Panomics

QuantiGene® 2.0

Gene Expression without the Limitations

Introduction

With QuantiGene 2.0 from Panomics, you can now combine exquisite sensitivity with a simplified workflow.

Stop using old fashioned approaches like PCR, with the hassle of RNA extraction, the inherent bias of target amplification and the need for costly instrumentation. Measure the RNA in your sample not some derivative. Simply lyse/homogenize your sample (cells, tissues, blood, FFPE) and go.

Our assays are easily automated and based on proven branched DNA (bDNA) technology that has been used for >10 years in a clinical setting as the diagnostic VERSANT 3.0 assays for HIV, HCV and HBV.

Highlights

No RNA Purification—
Work directly from cultured cell or whole blood lysates or fresh, frozen or formalin-fixed, paraffin-embedded (FFPE) tissue homogenates

No Reverse Transcription—
Eliminate biases against messages that do not reverse transcribe efficiently

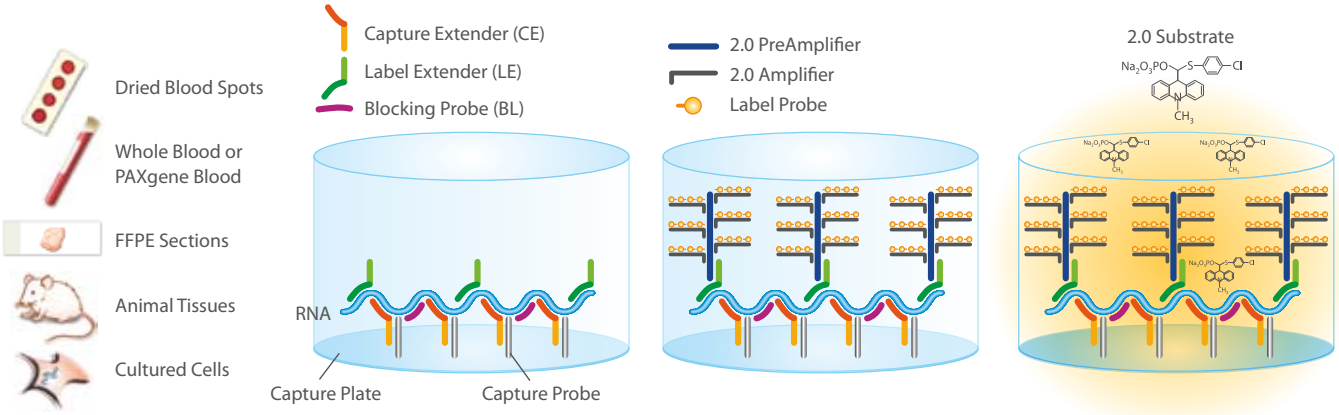
No Target Amplification—
Eliminate errors inherent to messages that do not amplify proportionately

Limit of Detection—
≤ 200 copies

Limit of Quantification—
≤ 500 copies

Precision
– Intra assay CV ≤ 10%
– Inter assay CV ≤ 15%

QuantiGene 2.0 Assay Overview



Step 1: Release Target RNA	Step 2: Target RNA Capture	Step 3: Signal Amplification	Step 4: Detection
Cells are lysed to release RNA.	Probe Set design determines the specificity of the target RNA capture. Probe Set oligonucleotides (CEs, LEs, BLs) bind a contiguous region of the target RNA and the CEs (Capture Extenders), by cooperative hybridization, selectively capture target RNA to the 96-well Capture Plate during an overnight incubation.	Signal amplification is performed via sequential hybridization of 2.0 Pre-Amplifier to Probe Set LEs (Label Extenders), 2.0 Amplifier to the 2.0 Pre-Amplifier and Label Probe to the 2.0 Amplifier. The number of LEs determines assay sensitivity.	Addition of a chemilumigenic 2.0 Substrate* generates a luminescent signal that is proportional to the amount of target mRNA present in the sample. *Lumigen® APS-5

"We have used the original QuantiGene bDNA technology for over three years to validate siRNA knockdowns and have always been pleased with the robustness of the assay and the level of precision. We recently had the opportunity to evaluate the new QuantiGene 2.0 technology and were astounded by the increase in sensitivity of the assay across many gene targets, in some cases over 30-fold. The assay now couples simple workflow with analytical sensitivity that is comparable with real time PCR."

