Assessing Laboratory Performance for Next Generation Sequencing Based Detection of Germline Variants through Proficiency Testing

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ARUP Laboratories Quarterly Webinar

September 27, 2018

Learning Objectives

- Explain the rationale for using a method-based PT approach for NGS testing for germline variants
- Describe analytical and annotation results evaluated in method-based PT for germline variants
- Relate trends in laboratory performance in method-based PT for germline variants
- Discuss how in silico PT increases options for assessment of laboratories performing NGS including complex scenarios presented by exome sequencing

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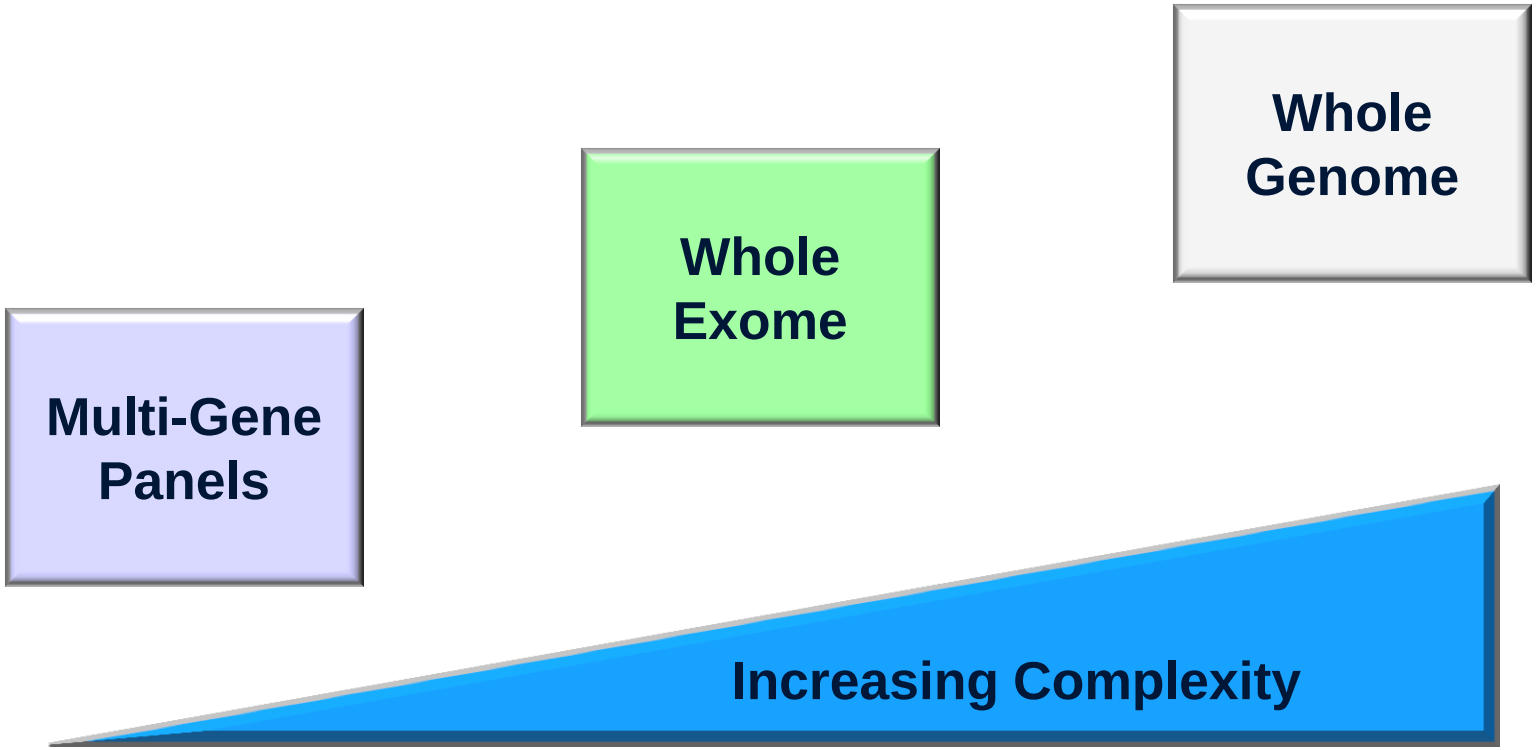
Current Diversity of NGS Germline Diagnostic Testing



Developing NGS PT for Germline Variants

Challenge: How to Create a Proficiency Testing Program To Assess Multi-Gene Panels to Exomes/Genomes for Germline Variants

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PERSPECTIVES

Methods-Based Proficiency Testing in Molecular Genetic Pathology

Iris Schrijver,*† Nazneen Aziz,‡ Lawrence J. Jennings,§¶ Carolyn Sue Richards,‖ Karl V. Voelkerding,**†† and Karen E. Weck‡‡§§

Concept of Methods Based PT for NGS

Assess the Laboratory's Ability to Accurately Detect Germline Variants In a Diversity of Genes

