Roche KAPA EvoPlus Boost Kit and KAPA EvoPlus V2 Kit on SPT Labtech firefly+

Overview

Roche KAPA EvoPlus Boost Kits and KAPA EvoPlus V2 Kits are ideally suited for low- and high-throughput Next-Generation Sequencing (NGS) library construction workflows. The KAPA EvoPlus Boost kit uses KAPA EvoAmp ReadyMix powered by KAPA HiFi to construct libraries from input DNA amounts of 0.1 ng to 500 ng. The kits are available in an automation-friendly 96-well plated format.

Full walkaway KAPA EvoPlus Boost (or KAPA EvoPlus V2) and KAPA EvoPlus Boost PCR-Free protocols have been developed for the SPT Labtech firefly+ using the 96-well plate format kits and the KAPA Unique Dual-Indexed (UDI) Adapter Kit. This poster provides supporting information for the firefly+ protocols listed below. These protocols are based on the Roche document - “Instructions for Use of KAPA DNA Library Prep Evolved Workflows with enzymatic fragmentation. For use with the KAPA EvoPlus V2 and KAPA EvoPlus Boost kits. Featuring KAPA EvoT4 DNA Ligase and KAPA EvoAmp ReadyMix. (October 2025, Version 5.0)” and are available to download from the firefly community.

Protocol highlights

- Full walkaway library preparation protocol for KAPA EvoPlus Boost (or KAPA EvoPlus V2)
- Full walkaway library preparation protocol for KAPA EvoPlus Boost PCR-Free
- On-deck thermocycler (ODTC) on firefly+ allows all incubation and thermocycling steps to take place on firefly+
- 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
- Additional protocols provide a flexible framework to enable multiple workflow options t o be automated, to include:
  - Low DNA input (0.1ng) workflows
  - An optional double-sided size selection can be run either at the post ligation or post amplification stage
  - Library Amplification can be run with either a single or double post PCR purification

firefly+ protocols

KAPA EvoPlus Boost or KAPA EvoPlus V2 – firefly+

Protocol name	firefly+ run time
3.1-3.3 Fragmentation and Adapter Ligation	1 hour 56 minutes (8 samples)
3.1-4.3a Fragmentation, Adapter Ligation and Amplification	2 hours 54 minutes (8 samples with 5 cycles PCR) to 3 hours 20 minutes (96 samples with 5 cycles PCR)
Appendix A. Double-sided Size Selection	42 minutes (8 samples)
4.1-4.3a Library Amplification and Single Purification	56 minutes (8 samples with 5 cycles PCR)
4.1-4.3b Library Amplification and Double Purification	1 hour 38 minutes (8 samples with 13 cycles of PCR)

Table 1. firefly+ protocol names and run times

firefly+ protocol - 3.1-3.3 Fragmentation and Adapter Ligation

This full walkaway protocol runs the KAPA EvoPlus Boost PCR-Free workflow on firefly+ following Chapter 3. *Prepare the Sample Library* of the Instructions for Use document.

The protocol adds FragTail ReadyMix to the input DNA and tip mixes these reagents. The input DNA plate is then placed on the On Deck ThermoCycler (ODTC) to run the Fragmentation and A-tailing. On completion of the Fragmentation and A-tailing, the protocol adds KAPA UDI Adapters and Ligation ReadyMix to the FragTail product and tip mixes these reagents. The plate is then incubated at 20°C for 15 minutes on the ODTC. Following the Adapter Ligation the protocol performs a 0.6X bead purification and then transfers the cleaned-up adapter ligated libraries to a fresh plate.

firefly+ protocol - 3.1-4.3a Fragmentation, Adapter Ligation and Amplification

This full walkaway protocol runs the KAPA EvoPlus Boost (or KAPA EvoPlus V2) workflow on firefly+ following Chapter 3. *Prepare the Sample Library* and Chapter 4 *Amplify the Sample Library* of the Instructions For Use document. This firefly+ protocol runs the same steps as the PCR-Free protocol and then carries on to the amplification and purification steps in Chapter 4 Steps 1-3a *Purification of amplified Sample Library* procedure.

