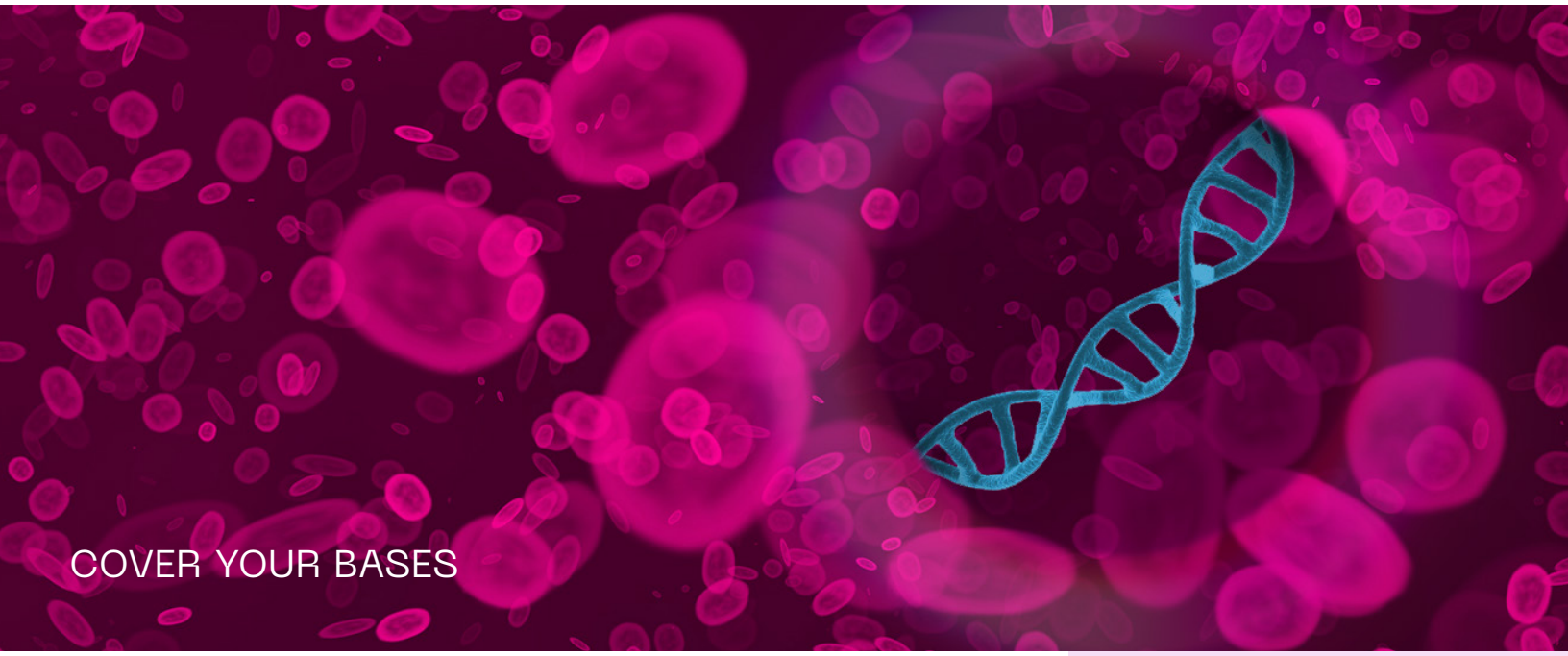
WATCHMAKER GENOMICS

WATCHMAKER DNA LIBRARY PREP KITS



Watchmaker DNA Library Prep Kits increase the conversion of template DNA into sequenceable library molecules to improve coverage and sequencing economy, especially with low-input cfDNA from liquid biopsy samples. The streamlined and automation-friendly workflow supports robust and flexible library preparation from fragmented DNA templates for a wide variety of sequencing applications.

Prepare high-complexity libraries from challenging samples, such as cfDNA, FFPE, and ChIP material, with minimal bias, artifacts, and polymerase errors. Pair with Equinox Uracil Tolerant Library Amplification Kits for methyl-sequencing applications.

