Improved RNA-Seq Library Prep: Further simplifying whole transcriptome library prep using modifications to the sparQ RNA-Seq HMR Kit

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Introduction

RNA-seq studies carried out using high-throughput sequencing of cDNA have provided tremendous insight into cellular transcript studies on a large and comprehensive scale. However, technical challenges such as laborious and lengthy workflows, affordability, compromised accuracy, read coverage biases and limited transcript diversity have impeded implementation of the technology in many labs. Here we present a simple, affordable, high-performance solution for directional RNA-seq library preparation: the sparQ RNA-Seq HMR Kit. The kit integrates depletion of ribosomal and globin transcripts (human, mouse, and rat) and RNA fragmentation into a single step and tube. The proprietary, highly optimized enzymes and streamlined workflow generates high quality, directional whole transcriptome NGS Barcodes from either intact or degraded RNA samples. Improvements to the workflow have allowed for a further reduction in processing time to 4.5 hours. Additionally, the introduction of a new Bead Booster component has improved yield and reduced adapter dimer formation.

The sparQ RNA-Seq HMR Kit features:

- Integrated riboglobin depletion technology
- Faster time to result (4.5 hours)
- Minimal hands-on time
- Fewer pipetting steps

Taken together, this workflow improves accessibility to RNA-seq technologies while allowing for faster turnaround time for sample-to-result.

Methods

Libraries were prepared using sparQ RNA-Seq HMR Kit with integrated rRNA/globin depletion. NEBNext® Ultra II Directional RNA Kit with NEBNext Globin & rRNA Depletion Kit (HMR), KAPA® HyperPrep Kit with RiboErase genome of each sample accordingly using the CLC Genomics Workbench bp). The sequenced reads were analyzed by aligning the reads to the reference then libraries were sequenced on the Illumina NextSeq 550 instrument (2x100 nt). cDNA libraries were analyzed and quantified using TapeStation 4200 (Agilent), as follows: UHRR (9.4), FFPE (3.5), Fresh Tissue (7.6) and Blood (8.5).

Results

The sparQ RNA-Seq HMR Kit shows excellent performance in a number of key areas:

- Simple and efficient workflow with results in 4.5 hours and 33% less hands-on time
- RNA and Globin Depletion: Fragmentation, 1st and 2nd strand synthesis, and end-polishing all take place in the same tube without purification steps
- Efficient removal of rRNA and globin improves sequencing results
- Improved library yield for samples with limited RNA quantity or poor quality
- Better overall coverage uniformity enables correct identification of full-length transcripts

For more information and ordering instructions, visit: www.quantabio.com