PROTOCOL

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SWIFT AMPLICON™ HS PANELS

Protocol for Cat. No:

- Swift Amplicon HS EGFR Panel (HS-51024)

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About This Guide

This guide provides instructions for the preparation of targeted NGS libraries from DNA samples using a Swift Amplicon HS panel with unique molecular identifiers (MIDs) to achieve improved specificity for variants at and below 1% allele frequency.

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

Applications

Swift Amplicon HS panels are suitable for the following targeted sequencing applications. This list is neither exhaustive nor meant to restrict applications.

Variant Type*	Applications	Sample Type
Somatic Mutation	• Oncology • Variant discovery	• Fresh frozen DNA • FFPE DNA • Cell-free DNA • Circulating tumor cell DNA • Whole genome amplification products • Peripheral blood lymphocytes (PBL) • Bone marrow
Identification of sub-populations	• Non Invasive Prenatal Testing (NIPT) • Graft integrity after organ transplant • Sample tracking	• Cell-free DNA

** Swift Amplicon HS panels may be used to interrogate the following types of variants in samples:*

- *base substitutions*
- *small insertions and deletions occurring in the target sequence*

Product Information

Swift Amplicon HS Panels enable the preparation of high quality targeted next-generation sequencing (NGS) libraries from a variety of sample types, including formalin-fixed paraffin-embedded (FFPE) and circulating cell-free DNA (cfDNA). Indexing primers are included for combinatorial dual indexing and multiplexing up to 96 samples on a sequencing run. The single-tube workflow from DNA to library can be completed within three hours.

The Swift Amplicon HS Panels include MIDs which allow tracking of amplification products. The MIDs can be used to generate a consensus sequence to eliminate errors that may have originated during amplification or sequencing.

Overview of Swift Amplicon HS MID Technology

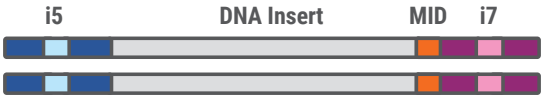


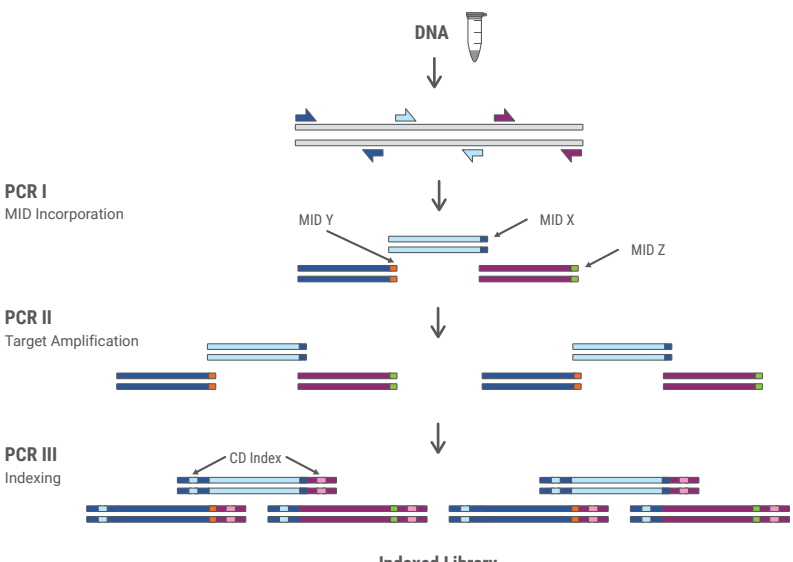
Figure 1: Swift Amplicon HS MID technology utilizes a 10-base random N sequence that enables greater than one million unique MIDs. These are positioned at the start of Read 2. Each amplicon receives a unique MID assigned to each original ssDNA template.

The kit utilizes Illumina®-compatible adapter sequences and has been validated on Illumina platforms.

Swift Amplicon HS Panel Workflow

This protocol contains three PCR steps, one for the incorporation of MIDs (PCR I), one for target amplification (PCR II), and one for the addition of combinatorial dual indexed adapters (PCR III), enabling multiplexing of up to 96 unique libraries.

