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PROTOCOL

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SWIFT 2S TURBO DNA LIBRARY KIT

with Enzymatic Fragmentation and Indexing PCR (Includes Swift Adapters)

Protocol for Cat. Nos. 44024 and 44096 for direct and targeted sequencing

To be used with Swift indexing kits:

- Swift 2S Turbo Single Indexing Primer Kit Set A (Cat. No. 46024)
- Swift 2S Turbo Combinatorial Dual Indexing Primer Kit (Cat. No. 48096)
- Swift 2S Turbo Set S1 Combinatorial Dual Indexing Primer Kit (Cat. No. 485192)
- Swift 2S Turbo Set S2 Combinatorial Dual Indexing Primer Kit (Cat. No. 486192)
- Swift 2S Turbo Set S3 Combinatorial Dual Indexing Primer Kit (Cat. No. 487192)
- Swift 2S Turbo Set S4 Combinatorial Dual Indexing Primer Kit (Cat. No. 488192)
- Swift 2S Turbo Set S1-S4 Combinatorial Dual Indexing Primer Kit (Cat. No. 489768)
- Swift 2S Turbo Unique Dual Indexing Primer Kit (Cat. No 49096)
- Swift 2S Turbo Unique Dual Indexing Primer Kit (Cat. No 490384)
- Swift 2S Turbo SureSelect Compatibility Module (Cat. No. 46424 and 46496)

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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 exome sequencing following enzymatic fragmentation and library construction. This kit is compatible for NGS library prep with single and dual indexing kits.

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 DNA Library Kits offer 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 a PCR workflow, as truncated adapters are attached during the ligation step of the protocol. PCR reagents are included. However, indexing primer kits are sold separately. This kit is validated for a wide range of DNA inputs, 50–250 ng for complex genomes (human DNA), and 1-250 ng for small genomes (i.e., bacterial).

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 instrument or consumables, but 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@swiftbiosci.com if you plan to use the Swift 2S Turbo DNA Library Kit with your particular automated liquid handling system.

Applications

The Swift 2S Turbo 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
- Hybridization capture of relevant genomic regions (i.e., the exome) or transcripts of interest
- Copy number variation detection
- Detection of germline inherited SNVs and Indels

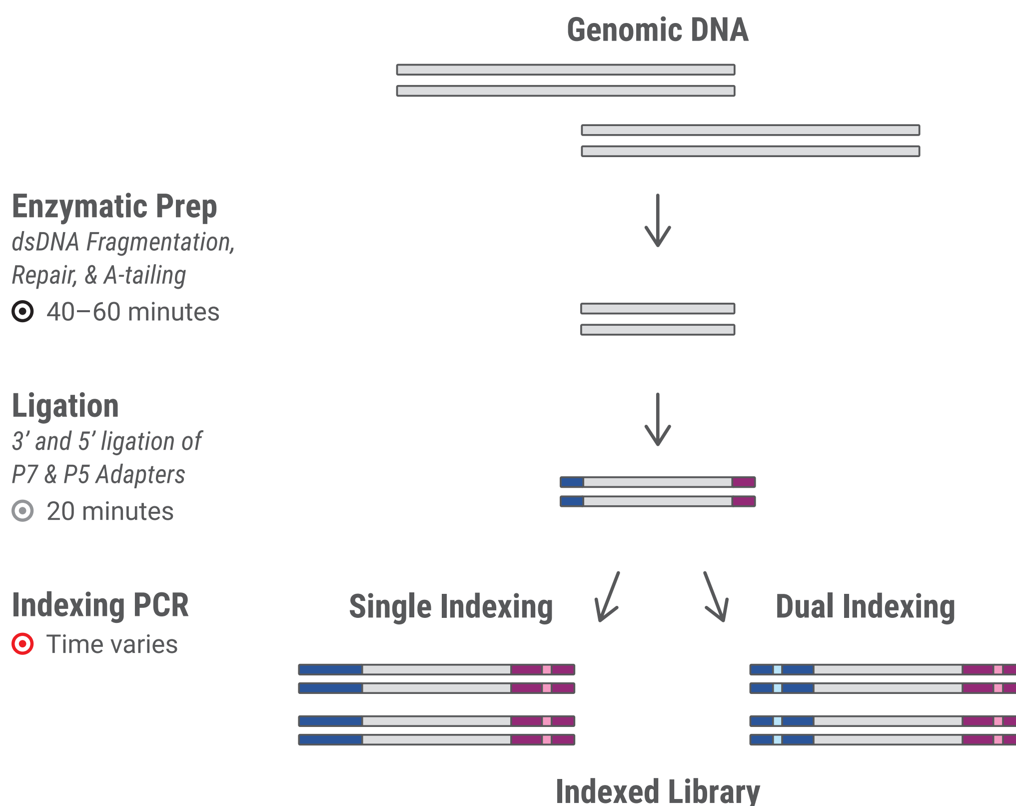
The Swift 2S Turbo DNA Library Kit is suitable for the following hybridization capture technologies:

- 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 truncated P5 and P7 adapters. The final indexing PCR step facilitates the completion of fully adapted indexed libraries.

Two bead-based clean-ups are used to remove oligonucleotides and small fragments.



The indexing PCR step is necessary to complete the indexed adapter sequences. This workflow applies to single or dual indexing of libraries for direct and targeted sequencing (using Swift, IDT, Twist, and Agilent hybridization capture workflows). For pre-hybridization amplification, the primers included in the indexing kit should be used in conjunction with the polymerase recommended with the capture probes of choice.

Kit Contents

The Swift 2S Turbo 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 supplied for direct sequencing applications. Index primers, Index X or R-XT (single indexing) or D50X/D7XX (dual indexing) are provided separately in one of the available Swift 2S Turbo indexing kits (see Appendix, Section B).

Reagents	Quantity (µl)		Storage (°C)
	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 W4	133	528	-20
Reagent R2	106	424	-20
Buffer R3	264	1056	-20
Enzyme R4	32	105	-20
ALU 115	540	540	-20
ALU 247	540	540	-20
Index X OR Reagent R-XT Turbo OR Index D50X/D7XX	*	*	-20

** Provided separately with an indexing kit.*

Reagents	Quantity (mL)	Storage (°C)
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

- A compatible Swift indexing kit
- 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 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; 50–250 ng for complex genomes (Human DNA), and 1-250 ng for small genomes (i.e., bacterial).

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 elu-tion 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.

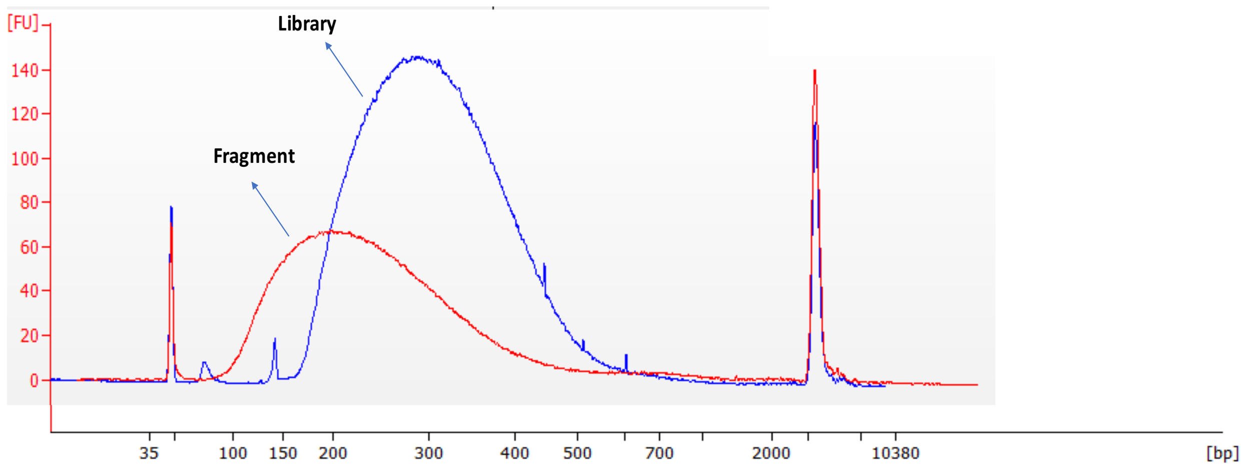
Recommended PCR Cycles

Below are 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.

