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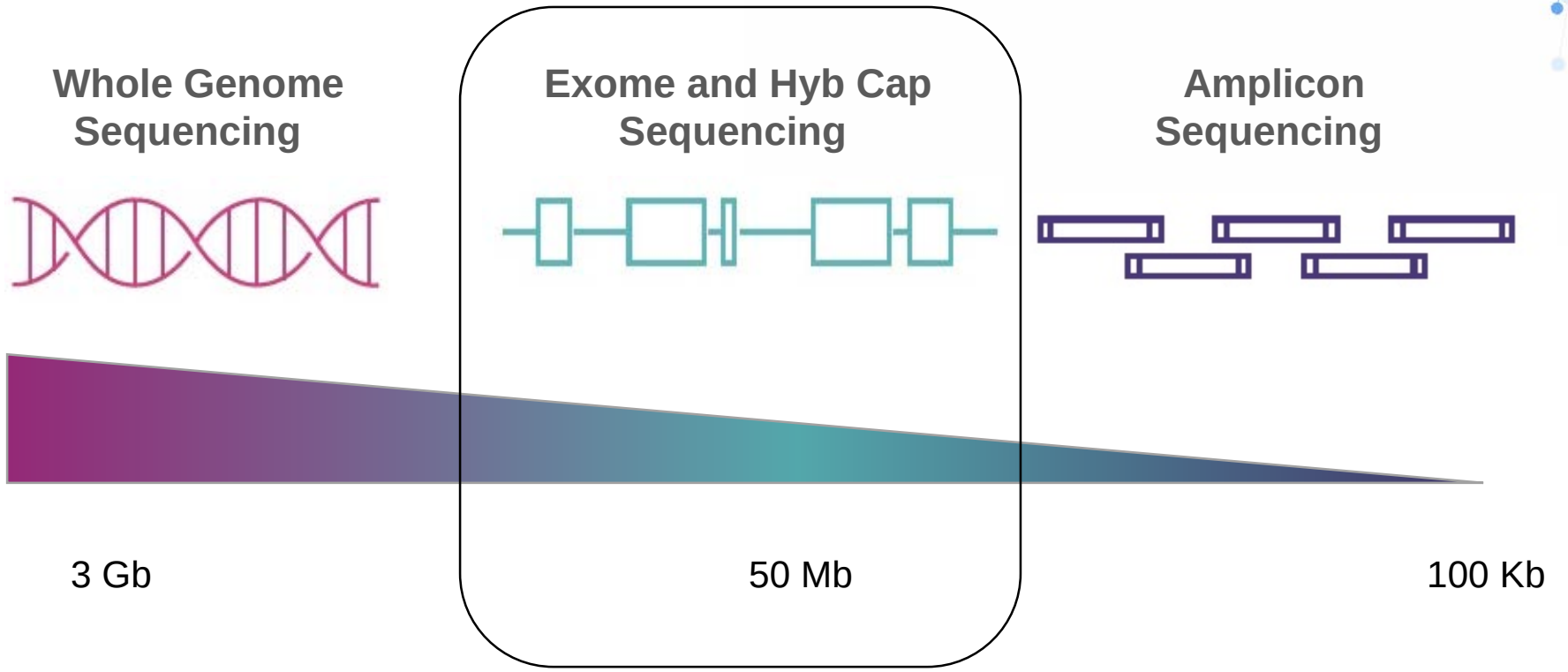
Swift Hybridization Capture Kits

Overview

Swift Now Offers Hybridization Capture Products!



- Swift Exome Hyb Panel
- Swift Pan-Cancer Hyb Panel
- Swift Inherited Diseases Hyb Panel

