firefly® technical note

QIAseq® FastSelect™ RNA Library Kit

Overview

- QIAseq® FastSelect™ RNA Library Kit including HMR rRNA removal (cat. 334235)
- The **QIAseq® FastSelect™ RNA Library Kit** including HMR rRNA removal enables 1-day NGS library prep from purified total RNA (1ng – 1µg) or enriched mRNA. This kit also enables the efficient removal of rRNA by including QIAseq FastSelect, which rapidly removes up to 99% of rRNA.
- This method was developed with 16 samples, using Eppendorf (100ng Human XpressRef Universal Total RNA, Cat no. / ID. 338112) 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: firefly 6-head, genomics.
- Development software version: v1.8.6
- Deviation(s) from published method:
  - Ethanol Additions during bead clean-up have been reduced to allow for transfer removal of ethanol with 125ul tips.

firefly protocols

Protocol number	Protocol name	firefly run time (minutes)	Thermocycler run time (minutes)
1 of 3	RNA fragmentation and FastSelect RNA removal	4	19
2 of 3	cDNA synthesis	108	107
3 of 3	Library amplification-indexing	55	50

Table 1. Overview of protocols to process 96 samples using QIAseq® FastSelect™ RNA Library Kit including HMR rRNA removal on SPT Labtech's firefly. Times may vary based on user and thermal cycler.

Input variables

Input Variable	Interval	Range	
		Minimum	Maximum
Number of Samples	8	8	96
Indexing reaction MM volume	1	25	26
QIAseq UX index volume	1	1	2

Table 2. General variables for using QIAseq® FastSelect™ RNA Library Kit including HMR rRNA removal on SPT Labtech firefly liquid handler.

sptlabtech

Master Mix Calculations

Protocol name	Reagent	Total Required Volume (µl)
1. RNA fragmentation and FastSelect RNA removal	FastSelect rRNA depletion	747.00
2. cDNA synthesis	cDNA synthesis master mix	699.00
3. Library amplification-indexing	QIAseq 2x HiFi master mix	2640.00

Table 3. Master Mix volumes for processing 96 samples with QIAseq® FastSelect™ RNA Library Kit including HMR rRNA removal 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)	050-096-EZ-FS (050-008-EZ-FS if <12 columns)	1x	—	—
125 µL Tips (Filtered)	125-096-EZ-FS (125-008-EZ-FS if <12 columns)	—	7×	3×

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.

Dispense Head Consumables Type	Product Number(s)	Protocol 1	Protocol 2	Protocol 3
Syringes (Ultra-low Retention)	4150-07208	—	—	—
Syringes (Standard)	4150-07200	1x	5×	5×
Reservoirs (Standard)	4150-07103	—	2×	3×
Reservoirs (Low Dead Volume)	4150-07202	1×	1×	—
Reservoirs (High Volume)	4150-07300	—	*2x	**2x

Table 5. This table outlines the Dispense Head consumables required for each protocol, with quantities adjusted for fewer columns.
*When running 4 columns or less, 2x standard reservoirs will be needed instead.
**When running 8 columns or less, 2x standard reservoirs will be needed instead.

Plates Type	Product Number(s)	Protocol 1	Protocol 2	Protocol 3
Eppendorf Twin.Tec 96 Skirted Plates	0030128648	1x	4×	2×
Fisherbrand 1ml Deep Well Plates	236600	—	1x	1×
SID-TS-96S	—	—	1x	—
QIAseq UX index Plate	—	—	—	1x

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	1x	7x	3x
Tip Loading Cassette	FFY-A-01-EZL-SL-5	1x	7x	3x
Strip Tip Insert - 8 Channel	FFY-A-01-EZL-096-SC-8	*1x	*7x	*3x
Alpaqua Magnum FLX	A000400	—	1x	1x
40mm Upper Deck Riser	3276-01838	—	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

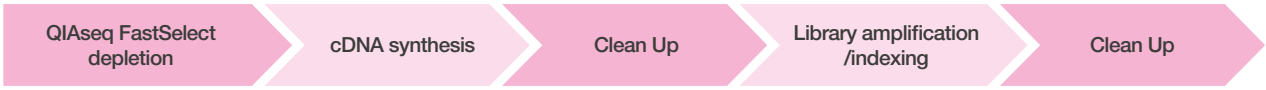

Figure 1. Overview of the steps performed by firefly to automate QIAseq® FastSelect™ RNA Library Kit including HMR rRNA removal.

Workflow details

Protocol 1 of 3 - 1. RNA fragmentation and FastSelect RNA removal

This protocol rapidly and efficiently removes rRNA by using FastSelect.

Prior to executing this protocol:

2. **a.** Thaw QIAseq FastSelect, 5x US RT Buffer and Nuclease-Free Water at room temperature.
   b. Dilute an aliquot of each FastSelect tube to 0.1x.
3. Prepare the FastSelect rRNA depletion reaction mix.

