Enhanced oligonucleotide microarray labeling and hybridization

Haider Ali Syed and David W. Threadgill
University of North Carolina, Chapel Hill, NC, USA

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Microarrays are a powerful tool for functional studies at the molecular level (1,2). Depending on the experiment, users have a variety of commercial and homemade choices. Most commercially available microarrays come with protocols already optimized for reproducibility and sensitivity. However, despite continued reductions in the cost of microarrays, commercially available labeling kits remain high and have now become one of the major expenses for performing microarray experiments. This article offers a source for improvement to reduce the associated costs, particularly that portion for the fluorescent dyes used by all two-color microarray platforms.

We describe a protocol that has been modified for significantly reduced amounts of reagents needed for amplification, labeling, and hybridization of probes to microarrays (detailed protocol is available at www.mouselab.org). Although optimized for the widely used Agilent Technologies microarray platform, this protocol is equally suitable for use on any other two-color microarray platforms.

For the modified protocol, 250 ng total RNA was mixed with T7 promoter primer (Agilent Technologies) in a total volume of 5.8 μL before heating to 65°C for 10 min. After placing the RNA/primer mixture on ice, 4.4 μL cDNA master mix containing 2 μL 5× first strand buffer, 1 μL 0.1 M dithiothreitol (DTT), 0.5 μL 10 mM dNTP mix, 0.6 μL Moloney murine leukemia virus reverse transcriptase...
Benchmarks

Table 1. Pearson Correlation Comparing Labeling Protocols

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<thead>
<tr>
<th>Reaction B Samples</th>
<th>Reaction A Samples</th>
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<tr>
<td>Colon</td>
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<tr>
<td>Liver</td>
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<td>Mammary Gland</td>
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**A**

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<tr>
<td>Mammary Gland</td>
<td>0.36</td>
<td>0.98</td>
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**B**

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<td>0.27</td>
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<tr>
<td>Liver</td>
<td>0.26</td>
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Colon, liver, and mammary gland samples were processed independently for reactions A (Agilent Technologies) and B (enhanced). (A) Samples were compared between labeling protocols and (B) between replicates of the same labeling protocol. Samples were log2 red/green (R/G) Lowess mean normalized with no centering. Bolded values are correlation coefficients using independent RNA samples from (A) the same tissue site or (B) the same RNA used in independent labeling reactions.

(MLLV RT), and 0.3 μL RNaseOUT™ (Agilent Technologies) were added, the contents gently mixed, and the reaction incubated at 40°C for 2 h. The reaction was stopped by heating to 65°C for 15 min before cooling and adding 0.5 μL of either 10 mM CyTM3-CTP or Cy5-CTP and 14.5 μL transcription master mix containing 3.8 μL nuclease-free water, 5 μL 4× transcription buffer, 2 μL NTP mix, 1.6 μL 50% polyethylene glycol (PEG), 0.12 μL RNaseOUT, 0.15 μL inorganic pyrophosphatase, and 0.3 μL T7 RNA polymerase. The contents were gently mixed and incubated at 40°C for 2 h. The resulting cRNA was purified using an RNeasy® kit (Qiagen, Valencia, CA, USA) followed by quantification of the cRNA by spectrosopy using an ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

In a new tube, 1 μg Cy3- and Cy5-labeled and amplified cRNA was mixed and fragmented according to the Agilent Technologies protocol before using for hybridization to a microarray. For both protocols, the overnight hybridization time was reduced from 17 h recommended in the commercial protocol to 10 h, and the final wash consisted of stabilization and drying solution diluted 1:1 (v/v) in acetonitrile, which gives a more even layer on the microarrays than using the concentrated solution that is provided with the hybridization kit.

After acquiring the raw images for the 10 samples (three tissue types from reactions A and B and the two independent B reactions, and two tissue types from reaction B replicates) from an Agilent Microarray Scanner, the images were processed using Feature Extraction software (both from Agilent Technologies). Genes were filtered to remove those that did not show mean signal intensity greater than twice the median background value in at least 70% of the microarrays before performing correlation experiments. The log2 for green (Cy3) over red (Cy5) intensity was calculated, and the corresponding samples from the two different protocols or replicates of the same protocol were compared using Pearson (4) or interclass correlation (5). Pearson correlation showed a very high level of correlation (≥0.98) between the two labeling protocols indicating that the enhanced protocol produced nearly identical results to the established commercial protocol (Table 1A). Likewise, replica reactions of the enhanced protocol were highly correlated (≥0.98) within tissue, but showed low cross-tissue correlation (<0.27) (Table 1B). Scatter plots supported the strong positive correlation among the samples for the three tissue types (Figure 1). We also did average linkage hierarchical clustering using cluster (6) and visualized the results in TreeView (7), which showed that the corresponding samples clustered together (data not shown).

The enhanced labeling and hybridization protocol reported here using Agilent microarrays can easily be adapted to any two-color microarray platform. The results using this protocol will be indistinguishable to established protocols, but with a substantial savings, averaging over U.S. $150 per microarray.

ACKNOWLEDGMENTS

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COMPETING INTERESTS

STATEMENT

The authors declare no competing interests.

REFERENCES


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Address correspondence to David Threadgill, Department of Genetics, CB#7264, University of North Carolina, Chapel Hill, NC 27599, USA. e-mail: dwt@med.unc.edu.

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