Following the Adapter Ligation Purification, the protocol adds Library Amplification Primer Mix and KAPA HiFi HotStart ReadyMix (2X) OR KAPA EvoAmp ReadyMix to the Adapter-ligated libraries and tip mixes. The plate is then run on the relevant user defined KAPA Library Amplification program on the ODTC. KAPA EvoPlus Boost workflows use the KAPA EvoAmp plate and cycling conditions. KAPA EvoPlus V2 workflows use the KAPA HiFi HotStart ReadyMix (2X) plate and cycling conditions.

Once the thermocycler program is complete, the plate is 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.

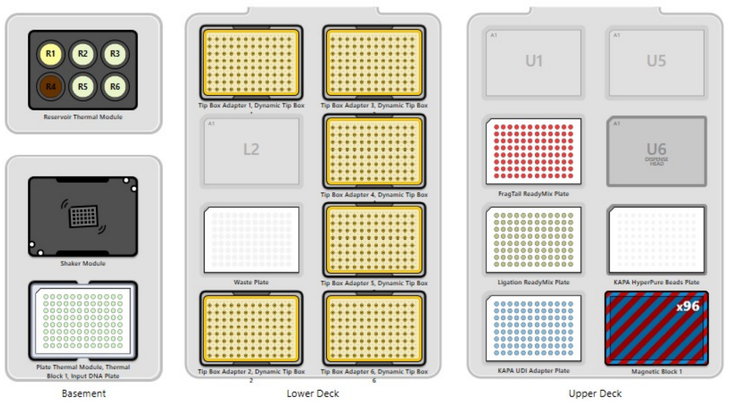

Figure 1. firefly starting deck layout for a 96-sample KAPA EvoPlus Boost (or KAPA EvoPlus V2) 3.1-3.3 Fragmentation and Adapter Ligation library prep run. Deck layout – Basement: Reservoir R1 – Elution Buffer, R2 +R3+R5+R6 – 80% Ethanol, R4 – KAPA HyperPure Beads, Plate Thermal Module - 96 sample Thermo Adapter Block and Input DNA Plate. Lower Deck: L1+L4+L5+L6+L7+L8 L7 - 100µL 96 format ATL tips on ATL 35-125µL Tip Stands, L3 – Waste Plate. Upper Deck: U2 – FragTail ReadyMix Plate, U3 – Ligation ReadyMix Plate, U4 – KAPA UDI Adapter Plate, U7 – KAPA HyperPure Beads Plate, U8 – Alpaqua Magnum FLX 96 sample Magnetic Block.

firefly+ protocol - Appendix A. Double-sided Size Selection

This optional Size Selection protocol can be run after either the firefly+ 3.1-3.3 Fragmentation and Adapter Ligation protocol to perform post-ligation sample purification or after the firefly+ 3.1-4.3a Fragmentation, Adapter Ligation and Amplification protocol to perform a post-amplification sample purification. Input volume is 50 µL, and output volume is 20 µL. 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.

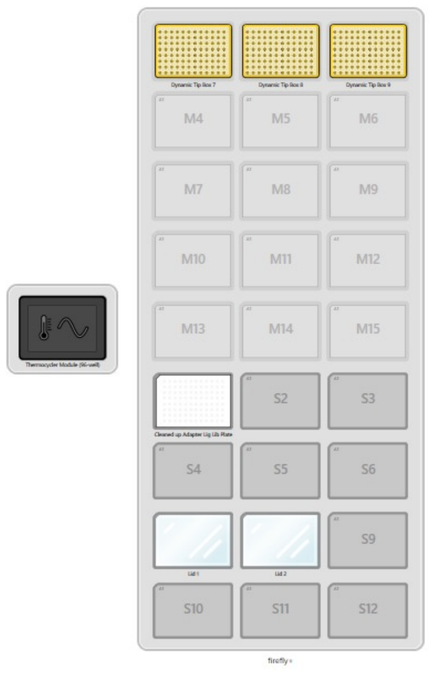

Figure 2. firefly+ starting layout for a 96-sample KAPA EvoPlus Boost (and KAPA EvoPlus V2) 3.1-3.3 Fragmentation and Adapter Ligation library prep run. M1-M3 - 100 µL 96 format ATL tips. S1 – Cleaned up Adapter Ligation Library Plate. S7, S8 – Plate Lids.

