ACCEL-AMPLICON™ PANELS

Amplicon sequencing has emerged as a reliable, cost-effective method for ultra-deep targeted sequencing. This highly adaptable approach is especially applicable for in-depth interrogation of numerous oncology-related targets in multiple samples in a single run. Swift Biosciences offers Accel-Amplicon Panels focused on comprehensive and hotspot screening of clinically-relevant variants significant in a variety of solid tumors, such as breast and lung cancers as well as hematological malignancies.

Accel-Amplicon Panels are comprised of tens up to thousands 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.

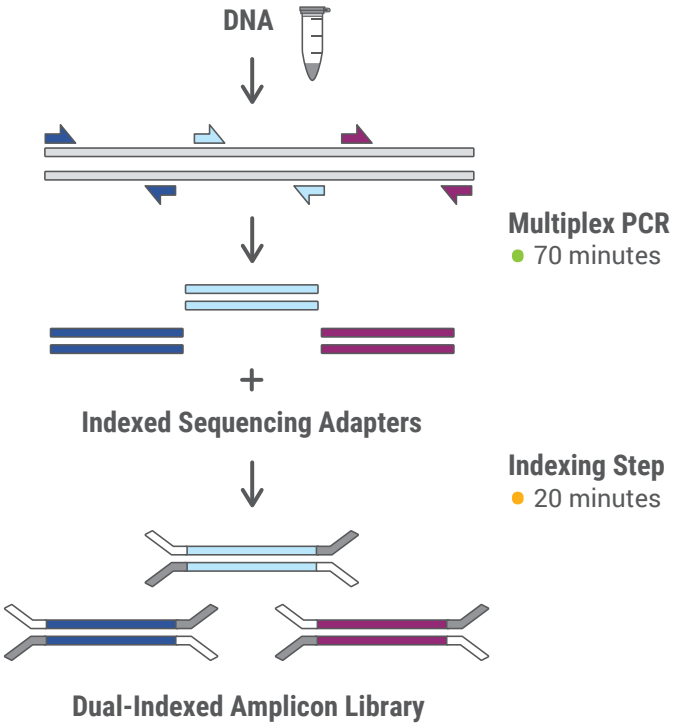
Features

- Single-tube assay
- Ready-to-sequence libraries in 2 hours
- 10 ng input DNA
- Average amplicon size of 138-149 bp
- Contiguous coverage
- Coverage uniformity > 95%
- On target > 95%
- Includes sequencing adapters

Publication Highlight

The following publication features a single-tube, 2-hour workflow and robust variant calling of the Accel-Amplicon CFTR Panel (coming soon). Swift's panel performs equivalently to the "gold standard" set by the Sanger Sequencing method and outperforms other currently available panels on the market. Read the full publication: "Newborn screening quality assurance program for CFTR mutation detection and gene sequencing to identify Cystic Fibrosis". Journal of Inborn Errors of Metabolism & Screening. 2016, Volume 4:1-11.

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.

Summary of Specifications of Accel-Amplicon Panels

Specification	Feature	56G Oncology v2	Comprehensive TP53	EGFR Pathway	BRCA1 and BRCA2	BRCA1, BRCA2, and PALB2	Sample_ID
Input	Input DNA required	10-25 ng					
	Sample types	FFPE, cfDNA, fresh frozen, genomic DNA					
Workflow	Time required	2 hours					
	Multiplexing on MiSeq® v2 Nano @ 5000X avg. depth	1 sample	19 samples	23 samples	1 sample	1 sample	34-90 samples**
	Multiplexing on MiSeq® v2 standard @ 5000X avg. depth	22 samples	285 samples*	352 samples*	24 samples	19 samples	N/A
Design	Number of amplicons	263	21	17	246	302	104
	Average amplicon size	138 bp	140 bp	136 bp	148 bp	149 bp	145 bp
	Number of genes covered	56	1	4	2	3	Exonic regions with minor allele frequency and gender ID
	Total target size	23.6kb	1.8kb	1.5kb	22.9kb	28.9kb	N/A; SNPs only
Performance	Limit of detection (at 10 ng base substitutions)	1-5%	1-5%	1-5%	1-5%	1-5%	Germline panel
	On target percentage	> 95%					
	Coverage uniformity (at > 20% of mean)	> 95%					

