Clinical Implementation of Whole Genome Sequencing

Madhuri Hegde, PhD, FACMG

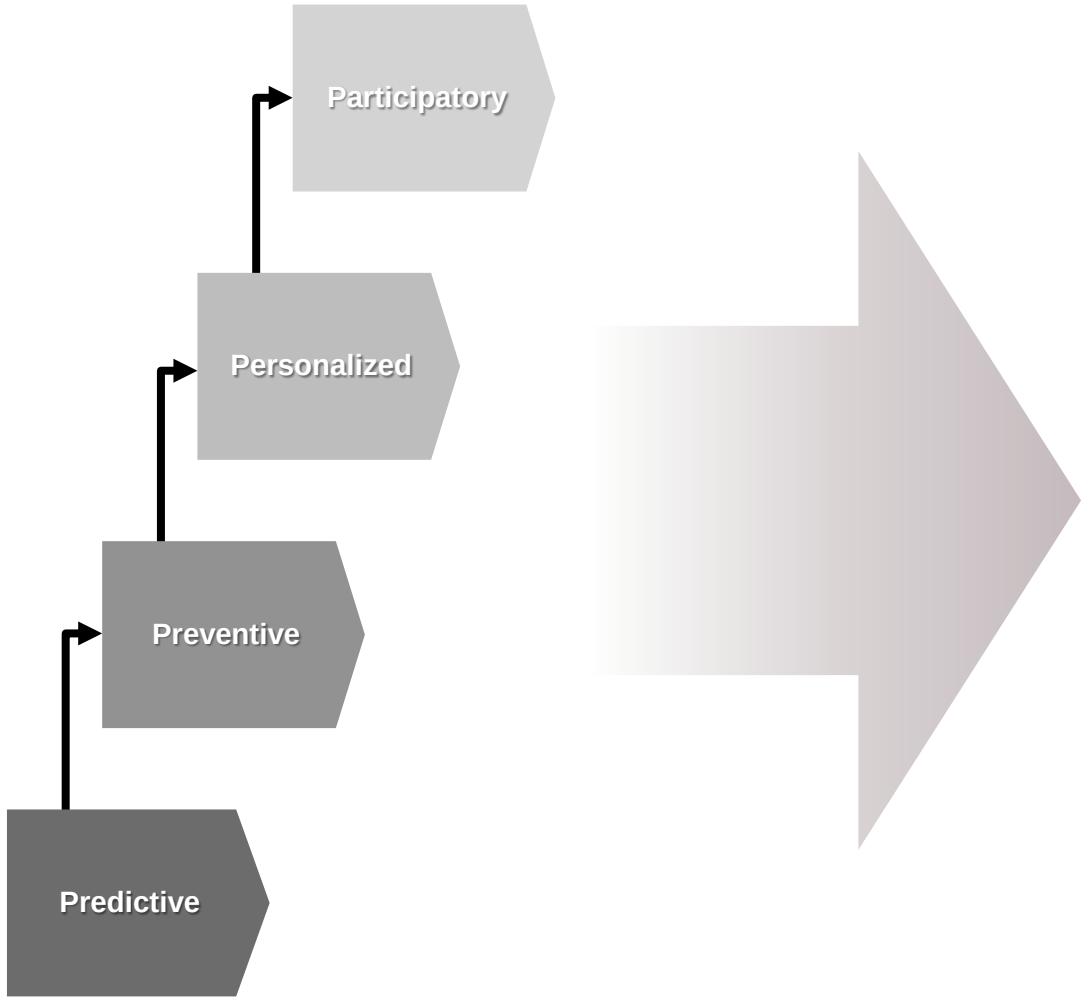
Adjunct Professor of Human Genetics and Pediatrics, Emory University and Georgia Institute of Technology

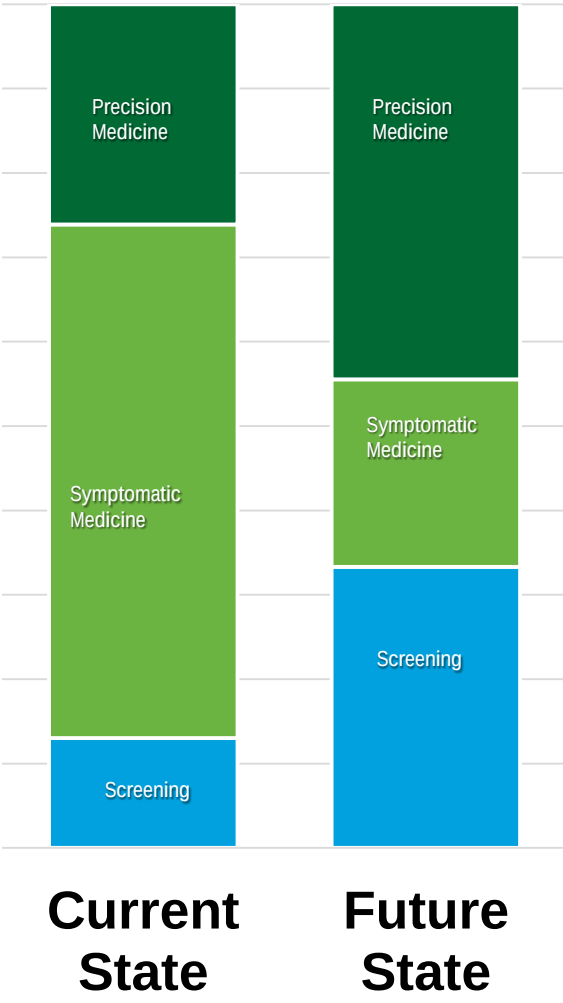
VP & CSO PerkinElmer Global Laboratories

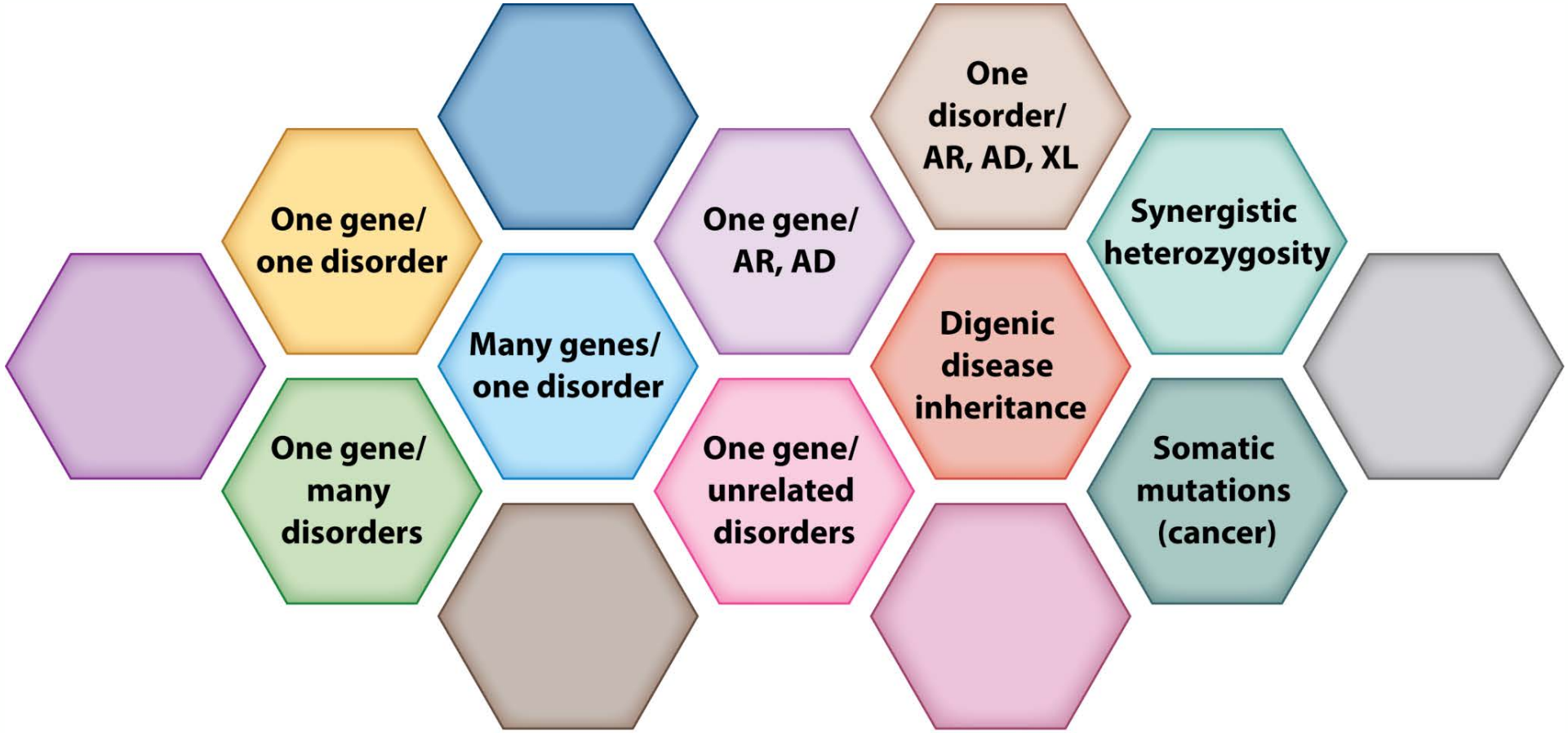
NEW ERA OF GENOMICS

Current and Future Direction of Genomic Testing

Personalized Medicine







Chakravorty S, Hegde M. 2017.
Annu. Rev. Genom. Hum. Genet. 18:229–56

Chakravorty S[1], Hegde M[1]. Annu Rev Genomics Hum Genet. 2017; 18:229-256.
doi: 10.1146/annurev-genom-083115-022545.

WGS Improves Diagnostic Yield

Official journal of the American College of Medical Genetics and Genomics **ORIGINAL RESEARCH ARTICLE** | Genetics inMedicine

Open

Improved diagnostic yield compared with targeted gene sequencing panels suggests a role for whole-genome sequencing as a first-tier genetic test

Whole-genome sequencing (WGS) provides a comprehensive testing platform that has the potential to streamline genetic assessments, but there are limited comparative data to guide its clinical use.

Methods: We prospectively recruited 103 patients from pediatric non-genetic subspecialty clinics, each with a clinical phenotype suggestive of an underlying genetic disorder, and compared the diagnostic yield and coverage of WGS with those of conventional genetic testing.

Results: WGS identified diagnostic variants in 41% of individuals, representing a significant increase over conventional testing results (24%; $P = 0.01$). Genes clinically sequenced in the cohort ($n = 1{,}226$) were well covered by WGS, with a median exonic coverage of $40\times \pm 8\times$ (mean $\pm$SD). All the molecular diagnoses made by conventional methods were captured by WGS. The 18 new diagnoses made with WGS included structural and non-exonic sequence variants not detectable with whole-exome sequencing, and confirmed recent disease associations with the genes *PIGG*, *RNU4ATAC*, *TRIO*, and *UNC13A*.

Conclusion: WGS as a primary clinical test provided a higher diagnostic yield than conventional genetic testing in a clinically heterogeneous cohort.

