

Table 1: Expression BeadChip Content

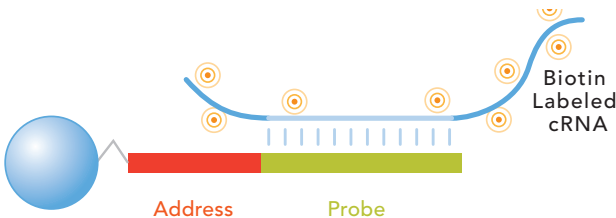
Probes	Description	Human HT-12 v4.0*	Mouse WG-6 v2.0	Mouse Ref-8 v2.0	Rat Ref-12	Human WG DASL HT*
		12-sample	6-sample	8-sample	12-sample	12-sample
RefSeq Content						
NM	Coding transcript, well-established annotation	28,688	26,766	24,854	6,277	27,253
XM	Coding transcript, provisional annotation	11,121	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 ⁹		2,371			
Total		47,231	45,281	25,697	22,523	29,285

* > 99.98% of the bead types are present on any HumanHT-12 array

Direct Hybridization Assay

The Direct Hybridization Whole-Genome Expression assay offers the 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.

Figure 2: Direct Hyb Gene Expression Profiling Bead Design



Illumina array-based technologies support a broad array of RNA expression profiling products for a variety of applications.

HumanHT-12 Expression BeadChip

The new HumanHT-12 v4.0 Expression BeadChip supports highly 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. Illumina guarantees that > 99.98% 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 applications. With the HumanHT-12 BeadChip, expression information can be easily incorporated into Infinium[®] assay-based genome-wide association studies (GWAS) or methylation studies.

MouseWG-6 and MouseRef-8 Expression BeadChips

The MouseWG-6 v2.0 and MouseRef-8 v2.0 Expression BeadChips 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	≤ 1:250,000
Dynamic Range	≥ 3 logs
Detectable Fold Change	≤ 1.35 fold
Reproducibility CV	< 10%
Input RNA Required	50–500 ng

Researchers can profile from six (MouseWG-6) to eight samples (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.

RatRef-12 Expression BeadChip

The RatRef-12 Expression BeadChip allows researchers to generate 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.

Whole-Genome DASL HT Assay

The Human Whole-Genome DASL HT assay is the only integrated 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 tested protocols from multiple vendors, which are often both costly 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.

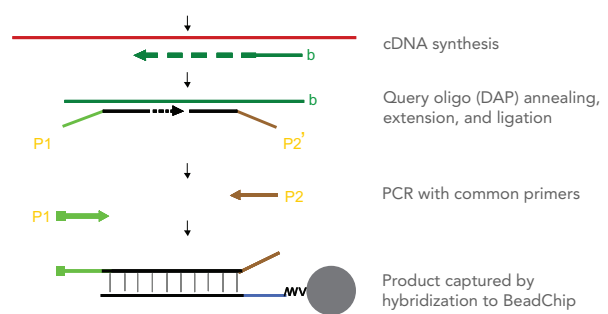
The assay combines the unique PCR and labeling steps from Illumina's proven DASL Assay with the whole-genome probe set of Illumina's Direct Hybridization assay (Figure 3). This greatly increases the DASL assay target set, which consists of well-characterized NCBI RefSeq (RefSeq Build 36.2, Release 38) 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.

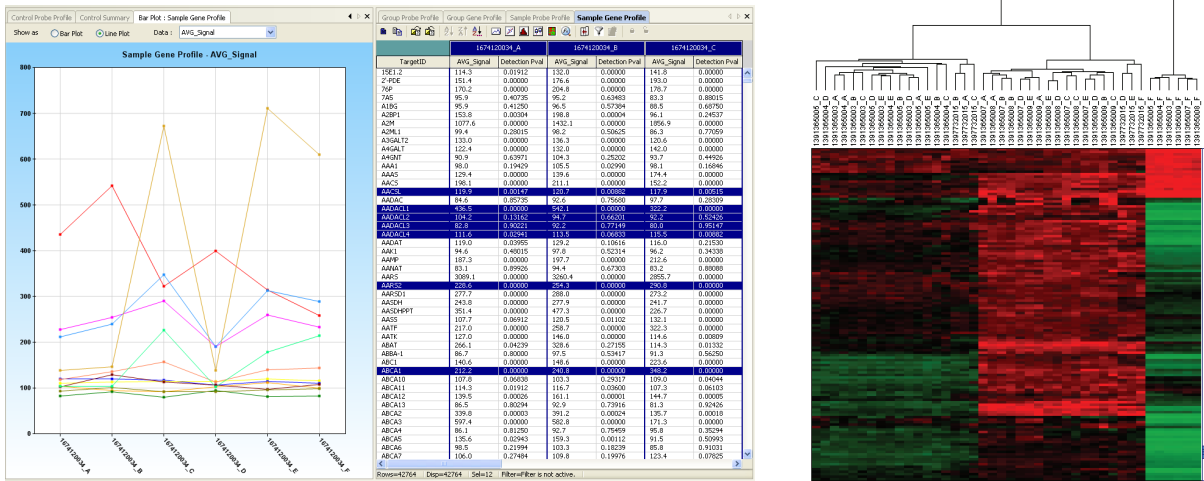
Figure 3: RNA Profiling with the Whole-Genome DASL HT Assay

Illumina array-based technologies support a broad array of RNA expression profiling products for a variety of applications.

Summary

Illumina's BeadArray-based technology enables cost-effective expression profiling solutions for human, mouse, and rat. Multi-sample 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.

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/](http://ftp.ncbi.nih.gov/refseq/release/)
- For more information about MEEBO, please go to <http://www.arrays.ucsf.edu/archives/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.dnafom.jp/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.
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- April C, et al. (2009) Whole-Genome Gene Expression Profiling of Formalin-Fixed, Paraffin-Embedded Tissue Samples. *PLoS ONE* 4 (12): e8162.