Bead-based SPRI clean-ups are used to purify the sample by removing unused oligonucleotides and changing buffer composition between steps.



Kit Contents

The kit contains enough reagents for the preparation of 24 libraries (10% excess volume provided).

Kit	Reagents	Quantity (µl) 24 rxn
MID Incorporation	Reagent B1*	53
	Enzyme B2	396
	Pre-PCR TE	1000
Target Amplification Reagents	Reagent G1	132
	Enzyme G2	660
Indexing Reagents	IPA_D50X	25 each of D501-D508
	IPA_D7XX	18 each of D701-D712
	Enzyme Y1	396
	Post-PCR TE	1000

** Reagent B1 is the panel-specific set of multiplex amplification primers.*

Kit	Reagent	Quantity 96 rxns	Storage (°C)
Additional Components Included	PEG NaCl Solution	20 ml	Room Temp
	Alu115 and Alu247 primers	540 µl	-20

Materials and Equipment Not Included

- Magnetic beads for clean-up steps, e.g., SPRIselect™ beads (Beckman Coulter™, Cat. No. B23317/ B23318/B23319)
- Invitrogen DynaMag™, Agencourt® SPRIPlate™ or similar magnetic rack for magnetic bead clean-ups
- qPCR-based input DNA quantification assay (for FFPE and cfDNA samples)
- Qubit® or similar fluorometric assay for high quality input DNA and library quantification
- Microcentrifuge
- Plastic consumables (0.2 mL PCR tubes, strips, 96-well plates, pipette tips, etc.)
- Programmable thermocycler operating within manufacturer's specifications
- 0.2 ml PCR tubes or 96-well plate
- Aerosol-resistant tips and pipette ranges from 1-1000 µl
- 200-proof/absolute ethanol (molecular biology grade)
- Nuclease-free water (molecular biology grade)

Storage and Usage Warning

Upon receipt, store the amplicon panel kit at -20 ˚C with the exception of PEG solution, which should be stored at room temperature.

Separate the MID Incorporation and Target Amplification Reagents (keep in pre-PCR area) and Indexing Reagents (keep in post-PCR area).

After thawing reagents at room temperature, briefly vortex (except the enzymes) to mix them well. Invert the enzyme tubes to mix well and ensure no precipitant is present before use. Spin all tubes in a microfuge to collect contents prior to opening.

Always add reagents to the master mix in the specified order as stated throughout the protocol.

The combinatorial dual indexing primers are the only reagents that are added individually to each sample.

IMPORTANT!

Assemble all reagent master mixes and reactions ON ICE and scale volumes as appropriate, using 5% excess volume to compensate for pipetting loss. To calculate the total volume of the master mixes, use our Swift Amplicon HS Master Mixing Volume Calculator and prepare them in advance to ensure the magnetic beads do not over dry during size selection steps while awaiting completion of master mix assembly.

Tips and Techniques

Avoiding Cross-Contamination

- Physically separate the laboratory space, equipment, and supplies where pre-PCR and post-PCR processes are performed.
- Separate the MID Incorporation and Target Amplification Reagents (keep in pre-PCR area) and Indexing Reagents (keep in post-PCR area).
- 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.
- Move samples to post-PCR area before opening tubes.

Swift Amplicon HS, like any amplicon enrichment technology, poses a risk of contamination of surfaces and other samples following the amplification step. Please use extreme caution when opening your sample tubes following the MID Incorporation and Target Amplification PCR steps. It is highly recommended that separate workspaces and pipettes be maintained for pre-PCR and post-PCR steps. If available, a positive pressure PCR hood should be used for pre-PCR steps and a negative pressure hood should be used for post-PCR steps. Clean lab areas using 0.5% sodium hypochlorite (10% bleach) and use specialty barrier pipette tips. Dispose of pipette tips and other disposables in sealed plastic bags.

Size Selection During Clean-up Steps

This protocol has been validated with SPRIselect beads (Beckman Coulter). Please perform validation if using other beads with this workflow.

