firefly® technical note

KAPA EvoPrep Automated Library Preparation

This technical note provides supporting information for the firefly protocols listed below. These protocols are based on the KAPA EvoPrep Kit Operators Manual v1.2 and are available to download from the firefly community. Here, we outline protocol run times, parts required and provide details on the steps performed in each protocol.

firefly protocols

Protocol number	Protocol name	firefly run time (minutes)	Thermocycler run time (minutes)	KAPA EvoPrep PCR-Free workflow	KAPA EvoPrep workflow
Protocol 1 of 4	3.1 End Repair and A-Tailing	5	60	✓	✓
Protocol 2 of 4	3.2 Adapter Ligation	5	5	✓	✓
Protocol 3 of 4	3.3 Post-Ligation Purification	32-38†	n/a	✓	✓
Protocol 4 of 4	4.1 - 4.3a Library Amp and Purification	34-40†	5-25		✓
Optional protocol*	Double-sided Size Selection	46-52†	n/a		

Table 1. Overview of firefly KAPA EvoPrep protocols

**Run the optional Double-sided Size Selection protocol after protocol 3 or after protocol 4.*
†firefly run times vary with the number of columns processed.
There is a safe stopping point after protocol 3.

Overview

The KAPA EvoPrep Kits are the latest high-performance, streamlined, and automation-friendly library preparation solutions from Roche.

SPT Labtech firefly protocols have been developed to run the KAPA EvoPrep and KAPA EvoPrep PCR-Free library preparation workflows - using the 96-well plate format of the KAPA EvoPrep Kit and the KAPA Unique Dual-Indexed Adapter Kit.

Protocol highlights

- Sheared input gDNA samples ranging from 0.1ng - 500ng can be processed into Illumina-compatible sequencing libraries with full-length, uniquely indexed adapters.
- Automated KAPA EvoPrep, KAPA EvoPrep PCR-Free and size selection workflows can be run with the flexibility to start from any step in these workflows.
- Protocol variables provide the flexibility to process 1 to 12 sample columns per run and to specify the starting column for the reagent plates (ReadyMix plates and UDI Adapters plates) - enabling multiple low-throughput runs using the same reagent plates and reducing waste.
- KAPA EvoPrep libraries produced on firefly are comparable in yield and fragment size to manually prepared libraries and are uniform in concentration and fragment size across a 96-well plate, with no detectable well-to-well contamination.

Protocol overview

firefly protocols are based on the steps described in the KAPA EvoPrep Kit Operators Manual v1.2 (Nov 2024) and firefly protocol names reflect the section numbering used in the manual.

Protocol 1 of 4

3.1 End Repair and A-Tailing

This protocol adds End Repair and A-Tailing ReadyMix to the input Fragmented DNA plate and tip mixes these reagents. The input Fragmented DNA plate should then be moved to a thermocycler to run the End Repair and A-tailing program.

Deck Layout - Basement: Thermal block and Fragmented DNA plate. Lower Deck: (L1) Tip Set 1 - 100µL pipetting head tips. Upper Deck: (U2) End Repair and A-Tailing ReadyMix plate.

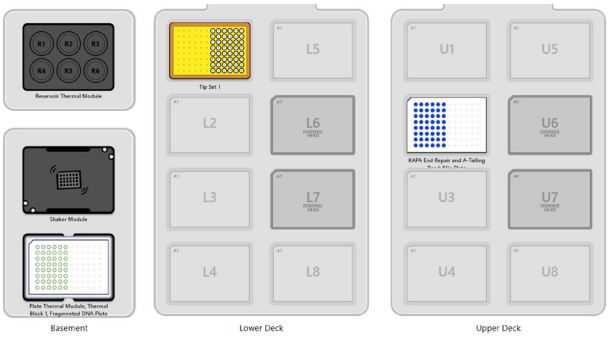

Figure 1. Starting deck layout for firefly KAPA EvoPrep Protocol 1 of 4: EvoPrep 3.1 End Repair and A-Tailing. Example shown is for 6 columns of samples.

Protocol 2 of 4

3.2 Adapter Ligation

This protocol follows on from the End Repair and A-tailing protocol. The input Fragmented DNA plate contains the End Repair and A-tailing reaction product. This protocol adds KAPA UDI Adapters and Ligation ReadyMix to the End Repair and A-tailing reaction product and tip mixes these reagents. The input Fragmented DNA plate should then be incubated on a thermocycler at 20°C for 5 minutes.

Deck Layout - Basement: Thermal block and Fragmented DNA plate. Lower Deck: (L5) Tip Set 2 - 100µL pipetting head tips. Upper Deck: (U3) Ligation ReadyMix plate, (U4) KAPA UDI Adapter plate.

