PROTOCOL

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SWIFT 2S® TURBO FLEXIBLE DNA LIBRARY KITS

with Enzymatic Fragmentation and Optional PCR

Protocol for Cat. Nos. 45024 and 45096 for direct and targeted sequencing.

Compatible with Illumina® adapters:

- TruSeq DNA Single Indexes (Cat. No. 20015960 for Set A and Cat. No. 20015961 for Set B)
- TruSeq DNA CD (combinatorial dual) (Cat. No. 20015949 for 96 indexes).

For custom adapters, please contact techsupport@swiftbiosci.com.
Visit swiftbiosci.com/protocols for updates.

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About This Guide

This guide provides instructions for the preparation of high complexity next generation sequencing (NGS) libraries from double-stranded DNA (dsDNA). The protocol is designed for users performing direct sequencing or hybridization capture following enzymatic fragmentation and library construction. This kit is validated for a wide range of DNA inputs, 1– 250 ng and may be used in conjunction with single or dual indexed adapters that are supplied by Illumina.

 IMPORTANT!
Read the entire Protocol before use, especially the sections pertaining to Product Information, Kit Contents, Material and Equipment Not Included, and Input Material Considerations. Also, read the certificate of analysis (CoA) provided with the kit or the label inside the product box to obtain recommended lot-specific fragmentation times for optimal performance.

Product Information

The Swift 2S Turbo Flexible DNA Library Kit offers a versatile solution to streamline NGS sample preparation of dsDNA for sequencing on Illumina platforms. The technology provides rapid DNA fragmentation and library construction for the production of libraries for sequencing across all Illumina instrument types. Although this prep can be used on damaged samples such as FFPE, optimization of fragmentation times may be required, as we have observed that FFPE samples require shorter fragmentation times. Better results may be obtained from our Accel-NGS® 2S library preparation kit that utilizes enhanced DNA repair steps that are ideal for damaged samples.

The workflow suggests fragmentation times for high quality DNA to obtain a mean 350 bp and 200 bp inserts for direct and targeted sequencing, respectively. Contents of the kit are for generating libraries via an optional PCR workflow, as full-length adapters are utilized during the ligation step of the protocol. PCR reagents are included for direct sequencing; however, for hybridization capture applications, use the polymerase and thermocycler conditions recommended by the hybridization capture reagents. Primers are included for optional amplification of libraries generated with full-length adapters.

The protocol is readily automatable. A 10% overage volume of reagents is supplied in both the 24 and 96 reaction kits to accommodate automation. Swift Biosciences does not supply automated liquid handling instrumentation or consumables, but collaborates with automation solution providers and customers to develop and qualify optimized automated scripts for use of our kits, in combination with liquid handling platforms routinely used in NGS library preparation. Please contact your instrument vendor or TechSupport@swiftbiosci.com if you plan to use the Swift 2S Turbo Flexible DNA Library Kit with your particular automated liquid handling system.

Applications

The Swift 2S Turbo Flexible DNA Library Kit is suitable for the following applications:

- Whole genome sequencing (WGS)
- Metagenomic sequencing
- Long-range PCR amplicons
- RNA-Seq starting with full length, double stranded cDNA input
- PCR-free sequencing
- Low frequency somatic variation detection of SNVs and Indels
- Copy number variation detection
- Detection of germline inherited SNVs and Indels
- Hybridization capture of relevant genomic regions (i.e., the exome) or transcripts of interest

