Direct Chemical Labeling of Microarray Samples with the *Label* IT® µArray™ Biotin Labeling Kit: A Versatile, Sensitive Improvement on Traditional Enzymatic Labeling Procedures

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Introduction

Microarrays allow thousands of genes to be simultaneously hybridized and quantified in a single experiment. A microarray consists of a two-dimensional array of known capture sequences immobilized on a solid support (e.g. glass slide). Nucleic acid samples must be highly labeled with a marker molecule for detection and quantification on microarrays. Mirus' *Label* IT® technology is designed to covalently attach marker molecules to nucleic acids in a simple one-step chemical reaction. *Label* IT® Reagents directly label nucleic acid bases within DNA or RNA, and the labels do not impact hybridization performance. The ability to simply, reproducibly, and uniformly label both RNA and DNA samples with a variety of detectable markers makes *Label* IT® Reagents ideal for hybridization applications.

The ***Label* IT® µArray™ Biotin Labeling Kit** has been optimized for the preparation of biotin-labeled nucleic acid samples for use in single-channel microarray hybridizations (Figure 1). The use of biotin as a marker molecule requires a post-hybridization detection procedure to introduce a fluorophore. As such, a biotin-labeled sample provides the flexibility to choose among several commercially available biotin detection reagents and amplification kits. Here, we demonstrate the performance of the *Label* IT® µArray™ Biotin Labeling Kit in a variety of microarray applications.

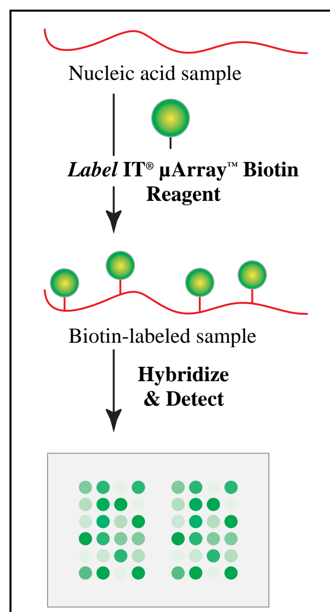

Figure 1. Overview of the *Label* IT® µArray™ Biotin Labeling Kit
The *Label* IT® µArray™ Biotin Labeling Kit offers a simple, versatile labeling procedure for microarray applications. Any nucleic acid sample (RNA or DNA) can be labeled with the *Label* IT® µArray™ Biotin Reagent (1 hour incubation at 37^{0}C). The chemical reaction covalently labels the nucleic acid sample without the need for enzymatic replication or the incorporation of labeled nucleotides, resulting in permanent and uniform biotin labeling of the original sample. The resulting biotin-labeled sample is then purified and hybridized to a microarray. A post-hybridization secondary detection step is used to introduce a fluorophore for signal generation. For example, the use of a streptavidin-Cy™3 conjugate is compatible with most microarray scanners.

