

# Next-generation sequencing: kansen en uitdagingen

Symposium Nefrogenetica  
12 oktober 2024



CENTRUM MEDISCHE  
GENETICA GENT



TO INTRODUCE GENETIC TEST  
STRATEGIES

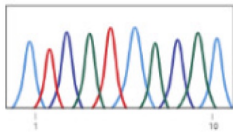


TO BETTER UNDERSTAND  
GENETIC TEST RESULTS

# Evolution in DNA sequencing

## First generation sequencing

Sanger sequencing



1977



Maxam-Gilbert sequencing

## Second generation sequencing

Illumina GA



Ion torrent PGM

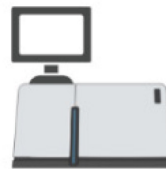


2005

2006

2007

2010



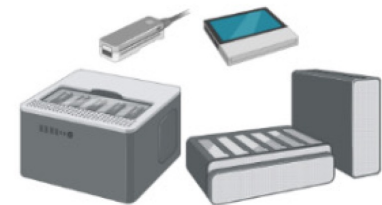
454 Pyrosequencing



ABI's SoLiD system

## Third generation sequencing

Nanopore sequencing family



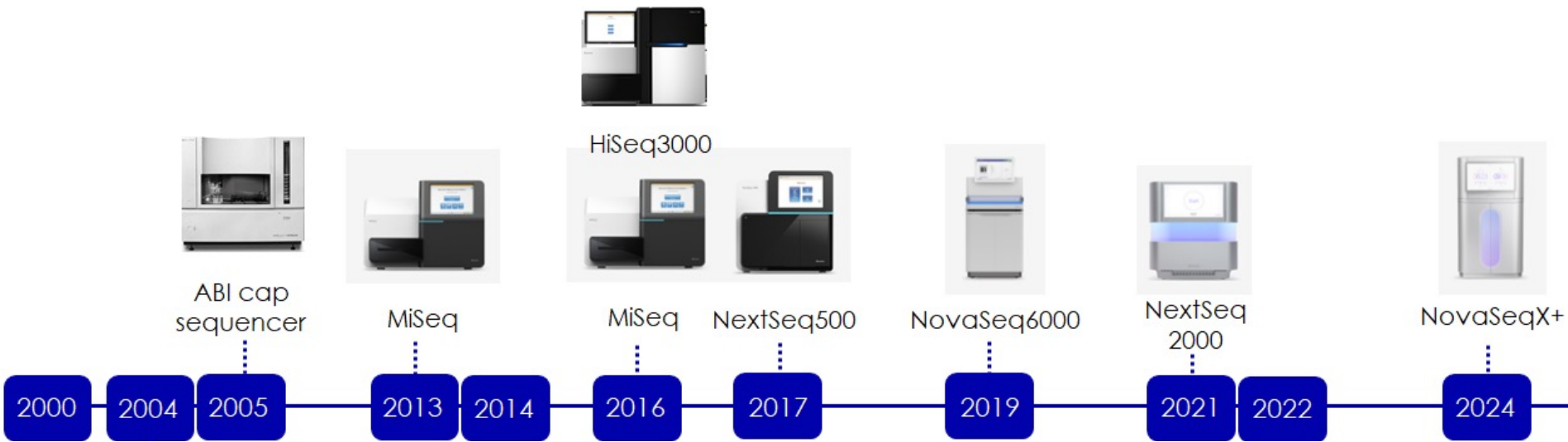
2011

2014

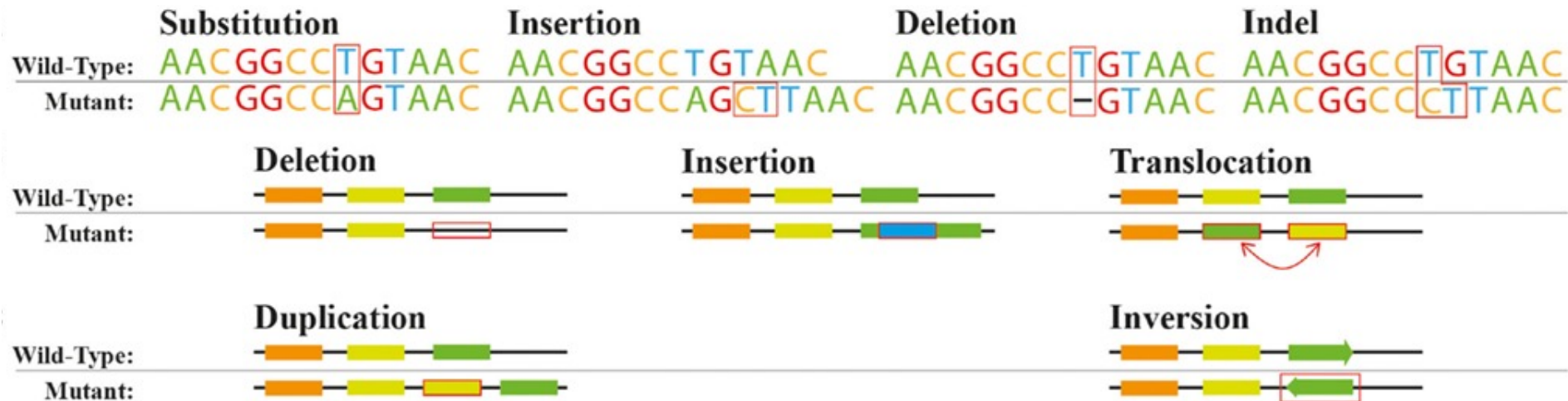


PacBio Sequel System

