illumına®

mRNA Expression Analysis

Sequencing- and array-based technologies support a broad range of RNA expression profiling products. These products provide workflows and features tailored to various applications, such as discovery, screening, or the use of traditionally difficult to analyze samples.

Highlights of Illumina MRNA Expression Analysis

- **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 or sequencing applications for discovery of any mRNA
- **Any Species:**
  Sequencing applications don't require prior gene identification or annotation
- **High Throughput:**
  Multi-sample BeadChips with automation options or high output sequencing of several gigabases per run

Figure 1: MRNA Discovery and Profiling Options

Illumina sequencing- and array-based technologies support a broad range of RNA expression profiling products. These products facilitate a wide range of applications, such as discovery, profiling, or the use of traditionally difficult to analyze samples.
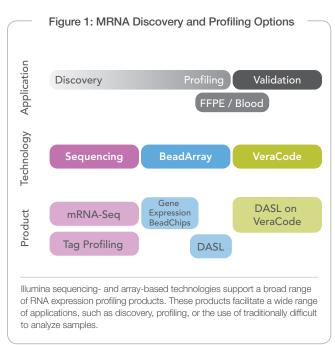
Introduction

Illumina leads the genetic analysis industry with innovative, flexible products designed for a broad scope of genetic research. For mRNA analysis and gene expression profiling, Illumina has created a range of products based on massively parallel sequencing and multiplex expression profiling assay technologies to provide benefits tailored to any study design (Figure 1). Each assay provides high-quality data, comprehensive gene coverage, and unique features for a wide spectrum of experiments from novel transcript and variant discovery to expression profiling in model organisms.

MRNA Discovery and Profiling

Illumina has a broad portfolio of gene expression analysis products to satisfy the needs of diverse experimental designs. By taking advantage of several innovative underlying technologies, an optimal solution is available regardless of which features are primary, such as highest sample throughput or maximal unbiased discovery.

Illumina sequencing determines several billion bases (gigabases, Gb) of sequence data per week. As a result of the massive output rates, this technology offers unparalleled benefits for discovery-stage applications. Nearly any poly-A transcript isoform in any species can be identified and quantified with mRNA-Seq. Tag Profiling uses sequencing technology to quantify expression levels of known and novel transcripts with a more traditional directed strategy, by sequencing short 3' transcript fragments. The efficient tag strategy of Tag Profiling provides a highersample throughput sequencing option.

Illumina also offers gene expression profiling products based on BeadArrayTM technology (Figure 2). The Direct Hyb and DASL® As-

says are deployed on BeadArray substrates. Both assays are highly accurate, with streamlined workflows and multi-sample formats for high-throughput multiplex gene expression profiling. Direct Hyb offers the highest multiplexing for whole-genome expression profiling of up to 48,000 transcripts. The DASL Assay can be used for high-multiplex whole-genome profiling or for more focused customizable panels. The DASL Assay is highly robust to degraded RNA and is ideal for profiling and screening formalin-fixed paraffin-embedded (FFPE) samples.

Maximal sample throughput with low- to mid-multiplex expression profiling is achieved by using the DASL assay with VeraCode® technology on the BeadXpress® platform.

MRNA-SEQ

mRNA sequencing can be performed on one of Illumina's stand-alone next generation sequencing systems, such as the HiSeq™, Genome AnalyzerIIx or Genome AnalyzerIIe ,or on its integrated HiScanSQ system that can perform gene expression analysis and next generation sequencing. The mRNA-Seq application uses Illumina sequencing technology to provide essentially unlimited discovery and profiling of the entire mRNA universe. With no probes or primers to design, mRNA-Seq is free to deliver unbiased and unparalleled information about the transcriptome. Researchers quickly generate full sequence information from any poly-A tailed RNA to analyze gene expression, cSNPs, novel transcripts, novel isoforms, alternative splice sites, allele specific expression, and rare transcripts in one experiment. The simple

Figure 2: Illumina BeadArray and Sequencing Technology

The BeadArray platform supports highly multiplexed gene expression profiling assays (left). High-throughput sequencing of samples is performed on a flow cell (right) with one of Illumina's stand-alone sequencing systems, such as the HiSeq™, Genome AnalyzerIIx, or Genome AnalyzerIIe, or on its integrated HiScan™SQ system.

sample preparation protocol and data analysis software support mRNA-Seq discovery in any species.

