

Improved Rapid Stranded RNA-Seq

Offers Higher Library Yields & Better Mapped Reads

Authors: Bradley Hehli^a

^a Bioo Scientific Corporation, 7050 Burleson Rd., Austin, TX 78744, USA

INTRODUCTION

Bioo Scientific's **NEXTflex™ Rapid Directional RNA-Seq Kit** offers researchers a cost-effective way to produce high quality, stranded RNA-Seq libraries. To assess the overall quality of our kit, we compared libraries prepared using **NEXTflex™ Poly(A) Beads** and NEXTflex Rapid Directional RNA-Seq Kit to those prepared with Competitor N's equivalent products. We examined rRNA removal, library yield, and overall quality metrics, and demonstrated the effectiveness of the NEXTflex Poly(A) Beads and the NEXTflex Rapid Directional RNA-Seq Kit for providing high quality library preparation solutions for next generation sequencing.

METHODS

Poly(A) selection of commercially available MCF-7 total RNA was performed using NEXTflex Poly(A) Beads or Competitor N's competitive product. Poly(A) selection was carried out according to the manufacturer's instructions using 1 µg total RNA. Libraries were prepared in triplicate using Poly(A) selection by NEXTflex Poly(A) Beads and the NEXTflex Rapid Directional RNA-Seq Kit (bb), or using Competitor N's Poly(A) beads and Competitor's N equivalent directional RNA-Seq kit (nn). Library quality was assessed by Bioanalyzer and yield was determined by qPCR. Each sample was quantified, pooled into equimolar ratios, and sequenced on a HiSeq 2500 in Rapid mode.

RESULTS

Library Quality and Quantity

Several metrics of library preparation were assessed prior to sequencing. All libraries contained insert sizes ranging from 200-400 bp as determined by Agilent Bioanalyzer, with minimal adapter contamination (Figure 1A). Quantification of library yield by qPCR revealed that library preparations utilizing NEXTflex Poly(A) Beads significantly outperformed those preparations created from Competitor N's equivalent poly(A) beads (Figure 1B). The concentration of libraries prepared with NEXTflex Poly(A) Beads was roughly four times higher than in those prepared with Competitor N's beads.

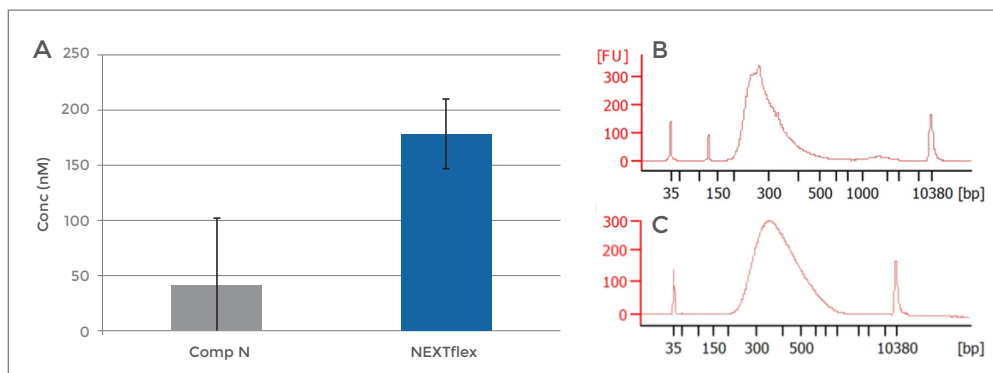


Figure 1. Library Yields
 (A) Averaged yields of 3 libraries of each treatment by qPCR. The qPCR was performed in triplicate on each sample at two dilutions.
 (B) Representative Bioanalyzer trace of Comp N prepared library.
 (C) Representative Bioanalyzer trace of 1:5 dilution of NEXTflex prepared library.

rRNA and Other Non-Coding RNA

In order to examine whether higher yields correspond to differences in the amount of rRNA depletion achieved, reads were mapped to rRNA sequences to determine rRNA contamination in each sample. In both sets of libraries, low levels of rRNA mapping to 5S, 5.8S, 18S or 28S rRNA were observed. However, libraries prepared with the NEXTflex Rapid Directional RNA-Seq Kit displayed consistently lower percentages of reads mapping to rRNA compared to Competitor N's kit (Figure 2). These data indicate Bioo Scientific's Rapid Directional RNA-Seq library preparation reagents provide higher library yield and greater levels of useful reads.

