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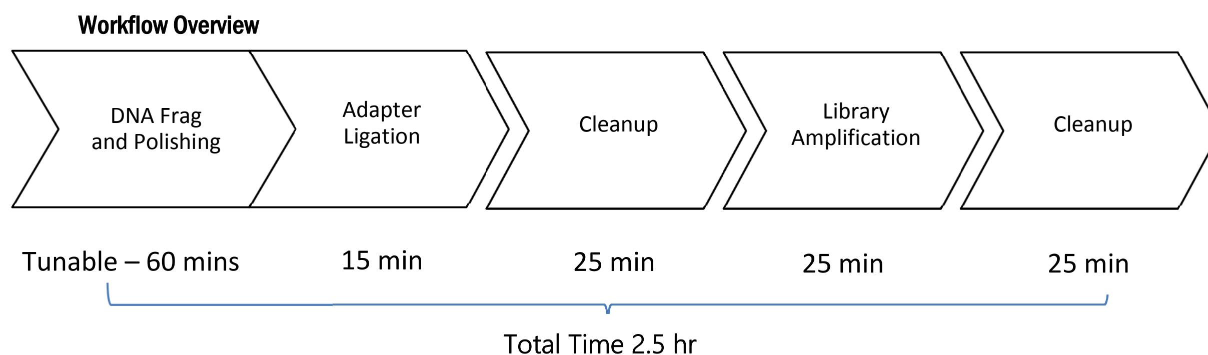
sparQ DNA Frag & Library Prep Kit

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 Illumina® NGS platforms. The streamlined workflow can be completed in under 3 hours with minimal hands-on time and can accommodate DNA input amounts from 1.0 ng to 1000 ng. The DNA fragmentation and 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 optional HiFi PCR Master Mix and Primer Mix allow unbiased amplification of fragments with appropriate adapters ligated to both ends. PCR-Free workflows are enabled from 100 ng of starting material.



Instrument Compatibility

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

Components

Component Description	Cap Color	Volume	
		24 reactions	96 reactions
DNA Frag & Polishing Enzyme Mix (5X)	Blue	1 x 240 µl	1 x 1.15 ml
DNA Frag & Polishing Buffer (10X)	Blue	1 x 120 µl	1 x 576 µl
DNA Frag Enhancer Solution	Blue	1 x 288 µl	1 x 288 µl
DNA Ligase	Orange	1 x 240 µl	1 x 1.15 ml
DNA Rapid Ligation Buffer (5X)	Orange	1 x 480 µl	2 x 1.15 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 288 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

- SPRI beads
  SPRI 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 1X AMPure® XP beads for post-amplification reaction cleanup. AMPure XP beads are available from Beckman Coulter, Inc.

- Adapters
  The sparQ DNA Frag & Library Prep Kit does not include adapters but is compatible with non-barcoded, single-barcoded, or dual-barcoded adapters routinely used in library construction workflows. Quantabio offers two sets of single-barcoded adapters available as companion products to the sparQ DNA Frag & Library Prep Kit. This kit is compatible with all adapters compatible with Illumina-based sequencing methods.

Part Number	Description	Kit Size	Adapter Barcodes Included *	Volume of each Adapter Barcode
95193-A96	sparQ Adapter Barcode Set A	96 reactions	2,4-7,12-16,18,19	0.015 ml
95193-B96	sparQ Adapter Barcode Set B	96 reactions	1,3,8-11,20-23,25,27	0.015 ml

* Twelve adapters with distinct barcode sequences are in each set. The barcode sequences are listed in Appendix A.

- NGS Library Quantification Kit
  Accurate quantification of DNA libraries is required for optimizing downstream processes such as target capture and sequencing. Quantabio offers the PerfeCTa® NGS Library Quantification Kit to specifically quantify library molecules with appropriate adapters at each end.

