More Robust Reagents for Use in the Whole-Genome DASL® Assay

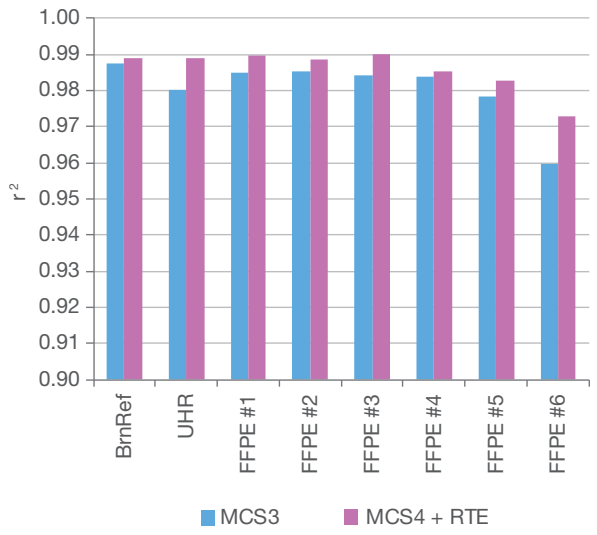
Reformatted cDNA synthesis reagents restore high levels of reproducibility across degradation-sensitive FFPE samples.

Introduction

The cDNA-mediated Annealing, Selection, extension, and Ligation (DASL) Assay allows for expression profiling of partially degraded RNA such as that commonly found in formalin-fixed, paraffin-embedded (FFPE) samples. The Whole-Genome DASL (WG-DASL) HT Assay (>29,000 gene targets) offers researchers the opportunity to analyze hundreds to thousands of RNA transcripts in FFPE samples from as little as 50 ng total RNA, delivering highly reproducible results[1].

Recent improvements to the cDNA synthesis reagents extend the reagent shelf-life, enabling researchers to continue obtaining the expected high reproducibility across samples. Specifically, this change splits the MCS3 reagent, which is a master mix containing buffer and reverse transcriptase enzyme (RTE), into an MCS4 buffer component and a separate RTE. This new format, MCS4 + RTE, produces equivalent performance to the MCS3 reagent throughout its entire shelf life.

Figure 1: High Reproducibility of MCS4 + RTE vs. MCS3



RNA from eight different samples used in the WG-DASL HT assay using either MCS3 or MCS4 + RTE reagents.

FFPE samples (Biochain)
UHR = Universal Human Reference RNA (Stratagene)
Brn Ref = Human Brain Reference RNA (Ambion)

High-Quality Performance

To evaluate and compare the performance of MCS4 + RTE with that of the MCS3 reagent, WG-DASL HT experiments using both methods were performed on eight different RNA samples, consisting of six Biochain FFPE samples and two intact MAQC samples [Universal Human Reference (UHR) and Human Brain Reference (BrnRef)]. FFPE RNA was extracted using the High Pure RNA Paraffin Kit (Roche), with total RNA input of 200 ng for each FFPE sample and 100 ng for each intact sample. BeadChips were scanned on the iScan System and raw data imported and analyzed using GenomeStudio® Data Analysis Software. Results are discussed below.

High Reproducibility

To assess the reproducibility of the new MCS4 + RTE reagents, all eight RNA samples were processed in duplicate and compared with MCS3 data generated in the same experiment. The raw signal intensities for all 29K probes were then compared between sample replicates for each reagent. The results demonstrate equivalent or improved levels of self-reproducibility for MCS4 + RTE as compared with MCS3, across a range of FFPE sample qualities, with r^2>0.97 for all samples tested (Figure 1).

High Gene Expression Profile Correlations

Direct comparisons of the raw signal intensities between experiments performed using MCS4 + RTE and MCS3 yielded correlations of r^2~0.99 for intact RNAs. Similar comparisons of FFPE RNAs yielded a range of correlations with r^2~0.92–0.95. Representative scatterplots are shown in Figure 2.

