

"Bridging the Genome to the Proteome. From Discovery to Diagnostics."

Increased Detection of Cystic Fibrosis Carriers in Hispanics and African Americans Using the xTAG CF70 Assay

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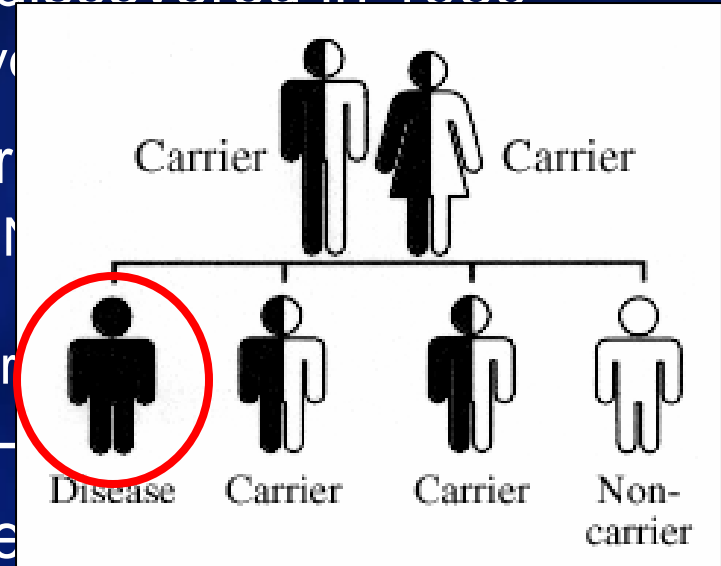
Molecular Genetics Laboratory

Outline

- Cystic Fibrosis background
- Diagnosis and genetic testing
- Treatments
- Mayo testing platform timeline
- Luminex (xTAG) process
- Need for ethnicity dependent panels
- Mayo testing/results
- Bonus material: Analysis software

Cystic Fibrosis (CF)

- Gene responsible (CFTR) first discovered in 1989
 - Sick Kids (Toronto) and the University of Toronto
- Multi-organ, multi-function disorder
 - Occurs mostly in Caucasians of Northern European descent
 - Present in all races and ethnic groups
- Caused by mutations in the CFTR transmembrane conductance regulator gene
- Located on chromosome 7 (7q)
- Autosomal recessive inheritance pattern
- 2500 babies are born with CF each year in the United States
 - 1 in 25 Caucasians is an unaffected carrier



Symptoms

- Disease severity variable
 - Underlying cause the same
 - Abnormality of secretory epithelia
 - Sweat cools the body
 - Pulmonary mucous prevents infection
- Loss of excessive amounts of sweat
 - Can upset the balance of minerals in the blood
- Thick accumulations of mucous
 - Frequent respiratory infections
 - Progressive to end-stage lung disease
- Lung Disease primary cause of death
 - Median life expectancy 30 - 35 years
 - Many die in adolescence due to non-compliance with treatment regimens

