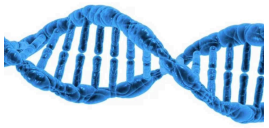
Jun 16, 2025

DNA Fragmentation & Library Construction

DOI
dx.doi.org/10.17504/protocols.io.bp2l6nr4zgqe/v1



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DOI: dx.doi.org/10.17504/protocols.io.bp2l6nr4zgqe/v1

Collection Citation: Marina McCowin 2025. DNA Fragmentation & Library Construction. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.bp2l6nr4zgqe/v1**

Protocol status: Working
We use this collection and it's working

Created: March 20, 2020

Last Modified: June 16, 2025

Collection Integer ID: 34600

Keywords: DNA Fragmentation, library construction, size selection, library amplification, ligation,

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

PDF

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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'-dTMP 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

SEARCH

Protocol

NAME

Library Construction Protocol

VERSION 1

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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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