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APPLICATION NOTE

# NEXTFLEX rapid DNA-seq kit 2.0: DNA library preparation that improves sequencing performance.

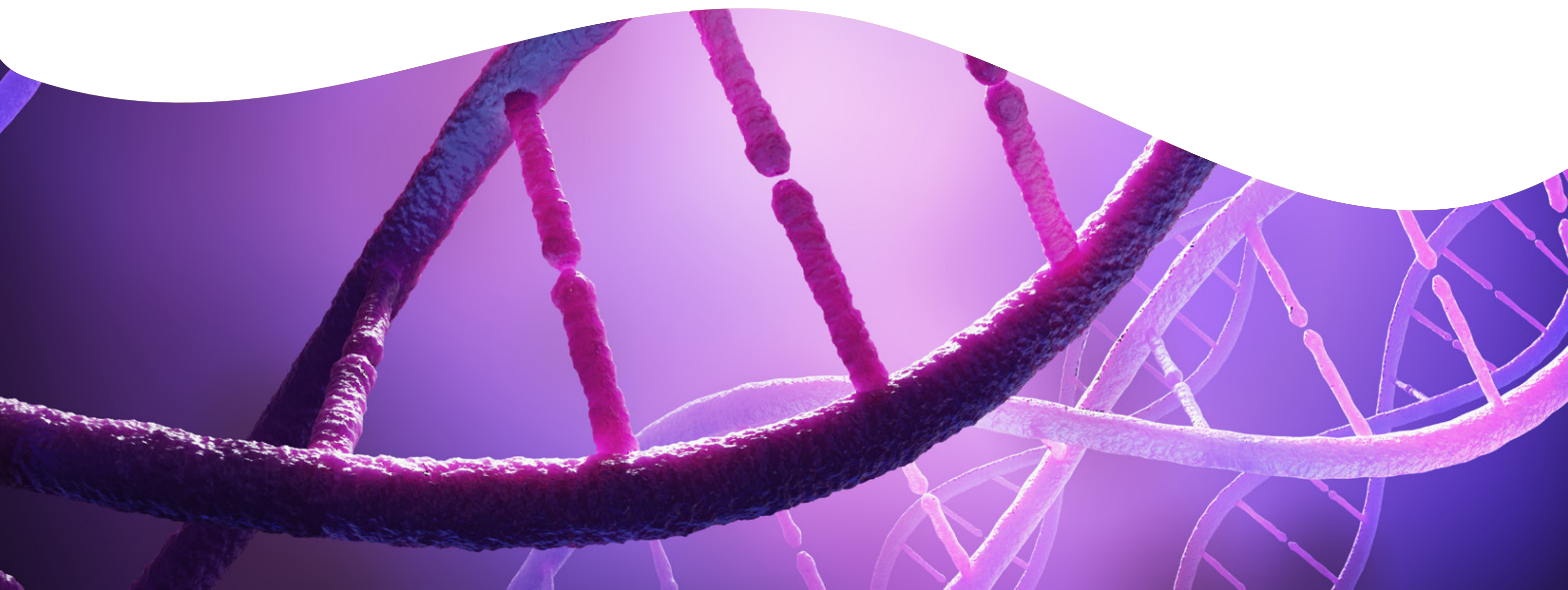
## Introduction

Many researchers rely on commercial next generation sequencing (NGS) DNA library preparation kits for their convenience, ease of use, and consistency in performance. Therefore, workflow and technical differences found in the various kits on the market can greatly affect performance in terms of yield and coverage, which can have a large impact on the quality of a researcher's data.

Library preparation involves the theoretically simple, but practically challenging steps of end-repair of fragmented DNA, ligation of adapters, and amplification. Each of these processes can introduce bias, resulting in uneven representation of some segments of the starting materials. Consequently, for successful library preparation, reliable reagents are necessary to give a consistent, accurate representation of the starting material. This can be achieved by optimizing reagents to increase efficiency and minimize bias throughout each step in the library preparation protocol.