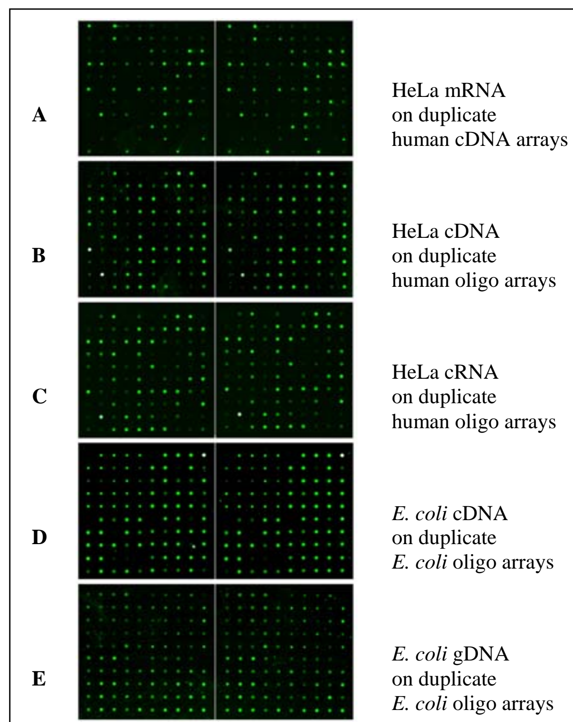

Figure 2. Direct Labeling of a Variety of Nucleic Acid Samples
The *Label* IT® µArray™ Biotin Reagent was used to label: (A) HeLa mRNA for hybridization to human cDNA arrays; (B) HeLa cDNA and (C) cRNA samples for hybridization to sense-strand human oligo arrays; and (D) *E. coli* cDNA and (E) genomic DNA (gDNA) for hybridization to sense-strand *E. coli* oligo arrays. A streptavidin-Cy™3 conjugate was used for fluorescent detection following hybridization.

Results and Discussion

The versatility of the *Label* IT® µArray™ Biotin Labeling Kit is illustrated by labeling a variety of nucleic acid samples followed by hybridization to microarrays (Figure 2). Traditional expression profiling applications require enzymatic replication of an RNA sample (to either cDNA or cRNA) in the presence of labeled nucleotides, followed by hybridization to a microarray. Since *Label* IT® µArray™ Reagents allow the direct chemical modification of nucleic acids, the original mRNA sample can be labeled and hybridized to a microarray (Figure 2A), as well as cDNA and cRNA derivatives (Figure 2B,C). The *Label* IT® µArray™ Biotin Labeling Kit can also be used to label prokaryotic mRNA (data not shown), prokaryotic cDNA (Figure 2D), and genomic DNA (Figure 2E) for alternate microarray applications.

The *Label* IT® µArray™ Biotin Labeling Kit, in direct comparison with enzymatic biotin labeling, generate more consistent and superior hybridization performance data, including more consistent technical replicates (Figure 3) and greater overall signal. Furthermore, mRNA samples that are labeled directly with the *Label* IT® µArray™ Biotin Labeling Kit do not require traditional enzymatic replication step(s), result in sensitive hybridizations, and represent the original sample without any enzymatic replication or incorporation biases. Direct labeling of mRNA allows detection of low copy number transcripts, as low as 10 copies per cell (Figure 4).

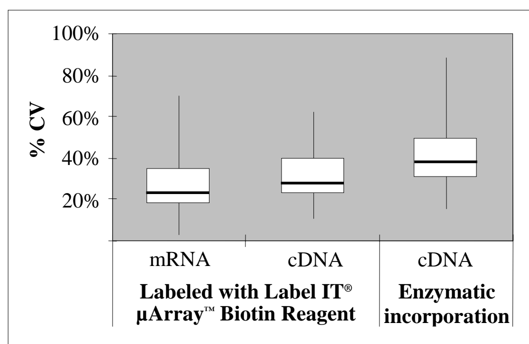

Figure 3. Reproducibility Between Technical Replicates
The reproducibility of gene expression quantification was determined for sets of five technical replicate hybridizations to human cDNA microarrays for the following labeled samples: HeLa mRNA (left) and cDNA (center) labeled with the *Label* IT® µArray™ Biotin Reagent and HeLa cDNA labeled with enzymatic incorporation of biotinylated nucleotides (right). The coefficient of variation (CV) was calculated for each gene based on background-corrected signals measured across the replicate sets (including four replicate features per gene per microarray). The box plot denotes the middle 50% of the distribution of CV values, the horizontal thick line represents the median CV value, and the vertical lines describe the full range of CV values for all genes quantified on the microarray.

