Table S1: Region of interest reported by the third-tier Archer CF assay

CFTR Exon[1]	Start (hg19)	Stop (hg19)	Reported Region Size
5'UTR	117,120,041	117,120,211	171
1			
2	117,144,297	117,144,427	131
3	117,149,078	117,149,206	129
4	117,170,943	117,171,178	236
5	117,174,320	117,174,429	110
6 (6a)	117,175,292	117,175,475	184
7 (6b)	117,176,597	117,176,737	141
intron 7 (6b)	117,179,036	117,179,049	14
8 (7)	117,180,144	117,180,410	267
9 (8)	117,182,060	117,182,172	113
10 (9)	117,188,690	117,188,887	198
11 (10)	117,199,508	117,199,719	212
intron 11 (10)	117,218,376	117,218,386	11
12 (11)	117,227,783	117,227,897	115
intron 12 (11)	117,229,516	117,229,535	20
13 (12)	117,230,397	117,230,503	107
14 (13)	117,231,978	117,232,721	744
15 (14a)	117,234,974	117,235,122	149
16 (14b)	117,242,870	117,242,927	58
17 (15)	117,243,576	117,243,846	271
18 (16)	117,246,718	117,246,817	100
19 (17a)	117,250,563	117,250,733	171
20 (17b)	117,251,605	117,251,872	268
21 (18)	117,254,657	117,254,777	121
22 (19)	117,267,566	117,267,834	269
intron 22 (19)	117,267,859	117,267,869	11
	117,280,010	117,280,020	11
23 (20)	117,282,482	117,282,657	176
24 (21)	117,292,886	117,292,995	110
25 (22)	117,304,732	117,304,924	193
26 (23)	117,305,503	117,305,628	126
27 (24)	117,306,952	117,307,262	311
3'UTR			

UTR – Untranslated region, *hg19* – human genome assembly GRCh37.

[1] Legacy *CFTR* nomenclature listed in parentheses, if different from HGVS nomenclature.

Table S3: *CFTR* TaqMan SNP genotyping assays

***CFTR* Variant[1]**	**SNP Genotyping Assay ID[2]**	**dbSNP ID**
p.Arg117His (R117H)	C__26083773_20	rs78655421
p.Phe508del (F508del)	C_151693869_10	rs113993960
p.Gly542* (G542X)	C__11399026_30	rs113993959
p.Gly551Asp (G551D)	C__32545127_20	rs75527207
p.Arg553* (R553X)	C__27861430_20	rs74597325
c.2988+1G>A (3120+1G>A)	C_100964958_20	rs75096551
p.Asn1303Lys (N1303K)	C__32544994_20	rs80034486
p.Trp1282* (W1282X)	C__32545014_20	rs77010898

[1]Legacy *CFTR* nomenclature listed in parentheses.

[2] ThermoFisher assay ID.

Table S4: Multiplex schema for del/dup qPCR assays

Assay Name[1]	*CFTR* Exon[1] with FAM probe	*CFTR* Exon[1] with VIC probe
E1+2	2	1
E3+4	3	4
E5+7 (E5+6b)	7 (6b)	5
E6+8 (E6a+7)	8 (7)	6 (6a)
E9+16 (E8+14b)	9 (8)	16 (14b)
E10+17 (E9+15)	10 (9)	17 (15)
E11+19 (E10+17a)	11 (10)	19 (17a)
E12+24 (E11+21)	12 (11)	24 (21)
E13+22 (E12+19)	13 (12)	22 (19)
E14+20 (E13+17b)	14 (13)	20 (17b)
E15+25 (E14a+22)	15 (14a)	25 (22)
E18+26 (E16+23)	18 (16)	26 (23)
E21+27 (E18+24)	21 (18)	27 (24)
E23 (E20)	23 (20)	N/A

del/dup – deletion/duplication.

All assays multiplexed with *RPPH1* reference assay (ThermoFisher, 4485714) with ABY reporter dye and QSY quencher. FAM probes have an MGB-NFQ quencher, and the VIC probes have a QSY quencher.

