WATCHMAKER GENOMICS

WATCHMAKER DNA LIBRARY PREP KITS WITH FRAGMENTATION

Watchmaker DNA Library Prep Kits with Fragmentation enable highly sensitive applications — accessing meaningful insights from a broad range of sample types, including ultra-low inputs and FFPE. It harnesses the benefits of enzymatic fragmentation, such as ease of automation, improved scalability, and preservation of low-input samples. It also reduces artifact formation, including false chimeric reads and hairpin artifacts, which can convolute variant calling in challenging applications such as translational oncology.

KEY FEATURES & BENEFITS

- Up to a 90% reduction in sequence artifacts improves assay accuracy — critical for highly sensitive applications such as low-frequency variant calling
- Highly tunable fragmentation delivers consistent library sizes over a wide input range (<1 ng to 500 ng) and between library batches
- Enzymatic fragmentation improves sequencing economy and accuracy with FFPE samples
- Streamlined workflow delivers PCR-free libraries in under 90 minutes and scales easily for high-throughput processing
- Built with automation in mind, including minimum transfer volumes and generous reagent overages to address automation dead volume requirements
- Custom formats available, including volumes to support 384-well processing
- Equinox polymerase delivers ultra-high-fidelity, low-bias library amplification

APPLICATIONS

- Human whole genome sequencing (WGS), including PCR-free
- Whole exome sequencing (WES)
- Somatic and germline variant detection
- FFPE analysis
- Copy number variation (CNV) analysis
- Structural variant analysis
- Microbial WGS
- Metagenomic analysis
- Viral genome sequencing
- Single cell analysis
- Bulk RNA-seq (from full-length cDNA)

READ. WRITE. EDIT.

HIGHLY TUNABLE AND CONSISTENT ENZYMATIC DNA FRAGMENTATION

Highly tunable DNA fragmentation is achieved through the simple modulation of reaction time and temperature, and consistent fragment sizes are attainable over a wide input range. Such flexibility supports a variety of applications — including challenging ones where DNA mass is limiting.

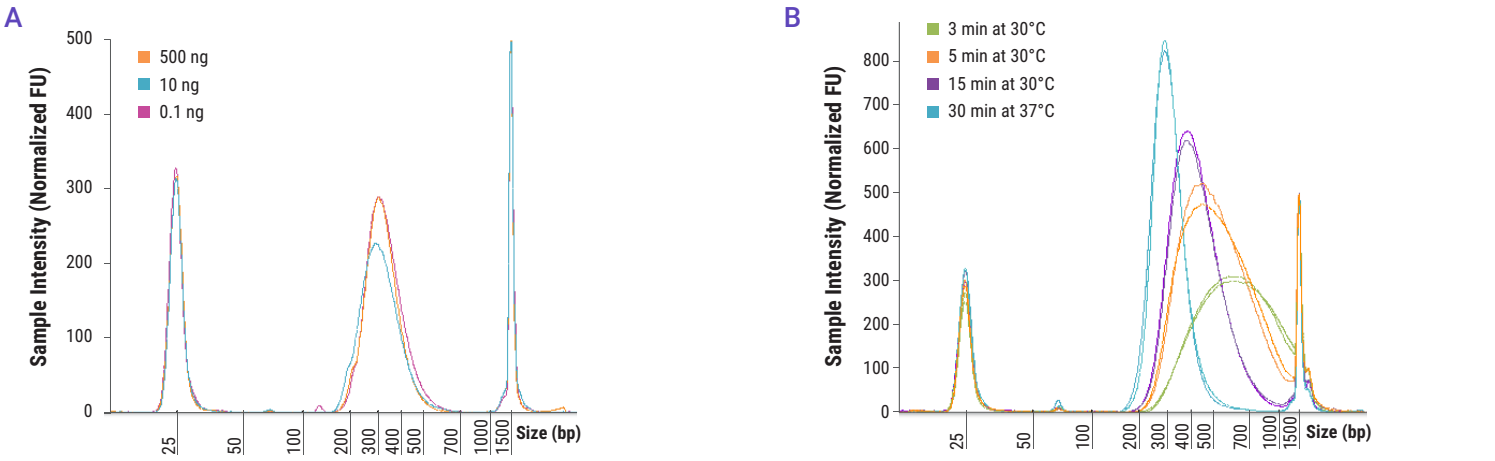

FIGURE 1. Robust enzymatic fragmentation for broad utility. (A) Libraries were constructed in duplicate from 500, 10, and 0.1 ng of human genomic DNA (hgDNA) fragmented for 20 minutes at 30°C. Consistent library sizes were achieved over this 5,000-fold input range. **(B)** Libraries were constructed from 50 ng hgDNA. A fragmentation reaction time titration was conducted using 30°C (3, 5, and 15 minutes) and 37°C (30 minutes) incubation temperature. A wide range of final library sizes was achieved. Final library distributions were assessed using a D1000 assay by TapeStation (Agilent).

