PROTOCOL

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SWIFT AMPLICON™ 16S+ ITS PANEL

Protocol for Cat. No. AL-51648

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About This Guide

This guide provides instructions for the preparation of targeted NGS libraries from DNA samples using the Swift Amplicon 16S+ITS Panel.

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

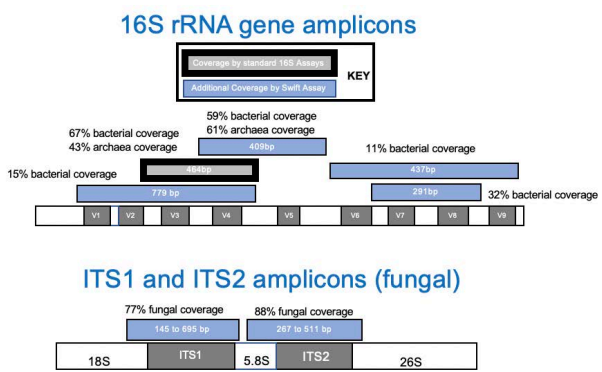
Swift Amplicon Panels are suitable for the following targeted sequencing applications. This list is not exhaustive nor meant to restrict applications.

- Identification of pathogenic isolates
- Microbial community analysis (e.g. gut microbiome, environmental matrices)

Product Information

Swift Amplicon Panels enable the preparation of high quality targeted next-generation sequencing (NGS) libraries from a variety of sample types. Adapters are included for dual indexing and multiplexing up to 96 samples on a sequencing run. The single-tube workflow from DNA to library can be completed within two hours.

The kit provides a single primer pool covering all the variable regions of the 16S rRNA gene (V1-V9) and the Fungal ITS1 and ITS 2 genes, library preparation reagents, and Illumina®-compatible adapters.



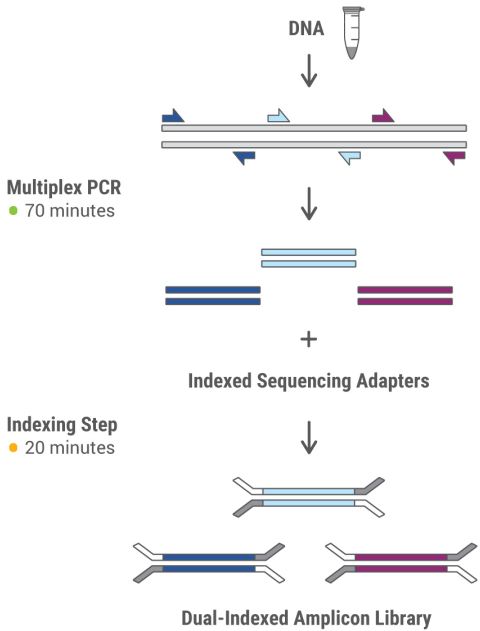
The table below lists key characteristics and typical performance of this panel.

Specification	Feature	16S + ITS Panel
Input	Input DNA required	10pg-50ng *depending on sample type and expected complexity
Workflow	Time required	2 hours
	Multiplexing on MiSeq® v2 standard	Up to 96
Design	Number of amplicons	7 5 16S rRNA + 2 Fungal (ITS 1 and ITS2)
	Average amplicon size	475 bp
	Genes covered	Bacterial 16S + Fungal ITS

Swift Amplicon Panel Workflow

This protocol contains a Multiplex PCR step for the simultaneous production of hundreds of amplicon targets in a single tube and an Indexing step for the addition of dual indexed adapters, enabling multiplexing of up to 96 unique libraries.

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



Kit Contents

Swift Amplicon Panels are available in a package size with reagents (10% excess volume) for the preparation of 48 libraries.

Kit	Reagents	Quantity (µl) 48 rxn	Storage (°C)
Multiplex PCR (Pre-PCR)	Reagent G1*	106	-20
	Reagent G2	160	-20
	Enzyme G3	800	-20
	Pre-PCR TE	1200	-20
Indexing Step (Post-PCR)	Index D50X	33 each of D501-D508	-20
	Index D7XX	44 each of D701-D712	-20
	Buffer Y1	1637	-20
	Enzyme Y2	53	-20
	Enzyme Y3	53	-20
	Enzyme Y4	106	-20
	Post-PCR TE	1200	-20

** Reagent G1 is the panel-specific set of multiplex amplification primers.*

Additional Components Included

Reagent	Quantity (µl) 48 rxns	Storage (°C)
PEG NaCl Solution	20,000	Room Temp

IMPORTANT!

