KAPA EvoPlus Kits Guide to Success

Streamlined sample prep workflow using the next generation of KAPA DNA Library Prep Reagents.

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 Amount	Number of amplification cycles for WGS to achieve 4 nM* (if using full length adapters)
500 ng	0 cycles PCR-free
100 ng	
75 ng	
50 ng	1 – 2 cycles
10 ng	5 - 7 cycles

* The number of cycles needed depends on the specific adapter and amplification primer design, as well as input type and quality.

1 INPUT DNA

Setup is at 4°C

2 ENZYMATIC FRAGMENTATION & A-TAILING

Variable time (see below)

Incubate at 37°C

30 min

Followed by 55°C

Keep going (assess size after ligation or amplification)

3 ADAPTER LIGATION

Incubate at 20°C

15 min

4 0.8X POST-LIGATION BEAD CLEANUP

5 LIBRARY AMPLIFICATION

Targeted Sequencing { Optional double-sided selection }

Whole Genome Sequencing (PCR-free)

6 1X POST-AMP BEAD CLEANUP

Move on to target capture.

Uh-oh… It's okay, it happens. That's what we're here for.
Visit us at sequencing.roche.com/support

NO

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

Let's sequence our library.

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

How much DNA do I need?

Application	Sample Type	Input
WGS	High quality gDNA	10 – 500 ng
WGS (PCR-free)	High quality gDNA	≥ 75 ng (no SS)* 500 ng (w/SS)*
WES**	High quality gDNA	100 ng
	Low quality FFPET derived DNA	> 100 ng

* SS = double-sided size selection; a requirement when performing WGS on patterned flow cells but may result in sample losses of 60 - 95%, irrespective of whether a bead- or gel-based technique is used.
** For this application, please refer to the KAPA HyperCap Workflow v3.x for guidelines.

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.

Estimated fragment length*	Fragmentation time at 37°C
550 – 640 bp	5 min
330 – 410 bp	10 min
240 – 320 bp	15 min
200 – 260 bp	20 min
170 – 230 bp	25 min
140 – 210 bp	30 min

* Sizes observed upon fragmentation of NA12878 (Coriell DNA). Size variation may be observed, depending on DNA type, DNA input and DNA elution buffers. We recommend optimizing the fragmentation time with a non-precious sample.

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*
500 ng	15 µM	20:1
100 ng	15 µM	100:1
75 ng	15 µM	135:1
50 ng	15 µM	200:1
10 ng	6 µM	400:1

* Adapter: insert molar ratio calculations are based on a mode DNA fragment length of 200 bp, and will be higher for longer DNA fragments, or slightly lower for DNA fragmented to a mode size <200 bp. The lower adapter: insert molar ratios recommended for inputs >100 ng represent a fair compromise between library construction efficiency and cost; higher library yields will be achieved if a higher adapter concentration is used.

Evolving together

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