SWIFT PROTOCOL

SWIFT 2S® SONIC DNA LIBRARY KITS

NGS Library Prep for Fragmented dsDNA

Protocol for Cat. Nos.:

Swift 2S® Sonic DNA Library Kit (Cat No. 42024, 42096) – includes truncated Y adapters

Swift 2S® Sonic Flexible DNA Library Kit (Cat No. 43024, 43096) – adapters not included, use with full-length, indexed Y adapters (not supplied by Swift)

The Swift 2S Sonic DNA Library Kits are compatible with the following Indexing Primer Kits supplied by Swift:

- Single Indexing Primer Kit Set A (12-plex)
  Cat. No. X6024
- Combinatorial Dual Indexing Primer Kit (96-plex)
  Cat. No. X8096
- Set S1-S4 Combinatorial Dual Indexing Primer Kits (192- to 768-plex),
  Cat. Nos. X85192, X86192, X87192, X88192, X89768
- Swift Unique Dual Indexing Primer Kits (24- to 96-plex),
  Cat. Nos. X9096, X90384, X9096-PLATE, X90384-PLATE, X92304-PLATE
- Swift Normalase Combinatorial Dual Indexing Primer Kit (96-plex)
  Cat. No. 68096 (Requires Swift Normalase® Kit for complete functionality, Cat. No. 66096)
- Swift Normalase Unique Dual Indexing Primer Kit (384-plex)
  Cat. No. X91384-PLATE
- Swift Normalase Unique Dual Indexing Primer Plates (96 to 384-plex)
  Cat. No. X91096-1-PLATE, X91096-2-PLATE, X91096-3-PLATE, X91096-4-PLATE

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Technical Support

For technical support please contact Swift at TechSupport@swiftbio.com, or by phone: 734.330.2568 (9:00 am – 5:500 pm ET, Monday through Friday).

Product Information

This protocol provides instructions for the preparation of high complexity next generation sequencing (NGS) libraries from double-stranded DNA (dsDNA) that has undergone DNA fragmentation by Covaris® shearing or other method. The protocol is designed for nanogram range inputs (1 ng – 1 µg) for direct sequencing or targeted sequencing using hybridization capture.

- The Swift 2S Sonic DNA Library Kits (Cat. Nos. 42024 and 42096) have an indexing PCR workflow and include truncated Y adapters for compatibility with Indexing Primer Kits supplied by Swift.
- The Swift 2S Sonic Flexible DNA Library Kits (Cat. Nos. 43024 and 43096) do not include adapters and are compatible with full-length, indexed Y adapters (not supplied by Swift). These kits support a PCR-free workflow or an optional library amplification step.
- If your samples are damaged or in the sub-nanogram range, we recommend using the Swift Accel-NGS 2S Kit, or the Swift Accel-NGS 1S kit for heavily damaged or single stranded DNA input. To assess sample quality, we recommend quantification by qPCR using Alu primer pairs (Cat. No. 90396; see Input DNA Quantification Assay).

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 collaborates with automation solution providers and customers to develop and qualify optimized automated scripts for use of our kits with liquid handling platforms routinely used in NGS library preparation. Please contact your instrument vendor or TechSupport@swiftbio.com if you plan to use this protocol with your particular automated liquid handling system.

IMPORTANT! Read the entire Protocol before use, especially the sections pertaining to Product Information, Kit Contents, and Materials and Equipment Not Included.

