Equinox® Prime Library Amplification Kit

Product Description

Equinox Prime Library Amplification Kits are designed for high-efficiency, ultra 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 (0.1 pg to 500 ng) and performance is maintained in the presence of a variety of paramagnetic beads. The mix is optimized for ultra high-fidelity, reduced homopolymer slippage, and uniform amplification of next-generation sequencing libraries across a broad range of GC content. The hot start functionality inhibits 5'→3' polymerase and 3'→5' exonuclease activities, ensuring robust performance when amplification reactions are set up at room temperature.

The Equinox Prime Library Amplification Kit is provided with or without amplification primers.

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Kit Contents

Kit	Kit code	Description	Component volume		
			24 rxn	96 rxn	384 rxn
Equinox Prime Library Amplification Kit	**7K0141-024** (24 rxn) **7K0141-096** (96 rxn) **7K0141-384** (384 rxn)	Equinox Prime Amplification Master Mix (2X)	690 µL	3 mL	12 mL
		P5/P7 Primer Mix (10X)	144 µL	600 µL	1.2 mL (x2)
Equinox Prime Library Amplification Kit (w/o primers)	**7K0140-024** (24 rxn) **7K0140-096** (96 rxn) **7K0140-384** (384 rxn)	Equinox Prime Amplification Master Mix (2X)	690 µL	3 mL	12 mL

For custom formats, contact the **Sales Team** at sales@watchmakergenomics.com.

Product Applications

The Equinox Prime Library Amplification Kit addresses the stringent demands of NGS applications such as rare variant detection, circulating free DNA (cfDNA) analysis and hybridization capture for oncology applications. In addition to uniform coverage, minimal bias, and the maintenance of library complexity, these applications require high yield, fidelity, and sensitivity.

Equinox Prime minimizes error rates and maximizes fidelity. By creating a formulation that maximizes fidelity, Equinox Prime is suited for applications that require a highly accurate readout of information from the target of amplification. It is ideally suited for NGS applications across all sequencing platforms along with the characterization of any downstream metrics such as SNP detection.

Microsatellites are highly repetitive, thus small insertions or deletions (indels) in these regions are difficult to distinguish from sequencing artifacts. Indels within microsatellites are biologically relevant and can be used as a biomarker to detect microsatellite instability (MSI), relevant for somatic oncology applications. The Equinox Prime polymerase was engineered to minimize errors introduced during the replication of repetitive sequences. By minimizing base misincorporation errors and polymerase slippage, the engineered polymerase ensures a more faithful amplification of the microsatellite regions, making it easier to detect true indels indicative of MSI.

The Equinox Prime Library Amplification kit is not compatible with intercalating dyes, such as those used for qPCR. For applications requiring the use of intercalating dyes, we recommend the Equinox Library Amplification kits (Kits 7K0014 or 7K0021).

The Equinox Prime Library Amplification Kit is ideally suited for:

- Efficient library amplification from a wide range of template amounts (0.1 pg – 500 ng) up to 1 kb in length
- 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 (WGS)
- Amplicon sequencing
- Indexing PCR
- RNA-Seq
- ChIP-Seq, ATAC-Seq, and associated epigenetic applications
- Illumina and non-Illumina sample preparation workflows

Storage and Handling

The Equinox Prime Library Amplification Kit is shipped on cold packs. Upon receipt, store all kit components at -20°C ± 5°C.

Equinox Prime Amplification Master Mix (2X) should be thawed prior to use at room temperature. It is stable at room temperature and can be kept at this temperature during reaction setup. Equilibrating fully to room temperature, followed by vortexing for 5 – 10 sec, will ensure the best product performance.

When stored and handled as indicated, the product will retain full performance until the expiry date printed on the kit box.

Required Materials Not Included

- PCR-grade water
- Thermocycler
- Ampure® XP Beads (Beckman Coulter, Inc. #A63881) or equivalent (see **Prior to Starting**)
- 80% Ethanol
- 200 µL thin-wall PCR tubes compatible with thermocycler, or 96-well 0.2 mL PCR Plates and Plate-Seals
- 10 mM Tris-HCl pH 8.0
- Magnetic rack compatible with 0.2 mL tubes and/or 96-well plate

These protocols are designed for use with the specified labware, consumables, and calibrated equipment.

Prior to Starting

Input DNA

Input Amount

This kit is compatible with a broad range of adapter-ligated library input amounts (0.1 pg – 500 ng) and GC content with templates up to 1 kb in length.

Ethanol Contamination

Ethanol can inhibit PCR. When using the Equinox Prime Library Amplification Kit after a magnetic bead purification, care must be taken to ensure ethanol used in bead washes is not carried over into the PCR reaction as even a few microlitres of carryover can affect performance.

After ethanol washes, ensure all ethanol is aspirated from the bead pellet. Bead pellet drying time will vary depending on ambient condition, volume of beads, etc. A visual indication that beads are sufficiently dry is when cracking within the pellet begins to appear. A shiny pellet is not sufficiently dried. When eluting, ensure the pellet is fully homogenized to facilitate full yield recovery.