Genet Med advance online publication 3 August 2017

Key Words: copy number variation; next-generation sequencing; noncoding; diagnostics; whole-genome sequencing

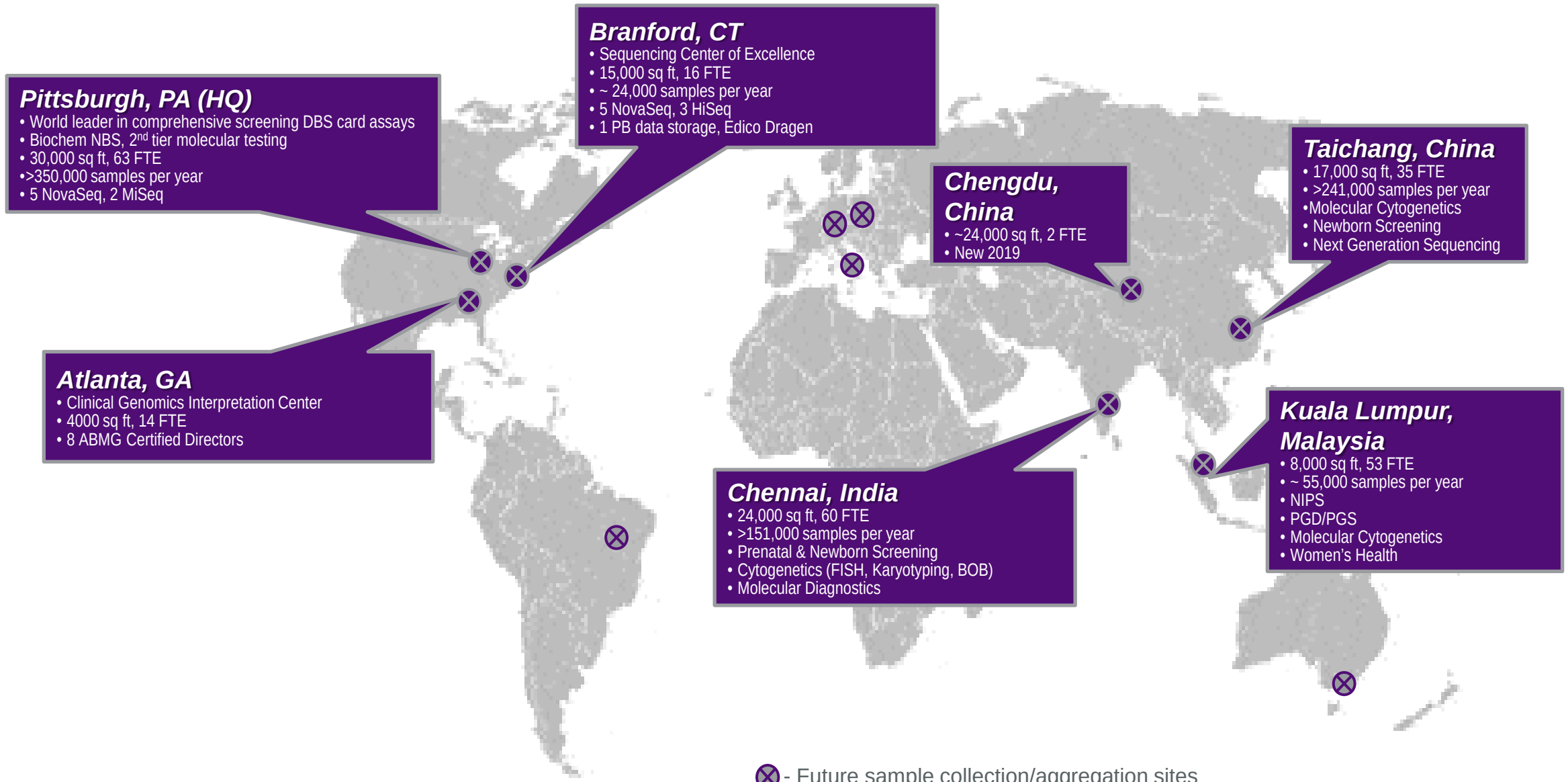
Benefits of Clinical WGS

- **Unbiased Sequencing (compared to WES)**
- **New disease-causing genes discovered frequently—on average 12 per month**
  - Previously unknown genes may be identified as contributing to a disease state.
    - Traditional genetic testing looks only at the common “troublemaker” genes
  - Ability to store and re-analyze genetic information over time to find new genetic causes
- **For >50% of all Mendelian disease genes, there is an indicated intervention**
  - Beneficial to detect thousands of individual conditions at an early stage rather than waiting until they become clinically apparent
- **Creating personalized plans to treat disease**
  - may be possible based not only on the mutant genes causing a disease, but also other genes in the patient’s genome
- **Shortening or preventing the diagnostic odyssey**
  - Look at the entire picture with WGS vs. looking at a single gene (or group of genes) at a time

A single test: Clinical WGS

- >98% of the 22,000 genes in the exome covered at >=20X
- Complete coverage of over 5900+ disease-causing genes
- Coverage of entire genomic sequence of a gene
- Mean coverage of >30X throughout the genome
- Reliable detection of genomic copy number variants (CNVs) with exon level resolution (smaller CNVs can be detected but follow-up confirmation strongly recommended)
  - **Replaces microarray**
- Mitochondrial Genome

PerkinElmer Genomics – A Global Laboratory

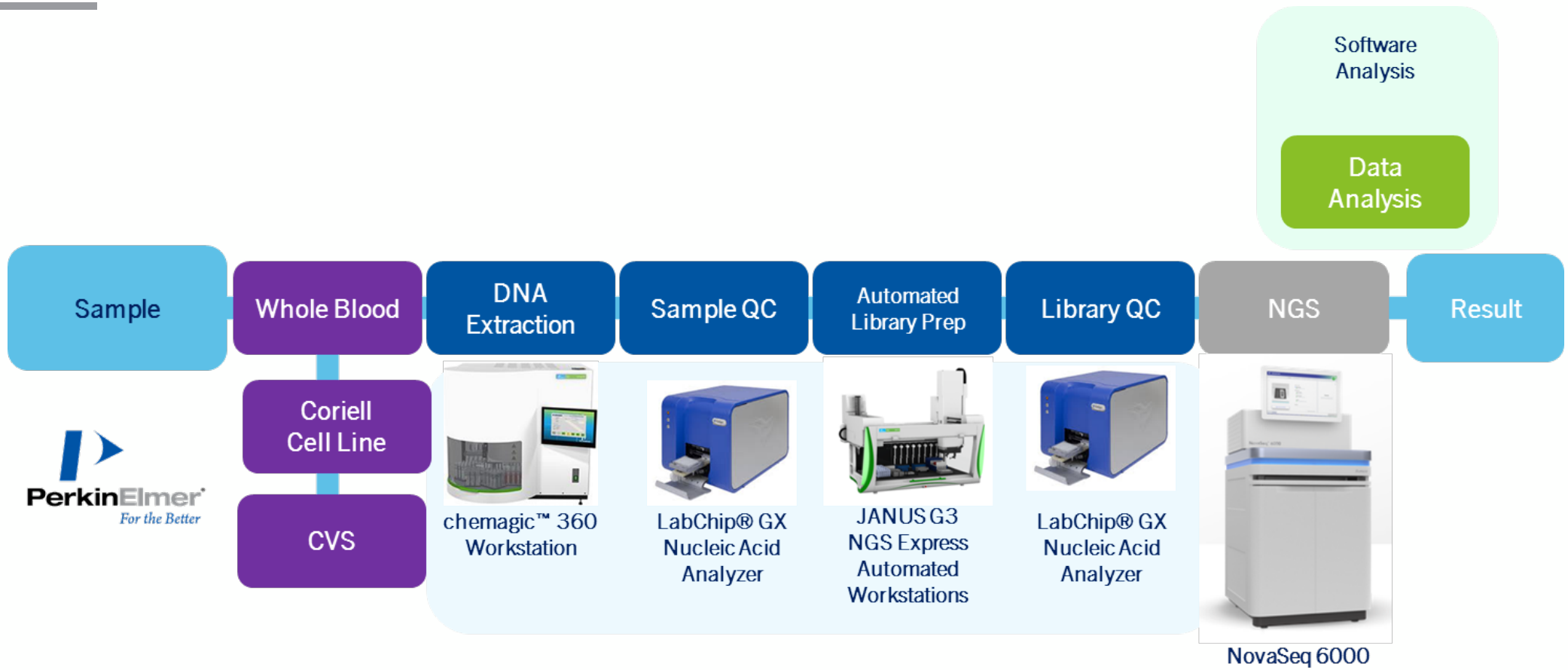


Developing a Continuum of Care Model

★ Coming Soon

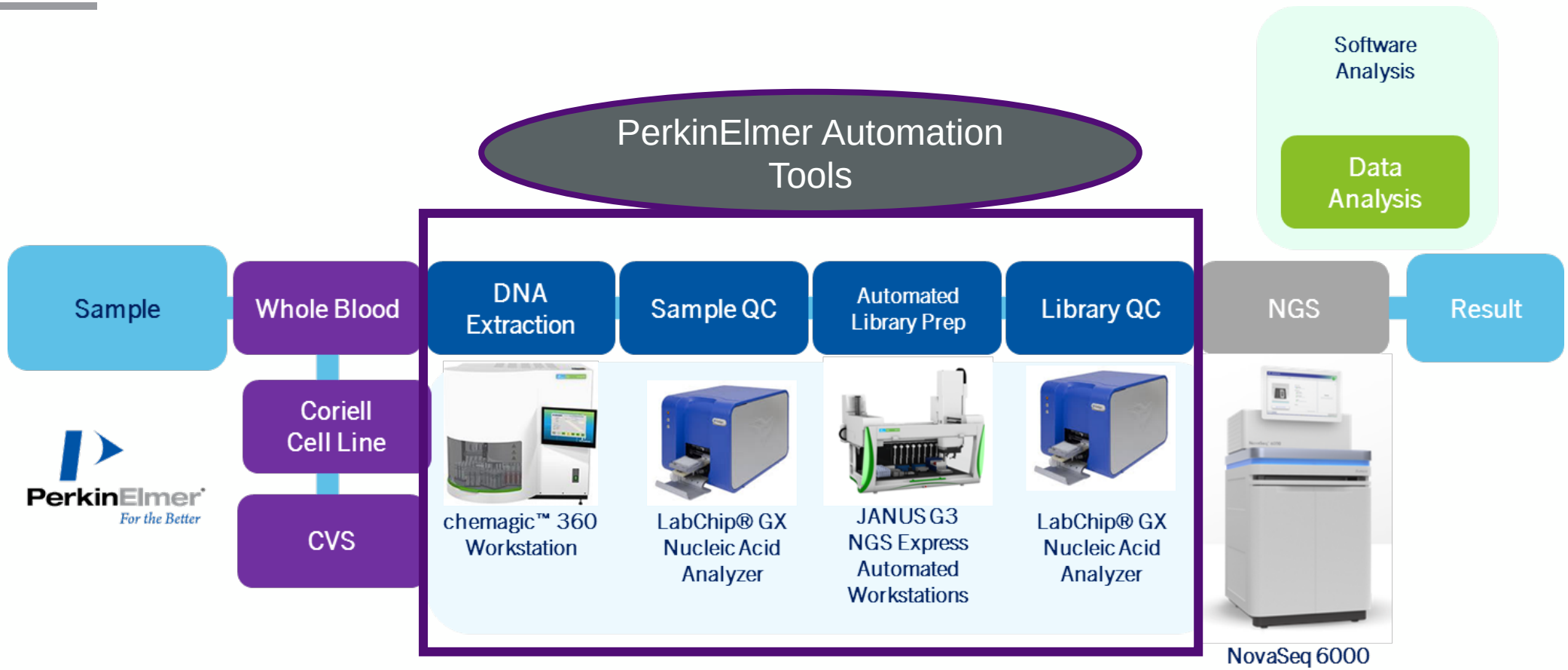
From Prenatal Testing, NBS to WGS
Utilizing Biochemical and Sequencing Data

WGS (5X and 30X) Methodology (Assay)



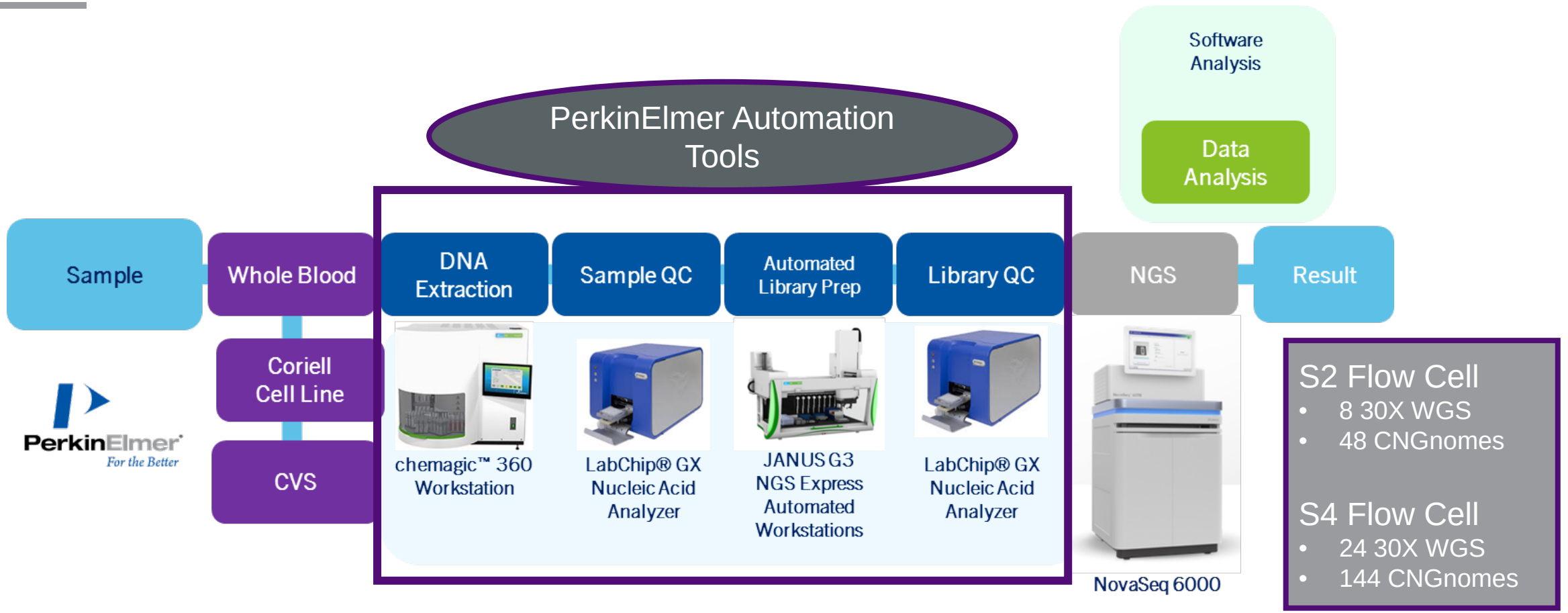
Easy and efficient workflow to generate 5X WGS data for CNGnome™

