

Total Solution for Quantitative Gene Expression Analysis & Protein Profiling –

Recent Advances & New Applications

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Panomics Solution

- 1. Gene Solution
 - > 1-plex (QuantiGene)
 - Multiplex (QuantiGene Plex)
 - ViewRNA (single cell, single copy RNA)
- 2. <u>Protein Solution</u>
 - Quantitation
 - Profiling



Problems in Real-time PCR

- 1. RNA extraction –RNA quality
- 2. RT,PCR somehow a random process
- 3. Sample limitation at least thousands of cells

QuantiGene totally different from Real-time PCR....



QuantiGene Assay: A "Single-Tube Solution"

Quantification Directly from the Source



NO Reverse Transcription!! NO PCR!!!



QuantiGene Assay: Starting Materials



QG 2.0 Assay Components



Technology – QuantiGene: Singleplex

Amplification calculation: amplifier no. (6,20) x label probe no. (4; 20) x tree no. (3; 6-8)



RNA Quantitation

QuantiGene 2.0 : 1 target gene / assay

incubator and Luminometer

QuantiGene Plex 2.0 : 2-36 target genes / assay

incubator and Luminex platform





QuantiGene Plex Assay

A Combination of bDNA technology and Luminex Platform

Beads-Based Technology

- Assay occurs on bead surface
- 5.6 µm bead diameter

HOW CAN MULTIPLEXING OCCUR?

→ BASED ON BEAD COLOR

- Each bead contains different concentration of RED and Infrared dyes
- Up to 100 uniquely-colored beads





Luminex xMAP Technology - Basic Assay Principle

5.6 Microns



The xMAP technology uses 5.6 micron polystyrene microspheres which are internally dyed with red and infrared fluorophores.

Using different intensities of the two dyes for different batches of microspheres, we have created 100 xMAPmicrosphere sets, each with a unique spectral signature determined by it's red/infrared dye mixture.

As each microsphere carries a unique signature, the xMAP detection system can identify to which set it belongs. Therefore, multiplexing up to 100 tests in a single reaction volume is possible.



The bead is now filled with a specific known ratio of the two dyes.



Beads Based Technology

100 color codes = 100 simultaneous tests



Luminex xMAP Technology - Basic Assay Principle



Many applications can be performed on the Luminex platform, including nucleic acid, immunological, receptor-ligand and enzyme assays.

Panomics currently provides both antibody-based and nucleic acidbased solutions.



Luminex xMAP Technology - Instrumentation



The Luminex platform is a robust flow cytometer based instrument, based on tried and trusted technology that is used in thousands of laboratories daily.

Our assays will work on any Luminex or Luminex based (e.g. Bio-Rad Bioplex) platform.

We have partnered with MiriaBio, a subsidiary of Hitachi, to provide a software solution.

It is also possible to fully automate the entire environment, and we can work with our customers to facilitate a complete walk-away solution.



Luminex xMAP Technology - Detection



As in any flow cytometer the stream of suspended particles (beads) is lined up in single file prior to passing through the detection chamber. This approach allows each particle to be measured as a discrete event.

Each particle is simultaneously exposed to a red (bead classifier) and green (reporter quantifier) laser which decodes both the signature of the individual bead and the code specific to the concentration of analyte associated with that bead.

QuantiGene Plex – Technology Overview

(Workflow Summary)



QuantiGene - Summary <u>Simple ... Accurate ... Precise ... Robust ... Flexible</u>

	QuantiGene	Real-time PCR
RNA extraction, RT, PCR	NO	YES
Sample Quantity required per assay	Single cell	Thousands of cells
Sample type	Normal samples and difficult samples (FFPE, Blood)	Normal sample
Specificity	Dozens of probes	Forward and reverse primers
Plex	QuantiGene – 1 gene QuantiGene Plex– 36 genes	1-5 genes
Precision and Accuracy	High	Low
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OVER 500 PUBLICATIONS



Data comparison between QuantiGene and qPCR



Gene expression in the spleen of LPS challenged mice.



AMGEN

•FDA Led Project with the participation of over 50 organizations addressing the concerns of microarray performance and analysis.

 Comprehensive study to compare expression data generated using a variety of microarray-based and alternative quantitative platforms, including

- Microarray: e.g. ABI, AFX, AGI, GEH, ILM
- Quantitative platform: e.g. QuantiGene, TaqMan, (sta)RT-PCR

 Unique opportunity to assess assay precision, accuracy and data compatibility across multiple platforms.

