

## Luminex® assays

### High-throughput, multiplex bead-based assays

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#### Gene expression analysis

- Measure up to 80 RNA transcripts in a single well

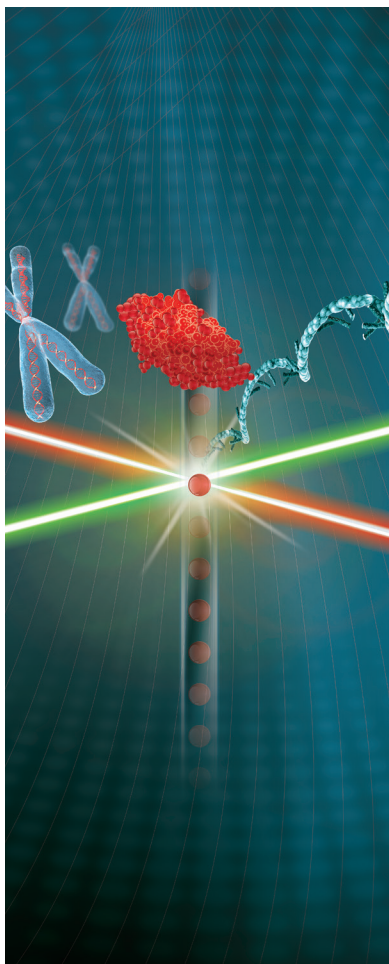
#### DNA copy number variation

- Analyze copy number variation in multiple DNA targets

#### Multiplex immunoassays




- Quantitate up to 80 analytes in only 25  $\mu$ L of serum/plasma sample or 50  $\mu$ L of cell culture supernatant
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Luminex xMAP® technology combines advanced fluidics, optics, and digital signal processing with fluorescently dyed microspheres to enable the quantitation of multiple nucleic acid or protein targets from a single sample. As an exclusive Luminex partner, Affymetrix offers QuantiGene® Plex Assays for nucleic acid quantitation and ProcartaPlex® Multiplex Immunoassays for measurement of protein expression.



# High-throughput immunoassays, gene expression analysis, and copy number variation—all from one supplier, on one instrument

As a certified partner of Luminex, Affymetrix has been providing Luminex users with multiplex reagents for over 10 years and has the most comprehensive offering of any Luminex partner. Luminex has been supplying instruments to the life science research market for almost 20 years, with over 13,000 units installed worldwide.

Assays	Analyte measured	Plex level	Targets available	Custom designs
ProcartaPlex® Multiplex Immunoassays		80	>700	✓
QuantiGene® Plex RNA Assays		80	>17,000	✓
QuantiGene® Plex DNA Assays		77	>700	✓

## ProcartaPlex® Multiplex Immunoassays

ProcartaPlex Multiplex Immunoassays are bead-based assays for protein detection using Luminex xMAP technology. ProcartaPlex assays are based on the principles of a sandwich ELISA, using two highly specific antibodies binding different epitopes of one protein.

## QuantiGene® Plex Assays for RNA and DNA quantitation

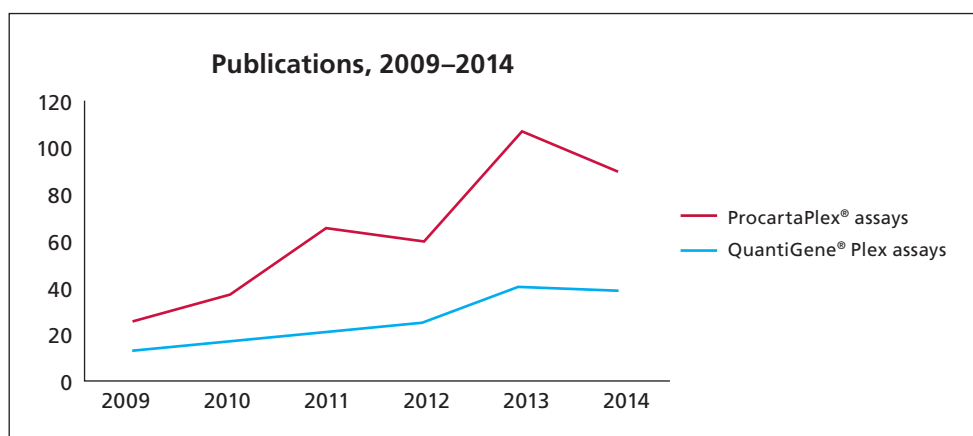
QuantiGene Plex assay is the highest throughput solution for multiplexed gene expression analysis and DNA copy number variation analysis, allowing researchers to measure up to 80 genes in a single well. This assay is fast, easy, and PCR-free—no RNA purification is needed, and there are no enzymes involved to cause bias, unlike qPCR. Additionally, custom-made panels can be designed, validated, and shipped within two weeks of a request.

