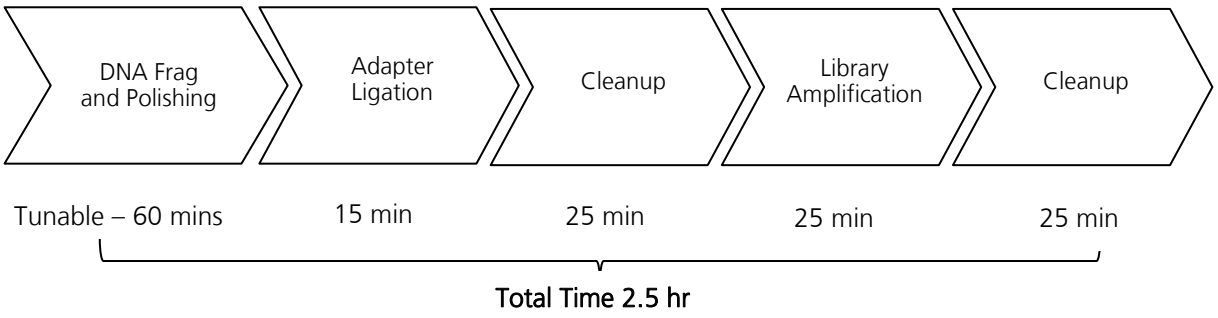
sparQ DNA Frag & Library Prep Kit for Element Biosciences AVITI™ Sequencing Platform

Cat. No. 95194-024 Size: 24 reactions **Store at -25°C to -15°C**
95194-096 96 reactions

Description

The sparQ DNA Frag & Library Prep Kit provides reagents essential for enzymatic fragmentation of DNA and the construction of libraries for sequencing on NGS platforms. The streamlined workflow can be completed in under 3 hours with minimal hands-on time and accommodates DNA input amounts from 1 ng to 1000 ng. The DNA fragmentation and end-polishing reactions are combined in a single step producing fragmented DNA that is taken through 5´-phosphorylation and 3´-dA-tailing polishing reactions. Fragment size is tunable based on reaction time. Subsequent ligation of sequencing adapters can be performed without the need for an intervening cleanup step. The HiFi PCR Master Mix allows unbiased amplification of fragments with appropriate adapters ligated to both ends. PCR-free workflows are enabled from as little as 100 ng of starting material when using Long UDI Adapters.

Workflow Overview



Instrument Compatibility

The kit is compatible with Illumina, Element and Singular sequencing instruments. This IFU is for use with Element Biosciences sequencing instruments.

Components

			Volume	
	Component Description	Cap Color	24 Reactions	96 Reactions
●	DNA Frag & Polishing Enzyme Mix (5X)	Blue	1 x 240 µl	1 x 960 µl
●	DNA Frag & Polishing Buffer (10X)	Blue	1 x 120 µl	1 x 528 µl
●	DNA Frag & Polishing Enhancer Solution	Blue	1 x 288 µl	1 x 264 µl
●	DNA Ligase	Orange	1 x 240 µl	1 x 960 µl
●	DNA Rapid Ligation Buffer (5X)	Orange	1 x 480 µl	2 x 960 µl
○	HiFi PCR Master Mix (2X)	White	1 x 600 µl	2 x 1.2 ml
○	Primer Mix	White	1 x 72 µL	1 x 144 µl

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

Adapters are not included with the kit and must be purchased separately. To use the sparQ DNA Frag & Library Prep Kit with the Element Biosciences AVITI Sequencing Platform, the compatible UDI adapters must be purchased from Element Biosciences or Integrated DNA Technologies (IDT).

To utilize PCR-free workflows (>100 ng input DNA), please order the following third party item from Element Biosciences:

Part Number	Description	Kit Size
830-00010	Elevate Long UDI Adapter Kit Set A	96 rxn

For PCR workflows (input DNA ≥ 1 ng), please order the following third party items from IDT:

Part Number	Description	Kit Size
10017036	xGen™ Stubby Adapter-UDI Primers for Element	16 rxn
10017037	xGen™ Stubby Adapter-UDI Primers for Element	96 rxn

NGS Library Quantification

Accurate quantification of DNA libraries is recommended for optimizing downstream processes such as target capture and sequencing. Quantabio offers the PerfeCTa SYBR Green FastMix which can be used for qPCR quantification of libraries.