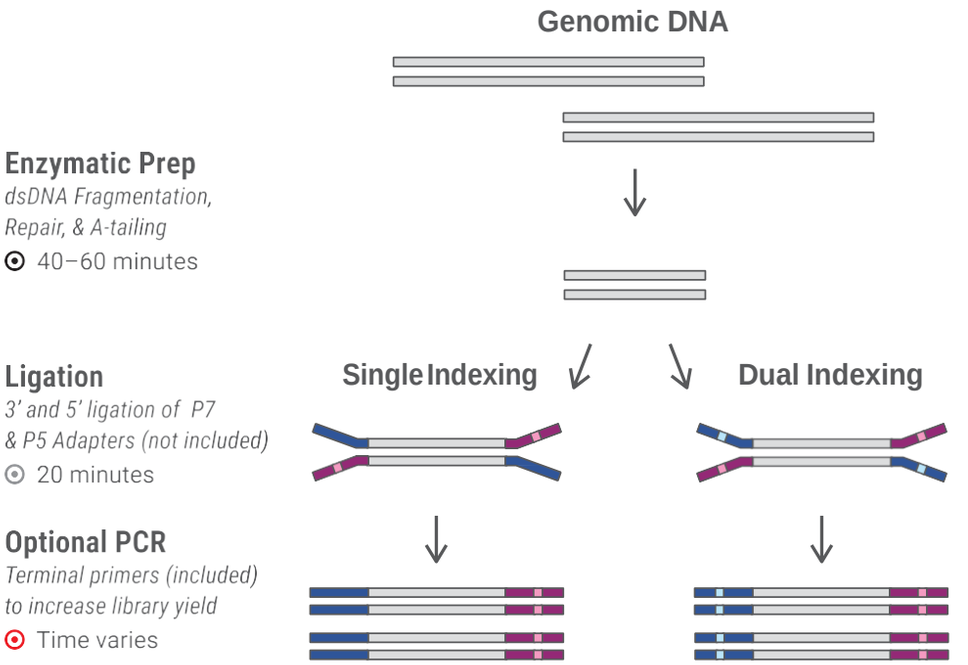
The Swift 2S Turbo Flexible DNA Library Kit is suitable for the following hybridization capture technologies:

- Swift Hybridization Capture Kits
- IDT xGen® Lockdown® probes
- Twist Bioscience panels
- Agilent SureSelect^{XT}

Swift 2S Turbo Workflow

This protocol contains minimal enzymatic incubations and bead-based purification steps, thereby reducing sample handling and overall library preparation time to under two hours prior to library amplification. The first step consists of enzymatic fragmentation of dsDNA, end-repair and dA-tailing, all performed in a single reaction. The fragmentation profile achieved is dependent on both temperature and time. The second step is ligation of the P5 and P7 adapters. The final PCR step is optional and can be used to increase library yield.

Following ligation and the optional PCR step, a bead-based clean-up is used to remove oligonucleotides and small fragments.



This workflow applies to single or dual indexing of libraries for direct and targeted sequencing (using Swift, IDT, Twist, and Agilent hybridization capture workflows). Primers are included for both direct sequencing and pre-hybridization capture amplification of libraries generated with full-length adapters. For hybridization capture, the primers should be used in conjunction with the polymerase recommended with the capture probes of choice.

Kit Contents

The Swift 2S Turbo Flexible DNA Library Kits are available in two sizes with reagents (10% excess volume) for the preparation of either 24 or 96 libraries. In addition to the reagent components in the table below, PCR reagents are also supplied for direct sequencing. The supplied PCR reagents are not compatible with hybridization capture.

Reagents	Quantity (µl)		Storage (ºC)
	24 rxn	96 rxn	
☐ Buffer K1	80	317	-20
☐ Reagent K2	121	476	-20
☐ Enzyme K3	160	634	-20
☐ Buffer W1	305	1218	-20
☐ Enzyme W3	106	424	-20
☐ Reagent R2	106	424	-20
☐ Buffer R3	264	1056	-20
☐ Enzyme R4	32	105	-20
☐ Reagent R1	132	528	-20
☐ ALU 115	540	540	-20
☐ ALU 247	540	540	-20

Reagents	Quantity (mL)	Storage (ºC)
Low EDTA TE	20	RT

☐ IMPORTANT!

Place the enzymes on ice, NOT in a cryo-cooler, for at least 20 minutes to allow enzymes to reach 4 ˚C prior to pipetting.

