Optimizing Library Preparation: Enhanced Adapter Ligation Technology

Introduction

Important factors to consider when choosing an NGS library preparation kit include ligation and amplification efficiency, bias and coverage, yield, cost, turnaround time and labor requirements. Adapter ligation is a critical library preparation consideration as greater ligation efficiency increases library diversity and complexity, thus allowing for use of degraded or smaller amounts of input DNA, which is crucial for research and clinical samples. Bioo Scientific incorporates our proprietary Enhanced Adapter Ligation Technology, which offers the best available ligation efficiency, into all of our library preparation kits including the new NEXTflex™ Rapid DNA-Seq Kit. The NEXTflex Rapid DNA-Seq Kit enables NGS libraries to be constructed in as little as two hours from 1 ng to 1 µg of input DNA.

In this study, we report a comparison between Bioo Scientific's NEXTflex Rapid DNA-Seq Kit, containing Enhanced Adapter Ligation Technology, and another commercial product claiming comparable ligation efficiency and reduction in library preparation time, Competitor N's kit. Since both kits are designed to require similarly minimal processing times compared to their standard counterparts, we decided to focus our analysis on the efficiency of adapter ligation prior to PCR amplification. Robust, unbiased ligation is necessary to minimize loss in overall coverage during sequencing, especially for low input samples. On the widely used Illumina sequencing platform, only "doubly-ligated" target DNA fragments (i.e. those containing adapters ligated at both the 5' and 3' ends) can serve as template for sequencing, so maximizing the yield of doubly-ligated product is essential for maximizing efficiency of library production.

Methods

To standardize the input DNA for the study, a single 280 bp amplicon was produced and used as input into both NEXTflex Rapid DNA-Seq and Competitor N's kits. Triplicate libraries were made for each kit.

The provided protocols for both kits were followed through ligation. All samples were then purified and size selected using an identical AMPure XP bead cleanup step. The workflow for the triplicate samples appears below:

100 ng Amplicon Input	
NEXTflex™ Rapid DNA-Seq Kit	**Competitor N's Kit**
STEP A: End Repair (Steps 1 – 2) 50 µL reaction volume 25°C, 20 minutes 72°C, 20 minutes 4°C hold	**STEP A: Competitor N End Prep (Steps 1 – 3)** 65 µL reaction volume 20°C, 30 minutes 65°C, 30 minutes 4°C hold
STEP B: Adapter Ligation (Steps 1 – 2) 100 µL reaction volume 25°C, 15 minutes	**STEP B: Adapter Ligation (Steps 1 – 3)** 83.5 µL reaction volume 20°C, 15 minutes
Cleanup 1.5x AMPure XP bead cleanup, eluted in 20 µL ddH_2O	**Cleanup** 1.5x AMPure XP bead cleanup, eluted in 20 µL ddH_2O

The protocol for Competitor N's kit was stopped at Step 3 of Adapter Ligation, as addition of the USER enzyme is not necessary for the Bioo Scientific DNA Barcodes used. DNA Barcode 1 (2.5 µL at 25 µM concentration) was used for all samples to ensure complete saturation of the amplicons.

Samples post-clean up were analyzed using a High-Sensitivity DNA Chip on an Agilent 2100 Bioanalyzer. Data was generated from the integration of peaks of the three expected products of ligation: non-ligated amplicon, singly-ligated amplicon and doubly-ligated amplicon.

Analysis and Results

Triplicate overlays of the three ligation product peaks for NEXTflex Rapid DNA-Seq and Competitor N's kits appear below.

