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Whole-Genome DASL® HT Assay for Expression Profiling in FFPE Samples

A high-throughput, sensitive, reproducible, and cost-effective method for whole-genome expression profiling of low abundance and partially degraded RNA samples.

Highlights

- **High Sample Throughput:**
  Processing of 12 samples per BeadChip
- **High Sensitivity:**
  Start with FFPE samples, brain tissue, or blood samples without globin reduction
- **Low-Sample Input:**
  Produce highly reproducible results with as little as 10–100 ng total RNA from fresh-frozen tissue or 50–200 ng total RNA from FFPE samples
- **High Multiplex Capabilities:**
  Analyze over 29,000 annotated transcripts at once
- **High-Quality Results:**
  Obtain high concordance with qPCR results
- **Low Per-Sample Cost:**
  Pay less than one-third the price of other methods

Figure 1: Whole-Genome DASL HT Assay Combines Two Proven Assays for Genome-Wide, Sensitive Expression Profiling

The Whole-Genome DASL HT Assay uses the standard DASL labeling strategy to generate highly multiplexed single-stranded Cy3-labeled probes which are directly hybridized via gene-specific complementation to the HumanHT-12 v4 Expression BeadChip.

Introduction

Tissue samples collected during surgeries and biopsies are often fixed in formalin, followed by embedding in paraffin for long-term preservation. There are estimated to be over 400 million of these FFPE (formalin-fixed paraffin-embedded) samples archived in North America for cancer alone. Many of these samples represent clinical outcomes with the potential to provide critical insight into expression profiles associated with complex disease development. Unfortunately, FFPE archival methods often lead to partial RNA degradation, limiting the amount of information that can be derived from such samples.

Current methods for analyzing FFPE samples, or other RNA samples available in limited supply, are time-consuming, costly, not scalable, and can produce unsatisfactory results. Illumina's Whole-Genome DASL HT Assay overcomes these limitations, processing 12 samples per BeadChip array to provide a cost-effective solution for simultaneous profiling of over 29,000 transcripts. In addition, expression profiles generated from as little as 50 ng total RNA using the Whole-Genome DASL HT Assay are highly reproducible ($r^2 > 0.97$) and similar to those obtained using qPCR.

How the Whole-Genome DASL HT Assay Works

The Whole-Genome DASL (cDNA-mediated Annealing, Selection, extension and Ligation) HT Assay combines the unique PCR and labeling steps of the original DASL Assay with gene-based hybridization and the whole-genome probe set of Illumina's Direct Hybridization Assay (Figure 1). This greatly increases the DASL Assay target set, while retaining the ability to accurately profile partially degraded RNA samples.

Proven DASL Labeling

The Whole-Genome DASL HT (WG-DASL HT) Assay begins with the conversion of total RNA to cDNA using biotinylated oligo (dT) and random nonamer primers. The biotinylated cDNA is then annealed to the DASL Assay Pool (DAP) probe groups. Probe groups contain oligonucleotides specifically designed to interrogate each target sequence in the transcripts. These probes span about 50 bases, making it possible to profile partially degraded RNA.

The assay probe sets consist of an upstream oligo containing a gene-specific sequence and a universal PCR primer sequence (P1) at the 5' end, and a downstream oligo containing a gene-specific sequence and a universal PCR primer sequence (P2') at the 3' end. The upstream oligo hybridizes to the targeted cDNA site, and then extends and ligates to its corresponding downstream oligonucleotide to create a PCR template that can be amplified with universal PCR primers (P1 and P2) (Figure 2).

The HumanHT-12 v4 BeadChip

The resulting PCR products are hybridized to the HumanHT-12 v4 Expression BeadChip to determine the presence or absence of specific genes. The HumanHT-12 v4 BeadChip features up-to-

Table 1: Additional RefSeq* Content on the Human WG-DASL HT Assay

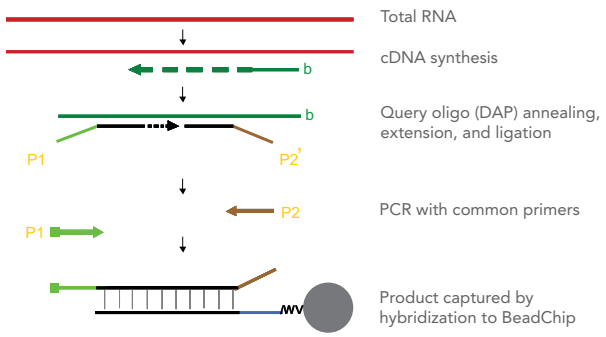
Probes	Description	Human WG-DASL Assay (HumanRef-8 v3 BeadChip)	Additional	Human WG-DASL HT Assay (HumanHT-12 v4 BeadChip)**
NM	Coding transcripts, well-established annotations	23,811	3442	27,253
XM	Coding transcripts, provisional annotations	426		426
NR	Non-coding transcripts, well-established annotations	263	1317	1,580
XR	Non-coding transcripts, provisional annotations	26		26
Total		24,526	4,759	29,285

