firefly® technical note

Twist Library Preparation EF 2.0 with Enzymatic Fragmentation and Twist Universal Adapter System

Overview

- Twist Library Preparation EF 2.0 with Enzymatic Fragmentation and Twist Universal Adapter System (DOC-001239 REV 6.0)
- The **Twist Library Preparation EF 2.0 Kit** with Enzymatic Fragmentation and Twist Universal Adapter System enables rapid, high-quality NGS library construction from as little as **1 ng of DNA**. Its streamlined, single-tube workflow integrates fragmentation, ligation, and amplification, making it ideal for whole-genome and targeted sequencing with high coverage uniformity and low chimera rates.
- This method was developed with 96 samples, using Eppendorf twin. Tec PCR 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 v1.8.6 for 96 samples.
- Instrument configuration: ff 6-head, genomics.
- Development software version: v1.8
- Deviation(s) from published method:
  - Some Master Mix volumes differ to accommodate reservoir dead volume. Sufficient reagent volumes supplied to process a full 96 well kit. Updated reservoir calculations can be found in Table C.
  - Ethanol Additions during bead clean-up have been reduced to allow for single transfer removal of ethanol with 125ul tips.

firefly protocols

Protocol number	Protocol name	firefly run time (minutes)	Thermocycler run time (minutes)
1 of 3	Step 1: DNA FRAGMENTATION, END REPAIR, AND dA-TAILING	4 Minutes	40 – 60 Minutes
2 of 3	Step 2: LIGATE TWIST UNIVERSAL ADAPTERS AND PURIFY	48 Minutes	15 Minutes
3 of 3	Step 3: PCR AMPLIFY USING TWIST UDI PRIMERS, PURIFY	43 Minutes	9 – 12 Minutes

Table 1. Overview of protocols to process 96 samples using Twist Library Preparation EF 2.0 with Enzymatic Fragmentation and Twist Universal Adapter System on SPT Labtech's firefly. Times may vary based on user.

Input variables

Input Variable	Interval	Range	
		Minimum	Maximum
Number of Samples	8	8	96

Table 2. General variables for using Twist Library Preparation EF 2.0 with Enzymatic Fragmentation and Twist Universal Adapter System on SPT Labtech firefly liquid handler.

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Master Mix Calculations

Protocol name	Reagent	Total Required Volume (ul)
Step 1: DNA FRAGMENTATION, END REPAIR, AND dA-TAILING	Fragmentation	1035
Step 2: LIGATE TWIST UNIVERSAL ADAPTERS AND PURI-FY	Ligation	2180
Step 2: LIGATE TWIST UNIVERSAL ADAPTERS AND PURI-FY	Universal Adaptors	580
Step 3: PCR AMPLIFY USING TWIST UDI PRIMERS, PURI-FY	Equinox Library Amp Mix (2x)	2650

Table 3. Master Mix volumes for processing 96 samples with Twist Library Preparation EF 2.0 with Enzymatic Fragmentation and Twist Universal Adapter System 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	Protocol 3
50 µL Tips (Filtered)	125-096-FF-FS (χ × 050-008-EZ-FS if <12 columns)	—	1×	1×
125 µL Tips (Filtered)	050-096-FF-FS (6χ × 125-008-EZ-FS if <12 columns)	—	6×	6×

Table 4. This table outlines the Pipette Head consumables required for each protocol, including product numbers and quantities based on the number of columns in use. *χ represents the number of columns.

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

Table 5. This table outlines the Dispense Head consumables required for each protocol, with quantities adjusted for fewer columns. *One fewer 4150-07103 reservoir and one more 4150-07202 reservoir is needed for protocols using fewer than 3 columns.

Plates Type	Product Number(s)	Protocol 1	Protocol 2	Protocol 3
Eppendorf Twin.Tec 96 Skirted Plates	0030128648	—	2×	3×
Abgene 0.8 mL Plates	AB-0765	—	—	2×
Twist UDI 96 Plate	N/A	—	—	1×

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

Accessory Type	Product Number(s)	Protocol 1	Protocol 2	Protocol 3
Tip Stand	3276-08075	—	7x	7x
Tip Loading Cassette	FFY-A-01-EZL-SL-5	—	7x	7x
Strip Tip Insert - 8 Channel	FFY-A-01-EZL-096-SC-8	—	*7x	*7x
Thermal Adapter for PCR Plate	3276-01065	1x	—	—
Alpaqua Magnum FLX	A000400	—	1x	1x

Table 7. This table lists the accessories needed for each protocol, with quantities based on column usage. *Required only when using columns.

Workflow overview

Figure 1. Overview of the steps performed by firefly to automate Twist Library Preparation EF 2.0 with Enzymatic Fragmentation and Twist Universal Adapter System. For the Target Enrichment step of this workflow please view Twist Target Enrichment Standard Hybridization v1 Workflow technical note.

Workflow details

Step numbers in protocol descriptions refer to steps in the Twist Protocol User Guide.

Protocol 1 of 3 - Step 1: DNA FRAGMENTATION, END REPAIR, AND dA-TAILING

This protocol performs enzymatic fragmentation of input gDNA and subsequent end repair and dA-tailing to generate dA-tailed DNA fragments.

Prior to executing this protocol:

- **1.1** Program the thermal cycler according to the manual with the correct conditions according to the Incubation Timetable to select conditions for fragmentation to achieve the desired insert size.
- **1.2** Mix gDNA by flicking the tube with a finger. Use the Qubit dsDNA Broad Range Quantitation Assay to determine the concentration of your genomic DNA (gDNA) samples.
- **1.2** Bring 50 ng of each gDNA sample to a total volume of 40 µl with water, 10 mM Tris-HCl pH 8, or buffer EB. Mix well with gentle pipetting.

