Using the QuantiGene Reagent System to Measure mRNA Levels Directly from Tissue Homogenates

Introduction

Quantitating and characterizing gene expression has required the development of tools to investigate the effects of drug treatment on disease, to compare different disease states to one another, and more generally to compare the effects of gene induction in different tissues. Such expression profiling is commonplace in drug discovery as a surrogate for disease, toxicity, or any response of a cell or tissue to its environment. Accuracy and precision in such measurements are critical; subtle changes in gene expression may produce dramatic biological impact.

Typically RNA expression is examined using quantitative PCR which requires RNA purification and amplification. These methods are time consuming, expensive and known to introduce errors and variability. An RNA quantitation method that does not require RNA purification may yield a truer representation of the signature profile of gene expression in live cells. In order to keep pace with the quick advance of scientific discovery and avoid the introduction of error and variability, a method that is independent of the requirement to amplify RNA is highly desired.

Here we demonstrate that the QuantiGene Reagent System can measure RNA directly from homogenized tissues without RNA purification and generate data that are consistent, reproducible and less prone to possible artifacts.

QuantiGene Reagent System Technology

Overview of the branched DNA assay

The QuantiGene Reagent System uses branched DNA (bDNA) technology to specifically bind and stabilize RNA/DNA duplexes that are then detected using an amplified chemiluminescence signal. The system's plate-based sandwich hybridization assay is specific and sensitive enough to measure RNA levels down to 6 thousand transcripts. A more detailed description of the QuantiGene technology can be found at www.panomics.com.

Materials and Methods

Materials Required

Item	Source	Cat. No.
QuantiGene Sample Processing Kit: Animal Tissues	Panomics	QG0104
RNAlater	Ambion	7020
Dounce Tissue Grinder	Fisher	06434
QuantiGene Discover Kit	Panomics	QG0002
QuantiGene Probe Sets	Panomics	Varies with probe set
Luminometer	Major Laboratory Supplier	
Incubator	Major Laboratory Supplier	

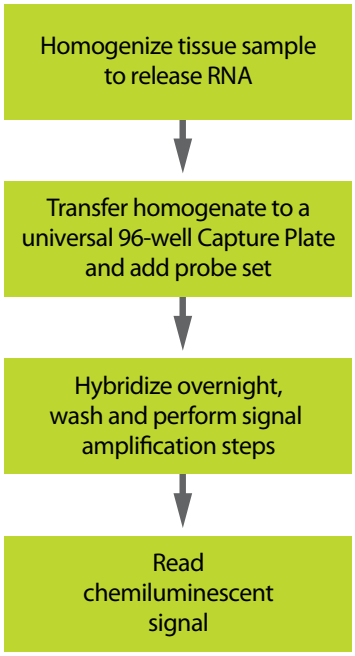

Figure 1. QuantiGene Reagent System workflow for the bDNA assay.

Animal Treatment and Tissue Harvest

Male Fisher 344 rats (150–200 grams) were treated for 4 hours, 24 hours or 14 days with either vehicle alone or with a compound known to alter liver gene expression. Following sacrifice, liver, kidney, and colon tissues were dissected and immediately placed in RNAlater buffer (Ambion).

Tissue Homogenate and Purified RNA Preparation

Step	Action
1	Place fresh tissue in five volumes of RNAlater.
2	Dispense 1 µL of 50 µg/µL Proteinase K solution into 300 µL of the Homogenizing Solution and add up 10 mg of tissue sample.
3	Homogenize the tissue sample and transfer the homogenate to a microcentrifuge tube.
4	Incubate the samples at 65°C for 30 minutes. Mix samples on a vortex mixer every 5 minutes.
5	Centrifuge to remove debris and transfer the supernatant to a new microcentrifuge tube.

To prepare purified RNA, follow Epicentre's RNA purification protocol.

Step	Action
1	Prepare the working probe set by adding 15 µL each of pooled CE, LE, and BL probes to 1455 µL of the Lysis Mixture.
2	Add 70 µL of diluted Lysis Mixture (1 volume of stock Lysis Mixture in 2 volumes of RNase-free water) to the Capture Plate wells.
3	Transfer 20 µL tissue homogenate or purified RNA (typically undiluted sample or a 1:10 dilution) to the Capture Plate well.
4	Add 10 µL of the appropriate working probe set.
5	Follow the QuantiGene user manual by sealing the plate and starting the 16–20 hour over night hybridization at 53°C.

Assay Results

RNA Recovery from Tissue Homogenates and Purified RNA

To determine the effects of the presence of tissue homogenates on mRNA measurements, we added one attomole of RNA expressed from an exogenous control gene, DAP (bacterial, accession number L38424) to tissue homogenates and to isolated total RNA from rat kidney, colon, and liver samples.

