sparQ DNA Library Prep Kit

Cat. No. 95191-024
95191-096

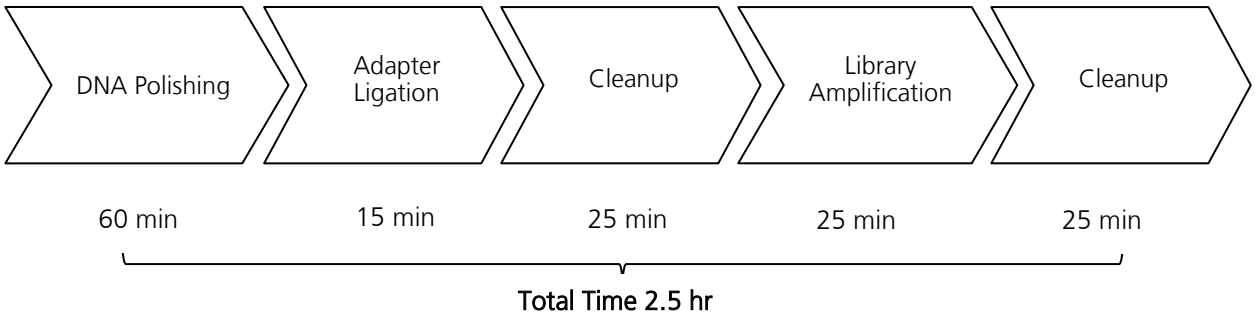
Size: 24 reactions
96 reactions

Store at -25°C to -15°C

Description

The sparQ DNA Library Prep Kit provides components for the rapid construction of DNA libraries from fragmented double-stranded DNA for sequencing on Illumina® NGS platforms. The streamlined workflow can be completed in under 3 hours with minimal hands-on time. The DNA polishing reactions are combined in a single step to convert fragmented DNA into 5´-phosphorylated and 3´-dA-tailed DNA fragments suitable for direct ligation of sequencing adapters without the need for an intervening cleanup. The HiFi PCR Master Mix and Primer Mix allow the optional, unbiased amplification of fragments with appropriate adapters ligated to both ends. The kit is compatible with multiple sample types and facilitates efficient and consistent library construction from a wide range of input amounts from 0.25 ng to 1000 ng DNA.

Workflow Overview



Instrument Compatibility

The kit is compatible with instruments of the Illumina sequencing platform.

Components

			Volume	
	Component Description	Cap Color	24 Reactions	96 Reactions
●	DNA Polishing Enzyme Mix (5X)	Blue	1 x 240 µl	1 x 0.96 ml
●	DNA Polishing Buffer (10X)	Blue	1 x 120 µl	1 x 0.48 ml
●	DNA Ligase	Orange	1 x 240 µl	1 x 0.96 ml
●	DNA Rapid Ligation Buffer (5X)	Orange	1 x 480 µl	2 x 0.96 ml
○	HiFi PCR Master Mix (2X)	White	1 x 600 µl	2 x 1.25 ml
○	Primer Mix	White	1 x 72 µL	1 x 0.144 ml

Storage and Stability

Store kit components 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

Purification Beads

Purification beads for post-ligation and post-amplification reaction cleanups are not included with the kit and must be purchased separately. This protocol has been validated using either sparQ PureMag Beads or AMPure® XP (Beckman Coulter). 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

The sparQ DNA Library Prep Kit does not include adapters, sparQ UDI adapters must be purchased separately. The sparQ DNA Library Prep Kit is also compatible with other Y-shaped adapters including non-barcoded, single-barcoded, or dual-barcoded adapters routinely used in library construction workflows.

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

NGS Library Quantification Kit

Accurate quantification of DNA 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 practices 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 where samples can be frozen at -20°C 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 to obtain the best results when performing protocol

Before You Begin

- Prepare a fresh solution of 80% ethanol, store at room temperature.
- Prepare a solution of 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 DNA Polishing Enzyme Mix, DNA Ligase, and HiFi PCR Master Mix to ensure even distribution of contents. Other tubes can be briefly vortexed to ensure mixing.
- Determine the amount of input DNA using standard methods.