WGS (5X and 30X) Methodology (Assay)

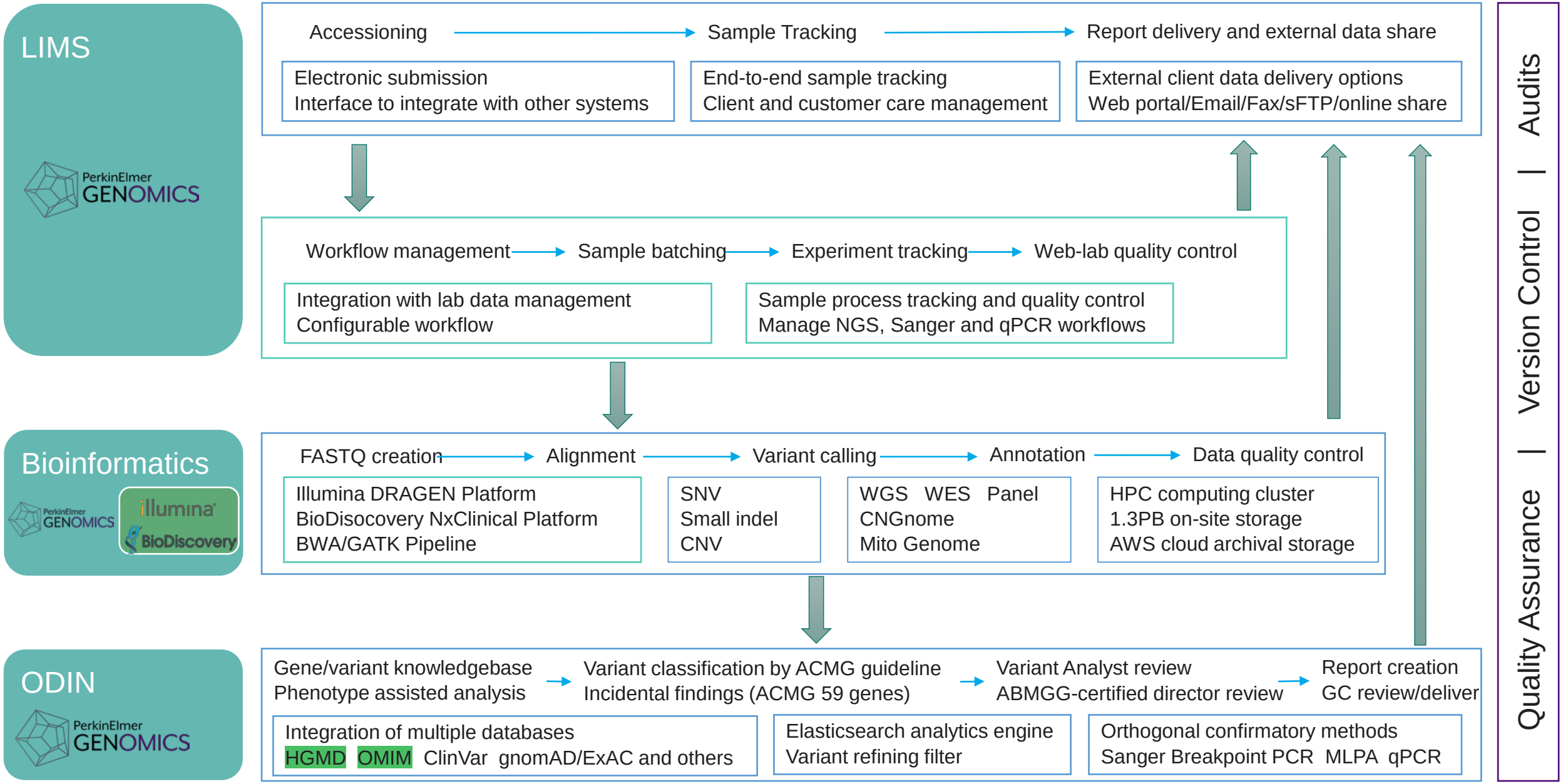


Easy and efficient workflow to generate 5X WGS data for CNGnome™

WGS (5X and 30X) Methodology (Assay)



*DNA extraction validated from Blood, Saliva, Dried blood spot card, amnio/CVS


LIMS
PerkinElmer GENOMICS
Accessioning
Sample Tracking
Report delivery and external data share
Electronic submission
Interface to integrate with other systems
End-to-end sample tracking
Client and customer care management
External client data delivery options
Web portal/Email/Fax/sFTP/online share
Workflow management
Sample batching
Experiment tracking
Web-lab quality control
Integration with lab data management
Configurable workflow
Sample process tracking and quality control
Manage NGS, Sanger and qPCR workflows
Bioinformatics
PerkinElmer GENOMICS
illumina BioDiscovery
FASTQ creation
Alignment
Variant calling
Annotation
Data quality control
Illumina DRAGEN Platform
BioDisocovery NxClinical Platform
BWA/GATK Pipeline
SNV
Small indel
CNV
WGS WES Panel
CNGnome
Mito Genome
HPC computing cluster
1.3PB on-site storage
AWS cloud archival storage
ODIN
PerkinElmer GENOMICS
Gene/variant knowledgebase
Phenotype assisted analysis
Variant classification by ACMG guideline
Incidental findings (ACMG 59 genes)
Variant Analyst review
ABMGG-certified director review
Report creation
GC review/deliver
Integration of multiple databases
HGMD OMIM ClinVar gnomAD/ExAC and others
Elasticsearch analytics engine
Variant refining filter
Orthogonal confirmatory methods
Sanger Breakpoint PCR MLPA qPCR
Quality Assurance | Version Control | Audits
PKIG Applications
External Applications
Commercially Licensed Resources

PerkinElmer GENOMICS

PKIG- WGS

Sample tracking and workflow management

Sample Accessioning	Wet-Lab	FASTQ Creation	Variant Calling	Variant Annotation	Data QC
• PKIG LIMS Data Entry • Demographics verification	• DNA Quality/ Quantity Evaluation • Automated WGS PCR Free library preparation	• Illumina DRAGEN & BioDiscovery NxClinical Platforms • BWA/GATK pipeline	• Alignment • SNV • Indels • CNV • Mitochondria	• WGS/WES • NMD Panel • CNGnome™ • Mito Genome	• HPC computing cluster • 1.3PB on-site storage • Cloud archival storage

ODIN

Variant Classification	Clinical Interpretation	Report Generation
• Automated ACMG Scoring • PKIG gene / disease/ variant knowledgebase • PGx knowledgebase	• Automated interpretation • ABMGG-certified director review • gene-disease-variant curation	• Clinician resource guide • Gene-disease information • Pre-scripted variant templates • Recommendation

Variant data

Clinical Validation Laboratory

Automated validation requests based on data concordance, pre-classification of variants, and variant prioritization scores