[1] Legacy *CFTR* nomenclature listed in parentheses, if different from HGVS nomenclature.

Table S5: Del/dup qPCR primer and probe sequences

***CFTR* Exon**[1]	**Forward primer sequence (5'>3')**	**Reverse primer sequence (5'>3')**	**Probe Sequence (5'>3')**
1	GCAGGCACCCAGAGTAGTAGGT	ACGCCCTCCTCTTTCGTG	TTTGGCATTAGGAGCTTGAGCCCAGA
2	AGCTGGACCAGACCAATTTTGA	CAGATAGATTGTCAGCAGAATCAACAG	CCTGGAATTGTCAGACATATA
3	TTTGCACATGCAACTTATTGGTC	TGATACATAATGAATGTACAAATGAGATCC	CTGGCTTCAAAGAAA
4	AGCCTCTCTTACTGGGAAGAATCATA	GTGATGAAGGCCAAAAATGGC	AGGCTTATGCCTTCTCTTTATTGTGAGGACACTG
5	TTAAAGCTGTCAAGCCGTGTTCT	GGAAAACTCCGCCTTTCCAG	CAACTTGTTAGTCTCCTTTCCAACAACCTGAACA
6 (6a)	CTTGCATTGGCAYATTTCGT	AATAGGTTGCTACCTGTACTTCATCATC	CAAGTGGCACTCCTCATGGGGCTAATCT
7 (6b)	TCAGAGAGCTGGGAAGATCAGTG	CCATTGCTTCTTCCCAGCAG	TGTGATTACCTCAGAAATGA
8 (7)	GAACTGAAACTGACTCGGAAGGC	AGTGCATAGGGAAGCACAGATAAAA	TGTGAGATACTTCAATAGCTCAG
9 (8)	AATCCTAGTGCTTGGCAAATTAACTTT	AGAAGGCTGTTACATTCTCCATCACTA	CAAGAATATAAGACATTGGAATAT
10 (9)	TGATGACAGCCTCTTCTTCAGTAATT	GAACTACCTTGCCTGCTCCAGT	ATTAATTTCAAGATAGAAAGAGGACAGTT
11 (10)	CTGGAGCCTTCAGAGGGTAAA	TGCTTTGATGAKGCTTCTGTATC	TCCTGGATTATGCCTGGCA
11 (10)	CTGGAGCCTTCAGAGGGTAAA	TGCTTTGATGAKGCTTCTGTATC	TCCTGGATTATGCCTGGCA
12 (11)	ACATCTCCAAGTTTGCAGAGAAAGA	CTTAACCCACTAGCCATAAAACCC	TGAATAACTAATTATTGGTCTAGCAAG
13 (12)	GCATGTAGTGAACTGTTTAAGGCAAAT	CATGAGCATTATAAGTAAGGTATTCAAAGAA	ACACTAGATGACCAGGAAA
14 (13)	AATTTTGCATGAAGGTAGCAGCT	GGTCGAAAGAATCACATCCCA	TCTACAGCCAGACTTTAG
15 (14a)	AATAAAACCACAATGGTGGCATG	ATGTAGTCACTGCTGGTATGCTCTC	AACTGTACTGTCTTATTGTAATAG
16 (14b)	TGTGTACCTTGATATTGGTACACACATC	GAGCCACAGCACAACCAAAGA	ACTGTGTCTTGTTCCATTCCAGGTGGCT
17 (15)	AACAGCTATGCAGTGATTATCACCAG	GACCTCTGAAGAATCCCATAGCA	TATGTGTTTTACATTTACGTGGGAGTAGCCGACA
18 (16)	TGCTAATTCTTATTTGGGTTCTGAATG	GGCAGAAGGTCATCCAAAATTG	CATCTTGTATATTATAGGTGGGATT
19 (17a)	CAGTTGTCGCAGTTTTACAACCC	TTACAAGATGAGTATCGCACATTCAC	TCACAGCAACTCAAACAACTGGAATCTGAAGGTAT
20 (17b)	TCACTCATCTTGTTACAAGCTTAAAAGG	AATGGAAATGAAGGTAACAGCAATG	TTCCACAAAGCTCTGAATTTACATACTGCCAACT
21 (18)	ACATTGCAGTGGGCTGTAAACTC	TCATGCTATTACTCATACTTTGTTACTTGTCT	ATGTGGATAGCTTGGTAAGT
22 (19)	CAAACCATACAAGAATGGCCAAC	AACAAAGCAAGCAGTGTTCAAATC	GCCAAATGACTGTCAAAGATCTCACAGCA
23 (20)	TTTACCTTATAGGTGGGCCTCTTG	TTCTGGCTAAGTCCTTTTGCTCA	AGCCTTTGGAGTGATACC
24 (21)	GAGGTAAGGCTGCTAACTGAAATGA	TGTTGCTCCAGGTATGTTAGGGTAC	AACTCATACCAACACAAATGGCTGATATAGCTGAC
25 (22)	ACATAAGCTTTCAGAACTCCTGTGTTT	CCATCCACAAGGACAAAGTCAA	TTCTCTAACTGCAGGTTGGGCTCAGATCTG
26 (23)	AAGCATTTGCTGATTGCACAGT	AAGGGCAATGAGATCTTAAGTAAAGTTATAA	AGGATAGAAGCAATGCTGGAATGCCAACA
27 (24)	AGGCTCTGGACATTGCATTCTT	TGCTTGAGTTCCGGTGGG	TCCTTTGAGCCTGTGCCAGTTTCTGTC

