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We use this protocol and it's working

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Labeling Protocol for Microarray Analysis

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ABSTRACT

Since an array can contain tens of thousands of probes, a microarray experiment can accomplish many genetic tests in parallel. Therefore, arrays have dramatically accelerated many types of investigation. In standard microarrays, the probes are synthesized and then attached via surface engineering to a solid surface by a covalent bond to a chemical matrix (via epoxy-silane, amino-silane, lysine, polyacrylamide or others).

Experiment Summary

1 Since an array can contain tens of thousands of probes, a microarray experiment can accomplish many genetic tests in parallel. Therefore, arrays have dramatically accelerated many types of

investigation. In standard microarrays, the probes are synthesized and then attached via surface engineering to a solid surface by a covalent bond to a chemical matrix (via epoxy-silane, amino-silane, lysine, polyacrylamide or others). The solid surface can be glass or a silicon chip, in which case they are colloquially known as an Affy chip when an Affymetrix chip is used. Other microarray platforms, such as Illumina, use microscopic beads, instead of the large solid support. Alternatively, microarrays can be constructed by the direct synthesis of oligonucleotide probes on solid surfaces. DNA arrays are different from other types of microarray only in that they either measure DNA or use DNA as part of its detection system.

Experimental Steps

2 1. Reverse Transcription

(1) Determine which product will be used (we will use our purity and gel data from the previous class) and labeled with the green dye and which will be labeled with the red dye.

(2) Second perform all calculations and then set up RNA-RT primer mix for each RNA sample in a seperate labeled 0.5 ml RNase-free tube:

1-12 ul total RNA (5 -10 ug)

1 µl of Cy3 or Cy5 RT primer, (Vial 2)

1 µl of Superase-In RNase inhibitor (Vial 4)

Add Nuclease Free Water (Vial 10) to make up a final volume of 14 ul

(3) Mix the samples well by vortexing and spin to the bottom. Note: To spin these small tubes make sure you insert them inside a large tube first or they will fall through the holes in the centrifuge.

(4) Heat to 75°C for 10 minutes in waterbath and place on ice immediatly after you take the tubes out of the bath. This denatures the RNA and primer to get rid of secondary structure. Make sure the temperature is not higher then this or you will destroy your RNA!

(5) Snap cool on ice for 2 min.

(6) In a seperate 0. 5 ml tube use careful pipetting to add each of the following ingrediants to make a master reaction mix. This reaction mix should be kept on ice at all times. The total volume of this solution is 12 ul.

8µl 5X RT buffer-Note we have exactly enough for the whole class-no extras.

2 µl 10 uM dNTP mix (Vial 3)

2 µl RT enzyme, 100 units (keep on ice at all times!!!!!)

(7) Mix gently by pipetting (do not vortex) then spin the contents to the bottom of the tube. Add 6 ul of this solution to your RNA/primer mix from step 4-mix gently). There should be 20 µl in the tube.

(8) Incubate 42°C for 2 hours (note we usually do this only for 1 hour and 30 minutes) in a waterbath.

(9) Stop the RT reactions by adding 3.5 µl of 0.5 M NaOH / 50 mM EDTA and incubating for 10 min at 65 degrees C. This denatures the RNA/DNA hybrids and degrades the RNA.

(10) Cool the reactions on ice and neutralize with 5 µl of 1 M Tris-HCl, pH 7.5 (Total volume = 29.5 µl).

(11) Combine your two RT products together into one of the two tubes. Rinse the tube that you took the first product out of with 73 ul of TE buffer pH = 8.0 and put the rinse solution in the tube

with the combined products. This should give you 130 ul of cDNAs.
2. Concentrating the cDNA
(1) To a YM30 microconcentrator into a 1.5 ml collection tube.
(2) Add 100 ul TE buffer pH = 8.0 to the resivour to wash the membrane. Do not touch the membrane with a pippette tip.
(3) Place the concentrator in a microfuge tube and spin for 3 minutes at 11K rpm (10-14K x g specified).
(4) Discard the TE buffer.
(5) Transfer the 130 ul of cDNA to the reservoir- do not touch the membrane with your pippette tip.
(6) Spin the concentrator for 9 minutes at 11K rpm.
(7) After the spin, remove the reservoir and add 5 ul of TE to Reservoir without touching the membrane. Tap sample to spread the TE accross the membrane.
(8) Invert the reservoir over a fresh tube and spin for two minus at 14K rpm (top speed).
(9) Discard the reservoir and measured eluate with a micropipette to find out how much you have. You should have between 3-10 ul. Add Nuclease Free Water (vial 10) to obtain a final volume of 10 ul. Freeze at -70 degrees Celcius.