Material and Equipment Not Included

- Single indexed adapters: TruSeq DNA Single Indexes (Cat. No. 20015960 for Set A and Cat. No. 20015961 for Set B), or Dual indexed adapters: TruSeq DNA CD (combinatorial dual) (Cat. No. 20015949 for 96 indexes)
- Magnetic beads for clean-up steps, e.g., SPRIselect™ beads (Beckman Coulter, Cat. No. B23317/B23318/B23319)
- Magnetic rack for clean-up steps, e.g., Invitrogen DynaMag™ or Agencourt® SPRIPlate™
- Library quantification kit
- Qubit® or other fluorometric-based assays for determining DNA concentration
- Microfuge
- Programmable thermocycler
- 0.2 mL PCR tubes
- 1.5 mL low retention microfuge tubes
- Aerosol-resistant, low retention pipettes and tips, 2 to 1000 µL
- 200-proof/absolute ethanol (molecular biology-grade)
- Nuclease-free water (molecular biology-grade)
- PCR reagents (including DNA polymerase) for hybridiztion capture of choice

Storage and Usage Warning

Upon receipt, store the Swift 2S Turbo Flexible DNA Library Kit products at -20 ˚C with the exception of Low EDTA TE solution, which is stored at room temperature.

The enzymes provided in this kit are temperature sensitive, and appropriate care should be taken during storage and handling. To maximize use of enzyme reagents when ready to use, remove enzyme tubes from -20 ˚C storage and place on ice, NOT in a cryocooler, for at least 20 minutes to allow enzymes to fully thaw and reach 4 ˚C prior to pipetting. Attempting to pipette enzymes at -20 ˚C may result in a shortage of enzyme reagents.

After thawing reagents to 4 ˚C, briefly vortex (except the enzymes) to mix them well. Spin all tubes in a microfuge to collect contents prior to opening.

 IMPORTANT!

- Keep all master mix reagents ON ICE, except for Buffer W1, as long as possible during handling and preparation. DO NOT proceed until all reagents, including the low EDTA TE solution have had enough time to equilibrate to an ice-cold temperature.
- Assemble all reagent master mixes ON ICE and scale volumes as appropriate, using 10% excess volume to compensate for pipetting loss.
- Always add reagents to the master mix *in the specified order* as stated throughout the Protocol.
- Buffer W1 added in the Ligation reaction master mix is very viscous and requires special attention during pipetting. Be sure to keep Buffer W1 at room temperature. Once ready for use, pipette it very slowly so that you accurately draw the desired quantity.

Tips and Techniques

Avoiding Cross-Contamination

To reduce the risk of DNA and library contamination, physically separate the laboratory space, equipment, and supplies where pre-PCR and post-PCR processes are performed. Follow the instructions below to avoid cross-contamination:

- Clean lab areas using 0.5% sodium hypochlorite (10% bleach).
- Use barrier pipette tips to avoid exposure to potential contaminants.
- Always change tips between each sample.

Size Selection During Clean-Up Steps

This protocol has been validated with SPRIselect beads (Beckman Coulter) for direct and targeted sequencing applications, but can be used with Agencourt AMPure® XP (Beckman Coulter). If other beads are used, solutions and conditions for DNA binding may differ.

Consider the information below for performing efficient size selection:

- Post enzymatic preparation, analyze the samples' size distribution by electrophoretic methods to determine the median fragment size of your fragmented dsDNA samples.
- The size selections utilized in this protocol perform a Left Side Size Selection, and are designed to produce an average insert size of 350 bp and 200 bp for direct and targeted sequencing, respectively. For customizing size selection, please use Beckman Coulter's SPRIselect User Guide for desired conditions not included in this protocol.

DNA Input Considerations

Quantification

For high quality samples, it is recommended to determine dsDNA concentration using Qubit, or a similar fluorometric method, as it will accurately represent the double-stranded, adaptable DNA content of your sample. For low quality DNA samples, we recommend quantification by qPCR using Alu primer pairs provided in this kit (see Input DNA Quantification Assay) to accurately assess the usable amount of DNA in the samples and their integrity.

High Quality gDNA	Quantify with Qubit or similar fluorometric method
FFPE DNA	Quantify by qPCR with Alu primer pairs (see Input DNA Quantification Assay)
Amplicons	Quantify with Qubit or similar fluorometric method
cDNA	Quantify with Qubit or similar fluorometric method

This kit has been validated for use with a wide range of DNA inputs; 1– 250 ng. For PCR-free sequencing, use a minimum of 100 ng.

 IMPORTANT!
Input quantities referenced in this Protocol refer to total DNA quantified prior to being used in DNA fragmentation reaction.

