NEXTFLEX® COMBO-SEQ mRNA/miRNA KIT

DNA-SEQ · RNA-SEQ · BARCODES · METAGENOMICS · AMPLICON PANELS · TARGET CAPTURE · EPIGENETICS · SMALL RNA-SEQ

Your RNA-seq preps haven't been telling you the whole story. *Find out what you've been missing!*

The NEXTFLEX® Combo-Seq™ mRNA/miRNA kit generates mRNA and small RNA libraries in a single workflow from 1 ng – 100 ng total RNA inputs for Illumina® sequencing. The protocol is completely gel-free and relies upon novel techniques that enable library construction for both mRNA and small RNA, all without the need for rRNA depletion or poly(A) selection. Building on the patented technology of the best-in-class NEXTFLEX® small RNA-seq kit v3, the adapters with randomized ends help to greatly reduce bias and ultimately enable more accurate representation of small RNAs in the starting material without compromising sequencing efficiency. The streamlined and automation-friendly protocol can generate quality libraries all within a single workday.

KEY FEATURES

- Combined library prep workflow for both small RNA and mRNA sequencing
- Completely gel-free protocol from inputs as low as 1ng
- Compatible with total RNA inputs, requiring no poly(A) selection or rRNA depletion
- Complete kit solution, including cleanup/size selection beads and barcodes
- Automation-ready on the PerkinElmer® Sciclone® G3 NGS/NGSx workstation

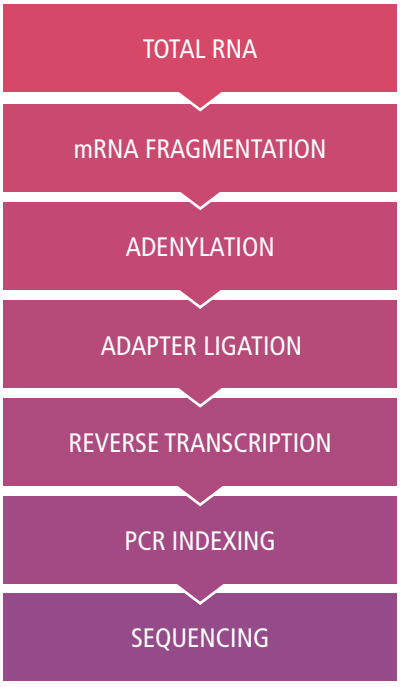

Figure 1. NEXTFLEX® Combo-Seq™ mRNA/miRNA Kit workflow.

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For research use only. Not for use in diagnostic procedures.

Reagent Attributes	NEXTFLEX® Combo-Seq™ Kit	Competitor N
mRNA and small RNA compatible	Yes	No
Poly(A) selection or rRNA depletion required	No	Yes
Minimum input	1 ng	10 ng
Automation	Yes	Poly(A) selection & fragmentation pose challenges to automation
cfRNA compatible	Yes	No
Barcodes included	Yes	No
Turn-around-time	7 hrs (one day)	10 hrs (two days)
All-inclusive kit (reagent, barcodes, beads, etc)	Yes	No
Adapter dilution required	No	Yes

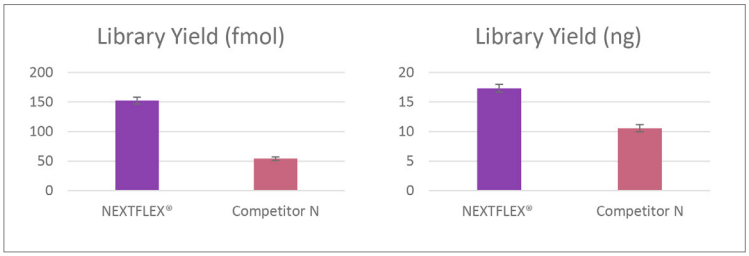

***Figure 2.* Consistently higher yield, both in weight and number of molecules, using the NEXTFLEX® Combo-Seq™ mRNA/miRNA kit.** Libraries were generated from 20 ng of Biochain Human Universal RNA (R4234565) using the NEXTFLEX® Combo-Seq™ mRNA/miRNA Kit and Competitor N's directional RNA library prep kit (requiring Poly(A) mRNA isolation module). 14 cycles of PCR were performed for both workflows. Yield and average size were determined by Qubit® instrument and the Agilent® Bioanalyzer workstation, respectively.