Part Number	Description	Kit Size
95154-500	PerfeCTa NGS Quantification Kit – Illumina	500 x 20 µl rxns
95155-500	PerfeCTa NGS Quantification Kit – Illumina, ROX	500 x 20 µl rxns
95156-500	PerfeCTa NGS Quantification Kit – Illumina, Low ROX	500 x 20 µl rxns

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General Guidelines

- Enzyme-based DNA fragmentation is sensitive to many factors, such as reaction temperature, time, and setup conditions, as well as input DNA. We strongly recommend users practice the fragmentation tuning protocol outlined in Appendix C and optimize the parameters (*i.e.*, reaction time) using the same or similar experimental DNA samples.
- 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 stopping points where samples can be frozen at -20ºC and plan your workflow accordingly.
- Different workflows require differing amounts of DNA Frag 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

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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, buffer EB, or LoTE (0.1X TE). If the DNA was dissolved in 1X TE or the EDTA concentration in the input DNA is not known, we strongly recommend purifying the DNA using AMPure XP beads following the instructions in Appendix B.
- 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 Frag & 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.
- 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 4°C, pause the program.

Step	Temperature	Incubation Time
1	4°C	1 min
2	32°C	Varies based on input amount *
3	65°C	30 min
4	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 C.*

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 C.

Protocol

Single-step DNA Frag and Polishing

1. Set up master mixes on ice per the tables 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 (do not vortex to mix).

For input DNA > 10 ng

	per reaction (µl)
DNA Frag & Polishing Buffer (10X)	5
Purified DNA	X
Nuclease-free H_2O	(35 - X)
Total	40

For input DNA 1 – 10 ng

	per reaction (µl)
DNA Frag & Polishing Buffer (10X)	5
Purified DNA	X
DNA Frag Enhancer Solution	2.5
Nuclease-free H_2O	(32.5 - X)
Total	40

2. Transfer 10 µl of the DNA Frag & Polishing Enzyme Mix to a new thin-walled PCR tube for each reaction. Add 40 µl of the master mix from step 1 and gently mix well by pipetting up and down 6-8 times. It is critical to keep the tubes on ice during reaction setup.
3. Pulse-spin the sample tubes and immediately transfer to the pre-chilled thermal cycler (4°C). Resume the cycling program.
4. 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.
5. Immediately proceed to the NGS library adapter ligation step.

Adapter Ligation

The following steps are for customers using Quantabio sparQ Adapter Sets. Customers using adapters from other sources may need to adjust the adapter dilutions 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 adapter dilutions to use for different amounts of 250 bp DNA fragments.

DNA sample (250 bp fragments)	Adapter dilution before use	Adapter concentration in ligation
1000 – 50 ng	No dilution	0.75 µM
25 – 10 ng	1:2 – 1:5 dilution	0.375 – 0.15 µM
5 – 1 ng	1:10 – 1:50 dilution	0.075 – 0.015 µM

6. Transfer 1.5 µl of appropriately diluted adapter 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.

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7. Prepare the ligation reaction 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	18.5
Total	48.5

8. Add **48.5 µl** of the ligation reaction mix to the **51.5 µl** fragmented DNA and adapter sample from step 6 and mix well by pipetting.
9. Incubate the ligation reaction at 20°C for 15 min using a thermal cycler without enabling a heated lid.
10. Proceed immediately to adapter ligation cleanup using paramagnetic SPRI beads.

Adapter Ligation Cleanup

This protocol has been validated using AMPure XP beads for the post-ligation reaction cleanup. The optimal bead to DNA sample ratio for libraries of fragments in the 150 – 350 bp size range is 0.8X. Conditions may differ if other beads are used.

11. Equilibrate the AMPure XP beads to room temperature (RT) for 20 min.
12. Thoroughly vortex the AMPure XP beads slurry and add **80 µl** (0.8X) to the ligation sample from step 9. Mix well by pipetting.
13. Incubate the mixture for 5 min at RT. Pellet the beads on a magnetic stand (e.g., DynaMag™) and carefully discard the supernatant.
14. Wash the beads with **200 µl** of the freshly-prepared 80% ethanol. Pellet the beads on the magnetic stand and discard the supernatant. Repeat the wash once.

