firefly® application note
Illumina DNA PCR-Free Prep

illumina®

Overview

The Illumina DNA PCR-Free Prep kit is an optimized, user-friendly workflow for preparing normalized and ready-to-sequence libraries in under three hours. The workflow is compatible with a broad range of sample types and DNA input amounts, delivering consistent insert sizes, highly uniform coverage across repetitive or uneven genome regions, and is highly compatible with automation[1].

Illumina DNA PCR-Free Prep relies on bead-based tagmentation technology where magnetic bead-based transposome complexes combine tagging and DNA fragmentation in a single 5-minute reaction. Unique dual indexes are then ligated with the DNA fragments and generate libraries compatible with all Illumina sequencing platforms. The overall process eliminates the need for PCR amplification steps and consequent bias, providing highly accurate sequence information for sensitive applications[1].

Here we show how the Illumina DNA PCR-Free Prep workflow, based on Illumina DNA PCR-Free Library Prep Reference Guide (1000000086922 v03), was automated on firefly®, in a two-part protocol with a single off-deck thermocycler step (Figure 1). Details of the firefly® protocol are described, and data is presented to demonstrate the performance of the automated workflow.

Reagents required

Kit	Catalog #
Illumina DNA PCR-Free Prep, Tagmentation (96 Samples)	20041795
Illumina DNA/RNA UD Indexes Set A, B, C or D, Tagmentation (96 Indexes, 96 Samples)	20091654 20091656 20091658 20091660

firefly protocols

Protocol number	Protocol name	Instrument run time
Protocol 1 of 2	Illumina DNA PCR-Free Prep Part 1	55 mins
Protocol 2 of 2	Illumina DNA PCR-Free Prep Part 2	95 mins

Table 1. firefly® protocols used to run the Illumina DNA PCR-Free Prep workflow – and are available to download from the firefly® community.

firefly workflow

Figure 1. Workflow and firefly® run times for the Illumina DNA PCR-Free Prep kit.

Workflow features

Minimal user interactions

- Users are only needed to set up the firefly® protocols and to transfer the plate to an off-deck thermocycler to run the ELM program.

The use of plastic consumables has been minimized

- Non-contact positive displacement technology of the firefly® dispense head enables the same reagent to be dispensed to multiple wells of a plate using a single syringe.
- Pipetting-head tips have been reused where appropriate to do so, with no detrimental effect on the library preparation.

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Protocol overview

Protocol 1 of 2
Illumina DNA PCR-Free Prep Part 1

Figure 2 shows the starting deck layout for this protocol. The firefly® dispense head is used to dispense Bead-Linked Transposomes PCR-Free (BLT-PF) and Tagmentation Buffer 1 (TB1) to the sample input plate LP1. Each sample well is tip-mixed and then LP1 is moved to the pre-heated on-deck thermal module for the Tagmentation incubation (42°C for 10 minutes).

On completion of this incubation, the dispense head delivers Stop Tagment Buffer 2 into LP1 and the pipetting head tip-mixes each sample well. LP1 is incubated at room temperature after which the BLT-PF beads are washed with Tagmentation Wash Buffer (TWB). The TWB reagent is dispensed by syringes 3 and 6 of the firefly® dispense head and then tip-mixed. The same set of tips is used for each bead resuspension and supernatant removal of the TWB wash.

Extension Ligation Mix (ELM) is dispensed to the LP1 plate using the dispense head, then Indexes are transferred to the sample plate using the pipetting head, and each sample well is tip-mixed. The user moves the LP1 plate from deck position U4 and to an off-deck thermocycler pre-programmed with the ELM program. On completion of the ligation program the sample plate is ready to proceed to the Illumina DNA PCR-Free Prep Part 2 protocol.