Input Material Considerations

Swift Amplicon HS panels enable the preparation of targeted NGS libraries from DNA templates.

The starting material should be quantified with the appropriate assay (qPCR-based for FFPE and cfDNA, Qubit for high quality DNA from whole blood, fresh frozen, or cultured cells) as described in the Input DNA Quantification section.

The optimal coverage uniformity, sensitivity, and specificity of this technology are achieved with qPCR-verified input amounts in the 10-50 ng range. Using less than 10 ng may reduce specificity of the assay and will affect variant calling for low frequency alleles. Consider the following example allele frequencies when determining the limit of detection:

Sample Quantity (ng)	Human Genome Equivalents (Total Copies)	Example Allele Frequency (%)	Example Allele Equivalents (Copies)	Feasibility of Calling Variant (High Quality DNA)
50	15,000	0.2	30	✓
50	15,000	0.1	15	✓
50	15,000	0.05	7.5	*X
20	6000	0.5	30	✓
20	6000	0.25	15	✓
20	6000	0.125	7.5	*X
10	3000	1.0	30	✓
10	3000	0.5	15	✓
10	3000	0.25	7.5	*X
1	300	5.0	15	✓
1	300	1.0	3	*X
1	300	0.5	1.5	*X

**Not supported, less than 10 allele copies may not be detectable because the probability of detection follows a normal distribution. That is, the fewer the copies of an allele in the input material, the less the chance it is present in the reaction.*

Prepare the DNA Sample

Input DNA Quantification

IMPORTANT!

Improper quantification of input material can lead to assay failure. Please read this section carefully and quantify the types of input material specified below appropriately to ensure success. The limit of detection, sensitivity, and specificity of Swift Amplicon HS Panels is highly dependent on accurate input quantification.

For high quality samples, it is recommended to determine DNA concentration using Qubit, or a similar fluorometric method, as it will accurately represent the double-stranded, amplifiable DNA content of your sample. For cfDNA or low quality DNA samples, we recommend quantification by qPCR using Alu primer pairs provided in this kit to accurately assess the usable amount of DNA in the samples and their integrity. Swift Amplicon HS Panels are designed with amplicons of approximately 140 bp for maximum compatibility with FFPE and cfDNA.

High Quality gDNA Quantify with Qubit or similar fluorometric method
cfDNA, FFPE DNA Quantify by qPCR with Alu primer pairs (see Tech Note online)

- We recommend using between 10-50 ng input DNA per library preparation keeping in mind the limit of detection is dependent on input amount.
- Input DNA should be re-suspended in 13 µl of Pre-PCR TE buffer.

Min. DNA input (ng)	Limit of detection (%)
10	0.5
20	0.25

If you have questions related to FFPE or cfDNA sample quality, please contact Swift Technical Support at TechSupport@swiftbiosci.com or tel: 734.330.2568.

Prepare the Reagent Master Mixes and Ethanol

- To create a master mix, scale reagent volumes as appropriate, using 5% excess volume to compensate for pipetting loss. To calculate the total volume of the master mixes based on the number of reactions of choice, use our Swift Amplicon HS Master Mixing Volume Calculator. This tool automatically incorporates 5% excess volume to compensate for pipetting loss.
- After thawing reagents at room temperature, briefly vortex (except the enzymes) to mix them well. Invert the enzyme tubes to mix well and ensure no precipitant is present before use. Spin all tubes in a microfuge to collect contents prior to opening.
- Separate the MID Incorporation and Target Amplification Reagents (keep in pre-PCR area) and Indexing Reagents (keep in post-PCR area).
- Prepare master mix reactions on ice before adding to samples and performing incubations.
- Before starting, prepare a fresh 80% ethanol solution using 200-proof/absolute ethanol and nuclease-free water (prepare 1.5 ml per sample).

Prepare the DNA Libraries

Pre-Program Thermocycler

See table below to pre-program the thermocycler for the MID Incorporation, Target Amplification and Indexing reactions. See insert (supplied in your shipment) for panel-specific information for your Swift Amplicon HS Custom NGS Panel. **NOTE:** Cycling conditions and data quality can vary based on input quality and quantity.