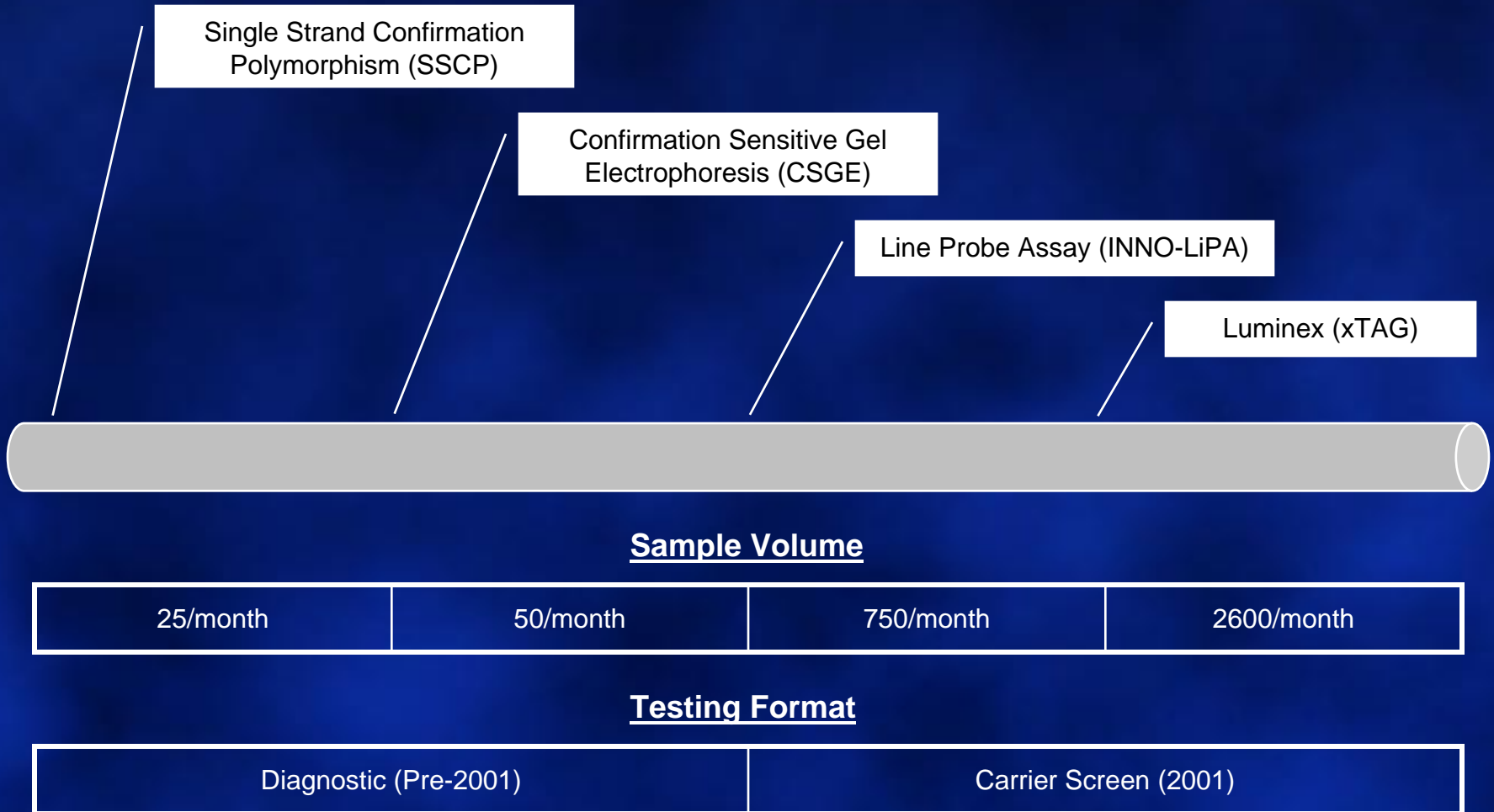
Diagnosis and Genetic Testing

- Sweat Chloride Test
- Immuno Reactive Trypsinogen (IRT)
 - Newborn screening first tier test (including MN)
 - Luminex xTAG 40 mutation panel F/U for positive IRT
- Additional testing available to establish clinical phenotype
 - Pulmonary function tests
 - Sputum
 - Stool
- Molecular Genetic Testing
 - Carrier Screening – Detection rates are ethnicity dependent
 - Direct DNA analysis – Sequencing
 - Over 1500 mutations have been discovered
 - Many present in single families only

