

## NGS CF Control Panel G211plus

### INTENDED USE:

The NGS CF Control Panel G211plus is intended for use as an external quality control to monitor the analytical performance of *in vitro* laboratory nucleic acid testing procedures for the qualitative detection of genetic variants found on the cystic fibrosis transmembrane conductance regulator (CFTR) gene, associated with cystic fibrosis (CF), including 100 CFTR variants recommended for carrier testing by the American College of Medical Genetics and Genomics (ACMG). Available testing procedures for the detection of CFTR variants include next-generation sequencing (NGS), Sanger sequencing, multiplex ligation-dependent probe amplification (MLPA), multiplex polymerase chain reaction (PCR) with oligonucleotide ligation, capillary electrophoresis, arrays, and mass spectrometry.

The NGS CF Control Panel G211plus can also be used as a method control to monitor the ability of an NGS test system to correctly identify challenging types of variants seen throughout the human genome. The control panel carries over 150 substitutions representing all possible nucleotide changes, 30 small deletions (1 to 22 bp), 4 complex insertion/deletions, 10 small insertions/duplications (1 to 4 bp), 2 large genomic rearrangements, and Poly-T/Poly-TG repeats. Appropriate primers are required to amplify the areas of interest.

Cystic fibrosis (CF), one of the most common autosomal recessive diseases in the United States, is caused by the presence of mutations in both copies of the gene for the cystic fibrosis transmembrane conductance regulator (CFTR)<sup>1</sup> protein. CF is recognized as a clinically heterogeneous multi-organ disease. While there is no cure for CF, newer mutation-specific targeted therapies are available<sup>2</sup>.

More than 2,000 variants have been reported in the CFTR coding and flanking sequence in all ethnic and racial populations at various frequencies. However, fewer than 10% of these reported variants have been interpreted to be clinically relevant variants. The 211 CFTR variants listed in Table 1 are contained in the 6 tubes of NGS CF Control Panel G211plus. The 178 asterisked (\*) variants have been identified as CF-causing by The Clinical and Functional Translation of CFTR (CFTR2) international project and are listed in the CFTR2 database at <http://cfr2.org> (April 7, 2023 version)<sup>3,4</sup>. Bolded variants in Table 1 are the 100 mutations recommended in 2023 by the ACMG and in 2011 by the American College of Obstetricians and Gynecologists (ACOG)<sup>5,6,7</sup>. Legacy nomenclature is used throughout this Package Insert.

Quality control materials should be used in accordance with local, state, and federal regulations and accreditation requirements.

### PRODUCT SUMMARY AND PRINCIPLE:

The NGS CF Control Panel G211plus is designed as a multiplex reference material to monitor the simultaneous detection of the CFTR mutations and variants associated with CF. The NGS CF Control Panel G211plus is composed of 6 tubes of CFTR DNA, each carrying multiple CFTR variants as described in Table 1. Legacy and Human Genome Variation Society (HGVS) nomenclature are both listed in Table 1. The control panel does not require extraction and can be added directly to the assay to monitor testing steps such as library preparation, target enrichment, amplification, sequencing and sequencing analysis using bioinformatics. Appropriate primers are required to amplify the areas of interest. Please consult with MMQCI regarding specific primers used in your laboratory.

Quality controls can be used for routine monitoring of test systems, evaluation, validation, verification, proficiency assessment, and training procedures. Quality controls that are consistent lot to lot assist the laboratory in identifying shifts, trends, and increased frequency of random errors caused by variations in the test system, such as failing reagents or malfunctioning equipment. Early investigation can prevent failed assay runs.

