

Microarray hybridization and wash conditions (1):

Prehybridization:

1. Prehybridization buffer: 5x SSC, 0.1% SDS and 1% BSA (Sigma #B-4287).
Heat to 50°C while stirring.
2. Slides to be analyzed are placed in a staining jar; prehybridization buffer is added, and incubation is carried out at 48°C for 45-60 min while stirring.
3. The slides are washed by dipping up and down approximately 10 times in two different staining jars of deionized water. Excess water is removed by shaking the slide rack up and down two times.
4. The slides are then dipped in an up and down motion approximately 10 times at room-temperature in isopropanol and spun dried. The slides are used immediately after prehybridization (less than 1 hr) as hybridization efficiency decreases rapidly if the slides are allowed to dry for more than that time.

Hybridization:

5. 2X hybridization buffer: 50% formamide, 10X SSC and 0.2% SDS. Incubate the solution until it reaches 48°C.
6. The Cy3 / Cy5 labeled mixtures are re-suspended in 9 μ l water, and heated to 95°C for 3 min to denature, and are centrifuged at maximum angular velocity for 1 min.
7. The following are added to each tube in order to block non-specific hybridization. Make a master-mix with the following ingredients for each tube:

- Calf Thymus DNA (1 μ g/ μ L) 8 μ l (Sigma; Cat #D 8661)
 - poly(A)-DNA (10mg/mL) 2 μ l (Sigma #P 9403)
 - yeast tRNA (4mg/mL) 2 μ l (Sigma #R 8759)
8. Then, 21 μ l 2X hybridization buffer that has been pre-heated to 48°C is added to the target mixture, mixed well, and centrifuged. The samples are kept at 48°C until placed on the slide.
 9. The labeled target is applied to a pre-hybridized microarray slide and covered with a 22 x 60 mm glass cover slip.
 10. The slide is placed in a sealed hybridization chamber (Corning, Acton, MA), and 12 μ l water is added to the small reservoirs at each end of the chamber.
 11. The sealed chamber is placed in a 48°C water bath and incubated for 40-60hr (2).

Post-hybridization washes:

12. The array is removed from the hybridization chamber with care taken not to disturb the coverslip.
13. The slide is placed in a rack for a staining dish containing 1X SSC, 0.1% SDS, and 0.1 mM DTT at 48°C.
14. The coverslip is gently removed while the slide is in solution and agitated for 15 min.
15. The slides are transferred to a staining dish containing 0.1X SSC, 0.1% SDS, and 0.1 mM DTT at 48°C and agitated for 5 min.
16. Repeat step 15 two more times.

17. The slides are transferred to a staining dish containing 0.1X SSC and 0.1 mM DTT at room-temperature and agitated for 5 min.
18. Repeat step 17 an additional time.
19. Slides are spun dried.

Notes

- The finished microarray slide is scanned and gridded using an Axon GenePix 4000 A or B with GenePixPro 5.0 software (Axon Instruments, Sunnyvale, CA).
- As described earlier, raw data from the microarray study are statistically analyzed in three steps: (i) the data are normalized, (ii) ANOVA for each gene is determined, and (iii) clustering analysis is carried out.
- The statistically analyzed datasets are merged into EASE computer programs to determine biological meaning.
- The datasets are placed in databases for accessibility by other biologists.

TROUBLESHOOTING

We have found that when a microarray slide is judged a failure as determined visually or by statistical analysis, in approximately 90% of the cases the failure is ultimately due to low quality RNA. Thus, it cannot be overstressed how important it is to carefully isolate the RNA and carry out quality control assays on the purified RNA. Other far less common sources of failure include missing or merged spots on the microarray slide, insufficient prehybridization or washing, leaks, and other mistakes due to human error.

REFERENCES

1. Hegde, P., Qi, R., Abernathy, K., Gay, C., Dharap, S., Gaspard, R., Hughes, J. E., Snesrud, E., Lee, N., and Quackenbush, J. (2000) *Biotechniques* **29**, 548-550, 552-544, 556.
2. Sartor, M., Schwanekamp, J., Halbleib, D., Mohamed, I., Karyala, S., Medvedovic, M., and Tomlinson, C. R. (2004) *Biotechniques* **36**, 790-796.