firefly® technical note

Watchmaker RNA Library Prep Kit with Polaris Depletion

This technical note provides supporting information for automating Watchmaker RNA Library Prep Kit with Polaris Depletion 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 8	A1 rRNA & Globin Depletion	5
Protocol 2 of 8	A2. Proble Digestion	5
Protocol 3 of 8	A3 - A4. Post-depletion Cleanup - Fragmentation & Priming	35
Protocol 4 of 8	A5 - A7. 1st & 2nd Strand Synthesis, Adapter Ligation	10
Protocol 5 of 8	A8. Post-Ligation Cleanup	35
Protocol 6 of 8	A9. 2nd Post-Ligation Cleanup (Optional)	35
Protocol 7 of 8	A10. Library Amplification & Strand Selection	10
Protocol 8 of 8	A11. Post-Amplification Cleanup	35

Table 1. Protocols & estimated run times used in Watchmaker RNA Library Prep Kit with Polaris Depletion on firefly.

Input variables

Protocol number	Protocol name	Variable ID	Default Value
Protocol 1 of 8	A1 rRNA & Globin Depletion	Number of Samples	96
Protocol 2 of 8	A2. Proble Digestion	Number of Samples	96
Protocol 2 of 8	A2. Proble Digestion	Standard reservoir dead volume (µL)	240
Protocol 2 of 8	A2. Proble Digestion	Number of Syringes/Capture Buffer	4
Protocol 3 of 8	A3 - A4. Post-depletion Cleanup - Fragmentation & Priming	number of samples	96
Protocol 4 of 8	A5 - A7. 1st & 2nd Strand Synthesis, Adapter Ligation	Number of Samples	96
Protocol 4 of 8	A5 - A7. 1st & 2nd Strand Synthesis, Adapter Ligation	Ligation MM overage (%)	0.1
Protocol 5 of 8	A8. Post-Ligation Cleanup	Number of Samples	96
Protocol 6 of 8	A9. 2nd Post-Ligation Cleanup (Optional)	Number of Samples	96
Protocol 7 of 8	A10. Library Amplification & Strand Selection	Number of Samples	96
Protocol 7 of 8	A10. Library Amplification & Strand Selection	Primer Plate Starting Column	1
Protocol 8 of 8	A11. Post-Amplification Cleanup	Number of Samples	96
Protocol 8 of 8	A11. Post-Amplification Cleanup	SPRI Bead Volume (µL)	50

Table 2. Variables used in Watchmaker RNA Library Prep Kit with Polaris Depletion 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 Watchmaker RNA Library Prep Kit with Polaris Depletion 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 8
A1 rRNA & Globin Depletion

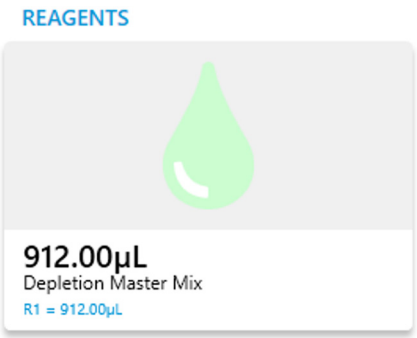

Figure 1. A1 rRNA & Globin Depletion minimum required reagent volumes.

Protocol 2 of 8
A2. Proble Digestion

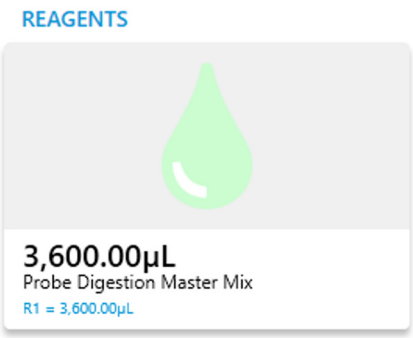

Figure 2. A2. Proble Digestion minimum required reagent volumes.

