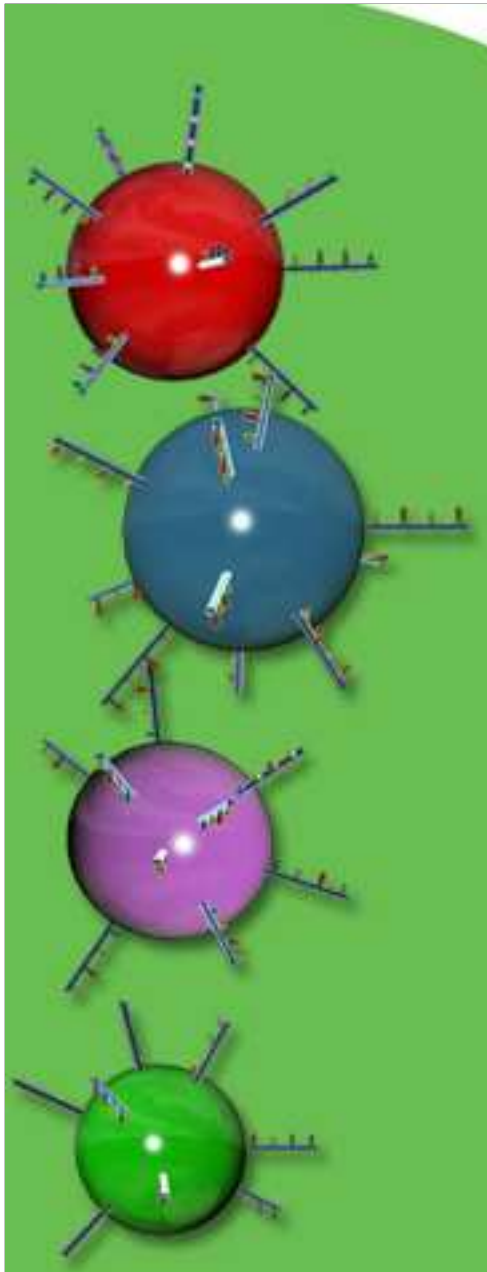


Genotyping with the Tag-It™ Mutation Detection Platform

Richard Janeczko, PhD
Salisbury, July 2004

MADRID TALK

- How to take penalty kicks

Overview

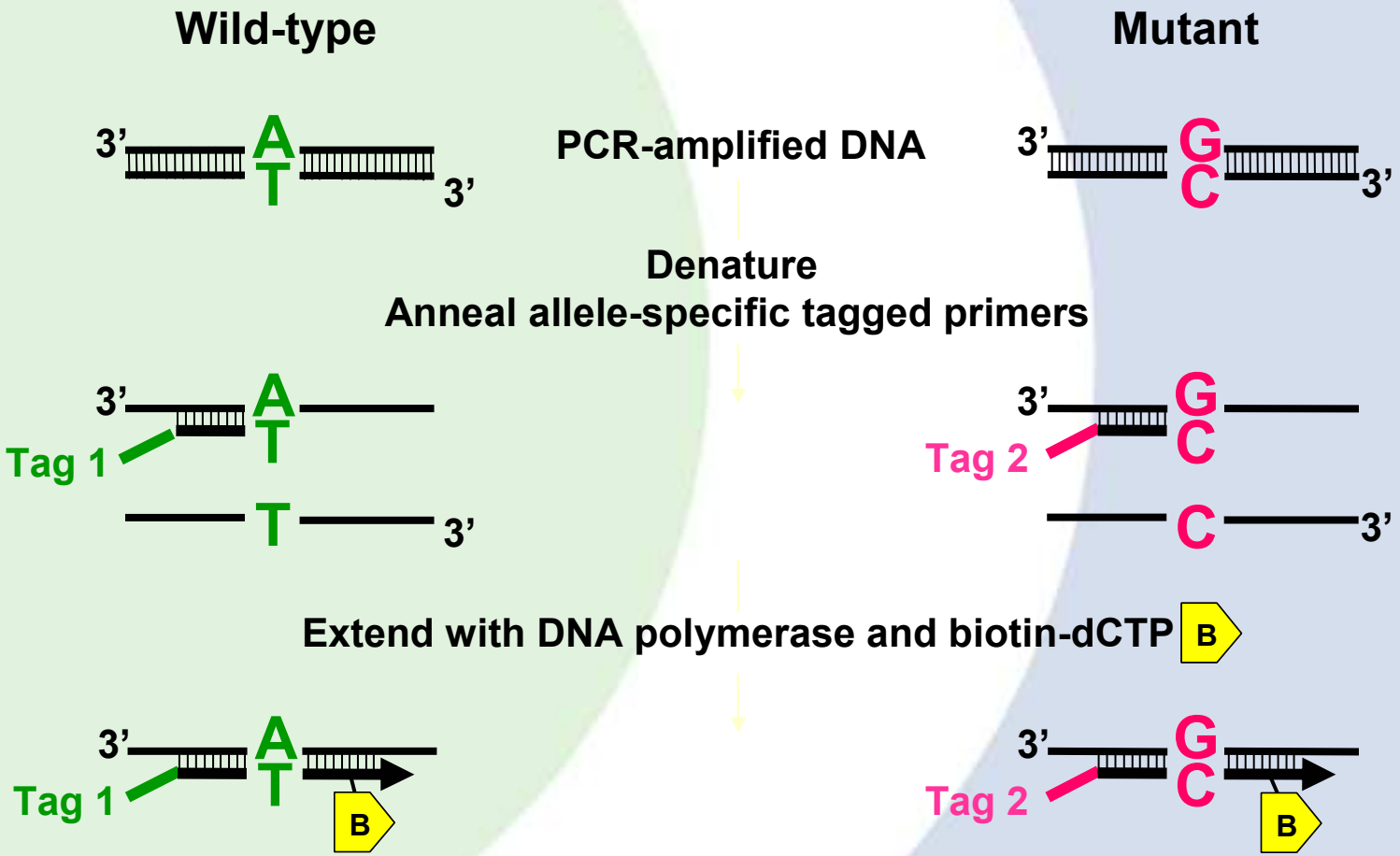
- **The Tag-It™ Genotyping Platform**
- **Tag-It™ Product Menu**
  - **Cystic fibrosis,**
  - **Ashkenazi Jewish**
  - **Thrombophilia**
  - **Drug metabolism (2D6, 2C9, 2C19)**
- **In Development**
  - **Fragile X**
  - **Infectious Disease (MRSA, viral genotypes)**

