New Jersey Department of Health New Disorders Webinar on Implementation of MPS1 and Pompe Lysosomal Storage Disease Newborn Screening

PRESENTED BY:

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MPS-1 and Pompe Phase 1 of Implementation

- Governor Christie signed legislation
  - **Emma's Law** (1/6/2012)- Pompe (Krabbe, Gaucher, Niemann-Pick A/B, Fabry)
  - **Let Them Be Little** (9/10/2014)- MPSI (MPSII)
- New Budgets- Christie allocated 1.6 million(July 2013) for new instrumentation and staff
- Lab upgrade- Sept. 2015- May 2016
  - More hoods, converted to house Nitrogen system, new Argon System
- 3500XL Genetic Analyzer for second tier sequencing was acquired June 2016
- Waters TQDs installed July 2016 and ancillary equipment ordered-received Feb. 2017 for biochemical analysis
- New staff hired July and Sept. 2016
- Reagents received October 2016
- Fee Increase- Proposed July 2016, implemented April 2017

MPS-1 and Pompe Phase 2 of Implementation

- Initial Screening using FIA-MS/MS and PE 6 Plex
  - Optimization of Instruments -December 2016-May 2017
  - Challenges:
    - Incorrect ancillary instrumentation
    - Buffer contamination
    - TQD issues
  - Validation and pilot study was initiated on August 2017 and completed on December 2017.
  - Problems/Challenges encountered during the validation phase:
    - Daily flow
    - Equipment choices
    - Setting cutoffs

MPS-1 and Pompe Phase 2 of Implementation

- Problems/Challenges encountered during the validation and pilot phase:
  - **Daily flow**
    - Too much time to process on heavy sample days
    - Purchased another Apricot Pipettor
  - **Equipment choices**
    - 1 Apricot PP5+1
    - 1 Apricot S2 – due to space issues
  - **Setting cutoffs**
    - Minimal amount of true positive data
    - Communicating with other states (TN, MO, NY, MN) and consultant group

Lessons Learned

- Evaluate how testing will be incorporated into current work flow specifically regarding timing, sample volume, and staffing
  - Lab space
  - Instrumentation
- Coordinating staffing
  - Time of hiring, amount of people, include communication with Follow up
- Communication with other labs
  - Validating protocols, assay set up and monitoring, cutoffs

Current Status for Full Implementation

- Final stages
  - LIMS setup
  - Training new staff
  - Follow up readiness

- Full implementation is dependent on staffing

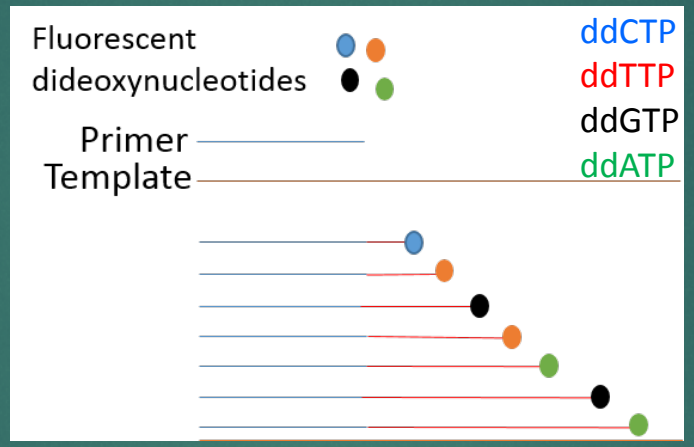
Sequencing as a second-tier assay for Pompe and MPS1 – WHY??

- Metabolic geneticists in NJ felt having sequencing part of NBS was critical
- Both disorders are known to have pseudodeficiency variants that can cause low biochemical results, but child will not have the disease
- Helps the physicians triage patient evaluation
- Eliminates weeks-long waiting time for sequencing results for diagnosis and treatment that must be started immediately
- Provides equitable healthcare for all newborns in NJ