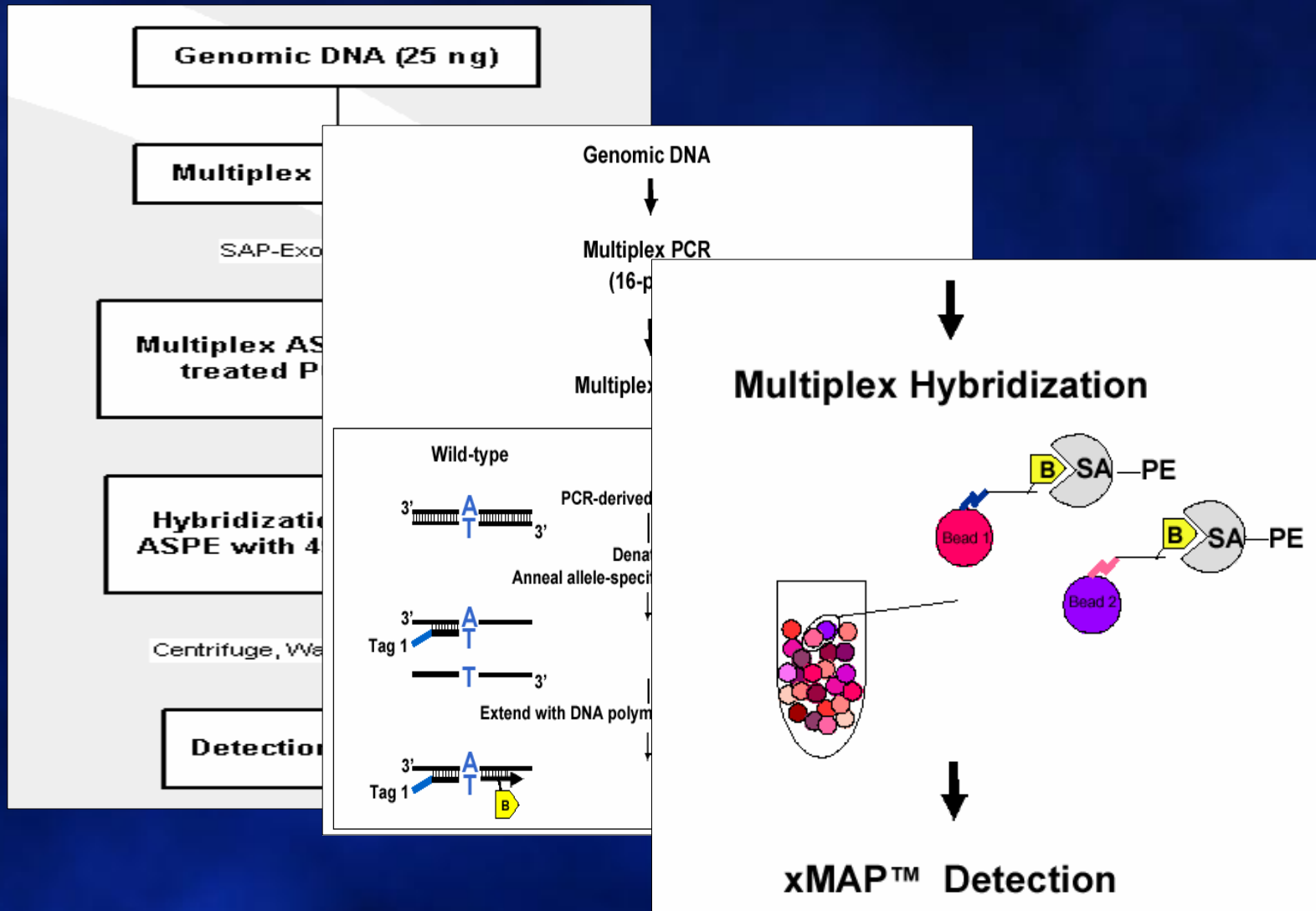
Treatment

- Once fatal in early childhood
- Only cure would be gene therapy at an early age
 - Gene Therapy not currently available
 - May one day be able to repair or replace defective gene
- Aim of treatment is to slow disease progression
 - Improve quality of life
 - Antibiotic therapy available to clear lungs
- Treatment types:
 - Chest Therapy
 - Bronchodilators, mucolytics, and decongestants
 - Pancreatic enzymes

Cystic Fibrosis Platform Timeline at Mayo



xTAG Process



Cystic Fibrosis Mutations: How Many are Enough? How Many are Too Many?

- Population-based carrier screening implemented in late 2001
 - Consensus conference at NIH in 1997
 - Recommended offering carrier screening to all pregnant couples and those planning a pregnancy
 - Details worked out at a 2nd conference in 1998 headed by a steering committee comprised of reps from ACMG, ACOG, and NHGRI.
 - Subcommittees were formed for:
 - a) Patient Education – Involvement of Genetic Counselors pre-testing
 - b) Laboratory Testing – What mutations should make up the Core Panel?
 - c) Provider education - Dr.'s would be more inclined to recommend testing if they better understood the purpose

Cystic Fibrosis Mutations: How Many are Enough?

- Subcommittees recommended a universal (pan-ethnic) screening panel of **25** mutations (those with an allele frequency in the affected population of $>$ or $= 0.1\%$).
- Residual Risk = Likelihood of carrying a CF mutation when testing negative using a given testing platforms detection rate

<u>Racial / Ethnic Group</u>	<u>Carrier Rate</u>	<u>Detection Rate</u>	<u>Residual Risk (1 in xxx)</u>
Caucasian	1/25	88%	208
Hispanic American	1/47	72%	167
African American	1/66	65%	200

Cystic Fibrosis Mutations: How Many are Too Many?

