ACCEL-AMPLICON™ CFTR PANEL

Get Fast, Comprehensive CFTR Gene Coverage from the Lowest Input

Highlights

- **Assess more CFTR mutations**
  Interrogate all ACMG-recommended mutations and rare variants on all CFTR exons and known pathological intronic variants.
- **Fast, easy, standardized workflow**
  Amplify all CFTR regions of interest in a single-tube reaction and have sequence-ready libraries in less than 2 hours.
- **Superior quality from the lowest amount of sample**
  Achieve high coverage from as little as 10 ng of DNA from complex or limited samples.

The Accel-Amplicon CFTR panel includes primer pairs, indexed sequencing adapters, and master mix to prepare 48 unique libraries for sequencing.

Introduction

Cystic Fibrosis (CF) is an autosomal recessive disorder impacting more than 70,000 children and adults worldwide.[1] Defects in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene lead to disruption in normal protein expression causing dysregulation of ion chloride channels affecting respiratory, digestive, and reproductive systems. Cystic Fibrosis affects both males and females, and people of all racial groups; however, it is most prevalent in Caucasians of Northern European descent. The disease is common among Latinos and Native Americans and, to a lesser extent, in people of African or Asian ancestry.

There are more than 2,000 known mutations in the CFTR gene, however most available genetic panels only screen for the most common CFTR mutations.[2] Although these assays can detect the majority of pathogenic variants, a negative result does not eliminate the possibility that a sample contains a rare pathogenic variant.

Swift Biosciences' Accel-Amplicon CFTR Panel is a comprehensive and sensitive next-generation sequencing (NGS)-based approach to screen disease-relevant mutations and variants in the CFTR gene. This panel offers coverage of all exons, including 5' and 3' UTRs, and regions of interest in introns 1, 12, 22, and 25. The Accel-Amplicon CFTR Panel captures all ACMG-recommended CFTR mutations and offers a standardized solution to identify additional variants, all in a single-tube assay format. The panel generates targeted libraries compatible with Illumina® sequencing platforms, and is a complete kit that includes all components necessary for generating ready-to-sequence libraries, including primer pairs and indexed sequencing adapters. This panel leverages a fast and easy single-tube workflow producing best-in-class performance for on-target percentage and coverage uniformity, enabling rare variant discovery and confirmation.

Access More CFTR Mutations

The Accel-Amplicon CFTR Panel captures all ACMG-recommended mutations and is the only NGS-based method that captures poly-T tracts in concordance with the Sanger sequencing method.

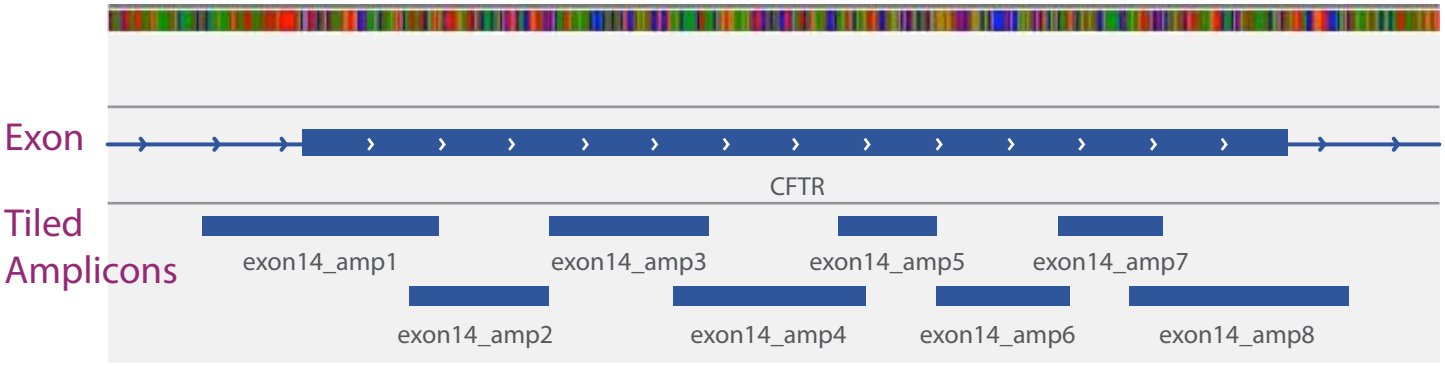
Table 1: Coverage of ACMG-recommended CFTR Mutations by Alternative Sequencing Method[3]

