ApplicationGuide

affymetrix eBioscience

QuantiGene® Plex Assay

Accurate and Precise Quantitation – Measure from 3 to 80 genes in a single well

Overview of QuantiGene® Plex Assay

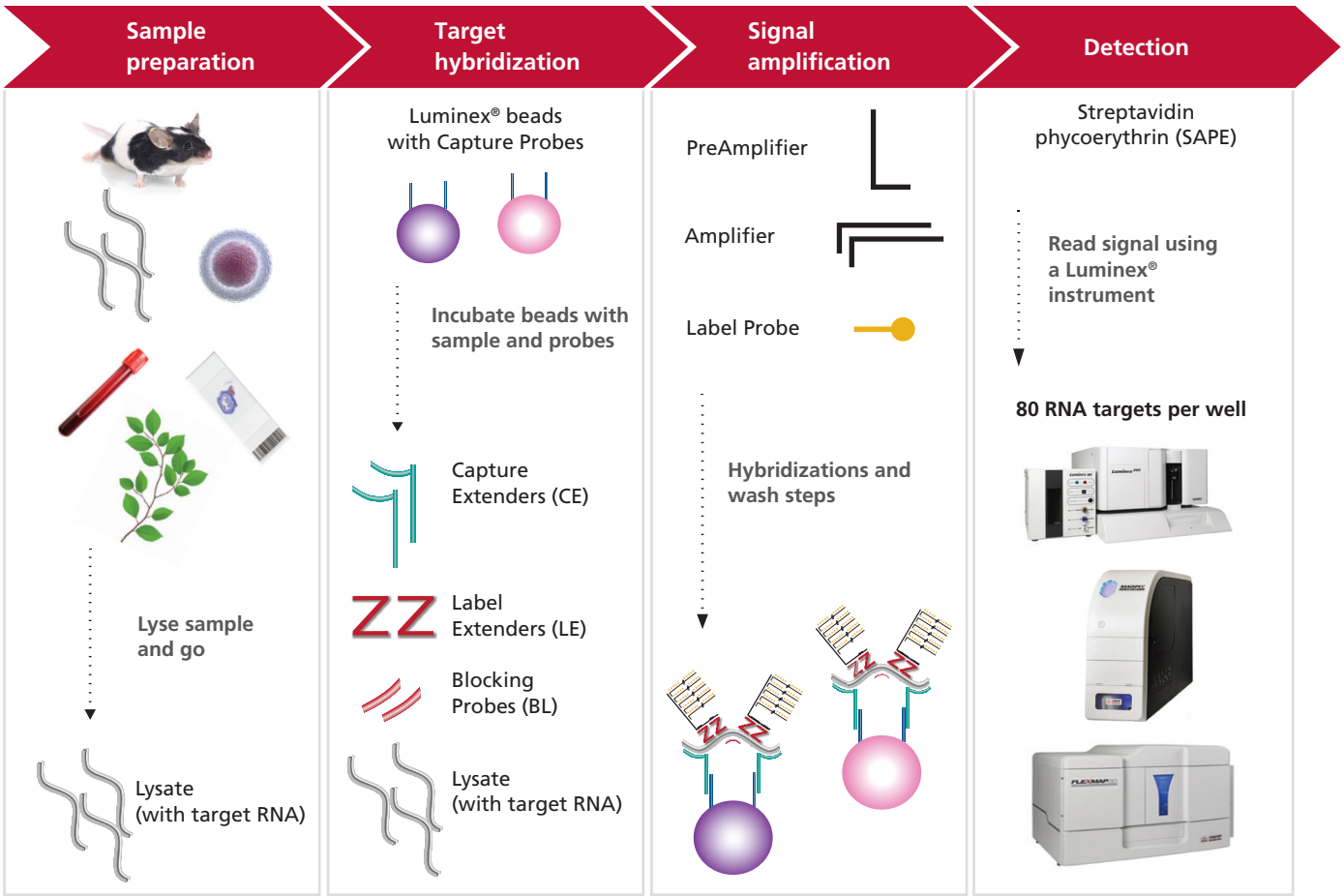
QuantiGene Plex Assay is the most accurate and precise assay for multiplexed gene expression quantitation. Using the Luminex® xMAP® technology, QuantiGene Plex Assays allow the simultaneous measurement of 3 to 80 mRNA species in every well of a 96-well plate. QuantiGene Plex Assay incorporates the exclusively licensed and clinically proven branched DNA technology from Siemens that is used in the Versant® viral load assays for HIV, HBV, and HCV. Branched DNA assays allow for the direct measurement of RNA transcripts by using signal amplification rather than template amplification.

The assay is simple and easy to use; QuantiGene Plex Assay does not require RNA extraction, cDNA synthesis, or PCR amplification. The QuantiGene Plex Assay is ideal to validate GeneChip® or next-generation sequencing data and to validate biomarkers for translational research.

