



A Laboratory Developed, 106 Mutation Cystic Fibrosis Carrier Screening Test Using the Agena MassARRAY®: Performance in the first 123,000 Tests

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Abstract

Introduction: In 2001 and 2004, the ACOG and ACMG recommended population-based carrier screening for cystic fibrosis (CF) using a panel of 23 mutations. Since that time, some have argued that expanded panels with additional mutations would be useful in some populations. We co-developed a CF screening test with Sequenom, Inc. that targets 106 mutations. Analytical verification of the assay has been presented (Farkas et al. JMD 2010;12:611-9). Here we report the data obtained from the first 123,172 test performed at Mayo Clinic, and ask whether extended carrier screening panels can increase the sensitivity of CF carrier detection.

Methods: The MassARRAY® CF screening test was validated according to CLIA guidelines. The workflow consists of 8 multiplex PCR reactions, removal of dNTPs and primers with Agencourt® AMPure® XP resin (Beckman-Coulter), 8 multiplex single base extension (SBE) reactions, desalting with an ion exchange resin, spotting the extended SBE products onto a MassARRAY chip, and analysis by MALDI-TOF mass spectrometry. 48 samples and controls are run per batch in 384 well plates. All steps are automated, and the test requires 9 hours to complete. The method requires only general purpose laboratory reagents, thus the test is inexpensive at under \$30 per reaction. Since validation, the MGL has tested 123,172 clinical samples.

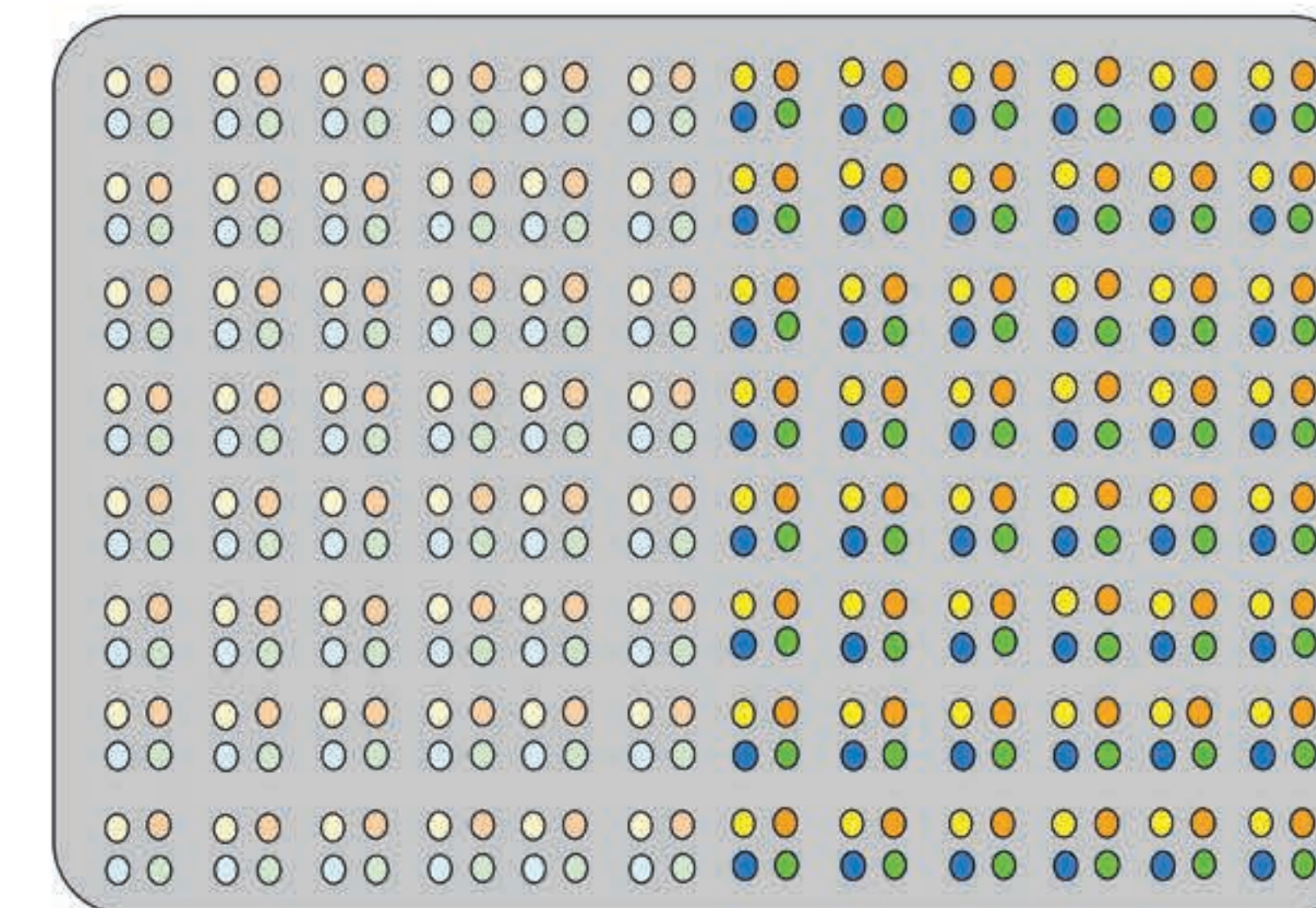
Results: Validation of accuracy included analysis of a set of 43 samples that contained 60 different mutations and potential interferences (such as E75Q and 711+3A>G), 28 of these samples were compound heterozygotes. In addition, 594 samples were tested in parallel (15 runs) with the current Luminex 70+5 commercial assay, 25 of these samples had one mutation detected by the Luminex assay. For both sets of samples, there were no discordant results.