Mutation (Legacy Name)	Sanger Sequencing "The Gold Standard"	Accel-Amplicon CFTR Panel	AmpliSeq™ CFTR Community Panel	CF 139-Variant Assay
F508del	+	+	+	+
I507del	+	+	+	+
G542X	+	+	+	+
G85E	+	+	+	+
R117H	+	+	+	+
621+1G>T	+	+	+	+
711+1G->T	+	+	+	+
R334W	+	+	+	+
R347P	+	+	+	+
A455E	+	+	+	+
1717-1G>A	+	+	+	+
R560T	+	+	+	+
R553X	+	+	+	+
G551D	+	+	+	+
1898+1G>A	+	+	+	+
2184delA	+	+	+*	+
2789+5G>A	+	+	+	+
3120+1G>A	+	+	+	+
R1162X	+	+	+	+
3659delC	+	+	+	+
3849+10kbC>T	+	+	+	+
W1282X	+	+	+	+
N1303K	+	+	+	+
F508C	+	+	+	CR
T5	+	+	+	CR
T7	+	+	ND	CR
T9	+	+	ND	CR

The Accel-Amplicon CFTR Panel detected ACMG-recommended variants (in bold) and other key variants in libraries prepared from 10-30 ng of DNA from DBS. The variants were called by FreeBays and GATK HaplotypeCaller (Broad Institute). The Accel-Amplicon CFTR Panel covers all exons, 5' and 3' UTRs, and regions of interest in introns 1, 12, 22, and 25 for the CFTR gene. Abbreviations: ACMG, American College of Medical Genetics; CF, cystic fibrosis; CFTR, CF transmembrane regulator; CR, conditionally reported with an R117H present; ND, not distinguishable. + mutations assayed but detectable by method. Boldface entries indicate ACMG mutations.*

Additionally, the Accel-Amplicon CFTR Panel covers all coding sequences, UTRs, and select regions in intron 1, 12, 22, and 25 increasing the likelihood of detecting rare or novel variants. To accomplish this, the Accel-Amplicon CFTR Panel leverages a proprietary tiled amplicon design strategy to deliver comprehensive coverage of 27 exons in the CFTR gene. The overlapping amplicons provide complete coverage of all bases in the 10 kb genomic target region while enabling simultaneous amplification of 87 primer pairs in a single reaction.

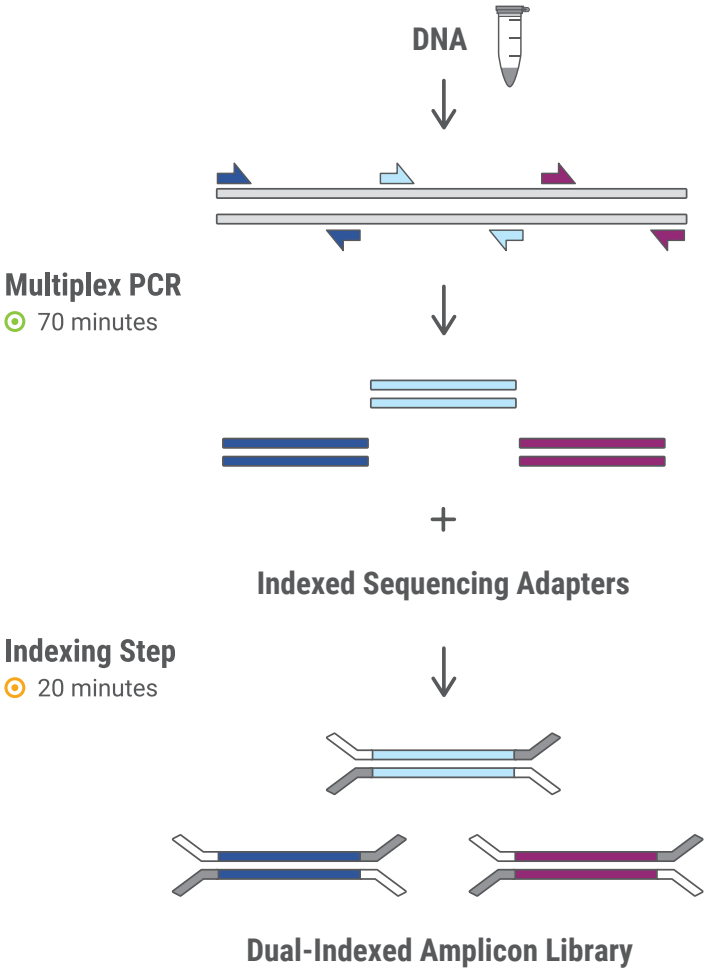
Figure 1: Accel-Amplicon CFTR Panel Leverages a Tiled Amplicon Design



