



MouseWG-6 v1.1 and MouseRef-8 v1.1 Expression BeadChips

The MouseWG-6 v1.1 and MouseRef-8 v1.1 Expression BeadChips allow researchers to generate genome-wide expression profiles for multiple samples in parallel on a single microarray, with outstanding performance and industry-leading pricing.

INTRODUCTION

The Illumina MouseWG-6 and MouseRef-8 v1.1 Expression BeadChips are genome-scale, gene expression microarrays built using the unique multi-sample Array of Arrays™ formats (Figure 1). BeadChip content was created by combining proven sources, including the Mouse Exonic Evidence Based Oligonucleotide¹ (MEEBO) set, the RIKEN FANTOM 2²⁻⁵ database, and the National Center for Biotechnology Information (NCBI) Reference Sequence (RefSeq) database. The MouseWG-6 and MouseRef-8 BeadChips are part of a complete gene expression solution that includes instrumentation, software, reagent kits, and access to an extended family of bead-based array products.

HIGHLIGHTS OF THE MOUSEWG-6 V1.1 AND MOUSEREF-8 V1.1 EXPRESSION BEADCHIPS

- **Confidence in Your Results**
Outstanding data quality with an average 30-fold redundancy and 100% hybridization-based array QC
- **Trusted Content**
Designed using NCBI RefSeq and RIKEN FANTOM 2 databases
- **Low RNA Input Requirements**
50–100ng total RNA needed
- **Freedom to Expand Your Science**
More samples for less than half the cost of other arrays

ARRAY CONTENT

Content for MouseWG-6 and MouseRef-8 BeadChips is based largely on the MEEBO set. This publicly-available set of 70mer oligonucleotide probe sequences was created by an international group of mouse researchers for use with spotted arrays. For the creation of BeadChip content, Illumina scientists designed 50mer subsequences based on the MEEBO oligonucleotides. The MEEBO set is largely derived from constitutively expressed exons, allowing the interrogation of almost 25,000 genes. The MEEBO set was designed to enable the study of mouse transcription patterns and, as broadly as possible, alternative splicing. An exon-centric design was selected to allow the differentiation of constitutively expressed versus alternatively expressed exons. In addition to the exon-centric probes, an extensive assortment of controls that facilitate accurate evaluation of expression results were included in the MEEBO set. The MouseWG-6 BeadChip contains probes corresponding to > 95.2% of all MEEBO 70mers.

The MEEBO content represented on the BeadChips is supplemented with more than 11,000 probes for additional targets from RIKEN FANTOM 2 and the NCBI RefSeq transcript databases. Greater than 98.3% of transcripts from RefSeq Release 5 and > 88% of coding transcripts from

FIGURE 1: MOUSEWG-6 V1.1 AND MOUSEREF-8 V1.1 EXPRESSION BEADCHIPS



The MouseWG-6 v1.1 Expression BeadChip (right) contains six whole-genome gene expression arrays allowing six samples to be hybridized to a single chip. The MouseRef-8 v1.1 Expression BeadChip (left) contains eight arrays for eight samples.

RIKEN FANTOM 2 are covered.

Descriptions of probe categories and the number of probes in each category appear in Table 1.

BEADARRAY™ TECHNOLOGY

The MouseWG-6 and MouseRef-8 BeadChips were designed using Illumina BeadArray technology. The BeadChips are constructed by introducing oligonucleotide-bearing, 3-micron beads to microwells etched into the surface of a slide-sized, silicon substrate. During the manufacturing process, beads self-assemble into the microwells of the

TABLE 1: CONTENT SOURCES

Category	Description	N (MouseWG-6)	N (MouseRef-8)
MEEBO-BASED PROBES			
Constitutive	Constitutive exons/islands based on the Rockefeller University MousDB3 and NCBI LocusLink databases.	24,334	16,287
Constitutive: Intron/Exon Boundaries	mRNA-derived probes that may span intron/exon boundaries.	4,963	4,154
Spliced/Skipped	A collection of alternative spliced/skipped exons generated through extensive curation of five published datasets by Max Diehn, Ash Alizadeh, Jean Yang, and Catherine Foo	4,143	3,169
BCR/TCR* Genic/Regional Probes	Probes recognizing transcripts from genes that undergo somatic rearrangement.	352	235
Miscellaneous	Probes recognizing mitochondrial DNA sequences, ribosomal RNA sequences, or syntenic orthologs of human loci exhibiting cis-antisense transcription, based on Yelin et al. ⁵	179	92
Murine Viral	Probes recognizing mouse viral sequences.	285	0
Transgenic Cassettes	Probes recognizing elements commonly used for transgenic constructs, e.g., GFP and Neo.	35	0
Positive Controls	Probes recognizing widely expressed mouse sequences.	9	9
Doped Controls	Probes recognizing non-mouse sequences that can be spiked into RNA samples.	220	0
SUPPLEMENTARY PROBES			
Supplementary RIKEN	Probes to transcripts present in the RIKEN FANTOM 2 database that are hit by no MEEBO probe with > 80% identity.	5,813	0
Supplementary RefSeq 5	Probes to transcripts present in the RefSeq database (Release 5) that are hit by no MEEBO probe with > 80% identity.	5,663	20
Supplementary RefSeq 19	Probes to transcripts present in the RefSeq database (Release 19) that are hit by no MEEBO probe or RefSeq 5 probe with > 80% identity.	647	647
Negative Controls	Random sequences used for the assessment of background noise.	1,675	822
TOTALS		48,318	25,435