REDUCED SEQUENCE ARTIFACTS ENABLE HIGH-SENSITIVITY APPLICATIONS

The workflow alleviates many issues associated with sonication — delivering ease of automation and improved scalability, while also mitigating the formation of sequence artifacts. These artifacts convolute the identification of true structural and single nucleotide variants and especially impact highly sensitive applications, such as low-frequency variant calling. The Watchmaker solution reduces the prevalence of both types of artifacts and delivers data quality on-par with sonication.

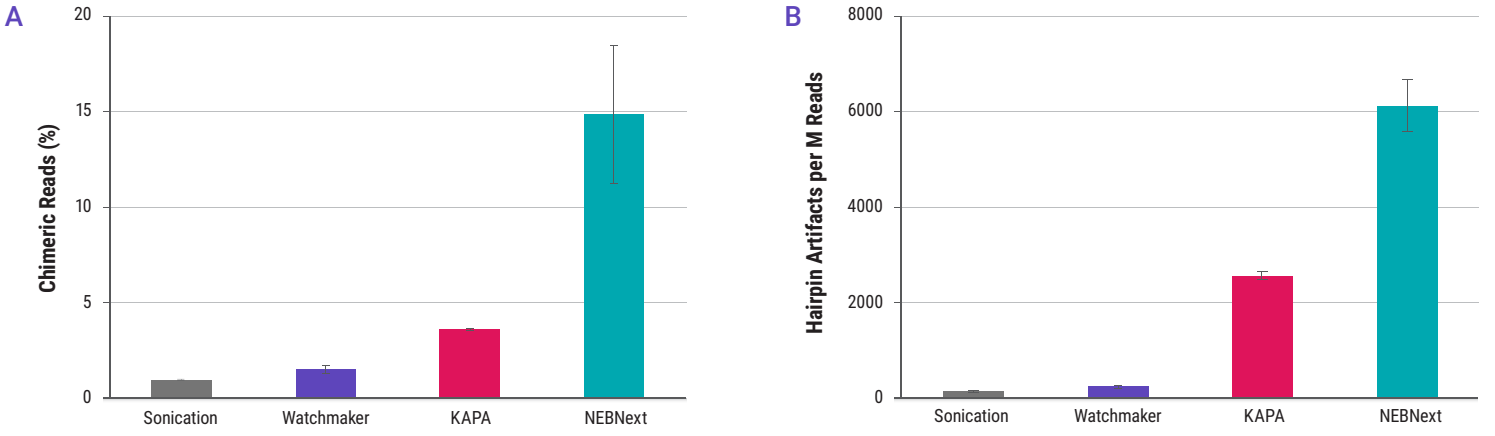

FIGURE 2. Reduced false chimeric reads and hairpin artifacts. Libraries were prepared in duplicate using either the Watchmaker DNA Library Prep Kit with Fragmentation, KAPA HyperPlus Kit, or NEBNext® Ultra™ II FS DNA Library Preparation Kit per manufacturer's recommendations. Sonication control library sets were also prepared. **(A)** Libraries used 1 ng of human genomic DNA (hgDNA) input. **(B)** Enzymatic fragmentation libraries used 10, 50, and 100 ng of hgDNA, and sonication libraries used 1 and 200 ng inputs. Data bars represent the mean across all DNA input amounts assessed per library preparation workflow.

APPLICATION HIGHLIGHT: PCR-FREE WHOLE GENOME SEQUENCING (WGS)

PCR-free WGS provides comprehensive genomic information with minimal bias, but is limited to high-input samples to ensure sufficient yields for sequencing. The Watchmaker DNA Library Prep Kit with Fragmentation delivers high library yields without the need for amplification, excellent sequence coverage, and robust variant calling — even with inputs as low as 75 ng. This broadens the addressable sample range and enables clinically relevant samples.