Discussion: Of the clinical samples tested, 3,153 were for a possible or known diagnosis of CF, pancreatitis, or male infertility; the remainder had indications for testing of routine carrier screening, a family history of CF, a partner that was a known CF carrier, or no indication given. Of the samples tested, 472 had two mutations (226 homozygotes and 246 compound heterozygotes). Of these, there were 203 deltaF508 homozygotes, and 184 cases with one deltaF508 with another mutation. There were 4,647 cases with one CF mutation identified. Of the 123,172 total cases tested, this represents a 3.8% carrier frequency, which is concordant with literature estimates. These numbers do not include 498 carriers of one copy of the R117H variant with a 5T allele. These results are reported, but not as CF carriers – rather carriers of a mild variant not typically associated with classical CF.

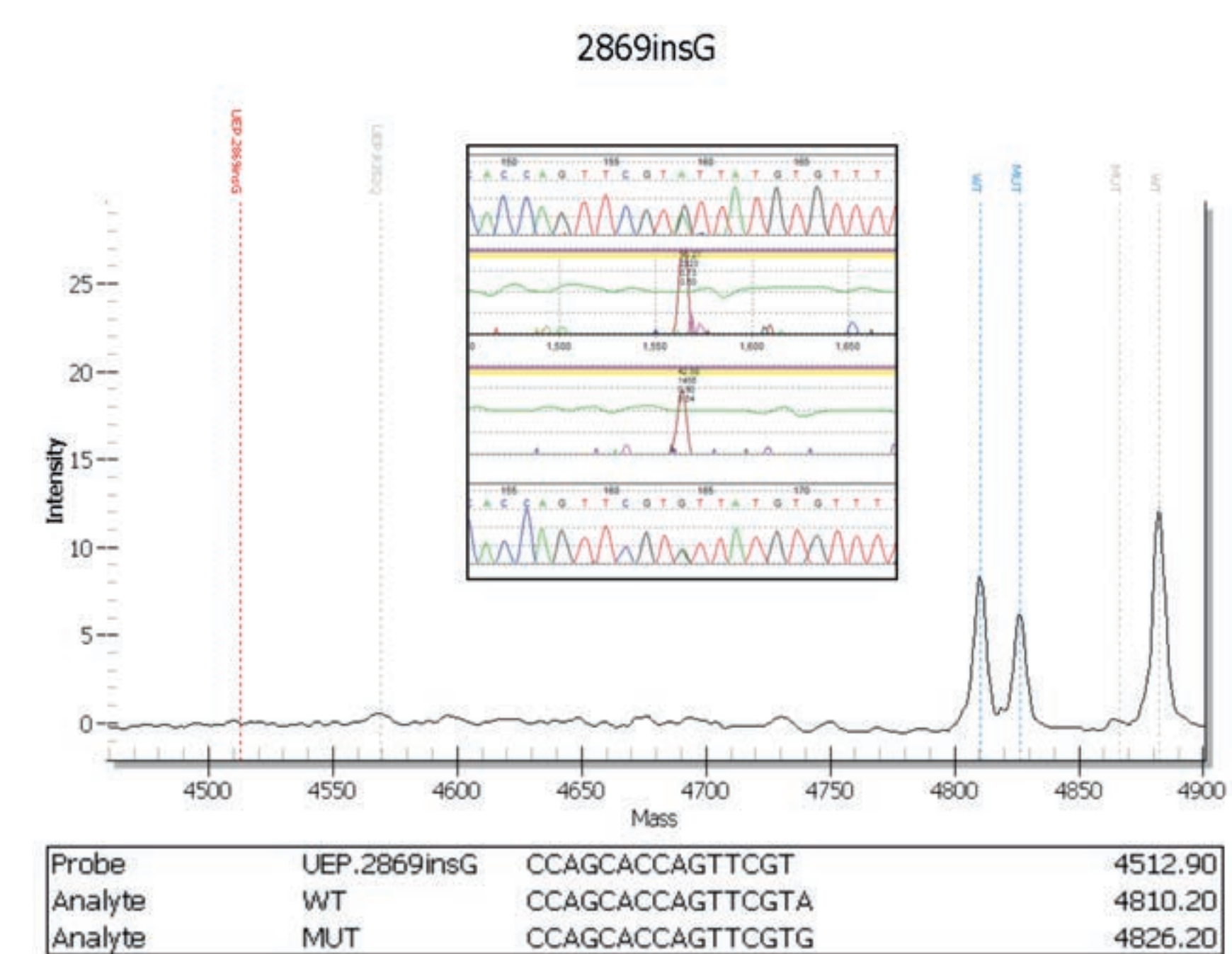
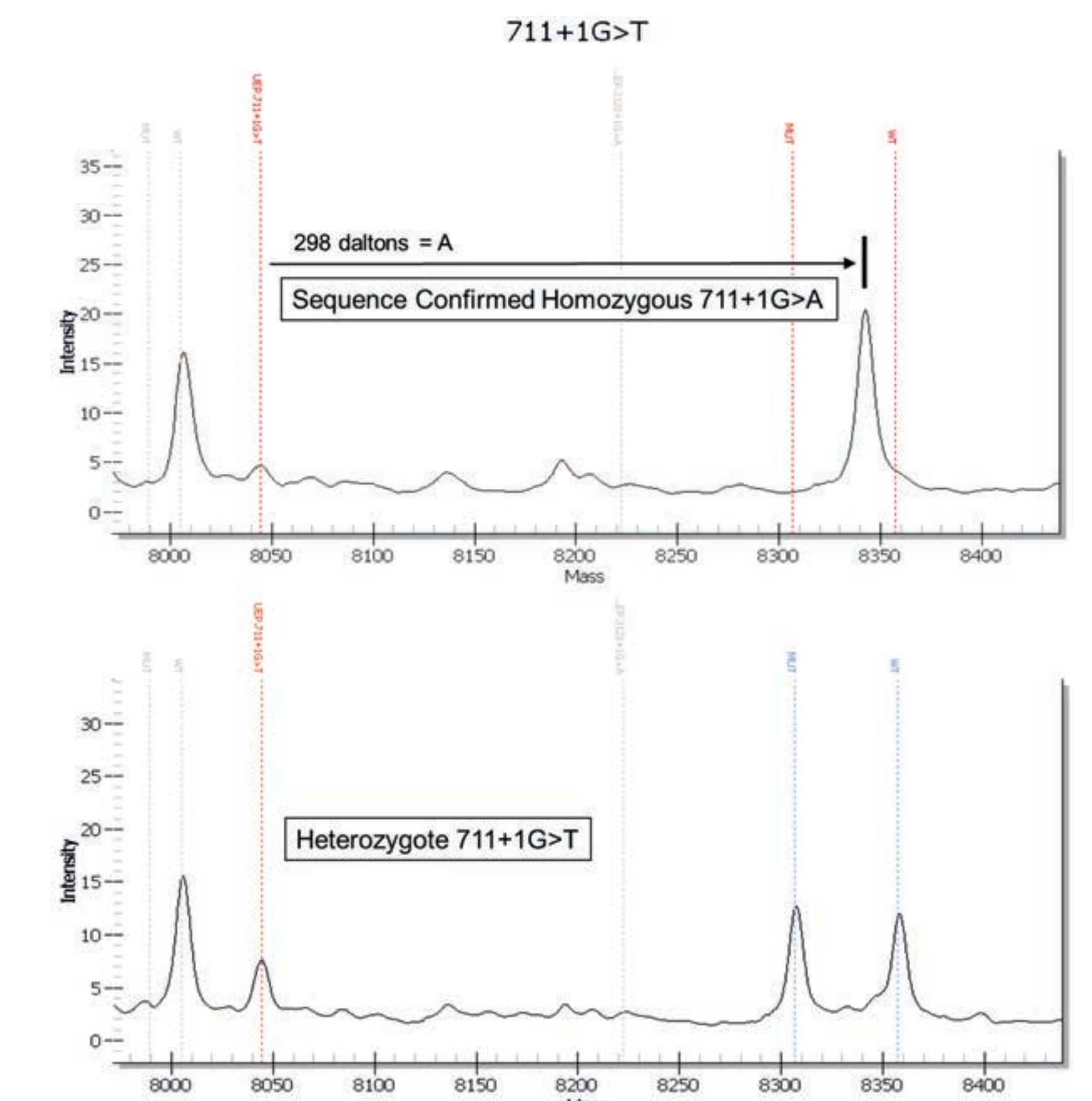
Methods