Key features and benefits of the assay

- **True multiplexing** – Measure up to 80 genes of interest and housekeeping genes in the same well with no cross-reactivity
- **Standardized platform** – 96-well plate format compatible with Luminex 100, 200, MagPix® and FlexMap3D® systems
- **Simple workflow** – ELISA-like workflow for direct hybridization of transcripts to beads and transcript labeling
- **No PCR** – No RNA purification, no reverse transcription, no PCR amplification and none of the associated artifacts
- **Works with difficult sample types** – Works with degraded and cross-linked RNA in FFPE tissues and directly with blood
- **Large inventory of validated genes** – Over 15,000 genes can be mixed to create pathway- and disease-themed panels
- **Fast customization** – If we don't have your gene(s), we can create your custom panel within 2 weeks
- **ISH compatible** – Same technology utilized in QuantiGene ViewRNA Kits for mRNA *in situ* hybridization

How it works



QuantiGene® Plex applications

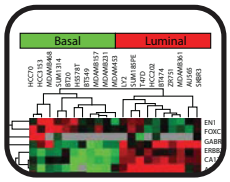
QuantiGene® Plex Assay for mRNA profiling and DNA copy number determination is ideal to support drug discovery and development efforts as well as clinical and translational research. The examples that follow highlight the capabilities and benefits of this powerful research tool.

Target discovery
Target and microarray validation cell-based assays

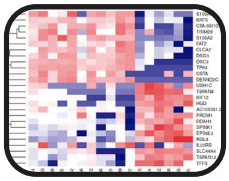
Lead optimization
High-throughput screening
Secondary screening

Preclinical
ADME
Toxicology

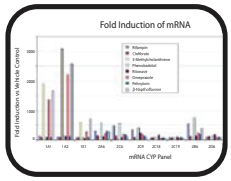
Clinical
Biomarker validation
Clinical trials (FFPE tissues)



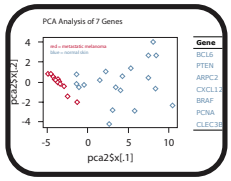
Tumor cell line characterization for drug compound screening



FFPE analysis of genes involved with cancer



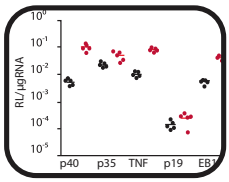
CYP genes used with human hepatocytes



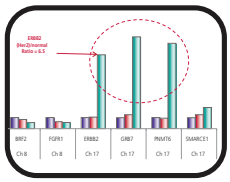
PCA analysis of genes involved with melanoma

Sample / Gene	Mouse Gene A
Background (no RNA)	3
Human Liver RNA	4
Mouse Spleen	3,989
Mouse Liver	392
Xenograft Spleen	3,321
Xenograft Liver	2,959

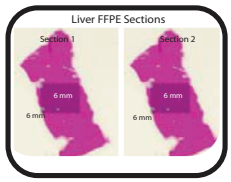
Patient Derived Xenograft (PDX) models



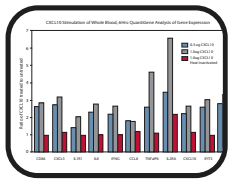
Validation of genes involved with inflammation



DNA copy number for breakpoint analysis



Validation of genes in FFPE samples



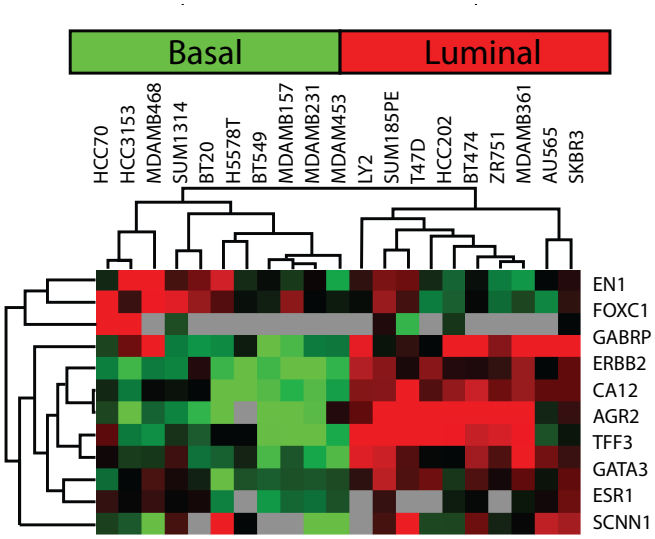
Expression analysis from whole human blood

Application 1: Patient Derived Xenograft (PDX) model

A 10-plex QuantiGene Plex panel was developed to simultaneously measure the expression of human and mouse genes in a PDX model. The panel comprised 3 human genes of interest and 2 human housekeeping genes as well as 3 mouse genes of interest and 2 mouse housekeeping genes. Patient derived tumors were implanted into JAX NSG (NOD SCID gamma) mice. After growth and metastasis of the tumor in the mice, both the liver and spleen from the mice were harvested and lysates from untreated and from xenografted mice were tested alongside human liver RNA. The table shows the mean fluorescence intensity values for the 6 genes of interest that were obtained with a Luminex instrument. The data demonstrated assay specificity: human samples were negative for mouse transcripts while untreated mice were negative for human transcripts. As expected, tissues from xenografted mice contained both human and murine transcripts.

