firefly® technical note

FlexPrep UHT Library Preparation with Enzymatic Fragmentation and Twist UDI Primers

This technical note provides supporting information for automating Twist FlexPrep UHT Library Preparation with Enzymatic Fragmentation and UDI Primers 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.

firefly protocols

Protocol number	Protocol name	Estimated run time (minutes)
Protocol 1 of 6	1.0 DNA Frag, End Repair & dA-tailing	40
Protocol 2 of 6	2.0A Ligate Normalization Adapters	25
Protocol 3 of 6	2.0B Ligate Normalization Adapters - Pool	10
Protocol 4 of 6	2.0C Ligate Normalization Adapters - Purify	30
Protocol 5 of 6	3.0A PCR Amplify	5
Protocol 6 of 6	3.0B PCR Purify	30

Table 1. Protocols & estimated run times used in Twist FlexPrep UHT Library Preparation with Enzymatic Fragmentation and UDI Primers on firefly.

Input variables

Protocol number	Protocol name	Variable ID	Default Value
Protocol 1 of 6	1.0 DNA Frag, End Repair & dA-tailing	Number of Samples	384
Protocol 1 of 6	1.0 DNA Frag, End Repair & dA-tailing	Number of Quadrants	4
Protocol 2 of 6	2.0A Ligate Normalization Adapters	Number of Samples	384
Protocol 2 of 6	2.0A Ligate Normalization Adapters	Number of Quadrants	4
Protocol 3 of 6	2.0B Ligate Normalization Adapters - Pool	Number of Samples	384
Protocol 3 of 6	2.0B Ligate Normalization Adapters - Pool	Number of Quadrants	4
Protocol 4 of 6	2.0C Ligate Normalization Adapters - Purify	Starting column	1
Protocol 4 of 6	2.0C Ligate Normalization Adapters - Purify	Number of samples	32
Protocol 5 of 6	3.0A PCR Amplify	Number of Samples	32
Protocol 5 of 6	3.0A PCR Amplify	UDI Primer Starting Column	1
Protocol 6 of 6	3.0B PCR Purify	Starting column	1
Protocol 6 of 6	3.0B PCR Purify	Number of Samples	32
Protocol 6 of 6	3.0B PCR Purify	Elution Volume (µL)	26
Protocol 6 of 6	3.0B PCR Purify	QC Aliquot Starting Column	2
Protocol 6 of 6	3.0B PCR Purify	cDNA Resuspension Volume (µL)	55

Table 2. Variables used in Twist FlexPrep UHT Library Preparation with Enzymatic Fragmentation and UDI Primers on firefly. Static variables, including those defined as algebraic expressions, are not shown.

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Reagent volumes

The reagent volumes required to run Twist FlexPrep UHT Library Preparation with Enzymatic Fragmentation and UDI Primers on SPT Labtech firefly depend on the number of samples being processed. Default required minimum volumes for these reagents, based on the number of samples shown in the **Input variables** table, are shown below and in the EXECUTE section of the firefly software.

Protocol 1 of 6
1.0 DNA Frag, End Repair & dA-tailing

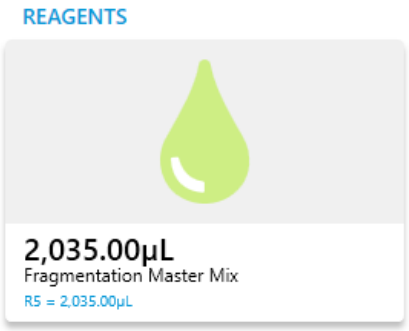

Figure 1. 1.0 DNA Frag, End Repair & dA-tailing minimum required reagent volumes.

Protocol 2 of 6
2.0A Ligate Normalization Adapters

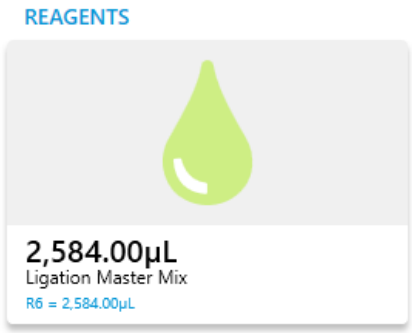

Figure 2. 2.0A Ligate Normalization Adapters minimum required reagent volumes.

