Automating KAPA EvoPlus library preparation kits on firefly®

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Overview

For the first time, we demonstrate that the Roche KAPA EvoPlus and KAPA EvoPlus 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 mitigating the risk of repetitive strain injuries (RSI) posed by such a substantial number of manual pipetting steps.

Protocol highlights

- Input gDNA samples ranging from 10 – 500 ng can be processed into Illumina-compatible sequencing libraries with full-length, uniquely indexed adapters.
- Automated KAPA EvoPlus, KAPA EvoPlus 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 Adapter plates), enabling multiple low-throughput runs using the same reagent plates and reducing waste.
- KAPA EvoPlus libraries produced on firefly® are comparable in yield and fragment size (~300-400 bp) to manually prepared libraries, and are uniform in concentration and fragment size across a 96-well plate, with no detectable well to well contamination.
- KAPA EvoPlus kits are available in automation-friendly plate format and the reagents have been optimized for stability.

Method

firefly® protocols and consumables

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	6	35-60	✓	✓	1	0	1 x DNA input plate
2 of 4	3.2 Adapter Ligation	6	15	✓	✓	1	0	n/a
3 of 4	3.3 Post-Ligation Purification	31-36†	n/a	✓	✓	6	6	1 x Elution plate 1 x Waste plate
4 of 4	4.1 - 4.3 Library Amplification and Purification	33-38†	5-25		✓	7	6	1 x Elution plate 1 x Waste plate
Optional◊	Double-sided Size Selection	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 *†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*

Input titration, size selection, high-throughput (HTP) performance and cross-contamination evaluation

Library preparations were run using human gDNA (Promega) as the input, with the following conditions:

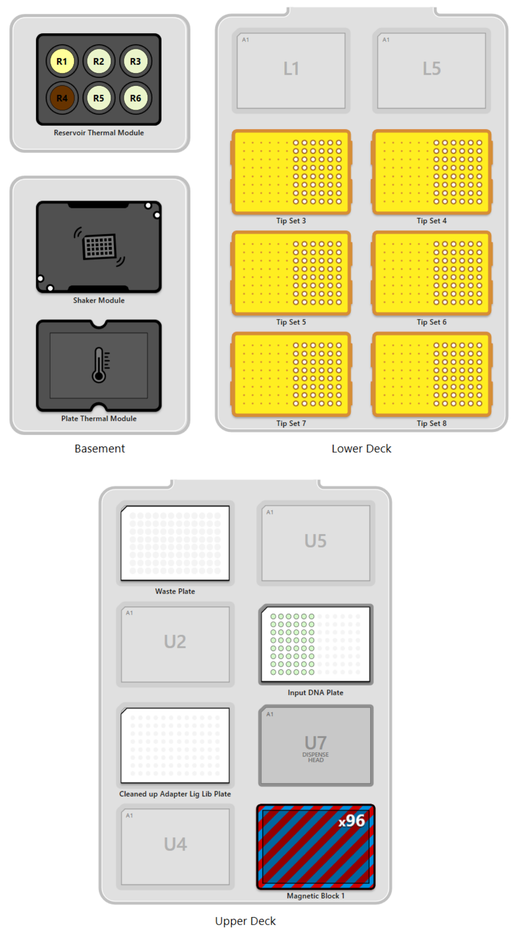
Workflow	Input	Fragmentation time (minutes)	Adapter Concentration	PCR Cycles	Manual run replicates	firefly® run replicates	firefly® HTP run 1 replicates	firefly® HTP run 2 replicates
KAPA EvoPlus	10 ng	20	6 µM	5	8	8	81x gDNA 15x NTC	81x gDNA 15x NTC
KAPA EvoPlus PCR-Free	100 ng	20	15 µM	0*	8	8	n/a	n/a
KAPA EvoPlus PCR-Free	500 ng	20	15 µM	0*	8	8	n/a	n/a
KAPA EvoPlus PCR-Free with size selection (0.5X – 0.7X)	500 ng	20	15 µM	0*	8	8	n/a	n/a

**5 PCR cycles were required to enable fragment size analysis*

Resulting libraries were:

- Quantified by qPCR using a Lightcycler 480 (Roche, KAPA Library Quantification kit)
- Sized using a Fragment Analyzer (Agilent, DNF-474 HS NGS Fragment Kit)

Example starting firefly® deck layout for protocol 3.3 Post Ligation Purification for 6 columns of samples



