

# Gene Spec III™

## Quantitative Analysis of Nucleic Acid Samples

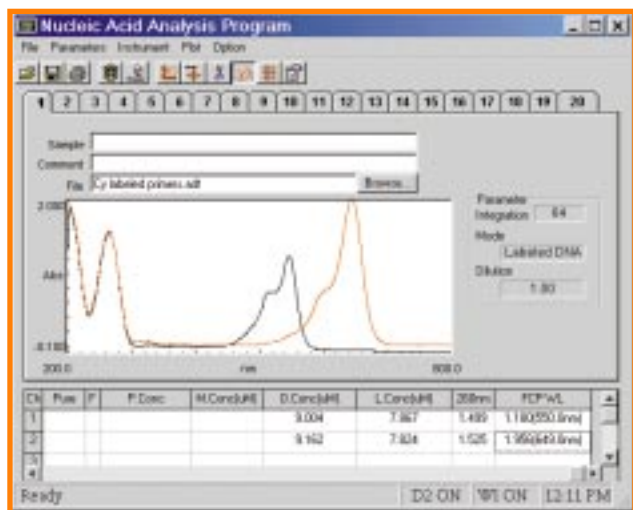
In recent years, the interrogation of m-RNA populations with microarray technology has become the method of choice for most cutting-edge laboratories. Despite the numerous advances in the approach, several technical difficulties persist. For example, quantitative and qualitative analyses of the nucleic acids from samples that are obtained by techniques such as laser micro-dissected tissue sample<sup>1</sup> or from batches of cells produced by immunochemical fractionation methods.

Absorbance spectra of the labeled yeast c-DNA, recorded with the Gene Spec III, shows both a nucleic acid peak at 260nm and a peak from either Cy3 ( $\lambda=550\text{nm}$ ) or Cy5 ( $\lambda=649\text{nm}$ ) indicating that the fluorescent dye has been incorporated into the c-DNA (Figure 1, a and b). The concentration of the DNA is automatically calculated and displayed, as well as is the concentration of the dye label and nucleic acid bases present in the



## Advantages

- Compact footprint
- Scans samples as small as 1  $\mu\text{L}$
- Broad spectral range
- Easy-to-use software



*Figure 1. Absorbance spectra (1  $\mu\text{L}$  cuvette, Gene Spec III) of both Cy3 and Cy5 labeled primers that were used for the reverse transcription of the m-RNA.*

sample (see Table 1). The concentration of the nucleic acid base is calculated from the absorbance at 260nm, assuming equal concentration of all four bases (0.25 is entered for each base and the average extinction coefficient for the four bases is calculated). From the ratio of the concentration of nucleic acid bases and dye label, the average molecular weight of the c-DNA can be calculated from the spectra of the c-DNA made with the dye-labeled primers. In this specific case, the average length of the transcript is calculated to be 142b from the Cy3-labeled primer and 163b from the Cy5-labeled

primer. The spectra of the c-DNA labeled with Cy-labeled dUTP yields information concerning the labeling efficiency of the labeling (the ratio of the concentration of dyes to the concentration of nucleic acid base) is, on average, 2.7 times higher using the triphosphate labeling method than the dye-labeled primer method.

Highly reproducible array hybridization results require the standardization of the concentration of RNA sample to be analyzed, the knowledge of the efficiency of dye labeling of the RNA and a measure of the average molecular weight of the c-DNA target. Since the sample volumes are often very small (e.g. 1 $\mu$ L), this data has been difficult to obtain until the introduction of the Gene Spec III, in the measurement of the concentration of nucleic acids.



1  $\mu$ L cuvette

Labeling reagent	b/Cy3	b/Cy5	532 mean intensity (RLU)	635 mean intensity (RLU)
CydUTP	60.7	51.4	820	1503
CydUTP	60.7	51.4	653	1082
Cy-T18	141.9	163.2	219	108
Cy-T18	141.9	163.2	316	270

*Table 1. Comparison of the labeling efficiency of Yeast c-DNAs measured with the Gene Spec III to the average signal intensity obtained after hybridization to a Yeast DNA microarray (in relative light units).*

#### References:

1. a) Schena, M. ed. DNA Microarray Technology, a Practical Approach. Oxford University Press, NY (1999) b) Schena, M. ed. Microarray Biochip Technology. Eaton Publishing, Natick, MA (2000).
2. Ohyama, H.; Zhang, X.; Kohno, Y.; Alevizos, I.; Posner, M.; Wong, D.T.; Todd, R. *Biotechniques* (2000) Vol. 29, No. 3, pg. 530.

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