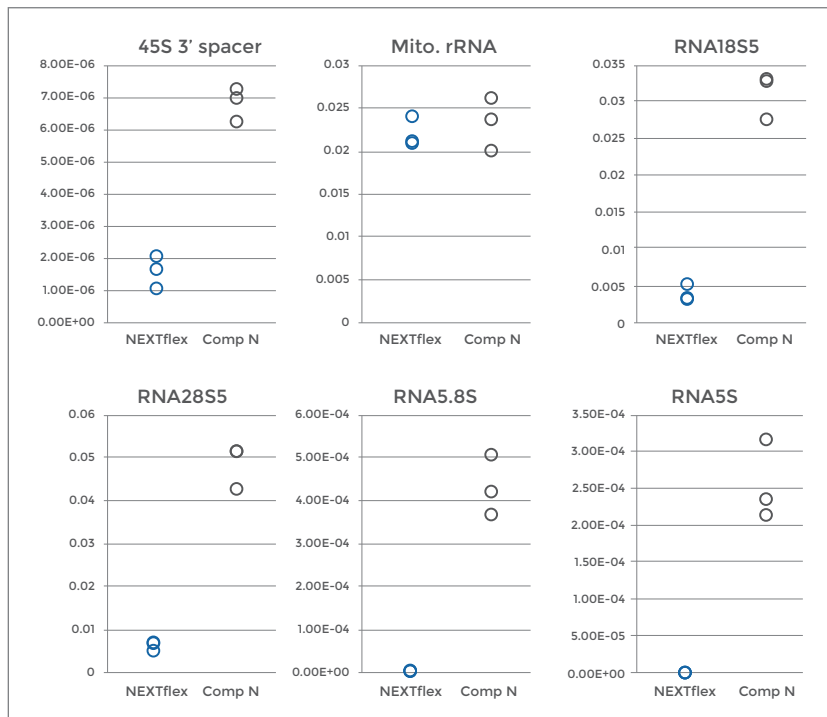


Figure 2. rRNA Mapping

Percentage of reads mapping to indicated rRNA sequence.

Read Mapping

Next, additional metrics of library quality were assessed by mapping reads to the human genome and examining the fraction of reads mapping to transcripts, as well as by examining duplication rates. Mapping metrics were not found to be statistically different between the two library prep methods. Each gave similar profiles of reads mapping to exons and introns, unaligned reads, and reads mapping to intergenic regions (Figure 3). Next, the percentage of libraries that contained uniquely mapping reads as well as percentage of reads that were completely unique was analyzed. The percentage of uniquely mapping reads, out of the total number of reads, was $53.49\% \pm 1.16\%$ for the NEXTflex Rapid Directional RNA-Seq Kit and $44.08\% \pm 3.95\%$ for Competitor N's kit, indicating that the NEXTflex Rapid Directional RNA-Seq Kit outperformed the competitor's kit in this metric (Table 1). Uniquely mapping reads are generally within the 50-65% range for the NEXTflex Rapid Directional RNA kits. The NEXTflex Rapid Directional RNA-Seq Kit performs better or similarly to Competitor N's kit in regard to percentage of duplicate reads (Figure 4). It is important to note that a duplication rate greater than 50% was observed, which is expected from libraries produced using any kit or platform that utilizes a PCR amplification step (Li et. al., 2014). Library complexity, as measured by examining duplication rates, reveals Competitor N's kit to have slightly higher numbers of duplicate reads.

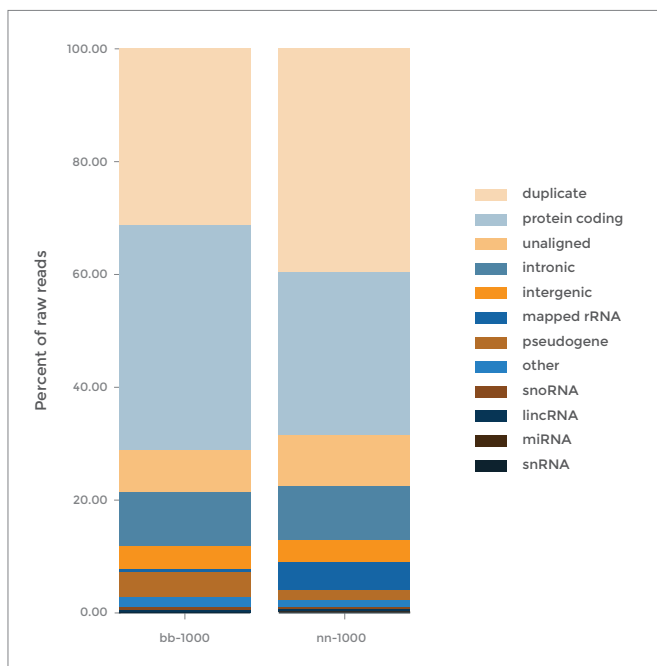


Figure 3. Reads Mapped to Genome
Percentage of reads mapping to classes of genes.

