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TECHNICAL NOTE # 21-001

RE: NGS CF Control Panel G211 v1.1 (p/n: G211v1.1)

ASSAY: TruSight™ Cystic Fibrosis Clinical Sequencing Assay

DATE: 12/08/2023

This Technical Note serves to explain 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 v1.1 Package Insert.

NGS CF Control Panel G211 v1.1 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° – 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 (< -20°C) immediately after use.

1. <u>Homopolymeric variant alignment:</u> 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 are 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 v1.1 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 v1.1 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 with different HGVSp	B	c.3063_3068delAGTGAT p.(Ile1023del)*	c.3067_3072delATAGTG p.(Ile1023_Val1024del)
Differing HGVSc with the same HGVSp (not shown)	D	c.312delA	c.313delA
	D	c.800delA	c.803delA
	D	c.927_929delCTT	c.929TCT[2]
	E	c.803_804delAT	c.805_806delAT
	E	c.1151_1152insAT	c.1155_1156dupTA
	E	c.1974delA	c.1976delA
	E	c.3037delC	c.3039delC
	E	c.3533_3536delCAAC	c.3536_3539delCCAA

**Note: Illumina Technical Support has confirmed that the correct HGVSp annotation is p.(Ile1023_Val1024del).*

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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 v1.1 variants not reported by the TruSight Cystic Fibrosis Clinical Sequencing Assay:** There are 3 variants present in the NGS CF Control Panel G211 v1.1 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 v1.1, as listed in Table 3.

Table 3. NGS CF Control Panel G211 v1.1 variants not reported by the TruSight Cystic Fibrosis Clinical Sequencing Assay software.

Tube	G211v1.1 HGVS variant (Legacy name)	G211v1.1 Sequence Configuration
A	c.1820_1903del84 (1949del84)	Large deletion in control overlaps with multiple primer binding sites of assay
E	c.3712C>T (Q1238X C>T)	Insufficient intronic sequence in control
F	c.3700A>G (I1234V)	Low allele frequency due to near neighbor interference with variant W1204X (G>A)

Please call us at 207-885-1072 x 1 if you have any questions.

Sincerely,
Maine Molecular Quality Controls, Inc.
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