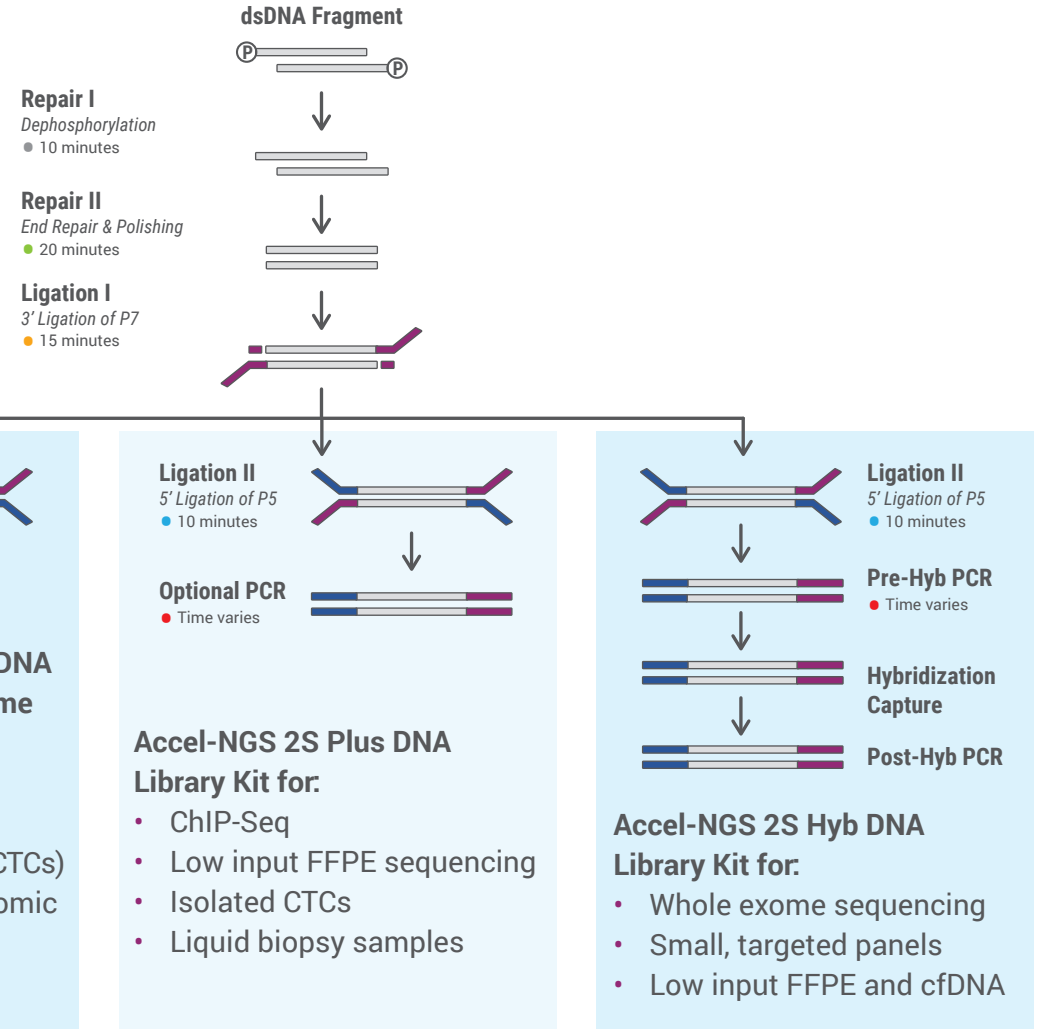
ACCEL-NGS® 2S DNA LIBRARY KITS

Accel-NGS 2S DNA Library Kits produce high quality libraries with an all-inclusive, easy-to-use format. The kits contain all reagents necessary to build high complexity libraries from a wide range of input quantities and a variety of sample types, including microbial samples, FFPE, and cell- free DNA (cfDNA). They can be used for PCR-free whole genome sequencing and ChIP-Seq, as well as targeted sequencing by hybridization capture. The Accel-NGS 2S Kits have been optimized for use with sample indices (barcodes) for multiplex sequencing, as well as with molecular identifiers (MIDs), which are used to filter duplicates during data analysis.