A full list of variants/mutations covered by the panel is available on our website at www.swiftbiosci.com or by email at TechSupport@swiftbiosci.com.

Fast, Easy, Standardized Workflow

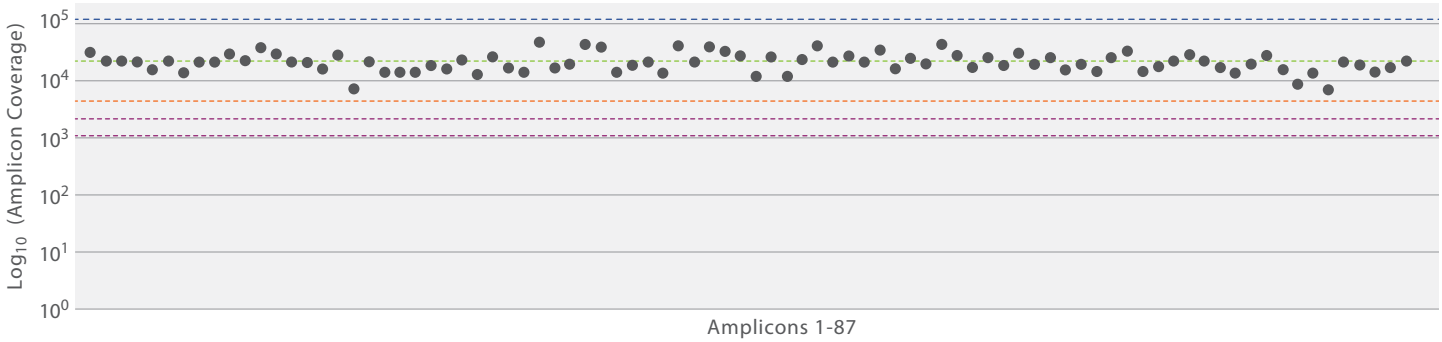
The Accel-Amplicon workflow uses an easy, fast single-tube approach consisting of a 70-minute target enrichment amplification step and a 20-minute adapter ligation step, yielding a 2-hour start-to-finish procedure. It enables simultaneous amplification of 87 primers in a single-tube reaction, minimizing hands-on time and sample processing errors. The Accel-Amplicon CFTR Panel contains a multiplex of pre-designed and concentration-optimized amplification primers, enzymes, buffers, and indices to generate 48 unique libraries per kit.



Superior Quality with the Lowest Amount of Sample

The Accel-Amplicon CFTR assay requires sample input as low as 10 ng per sample and is compatible with multiple sample types, including genomic DNA from reference DNA, previously whole-genome amplified samples, whole blood, dried blood spots (DBS/Guthrie cards), saliva, and buccal swabs. The panel is optimized to generate high quality data with low errors, including > 95% on-target specificity and coverage uniformity.

Figure 2: Accel-Amplicon CFTR Panel Provides High Coverage Uniformity Across Amplicons



The panel was used to prepare libraries using 10 ng input of high quality Coriell NA11496 gDNA. Representative plot demonstrates amplicon coverage for each amplicon. Red and blue dashed lines represent 20% mean coverage and 5x mean coverage, respectively.

