



firefly® technical note

Illumina Stranded mRNA Prep - Ligation

Overview

- This technical note provides supporting information for automating Illumina Stranded mRNA Prep on SPT Labtech firefly+ liquid handler. 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.
- This protocol performs the following sections described in the Illumina Stranded mRNA Prep, Ligation Handbook (Document#1000000124518v03) to enable next-generation sequencing of stranded mRNA.
- The whole workflow from mRNA capture to library cleanup is split into two protocols on firefly+ with on deck thermo cycler. No user interaction needed.
- This protocol has been written to be compatible with v2.0.3 and tested for 24 samples of 100 ng RNA samples with different quality. The Index anchor primers were diluted 5 times, and 14 PCR cycles were used.
- This protocol produces libraries of expected size and yield. Sequencing data was not collected.
- This method has been optimized using Eppendorf twin.tec PCR plates and the Alpaqua Magnum FLX magnet. The use of alternative labware may require further optimization.
- Instrument configuration: firefly plus 6-head, genomics, ATL head, 96 ODTs.
- Variables are setup for NUMBER OF SAMPLES, SAMPLE STARTING COLUMN, INDEX ANCHOR VOLUME in the well and PCR cycles for amplification.

firefly protocols

Protocol number	Protocol name	firefly+ run time (minutes)	Thermocycler run time (minutes)
Protocol 1 of 2	Illumina stranded mRNA-FIREFLY+ Part1	2 h 35 min*	2 h
Protocol 2 of 2	Illumina stranded mRNA-FIREFLY+ Part2	2 h 35 min*	1 h 30 min**

Table 1. Protocols & estimated run times used in Illumina Stranded mRNA Prep on SPT Labtech's firefly+. Times may vary based on sample number.

*time shown in the execution page is longer than indicated here, due to Pauses setup to wait for the long ODT program to finish before resuspension of settled beads, no user interaction needed.

**Run time will vary depending on the amplification cycles required.

Input variables

Illumina stranded mRNA-firefly+ Part 1			
Input variable	Interval	Minimal	Maximal
Number of samples	8	8	96
Sample starting column	1	1	12

Table 2. Variables used in Protocol Illumina Stranded mRNA-firefly+ Part 1.

Illumina stranded mRNA-firefly+ Part 2			
Input variable	Interval	Minimal	Maximal
Number of samples	8	8	96
Index anchor volume (µL)	-	6	-
Sample starting column	1	1	1
PCR cycles for amplification	-	-	-

Table 3. Variables used in Protocol Illumina Stranded mRNA-FIREFLY+ Part2

Reagent volumes

The reagent volumes required to run Illumina Stranded mRNA Prep on SPT Labtech firefly+ depend on the number of samples being processed. Required minimum

volumes for these reagents are shown in the EXECUTE section of the firefly software.

Protocol 1 of 2

Illumina stranded mRNA-FIREFLY+ Part 1	
Reagent	Total required volume (µL) in reservoir
ELB	675
BBB	675
Fragmentation master mix	531
First Strand Synthesis master mix	297
Second Strand Synthesis master mix	705
RPBX	675
Reagent	Total required volume (µL) in reagent plate
Ethanol	350 µL/well x Nr. of columns
BWB	220 µL/well x Nr. of columns
RSB	25 µL/well x Nr. of columns
SPRI beads	100 µL/well x Nr. of columns

Table 4. Reagent volumes for processing 24 samples with Protocol Illumina Stranded mRNA-FIREFLY+ Part1 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).

Protocol 2 of 2

Illumina stranded mRNA-FIREFLY+ Part 2	
Reagent	Total required volume (µL) in reservoir
ATL	375
EPM	605
LIGX	150
RSB	1011
SPRI	2760
STL	195
Reagent	Total required volume (µL) in reagent plate
Ethanol	800 µL/well x Nr. of columns

Table 5. Reagent volumes for processing 24 samples with Protocol Illumina Stranded mRNA-FIREFLY+ Part2 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).

Protocol overview

Protocol 1 of 2



Figure 1. Schematic illustration of the workflow enabled in Protocol Illumina Stranded mRNA-Firefly+ Part1 on firefly+. mRNA is isolated, purified, and converted into high-quality double-stranded cDNA through reverse transcription and cleanup steps.

Illumina Stranded mRNA Prep-FIREFLY+ Part 1

This protocol performs the following sections described in the Illumina Stranded mRNA Prep, Ligation Handbook (Document#1000000124518v03):

- Purify and Fragment mRNA
- Synthesize First strand cDNA
- Synthesize Second strand cDNA
- cDNA Cleanup

Prior to executing this protocol:

- The Illumina Stranded mRNA Prep-FIREFLY+ (96) Part 1 protocol requires a pre-filled RNA sample input plate containing 25 μ L RNA samples (25-1000 ng), sitting on pre-cooled thermo block.