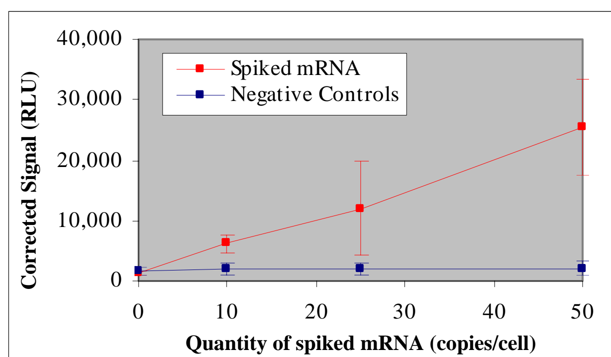

Figure 4. Sensitivity of mRNA Detection
Sensitivity of detection using the *Label* IT® µArray™ Biotin Labeling Kit was determined by adding specific quantities of an Arabidopsis mRNA sequence to a HeLa mRNA sample before labeling. An estimated mRNA copy number of ~10 copies per cell (30 pg of the spiked Arabidopsis mRNA in 1 µg HeLa mRNA) can be detected above non-specific hybridization signal (negative controls). Fluorescent signal detected for the spiked mRNA and negative controls is corrected by subtracting the median local background value; error bars represent one standard deviation.

Slight differences in relative gene expression levels quantified with labeled mRNA and cDNA samples (Figure 5: genes A04, C09) may reflect enzymatic replication bias inherent with cDNA sample preparation, demonstrating the advantage of directly labeling mRNA with the *Label* IT® µArray™ Biotin Labeling Kit for microarray analysis.

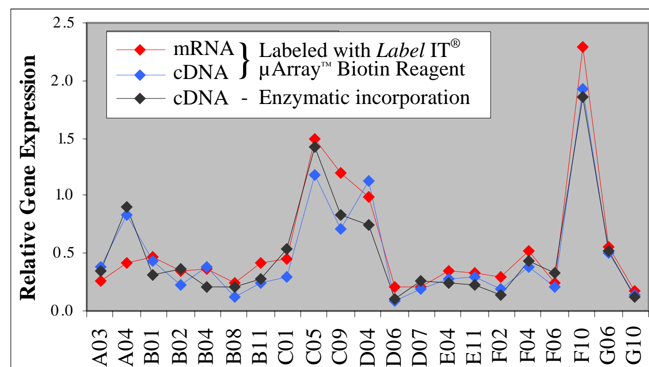

Figure 5. Gene Expression Profiles of Labeled mRNA and cDNA Samples
HeLa mRNA and cDNA samples labeled with the *Label* IT® µArray™ Biotin Reagent generate the same gene expression profiles as cDNA samples labeled with enzymatic incorporation of biotinylated nucleotides. Relative gene expression values for each labeling method represent the average of five technical replicate hybridizations to human cDNA microarrays (data from each microarray hybridization were normalized before averaging), and are shown for 21 genes on the microarray (indicated by Research Genetics well number).

Summary

The *Label* IT® µArray™ Biotin Labeling Kit, optimized for the preparation of biotin-labeled nucleic acid samples for microarray analysis, offers a versatile and sensitive alternative to traditional enzymatic labeling procedures. Samples labeled with this kit generate high signal-to-noise ratios, consistent quantification of gene expression, and sensitive detection of low-copy number transcripts in microarray hybridizations.

We demonstrate the robust performance of the *Label* IT® µArray™ Biotin Labeling Kit in a variety of microarray formats and highlight the utility of *Label* IT® µArray™ Reagents in the direct labeling and hybridization of mRNA samples. Non-enzymatic chemical labeling with *Label* IT® µArray™ Reagents eliminates the need for enzymatic replication and the incorporation of labeled nucleotides, allowing the original sample to be analyzed without the introduction of enzymatic biases. The *Label* IT® µArray™ Biotin Labeling Kit offers significant advantages for single-channel microarray labeling applications, with a simple and versatile chemical labeling procedure that generates high-quality microarray data.

Methods

Microarrays

All microarrays were printed at Mirus Corporation using the QArray^{lite} robotic arrayer (Genetix Ltd., www.genetix.co.uk) and standard protocols. Human cDNA microarrays consisting of a panel of human housekeeping gene I.M.A.G.E. clones (library of cDNA clones from Research Genetics/Invitrogen Life Technologies, www.invitrogen.com) were printed on Takara Slide Glass (amine-coated slides; Takara.Mirus.Bio, www.takaramirusbio.com). Human and *E. coli* sense-strand oligo microarrays (oligo test sets from MWG Biotech, www.mwg-biotech.com) were printed on Takara Hubble Glass slides (Takara.Mirus.Bio).