Part Number	Description	Kit Size
95074-250	PerfeCTa SYBR Green FastMix Low ROX	250 x 20 µL rxns (2 x 1.25 mL)

General Guidelines

- Enzyme-based DNA fragmentation is sensitive to many factors, such as reaction temperature, time, setup conditions, as well as the physical properties and amount of and input DNA. We strongly recommend practicing the fragmentation tuning protocol outlined in **Appendix B** and optimizing parameters (*i.e.* reaction time) using a DNA that is representative of experimental DNA samples.
- Use good laboratory practices to minimize cross-contamination of nucleic acid products.
- Use PCR-grade, nuclease-free water. Do not use DEPC-treated water.
- Always use PCR tubes, microfuge tubes, and pipette tips that are certified sterile, DNase- and RNase-free.
- Thin-walled tubes with good heat transfer are recommended for the fragmentation reactions.
- For consistent reaction conditions, 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.
- Different workflows require differing amounts of DNA Frag & Polishing Enhancer Solution. Discard any unused Enhancer Solution at completion of kit usage.

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

- It is important to remove all cations and chelators from DNA preparations. Make sure input DNA is in water, 10 mM Tris-HCl (pH 8.0), buffer EB, or 0.1X TE buffer. If the DNA was dissolved in 1X TE or the EDTA concentration in the input DNA solution is unknown, we strongly recommend purifying the DNA using the sparQ PureMag Beads following the instructions in **Appendix A**.
- 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.
- Prepare UDI Dilution Buffer (10 mM Tris-HCl, pH 8.0, 50 mM NaCl, 0.5 mM EDTA) if adapter dilution is needed.
- 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 Frag & Polishing Enzyme Mix, DNA Ligase, Cleave Enzyme, and HiFi PCR Master Mix to ensure even distribution of contents. Other tubes can be briefly vortexed to ensure mixing.
- Determine the concentration of input DNA using standard methods.

Protocol

This protocol involves the preparation of master mixes for each reaction step. We recommend each master mix is prepared immediately before use. However, master mixes may be prepared in advance if used on the same day. Keep all master mixes on ice until use.

Single-Step DNA Frag and Polishing

1. Program the thermal cycler with the parameters in the table below. If possible, set the heated lid to 70°C. When the thermal cycler block reaches 32°C, pause the program.

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

Step	Temperature (lid 70°C)	Incubation Time
1	32°C	Varies based on input amount *
2	65°C	30 min
3	4°C	Hold

** Average fragment size is tunable based on incubation time and DNA input amount. The table below provides guidelines to achieve the desired fragment peak size for different input amounts.*
To determine your optimal fragmentation time, refer to the detailed protocol in Appendix B

Guidelines for Choosing Fragmentation Time

	Fragmentation Time (min) at 32°C			
Fragment Peak Size	**250 bp**	**350 bp**	**450 bp**	**550 bp**
10 ng input DNA	24	16	14	10
100 ng input DNA	16	10	8	6
1000 ng input DNA	14	8	6	4

Note: *The exact reaction time may need to be optimized by the end user. Detailed guidance is provided in* ***Appendix B****. Larger insert sizes can be achieved by incubating at 25°C instead of 32°C. Please refer to the application note, "Increased control over longer DNA fragmentation patterns using lower temperature with sparQ DNA Frag & Library Prep Kit", for further instructions.*

Note: *This protocol is suitable for 1 - 1000 ng input purified DNA. We recommend that the DNA be in 10 mM Tris-HCl, pH 8.0 buffer or buffer EB, but LoTE and nuclease-free water are also acceptable. If the input DNA volume is less than 35 µL, add nuclease-free water to a final volume of 35 µL (32.5 µL when using input <10 ng).*

2. Prepare the DNA Frag and Polishing Master Mix on ice according to the relevant table below. For DNA input amounts <10 ng, the indicated volume of DNA Frag Enhancer Solution should be added to the reaction. Volumes can be scaled as needed for the desired number of samples. Mix well by gently pipetting.

For Input DNA > 10 ng

	Components	Per Reaction (µl)
●	DNA Frag & Polishing Buffer (10X)	5
●	DNA Frag & Polishing Enzyme Mix (5X)	10
	Total	**15**

For Input DNA 1 – 10 ng

	Components	Per Reaction (µl)
●	DNA Frag & Polishing Buffer (10X)	5
●	DNA Frag & Polishing Enzyme Mix (5X)	10
●	DNA Frag & Polishing Enhancer Solution	2.5
	Total	**17.5**

3. **For input >10 ng**: Transfer **35 µl** DNA to a new thin-walled PCR tube for each reaction. Add **15 µl** of the DNA Frag & Polishing Master Mix from step 2 and gently mix well by pipetting up and down 10-12 times. It is critical to keep the tubes on ice during reaction setup.