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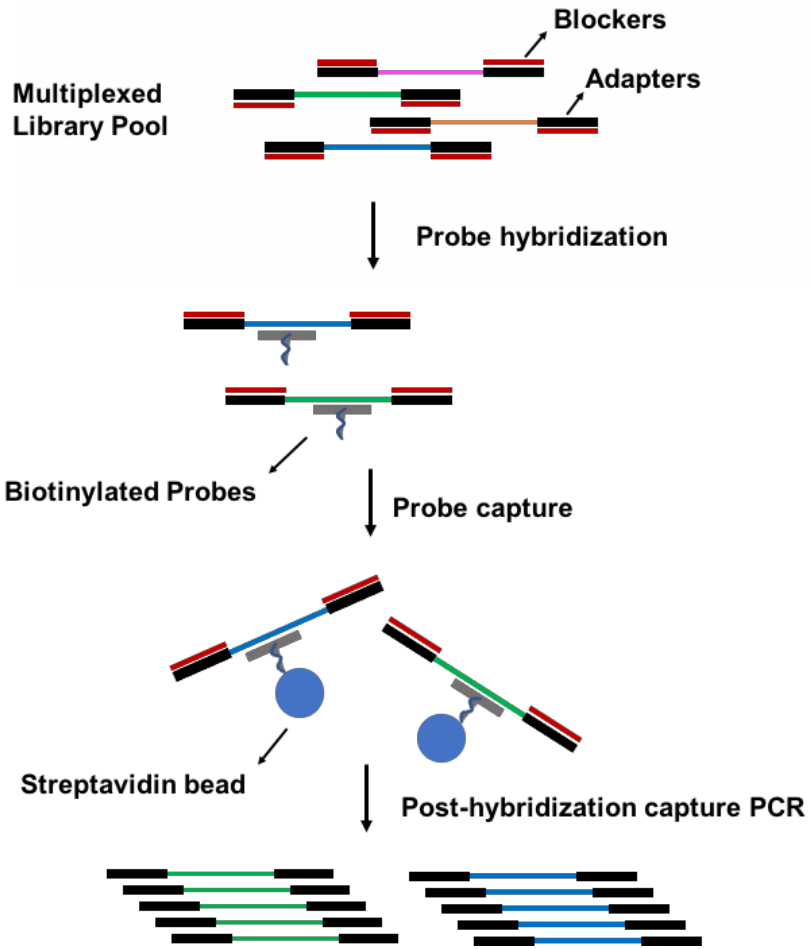
Highlights

- **Enables enrichment of the human exome or subsets of disease-related genes**
  - Superior on-target performance and comprehensive coverage of human coding sequences from the RefSeq database, probes designed to version hg19
- **Saves sequencing costs**
  - Pre-capture multiplexing facilitates orders of magnitude more efficient next generation sequencing by targeting genes of interest while conserving enrichment reagents
- **Provides high quality data**
  - Probes achieve deep and uniform coverage even across GC-rich regions such as first exons

Workflow

- The libraries are pooled and combined with blocking oligos and human Cot DNA.
- The libraries are hybridized to the Swift probes.
- Capture beads pull down the library-probe complexes and unbound fragments are removed by washing.
- The enriched library pool is amplified by PCR, ready for multiplexed Illumina® Sequencing.



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Applications & Sample Types

- Hybridization capture of relevant genomic regions (i.e., exome) or transcripts
- Detection of germline inherited SNVs and Indels
- Low frequency somatic variant detection of SNVs and Indels
- Copy number variant detection

- Compatible with the following DNA library kits:
  - Swift 2STM Turbo and Swift 2S Turbo Flexible, for high quality genomic DNA and FFPE
  - Accel-NGS® 2S Hyb for FFPE, cfDNA, and incorporation of molecular identifiers (MIDs) for ultra-low frequency variant detection
  - Accel-NGS® 1S Plus for heavily nicked or denatured samples
  - Swift Normalase™ for post-hybridization library normalization

Product Details

Feature	Swift Exome	Swift Pan-Cancer	Swift Inherited Diseases
Human Target Region	39 Mb	0.8 Mb	11.1 Mb
Genes Covered	Coding region of 19,396 coding genes	127 oncology-related genes* implicated in 12 tumor types	4,503 genes* based on the Human Gene Mutation Database (HGMD®)
Input DNA and Multiplexing	500 ng per library, 1-12-plex (up to 6 µg)		
Depth Recommendations	~100x, Detecting germline variants (SNV, Indel, CNV)	~1000x to 10,000x (+MIDs), Detecting somatic variants (SNV, Indel, CNV)	~100x, Detecting germline variants (SNV, Indel, CNV)
Compatible Platforms	All Illumina Platforms		

