Thermo Fisher SCIENTIFIC

Gene expression analysis

QuantiGene assays for analysis of gene expression utilizing branched DNA (bDNA) technology

Quantitate up to 80 genes in a single well directly from a variety of sample types, including blood, cell lysates, and tissue homogenates, including FFPE

invitrogen

QuantiGene Singleplex Assays

Accurate and precise RNA quantitation in a simple workflow

QuantiGene Singleplex Assays

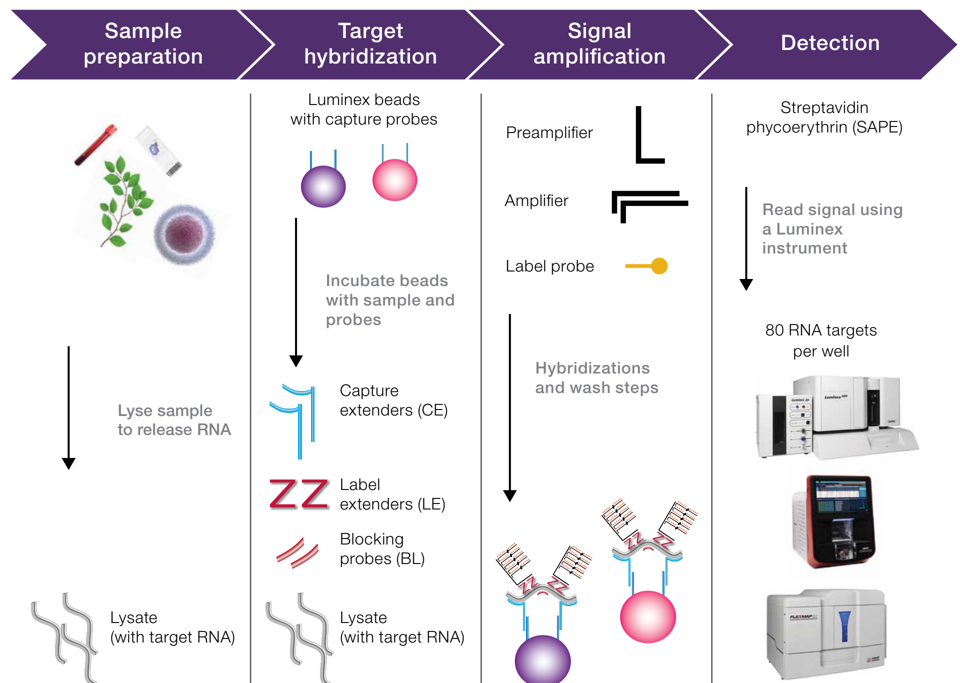
By quantitating RNA directly from your starting sample and using a signal amplification assay, Invitrogen™ QuantiGene™ Singleplex Assays are an accurate and precise method of quantitating gene expression with a simple workflow.

Technical overview and workflow

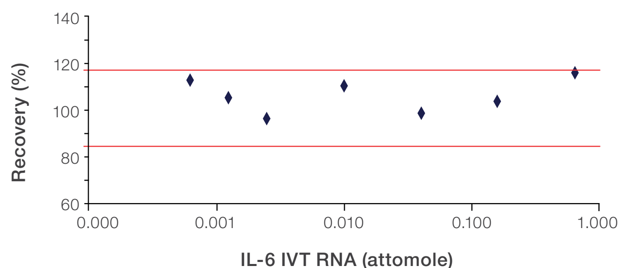
QuantiGene assays utilize branched DNA (bDNA) technology that has been used for decades in the VERSANT™ 3.0 diagnostic assays for HIV-1, HCV, and HBV. First, samples are homogenized to release the target RNA. Second, the oligonucleotide probe set is incubated with the target RNA and capture plate overnight. During this incubation, the probes cooperatively hybridize to the target and capture probes bound to the plate, capturing the target RNA. Third, signal amplification is performed by sequential hybridization of the bDNA preamplifier, amplifier, and label-probe molecules to the target. Addition of a chemiluminescent substrate generates a luminescence signal directly proportional to the amount of target RNA present in the sample.