Protocol 3 of 8
A3 - A4. Post-depletion Cleanup - Fragmentation & Priming

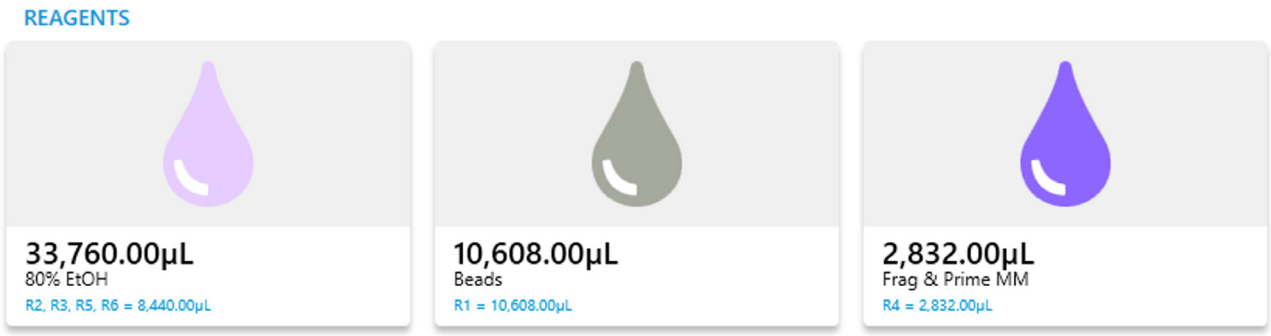

Figure 3. 3 - A4. Post-depletion Cleanup - Fragmentation & Priming minimum required reagent volumes.

Protocol 4 of 8
A5 - A7. 1st & 2nd Strand Synthesis, Adapter Ligation

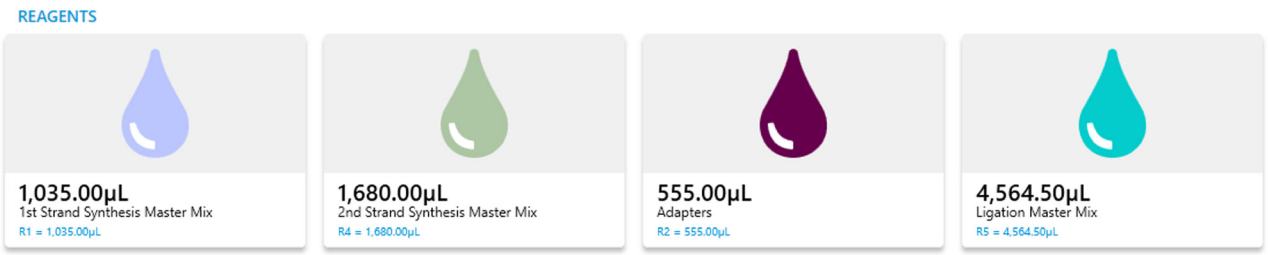

Figure 4. A5 - A7. 1st & 2nd Strand Synthesis, Adapter Ligation minimum required reagent volumes.

Protocol 5 of 8
A8. Post-Ligation Cleanup

Figure 5. A8. Post-Ligation Cleanup minimum required reagent volumes.

Protocol 6 of 8
A9. 2nd Post-Ligation Cleanup (Optional)

Figure 6. A9. 2nd Post-Ligation Cleanup (Optional) minimum required reagent volumes.

Protocol 7 of 8
A10. Library Amplification & Strand Selection

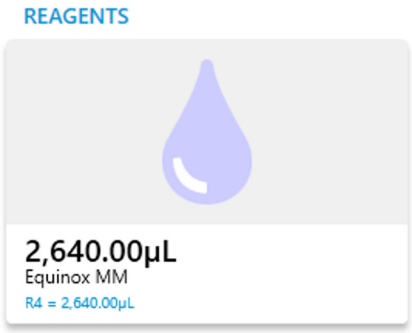

Figure 7. A10. Library Amplification & Strand Selection minimum required reagent volumes.

Protocol 8 of 8
A11. Post-Amplification Cleanup

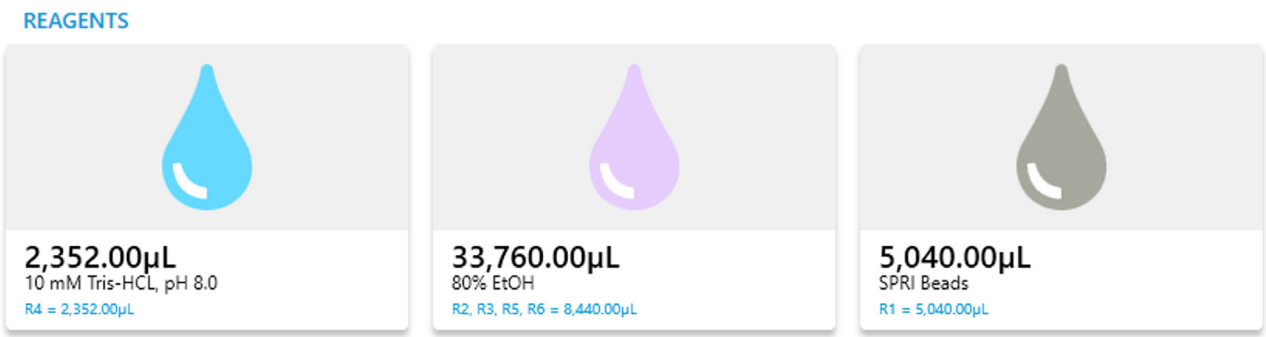

Figure 8. A11. Post-Amplification Cleanup minimum required reagent volumes.