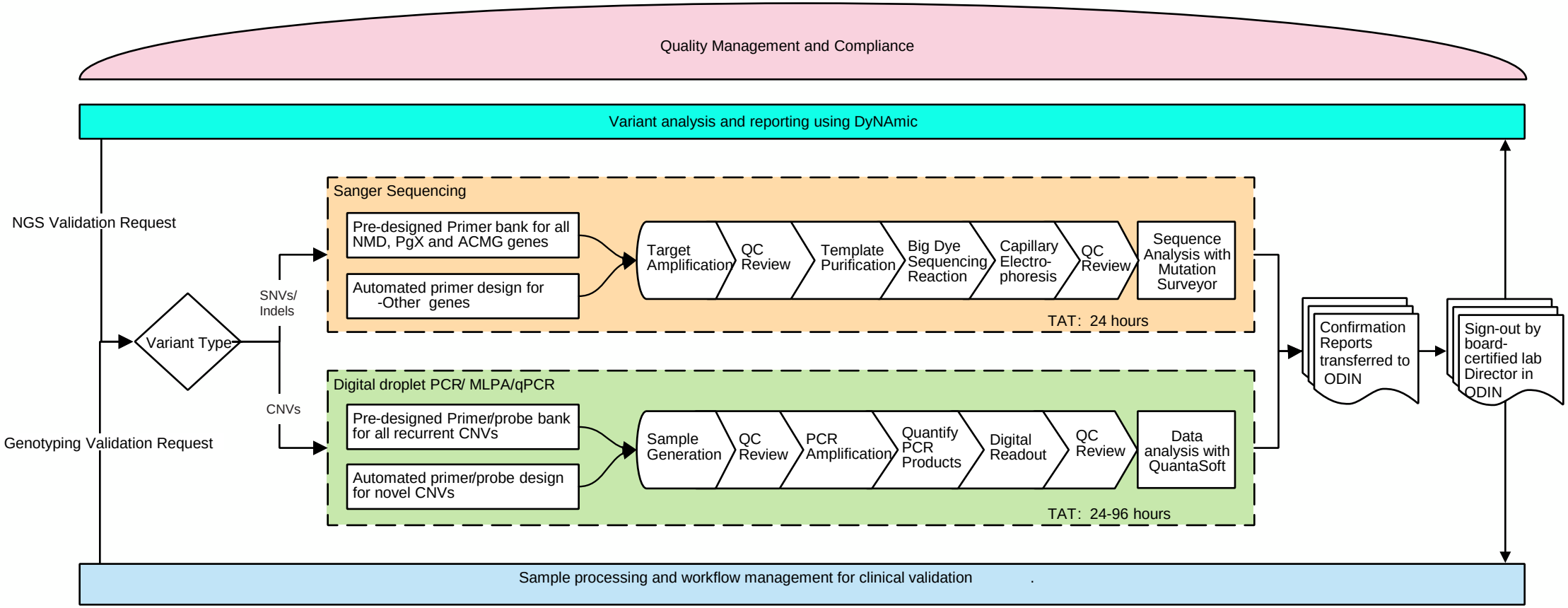
Biobank

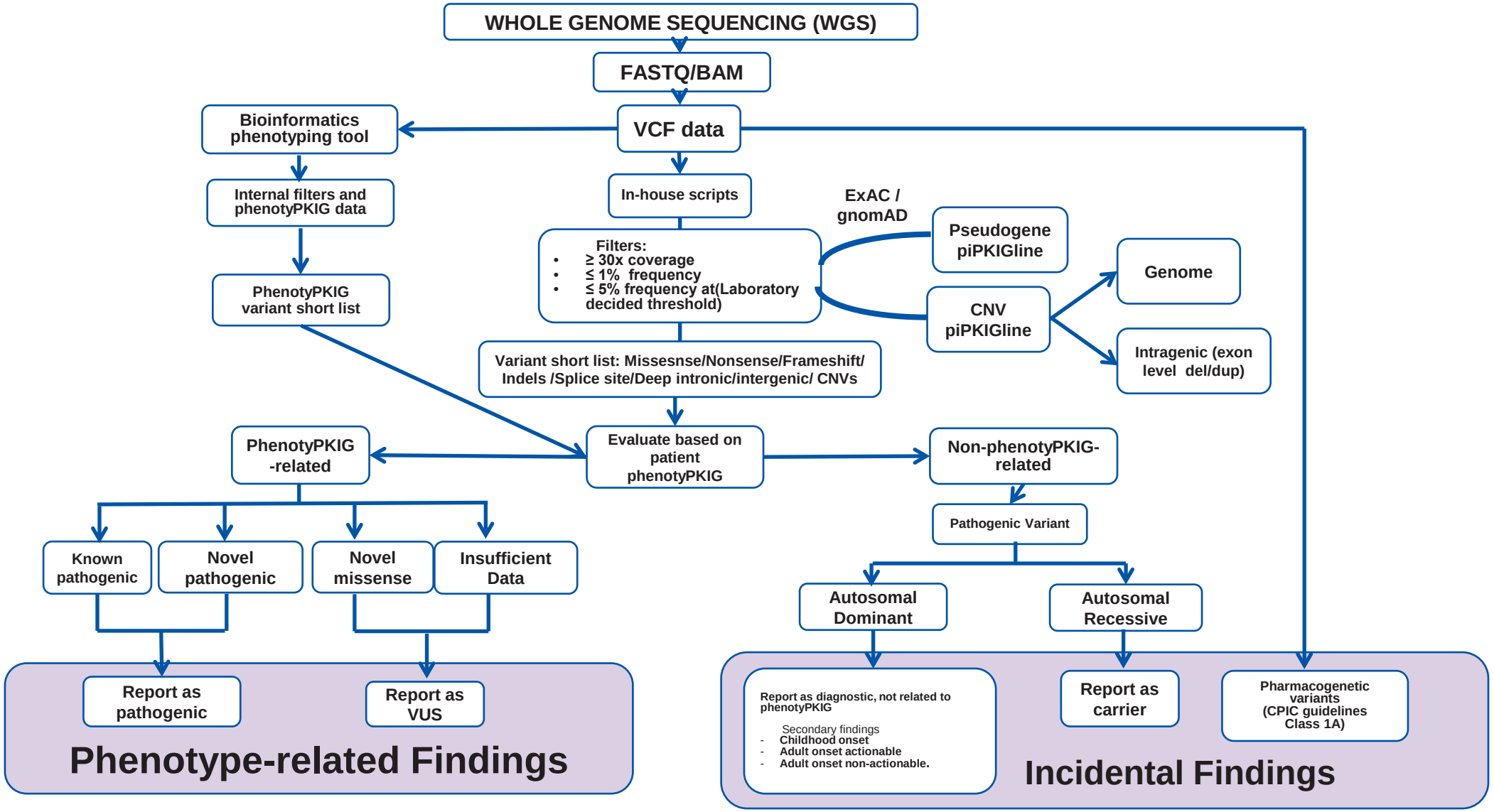
Genetic Counseling Resource Center

Data upload to servers

Variant submission to ClinVar

Electronic report delivery to Coordinating Center

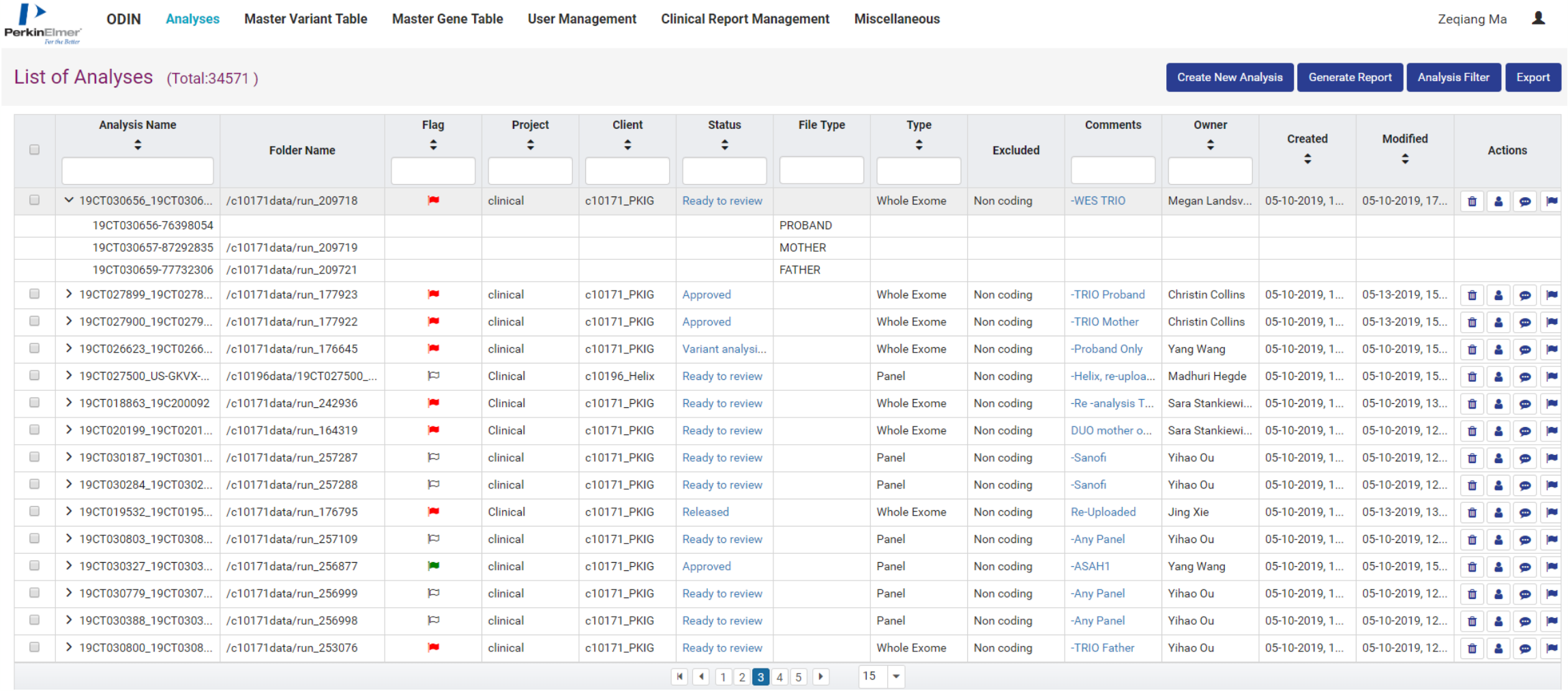

Quality Management and Compliance
Variant analysis and reporting using DyNAmic
NGS Validation Request
Genotyping Validation Request
Variant Type
SNVs/ Indels
CNVs
Sanger Sequencing
Pre-designed Primer bank for all NMD, PgX and ACMG genes
Automated primer design for -Other genes
Target Amplification
QC Review
Template Purification
Big Dye Sequencing Reaction
Capillary Electro-phoresis
QC Review
Sequence Analysis with Mutation Surveyor
TAT: 24 hours
Digital droplet PCR/ MLPA/qPCR
Pre-designed Primer/probe bank for all recurrent CNVs
Automated primer/probe design for novel CNVs
Sample Generation
QC Review
PCR Amplification
Quantify PCR Products
Digital Readout
QC Review
Data analysis with QuantaSoft
TAT: 24-96 hours
Confirmation Reports transferred to ODIN
Sign-out by board-certified lab Director in ODIN
Sample processing and workflow management for clinical validation .


WHOLE GENOME SEQUENCING (WGS)
FASTQ/BAM
VCF data
Bioinformatics phenotyping tool
Internal filters and phenotyPKIG data
PhenotyPKIG variant short list
In-house scripts
ExAC / gnomAD
Filters:
• ≥ 30x coverage
• ≤ 1% frequency
• ≤ 5% frequency at(Laboratory decided threshold)
Pseudogene piPKIGline
CNV piPKIGline
Genome
Intragenic (exon level del/dup)
Variant short list: Missesnse/Nonsense/Frameshift/ Indels /Splice site/Deep intronic/intergenic/ CNVs
Evaluate based on patient phenotyPKIG
PhenotyPKIG -related
Non-phenotyPKIG-related
Known pathogenic
Novel pathogenic
Novel missense
Insufficient Data
Pathogenic Variant
Autosomal Dominant
Autosomal Recessive
Report as pathogenic
Report as VUS
Report as diagnostic, not related to phenotyPKIG
Secondary findings
- Childhood onset
- Adult onset actionable
- Adult onset non-actionable.
Report as carrier
Pharmacogenetic variants (CPIC guidelines Class 1A)
Phenotype-related Findings
Incidental Findings

ODIN

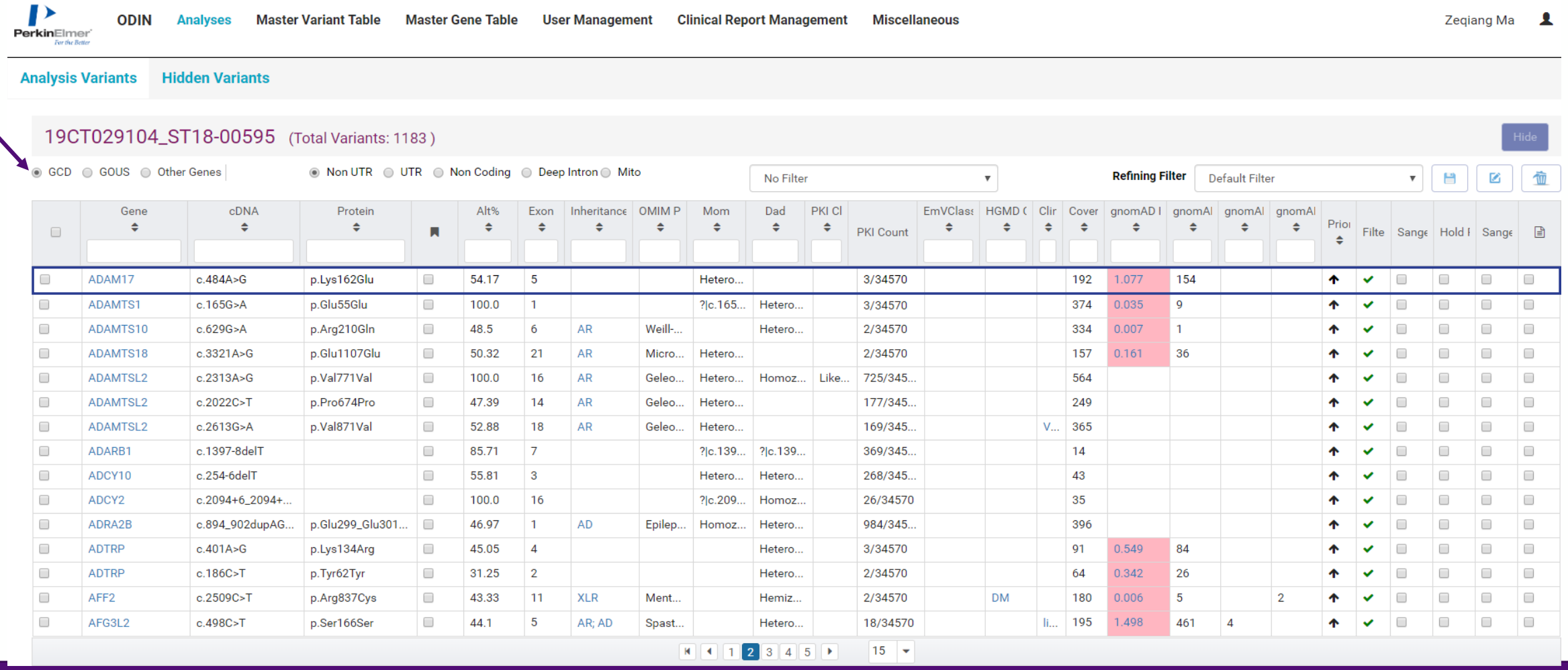
(Ordered Data Interpretation Network)

