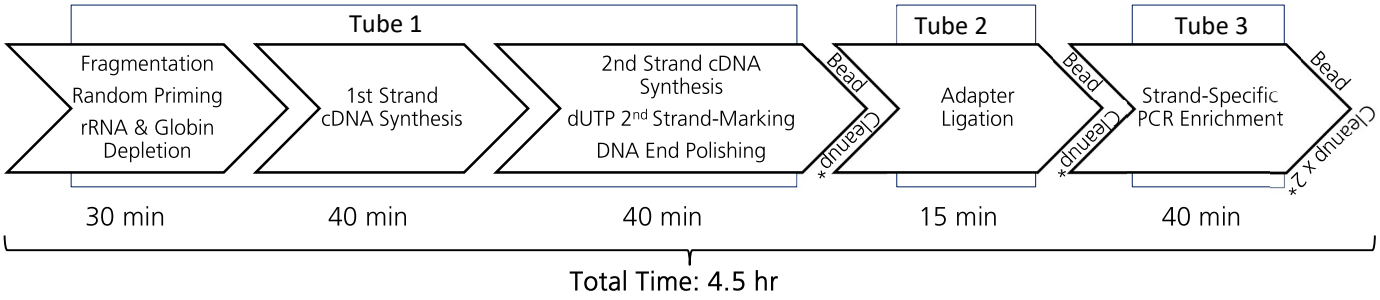
sparQ RNA-Seq HMR Kit

Cat. No.	95216-008 (Sample size only)	Size	8 reactions	Box 1: Store at -25°C to -15°C Bag 2: Store at 2°C to 8°C
	95216-024		24 reactions	
	95216-096		96 reactions	

Description

The sparQ RNA-Seq HMR Kit provides reagents essential for construction of stranded RNA libraries with rRNA and globin mRNA depletion for Human/Mouse/Rat (HMR) samples on NGS platforms. The kit uses proprietary, highly optimized enzymes in a streamlined workflow with integrated ribosomal depletion for RNA library. High quality whole transcriptome libraries can be prepared from either intact or degraded RNA samples at varying input quantities (from 1 ng of high-quality RNA and 10 ng of degraded RNA up to 1000 ng RNA input). The protocol is simplified into 3 reaction tubes and 9 steps to minimize hands-on and total workflow time to prepare sequencer-ready libraries in a single day.

Workflow Overview



* Each bead cleanup takes approximately 25 minutes and is included in the total time.

Instrument Compatibility: The kit is compatible with Illumina®, Element and Singular sequencing instruments. This IFU is for use with Illumina sequencing instruments.

Components

				Volume		
	Component Description	Cap Color		8 reactions	24 reactions	96 reactions
Box 1 Store at -25°C to -15°C	Frag Prime RG Depletion Mix	●	Blue	1 x 32 µl	1 x 96 µl	1 x 384 µl
	1st Strand Enzyme Mix	●	Brown	1 x 32 µl	1 x 96 µl	1 x 384 µl
	2nd Strand Buffer	●	Green	1 x 160 µl	1 x 480 µl	2 x 960 µl
	2nd Strand Enzyme Mix	●	Green	1 x 80 µl	1 x 240 µl	1 x 960 µl
	Rapid Ligation Buffer (5X)	●	Orange	1 x 160 µl	1 x 480 µl	2 x 960 µl
	T4 DNA Ligase	●	Orange	1 x 80 µl	1 x 240 µl	1 x 960 µl
	HiFi Plus Master Mix (2X)	○	White	1 x 200 µl	1 x 600 µl	2 x 1.2 ml
	Primer Mix	○	White	1 x 12 µl	1 x 36 µl	1 x 144 µl
Bag 2 Store at 2°C to 8°C	Bead Booster		Clear	1 x 48 µl	1 x 144 µl	1 x 576 µl
	UDI Dilution Buffer		Clear	1 x 480 µl	1 x 1.44 ml	1 x 5.76 ml

Storage and Stability

Store Box 1 in a constant temperature freezer at -25°C to -15°C upon receipt.
Store Bag 2 in a constant temperature refrigerator at 2 - 8°C upon receipt.
For lot specific expiry date, refer to package label, Certificate of Analysis or Product Specification Form.

Additional reagents that are not supplied

- Elution Buffer: 10 mM Tris-HCl pH 8.0
- Nuclease-free water
- 80% freshly prepared ethanol
- Purification Beads for post-second strand, post-ligation and post-amplification reaction cleanups are not included with the kit and must be purchased separately. We recommend using sparQ PureMag Beads from Quantabio.

