

sparQ DNA Library Prep Kit

Cat. No. 95191-024
95191-096

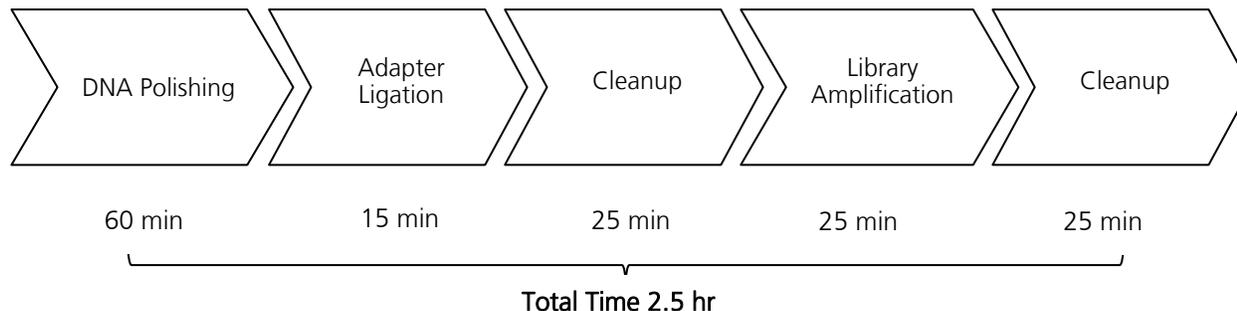
Size: 24 reactions
96 reactions

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 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 Illumina, Element and Singular sequencing instruments. This IFU is for use with Illumina sequencing instruments.

Components

	Component Description	Cap Color	Volume	
			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, stubby, single-barcoded, or dual-barcoded adapters (with or without unique molecular identifiers) routinely used in library construction workflows. 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

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
95210-15B	sparQ Library Dilution Buffer	15 ml

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 reaction.

Step	Temperature (lid 70°C)	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 Buffer (10X)	5
●	DNA Polishing Enzyme Mix (5X)	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.

- Pulse-spin the sample tubes and immediately transfer to the pre-chilled thermal cycler (4°C). Resume the cycling program.
- 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.
- Immediately proceed to the adapter ligation step.

Adapter Ligation

Adapters may need to be diluted using UDI Dilution Buffer (10 mM Tris-HCl, pH 8.0, 50 mM NaCl, 0.5 mM EDTA) 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 or third party stubby (truncated) adapter concentrations for 250 bp DNA fragments.



Detailed guidance is provided in Appendix B.

Total DNA Input (250 bp fragments)	Dilution of sparQ UDI Adapters	Dilution of 15 µM Stubby Adapters
500-1000 ng	None	None
100-499 ng	1:2	None
10-99 ng	1:25	None
1-9 ng	1:500	1:10
0.25-0.99 ng	1:500	1:50

- Transfer **4 µl** of appropriately diluted adapters into the PCR tube with **50 µl** of 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.

- 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 ₂ O	16
	Total	46

- Add **46 µl** of the Ligation Master Mix to the **54 µl** DNA and adapters sample from step 8 and mix well by pipetting.
- Incubate the ligation reaction according to below table without enabling the heated lid.

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

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 C**. 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 3 - 5 min or until the beads pellet appears matte and no longer shiny. Over-drying of beads may result in lower DNA recovery.
19. Full-length Y-shaped adapters such as the sparQ UDI Adapters allow for PCR-free library preparation when starting from DNA input amounts >100 ng. Library amplification is required for stubby adapters. Please choose carefully from the following options depending on the adapter type used for this preparation.

If full-length Y-shaped adapters (i.e., sparQ UDI Adapters) were used and 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 full-length Y-shaped adapters (i.e., sparQ UDI Adapters) were used and 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.



If stubby (truncated) adapters were used in adapter ligation: Resuspend the dried beads in **22 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 **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 -20°C.

Library Amplification



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 prepared with full-length Y-shaped adapters (i.e., sparQ UDI Adapters) and flanked by the standard P5 and P7 adapter 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.

Library Amplification for Full-Length Y-shaped Adapters

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	Per Reaction (µl)
○	HiFi PCR Master Mix (2X)	25
○	Primer Mix (10 µM)	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. Proceed to step **22**.

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 (lid 105°C)	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

Library Amplification for Stubby Adapters

20. 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**. The table below shows the volumes of each reagent in the final reaction for convenience.