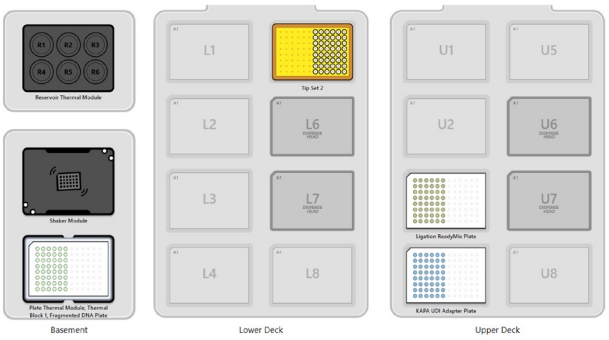

Figure 2. Starting deck layout for firefly KAPA EvoPrep Protocol 2 of 4: EvoPrep 3.2 Adapter Ligation. Example shown is for 6 columns of samples.

Protocol 3 of 4

3.3 Post-Ligation Purification

This protocol follows on from the Adapter Ligation protocol. The input Fragmented DNA plate contains the ligation reaction product. This protocol performs a 0.93X bead purification and then transfers the cleaned-up adapter ligated libraries to a fresh plate.

Deck Layout - Basement: Reservoirs R1 – Tris; R2, R3, R5, R6 – 80% Ethanol; R4 – KAPA HyperPure Beads. Lower Deck: (L2, L3, L4, L6, L7, L8) Tip Sets 3-8 - 100µL pipetting head tips. Upper Deck: (U1) Waste plate, (U3) Clean plate, (U6) Input Fragmented DNA plate, (U8) Alpaqua Magnum FLX (x96) Magnetic Block.

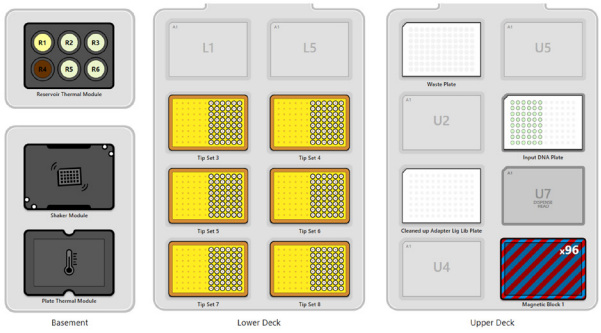

Figure 3. Starting deck layout for firefly KAPA EvoPrep Protocol 3 of 4: EvoPrep 3.3 Post Ligation Purification. Example shown is for 6 columns of samples.

Protocol 4 of 4

4.1 - 4.3a Library Amp and Purification

This protocol follows on from the Post Ligation Purification protocol. The Cleaned-up Adapter Ligated Library plate contains Adapter-ligated library. This protocol adds KAPA Library Amplification Primer Mix (10X) and KAPA HiFi HotStart ReadyMix (2X) to the Adapter-ligated libraries and tip mixes. The plate should then be moved to a thermocycler and the relevant KAPA Library Amplification program run. Once the thermocycler program is complete the plate should be returned to the firefly deck. The protocol then performs a 1.0X bead purification and transfers the cleaned up amplified libraries to a fresh plate.

Deck Layout - Basement: Reservoirs R1 – Tris; R2, R3, R5, R6 – 80% Ethanol; R4 – KAPA HyperPure Beads. Lower Deck: (L1-L7) Tip Sets 1-7 - 100µL pipetting head tips. Upper Deck: (U1) Waste plate, (U2) KAPA HiFi HotStart ReadyMix (2X) plate, (U3) KAPA Library Amp Primer Mix plate, (U4) Clean plate, (U6) Cleaned up Adapter Ligation Library plate, (U8) Alpaqua Magnum FLX (x96) Magnetic Block.

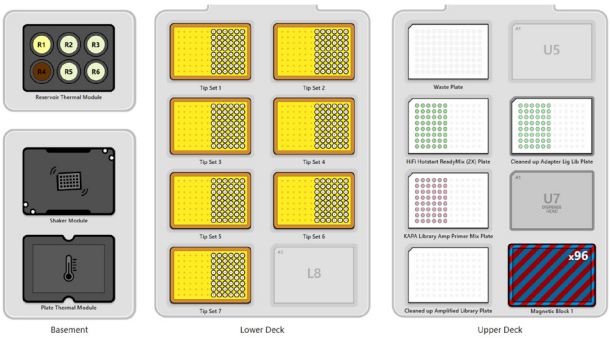

Figure 4. Starting deck layout for firefly KAPA EvoPrep Protocol 4 of 4: EvoPrep 4.1-4.3a Library Amp and Purification. Example shown is for 6 columns of samples.

Optional protocol

Double-sided Size Selection

Appendix A of the KAPA EvoPrep Operators Manual. This optional Size Selection protocol can be run after either the post-ligation sample purification (after KAPA EvoPrep protocol 3) or after the post-amplification sample purification (after KAPA EvoPrep protocol 4). Input volume is 50µL and output volume is 20µL. This protocol performs a double-sided size selection purification where the user selects the SPRI 1 and SPRI 2 ratios. The size selected libraries are then transferred to a fresh output plate.

Deck Layout - Basement: Reservoirs R1 – Tris; R2, R3, R5, R6 – 80% Ethanol; R4 – KAPA HyperPure Beads. Lower Deck: (L1-L7) Tip Sets 1-7 - 100µL pipetting head tips. Upper Deck: (U1) Waste plate, (U2) Intermediate plate, (U3) Output plate, (U6) Input plate, (U8) Alpaqua Magnum FLX (x96) Magnetic Block.