Landing page



Analysis Name	Folder Name	Flag	Project	Client	Status	File Type	Type	Excluded	Comments	Owner	Created	Modified	Actions
19CT030656_19CT0306...	/c10171data/run_209718		clinical	c10171_PKIG	Ready to review		Whole Exome	Non coding	-WES TRIO	Megan Landsv...	05-10-2019, 1...	05-10-2019, 17...	
19CT030656-76398054						PROBAND							
19CT030657-87292835	/c10171data/run_209719					MOTHER							
19CT030659-77732306	/c10171data/run_209721					FATHER							
19CT027899_19CT0278...	/c10171data/run_177923		clinical	c10171_PKIG	Approved		Whole Exome	Non coding	-TRIO Proband	Christin Collins	05-10-2019, 1...	05-13-2019, 15...	
19CT027900_19CT0279...	/c10171data/run_177922		clinical	c10171_PKIG	Approved		Whole Exome	Non coding	-TRIO Mother	Christin Collins	05-10-2019, 1...	05-13-2019, 15...	
19CT026623_19CT0266...	/c10171data/run_176645		clinical	c10171_PKIG	Variant analysi...		Whole Exome	Non coding	-Proband Only	Yang Wang	05-10-2019, 1...	05-10-2019, 15...	
19CT027500_US-GKVX-...	/c10196data/19CT027500_...		Clinical	c10196_Helix	Ready to review		Panel	Non coding	-Helix, re-uploa...	Madhuri Hegde	05-10-2019, 1...	05-10-2019, 15...	
19CT018863_19C200092	/c10171data/run_242936		Clinical	c10171_PKIG	Ready to review		Whole Exome	Non coding	-Re -analysis T...	Sara Stankiewi...	05-10-2019, 1...	05-10-2019, 13...	
19CT020199_19CT0201...	/c10171data/run_164319		Clinical	c10171_PKIG	Ready to review		Whole Exome	Non coding	DUO mother o...	Sara Stankiewi...	05-10-2019, 1...	05-10-2019, 12...	
19CT030187_19CT0301...	/c10171data/run_257287		clinical	c10171_PKIG	Ready to review		Panel	Non coding	-Sanofi	Yihao Ou	05-10-2019, 1...	05-10-2019, 12...	
19CT030284_19CT0302...	/c10171data/run_257288		clinical	c10171_PKIG	Ready to review		Panel	Non coding	-Sanofi	Yihao Ou	05-10-2019, 1...	05-10-2019, 12...	
19CT019532_19CT0195...	/c10171data/run_176795		Clinical	c10171_PKIG	Released		Whole Exome	Non coding	Re-Uploaded	Jing Xie	05-10-2019, 1...	05-13-2019, 13...	
19CT030803_19CT0308...	/c10171data/run_257109		clinical	c10171_PKIG	Ready to review		Panel	Non coding	-Any Panel	Yihao Ou	05-10-2019, 1...	05-10-2019, 12...	
19CT030327_19CT0303...	/c10171data/run_256877		clinical	c10171_PKIG	Approved		Panel	Non coding	-ASAH1	Yang Wang	05-10-2019, 1...	05-10-2019, 15...	
19CT030779_19CT0307...	/c10171data/run_256999		clinical	c10171_PKIG	Ready to review		Panel	Non coding	-Any Panel	Yihao Ou	05-10-2019, 1...	05-10-2019, 12...	
19CT030388_19CT0303...	/c10171data/run_256998		clinical	c10171_PKIG	Ready to review		Panel	Non coding	-Any Panel	Yihao Ou	05-10-2019, 1...	05-10-2019, 12...	
19CT030800_19CT0308...	/c10171data/run_253076		clinical	c10171_PKIG	Ready to review		Whole Exome	Non coding	-TRIO Father	Yihao Ou	05-10-2019, 1...	05-10-2019, 12...	

Variant Table



Variant Detail

Selected Variant is : ACADM c.985A>G(p.Lys329Glu)

Add Variant | Create Clinical Report

Details | JBrowse | Other Analyses

CLINICAL REPORT DATA

Notes

Select Variant Interpretation Template: Select Template

The c.985A>G (p.Lys329Glu) missense variant results in the substitution of the lysine codon at amino acid position 329 with a glutamic acid codon. This variant is a common *ACADM* pathogenic variant in individuals of northern European ancestry (previously referred to as p.Lys304Glu) (PMID:

PKI Classification:	Pathogenic
Frequent Artifact:	
ACMG Calculator:	
Status:	Edited
Last Modification:	6-Dec-2018 10:43:21
Modified by:	Yang Wang

Internal Comments

VARIANT

Chromosome:	1
Position:	76226846-76226846
Ref:	A
Alt:	G
Ref(%):	50.5%
Alt(%):	49.5%
Read Depth (DP):	103
Type:	snv
Filter:	PASS
Call Quality:	1429.54
Genotype Quality (gq):	99.0
dbSNP ID:	rs77931234
Zygosity:	Heterozygous

Sanger Notes

VARIANT ANNOTATIONS

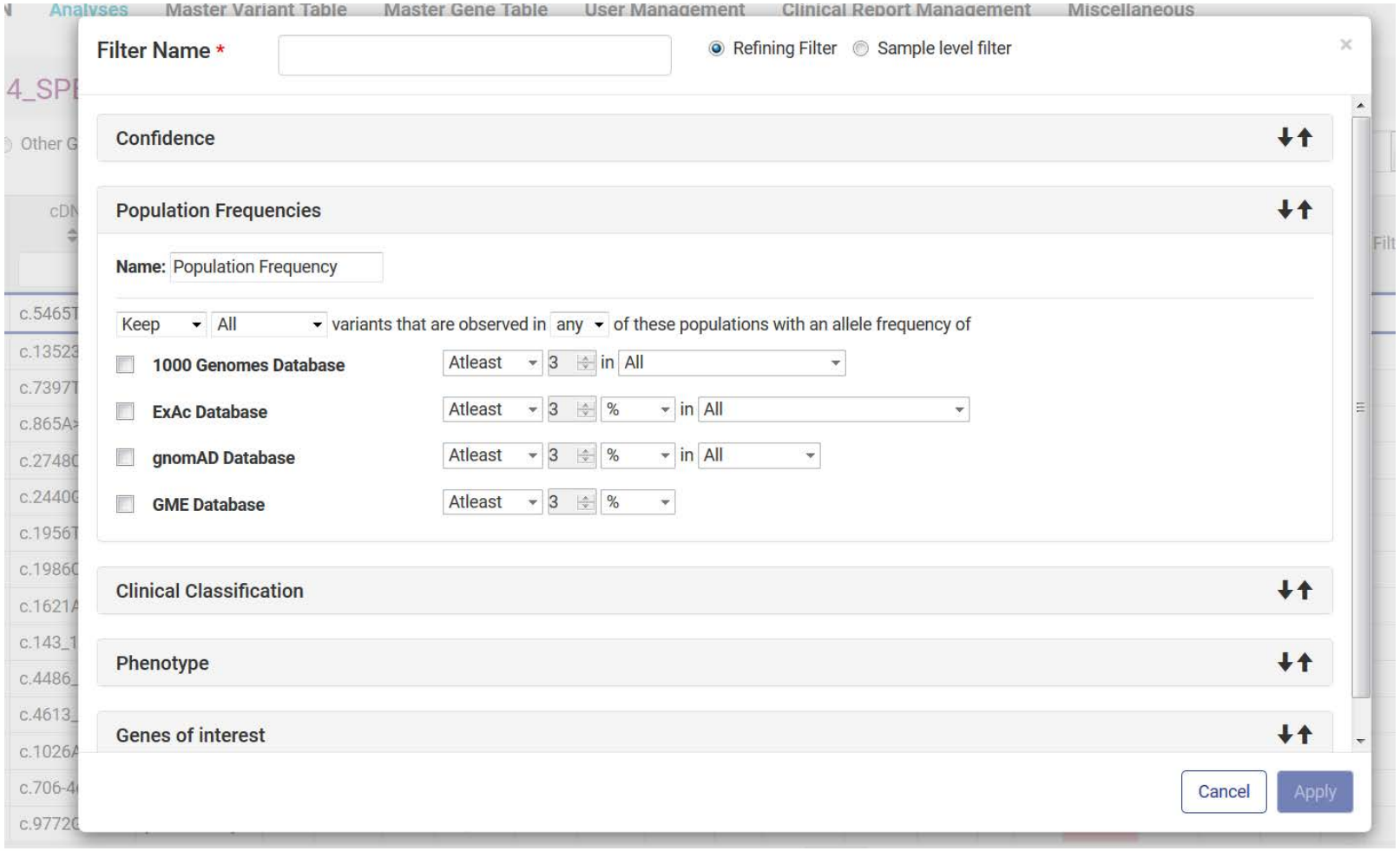
Transcript:	NM_000016.4
HGVS.c:	c.985A>G
HGVS.p:	p.Lys329Glu
Mother:	Heterozygous|c.985A>G
Father:	
Sibling 1:	
Sibling 2:	
SnpEff Type:	missense_variant
1000Genomes AF:	All=0.229%
gnomAD AF|AC:	PopMax:0.626%|699 | NFE
ExAC AF|AC:	All=0.331%|402
GME AF|AC:	0.05 % | 11985
dbSNP AF:	
PolyPhen-2:	P,B,B,B,B
SIFT:	T,T,T,T,T
Mutation Taster:	D,D,D,D,D
EmVClass:	Pathogenic,Pathogenic
HGMD:	DM

GENE ANNOTATIONS

Gene Symbol:	ACADM
Strand:	+
ENSEMBL ID:	ACADM
Inheritance:	AR
OMIM Phenotype:	Acyl-CoA dehydrogenase, medium chain, deficiency of

SAMPLE NOTES

Variant Filter

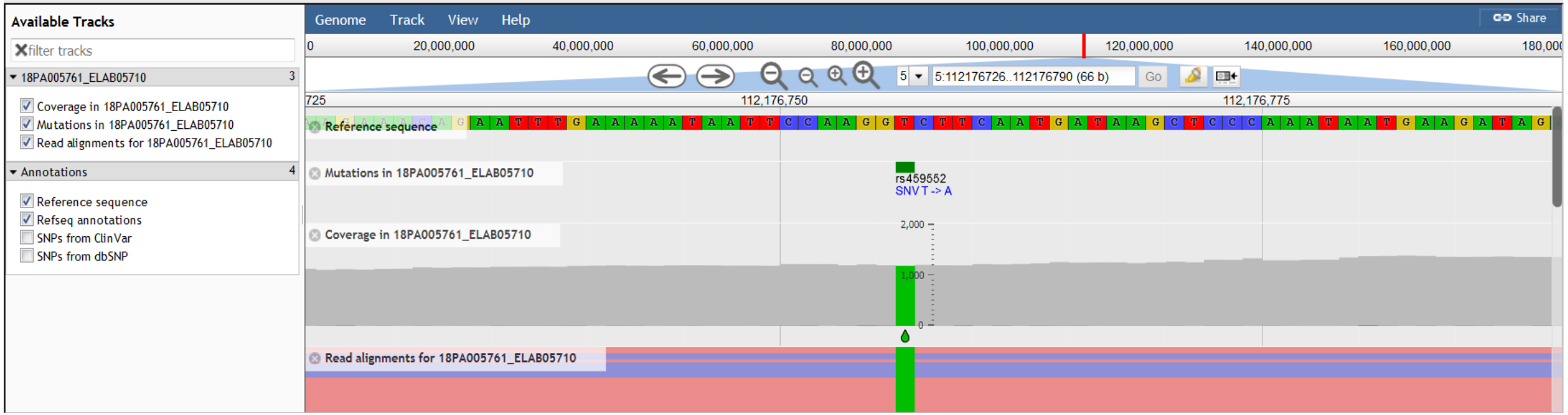


Genome Browser

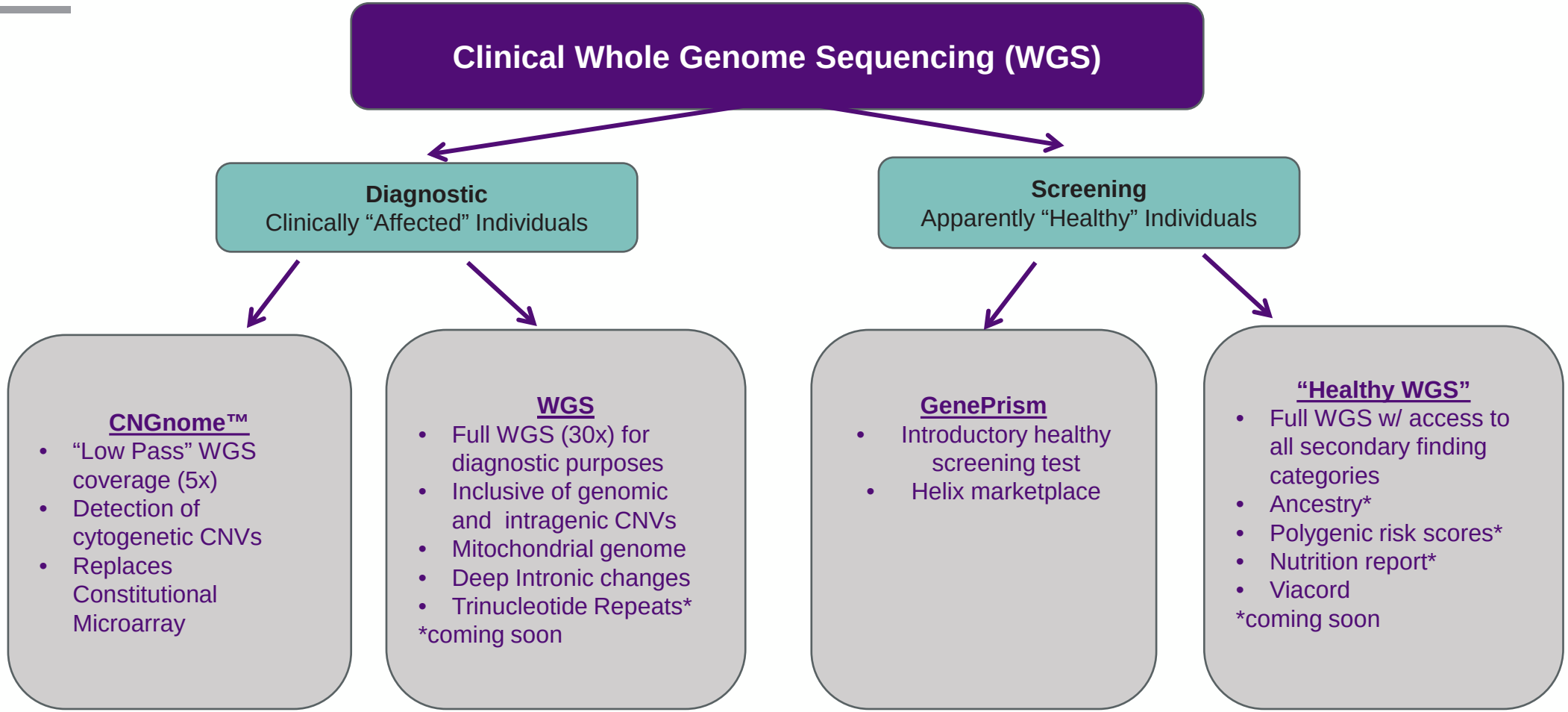
Selected Variant is : APC c.5465T>A(p.Val1822Asp)