Protocol performance

The KAPA EvoPlus Boost library preparation protocols were run on firefly+ and manually. Three tests were performed: Test 1 was a high-throughput run, using 96 samples at 10ng input, including 15 NTCs; Test 2 was a PCR-Free run at was 500 ng input (and then later a Size Selection), and Test 3 was a 0.1 ng low input run. See Table 2 for details. The ODTC was used for thermocycling steps for both the firefly+ and manual runs.

The resulting libraries were analyzed to determine their concentration by qPCR and average fragment size – using a LightCycler 480 System (Roche, KAPA Library Quantification kit) and a Fragment Analyzer (Agilent, DNF-474 HS NGS Fragment kit) respectively. See Table 3 for a summary of the results. Figures 3-6 show a further breakdown of the results. No library was detected in the NTCs – see Figure 5.

Experiment	Input (ng)	firefly+ replicates	Manual replicates	Adapter Conc (µM)	PCR Cycles	Post Amplification cleanup
Test 1 high-throughput	10	96	8	6	5	Chapter 4 Step 3a (Single 1.0X cleanup)
Test 2 (PCR-Free)	500	8	4	15	0*	N/A
Test 3 low input	0.1	8	8	0.6	13	Chapter 4 Step 3b (Double 1.0X cleanup)

Table 2. Experiments run to assess protocol performance of KAPA EvoPlus V2 (with EvoBoost) on firefly+. *5 cycles of PCR run for sizing purposes.

Experiment	Number samples sized	Average Size (bp)	Average Size CV%	Number of Samples quantified	Concentration (nM)	Concentration CV%
Test 1 high-throughput – firefly+	16	351	1.3	80	6.1	13.6
Test 1 manual + ODTC	8	347	2.6	8	7.15	13.4
Test 2 PCR-Free (Post Ligation) – firefly+	8	332	1.6	8	49.78	10.5
Test 2 PCR-Free (Post Ligation) - manual	4	342	3.8	4	33.73	12.0
Test 3 low input – firefly+	8	362	2.1	8	15.67	28.6
Test 3 low input – manual + ODTC	8	394	4.5	8	11.47	27.7

Table 3. Summary of sizing and concentration data for KAPA EvoPlus V2 (with EvoBoost) firefly+ and manual runs.

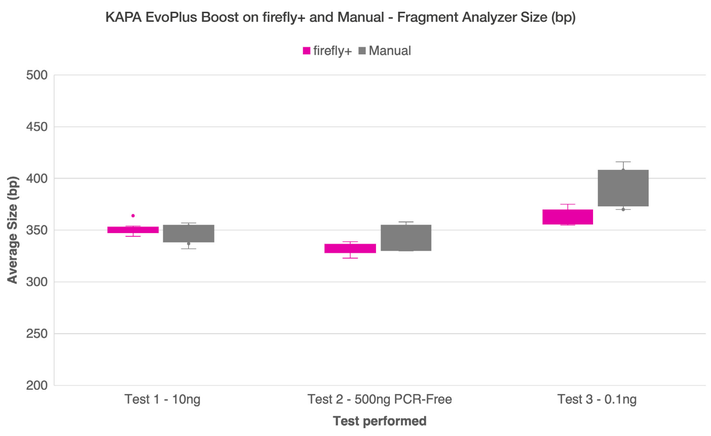

Figure 3. Fragment Analyzer average size (bp) results for KAPA EvoPlus Boost libraries for each of the 3 tests – 10ng input with 5 cycles of PCR, 500ng input PCR-Free and 0.1ng input with 13 cycles of PCR. The comparison between libraries generated on the firefly+ and manually is shown.

