

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

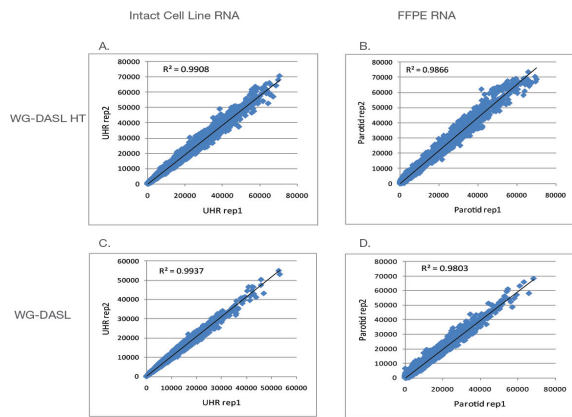
WG-DASL HT Assay (based on the HumanHT-12 v4 BeadChip) preserves all of the content of our earlier WG-DASL Assay (based on the HumanRef-8 v3 BeadChip). To obtain a more direct and quantitative assessment of the performance of the WG-DASL HT Assay compared to the earlier WG-DASL Assay, fold-change correlations 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 ($r^2 \sim 0.93$) and FFPE ($r^2 \sim 0.83$) 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.

Low Input RNA Requirement

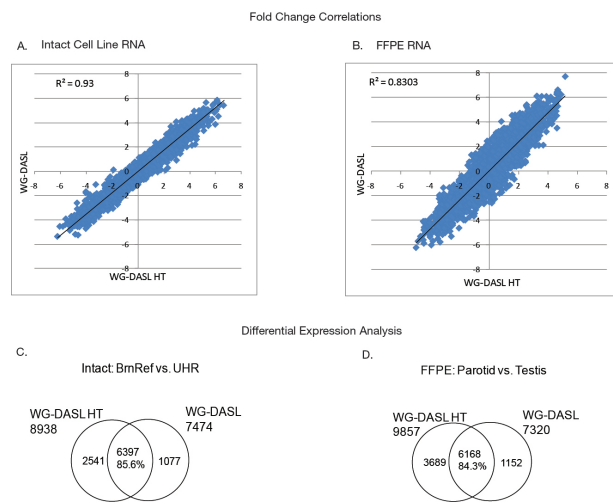
Even with as little as 10 ng total RNA, the WG-DASL HT Assay produces accurate, reproducible expression profiling consistent with the previous WG-DASL Assay (Table 2). High transcript detection rates (>95%) are maintained with 10 ng input RNA rather than the standard 100 ng required by other assays.

Figure 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).

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.