Table 1. Unique Mapping Rate and Number of Unique Reads.

The percentage of uniquely mapped reads and the number in millions of total unique reads from n=3 replicate libraries for each kit.

Kit Name	Unique Mapped	Unique Mapped Reads (millions)
NEXTflex™ Rapid Directional RNA-Seq Kit	53.49% ± 1.16%	1604 ± 35
Competitor N Directional RNA-Seq Kit	44.08% ± 3.95%	1321 ± 118

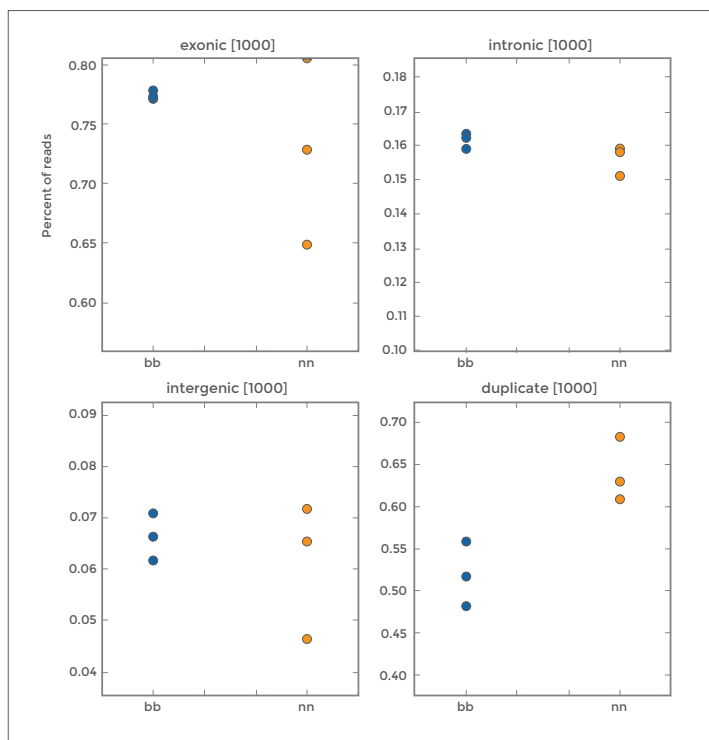


Figure 4. Reads Mapping Metrics
Percentage of reads mapping to exonic, intronic, and intergenic regions, as well as sample duplication rate.

CONCLUSIONS

The NEXTflex Poly(A) Beads and NEXTflex Rapid Directional RNA-Seq Kit are high quality library preparation tools that outperform Competitor N's similar product in several metrics. Bioo Scientific's NEXTflex Poly(A) Beads show a clear advantage in performance, leading to better yields enabling the construction of libraries with input amounts lower than typically recommended. The NEXTflex Rapid Directional RNA-Seq Kit outperformed the competitor's kit when mapping contaminating reads such as rRNA, offering researchers confidence that the NEXTflex libraries consist of useful sequences. Both sequencing kits performed similarly in terms of read mapping, with the NEXTflex kit providing an advantage in unique reads. Bioo Scientific can strongly recommend the NEXTflex Rapid Directional RNA-Seq Kit to researchers for generating high quality RNA-Seq libraries in a cost efficient manner.

References

Li S, Tighe SW, Nicolet CM, Grove D, Levy S, Farmerie W, Viale A, Wright C, Schweitzer PA, Gao Y, Kim D, Boland J, Hicks B, Kim R, Chhangawala S, Jafari N, Raghavachari N, Gandara J, Garcia-Reyero N, Hendrickson C, Roberson D, Rosenfeld J, Smith T, Underwood JG, Wang M, Zumbo P, Baldwin DA, Grills GS, Mason CE. Multi-platform assessment of transcriptome profiling using RNA-seq in the ABRF next-generation sequencing study. Nat Biotechnol. 2014 Sep;32(9):915-25.



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Barcelona

93 645 50 28
barcelona@rafer.es

Bilbao

94 499 85 80
bilbao@rafer.es

La Coruña

981 93 89 26
galicia@rafer.es

Madrid

91 365 15 70
madrid@rafer.es

Málaga

639 359 792
malaga@rafer.es

Sevilla

954 369 334
sevilla@rafer.es

Valencia

96 340 48 00
levante@rafer.es

Zaragoza

976 23 74 00
rafer@rafer.es

Lisboa

21 154 19 98
lisboa@rafer.es