#### References:

1. Moskowitz SM, Chmielewski JF, Sternan DL, Cheng E, Gibson RL et al. Clinical practice and genetic counseling for cystic fibrosis and CFTR-related disorders. *Genet Med*. 2008;10:851–868
2. Grasemann H, Ratjen F. Cystic Fibrosis. *N Engl J Med* 2023;389:1693–1707
3. The Clinical and Functional Translation of CFTR (CFTR2); available at <http://cfr2.org>
4. Sosnay PR, Siklosi KR, Van Goor F, Kaniecki K, Yu H, et al. Defining the disease liability of variants in the cystic fibrosis transmembrane conductance regulator gene. *Nat Genet*. 2013;45:1160–1167.
5. Deignan, Joshua L et al. "Updated recommendations for CFTR carrier screening: A position statement of the American College of Medical Genetics and Genomics (ACMG)." *Genetics in medicine: official journal of the American College of Medical Genetics* vol. 25,8 (2023): 100867. doi:10.1016/j.jim.2023.100867
6. Update on Carrier Screening for Cystic Fibrosis. Committee Opinion Number 486. American College of Obstetricians and Gynecologists. *Obstet Gynecol* 2011;117:1028–31
7. Bobadilla JL, Macek M, Fine JP, and Farrell PM. Cystic fibrosis: A worldwide analysis of CFTR mutations correlation with incidence data and application to screening. *Human Mutation* 2002;19(6):575–606

### COMPOSITION:

NGS CF Control Panel G211plus is comprised of synthetic CFTR DNA suspended at a concentration similar to that of extracted human whole blood samples in a non-infectious buffer with stabilizers. The CFTR DNA is composed of all 27 CFTR gene exons plus intronic borders and contains CF-associated variants divided among 6 tubes (G211Aplus, G211Bv1.1, G211Cplus, G211Dv1.1, G211Eplus and G211Fplus), described in Table 1. All DNA in the NGS CF Control Panel G211plus, wild type and variant, has been Sanger sequenced, such that 100% of the sequence in the NGS CF Control Panel G211plus has been confirmed.

### INSTRUCTIONS FOR USE:

*Controls do not require extraction before use.*

1. Thaw all controls completely.
2. Allow the controls to be tested to come completely 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. Pipette the same volume (usually 5–10µL) pipetted for extracted patient sample DNA for each control tube and treat just as you would genomic DNA.
6. Follow all other procedures as described by test manufacturer's product insert.
7. Opened material should be tightly capped and returned to freezer (-25°C to -15°C) immediately after use.

**Note:** The NGS CF Control Panel G211plus cannot be quantified or measured by standard methods such as spectrophotometry (A260nm/A280nm) because synthetic DNA has significantly less mass than genomic DNA. However, the controls are manufactured to have approximately equivalent copy numbers of the target gene as genomic DNA. Please contact MMQCI if guidance on volume to add to assay is desired.

### STORAGE and STABILITY:

Upon receipt and after opening, the material should be stored at -25°C to -15°C. Unopened NGS CF Control Panel G211plus is stable through the expiration date printed on each tube when consistently stored frozen (-25°C to -15°C). Opened material should be tightly capped and returned to the freezer (-25°C to -15°C) immediately after use. The material is stable for five freeze/thaw cycles.

### PRECAUTIONS, WARNINGS, and LIMITATIONS:

- Do not dilute.
- Do not transfer control material to a new tube.
- Do not quantify by spectrophotometry or other standard methods.
- Do not freeze/thaw more than 5 times.
- This product does not contain any biological material of human origin and is not infectious. Universal Precautions are NOT required when handling this product.
- NGS CF Control Panel G211plus is provided for Research Use Only (RUO). It cannot be cloned, sold, or transferred without the explicit written consent of MMQCI.

### EXPECTED VALUES:

The genotype for each CFTR variant found in tubes A-F of NGS CF Control Panel G211plus is listed in Table 1. The sequence of NGS CF Control Panel G211plus has been confirmed by bi-directional Sanger sequencing. For an NGS, or any multiplex CFTR assay to detect all genotypes listed, the assay primers must have sufficient control sequence on either side of the variant to anneal and amplify the area of interest. If unexpected calls are reported, or variants not detected, your laboratory's assay primers may require sequence not present in NGS CF Control Panel G211plus, or they may anneal poorly due to the presence of a nearby variant. Please contact MMQCI for interpretation assistance as needed.

The laboratory should follow Good Laboratory Practice (GLP) and establish its own performance characteristics for the NGS CF Control Panel G211plus in demonstrating adequate system performance.

