Molecular technologies for acquired and constitutional diagnosis

SOFIE SYMOENS

ANNELIES DHEEDENE

Searching for a needle in a haystack = Finding the genetic cause of disease

- The diploid **human** genome consists of 6 billion nucleotides
- A mistake in 1 nucleotide can be causal to severe disease
- countless strategies and techniques developed to find this 'needle'

What material do we need?

Isolated gDNA

EDTA-blood sample / umbilical cord blood sample

- To isolate gDNA
- To generate EBV cell lines
- To isolate mitochondrial DNA

Skin biopsy – cultured fibroblasts

Buccal swap

Prenatal samples: blood sample mother – chorion villus – amniotic fluid – skin/rib/muscle biopsy (terminated pregnancy)

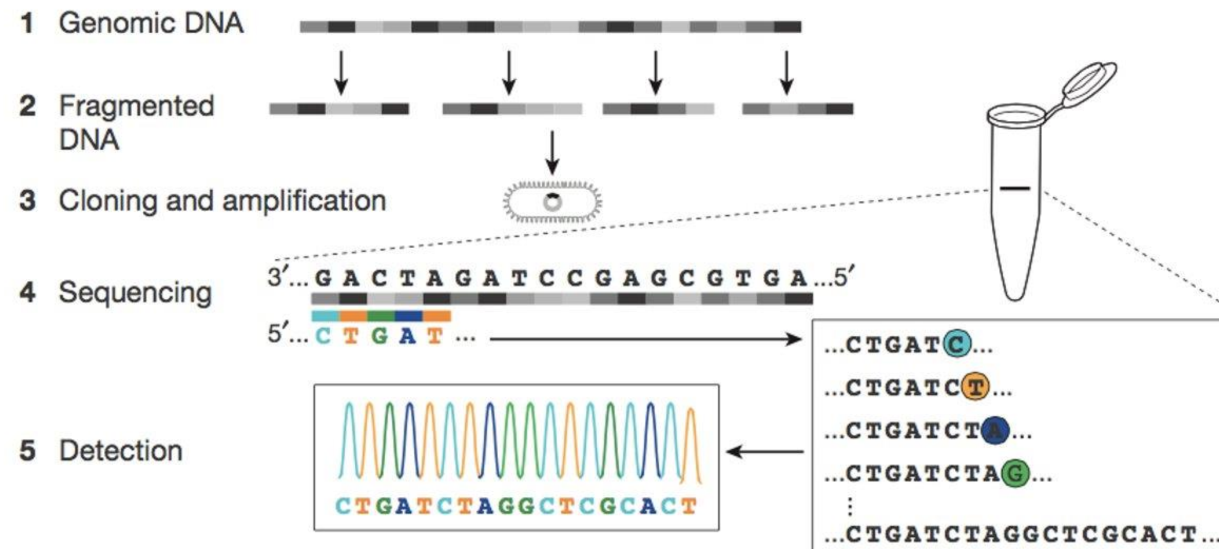
Paraffin sections (FFPE)

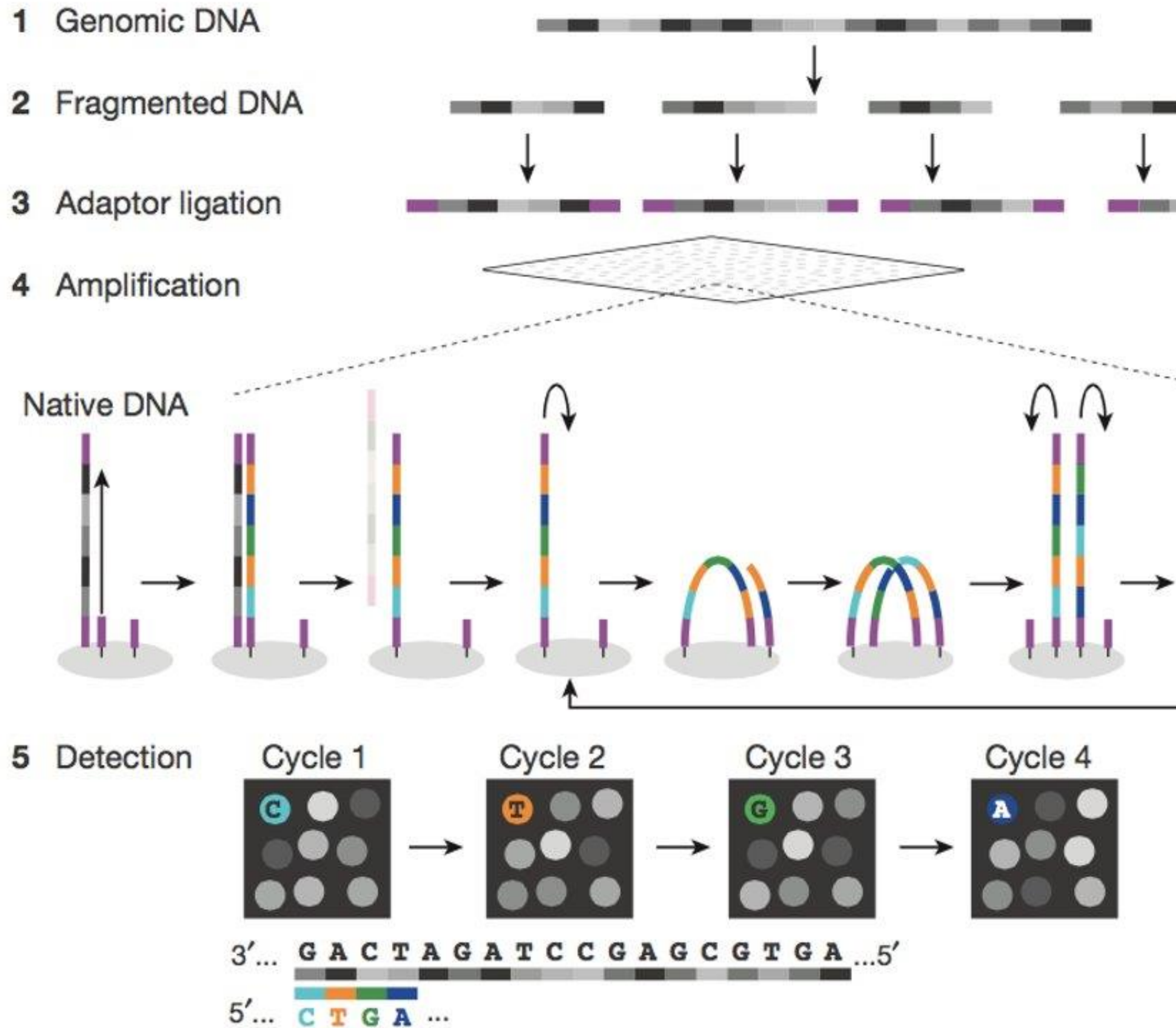
First Generation	Second Generation (Next Generation Sequencing)	Third Generation
Sanger Sequencing Maxam and Gilbert Sanger Chain-termination	454, Solexa, Ion Torrent Illumina	PacBio Oxford Nanopore
- Infer nucleotide identity using dNTPs then visualize with electrophoresis - 500-1000 bp fragments	- High throughput from the parallelization of sequencing reactions - ~50-500 bp fragments	- Sequence native DNA in real time with single-molecule resolution - Tens of kb fragments, on average