** Requires custom indexing solution. Please inquire.*
*** Only 200-500X required.*

Coming Soon:

- **Accel-Amplicon CFTR Panel** – Contains ~ 80 amplicons, covering all exons for the CFTR gene, as recently published.
- **Accel-Amplicon ADME Panel** – Contains 364 amplicons covering hotspots for common drug metabolism genes.

Contact Swift Biosciences at TechSupport@swiftbiosci.com if you are interested in BED files or early access to these panels.

Accel-Amplicon 56G Oncology Panel v2

This extensive panel 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.

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.*

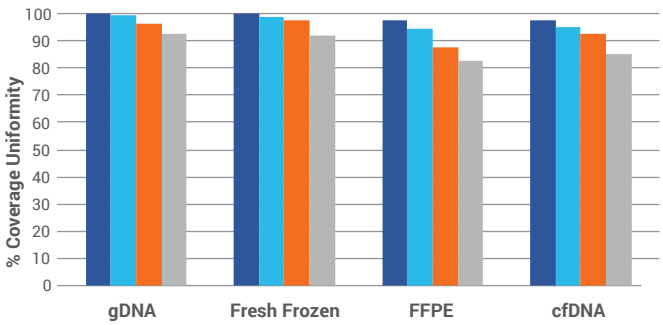
Reproducible Variant Calling from Q-Seq HDx™ Quantitative Standards

Libraries prepared with the 56G primer sub-set of 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.

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
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 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.

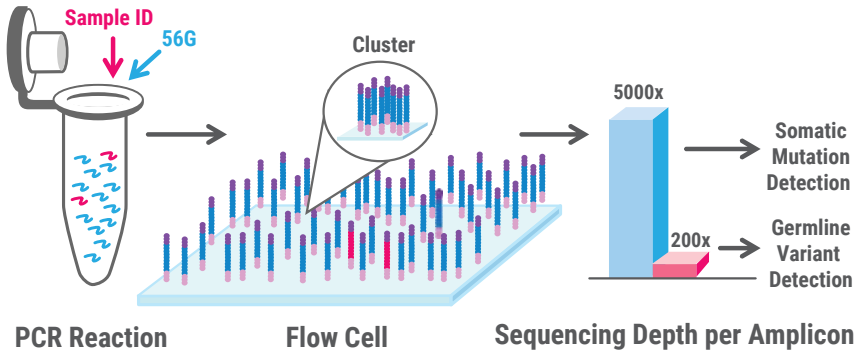
Detection of Somatic Mutations in cfDNA and FFPE

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.

Accel-Amplicon BRCA1 and BRCA2 Panel

This complete panel contains 246 amplicons with an average size of 148 bp that covers the entire coding sequence of *BRCA1* and *BRCA2* genes. This panel includes all components necessary for generating ready-to-sequence libraries, including primer pairs and indexed sequencing adapters.

The data below demonstrates the performance of the Accel-Amplicon BRCA1 and BRCA2 Panel on a variety of sample types.

Input DNA	Sample Type	Reads Aligned	% Bases On Target Aligned	Mean Coverage	% Coverage Uniformity
HD701	Horizon Diagnostics	165925	98.9	990.0	99.2
NA12878	Coriell	170687	98.9	1005.9	99.2
NA24143 (Ashkenazi)	Coriell	169613	97.5	948.0	96.7
NA24695 (Han Chinese)	Coriell	170799	98.6	997.4	99.2
NA19240 (Yoruban)	Coriell	171228	98.4	997.3	99.1
FFPE_6hr	FFPE	171323	98.3	996.3	95.2
FFPE_24hr	FFPE	171459	98.4	1009.8	95.9
FFPE_48hr	FFPE	171074	98.1	999.5	95.2