Supported Applications

- Whole genome sequencing (WGS)
- Hybridization capture of targeted genomic regions (exome)
- Metagenomic sequencing
- Detection of germline inherited SNVs and Indels
- Low frequency somatic variant detection of SNVs and Indels
- Copy number variation detection

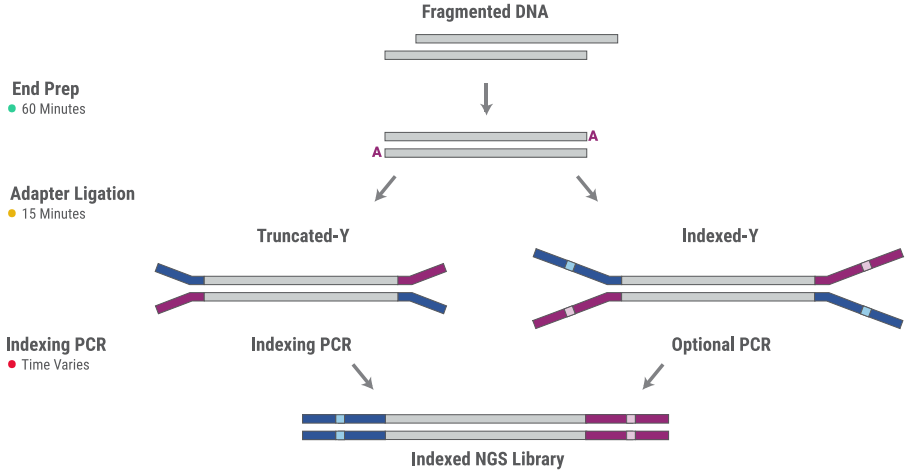
The Swift 2S Sonic DNA Library Kits can be used for targeted hybridization capture, where the Swift HiFi Polymerase Master Mix supplied in the kits is suitable for pre-hyb PCR to produce yields of 500 ng or greater from as low as 1 ng DNA input. The Swift 2S Sonic DNA Library kits are compatible with:

- Swift Hybridization Capture Panels
- IDT xGen® Lockdown Panels
- Twist Bioscience Panels

Swift 2S Sonic Workflow

This protocol contains two enzymatic incubations with an optional PCR, and bead-based purification steps. This minimizes sample handling and overall library preparation time to two hours (with PCR). The first incubation consists of end repair, polishing, and dA-tailing, all performed in a single End Prep reaction. This is followed by ligation of either a truncated Y adapter (Swift 2S Sonic, left) or full-length indexed Y adapter (Swift 2S Sonic Flexible, right). The ligation step is followed by either an Indexing PCR step if using truncated Y adapters (left) or an optional library amplification step if using full length, indexed adapters (right).

NOTE: Both Swift 2S Sonic and 2S Sonic Flexible are compatible with Normalase. (see Appendix Section D and the Normalase Kit protocol for instructions **before starting your PCR setup**).



Kit Contents

These kits contain enough reagents for the preparation of either 24 or 96 libraries (10% excess volume provided).

Protocol Stage	Component	Volume (µl)		Storage
		24 rxn	96 rxn	
End Prep	• Buffer E1	124	496	-20 °C
	• Enzyme E2	26	104	
	• Enzyme E3	26	104	
	• Enzyme E4	20	35	
Adapter Ligation	• Buffer L1	475	1,900	
	• Enzyme L2	158	632	
	• Reagent L3*	132	528	
PCR Amplification	• PCR Master Mix	660	2,640	
	• Reagent P2**	132	528	
Additional Reagents	Low EDTA TE	20 mL	20 mL	Room Temperature

*Reagent L3 is the truncated Y adapter and is only supplied with Swift 2S Sonic DNA Library Kits (Cat. Nos. 42024 / 42096)
**Reagent P2 is the amplification primers and is only supplied with Swift 2S Sonic Flexible DNA Library Kits (Cat. Nos. 43024 / 43096)

Storage and Usage Recommendations

- Upon receipt, store the 2S Sonic reagents at -20 ºC with the exception of Low EDTA TE bottle, which is stored at room temperature.
- To maximize use of enzyme reagents when ready to use, remove enzyme tubes from -20 ºC storage and place on ice for at least 10 minutes prior to pipetting. Attempting to pipette enzymes at -20 ºC may result in a shortage of enzyme reagents.
- After thawing reagents to 4 ºC, briefly vortex (except the enzymes) to mix them well. Spin all tubes in a microfuge to collect contents prior to opening.

