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 libraries 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 (HMR) and Illumina® Stranded Total RNA Prep with Ribo-Zero Plus from a range of input samples. RNA samples used in this study were Agilent Universal Human Reference (UHR) RNA (1 - 100 ng), human adult normal liver tissue FFPE total RNA (BioChain) (10 - 100 ng) and total RNA from mouse fresh liver tissue and human blood (Innovative Research) (10 - 300 ng). RIN scores were as follows: UHRR (9.4), FFPE (3.5), Fresh Tissue (7.6) and Blood (8.5).

cDNA libraries were analyzed and quantified using TapeStation 4200 (Agilent), then libraries were sequenced on the Illumina NextSeq 550 instrument (2x100 bp). The sequenced reads were analyzed by aligning the reads to the reference genome of each sample accordingly using the CLC Genomics Workbench 20.0.4 software (QIAGEN). The sequencing data were processed to determine the read quality metrics that include GC-content, strand specificity, unique mapped fragments, RNA biotypes and 5'-3' transcript coverage.

Results

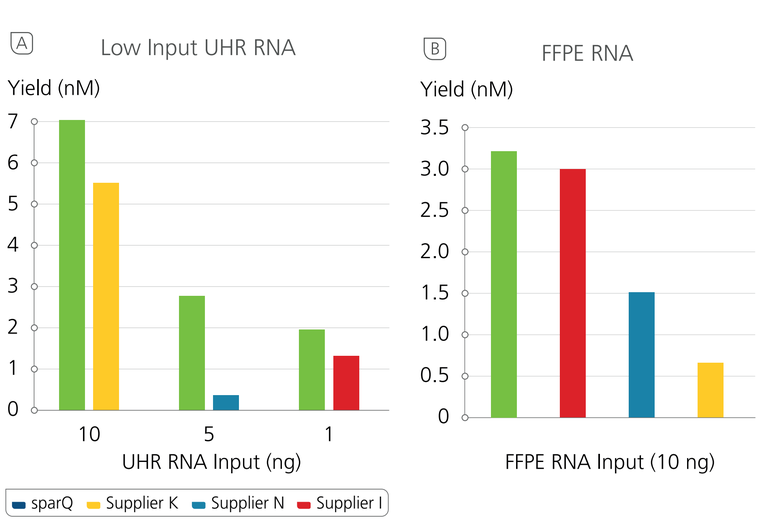

Figure 1 Higher library yield observed with sparQ RNA-Seq HMR Kit for low input and degraded input RNA. For low input quantities of UHR RNA, the lowest recommended RNA input amount for each kit was used. (**A**) The sparQ RNA-Seq HMR Kit generated consistently higher yields at low UHR RNA inputs and (**B**) with low quality FFPE RNA (RIN 3.5).

