NEWBORN SCREENING
Yesterday, Today, and Tomorrow
(Impact on Plain Community)

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and
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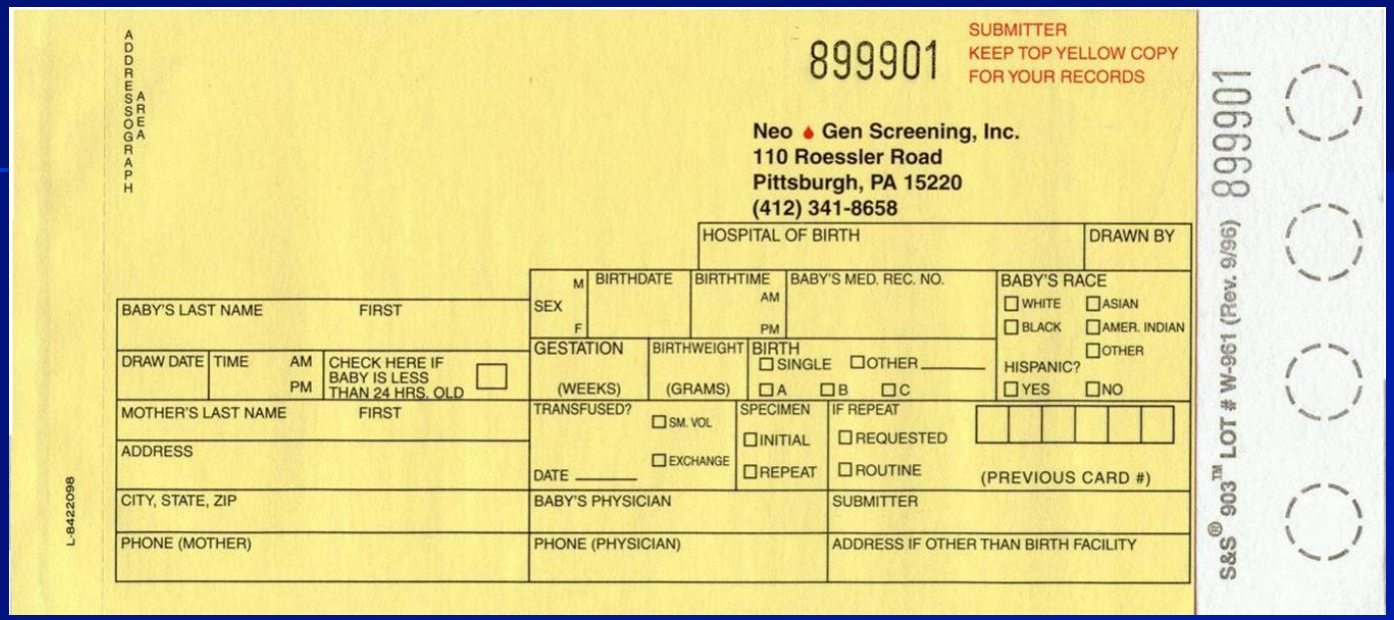
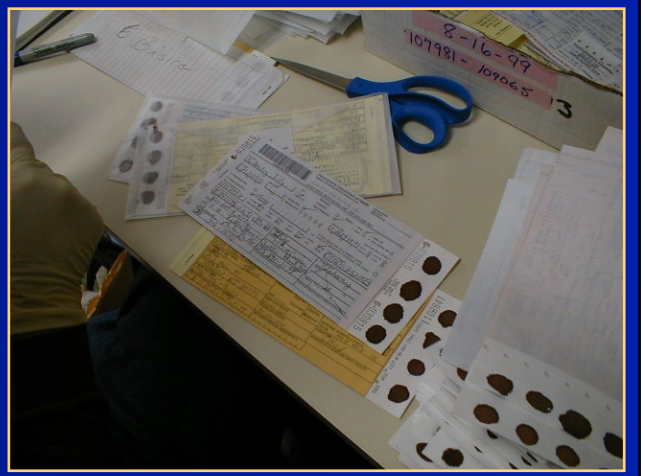
The PKU Card



3/16”punch

Fits in a standard business envelope



History of Mandatory Newborn Screening in Pennsylvania

- 1965 – Phenylketonuria
- 1975 – Congenital Hypothyroidism
- 1992 – Sickle Cell Disease
- 1993 – Maple Syrup Urine Disease
- 2000 – Galactosemia
- 2000 – Congenital Adrenal Hyperplasia

History of Expanded Newborn Screening in Pennsylvania

- 1985 – Supplemental program began at Magee-Womens Hospital
  - 10,000 annual births
  - CAH, Gal, Bio, Sickle Cell
- 1987 – Supplemental program expanded to 10 disorders
  - MSUD, HCys, CF, G6PD, Arg, PyroGlu

History of Expanded Screening in Pennsylvania (Con't)

- 1989 – 2nd Tier DNA screen for CF
- 1992 – MS/MS screening introduced
- 1994 – Neo Gen Screening spun off from Magee
- 2003 – Neo Gen acquired by Pediatrix

Traditional Testing Methods

- For nearly 20 years, BIA was used to test for a disease from a dried blood specimen.
  - Method relies on growth of bacteria in presence of phenylalanine.
  - Poor precision.
  - False + of 1%
- Many laboratories replaced with Fluorometric assays.
  - Improvements noted
  - False positive rates 0.1%

Expanded Screening Technology (1993)

- DNA- for specific mutations (ex. CF) 2nd tier analysis
- Tandem Mass Spectrometry (MS/MS) - measures the *weight* of molecules in a specimen

METHODOLOGY

Automated Sample Preparation

One 3/16th in. blood disk

Butyl ester derivatization for enhance sensitivity, selectivity, and throughput.

Batched microtiter plate automated sample handling and preparation.

Automated Sample Injection

Two minute analysis time.

Microtiter plate format.

Small sample volume automated injection (10 µL sample loop)

Low sample carry-over, versatile multiple platforms

High Throughput Electrospray MS/MS

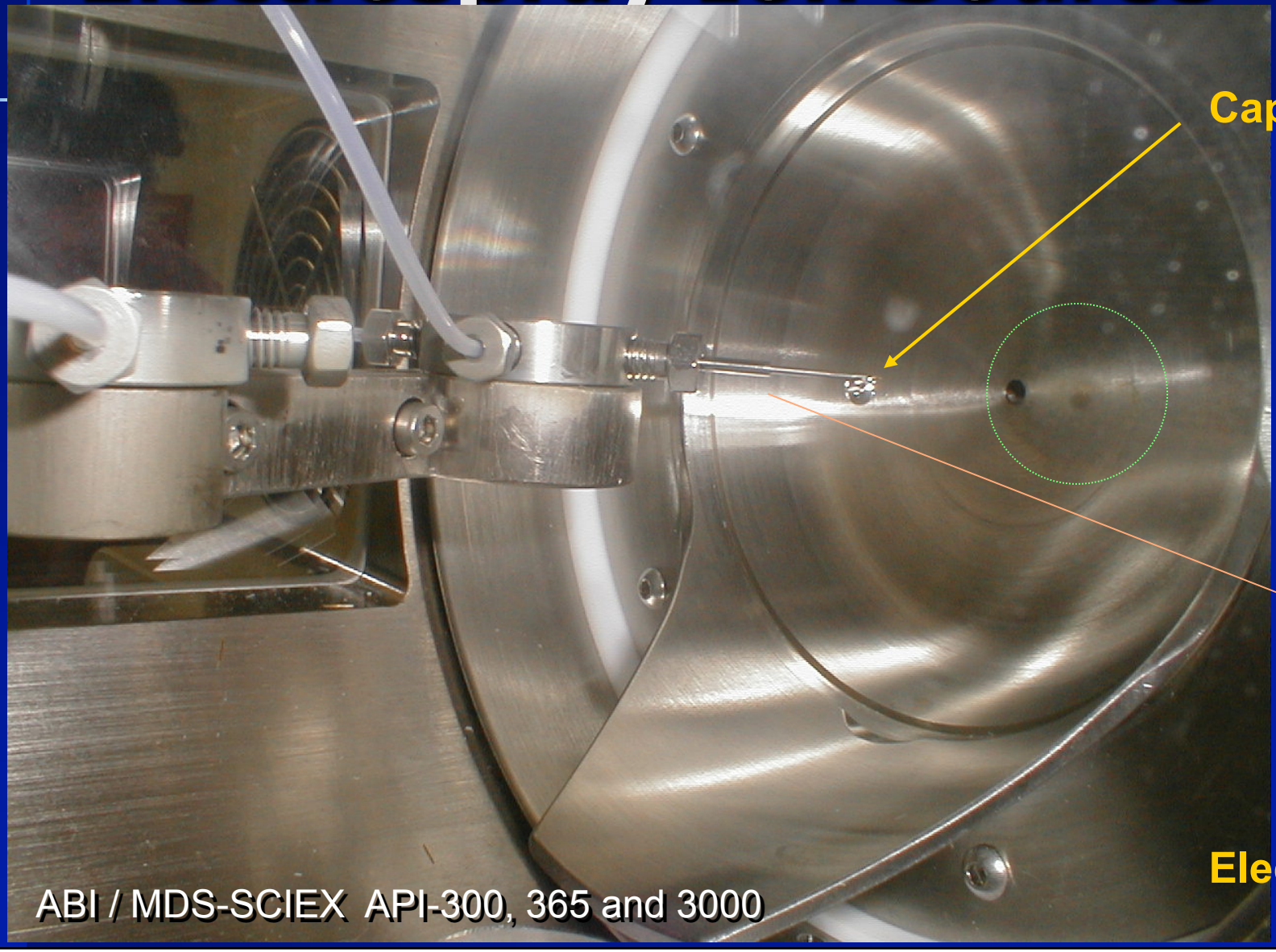
Low Flow Rate (<20 µL/min) for enhanced sensitivity.