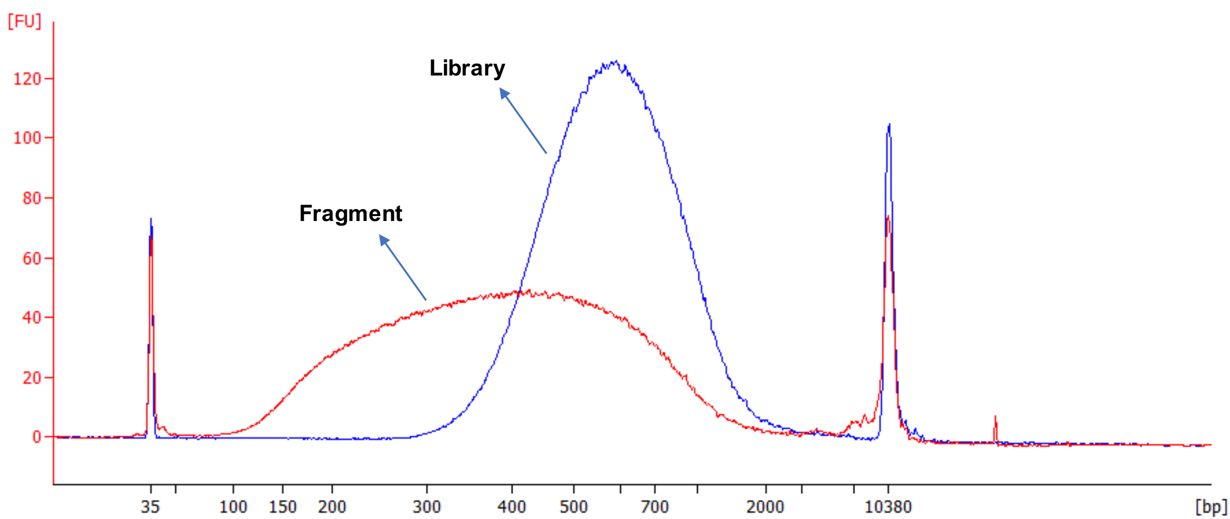
Input Material (ng)	Minimum PCR Cycles	Average Yield (nM)
≥ 100	3	≥ 4
25	5	≥ 4
10	7	≥ 4
1	10	≥ 4

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).



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 master mixes in advance (refer to page 4 for the guidelines on proper reagent handling). Always add reagents to the master mix in specified order. This applies to all reagents except for the indexing primers, provided separately in the indexing primer kits 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 human genomic DNA inputs ≥ 50 ng and 1-250 ng of low complexity DNA (i.e., bacterial) 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. Optimi-zation 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.

Application	Average Insert Size	Desired fragmentation time (min) for DNA ≥ 25 ng*	Desired fragmentation time (min) for DNA < 25 ng*
Direct Sequencing	350 bp	8 ± 4	10 ± 4
Hybridization Capture	200 bp	18 ± 10	20 ± 10

**** See your 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 °C:

- Hold at 4 °C
- 32 °C for the desired fragmentation time
- 65 °C for 30 minutes
- Hold at 4 °C — 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 °C 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 the thermocycler program for 20 minutes at 20 °C with lid heating OFF or set at 40 °C.
9. Add 30 µl of pre-mixed Ligation Master Mix (listed in the table below) to the same tubes in which Enzymat-ic Prep was performed (30 µl). Mix by low-to-moderate vortexing for 5 seconds. Buffer W1 is very viscous (use at room temperature). Care should be taken to ensure slow pipetting during Master Mix preparation and drawing.

Reagents	Volume per Sample
Low EDTA TE	9 µl
Buffer W1	12 µl
Enzyme W3	4 µl
Reagent W4*	5 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 Reagent W4 (truncated adapter) 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)	Reagent W4
≥ 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
Hybridization Capture	200 bp	60 µl	48 µl (ratio: 0.8)	22 µl

12. At the end of the clean-up, re-suspend the beads in 22 µl of Low EDTA TE buffer.
13. Place the sample tubes on a magnetic rack and wait 2 minutes.
14. Carefully transfer 20 µl of the supernatant to a clean tube without carrying any beads.

