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Molecular Analysis to Enhance Newborn Screening……..

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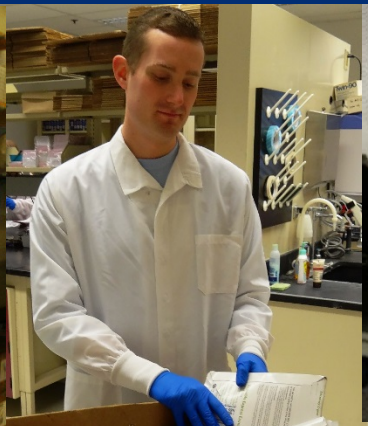
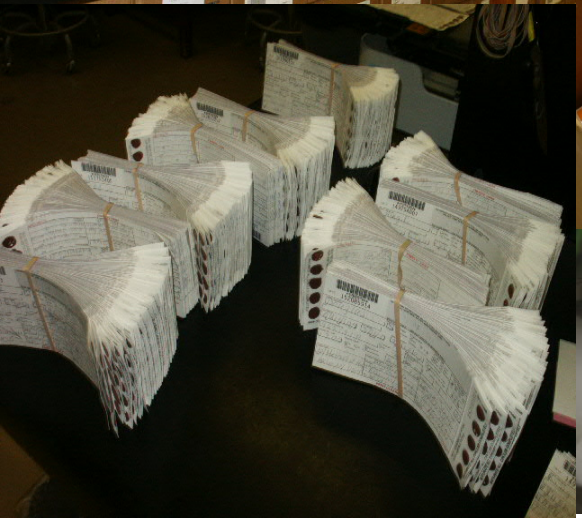
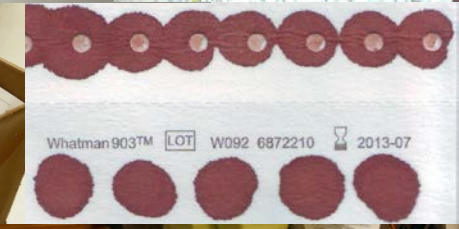
February 29, 2016

….Are You Ready to Jump In??

Population – Based Risk Assessment







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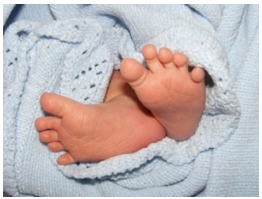
Characteristics of Newborn Disorders Include

- Significant disease
- Treatment possible
- Not evident until harm is done
- Mass testing methods available
- Benefits justify costs



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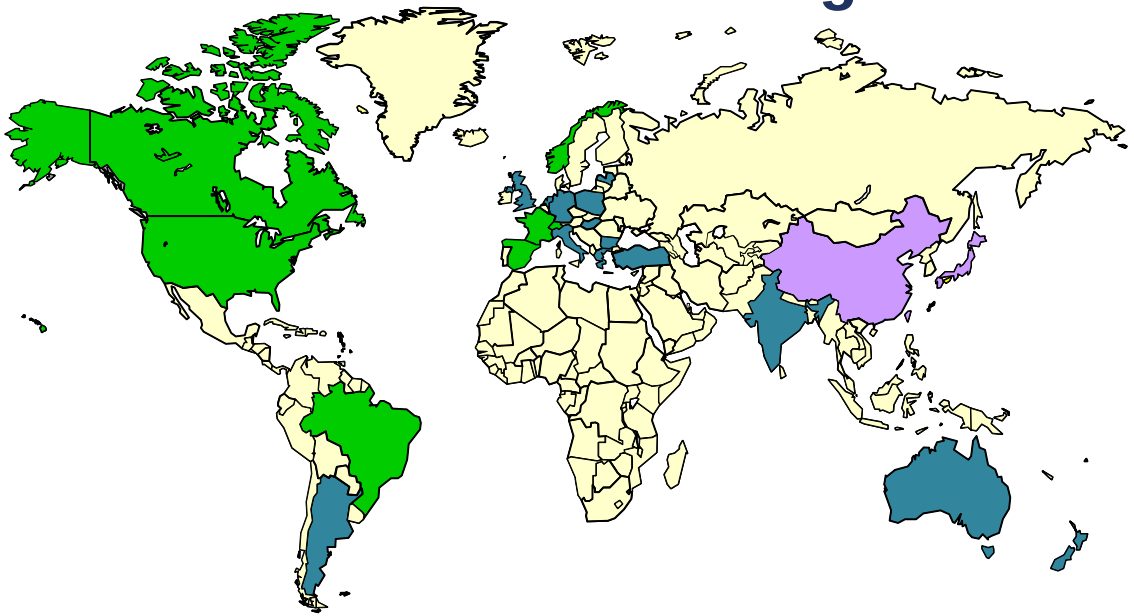
Slide from Mike Glass - Washington State Newborn Screening Program