*Release 38

**Illumina guarantees that >99.99% of the bead types will be present on any given HumanHT-12 array.

date content covering > 47,000 annotated transcripts derived from the National Center for Biotechnology Information Reference Sequence (RefSeq) database (Release 38, November 2009)[1]. The WG-DASL HT assay targets >29,000 of those probes (Table 1). Illumina guarantees that >99.99% 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. This high-value content provides genome-wide transcriptional coverage of well-characterized genes, gene candidates, and splice variants. The higher throughput format of the Human HT-12 v4 BeadChip enables researchers to perform these studies more quickly and economically, without the need for expensive, specialized automation.

Figure 2: RNA Profiling with the WG-DASL HT Assay



Accurate Imaging

After hybridization, HumanHT-12 v4 BeadChips are scanned on HiScan™SQ, iScan, or BeadArray™ Reader. These systems incorporate high-performance lasers, optics, and detection systems for rapid, quantitative scanning of Illumina's BeadChips. All offer high signal-to-noise ratio, high sensitivity, low limit of detection, and broad dynamic range, leading to exceptional data quality.

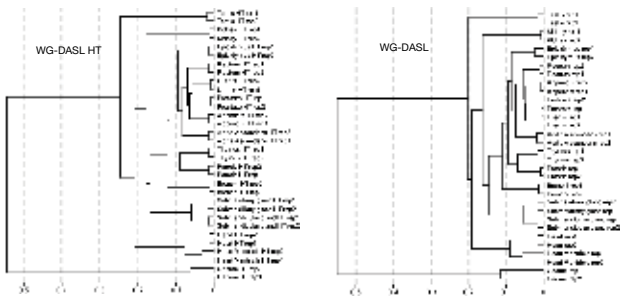
High-Quality Performance

To evaluate and compare the performance of the WG-DASL HT to the WG-DASL Assay, experiments were performed with 16 commercially obtained FFPE samples (BioChain). RNA was extracted using the High Pure RNA Paraffin Kit (Roche) with total RNA inputs of 200 ng. Duplicates samples were run on both WG-DASL HT and WG-DASL Assays. The results shown in Figure 3 illustrate good technical reproducibility for both assays. Importantly, they show that the relative relationships among all 16 FFPE samples are highly conserved across the two assays, suggesting that the gene expression profiles captured are very similar.

High Reproducibility

The WG-DASL HT Assay produces highly reproducible expression profiles with RNA derived from a Universal Human Reference (UHR, Agilent) sample ($r^2 > 0.98$) and a commercially available FFPE (parotid, BioChain) tissue ($r^2 > 0.97$) (Figure 4, top row). This level of self-reproducibility is comparable with that obtained for the previous WG-DASL Assay (Figure 4, bottom row). While such consistency brings reliability to an assay frequently performed on compromised samples, it should also be noted that assay performance is dependent on sample quality.[3]

Figure 3: Unsupervised Hierarchical Clustering of FFPE Samples



Unsupervised hierarchical clustering of 16 FFPE tissues for the WG-DASL HT (left) and WG-DASL (right) Assays. The samples were clustered using 24,526 transcripts that are common to both assays. Units on the x-axis are Pearson's correlation coefficient (R).

Table 2: WG-DASL HT Assay Performance as a Function of Intact RNA input

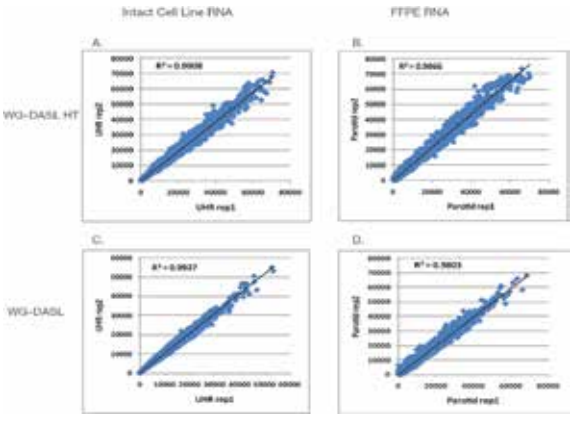
Metric	100 ng	50 ng	10 ng
WG-DASL HT Assay: Average self-reproducibility (r^2)	0.985	0.982	0.981
WG-DASL Assay: Average self-reproducibility (r^2)*	0.989	0.988	0.987
WG-DASL HT Assay: Average correlation (r^2) compared to 100 ng total RNA	0.985	0.979	0.939
WG-DASL Assay: Average correlation (r^2) compared to 100 ng total RNA*	0.989	0.987	0.976
WG-DASL HT Assay: % retention of transcripts detected (p<0.01) in 100 ng total RNA	98.9	98.2	96.4
WG-DASL Assay: % retention of transcripts detected (p<0.01) in 100 ng total RNA*	99.2	99.1	98.5

*April C, et al. (2009) Whole-Genome Gene Expression Profiling of Formalin-Fixed, Paraffin-Embedded Tissue Samples. PLoS One 4 (12): e8162.

