

KAPA HYPERPLUS

Guide to Success

KAPA HyperPlus: single-tube fragmentation and library preparation workflow in less than 3 hours
 Contact Technical Support at sequencing.roche.com/support



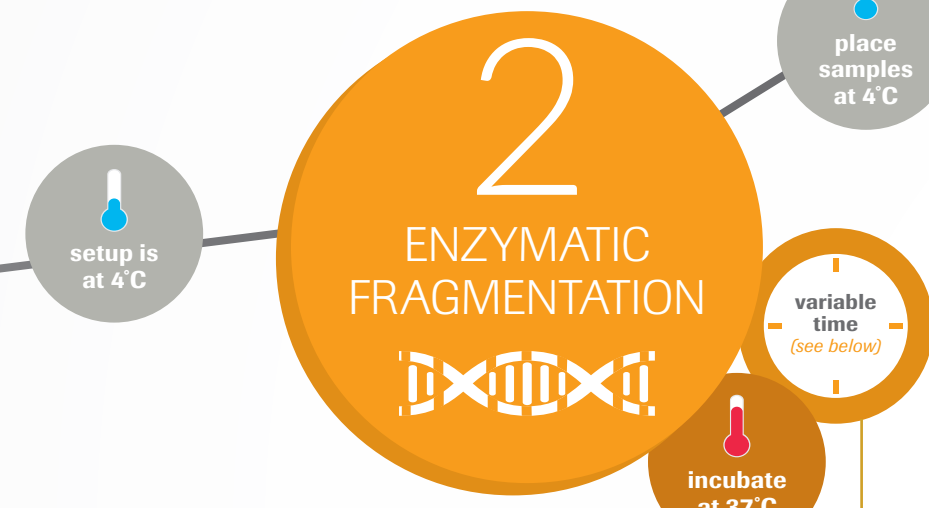
Got EDTA?

- The enzymatic fragmentation reaction is sensitive to EDTA.
- The best strategy is to remove EDTA by means of a cleanup step before fragmentation.
- If your DNA contains EDTA, please see **Appendix 2** (p. 16) of the Technical Data Sheet (TDS).

How much DNA do I need?

Application	Sample type	Recommended input
WGS	Complex gDNA (high quality)	50 ng – 1 µg
Target capture (WES, custom panels)	Complex gDNA (high quality)	10 ng – 1 µg
WGS, target capture	FFPE DNA	≥50 ng (quality dependent)
WGS	Microbial DNA	1 ng – 1 µg
WGS (PCR-free)	High-quality DNA	≥50 ng (no SS)* ≥500 ng (w/SS)*
Targeted sequencing	Long amplicons	≥1 ng

*SS = size selection



- Get to chopping.**
- Mode and size distribution of DNA is controlled by fragmentation time and temperature.
 - Try a range of fragmentation times to determine optimal insert size.
 - For ease of sample processing, place samples with the longest fragmentation time in the thermal cycler first. Add samples with shorter fragmentation times at appropriate intervals.

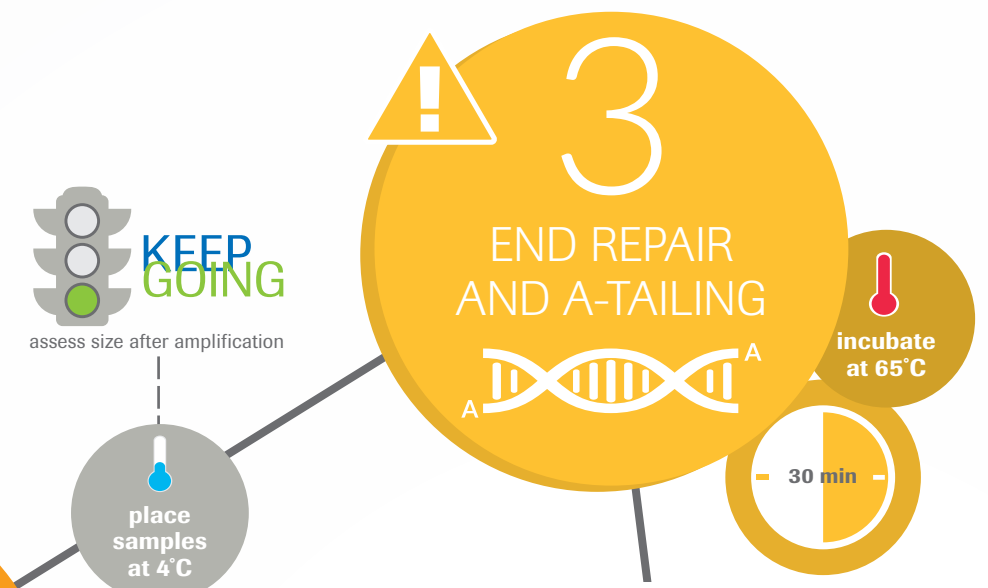
Mode	fragment length	Incubation time at 37°C*	Optimization range
	600 bp	5 min	3 – 10 min
	350 bp	10 min	5 – 20 min
	200 bp	20 min	10 – 25 min
	150 bp	30 min	20 – 40 min

*These parameters are a good starting point for high-quality genomic DNA. Please refer to Appendix 2: Optimization of Fragmentation Parameters of the TDS for guidelines on how to optimize fragmentation time and temperature, if needed.