— Angela Reynolds
Associate Scientist, Dharmacon Inc.

Assay Specifications

Limit of Detection: ≤ 200 transcripts

Limit of Quantitation: ≤ 500 transcripts

Linear Dynamic Range: ≥ 3.5 logs

Assay CV: ≤ 10% intra-assay,
≤ 15% inter-assay

Compatible Sample Types:
Cultured cells; whole blood, PAXgene® blood or dried blood spots; fresh/frozen animal tissues; FFPE samples; purified RNA

Assay Format: 96-well plate

Targets/Well: 1

Hardware Requirements:
Microplate Luminometer

Applications

Quantification of RNAi Knockdown

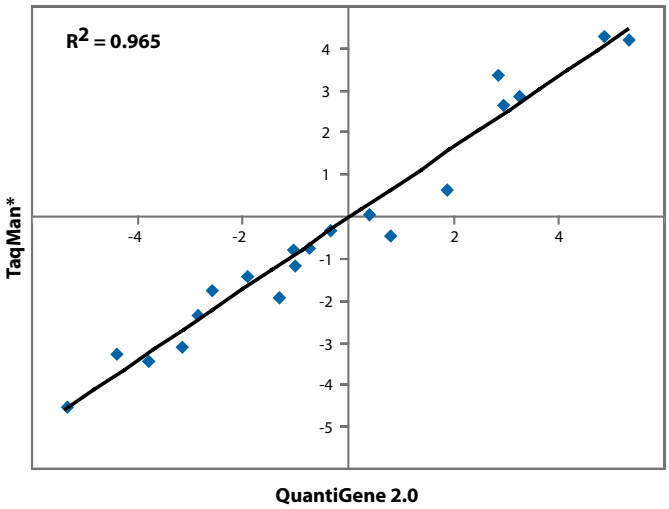
Biomarker Studies

Secondary Screening

Predictive Toxicology

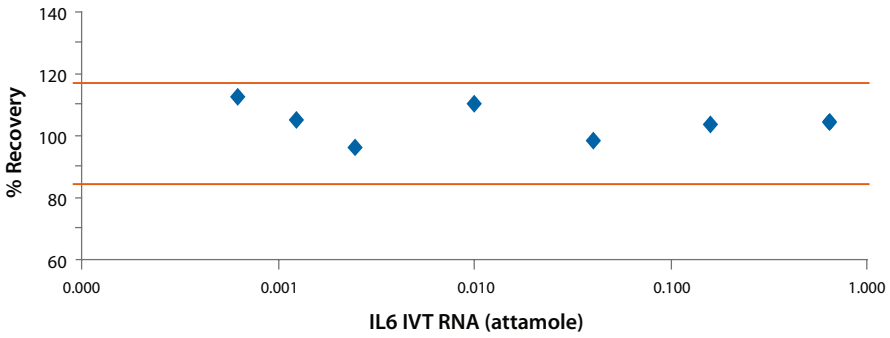
Microarray Validation

Prospective/Retrospective Analysis of Clinical Samples
- **Whole Blood**
- **FFPE Samples**



QuantiGene 2.0 Data Show Excellent Correlation with TaqMan® Data

Data shown are log2 transformed ratios of signals from twenty RNAs of varied expression level measured in two reference RNA samples. The TaqMan data shown are results from the MAQC study, an FDA sponsored initiative aimed at understanding the role microarray technologies play in the drug development cycle (Canales, R.D., et al., Evaluation of DNA microarray results with quantitative gene expression platforms. Nat Biotechnol, 2006. 24(9): p. 1115-22).