Does Molecular Testing Add Value??

OR

- Increase in sensitivity of a primary test, effect on specificity
- Identification of carriers; teaching moments
- Predictions regarding phenotype
- Clinicians' perception, diagnostic tool
- Timeliness??

It Is Here – 23 Countries Participate in CDC's Molecular PT Programs in 2015



Countries Participating only in CF PT (14)
Countries Participating only in SCID PT (3)
Countries Participating in CF & SCID PT (6)

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Note that not all NSQAP PT participating countries offer universal screening

Current Molecular Testing in Newborn Screening Laboratories

- **Second tier molecular tests**
  - Increase sensitivity or specificity of primary assay
    - Cystic Fibrosis (CF)
  - Clarify an ambiguous result
    - Hemoglobinopathies
  - Supplemental “Just in Time” assay
    - Galactosemia

- **Primary molecular test**
  - When no other assay is available – e.g. severe combined immunodeficiency; spinal muscular atrophy

Will Molecular Testing Take NBS by Storm?

Or Will We Ride the Wave?

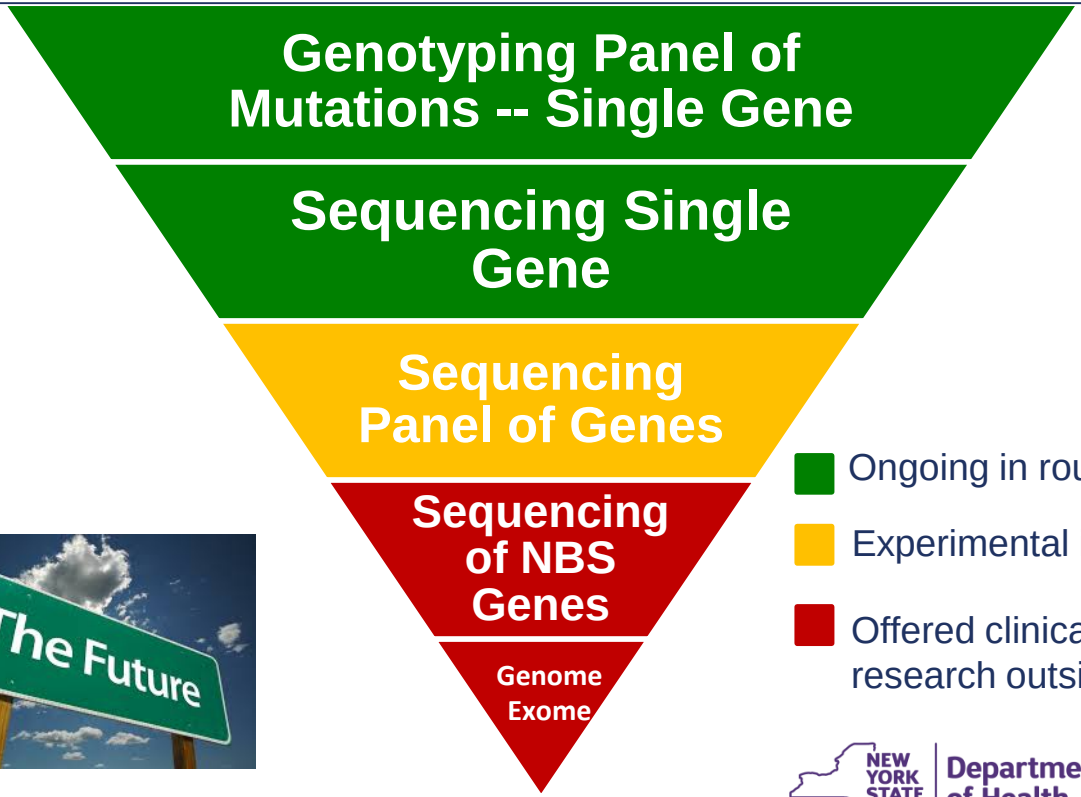
Either way, we are going to get wet…

What Must We Consider??

- Cost
- Value added?
- Impact on TAT; timeliness big concern
- Staff time and qualifications
- Bioinformatics needs
- Instrumentation requirements
- Practical issues
- Are we now diagnostic laboratories?

Technology and Redundancy Considerations

Molecular Analysis in Newborn Screening
A Staged Approach



Ongoing in routine NBS

Experimental in NBS

Offered clinically and research outside NBS

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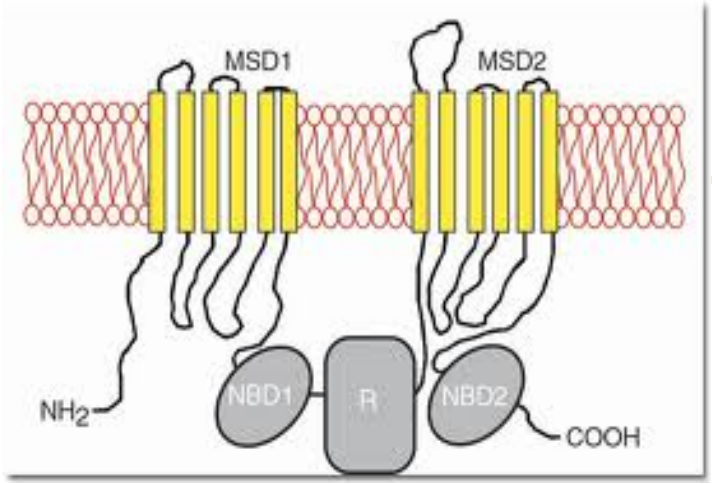
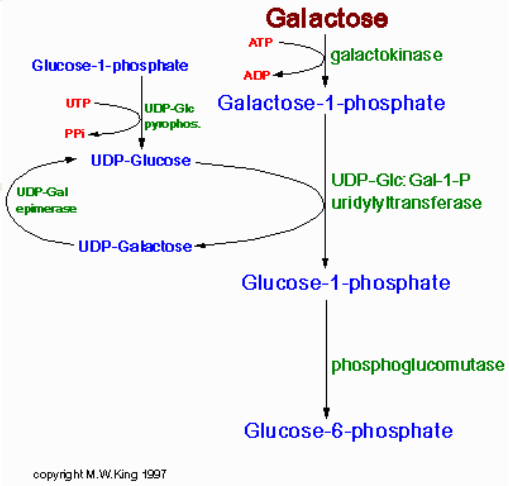
Targeted mutation panels – population-specific?

Cystic Fibrosis



CFTR2 panel of
disease causing mutations

Galactosemia



5-9 mutations
commonly tested

First Level

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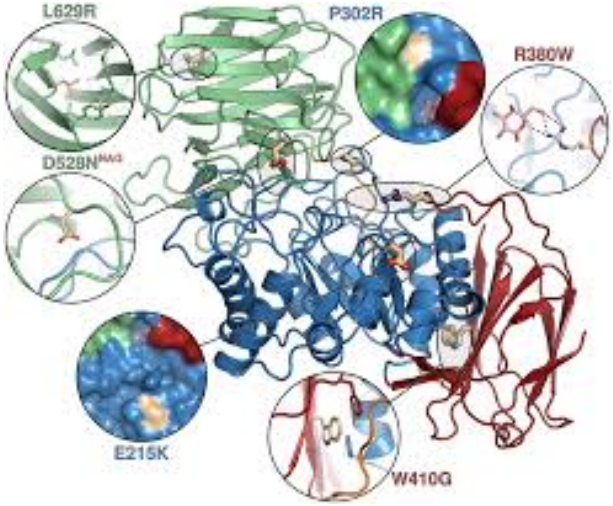
Entire coding sequence of an entire gene