## Why use Luminex technology?

**Profile more biomarkers with less starting sample:** Quantitate up to 80 analytes simultaneously with the FLEXMAP 3D® or Luminex 200 and up to 50 analytes with the MAGPIX® instrument. All of our ProcartaPlex assays are compatible with as little as 25 µL of plasma or serum samples or 50 µL of cell culture supernatant sample.

**High-throughput analysis:** QuantiGene Plex and ProcartaPlex assays are available in 96-well and 384-well formats for high-throughput analysis.

**Proven technology on trusted platform:** Hundreds of peer-reviewed publications have cited ProcartaPlex and QuantiGene Plex assays, demonstrating their value to researchers.



# Luminex xMAP technology

## Quick, cost-effective, and accurate multi-analyte profiling system

The open-architecture xMAP technology uses flow cytometry, microspheres, lasers, digital signal processing, and traditional chemistry, combining proven technologies in a unique way.

### Advantages

- Reduced cost and labor by multiplexing
- Smaller sample size requirements
- Faster, more reproducible results from favorable kinetics of liquid-bead array approach
- Broad coverage of applications including protein expression and gene expression profiling

### Luminex instruments



System	MAGPIX® system: Affordable, efficient, and compact	Luminex® 100/200™ system: Versatile industry standard for multiplexing	FLEXMAP 3D® system: High-throughput and automation compatible
Advantages	Low cost	Higher multiplex capacity	Automation/LIS compatibility
	Easy-to-use	Easy-to-use	Ultra-fast
	Small footprint and portable	Scalable with bar code reader included	96- and 384-well capability Extended dynamic range
Hardware and optics	Fluorescent imager	Flow cytometry-based	Flow cytometry-based
	LED/CCD camera	Lasers/APDs/PMTs	Lasers/APDs/PMTs
Multiplex capacity	50	80	80
Read time	~60 min/96-well plate	~40 min/96-well plate	~18 min/96-well plate
Dynamic range	3.5 logs	3.5 logs	4.5 logs (up to 400,000 MFI)
Microtiter plate	96-well	96-well	96-well and 384-well

### How xMAP technology works

Sets of magnetic microspheres are color-coded with combinations of red and infrared fluorophores. Each bead set is coated with a reagent specific to a particular bioassay. This allows the capture and detection of several specific analytes from a single sample. Lasers in the compact analyzer excite the internal dyes that identify each microsphere as well as any reporter dye captured. This allows quantification of individual analytes on each microsphere. The analyzer reads many beads from each set, delivering rapid and precise results for several targets within a single sample.

# Get the immunoassay panel you want

## ProcartaPlex® Multiplex Immunoassays

ProcartaPlex Multiplex Immunoassays allow simultaneous quantification of up to 80 analytes in a single sample of serum, plasma cell/tissue lysates, cell culture supernatants, or bodily fluids. Assays can be easily combined to create custom panels that will achieve reproducible results throughout the course of a study.

- More than 90% of ProcartaPlex assays can be combined with one another
- High correlation to our Platinum ELISA assays ( $R^2 > 0.9$ )
- Scalable and reproducible performance regardless of plex size
- Largest published panel for quantitation of up to 63 analytes<sup>1,2,3,4</sup>

ProcartaPlex assays are provided in multiple formats across six species: human, mouse, rat, non-human primate, porcine, and canine.

## ProcartaPlex® Simplex kits

The ready-made ProcartaPlex Simplex kits detect individual analytes and are designed to be added to ProcartaPlex panels to increase customization. Alternatively, multiple ProcartaPlex Simplex sets can be combined together.

## ProcartaPlex® panels

More than 50 pre-mixed panels are available for immediate delivery. Multiple panels can be combined with one another or with ProcartaPlex Simplex kits in order to provide greater flexibility in panel design.

## ProcartaPlex® Mix&Match panels (Custom mixed)

You pick, we mix. Choose your analytes of interest from our portfolio list, and we will build your panel for you.

