firefly® technical note

Illumina DNA PCR-Free Prep

This technical note provides supporting information for the firefly protocols listed below. These protocols 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	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 kit.

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].

Workflow

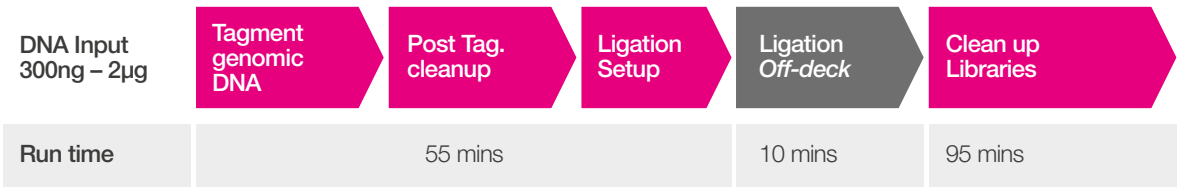

Figure 1. Workflow and firefly® run times for the Illumina DNA PCR-Free Prep kit.

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.

Deviations from the standard manual protocol

- Tagmentation incubation is performed on the firefly® thermal plate module with the sample plate unsealed, instead of being executed in a thermal cycler with a sealed plate.
- Tagmentation incubation time was increased to 10 minutes.
- HP3 room-temperature incubation time is 3 minutes instead of 2 minutes.
- Final bead pelleting before Final Library transfer is 10 minutes instead of 2 minutes.
- The volume library supernatant transferred into the Final Library Plate is 19µL rather than 20µL per sample.

The firefly® protocols (listed in Table 1) are set up to process 1 to 12 columns worth of samples per run. One Illumina DNA PCR-Free Prep (96 samples) kit has successfully been used to process 96-samples. Reagent volumes may become limited if a single 96-sample kit is used to start multiple runs.

Reagent volumes and master mixes

Table 2 below outlines the reagent volumes the dispense head uses in a single run of 1 to 12 columns of samples. The number of columns to be processed can be adjusted as a variable in the firefly® protocol, which will update the volumes required in each reservoir and will be displayed in the Execute screen of the firefly® software. If processing less than 12 columns, the Tip Box Assets must first be updated to Use Strips in the Protocol Steps section before attempting to execute a protocol. There are 3 tip sets in the Part 1 protocol and 7 tip sets in the Part 2 protocol.

Reservoirs are filled and loaded onto firefly® immediately before starting each protocol. We recommend manually tip-mixing each reagent gently in the reservoir to avoid bubble formation, and at least 20 times.

	Number of Columns											
Reagent	1	2	3	4	5	6	7	8	9	10	11	12
BLT-PF	195	315	435	555	675	795	915	1,035	1,155	1,275	1,395	1,680
TB1	155	235	315	395	475	555	635	715	795	875	955	1,035
ST2	171	267	363	459	555	651	747	843	939	1,035	1,131	1,227
TWB	2,260	4,220	6,510	8,470	10,760	12,720	14,680	16,640	18,600	20,560	22,520	24,480
ELM	475	875	1,275	1,840	2,240	2,640	3,040	3,440	3,840	4,240	4,640	5,040
HP3 1X	475	875	1,275	1,840	2,240	2,640	3,040	3,440	3,840	4,240	4,640	5,040
IPB	851	1,792	2,568	3,344	4,120	4,896	5,672	6,448	7224	8,000	8,776	9,552
RSB	291	507	723	939	1,155	1,371	1,752	1,968	2,184	2,400	2,616	2,832

Volume (µL)*

Table 2. Dispense head reservoir volumes required to process 8 to 96 samples. BLT-PF – Bead-Linked Transposomes PCR-Free; ELM – Enhanced Ligation Mix; HP3 1X – 0.2N Sodium Hydroxide; IPB – Illumina Purification Beads; RSB – Resuspension Buffer; ST2 - Stop Tagment Buffer 2 - TB1 – Tagmentation Buffer 1; TWB – Tagmentation Wash Buffer.

