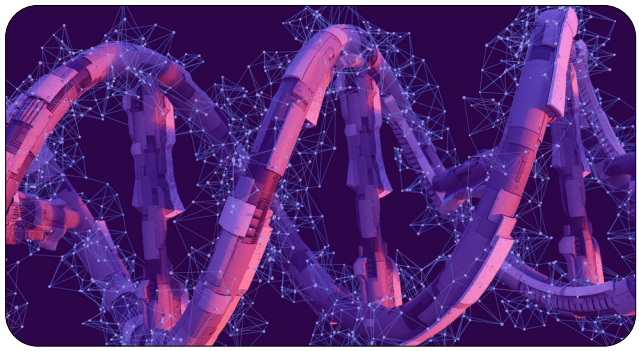
revvity

New and improved library preparation kit for your RNA sequencing needs

NEXTFLEX® Rapid Directional RNA-Seq kit 2.0.

The NEXTFLEX® rapid directional RNA-seq kit 2.0 produces libraries for Illumina® sequencing with high coverage uniformity, low duplication rates, >99% strand specificity and minimal rRNA contamination when used with the NEXTFLEX® Poly(A) Beads 2.0. This kit includes reverse transcriptase, necessary library preparation reagents, and cleanup/size selection beads optimized to ensure reliable performance. The kit involves a simple library preparation protocol that has been validated with the updated NEXTFLEX® poly(A) beads 2.0 to accommodate total RNA as input. The NEXTFLEX® rapid directional RNA-seq kit 2.0 is designed to be used with NEXTFLEX® barcodes, which will be provided to early access users. All NEXTFLEX® barcodes are color-balanced and have undergone proprietary purity QC metrics to give you reliable sequencing results for every sample.



gDNA

Fragmentation + End Repair + Adenylation

Adapter Ligation

Optional Size Selection

PCR

Sequencing

Are you looking for an RNA-Seq analysis solution? Contact www.revvity.com for software platform recommendations

- Reliable: High coverage uniformity with low duplication rate
- Convenient: Optimized with reverse transcriptase and cleanup/size selection beads
- Streamlined: Simple protocol validated with NEXTFLEX® poly(A) beads 2.0
- Flexible: Designed to work with NEXTFLEX® color-balanced barcodes that allow a wide range of multi-plexing (2 up to 384 samples in one run)
- Efficient: Automated on the Sciclone® G3 NGSx, Sciclone® G3 NGSx iQ™, and Zephyr® G3 NGS workstations