Swift Hyb Panels Workflow

1	•Combine DNA with Blockers •Dry Down DN STOP •Perform Hybridization reaction	15 minutes Variable 4-16 hours
2	•Prepare buffers	15 minutes*
3	•Wash streptavidin beads	15 minutes*
4	•Perform bead capture	45 minutes
5	•Perform washes	30 minutes
6	•Perform post-capture PCR STOP	30 minutes
7	•Purify post-capture PCR fragm STOP	30 minutes

* Perform during hybridization reaction

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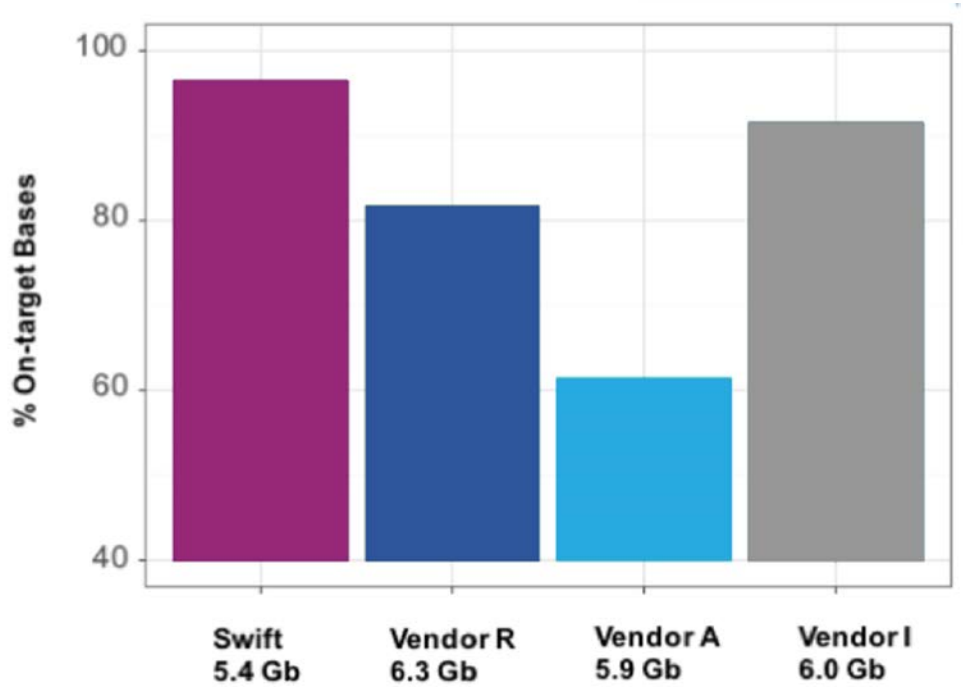
Performance Data

Swift Exome Enables High Coverage Uniformity and On-Target Performance

	Input (ng)	%Duplication	Mean Bait Cov	%Covered 20x	%Covered 50x	%Covered 100x	%Bases on Target
NA12878	100	18.2	154	97.9	87.7	53.0	93.6
	50	17.5	151	98.0	89.0	50.7	94.2
	25	17.4	146	97.9	89.0	50.4	94.1
	10	18.8	149	98.0	87.5	51.5	94.2
	1	47.6	151	97.2	74.3	14.1	94.3

Range of input NA28178 DNA were used as input to the 2S Turbo Library Kit and Swift Exome Hyb Panel for enrichment to prepare and sequenced on ILMN HiSeq4000.

Highest On-Target Delivered by Swift Exome Hyb Panel



In this study, an independent, large genome center compared the Swift Exome Hyb Panel to exome panels from other vendors. Results demonstrated that the Swift Exome Hyb Panel delivered the most uniform coverage (data not shown, but available upon request) and highest on-target mapping of the exome panels tested. The percentage of bases on target for each vendor's panel was calculated across a 500 bp flanking region.

Better Coverage, Lower Duplicates with Swift 2S Turbo and Swift Exome at Low Input

Prep	Panel	%Duplication	Mean Bait Cov	%Covered 20x	%Covered 50x	%Covered 100x	%Bases on Target
Turbo	Swift Exome	47.6	151	97.2	74.3	14.1	94.3
Comp-N		79.1	155	74.1	1.01	0.03	93.9
Comp-K		73.1	147	66.1	21.0	0.05	94.3
Comp-M		57.4	77	25.1	0.11	0.02	56.0

Swift 2S Turbo was evaluated for enrichment via Swift Exome Hyb Panel using 1 ng of DNA and compared to other library prep kits. Coverage uniformity and complexity was significantly higher with Turbo libraries compared to competitors.

