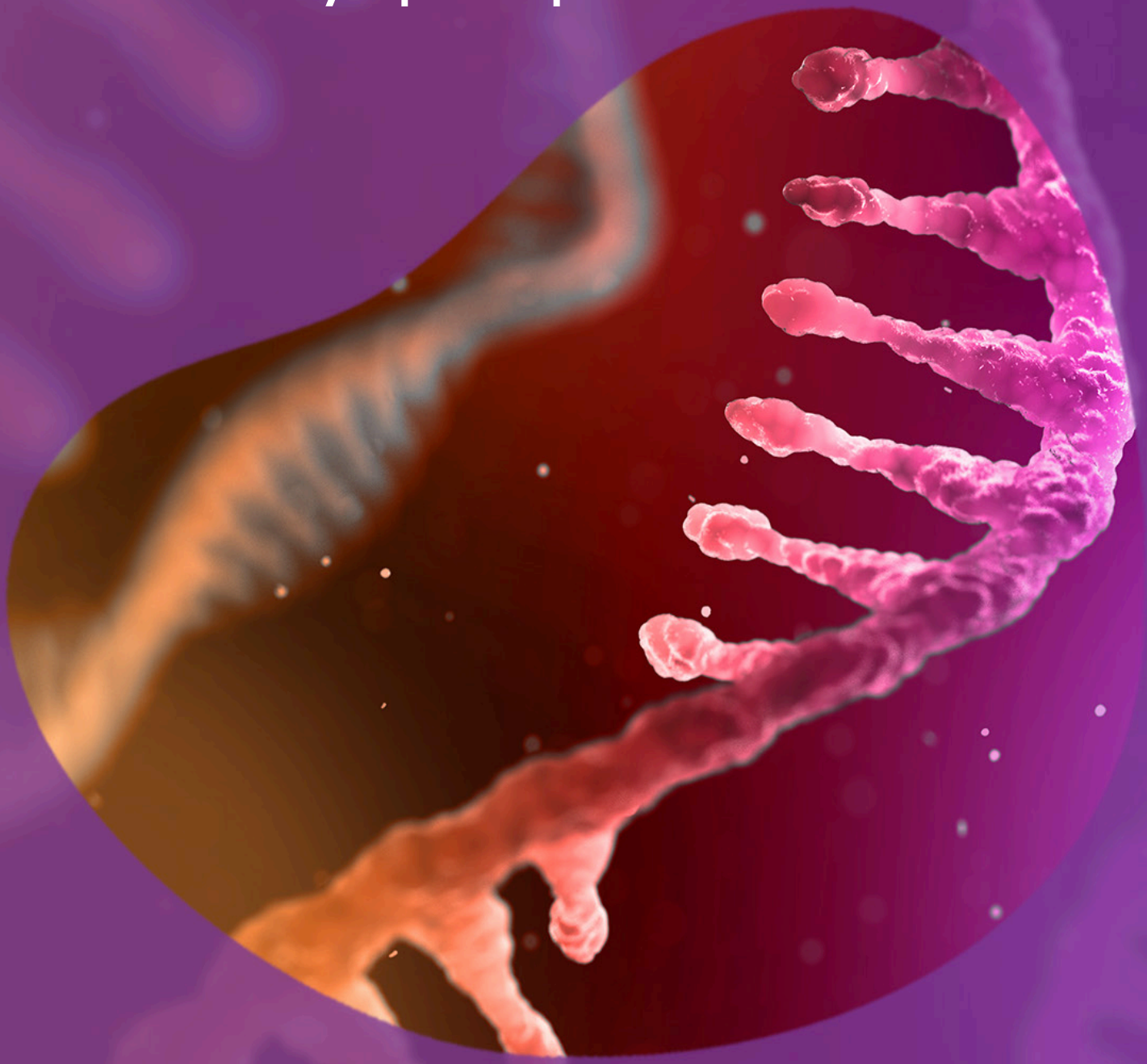
High-performing, automated RNA-SEQ library prep.



