Swift BIOSCIENCES™

Protocol

Accel-NGS® 2S Hyb DNA Library Kit

NGS Prep for Hybridization Capture

Cat. No. 23024/23096

Contents

Introduction

The Accel-NGS 2S Hyb DNA Library Kit designed for Illumina® platforms enables the preparation of high complexity next-generation sequencing (NGS) libraries from double-stranded DNA. This kit is designed for users performing hybridization capture following library construction. The Accel-NGS 2S Hyb Kit utilizes Illumina-compatible adapter sequences and has been validated on the MiSeq® and HiSeq® 2500 platforms.

The Accel-NGS 2S Hyb DNA Library Kit is suitable for the following applications:

Applications
Hybridization Capture • Agilent SureSelect^{XT} and SureSelect^{XT2}* • NimbleGen™ SeqCap™ EZ • IDT xGen® Lockdown® Probes† • Other hybridization capture technologies, please contact Technical Support for details.

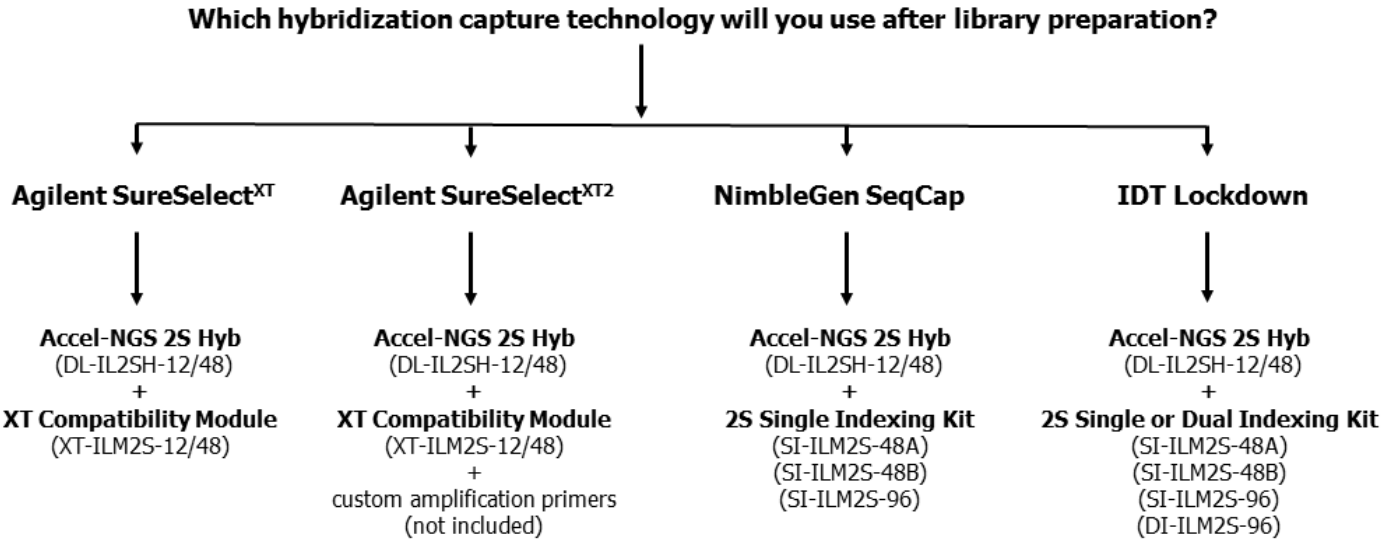
*For compatibility with SureSelect^{XT2} target enrichment, custom amplification primers are also required. Please see the SureSelect^{XT} and SureSelect^{XT2} Hybridization Capture Compatibility with the Accel-NGS 2S Hyb Library Kit Technical Note for details.

†xGen Lockdown capture does not recommend a library insert size. This kit has been validated with a 200 bp insert size.

For pre-hybridization amplification, please use the amplification primers supplied in the Accel-NGS 2S Hyb Kit with the polymerase recommended with the hybridization capture reagents.

Our Technical Support team may be reached at TechnicalSupport@Swiftbiosci.com or by calling 734.330.2568 and pressing 2 when prompted.

To determine the appropriate Accel-NGS 2S Hyb Indexing Adapter Kit for your hybridization capture application, please consult the decision tree below:



Experienced User Protocol

Repair I (page 7)

Transfer sample to 0.2 ml PCR tube	
Low EDTA TE	13 µl
Buffer W1	6 µl
Enzyme W2	1 µl
Reaction Mix	**20 µl**
Sample	40 µl
Total	**60 µl**

Thermocycler Conditions (cfDNA)
37 °C for 5 minutes, lid heating ON
65 °C for 2 minutes, lid heating ON
37 °C for 5 minutes, lid heating ON

Thermocycler Conditions (All Other Inputs)
37 °C for 10 minutes, lid heating OFF

Post-Repair I SPRI (page 7)

Application	Insert Size	Sample Volume	Bead Volume	PEG NaCl Volume
SureSelect^{XT}	150 bp	60 µl	108 µl (ratio: 1.8)	---
SureSelect^{XT2}				
xGen Lockdown	200 bp*			
SeqCap EZ				

*xGen Lockdown capture does not recommend a library insert size. This kit has been validated with a 200 bp insert size.