# Evolution in DNA sequencing



# Types of genetic variation



# Evolution in DNA sequencing

## First generation sequencing

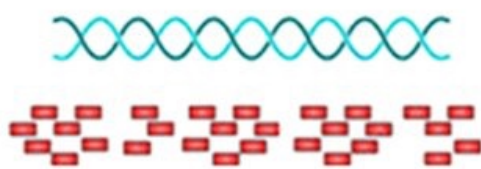
- ▶ High sequencing cost / base
- ▶ Small-scale: 1 run = 96 exons/amplicons
- ▶ Serial: gene by gene sequencing
- ▶ Sequence variants (SNVs)

## Second generation sequencing

- ▶ Expensive infrastructure
- ▶ Low sequencing cost / base
- ▶ Parallel: many targets at the same time, high-throughput
- ▶ Sequence variants (SNVs) but also copy number variants (CNVs)

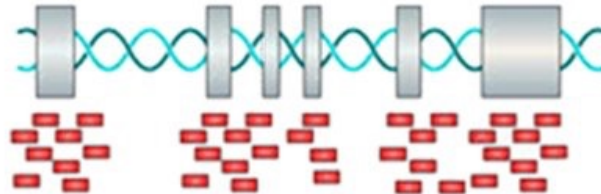
# Second Generation Sequencing = Massively Parallel Sequencing (MPS) or Next Generation Sequencing (NGS)

## Whole genome sequencing



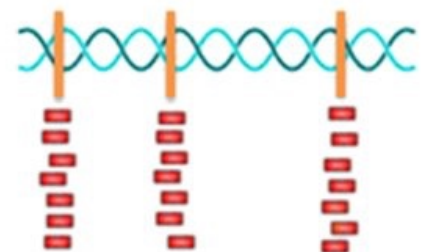
- Sequencing region : whole genome
- Sequencing Depth : >30X
- Covers everything – can identify all kinds of variants including SNPs, INDELs and SV.