Place the enzymes on ice, NOT in a cryocooler, for at least 10 minutes to allow enzymes to reach 4 ˚C prior to pipetting.

Materials and Equipment Not Included

- Magnetic beads for clean-up steps, e.g., SPRIselect™ beads (Beckman Coulter, Cat. No. B23317/ B23318/B23319) or AMPure® XP Beads (Beckman Coulter, Cat. No. A63880/ A63881/A63882)
- Magnetic rack for clean-up steps, e.g., Invitrogen DynaMag™ or Agencourt® SPRIPlate™
- Library quantification kit (qPCR-based)
- Qubit® or other fluorometric-based assays for determining input DNA concentration
- Plastic consumables (0.2 mL PCR tubes, strips, 96-well plates, pipette tips, etc.)
- Centrifuge compatible with format of plastic consumables
- Programmable thermocycler
- Aerosol-resistant, low retention pipettes and tips, 2 to 1000 µL
- 200-proof/absolute ethanol (molecular biology-grade)
- Nuclease-free water (molecular biology-grade)

Storage and Usage Warning

Upon receipt, store the Swift Amplicon Panel Kit at -20 ˚C with the exception of PEG solution, which should be stored at room temperature.

Separate the Multiplex PCR Reagents (keep in pre-PCR area) and Indexing Reagents (keep in post-PCR area).

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 10 minutes to allow enzymes 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 to 4 ˚C, briefly vortex (except the enzymes) to mix them well. Enzyme G3 is the only enzyme that may be vortexed. 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 dual indexed adapters 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 Master Mixing Volume Calculator and prepare them in advance to ensure the magnetic beads do not overdry during size selection steps while awaiting completion of master mix assembly. Neglecting to store master mixes and reagents on ice prior to incubations reduces yields and performance of this product.

Tips and Techniques

Avoiding Cross-Contamination

- Physically separate the laboratory space, equipment, and supplies where pre-PCR and post-PCR processes are performed, including appropriate reagent boxes for Multiplex PCR and Indexing Step.
- 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, 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 Multiplex PCR step. It is highly recommended that separate workspaces and pipettes be maintained for pre-PCR and post-PCR steps. A negative pressure hood should be used for post-PCR steps if available. 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) but can be used with Agencourt AMPure XP (Beckman Coulter). If other beads are used, solutions and conditions for DNA binding may differ.

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 Panels is highly dependent on accurate input quantification.

It is recommended that input DNA concentration be determined using Qubit, or a similar fluorometric method, as it will accurately represent the double-stranded DNA content of your sample. Sample purity should be determined by spectrophotometry (260/280).

The Swift 16S+ITS kit has been tested with DNA input between 10pg and 50ng. When considering input amounts, please take into account the expected complexity of your sample and sensitivity required.

The assay produces sequence-able yields for Illumina sequencing from picogram to nanogram range. If your sample does not produce yields and you suspect sample impurities to be an inhibitory factor, a bead-based cleanup may improve amplifiability of samples.

- Use 10pg-50 ng input DNA per library preparation.
- Input DNA should be re-suspended in 10 µl of Pre-PCR TE buffer.

Prepare the Reagent Master Mixes and Ethanol

1. 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 Master Mixing Volume Calculator. This tool automatically incorporates 5% excess volume to compensate for pipetting loss.
2. To assemble reagent master mixes for the Multiplex PCR and Indexing steps, ensure the reagent vials are thawed and then stored on ice. After thawing reagents, briefly vortex (except the enzymes) to mix them well. Enzyme G3 is the only enzyme that may be vortexed. If a precipitate is observed in Enzyme G3, warm it gently to room temperature and vortex to dissolve solutes. Spin tubes in a microfuge to collect contents prior to opening. Add reagents in order listed when preparing master mix. Master mixes should be prepared and stored ON ICE until used.