Robust, sensitive, low maintenance systems. 120,000 specimens analyzed per year per instrument.

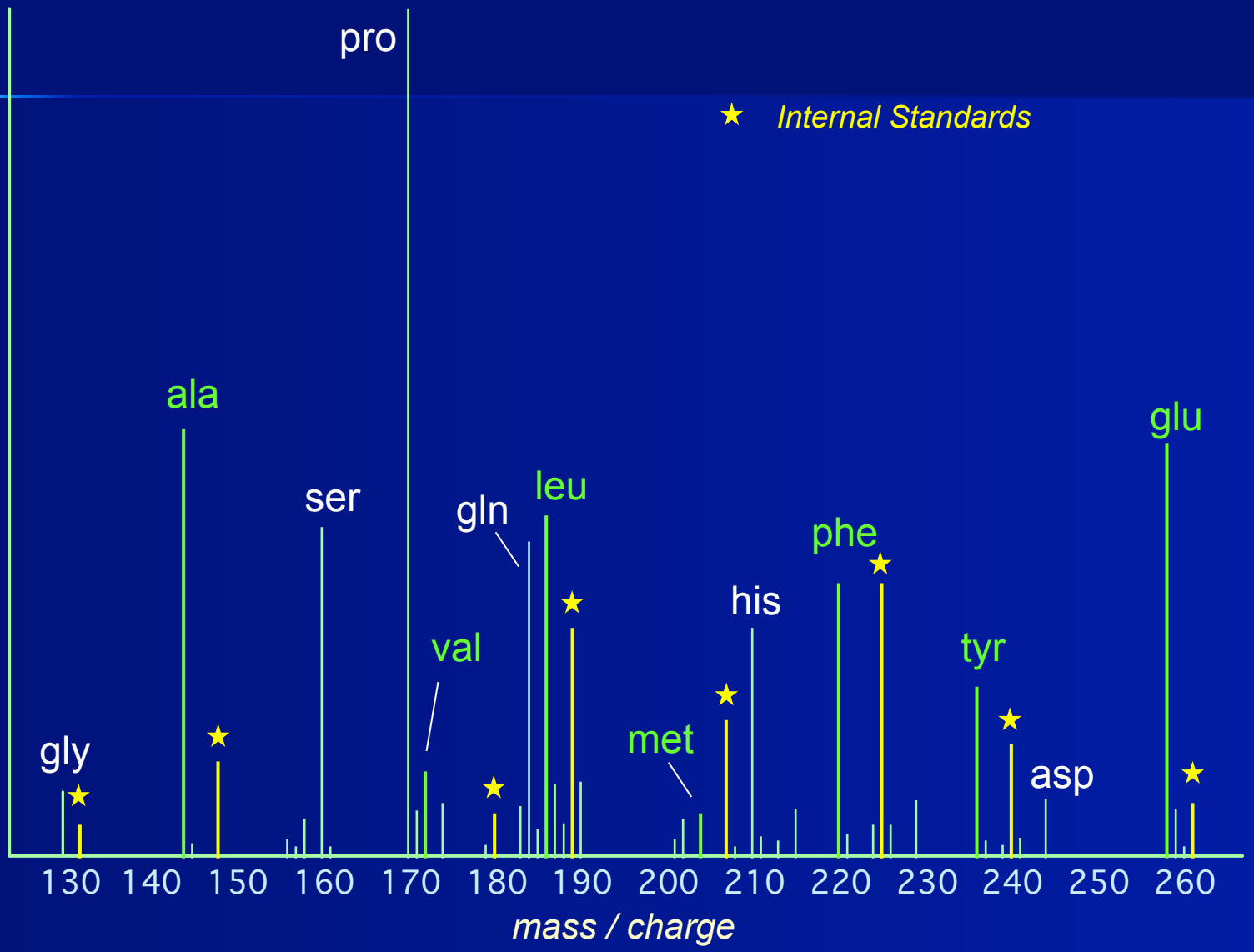
Comprehensive multi-function MS/MS panel

Automated data and result processing.

Electrospray Ion Source



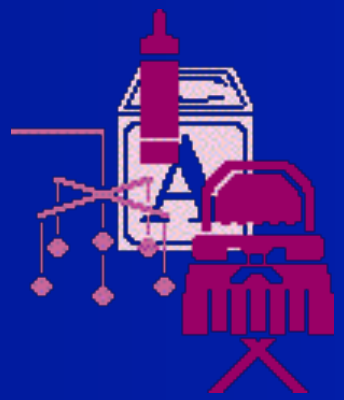
Amino Acid Profile (Normal)