Repair II (page 8)

Low EDTA TE	30 µl
Buffer G1	5 µl
Reagent G2	13 µl
Enzyme G3	1 µl
Enzyme G4	1 µl
Reaction Mix	**50 µl**
Sample	beads
Total	**50 µl**

Thermocycler Conditions
20 °C for 20 minutes, lid heating OFF

Post-Repair II SPRI (page 8)

Application	Insert Size	Sample Volume	Bead Volume	PEG NaCl Volume
SureSelect^{XT}	150 bp	50 µl	---	82.5 µl (ratio:1.65)
SureSelect^{XT2}				
xGen Lockdown	200 bp			
NimbleGen				

Ligation I (page 8)

Low EDTA TE	20 µl
Buffer Y1	3 µl
Enzyme Y3	2 µl
Reaction Mix	**25 µl**
Reagent Y2*† -OR- Reagent Y-XT‡	5 µl
Sample	beads
Total	**30 µl**

Thermocycler Conditions
25 °C for 15 minutes, lid heating OFF

*For single indexing, Reagent Y2 is the indexed adapter and, if multiplexing, should be added individually to each sample.
†For dual indexing, Reagent Y2 is the truncated adapter and can be added to the reaction mix.
‡For SureSelect, Reagent Y-XT is the truncated SureSelect-compatible adapter and can be added to the reaction mix.

Post-Ligation I SPRI (page 9)

Application	Insert Size	Sample Volume	Bead Volume	PEG NaCl Volume
SureSelect^{XT} SureSelect^{XT2}	150 bp	30 µl	---	49.5 µl (ratio: 1.65)
xGen Lockdown NimbleGen	200 bp			

Ligation II (page 9)

Low EDTA TE	30 µl
Buffer B1	5 µl
Reagent B2	2 µl
Reagent B3	9 µl
Enzyme B4	1 µl
Enzyme B5	2 µl
Enzyme B6	1 µl
Reaction Mix	**50 µl**
Sample	beads
Total	**50 µl**

Thermocycler Conditions
40 °C for 10 minutes, lid heating OFF
25 °C hold

Post-Ligation II SPRI (page 9)

Application	Insert Size	Sample Volume	Bead Volume	PEG NaCl Volume	Elution Volume
SureSelect^{XT} SureSelect^{XT2}	150 bp	50 µl	---	82.5 µl (ratio: 1.65)	20 µl
xGen Lockdown NimbleGen	200 bp				

Pre-Hybridization PCR – Library Amplification (page 10)

Final libraries should be amplified to obtain adequate input for the hybridization capture. Please use the following PCR primers – Reagent R-XT (for SureSelect^{XT}), custom amplification primers (for SureSelect^{XT2}), Reagent R1 (for single indexing), or Index D50X/Index D7XX (for dual indexing) – with the polymerase recommended with the hybridization capture reagents.

A detailed description of the PCR primers can be found on page 10.

Post-PCR SPRI (page 10)

Application	Insert Size	Sample Volume	Bead Volume	PEG NaCl Volume	Elution Volume
SureSelect^{XT} SureSelect^{XT2}	150 bp	50 µl	90 µl (ratio: 1.8)	---	25 µl of H_2O*
xGen Lockdown NimbleGen	200 bp				

***It is important to elute in water to prevent residual EDTA from interfering with downstream applications.**

Before You Start

- Upon receipt, store the Accel-NGS 2S Hyb DNA Library Kit at -20 ºC.
- Please read this manual carefully before starting.

Kit Contents

Kit contains enough reagents for the preparation of either 24 or 96 libraries (10% excess volume provided).

