ACCEL-NGS® 2S HYB DNA LIBRARY KIT

The Accel-NGS 2S Hyb DNA Library Kit constructs high complexity libraries from low inputs for hybridization capture applications. This high efficiency library preparation enables targeted sequencing of samples of limited quality or quantity. Now meaningful targeted sequencing data can be obtained from samples that would fail with less efficient library preparations, expanding the sample types that can be successfully analyzed.

Features

- Validated as low as 1 ng human DNA
- 5′ and 3′ end repair steps maximize recovery of damaged samples
- Increased library complexity
- Balanced coverage of AT-/GC-rich regions

Supported DNA Sequencing Applications and Sample Types

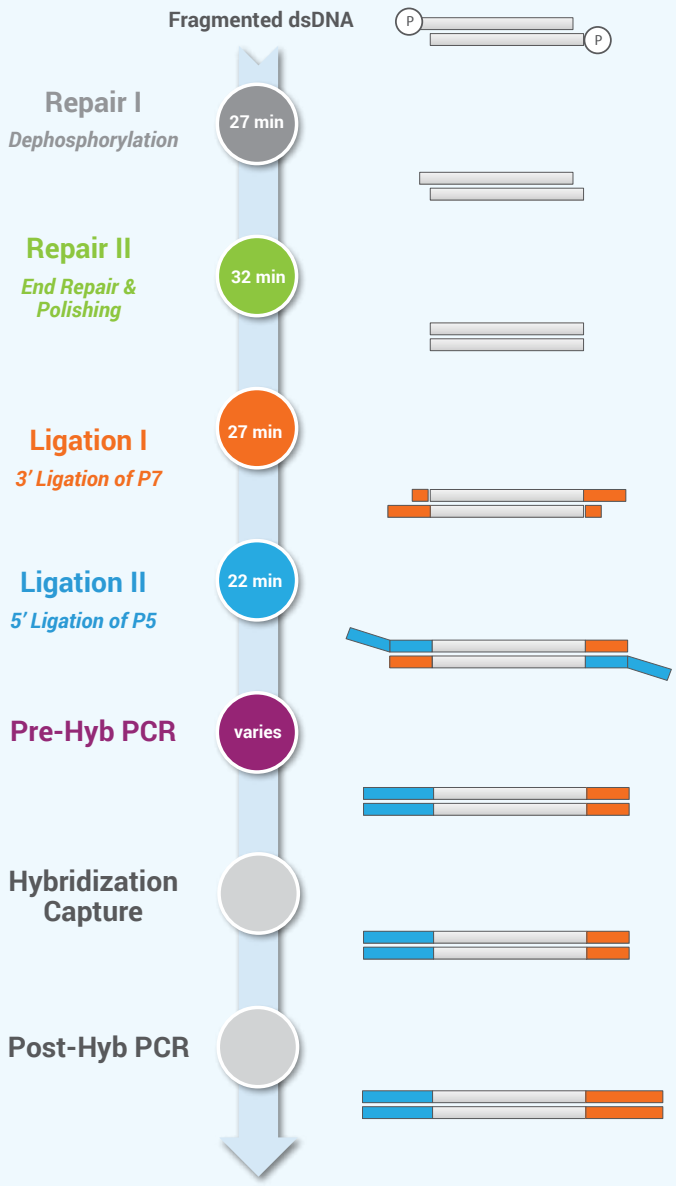
- Whole exome sequencing
- Specific gene panel targeted sequencing
- Low input FFPE samples
- Low input circulating, cell-free DNA (cfDNA)

Compatibility

Accel-NGS 2S Hyb libraries can be constructed with one of several available 2S Indexing Kits that are designed to match commercially-available panels. This enables compatibility with:

- Agilent SureSelect^{XT} and SureSelect^{XT2}
- NimbleGen™ SeqCap™ EZ
- IDT xGen® Lockdown® Probes
- Custom hyb-based panels

Hybridization Capture Workflow



Low Input Performance with Cancer Panels

FFPE Fixation Time Course with IDT xGen Pan-Cancer Panel

Input Quantity	Sample Type	% Aligned	% Duplication	Mean Bait Coverage	% Covered ≥ 1X	% Covered ≥ 20X	% Bases on Target
100 ng	Frozen	96	1	42X	99	91	80
	6 hr. Fix	96	1	43X	99	93	81
	24 hr. Fix	97	1	44X	99	93	82
	48 hr. Fix	97	1	45X	99	88	82
10 ng	Frozen	96	3	42X	99	90	80
	6 hr. Fix	96	5	41X	99	92	80
	24 hr. Fix	97	4	42X	99	93	81
	48 hr. Fix	97	8	42X	99	86	81
1 ng	Frozen	95	18	33X	99	85	77
	6 hr. Fix	94	32	26X	99	77	74
	24 hr. Fix	95	31	27X	100	79	76
	48 hr. Fix	95	44	22X	99	53	73