Protocol

DNA Polishing

DNA Polishing combines DNA end-repair and dA-tailing into one step.

1. Program the thermal cycler with the parameters in the table below. If possible, set the temperature of the instrument's heated lid to 70°C.

Note: *Use of heated lid at >70°C may affect the temperature of the fragmentation reaction.*

Step	Temperature	Incubation Time
1	4°C	1 min
2	20°C	30 min
3	65°C	30 min
4	4°C	Hold

2. Run the thermal cycler program. When the thermal cycler block reaches 4°C, pause the program.

This protocol is suitable for 0.25 - 1000 ng input fragmented DNA. We recommend that the DNA be in 10 mM Tris-HCl pH 8.0 buffer, nuclease-free water or 1X TE buffer. If the input DNA volume is less than 35 µL, add nuclease-free water to a final volume of 35 µL.

3. Prepare the DNA Polishing Master Mix in a new thin-walled PCR tube on ice by combining the DNA Polishing Enzyme Mix and the DNA Polishing Buffer per the volumes in the table below. Mix well by gently pipetting. The input DNA (0.25 ng – 1000 ng) should be in water, 10 mM Tris-HCl pH 8.0 buffer, or 1X TE buffer.

	Component	Volume for 1 Reaction (µl)
●	DNA Polishing Enzyme Mix (5X)	5
●	DNA Polishing Buffer (10X)	10
	Total	**15**

4. Transfer **35 µl** DNA to a new thin-walled PCR tube for each reaction. Add **15 µl** of the DNA Polishing Master Mix from step 3 and gently mix well by pipetting up and down 10-12 times. It is critical to keep the tubes on ice during reaction setup.

5. Pulse-spin the sample tubes and immediately transfer to the pre-chilled thermal cycler (4°C). Resume the cycling program.

6. When thermal cycler program is complete and block temperature has returned to 4°C, remove the sample tubes from the block and place on ice.

7. Immediately proceed to the adapter ligation step.

Adapter Ligation

Adapters may need to be diluted before use. To achieve optimal adapter ligation efficiency for various input DNA amounts, it is recommended to adjust insert/adapter molar ratio accordingly. The following table provides general guidance on recommended sparQ UDI adapter concentrations for 250 bp DNA fragments.

Detailed guidance is provided in Appendix C.

DNA Sample (250 bp Fragments)	Dilution of Adapter stock solution	Final Adapter Concentration in Ligation
500 ng	None	500 nM
100 ng	1:2 (2 fold)	250 nM
10 ng	1:25 (25 fold)	20 nM
1 ng	1:500 (500 fold)	1 nM

8. Transfer **4 µl** of appropriately diluted adapters into the PCR tube with **50 µl** of fragmented DNA from step 6. Mix gently by pipetting and cool on ice.

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

9. Prepare the Ligation Master Mix in a separate tube on ice per the table below. Mix well by pipetting. Volumes can be scaled as needed for the desired number of samples.

	Components	Volume for 1 Reaction (µl)
●	DNA Rapid Ligation Buffer	20
●	DNA ligase	10
	Nuclease-free H_2O	16
	Total	**46**

10. Add **46 µl** of the Ligation Master Mix to the **54 µl** fragmented DNA and adapters sample from step 8 and mix well by pipetting.

11. Incubate the ligation reaction at 20°C for 15 min using a thermal cycler without enabling the heated lid.

12. Proceed immediately to adapter ligation cleanup using sparQ PureMag Beads

Adapter Ligation Cleanup

Adapter ligation 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 library amplification following cleanup, the final elution must be collected in to a thin-walled PCR tube.

*If size selection is required, follow instructions in **Appendix B**. Size selection is not recommended for inputs <100 ng.*

13. Equilibrate the sparQ PureMag Beads to room temperature (RT) for 20 min.

14. Thoroughly vortex the sparQ PureMag Beads slurry and add **80 µl** (0.8X) to the ligation sample from step 11. Mix well by pipetting.