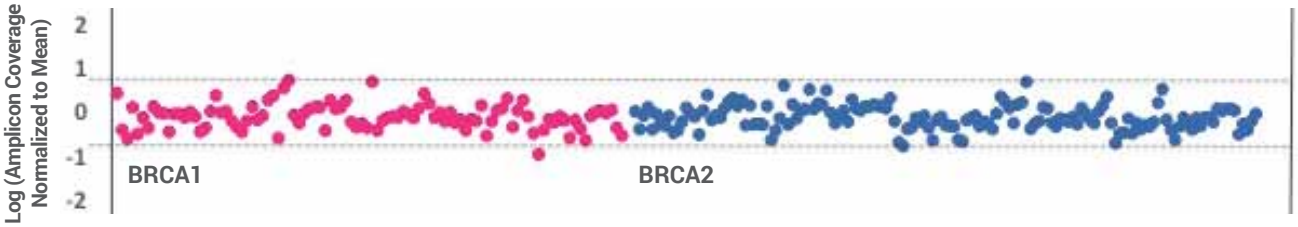
Libraries using the Accel-Amplicon BRCA1 and BRCA2 Panel were prepared from 10 ng input of high quality Coriell (NA12878, NA24143, NA24695, and NA19240) gDNA, Horizon Diagnostics Quantitative Multiplex DNA Reference Standards HD701, and FFPE DNA. Sequencing was performed using MiniSeq® reagents.

Variant Calling by Accel-Amplicon BRCA1 and BRCA2 Panel

Input DNA	Variant	Expected AF (%)	Observed AF (%)
Horizon Diagnostics HD701	BRCA2 A1689fs	33.0	32.9
Coriell NA14684-21	BRCA1 916delTT	validated germline variant	48.8
Coriell NA14805-26	BRCA1 810G>A	validated germline variant	99.4
Coriell NA14788-27	BRCA2 983del4	validated germline variant	99.4

The Accel-Amplicon BRCA1 and BRCA2 Panel consistently detected validated variants at the expected frequency in replicates from 10 ng of the Horizon Diagnostics Quantitative Multiplex DNA Reference Standards HD701 and Coriell (NA14684-21, NA14805-26 and NA14788-27). The variants were called by GATK HaplotypeCaller (Broad Institute).

Accel-Amplicon BRCA1 and BRCA2 Panel Coverage Uniformity



The Accel-Amplicon BRCA1 and BRCA2 Panel provides high coverage uniformity across amplicons. This panel was used to prepare libraries using 10 ng input of high quality Coriell NA12878 gDNA. The representative plot demonstrates amplicon coverage normalized to mean. Gates were drawn to represent 15-500% of the mean.

Accel-Amplicon BRCA1, BRCA2, and PALB2 Panel

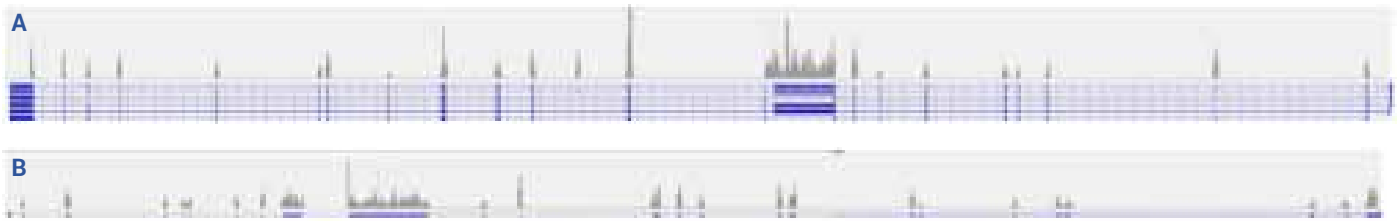
This comprehensive panel contains 302 amplicons with an average size of 149 bp that covers the entire coding sequence of *BRCA1* and *BRCA2* genes, as well as all coding exons and 5′ and 3′ UTR regions of PALB2. This product is a complete kit that includes all components necessary for generating ready-to-sequence libraries, including primer pairs and indexed sequencing adapters.