Table 2: High Coverage Uniformity in Ethnically Diverse Samples

Input DNA	Source	Sample Type	Reads Aligned	% Bases On Target Aligned	Mean Coverage	% Coverage Uniformity
NA00897		Coriell	38,843	99.2	448	100.0
NA11496	Caucasian	Coriell	38,297	99.4	443	100.0
NA12878		Coriell	39,848	99.4	460	100.0
NA19240	Yoruban	Coriell	43,318	99.6	504	99.8
NA24143	Ashkenazi	Coriell	42,759	99.6	499	99.7
NA24695	Han Chinese	Coriell	43,171	99.5	501	98.1

The data demonstrates the performance of the Accel-Amplicon CFTR Panel on diverse ethnic backgrounds. The Accel-Amplicon CFTR Panel was used to prepare libraries from 10 ng input of high quality Coriell gDNA. Sequencing was performed using MiniSeq® Reagents.

Table 3: Reproducible Variant Calling of Reference DNA

					Allele Frequency			
Chr	Position Crch37	Ref	Alt	AA Change	Coriell NA12878 (Negative Control)	Coriell NA00897 (c.1521_1523delCTT, F508del, c.1172C>G, R347P)	Coriell NA11496 (c.1756G>T, G542*)	Clinical Significance (ClinVar)
7	117176738	C	T	Intron	N/A	51%	100%	Benign
7	117180324	G	C	R347P	N/A	47%	N/A	**Pathogenic**
7	117199457	A	G	Intron	N/A	46%	100%	N/A
7	117199533	G	A	V470M	N/A	45%	100%	Benign
7	117199644	ATCT	A	IF508del	N/A	50%	N/A	**Pathogenic**
7	117227832	G	T	G542*	N/A	N/A	100%	**Pathogenic**
7	117306991	C	T	Y1424Y	N/A	52%	N/A	Benign

Expected Variants

The Accel-Amplicon CFTR Panel consistently detected validated variants. The libraries were prepared from 10 ng of the Coriell (NA00897 and NA11496) with known pathogenic mutations (highlighted in blue). The panel also detected additional CFTR-relevant variants present in the reference sample. DNA from Coriell NA12878 was used as a negative control. The variants were called by GATK HaplotypeCaller (Broad Institute).

Table 4: Accel-Amplicon CFTR Panel Specifications

Specification	Feature	Accel-Amplicon CFTR Panel
Input	Input DNA required	10 ng
	Sample types	Genomic DNA extracted from whole blood, DBS, saliva, and buccal
Workflow	Assay time	2 hours
	Multiplexing on MiSeq® v2 Nano @ 500X avg. depth	43 samples
	Multiplexing on MiSeq v2 @ 500X avg. depth	384* samples
Design	Number of amplicons	87
	Amplicon size	Average 143 bp
	Regions covered	All exons including 5′ and 3′ UTRs, select intronic regions (1, 12, 22, and 25)
	Total target size	10.01 kb
Performance	On target percentage	> 95%
	Coverage uniformity at > 20% of mean	> 95%

** Coming soon.*

Table 5: Accel Amplicon CFTR Panel Contents

The following items are included in the kit.

Kit	Reagents	Quantity (µl) 48 rxn	Storage (°C)
Multiplex PCR (Pre-PCR)	Reagent G1*	106	-20
	Reagent G2	160	-20
	Enzyme G3	800	-20
	Pre-PCR TE	1200	-20
Indexing Step (Post-PCR)	Index D50X	33 each of D501-D508	-20
	Index D7XX	44 each of D701-D712	-20
	Buffer Y1	1637	-20
	Enzyme Y2	53	-20
	Enzyme Y3	53	-20
	Enzyme Y4	106	-20
	Post-PCR TE	1200	-20

** Reagent G1 is the panel-specific set of multiplex amplification primers.*

Kit	Reagent	Quantity (µl) 48 rxns	Storage (°C)
Additional Components Included	PEG NaCl Solution	20,000	Room Temp
	Alu115 and Alu247 primers	290 of each	-20

Ordering Information

Product Name	Reactions	Catalog No.
Accel-Amplicon CFTR Panel	48	AL-55048

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

References

1. Cystic Fibrosis Foundation website, What is Cystic Fibrosis? https://www.cff.org/What-is-CF/CF-Genetics/
2. Cystic Fibrosis Mutation Database http://www.genet.sickkids.on.ca/app
3. Hendrix et. al. 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.*

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