KAPA HYPERPLUS
Guide to Success

KAPA HyperPlus: single-tube fragmentation and library preparation workflow in less than 3 hours

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KEEP GOING
assess size after amplification

1 INPUT DNA

setup is at 4˚C

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

2 ENZYMATIC FRAGMENTATION

place samples at 4˚C

variable time *(see below)*

incubate at 37˚C

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**

3 END REPAIR AND A-TAILING

incubate at 65˚C

30 min

4 ADAPTER LIGATION

incubate at 20˚C

15 min

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

5 0.8X POST-LIGATION CLEANUP

optional size selection

6 LIBRARY AMPLIFICATION

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

7 1X POST- AMP CLEANUP

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.

YES

Do you have adapter-dimers?

NO

YES

Repeat Step 7

Uh-oh...it's okay, it happens. That's what we're here for.

Visit us at
sequencing.roche.com/support

8 TARGET CAPTURE OR SEQUENCING

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

KAPABIOSYSTEMS

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

input DNA | 4˚C | enzymatic fragmentation | 4˚C | end repair & A-tailing | adapter ligation | 0.8X post-ligation cleanup | optional size selection | library amplification | 1X post-amp cleanup | target capture or sequencing