Detection Rates

<u>Ethnic Group</u>	<u>CF 23</u>	<u>CF39+5</u>	<u>CF70+6</u>
Caucasian	88%	90%	91%
Hispanic American	72%	73%	81%
African American	65%	69%	78%

Residual Risk (1 in xxx)

<u>Ethnic Group</u>	<u>CF 23</u>	<u>CF39+5</u>	<u>CF70+6</u>
Caucasian	208	250	278
Hispanic American	167	167	250
African American	200	213	303

Risk Calculations - Expanded

Northern European Caucasian

The Risk of a Negative x Positive Couple Having a Child with CF is

$$1/250 \times 1 \times 1/4 = 1/1000 \longrightarrow 1/1112$$

Hispanic American

The Risk of a Negative x Positive Couple Having a Child with CF is

$$1/108 \times 1 \times 1/4 = 1/432 \longrightarrow 1/1000$$

African American

The Risk of a Negative x Positive Couple Having a Child with CF is

$$1/220 \times 1 \times 1/4 = 1/880 \longrightarrow 1/1212$$

25 Mutation Panel

70 Mutation Panel

2000 United States Census

	<u># of People over 18 years</u>	<u>% of US Population</u>
African American	23.7 million	11.4
Hispanic American	23.0 million	11.0

% Change in the Population from 1990 to 2000

Caucasian	5.9
African American	15.6
Hispanic	57.9

Published Articles

- “CF Carrier Screening – Making it Meaningful”
 - CAP Today – January 2003
 - Discussed how and why original panel was derived
- “The Cystic Fibrosis mutation “arms race”: When less is more”
 - Genetics in Medicine – November 2007
 - Commentary questioning the relevance of expanded panels

**CFTR Mutation Detection Rates in Different Ethnic Groups Using the
Luminex® Tag-It™ CFTR 70+6 assay**

Tad Holtegaard, CLSp(MB), and W. Edward Highsmith Jr., PhD.
Mayo Clinic, Rochester, MN

The proof is in the pudding!!

Mayo Testing/Results

- 16,691 samples tested from January 2008 – June 2008
 - Caucasian/Mixed European – 11856 (69.9%)
 - Hispanic – 1453 (8.6%)
 - African American – 1127 (6.6%)
 - Not Specified – 2255 (13.3%)

Mayo Positives (Overall)				
	<u>CF23</u>	<u>CF16</u>	<u>CF31</u>	<u>Total</u>
Caucasian/Mixed European	524 (93.7%)	7 (1.3%)	28 (5.0%)	559 (509)
Northern European	158 (28.3%)	3 (0.54%)	7 (1.3%)	
Southern European	3 (0.54%)	0 (0.0%)	0 (0.0%)	
Hispanic	30 (85.7%)	1 (2.9%)	4 (11.4%)	35 (30)
African-Am	16 (80.0%)	0 (0.0%)	4 (20.0%)	20 (18)
Not specified	131 (89.1%)	4 (2.7%)	12 (8.2%)	147 (102)
Total	701	12	49	762

Luminex Data Resulter (LDR)