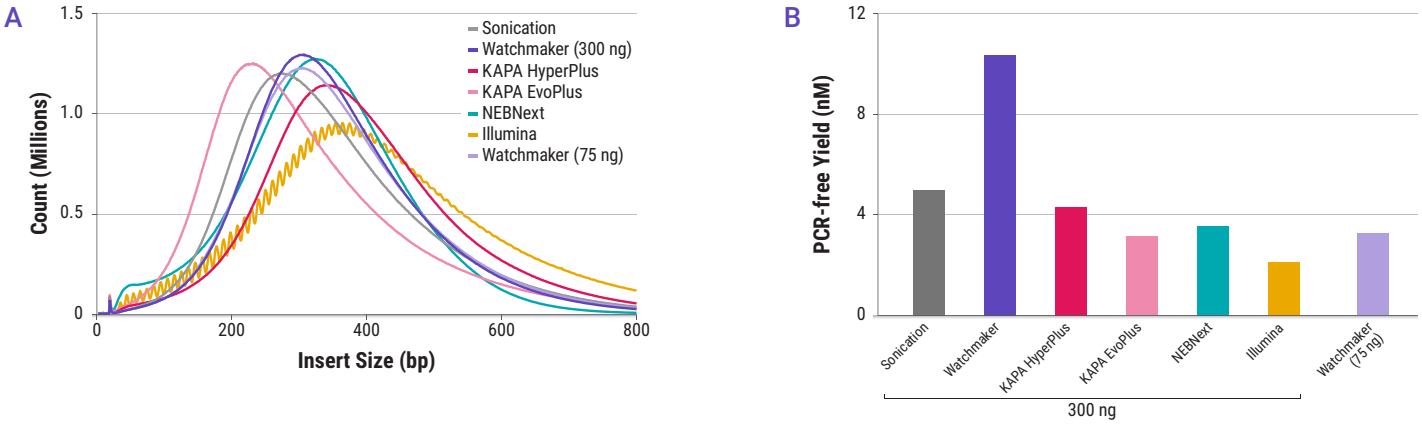

FIGURE 3. Efficient conversion delivers high library yields without amplification. PCR-free WGS libraries were constructed using a variety of commercially available solutions: KAPA HyperPrep (sonication control), Watchmaker DNA Library Prep with Fragmentation, KAPA HyperPlus, KAPA EvoPlus, NEBNext Ultra II FS DNA Library Prep, and Illumina DNA Prep. Either 300 ng (all evaluated kits) or 75 ng (Watchmaker only) of NA12878 human genomic DNA was used as input. Workflows were optimized to deliver similarly sized final libraries by tuning fragmentation and post-ligation cleanup parameters. **(A)** Insert size distributions were plotted using NovaSeq 6000 output data. **(B)** Final PCR-free library yields as measured by qPCR. The Watchmaker solution delivers insert sizes appropriate for WGS without a sawtooth patterning and yields that exceed other preps, including sonication.