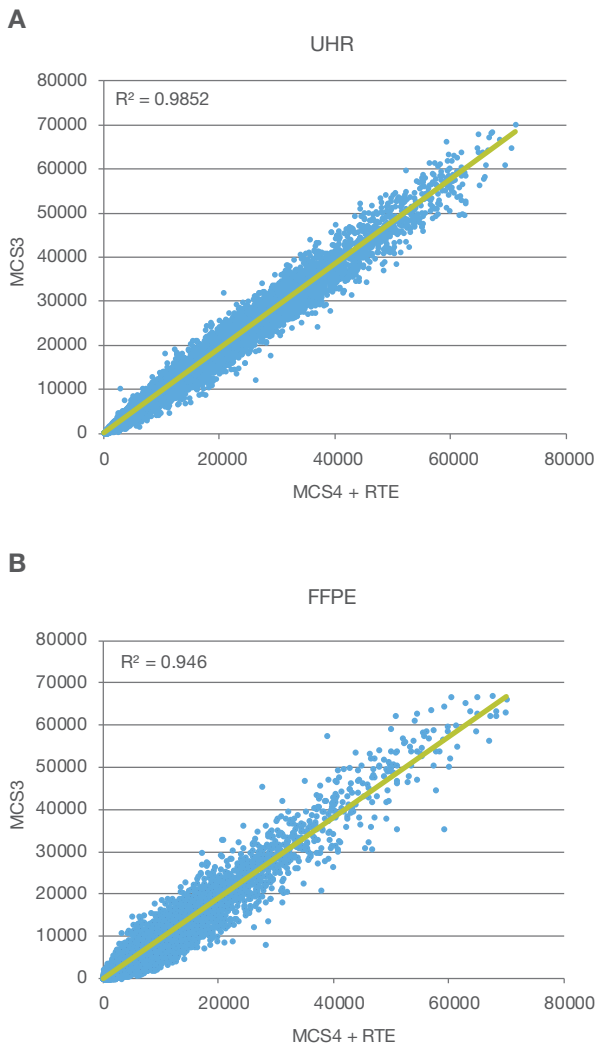
Equivalent Sensitivity

Using intact and FFPE samples, a similar level of sensitivity was obtained on assays done with MCS4 + RTE when compared with those using MCS3. Equivalent numbers of genes were also detected in each sample (Figure 3). Additionally, a high degree of overlap was observed between detected transcripts in samples processed with either MCS4 + RTE or MCS3, with ~99% and ~96% of the detected transcripts shared in intact and FFPE samples, respectively (Figure 4).

High Cross-Platform Concordance

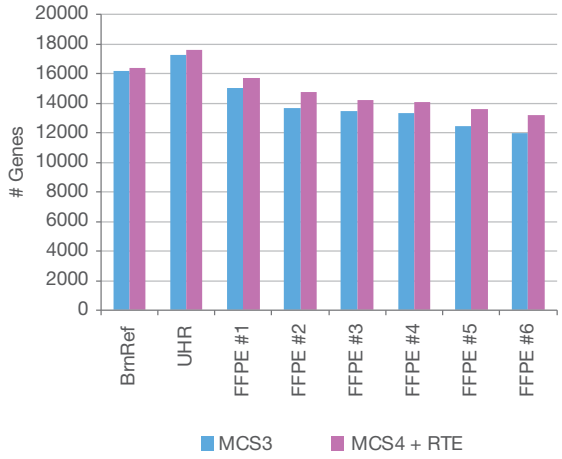
To independently validate gene expression profiles generated using the MCS4 + RTE reagents in the WG-DASL HT assay, fold-change comparisons with MAQC data derived from mRNA-Seq on the HiScan® and TaqMan[2] platforms were performed. Scatterplot comparisons of the WG-DASL HT assay vs. TaqMan (653 transcripts)

Figure 2: High Expression Profile Correlations Between MCS4 + RTE and MCS3



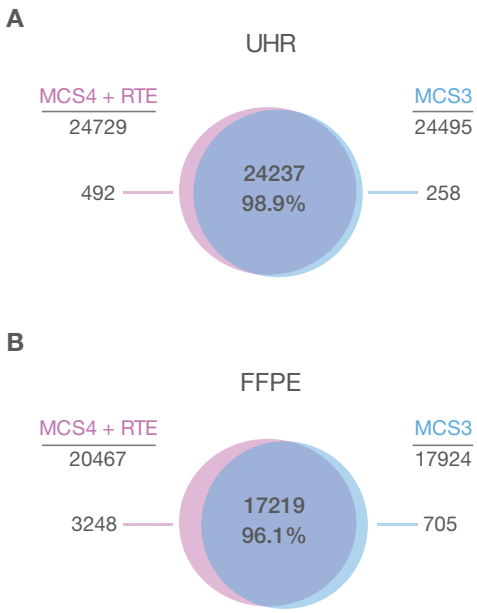
Raw signal intensity scatterplots comparing results obtained using the WG-DASL HT assay with MCS4 + RTE or MCS3 reagents using intact (A) or FFPE (B) RNA. Data is shown for all 29K probes.