Key benefits

- **Fast sample prep**—perform the assay directly on sample homogenates or lysates; no need for RNA purification
- **Precisely detect subtle changes**—detect gene expression changes smaller than 10%
- **Works with difficult sample types**—easily quantitate heavily degraded and cross-linked RNA in formalin-fixed, paraffin-embedded (FFPE) tissues, or quantitate RNA in blood
- **Quick turnaround**—thousands of probe designs stocked; custom probes designed to any sequence, and ready to ship within weeks
- **Superior specificity**—delivers greater specificity than traditional RNA quantification technologies, and distinguishes between closely related genes because of probe design with several capture points along the target RNA of interest
- **Flexibility**—easily automated for use in routine compound screening
- **Compatibility**—works with a variety of sample types: cultured cells, whole blood, PAXgene™ blood, dried blood spots, fresh or frozen animal or plant tissues, FFPE samples, and purified RNA, using standard instrumentation (microplate luminometer and a horizontal airflow oven)

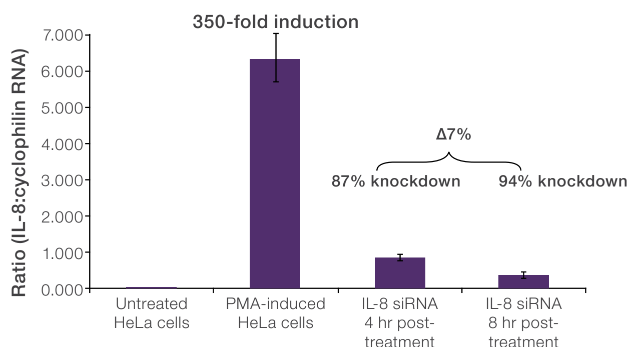


Applications include compound screening of drug candidates, biomarker verification, siRNA knockdown efficiency, prospective and/or retrospective analysis of clinical samples, miRNA profiling, microarray verification, predictive toxicology, and detection of translocations and fusion genes.



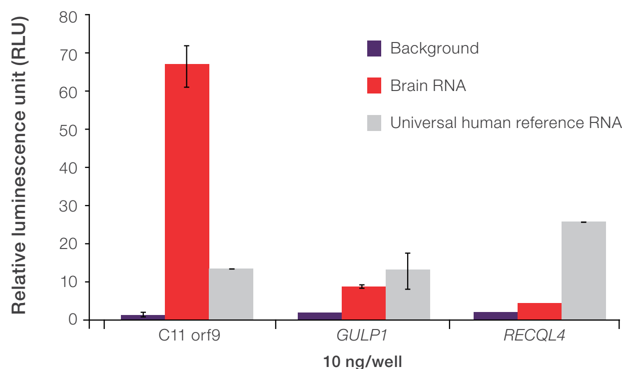
Exceptional accuracy of measurement—spike recoveries of 85–115%

A wide range of concentrations (0.001–1.0 attomoles) of an IL-6 *in vitro*–transcribed (IVT) RNA was spiked into a cell lysate with undetectable levels of endogenous expression. Percent recovery of the spike was calculated based on the signal from the IVT RNA in the presence of lysate.



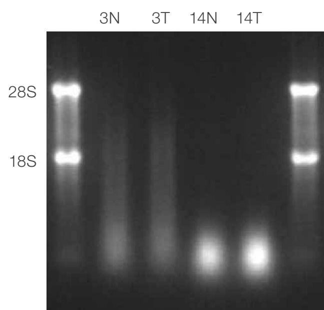
siRNA knockdown screening and verification

Phorbol myristate acetate (PMA) induction of HeLa cells led to a spike in IL-8 RNA, which was then knocked down by siRNA treatment. Knockdown efficiency was measured at two time points, and a 7% change was accurately detected by QuantiGene assays.