The NEXTFLEX® rapid DNA-seq kit 2.0 improves the efficiency of each step to generate high yields of DNA sequencing libraries that exhibit even coverage. Furthermore, the NEXTFLEX® rapid DNA-seq kit 2.0 features a straightforward protocol that generates a set of libraries within 3 hours, with 1.5 hours of hands-on time. In all metrics considered critical in DNA library prep, such as yield, mapping rate, coverage, and GC bias, our results demonstrate that the NEXTFLEX® rapid DNA-seq kit 2.0 is comparable or superior in performance to the leading market product, competitor K's kit.

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



## Materials and methods

Libraries were generated in duplicate or triplicate using the NEXTFLEX® rapid DNA-seq kit 2.0 and Competitor K's kit from 1 µg, 250 ng, and 1 ng of human, male genomic DNA, 250 ng and 1 ng of E. coli genomic DNA, and 100 ng of S. cerevisiae, E. coli, and Bordetella genomic DNA. All libraries were prepared according to the manufacturer instructions, with no size selection steps performed. All genomic DNA were fragmented using the Covaris® S2 Focused-ultrasonicator to approximately 350 bp. 3 PCR cycles were performed with 250 ng inputs, 5 PCR cycles were performed with 100 ng inputs, and 12 PCR cycles were performed with 1 ng inputs. Quantitative PCR was performed with libraries prepared with 1 µg inputs using a commercial library quantitation kit.

Libraries from S. cerevisiae, E. coli, and Bordetella genomic DNA were sequenced on an Illumina® MiSeq® sequencing platform with 150 bp singleend reads, with the reads corresponding to approximately 20 X coverages of each genome. Analysis was performed according to the following steps: first, adapter sequences were trimmed from the ends of the reads and any adapter-dimer reads were discarded. Then, the remaining reads in each library were randomly sampled such that each analysis involved the same number of reads. Next, reads were aligned to the S. cerevisiae, E. coli, and Bordetella genome with Bowtie 2. Lastly, following alignment, GC bias andcoverage were analyzed with Picard and mapping rate data determined by Flagstat.

## Results

Final library yield was used to measure the efficiency of library preparation kits. As shown in Figure 1A, the final yield from the NEXTFLEX® rapid DNA-seq kit 2.0 was higher than libraries constructed using Competitor K's kit in both 250 ng and 1 ng of inputs of fragmented human male and E. coli genomic DNA. qPCR was performed to quantitate adapter-ligated molecules in the libraries prepared from 1 µg of fragmented human genomic DNA without a PCR amplification step. The quantitation values, which were normalized to the conversion rate for the NEXTFLEX® rapid DNA-seq kit 2.0, showed comparable conversion rates between the two kits (Figure 1B).