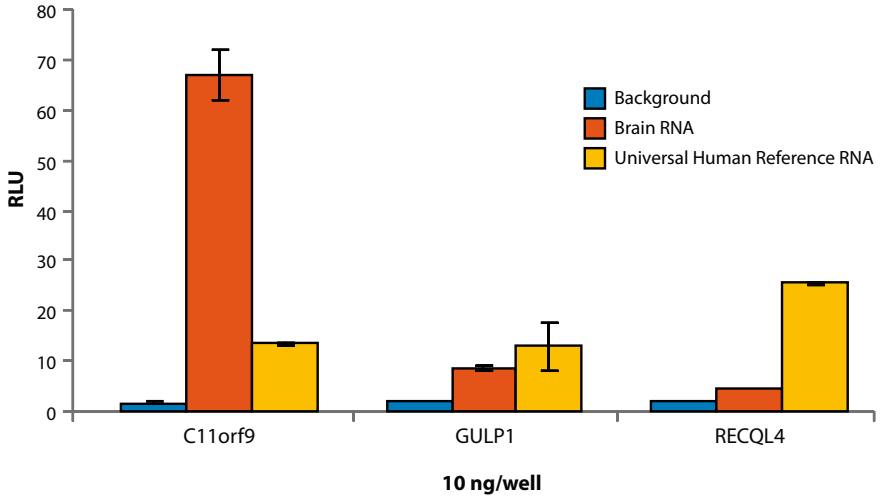
Unparalleled Accuracy of Measurement—Spike Recoveries of 85-115%

A wide range of concentrations (0.001–1.0 attamoles) of an IL6 *in vitro* transcribed (IVT) RNA was spiked into a cell lysate with undetectable levels of endogenous expression. Spike recovery was calculated as signal from the IVT RNA in the presence of lysate divided by the signal in the absence of lysate multiplied by 100.

Detection of Low Abundance Genes Not Detected by PCR

Our QuantiGene 2.0 technology was able to detect 3 RNAs of low abundance in two reference samples, Brain RNA and Universal Human Reference RNA, from the MAQC study. These genes were undetectable (Ct >35) by PCR.

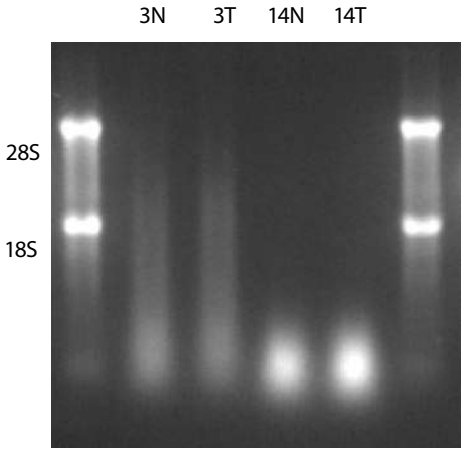
This exquisite sensitivity allows for the basal measurement of low expression genes.



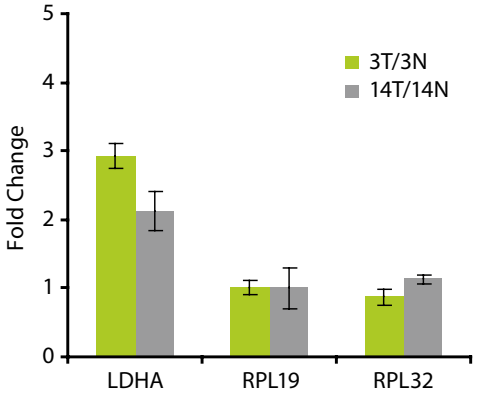
Quantitative Gene Expression Data from Archived FFPE Samples

QuantiGene assay profiling of lactate dehydrogenase RNA in 14-year old FFPE lung tumor and adjacent normal tissue demonstrated 2–3 fold induction of this advanced-stage cancer biomarker, in agreement with published data[1]. RNA purified from these samples was extremely degraded and failed to produce quantifiable signals in qPCR experiments.

1 Beer DG et. al., Gene-expression profiles predict survival of patients with lung adenocarcinoma. Nat Med. 2002 Aug;8(8):816-24.



RNA from 3 and 14 year old FFPE samples of tumor (T) and adjacent normal (N) tissue from lung cancer patients as visualized by agarose gel electrophoresis The positions of the 28S and 18S rRNAs are indicated.

