

Gene Spec™ III

Dye Labeling Efficiency for Microarrays

Highly reproducible results from array hybridization experiments require well-controlled preparation and analysis of the dye-labeled c-DNA sample. This includes standardization of the concentration of the m-RNA sample to be reverse transcribed and measurement of the labeling efficiency of the c-DNA. Since the sample volumes used in microarray experiments are often very small (e.g. 1 μL), this data has been very difficult to obtain. Here we demonstrate the utility of the Gene Spec III micro-absorbance spectrophotometer in the measurement of the concentration of nucleic acids and dye labels contained in samples as small in volume as 1 μL .

Yeast m-RNA was reverse transcribed, and the product c-DNA was purified by ultrafiltration. The concentration of the m-RNA was measured and standardized with the Gene Spec III using a 1 μL cuvette.

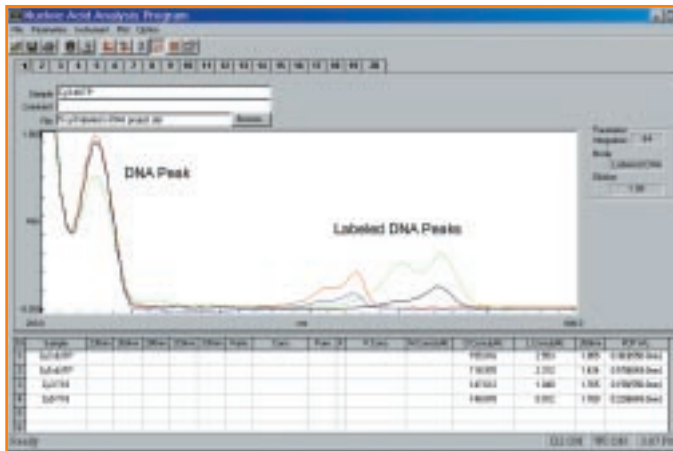
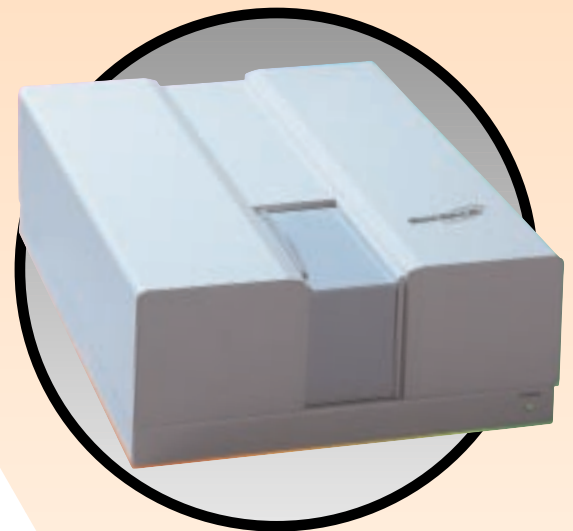


Figure 1. Absorbance spectra (1 μL cuvette, Gene Spec III) of the dye-labeled c-DNA product of the reverse transcription of yeast m-RNA with Cy3-dUTP (red), Cy5-dUTP (green), Cy3-T18 (blue) and Cy5-T18 (black). The data column O. Conc. (μM) lists the concentration of the nucleic acid base in each sample. The data column L. Conc. (μM) lists the concentration of the dye label. The ratio of the two concentrations O. Conc. is a highly accurate measure of the labeling efficiency of the reverse transcription reaction used to make the c-DNA.



Advantages

- Minimize experimental error
- Increase array reproducibility
- Maximize data usefulness

The c-DNA was labeled during the reverse transcription using either dye-labeled triphosphates or dye-labeled primers. Absorbance spectra of the labeled yeast c-DNA, recorded with the Gene Spec III, shows both a peak from the nucleic acid at 260 nm and a peak from either the CyTM3 (550nm) or Cy5 (649nm) label (Figure 1). The observance of this dye peak indicates that the fluorescent dye has been incorporated into the c-DNA.

The concentration of the nucleic acid base is calculated from the absorbance at 260 nm. This concentration is automatically calculated and displayed inside the table of the Gene Spec software as O.Conc (μM) (Figure 1). The concentration of the dye label is also automatically listed in the software in column L.Conc (μM).

The ratio of these two concentrations, 0.Conc/L.Conc is a highly accurate measurement of the labeling efficiency of the reverse transcription reaction used to make the c-DNA (Table 1). This data shows that the efficiency of labeling of the c-DNA with the dye-labeled triphosphates (60.7) is 2.7 times higher than the dye-labeled primer method (141.9).

The c-DNAs made using the two different labeling methods were hybridized to complementary DNA microarrays that were printed with the Hitachi Genetic Systems SPBIO™ microarray spotter. The arrays were processed and the fluorescence intensity of each spot was determined by fluorescence scanning (Figure 2). The average signal intensity of the spots on each array was calculated and is presented in Table 1 along with labeling efficiency data. Notice that as labeling efficiency increases, the average signal intensity measured on the array also increases. This direct relationship between the labeling efficiency and the signal intensity allows the Gene Spec III measurement to be used to predict the average signal intensity in an array experiment. Since this average signal intensity partially determines the coefficient of variance within an experiment, especially at low signal intensity levels, the Gene Spec III measurement gives the researcher the ability to predict error in an array result.

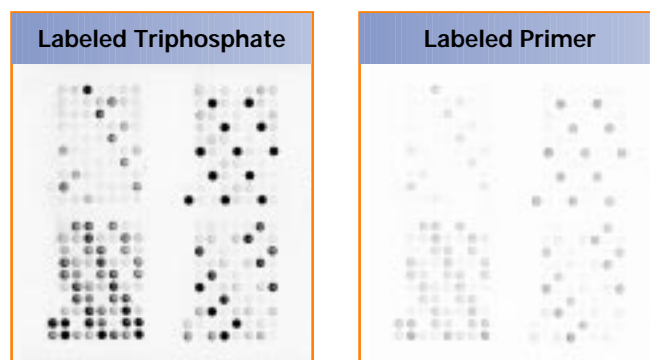


Figure 2. Labeled c-DNA samples were hybridized to a microarray of complementary DNA sequences that were printed with the SPBIO microarray spotter. From these images, the average fluorescent signal intensity was measured and is reported in Table 1.

Labeling reagents		Base/Cy3	Base/Cy5	Mean intensity (RLU)	
				532	635
Dye Labeled Triphosphate	CydUTP	60.7	51.4	820	1503
	CydUTP	60.7	51.4	653	1082
Dye Labeled Primer	Cy-T18	141.9	163.2	219	108
	Cy-T18	141.9	163.2	316	270

Table 1. Comparison of the labeling efficiency of reverse transcription reactions measured with the Gene Spec III to the average signal intensity (after background subtraction) obtained after hybridization to a yeast c-DNA microarray (in relative light units). The results of two identical hybridization experiments, run side-by-side, are reported.

www.miraibio.com

HITACHI Genetic Systems
By Hitachi Software Engineering

MiraiBio Inc.

1201 Harbor Bay Parkway, Suite 150

Alameda, CA 94502

Tel: 800-624-6176 · 510-337-2000 · Fax: 510-337-2099