About This Program

Raw Data File Location

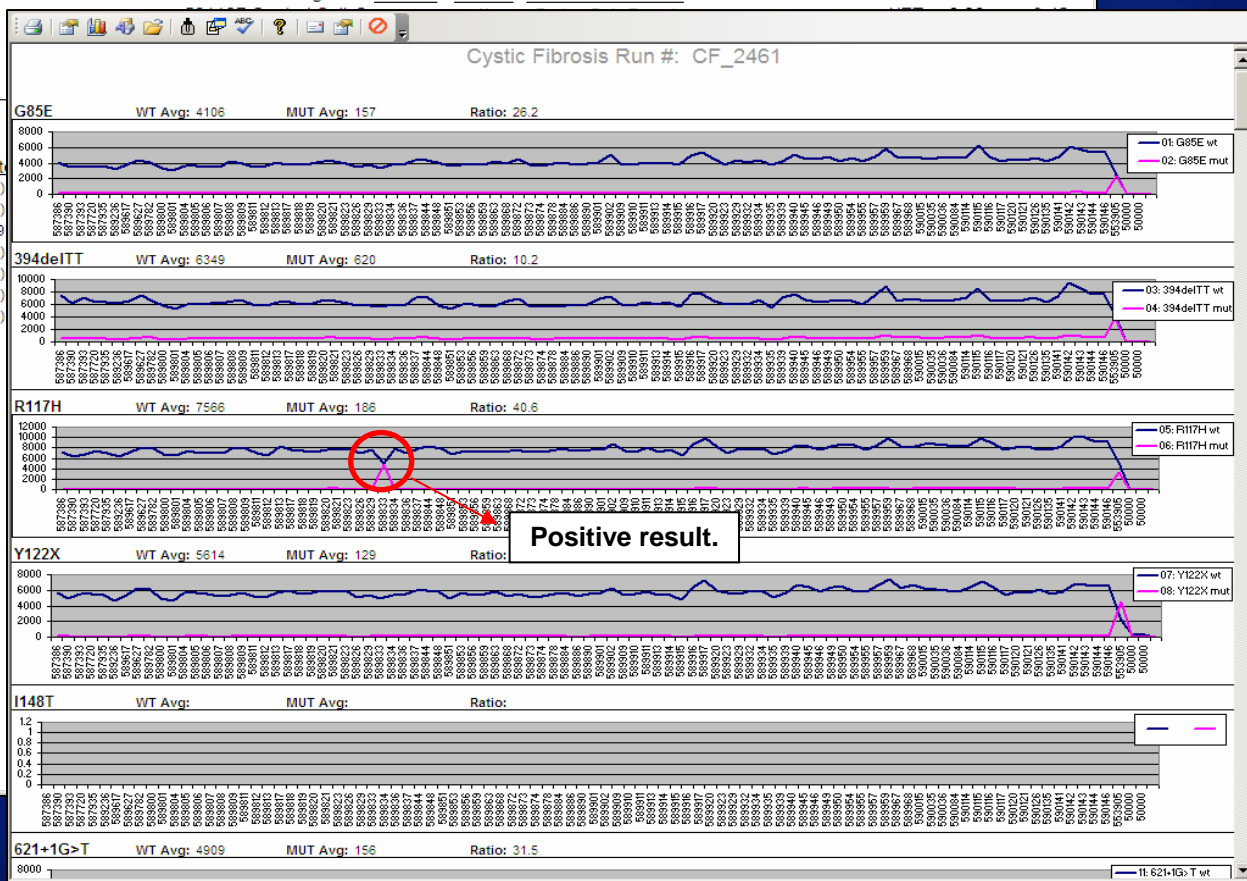
Send Daily Email

Change Control I.D.'s

Assay:	Tag-It CF70 Assay	Controls Within Range:	Yes	No	Comments	Run Acceptance Values:	Limits	This Run
Experiment:	CF_2461	553905 Control Signals?	X			50000 (NoDNA) Max	223	222
Run on:	11/14/2007	553905 Control Calls?		X	Review Data Report	Min. Signal	700	936
Run by:	CLS	581187 Control Signals?	X			Min. Allelic Ratios:	WT	0.85
# of Samples:	95							0.89

Positives:

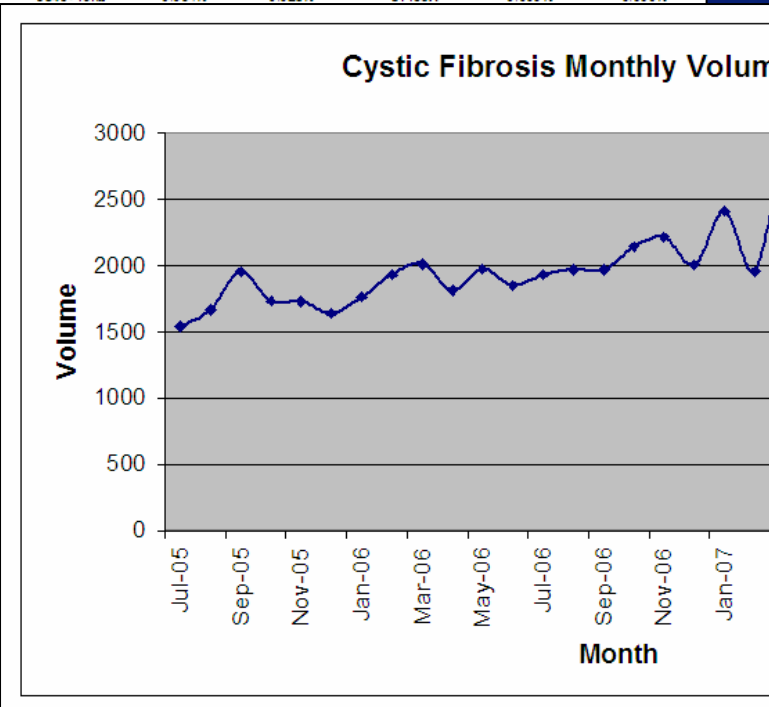
Lab ID	Heterozygote
587935	dF508 (CF39)
589808	dF508 (CF39)
589833	R117H (CF39)
589872	dF508 (CF39)
589873	dF508 (CF39)
589910	dF508 (CF39)
589916	dF508 (CF39)



Luminex Data Resulter (LDR)

Molecular Genetics Cystic Fibrosis Detection Rates

Detection Rates			Detection Rates			Detection Rates		
Mutation	Overall	Current	Mutation	Overall	Current	Mutation	Overall	Current
G85E	0.026%	0.046%	3659delC	0.009%	0.023%	D1152H	0.010%	0.000%
384delTT	0.019%	0.023%	S1295X(19)	0.000%	0.000%	R1158X	0.000%	0.000%
R117H	0.486%	0.481%	S1295X(20)	0.000%	0.000%	3791delC	0.000%	0.000%
Y122X	0.000%	0.000%	3849+10kb	0.054%	0.023%	S1196X	0.000%	0.000%
I148T	0.000%	N/A						
621+1G>T	0.040%	0.046%						
711+1G>T	0.005%	0.023%						
1078delT	0.003%	0.000%						
R334I	0.016%	0.023%						
R347P	0.024%	0.000%						
R347H	0.024%	0.046%						
A455E	0.022%	0.000%						
dI507	0.014%	0.000%						
dF508	2.808%	2.810%						
V520F	0.007%	0.000%						
1717-1G>A	0.035%	0.000%						
G542X	0.095%	0.046%						
S543N	0.016%	0.023%						
S543R	0.003%	0.000%						
G551D	0.090%	0.114%						
R553X	0.042%	0.000%						
A559T	0.007%	0.000%						
R560T	0.016%	0.000%						
1898+1G>A	0.016%	0.000%						
1898+5G>T	0.000%	0.000%						
2183AA>G	0.012%	0.000%						
2184delA	0.019%	0.046%						
2307insA	0.000%	0.000%						
2789+5G>A	0.026%	0.023%						
3120+1G>A	0.022%	0.023%						
Y1092XC>G	0.000%	0.000%						
Y1092XC>A	0.012%	0.023%						
MH101K	0.000%	0.000%						
R1162X	0.017%	0.023%						