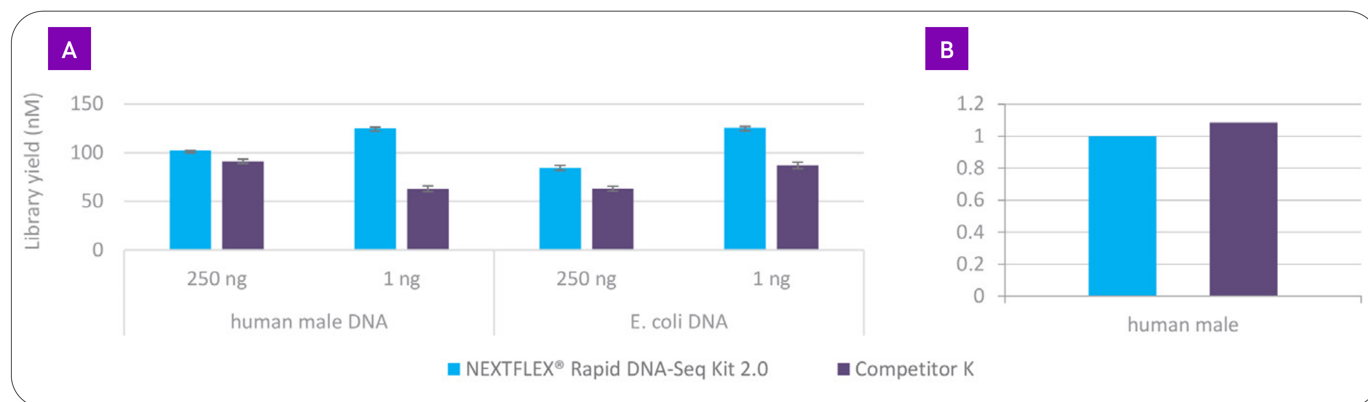



Figure 1: **Libraries prepared with NEXTFLEX® rapid DNA-seq kit 2.0 show higher yield than libraries prepared with Competitor K's kit.** **(A)** Average yield of DNA libraries. Libraries were prepared with 350 bp-fragmented E. coli and human male genomic DNA. 3 and 12 PCR cycles were performed for 250 ng and 1 ng inputs, respectively. The error bars indicate standard deviation based on triplicates. **(B)** Conversion rate of input DNA to adapter-ligated product. Libraries were prepared with 1 µg of 350 bp-fragmented human male genomic DNA. Quantitative PCR was performed and the quantitation values were then normalized to the conversion rate for the library prepared with NEXTFLEX® rapid DNA-seq kit 2

To obtain more usable data from the same number of sequencing reads, any bias during library preparation must be avoided to ensure there is no reduction in the high complexity of libraries. To determine the GC bias, libraries were prepared with three different organisms which have different GC contents: S. cerevisiae (low GC-content), E. coli (middle GC-content), and Bordetella (high GC-content). As shown in Figure 2, the NEXTFLEX® rapid DNA-seq kit 2.0 has comparable or less GC-bias from all three tested organism genomes. Less GC bias was clearly observed at high GC genome regions in the high GC-content Bordetella genome. Less bias translates to high genome coverage. As expected, the libraries prepared with the NEXTFLEX® rapid DNA-seq kit 2.0 show higher genome coverage than libraries prepared with Competitor K's kit. This coverage difference is more pronounced in genomic regions with 10X coverage (Figure 3).

Figure 2: **Libraries prepared with NEXTFLEX® rapid DNA-seq kit 2.0 show less GC bias than libraries prepared with Competitor K's kit. Picard GC bias analysis.** Read coverage over the indicated genome with respect to GC content, shown as a fraction of normalized coverage (blue) calculated in 100 bp windows (red) and plotted against the left y-axis. Mean base quality at each window (green) is calculated and plotted against the right y-axis.

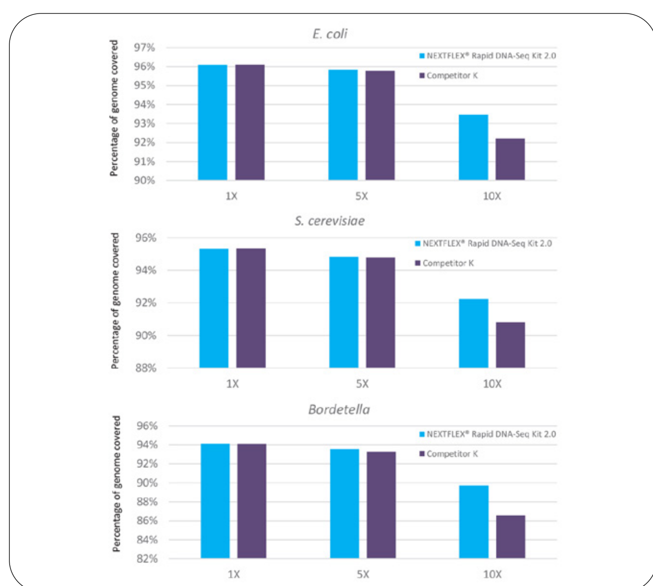



Figure 3: **Libraries prepared with NEXTFLEX® rapid DNA-seq kit 2.0 show higher genome coverage than libraries prepared with Competitor K's kit.** The percentage of genome bases covered at a minimum depth of 1X, 5X, and 10X.

High mapping rate and low duplication rate are also common metrics to define the quality of the prepared library. We observed slightly better mapping rates and lower duplication rates in libraries prepared with the NEXTFLEX® rapid DNA-seq kit 2.0 compared to Competitor K's kit, though the difference is trivial (Figure 4).