Part Number	Description	Kit Size
95196-005	sparQ PureMag Beads	5 ml
95196-060	sparQ PureMag Beads	60 ml
95196-450	sparQ PureMag Beads	450 ml

- Adapters are not included with the kit and must be purchased separately. The sparQ RNA-Seq HMR Kit is compatible with Y-shaped adapters including non-barcoded, stubby, single-barcoded, or dual-barcoded adapters (with or without unique molecular identifiers) routinely used in library construction workflows. We recommend using sparQ UDI Adapters from Quantabio. If using third party stubby (truncated) adapters, uniquely indexed PCR primers must also be purchased separately.

Part Number	Description	Kit Size
95211-096	sparQ UDI Adapters	96 rxn

- NGS Library Quantification Kit is not included with the kit and must be purchased separately. Accurate quantification of RNA-seq libraries is recommended for optimizing downstream processes such as target capture and sequencing. Quantabio offers the sparQ Universal Library Quant Kit that uses qPCR to quantify library molecules with appropriate Illumina P5/P7 adapters at each end.

Part Number	Description	Kit Size
95210-100	sparQ Universal Library Quant Kit	100 rxn
95210-500	sparQ Universal Library Quant Kit	500 rxn
95210-15B	sparQ Library Dilution Buffer	15 ml

- To check NGS library size and quality, we recommend using automated electrophoresis systems, such as Agilent 2100 Bioanalyzer™, Agilent 2200 TapeStation® or QIAxcel® Connect from QIAGEN®.

General Guidelines

- Use good laboratory practice to minimize cross-contamination of nucleic acid products.
- Always use PCR tubes, microfuge tubes, and pipette tips that are certified sterile, DNase- and RNase-free.
- For consistent library amplification, ensure the thermal cycler used in this protocol is in good working order and has been calibrated to within the manufacturer's specifications.
- Briefly centrifuge tubes prior to opening to avoid loss of material.
- Read the entire protocol before beginning. Take note of stopping points and plan your workflow accordingly.

Point in protocol where procedure can be stopped, stored at appropriate conditions outlined, and continued within 24 hours

Take note of recommendations in protocol

Use caution when performing protocol to obtain the best results

Before You Begin

- Prepare beads by mixing 300 µl of magnetic cleanup beads (sparQ PureMag Beads) with 6 µl Bead Booster for each library preparation. This mixture generates enough beads to take 1 sample through the entire library preparation process and should be kept at room temperature during preparation process.
- Prepare a fresh solution of 80% ethanol, store at room temperature.
- Prepare 10 mM Tris-HCl pH 8.0, store at room temperature.
- Wipe down work areas and pipettes with an RNase and DNA cleaning product.
- Thaw reagents on ice. Once thawed, finger flick (do not vortex) the tubes containing 1st Strand Enzyme Mix, 2nd Strand Enzyme Mix, T4 DNA Ligase and HiFi Plus Master Mix (2X). Briefly centrifuge to ensure even distribution of contents. Other tubes can be briefly vortexed and centrifuged to ensure mixing.
- Determine the amount of input RNA using standard methods.
- High-quality RNA is recommended for successful library preparation and sequencing.
    - High quality RNA has an A260:A280 ratio of 1.9–2.1 in 10 mM Tris-HCl, pH 7.5.
    - RNA integrity: Total RNA extracts from cells and tissues can be checked for quality using an automated analysis system (such as Agilent TapeStation or QIAxcel Connect from QIAGEN) that provides an RNA integrity number (RIN). Ideally, RIN value ≥ 9 is recommended for low input, such as 1 ng. For higher amounts, RIN ≥ 7 is recommended. However, sparQ RNA-Seq HMR Kit shows a high rate of successful libraries with samples with lower RIN values. For optimal performance with degraded and/or FFPE RNA samples, a minimum of 10 ng total RNA is recommended.

Protocol

RNA Fragmentation and Ribo-Globin Depletion

This step combines RNA fragmentation, random priming and ribo-globin depletion into one step.

1. Thaw RNA on ice. Gently mix, briefly centrifuge and return to ice.
2. Thaw Frag Prime RG Depletion Mix on ice.
3. Prepare the fragmentation and depletion reaction on ice per Table 1.

***Note**: The master mix combining 'Frag Prime RG Depletion Mix' and 'nuclease-free water' can be prepared for multiple reactions and stored on ice until use.*

The master mix should be mixed by medium speed vortexing for at least 5 seconds, then purified RNA added and mixed by vortexing or pipetting.