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

21. To the **20 µl** of purified, adapter-ligated DNA from step 19, add **25 µl** HiFi PCR Master Mix and **5 µl** of the specific indexed primer for a total reaction volume of 50 µl. Mix gently by pipetting up-and-down 8-10 times. Keep the PCR tube on ice during reaction setup. Proceed to step **22**.



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 Amount (ng)	Suggested Number of Cycles
500 - 1000	2 - 3
100 - 499	4 - 5
50 - 99	5 - 6
10 - 49	8 - 10
1 - 9	13 - 15
0.25 - 0.99	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 **45 µl** (0.9X) 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 3 - 5 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. Special care must be taken for accurate validation of PCR-free libraries. For more information and step-by-step instructions, please refer to the application note, “Quality Control for PCR-Free WGS Libraries: A Guideline for TapeStation Analysis of PCR-Free Library Preparation with Y-Shaped Adapters.”

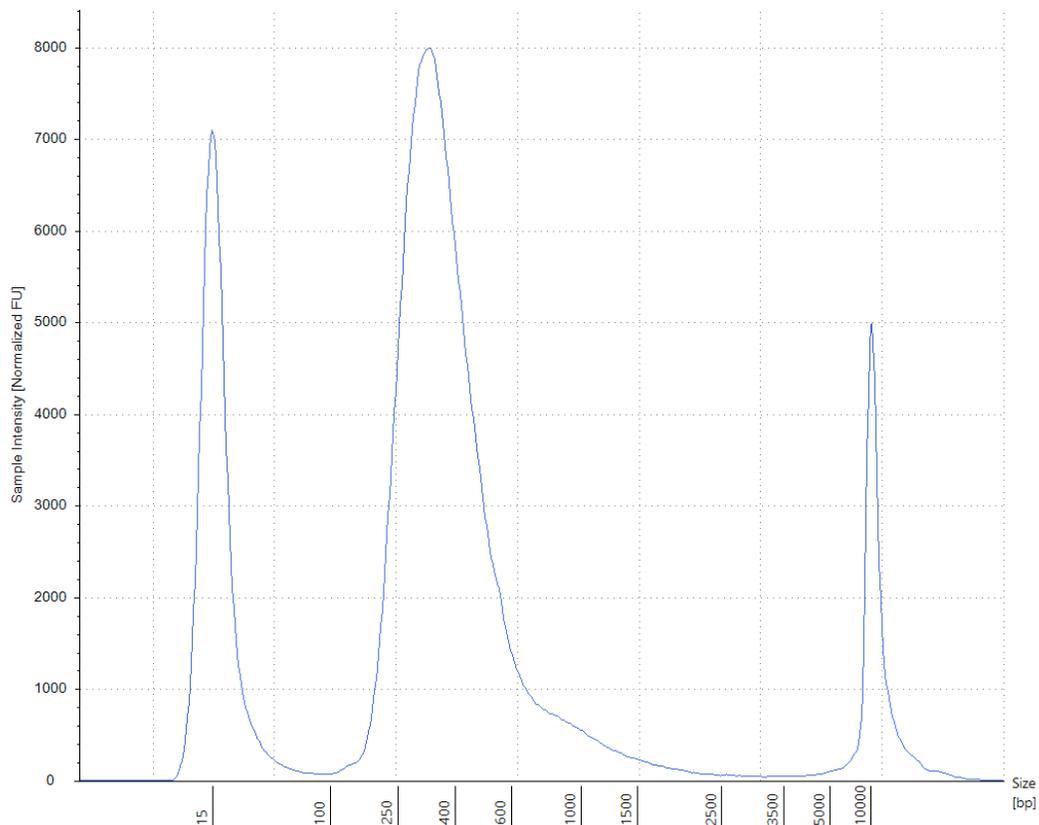


Figure 1: Typical Agilent TapeStation trace of prepared library using sparQ DNA Library Prep Kit. Library was prepared using 100 ng mechanically sheared FFPE DNA and ligated with sparQ UDI adapters. Following post-ligation cleanup, libraries were amplified with 5 PCR cycles. Peaks at 15 and 10000 bp 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 library preparation 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 μ l, adjust the volume to **50 μ l** with nuclease-free water.
2. Add **90 μ l** 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 μ l, 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 μ l** 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 μ L 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 μ l** 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 μ l** 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

It is recommended to perform serial dilutions of the adapters. UDI Dilution Buffer or 10 mM Tris-HCl pH 8.0, 50 mM NaCl, 0.5 mM EDTA can be used for adapter dilution. Optimal dilutions may vary depending on the nature of your samples and NGS sequencing operations. Use the following example showing preparation of 1:2, 1:25 and 1:500 dilutions as a general guideline.

