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DNA Fragmentation & Library Construction

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Protocol status: Working

We use this collection and it's working

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Keywords: DNA Fragmentation, library construction, size selection, library amplification, ligation, streamlined dna fragmentation, library construction the kapa hyperplus kit, dna fragmentation, sheared dna, kapa hyperplus kit, library construction protocol for the rapid preparation, kapa hyperprep kit from covari, kapa hyperprep kit, library construction protocol, libraries for illumina, dna,

Abstract

The KAPA HyperPlus Kit provides a versatile, streamlined DNA fragmentation and library construction protocol for the rapid preparation of libraries for Illumina sequencing. The one-tube chemistry and protocol improves the efficiency and consistency of library construction, and yields libraries of similar or better quality than those produced with the KAPA HyperPrep Kit from Covaris sheared DNA. It outperforms tagmentation-based workflows in terms of robustness, flexibility and sequence coverage and uniformity.

Attachments



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Guidelines

The workflow combines enzymatic steps and employs minimal bead-based cleanups, thereby reducing sample handling and overall library preparation time to 1.5 – 3 hrs. The kit contains all of the enzymes and reaction buffers required for:

1. enzymatic fragmentation to produce dsDNA fragments;
2. end repair and A-tailing to produce end-repaired, 5'-phosphorylated, 3'-dA-tailed dsDNA fragments;
3. adapter ligation, during which dsDNA adapters with 3'-dTTP overhangs are ligated to 3'-dA-tailed molecules; and
4. library amplification (optional), which employs highfidelity, low-bias PCR to amplify library fragments carrying appropriate adapter sequences on both ends.



Materials

MATERIALS

 KAPA mRNA HyperPrep Kit **Kapa Biosystems Catalog #KK8514**

Safety warnings

Safe Stopping Points

The library construction process, from enzymatic fragmentation to final library, can be performed in 1.5 to 3 hrs—depending on experience, the number of samples being processed, and whether or not library

amplification is performed. If necessary, the protocol may be paused safely after completion of the Post-ligation

Cleanup (step 4.17; the end of the protocol for PCR-free workflows). Purified, adapter-ligated library DNA may be stored at 2°C to 8°C for 1 – 2 weeks, or at -15°C to -25°C for ≤1 month before amplification, target capture and/or sequencing.

To avoid degradation, always store DNA in a buffered solution (10 mM Tris-HCl, pH 8.0 – 8.5) when possible,

and minimize the number of freeze-thaw cycles.

Notes:

- First-time users should refer to Appendix 2: Optimization of Fragmentation Parameters (p. 16) before trying this kit, as standard fragmentation parameters may not result in the optimal size distribution for libraries prepared from your specific DNA samples. Precious samples should not be used when evaluating this kit. Instead, parameters should be optimized with a non-precious, bulk DNA sample that is representative of the actual samples to be processed.
- If your DNA samples contain EDTA, please consult the Appendix 2: Handling of DNA Samples Containing EDTA (p. 16), as well as Important Parameters: Input DNA (p. 4) before starting this protocol.
- This protocol does not include size selection. Please refer to Appendix 1 (p. 15) for a detailed double-sided size selection protocol that may be included after ligation or after amplification.
- Always ensure that KAPA cleanup beads are fully equilibrated to room temperature and fully resuspended before use.



Before start

Shipping and Storage

The enzymes provided in this kit are temperature sensitive, and appropriate care should be taken during shipping and

storage. KAPA HyperPlus Kits are shipped on dry ice or ice packs, depending upon country of destination. Upon receipt, immediately store enzymes and reaction buffers at -15°C to -25°C in a constant-temperature freezer.

When

stored under these conditions and handled correctly, the kit components will retain full activity until the expiry date

indicated on the kit label.

Handling

Always ensure that KAPA HyperPlus Kit components have been fully thawed and thoroughly mixed before use.

The End Repair & A-Tailing Buffer and Ligation Buffer may contain precipitates when thawed at 2°C to 8°C. These buffers must be thawed at room temperature and vortexed thoroughly before use. KAPA HiFi HotStart ReadyMix (2X)

contains isostabilizers and may not freeze completely, even when stored at -15°C to -25°C. Nevertheless, always ensure that the ReadyMix is fully thawed and thoroughly mixed before use. Reaction master mixes prepared from the enzymes and buffers for fragmentation, end repair and A-tailing, as well as for ligation, are very viscous and require special attention during pipetting. Keep all enzyme components and master mixes on ice as long as possible

during handling and preparation.

Quality Control

All kit components are subjected to stringent functional quality control, are free of detectable contaminating exoand

endonuclease activities, and meet strict requirements with respect to DNA contamination.



Attachments



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Files

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Protocol



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Library Construction Protocol

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Appendix 1: Size Selection

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Appendix 2: Optimization of Fragmentation Parameters

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Optimization of Fragmentation Temperature

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