712-1G>T_W1_E Y1092X(C>A-G)_W1_E G330X_W1_E S1196X_W1_E DF508-4506V#1_W1_E 2307insA_W1_E R75X-Q#1_W1_E 406-1G>A_W1_E 457TAT>G_W1_E S1251N_W1_E 2043delG_W1_E 1349del84_W1_E 3171delC_W1_E R1158X_W1_E P574H_W1_E Q552X_W1_E N1303K(C>A-G)_W1_E 621-1G>T_W1_E	R764X_W3_E A455E_W3_E 852del22_W3_E W1282X_W3_E 2789+5G>A_W3_E Q890X_W3_E P205S_W3_E CFTRdele2.3_W3_E R553X_W3_E 3876delA_W3_E D1507-4507V_W3_E 711+5G>A_W3_E 2055del9>A_W3_E 2184delA_W3_E 1078delT_W3_E 663delT_W3_E	R1066C_W5_E DF311_W5_E T338L_W5_E R117C-H#1_W5_E 1812-1G>A_W5_E 1506T-F508C#1_W5_E 3667delA_W5_E 1506T-F508C#2_W5_E G551D_W5_E K710X_W5_E 296(+2)T>A_W5_E W1089X_W5_E R117C-H#2_W5_E G85E_W5_E	R347P/H_W7_X CFTR_W7_E3 H199Y_W7_X G542X_W7_E CFTR_W7_E2 L997F_W7_X M1101K/R_W7_X 1898+5G>T_W7_X 405+3A>C_W7_X
2869insG_W2_E R352Q_W2_E S549N-R(T>G)#1_W2_E Q1238X_W2_E S549N-R(T>G)#2_W2_E L206W_W2_E 2183delAAA>G_W2_E Y122X_W2_E Q493X_W2_E E92X_W2_E 2108delA_W2_E 1677delTA_W2_E S1255X_W2_E 936delTA_W2_E D1152H_W2_E 3659delC_W2_E S466X(C>A-G)_W2_E L288insTA_W2_E	R1162X_W4_E 3199del6_W4_E 3849+10kbC>T_W4_E 1717-1G>A_W4_E G178R_W4_E E60X_W4_E 444delA_W4_E C524X_W4_E R334W_W4_E R560T_W4_E 3791delC_W4_E 1898-1G>A-T-C_W4_E G480C_W4_E 935delA_W4_E 405+1G>A_W4_E 711+1G>T_W4_E 3120+1G>A_W4_E	Q359K_W6_X 2143delT_W6_E W1204X_W6_Ev2 574delA_W6_E T360K_W6_X DF508-4506V#2_W6_E R709X_W6_E A559T_W6_E 394delTT_W6_E	2184insA_W8_X 3905insT_W8_X 4016insT_W8_X 5T_7T_9T_W8_X

