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Incorporate more fluorescence into cDNA for enhanced microarray screening

The perfect outcome depends on the right strategy. For successful microarray analysis, you need effective fluorescent targets. But the multiple operations required to generate labeled cDNA—reverse transcription, incorporation of fluorescently labeled nucleotides, purification—can present a formidable task. You need reliable execution every step of the way, yet you want a streamlined protocol to speed your work. A systematic approach, yet easy to follow. And why not? Get it all with the FluoroScript™ cDNA Labeling System.

Superior results all-around

Many cDNA labeling kits provide efficient integration of fluorescently labeled nucleotides, but can't generate cDNA with sufficient yield and length. Other systems offer acceptable RT performance, but fail to incorporate fluorescent labels efficiently. With the FluoroScript™ cDNA Labeling System, there's no such trade-off. You get it all:

- fluorescent nucleotide incorporation of ~10-20%
- first-strand cDNA yield of 25-30%
- cDNA length between 0.5 and 6 kb

FluoroScript™ is optimized to incorporate fluorescent nucleotides with exceptional efficiency. You get great yield and length—with the fluorescence incorporation that's essential for your microarray screening (Table 1).

Table 1 - Performance of FluoroScript™ RT

RNA source	Average cDNA synthesis yield (%)	Average Cy3-dUTP incorporation (%)	cDNA size (kb)
2.3 kb mRNA	47.3	24.3	2.3
HeLa total RNA	34.8	12.8	0.5-7.0

RNA (1 µg of 2.3 kb mRNA or 20-50 µg of HeLa total RNA) was reverse transcribed using FluoroScript™ RT in the presence of oligo $(dT)_{12-18}$ primer, dNTP mix, and fluorescent Cy3-dUTP following the FluoroScript™ cDNA Labeling System protocol. Average cDNA synthesis yield, average Cy3-dUTP incorporation, and cDNA size are shown.

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An RT engineered to perform

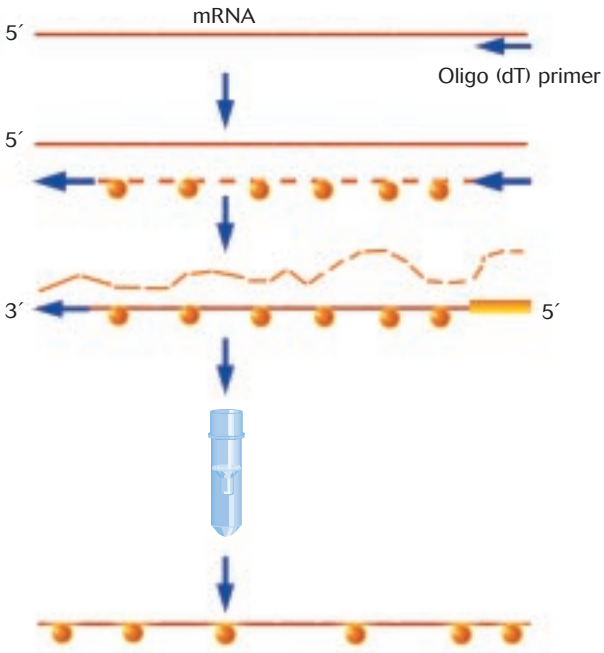
FluoroScript™ RT, an RNase H⁻ avian reverse transcriptase, is engineered for optimized fluorescent cDNA generation:

- Efficiently synthesizes fluorescent cDNA from either total RNA or mRNA, since FluoroScript™ RT is not inhibited by rRNA or tRNA
- Increased thermostability allows use at temperatures up to 55°C, which reduces RNA secondary structure and increases primer specificity
- RNase H⁻ prevents RNA degradation, for cDNA with superior yield and length

Protocol designed for speed

The FluoroScript™ System provides an efficient direct-labeling method to generate fluorescently labeled cDNA from 25 µg total RNA or 1 µg mRNA. Oligo (dT) is annealed to a denatured RNA template and reverse transcribed in the presence of fluorescent dNTP to directly produce fluorescent cDNA. FluoroScript™ RT catalyzes the reverse transcription reaction. The RNA template is degraded by alkaline hydrolysis, and the fluorescent cDNA is purified by a one-step procedure using a S.N.A.P.™ column, further speeding production. Figure 1 outlines these steps.

Figure 1 - The FluoroScript™ cDNA Labeling System process



1. Anneal oligo (dT) primer to mRNA or total RNA preparation.
2. Incorporate fluorescent dNTP while copying mRNA into cDNA with FluoroScript™ RT.
3. Degrade RNA with NaOH.
4. Purify labeled cDNA with S.N.A.P.™ column.
5. Concentrate labeled cDNA and proceed to hybridization.