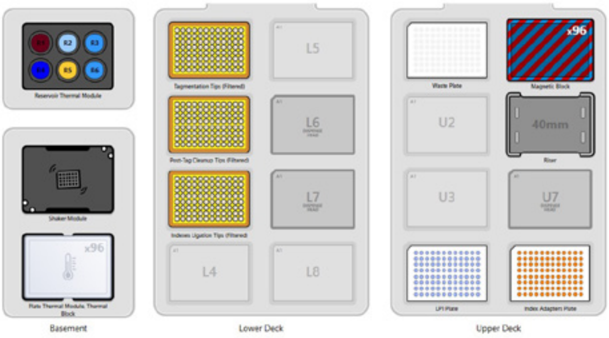


Figure 2. Starting deck layout for firefly® Illumina DNA PCR-Free Prep Part 1. Lower deck: (L1) Tagmentation Tips (Filtered) - 100µL pipetting head tips; (L2) Post-Tag Cleanup Tips (Filtered) - 100µL pipetting head tips; (L3) Indexes Ligation Tips (Filtered) - 100µL pipetting head tips. Upper deck: (U1) Waste Plate - Fisherbrand 1mL Deep Well Thermo Fisher Scientific; (U4) LP1 Plate - Hard Shell Plate Bio-Rad with 25µL input DNA per well; (U5) Alpaqua Magnum FLX Magnet; (U6) SPTLabtech 40mm Upper Deck; (U8) Index Adapters Plate - twin.tec PCR Eppendorf plate. Dispense head reservoirs: (R1) Bead-Linked Transposomes PCR-Free; (R2) Tagmentation Buffer 1; (R3, R6) Tagmentation Wash Buffer; (R4) Stop Tagment Buffer 2; (R5) Extension Ligation Mix. Plate Thermal Module: 96 Thermal Block.

Protocol 2 of 2
Illumina DNA PCR-Free Prep Part 2

On completion of the ELM thermocycler program, the user returns LP1 to position U4 on the firefly® deck and starts the Illumina DNA PCR-Free Prep Part 2 protocol, which performs an alkaline-based library elution and double-sided bead purification. See Figure 3 for the starting deck layout.

Whilst LP1 is incubated on the magnet, Illumina Purification Beads (IPB) are dispensed into an intermediary bead reservoir plate and to a second library plate (LP2) using syringe 1 of the firefly® dispense head. The BLT-PF beads in LP1 are washed with TWB dispensed from syringes 3 and 6, followed by a library elution step using the diluted HP3 reagent dispensed from syringe 2. IPB are transferred from the intermediary bead plate into LP1 using the pipetting head and are tip-mixed. After incubating LP1 on the magnet, the supernatant is transferred into LP2 and tip-mixed with purification beads. Following an incubation and bead pelleting period, the supernatant in LP2 is removed into waste and the beads are washed twice with 80% Ethanol. The pipetting head is used to transfer ethanol from the deep well reservoir plate to LP2, and to r emove the wash supernatant into the waste plate.

After air-drying the beads for 2 minutes, the dispense head is used to dispense Resuspension Buffer to LP2. The beads are resuspended by using tip-mixing and the libraries are eluted at room temperature. Following a bead pelleting step on the magnet, the pipetting head is used to transfer the eluted libraries into the Final Libraries Plate (FLP). A User Interaction prompt indicates the end of the protocol and that the libraries can be collected from position U8 of the Upper Deck.

