



firefly® technical note

Watchmaker DNA Library Prep Kit with Fragmentation (PCR free)

This technical note provides supporting information for automating Watchmaker DNA Library Prep Kit with Fragmentation (PCR free) 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 3	1.0 FragERAT - PCRfree	5
Protocol 2 of 3	2.0 Adapter Ligation - PCRfree	20
Protocol 3 of 3	3.0 Post Ligation Cleanup - PCRfree	30

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

Input variables

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

Table 2. Variables used in Watchmaker DNA Library Prep Kit with Fragmentation (PCR free) 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 (PCR free) 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 3

1.0 FragERAT - PCRfree

REAGENTS



1,035.00µL
5x Frag/AT MM
R1 = 1,035.00µL

Figure 1. 1.0 FragERAT - PCRfree minimum required reagent volumes.

Protocol 2 of 3

2.0 Adapter Ligation - PCRfree

REAGENTS



1,995.00µL
Ligation MM
R4 = 1,995.00µL

Figure 2. 2.0 Adapter Ligation - PCRfree minimum required reagent volumes.

Protocol 3 of 3

3.0 Post Ligation Cleanup - PCRfree

REAGENTS



2,352.00µL
10 mM Tris-HCl pH 8.1
R4 = 2,352.00µL



37,760.00µL
80% EtOH
R2, R3, R5, R6 = 9,440.00µL



6,000.00µL
Beads
R1 = 6,000.00µL

Figure 3. 3.0 Post Ligation Cleanup - PCRfree 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	2	Reservoir types needed are dependent on the number of columns processed
SPT Labtech	dragonfly® discovery Sterile Reservoirs	4150-07204	6	
Alpaqua Engineering	Alpaqua Magnum FLX	A000400	1	
Thermo Fisher Scientific	Abgene 0.8mL Deep Well	AB0765	1	Number required depends on the number of columns processed
Bio-Rad	Hard Shell Plate (HSP)	HSP-9601	5	
nan	nan	nan	8	Waste plate
nan	nan	nan	4	
nan	nan	nan	2	

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

Protocol overview

This suite of protocols performs Watchmaker's DNA Library Prep Kit with Fragmentation, PCR-free, 7K0022 v1.1.1021.

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

This protocol has been updated to be compatible with v1.4 for 96 samples.

Protocol 1 of 3

1.0 FragERAT - PCRfree

This protocol performs section 1 of Watchmaker DNA Library Prep Kit with Fragmentation, PCR-free, 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 μ L. Dilute PCR-grade H₂O, 10 mM Tris-HCl 8.0 or 0.1 mM EDTA
- **1.6** Preheat thermalcycler

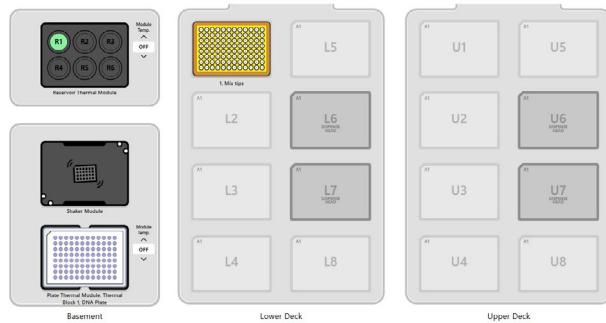


Figure 4. 1.0 FragERAT - PCRfree deck layout.

Protocol 2 of 3

2.0 Adapter Ligation - PCRfree

This protocol performs section 2 of Watchmaker DNA Library Prep Kit with Fragmentation, PCR-free, 7K0022 v1.1.1021.

Prior to executing this protocol:

- Prepare adapter stock solutions
- Thaw and invert Ligation Master Mix to homogenize (DO NOT MIX)
- Thaw UMI adapter plate

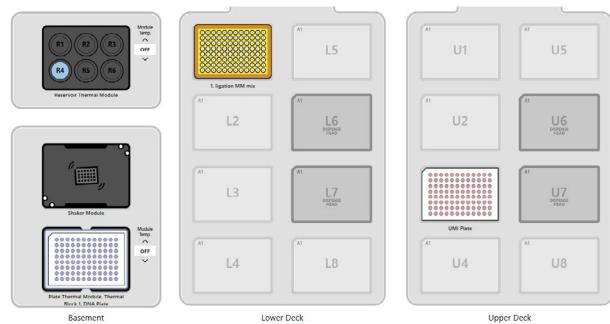


Figure 5. 2.0 Adapter Ligation - PCRfree deck layout.

Protocol 3 of 3

3.0 Post Ligation Cleanup - PCRfree

This protocol performs section 3 of Watchmaker DNA Library Prep Kit with Fragmentation, PCR Free, 7K0022 v1.1.1021.

Prior to executing this protocol:

- Bring beads to room temperature
- Prepare fresh 80% EtOH

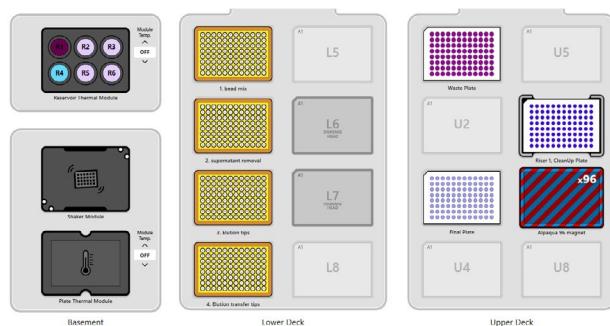


Figure 6. 3.0 Post Ligation Cleanup - PCRfree deck layout.