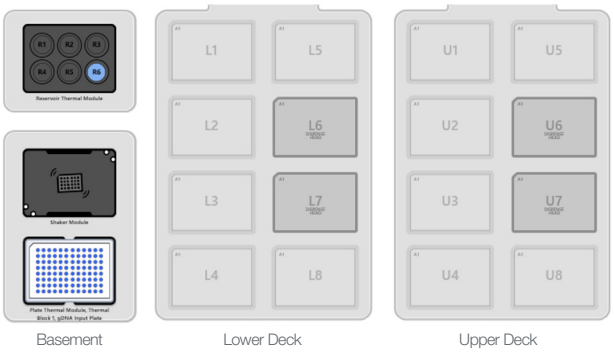

Figure 2. Protocol 1 of 3 - Step 1: DNA FRAGMENTATION, END REPAIR, AND dA-TAILING initial deck layout.

Protocol 2 of 3 - Step 2: LIGATE TWIST UNIVERSAL ADAPTERS AND PURIFY

This protocol performs the Ligation of Twist Universal Adapters to the dA-tailed DNA fragments from Step 1 and purify to generate gDNA libraries ready for index introduction through amplification in Step 3.

Prior to executing this protocol:

Program a thermal cycler to incubate samples at 20°C with the heated lid set to minimum temperature or turned off. Start the program so that the cycler has reached 20°C when the samples are done being prepared.

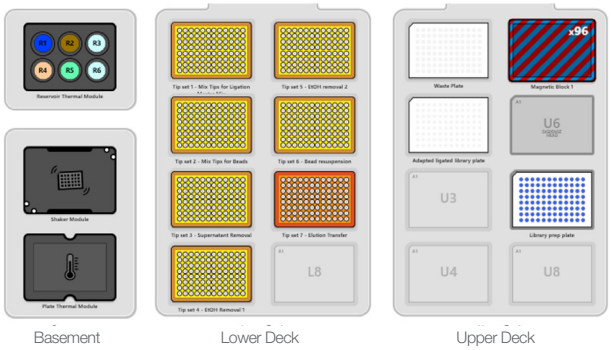

Figure 3. Protocol 2 of 3 - Step 2: LIGATE TWIST UNIVERSAL ADAPTERS AND PURIFY initial deck layout.

Protocol 3 of 3 - Step 3: PCR AMPLIFY USING TWIST UDI PRIMERS, PURIFY

This protocol performs the amplification of the adapted gDNA libraries with Twist UDI or Twist HT UDI Primers, and purify them. This protocol does not include QC to be performed after library preparation is complete.

Prior to executing this protocol:

- **2.1** Program a thermal cycler with the conditions advised within the manual.

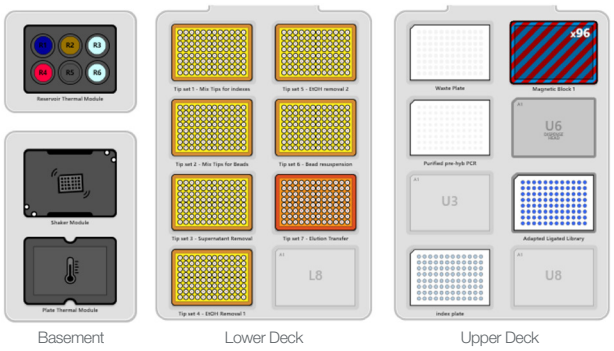

Figure 4. Protocol 3 of 3 - Step 3: PCR AMPLIFY USING TWIST UDI PRIMERS, PURIFY initial deck layout.

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

Appendix 1:
Consumables and Accessories:

Supplier	Part Name	Part Number	96 Samples	<96 samples
SPT Labtech	dragonfly® discovery LDV Reservoirs	4150-07202	2	*2
SPT Labtech	dragonfly® Reservoirs	4150-07103	10	*10
SPT Labtech	dragonfly® discovery Ultra Low Retention Syringes	4150-07208	7	7
SPT Labtech	dragonfly® discovery Syringes	4150-07200	6	6
SPT Labtech	EZ-Load Strip Tips, 050µL, Sterile 8 Tips Per Strip	050-008-EZ-S	0	**2
SPT Labtech	EZ-Load Strip Tips, 100µl, with Filters, Sterile, 8 Tips Per Strip	125-008-EZ-FS	0	**12
SPT Labtech	firefly® Pipette Tips, 100µL, with Filters, Sterile, 96 Tips per Rack	125-096-FF-FS	12	0
SPT Labtech	firefly® Pipette Tips, 35µL, with Filters, Sterile	050-096-FF-FS	2	0
SPT Labtech	Strip Tip Insert - 8 Channel Offset	FFY-A-01-EZL-096-SC-8	7	7
SPT Labtech	Universal Tip Loading Cassette	FFY-A-01-EZL-SL-5	7	7
SPT Labtech	Universal Tip Stand	3276-08075	7	7
SPT Labtech	Thermal Adapter for PCR Plate, 384	3276-01066	5	5
Eppendorf	twin.tec PCR Skirted 96 well plate	30128648	5	5
Thermo Fisher Scientific	Abgene 0.8ml 96 deep well plate	AB-0765	2	2

Table 10. Overview of protocols for processing 96 samples using Twist Library Preparation EF 2.0 Kit on SPT Labtech's firefly. Times may vary based on user.
*One fewer 4150-07103 reservoir and one more 4150-07202 reservoir is needed for protocols using fewer than 3 columns.
**multiplied by the number of columns used.