Add Variant | Create Clinical Report

Details | JBrowse | Other Analyses



Clinical Whole Genome Sequencing



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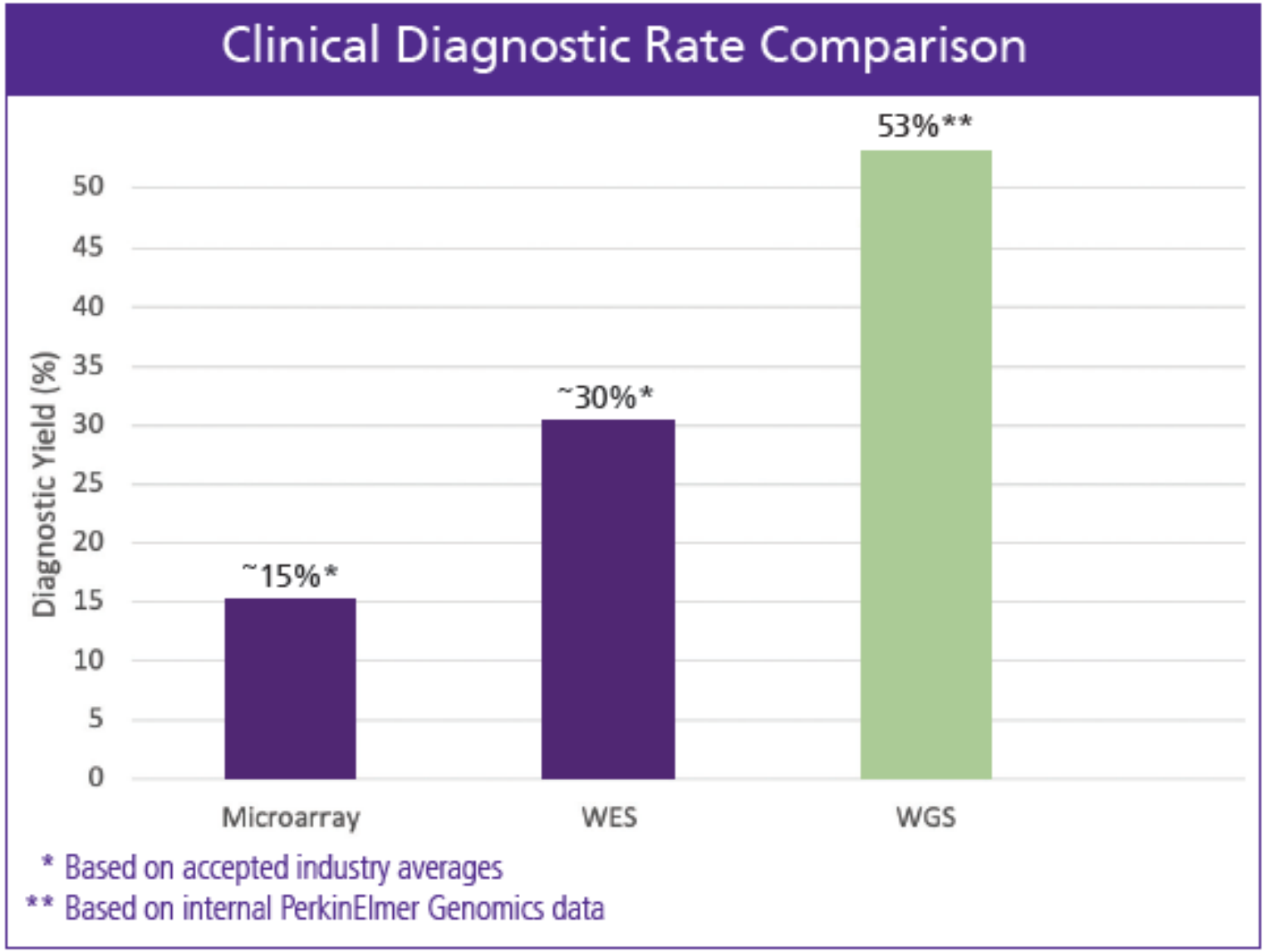
WGS (30X)- Complete Genomic Solution

Experience from first 150 WGS cases

- All clinical/diagnostic cases were subjected to 30x WGS assay (aligned to human reference genome hg19).
- 79 (53%) cases were classified with known pathogenic SNV / CNVs of clinical significance.
- 35 cases were NICU and 115 cases were pediatric.
- Pathogenic variants, SNV and CNV detected in 81 genes and two or more cases in *WDR45* and *KCNQ1* genes.
- **Dual Mendelian Diagnoses:** 2 cases with dual clinical diagnoses resulting from 2 pathogenic CNVs were identified.
- Mitochondrial pathogenic variant identified in one case.
- CNV identified in 4 cases- In addition, two cases of Trisomy 21.
- Two deep intronic pathogenic variants identified.

Increase in Diagnostic Yield



WGS case 1

7-year-old male with history of vomiting for 24 hours develops:

- Progressive encephalopathy
- Acute hyperammonemia

Previous Testing:

- Biochemical analysis consistent with OTC deficiency
- Negative *OTC* molecular studies

WGS Case 1 Results

Test Performed: Whole Genome Sequencing, Proband only

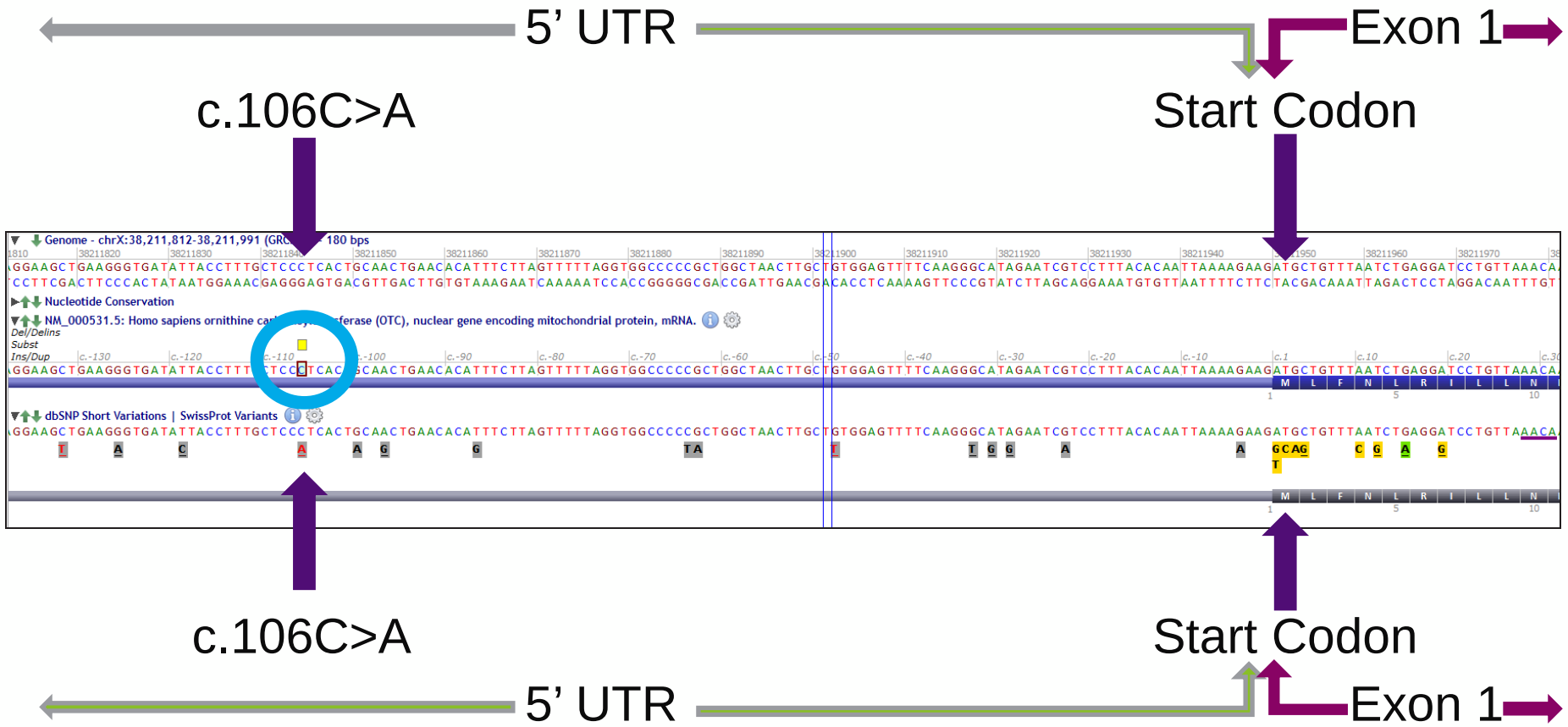
TEST RESULT SUMMARY
A hemizygous c.-106C>A *OTC* variant of unknown significance was identified in this sample. Clinical and biochemical correlation is required.

Diagnostic findings:

Gene OMIM	Associated Disease (Inheritance)	Exon/ Intron	DNA Change*	Protein Change	Zygosity	Classification
OTC 300461	Ornithine transcarbamylase deficiency (XLR)	5'-untranslated region	c.-106C>A	-	Hemizygous	Variant of Unknown Significance

XLR= X-linked recessive
* Sanger confirmation is pending.

WGS Case 1:
Finding in the 5’ Untranslated Region



From Alamut Visual

WGS Case 1:
Finding in the 5' Untranslated Region

The c.-106C>A change in the *OTC* gene is a substitution of an A for a C 106 nucleotides prior to the start codon. This change is located in the 5' untranslated region of the *OTC* gene. In general, sequences in the 5' untranslated region may be part of promoter or enhancer elements. Changes in this region have the potential to regulate DNA transcription and mRNA processing. To our knowledge, the c.-106C>A variant has not been reported in individuals with OTC deficiency, and it is rare in the general population with the minor allele frequency at about 0.03% in 1000 Genomes[1]. An *OTC* alteration upstream of the c.-106C>A variant has been reported in a female individual with signs of OTC deficiency; functional studies indicated that this change disrupted the interaction of the promoter with the enhancer[2]. There is currently insufficient evidence to determine the pathogenicity of this variant; the c.-106C>A *OTC* variant is therefore classified as a variant of unknown significance. Clinical and biochemical correlation is required.

1. https://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?type=rs&rs=rs749748052
2. Lukson et al., Hum Mutat. 2010 Apr;31(4):E1294-303. doi: 10.1002/humu.21215.