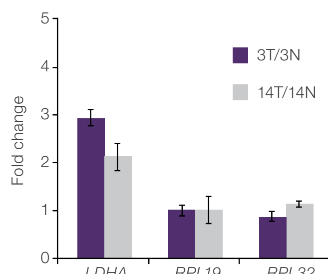


Detection of low-abundance genes

QuantiGene assays were able to detect RNAs of 3 low-abundance genes in two reference samples: brain RNA and universal human reference RNA. These genes were undetectable using other technologies. This exquisite sensitivity allows for the basal measurement of low-expression genes.



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 RNAs are indicated.



In agreement with the literature [1], the QuantiGene assay detected at least a 2-fold induction of *LDHA* in tumor relative to the normal tissue, even in highly degraded 14-year-old samples.

Quantitative gene expression data from archived FFPE samples

Profiling with a QuantiGene Singleplex Assay for lactate dehydrogenase (LDHA) RNA in 14-year-old FFPE lung tumor (14T) and adjacent normal tissue (14N) demonstrated a 2–3 fold induction of this advanced-stage cancer biomarker, in agreement with published data [1].

QuantiGene Plex Assays

Accurate and precise RNA quantitation of up to 80 genes in a single well

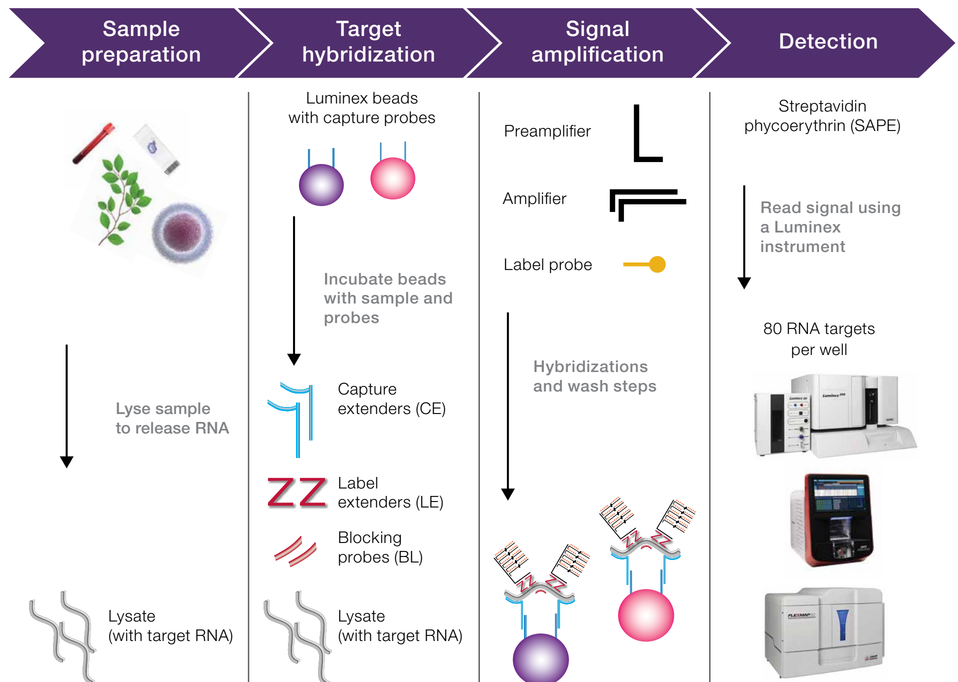
Overview of QuantiGene Plex Assays

The Invitrogen™ QuantiGene™ Plex Assays provide an accurate and precise method for multiplexed gene expression quantitation. Using Luminex® xMAP® technology, QuantiGene Plex Assays allow for simultaneous measurement of 3–80 mRNA transcripts in every well of a 96- or 384-well plate. QuantiGene Plex Assays incorporate branched DNA technology, which allows for the direct measurement of RNA transcripts by using signal amplification rather than template amplification.