Indexing PCR

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

    For pre-hybridization capture PCR, use the primers supplied (at 6 µM, used at 600 nM working concentration) as part of the indexing kit 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) for hybridization capture.

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	3	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	5	
10 ng	7	
1 ng	10	

16. Add indexing reagents (5 µl) directly to the entire eluted library (20 µl). Mix by pipetting. Note: Index primers are provided separately in the indexing kit of choice. The combinatorial dual indices are not pre-mixed and are provided as 20 individual primers for generating 96 combinations.

Indexing Kit	Reagents	Volume per Sample
Single Indexing	Index X or R-XT*	5.0 µl
Unique Dual Indexing	U0XX	5.0 µl
Combinatorial Dual Indexing	D50X D7XX	2.5 µl 2.5 µl
	Sample	**20.0 µl**
	Sample + Primer Mix	**25.0 µl**

** For single indexing, Reagent R-XT is the truncated SureSelect^{XT} compatible adapter and can be added to the reaction mix.*

17. Add 25 µl of the pre-mixed Indexing PCR Master Mix (listed in the table below) to the entire eluted sample and primer mix (25 µl). Mix by vortexing.

Reagents	Volume per Sample
Low EDTA TE	10 µl
⦿ Reagent R2	4 µl
⦿ Buffer R3	10 µl
⦿ Enzyme R4	1 µl
Total Volume	**25 µl**

18. Spin down the sample tube in a microfuge and run it in the indexing PCR pre-programmed thermocycler. Samples can be stored in thermocycler overnight at 4 °C.
19. Purify the Indexing 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

20. At the end of the clean-up, re-suspend the beads in 22 µl of Low EDTA TE buffer.
21. Place the sample tubes on a magnetic rack and wait 2 minutes.

22. 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, HiSeq X Ten), perform the following second clean-up to ensure optimal removal of the unincorporated primers. Unincorporated primers can increase indexing hopping on patterned flow cells. This second purification is not necessary for targeted sequencing as unincorporated primers will not be retained during hybridization capture.

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23. Perform a second clean-up 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

24. At the end of the clean-up, re-suspend the beads in 22 µl of Low EDTA TE buffer.
25. Place the sample tubes on a magnetic rack and wait 2 minutes.
26. Carefully transfer 20 µl of the supernatant containing the final library to a clean tube without carrying 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. 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.
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) or 20 (post-PCR clean-up), or 24 (post-PCR clean-up II) to complete the clean-up step.

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
Hybridization Capture	200 bp	60 µl	48 µl (ratio: 0.8)	22 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)	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: Indexing Kits

Swift 2S Turbo Single Indexing Primer Kit Set A (Cat. No. 46024)

Libraries made with uniquely indexed primers may be multiplexed during cluster generation and co-sequenced on the same Illumina flow cell. The full-length adapter sequences where X is replaced by the index sequences in the tables below are as follows:

P5 TruSeq® Universal Adapter:

5' AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 3'

P7 TruSeq Adapter:

5' GATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXXXXCTCGTATGCCGTCTTCTGCTTG 3'

The number on the product tube label indicates which indexed primer is provided in the tube. The bases in parentheses (provided in the table below) are not considered part of the six bp index sequences, but can be used for 8 bp index reads (dual indexing).

Set A Indexes	Sequence	46024
Index 2, A002	CGATGT(AT)	22 µl
Index 4, A004	TGACCA(AT)	22 µl
Index 5, A005	ACAGTG(AT)	22 µl
Index 6, A006	GCCAAT(AT)	22 µl
Index 7, A007	CAGATC(AT)	22 µl
Index 12, A012	CTTGTA(AT)	22 µl
Index 13, A013	AGTCAA(CA)	22 µl
Index 14, A014	AGTTCC(GT)	22 µl
Index 15, A015	ATGTCA(GA)	22 µl
Index 16, A016	CCGTCC(CG)	22 µl
Index 18, A018	GTCCGC(AC)	22 µl
Index 19, A019	GTGAAA(CG)	22 µl

During library prep, make sure to note which indexed primer you are using with your sample and do not use the same indexed primer on two different samples you plan to multiplex together.

