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REVIEW ARTICLE OPEN

Check for updates

Best practices for the interpretation and reporting of clinical whole genome sequencing

Christina A. Austin-Tse[1,2,3,16]✉, Vaidehi Jobanputra[4,5,16], Denise L. Perry[6], David Bick[7], Ryan J. Taft[6], Eric Venner[8], Richard A. Gibbs[8], Ted Young[9], Sarah Barnett[10], John W. Belmont[6], Nicole Boczek[10,11], Shimul Chowdhury[12], Katarzyna A. Ellsworth[12], Saurav Guha[4], Shashikant Kulkarni[13,14], Cherisse Marcou[10], Linyan Meng[13,14], David R. Murdock[8,14], Atteeq U. Rehman[4], Elizabeth Spiteri[15], Amanda Thomas-Wilson[4], Hutton M. Kearney[10,16], Heidi L. Rehm[1,3,16] and Medical Genome Initiative*

Speaker：R2 張嘉文

Supervisor: 張家銘 醫師

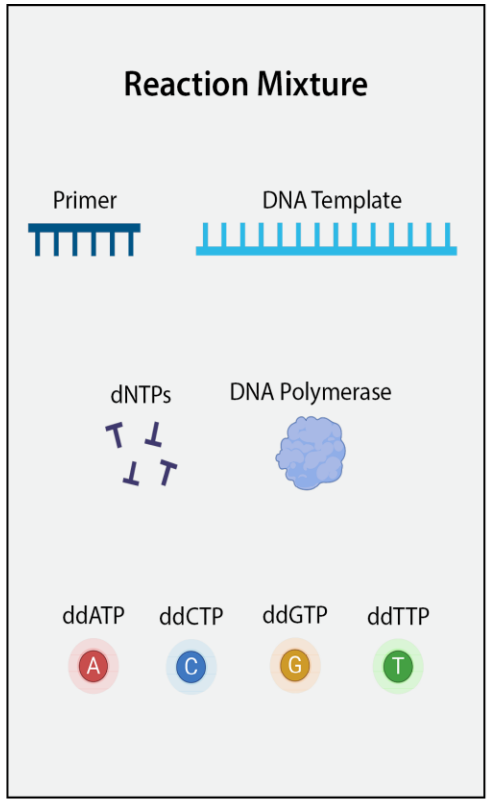
Introduction

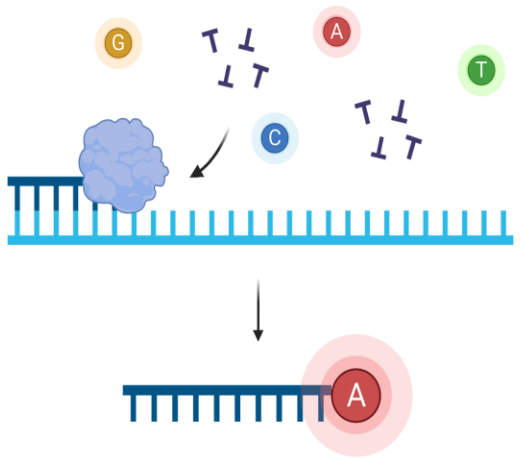
- **Whole genome sequencing (WGS)** is emerging as a first-tier diagnostic test for rare genetic diseases
- However, standards addressing the definition and deployment practice of test are lacking

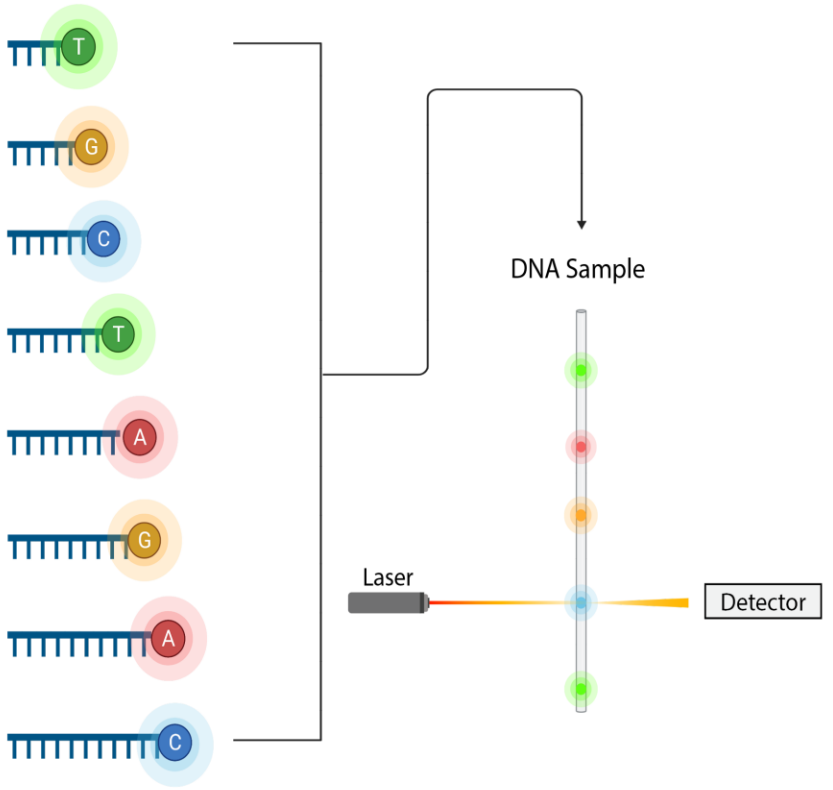
- “Medical Genome Initiative” was formed to expand access to high quality clinical WGS

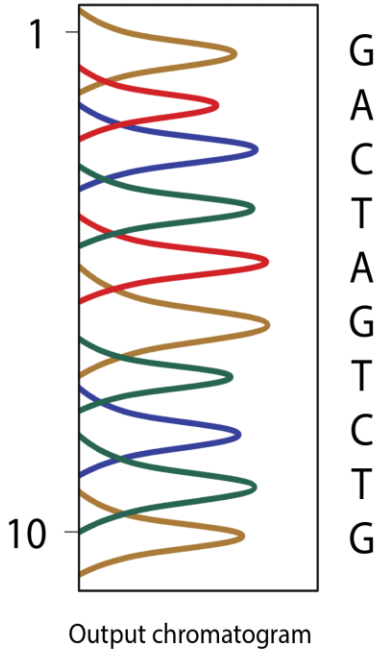
Sanger sequencing

Sanger Sequencing



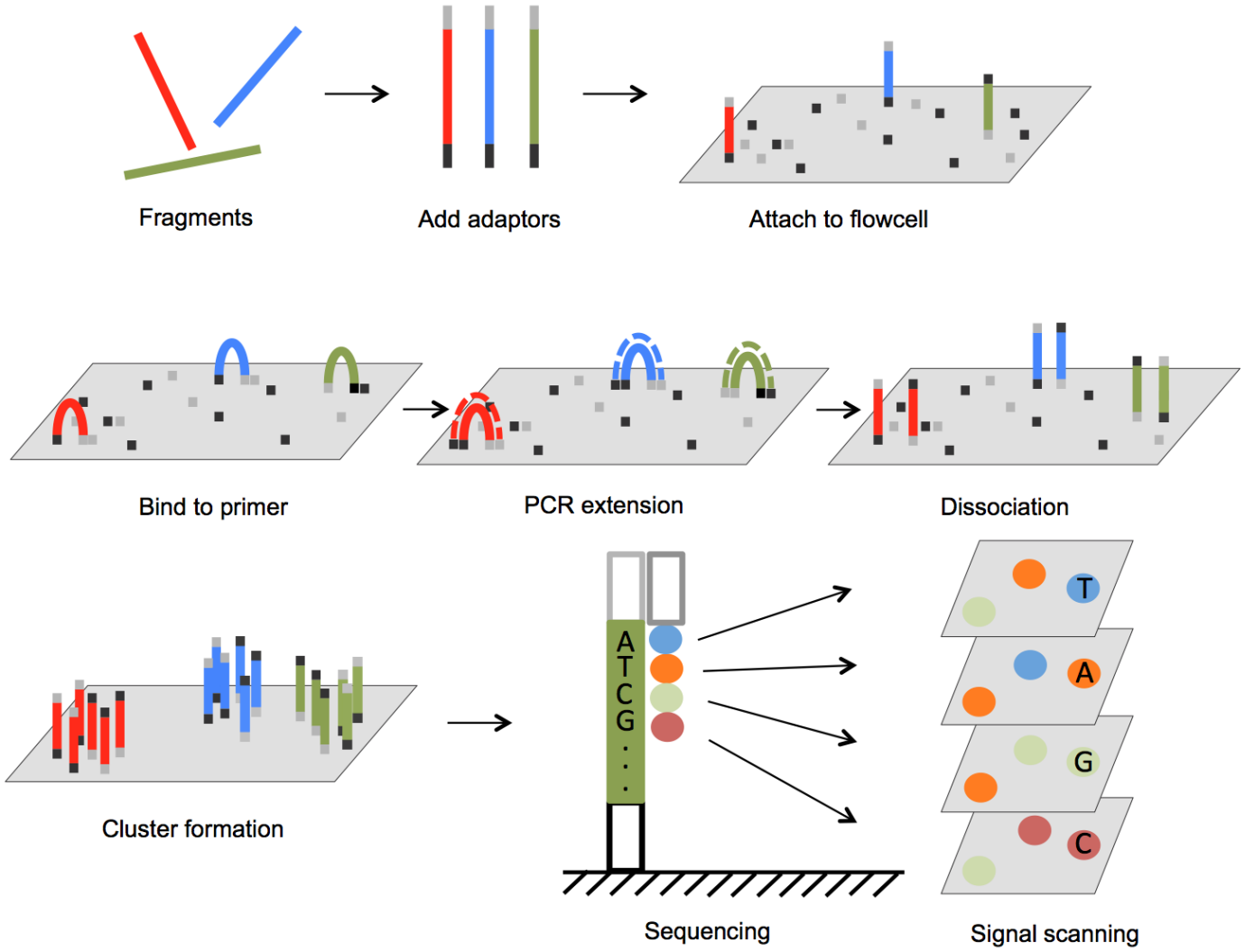






1. Chain-termination PCR using fluorescent ddNTPs
2. Size separation and sequence analysis using capillary gel electrophoresis and fluorescence detection