Accel-NGS 2S PCR-Free* DNA Library Kit for whole genome sequencing of:

- FFPE
- cfDNA
- Circulating tumor cells (CTCs)
- Microbial and metagenomic samples

Accel-NGS 2S Plus DNA Library Kit for:

- ChIP-Seq
- Low input FFPE sequencing
- Isolated CTCs
- Liquid biopsy samples

Accel-NGS 2S Hyb DNA Library Kit for:

- Whole exome sequencing
- Small, targeted panels
- Low input FFPE and cfDNA

**PCR-free capability is determined by sample quality and input quantity. Accel-NGS 2S PCR-free libraries can be generated from as low as 100 ng of high quality genomic DNA or 10 ng of circulating, cfDNA.*

Accel-NGS 2S Kit Overview

Swift Biosciences offers the following Accel-NGS 2S Kits:

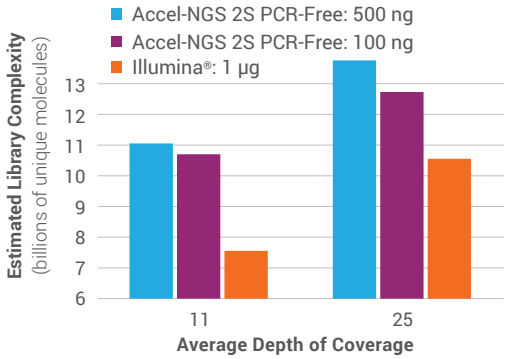
- Accel-NGS 2S PCR-Free
- Accel-NGS 2S Plus
- Accel-NGS 2S Hyb

These DNA Library Kits utilize the same proprietary technology to efficiently convert input DNA into sequencable library molecules. These kits enable high quality next-generation sequencing (NGS) analysis of degraded and ultra low inputs of nucleic acid. PCR-free libraries can be constructed from cfDNA ≥10 ng and gDNA ≥ 100 ng.

Features and Benefits

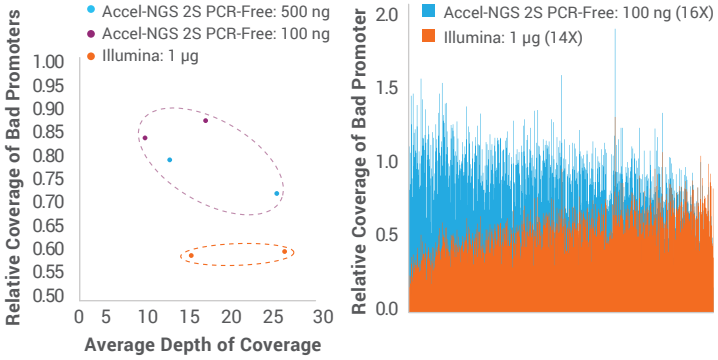
Features	Benefits
5′ and 3′ repair steps	Recovery of damaged samples
Balanced coverage of AT-/GC-rich regions	Suitable for a diverse set of sample types
Superior library preparation efficiency	More unique molecules available for capture
No adapter titration or heat steps involved	Readily automatable
PCR-free library construction from ≥ 10 ng of DNA	Minimized amplification bias
“With bead” workflow	Reduce workflow costs and time

Higher Complexity Libraries From Less DNA



Library complexity was obtained at various sequencing depths for Accel-NGS 2S PCR-free libraries compared to libraries made with the leading kit.

Cover High GC-Rich Regions Better



Relative coverage of the GC-rich 1000 bad promoters obtained at various sequencing depths of Accel-NGS 2S PCR-free libraries compared to libraries made with the leading kit.