Swift 2S Turbo Combinatorial Dual Indexing Primer Kit (Cat. No. 48096)

During the Indexing PCR step, you must use a unique combination of index primers to label each library. Libraries made with uniquely indexed adapters may be multiplexed during cluster generation and co-sequenced on the same Illumina flow cell. The full-length adapter sequences where X is replaced by the indexed adapter sequences in the tables below are as follows:

P5 TruSeq Adapter:

5' AATGATACGGCGACCACCGAGATCTACACXXXXXXXXACACTCTTTCCCTACACGACGCTCTTCCGATCT 3'

P7 TruSeq Adapter:

5' GATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXXXXATCTCGTATGCCGTCTTCTGCTTG 3'

The number on the product tube label indicates which indexing primer is provided in the tube. Unique indexing primers in quantity ordered (see table below), which should be used where this manual calls for 2.5 µl of each Index Primer in the Indexing PCR step:

D5 Indexes*	Sequence	48096
D501	TATAGCCT	33 µl
D502	ATAGAGGC	33 µl
D503	CCTATCCT	33 µl
D504	GGCTCTGA	33 µl
D505	AGGCGAAG	33 µl
D506	TAATCTTA	33 µl
D507	CAGGACGT	33 µl
D508	GTACTGAC	33 µl

D7 Indexes	Sequence	48096
D701	ATTACTCG	22 µl
D702	TCCGGAGA	22 µl
D703	CGCTCATT	22 µl
D704	GAGATTCC	22 µl
D705	ATTCAGAA	22 µl
D706	GAATTCGT	22 µl
D707	CTGAAGCT	22 µl
D708	TAATGCGC	22 µl
D709	CGGCTATG	22 µl
D710	TCCGCGAA	22 µl
D711	TCTCGCGC	22 µl
D712	AGCGATAG	22 µl

**i5 bases shown are for sample sheet: NovaSeq, MiSeq, HiSeq 2500. i5 bases for sample sheet: iSeq, MiniSeq, NextSeq, HiSeq 4000 are the reverse complement (not shown).*

During library prep, make sure to note which indexing primers you are using with your sample and do not use the same indexing primers on two different samples you plan to multiplex together.

Swift 2S Turbo Set S1, S2, S3, S4, and Set S1-S4 Combinatorial Dual Indexing Primer Kit (Cat. No. 485192, 486192, 487192, 488192, and 489768)

Libraries made with dual indexing primers may be multiplexed during cluster generation and co-sequenced on the same Illumina flow cell. The full-length adapter sequences where X is replaced by the index sequences in the tables below are as follows:

P5 TruSeq Indexed Adapter:

5' AATGATACGGCGACCACCGAGATCTACACXXXXXXXXACACTCTTTCCCTACACGACGCTCTTCCGATCT 3'

P7 TruSeq Indexed Adapter:

5' GATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXXXXATCTCGTATGCCGTCTTCTGCTTG 3'

The number on the product tube label indicates which indexing primer is provided in the tube.

D5 Indexes*	Sequence	485192	486192	487192	488192	489768
Index D501	TATAGCCT	66 µl	66 µl	66 µl	66 µl	300 µl
Index D502	ATAGAGGC	66 µl	66 µl	66 µl	66 µl	300 µl
Index D503	CCTATCCT	66 µl	66 µl	66 µl	66 µl	300 µl
Index D504	GGCTCTGA	66 µl	66 µl	66 µl	66 µl	300 µl
Index D505	AGGCGAAG	66 µl	66 µl	66 µl	66 µl	300 µl
Index D506	TAATCTTA	66 µl	66 µl	66 µl	66 µl	300 µl
Index D507	CAGGACGT	66 µl	66 µl	66 µl	66 µl	300 µl
Index D508	GTACTGAC	66 µl	66 µl	66 µl	66 µl	300 µl

**i5 bases shown are for sample sheet: NovaSeq, MiSeq, HiSeq 2500. i5 bases for sample sheet: iSeq, MiniSeq, NextSeq, HiSeq 4000 are the reverse complement (not shown).*

