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Appendix 2: Optimization of Fragmentation Parameters

In 1 collection

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

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Abstract

Fragmentation guidelines provided in the Library Construction Protocol: Enzymatic Fragmentation (step 1) may not result in the optimal library size distribution for your specific DNA samples. For this reason, precious samples should not be used when evaluating the KAPA HyperPlus Kit for the first time. Instead, fragmentation parameters should be optimized in the context of the KAPA HyperPlus workflow, using a non-precious, bulk DNA sample that is representative of the actual samples to be processed.

The information in this Appendix should be considered during the experimental design for your evaluation of the KAPA HyperPlus Kit.

Attachments

PDF

Marina-McCowin-Proto...

706KB

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.

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Note

Fragmentation guidelines provided in the Library Construction Protocol: Enzymatic Fragmentation (step 1) may not result in the optimal library size distribution for your specific DNA samples. For this reason, precious samples should not be used when evaluating the KAPA HyperPlus
Kit for the first time. Instead, fragmentation parameters should be optimized in the context of the KAPA HyperPlus workflow, using a non-precious, bulk DNA sample that is representative of the actual samples to be processed.

The information in this Appendix should be considered during the experimental design for your evaluation of the KAPA HyperPlus Kit.

2 **Quantification of Input DNA**

The Qubit fluorometer is recommended for the quantification of high-quality DNA, whereas the
KAPA hgDNA Quantification and QC Kit provides both concentration and quality information for FFPE DNA.

3 **Handling of DNA Samples Containing EDTA**

Note

If the DNA samples contain EDTA, perform a 3X beadbased cleanup with KAPA cleanup beads to remove EDTA prior to fragmentation. Please refer to the relevant Technical Data Sheet (KR1705 or KR1245) for a detailed DNA cleanup protocol.

4 **For example:**
• If your DNA samples are in TE buffer, and your input into library construction is 100 ng, dilute 100 ng of each sample into a final volume of 30 µL (i.e., to 3.33 ng/µL) using TE buffer.

• All samples will now contain the same final EDTA concentration once diluted to 50 µL for fragmentation.
This concentration is: EDTA concentration in TE buffer x (30 µL/50 µL) = 1 mM x (30 µL/50 µL) = [M] 0.6 millimolar (mM)

• Make a 2.2-fold dilution of the Conditioning Solution (as per Table 3 on p. 4), and follow the Library
Construction Protocol (second half of step 1).

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Note

If you are unsure about the presence or concentration of EDTA in your DNA samples, remove the EDTA by performing a column- or bead-based purification or buffer exchange prior to enzymatic fragmentation, or follow the procedure outlined below:

5.1 Set up a series of test reactions with the appropriate amount of input DNA, and different final concentrations
of Conditioning Solution.

5.2 Include at least one reaction with the same input of control DNA known to be EDTA-free. The control DNA
should preferably be of the same type and quality as the test samples.

5.3 Fragment the DNA using the appropriate parameters, as outlined in the Library Construction Protocol:
Enzymatic Fragmentation (step 1). Complete the library construction process, and compare library size
distributions for the test and control samples using an electrophoretic system (see Important Parameters:
Evaluating the Success of Library Construction, p. 8).

5.4 Titrate the final concentration of Conditioning Solution in the reaction until the test samples yield similar
fragmentation profiles as the EDTA-free control sample, or until the desired library size distribution has been
achieved.

5.5 A two-step strategy may be the best. Start with 3 – 4 test samples covering a broad range of final Conditioning
Solution concentrations, then perform a finer titration over a narrower concentration range.