15. Incubate the mixture for 5 min at RT. Pellet the beads on a magnetic stand (e.g., DynaMag™) and carefully remove and discard the supernatant.

16. Keeping the tube on the magnetic stand, add **200 µl** of the 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 for a total of two washes.

17. Use 10 µl pipette to remove any excess ethanol from the bottom of the tube.

18. Air-dry the beads on the magnetic stand for 5 - 10 min or until the beads pellet appears matte and no longer shiny. Over-drying of beads may result in lower DNA recovery.

19. If optional library amplification is intended: Resuspend the dried beads in **25.5 µl** of 10 mM Tris-HCl, pH 8.0. 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 -20°C.

    If library amplification is not intended: Resuspend the dried beads in **12.5 µl** of 10 mM Tris-HCl, pH 8.0. Incubate the beads at room temperature for 2 - 3 min. Pellet the beads on the magnetic stand. Carefully transfer **10 µl** of supernatant into a new thin-walled PCR tube and proceed to validation and quantification of the library using gel electrophoresis, qPCR and/or Bioanalyzer. An additional 1X sparQ PureMag Beads purification may be added if significant adapter and/or adapter dimer are detected. If not proceeding immediately, the sample can be stored at -20°C.

Library Amplification (Optional)

Library amplification is generally recommended if the input DNA is below 100 ng. 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 flanked by the standard P5 and P7 adapter sequences. If a different primer mix is preferred, please follow the supplier's instructions.

20. Prepare the PCR Master Mix in a separate tube on ice by combining the HiFi PCR Master Mix (2X) and Primer Mix per the table below. Mix well by pipetting. Volumes can be scaled as needed for the desired number of reactions.

	Components	Volume for 1 Reaction (µl)
○	HiFi PCR Master Mix (2X)	25
○	Primer Mix	1.5
	Total	**26.5**

21. Add **26.5 µl** of the PCR Master Mix from step 20 to the **23.5 µl** of DNA sample from step 19 in the thin-walled PCR tube and mix gently by pipetting up-and-down 8 - 10 times. Keep the PCR tube on ice during reaction setup.
22. Program a thermal cycler with the parameters listed in the table below. Set the instrument's heated lid to 105°C. When the thermal cycler block reaches 98°C, pause the program.

Step	Temperature	Incubation Time	Cycles
1	98°C	2 min	1
2	98°C	20 sec	Varies based on input amount – see table on page 9
3	60°C	30 sec	
4	72°C	30 sec	
5	72°C	1 min	1
6	4°C	Hold	1

Note: *Excessive library amplification increases the likelihood of amplification bias and the generation of unwanted artifacts. Therefore, it is recommended to limit the number of amplification cycles to the minimum needed to achieve acceptable yield for downstream processes. Yields in the range of 250 – 1,000 ng are typically sufficient for target capture and sequencing applications. The table below provides guidelines on the number of cycles to yield 500 ng of DNA library from various sample input amounts.*

Input DNA Sample (ng)	Suggested Number of Cycles to Yield 500 ng of DNA Library
1000	0 - 1
500	1 - 2
100	4 - 5
50	5 - 6
10	8 - 10
1	13 - 15
0.25	16 - 18

23. Pulse-spin the sample tube and immediately transfer to the pre-heated thermal cycler (98°C). Resume the cycling program.
24. When the thermal cycler program is complete and sample block has returned to 4°C, remove the sample from the block and proceed immediately to post-amplification cleanup using sparQ PureMag Beads.

Post-Amplification Cleanup

25. Equilibrate the sparQ PureMag Beads to RT for 20 min.
26. Thoroughly vortex the sparQ PureMag Beads slurry and add 50 µl (1X) to the PCR reaction. Mix well by pipetting.
27. Incubate the mixture for 5 min at RT. Pellet the beads on a magnetic stand (e.g., DynaMag) and carefully discard the supernatant.