Simple and effective purification

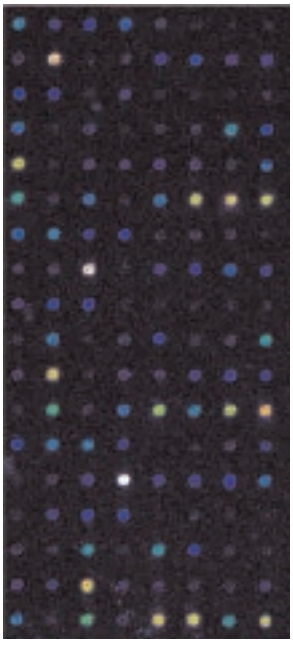
A one-step purification method provides the speed and convenience you desire without compromising the results of your analysis. S.N.A.P.™ columns are made with a silica-based resin in a spin column format, allowing efficient binding of labeled cDNA while easily removing contaminants—including the RT, dNTPs, and oligo (dT) primer. You'll get great results, but with less effort than two-step methods demand.

Achieve the best results

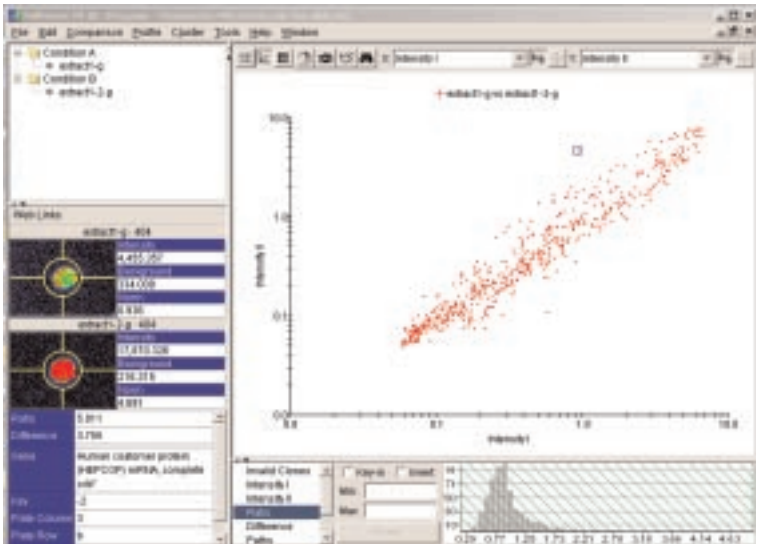
Once purified, the labeled cDNA is ready for microarray screening. To demonstrate performance, we generated fluorescent cDNA with the FluoroScript™ cDNA Labeling System and screened a glass microarray (Figure 2). The images portray bright signal intensities and tight scatter plot—precisely the results you want from your microarray experiments.

Figure 2 - Microarray screening results using fluorescent cDNA generated using the FluoroScript™ cDNA Labeling System

A – Signal intensity



B – Scatter plot



To generate Cy3- and Cy5-labeled cDNA, 25 µg total RNA was isolated from control and resveratrol induced HCT116 cells following the FluoroScript™ cDNA Labeling System protocol. Labeled cDNA was co-hybridized to a glass microarray. Slides were scanned (A) and the image was analyzed using Pathways™ 4-Universal microarray analysis software (B). A signal indicating upregulation was selected on the scatter plot for analysis. Details are shown on the left side of figure B.

Complete, integrated system

Each FluoroScript™ cDNA Labeling System comes with everything you need to generate purified fluorescent cDNA to screen microarrays (except fluorescent nucleotides) (Table 2). All reagents are tested for quality to ensure reproducible fluorescent cDNA preparation. You won't have to locate, order, or test items from different vendors. Nor will you have to repeat experiments because reaction components are incompatible with one another. What you will get is a state-of-the-art method to produce labeled cDNA.

Table 2 - System components

FluoroScript™ RT	S.N.A.P.™ columns
5X FluoroScript™ buffer	S.N.A.P.™ collection tubes
Dithiothreitol (DTT)	Loading buffer
dNTP mix	Wash buffer
Oligo(dT)$_{12-18}$ primer	Control RNA (2.3 kb)
DEPC-treated water	

Lay the groundwork for successful microarray analysis

The FluoroScript™ cDNA Labeling System combines power and simplicity in a single, complete package. Get enhanced fluorescent cDNA target generation, bright signals from microarray hybridization, sensitive detection of gene expression. A logical sequence, but you have to take the first step—order the FluoroScript™ cDNA Labeling System today.

Product	Reactions	Cat. no.
FluoroScript™ cDNA Labeling System	20	L1013-01

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