The data below demonstrates the performance of the Accel-Amplicon BRCA1, BRCA2, and PALB2 Panel on a variety of sample types.

Input DNA	Sample Type	Reads Aligned	% Bases On Target Aligned	Mean Coverage	% Coverage Uniformity
NA12878	Coriell	464,833	98.4	97.6	98.2
HD710	Horizon Diagnostics	462,032	98.5	97.9	98.0

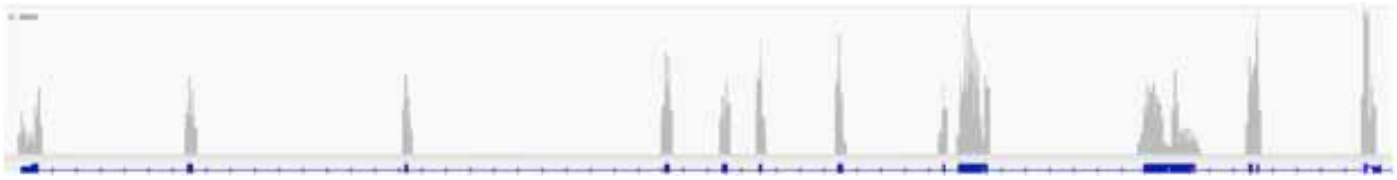
The data demonstrates the performance of the Accel-Amplicon BRCA1, BRCA2, and PALB2 Panel on a variety of sample types. The Accel-Amplicon BRCA1, BRCA2, and PALB2 Panel was used to prepare libraries from 10 ng input of high quality Coriell NA12878 gDNA. Sequencing was performed using MiniSeq® Reagents.

Comprehensive Coverage of Coding Sequences of BRCA1 and BRCA2



Coverage of all coding exons of the BRCA1 *(A) and* BRCA2 *(B) genes by the Accel-Amplicon BRCA1 and BRCA2 Panel are represented in a Sashimi plot (IGV; Broad Institute).*

Comprehensive Coverage, Including UTRs of *PALB2* Gene



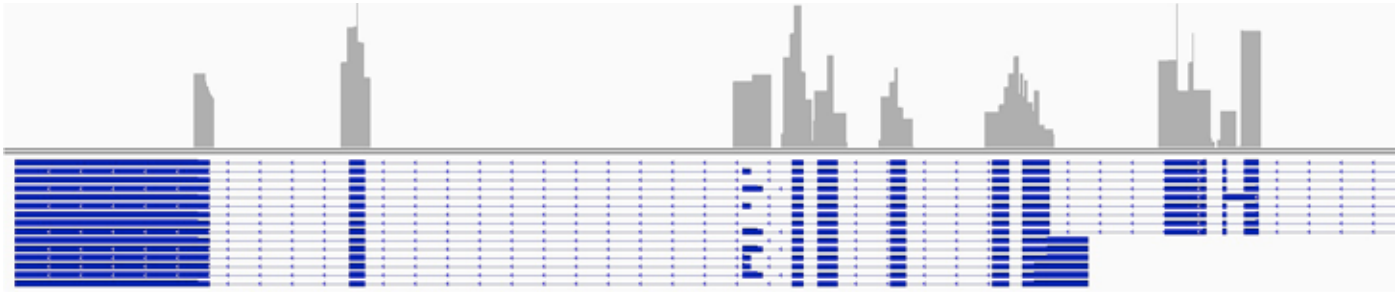
Coverage of all coding exons and 5′ and 3′ UTR regions of the PALB2 *gene by Accel-Amplicon BRCA1, BRCA2, and PALB2 Panel are represented in a Sashimi plot (IGV; Broad Institute).*

Accel-Amplicon Comprehensive TP53

This extensive panel contains 21 amplicons with an average size of 140 bp that provides comprehensive coverage of all coding regions of TP53.