Increased Coverage at Low Inputs

	1 ng				100 pg			
	PCR Cycles	Reads (Millions)	Average Coverage	Duplication Rate (%)	PCR Cycles	Reads (Millions)	Average Coverage	Duplication Rate (%)
Swift	9	11.3	16X	0.4	12	14.5	20X	1.2
Kapa	12	11.3	14X	7.9	16	14.5	14X	10.4
NEB	14	11.3	15X	1.2	17	14.5	14X	10.4
Nextera	12	11.3	13X	3.4	—	—	—	—

A sample representing a broad range of base compositions (19-71% GC) was constructed using a genomic equivalent mixture of six microbial genomes: B. pertussis *(68% GC),* R. sphaeroides *(69% GC),* S. avermitilis *(71% GC),* P. falciparum *(19% GC),* S. aureus *(32% GC), and* E. coli *(50% GC). NGS libraries were constructed from 1 ng and 100 pg of this sample, and sequencing metrics were compared across four commercially-available library kits: Swift Biosciences' Accel-NGS 2S Plus, Kapa Biosystems' Hyper, New England BioLabs® NEBNext® Ultra™, and Illumina's Nextera. Data shown is the average of duplicate libraries. Nextera chemistry restricts library construction to only 1 ng of input DNA.*

Molecular Identifiers for Accel-NGS 2S Library Kits

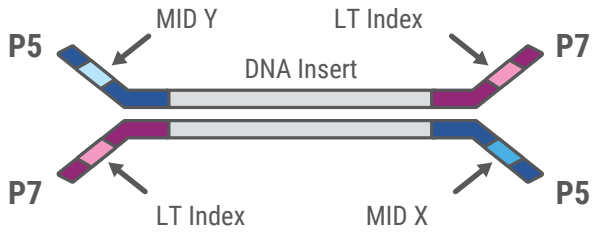
Swift Biosciences offers Molecular Identifiers (MIDs) that have been designed, optimized, and validated for use with the Accel-NGS 2S DNA Library Kits. The use of MIDs improves the detection of low frequency alleles by distinguishing low frequency mutations from synthetic mutations generated during PCR amplification and sequencing. Additionally, these strand-specific MIDs allow accurate filtering of PCR duplicates from fragmentation and strand duplicates. Accurate identification of duplicate reads is especially useful with samples that have undergone non-random fragmentation or are sequenced as a single read. Within cfDNA or ChIP libraries, uniquely derived fragments of the same sequence and length may exist in the final dataset. Previously, these unique fragments would be identified as PCR duplicates. The only way to differentiate between unique fragment reads and reads from PCR duplicates is by using MIDs. The use of MIDs in these samples will improve identification of rare somatic mutations in oncology samples, as well as finding protein factor binding sites in ChIP-Seq samples.

Features

- Incorporate molecular identifiers
- Identify unique library molecules
- Retain more sequencing data
- Improve detection of low frequency alleles
- Readily automate library processing

Applications

- Exome sequencing
- Deep targeted sequencing
- Cell-free DNA sequencing
- Single-end sequencing, such as ChIP-Seq



Accel-NGS 2S MID libraries are constructed with a strand-specific MID sequence on the P5 adapter, and a sample index sequence on the P7 adapter. Each dsDNA substrate receives two independent P5 MID adapters "X" and "Y".

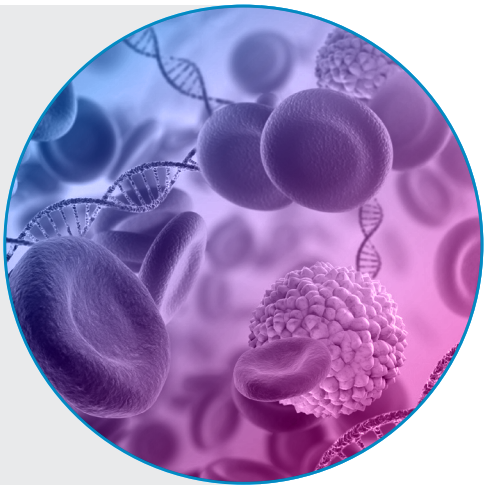
Accel-NGS 2S MID Validation

The 9 bp random sequence MID utilized by Accel-NGS 2S Kits has a maximum of 262,144 theoretical MID sequence combinations. Both PCR-free and amplified libraries were analyzed for number of unique MID sequences and MID copy uniformity. These validation metrics are important indicators of the technology's ability to uniquely identify library molecules.

