Clinical Whole Genome Sequencing in Rare Disease

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Agenda

SECTIONS

1. The Illumina Clinical Vision
2. Burden of Rare and Undiagnosed Genetic Disease
3. Clinical Whole Genome Sequencing (WGS) Technical Overview
4. Clinical Evidence
5. TruSight Software Suite

Illumina Clinical Vision

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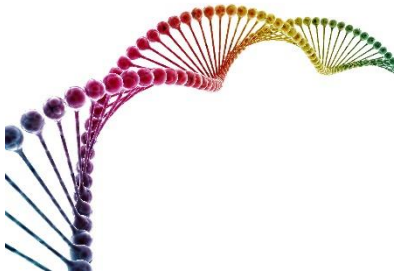
The Illumina Clinical Vision

Drive Adoption of Next-Generation Sequencing into Medical Practice

Our Driving Hypotheses

Early deployment of **whole-genome sequencing** has the potential to **reduce** the number of **unresolved complex**, **costly**, and **chronic disease cases** in newborn, pediatric, and adult medicine



The proportion of patients who would benefit from **agnostic whole-genome diagnostic testing is higher** than the number currently **receiving any form of molecular testing**

Burden of Rare and Undiagnosed Genetic Disease

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What is a Rare Disease?

Definition

United States of America

A disease or disorder that affects **fewer than 200,000** Americans at any given time

European Union

A disease affecting **fewer than 1 in 2000**

As many as

350 million people worldwide affected by rare disease

Up to 25M in U.S. alone (6-8% of population)

7000+
known rare diseases

80%
are genetic in origin

New Rare Diseases
Continue to Be Discovered

Sources:
Global Genes. https://globalgenes.org/rare-diseases-facts-statistics/
Rare Disease Day. https://www.rarediseaseday.org/
Eurodis. https://www.eurordis.org/
NIH Genetics and Rare Disease (GARD): https://rarediseases.info.nih.gov/diseases/pages/31/faqs-about-rare-diseases

Rare Disease

A major unmet need

Children are Significantly Impacted Patient Population

50%
of rare diseases affect children

33.3%
admitted to NICU have an underlying genetic disease

30%
affected children die by age five

4.5%
children annually present with a genetic condition

Average pathway to diagnosis for patient with RUGD to receive a proper diagnosis

8 physicians* **7.6** years **2–3** misdiagnoses

*(4 primary care, 4 specialists)

Sources:
Global Genes. https://globalgenes.org/rare-diseases-facts-statistics/

Clinical WGS Technical Overview

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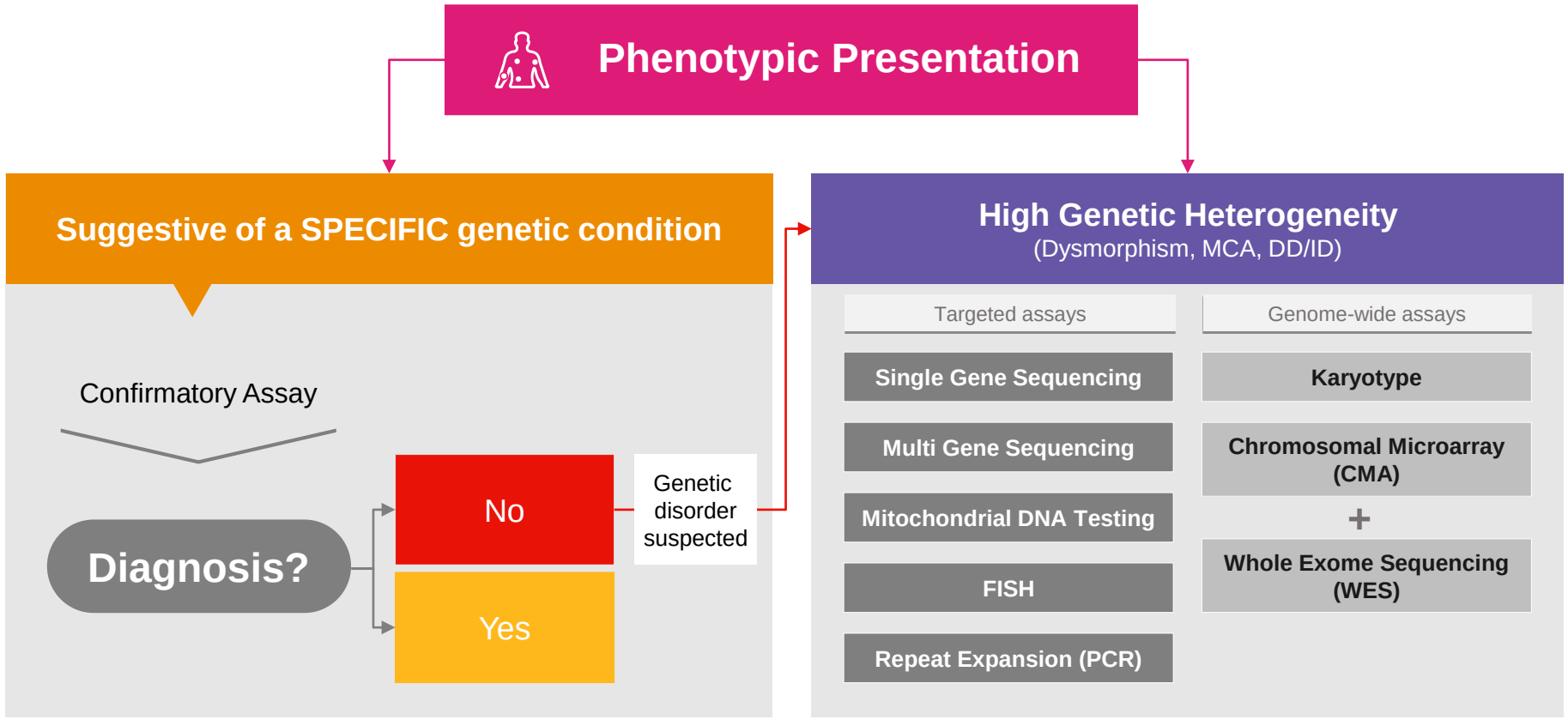
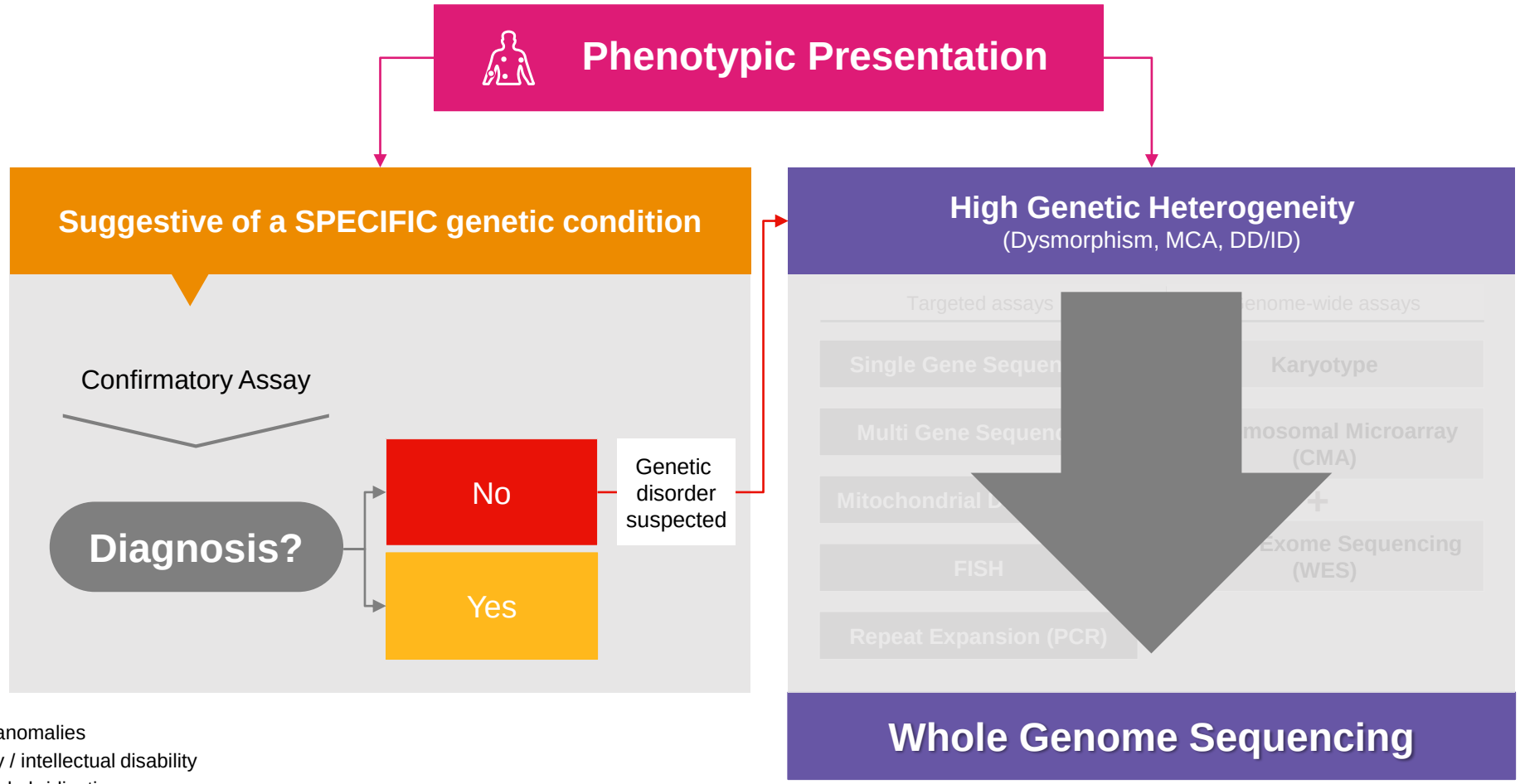
Rapid End to the Diagnostic Odyssey



MCA: Multiple congenital anomalies
DD/ID: Development delay / intellectual disability
FISH: Fluorescence in situ hybridization

Sun F, Oristaglio J, Levy SE, et al. Genetic testing for developmental disabilities, intellectual disability, and autism spectrum disorder. Technical Brief Number. 2015;23,(2):531.