KRABBE DISEASE

emergent results

- VOUS
- Phenotype predictions
- Timeliness
- 41.3% reduction in referrals

Other LSDs? -- pseudodeficiency

Second Level

Next Gen Sequencing and Cystic Fibrosis Newborn Screening

94% of referred CF screens are false positives in NYS

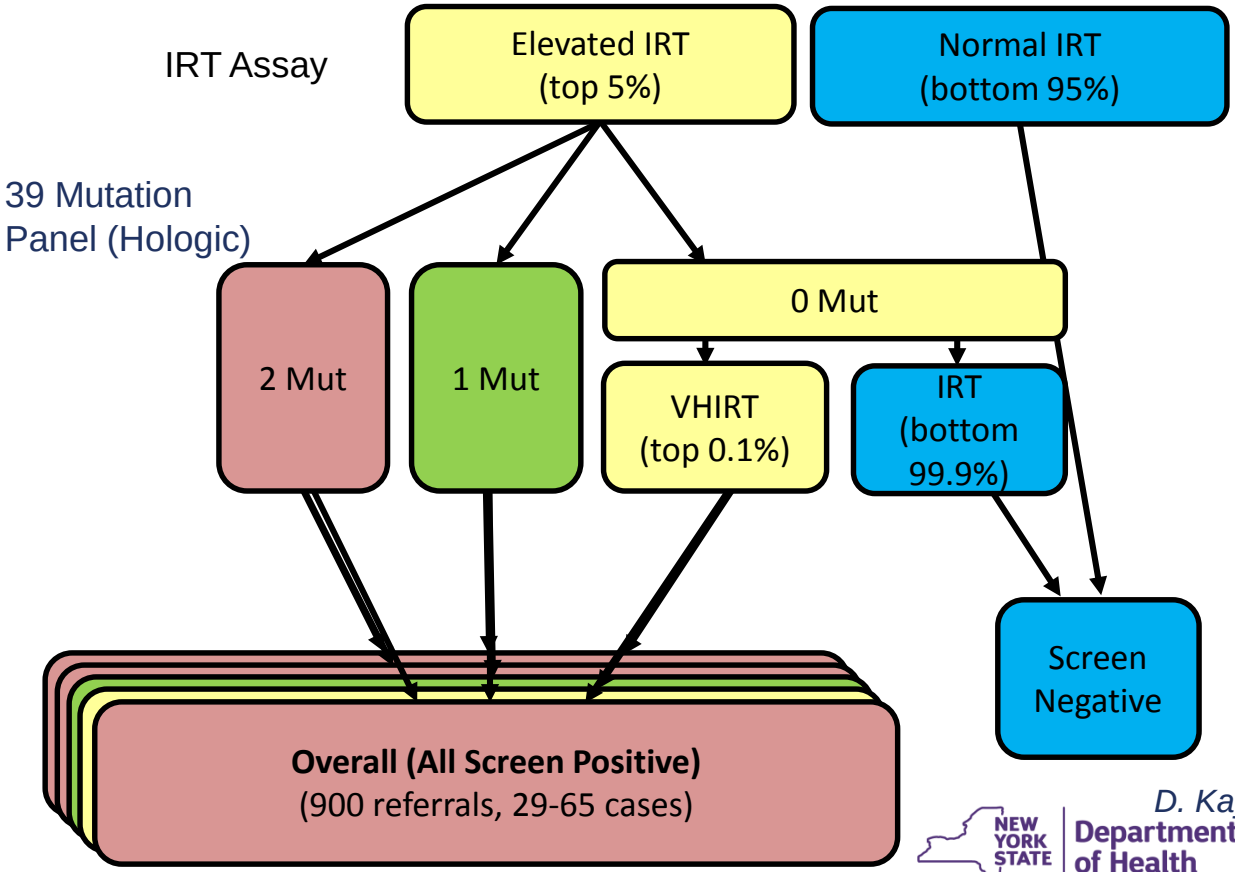
- Screen positive – ↑IRT and at least 1 CF causing mutation
  - Most assays detect a panel of mutations that cause CF
  - >2000 known mutations/variants in CFTR gene

- Not all CFTR mutations cause classic CF
  - Will identify CF related metabolic syndrome or unknown variants
  - Can limit sequence detection to known mutations but will miss cases?
  - How many missed cases can we live with?
  - Can't we do better?