15. Air-dry the beads on the magnetic stand for 5 - 10 min or until the beads are dry. Over-drying of beads may result in lower DNA recovery.
16. If optional library amplification is intended: Resuspend the dried beads in **25.5 µl** of 10 mM Tris-HCl, pH 8.0. 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. 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. If size selection is required, please use your choice of method and follow the corresponding protocols. An additional 1X AMPure XP 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.

17. Prepare the PCR reaction 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

18. Add **26.5 µl** of the master mix from step 17 to the **23.5 µl** of DNA sample from step 16 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.

19. 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 8
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.

To yield 500 ng of DNA library from your input DNA, select from number of cycles recommended in the table below.

Input DNA sample (ng)	To yield 500 ng of DNA library - Suggested number of cycles
1000	0 - 1
500	1 - 2
100	4 - 5
50	5 - 6
10	8 - 10
1	13 - 15

20. Pulse-spin the sample tube and immediately transfer to the pre-heated thermal cycler (98°C). Resume the cycling program.
21. 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 paramagnetic SPRI beads (step 22).

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Post-Amplification Cleanup

This protocol has been validated using 1X AMPure XP beads for post-amplification reaction cleanup. Conditions may differ if other beads are used.

22. Equilibrate the AMPure XP beads to RT for 20 min.
23. Thoroughly vortex the AMPure XP beads slurry and add 50 µl (1X) to the PCR reaction. Mix well by pipetting.
24. Incubate the mixture for 5 min at RT. Pellet the beads on a magnetic stand (e.g., DynaMag) and carefully discard the supernatant.
25. Wash the beads with 200 µl of 80% ethanol. Pellet the beads on the magnetic stand and discard the supernatant. Repeat the wash once.

26. Air-dry the beads on the magnetic stand for 5 - 10 min or until the beads are dry. Over-drying of beads may result in lower DNA recovery.

27. Resuspend the dried beads in 32.5 µl of 10 mM Tris-HCl, pH 8.0. 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.

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 NGS Library Quantification Kits (95154-500, 95155-500, 95156-500) 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: Barcode Sequences of sparQ Adapter Barcodes

sparQ Adapter Barcode Set A	
Adapter Barcode Number	Barcode Sequence
2	CGATGT
4	TGACCA
5	ACAGTG
6	GCCAAT
7	CAGATC
12	CTTGTA
13	AGTCAA
14	AGTTCC
15	ATGTCA
16	CCGTCC
18	GTCCGC
19	GTGAAA

sparQ Adapter Barcode Set B	
Adapter Barcode Number	Barcode Sequence
1	ATCACG
3	TTAGGC
8	ACTTGA
9	GATCAG
10	TAGCTT
11	GGCTAC
20	GTGGCC
21	GTTTCG
22	CGTACG
23	GAGTGG
25	ACTGAT
27	ATTCCT

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

Input DNA for the fragmentation reaction should be in water, 10 mM Tris, 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 AMPure XP 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 AMPure XP 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 AMPure XP 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. Wash the beads with 200 µl of 80% ethanol. Pellet the beads on the magnetic stand and discard the supernatant. Repeat the wash once.
5. Air-dry the beads on the magnetic stand for 10 min or until the beads are dry.
6. Thoroughly resuspend the dried beads in 45 µl of 10 mM Tris-HCl, pH 8.0. Pellet the beads on the magnetic stand for 2 min. Carefully transfer 42.5 µl of supernatant into a new tube.
7. Determine the concentration of the purified DNA using Qubit, Picogreen or other methods.

Appendix C: 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 1 (page 14) provide 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 1. 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 profile.