48 Samples per 384-Well Plate
*46 Patients/1 Rotating Multiplex Control/1 Blank
*PCR and SBE Plates (prepared quarterly and stored @ -80°C)

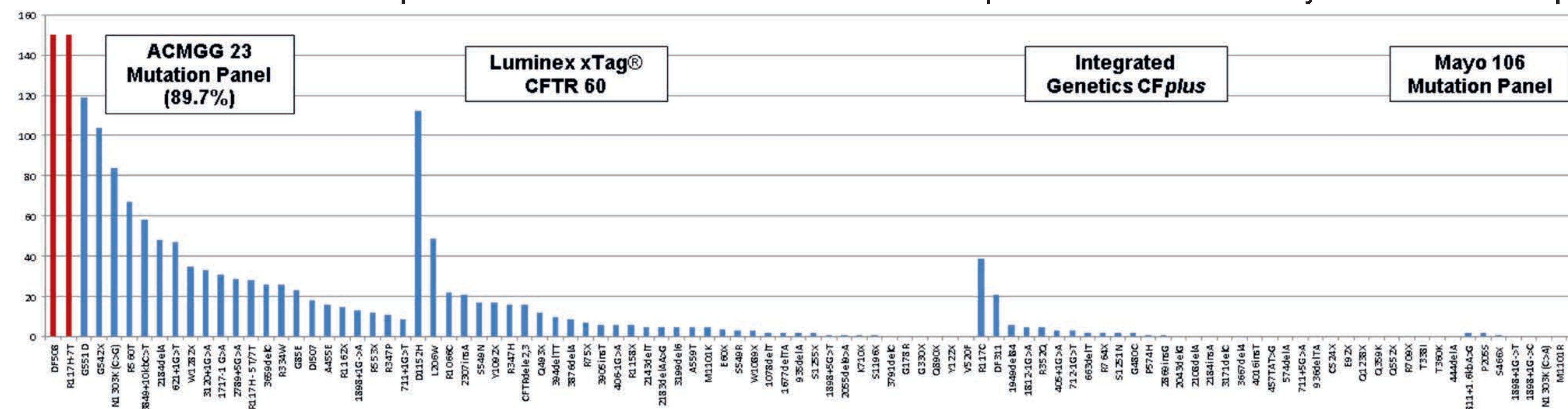


Example Interesting Cases



Results

Distribution of mutations identified compared to the ACMGG recommended 23 mutation panel and two commercially available extended panels.



Note: dF508 (3276) and R117H-7T (498) were truncated to fit the scale of the chart (red bars). Heterozygous results for R117H-7T are reported, not as positive carrier status but as a mild variant.

Discussion

We collaborated with Sequenom (now Agena Biosciences) to develop a novel, 106 mutation assay designed for high throughput, cystic fibrosis carrier screening using the Mass Array® MALDI-TOF platform. The method has proven to be robust, user friendly, and compatible with a high degree of automation. We have used it to process over 120,000 samples. The observed carrier rate of 3.8% is in agreement with published values.

We confirm the value of extended panel testing for CF carrier screening, with slightly over 10% of the positives being outside of the ACMGG 23 mutation panel.