Rapid End to the Diagnostic Odyssey



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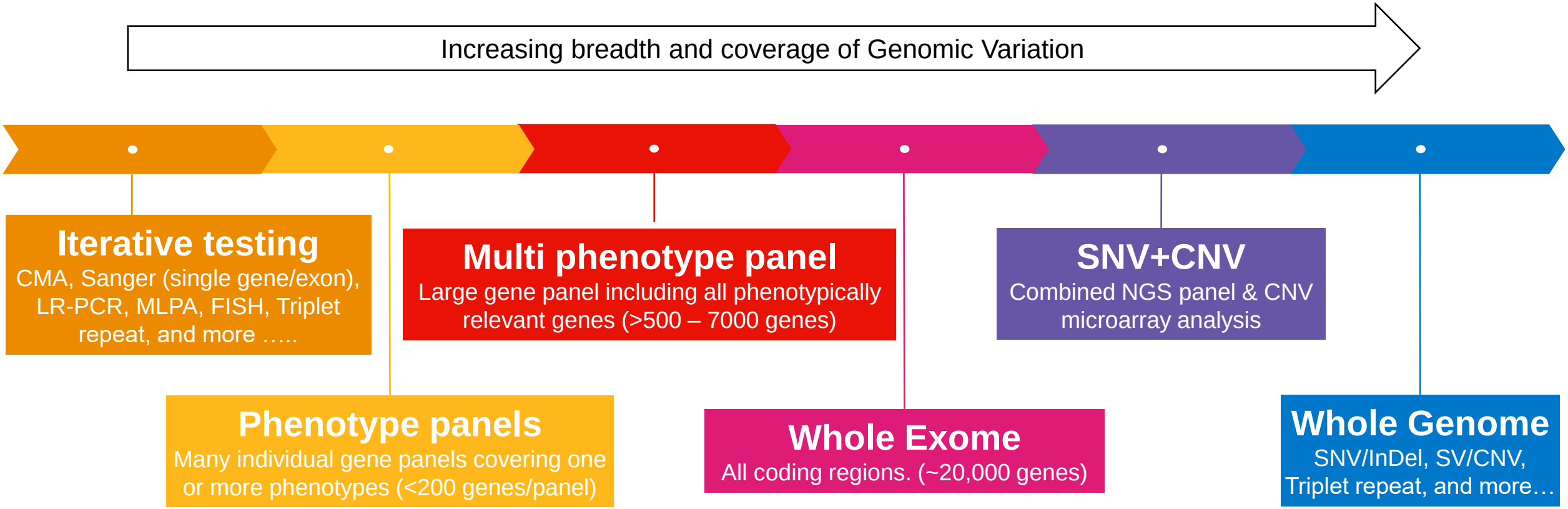
Evolution of Genetic Disease Testing



WGS Offers the Broadest Scope of Detectable Variants to Provide Comprehensive Variant Analysis

Current testing options	SNVs and indels	CNVs	Repeat expansions	Structural variants	Mitochondrial variants
Sanger	●				●
Targeted NGS	●	LIMITED			●
PCR	●	●	●		
FISH		●		●	
Karyotype				●	
CMA		●			
WES	●	LIMITED		LIMITED	●
WGS	●[1]	●[1]	●[2]	●[3]	●[1]

CMA=chromosomal microarray; CNV=copy number variant; FISH=fluorescence in situ hybridization; Indel=small insertion/deletion; NGS=next-generation sequencing; PCR=polymerase chain reaction; SNV=single nucleotide variant; WES=whole-exome sequencing; WGS=whole-genome sequencing.

References: 1. Lionel AC, Costain G, Monfared N, et al. Improved diagnostic yield compared with targeted gene sequencing panels suggests a role for whole-genome sequencing as a first-tier genetic test. *Genet Med*. 2018 Apr;20(4):435-443. doi: 10.1038/gim.2017.119. Epub 2017 Aug 3. **2.** Dolzhenko E, van Vugt JJFA, Shaw RJ, et al. Detection of long repeat expansions from PCR-free whole-genome sequence data. *Genome Res*. 2017;27(11):1895-1903. doi: 10.1101/gr.225672.117. **3.** Chen X, Schulz-Trieglaff O, Shaw R, et al. Manta: rapid detection of structural variants and indels for germline and cancer sequencing applications. *Bioinformatics*, 2016;32(8):1220–1222. http://doi.org/10.1093/bioinformatics/btv710.

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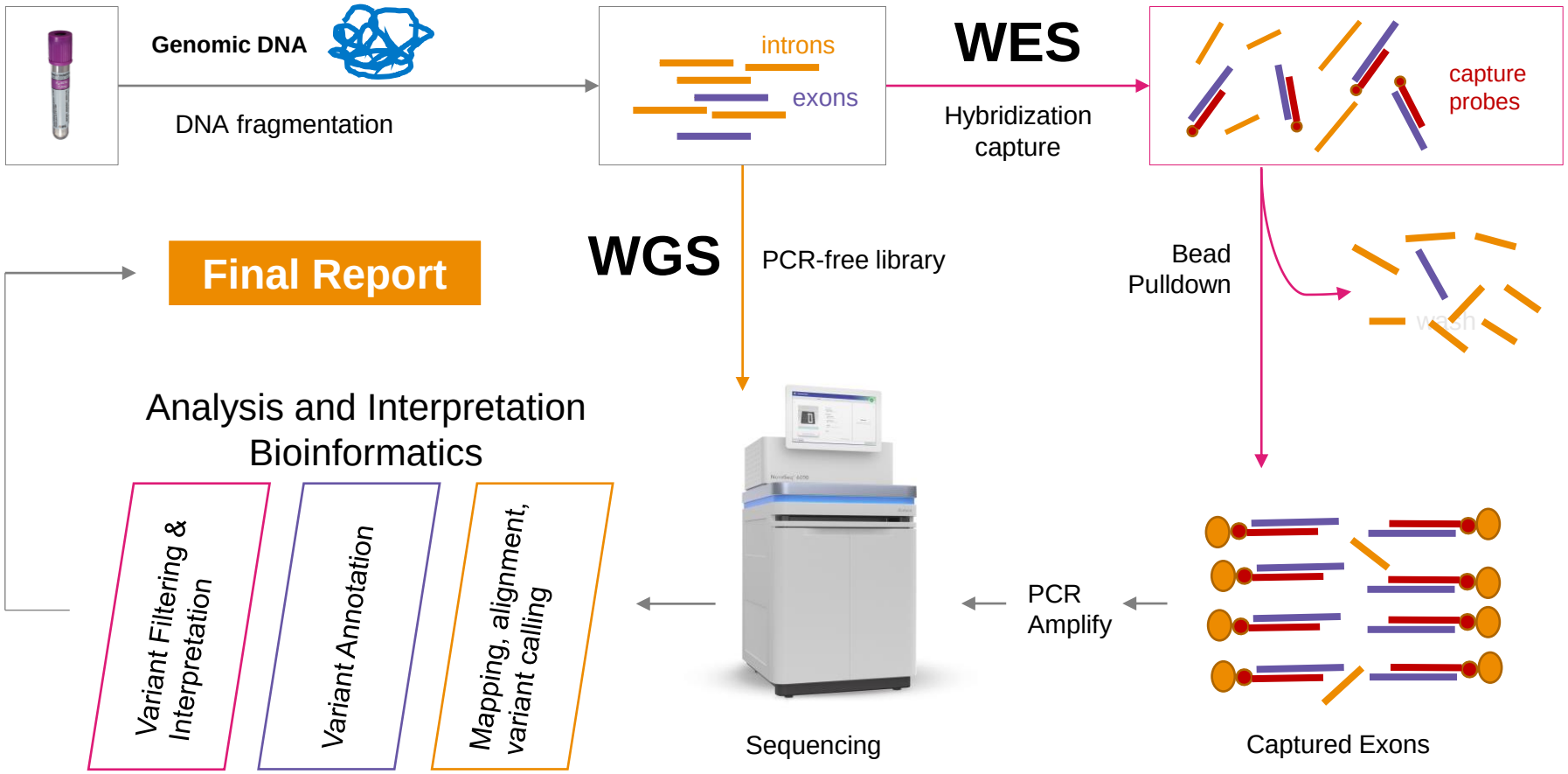
Genome vs Exome Sequencing

Simplified illustration of contrasting workflows



A PCR-Free Library Method Makes WGS Workflow Simpler and Faster than Conventional Approach

WES

STEP 1 Sample

STEP 2 DNA fragmentation

STEP 3 Hybridization capture

STEP 4 Bead pulldown/wash

STEP 5 Captured exons

STEP 6 PCR amplification

STEP 7 Sequencing

STEP 8 Analysis and interpretation bioinformatics

STEP 9 Final report

WGS

STEP 1 Sample

STEP 2 DNA fragmentation

STEP 3 Sequencing

STEP 4 Analysis and interpretation bioinformatics

STEP 5 Final report

PCR=polymerase chain reaction; WES=whole-exome sequencing; WGS=whole-genome sequencing.