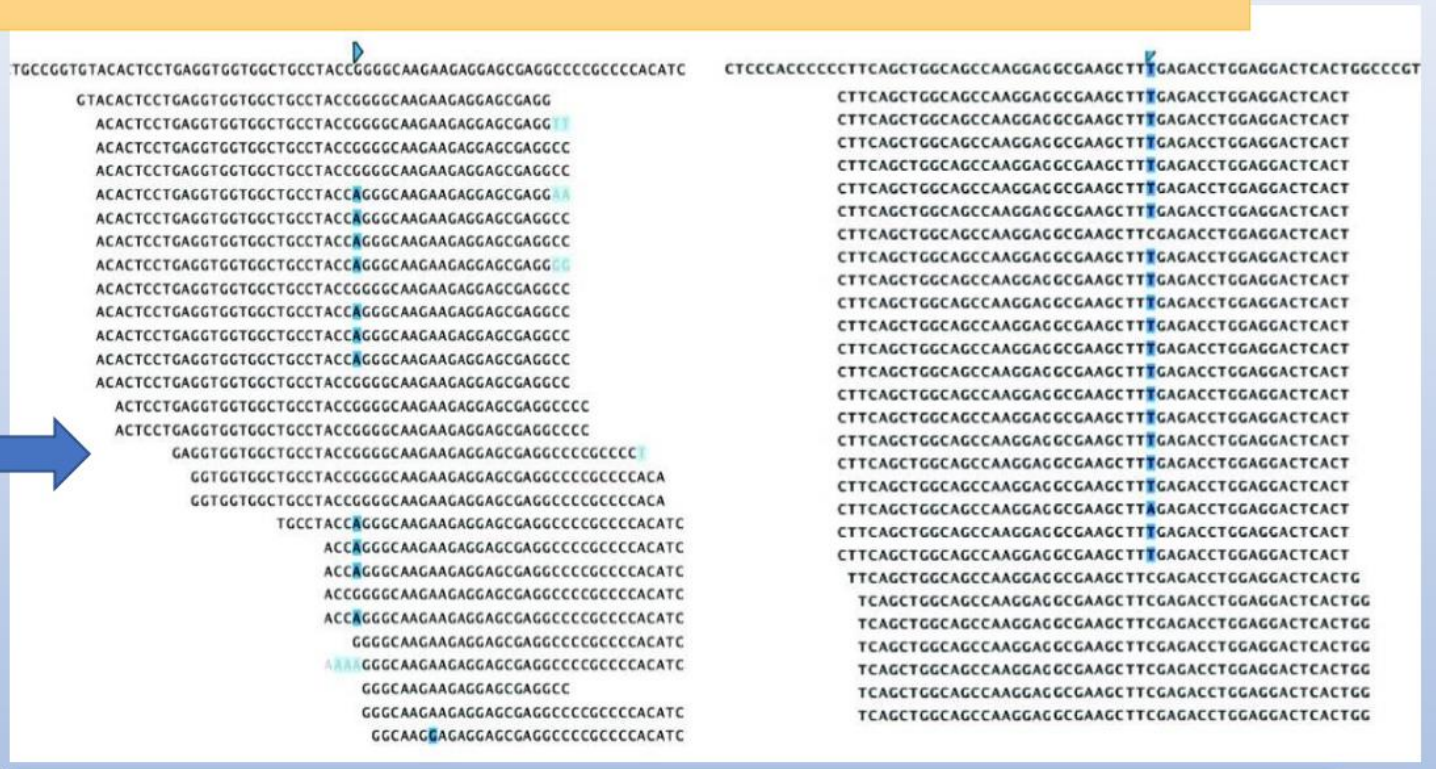
Next Generation Sequencing(NGS) Illumina

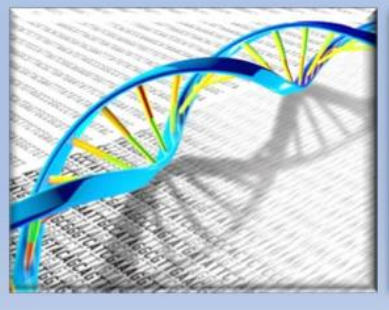


NGS：透過reads排列分析變異點

Reference sequence

reads



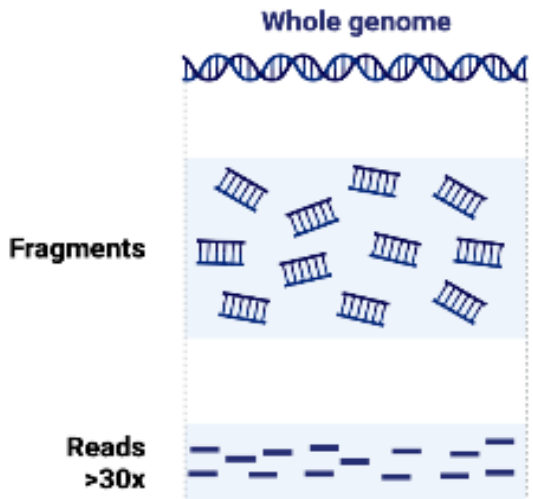


Genome-wide variants

https://medicalxpress.com/

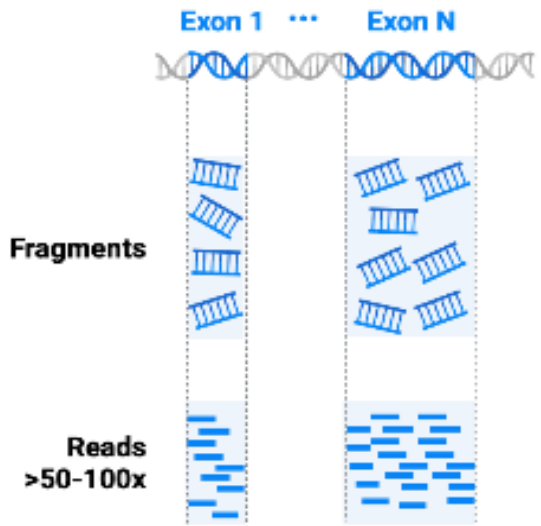
Next Generation Sequencing

Genome Sequencing



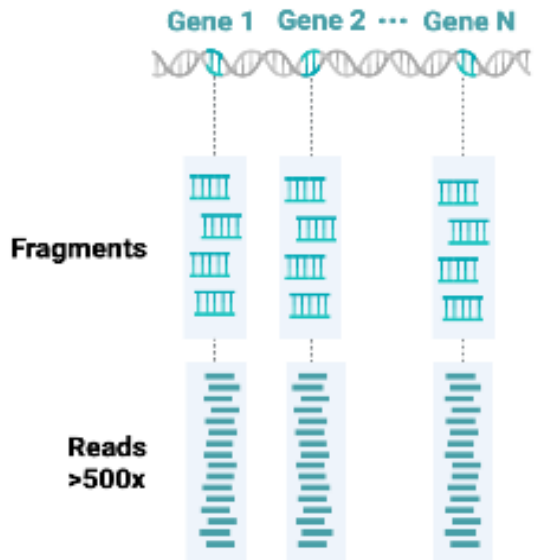
Coverage: All genes and non-coding DNA
Acuracy: Low
Time: Longest turnaround time
Cost: Most expensive
Depth: >30X

Exome Sequencing



Coverage: Entire exome (20-25k genes)
Acuracy: Good
Time: Long turnaround time
Cost: Cost-effective
Depth: >50-100X

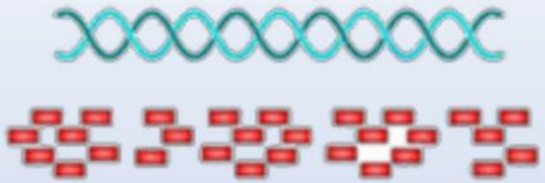
Targeted Gene Panel



Coverage: 10-500 genes
Acuracy: High
Time: Rapid turnaround time (few days)
Cost: Most cost-effective
Depth: >500X

Template adapted from: *Dr. Roshini Abraham*
Clinical Immunologist at Nationwide Children's Hospital

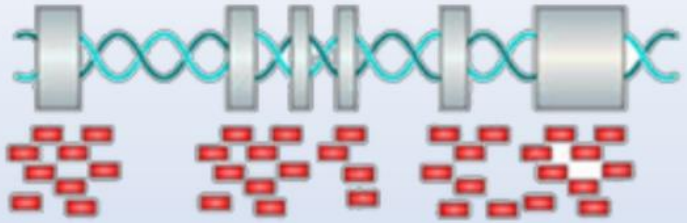
Whole genome sequencing



- Sequencing region : whole genome
- Sequencing Depth: >30X
- Covers everything – can identify all kinds of variants including SNPs, INDELs and SV.

全基因定序 (WGS)

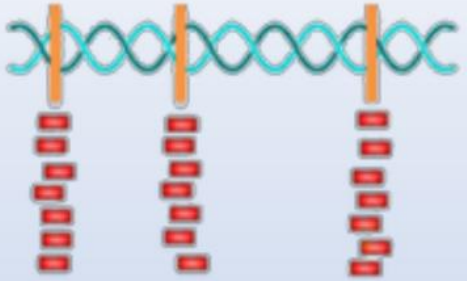
Whole exome sequencing



- Sequencing region: whole exome
- Sequencing Depth : >50X ~ 100X
- Identify all kinds of variants including SNPs, INDELs and SV in coding region.
- Cost effective

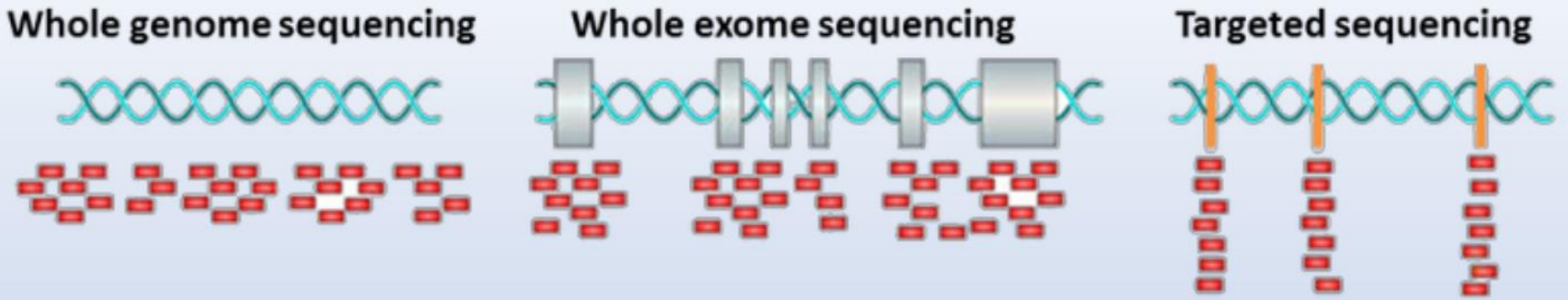
全基因外顯子定序 (WES)

Targeted sequencing



- Sequencing region: specific regions (could be customized)
- Sequencing Depth : >500X
- Identify all kinds of variants including SNPs, INDELs in specific regions
- Most Cost effective

目標定序 (TPS, Targeted Panel seq)



- Sequencing region : whole genome
- Sequencing Depth: >30X
- Covers everything – can identify all kinds of variants including SNPs, INDELs and SV.