Materials and Equipment Not Included

- A compatible indexing primer kit if using truncated Y adapters (See Appendix, Section D)
- Full-length indexed Y adapters (15 uM stock concentration) if using Swift 2S Sonic Flexible (not supplied by Swift)
- Normalase Kit if using Normalase indexing primers (Cat. No. 66096)
- Method for fragmentation of input DNA by mechanical shearing (i.e., Covaris)
- SPRIselect beads (Beckman Coulter, Cat. No. B23317/B23318/B23319)
- Invitrogen DynaMag™, Agencourt® SPRIPlate® or similar magnetic rack for magnetic bead clean-ups
- Qubit® or similar fluorometric DNA quantification assay (for input DNA and library quantification)
- Electrophoretic chip consumables and instrument to determine DNA fragment and library size (Bioanalyzer etc.)
- qPCR-based library quantification assay for Illumina libraries (optional)
- Microcentrifuge
- Vortex
- Programmable thermocycler
- 0.2 ml PCR tubes or 96-well plate
- Aerosol-resistant tips and pipette ranges from 2-1000 µl
- 200-proof/absolute ethanol (molecular biology grade)
- Nuclease-free water (molecular biology grade)

Tips and Techniques

IMPORTANT! Assemble all reagent master mixes ON ICE and scale volumes as appropriate, using 5% excess volume to compensate for pipetting loss. Always add reagents to the master mix in the specified order as stated throughout the protocol.

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

Prepare the DNA Sample

Input DNA Quantification

- 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.
- This kit has been validated for use with a wide range of DNA inputs; 1 ng - 1 µg.

DNA Sonication

If working with samples that have already undergone fragmentation, this step is omitted. This protocol supports 200 bp and 350 bp sheared DNA in Swift Low EDTA TE buffer. If the DNA volume post shearing is less than 50 µl, add TE buffer to a final volume of 50 µl. Alternatively, samples can be diluted with Swift Low EDTA TE buffer. Other shear sizes are compatible, please contact Swift at TechSupport@swiftbio.com.

Prepare the DNA Libraries

End Prep

1. Transfer fragmented dsDNA samples to a sterile 0.2 mL PCR tube and adjust the volume of the sample to a final volume of 50 µl using Low EDTA TE, if necessary.

2. Pre-set a thermocycler according to the program listed below with the heated lid set to 70 °C (a heated lid is required).

Thermocycler conditions, lid kept at 70 °C:
Hold @ 4 °C
30 minutes @ 20 °C
30 minutes @ 65 ℃
Hold @ 4 °C

3. Prepare the End-Prep Master Mix in the order listed in the table below.

Reagents	Volume per Sample
Low EDTA TE	3 µl
• Buffer E1	4.7 µl
• Enzyme E2	1 µl
• Enzyme E3	1 µl
• Enzyme E4	0.3 µl
Total Master Mix	**10 µl**
Fragmented DNA	50 µl
Total Volume	**60 µl**

4. Thoroughly mix the Master Mix by moderate vortexing for 5 seconds.

5. Add 10 µl of pre-mixed Master Mix to each sample and mix thoroughly by moderate vortexing for 5 seconds.

6. Spin down the sample tubes in a microfuge and place them in the chilled thermocycler and advance the program to the 20 °C step.

7. Prior to completion of the thermocycler program, begin to prepare your master mix for the adapter ligation step. The samples should be kept at 4 °C for no more than one hour.

It is recommended to proceed to the Adapter Ligation step within one hour to avoid a loss in yield. However, if necessary, samples can be stored at -20 ºC overnight.

Adapter Ligation

8. Pre-set a thermocycler program for 15 minutes at 20 °C with lid heating OFF.

Thermocycler conditions, lid heating OFF:

15 minutes @ 20 °C

IMPORTANT! When using DNA input < 50 ng, Reagent L3 or full-length indexed Y adapters must be diluted 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. For certain applications, adapter dilution may be adjusted to achieve best results.

For intermediate inputs scale the adapter dilution accordingly. Optimization may be required.