Tunable Sensitivity

mRNA-Seq offers a new level of assay customizability to optimize each experiment to a study's specific design and purposes. Sequencing data are essentially digital (numerical frequency of discrete base strings), so coverage depth can be tuned by the user and increased simply by sequencing more of the sample. This customizable depth facilitates the entire range of applications, from expression profiling and sample classification with low read count to rare transcript or variant discovery with deep coverage.

Persistent Data and Novel Discovery

Also unique to sequencing-based RNA discovery is the persistence of the data and the ability to record the presence of novel transcripts. Since the expression level data set is in terms of the actual sequence of mRNA, rather than probe IDs, researchers don't miss out on analyzing as-yet unknown exons. Future identified mRNA can be located in a previous data set without re-running the samples. Novel transcripts can be identified with mRNA-Seq because there are no probes to design from prior sequence knowledge.

High-Quality Data

The high accuracy of Illumina sequencing contributes to the high data quality generated with mRNA-Seq. Illumina's sequencing systems generate per-base read accuracy greater than 98.5%, and consensus accuracy of 99.99% at greater than 3× coverage.

Illumina scientists rigorously test all products during development to ensure consistent high quality performance. High accuracy and sensitivity allow researchers to quantify expression differences between samples (Figure 3). High concordance between results generated with mRNA-Seq and qPCR provide an assay-independent confirmation of the accuracy of mRNA-Seq data (Figure 4).

Differential expression is determined with high confidence since the robust mRNA-Seq protocol is highly reproducible. Technical reproducibility experiments have calculated the correlation (r) to be greater than 0.99.

Sequencing generates single base resolution data, enabling high resolution identification of splice sites or SNPs in transcripts (*Figure* 5).

Simple Automated Assay Workflow

Sample preparation for mRNA-Seq is straightforward and uses standard molecular biology techniques requiring minimal hands-on time (Figure 6). As a result, researchers can progress from RNA collection to analyzing their data in less than a week.
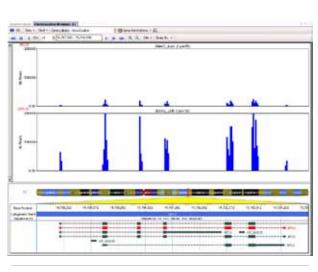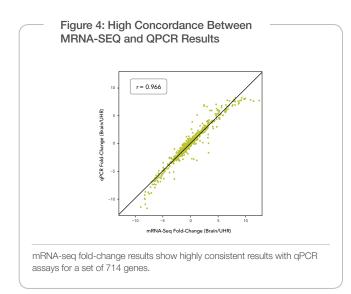
Figure 3: Tissue-Specific Expression Detected with MRNA-SEQ

Comprehensive assay designer, streamlined workflow, and intuitive analysis tools support flexible custom assay development.

Figure 4: High Concordance Between MRNA-SEQ and QPCR Results

r = 0.966

qPCR Fold-Change (Brain/UHR)

mRNA-Seq Fold-Change (Brain/UHR)

mRNA-seq fold-change results show highly consistent results with qPCR assays for a set of 714 genes.

First, cellular poly-A tailed RNA is isolated. RNA is fragmented and randomly primed for reverse transcription to generate double stranded cDNA fragments to be sequenced. Supplied sequencing adaptors are ligated to the cDNA fragments, which are size-selected by gel electrophoresis and excision. A limited cycle PCR step ensures minimal contamination of RNA and unligated cDNA remains in the sample.

These mRNA template libraries are sequenced just as any DNA sample using Illumina sequencing. They are loaded onto the fully automated Cluster Station where they bind to complementary adapter oligos grafted onto a proprietary flow cell surface. The Cluster Station isothermally amplifies these cDNA constructs to create clonal clusters of ~1000 copies each. The resulting high-density array of template clusters is then directly sequenced on one of Illumina's sequencing systems. Illumina sequencing technology uses four proprietary, fluorescently labeled, reversibly terminated nucleotides and a specialized polymerase to rapidly sequence the tens of millions of clusters base by base in parallel.