Accel-Amplicon Comprehensive TP53 Panel is compatible with short DNA fragments from both FFPE and cfDNA samples. This product is a complete kit that includes all elements necessary for generating ready-to-sequence libraries, including primer pairs and indexed sequencing adapters.

Comprehensive Coverage of Coding Regions 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).

Reproducible Variant Calling from AcroMetrix® Oncology Hotspot Control

Libraries prepared with the Accel-Amplicon Comprehensive TP53 Panel accurately detected validated variants within the expected frequency range from 10 ng of AcroMetrix Oncology Hotspot Control DNA.

Cosmic-ID	SNP_ID	Amino Acid Change	POS	REF	ALT	Expected Allele Frequency (%)	Detected Allele Frequency (%)
COSM13747	N/A	R72P	7579472	G	C	Genomic variant	92.14
COSM10663	rs121913344	E56*	7579521	C	A	5-15	6.83
COSM10710	rs201744589	Q38*	7579575	G	A	5-15	6.83
COSM10660	rs28934576	E51*	7579536	C	A	5-15	6.67
COSM10662	rs11540652	Y234C	7577580	T	C	5-15	6.46
COSM6932	rs28934575	R306*	7577022	G	A	5-15	6.40
COSM10812	rs28934573	R273H	7577120	C	T	5-15	6.25
COSM10808	rs148924904	Y220C	7578190	T	C	5-15	5.56
COSM10739	rs193920817	E339*	7574012	C	A	5-15	5.46
COSM10670	rs121912654	R342*	7574003	G	A	5-15	5.41
gDNA26-COSM250061	rs1042522	G245S	7577548	C	T	5-15	4.08

The variants were called by LoFreq 2.1.1 (Genome Institute of Singapore) and GATK Haplotype-Caller (Broad Institute).

Accel-Amplicon EGFR Pathway Panel

This targeted panel contains 17 amplicons with and average size of 136 bp that cover hotspots in BRAF, KRAS, NRAS, and contiguous regions of EGFR.

EGFR Pathway Panel Performance with Horizon's 1% Multiplex I cfDNA Reference Standard

The Accel-Amplicon EGFR Pathway Panel consistently detected validated variants at the expected frequency in three replicates from 10 ng of the Horizon Diagnostics Multiplex cfDNA Reference Standards HD780.

Gene	AA	CHR	POS	REF	ALT	Expected Allele Frequency	Detected Allele Frequency (N=3)	Standard Deviation
NRAS	A59T	1	115256536	C	T	1.3	1.0	0.5
NRAS	Q61K	1	115256530	G	T	1.3	1.3	0.3
KRAS	G12D	12	25398284	C	T	1.3	1.3	0.3
EGFR	L858R	7	55259515	T	G	1.0	0.8	0.0
EGFR	T790M	7	55249071	C	T	1.0	1.1	0.1
EGFR	ΔE746-A750	7	55242465-55242479	Del15bp	–	1.0	0.5	0.2
EGFR	V769-D770insASV	7	55248998	A	TGGC-CAGCG	1.0	0.8	0.2

The variants were called by LoFreq 2.1.1 (Genome Institute of Singapore) and GATK Haplotype-Caller (Broad Institute). When examining sporadic variants among the replicates, the majority of background variants were present at less than 0.6%. No sporadic variants greater than 0.6% were detected. ***None of these variants were detected in the 100% Multiplex I Wild Type cfDNA Reference Standard.***

Ordering Information

Product Name	Reactions	Catalog No.
Accel-Amplicon 56G Oncology Panel v2	48	AL-56248
Accel-Amplicon BRCA1 and BRCA2 Panel	48	AL-52048
Accel-Amplicon BRCA1, BRCA2, and PALB2 Panel	48	AL-57048
Accel-Amplicon Comprehensive TP53 Panel	48	AL-53048
Accel-Amplicon EGFR Pathway Panel	48	AL-51048
Accel-Amplicon Sample_ID Panel	48	AL-50048

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