- **Previous molecular testing likely negative because sequencing didn't go this far into 5' UTR**

WGS Case 2

Proband with mild lateral and ventricular dilation suggestive of diffuse atrophy (*de novo* duplication)

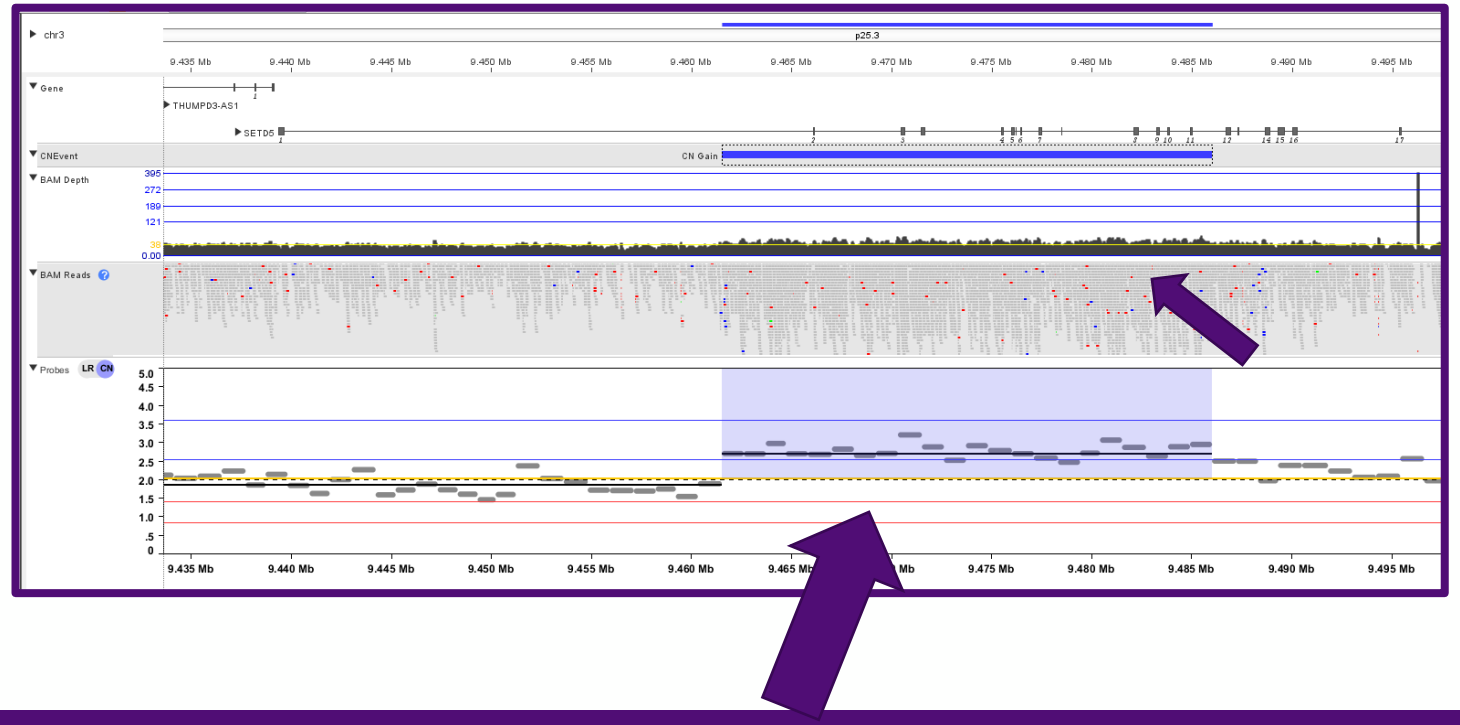
TEST RESULT SUMMARY

Preliminary Report: A duplication of *SETD5* including exons 3-14 was identified in this sample. Clinical and biochemical correlation is required.

Diagnostic findings:

Gene OMIM	Associated Disease (Inheritance)	Location of Most 5' Abnormal Probe[a]	Location of Most 3' Abnormal Probe[a]	Classification
SETD5 615761	Mental retardation, autosomal dominant 23 (AD)	g.9470573 c.-50 (5'UTR)	g.9489001 c.1782+10 (intron 14)	Variant of Unknown Significance

AD=autosomal dominant
a. Probe location does not indicate precise breakpoint.



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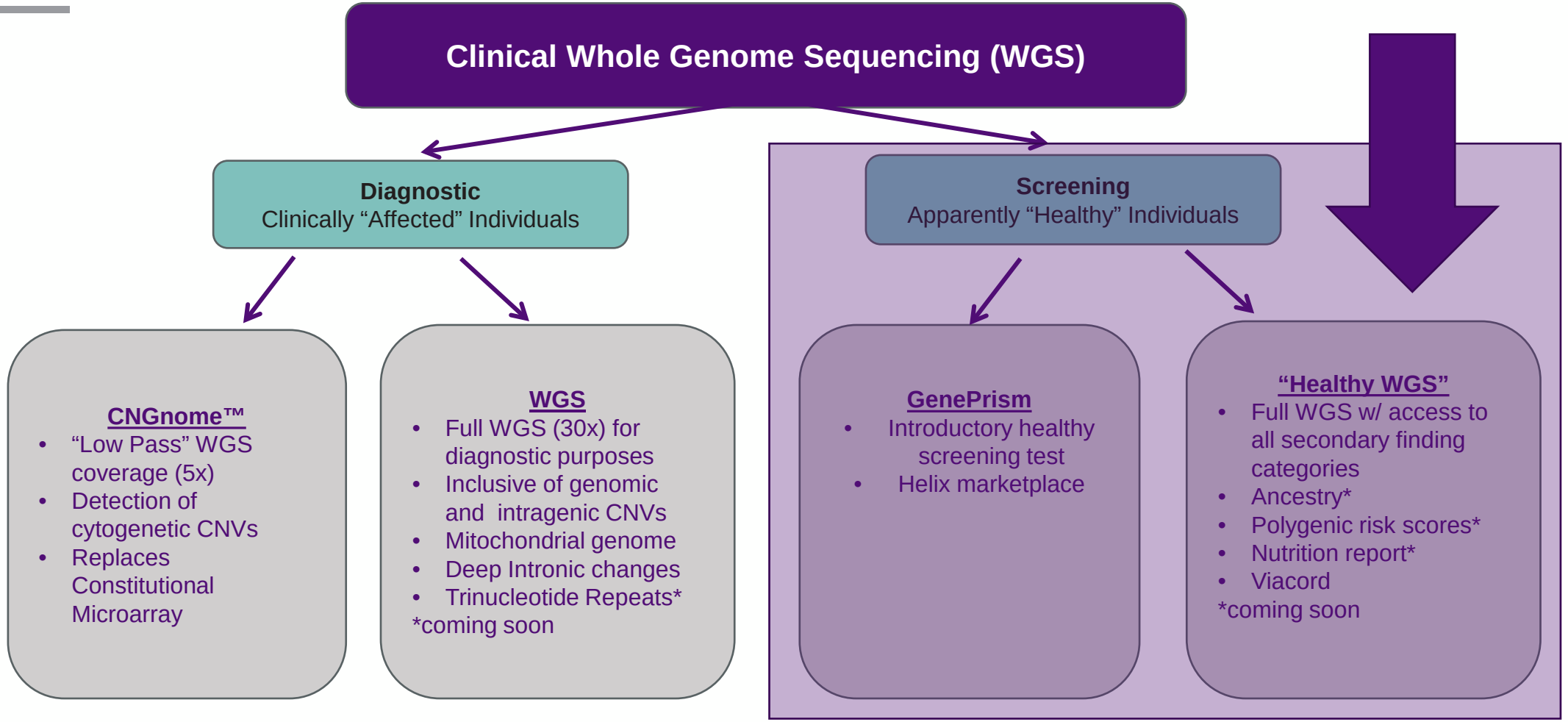
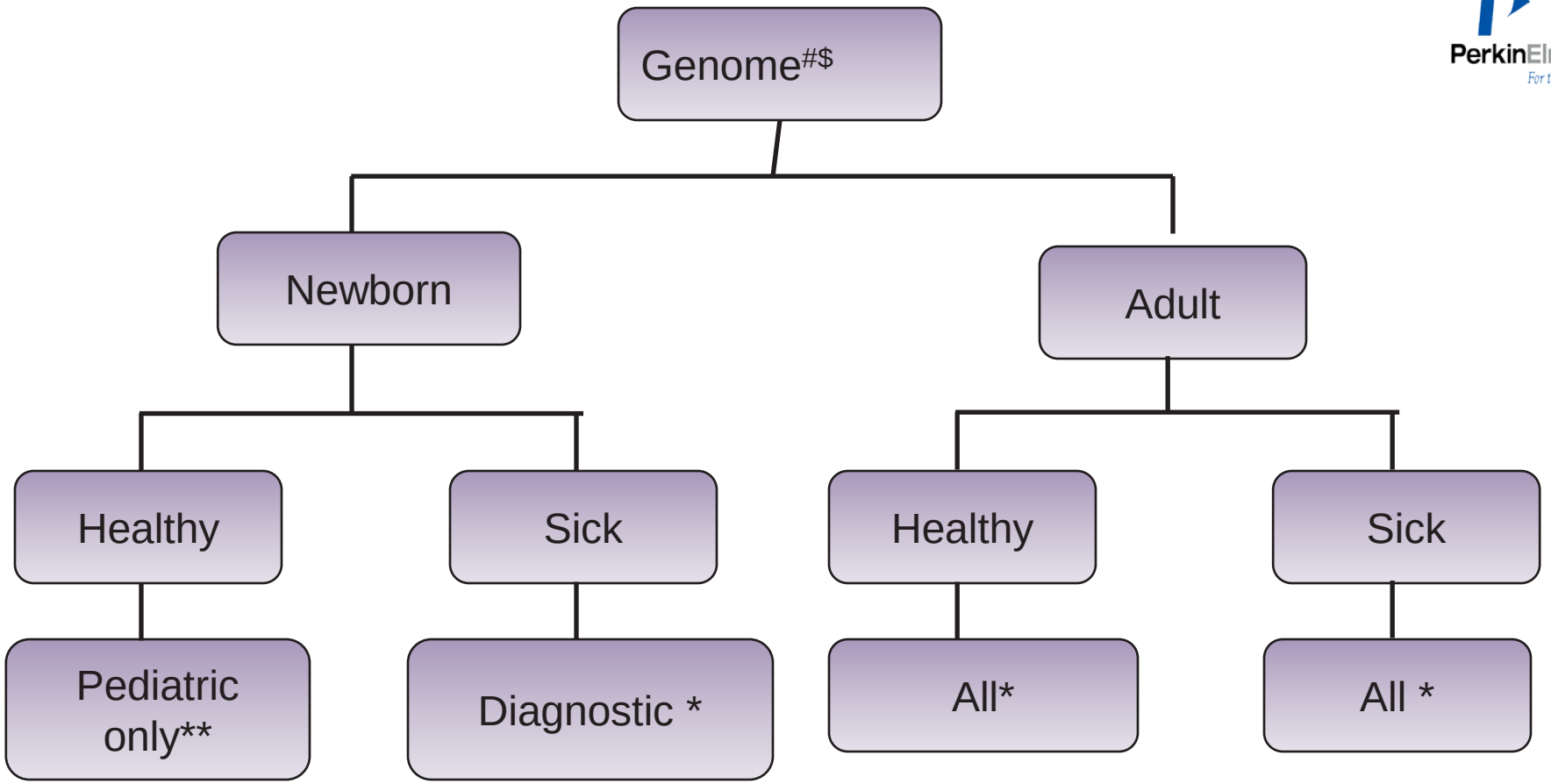
Clinical Whole Genome Sequencing