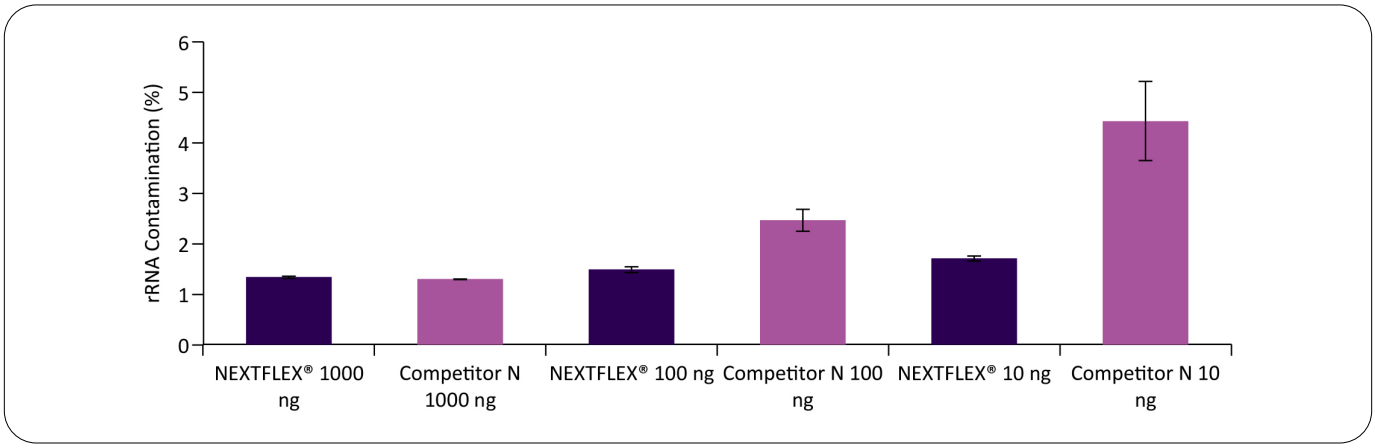


Figure 4: The NEXTFLEX® Rapid Directional RNA-Seq kit 2.0 delivers libraries containing lower levels of rRNA contamination than the Competitor N kit. Poly(A) mRNA was isolated from Universal Human Reference RNA (Agilent #740000) using the NEXTFLEX® Poly(A) Beads 2.0 and the Competitor N Poly(A) enrichment kit. Libraries were generated using the NEXTFLEX® Rapid Directional RNA-Seq kit 2.0 and the Competitor N's library preparation kit. The resulting libraries were sequenced on the Illumina® MiSeq® sequencer using paired-end mode (2x76 bp). The reads were trimmed using cutadapt and the percent of rRNA was determined by using bowtie2 to map reads to human rRNA. The NEXTFLEX® Rapid Directional RNA-Seq kit 2.0 demonstrated superior removal of 5S, 5.8S, 12S, 16S, 18S, and 28S rRNA species compared to the Competitor N kit.

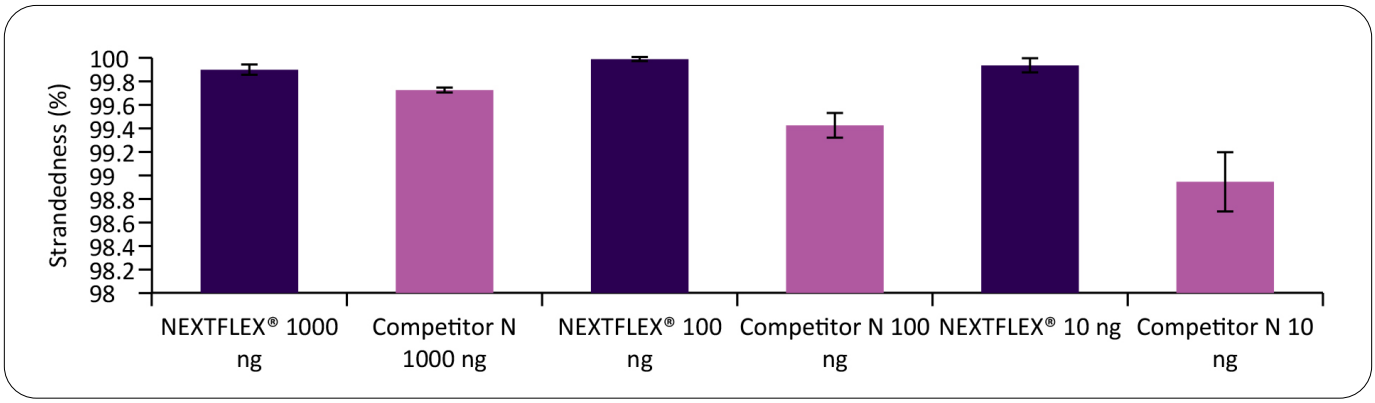


Figure 3: The NEXTFLEX® Rapid Directional RNA-Seq kit 2.0 demonstrate superior strandedness than the Competitor N kit. Poly(A) mRNA was isolated from Universal Human Reference RNA (Agilent #740000) containing ERCC RNA Spike-In mix (Thermo Fisher® Scientific #4456740) using the NEXTFLEX® Poly(A) Beads 2.0 and the Competitor N Poly(A) enrichment kit. Libraries were generated using the NEXTFLEX® Rapid Directional RNA-Seq kit 2.0 and the Competitor N's library preparation kit. The resulting libraries were sequenced on the Illumina® MiSeq® sequencer using paired-end mode (2x76 bp). Reads were trimmed using cutadapt and mapped to the ERCC92 reference using bowtie2. Strandedness was calculated using SAMtools.