28. Keeping the tube on the magnetic stand, add 200 µl of the 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 for a total of two washes.
29. Use 10 µl pipette to remove any excess ethanol from the bottom of the tube.
30. Air-dry the beads on the magnetic stand for 5 - 10 min or until the bead pellet appears matte and no longer shiny. Over-drying of beads may result in lower DNA recovery
31. Resuspend the dried beads in **32.5 µl** of 10 mM Tris-HCl, pH 8.0. Incubate the beads at room temperature for 2 - 3 min. Pellet the beads on the magnetic stand. Carefully transfer **30 µl** of supernatant into a new tube. The sample can be stored at -20°C if not proceeding immediately to library quantification or other downstream processes.

Library Validation and Quantification

DNA libraries constructed using the above instructions 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 instructions.

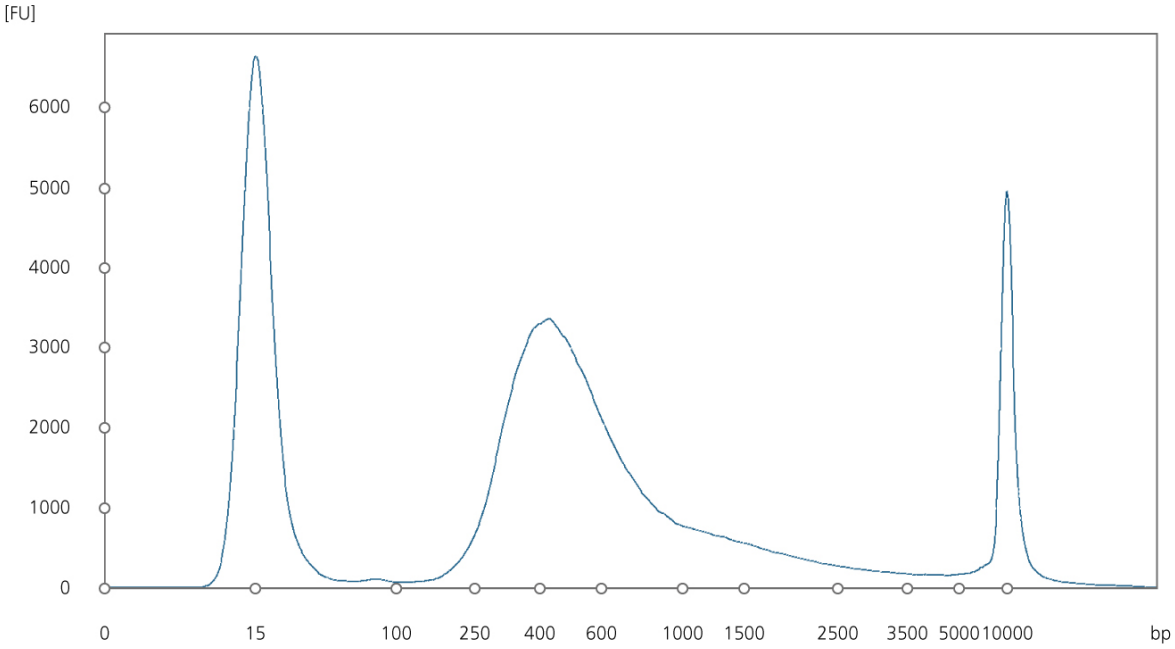


Figure 1: Typical Agilent TapeStation trace of prepared library using sparQ DNA Frag and Library Prep Kit. Library was prepared using 500 ng input hgDNA, fragmented for 15 min and ligated with sparQ UDI adapters. Following post-ligation cleanup, libraries were amplified with 2 PCR cycles. Peaks at 15 and 10000 bp represent low and high MW markers.

An estimate of library concentration can be assessed using Qubit or another fluorometric method.

More accurate library quantification can be obtained using a qPCR-based assay. Quantabio offers the sparQ Universal Library Quant Kit (95210-100, 95210-500) for accurate quantification of DNA library molecules suitable for sequencing on Illumina NGS platforms.

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.