IMPORTANT!

Prepare the reagents in advance to ensure the magnetic beads do not dry out during size selection steps. Always add reagents in specified order. This applies to all reagents except for the indexed adapters that should be added individually to uniquely index each library.

Ensure PEG NaCl solution is at room temperature.

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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.

Prepare the DNA Libraries

Pre-Program Thermocycler

See table below to pre-program the thermocycler for the Multiplex PCR (Note: panel-specific PCR cycles) and Indexing steps.

IMPORTANT!
Work in pre-PCR area.

PCR STEP	CYCLING CONDITIONS		
Multiplex PCR Thermocycler Program	**Lid heating ON**		
	30 sec	98 °C	
	10 sec	98 °C	**4 cycles**
	5 min	63 °C	
	1 min	65 °C	
	10 sec	98 °C	**22 cycles**
	1 min	64 °C	
	1 min	65 °C	
	Hold	4 °C	
Indexing Thermocycler Program	**Lid heating OFF**		
	20 min	37 °C	

Multiplex PCR Step

1. Load the Multiplex PCR program and allow the block to reach 98 °C before loading samples (confirm lid heating is turned ON and is set to reach 105 °C).
2. Load 10 µl of sample DNA (adjust with Pre-PCR TE) into each PCR tube. Keep on ice.
3. **Keep all tubes on ice during assembly of the master-mix and the reaction until placed in thermocycler.** Make the Multiplex PCR Reaction Mix. Components G1, G2, and G3 should be vortexed first and may be master-mixed when running multiple samples in parallel. If any
salt precipitant is present in Enzyme G3, allow the vial to reach room temperature and gently vortex to dissolve solids. Place on ice for remainder of use.

Panel-Specific Multiplex PCR Reaction Mix

To calculate the total volume of the master mixes based on the number of reactions of choice, use our Swift Amplicon Master Mixing Volume Calculator.

Component	Volume (1 Reaction)
Reagent G1*	2 µl
Reagent G2	3 µl
Enzyme G3	15 µl
Reaction Mix	**20 µl**

**Reagent G1 is the panel-specific set of amplification primers.*

4. Mix the master mix well and then add 20 µl of the Multiplex PCR Reaction Mix to each 10 µl input DNA sample. Place in the thermocycler and run the program.

IMPORTANT!
Move samples to post-PCR area before opening tubes. Keep samples at room temperature. At no time should 'with bead' samples be stored on ice, as this affects binding to magnetic beads.

5. Near the completion of the thermocycler run, prepare the Indexing Reaction Mix in the post-PCR area with the following components. **Assemble this reaction mix on ice and keep cold until adding it to samples in the Indexing Step**. All components may be master-mixed when running multiple samples in parallel.

Indexing Step

To calculate the total volume of the master mixes based on the number of reactions of choice, use our Swift Amplicon Master Mixing Volume Calculator.

Component	Volume (1 Reaction)
Buffer Y1	31 µl
Enzyme Y2	1 µl
Enzyme Y3	1 µl
Enzyme Y4	2 µl
Reaction Mix	**35 µl**

Size Selection and Clean-Up Step 1

6. Ensure beads and samples are at room temperature. Briefly vortex beads to homogenize before use.
7. Add 36 µl (ratio: 1.2) of magnetic beads to each 30 µl sample. Mix by vortexing. Pulse-spin the samples in a microfuge. Ensure no bead-sample suspension droplets are left on the sides of the tube.
8. Incubate the samples for 5 minutes at room temperature off the magnet.
9. Place the sample tubes on a magnetic rack until the solution clears and a pellet is formed (≈ 5 minutes).
10. 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.
11. 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.
12. Repeat step 11 once for a second wash with the ethanol solution.
13. Pulse-spin the samples in a microfuge, place back onto the magnet and remove any residual ethanol solution from the bottom of the tube with a small volume tip. Proceed to the Indexing Step.

IMPORTANT!
Continue working in the post-PCR area.

Indexing Step

14. Load the Indexing Thermocycler Program and allow the block to reach 37 °C before loading samples.
15. Add a unique combination of 5 µl Index D50X + 10 µl Index D7XX to each sample bead pellet.