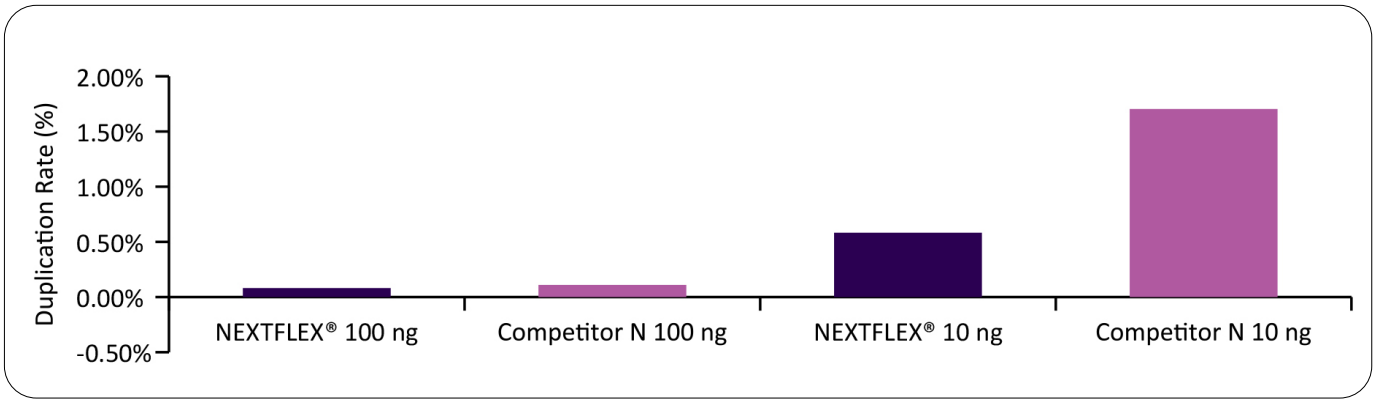


Figure 2: The NEXTFLEX® Rapid Directional RNA-Seq kit 2.0 demonstrate lower duplication rate compared to the Competitor N kit. Poly(A) mRNA was isolated from Universal Human Reference RNA (Agilent #740000) using the NEXTFLEX® Poly(A) Beads 2.0 and the Competitor N Poly(A) enrichment kit. Libraries were generated using the NEXTFLEX® Rapid Directional RNA-Seq kit 2.0 and the Competitor N's library preparation kit. The resulting libraries were sequenced on the Illumina® MiSeq® sequencer using paired-end mode (2x76 bp). Reads were trimmed using cutadapt, mapped to the Gencode v30 reference using bowtie2, and randomly downsampled to 100k reads. Duplication rate was calculated using the fastp all-in-one FASTQ preprocessor.