EDTA in Elution Buffers

- The enzymatic reaction is very sensitive to high concentrations of EDTA. EDTA is usually introduced via elution buffers used in the final steps of the DNA extraction or purification process.
    - Our standard enzymatic prep recommends 0.1 mM EDTA TE (as provided in this kit) and requires only 1.5 µl of Reagent K2 for fragmentation to maximize fragmentation efficiency.
    - If DNA was eluted in standard TE with 1 mM EDTA, perform a buffer exchange using a column or bead-based purification. Alternatively, you can adjust the amount of Reagent K2 used in the Enzymatic Prep step to no more than 3x to achieve the desired fragment length (up to 4.5 uL of Reagent K2 per reaction).
    - Please contact TechSupport@swiftbiosci.com if your DNA is resuspended in 10 mM Tris or water (e.g., Buffer EB, Qiagen, 10 mM Tris-HCl, pH 8.5).

Fragmentation Parameters

We have observed minor lot to lot variation with the fragmentation mix. When utilizing a new lot, read the CoA provided with your kit or the label inside the product box for recommended lot-specific fragmentation times for optimal performance. These fragmentation times were determined by sequencing the libraries on Illumina MiSeq and assessing aligned mean insert size.

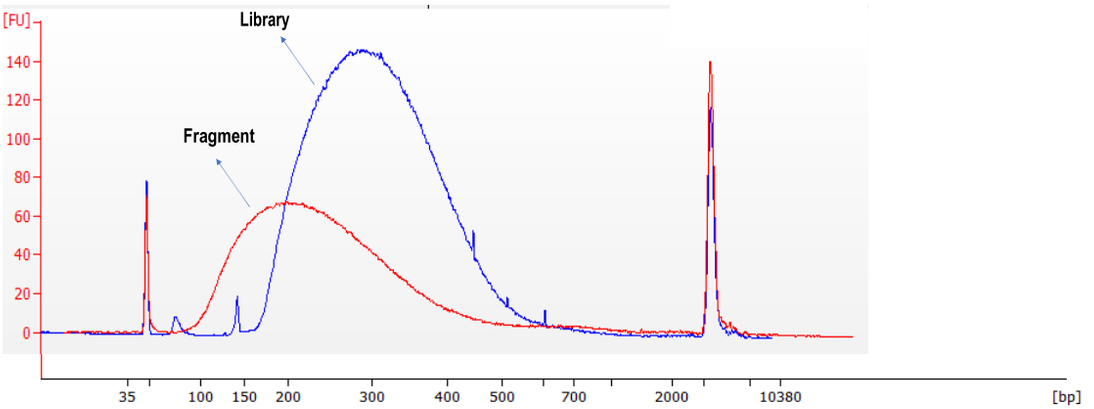
Recommended PCR Cycles

Below are the recommended minimum PCR cycles for direct sequencing of libraries prepared from high quality genomic DNA. For samples of compromised quality, additional cycles may be required. Yields are approximate and will vary between sample types. For hybridization capture, consult the capture platform of choice for recommended thermocycler conditions.

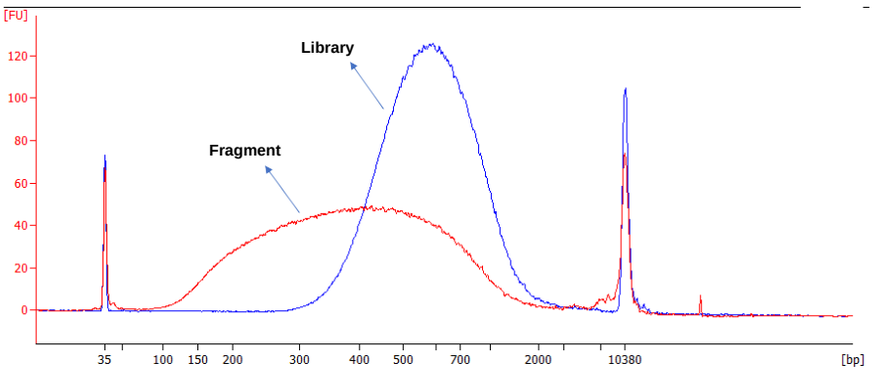
Input Material (ng)	Minimum PCR Cycles	Average Yield (nM)
≥ 100	0	≥ 4
25	4	≥ 4
10	6	≥ 4
1	9	≥ 4

Expected Results

Agilent High Sensitivity DNA trace of samples prepared from NA12878 Coriell DNA, for mean library insert of 200 bp (post library amplification). Notice, obtaining a mean aligned insert of 200 bp requires libraries with a peak height of ~320 bp on the Agilent system to account for the adapter length (~125 bp).