Pre-program a thermal cycler according to Table 2 with heated lid set to 105°C. Start program to pre-heat block to 94°C and pause.

Table 1

	Component	Per Reaction (µl)
	Purified RNA (1 - 1000 ng)	x
●	Frag Prime RG Depletion Mix	4
	Nuclease-free water	6 - x
	Total	10

Note: *Recommended input volume for Purified RNA is between 2 µl and 6 µl depending on RNA amount and concentration.*

4. Mix well by pipetting up and down 5 times and briefly centrifuge.
5. Load the reactions into the pre-programmed cycler, resume the incubation at 94°C followed by cycling according to Table 2.

Table 2

Step	Temperature (lid 105°C)	Incubation Time		
		High Quality Input RNA (RIN > 7)	Partially Degraded Input RNA (RIN < 7)	FFPE RNA (10 - 1000 ng)
1	94°C	3 min for 500 - 1000 ng 2 min for < 500 ng	2 min	none
2	75°C	2 min		
3	70°C	2 min		
4	65°C	2 min		
5	60°C	2 min		
6	55°C	2 min		
7	37°C	5 min		
8	25°C	5 min		
9	4°C	Hold		

6. After fragmentation, briefly centrifuge the fragmentation reactions and proceed immediately to First Strand Synthesis.

First Strand Synthesis

7. Remove 1st Strand Enzyme Mix from the freezer just before use. Thaw on ice.
8. Prepare First Strand Synthesis Master Mix on ice according to Table 3.

Note: *First Strand Synthesis Master Mix can be prepared for multiple reactions and stored on ice until use.*

Pre-program a thermal cycler according to Table 4 with heated lid set to 75°C. Start program to pre-heat block to 25°C and pause.

Table 3: First Strand Synthesis Master Mix

	Component	Per Reaction (µl)
●	1st Strand Enzyme Mix	4
	Nuclease-free water	6
	Total	10

9. Add **10 µl** of the First Strand Synthesis Master Mix to the **10 µl** of fragmented, depleted, primed RNA from step 6 for a total reaction volume of 20 µl.

10. Mix well by pipetting up and down 5 times and briefly centrifuge. Incubate as described in Table 4.

Table 4

Step	Temperature (lid 75°C)	Incubation Time
1	25°C	10 min
2	42°C	15 min
3	70°C	15 min
4	4°C	Hold

11. After First Strand Synthesis, briefly centrifuge the reaction and place on ice. Proceed immediately to Second Strand Synthesis and End Polishing.

Second Strand Synthesis and End Polishing

12. Thaw 2nd Strand Buffer on ice. Remove 2nd Strand Enzyme Mix from the freezer just before use.

13. Prepare Second Strand Synthesis Master Mix on ice according to Table 5.

Note: *Second Strand Synthesis Master Mix can be prepared for multiple reactions and stored on ice until use.*

Pre-program a thermal cycler according to Table 6 with heated lid set to 75°C. Start program to pre-set block to 16°C and pause.

Table 5: Second Strand Synthesis Master Mix

	Components	Per Reaction (µl)
●	2nd Strand Buffer	20
●	2nd Strand Enzyme Mix	10
	Total	30

14. Add **30 µl** of the Second Strand Synthesis Master Mix to the **20 µl** of First Strand Synthesis product from step 11 for a total reaction volume of 50 µl.

15. Mix well by pipetting up and down 5 times and briefly centrifuge. Incubate as described in Table 6.

Table 6

Step	Temperature (lid 75°C)	Incubation Time
1	16°C	30 min
2	62°C	10 min
3	4°C	Hold

16. Briefly centrifuge and proceed to Post-Second Strand Cleanup.

Post-Second Strand Cleanup

Post-Second Strand Cleanup can be carried out in the PCR tube if a suitable magnetic stand is available. Alternatively, the full reaction volume can be transferred to low-bind 1.5 ml tubes for cleanup. If proceeding to adapter ligation following cleanup, the final elution must be collected into a thin-walled PCR tube.

