

Human-6 v2 and HumanRef-8 v2 Expression BeadChips

The Human-6 v2 and HumanRef-8 v2 Expression BeadChips allow researchers to generate whole-genome expression profiles for six or eight samples in parallel on a single BeadChip, with industry-leading price and performance.

INTRODUCTION

The Human-6 v2 and HumanRef-8 v2 Expression BeadChips (Figure 1) enable large-scale biological experimentation, opening doors to differential expression analysis, disease classification, and pathway analysis. Researchers can generate whole-genome expression profiles for multiple samples on a single BeadChip (Figure 2), which is key for better experimental design. Researchers can profile six or eight samples per BeadChip, with more than 48,000 transcript probes per sample using the Human-6 v2 and more than 22,000 transcript probes using the HumanRef-8 v2. Low per-sample

array pricing means that researchers can do more for less, generate more accurate statistics, and feel confident about their conclusions.

These Expression BeadChips are part of a complete Illumina Gene Expression Solution, which includes instrumentation, software, reagent kits, and access to an extended family of bead-based Array of Array™ products.

BEADCHIP CONTENT

Illumina BeadArray technology has 3-micron features, configurable geometries, and oligo-based content flexibility, making it ideal for a wide range of applications.

Each array on an Expression BeadChip provides genome-wide transcriptional coverage of well-characterized genes, gene candidates, and splice variants.

PROBE DESIGN STRATEGY

T_m score, self-complementarity, and a specificity score were used to construct probe content. Content includes probes for genes with unique locations in the human genome. Probes were specifically designed to avoid querying pseudo-genes and SNP sites.

The curated content on the HumanRef-8 v2 BeadChip is based on the curated content from the National Center for Biotechnology

HIGHLIGHTS OF THE HUMAN-6 V2 AND HUMANREF-8 V2 EXPRESSION BEADCHIPS

- Low Array-to-Array Variability: multi-sample format
- High Sensitivity: optimized 50mer probes
- Industry's Highest-Density Arrays: low reagent volumes
- Low Sample Volume Required: 50-100ng total RNA input
- Low Per-Sample Cost: less than half the price of other commercial arrays
- Easy to Use: improved coverseal technology with IntelliHyb Seal for faster processing

FIGURE 1: HUMAN-6 V2 AND HUMANREF-8 V2 EXPRESSION BEADCHIPS



Profile six or eight individual samples in parallel on the Human-6 v2 (right) and HumanRef-8 v2 (left) Expression BeadChips, respectively.

FIGURE 2: MULTI-SAMPLE FORMAT



Each sample on the Human-6 v2 BeadChip is probed by a pair of arrays.

Information (NCBI) Reference Sequence (RefSeq) database¹, Release 17, with probe content that is well-annotated and widely accepted. The Human-6 v2 contains the probes from the HumanRef-8 v2 with additional probes designed based on UniGene release 188.

To quantitate mRNA levels for each sample, 1.8 million beads are available on the Human-6 v2 BeadChip, 900,000 on the HumanRef-8 v2 BeadChip. In total, the Human-6 v2 BeadChip contains over ten million features, and the HumanRef-8 v2 contains over seven million features.

BEAD DESIGN

Optimized full-length 50mer oligo probes combine with redundant feature representation to deliver a combination of high sensitivity, selectivity, and measurement precision. Illumina Expression beads contain 50mer gene-specific probes and harbor several hundred thousand copies of the probe across the bead surface. Concatenated to the probe is a short “address sequence,” which is used during the array manufacturing process to identify the bead (Figure 3).

BEADCHIP PERFORMANCE

Illumina has compiled performance data for the Human-6 v2 and HumanRef-8 v2 Expression BeadChips from both internal and external sources (Table 1). Reproducibility has been demonstrated by high concordance and precision between hybridization replicates. These industry-leading performance specifications minimize false discovery rates for differential expression.