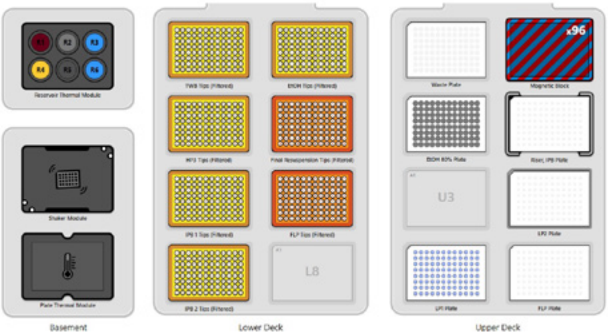


Figure 3. Starting deck layout for firefly® Illumina DNA PCR-Free Prep Part 2. Lower deck: (L1) TWB Tips (Filtered) - 100µL pipetting head tips; (L2) HP3 Tips (Filtered) - 100µL pipetting head tips, (L3) IPB 1 Tips (Filtered) - 100µL pipetting head tips; (L4) IPB 2 Tips (Filtered) - 100µL pipetting head tips; (L5) EtOH Tips (Filtered) - 100µL pipetting head tips; (L6) Final Resuspension Tips (Filtered) - 35µL pipetting head tips; (L7) FLP Tips (Filtered) - 35µL pipetting head tips. Upper deck: (U1) Waste Plate - Fisherbrand 1mL Deep Well Thermo Fisher Scientific; (U2) EtOH 80% Plate - Fisherbrand 1mL Deep Well Thermo Fisher Scientific; (U4) LP1 Plate - Hard Shell Plate Bio-Rad; (U5) Alpaqua Magnum FLX Magnet; (U6)IPB Plate - Hard Shell Plate Bio-rad stacked on SPTLabtech 40mm Upper Deck; (U6) LP2 Plate - Hard Shell Plate Bio-Rad; (U4) FLP Plate - Hard Shell Plate Bio-Rad. Dispense head reservoirs: (R1) Illumina Purification Beads; (R2) HP3 diluted 10-fold; (R3, R6) Tagmentation Wash Buffer; (R4) Elution Buffer.

Protocol performance

High throughput performance and well-to-well contamination analysis

The Illumina DNA PCR-Free Prep kit with DNA/RNA UD Indexes set D was run on firefly® with 86 samples of human genomic DNA NA12878 (Coriell Institute for Medical Research) at 300ng input amount, and with 10 no-template negative controls. Library yields were determined using the KAPA Library Quantification kit and a Lightcycler® 480 (Roche) assuming an average library size of 450bp. Sixteen libraries were randomly selected and sequenced using a NovaSeqTM 6000 Sequencing System, with a run configuration of 2 × 151bp and an S4 flow cell.

Libraries generated using the Illumina DNA PCR-Free chemistry are expected to yield at least 2.8nM when using inputs higher than 300ng2. Figure 4 shows that libraries prepared using the Illumina DNA PCR-Free Prep workflow on firefly® (3.2–7.5nM, average 4.3nM, %CV 19.5%) meet this requirement. Additionally, all no-template wells were negative for well-to-well contamination events.

Table 2 summarizes the sequencing performance of a pool of 16 libraries generated from 300ng Coriell NA12878 gDNA on firefly®. All metrics meet their respective criteria.

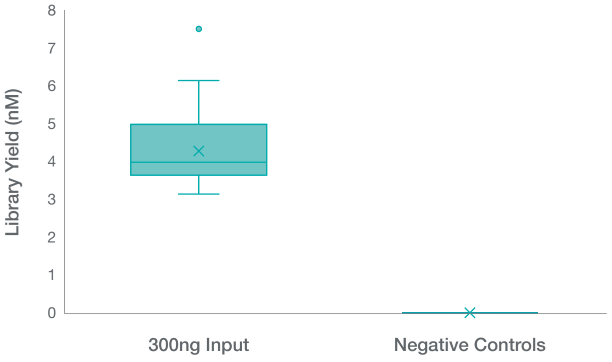


Figure 4. Final libraries yield range from 86 samples of NA12878 and 10 negative controls processed on firefly® using the Illumina DNA PCR-Free Prep kit.

Metrics	Criteria	Results
NovaSeq6000 S4 Flow Cell		
Yield	≥3TB	3.76TB
%PF	≥70%	77.33%
%Q30	≥85%.	90.96%
Index %CV	≤20% with correction factors	17.28%
Samples (Average)		
Yield	≥100Gbp for ≥95% of samples	208.7Gbp
Autosomal coverage	≥30x for ≥95% of samples	59.1x
Insert size	450±75bp	432bp
Q30 bases (excluding duplicates & clipped bases)	≥85Gbp for ≥95% of samples	170.1Gbp
Duplicate read rates	≤15% for ≥95% of samples	8.43%
Percentage of genome with at least 15x coverage	≥90% for ≥95% of samples	93.76%

Table 2. Sequencing metrics for 16-plexity library run on a NovaSeq 6000 with an S4 flow cell. Libraries were prepared on firefly® using 300ng of Coriell NA12878 DNA and Illumina DNA/RNA UD Indexes Set D.