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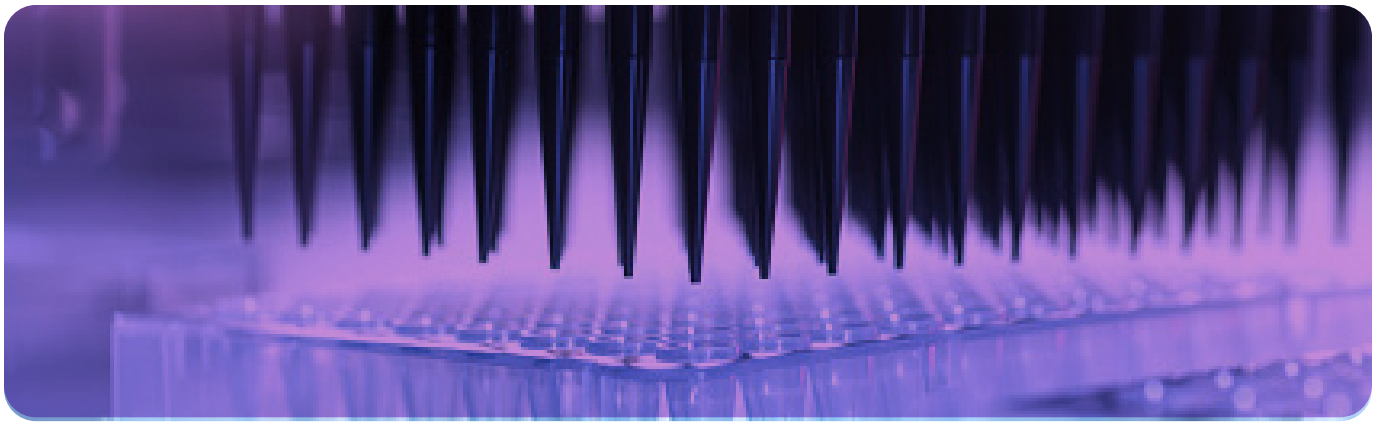
NEXTFLEX® Rapid Directional RNA-Seq Kit 2.0

DNA-SEQ · RNA-SEQ · BARCODES · METAOmics · AMPLICON PANELS·
NUCLEIC ACID ISOLATION · TARGET CAPTURE · EPIGENETICS · SMALL RNA-SEQ



Simple, validated and automated RNA sequencing at your finguretips

New and improved library preparation kit for your RNA sequencing needs

The NEXTFLEX® rapid directional RNA-seq kit 2.0 produces libraries for Illumina® sequencing instruments with high coverage uniformity, low duplication rates, strand specificity and minimal rRNA contamination when used with the NEXTFLEX® Poly(A) Beads 2.0 (10 ng - 5 µg) or NEXTFLEX® RiboNaut™ rRNA depletion kit (human, mouse, rat) (5 ng - 1 µg). 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 and NEXTFLEX® RiboNaut™ rRNA depletion kit (human, mouse, rat) to accommodate total RNA as input. The NEXTFLEX® rapid directional RNA-seq kit 2.0 is designed to be used with NEXTFLEX® RNA-Seq 2.0 Unique Dual Index Barcodes (6.25µM), which are color-balanced and have undergone proprietary purity QC metrics to generate reliable sequencing results for every sample.

AUTOMATED RNA-SEQ WORKFLOW

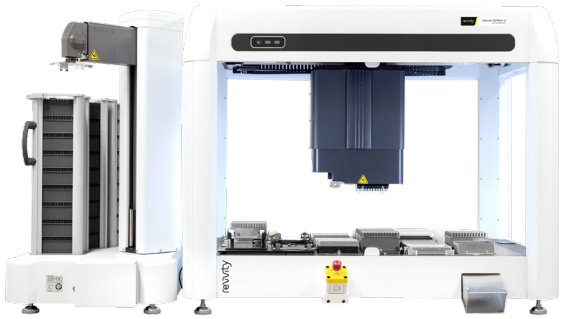
Total RNA	Poly(A) Enrichment or rRNA Depletion	Fragmentation & cDNA Synthesis	Adenylation & Adapter Ligation	PCR	Sequencing