Sample Preparation

Total RNA was extracted from confluent HeLa cells (ATCC# CCL-13) using TriReagent (Molecular Research Center, Inc., www.mrcgene.com), followed by RNeasy column purification (Qiagen Inc., www.qiagen.com). For mRNA labeling, polyA$^+$ RNA (mRNA) was extracted from HeLa total RNA using the Poly(A)Purist™ mRNA Purification Kit (Ambion Inc., www.ambion.com). Total RNA was used to synthesize first-strand cDNA using an oligo dT primer and SuperScript™ II or III RNaseH$^-$ reverse transcriptase (Invitrogen). The RNA template was hydrolyzed and the cDNA purified prior to biotin labeling (as directed in the *Label* IT® µArray™ Biotin Labeling Kit, protocol #ML031). Similarly, cRNA was prepared and labeled with biotin as per the kit's instructions. Enzymatically labeled cDNA and cRNA used in the comparison experiments were prepared by the incorporation of biotinylated nucleotides in the reverse transcription or RNA polymerase amplification step, respectively, according to established protocols. Technical replicates (biotin-labeled mRNA and cDNA samples) were independently generated from the same HeLa total RNA sample.

For the sensitivity of detection experiments, specific amounts of an exogenous *Arabidopsis thaliana* mRNA sequence (Stratagene SpotReport™ Array Validation System, www.stratagene.com) were spiked into HeLa mRNA samples before labeling. The spike-in amounts were selected using a transcript copy number per cell calculation according to Kane *et al*. (NAR 2000 28(22):4552-4557).

Total RNA was isolated from *E. coli* DH10b using the RNAprotect™ Reagent and the RNeasy Kit (Qiagen). *E. coli* mRNA was prepared using the MICROB*Express*™ Kit (Ambion). Bacterial first-strand cDNA was synthesized using random hexamers and SuperScript™ II or III RNaseH$^-$ reverse transcriptase (Invitrogen). The RNA template was hydrolyzed and the cDNA purified prior to biotin labeling (as directed in the *Label* IT® µArray™ Biotin Labeling Kit protocol).

Genomic DNA was isolated from *E. coli* DH10b using the MasterPure™ DNA Purification Kit (Epicentre Technologies, www.epicentre.com), digested with the restriction enzyme T*sp*509I (New England Biolabs Inc., www.neb.com), and labeled with biotin according to the cDNA labeling protocol of the *Label* IT® µArray™ Biotin Labeling Kit.

Hybridization and Biotin Detection

The microarray hybridizations and biotin detection were performed as recommended in the *Label* IT® µArray™ Biotin Labeling Kit protocol. The cDNA and oligo microarray hybridizations involved different hybridization and wash conditions. All biotin microarray hybridizations were detected using Cy™3 conjugated streptavidin (Jackson Immuno-Research Labs Inc., www.jacksonimmuno.com). All scans were acquired using the GenePix® 4000B microarray scanner (Axon Instruments Inc., www.axon.com). Data analysis was performed using GenePix® Pro Microarray Image Analysis (Axon) and Microsoft Excel software.

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Mirus Bio's *Label* IT® Technologies are covered by U.S. Patent No. 6,262,252 and No. 6,593,465.

Ordering Information:

Label IT® µArray™ Biotin Labeling Kit

Product #	Quantity
MIR 8010	10 reactions*
MIR 8050	50 reactions*

*Each reaction labels 1µg of nucleic acid.
Biotin detection reagents are not included.
Protocol: ML031

Related Products:

Label IT® µArray™ Dual Labeling Kit
with Biotin and Fluorescein labeling reagents

Product #	Quantity
MIR 8010	2 x 5 reactions*
MIR 8050	2 x 25 reactions*

*Each reaction labels 1 µg of nucleic acid.
Secondary detection reagents are not included.
Protocol: ML032

For Customer and Technical Support,
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