WGS is the Best Tool to Aid Diagnosis of Genetic Syndromes

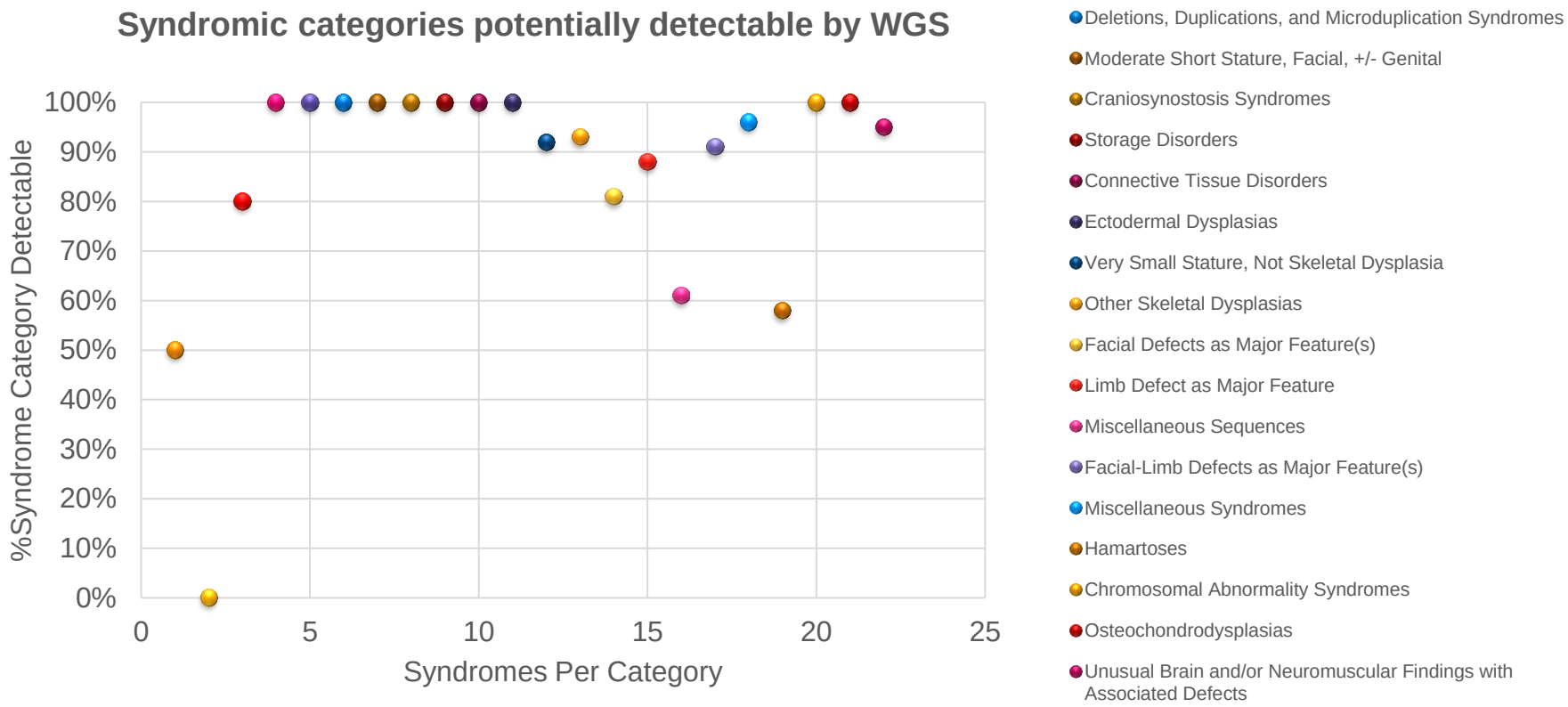
Leveraging the most comprehensive clinical genetic test

WGS can detect genetic variants associated with

88%

of non-teratogenic syndromes represented in Smith's Recognizable Patterns of Human Malformation

Internal source, courtesy of John Belmount



WGS May Capture Common Types of Pathogenic Variation

Broad scope of variants beyond the coding regions

	Coding/ Non-coding	Disruptive or Loss of Function (LOF)	Found by exome sequencing	Found by genome sequencing
5′/3′ UTR variation	Non-coding	No	Yes	Yes
Synonymous variation	Coding	No	Yes	Yes
Non-synonymous variation	Coding	Yes	Yes	Yes
Out-of-frame insertion/deletions	Coding	Yes, LOF	Yes	Yes
Stop loss/gains	Coding	Yes, LOF	Yes	Yes
In-frame insertion/deletions	Coding	Yes	Yes	Yes
Splice donor/acceptors	Coding	Yes, LOF	Yes	Yes
Intronic variation	Non-coding	No	Sometimes	Yes
Upstream promoter variation	Non-coding	No	Sometimes	Yes
Proximal downstream variation	Non-coding	No	Sometimes	Yes
Distal enhancer/repressor variation	Non-coding	No	No	Yes
microRNA variation	Non-coding	No	No	Yes
cis-Regulatory element (CRE) variation	Non-coding	No	No	Yes
Large structural variation	Both	Yes	Sometimes	Yes

29% of variants curated within Online Mendelian Inheritance in Man (OMIM) and ClinVar databases are estimated to be either upstream or downstream of their target gene.[1]

1. Zappala Z, & Montgomery,SB. Non-coding loss-of-function variation in human genomes. *Human Heredity*, 2017; 81(2): 78–87. http://doi.org/10.1159/000447453

WGS Effectively Calls CNVs

Studies comparing CNV yield from CMA and WGS

All previously reportable CNVs were identified with WGS[1]

Prospective study of >100 pediatric patients

WGS identified all clinically relevant CNVs identified by CMA, & additional potentially pathogenic CNVs < 20 kb[2]

Direct comparison for detection of pathogenic CNVs from 17 Coriell cell lines

	Coriell Variants	Size Range	Detected by Microarray	Detected by WGS
Deletion	19	~13kb – >1MB	15 (79%)	**17 (89%)**
Duplication	14	~10kb - triploidy	10 (71%)	**14 (100%)**
All	33		23 (70%)	**31 (94%)**

Illumina,Inc. Data on File

*Based on 50% overlap of coordinate calls. a) Excluding 3 CNVs suspected to be falsely annotated for the Coriell sample

1. Lionel, A. C., Costain, G., Monfared, N., Walker, S., Reuter, M. S., Hosseini, S. M., … Marshall, C. R. (2017). Improved diagnostic yield compared with targeted gene sequencing panels suggests a role for whole-genome sequencing as a first-tier genetic test. *GENETICS in MEDICINE*, *0*(August), 1–9. https://doi.org/10.1038/gim.2017.119
2. Trost, B., Walker, S., Wang, Z., Thiruvahindrapuram, B., MacDonald, J. R., Sung, W. W. L., … Scherer, S. W. (2018). A Comprehensive Workflow for Read Depth-Based Identification of Copy-Number Variation from Whole-Genome Sequence Data. *The American Journal of Human Genetics*, 102(1), 142–155. https://doi.org/10.1016/j.ajhg.2017.12.007

Clinical WGS Published Evidence Base

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Some Challenges for WGS Implementation

Areas of focus for future test adoption

Informatics

Supporting evidence for validated algorithms

Data storage bottlenecks

Interpretation

Incidental findings, variants of unknown significance

Periodic data reanalysis

Professional Governance

Recommendations and guidelines for use

Evidence for clinical utility and health economic benefits

Access to Testing

Access to testing facility offering clinical WGS

Payer reimbursement

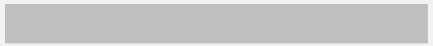
In NICU and PICU Patients, WGS Shortened Median Time to Diagnosis by 87.8% vs Standard Testing

Results from NSIGHT1

Median time to diagnosis:

13 days with WGS

vs **107 days** with standard testing

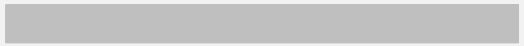


- WGS: 1-84 days
- Standard testing: 21-429 days

Median day of life (DOL) at diagnosis:

25 days with WGS

vs **130 days** with standard testing



- WGS: 14-90 days old at diagnosis
- Standard testing: 37-451 days old at diagnosis

Faster time to diagnosis can facilitate decision-making, alter management recommendations, and contribute to care plans

Study design: Infants of age <4 months from level IV neonatal and pediatric intensive care units with clinical features suggestive of genetic disease were randomized to 1 of 2 cohorts: standard diagnostic tests (n=33) vs standard tests and trio rapid WGS (n=32). Day of life at enrollment was 22.8 for the WGS arm and 22.0 for the standard test arm. Standard testing included chromosomal microarray, multi-gene panel, whole-exome sequencing, and methylation analysis.