AUTOMATED ON THREE REVVITY PLATFORMS

Zephyr® G3 NGS Workstation

Sciclone® G3 NGSx Workstation

Sciclone® G3 NGSx iQ™ Workstation



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 and NEXTFLEX® RiboNaut™ rRNA depletion kit (human, mouse, rat)

Flexible: Designed to work with NEXTFLEX® RNA-Seq 2.0 Unique Dual Index Barcodes that allow a wide range of multiplexing (2 up to 384 samples in one run)

Efficient: Automated on the Sciclone® G3 NGSx, Sciclone® G3 NGSx iQ™, and Zephyr® G3 NGS workstations

NEXTFLEX vs. competitor N

NEXTFLEX® rapid directional RNA-seq kit 2.0 with NEXTFLEX® Poly(a) beads 2.0

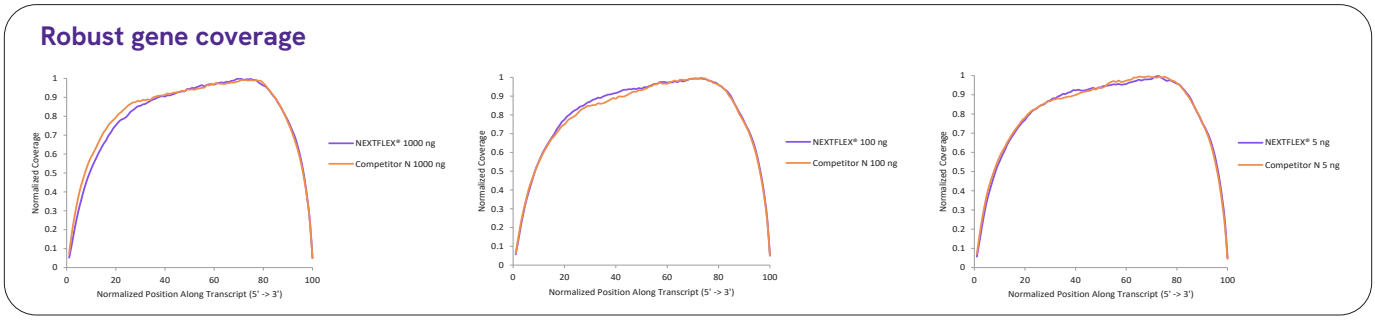


Figure 1: The NEXTFLEX® Rapid Directional RNA-Seq kit 2.0 demonstrates even coverage along transcripts compared to the Competitor N kit. Poly(A) mRNA was isolated from Universal Human Reference RNA (Agilent® #740000) using the NEXTFLEX® Poly(A) Beads 2.0 and the Competitor N Poly(A) enrichment kit. Libraries 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 (2×76 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.

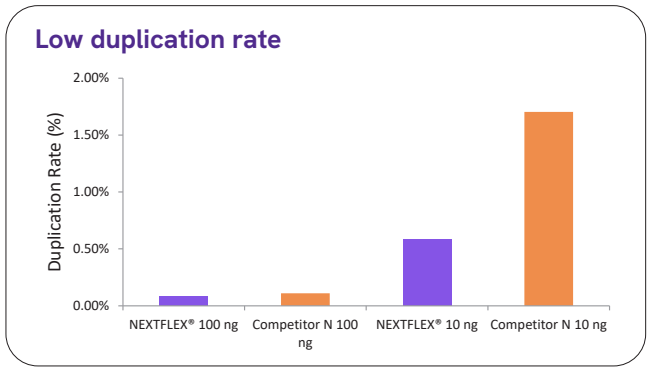


Figure 2: The NEXTFLEX® Rapid Directional RNA-Seq kit 2.0 demonstrate low duplication rate compared to the Competitor N kit. Poly(A) mRNA was isolated from Universal Human Reference RNA (Agilent #740000) using the NEXTFLEX® Poly(A) Beads 2.0 and the Competitor N Poly(A) enrichment kit. Libraries were 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.