### ORDERING INFORMATION:

NGS CF Control Panel G211plus

**Part Number: G211plus**

Kit Contains: 6 tubes x 50µL

**Table 1. NGS CF Control Panel G211plus** Variants are listed in genomic coordinate order. **Bold** = ACMG recommended panel of 100 variants.  
Asterisk\* = clinically relevant CFTR2 variants.

| CFTR Legacy Name | cDNA Name (HGVS)           | Tube A        | Tube B        | Tube C        | Tube D        | Tube E        | Tube F        |
|------------------|----------------------------|---------------|---------------|---------------|---------------|---------------|---------------|
| 1. M1V*          | c.1A>G                     | WT            | WT            | WT            | WT            | WT            | HET           |
| 2. Q2X*          | c.4C>T                     | WT            | WT            | HET           | WT            | WT            | WT            |
| 3. R31C          | c.91C>T                    | WT            | WT            | HET           | WT            | WT            | WT            |
| 4. CFTRdele2,3*  | c.54-5940_273+10250del21kb | HET           | WT            | WT            | WT            | WT            | WT            |
| 5. Q39X*         | c.115C>T                   | WT            | WT            | WT            | WT            | WT            | HET           |
| 6. 296+2T>A      | c.164+2T>A                 | WT            | WT            | WT            | WT            | HET           | WT            |
| 7. E60X*         | c.178G>T                   | WT            | HET           | WT            | WT            | WT            | WT            |
| 8. P67L*         | c.200C>T                   | WT            | WT            | WT            | WT            | HET           | WT            |
| 9. R75X*         | c.223C>T                   | WT            | WT            | WT            | HET           | WT            | WT            |
| 10. G85E*        | c.254G>A                   | WT            | HET           | WT            | WT            | WT            | WT            |
| 11. 394delTT*    | c.262_263delTT             | WT            | WT            | HET           | WT            | WT            | WT            |
| 12. G91R*        | c.271G>A                   | WT            | WT            | WT            | WT            | WT            | HET           |
| 13. G91G         | c.273G>A                   | WT            | WT            | WT            | WT            | HET           | WT            |
| 14. 405+1 G>A*   | c.273+1G>A                 | WT            | WT            | WT            | WT            | HET           | WT            |
| 15. 405+3A>C*    | c.273+3A>C                 | WT            | WT            | WT            | HET           | WT            | WT            |
| 16. 406-1 G>A*   | c.274-1G>A                 | WT            | WT            | WT            | HET           | WT            | WT            |
| 17. E92K*        | c.274G>A                   | WT            | WT            | WT            | WT            | WT            | HET           |
| 18. E92XG>T*     | c.274G>T                   | WT            | WT            | WT            | WT            | HET           | WT            |
| 19. Q98X*        | c.292C>T                   | WT            | WT            | HET           | WT            | WT            | WT            |
| 20. Q98R*        | c.293A>G                   | WT            | WT            | HET           | WT            | WT            | WT            |
| 21. 444delA*     | c.313delA                  | WT            | WT            | WT            | HET           | WT            | WT            |
| 22. 457TAT>G*    | c.325_327delinsG           | WT            | WT            | WT            | WT            | HET           | WT            |
| 23. D110H*       | c.328G>C                   | WT            | WT            | WT            | WT            | WT            | HET           |
| 24. R117C*       | c.349C>T                   | WT            | WT            | WT            | HET           | WT            | WT            |
| 25. R117H        | c.350G>A                   | WT            | WT            | HET           | WT            | WT            | WT            |
| 26. Y122X*       | c.366T>A                   | HET           | WT            | WT            | WT            | WT            | WT            |