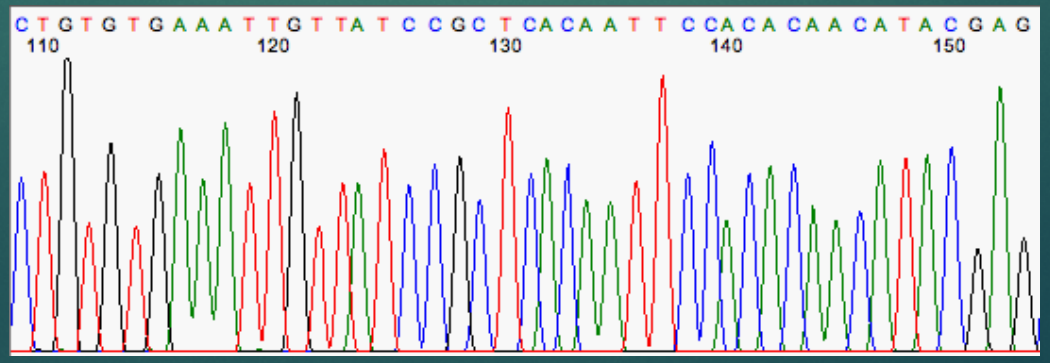
Sanger sequencing determines the genetic code of the entire gene of interest, typically all exons and exon/intron boundaries. Assay takes 1.5 days, detects 95+% of potentially deleterious genetic variants.

1. DNA extracted from dried blood spots undergoes PCR to amplify all gene regions to be sequenced.

2. A second round of PCR incorporates fluorescently labeled DNA building blocks into the regions of interest.

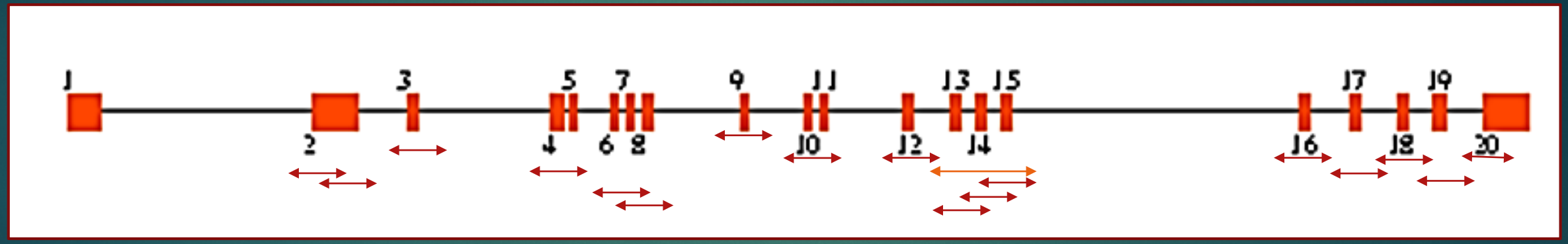


3. Products are separated by size; fluorescent tags detected by laser. Chromatogram output analyzed by software.



GAA: Pompe Sequencing

- Acid alpha glucosidase (GAA)
- DNA: 18.4 kB
- mRNA: 3847 bp, CDS: 2859
- 20 exons
- 17 PCR amplicons
- Exon 1: Not part of CDS; therefore, not sequenced
- Majority of Exon 20 non-coding