General Guidelines

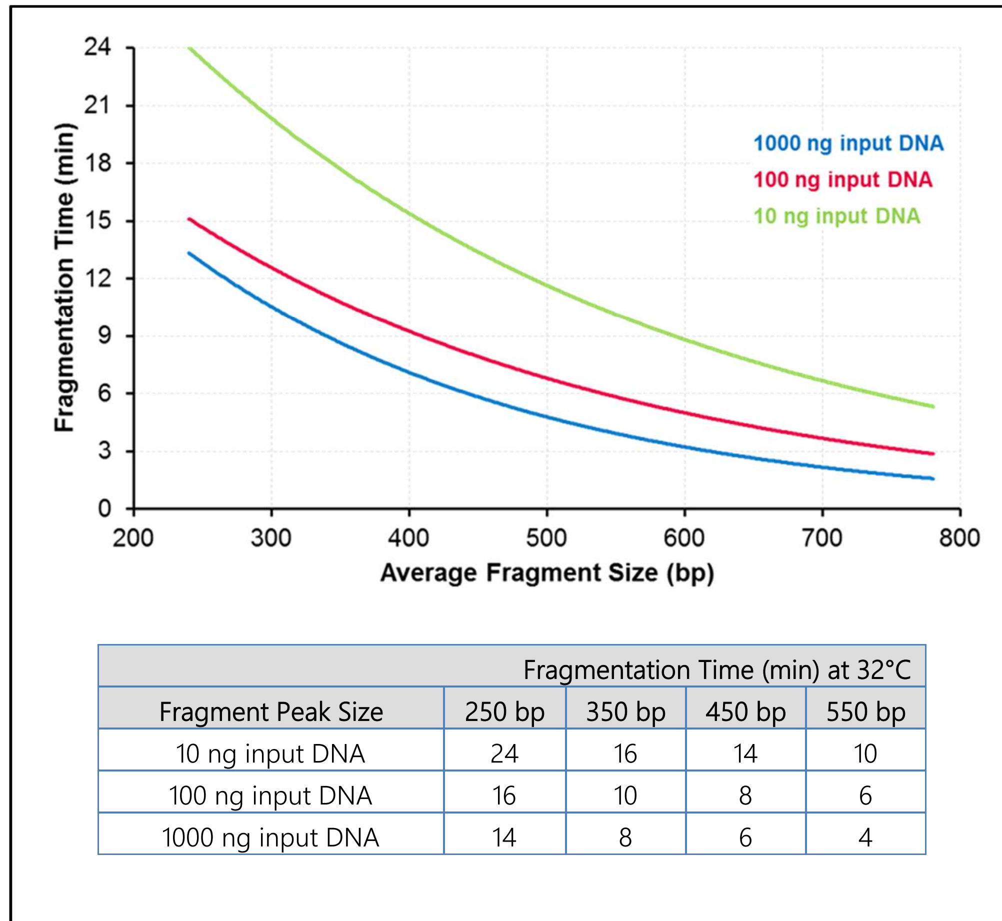
- Enzyme-based DNA fragmentation is sensitive to many factors, such as reaction temperature, time, and setup conditions. In tests using your experimental sample, follow the optimization guidelines in this protocol.
- Use good laboratory practices to minimize cross-contamination of nucleic acid products.
- 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 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 1: Guidelines for Tuning Fragmentation Size

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Optimizing Fragmentation Time for Experimental DNA Samples

Step 1: Single-step Fragmentation of experimental sample DNA.

1. Fragmentation of the 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 1** provide guidelines of incubation times and expected sizes based on input amount.

1.1 Using the table and chart in **Figure 1**, 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 1**
T-short	T-opt – 3 minutes
T-long	T-opt + 3 minutes

*Use the last row in the table below to **write in your selected input, fragmentation size, and time points**:*

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 4°C, pause the program.

Step	Temperature	Incubation Time
1	4°C	1 min
2	32°C	T-long *
3	65°C	30 min
4	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).

