ACCEL-AMPLICON™ PANELS

Accel-Amplicon panels are based on a unique molecular biology that provides powerful solutions for detecting and screening clinically-relevant mutations. Swift Biosciences' multiplex amplicon panels are comprised of 10's to 100's of primer pairs in a single-tube format which are optimized for sequencing on Illumina® platforms. Primer pairs in the panels are designed for compatibility with the short DNA fragments from both formalin-fixed, paraffin-embedded (FFPE) and circulating, cell-free DNA (cfDNA) samples. A fast and easy single-tube workflow produces the best-in-class performance for on-target percentage and coverage uniformity, enabling variant discovery and confirmation.

Features

- Single-tube, 2-hour workflow
- Inputs as low as 10 ng
- Amplicons sized 120-160 bp for compatibility with cfDNA and FFPE
- Limit of detection as low as 1%
- On-target specificity and coverage uniformity > 95%
- Leverages the high fidelity performance of the Illumina platform
- Includes sequencing adapters

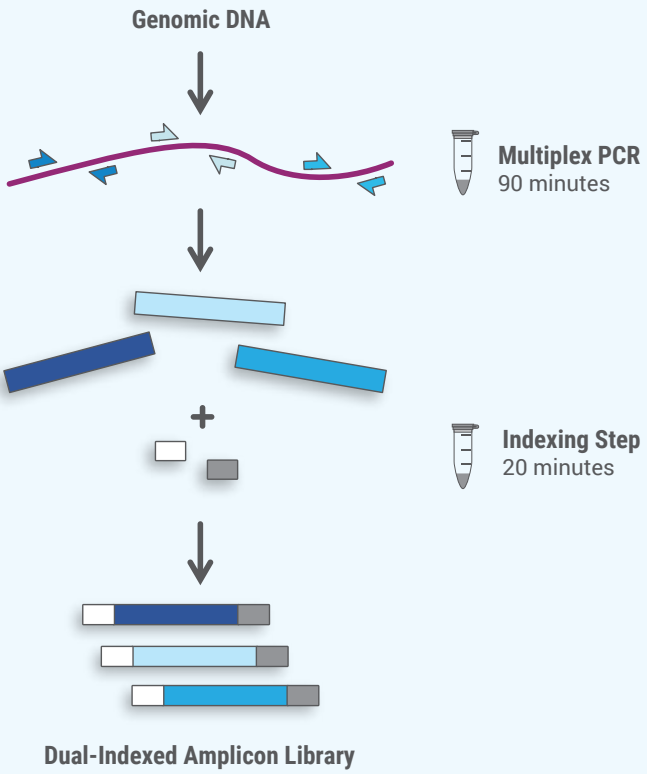
Supported Panels

- Comprehensive TP53 Panel
- 56G Oncology Panel
- Pharmacogenetics Panel
- EGFR Pathway Panel
- Sample_ID Panel
- Custom panels

Sample Types

- FFPE
- cfDNA
- Fresh frozen
- Genomic DNA

Single-Tube, 2-Hour Workflow



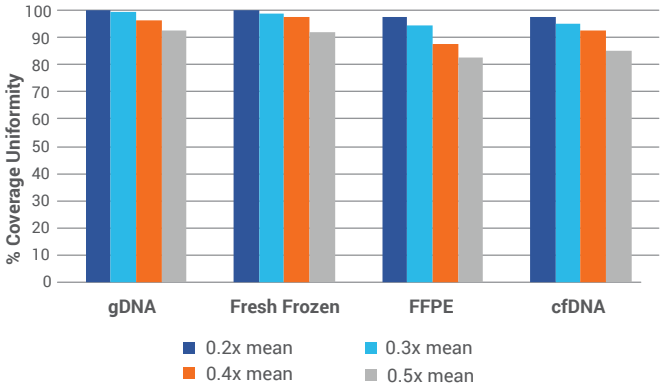
The single-tube workflow includes two brief incubations to generate the multiplex amplicon targets and add a unique combination of Illumina-compatible indexed adapters, creating up to 96 uniquely-indexed libraries for multiplexing on a single sequencing run.