The assays are simple and easy to use; QuantiGene Plex Assays do not require RNA purification, cDNA synthesis, or PCR amplification. QuantiGene Plex Assays are ideal for verifying Applied Biosystems™ GeneChip™ analysis or next-generation sequencing (NGS) data and for verifying biomarkers for translational research.

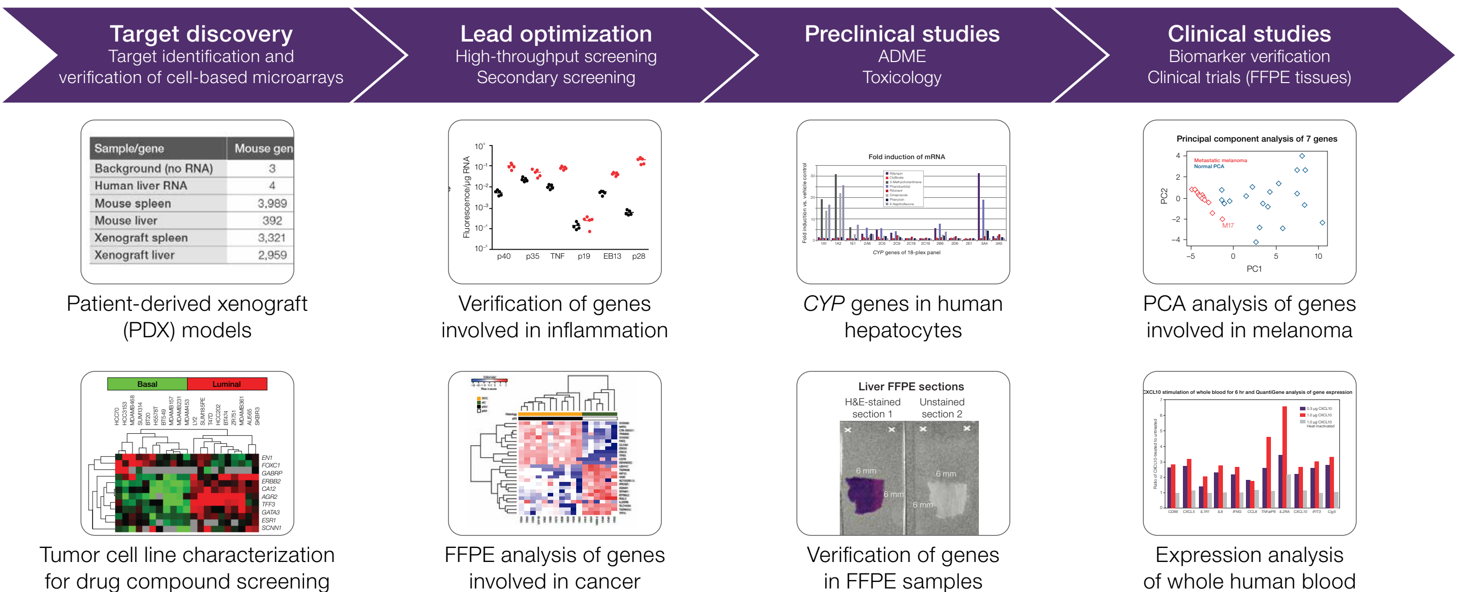
Key features and benefits of the assay

- **True multiplexing**—measure up to 80 genes of interest, including housekeeping genes, in the same well with no cross-reactivity
- **Standardized platform**—96- and 384-well plate format compatible with MAGPIX®, Luminex® 100™, 200™, FLEXMAP 3D®, and xMAP INTELLIFLEX® systems
- **Simple workflow**—ELISA-like workflow for direct hybridization of transcripts to beads and transcript labeling
- **Works with difficult sample types**—works with degraded and cross-linked RNA in FFPE tissues, and directly with blood
- **Large inventory of verified predesigned panels and combinable genes to build your own unique panel**—over 25,000 genes can be mixed to create pathway- and disease-themed panels
- **Fast customization**—if we do not have your gene(s), we can create and perform QC on your custom panel within 2–3 weeks
- **ISH compatible**—same technology utilized in the Invitrogen™ QuantiGene™ ViewRNA™ Kits for RNA *in situ* hybridization (ISH)



QuantiGene Plex Assay applications

QuantiGene Plex Assays are ideal for supporting drug discovery and development efforts, as well as translational and clinical research. The examples that follow highlight the capabilities and benefits of this powerful research tool.