Detection of a broad range of variant types in a single assay

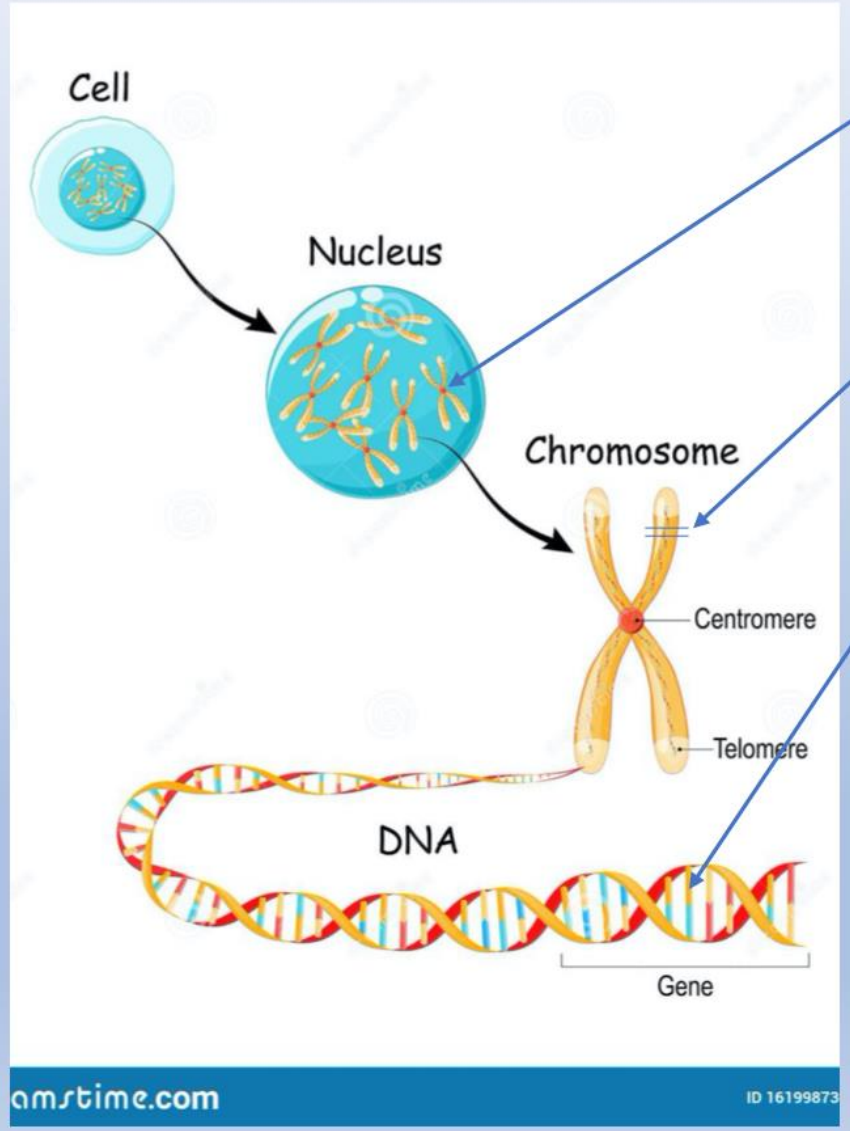
Untargeted
Add coverage of intronic, intergenic and regulatory regions

全基因定序 (WGS)

全基因外顯子定序 (WES)

目標定序 (TPS, Targeted Panel seq)

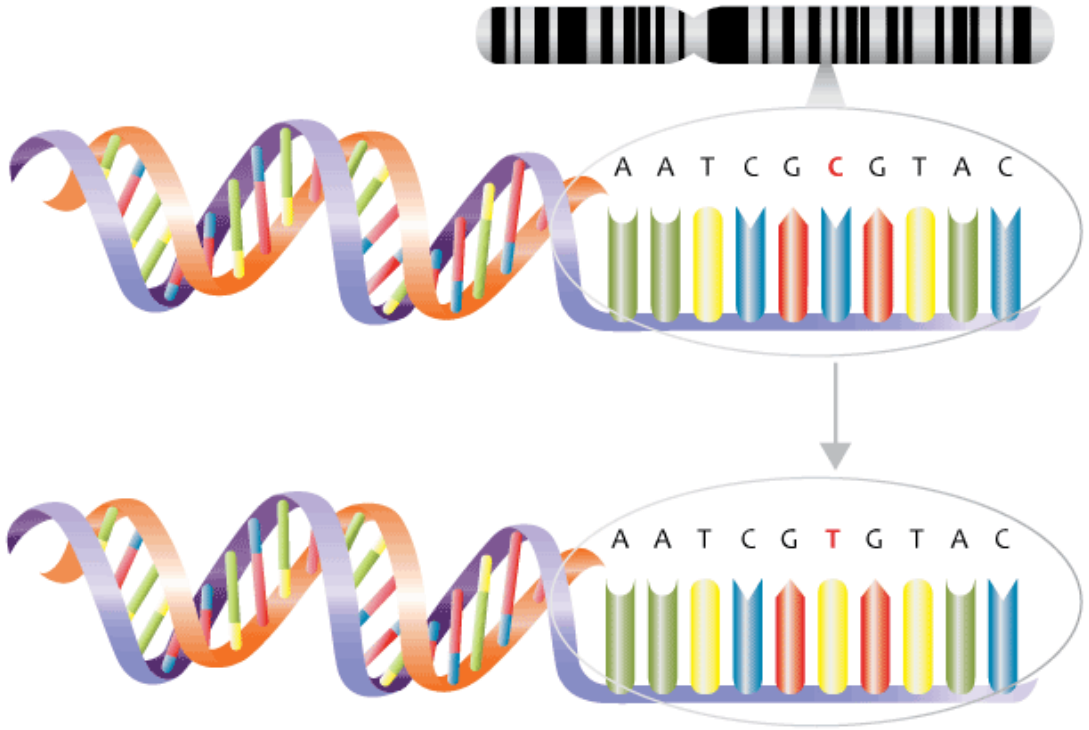
基因體醫學進展；Omics techniques



Chromosome number, structure	>10MB	karyotyping	I
Copy number variation	>700bp	Array CGH SNP array	I
SNP, indel	1~100 bp	NGS Exome, Whole genome Targeted panel seq	II
SNP, indel, large indel, structure	1~20000 bp	Long read seq (3rd generation)	II

Single-nucleotide polymorphism (SNP)

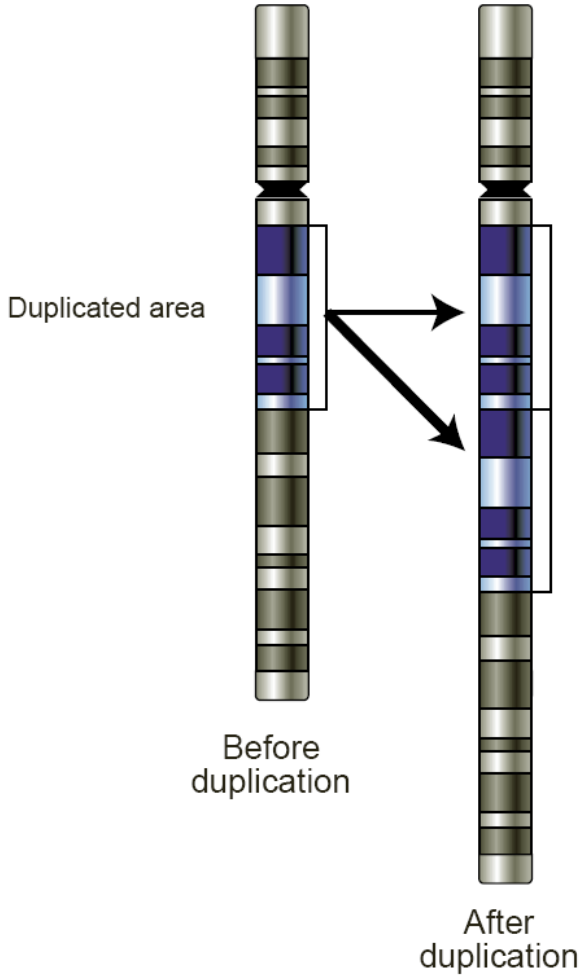
- Variation at a single position in a DNA sequence among individuals



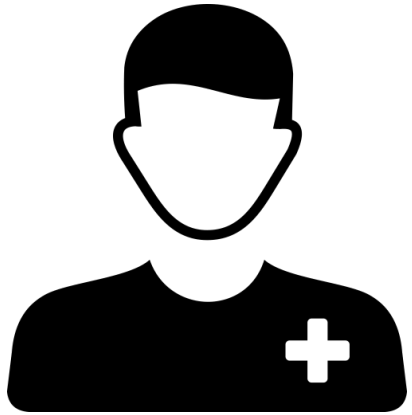
Indel (insertion/deletion)

- Insertion and/or deletion of nucleotides into genomic DNA and include events less than 1 kb in length

Copy number variation



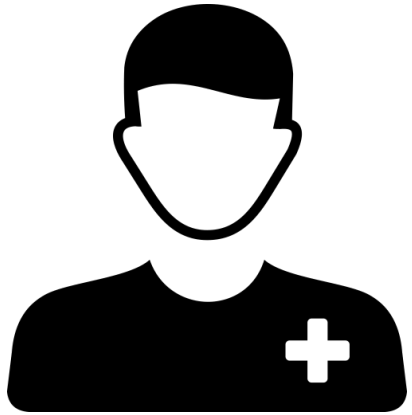
- Type of structural variation
- sections of the genome are repeated and the number of repeats in the genome varies between individuals