Total Newborns Screened Using MS/MS

2,117,013

Through August 31th, 2004



Total Disorders Detected Using MS/MS

705

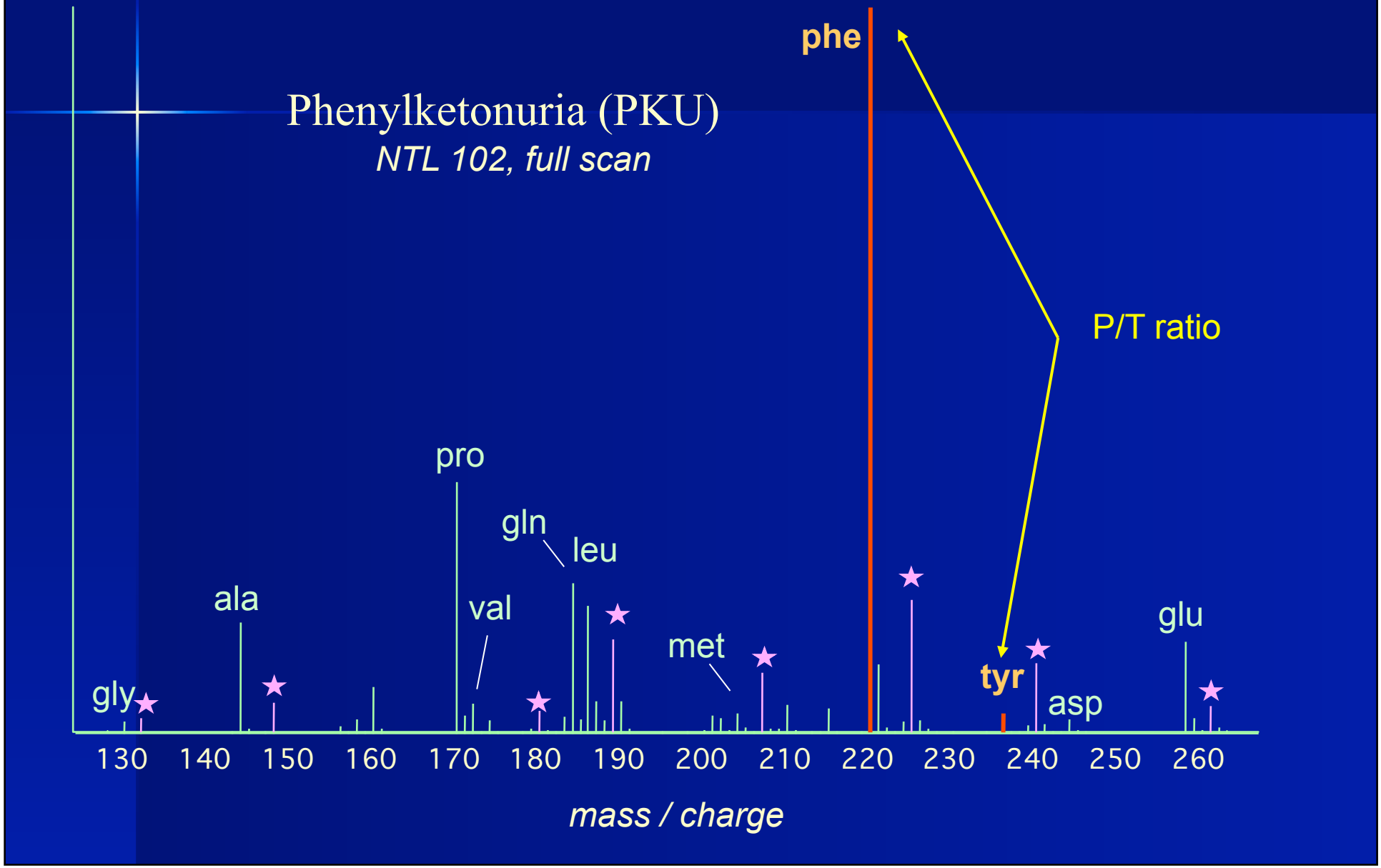
Frequency:

1 positive case per 3,003 newborns

Amino Acid Disorders

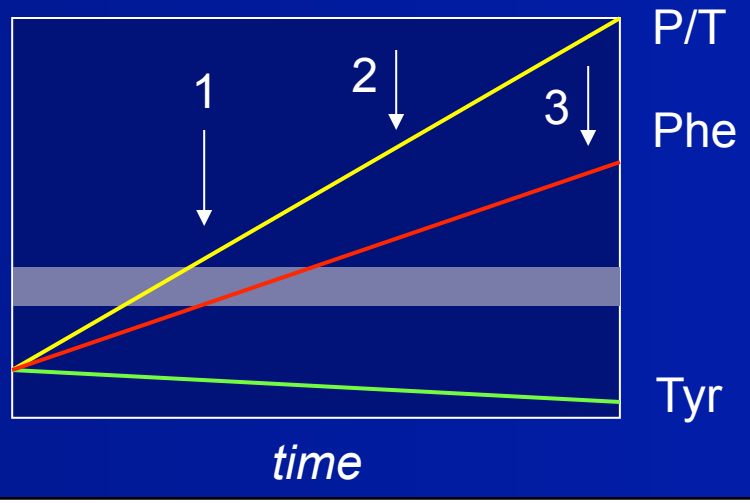
Phenylketonuria	255
Maple Syrup Urine Disease	55
Citrullinemia	13
Argininosuccinic Aciduria	5
Homocystinuria	3
Tyrosinemia (Type 1 & 3)	2
Arginase Deficiency	1

Phenylketonuria

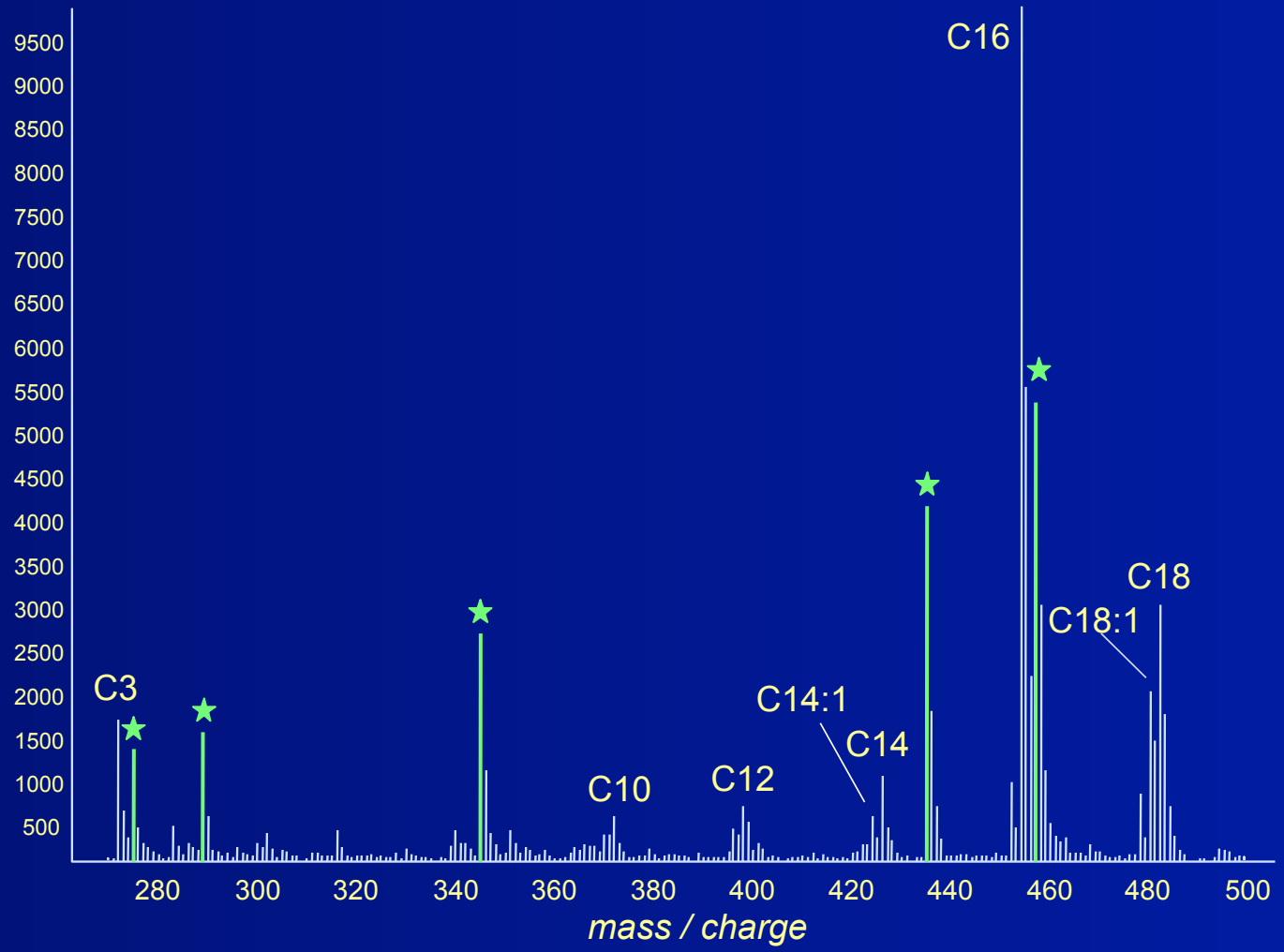


MS/MS and PKU Detection

- Measure both Phenylalanine and Tyrosine
- Obtain a "Molar" Ratio for Diagnosis.
- Higher Precision and accuracy enables detection of PKU less than 24 hours.
- False + rate <0.01%