For DNA input > 10 ng

Component	Per reaction (µl)	For 3 reactions
DNA Frag & Polishing Buffer (10X)	5	15
Experimental sample DNA	X	X
Nuclease-free water	35-X	105 - X
Total	40	120

For DNA input 1 - 10 ng

Component	Per reaction (µl)	For 3 reactions
DNA Frag & Polishing Buffer (10X)	5	15
Experimental sample DNA	X	X
DNA Frag & Polishing Enhancer Buffer	2.5	7.5
Nuclease-free water	32.5 - X	97.5 - X
Total	40	120

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

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 6-8 times. It is critical that all tubes are kept on ice during reaction setup.

1.5 Transfer tube S1 to the pre-chilled thermal cycler (4°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 2.

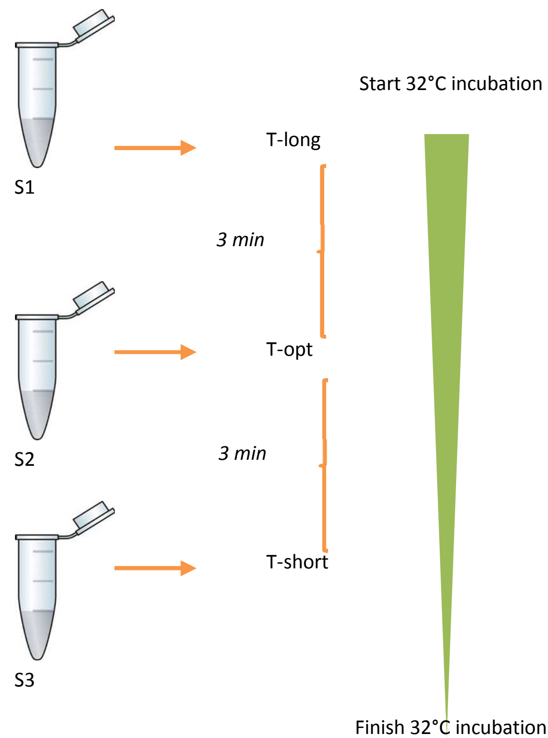
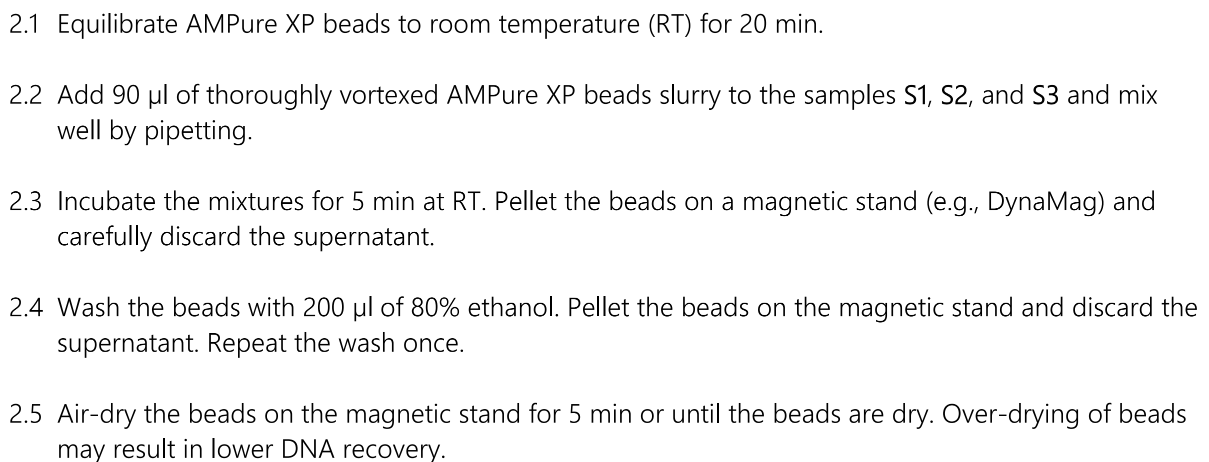


Figure 2: Testing three fragmentation times for the experimental DNA sample

2: Post-Fragmentation Purifications of Samples

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



2.1 Equilibrate AMPure XP beads to room temperature (RT) for 20 min.

2.2 Add 90 µl of thoroughly vortexed AMPure XP 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 Wash the beads with 200 µl of 80% ethanol. Pellet the beads on the magnetic stand and discard the supernatant. Repeat the wash once.