Protocol 4 of 6
2.0C Ligate Normalization Adapters - Purify

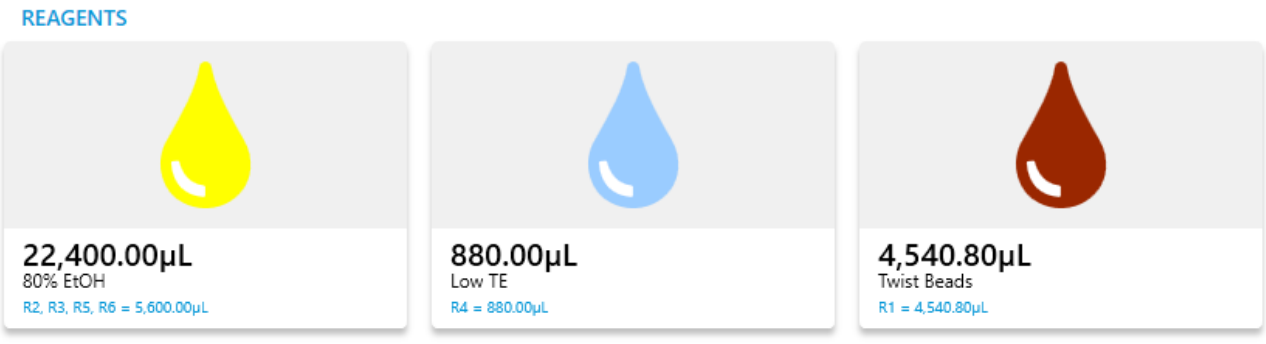

Figure 3. 2.0C Ligate Normalization Adapters - Purify minimum required reagent volumes.

Protocol 5 of 6
3.0A PCR Amplify

Figure 4. 3.0A PCR Amplify minimum required reagent volumes.

Protocol 6 of 6
3.0B PCR Purify

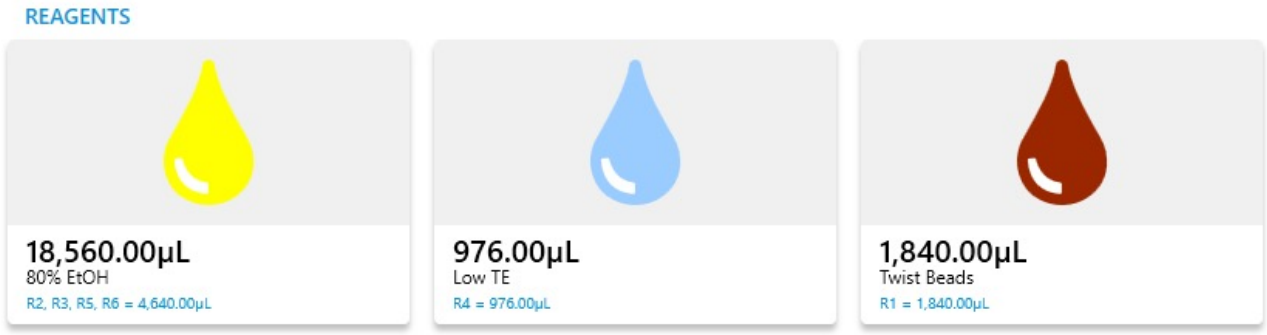

Figure 5. 3.0B PCR Purify minimum required reagent volumes.

Consumables

Supplier	Part Name	Part Number	Number Required	Note
SPT Labtech	40mm Upper Deck Riser	3276-01838	1	
SPT Labtech	dragonfly® discovery Sterile LDV Reservoirs	4150-07203	4	Reservoir types needed are dependent on the number of columns processed
SPT Labtech	dragonfly® discovery Sterile Reservoirs	4150-07204	14	
SPT Labtech	dragonfly® discovery Sterile Syringes	4150-07201	10	
SPT Labtech	dragonfly® discovery Sterile, Ultra Low Retention Syringes	4150-07209	8	Number required depends on the number of columns processed
SPT Labtech	firefly® Pipette Tips, 35µL, with Filters, Sterile	050-384-FF-FS	1	
SPT Labtech	firefly® Strip Tips, 100µl, with Filters, Sterile	125-008-FF-FS	13	Waste plate
SPT Labtech	firefly® Pipette Tips, 35µL, with Filters, Sterile	050-096-FF-FS	4	
SPT Labtech	firefly® Pipette Tips, 125µL, Sterile, 96 Tips per Rack	125-096-FF-S	4	
Alpaqua Engineering	Alpaqua Magnum FLX	A000400	1	
Thermo Fisher Scientific	Fisherbrand 1ml Deep Well	236600	1	
Bio-Rad	Hard Shell Plate (HSP)	HSP-3801	1	
Greiner	V-bottom Chimney Well	651261	2	
Eppendorf	twin.tec PCR	30128648	2	

Table 3. Consumables & labware required for Twist FlexPrep UHT Library Preparation with Enzymatic Fragmentation and UDI Primers on firefly.