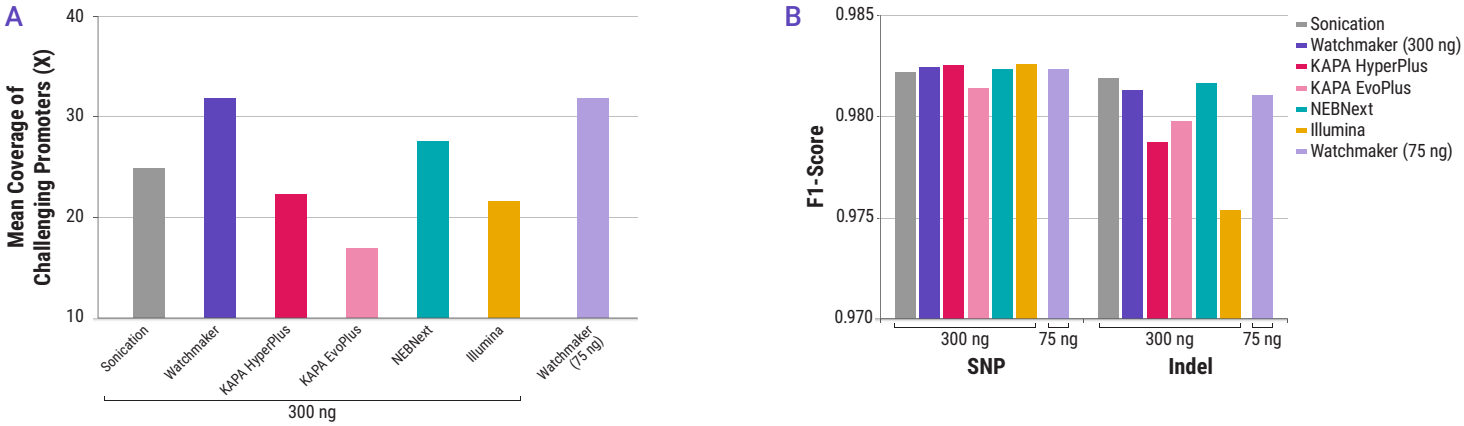

FIGURE 4. Improved coverage of challenging promoter regions and robust variant calling. All libraries were sequenced on a NovaSeq 6000 and subsampled to 387M read pairs. **(A)** Mean coverage across challenging, GC-rich promoters.[1] **(B)** SNP and Indel F1-scores. The Watchmaker solution delivers the highest mean coverage of GC-rich promoters, even when only 75 ng of DNA is used for library prep. Further, Watchmaker libraries deliver variant calling performance on-par with the sonication control.

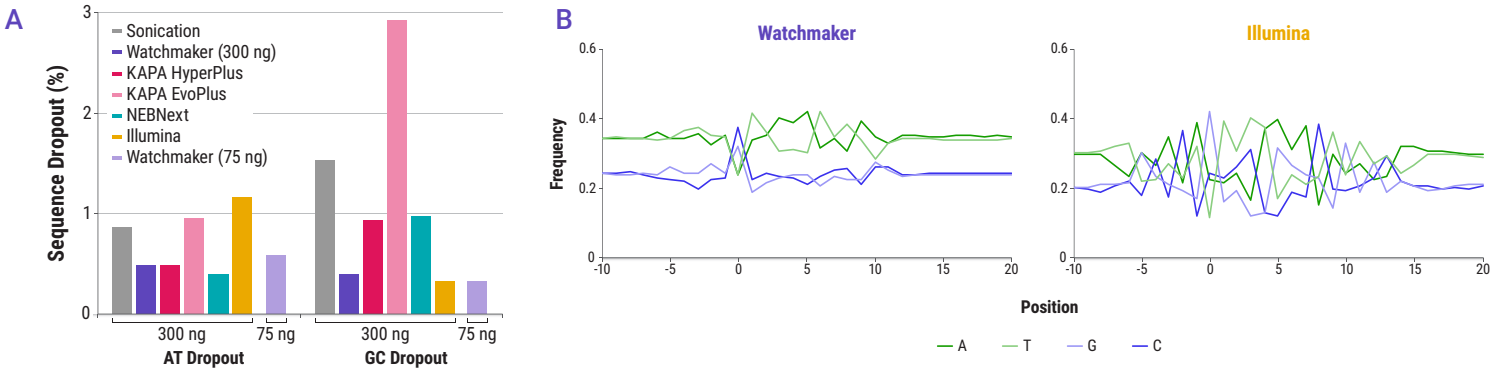

FIGURE 5. Even sequence coverage with minimal bias. All libraries were assessed with respect to **(A)** AT and GC dropout rates and **(B)** start site bias. The Watchmaker solution delivered libraries with minimal sequence dropout in comparison to other workflows. Additionally, the Watchmaker solution, along with KAPA HyperPlus and NEBNext Ultra II FS (data not shown), delivered minimal start site bias in comparison to KAPA EvoPlus and Illumina DNA Prep.

[1]Michael G. Ross et al. "Characterizing and measuring bias in sequence data." *Genome Biology*, vol. 14, no. 5, 2013, article R51. doi: 10.1186/gb-2013-14-5-r51

INCREASE ACCURACY AND SEQUENCING ECONOMY WITH FFPE SAMPLES

FFPE samples are particularly challenging to sequence due to high damage, residual crosslinks, and exposed single-stranded ends. Sonication library prep results in artifacts and elevated soft-clipping (where read ends are trimmed to improve alignment). Watchmaker's solution minimizes both artifact formation, as well as bases wasted to soft-clipping.

