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Molecular Analysis to Enhance Newborn Screening……..

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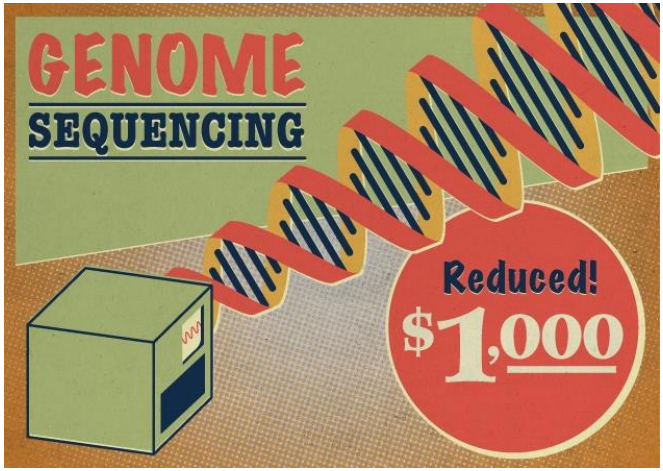
August 25, 2016

Population – Based Risk Assessment

Tests must be universally available and timely

The Declining Cost of Genome Sequencing



http://www.nature.com/news/technology-the-1-000-genome-1.14901

HiSeq X Ten Next Gen Sequencer

- **Produces 16 human genomes in 3 days at 30x coverage**
- **Projected costs per genome**
  - Reagents $797
  - Machine depreciation $137
  - Technician $55–65
- **Does not include overhead, infrastructure and analysis costs**
- **Instrument cost $10 Million USD**

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??

Where Are We Currently??

- **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

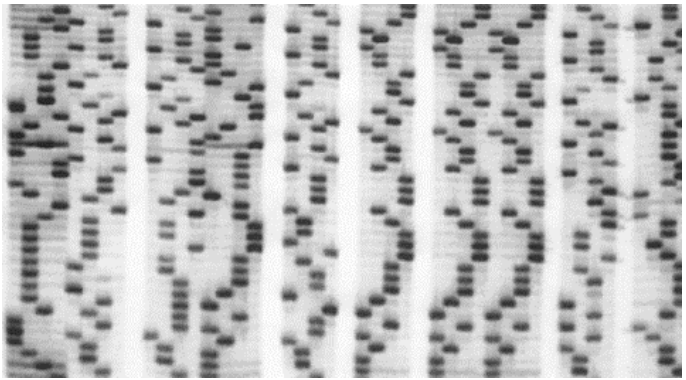
In 2015, 23 countries participated in CDC PT

What Must We Consider??

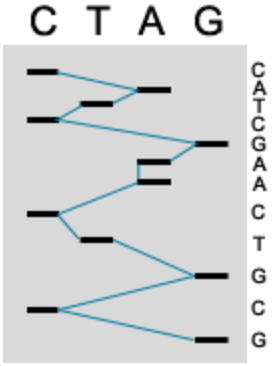
- Cost
- Value added?
- Impact on TAT; timeliness big concern
- Staff time and qualifications
- Bioinformatics needs
- Instrumentation requirements
- Practical issues
- Are we now diagnostic laboratories?