Accel-Amplicon 56G Oncology Panel

The Accel-Amplicon 56G Oncology Panel offers hotspot coverage of 56 clinically-relevant, oncology-related genes, using a 263-amplicon design to generate multiplex libraries compatible with Illumina sequencing platforms.

Product Specifications	
Input DNA Required	10-25 ng
Time Required	2 hours
Number of Amplicons	263
Amplicon Size	92-184 bp (average 138 bp)
Number of Genes Covered	56
Total Target Size	23.6 kb
FFPE/cfDNA Compatible	Yes
On Target Percentage	> 95%
Coverage Uniformity at > 20% of Mean	> 95%
Limit of Detection (at 10 ng for Base Substitutions)	1-5%
Multiplexing on MiSeq® v2 Nano @ 5000X Avg. Depth	1
Multiplexing on MiSeq v2 @ 5000X Avg. Depth	22

High Coverage Uniformity Across Sample Types



10 ng of input DNA from a variety of sample types was used to generate libraries with the Accel-Amplicon 56G Oncology Panel. The coverage uniformity, as the percentage of the bases covered at least 20%, 30%, 40%, or 50% of the average depth, was determined across four sample types. The percentage of reads on target was > 95% for all sample types.

Genes Represented in the 56G Oncology Panel

ABL1	5	CSF1R	2	FBXW7	6	GNAS	2	KIT	14	NPM1	1	STK11	5
AKT1	2	CTNNB1	1	FGFR1	2	HNF1A	4	KRAS	3	NRAS	3	SMAD4	10
ALK	2	DDR2	1	FGFR2	4	HRAS	2	MAP2K1	5	PDGFRA	4	SMARCB1	4
APC	9	DNMT3A	1	FGFR3	6	IDH1	1	MET	6	PIK3CA	11	SMO	5
ATM	19	EGFR	9	FLT3	4	IDH2	2	MLH1	1	PTEN	14	SRC	1
BRAF	2	ERBB2	4	FOXL2	1	JAK2	2	MPL	1	PTPN11	2	TP53	21
CDH1	3	ERBB4	8	GNA11	2	JAK3	3	MSH6	4	RB1	12	TSC1	1
CDKN2A	2	EZH2	1	GNAQ	2	KDR	9	NOTCH1	3	RET	6	VHL	3

The Accel-Amplicon 56G Oncology Panel includes both clinically-relevant hotspot loci and regions of contiguous coverage, depending on the allele distribution across each target gene. The table depicts the genes represented, followed by the number of amplicons for each gene.

Contiguous, overlapping coverage is included for APC, ATM, EGFR, FBXW7, FGFR3, HNF1A, KIT, MSH6, PIK3CA, PTEN, and SMAD4.

Comprehensive coding exon coverage is included for TP53.

Reproducible Variant Calling from Q-Seq HDx™ Quantitative Standards

Gene	AA	CHR	POS	REF	ALT	Expected Allele Frequency	Detected Allele Frequency (N=10)	Standard Deviation
EGFR	G719S	7	55241707	G	A	**24.5**	23.8	1.5
PIK3CA	H1047R	3	178952085	A	G	**17.5**	17.5	1.3
KRAS	G13D	12	25398281	C	T	**15.0**	15.0	1.8
NRAS	Q61K	1	115256530	G	T	**12.5**	13.4	1.2
BRAF	V600E	7	140453136	A	T	**10.5**	9.9	0.3
KIT	D816V	4	55599321	A	T	**10 .0**	10.3	1.1
PIK3CA	E545K	3	178936091	G	A	**9.0**	8.5	1.1
KRAS	G12D	12	25398284	C	T	**6.0**	6.6	1.2
EGFR	L858R	7	55259515	T	G	**3.0**	2.7	0.5
EGFR	ΔE746-A750	7	55242465-55242479	Del15bp		**2.0**	1.4	0.5
EGFR	T790M	7	55249071	C	T	**1.0**	1.0	0.3

