



firefly® + technical note

Illumina DNA Prep

Overview

- Illumina DNA Prep (document #1000000025416 v11)
- **The Illumina DNA Prep (IDP)** is a library prep kit that uses fragmentation to rapidly fragment DNA and add adapter sequences. The kit may be used with as little as 1ng input.
- This method has been optimised using Biorad Hard Shell 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 + 6-head, genomics, ATL head, 96 ODTC
- Development software version: v2.0.1
- Deviation(s) from the published method:
 - Ethanol Additions during bead clean-up have been slightly reduced to allow for transfer removal of ethanol with 125µl tips.

firefly protocols

| Protocol number | Protocol name | firefly run time (minutes) | Thermocycler run time (minutes) |
|-----------------|-------------------|----------------------------|---------------------------------|
| 1 of 1 | Illumina DNA Prep | 143 | 30 |

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

Input variables

| Input Variable | Interval | Range | |
|--------------------------------|----------|---------|---------|
| | | Minimum | Maximum |
| Number of columns (1-12) | 1 | 1 | 12 |
| Starting column on Index plate | 1 | 1 | 12 |

Table 2. General variables for using Illumina DNA Prep on SPT Labtech firefly liquid handler.

Master Mix Calculations – Reservoir Volumes

| Reagent | Total Required Volume (ul) |
|--|----------------------------|
| Bead- Linked Transposomes (BLT)/Fragmentation Buffer 1 (TB1) 1:1 Mix | 2320 |
| Enhanced PCR Mix (EPM)/Nuclease-free water (NFW) 1:1 Mix | 4640 |
| Nuclease-free water | 5000 |
| Resuspension Buffer (RSB) | 3600 |
| Fragment Stop Buffer (TSB) | 1240 |

Table 3. Master Mix volumes for processing 96 samples with Illumina DNA 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 |
|------------------------------|-------------------|------------|
| 50 µL Tips (Filtered) | 050-96-FF-AL-FS | — |
| 125 µL Tips (Filtered) | 125-96-FF-AL-FS | 8x |
| ATL Tip Stand | 3276-08335 | 3x |

Table 4. This table outlines the Pipette Head consumables required for the protocol, including product numbers and quantities.

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

Table 5. This table outlines the Dispense Head consumables required for the protocol, including product numbers and quantities.

| Plate Type | Product Number(s) | Protocol 1 |
|-----------------------------------|-------------------|------------|
| Biorad Hard Shell Plate | HSP-9601 | 5x |
| Abgene 0.8ml Deep Well | AB0765 | 2x |
| Abgene 1.2ml Round Deep Well | AB0564 | 1x |
| Index Adapters Plate | — | 1x |
| Azenta Auto-Sealing PCR Plate Lid | 4ti-0291 | 1x |

Table 6. Table detailing the types and quantities of plates required for the protocol.

| Accessory Type | Product Number(s) | Protocol 1 |
|-----------------------|-------------------|------------|
| Alpaqua Magnum FLX | A000400 | 1x |
| 40mm Upper Deck Riser | 3276-01838 | 1x |
| Thermo Adapter Block | 3276-01065 | 1x |

Table 7. This table lists the accessories needed for the protocol.

Workflow overview



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

Workflow details

Protocol 1 of 1 - Illumina DNA Prep

This firefly + protocol performs the following steps from the Illumina DNA Prep (document #1000000025416 v11):

- Tagment Genomic DNA
- Post Tagmentation Clean Up
- Amplify Tagmented DNA
- Clean Up Libraries

Prior to executing this protocol:

- Prepare the Tagmentation master mix (BLT/TB1 1:1)
- Prepare the DNA amplification master mix (EPM/NFW 1:1)
- Prepare reagent plates (IPB, TWB and fresh 80% EtOH)

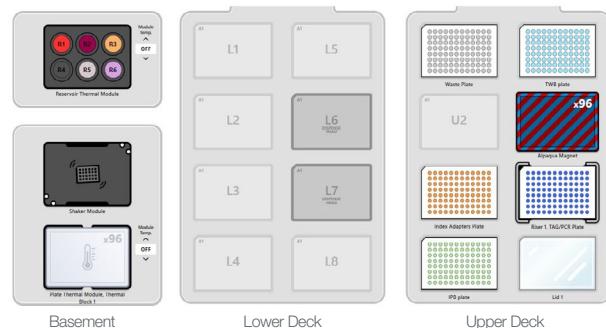


Figure 2a. Illumina DNA Prep of firefly initial deck layout.



Figure 2b. Illumina DNA Prep of firefly + module initial layout.

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

Appendix 1: Consumables and Accessories

| Supplier | Part Name | Part Number |
|--------------------------|---|-----------------|
| SPT Labtech | dragonfly® discovery LDV Reservoirs | 4150-07202 |
| SPT Labtech | dragonfly® Reservoirs | 4150-07103 |
| 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 | Thermal Adapter for PCR Plate, 96 | 3276-01065 |
| Biorad | Hard Shell Plate | HSP-9601 |
| Thermo Fisher Scientific | Abgene 0.8ml Deep Well | AB0765 |
| Thermo Fisher Scientific | Abgene 1.2ml Round Deep Well | AB0564 |
| Azenta | Azenta Auto-Sealing PCR Plate Lid | 4ti-0291 |

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