Patient-derived xenograft (PDX) models

Verification of genes involved in inflammation

CYP genes in human hepatocytes

PCA analysis of genes involved in melanoma

Tumor cell line characterization for drug compound screening

FFPE analysis of genes involved in cancer

Verification of genes in FFPE samples

Expression analysis of 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™ NOD *scid* gamma (NSG) mice. After growth and metastasis of the tumor in mice, both the liver and spleen from the mice were harvested, and lysates from untreated and xenografted mice were tested alongside human liver RNA. The table below 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 samples were negative for human transcripts. As expected, tissues from xenografted mice contained both human and murine transcripts.

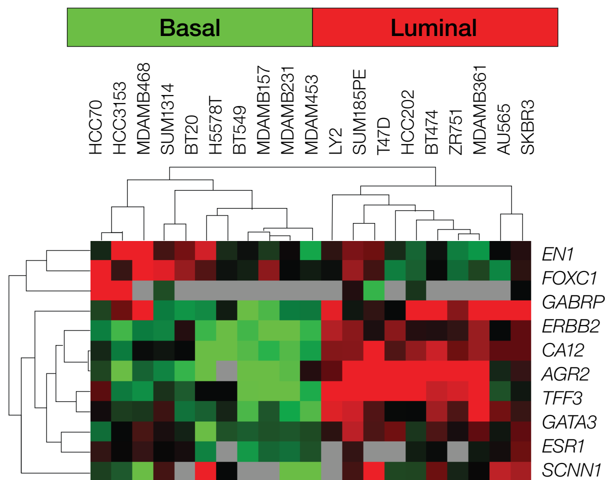
Benefit: The specificity of each component assay within a customized multiplexed QuantiGene Plex panel allows the assay to be tailored to address complex biological questions.

Sample or 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 being 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 & Science University.

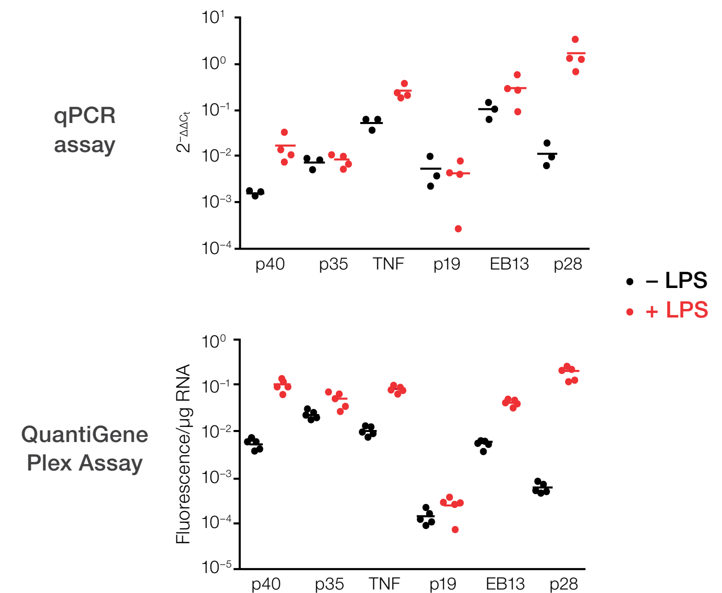
Benefit: Multiplexing allows for rapid classification of cell lines that can then be used for compound screening.