Tag Profiling

For a slightly more directed search through genome-wide mRNA, Tag Profiling uses Illumina sequencing technology to identify and quantify transcripts by sequencing short tags rather than entire transcripts. The Illumina Tag Profiling protocol identifies mRNA transcripts by their unique, positionally known 20- or 21-base pair cDNA tag. These tags are compared to a species' reference genome to identify the genes expressed.

Tag Profiling provides similar benefits to mRNA-Seq, such as digital data for tunable depth and coverage, no need for probe design to support unbiased discovery, and persistent data that can be reannotated as genome databases evolve. Since
Tag Profiling does not attempt to sequence the entire length of all expressed genes, its sensitivity is higher with fewer total reads. For example, four million tags per sample yields an average of 12 counts for transcripts present at one copy per cell. Researchers can make use of the tunable depth of coverage for an almost unlimited dynamic range, to accomplish rare transcript discovery and quantification.

Tag Profiling also offers researchers an ideal global orthogonal validation method for hybridization arrays.

High Quality Data

Tag Profiling creates and sequences positionally registered tags of 20 or 21 base pairs. Tag sequence information is then used for confident identification of novel transcripts from any eukaryotic genome. Theoretical calculations suggest that over 99.8% of 21-base pair tags occur only once in genomes the size of the human genome1. Analyses based on actual sequence information from ~16,000 known genes suggest that more than 75% of 21-base pair tags are expected to occur only once in the human
genome1. The remaining tags match duplicated genes or repeat sequences.
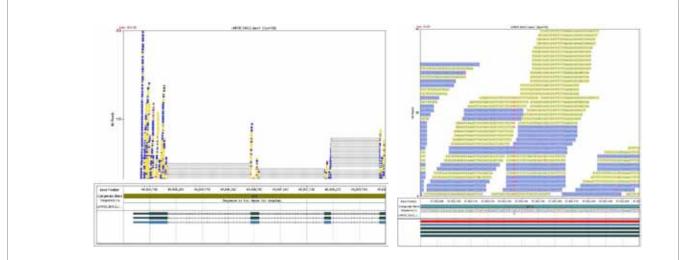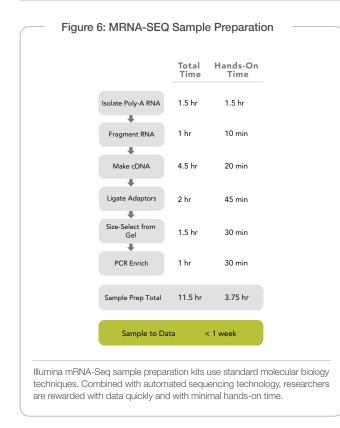
Figure 5: Splice Junctions and CSNPS Detected with Single E-Base Resolution

The BeadArray platform supports highly multiplexed gene expression profiling assays (left). High-throughput sequencing of samples is performed on a flow cell (right) with one of Illumina's stand-alone sequencing systems, such as the HiSeq™, Genome AnalyzerIIx, or Genome AnalyzerIIe, or on its integrated HiScan™SQ system.

Figure 6: MRNA-SEQ Sample Preparation

	Total Time	Hands-On Time
Isolate Poly-A RNA	1.5 hr	1.5 hr
Fragment RNA	1 hr	10 min
Make cDNA	4.5 hr	20 min
Ligate Adaptors	2 hr	45 min
Size-Select from Gel	1.5 hr	30 min
PCR Enrich	1 hr	30 min
Sample Prep Total	11.5 hr	3.75 hr
Sample to Data	< 1 week	

Illumina mRNA-Seq sample preparation kits use standard molecular biology techniques. Combined with automated sequencing technology, researchers are rewarded with data quickly and with minimal hands-on time.

Tag Profiling opens up the entire mRNA spectrum for characterization. With 20 times the sensitivity of SAGE, Tag Profiling delivers an unprecedented depth of coverage. Analyzing millions of tags per sample per flow cell channel, Tag Profiling has effectively unlimited dynamic range with excellent reproducibility, allowing researchers to probe even very low level expressers (Figure 7).