Short-read sequencing (First and Second Generation)

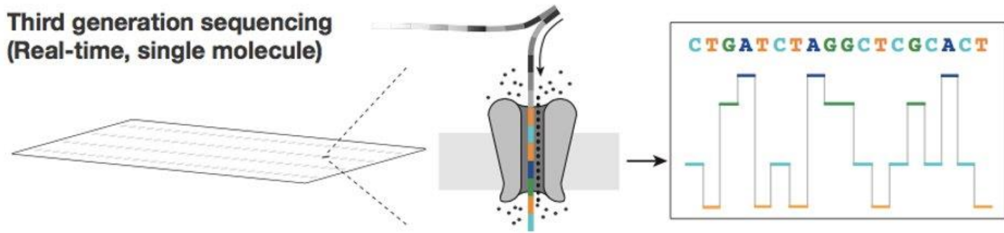
Long-read sequencing (Third Generation)

First generation sequencing: Sanger sequencing



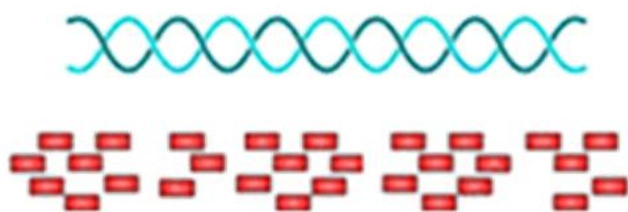
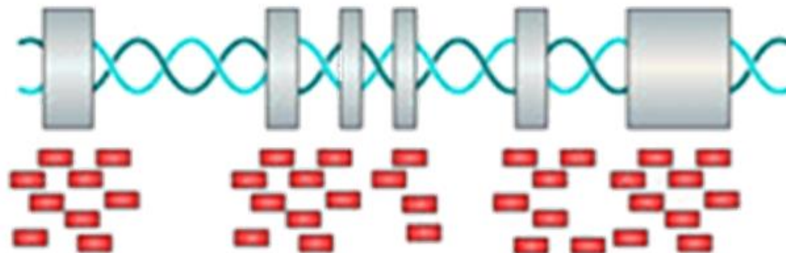
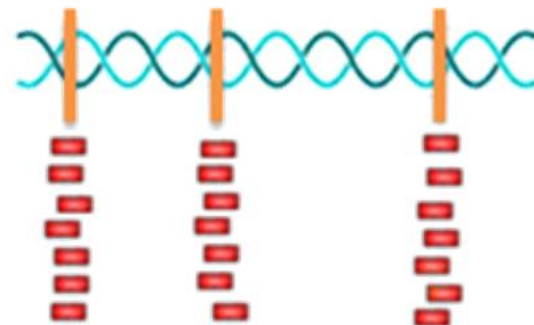


Second generation sequencing: massively parallel sequencing (MPS)



Third generation sequencing

Rare Mutation Matters!
Find it by deep sequencing of whole exome or candidate genes
BGI'S GENOMICS BAR
Here you go!
What are the best ones for rare mutation?
BGI
Exome
Candidate genes
WINELIST
Candidate genes
Exome
DGE
Transcriptome
de novo
Epigenome
Metagenome
Resequencing
Cloud computing
华大基因
BGI
tech@genomics.cn
www.genomics.cn
86-755-25273395

Whole genome sequencing	Whole exome sequencing	Targeted sequencing
		
■ Sequencing region : whole genome ■ Sequencing Depth: >30X ■ Covers everything – can identify all kinds of variants including SNPs, INDELs and SV.	■ Sequencing region: whole exome ■ Sequencing Depth : >50X ~ 100X ■ Identify all kinds of variants including SNPs, INDELs and SV in coding region. ■ Cost effective	■ Sequencing region: specific regions (could be customized) ■ Sequencing Depth : >500X ■ Identify all kinds of variants including SNPs, INDELs in specific regions ■ Most Cost effective