   For input DNA 1 -10 ng: Transfer **32.5 µl** DNA to a new thin-walled PCR tube for each reaction. Add **17.5 µl** of the DNA Frag & Polishing Master Mix from step 2 and gently mix well by pipetting up and down 10-12 times. It is critical to keep the tubes on ice during reaction setup.

4. Pulse-spin the sample tubes and immediately transfer to the pre-heated thermal cycler (32°C). Resume the cycling program.
5. 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.
6. 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.

Detailed guidance is provided in Appendix C.

Total DNA Input	Dilution of 15 µM Element Elevate Long UDI Adapters for PCR-free Workflows	Dilution of 15 µM Stubby Adapters for PCR workflows
500 - 1000 ng	None	None
100 - 499 ng	1:2	None
10 - 99 ng	Not recommended	None
1 - 9 ng	Not recommended	1:10

7. Transfer **4 µl** of appropriately diluted adapters into the PCR tube with **50 µl** of fragmented DNA from step 5. 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.

8. 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 (5X)	20
●	DNA Ligase	10
	Nuclease-free H_2O	16
	Total	**46**

9. Add **46 µl** of the Ligation Master Mix to the **54 µl** fragmented DNA and adapters sample from step 7 and mix well by pipetting.
10. 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

11. 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 into a thin-walled PCR tube.

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

12. Equilibrate the sparQ PureMag Beads to room temperature (RT) for 20 min.
13. Thoroughly vortex the sparQ PureMag Beads slurry and add **80 µl** (0.8X) to the ligation sample from step 10. Mix well by pipetting.
14. 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.
15. Keeping the tube on the magnetic stand, gently pipette **200 µl** of the freshly-prepared 80% ethanol over the bead pellet to wash. Pellet the beads on the magnetic stand for 30 s then carefully remove and discard the supernatant. Repeat step 16 for a total of two washes.
16. Use a 10 µl pipette to remove any excess ethanol from the bottom of the tube.

17. 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.

18. Full-length adapters such as the Element Elevate Long 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.

    a. **If full-length adapters (ie. Element Elevate Long 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 TapeStation®. An additional 0.9X 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.

    b. **If stubby (truncated) adapters were used in adapter ligation:** Resuspend the dried beads in **22 µl** of 10 mM Tris-HCl, pH 8.0. 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 required if stubby adapters were used during ligation.

19. 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
	xGen UDI Primers for Element (10 µM)	5
	Purified, adapter-ligated DNA	20
	Total	**50**

20. To the **20 µl** of purified, adapter-ligated DNA from step 18b, 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 **21**.

21. 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 next page
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 – 1000 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

22. Pulse-spin the sample tube and immediately transfer to the pre-heated thermal cycler (98°C). Resume the cycling program.
23. 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 (step 25).

Post-Amplification Cleanup

24. Equilibrate the sparQ PureMag Beads to RT for 20 min.
25. Thoroughly vortex the sparQ PureMag Beads slurry and add **45 µl** (0.9X) to the PCR reaction. Mix well by pipetting.
26. Incubate the mixture for 5 min at RT. Pellet the beads on a magnetic stand (e.g., DynaMag) and carefully discard the supernatant.
27. Keeping the tube on the magnetic stand, gently pipette **200 µl** of the freshly-prepared 80% ethanol over the bead pellet to wash. Pellet the beads on the magnetic stand for 30 s then carefully remove and discard the supernatant. Repeat step 27 for a total of two washes.
28. Use a 10 µl pipette to remove any excess ethanol from the bottom of the tube.
29. 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.
30. 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".