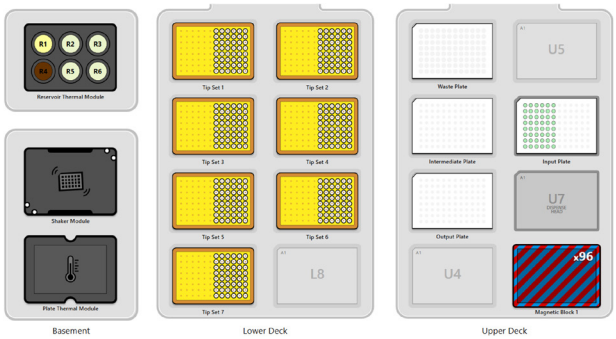

Figure 5. Starting deck layout for firefly KAPA EvoPrep Optional Protocol: EvoPrep Double-sided Size Selection. Example shown is for 6 columns of samples.

Variables

- Input variables allow between 1 to 12 columns of samples to be processed per run and the starting column for the KAPA EvoPrep reagent plates to be set.
- Final elution volume can be adjusted following both post ligation purification and post amplification purification protocols to allow for the appropriate 50µL input to the optional double-sided size selection protocol or if an elution volume other than the standard 20µL is required.
- The SPRI 1 and SPRI 2 ratios for the double-sided size selection can be set by the user.

Reagent volumes

Reagent volumes for 80% ethanol, KAPA HyperPure Beads and 10mM Tris-HCl (pH 8.0) are dependent on user input variables. The required volume of these reagents is shown in the Execute section of the firefly software.

Protocol	KAPA HyperPure Beads volume (µL)	80% Ethanol volume (µL)	10mM Tris-Hcl (pH 8.0) volume (µL)
3.3 Post Ligation Purification (20µL elution)	6,960	30,360	2,640
4.1 - 4.3a Library Amp and Purification (20µL elution)	5,040	30,360	2,640
Double-sided Size Selection (0.5X-0.7X)	3,700	30,360	2,640

Table 2. Example of user supplied reagent volumes required for a 96 sample KAPA EvoPrep run on firefly

Consumables

Protocol name	100µL filtered strip tip sets required*	Standard dispense head syringes required	Additional plates required
3.1 End Repair and A-Tailing	1	0	1 x Fragmented DNA input plate
3.2 Adapter Ligation	1	0	n/a
3.3 Post Ligation Purification	6	6	1 x Elution plate 1 x Waste plate
4.1 - 4.3a Library Amp and Purification	7	6	1 x Elution plate 1 x Waste plate
Double-sided Size Selection	7	6	1 x Elution plate 1 x Intermediate plate 1 x Waste plate

Table 3. Consumables required to run firefly KAPA EvoPrep protocols. Excludes parts provided in the kit.

**Where (number of strip tip sets) x (number of sample columns processed) = number of strip tips needed.*
e.g. to process 5 columns of samples through protocol 3 of 4 (3.3 Post-ligation purification): (6 strip tip sets) x (5 columns) = 30 strip tips are required.

Parts required for a 96 sample KAPA EvoPrep run on firefly

Supplier	Part	Part number	Number required
Roche	KAPA EvoPrep Kit + KAPA Library Amplification Primer Mix (10X), 96 rxn, plate format	10212276702	1
Roche	KAPA Unique Dual-Indexed Adapter Kit (15µM)	08861919702	1
Roche	KAPA HyperPure Beads (60mL)	08963851001	1
eppendorf	twin.tec® PCR Plate 96	30128648	3
Thermo Fisher Scientific	Fisherbrand 1ml Deep Well (waste plate)	236600	2
SPT Labtech	8-channel EZ-Load Strip Tips, 100µL, with Filters, Sterile (Pack of 40)	125-008-EZ-FS	15 tip boxes
SPT Labtech	dragonfly® discovery syringes (pack 100 syringes / plungers	4150-07200	12 syringes*
SPT Labtech	dragonfly® reservoirs (pack/50)	4150-07103	12 reservoirs*
SPT Labtech	dragonfly® discovery low dead volume reservoirs (pack 25)	4150-07202	0 (required in place of standard reservoirs for runs of ≤7 columns)
Alpaqua Engineering	Alpaqua Magnum FLX (96 well ring magnet)	A000400	1
SPT Labtech	firefly Thermo block 96	3276-01065	1

Table 4. Parts required to process 96 samples (12 columns) through KAPA EvoPrep protocols 1-4 on firefly.

**If the same syringes and reservoirs are used for protocols 3 and 4, this number can be reduced to 6.*

Low dead volume reservoirs will be automatically selected when running fewer than 7 columns per run to save on reagents required. Syringes and reservoirs used in protocol 3 (3.3 Post Ligation Purification) can be re-used in subsequent protocols such as protocol 4 (4.1-4.3a Library Amp and Purification) and the Optional protocol (Double-sided Size Selection) if they are not removed in between protocols. See table 2 for an example of the reagent volumes required for a 96-sample run.

sptlabtech