Solving the molecular diagnostic testing conundrum for Mendelian disorders in the era of next-generation sequencing: single-gene, gene panel, or exome/genome sequencing

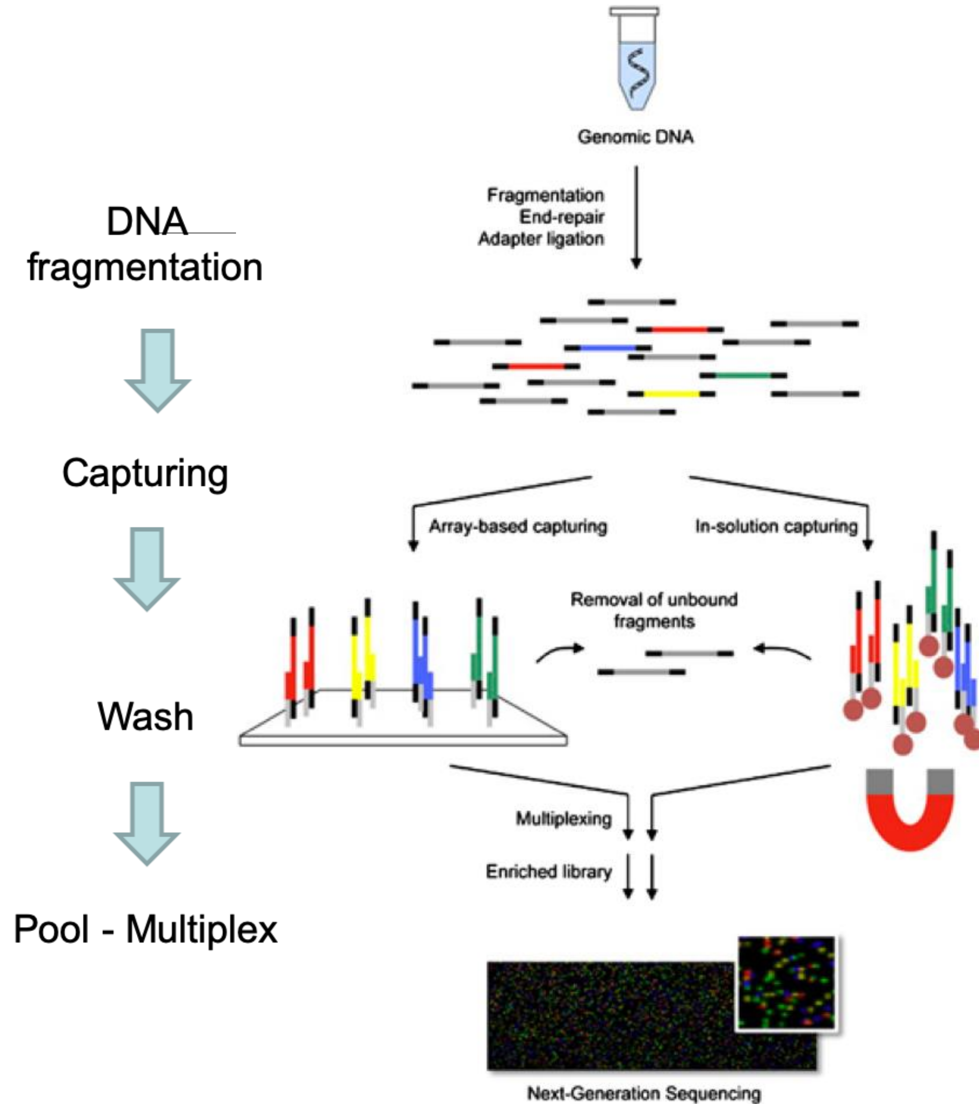
Yuan Xue, PhD, FACMG1, Arunkanth Ankala, PhD1, William R. Wilcox, MD, PhD2 and Madhuri R. Hegde, PhD, FACMG1, Genetics in Medicine doi:10.1038/gim.2014.122

Table 1 Indications for single-gene, gene panel, and ES tests

Testing option	Indications	Examples	Reference
Single-gene test	Minimal locus heterogeneity	*CFTR* for CF	32
	Distinctive clinical findings (e.g., X-ray, biochemical evaluation) clearly point to a specific gene	*FGFR3* for achondroplasia; *PAH* for PKU	4,33
	Limitations of NGS sequencing technology to detect trinucleotide repeat disorders and disorders with epigenetic abnormalities	Fragile X; Prader-Willi and Angleman syndrome	34,35
Gene panel	Heterogeneity	Muscular dystrophies panel	16
	Disorders with overlapping phenotype—differential diagnosis	Cardiomyopathy panel	36
	Disorders share one manifestation but may have completely different overall presentation	Epilepsy panel	37
	Diseases associated with genes from a common pathway or structure	RASopathies panel	38
ES/GS[a]	Extreme heterogeneity and de novo changes are the major mutations	Autism, ID	39
	Two or more likely unrelated phenotypes in one patient	Oculocutaneous albinism and neutropenia	40
	No key phenotypic feature is present at the time when the test is ordered	Kabuki syndrome	41
	Phenotype is indistinct, and the real underlying cause is not easy to identify	Congenital diarrhea; Zellweger syndrome	42,43

CDG, congenital disorder of glycosylation; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; ES, exome sequencing; FGFR3, fibroblast growth factor receptor 3; GS, genome sequencing; ID, intellectual disability; NGS, next-generation sequencing; PAH, phenylalanine hydroxylase; PKU, phenylketonuria; RASopathies, a group of genetic disorders caused by pathogenic variants in genes that encode components of the Ras/mitogen-activated protein kinase (MAPK) pathway.

