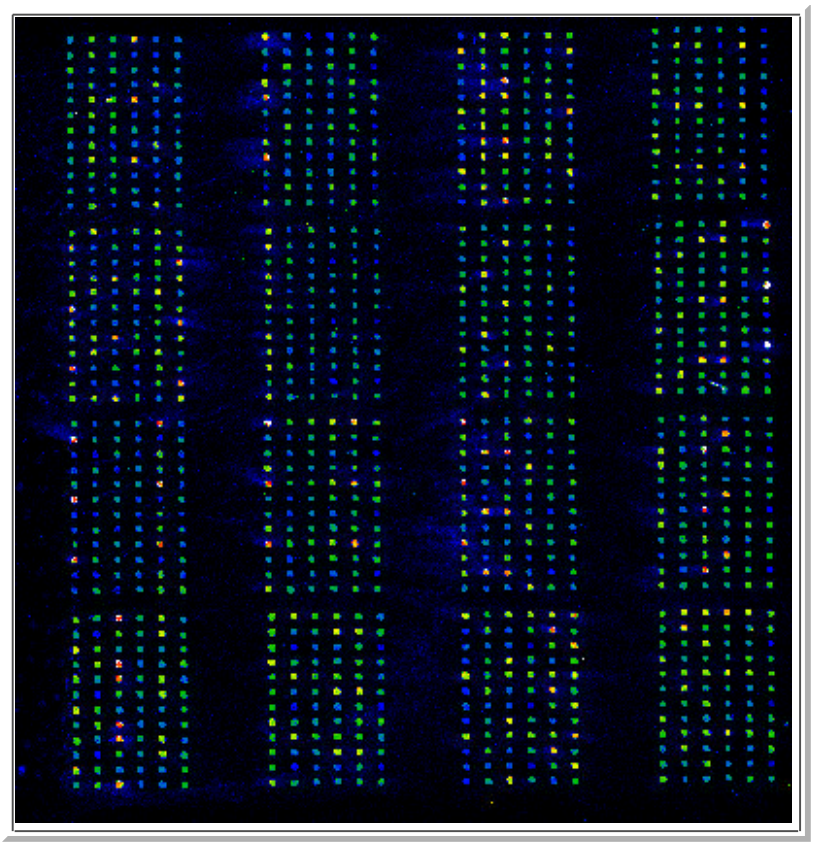
for 20 ul 9-mer Hyb Mix :

Target	Amount	Stock
4x SSC	4 ul	20x
1 mg/ml poly-dA	2 ul	10 mg/ml
50 mM HEPES pH 7 (or Tris pH 7.5)	1 ul	1 M
0.2% SDS	0.4 ul	10% SDS
7.5 uM Cy3 random 9-mer	150 pmols	?
Total Volume to 20 ul with H_2O		

1. Briefly heat probe to 90 deg C.
2. Cool by Spinning in Microfuge (don't place on ice or SDS will precipitate)
3. Pipet probe onto slide and use coverslip as you would for a normal hybridization.
4. Allow to incubate at room temperature for 3 to 5 minutes.
5. Wash slide in 2X SSC 0.2% SDS
6. Wash slide in 0.05X SSC
7. Dry slide by spinning slide rack or by placing slide in 50 ml Falcon tube, and spinning at 500-1000 RPM for 5 min.

Notes :

The Tm for a 9-mer ranges from about 4 to 40 degrees Celsius. The average will likely be below room temperature. So the things to consider with this protocol are Temperature, salt concentration, and oligo concentration.



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