DNA Input	Adapter*
≥ 50 ng	No dilution
10 ng	10-fold (1:10)
1 ng	20-fold (1:20)

***** For the Flexible kit, full-length indexed Y adapters are used at a 15uM stock concentration.

9. Refer to the tables below for preparing the ligation Master Mix when using either a truncated Y adapter (left table, Reagent L3) or full-length indexed Y adapter (right table).

Swift 2S Sonic (Cat. Nos. 42024/42096)	
Reagents	**Volume per Sample**
• Buffer L1	18 µl
• Enzyme L2	6 µl
• Reagent L3*	5 µl
Total Master Mix	**29 µl**
End-Repair Sample	60 µl
Total Volume	**89 µl**

Swift 2S Sonic Flexible (Cat. Nos. 43024/43096)	
Reagents	**Volume per Sample**
• Buffer L1	18 µl
• Enzyme L2	6 µl
Total Master Mix	**24 µl**
End-Repair Sample	60 µl
Full-length indexed Y adapter**	5 µl (uniquely added to each sample)
Total Volume	**89 µl**

***** The Ligation Master Mix without the truncated adapter (Reagent L3) can be prepared ahead of time and kept on ice when making the End Prep Master Mix. Add the adapter to the Master Mix just before use.

** Full-length indexed adapters should be added individually to uniquely index each library. Use Y adapters at a 15 µM stock concentration.

10. For Swift 2S Sonic Flexible ONLY, add the full-length indexed Y adapters individually to each sample.

11. Add the pre-mixed Ligation Master Mix (29 µl for Sonic, 24 µl for Sonic Flexible) directly to the End Prep reaction mixture.

12. Thoroughly mix the Ligation reactions by moderate vortexing for 5 seconds and spin down the sample tubes in a microfuge.

13. Place in the thermocycler, programmed at 20 °C for 15 minutes with lid heating OFF.

14. Purify the Adapter Ligation reaction using a SPRI bead suspension, magnetic rack 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	89 µl	72 µl (ratio: 0.8)	21 µl
Hybridization Capture	200 bp	89 µl	72 µl (ratio: 0.8)	21 µl

15. At the end of the clean-up, re-suspend the beads in 21 µl of Low EDTA TE buffer.

16. Place the sample tubes on a magnetic rack and wait 2 minutes.

17. Carefully transfer 20 µl of the supernatant to clean PCR tubes without carry over of any beads.

18. Store freshly prepared PCR-free libraries at -20 ºC.

SAFE STOPPING POINT! Store the purified libraries post ligation at -20 °C if not proceeding to amplification, target capture or sequencing.
PCR-free libraries are now ready for quantification, which must be performed by qPCR to ensure accuracy. Note that PCR-free libraries cannot be accurately quantified or assessed for library size by electrophoretic methods.

IMPORTANT! A second clean-up (using a 0.8X or a different bead ratio) may be beneficial if post-ligation analysis reveals unacceptable levels of adapter-dimer carry-over or when PCR-free libraries are prepared for direct sequencing on patterned flow cells with the potential for index hopping.

PCR Amplification

The ligation step is followed by either an Indexing PCR step if using truncated Y adapters (Swift 2S Sonic) or an optional library amplification step if using full length, indexed Y adapters (Swift 2S Sonic Flexible).

Use the Swift HiFi Polymerase Master Mix supplied in the kits for both direct sequencing and pre-hybridization capture PCR workflows.

DNA Input	Minimum Recommended Cycles*	
	Swift 2S Sonic (Cat. Nos. 42024/42096)	Swift 2S Sonic Flexible (Cat. Nos. 43024/43096)
1 µg	3**	0 – 3
100 ng	3**	0 – 3
10 ng	6 – 7	6 – 7
1 ng	9 – 10	9 – 10

*These recommendations are for direct sequencing. For pre-hybridization capture PCR, you will need more PCR cycles to generate required quantities (200-500 ng) for hybridization capture. Refer to Appendix, section B for recommended cycle numbers to generate 1 µg of DNA.