KEY FEATURES & BENEFITS

- **Convert more DNA** into sequenceable library molecules for enhanced sensitivity — particularly with clinically relevant cfDNA from liquid biopsies
- Prepare highly complex libraries from **as little as 500 pg** (and up to 1 µg)
- Generate libraries in **under 2 hours** with a simple, **automatable** workflow
- Supports **DNA methylation** analysis when paired with **Equinox® Uracil Tolerant Amplification Kits** for superior yield and uniformity

APPLICATIONS

- cfDNA and ctDNA analysis
- Fragmentomics
- DNA methylation analysis*
- ChIP-seq
- Somatic mutation calling and other low-frequency variant detection assays
- Inherited disease sequencing
- Human whole genome sequencing (WGS), including PCR-free
- Whole exome sequencing (WES)
- Microbial and metagenomic sequencing
- Viral genome sequencing
- Amplicon sequencing

*In combination with Equinox Uracil Tolerant Library Amplification Kits.

INDUSTRY-LEADING PERFORMANCE WITH CELL-FREE DNA

Naturally fragmented cell-free DNA (cfDNA) is an emerging, noninvasive biomarker for a wide range of diseases and conditions that is revolutionizing many fields of research — from oncology to prenatal and transplantation medicine. The Watchmaker DNA Library Prep Kit offers a streamlined, high-performance workflow compatible with both direct and targeted cfDNA sequencing that minimizes sample loss, adapter-dimer contamination, biases, and artifacts.

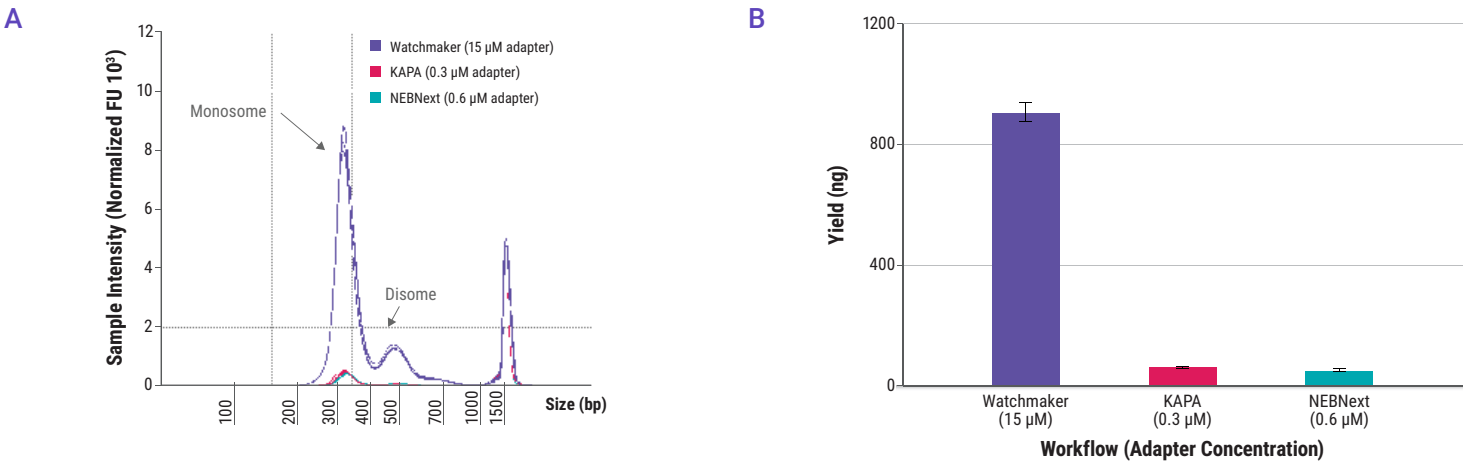


FIGURE 1. Improve yields with cfDNA. (A) TapeStation traces of final libraries. **(B)** Final library yield as assessed by TapeStation analysis. Watchmaker library yields were 9-fold higher compared to libraries prepared with other kits. No adapter-dimers were observed with any protocol. Libraries were prepared in triplicate from 1 ng of Isopure cfDNA with the Watchmaker DNA Library Prep Kit, KAPA HyperPrep Kit, or NEBNext® Ultra™ II for DNA Library Prep according to each manufacturer's recommended protocol.