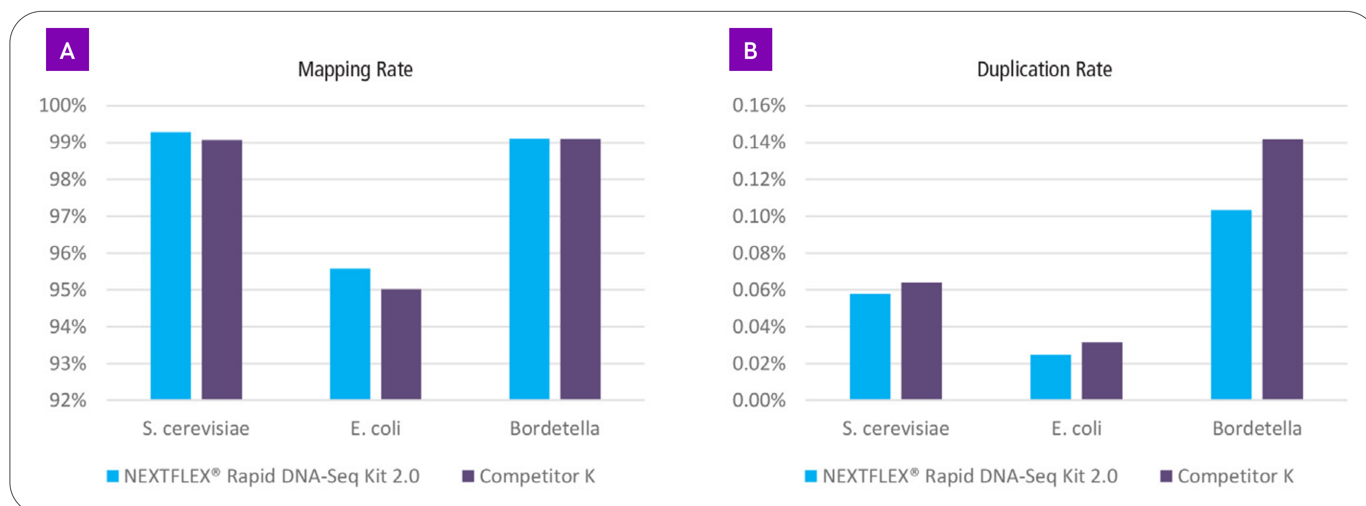


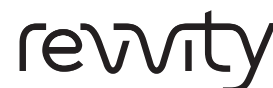

Figure 4: **Libraries prepared with NEXTFLEX® rapid DNA-seq kit 2.0 show better mapping rates and lower duplication rates than libraries prepared with Competitor K's kit. (A)** Mapping rates. The percentage of reads which aligned to the indicated genome. **(B)** Duplication rates. The percentage of reads predicted to be PCR duplicates by Picard MarkDuplicates.

## Results

NGS DNA library preparation kits are critical components in various applications including but not limited to whole genome sequencing (WGS), exome sequencing (WES), target capture sequencing, and chromatin immunoprecipitation (ChIP) sequencing. It is important to choose the best overall kit that is both convenient and reliable in providing robust, quality data for every sample. Of the multiple metrics deemed important in the field when analyzing the quality of a library preparation method, the NEXTFLEX® rapid DNA- seq kit 2.0 clearly outperformed or performed comparably to Competitor K's kit in terms of yield, GC bias, genome coverage, mapping rate, and duplication rate.

Ease of use and a streamlined workflow, considerations for automation, and availability of reagents in a convenient, all-inclusive offering are other important factors to consider when choosing a NGS DNA library preparation kit for their needs. The streamlined workflow of the NEXTFLEX® rapid DNA-seq kit 2.0 allows DNA sequencing libraries to be prepared in less than 3 hours with 1.5 hours of hands- on time. The NEXTFLEX® rapid DNA-seq kit 2.0 contains cleanup beads and barcoded adapters as part of a bundle tied to a single lot, which provides significant amounts of convenience, reduces the confusion of reagent compatibility, and mitigates the burden of tracking several lots.

In conclusion, the NEXTFLEX® rapid DNA-seq kit 2.0 outperforms Competitor K's kit on a variety of quality metrics and reliably delivers accurate, high-yield, low bias DNA sequencing libraries.



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