De Hart & Vaatgroep

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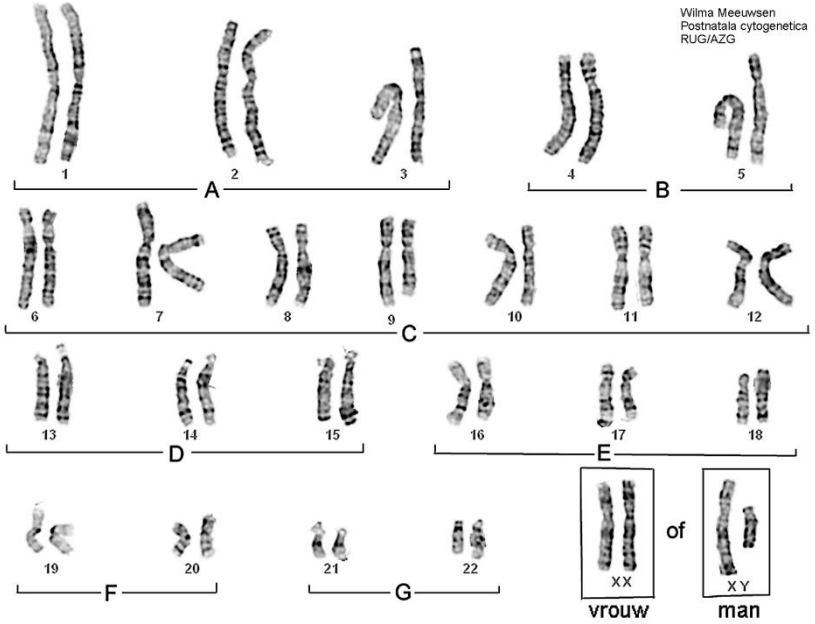
STICHTING GENETISCHE HARTSPIERZIEKTE PLN

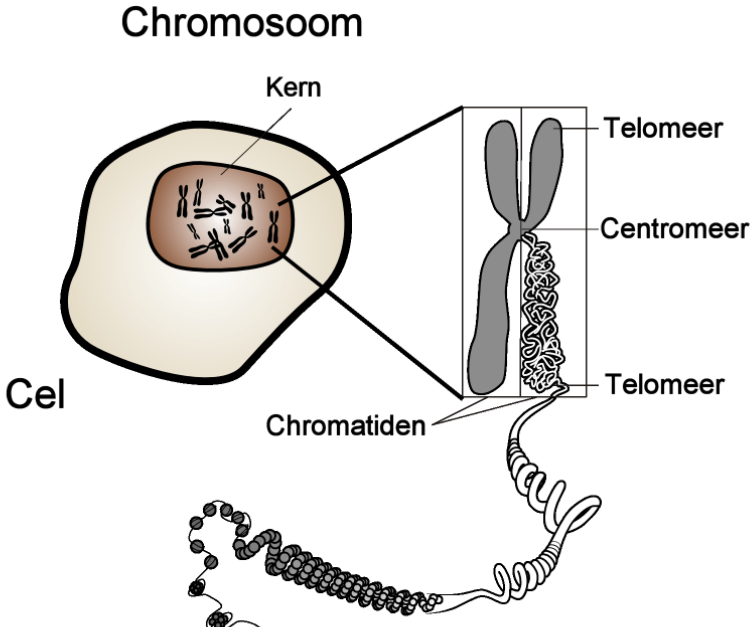
Sectie Genoom Diagnostiek

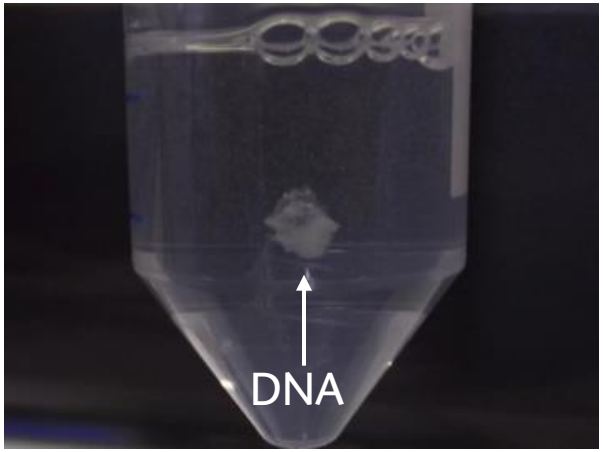
12 laboratorium specialisten, 75 analisten