| 27. 574delA*     | c.442delA                  | WT            | WT            | WT            | WT            | HET           | WT            |
| 28. I148T        | c.443T>C                   | WT            | HET           | WT            | WT            | WT            | WT            |
| 29. 621+1 G>T*   | c.489+1G>T                 | WT            | HET           | WT            | WT            | WT            | HET           |
| 30. 663delT*     | c.531delT                  | WT            | WT            | WT            | WT            | HET           | WT            |
| 31. G178R*       | c.532G>A                   | WT            | WT            | WT            | HET           | WT            | WT            |
| 32. F191V*       | c.571T>G                   | WT            | WT            | HET           | WT            | WT            | WT            |
| 33. 711+1 G>T*   | c.579+1G>T                 | WT            | HET           | WT            | WT            | WT            | WT            |
| 34. 711+3 A>G*   | c.579+3A>G                 | WT            | WT            | WT            | WT            | WT            | HET           |
| 35. 711+5 G>A*   | c.579+5G>A                 | WT            | WT            | HET           | WT            | WT            | WT            |
| 36. 712-1 G>T*   | c.580-1G>T                 | WT            | WT            | WT            | WT            | HET           | WT            |
| 37. H199Y*       | c.595C>T                   | WT            | HET           | WT            | WT            | WT            | WT            |
| 38. P205S C>T*   | c.613C>T                   | WT            | WT            | HET           | WT            | WT            | WT            |
| 39. L206W*       | c.617T>G                   | WT            | WT            | WT            | HET           | WT            | WT            |
| 40. L218X*       | c.653T>A                   | HET           | WT            | WT            | WT            | WT            | WT            |
| 41. Q220X*       | c.658C>T                   | WT            | WT            | WT            | WT            | WT            | HET           |
| 42. L227R*       | c.680T>G                   | WT            | WT            | HET           | WT            | WT            | WT            |
| 43. V232D*       | c.695T>A                   | WT            | WT            | HET           | WT            | WT            | WT            |
| 44. 852del22*    | c.723_743+1del             | WT            | WT            | HET           | WT            | WT            | WT            |
| 45. 935delA*     | c.803delA                  | WT            | WT            | WT            | HET           | WT            | WT            |
| 46. 936delTA*    | c.805_806delAT             | WT            | WT            | WT            | WT            | HET           | WT            |
| 47. Q290X*       | c.868C>T                   | HET           | WT            | WT            | WT            | WT            | WT            |
| 48. delF311      | c.929TCT[2]                | WT            | WT            | WT            | HET           | WT            | WT            |
| 49. 1078delT*    | c.948delT                  | WT            | HET           | WT            | WT            | WT            | WT            |
| 50. G330X*       | c.988G>T                   | WT            | WT            | WT            | HET           | WT            | WT            |
| 51. R334W*       | c.1000C>T                  | WT            | HET           | WT            | WT            | WT            | WT            |
| 52. I336K*       | c.1007T>A                  | WT            | WT            | WT            | WT            | WT            | HET           |
| 53. T338I*       | c.1013C>T                  | WT            | WT            | WT            | WT            | HET           | WT            |
| 54. S341P*       | c.1021T>C                  | WT            | WT            | HET           | WT            | WT            | WT            |
| 55. 1154insTC*   | c.1021_1022dupTC           | HET           | WT            | WT            | WT            | WT            | WT            |