17. Ensure that the sparQ PureMag Beads and Bead Booster mixture has been kept at room temperature (RT) for at least 20 min before use.
18. Thoroughly vortex the sparQ PureMag Beads slurry and add **90 µl** (1.8X) to the Second Strand Synthesis product from step 16. Mix well by pipetting.
19. Incubate the mixture for 5 min at RT. Pellet the beads on a magnetic stand and carefully remove and discard the supernatant.
20. Keeping the tube on the magnetic stand, add 200 µl of freshly-prepared 80% ethanol to wash the beads. Pellet the beads on the magnetic stand for 30 s then carefully remove and discard the supernatant. Repeat the wash once.
21. Air-dry the beads on the magnetic stand for 3 - 5 min or until the beads appear matte and no longer shiny. Over-drying of beads may result in lower cDNA recovery.
22. Remove the tube from the magnetic stand, resuspend the dried beads by adding 68 µl of 10 mM Tris-HCl pH 8.0, and mix well by pipetting up and down at least 5 times. Incubate the beads at RT for 2-3 min. Pellet the beads on the magnetic stand. Carefully transfer **65 µl** of supernatant into a new thin-walled PCR tube and proceed to Adaptor Ligation. Alternatively, the sample can be stored at 4ºC for up to 24 hours.

Adapter Ligation

Adapters may need to be diluted using UDI Dilution Buffer before use. To achieve optimal adapter ligation efficiency for various input RNA amounts, it is recommended to adjust insert/adapter molar ratio accordingly. Table 7 and Appendix A provide general guidance on recommended sparQ UDI or third party stubby (truncated) adapter concentrations.

Table 7

Total RNA Input	Adapter Dilution*	Dilution of 15 µM Stubby Adapters
501 - 1000 ng	1:10	1:10
100 - 500 ng	1:50	1:10
51 – 99 ng	1:50	1:50
1 - 50 ng	1:200	1:50

* Serial dilution of the adapters is recommended.
For further guidance, refer to Appendix B.

23. Thaw Rapid Ligation Buffer (5X) on ice. Remove T4 DNA Ligase from the freezer just before use.
24. Prepare Ligation Master Mix on ice according to Table 8.

Note: Ligation Master Mix can be prepared for multiple reactions and stored on ice until use.

Pre-program a thermal cycler according to Table 9 with heated lid off. Start program to pre-set block to 20°C and pause.

Table 8: Ligation Master Mix

	Components	Per Reaction (µl)
●	Rapid Ligation Buffer (5X)	20
●	T4 DNA Ligase	10
	Total	30

Use caution when pipetting adapters. Avoid touching the tube with any part of your pipette other than pipette tips to minimize potential cross contamination.

25. Add 5 µl of diluted unique adapter to each sample from step 22.
26. Transfer 30 µl of Ligation Master Mix to each PCR tube containing sample plus adapter from step 25.
27. Mix well by pipetting up and down 5 times, gently vortexing if necessary, then briefly centrifuge. Incubate as described in Table 9.

Note: Heated lid must be OFF and < 30°C.

Table 9

Step	Temperature (lid off)	Incubation Time
1	20°C	15 min
2	4°C	hold

28. Briefly centrifuge and proceed to Post-Ligation Cleanup.

Post-Ligation Cleanup

29. Ensure that the sparQ PureMag Beads and Bead Booster mixture has been kept at room temperature (RT) for at least 20 min before use.
30. Thoroughly vortex the sparQ PureMag Beads slurry and add 70 µl (0.7X) to the sample from step 28. Mix well by pipetting.
31. Incubate the mixture for 5 min at RT. Pellet the beads on a magnetic stand and carefully remove and discard the supernatant.
32. Keeping the tube on the magnetic stand, add 200 µl of freshly-prepared 80% ethanol to wash the beads. Pellet the beads on the magnetic stand for 30 s then carefully remove and discard the supernatant. Repeat the wash once.

33. Air-dry the beads on the magnetic stand for 3 - 5 min or until the beads appear matte and no longer shiny. Over-drying of beads may result in lower cDNA recovery.

34. Remove the tube from the magnetic stand. Choose from the following options for resuspension volumes based on the adapter type used for this preparation.

    a. If full-length Y-shaped adapters (ie. sparQ UDI Adapters) were used: Resuspend the dried beads in **25 µl** of 10 mM Tris-HCl, pH 8.0 and mix well by pipetting up and down at least 5 times. Incubate the beads at room temperature for 2 – 3 min. Pellet the beads on the magnetic stand. Carefully transfer **23.5 µl** of supernatant into a new thin-walled PCR tube and proceed to library amplification. If not proceeding immediately, the sample can be stored at 4°C for up to 24 hours.

    b. If stubby (truncated) adapters were used in adapter ligation: Resuspend the dried beads in **22 µl** of 10 mM Tris-HCl, pH 8.0 and mix well by pipetting up and down at least 5 times. Incubate the beads at room temperature for 2 – 3 min. Pellet the beads on the magnetic stand. Carefully transfer **20 µl** of supernatant into a new thin-walled PCR tube and proceed to library amplification. If not proceeding immediately, the sample can be stored at 4°C for up to 24 hours.