Protocol overview

These protocols were developed in v1.9 of the firefly software using 384 samples with an input range of 30 – 300 ng. Development was performed using Biorad 384-Well PCR (HSP3805), Greiner 96 v-bottom chimney well (651201), Eppendorf 96 twin.tec PCR (951020401) plates, and Alpaqua's Magnum FLX 96 magnet. The use of alternative labware may require further optimization.

Protocol 1 of 6
1.0 DNA Frag, End Repair & dA-tailing

This protocol performs step 1 of Twist Bioscience's FlexPrep UHT Library Preparation with Enzymatic Fragmentation and Twist UDI Primers (#109224, DOC-001511 REV 1.0)

Prior to executing this protocol:

- Prepare gDNA samples, 30 – 300 ng in total volume of 5 µL Thaw or place the following on ice:
  - Molecular biology grade water
  - gDNA samples
  - 10x Twist FlexPrep Fragmentation Enzyme Mix
  - 5x Twist FlexPrep Fragmentation Buffer 1.1 Program thermal cycler(s)

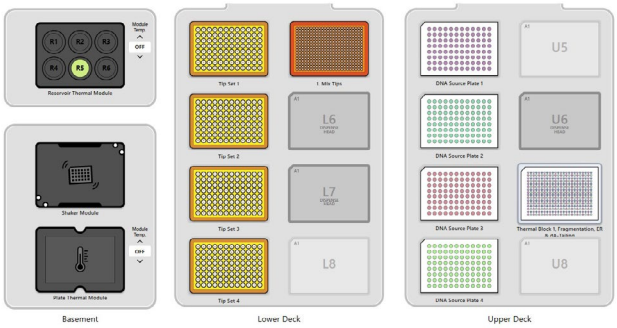

Figure 6. 1.0 DNA Frag, End Repair & dA-tailing deck layout.

Protocol 2 of 6
2.0A Ligate Normalization Adapters

This protocol performs step 1 of Twist Bioscience's FlexPrep UHT Library Preparation with Enzymatic Fragmentation and Twist UDI Primers (#109224, DOC-001511 REV 1.0)

Prior to executing this protocol:

- Thaw or place the following on ice:
  - Twist FlexPrep Normalization Adapters (96-well plate; multiple reactions in each well)
  - 20x Twist FlexPrep Ligation Mix
  - 4x Twist FlexPrep Ligation Buffer Prepare 2 ml 80% ethanol for every 12 libraries generated (for use in both Steps 2 and 3 of the protocol). Equilibrate DNA Purification Beads to room temperature for at least 30 minutes (for use in both Steps 2 and 3 of the protocol)
- **2.1** Program thermal cycle
- **2.2** Vortex the Twist FlexPrep Normalization Adapter plate for 5 seconds. Pulse-spin to collect all liquid in the bottom of each well

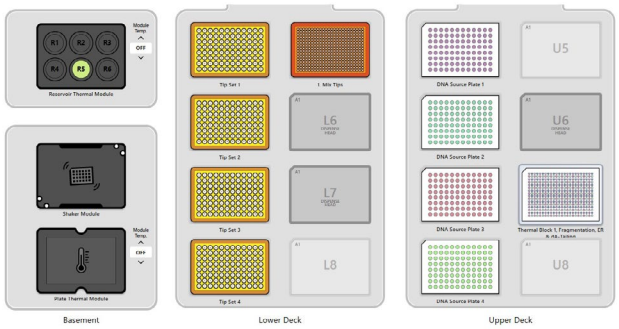

Figure 7. 2.0A Ligate Normalization Adapters deck layout.