*B-Cell Receptor/T-Cell Receptor

BeadChips. Each bead contains hundreds of thousands of copies of covalently-attached, full-length oligonucleotide probes and is represented with an average 30-fold

redundancy. This means each reading is taken multiple times across the array, increasing the precision of the measurement. The high quality inherent in this redundancy is bol-

stered by the Illumina manufacturing process. After random bead assembly, 29mer address sequences present on each bead are used for a hybridization-based procedure

TABLE 2: PRODUCT SPECIFICATIONS

Specification	Value
Probe Length	50mer gene-specific probe plus 29mer address sequence ^a
Probes	48,318 (MouseWG-6) 25,435 (MouseRef-8)
Sensitivity ^{b,c}	≤ 1:250,000
Dynamic Range ^{b,d}	≥ 3 logs
Precision ^{b,e}	≤ 1.35 fold
Starting Material ^f	50–100 ng
Hybridization Volume	30 µl (MouseWG-6) 15 µl (MouseRef-8)

^a The address sequence is used to map and decode the array

^b Determined by spiking experiments in a labeled mouse RNA background using pre-labeled heterologous targets of defined concentration

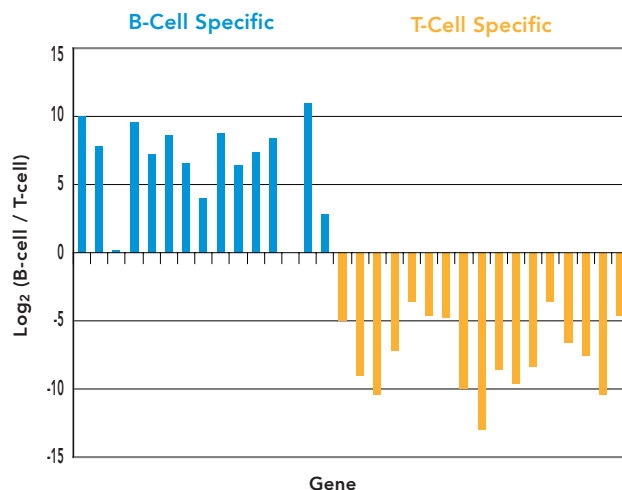
^c 99% confidence, based on non-parametric statistical assessment of the distribution of > 1,600 negative controls for MouseWG-6, > 800 for the MouseRef-8

^d Defined as the concentration range over which concentration changes of ≤ 2-fold can be determined with 95% confidence

^e Median measurement precision within the dynamic range, 95% confidence

^f Total RNA with a single round of IVT amplification; range may vary based on input material source and quality

used to map the array, identifying the location of each bead. This final process validates the hybridization performance of every bead on all BeadChips and provides 100% array QC. The MouseWG-6 and MouseRef-8 BeadChips are arranged in an Array of Arrays format, providing six arrays on the MouseWG-6 BeadChip, eight on the MouseRef-8 BeadChip. The arrays are separated by a seal so that a different sample can be hybridized to each array. All steps downstream of hybridization are performed in parallel on each BeadChip, reducing experimental variation and decreasing handling. MouseWG-6 and MouseRef-8 BeadChips are scanned on the Illumina BeadArray Reader, a

FIGURE 2: BIOLOGICAL VALIDATION OF MOUSEWG-6 BEADCHIP PERFORMANCE

Messenger RNA was purified from cultured mouse B-cell lymphoma line A-20 or T-cell lymphoma line R1.1 (both lines purchased from American Type Culture Collection) using RNeasy (Qiagen), then amplified and labeled. The labeled product was hybridized to each of four arrays (1.5 µg/array) on a MouseWG-6 BeadChip. Log₂ ratios of normalized averaged intensity values for the 32 genes examined are shown. Bars are coded as blue (B-cell specific) or orange (T-cell specific) based on literature searches performed prior to the experiment. All genes selected by the literature searches are shown, irrespective of array results.

sub-micron resolution scanner that can scan the 3-micron, high-density features of the MouseWG-6 and MouseRef-8 BeadChips. A special adapter tray allows automated scanning of three BeadChips (18 to 24 arrays) in a single scan session.