Hughes EE et al., Hum Mutat, 37:201-208

Second Level

NYS CF Newborn Screening Algorithm (2010-2013)



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# Infants Referred	Hologic 39-Mut 79.8%	Illumina 139-Mut 86.6%	Illumina CSA+ 98.2%
350	2 MUT N=256	2 MUT N=300	2 MUT N=378
6,851	1 MUT N=114	1 MUT N=79	1 MUT N=14
6,341	VHIRT N=22	VHIRT N=13	VHIRT N=0

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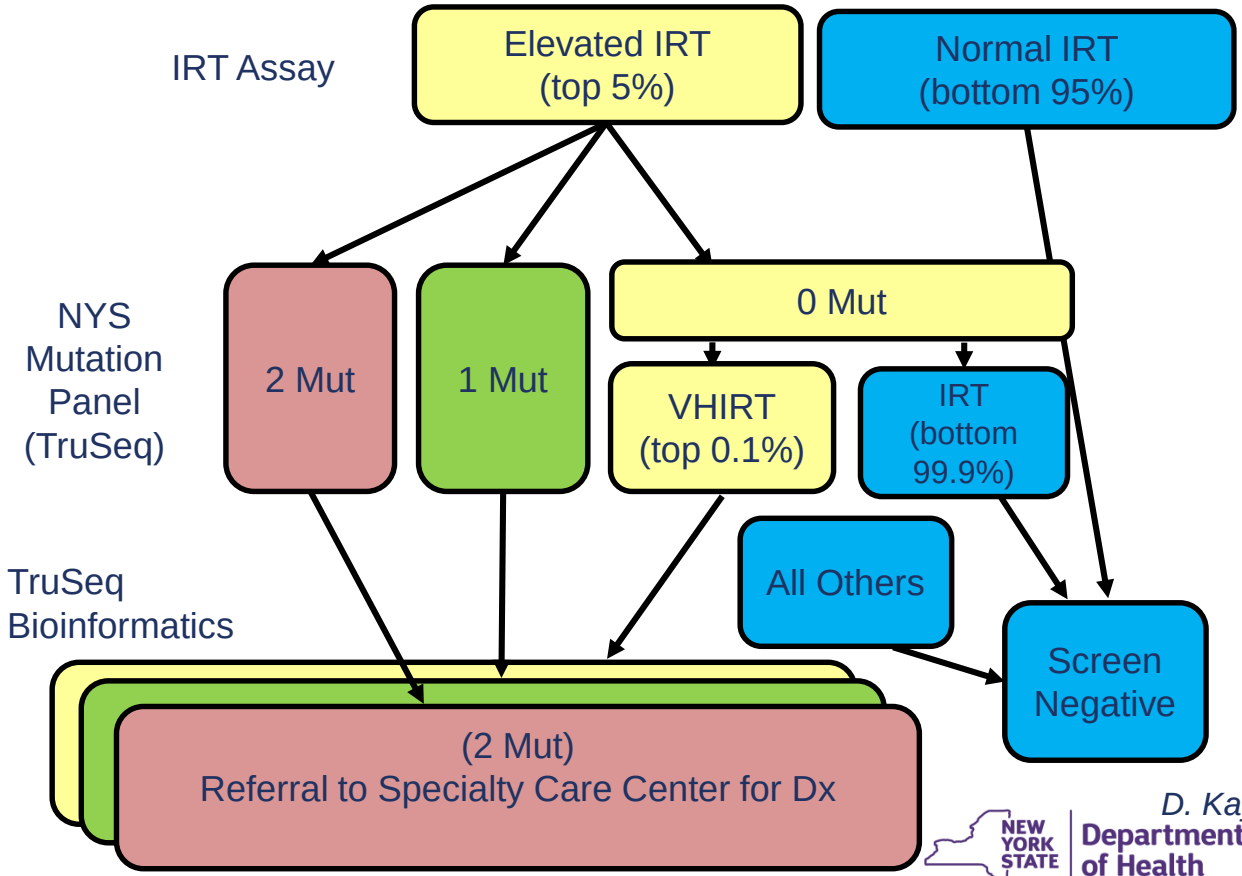
Hughes EE et al., Hum Mutat, 37:201-208