del/dup – deletion/duplication.

[1] Legacy *CFTR* nomenclature listed in parentheses, if different from HGVS nomenclature.

Table S6: Del/dup qPCR assay relative quantity reference intervals

Average RQ	Copy Number	Interpretation
Undetermined	0	Homozygous for *CFTR* exon deletion
0.001 – 0.299	Unknown	Equivocal results
0.300 – 0.599	1	Heterozygous for *CFTR* exon deletion
0.600 – 0.799	Unknown	Equivocal results
0.800 – 1.200	2	Normal
1.201 – 1.249	Unknown	Equivocal results
≥1.250	≥3	Amplification[1]

RQ – relative quantity.

[1] Positive deletion and duplication controls were not available for all exons. qPCR assays were validated to detect heterozygous deletions including exons: 1-9,12,14,19-21 and 25-26 (1-8,11,13,17a-18 and 22-23), and duplications/amplifications including exons: 1-3,7-8,18-22 and 25-27 (1-3,6b-7,16-19 and 22-24).

Table S7: Recurrent artifacts in 58 of 181 specimens analyzed using the SV algorithm

CFTR Exon(s) Affected [1]	Del/dup	Number Start Sites[2]	Number Reads[2]	% Reads[2]	Strong SV[2]	Breakpoint (hg19)[2]	Breakpoint (hg19)[2]	Size (bp)[3]
intron 20 (intron 17b)	Del	5 – 126	6 – 457	2.1 – 92.6	Y	Variable. minimum observed: 117,252,060	Variable. maximum observed: 117,252,122	46 – 52
27 - 3'UTR (24 - 3'UTR)	Del	4 – 19	6 – 35	0.5 – 12.1	Y	117,307,228	117,307,260	33
27 - 3'UTR (24 - 3'UTR)	Del	4 – 14	5 – 29	0.7 – 8.3	Y	117,307,194	117,307,226	33

Del – deletion, *Dup* – duplication, *hg19* – human genome assembly GRCh37, *bp* – base pair, *UTR* – untranslated region.

[1]Legacy exon *CFTR* nomenclature listed in parentheses. [2] Output from SV algorithm. [3] Size determined using the SV algorithm.