Accel-NGS 2S Hyb libraries were constructed with 100, 10, and 1 ng of DNA. DNA extracted from the same normal kidney sample which had either been fresh-frozen or fixed for 6, 24, or 48 hours before being paraffin-embedded. Amplified libraries were enriched with the IDT xGen Pan-Cancer Panel. The xGen Pan-Cancer Panel is 0.9Mb and all samples were normalized to 0.6M reads.

Formalin-Compromised DNA with IDT xGen Pan-Cancer Panel

Input Quantity	Sample Type	% Aligned	% Duplication	Mean Bait Coverage	% Covered ≥ 1X	% Covered ≥ 20X	% Bases on Target
5 ng	HD701	97	4	41X	99	94	82
	HD-C749	97	5	43X	99	95	82
	HD-C751	95	34	28X	99	57	74
1 ng	HD701	97	20	33X	99	90	79
	HD-C749	97	19	35X	99	91	80
	HD-C751	92	69	8X	97	6	50

Accel-NGS 2S Hyb libraries were constructed from 5 and 1 ng of Horizon Discovery standards. HD701 is not a formalin-compromised sample. HD-C749 and HD-C751 are formalin-compromised versions of the same DNA present in HD701. Libraries were enriched with the IDT xGen Pan-Cancer Panel. The Pan-Cancer Panel is 0.9Mb and samples were normalized to 0.6M reads.

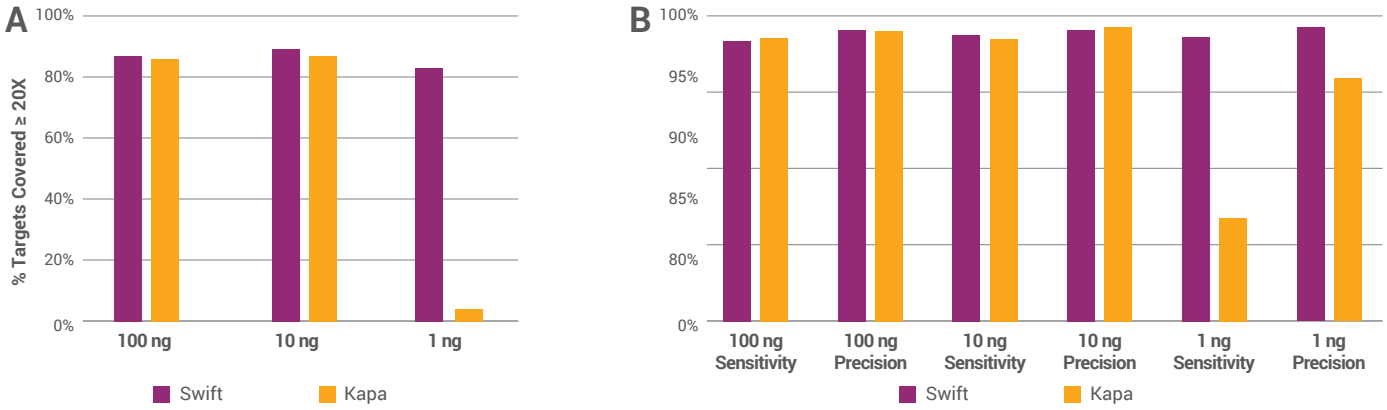
FFPE and cfDNA Samples with Agilent SureSelect^{XT} Custom Cancer Panel

Sample Type	Input Quantity	% Aligned	% Duplication	Mean Bait Coverage	% Covered ≥ 1X	% Covered ≥ 20X	% Bases on Target
FFPE	20 ng	97	18	121X	99	99	89
	10 ng	96	30	96X	99	98	88
cfDNA	20 ng	97	20	111X	99	98	80
	10 ng	96	38	95X	99	98	80

The Accel-NGS 2S Hyb Library Kit was used to make libraries of two different inputs from FFPE lung tumor sample and cfDNA. Libraries were enriched using the Agilent SureSelect^{XT} Quintiles Comprehensive Cancer Panel (QCCP). Thank you to Q^2 Lab Solutions for generation and sequencing of libraries.