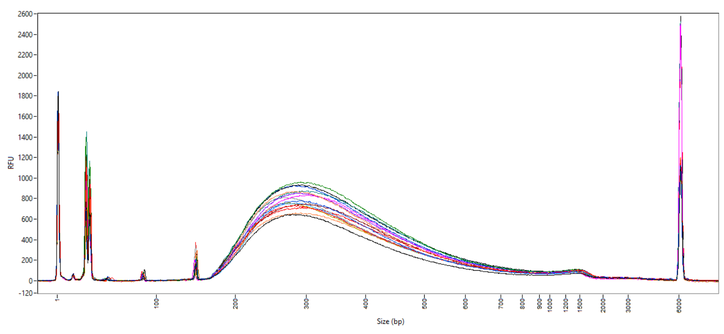

Figure 4. Fragment Analyzer traces for samples run on test 1 10ng input firefly+ n=16.

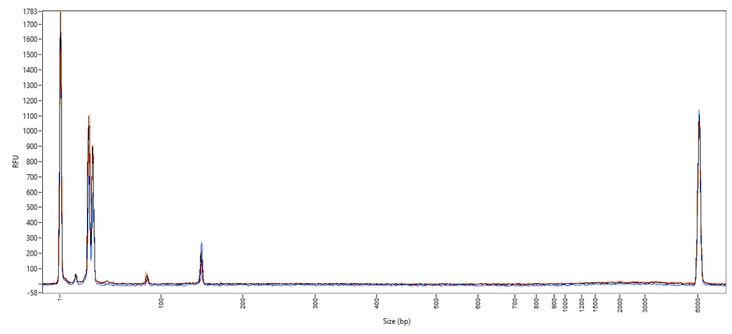

Figure 5. Fragment Analyzer traces for NTCs run on test 1 10ng input firefly+ n=4.

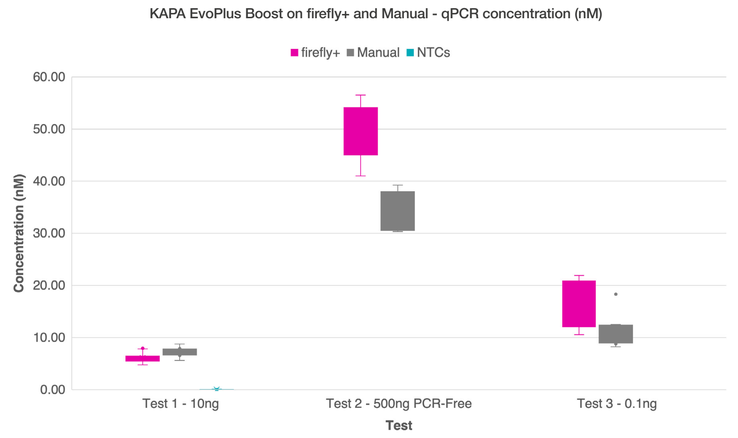

Figure 6. KAPA Library Quantification size adjusted concentration (nM) results for KAPA EvoPlus Boost libraries for each of the 3 tests – 10ng input with 5 cycles of PCR, 500ng input PCR-Free and 0.1ng input with 13 cycles of PCR. The comparison between libraries generated on the firefly+ and manually is shown. NTCs run as part of firefly+ test 1 are also shown.

Size Selection

As part of Test 2, the 500 ng input PCR-Free samples underwent 5 cycles of PCR, a post-PCR cleanup, and then an additional double-sided Size Selection cleanup following the post-PCR cleanup, with the first cut at 0.5X and the second cut at 0.7X. Library Size Pre and Post Size Selection was measured for the firefly+ and manual runs using Fragment Analyzer, and the results are shown in Table 4 and Figures 7-8. The results show that a double-sided size selection was able to be run successfully on firefly+ with comparable results to manual size selection.