High Concordance with Previous DASL Assay

WG-DASL HT Assay (based on the HumanHT-12 v4 BeadChip) preserves all of the content of our earlier WG-DASL Assay (based on the HumanRef-8 v3 BeadChip). To obtain a more direct and quantitative assessment of the performance of the WG-DASL HT Assay compared to the earlier WG-DASL Assay, fold-change correlations were performed using sample pairs for both intact (Figure 5A) and FFPE (Figure 5B) samples. For the intact samples, 100 ng of UHR and Human Brain Reference (BrnRef, Ambion) RNAs were used, whereas 200 ng of total RNA extracted from the parotid and testis (BioChain) were used for the FFPE comparisons. Good fold-change correlations were obtained for both the intact (r^2 ~ 0.93) and FFPE (r^2 ~ 0.83) samples. Moreover, differential expression analysis of the same set of intact and FFPE samples yielded good overlap between the WG-DASL HT and WG-DASL Assays. Approximately 85% of the differentially expressed transcripts identified in the WG-DASL Assay for the intact samples were also identified in the WG-DASL HT Assay (Figure 5C), and ~ 84% of the differentially expressed transcripts identified in the WG-DASL Assay for the FFPE samples overlapped with those identified in the WG-DASL HT Assay (Figure 5D). The WG-DASL HT assay is also able to detect 1.5-fold change with 95% confidence and has a dynamic range of ~ 3 logs. Taken together, these data indicate an equivalent level of performance between the WG-DASL HT and WG-DASL Assays.

Figure 4: WG-DASL HT vs. WG-DASL Self-Reproducibility

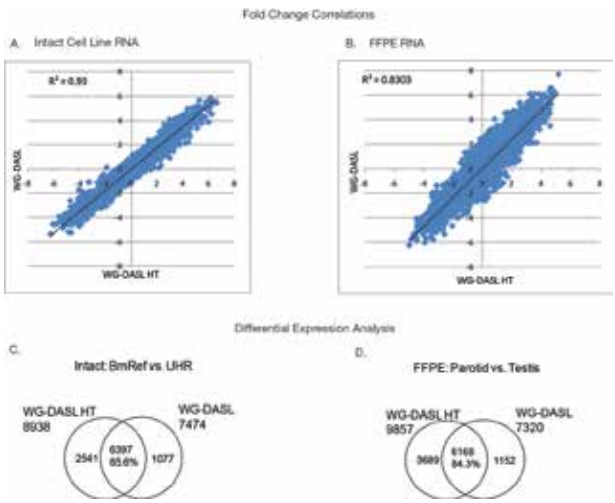


The Whole-Genome DASL HT Assay (29K probes) was used to generate expression profiles for 100 ng intact total RNA from a cell line (UHR) (A) and 200 ng total RNA from an FFPE sample (B). For comparison purposes, the previous Whole-Genome DASL Assay (24K probes) was used to generate expression profiles for the same 100 ng intact total RNA from the same cell line (UHR) (C) and 200 ng total RNA from the same FFPE sample (D).

Low Input RNA Requirement

Even with as little as 10 ng total RNA, the WG-DASL HT Assay produces accurate, reproducible expression profiling consistent with the previous WG-DASL Assay (Table 2). High transcript detection rates (>95%) are maintained with 10 ng input RNA rather than the standard 100 ng required by other assays.

Figure 5: WG-DASL HT vs. WG-DASL Assay: Fold-Change Correlations and Differential Expression Analyses

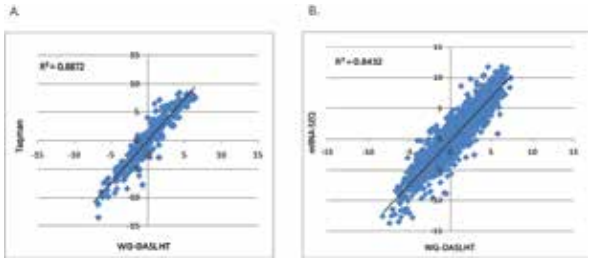


High concordance was obtained for fold-change correlations between the WG-DASL HT (x-axis) and WG-DASL (y-axis) Assays for both intact (A: BrnRef/UHR) and FFPE (B: parotid/testis) samples. Logarithmic fold-differences in transcript abundance between samples pairs were derived from signal intensities for detected probes (p<0.01) across 24,526 common transcripts. Good overlap was obtained for differentially expressed transcripts between the WG-DASL HT and WG-DASL Assays for both intact (C) and FFPE (D) samples. Differentially expressed probes that exhibited > 1.5-fold change between sample groups and that were detected (p<0.01) across all samples for the 24,526 common transcripts are shown.

