A novel, streamlined workflow for high-throughput whole transcriptome RNAseq library preparation

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RNA sequencing (RNA-seq) has become an invaluable tool in the study of biology, but often requires multiple days to process samples from total RNA to final libraries that can be loaded on to a sequencer. When working with samples in a high-throughput capacity in particular, the time to prepare libraries can be a great hindrance for processing samples in a timely manner.

Here, we present a new protocol that allows users to easily generate RNA-seq libraries in a single day from 25-250 ng of total RNA. Compatible with poly(A) enrichment or ribosomal RNA depletion, this new workflow has reduced preparation time by about 40% compared to current library prep kits without sacrificing quality of libraries or any sequencing metrics, including 5'-3' coverage, GC bias. In addition to master mixed components and reduced incubation times, the reduction in bead cleanup steps significantly reduces hands-on time for library preparation, simplifying and streamlining the process.

This new library preparation method is also ideal for processing samples of various inputs with a single condition at every step throughout the protocol, without modifying cycling conditions or dilutions based on input amount. This workflow provides a great advantage in efficiently generating RNA-seq libraries while simultaneously maintaining high quality sequencing results and compatibility with both high- and low-quality RNA such as FFPE.