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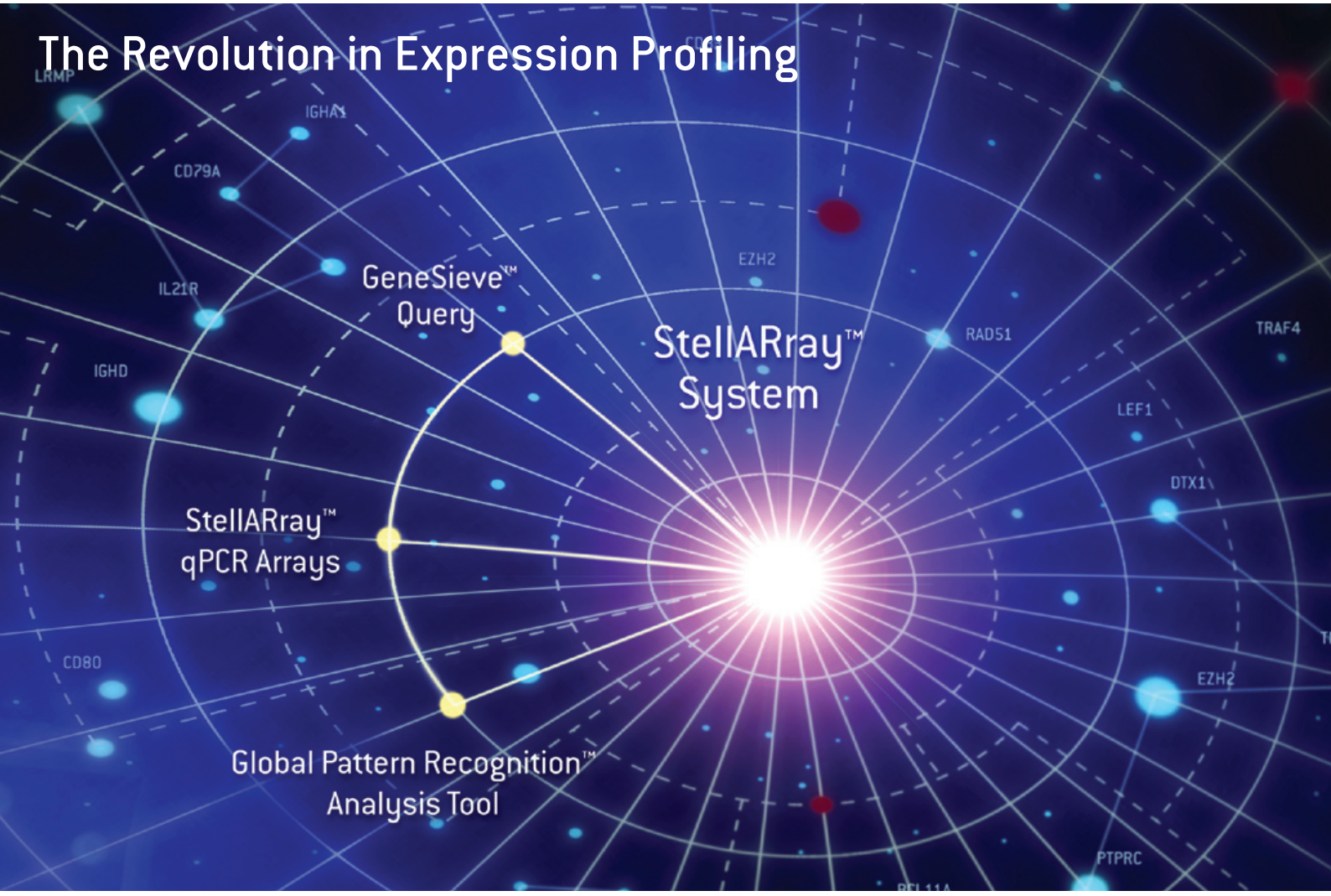