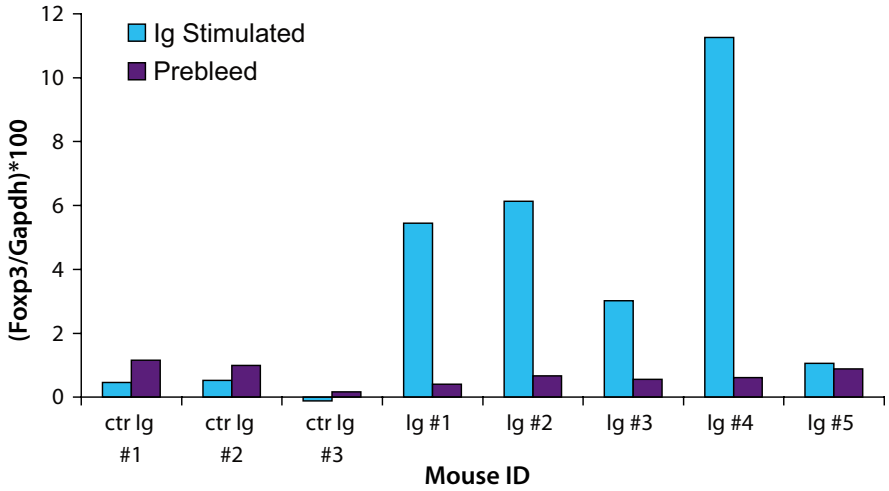


In agreement with the literature (Beer et al 2002), QuantiGene detected 2-fold induction of LDHA RNA in tumor relative to normal tissue even in highly-degraded 14 year old samples.

Expression Analysis of Foxp3 Gene in Regulatory T Cells

This study shows the expression of the Foxp3 gene, a marker for regulatory T cells, after stimulation by an experimental antibody.

Five mice were treated with the experimental antibody (Ig) and three mice were treated with a control antibody (ctrl Ig). Blood was drawn before stimulation (prebleed) and 6 days after stimulation (Ig stimulated) from the same animal. Data shown are direct measurements of Foxp3 RNA levels, from 10µL whole blood lysate, normalized to Gapdh.



For pricing and more information visit our website at www.panomics.com or call us at 1.877.726.6642.

QuantiGene 2.0 Ordering Information

The QuantiGene 2.0 Reagent System is comprised of 2 or 3 modules (each sold separately):

- QuantiGene 2.0 Assay Kit
- QuantiGene 2.0 Target-specific Probe Set(s)
- QuantiGene Sample Processing Kit (not required for purified RNA samples)

Contact your local representative about our evaluation kit available to new users.

Evaluation Kits enable new users to evaluate the QuantiGene 2.0 Reagent System's sensitivity, simple assay workflow and the assay's high precision, accuracy and robustness. The evaluation kit contains all necessary materials and reagents for 192 QuantiGene 2.0 assays (two, 96-well plates), including Probe Sets for a customer specified target and housekeeping gene.

Product	Size	Cat. #
QuantiGene 2.0 Evaluation Kits		
Animal Tissues	2 plates	QS0013
Purified RNA	2 plates	QS0014
Blood Samples	2 plates	QS0015
FFPE Samples	2 plates	QS0017
FFPE Method Proficiency Kit	2 plates	QS0016

Product	Size	Cat. #
QuantiGene 2.0 Assay Kits		
QuantiGene 2.0 Assay Kit	2-plate	QS0008
	10-plate	QS0009
	5 x 10-plate*	QS0010
	50-plate**	QS0011
QuantiGene 2.0 Probe Sets		
QuantiGene 2.0 Probe Set, Catalog	Multiple	Inquire
QuantiGene 2.0 Probe Sets, By Request	200 rxn	QS0050
QuantiGene 2.0 Probe Sets, By Request	1,000 rxn	QS0051
QuantiGene 2.0 Probe Sets, By Request	5,000 rxn	QS0052
QuantiGene Sample Processing Kits		
Cultured Cells	2-plates	QS0100
	10 plates	QS0101
	5 x 10 plates	QS0102
	50 plates	QS0103
Animal Tissues	10 samples	QS0104
	25 samples	QS0105
	100 samples	QS0106
Blood Samples	2 plates	QS0110
	10 plates	QS0111
	5 x10 plates	QS0112
FFPE Samples	10 samples	QS0107
	25 samples	QS0108
	100 samples	QS0109

* Configured for the processing of one or more plates/run.

** Configured for high-throughput users that prepare a minimum of 10 plates/run.

Panomics

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