Cross-Platform Concordance with Taqman and mRNA-SEQ Assays

Independent comparisons, using BrnRef and UHR intact RNAs, in which the WG-DASL HT Assay was compared with both the Taqman (Figure 6A) and mRNA-SEQ (Figure 6B) assays yielded high fold-change correlations, with r^2 = 0.88 and r^2 =0.84, respectively. Taqman data were derived from the MAQC consortium study,[4] whereas mRNA-SEQ data were obtained from HiScan SQ runs. These results are indicative of a high degree of measurement concordance across different gene expression platforms.

Figure 6: High Concordance with Taqman and mRNA-SEQ

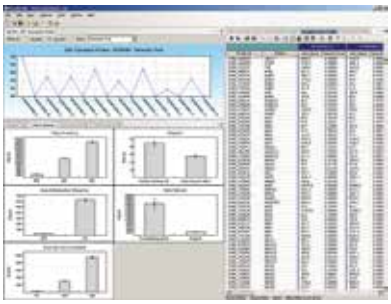


High concordance was obtained between the WG-DASL HT (x-axis) and Taqman (y-axis) assays (A: r2 = 0.88) and WG-DASL HT (x-axis) and mRNA-SEQ (y-axis) assays (B: r2 = 0.84). Logarithmic fold-differences in transcript abundance between the two MAQC samples (BrnRef/UHR) were derived from Ct numbers (Taqman, 703 genes) and raw counts (mRNA-SEQ, 13K genes). For the data in 6A, only genes which had present calls in the Taqman assay and were detected ($p<0.01$) in the WG-DASL HT assay are plotted. For the data in 6B, only genes that were detected ($p<0.01$) in the WG-DASL HT assay are plotted.

User-Friendly Analysis

The WG-DASL HT Assay is fully supported by the Gene Expression Module in Illumina's GenomeStudio® Software Package (Figure 7). Using this intuitive software, image data scanned by the HiScanSQ, iScan, or BeadArray Reader is analyzed for expression levels of specific mRNAs or differential mRNA expression between two experimental samples. Simplified data management tools enable hierarchical organization of samples, groups, group sets, and all associated project analyses. The software also offers gene-level statistical analysis for differential analysis, heat map visualization, and sample clustering. Output files are easily exported to third-party gene expression analysis software.

Figure 7: Data Analysis Using GenomeStudio Software



Data generated using the WG-DASL HT Assay is analyzed with GenomeStudio Data Analysis Software. The user-friendly GenomeStudio interface displays p-values and a summary dashboard highlighting controls available for analyzing data from the WG-DASL HT Assay.

References

1. www.ncbi.nlm.nih.gov/RefSeq
2. April C, et al. (2009) Whole-Genome Gene Expression Profiling of Formalin-Fixed, Paraffin-Embedded Tissue Samples. PLoS ONE 4 (12): e8162.
3. www.illumina.com/documents/products/technotes/technote_dasl_mcs3.pdf.
4. MAQC Consortium (2006) The MicroArray Quality Control (MAQC) Project Shows Inter- and Intraplatform Reproducibility of Gene Expression Measurements. Nat. Biotechnol. 24 (9):1151-1161.

Ordering Information

Catalog No.	Product	Description
DA-905-0024	Whole-Genome DASL HT Assay (24 samples)	Includes a Whole-Genome DASL DAP, two HumanHT-12 BeadChips, each able to analyze 12 samples, plus reagents for amplifying, hybridizing, washing, and processing 24 RNA samples.
DA-905-1024	Whole-Genome DASL HT Assay with UDG (24 samples)	Includes a Whole-Genome DASL DAP, two HumanHT-12 BeadChips, each able to analyze 12 samples, plus reagents for amplifying, hybridizing, washing, and processing 24 RNA samples (with UDG).
DA-905-0096	Whole-Genome DASL HT Assay (96 samples)	Includes a Whole-Genome DASL DAP, eight HumanHT-12 BeadChips, each able to analyze 12 samples, plus reagents for amplifying, hybridizing, washing, and processing 96 RNA samples.
DA-905-1096	Whole-Genome DASL HT Assay with UDG (96 samples)	Includes a Whole-Genome DASL DAP, eight HumanHT-12 BeadChips, each able to analyze 12 samples, plus reagents for amplifying, hybridizing, washing, and processing 96 RNA samples (with UDG).

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