WATCHMAKER GENOMICS

WATCHMAKER DNA LIBRARY PREP KITS WITH FRAGMENTATION

RAISING THE BAR IN SCALABILITY AND ACCURACY

Watchmaker DNA Library Prep Kits with Fragmentation enable highly sensitive clinical and translational applications to access meaningful insights from a broad range of biological sample types—including ultra-low inputs and FFPE—with an unparalleled combination of accuracy and scalability.

The workflow harnesses the process benefits of enzymatic fragmentation, such as ease of automation, increased scalability, and preservation of low-input samples, while mitigating the formation of associated library preparation artifacts—including false chimeric reads and hairpin artifacts—that can convolute variant calling. Further, use of the Equinox polymerase delivers ultra-high fidelity, low-bias library amplification.

KEY FEATURES & BENEFITS

- Up to a 90% reduction in sequence artifacts improves assay accuracy—critical for highly sensitive applications
- Improved library amplification polymerase error rates and even UMI family coverage enable rare mutation detection
- Robust fragmentation and library preparation efficiency support the use of clinically relevant sample types, such as low inputs and degraded material
- Highly tunable fragmentation delivers consistent library sizes over a wide input range (<1 ng to 500 ng) and between library batches
- Streamlined workflow delivers PCR-free libraries in under 90 minutes and scales easily to high sample numbers and automation platforms
- Uniform sequence coverage improves sequencing efficiency

APPLICATIONS

- Somatic mutation calling and other low-frequency variant detection NGS assays, including those utilizing challenging samples such as FFPE
- Inherited disease sequencing
- Human whole genome sequencing (WGS), including PCR-free
- Whole exome sequencing (WES)
- Single cell analysis
- Metagenomic analysis
- Bulk RNA sequencing (using cDNA as input)
- Viral genome sequencing
- Microbial WGS

READ. WRITE. EDIT.

REDUCED SEQUENCE ARTIFACTS ENABLES HIGH-SENSITIVITY APPLICATIONS

The Watchmaker DNA Library Prep Kit with Fragmentation alleviates many issues associated with sonication, delivering ease of automation and improved scalability, while also mitigating the formation of sequence artifacts, such as false chimeric reads and false SNVs resulting from hairpin artifacts,[1] that are often associated with enzymatic fragmentation. These artifacts convolute the identification of true structural and single nucleotide variants in a sample and especially impact highly sensitive applications, such as low-frequency variant calling.