Consumables

Supplier	Part Name	Part Number	Number Required
SPT Labtech	40mm Upper Deck Riser	3276-01838	1
SPT Labtech	dragonfly® discovery Sterile Reservoirs	4150-07204	31
SPT Labtech	dragonfly® discovery Sterile Syringes	4150-07201	30
SPT Labtech	dragonfly® discovery Sterile, Ultra Low Retention Syringes	4150-07209	1
SPT Labtech	firefly® Pipette Tips, 100µL, with Filters, Sterile, 96 Tips per Rack	125-096-FF-FS	19
SPT Labtech	firefly® Pipette Tips, 125µL, Sterile, 96 Tips per Rack	125-096-FF-S	2
Alpaqua Engineering	Alpaqua Magnum FLX	A000400	1
Thermo Fisher Scientific	Fisherbrand 1ml Deep Well	236600	4
Bio-Rad	Hard Shell Plate (HSP)	HSP-9601	2
Eppendorf	twin.tec PCR	30128648	4

Table 3. Consumables & labware required for Watchmaker RNA Library Prep Kit with Polaris Depletion on firefly.

Protocol Overview

This suite of protocols execute Watchmaker's RNA Library Prep Kit with Polaris Depletion – rRNA/Globin(HMR), 7K0077/78-096, v2.0.0523, Protocol A: High Quality and partially degraded samples.

This method was developed with an EZ-load 6 head genomics (v1.5.4 software) with firefly 16 samples and published for use with 96 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 8
A1 rRNA & Globin Depletion

This protocol performs section 1 of the Watchmaker RNA Library Prep Kit with Polaris Depletion – rRNA/Globin(HMR), 7K0077/78-096, v2.0.0523, Protocol A: High Quality and partially degraded samples.

Prior to executing this protocol:

- **A1.1** Program & preheat thermocycler
- **A1.2** Prepare Depletion Reaction Mix
- **A1.4** On ice, prepare input RNA in a total volume of 18 µL using RNase-free water

This protocol is compatible with 8 – 96 samples as written and has been updated to v1.8.6 firefly software.

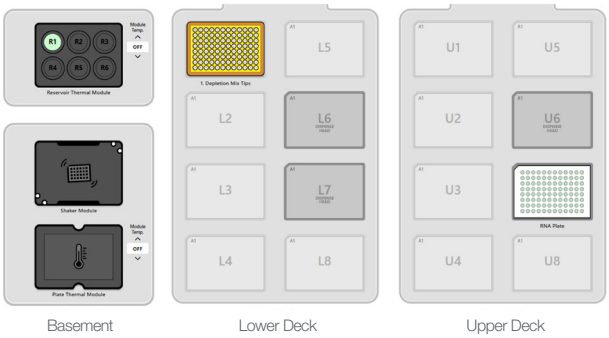

Figure 9. A1 rRNA & Globin Depletion deck layout.

Protocol 2 of 8
A2. Proble Digestion

This protocol performs section 2 of the Watchmaker RNA Library Prep Kit with Polaris Depletion – rRNA/Globin(HMR), 7K0077/78-096, v2.0.0523, Protocol A: High Quality and partially degraded samples.

Prior to executing this protocol:

- **A2.1** Program & preheat thermocycler

This protocol is compatible with 8 – 96 samples as written and has been updated to v1.8.6 firefly software.

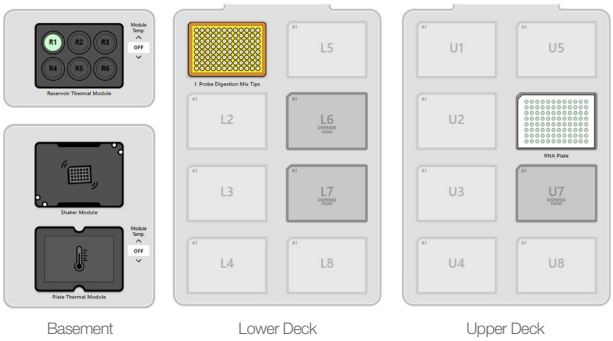

Figure 10. A2. Proble Digestion deck layout.