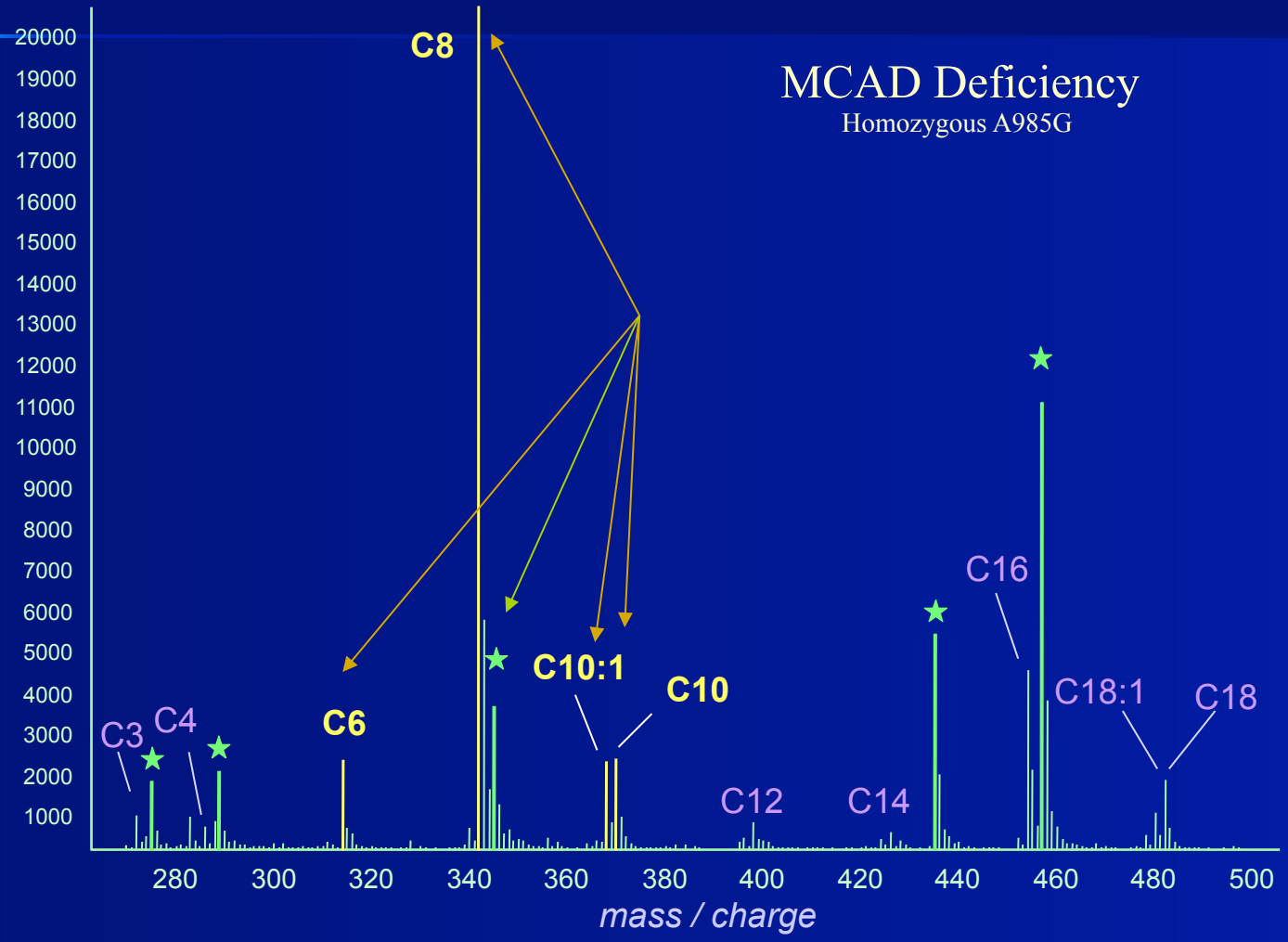
Acylcarnitine Profile (Normal)



Fatty Acid Oxidation Disorders

- MCAD Deficiency 156
- VLCAD Deficiency 33
- LCHAD/TFP Deficiencies 18
- SCAD Deficiency 12
- CPT II / Translocase Deficiencies 15
- MADD Deficiencies 11

