# Equinox Library Amplification Kit

## Product Description

The Equinox Library Amplification Kit is 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 (0.1 pg to 500 ng) and GC contents, and performance is maintained in the presence of a variety of paramagnetic beads. The hot start functionality inhibits polymerase and 3' → 5' exonuclease activities, ensuring robust performance when amplification reactions are set up at room temperature. This product is not compatible with uracil-containing templates.

## Table of Contents



## Kit Contents

| Kit | Kit Code | Description | Component Volume | | |
|---|---|---|---|---|---|
| | | | 24 rxn kit | 96 rxn kit | 384 rxn kit |
| Equinox Library Amplification Kit[1] | **7K0014-024** (24 rxns)<br>**7K0014-096** (96 rxns)<br>**7K0014-384** (384 rxns) | Equinox Amplification Master Mix (2X)[2] | 690 µL | 3 mL | 12 mL |
| | | P5/P7 Primer Mix (10X) | 144 µL | 600 µL | 1.2 mL (x2) |
| Equinox Library Amplification Kit (w/o primers)[3] | **7K0021-024** (24 rxns)<br>**7K0021-096** (96 rxns)<br>**7K0021-384** (384 rxns) | Equinox Amplification Master Mix (2X)[2] | 690 µL | 3.0 mL | 12 mL |

[1]Previously named **Library Amplification Kit for Illumina sequencing**
[2]Previously named **Library Amplification Hot Start Master Mix (2X)**
[3]Previously named **Library Amplification Master Mix**

## Product Applications

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

**Equinox Library Amplification Kits are 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
- RNA-Seq
- ChIP-Seq, ATAC-Seq, and associated epigenetic applications
- Illumina and non-Illumina sample preparation workflows

## Storage and Handling

Equinox Library Amplification Kits are shipped on dry ice or ice packs. Upon receipt, store all kit components at -20°C ± 5°C.

Reagents may be temporarily stored at 4°C for up to 2 weeks. Reagents are stable through at least 25 freeze-thaw cycles. Keep solutions on ice and avoid vortexing during regular use.

When stored and handled as such, kits will retain full activity until the expiry date printed on the kit label.

## Required Materials not Included

- PCR-grade water
- Thermocycler
- SPRI purification beads (see **Prior to Starting** section)
- 100% ethanol
- 10 mM Tris-HCl pH 8.0
- Magnetic rack compatible with 0.2 mL tubes and/or 96-well plate

## 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 templates up to 1 kb in length.

*Ethanol Contamination*

Ethanol is a potent inhibitor of PCR. When using the Equinox 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 microliters 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 0.5 to 2 µM each. A final concentration of 0.5 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 70°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 Amplification Protocol

**Note:**

- 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, please review **Appendix A** prior to proceeding.

### 1. Library Amplification

1.1 Thaw and equilibrate the Equinox Amplification Master Mix (2X) and primers—either supplied P5/P7 Primer Mix (10X) or user-supplied—on ice.

1.2 Once thawed, invert the Equinox Amplification Master Mix (2X) several times or swirl vigorously to mix (DO NOT VORTEX). Vortex primers and briefly centrifuge.

1.3 Program a thermocycler according to the table below:

| Step | Temperature (°C) | Time (sec) | Cycles |
|---|---|---|---|
| Initial denaturation | 98 | 45 | 1 |
| Denaturation | 98 | 15 | See Table 1 |
| Annealing[1] | 60 | 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 – 70°C) experiment.

[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 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 Amplification Master Mix (2X) | 1X | 25 µL |
| Each primer (Fwd/Rev)[1] | 0.5 – 2 µM | Variable |
| Template DNA | 0.1 pg – 500 ng | Variable |
| PCR-grade water | – | Up to 50 µL |

[1]See **Prior to Starting** section 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 immediately into **Post-amplification Clean-up**.

**2. Post-amplification Clean-up**

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

2.2 Vortex SPRI beads at room temperature 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 to not 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 hybridization 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). The Equinox Amplification Master Mix (2X) and other library amplification systems are not compatible with the extreme inhibition characterized by Group III beads. Table 2 details the various paramagnetic bead types evaluated.

**Table 2. 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).

For Technical Support, please contact the **Scientific Support Team** at support@watchmakergenomics.com.

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