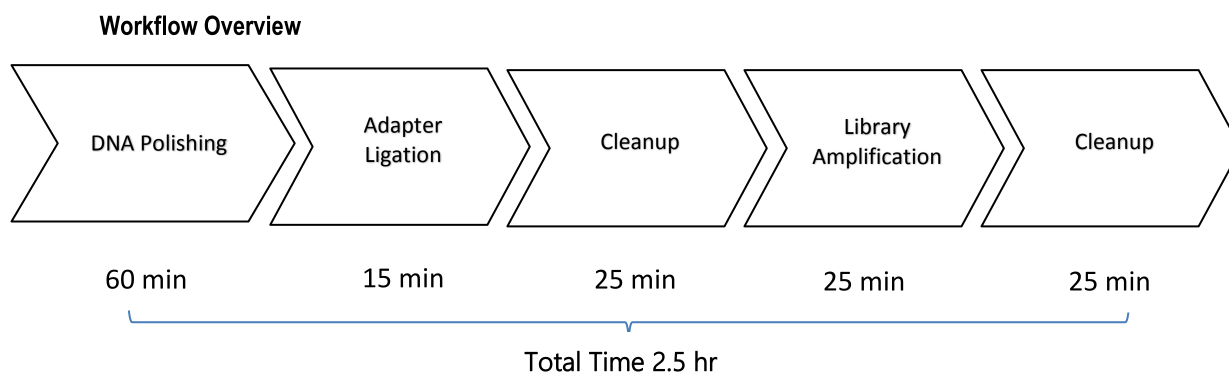
Quantabio

# sparQ DNA Library Prep Kit

| Cat. No. | 95191-024 | Size: | 24 reactions | Store at -25°C to -15°C |
|---|---|---|---|---|
| | 95191-096 | | 96 reactions | |

## Description

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




## Instrument Compatibility

The kit is compatible with instruments of the Illumina sequencing platform

## Components

| | | Volume | |
|---|---|---|---|
| Component Description | Cap Color | 95191-024 | 95191-096 |
| DNA Polishing Enzyme Mix | Blue | 1 x 0.24 ml | 1 x 0.96 ml |
| DNA Polishing Buffer | Blue | 1 x 0.12 ml | 1 x 0.48 ml |
| DNA Ligase | Orange | 1 x 0.24 ml | 1 x 0.96 ml |
| DNA Rapid Ligation Buffer | Orange | 1 x 0.48 ml | 2 x 0.96 ml |
| HiFi PCR Master Mix (2X) | White | 1 x 0.60 ml | 2 x 1.25 ml |
| Primer Mix | White | 1 x 0.036 ml | 1 x 0.144 ml |

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## 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.

- Adapter Barcodes
  The sparQ DNA 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 recommends two sets of single-barcoded adapters for purchase as companion products to the sparQ DNA Library Prep Kit.

| 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

## 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 -20ºC and plan your workflow accordingly.

Point in protocol where procedure can be stopped and stored at appropriate conditions outlines

Take note of recommendations in protocol

Use caution to obtain the best results when performing protocol

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## 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.
- Program a thermal cycler with the parameters listed in the table below:

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

*It is recommended to use the thermocycler's heated lid with temperature set to 70°C.*

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

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# Protocol

### DNA Polishing

DNA Polishing combines DNA End-Repair and dA-Tailing into one step.

1. Prepare a reaction mix in a new thin-walled PCR tube on ice by combining the DNA Polishing Buffer, DNA sample, and nuclease-free water per the volumes in the table below. Mix well by gently pipetting (do not vortex to mix). 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 | 5 |
| DNA sample | X |
| Nuclease-free water | (35 - X) |
| Total | 40 |

2. Add 10 µl of DNA Polishing Enzyme Mix to each reaction and gently mix well by pipetting up and down 8-10 times. It is recommended to keep the PCR tube on ice for the entire time during reaction setup.
3. Pulse-spin the sample tube and immediately transfer to the pre-chilled thermal cycler (4°C). Resume the cycling program.
4. When thermal cycler program is complete and sample block has returned to 4°C, remove samples from block and place on ice.
5. Immediately proceed to the adapter ligation step.

### Adapter Ligation

*The following steps are for customers using Quantabio sparQ Adapter Barcode 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 – 2.5 ng | 1:10 – 1:20 dilution | 0.075 – 0.0375 µM |
| 1 – 0.25 ng | 1:50 – 1:100 dilution | 0.015 – 0.0075 µM |

6. Transfer 1.5 µl of appropriately diluted adapter into the PCR tube with 50 µl of polished DNA from step 5. Mix gently by pipetting and cool on ice.

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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 | 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 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. 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.

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**Library Amplification (optional)**

*Library amplification is generally recommended if the input DNA is below 100 ng. The PCR reagents (2X 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 2X HiFi PCR Master Mix 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 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 below |
| 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 (ng) | Suggested number of cycles to yield 500 ng of DNA library |
|---|---|
| 1000 | 0 - 1 |
| 500 | 1 - 2 |
| 100 | 4 - 5 |
| 50 | 5 - 6 |
| 10 | 8 - 10 |
| 1 | 13 - 15 |
| 0.25 | 16 - 18 |

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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.

**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 either of the above two protocols 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 (95155-500, 95156-500) for accurate quantification of DNA library molecules suitable for sequencing on Illumina NGS platforms.

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## 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: 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 |

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## Related NGS Products Sold Separately

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.

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