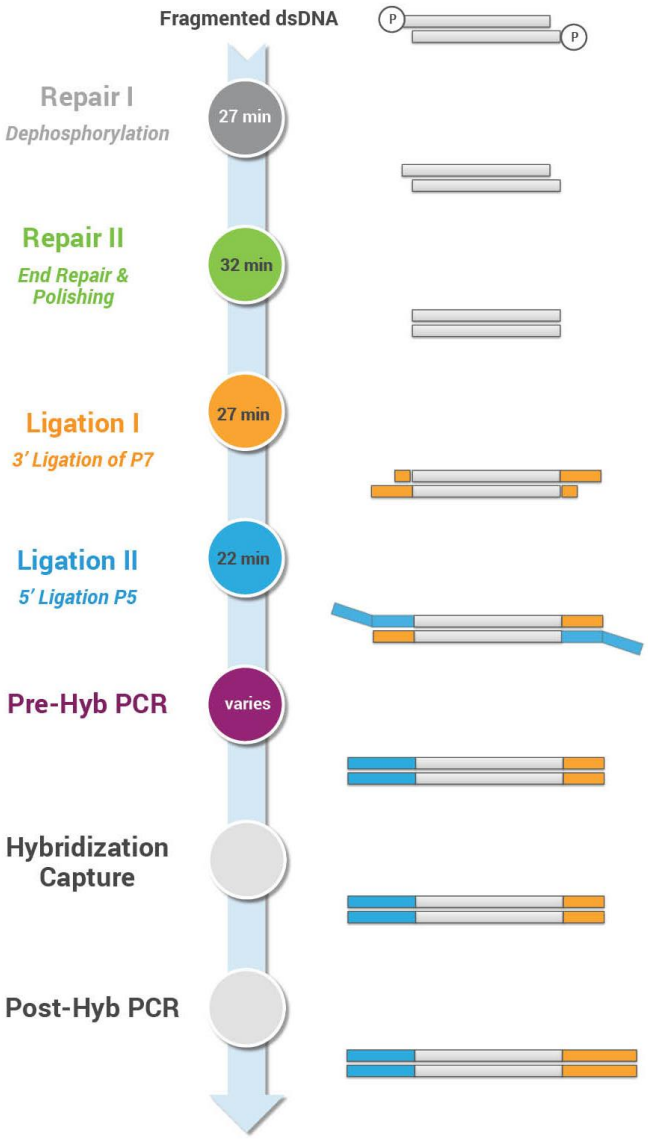
Kit	Reagents	24 Reactions	96 Reactions	Kit	Reagents	24 Reactions	96 Reactions
Repair I Reagents	Buffer W1	158 µl	634 µl	Ligation I Reagents	Buffer Y1	80 µl	316 µl
	Enzyme W2	26 µl	106 µl		Reagent Y2* or Reagent Y-XT *	-	-
Repair II Reagents	Buffer G1	132 µl	528 µl			-	-
	Reagent G2	344 µl	1374 µl		Enzyme Y3	52 µl	212 µl
	Enzyme G3	26 µl	106 µl	Ligation II Reagents	Buffer B1	132 µl	528 µl
	Enzyme G4	26 µl	106 µl		Reagent B2*	-	-
Pre-Hyb PCR Reagents	Reagent R1* or Reagent R-XT* or Index D50X/D7XX*	-	-		Reagent B3	238 µl	950 µl
		-	-		Enzyme B4	26 µl	106 µl
		-	-		Enzyme B5	52 µl	212 µl
		-	-		Enzyme B6	26 µl	106 µl

*Reagent Y2 or Y-XT (the adapter); Reagent R-XT, Reagent R1, or Index D50X/D7XX (the PCR primer mix); and Reagent B2 (the adapter) are provided separately in one of the available Accel-NGS 2S Indexing Adapter Kits (see Appendix).

Required Materials Not Supplied

- A compatible Accel-NGS 2S Indexing Adapter Kit (Reagents Y2 or Y-XT; R-XT, R1, or Index D50X/D7XX; and B2) (see Appendix)
- PEG-8000 (Sigma Aldrich Cat. No. P5413) and NaCl (JT Baker Cat. No. 3624-19) (see Appendix)
- DNA Suspension Buffer (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA, pH 8.0) (Teknova Cat. No. T0227)
- 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®
- Qubit® or other fluorometric method for determining dsDNA and library concentration
- Bioanalyzer or other electrophoretic device for assessing library size and quantity
- Method for fragmentation of input DNA by mechanical shearing or enzymatic shearing
- Microfuge
- Programmable thermocycler
- 0.2 ml PCR tubes
- 1.7 ml low retention microfuge tubes
- Aerosol-resistant, low retention tips and 2 to 1000 µl pipettes
- 200-proof/absolute ethanol (molecular biology grade)
- Nuclease-free water (molecular biology grade)

Protocol Overview



- Using four incubations, this protocol repairs both 5' and 3' termini and sequentially attaches Illumina adapter sequences to the ends of fragmented dsDNA.
- Bead-based SPRI clean-ups are used to remove oligonucleotides and small fragments, and to change enzymatic buffer composition between steps.
- The Pre-Hybridization PCR step increases library yield. For pre-hybridization amplification, please use the amplification primers supplied in the Accel-NGS 2S Hyb Kit with the polymerase recommended with the hybridization capture reagents.

Notes on Starting Input Material

- Consider genome complexity and sample quality when choosing input DNA quantity. Although libraries may be successfully prepared from ultra-low inputs, reduced representation of genome complexity may occur.
- To reduce the risk of DNA and library contamination, particularly at ultra-low input:
  1. Physically separate the laboratory space, equipment, and supplies where pre-PCR and post-PCR processes are performed.
  2. Clean lab areas using 0.5% sodium hypochlorite (10% bleach).
  3. Use specialty barrier pipette tips to avoid exposure to potential contaminants.
- Starting material should be in 1-40 µl of Low EDTA TE buffer. Contact TechnicalSupport@swiftbiosci.com if you would like to work with larger volumes.
- For best results, it is recommended to determine dsDNA concentration using Qubit, or a similar fluorometric method, as it will more accurately represent the double-stranded, adaptable DNA content of your sample. For low quality samples including FFPE, it is important to use a quality control metric to analyze DNA integrity and purity. A qPCR sample QC assay will provide the best results as it will quantify the usable amount of DNA in the sample. Several kits are commercially available. If you have questions related to FFPE sample quality, please contact TechnicalSupport@swiftbiosci.com.