Reflects Primary Analytical Goal of Germline Panel, Exome and Genome NGS Testing

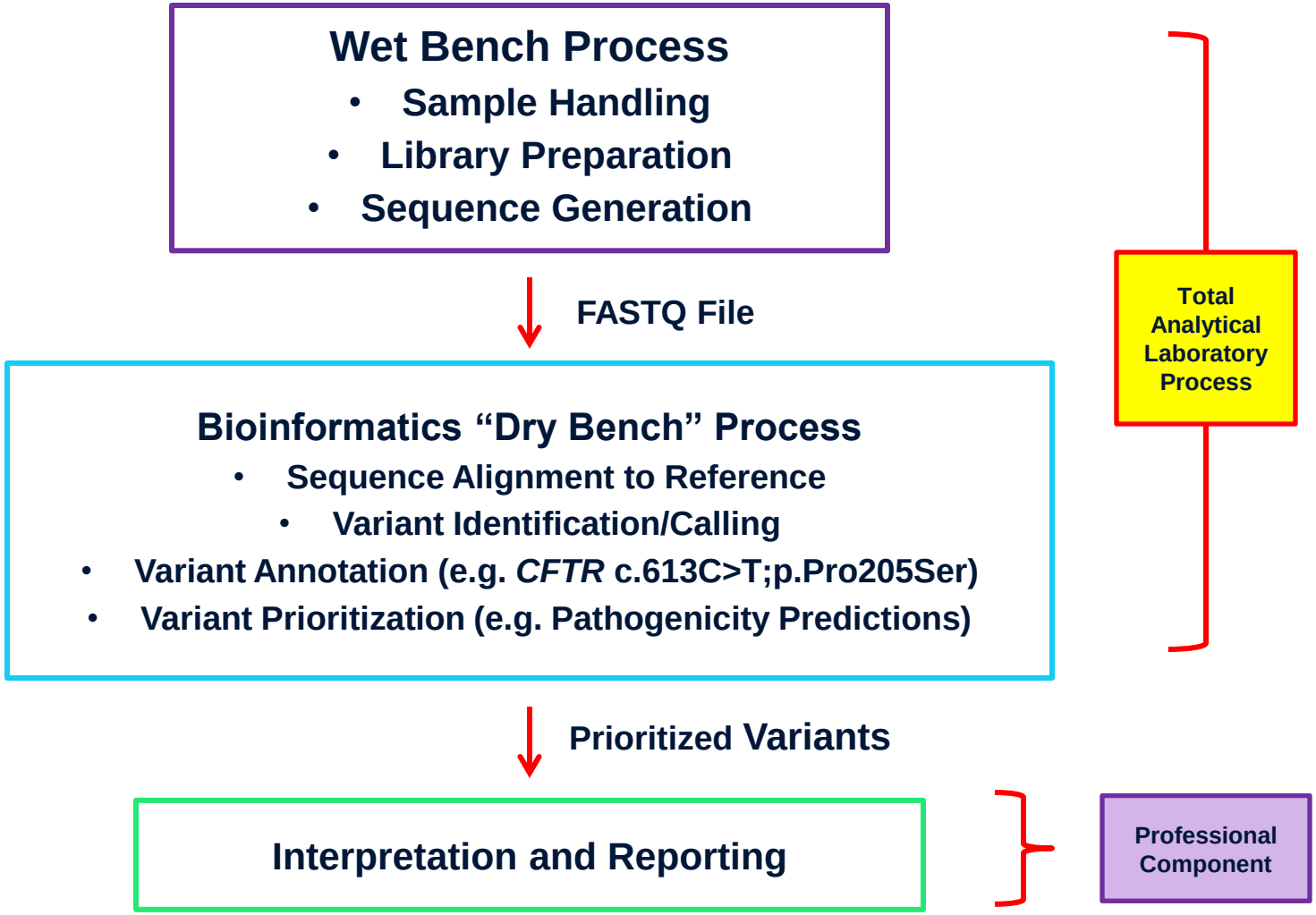
- Auto-Inflammatory Syndromes
- Autism
- Cardiomyopathies
- Congenital Hearing Loss
- Glycosylation Disorders
- Hereditary Predisposition to Cancer
- Immune Deficiencies
- Intellectual Disability
- Mitochondrial Disorders
- Neuropathies
- Renal Disorders
- Retinopathies

NGS Panels
10-100s
Genes Tested

Concept of Methods Based PT for NGS

What is the NGS Analytical Process That Needs to be Assessed?

CAP Definition of a NGS Test



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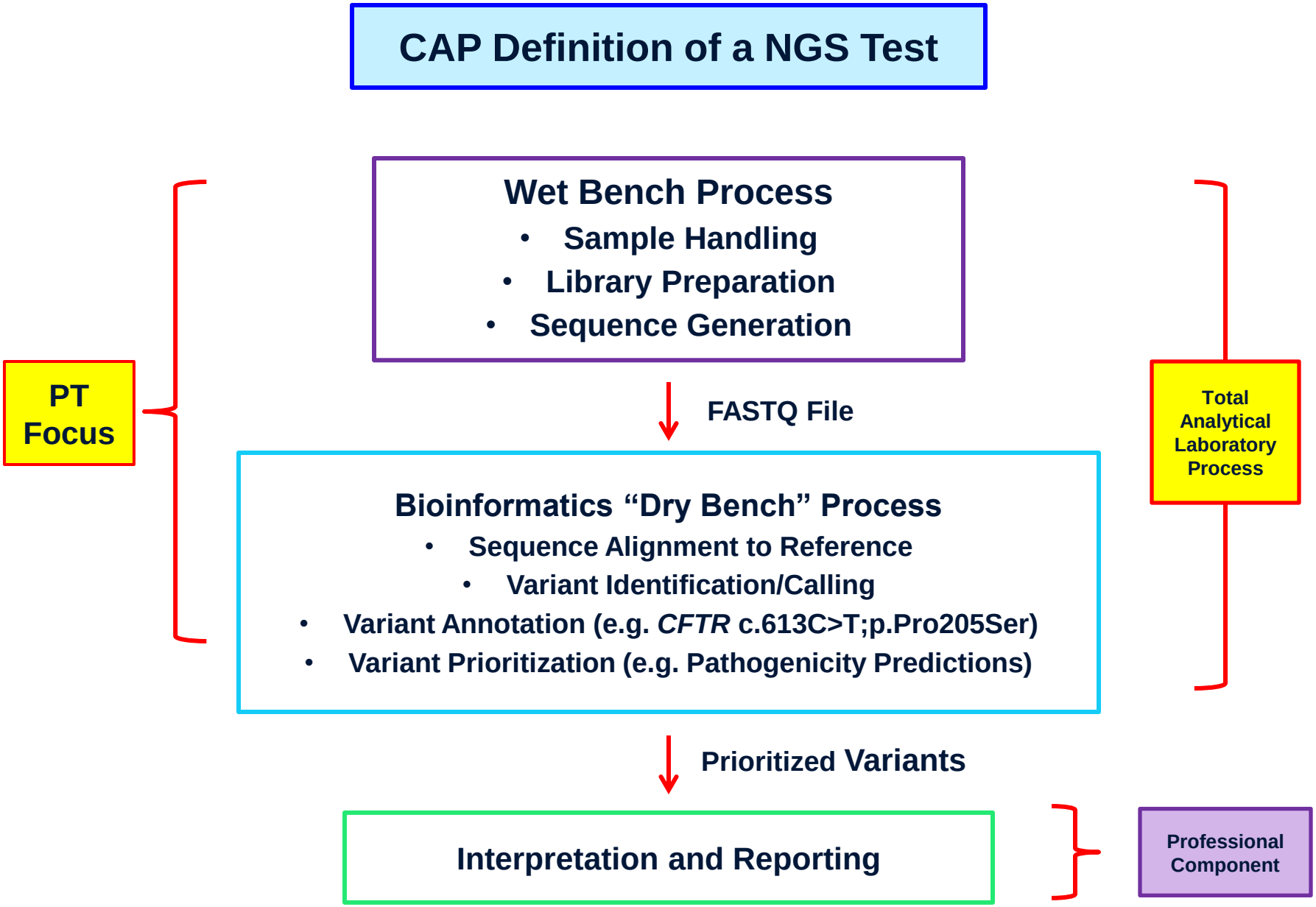

