

# Gene Expression Analysis Using Infrared Fluorescent Labeling and Detection Methods

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## Abstract

cDNA arrays and Northern blotting are standard techniques for evaluating gene expression. New infrared fluorescent labeling methods coupled with detection using an infrared imaging instrument have yielded highly reproducible gene expression analysis data. Commercially available mouse cell cycle gene arrays on membranes were used to screen cell cycle genes that are expressed in mouse heart and brain tissues. Incorporation of Biotin-16-dUTP into the cDNA probe and detection with infrared-fluorophore-labeled streptavidin were used to analyze the arrays. Cyclin D3 and Cyclin G gene expression were confirmed by Northern blot analysis. cDNA clones of these genes were PCR amplified to incorporate biotin-16-dUTP or aminoallyl-dUTP into the probe sequence. Aminoallyl-modified probes were dye coupled with succinimidyl esters of one of two spectrally distinct infrared dyes.

Results indicate that infrared fluorescent detection provides a complete, reproducible system for evaluating gene expression. Clear advantages include the ability to simultaneously detect two targets on Northern blots using two different fluorophore labels, and reduction of the time needed to obtain reliable data. This method also allows easy normalization of Northern blot data against an internal control, which is not possible with chemiluminescence or radioactive methods. The use of biotin or aminoallyl chemistry coupled with infrared fluorescent detection provides a new and useful tool to evaluate gene expression.

## Introduction

The LI-COR® Biosciences Odyssey® Infrared Imaging System provides a complete two-color system for gene expression analysis. Near-infrared fluorophores (700 nm and 800 nm) are used to detect biotin or amino-allyl modified probes on cDNA Arrays and Northern Blots. Infrared wavelengths provide low background, wide dynamic range, and a highly sensitive alternative to chemiluminescence. This system also provides direct detection of the membrane, eliminating the need for expensive substrates and darkroom usage.

## Materials & Methods

### GEArray® (SuperArray Bioscience Corporation) Analysis

- Probe Labeling** – Total RNA was labeled using the GEArray® Q Series Mouse Cell Cycle Gene Array Non-Radioactive Kit (SuperArray, Cat. # MM-001N). Biotin-16-dUTP (Roche,

Cat. No. 1-093-070) was incorporated into the probe via reverse transcription. An enzyme-linked streptavidin–Alexa Fluor® 680 (Molecular Probes, Cat. No. S-21378) or streptavidin–IRDye™ 800 (Rockland, Cat. No. S000-32) was used to detect the biotin-modified probe. Six micrograms of mouse brain, heart, and liver total RNA was labeled for tissue comparisons.

- Washing and Detection** – Arrays were washed according to the SuperArray protocol. An additional 0.5% SDS wash was done to reduce background. The arrays were scanned on the Odyssey Infrared Imaging System (LI-COR Biosciences).

- Analysis** – Quantification was performed using Odyssey software. Integrated intensity values with median background subtracted were imported into Excel for ANOVA, regression and Pearson correlation analyses.

### Northern Blot Analysis

- Northern blotting** – All Northern blots were performed using a NorthernMax® Kit (Ambion, Cat.No. 1940) and Ambion mouse total RNAs of various origins.

- Probe Labeling and Hybridization** – Biotin-16-dUTP (Roche, Cat. No. 1-093-070) was incorporated into cDNA clone inserts by PCR amplification using M13 forward and M13 reverse primers. DECAtemplate™-GAPDH-mouse (Ambion, Cat. No. 7330) was labeled using an IRDye 800 DNA Labeling Kit (LI-COR, Cat. No. 928-10020). Ares Alexa Fluor 680 DNA Labeling Kit (Molecular Probes, Cat. No. A-21672) was used for aminoallyl labeling of probe inserts. Blots were hybridized in Ultrahyb™-OS (Ambion, Cat. No. 8677) at 42°C overnight. Stringency washes were done according to NorthernMax™ Kit Protocol.