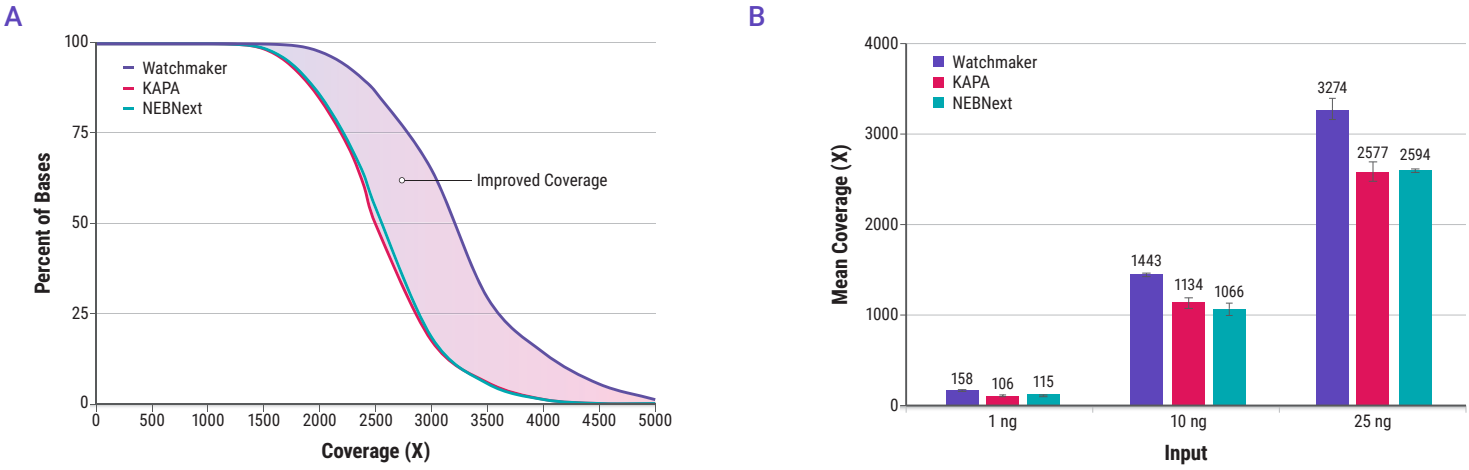


FIGURE 2. Increase coverage of liquid biopsy samples. (A) Cumulative coverage across the entire target region for 25 ng libraries. The shaded region indicates improved coverage of the Watchmaker libraries in comparison to other vendors. **(B)** Deduplicated UMI coverage for libraries sequenced to near-saturation (duplication rates ≥95%). Mean coverage for Watchmaker libraries was 20 – 30% higher compared to libraries prepared with other kits.

Cell-free DNA libraries were constructed in triplicate from 1 ng, 10 ng, or 25 ng of a reference sample (HD779, Horizon Discovery) containing eight mutations at a verified allele frequency (AF) of 0.1%. Libraries were prepared with the Watchmaker DNA Library Prep Kit, KAPA HyperPrep Kit, or NEBNext® Ultra™ II for DNA Library Prep according to each manufacturer's recommended protocol. Twelve-plex target enrichment was performed using a 37 kb custom oncology panel. Sequencing was performed on a NovaSeq™. Data were randomly subsampled to 25 million read pairs per library.

IMPROVE VARIANT CALLING SENSITIVITY FOR LIQUID BIOPSY SAMPLES

Liquid biopsy applications require the detection of low-abundance variants (typically <1%) from very limited inputs. Increased conversion of cfDNA template into sequenceable library results in higher library complexity from limited inputs, which translates to deeper coverage and highly sensitive and reliable variant calling from cfDNA samples.