Development of NGS Methods Based PT Germline Variants

Source CAP Genome Cell Line Sequence With Multiple Technologies

Generate Consensus Variants

Select Germline Variants For PT

Conduct Pilot PT Volunteer Laboratories

Conduct 2015 Educational PT A and B Mailings

Launch 2016 PT A and B Mailings

Learning Objectives

- Explain the rationale for using a method-based PT approach for NGS testing for germline variants
- **Describe analytical and annotation results evaluated in method-based PT for germline variants**
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CAP Catalogue NGS Germline PT

Next-Generation Sequencing

Analytes/procedures in **bold** type are regulated for proficiency testing by the Centers for Medicare & Medicaid Services (CMS).

Next-Generation Sequencing NGS		
Procedure	**Program Code**	**Challenges/Shipment**
	NGS	
Next-generation sequencing	▮	1

Additional Information

Laboratories will have the ability to test up to 200 preselected chromosomal positions within various genes; for a full list of genes in this program, please go to cap.org. Under the Laboratory Improvement tab, click on Catalog and Ordering Information. The list is located under the PT Order Supplements header.

Program Information

- One 10.0-µg extracted DNA specimen
- Methods-based challenge for germline variants for laboratories using gene panels, exome, and whole genome sequencing
- Results for this program must be submitted online through e-LAB Solutions Suite
- Two shipments per year

Labs Can Analyze Up To 200 Chromosomal Positions Chosen in Genes Involved in Inherited Disorders Included Reference (Wild Type) Positions, SNVs and Indels

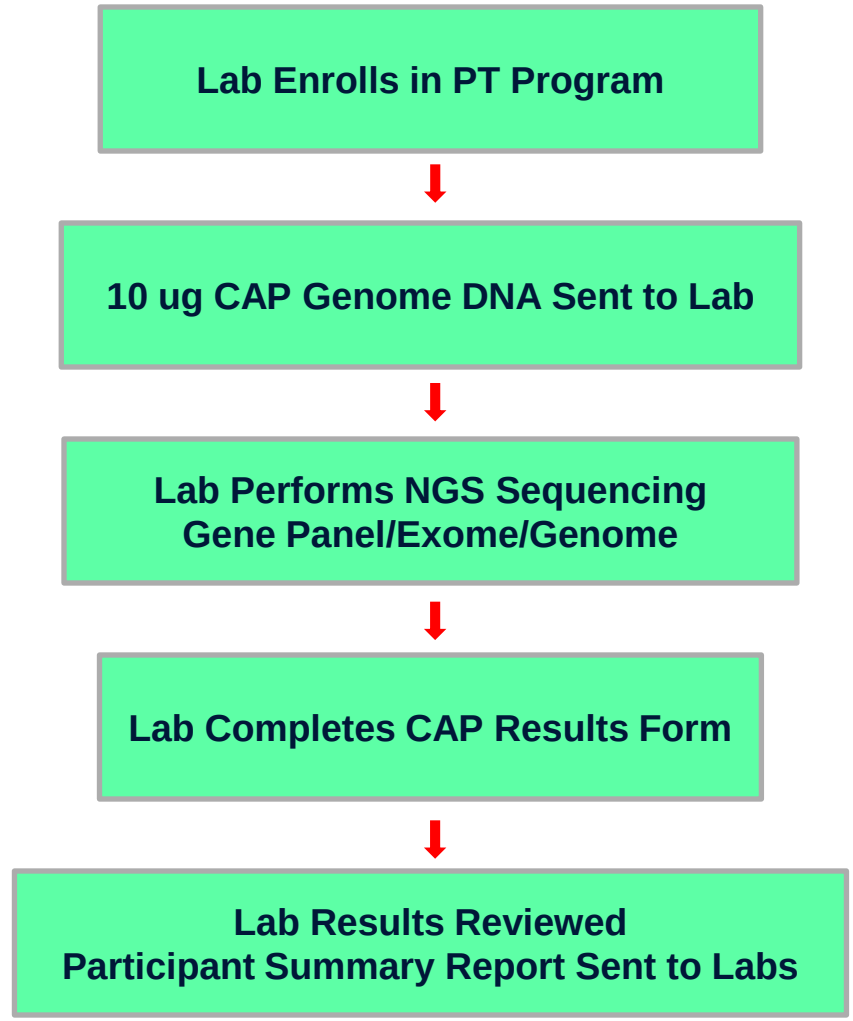
200 Chromosomal Positions Chosen for PT from CAP Genome Located in Disease Relevant Genes – Listed in CAP Catalogue

Disease areas
Autism
Arrhythmogenic disorders
Cancer
Cardiomyopathy
Ciliopathies
Congenital disorders
Epilepsy
Eye disorders
Familial hypercholesterolemia
Hearing loss
Hereditary Liver Disease
Hereditary Red Cell Disorder
Heterotaxy
Long QT
Marfan syndrome
Neuromuscular disorders
Noonan and related Syndrome
Nuclear mitochondrial
Periodic Fever Syndrome
Renal
Respiratory disease
Short Stature
Severe combined immunodeficiency
X linked intellectual disability

Positions Chosen Contain

Reference (Wild Type) Sites
SNVs
Indels

CAP NGS Germline PT Process for Laboratories



Results Form for CAP PT for Germline Variants

NGS-01 Results

Exception Code 090 ○ 33

Gene Name (HGNC ID) Chromosomal Position (HG19, Genome Reference Consortium GRCh37) Transcript Number	Variant Type	Zygosity (if applicable)	Variant Description* (if applicable)
100 ○ (1000) *ABCC9* (HGNC:60) chr12 22086810 NM_020297.2	110 ○ 420 Insertion ○ 421 Deletion ○ 422 SNV ○ 297 DelIns (Indels) ○ 423 No variant detected Cannot be evaluated: ○ 298 Low quality data ○ 299 Coverage below threshold ○ 010 Other, specify: 120	130 ○ 730 Heterozygous ○ 729 Homozygous	140
150 ○ (1001) *ABCD1* (HGNC:61) chrX 153001860 NM_000033.3	160 ○ 420 Insertion ○ 421 Deletion ○ 422 SNV ○ 297 DelIns (Indels) ○ 423 No variant detected Cannot be evaluated: ○ 298 Low quality data ○ 299 Coverage below threshold ○ 010 Other, specify: 170	180 ○ 730 Heterozygous ○ 729 Homozygous	190

200 Chromosomal Positions or Intervals Listed

Laboratories Requested to Provide What Genes Their Assay(s) Cover

HGVS Nomenclature (c. and p.)