## 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

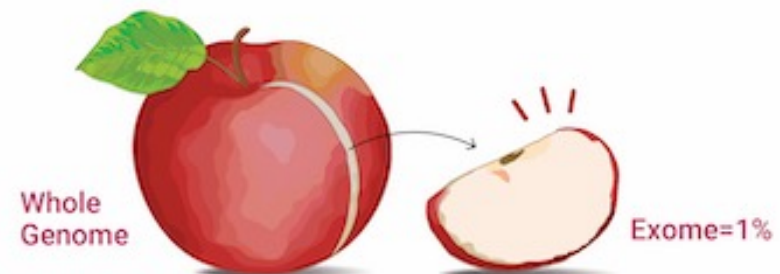
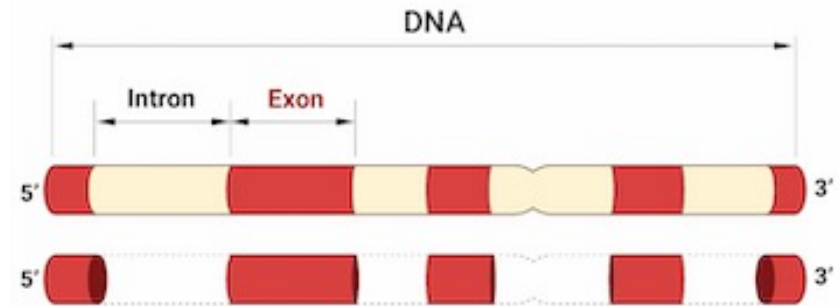
## Targeted sequencing



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

Coverage = number of reads that align to a specific base

# Screening of the nephropathy gene panel @ UZ Gent: Whole Exome Sequencing (WES) approach



# Screening of the nephropathy gene panel @ UZ Gent: Whole Exome Sequencing (WES) approach

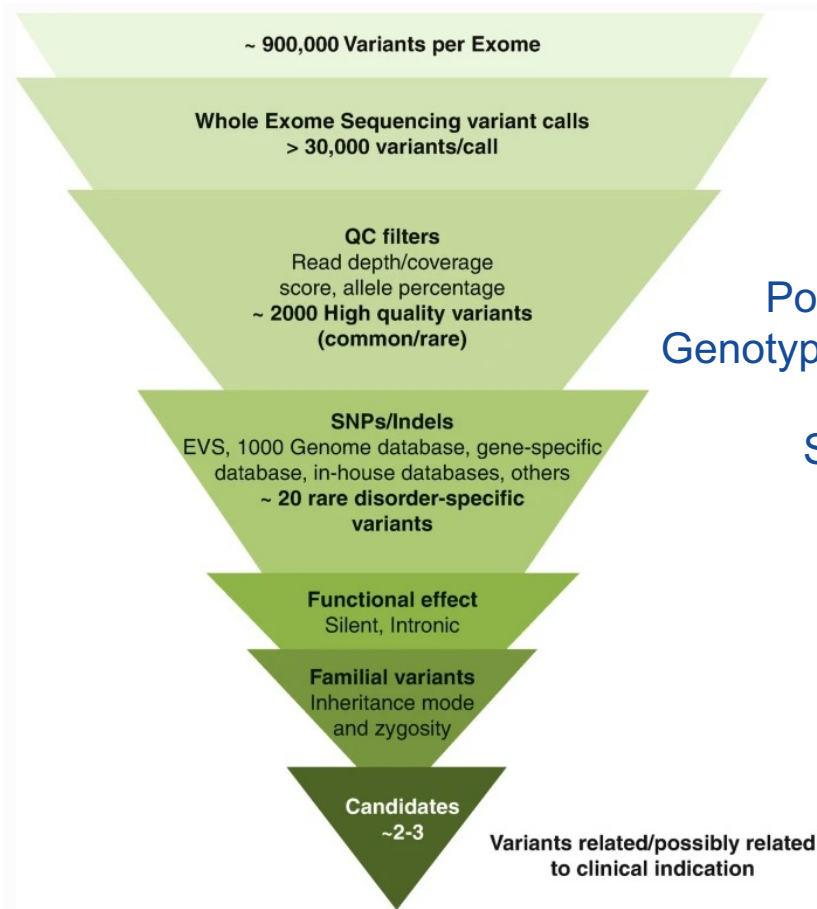
- ▶ WES: virtual Nefro panel
  - ▶ Annual update
  - ▶ 343 genes (version 2)
  - ▶ Content: CMGG website
  - ▶ Literature (pubmed), PanelApp ([Genomics England PanelApp](#)), finetuning with commercial websites such as Invitae
- ▶ Lab: Jozefien Weytens, Griet Vermeulen and Petra Vermassen
- ▶ [Constitutioneel genetische aandoeningen - Centrum Medische Genetica \(cmgg.be\)](#)