For samples with <10ng input an additional 1 or 2 PCR cycles in the Target Amplification step is expected to increase yields.

PCR Step	Cycling conditions		
MID Incorporation Thermocycler Program	45 sec	98 °C	3 Cycles
	30 sec	98 °C	
	2 min	64 °C	
	2 min	62 °C	
	4 min	60 °C	
	2 min	58 °C	
	1 min	65 °C	
	1 min	65 °C	
	Hold	4 °C	
Target Amplification Thermocycler Program	45 sec	98 °C	22 cycles *EGFR-MID Panel
	10 sec	98 °C	
	15 sec	60 °C	
	1 min	66 °C	
	Hold	4 °C	
Indexing Thermocycler Program	45 sec	98 °C	7 Cycles
	10 sec	98 °C	
	15 sec	60 °C	
	1 min	66 °C	
	Hold	4 °C	

MID Incorporation Step

IMPORTANT! Work in pre-PCR area.

1. Load the MID Incorporation PCR program and allow the block to reach 98 °C before loading samples (confirm lid heading is turned ON and is set to reach 105 °C).
2. Load 13 µl of sample DNA (adjust with Pre-PCR TE) into each PCR tube.
3. Make the Reaction Mix with the following components. Assemble on ice. Components B1 and B2 may be master-mixed when running multiple samples in parallel.

Component	Volume (1 Reaction)
Reagent B1*	2 µl
Enzyme B2	15 µl
Reaction Mix	**17 µl**

** Reagent B1 is the panel-specific set of multiplex amplification primers.*

4. Mix well and then add 17 µl of the Reaction Mix to each 13 µl sample. Place in the thermocycler and run the program.

DNA Clean-up

5. Ensure beads and samples are at room temperature. Briefly vortex beads to homogenize before use.
6. Add 36 µl (ratio: 1.2) of SPRIselect beads to each 30 µl sample. Mix by vortexing. Ensure no bead-sample suspension droplets are left on the sides of the tube.
7. Incubate the samples for 5 minutes at room temperature off the magnet.
8. Pulse-spin the samples in a microfuge. Place the sample tubes on a magnetic rack until the solution clears and a pellet is formed (≈ 5 minutes).
9. While leaving your sample on the magnet, remove and discard the supernatant without disturbing the pellet (approximately 5 µl may be left behind). Leave tubes on the magnet.
10. Add 200 µl of freshly prepared 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.
11. Repeat step 10 once for a second wash with the ethanol solution.
12. Pulse-spin the samples in a microfuge, place back onto the magnet and remove any residual ethanol solution from the bottom of the tube.
13. Add 20 µl of Pre-PCR TE buffer and resuspend the pellet, mixing well by pipetting up and down until homogenous. Incubate at room temperature for 2 minutes off the magnet. Then place the sample back on the magnet and transfer the clean 20 µl library eluate to a fresh tube.

Target Amplification

14. Load the Target Amplification Thermocycler Program and allow the block to reach 98 °C before loading samples (confirm lid heating is turned ON).
15. Make the Reaction Mix with the following components. Assemble on ice. Components G1 and G2 may be master-mixed when running multiple samples in parallel.

Component	Volume (1 Reaction)
Reagent G1	5 µl
Enzyme G2	25 µl
Reaction Mix	**30 µl**

16. Mix well and then add 30 µl of the PCR Reaction Mix to each 20 µl sample. Place in the thermocycler and run the program.

IMPORTANT!
Move samples to post-PCR area before opening tubes.

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17. Load the Indexing Thermocycler Program and allow the block to reach 98 °C before loading samples (confirm lid heating is turned ON).

