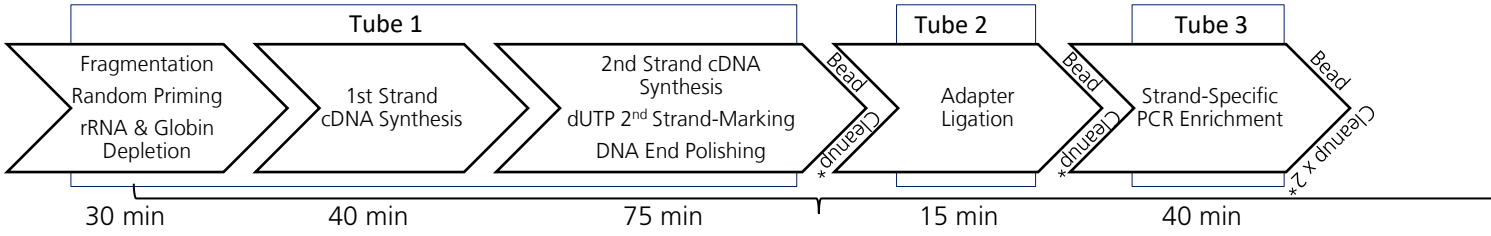
sparQ RNA-Seq HMR Kit

Cat. No.	95216-008 (Sample size only)	Size	8 reactions	**Store at -25°C to -15°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 Illumina® 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 (1 - 1000 ng). The protocol is simplified into 3 reaction tubes and 9 steps to minimize hands-on and total workflow time for sequencer-ready libraries in a single day.

Workflow Overview



Total Time: 5 hr

* Each bead cleanup takes approximately 25 minutes.

Instrument Compatibility: This product is compatible with all Illumina sequencing platforms.

Components

			Volume		
Component Description		**Cap Color**	**8 reactions**	**24 reactions**	**96 reactions**
●	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
	UDI Dilution Buffer	Clear	1 x 480 µl	1 x 1.44 ml	1 x 5.76 ml

Storage and Stability

Store the product in a constant temperature freezer at -25°C to -15°C upon receipt.
Once the 'UDI Dilution Buffer' has been thawed for use, it should be stored at -2 - 8°C.
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, 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.

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 to quantify library molecules with appropriate 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

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 and stored at appropriate conditions outlined

Take note of recommendations in protocol

Use caution when performing protocol to obtain the best results

Before You Begin

- 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.
- We recommend preparing RNA dilutions and quantifying RNA at specific pH.
    - 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 the Agilent TapeStation) 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, with maximum of 30 minutes in advance, 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	8 min for 500 - 1000 ng 4 min for < 500 ng	6 min for 500 - 1000 ng 3 min for < 500 ng	1 min
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, with maximum of 30 minutes in advance, 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

	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, with maximum of 30 minutes in advance, 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-heat block to 16°C and pause.

Table 5

	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	45 min
2	65°C	30 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. Equilibrate the sparQ PureMag Beads to room temperature (RT) for 20 min.
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 the Appendix provide general guidance on recommended sparQ UDI Adapter concentrations.

Table 7

Total RNA Input	Adapter Dilution
501 – 1000 ng	1:10
1 – 500 ng	1:50

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, with maximum of 30 minutes in advance, and stored on ice until use.*

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

Table 8

	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. Equilibrate the sparQ PureMag Beads to room temperature (RT) for 20 min.
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, resuspend the dried beads by adding 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 RT 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 immediately to library amplification. Alternatively, samples can be stored at 4ºC for up to 24 hours.

Library Amplification

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

35. Thaw HiFi Plus Master Mix (2X) and Primer Mix on ice.
36. Prepare PCR Master Mix according to Table 10.

Table 10

	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 **23.5 µl** of purified, adapter-ligated DNA from step 34 for a total reaction volume of 50 µl.
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	8-14*
	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 to yield > 1 nM of cDNA library.

Table 12

Total RNA Input Amount (ng)	Number of Cycles
800 – 1000	8 – 10
400 – 799	12 – 13
100 – 399	13 – 14
1 – 99	14

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

Post-Amplification Cleanup

40. Equilibrate the sparQ PureMag Beads to room temperature (RT) for 20 min.
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 32 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 **30 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 a digital electrophoresis system such as the Agilent 2100 Bioanalyzer or Agilent 2200 TapeStation per manufacturer's instructions.

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.

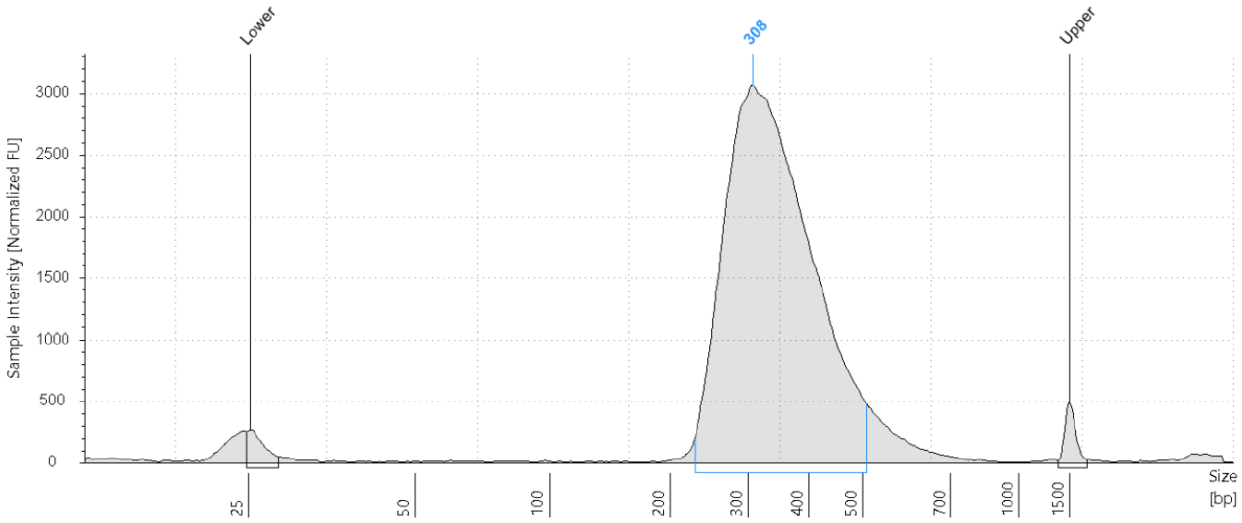


Figure 1: Example of cDNA library prepared using 100 ng of Universal Human Reference (UHR) RNA. Analyzed using Agilent TapeStation 4200 instrument.

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: 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 increase 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 150-175 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.

Adapter dimers can be removed from the final library products via 2 rounds of bead cleanup. Products <157 bp typically represent adapter dimers. Adapter dimers can be removed with 0.9X (beads to sample) sparQ PureMag Beads clean up.

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

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

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.