** When indexing by PCR, a minimum of 3 cycles is required to complete adapter sequences, irrespective of whether a sufficient amount of library is available following ligation.

19. Pre-set the following thermocycler program, adjusting the number of cycles depending on the input amount and workflow (see table above).

Thermocycler program, lid kept at 105 °C:
98 °C for 2 minutes
PCR Cycles:
    98 °C for 20 seconds
    60 °C for 30 seconds
    72 °C for 30 seconds
72 °C for 1 minute
Hold @ 4 °C – proceed to clean-up step

20. This step differs depending on which 2S Sonic Kit is used:

Use step 20-A if using truncated Y adapters and indexing primers (Swift 2S Sonic).

Use step 20-B if using full length, indexed Y adapters and Reagent P2 (Swift 2S Sonic Flexible).

20-A For Indexing PCR, add indexing primers directly to the eluted library following the table below as a guideline. Add 25 µl PCR Master Mix to each sample and mix by moderate vortexing for 5 seconds and spin down in a microfuge.

> **NOTE:** If using Normalase Indexing Primers, see Appendix Section C and the Normalase Kit Protocol for specific instructions.

Indexing Options	Reagents	Volume per Sample
Single Indexing	Pre-mixed primer pair	5.0 µl
Unique Dual Indexing (UDI)	Pre-mixed primer pair	5.0 µl
Combinatorial Dual Indexing (CDI)	i5 primer i7 primer	2.5 µl 2.5 µl

Swift 2S Sonic (Cat. Nos. 42024/42096)	
Reagents	**Volume per Sample**
• PCR Master Mix	25 µl
Sample + Primer Mix	25 µl
Total Volume	**50 µl**

20-B For optimal amplification of fully indexed libraries, add Reagent P2 to the PCR Master Mix (see table below). Add 30 µl of the prepared PCR Master Mix to the eluted samples and mix by moderate vortexing for 5 seconds and spin down in a microfuge.

Swift 2S Sonic Flexible (Cat. Nos. 43024/43096)	
Reagents	**Volume per Sample**
• PCR Master Mix	25 µl
• Reagent P2	5 µl
Total Master Mix	**30** µl
Eluted Sample	20 µl
Total Volume	**50 µl**

21. Place in the thermocycler and run the program.

22. Purify the PCR reaction using a SPRI bead suspension, magnetic rack 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)	21 µl
Hybridization Capture	200 bp	50 µl	75 µl (ratio: 1.5)	21 µl

At the end of the clean-up, re-suspend the beads in 21 µl of Low EDTA TE buffer.

23. Place the sample tubes on a magnetic rack and wait 2 minutes.

24. Carefully transfer 20 µl of the supernatant containing the final library to a clean tube without carrying any beads.

IMPORTANT! If direct sequencing on patterned flow cells (i.e., NovaSeq HiSeq 4000, iSeq 100, NextSeq2000), perform the following second clean-up to ensure optimal removal of the unincorporated primers which can increase index hopping on patterned flow cells. This second purification is not necessary if using Normalase or hybridization capture as unincorporated primers will not be retained during either workflow.

25. If direct sequencing, perform an optional second clean-up using a magnetic rack, bead suspension and freshly prepared 80% ethanol. See Appendix, Section A for instructions. Follow clean-up instructions below:

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

26. At the end of the clean-up, re-suspend the beads in 20 µl Low EDTA TE buffer.

27. Place the sample tubes on a magnetic rack and wait 2 minutes.

28. Carefully transfer 20 µl of the supernatant containing the final library to a clean tube without carrying any beads.

Library Quantification

The library is now ready for quantification, which can be performed using fluorometric methods (i.e., Qubit) or qPCR. An electrophoretic chip (i.e., high sensitivity DNA Agilent Bioanalyzer kit) can be used to ensure desired library size and confirm quantification. Store libraries at -20 ºC.