Results Form for CAP PT for Germline Variants

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200 Chromosomal Positions or Intervals Listed

- **Labs Performing Gene Panels Answer Chromosomal Positions in Their Test**
- **Labs Performing Exomes/Genomes Answer 50 Required Positions**

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Data Summary 2016 A and B Mailing Surveys

First Graded
CAP NGS Methods Based Proficiency Test
Germline Variants

Laboratory Enrollment in CAP NGS Germline MBPT Surveys

Participant Laboratory Data	2016 A Mailing	2016 B Mailing
Number of Labs Enrolled	130	142
Number of Labs Returning Results*	125 (91%)	128 (90%)

*Number and (Percentage) of Labs Returning Results
At Time of Data Summarization

Assay Types Performed in CAP NGS Germline MBPT Surveys

Assays Performed by Participating Laboratories*	2016 A Mailing	2016 B Mailing
Multi-Gene Panels	72	71
Exome	42	47
Whole Genome	5	6

*Multiple Responses per Laboratory Allowed
Not All Laboratories Responded

Platform Usage* in CAP NGS Germline MBPT Surveys

Platform	2016 A Mailing Responses from 115 Labs	2016 B Mailing Responses from 115 Labs
Illumina HiSeq 2500	37	35
Illumina MiSeq	33	34
Illumina NextSeq 500	22	24
Illumina HiSeq 3000/4000	6	6
Illumina HiSeq X Five/Ten	3	4
Illumina HiSeq 2000	1	0
Illumina MiSeqDx	1	0
Ion Torrent PGM	9	8
Ion Torrent Proton	6	5
Ion Torrent S5/S5 XL	2	3
Pacific Biosciences RS/RS II	0	0
Roche 454 GS Junior/FLX+	1	0

***Multiple Responses per Laboratory Allowed**
Not All Laboratories Responded

Types of Chromosomal Positions in CAP NGS Germline MBPT Surveys

Chromosomal Position Type	2016 A Mailing	2016 B Mailing
Reference	140 (1)*	90
Single Nucleotide Variant	57 (7)	109 (1)
Insertion	1	0
Deletion	2	1 (1)

(1)* Position is Non-Coding and Omitted from Data Analysis

Red Parentheses are Non-Graded Positions
Discordance Between Genomic and Transcript Reference Sequences

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Reference Position Analysis for 2016 A and B Mailing Surveys

2016 A Mailing	Number of Labs Analyzing Each of the 139 Graded Reference Positions	Mean 26 Median 24	Range 12-68
2016 B Mailing	Number of Labs Analyzing Each of the 90 Graded Reference Positions	Mean 30 Median 24	Range 15-59

Reference Position Analysis for 2016 A and B Mailing Surveys

	Analysis Responses for All 139 Graded Reference (WT) Positions	% No Variant Detected	% Variant Detected	% Cannot Evaluate
2016 A Mailing		Mean 98.0% Median 100% Range 81.8-100%	Mean 1.53% Median 0.0% Range 0.0-9.5%	Mean 0.49% Median 0.0% Range 0.0-13.6%
2016 B Mailing	Analysis Responses for All 90 Graded Reference (WT) Positions	% No Variant Detected	% Variant Detected	% Cannot Evaluate
		Mean 98.9% Median 100% Range 83.3-100%	Mean 0.0% Median 0.0% Range 0.0-0.0%	Mean 1.02% Median 0.0% Range 0.0-16.7%

Reference Position Analysis for 2016 A and B Mailing Surveys

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Intended Response

Types of Chromosomal Positions in CAP NGS Germline MBPT Surveys

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Discordance Between Genomic and Transcript Reference Sequences

SNV Position Analysis for 2016 A and B Mailing Surveys

2016 A Mailing	Number of Labs Analyzing Each of the 50 Graded SNV Positions	Mean 43 Median 46	Range 17-75
2016 B Mailing	Number of Labs Analyzing Each of the 108 Graded SNV Positions	Mean 29 Median 23	Range 16-51

SNV Position Analysis for 2016 A and B Mailing Surveys

A

Analysis Responses for All 50 Graded SNV Positions	% Variant Detected	% Not Detected	% Cannot Evaluate
	Mean 93.6% Median 94.0% Range 82.4-100%	Mean 5.6% Median 5.4% Range 0.0-16.2%	Mean 0.83% Median 0.0% Range 0.0-6.7%

B

Analysis Responses for All 108 Graded SNV Positions	% Variant Detected	% Not Detected	% Cannot Evaluate
	Mean 97.8% Median 100% Range 87.5-100%	Mean 1.8% Median 0.0% Range 0.0-10.3%	Mean 0.40% Median 0.0% Range 0.0-10.5%

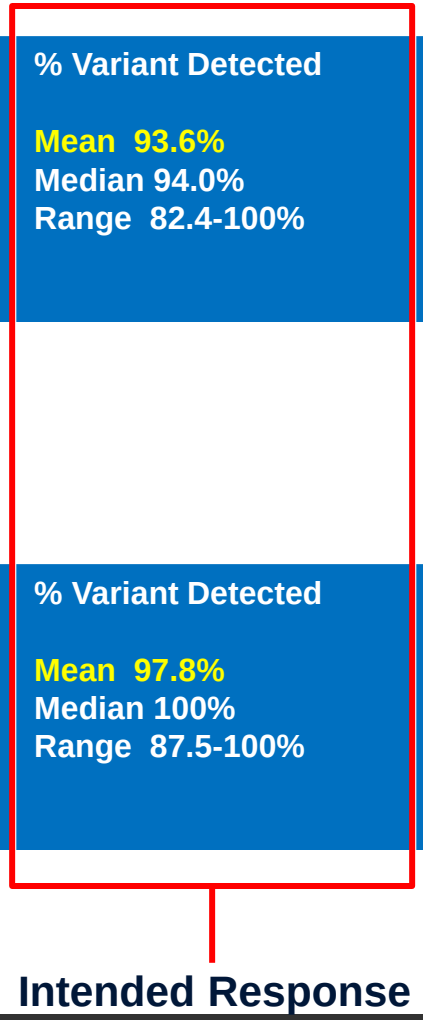
SNV Position Analysis for 2016 A and B Mailing Surveys

