



firefly® technical note

Watchmaker DNA Library Prep Kit with Fragmentation

This technical note provides supporting information for automating Watchmaker DNA Library Prep Kit with Fragmentation 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 5	1.0 FragERAT	5
Protocol 2 of 5	2.0 Adapter Ligation	20
Protocol 3 of 5	3.0 Post Ligation Cleanup	30
Protocol 4 of 5	4.0 LibraryAmp	5
Protocol 5 of 5	5.0 PostAmp Cleanup	30

Table 1. Protocols & estimated run times used in Watchmaker DNA Library Prep Kit with Fragmentation on firefly.

Input variables

Protocol number	Protocol name	Variable ID	Default Value
Protocol 1 of 5	1.0 FragERAT	Number of Samples/Wells	96
Protocol 1 of 5	1.0 FragERAT	LDV Reservoir Volume (µL)	75
Protocol 1 of 5	1.0 FragERAT	Standard Reservoir Volume (µL)	240
Protocol 1 of 5	1.0 FragERAT	Frag/AT MM volume (µL)	10
Protocol 1 of 5	1.0 FragERAT	Sample Volume (µL)	40
Protocol 2 of 5	2.0 Adapter Ligation	Number of samples/wells	96
Protocol 2 of 5	2.0 Adapter Ligation	LDV reservoir volume (µL)	75
Protocol 2 of 5	2.0 Adapter Ligation	Standard reservoir volume (µL)	240
Protocol 2 of 5	2.0 Adapter Ligation	Sample input volume (µL)	50
Protocol 2 of 5	2.0 Adapter Ligation	Adapter volume (µL)	5
Protocol 2 of 5	2.0 Adapter Ligation	Ligation Master Mix Volume (µL)	20
Protocol 3 of 5	3.0 Post Ligation Cleanup	Number of Samples	96
Protocol 3 of 5	3.0 Post Ligation Cleanup	LDV Reservoir Volume (µL)	75
Protocol 3 of 5	3.0 Post Ligation Cleanup	Standard Reservoir Volume (µL)	240
Protocol 3 of 5	3.0 Post Ligation Cleanup	Ligated DNA Sample Input Volume (µL)	75
Protocol 3 of 5	3.0 Post Ligation Cleanup	Cleanup Ratio (X)	0.8
Protocol 3 of 5	3.0 Post Ligation Cleanup	Elution Volume (µL)	20
Protocol 3 of 5	3.0 Post Ligation Cleanup	Number of EtOH Syringes/Reservoirs	4
Protocol 4 of 5	4.0 LibraryAmp	Number of Samples	96
Protocol 4 of 5	4.0 LibraryAmp	LDV reservoir volume (µL)	75

Protocol number	Protocol name	Variable ID	Default Value
Protocol 4 of 5	4.0 LibraryAmp	Standard reservoir volume (μ L)	240
Protocol 4 of 5	4.0 LibraryAmp	Post Ligation Cleanup Volume (μ L)	20
Protocol 4 of 5	4.0 LibraryAmp	Equinox Amp MM Volume (μ L)	25
Protocol 4 of 5	4.0 LibraryAmp	Index Primer Volume (μ L)	2
Protocol 4 of 5	4.0 LibraryAmp	Index Primer Start Column	1
Protocol 5 of 5	5.0 PostAmp Cleanup	Number of Samples	96
Protocol 5 of 5	5.0 PostAmp Cleanup	LDV Reservoir Volume (μ L)	75
Protocol 5 of 5	5.0 PostAmp Cleanup	Standard Reservoir Volume (μ L)	240
Protocol 5 of 5	5.0 PostAmp Cleanup	Amplified Library Volume (μ L)	50
Protocol 5 of 5	5.0 PostAmp Cleanup	Cleanup Ratio (X)	1
Protocol 5 of 5	5.0 PostAmp Cleanup	Elution Volume (μ L)	20
Protocol 5 of 5	5.0 PostAmp Cleanup	Number of EtOH Syringes/Reservoirs	4
Protocol 5 of 5	5.0 PostAmp Cleanup	Elution Overage Volume (μ L)	2

Table 2. Variables used in Watchmaker DNA Library Prep Kit with Fragmentation on firefly.
Static variables, including those defined as algebraic expressions, are not shown.

Reagent volumes

The reagent volumes required to run Watchmaker DNA Library Prep Kit with Fragmentation 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 5

1.0 FragERAT

REAGENTS



Figure 1. 1.0 FragERAT minimum required reagent volumes.