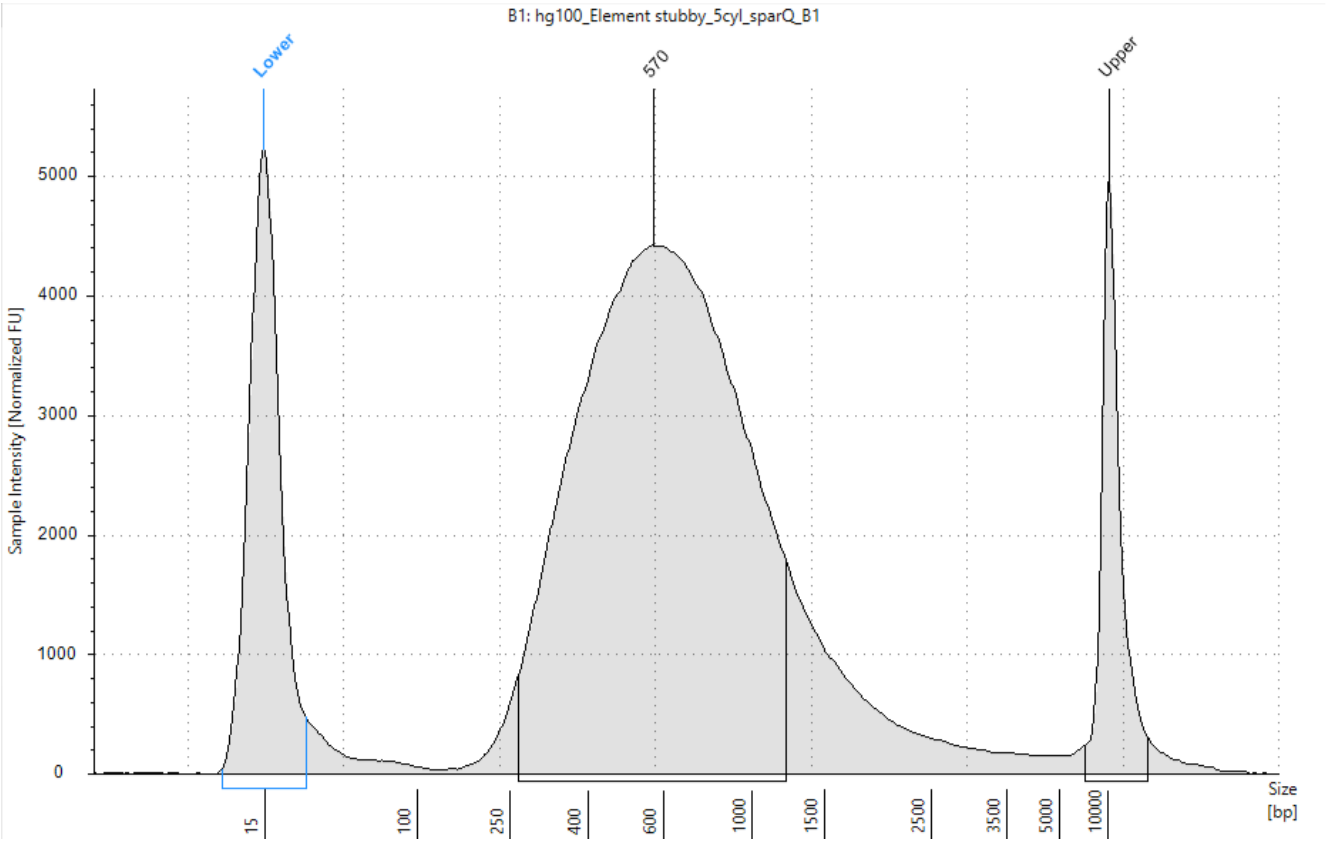


Figure 1: Typical Agilent TapeStation trace of prepared library using sparQ DNA Frag and Library Prep Kit. Library was prepared using 100 ng input hgDNA, fragmented for 5 min and ligated with stubby adapters. Following post-ligation cleanup, libraries were amplified with 5 PCR cycles. Peaks at 15 and 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 PerfeCTa SYBR Green FastMix for qPCR quantification of DNA library molecules suitable for sequencing.