STANDARD ASSAY PROTOCOL

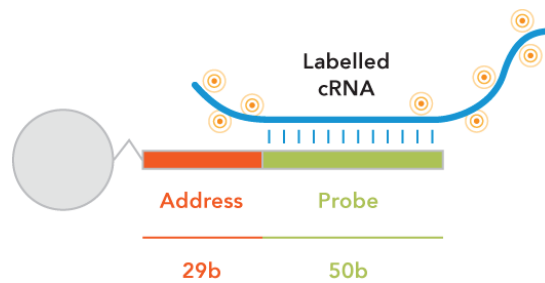
The Human-6 v2 and the HumanRef-8 v2 BeadChips employ a standard assay protocol for sample preparation and labeling^{2,3}. RNA isolation, cDNA synthesis, and *in vitro* transcription amplification are followed by hybridization. With the Illumina TotalPrep RNA Amplification Kit from Ambion, just 50–100ng of total RNA and one round of amplification are required for each sample. The new BeadChips are imaged using Illumina’s BeadArray Reader.

TABLE 1: ARRAY PERFORMANCE*

Precision (fold-change)	≤ 1.35 fold
Dynamic Range	≥ 3 logs
Sensitivity	< 1:250K
Array-to-Array Variation (CV)	< 10%

*Values were obtained from dose-response spiking experiments on Expression BeadChips. Values are the median response of all genes.

FIGURE 3: BEAD DESIGN



Gene-specific probes are concatenated with a short "address sequence."

BEADSTUDIO GENE EXPRESSION MODULE

Output files can be exported to third-party software for analysis, or they can be conveniently analyzed using Illumina's BeadStudio software (Figure 4). Gene-level statistical analysis tools can be used for differential analysis, heat map visualization (Figure 5), and clustering. The BeadStudio Gene Expression Module enables simplified data management for hierarchical organization of samples, groups, groupsets, and all associated project analysis.

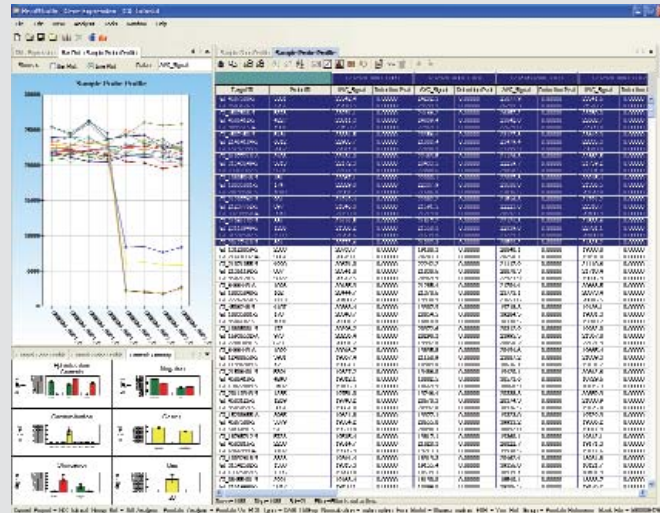
ILLUMINA SOLUTIONS FOR GENE EXPRESSION

High-quality data and low per-sample cost of the Human-6 v2 and HumanRef-8 v2 Expression Bead-Chips are part of the powerful Illumina Gene Expression Solution. The combination of Illumina's assay technologies and flexible content deployment delivers the most comprehensive solution for gene expression now available. Illumina Gene Expression products, with standard or custom content, can be accessed via the Illumina Customer Sample Evaluation (CSE) Program or one's own Illumina BeadStation. Illumina solutions provide industry-leading levels of accuracy, flexibility, and affordability.