Figure 3: Equivalent Sensitivity



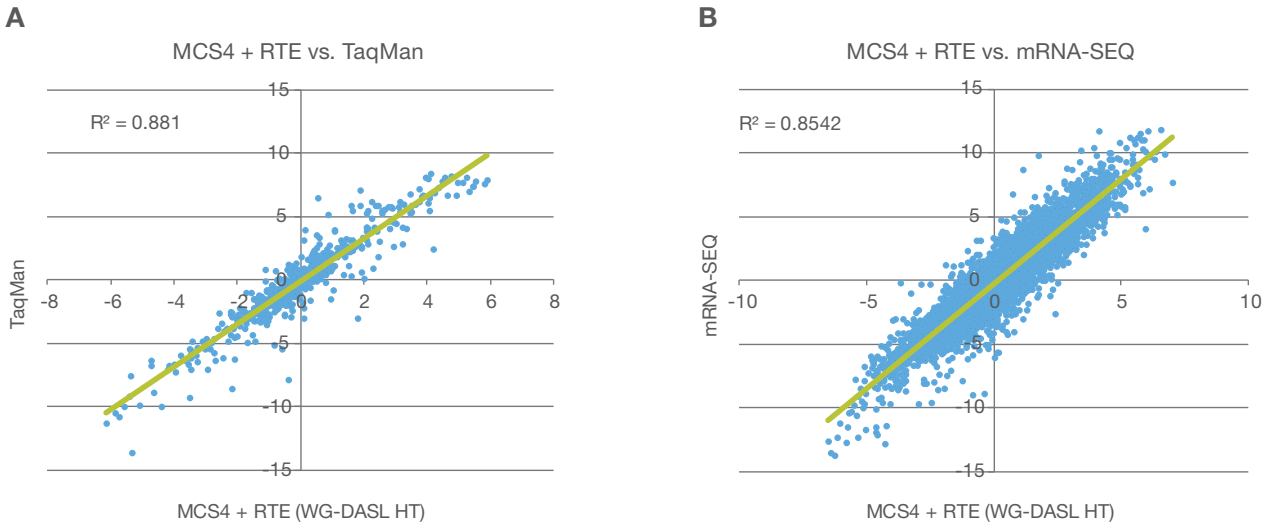
Equivalent numbers of genes were detected in each sample for both intact and FFPE RNA samples. Data shown is for P value <0.01.

Figure 4: High Overlap of Detected Transcripts



Overlap of genes detected (P value <0.01) using the WG-DASL HT assay with either MCS4 + RTE or MCS3 and intact (A) or FFPE (B) RNA samples.

Figure 5: High Cross-Platform Concordance



MAQC samples were analyzed using the WG-DASL HT assay with MCS4 + RTE, mRNA-Seq, and TaqMan platforms. A comparison of cross-platform results shows high concordance of the WG-DASL HT assay with both TaqMan (A) and mRNA-Seq (B). Data shown includes genes with WG-DASL HT detection for P value <0.01 only.

and WG-DASL HT assay vs. mRNA-Seq (13,600 transcripts) data yielded robust correlations of $r^2 \sim 0.88$ and $r^2 \sim 0.85$, respectively, indicating a high degree of measurement concordance between the WG-DASL HT assay (MCS4 + RTE reagent) and the other two gene expression platforms (Figure 5).

Conclusions

Splitting the MCS3 reagent in the WG-DASL HT assay into two components, MCS4 buffer and RTE, maintains high assay performance and data quality throughout the product shelf life. Because the gene expression profiles of WG-DASL HT assays performed with MCS3 and MCS4 + RTE are similar, samples processed with both reagents can be analyzed in a single experiment.

References

1. April C, Klotzle B, Royce T, Wickham-Garcia E, Boyaniwsky T et al. (2009) Whole-genome gene expression profiling of formalin-fixed, paraffin-embedded tissue samples. PLoS ONE 4(12): e8162.

2. MAQC Consortium, Shi L, Reid LH, Jones WD, Shippy R, et al. (2006) The Microarray Quality Control (MAQC) project shows inter- and intraplatform reproducibility of gene expression measurements. Nat Biotechnol. 24:1151–61.

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