Streamlined library preparation with enzymatic fragmentation: Automating KAPA EvoPlus V2 Kits on firefly®

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Overview

For the first time, we demonstrate that the Roche KAPA EvoPlus V2 and KAPA EvoPlus V2 PCR-free DNA library preparation workflows can be successfully automated on firefly to create Illumina-compatible NGS libraries comparable to those prepared manually.

The combination of these technologies enables significant time savings in the laboratory for whole genome sequencing applications, whilst also reducing operator variability.

Protocol highlights

- Input gDNA samples ranging from 0.1 – 500 ng can be processed into Illumina-compatible sequencing libraries with full-length, uniquely indexed adapters.
- Automated KAPA EvoPlus V2, KAPA EvoPlus V2 PCR-Free and size selection workflows can be run with the flexibility to start the protocol from any step.
- Protocol variables provide the flexibility to process 1 – 12 sample columns per run and to specify the starting column for reagent plates (ReadyMix plates and UDI Adapters plates), enabling multiple low-throughput runs using the same reagent plates and reducing waste.
- KAPA EvoPlus V2 libraries produced on firefly are comparable in yield and fragment size (~300-400 bp) to manually prepared libraries and show uniformity in concentration and fragment size.

Method

Table 1. firefly® protocols and consumables required for automated KAPA EvoPlus V2 Kits.

Protocol number	Protocol name	firefly® run time (mins)	Thermocycler run time (mins)	KAPA EvoPlus PCR-Free workflow	KAPA EvoPlus workflow	125 µL filtered strip tip-sets required*	Standard dispense head syringes required	Additional plates required
1 of 4	3.1 Fragmentation and A-Tailing V2	6	35-60	✓	✓	1	0	1 x DNA input plate
2 of 4	3.2 Adapter Ligation V2	6	20	✓	✓	1	0	n/a
3 of 4	3.3 Post-Ligation Purification V2	31-36†	n/a	✓	✓	6	6	1 x Elution plate 1 x Waste plate
4a of 4	4.1 - 4.3a Library Amplification and Purification V2	34-40†	5-25		✓	7	6	1 x Elution plate 1 x Waste plate
4b of 4	4.1 - 4.3b Library Amplification and Purification V2	62-73†	5-25		✓	7	6	1 x Elution plate 1 x Intermediate plate 1 x Waste plate
Optional♦	Double-sided Size Selection V2	46-52†	n/a			7	6	1 x Elution plate 1 x Intermediate plate 1 x Waste plate

♦ *Run the optional Double-sided Size Selection protocol after protocol 3 or after protocol 4 Use protocol 4a for DNA input >0.1 ng and use protocol 4b for DNA inputs of 0.1 ng*

† *firefly run times vary with the number of columns processed*

* *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*

There is a safe stopping point after protocol 3

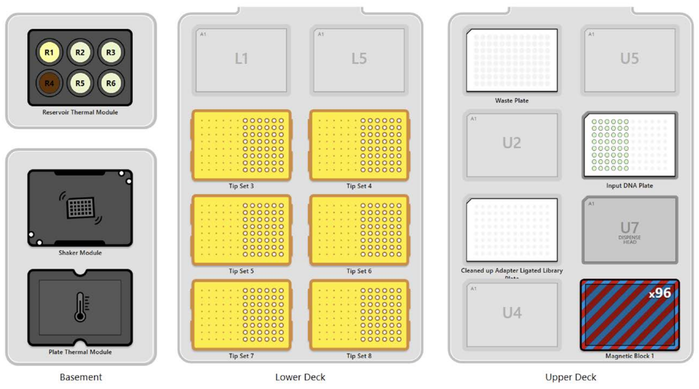


Figure 1: Example starting firefly deck layout for protocol 3.3 Post Ligation Purification V2 for 6 columns of samples

The KAPA EvoPlus V2 library preparation was run manually and on firefly using human gDNA (Promega) as the input. See Table 2 for the workflows tested. Each run used a gDNA input of 10 ng or 0.1 ng, with 8 replicates at each input level. All runs used a 20-minute fragmentation time and a 0.6X post-ligation clean up. Samples with a 10 ng input were processed with 5 cycles of PCR, followed by a 1.0X post-amplification clean up. Samples with a 0.1 ng input were processed with 13 cycles of PCR followed by two 1.0X post-amplification clean ups, as specified in the KAPA EvoPlus V2 *Instructions for Use* document.