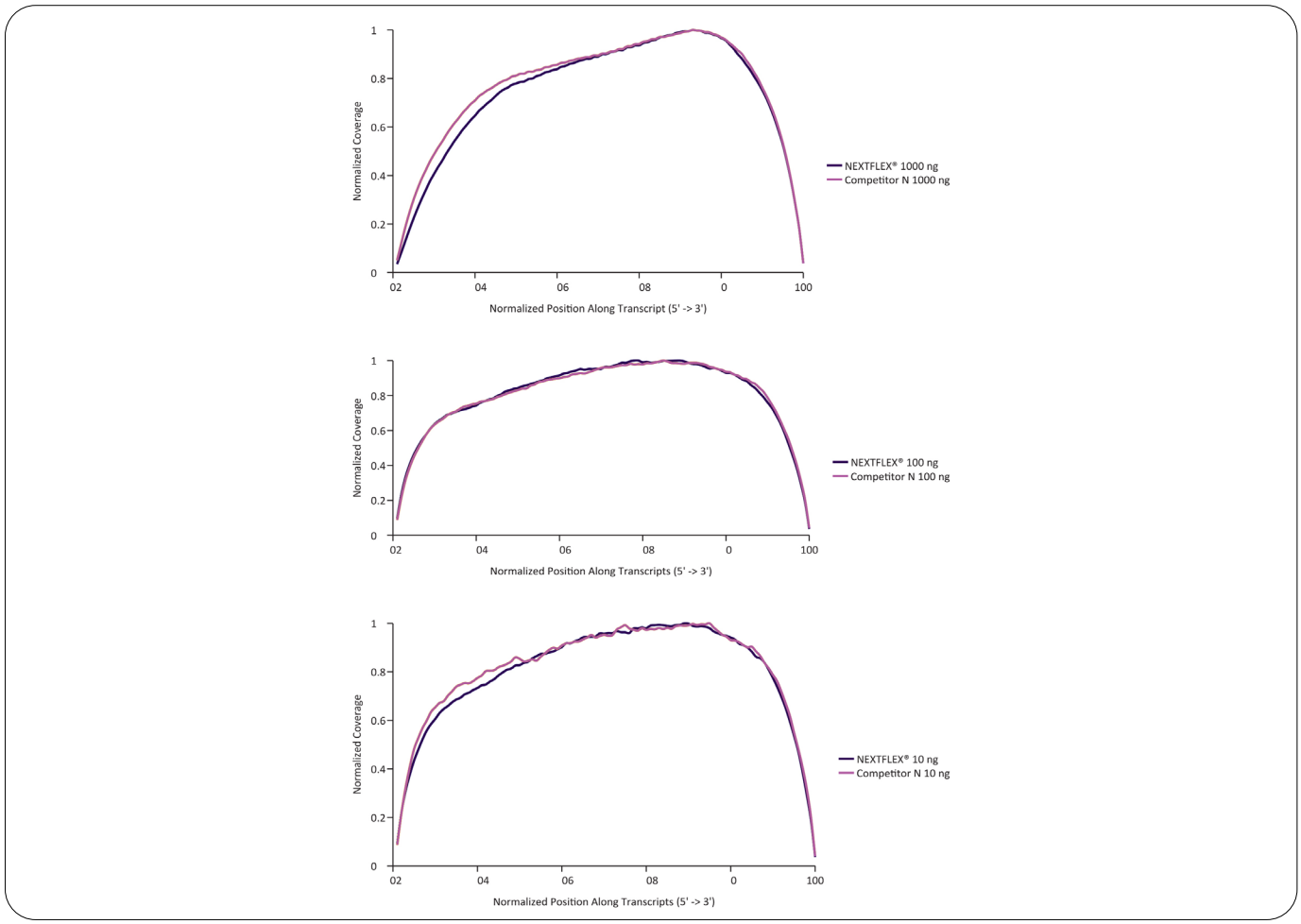


Figure 1: The NEXTFLEX® Rapid Directional RNA-Seq kit 2.0 demonstrates even coverage along transcripts compared to the Competitor N kit. Poly(A) mRNA was isolated from Universal Human Reference RNA (Agilent® #740000) using the NEXTFLEX® Poly(A) Beads 2.0 and the Competitor N Poly(A) enrichment kit. Libraries were generated using the NEXTFLEX® Rapid Directional RNA-Seq kit 2.0 and the Competitor N's library preparation kit. The resulting libraries were sequenced on the Illumina® MiSeq® sequencer using paired-end mode (2x76 bp). Reads were trimmed using cutadapt and mapped to the Gencode v30 reference using bowtie2. The coverage along transcripts was calculated using the BBMap pileup tool.