NICU=neonatal intensive care units; PICU=pediatric intensive care units; WGS=whole-genome sequencing.

Reference: 1. Petrikin JE, Cakici JA, Clark MM, et al. The NSIGHT1-randomized controlled trial: rapid whole-genome sequencing for accelerated etiologic diagnosis in critically ill infants. *NPJ Genom Med.* 2018 Feb 9;3:6. doi: 10.1038/s41525-018-0045-8.

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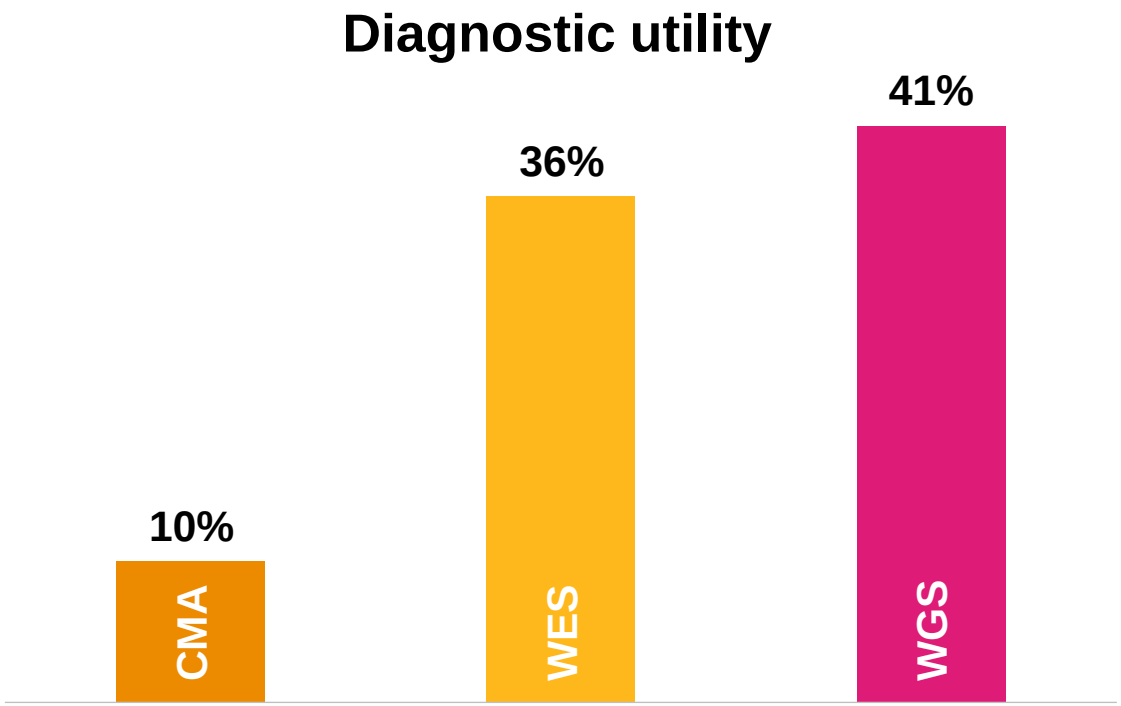
WGS and WES Offer Significant Improvements in Diagnostic Success vs CMA

8.3X GREATER ODDS OF DIAGNOSIS WITH WGS/WES*

In a meta-analysis of literature from January 2011 to August 2017, 37 studies comprising 20,068 children were included for review of diagnostic utility of 3 testing approaches: CMA, WES, and WGS.



*95% CI: 4.7-14.9, *P*<0.0001.

CMA=chromosomal microarray; WES=whole-exome sequencing; WGS=whole-genome sequencing.

Reference: 1. Clark MM, Stark Z, Farnaes L, et al. Meta-analysis of the diagnostic and clinical utility of genome and exome sequencing and chromosomal microarray in children with suspected diseases. *NPJ Genom Med*. 2018 Jul 9;3:16. doi: 10.1038/s41525-018-0053-8.

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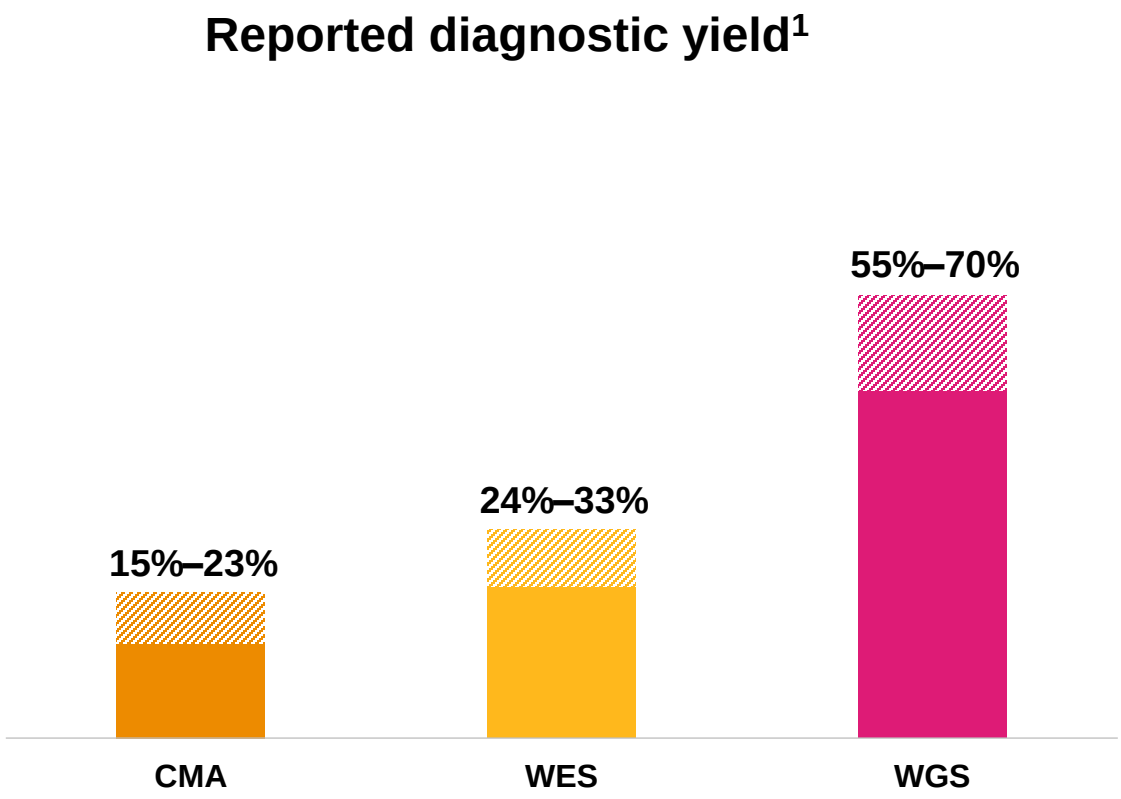
WGS Has Been Shown to Improve Diagnostic Yield vs WES and CMA



- **WGS** improved diagnostic yield by **32%–55%** vs CMA[1]
- **WES** improved diagnostic yield by up to **18%** vs CMA[1]

CMA=chromosomal microarray; WES=whole-exome sequencing; WGS=whole-genome sequencing.

Reference: 1. Vissers LE, Gilissen C, Veltman JA. Genetic studies in intellectual disability and related disorders. *Nat Rev Genet.* 2016;17:9-18.

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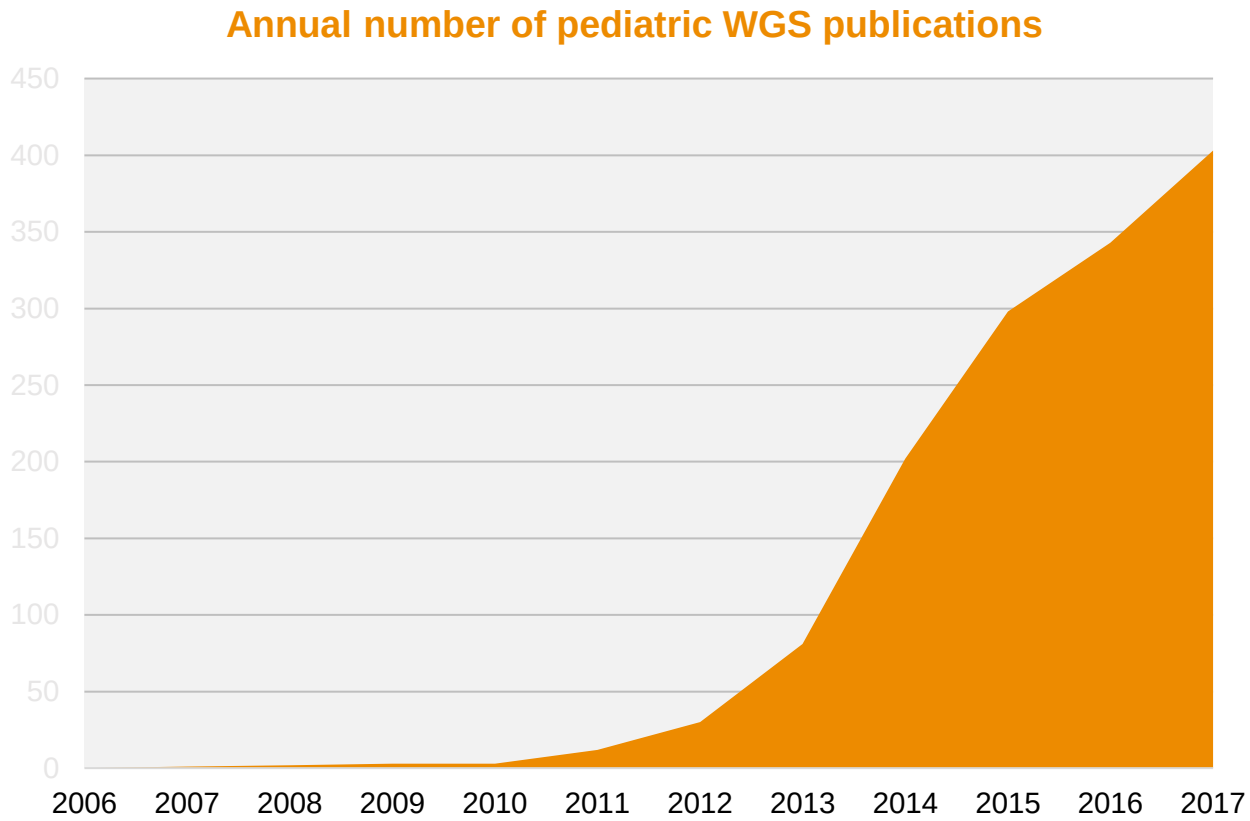
Since 2012, Interest in WGS Pediatric Application has Grown by Over 1300%[1]