Cystic Fibrosis Positives Report

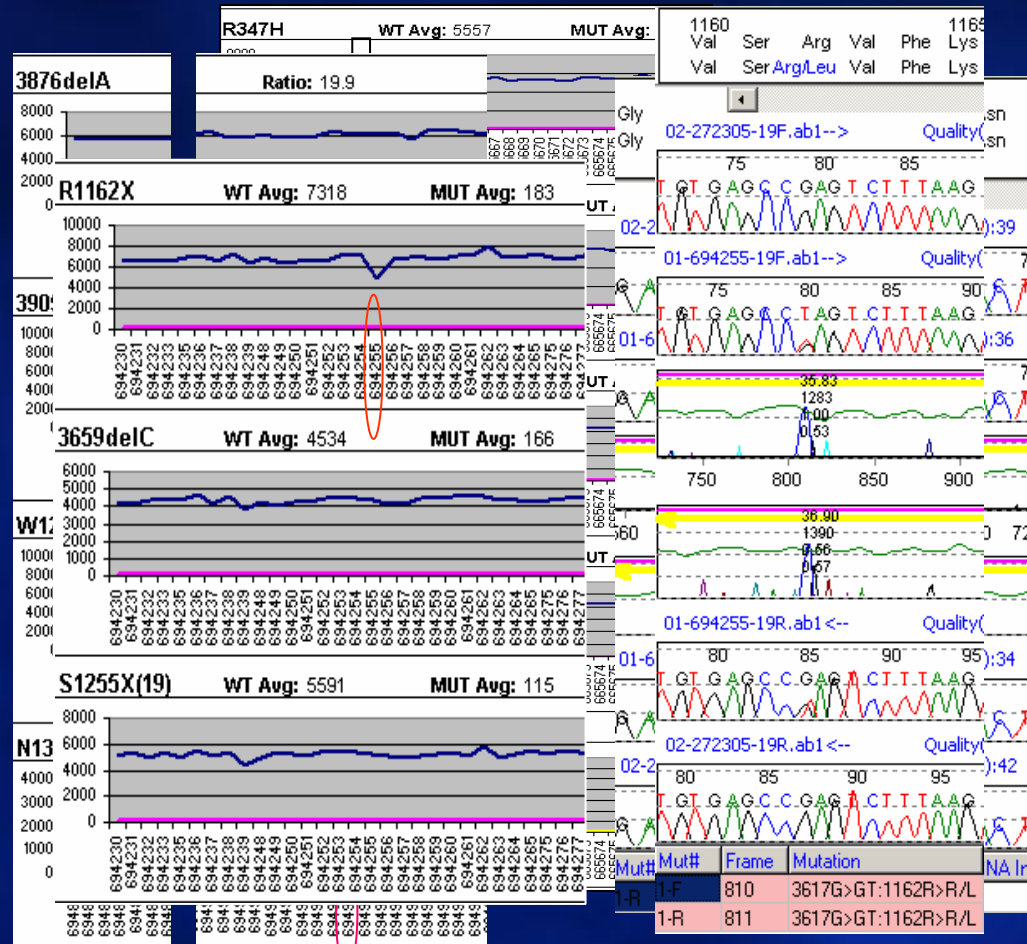
Selected Range: Between January 1, 2007 & March 1, 2007

Run #:	Lab ID:	HETS:	MUTS:	Checked:
CF2179	518478	dF508 (ND287)		☐
CF2180	518750	dF508		☐
CF2180	518757	dF508		☐
CF2180	518774	N1303K		☐
CF2180	518790	R117H dF508		☐
CF2181	518390	R117H		☐
CF2181	518494	R117H		☐
CF2181	518734	dF508		☐
CF2183	519281	dF508		☐
CF2183	519318	R117H		☐
CF2183	519320	dF508		☐
CF2184	519367	dF508		☐
CF2184	519402	dF508		☐
CF2185	519285	G85E		☐
CF2185	519580	dF508		☐
CF2185	519595	G551D		☐
CF2186	519716	dF508		☐
CF2186	519722	R347H		☐
CF2186	519726	N1303K		☐
CF2186	519826	dF508		☐
CF2186	519874	dF508		☐
CF2186	519924	dF508		☐
CF2187	519972	G85E		☐
CF2188	520254	W1282X		☐
CF2188	520261	2184delA		☐
CF2188	520316	dF508		☐
CF2189	520429	dF508		☐
CF2189	520497	dF508		☐
CF2190	520699	dF508		☐
CF2190	520702		dF508	☐
CF2190	520766	dF508		☐
CF2190	520781	dF508		☐
CF2190	520805	dF508		☐
CF2191	520889	R117H		☐
CF2191	520927	dF508		☐
CF2191	520970	621+1G>T		☐
CF2191	520974	dF508		☐

Luminex Data Result (LDR)

Repeats:

Lab I.D.	Reason
587720	Possible R75Q
589236	Possible R75Q



Summary

- Increased detection rates in Hispanics and African Americans by over 10%
- African American population grew at a rate of over 15% in the United States from 1990 - 2000
 - Compared to 5% growth in Caucasians
 - CF23 to CF 70 decreased risk by slightly less than 50%
- Hispanic population grew at a rate of almost 60% over the same time-span
 - CF23 to CF70 decreased risk by more than 50%
- From January 2008 to June 2008 15% of patient base was either African American or Hispanic

Acknowledgments

- **Dr. Ed Highsmith, Ph.D.**
- **Kent Kruckeberg**
- **Molecular Genetics Laboratory**