Constitutioneel genetische aandoeningen

| ANALYSES                    |  | TOELICHTING                                                                                                                                                                        |           |
|-----------------------------|--|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| Filter:                     |  | nefro                                                                                                                                                                              |           |
| Aandoening                  |  | Gen / Locus / Chromosoom                                                                                                                                                           | Duur      |
| <a href="#">Nefropathie</a> |  |  Nefrogenetica panel:<br><a href="#">genpanel_versie 1</a><br><a href="#">genpanel_versie 2</a> | 6 maanden |

**Gene panel update is coming!**  
**Version 3 will be launched**  
**November 2024**  
**392 genes**

# How to determine the pathogenicity of a variant?



## Evidence

Population frequency  
Genotype-phenotype correlation  
Functional data  
Segregation data

5 classes



Benign



Likely benign  
> 90% certainty



Uncertain  
significance

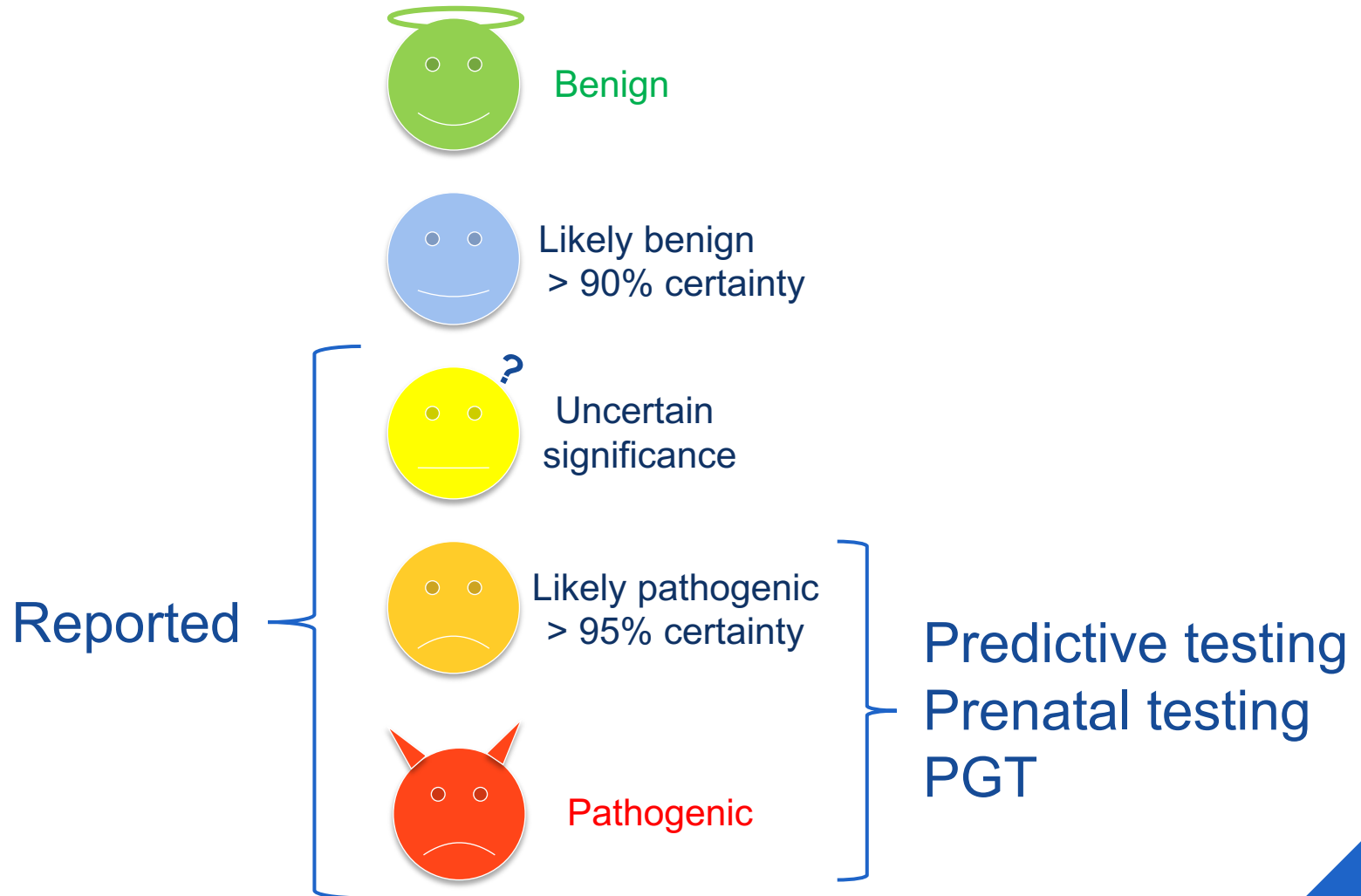


Likely pathogenic  
> 95% certainty



Pathogenic

# How to determine the pathogenicity of a variant?



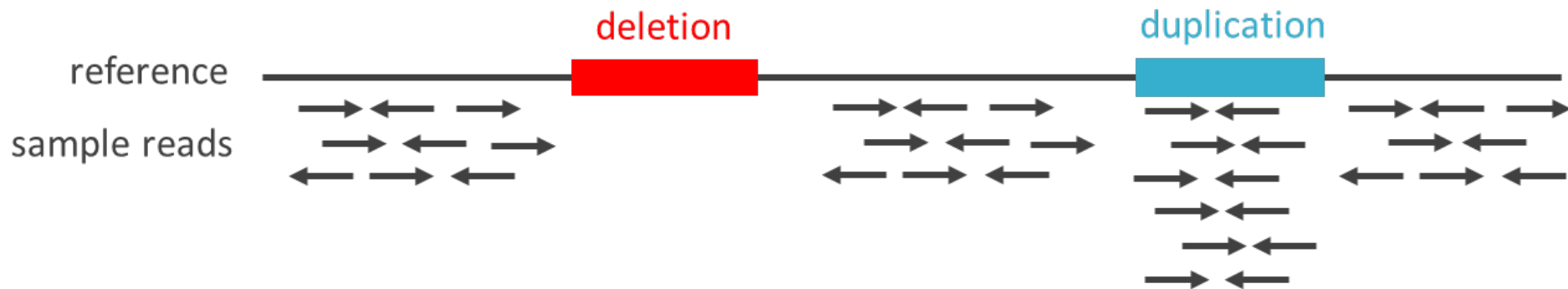
# When is a variant of unknown significance reported?



- ▶ *De novo* variant in a gene fitting with the phenotype
- ▶ Autosomal recessive disorder: in combination with one (likely) pathogenic variant in this gene
- ▶ Autosomal recessive disorder: homozygous variant
- ▶ "Hot" VUS but insufficient evidence to reach variant classification likely pathogenic due to absence of familial samples or functional evidence

# Screening of the nephropathy gene panel @ UZ Gent: Whole Exome Sequencing (WES) approach

CNVs: ExomeDepth: algorithm for the detection of CNVs in  
WES data based on read depth