Application 3: Target verification

Gene expression signatures for inflammation pathway activation were identified using Applied Biosystems™ GeneChip™ Arrays. The signatures were further verified by testing samples from lipopolysaccharide (LPS)-treated mice using 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. Both the QuantiGene Plex and qPCR assays showed similar patterns of up- and down-regulation upon treatment. Data courtesy of Amgen, Inc [2].

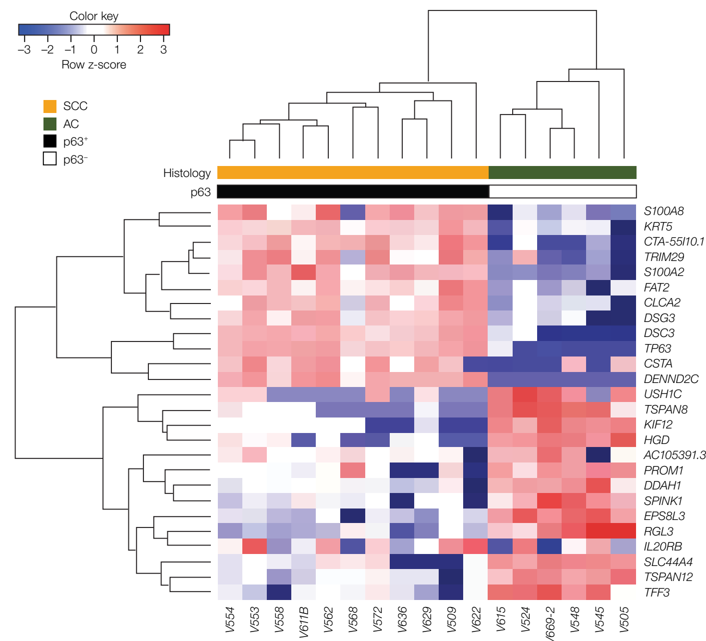
Benefit: The ability to test simple lysates makes the QuantiGene Plex Assays robust, accurate, and precise. Multiplexing increases efficiency and conserves sample.



Application 4: Verification of GeneChip array signatures from FFPE samples

Using Applied Biosystems™ 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 for an average of 12 (range 10–16) years. After analysis of the exon array data, a QuantiGene 26-plex panel was constructed and successfully verified against the 19 cervical SCC and 9 cervical AC FFPE samples. Reprinted with permission from Macmillan Publishers Ltd on behalf of Cancer Research UK [3].

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 work well for FFPE samples because the assay is based on hybridization. The assay works directly with FFPE tissues without the need for RNA purification or amplification.

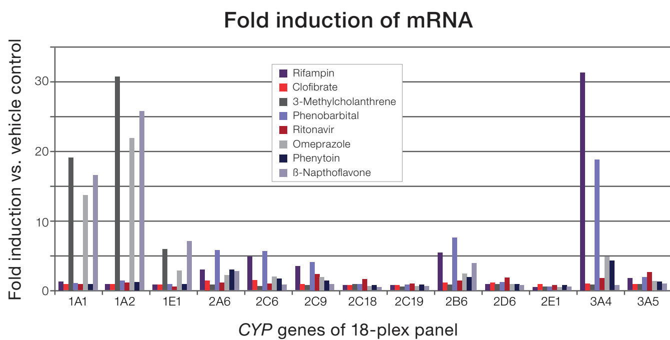


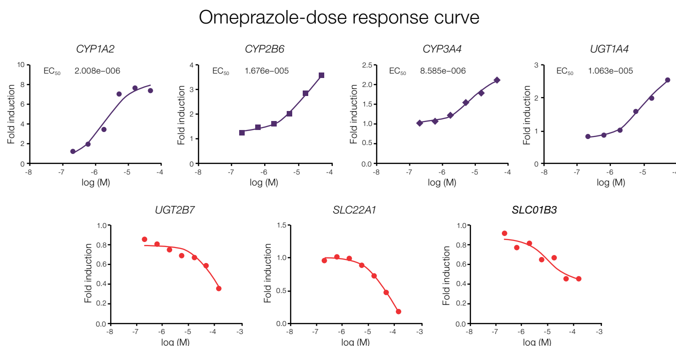
Application 5: ADME and toxicology screening

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 Invitrogen™ QuantiGene™ Plex lysis reagent, and the lysate was used directly in the QuantiGene Plex Assay. The investigators 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 [4].