Protocol Reproducibility

Protocol reproducibility was evaluated by preparing 3 plates of 96 samples at 300ng input amount of Human Genomic Mix DNA (Promega, G3041). Each plate was freeze-thawed once and processed using the Illumina DNA PCR-Free Prep kit with DNA/RNA UD Indexes Set C on firefly®. Library yields were determined using the KAPA Library Quantification kit and a Lightcycler® 480 (Roche) assuming an average library size of 450bp.

All 3 runs generated libraries with similar average yields (Figure 5) and cross plate yield patterns (Tables 3–5) demonstrating the reproducibility of the Illumina DNA PCR-Free Prep kit on firefly® workflow.

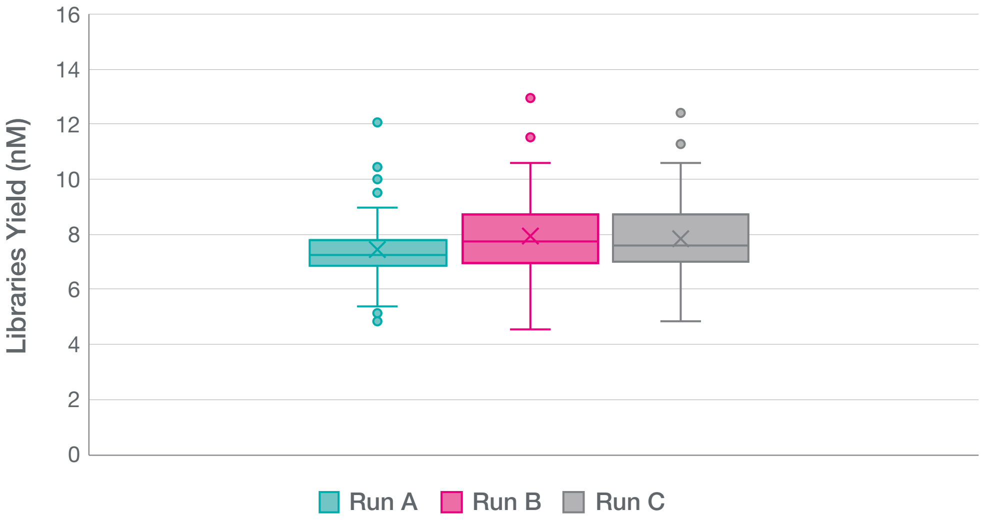
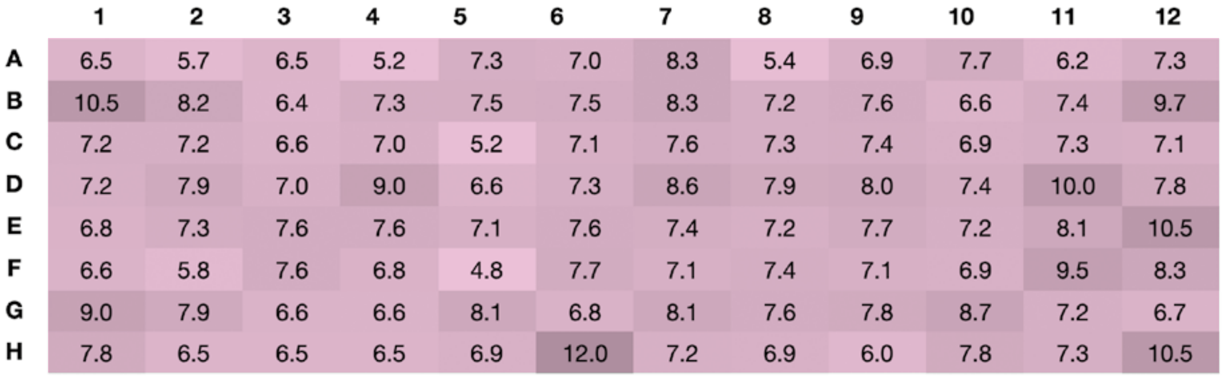