Agilent High Sensitivity DNA trace of samples prepared from NA12878 Coriell DNA, for mean library insert of 350 bp (post-library amplification). Notice, obtaining a mean aligned insert size of 350 bp requires libraries with a peak height of ~560 bp on the Agilent system, due to the broad size distribution of the library and accounting for the adapter length (~125 bp).



If preparing PCR-free libraries, migration may not run true to size. See Appendix, Section B for more information.

Prepare the Reagent Master Mixes and Ethanol

1. To create the master mix, scale reagent volumes as appropriate, using 10% excess volume to compensate for pipetting loss.
2. To assemble reagent master mixes for the Enzymatic Prep, Ligation, and Indexing PCR steps, ensure the reagent vials and enzymes are at 4 °C (except Buffer W1 that should be kept at room temperature). After thawing reagents, briefly vortex (except the enzymes) to mix them well. Spin tubes in a microfuge to collect contents prior to opening. Add reagents in order listed when preparing master mixes. Once prepared, master mixes should be stored ON ICE until used.

 IMPORTANT!
Prepare the reagents in advance (refer to page 4 for the guidelines on proper reagent handling). Always add reagents in specified order. This applies to all reagents except for the indexed adapters, provided separately in the indexed adapter kit that should be added individually to uniquely index each library.

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3. Prepare a fresh 80% ethanol solution using 200-proof/absolute ethanol and nuclease-free water. Approximately 1 mL of 80% ethanol solution will be used per sample.

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Prepare the DNA Libraries

Follow the protocol in this section for genomic DNA inputs of 1-250 ng to construct libraries with an average insert size of 200 and 350 bp, for targeted enrichment and direct sequencing, respectively. Fragmentation times provided are for high quality samples. Optimization of fragmentation time may be required for samples of compromised quality (e.g., FFPE).

Please refer to page 6, *EDTA in Elution Buffers,* for instructions on enzymatic prep set up using DNA inputs in Low EDTA TE, TE, or Tris DNA elution buffers.

□ Enzymatic Prep

1. Transfer the DNA sample to a 0.2 mL PCR tube and adjust the volume of the sample to a total of 19.5 µl using Low EDTA TE, if necessary.

Reagents	Volume per Sample
Low EDTA TE	(19.5 - x) µl
DNA	x µl
Total Volume	**19.5 µl**

□ IMPORTANT!
Ensure that the enzymatic prep master mix is mixed THOROUGHLY prior to and following addition of DNA samples. Failure to mix thoroughly will result in incomplete fragmentation.

In addition, ensure that the enzymatic prep master mix and the DNA samples are kept on ice until placed in the thermocycler to ensure desired fragmentation. Enzymes are active at room temperature and may fragment DNA to undesired sizes.

Next, determine the appropriate enzymatic fragmentation time for your application and input amount.

To do this, see your Certificate of Analysis (CoA) or the label inside the product box for specific fragmentation time recommendations for the lot number you receive.

2. Pre-set the thermocycler according to the program in the order listed below. A heated lid set at 70 °C is required for this step. Use the recommendations below to determine the optimal reaction time required to generate the desired fragment size. Reaction times may need to be optimized for individual samples. Prior to mixing, start the program to allow cycler lid to reach 70 °C and temperature block to reach 4 °C.

Thermocycler Conditions, lid kept at 70 ºC:

- Hold at 4 ºC
- 32 ºC for the desired fragmentation time
- 65 ºC for 30 minutes
- Hold at 4 ºC — proceed to the Ligation step

3. Prepare the Enzymatic Prep Master Mix in the order listed in the table below. Thoroughly mix the Master Mix reagents by low-to-moderate vortexing for 5 seconds.

Reagents	Volume per Sample
□ Buffer K1	3 µl
□ Reagent K2	1.5 µl
□ Enzyme K3	6 µl
Total MasterMix	**10.5 µl**

If samples are in 1 mM EDTA, using 2-3x volume of Reagent K2 will reduce EDTA-induced under-fragmentation.