DNA Clean-up

18. Ensure beads and samples are at room temperature. Briefly vortex beads to homogenize before use.
19. Add 60 µl (ratio: 1.2) of SPRIselect beads to each 50 µl sample. Mix by vortexing. Ensure no bead-sample suspension droplets are left on the sides of the tube.
20. Incubate the samples for 5 minutes at room temperature off the magnet.
21. Pulse-spin the samples in a microfuge. Place the sample tubes on a magnetic rack until the solution clears and a pellet is formed (≈ 5 minutes).
22. While leaving your sample on the magnet, remove and discard the supernatant without disturbing the pellet (approximately 5 µl may be left behind). Leave tubes on the magnet.
23. Add 200 µl of freshly prepared 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.
24. Repeat step 23 once for a second wash with the ethanol solution.
25. Pulse-spin the samples in a microfuge, place back onto the magnet and remove any residual ethanol solution from the bottom of the tube.
26. Proceed to the Indexing Step for resuspension without delay.

Indexing Step

Continue working in the post-PCR area.

27. Add without delay a unique combination of 7.5 µl IPA_D50X + 7.5 µl IPA_D7XX to each sample bead pellet, ensure that the beads do not over-dry.
28. Add 15 µl of Enzyme Y1 to each sample and resuspend the pellet (total volume 30 µl).

Component	Volume (1 Reaction)
IPA_D50X	7.5 µl
IPA_D7XX	7.5 µl
Enzyme Y1	15 µl
Reaction Mix	**30 µl**

29. Place in the thermocycler and run the program.

DNA Clean-up

30. Ensure PEG NaCl solution is at room temperature. Briefly vortex the PEG NaCl solution to homogenize before use.
31. Add 26 µl (ratio: 0.85) of PEG NaCl solution to each 30 µl sample. Mix by vortexing. Ensure no bead-sample suspension droplets are left on the sides of the tube.
32. Incubate the samples for 5 minutes at room temperature off the magnet.
33. Pulse-spin the samples in a microfuge. Place the sample tubes on a magnetic rack until the solution clears and a pellet is formed (≈ 5 minutes).
34. While leaving your sample on the magnet, remove and discard the supernatant without disturbing the pellet (approximately 5 µl may be left behind). Leave tubes on the magnet.
35. Add 200 µl of freshly prepared 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.
36. Repeat step 35 once for a second wash with the ethanol solution.
37. Pulse-spin the samples in a microfuge, place back onto the magnet and remove any residual ethanol solution from the bottom of the tube.
38. Add 20 µl of Post-PCR TE buffer and resuspend the pellet, mixing well by pipetting up and down until homogenous. Incubate at room temperature for 2 minutes off the magnet. Then place the sample back on the magnet and transfer the clean 20 µl library eluate to a fresh 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.

Library Quantification

Library quantification can be performed by qPCR, a fluorometric method such as Qubit, or Bioanalyzer®.

For qPCR, quantify a 1:10,000 or 1:100,000 dilution of your library in triplicate using an assay based upon a library size of 275 bp. Upon calculating library concentration, be sure to adjust for proper library size of the standards in your library quantification kit. Variation in length of DNA in the standards from the kit and your library size may lead to improper estimation of DNA concentration.

Sequence the DNA Libraries

Please refer to the latest version of Illumina Experiment Manager for detailed instructions on how to set up a sample sheet. Be sure to select the appropriate workflow parameters as noted below:

- Read Type: "Paired End"
- Cycle Read 1: "151", Cycles Read 2: "151"

Make sure the "Use Adapter Trimming" and "Use Adapter Trimming Read 2" are selected. Please ensure that adapter trimming is enabled while setting up the sequencing run. Failure to trim adapter sequences will result in incorrect primer trimming and will lead to inaccurate variant calling. To overcome this issue, enable automatic trimming by the sequencer software or perform adapter trimming by Trimmomatic during data analysis. For more information, please consult our Bioinformatics Resources page at swiftbiosci.com/biofx.

MiSeq Loading Recommendations

Recommended Loading Concentration for MiSeq® v2 and v3 Reagent Kits

Chemistry	Final Library Pool Concentration	Recommended Loading Concentration
MiSeq Reagent Kit v2	2-4 nM	10-12 pM
MiSeq Reagent Kit v3	4 nM	20 pM

Amplicon libraries can be pooled together to obtain a 2 nM or 4 nM final concentration mix. Denaturation of libraries with freshly diluted 0.2 N NaOH is required before loading on the MiSeq.

