MMQCI
MAINE MOLECULAR QUALITY CONTROLS, INC.

23 Mill Brook Road
Saco, ME 04072 USA
Phone: 207-885-1072, Fax: 207-885-1079
Web: www.mmqci.com Email: info@mmqci.com

TECHNICAL NOTE # 25-002

RE: NGS CF Control Panel G211*plus* (p/n: G211*plus*)
ASSAY: TruSight™ Cystic Fibrosis Clinical Sequencing Assay
DATE: 10/09/2025

This Technical Note describes the differences in variant nomenclature and variant reporting between Illumina's TruSight Cystic Fibrosis Clinical Sequencing Assay report and the NGS CF Control Panel G211*plus* Package Insert.

NGS CF Control Panel G211plus Protocol for use with the TruSight Cystic Fibrosis Clinical Sequencing Assay:

Controls are ready to use; no extraction is needed.

1. Thaw all controls completely.
2. Allow the controls to be tested to come to room temperature (18°C to 25°C).
3. Mix well by flicking the control tubes and vortex immediately before using.
4. Quick spin to remove droplets from the tube cap before opening.
5. Add 5µL of each control to the appropriate wells in the **HYB** plate of Illumina's TruSight Cystic Fibrosis Clinical Sequencing Assay (as described in the TruSight Cystic Fibrosis Clinical Sequencing Assay protocol). The controls cannot be quantified; they are manufactured to have approximately equivalent copy numbers of the target gene as genomic DNA.
6. Follow all other procedures as outlined in the TruSight Cystic Fibrosis Clinical Sequencing Assay protocol.
7. Opened material should be tightly capped and returned to freezer (-25°C to -15°C) immediately after use.

1. **Homopolymeric variant alignment:** The TruSight Cystic Fibrosis Clinical Sequencing Assay Package Insert states that insertion or deletion mutations within homopolymeric regions are, by default, left-aligned. The exception to this rule is disease-associated variants listed in the CFTR2 database, such as all CF-139 assay variants, which are right-aligned according to HGVS nomenclature. All variants listed in the NGS CF Control Panel G211*plus* Package Insert follow right-aligned, HGVS nomenclature and appear in CFTR1 and/or CFTR2 databases. As such, homopolymeric insertion or deletion variants that are left-aligned by Illumina's software will not match the NGS CF Control Panel G211*plus* Package Insert. Those variants that do not match are listed in Table 1. All variants with differing HGVS coding sequence names (HGVSc) have the same HGVS protein sequence names (HGVSp) except for one variant. See Table 1.

Table 1. Variants whose HGVS names differ.

	Tube	Illumina Clinical Sequencing Report	MMQCI Package Insert
Differing HGVSc and HGVSp	B	c.3063_3068delAGTGAT p.(Ile1023del)	c.3067_3072delATAGTG p.(Ile1023_Val1024del)
Differing HGVSc with the same HGVSp (not shown)	A	c.1022_1023insTC	c.1021_1022dupTC
	A	c.1372delG	c.1373delG
	A	c.2175_2176insA	c.2175dupA
	A	c.4077_4080delTGTTinsAA	c.4077_4080delinsAA
	B	c.3063_3068delAGTGAT	c.3067_3072delATAGTG
	B	c.3717+12191C>T	c.3718-2477C>T
	B	c.3773_3774insT	c.3773dupT
	C	c.720_741delAGGGAGAATGATGATGAAGTAC	c.723_743+1del
	C	c.1519_1521delATC	c.1516ATC[1]
	C	c.2051_2052delAAinsG	c.2051_2052delinsG
	D	c.312delA	c.313delA
	D	c.800delA	c.803delA
	D	c.927_929delCTT	c.929TCT[2]
	D	c.1519_1521delATC	c.1516ATC[1]
	E	c.325_327delTATinsG	c.325_327delinsG
	E	c.803_804delAT	c.805_806delAT
	E	c.1151_1152insAT	c.1155_1156dupTA
	E	c.1690delA	c.1692delA
	E	c.1974delA	c.1976delA

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Differing HGVSc with the same HGVSp (not shown)	E	c.2052_2053insA	c.2052dupA
	E	c.3037delC	c.3039delC
	E	c.3533_3536delCAAC	c.3536_3539delCCAA
	E	c.3884_3885insT	c.3889dupT
	F	c.1127_1128insA	c.1130dupA
	F	c.1329_1330insAGAT	c.1327_1330dupGATA
	F	c.1679+1.6kbA>G	c.1680-886A>G

2. **Complex deletion-insertion (delins) variant called as 2 variants:** Although the c.1923_1931del9insA is a single variant that appears on one plasmid within the control, it is reported as two separate calls (Table 2). Since Illumina's variant caller does not perform haplotype phasing and does not recognize this complex variant is on a single copy of plasmid DNA (synthetic chromosome) with an A-homopolymer, it undergoes an alternative alignment, resulting in 2 variant calls instead of 1. The raw data is expected to reflect the c.1923_1931del9insA variant at approximately 50% frequency.

Table 2. c.1923_1931del9insA is reported as two distinct variants by the Illumina TruSight Cystic Fibrosis_Clinical Sequencing Assay software.

Tube	Illumina Clinical Sequencing Report	MMQCI Package Insert
D	c.1923_1928delCTCAAA	c.1923_1931del9insA HET
	c.1930_1931delCT	

3. **NGS CF Control Panel G211*plus* variants not reported by the TruSight Cystic Fibrosis Clinical Sequencing Assay:** There is 1 variant present in the NGS CF Control Panel G211*plus* which will not be reported when using the TruSight Cystic Fibrosis Clinical Sequencing Assay due to the sequence configuration of NGS CF Control Panel G211*plus*, as listed in Table 3.

Table 3. NGS CF Control Panel G211*plus* variants not reported by the TruSight Cystic Fibrosis Clinical Sequencing Assay software.

Tube	G211*plus* HGVS variant (Legacy name)	G211*plus* Sequence Configuration
F	c.3700A>G (I1234V)	Low allele frequency due to near neighbor interference with variant W1204X (G>A)

4. **Skewed allele frequencies:** The variants in Table 4 are reported at consistently high or consistently low allele frequencies.

Table 4: Causes for skewed allele frequencies.

Variant	Control	AF Skew	Cause of Skew	Details
c.1022_1023insTC	G211A*plus*	High	Illumina bioinformatics	Allele frequency is elevated resulting from a population of unpaired reads containing the TC-insertion.
c.3302T>A	G211A*plus*	Low	Illumina bioinformatics	The presence of the T>A substitution, which is adjacent to a poly-A homopolymer, causes a shift, or misalignment, at the end of reads. The shift is counted as a "deletion" rather than the 'A' allele.
c.1516A>G	G211C*plus*	High	Overlapping variants in the control	c.1516A>G (I506V) reports with a homozygous-like frequency because it is paired with deletion c.1516ATC[1] (I507del) in the same control. One allele is G, the second allele is deleted.
c.1013C>T	G211E*plus*	Low	Illumina bioinformatics	When present as the last base at the end of reads, the allele is removed by bioinformatic soft-trimming which significantly reduces the frequency.