April 2026

Supplementary Protocol

QIAseq® FastSelect® RNA Library Kit – HMR

The QIAseq FastSelect RNA Library Kit HMR ships in multiple boxes. QIAseq RNA Lib Enzymes & Buffers, NGS 2× Hi-Fi Master Mix Kit, and QIAseq FastSelect –rRNA HMR, are shipped on dry ice and should be stored at –30°C to –15°C.

QIAseq Beads are shipped at 4°C and should be stored at 2–8°C in a refrigerator.

Important: Do not freeze the QIAseq Beads.

Equipment and reagents to be supplied by the user

- QIAseq UX Index Kits IL UDI (depending on the multiplexing).

  For further plates and higher multiplexing QIAseq UX Index Kits IL UDI, visit **www.qiagen.com/QIAseqFastSelectRNALibKits**
- 100% ethanol, ACS-grade
- Nuclease-free pipette tips and tubes
- Microcentrifuge tubes (2 mL)
- PCR tubes (0.2 mL individual tubes/tube strips) (VWR, cat. no. 20170-012 or 93001-118)
- Ice
- Microcentrifuge

Sample to Insight

- Thermal cycler: recommended with heated lid, and maximal heating and cooling rate of 2.5°C/s. Temperature uniformity ±0.4°C well-to-well within 10 s of arrival at 90°C.
- Magnet for QIAseq Bead Cleanups: MagneSphere® Technology Magnetic Separation Stand (Promega, cat. no. Z5342), DynaMag™-2 magnetic rack (Thermo Fisher Scientific, cat. no. 12321D), DynaMag-96 Side Magnet (Thermo Fisher Scientific, cat. no. 12331D), or similar.

Protocol: RNA-seq library using FastSelect rRNA removal

This protocol is for building an RNA-seq library using FastSelect rRNA removal and random oligos for the reverse transcription reaction, when starting from total RNA concentration of 5 ng/μL or higher, and an RIN greater than 3.

Important points before starting

- Review the *QIAseq FastSelect RNA Library Kit Handbook*, which can be found at **www.qiagen.com/HB-3152**
- Check your supply of QIAseq UX Index Kits (sold separately).
- Refer to "Appendix: Reference Guide" for specific thermal cycling conditions based on RNA input and quality.
- Keep samples, enzymes, master mixes, and buffers on ice when not in use.
- Nuclease-Free Water (cat. no. 129115) used for elution from beads should be at room temperature (15–25°C).
- Keep the QIAseq beads at room temperature while in use. Always mix beads thoroughly before use and make sure QIAseq beads are a homogeneous solution when pipetting.
- Safe stopping points are after cDNA cleanup, after PCR amplification, and after PCR cleanup. The final library is stable for 90 days when stored at –20°C or colder.

Procedure

1. Prepare the SID-TS RT Primer for the reverse transcription reaction by resuspending each well of dried oligo in the SID-TS Plate with 2.5 µL Nuclease-Free Water. Incubate for at least 10 min at room temperature to fully dissolve the oligos.
2. Dilute samples to 5 µL with Nuclease-Free Water.
3. Make QIAseq FastSelect HMR 0.1× dilution (e.g., Mix 3 µL FastSelect tube + 27 µL Nuclease-Free Water).
4. Prepare the **RNA Frag and FastSelect depletion master mix** using the formula below:

   Component volume per sample × Number of samples = V_T + 1 sample

Component	Volume per sample		No. of samples		Total volume
QIAseq FastSelect 0.1×	1.0 µL	×		=	
N6-T RT primer	1.0 µL	×		=	
US RT Buffer 5×	4.0 µL	×		=	

5. Add 6 µL of RNA Fragment and FastSelect depletion master mix to each sample and mix well.
6. Put your sample tube in the thermocycler and initiate the "RNA Fragmentation and FastSelect depletion incubation" in "Appendix: Reference Guide" at the end of the document.
7. Add 2.5 µL of the resuspended SID-TS primer to each sample. Each sample requires a different SID-TS primer.

8. Prepare the **cDNA master mix** using the formula below:

Component volume per sample × Number of samples = V_T + 1 sample

Component	Volume per sample		No. of samples		Total volume
Nuclease-Free Water	2.0 µL	×		=	
DTT (100mM)	0.5 µL	×		=	
dNTP (10mM)	2.0 µL	×		=	
RNase Inhibitor	0.5 µL	×		=	
EZ Reverse Transcriptase	1.5 µL	×		=	

9. Add 6.5 µL of the cDNA mastermix to each sample. The final volume of the cDNA reaction is 20 µL.
10. Put your sample tube in the thermocycler and initiate the "cDNA synthesis incubation" in "Appendix: Reference Guide" at the end of the document.
11. Combine 24 µL of room temperature QIAseq beads with 11 µL of Nuclease-Free Water in a new tube or 96-well plate.
12. Add 19 µL of the cDNA synthesis reaction to the QIAseq Beads. Mix and incubate for 5 min on the bench.
13. Place the tube on the magnet and wait 2 min for the pellet of beads to migrate to the side of the tube. Remove and discard the supernatant without disturbing the beads.
14. Wash beads 2 times with 200 µL of 80% ethanol. After the second wash, remove all ethanol and let the beads dry for 5–10 min.

