

Microarray hybridization

We have prepared cDNA from our RNA samples. We will now hybridize the cDNA to the chips. We then do a second hybridization in order to develop the color and then send the microarray off to be analyzed. We will obtain a “listing” of which spots on the microarray were seen. This data is then analyzed to determine which genes were affected by HCV.

Materials

Genisphere 3DNA Submicro Expression Array Detection Kit

55°C water bath or incubator

42°C water bath or incubator

3X SSC + 0.1% SDS

2X SSC + 0.2% SDS

2X SSC

0.2X SSC

Glass coverslips (Corning brand, e.g. Fisher or VWR; 24 x 50)

Procedures

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

- 0) Add 26 µl of water to the cDNA from step 21 of the previous handout to dissolve the DNA.
[Stop here and put the sample in the freezer if the first hybridization won't be done today]

Hybridization of the cDNA/3DNA mixture to the microarray

- 1) Put on gloves.
- 2) Thaw the 2X hybridization buffer (vial 7) by heating it to 55°C for 10 or more minutes. *While the buffer is incubating, continue with step 4.* Vortex it and make sure that it is completely resuspended. If it is necessary, heat and vortex the vial again.
- 3) Microfuge the buffer for 1 minute to pellet insoluble material.
- 4) Prepare a 50 ml tube of warm 3X SSC and 0.1% SDS. If this prehybridization solution has crystals in it, warm until they dissolve. Put the microarray into the tube so that the solution covers the array. It should be agitated gently at room temperature 30-60 minutes. Two slides can be treated back-to-back. *While the slide is incubating, continue with step 8.*
- 5) Dip the slide in distilled water several times and dry with air. If it won't be used immediately, store it away from dust until ready to use.
- 6) Warm the microarray to 42°C.
- 7) Wash the glass coverslips by briefly submerging the coverslip in water. Remove excess liquid with a kimwipe and let it air dry.
- 8) Mix together the following in a PCR tube:

Solution	Volume (μl)
cDNA	25.4
Hybridization buffer (Vial 7)	27.4 (of the supernatant)
LNA dT blocking reagent (Vial 9 or 9b)	2

- 9) Gently mix the components.
- 10) Incubate the mix in the thermocycler (PCR machine) at 75-80°C for 10 minutes, and then at 42°C until ready to use.
- 11) Put the slide on a clean bench, with the label side facing up. Add the entire cDNA mixture to the microarray, taking care not to touch the surface and by making a liquid line down the length of the spots. Using gloved hands, gently lower the coverslip onto the microarray, stopping one end with fingers, supporting the other on a needle. Avoid bubbles if at all possible; if not, use gentle pressure with the needle to guide them to the edge of the array.
- 12) Place the slide into a hybridization chamber with 15 μ l of water in the two wells. Seal the chamber and incubate it in the dark at 37°C for at least 16 hours (up to two days).

Post-hybridization wash

- 13) 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.
- 14) Transfer the slide to a Coplan jar that has been pre-warmed to 50-55°C and which contains 2X SSC, 0.2% SDS and let it incubate for 10 minutes.
- 15) Transfer the slide to a Coplan jar containing 2X SSC at room temperature and let it sit for 10 minutes.
- 16) Transfer the slide to a Coplan jar containing 0.2X SSC at room temperature and let it sit for 10 minutes.
- 17) Immediately put the slide into a fresh 50 ml tube that has a kimwipe on the bottom, and centrifuge it at 1500 rpm for five minutes to dry the slide. Do not touch the array surface.
- 18) Store the slide in the dark in a fresh 50 ml tube until the next hybridization.

Analysis

When finished, 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 Array 900 Expression Array Detection Kit for Microarrays (12/16/03).
GCAT 3DNA method (11/17/2003).