WATCHMAKER GENOMICS

EQUINOX LIBRARY AMPLIFICATION KITS

SETTING A NEW STANDARD IN NGS LIBRARY AMPLIFICATION

Equinox Library Amplification Kits deliver excellent fidelity, uniform sequence coverage, and high library complexity to specifically address the stringent demands of applications such as rare variant detection, circulating cell-free DNA (cfDNA) analysis, single-cell analysis, and hybridization capture. Kits contain a uniquely engineered, ultra-high-fidelity DNA polymerase in an optimized hot start PCR master mix formulated for high-efficiency, low-bias NGS library amplification.

KEY FEATURES & BENEFITS

- Improve overall assay sensitivity with ultra-high-fidelity amplification, reducing misincorporation events by up to 40%
- Enable low-input applications and automated workflows with an antibody-based hot start formulation
- Amplify libraries efficiently from a wide range of inputs (0.1 pg – 500 ng) and GC content (15% to 85%)
- Optimize sequencing economy with highly uniform sequence coverage
- Achieve robust performance in hybridization capture workflows due to compatibility with paramagnetic beads
- Leverage the performance benefits of Equinox DNA Polymerase for bisulfite converted DNA with Equinox Uracil Tolerant Library Amplification Kits

APPLICATIONS

- Low-frequency variant detection NGS assays, including those utilizing challenging samples such as FFPE and cfDNA
- Hybridization-capture workflows
- Single-cell analysis
- Whole-genome sequencing
- RNA-Seq
- Amplicon sequencing
- ChIP-Seq, ATAC-Seq, and associated epigenetic applications
- Illumina and non-Illumina sample preparation workflows

Equinox Uracil Tolerant Library Amplification Kits for:

- Bisulfite-converted DNA
- Damaged DNA samples or templates containing modified bases

READ. WRITE. EDIT.

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

Equinox Library Amplification Kits are designed for high-efficiency, high-fidelity amplification of next generation sequencing (NGS) libraries. The ready-to-use mix contains an optimized PCR buffer and hot start enzyme formulation that enables library amplification with minimal bias and error across a broad range of input amounts and GC contents, and performance is maintained in the presence of a variety of paramagnetic beads.

Three different Equinox Library Amplification Kits are available, each provided with or without amplification primers:

2X

Equinox Library Amplification Kit ultra-high-fidelity polymerase enables highly sensitive applications, in a convenient 2X master mix format

4X

Equinox HC (High Concentration) Library Amplification Kit is provided at a more concentrated 4X version, for library amplification reactions using more dilute inputs.

U+

Equinox Uracil Tolerant Library Amplification Kit for amplification of uracil-containing templates including bisulfite-converted, deaminated, or damaged DNA (e.g., FFPE).

LIBRARY AMPLIFICATION WITH EQUINOX ENABLES RARE MUTATION DETECTION APPLICATIONS

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.

To further improve variant calling in low-input applications, Unique Molecular Indices (UMIs) are added prior to library amplification for accurate identification of PCR duplicates. 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.

ULTRA-HIGH FIDELITY

Enables sensitive variant detection by reducing false variant calls, especially C>T substitutions, one of the most common mutation types in cancers.

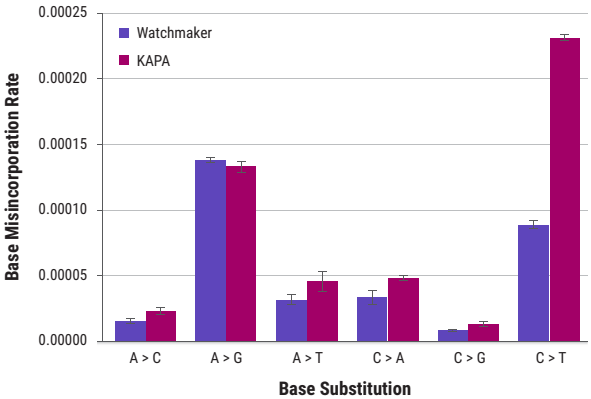

FIGURE 1. Up to 40% reduction in overall polymerase error rate. Error rates were measured after >9 million base incorporation events in three separate reactions, using a proprietary NGS-based assay. The Equinox Library Amplification Kit displayed a 40% reduction in overall polymerase error rate in comparison to KAPA HiFi HotStart ReadyMix.