Protocol 3 of 8
A3 - A4. Post-depletion Cleanup - Fragmentation & Priming

This protocol performs sections 3-4 of Watchmaker RNA Library Prep Kit with Polaris Depletion – rRNA/Globin(HMR), 7K0077/78-096, v2.0.0523, Protocol A: High Quality and partially degraded samples.

Prior to executing this protocol:

- **A3.1** Freshly prepare 80% EtOH
- **A3.2** Vortex room temperature SPRI beads to thoroughly mix
- **A4.1** Prepare the Frag & Primer Master Mix
- **A4.5** Program & preheat thermocycler

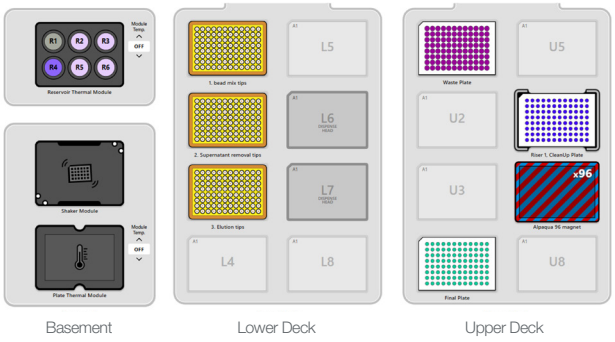

Figure 11. A3 - A4. Post-depletion Cleanup - Fragmentation & Priming deck layout.

This protocol is compatible with 48 – 96 samples as written and has been updated to v1.8.6 firefly software. To process > 48 samples, update 80% Ethanol reservoir asset and Aspirate steps.

Protocol 4 of 8
A5 - A7. 1st & 2nd Strand Synthesis, Adapter Ligation

This protocol performs sections 5-7 of the Watchmaker RNA Library Prep Kit with Polaris Depletion – rRNA/Globin(HMR), 7K0077/78-096, v2.0.0523, Protocol A: High Quality and partially degraded samples.

Prior to executing this protocol:

- **A5.1** Program and preheat thermocycler
- **A5.2** Prepare the 1st Strand Master Mix
- **A6.1** Program and preheat thermocycler
- **A6.2** Prepare 2nd Strand Master Mix
- **A7.1** Program and preheat thermocycler
- **A7.3** Dilute appropriate adapters
- **A7.5** Prepare Ligation Master Mix

This protocol is compatible with 8 – 96 samples as written and has been updated to v1.8.6 firefly software.

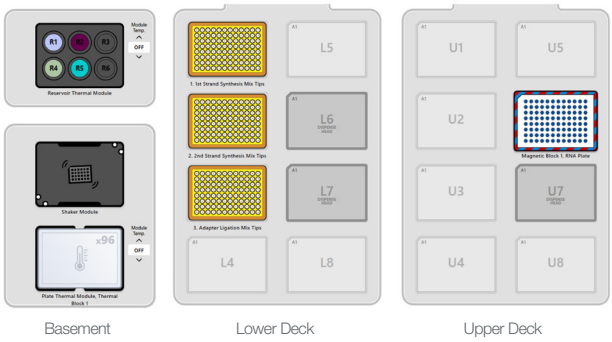

Figure 12. A5 - A7. 1st & 2nd Strand Synthesis, Adapter Ligation deck layout.

Protocol 5 of 8
A8. Post-Ligation Cleanup

This protocol performs section 8 of the Watchmaker RNA Library Prep Kit with Polaris Depletion – rRNA/Globin(HMR), 7K0077/78-096, v2.0.0523, Protocol A: High Quality and partially degraded samples.

Prior to executing this protocol:

- **A8.1** Freshly prepare 80% EtOH
- **A8.2** Vortex room temperature SPRI beads to thoroughly mix

This protocol is compatible with 48 – 96 samples as written and has been updated to v1.8.6 firefly software. To process > 48 samples, update 80% Ethanol reservoir asset and Aspirate steps.

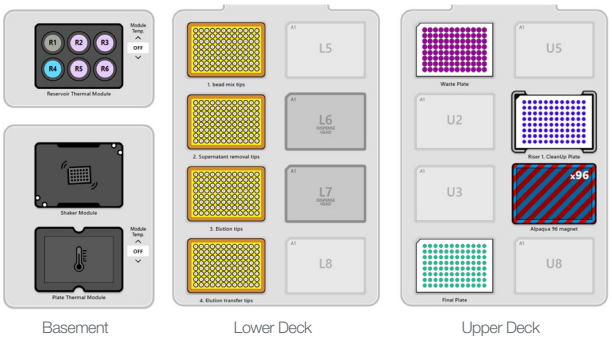

Figure 13. A8. Post-Ligation Cleanup deck layout.

