Quantabio

# sparQ mRNA-Seq Kit

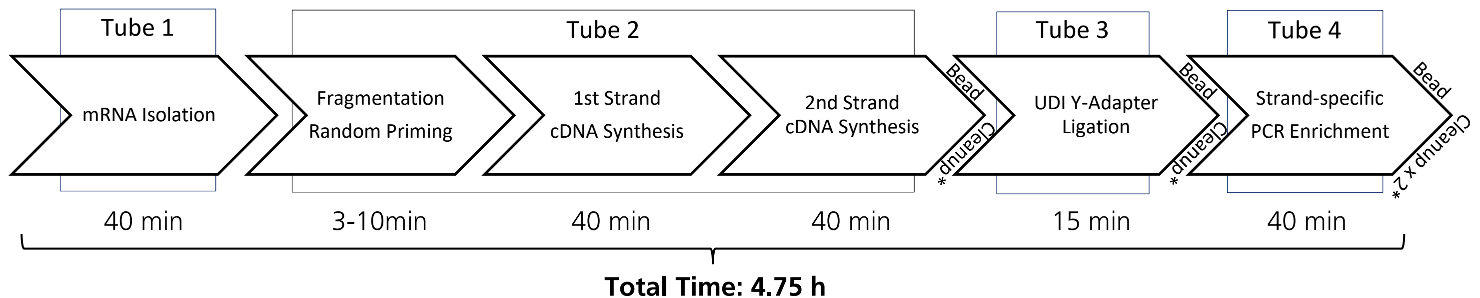
| Cat. No. | | Size | |
|---|---|---|---|
| | 95218-008 (Sample size only) | | 8 reactions |
| | 95218-024 | | 24 reactions |
| | 95218-096 | | 96 reactions |

**Box 1: Store at 2°C to 8°C**
**Box 2: Store at -25°C to -15°C**

## Description

The sparQ mRNA-Seq Kit provides reagents essential for construction of stranded RNA libraries with total RNA input from eukaryotes for Illumina® NGS platforms. The kit uses proprietary, highly optimized enzymes in a streamlined workflow with integrated poly-A enrichment for RNA library preparation in 4.75 hours. High quality mRNA libraries can be prepared from RNA with RIN ≥ 7 at varying input quantities (5 - 1000 ng).

## Workflow Overview




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

## Components

| | Component Description | Cap Color | Volume 8 Reactions | Volume 24 Reactions | Volume 96 Reactions |
|---|---|---|---|---|---|
| **Box1** Store at 2°C to 8°C | Oligo-dT Beads | Yellow | 1 x 280 µl | 1 x 840 µl | 3 x 1.12 ml |
| | Binding Buffer | Yellow or Clear (Bottle)* | 1 x 968 µl | 3 x 968 µl | 1 x 11.7 ml |
| | Suspension Buffer | Yellow | 1 x 400 µl | 1 x 1.2 ml | 4 x 1.2 ml |
| | Wash Buffer | Yellow or Clear (Bottle)* | 2 x 1.44 ml | 1 x 8.64 ml | 1 x 34.6 ml |
| | 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 |

* The 'Binding Buffer' is in a clear-capped bottle for the 96 reaction kit. The 'Wash Buffer' is in a clear-capped bottle for the 24 and 96 reaction kits. Otherwise, they are in a tube with a yellow cap.

| | Component Description | Cap Color | Volume 8 Reactions | Volume 24 Reactions | Volume 96 Reactions |
|---|---|---|---|---|---|
| **Box 2** Store at -25°C to -15°C | RNase Inhibitor | Yellow | 1 x 24 µl | 1 x 24 µl | 1 x 96 µl |
| | mRNA Frag Prime Buffer | 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 |

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

## Storage and Stability

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

## Additional reagents and materials 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 mRNA-Seq 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.

| 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 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 |

- To check NGS library size and quality, we recommend using automated electrophoresis systems such as Agilent 2100 Bioanalyzer or 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 (EB), 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 RNase Inhibitor, 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 briefly 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 the Agilent TapeStation) that provides an RNA integrity number (RIN). Ideally, RIN value ≥ 9 is recommended for low input, such as 5 ng. For higher amounts, RIN ≥ 7 is recommended.

