

Microarrays, second hybridization

This continues the 3DNA microarray procedure. In the first part, cDNA was made, and then hybridized to the microarray slide. This part now hybridizes the dye to the cDNA, allowing detection of the samples.

Materials

Genisphere 3DNA Submicro Expression Array Detection Kit
0.2% SDS
Glass coverslips (Corning brand, e.g. Fisher or VWR)
2X SSC + 0.2% SDS (Day 2)
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Procedures

Note: use autoclaved or sterile tubes, tips, and solutions. Wear gloves to protect your samples!

Note: The Cy3 and Cy5 dyes are light sensitive. Keep the vials containing the dyes **in the dark** as much as it is possible.

Second hybridization

- 1) Put on gloves.
- 2) Wash the glass coverslip by briefly submerging it in 0.2% SDS followed by a brief rinse in water. Remove excess liquid with a kimwipe and let it air dry. Place your microarray into the 55°C incubator to warm it up.
- 3) Heat the alternate hybridization buffer (Vial 7) at 55°C for 10 minutes to thaw it. Mix by inversion to ensure that the buffer is evenly resuspended.
- 4) Prepare stock solutions of the following:
 - Anti-fade reagent (Vial 8): This reduces fading of the fluorescent dyes. Prepare it by adding 1 µl of Vial 8 to 100 µl of the hybridization buffer (Vial 7). Store it at -20°C for up to two weeks.
 - High-end differential enhancer (Vial 10): This increases the differential between labeled samples run on the same array, especially if they differ by more than 10-fold.
- 5) Prepare the 3DNA Capture reagent (Vial 1) as follows:

Note that it is necessary to break up aggregates that may form from the freezing.

 - a) Thaw the 3DNA Capture reagent (Vial 1) in the dark at room temperature for 20 minutes.
 - b) Vortex at the maximum setting for 3 seconds and microfuge briefly (1 second).
 - c) Incubate at 50 to 55°C for 10 minutes.
 - d) Vortex at the maximum setting for 3 to 5 seconds.
 - e) To collect the contents at the bottom of the tube, briefly centrifuge the tube.
 - f) Check for aggregates. If they are present, vortex and centrifuge again.
- 6) Mix together the following in a new PCR tube.

Solution	Vial	Volume (µl)
Hybridization buffer with anti-fade	Prepared from 7	34
3DNA Capture reagent #1	1 (Cy3)	2.5
3DNA Capture reagent #2	1 (Cy5)	2.5
High-end differential enhancer	10	1

This is the **Hybridization Mix**.

- 7) Incubate the Hybridization Mix at 75-80°C for 10 minutes, then at 45-50°C for 15-20 minutes. If possible, use the thermocycler.
- 8) Pre-warm the microarray to 55-65°C for 10 to 15 minutes.
- 9) Remove the microarray, wait one minute, and add the Hybridization mix to the microarray.
- 10) Apply a washed glass coverslip (Step 1) to the array and incubate the microarray for 2 to 3 hours in a dark sealed tube that has 300 µl of water in it.

Post hybridization wash

Notes:

- (a) Perform the following steps **in the dark** to avoid photobleaching and fading of the dyes.
 - (b) Do not use MilliQ water, which damages the Cy5 dye.
 - (c) Do not use DEPC-treated water (to make it nuclease free), as it oxidizes the dyes.
- 11) Remove the cover slip by placing the slide in a Coplan jar containing 2X SSC and 0.2% SDS at room temperature for up to 5 minutes. The cover slip should slide right off.
 - 12) Transfer the slide to a Coplan jar that has been pre-warmed to 60-65°C and which contains 2X SSC, 0.2% SSC and let it incubate for 10 minutes.
 - 13) Transfer the slide to a Coplan jar that contains 2X SSC at room temperature, and let it sit for 10 minutes.
 - 14) Transfer the slide to a Coplan jar that contains 0.2X SSC at room temperature, and let it sit for 10 minutes.
 - 15) Immediately put the slide into a fresh 50 ml tube and centrifuge it at 1500 rpm for five minutes to dry the slide. Do not touch the array surface.
 - 16) Transfer the tube to a new 50 ml tube. Cover with aluminum foil to keep it dark.

Note: The samples will now be mailed for the reading of the results (colors).

Analysis

We will use special software to analyze the data. You need to compile a list of the genes with altered expression. Which went up or down? Are there any conclusions that you can make about the genes?

References

3DNA Submicro Expression Array Detection Kit (9/2002)