KAPA HyperCap Workflow v3.4 Guide to Success

Using KAPA HyperPrep, KAPA HyperPlus or KAPA EvoPlus Kits

Sample prep workflow using superior probe technology with optimized Library Preparation and Target Enrichment.

Covaris Shearing
Fragment the gDNA sample to 180 – 220 bp average length.

Incubation Conditions
Remember the additional first incubation step when using KAPA HyperPrep Kits.
30 min at 20°C first followed by 30 mins at 65°C.

15 min

Incubate at 20°C

10 µL (for KAPA HyperPrep or KAPA HyperPlus) 5 µL (for KAPA EvoPlus)

How many cycles of amplification in total?

KAPA HyperPrep Kit	8 cycles
KAPA HyperPlus Kit	6 cycles
KAPA EvoPlus Kit	7 cycles

So, did we make a good library?
Determine the concentration (using Qubit), the size distribution and quality (using the Agilent Bioanalyzer) of the amplified library. Is the library good enough to proceed to target capture?

NO

YES

We can help...
That's what we're here for

Visit us at **sequencing.roche.com/support**

HyperDesign Tool
Intuitive online custom design tool with Roche's renowned probe-design algorithm in a simple, easy-to-use interactive web interface.
www.hyperdesign.com

100 ng gDNA

1 INPUT DNA

2A MECHANICAL FRAGMENTATION (for KAPA HyperPrep)

2B ENZYMATIC FRAGMENTATION (for KAPA HyperPlus)

Set up at 4°C; incubate at 37°C

3A END REPAIR & A-TAILING (for KAPA HyperPrep)

3B END REPAIR & A-TAILING (for KAPA HyperPlus)

2C+3C ENZYMATIC FRAGMENTATION & A-TAILING (for KAPA EvoPlus)

100 ng gDNA

4 (A or B) ADAPTER LIGATION

4 ADAPTER LIGATION

5 0.8X POST-LIGATION CLEANUP

6 PRE-CAPTURE LIBRARY AMPLIFICATION

7 1.4X POST-AMP CLEANUP

Optional pre-capture multiplexing

8 TARGET CAPTURE HYBRIDIZATION-BASED

1 µg (single-plex) or 1.5 µg (multiplex) input

95°C for 5min, 55°C for 16 – 20 hr

How much Hybridization Component H to add?
Hybridize your KAPA HyperCap Probes to your single/multiplex DNA sample library, where the amount of Hybridization Component H depends on the capture target size.

≤40 Mbp Capture Target Size	12 µL
≥40 Mbp Capture Target Size	9 µL

55°C for 5min

9 WASH & RECOVERY

10 POST-CAPTURE LIBRARY AMPLIFICATION

11 1.4X POST-AMP CLEANUP

Get to chopping
Fragmentation of high quality gDNA has been optimized to 180 – 220 bp average fragment length using 22 min at 37°C. Look for further optimization instructions in the KAPA HyperPlus Kit TDS.

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

Got EDTA?
- If using the KAPA HyperPlus Kits (proceed to step 2B), the enzymatic fragmentation reaction is sensitive to EDTA.
- If your DNA contains EDTA, please refer to the KAPA HyperPlus Kit Technical Data Sheet (TDS) on how to easily remove it with KAPA HyperPure Beads, or mitigate it (in case you know its concentration) by using the Conditioning Solution provided in the kit.
- If using the KAPA EvoPlus Kits, no need to worry – the enzymatic fragmentation reaction **is insensitive to EDTA (up to 2 mM)**, as well as numerous other inhibitors.

The KAPA FragTail ReadyMix is viscous and requires special attention during pipetting.

Fragmentation at 37°C for 18 minutes followed by A-tailing at 55°C for 30 minutes.

Which End Repair and A-Tailing Enzyme Mix to use?
The KAPA HyperCap Workflow v3.4 has been validated with the updated HyperPlus ERAT Enzyme Mix (orange cap) for improved performance.

Do not use the HyperPlus ERAT Enzyme Mix (orange cap) if you are using Covaris sonicated or mechanically sheared gDNA.

The KAPA Universal Adapter must be added prior to addition of the Ligation Master Mix to limit the formation of adapter dimers.

Great!
Need more KAPA Sample Prep Reagents?
Contact your local sales representative to order more.

Prepare your wash buffers to create working solutions before you start the day.

Prepare your Capture Beads based on the capture target size.

Capture Target Size

<40 Mbp	50 µL per rxn
≥40 Mbp	100 µL per rxn

How many total cycles of amplification are required?

Capture target*	Total cycles
<100 kb	18
100 kb – 2 Mb	16
>2 Mb – 40 Mb	10
>40 Mb	8

* Check the "Coverage summary" file (HyperDesign) for the "Total capture space" value.

Get the latest Operators Manuals (Method Sheets, Users Guides and Instructions for Use) at navifyportal.roche.com

INPUT DNA · FRAGMENTATION · END REPAIR & A-TAILING · ADAPTER LIGATION · 0.8X POST-LIGATION CLEANUP · PRE-CAPTURE LIBRARY AMPLIFICATION · 1.4X POST-AMP CLEANUP · OPTIONAL PRE-CAPTURE MULTIPLEXING · TARGET CAPTURE · WASH & RECOVERY · POST-CAPTURE LIBRARY AMPLIFICATION · 1.4X POST-AMP CLEANUP · SEQUENCING

Contact KAPA