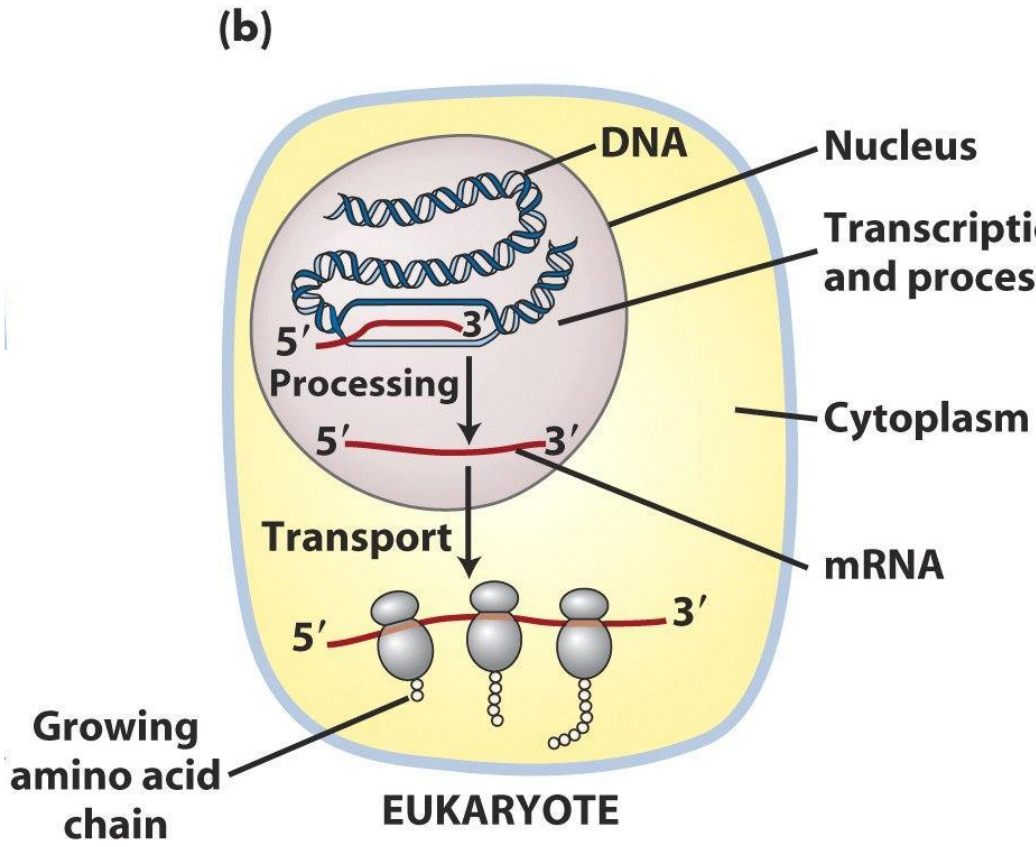
>20,000 genetics tests

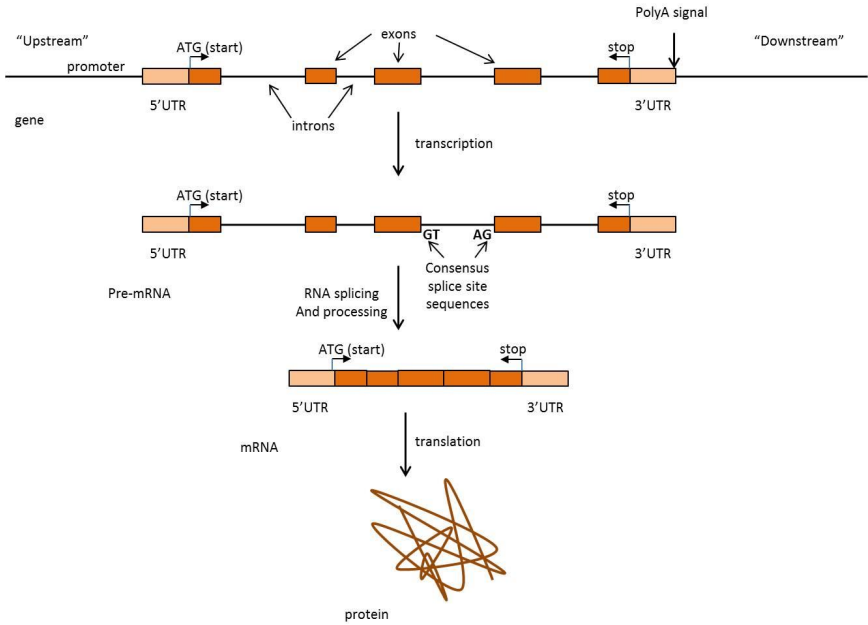
- Karyotypering
- SNP & Oligo Arrays
- FISH
- Sequentie analyse


Wilma Meeuwsen
Postnatala cytogenetica
RUG/AZG
1
2
3
A
4
5
B
6
7
8
9
C
10
11
12
13
14
15
D
16
17
18
E
19
20
F
21
22
G
XX
vrouw
of
XY
man


Chromosoom
Kern
Telomeer
Centromeer
Telomeer
Cel
Chromatiden
Basenpaar
Histonen
DNA-
dubbelstreng


DNA


(b)
DNA
Nucleus
Transcription and processing
5′
3′
Processing
5′
3′
Cytoplasm
Transport
mRNA
5′
3′
Growing amino acid chain
EUKARYOTE


"Upstream"
promoter
ATG (start)
exons
stop
PolyA signal
"Downstream"
gene
5′UTR
introns
3′UTR
transcription
ATG (start)
stop
5′UTR
GT
AG
3′UTR
Pre-mRNA
RNA splicing And processing
Consensus splice site sequences
ATG (start)
stop
5′UTR
3′UTR
mRNA
translation
protein

rijksuniversiteit groningen

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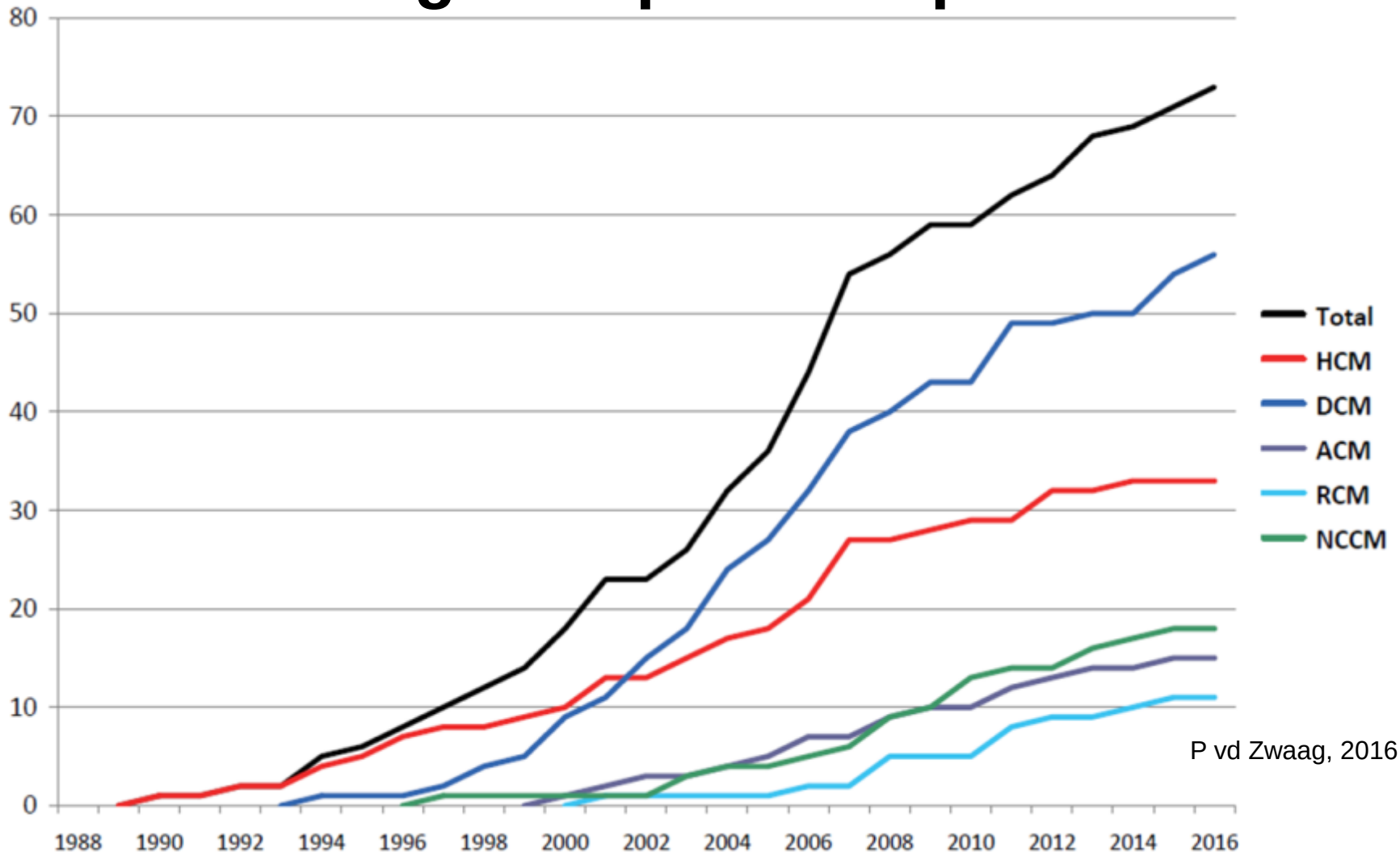
Aantal genen per hartspierziekte



P vd Zwaag, 2016

2005

LMNA/C

MYH7

TNNI3

(PKP2)

2012

CSRP3

MYBPC3

LMNA/C

TNNT2

TPM1 (exon6B)