Library Amplification

The PCR reagents (HiFi PCR Master Mix and Primer Mix) can be used for high-fidelity amplification of the DNA library. The Primer Mix contains both forward and reverse primers and is compatible with libraries prepared with full-length Y-shaped adapters (ie. sparQ UDI Adapters) and flanked by the standard P5 and P7 sequences. Follow the steps on the left to prepare the amplification reaction.

If stubby (truncated) adapters were used during ligation, unique index primers must be purchased separately for use during amplification. Follow the steps on the right to prepare the amplification reaction.

35. Thaw HiFi Plus Master Mix (2X) and Primer Mix on ice.

Pre-program a PCR cycler according to Table 11 with heated lid set to 105°C. Start program to pre-heat block to 37°C and pause.

Library Amplification for Full-Length Y-shaped Adapters

36. Prepare PCR Master Mix in a separate tube on ice by combining the HiFi Plus Master Mix (2X) and Primer Mix according to Table 10a. Mix well by pipetting. Volumes can be scaled as needed for the desired number of reactions and stored on ice until use.

Table 10a: PCR Master Mix

	Components	Per Reaction (µl)
◯	HiFi Plus Master Mix (2X)	25
◯	Primer Mix	1.5
	Total	26.5

37. Add **26.5 µl** of the PCR Master Mix to the **23.5 µl** of purified, adapter-ligated DNA from step 34 for a total reaction volume of 50 µl. Proceed to step 38.

Library Amplification for Stubby Adapters

36. Due to the need for unique index primers for each sample when using stubby (truncated) adapters, it is not recommended to prepare a master mix for library amplification. Table 10b shows the volumes of each reagent in the final reaction for convenience.

Table 10b: PCR Master Mix

	Components	Per Reaction (µl)
◯	HiFi Plus Master Mix (2X)	25
	Indexed Primer Mix (10 µM)	5
	Purified, adaper-ligated DNA	20
	Total	50

37. To the **20 µl** of purified, adapter-ligated DNA from step 34, add **25 µl** HiFi Plus Master Mix and **5 µl** of the specific indexed primer for a total reaction volume of 50 µl. Proceed to step 38.

38. Mix well by pipetting up and down 5 times, then briefly centrifuge. Incubate as described in Table 11.

Table 11

Step	Temperature (lid 105°C)	Incubation Time	Cycles
1	37°C	10 min	1
2	98°C	45 sec	1
3	98°C	20 sec	10-16*
	60°C	30 sec	
	72°C	30 sec	
4	72°C	1 min	1
5	4°C	Hold	1

* Refer to Table 12 for recommended number of PCR cycles.

Table 12

Total RNA Input Amount (ng)	Number of Cycles
800 – 1000	10
400 – 799	13
100 – 399	14
1 – 99	16

39. Briefly centrifuge and proceed to Post-Amplification Cleanup. Alternatively, the sample can be stored at 4°C for up to 24 hours.

Post-Amplification Cleanup

40. Ensure that the sparQ PureMag Beads and Bead Booster mixture has been kept at room temperature (RT) for at least 20 min before use.
41. Thoroughly vortex the sparQ PureMag Beads slurry and add **45 µl** (0.9X) to the sample from step 39. Mix well by pipetting.
42. Incubate the mixture for 5 min at RT. Pellet the beads on a magnetic stand and carefully remove and discard the supernatant.
43. Keeping the tube on the magnetic stand, add 200 µl of freshly-prepared 80% ethanol to wash the beads. Pellet the beads on the magnetic stand for 30 s then carefully remove and discard the supernatant. Repeat the wash once.

44. Air-dry the beads on the magnetic stand for about 3 - 5 min or until the beads appear matte and no longer shiny. Over-drying of beads may result in lower cDNA recovery.
45. Remove the tube from the magnetic stand, resuspend the dried beads by adding 52 µl of 10 mM Tris-HCl, pH 8.0. and mix well by pipetting up and down at least 5 times. Incubate the beads at RT for 2-3 min. Pellet the beads on the magnetic stand. Carefully transfer **50 µl** of supernatant into a new thin-walled PCR tube.
46. Repeat steps **41 to 44** for a total of two bead cleanups.
47. Remove the tube from the magnetic stand, resuspend the dried beads by adding 22 µl of 10 mM Tris-HCl, pH 8.0. and mix well by pipetting up and down at least 5 times. Incubate the beads at RT for 2-3 min. Pellet the beads on the magnetic stand. Carefully transfer **20 µl** of supernatant into a new thin-walled PCR tube. The sample can be stored at 4°C for up to 24 hours or at -20°C for longer term storage.