PROBE DESIGN ALGORITHMS

Each address and probe sequence combination has been carefully selected bioinformatically. Gene-specific probes were designed using a multi-step algorithm scoring the following parameters:

- 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

OLIGONUCLEOTIDE SYNTHESIS/QC

The the Illumina proprietary Oligator[®] technology is used to manufacture oligonucleotides incorporated into the arrays. Illumina utilizes multiple methods to assure oligonucleotide quality including:

- Real-Time Digital Trityl Monitoring: Real-time monitoring of the coupling success of each base addition for every oligo synthesized
- Capillary Electrophoresis (CE): CE is used to achieve single-base resolution of synthesis success for long oligos. Precise abundance measurements with stepwise coupling efficiency values are gathered
- Optical Density (OD₂₆₀) Analysis: Oligo yield is quantitated by measurement of UV absorbance at 260 nm

ORDERING INFORMATION

CATALOG NO.	PRODUCT	DESCRIPTION
BD-26-113	MouseWG-6 v1.1 Expression Beadchip	Two-pack of the MouseWG-6 v1.1 Expression BeadChips for the analysis of > 46,000 mouse targets based on the RefSeq, RIKEN FANTOM 2 databases, and other data sources. Includes hybridization buffers, wash buffers, and wash trays.
BD-26-213	MouseRef-8 v1.1 Expression Beadchip	Two-pack of the MouseRef-8 v1.1 Expression BeadChips for the analysis of > 24,000 mouse targets based on the NCBI mouse RefSeq database. Includes hybridization buffers, wash buffers, and wash trays.
BD-26-111-CSE	MouseWG-6 v1.1 Expression Beadchip Customer Sample Evaluation	Illumina scientists will run up to 10 customer samples using two MouseWG-6 v1.1 BeadChips. All standard data output files will be supplied to customer.
BD-26-211-CSE	MouseRef-8 v1.1 Expression Beadchip Customer Sample Evaluation	Illumina scientists will run up to 14 customer samples using two MouseRef-8 v1.1 BeadChips. All standard data output files will be supplied to customer.

RELATED PRODUCTS

SC-16-103	Illumina BeadStation 500	A flexible system for genetic analysis, supporting an expanding portfolio of applications. Includes hardware, software, training and warranty (available in 110V and 220V).
IL1791	Illumina TotalPrep RNA Amplification Kit	Available from Ambion (www.ambion.com), telephone: 1.800.888.8804 or 1.512.651.0201

PERFORMANCE

BeadChip performance has been tested for fundamental assay dose-response characteristics by techniques previously described^{6,7}. Performance specifications are listed in *Table 2*.

Gene expression differences in mouse B and T cells were used to validate biological performance of the MouseWG-6 BeadChip (*Figure 2*). Expression ratios (B cell/T cell) were examined for 32 genes previously determined, in peer-reviewed publications, to be B- or T-cell-specific. Thirty-one genes produced ratios corresponding to the literature-based predictions; the remaining gene showed no evidence of expression by either cell type.

MOUSE WHOLE-GENOME SOLUTION

The Illumina comprehensive coverage of the mouse genome in multiple array formats enables flexibility, while low-volume hybridization supports cost-effectiveness. Use the Illumina Whole-Genome Expression Solution, from BeadChips to BeadStudio analysis software, to find answers to gene expression-related questions. Low sample input and high-quality data with 100% QC on every feature deliver the most comprehensive solution currently available to mouse researchers.

ADDITIONAL INFORMATION

To learn more about the MouseWG-6 v1.1 or MouseRef-8 v1.1 Expression BeadChip visit www.illumina.com, contact your local sales representative, or Customer Solutions.

REFERENCES

- (1) For more information about MEEBO, please go to <http://www.arrays.ucsf.edu/meebo.html>.
- (2) The FANTOM Consortium and The RIKEN Genome Exploration Research Group Phase I and II Team. Analysis of the mouse transcriptome based on functional annotation of 60,770 full-length cDNAs. (2002) *Nature* 420: 563-573.
- (3) For more information about RIKEN FANTOM 2, please go to <http://fantom2.gsc.riken.jp/>.
- (4) To obtain FANTOM 2 clones, please go to http://www.dnaform.jp/index_e.html.
- (5) Yelin R, Dahary D, Sorek R, Levanon E Y, Goldstein, et al. (2003) Widespread occurrence of antisense transcription in the human genome. *Nature Biotechnol* 21: 379-386.
- (6) Kuhn K, Baker SC, Chudin E, Lieu M, Oeser S, et al. (2004) A novel, high-performance random array platform for quantitative gene expression profiling. *Genome Res* 14: 2347-2356.
- (7) Whole-genome gene expression analysis using the Human-6 and HumanRef-8 Expression BeadChips Technical Bulletin at www.illumina.com.

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