Please use the plate layout guide below to generate 48 unique index combinations

	1	2	3	4	5	6
A	501/701	501/702	501/703	501/704	501/705	501/706
B	502/701	502/702	502/703	502/704	502/705	502/706
C	503/701	503/702	503/703	503/704	503/705	503/706
D	504/701	504/702	504/703	504/704	504/705	504/706
E	505/707	505/708	505/709	505/710	505/711	505/712
F	506/707	506/708	506/709	506/710	506/711	506/712
G	507/707	507/708	507/709	507/710	507/711	507/712
H	508/707	508/708	508/709	508/710	508/711	508/712

If you have 2 kits (48 reactions each), use the guide below to generate 96 unique index combinations

	1	2	3	4	5	6	7	8	9	10	11	12
A	501/701	501/702	501/703	501/704	501/705	501/706	501/707	501/708	501/709	501/710	501/711	501/712
B	502/701	502/702	502/703	502/704	502/705	502/706	502/707	502/708	502/709	502/710	502/711	502/712
C	503/701	503/702	503/703	503/704	503/705	503/706	503/707	503/708	503/709	503/710	503/711	503/712
D	504/701	504/702	504/703	504/704	504/705	504/706	504/707	504/708	504/709	504/710	504/711	504/712
E	505/701	505/702	505/703	505/704	505/705	505/706	505/707	505/708	505/709	505/710	505/711	505/712
F	506/701	506/702	506/703	506/704	506/705	506/706	506/707	506/708	506/709	506/710	506/711	506/712
G	507/701	507/702	507/703	507/704	507/705	507/706	507/707	507/708	507/709	507/710	507/711	507/712
H	508/701	508/702	508/703	508/704	508/705	508/706	508/707	508/708	508/709	508/710	508/711	508/712

1st kit
2nd kit

16. Add 35 µl of the cold Indexing Reaction Mix to each sample (total volume 50 µl).
17. Place in the thermocycler and run the program (37 °C for 20 minutes).

Size Selection and Clean-Up Step 2

18. Ensure PEG NaCl solution is at room temperature. Briefly vortex the PEG NaCl solution to homogenize before use.
19. Add 42.5 µl (ratio: 0.85) of PEG NaCl solution 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 180 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 with a small volume tip.
26. Proceed immediately to 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

Quantify a 1:10,000 dilution of the library in triplicate using a qPCR-based assay based upon a library size of 475 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.

If quantification of individual libraries by qPCR is not an option due to workflow constraints, Swift recommends pooling all libraries at equal volumes and performing one qPCR quantification of the final pool to generate a library pool that will result in the correct sequencer loading and clustering density. With this method, it is important to first establish familiarity with sample input amount consistency and performance so as to minimize differences in representation across libraries.

Improper library quantification by other methods will lead to uneven pooling and sub-optimal cluster density, impacting sequencing data.

It is not recommended to use an electrophoretic method (such as Agilent Bioanalyzer® or TapeStation®) for quantifying libraries because:

- As there is no PCR enrichment of the library following the Indexing Step, using an electrophoretic method will not accurately quantify fully adapted library vs. other DNA.
- Library adapters have secondary structure which exhibits migration artifacts when analyzed using an electrophoretic method.

It is not recommended to use a fluorometric method (such as Qubit) for quantifying libraries because:

- As there is no PCR enrichment of the library following the Indexing Step, a fluorometric method will not accurately quantify fully adapted library vs. other DNA.

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 Requirements: Either 151X151 or 300X300 depending on sequencing needs. If using 151X151, Please test compatibility with your samples first by running a small representative sample. The Swift 16S + ITS Panel has been validated on the Illumina MiniSeq® and MiSeq® platforms.

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 lead to inaccurate read assignment. 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 the Swift Amplicon 16S + ITS Panel do not require a PhiX spike-in because they inherently have sufficient complexity to yield optimal sequencing data on Illumina MiniSeq® and MiSeq® platforms. For other Illumina platforms, please consider instrument-specific library complexity requirements.

