

Array-Based Gene Expression Analysis

Expression profiling products tailored for a variety of genetic research applications.

Highlights

- **High-Quality Data:**
Rigorously tested assays with internal controls on each array
 - **Broad Range of Products:**
Supports discovery, screening, and profiling FFPE samples
 - **Comprehensive Coverage:**
Carefully selected array content for discovery of any mRNA
 - **Multiple Species**
Expression profiling solutions for human, mouse, and rat

Illumina leads

products designed for a broad range of genetic research. Its RNA expression profiling assays are performed on Illumina's BeadArray™ technology-based systems (Figure 1), providing comprehensive gene coverage on a wide range of sample types.

Specifically, Illumina's Direct Hybridization Whole-Genome Expression

assays can be used for analyzing intact RNA samples, while its **DASL** (cDNA-mediated Annealing, Selection, Extension, and Ligation) assays enable analysis of difficult or degraded RNA samples, such as formalin-fixed, paraffin-embedded (FFPE) tissues. These proven assays offer highly accurate data, streamlined workflows, and multi-sample formats for high-throughput multiplex gene expression profiling.

Data generated with either of these assays can be visualized and

analyZEU with Illumina's GenomeStudio™ data analysis software. The performance-optimized tools and user-friendly graphical interface of GenomeStudio software allows researchers to quickly and easily convert data into meaningful results.

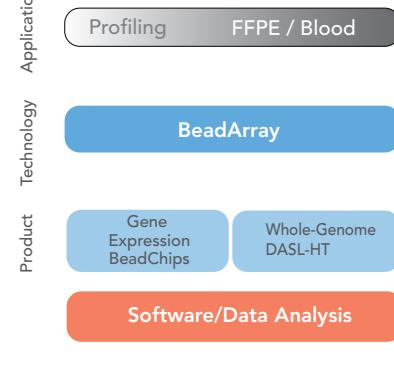
Illumina Whole-Genome Gene Expression
oligonucleotides immobilized to beads

surface of an array substrate (Figure 2). Data quality and reproducibility are supported in part by the high level of bead-type redundancy (up to an average of 30 beads per probe) on every array. After randomly distributing the beads across the substrate surface, 29-mer address sequences present on each bead are used for a hybridization-based procedure to map the array, identifying the location of each bead. This final process also validates the hybridization performance of every bead on every BeadChip, ensuring 100% array quality control.

Illumina Expression BeadChip arrays are arranged in a multi-sample format for higher throughput and reduced sample-to-sample variability.

Labeled sample cRNA are detected by hybridization to 50-mer probes on the BeadChip. After washing and staining steps, BeadChips are scanned on a HiScan™SQ, iScan, or BeadArray™ Reader. For the

Figure 1: Gene Expression Profiling and Validation Options



expression profiling products for a variety of applications.

can automate BeadChip loading and scanning with the AutoLoader, AutoLoader2, or AutoLoader 2.x.

Each probe location (address) and see

- Lack of similarity to other genes

- Absence of highly repeated sequence in the genome
 - Sequence complexity
 - Self-complementarity for hairpin structure prediction
 - Melting temperature for hybridization uniformity
 - Distance from 3' end of the transcript

Illumina expression array solutions include complete kits
for gene and transcript expression analysis applications.

up-to-date content selected from widely used databases such as the National Center for Biotechnology Information Reference Sequence (NCBI RefSeq¹) and more specialized sources (Table 1).

Reproducibility has been demon

technical replicates. Exceptional performance specifications for sensitivity, dynamic range, and fold-change detection precision maximize the impact of differential expression analysis (Table 2).

