

MEMBRANE BASED MICROARRAY HYBRIDIZATION

Purpose: to hybridize radiolabeled cDNA probe to microarray filters.

Materials Needed:

- 20X SSC
- 100ml 50% Dextran Sulfate in 500ml bottle, (Serologicals Corp. cat# S4030)
- 50ml conical tubes (Falcon, cat# 352098)

- Microhyb (Research Genetics, cat# HYB250.gf)
- Human COT1 DNA (Invitrogen, cat# 15279-011)
- Poly A 8mg/ml (Sigma, cat# P-9403)
- 33-P alpha-dCTP (ICN, cat# 58430)
- 20% SDS (Quality Biological Inc., cat# 351-066-101)
- Glass or metal plate (we recommend custom cut stainless steel backing plates)
- Alternative tube: 5 1/2" x 1 1/8" (OD) plastic tube (#16B), with bottom. 1" vinyl cap. From North American Plastics Technology Corp. (Driftwood, PA 1-800-397-2366 <http://www.northamericanplastic.com/>)

Preparation/Pre-hybridization conditions:

A: With Dextran Sulfate

1. Add 400ml Microhyb, 1.25ml Human COT1 DNA, and 1.25ml Poly A to the 100ml 50% Dextran Sulfate, mix gently but well.
2. Warm the solution, but not boil, mix gently but well.
3. Aliquot the solution to 15ml conical tubes and store at 4 °C degrees.
4. Load micro filters into 50 ml Conical tubes lengthwise with the spots/writing towards the inside of the tube, make sure no filters overlap when hybing multiple filters. If using large format filters, make sure to roll them all the same way.
5. Pre-wet micro filters w/ 40 mls 2X SSC, then decant.
6. Heat 4mls of hyb mix with Dextran per sample to 95 °C for 5 minutes to denature blocking agents.
7. Add 4 mls to each tube containing filters and rotate in the hyb oven at 55°C for between 2-4 hrs. Decant and dispose of pre-hyb just before moving on to the hybridization step.
8. ***For hybridization temperatures above 50 °C, tubes should be allowed to come to temperature for 5-10 minutes standing upright before being placed in rotator! This prevents most leakage.***

B: Without Dextran Sulfate

1. Load micro filters into 50 ml Conical tubes lengthwise with the spots/writing towards the inside of the tube, make sure no filters overlap when hybing multiple filters. If using large format filters, make sure to roll them all the same way.
2. Pre-wet micro filters w/ 40 mls 2X SSC, then decant.
3. Heat denature blocking agents @ 95°C 5 minutes. Add 10ul of Human Cot-1 DNA and 10ul of poly-A to 8mls Microhyb per sample.
4. Alternatively the blocking reagents can be added to Microhyb in 50 ml tubes and placed in boiling water for enough time to heat the Microhyb to 95°C.
5. Allow the pre-hyb mixture to cool slightly, add 4 mls to each tube containing filters and rotate in the hyb oven at 55°C for between 2-4 hrs. The additional 4mls of hyb mix with blockers will be used for the hybridization. Decant and dispose of pre-hyb just before moving on to the hybridization step.

Procedure:

Hybridization—

1. Using 4 mls of fresh hyb mix with dextran, or the 4mls of leftover Microhyb with blockers, add to a 15 ml conical tube for each sample and place in boiling water for 5 minutes.
2. Heat denature probe @ 95°C for 5 min (critical step!).
3. Add probe, (~75 ul) to 15 ml tubes. Probe count should be *at least* 1×10^6 count per ml of hyb solution. The probe can also be added to the 15 ml tube without prior heating and the 15 mls tube boiled for 5 minutes.
4. Add hyb mix with probe to tubes with pre-hybed filters and rotate overnight (16-18h) at 55°C.
5. ***For hybridization temperatures above 50 °C, tubes should be allowed to come to temperature for 5-10 minutes standing upright before being placed in rotator! This prevents most leakage.***
6. If hybing the same sample with filters in two different tubes, 8 mls of hyb mix should be used, the probe added and 4 mls dispensed to each tube containing a filter.

Washing--

1. Pour hyb solution into 15 ml tubes from the day before if you are saving the probe, or the radioactive liquid waste if not.
2. Do a quick wash in the hybridization tube with ~40 mls of 2xSSC with 0.1%SDS at 65°C. Swirl tube to break filter from wall and decant into liquid rad. waste.
3. Continue washing in hyb oven @ 65°C with ~40 mls 2xSSC with 0.1%SDS
4. Wash 2 times at 65° C for 15min each.
5. Survey filters w/ survey meter. If necessary, continue wash.
6. Wash 2 times at 65°C in 1xSSC with 0.1% SDS for 15min each.
7. Survey filters w/ survey meter. If necessary, continue wash.

8. The exact extent of washing must be determined empirically.
9. It's best to monitor the membrane after the first stringent condition, if the probe activity is down, one wash may be enough)
- 10. Do not let the membrane dry out!!**

Exposure—

1. Wet filters in 2X SSC and place them “dot side up” on a clean dry glass or metal plate.
2. Cover plate with plastic wrap. Pull plastic wrap very tightly and tape 3 sides to the back of the plate. Turn over and roll out excess air and liquid on to paper towels with a rubber roller, tape 4th side down and roll a final time. Check with a survey meter to determine the exposure time. (Lots of counts shorter exposure)
3. Place plate, filter side up, in a phosphoimager cassette and place a bleached phosphoimager storage screen face down and close cassette.
4. Expose at least overnight, and scan on a Molecular Dynamics STORM phosphoimager.
5. The scanner must be set to 50-micron resolution each time; the machine will default to a setting of 200 microns.
6. Determine the need for a longer exposure, 3-day, 5-day, etc.

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For frequently asked questions go to the following address:

<http://www.grc.nia.nih.gov/branches/rrb/dna/protocolFAQs.htm>

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