A

Analysis Responses for All 50 Graded SNV Positions	% Variant Detected	% Not Detected	% Cannot Evaluate
	Mean 93.6% Median 94.0% Range 82.4-100%	Mean 5.6% Median 5.4% Range 0.0-16.2%	Mean 0.83% Median 0.0% Range 0.0-6.7%

B

Analysis Responses for All 108 Graded SNV Positions	% Variant Detected	% Not Detected	% Cannot Evaluate
	Mean 97.8% Median 100% Range 87.5-100%	Mean 1.8% Median 0.0% Range 0.0-10.3%	Mean 0.40% Median 0.0% Range 0.0-10.5%



Intended Response

HGVS Nomenclature Assessment in 2016 A and B Mailing Surveys

NGS-01 Results

Exception Code 090 ○ 33

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Standardized Nomenclature
Currency of Genetic Information

HGVS
HUMAN GENOME VARIATION SOCIETY

HGVS Nomenclature (c. and p.)

HGVS Nomenclature Assessment in 2016 A and B Mailing Surveys

Five Assessment Categories for HGVS Nomenclature Usage

- Preferred: Complete HGVS Nomenclature: NF1 c.7546C>T; p.Pro2516Ser
- Acceptable: Acceptable HGVS Nomenclature: NF1 c.7546C>T; p.P2516S
- Incomplete: Incomplete HGVS Nomenclature: NF1 c.7546C>T
- Unacceptable: Incorrect Annotation
- Not Evaluated/Graded*

*Discordance Between Population Allele Frequency
Of Human Genome Reference Sequence (Minor Allele)
And Reference Transcript Provided (Major Allele)

SNV Position Analysis for 2016 A and B Mailing Surveys

A

Percentage of Labs Providing Complete Intended Responses (Including Variant Type, Zygosity and Preferred or Acceptable Nomenclature) For Each of the 50 Graded SNV Positions	Mean 93.3% Median 94.0%	Range 81.0-100%

B

Percentage of Labs Providing Complete Intended Responses (Including Variant Type, Zygosity and Preferred or Acceptable Nomenclature) For Each of the 108 Graded SNV Positions	Mean 96.2% Median 97.1%	Range 55.6-100%

Types of Chromosomal Positions in CAP NGS Germline MBPT Surveys

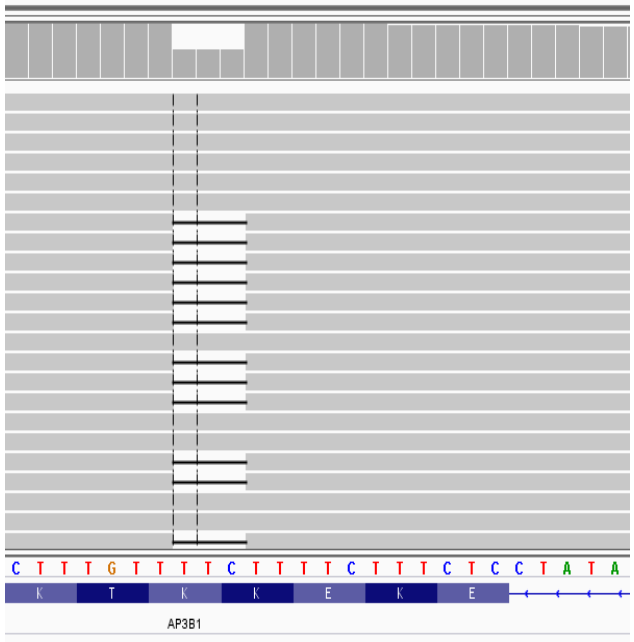
Chromosomal Position Type	2016 A Mailing	2016 B Mailing
Reference	140 (1)*	90
Single Nucleotide Variant	57 (7)	109 (1)
Insertion	1	0
Deletion	2	1 (1)

(1)* Position is Non-Coding and Omitted from Data Analysis

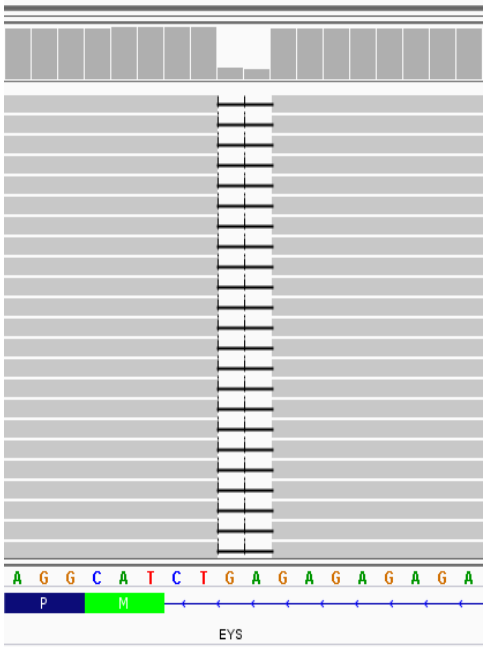
Red Parentheses are Non-Graded Positions
Discordance Between Genomic and Transcript Reference Sequences

Insertion and Deletion Analysis for 2016 A Mailing Survey

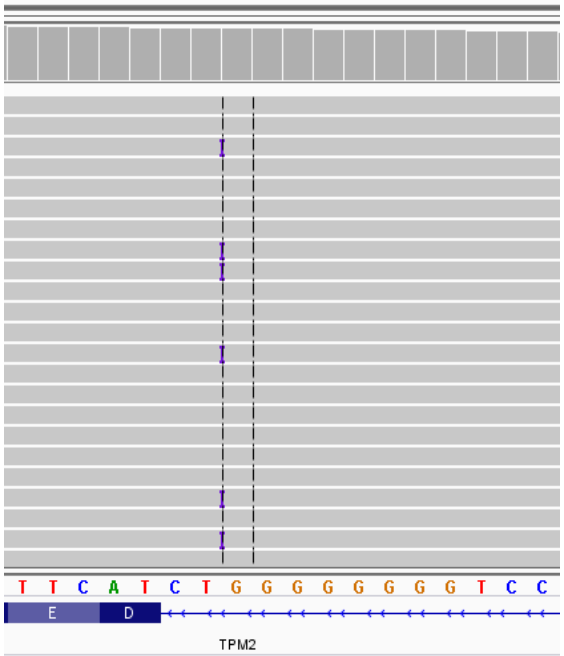
AP3B1 Deletion	*EYS* Deletion	*TPM2* Insertion
Heterozygous c.2409_2411delGAA p.Lys804del	Homozygous c.6079-4_6079-3delTC	Heterozygous c.773_3dupC