The Accel-Amplicon 56G Oncology Panel consistently detected validated variants at the expected frequency in replicates by five different users from 10 ng of the Horizon Diagnostics Quantitative Multiplex DNA Reference Standards HD701. The variants were called by LoFreq 2.1.1 (Genome Institute of Singapore) and GATK HaplotypeCaller (Broad Institute). When examining sporadic variants among the 10 replicates, the majority of background variants were present at less than 0.6%. No sporadic variants greater than 0.6% were detected.

Detection of Somatic Mutations in cfDNA and FFPE

Matched FFPE tumor, FFPE normal-adjacent, and cfDNA samples were obtained from Spectrum Health for analysis with the Accel-Amplicon 56G Oncology Panel. The data below shows concordance in variant allele frequencies across these matched samples.

Cancer Type	Gene	hg19 Coordinate	Amino Acid Change	% Mutant in FFPE Normal Adjacent	% Mutant in FFPE Tumor	% Mutant in cfDNA
Metastatic colorectal adenocarcinoma	PIK3CA	chr3:178936091	E545K	0	23	11
	APC	chr5:112175576	Q1429*	0	20	5
	TP53	chr17: 7579575	Q38* or intron	0	21	14
	KRAS	chr12: 25398281	G13D	0	22	5
Mammary carcinoma	PIK3CA	chr3:178952085	H1047R	0	17	0
	TP53	chr17:7578488	D148H	0	0	9
Ovarian cystadenofibroma	BRAF	chr7:140453136	V600E	0	23	1
Fallopian tube adenocarcinoma	TP53	chr17:7577085	E285K	0	48	0
	TP53	chr17:7578488	D148H	0	0	5

In the above, cfDNA was extracted from 10 ml of blood and gDNA was obtained from FFPE normal or tumor tissues. The Accel-Amplicon 56G Oncology Panel was used to create libraries from 10 ng of cfDNA and 15 ng of FFPE gDNA. Sequencing was performed using V2 reagents on an Illumina MiSeq. Coverage uniformity and percentage of reads on target were greater than 95%. The average depth of coverage per base ranged from 2,500-5,000X. Somatic mutations were called using LoFreq 2.1.1 (Genome Institute of Singapore) and GATK HaplotypeCaller (Broad Institute).

**Signifies a substitution leading to a nonsense mutation.*

Accel-Amplicon Comprehensive TP53

The Accel-Amplicon TP53 Panel offers comprehensive, full-exon coverage of the TP53 gene.

Product Specifications	
Input DNA Required	10-25 ng
Time Required	2 hours
Number of Amplicons	21
Amplicon Size	106-154 bp (average 140 bp)
Total Target Size	1.8 kb
FFPE/cfDNA Compatible	Yes
On Target Percentage	> 95%
Coverage Uniformity at > 20% of Mean	> 95%
Limit of Detection (at 10 ng for Base Substitutions)	1-5%
Multiplexing on MiSeq® v2 Nano @ 5000X Avg. Depth	19
Multiplexing on MiSeq v2 @ 5000X Avg. Depth	285

Comprehensive Coverage of TP53

Coverage of all coding regions of the TP53 gene by the Accel-Amplicon Comprehensive TP53 Panel are represented in a Sashimi plot (IGV; Broad Institute).

Ordering Information

Product Name	Reactions	Catalog No.
Accel-Amplicon Sample_ID Panel	48	AL-50048
Accel-Amplicon EGFR Pathway Panel	48	AL-51048
Accel-Amplicon Comprehensive TP53 Panel	48	AL-53048
Accel-Amplicon 56G Oncology Panel	48	AL-56048

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

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