MIDs Enable Ultra-Low Frequency Variant Detection Using Swift Pan-Cancer Hyb Panel

gDNA Spike-in Variants

Chr: Position	ALLELE FREQUENCY			
	Sample 4		Sample 5	
	Expected	Observed	Expected	Observed
2: 212243011	1.0%	**1.1%**	0.5%	**0.6%**
2: 212244761	1.0%	**0.9%**	0.5%	**0.3%**
2: 212245090	1.0%	**0.5%**	0.5%	**0.4%**
2: 212245489	1.0%	**1.3%**	0.5%	**0.7%**
3: 176738798	1.0%	**1.1%**	0.5%	**0.6%**
3: 176739663	1.0%	**1.2%**	0.5%	**0.7%**

cfDNA Spike-in Variants

Chr: Position	ALLELE FREQUENCY					
	Sample 1		Sample 2		Sample 3	
	Expected	Observed	Expected	Observed	Expected	Observed
2: 212244718	1.0%	**1.05%**	1.0%	**0.87%**	1.0%	**0.77%**
12: 25361074	1.0%	**1.15%**	1.0%	**1.16%**	1.0%	**1.01%**
12:25361142	1.0%	**1.40%**	1.0%	**0.97%**	1.0%	**0.66%**
12: 25361646	1.0%	**1.39%**	1.0%	**1.40%**	1.0%	**0.59%**
12: 40688695	1.0%	**0.71%**	1.0%	**0.97%**	1.0%	**0.55%**
12: 115108136	1.0%	**0.90%**	1.0%	**1.96%**	1.0%	**0.70%**

cfDNA was extracted from blood of four individuals with unique genetic background and Coriell gDNA samples from different genetic backgrounds were obtained. To determine the effect of MIDs on low frequency variant calling, sample spike-ins were performed at 1% or 0.5% frequency into 10 ng cfDNA or 100 ng gDNA. Libraries were prepared with the Swift 2S Hyb kit with MIDs, enriched with the Swift Pan-Cancer Hyb Panel that covers an 800kb target containing 127 genes, and sequenced on an Illumina HiSeq ® to a minimum of 8000x coverage. A consensus sequence was generated for each MID family (BMFtools) and data were analyzed for homozygous SNPs present in the spike-in sample only. 6/6 known variants were present in all three 1% cfDNA samples and 27/27 known variants were present in both 1% and 0.5% gDNA samples depicting the power of MIDs for low frequency variant calling (6 shown in this slide).

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High On-Target Performance with Swift Inherited Diseases and Pan-Cancer Hyb Panels

Library	Sample Type	Sample Input	Mean Insert Size	%Duplication	Mean Bait Coverage	%Covered > 20X	%Covered > 40X	%Bases On-Target
Inherited Diseases_1	NA12878	50 ng	194.9	2.1	61x	99.3	60	95
Inherited Diseases_2			193.9	1.8	61x	99.3	61	95
Inherited Diseases_3			193.0	2.0	61x	99.3	60	95
Inherited Diseases_4			192.5	2.1	61x	99.3	60	95
PanCan_1			189.6	1.7	77x	98.9	96.2	78
PanCan_2		250 ng	196.5	2.1	79x	98.7	95.7	79

Table 1. *Swift 2S Turbo DNA Library Kit was evaluated for enrichment using Swift Inherited Diseases Panel and Pan-Cancer Panel. Comparable target coverage and complexity was observed with varied input Turbo libraries regardless of the panel size.*

Product Use Information

Ordering Information

Product Name	Reactions	Catalog No.
Swift Exome Hyb Panel	16	83216
Swift Pan-Cancer Hyb Panel	16	83816
Swift Inherited Diseases Hyb Panel	16	83416
Swift Hyb and Wash Kit	16	88016
Swift Hyb, Wash, and Universal Blocker Kit	16	89016
Swift Library Amplification Primer Mix	96	88196

Frequently Asked Questions

Swift Hybridization Capture Kits_ FAQs

What are the common applications for Swift Hybridization Capture Kits?