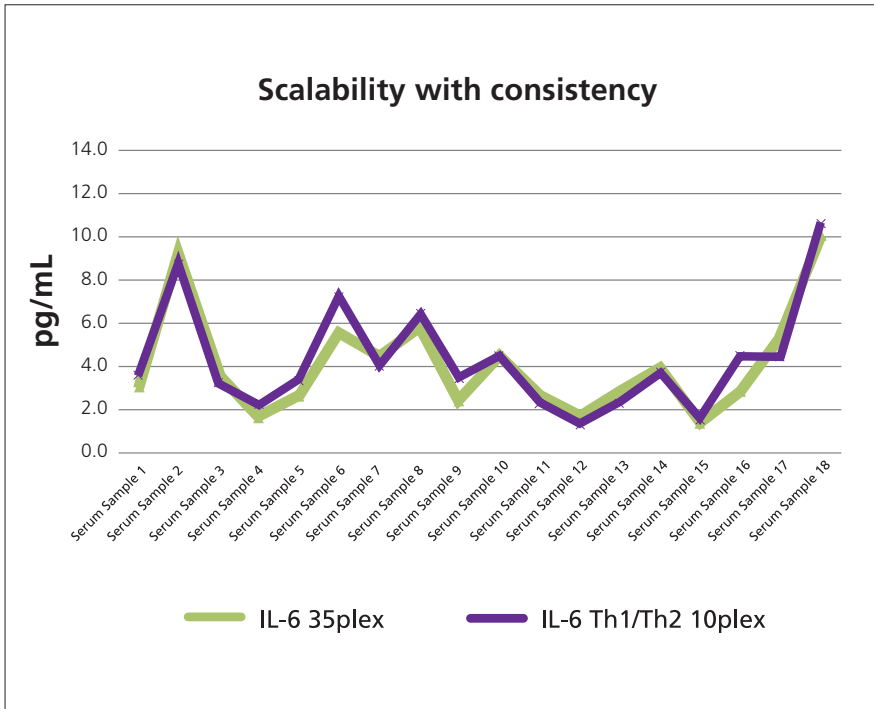
## Applications

ProcartaPlex Multiplex Immunoassays are available for key targets in many research fields:

- Angiogenesis
- Apoptosis
- Atopic dermatitis
- Bone markers
- Cancer
- Cardiology
- Cell stress
- Diabetes
- Hematology
- Immune regulation
- Immuno-oncology
- Inflammation
- Isotyping
- Metabolism
- Myokines
- Preeclampsia
- Rheumatoid arthritis
- Sepsis
- Transplantation/GvH disease