Prepare the Library

For best results, please follow these recommendations:

- To maximize efficient use of enzyme reagents, remove enzyme tubes from -20 °C storage and place on ice, **NOT in a cryocooler**, for at least 10 minutes to allow reagents to reach 4 °C prior to pipetting. **Attempting to pipette enzymes at -20 °C may result in a shortage of enzyme reagents.**
- After thawing reagents, briefly vortex (except enzymes) to mix them well. Spin tubes in a microfuge to collect contents prior to opening.
- Before starting, prepare a fresh 80% ethanol solution using 200-proof/absolute ethanol and nuclease-free water (approximately 2.0 ml will be used per sample).
- This "with bead" protocol utilizes a PEG NaCl solution in Post-Repair II, Post-Ligation I, and Post-Ligation II SPRI Steps to bind DNA to SPRIselect beads already in the tube rather than adding fresh SPRIselect beads for each step. PEG NaCl solution can be used from Swift Biosciences (Cat. No. 90148). Alternatively, see the Appendix for protocol to prepare a solution of 20% polyethylene glycol (PEG-8000) and 2.5 M NaCl.
- Assemble reagent master mixes for the Repair I, Repair II, Ligation I, Ligation II, and Pre-Hyb PCR steps **ON ICE** and scale volumes as appropriate, using 5% excess volume to compensate for pipetting loss. **Add the reagents in the specified order.**

DNA Fragmentation

1. If necessary, fragment the DNA. Multiple fragmentation methods are available; this kit has specifically been validated on Covaris®-fragmented DNA. However, DNA fragmented by enzymatic means is also suitable for processing with the Accel-NGS 2S Hyb Kit. Consult the Enzymatic Fragmentation of Input DNA Technical Note at www.swiftbiosci.com.

Repair I

1. Transfer the fragmented DNA sample to a 0.2 ml PCR tube and adjust the volume of the sample to 40 µl using Low EDTA TE, if necessary.
2. Add 20 µl of the pre-mixed Repair I Reaction Mix to each PCR tube containing the 40 µl DNA sample. Mix by pipetting. Place in the thermocycler and run the Repair I Thermocycler Program.

Reagent	Volume (1 Reaction)
Low EDTA TE	13 µl
Buffer W1	6 µl
Enzyme W2	1 µl
Reaction Mix	**20 µl**
Sample	40 µl
Total	**60 µl**

Repair I Thermocycler Program (cfDNA)
37 °C for 5 minutes, lid heating ON
65 °C for 2 minutes, lid heating ON
37 °C for 5 minutes, lid heating ON

Repair I Thermocycler Program (All Other Inputs)
37 °C for 10 minutes, lid heating OFF*

***Alternatively, the thermocycler lid may be left open.**

Post-Repair I SPRI

1. Clean up the Repair I Reaction using SPRIselect beads (refer to Appendix) and freshly prepared 80% ethanol.

Application	Insert Size	Sample Volume	Bead Volume	PEG NaCl Volume
SureSelect^{XT}	150 bp	60 µl	108 µl (ratio: 1.8)	---
SureSelect^{XT2}				
xGen Lockdown	200 bp*			
SeqCap EZ				

***xGen Lockdown capture does not recommend a library insert size. This kit has been validated with a 200 bp insert size.**

Repair II

1. Add 50 µl of the pre-mixed Repair II Reaction Mix to the beads for each sample and resuspend by pipetting.
2. Place in the thermocycler and run the Repair II Thermocycler Program.

Reagent	Volume (1 Reaction)
Low EDTA TE	30 µl
Buffer G1	5 µl
Reagent G2	13 µl
Enzyme G3	1 µl
Enzyme G4	1 µl
Reaction Mix	**50 µl**
Sample	beads
Total	**50 µl**

Repair II Thermocycler Program
20 °C for 20 minutes, lid heating OFF*

***Alternatively, the thermocycler lid may be left open.**

Post-Repair II SPRI

1. Clean up the Repair II Reaction using PEG NaCl solution (refer to Appendix) and freshly prepared 80% ethanol.

Application	Insert Size	Sample Volume	Bead Volume	PEG NaCl Volume
SureSelect^{XT}	150 bp	50 µl	---	82.5 µl (ratio: 1.65)
SureSelect^{XT2}				
xGen Lockdown	200 bp			
SeqCap EZ				

Ligation I

1. For single indexing, add 25 µl of the pre-mixed Ligation I Reaction Mix to the beads for each sample. Then add 5 µl of the appropriate indexed Reagent Y2 to each sample, and resuspend by pipetting.
2. For dual indexing and SureSelect, 5 µl of Reagent Y2 or Reagent Y-XT can be added to the reaction mix rather than each sample individually. Add 30 µl of the pre-mixed Ligation I Reaction Mix to the beads for each sample and resuspend by pipetting.
3. The final reaction volume for each sample is 30 µl. Place in the thermocycler and run the Ligation I Thermocycler Program.