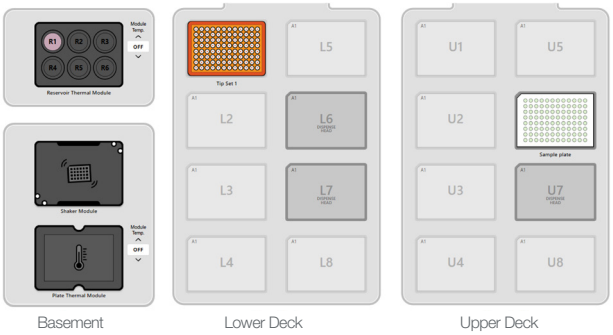

Figure 2. Protocol 1 of 3 - 1. RNA fragmentation and FastSelect RNA removal initial deck layout.

Protocol 2 of 3 - 2. cDNA synthesis

This protocol performs the cDNA synthesis and purification. A random hexamer (N6-T RT) or an oligo-dT (ODT-T RT) primer may be used to perform a complete transcriptome analysis or 3' RNA-seq analysis, respectively. Both primers may also be combined.

Prior to executing this protocol

Equilibrate beads to room temperature 20-30 minutes before use. Vortex thoroughly.

1. **a.** Thaw DTT (100mM), dNTP (10mM), and Nuclease-Free Water at room temperature.
   b. Keep RNase Inhibitor and EZ Reverse Transcriptase on ice.
   c. Process the SID-TS-96S plate as described in step 5 of the RNA Library Kit Handbook and transfer the dissolved content of the wells that will be used to a new plate.
2. Prepare the cDNA synthesis reaction mix on ice.

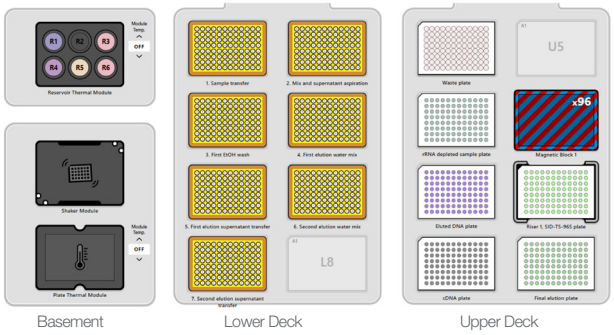

Figure 3. Protocol 2 of 3 - 2. cDNA synthesis initial deck layout.

Protocol 3 of 3 - 3.
Library amplification-indexing

This protocol performs the amplification of the libraries using QIAseq UX Index kits (up to 768 UDIs are available) and its purification.

Prior to executing this protocol:

Equilibrate beads to room temperature 20-30 minutes before use. Vortex thoroughly.

1. **a.** Thaw QIAseq UX index plates at room temperature.
   b. Thaw the QIAseq 2x HiFi MM on ice.
2. Prepare the library amplification/ indexing reaction mix on ice.

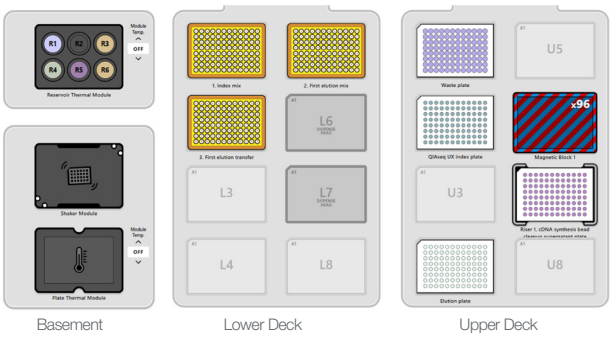

Figure 4. Protocol 3 of 3 - 3. Library amplification-indexing initial deck layout.

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
SPT Labtech	dragonfly® discovery LDV Reservoirs	4150-07202
SPT Labtech	dragonfly® Reservoirs	4150-07103
SPT Labtech	dragonfly® discovery Ultra Low Retention Syringes	4150-07208
SPT Labtech	dragonfly® discovery Syringes	4150-07200
SPT Labtech	EZ-Load Strip Tips, 050µL, Sterile 8 Tips Per Strip	050-008-EZ-S
SPT Labtech	EZ-Load Strip Tips, 100µl, with Filters, Sterile, 8 Tips Per Strip	125-008-EZ-FS
SPT Labtech	firefly® Pipette Tips, 100µL, with Filters, Sterile, 96 Tips per Rack	125-096-FF-FS
SPT Labtech	firefly® Pipette Tips, 35µL, with Filters, Sterile	050-096-FF-FS
SPT Labtech	Strip Tip Insert - 8 Channel Offset	FFY-A-01-EZL-096-SC-8
SPT Labtech	Universal Tip Loading Cassette	FFY-A-01-EZL-SL-5
SPT Labtech	Universal Tip Stand	3276-08075
SPT Labtech	Thermal Adapter for PCR Plate, 96	3276-01065
Eppendorf	twin.tec PCR Skirted 96 well plate	30128648
Thermo Fisher Scientific	Fisherbrand 1ml Deep Well Plates	236600

Table 8. Overview of consumables and accessories required for processing 96 samples using QIAseq® FastSelect™ RNA Library Kit on SPT Labtech's firefly indicating their part number.