Approaching Theoretical Copy Uniformity of Unique MIDs at High Sequencing Depth

DNA Library Kit	PCR Cycles	Number of Unique MIDs	Average MID Copy Number	MID Copy Uniformity (%)	Copy Number of Most Prevalent MID	Total Index 2 (I5) Reads
Accel-NGS 2S PCR-Free	0	262,104	20.98	98	464	5,498,922
Accel-NGS 2S Plus	9	262,036	18.50	99	447	4,848,690

Accel-NGS 2S MID libraries attain close to theoretical representation of MID combinations with high copy uniformity (percentage of MIDs that are within 20% of the average MID copy number).

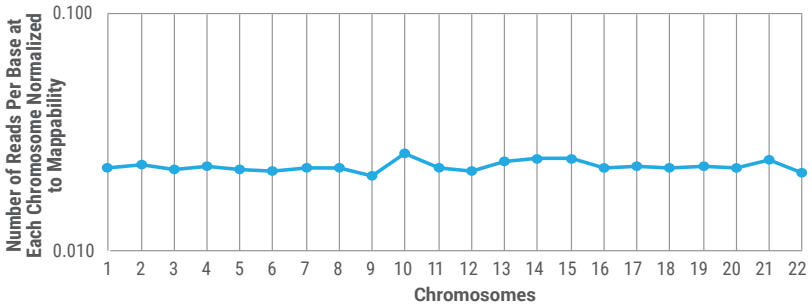


Sequencing Liquid Biopsy Samples

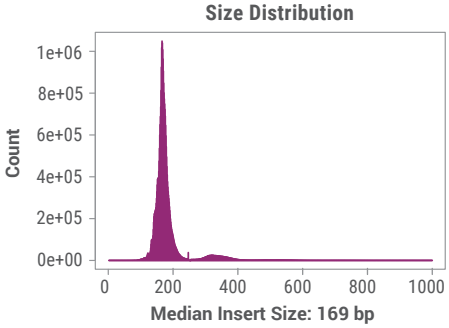
Liquid biopsy assays enable monitoring of disease progression and treatment response of oncology patients in research studies. The Accel-NGS 2S DNA Library Kits efficiently convert input cfDNA from liquid biopsies into sequencable, high complexity libraries to aid in detection of low frequency mutations. From whole genome sequencing to targeted oncology capture, Accel-NGS 2S Kits deliver conversion rates as high as 90% with cfDNA samples.

PCR-Free Prep from 10 ng of Cell-Free DNA

# Reads	% Mapped to Human Genome	Estimated Library Size	Chimera	Dimers
69,853,401	99.5	1.2 x 10^9	2.6%	0.02%



Even at low sequencing depth, the minimal sequence-dependent bias of the Accel-NGS 2S adapter attachment results in even coverage across the genome. Data was normalized to mappable chromosome content. Mappability and read length, Li W. Freudenberg, J.; Front Genet *2014 Nov*



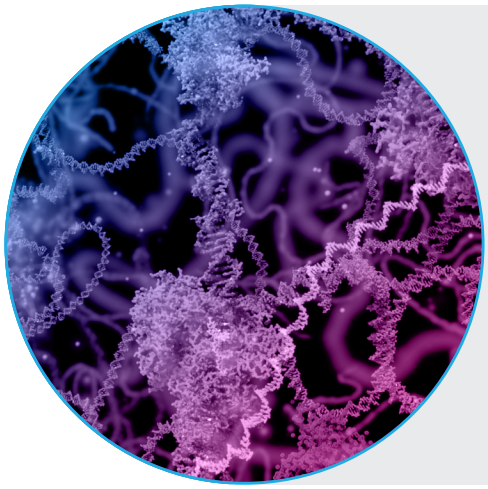
The inherently narrow size distribution of cfDNA, centering around 165 bp, reduces loss of DNA during library prep size selection steps.