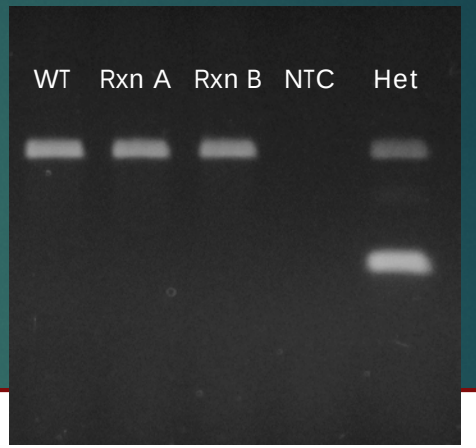
↔ = approximate PCR coverage

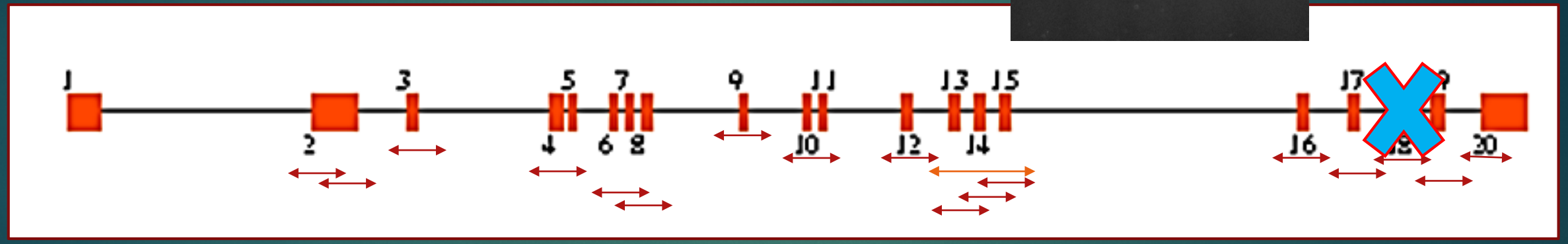
Sequence Analysis performed on:

- All coding exon sequences
- 10 intronic base pairs before and after each exon to detect potential splicing changes

GAA: Pompe Deletion

- Deletion of Exon 18





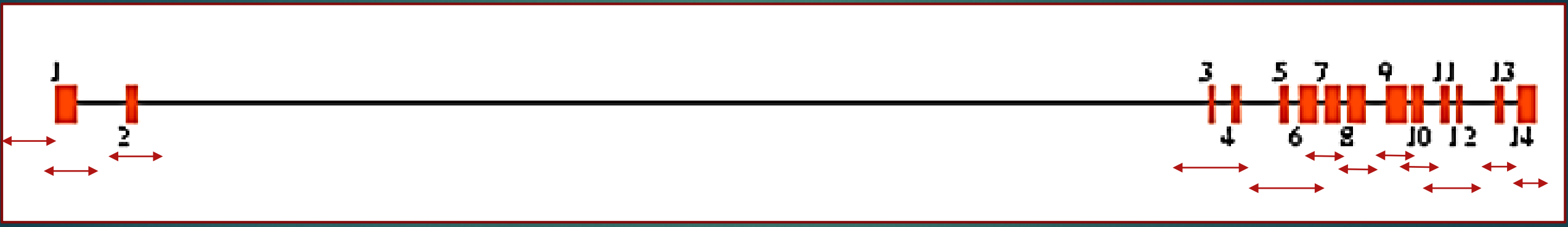
= approximate PCR coverage

Deletion Analysis performed on:

- Exon 18
- Any other large deletion/insertions will not be detected

IDUA: MPS I Sequencing

- Alpha- L- iduronidase
- DNA: 17.5 kB
- mRNA: 2155 bp
- 14 exons + Promoter (~450 bp)
- 12 PCR amplicons
- No known common large deletions