UNIFORM COVERAGE OF UMI FAMILIES

Equinox delivers even coverage across UMI families for bioinformatic error correction, critical for low frequency variant detection.

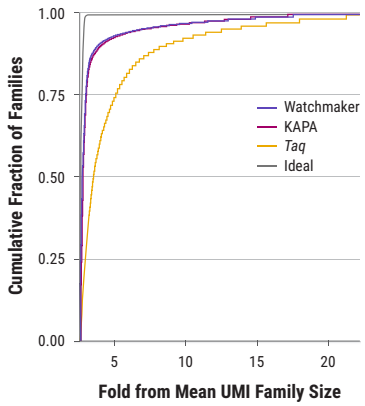

FIGURE 2. Low-bias UMI amplification. Whole genome libraries were prepared using UMI-containing adapters. A limiting dilution of 80,000 library molecules were used for polymerase amplification, as indicated, and sequencing. Equinox Library Amplification Kits support coverage for >75% of all read families (and >90% of read families with GC content from 25 – 75%) within 3X of the mean family depth. 'Ideal' line indicates a completely uniform coverage across UMI families modeled with a Poisson distribution.

EFFECTIVE HOT START FORMULATION

Inhibits both polymerase and 3' → 5' exonuclease activities to mitigate sample and primer degradation and facilitate automated library construction.

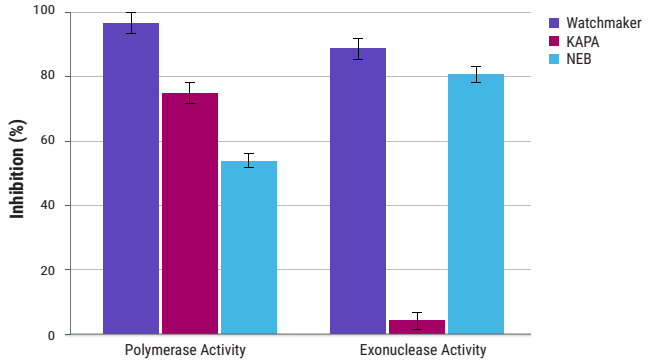

FIGURE 3. Improved hot start functionality. Polymerase and exonuclease activities of the Polymerase and exonuclease activities of the Equinox Library Amplification polymerase, KAPA HiFi HotStart DNA Polymerase and NEB Q5 DNA Polymerase were assessed by the detection of dNTP incorporation or dNMP release, respectively, after incubation at 25°C. Percent inhibition is reported relative to uninhibited formulations.

HIGH-EFFICIENCY AMPLIFICATION

Limits the number of PCR cycles required, which minimizes associated bias and artifacts, even in high-yield demanding workflows such as hybridization capture.

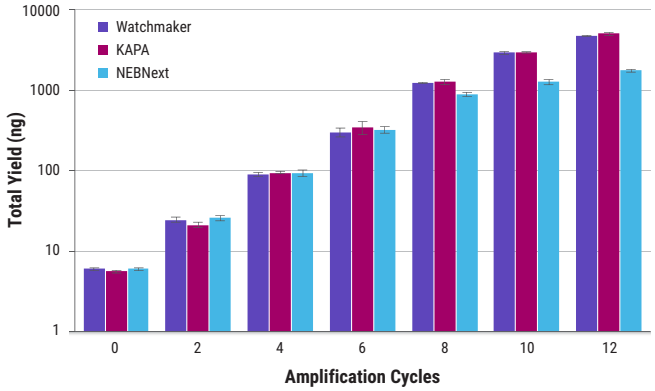

FIGURE 4. Highly efficient library amplification. Human whole-genome libraries (10 ng) were amplified in triplicate with the Equinox Library Amplification Kit, KAPA HiFi HotStart ReadyMix, and NEBNext Ultra II Q5 Master Mix. Yields were determined by qPCR-based library quantification at 2-cycle intervals.