AP3B1



EYS



TPM2

Insertion and Deletion Analysis for 2016 A Mailing Survey

Analysis Responses PSR Table 1 and 2	*AP3B1* Deletion Heterozygous c.2409_2411delGAA p.Lys804del	*EYS* Deletion Homozygous c.6079-4_6079-3delTC	*TPM2* Insertion Heterozygous c.773-3dupC
Number of Labs Providing Responses and Response Types	52	46	45
Cannot Evaluate	4 (7.7%)	4 (8.7%)	5 (11.1%)
Variant Not Detected	4 (7.7%)	5 (10.9%)	5 (11.1%)
Variant Detected	44 (84.6%)	37 (80.4%)	35 (77.8%)
Complete Intended Response (Variant Type, Zygosity, Preferred or Acceptable Nomenclature)	37 of 44 (84.1%)	24 of 37 (64.9%)	30 of 35 (85.7%)

Insertion and Deletion Analysis for 2016 A Mailing Survey

Analysis Responses PSR Table 1 and 2	*AP3B1* Deletion Heterozygous c.2409_2411delGAA p.Lys804del	*EYS* Deletion Homozygous c.6079-4_6079-3delTC	*TPM2* Insertion Heterozygous c.773-3dupC
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AP3B1 Gene
Heterozygous Deletion c.2409_2411delGAA, p.Lys804del

Gene Name/ Chromosomal Position	Transcript Number	Variant Type	Zygosity	Description	Nomenclature Grade Assignment	No. Labs	Percent
AP3B1 (HGNC:566)							
chr5 77396832-77396841	NM_003664.4	Deletion	Heterozygous	c.2409_2411delGAA, p.Lys804del	Preferred	28	63.7%
				c.2262_2264delGAA, p.Lys755del	Unacceptable	1	2.3%
				c.2409_2411del In-frame Unknown pathogenicity	Incomplete (a)	1	2.3%
				c.2409_2411del, p.Lys804del	Acceptable	9	20.4%
				c.2409_2411delGAA, p.Lys803_Lys804del	Unacceptable	2	4.5%
				c.2409_2411delGAA;p.Lys806delLys	Unacceptable	1	2.3%
				c.2409_2411delNNN p.Lys804del	Unacceptable	1	2.3%
		SNV	Homozygous	c.1754T>A, p.Val585Glu	Unacceptable	1	2.3%

Variable Use of HGVS Nomenclature – Observed in Other CAP PT Programs

Performance Summary
2016 NGS MBPT Germline Graded Surveys

Observations

- Analysis of Reference Positions and SNVs Positions is Solid
- Indel Detection Sensitivity is Lower than SNVs (Numbers of Indels are Low)
- Harmonization of HGVS Nomenclature Usage is a Future Goal

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NGS Proficiency Testing

Current Proficiency Testing with Physical DNA Samples is Constrained

- **Limited Number and Types of Variants in Any Given Physical Sample**

Approaches to Increasing the Diversity of Physical DNA Samples

- **Cell Line DNAs with Spiked In Synthesized DNAs Containing Variants**
- **Modify Cell Lines via Genome Editing (eg, CRISPR-Cas9)**

In Silico Mutagenesis Based PT for NGS Diagnostics

Advantages

Current Ability: Simulation of SNVs and Indels with Different Variant Allele Fractions

In Development: Simulation of Copy Number and Structural Variation

Applicable to Diverse Testing Areas (Germline, Somatic, Infectious Diseases)

PT Samples Are Data Files that Laboratories Download and Process - Portability

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PT Samples Are Data Files that Laboratories Download and Process - Portability