Protocol 6 of 8
A9. 2nd Post-Ligation Cleanup (Optional)

This protocol performs section 9 of the Watchmaker RNA Library Prep Kit with Polaris Depletion – rRNA/ Globin(HMR), 7K0077/78-096, v2.0.0523, Protocol A: High Quality and partially degraded samples.

Prior to executing this protocol:

- **A9.1** Freshly prepare 80% EtOH
- **A9.2** Vortex room temperature SPRI beads to thoroughly mix

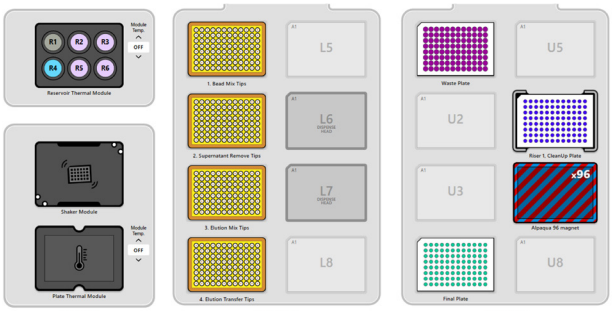

Figure 14. A9. 2nd Post-Ligation Cleanup (Optional) deck layout.

This protocol is compatible with 48 – 96 samples as written and has been updated to v1.8.6 firefly software. To process > 48 samples, update 80% Ethanol reservoir asset and Aspirate steps.

Protocol 7 of 8
A10. Library Amplification & Strand Selection

This protocol performs section 10 of the Watchmaker RNA Library Prep Kit with Polaris Depletion – rRNA/ Globin(HMR), 7K0077/78-096, v2.0.0523, Protocol A: High Quality and partially degraded samples.

Prior to executing this protocol:

- **A10.1** Thaw and equilibrate the Equinox Amplification Master Mix (2X) on ice. Once thawed, invert several times or swirl vigorously to mix. DO NOT VORTEX.
- **A10.2** Program and preheat thermocycler

This protocol is compatible with 8 – 96 samples as written and has been updated to v1.8.6 firefly software.

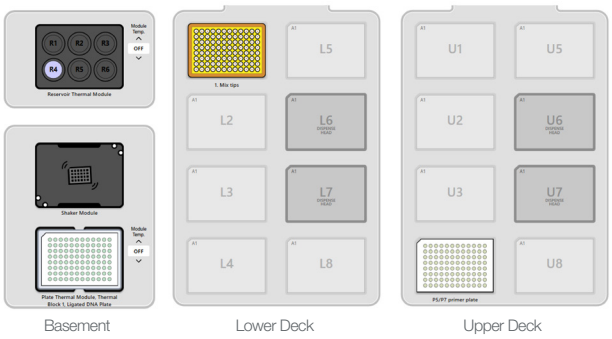

Figure 15. A10. Library Amplification & Strand Selection deck layout.

Protocol 8 of 8
A11. Post-Amplification Cleanup

This protocol performs section 11 of the Watchmaker RNA Library Prep Kit with Polaris Depletion – rRNA/ Globin(HMR), 7K0077/78-096, v2.0.0523, Protocol A: High Quality and partially degraded samples.

Prior to executing this protocol:

- **A11.1** Freshly prepare 80% EtOH
- **A11.2** Vortex room temperature SPRI beads to thoroughly mix

This protocol is compatible with 48 – 96 samples as written and has been updated to v1.8.6 firefly software. To process > 48 samples, update 80% Ethanol Reservoir Asset and Aspirate steps.

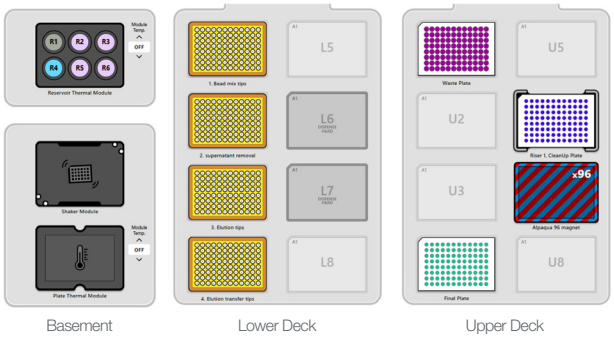

Figure 16. A11. Post-Amplification Cleanup deck layout.