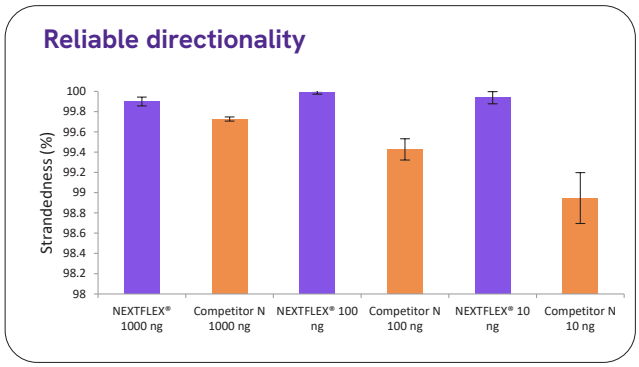


Figure 3: The NEXTFLEX® Rapid Directional RNA-Seq kit 2.0 demonstrates comparable directionality relative to the Competitor N kit. Poly(A) mRNA was isolated from Universal Human Reference RNA (Agilent #740000) containing ERCC RNA Spike-In mix (Thermo Fisher® Scientific #4456740) using the NEXTFLEX® Poly(A) 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.

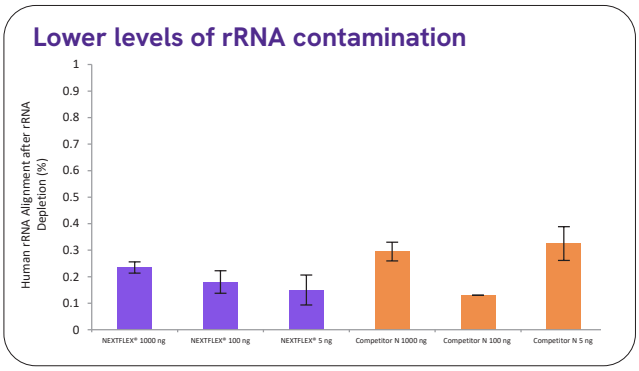


Figure 4: The NEXTFLEX® Rapid Directional RNA-Seq kit 2.0 delivers libraries containing lower levels of rRNA contamination than the Competitor N kit. Poly(A) mRNA was isolated from Universal Human Reference RNA (Agilent #740000) using the NEXTFLEX® Poly(A) Beads 2.0 and the Competitor N Poly(A) enrichment 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.

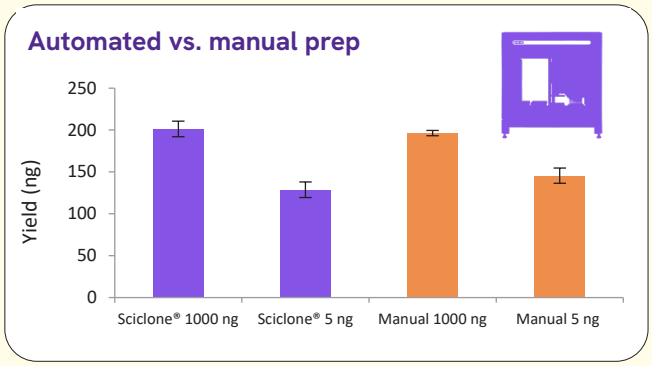


Figure 5: Libraries prepared using the Zephyr® G3 NGS workstation and manually deliver comparable yields using the NEXTFLEX® Rapid Directional RNA-Seq kit 2.0. Poly(A) mRNA was isolated from Universal Human Reference RNA (Agilent® #740000) using the NEXTFLEX® Poly(A) Beads 2.0. Libraries were generated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

NEXTFLEX vs. competitor N

NEXTFLEX® rapid directional Rna-seq kit 2.0 with NEXTFLEX® RiboNaut™ rRNA depletion kit