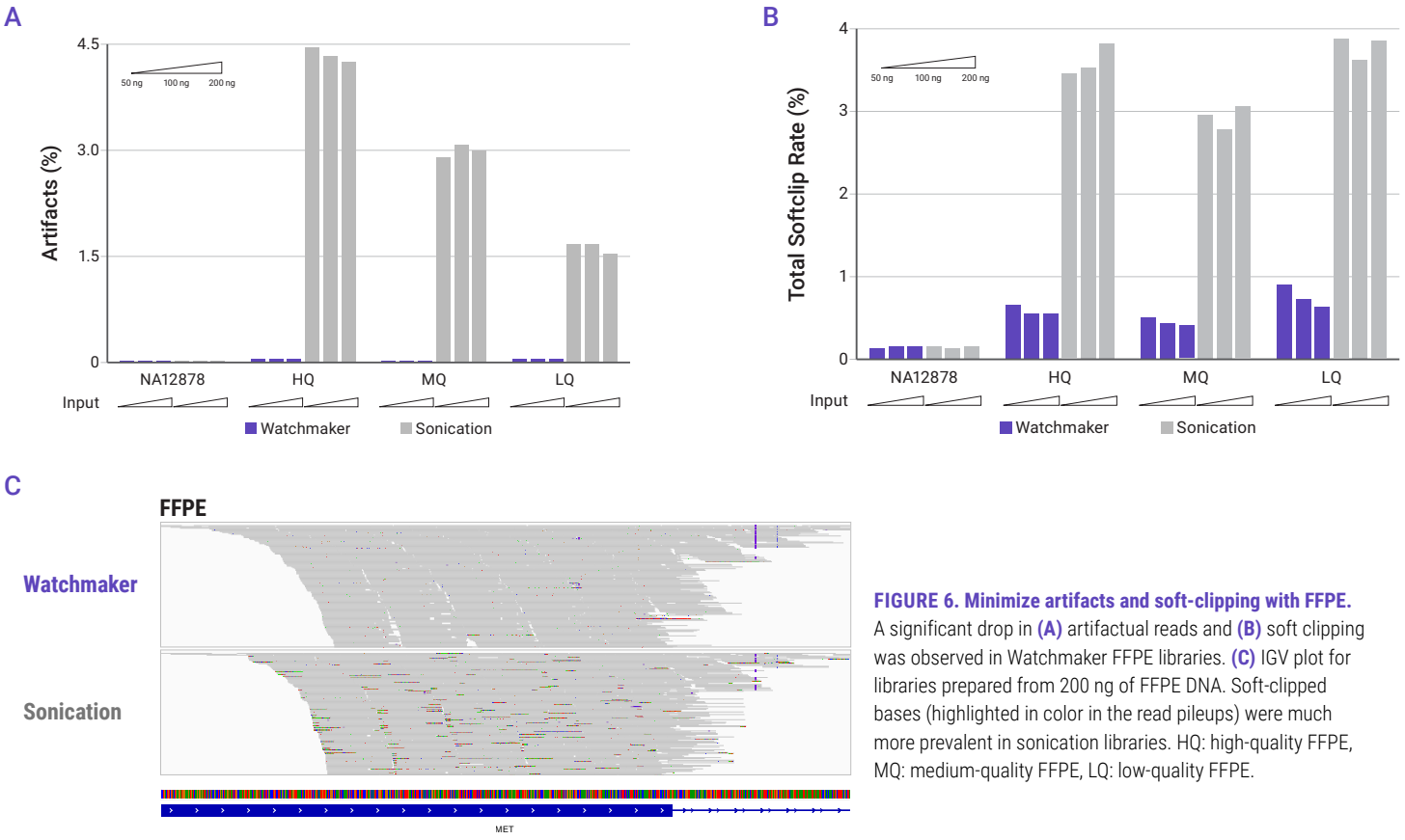

FIGURE 6. Minimize artifacts and soft-clipping with FFPE. A significant drop in **(A)** artifactual reads and **(B)** soft clipping was observed in Watchmaker FFPE libraries. **(C)** IGV plot for libraries prepared from 200 ng of FFPE DNA. Soft-clipped bases (highlighted in color in the read pileups) were much more prevalent in sonication libraries. HQ: high-quality FFPE, MQ: medium-quality FFPE, LQ: low-quality FFPE.

Contact sales@watchmakergenomics.com
or visit watchmakergenomics.com/DNAprep-frag to learn more.

PRODUCT	24 RXN	96 RXN
Watchmaker DNA Library Prep Kit with Fragmentation *includes Equinox Library Amplification Master Mix (2X) and P5/P7 Primer Mix (10X)*	7K0019-024	7K0019-096
Watchmaker DNA Library Prep Kit with Fragmentation *(PCR-free)*	7K0013-024	7K0013-096
Watchmaker DNA Library Prep Kit with Fragmentation *(w/o primers); includes Equinox Library Amplification Master Mix (2X)*	7K0022-024	7K0022-096

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