Set S1 Indexes	Sequence	485192
Index S701	CAACACAG	22 µl
Index S702	ACACCTCA	22 µl
Index S703	ACCATAGG	22 µl
Index S704	CAGGTAAG	22 µl
Index S705	AACGCACA	22 µl
Index S706	TAGTCTCG	22 µl
Index S707	CAGTCACA	22 µl
Index S708	CCAACACT	22 µl
Index S709	ACATGCCA	22 µl
Index S710	ATTCCGCT	22 µl
Index S711	CAAGGTAC	22 µl
Index S712	CCATGAAC	22 µl
Index S713	TCAGCCTT	22 µl
Index S714	CAGTGCTT	22 µl
Index S715	CTCGAACA	22 µl
Index S716	ACAGTTCG	22 µl
Index S717	ATCCTTCC	22 µl
Index S718	CGAAGTCA	22 µl
Index S719	CTCTATCG	22 µl
Index S720	ACTCTCCA	22 µl

Set S1 Indexes	Sequence	485192
Index S721	TCCTCATG	22 µl
Index S722	AACAACCG	22 µl
Index S723	CTCGTTCT	22 µl
Index S724	TCAGTAGG	22 µl

Set S2 Indexes	Sequence	486192
Index S725	GCTTCACA	22 µl
Index S726	CGATGTTT	22 µl
Index S727	TTAGGCAT	22 µl
Index S728	ACAGTGGT	22 µl
Index S729	GCCAATGT	22 µl
Index S730	CAGATCTG	22 µl
Index S731	ACTTGATG	22 µl
Index S732	TAGCTTGT	22 µl
Index S733	TGGTTGTT	22 µl
Index S734	TGTACCTT	22 µl
Index S735	TCTGCTGT	22 µl
Index S736	TTGGAGGT	22 µl
Index S737	TCGAGCGT	22 µl
Index S738	TGATACGT	22 µl
Index S739	TGCATAGT	22 µl
Index S740	TGCGATCT	22 µl
Index S741	TTCCTGCT	22 µl
Index S742	TACAGGAT	22 µl
Index S743	TGTGGTTG	22 µl
Index S744	TTCCATTG	22 µl
Index S745	TAACGCTG	22 µl
Index S746	TTGGTATG	22 µl
Index S747	TGAACTGG	22 µl
Index S748	TACTTCGG	22 µl

Set S3 Indexes	Sequence	487192
Index S749	TCCAGTCG	22 µl
Index S750	TGTATGCG	22 µl
Index S751	TCATTGAG	22 µl
Index S752	TGGCTCAG	22 µl
Index S753	TATGCCAG	22 µl
Index S754	TCAGATTC	22 µl
Index S755	GGTTGGAC	22 µl
Index S756	GACACTTA	22 µl
Index S757	GCTATGGA	22 µl
Index S758	GTAACCGA	22 µl

Set S3 Indexes	Sequence	487192
Index S759	GGCAAGCA	22 µl
Index S760	GAACGACA	22 µl
Index S761	GCGTCGAA	22 µl
Index S762	AAGGCGAT	22 µl
Index S763	CAGGCATT	22 µl
Index S764	AACTGTAT	22 µl
Index S765	ATGCTTGA	22 µl
Index S766	AGTATCTG	22 µl
Index S767	ATGTAATG	22 µl
Index S768	ACACATGT	22 µl
Index S769	ATAGCACG	22 µl
Index S770	ATATTGTA	22 µl
Index S771	CAATTGAT	22 µl
Index S772	CACGTCGT	22 µl

Set S4 Indexes	Sequence	488192
Index S773	AGTCTGTA	22 µl
Index S774	CCGTATCT	22 µl
Index S775	CGCTTCCT	22 µl
Index S776	CAAGACCT	22 µl
Index S777	CCTAGTAT	22 µl
Index S778	CCACCGAT	22 µl
Index S779	CTATCATG	22 µl
Index S780	CATGAATG	22 µl
Index S781	CTGTACGG	22 µl
Index S782	CACTCGAG	22 µl
Index S783	CCGACAAG	22 µl
Index S784	CTTGCTTC	22 µl
Index S785	CGCCTTAT	22 µl
Index S786	GCAACCAT	22 µl
Index S787	TGACCGTT	22 µl
Index S788	TTGAGCTC	22 µl
Index S789	CCACATTG	22 µl
Index S790	AGCCAACT	22 µl
Index S791	ATCACGTT	22 µl
Index S792	TCTCGGTT	22 µl
Index S793	TTGACTCT	22 µl
Index S794	TCGAAGTG	22 µl
Index S795	CACCCAAA	22 µl
Index S796	CTTCACAT	22 µl

During library prep, make sure to note which indexing primers you are using with your sample and do not use the same indexed primers on two different samples you plan to multiplex together.