Protocol 2 of 5

2.0 Adapter Ligation

REAGENTS

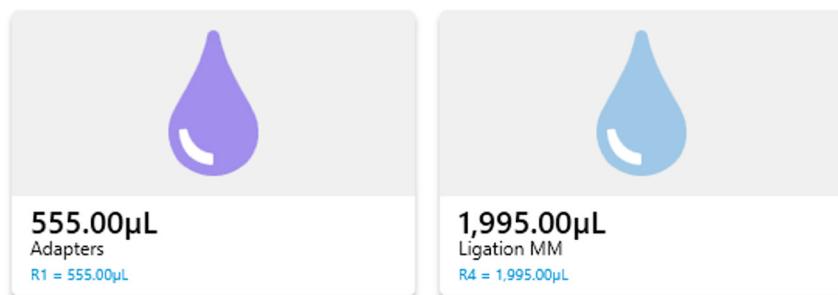


Figure 2. 2.0 Adapter Ligation minimum required reagent volumes.

Protocol 3 of 5

3.0 Post Ligation Cleanup

REAGENTS



Figure 3. 3.0 Post Ligation Cleanup minimum required reagent volumes.

Protocol 4 of 5

4.0 LibraryAmp

REAGENTS

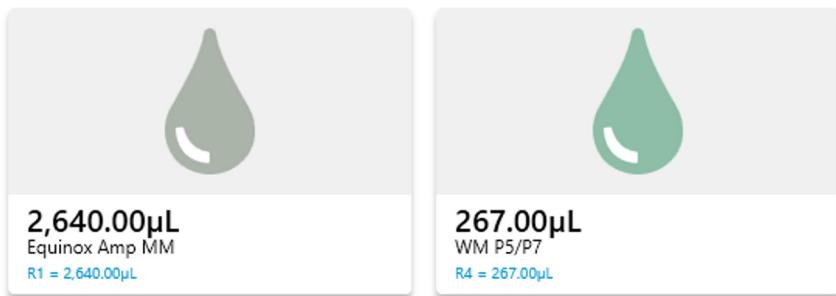


Figure 4. 4.0 LibraryAmp minimum required reagent volumes.

Protocol 5 of 5

5.0 PostAmp Cleanup

REAGENTS



Figure 5. 5.0 PostAmp Cleanup minimum required reagent volumes.

Consumables

Supplier	Part Name	Part Number	Number Required
SPT Labtech	40mm Upper Deck Riser	3276-01838	1
SPT Labtech	dragonfly® discovery Sterile LDV Reservoirs	4150-07203	5
SPT Labtech	dragonfly® discovery Sterile Reservoirs	4150-07204	12
Alpaqua Engineering	Alpaqua Magnum FLX	A000400	1
Thermo Fisher Scientific	Abgene 0.8mL Deep Well	AB0765	2
Bio-Rad	Hard Shell Plate (HSP)	HSP-9601	3
SPT Labtech	firefly® Pipette Tips, 125µL, Sterile, 96 Tips per Rack	125-096-FF-S	17
SPT Labtech	dragonfly® discovery Sterile Syringes	4150-07201	5
SPT Labtech	dragonfly® discovery Sterile, Ultra Low Retention Syringes	4150-07209	12

Table 3. Consumables & labware required for Watchmaker DNA Library Prep Kit with Fragmentation on firefly.

Protocol overview

This suite of protocols perform Watchmaker's DNA Library Prep Kit with Fragmentation, 7K0022 v1.1.1021

These protocols were developed with 16 samples, using Biorad HSP-9601 PCR plates and the Alpaqua Magnum FLX magnet. The use of alternative labware may require further optimization.

Protocol 1 of 5

1.0 FragERAT

Protocol 1 of 5. Enzymatic Fragmentation, End Repair and A-tailing (Frag/AT) This protocol performs section 1 of Watchmaker DNA Library Prep Kit with Fragmentation, 7K0022 v1.1.1021.

Prior to executing this protocol:

- 1.1-1.3 Prepare Frag/AT Master Mix on ice
- 1.4 Prepare input DNA in total volume of 40 µL. Dilute PCR-grade H2O, 10 mM Tris-HCL 8.0 or 0.1 mM EDTA
- 1.6 Preheat thermalcycler

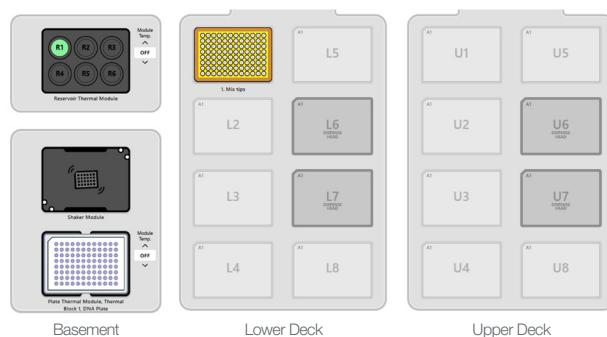


Figure 6. 1.0 FragERAT deck layout.