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## Protocol

### mRNA Isolation

1. Bring Oligo-dT Beads, Binding Buffer, Suspension Buffer and Wash Buffer to room temperature (RT) prior to use.
2. Remove the mRNA Frag Prime Buffer and RNase Inhibitor from -20°C and thaw on ice.

*Pre-program a thermal cycler according to Table 2 with heated lid set to **105°C**. Start program to pre-heat block to 70°C and pause. Enter the reaction volume of **150 µl** or enter the maximum volume allowed in the cycler.*

3. Prepare RNA Binding Master Mix as per Table 1. Mix by pipette and briefly centrifuge. Store on ice.
4. Thoroughly vortex **Oligo-dT Beads**. Transfer **35 µl** into a new thin-walled PCR tube for each reaction.

***Note**: The RNA Binding Master Mix (Table 1) can be prepared for multiple reactions and stored on ice until use. The master mix should be mixed by pipetting, then purified RNA added and mixed again by pipetting.*

**Table 1:** RNA Binding Master Mix

| | Component | Per Reaction (µl) |
|---|---|---|
| | Nuclease-free water | 38 |
| ● | Binding Buffer | 71 |
| ● | RNase Inhibitor | 1 |
| | **Total** | **110** |

5. Add 110 µl of RNA Binding Master Mix to each tube from Step 4.
6. Add 5 µl of RNA for a total reaction volume of 150 µl. Mix by pipette and briefly centrifuge. Incubate according to Table 2.

**Table 2**

| Step | Temperature (lid 105°) | Incubation Time |
|---|---|---|
| 1 | 70°C | 3 min |
| 2 | 25°C | 10 min |

*Pre-program a thermal cycler according to Table 2 once again with heated lid set to **105°C.** Start program to pre-heat block to 70°C and pause.*

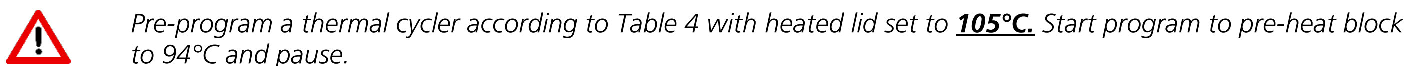
7. After incubation, briefly centrifuge, place on magnet until clear, and discard supernatant.
8. Add **180 µL** of **Wash Buffer**, mix by pipetting, place on magnet until clear, and discard supernatant.
9. Add **50 µL** of **Suspension Buffer**. Vortex, centrifuge, and incubate in thermocycler according to Table 2 (above).
10. Add **50 uL** of **Binding Buffer**, vortex. Incubate for 10 min at room temperature (20-25°C). Place on magnet until clear and discard supernatant.

11. Add **180 µL** of **Wash Buffer**, mix by pipetting, place on magnet until clear, and discard supernatant.

***Note***: *These tubes contain purified mRNA. Remove the tube from the magnet with the cap closed and place on ice. Keep the tube on ice while preparing* Frag & Prime Master Mix *in step 13.*

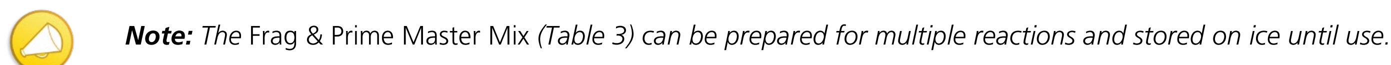
12. Proceed to fragmentation and priming step.

**RNA Fragmentation and Priming**



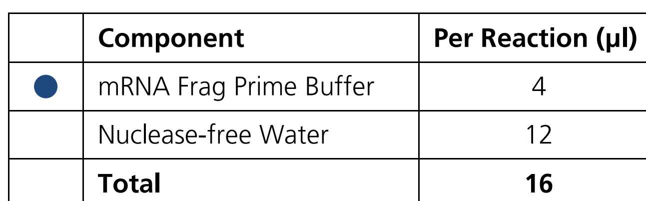
*Pre-program a thermal cycler according to Table 4 with heated lid set to **105°C.** Start program to pre-heat block to 94°C and pause.*

13. Prepare Frag & Prime Master Mix on ice according to Table 3.



***Note:*** *The* Frag & Prime Master Mix *(Table 3) can be prepared for multiple reactions and stored on ice until use.*

**Table 3:** Frag & Prime Master Mix



| | Component | Per Reaction (µl) |
|---|---|---|
| ● | mRNA Frag Prime Buffer | 4 |
| | Nuclease-free Water | 12 |
| | **Total** | **16** |

14. Add **16 µL** of the Frag & Prime Master Mix to each sample with beads from Step 11. Mix well.

***Note:*** *To resuspend the beads, pipette or vortex until fully mixed.*

15. Briefly centrifuge the reactions from Step 14.
16. Load the reactions into the pre-programmed cycler. Incubate according to Table 4.