- The PPBX, ELB, BBB, Reaction mix for fragmentation (water: EPH3= 1:1), First strand synthesis MM: (FSA + RVT =9:1) and Second Strand Synthesis MM are loaded in reservoir.
- The following reagents need to be prepared in reagent plates:
 - Ethanol: 350 μ L/well x Nr. of columns
 - BWB: 220 μ L/well x Nr. of columns
 - RSB: 25 μ L/well x Nr. of columns
 - SPRI beads: 100 μ L/well x Nr. of columns

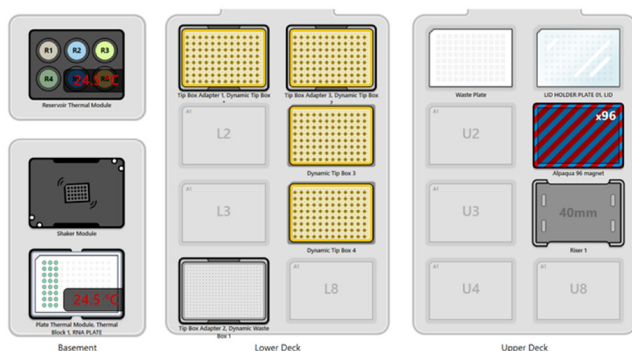


Figure 2. Illumina Stranded mRNA-firefly+ Part 1 initial deck layout for 24 samples.

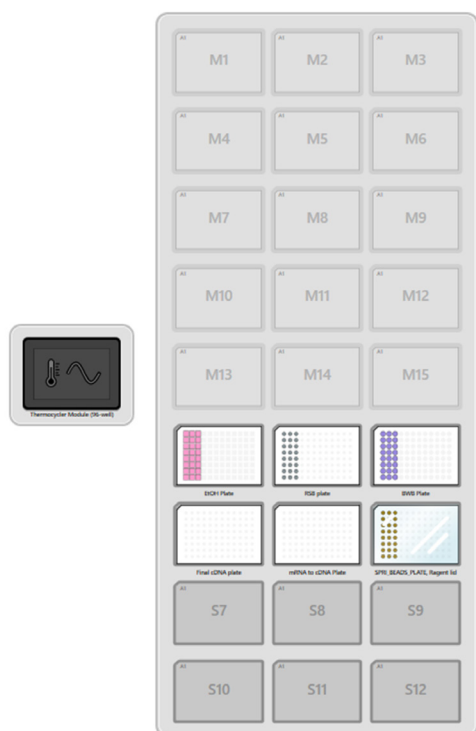


Figure 3. Illumina Stranded mRNA- firefly+ Part 1 plus module initial layout for 24 samples.

Appendix 1:

Consumables and accessories

Supplier	Part name	Part number	Part 1	Part 2
SPT Labtech	AT-Load firefly® Pipette Tips, 100 µL with filters, Sterile 96 Tips Per Rack	125-096-FF-AL-FS	4	4
SPT Labtech	Empty Tip Rack	non	1	1
SPT Labtech	dragonfly® discovery Sterile Syringes	4150-07201	5	6
SPT Labtech	dragonfly® discovery Sterile, Ultra Low Retention Syringes	4150-07209	1	0
SPT Labtech	Reservoirs (Standard)	4150-07103	0	1
SPT Labtech	Reservoirs (Low Dead Volume)	4150-07202	6	5
SPT Labtech	ATL 35-125uL Tip Stand	3276-08335	3	3
SPT Labtech	40mm Upper Deck Riser	3276-01838	1	1
SPT Labtech	Thermo Adapter Block	3276-01065	1	1

Table 6. SPT Labtech consumables required for processing 24 samples, including product numbers and quantities for Illumina Stranded mRNA Prep on firefly+.

Supplier	Part name	Part number	Part 1	Part 2
Azenta	Auto-Sealing PCR Plate Lid	4ti-0291	2	2
Thermo Fisher Scientific	Abgene 1.2mL Deep Well	AB-1127	1	1
Corning	Axygen V-bottom	P-96-459V-C	1	0
Corning	Axygen round-bottom 2.2mL deep well	P-2ML-SQ-C-S	1	1
Eppendorf	twin.tec PCR	30128648	6	7

Table 7. SPT Labtech consumables required for processing 24 samples, including product numbers and quantities for Illumina Stranded mRNA Prep on firefly+.

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