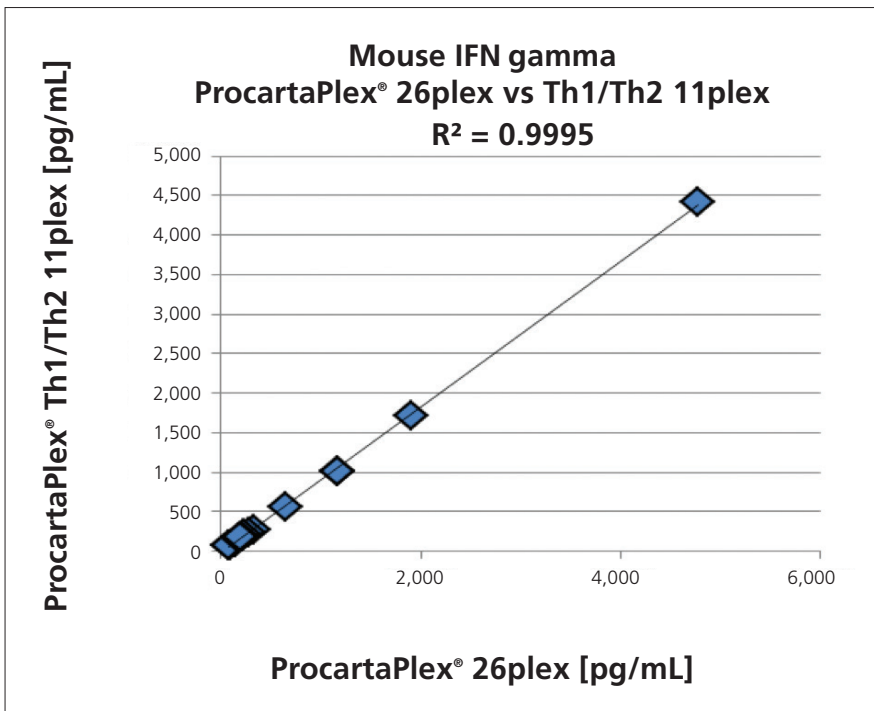
# Scalability of ProcartaPlex assays

Researchers need the ability to scale the number of analytes investigated in multiplex experiments in order to correlate their data across the different stages of their projects as they narrow down their targets of interest. Most multiplex assays from other suppliers are not developed to give consistent results between large panels, small panels, and simplex assays. Data below demonstrates the scalability of ProcartaPlex assays from a large panel down to a single analyte. This excellent correlation between panel sizes and correlation with Platinum ELISAs from Affymetrix allow researchers to confidently compare data between experiments.



## High correlation between large and small plexes

To illustrate the scalability of ProcartaPlex® Multiplex Immunoassays, diseased human serum samples were run in parallel using both ProcartaPlex® Human Cytokine and Chemokine Panel 1A (34 plex) and Human Th1/Th2 Cytokine Panel (11 plex). This experiment demonstrates consistency achieved in both large and small scale multiplex panels.



## High correlation with Platinum ELISA

Results of an experiment measuring IFN gamma in stimulated human peripheral blood mononuclear cells (PBMCs). Supernatants were serially diluted two-fold in normal human serum to allow for differential cytokine levels in a serum matrix.

# PCR-free multiplexed gene expression

## QuantiGene® Plex Assay

QuantiGene Plex Assays provide the fastest and highest throughput solution for multiplexed gene expression quantitation, allowing the simultaneous measurement of up to 80 genes of interest in a single well of a 96- or 384-well plate. QuantiGene Plex Assay is hybridization-based and incorporates branched DNA (bDNA) technology, which uses signal amplification rather than target amplification for direct measurement of RNA or DNA transcripts. The assay is extremely easy to use with a simple, ELISA-like workflow, and unlike qPCR, does not require RNA purification.

### True multiplexing

Measure up to 80 genes of interest in a single well with no cross-reactivity, enabling normalization to housekeeping genes in the same reaction and minimizing the total number of plates that need to be run.

### Fast, easy, and PCR-free

Requires no RNA purification, no reverse transcription, and no PCR amplification, avoiding enzymatic bias and saving you valuable time. Just lyse and run!

### Perfect for formalin-fixed, paraffin-embedded (FFPE) tissues

Works well with heavily degraded RNA in archived samples and is insensitive to chemical modification of RNA such as protein cross-linking due to formalin fixation.

### Speedy customization

Custom panels are designed, quality tested, and shipped within two weeks of your request, with no need for extensive assay optimization on your part.

## Applications

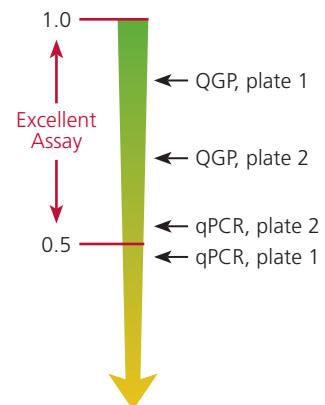
QuantiGene Plex Assays are the ideal platform for a wide range of applications, from compound screening in the drug discovery process through biomarker validation in archival FFPE tissue samples or whole blood. Following are several examples that highlight the capabilities and benefits of this powerful research tool.

### Drug discovery

QuantiGene Plex Assays are well-suited for use in various stages of drug discovery and make compound screens more efficient by providing robust, accurate data. Here are other common QuantiGene Plex applications throughout the drug discovery process:

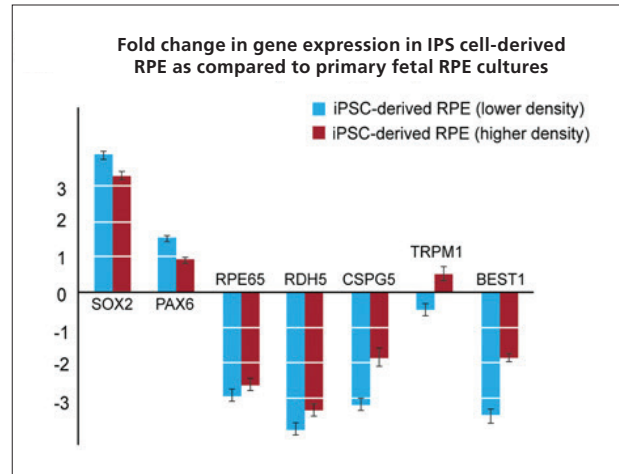
Target identification & validation	Lead optimization	Preclinical	Clinical
<ul style="list-style-type: none"><li>Microarray data validation</li><li>RNAi knockdown</li><li>Target validation (<i>in vitro</i>)</li></ul>	<ul style="list-style-type: none"><li>Gene expression-based, high-throughput screening</li><li>Primary and secondary compound screening</li><li>RNAi off-target effects</li></ul>	<ul style="list-style-type: none"><li>ADME</li><li>Toxicology</li></ul>	<ul style="list-style-type: none"><li>Biomarker validation</li><li>Clinical trials (FFPE tissues, whole blood, hair follicles)</li></ul>