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.

Pico-Scale ChIP-Seq



Accel-NGS 2S Kits allow you to push the lower limits of input for your ChIP-seq applications by efficiently converting low input DNA into highly diverse and complex libraries. The proprietary chemistry, which polishes both the 5′ and 3′ ends, produces libraries with an unsurpassed conversion efficiency and balanced representation of both AT-/GC-rich regions. It is possible to construct libraries from as little as 1,000 cells, minimizing the impact of cellular heterogeneity on genome-wide epigenetic profiles for chromatin proteins and transcription factors.

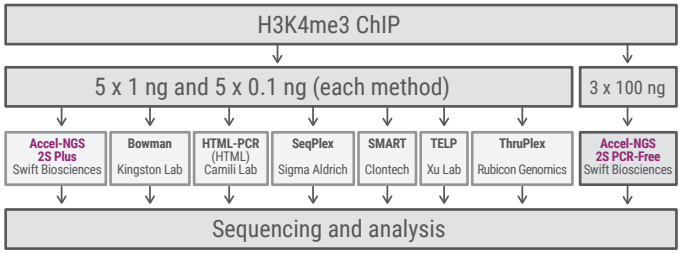
By constructing a more complex NGS library, Swift Biosciences' Accel-NGS 2S Plus Library Kit enables the use of low input samples for ChIP-Seq applications. A recent technology comparison of seven different library preparations by Oslo University demonstrates the superior performance of Swift's Accel-NGS 2S library technology.

Better Library Performance Metrics

- Lower duplicate reads
  - Fewer PCR cycles
  - Ability to retain fragment duplicates
  - Highest library complexity at picogram inputs
- Low base composition bias
  - High coverage of GC-rich promoters
  - Improved coverage uniformity
- Sharper peak calling