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

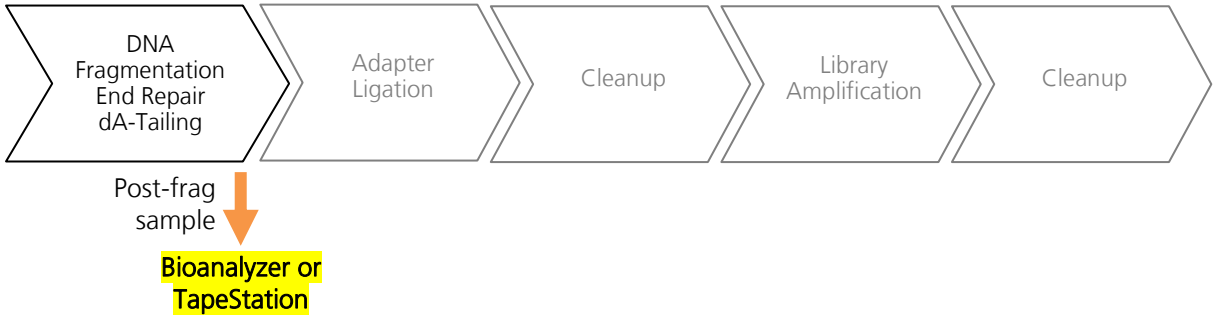
Input DNA for the fragmentation reaction should be in nuclease-free water, 10 mM Tris-HCl (pH 8.0), buffer EB, or LoTE (0.1X TE). If the DNA was dissolved in 1X TE or the concentration of cations and chelators is not known, we strongly recommend purifying the DNA using sparQ PureMag Beads per the instructions 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 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 3 - 5 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: Recommended Fragmentation Tuning Protocol

Overview

A key feature of the sparQ DNA Fragmentation & Library Preparation Kit is that the average size of DNA fragments is tunable based on sample type, reaction time, and input amount. Since the source and amount of your experimental sample DNA will differ from any control DNA, optimal reaction conditions should be determined empirically. **Figure 2** (page 12) provides guidance on initial incubation times to test based on DNA input amount. To find the optimal conditions that yield the desired fragment size, we recommend testing timepoints at and around the time determined from **Figure 2**. This fragmentation tuning protocol involves the testing of three incubation times. This is done by adding samples to a thermal block at each test time so that all reactions are completed simultaneously. Samples are collected and purified immediately after the fragmentation and DNA polishing reaction. Bioanalyzer analysis of the collected samples will confirm if one of the timepoints yielded the desired fragmentation.



General Guidelines

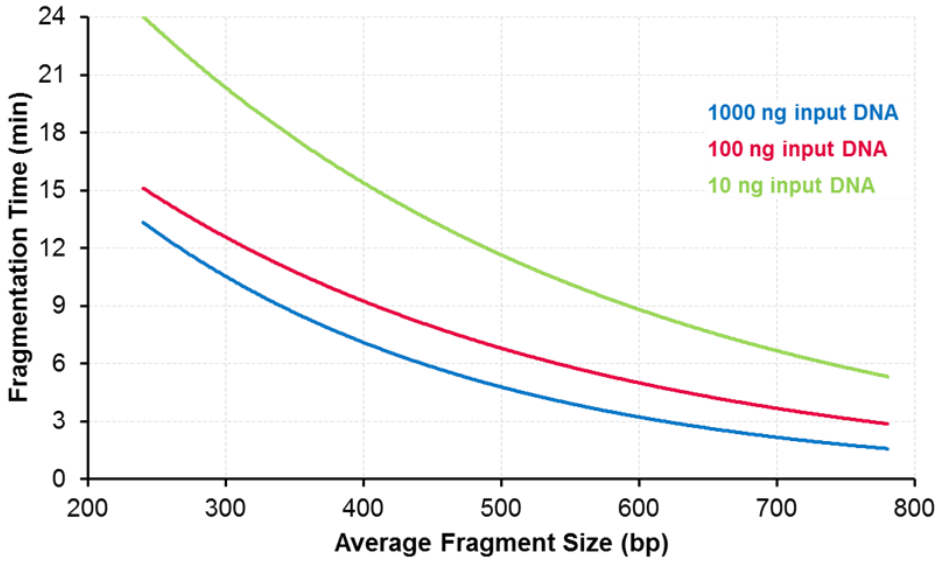
- Enzyme-based DNA fragmentation is sensitive to many factors, such as reaction temperature, time, and setup conditions. In tests using your experimental sample, follow the optimization guidelines in this protocol.
- Use good laboratory practices to minimize cross-contamination of nucleic acid products.
- Always use PCR tubes, microfuge tubes, and pipette tips that are certified sterile, DNase- and RNase-free.
- For consistent reaction conditions, 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 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 tube containing DNA Frag & Polishing Enzyme Mix to ensure even distribution of contents. DNA Frag & Polishing Buffer tube can be briefly vortexed to ensure mixing.



