



firefly[®]+ technical note

Illumina DNA PCR free

Overview

- Illumina DNA PCR-Free Library Prep, Tagmentation (document # 1000000086922v03).
- Illumina DNA PCR-Free Library Prep enables to prepare up to 96 dual-indexed paired-end single-stranded libraries from DNA. This kit accepts purified DNA inputs of 25–2000ng.
- This method has been optimized using Bio-Rad Hard Shell, Fisherbrand Deep well plates and the Alpaqua Magnum FLX magnet. The use of alternative labware may require further optimization. This protocol has been written to be compatible with v2.0.1 for 96 samples.
- Instrument configuration: firefly plus 6-head, genomics, ATL head, 96 ODT Development software version: v2.0.1
- Deviation(s) from published method:
 - Ethanol Additions during bead clean-up have been slightly reduced to allow for transfer removal of ethanol with 125ul tips.

firefly protocols

Protocol number	Protocol name	firefly run time	Thermocycler run time
1 of 2	Illumina DNA PCR-Free Plate Setup	23min	0
2 of 2	Illumina DNA PCR-Free Prep	2h 7min	15min

Table 1. Overview of the protocol to process 96 samples using Illumina DNA PCR-Free Library Prep on SPT Labtech's firefly. Times may vary based on user.

Input variables

Illumina DNA PCR-Free Plate Setup			
Input Variable	Interval	Range	
		Minimum	Maximum
Number of columns (1-12)	1	1	12

Table 2: General variables for using Illumina DNA PCR-Free Plate Setup on SPT Labtech firefly liquid handler.

Illumina DNA PCR-Free Prep			
Input Variable	Interval	Range	
		Minimum	Maximum
Number of columns (1-12)	1	1	12

Table 3: General variables for using Illumina DNA PCR-Free Prep on SPT Labtech firefly liquid handler.

Reagent Calculations

Illumina DNA PCR-Free Plate Setup	
Reagent	Total Required Volume (µl)
IPB	8400.00
HP3	5040.00
RSB	4080.00
EtOH 80%	49020.00

Table 4: Reagent volumes for processing 96 samples with Illumina DNA PCR-Free Plate Setup on SPT Labtech firefly liquid handler. For details regarding the volumes required for variable sample inputs please reach out directly to SPT Labtech (see contact details below).

Illumina DNA PCR-Free Prep	
Reagent	Total Required Volume (µl)
BLT-PF	1680.00
ELM	5040.00
ST2	1227.00
TB1	1035.00
TWB	23240.00

Table 5: Reagent volumes for processing 96 samples with Illumina DNA PCR-Free Prep on SPT Labtech firefly liquid handler. For details regarding the volumes required for variable sample inputs please reach out directly to SPT Labtech (see contact details below).

Consumables and Accessories:

Pipette Head Consumable Type	Product Number(s)	Protocol 1	Protocol 2
50 µL Tips (Filtered)	050-96-FF-AL-FS	-	2
125 µL Tips (Filtered)	125-96-FF-AL-FS	-	8
ATL Tip Stand	3276-08335	-	3

Table 6: This table outlines the Pipette Head consumables required for processing 96 samples including product numbers and quantities.

Dispense Head Consumables Type	Product Number(s)	Protocol 1	Protocol 2
Syringes (Ultra-low Retention)	4150-07208	1	4
Syringes (Standard)	4150-07200	5	2
Reservoirs (Standard)	4150-07103	3	2
Reservoirs (Low Dead Volume)	4150-07202	-	2
Reservoirs (High Volume)	4150-07300	3	2

Table 7: This table outlines the Dispense Head consumables required for processing 96 samples, including product numbers and quantities (quantities refer to the individual item).

Plate and Lid Type	Product Number(s)	Protocol 1	Protocol 2
Index Adapter plate	-	-	1
Bio-Rad Hard Shell (96)	HSP-9601	4*	6 (4*)
Fisherbrand 1ml deep well plate	236600	1*	2 (1*)
Azenta Auto-Sealing PCR Plate Lid	4ti-0291	-	3
Whatman Universal Microplate Lid	7704-1001	-	1

Table 8: Table detailing the types and quantities of plates required for the protocol.
*Indicates plates that are used in both protocols.

Accessory Type	Product Number(s)	Protocol 1	Protocol 2
Alpaqua Magnum FLX	A000400	-	1
firefly HV Reservoir Tray firefly	3276-01928	1	1
HV Reservoir Tray LDV Adaptor - LDV inserts	3276-01898	-	2
firefly HV Reservoir Tray 10 mL Adaptor - Standard Insert	3276-01897	3	2

Table 9: Table detailing the accessories needed for the protocol.

Workflow overview



Figure 1. Overview of the steps performed by firefly to automate Illumina DNA PCR-Free Prep.

Workflow details

Protocol 1 of 2 - Illumina DNA PCR-Free Plate Setup

This protocol dispenses the following reagents in distinct plates, required in the Illumina DNA PCR- Free Prep protocol:

- 1) 80% Ethanol, 500µl per well
- 2) IPB, 43µl per well
- 3) LP2, 42µl per well
- 4) HP3, 50µl per well
- 5) RSB, 40µl per well