4. Add 10.5 µl of pre-mixed Master Mix to each sample containing the DNA sample and Low EDTA TE to a final volume of 30 µl.
5. Mix thoroughly by vortexing for 5 seconds.
6. Spin down the sample tube in a microfuge and immediately place in the chilled thermocycler, and advance the program to the 32 ºC step.
7. Prior to completion of the thermocycler program, begin to prepare your master mix for the Ligation step. Fragmented samples can be kept at 4 °C for no more than one hour.

□ Ligation

8. Pre-set a thermocycler program for 20 minutes at 20 °C with lid heating OFF or set at 40 degree °C.
9. Add 30 µl of pre-mixed Ligation Master Mix (listed in the table below) to the same tubes in which Enzymatic Prep was performed (30 µl). Mix by low-to-moderate vortexing for 5 seconds. Buffer W1 is very viscous and should be used at room temperature. Care should be taken to ensure slow pipetting during Master Mix preparation and drawing.

Reagents	Volume per Sample
Low EDTA TE	10 µl
□ Buffer W1	12 µl
□ Enzyme W3	4 µl
Illumina Adapters	4 µl
Total MasterMix	**30 µl**
Sample	30 µl
Total Volume	**60 µl**

**** For direct sequencing applications, all DNA inputs < 25 ng, adapters should be titrated as suggested below.***

 IMPORTANT!
For direct sequencing using an input of DNA < 25 ng, dilute adapters in Low EDTA TE buffer to the appropriate concentrations, as outlined in the Table below. Adapter dilution is necessary to achieve low levels of adapter dimer. Careful quantification of your sample input is necessary for achieving optimal results.

DNA input (ng)	Illumina Adapter
≥ 25	No dilution
10	10-fold (1:10)
1	20-fold (1:20)

10. Place the samples in the thermocycler, programmed at 20 ˚C for 20 minutes with lid heating OFF or set at 40 ˚C.
11. Purify the Ligation reaction using a magnetic rack, bead suspension, and freshly prepared 80% ethanol. See Appendix, Section A for instructions. Follow the clean-up instructions below.

Application	Average Insert Size	Sample Volume	Bead Volume	Elution Volume
Direct Sequencing	350 bp	60 µl	48 µl (ratio: 0.8)	22 µl
PCR-free direct sequencing (≥ 100 ng)	350 bp	60 µl	48 µl (ratio: 0.8)	50 µl*
Hybridization Capture	200 bp	60 µl	48 µl (ratio: 0.8)	22 µl

* ***Transfer eluate and perform a second clean-up for size selection using 32.5 µl Bead Volume (ratio: 0.65)***

01- At the end of the clean-up, re-suspend the beads in 22 µl of Low EDTA TE buffer.
02- Place the sample tubes on a magnetic rack and wait 2 minutes.
03- Carefully transfer 20 µl of the supernatant to a clean tube without carry over of any beads. For PCR-free applications, skip steps 15 –21. If sequencing on patterned flow cells proceed with step 22; otherwise, libraries are ready for PCR or to be quantified and sequenced. Refer to the “Safe Stopping Point” instructions on page 13.

Optional PCR

15. For direct sequencing, pre-set the thermocycler in accordance with the cycles required due to your DNA input and the program in the order listed below. Utilize the primers and the polymerase supplied with our library preparation kits and thermocycler conditions below.

    For pre-hybridization capture PCR, use the primers supplied (at 6 µM, used at 600 nM working concentration) with the polymerase and thermocycler conditions recommended by the hybridization capture reagents. Note that you will need significantly more PCR cycles to generate required quantities (500 ng - > 1 µg).

 IMPORTANT!
The number of cycles required to produce sufficient library for sequencing will depend on input quantity and quality. In the case of low quality samples including FFPE, the number of cycles required may vary based on the quality of the sample and amount of usable DNA present. Approximate guidelines for high quality DNA are as indicated below, but the exact number of cycles required must be determined by the user.