Functional specifications: QC Library length must be within 15% of the reference library length. Concentration of the QC library generated from 100 ng input DNA (average ~300 bp fragments) is >60 nm with mapped reads >90%. For QC library, normalized coverage should be within 0.7 to 1.3 for most of the genome (10% - 80% GC content).

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Appendix A: Removal of Divalent Cations and EDTA from Input Nucleic Acid

Input DNA for the fragmentation reaction should be in nuclease-free water, 10 mM Tris-HCl (pH 8.0), buffer EB, or LoTE (0.1X TE). If the DNA was dissolved in 1X TE or the concentration of cations and chelators is not known, we strongly recommend purifying the DNA using sparQ PureMag Beads per the instructions below.

1. If DNA is in a volume of less than 50 µl, adjust the volume to **50 µl** with nuclease-free water.
2. Add **90 µl** of thoroughly vortexed sparQ PureMag Beads slurry to the reaction for a ratio of 1.8X and mix well by pipetting. If DNA is in a volume greater than 50 µl, scale the volume of sparQ PureMag Beads appropriately such that the ratio of beads to DNA is 1.8X.
3. Incubate the mixture for 5 min at room temperature. Pellet the beads on a magnetic stand for 2-4 min and carefully discard the supernatant without disturbing the beads.
4. Keeping the tube on the magnetic stand, gently pipette **200 µl** of the freshly prepared 80% ethanol to over the bead pellet to wash. Pellet the beads on the magnetic stand for 30 s then carefully remove and discard the supernatant. Repeat the wash for a total of two washes.
5. Use a 10 µL pipette to remove any excess ethanol from the tube.
6. Air-dry the beads on the magnetic stand for 10 min or until the bead pellet appears matte and no longer shiny. Over-drying of beads may result in lower DNA recovery.
7. Thoroughly resuspend the dried beads in **45 µl** of 10 mM Tris-HCl, pH 8.0 and incubate at RT for 2-3 min. Pellet the beads on the magnetic stand for 2 min or until the solution is clear. Carefully transfer **42.5 µl** of supernatant into a new tube.
8. Determine the concentration of the purified DNA using Qubit, Picogreen or other methods.

Appendix B: 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 DNA input amounts and range of tunable insert sizes, it is recommended that the adapter concentration be adjusted accordingly. Generally, increasing the input amount of DNA and/or tuning to smaller insert sizes, increase the concentration of adapters required. Both result in an increase concentration of DNA available for ligation. Decreasing the input amount of DNA 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.

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 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 bead cleanup. Products >200 bp typically represent adapter dimers. Adapter dimers can be removed with 0.8X (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

Below are recommendations of for adapter concentrations of various vendors at 250 bp inserts.

DNA Input (250 bp Fragments)	Adapter concentration in Ligation **(sparQ UDI)**
500 ng	125 to 500 nM
100 ng	75 to 300 nM
10 ng	10 to 25 nM
1 ng	0.2 to 1 nM

DNA Input (250 bp Fragments)	Adapter concentration in Ligation **(IDT UDI)**
500 ng	375 to 750 nM
100 ng	375 to 750 nM
10 ng	25 to 50 nM
1 ng	0.2 to 1 nM

DNA Input (250 bp Fragments)	Adapter volume in Ligation **(Illumina TruSeq)**
500 ng	5 to 7.5 µL*
100 ng	5 to 7.5 µL*
10 ng	1 to 2.5 µL
1 ng	2.5 µL **

*One or two extra cycles of PCR recommended.
** For 1 ng input, it is recommend to dilute Illumina TruSeq adapters to 0.2x-0.1x for the ligation step.

Appendix C: Size selection with sparQ PureMag Beads

Depending on the specific application, size selection may be required to ensure the DNA insert size falls into a certain range. The ligation reaction buffer can greatly influence the size selection range so initial purification using the sparQ PureMag Beads before size selection is recommended (step 1.1- 1.7 below). If size selection without initial purification is preferred, the required amount of beads should be determined empirically.