DNA Sequencing
1975 to 2015 and Beyond

Radioactive Sanger Sequencing

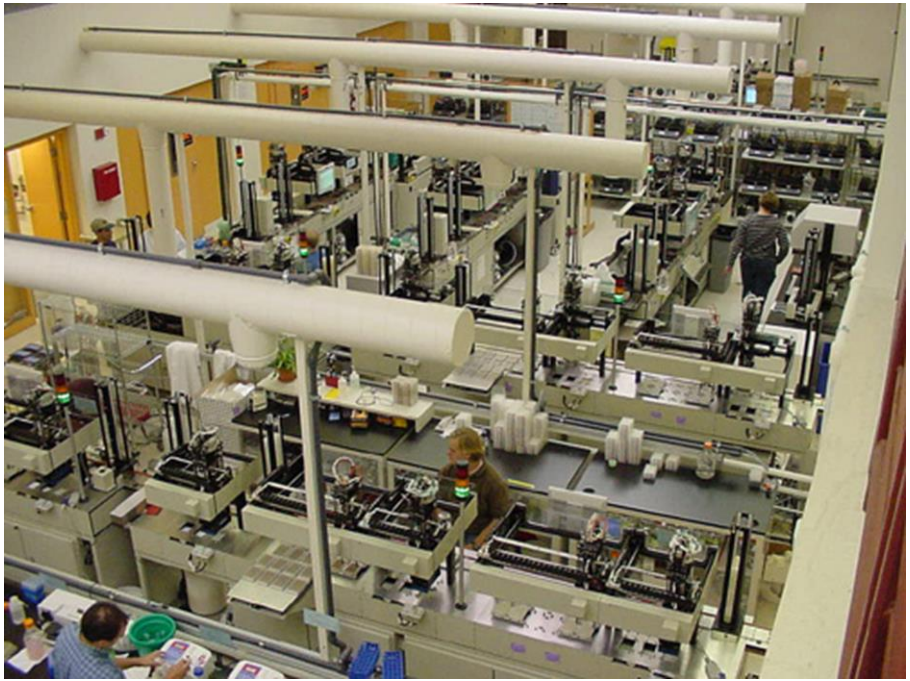


http://molecularstaging.aussieblogs.com.au/category/dna/



http://www.uvm.edu/~cgep/Education/Sequence.html

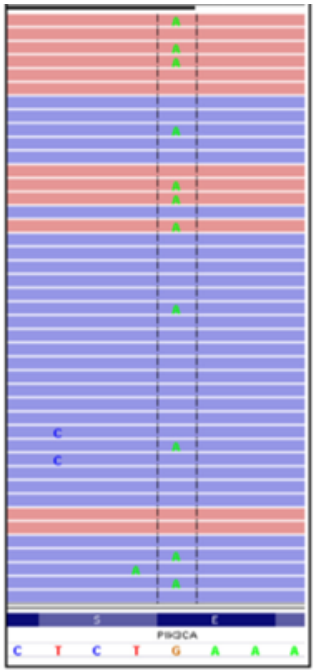
DNA Sequencing
1975 to 2015 and Beyond



The Broad Institute of MIT and Harvard large-scale Sanger DNA sequencing center

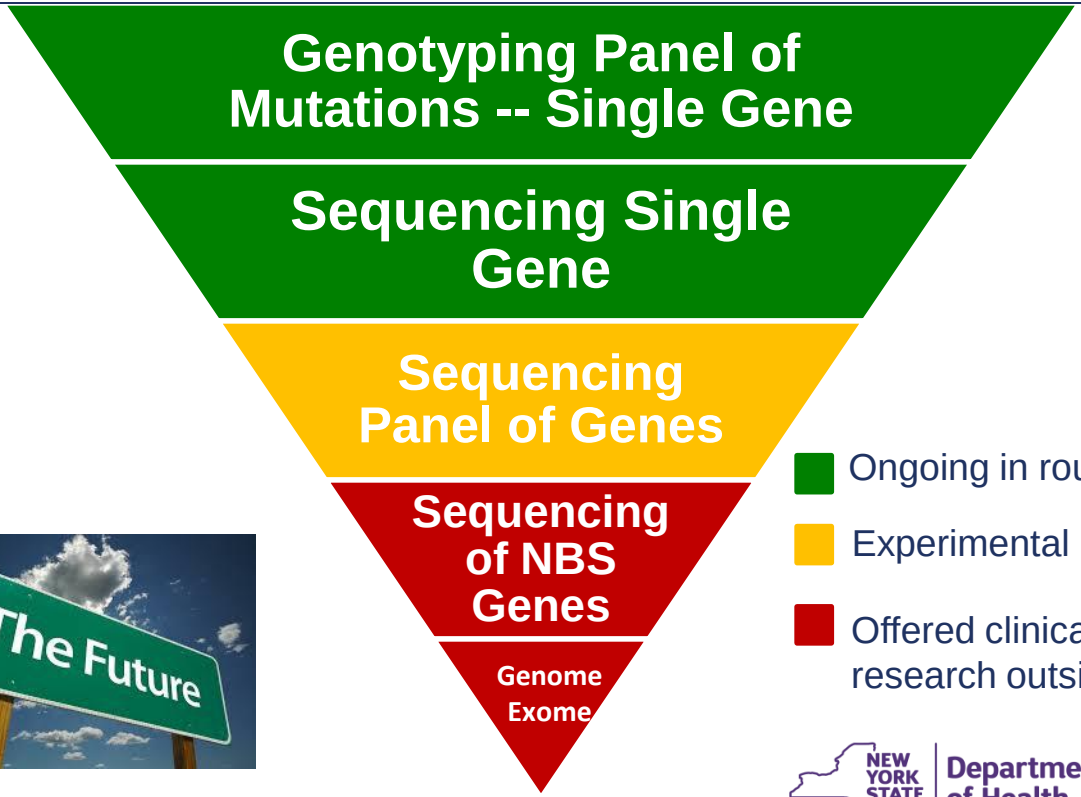
DNA Sequencing
1975 to 2015 and Beyond

Next G encing