Amplification Primers

Storage and Handling

Use a buffered solution, such as 10 mM Tris-HCl, pH 8.0, to store and dilute primers. Limit the number of freeze-thaw cycles.

Primer Concentration

Always use equimolar concentrations of forward and reverse primers at a final concentration of 1 µM to 2 µM each. A final concentration of 1 µM is sufficient to yield 500 ng of library (approximately 40 nM in a 50 µL reaction for a library with an average fragment length of 400 bp), which is typically adequate for library QC, quantification, and sequencing. If the total library yield must exceed 500 ng or 40 nM, use 2 µM final concentration.

The P5/P7 Primer Mix (10X) is supplied at 20 µM (each primer) for a 2 µM final concentration.

Primer Design

Protection of the 3'-ends of amplification primers through the incorporation of one or more phosphorothioate bonds is highly recommended to achieve optimal specificity and library yields.

Library Amplification Optimization

Annealing Temperature

For user-supplied primers, the optimal annealing temperature in this proprietary buffer should be determined empirically in an annealing temperature gradient (55°C to 65°C) experiment.

Extension Time

Longer extension times may be employed to ensure efficient amplification of longer-insert libraries. A 30 sec extension is sufficient for libraries with a mode fragment size up to 500 bp. A 45 sec extension time is recommended for libraries with mode fragment sizes >500 bp. The optimal condition for each application may need to be determined empirically.

Cycle Number

Since bias and amplification artifacts are compounded with each cycle of PCR, it is desirable to use the lowest number of PCR cycles required for the application to maximize coverage uniformity and fidelity. Table 1 (page 4) provides a starting point for PCR cycle number optimization based on DNA input into library amplification. Note that this may be significantly less than the amount of DNA input into upstream library construction. Imperfect enzyme efficiencies, SPRI cleanups, and/or size selections result in a reduction of material available for library amplification. Adapter-ligated library may be quantified by qPCR or estimated by other means or methods to determine the optimal number of amplification cycles for the desired library yield.

SPRI Purification

Instructions are provided in this protocol for a bead clean-up to purify the amplified library. SPRI-to-sample bead ratios may be optimized for different applications. A 1X ratio is recommended as a starting point when using standard P5/P7 amplification primers.

The protocol outlined below assumes the use of AMPure XP (Beckman Coulter) reagent for bead purification steps.

Other SPRI bead brands may be used, assuming they are nuclease-free and deliver equivalent sizing performance at the given bead ratio (1X). Otherwise, bead purification ratios will need to be optimized for the bead brand used.

Ensure beads are equilibrated to room temperature and thoroughly resuspended via vortexing prior to use.

Library Construction Protocol

Note:

- Equinox Prime Amplification Master Mix should be thawed and equilibrated at room temperature prior to use.
- If planning to bead-purify the amplified library, ensure SPRI purification beads are fully equilibrated to room temperature and thoroughly resuspended via vortexing prior to use.
- If amplifying in the presence of paramagnetic beads and/or streptavidin beads, please review **Appendix A** prior to proceeding.

1. Library Amplification

1.1 Equilibrate the Equinox Prime Amplification Master Mix (2X) to room temperature, and the primers — either supplied P5/P7 Primer Mix (10X) or user-supplied — on ice.

1.2 Once thawed, vortex the Equinox Prime Amplification Master Mix for 10 sec and primers for 5 sec and briefly centrifuge.

1.3 Program a thermocycler as indicated below:

Step	Temperature (°C)	Time (sec)	Cycles
Initial denaturation	98	45	1
Denaturation	98	15	See Table 1
Annealing[1]	P5/P7 primers: 60°C Indexed primers: 55°C	30	
Extension[2]	72	30	
Final extension	72	60	1
Final hold	4 – 12	Hold	–

[1]An annealing temperature of 60°C is recommended for standard Illumina® "P5" and "P7" primers (P5: AATGATACGGCGACCACCGA; P7: CAAGCAGAAGACGGCATACGAGAT). For other amplification primers, the optimal annealing temperature should be determined empirically in an annealing temperature gradient (55°C – 65°C) experiment. An annealing temperature of 55°C is recommended for all indexing primers.

[2]Longer extension times may be employed to ensure efficient amplification of longer-insert libraries. A 30 sec extension is sufficient for libraries with a mode fragment size up to 500 bp; a 45 sec extension time is recommended for libraries with mode fragment sizes >500 bp. The optimal condition for each application may need to be determined empirically.

Table 1. Recommended PCR cycle numbers based on DNA input

Adapter-ligated DNA (ng)	PCR cycles[1] to generate:	
	40 nM library	1 µg library
500	1	2
100	3	4
50	4	5
10	7	8
5	8	9
1	11	12
0.5	12	13
0.1	15	16
0.01	18	19
0.001	22	23
0.0001	26	27

[1]Cycle number recommendations are appropriate for libraries with mean fragment length of approximately 300 – 600 bp. Cycle numbers may be adjusted accordingly if mean fragment length differs significantly from this range.