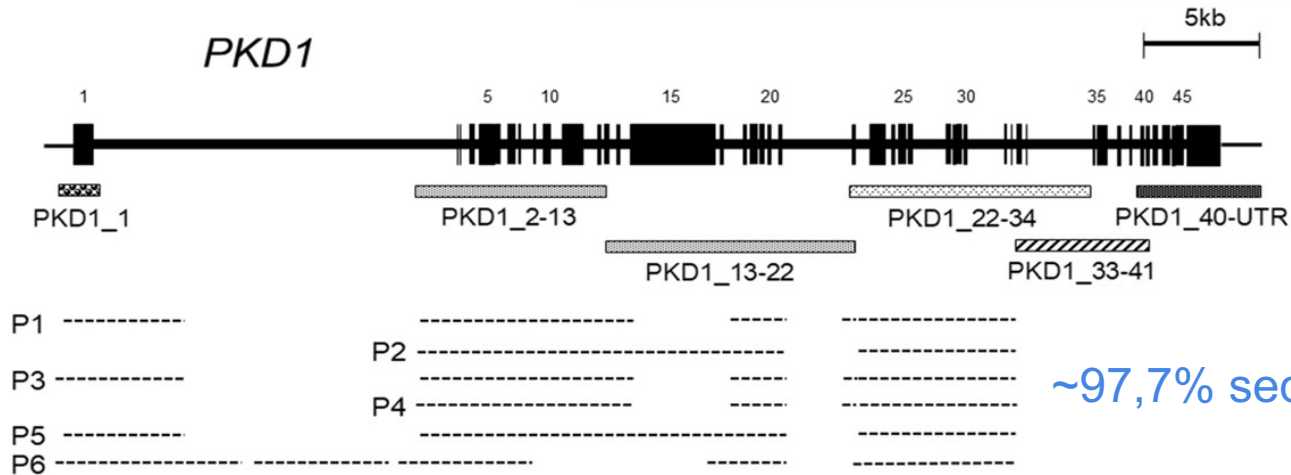


# Challenges and limitations of WES

- Variants that are not linked to the clinical phenotype, but do have clinical implications (secondary/unsolicited findings): guidelines ACMG: 'actionable' genes
- Technical limitations:
  - Not all types of genetic alterations are detectable
    - Triplet repeats
    - Structural variants - uniparental disomy
    - Deep intronic variants
    - Non-coding variants
  - Uninsufficient coverage – regions not captured
  - Mitochondrial DNA changes
  - Novel genes
  - Digenic/oligogenic inheritance
  - Mosaicism
  - Not a genetic cause

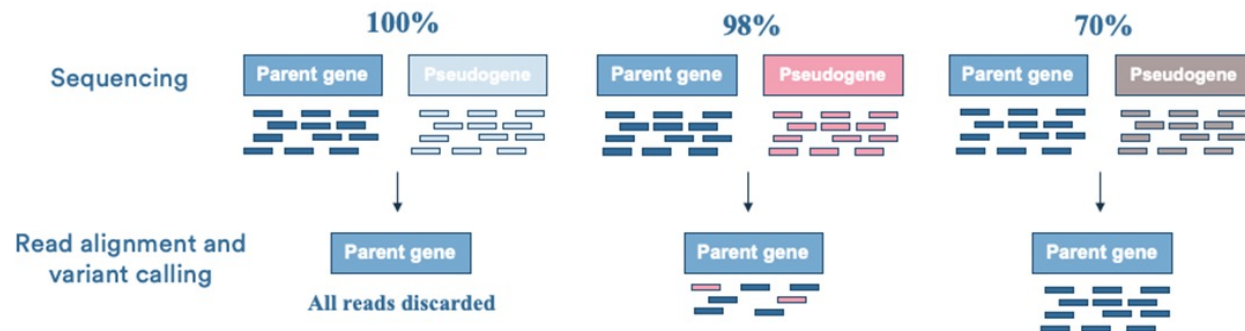
# WES challenge: *PKD1*: pseudogenes...

## 1. PCR amplification



~97,7% sequence homology

## 2. Data analysis



# Whole Genome Sequencing (WGS)

- ▶ Better and more uniform coverage
  - ▶ Detection structural variants, non-coding regions, ...
  
