Nucleic Acid and Fluorescent Dye Concentration Measurement

Key Words
NanoDrop Spectrophotometer, Cancer, DNA, Europroteome, Fluorescent Dye, Gene Expression, Nucleic Acid, RNA

Introduction

Europroteome is a leading biomedical firm focusing on R&D and product development in the area of epithelial cancers. Gene expression analysis at Europroteome is performed either by real-time quantitative PCR or with the DNA-microarray technology. Information quality is dependent on accurate and reproducible measurement of RNA concentrations. In both cases, total RNA from paired non-tumor and tumor samples are systematically analyzed in parallel and the relative levels of expression of one or thousands of genes are compared.

Nucleic Acid Measurement

The proteomics and gene expression laboratory in Hennigsdorf, Germany, utilizes immunopurified epithelial cells extracted from already limited human tissue samples resected at one of the networking hospitals. Samples are screened, at a proteomics and transcriptomics level, to identify cancer markers and diagnostic targets. To accomplish this, Europroteome needed to establish procedures that limit sample consumption without interfering with the data quality. One aspect of this optimization process was the quantification of extracted total RNA for rt-PCR and cDNA microarray experiments. Table 1 shows the concentrations (mean +/- SD) calculated by both instruments for each of the measured samples. (1) is the non diluted sample, that could not be measured directly with the Amersham™ Ultrospec™ 3100 Pro due to the high concentration. As it can be seen, results from the Thermo Scientific™ NanoDrop™ 1000 spectrophotometer show a better linearity and in a higher range of RNA concentrations than those from the Ultrospec 3100 Pro.

Fluorescent Dye Labeling Efficiency

For gene expression analysis on cDNA microarrays, about 10 µg total RNA corresponding to only 100 ng mRNA is used as starting material. During the labeling process, the mRNA is reverse transcribed into cDNA with concurrent Cy-dye labeled nucleotide incorporation. The two samples, derived from the tumor and nontumor specimen, are combined and hybridized to the array.

With the NanoDrop 1000 spectrophotometer, for the first time, we now have the possibility to check the efficiency of the cDNA labeling process and to control the quality of the hybridization probes in our microarray experiments. Before the NanoDrop 1000 spectrophotometer was used it was not possible to quality control the labeling procedure. The sample volume of the hybridization solution must not exceed 25 µL. For an analysis in a regular spectrophotometer at least about 50 µL are needed. The NanoDrop 1000 spectrophotometer enables the researcher to check for Cy3 and Cy5 labeling efficiencies by using only 1 µL of the hybridization solution. The quality and quantity

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Table 1

	NanoDrop 1000 Spectrophotometer		Amersham Ultrospec 3100 Pro	
n=2 Rel. Dil.	Mean	SD	Mean	SD
0.0009375	1.90	0.15	3.80	
0.001875	3.78	0.21	10.65	0.92
0.00375	7.65		14.83	1.10
0.0075	16.75		27.43	0.11
0.015	35.98	0.48	47.38	1.52
0.03	75.43	1.21	85.70	
0.06	153.85	3.06	150.20	2.47
1	2292.46	1.22		

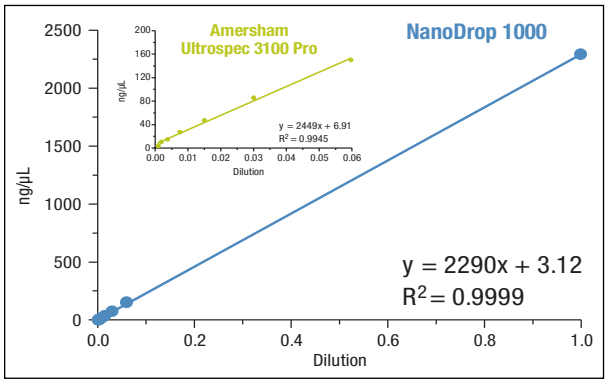

Figure 1/Table 1: A total RNA sample was serially diluted twice independently and the resulting standard dilutions (including the original, non diluted sample) were measured in parallel using both the NanoDrop 1000 and the Amersham Ultrospec 3100 Pro spectrophotometers.

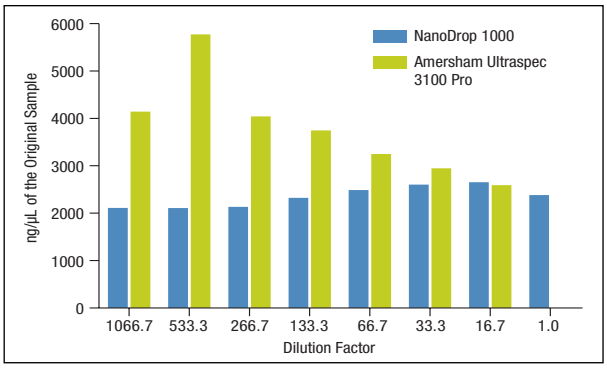

Figure 2: Uniformity of the calculated concentrations after applying the Dilution Factor.

control of the hybridization probes is of great value because the experiment can be stopped if one of the labeling reactions did not perform well therefore saving the costs of an expensive cDNA microarray chip which would have been hybridized with a bad sample.

In addition to the nucleic acid and microarray applications, the NanoDrop 1000 spectrophotometer can also be used as an UV-Vis spectrophotometer. Collectively, the NanoDrop 1000 spectrophotometer is of great value for us. It is very easy to use. A major advantage is very low sample consumption, which is especially important when using precious materials like human tissue samples. Also significant is elimination of cuvettes and, in many cases, no need to dilute the samples because the NanoDrop 1000 spectrophotometer can measure higher concentrations than any other commercially available spectrophotometers. Thus, costs are reduced and measurements are much faster.

Table 2

	NanoDrop 1000 Spectrophotometer		Final Concentration		Amersham Ultrospec 3100 Pro		Final Concentration	
Dilution Factor	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1066.7	1.9	0.1	2021.3	158.4	3.8		4053.3	
533.3	3.8	0.2	2016.0	113.1	10.7	0.9	5680.0	490.3
266.7	7.7		2040.0		14.8	1.1	3953.3	292.3
133.3	16.8		2233.3		27.4	0.1	3656.7	14.1
66.7	36.0	0.5	2398.7	32.1	47.4	1.5	3158.3	101.4
33.3	75.4	1.2	2514.2	40.3	85.7		2856.7	
16.7	153.8	3.1	2564.1	51.0	150.2	2.5	2503.3	41.2
1.0	2292.5	1.2	2292.5	1.2				
Average Mean			2260.0				3694.5	
Average SD				221.3				1044.6
CV (%)			9.8				28.3	

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