- The Swift Hybridization Capture Kits can be applied to:
  - Low frequency somatic variation detection of SNVs and Indels
  - Copy number variation detection
  - Detection of germline inherited SNVs and Indels
  - Hybridization capture of relevant genomic regions (i.e., exome) or transcripts of interest

What library preparation kits does this protocol support?

- This protocol have been validated with TruSeq libraries constructed using the Swift 2S Turbo, Accel-NGS 2S DNA Library Kits, KAPA Hyper Prep kits, NEB Ultra Kits, and Nextera DNA Library Prep Kits from Illumina.

What are the fragment size ranges for working with this protocol?

- For optimal results, we recommend using fragmented DNA between 150-350 bp.

Swift Hybridization Capture Kits_ FAQs

What is the shelf life of the Swift Hybridization Capture Kits?

- The shelf life of the Swift Hybridization Capture Kits is 6 months, when stored at -20 ºC.

Can I use my favorite polymerase to amplify libraries prior to hybrid capture?

- No, This protocol has been validated with KAPA HiFi HotStart ReadyMix from Kapa Biosystems (Cat# KK2601). We recommend performing the pre-capture PCR using KAPA polymerase to produce at least 500 ng of each prepared library for hybridization capture.

What is the recommended method for quantifying amplified libraries prior to performing hybridization capture?

- Pre-capture libraries can be quantified by Qubit and assessed for library size on the Bioanalyzer.

How many libraries can be multiplexed into each hybridization capture?

- For hybridization capture, multiplexing has been tested on up to 12 libraries (6 µg total DNA) with limited impact on data quality.

Swift Hybridization Capture Kits_ FAQs

How do I select the appropriate blocking oligos for my libraries?

- Using the correct blocking oligos can significantly improve overall capture performance. Swift blocking oligos are compatible with libraries and can be readily used with other Swift hybridization capture products. These blockers are designed for both single and dual indexing strategies when the index sequences are 6 to 8 bases in length or when using the Swift 9 base molecular identifiers (MIDs, Cat. No. 27024, 27096). Contact TechSupport@swiftbiosci.com, for compatibility with alternate adapter sequences or indexing strategies.
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What is the minimum amount of library required for capture?

- We recommend using 500 ng of each prepared library for hybridization capture. For exome captures, Using less input for capture can result in higher duplicate rates, lower mean coverage, and poor coverage uniformity.

What is the most optimal method to concentrate libraries with blocking oligos prior to hybridization capture?

- For optimal results, use a SpeedVac™ system (Savant) for concentrating DNA. Although the optional Appendix: AMPure XP Bead DNA concentration protocol can be used, our testing has found reproducible, though minor, adverse impact on GC bias during bead-based concentration.
- Note: To multiplex a high quantity of samples, we recommend using a SpeedVac system; if you require a quicker turnaround, you may prepare the DNA samples following the instructions in the Appendix for the bead-based DNA concentration protocol.

Swift Hybridization Capture Kits FAQs

What Cot DNA should I use if my DNA samples are non-human?

- If your experiment focuses on non-human library captures, realize that Human Cot DNA included in the Swift Hybridization and Wash Kits might not be optimal. For the best results, use alternatives like mouse Cot DNA, or Salmon sperm DNA.

When and how do you recommend using the plate protocol?

- We recommend using the plate protocol when processing more than 6 captures. Using the plate protocol (versus the tube protocol) shows lower sample-to-sample variability within one experiment. When working with plates, avoid using the wells on the perimeter of the plate's edges. The plate protocol is optimized for a maximum of 4 columns of captures in standard 96-well plate format. We do not recommend running more than 32 captures at a time because the timing and temperature of washes will be impacted.

What is the optimal hybridization reaction incubation time?