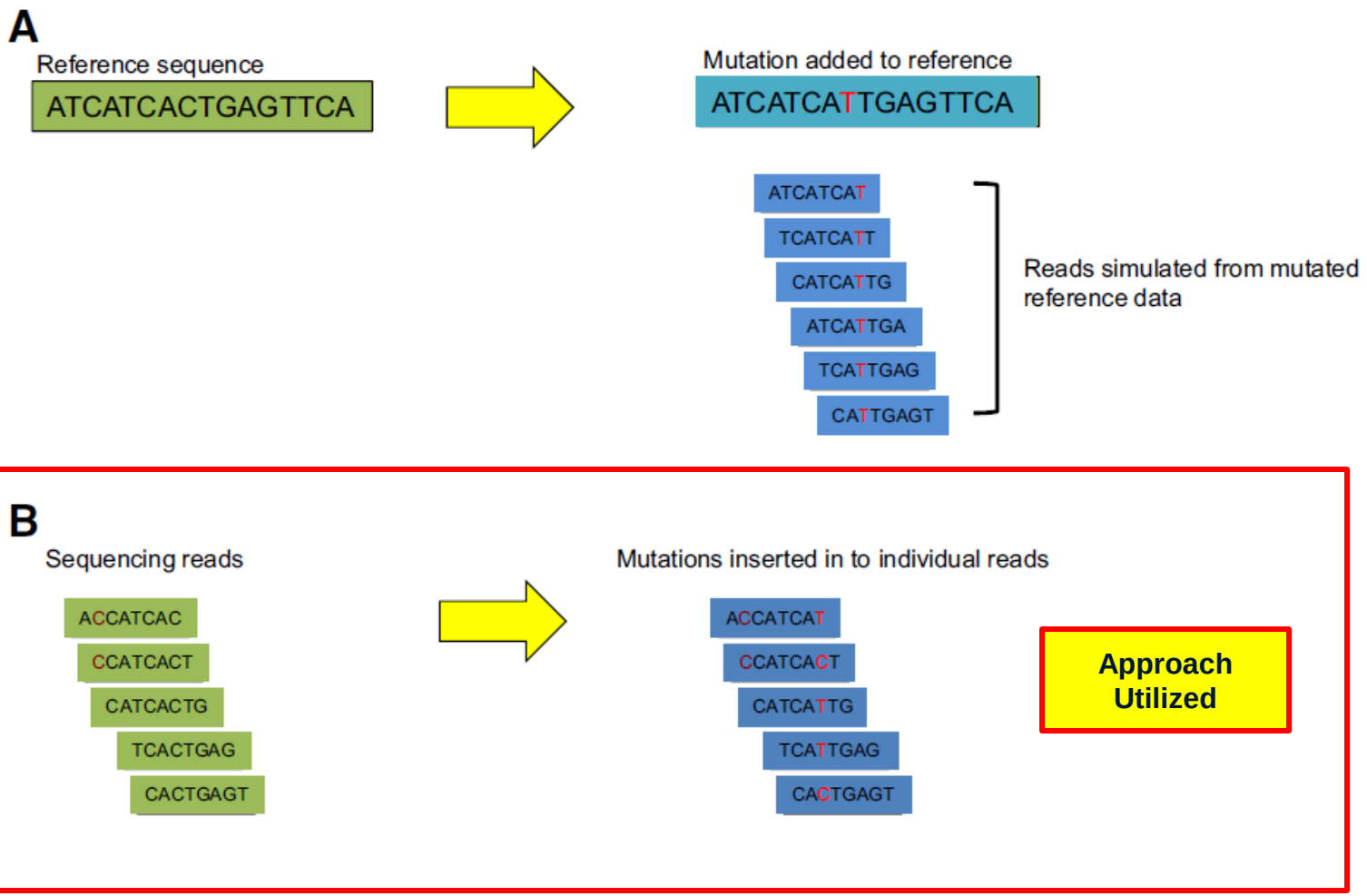
Limitations

Only Evaluates the Bioinformatics Pipeline

Requires Laboratory Expertise in Managing File Sharing Protocols

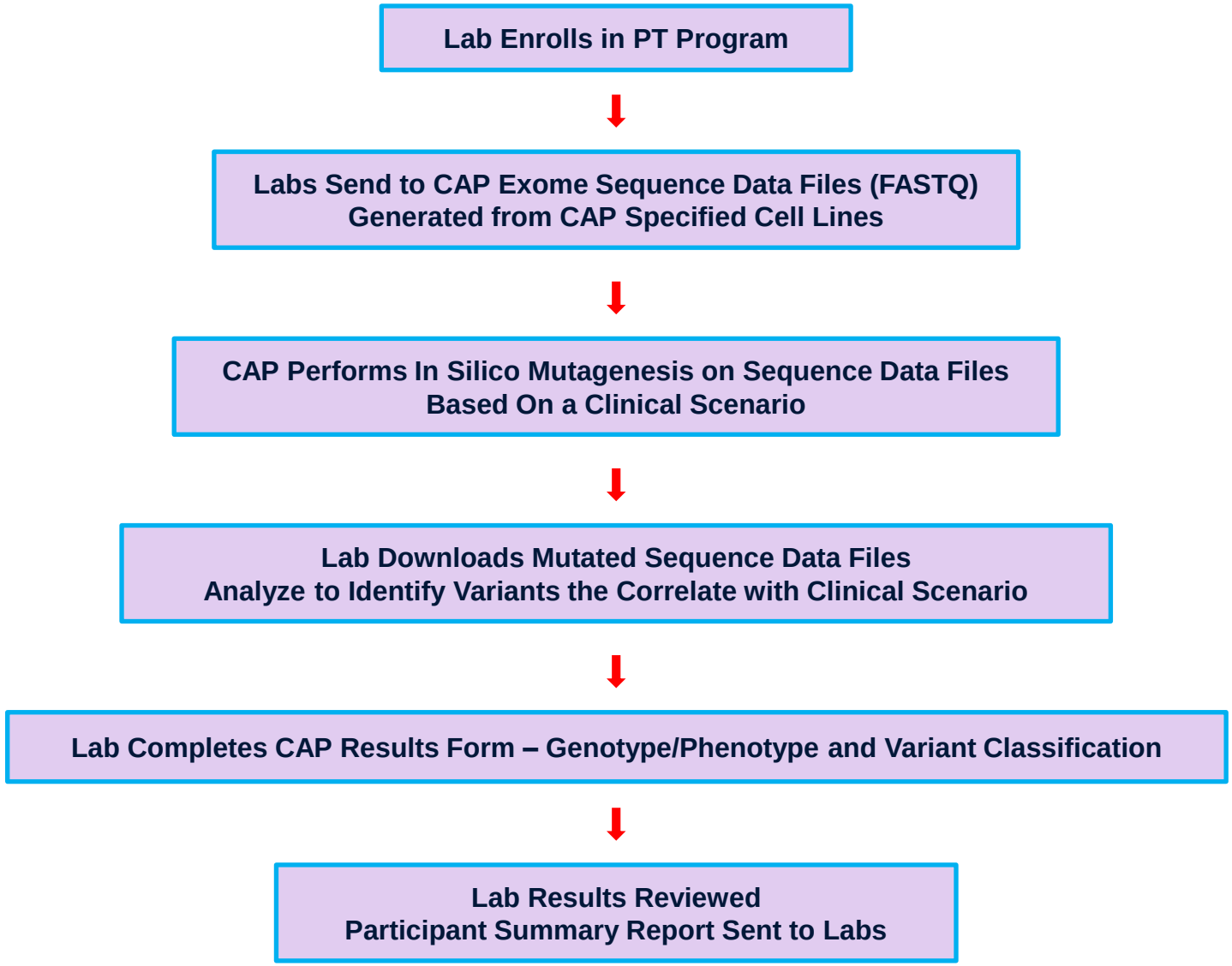
Uncertainty Persists in Biases in In Silico Manipulated Sequence Files

Two Major Approaches for In Silico Mutagenesis



Duncavage et al. 2017 J Mol Diag Vol 19 (1)

In Silico Based PT for Exome Sequencing for Undiagnosed Disorders



In Silico Based PT for Exome Sequencing for Undiagnosed Disorders
Educational PT Launch 2018

Next-Generation Sequencing Undiagnosed Disorders—Exome NGSE

Analyte/Procedure	Program Code	Challenges per Shipment
	NGSE	
Exome analysis for germline undiagnosed disorders	▮	1

Additional Information/Minimum Requirements

- This in silico based Survey will assess the ability of the laboratory to identify germline variants responsible for a provided clinic phenotype as is encountered in an undiagnosed disease scenario. In addition to analyzing the in silico mutagenized file to identify a genetic diagnosis for the provided clinical scenario, pathogenic or likely pathogenic ACMG secondary findings may also be reported.
- Laboratories must provide an exome sequencing data file that has been generated using one of the following sources: a specimen from the NGS Survey program (see page 252) or from one of the NIST Reference Material cell lines: RM 8398 (NA12878), RM 8391, RM 8392, or RM 8393. Specimens from the NGSST and NGSHM Surveys cannot be used for this program.
- FASTQs or unaligned BAMs must be submitted along with a BED file describing the regions targeted and interrogated by your laboratory. Additionally, >90% of exons targeted and interrogated by your laboratory must have a minimum read coverage of 10X.

