Help, mijn kind wordt geel
een wereld met NGS

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Human Genetics

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Variant collection

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From art….

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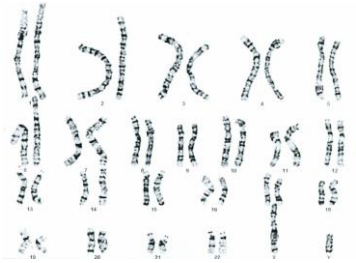
The humane genome



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To industry….

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Finding the answer in the genome

6 billion nucleotides
46 chromosomes

2 people differ
at ???? positions

**1 variation (mutation)
can result in disease**

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Variation in our genomes (# per genome)

- SNVs (single nucleotide variants):
  - **~ 3-3.5Mio SNVs**
    - **of which vast majority SNPs – single n**
    - ~ 500 private/rare coding varia
    - ~50-100 de novo mutati coding)
- Indels (insertions/deleti
  - **~500,000 inde**
  - Larges
- CNV its)
  - **per genome**
  - 30/genome
  - *De novo*, >100kb: <1 per genome
  - Inversions/translocations?

And often we do not know which type of mutation is causing disease in a given individual

(e.g. hereditary breast cancer can be caused by SNVs, Indels or CNVs of BRCA1)

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Looking for a needle in a haystack?

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Questions parents and patients have?

What is happening in our child?
What is the cause (am I to blame)?
What can we do?
What kind of complications can we expect and if possible prevent?
Is there a recurrence risk?
Are there other children with similar disorders from which we can learn?

Human Genetics Nijmegen

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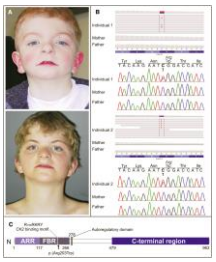
Recurrent De Novo Mutations in PACS1 Cause Defective Cranial-Neural-Crest Migration and Define a Recognizable Intellectual-Disability Syndrome

Schuurs-Hoeijmakers et al, AJHG, 91, 1122–1127, December 7, 2012



Human Genetics Nijmegen

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Illumina, meet the dominant technology

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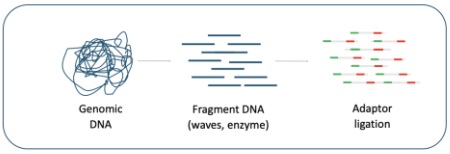
- NGS basics -

Library preparation



Adaptors = double-stranded universal sequencing primers

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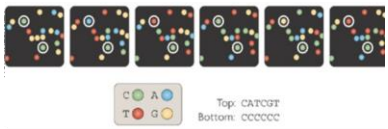
Next generation sequencing basics

Main principles
1.) Massively parallel (millions to billions of molecules in parallel)
2.) Non-terminated fluorescently labeled bases added to the sequencer over time
a) (Fluorescent) Intensity is measure for the number of bases incorporated (e.g. 454, Iontorrent)
b) Sequential pictures are taken – multiple colors (e.g. Illumina, BGI, PacBio)



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Mapping sequencing reads

Mapping the reads to a **reference genome**

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Variant detection - Theory

Wildtype sequence
AGCTGGCTTAGATAACCATT G ACTAGA A CTTTCCGGATCG

Sequence reads
CTGGCTTAGATAACCATT G ACTAGA T CTTTCCGG
TGGCTTAGATAACCATT G ACTAGA T CTTTCCGGA
GCTTACATAACCATT G ACTAGA T CTTTCCGGATC
TTAGATAACCATT C ACTAGA T CTTTCCGGATCTA
AGATAACCATT C ACTAGA T CTTTCCGGATCGA
ATAACCATT C ACTAGA T CTTTCCGGATCGA

Heterozygous variant
Homozygous variant
Error (PCR or sequencing) – main reason we need high coverage

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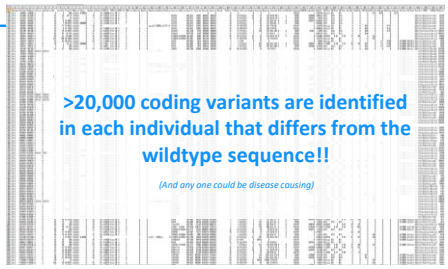
Variant calling and variant annotation



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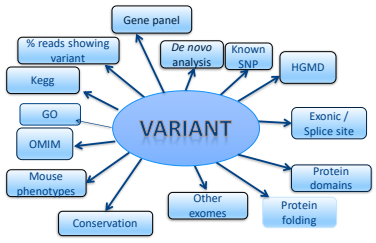
Annotation pipeline



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Filtering out inherited variation greatly reduces amounts of variants for follow-up

Filtering step	er
Total called	
After exclusion of & synonymo	10
Priva	143
A ants	5
Val equencing	0-2

Identification gets 'easy' - proof of causality not trivial

Vissers et al. 2010 Nat Genet

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Diagnostics (Basel). 2021 Oct; 11(10): 1904.

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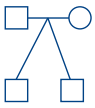
Casus non-invasive prenatal exome sequencing

- 35-jarige zwangere vrouw - 32+3 weken
- tweeling zwangerschap vanuit ivf behandeling
- biochemisch onderzoek verdenking

acute fatty liver disease of pregnancy (AFLP)



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- AFLP is zeldzame aandoening (1:10.000), vaak tijdens derde trimester
- *LCHAD* gen

- ICP aandoening in tweede of derde trimester bij 3-5% van de zwangerschappen

betrokken genen

ABC membraaneiwit complex (13 genen),

ATP8B1, TJB2, NR1H4, ANO8

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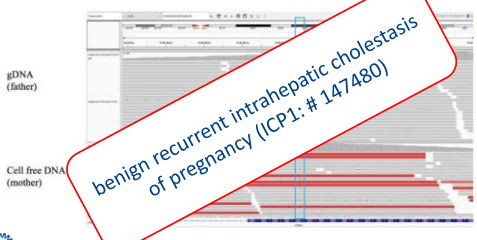
ATP8B1 : missense variant c.[913T > A] + [=] p.[Phe305Ile] + [=]



benign recurrent intrahepatic cholestasis of pregnancy (ICP1: # 147480)

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Take Home message

WES/WGS zijn "unbiased" en helpt bij lastig te onderscheiden phenotypes

Een snelle en correcte diagnose heeft impact op management van zowel patiënt, pasgeborene, en prenataal

Lever aandoeningen komen tijdens zwangerschap met overlappende verschijnselen. Essentieel om snel en precies diagnoses te stellen.

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