Protocol 2 of 5

2.0 Adapter Ligation

Protocol 2 of 5. Adapter Ligation This protocol performs section 2 of Watchmaker DNA Library Prep Kit with Fragmentation, 7K0022 v1.1.1021.

Prior to executing this protocol:

- 1. Prepare adapter stock solutions
- 2. Thaw and invert Ligation Master Mix to homogenize (DO NOT MIX)

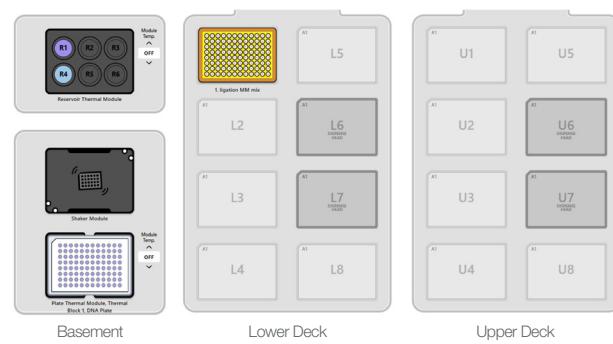


Figure 7. 2.0 Adapter Ligation deck layout.

Protocol 3 of 5

3.0 Post Ligation Cleanup

Protocol 3 of 5. Post-Ligation Cleanup This protocol performs section 3 of Watchmaker DNA Library Prep Kit with Fragmentation, 7K0022 v1.1.1021.

Prior to executing this protocol:

- 1. Bring beads to room temperature
- 2. Prepare fresh 80% EtOH

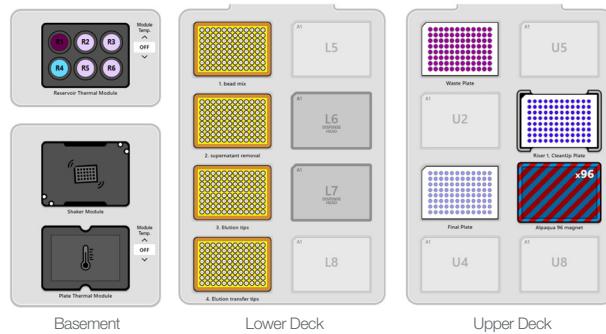


Figure 8. 3.0 Post Ligation Cleanup deck layout.

Protocol 4 of 5

4.0 LibraryAmp

Protocol 4 of 5. Library Amplification This protocol performs section 4 of Watchmaker DNA Library Prep Kit with Fragmentation, 7K0022 v1.1.1021.

Prior to executing this protocol:

- 4.2 Thaw Equinox Amplification Master Mix, mix by inversion - DO NOT VORTEX, load to R1
- 4.3 Program and preheat thermalcycler

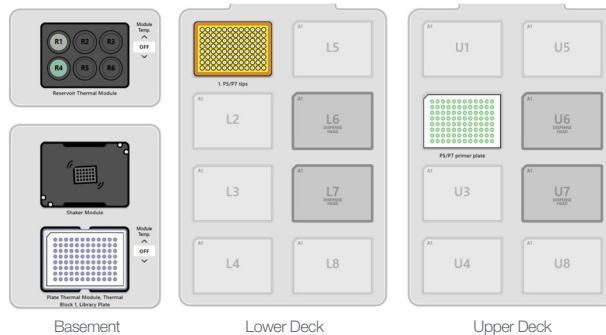


Figure 9. 4.0 LibraryAmp deck layout.

Protocol 5 of 5

5.0 PostAmp Cleanup

Protocol 5 of 5. Post-Ligation Cleanup This protocol performs section 3 of Watchmaker DNA Library Prep Kit with Fragmentation, 7K0022 v1.1.1021.

Prior to executing this protocol:

- 1. Bring beads to room temperature
- 2. Prepare fresh 80% EtOH

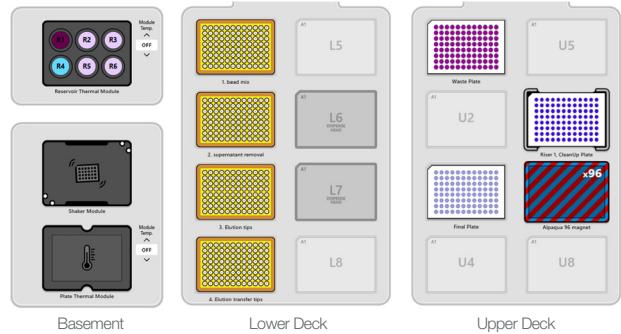


Figure 10. 5.0 PostAmp Cleanup deck layout.