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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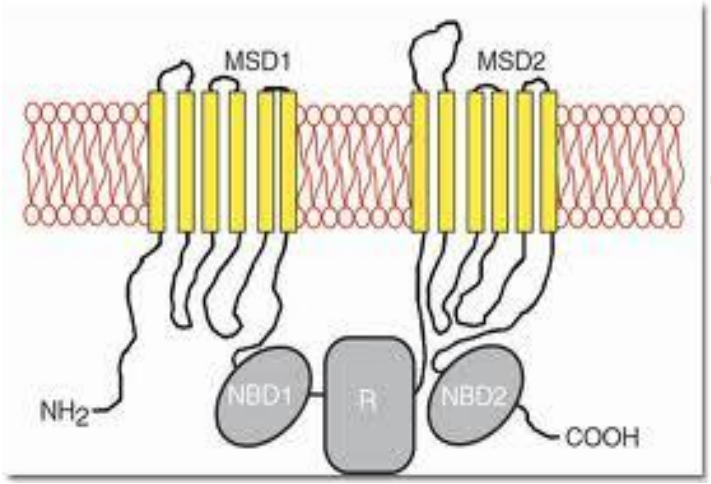
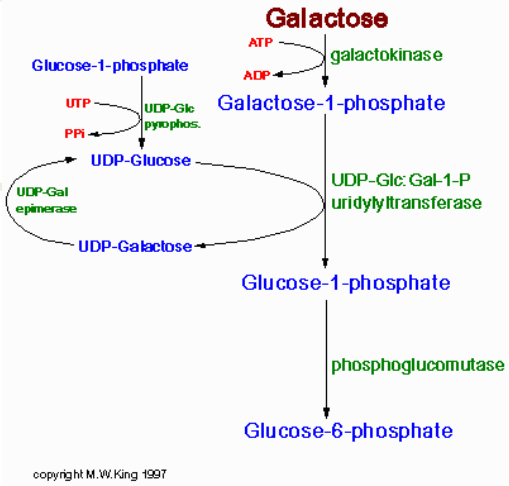
Targeted mutation panels – population-specific?

Cystic Fibrosis



CFTR2 panel of
disease causing mutations

Galactosemia



5-9 mutations
commonly tested

First Level

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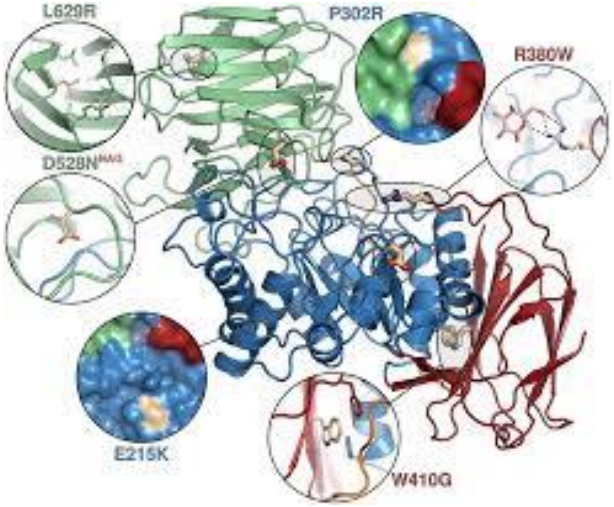
Example: Increasing Specificity – DNA Sequence Analysis Without A Loss of Timeliness