| 56. 1161delC*    | c.1029delC                 | WT            | WT            | HET           | WT            | WT            | WT            |
| 57. R347H*       | c.1040G>A                  | WT            | WT            | HET           | WT            | WT            | WT            |
| 58. R347P*       | c.1040G>C                  | WT            | HET           | WT            | WT            | WT            | WT            |
| 59. R352Q*       | c.1055G>A                  | WT            | WT            | WT            | HET           | WT            | WT            |
| 60. 1213delT*    | c.1081delT                 | WT            | WT            | WT            | WT            | WT            | HET           |
| 61. S364P        | c.1090T>C                  | WT            | WT            | WT            | HET           | WT            | WT            |
| 62. 1248+1G>A*   | c.1116+1G>A                | HET           | WT            | WT            | WT            | WT            | WT            |
| 63. 1259insA*    | c.1130dupA                 | WT            | WT            | WT            | WT            | WT            | HET           |
| 64. 1288insTA*   | c.1155_1156dupTA           | WT            | WT            | WT            | WT            | HET           | WT            |
| 65. W401X (TAG)* | c.1202G>A                  | HET           | WT            | WT            | WT            | WT            | WT            |
| 66. W401X (TGA)* | c.1203G>A                  | WT            | WT            | WT            | HET           | WT            | WT            |
| 67. 1341+1G>A*   | c.1209+1G>A                | WT            | WT            | HET           | WT            | WT            | WT            |
| 68. PolyT        | PolyT                      | 7T/7T         | 9T/9T         | 7T/5T         | 5T/9T         | 7T/9T         | 9T/9T         |
| 69. PolyTG       | PolyTG                     | (TG)11/(TG)12 | (TG)12/(TG)12 | (TG)11/(TG)12 | (TG)11/(TG)12 | (TG)11/(TG)12 | (TG)11/(TG)12 |
| 70. 1461ins4*    | c.1327_1330dupGATA         | WT            | WT            | WT            | WT            | WT            | HET           |
| 71. A455E*       | c.1364C>A                  | WT            | HET           | WT            | WT            | WT            | WT            |
| 72. V456A*       | c.1367T>C                  | WT            | WT            | HET           | WT            | WT            | WT            |
| 73. 1504delG*    | c.1373del                  | HET           | WT            | WT            | WT            | WT            | WT            |
| 74. 1525-1G>A*   | c.1393-1G>A                | WT            | WT            | WT            | WT            | WT            | HET           |
| 75. S466X (C>A)* | c.1397C>A                  | WT            | WT            | WT            | WT            | HET           | WT            |
| 76. S466X (C>G)* | c.1397C>G                  | HET           | WT            | WT            | WT            | WT            | WT            |
| 77. L467P*       | c.1400T>C                  | WT            | WT            | HET           | WT            | WT            | WT            |
| 78. M470V        | c.1408G>A                  | HET           | HOM           | HET           | HOM           | HET           | HET           |
| 79. 1548delG*    | c.1418delG                 | WT            | WT            | WT            | HET           | WT            | WT            |
| 80. G480C        | c.1438G>T                  | WT            | WT            | WT            | HET           | WT            | WT            |
| 81. S489X*       | c.1466C>A                  | WT            | WT            | HET           | WT            | WT            | WT            |
| 82. S492F*       | c.1475C>T                  | HET           | WT            | WT            | WT            | WT            | WT            |
| 83. Q493X*       | c.1477C>T                  | HET           | WT            | WT            | WT            | WT            | WT            |
| 84. I506V        | c.1516A>G                  | WT            | WT            | HET**         | WT            | WT            | WT            |
| 85. I507V        | c.1519A>G                  | HET           | WT            | WT            | WT            | WT            | WT            |