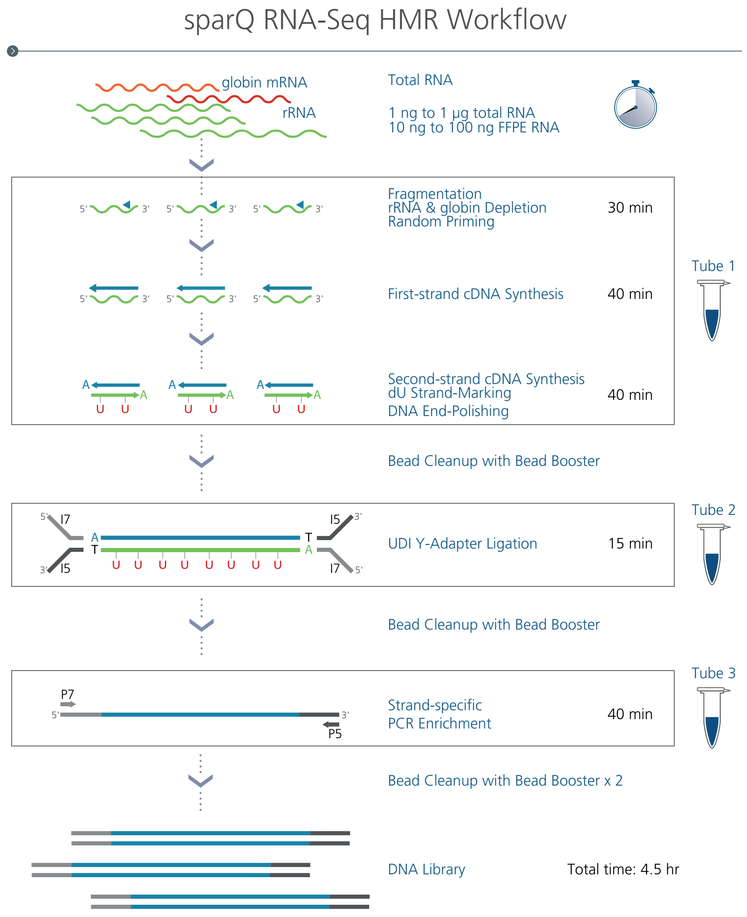

Figure 2 sparQ RNA-Seq HMR Kit streamlined workflow. The sparQ RNA-Seq HMR Kit is suitable for either intact or degraded samples at varying input quantities (from 1 ng of high-quality RNA and 10 ng of degraded RNA up to 1000 ng RNA input). High coverage, riboglobin depleted, strand-specific RNA-seq libraries can be prepared from total RNA in 4.5 hr using the sparQ RNA-Seq HMR Kit.

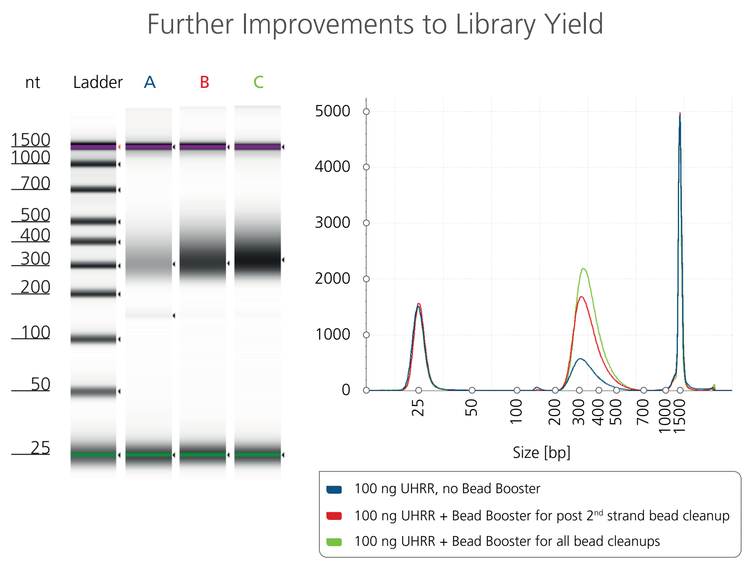

Figure 3 Use of Bead Booster during bead cleanups improves overall yield. Libraries were prepared using 100 ng UHR RNA with (A) no Bead Booster, (B) Bead Booster used only for cleanup post-2nd strand synthesis and (C) with Bead Booster used during all bead cleanup steps. The use of Bead Booster greatly improved yield and handling of bead cleanups steps (data not shown).

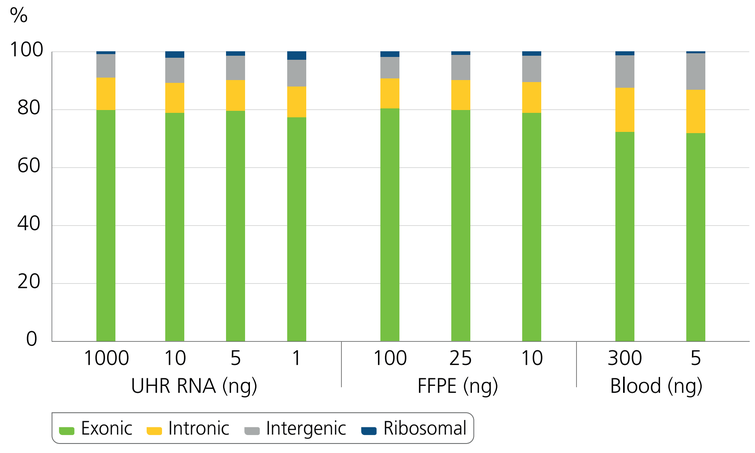

Figure 4 High proportion of reads mapped to protein coding regions. Libraries were prepared from various sample types at varying input amounts, then sequenced. Reads were mapped to the human transcriptome RNA biotypes. Libraries prepared with sparQ RNA-Seq HMR Kit showed the highest proportion of protein coding reads, when compared with other supplier kits, and very low rRNA reads (0–5%).