Program Information

- One exome sequencing data file, originating from your laboratory and provided to the CAP, for in silico mutagenesis. The mutagenized exome sequencing data file is to be downloaded and analyzed by your bioinformatics pipeline
- The mutagenized exome sequencing file will be accompanied by a clinical history, relevant laboratory data, and results of ancillary studies, where appropriate
- Two online activities per year; your CAP shipping contact will be notified via email when the activity is available

CLINICAL SCENARIO for 2018 A Mailing for Exome PT

The patient presented in the first year of life with failure to thrive, hypotonia, group B streptococcus bacteremia, and hypoalbuminemia. By the third year of life, additional features included strabismus, seizures and ataxia

Table 1: Indicate the gene(s) and variant(s) causative for patient phenotype(s). Carrier states in genes that could be causative for the patient phenotype should also be reported. Refer to the kit instructions for examples of reporting format.

Variant 1

Gene Symbol (HGNC)	Genomic Coordinates (Chromosome and chromosomal position or interval)	Transcript Used for Annotation (Indicate only 1 NCBI (NM) Ref Seq)	HGVS-Based Variant Description	
			Nucleotide Change (c.)	Predicted Protein Change (p.)
060	070	080	090	100

Zygosity

110
- ○ 728 Hemizygous
- ○ 730 Heterozygous
- ○ 729 Homozygous

Causative For (Select all that apply.)

120
- ○ 1063 Ataxia
- ○ 1064 Failure to thrive
- ○ 1169 Group B streptococcus bacteremia
- ○ 1170 Hypoalbuminemia
- ○ 1065 Hypotonia
- ○ 1066 Seizures
- ○ 1171 Strabismus

Mode of Inheritance for Variant

190
- ○ 1067 Autosomal dominant
- ○ 1068 Autosomal recessive
- ○ 1069 X-linked dominant
- ○ 1070 X-linked recessive

Variant Classification

200
- ○ 1071 Pathogenic
- ○ 1072 Likely pathogenic
- ○ 1073 Variant of uncertain significance

Fields for Additional Variants Causative for Phenotypic Features
Separate Section for Secondary Findings

CLINICAL SCENARIO for 2018 A Mailing for Exome PT

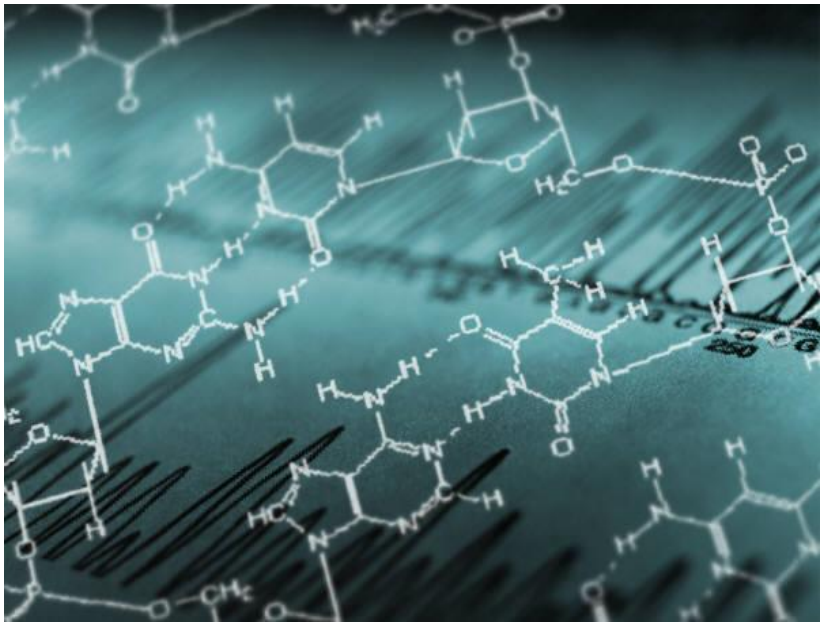
The patient presented in the first year of life with failure to thrive, hypotonia, group B streptococcus bacteremia, and hypoalbuminemia. By the third year of life, additional features included strabismus, seizures and ataxia

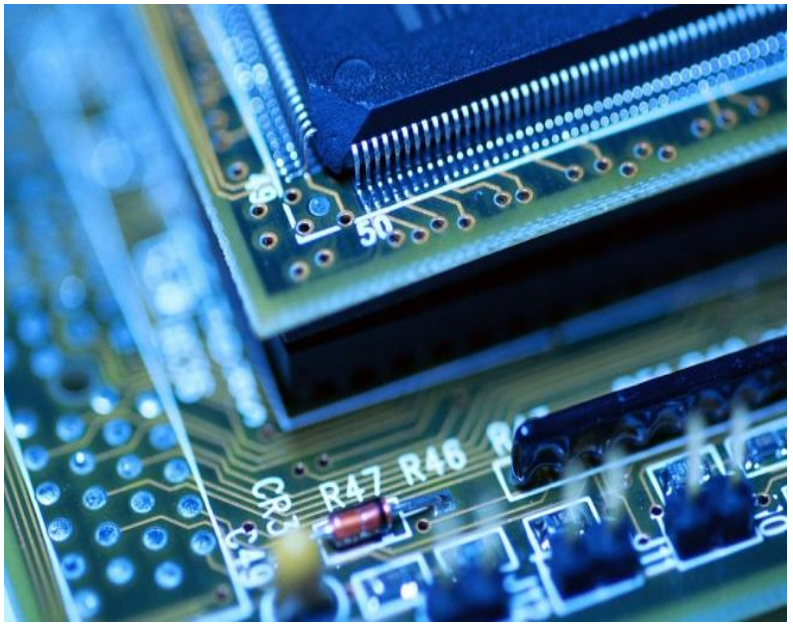
Results from 2018 A Mailing for Exome PT Currently Being Analyzed and Summarized

Learning Objectives

- Explain the rationale for using a method-based PT approach for NGS testing for germline variants
- Describe analytical and annotation results evaluated in method-based PT for germline variants
- Relate trends in laboratory performance in method-based PT for germline variants
- Discuss how in silico PT increases options for assessment of laboratories performing NGS including complex scenarios presented by exome sequencing

Thank You





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