Panomics

QuantiGene Plex

Setting the New Standard in mRNA Quantitation

Introduction

Identifying and assessing gene expression signatures allows researchers to identify targets and optimize lead compounds by tracking multiple genes associated with specificity, potency and toxicity. Gene expression profiling can also provide important information for tracking disease type and progression in biomarker validation studies. Now, there is a cost-effective way to generate high-confidence, high-quality, reproducible gene expression results through parallel analysis of multiple targets across large numbers of samples.

One well. Multiple assays. Performance you can count on.

QuantiGene® Plex offers high reproducibility and ease-of-use that make it the perfect assay to bridge the technology gap when studying many genes in a limited number of samples and studying a few genes in a large number of samples.

With QuantiGene Plex, researchers can easily perform multiplexed analyses from rare or volume-limited samples and can compare results across different samples, experiments and laboratories. Profiling many genes simultaneously in a single reaction directly from crude cell lysates or tissue homogenates, can be accomplished without the need for RNA purification, reverse transcription, or amplification.

Highlights

High Content—quantitate multiple mRNA targets simultaneously

Simple—no RNA purification, reverse transcription, or amplification

Accurate & Precise—detects percent differences in expression

High Throughput Detection—walkaway robotic sampling from 96-well plate

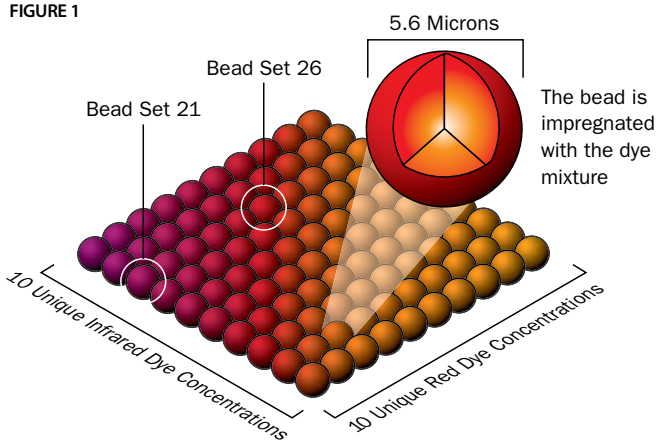
Flexible—use cell lysates or tissue homogenates

Robust—more reliable information from less sample

Efficient—sample sharing across multiple analytes is ideal for low volume or rare samples

Cost-Effective—saves time, labor, reagent, and compound costs

FIGURE 1



QuantiGene Plex Performance Highlights

Sensitivity
Single copy gene expression from a sample of 25,000 cells

Precision
Intra-plate CV <10%
Inter-plate CV <15%

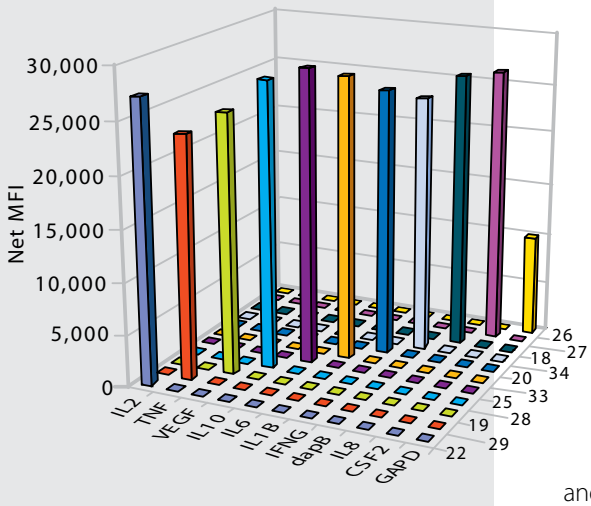
Accuracy
Cross-reactivity <0.2%
Cross-hybridization negligible

Linear Dynamic Range
Over 3 logs

Throughput
~15 seconds per well*

*Depends on experimental conditions

Specificity & Cross-reactivity



A cytokine panel was evaluated for cross-reactivity. When 40 amol of individual IVT RNA transcripts (represented by gene name in x-axis) was added into a mixture of 11 CP-conjugated beads, only the corresponding bead (represented by the number in y-axis) gives a strong fluorescent signal (z-axis). Net MFI is the median fluorescent intensity from 100 counted beads minus background.

Proven Technologies: xMAP + bDNA

Introducing QuantiGene Plex—the perfect marriage of two ground-breaking technologies—xMAP® (multi-analyte profiling beads) and bDNA (branched DNA signal amplification technology). Now, you can measure multiple mRNA targets simultaneously—directly from cell lysates or tissue homogenates—without the need for RNA purification, reverse transcription, or amplification!

And, you can count on the great accuracy, precision, and speed you've come to expect from the QuantiGene brand.

xMAP

The QuantiGene Plex system is based on proven technology—branched-DNA (bDNA) plus the xMAP system that combines flow cytometry, hard-dyed microspheres (beads), fluorescence, and digital signal processing. xMAP is made up of discrete microspheres each of which contains a unique spectral signature determined by the concentration of each of the internal red and infrared dyes (see Figure 1).