DNA Input (ng)	Minimum Recommended PCR Cycles	Thermocycler Program for Direct Sequencing, Heated Lid at 105 °C
≥ 100 ng	0	98 ºC for 30 seconds PCR Cycles: • 98 ºC for 10 seconds • 60 ºC for 30 seconds • 68 ºC for 60 seconds Hold at 4 ºC — proceed immediately to clean-up step
25 ng	4	
10 ng	6	
1 ng	9	

16. Add 30 µl of the pre-mixed PCR Master Mix (listed in the table below) to the entire eluted sample (20 µl). Mix by vortexing.

Reagents	Volume per Sample
Low EDTA TE	10 µl
□ Reagent R1	5 µl
□ Reagent R2	4 µl
□ Buffer R3	10 µl
□ Enzyme R4	1 µl
Total Volume	**30 µl**

17. Spin down the sample tube in a microfuge and run it in the PCR pre-programmed thermocycler. Samples can be stored in thermocycler overnight at 4 °C.
18. Purify the PCR reaction using a magnetic rack, bead suspension, and freshly prepared 80% ethanol. See Appendix, Section A for instructions. Follow the clean-up instructions below.

Application	Average Insert Size	Sample Volume	Bead Volume	Elution Volume
Direct Sequencing	350 bp	50 µl	32.5 µl (ratio: 0.65)	22 µl
Hybridization Capture	200 bp	50 µl	90 µl (ratio: 1.8)	22 µl

19. At the end of the clean-up, re-suspend the beads in 22 µl of Low EDTA TE buffer.
20. Place the sample tubes on a magnetic rack and wait 2 minutes.
21. Carefully transfer 20 µl of the supernatant containing the final library to a clean tube without carry over of any beads.

□ IMPORTANT!
If direct sequencing on patterned flow cells (i.e. NovaSeq, HiSeq 4000, iSeq 100, HiSeq X Ten), perform the following second clean-up to ensure optimal removal of the unincorporated adapters. Unincorporated adapters can increase indexing hopping on patterned flow cells. This second purification is not necessary for targeted sequencing as unincorporated adapters will not be retained during hybridization capture.

22. Perform a second purification using a magnetic rack, bead suspension and freshly prepared 80% ethanol. See Appendix, Section A for instructions. Follow clean-up instruction below.

Application	Average Insert Size	Sample Volume	Bead Volume	Elution Volume
Direct Sequencing	350 bp	20 µl	24 µl (ratio: 1.2)	22 µl

23. At the end of the clean-up, re-suspend the beads in 22 µl of Low EDTA TE buffer.
24. Place the sample tubes on a magnetic rack and incubate at room temperature for 2 minutes.
25. Carefully transfer 20 µl ofthe supernatant containing the final library to a clean tube without carry over of any beads.

Safe Stopping Point

Store freshly prepared libraries at 4 ˚C (or long term at -20 ˚C). The library is now ready for quantification, which can be performed using fluorometric methods (i.e., Qubit) or qPCR. For PCR-fee libraries, qPCR is required as fluorometric methods cannot distinguish fully from partially ligated molecules. A high sensitivity DNA Agilent Bioanalyzer kit can be used to ensure desired library size.

Appendix

Section A: Clean-Up Protocol

Please use the following protocol for each clean-up step, substituting the correct **Bead Volume** and **Elution Volume** based on the table provided for each section.

1. Ensure the magnetic beads are at room temperature and vortex beads to homogenize the suspension before use.
2. Add the specified Bead Volume to each sample. Mix by vortexing. Quick spin the samples in a tabletop microcentrifuge.
3. Incubate the samples for 5 minutes at room temperature.
4. Place the sample on a magnetic rack until the solution clears and a pellet is formed (~ 2 minutes).
5. Remove and discard the supernatant without disturbing the pellet (less than 5 µl may be left behind).
6. Add 180 µl of freshly prepared 80% ethanol solution to the sample while it is still on the magnetic rack. Use care not to disturb the pellet. Incubate for 30 seconds and then carefully remove the ethanol solution.
7. Repeat step 6 once more for a second wash with the 80% ethanol solution.
8. Quick spin the samples in a tabletop microcentrifuge and place back on the magnetic rack. Remove any residual ethanol solution from the bottom of the tube using a smaller pipette tip.
9. Add the specified elution volume of Low EDTA TE buffer and re-suspend the pellet. Mix well by pipetting up and down until homogenous.
10. Proceed with step 12 (post-Ligation clean-up), 19 (post-PCR clean-up), or 23 (post-PCR clean-up).