Proband

Provider

Laboratory



Proband

ADVICE

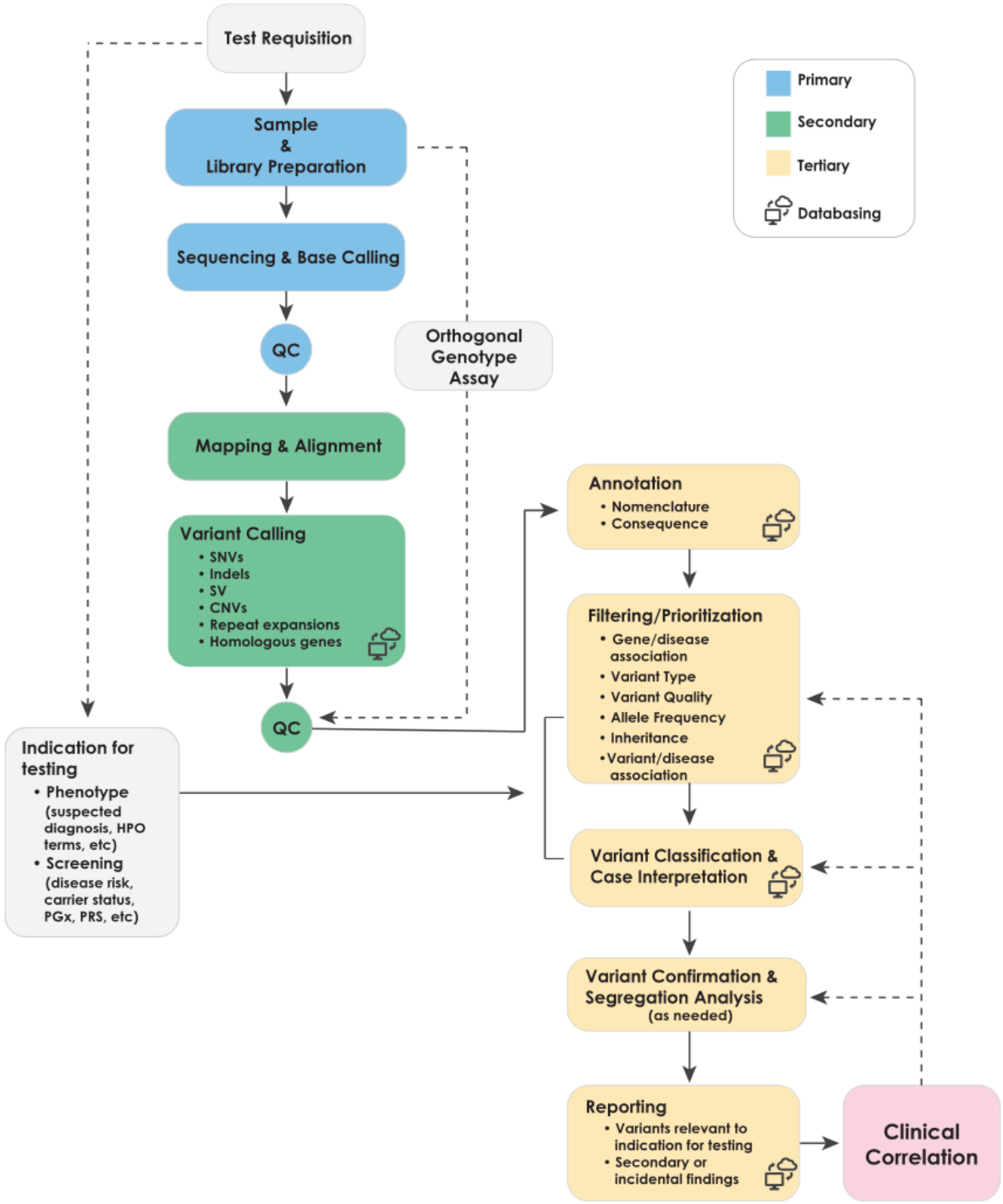
REQUISITION/ CONSENT

Provider

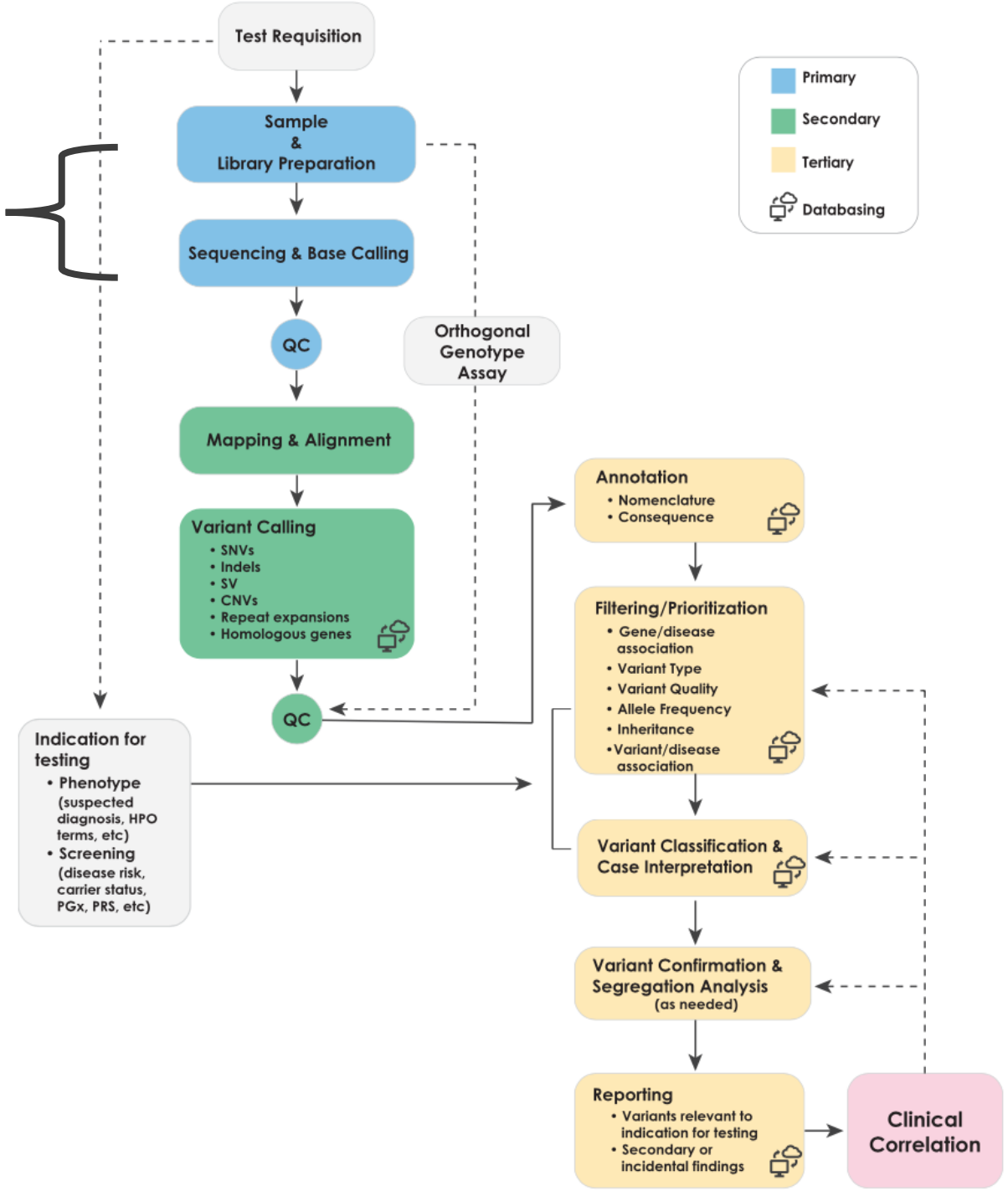
ELECTRONIC MEDICAL RECORD

ANALYSIS/ REPORTING

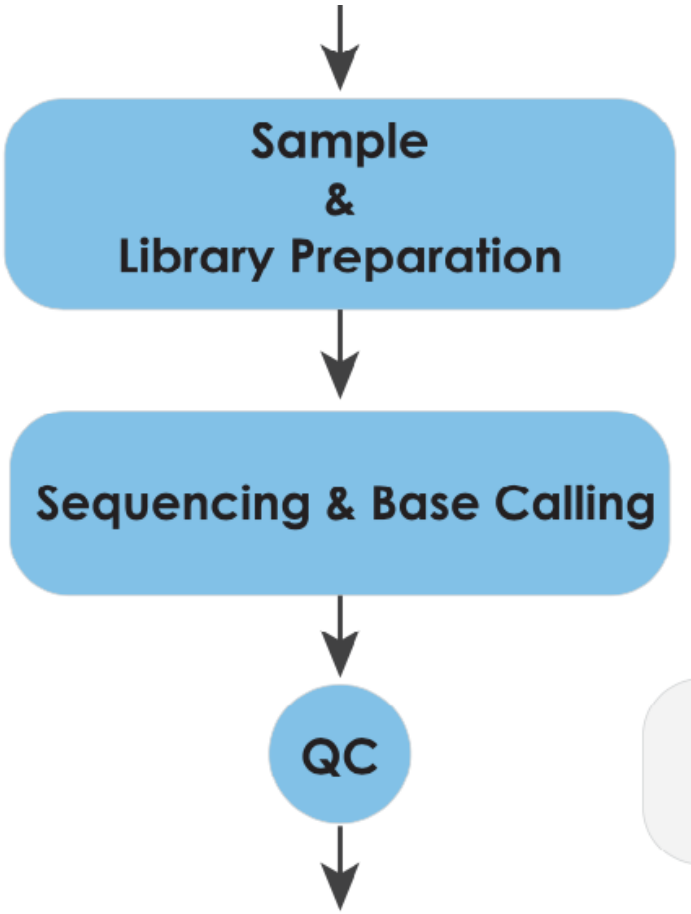
Laboratory


Test Requisition
Sample & Library Preparation
Sequencing & Base Calling
QC
Mapping & Alignment
Variant Calling
• SNVs
• Indels
• SV
• CNVs
• Repeat expansions
• Homologous genes
QC
Orthogonal Genotype Assay
Indication for testing
• Phenotype (suspected diagnosis, HPO terms, etc)
• Screening (disease risk, carrier status, PGx, PRS, etc)
Annotation
• Nomenclature
• Consequence
Filtering/Prioritization
• Gene/disease association
• Variant Type
• Variant Quality
• Allele Frequency
• Inheritance
• Variant/disease association
Variant Classification & Case Interpretation
Variant Confirmation & Segregation Analysis (as needed)
Reporting
• Variants relevant to indication for testing
• Secondary or incidental findings
Clinical Correlation
Primary
Secondary
Tertiary
Databasing