Tag-It™ Product Specifications

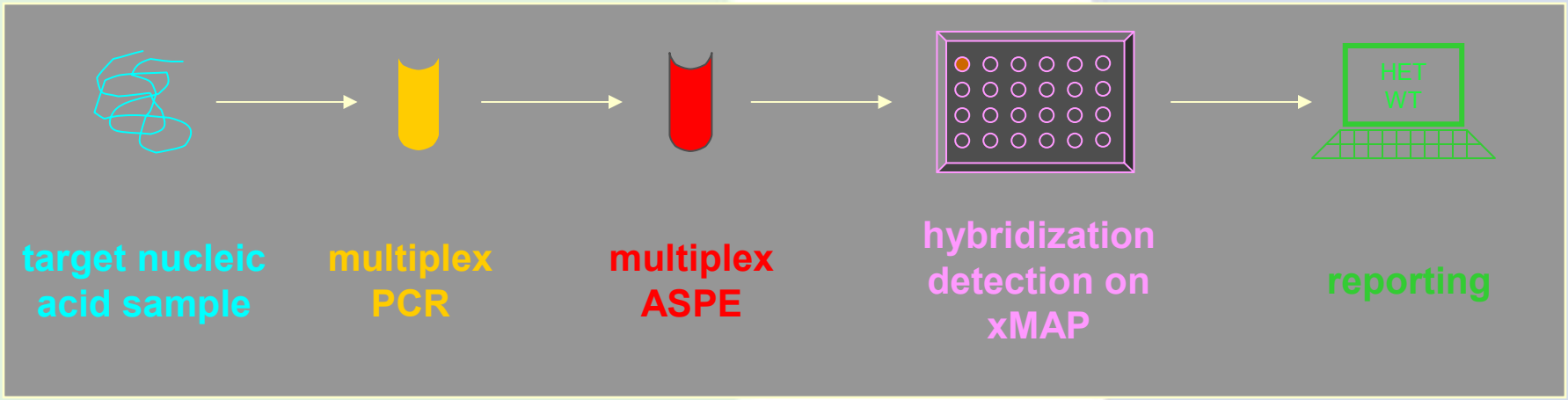
- Accurate, robust, reproducible
- Flexible open system allows for
  - Easy addition and subtraction of specific mutations by testing lab
  - Open home-brew platform
- Variable sample throughput capability
- Software:
  - Reports both wild-type and mutant alleles
  - Integrates with LIMS and generates clinical reports
- Cost-effective

ASPE Genotyping with 'Tag-It'

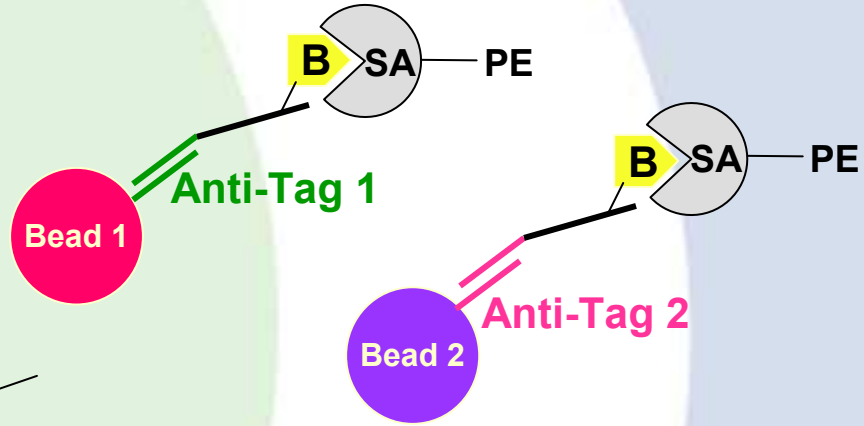
or how to use the Tag-It Array

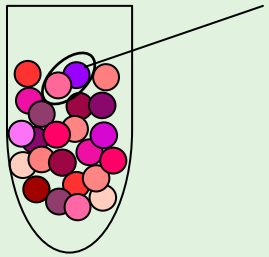


Tag-It platform: product format



Tag-It, Universal Array Sorting





86 Luminex Bead Populations

Tag-It™ Cystic Fibrosis Products: Mutation Panels

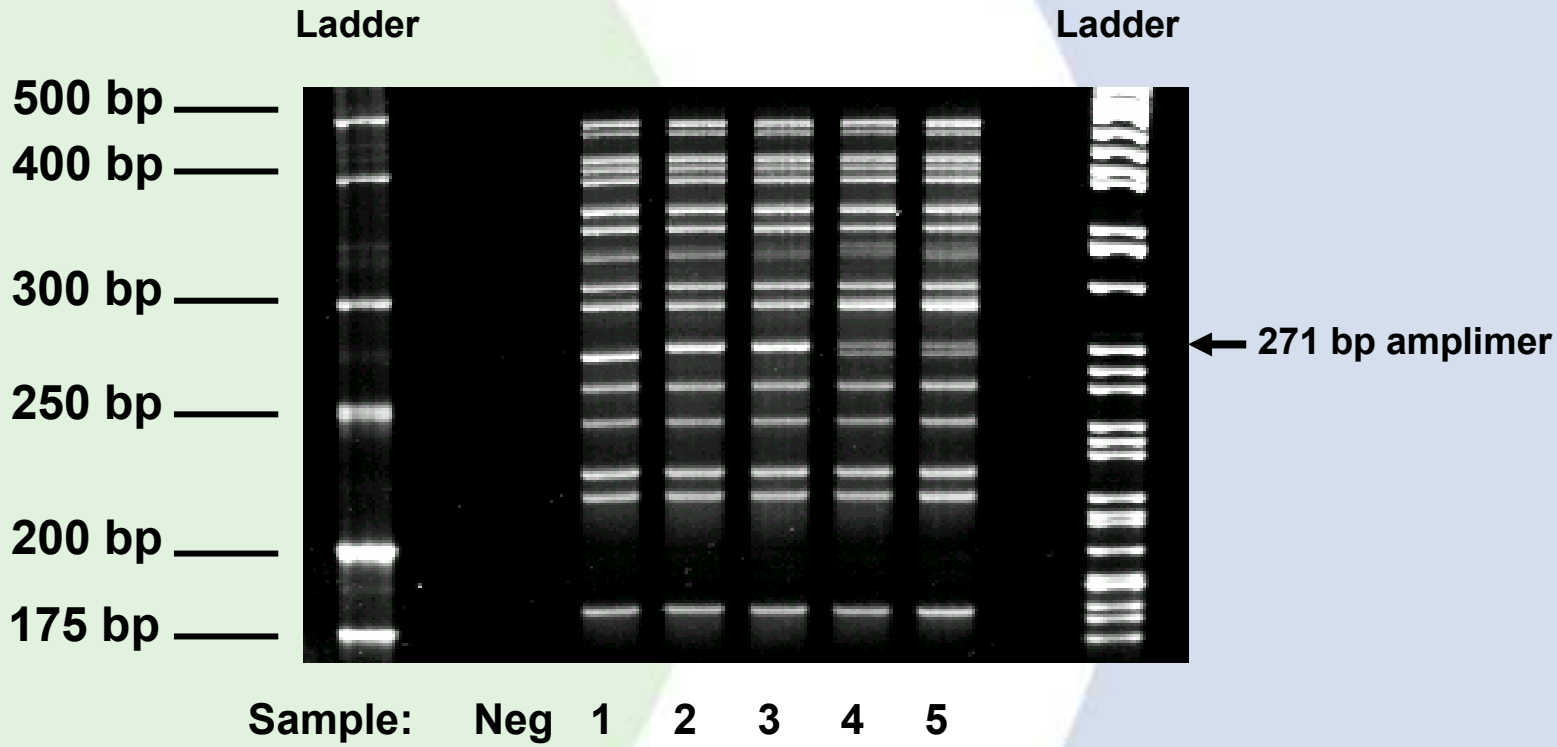
- CFTR 23+4 (available March 5th 2004)
- CFTR 25+4
  - 25 most prevalent (ACMG set)+ 4 reflex tests, Q3 2003
- CFTR 40+4 set
  - ACMG set + 15 world's most common + 4 reflex tests, Q3 2003
- CFTR 71+4
  - CFTR 40+4 set + 31 expanded US and European coverage (in development), Q4 2004