Benefit: The specificity of each component assay within a customized multiplexed QuantiGene Plex panel allows the assay to be tailored to address complex biological questions.

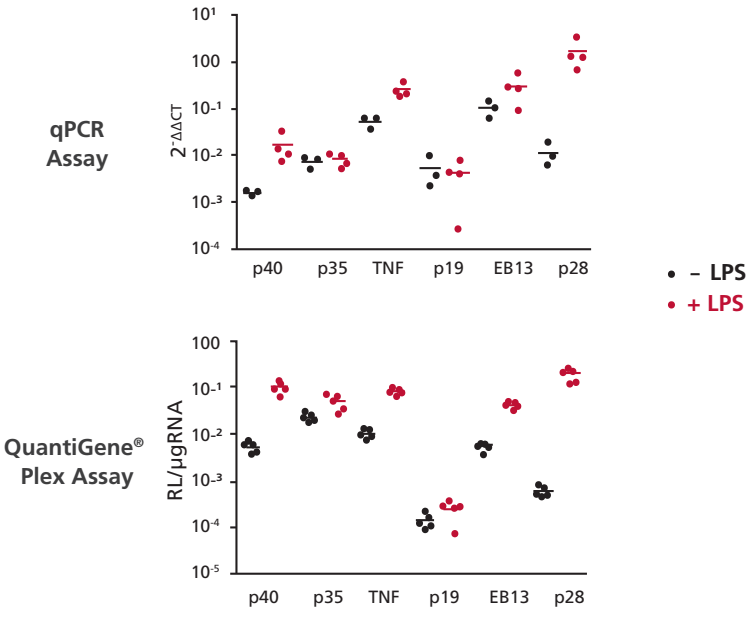
Sample/gene	Mouse gene A	Human gene 1	Mouse gene B	Human gene 2	Mouse gene C	Human gene 3
Background (no RNA)	3	3	7	8	7	7
Human liver RNA	4	24,788	9	24,882	3	1,084
Mouse spleen	3,989	19	7,655	1	18,023	4
Mouse liver	392	4	1,488	2	26,586	2
Xenograft spleen	3,321	27,642	472	24,605	24,563	6,547
Xenograft liver	2,959	21,790	2,114	7,058	27,826	1,702



Application 2: Cell line characterization for compound screening

Breast cancer cell lines were created from primary tumors that contained recurrent genetic abnormalities. A Quantigene® 12-plex Assay was developed to differentiate patterns of RNA expression across the cell lines. When the normalized levels of gene expression were plotted on a heat map, the data showed that the pattern of expression for the 12 genes could be used to classify the cell lines as either the basal or the luminal subtype of breast cancer. The cell lines were then used for further screening of drug compounds based on the specific subtypes. Data courtesy of Joe Gray and Nick Wang, Oregon Health Sciences University.

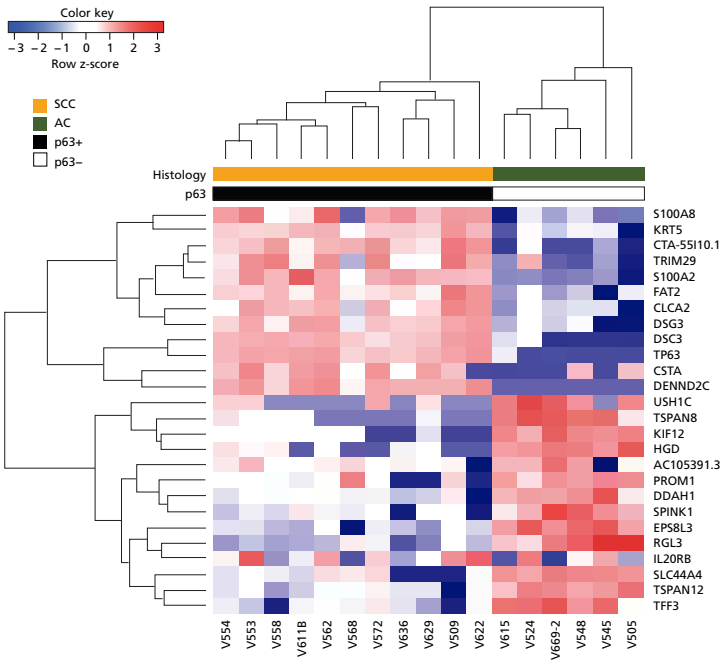
Benefit: Multiplexing allows the rapid classification of cell lines that can then be used for compound screening.