	Fragmentation Time (min) at 32°C			
Fragment Peak Size	250 bp	350 bp	450 bp	550 bp
10 ng input DNA	24	16	14	10
100 ng input DNA	16	10	8	6
1000 ng input DNA	14	8	6	4

Figure 2: Guidelines for Tuning Fragmentation Size

Optimizing Fragmentation Time for Experimental DNA Samples

1. Single-Step Fragmentation of Experimental Sample DNA

The optimal incubation time to obtain the desired fragment size for the experimental sample DNA must be determined. The chart and table of **Figure 2** provide guidelines of incubation times and expected sizes based on input amount.

1.1 Using the table and chart in **Figure 2**, select an incubation time likely to generate fragments of the desired size of your experimental DNA sample. Refer to this time as T-opt. Include two additional time points at 3 minutes shorter and 3 minutes longer than T-opt. Refer to these as T-short and T-long, respectively.

T-opt	Optimal incubation time determined from Figure 2
T-short	T-opt – 3 minutes
T-long	T-opt + 3 minutes

Example fragmentation times, determined from **Figure 2**, are shown in the table below.

Sample Input DNA	Desired Fragmentation Size	T-opt	T-short	T-long
100 ng	450 bp	8 minutes	5 minutes	11 minutes
1000 ng	300 bp	11 minutes	8 minutes	14 minutes

1.2 Program a thermal cycler with the parameters in the table below. If possible, set the temperature of the instrument's heated lid to 70°C. When the thermal cycler block reaches 32°C, pause the program.

Step	Temperature (lid 70°C)	Incubation Time
1	32°C	T-long *
2	65°C	30 min
3	4°C	Hold

** T-long is the optimal incubation time plus 3 minutes as described in 1.1 and selected by you.*

1.3 Prepare a master mix on ice for three identical fragmentation reactions by combining the components listed in the appropriate table below. For DNA input amounts >10 ng, follow the upper table. For DNA input amounts <10 ng, DNA Frag & Polishing Enhancer Buffer should be added to the reaction as per the lower table. Mix well by gently pipetting (do not vortex to mix).

DNA input > 10 ng

	Component	Per Reaction (µl)	For 3 Reactions (+10%)
●	DNA Frag & Polishing Buffer (10X)	5	16.5
	Experimental sample DNA	X	X
	Nuclease-free water	35-X	115.5 - X
	Total	**40**	**132**

DNA input 1 - 10 ng

	Component	Per Reaction (µl)	For 3 Reactions (+10%)
●	DNA Frag & Polishing Buffer (10X)	5	107.25
	Experimental sample DNA	X	X
●	DNA Frag & Polishing Enhancer Solution	2.5	8.25
	Nuclease-free water	32.5 - X	97.5 - X
	Total	**40**	**132**

1.4 Transfer **10 µl** of the DNA Frag & Polishing Enzyme to each of three new thin-walled PCR tubes, labeled as **S1, S2, and S3**. Add **40 µl** of the master mix from step 1.3 to each tube for a final reaction volume of **50 µl** and gently mix well by pipetting up and down 10-12 times. It is critical that all tubes are kept on ice during reaction setup.

1.5 Transfer tube **S1** to the pre-heated thermal cycler (32°C). This will be incubated for the time corresponding to T-long. Keep the other tubes on ice. Resume the cycling program and monitor the countdown for the 32°C incubation time.

1.6 Three minutes later, open the lid and add tube **S2** to the thermal cycler. This will be incubated for the time corresponding to T-opt.

1.7 Wait another three minutes and open the lid again and add tube **S3** to the thermal cycler. This will be incubated for the time corresponding to T-short.

1.8 Allow the remainder of the program to complete. When the thermal cycler temperature has returned to 4°C, remove all tubes from the block and place on ice.

A diagram of the above experimental sample DNA fragmentation procedures is shown in **Figure 3**.