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. Prepare a fresh 80% ethanol solution using 200-proof/absolute ethanol and nuclease-free water. Approximately 2 mL of 80% ethanol solution will be used per sample.
2. Ensure the magnetic beads are at room temperature and vortex beads to homogenize the suspension before use.
3. Add the specified Bead Volume to each sample. Mix by vortexing. Quick spin the samples in a microcentrifuge.
4. Incubate the samples for 5 minutes at room temperature.
5. Place the samples on a magnetic rack until the solution clears and a pellet is formed (~ 2 minutes).
6. Remove and discard the supernatant without disturbing the pellet (less than 5 µl may be left behind).
7. 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.
8. Repeat step 6 once more for a second wash with the 80% ethanol solution.
9. Quick spin the samples in a microcentrifuge and place back on the magnetic rack. Remove any residual ethanol solution from the bottom of the tube.
10. Remove the samples from the magnetic rack.
11. Add the specified elution volume of Low EDTA TE buffer and re-suspend the pellet. Mix well by pipetting up and down until homogenous.
12. Place the samples back on a magnetic rack until the solution clears and a pellet is formed (~ 2 minutes).
13. Transfer the specified eluate volume to a new 0.2 mL PCR tube. Ensure that eluate does not contain magnetic beads (indicated by brown coloration in eluate). If magnetic beads are present, pipette eluate into a new tube, place on magnet, and transfer eluate again.

Post-Adapter Ligation Clean-Up

Application	Average Insert Size	Sample Volume	Bead Volume	Elution Volume
Direct Sequencing	350 bp	89 µl	72 µl (ratio: 0.8)	21 µl
Hybridization Capture	200 bp	89 µl	72 µl (ratio: 0.8)	21 µl

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)	21 µl
Hybridization Capture	200 bp	50 µl	75 µl (ratio: 1.5)	21 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)	20 µl

Section B: Recommended Cycle Numbers for Generating 1 µg of Amplified DNA

Input DNA into End Prep	Number of cycles required to generate	
	100 ng Library	1 µg Library
1 µg	0 – 3*	2 – 3*
100 ng	2 – 3*	6 – 7
10 ng	7 – 9	11 – 13
1 ng	13 – 15	17 – 19

*When using the Swift 2S Sonic Kit and indexing by PCR, a minimum of 3 cycles is required to complete adapter sequences, irrespective of whether a sufficient amount of library is available following ligation.

Section C: Normalase Instructions

Please review this section and the Normalase Kit protocol before setting up your PCR. In order to achieve expected results, amplify each library using Normalase primers with the appropriate number of cycles and thermocycling conditions shown below to obtain a library yield of 12 nM or greater in a 20 µl eluate.

For Swift 2S Sonic, use Normalase Indexing Primers:

Normalase indexing primers complete the adapter sequences, amplify and condition libraries for downstream Normalase steps. Assemble using standard PCR reagents as shown below, except substitute standard indexing primers with i5 and i7 Normalase Indexing Primers.

1. To each sample, add 2 µl of each Normalase combinatorial dual index primer or 4 µl of Normalase unique index primer pair for a total volume of 24 µl.

Indexing Options	Reagents	Volume per Sample
Normalase UDI	Pre-mixed primer pair	4.0 µl
Normalase CDI	i5 primer	2.0 µl
	i7 primer	2.0 µl

Note: See Appendix Section D for Index information.

2. Assemble the PCR master mix on ice. Mix thoroughly and pulse spin to collect contents. Add 26 µl of the mix to each sample tube, mix thoroughly and pulse spin to collect contents (50 µl total reaction volume) and place in the thermocycler.

Normalase UDI	
Component	**Volume per reaction**
• PCR Master Mix	25 µl
• Reagent R7	1 µl
Total Master Mix	**26 µl**
Eluted Sample + Primers	24 µl
Total Volume	**50 µl**

Normalase CDI	
Component	**Volume per reaction**
• PCR Master Mix	25 µl
• Reagent R6	1 µl
Total Master Mix	**26 µl**
Eluted Sample + Primers	24 µl
Total Volume	**50 µl**

3. Run the following thermocycler program, adjusting the number of cycles depending on the input amount and sample quality. Lid heating set to 105 °C.