Reagent	Volume (1 Reaction)
Low EDTA TE	20 µl
Buffer Y1	3 µl
Enzyme Y3	2 µl
Reaction Mix	**25 µl**
Sample	beads
Reagent Y2*† -OR- Reagent Y-XT‡	5 µl
Total	**30 µl**

Ligation I Thermocycler Program
25 °C for 15 minutes, lid heating OFF*

***Alternatively, the thermocycler lid may be left open.**

***For single indexing, Reagent Y2 is the indexed adapter and, if multiplexing, should be added individually to each sample.**
†For dual indexing, Reagent Y2 is the truncated adapter and can be added to the reaction mix.
‡For SureSelect, Reagent Y-XT is the truncated SureSelect-compatible adapter and can be added to the reaction mix.

Post-Ligation I SPRI

1. Clean up the Ligation I Reaction using PEG NaCl solution (refer to Appendix) and freshly prepared 80% ethanol.

Application	Insert Size	Sample Volume	Bead Volume	PEG NaCl Volume
SureSelect^{XT}	150 bp	30 µl	---	49.5 µl (ratio: 1.65)
SureSelect^{XT2}				
xGen Lockdown	200 bp			
SeqCap EZ				

Ligation II

1. Add 50 µl of the pre-mixed Ligation II Reaction Mix to the beads for each sample and resuspend by pipetting.
2. Place in the thermocycler and run the Ligation II Thermocycler Program.
3. Proceed immediately to Post-Ligation II SPRI.

Reagent	Volume (1 Reaction)
Low EDTA TE	30 µl
Buffer B1	5 µl
Reagent B2*	2 µl
Reagent B3	9 µl
Enzyme B4	1 µl
Enzyme B5	2 µl
Enzyme B6	1 µl
Reaction Mix	**50 µl**
Sample	Beads
Total	**50 µl**

***Reagent B2 (the adapter) is provided separately in the Indexed Adapter kit.**

Ligation II Thermocycler Program
40 °C for 10 minutes, lid heating OFF*
25 °C hold

***Alternatively, the thermocycler lid may be left open.**

Post-Ligation II SPRI

1. Clean up the Ligation II Reaction using PEG NaCl solution (refer to Appendix) and freshly prepared 80% ethanol.

Application	Insert Size	Sample Volume	Bead Volume	PEG NaCl Volume	Elution Volume
SureSelect^{XT}	150 bp	50 µl	---	82.5 µl (ratio: 1.65)	20 µl
SureSelect^{XT2}					
xGen Lockdown	200 bp				
SeqCap EZ					

2. At the end of the SPRI clean-up, resuspend the beads in 20 µl of Low EDTA TE buffer.
3. Put on the magnet.
4. Carefully transfer the supernatant to a clean tube without carrying any beads.

Store freshly prepared libraries at 4 °C (or long term at -20 °C).

Pre-Hybridization PCR – Library Amplification

1. Final libraries should be amplified to obtain adequate input for the hybridization capture. Please use the PCR primers provided separately as part of the Indexing Kit with the polymerase recommended with the hybridization capture reagents (see the decision tree on page 1). The PCR primers provided are at a concentration of 6 µM.

   For SureSelect^{XT}:

   Reagent R-XT contains the following PCR primers:

   Primer 1: 5'-GTGACTGGAGTTCAGACGTG-3'
   Primer 2: 5'-AATGATACGGCGACCACCGAGATC-3'

   For SureSelect^{XT2}:

   Custom amplification primers should be designed as follows and used at 600 nM (final concentration in the PCR reaction):

   Primer 1: 5'-AATGATACGGCGACCACCGAGATC-3'
   Primer 2: 5'-CAAGCAGAAGACGGCATACGAGAT**XXXXXXXX**GTGACTGGAGTTCAGACGTG-3'

   where **XXXXXXXX** indicates the 8 bp index sequence. Please see the SureSelect^{XT} and SureSelect^{XT2} Hybridization Capture Compatibility with the Accel-NGS 2S Hyb Library Kit Technical Note for details.

   For xGen Lockdown and SeqCap EZ:

   Reagent R1 for single indexing contains the following PCR primers:

   Primer 1: 5'-AATGATACGGCGACCACCGAGATC-3'
   Primer 2: 5'-CAAGCAGAAGACGGCATACGA-3'

   For xGen Lockdown only:

   Index D50X/D7XX for dual indexing contains the following PCR primers:

   Primer 1: 5'-AATGATACGGCGACCACCGAGATCTACAC**XXXXXXXX**ACACTCTTTCCCTACACGAC-3'
   Primer 2: 5'-CAAGCAGAAGACGGCATACGAGAT**XXXXXXXX**GTGACTGGAGTTCAGACGTG-3'

   where **XXXXXXXX** indicates the 8 bp index sequence. Please see the Appendix for details.