MCAD Deficiency

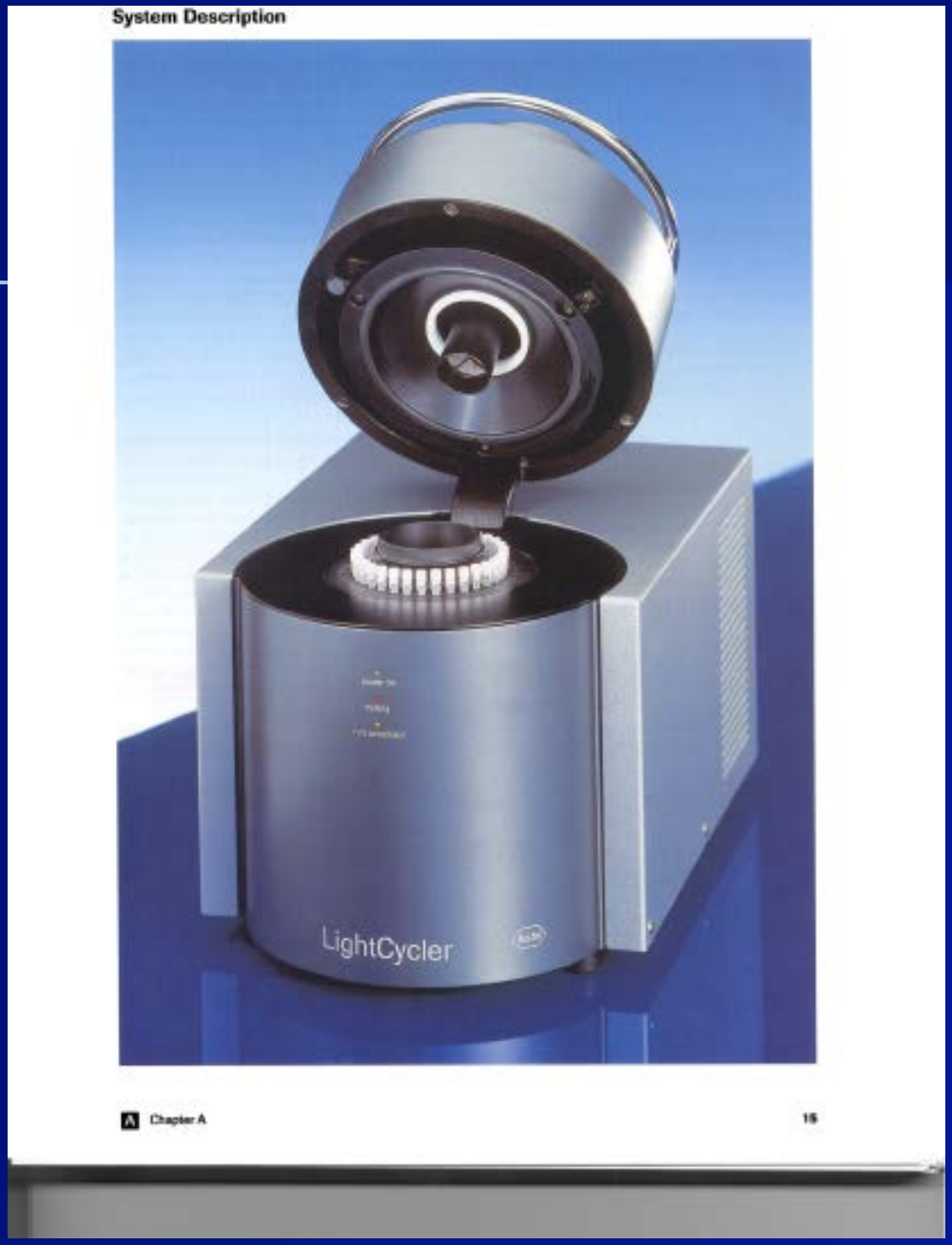


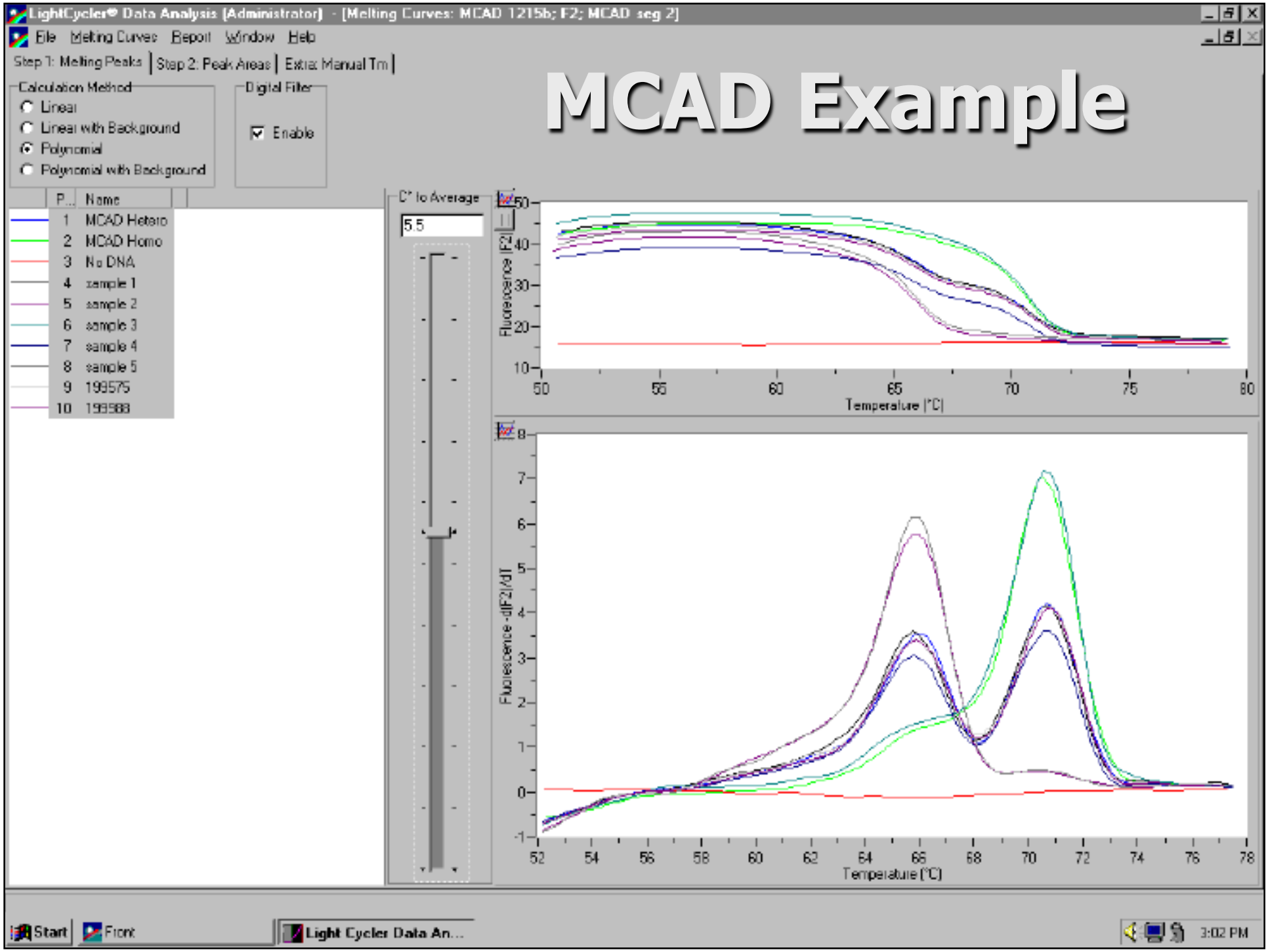
MCAD Deficiency

2nd-Tier DNA Molecular Results

A985G / A985G	138
A985G / T199C	19
A985G / Other	49
Other / Other	5
Other / ?	4
? / ?	10

The Light Cycler

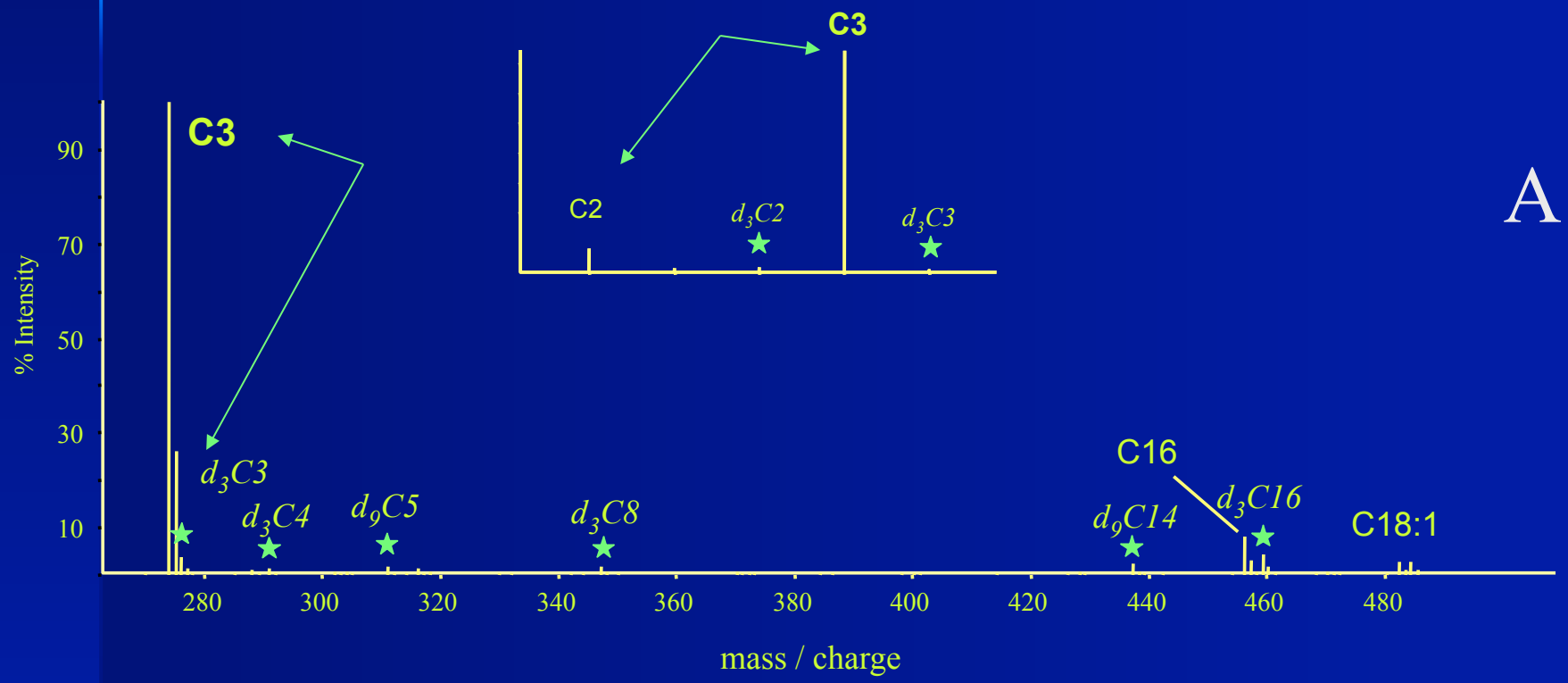



LightCycler® Data Analysis (Administrator) - [Melting Curves: MCAD 1215b; F2; MCAD seg 2]
File Melting Curves Report Window Help
Step 1: Melting Peaks | Step 2: Peak Areas | Extra: Manual Tm
Calculation Method
Linear
Linear with Background
Polynomial
Polynomial with Background
Digital Filter
Enable
MCAD Example
P... Name
1 MCAD Hetero
2 MCAD Homo
3 No DNA
4 sample 1
5 sample 2
6 sample 3
7 sample 4
8 sample 5
9 199575
10 199588
C° to Average
5.5
Fluorescence (F2)
Temperature (°C)
Fluorescence -d(F2)/dT
Temperature (°C)
Start
Front
Light Cycler Data An...
3:02 PM