The following size selection protocol is for libraries with a fragment size range of 250-500 bp. For libraries with different size distribution, refer to the table below for adjustments to bead ratios. The protocol and guidelines are based on double-sided size selection of adapter ligated DNA with Y-shaped adapters attached in a starting volume of 100 µl.

The size range reflects the final fragment size and insert size based on sparQ UDI Adapters. If calculating the insert size with different adapters, please take into account that the adapter size and configuration both have a significant impact on the size of the fragment appearing on the Bioanalyzer or other size differentiation and detection instruments.

	Target Insert Size	200 bp	350 bp	550 bp
Volume sparQ PureMag Beads (µL)	Initial cleanup	80	80	80
	1st binding	32.5	27.5	25
	2nd binding	10	7.5	7.5
	Final DNA fragment range	250-500 bp	450-600 bp	500-800 bp

1. Initial cleanup
   1.1. Equilibrate the sparQ PureMag Beads to room temperature (RT) for 20 min.
   1.2. Thoroughly vortex the sparQ PureMag Beads slurry and add **80 µl** (0.8X) to the ligation sample from step 11 (main protocol). Mix well by pipetting.
   1.3. Incubate the mixture for 5 min at RT. Pellet the beads on a magnetic stand and carefully remove and discard the supernatant.
   1.4. Keeping the tube on the magnetic stand, gently pipette **200 µl** of the freshly prepared 80% ethanol to over the bead pellet to wash. Pellet the beads on the magnetic stand for 30 s then carefully remove and discard the supernatant. Repeat the wash for a total of two washes.
   1.5. Use a 10 µl pipette to remove any excess ethanol from the bottom of the tube.
   1.6. Air-dry the beads on the magnetic stand for 5 - 10 min or until the beads the bead pellet appears matte and no longer shiny. Over-drying of beads may result in lower DNA recovery.
   1.7. Resuspend the dried beads in **53 µl** of 10 mM Tris-HCl, pH 8.0. Incubate the beads at room temperature for 2 - 3 min. Pellet the beads on the magnetic stand. Carefully transfer **50 µl** of supernatant into a new tube.

2. First Binding
   2.1. Thoroughly vortex the sparQ PureMag Beads slurry and add **32.5 µl** (0.65X) to the eluted DNA from step 1.7. Mix well by pipetting.
   2.2. Incubate the mixture for 5 min at RT. Pellet the beads on the magnetic stand until the liquid is clear. **DO NOT discard supernatant**.
   2.3. Transfer all supernatant to a new tube. Be careful not to carry any beads into the new tube. Discard the beads

3. Second Binding

3.1. Add **10 µl** (0.85X) of new beads into the tube containing the supernatant from step 2.3 and mix thoroughly by vortexing or pipetting up and down several times with pipette set at 100 µl. To ensure that the accurate amount of beads is added, take extra caution during pipetting and mixing as the solution can be viscous to handle.

3.2. Incubate the mixture for 5 min at RT. Pellet the beads on the magnetic stand until the liquid is clear. Carefully **discard the supernatant**. Retain the beads.

3.3. Keep the tube on the magnetic stand gently pipette **200 µl** of the freshly-prepared 80% ethanol to over the bead pellet to wash. Incubate for 30 s to allow the beads to re-form into a pellet.

3.4. Carefully remove and discard the supernatant. Repeat the wash for a total of two washes.

3.5. Use a pipette set to 10 µL to remove any remaining ethanol from the bottom of the tube.

3.6. Air-dry the beads on the magnetic stand for 5 min or until the beads appear matte and not glossy. Over-drying of beads may result in lower DNA recovery.

3.7. **If optional library amplification is intended:** Resuspend the dried beads in 25.5 µl of 10 mM Tris-HCl, pH 8.0. 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 (step 20, main protocol). If not proceeding immediately, the sample can be stored at -20°C.