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.

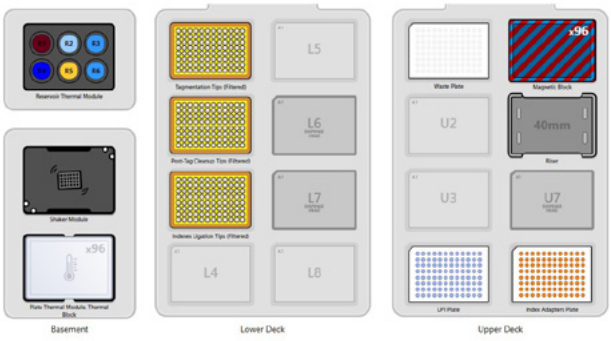

Figure 2. Starting deck layout for firefly® Illumina DNA PCR-Free Prep Part 1. Lower deck: (L1) Tagmentation Tips (Filtered) - 100µL pipetting head tips; (L2) Post-Tag Cleanup Tips (Filtered) - 100µL pipetting head tips: (L3) Indexes Ligation Tips (Filtered) - 100µL pipetting head tips. Upper deck: (U1) 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 remove 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.

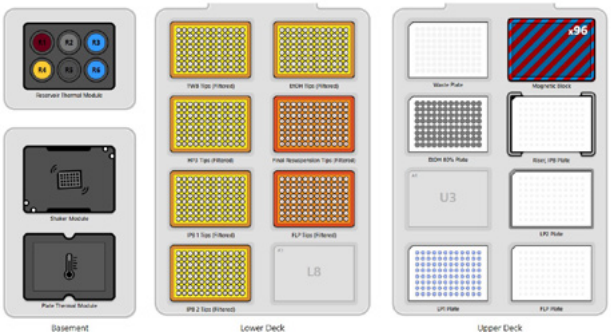

Figure 3. Starting deck layout for firefly® Illumina DNA PCR-Free Prep Part 2. Lower deck: (L1) TWB Tips (Filtered) - 100µL pipetting head tips; (L2) HP3 Tips (Filtered) - 100µL pipetting head tips, (L3) IPB 1 Tips (Filtered) - 100µL pipetting head tips; (L4) IPB 2 Tips (Filtered) - 100µL pipetting head 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.

Parts required

Supplier	Part	Part number	Number required
Illumina	Illumina DNA PCR-Free Prep, Tagmentation (96 Samples)	20041795	1
Illumina	Illumina DNA/RNA UD Indexes Tagmentation (96 Indexes, 96 Samples), Sets A-D	20091654 20091656 20091658 20091660	1
SPT Labtech	EZ-Load Pipette Tips, 100µL, with Filters, Sterile, 96 Tips per Rack	125-096-FF-FS	8 tip boxes
SPT Labtech	EZ-Load Pipette Tips, 35µL, with Filters, Ster-ile, 96 Tips per Rack	050-096-FF-FS	2 tip boxes
SPT Labtech	dragonfly® discovery syringes	4150-07200	11 syringes
SPT Labtech	dragonfly® reservoirs	4150-07103	9 reservoirs
SPT Labtech	dragonfly® discovery low dead volume reser-voirs	4150-07202	2 reservoirs
SPT Labtech	Thermo Adapter Block	3276-01065	1
SPT Labtech	40mm Upper Deck Riser	3276-01838	1
Alpaqua Engineering	Alpaqua Magnum FLX Magnet	A000400	1
Bio-Rad	Hard Shell Plate	HSP-9601	4
Thermo Fisher Scientific	Fisherbrand 1ml Deep Well	236600	3

Table 4. **Parts required to process 96 samples on firefly® using Illumina DNA PCR-Free Prep kit protocols 1 and 2, with no reuse of tips, syringes or reservoirs between protocols.**

illumina®

In partnership with sptlabtech