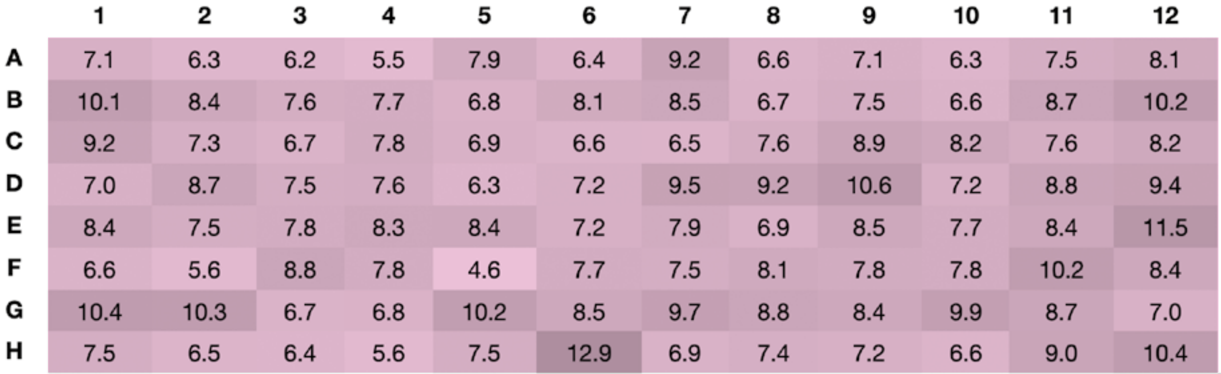
Figure 5. Final libraries yield for 3 high throughput runs of Illumina DNA PCR-Free Prep on firefly®. Each run consisted of 96 samples of Promega Human Genomic Mix DNA at 300ng input amount and Illumina DNA/RNA UD Indexes Set C.

[1] *For life science research only. Not for use in diagnostic procedures.*



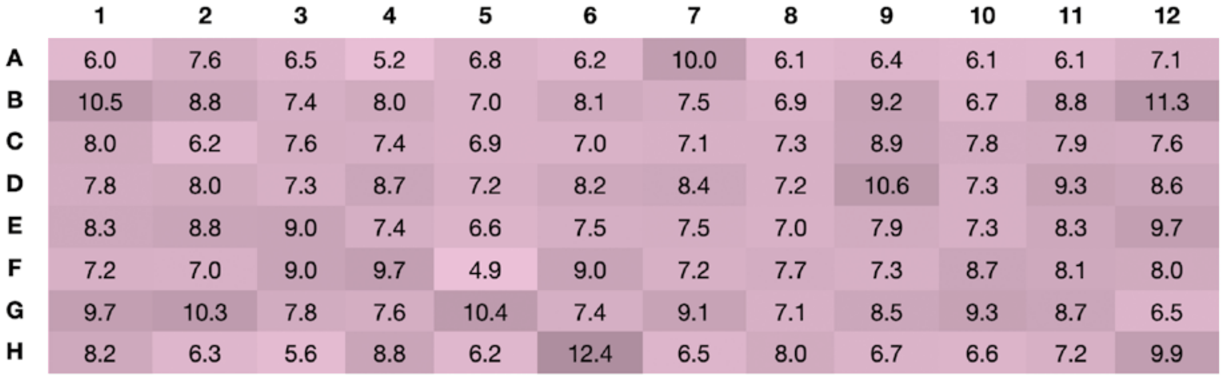
	1	2	3	4	5	6	7	8	9	10	11	12
A	6.5	5.7	6.5	5.2	7.3	7.0	8.3	5.4	6.9	7.7	6.2	7.3
B	10.5	8.2	6.4	7.3	7.5	7.5	8.3	7.2	7.6	6.6	7.4	9.7
C	7.2	7.2	6.6	7.0	5.2	7.1	7.6	7.3	7.4	6.9	7.3	7.1
D	7.2	7.9	7.0	9.0	6.6	7.3	8.6	7.9	8.0	7.4	10.0	7.8
E	6.8	7.3	7.6	7.6	7.1	7.6	7.4	7.2	7.7	7.2	8.1	10.5
F	6.6	5.8	7.6	6.8	4.8	7.7	7.1	7.4	7.1	6.9	9.5	8.3
G	9.0	7.9	6.6	6.6	8.1	6.8	8.1	7.6	7.8	8.7	7.2	6.7
H	7.8	6.5	6.5	6.5	6.9	12.0	7.2	6.9	6.0	7.8	7.3	10.5