REFERENCES

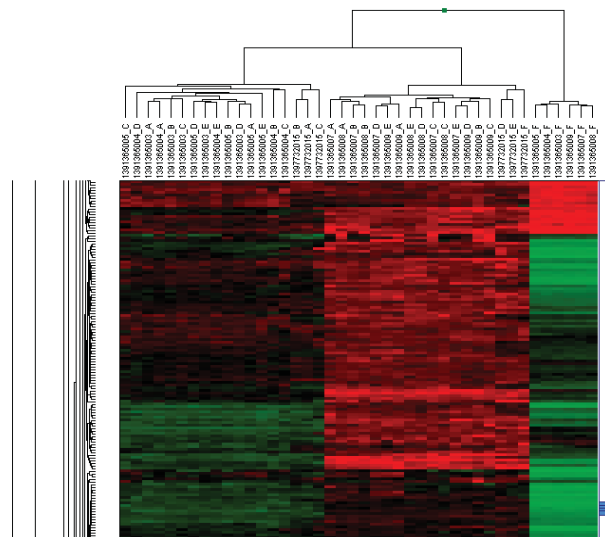
- (1) For NCBI RefSeq information, visit <ftp://ftp.ncbi.nih.gov/refseq/release/>.
- (2) Kuhn K, Baker SC, Chudin E, M Lieu, S Oeser, et al. (2004) A novel, high-performance random array platform for quantitative gene expression profiling. *Genome Research* 14: 2347-2356.
- (3) Van Gelder RN, von Xastrow MF, Yool A, Dement DC, Barchas JD, et al. (1990) Amplified RNA synthesized from limited quantities of heterogeneous cDNA. *Proc Natl Acad Sci USA* 87: 1663-1667.

FIGURE 4: THE BEADSTUDIO GENE EXPRESSION MODULE



The BeadStudio software interface provides user flexibility for array selection, imaging, and expression analysis.

FIGURE 5: THE BEADSTUDIO GENE EXPRESSION MODULE: HEAT MAP



Differential expression is represented using the Heat Map tool in the Gene Expression Module.

ORDERING INFORMATION

CATALOG NO.	PRODUCT	DESCRIPTION
BD-25-113	Human-6 v2 Expression BeadChipKit (2)	Each Human-6 v2 BeadChip simultaneously assays six samples with > 48,000 probes per sample, targeting genes and known alternative splice variants from the RefSeq database release 17 and UniGene build 188. Includes two BeadChips, hybridization and wash buffers, and wash trays.
BD-25-213	HumanRef-8 v2 Expression BeadChip Kit (2)	Each HumanRef-8 v2 BeadChip simultaneously assays eight samples with > 22,000 probes per sample targeting genes and known alternative splice variants from the RefSeq database release 17. Includes two BeadChips, hybridization and wash buffers, and wash trays.
BD-25-111-CSE	Human-6 v2 Expression BeadChip Customer Sample Evaluation	Illumina scientists will run up to 10 customer samples using two Human-6 v2 BeadChips. All standard data output files will be supplied to the customer.
BD-25-211-CSE	HumanRef-8 v2 Expression BeadChip Customer Sample Evaluation	Illumina scientists will run up to 14 customer samples using two HumanRef-8 v2 BeadChips. All standard data output files will be supplied to the customer.
RELATED PRODUCTS		
UG-10-301	IntelliHyb Gene Expression Hyb Chamber Set	Hyb chamber upgrade kit for use with Expression BeadChips with IntelliHyb seals. Includes hyb chamber, hyb chamber gasket, and four hyb chamber BeadChip inserts.
BD-60-503	Rocker Attachment for Hybridization Oven	Rocker unit for the Hybridization Oven, used with Whole-Genome Gene Expression and Infinium Whole-Genome Genotyping
SC-16-103	Illumina BeadStation 500	A flexible system for genetic analysis, that supports 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)

ADDITIONAL INFORMATION

To learn more about the Human-6 v2 and HumanRef-8 v2 Expression BeadChips, visit our website or contact us.

Illumina, Inc.
Customer Solutions
 9885 Towne Centre Drive
 San Diego, CA 92121-1975
 1.800.809.4566 (toll free)
 1.858.202.4566 (outside the U.S.)
 expression@illumina.com
 www.illumina.com

FOR RESEARCH USE ONLY

© 2006 Illumina, Inc.
 Illumina, Making Sense Out of Life, Sentrix, GoldenGate, DASL, BeadArray, Array of Arrays, Infinium, BeadXpress, VeraCode, IntelliHyb, iSelect, and CSPPro are registered trademarks or trademarks of Illumina. All other brands and names contained herein are the property of their respective owners.
 Pub. No. 470-2006-010 23Dec06