Data Sheet: Gene Expression

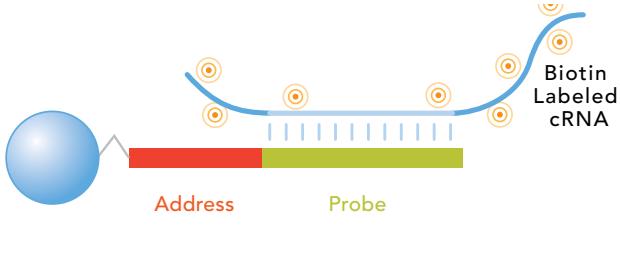
Table 1: Expression BeadChip Content

Probes	Description	Human HT-12 v4.0*	Mouse WG-6 v2.0	Mouse Ref-8 v2.0	Rat Ref-12	Human WG DASL HT*
		12-sample	6-sample	8-sample	12-sample	12-sample
RefSeq Content						
NM	Coding transcript, well-established annotation	28,688	26,766	24,854	6,277	27,253
XM	Coding transcript, provisional annotation	11,121	6,856	796	15,983	426
NR	Non-coding transcript, well-established annotation	1,752	56	47	1	1,580
XR	Non-coding transcript, provisional annotation	2,209			12	26
Source	RefSeq source release	Human RefSeq Rel 38		Mouse RefSeq Rel 22	Rat RefSeq Rel 16	Human RefSeq Rel 38
Supplementary Content						
UniGene	Experimentally confirmed mRNA sequences that align to EST clusters	3,461			250	
RIKEN FANTOM2	Exemplar protein-coding sequences from the RIKEN FANTOM2 database		5,659			
RefSeq Release 5	Transcripts with NM and XM annotation in RefSeq Release 5 (Build 33.1)		3,573			
MEEBO	Probes to transcripts that do not align with 100% accuracy to RefSeq, but are confirmed as valid mRNA mapping to clusters in Expressed Sequence Tag databases ⁶		2,371			
Total		47,231	45,281	25,697	22,523	29,285

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The Direct Hybridization Whole-Gene E

highest multiplexing capabilities for whole-genome gene expression, simultaneously profiling more than 47,000 transcripts. By combining the most up-to-date genomic content with low-cost, high-throughput array processing, Illumina Expression BeadChips efficiently and economically deliver high-quality data for gene expression studies of any size. They are ideal for applications such as differential expression analysis, disease classification, pathway analysis, and expression-based quantitative trait loci (eQTL) studies.



The new HumanHT-12 v4.0 Expression B

efficient human whole-genome gene expression profiling studies with updated content and industry-leading pricing. Its high-value content provides genome-wide transcriptional coverage of well-characterized genes, gene candidates, and splice variants, with a significant portion targeting well-established sequences supported by peer-reviewed literature.

Each array on this BeadChip targets more than 47,000 probes.

Mammal guarantees that 7-33.3% of the bead types will be present on any given HumanHT-12 array. This means up to five probes may be represented with only 0, 1, or 2 copies on each HumanHT-12 array. Probes were designed to cover content from NCBI RefSeq Release 38 (November 7, 2009), as well as legacy UniGene content.

The 12-sample format facilitates large-scale gene expression

information can be easily incorporated into Infinium® assay-based genome-wide association studies (GWAS) or methylation studies.

are Illumina's genome-wide gene expression profiling solutions for

researchers using the mouse as their model organism. The MouseRef-8 BeadChip features recent content derived from the NCBI RefSeq database. In addition to RefSeq content, the MouseWG-6 BeadChip is supplemented with probes that target the Mouse Exonic Evidence Based Oligonucleotide (MEEBO) set², as well as exemplar protein-coding sequences described in the RIKEN FANTOM24-6 database³⁻⁵.

Table 2: Direct Hybridization Gene Expression Assay Product Specifications

Parameter	Specification
Probe-Length	50-mer gene-specific probe, plus 29-mer address sequence
Sensitivity	$\leq 1:250,000$
Dynamic Range	≥ 3 logs
Detectable Fold Change	≤ 1.35 fold
Reproducibility CV	< 10%
Input RNA Required	50–500 ng

(MouseRef-8) in parallel on a single BeadChip, dramatically increasing throughput while decreasing experimental variability. Illumina's 100% hybridization-based QC on every probe ensures that Mouse Expression BeadChips deliver the industry's best performance and reproducibility.

The RatRef-12 Expression BeadChip al

genome-wide expression profiles for twelve samples in parallel on a single BeadChip, with outstanding performance and excellent throughput. BeadChip content (22,523 probes per array) was selected primarily from the NCBI RefSeq database. Low sample input and high-quality data with 100% QC on every feature make the RatRef-12 Expression BeadChip the most comprehensive solution currently available to researchers who are using the rat as a model organism.

The Human Whole-Genome DASL HT assay is

system optimized to produce genome-wide expression profiles from low-abundance or partially degraded human RNA samples, especially those from formalin-fixed, paraffin-embedded (FFPE) tissues. It streamlines the discovery process, allowing researchers to identify new biomarkers by generating expression profiles from archived samples and correlating those profiles with known clinical outcomes.

Competing methods for analyzing FFPE samples are based on lightly treated tissue from which more mRNA can be extracted, or on

and unreliable. Illumina's Whole-Genome DASL HT assay overcomes these limitations through an innovative combination of two proven assays for genome-wide, highly-sensitive expression profiling.

