Set S3 Adapters	Sequence	26796
Reagent Y2 (Index S749)	TCCAGTCG	24 µl
Reagent Y2 (Index S750)	TGTATGCG	24 µl
Reagent Y2 (Index S751)	TCATTGAG	24 µl
Reagent Y2 (Index S752)	TGGCTCAG	24 µl
Reagent Y2 (Index S753)	TATGCCAG	24 µl
Reagent Y2 (Index S754)	TCAGATTC	24 µl
Reagent Y2 (Index S755)	GGTTGGAC	24 µl
Reagent Y2 (Index S756)	GACACTTA	24 µl
Reagent Y2 (Index S757)	GCTATGGA	24 µl
Reagent Y2 (Index S758)	GTAACCGA	24 µl
Reagent Y2 (Index S759)	GGCAAGCA	24 µl
Reagent Y2 (Index S760)	GAACGACA	24 µl
Reagent Y2 (Index S761)	GCGTCGAA	24 µl
Reagent Y2 (Index S762)	AAGGCGAT	24 µl
Reagent Y2 (Index S763)	CAGGCATT	24 µl
Reagent Y2 (Index S764)	AACTGTAT	24 µl
Reagent Y2 (Index S765)	ATGCTTGA	24 µl
Reagent Y2 (Index S766)	AGTATCTG	24 µl
Reagent Y2 (Index S767)	ATGTAATG	24 µl
Reagent Y2 (Index S768)	ACACATGT	24 µl
Reagent Y2 (Index S769)	ATAGCACG	24 µl
Reagent Y2 (Index S770)	ATATTGTA	24 µl
Reagent Y2 (Index S771)	CAATTGAT	24 µl
Reagent Y2 (Index S772)	CACGTCGT	24 µl

Set S4 Adapters	Sequence	26896
Reagent Y2 (Index S773)	AGTCTGTA	24 µl
Reagent Y2 (Index S774)	CCGTATCT	24 µl
Reagent Y2 (Index S775)	CGCTTCCT	24 µl
Reagent Y2 (Index S776)	CAAGACCT	24 µl
Reagent Y2 (Index S777)	CCTAGTAT	24 µl
Reagent Y2 (Index S778)	CCACCGAT	24 µl
Reagent Y2 (Index S779)	CTATCATG	24 µl
Reagent Y2 (Index S780)	CATGAATG	24 µl
Reagent Y2 (Index S781)	CTGTACGG	24 µl
Reagent Y2 (Index S782)	CACTCGAG	24 µl

Set S4 Adapters	Sequence	26896
Reagent Y2 (Index S783)	CCGACAAG	24 µl
Reagent Y2 (Index S784)	CTTGCTTC	24 µl
Reagent Y2 (Index S785)	CGCCTTAT	24 µl
Reagent Y2 (Index S786)	GCAACCAT	24 µl
Reagent Y2 (Index S787)	TGACCGTT	24 µl
Reagent Y2 (Index S788)	TTGAGCTC	24 µl
Reagent Y2 (Index S789)	CCACATTG	24 µl
Reagent Y2 (Index S790)	AGCCAACT	24 µl
Reagent Y2 (Index S791)	ATCACGTT	24 µl
Reagent Y2 (Index S792)	TCTCGGTT	24 µl
Reagent Y2 (Index S793)	TTGACTCT	24 µl
Reagent Y2 (Index S794)	TCGAAGTG	24 µl
Reagent Y2 (Index S795)	CACCCAAA	24 µl
Reagent Y2 (Index S796)	CTTCACAT	24 µl

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.

Reagents	26596	26696	26796	26896	269384
Reagent B2	211 µl	211 µl	211 µl	211 µl	844 µl
Reagent R1	528 µl	528 µl	528 µl	528 µl	2,112 µl

For questions, please contact TechSupport@swiftbiosci.com.

Section C: Helpful Information and Troubleshooting

Problem	Possible Cause	Suggested Remedy
Library migrates unexpectedly on Bioanalyzer.	When analyzed on the Agilent High Sensitivity chip, migration behavior overestimates library size of PCR-free libraries made from DNA fragmented to the 200–300 base range (as required in this protocol).	• "200 bp insert" PCR-free libraries should migrate to a ≈500 bp peak and "350 bp insert" PCR-free libraries should migrate to a ≈800 bp peak on the High Sensitivity Chip. • Consult the Expected Results section and the application note released by Covaris titled "Analysis of DNA Fragments Using the Agilent 2100 Bioanalyzer" to ensure proper analysis of library size.
	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: broad or lopsided (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 TechSupport@swiftbiosci.com, or by phone at 734.330.2568 (9:00 am-5:00 pm ET, Monday-Friday).

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