Contact TechnicalSupport@swiftbiosci.com with any questions relating to library amplification.

Post-PCR SPRI

1. Clean up the **PCR Reaction** using SPRIselect beads (refer to Appendix) and freshly prepared 80% ethanol.

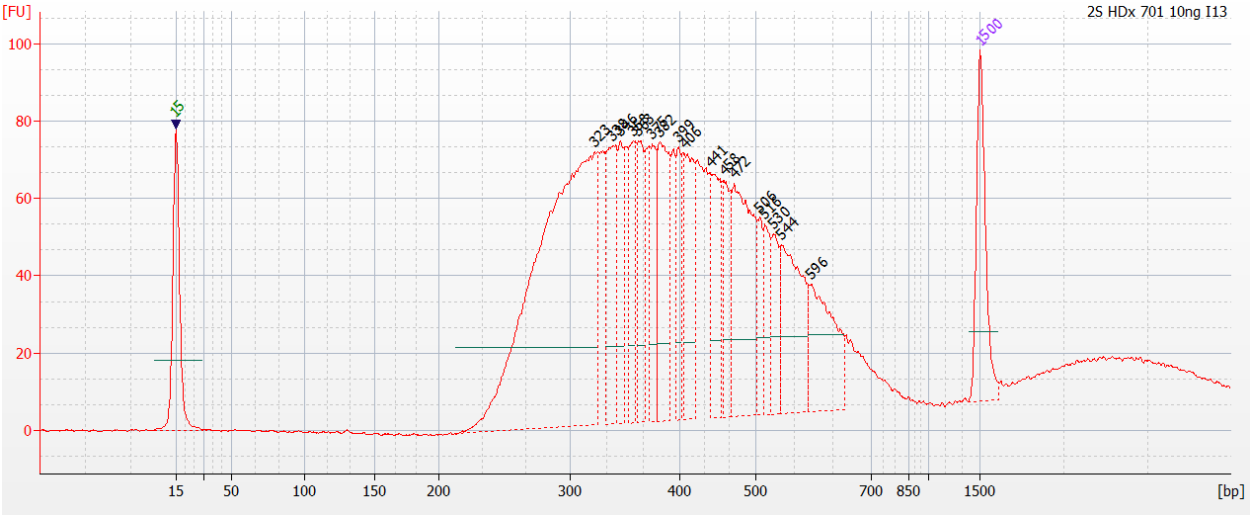
Application	Insert Size	Sample Volume	Bead Volume	PEG NaCl Volume	Elution Volume
SureSelect^{XT}	150 bp	50 µl	90 µl (ratio: 1.8)	---	25 µl of H_2O*
SureSelect^{XT2}					
xGen Lockdown	200 bp				
SeqCap EZ					

***It is important to elute in water to prevent residual EDTA from interfering with downstream applications.**

Store freshly prepared libraries at 4 °C (or long term at -20 °C). The library is now ready for quantification and assessment of size and purity. Please follow instructions provided with the hybridization reagents of choice.