| CFTR Legacy Name   | cDNA Name (HGVS)              | Tube A | Tube B | Tube C | Tube D | Tube E | Tube F |
|--------------------|-------------------------------|--------|--------|--------|--------|--------|--------|
| 86. I507del*       | c.1516ATC[1] (c.1519 1521del) | WT     | WT     | HET    | HET    | WT     | WT     |
| 87. F508del*       | c.1521 1523delCTT             | WT     | HET    | WT     | WT     | WT     | WT     |
| 88. F508C          | c.1523T>G                     | HET    | WT     | WT     | WT     | WT     | WT     |
| 89. I677delTA*     | c.1545 1546delTA              | WT     | WT     | HET    | HET    | WT     | WT     |
| 90. V520F*         | c.1558G>T                     | WT     | HET    | WT     | WT     | WT     | WT     |
| 91. C524X*         | c.1572C>A                     | WT     | WT     | HET    | WT     | WT     | WT     |
| 92. Q525X*         | c.1573C>T                     | WT     | WT     | WT     | WT     | WT     | HET    |
| 93. I716+1G>A      | c.1584+1G>A                   | WT     | WT     | WT     | WT     | HET    | WT     |
| 94. I717-1G>A*     | c.1585-1G>A                   | WT     | HET    | WT     | WT     | WT     | WT     |
| 95. I717-8G>A*     | c.1585-8G>A                   | WT     | WT     | WT     | WT     | WT     | HET    |
| 96. G542X*         | c.1624G>T                     | WT     | HET    | WT     | WT     | WT     | WT     |
| 97. S549R*         | c.1645A>C                     | WT     | WT     | HET    | WT     | WT     | WT     |
| 98. S549N*         | c.1646G>A                     | WT     | WT     | WT     | HET    | WT     | WT     |
| 99. S549R*         | c.1647T>G                     | HET    | WT     | WT     | WT     | WT     | WT     |
| 100. G551S*        | c.1651G>A                     | HET    | WT     | WT     | WT     | WT     | WT     |
| 101. G551D*        | c.1652G>A                     | WT     | HET    | WT     | WT     | WT     | WT     |
| 102. Q552XC>T*     | c.1654C>T                     | WT     | WT     | WT     | WT     | HET    | WT     |
| 103. R553X*        | c.1657C>T                     | WT     | WT     | HET    | WT     | WT     | WT     |
| 104. I558S*        | c.1673T>C                     | WT     | WT     | WT     | WT     | WT     | HET    |
| 105. A559T*        | c.1675G>A                     | HET    | WT     | WT     | WT     | WT     | WT     |
| 106. R560T*        | c.1679G>C                     | WT     | HET    | WT     | WT     | WT     | WT     |
| 107. R560K*        | c.1679G>A                     | WT     | WT     | WT     | HET    | WT     | WT     |
| 108. I811+1G>A*    | c.1679+1G>A                   | WT     | WT     | HET    | WT     | WT     | WT     |
| 109. I811+1. 6 kb* | c.1680-886A>G                 | WT     | WT     | WT     | WT     | WT     | HET    |
| 110. R560S*        | c.1680A>C                     | HET    | WT     | WT     | WT     | WT     | WT     |
| 111. I812-1 G>A*   | c.1680-1G>A                   | WT     | WT     | WT     | HET    | WT     | WT     |
| 112. A561E*        | c.1682C>A                     | WT     | WT     | HET    | WT     | WT     | WT     |
| 113. I824delA*     | c.1692delA                    | WT     | WT     | WT     | WT     | HET    | WT     |
| 114. Y569D*        | c.1705T>G                     | WT     | WT     | WT     | WT     | WT     | HET    |
| 115. G576A         | c.1727G>C                     | WT     | WT     | WT     | HET    | WT     | WT     |
| 116. E585X*        | c.1753G>T                     | WT     | WT     | HET    | WT     | WT     | WT     |
| 117. I898+1 G>A*   | c.1766+1G>A                   | WT     | HET    | WT     | WT     | WT     | WT     |
| 118. I898+3 A>G*   | c.1766+3A>G                   | WT     | WT     | WT     | WT     | HET    | WT     |
| 119. I898+5G>T*    | c.1766+5G>T                   | HET    | WT     | WT     | WT     | WT     | WT     |
| 120. A613T*        | c.1837G>A                     | HET    | WT     | WT     | WT     | WT     | WT     |
| 121. D614G         | c.1841A>G                     | WT     | WT     | WT     | WT     | WT     | HET    |
| 122. G622D         | c.1865G>A                     | WT     | WT     | WT     | HET    | WT     | WT     |
| 123. G628R*        | c.1882G>A                     | HET    | WT     | WT     | WT     | WT     | WT     |
| 124. 2055del9>A*   | c.1923 1931delinsA            | WT     | WT     | WT     | HET    | WT     | WT     |
| 125. 2108delA*     | c.1976delA                    | WT     | WT     | WT     | WT     | HET    | WT     |
| 126. R668C         | c.2002C>T                     | WT     | WT     | WT     | WT     | WT     | HET    |
| 127. 2143delIT*    | c.2012delIT                   | WT     | HET    | WT     | WT     | WT     | WT     |
| 128. 2183AA>G*     | c.2051 2052delinsG            | WT     | WT     | HET    | WT     | WT     | WT     |