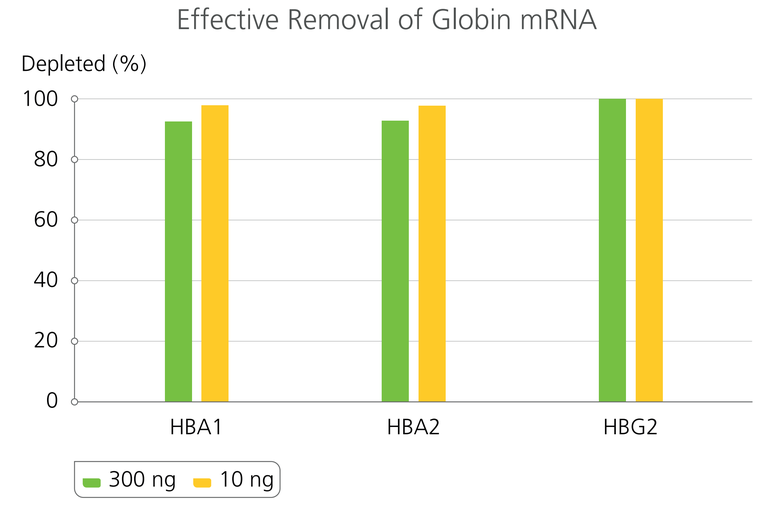

Figure 5 Consistent, high percentage of globin mRNA depletion. Libraries were generated with 300 ng and 10 ng input of blood samples according to the standard protocol, then compared against a library generated without globin mRNA depletion at 300 ng input. Globin mRNA levels were measured using TPM (transcripts per million) count.

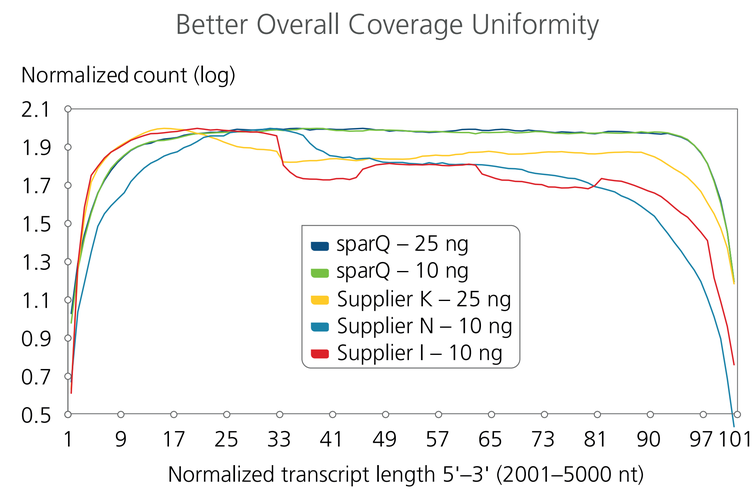

Figure 6 Uniform 3' transcript coverage. sparQ RNA-Seq HMR Kit was uniquely able to retain uniform 3' coverage for FFPE RNA, a feature that will help correctly identify full-length genes in low quality samples. For UHR RNA, all RNA-seq kits showed comparable uniformity.

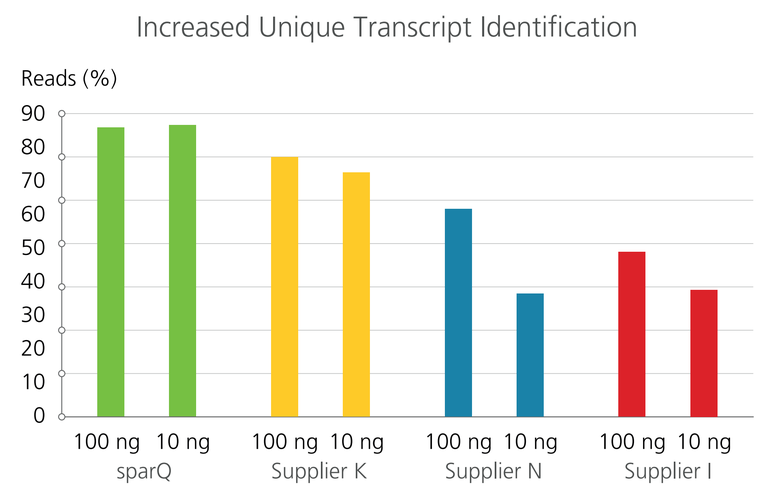

Figure 7 Comparison of unique fragments. The sparQ RNA-Seq HMR Kit consistently demonstrated higher rates of unique fragments indicating the highest library diversity regardless of RNA input quantity and sample type.

Conclusions

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
- rRNA 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