

Procedure 2.1.4.1 – Gene Expression Analysis Using cDNA Microarrays (Direct Labeling)

Date: _____

Experiment ID: 2.1.4.1-*yyyymmdd*

Technician: _____

Project: _____

Client Signature: _____

Prepare Spike A Mix and Spike B Mix

1. Assign stock solutions of Stratagene SpotReport Controls
LMT numbers (Appendix 7.3). 1 _____

2 _____

7 _____

9 _____

2. Prepare yeast tRNA solution at 400 ng/ul.
Record in Reagent LogBook and assign LMT number. _____

3. Stock concentration of spike solutions is 10,000 pg/ul.
Dilute with tRNA solution to 100 pg/ul. Assign LMT numbers. 1 _____

2 _____

7 _____

9 _____

3. Prepare Spike Mix A by combining the following.

Spike solution 1	400ul
Spike solution 2	100ul
Spike solution 7	200ul
Spike solution 9	150ul
tRNA solution	150ul

Assign LMT number and transfer to PCR tubes in 10ul aliquots. _____

4. Prepare Spike Mix B by combining the following.

Spike solution 1	100ul
Spike solution 2	400ul
Spike solution 7	200ul
Spike solution 9	300ul
tRNA solution	0 ul

Assign LMT numbers and transfer to PCR tubes in 10ul aliquots. A _____

B _____

5. Store aliquots in -80C freezer boxes designated <cDNA spike mix>.

Anneal Primer to RNA targets.

1. Combine the following in a PCR tube or plate.

Total RNA (5.5ug/ul)	9.0 ul	
Spike mix A or B	1.0 ul	_____
Oligo-dT Primer (1ug/ul)	1.0 ul	_____

2. Place in thermocycler and run <DIRECT1>.

Assemble Labeling Reactions.

1. Prepare a master mix of the following by multiplying by n.5, where n is the number of reactions.

5X Reaction Buffer	5.0 ul	
0.1M DTT	2.5 ul	
lowC dNTP mix	2.0 ul	
Cy3 or Cy5 dCTP	2.0 ul	
Rnase inhibitor	0.5 ul	
SuperScript RT	2.0 ul	_____

2. Add 14 ul master mix to each reaction. Mix.

3. Place in thermocycler and run <DIRECT2>.

Degrade Template RNA

1. Combine Cy3 and Cy5 reactions.
2. Prepare a master mix of the following by multiplying by n.5, where n is the number of reactions.

Nuclease-free water	38 ul	_____
Rnase One Buffer	10 ul	_____
Rnase One Enzyme	2 ul	_____

3. Add 50 ul master mix each reaction pair.
 4. Place in thermocycler and run <DIRECT3>.
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Purify Labeled cDNA

1. Assign QIAquick PCR purification kit an LMT number. _____
 2. Add 500 ul buffer PB to each sample.
 3. Transfer to QIAquick spin column.
 4. Centrifuge at 500 rcf for one minute.
 5. Centrifuge at 10,000 rcf for 30 seconds.
 6. Discard the flowthrough.
 7. Add 700 ul buffer PE to each column.
 8. Centrifuge at 10,000 rcf for one minute.
 9. Discard the flowthrough.
 10. Centrifuge for one minute at 10,000 rcf to dry the column.
 11. Elute the cDNA with 50 ul nuclease free water by centrifuging at 10,000 rcf for two minutes. _____
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Concentrate the Purified cDNA

1. Transfer eluted cDNA to Microcon-50. _____
2. Centrifuge at 12,400 rcf for two minutes.
3. Invert the microcon into a clean micro centrifuge tube.
4. Centrifuge at 3000 rcf for two minutes to collect the cDNA.

5. Measure the volume of the cDNA with a pipettor.
 6. Add nuclease free water to a volume of 15 ul. _____
 7. Quantitate labeled cDNA using the NanoDrop spectrophotometer. (Appendix 7.1).
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Prepare Hybridization Samples

1. Add the following to each sample.

20X blocking solution	2.3 ul	(Procedure 6.3.1)	_____
Hybridization solution	28.8 ul	(Procedure 6.3.1)	_____

Mix carefully to avoid air bubbles forming.

2. Incubate at 95C for two minutes.
 3. Incubate at 50C for one hour.
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Prehybridize Microarrays

1. Place microarrays in Corning hybridization cassettes.
 2. Scan barcodes into an Excel sheet. Add corresponding sample Ids and save as < \dnamicroarray\Procedure_2.1.4.2\2.1.4.2_yyymmdd>
 2. Set glass cover slips on bench so that they overhang the edge of the bench.
 3. Pipette 35 ul 42C prehybridization solution onto each coverslip. (Procedure 6.3.1). _____
 4. Invert the cover slips with a wafer tool and carefully place onto the arrays.
 5. Place the tops on the hybridization cassettes and seal with clamps.
 6. Incubate in 42C water bath for at least one hour.
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Hybridize Microarrays

1. Remove hybridization samples and prehybridized microarrays from water baths.
2. Gently dry cassettes off as much as possible.
3. Remove clamps and tops of cassettes. Set aside.

4. Briefly centrifuge samples to collect condensation.
 5. Carefully remove coverslip from one array by prying away from the microarray with a razor blade with the long edge of the microarray resting on a paper towel. This will blot the prehybridization solution away from the microarray.
 6. Replace the microarray in the cassette.
 7. Pipette 35ul of hybridization sample onto a clean cover slip as before.
 8. Invert the cover slip with a wafer tool and place onto the microarray.
 9. Reassemble the cassette and incubate in 50C water bath for 16 hours.
 10. Repeat for all remaining microarrays.
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Microarray Wash

1. Remove microarrays from water bath and gently dry off as much as possible.
 2. Disassemble the cassettes.
 3. Remove the cover slips by immersing the microarrays in 50C Wash solution 1 in a 250mL beaker. (Procedure 6.3.1) _____
 4. After removing coverslip place microarray into a rack in a glass histology dish containing 50C wash solution 1.
 5. Place on orbital shaker and rotate at speed 3 for 5 minutes in the dark.
 6. Transfer microarrays to a second dish containing wash solution 2. _____
 7. Rotate for 5 minutes.
 8. Transfer microarrays to a third dish containing wash solution 3. _____
 10. Rotate for 5 minutes.
 11. Transfer microarrays to a fourth dish containing wash solution 4. _____
 12. Rotate for 5 minutes.
 12. Dry microarrays by centrifuging in 50ml conical tubes with the barcode down for 5 minutes at 600 rpm.
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Scan the microarrays as described in Appendix 7.4