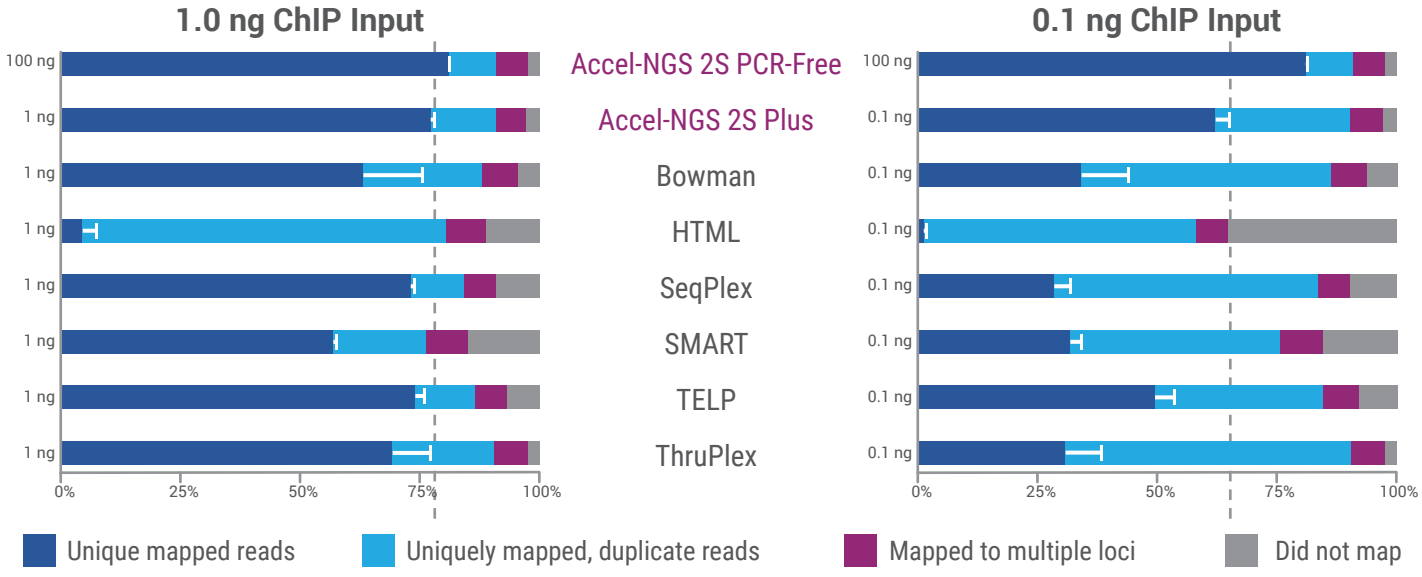
Experimental Design

Oslo University Hospital

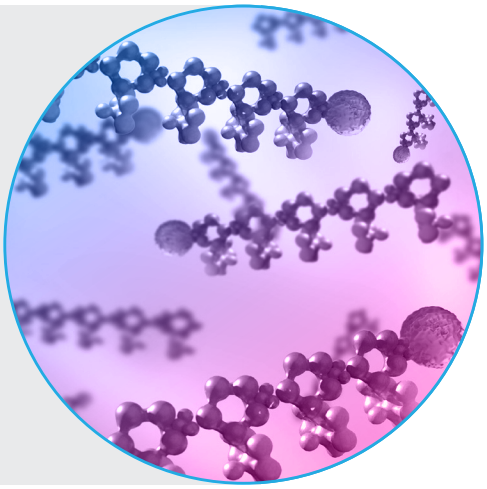


Taken from Sundaram, et al, BMC Genomics (2016) 17:816.

Accel-NGS 2S technology enables the use of ultralow input samples for ChIP-Seq applications by utilizing a unique chemistry for the most efficient preparation of NGS libraries.

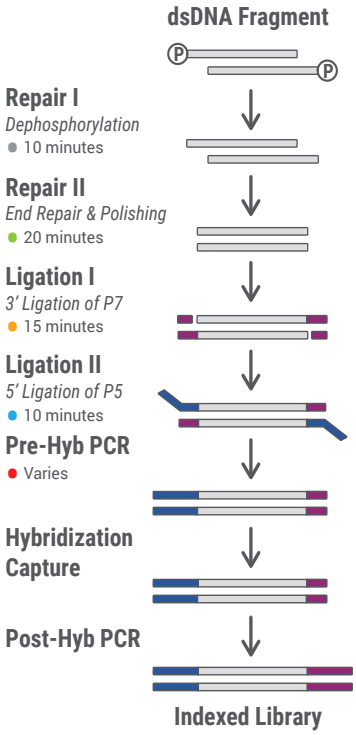


Results shown are the mean of 5 replicates for each method, using 25 million reads per replicate.



Hybridization Capture

The Accel-NGS 2S Hyb DNA Library Kit has been developed for construction of high complexity libraries from low inputs for hybridization and bait capture applications. The kit is ideal for exome sequencing with DNA isolated from FFPE samples. The 5′ and 3′ steps repair the damaged DNA from the FFPE samples, enabling the construction of high quality libraries from nicked, degraded, and damaged samples. The Accel-NGS 2S Hyb Kit works with Agilent, NimbleGen™ and IDT exome and smaller panels. Indexing kits are available that allow for multiplexing samples within a lane on Illumina sequencers such as the HiSeq.



The Accel-NGS 2S Hyb kit is compatible with Agilent SureSelect, NimbleGen SeqCap EZ, and IDT xGen Lockdown probes. This figure depicts a SureSelect^{XT}-specific workflow.

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

Publication Highlight

Libraries were prepared from 13 ng of DNA using the Accel-NGS 2S Hyb DNA Library Kit. Whole exome sequencing was performed and analysis of ctDNA and tumor samples provided insight into mechanisms of resistance in many patients. Read the full publication: “Application of Sequencing, Liquid Biopsies, and Patient-Derived Xenografts for Personalized Medicine in Melanoma.”, Girotti MR., et al. Cancer Discov. (2016) Mar; 6(3):286-99.