We then measured DAP RNA levels in these samples and compared with control DAP (RNA in buffer). As shown in Figure 2, the presence of crude tissue homogenates or total RNA from kidney, colon or liver did not interfere with the quantitation of exogenously added DAP RNA, compared to the measurement of control samples in the absence of cellular RNA (95% and 93% of the control, respectively). The average CVs of the assay were 2.6%. The spike-recovery experiments indicate there is no significant difference between tissue homogenates, purified RNA or buffer.

Tissue Homogenate vs. Purified RNA

We next compared mRNA measurements in the QuantiGene system for an endogenous glycolytic gene, Gapd (Accession number: NM_017008). The liver tissue homogenate was split; one-half of the sample was used directly, and the other half was used to prepare total RNA. A dilution series of total RNA from 230 ng to 0.69 ng and an equivalent amount of tissue homogenates was used to determine the expression of Gapd. Linear response of luminescent signals (RLU-relative light unit) from the QuantiGene Reagent System was observed for both samples (data not shown). The two sets of results correlated very well (Figure 3), with an R-squared value of 0.999 when the luminescent signal from total RNA was plotted against the results from tissue homogenates. Linear curve fit shows a slope of 1.11, indicating that the tissue homogenate generates 11% higher signal than total RNA. Gapd expression was also determined in tissue homogenate and total RNA of kidney and colon samples and showed comparable results between fresh tissue and purified RNA (data not shown). The higher signal in tissue homogenates suggests that direct quantitation of RNA from tissue lysates may produce a more accurate signal and a higher yield.

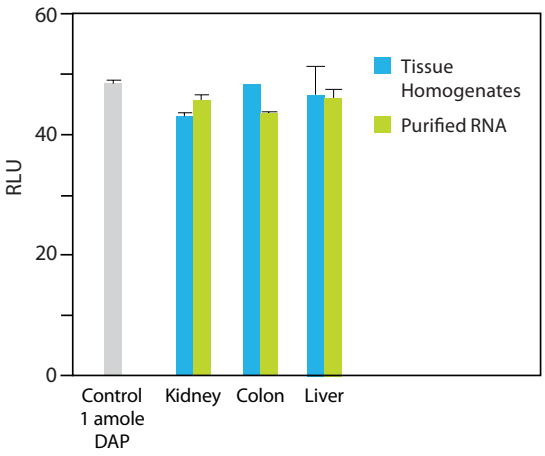

Figure 2. Comparison of RNA quantitation between various tissue homogenates and purified RNA. One attomole of bacterial DAP RNA was added to tissue homogenates (blue) and purified (green) RNA. The control (gray) is the value obtained assaying the DAP RNA in the absence of cellular RNA.

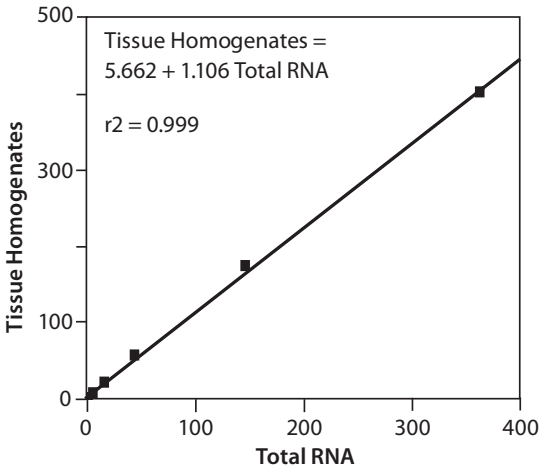

Figure 3. Comparison of Gapd RNA quantitation between liver homogenate and purified RNA using the QuantiGene assay.

Induction of Liver Genes

In a study of drug toxicity and response, rats were treated with either vehicle alone or with a drug for variable lengths of time (4 hours, 24 hours, or 14 days). Six animals (three control, three drug-treated) were sacrificed and liver tissue was prepared as described. Gene expression of five genes (Gapd, Pgm1, Cpt1a, Fiaf, and P4hb (5-MD)) in tissue homogenate or total RNA was measured using the QuantiGene Reagent System.

Three of the genes (Pgm1, Cpt1a, and Fiaf) showed low levels of expression in the control animals but strong induction in the compound-treated animals. Of these, time-dependent changes of gene expression were observable in two of the genes, Cpt1a and Fiaf, with maximal gene induction at 24 hr. In contrast, P4hb (5-MD) mRNA levels were either modestly affected or even slightly inhibited by the drug at day 14.

In this experiment, a tight correlation of gene expression was observed between tissue homogenates and total RNA of all the samples (Table 1), with the average signal from total RNA about 88% (± 14%) of the signal from tissue homogenates. The 14% CV largely reflects the variation of total RNA isolation among different tissue samples. The average assay CV for all tissue homogenate samples was 3.9% and for all total RNA samples is 3.2%.