Variants Not Detected on Clinical Sequencing Assay

CF ID	Mut 1	Mut 2	Final Dx	Sweat Test (mmol/L)	Race/ Ethnicity	Explanation
30	4382delA	1949del84	CF	108	Caucasian	Supplemental Assay
52	R347P	CFTRdele17a-18	CF	89	Caucasian	Supplemental Assay
84	1482ins4	1811+1.6kbA>G	CF	85.9	Hispanic	Bioinformatics
98	3876delA	1811+1643 G>T	CF	78.1	Caucasian	Bioinformatics
102	1949del84	1949del84	CF	103	Hispanic	**Bioinformatics**
110	R347P	CFTRdele17a-18	CF	95	Caucasian	Supplemental Assay
115	1811+1643G>T	1811+1643 G>T	CF	112	Hispanic	**Bioinformatics**
130	1949del84	1949del84	CF	109	Hispanic	**Bioinformatics**
163	S549N	1949del84	CF	79	Hispanic	Supplemental Assay

NYS CF Newborn Screening Algorithm



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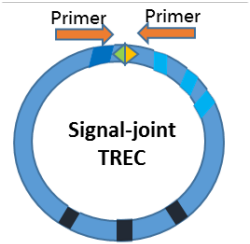
Next Gen Sequencing and SCID Newborn Screening

Issue: SCID is a spectrum of disorders that can only be differentiated by identifying causative mutations

- Many genes involved in SCID
- Immunologists can provide better care when SCID causative mutations are known quickly
- Screening labs can provide timely mutation analysis
- When public health provides mutational analysis, ensures health equality



S. Cordovado, Ph.D.

Third Level

Entire coding sequence of all known genes catalogued as disease-causing

Current NBS for severe combined immunodeficiency:

- **Measure T-cell receptor excision circles**
- **<125 TRECs constitutes a referral**
- **Immunologists order CBC, flow, mitogen studies**
- **Molecular tests order by candidacy, multi-gene panel(s), insurance issues, available labs**
- **Becomes iterative, slow, stressful process**

Third Level

Specific Aims

- Validate 2 platforms for 39-gene NGS immunodeficiency panel

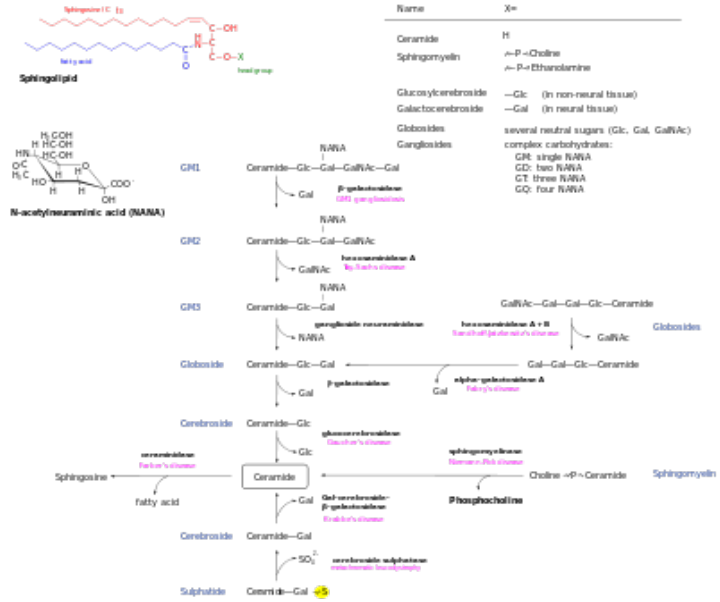
- Evaluate Next Gen Sequencing Utility and TAT
  - Shortened time to diagnosis?
  - Fewer visits to Specialist?
  - Earlier, targeted treatment?
  - Long-term follow-up