Table S8: Del/dup detected by each algorithm

CFTR Exon(s) Affected	Del/ Dup	HGVS Nomenclature[1,4]	# NYS alleles tested[3]	Vision/ FreeBayes[2,3]	SV[3,4]	CNV[3,4]	Comments
14 (13)	Del	c.1820_1903del	12	12/12	12/12	0/12	Legacy=1949del84. Too small to be detected by CNV algorithm.
1-9 (1-8)	Del	c.(?_1)_(1209+1_1210-1)del	1	0/1	0/1	1/1	Breakpoints not included in assay design.
2	Del	c.(53+1_54-1)_(164+1_165-1)del	2	0/2	0/2	2/2	Breakpoints for several reported deletions in exon 2 were targeted; but the exact exon 2 breakpoints in these samples (unknown) may not have been included.
2-3	Del	c.54-5940_273+10250del	3	0/3	3/3	3/3	Called using SV and CNV algorithms.
2-4	Del	c.(53+1_54-1)_(489+1_490-1)del	1	0/1	1/1	1/1	Called using SV and CNV algorithms.
18-21 (16-18)	Dup	c.(2908+1_2909-1)_(3468+1_3469-1)dup	1	0/1	0/1	1/1	Breakpoints not included in assay design.
19-21 (17a-18)	Del	c.2988+1168_3468+2108del	2	0/2	2/2	2/2	Reliably called using SV and CNV algorithms.
25-26 (22-23)	Del	c.3964-82_4242+575del	2	0/2	2/2	1/2	Likely too small to be reliably detected by CNV algorithm.

Del – deletion, *Dup* – duplication, *SV* – structural variant algorithm, *CNV* – copy number variant algorithm.

[1] HGVS nomenclature relative to NC_000007.13 and NM_000492.3.

[2] Vision and FreeBayes both consistently detected c.1820_1903del (1949del84).

[3] Number detected / number tested using each algorithm. Counts include replicate samples.

[4] Exact breakpoints were unknown for most del/dup, except where specified. Breakpoints reported by the Archer Analysis SV algorithm were slightly different than reported in literature for some del/dup. For example, the deletion at exons 25-26 is reported in CFTR2 as c.3964-78_4242+577del.

Table S9: Unique Tier 2 TMF variants identified during the first year of NBS using the Archer CF assay