Lab Requirements

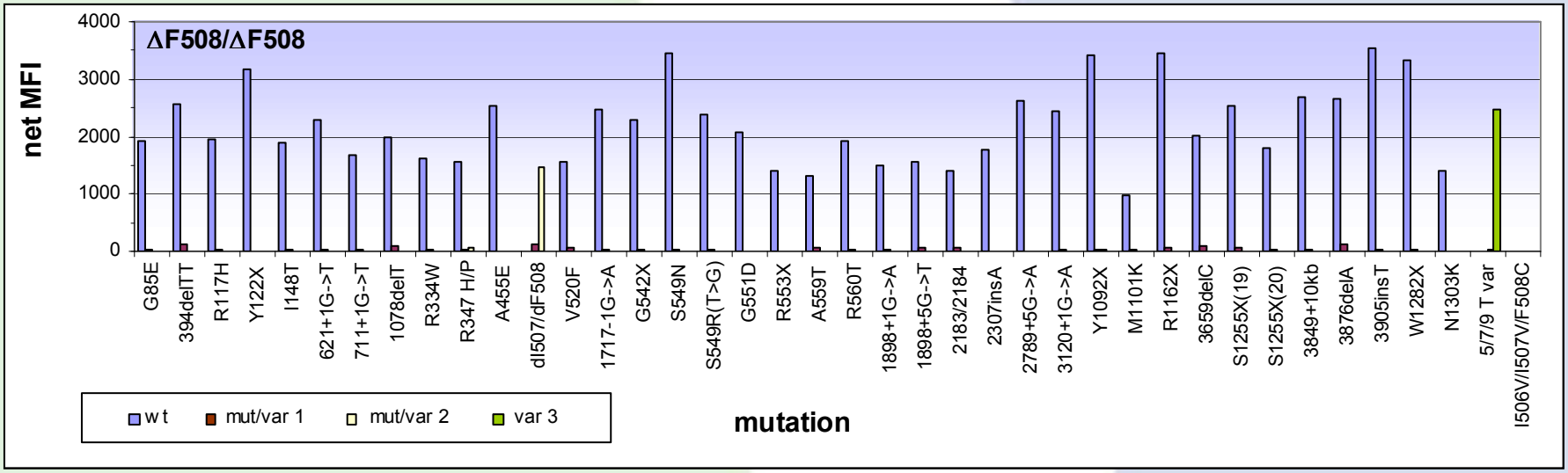
4 Major Steps	Schematic	Hands On Time
I Multiplex PCR	Genomic DNA (25 ng)	1 hr
	SAP-EXO Treatment	
	5 µL of Treated PCR Reaction	
II Multiplex ASPE		15 min
	5 µL of ASPE Reaction	
III Bead Hybridization		45 min
	Filtration and Wash	
IV Data Acquisition on xMAP		5 min
	Data Analysis	