The assay combines the unique PCR and labeling steps from Illumina's proven DASL Assay with the whole-genome probe set of Illumina's

Direct Hybridization assay (Figure 3). This greatly increases the DASL assay target set, which consists of well-characterized NCBI RefSeq (RefSeq Build 36.2, Release 38) genes, while retaining the ability to accurately profile partially-degraded RNA samples.

The Whole-Genome DASL HT assay provides a high-multiplex, low-cost array solution for the simultaneous profiling of over 29,000 transcripts.

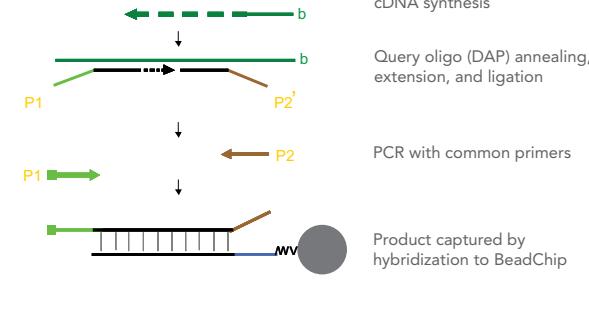
Highly reproducible expression profiles ($R^2 > 0.97$) can be produced from as little as 10–100 ng total RNA from fresh-frozen tissue or 50–200 ng total RNA from FFPE samples.

Expression Array Data Analysis

Illumina Expression BeadChips are part of a complete gene expression solution that includes instrumentation, software, and reagent kits. Data analysis is straightforward, since known biologically relevant transcripts are annotated from well-curated databases and probes are designed and validated by Illumina scientists.

illumina's GenomeStudio Gene Expression Module (Figure 4) enables simplified data management for hierarchical organization of samples, groups, group sets, and all associated project analysis. It offers probe-level and gene-level statistical analysis tools for differential analysis, heat map visualization, and clustering.

DASL HT Assay



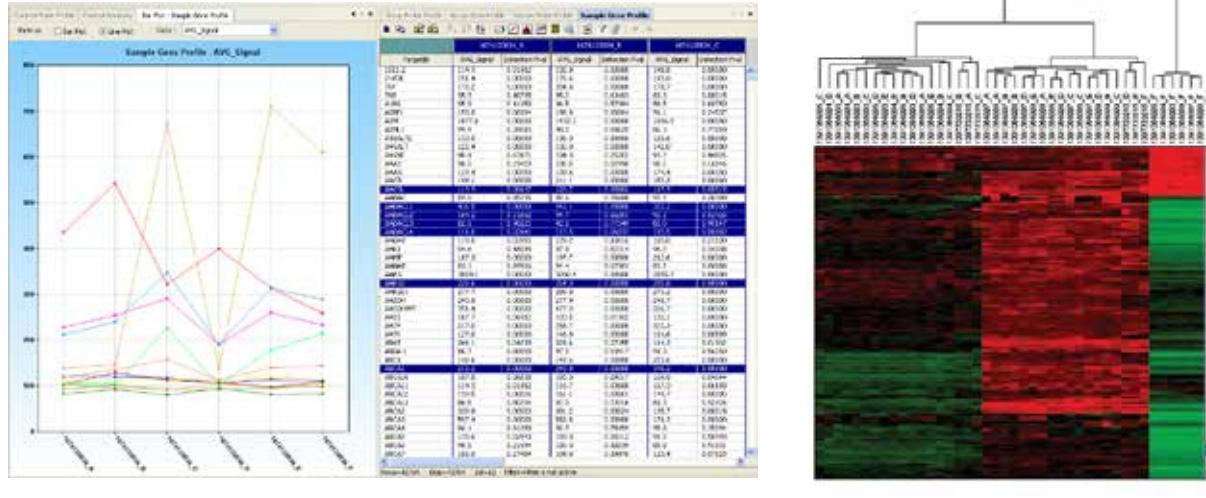
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Illumina's Bead

BeadChips increase throughput, supporting streamlined experiments. These BeadChips contain comprehensive, up-to-date content derived from several important sources. The DASL assay supports expression profiling from FFPE or RNA-limited samples, and is available as a whole-genome gene expression BeadChip on the BeadArray platform. For any experimental design, Illumina products provide the fastest and most cost-effective path to discoveries and publication.