Primary analysis

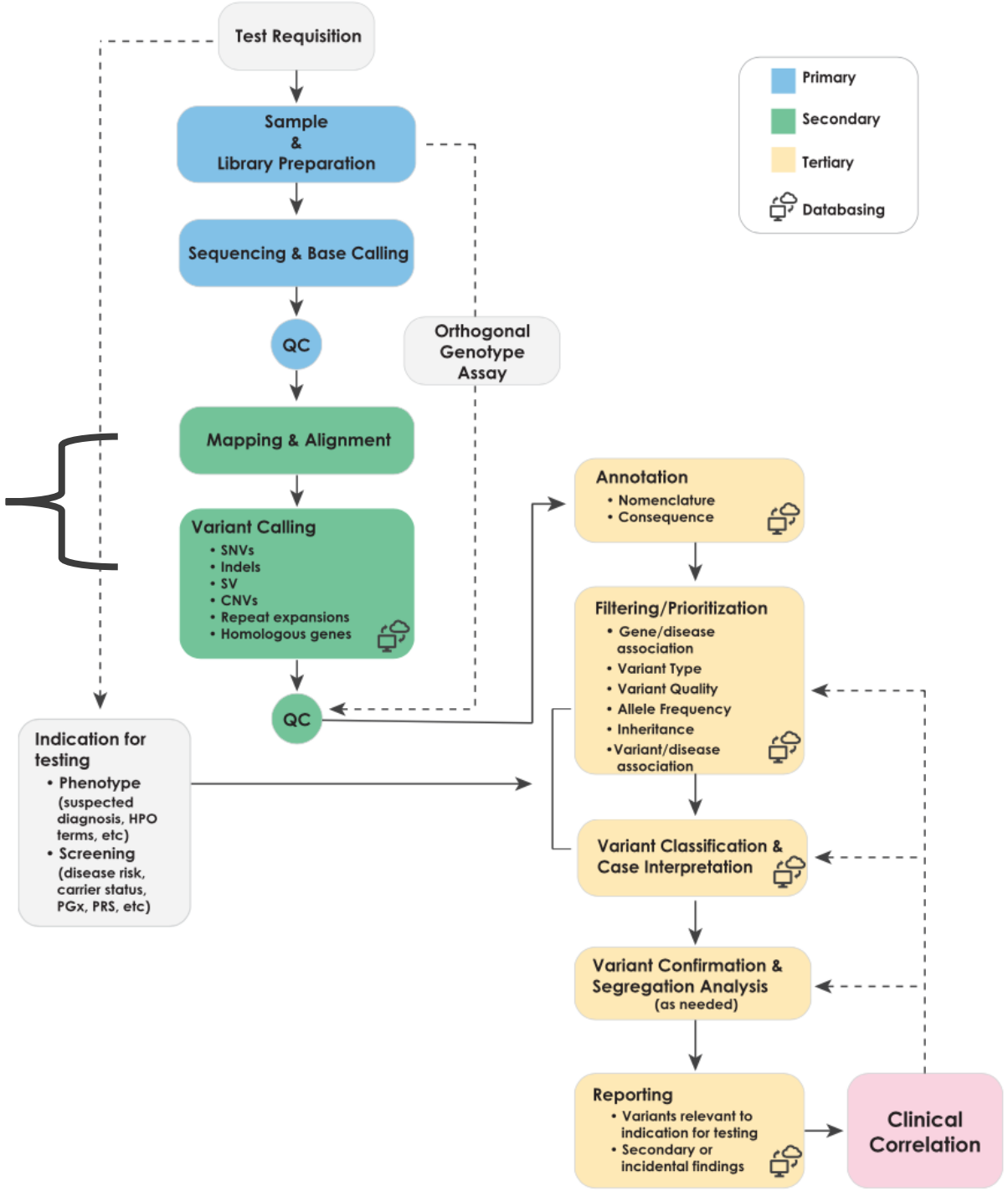


Primary analysis

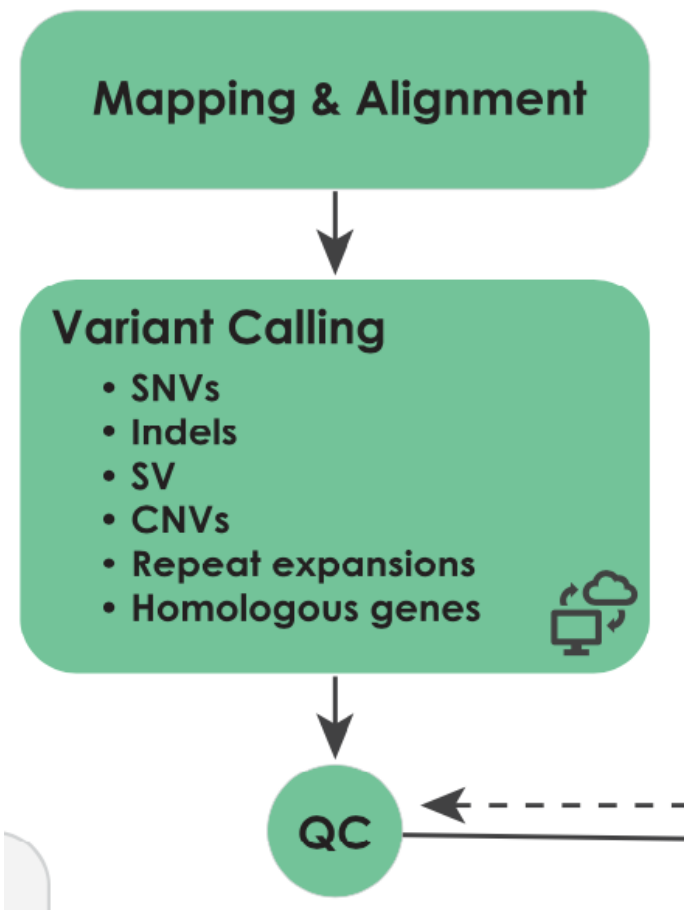


- DNA extraction
- Library preparation
- Sequence generation
- Preliminary data quality control (QC)

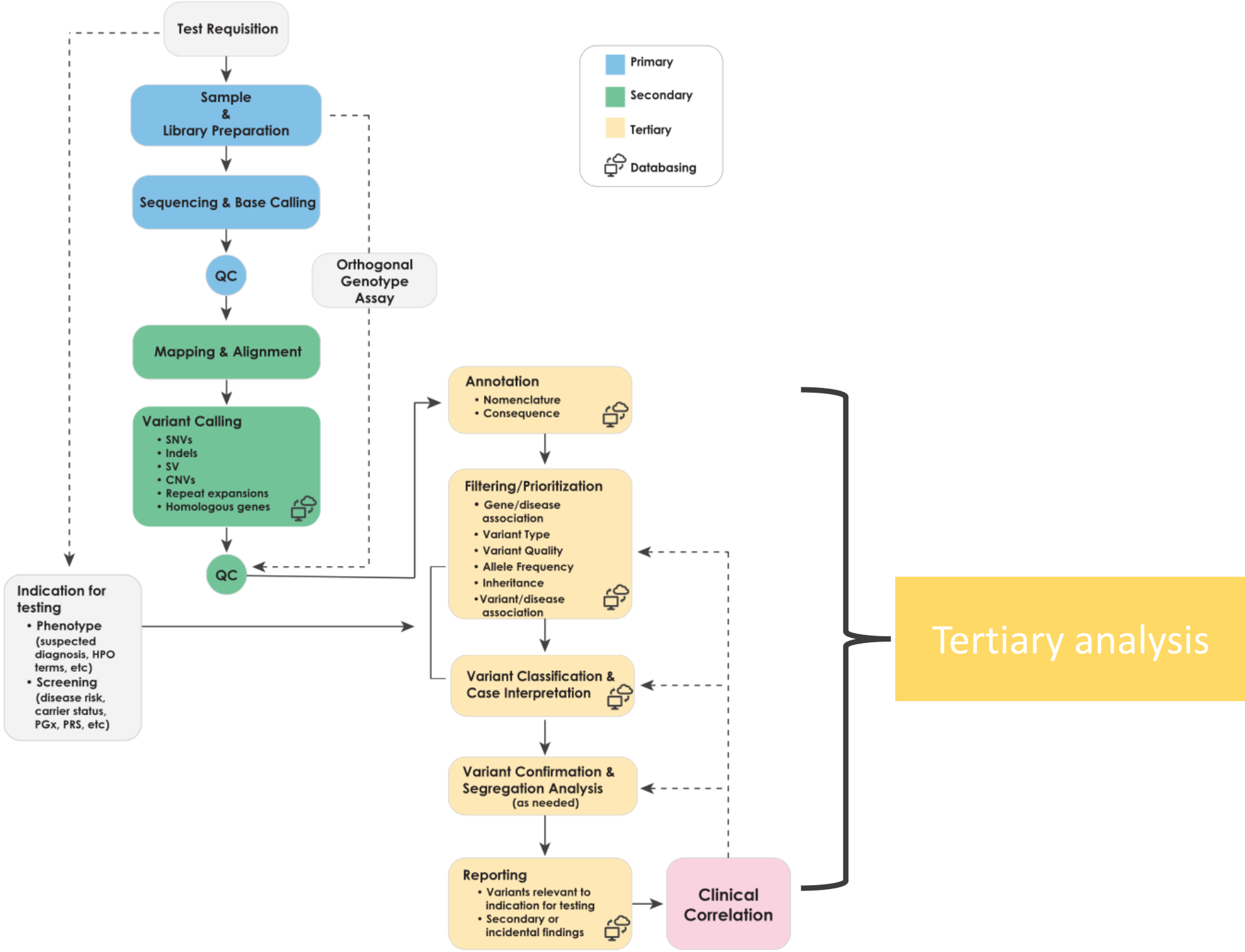
Secondary analysis



Secondary analysis



- Alignment of the raw sequence data to a genome reference
- Variant calling
- Further data QC operations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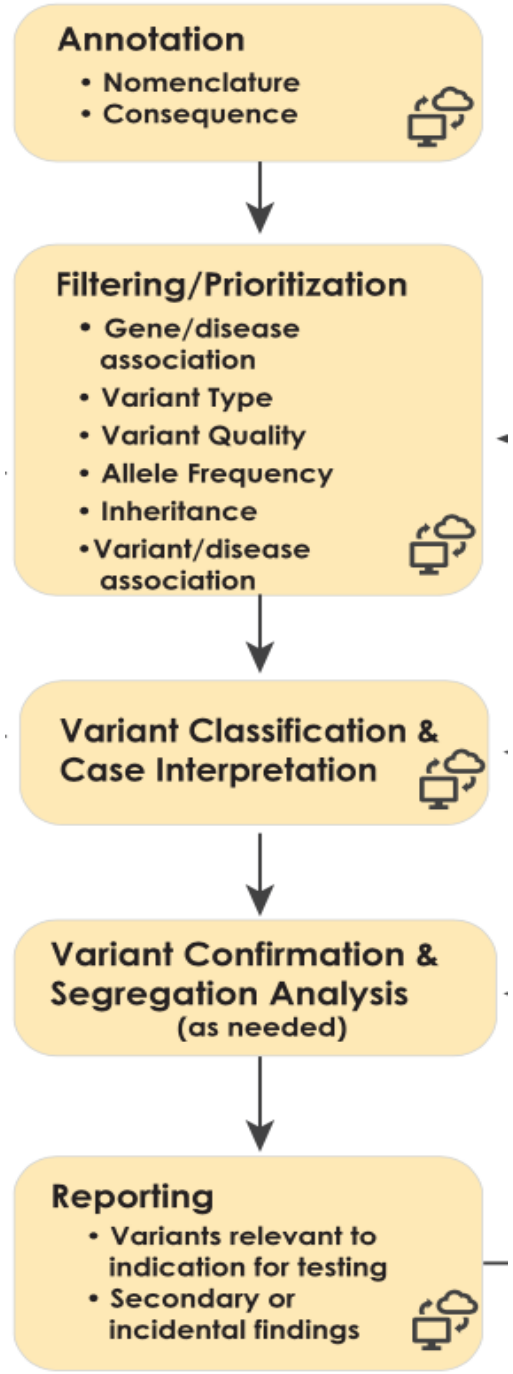
• Variant/disease association
Variant Classification & Case Interpretation
Variant Confirmation & Segregation Analysis (as needed)
Reporting
• Variants relevant to indication for testing
• Secondary or incidental findings
Clinical Correlation
Tertiary analysis

Tertiary analysis

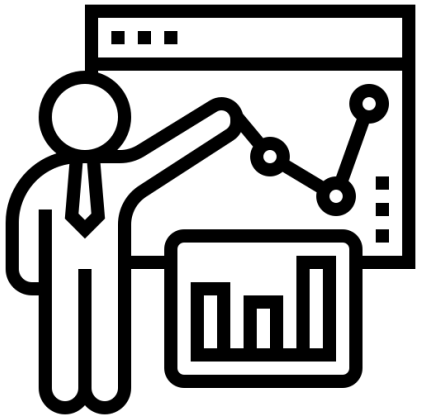
- Annotation
- Filtering
- Prioritization
- Classification of variants
- Case interpretation and reporting



REQUISITION
& CONSENT

ANALYSIS

ANNOTATION

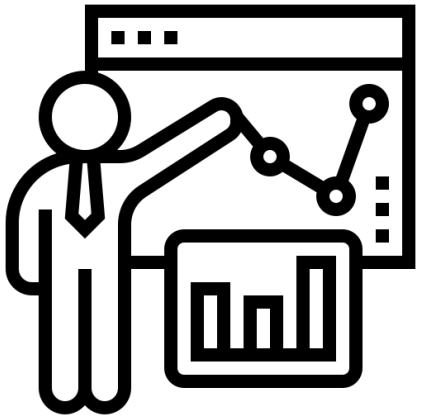


INTERPRETATION
& REPORT

REQUISITION
& CONSENT

ANALYSIS

ANNOTATION



INTERPRETATION
& REPORT

- **Understanding drives selection of analysis strategy**
  - Phenotype
  - Family history
  - Desired result
- **Phenotype capture**
  - Staff extraction
  - Digital tools
    - ✓ Human Phenotype Ontology(HPO), OMIM, Disease Ontology…
- **Secondary/ Incidental findings**

SAMPLE Whole Genome Sequencing Test Requisition

DISCLAIMER: This sample form is provided for informational purposes only and does not represent requisition forms in use at MGI participating institutions.