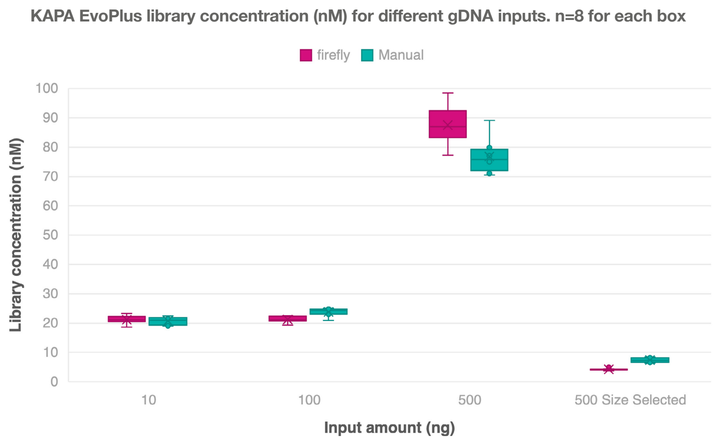
Summary

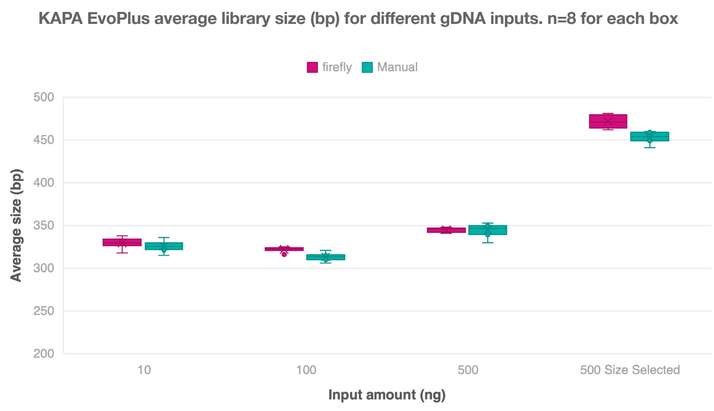
Input (ng)	Prep	No. replicates	Average size (bp)	Size CV%	Average Conc (nM)	Conc CV%
10	firefly®	8	330	1.85	21.16	6.53
10	Manual	8	326	1.91	20.76	6.23
100	firefly®	8	322	0.88	21.23	4.89
100	Manual	8	313	1.43	23.80	5.77
500	firefly®	8	345	0.80	87.54	7.59
500	Manual	8	345	2.24	76.79	7.63
500	firefly® Size selected	8	472	1.56	4.22	7.83
500	Manual Size selected	8	453	1.35	7.39	10.43
10	firefly® HTP 1	81	323	0.97	24.77	10.83
10	firefly® HTP 2	81	344	1.15	27.84	11.01

NTCs showed no detectable library contamination by qPCR or Fragment Analysis

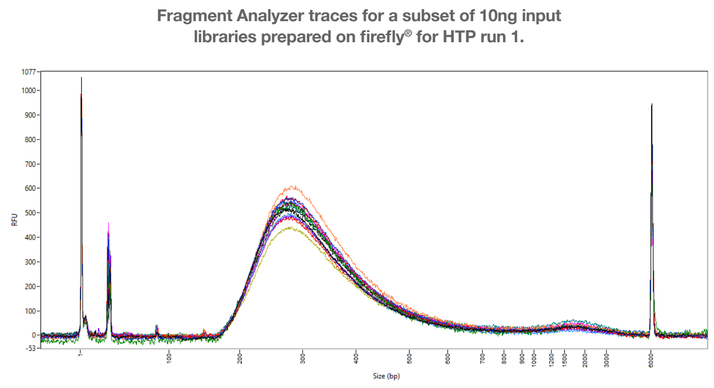
Results

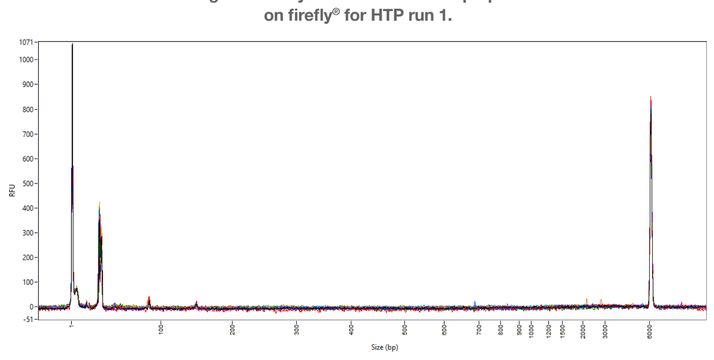
Input titration and size selection

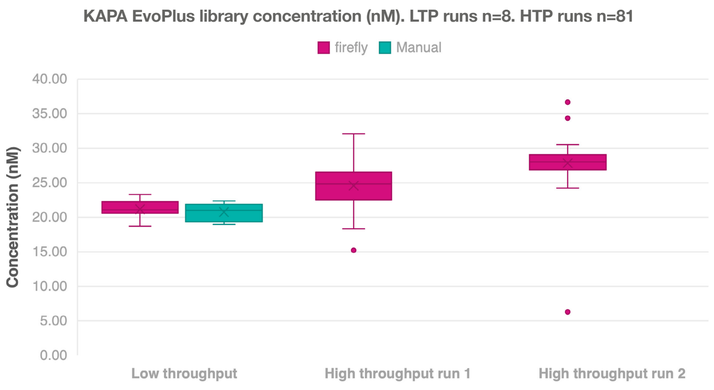


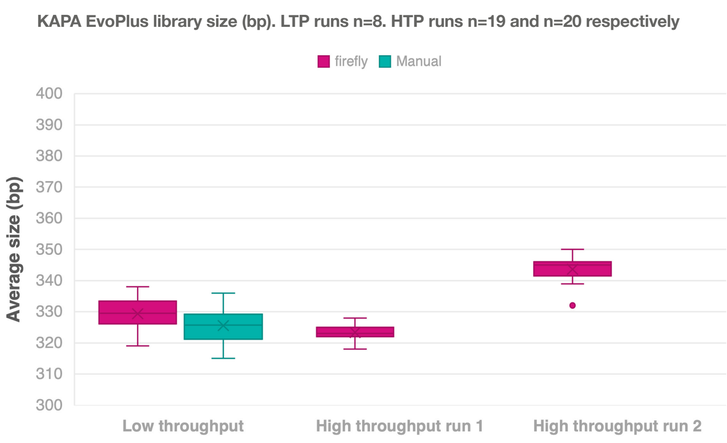


High-throughput performance and cross contamination evaluation









Conclusions

- These results demonstrate that the KAPA EvoPlus and KAPA EvoPlus PCR-free workflows can be successfully automated on firefly® to generate Illumina-compatible sequencing libraries.
- KAPA EvoPlus 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 (%CV ≤ 11%) and fragment size (%CV ≤ 1.2%) across a 96-well plate and show no detectable well to well contamination.

Data on file. Project: KAPA EvoPlus on SPT Labtech firefly

sptlabtech.com