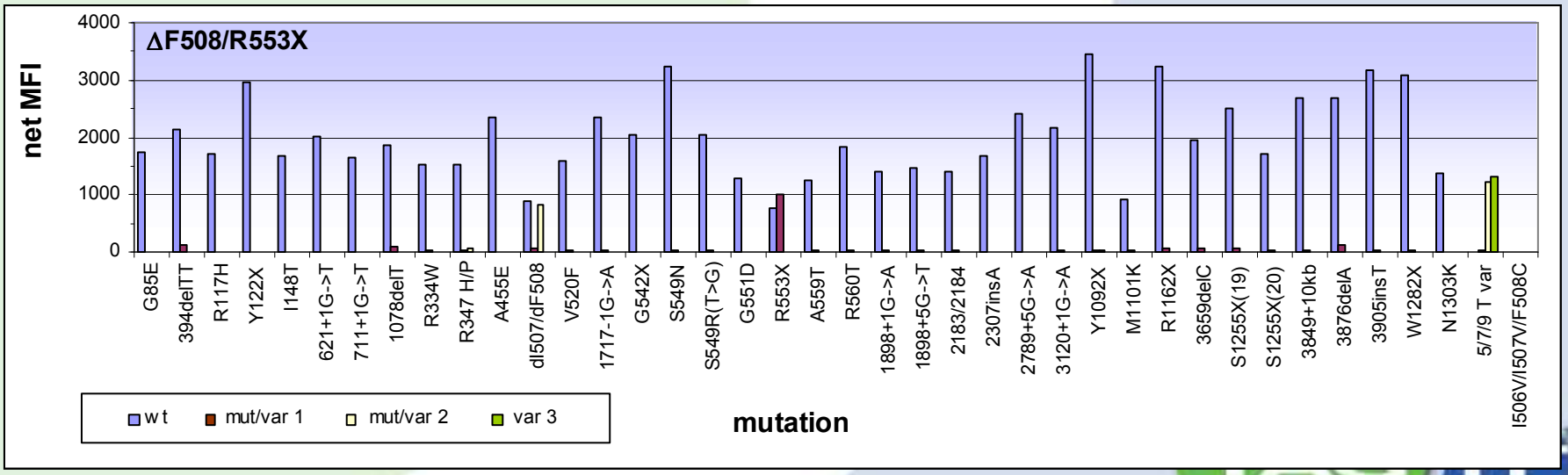
TAG IT™

Tag-It Multiplex (16-plex) PCR

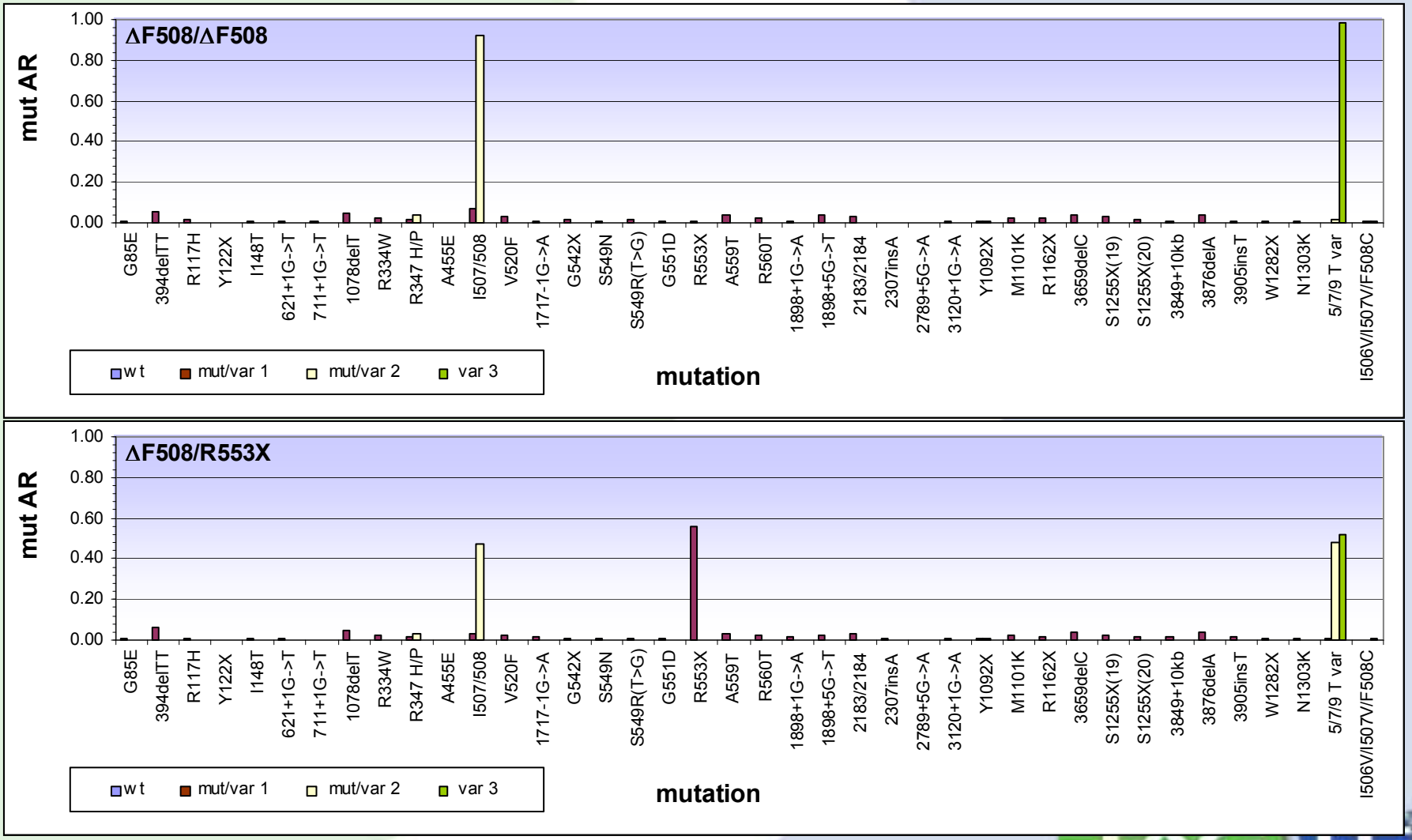


CFTR 40+4: Net Signals (MFI)