Prepare a **1:2 dilution** by adding 5 µl of the adapters (stock) to 5 µl of UDI Dilution Buffer.

Prepare a **1:25 dilution** by adding 2 µl of the adapters (stock) adapters to 48 µl of UDI Dilution Buffer.

Prepare a **1:500 dilution** by adding 2 µl of the diluted (1:25) adapters to 38 µl of UDI Dilution Buffer.

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 increased 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 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.

Adapter dimers can be removed from the final library products via bead cleanup. 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	17	TGAACGTTGT	GAGCCAAGTT	TGCATAGCTT	CACTGCTATT	GATTGAGTTC	AGGCCTACAT	AGGATGTCCA	CCTCGGAATG	ATGACTCGAA	ACGTTGACTC	CCTCCATTAA	CTGATGATCT
	15	ATGGCCGACT	AGGCACCTTC	AGCCATAACA	CATTCTTGGA	GCCAGCTGTT	AGAATTCTGG	TTGTAGAAGG	CGCTAAGGT	CGCTCGTTAG	GTTGAGATTC	ATGGAATGGA	TTGTACTCCA
B	17	ACCAGACTTG	AAGGCCGTAG	AACCTTCTCG	AATGGTAGGT	GTAATGCCAA	TGTGAACCG	CACCTTATGT	GTCTGGAAC	GAACAATCCT	CCACTTAACA	AGTCGCGGTT	ACTAGGTGTT
	15	CGATGAGCAC	CTGTGGTAA	CCACAAGTGG	ATGCAAGGTT	TGCCCTGTGC	CATTGACTCT	CCTAGCCTA	GAGGTGAACA	AACAACGCTG	TGTGTGCGGA	CATTCTCTAC	GTGCACATAA
C	17	ACTGGCAAC	TTAGAGAAGC	AAGAGATCAC	GATACCTATG	TCGTTGCCT	CGTATTAAGC	AAGCGGCTGT	AGATTCACCA	TGGCAAGGAG	AGCAGTTCCT	CTCATCCAGG	CTGTAGCGG
	15	GATAAGTCGA	GCTGGTACTC	GTTATCACAC	CGCCAGACAA	CTATCCGCTG	GCGGCTCAA	ATCGTGTCT	TCAGAACTAC	C GCGGCTATT	GTTGCGGAA	GCATAGGAAG	AGGACAAGTA
D	17	GCGTTAGGCA	TCTAAGACCA	GCCTGAAGGA	CACTAGGTAC	AGGTGAGTAT	CCAGTGGTTA	TTCTGTGAG	TCGGTCAGAT	GAATATTGGC	TCGCCCTCGT	TGTGGTTGAA	ATCGACCAA
	15	TCACGCCCTG	TAAGGAGCGG	TACCGTCTT	GAAGGTTGGC	AATGCCGGAA	TTATGGTCTC	CCAACCTATC	CGGATATTGA	GCTCGACACA	AGCTGTATTG	TGTTCTGTT	CCGATTCGAG
E	17	TTATCGGCT	TGTAACCACT	ATTGTGCCCT	AGCTCGTTCA	TCGATAATGG	GCGTTCGAGT	AGTACAGTTC	CACTCTCGCT	CCGGAACCTA	TAGGACTGCG	TTATCGTGG	CTTACTTGGT
	15	AGGAACACAA	AATCGCTCCA	AGGCGTTAGG	TCGCATCACG	CGGTTATCCG	CGTAACCAGG	GAAGCCAAGG	AGGAGTAGAT	TTCTTCCAAC	CAGCGGATGA	TAAGACCGTT	GTAGGAACCT
F	17	GAGGTATAAG	CCGACACAAG	TCCTCTACCG	TGTCAGTCTT	GCGTCTCTC	CCTCCGGTT	TACAGCTCA	GTTGTCCAG	ACTTGTTCGG	TCCGAGCGAA	GCGAATGAT	CCTTAATCGG
	15	CTCAGTAGGC	CTCCTAATTG	CCGTAACGTC	CCGGTCTATG	GCGGAAGAGT	AGCTCAGATA	TGGAGTTCAA	CCGCCGAATA	TTGGCGGTTG	GTCCTTGGAT	ATGGTACCAG	TACACTACGA
G	17	TCAAGGATTC	CTCTGATGCG	TACCATGAAC	GATGAACAGT	GTCTCTGCA	CACAAGACGG	GTTCTATTGG	AGCTCGAAGC	CAAGTCCAAT	TTCGGTTGTT	GTCGAAGTCG	TCTCGCCTAG
	15	GAAGTGCCCTG	GCCTCATAAT	GTAATAGCCA	ATTCACAAGC	TTGGTTAGTC	CCGGTGTTC	CTTCAATCCT	GAGTCTATAC	AACAGGCAAT	TCTAGATGCT	CCGACAGCTT	ATGACCTTGA
H	17	CGAACCGAGA	CGGCCTGTTA	CATTGGCAGA	ACAATCGGCG	GAGCTTCATT	GCTTACACAC	ATATACCGGT	AGAGGTTCTA	AACCGCAAGG	ACAGGAGGAA	TAGAGTTGGA	TCTTCAGAGA
	15	TCTCTCGCCT	TGTATTGAGC	TAGCGCCGAT	CAACCTGTAA	TTCAGTGTGA	GACCTAACCT	ATCTTGGCTG	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 μ L*
100 ng	5 to 7.5 μ L*
10 ng	1 to 2.5 μ L
1 ng	2.5 μ L**