The resulting libraries were analyzed to determine their concentration by qPCR and average fragment size using a LightCycler 480 System[1] (Roche, KAPA Library Quantification kit) and a Fragment Analyzer (Agilent, DNF-474 HS NGS Fragment Kit) respectively.

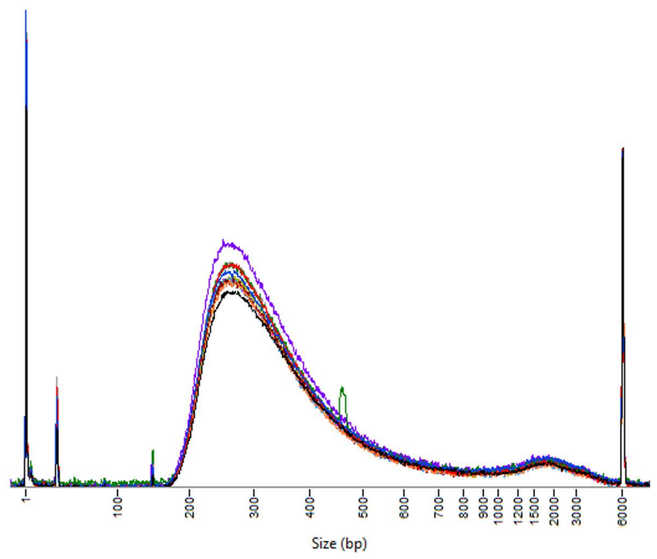
Table 2. Summary of KAPA EvoPlus V2 Kits workflows automated on firefly.

Platform	Number of Samples	Input DNA (ng)	Adapter concentration (µM)	PCR cycles	Post PCR cleanup
firefly	8	10	6	5	Protocol 4.3a Single clean up
firefly	8	0.1	0.6	13	Protocol 4.3b Double clean up
manual	8	10	6	5	Protocol 4.3a Single clean up
manual	8	0.1	0.6	13	Protocol 4.3b Double clean up

Results

Fragment Analyzer traces

A.



B.

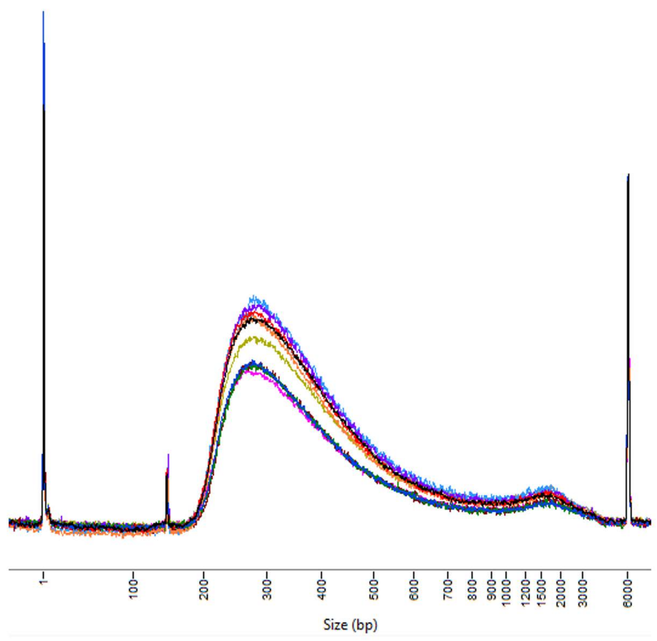


Figure 2: Fragment Analyzer traces for KAPA EvoPlus V2 libraries prepared on firefly using (A) 10 ng input gDNA and (B) 0.1ng input gDNA