MYH7

DSC2

DSP

TMEM43

DES

MYH7

TNNI3

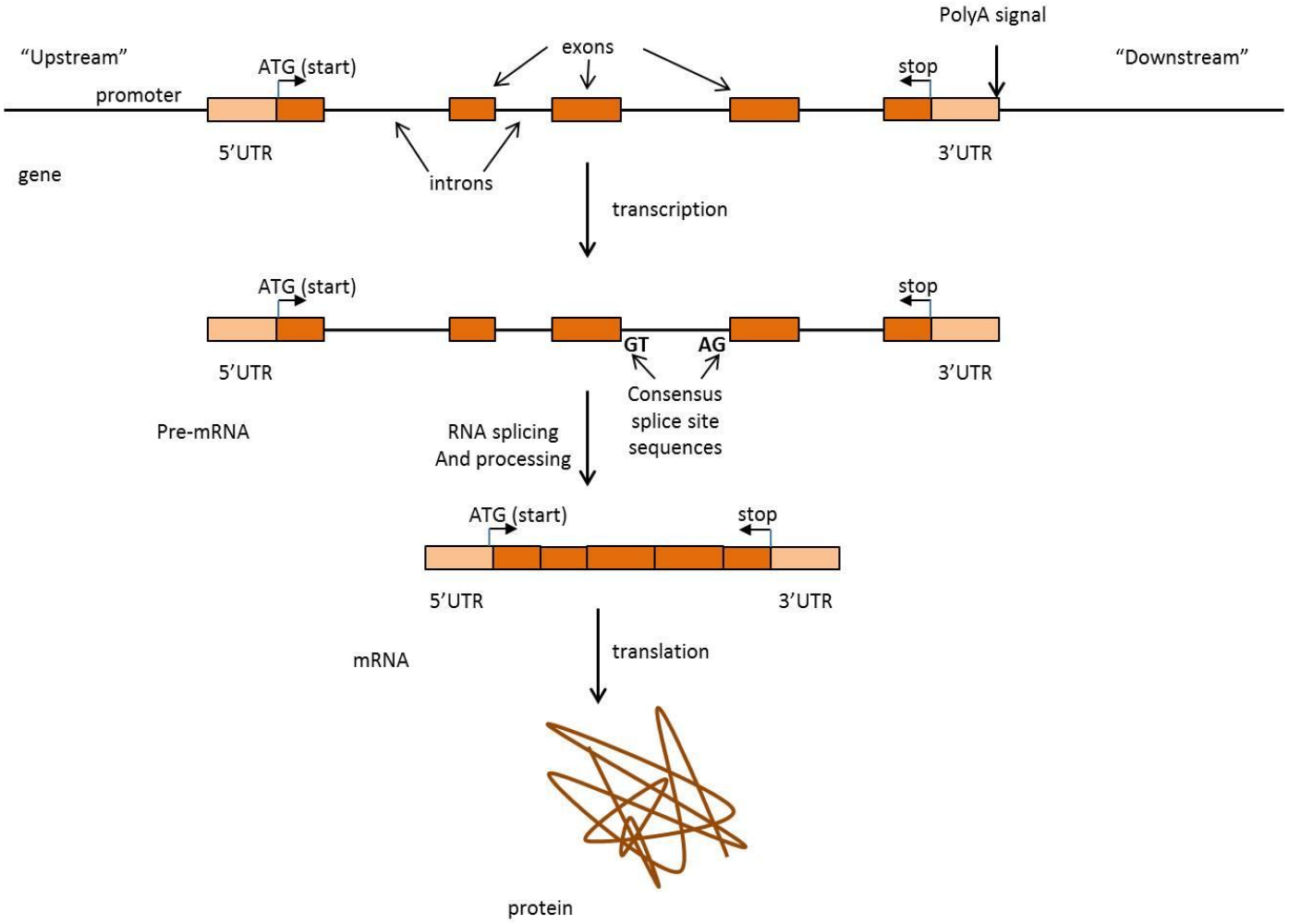
PLN

KBTBD13

PKP2

DSG2

JUP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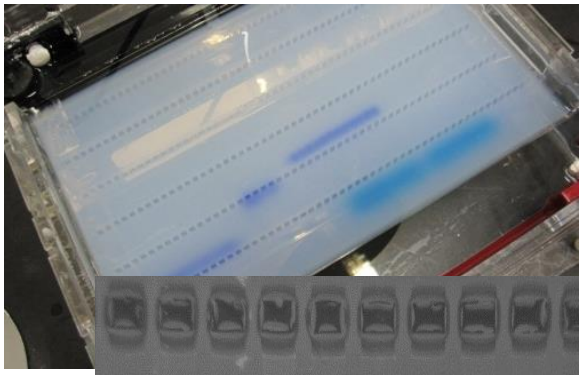
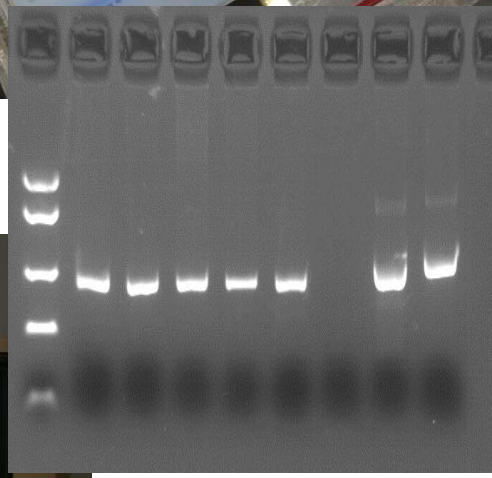
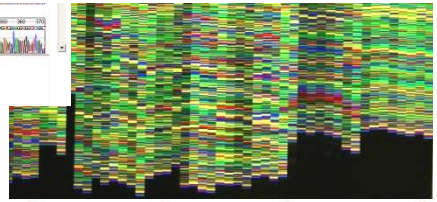
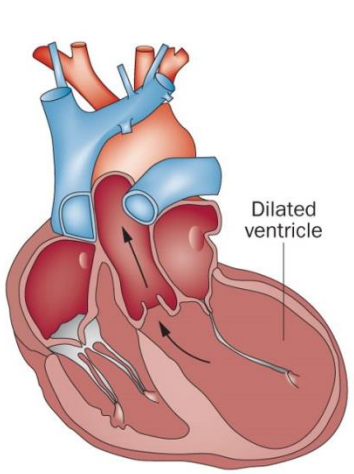




http://www.allesoverdna.nl/woordenboek/dna-sequencen.html



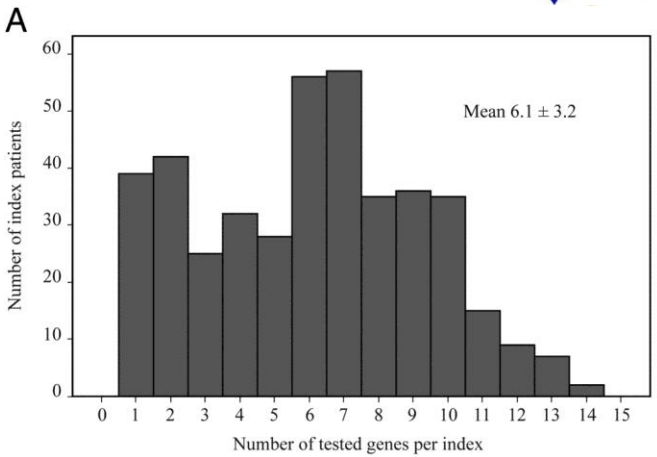
DCM:

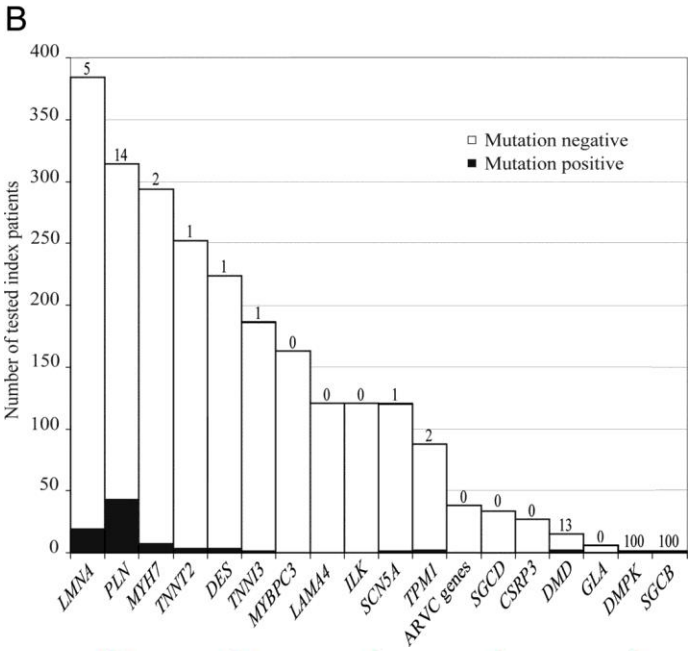


European Journal of Heart Failure
doi:10.1093/eurjhf/hft013

Genetic analysis in 418 index patients with idiopathic dilated cardiomyopathy: overview of 10 years' experience

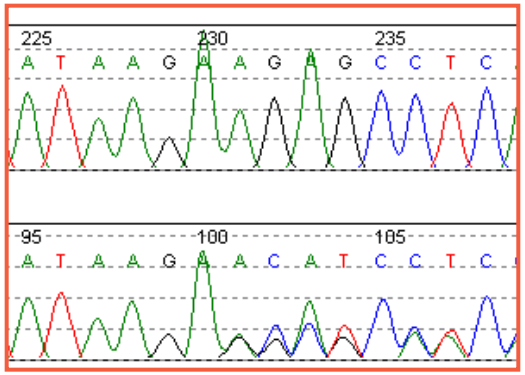
Karin Y. van Spaendonck-Zwarts[1,2*†], Ingrid A.W. van Rijsingen[3†], Maarten P. van den Berg[4], Ronald H. Lekanne Deprez[2], Jan G. Post[5], Anneke M. van Mil[6], Folkert W. Asselbergs[7], Imke Christiaans[2], Irene M. van Langen[1], Arthur A.M. Wilde[3], Rudolf A. de Boer[4], Jan D.H. Jongbloed[1], Yigal M. Pinto[3†], and J. Peter van Tintelen[1,8†]



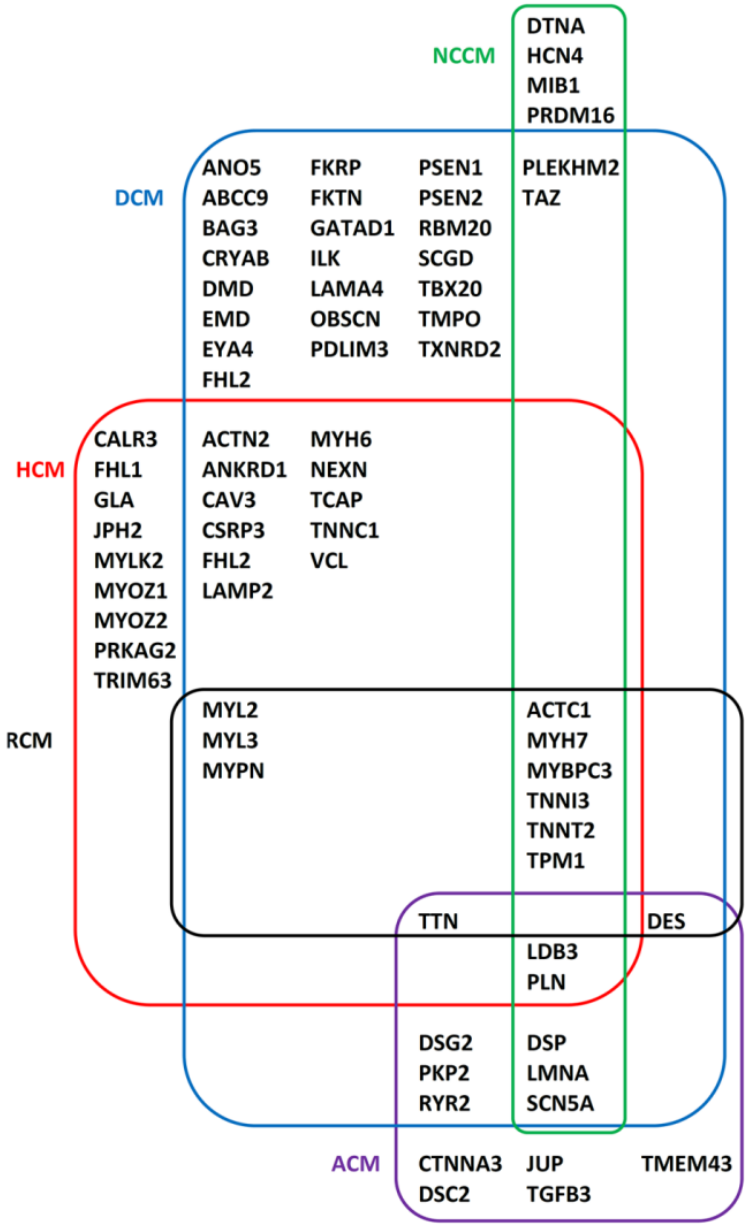


van Spaendonck (2012) Eur J Heart Fail 15:376-84

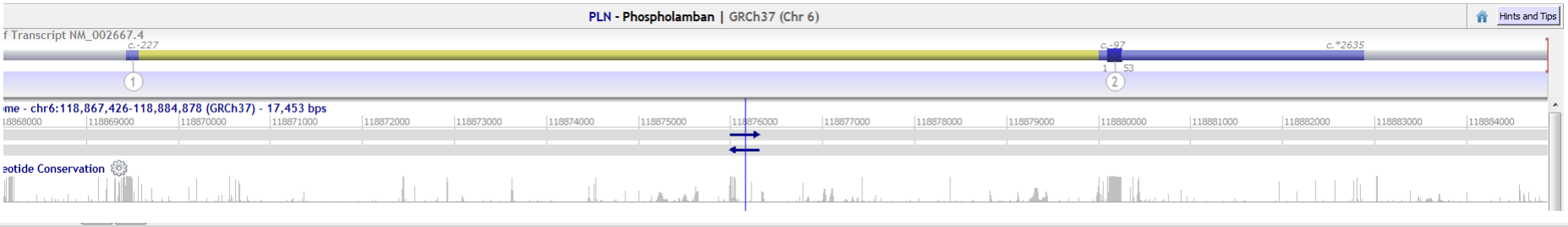
Screening
Kandidaat Gen(en)

