A Performance Comparison of the Enzo Single Round RNA Amplification and Biotin Labeling System

A Technical Note comparing performance of the Enzo Single-Round RNA Amplification and Biotin Labeling System to the Affymetrix GeneChip 3' IVT Express and the Affymetrix One-Cycle target labeling kits.

Introduction

The discontinuation of the One-Cycle kit necessitated the identification of a suitable replacement target preparation kit. Our initial evaluation of the GeneChip 3' IVT Express Assay found its performance to be comparable to the One-Cycle kit, albeit with some differences, notably, 3'/5' Actin ratios. Significant lot-to-lot variability associated with the 3' IVT Express Assay has necessitated Expression Analysis' (EA) evaluation of a number of alternative target preparation methods in order to identify methods with higher levels of comparability to the One-Cycle method. This Technical Note summarizes the performance of one of these methods, the Enzo Single-Round RNA Amplification and Biotin Labeling System.

Results

EA evaluated the Enzo kit using the Universal Human Reference RNA (UHRR) and the Human Brain Reference RNA (HBRR) samples featured in the MicroArray Quality Control (MAQC) project. Three independent replicates of each sample were prepared with the Enzo kit and hybridized to HG-U133A_2 GeneChips. Signal was derived using MAS5 and compared to data originating from the EA Microarray Proficiency Testing program (One-Cycle) and to 3' IVT Express data created during our initial evaluation of that amplification and labeling system.

In MAQC-I, two of the primary measures of similarity between assays were the overlap in gene lists using joint rules (significance and magnitude) for differential detection and the correlation of the Log Ratio values (for transcripts detected by each assay). Using the MAQC reference samples, Figure 1 compares lists of probe sets found to be differentially expressed by assay type using joint rules while Figure 2 illustrates the similarity in Log Ratio values for the assays.

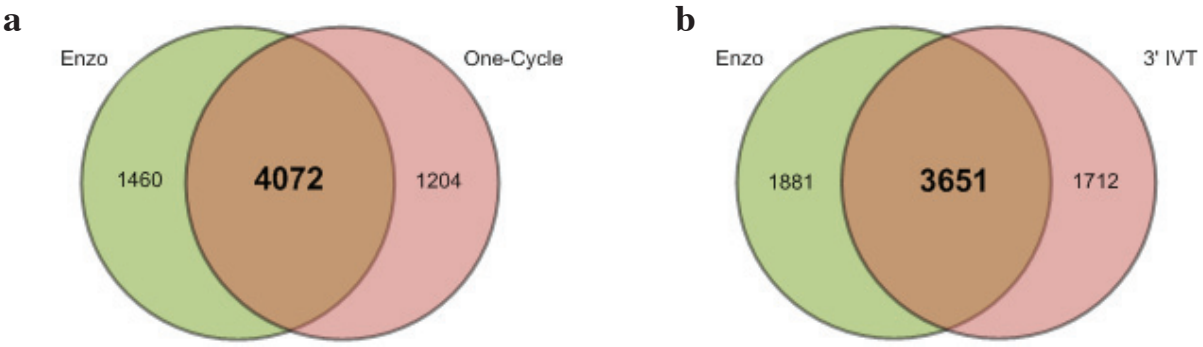
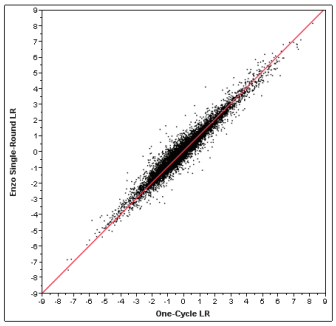


Figure 1. Differential expression in UHRR vs. HBRR compared between the Enzo kit and the One-Cycle kit (a) and the 3' IVT kit (b). The Enzo kit identifies a greater number of differentially expressed genes compared to either alternative and has a higher degree of overlap with the One-Cycle kit. The overlap between lists of significantly different probe sets ($p < 0.001$, fold-change ≥ 2.0) is displayed using Venn diagrams. More than 75% of significantly different probe sets detected using the One-Cycle kit are also detected using the Enzo kit. In contrast, only 68% of significantly different probe sets detected using the 3' IVT kit are also detected using the Enzo kit.

a



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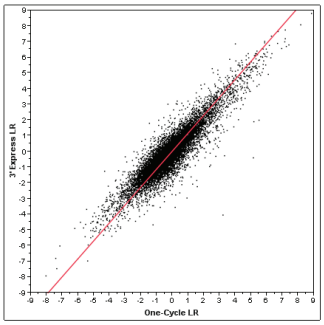

Figure 2. Correlation of UHRR vs. HBRR Log Ratio values between the Enzo kit and the One-Cycle kit (a) and the 3' IVT kit (b). The Enzo kit produces Log Ratio values that are highly similar to the One-Cycle kit. The correlation of the Log Ratio values produced by the Enzo kit and the One-Cycle kit (a) is 0.97. In contrast, a comparison between the Enzo kit and the 3' IVT kit (b) yields a correlation of 0.92.