Papers published in Sept. 06 issue of Nature Biotechnology

Data publicly available for independent analysis. http://www.fda.gov/nctr/science/centers/toxicoinformatics/magc/



MAQC: Experimental Design

 Assaying four pools of two RNA samples repeatedly.

Four pools of RNA samples:

•A: Universal Human Reference RNA (UHRR) from Stratagene
•B: Human Brain Reference RNA (HBRR) from Ambion
•C: 75% A + 25% B
•D: 25% A + 75% B

Assessing performance metrics:

Precision – assay CV

Relative accuracy

- Predicted: C' = 75% A + 25% B
- Predicted: D' = 25% A + 75% B
- RA = (C C')/C' or (D D')/D'

Fold change correlation – Log₂ (A/B)



Relative accuracy score (RA) analyzed based on two titration samples C and D



MAQC: Experimental Design Difference



Technical Replicates on genes



Assay Precision

All 224 shared genes between QGN and TAQ



Assay Accuracy



Percent Difference between actual & predicted for sample C and D



Summary for MAQC

QuantiGene[®] outperforms qPCR in precision and accuracy





Procarta:

Cytokine, Chemokine, Growth Factor Assays

Luminex xMAP Technology - Basic Assay Principle



Peptides Oligonucleotides

Many applications can be performed on the Luminex platform, including nucleic acid, immunological, receptor-ligand and enzyme assays.

Panomics currently provides both antibody-based and nucleic acidbased solutions.



How it works



A 2.5-hour assay



Applications for Multiplexed Cytokine

- TH1/TH2 Profiling
- Inflammation
- Biomarker Discovery/Validation
- Cancer Marker Profiling
- Immune monitoring
- ADME/TOX





ELISA vs Procarta Luminex

Throughput





Overview of Procarta Cytokine Kits

- Optimized for sample type: Supernatant, serum, plasma and tissues
- Assay Buffer is matrixed matched for human, mouse, rat
- Excellent Standard/Spike recovery for all matrices.
- Easy to use- No sample dilutions needed, all 1X dilutions
- Low background with better assay range
- Create your own panel from current list of cytokines
- Limit of Detection <1 pg/ml Limit of Quantitation <10 pg/ml
- Certificate of Analysis available for every order
- Highly Competitive Price



47 Human Cytokine/Chemokine

Adiponectin	IL- 1RA	IL-15	PDGF-BB
BLC	IL- 2	IL-17A	RANTES
ENA-78	IL- 4	IL-17F	TGF-beta**
Eotaxin	IL- 5	IL-23	TNF-alpha
FGFbasic	IL- 6	IP-10	TNF-beta
GCSF	IL- 7	Leptin	VEGF
GM-CSF	IL- 8	MCP-1	CRP**
GRO-alpha	IL-10	MCP-3	
IFN-g	IL-12p40	MIP-1a	
IL-1-alpha	IL-12p70	MIP-1b	
IL-1-betea	IL-13	NGF	

Specifications LOD <1 pg/ml LOQ < 10 pg/ml Dynamic Range > 2 logs Mix and Match up to 30

MMP-1 MMP-2 MMP-3 MMP-9 MMP-13

Her-2	
Beta-actin	



28 Mouse Cytokines/Chemokine

IFN-alpha

IFN-beta

Eotaxin	IL-5	IP-10
G-CSF	IL- 6	КС
GM-CSF	IL- 9	MCP-1
IFNgamma	IL-10	MCP-3
IL- 1-alpha	IL-12p40	MIP-1alpha
IL- 1-beta	IL-12p70	RANTES
IL- 2	IL-13	TNFalpha
IL- 3	IL-17	VEGF
IL- 4	IL-23	

Specifications LOD <1 pg/ml LOQ < 10 pg/ml Dynamic Range > 2 logs Mix and Match up to 25

*IFN-alpha and IFN-beta is offered as a two plex

Panomics

22 Rat Cytokines

Eotaxin	IL- 1alpha	КС	RANTES
G-CSF	IL- 1-beta	MCP-1	TGF-beta**
GM-CSF	IL- 4	MCP-3	TNF-alpha
ICAM	IL- 6	MIP-1alpha	VCAM
IFN-gamma	IL-10	NGF	VEGF
IL- 1alpha	IL-12(p40)	RANTES	CRP