Application 3: Target validation

Gene expression signatures for inflammation pathway activation were identified using Affymetrix GeneChip® Arrays. The signatures were further validated by testing samples from LPS-treated mice with QuantiGene Plex and qPCR Assays. The spleens from 52 treated and untreated mice were harvested to prepare tissue lysates for the QuantiGene Plex Assay or to purify RNA for qPCR testing. Both QuantiGene Plex and qPCR Assays showed similar patterns of up- and down-regulation upon treatment. However, the QuantiGene Plex Assay provided better accuracy and precision than did qPCR. In this study, 14 qPCR plates had to be processed to provide as much information as one QuantiGene Plex plate. Data courtesy of Amgen, Inc[1].

Benefit: By testing simple lysates, QuantiGene Plex Assays avoid the expense and errors associated with the time-consuming steps of RNA purification and reverse transcription that qPCR requires. QuantiGene Plex Assays are robust, accurate, and precise. Multiplexing increases efficiency and conserves sample.



Application 4: Validation of GeneChip® signatures from FFPE

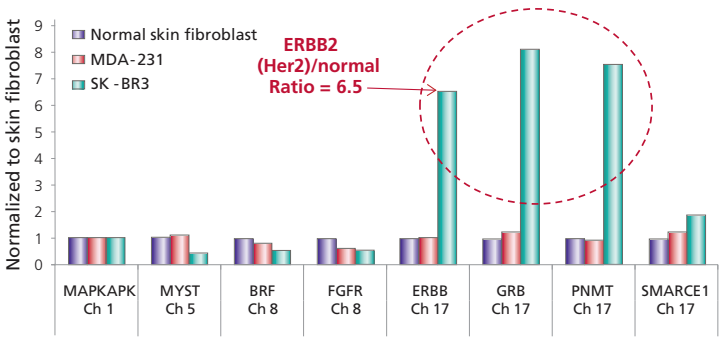
Using GeneChip® Human Exon 1.0 ST Arrays, 19 cervical squamous cell carcinoma (SCC) and 9 adenocarcinoma (AC) FFPE samples were screened to find a gene signature that would differentiate SCC from AC. The FFPE blocks had been stored an average of 12 (range 10–16) years. After analysis of the exon array data, a QuantiGene® 26-plex panel was constructed and successfully validated against the 19 cervical SCC and 9 cervical AC FFPE samples. Reprinted by permission from Macmillan Publishers Ltd on behalf of Cancer Research UK: Hall, *et al. British Journal of Cancer* **104**:971-981 (2011).

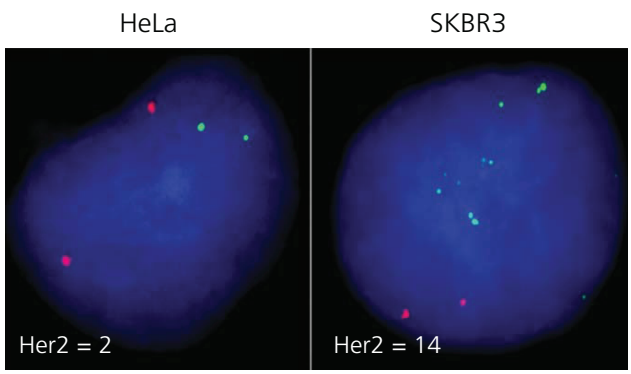
Benefit: Formalin has been known to degrade and chemically modify RNA, which can be an issue for assays that use DNA polymerases as part of their template amplification step. QuantiGene® Plex Assays have no issues with FFPE samples because the assay is based on hybridization. The assay works directly with FFPE tissues without the need for RNA purification or amplification.