Library Validation and Quantification

RNA-Seq libraries constructed using this protocol should be validated and quantified to ensure optimal input for sequencing reactions.

Average fragment length can be measured using automated electrophoresis systems such as Agilent 2100 Bioanalyzer, Agilent 2200 TapeStation or QIAxcel Connect from QIAGEN per each manufacturer's instructions. Due to average product size, we recommend read lengths no longer than 100 bp. If a longer read length is used for sequencing, additional *in silico* adapter trimming may be necessary.

If an adapter-dimer peak at 150-157 bp is present at more than 25% of the library peak, an additional 0.9X bead clean up step should be performed according to steps 40-44 and 47 in the protocol before sequencing.

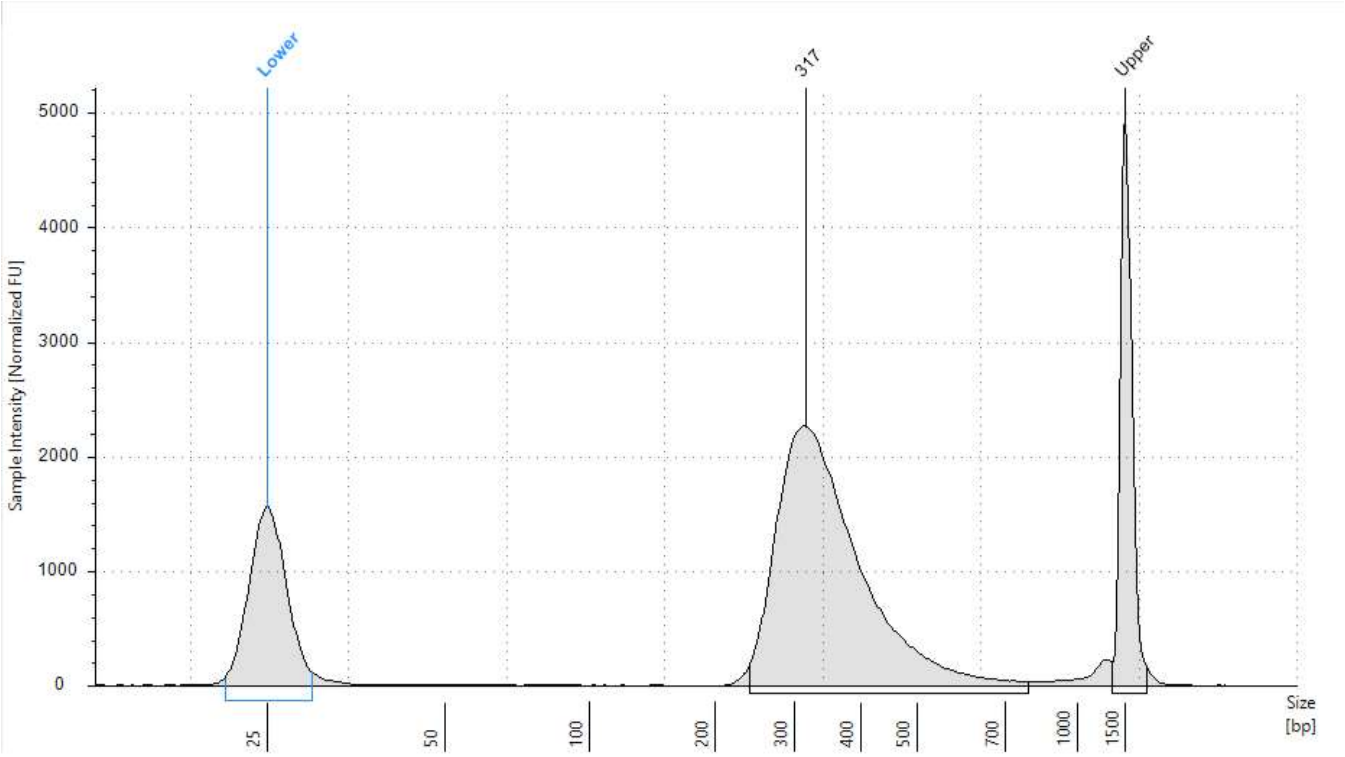


Figure 1: Example of cDNA library prepared using 100 ng of Universal Human Reference (UHR) RNA. Analyzed using Agilent TapeStation 4200 instrument.

Quality Control

Contamination specifications: Kit enzyme components were tested prior to assembly and found free of contaminating endonucleases and exonucleases. Enzyme purity was >95% as determined by SDS-PAGE and negligible *E.coli* genomic DNA contamination was confirmed by qPCR.