KRABBE DISEASE

emergent results

- Biochemistry first
- Molecular second
- Phenotype predictions
- 41.3% reduction in referrals

Familial anxiety decreases with increased specificity

Second Level

Challenges of Sequencing – 1 Gene, Several Genes, or Genome/Exome

- **Major Challenge: Determining whether any given variant is pathogenic**
- **ACMG determined 5 categories to classify variants:**
  - **Known pathogenic**
  - ***Likely to be pathogenic***
  - ***Unknown significance***
  - ***Likely to be benign***
  - **Benign**

- **Knowledge accruing daily, however the medical impact of most variants is unknown**

Example: Increasing Specificity – DNA Sequence Analysis With A Loss of Timeliness

Issue: Most referrals for cystic fibrosis don't have disease – high rate of false positive results

Screen positive – ↑Immunoreactive trypsin (IRT) and at least 1 CF causing mutation

- Most assays detect a panel of 39-100+ mutations that cause CF
- >2000 known mutations/variants in CFTR gene

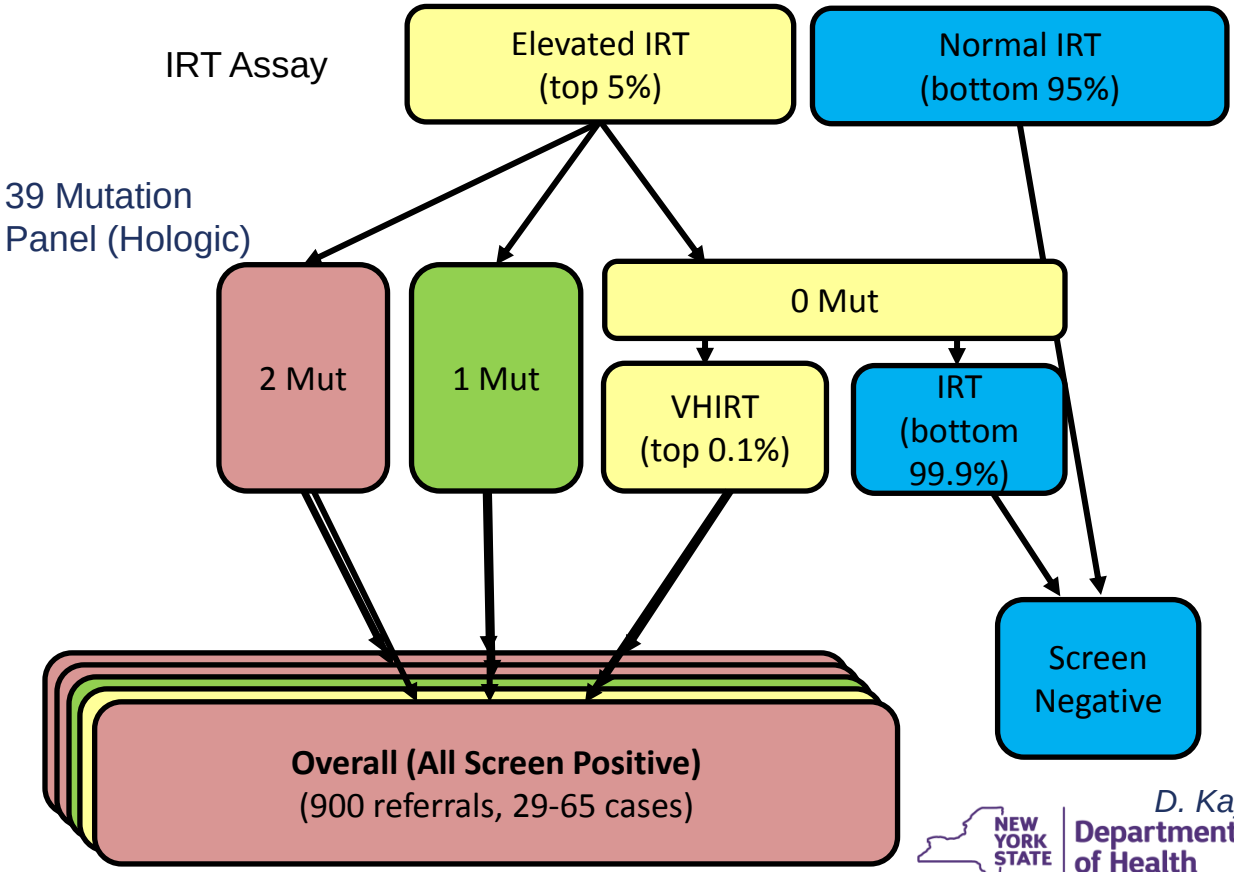
… And not all CFTR mutations cause classic CF