GeneChip QC metrics were also found to be more similar between the Enzo kit and the One-Cycle Kit. As illustrated in Figure 3, detection rates overall were similar between all of the kits, though a comparison of means (Tukey-Kramer HSD) did identify significantly more detected transcripts with the Enzo kit than with the 3' IVT Express kit ($p = 0.003$). The same test detected a slight difference between the Enzo kit and the One-Cycle kit ($p = 0.055$), and no difference between the One-Cycle kit and the 3' IVT Express kit ($p = 0.338$). Differences between the kits for 3'/5' ratios further demonstrate similarities between the Enzo kit and the One-Cycle kit. While no significant differences were seen for GAPDH, Actin ratios showed highly significant differences between either the Enzo or One-Cycle kit when compared to the 3' IVT Express kit ($p = 0.002$ and $p = 0.004$, respectively), yet no significant difference between the Enzo kit and the One-Cycle kit ($p = 0.338$). Not surprisingly, a portion of the 3'/5' Actin ratio variance is attributable to RNA source. However, the target labeling method is estimated to contribute to more than 80% of the variance.

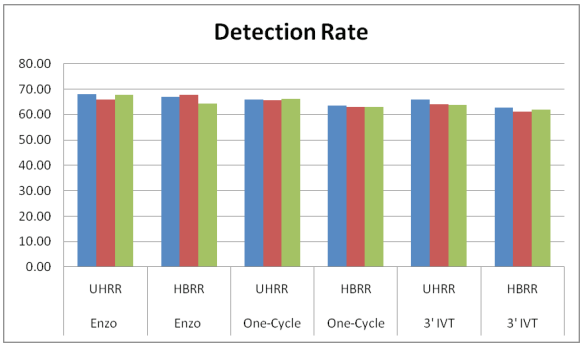

Figure 3. Detection rates (Percent Present) for each kit. The proportion of probe sets called Present by each method is highly similar.

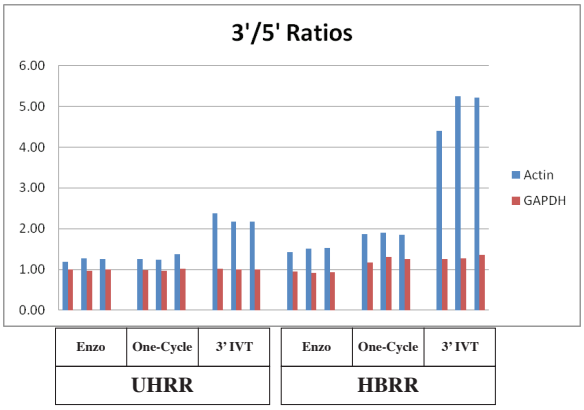

Figure 4. Actin and GAPDH 3'/5' ratios according to source RNA and target labeling method. Like the One-Cycle kit, the Enzo kit produces 3'/5' Actin ratios that are significantly lower than those produced by the 3' IVT kit. Actin and GAPDH ratios are influenced by the source RNA but the target labeling method is a larger contributor to variance.

Summary

Prior to its discontinuation, the One-Cycle method was the method of choice for Affymetrix GeneChip analyses. EA has investigated several methods and compared the results to existing One-Cycle data. While all target preparation methods introduce some level of bias, we find that the Enzo Single-Round RNA Amplification and Biotin Labeling kit produces data that is most closely aligned with One-Cycle data. Detection rates and 3'/5' ratios have a high degree of similarity, and differential expression—as measured by the Log Ratio of probe sets that are significantly different between UHRR and HBRR samples—display a correlation exceeding 0.97. Overall performance of the Enzo kit was found to be superior to that of the 3' IVT Express kit. Notwithstanding these performance differences, the Log Ratio correlation between the Enzo kit and the 3' IVT kit (0.92) was found to be similar to the Log Ratio correlation between the One-Cycle kit and the 3' IVT Express kit (0.90, EA Technical Note, September 2009). In addition to the results presented herein, EA has also compared data from a number of non-reference samples, with similar findings (data not shown).

Taken together, our results indicate that the Enzo kit is the best choice for routine Affymetrix GeneChip experiments.