*One or two extra cycles of PCR recommended.

** For 1 ng input, it is recommended 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 μ L.

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 (μ L)	Initial cleanup	80	80	80
	1 st binding	32.5	27.5	25
	2 nd 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 μ L** (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 μ L** 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 μ L pipette to remove any excess ethanol from the bottom of the tube.
-  1.6. Air-dry the beads on the magnetic stand for 3 - 5 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 μ L** 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 μ L** of supernatant into a new tube.

2. First Binding

- 2.1. Thoroughly vortex the sparQ PureMag Beads slurry and add **32.5 μ L** (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 μ l** (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 μ l. 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 μ l** 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 μ L to remove any remaining ethanol from the bottom of the tube.
- 3.6. Air-dry the beads on the magnetic stand for 3 - 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 μ l** 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 μ l 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 μ l** 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 μ l 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 .

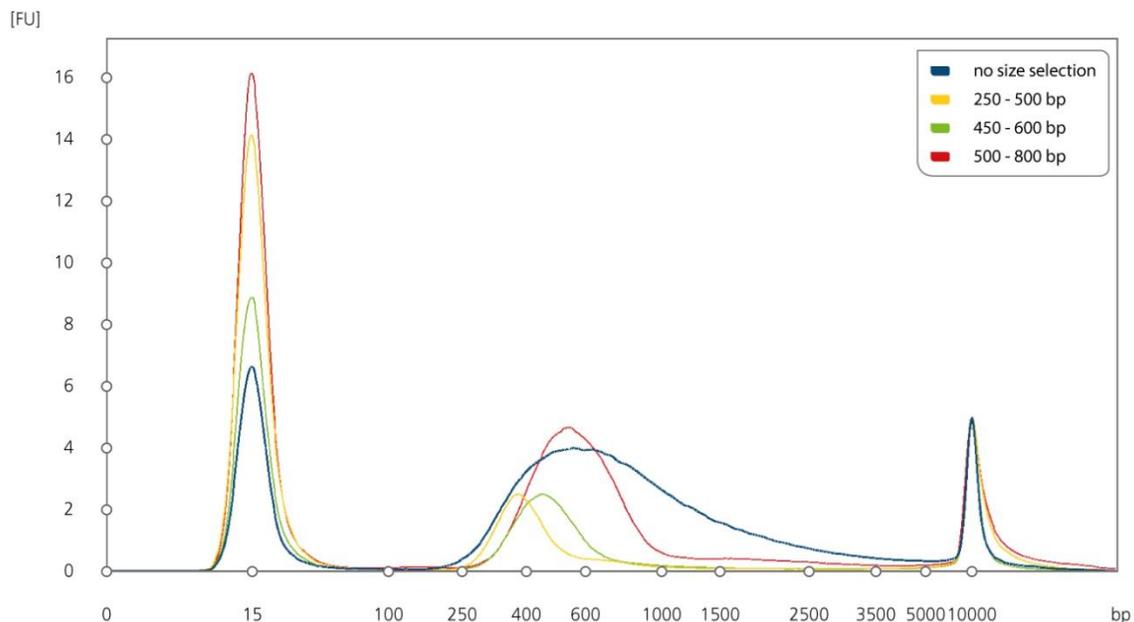


Figure 2: Example TapeStation traces of size selected DNA. Initial clean-up (0.8X) was carried out on adapter-ligated DNA (blue), then size-selected according to the volumes listed on page 13 for a target fragment range of 250- 500 bp (yellow), 450- 600 bp (green) or 500- 800 bp (red).



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 UDI Adapters (1 - 96)

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

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.