In compound screening, Z-factors are calculated to determine the robustness of an assay. Z-factors closer to 1.0 are more ideal, and Z-factors below 0.5 indicate the assay is not suitable for screening. In this experiment, HUVEC cells were seeded at 10,000 cells/well and treated with IL-1 beta at various doses for 24 hours. Lysates were run directly with QuantiGene Plex Assay (QGP) and RNA was extracted and run with qPCR. Z-factors were calculated for nine genes across two plates, and average Z-factors for each plate are shown to the right.



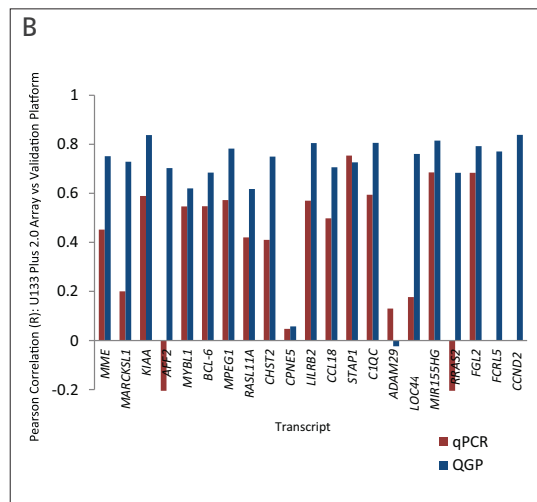
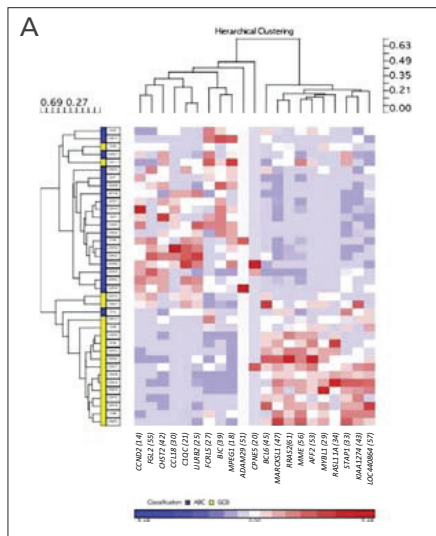
### Optimization of induced pluripotent stem cell (iPSC) differentiation protocols

Differentiated iPSCs can have the same phenotype as mature cells of the desired lineage, but may still possess fetal gene expression patterns. In this study, genes that measure cell development and differentiation, function, and pathology were selected.<sup>5</sup> Their expression was monitored in iPSC-derived retinal pigment epithelium (RPE) using QuantiGene Plex Assays and compared to expression in iPSCs and primary fetal RPE cells. When monitoring expression within a sample over multiple time points, increased efficiency through true multiplexing and the ability to measure subtle changes become extremely beneficial.



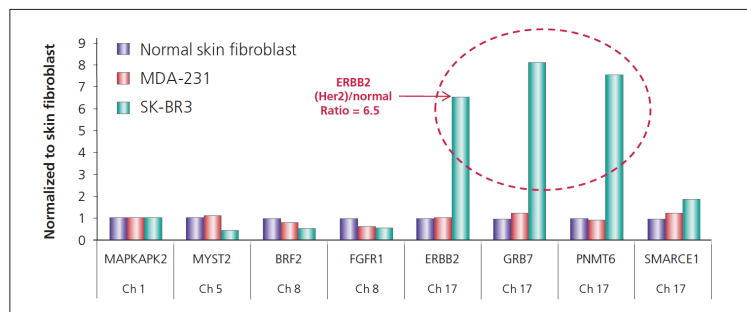
### Subtyping of diffuse large B-cell lymphoma (DLBCL) in FFPE tissue samples