Post-Ligation Clean-Up

Application	Average Insert Size	Sample Volume	Bead Volume	Elution Volume
Direct Sequencing	350 bp	60 µl	48 µl (ratio: 0.8)	22 µl
PCR-free direct sequencing	350 bp	60 µl	48 µl (ratio: 0.8)	50 µl*
Hybridization Capture	200 bp	60 µl	48 µl (ratio: 0.8)	22 µl

**** Transfer eluate and perform a second clean-up for size selection using 32.5 µl Bead Volume (ratio: 0.65)***

Post-PCR Clean-Up

Application	Average Insert Size	Sample Volume	Bead Volume	Elution Volume
Direct Sequencing	350 bp	50 µl	32.5 µl (ratio: 0.65)	22 µl
Hybridization Capture	200 bp	50 µl	90 µl (ratio: 1.8)	22 µl

Post-PCR Clean-Up II (Recommended for direct sequencing on patterned flow cells)

Application	Average Insert Size	Sample Volume	Bead Volume	Elution Volume
Direct Sequencing	350 bp	20 µl	24 µl (ratio: 1.2)	22 µl

Section B: Helpful Information and Troubleshooting

Problem	Possible Cause	Suggested Remedy
Library migrates unexpectedly on Bioanalyzer	Broad library size distribution for 350 bp fragmentation profile	Agilent High Sensitivity DNA traces for a library insert of 350 bp fragmentation run larger than expected at a ~560 bp mode. This is due to the broad size distribution of the library; however, smaller inserts will preferentially cluster. When sequenced, an aligned insert size of 350 bp will be obtained.
	Over-amplification of library leads to the formation of heteroduplex structures that migrate abnormally.	• Quantify library by qPCR, as other quantification methods will not accurately detect heteroduplex library molecules. • Perform the minimum number of PCR cycles necessary to avoid over-amplification.
	Migration behavior over-estimates library size of PCR-free libraries due to partially single stranded adapters.	• "200 bp insert" PCR-free libraries should migrate to a ~ 500 bp peak and "350 bp insert" PCR-free libraries should migrate to a ~800 bp peak on the High Sensitivity Chip.
DNA does not fragment properly:		
Under digestion: high molecular weight profile of fragmented DNA	Input DNA was in a buffer with greater than 0.1 mM EDTA	Buffer exchange column or bead-based clean-up before fragmentation or use 2-3x volume of Reagent K2 in your fragmentation reaction.
	Improper mixing of reagents	Ensure fragmentation mix is adequately mixed prior to and after adding to sample input.
Over digestion:	Reaction left at room temperature	Ensure the Enzymatic Prep master mix and the DNA sample are kept on ice until placed onto the pre-chilled thermocycler.
	Sample integrity compromised	Fragmentation time must be optimized for DNA samples that are not high quality (e.g., FFPE). We have observed more rapid digestion for FFPE samples.
Incomplete resuspension of beads after ethanol wash during purification steps.	Over-drying of beads.	Continue pipetting the liquid over the beads to break up clumps for complete resuspension. To avoid over-drying, resuspend beads immediately after the removal of residual ethanol.
Shortage of enzyme reagents.	Pipetting enzymes at -20 °C instead of 4 °C.	Allow ligation enzyme reagents to equilibrate to 4 °C for 10 minutes prior to pipetting.
Retention of liquid in pipette tip	Viscous reagents (i.e., Buffer W1) may stick to pipette tip, especially for non-low retention tips.	Pipette up and down several times to ensure all liquid is released from the pipette tip.

If you experience problems with your library prep, please contact us at TechSupport@swiftbiosci.com, or by phone at 734.330.2568 (9:00 am-5:00 pm ET, Monday-Friday).

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The warranties identified in this paragraph are Swift’s sole and exclusive warranties with respect to the products and are in lieu of all other warranties, statutory, express or implied, all of which other warranties are expressly disclaimed,

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Swift Biosciences, Inc.
674 S. Wagner Road, Suite 100 • Ann Arbor, MI 48103 • 734.330.2568 • www.swiftbiosci.com

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