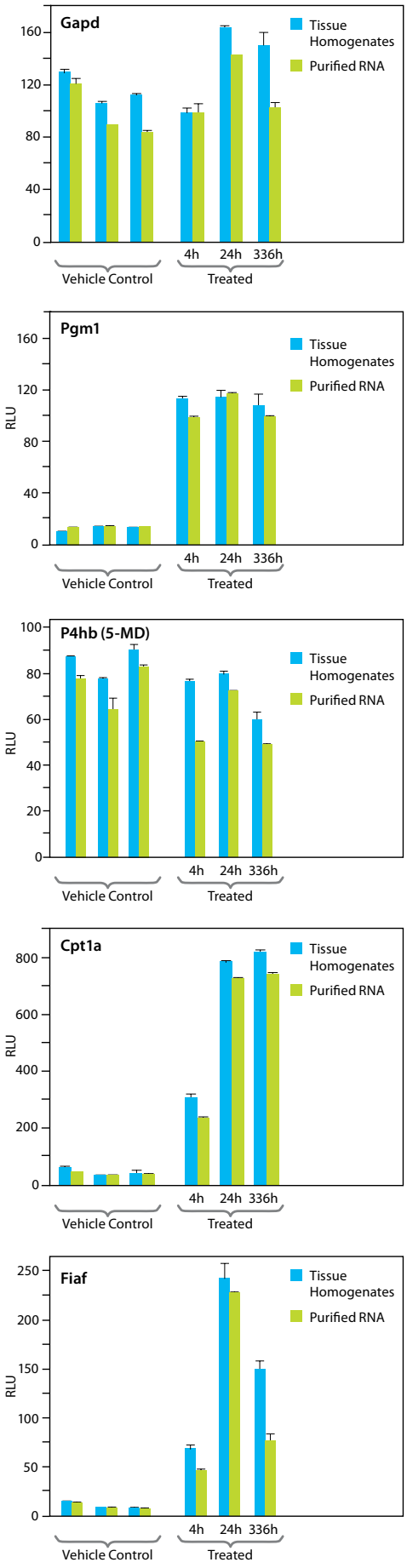

Figure 4. Comparison of tissue homogenate (blue) and purified RNA (green) for five liver genes (Gapd, Pgm1, Cpt1a, Fiaf, P4hb (5-MD)). RNA was quantitated after a time course (4 hours, 24 hours and 14 days (336 hours)) of drug treatment in six rats (three control and three treated).

Discussion

The data presented in this application note demonstrates the following:

- A comparison of RNA quantitation between tissue homogenates and purified RNA demonstrates that the QuantiGene Reagent System yields nearly identical results.
- Very low CVs in the data indicate that the QuantiGene Reagent System is a reliable and reproducible method for RNA quantitation directly from crude tissue homogenates.
- The QuantiGene Reagent System can accurately quantitate RNA in a variety of tissue homogenates.
- In tissue homogenates, the QuantiGene Reagent System can easily detect even modest changes in drug-induced gene expression of a variety of genes in liver.

The performance of the QuantiGene Reagent System demonstrates that high quality and precise quantitation of mRNA levels can be obtained without the need for RNA purification. The capability to measure target-specific RNA in tissue lysates saves time and expense while enabling higher sample throughput. Perhaps more significantly, the capability to quantitate target-specific RNA directly from cell or tissue homogenates may help to eliminate error and variability that occurs in assays which rely on gene RNA purification and amplification procedures.

Treatment Group	Gene	% CV			
		Homogenates		Purified RNA	
		Mean	Range	Mean	Range
Control	Gapd	1.40	(0.28–2.26)	2.38	(2.12–2.88)
Treated		3.67	(1.28–6.84)	3.40	(0.18–6.34)
Control	Pgm1	2.56	(0.81–4.68)	1.02	(0.04–2.0)
Treated		8.95	(2.89–15.64)	1.45	(0.92–2.0)
Control	Cpt1a	3.68	(1.02–8.31)	2.24	(2.11–2.45)
Treated		0.24	(0.2–0.29)	0.84	(0.36–1.8)
Control	Fiaf	2.54	(1.48–4.61)	5.34	(4.01–7.64)
Treated		5.56	(5.0–5.98)	4.39	(0.1–8.36)
Control	P4hb (5-MD)	1.37	(0.86–2.3)	3.28	(0.86–7.5)
Treated		1.09	(0.78–1.4)	0.53	(0.03–1.35)
Overall Average CV		**3.11**		**2.49**	

Table 1. Comparison of the CVs between liver tissue homogenates and purified RNA from six rats (three vehicle control, three drug-treated) for five genes. RNA quantitation is shown in Figure 4.

Ordering Information

Product	Size	Catalog No.
QuantiGene Explore Kit	2 – 96-well plate (192 assays)	QG0001
QuantiGene Discover Kit	10 – 96-well plate (960 assays)	QG0002
QuantiGene Discover XL Kit*	50 – 96-well plate (4,800 assays)	QG0003
QuantiGene Screen Kit**	50 – 96-well plate (4,800 assays)	QG0004

* Configured to allow processing of 1 or more plates/run

** Configured for high throughput applications requiring the processing of 10 plates/run

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