Variant Frequency	Protein (NP_000483.3)	cDNA (NM_000492.3)	Legacy Name
Common	p.Phe508del	c.1521_1523delCTT[1,2]	F508del
Recurrent (≥5 alleles)	p.Arg117His	c.350G>A[1,2]	R117H
	p.Trp1282*	c.3846G>A[1,2]	W1282X
	N/A	c.2988+1G>A[1,2]	3120+1G>A
	p.Gly542*	c.1624G>T[1,2]	G542X
	N/A	c.3718-2477C>T[1,2]	3849+10kbC>T
	p.Gly551Asp	c.1652G>A[1,2]	G551D
	N/A	c.2657+5G>A[1,2]	2789+5G>A
	p.Ala559Thr	c.1675G>A[2]	A559T
	p.Asn1303Lys	c.3909C>G[1,2]	N1303K
	p.Ser549Asn	c.1646G>A[2]	S549N
	p.Val456Ala	c.1367T>C	V456A
	p.Leu206Trp	c.617T>G	L206W
	p.Arg334Trp	c.1000C>T[1,2]	R334W
	p.Arg553*	c.1657C>T[1,2]	R553X
Rare (2-4 alleles)	p.Arg658Lysfs*4	c.1973_1985delGAAATTCAATCCTinsAGAAA	2105-2117del13insAGAAA
	p.Ile507del	c.1519_1521delATC[1,2]	I507del
	p.Arg1162*	c.3484C>T[1,2]	R1162X
	p.Arg709*	c.2125C>T	R709X
	N/A	c.1585-1G>A[1,2]	1717-1G>A
	p.Gln685Thrfs*4	c.2052dupA	2184insA
	N/A	c.489+1G>T[1,2]	621+1G>T
	p.Gly85Glu	c.254G>A[1,2]	G85E
	p.Leu15Pro	c.44T>C	L15P
	p.Arg1158*	c.3472C>T	R1158X
	p.Arg117Cys	c.349C>T	R117C
	p.Arg560Thr	c.1679G>C[1,2]	R560T
	p.Glu726Argfs*4	c.2175dupA[2]	2307insA
	N/A	c.3140-26A>G	3272-26A>G
	p.Lys1250Argfs*9	c.3744delA[2]	3876delA
	N/A	c.579+3A>G	711+3A>G
	p.Phe312del	c.935_937delTCT	F312del or F311del
	p.Leu1254*	c.3761T>G	L1254X
	p.Arg347Pro	c.1040G>C[1,2]	R347P
	p.Arg352Gln	c.1055G>A	R352Q
	p.Thr1036Asn	c.3107C>A	T1036N
Private (1 allele)	p.Cys343*	c.1029delC	1161delC
	N/A	c.1209+1G>A	1341+1G>A
	p.Ile444Argfs*3	c.1327_1330dupGATA	1461ins4
	p.Lys447Argfs*2	c.1340delA	1471delA
	p.Gly473Glufs*54	c.1418delG	1548delG
	N/A	c.1680-877G>T	1811+1643G>T
	N/A	c.1766+1G>A[1,2]	1898+1G>A
	p.Lys684Serfs*38	c.2051_2052delAAinsG[2,3]	2183AA>G
	N/A	c.2490+1G>A	2622+1G>A
	N/A	c.164+1G>T	296+1G>T
	N/A	c.165-3C>T	297-3C>T
	p.Thr1220Lysfs*8	c.3659delC	3791delC
	p.Trp79Leufs*32	c.233dupT	365-366insT
	p.Leu1258Phefs*7	c.3773dupT[2]	3905insT
	p.Ile1295Phefs*32	c.3883_3886delATTT	4010del4
	p.Glu1418Argfs*14	c.4251delA	4382delA

Variant Frequency	Protein (NP_000483.3)	cDNA (NM_000492.3)	Legacy Name
	N/A	c.579+1G>T[1,2]	711+1G>T
	p.Asn268Ilefs*17	c.803delA	935delA
	p.Asn287Lysfs*19	c.861_865delCTTAA	991del5
	p.Ala455Glu	c.1364C>A[1,2]	A455E
	p.Ala613Thr	c.1837G>A	A613T
	p.Cys276*	c.828C>A	C276X
	p.Asp110His	c.328G>C	D110H
	p.Glu60*	c.178G>T	E60X
	p.Gly1244Glu	c.3731G>A	G1244E
	p.Lys710*	c.2128A>T	K710X
	p.Pro67Leu	c.200C>T	P67L
	p.Gln98*	c.292C>T	Q98X
	p.Gln996=	c.2988G>A	3120G>A or Q996Q
	p.Arg1066Cys	c.3196C>T	R1066C
	p.Arg75*	c.223C>T	R75X
	p.Ser1159Phe	c.3476C>T	S1159F
	p.Ser1255Pro	c.3763T>C	S1255P
	p.Ser466*	c.1397C>G	S466X(TAG)
	p.Val232Asp	c.695T>A	V232D
	p.Trp1098*	c.3293G>A	W1098X(TAG)
	p.Trp57*	c.171G>A	W57X
	p.Tyr1092*	c.3276C>A	Y1092X

N/A – Not applicable. Variants are ordered by frequency, including carriers and infants referred.

[1] ACMG-23 variant.

[2] Luminex xTAG CF39v2 variant.

[3] Vision variant caller reports both c.2052delA and c.2051_2052delAAinsG for samples that are heterozygous for c.2051_2052delAAinsG; FreeBayes variant caller and Sanger sequencing confirm the correct call is only c.2051_2052delAAinsG.