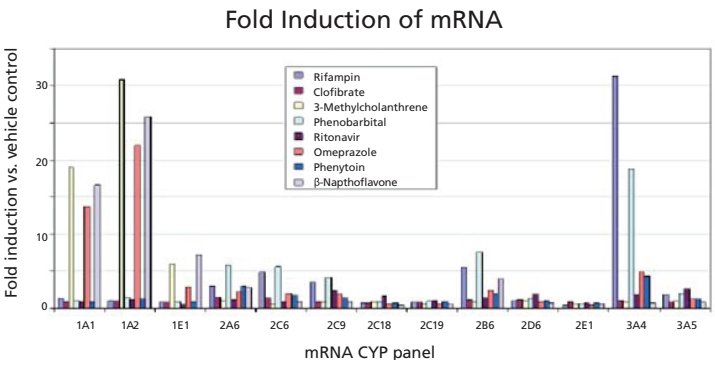
Application 5: DNA breakpoint analysis

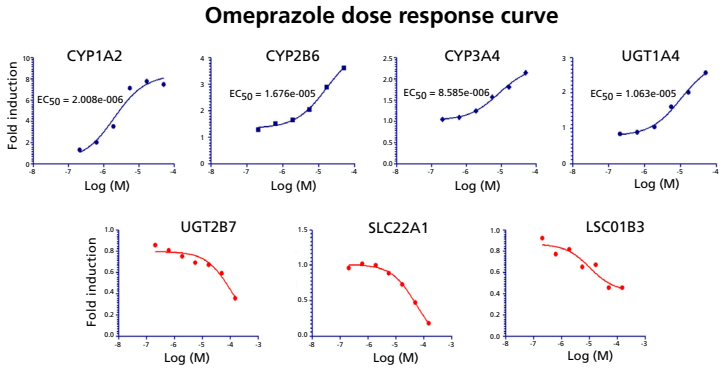
The QuantiGene Plex DNA Copy Number Assay was used to detect DNA breakpoints for Her2 and adjacent genes on chromosome 17 as well as control genes on chromosomes 1, 5, and 8 in SKBR3 breast cancer cells and control cell lines (normal skin fibroblasts and MDA-231 cells). As expected, a 7-fold amplification (6.5 normalized ratio) of Her2 was detected. The QuantiGene Plex result was concordant with that of a bDNA FISH assay (note: the DNA assay is not currently commercially available), in which 14 copies can be counted in SKBR3 cells vs. 2 copies in the HeLa cells as expected, again showing a 7-fold amplification. Amplification of PNMT6 and GRB7, 2 genes adjacent to Her2, were also detected at 7- to 8-fold, whereas control genes were detected at the expected single copy number.

Benefit: The QuantiGene Plex DNA Assay has the ability to interrogate single copy DNA changes across a given chromosome to further analyze DNA breakpoint changes. The assay has the ability to measure up to 33 regions per well of a 96-well plate.









Application 6: ADME/toxicology screening

Transcripts for 13 different cytochrome p450 genes were measured in toxicant-treated human hepatocytes at a single dose. Cryopreserved human donor hepatocytes were cultured for 2 days followed by treatment with 1 of the 8 toxicants or vehicle control. After 48 hours, the cells were lysed using the QuantiGene® Plex lysis reagent and the lysate was used directly in the QuantiGene® Plex Assay. The investigators also found that there was a strong correlation between the levels of mRNA expression and the levels of enzyme activity of the encoded proteins. Data courtesy of Genentech[2].

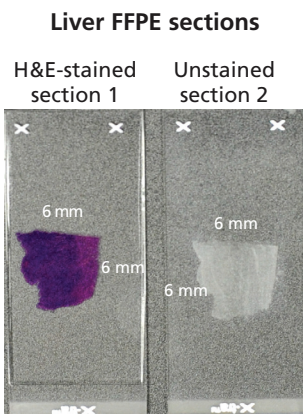
Cryopreserved hepatocytes from a single donor were treated with Omeprazole and dosed between 150 and 0.21 µM. A QuantiGene 18-plex panel consisting of CYP and transporter genes were used to investigate mRNA response. The EC_{50} calculations and the dose response curves were prepared for the selected CYP and transporter genes. Data courtesy of Celsis IVT[3].

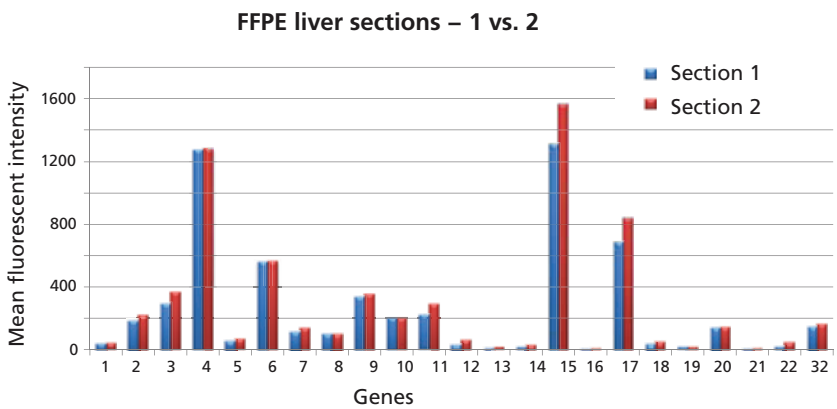
Benefit: The protocol for the QuantiGene Plex Assay is invariant regardless of the panel of genes that is measured. Unlike enzyme assays, no further optimization is required when going from 1 panel to the next. The specificity of QuantiGene Plex Assays allows closely related genes to be measured simultaneously in the same well.

Application 7: H&E- and non-H&E stained FFPE slides

Gene expression analysis was performed on H&E-stained and non-stained formalin-fixed paraffin-embedded (FFPE) tissue sections. Two slides were obtained and a 6 mm x 6 mm portion of tissue (5 µm thick) was removed from each slide and homogenized in the QuantiGene lysis buffer. A 23-plex gene panel consisting of 20 target genes and 3 housekeeping genes was used with the 2 samples. Comparable expression was observed in both sections for all 23 genes with an overall CV of 8.6%.

Benefit: The assay allows the use of H&E-stained slides. In this example, a 6 mm x 6 mm size section of tissue provides users with the ability to test small samples in previously stained H&E-stained slides. Areas of interest identified in H&E-stained FFPE sections are viable samples for subsequent expression analysis.