**Table 4**

| Step | Temperature (lid 105°C) | Incubation Time | | |
|---|---|---|---|---|
| | | Desired Insert size > 320 bp | Desired Insert size 280 – 320 bp | Desired Insert size ~260 bp |
| 1 | 94°C | 3 min | 6 min | 10 min |
| 2 | 4°C | Hold | | |

17. After fragmentation, briefly centrifuge the tube, place on magnet until clear and transfer supernatant (~16 µL) to new tube.
18. Proceed immediately to First Strand Synthesis.

**First Strand Synthesis**

19. Remove 1st Strand Enzyme Mix from the freezer just before using. Thaw on ice.

*Pre-program a thermal cycler according to Table 5 with heated lid set to **75°C.** Start program to pre-set block to 25°C and pause.*

20. Add **4 µl** of the 1st Strand Enzyme Mix ( ● ) to the **16 µl** of fragmented and primed mRNA from Step 17 for a total reaction volume of 20 µl.

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

**Table 5**

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

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

### Second Strand Synthesis and End Polishing

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

25. Prepare Second Strand Synthesis Master Mix on ice according to Table 6.

***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 7 with heated lid set to **75°C.** Start program to pre-chill block to 16°C and pause.*

**Table 6**: Second Strand Synthesis Master Mix

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

26. Add **30 µl** of the Second Strand Synthesis Master Mix to the **20 µl** of First Strand Synthesis product from Step 22 for a total reaction volume of 50 µl.

27. Mix well by pipetting up and down 5 times and briefly centrifuge. Incubate as described in Table 7.

**Table 7**

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

28. 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 to cDNA following cleanup, the final elution must be collected in to a thin-walled PCR tube.*

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 **90 µl** (1.8X) to the Second Strand Synthesis product 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 **69 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 **67 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 8 and Appendix A provide general guidance on recommended sparQ UDI Adapter concentrations.*

**Table 8**

| Total RNA Input | Adapter Dilution* |
|---|---|
| 101 – 1000 ng | 1:10 |
| 51– 100 ng | 1:100 |
| 5 - 50 ng | 1.:200 |

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

35. Thaw Rapid Ligation Buffer (5X) on ice. Remove T4 DNA Ligase from the freezer just before use.
36. Prepare Ligation Master Mix on ice according to Table 9.

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

*Pre-program a PCR cycler according to Table 10 with heated lid off. Start program to pre-set block to 20°C and pause.*

**Table 9:** 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.*

37. Add **3 µl** of diluted unique adapter to each sample from step 34.
38. Transfer **30 µl** of Ligation Master Mix to each PCR tube containing sample plus adapter from step 37 for a total reaction volume of 100 µl. Mix well by pipetting up and down 5 times, gently vortexing if necessary, then briefly centrifuge.
39. Load the ligation reactions into the pre-programmed cycler. Resume the incubation as described in Table 10. Lid must be OFF and < 30°C.

***Note:*** *Heated lid must be OFF and ensure the lid temperature is <30°C during incubation.*

**Table 10**

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

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

### Post-Ligation Cleanup

41. Ensure that the sparQ PureMag Beads and Bead Booster mixture has been kept at room temperature (RT) for at least 20 min before use.
42. Thoroughly vortex the sparQ PureMag Beads slurry and add **70 µl** (0.7X) to the sample from Step 40. Mix well by pipetting.
43. Incubate the mixture for 5 min at RT. Pellet the beads on a magnetic stand and carefully remove and discard the supernatant.
44. 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.
45. 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 DNA recovery.

46. 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 4°C for up to 24 hours.