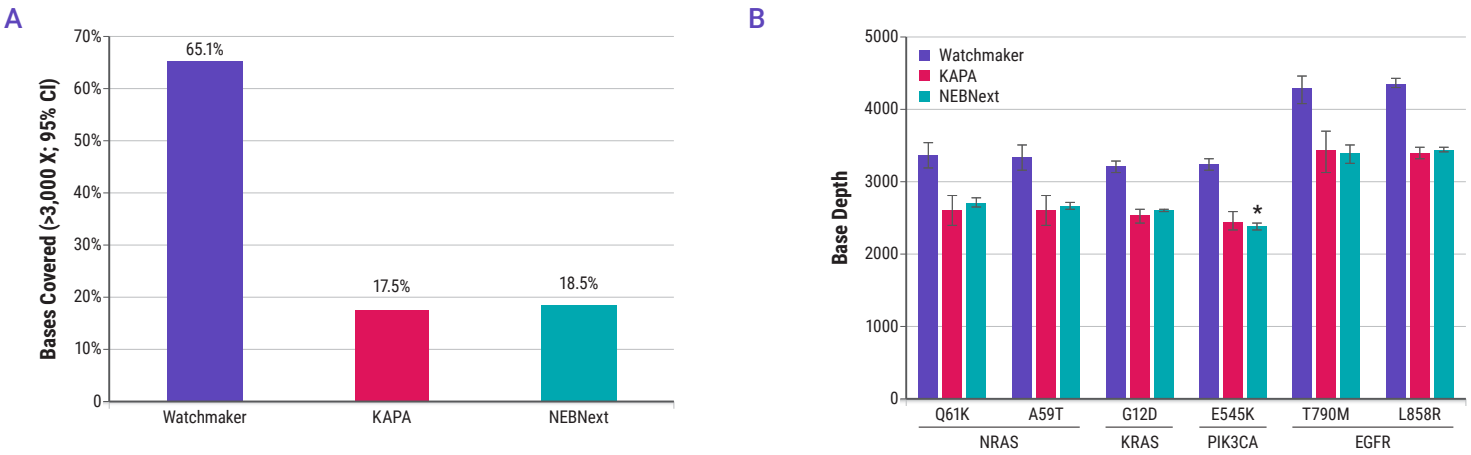


FIGURE 3. High-depth coverage of rare variants. (A) Percentage of bases covered at ≥3,000X. The Watchmaker kit offered a 3.5-fold improvement over other kits, which has significant implications for variant detection sensitivity and sequencing economy from cfDNA samples. Statistical modeling determined 3,000X to be the minimum coverage needed to call very rare variants (AF=0.1%) within a 95% confidence interval, with an alternative allele count ≥1). **(B)** Base coverage depth for the six SNVs expected at an AF of 0.1%. All expected variants were detected in all replicates, except for one true positive (*), missed in one of the NEB replicate libraries. See Figure 2 for sample, library preparation, target enrichment, and sequencing information.

INCREASE COVERAGE WITH FFPE SAMPLES

FFPE samples are ubiquitously used in NGS analysis of clinical samples but continue to pose challenges due to highly variable quality and limited inputs. The Watchmaker DNA Library Prep Kit with Fragmentation is recommended for FFPE samples, but the standard DNA Prep Kit offers similar workflow and performance advantages for FFPE samples that are already fragmented or in settings where sonication is the preferred shearing method.

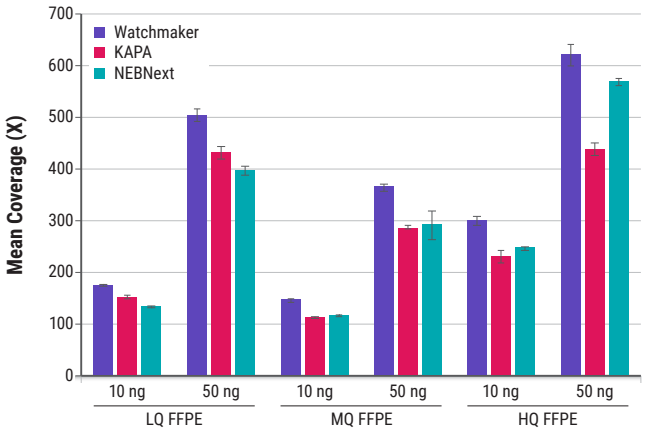


FIGURE 4. Improved coverage from FFPE samples of variable quality. Coverage for libraries prepared with the Watchmaker solution was 10 – 50% higher in comparison to other kits, with the most significant differences observed for low- and medium-quality samples. FFPE libraries were prepared from 10 ng or 50 ng of low- (LQ), medium- (MQ), and high-quality (HQ) FFPE samples using the Watchmaker DNA Library Prep Kit, KAPA HyperPrep Kit, or NEBNext® Ultra™ II for DNA Library Prep according to each manufacturer's recommended protocol. Twelve-plex target enrichment was performed using a 37 kb custom oncology panel. Sequencing was performed on a NovaSeq™. Data were randomly subsampled to 16 million read pairs per library.