- Will identify CF related metabolic syndrome (CRMS) or unknown variants

Hughes EE et al., Hum Mutat, (2016), 37:201-208.
S. Cordovado, Ph.D.

Second Level

NYS CF Newborn Screening Algorithm (2010-2013)



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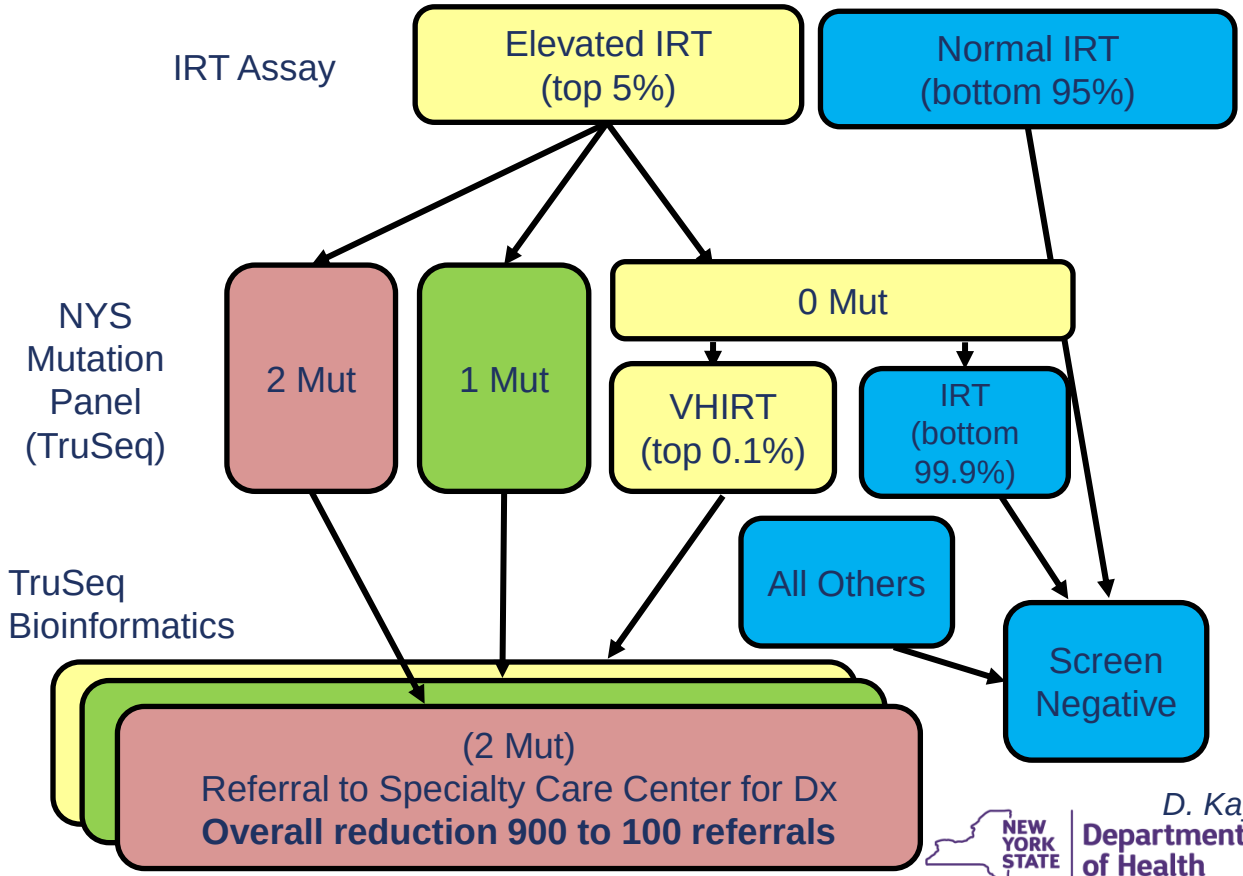
# Infants Referred	Hologic 39-Mut 79.8%	Illumina 139-Mut 86.6%	Illumina CSA+ 98.2%
350	2 MUT N=256	2 MUT N=300	2 MUT N=378
6,851	1 MUT N=114	1 MUT N=79	1 MUT N=14
6,341	VHIRT N=22	VHIRT N=13	VHIRT N=0