LOW-BIAS AMPLIFICATION

Ensures high coverage uniformity across complex genomes, even in the presence of paramagnetic beads, to optimize sequencing economy.

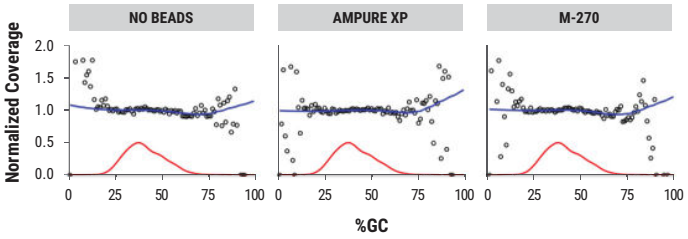

FIGURE 5. Highly uniform sequence coverage in the presence of paramagnetic beads. Human whole-genome libraries (0.04 pg) were amplified for 26 cycles with the Equinox Library Amplification Kit in the absence or presence of paragmagnetic beads: AMPure XP Reagent (100 µL slurry; relevant to reaction purification) and Dynabeads™ M-270 Streptavidin (500 µg; relevant to hybridization capture). Coverage plots were normalized to those for unamplified libraries. Blue lines represent locally weighted smoothed (LOESS) normalized coverage.

ROBUST AMPLIFICATION THROUGH URACIL

Many proofreading B-family polymerases stall replication in response to uracil bases in DNA templates. Equinox Uracil Tolerant Polymerase is an engineered polymerase that reads through uracil-containing templates for bisulfite sequencing.

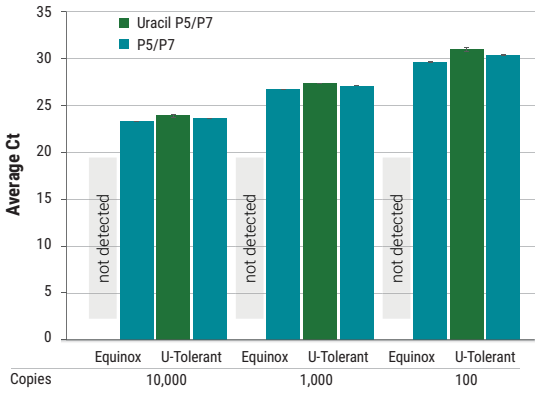

FIGURE 6. Equinox Uracil Tolerant Library Amplification Kits efficiently amplify uracil-containing templates. As expected, Equinox Library Amplification Kits were inhibited by uracil-containing primers, while Equinox Uracil Tolerant Library Amplification Kits show no inhibition across a range of inputs.

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PRODUCT	24 RXN	96 RXN	384 RXN
Equinox Library Amplification Kit *Includes P5/P7 Primer Mix (10X)*	7K0014-024	7K0014-096	7K0014-384
Equinox Library Amplification Kit *(w/o primers)*	7K0021-024	7K0021-096	7K0021-384
Equinox HC Library Amplification Kit *Includes P5/P7 Primer Mix (10X)*	–	7K0094-096	7K0094-384
Equinox HC Library Amplification Kit *(w/o primers)*	–	7K0065-096	7K0065-384
Equinox Uracil Tolerant Library Amplification Kit *Includes P5/P7 Primer Mix (10X)*	7K0023-024	7K0023-096	7K0023-384
Equinox Uracil Tolerant Library Amplification Kit *(w/o primers)*	7K0028-024	7K0028-096	7K0028-384

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