[a]ES/GS selection is based on cost and ability to performance analysis. GS is typically performed at a lower depth than ES; the cost of performing the assay, performing analysis, and storage is more than that for ES.



Target selection/hybridisation strategies

PCR based approaches

- Singleplex
- Multiplex

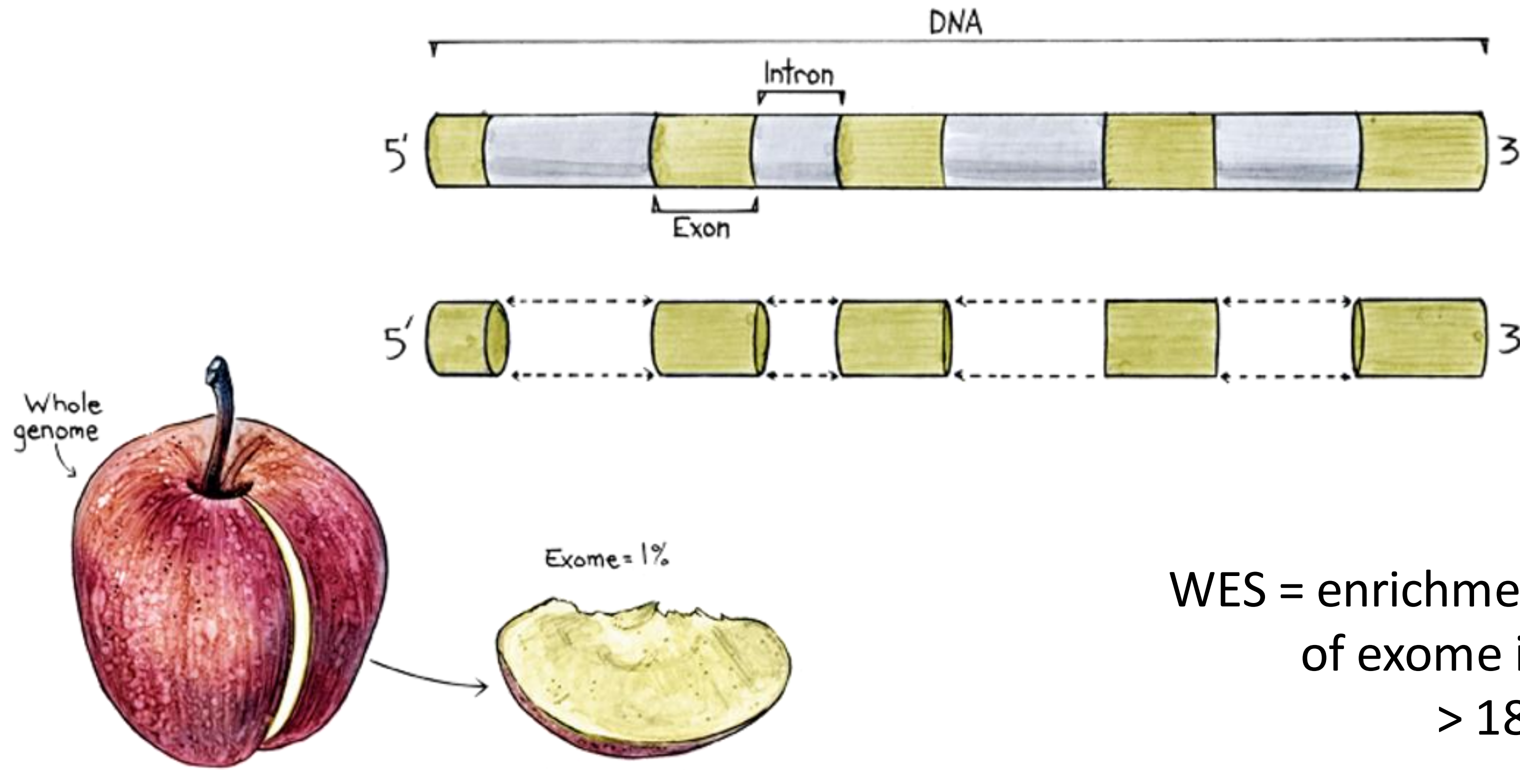
Hybridisation based approaches

- Array Capture
- In **solution followed by bead capture**

Molecular Inversion probes

Whole Exome Sequencing (WES)