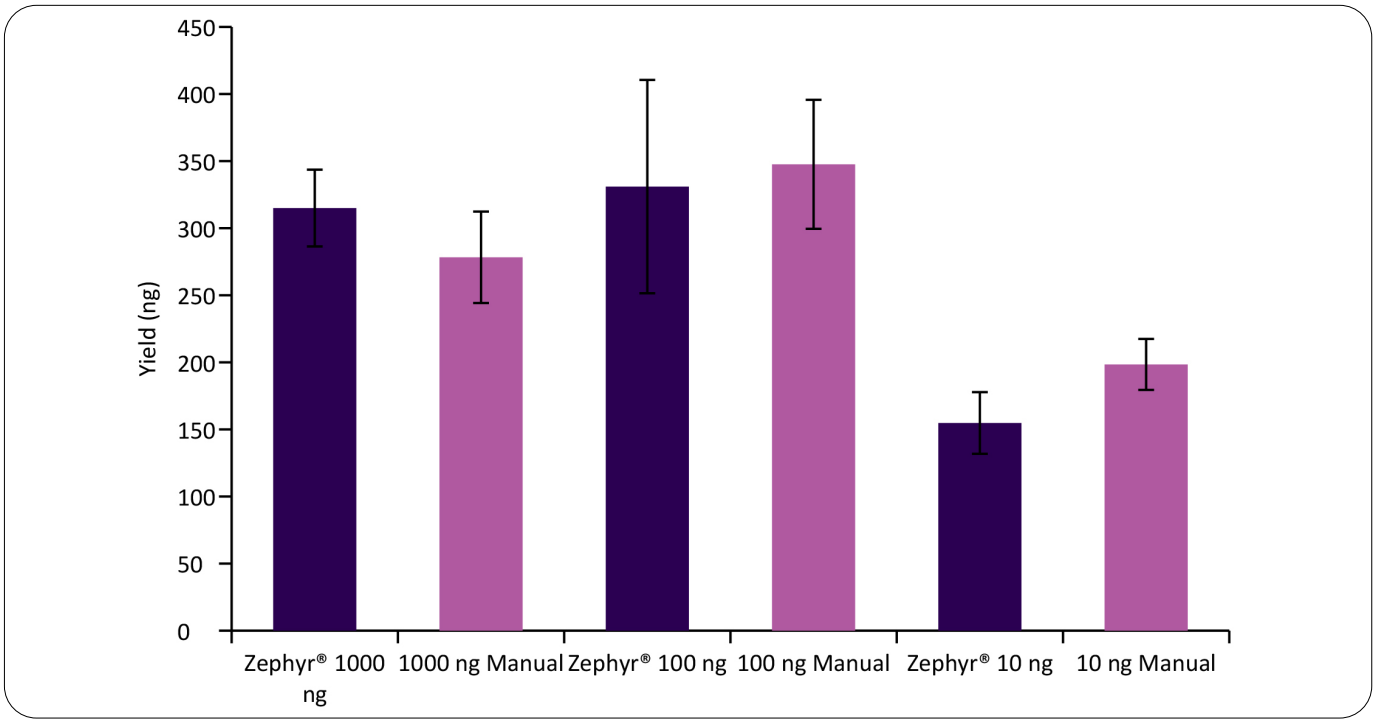


Figure 5: Libraries prepared using the Zephyr® G3 NGS workstation and manually deliver comparable yields using the NEXTFLEX® Rapid Directional RNA-Seq kit 2.0. Poly(A) mRNA was isolated from Universal Human Reference RNA (Agilent® #740000) using the NEXTFLEX® Poly(A) Beads 2.0. Libraries were generated using the NEXTFLEX® Rapid Directional RNA-Seq kit 2.0. Final library concentrations were quantified using the Qubit® 2.0 fluorometer (Thermo Fisher® Scientific #Q32866).