Data Sheet: Gene Expression

Figure 4: GenomeStudio Gene Expression Module



The GenomeStudio software interface (left) provides a flexible graphical interface for data and controls display. GenomeStudio software contains powerful built-in data display tools, such as line graphs, tables, and heat maps (right) for expression analysis.

References

- For more information about NCBI RefSeq, please go to <ftp://ftp.ncbi.nih.gov/refseq/release/>
- For more information about MEEBO, please go to <http://www.arraysexp.org/archive/meebo.html>
- For more information about RIKEN FANTOM 2, please go to <http://fantom2.gsc.riken.jp/>
- To obtain FANTOM 2 clones, please go to http://www.dnaform.jo/index_e.html
- The FANTOM Consortium and The RIKEN Genome Exploration Research Group Phase I and II Team (2002) Analysis of the mouse transcriptome based on functional annotation of 60,770 full-length cDNAs. *Nature* 420: 563–573.
- Kuhn K, et al. (2004) A novel , high-performance random array platform for quantitative gene expression profiling. *Genome Res* 14:2347–2356.
- Goring HH, et al. (2007) Discovery of Expression QTLs Using Large-Scale Transcriptional Profiling in Human Lymphocytes. *Nat Genet* 39: 1208–1216.
- Stranger BE, et al. (2007) Relative Impact of Nucleotide and Copy Number Variation on Gene Expression Phenotypes. *Science* 315: 848–853.
- Weinsheimer S, et al. (2007) Integration of Expression Profiles and Genetic Mapping Data to Identify Candidate Genes in Intracranial Aneurysm. *Physiol Genomics* 32: 45–57.
- Idaghdour Y, et al. (2008) A Genome-Wide Gene Expression Signature of Environmental Geography in Leukocytes of Moroccan Amazighs. *PLoS Genet.* 4: e1000052.
- Mutarelli M, et al. (2008) Time-Course Analysis of Genome-Wide Gene Expression Data from Hormone-Responsive Human Breast Cancer Cells. *BMC Bioinformatics* 9 Suppl 2: S12.
- Integrating Gene Expression Analysis into Genome-Wide Association Studies (PDF) www.illumina.com/Documents/products/technotes/technote_integrating_expression_analysis.pdf
- RNA Profiling with the DASL Assay Tech Bulletin (PDF) www.illumina.com/Documents/products/techbulletins/techbulletin_dasl.pdf
- April C, et al. (2009) Whole-Genome Gene Expression Profiling of Formalin-Fixed, Paraffin-Embedded Tissue Samples. *PLoS ONE* 4 (12): e8162.

Data Sheet: Gene Expression

Ordering Information

Product	Samples	Catalog No.
Human Expression BeadChips		
HumanHT-12 v4.0 Expression BeadChip Kit	72	BD-103-0604
<ul style="list-style-type: none"> • 12 samples per BeadChip • > 47,000 human targets per sample • Includes hybridization buffers, wash buffers, and wash trays 	24	BD-103-0204
Human Whole-Genome DASL HT Assay Kit	24	DA-905-0024 or DA-905-1024
<ul style="list-style-type: none"> • 12 samples per BeadChip • > 29,000 human targets per sample • Includes reagents for amplifying, hybridizing, washing, and processing 	96	DA-905-0096 or DA-905-1096
Mouse Expression BeadChips		
MouseWG-6 v2.0 Expression BeadChip Kit	12	BD-201-0202
<ul style="list-style-type: none"> • 6 samples per BeadChip • > 45,000 mouse targets per sample • Includes hybridization buffers, wash buffers, and wash trays 	36	BD-201-0602
MouseRef-8 v2.0 Expression BeadChip Kit	16	BD-202-0202
<ul style="list-style-type: none"> • 8 samples per BeadChip • > 25,000 mouse targets per sample • Includes hybridization buffers, wash buffers, and wash trays 	48	BD-202-0602
Rat Expression BeadChips		
RatRef-12 Expression BeadChip Kit	24	BD-27-303
<ul style="list-style-type: none"> • 12 samples per BeadChip • > 22,000 rat targets per sample • Includes hybridization buffers, wash buffers, and wash trays 	72	BD-27-302
Related Products		
Illumina TotalPrep RNA Amplification Kit	24	AMIL1791
<ul style="list-style-type: none"> • Available from Ambion: 1-800-888-8804 (U.S.) 	96	4393543
TargetAmp Nano-g Biotin-aRNA Labeling Kit	TAN07924-142	
<ul style="list-style-type: none"> • Available from Epicentre Biotechnologies: 1.800.284.8474 (U.S.) 		

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