It is generally accepted that one transcript per cell can be equated to one copy in 350,000 transcripts. Displaying sequencing data in templates per million (TPM), Illumina sequencing systems register a single transcript copy as a signal of about three TPM. Therefore, if

researchers tune the sequencing depth to an easily attainable four million tags per sample, a single-copy transcript will be called 12 times. If greater confidence is desired for calling low and zero expression levels, the investigator can read more tags from the sample.

Illumina scientists confirmed that the results of Tag Profiling were equivalent with that of qPCR assays for more than 600 genes using MAQC samples (Figure 8). Fold-change ratios were highly concordant between the two independent assay types, and no ratio compression was seen.

Assay Workflow

For Tag Profiling, Illumina scientists have designed a simple sample preparation protocol for sequencing a short region of any mRNA. The analysis strategy uniquely maps these short tags to genes by their sequence and position relative to a specific restriction site. This protocol does not require any transcript-specific probes, so Tag Profiling enables researchers to discover and quantify transcripts in any organism, irrespective of the available annotation.

The Illumina Tag Profiling sample preparation protocol builds constructs comprised of a unique 20- or 21-base pair cDNA tag with defined oligonucleotide adapters ligated to both ends. Tag Profiling provides access to any messenger RNA with two different restriction enzyme tag construction options. With a restriction site every 256 base pairs on average, digestion with NlaIII captures most mRNA species. For transcripts that are not addressed with NlaIII tag construction, Illumina offers an alternate method to anchor tags using DpnII restriction.

After template construction, automated cluster generation and sequencing proceed in the same manner as other sequencing applications, such as mRNA-Seq, described above.

Whole-Genome Gene Expression BeadChips

Hybridization-based arrays offer researchers a more economical method to quickly perform expression screening and classification of a few or a large set of samples. Illumina expression arrays feature up-to-date
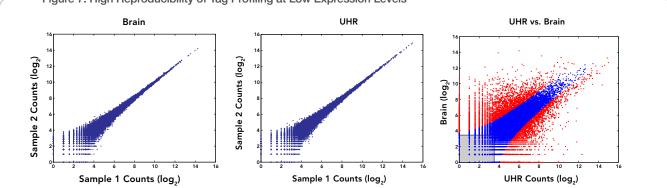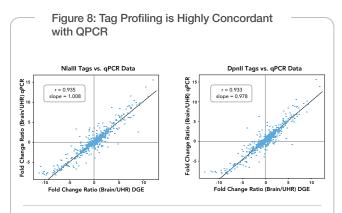
Figure 7: High Reproducibility of Tag Profiling at Low Expression Levels

Two samples of MAQC human brain specimen were prepared using the Tag Profiling protocol. Each sample was run on a single flow cell lane. The resulting data, plotted as Sample 1 versus Sample 2, clearly demonstrate the outstanding reproducibility Tag Profiling delivers. Similar results were seen for the same experiment using two MAQC universal human reference (UHR) samples. Distinct differential expression is observed when the two lanes of brain and UHR data are compared. Tags plotted in blue are identical in each specimen. Tags shown in red reflect a greater than two-fold change in expression (p = 0.95). On the UHR versus Brain scatter plot, the data in the shaded box show the signals observed at a level of fewer than one transcript per cell (three templates per million) for both samples.

Figure 8: Tag Profiling is Highly Concordant with QPCR

NlaIII Tags vs. qPCR Data

r = 0.935
slope = 1.008

Fold Change Ratio (Brain/UHR) qPCR

Fold Change Ratio (Brain/UHR) DGE

DpnII Tags vs. qPCR Data

r = 0.933
slope = 0.978

Fold Change Ratio (Brain/UHR) qPCR

Fold Change Ratio (Brain/UHR) DGE

Experiments using MAQC samples show that the data correlation between qPCR and the Tag Profiling protocol is greater than 0.93. After being assayed by qPCR, 629 and 625 RefSeq genes were quantified using the NlaIII and DpnII protocols, respectively. Unlike microarray data, there is no observed "ratio compression" in the data as evidenced by the slopes being approximately 1.

content selected from widely used databases such as NCBI RefSeq, and more specialized sources. Illumina Whole-Genome Expression BeadChips are ideal for applications such as differential expression analysis, disease classification, pathway analysis, and eQTL studies when discovery of novel transcripts is not essential and samples are from human, mouse, or rat.