Patient Information

First name: _____ MI:____ Last name: _____

Date of Birth: (mm/dd/yyyy) _____ /_____ / _____ Gender: ❑ Male ❑ Female ❑ Unknown/Unspecified

Address:_____

City: _____ State: _____ Zip Code: _____ Phone:_____

Email: _____

Institution:_____ Medical Record Number: _____

Is the patient adopted? ❑ Yes ❑ No Is the patient deceased? ❑ Yes ❑ No *if yes,* date:_____

Has the patient undergone bone marrow transplant? ❑ Yes ❑ No *if yes,* date: _____

Testing for patients who have received an allogenic bone marrow transplant must be completed on a pre-transplant sample or a non-hematologic sample.

Has the patient received a blood transfusion? ❑ Yes ❑ No *if yes,* date: _____

Blood obtained for genetic testing should ideally be collected at least 2-4 weeks after the date of the last transfusion

Race and Ethnicity (please select all that apply):

❑ White ❑ Ashkenazi Jewish ❑ Hispanic ❑ Asian ❑ Black/African American ❑ American Indian/Native Alaskan

❑ Native Hawaiian or other Pacific Islander ❑ Other

Test Order

❑Proband Only Clinical Genome Sequencing + Interpretation.

❑Trio (or other family-based analysis) Clinical Genome Sequencing + Interpretation.

For family-based analysis, submit a test requisition form for each patient submitted for testing.
Number of family members submitted for testing _____

	Affected Status		
If axillary family member, relationship to the proband:	Affected	Unaffected	Unknown
❑ Biological mother of the proband	❑	❑	❑
❑ Biological father of the proband	❑	❑	❑
❑ Full brother of the proband	❑	❑	❑
❑ Full sister of the proband	❑	❑	❑
❑ Other [describe relationship to the proband specifically (eg, maternal half-sister of the proband)]	❑	❑	❑

❑Reanalysis/Reinterpretation of Clinical Sequencing Data

❑Reanalysis of whole genome

❑Reanalysis of selected genes of interest (provide details below or contact the laboratory)

Genes requested: _____

The WGS report will contain any identified variants that are suspicious for causing the symptoms/diseases provided. If there is a specific condition that might cause your symptoms but you do NOT wish to learn about, please discuss it with your doctor and/or genetic counselor and list here:

_____

_____

Detailed phenotype information (select at least one option below):

❑Option 1: Include a recent clinic note, including family history; Photo(s) of the patient may be helpful if available.

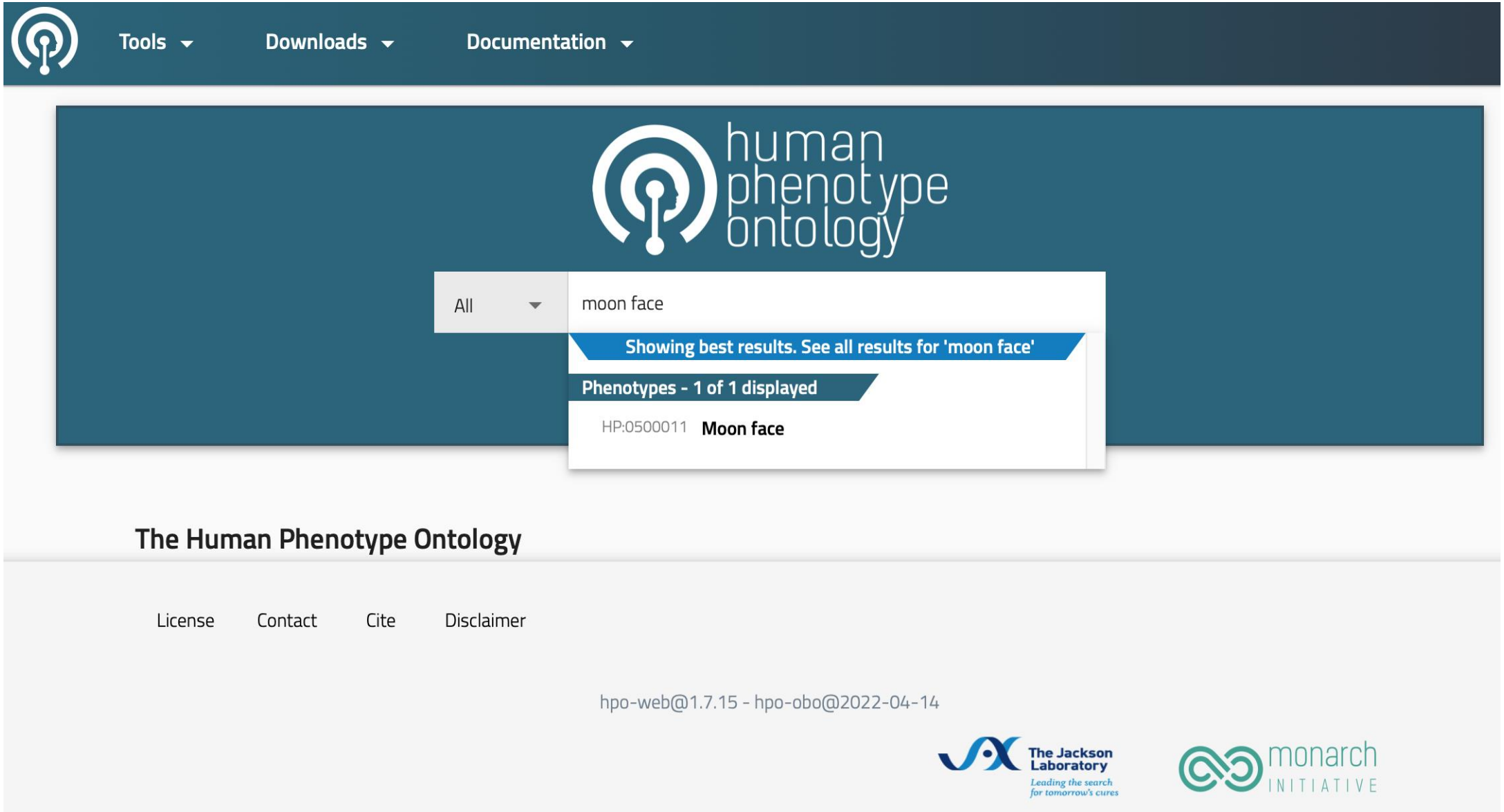
❑Option 2: Submit clinical information via [phenotype interface of choice, e.g. PhenoTips]

❑Option 3: Complete section below

Abnormality of:

Head or neck	
Eye	
Ear	
Voice	
Thoracic cavity	
Cardiovascular system	
Breast	
Respiratory system	
Limbs	
Musculature	
Skeletal system	
Connective tissue	
Digestive system	

Nervous system	
Genitourinary system	
Immune system	
Endocrine system	
Blood and blood-forming tissues	
Metabolism/homeostasis	
Integument	
Growth abnormality	
Prenatal development or birth	
Neoplasm	
Other	


Tools
Downloads
Documentation
human phenotype ontology
All
moon face
Showing best results. See all results for 'moon face'
Phenotypes - 1 of 1 displayed
HP:0500011 Moon face
The Human Phenotype Ontology
License
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Cite
Disclaimer
hpo-web@1.7.15 - hpo-obo@2022-04-14
The Jackson Laboratory
Leading the search for tomorrow's cures
monarch INITIATIVE

Moon facies HP:0500011

A rounded, puffy face with fat deposits in the temporal fossa and cheeks, a double chin.

Synonyms: *Puffy facies, Moon face, Puffy face*

Comment: Moon facies can occur in persons treated with high-dose corticosteroids or with Cushing disease.

Export Associations

Disease Associations | Gene Associations

Disease Id	Disease Name	Associated Genes
ORPHA:1359	Carney Complex	PRKAR1A [5573]
ORPHA:226313	Congenital Hypothyroidism Due To Maternal Intake Of Antithyroid Drugs	
ORPHA:95715	Congenital Hypothyroidism Due To Transplacental Passage Of Tsh-binding Inhibitory Antibodies	
ORPHA:96253	Cushing Disease	USP8 [9101] CDH23 [64072]
ORPHA:99889	Cushing Syndrome Due To Ectopic Acth Secretion	
ORPHA:189427	Cushing Syndrome Due To Macronodular Adrenal Hyperplasia	GNAS [2778] ARMC5 [79798]

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Human Genetics Knowledge for the World

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Online Mendelian Inheritance in Man®

An Online Catalog of Human Genes and Genetic Disorders

Updated May 11, 2022

marfan syndrome

- **marfan syndrome**
- **marfan syndrome** affect
- **marfan syndrome** allele
- **marfan syndrome** arose
- **marfan syndrome** based
- **marfan syndrome** cases
- **marfan syndrome** children
- **marfan syndrome** combined
- **marfan syndrome** compared
- **marfan syndrome** examined

ls

ibutions from people like you.

154700

MARFAN SYNDROME; MFS

Alternative titles; symbols

MARFAN SYNDROME, TYPE I; MFS1

Phenotype-Gene Relationships

Location	Phenotype	Phenotype MIM number	Inheritance	Phenotype mapping key	Gene/Locus	Gene/Locus MIM number
15q21.1	Marfan syndrome	154700	AD	3	FBN1	134797

Clinical Synopsis | PheneGene Graphics

TEXT

A number sign (#) is used with this entry because all cases of the Marfan syndrome appear to be due to heterozygous mutation in the fibrillin-1 gene (FBN1; 134797) on chromosome 15q21.

Description

A heritable disorder of fibrous connective tissue, Marfan syndrome shows striking pleiotropism and clinical variability. The cardinal features occur in 3 systems--skeletal, ocular, and cardiovascular (McKusick, 1972; Pyeritz and McKusick, 1979; Pyeritz, 1993). It shares overlapping features with congenital contractural arachnodactyly (121050), which is caused by mutation in the FBN2 gene (612570).