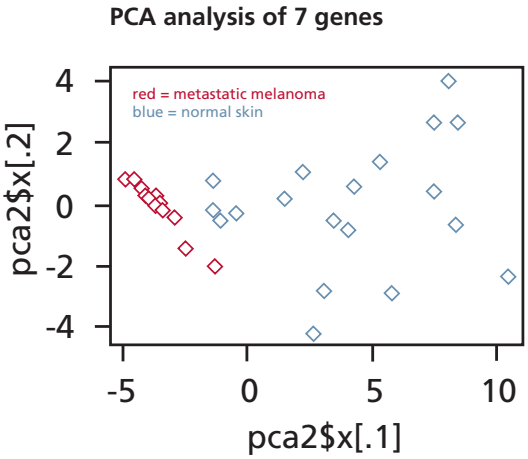
Application 8: Classification of melanoma subgroups using QuantiGene® Plex gene signatures

62 genes relevant to melanoma were incorporated in a QuantiGene® Plex Assay as a proof of concept to demonstrate the capability to discover biomarkers. Tissue lysates were prepared from frozen sections from 20 cases of metastatic melanoma alongside the corresponding normal skin counterparts. Data analysis identified those genes most closely linked with the disease.

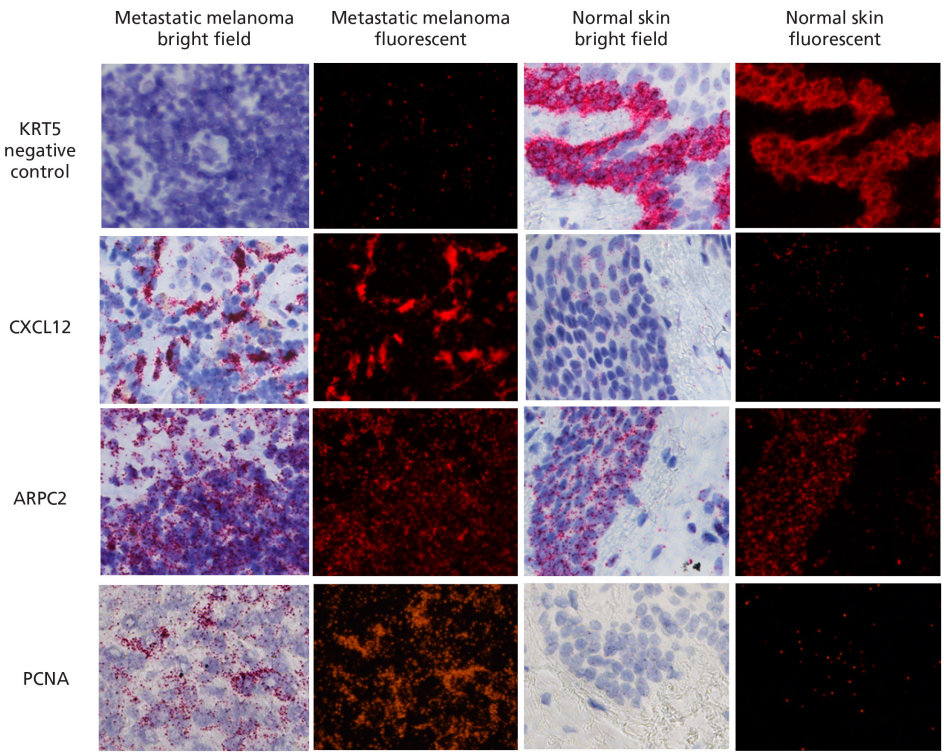
Gene expression measurements were made against the 62 genes, and each gene of interest was normalized by the geometric mean of 5 housekeeping genes. The p-value for each gene was calculated using a supervised Student T-test. The genes with the best 7 p-values were analyzed using an unsupervised Principle Component Analysis (PCA).

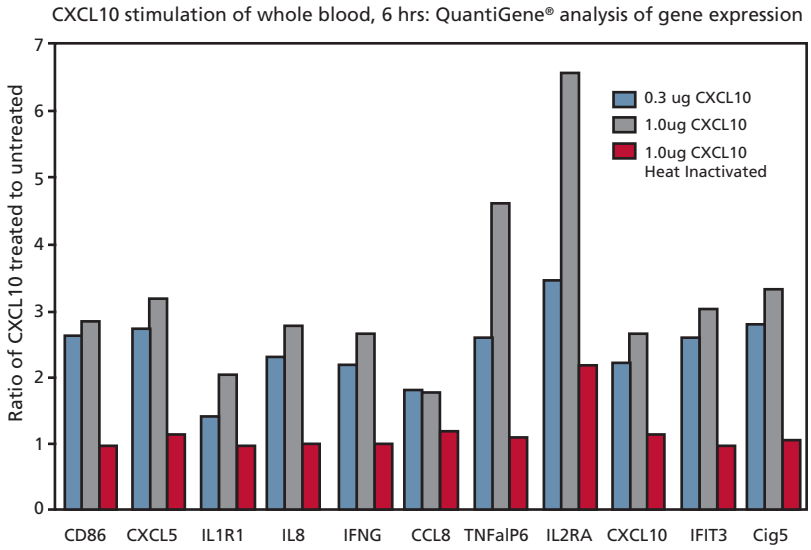
In situ gene analysis of these genes was performed using the QuantiGene ViewRNA® analysis. Metastatic and normal skin sections were probed for the gene transcripts of KRT5, CXCL12, ARPC2, and PCNA. The dye used in the assay is colorimetric but can also be viewed under fluorescence. Data courtesy of California Pacific Medical Center/UCSF[4].