AMPLIFY CHALLENGING SAMPLES — INCLUDING URACIL-CONTAINING TEMPLATES — WITH HIGH FIDELITY

DNA methylation is a critical biomarker that is used to study the epigenetic mechanisms that help regulate gene expression. Understanding how DNA methylation patterns are established and modified can provide insights into the regulation of genes during development, differentiation, and disease. Methyl sequencing workflows often leverage bisulfite conversion, where unmethylated cytosines are converted to uracils, necessitating the use of a uracil-tolerant polymerase. The **Equinox Uracil Tolerant Library Amplification Kit** harnesses the fidelity benefits of our original Equinox polymerase, while being engineered specifically for compatibility with uracils.

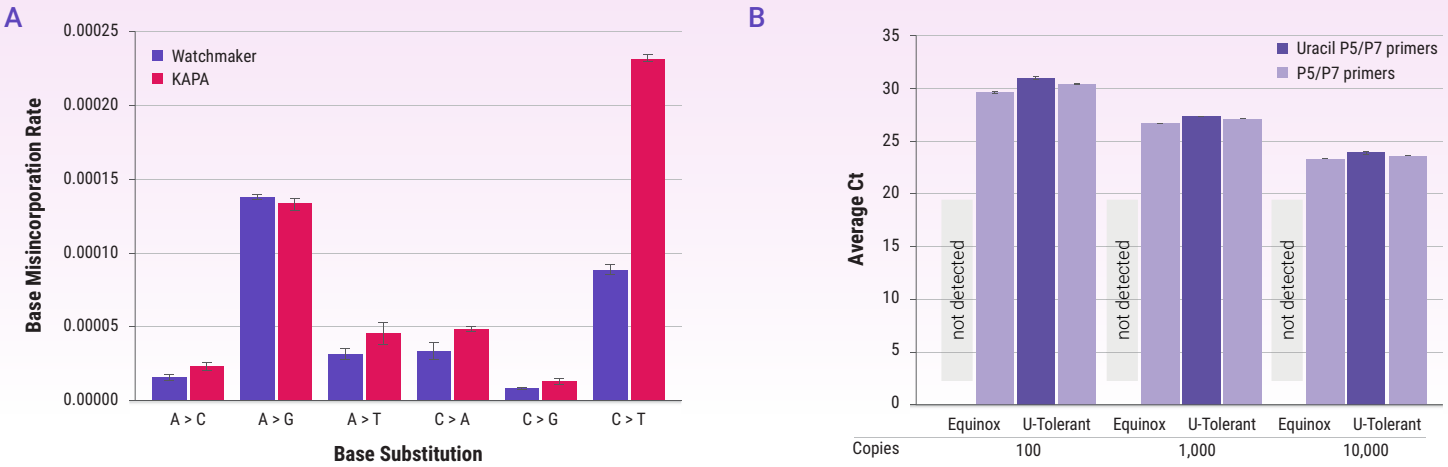


FIGURE 5. Library amplification designed for challenging applications. (A) Equinox improves overall fidelity, and reduces C to T base misincorporation events that are relevant for oncology and methylation applications. Error rates of the Equinox Library Amplification Kit and KAPA HiFi HotStart ReadyMix were measured after >9 million base incorporation events in three separate reactions, using a proprietary NGS-based assay. Base substitution profiles were examined in triplicate over 5.4 million G/C incorporation events and 4.0 million A/T incorporation events. Data not shown for NEB Q5 polymerase as it did not produce sufficient yield for the base misincorporation assay. **(B)** As expected, Equinox Library Amplification Kits were inhibited by uracil-containing primers, while Equinox Uracil Tolerant Library Amplification Kits show no inhibition across a range of inputs.

PRODUCT	24 RXN	96 RXN
Watchmaker DNA Library Prep Kit *Includes Equinox Library Amplification Master Mix (2X) and P5/P7 Primer Mix (10X)*	7K0102-024	7K0102-096
Watchmaker DNA Library Prep Kit *(PCR-free)*	7K0101-024	7K0101-096
Watchmaker DNA Library Prep Kit *(w/o primers); includes Equinox Library Amplification Master Mix (2X)*	7K0103-024	7K0103-096
Equinox Uracil Tolerant Library Amplification Kit *Includes P5/P7 Primer Mix (10X)*	7K0023-024	7K0023-096
Equinox Uracil Tolerant Library Amplification Kit *(w/o primers)*	7K0028-024	7K0028-096

Contact sales@watchmakergenomics.com
or visit watchmakergenomics.com/DNA-LPK to learn more.

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