- 2 nM library denaturation (supports 10 pM loading)
  - 2 nM library pool (5 µl) + 0.2N NaOH (5 µl).
  - Mix and incubate 5 minutes at room temperature.
  - Add 990 µl of pre-chilled HT1 to obtain a 10 pM denatured library mix, mix well.
  - Load 600 µl in the cartridge.

- 4 nM library denaturation (supports 10 pM-20 pM loading)
  - 4 nM library pool (5 µl) + 0.2N NaOH (5 µl).
  - Mix and incubate 5 minutes at room temperature.
  - Add 990 µl of pre-chilled HT1 to obtain a 20 pM denatured library pool, mix well.
  - Dilute the denatured DNA to load the cartridge:

Final Concentration	10 pM	11 pM	12 pM	20 pM
20 pM Denatured Libraries	300 µl	330 µl	360 µl	600 µl
Pre-chilled HT1	300 µl	270 µl	240 µl	0 µl

  - Load 600 µl of the desired dilution into the cartridge.

When working with other Illumina instruments (i.e. NextSeq®, MiniSeq®, etc.), please refer to manufacturer's guide for recommendations regarding reagents and loading.

PhiX Spike-In

Libraries prepared from Swift Amplicon HS panels with at least 17 amplicons do not require a PhiX spike-in when sequencing on the Illumina MiSeq and MiniSeq instruments because they inherently have sufficient complexity to yield optimal sequencing data.

Please consult TechSupport@swiftbiosci.com Tel: 734-330-2568 if you plan to sequence these libraries, without addition of higher complexity samples, on any other Illumina instruments.

Bioinformatics Options

Adapter and Primer Trimming

As noted in the earlier section of this manual titled Sequence the DNA libraries, please ensure that adapter trimming is enabled while setting up the sequencing run. Alternatively, adapter trimming could be performed bioinformatically prior to analysis. In addition, Swift Amplicon HS Panels are designed with overlapping amplicons to allow for contiguous regions of coverage in a single-tube format. Therefore, synthetic primer sequences will be encountered both at the beginning and end of some reads. These primer sequences must be trimmed during the data analysis. This can be done using Swift's publicly available tool called Primerclip (http://github.com/swift-biosciences/primerclip). For more information, please consult our Bioinformatics Resources page at swiftbiosci.com/biofx. Download panel specific files through our website. A target BED file is provided with purchase of the Swift Amplicon HS Panel.

There are a few key considerations when analyzing sequencing data generated from the Swift Amplicon HS Panels with MIDs:

- The first 10 bases in front of Read 2 constitute an MID (see Figure 1). Hence, trim (CROP) these first 10 bases from Read 2 (using Trimmomatic http://www.usadellab.org/cms/?page=trimmomatic) to make an MID/UMI fastq file for use with MID pipeline based on fgbio package from Fulcrum Genomics (https://github.com/fulcrumgenomics/fgbio).
- In addition, prior to aligning the reads, make sure that the 10bp MID (which contains random bases) has been trimmed off from 5' of Read 2. If additional informatics pipeline advice is needed to use these instructions, please contact Swift Technical Support at TechSupport@swiftbiosci.com or tel: 734.330.2568.

Swift Amplicon HS Data Analysis on Genialis Platform

Sequencing data from all standard and custom Swift Amplicon HS panels can be analyzed through a web-based software solution supported by Genialis. This data analysis solution has been designed to integrate alignment, trimming, QC and variant calling into one simple pipeline. The Genialis App also automates an algorithm optimized for accurate variant detection, including low frequency variants. It is fast and easy to use, as it offers data visualization and annotated variants using direct links to publicly available databases such as COSMIC and NCBI.

Please visit genial.is/swiftbio/ to learn more about this service.