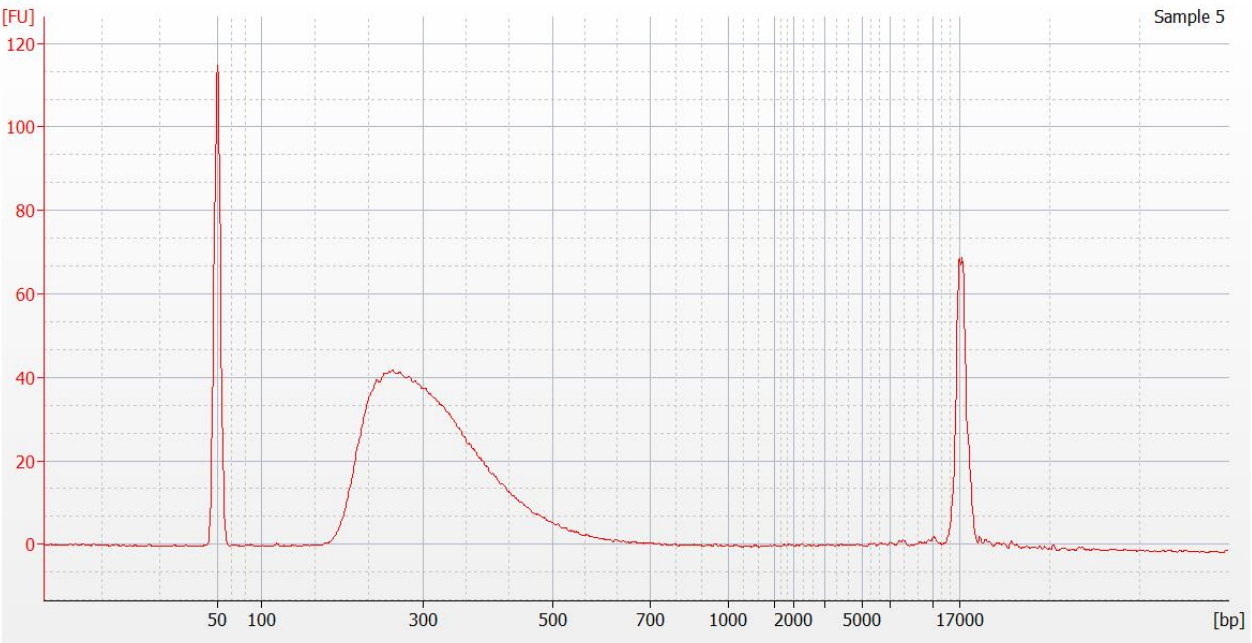
Expected Results

Agilent DNA 1000 Bioanalyzer Trace of xGen Lockdown/SeqCap EZ Library Prepared from Horizon Discovery Reference Standard HD701, 200 bp fragment size.



It is normal and expected to observe a small peak of library migrating at >1500 bp, as these are abnormally migrating heteroduplex structures resulting from the number of PCR cycles needed to achieve the required input for the hybridization capture.

Agilent DNA 1000 Bioanalyzer Trace of a SureSelect^{XT}-ready Library Prepared from Coriell Human Genomic DNA (NA12878), 150 bp fragment size.



Appendix

SPRIselect Clean-Up Protocol

Use the following protocol for each SPRI Step, substituting in the correct **Bead Volume**, **PEG NaCl Volume,** and **Elution Volume,** as indicated in the table for each step. For instructions on preparation of PEG NaCl solution, see Page 13:

1. Ensure beads are at room temperature and briefly vortex beads to homogenize the suspension before use.
2. Add the specified **Bead Volume** of beads or **PEG NaCl Volume** to each sample. Mix by vortexing. Pulse-spin the samples in a microfuge.
3. Incubate the samples for 5 minutes at room temperature.
4. Place the sample tubes on a magnetic rack until the solution clears and a pellet is formed (≈ 5 minutes).
5. Remove and discard the supernatant without disturbing the pellet (< 5 µl may be left behind).
6. Add 180 µl of freshly prepared 80% ethanol solution to the pellet while it is still on the magnet. Use care not to disturb the pellet. Incubate for 30 seconds and then carefully remove the ethanol solution.
7. Repeat step 6 once for a second wash with the ethanol solution.
8. Pulse-spin the samples in a microfuge, place back onto the magnet, and remove any residual ethanol solution from the bottom of the tube.
9. Air-dry the pellet, watching the pellet to avoid cracking or over-drying.
10. Add the specified volume of Reaction Mix after Post-Repair I, Post-Repair II, and Post-Ligation I SPRI steps; or the **Elution Volume** of Low EDTA TE after the Post-Ligation II SPRI step; or water after the Post-PCR SPRI step and resuspend the pellet, mixing well by pipetting up and down until homogenous.

Post-Repair I SPRI

Application	Insert Size	Sample Volume	Bead Volume	PEG NaCl Volume
SureSelect^{XT}	150 bp	60 µl	108 µl (ratio: 1.8)	---
SureSelect^{XT2}				
xGen Lockdown	200 bp			
SeqCap EZ				

Post-Repair II SPRI

Application	Insert Size	Sample Volume	Bead Volume	PEG NaCl Volume
SureSelect^{XT}	150 bp	50 µl	---	82.5 µl (ratio:1.65)
SureSelect^{XT2}				
xGen Lockdown	200 bp			
SeqCap EZ				

Post-Ligation I SPRI

Application	Insert Size	Sample Volume	Bead Volume	PEG NaCl Volume
SureSelect^{XT}	150 bp	30 µl	---	49.5 µl (ratio: 1.65)
SureSelect^{XT2}				
xGen Lockdown	200 bp			
SeqCap EZ				

Post-Ligation II SPRI

Application	Insert Size	Sample Volume	Bead Volume	PEG NaCl Volume	Elution Volume
SureSelect^{XT}	150 bp	50 µl	---	82.5 µl (ratio: 1.65)	20 µl
SureSelect^{XT2}					
xGen Lockdown	200 bp				
SeqCap EZ					

Post-PCR SPRI Step

Application	Insert Size	Sample Volume	Bead Volume	PEG NaCl Volume	Elution Volume
SureSelect^{XT}	150 bp	50 µl	90 µl (ratio: 1.8)	---	25 µl H_2O*
SureSelect^{XT2}					
xGen Lockdown	200 bp				
SeqCap EZ					

***It is important to elute in water to prevent residual EDTA from interfering with downstream applications.**

Preparation of PEG NaCl Solution

Add 10 g of PEG-8000 (Sigma-Aldrich Cat. No. P5413) and 7.3 g of NaCl to a 50 ml conical tube. Add 20-25 ml of ultrapure water and mix. When completely dissolved, transfer the solution to a graduated cylinder and adjust the volume to 50 ml with ultrapure water. Carefully prepare this solution. Improper ratios of PEG and NaCl in this solution could affect the overall insert size and percentage of adapter dimers seen in your sequencing data.