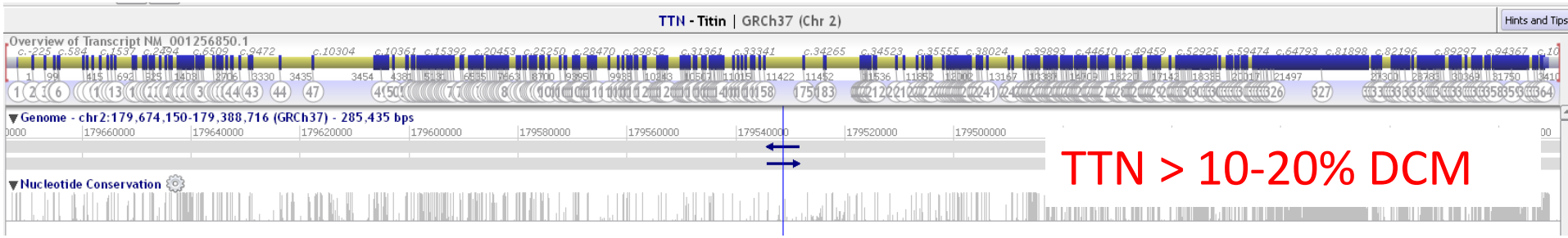


Next Generation
Sequencing



PA vd Zwaag, 2016





TTN > 10-20% DCM

Fun facts:

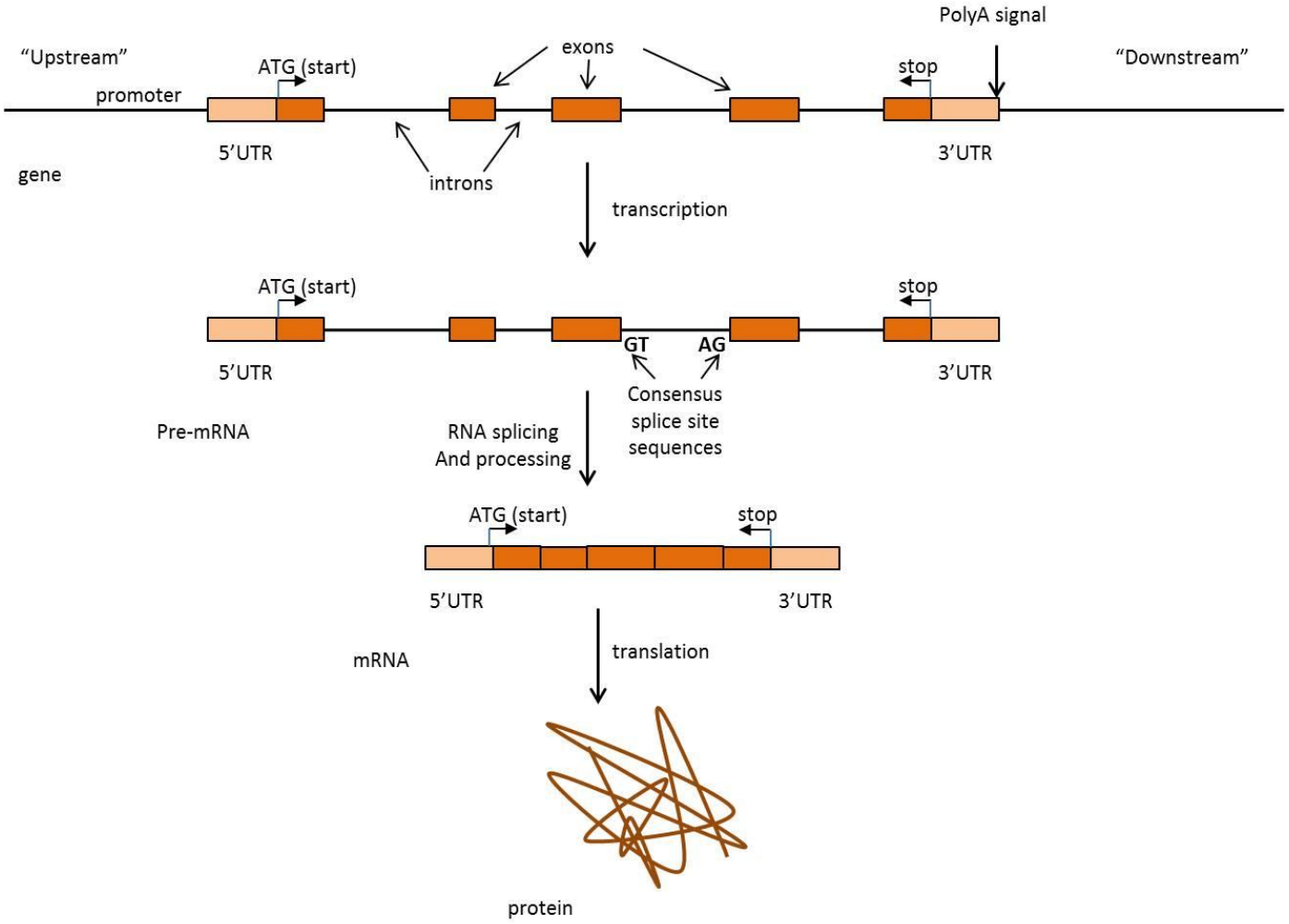
- *PLN* heeft 1 exon
- *PLN* heeft 158 coderende nucleotiden
- Het grootste exon is 158 nucleotiden

Fun facts:

- *TTN* heeft 363 exonen
- *TTN* heeft 107 973 coderende nucleotiden (680x PLN)
- Het grootste exon is 17 402 nucleotides
- *RYR2* is 14 904 nucleotides (95x PLN)

12 patiënten
1 run
50-200 genen

Gerichte (panel analyse):
55/60 genen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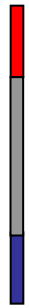
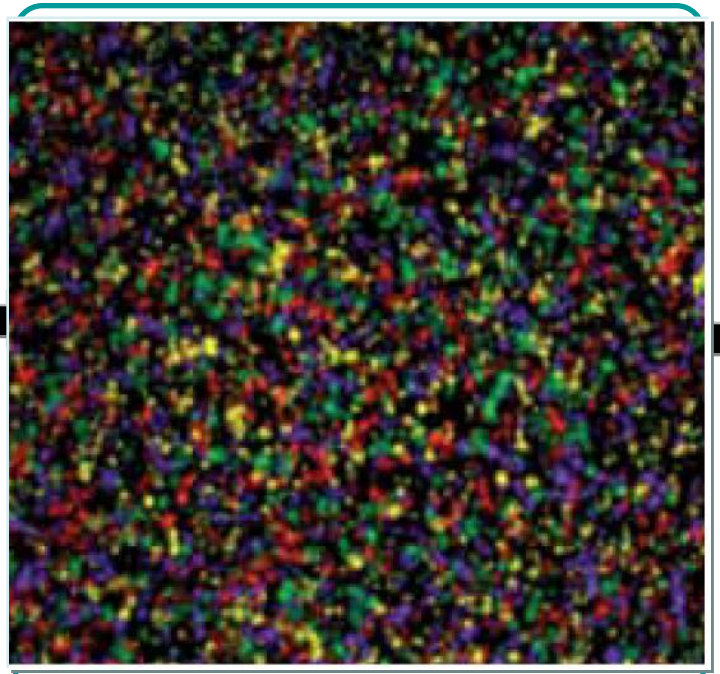
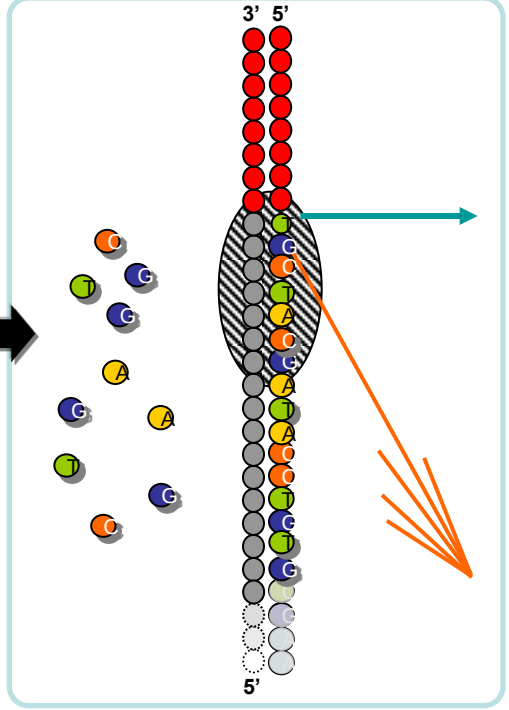
3'UTR
Consensus
splice site
sequences
Pre-mRNA
RNA splicing
And processing
ATG (start)
stop
5'UTR
3'UTR
mRNA
translation
protein