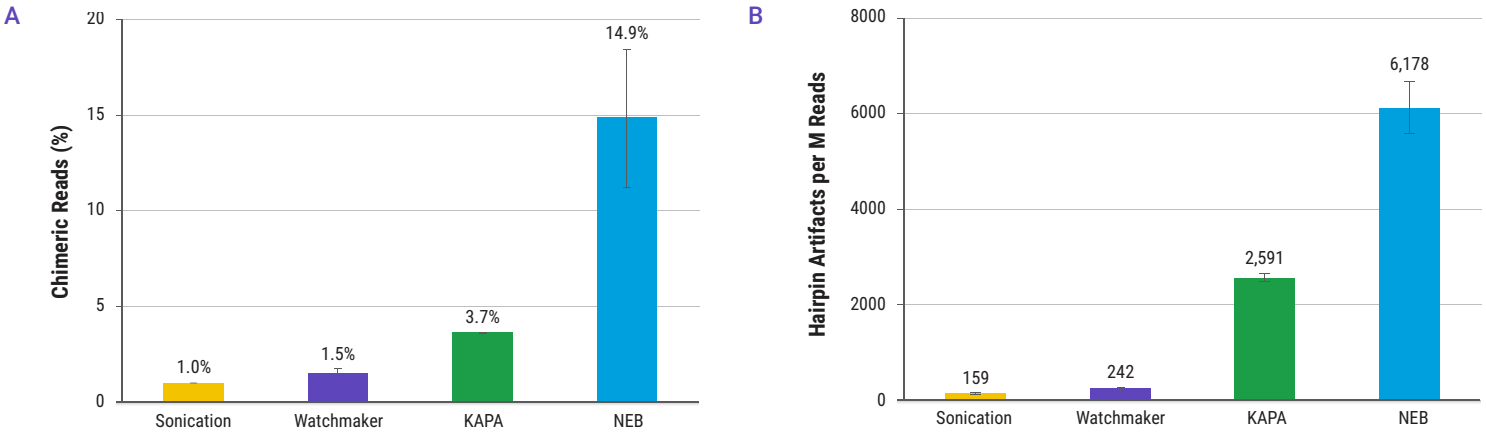


FIGURE 1. Reduced false chimeric reads and hairpin artifacts improve assay sensitivity. Libraries were prepared in duplicate using either the Watchmaker DNA Library Prep Kit with Fragmentation, KAPA HyperPlus Kit, or NEBNext Ultra II FS DNA Library Preparation Kit per manufacturer's recommendations.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

1Thomas Gregory, Apollinaire Ngankeu, Shelley Orwick, Esko A Kautto, Jennifer A Woyach, John C Byrd, James S Blachly, Characterization and mitigation of fragmentation enzyme-induced dual stranded artifacts, NAR Genomics and Bioinformatics, Volume 2, Issue 4, December 2020, lqaa070, https://doi.org/10.1093/nargab/lqaa070

ROBUST PERFORMANCE IMPROVES UTILITY OF CLINICALLY RELEVANT SAMPLE TYPES

Challenging clinically relevant sample types have historically been difficult to reproducibly process. Vanishingly small input amounts raise the issue of inherent sample loss when using sonication to shear genomic DNA. Formalin-fixed paraffin-embedded (FFPE) samples, while critical to oncology research, are typically highly damaged as a result of the fixation process. The Watchmaker DNA Library Prep Kit with Fragmentation delivers high-quality libraries, exhibiting exceedingly small amounts of adapter-dimer contamination, with ultra-low input amounts and poor sample qualities, improving researchers' ability to derive meaningful biological interpretations from sequence data.

