

MCS3 Reagent Improves DASL® Assay Performance

Reformulated cDNA synthesis reagent delivers better reproducibility across FFPE samples.

Introduction

Illumina's Whole-Genome DASL® (cDNA-mediated Annealing, Selection, Extension, and Ligation) and VeraCode® DASL assays provide highly sensitive and cost-effective solutions for gene expression profiling from difficult or degraded RNA samples, especially those from formalin-fixed, paraffin-embedded (FFPE) tissues. Employed on Illumina's BeadArray™ and VeraCode platforms respectively, these assays begin with the conversion of total RNA to cDNA using biotinylated oligo (poly-dT) and random nonamer primers. The cDNA synthesis reagent, known as MCS2, enables this to occur.

During in-house quality testing of these DASL products, Illumina discovered that in some cases the MCS2 reagent was not providing an acceptable level of reproducibility when applied to RNA extracted from certain FFPE samples. RNA extracted from non-FFPE sources was not affected and continued to give acceptable levels of reproducibility. As a result, Illumina has reformulated the cDNA synthesis reagent that is used in this initial step. Compared to MCS2, the new MCS3 reagent delivers distinctly better reproducibility across a wider range of FFPE samples.

Analysis of Self-Reproducibility

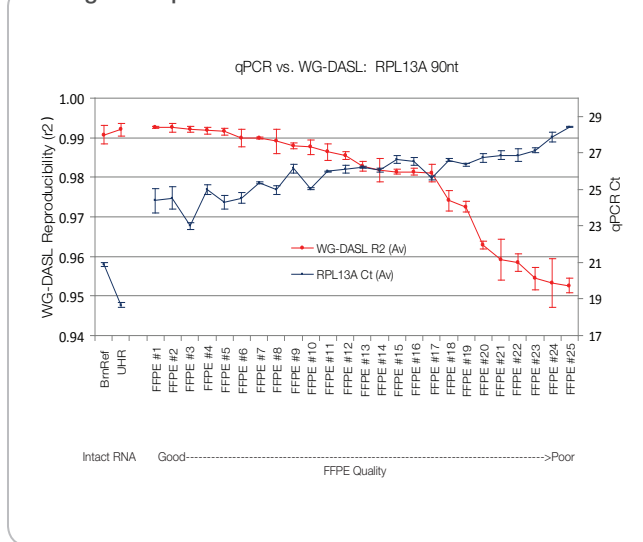
To evaluate and compare the self-reproducibility of data produced with each reagent, analytical experiments were performed with eight samples, consisting of RNA extracted from six Biochain FFPE samples and two intact MAQC samples [Universal Human Reference (UHR) and Human Brain Reference (BrnRef)]. RNA was extracted using the High Pure RNA Paraffin Kit (Roche), with total RNA inputs of 200 ng for the FFPE samples and 100 ng for the intact samples. Samples

Figure 1: Self-Reproducibility of MCS3 vs. MCS2



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

Figure 2: qPCR vs. Whole-Genome DASL



were chosen to demonstrate the performance of the assay with MCS2 or MCS3 using RNA from both poor-quality FFPE samples and better-quality FFPE samples, as assessed by the reproducibility achieved with the Whole-Genome DASL Assay. Samples were assayed in triplicate and scanned on the iScan System. All 24,526 probes were interrogated and the averaged raw unnormalized correlation (r^2) values plotted for each sample type (error bars = standard deviation of the mean).

The results shown in Figure 1 demonstrate improved self-reproducibility for poor-quality FFPE samples with MCS3 compared to MCS2. While both reagents delivered high reproducibility for better quality FFPE and intact RNA samples, only MCS3 delivered good self-reproducibility correlations ($r^2 > 0.97$) for all samples tested.

Pre-Qualification Protocol for cDNA Samples

To provide additional guidance on the use of the optional pre-qualification protocol for cDNA samples, we analyzed a larger set of 25 Biochain FFPE samples and two intact MAQC samples in triplicate using both qPCR and Whole-Genome DASL. The qPCR assay was based on the amplification of a 90 bp fragment of the highly expressed RPL13A ribosomal protein gene (GenBank accession #NM_012423.2) with SYBR Green detection. The RPL13A primers are designed to span an intron and should only generate a correctly amplified product from cDNA, not from genomic DNA. We then compared the WG-DASL self-reproducibility (r^2), and qPCR crossover threshold (Ct) values. The 25 FFPE samples ranged from very high quality to very poor quality, with archival ages between three and nine years.