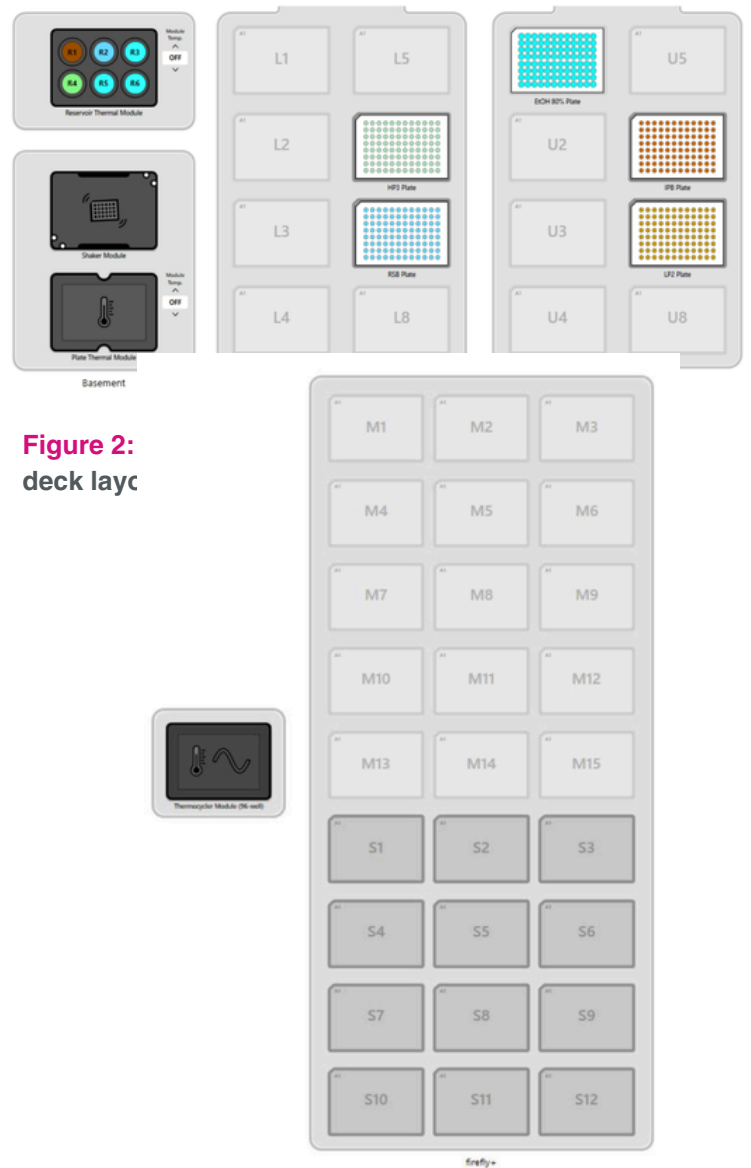


Figure 2: deck layout

Figure 3: Illumina DNA PCR-Free Plate Setup plus module initial layout.

Workflow details

Protocol 2 of 2 - Illumina DNA PCR-Free

This protocol performs the following sections described in the Illumina DNA PCR-Free Library Prep Reference Guide 1000000086922 v03.

- Tagment Genomic DNA
- Post Tagmentation Clean Up
- Ligate Indexes
- Clean up libraries

Prior to executing this protocol:

- The Illumina DNA PCR-Free Prep protocol requires pre-filled plates, previously prepared with the Illumina DNA PCR-Free Plate Setup protocol.

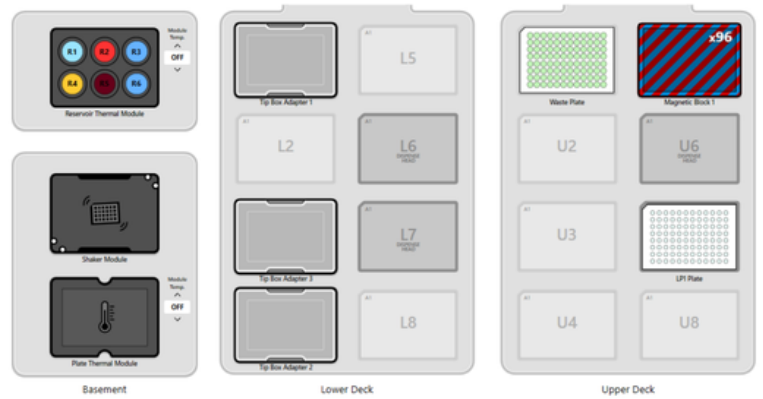


Figure 4: Illumina DNA PCR-Free initial deck layout.



Figure 5: Illumina DNA PCR-Free plus module initial layout.

Appendix 1:

Consumables and Accessories

Supplier	Part Name	Part Number
SPT Labtech	dragonfly® discovery LDV Reservoirs	4150-07202
SPT Labtech	dragonfly® Standard Reservoirs	4150-07103
SPT Labtech	dragonfly® HV Reservoirs	4150-07300
SPT Labtech	dragonfly® discovery Ultra Low Retention Syringes	4150-07208
SPT Labtech	dragonfly® discovery Syringes	4150-07200
SPT Labtech	firefly® Pipette Tips, 100µL, with Filters, Sterile	050-96-FF-AL-FS
SPT Labtech	firefly® Pipette Tips, 35µL, with Filters, Sterile	125-96-FF-AL-FS
SPT Labtech	firefly® HV Reservoir Tray	3276-01928
SPT Labtech	firefly® HV Reservoir Tray LDV Adaptor - LDV inserts	3276-01898
SPT Labtech	firefly® HV Reservoir Tray 10 mL Adaptor - Standard Insert	3276-01897
Alpaqua	Alpaqua Magnum FLX	A000400
Bio-Rad	Hard Shell 96	HSP-9601
Thermofisher	Fisherbrand 1ml Deep well plate 96	236600
Azenta	Azenta Auto-Sealing PCR Plate Lid	4ti-0291
Cytiva	Whatman Universal Microplate Lid	7704-1001

Table 8. Overview of consumables and accessories required for processing 96 samples using Illumina DNA PCR- Free on SPT Labtech's firefly indicating their part number.

For questions regarding SPT Labtech's firefly, contact our support team through fireflysupport@sptlabtech.com