15. Resuspend the beads with 32 µL of Nuclease-Free Water. Wait 2 min. Place the tube back on the magnet and wait for the solution to clear.
16. Transfer 30 µL of the supernatant to a clean tube/well. Mix with 24 µL of room temperature QIAseq beads and incubate for 5 min.
17. Place the tube on the magnet and wait 2 min for the pellet of beads to migrate to the side of the tube. Remove and discard the supernatant without disturbing the beads.
18. Wash beads 2 times with 200 µL of 80% ethanol. After the second wash, remove all ethanol and let the beads dry for 5–10 min.
19. Resuspend the beads in 25 µL of Nuclease-Free Water. Wait 2 min. Place the tube back on the magnet and wait for the solution to clear. Your cDNA is in the supernatant.
20. Transfer 23 µL of the supernatant to a new tube. This is your cDNA sample.

	This is a safe stopping point. Freeze at –20°C overnight. Continue processing next day.

21. Add 25 µL of QIAseq 2× HiFi Mastermix to each sample.
22. Add 2 µL of a unique UDI primer pair to each sample from the QIAseq UX Index Kits.
23. Initiate the "Library amplification and indexing cycling program" in "Appendix: Reference Guide" at the end of the document.

	This is a safe stopping point. Samples can stay in the PCR instrument at 4°C overnight, and stored at –20°C or colder for 90 days.

24. Add 40 µL of room temperature QIAseq Beads to a fresh tube.
25. Add 50 µL of the PCR amplified library to the beads. Mix and incubate for 5 min on the bench.
26. Place the tube on the magnet and wait 2 min for the pellet of beads to migrate to the side of the tube. Remove and discard the supernatant without disturbing the beads.
27. Wash beads 2 times with 200 µL of 80% ethanol. After the second wash, remove all ethanol and let the beads dry for 5–10 min.

28. Add 24 µL of Nuclease-Free Water to the beads. Wait 2 min. Place the tube back on the magnet and wait for the solution to clear.
29. Transfer 22 µL of the supernatant to a new tube.

Once finished, the result will be your RNA-seq library.

The final library can be stored at –20°C for 90 days before sequencing.

Appendix: Reference Guide

This guide is used for customizing the RNA Fragmentation, FastSelect depletion time, and customize PCR cycles used for the Library amplification and indexing reaction based on the amount and quality of starting RNA, and when working with N6-T RT Primer-only, or N6-T RT + ODT-T primer in the Reverse Transcription reaction.

For Table 1, set the heated lid to 75°C.

Table 1. RNA Fragmentation and FastSelect depletion incubation

Step	Temperature (°C)	Duration
1	94	See Table 4
2	75	2 min
3	70	2 min
4	65	2 min
5	60	2 min
6	55	2 min
7	37	2 min
8	25	2 min
9	4	2 min
10	4	Hold

For Table 2, set the cycler heated lid to 75°C.

Table 2. cDNA synthesis incubation

Step	Temperature (°C)	Duration
1	4	1 min
2	25	5 min
3	42	90 min
4	70	10 min
5	4	1 min
6	4	Hold

For Table 3, set the heated lid to 105°C (or max).

Table 3. Library amplification and indexing cycling program

No. of cycles	Temperature (°C)	Duration
1	98	3 min
X (See Table 5)	98	5 s
	55	10 s
	72	20 s
1	72	2 min
6	4	1 min
–	4	Hold

Table 4. RNA Fragmentation and FastSelect depletion times

RNA quality (RIN)	Input (ng)	Fragmentation time at 94°C (min)
Less than 3	Less than 10	None
	10–250	1
	Greater than 250	2
3-5	Less than 10	1
	10–250	3
	Greater than 250	3
5-8	Less than 10	2
	10–250	3
	Greater than 250	3
Greater than 8	Less than 10	3
	10–250	3
	Greater than 250	5

Table 5. PCR cycle number based on RNA input

Total RNA Input (ng)	Number of amplification cycles
1	27
10	23
100	20
1000	17
Enriched poly *A*+ RNA from 100 ng of Total RNA	22

Notes.

Notes.

Document Revision History

Date	Changes
04/2026	Initial release.

Scan QR code for handbook.

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