Organic Acidurias

Methylmalonic Aciduria	31
Propionic Aciduria	28
Glutaric Aciduria-Type 1	27
Isovaleric Aciduria	19
3-Methylcrotonyl Glycinuria	17
HMG CoA Lyase Deficiency	2
β-Ketothiolase Deficiency	1
Multiple CoA Carboxylase Deficiency	1

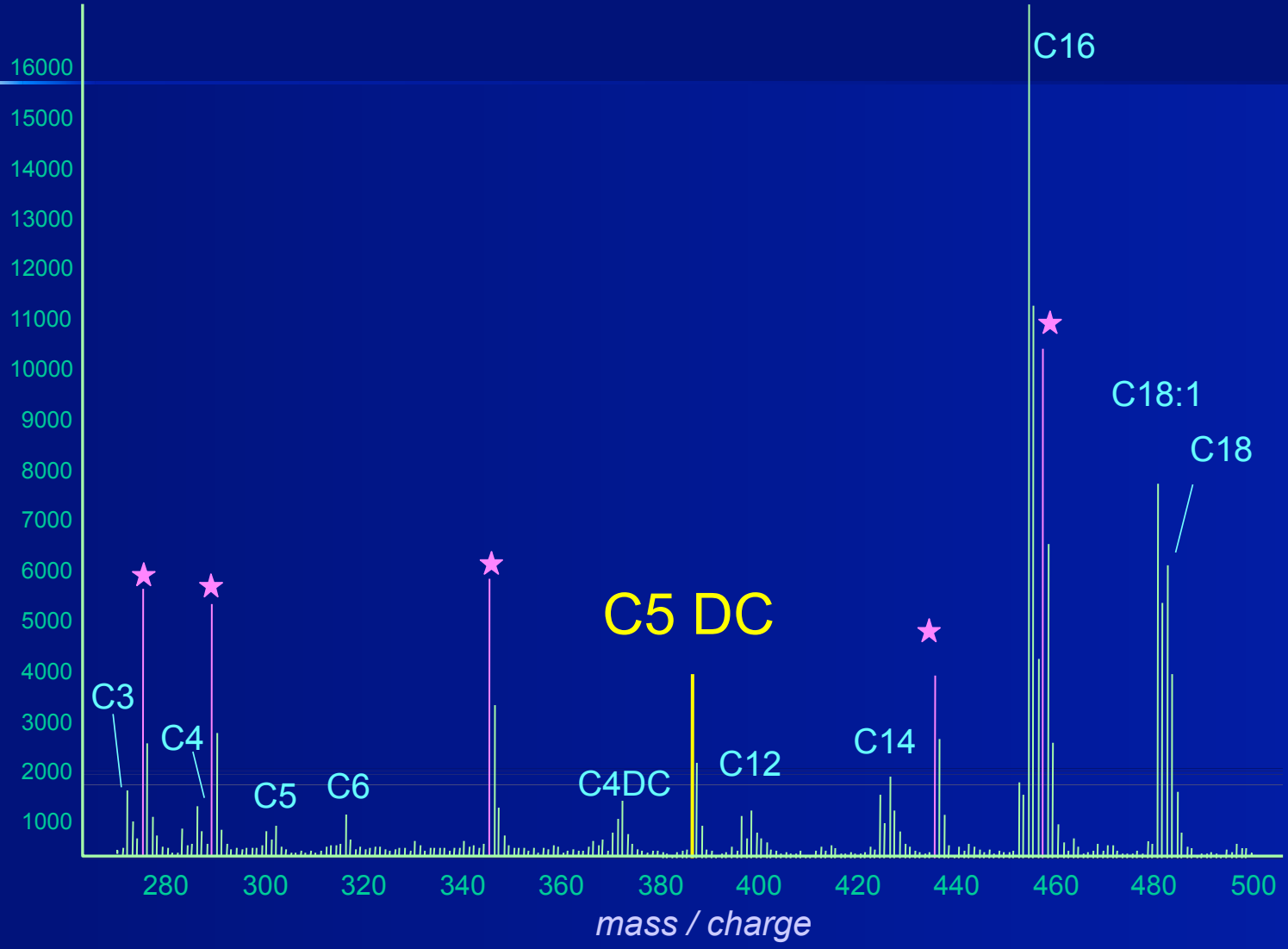
Propionic Acidemia



PROPIONIC ACIDEMIA
Second Tier Molecular Results

6	Del14Ins12 / ?	(US & Canada)
2	Del14Ins12 / Del14Ins12	(Chile)
2	E168K / E168K	(Chile)
1	E168K / ?	(Chile)
6	N536D / N536D	(Amish)
32	? / ?	

Glutaric Acidemia



Why MS can make a difference.

Not Screened
Affected
GA-I

Screened
Well
GA-I

Screened
Well
GA-I

Glutaric Aciduria – Type 1

2nd Tier DNA Molecular Results
Newborns (n=30)

A421V / A421V	23	(Amish)
A421V / R313W	1	(Amish)
A421V / R88C	1	(Unknown)
R227P / G390R	1	(Unknown)
Wt (2) / wt (2)	3	(Unknown)
? / ?	1	(Arab)

Glutaric Aciduria – Type 1

2nd Tier DNA Molecular Results

Older High Risk Cases

A421V / A421V	11	(Amish)
A421V / R88S	2	(Unknown)
A421V / V400M	1	(Unknown)
A421V / 219delC	1	(Unknown)
A421V / A349T	1	(Amish)
A421V / R294Q	1	(Unknown)
A421V / M191T	1	(Unknown)
A421V / wt (2)	1	(Amish)

Organic Acidemias

3-Methyl-Crotonyl-CoA Carboxylase Deficiency (3-Methylcrotonylglycinuria)

Maternal (11)
Isolated (4)

Cystic Fibrosis

2nd-Tier DNA Molecular Results

df508 / df508	225
df508 / G551D	12
df508 / G542X	12
df508 / N1303K	8
df508 / W1282X	6
df508 / 621+1 G->T	5
df508 / R1162X	5
df508 / 2183del AA^{-}	5
df508 / E60X	4
df508 / other	31

Cystic Fibrosis (Con't)

- df508 / R117H 29
  - Poly T (7,9) 15
  - Poly T (7) 4
  - Poly T (5,9) 2
  - Poly T (5) 1
  - Poly T (?) 7
- df508 / df508C 6
- df508 / I148T 4

Galactosemia

Mutations Detected by 2nd-Tier Testing

- Q188R
- S135L
- K285N
- L195P
- Exon 10
- N314D (Duarte)

Galactosemia (Classical)
2nd-Tier DNA Molecular Results

33	Q188R / Q188R
3	Q188R / K285N
2	Q188R / L195P
1	Q188R / S135L
2	K285N / K285N
2	S135L / S135L
1	S135L / K285N
5	Exon 10 / Exon 10
3	Q188R / Exon 10
3	S135L / Exon 10
7	Q188R / ?
2	S135L / ?
1	L195P / ?
17	? /?
37	wt (5) / wt (5) (? Kin & Epi)

Biotinidase Deficiency

Mutations Detected by 2nd-Tier Testing

- Q456H
- D444H:A171T
- G98:d7i3
- R538C
- D444H
- R157H
- D252G
- D444H:F403V
- D444H:Q456H

Biotinidase Deficiency (Complete)