Robust gene coverage

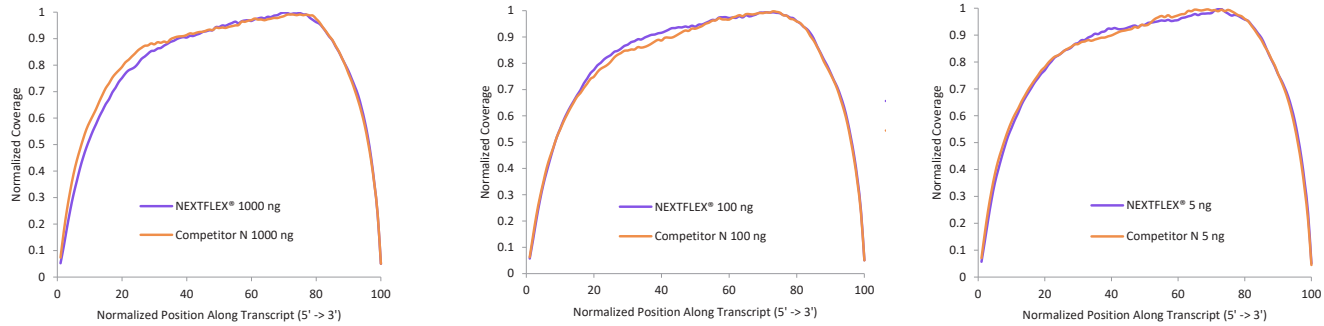


Figure 6: The NEXTFLEX® Rapid Directional RNA-Seq kit 2.0 demonstrates even coverage along transcripts compared to the Competitor N kit. rRNA-depleted total RNA was isolated from Universal Human Reference RNA (Agilent® #740000) using the NEXTFLEX® RiboNaut™ rRNA depletion kit (human, mouse, rat) and the Competitor N rRNA-depletion 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 single-end mode (1x151 bp). Reads were trimmed using cutadapt, mapped to the Gencode v30 reference transcriptome using bowtie2, and randomly downsampled to 720k reads. The coverage along transcripts was calculated using the BBMap pileup tool.

Low duplication rate

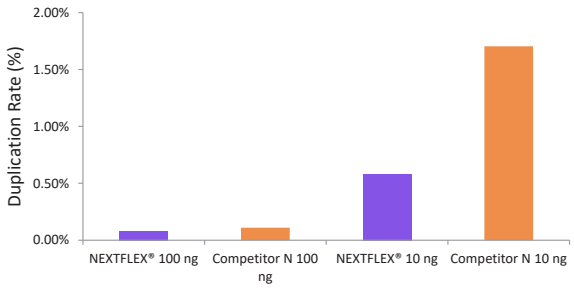


Figure 7: The NEXTFLEX® Rapid Directional RNA-Seq kit 2.0 demonstrate low duplication rates compared to the Competitor N kit. rRNA-depleted total RNA was isolated from Universal Human Reference RNA (Agilent® #740000) using the NEXTFLEX® RiboNaut™ rRNA depletion kit (human, mouse, rat) and the Competitor N rRNA-depletion kit. Libraries were generated using the NEXTFLEX® Rapid Directional RNA-Seq kit 2.0 and the Compeatitor N's library preparation kit. The resulting libraries were sequenced on the Illumina® MiSeq® sequencer using single-end mode (1x151 bp). Reads were trimmed using cutadapt, mapped to the Gencode v30 reference transcriptome using bowtie2, and randomly downsampled to 28k reads. Duplication rate was calculated using the fastp all-in-one FASTQ preprocessor.

Reliable directionality

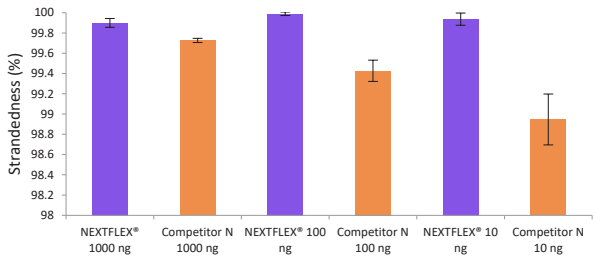


Figure 8. The NEXTFLEX® Rapid Directional RNA-Seq kit 2.0 demonstrates comparable directionality relative to the Competitor N kit. rRNA-depleted total RNA was isolated from Universal Human Reference RNA (Agilent® #740000) using the NEXTFLEX® RiboNaut™ rRNA depletion kit (human, mouse, rat) and the Competitor N rRNA-depletion 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 single-end mode (1x151 bp). Reads were trimmed using cutadapt and mapped to the Gencode v30 reference transcriptome using bowtie2. Reads from respective samples were combined and downsampled for a total of 800k reads each. Strandedness was calculated using the fastp all-in-one FASTQ preprocessor.