Benefit: Ability to work directly with melanoma tissues without RNA purification or concerns about melanin inhibition of DNA polymerase.



Gene	P value
BCL6	1.28E -09
PTEN	1.03E -08
ARPC2	2.17E -08
CXCL12	1.37E -07
BRAF	4.18E -07
PCNA	6.33E -07
CLEC3B	1.13E -06





Application 9: Stimulation of whole human blood

CXCL10-responsive gene induction was confirmed using a 12-plex QuantiGene® Plex panel to test RNA isolated from healthy donor blood treated *ex vivo* with CXCL10. Gene induction was shown to be dose dependent and was eliminated upon heat inactivation of the chemokine. Gene expression was normalized to the expression level of the GAPDH housekeeping gene and presented as the ratio of treated/untreated. Data courtesy of Medarex Inc, a BMS Company[5].

Benefit: The QuantiGene® Plex Assay works directly with whole blood without the need for globin depletion or RNA purification.

1. Ebsworth K. Gene expression analysis by Quantigene Plex: validation and applications. Paper presented at: Planet xMAP USA; 2007 March 12-14; Laguna Hills, CA.
2. Wong S., *et al*. New methodology for measuring mRNA levels along with monitoring CYP activity in induction studies using human plateable cryopreserved hepatocytes. Paper presented at: The 8th International Society for the Study of Xenobiotics; 2007 Oct 9-12; Sendai, Japan.
3. Moeller, T., *et al.* Celsis IVT – Transcript regulation of 18 ADME genes by prototypical inducers in human hepatocytes. Paper presented at: The 18th International Society for the Study of Xenobiotics. 18th North American Regional Meeting; 2012 Oct 14-18; Dallas, TX.
4. Leong S. P., *et al.* Manuscript is in preparation. Paper presented at: The 4th International Symposium on Cancer Metastasis and the Lymphovascular System: Basis for Rational Therapy; 2011 May 12-14; New York, NY.
5. Witte A., *et al.* Medarex – CXCL10 expression and biological activities in inflammatory bowel disease. Paper presented at: The Digestive Disease Week Meeting; 2008 May 19-21; San Diego, CA.

Specifications

QuantiGene Plex Assay can be used for both mRNA profiling and DNA copy number variation.

Limit of detection	≤1,000–2,000 transcripts/assay well
Limit of quantitation	≤2,000–4,000 transcripts/assay well
Linear dynamic range	≥3.0 logs
Assay CV	≤15% intra-assay; ≤20% inter-assay
Compatible sample types	Cultured cells, bacteria, whole bood, PAXgene, Tempus, dried blood spots, fresh/frozen tissues (animal or plant), FFPE samples, purified RNA
Assay format	96- or 384-well plate
Targets/well	3–80 for RNA; 3–33 for DNA

Off the shelf panels

Affymetrix has also developed over 1,600 panels that cover over 15,000 genes. These available panels cover a wide range of host species that include: human, mouse, rat, canine, monkey, porcine, soy, maize and many others. Any combination of existing genes can be combined to create a new panel. To find the latest panels and genes, please go to www.panomics.com or contact one of our probe designers at probes@affymetrix.com.

Custom panels

Custom Plex Sets are available for any gene and any species. Custom panels ranging from 3- to 80-plex can be designed and shipped within 2 weeks. Customers simply provide Affymetrix with a gene list, RefSeq IDs, or DNA sequence.

Available human 80-plex panels

Apoptosis
Cancer
Cytokine
Inflammation
Angiogenesis
Breast Cancer-ER
Cell cycle
Immunology
Jak-Stat
Kinase
p53
Stem cell
Toxicity
Wnt signalling
Drug metabolism

Available mouse 80-plex panels

Angiogenesis
Apoptosis
Cell cycle
Immunology
Jak-Stat
Kinase
Stem cell
Cytokine
Kinase
Drug metabolism

Below are some examples of the genes included in the 80 plex panels

The list of our 80-plex panels as well as the rest of our 1,600 panels can be found on our website at www.panomics.com.