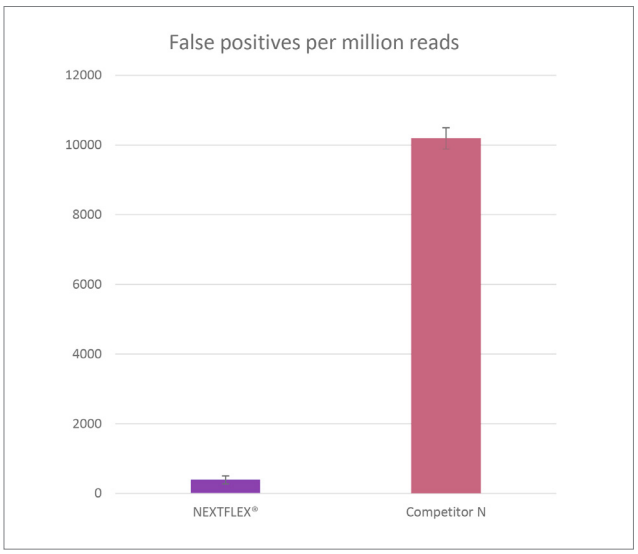

***Figure 3.* The NEXTFLEX® Combo-Seq™ mRNA/miRNA kit gives higher confidence in strand calls compared to Competitor N's kit.** Reads were mapped to synthetic ERCC's to determine orientation. NEXTFLEX® chemistry has >99.9% directionality whereas Competitor N is only ~99% directional (data not shown), which allows for higher directionality/strandedness and lower rate of false-positives regarding directionality.

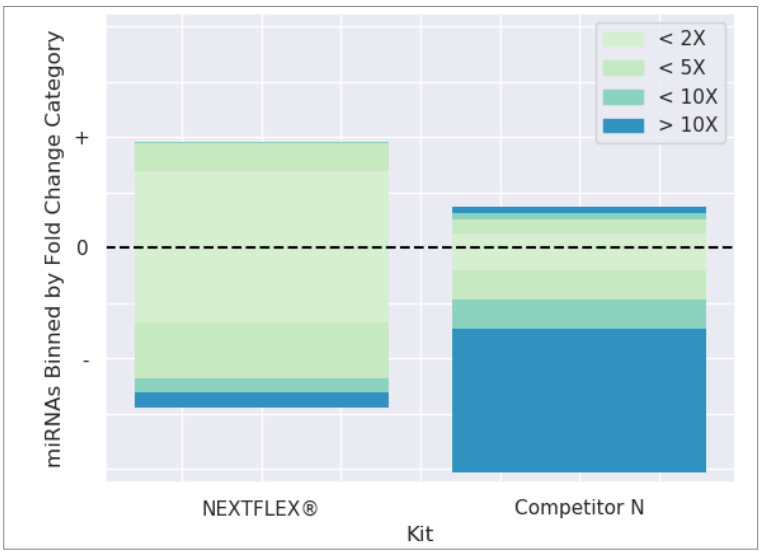

***Figure 4.* The NEXTLFEX® Combo-Seq ™ mRNA/miRNA kit gives more accurate representation of miRNAs than Competitor N's kit.** In order to assess bias, libraries were generated from a pool of 963 equimolarly pooled miRNAs using the NEXTFLEX® Combo-Seq™ mRNA/miRNA kit and a commercially available small RNA-seq kit from Competitor N. Individual miRNAs were binned into categories based on their representation in the sequencing library in terms of fold-change from expected. With the NEXTFLEX® Combo-Seq™ mRNA/miRNA kit, only 5.8% of miRNAs deviate more than 10-fold from the expected value, compared with 30.1% of miRNAs with Competitor N.

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