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 heavily curated databases and probes are designed and validated by Illumina scientists. With a streamlined workflow and multi-sample BeadChip format, researchers can profile up to twelve samples in parallel on a single BeadChip, dramatically increasing throughput while decreasing experimental variability. The 100% hybridization-based QC of every probe ensures that BeadChips deliver outstanding performance and reproducibility. In addition, this unrivaled data quality comes at a lower cost per sample than other microarrays, allowing researchers to expand the scope of their science.

Comprehensive Array Content

Each address and probe sequence combination on Illumina Expression BeadChips was carefully selected bioinformatically. Gene-specific probes were designed using a multi-step algorithm to optimize several 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**
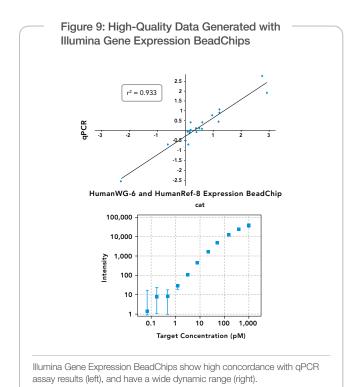- **Distance from 3' end of the transcript**

Table 1: Expression BeadChip Content

PROBES	DESCRIPTION	HUMAN WG-6	HUMAN REF-8	HUMAN HT-12[†]	MOUSE WG-6	MOUSE REF-8	RAT REF-12
RefSeq Content*							
NM	Coding transcript, well-established annotation	27,455	23,811	27,455	26,766	24,854	6,277
XM	Coding transcript, provisional annotation	7,870	426	7,870	6,856	796	15,983
NR	Non-coding transcript, well-established annotation	446	263	446	56	47	1
XR	Non-coding transcript, provisional annotation	196	26	196			12
Supplementary Content							
UniGene (Build 199)	Experimentally confirmed mRNA sequences that align to EST clusters	12,837		12,837			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		**48,804**	**24,526**	**48,804**	**45,281**	**25,697**	**22,523**

*Human RefSeq Build 36.2 Rel 22, mouse RefSeq Build 36 Rel 22, rat RefSeq Rel 16

[†] > 99.99% of the bead types are present on any HumanHT-12 array

Table 2: Whole-Genome Expression BeadChips Product Specifications

Parameter	Specification
Probe Length	50-mer gene-specific probe plus 29-mer address sequence
Sensitivity	≤ 1:250,000
Dynamic Range	≥ 3 logs
Precision	≤ 1.35 fold
Input RNA Required	50–100 ng

All Illumina Expression BeadChips feature up-to-date content largely derived from a recent release of the RefSeq database. Regularly curated and updated by field experts and annotated according to strict guidelines, this widely used database serves as the scientific community's most comprehensive and stable reference for genomic DNA, transcript, and protein products. The HumanRef-8, MouseRef-8, and RatRef-12 BeadChips are developed exclusively from the RefSeq database content and target 18,631, 18,122, and 21,910 unique genes, respectively (Table 1).

The HumanWG-6 and MouseWG-6 BeadChips contain the full set of HumanRef-8 and MouseRef-8 BeadChip probes plus supplemental content derived from additional databases. The HumanWG-6 BeadChip includes 12,837 probes targeting EST clusters from the UniGene database (Build 199). As a result, the HumanWG-6 BeadChip targets a total of 25,440 annotated genes with more than 48,000 probes (Table 1). The MouseWG-6 BeadChip includes 11,603 ad-

Figure 9: High-Quality Data Generated with Illumina Gene Expression BeadChips

HumanWG-6 and HumanRef-8 Expression BeadChip

Illumina Gene Expression BeadChips show high concordance with qPCR assay results (left), and have a wide dynamic range (right).

Figure 10: High Concordance Between HumanHT-12 and HumanWG-6 Data

Data generated from the higher throughput HumanHT-12 BeadChip are highly concordant with the HumanWG-6 BeadChip.

ditional probes derived from RIKEN FANTOM2, RefSeq rel 5, and MEEBO databases.