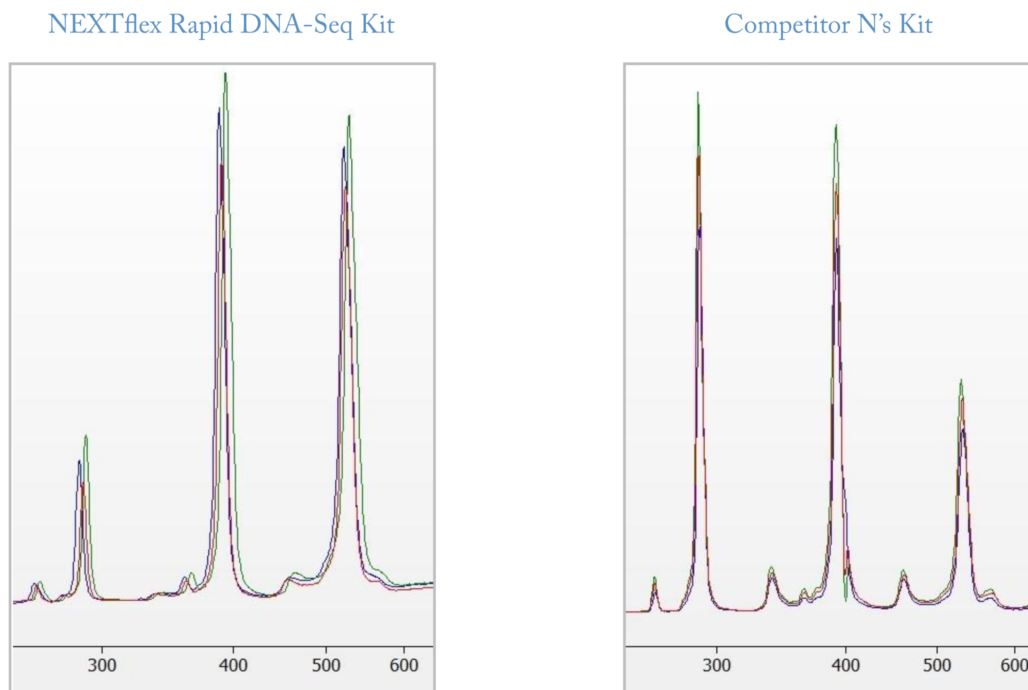

Figure 1. Overlays of the triplicate samples of NEXTflex Rapid DNA-Seq (left) and Competitor N's kit (right).

Integration of the peaks by the Agilent 2100 Bioanalyzer software produces size-independent values for percentage of product comprising each peak. These values were averaged for the triplicate ligation reactions for each sample. The resulting comparison of unligated, singly-ligated, and doubly-ligated products was graphed.

Sample	% Representation Non-ligated Peak 1	Singly-ligated Peak 2	Doubly-ligated Peak 3
NEXTflex Rapid DNA-Seq	10.9	42.1	47.1
Competitor N's Kit	38.7	39.8	21.5

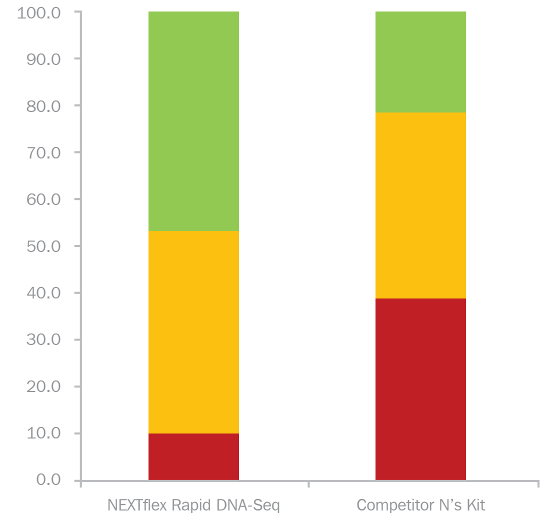

Figure 2. Direct comparison of NEXTflex Rapid DNA-Seq to Competitor N's kit by analysis of proportions of ligation products. Red portions of the graph indicate non-ligated product proportion; yellow, singly-ligated; green, the desired doubly-ligated amplicon.

Discussion

A concern with using rapid NGS library preparation methods is whether they result in compromised yields of the desired product. Reducing the preparation time places more reliance upon the critical ligase enzymes to catalyze their reactions efficiently. Bioo Scientific's Enhanced Adapter Ligation Technology has been designed for exactly this purpose. Here the ligation efficiency of two accelerated library prep kits, NEXTflex Rapid DNA-Seq and Competitor N's kit, was compared. The results of the study revealed a higher proportional yield of the desired doubly-ligated product using the NEXTflex Rapid DNA-Seq Kit. The NEXTflex Rapid DNA-Seq Kit demonstrated more than twice the proportional yield of doubly ligated product compared to Competitor N's kit.

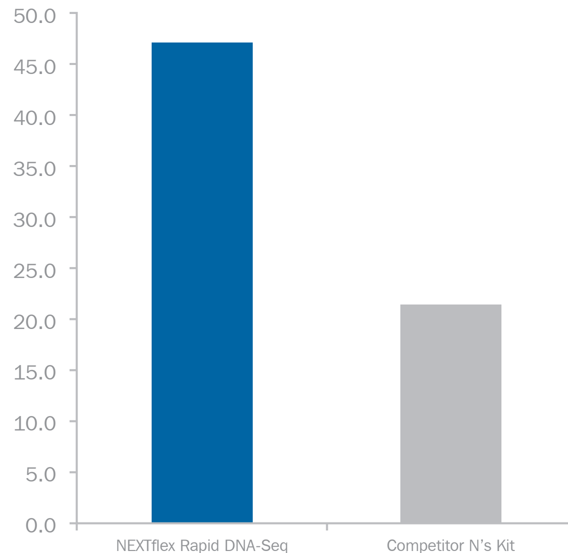

Figure 3. Average triplicate values for the desired doubly-ligated products for NEXTflex Rapid DNA-Seq and Competitor N prepared libaries. The NEXTflex libaries produced 47.1% relative conversion compared to Competitor N's 21.5%.

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