- Detection** – Hybridized blots were blocked in Odyssey Blocking Buffer (LI-COR, Cat. No. 927-40000) + 1%SDS and conjugated with streptavidin–Alexa Fluor 680 (Molecular Probes, Cat. No. S-21378).

## Results

### GEArray on Odyssey Infrared Imaging System

By replacing the AP-Streptavidin in the non-radioactive detection GEArray Q Series Kit with Streptavidin-Alexa Fluor 680, we were able to detect gene expression equivalent to the standard chemiluminescent detection method (Figure 1). The image was clear and easily quantified. Using this same method, variable expression levels were determined between mouse brain, liver, and heart tissues (Figure 2). From this data, Cyclin G and Cyclin D3 were chosen to evaluate by Northern hybridization methods.

### Northern Blots

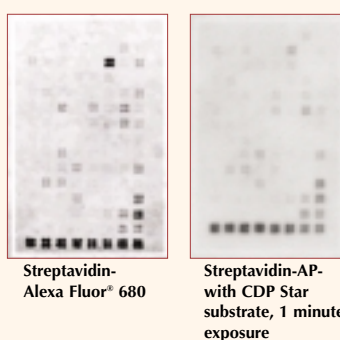
The sensitivity of the Northern Blotting procedure on the Odyssey Infrared Imaging System using Biotin/Streptavidin Alexa Fluor 680 is shown in Figure 3. Analysis of various mouse tissue RNAs indicates Northern blot analysis on the Odyssey System provides similar data to the array analysis (Figure 4). Two-color imaging using a GAPDH probe as a control is feasible. However, due to various expression of GAPDH across mouse tissues, normalization using this probe was not possible. In order to use GAPDH for normalization, the same tissues should be compared. Overall, the Odyssey Infrared Imaging System provides a complete system for gene expression analysis using GEArrays and Northern blot analysis.

## Conclusions

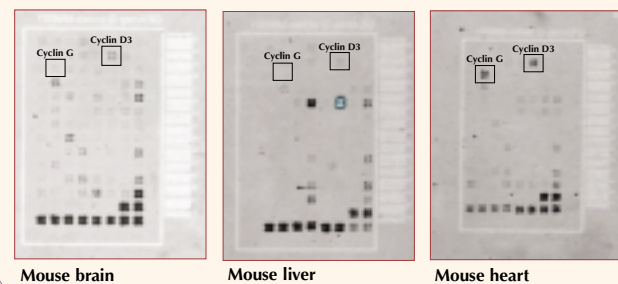
- Biotin labeled probes produced highly reproducible results with low intra- and inter-assay variability on GEArray Q Series Mouse Cell Cycle Gene Arrays (data not presented).
- GEArray Q Series Mouse Cell Cycle Gene Arrays were successfully used to identify variable gene expression in mouse tissues. Gene expression confirmation using Northern blot analysis was also completed using the Odyssey Infrared Imaging System.
- Northern blot analysis using two different fluorophore labels allows easy normalization of data against an internal control increasing the accuracy of the analysis, which is not possible with chemiluminescence or radioactive methods.

## GEArray® Analysis

**Figure 1.** Odyssey detection of GEArray® Q Series Mouse Cell cycle Gene Arrays compared to chemiluminescence labeling and detection.

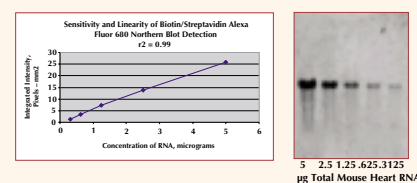


**Figure 2.** GEArray Q Series Mouse Cell cycle Gene Arrays hybridized with mouse brain, liver and heart total RNA detected using biotin-linked streptavidin-Alexa Fluor® 680.



## Northern Blot Analysis

**Figure 3.** Sensitivity and linearity of Cyclin G in mouse heart total RNA using the Odyssey Infrared Imaging System to quantify Northern Blots.



**Figure 4.** Two-color Mouse Cyclin G and Cyclin D3 expression analysis on various mouse tissue RNA.