It's not a typo!

- Ensure that you are adding the correct volume of KAPA Frag Buffer (5 µL) and KAPA Frag Enzyme (10 µL) to each reaction.

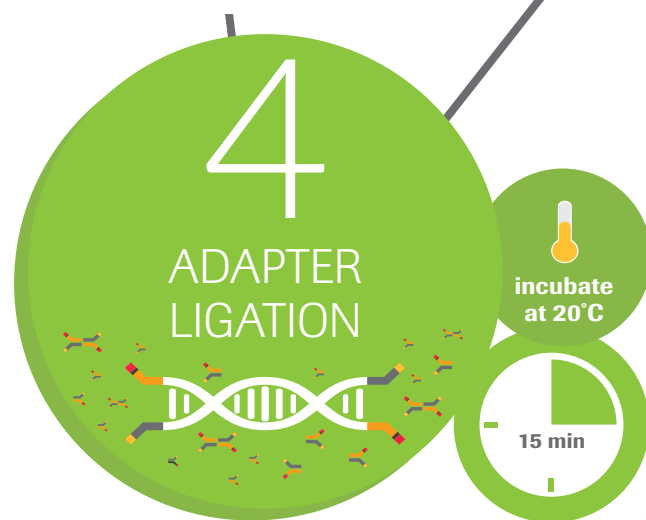
Component	Volume
Double-stranded DNA (with KAPA Frag Conditioning Solution, if needed)	35 µL
KAPA Frag Buffer (10X)	5 µL
KAPA Frag Enzyme	10 µL
Total volume	50 µL



- Which End Repair and A-Tailing Enzyme Mix to use?**
- The formulation of the End Repair and A-Tailing (ERAT) Enzyme Mix has been enhanced to improve performance for more sensitive assays.
 - The user must choose which ERAT Enzyme Mix to use:

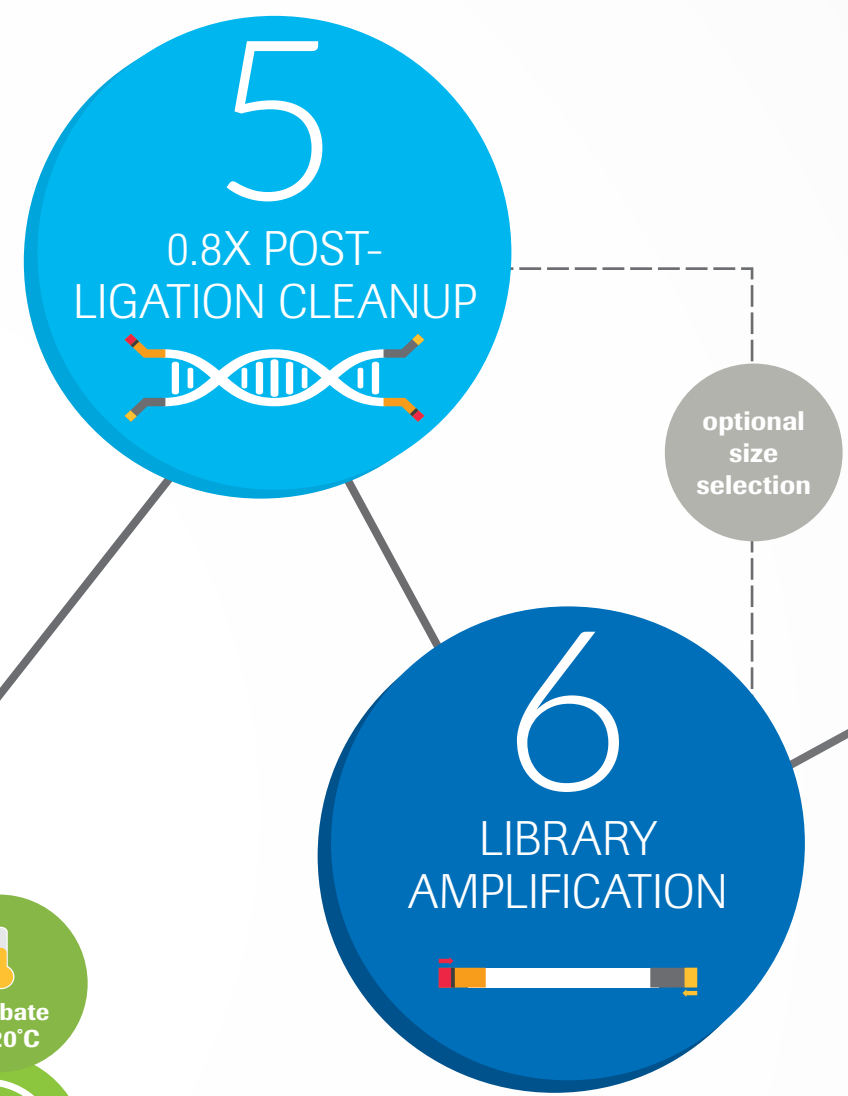
Enzyme name	Cap color	When to use
HyperPrep ERAT Enzyme Mix	Purple	Existing, validated workflows
HyperPlus ERAT Enzyme Mix	Orange	New workflows; more sensitive applications