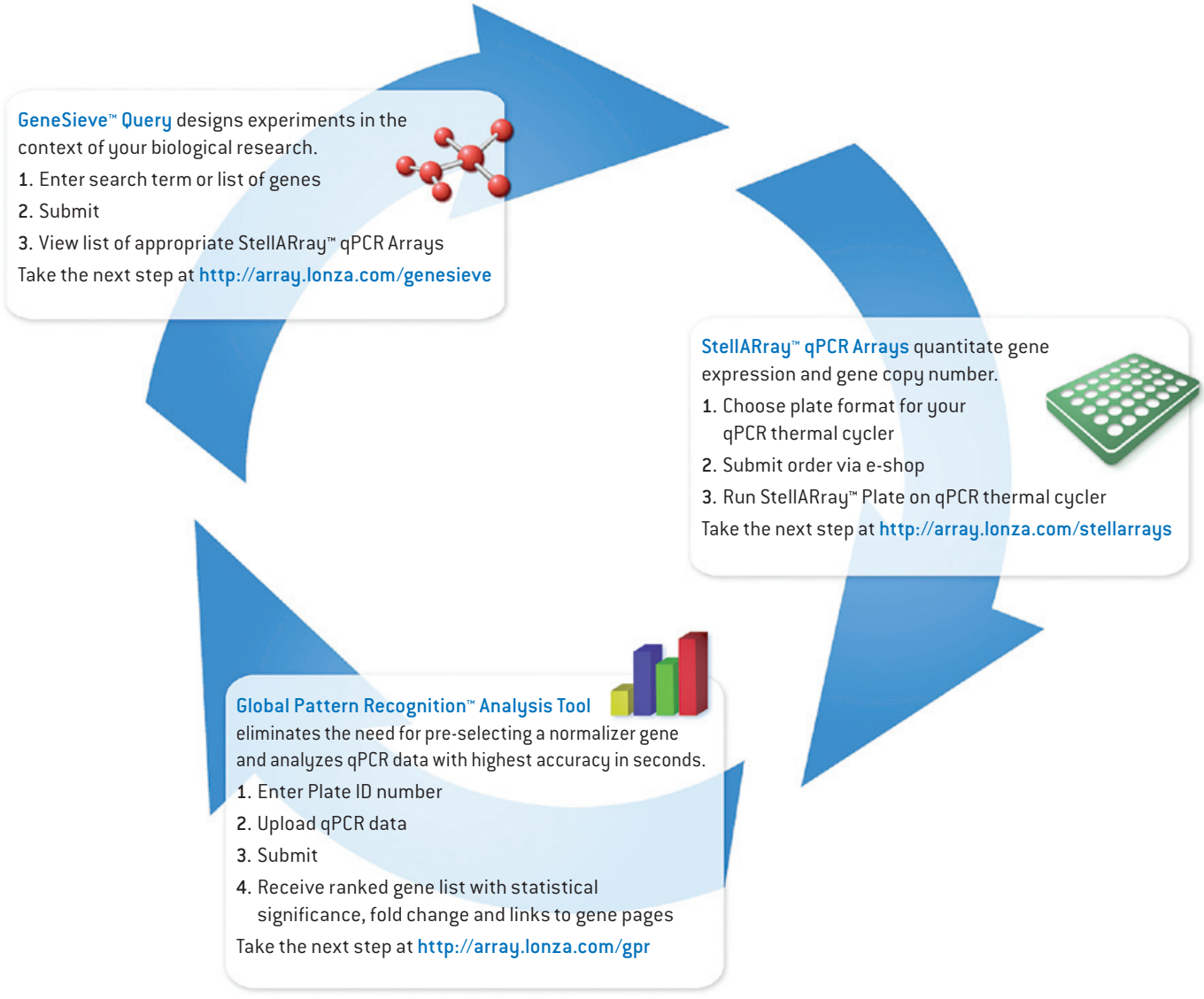
The StellARray™ Gene Expression System