### Library Amplification

47. Thaw HiFi Plus Master Mix (2X) and Primer Mix on ice.
48. Prepare PCR Master Mix according to Table 11.

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

*Pre-program a PCR cycler according to Table 12 with heated lid set to 105°C. Start program to pre-heat block to 37°C and pause.*

**Table 11:** PCR Master Mix

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

49. Add **26.5 µl** of the PCR Master Mix to **23.5 µl** of purified, adapter-ligated DNA from Step 46 for a total reaction volume of 50 µl.
50. Incubate as described in Table 12.

**Table 12**

| 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 | 8-16* |
| | 60°C | 30 sec | |
| | 72°C | 30 sec | |
| 4 | 72°C | 1 min | 1 |
| 5 | 4°C | Hold | 1 |

* Refer to Table 13 for recommended number of PCR cycles.

**Table 13**

| Total RNA Input Amount (ng) | Number of Cycles |
|---|---|
| 800 – 1000 | 8 – 10 |
| 400 – 799 | 12 – 13 |
| 100 – 399 | 13 – 14 |
| 5 – 99 | 15-16 |

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

**Post-Amplification Cleanup**

52. Ensure that the sparQ PureMag Beads and Bead Booster mixture has been kept at room temperature (RT) for at least 20 min before use.
53. Thoroughly vortex the sparQ PureMag Beads slurry and add **45 µl** (0.9X) to the sample from Step 51. Mix well by pipetting.
54. Incubate the mixture for 5 min at RT. Pellet the beads on a magnetic stand and carefully remove and discard the supernatant.
55. 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.
56. 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 recovery.
57. 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.
58. Repeat steps 53 to 56 for a total of two bead cleanups.
59. 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.
60. The sample can be stored at 4ºC for up to 24 hours or at -20ºC for longer term storage.

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**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.

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 **52 – 57** and **59** in the protocol before sequencing.

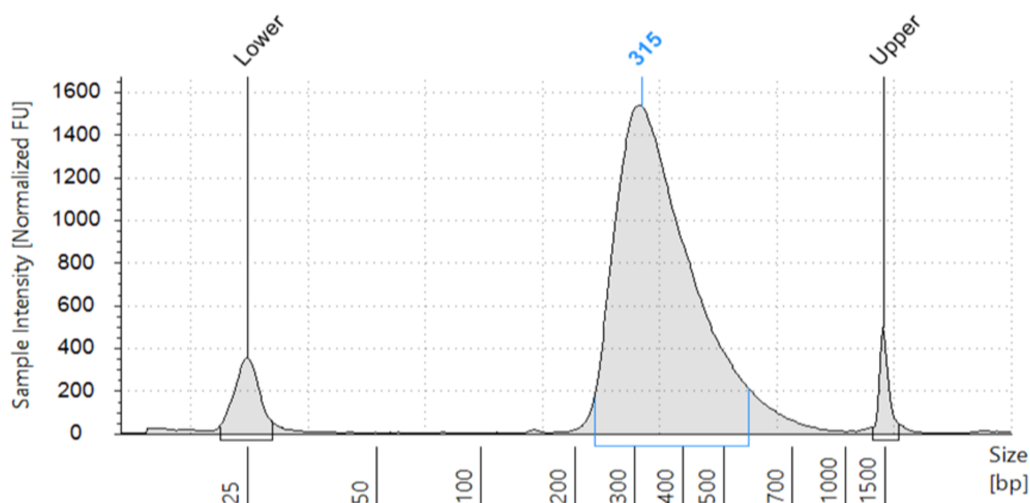


**Figure 1:** Example of cDNA library prepared using mRNA isolated from 100 ng of UHR RNA (RIN 9.7). Analyzed using Agilent TapeStation 4200 instrument.

## 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 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.

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, 1:100 and 1:200 dilutions as a general guideline.

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

Prepare a **1:100 dilution** by adding 2 µl of the diluted (1:10) adapters to the 18 µl of UDI Dilution Buffer.

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

Quantabio

## NGS Products Used in Protocol Sold Separately

### sparQ PureMag Beads

| 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 |

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)

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

The sparQ UDI Adapters are 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

| Part Number | Description | Kit Size |
|---|---|---|
| 95210-100 | sparQ Universal Library Quant Kit | 100 rxn |
| 95210-500 | sparQ Universal Library Quant Kit | 500 rxn |

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.