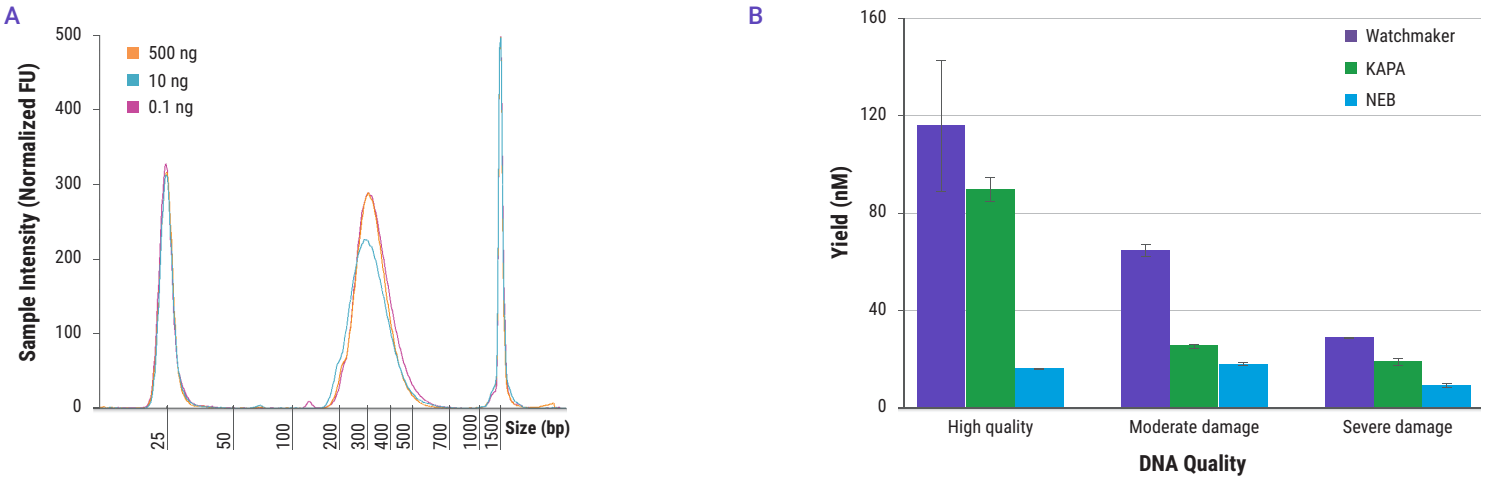


FIGURE 2. Consistent and efficient library preparation across a wide range of sample quantities and qualities. A) Libraries were constructed in duplicate from 500, 10, and 0.1 ng of human genomic DNA fragmented for 20 minutes at 30ºC. Final library distributions were assessed using a D1000 assay by TapeStation (Agilent). **B)** Libraries were constructed in duplicate from 25 ng of either high-quality or formalin-compromised DNA of moderate to severe damage (HD799 and HD803, respectively; Horizon Discovery) using the Watchmaker DNA Library Prep Kit with Fragmentation, KAPA HyperPlus Kit, or NEBNext Ultra II FS DNA Library Preparation Kit. High-quality DNA fragmentation times were selected to target final library sizes of approximately 500 bp per manufacturer's published recommendations (5, 10, and 10 minutes at 37ºC, respectively). Damaged DNA fragmentation times were fixed at 10 minutes at 37ºC. All libraries were amplified for 6 PCR cycles. Final library distributions and yields were assessed using a D1000 assay by TapeStation (Agilent).

TUNABLE FRAGMENTATION

Ensures compatibility with a broad range of applications with insert sizes easily modulated through reaction time and temperature.

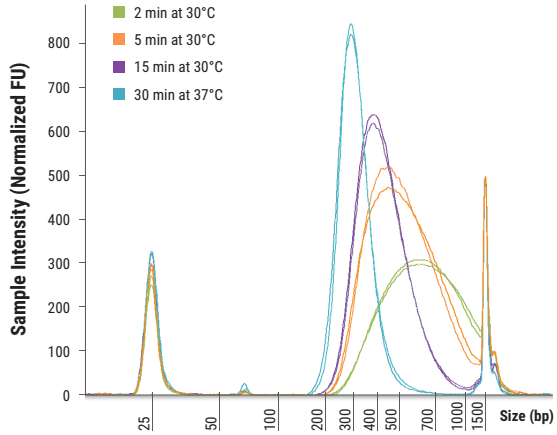


FIGURE 3. Library sizes are easily tailored to application-specific needs. Libraries were constructed from 50 ng of human genomic DNA. A fragmentation reaction time titration was conducted using 30°C (3, 5, and 15 minutes) and 37°C (30 minutes) incubation temperatures. Final library distributions were assessed using a D1000 assay by TapeStation (Agilent).

UNIFORM SEQUENCE COVERAGE

Improves sequencing economy by reducing the overall amount of sequencing—and associated costs—needed to achieve desired coverage depth for all regions of interest.