Accurate Variant Calls with NimbleGen SeqCap EZ Medexome Panel

Input Quantity	Method	% Aligned	Estimated Library Size (M)	% Duplication	Mean Bait Coverage	% Bases on Target
100 ng	**SWIFT**	93	1,125	1	50X	67
	Kapa	93	240	6	51X	74
10 ng	**SWIFT**	93	275	5	52X	67
	Kapa	93	97	13	47X	68
1 ng	**SWIFT**	93	45	26	37X	65
	Kapa	90	7	71	10X	65



Accel-NGS 2S Hyb Kit and Roche Kapa Library Preparation Kit performance was compared with the NimbleGen SeqCap EZ MedExome Panel. High quality Coriell NA12878 gDNA at 100, 10, and 1 ng inputs were evaluated. Reads were normalized to 39M for comparison of coverage metrics. Sensitivity (TP/TP+FN) and precision (TP/TP+FP) metrics refer to SNP variant calls. SNP concordance with the NIST GIAB truth list in high-confidence regions was ≥ 99% for all inputs and library preparation methods, with the exception of Kapa 1 ng, which was 98% (Zook et al. Nature Biotechnology 2014). Kapa Library Preparation performance drops significantly at 1 ng, illustrated by a sharp rise in % duplication rates and dramatic decreases in mean coverage depth (as shown in the table above), % targets covered ≥ 20X (A), and sensitivity/precision of SNP variant calls (B).

Limit of Detection Analysis with 10 ng cfDNA

CHR:POS	Allele: Sample 1 (Homozygous)	Allele: Samples 2 & 3 (Homozygous)	Allele Frequency (Mix 1% Sample 1 + 99% Sample 2)		Allele Frequency (Mix 1% Sample 1 + 99% Sample 3)	
			Expected	Observed	Expected	Observed
2: 212244718	C	T	C=1.0%	C=0.6%	C=1.0%	C=1.0%
12: 25361074	A	G	A=1.0%	A=1.6%	A=1.0%	A=1.9%
12: 25361142	G	A	G=1.0%	G=1.1%	G=1.0%	G=0.9%
12: 25361646	C	T	C=1.0%	C=1.9%	C=1.0%	C=1.6%
12: 40688695	C	T	C=1.0%	C=0.5%	C=1.0%	C=1.1%
12: 115108136	C	T	C=1.0%	C=0.7%	C=1.0%	C=2.0%

cfDNA was extracted from the blood of three individuals with unique genetic backgrounds using the PerkinElmer chemagic™ 360, and libraries were constructed with the Accel-NGS 2S Hyb DNA Library Kit. To detect mutations, 1% of cfDNA from Sample 1 was spiked into 10 ng cfDNA from Samples 2 and 3 (~30 into 3,000 chromosomal copies). Libraries were enriched using the IDT xGen Pan-Cancer Panel and were sequenced on a HiSeq® 2500.

Ordering Information

Product Name	Reactions	Catalog No.
Accel-NGS 2S Hyb DNA Library Kit	24	23024
Accel-NGS 2S Hyb DNA Library Kit	96	23096

An Accel-NGS 2S Hyb Indexing Kit compatible with your hybridization capture technology is required for complete functionality of the library kit.

Agilent SureSelect^{XT} and SureSelect^{XT2}* Target Enrichment

Indexing Adapter Kit	Reactions	Catalog No.
SureSelect^{XT} Compatibility Module	24	26424
SureSelect^{XT} Compatibility Module	96	26496

**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 Accel-NGS 2S Hyb Library Kit Technical Note for details.*

NimbleGen SeqCap EZ Target Enrichment

Indexing Adapter Kit	Reactions	Catalog No.
2S Set A Indexing Kit (12 indices)	48	26148
2S Set B Indexing Kit (12 indices)	48	26248
2S Set A+B Indexing Kit (24 indices)	96	26396

IDT xGen Lockdown Probes

Indexing Adapter Kit	Reactions	Catalog No.
2S Set A Indexing Kit (12 indices)	48	26148
2S Set B Indexing Kit (12 indices)	48	26248
2S Set A+B Indexing Kit (24 indices)	96	26396
2S Dual Indexing Kit (96 combinations)	96	28096

Visit **www.swiftbiosci.com** for easy ordering.

Swift Biosciences, Inc.
58 Parkland Plaza, Suite 100 • Ann Arbor, MI 48103 • 734.330.2568 • www.swiftbiosci.com