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Appendix A: Guidance on Adapter Concentrations

Determining the correct concentration of adapters during ligation is critical for high quality sequencing data. A low concentration of adapters during ligation will impact overall library yields. A high concentration of adapters results in the dimerization of adapters as they ligate to one another. Presence of adapter dimers in the final library may negatively impact sequencing run data quality and lower sample data yield.

With the range of RNA input amounts and range of tunable insert sizes, it is recommended that the adapter concentration be adjusted accordingly. Generally, increasing the input amount of RNA and/or tuning to smaller insert sizes, increase the concentration of adapters required. Both result in an increased concentration of cDNA available for ligation. Decreasing the input amount of RNA and/or tuning to larger insert sizes will require lower adapter concentration in comparison.

Titration of adapters is recommended to find the optimal adapter concentration to your specified insert size and input amount. UDI Dilution Buffer or 10 mM Tris-HCl pH 8.0, 50 mM NaCl, 0.5 mM EDTA can be used for adapter dilution.

Libraries with adapters added at low concentrations in the ligation step will report lower than expected yields in qPCR when compared to yields estimated from trace analysis and Qubit™. Sequencing of these libraries may result in lower than expected coverage, depth of coverage and uniformity.

Libraries with adapters added with high concentrations in ligation step are characterized with defined peak at 120-150 bp in fragment analysis traces. Sequencing of libraries with adapter dimers present may negatively impact sequencing run data accuracy, or even cause an instrument error, terminating the run. Completed run data sets will see an over-representation of the adapter reads, a smaller DNA fragments preferentially cluster to the flow cell.

If additional adapter dimers remain, another round of 0.9X (beads to sample) bead cleanup with sparQ PureMag Beads is recommended.

[sparQ UDI barcodes by plate location]

		1	2	3	4	5	6	7	8	9	10	11	12
A	I7	TGAACGTTGT	GAGCCAAGTT	TGCATAGCTT	CACTGCTATT	GATTGAGTTC	AGGCCTACAT	AGGATGTCCA	CCTCGGAATG	ATGACTCGAA	ACGTTGACTC	CCTCCATTAA	CTGATGATCT
	I5	ATGGCCGACT	AGGCACCTTC	AGCCATAACA	CATTCTTGGA	GCCAGTCGTT	AGAATTCTGG	TTGTAGAAGG	CGTCTAAGGT	CGCTCGTTAG	GTTGAGATTC	ATGGAATGGA	TTGTACTCCA
B	I7	ACCAGACTTG	AAGGCCGTAG	AACCTTCTCG	AATGGTAGGT	GTAATGCCAA	TGTGGAACCG	CACCTTATGT	GTTCTGGAAC	GAACAATCCT	CCACTTAACA	AGTCGCGGTT	ACTAGGTGTT
	I5	CGATGAGCAC	CTGTTGGTAA	CCACAAGTGG	ATGCAAGGTT	TGCCTTGTCG	CATTGACTCT	CCTAGCACTA	GAGGTGAACA	AACAACGCTG	TGTGTGCGGA	CATTCCTCAC	GTGCACATAA
C	I7	ACTGGCGAAC	TTAGAGAAGC	AAGAGATCAC	GATACCTATG	TCGTTGCGCT	CGTATTAAGC	AAGCGGCTGT	AGATTCACCA	TGGCAAGGAG	AGCAGTTCCT	CTCATCCAGG	CTGTTAGCGG
	I5	GATAAGTCGA	GCTGGTACCT	GTTATCACAC	CGCCAGACAA	CTATCCGCTG	GCGGCTTCAA	ATCGTGTTCT	TCAGAACTAC	CGCGGCTATT	GTTCGGCGAA	GCATAGGAAG	AGGACAAGTA
D	I7	GCGTTAGGCA	TCTAAGACCA	GCCTGAAGGA	CACTAGGTAC	AGGTGAGTAT	CCAGTGGTTA	TTCCTGTGAG	TCGGTCAGAT	GAATATTGGC	TCGCCTTCGT	TGTGGTTGAA	ATCGCACCAA
	I5	TCACGCCTTG	TAAGGAGCGG	TACCGTTCTT	GAAGGTTGGC	AATGCCGGAA	TTATGGTCTC	CCAACTTATC	CGGATATTGA	GCTCGACACA	AGCTGTATTG	TGTTCGTGTT	CCGATTCGAG
E	I7	TTATCGGCCT	TGTAACCACT	ATTGTGCCTT	AGCTCGTTCA	TCGATAATGG	GCGTTCGAGT	AGTACAGTTC	CACTCTCGCT	CCGGAACCTA	TAGGACTGCG	TTATGCGTGG	CTTACTTGGT
	I5	AGGAACACAA	AATCGCTCCA	AGGCGTTAGG	TCGCATCACG	CGGTTATCCG	CGTAACCAGG	GAAGCCAAGG	AGGAGTAGAT	TTCTTCCAAC	CAGCGGATGA	TAAGACCGTT	GTAGGAACTT
F	I7	GAGGTATAAG	CCGACACAAG	TCCTCTACCG	TGTCAGTCTT	GCGTCTCTTC	CCTTCCGGTT	TACAGCCTCA	GTTGGTCCAG	ACTTGTTCGG	TCCGAGCGAA	GCGAATGTAT	CCTTAATGCG
	I5	CTCAGTAGGC	CTCCTAATTG	CCGTAACGTC	CCGGTCATGA	GCGGAAGAGT	AGCTCAGATA	TGGAGTTCAA	CCGCCGAATA	TTGGCGGTTG	GTCCTTGGAT	ATGGTACCAG	TACACTACGA
G	I7	TCAAGGATTC	CTCTGATGGC	TACCATGAAC	GATGAACAGT	GTCTCCTGCA	CACAAGACGG	GTTCTATTGG	AGCTCGAAGC	CAAGTCCAAT	TTCGGTTGTT	GTCAAGCTCG	TCTCGCCTAG
	I5	GAAGTGCCTG	GCCTCATAAT	GTAATAGCCA	ATTCACAAGC	TTGGTTAGTC	CCGGTGTTAC	CTTCAATCCT	GAGTCTATAC	AACAGGCAAT	TCTAGATGCT	CCGACAGCTT	ATGACCTTGA
H	I7	CGAACCGAGA	CGGCCTGTTA	CATTGGCAGA	ACAATCGGCG	GAGCTTCATT	GCTTACACAC	ATATACCGGT	AGAGGTTCTA	AACCGCAAGG	ACAGGAGGAA	TAGAGTTGGA	TCTTCAGAGA
	I5	TCTCTCGCCT	TGTATTGAGC	TAGCGCCGAT	CAACCTGTAA	TTCAGTGTGA	GACCTAACCT	ATCTTGCGTG	TTATTACCGG	CAGAATGGCG	CGAGCCACAT	GACGATATGA	CTACGTGACG