In clinical research, FFPE tissue samples can often be difficult to assess with traditional methods such as qPCR. In this study, 40 DLBCL FFPE tumors of known subtype were reclassified using QuantiGene Plex Assay and qPCR with a 21-gene signature.<sup>6</sup> QuantiGene Plex Assay is able to subtype DLBCL into activated B cell and germinal center B cell subtypes with 92.1% classification accuracy (Figure A), as opposed to only 78.9% accuracy with qPCR. QuantiGene Plex data also shows a significantly higher correlation with gold standard microarray (GeneChip® Human Genome U133 Plus 2.0 Array) data than does qPCR data (Figure B).



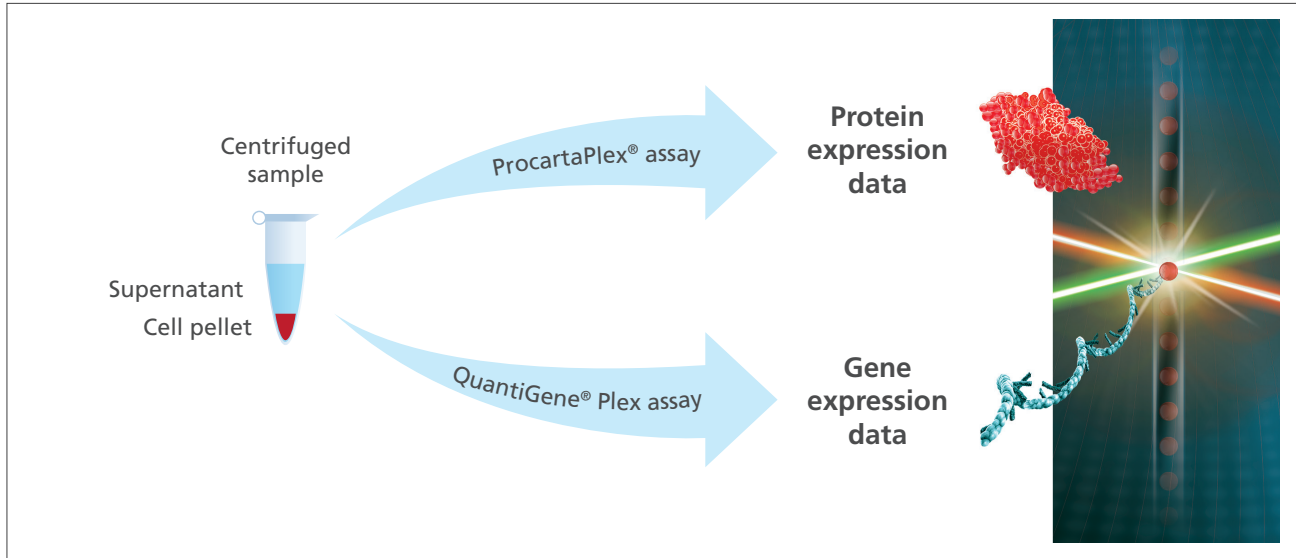
### Analysis of DNA copy number amplification in HER2+ breast cancer

QuantiGene Plex DNA Assay allows simultaneous detection of multiple copy number alterations in a single well, which is especially useful for the analysis of "C" class tumors (tumors driven by copy number alterations). In this example, QuantiGene Plex DNA Assay was used to quantify 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).



## Protein and gene expression analysis from a single sample

Protein and gene expression data can be generated from the same sample, giving researchers more information about their samples. After a cell culture is centrifuged, the supernatant is analyzed for multiple cytokines using the ProcartaPlex assay. The cell pellet is then lysed and run using the QuantiGene Plex assay in order to obtain gene expression data.



## References

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4. Siebert J. C., *et al.* The Stanford Data Miner: a novel approach for integrating and exploring heterogeneous immunological data. *Journal of Translational Medicine* **10**:62 (2012).
5. Ferrer M., *et al.* A Multiplex High-Throughput Gene Expression Assay. *Stem Cells Translational Medicine* **3**:1–12 (2014).
6. Hall, J. S. QuantiGene Plex Represents a Promising Diagnostic Tool for Cell-of-Origin Subtyping of Diffuse Large B-Cell Lymphoma. *Journal of Molecular Diagnostics* **17**(4):402–411 (2015).



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