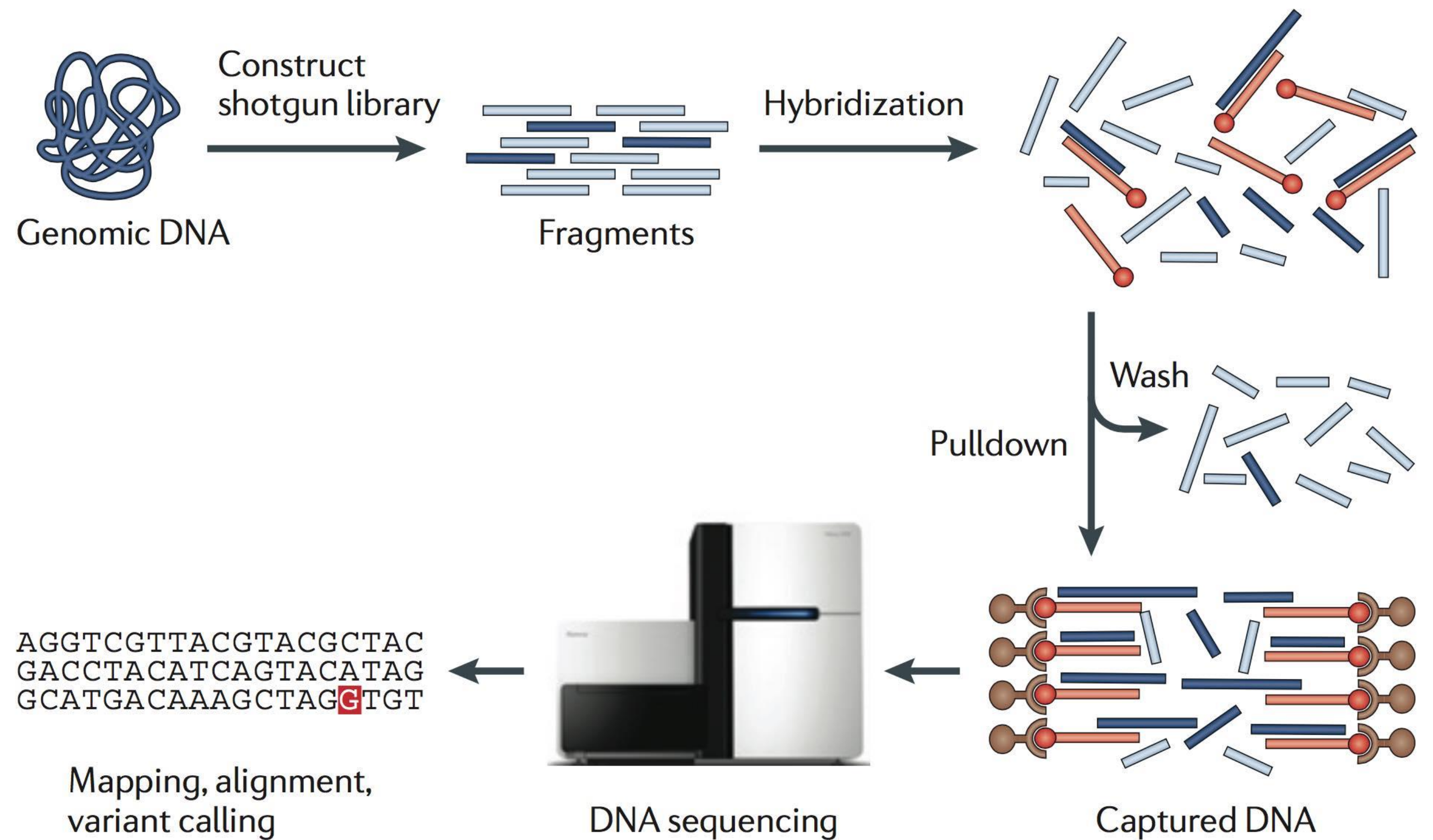
Exome = all coding sequences or exons of a genome, only 1-2% of the genome, 30 Mb

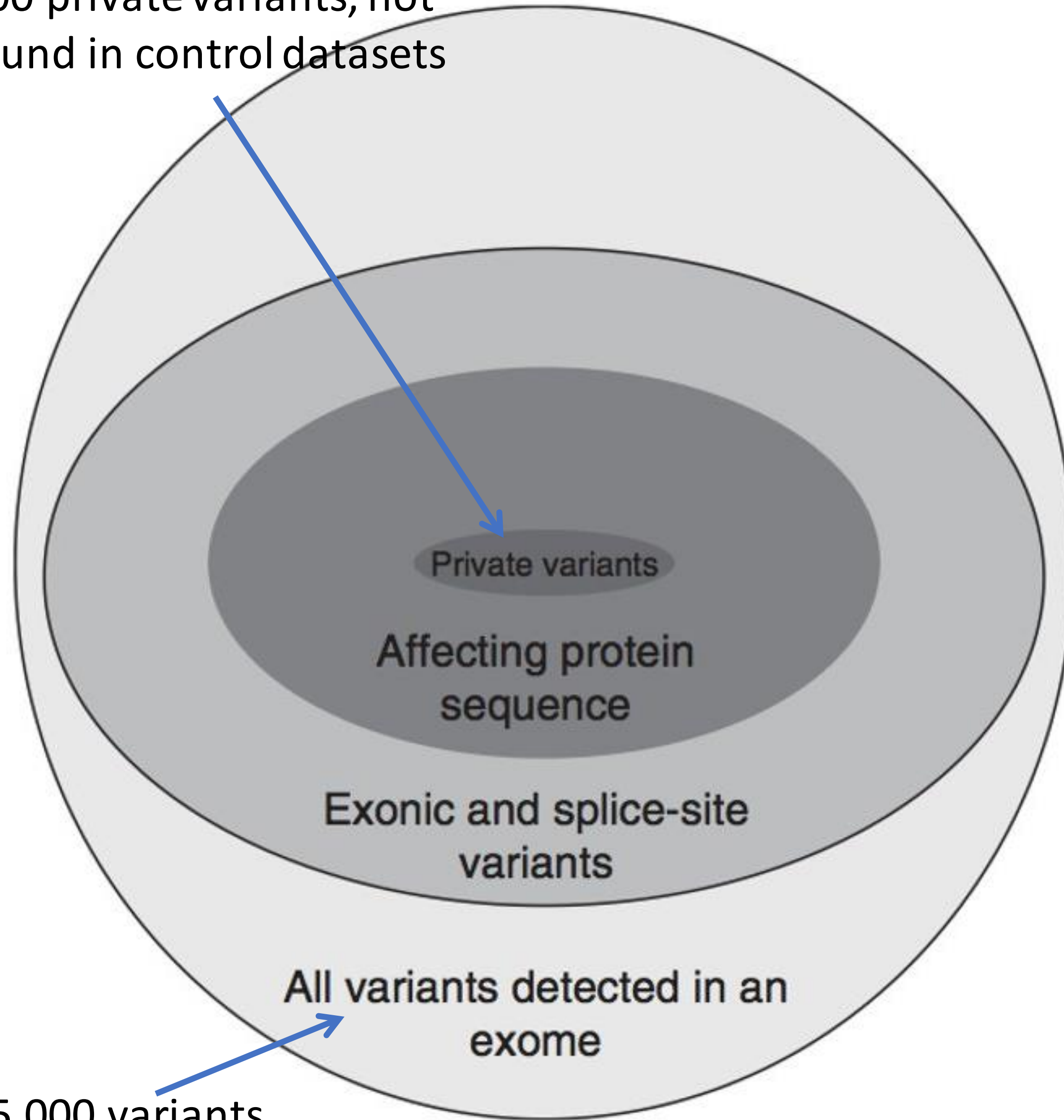


WES = enrichment of exome, sequencing of exome in one experiment

> 180 000 exons

Whole exome sequencing (WES)