Specifications LOD <1 pg/ml LOQ < 10 pg/ml Dynamic Range > 2 logs Mix and Match up to 25



Other Analytes

20 Non Human Primates

6 Porcine

Granzyme B	IL-10	INF gamma	Rantes
IL- 1 beta	IL-12 (p70)	IP10	TNF alpha
IL- 2	IL-12 (p40)	MCP-1	
IL- 4	IL-13	MCP-3	
IL- 5	IL-17A	MIP-1 alpha	
IL- 6	IL-23	MIP-1 beta	

IL-1-beta
IL-6
IL-8
IL-10
TNF-alpha



Work Flow Outline





Procarta Human Cytokine Kit Configuration



Cytokine Assay format - 1 PLATE SIZE

 \star Sample buffer and standard buffer are required <u>only</u> for serum or plasma samples.

- $\sqrt{}$ Reagents supplied at 1x ready to use concentration
- $\sqrt{}$ Also offered in 10 plates size
- ✓ Optional High and Low Controls



Human Cytokines: IL-1 Beta Standard Recovery

Serum

Media

Obs Conc	Exp Conc	(Obs/Exp) x 100
29754	25000	119
4534	5000	91
1032	1000	103
202	200	101
38.96	40	97
8.05	8	101
1.63	1.6	102

Obs Conc	Exp Conc	(Obs/Exp) x 100
29636	25000	119
4867	5000	97
990	1000	99
216	200	108
38.42	40	96
6.94	8	87
2.01	1.6	126

Plasma

Obs Conc	Exp Conc	(Obs/Exp) x 100
31546	25000	126
4748	5000	95
987	1000	99
231	200	116
34.8	40	87
7.47	8	93
2.04	1.6	127

% of Standard Recovery meets specs: 80% - 120% Similar recoveries for Mouse and Rat Cytokines



Comparison of ELISA and Procarta Assays





Comparison using Lysate Samples



TNF-alpha Luminex Assay



IL-4 Luminex Assay



Procarta Transcription Factor Plex Assay



Gene Expression Regulated by Multiple TFs



Why TF Plex





Diagram of TF Plex Assay- Part 1



Procedures

- Mix nuclear extracts and cell extracts with a library of biotin-labeling ciselement probes (TF binding sequences)
- 2) Separate Protein/DNA complexes from free probes with a 96 well separation plate
- (3) Denaturing Protein DNA complex will release the ds oligos and are captured in a collection plate



Diagram of TF Plex Assay- Part 2



Key Benefits

- Panel 1= 40 most popular TFs, Panel 2= 43 more TF's
- Easy separation of TF/Probe complexes from free probes with 96 well filter plates
- Most sensitive TF assay
- high throughput assay for measuring both multiple samples and multiple targets simultaneously
- Simple procedure
- Cost effective



Panel 1: 40 Most Popular TF Targets

RUNX/AML	E2F1	ISRE	PPAR
AP1	ELK1	MEF-2	PAX3
AP2	ER	MYOD	SMAD
AR	ETS/PEA	NF-1	STAT1
ATF2	FKHR-1	NFATc	STAT3
NF-Y	GATA-1	NF-E1(YY1)	STAT4
CEBP	GR/PR	NF-E2	STAT5
FAST1	HIF-1	NFKB1	NKX-2.5
C-MYB	HNF1	ОСТ	PAX5
CREB	IRF1	P53	BRN3



Panel 2: 43 More TF Targets

AP3	HiNF	Pit1
AP4	HNF-3	Pur-1
CAR	KPF1	PXR
CDP	LEF1	PYR
CEF1	LF-A1	RB
CEF2	LyF	RXR
E12	MTF	SRY
E47	NPAS2	TCF/LEF
Elf-1	NRF-1	TFE3
EVI-1	Pax4	TREF1, 2
Gfi-1	Pax6	TTF1
GKLF	Pax8	USF
H4TF	Pbx1	WT1
HFH-1		



Summary

Transcription Factor Assays

- Increased throughput for TF Profiling vs EMSA
- Create your own plex set two panels of TFs (83 TFs total)
- Custom Assay development available

Additional Multiplexed Assays

- Gene Expression
- Cytokine Assays
- SH2 Protein Binding Domains





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