2.5 Air-dry the beads on the magnetic stand for 5 min or until the beads are dry. Over-drying of beads may result in lower DNA recovery.

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2.6 Resuspend the dried beads in 25 µl of 10 mM Tris-HCl, pH 8.0. Pellet the beads on the magnetic stand. 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.

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 15. This is the time you will use in your experimental protocol.

3.3 As an example, 1000 ng of control DNA was taken through the fragmentation tuning protocol at varied time points and bioanalyzer profiles are shown in Figure 3 as a reference.

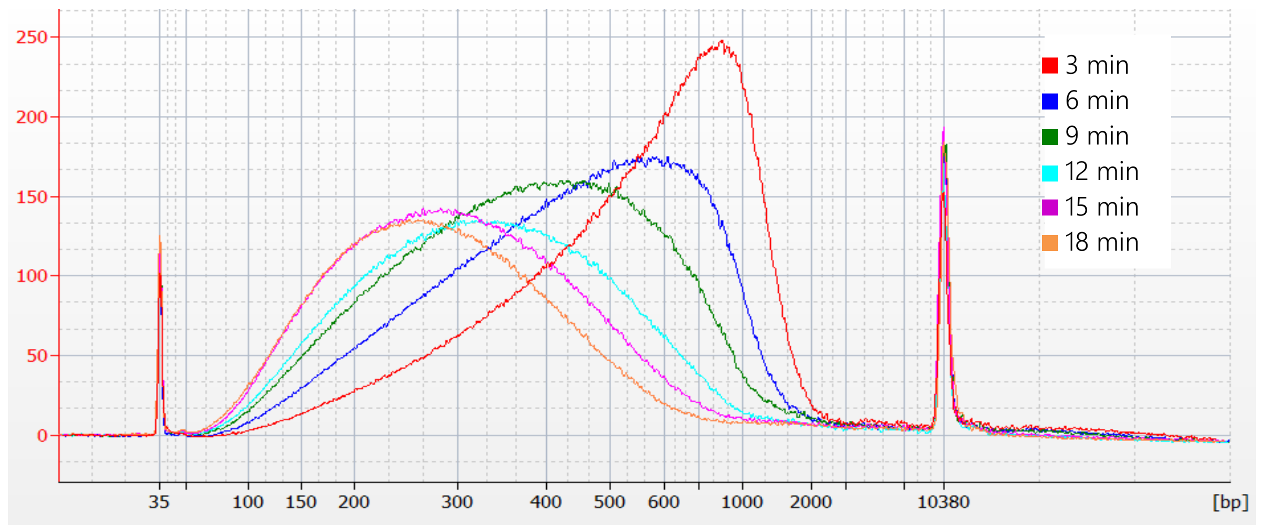

Figure 3: Tuning profile of 1000 ng input control human gDNA run through a fragmentation time course experiment (individual sample fragmentation profiles may vary).

Related NGS Products Sold Separately

sparQ DNA Library Prep Kit

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

sparQ Adapter Barcodes Set A

Cat. No.	95193-A96	Size:	96 reactions

sparQ Adapter Barcodes Set B

Cat. No.	95193-B96	Size:	96 reactions

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.

PerfeCTa NGS Quantification Kit – Illumina

Cat. No.	95154-500	Size:	500 reactions

PerfeCTa NGS Quantification Kit – Illumina, ROX

Cat. No.	95155-500	Size:	500 reactions

PerfeCTa NGS Quantification Kit – Illumina, Low ROX

Cat. No.	95156-500	Size:	500 reactions

The PerfeCTa NGS Quantification Kits use real-time PCR to enable accurate quantification of DNA Libraries compatible with sequencing on Illumina NGS platforms. The included stabilized pre-diluted standards and pre-qualified primer set ensures reproducible and precise results.

Trademarks
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