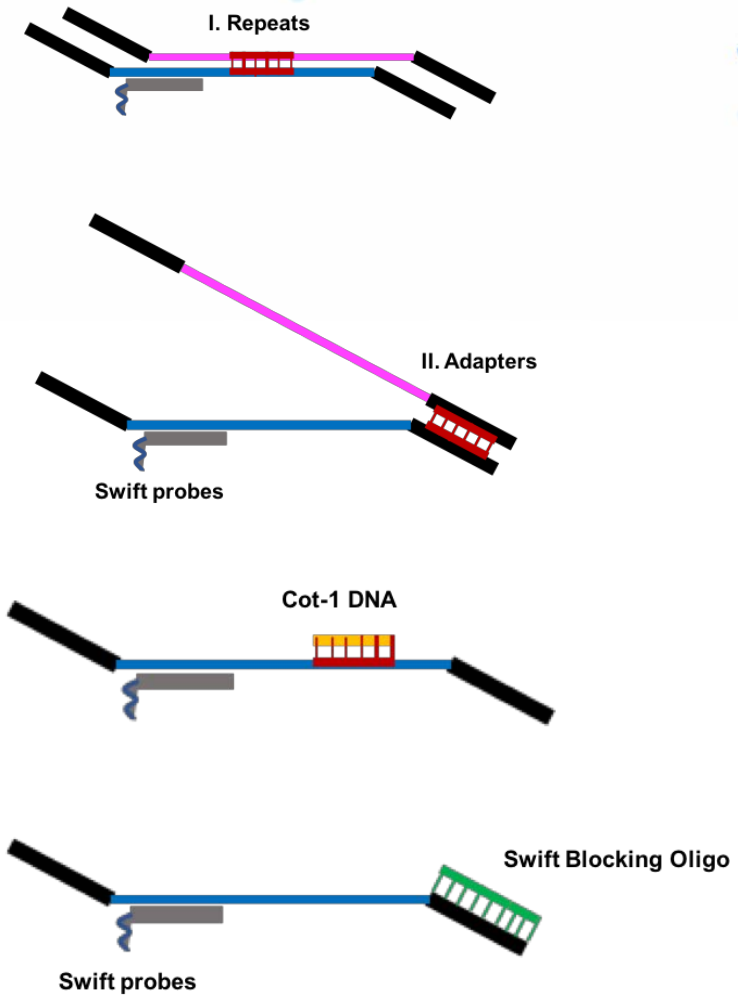
- We recommend a 4 hour hybridization incubation duration which the hybridization reaction tube or plate should be kept tightly sealed. Extending hybridization incubation time from 4 to 16 hours may improve panel performance, particularly for GC-rich or smaller panels (<1000 probes).

Swift Hybridization Capture Kits FAQs

What types of standard blocking oligos are needed and what are their function?

- They are two major issues when capturing DNA with the Swift probes.
  - The contribution of the repeat elements (I) even if you do not design your probes to target these sequences. The repeat can be within the fragment that you capture pulling down off-target material.
  - The other problem is with the adapter-adapter hybridization (II). The adapters are single sequence oligos that have the ability to hybridize to other adapters pulling down off-target DNA regions.

- The solutions are:
  - I) Cot-1 DNA hybridizes to the repeat elements and both to your target as well as the off target regions inhibiting the pulling down of the off target fragment with the on-target fragment
  - II) Swift blocking oligos are single oligo sequences. They are complementary to the TruSeq adapter sequences and have a 3' modification to inhibit extension. They bind to adapter sequences to inhibit the hybridization of the adapters to one another.



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Swift Hybridization Capture Kits_ FAQs

Do I need extra Cot DNA if performing the optional AMPure XP Bead DNA concentration protocol?

- Yes, understand that the optional Appendix: AMPure XP Bead DNA concentration protocol requires more Human Cot DNA than the standard SpeedVac method outlined in the protocol. Additional Human Cot DNA can be purchased through IDT (IDT Cat# 1080768; 1080769). Using the standard amount of Human Cot DNA with AMPure XP Bead DNA concentration protocol can lead to lower flanked on-target percentage due to loss of small human Cot DNA fragments (50-300 bp fragment size) during AMPure XP concentration.

What are the critical steps to avoid capture failure?

- Preheat the “heated wash buffers” a minimum of 15 minutes before use. Preheating helps ensure there is sufficient time to heat the buffers to 65 ºC at critical steps during the protocol.
- Vortex every 10 to 12 minutes during the 45-minute bead capture to improve the kinetics of the capture.
- Do not let the Streptavidin beads dry out at any point during the protocol. If necessary, extend washes slightly rather than let the beads dry out.
- Ensure the Streptavidin beads remain fully re-suspended during room temperature washes. Vortex vigorously and adhere to the incubation guidelines during room temperature washes to improve data quality.
- Always use fresh seals in each step of the protocol that calls for adhesive seals on plates. Using fresh seals avoids possible sample cross-contamination during the plate protocol.

THANK YOU

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