ERAT = End Repair and A-Tailing



- How much adapter do I need?**
- Adapter concentration affects ligation efficiency, as well as adapter and adapter-dimer carry-over during the post-ligation cleanup.

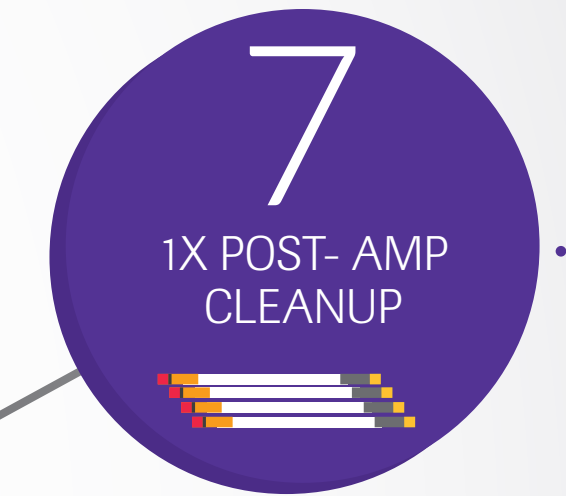
Input DNA	Adapter stock concentration	Adapter:insert molar ratio
1 µg	15 µM	10:1
500 ng	15 µM	20:1
250 ng	15 µM	40:1
100 ng	15 µM	100:1
50 ng	15 µM	200:1
25 ng	7.5 µM	200:1
10 ng	3 µM	200:1
5 ng	1.5 µM	200:1
2.5 ng	750 nM	200:1
1 ng	300 nM	200:1



Don't do more work than you have to.

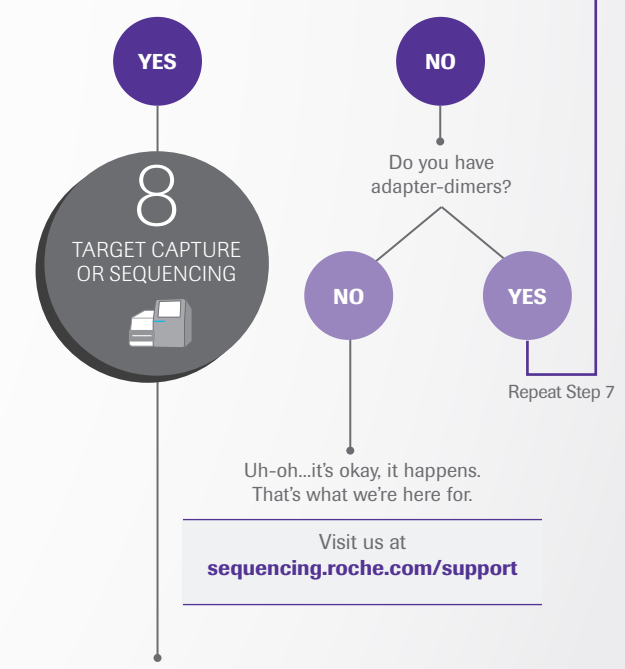
- Determine how much final library material is required for your downstream application (e.g., capture, sequencing).

Input into library construction	Number of cycles required to generate	
	100 ng library	1 µg library
1 µg	0	0 – 1
500 ng	0	2 – 3
250 ng	0 – 1	3 – 5
100 ng	0 – 2	5 – 6
50 ng	3 – 5	7 – 8
25 ng	5 – 6	8 – 10
10 ng	7 – 9	11 – 13
5 ng	9 – 11	13 – 14
2.5 ng	11 – 13	14 – 16
1 ng	13 – 15	17 – 19



So did you make a good library?

This is where you decide if you made a good library by assessing both the size and concentration with an appropriate quantification method.



Great! Now you want more HyperPlus, don't you? Contact your local sales representative to order more.

Data on file. For Research Use Only. Not for use in diagnostic procedures.