Run type	Average Size (bp) Pre Size Selection	Average Size (bp) Post Size Selection	Number of Samples
firefly+	368	429	8
Manual	362	408	3

Table 4. Summary of the Fragment Analyzer sizing data for the KAPA EvoPlus V2 (EvoAmp) libraries undergoing double sided size selection.

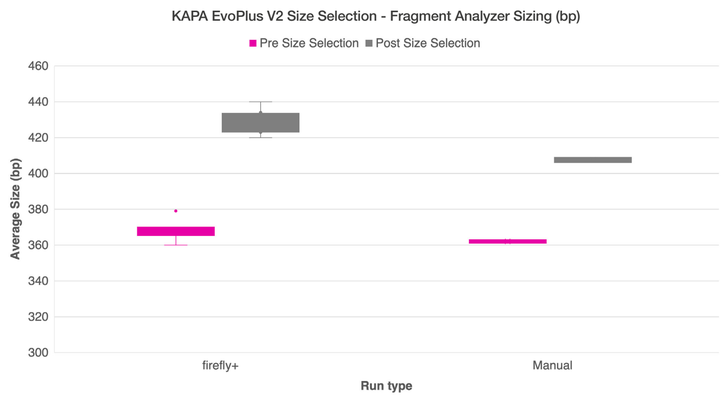

Figure 7. Fragment Analyzer average size (bp) for KAPA EvoPlus V2 (EvoAmp) libraries undergoing double-sided size selection on firefly+ and run manually.

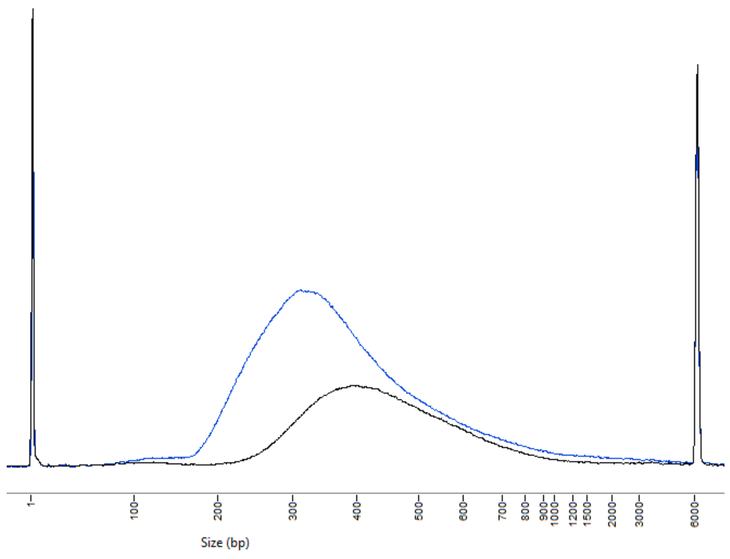

Figure 8. Test 2 Size Selection on firefly+. Fragment Analyzer traces shown are for sample in position A1 pre and post size selection. Size selection was performed following a 5 cycle PCR and cleanup. 1 in 10 dilution run on the fragment analyzer.

Summary

These results demonstrate that the KAPA EvoPlus Boost, KAPA EvoPlus Boost PCR-Free and KAPA EvoPlus V2 workflows have been successfully automated on firefly+ to generate Illumina-compatible sequencing libraries. Full walkaway firefly+ protocols are available for:

- 3.1-3.3 Fragmentation and Adapter Ligation
- 3.1-4.3a Fragmentation, Adapter Ligation and Amplification

Additional firefly+ protocols are available to perform optional double-sided size selection and alternative versions of the library amplification and cleanup steps. Plate-based reagents and user input variables in the firefly+ protocols allow flexibility to run varying numbers of columns of samples and to start at varying columns in the reagent plates – allowing both high-throughput or multiple low-throughput run approaches from a single kit.

Libraries prepared on firefly+ are in line with manually prepared libraries in terms of average fragment size and concentration. The libraries show consistent fragment sizing and concentration across a 96-well plate and show no detectable well-to-well contamination. The firefly+ size selection workflow also shows expected results consistent with manually prepared libraries.

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