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 the mRNA response. The EC_{50} calculations and the dose response curves were prepared for the selected *CYP* and transporter genes. Data courtesy of CelsisIVT [5].

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.

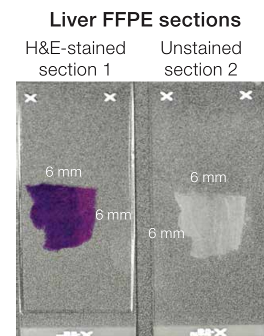


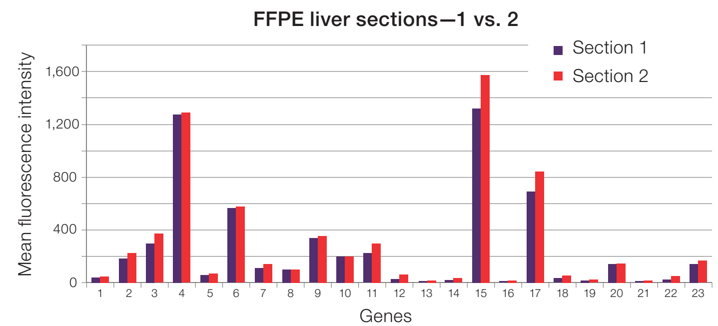


Application 6: H&E-stained and unstained FFPE samples

Gene expression analysis was performed on H&E-stained and unstained FFPE tissue sections. Two slides were obtained and a 6 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 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 7: Classification of melanoma subgroups using QuantiGene Plex gene signatures

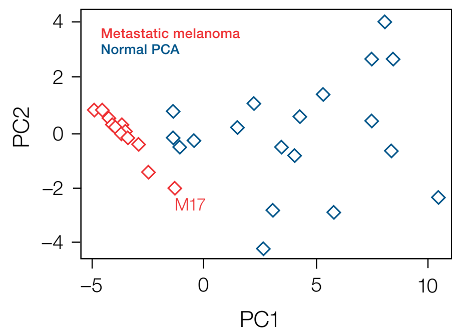
62 genes relevant to melanoma were incorporated in a QuantiGene Plex Assay as a proof of concept to demonstrate the capability to discover biomarkers. Tissue lysates were prepared from frozen sections from 20 cases of metastatic melanoma alongside the corresponding normal skin counterparts. Data analysis identified those genes most closely linked to 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's *t*-test. The 7 genes with the best *P* values were analyzed using an unsupervised principle component analysis (PCA).