Secondary Findings

The primary goal of the WGS test is to find the genetic basis of your/your child's disorder. However, a secondary findings analysis is also available. This analysis includes a targeted screen for disease-causing variants in medically actionable genes that may be unrelated to your/your child's disorder but are recommended for reporting by the American College of Medical Genetics and Genomics (ACMG). This analysis is optional.

❑Patient **OPTS IN** to secondary findings analysis of the ACMG recommended genes

❑Patient **OPTS OUT** of secondary findings analysis

Important points to consider:

- Opting out of secondary findings analysis means that a targeted search for variants in the list of genes recommended by the ACMG for reporting of secondary findings will not be performed.
- If an individual opts out of the analysis, **variants in secondary findings genes may still be reported if they have possible relevance to the indication for testing**.
- In the case of a family-based analysis (e.g., trio sequencing), identification of secondary findings in family members who opt in for the analysis may inform carrier status of other members of the family, even those who choose to opt out of the analysis.

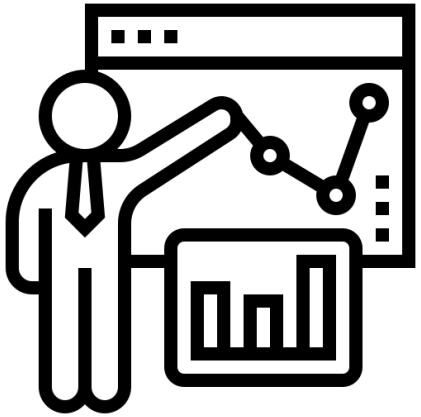
Incidental Findings

Incidental findings are defined as clinically significant variants found in genes associated with phenotypes that are unrelated to the patient's primary indication for testing. Unlike Secondary Findings, these variants are not actively sought, but may be noted during analysis. Variants in genes that are not part of the ACMG recommended gene list for Secondary Findings but have the potential to influence medical management [insert language for how these variants will be handled/reported by the laboratory per the laboratory's incidental findings policy].

REQUISITION
& CONSENT

ANALYSIS

ANNOTATION



INTERPRETATION
& REPORT

- The first step of tertiary analysis is data annotation
- Data annotations determine which variants undergo expert review
- Annotation source: dynamic database (Clinvar)
  - Database should be update quarterly
- Extensive functional analysis of variant
  - Noncoding RNAs
  - Regulatory elements
  - Deep intronic region

National Library of Medicine
National Center for Biotechnology Information

ClinVar | ClinVar | Lynch syndrome | Search
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ClinVar

ClinVar aggregates information about genomic variation and its relationship to human health.

Using ClinVar

- About ClinVar
- Data Dictionary
- Downloads/FTP site
- FAQ
- Contact Us
- Factsheet

Tools

- ACMG Recommendations for Reporting of Incidental Findings
- ClinVar Submission Portal
- Submissions
- Variation Viewer
- Clinical Remapping - Between assemblies and RefSeqGenes
- RefSeqGene/LRG

Related Sites

- ClinGen
- GeneReviews ®
- GTR ®
- MedGen
- OMIM ®
- Variation

C

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Tabular | 100 per page | Sort by Location | Download:

(767)

Search results

'75)

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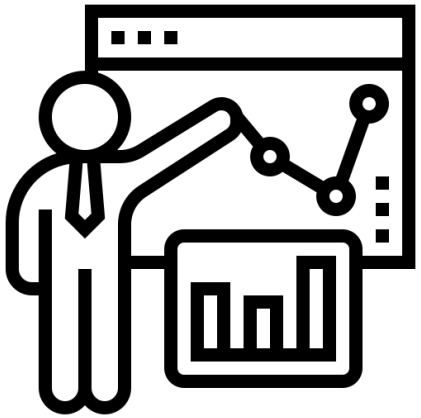
	Variation *Location*	Gene(s)	Protein change	Condition(s)	Clinical significance (Last reviewed)	Review status	Accessio
1.	NM_006015.6(ARID1A):c.1653C>G (p.Tyr551Ter) *GRCh37:* Chr1:27057945 *GRCh38:* Chr1:26731454	ARID1A	Y551*	Colorectal cancer	association	no assertion criteria provided	VCV0005236
2.	NM_006015.6(ARID1A):c.4689del (p.Pro1563_Met1564insTer) *GRCh37:* Chr1:27101402 *GRCh38:* Chr1:26774911	ARID1A		Colorectal cancer	Pathogenic	no assertion criteria provided	VCV0009981
3.	NM_006015.6(ARID1A):c.5715del (p.Lys1905fs) *GRCh37:* Chr1:27106100 *GRCh38:* Chr1:26779609	ARID1A	K1688fs, K1905fs	Colorectal cancer	Pathogenic	no assertion criteria provided	VCV000998
4.	NM_001048174.2(MUTYH):c.1562del (p.Gln521fs)	MUTYH	Q406fs, Q429fs, Q521fs, Q522fs, Q532fs, Q536fs,	Colorectal cancer	Pathogenic	no assertion criteria provided	VCV0009981