DNA volgordes bepalen:

DNA fragmenten



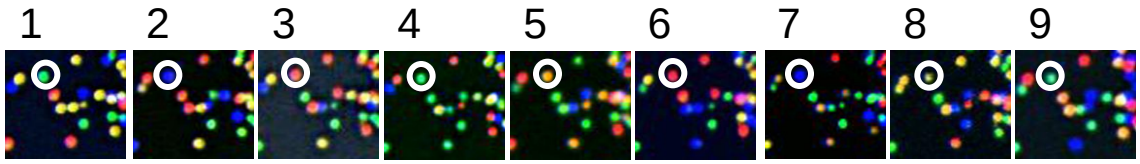




DNA voorbereiding

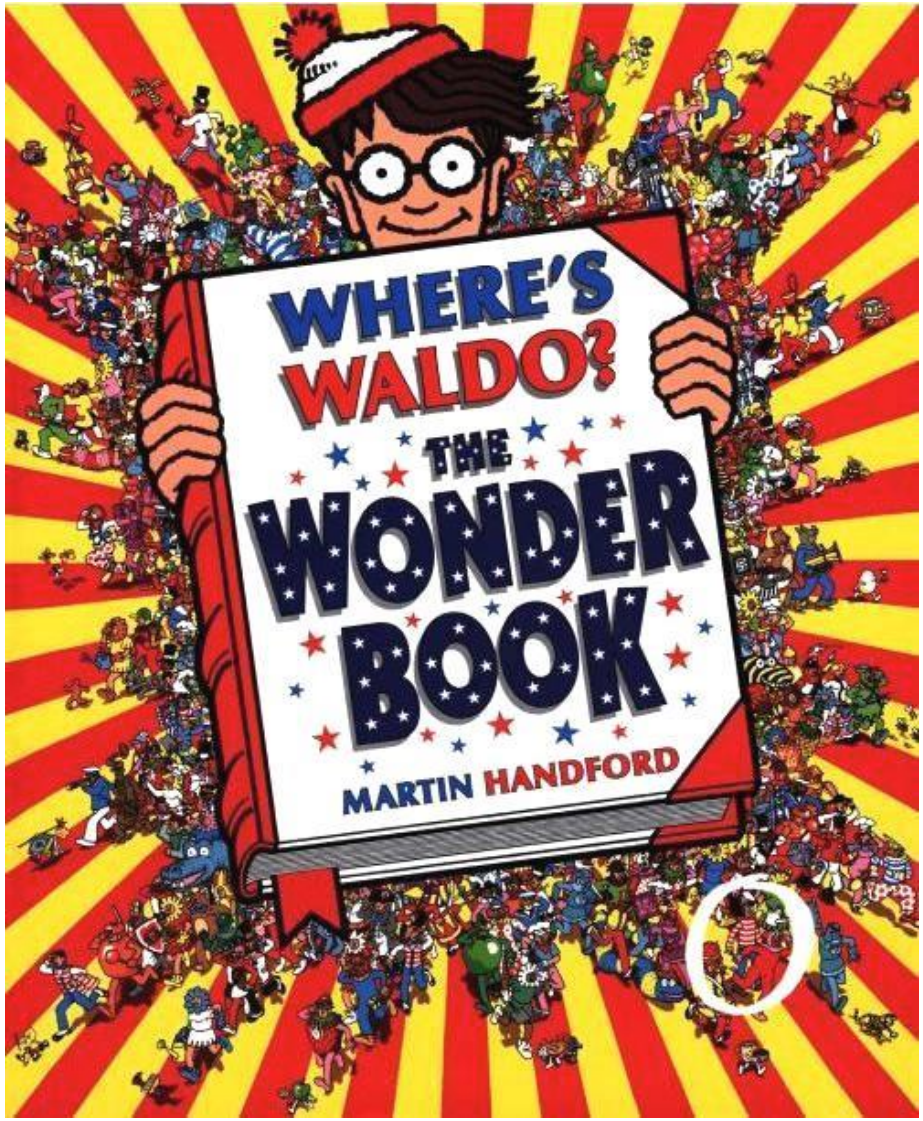
Clusters genereren

Sequentie analyse

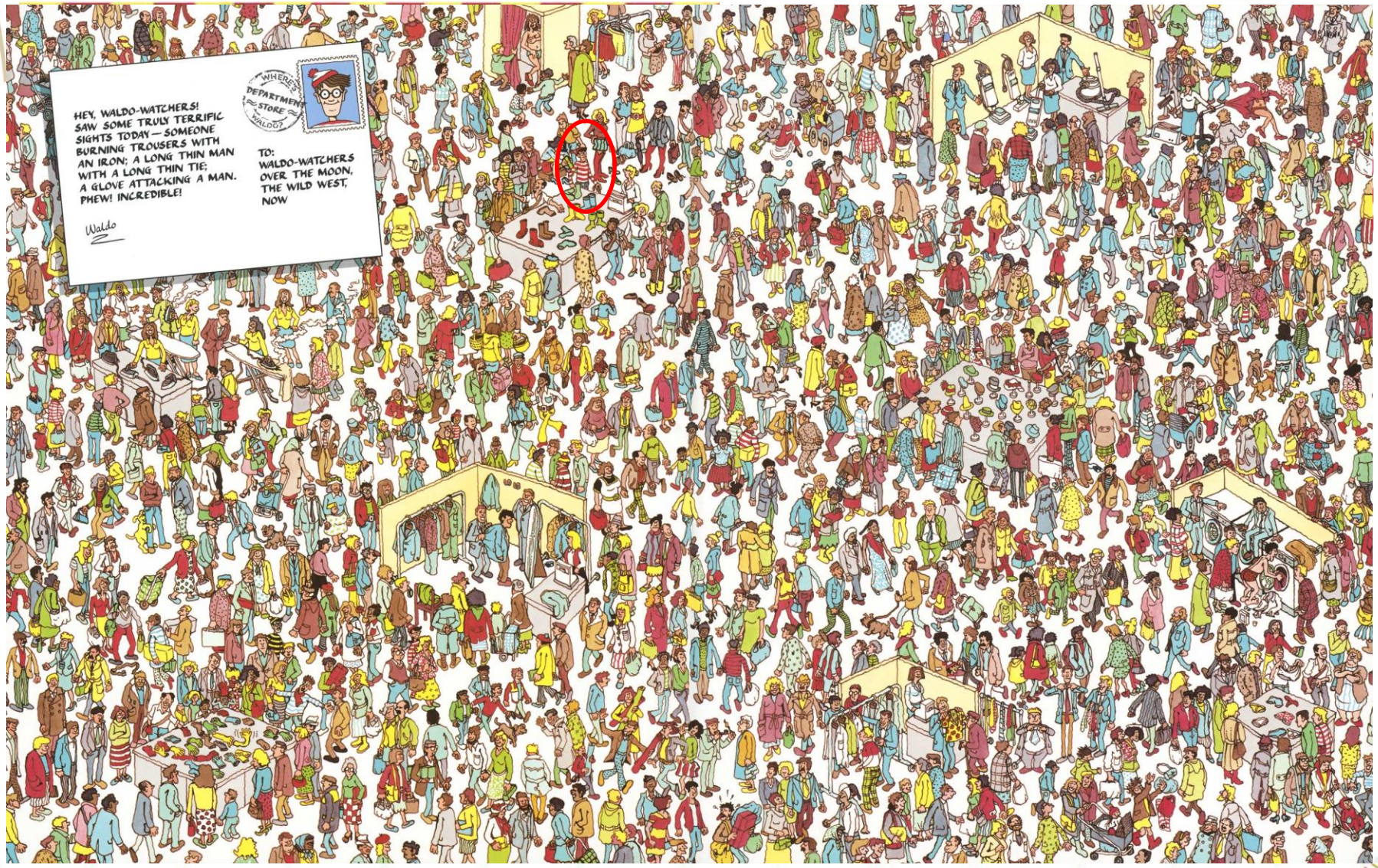


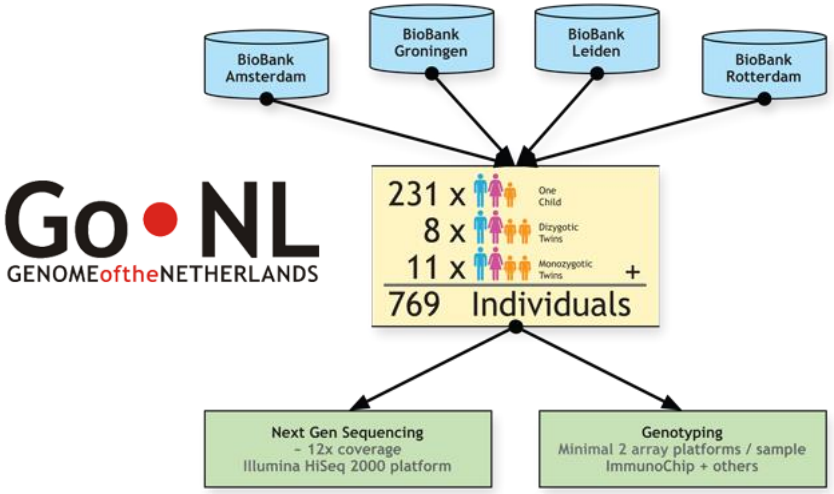
Plaatjes maken

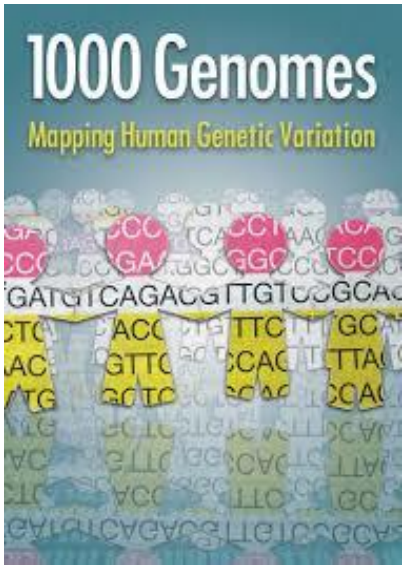
Basen bepalen



Ziekteverwekkende Variant vinden


HEY, WALDO-WATCHERS! SAW SOME TRULY TERRIFIC SIGHTS TODAY — SOMEONE BURNING TROUSERS WITH AN IRON; A LONG THIN MAN WITH A LONG THIN TIE; A GLOVE ATTACKING A MAN. PHEW! INCREDIBLE!