- Create and disseminate educational materials for parents and providers to state programs

Severe Combined Immunodeficiency 39 – Gene Panel

ADA	AK2	ATM	BLNK	BTK	CD3D	CD3E
CD3G	CD247	CD40LG	PTPRC	CHD7	CORO1A	DCLRE1C
DKC1	DOCK2	DOCK8	FOXN1	GATA2	IGHM	IL2RG
IL7R	JAK3	LIG4	MTHFD1	MTR	NHEJ1	NBN
PNP	PRKDC	RAC2	RAG1	RAG2	RMRP	SLC46A1
STAT5B	TBX1	WAS	ZAP70			

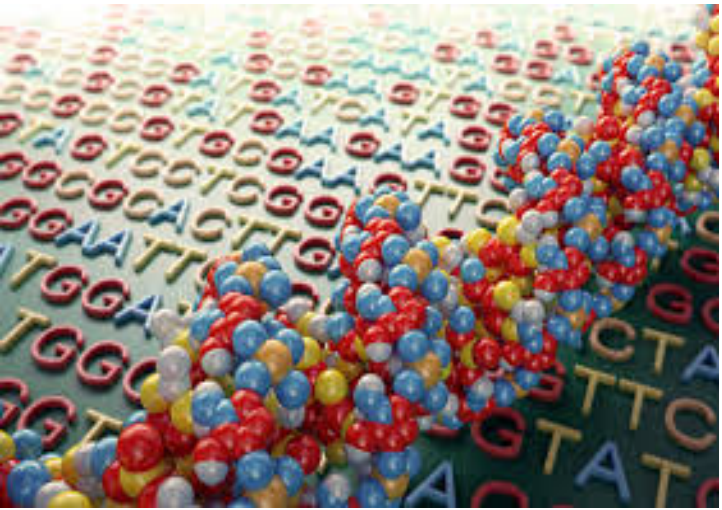
Entire coding sequence of all known genes in a given biochemical pathway



- **Modifiers**
- **Phenotype predictions**
- **Infantile, juvenile, late**

Third Level

Entire coding sequence of all known NBS genes



- Complete
- Only looking at NBS
- Can turn off analysis
- Easily modifiable
- Similar information
- Economy of scale
- Still ‘manageable’

- *Under consideration in NY*
- *Establishment of NBS core*

Fourth Level

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Whole exome or whole genome analyses

- Complete
- All disease / onset
- VOUS
- Screening v. diagnostic
- No phenotype yet
- Consent
- No longer ‘manageable’ currently

Fifth Level

Points to Consider

- Will we make it easier for families?
- Will we alleviate or increase burden?
- Variants of unknown significance
- Misclassified variants
- Screening programs become diagnostic
- Molecular diagnosis may not result in phenotype – patients in waiting
- Providers need education to relay information
- Availability of genetic counseling

We Can Do This Right

- Molecular subcommittee
- Expertise exists in NBS
- Community of collaboration
- Be smart about implementation
- Tools can help families
  - -- reduce # of referred
  - -- provide data for future
- Health care equality
- Information at time of referral

Acknowledgements

- Suzanne Cordovado, Ph.D.
- The late Mike Glass, M.S.
- Erin Hughes, M.L.S.
- Denise Kay, Ph.D.
- New York State Krabbe Consortium
- Immunology Specialty Care Center Directors
- CF Specialty Care Center Directors
- Applied Genomic Technologies Core [WC]
- Staff in the NBS DNA Laboratory
- Jill Taylor, Ph.D.

Thank You !!


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AATATGCTCTAT
CTAAGCATCCTCC
GTGCTATGAATC
INFANT'S