The HumanHT-12 contains the same comprehensive panel of probes as the HumanWG-6 BeadChip, but provides higher throughput processing of 12 samples per BeadChip. With this BeadChip, expression information can easily be incorporated in genome-wide association studies (GWAS), and large gene expression studies can be completed more quickly and economically. Illumina guarantees that more than 99.99% of the bead types will be present on any given HumanHT-12 array. This means up to five HumanWG-6 probes may be represented with only 0, 1, or 2 copies on each HumanHT-12 array.

In sum, the high-value content on human, mouse, and rat Expression BeadChips provides genome-wide transcriptional coverage of well-characterized genes, gene candidates, and splice variants, targeting well-established sequences supported by peer-reviewed literature.
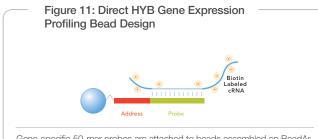
High-Quality Data

Illumina has compiled performance data for all Expression Bead-Chips2. Reproducibility has been demonstrated by high concordance between hybridization replicates. Industry-leading performance specifications for sensitivity, dynamic range, and fold-change detection precision dramatically minimize false discovery rates for differential expression analysis (Table 2, Figure 9).

As expected, HumanHT-12 assay performance is equally high, and shows very high concordance with HumanWG-6 BeadChip data (Figure 10).

Streamlined Assay Workflow

Illumina Expression BeadChips are designed using BeadArray technology. BeadChips consist of bead-linked oligonucleotides held in microwells on the surface of a slide-sized substrate. During the manufacturing process, beads self-assemble into the micro-wells of the BeadChips. Each bead type contains hundreds of thousands of copies of a covalently attached, full-length oligonucleotide probe. 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 random bead assembly, 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

Figure 11: Direct HYB Gene Expression Profiling Bead Design

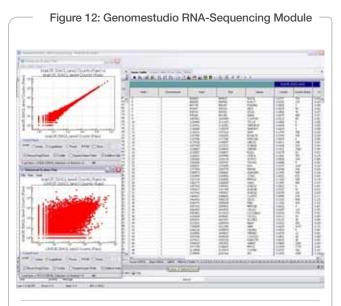Gene-specific 50-mer probes are attached to beads assembled on BeadArray substrates.

final process also validates the hybridization performance of every bead on every BeadChip, ensuring 100% array QC.

Expression BeadChip arrays are arranged in a multi-sample format for higher throughput and virtual elimination of array-to-array variability. All steps after hybridization are performed in parallel on each BeadChip, significantly reducing experimental variation and handling requirements. Labeled sample cRNA are detected by hybridization to 50-mer probes on the BeadChip. After washing and staining steps, Bead-Chips are scanned on the Illumina iScanTM, HiScanSQ, or BeadArray Reader.

For the highest sample throughput (up to 216 arrays at a time), researchers can automate BeadChip loading and scanning with the Illumina AutoLoader.

DASL Assay

The DASL Assay (cDNA-mediated annealing, selection, extension, and ligation) is highly robust for gene expression profiling of traditionally difficult to assay samples, such as those having low RNA abundance or with RNA degradation due to FFPE (formalin-fixed paraffin-embedded) processing.

Figure 12: Genomestudio RNA-Sequencing Module

The GenomeStudio RNA-Sequencing Module includes tools for data visualization, including graphical plotting and table views for analyzing genes, exons, exon junctions, and alleles (for SNP detection).

The Whole-Genome DASL Assay5 covers more than 24,000 transcripts, using same content as HumanRef-8 v3.0 BeadChip.

For focused panels of up to 1,536 genes, the DASL Assay is deployed on multi-sample Universal Arrays3. Very high throughput of low- to mid-multiplex assays are possible when the DASL Assay is used in combination with VeraCode technology4.