Swift 2S Turbo Unique Dual Indexing Primer Kit (Cat. No. 49096 and 490384)

During the Indexing PCR step, you must use a unique combination of index primers to label each library. Libraries made with unique indexing primers may be multiplexed during cluster generation and co-sequenced on the same Illumina flow cell. The full-length adapter sequences where X is replaced by the indexed adapter sequences in the tables below are as follows:

Index 1 (i7) Adapter:

5' GATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXXXXATCTCGTATGCCGTCTTCTGCTTG 3'

Index 2 (i5) Adapter:

5' AATGATACGGCGACCACCGAGATCTACACXXXXXXXXACACTCTTTCCCTACACGACGCTCTTCCGATCT 3'

The number on the product tube label indicates which indexing primers are provided in the tube. Unique indexing primers in quantity ordered (see table below), which should be used where this manual calls for 5 µl of each dual index primer mix in the Indexing PCR step.

UDI #	i7 Index Sequence	i5 Index Sequence*	49096	490384
U001	CAACACAG	CTTCACAT	22 µl	22 µl
U002	ACACCTCA	CACCCAAA	22 µl	22 µl
U003	ACCATAGG	TCGAAGTG	22 µl	22 µl
U004	CAGGTAAG	TTGACTCT	22 µl	22 µl
U005	AACGCACA	TCTCGGTT	22 µl	22 µl
U006	TAGTCTCG	ATCACGTT	22 µl	22 µl
U007	CAGTCACA	AGCCAACT	22 µl	22 µl
U008	CCAACACT	CCACATTG	22 µl	22 µl
U009	ACATGCCA	TTGAGCTC	22 µl	22 µl
U010	ATTCCGCT	TGACCGTT	22 µl	22 µl
U011	CAAGGTAC	GCAACCAT	22 µl	22 µl
U012	CCATGAAC	CGCCTTAT	22 µl	22 µl
U013	TCAGCCTT	CTTGCTTC	22 µl	22 µl
U014	CAGTGCTT	CCGACAAG	22 µl	22 µl
U015	CTCGAACA	CACTCGAG	22 µl	22 µl
U016	ACAGTTCG	CTGTACGG	22 µl	22 µl
U017	ATCCTTCC	CATGAATG	22 µl	22 µl
U018	CGAAGTCA	CTATCATG	22 µl	22 µl
U019	CTCTATCG	CCACCGAT	22 µl	22 µl
U020	ACTCTCCA	CCTAGTAT	22 µl	22 µl
U021	TCCTCATG	CAAGACCT	22 µl	22 µl
U022	AACAACCG	CGCTTCCT	22 µl	22 µl
U023	CTCGTTCT	CCGTATCT	22 µl	22 µl
U024	TCAGTAGG	AGTCTGTA	22 µl	22 µl
U025	GCTTCACA	CACGTCGT	–	22 µl
U026	CGATGTTT	CAATTGAT	–	22 µl
U027	TTAGGCAT	ATATTGTA	–	22 µl
U028	ACAGTGGT	ATAGCACG	–	22 µl
U029	GCCAATGT	ACACATGT	–	22 µl
U030	CAGATCTG	ATGTAATG	–	22 µl
U031	ACTTGATG	AGTATCTG	–	22 µl
U032	TAGCTTGT	ATGCTTGA	–	22 µl
U033	TGGTTGTT	AACTGTAT	–	22 µl
U034	TGTACCTT	CAGGCATT	–	22 µl
U035	TCTGCTGT	AAGGCGAT	–	22 µl
U036	TTGGAGGT	GCGTCGAA	–	22 µl
U037	TCGAGCGT	GAACGACA	–	22 µl
U038	TGATACGT	GGCAAGCA	–	22 µl
U039	TGCATAGT	GTAACCGA	–	22 µl
U040	TGCGATCT	GCTATGGA	–	22 µl
U041	TTCCTGCT	GACACTTA	–	22 µl
U042	TACAGGAT	GGTTGGAC	–	22 µl
U043	TGTGGTTG	TCAGATTC	–	22 µl
U044	TTCCATTG	TATGCCAG	–	22 µl
U045	TAACGCTG	TGGCTCAG	–	22 µl
U046	TTGGTATG	TCATTGAG	–	22 µl
U047	TGAACTGG	TGTATGCG	–	22 µl
U048	TACTTCGG	TCCAGTCG	–	22 µl
U049	TCCAGTCG	TACTTCGG	–	22 µl
U050	TGTATGCG	TGAACTGG	–	22 µl
U051	TCATTGAG	TTGGTATG	–	22 µl
U052	TGGCTCAG	TAACGCTG	–	22 µl
U053	TATGCCAG	TTCCATTG	–	22 µl
U054	TCAGATTC	TGTGGTTG	–	22 µl
U055	GGTTGGAC	TACAGGAT	–	22 µl
U056	GACACTTA	TTCCTGCT	–	22 µl