Library Size

KAPA EvoPlus V2 library size (bp)

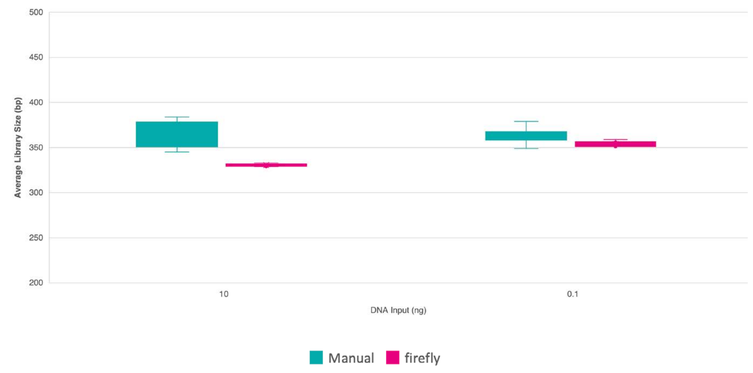


Figure 3: Average size of KAPA EvoPlus V2 Kits libraries generated manually and on firefly for gDNA inputs of 10 ng and 0.1 ng. The 10 ng input samples received 5 cycles of PCR. The 0.1 ng input samples received 13 cycles of PCR.

Library Concentration

KAPA EvoPlus V2 library concentration (nM)

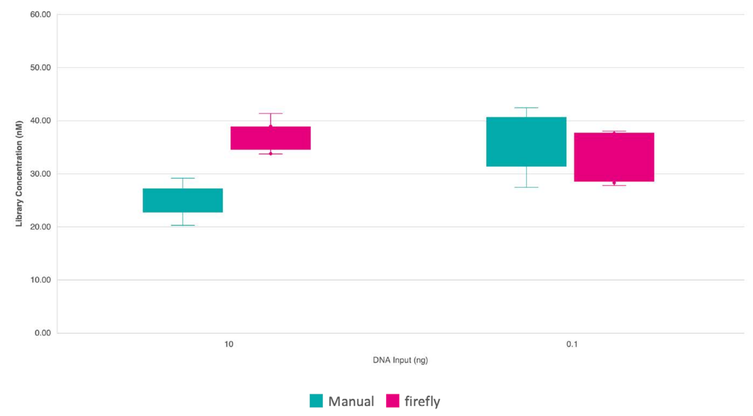


Figure 4: Library concentration (nM) of KAPA EvoPlus V2 libraries generated manually and on firefly for gDNA inputs of 10 ng and 0.1 ng. The 10 ng input samples received 5 cycles of PCR. The 0.1 ng input samples received 13 cycles of PCR.

Summary

Table 3. Summary of Sizing and Concentration data for KAPA EvoPlus V2 Kits libraries prepared on firefly. n=8 for each run condition.

Run condition	Average Size (bp)	%CV Size	Average concentration (nM)	%CV Concentration
Manual 10 ng input	364	3.69	25.20	11.38
firefly 10 ng input	331	0.52	37.57	7.01
Manual 0.1 ng input	364	2.15	35.52	14.42
firefly 0.1 ng input	354	0.85	33.46	13.56

Conclusion

- These results demonstrate that the KAPA EvoPlus V2 and KAPA EvoPlus V2 PCR-free workflows can be successfully automated on firefly to generate Illumina-compatible sequencing libraries.
- KAPA EvoPlus V2 sequencing libraries generated on firefly are in line with manually prepared libraries in terms of final library concentration and average fragment sizes.
- Libraries prepared on firefly show consistent library concentration and fragment size (%CV ≤ 0.9%) across runs.

Data on file with Roche and SPT Labtech.
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