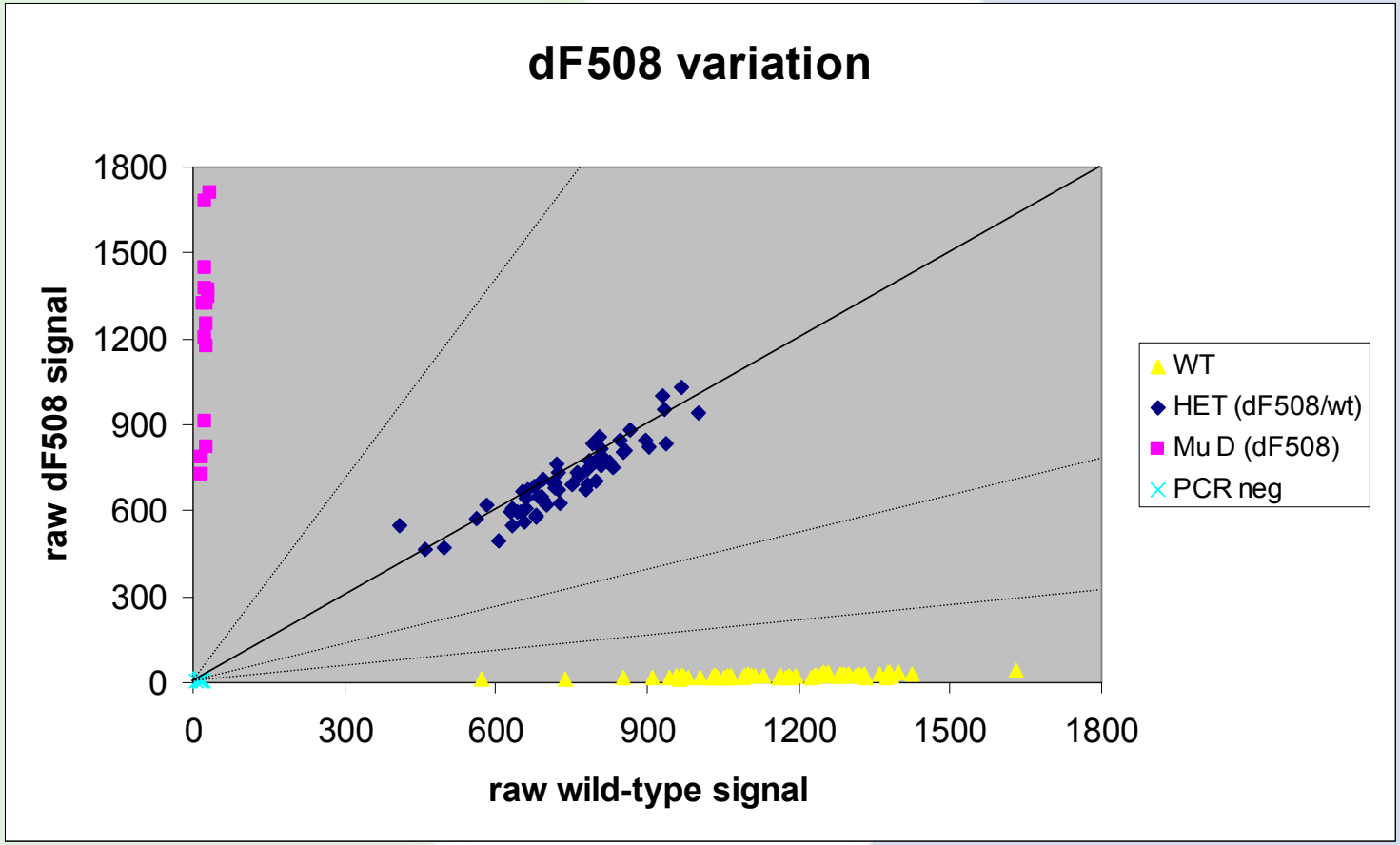




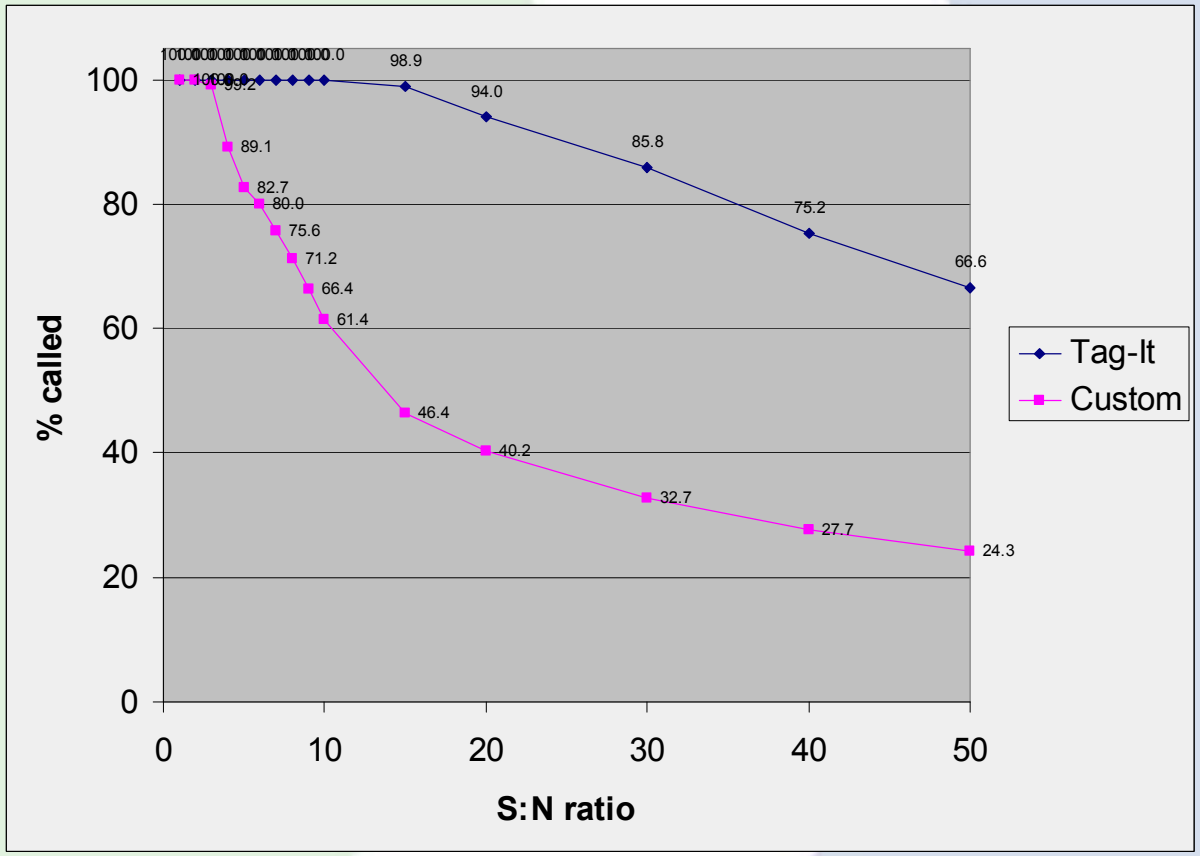
CFTR 40+4: Allelic Ratios



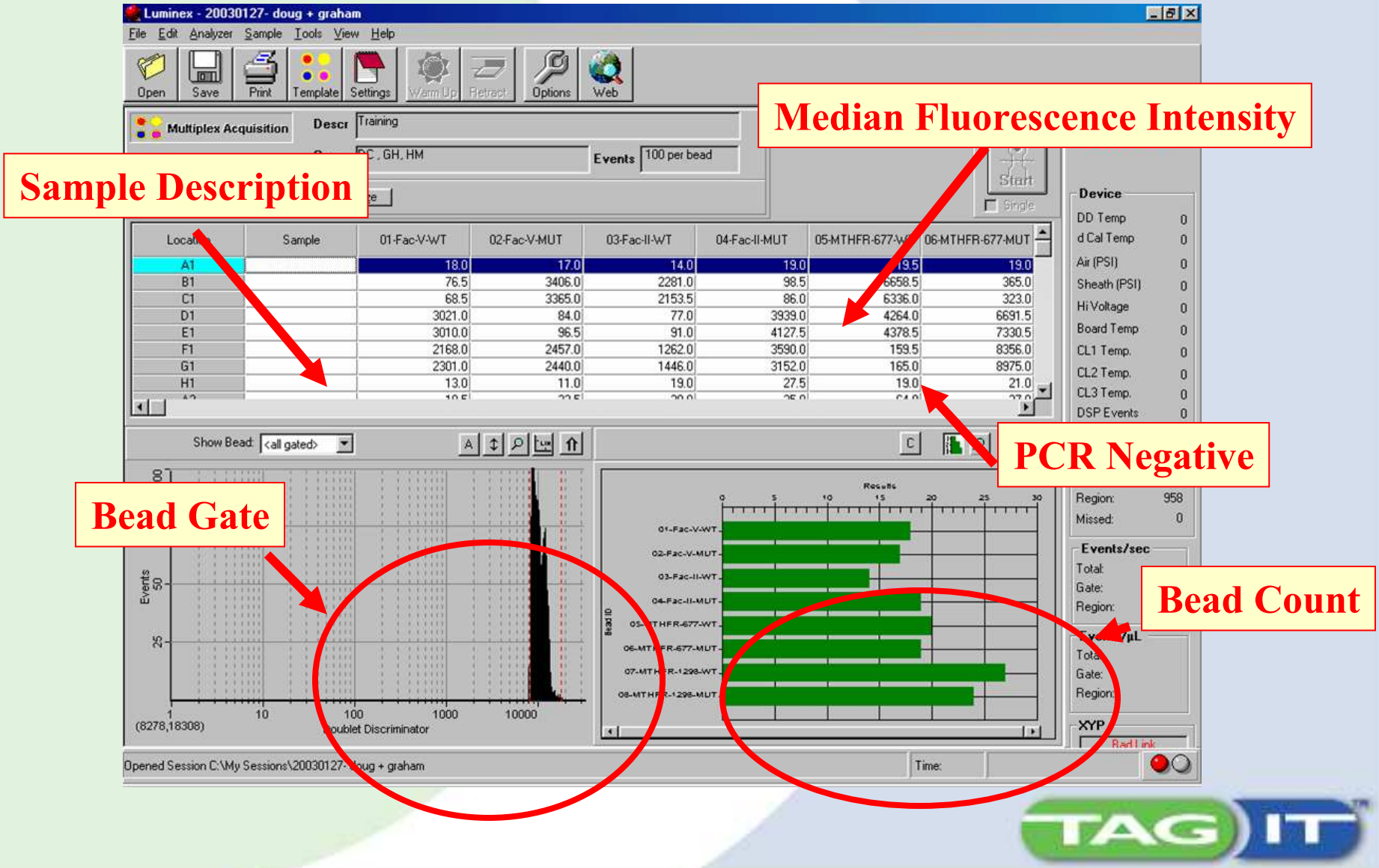
Tag-It Specificity



Correct Calls as a Function of S:N

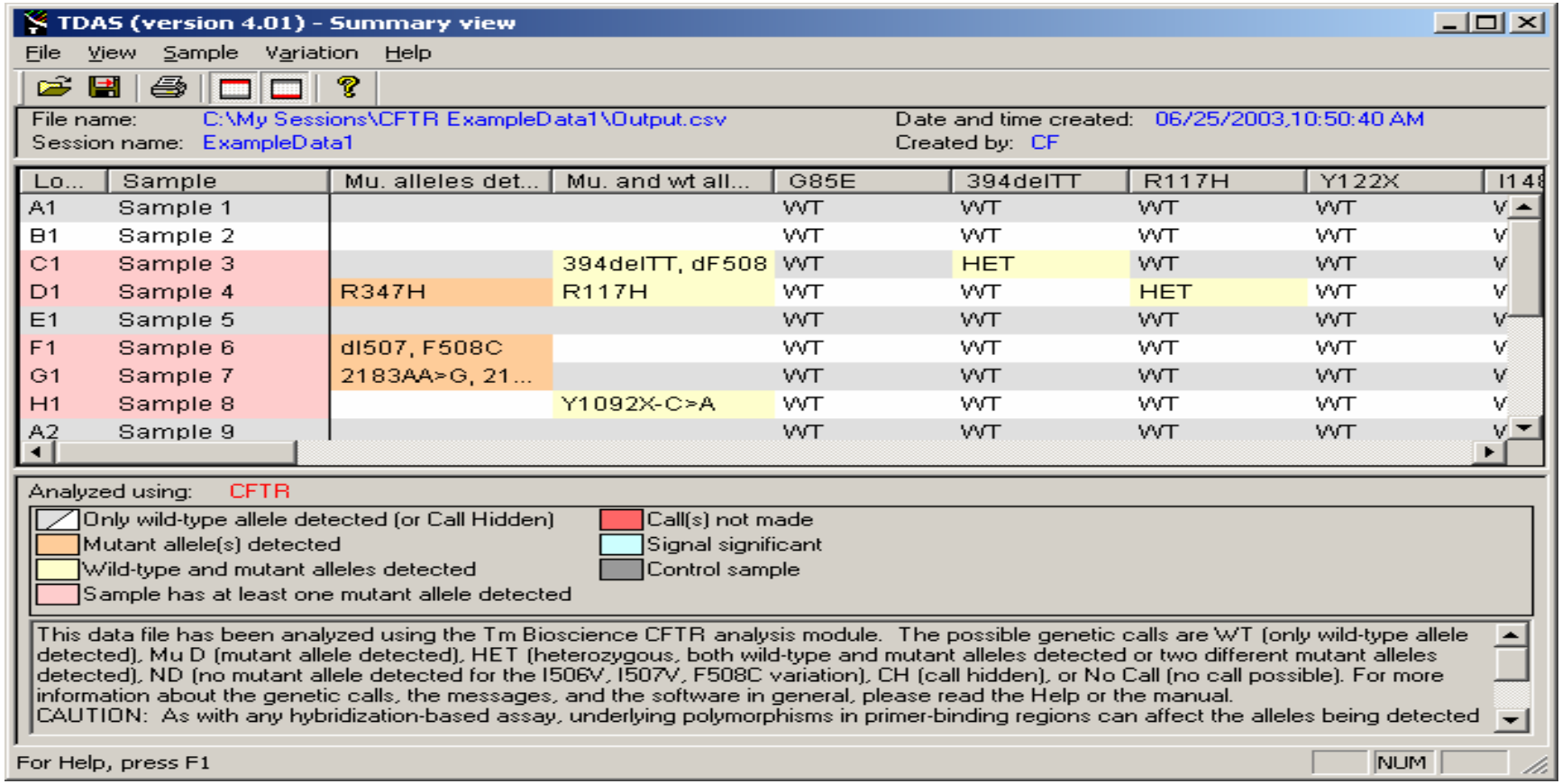


Tag-It Data Acquisition



Location	Sample	01-Fac-V-WT	02-Fac-V-MUT	03-Fac-II-WT	04-Fac-II-MUT	05-MTHFR-677-WT	06-MTHFR-677-MUT
A1		18.0	17.0	14.0	19.0	19.5	19.0
B1		76.5	3406.0	2281.0	98.5	6658.5	365.0
C1		68.5	3365.0	2153.5	86.0	6336.0	323.0
D1		3021.0	84.0	77.0	3939.0	4264.0	6691.5
E1		3010.0	96.5	91.0	4127.5	4378.5	7330.5
F1		2168.0	2457.0	1262.0	3590.0	159.5	8356.0
G1		2301.0	2440.0	1446.0	3152.0	165.0	8975.0
H1		13.0	11.0	19.0	27.5	19.0	21.0

TDAS Summary View



Genetic calls are displayed for each variation, for all samples.