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

NYS CF Newborn Screening Algorithm



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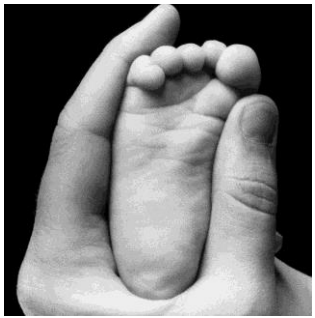
Cystic Fibrosis Newborn Screening Summary

NY Annual birth rate: ~250,000

1st tier — Babies in upper 5% IRT: ~12,500

2nd tier — Babies with 1 or 2 *CFTR* muts or VHIRT: ~900

3rd tier — Babies with 2 *CFTR* muts: ~100



⇦ Only these babies are sent for diagnostic evaluation and testing

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Increased Turnaround Time
89% Decrease in Referrals

- Accessioning (1)
- IRT test (1)
- Abnormal (2)
- Repeat IRT test (2)
- Extract DNA (2)
- 39-mutation screen (3)
- Extract fresh punch (3)
- 39-mutation screen (3)
- Enter results (4)
- Mailer (5)

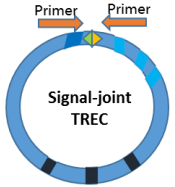
- Accessioning (1)
- IRT test (1)
- Abnormal (2)
- Repeat IRT test (2)
- Extract DNA (2)
- 39-mutation screen (3)
- Extract fresh punch (3)
- Next Gen (3-5)
- Sanger / Suppl (5-6)
- Enter results (6)
- Mailer (7)*

*These times don't account for any batching

Next Gen Sequencing and SCID Newborn Screening – Post-analytic to Analytic?

Severe Combined Immunodeficiency (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; now done post-analytically
- Screening labs can provide timely mutation analysis
- When public health provides mutation analysis, health care quality ensured



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Third Level

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Moving DNA Analysis to the Analytic Phase – Impact on Timeliness; Improved Clinical Sensitivity

Current NBS for severe combined immunodeficiency:

- **Measure T-cell receptor excision circles (TRECs)**
- **<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**

Specific Aims

- Validate 2 platforms for 39-gene NGS immunodeficiency panel

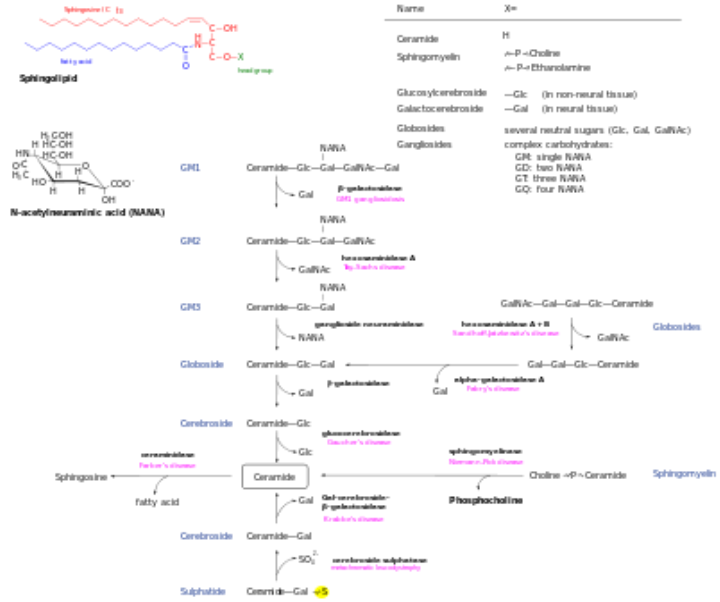
- Evaluate Next Gen Sequencing Utility and TAT
  - Shortened time to diagnosis?
  - Fewer visits to Specialist?
  - Earlier, targeted treatment?
  - Long-term follow-up