Table 3. Library yields (nM) from high throughput Run A of Illumina DNA PCR-Free Prep on firefly® using Promega Human Genomic Mix DNA at 300ng input amount and Illumina DNA/RNA UD Indexes Set C.



	1	2	3	4	5	6	7	8	9	10	11	12
A	7.1	6.3	6.2	5.5	7.9	6.4	9.2	6.6	7.1	6.3	7.5	8.1
B	10.1	8.4	7.6	7.7	6.8	8.1	8.5	6.7	7.5	6.6	8.7	10.2
C	9.2	7.3	6.7	7.8	6.9	6.6	6.5	7.6	8.9	8.2	7.6	8.2
D	7.0	8.7	7.5	7.6	6.3	7.2	9.5	9.2	10.6	7.2	8.8	9.4
E	8.4	7.5	7.8	8.3	8.4	7.2	7.9	6.9	8.5	7.7	8.4	11.5
F	6.6	5.6	8.8	7.8	4.6	7.7	7.5	8.1	7.8	7.8	10.2	8.4
G	10.4	10.3	6.7	6.8	10.2	8.5	9.7	8.8	8.4	9.9	8.7	7.0
H	7.5	6.5	6.4	5.6	7.5	12.9	6.9	7.4	7.2	6.6	9.0	10.4

Table 4. Library yields (nM) from high throughput Run B of Illumina DNA PCR-Free Prep on firefly® using Promega Human Genomic Mix DNA at 300ng input amount and Illumina DNA/RNA UD Indexes Set C.at 300ng input amount and Illumina DNA/RNA UD Indexes Set C.



	1	2	3	4	5	6	7	8	9	10	11	12
A	6.0	7.6	6.5	5.2	6.8	6.2	10.0	6.1	6.4	6.1	6.1	7.1
B	10.5	8.8	7.4	8.0	7.0	8.1	7.5	6.9	9.2	6.7	8.8	11.3
C	8.0	6.2	7.6	7.4	6.9	7.0	7.1	7.3	8.9	7.8	7.9	7.6
D	7.8	8.0	7.3	8.7	7.2	8.2	8.4	7.2	10.6	7.3	9.3	8.6
E	8.3	8.8	9.0	7.4	6.6	7.5	7.5	7.0	7.9	7.3	8.3	9.7
F	7.2	7.0	9.0	9.7	4.9	9.0	7.2	7.7	7.3	8.7	8.1	8.0
G	9.7	10.3	7.8	7.6	10.4	7.4	9.1	7.1	8.5	9.3	8.7	6.5
H	8.2	6.3	5.6	8.8	6.2	12.4	6.5	8.0	6.7	6.6	7.2	9.9

Table 5. Library yields (nM) from high throughput Run C of Illumina DNA PCR-Free Prep on firefly® using Promega Human Genomic Mix DNA at 300ng input amount and Illumina DNA/RNA UD Indexes Set C.

Summary

The data presented here demonstrates that high quality libraries can be prepared consistently from genomic DNA on firefly® using the Illumina DNA PCR-Free Prep kit.

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References

(1) Illumina DNA PCR-Free Prep, Tagmentation Data Sheet (M-GL-00679 v2.0)
(2) Illumina DNA Library Pre FAQ