Blueprint: Kapa Hyper Prep Kit

Library Construction Protocol

1. End Repair and A-Tailing

Assemble each End Repair & A-Tailing reaction as follows in a PCR tube:

Component	Volume
Fragmented, double-stranded DNA	50 µl
End Repair & A-Tailing Buffer	7 µl
End Repair & A-Tailing Enzyme Mix	3 µl
Total volume	**60 µl**

Mix thoroughly and centrifuge briefly.

Incubate in a thermocycler with the following thermal profile:

Step	Temp	Time
End Repair & A-Tailing	20 °C	30 min
	65°C	30 min
HOLD	12°C	∞

Proceed immediately to the next step.

2. Adapter Ligation

2.1 Dilute adapter stocks to the appropriate concentration, as outlined in the table below.

Input DNA	Adapter stock concentration	Final adapter concentration
2-5 ng	600 nM*	28 nM
Less than 2 ng	300 nM	14 nM

*Original stock NextFlex adapters is 600 nM

2.2 Assemble each Adapter Ligation reaction in a 1,5 ml tube as follows:

Component	Volume
End Repair & A-Tailing reaction product	60 µl
PCR-grade water (NF H_2O)	5 µl
Ligation Buffer	30 µl
DNA Ligase	10 µl
Adapter stock	5 µl
Total volume	**110 µl**

2.3 Mix thoroughly and centrifuge briefly.
2.4 Incubate at 20°C for 15 min in thermo-shaker.
2.5 Proceed immediately to the next step.

3. Post-ligation Cleanup

3.1 Perform a 0.8X SPRI cleanup by combining the following:

Component	Volume
Adapter Ligation reaction product	110 µl
Agencourt AMPure XP reagent	88 µl
Total volume	**198 µl**

3.2 Thoroughly resuspend the beads by pipetting up and down multiple times.
3.3 Incubate the tube at room temperature for 5-15 min to bind DNA to the beads.
3.4 Place the tube on a magnet to capture the beads. Incubate until the liquid is clear.
3.5 Carefully remove and discard the supernatant.
3.6 Keeping the tube on the magnet, add 200 µl of fresh 80% ethanol.
3.7 Incubate the tube at room temperature for ≥30 sec.
3.8 Carefully remove and discard the ethanol.
3.9 Keeping the tube on the magnet, add 200 µl of fresh 80% ethanol.
3.10 Incubate the tube at room temperature for ≥30 sec.
3.11 Carefully remove and discard the ethanol. Try to remove all residual ethanol without disturbing the beads.
3.12 Dry the beads at room temperature, until all of the ethanol has evaporated. **Caution: over-drying the beads may result in dramatic yield loss.**
3.13 Remove the tube from the magnet.
3.14 Resuspend the beads in 22,5 µl of elution buffer (10 mM Tris-HCl, pH 8.0).
3.15 Incubate the tube at room temperature for 2 min to elute DNA off the beads.
3.16 Place the tube on a magnet to capture the beads. Incubate until the liquid is clear.
3.17 Transfer 20 µl of the clear supernatant to a new tube.

4. Library Amplification

4.1 Assemble each library amplification reaction as follows:

Component	Volume
2X KAPA HiFi Hotstart ReadyMix	25 µl
10X Library Amplification Primer Mix	5 µl
Adapter-ligated library	20 µl
Total volume	**50 µl**

4.2 Mix thoroughly and centrifuge briefly.
4.3 Amplify using the following cycling protocol:

Step	Temp	Duration	Cycles
Initial denaturation	98°C	45 sec	1
Denaturation	98°C	15 sec	10
Annealing	60°C	30 sec	
Extension	72°C	30 sec	
Final extension	72°C	1 min	1
HOLD	12°C	∞	1

4.4 Store the tube at 4°C or -20°C for up to 72 hours, or proceed directly to Step 5: Post-amplification Cleanup.

5. Post-amplification Cleanup

5.1 Perform a QIAquick MinElute PCR Purifaction:

- Add 5 volumes PB buffer to the library amplification reaction and mix.
- Load mix onto a MinElute column.
- Centrifuge 1 minute at 13.000 rpm, remove flow through.
- Wash with 700 µl PE buffer.
- Centrifuge 1 minute at 13.000 rpm, remove flow through.
- Centrifuge again 1 minute at 13.000 rpm. Put the column in a new tube.
- Add 10 µl EB to elute.
- Incubate the tube 1 minute at 37°C.
- Centrifuge 1 minute at 13.000 rpm.
- Transfer sample to a new tube and add again 10 µl EB to the column.
- Incubate the tube 1 minute at 37°C.
- Centrifuge 1 minute at 13.000 rpm.
- Combine the two elutes, the final volume is 20 µl.

Recommended is to perform a Qubit measurement at this step, to know the amount of DNA before size selection. Do not load more than 6000 ng DNA per lane on an E-gel.

6. Size selection with E-gel

6.1 Remove the gel from the package and gently remove the combs from the SizeSelect cassette.

6.2 Insert the gel into the E-Gel iBase Power System right edge first. Press firmly at the left edge of the cassette to seat the gel in the base. A steady light illuminates on the iBase when the cassette is correctly inserted.

6.3 Dilute the 100 bp marker 1:10 with **EB buffer**. The final concentration is 50 ng/µl.

6.4 Load gel as follows:

- 20 µl sample into each well of top row.
- 5 µl appropriately diluted DNA ladder into middle well (lane M).
- 20 µl **EB buffer** into all empty wells (top row).
- 25 µl **EB buffer** into all large bottom wells (collection wells) and 10 µl **EB buffer** for lane M of the bottom row.

6.5 Place the amber filter over the E-Gel iBase.

6.6 Select Run SizeSelect 2% (program 8) and set time to 14 minutes and 30 seconds for a 300 bp band, please check if the 300 bp band reaches the collection well.

6.7 Press the Go button on the iBase. The red light turns to a green light indicating the start of the run.

6.8 The end of the run is signaled with a flashing red light and rapid beeping.

6.9 Collect all DNA (10-15 µl) from the wells using a pipette without piercing the bottom of the well. Recommendation: Collect 2 to 4 times to increase the yield, by adding 7,5 ul NF water to the collection well and run for another 10 seconds.

6.11 Combine all collections and measure the concentration of the sample.

Afterwards a qPCR with specific primers have to be performed as a quality control. And the samples has to be checked on the BioAnalyzer.

Questions and/or remarks:

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