1.4 Assemble each amplification reaction according to the tables below, following the recipe that corresponds to primer source:

Reaction setup – P5/P7 Primer Mix (10X):

Component	Final concentration	Volume (50 µL reaction)
Equinox Prime Amplification Master Mix (2X)	1X	25 µL
P5/P7 Primer Mix (10X)	2 µM	5 µL
Template DNA	0.1 pg – 500 ng	Variable
PCR-grade water	–	Up to 50 µL

Reaction setup – user-supplied primers:

Component	Final concentration	Volume (50 µL reaction)
Equinox Prime Amplification Master Mix (2X)	1X	25 µL
Each primer (Fwd/Rev)[1]	1 – 2 µM	Variable
Template DNA	0.1 pg – 500 ng	Variable
PCR-grade water	–	Up to 50 µL

[1]See **Prior to Starting** for more information on user-supplied primers.

1.5 Mix thoroughly by pipetting and centrifuge briefly.

1.6 Place reactions in the thermocycler (programmed in **Step 1.3**) and run the program.

1.7 If purifying amplified material, proceed into **Post-amplification Cleanup**.

Safe stopping point. Samples can be stored at 4°C overnight and at -20°C for up to 1 month.

2. Post-amplification Cleanup

2.1 Freshly prepare at least 0.4 mL of an 80% ethanol solution for each reaction.

2.2 Vortex room-temperature SPRI beads to thoroughly mix. Add 50 µL (1X) of beads to each amplification reaction and mix thoroughly by pipetting.

NOTE: SPRI-to-sample bead ratios may be optimized for different applications. A 1X ratio is recommended as a starting point when using standard P5/P7 amplification primers.

2.3 Incubate the library-bead mixtures at room temperature for at least 5 min to maximize library recovery.

2.4 Place sample tubes on a magnet for at least 5 min, or until all beads have collected on the tube wall and the solution is clear.

2.5 Carefully remove and discard the supernatant from each tube.

2.6 Add 200 µL of freshly prepared 80% ethanol to each tube, taking care not to disturb the bead pellet on the tube wall.

2.7 Incubate tubes at room temperature for at least 30 sec without removing the tube from the magnet or disturbing the bead pellet. Carefully remove and discard the ethanol.

2.8 Repeat **Steps 2.6 – 2.7** for a total of two washes.

2.9 Allow remaining ethanol to evaporate by allowing the pellets to air dry for 3 – 5 min.

NOTE: Beads have been sufficiently dried when cracks appear in the surface of the bead pellet and no liquid is visible in the tube.

2.10 Remove sample tubes from the magnet and carefully resuspend each bead pellet in 22 µL of 10 mM Tris-HCl pH 8.0. Pipetting carefully will minimize bubbling and allow for greater library recovery.

2.11 Incubate tubes at room temperature for at least 2 min before placing back on the magnet.

2.12 Leave sample tubes on the magnet for at least 2 min, or until all beads have collected on the tube wall and the solution is clear.

2.13 Carefully transfer each library-containing supernatant to a new tube.

2.14 At this point, libraries are ready for quantification, normalization, pooling, hybridization capture, and/ or sequencing.

NOTE: We recommend quantifying libraries using qPCR and analyzing quality and sizing using capillary electrophoresis prior to preparing the libraries for sequencing

Appendix A: Amplification with Paramagnetic Purification Beads

For some library preparation workflows, it may be preferred or advantageous to perform PCR in the presence of paramagnetic purification beads. For example, in hybrid capture workflows, post-capture amplification may be performed by adding PCR mix directly to the capture beads with bound library, eliminating an elution step. Alternatively, libraries may be eluted directly from SPRI beads in the PCR reaction mixture.

We have found paramagnetic purification beads to fall into three categories of compatibility with PCR and sequencing based on their surface chemistry: Group I—fully compatible, Group II—potentially inhibitory, and Group III—incompatible. While Group II beads are inhibitory to non-optimized PCR systems, the Equinox Amplification Master Mix (2X) has been optimized to allow amplification in the presence of both Group I and Group II beads, with no observable loss in performance (e.g., efficiency, uniformity, or fidelity). Equinox Amplification Master Mix (2X) and other library amplification systems are not compatible with the extreme inhibition characterized by Group III beads. Table A details the various paramagnetic bead types evaluated.

Table A. Paramagnetic purification bead types

Bead Type	Vendor	Catalog Number	Compatibility/amount tested[1]
Group I (Tosyl-activated beads)			
Dynabeads™ M280 Streptavidin	Thermo Fisher	11205D	500 µg
Dynabeads MyOne™ Streptavidin T1	Thermo Fisher	65601	500 µg
Group II (Carboxylic acid-activated beads)			
Dynabeads M270 Streptavidin[2]	Thermo Fisher	65305	500 µg
SPRI	Various, incl. Beckman Coulter	A63882	100 µL
Dynabeads MyOne Streptavidin C1	Thermo Fisher	65001	500 µg
Group III (Not compatible with PCR)			
Dynabeads M270 Carboxylic Acid	Thermo Fisher	14305D	500 µg

[1]Volume of slurry or mass of beads per 50 µL amplification reaction.
[2] Used in xGen Hybridization and Wash Kit (Integrated DNA Technologies).

Revision History

Version	Description	Date
1.0	• First release	02/2026

WATCHMAKER GENOMICS

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