  - ▶ BUT.... Not the technology itself, other factors limit its implementation:
  - ▶ Variant analysis / interpretation: Need for trio-analysis
  - ▶ Information management: data-analysis and data-storage
  - ▶ Need for bioinformatics, genetic counselors
  - ▶ Actual cost
- 
- A solid blue triangle is located in the bottom right corner of the slide, pointing towards the top right.

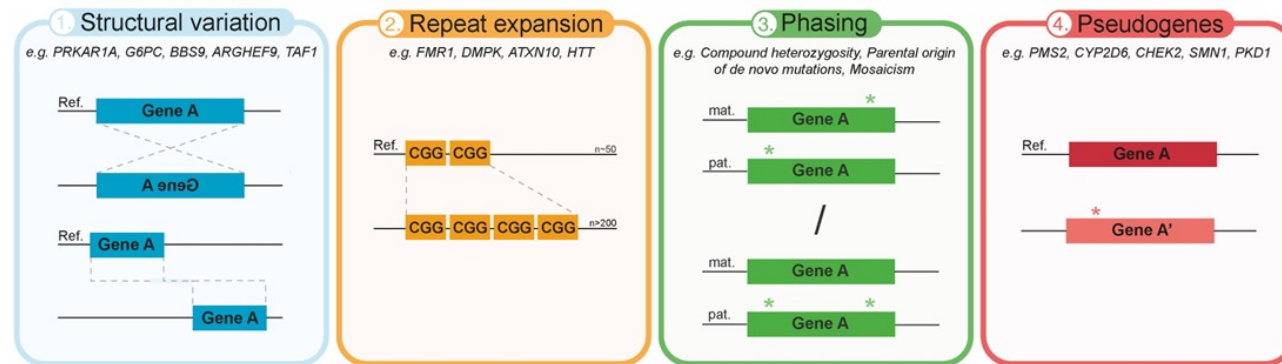
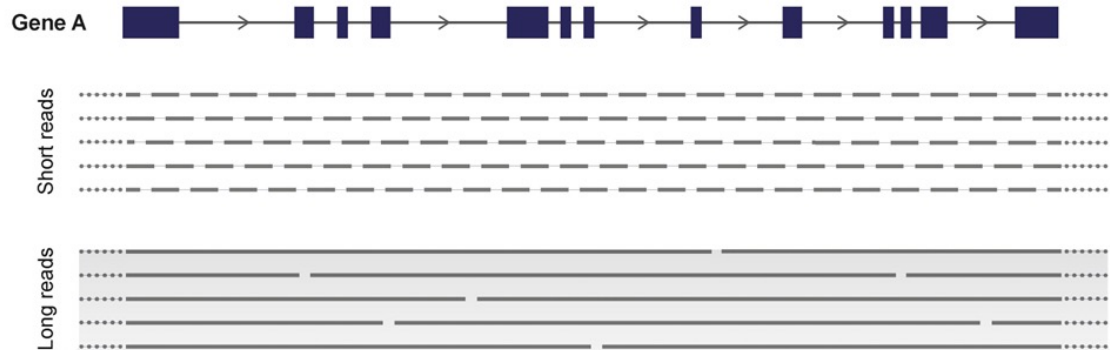
- Long-read sequencing (>10kb)
- Two primary long-read technologies
  - Pacific biosciences (PacBio)
  - Oxford nanopore technology (ONT)
- Genome assembly
- Variant detection
  - Large and complex rearrangements
  - CNVs
  - Repetitive regions
- Haplotype phasing
- Pseudogenes



Gene A

Short reads

Long reads



# Take home messages...

- ✓ NGS: several technologies
- ✓ Genetic testing kidney disorders: virtual nephropathy panel on WES data
- ✓ Each test has limitations, think of hidden variations
- ✓ Communicate accurate clinical and familial information to the lab as this information is essential for the correct interpretation of sequence variants

**SOFIE SYMOENS**  
Laboratoriumsupervisor  
CMGG

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Volg ons op