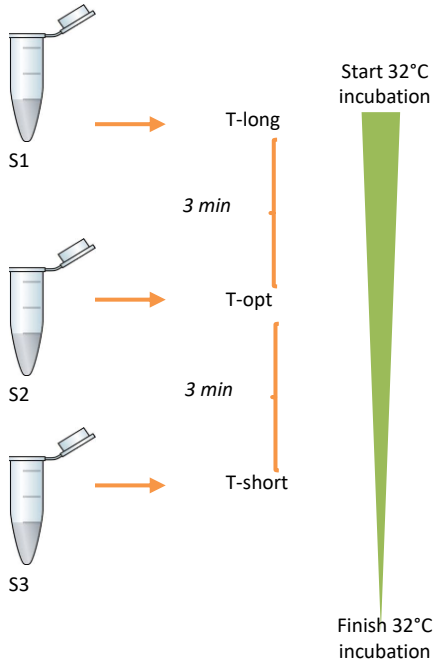


Figure 3: Testing three fragmentation times for the experimental DNA sample

2. Post-Fragmentation Purification of Samples

Upon completion of fragmentation, each sample is taken for purification. These purified samples will be for assessment of the fragmentation size profiles.

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

2.2 Add **90 µl** of thoroughly vortexed sparQ PureMag Beads slurry to the samples **S1**, **S2**, and **S3** and mix well by pipetting.

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

2.4 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.

2.5 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.

2.6 Resuspend the dried beads in **25 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 for 2 min or until the solution is clear. Carefully transfer **22.5 µl** of each supernatant into new tubes labeled, **S1-long**, **S2-opt**, and **S3-short** and keep on ice until ready to perform Bioanalyzer analysis.

3. Bioanalyzer Analysis of all collected samples

3.1 Apply **1 µl** of each purified sample on a Bioanalyzer with Agilent High Sensitivity DNA kit to visualize the sample profiles. DNA may need to be diluted before loading. Other automated gel electrophoresis systems, such as the Agilent TapeStation, may also be used.

3.2 Examine the Bioanalyzer profiles of samples S1-long, S2-opt, and S3-short. Select the fragmentation time that corresponds to your target fragment size and circle that time in section 1.1 on page 12. This is the time you will use in your experimental protocol.

3.3 As an example, 1,000 ng of control DNA was taken through the fragmentation tuning protocol at varied time points and Bioanalyzer profiles are shown in **Figure 4** as a reference.

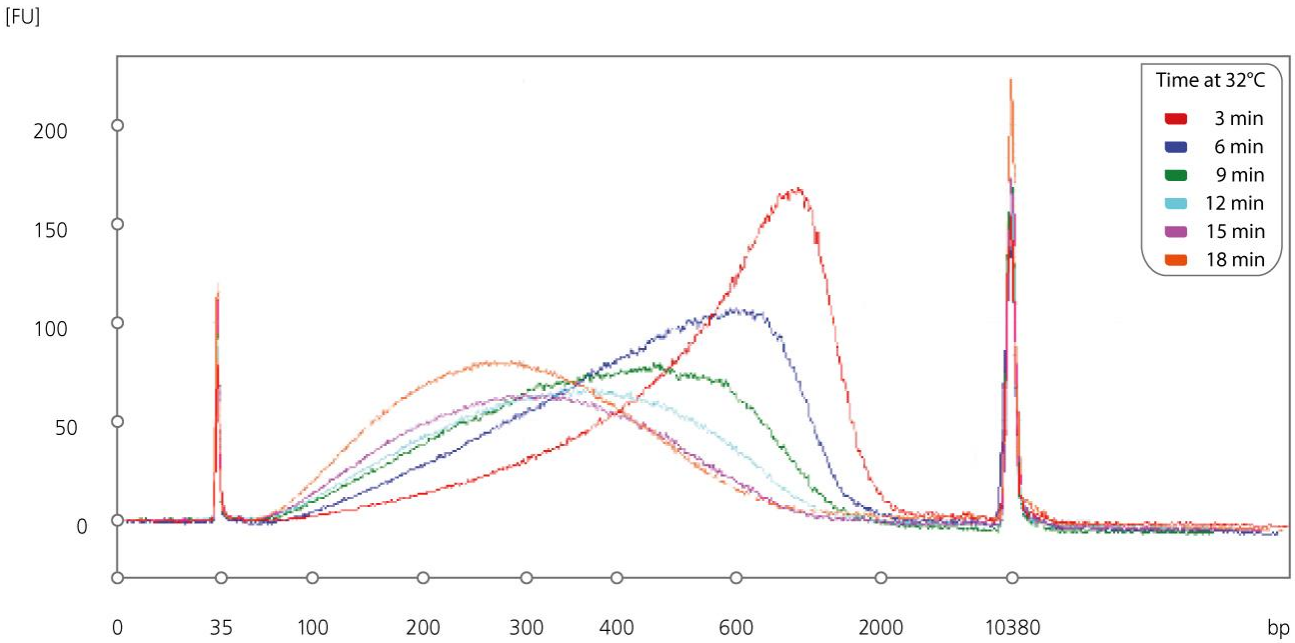


Figure 4: Tuning profile of 1,000 ng input control human gDNA run through a fragmentation time course experiment (individual sample fragmentation profiles may vary).