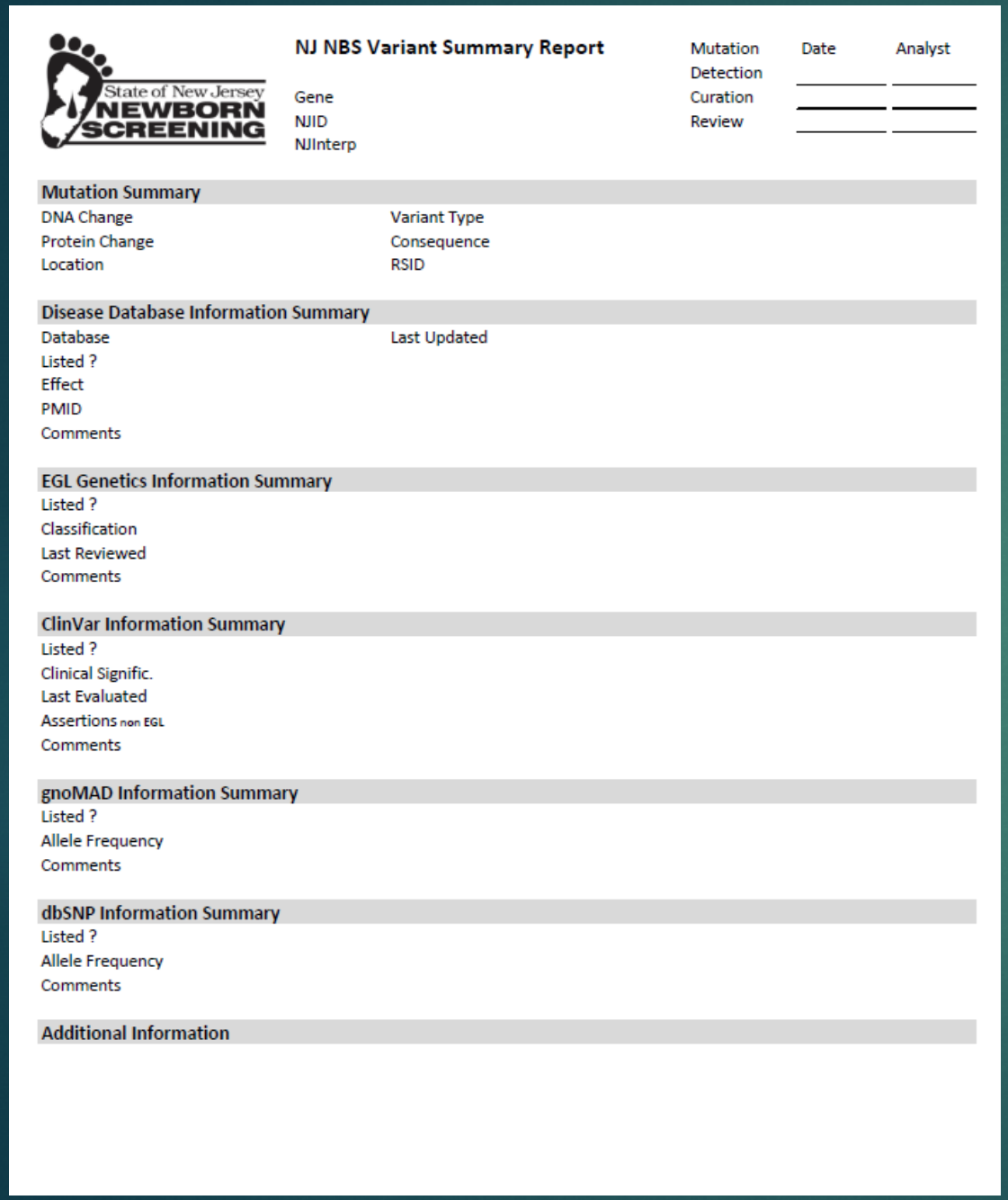


Sequence Analysis performed on:

- All coding exon sequences
- 10 intronic base pairs before and after each exon to detect potential splicing changes

Current Status

- Instrumentation and reagents acquired
- Staff trained
- SOPs written
- Validations completed – 20 specimens run for each disorder with an assortment of mutations and locations
- In the process of LIMS configuration
- Currently developing variant interpretation workflow



State of New Jersey NEWBORN SCREENING

NJ NBS Variant Summary Report

Gene
NJID
NJInterp

	Mutation Detection	Curation	Review
Date			
Analyst			

Mutation Summary

DNA Change	Variant Type
Protein Change	Consequence
Location	RSID

Disease Database Information Summary

Database	Last Updated
Listed ?	
Effect	
PMID	
Comments	

EGL Genetics Information Summary

Listed ?
Classification
Last Reviewed
Comments

ClinVar Information Summary

Listed ?
Clinical Signific.
Last Evaluated
Assertions non EGL
Comments

gnoMAD Information Summary

Listed ?
Allele Frequency
Comments

dbSNP Information Summary

Listed ?
Allele Frequency
Comments

Additional Information

Workflow in Progress

- Summary of DNA and protein changes
- Utilizing ACMG guidelines for interpretation
  - Searches of publicly available databases including disease-specific databases, EGL Genetics, ClinVar, gnoMAD for frequency
  - Pubmed searches for published literature to evaluate clinical and functional studies
  - Looking to add computational/predictive component
- Will be reporting all variants, interpretation will begin with well-characterized variants, with more to follow

Lessons Learned

- Different genes behave differently – be open to different methods for different genes!
  - We needed to use different DNA extraction methods for GAA and IDUA
- LIMS configuration takes longer than expected
- Getting the lab test running is the "easy" part – it's development of the variant interpretation workflow that is new and challenging
  - Make sure to devote enough time to variant interpretation. This sort of data analysis is new to NBS