Appendix

Section A: Library Multiplexing Options for MiSeq

Use the following equation to determine possible number of libraries to multiplex per sequencing run:

Level of multiplexing = (number of paired-end reads) / (number of amplicons * intended average read depth)

MiSeq Multiplexing guidelines for the Swift Amplicon HS EGFR-MID panel are as follows:

			Level of MiSeq Multiplexing			
Input	**LOD (%)**	**Minimum # Recommended Reads**	**v2 Nano (300 cycles) (2M paired-end reads)**	**v2 Micro (300 cycles) (8M paired-end reads)**	**v2 (300 cycles) (30M paired-end reads)**	**v3 (600 cycles) (50M paired-end reads)**
10 ng	1	0.5M	4	16	60	96*
	0.5	1M	2	8	30	50
20 ng	1	0.75M	2	10	40	66
	0.5	1M	2	8	30	50
	0.25	1.5M	1	5	20	33

** Higher level of multiplexing is possible with a custom solution. Please inquire.*

Section B: Indexed Adapter Sequences

During the Indexing PCR Step in the protocol, you must use a unique combination of indexing primers to re-suspend and label each library. Libraries made with unique index combinations may be multiplexed during cluster generation and co-sequenced on the same Illumina flow cell or lane.

D5 Adapters	**Sequence MiSeq, HiSeq® 2000/2500, NovaSeq**	**Sequence MiniSeq, NextSeq, HiSeq 3000/4000**
D501	TATAGCCT	AGGCTATA
D502	ATAGAGGC	GCCTCTAT
D503	CCTATCCT	AGGATAGG
D504	GGCTCTGA	TCAGAGCC
D505	AGGCGAAG	CTTCGCCT
D506	TAATCTTA	TAAGATTA
D507	CAGGACGT	ACGTCCTG
D508	GTACTGAC	GTCAGTAC

NOTE: Include reverse complement sequences provided in the table above when using Illumina MiniSeq, NextSeq, or HiSeq 3000/4000 systems.

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

The number on the product tube label indicates which indexing primer is provided in the tube. During library prep, make sure to note which index combination you are using with your sample. Please do not use the same index combination on two different samples you plan to co-sequence.

Section C: Helpful Information and Troubleshooting

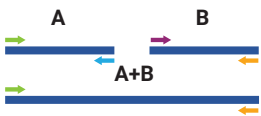
Problem	Possible Cause	Suggested Remedy
Lower than expected yields	Inadequate sample quality and/or quantity, incorrect input quantification method, or incorrect SPRI methods.	Use qPCR-quantified input. Perform SPRI carefully to avoid over drying or cracking of beads.
Incomplete resuspension of beads after ethanol wash during SPRI steps	Over-drying of beads.	Continue pipetting the liquid over the beads for complete resuspension.
Lower than expected cluster density	Error in library quantification.	Quantify library with a qPCR-based method.
Precipitates in Enzymes B2, G2 or Y1	Salt precipitation.	Allow the vial to reach room temperature and gently rock until precipitation dissolves. Place on ice for remainder of use.

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).

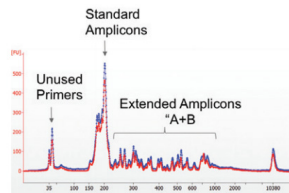
Section D: Structure of Swift Amplicon HS Libraries and Migration Behavior of Swift Amplicon HS Libraries on Agilent Bioanalyzer or TapeStation®

Swift Amplicon HS libraries exhibit a feature which should be understood if analyzed using electrophoretic methods such as Agilent Bioanalyzer or TapeStation:

If using high quality DNA, "extended amplicons" can be observed. These are formed from the forward primer and the reverse primer of two adjacent amplicons. Note that these extended amplicons are not usually formed when using fragmented or cross-linked (FFPE) DNA, or cell-free DNA. Coverage uniformity is not affected by the presence or absence of extended amplicons.



"A+B" is an extended amplicon, which is a contiguous alignable sequence



Section E: Safety Data Sheet

Please refer to Swift Amplicon HS products Safety Data Sheet (SDS) for more information about the potential hazards for each component (reagents, buffers and enzymes) and instructions on safe use.

General Warranty

Swift Biosciences, Inc. ("Swift") warrants that its products meet Swift's specifications at the time of delivery. Any sample or model used in connection with Swift's product literature is for illustrative purposes only and does not constitute a warranty that the products will conform to the sample or model.

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