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Optimization of Fragmentation Temperature

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

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Abstract

The standard fragmentation temperature is 37°C. If you are fragmenting high-quality genomic DNA, any other high-complexity DNA sample, or FFPE DNA to a mode fragment length <500 bp, it is unlikely that you will have to change or optimize the fragmentation temperature.

Low-complexity samples (e.g., small viral genomes, plasmids, long amplicons and cDNA) may, however, be over-fragmented at 37°C, even with short incubation times. The likelihood of over-fragmentation depends on the nature, molecular weight/length of the input DNA, the desired size distribution after fragmentation and, to a lesser
degree, the DNA input into fragmentation. For example, 100 ng of a 1.8 kb PCR product will yield a similar mode fragment length (~300 bp) as 100 ng E. coli or human genomic DNA when fragmented at 37°C for 10 min, whereas 1 ng of a 1 kb PCR product will be fragmented to a mode size <250 bp using the same parameters.

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.

1 To determine the optimal fragmentation parameters for low-complexity samples, or high-complexity samples
when the desired mode fragment length is >500 bp:

1.1 Set up four replicate reactions with a non-precious, bulk sample that is representative of the actual samples
to be processed.

1.2 Fragment two of the samples at 37 °C, for 00:05:00 and 00:10:00, respectively. Repeat these fragmentations with the other two samples, but at 30 °C.

1.3 Complete the library construction process, and evaluate the size distribution of the final libraries
electrophoretically.

Note

-- If the mode fragment length obtained with a 00:10:00 incubation at 37 °C is too long, continue optimizing (increasing) the fragmentation time at 37 °C.

-- If the mode fragment length obtained with a 00:10:00 incubation at 30 °C is too long, but 00:05:00 at 37 °C esulted in over-fragmentation, continue optimizing (increasing) the fragmentation time at 30 °C.

-- If a 00:05:00 incubation at 30 °C
resulted in overfragmentation, perform a second set of reactions (e.g., for 5 min, 10 min, 15 min, and 20 min) at 25 °C, and fine-tune the fragmentation time if needed.