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.	• Perform the minimum number of PCR cycles necessary to avoid over-amplification.
DNA does not fragment properly: broad or lop-sided (high molecular weight) sonication profile of fragmented DNA.	Impure DNA or fragmentation device malfunction.	• Isopropanol purification, bead clean-up, column purification, or other method before fragmentation. • Ensure fragmentation device is functioning within manufacturer's parameters.
Incomplete resuspension of beads after ethanol wash during SPRI steps.	Over-drying of beads.	• Continue pipetting the liquid over the beads to break up clumps for complete resuspension.
Shortage of enzyme reagents.	Pipetting enzymes at -20 °C instead of 0-4 °C.	• Allow enzyme reagents to equilibrate to 0-4 °C for 10 minutes prior to pipetting.
Retention of liquid in pipette tip.	Viscous reagents may stick to pipette tip, especially for non-low retention tips.	• Pipette up and down several times to ensure all liquid and/or beads are released from the pipette tip.

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

Indexing Kit Contents

For single indexing, during **Ligation I** in the protocol, you must use a unique indexed adapter (Reagent Y2) 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.

Set A Adapters	Sequence*	26148	26248	26396
Reagent Y2 (I2)	CGATGT(A)	22 µl	-	22 µl
Reagent Y2 (I4)	TGACCA(A)	22 µl	-	22 µl
Reagent Y2 (I5)	ACAGTG(A)	22 µl	-	22 µl
Reagent Y2 (I6)	GCCAAT(A)	22 µl	-	22 µl
Reagent Y2 (I7)	CAGATC(A)	22 µl	-	22 µl
Reagent Y2 (I12)	CTTGTA(A)	22 µl	-	22 µl
Reagent Y2 (I13)	AGTCAA(C)	22 µl	-	22 µl
Reagent Y2 (I14)	AGTTCC(G)	22 µl	-	22 µl
Reagent Y2 (I15)	ATGTCA(G)	22 µl	-	22 µl
Reagent Y2 (I16)	CCGTCC(C)	22 µl	-	22 µl
Reagent Y2 (I18)	GTCCGC(A)	22 µl	-	22 µl
Reagent Y2 (I19)	GTGAAA(C)	22 µl	-	22 µl

Set B Adapters	Sequence*	26148	26248	26396
Reagent Y2 (I1)	ATCACG(A)	-	22 µl	22 µl
Reagent Y2 (I3)	TTAGGC(A)	-	22 µl	22 µl
Reagent Y2 (I8)	ACTTGA(A)	-	22 µl	22 µl
Reagent Y2 (I9)	GATCAG(A)	-	22 µl	22 µl
Reagent Y2 (I10)	TAGCTT(A)	-	22 µl	22 µl
Reagent Y2 (I11)	GGCTAC(A)	-	22 µl	22 µl
Reagent Y2 (I20)	GTGGCC(T)	-	22 µl	22 µl
Reagent Y2 (I21)	GTTTCG(G)	-	22 µl	22 µl
Reagent Y2 (I22)	CGTACG(T)	-	22 µl	22 µl
Reagent Y2 (I23)	GAGTGG(A)	-	22 µl	22 µl
Reagent Y2 (I25)	ACTGAT(A)	-	22 µl	22 µl
Reagent Y2 (I27)	ATTCCT(T)	-	22 µl	22 µl

Reagents	26148	26248	26396
Reagent B2	106 µl	106 µl	212 µl
Reagent R1	264 µl	264 µl	528 µl

* The base in parentheses is read during a seventh cycle but is not considered part of the index sequence.

The number on the product tube label indicates which indexed adapter is provided in the tube. During library prep, make sure to note which indexed adapter you are using with your sample and do not use the same indexed adapter on two different samples you plan to multiplex together.

For SureSelect^{XT} and SureSelect^{XT2}, during **Ligation I** in the protocol, you must use a truncated adapter (Reagent Y-XT) for each library. SureSelect^{XT} libraries will be labeled with an index sequence in the Post-Hybridization Capture Amplification Step with primers supplied by Agilent. SureSelect^{XT2} libraries will be labeled with an index sequence in the **Pre-Hybridization PCR Step** with custom primers designed and purchased separately by the user. Please see the SureSelect^{XT} & SureSelect^{XT2} Hybridization Capture Compatibility with Accel-NGS 2S Hyb Library Kit Technical Note for details. Libraries made with uniquely indexed adapters may be multiplexed during cluster generation and co-sequenced on the same Illumina flow cell.

XT Compatibilty Module	26424	26496
Reagent Y-XT	66 µl	264 µl
Reagent R-XT	66 µl	264 µl
Reagents	**26424**	**26496**
Reagent B2	27 µl	106 µl

For dual indexing, during **Pre-Hybridization PCR** in the protocol, 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.

D5 Adapters	Sequence	28096
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 Adapters	**Sequence**	**28096**
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
Reagents		**28096**
Reagent Y2		528 µl
Reagent B2		212 µl

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Notes

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