- 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			

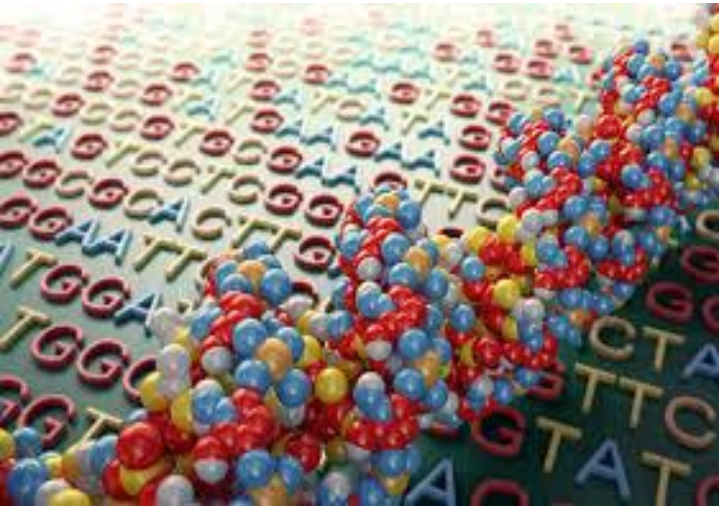
Entire coding sequence of all known genes in a given biochemical pathway