| 129. 2184insA*     | c.2052dupA                    | WT     | WT     | WT     | WT     | HET    | WT     |
| 130. 2184delA*     | c.2052delA                    | WT     | HET    | WT     | WT     | WT     | WT     |
| 131. R709XC>T*     | c.2125C>T                     | WT     | WT     | WT     | WT     | HET    | WT     |
| 132. K710X*        | c.2128A>T                     | WT     | WT     | WT     | HET    | WT     | WT     |
| 133. 2307insA*     | c.2175dupA                    | HET    | WT     | WT     | WT     | WT     | WT     |
| 134. L732X*        | c.2195T>G                     | WT     | WT     | WT     | WT     | WT     | HET    |
| 135. 2347delG*     | c.2215delG                    | WT     | WT     | WT     | HET    | WT     | WT     |
| 136. V754M         | c.2260G>A                     | WT     | WT     | WT     | WT     | WT     | HET    |
| 137. R764XC>T*     | c.2290C>T                     | WT     | WT     | WT     | WT     | HET    | WT     |
| 138. R785X         | c.2353C>T                     | HET    | WT     | WT     | WT     | WT     | WT     |
| 139. R792X         | c.2374C>T                     | WT     | WT     | WT     | WT     | HET    | WT     |
| 140. 2585delIT*    | c.2453delIT                   | WT     | WT     | WT     | WT     | WT     | HET    |
| 141. E822X*        | c.2464G>T                     | WT     | WT     | WT     | HET    | WT     | WT     |
| 142. 2622+1G>A*    | c.2490+1G>A                   | WT     | HET    | WT     | WT     | WT     | WT     |
| 143. E831X*        | c.2491G>T                     | WT     | WT     | WT     | WT     | WT     | HET    |
| 144. W846X*        | c.2537G>A                     | HET    | WT     | WT     | WT     | WT     | WT     |
| 145. W846X(2670)*  | c.2538G>A                     | WT     | HET    | WT     | WT     | WT     | WT     |
| 146. R851X*        | c.2551C>T                     | WT     | WT     | WT     | WT     | WT     | HET    |
| 147. 2711delIT*    | c.2583delIT                   | WT     | WT     | WT     | HET    | WT     | WT     |
| 148. 2789+2insA    | c.2657+2 2657+3insA           | WT     | WT     | WT     | WT     | WT     | HET    |
| 149. 2789+5G>A*    | c.2657+5G>A                   | WT     | HET    | WT     | WT     | WT     | WT     |
| 150. Q890X*        | c.2668C>T                     | WT     | WT     | WT     | HET    | WT     | HET    |
| 151. 2869insG*     | c.2737 2738insG               | WT     | WT     | WT     | HET    | WT     | WT     |
| 152. Y913X*        | c.2739T>A                     | HET    | WT     | WT     | WT     | WT     | WT     |
| 153. L927P*        | c.2780T>C                     | WT     | WT     | WT     | WT     | WT     | HET    |
| 154. S945L*        | c.2834C>T                     | WT     | WT     | WT     | WT     | WT     | HET    |
| 155. 3007delG*     | c.2875delG                    | WT     | HET    | WT     | WT     | WT     | WT     |
| 156. G970R*        | c.2908G>C                     | WT     | WT     | WT     | WT     | WT     | HET    |
| 157. G970D*        | c.2909G>A                     | HET    | WT     | WT     | WT     | WT     | WT     |
| 158. S977F         | c.2930C>T                     | WT     | WT     | WT     | WT     | WT     | HET    |
| 159. 3120G>A*      | c.2988G>A                     | WT     | WT     | WT     | HET    | WT     | WT     |
| 160. 3120+1G>A*    | c.2988+1G>A                   | WT     | HET    | WT     | WT     | WT     | WT     |
| 161. 3121-1G>A*    | c.2989-1G>A                   | WT     | WT     | WT     | WT     | WT     | HET    |
| 162. L997F (G>C)   | c.2991G>C                     | WT     | WT     | WT     | WT     | HET    | WT     |
| 163. 3171delC*     | c.3039delC                    | WT     | WT     | WT     | WT     | HET    | WT     |
| 164. 3199del6*     | c.3067 3072delATAGTG          | WT     | HET    | WT     | WT     | WT     | WT     |
| 165. I1027T        | c.3080T>C                     | WT     | WT     | WT     | WT     | WT     | HET    |
| 166. T1036N*       | c.3107C>A                     | HET    | WT     | WT     | WT     | WT     | WT     |
| 167. 3272-26A>G*   | c.3140-26A>G                  | WT     | WT     | HET    | WT     | WT     | WT     |
| 168. L1065P*       | c.3194T>C                     | WT     | WT     | WT     | WT     | HET    | WT     |
| 169. R1066C*       | c.3196C>T                     | WT     | WT     | WT     | HET    | WT     | WT     |
| 170. R1066H*       | c.3197G>A                     | WT     | WT     | HET    | WT     | WT     | WT     |
| 171. R1070W        | c.3208C>T                     | WT     | HET    | WT     | WT     | WT     | WT     |
| 172. R1070Q        | c.3209G>A                     | HET    | WT     | WT     | WT     | WT     | WT     |
| 173. L1077P*       | c.3230T>C                     | WT     | HET    | WT     | WT     | HET    | WT     |