Waldo
WHERE'S DEPARTMENT STORE WALDO?
TO: WALDO-WATCHERS OVER THE MOON, THE WILD WEST, NOW





Controle groepen gebruiken

Exomen van >60.000 mensen

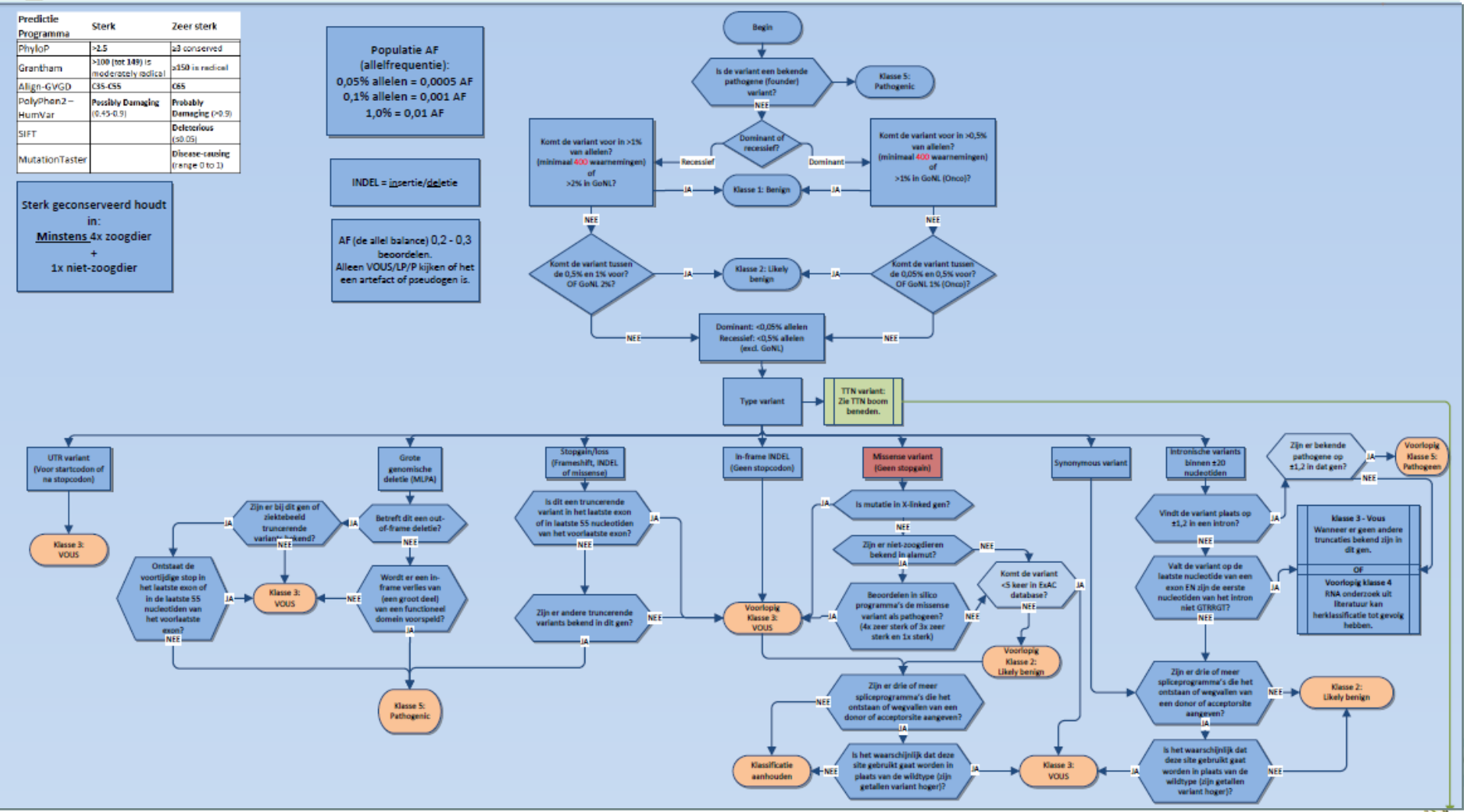
Predictie Programma	Sterk	Zeer sterk
PhyloP	>2.5	≥3 conserved
Grantham	>100 (tot 149) is moderately radical	≥150 is radical
Align-GVGD	C35-C55	C65
PolyPhen2 – HumVar	Possibly Damaging (0.45-0.9)	Probably Damaging (>0.9)
SIFT		Deleterious (≤0.05)
MutationTaster		Disease-causing (range 0 to 1)