Systems and Software Sequencing Data Analysis

mRNA-Seq and Tag Profiling generate data using open architecture software, allowing researchers to tailor Illumina sequencing system data analysis to address their specific needs. The Analysis Pipeline is responsible for performing primary data acquisition, determining base calls, and calculating confidence scores from the fluorescence signals on Illumina's sequencing systems. Higher level analyses, such as aligning reads to a reference, determining expression levels of genes and exons, and identifying variants, are performed using the tools in the Analysis Pipeline and integrated algorithms. The results from these analyses are parsed to the RNA-Sequencing Module in Illumina's GenomeStudioTM analysis software for display (Figure 12). Results are easily visualized on the integrated graphical genome viewer that includes annotation information (Figure 3). Users can also zoom down to single-base sequence resolution to identify cSNPs and exon-exon junctions (Figure 5). Exon and gene table views allow for the examination of expression levels in unprecedented detail (Figure 12).
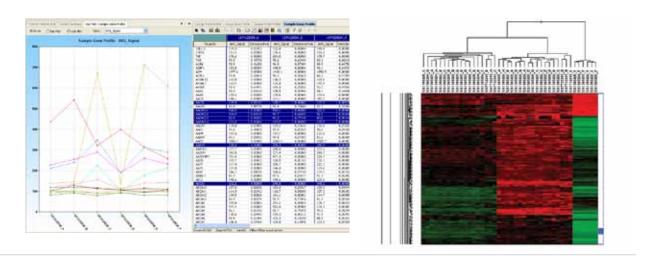
For the Tag Profiling application, image analysis, base calling, and standard filtering by the Analysis Pipeline generate a list of sequence tags and counts. This list of tags can be annotated with genomic information and used to analyze differential gene expression. The software provides canonical sequences for human and mouse genomes. Expression profiles for other species can be compared easily against public databases like the NCBI RefSeq database and the University of California Santa Cruz (UCSC) genome browser.

Expression Array Data Analysis

Illumina's GenomeStudio Gene Expression Module (Figure 13) enables simplified data management for hierarchical organization of samples, groups, groupsets, and all associated project analysis. It offers gene-level statistical analysis tools for differential analysis, heat map visualization, and clustering. With Gene Expression Modules 3.6.2 or higher, researchers can combine Expression BeadChip data generated from different major product versions (e.g., HumanRef-8 v2.0 and HumanRef-8 v3.0).

Researchers can easily combine Expression BeadChip data with either methylation or miRNA profiling data in a single GenomeStudio gene expression project. This enables powerful integrated approaches to studying epigenetic impacts on gene expression. Important for eQTL studies, the flexible data management architecture of the GenomeStudio software supports integrating gene expression probe annotation information with SNP location coordinates. Using APIs, researchers can export genotyping and expression data from GenomeStudio software to third-party applications to perform eQTL-like integrated analysis.

Figure 13: 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.

Services

Illumina FastTrack Sequencing Services are available to analyze samples in a timely fashion at a reasonable cost for full-length cDNA sequencing and digital expression profiling. This option allows researchers to acquire high-quality data for limited studies or before purchasing their own equipment. Data and a concise summary report are provided, and Illumina scientists offer a range of consultative and analytical support, which can be tailored to meet your needs.

Summary

Illumina technologies support a broad portfolio of gene expression analysis products. mRNA-Seq and Tag Profiling identify transcripts and determine expression levels using sequencing. This powerful technology allows unbiased transcript discovery in nearly any species. Illumina sample preparation kits are designed for industry-leading ease of use, requiring the least hands-on time to generate several gigabases of sequence.

Illumina BeadArray-based assays are streamlined expression profiling solutions for human, mouse, and rat. These BeadChips contain comprehensive, up-to-date content derived from several important sources. The DASL Assay supports expression profiling from FFPE or limited samples. For any experimental design, Illumina products provide the fastest path to discoveries and publication.

References

1. Saha S, Sparks AB, Rago C, Viatcheslav A, Wang CJ, et al. (2002) Using the transcriptome to annotate the genome. Nat Biotech 20: 508-512.
2. Whole-Genome Expression Analysis Using the Human-6 and HumanRef-8 Expression BeadChips Tech Bulletin (PDF) http://www.illumina.com/downloads/WholeGenomeExpressionTechnicalBulletin.pdf
3. RNA Profiling with the DASL Assay Tech Bulletin (PDF) http://www.illumina.com/downloads/DASLTECHBULLETIN.pdf
4. DASL Gene Expression Profiling with VeraCode Technology (PDF) http://www.illumina.com/downloads/DASLVeraCode.pdf
5. Whole-Genome DASL Assay for Expression Profiling Data Sheet (PDF) http://www.illumina.com/downloads/WGDASLAssay_Datasheet.pdf