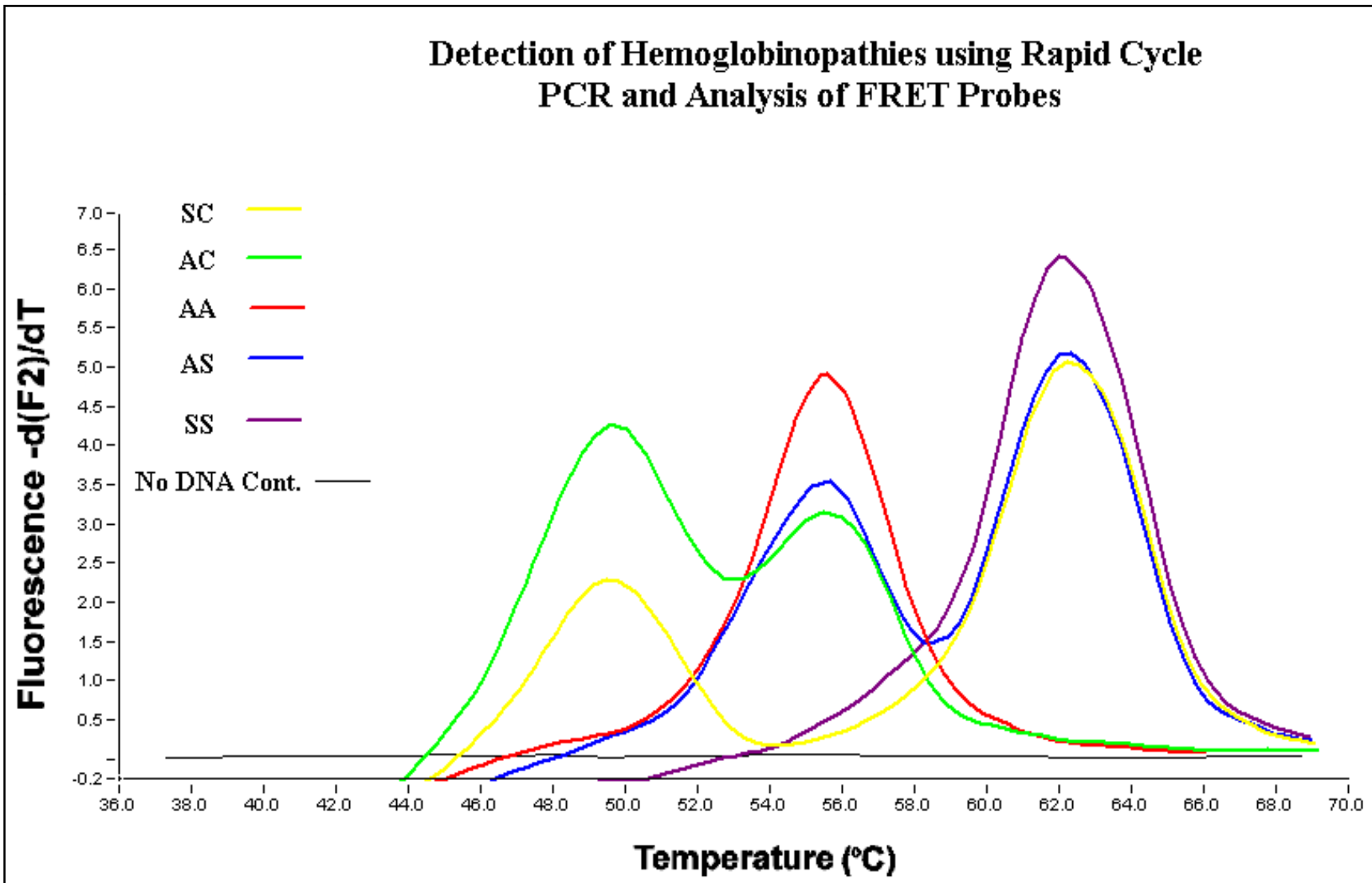
2nd-Tier DNA Molecular Results

G98:d7i3 / G98:d7i3	4
Q456H / Q456H	2
D444H:A171T / D444H:A171T	2
D444H:A171T / R538C	1
D444H:A171T / Q456H	1
D444H:A171T / R157H	1
Q456H / D252G	1
Q456H / R157H	1
R538C / R157H	1
D444H:F403V / D444H:F403V	1
Q456H / ?	4
G98:d7i3 / ?	3
D444H:A171T / ?	1
R157H / ?	1
? / ? or wt / wt	2

Sickle Cell Disease

Mutations Detected by 2nd-Tier Testing

Sickle Cell Disease		β -Thallassemia
Hb S	A173T	A (- 29) G
Hb C	G172A	C (- 88) T
Hb E	G232A	IVS 1



Sickle Cell Disease
2nd-Tier DNA Molecular Testing

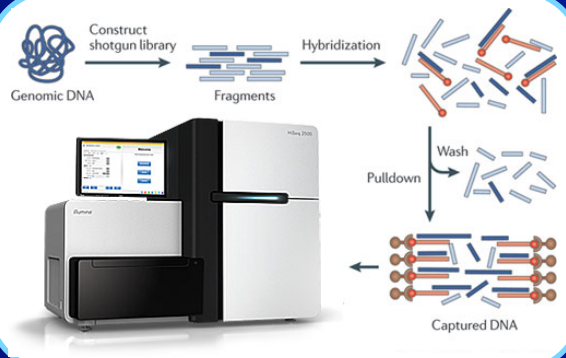
Benefits of 2nd Tier Light Cycler Screening

- Useful if common mutations are present
- Improves Sensitivity and Specificity
- Permits Genotype / Phenotype correlations
- Lays foundation for future primary DNA screening programs

Workflow for 2nd Tier Next Generation Sequencing Newborn Screening

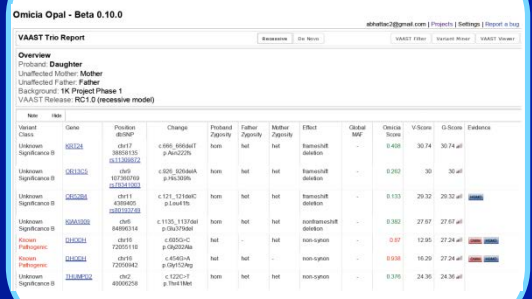
Sample Isolation 2h

DNA Capture & Sequencing 92h



Raw Data Management 10h

Analysis & Interpretation 1h+



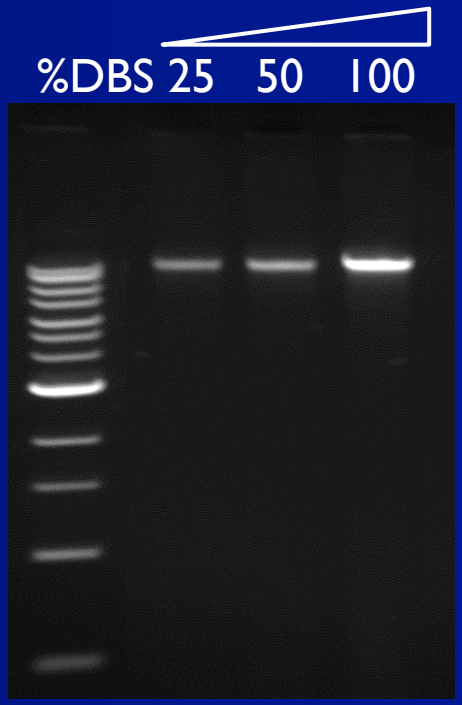
8 samples, 105 Hrs, <$10,000 = Real Neonatal Genomics!

Workflow for 2nd Tier Next Generation Sequencing Newborn Screening

Sample Isolation

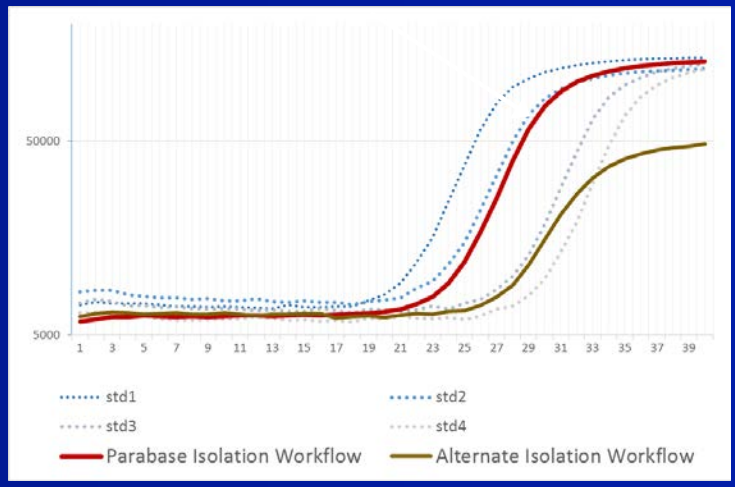
2h

- High M.Wt. DNA
- PCR Amplifiable and NGS ready
- Processing Time ~ 2 hrs
- Amenable to automation