Lower levels of rRNA contamination

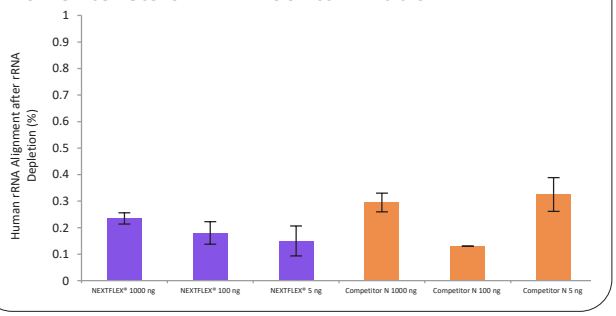


Figure 9: The NEXTFLEX® Rapid Directional RNA-Seq kit 2.0 delivers libraries containing low levels of rRNA contamination compared to the Competitor N kit. rRNA-depleted total RNA was isolated from Universal Human Reference RNA (Agilent #740000) using the NEXTFLEX® RiboNaut™ rRNA depletion kit (human, mouse, rat) and the Competitor N rRNA-depletion 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 single-end mode (1x151 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.

Automated vs. manual prep

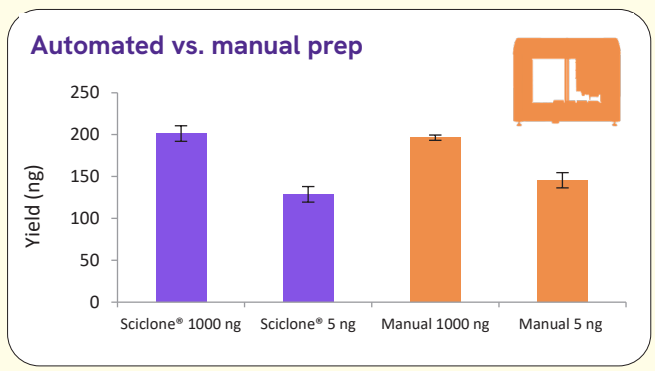


Figure 10: Libraries prepared using the Sciclone® G3 NGSx workstation and manually deliver comparable yields using the NEXTFLEX® Rapid Directional RNA-Seq kit 2.0. rRNA-depleted total RNA was isolated from Universal Human Reference RNA (Agilent #740000) using the NEXTFLEX® RiboNaut™ rRNA depletion kit. 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).

Ordering information

Catalog #	Kit name	Quantity
NOVA-5198-0X	NEXTFLEX® Rapid Directional RNA-Seq Kit 2.0	8, 48 or 96 reactions
NOVA-51296X	NEXTFLEX® RiboNaut rRNA depletion (Human, Mouse, Rat)	8, 48 or 96 reactions
NOVA-51299X	NEXTFLEX® Poly(A) Beads 2.0	8, 48 or 96 reactions
NOVA-512920	NEXTFLEX® RNA-Seq 2.0 Unique Dual Index Barcodes (barcode 1-96)	192 reactions
NOVA-512921	NEXTFLEX® RNA-Seq 2.0 Unique Dual Index Barcodes (barcode 97-192)	192 reactions
NOVA-512922	NEXTFLEX® RNA-Seq 2.0 Unique Dual Index Barcodes (barcode 193-289)	192 reactions
NOVA-512923	NEXTFLEX® RNA-Seq 2.0 Unique Dual Index Barcodes (barcode 289-384)	192 reactions

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