If library amplification is not intended: Resuspend the dried beads in 12.5 µl of 10 mM Tris-HCl, pH 8.0. Incubate the beads at room temperature for 2 - 3 min. Pellet the beads on the magnetic stand. Carefully transfer 10 µl of supernatant into a new thin-walled PCR tube and proceed to validation and quantification of the library using gel electrophoresis, qPCR and/or Bioanalyzer. An additional 1X sparQ PureMag Beads purification may be added if significant adapter and/or adapter dimer are detected. If not proceeding immediately, the sample can be stored at -20°C.

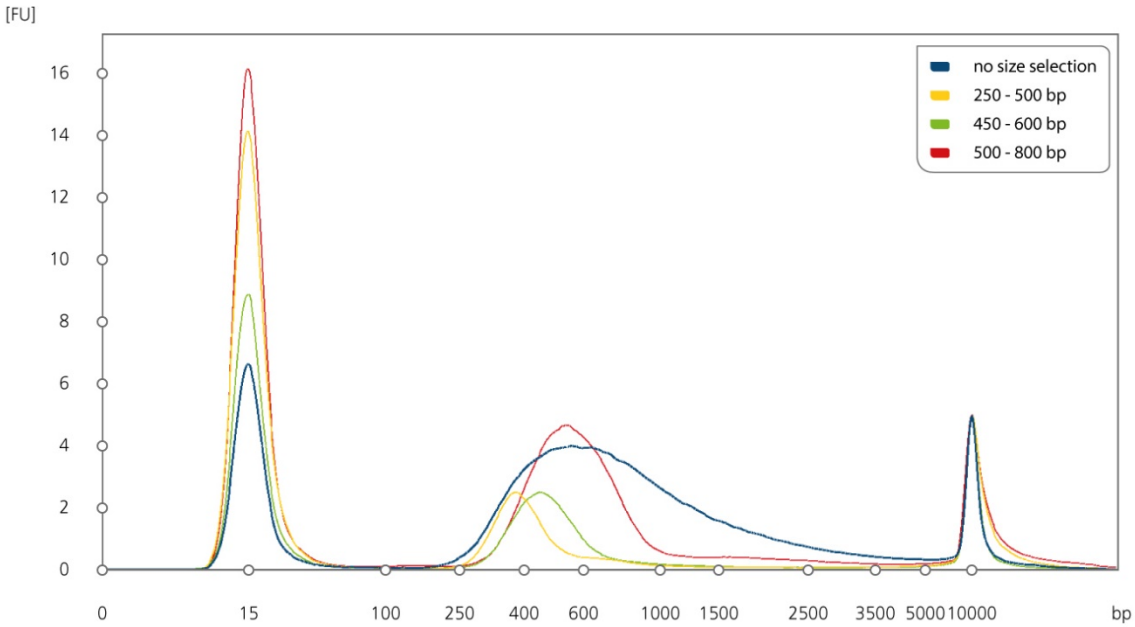

Figure 2: Example TapeStation traces of size selected DNA. Initial clean-up (0.8X) was carried out on adapter-ligated DNA (blue), then size-selected according to the volumes listed on page 13 for a target fragment range of 250- 500 bp (yellow), 450- 600 bp (green) or 500- 800 bp (red).

NGS products utilized 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 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.

Related NGS Products Sold Separately

sparQ DNA Frag & Library Prep Kit

Cat. No.	95194-024	Size:	24 reactions
	95194-096		96 reactions

The sparQ DNA Frag & Library Prep Kit is optimized for enzymatic fragmentation of DNA and streamlined construction
of high quality libraries for sequencing on Illumina® NGS platforms. The simple, convenient 2-step workflow can be completed in 2.5 hours with minimal hands-on time and accommodates DNA input amounts from 1 ng to 1000 ng.

sparQ HiFi PCR Master Mix

Cat. No.	95192-050	Size:	50 reactions
	95192-250		250 reactions

The sparQ HiFi PCR Master Mix is a high efficiency, high-fidelity, and low-bias PCR master mix for NGS workflows requiring DNA library amplification prior to sequencing. The included primer mix allows amplification of DNA libraries flanked by adapters containing the P5 and P7 Illumina® flow cell sequences.