Detailed Sample View

Complete data for sample D1 (Sample 4) on session ExampleData1

Variation	Call	Raw Signal (MFI)		Background (MFI)		Net Signal (MFI)		Allelic Ratio		AR Thresholds		
		Wt Allele	Mu Allele	Wt Allele	Mu Allele	Wt Allele	Mu Allele	Wt Allele	Mu Allele	WT Call	Wt Present	Mu Present
G85E	WT	2224.5	241.0	36.5	33.5	2188.0	207.5	0.91	0.09	0.85 - 1.00	0.30 - 1.00	0.30 - 1.00
394delTT	WT	1380.5	63.5	35.0	47.5	1345.5	16.0	0.99	0.01	0.85 - 1.00	0.30 - 1.00	0.30 - 1.00
R117H	HET	601.0	757.0	23.0	30.5	578.0	726.5	0.44	0.56	0.85 - 1.00	0.30 - 1.00	0.30 - 1.00
Y122X	WT	1028.0	54.0	37.0	45.0	991.0	9.0	0.99	0.01	0.85 - 1.00	0.30 - 1.00	0.30 - 1.00
I148T	WT	1430.0	69.0	25.0	37.0	1405.0	32.0	0.98	0.02	0.85 - 1.00	0.30 - 1.00	0.30 - 1.00
621+1G>T	WT	1548.5	52.0	37.0	49.0	1511.5	3.0	1.00	0.00	0.85 - 1.00	0.25 - 1.00	0.30 - 1.00
711+1G>T	WT	1492.0	42.0	27.0	30.0	1465.0	12.0	0.99	0.01	0.85 - 1.00	0.30 - 1.00	0.30 - 1.00
1078delT	WT	1701.0	93.5	29.0	36.0	1672.0	57.5	0.97	0.03	0.85 - 1.00	0.30 - 1.00	0.30 - 1.00
R334W	WT	955.0	75.0	43.0	30.5	912.0	44.5	0.95	0.05	0.85 - 1.00	0.30 - 1.00	0.30 - 1.00
R347P	Mu D (H)	56.0	67.0	33.0	40.0	23.0	27.0	0.01	0.02	0.85 - 1.00	0.30 - 1.00	0.30 - 1.00
└ R347H			1673.5		32.0		1641.5		0.97			0.30 - 1.00
A455E	WT	1924.0	49.0	29.0	39.0	1895.0	10.0	0.99	0.01	0.85 - 1.00	0.30 - 1.00	0.30 - 1.00

Analyzed using: CFTR

- Only wild-type allele detected (or Call Hidden)
- Mutant allele(s) detected
- Wild-type and mutant alleles detected
- Sample has at least one mutant allele detected
- Call(s) not made
- Signal significant
- Control sample

This data file has been analyzed using the Tm Bioscience CFTR analysis module. The possible genetic calls are WT (only wild-type allele detected), Mu D (mutant allele detected), HET (heterozygous, both wild-type and mutant alleles detected or two different mutant alleles detected), ND (no mutant allele detected for the I506V, I507V, F508C variation), CH (call hidden), or No Call (no call possible). For more information about the genetic calls, the messages, and the software in general, please read the Help or the manual.

Print | Help | Close | Close all sample data windows

All data and genetic calls are displayed for the selected sample.

Detailed Variation View

Complete data for variation R117H on session ExampleData1

Sample		Call	Raw Signal (MFI)		Background (MFI)		Net Signal (MFI)		Allelic Ratio		AR Thresholds		
Location	Name		Wt Allele	Mu Allele	Wt Allele	Mu Allele	Wt Allele	Mu Allele	Wt Allele	Mu Allele	WT Call	Wt Present	Mu Present
A1	Sample 1	WT	1037.5	57.0	23.0	30.5	1014.5	26.5	0.97	0.03	0.85 - 1.00	0.30 - 1.00	0.30 - 1.00
B1	Sample 2	WT	3000.0	65.0	23.0	30.5	2977.0	34.5	0.99	0.01	0.85 - 1.00	0.30 - 1.00	0.30 - 1.00
C1	Sample 3	WT	1757.0	60.0	23.0	30.5	1734.0	29.5	0.98	0.02	0.85 - 1.00	0.30 - 1.00	0.30 - 1.00
D1	Sample 4	HET	601.0	757.0	23.0	30.5	578.0	726.5	0.44	0.56	0.85 - 1.00	0.30 - 1.00	0.30 - 1.00
E1	Sample 5	WT	3162.0	64.5	23.0	30.5	3139.0	34.0	0.99	0.01	0.85 - 1.00	0.30 - 1.00	0.30 - 1.00
F1	Sample 6	WT	1757.0	60.0	23.0	30.5	1734.0	29.5	0.98	0.02	0.85 - 1.00	0.30 - 1.00	0.30 - 1.00
G1	Sample 7	WT	1757.0	60.0	23.0	30.5	1734.0	29.5	0.98	0.02	0.85 - 1.00	0.30 - 1.00	0.30 - 1.00
H1	Sample 8	WT	1757.0	60.0	23.0	30.5	1734.0	29.5	0.98	0.02	0.85 - 1.00	0.30 - 1.00	0.30 - 1.00
A2	Sample 9	WT	2613.5	49.0	23.0	30.5	2590.5	18.5	0.99	0.01	0.85 - 1.00	0.30 - 1.00	0.30 - 1.00
B2	Sample 10	WT	2559.0	50.0	23.0	30.5	2536.0	19.5	0.99	0.01	0.85 - 1.00	0.30 - 1.00	0.30 - 1.00
C2	Sample 11	WT	2948.0	58.5	23.0	30.5	2925.0	28.0	0.99	0.01	0.85 - 1.00	0.30 - 1.00	0.30 - 1.00
D2	Sample 12	WT	3007.5	59.0	23.0	30.5	2984.5	28.5	0.99	0.01	0.85 - 1.00	0.30 - 1.00	0.30 - 1.00
E2	Sample 13	HET	1253.0	1531.5	23.0	30.5	1230.0	1501.0	0.45	0.55	0.85 - 1.00	0.30 - 1.00	0.30 - 1.00
F2	Sample 14	Mu D	32.0	2400.0	23.0	30.5	9.0	2369.5	0.00	1.00	0.85 - 1.00	0.30 - 1.00	0.30 - 1.00