Yield 400ng per Spot

More yield and DNA purity than alternate workflow



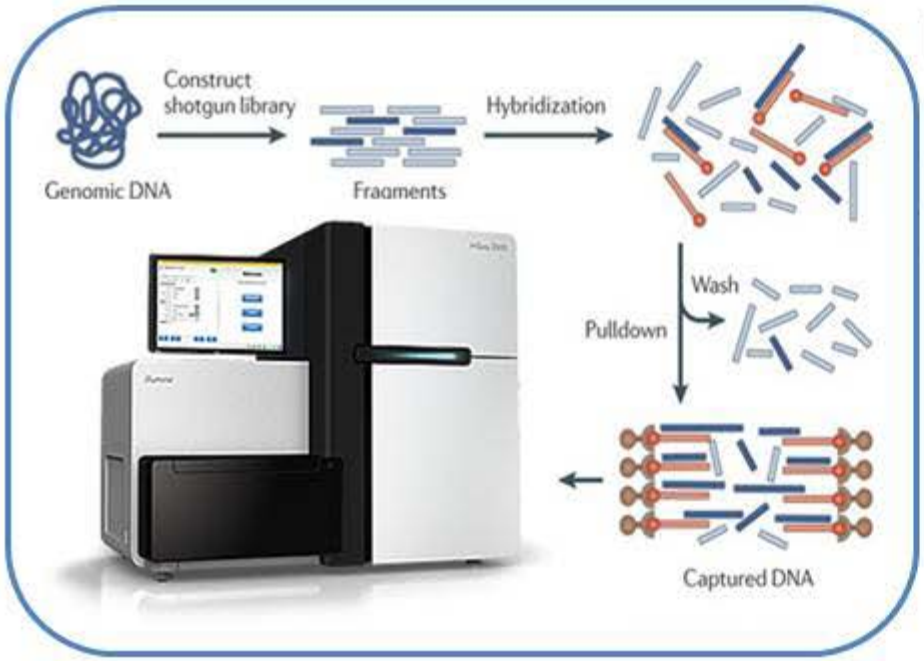
Workflow for 2nd Tier Newborn Screening

DNA Capture*
& Sequencing**

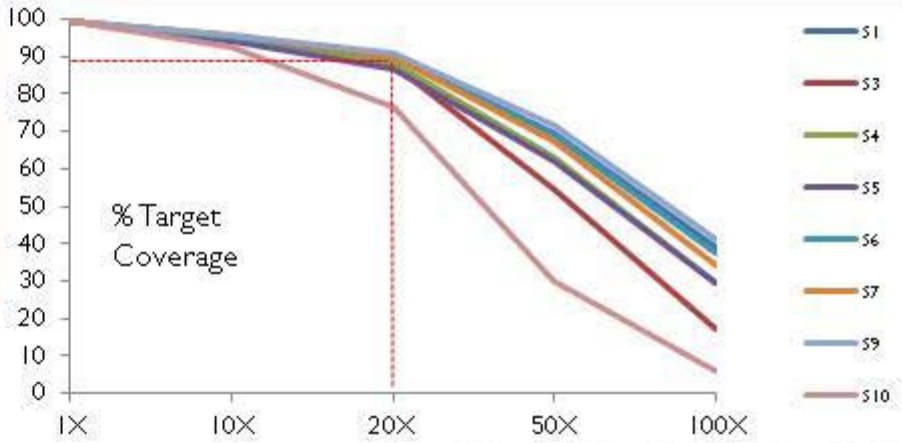
92h

** Room for improvement*
*** Process reduced from 19* days to 92 hrs*

- Library Prep ~ 5 hrs
- Capture ~ 66 hrs
- HiSeq 2500 PE 75 ~ 21 hrs
- Fast turnaround & High quality data



	S1	S3	S4	S5	S6	S7	S9	S10
TOTAL READS (MIn)	92	68	77	75	90	85	96	49
MAPPED READS (MIn)	89	58	72	72	86	79	91	37
% READS MAPPED	96	86	95	96	95	95	95	76
READS ON TARGET (MIn)	69	38	56	56	66	61	71	21
% READS ON TARGET	74	56	73	75	73	73	74	42



Data generated on Nimblegen Exome and HiSeq2500
Variant calling GATK2 (Broad Institute, MA) by CFI and Real Time Genomics
2 Known (MSUD and PA) & 8 blinded samples were provided by Clinic for Special Children, PA (S1-S10)
*Saunders et al., (2012) Rapid Whole Genome Sequencing for Genetic Disease Diagnosis in NICUs

PARABASE GENOMICS

Detecting Known Cases

Identifying causal variants using population frequency and disease category as filters

Type	Sample	Disease	Exome Variants	Protein Impact (PI)	PI+ Probably Damaging	PI+PD Hom.Read>4 MAF <5%	126 Gene Panel Read >4 MAF<5%	126 GP,Common Hom., Read >4 MAF<5%	Gene	Reads	Transcript Variant	Protein Variant	Status
Amish*	whole blood	Propionic Acidemia	71,261	10,127	1,013	11	19	2	PCCB	18	c.1606A>G	p.Asn536Asp	Hom.
Amish*	DNA	Propionic Acidemia	64,003	10,451	1,037	15	16	2	PCCB	5	c.1606A>G	p.Asn536Asp	Hom.
Mennonite*	DNA	Maple Syrup Urine Disease	68,703	10,217	1,031	19	19	3	BCKDHA	35	c. 1312 T>A	p.Tyr438Asn	Hom.
Mennonite*	DNA	Mental Retardation NS	69,946	10,329	1,086	21	ND	ND	CRADD	15	c. 382G>C	p.Gly128Arg	Hom.

Validation of False Positive/ False Negatives by comparing different Methods

CRADD Sample, Broad**	Exome Variants	Panel (*in silico*)
SYNONYMOUS	11219	81
MISSENSE	10518	65
NONSENSE	91	0
SMALL INDELS	889	4
INTRON, UTRs	26083	232
SPLICE SITE	160	1

Data generated on Nimblegen Exome; variant calling GATK (Broad Institute, MA); Omicia (Emeryville, CA)

•*Samples provided by Clinic for Special Children

•** Puffenberger *et al.*, 2012. PLoS ONE 7(1): e28936. Agilent Exome using Broad Pipeline

PARABASE GENOMICS

Results of 8 Blinded and 2 Known Samples

1.	Phenylketonuria	A/M	PAH	782 G>A / 280-282 del ATC	R261Q I95 del
2.	MSUD	M	BCKDHA	1312 T>A	Y438N
3.	MCAD	M	ACADM	985 A>G	K329E
4.	Cystic Fibrosis	A/M	CFTR	1522-4 del TTT	Δf508
5.	SCID	M	IL7R	2 T>G	M1R
6.	11-β-Hydroxylase	A	CYP11B1	1343 G>A	R448H
			ADA	646 G>A	G216R
7.	Galactosemia	A	GALT	563 A>G	Q188R
8.	Biotinidase	M	BTD	1459 T>C	W487R
9.	Homocystinuria	A	MTHFR	1129 C>T	R377C
10.	Propionic Acidemia B	A/M	PCCB	1606 A>G	N536D

Summary: Targeted Next Generation Sequencing

- Can be used for 2nd-tier newborn screening
- Can be used for high risk and NICU screen
- Causal variants identified rapidly
- Lower cost than WES or WGS (<$1,000)
- Faster than WES or WGS (10 run in 100 h)
- >500 genes per panel
- Lower exon drop out rate (<2%)

Acknowledgements

- Parabase Genomics (Sample Isolation)
  - Andy Bhattachrjee, Ph.D.
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  - Alexander Zaranek, Ph.D.
- RealTime Genomics, Inc (Raw Data Management)
  - Francisco De-LaVega, D.Sc.
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  - Martin Reese, Ph.D.
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  - Kevin Strauss, M.D.
  - Erick Puffenberger, Ph.D.