| CFTR Legacy Name       | cDNA Name (HGVS)      | Tube A | Tube B | Tube C | Tube D | Tube E | Tube F |
|------------------------|-----------------------|--------|--------|--------|--------|--------|--------|
| 174. W1089X*           | c.3266G>A             | WT     | WT     | WT     | HET    | WT     | WT     |
| 175. Y1092X (C>A)*     | c.3276C>A             | HET    | WT     | WT     | WT     | WT     | WT     |
| 176. Y1092X (C>G)*     | c.3276C>G             | WT     | WT     | HET    | WT     | WT     | WT     |
| 177. W1098C*           | c.3294G>C             | WT     | WT     | WT     | WT     | WT     | HET    |
| 178. M1101K*           | c.3302T>A             | HET    | WT     | WT     | WT     | WT     | WT     |
| 179. E1104X*           | c.3310G>T             | WT     | WT     | WT     | WT     | WT     | HET    |
| 180. S1118F*           | c.3353C>T             | WT     | WT     | WT     | WT     | WT     | HET    |
| 181. D1152H            | c.3454G>C             | WT     | HET    | WT     | WT     | WT     | WT     |
| 182. R1158X*           | c.3472C>T             | WT     | WT     | WT     | HET    | WT     | WT     |
| 183. R1162X*           | c.3484C>T             | WT     | HET    | WT     | WT     | WT     | WT     |
| 184. R1162L            | c.3485G>T             | WT     | WT     | WT     | WT     | WT     | HET    |
| 185. 3659delC*         | c.3528delC            | WT     | HET    | WT     | WT     | WT     | WT     |
| 186. 3667del4*         | c.3536-3539delCCAA    | WT     | WT     | WT     | WT     | HET    | WT     |
| 187. S1196X*           | c.3587C>G             | WT     | WT     | WT     | HET    | WT     | WT     |
| 188. W1204X (3743G>A)* | c.3611G>A             | WT     | WT     | WT     | WT     | HET    | WT     |
| 189. W1204X (3744G>A)* | c.3612G>A             | WT     | WT     | WT     | WT     | WT     | HET    |
| 190. 3791delC*         | c.3659delC            | WT     | WT     | WT     | HET    | WT     | WT     |
| 191. I1234V*           | c.3700A>G             | WT     | WT     | WT     | WT     | WT     | HET    |
| 192. S1235R            | c.3705T>G             | WT     | HET    | WT     | WT     | WT     | WT     |
| 193. 3849+5G->A*       | c.3717+5G>A           | HET    | WT     | WT     | WT     | WT     | WT     |
| 194. 3849+10kb C>T*    | c.3718-2477C>T        | WT     | HET    | WT     | WT     | WT     | WT     |
| 195. 3849+4A>G*        | c.3717+4A>G           | HET    | WT     | WT     | WT     | WT     | WT     |
| 196. G1244E*           | c.3731G>A             | WT     | WT     | WT     | WT     | HET    | HET    |
| 197. 3876delA*         | c.3744delA            | WT     | HET    | WT     | WT     | WT     | WT     |
| 198. S1251N*           | c.3752G>A             | HET    | WT     | WT     | WT     | HET    | WT     |
| 199. S1255XA>G         | c.3607A>G             | WT     | WT     | HET    | WT     | WT     | WT     |
| 200. S1255XC>A*        | c.3764C>A             | WT     | WT     | HET    | WT     | WT     | WT     |
| 201. 3905insT*         | c.3773dupT            | WT     | HET    | WT     | WT     | WT     | WT     |
| 202. 3940delG*         | c.3808del             | HET    | WT     | WT     | WT     | WT     | WT     |
| 203. D1270N            | c.3808G>A             | WT     | WT     | WT     | HET    | WT     | WT     |
| 204. W1282X*           | c.3846G>A             | WT     | HET    | WT     | WT     | WT     | WT     |
| 205. 4005+1G>A*        | c.3873+1G>A           | WT     | WT     | WT     | WT     | WT     | HET    |
| 206. 4016insT*         | c.3889dupT            | WT     | WT     | WT     | WT     | HET    | WT     |
| 207. N1303K*           | c.3909C>G             | WT     | HET    | WT     | WT     | WT     | HET    |
| 208. Q1313X*           | c.3937C>T             | HET    | WT     | WT     | WT     | WT     | WT     |
| 209. CFTRdel22,23*     | c.3964-78-4242+577del | WT     | WT     | WT     | WT     | WT     | HET    |
| 210. 4209TGTT>AA*      | c.4077-4080delinsAA   | HET    | WT     | WT     | WT     | WT     | WT     |
| 211. 4382delA*         | c.4251delA            | HET    | WT     | WT     | WT     | WT     | WT     |