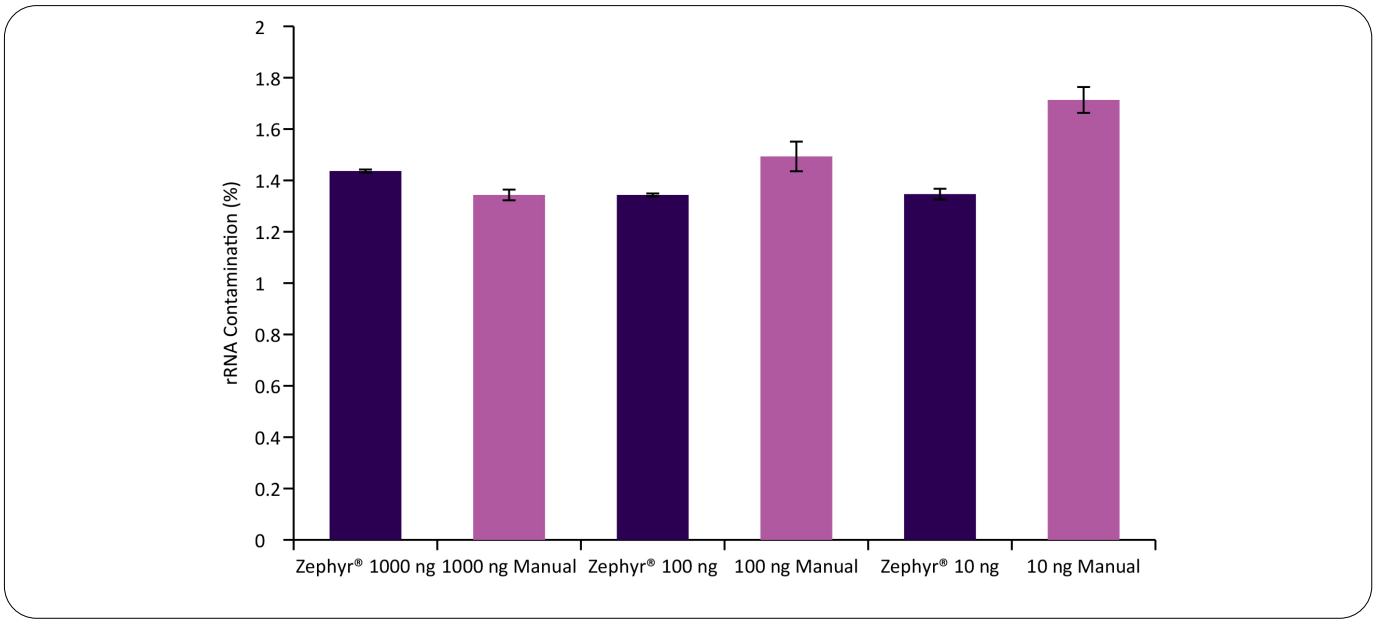


Figure 7: Libraries prepared using the Zephyr® G3 NGS workstation and manually have comparably low levels of rRNA contamination. Poly(A) mRNA was isolated from Universal Human Reference RNA (Agilent® #740000) using the NEXTFLEX® Poly(A) Beads 2.0. Libraries were generated using the NEXTFLEX® Rapid Directional RNA-Seq kit 2.0. The resulting libraries were sequenced on the Illumina® MiSeq® sequencer using paired-end mode (2x76 bp). The reads were trimmed using cutadapt and the percent of rRNA was determined by using bowtie2 to map reads to human rRNA.

