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Appendix 1: Size Selection

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

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Abstract

Any commonly used size selection technique (e.g., the double-sided size selection described here, or an electrophoretic
method) may be integrated into the KAPA HyperPlus library construction workflow. Size selection should preferably be
carried out after the post-ligation cleanup, or after library amplification. Whether or not size selection is performed, which technique is used, and at what stage of the library construction process it is performed, depends on the nature of the sample, input into library construction, and the sequencing application and read length. For more information on size selection, refer to Important Parameters: Size Selection (p. 7).

Attachments

PDF

Marina-McCowin-Proto...

706KB

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.

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Note

The double-sided size selection protocol outlined in this appendix is designed for the selection of library molecules (inclusive of adapter) in the range of 250 – 450 bp. To obtain a population of shorter or longer molecules, the protocol may be modified as follows:

	A	B	C	D
	Upper size limit	**Modification**	**Lower size limit**	**Modification**
	Increase	Decrease the ratio of the first cut	Increase	Decrease the ratio of the second cut*
	Decrease	Increase the ratio of the first cut	Decrease	Increase the ratio of the second cut*

* The second size cut should be performed with at least 0.2 volumes of KAPA cleanup beads reagent. Please note that the volume of KAPA cleanup beads needed for the second cut is calculated relative to the volume of the DNA at the start of the size selection procedure, not the volume of the DNA-containing supernatant transferred after the first cut. DNA recovery is dramatically reduced if the difference between first and second cuts is less than ~0.2 volumes. To increase the amount of DNA recovered, >0.2 volumes of KAPA cleanup beads may be used for the second cut, but note that this may result in the recovery of smaller library fragments and/or a broader size distribution. For more information on double-sided size selection, please refer to the KAPA NGS Library Preparation Technical Guide (available on request from Technical Support at sequencing.roche.com/support).

2 Perform the first (0.7X) size cut (to exclude library molecules larger than ~450 bp) by combining the
following:

	A	B
	Component	**Volume**
	DNA to be size selected	50 µL
	KAPA cleanup beads	35 µL
	Total volume per well/tube:	85 µL

3 Mix thoroughly by vortexing and/or pipetting up and down multiple times.

4 Incubate the plate/tube(s) at room temperature for 00:05:00 – 00:15:00 in to bind library molecules larger than ~450 bp to the beads.

5 Place the plate/tube(s) on a magnet to capture the beads. Incubate until the liquid is clear.

6 Carefully transfer ~ 80 µL of supernatant containing library molecules smaller than ~450 bp to a new plate/tube. It is critical that no beads are transferred with the supernatant. Discard the plate/tube(s) with the beads to which library molecules larger than ~450 bp were bound.

7 Perform the second size cut (0.9X), to retain library molecules >250 bp) by combining the following:

	A	B
	Component	**Volume**
	Supernatant from first size cut	80 µL
	KAPA cleanup beads	10 µL
	Total volume per well/tube:	90 µL

8 Mix thoroughly by vortexing and/or pipetting up and down multiple times.

9 Incubate the plate/tube(s) at room temperature for 00:05:00 – 00:15:00 n to bind library molecules larger than ~250 bp to the beads.

10 Place the plate/tube(s) on a magnet to capture the beads. Incubate until the liquid is clear.

11 Carefully remove and discard the supernatant, which contains library molecules smaller than ~250 bp.

12 Keeping the plate/tube(s) on the magnet, add 200 µL of 80% ethanol.

13 Incubate the plate/tube(s) on the magnet at room temperature for ≥ 00:00:30 .

14 Carefully remove and discard the ethanol.

15 Keeping the plate/tube(s) on the magnet, add 200 µL of 80% ethanol.

16 Incubate the plate/tube(s) on the magnet at room temperature for ≥ 00:00:30 .

17 Carefully remove and discard the ethanol. Try to remove all residual ethanol without disturbing the beads.

18 Dry the beads for 00:03:00 – 00:05:00 at Room temperature , or until all of the ethanol has evaporated. Caution: over-drying the beads may result in reduced yield.

19 Remove the plate/tube(s) from the magnet.

20 Thoroughly resuspend the beads in the required volume of elution buffer ([M] 10 millimolar (mM) Tris-HCl, pH 8.0 – 8.5).

21 Incubate the plate/tube(s) at room temperature for 00:02:00 to elute DNA off the beads.

22 Place the plate/tube(s) on a magnet to capture the beads. Incubate until the liquid is clear.

23 Transfer the clear supernatant with size-selected DNA to a new plate/tube(s) and proceed with the next step in your workflow, or store DNA at 2 °C to 8 °C for 1 – 2 weeks, or at -15 °C to -25 °C .