Exceptional Coverage Uniformity Achieved for Low Input Coriell Samples

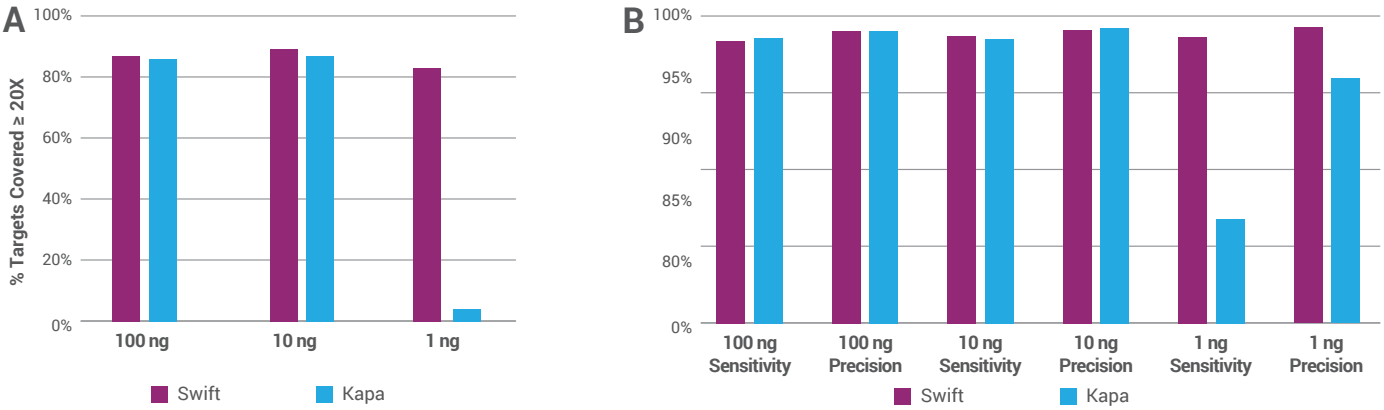
Sample	Input (ng)	Mean Coverage	% Duplication	% Covered 10X	% Covered 50X	% Covered 100X	% Aligned
NA12878	60	35X	1.8	93.4	—	—	97.6
		100X	5.4	98.6	82.8	—	97.6
		250X	12.0	99.1	97.2	88.8	97.6
NA24385	60	35X	1.7	93.1	—	—	97.6
		100X	5.0	98.7	81.3	—	97.6
		250X	11.5	99.2	97.2	88.5	97.6

The Accel-NGS 2S Hyb DNA Library Kit was evaluated with the Agilent SureSelect V5 Exome Panel and 60 ng DNA from two distinct high quality Coriell DNA samples. Libraries were sequenced on a HiSeq 2500 Rapid Run with 100 bp PE reads.

Hybridization Capture (continued)

Accurate Variant Calls with Roche 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
	Roche Kapa	93	240	6	51X	74
10 ng	**SWIFT**	93	275	5	52X	67
	Roche Kapa	93	97	13	47X	68
1 ng	**SWIFT**	93	45	26	37X	65
	Roche 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).

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.

Ordering Information

Product Name	Reactions	Catalog No.
Accel-NGS 2S PCR-Free DNA Library Kit	24	20024
Accel-NGS 2S PCR-Free DNA Library Kit	96	20096
Accel-NGS 2S Plus DNA Library Kit	24	21024
Accel-NGS 2S Plus DNA Library Kit	96	21096
Accel-NGS 2S Hyb DNA Library Kit	24	23024
Accel-NGS 2S Hyb DNA Library Kit	96	23096

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
SureSelect Compatibility Module	24	26424
SureSelect Compatibility Module	96	26496
2S Set A MID Indexing Kit (12 indices)	48	27148
2S Set B MID Indexing Kit (12 indices)	48	27248
2S Set A+B MID Indexing Kit (24 indices)	96	27396
SureSelect MID Compatibility Module	24	27424
SureSelect MID Compatibility Module	96	27496
2S Dual Indexing Kit (96 combinations)	96	28096

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