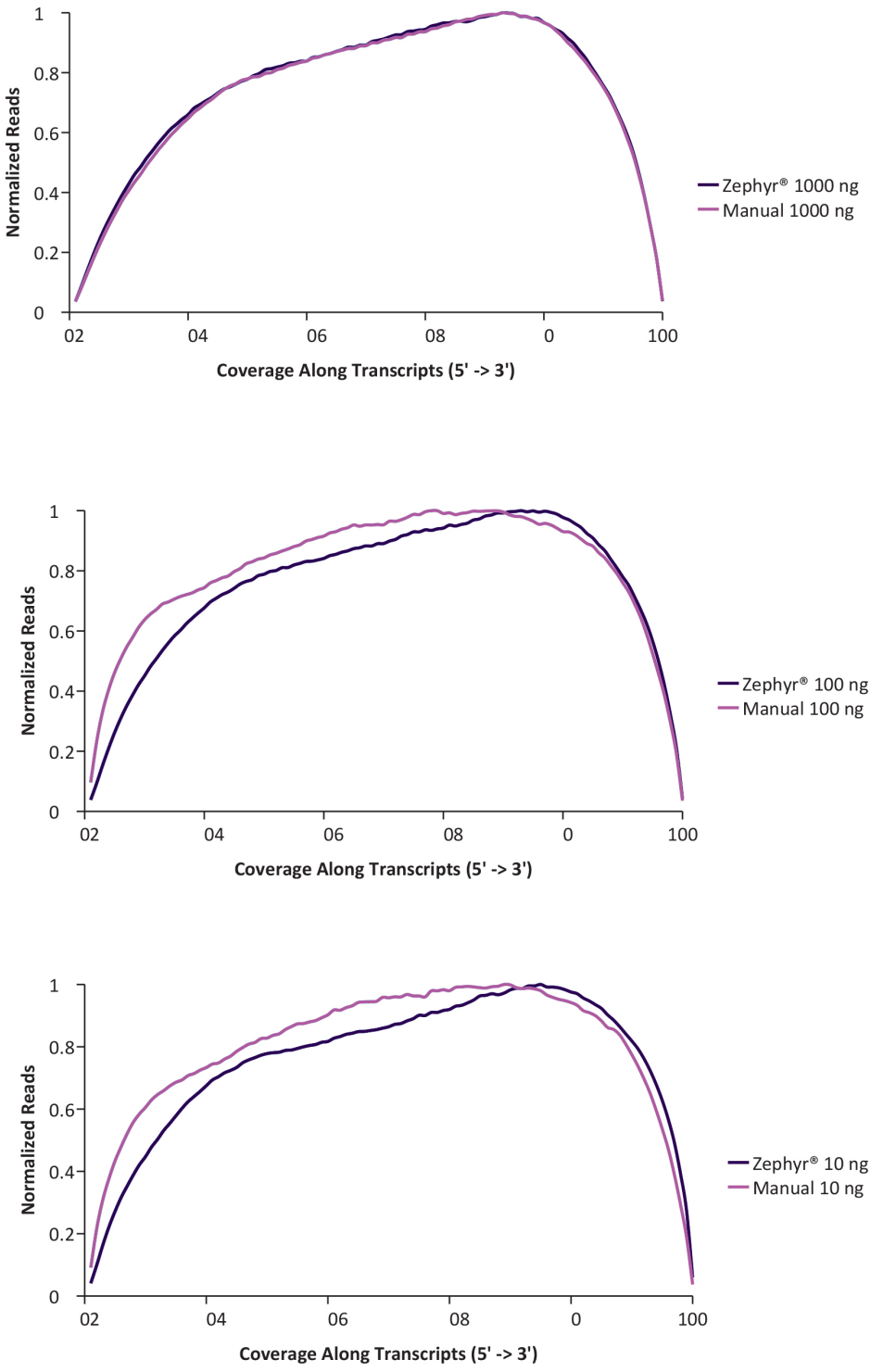


Figure 6: Libraries prepared using the Zephyr® G3 NGS workstation and manually both contain even coverage of transcripts. Poly(A) mRNA was isolated from Universal Human Reference RNA (Agilent® #740000) using the NEXTFLEX® Poly(A) Beads 2.0. Libraries were generated using the NEXTFLEX® Rapid Directional RNA-Seq kit 2.0. The resulting libraries were sequenced on the Illumina® MiSeq® sequencer using paired-end mode (2x76 bp). Reads were trimmed using cutadapt and mapped to the Gencode v30 reference using bowtie2. The coverage along transcripts was calculated using the BBMap pileup tool.

Ordering information

Catalogue #	Product name	Quantity
NOVA-5198-01	NEXTFLEX® Rapid Directional RNA-Seq Kit 2.0	8 rxn
NOVA-512991	NEXTFLEX® Poly(A) Beads 2.0	8 rxn

NEXTFLEX® adapter barcodes will be provided for early access reagents. To discuss NEXTFLEX® library preparation products for your Illumina® sequencing platform application, please contact ngs@revvity.com

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