Multi-site Evaluation of Next-Generation Methods for Small RNA Quantification

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Abstract

Small RNAs (smRNA) have been identified as important regulators of many biological processes and are now most frequently characterized using Illumina sequencing. However, while standard RNAseq library preparation has become routine in most sequencing facilities, small RNA sequencing library preparation has historically been challenging due to high input amount requirements, laborious protocols involving gel purifications, inability to automate, and a lack of benchmarking standards. Additionally, studies have suggested that many of these methods are non-linear and do not reflect the accurate levels of small RNAs in vivo. Recently, a number of new kits have become available that permit lower input amount along with less laborious gel-free protocol options. Many also claim to reduce RNA ligation-dependent bias through novel adapter modifications and to deplete adapter-dimer contaminants in the resulting libraries. With the increasing number of smRNA kits available, understanding the relative strengths of each method is critical for appropriate experimental design. In this study, we systematically compared ten commercially available small RNA library preparation kits as well as handforming probe hybridization across multiple study sites. We observe that, while new methodologies do reduce the amount of heavily oversequenced microRNAs, none of the methods are able to remove all of the bias in the library preparation. Identical samples prepared with different methods showed highly varied levels of different microRNAs. Even so, many methods excel in ease of use, lower input requirement, fraction of usable reads, and on reproducibility across sites. These differences may help users select the most appropriate methods for their specific question of interest.

Conclusions

All kits demonstrated high reproducibility between replicates, across site, and notably between the MUR and MUR-D sample despite the complex biological background in the Dicer1 cell line. However, despite high intra-kit reproducibility, all methods exhibited quantitative biases – no kit preserved even 50% of the miRNAs in the MUR spike-in within 2 fold of the median. Consequently, small RNA method selection should be based on multifactorial considerations including small RNA features of interest, input amount, ease of use, size selection requirements, cost, and methods used to generate pre-existing data.

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