Appendix B: Preparation of Adapter Dilution for Adapter Ligation

It is recommended to perform serial dilutions of the adapters.

Optimal dilutions may vary depending on the nature of your samples and NGS sequencing operations. Use the following example showing preparation of 1:10, 1:50 and 1:200 dilutions as a general guideline.

Prepare a 1:10 dilution by adding 2 µl of the adapters (stock) to 18 µl of UDI Dilution Buffer.

Prepare a 1:50 dilution by adding 4 µl of the diluted (1:10) adapters to 16 µl of UDI Dilution Buffer.

Prepare a 1:200 dilution by adding 2 µl of the diluted (1:10) adapters to 38 µl of UDI Dilution Buffer.

NGS Products Used in Protocol Sold Separately

sparQ PureMag Beads

Cat. No.	95196-005	Size:	5 ml
	95196-060		60 ml
	95196-450		450 ml

The sparQ PureMag Beads is a fast and reliable nucleic acid purification system for reaction cleanup and size selection in NGS workflows. It can be used to quickly remove primers, primer-dimers, unincorporated nucleotides, salts, adapters and adapter-dimers from NGS library prep reactions to improve downstream sequencing performance.

sparQ UDI Adapters (1 - 96)

Cat. No.	95211-096	Size:	1 - 96

The sparQ UDI Adapters are unique dual-indexed barcoded adapters for DNA and RNA libraries for Illumina sequencing platforms. It allows flexible pooling with improved performance by preventing index hopping and enhancing demultiplexing accuracy. The adapters are compatible with both DNA and RNA NGS libraries for various applications.

sparQ Universal Library Quant Kit

Cat. No.	95210-100	Size:	100 reactions
	95210-500		500 reactions
	95210-15B (dilution buffer)		15 ml

The sparQ Universal Library Quant Kit is optimized for rapid, sensitive, and accurate quantification of NGS libraries of various sizes and GC-contents. The kit uses fast cycling protocol, allowing results to be achieved in 40 minutes versus 1 hour and 20 minutes with other NGS library quantification kits.