Bioinformatics Options

Data from this panel can be analyzed using the following tools:

- Illumina's 16S Metagenomics BaseSpace™ App. Please find instructions here.
- Illumina's ITS Metagenomics BaseSpace™ App.
- RDP Tools Classifier. Citation: Wang, Q, G. M. Garrity, J. M. Tiedje, and J. R. Cole. 2007. Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. Appl Environ Microbiol. 73(16):5261-5267; doi: 10.1128/AEM.00062-07 [PMID: 17586664]. Please Note: Other analysis tools are available; however, these have not yet been internally validated.

APPENDIX

Section A: Indexed Adapter Sequences

During the Indexing Step in the protocol, you must use a unique combination of Index Adapters to re-suspend and label each library. Libraries made with uniquely indexed adapter combinations may be multiplexed during cluster generation and co-sequenced on the same Illumina flow cell.

CONTENTS: Unique indexed adapters, which should be used where this manual calls for 5 or 10 µl of each Index Primer:

D5 Adapters	Sequence MiSeq, HiSeq® 2000/2500	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 compliment 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 indexed adapter is provided in the tube.

During library prep, make sure to note which indexed adapter combination you are using with your sample and do not use the same indexed adapter combination on two different samples you plan to co-sequence.

Section B: Helpful Information and Troubleshooting

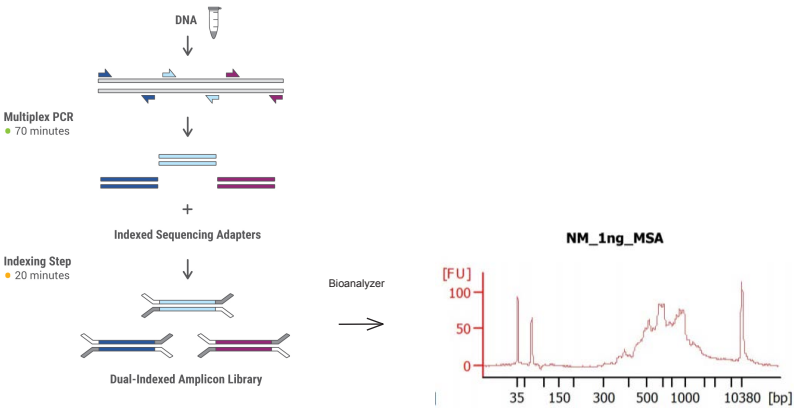
Problem	Possible Cause	Suggested Remedy
Lower than expected yields	Inadequate sample quality and/or quantity, incorrect input quantification method, or incorrect SPRI™ methods.	Increase multiplex PCR cycles from 22 to 23-24 cycles. Further purify the input DNA with a SPRI cleanup at 1.8X ratio.
Unusual electrophoretic trace	Secondary structure of adapters and lack of PCR enrichment of the library following the Indexing Step exhibit characteristics of migration artifacts.	Quantify library with a qPCR-based method; if you need to ascertain amplicon insert size from the sequencing data. (Review full explanation in "Structure of Amplicon Libraries and Migration Behavior" section.)
Precipitates in Enzyme G3	Salt precipitation.	Allow the vial to reach room temperature and gently vortex to dissolve solids. Place on ice for remainder of use.
Lower than expected cluster density	Error in library quantification. Bioanalyzer and Qubit do not accurately quantify fully adapted library vs. other DNA.	Quantify library with a qPCR-based method for flow cell loading calculations.
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 TechSupport@swiftbiosci.com, or by phone at 734.330.2568 (9:00 am-5:00 pm ET, Monday-Friday).

Section C: Structure of Swift Amplicon Libraries and Migration Behavior

Please note that qPCR-based methods are most accurate for quantifying Swift Amplicon libraries, however, this section provides an overview of expected results when using an electrophoretic method.

The secondary structure of Swift Amplicon libraries exhibits a feature which should be understood if analyzed using electrophoretic methods such as Agilent Bioanalyzer or TapeStation: After indexing, the library is partially single-stranded, and the migration is impaired, causing the library to appear large on the Bioanalyzer; therefore, the traces should not be used to accurately determine the size or the quantity of the library.



Section D: Material Safety Data Sheet

Please refer to Swift Amplicon products material safety data sheet (MSDS) for more information about the potential hazards for each component (reagents, buffers and enzymes) and instructions on safe use.

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