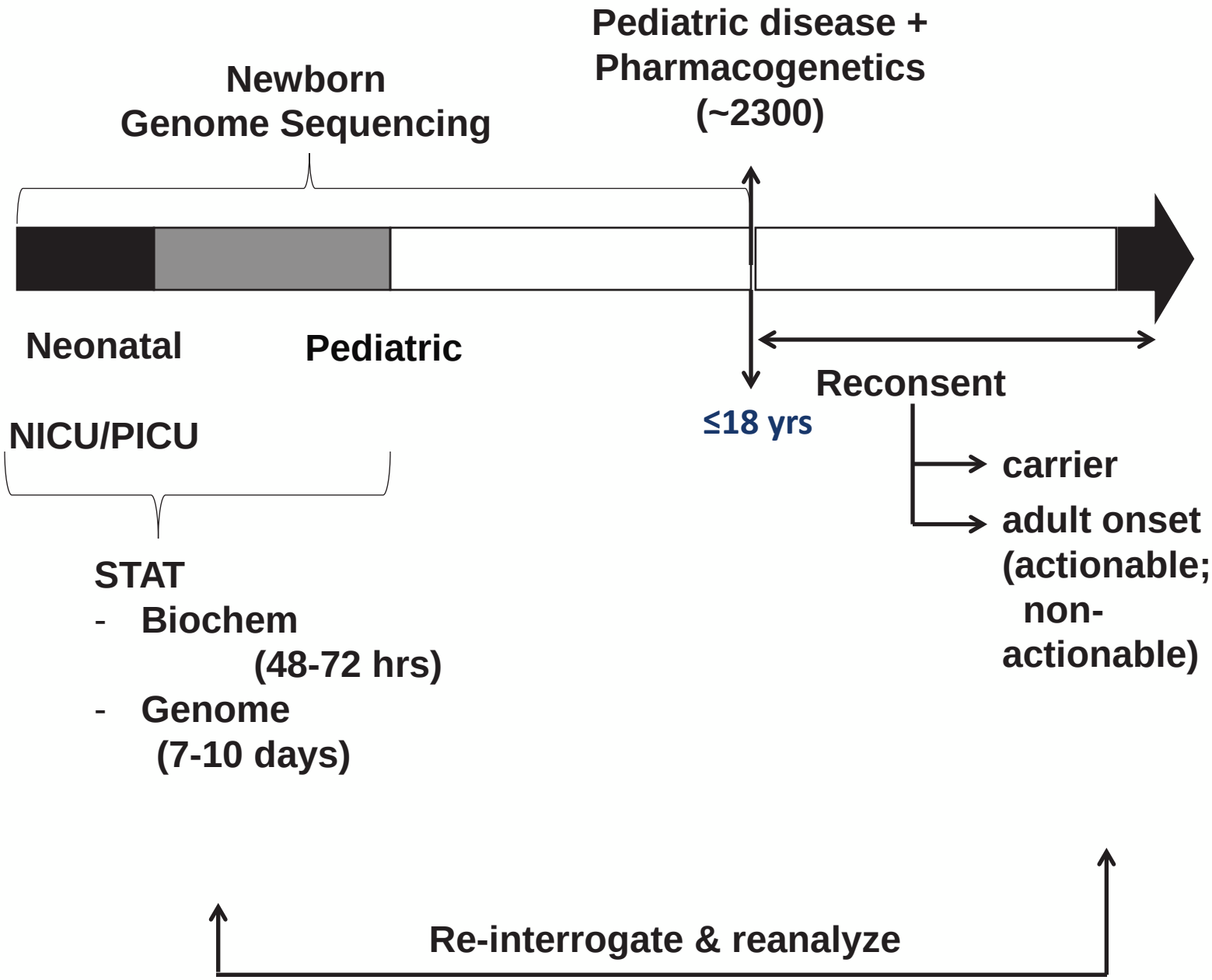




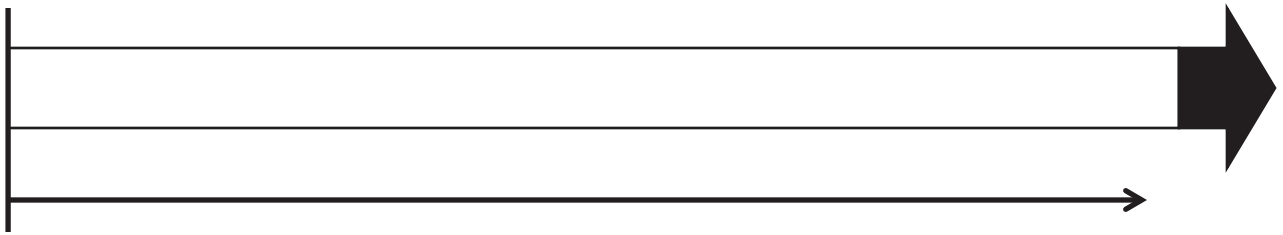
**ViaCord- Phase 1 – 386,000 units (300,000 unique families)

Phase II- Live recruitment- 25,000-30,000

*consent form
DBS, Saliva, Whole blood- Validation included Coriell samples (GAIB), 60 known positive controls, All three sample types from same individual
$ Validated on TruSeq, NovaSeq and supplemented with ancillary methods


Pediatric disease +
Pharmacogenetics
(~2300)
Newborn
Genome Sequencing
Neonatal
Pediatric
NICU/PICU
≤18 yrs
Reconsent
carrier
adult onset
(actionable;
non-
actionable)
STAT
- Biochem
(48-72 hrs)
- Genome
(7-10 days)
Re-interrogate & reanalyze

Adult
genome sequencing



≤18 yrs

Consent form driven

- Diagnostic
- Carrier
- Actionable
- Non-actionable

Re-interrogate & reanalyze

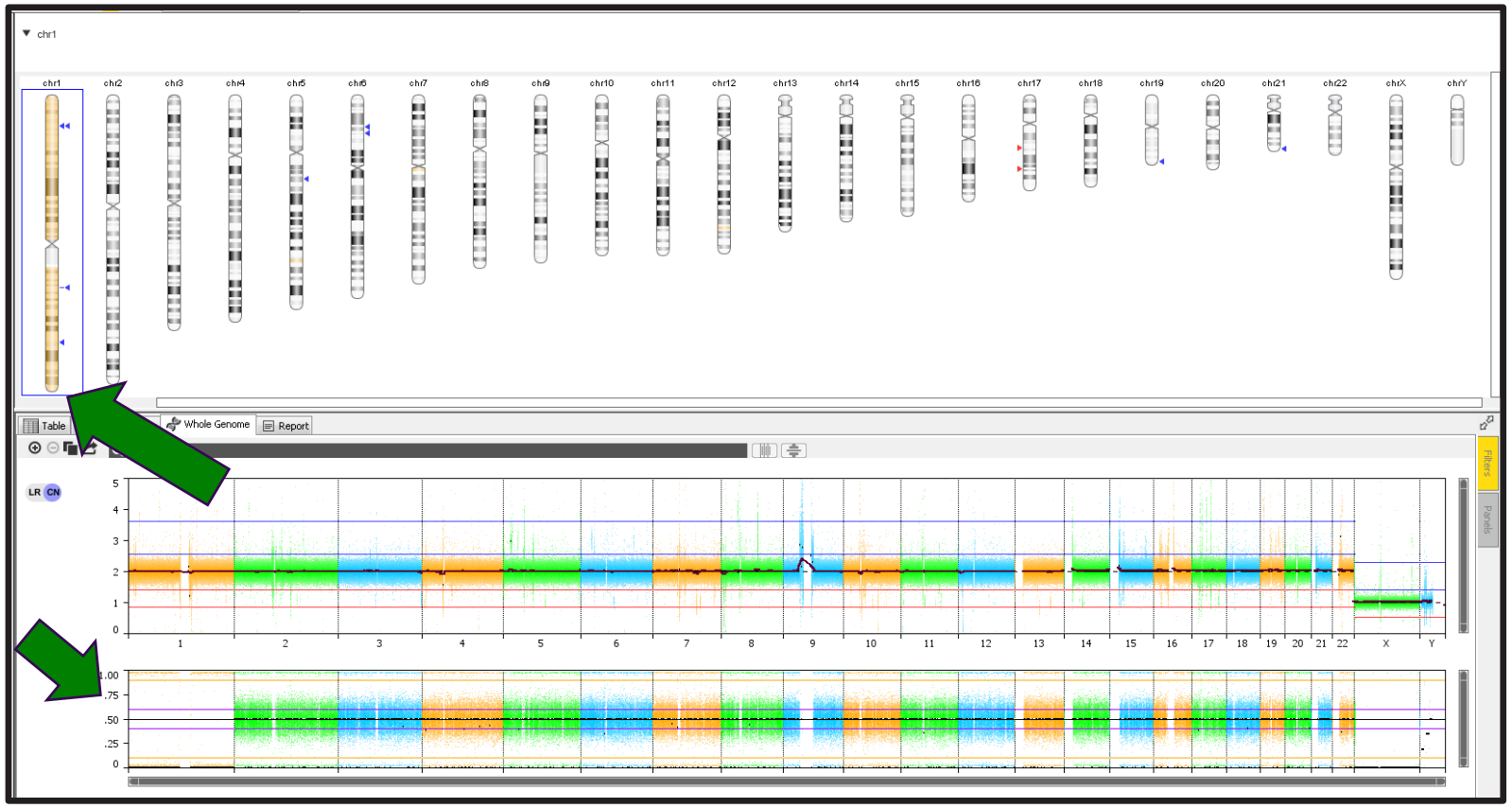
UPD 1 (isodisomy) in healthy male

48 yr old healthy Caucasian male. No clinical information

Diagnostic genomic finding- UPD1

Chromosome 1: non-imprinted

UPD1: reported with unmasking of AR disorders in literature



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UPD may or may not cause phenotypic consequences

New Results

Comment on this paper

Characterization of prevalence and health consequences of uniparental disomy in four million individuals from the general population

Priyanka Nakka, Samuel Pattillo Smith, Anne H O'Donnell-Luria, Kimberly F McManus, 23andMe Research Team, Joanna L Mountain, Sohini Ramachandran, Fah Sathirapongsasuti

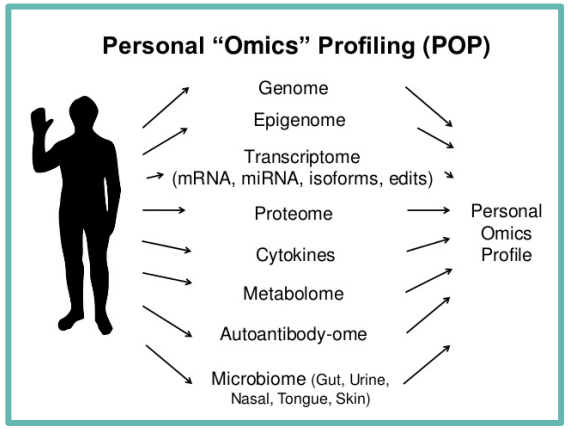
doi: https://doi.org/10.1101/540955

Phenotypic Consequences of UPD

UPD can cause phenotypic consequences in multiple ways, including 1) disrupting imprinting and 2) uncovering recessive alleles in blocks of isodisomy. We tested for phenotypic associations between UPD of each of the 23 chromosomes in true positives in the 23andMe dataset and 206 phenotypes across five categories (cognitive, personality, morphology, obesity and metabolic traits) obtained from self-reported survey answers. We found 23 nominally significant (p-value < 0.01) phenotype associations with UPD of chromosomes 1, 3, 6, 7, 8, 15, 16, 21 and 22 (Supplementary Table 2). While some of these 23 associations were driven by a single UPD case, three associations had multiple cases (or multiple measurements, in the case of quantitative traits), representing a more robust signal: we found that UPD6 is associated with lower weight (p-value = 0.0038) and shorter height (p-value = 0.0055), and UPD22 is associated with a higher risk for autism (p-value = 2.557×10^{-5}) (Table 1).

What after Genomics?

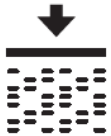
Complimenting Genomics results with other “OMIC” profiles



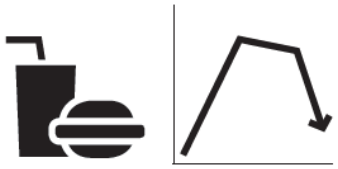
Advantages of integrating metabolomics and whole-genome sequencing

Interpret WGS-based findings
Metabolomics + adult sequencing: test molecular effects of rare mutations, including phenotype penetrance and overall burden of multiple mutations.

Newborn screening
Metabolomics + WGS for newborns: molecular readout of lipid, carbohydrate and amino acid disorders such as familial hypercholesteremia.



Catalogue comprehensive metabolic effects
Deep assessment of the molecular impact of mutations, e.g loss-of-function variants that are not fully penetrant.



Assess molecular response to exposures
Track impact of mutations in response to diet and other challenges.

PerkinElmer Genomics Global Team

Dr Madhuri Hegde
VP & CSO Global Labs

Dr Alice Tanner
Director, Laboratory Testing & Clinical Education

Dr Alka Chaubey
Head of Cytogenomics

Dr Joseph Quashnock
Laboratory Director

Dr Zeq Ma
Director Clinical Informatics

Justin Leighton
Director of Genomic Marketing

Jason Priar
Head of Global Sales & Marketing

Dr Raghu Basiboina
Director, Dx Lab Services

Nicky Xu
General Manager ICL

Calvin Thien
Director Lab Services

PJ Borandi
Site Leader

Dr Taraka Donti
Laboratory Director

Dr Jing Xie
Laboratory Director

Adam Bennett
Business Development Leader

Dr Zhili Lin
Laboratory Director

Dr Ramesh Nallamilli
Laboratory Director

Dr Akanchha Kesari
Laboratory Director

Dr Yang Wang
Laboratory Director

Dr Christin Collins
Laboratory Director

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Questions?

www.PerkinElmerGenomics.com

Genomics@Perkinelmer.com