Temperature	Time
98 °C	2 min
98 °C	20 sec
60 °C	30 sec
72 °C	30 sec
Perform X cycles*	
72 °C	5 min
4 °C	hold

* The recommended minimum number of cycles for each input in order to provide ≥ 12 nM yields suitable for the Normalase workflow is as follows:

DNA Input (ng)	Minimum number of cycles for ≥ 12 nM
≥ 100	3
25	5
10	7
1	10

The number of cycles required may vary based on the input amount, as detailed above. Recommendations above are for high-quality input DNA.

4. Proceed to post-PCR SPRI clean-up (step 22 on page 10 of this protocol).

5. Proceed to Normalase I, Pooling, and Normalase II in the Normalase Kit Protocol.

For Swift 2S Sonic Flexible, use Reagent R5 for Normalase PCR: Library Conditioning

1. If you typically obtain the required minimum threshold (i.e. ≥ 12 nM following library amplification), simply utilize Normalase primers (Reagent R5) and add one additional PCR cycle to your program.

If prior to amplification your library yields are ≥ 12 nM, a minimum of 3 cycles is still required to condition the libraries for downstream Normalase enzymology.

2. Assemble your PCR Master Mix using standard PCR reagents as shown in the table below, except substitute standard primers (Reagent P2) with 5 µl of Reagent R5. Thoroughly mix by moderate vortexing, pulse spin to collect contents and place in the thermocycler.

Component	Volume per reaction
• PCR Master Mix	25 µl
• Reagent R5	5 µl
Total Master Mix	**30 µl**
Eluted Sample	20 µl
Total Volume	**50 µl**

3. Repeat steps 3 - 5 of the above protocol for Swift 2S Sonic on page 14.

Section D: Indexed Adapter Sequences

For Swift 2S Sonic's indexing PCR step, use a unique indexing primer Index X (single index), D50X and D7XX/S7XX (combinatorial dual indexes), or U001-U096/SU001-SU384 (unique dual indexes) to label each library. Libraries made with uniquely indexed adapters may be pooled prior to cluster generation and co-sequenced on the same Illumina flow cell.

During library prep, make sure to note which indexing primer(s) is used with each sample and do not use the same indexing primer set on two different samples that will be multiplexed together.

Single Indexing and Unique Dual Indexing primer kits provide the appropriate Universal or i5 and i7 primers mixed together in either tubes or single use plates. Combinatorial Dual Indexing Primer Kits provide the i5 and i7 primers in separate tubes. Please reference the table below and see PCR amplification on page 9 or Normalase PCR instructions on page 13 of this protocol.

Indexing Kit	Reagent	Reagent Contents	Volume added to each sample
Single Indexing	Index X	Pre-mixed Universal and i7 primers	5 µl
Combinatorial Dual Indexing	D50X	i5 primer	2.5 µl
	D7XX / S7XX	i7 primer	2.5 µl
Normalase Combinatorial Dual Indexing	D50XN	i5 primer	2 µl
	D7XXN	i7 primer	2 µl
Unique Dual Indexing	U001-U096	Pre-mixed i5 and i7 primers	5 µl
Normalase Unique Dual Indexing	U001-U384	Pre-mixed i5 and i7 primers	4 µl

For access to all index sequences to set up your sample sheet for your sequencing run, please go to 2S Sonic Product page at www.swiftbiosci.com and click on Protocols and Tools tab.

The full-length adapter sequences of the single, combinatorial dual, and unique dual indices are below. The underlined text indicates the location of the index sequences, as detailed in the tables below.

Single Indexing (Set A Indexing Kit, Cat. No. X6024)

TruSeq Universal Adapter:
5' AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT

TruSeq Index Adapter (I2, I4, I5, I6, I7, I12):
5' GATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXX(AT)CTCGTATGCCGTCTTCTGCTTG

TruSeq Index Adapter (I13, I14, I15, I16, I18, I19):
5' GATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXX(XX)ATCTCGTATGCCGTCTTCTGCTTG

The number on the product tube label indicates which indexing primer is provided in the tube. The bases in parentheses are not considered part of the 6-base index sequences but can be used for 8-base index reads.