A Complete Solution —
From Selection of Research Area-specific Gene Panels to Comprehensive Data Analysis



Custom qPCR Arrays Tailored for Your Research

StellARray™ qPCR Arrays are available as pre-made arrays, as well as custom arrays, customized with the gene list of your preference. StellARray™ Custom qPCR Arrays enable you to take advantage of the benefits provided by the StellARray™ System for the exact set of genes you want to analyze. For more details, please refer to **www.array.lonza.com** or contact our Scientific Support Team.

Continue to Discover

The challenges in gene expression research can be compared to astronomers investigating the myriad of stellar constellations. The StellARray™ Gene Expression System offers life scientists a similar approach: a revolutionary qPCR system that helps you to focus on the gene "constellations" relevant for your individual research area and frees your gene expression profiling from unwanted bias.

This unique system makes gene-by-gene expression analysis, microarray data validation, biomarker discovery and siRNA knock-down verification simple, fast and reliable.

Benefit from:

— **Reliable normalization without predefined normalizers** – StellARray™ Proprietary GPR Software automatically defines the best normalizer amplicons, based on lowest variance, within seconds

— **More than 150 pre-validated pathway or disease area-specific qPCR arrays available off-the-shelf** – Just order and start comprehensive array experiments for your research area using your standard qPCR equipment

— **Copy number analysis and expression profiling using the same array plate** – effortlessly compare gene copy numbers and gene expression profiles for exactly the same sets of genes

Let Your Experiment Define the Best Normalizer Gene

Currently, the most common approach is to use housekeeping genes as normalizer genes, assuming that the expression level of the housekeeping gene does not change in the context of the experiment. However, housekeeping gene expression often changes when tissues or cells in different states are compared. Any small variation in the housekeeping gene expression compromises the analysis of the complete real-time PCR data set (Figure 1).

— The StellARray™ System does not work with housekeeping genes as predefined normalizers

— Proprietary Global Pattern Recognition™ (GPR) Analysis Software algorithms compare within and between test and control amplicons to determine the best set of normalizer genes and produce more accurate fold change expression values

— GPR Software also enables comparison of replicates between different biological samples to further increase the accuracy of the statistical ranking of fold changes

— The GPR tool not only tabulates statistical significance (p-value) of gene expression changes, but also lists related genes and links to MGI and NCBI gene pages

Which data would you trust?

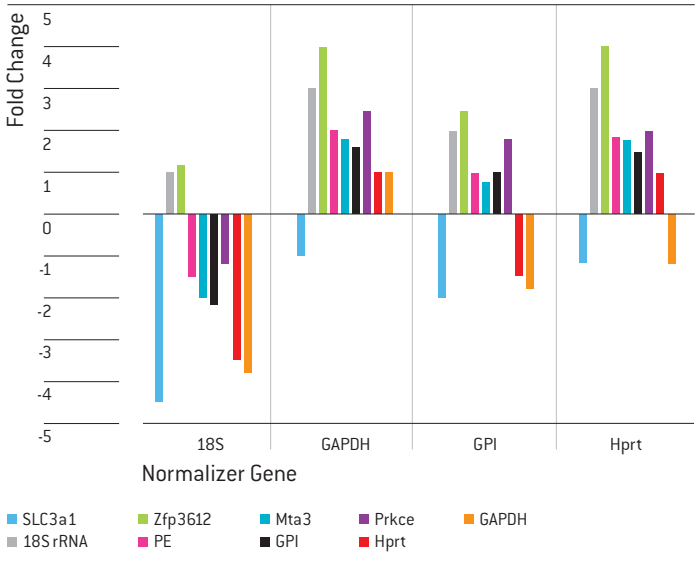


Figure 1. Using the conventional ΔΔCt method for analysis of qPCR data, the same experimental qPCR data set was analyzed four times by normalization to each 18S, GAPDH, GPI or HPRT as traditional housekeeping genes. Depending on the selection of the housekeeping gene for normalization, very different gene expression profiles were obtained. The StellARray™ System does not work with predefined normalizers or housekeeping genes. By using the StellARray™ System, the experiment defines the best set of genes for normalization to ensure highest accuracy of analysis.

A Broad Panel of More Than 150 Research Area-specific StellARray™ qPCR Arrays

Find the gene panels most relevant for your research area from more than 150 pathway or disease area-specific StellARray™ qPCR Arrays in 96- and 384-well plate formats available off-the-shelf.

- Allergy
- Angiogenesis
- Blood disease
- Cancer
- Cardiovascular disease
- CNS disorder
- Developmental biology
- Immune disorder
- Immunology
- Infectious disease
- Mental disorder
- Metabolism
- Obesity
- Signal transduction
- Stem Cells
- Toxicology
- Wound healing

The selection of the genes on each StellARray™ qPCR Array involves extensive "sieving" of data using the advanced capabilities of the GeneSieve™ Bioinformatics System. The GeneSieve™ System is composed of several modules that work together to assemble 'constellations' of genes associated with specific biological pathways and processes. This system identifies relevant genes using a multifaceted approach involving:

- Sorting of genes by relevance to biological processes
- Merging of databases containing "gene-centric" biological information
- Searching of over 16 million abstracts for genes involved in related processes; genes are subsequently ranked by multiple fields including quantity and quality of literature references
- Plus other filter modules, including analysis of canonical signaling pathways, microarray data, gene ontology classifications and the downstream targets of transcription

Analyze Both Gene Copy Numbers and Gene Expression for the Same Set of Genes

The importance of determining gene copy number or copy number variation (CNV) is increasing, as it is believed to be responsible for:

- Phenotypical variability seen in humans during drug treatment
- Disease susceptibility, including HIV infection and lupus (SLE)
- Complex behavioral traits, including autism and schizophrenia
- Development of certain cancers

StellARray™ qPCR Arrays and GPR Analysis Software are designed to be used for expression profiling, as well as the determination of gene copy number. By applying genomic DNA as template, the StellARray™ System easily and accurately determines the number of copies of a gene which is/are responsible for the expression levels observed.

Rank	Gene Name	p-Value	Fold Change	Chromosome
1	Cdx4	0.00002	2.1984	X
2	Rhox6	0.00002	2.0614	X
3	Tro	0.00002	2.0355	X
4	Cd40lg	0.00006	2.0013	X
5	Ikbkg	0.00007	1.7857	X
6	Hprt1	0.00009	1.9999	X
7	Btk	0.00014	1.9606	X
8	Gata1	0.00016	2.0157	X
9	Mecp2	0.00037	2.0405	X
10	Suv39h1	0.00044	1.9456	X
11	Mageh1	0.00068	2.1936	X
12	genomic3	0.00099	1.9252	X
13	Hdac1	0.0027	1.2972	8
14	Tert	0.0398	-1.2339	13
15	Irf2	0.0538	-1.1347	8
16	Il6st	0.0571	-1.1236	11
17	Hmgb2	0.0657	1.1361	11
18	Tnfrsf13b	0.0748	-1.1386	5
19	Exh1	0.0802	-1.1468	12
20	Shh	0.0871	-1.5049	10
21	Max	0.0878	1.1223	4

Figure 2. GPR-based genomic DNA copy number variation (CNV) analysis. Using the 384-well Lymphoma and Leukemia StellARray™ Product, individual gDNA samples (biological replicates) from five male C57BL/6J and five female C57BL/6J mice were compared for a GPR-based genomic DNA copy number variation (gDNA CNV) analysis. The gene content of this StellARray™ Plate includes 12 genes physically located on the mouse X chromosome. The expected CNV is two fold due to the females having XX (2 copies of X) and males having XY (one copy of X). This '5 vs. 5' example identified all 12 X-specific genes with statistical significance, ranking them as the top 12 'hitters', and providing an average Fold Change value of 2.0136 with a standard deviation of 0.112. Additionally, there are no other X chromosome genes within this gene set thus GPR was highly efficient at determining 2 fold differences.

Reliable qPCR Data Through Validated PCR Primers

StellARray™ Primer design and validation is done by using a constantly growing database of 4.8 M PCR primers, state-of-the-art design software and more than a decade of experience in qPCR development. Using standardized DNA templates, every primer pair of each StellARray™ qPCR Array is tested by real, "wet lab" qPCR reactions, not just *in silico* tests, for organism specificity with minimized SNP interference, gene specificity and amplicon efficiency, in order to ensure reliable results in your experiments.