The xMAP detection system will identify and map each microsphere to a specific address or "zip code" based on its unique fluorescence signature—allowing multiple discrete microspheres to be combined into 1 assay, forming a "suspension array" of multiple analytes.

bDNA

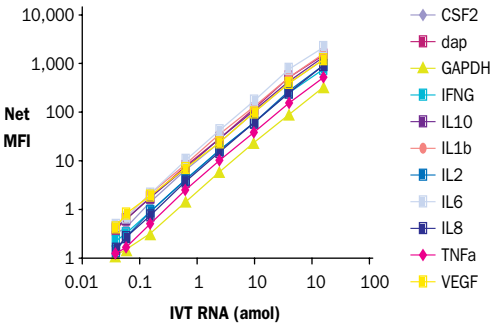
Branched DNA technology is a sandwich nucleic acid hybridization assay that provides a unique approach for RNA detection and quantification by amplifying the reporter signal rather than the sequence. By measuring RNA directly from crude cell lysates or tissue homogenates, the assay avoids variations or errors inherent to extraction and amplification of target sequences. Branched DNA technology is the basis of clinically proven vial load tests commercialized by Bayer Corporation and has been in practice for over a decade in drug discovery and development applications.

Principle of the Assay

The QuantiGene Plex system utilizes fluorescent microspheres as a support to capture the mRNA species. As in singleplex QuantiGene, each target-specific probe set contains Capture Extenders (CE), Label Extenders (LE), and Blockers (BL) that recognize a particular mRNA. The tails of the CEs discriminate between the different beads within the bead array while quantitatively capturing, via cooperative hybridization, the associated target mRNA.

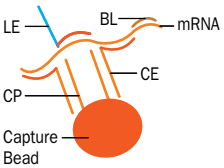
Signal amplification occurs when the bDNA molecule hybridizes to the Label Extender. The bDNA molecule contains hybridization sites for 45 biotinylated Label Probes. This is then visualized through the addition of Streptavidin-conjugated Phycoerythrin and the resulting fluorescence signal is proportional to the amount of mRNA captured on the bead surface by the target-specific probe set.

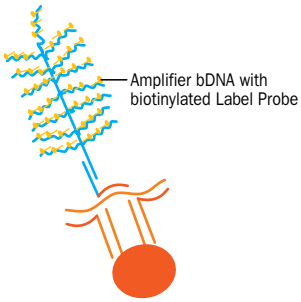
Sensitivity & Dynamic Range



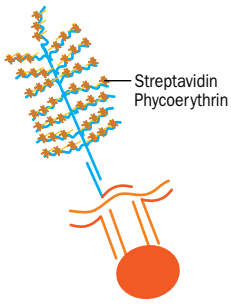
A cytokine panel was evaluated for assay sensitivity and dynamic range. An IVT dilution series of IVT RNA for all genes in the panel was assayed. All genes showed a linear response with average coefficients of correlation (R2) 0.99.

QuantiGene Plex Workflow

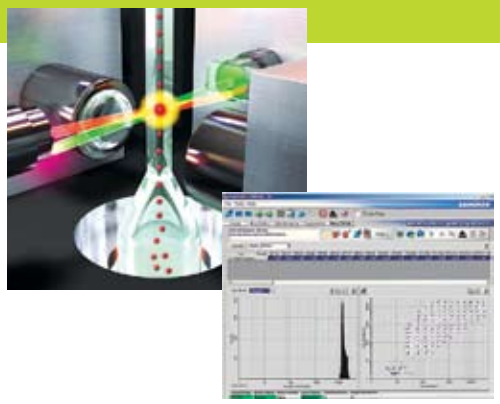




Illus 2a



Illus 2b



Step 1: Bead Capture

Specific mRNA transcripts are captured to their respective beads through Capture Extender (CE) Capture Probe (CP) interaction during an overnight hybridization at 53°C.

Step 2: Signal Amplification

a) Sequential hybridization of the bDNA amplification molecule and biotinylated Label Probe, respectively, for an hour at 46°C.

b) Binding with Streptavidin-conjugated Phycoerythrin (SA-PE) at room temperature for 30 minutes.

Step 3: Detection & Analysis

The sample is analyzed on a Luminex* instrument. The level of SA-PE fluorescence is proportional to the amount of mRNA transcripts captured by the bead.

*Bio-Plex suspension array system or other Luminex-based array systems.

Get QuantiGene Plex and begin to flex your plex

Select either fixed or mixed-to-order panels from our evolving QuantiGene Plex panel menu. To tailor panels to your specific research, Panomics will customize QuantiGene Plex panels that fulfill your unique gene expression profiling needs.

Check our website, www.panomics.com, for the most up-to-date listing of panels and available probe sets. Or contact your local representative to discuss how QuantiGene Plex can work for you.

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