Analyzed using: CFTR

- Only wild-type allele detected (or Call Hidden)
- Mutant allele(s) detected
- Wild-type and mutant alleles detected
- Sample has at least one mutant allele detected
- Call(s) not made
- Signal significant
- Control sample

This data file has been analyzed using the Tm Bioscience CFTR analysis module. The possible genetic calls are WT (only wild-type allele detected), Mu D (mutant allele detected), HET (heterozygous, both wild-type and mutant alleles detected or two different mutant alleles detected), ND (no mutant allele detected for the I506V, I507V, F508C variation), CH (call hidden), or No Call (no call possible). For more information about the genetic calls, the messages, and the software in general, please read the Help or the manual.

Print | Help | Close | Close all variation data windows

All data and genetic calls are displayed for the selected variation.

Ashkenazi Jewish Panel

- Bloom Syndrome
- Canavan Disease
- Familial Dysautonomia
- Fanconi Anemia Group C
- Gaucher Disease
- Mucolipidosis Type IV (ML-IV)
- Niemann-Pick Disease
- Tay-Sachs disease

Additional Products

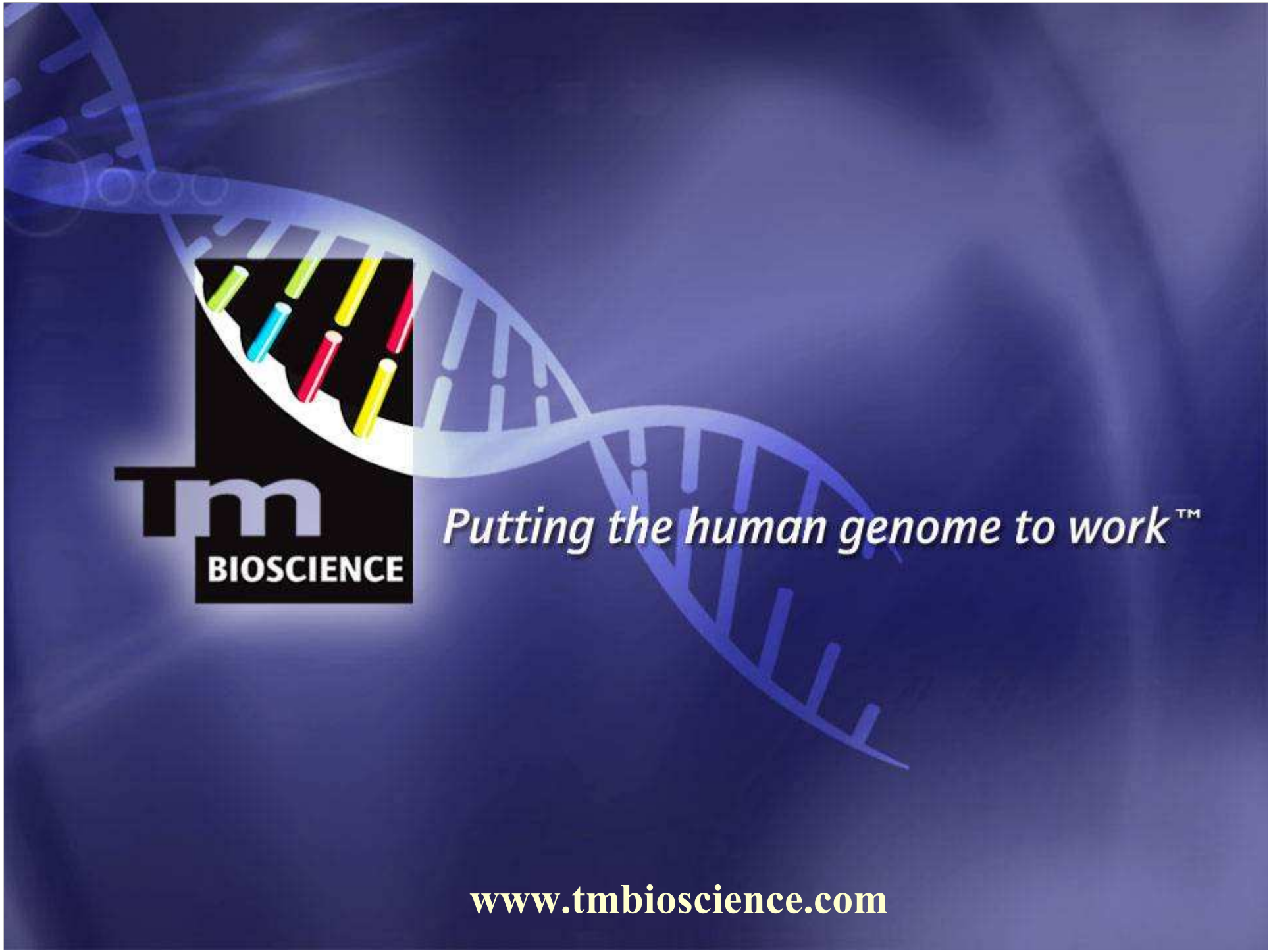
- PGx Panels (2D6, 2C9, 2C19)
- Fragile X
- Gene Dosage (aneuploidy)
- ID (MRSA, HPV)

Tag-It™ Advantage

- Multiplexed—entire assay (86 alleles) tested in one well
- Complete genotyping—analyzes both wildtype & mutant at each locus in each assay
- Accuracy—S:N of 20:1 to 50:1 for each allele
- Automation—Up to 1200 samples/day/technician in a 96-well format
- Menu—CF40, Jewish panel (8 diseases), P450 testing (2D6, 2C9, 2C19), others

Tag-It™ Advantage

- No more reflexing—reflex tests included in each assay, but results only shown if needed
- Full data analysis software provided; makes summary calls; can easily interface with LIS
- Low-cost instrument with ability to process 96 samples/40 minutes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