WGS continues to be a rapidly evolving and dynamic area for innovation in pediatric clinical care

WGS=whole-genome sequencing.

Reference: 1. Alexandru Dan Corlan. Medline trend: automated yearly statistics of PubMed results for “pediatric whole-genome sequencing”, 2018. Web resource at URL:http://dan.corlan.net/medline-trend.html. Accessed June 13, 2018.

WGS as a Better Exome Than WES

University of Zurich

Key Author Conclusions and Takeaways

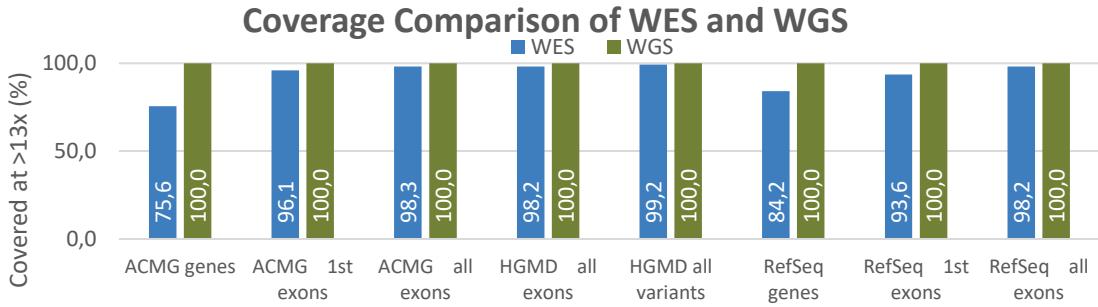
- WGS, which forgoes capturing, is less sensitive to GC content and more likely to provide complete coverage of the entire coding region of the genome.
- WGS will likely replace array techniques in CNV detection whereas WES may not.

Methods

sample comparison of optimal WES (using Agilent SureSelect v5 + UTR capturing) to WGS (using Illumina's TruSeq PCR-free WGS library preparation).

Evidence and Proof Points

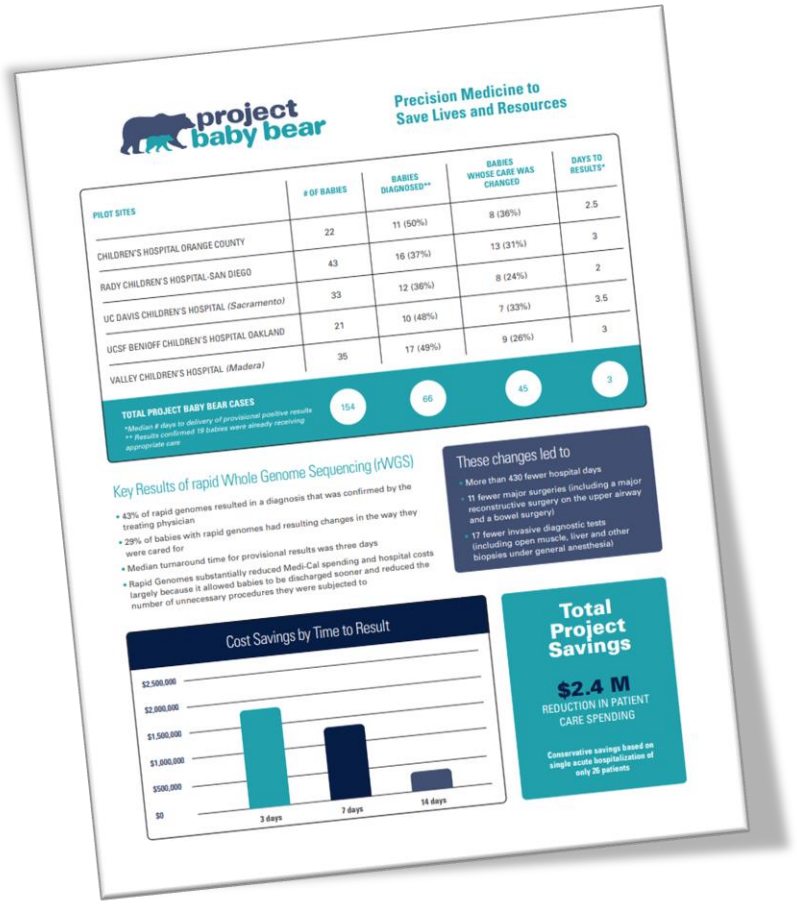
- Since PCR-free WGS is much less sensitive to GC content, it provides more uniform coverage than WES and is not susceptible to the biases of capturing design and enrichment.
- WES may miss 0.81 % (863/106,819) of disease mutations detectable by WGS.



Meienberg J, Bruggmann R, Oexle K, Matyas G. Clinical sequencing: is WGS the better WES? *Hum Genet*. 2016;135(3):359-362.

Project Baby Bear

In 2018 the State of California launched this $2M program to provide rapid whole-genome sequencing to critically-ill newborns



PILOT SITES	# OF BABIES	BABIES DIAGNOSED**	BABIES WHOSE CARE WAS CHANGED	DAYS TO RESULTS*
CHILDREN'S HOSPITAL ORANGE COUNTY	22	11 (50%)	8 (36%)	2.5
RADY CHILDREN'S HOSPITAL-SAN DIEGO	43	16 (37%)	13 (31%)	3
UC DAVIS CHILDREN'S HOSPITAL *(Sacramento)*	33	12 (36%)	8 (24%)	2
UCSF BENIOFF CHILDREN'S HOSPITAL OAKLAND	21	10 (48%)	7 (33%)	3.5
VALLEY CHILDREN'S HOSPITAL *(Madera)*	35	17 (49%)	9 (26%)	3
TOTAL PROJECT BABY BEAR CASES	154	66	45	3

**Median # days to delivery of provisional positive results*
*** Results confirmed 19 babies were already receiving appropriate care*

These changes led to

- More than 430 fewer hospital days
- 11 fewer major surgeries (including a major reconstructive surgery on the upper airway and a bowel surgery)
- 17 fewer invasive diagnostic tests (including open muscle, liver and other biopsies under general anesthesia)

Total Project Savings

$2.4 M
REDUCTION IN PATIENT CARE SPENDING

Conservative savings based on single acute hospitalization of only 26 patients

1. Project Baby Bear Interim Outcomes. March 11, 2020. https://www.radygenomics.org/wp-content/uploads/2020/03/PBB_TwoPager_Overview_v3.pdf