Human Apoptosis 80-plex panel

CASP6	CARD8	BNIP2	DAPK1	BAG3	HPRT1	TNFRSF10A	BID	TNFRSF7	LTBR	TNFRSF9	BIRC8	CASP3	TP53	BIRC3	TNFRSF1A
PYCARD	AKT1	BRAF	TP53BP2	BAD	BCL2L1	TP73	GADD45A	FADD	TNFRSF21	BCL2L2	NOL3	FASLG	CASP8	PPIB	CASP4
CASP1	CASP2	BCLAF1	ABL1	TNF	TNFSF10	TNFRSF11B	APAF1	TNFSF8	CASP14	IGF1R	BCL10	LTA	TNFRSF10B	CASP9	BCL2L11
TRAF4	BFAR	MCL1	BIRC6	TNFSF5	BAG1	BCL2A1	BAK1	CIDEB	TRAF2	TRAF3	CFLAR	CASP10	CD40	BNIP3L	RIPK2
CASP7	BAG4	BIRC1	BCL2L10	TRADD	BNIP1	TNFRSF25	BAX	BNIP3	BIRC2	CARD4	CARD6	CIDEA	FAS	BCL2	BIK

Human Cancer 80-plex panel

NUSAP1	ERBB3	WNT2	SPARC	RAB17	PRAME	GJB1	SLC7A1	MCAM	RNF157	ACSL3	TRPM4	CACNA1D	TOP2A	BRAF	SPARCL1
MYLK	BIRC5	ARPC2	CDKN1B	POU2F3	CCNE1	FN1	E2F1	ZNF577	PHIP	HIF1A	KIF20A	MAGEA1	C10orf137	HPRT1	BUB1
CASP8	KLK3	CDKN2B	CCND1	RB1	CDH2	YWHAZ	MCM6	DSC1	MCM4	MITF	VCAN	SPP1	MKI67	MYRIP	PCSK6
TP53	NCOA3	PCNA	TFRC	F10	ITGB1	CCR7	PGK1	PTEN	ITGB3	MMP10	MMP9	GSTP1	MMP2	MLANA	BCL2A1
RAD54B	MET	GCNT1	AQP3	MCM10	WNT5A	DCT	TBP	GPC3	CDKN2A	PRKCA	ITGB4	CRISP3	CCNA1	GUSB	PLAUR

Human Cytokine 80-plex panel

IL1F6	IL1F7	IFNA5	IFNA8	IL1F8	IFNB1	IL19	CD70	IL3	IL1F10	IL17E	FAM3B	NODAL	TNFSF13	GDF3	TNFSF4
BMP4	INHBA	TNFSF12	BMP2	TGFB3	CSF1	BMP1	BMP6	GDF2	GDF5	GDF9	BMP7	TXLNA	BMP3	TNFSF13B	IL16
TNFSF14	IL1F5	GDF10	BMP8B	IL24	IL9	TNF	IL12A	IL22	IL13	IL17A	TNFSF10	TNFSF11	TGFB1	IL7	TGFA
PDGFA	IL12B	TGFB2	TNFRSF11B	IL10	BMP5	IL5	GAPDH	IL18	GDF11	IFNA2	IL2	CSF2	IL20	FASLG	IL1F9
IFNA1	IL4	IFNG	LTA	IL6	FIGF	ACTB	IL1B	IL1A	GDF8	IL15	LTB	IFNK	PPIB	IL8	IL21

Human Inflammation 80-plex panel

PYCARD	MAPK3	SUGT1	MAP3K7IP1	MAP3K7	MEFV	MAPK12	PSTPIP1	MAPK13	TNFSF14	PEA15	RIPK2	PANX1	NLRX1	NALP1	NLRP9
TNFSF4	P2RX7	NLRP4	MAP3K7IP2	BIRC1	NLRC5	BIRC4	NLRP3	NLRP5	AIM2	BIRC2	CARD6	CASP1	CASP5	CHUK	CIITA
CTSB	HSP90AA1	TRA1	IKBKB	IKBKG	IRF2	MAPK9	NFKB1	MAPK8	NFKBIA	RAGE	TNF	TNFSF5	IL12A	BCL2	TNFSF11
ICEBERG	CASP4	IRF1	IL12B	MAPK1	GAPDH	CXCL1	CASP8	TRAF6	CXCL2	CCL5	CCL7	RELA	PTGS2	IFNG	IL6
TIRAP	IRAK1	ACTB	IL1B	TXNIP	MYD88	CARD12	BIRC3	CCL2	PPIB	IFNB1	NOD2	IL18	BCL2L1	HSPCB	NALP12

eBioscience (US) Tel: +1-888-999-1371 ▪ Tel: +1-858-642-2058 ▪ eBioscience (EU) Tel: +43 1 796 40 40 305 ▪ info@ebioscience.com
Affymetrix, Inc. Tel: +1-888-362-2447 ▪ Affymetrix UK Ltd. Tel: +44-(0)1628-552550 ▪ Affymetrix Japan K.K. Tel: +81-(0)3-6430-4020
Panomics Solutions Tel: +1-877-726-6642 panomics.affymetrix.com ▪ USB Products Tel: +1-800-321-9322 usb.affymetrix.com

www.ebioscience.com Please visit our website for international distributor contact information.

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

P/N QG2037 Rev. 3