# 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

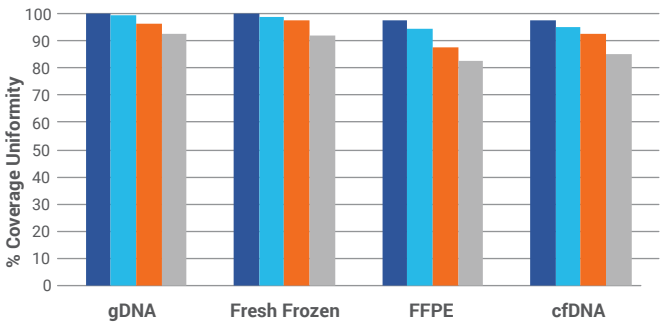
Libraries prepared with the Accel-Amplicon 56G Oncology Panel v2 consistently detected validated variants at the expected frequency in replicates by five different users from 10 ng of the Horizon Diagnostics Quantitative Multiplex DNA Reference Standards, HD701.

***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.*

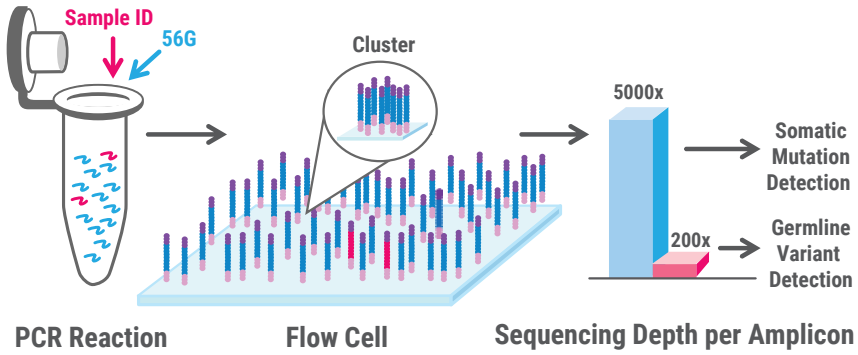
## Detection of Somatic Mutations in cfDNA and FFPE

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 |

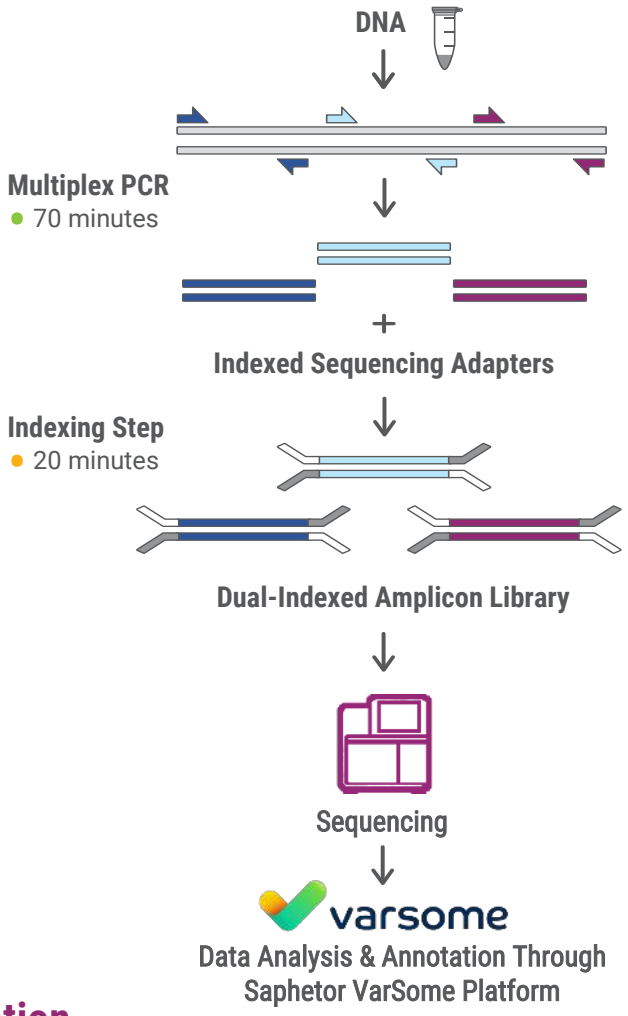
*In the above, cfDNA was extracted from 10 ml of blood and gDNA was obtained from FFPE normal or tumor tissues. The Accel-Amplicon 56G Oncology Panel v2 was used to create libraries from 10 ng of cfDNA and 15 ng of FFPE gDNA. Sequencing was performed using v2 reagents on an Illumina MiSeq. Coverage uniformity 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** for easy ordering.

**Swift Biosciences, Inc.**
674 S. Wagner Road, Suite 100 • Ann Arbor, MI 48103 • 734.330.2568 • www.swiftbiosci.com