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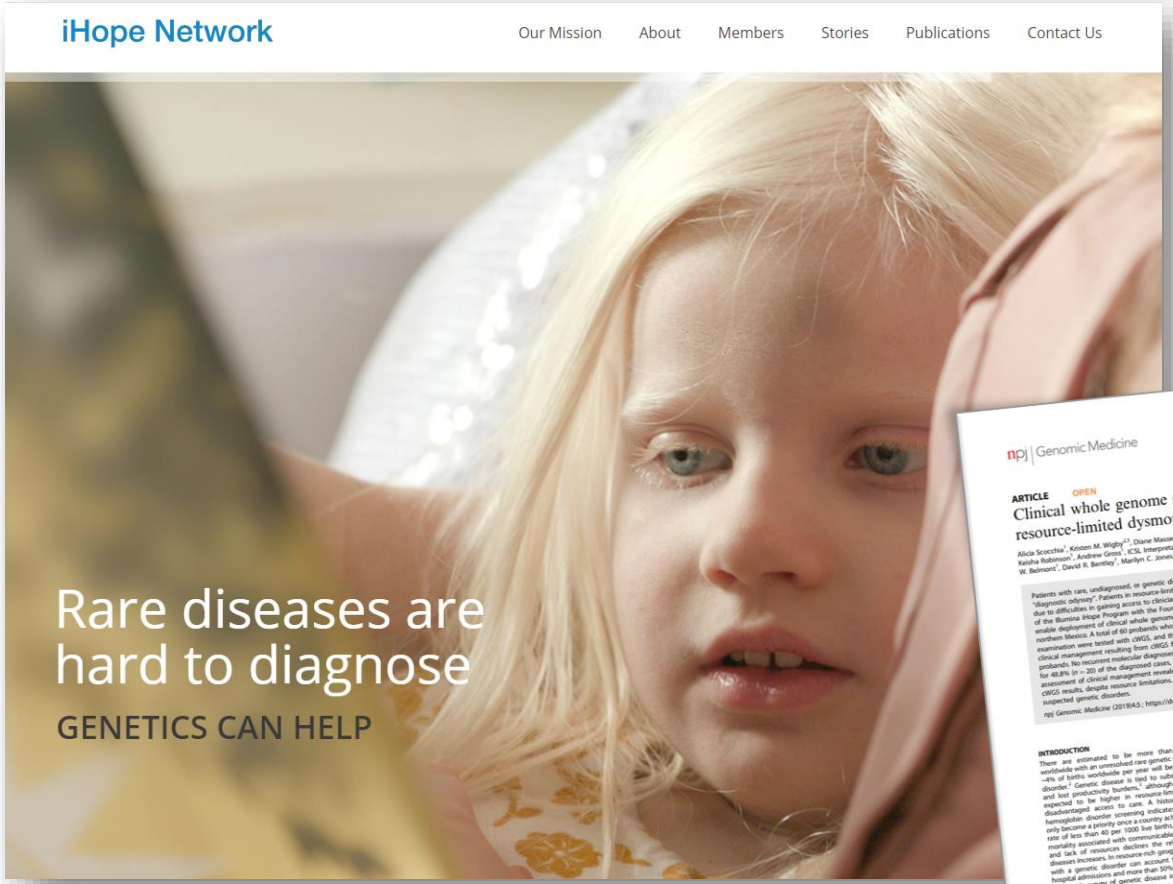
The iHope Network



Mission – *Every Diagnosis Matters*

Donated whole-genome sequencing (WGS) tests can give underserved children and their families a way to find answers about their rare and undiagnosed genetic diseases.

Clinical whole genome sequencing as a first-tier test at a resource-limited dysmorphology clinic in Mexico

February 2019

- n = 60 (1 proband-only, 14 duos, 42 trios, 3 quads)
- Minimal prior genetic testing
- Diagnostic yield = 68.3% (41 cases)
  - SNVs: 43.9% (18)
  - CNVs <18Mb: 24.4% (10)
  - CNVs >18Mb: 12.2% (5)
  - UPD: 2.4% (1)
  - Indel: 4.9% (2)
  - Multiple types: 7.2% (3)

1. The iHope Network Homepage: https://ihopenetwork.org/
2. Scocchia A, Wigby K, et al. *Genomic Medicine*. 2019 https://www.nature.com/articles/s41525-018-0076-1

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Undiagnosed Diseases Network (UDN)

The UDN is a study funded by the NIH to bring together clinical and research experts to solve the most challenging medical mysteries using advanced technologies.

Network

- 12 clinical sites (incl. University of Utah)
- 1 biorepository
- 1 coordinating center
- 1 metabolomics core
- 2 model organism screening centers
- 1 sequencing core

Results (Updated April 27, 2020)

- Participants with WES/WGS: 1222
- Participants diagnosed: 408
- Manuscripts published: 87
- ClinVar submissions: 357

1. The UDN Homepage: https://undiagnosed.hms.harvard.edu/
2. UDN Facts and Figures. 27 Apr 2020. https://undiagnosed.hms.harvard.edu/about-us/facts-and-figures/

Genomics England

Whole-genome sequencing of rare disease patients in a national healthcare system

- 7,065 rare disease patients with phenotype and WGS
- 1.1.2019 – 1,040 received a genetic diagnosis
- 2.18.2020 - 1,138 received a genetic diagnosis

To date, actionable findings have been found for **1 in 4/1 in 5** rare disease patients, and around **50%** of cancer cases contain the potential for a therapy or a clinical trial.

1. Genomics England Website: https://www.genomicsengland.co.uk/about-genomics-england/the-100000-genomes-project/
2. Ouweand et al. bioRxiv. 01 Jan 2019. https://www.biorxiv.org/content/10.1101/507244v1
3. Ouweand et al. bioRxiv. 18 Feb 2020. https://www.biorxiv.org/content/10.1101/507244v3

Clinical Implementation in European Countries

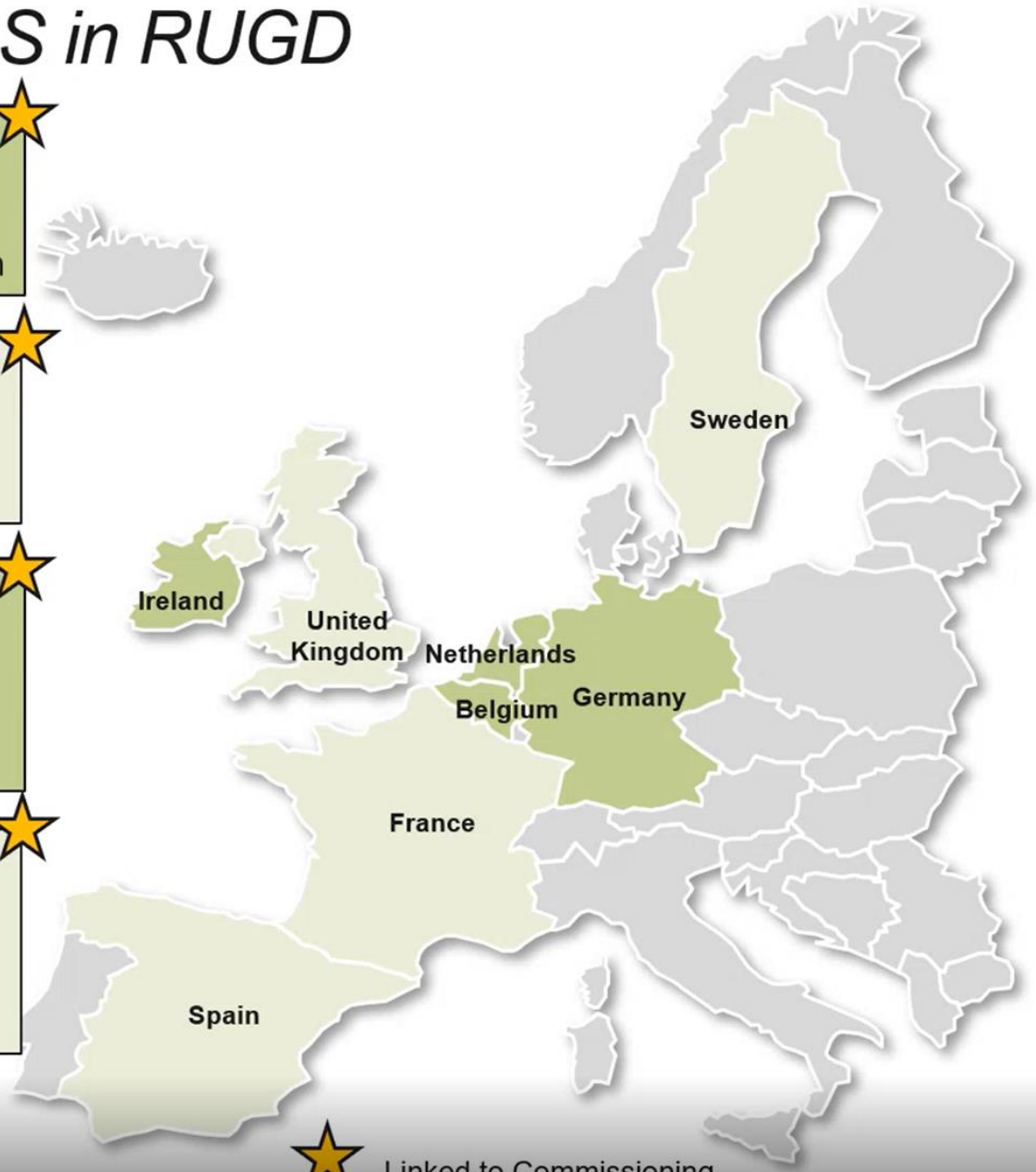
Examples for WGS in RUGD

Radboudumc (Netherlands)
- Comparative eval against SoC
- Workflow & cost-effectiveness
- Establish WGS as a complete solution

NHS Genomic Medicine Services
- Single national test directory
- 22 RD conditions identified for WGS
- Centralization

Belgian SolveRD (nationwide)
- 2-arm RCT against array/WES
- Technical validation
- Clinical utility
- HE impact, reimbursement

Navarra Project
- Centralization
- Feasibility of implementation of genomics in routine
- RUGD (potentially oncology)



Linked to Commissioning

GMI Ireland
- Clinical utility
- HE impact, reimbursement

GMCK-RD, Sweden
- Clinical routine since 2015 for selected patient categories
- 160+ samples/months (Stockholm area), 34% Dx yield
- 84% singletons, 16% trios

Genome First / Ge-Med (Germany)
- Genome first approach for all RD and familial Cx
- Efficacy in a routine diagnostic setting
- Supported by PRS

France Génomique 2025
- 62 indications
- Nationwide
- Clinical utility
- HE impact, reimbursement

Project Baby Gazelle (Israel)
- Genome first approach
- Efficacy in a routine diagnostic setting
- NICU focused