1. Data Production

Millions of raw sequence reads are produced for a patient tumor.

2. Processing and Event Detection

Sequences are aligned to the reference genome and tumor-specific events predicted.

3. Filtering, Review, and Validation

Data are reviewed and validation experiments performed to identify high quality events.

4. Annotation and Functional Prediction

Events are annotated and scored in an effort to predict events of functional significance.

5. Interpretation and Report Generation

A genome analyst attempts to interpret, prioritize, and summarize functionally significant events in the context of published literature, clinical trials, and a multitude of knowledgebases.

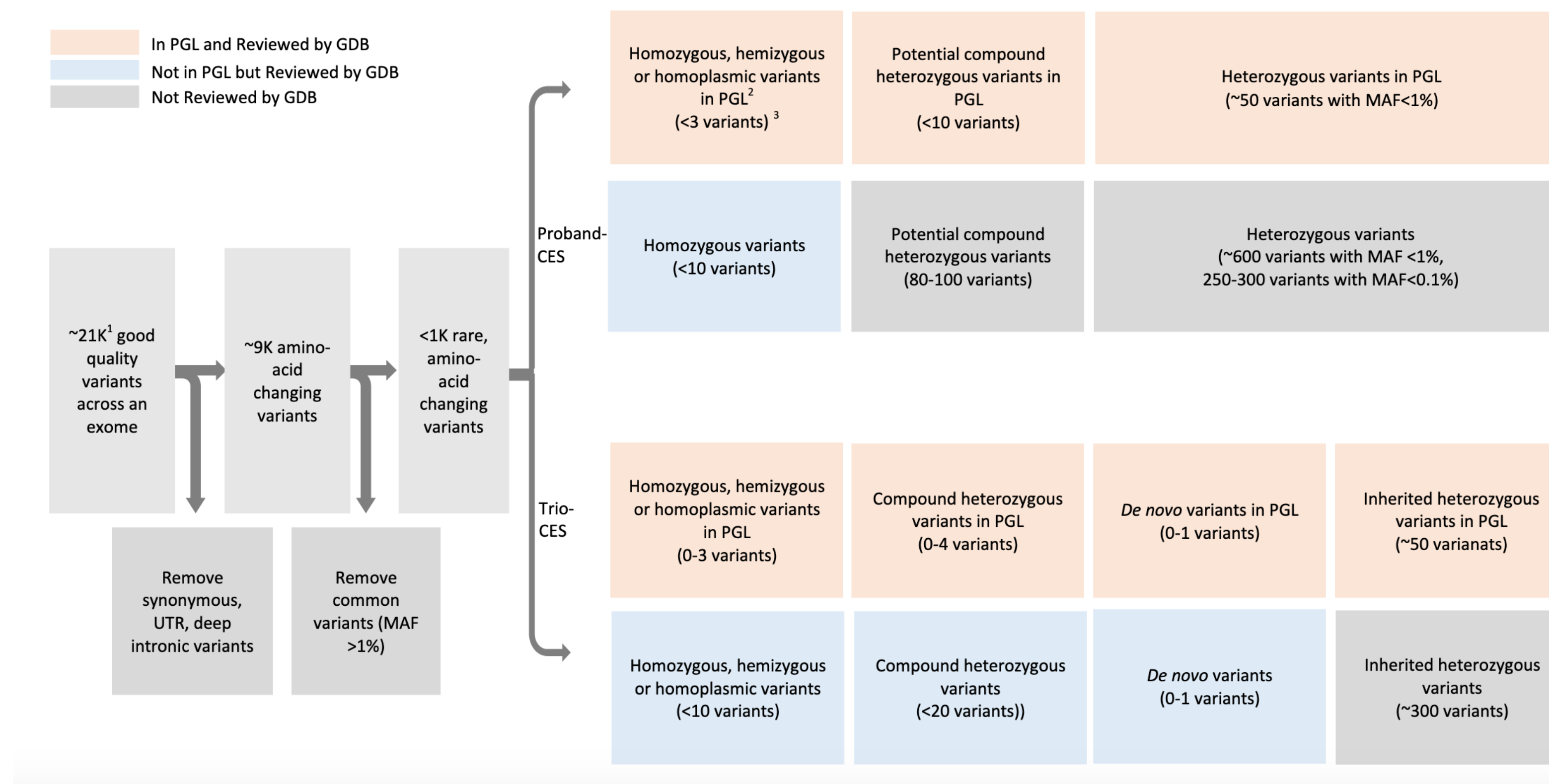
6. Clinical Application

Pathologists and oncologists evaluate the significance of potentially clinically actionable events, and incorporate their research into patient care.