REQUISITION
& CONSENT

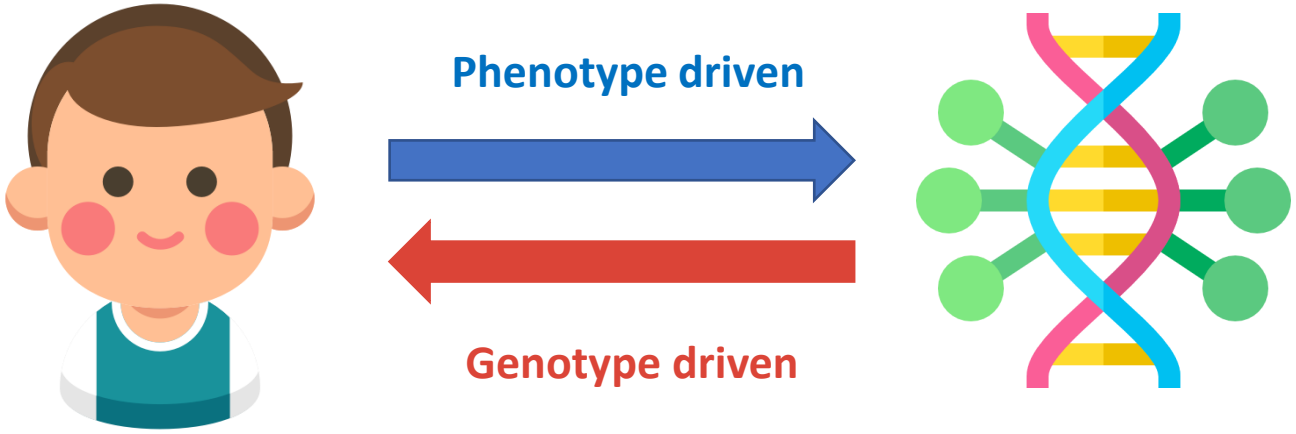
ANALYSIS

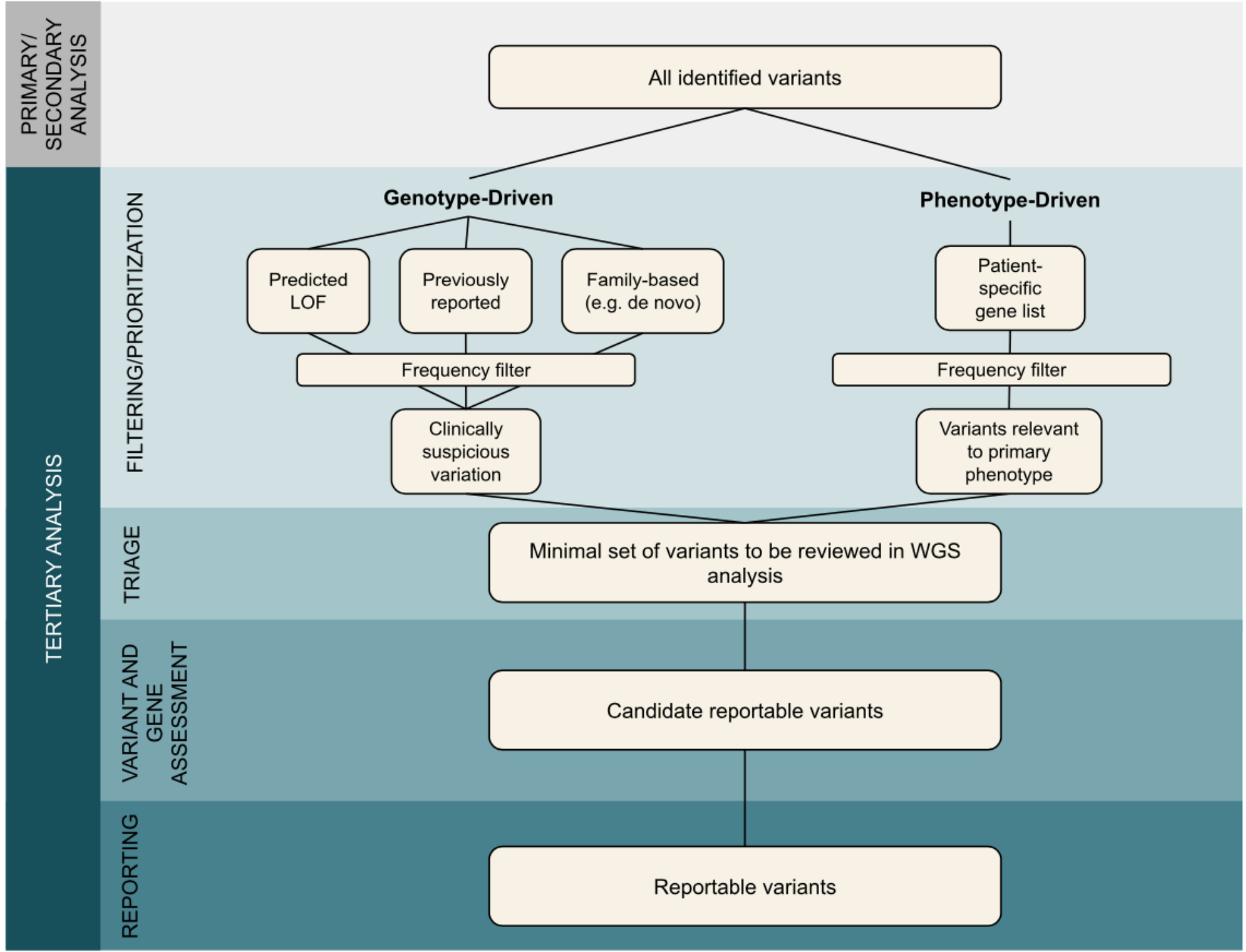
ANNOTATION

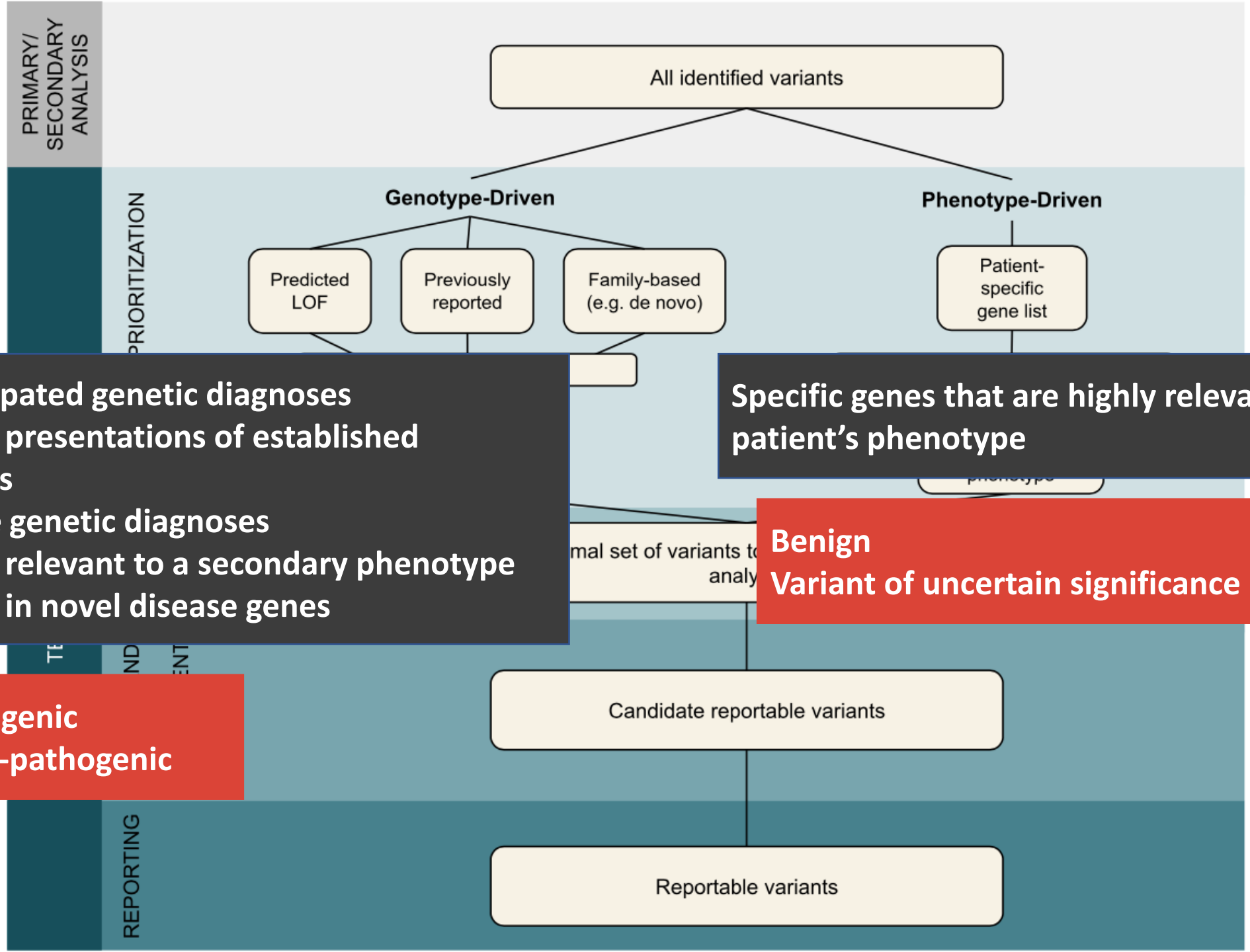


INTERPRETATION
& REPORT

- Filtering
- Prioritization
- Genotype driven vs. Phenotype driven



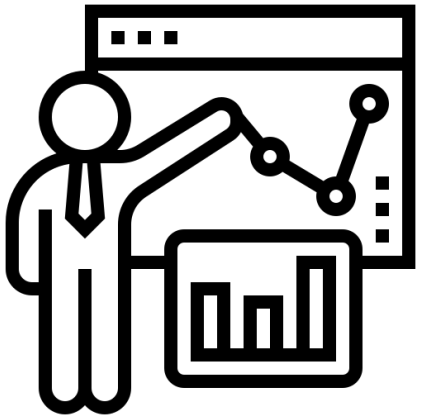

PRIMARY/ SECONDARY ANALYSIS
TERTIARY ANALYSIS
FILTERING/PRIORITIZATION
TRIAGE
VARIANT AND GENE ASSESSMENT
REPORTING
All identified variants
Genotype-Driven
Phenotype-Driven
Predicted LOF
Previously reported
Family-based (e.g. de novo)
Patient-specific gene list
Frequency filter
Frequency filter
Clinically suspicious variation
Variants relevant to primary phenotype
Minimal set of variants to be reviewed in WGS analysis
Candidate reportable variants
Reportable variants


PRIMARY/ SECONDARY ANALYSIS
All identified variants
Genotype-Driven
Phenotype-Driven
Predicted LOF
Previously reported
Family-based (e.g. de novo)
Patient-specific gene list
(1) Unanticipated genetic diagnoses
(2) Unusual presentations of established disorders
(3) Multiple genetic diagnoses
(4) Variants relevant to a secondary phenotype
(5) Variants in novel disease genes
Specific genes that are highly relevant to the patient's phenotype
Benign
Variant of uncertain significance
Pathogenic
Likely-pathogenic
Candidate reportable variants
Reportable variants
REPORTING

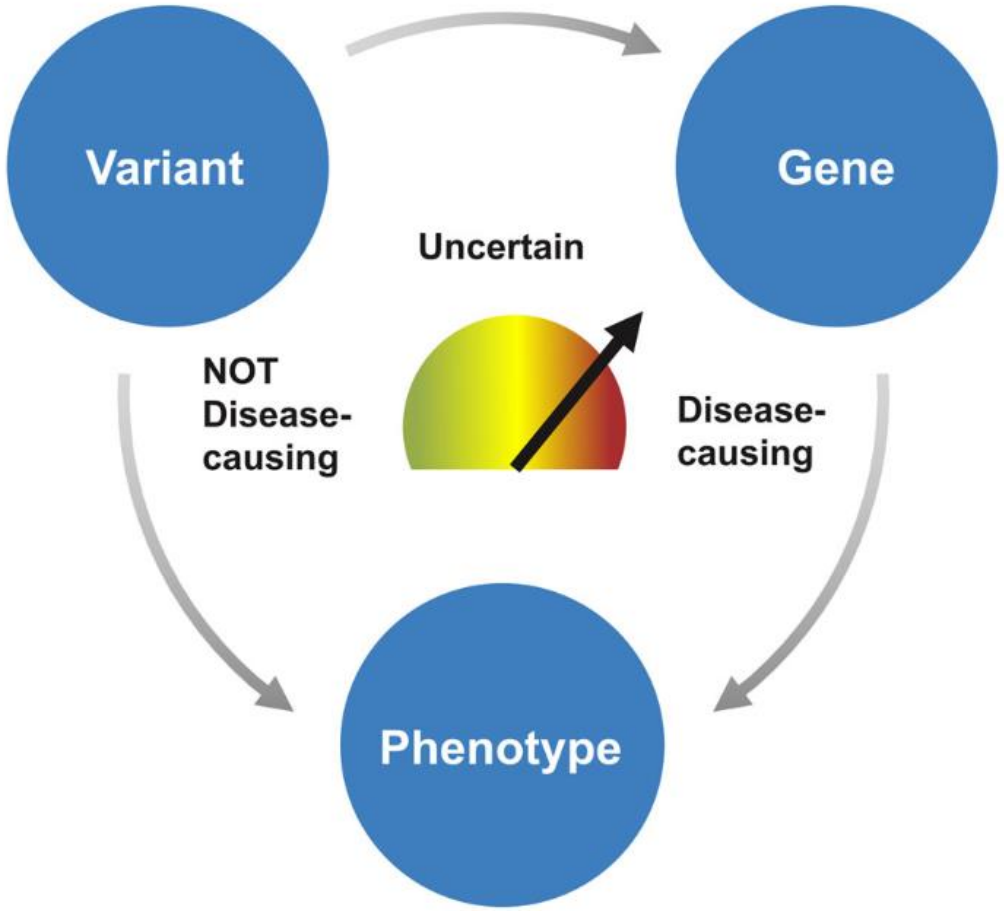
REQUISITION
& CONSENT

ANALYSIS

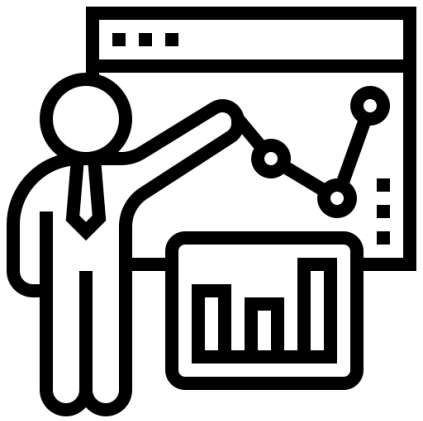
ANNOTATION



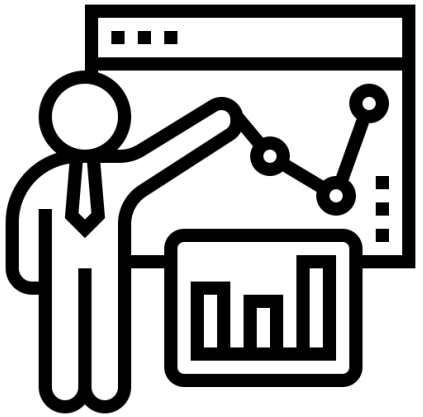
INTERPRETATION
& REPORT



- What gene(s) does the variant impact?
- Is the variant expected to impact the function of the gene(s) and if so, does it cause a human phenotype?
- How well does the variant or gene's disease association match that of the patient?
- Has this particular variant (or this variant type, e.g. LOF variants) been shown to cause a phenotype?
- Is the variant returnable as a secondary or incidental finding?



- Triage efficiency can be influenced by the software used to support analysis
- Variant prioritization can be considered as a means to improve overall analytical efficiency
- Time-delay between publication and database



Reporting

- The lab should establish policies defining the types of findings that are considered for return
- High-level descriptive statements are essential
  - “Positive”
  - “Positive: Findings explain indication for testing”
- Integration of WGS result into medical record

Reanalysis

- It is recommended that lab provide an option for reanalysis of finalized WGS cases

Table 2. Suggested steps of reanalysis based on events that have occurred since initial analysis.

Change since initial analysis	Primary analysis (sample/ library prep and sequencing)	Secondary analysis (mapping, alignment, variant calling, QC)	Tertiary analysis			
			Annotation	Variant stratification	Variant and gene assessment	Reporting
Significant improvements in library prep/sequencing technology	✓	✓	✓	✓	✓	✓
Bioinformatics improvements		✓	✓	✓	✓	✓
>1 year lapsed since initial analysis			✓	✓	✓	✓
Additional patient phenotypes or family history				✓	✓	✓
Improved understanding of the genetic etiology of patient condition				✓	✓	✓
New methodology or resource for variant assessment					✓	✓

Thank you !!