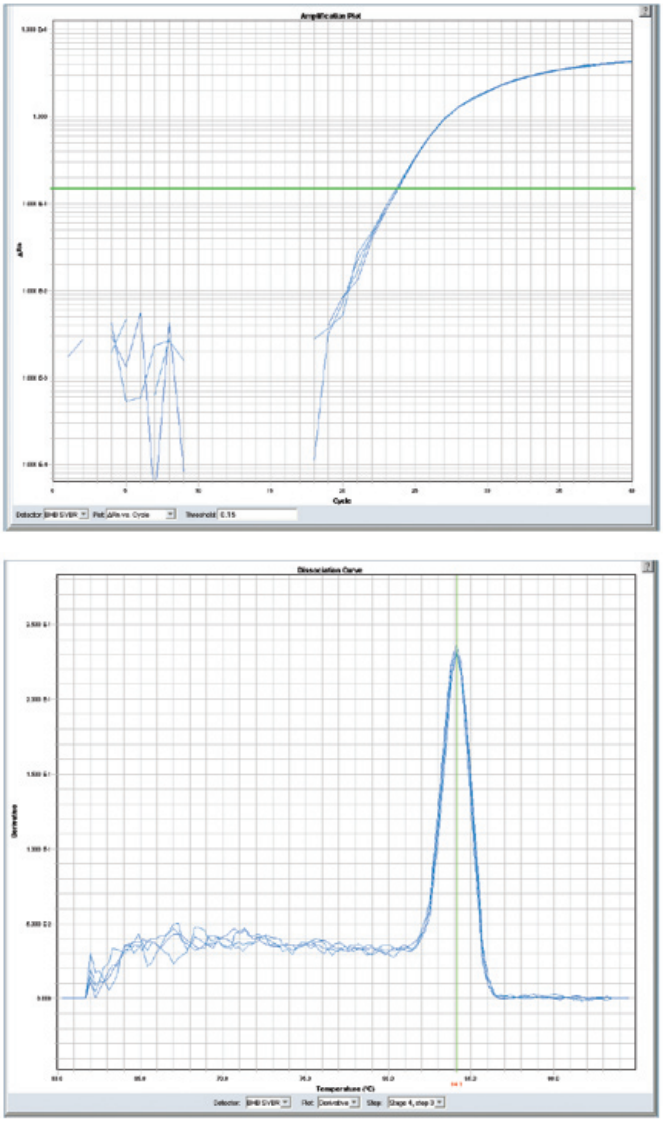


Figure 3. Extensive validation of PCR primer pairs by "wet lab" tests. Every StellARray™ PCR primer pair is checked in quadruplicates [top] via amplification plot analysis for consistent amplification characteristics and [bottom] via melting curve analysis for generation of single-peak dissociation curves.

Benefit from Broad Compatibility with Most Common Real-time PCR Devices

Supported PCR Instruments

Applied Biosystems (ABI®)	5700, 7300, 7500, 7700, 7900HT.
Bio-Rad	My iQ®, iCycler®, iQ5®
Stratagene	MX3000p®, MX3005p®
Eppendorf	Mastercycler® *ep realplex*
Roche	LightCycler® 480 (on request)

Please contact our Scientific Support Team if your device is not listed.

Selected Publications

1. Akilesh S, Shaffer DJ, Roopenian D. Customized molecular phenotyping by quantitative gene expression and pattern recognition analysis. *Genome Res.* 2003 Jul; 13(7): 1719–27.
2. Akilesh S, Petkova S, Sproule TJ, Shaffer DJ, Christianson GJ, Roopenian D. The MHC class I-like Fc receptor promotes humorally mediated autoimmune disease. *J Clin Invest.* 2004 May; 113(9): 1328–33.
3. Hart GT, Shaffer DJ, Akilesh S, Brown AC, Moran L, Roopenian DC, Baker PJ. Quantitative gene expression profiling implicates genes for susceptibility and resistance to alveolar bone loss. *Infect Immun.* 2004 Aug; 72(8): 4471–9.
4. Wo Y, Wright SM, Maas SA, Alley TL, Caddle LB, Kadmar S, Affourti J, Foreman O, Shaffer D, Bronson RT, Roopenian DC, Mills KD. The nonhomologous end joining factor Artemis suppresses multi-tissue tumor formation and prevents loss of heterozygosity. *Oncogene.* 2007 Sep 6; 26(41): 6010–20.
5. Seymour RE, Hasham MG, Cox GA, Shultz LD, Hogenesch H, Roopenian DC, Sundberg JP. Spontaneous mutations in the mouse Sharpin gene result in multiorgan inflammation, immune system dysregulation and dermatitis. *Genes Immun.* 2007 Jul; 8(5): 416–21.
6. Review – Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. SA Bustin, *Journal of Molecular Endocrinology* (2000) 25, 169–193. Paper by reviewing qPCR including an example of GAPDH expression variability.
7. Chang, TJ, Juan, CC, Yin, PH, Chi, CW, and Tsay, HJ (1998) Up-regulation of beta-actin, cyclophilin and GAPDH in N1S1 rat hepatoma. *Oncol Rep. 5*, 469–471.

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BR-StellARray-1 05/09