Ordering Information

Product	Enzyme	Quantity	Catalog No
mRNA-Seq			
mRNA-Seq 8-Sample Prep Kit		8 Samples	RS-100-0801
mRNA-Seq Cluster Generation Kit (GAII)		1 Flow Cell	RS-110-0101
		10 Flow Cells	RS-110-1001
mRNA-Seq 36-Cycle Sequencing Kit		1 Flow Cell	RS-120-3601
Tag Profiling			
Tag Profiling Sample Prep Kit	NlaIII	8 Samples/1 Flow Cell	FC-102-1005
		40 Samples/5 Flow Cells	FC-102-1006
	DpnII	8 Samples/1 Flow Cell	FC-102-1007
		40 Samples/5 Flow Cells	FC-102-1008
Tag Profiling Cluster Generation Kit	NlaIII	Up to 8 Samples	FC-103-1004
• Reagents		Up to 80 Samples	FC-103-1005
• Flow Cell			
• Amplification Manifold	DpnII	Up to 8 Samples	FC-103-1006
• Hybridization Manifold		Up to 80 Samples	FC-103-1007
18-cycle Illumina Sequencing Kit		1 Flow Cell	FC-104-1001
Sequencing Instruments			
Illumina Cluster Station			SY-301-2001
• Includes computer, software, installation, training, and 1-year warranty			
Illumina Genome Analyzer			SY-301-1001
• Includes computer, software, installation, training, and 1-year warranty			
Illumina HiScanSQ			SY-301-1001-PRE
• Includes computer, software, installation, training, and 1-year warranty			

Ordering Information (Continued)

Product	Samples	Catalog No
Human Expression BeadChips		
HumanWG-6 v3.0 Expression BeadChip Kit	12	BD-101-0203
• 6 samples per BeadChip		
• > 45,000 human targets per sample	36	BD-101-0603
• Includes hybridization buffers, wash buffers, and wash trays		
Customer Sample Evaluation Using HumanWG-6 v3.0 BeadChip	10	BD-101-203-CSE
• All standard data output files supplied		
HumanRef-8 v3.0 Expression BeadChip Kit	16	BD-102-0203
• 8 samples per BeadChip		
• > 25,000 human targets per sample	48	BD-102-0603
• Includes hybridization buffers, wash buffers, and wash trays		
Customer Sample Evaluation Using HumanRef-8 v3.0 BeadChip	14	BD-102-203-CSE
• All standard data output files supplied		
HumanHT-12 v3 Expression BeadChip Kit (6-pack)	144	BD-103-0603
• 12 samples per BeadChip		
• > 45,000 human targets per sample	24	BD-103-0203
• Includes hybridization buffers, wash buffers, and wash trays		
Mouse Expression BeadChips		
MouseWG-6 v2.0 Expression BeadChip Kit	12	BD-201-0202
• 6 samples per BeadChip		
• > 45,000 mouse targets per sample	36	BD-201-0602
• Includes hybridization buffers, wash buffers, and wash trays		
Customer Sample Evaluation Using MouseWG-6 v2.0 BeadChip	10	BD-201-0202-CSE
• All standard data output files supplied		
MouseRef-8 v2.0 Expression BeadChip Kit	16	BD-202-0202
• 8 samples per BeadChip		
• > 25,000 mouse targets per sample	48	BD-202-0602
• Includes hybridization buffers, wash buffers, and wash trays		
Customer Sample Evaluation using MouseRef-8 v2.0 BeadChip	14	BD-202-0202-CSE
• All standard data output files supplied		
Rat Expression BeadChips		
RatRef-12 Expression BeadChip Kit	24	BD-27-303
• 12 samples per BeadChip		
• 22,523 rat targets per sample	72	BD-27-302
• Includes hybridization buffers, wash buffers, and wash trays		
Customer Sample Evaluation using RatRef-12 BeadChip	22	BD-27-301-CSE
• All standard data output files supplied		
Related Products		
Illumina TotalPrep RNA Amplification Kit	24	AMIL1791
• Available from Ambion: 1-800-888-8804 (U.S.)	96	4393543
TargetAmp Nano-g Biotin-aRNA Labeling Kit		TAN07924-142
• Available from Epicentre Biotechnologies: 1-800-284-8474 (U.S.)		