Each tube contains 11 μl of the appropriate indexing primer set.

Combinatorial Dual Indexing (CD Indexing Kit, Cat. No. X8096; Swift S1-S4 Indexing Kit, Cat. Nos. X85192, X86192, X87192, X88192, X89768) and Normalase Combinatorial Dual Indexing Kit (Cat. No. 68096)

TruSeq Index 1 (i7) Adapter (D701-D712 for 96-plex kits; S701-S796 for 192 to 768-plex kits):
5' GATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXXXXATCTCGTATGCCGTCTTCTGCTTG

TruSeq Index 2 (i5) Adapter (D501-D508):
5' AATGATACGGCGACCACCGAGATCTACACXXXXXXXXACACTCTTTCCCTACACGACGCTCTTCCGATCT

For 96-plex Combinatorial Dual Indexing (Cat. No. X8096), each D50X tube contains 33 μl and each D7XX tube contains 22 μl of the appropriate indexing primer.

For 96-plex Normalase Combinatorial Dual Indexing (Cat. No. 68096), each D50XN tube contains 27 μl and each D7XXN tube contains 18 μl of the appropriate indexing primer. The Reagent R6 tube contains 106 μl.

For 192-plex Combinatorial Dual Indexing (Cat. Nos. X85192, X86192, X87192, X88192), each D50X tube contains 66 μl and each S7XX tube contains 22 μl of the appropriate indexing primer. For 768-plex Combinatorial Dual Indexing (Cat. No. X89768), each D50X tube contains 300 μl and each S7XX tube contains 22 μl of the appropriate indexing primer.

Swift Unique Dual Indexing (UDI Kit, Cat. No. X9096, X90384, X9096-PLATE, X90384-PLATE, & X92304-PLATE) and Swift Normalase Unique Dual Indexing Primer Plates (UDI Kit, Cat. No. X91384-PLATES, X91096-1-PLATE, X91096-2-PLATE, X91096-3-PLATE, X91096-4-PLATE).

The UDI 96-plex Set are 8 base index sequences, whereas the UDI 96 to 384-plex set are 10 base index sequences as depicted by the two additional (XX) bases.

TruSeq Index 1 (i7) Adapters:

5' – GATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXXXX(XX)ATCTCGTATGCCGTCTTCTGCTTG – 3'

TruSeq Index 2 (i5) Adapters:

5' – AATGATACGGCGACCACCGAGATCTACACXXXXXXXX(XX)ACACTCTTTCCCTACACGACGCTCTTCCGATCT – 3'

Each U0XX tube contains 22 µl of the appropriate indexing primer set. Each U0XX/SUXXX plate well contains 12 µl.

Section E: Helpful Information and Troubleshooting

Problem	Possible Cause	Suggested Remedy
Library migrates unexpectedly on Bioanalyzer	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.
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, re-suspend beads immediately after the removal of residual ethanol.
Shortage of enzyme reagents.	Pipetting enzymes at -20 °C.	Place enzyme reagents on ice for 10 minutes prior to pipetting.
Retention of liquid in pipette tip	Viscous reagents (i.e., Buffer L1) 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.
Unexpected increase in adapter dimers	Improper adapter dilution Improper bead purification Reagent L3 not added to the ligation master mix just before use	Use the specified dilution for your input quantity Use the specified bead volume particularly for the post-PCR purification Add Reagent L3 (truncated Y adapter) to your ligation master mix just before use

Technical Support

For technical support please contact Swift at TechSupport@swiftbio.com, or by phone: 734.330.2568 (9:00 am – 5:500 pm ET, Monday through Friday).

Revision History

Document #	Revision	Date	Description of Change
PRT-026	Version 1	10/02/2020	Initial release.

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