Single case (proband) vs Trio-exome

Clinical Exome Sequencing for Genetic Identification of Rare Mendelian Disorders. JAMA. 2014 November 12; 312(18): 1880–1887. doi:10.1001/jama.2014.14604. Hane Lee, PhD, Joshua L. Deignan, PhD, Naghmeh Dorrani, MS, CGC, Samuel P. Strom, PhD, Sibel Kantarci, PhD, Fabiola Quintero-Rivera, MD, Kingshuk Das, MD, Traci Toy, BS, Bret Harry, BS, Michael Yourshaw, PhD, Michelle Fox, MS, CGC, Brent L. Fogel, MD, PhD, Julian A. Martinez-Agosto, MD, PhD, Derek A. Wong, MD, Vivian Y. Chang, MD, MS, Perry B. Shieh, MD, PhD, Christina G. S. Palmer, PhD, CGC, Katrina M. Dipple, MD, PhD, Wayne W. Grody, MD, PhD, Eric Vilain, MD, PhD, and Stanley F. Nelson, MD

Clinical exome sequencing was performed on 814 consecutive patients with undiagnosed, suspected genetic conditions at the University of California, Los Angeles, Clinical Genomics Center between January 2012 and August 2014.



The molecular diagnosis rate for **trio-CES was 31%** and **22% for proband-CES**.
In cases of developmental delay in children (<5 years, n = 138), the molecular diagnosis rate was **41% for trio-CES cases** and **9% for proband-CES cases**.
The significantly higher diagnostic yield of trio-CES was due to the **identification of de novo and compound heterozygous variants**.

--> With the introduction of large gene panels, the **interaction between lab and clinical geneticists**: more and more important and necessary.

10 reasons why WES fails....

1. “Holes”. Regions not enriched
2. Mitochondrial mutations
3. Triplet repeat disorders
4. Regulatory mutations (UTRs, promoter, *cis*-regulatory elements)
5. Deep intronic changes
6. Structural variants (translocations and inversions)
7. Copy number variations
8. Noncoding RNAs
9. Uniparental disomy
10. Epigenetic changes, imprinted genes

What's next?

PRENATAL WHOLE EXOME SEQUENCING

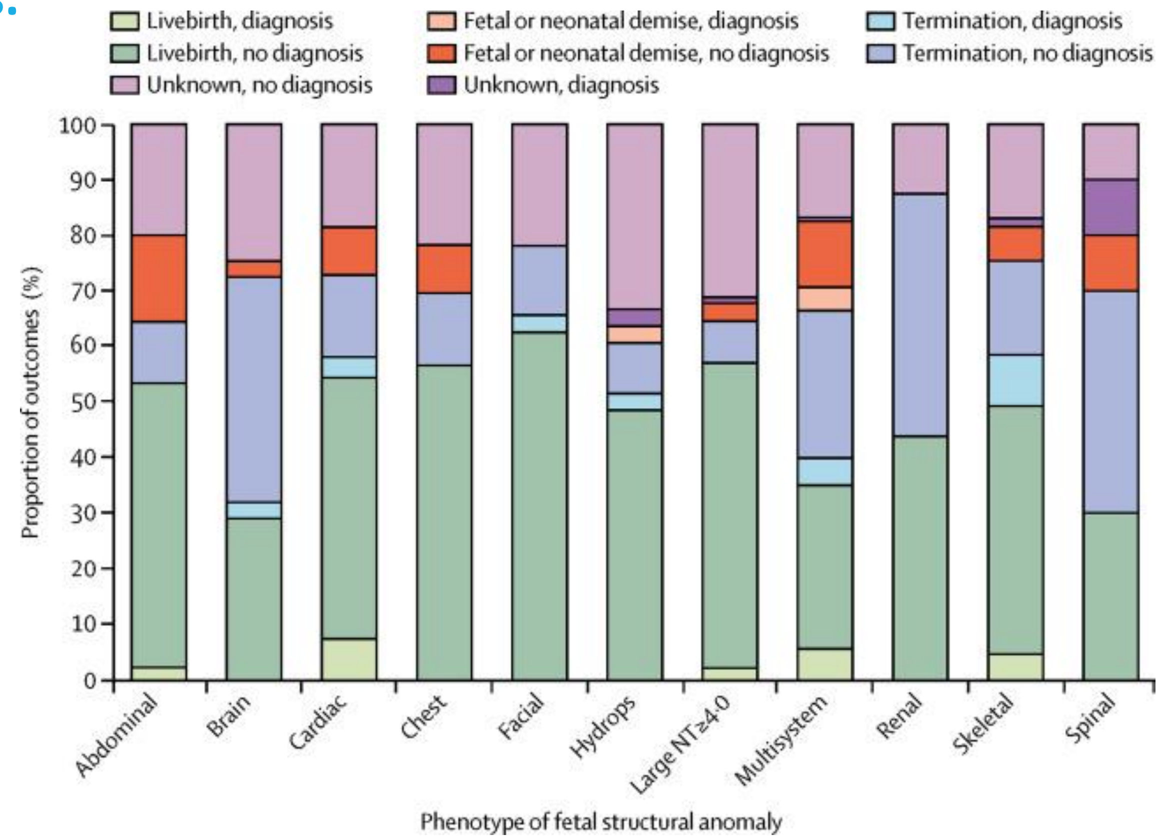
Prenatal exome sequencing analysis in fetal structural anomalies detected by ultrasonography (PAGE): a cohort study

Lancet 2019; 393: 747–57. Jenny Lord*, et al., the Prenatal Assessment of Genomes and Exomes Consortium‡

http://dx.doi.org/10.1016/

610 fetuses with structural anomalies and 1202 matched parental samples (analysed as 596 fetus-parental trios, including two sets of twins, and 14 fetus-parent dyads) were analysed by WES