Appendix C: 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 and 1:10 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 **1:10 dilution** by adding 2 µl of the adapters (stock) adapters to 18 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. For more information regarding adapter and index sequences, please refer to the product documents provided by the supplier (ie. Element Biosciences or IDT).

Appendix D: 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 10 (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 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 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 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 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 18, 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.

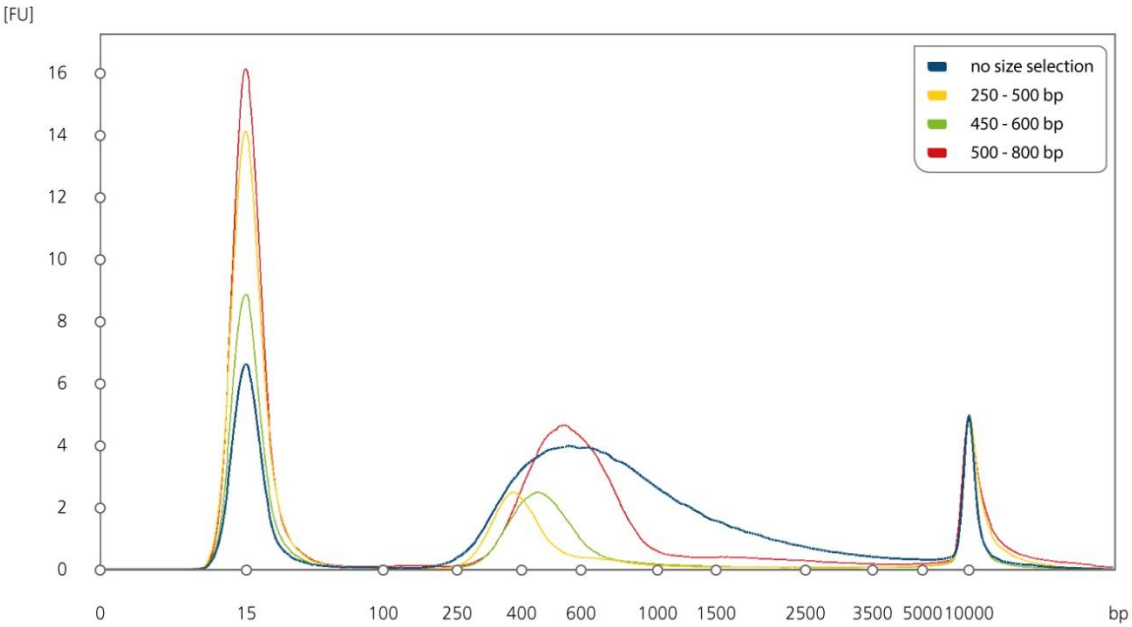


Figure 5: Example TapeStation traces of size selected DNA. Initial clean-up (0.8X) was carried out on adapter-ligated DNA (blue), then size-selected according to the volumes listed on page 17 for a target fragment range of 250- 500 bp (yellow), 450- 600 bp (green) or 500- 800 bp (red).

NGS products used in protocol sold separately

sparQ PureMag Beads

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

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

PerfeCTa SYBR Green FastMix Low ROX

Cat. No.	95074-250	Size:	250 x 20 µL rxns (2 x 1.25 mL)

PerfeCTa SYBR® Green FastMix is a 2X qPCR master mix designed for high-sensitivity, high-specificity, and fast cycling. It includes hot-start Taq, SYBR Green dye, and a proprietary buffer for reliable and efficient amplification. Compatible with fast and standard thermal cycling protocols, it supports high-throughput, accurate quantification.

Related NGS Products Sold Separately

sparQ DNA Library Prep Kit

Cat. No.	95191-024	Size:	24 reactions
	95191-096		96 reactions

The sparQ DNA Library Prep Kit is optimized for the rapid construction of DNA libraries from fragmented double-stranded DNA for sequencing on NGS platforms. The simplified protocol speeds up library prep to 2.5 hours with minimal hands-on time and accommodates DNA input amounts from 250 pg to 1 µg.

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.