TruSight Software Suite

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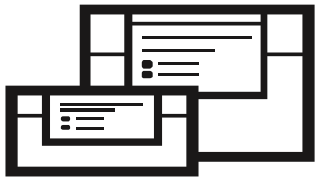
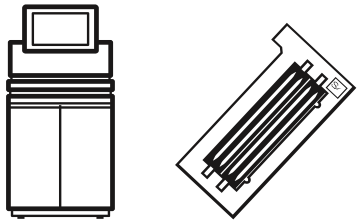
Illumina's WGS Sample-to-Report Workflow

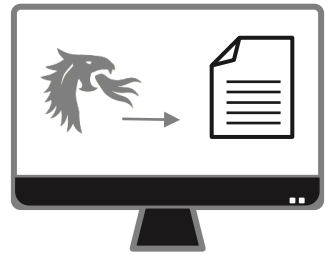
Illumina DNA PCR-Free Prep, Tagmentation



NovaSeq™ 6000



TruSight Software Suite



PREP
from sample to sequencer

SEQUENCE
from library to raw data (FASTQ)

ANALYZE & REPORT
from FASTQ to report

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Sequencer Integration with TruSight Software Suite

Seamless Transfer of Sequencing Data

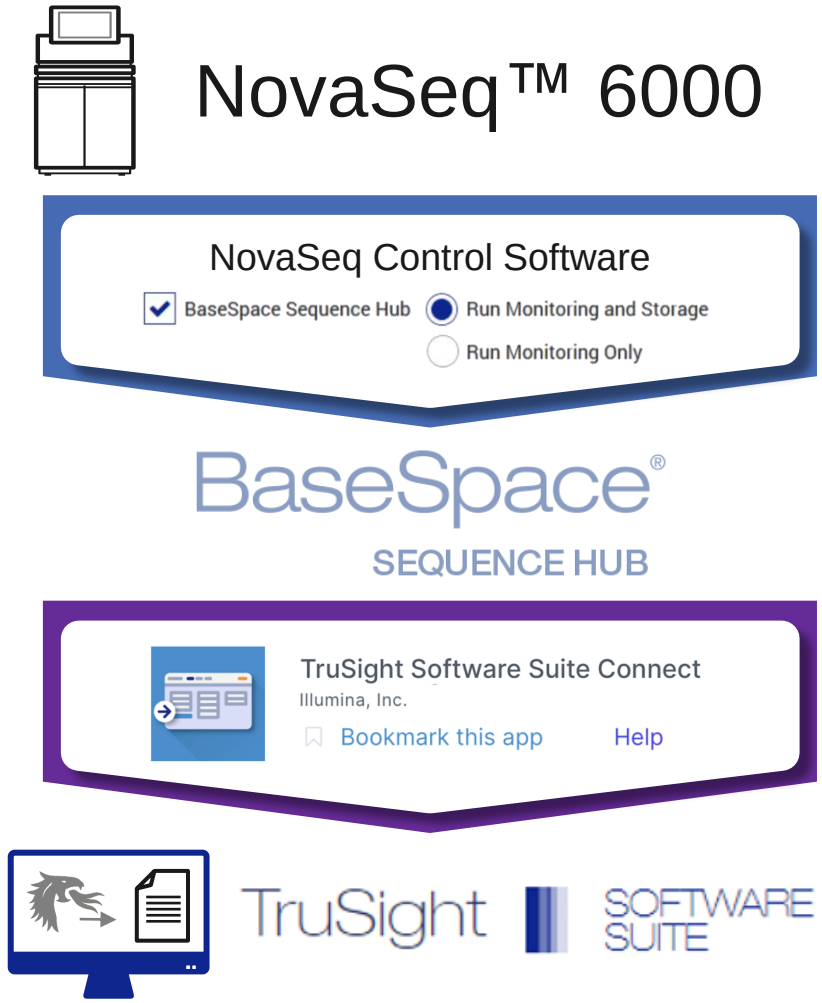
From NovaSeq 6000 to TruSight Software Suite

BSSH Enterprise License Included

with any TruSight Software Suite sample pack purchase to support upload of sequencing data to Sequence Hub

New BaseSpace Sequence Hub App

Supports push-button data transfer from BaseSpace Sequence Hub to TruSight Software Suite



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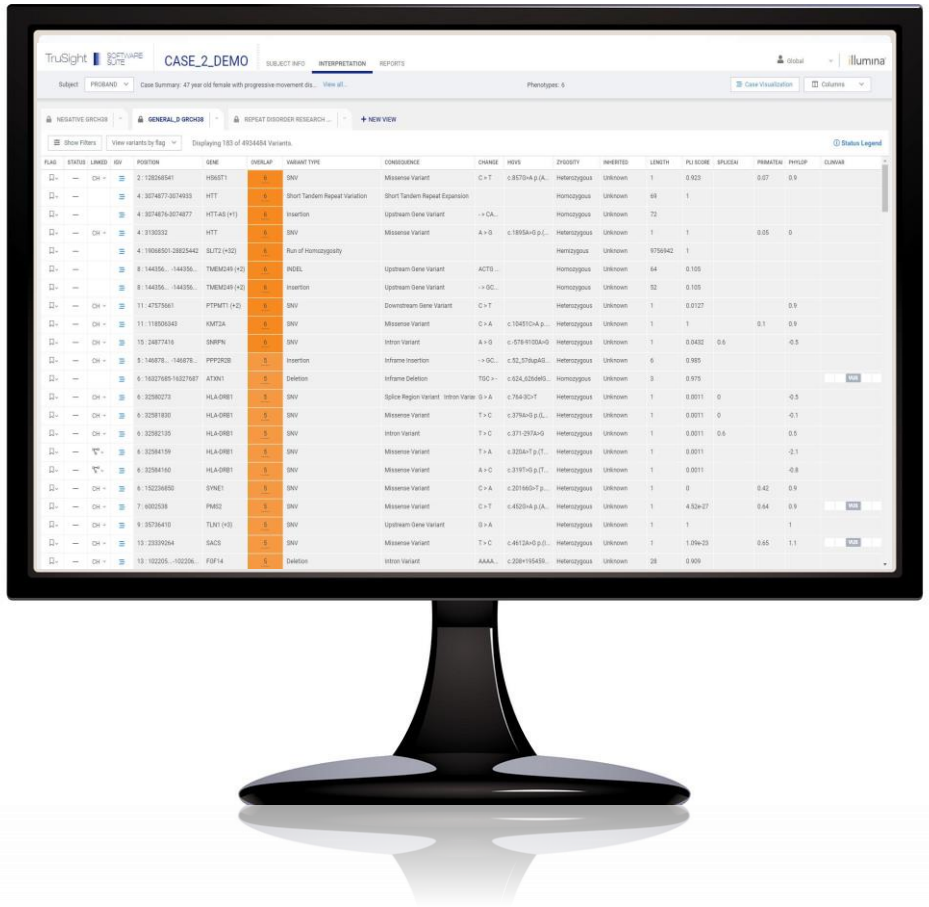
Analyze & Report: TruSight Software Suite



Integrated Secondary Analysis

DRAGEN™ integrated into TruSight Software Suite for hardware-accelerated variant calling from bases to variants in thirty minutes

Comprehensive Variant Classes

ACGTAT
ACCTAT
ACTAT
ACCACC
A

Fully-integrated calling, interpretation, and reporting of single nucleotide variants, insertions, deletions, copy number variants, repeat expansions, structural variants, mitochondrial variants and paralogs

One Interface

Everything in one location. Case management, visualization, annotation, interpretation, machine learning, test configuration, case database all within a single tool.

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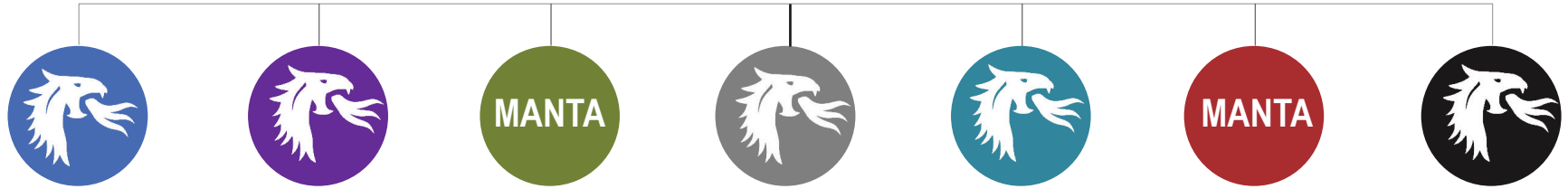
TSS Variant Types Detected by WGS



Small Variants
SNVs
Insertions
Deletions

CNV
Copy Number Variation

SV
Structural Variants
Translocations
Transversions
Insertions
Deletions

mtV
Mitochondrial Variants

STR
Repeat expansions, Short tandem repeats

ROH
Region of Homozygosity

SMN1
Spinal Muscular Atrophy calling

DRAGEN™ accuracy: FP+FN, recall, and precision Application Note: Germline Small Variant Calling with the DRAGEN Platform

Manta Methods and Benchmarking Paper:
Chen, X. et al. (2016) Manta: rapid detection of structural variants and indels for germline and cancer sequencing applications. Bioinformatics, 32, 1220-1222. doi:10.1093/bioinformatics/btv710