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

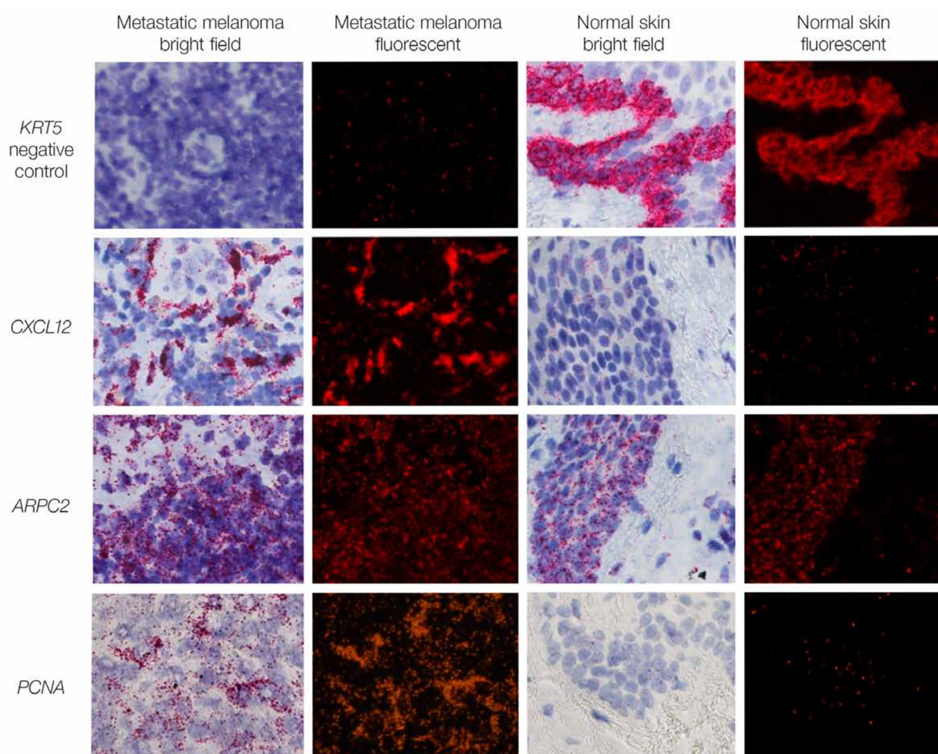
Benefit: The assays work directly with melanoma tissues without RNA purification or concerns about melanin inhibition of DNA polymerase.

Principal component analysis of 7 genes



Gene	*P* value
BCL6	1.28×10^{-9}
PTEN	1.03×10^{-8}
ARPC2	2.17×10^{-8}
CXCL12	1.37×10^{-7}
BRAF	4.18×10^{-7}
PCNA	6.33×10^{-7}
CLEC3B	1.13×10^{-6}

M17: Not melanoma; lymph node with pigmented histiocytes

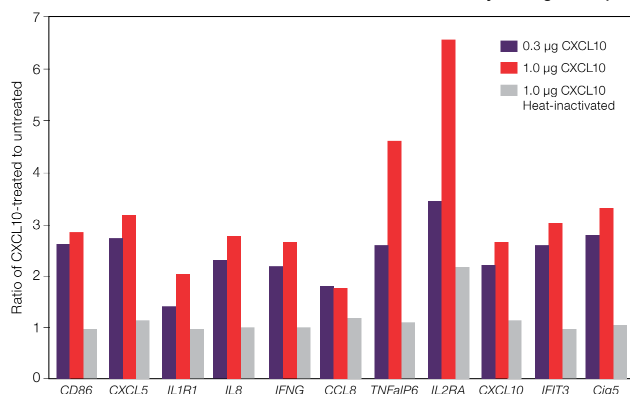


Application 8: Stimulation of whole human blood

CXCL10-responsive gene induction was confirmed using a QuantiGene 12-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 [6].

Benefit: QuantiGene Plex Assays work directly with whole blood without the need for globin depletion or RNA purification.

CXCL10 stimulation of whole blood for 6 hr and QuantiGene analysis of gene expression



Specifications

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

QuantiGene Plex panel configurator

Don't know which targets to choose for your research? Use the Invitrogen™ QuantiGene™ Plex Gene Expression Panel Configurator to browse hundreds of panels using name, keyword, category, or gene target search. Most panels also include a link to a publication where you can see how your peers used this panel in their research. You can also search for individual gene targets or search through curated pathway lists. In just a few easy steps, you select your target and housekeeping genes, then review and refine your panel. Our bioinformatics team then performs *in silico* panel validation to help ensure your panel will work appropriately. You can expect to receive your panel within 3–4 weeks of submission.

Genes included in selected 80-plex panels

Information on hundreds of preconfigured and published panels can be found in our QuantiGene Plex panel configurator at **thermofisher.com/qgp-panelconfigurator**.

Human apoptosis 1 panel (80-plex)

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 pan cancer 1 panel (80-plex)

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 cytokines 1 panel (80-plex)

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 1 panel (80-plex)

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

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