Protocol 3 of 6
2.0B Ligate Normalization Adapters - Pool

This protocol performs step 2.11 of Twist Bioscience's FlexPrep UHT Library Preparation with Enzymatic Fragmentation and Twist UDI Primers (#109224, DOC-001511 REV 1.0)

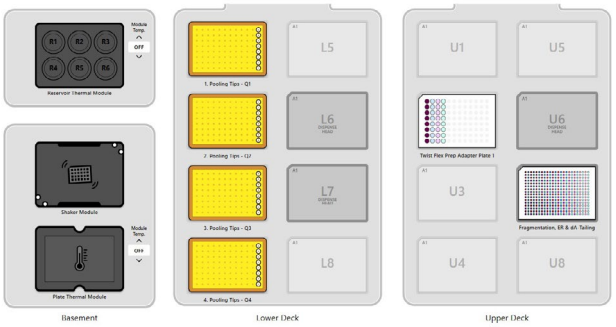

Figure 8. 2.0B Ligate Normalization Adapters - Pool deck layout.

Protocol 4 of 6
2.0C Ligate Normalization Adapters - Purify

This protocol performs steps 2.14 – 2.25 of Twist Bioscience's FlexPrep UHT Library Preparation with Enzymatic Fragmentation and Twist UDI Primers (#109224, DOC-001511 REV 1.0)

Prior to executing this protocol:

- Prepare 2 ml 80% ethanol for every 12 libraries generated (for use in both Steps 2 and 3 of the protocol)
- Equilibrate DNA Purification Beads to room temperature for at least 30 minutes (for use in both Steps 2 and 3 of the protocol)

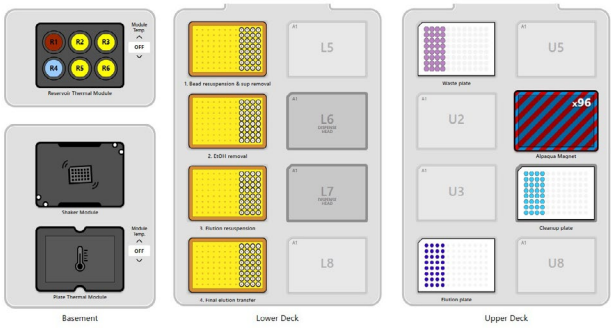

Figure 9. 2.0C Ligate Normalization Adapters - Purify deck layout.

Protocol 5 of 6
3.0A PCR Amplify

This protocol performs steps 3.2 – 3.3 of Twist Bioscience's FlexPrep UHT Library Preparation with Enzymatic Fragmentation and Twist UDI Primers (#109224, DOC-001511 REV 1.0)

Prior to executing this protocol:

- Thaw or place on ice:
  - 2x Twist Library Amp Mix
  - Twist UDI Primers 3.1 Program thermal cycler

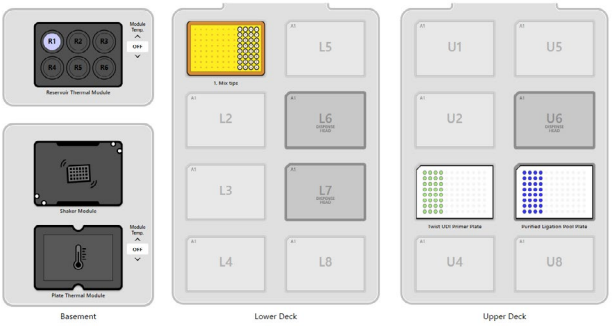

Figure 10. 3.0A PCR Amplify deck layout.

Protocol 6 of 6
3.0B PCR Purify

This protocol performs steps 3.7 – 3.18 of Twist Bioscience's FlexPrep UHT Library Preparation with Enzymatic Fragmentation and Twist UDI Primers (#109224, DOC-001511 REV 1.0)

Prior to executing this protocol:

- Vortex pre-equilibrated DNA Purification Beads

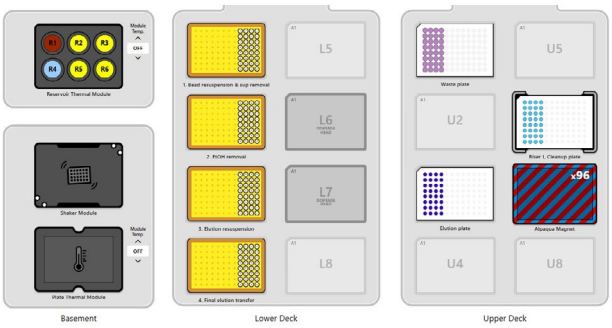

Figure 11. 3.0B PCR Purify deck layout.