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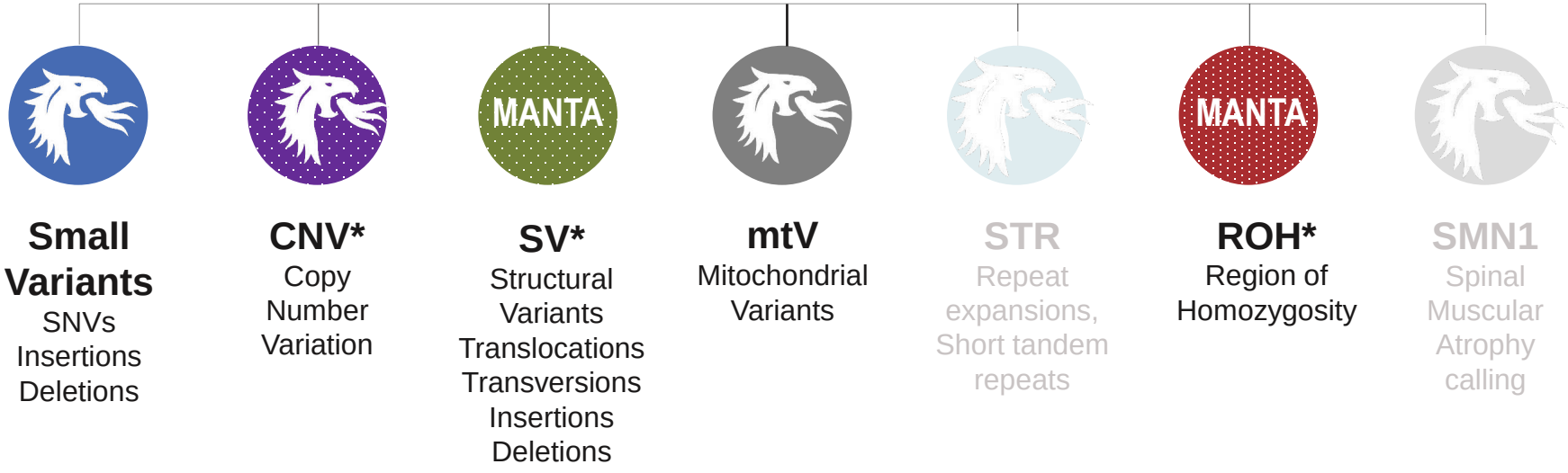
TSS Variant Types Detected by WES



Small Variants
SNVs
Insertions
Deletions

CNV*
Copy Number Variation

SV*
Structural Variants
Translocations
Transversions
Insertions
Deletions

mtV
Mitochondrial Variants

STR
Repeat expansions, Short tandem repeats

ROH*
Region of Homozygosity

SMN1
Spinal Muscular Atrophy calling

* Possible after optimization

DRAGEN™ accuracy: FP+FN, recall, and precision Application Note: Germline Small Variant Calling with the DRAGEN Platform

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CaseLog: TruSight Software Suite's Learning Engine

Search for variants in all prior cases and federated cohorts to improve decision-making

Leverage Prior Knowledge

Review against variants federated from other genomic studies

Review against variants previously seen and annotated in your lab

Use Public Data and Predictive Tools

Review the location of your variant against known variants from ClinVar

Review against predictions from PrimateAI

Evaluate the Gene

Determine the probability that the gene is associated with the subject phenotypes using PheWAS

Review the expression patterns in tissues of interest

Pre-Loaded Federated Data

Publication	Year	Samples	Phenotype
McRae et al. Nature 19:1194-1196	2017	4293 (3664/629)	ID
De Ligt et al. N Engl J Med 367:1921-1929	2012	100	ID
Rauch et al. Lancet 380:1674-1682	2012	51	ID
Lelieveld et al. Nature Neuro 19:1194-1196	2016	821	ID
Sanders et al. Neuron 87:1215-1233	2015	750 (314/436)	ASD
Sanders et al. Nature 485:237-241	2012	452 (238/214)	ASD
De Rubeis et al. Nature 515:209-215	2014	1604 (1443/161)	ASD
Iossifov et al. Nature 498:216-221	2014	3095 (1726/1369)	ASD
Iossifov et al. Neuron 74:285-299	2014	686 (343/343)	ASD
O'Roak et al. Nature 485:246-250	2012	206	ASD
Epi4K Consortim, Nature 501:217-221	2013	264	Epilepsy
EuroEPINOMICS-RES Consortium. AJHG95:360-370	2014	92	Epilepsy
Homsy et al. Science 350:1262-1266	2015	1213	Cong. Heart Disease

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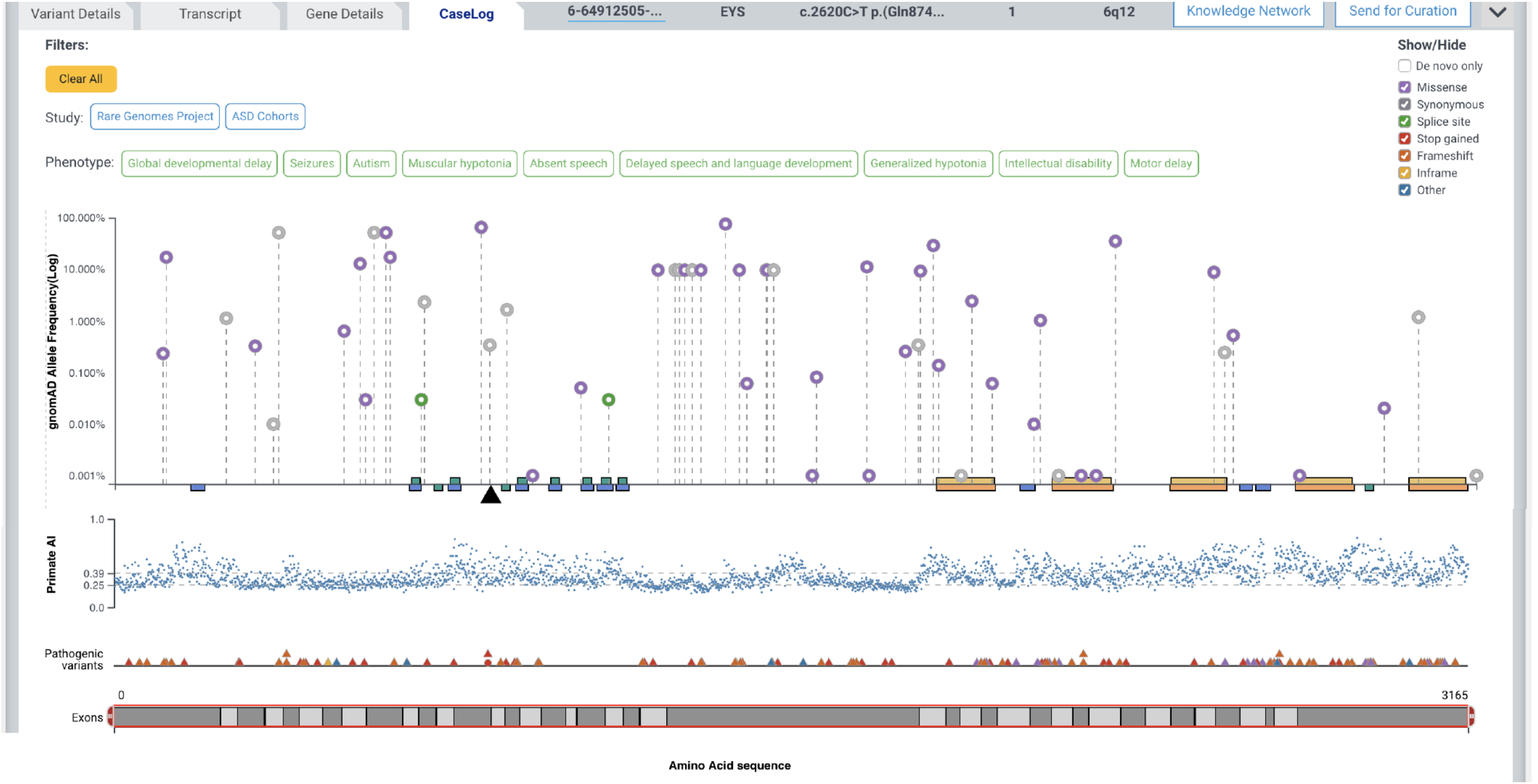

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TruSight Software Suite

Integration
Integration with Illumina Analytic Platform for storage and DRAGEN for secondary analysis. Integration with NovaSeq.

Intelligence
Machine learning and AI tools for variant prediction includingSpliceAI and PrimateAI, literature search

Variant Calling
Integration of MANTA, ExpansionHunter, DRAGEN, SMN1/2, and CNV callers

Variant Prioritization
Prioritization of variants by phenotype overlap and prediction of pathogenicity with machine learning

Variant Details
Integration of external resources including gnomAD, ClinVar, OMIM, IGV

Storage
Integration with Illumina Analytics Platform allows users to manage genome storage directly from TruSight Software Suite

Learning
CaseLog enables gene and variant-level visualization across all prior cases and datasets

Case Management
Case dashboard allows visualization of all cases in the pipeline and assign cases to reviewers

Visualization
Integrative Genomics Viewer (IGV), repeat expansion viewer, CaseLog, and PrimateAI

Security
ISO-27001, ISO-13485, HIPAA (third-part audited), and GDPR principles

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Thank You

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