UDI #	i7 Index Sequence	i5 Index Sequence*	49096	490384
U057	GCTATGGA	TGCGATCT	—	22 µl
U058	GTAACCGA	TGCATAGT	—	22 µl
U059	GGCAAGCA	TGATACGT	—	22 µl
U060	GAACGACA	TCGAGCGT	—	22 µl
U061	GCGTCGAA	TTGGAGGT	—	22 µl
U062	AAGGCGAT	TCTGCTGT	—	22 µl
U063	CAGGCATT	TGTACCTT	—	22 µl
U064	AACTGTAT	TGGTTGTT	—	22 µl
U065	ATGCTTGA	TAGCTTGT	—	22 µl
U066	AGTATCTG	ACTTGATG	—	22 µl
U067	ATGTAATG	CAGATCTG	—	22 µl
U068	ACACATGT	GCCAATGT	—	22 µl
U069	ATAGCACG	ACAGTGGT	—	22 µl
U070	ATATTGTA	TTAGGCAT	—	22 µl
U071	CAATTGAT	CGATGTTT	—	22 µl
U072	CACGTCGT	GCTTCACA	—	22 µl
U073	AGTCTGTA	TCAGTAGG	—	22 µl
U074	CCGTATCT	CTCGTTCT	—	22 µl
U075	CGCTTCCT	AACAACCG	—	22 µl
U076	CAAGACCT	TCCTCATG	—	22 µl
U077	CCTAGTAT	ACTCTCCA	—	22 µl
U078	CCACCGAT	CTCTATCG	—	22 µl
U079	CTATCATG	CGAAGTCA	—	22 µl
U080	CATGAATG	ATCCTTCC	—	22 µl
U081	CTGTACGG	ACAGTTCG	—	22 µl
U082	CACTCGAG	CTCGAACA	—	22 µl
U083	CCGACAAG	CAGTGCTT	—	22 µl
U084	CTTGCTTC	TCAGCCTT	—	22 µl
U085	CGCCTTAT	CCATGAAC	—	22 µl
U086	GCAACCAT	CAAGGTAC	—	22 µl
U087	TGACCGTT	ATTCCGCT	—	22 µl
U088	TTGAGCTC	ACATGCCA	—	22 µl
U089	CCACATTG	CCAACACT	—	22 µl
U090	AGCCAACT	CAGTCACA	—	22 µl
U091	ATCACGTT	TAGTCTCG	—	22 µl
U092	TCTCGGTT	AACGCACA	—	22 µl
U093	TTGACTCT	CAGGTAAG	—	22 µl
U094	TCGAAGTG	ACCATAGG	—	22 µl
U095	CACCCAAA	ACACCTCA	—	22 µl
U096	CTTCACAT	CAACACAG	—	22 µl

**i5 bases shown are for sample sheet: NovaSeq, MiSeq, HiSeq 2500. i5 bases for sample sheet: iSeq, MiniSeq, NextSeq, HiSeq 4000 are the reverse complement (not shown).*

Section C: 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.
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).

Notes

General Warranty

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does not constitute a warranty that the products will conform to the sample or model.

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