



## ACCEL-AMPLICON® 56G ONCOLOGY PANEL V2

The Accel-Amplicon 56G Panel v2 offers comprehensive and hotspot coverage of 56 clinically-relevant, oncology-related genes. The v2 panel contains a 263-amplicon design to generate multiplex libraries compatible with Illumina® sequencing platforms, and 104 exonic and gender Sample\_ID amplicons for gender identification for tracking tumor-normal pairs and samples in longitudinal studies. This panel's primer is designed and validated with cfDNA, liquid biopsy, and FFPE samples.



### Genes Represented in the 56G Oncology Panel v2

ABL1	CSF1R	FBXW7	GNAS	KIT	NPM1	STK11
AKT1	CTNNB1	FGFR1	HNF1A	KRAS	NRAS	SMAD4
ALK	DDR2	FGFR2	HRAS	MAP2K1	PDGFRA	SMARDCB1
APC	DNMT3A	FGFR3	IDH1	MET	PIK3CA	SMO
ATM	EGFR	FLT3	IDH2	MLH1	PTEN	SRC
BRAF	ERBB2	FOXL2	JAK2	MPL	PTPN11	TP53
CDH1	ERBB4	GNA11	JAK3	MSH6	RB1	TSC1
CDKN2A	EZH2	GNAQ	KDR	NOTCH1	RET	VH1

The Accel-Amplicon 56G Oncology Panel v2 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.

- 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

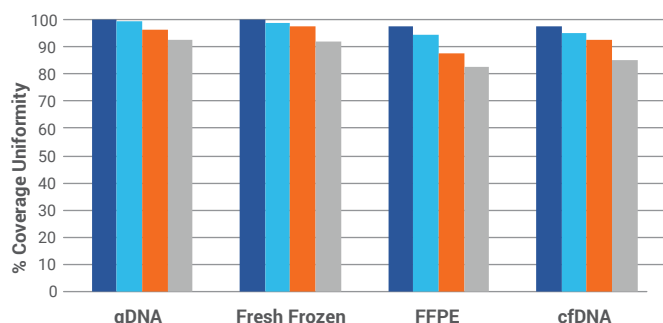
Libraries prepared with the Accel-Amplicon 56G Oncology Panel v2 consistently detected validated variants at the expected frequency in replicates by five different users from 10 ng of the Horizon Diagnostics Quantitative Multiplex DNA Reference Standards, HD701.

#### Representative Examples:

Gene	AA	CHR	POS	REF	ALT	Expected Allele Frequency (%)	Detected Allele Frequency (%)	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
NRAS	Q61K	1	115256530	G	T	12.5	13.4	1.2
BRAF	V600E	7	140453136	A	T	10.5	9.9	0.3
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	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 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.

## High Coverage Uniformity Across Sample Types



10 ng of input DNA from a variety of sample types was used to generate libraries with the 56G primer sub-set of the Accel-Amplicon 56G Oncology Panel v2. 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.

■ 0.2x mean      ■ 0.3x mean  
 ■ 0.4x mean      ■ 0.5x mean

## Detection of Somatic Mutations in cfDNA and FFPE

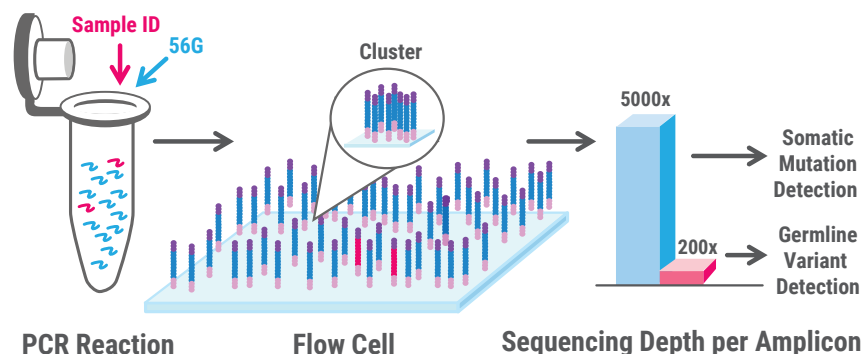
Matched FFPE tumor, FFPE normal-adjacent, and cfDNA samples were obtained from Spectrum Health for analysis with the Accel-Amplicon 56G Oncology Panel v2. The data below shows concordance in variant allele frequencies across these matched samples.

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 v2 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).

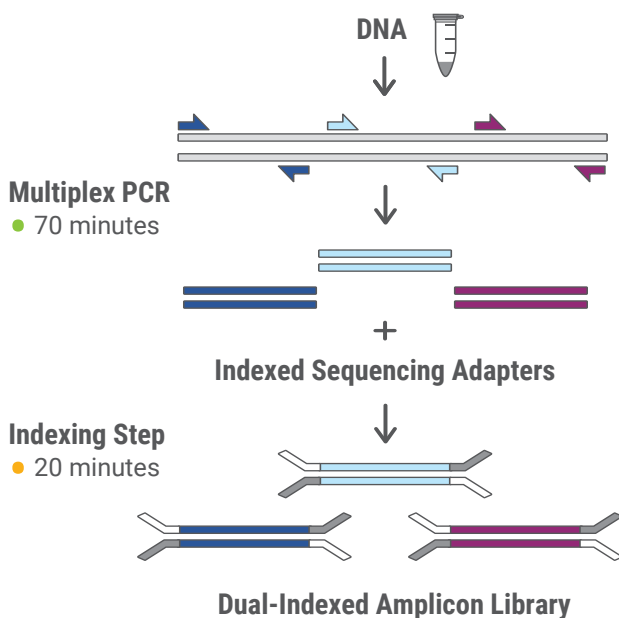
\*Signifies a substitution leading to a nonsense mutation.

## Sample\_ID as a Spike-in to the 56G Oncology Panel v2



The Accel-Amplicon Sample\_ID primers have been manufactured as spiked-in to the 56G Oncology Panel primer pool at a low percentage (2-4% of reads). This enables a sequencing depth of 200X for the germline Sample\_ID targets and 5000X for the 56G Oncology targets.

## 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 384 uniquely-indexed libraries for multiplexing on a single sequencing run.

## Ordering Information

Product Name	Indexing Included	Catalog No.
Accel-Amplicon 56G Oncology Panel v2 (48 rxns)	8 i5* X12 D701-712	AL-56248
Accel-Amplicon 56G Oncology Panel v2 (96 rxns)	Not Included - Choose 1 of the 4 Below	AL-56296
Swift Amplicon Combinatorial Dual Indexing Kit (Set 1A, 96 rxns)	8 i5 X12 S701-712	AL-S1A96
Swift Amplicon Combinatorial Dual Indexing Kit (Set 1B, 96 rxns)	8 i5 X12 S701-724	AL-S1B96
Swift Amplicon Combinatorial Dual Indexing Kit (Set 2A, 96 rxns)	8 i5 X12 S725-736	AL-S2A96
Swift Amplicon Combinatorial Dual Indexing Kit (Set 2B, 96 rxns)	8 i5 X12 S737-748	AL-S2B96
VarSome Data Analysis Token (48 Samples)	N/A	AL-VS48

i5\* Illumina Truseq CD indexes D501-D508

Visit [www.swiftbiosci.com](http://www.swiftbiosci.com) for easy ordering.



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