WES facilitates genetic diagnosis of fetal structural anomalies, which enables more accurate predictions of fetal prognosis and risk of recurrence in future pregnancies. However, the overall detection of diagnostic genetic variants in a prospectively ascertained cohort with a broad range of fetal structural anomalies is lower than that suggested by previous smaller-scale studies of fewer phenotypes. **WES improved the identification of genetic disorders in fetuses with structural abnormalities; however, before clinical implementation, careful consideration should be given to case selection to maximise clinical usefulness.**

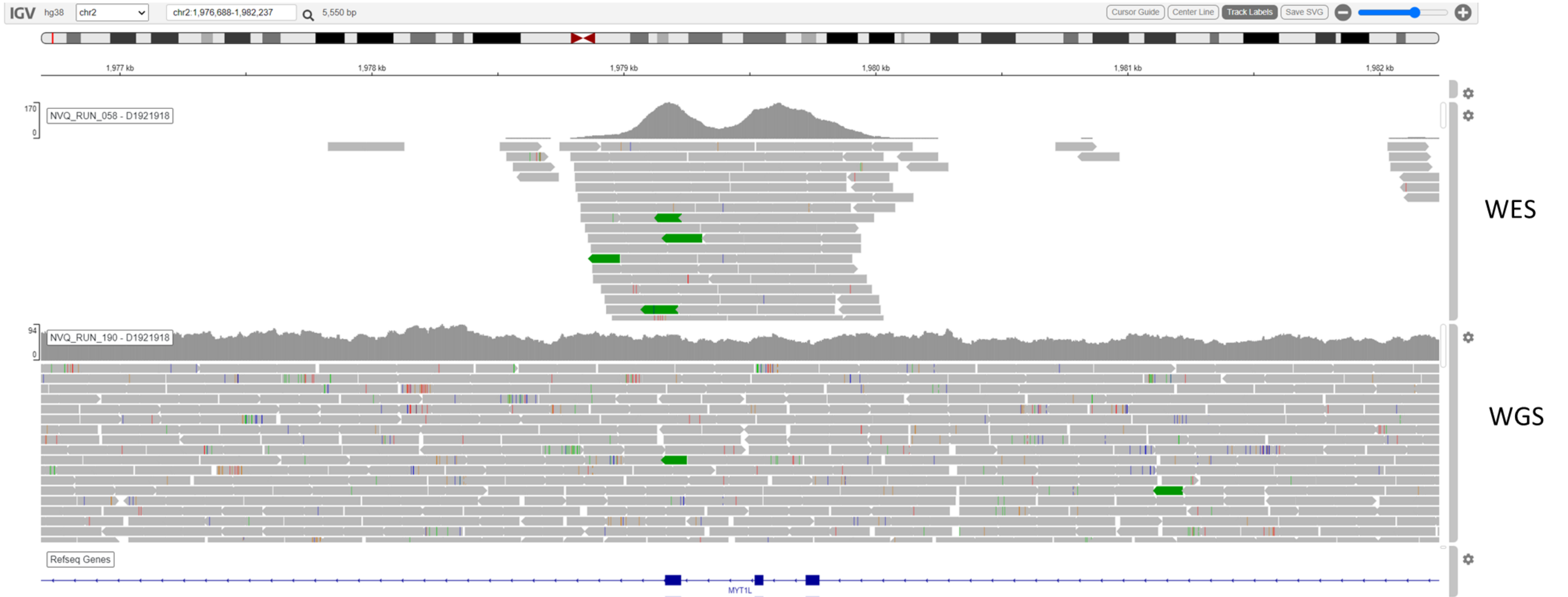


WES or WGS?

WGS data can typically be generated in less than a month for approximately $2000 or less on the latest platforms. However, the assembly of the genome is computationally laborious and most of the non-coding sequence is difficult to interpret. Whole-exome sequencing (WES) can be completed in a similar timeframe and interrogates approximately 95% of the coding region of the genome, comprising ~20,000 genes.

But solution for:

- Regulatory mutations (UTRs, promoter, *cis*-regulatory elements)
- Deep intronic changes
- Noncoding RNAs?
- Holes? Long read sequencing but in not really implemented in diagnostics (yet)

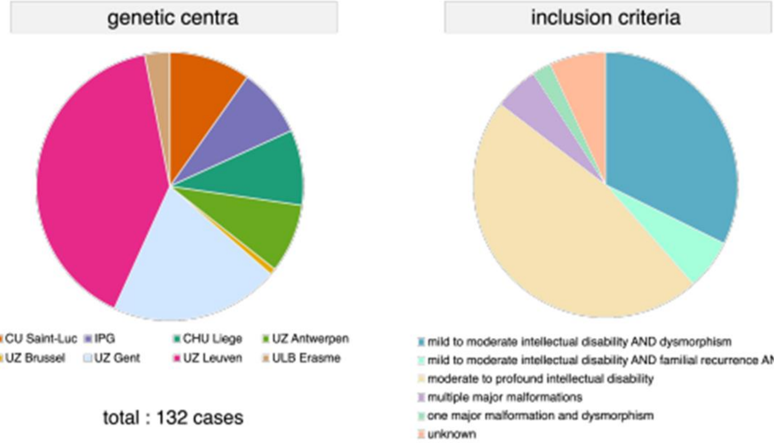


- more even coverage
- reads in “difficult” regions
- lower coverage per locus
- Intronic & intergenic regions covered

BeSolveRD: The Belgian Genome Resource to Resolve Rare Diseases

MULTICENTRIC PROSPECTIVE RANDOMISED TRIAL

INCLUSION STATUS



May 2022

https://beshg.be/workgroups/besolverd

BAD NEWS, YOU HAVE A PREDISPOSITION TO CURIOSITY
DNA TEST RESULTS