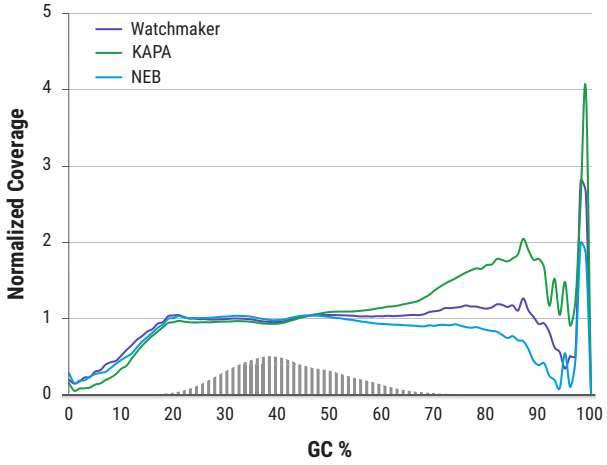


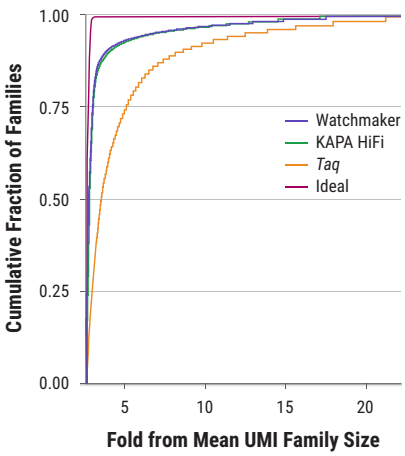
FIGURE 4. Even coverage of complex genomes. Libraries were prepared from 1 ng of human genomic DNA using either the Watchmaker DNA Library Prep Kit with Fragmentation, KAPA HyperPlus Kit, or NEBNext Ultra II FS DNA Library Preparation Kit per manufacturer's recommendations.

EQUINOX FOR LIBRARY AMPLIFICATION ENABLES RARE MUTATION DETECTION APPLICATIONS

Unique Molecular Indices (UMIs) are added prior to library amplification for accurate identification of PCR duplicates and improved variant calling in low-input applications. Biased amplification, where a small number of molecules are preferentially amplified, results in uneven UMI family representation and generates large numbers of singleton UMIs (families represented by only one read) that cannot be error corrected. Equinox enables uniform UMI family amplification, supporting coverage for >75% of all read families (and >90% of read families with GC content from 25 – 75%) within 3X of the mean family depth.

When error correction is not feasible, ultra-high fidelity library amplification is critical for sensitive applications. Equinox delivers a 40% reduction in overall polymerase error rate in comparison to KAPA HiFi HotStart. This enables sensitive variant detection by minimizing overall error rates and reducing false variant calls.

A



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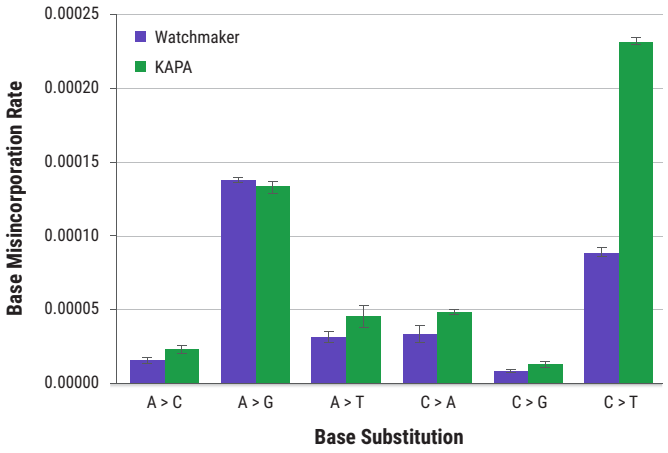

FIGURE 5. Library amplification optimized for high-stringency applications. A) Whole genome libraries were prepared from human gDNA using UMI-containing adapters, quantified by qPCR, and diluted such that 80,000 molecules were template for 26 cycles of amplification using Equinox Library Amplification Kit, KAPA HiFi HotStart ReadyMix, or NEB 2X Taq Master Mix. Libraries were sequenced on Illumina NovaSeq and subsampled to 9 million clusters. 'Ideal' line indicates completely uniform coverage across UMI families as modeled from the Poisson distribution. **B)** Error rates of the Equinox Amplification Master Mix (purple) and KAPA HiFi HotStart ReadyMix (green), were measured after >9 million base incorporation events in three separate reactions, using a proprietary NGS-based assay.

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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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