- **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

NBS Molecular Subcommittee

MISSION

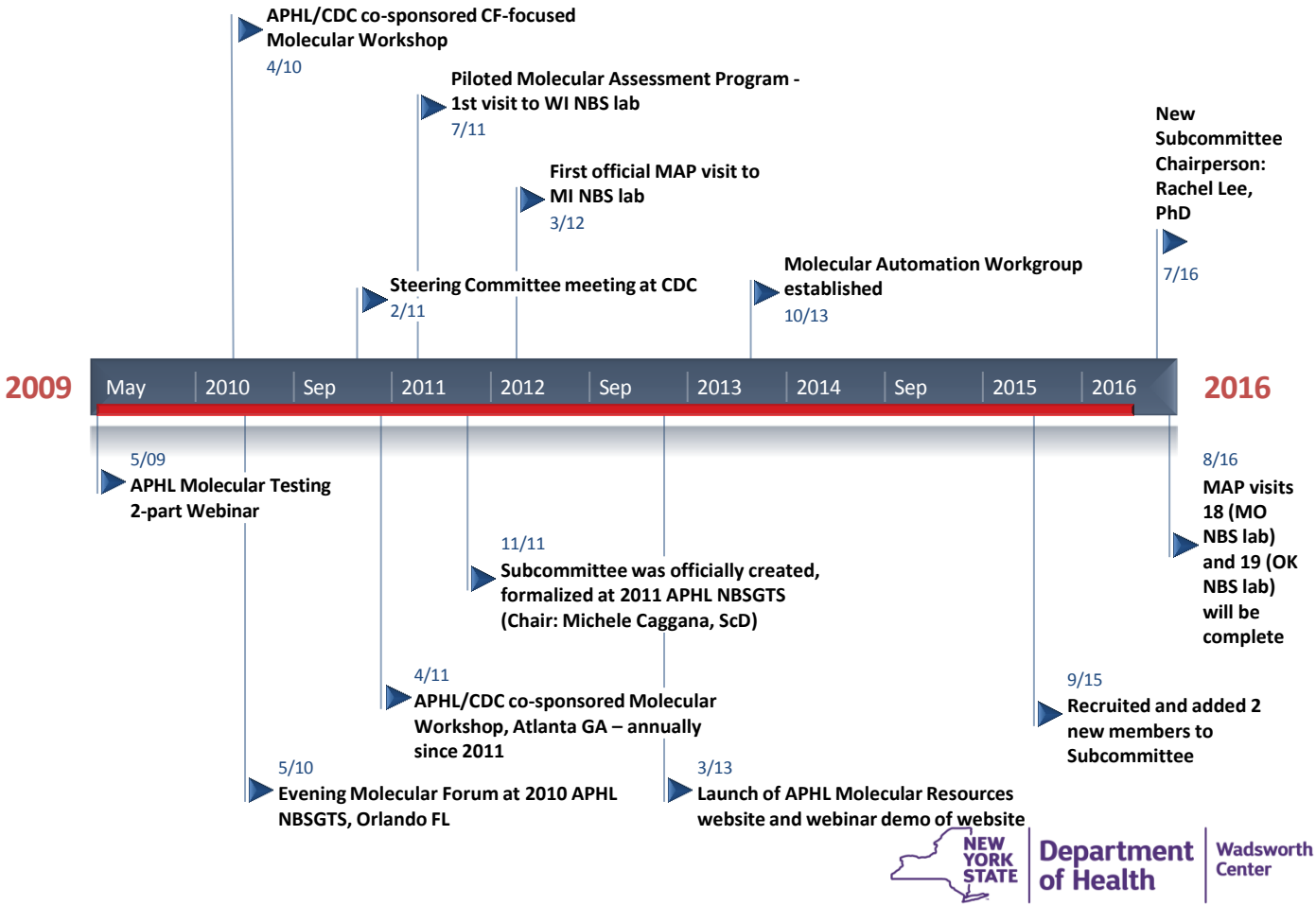
- The mission of the subcommittee is to ensure continuity and responsible growth of emerging molecular technologies within the newborn screening public health environment.

- WA, MI, CA, NY, MN, IA, WI, TX, MA, PR, CA(2)

NBS Molecular Subcommittee

OBJECTIVES:

- To facilitate a collaborative environment for transfer of knowledge about emerging technology among newborn screening laboratories.
- To provide input to CDC's Molecular Quality Improvement Program (MQIP) on procedures, policies and activities for molecular testing.
- To provide input to state newborn screening public health laboratories on procedures, policies and activities for molecular screening.
- To serve as a communications conduit between MQIP and newborn screening systems.
- To assist laboratories in improving newborn disorder detection sensitivity and specificity with molecular testing.
- To collaborate with newborn screening laboratories to anticipate future molecular assays and needs
- To serve as a liaison to organizations, programs and activities in order to address issues concerning molecular testing in newborn screening.



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NBS Molecular Subcommittee

- Molecular Quality Improvement Program
- NBS Molecular Workshops
- Molecular Assessment Program
- Molecular Resources Website
- Paradigm for NBS Molecular Pilots
- Presentations to the Community

Molecular Subcommittee Meeting

- **Meeting Objective:**
  - Discuss current status of gene sequencing in NBS
  - Discuss laboratory and follow-up needs, barriers and solutions
  - Provide state experience in implementation and practice
- **Target Audience – NBS Lab and Follow-up Managers**
- **Planned for first quarter 2017**
- **laura.russell@aphl.org or snc4@cdc.gov for more information**

Molecular Survey

- **Goals:**
  - To assess the status of molecular testing in US NBS laboratories currently and in the near future
  - To identify states actively or planning to use sequencing for certain disorders and to identify the platforms used or under consideration

Contact laura.russell@aphl.org or snc4@cdc.gov for more information

Acknowledgements

- Suzanne Cordovado, Ph.D.
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- CF Specialty Care Center Directors
- Applied Genomic Technologies Core [WC]
- Staff in the NBS DNA Laboratory
- Jill Taylor, Ph.D.

APHL

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Thank You !!


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