Sterk geconserveerd houdt in:
Minstens 4x zoogdier
+
1x niet-zoogdier

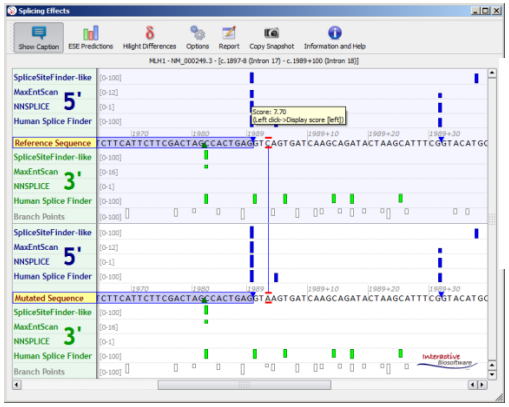
Populatie AF (allelfrequentie):
0,05% allelen = 0,0005 AF
0,1% allelen = 0,001 AF
1,0% = 0,01 AF

INDEL = insertie/deletie

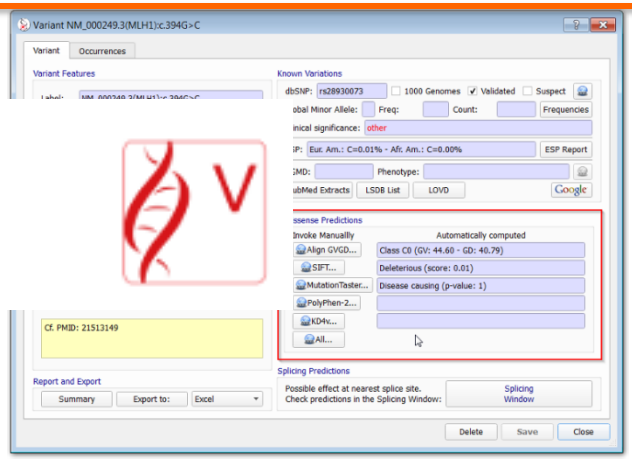
AF (de allel balance) 0,2 - 0,3 beoordelen.
Alleen VOUS/LP/P kijken of het een artefact of pseudogen is.

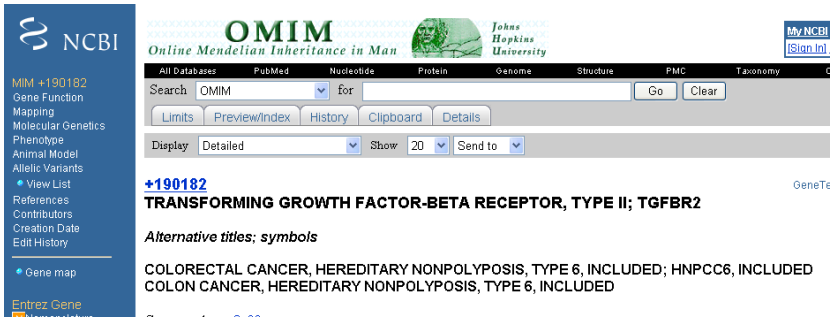


Welke mutatie is daadwerkelijk ziekteverwekkend?



alamut visual





PubMed

HGMD®
The Human Gene Mutation Database
Cardiff

Welke mutatie is daadwerkelijk ziekteverwekkend?

- **Pathogeen**
- **Waarschijnlijk pathogeen**
- **Onbekende betekenis**
- **Waarschijnlijk onschuldig**
- **Onschuldig**

ABCC9, ACTC1, ACTN2, ANKRD1, BAG3, CALR3, CAV3, CRYAB, CSRP3/MLP,

Sinds September 2012 in Routine Diagnostiek:

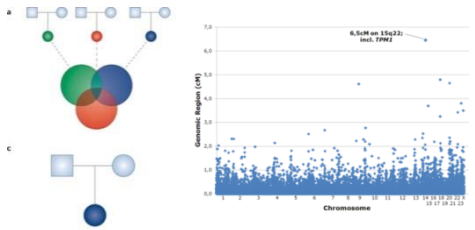
1150 patiënten 55 genen panel
1020 patiënten 60 genen panel
2170 patiënten totaal

TCAP, TMEM43, TNNC1, TNNI3, TNNT2, TPM1, TTN, TXNRD2, VCL, ZASP/LDB3

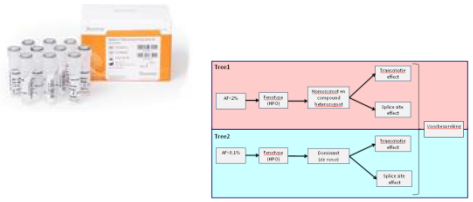
Gene panel based resequencing

ABCC9, ACTC1, ACTN2, ANKRD1, BAG3, CALR3, CRYAB, SRP3/MLP, DES, DMD, DSC2, DSG2, DSP, EMD, GLA, JPH2, JUP, LAMA4, LAMP2, LMNA, MYBPC3, MYH6, MYH7, MYL2, MYL3, MYPN, MYOZ1, MYOZ2, PKP2, PLN, PRKAG2, PSEN1, PSEN2, RBM20, RYR2, SCN5A, SGCD, TAZ, TBX20, TCAP, TMEM43, TNNC1, TNNI3, TNNT2, TPM1, TTN, VCL, ZASP

Exome Sequencing



Whole Genome Sequencing



Exoom (WES) vs Whole genome (WGS)

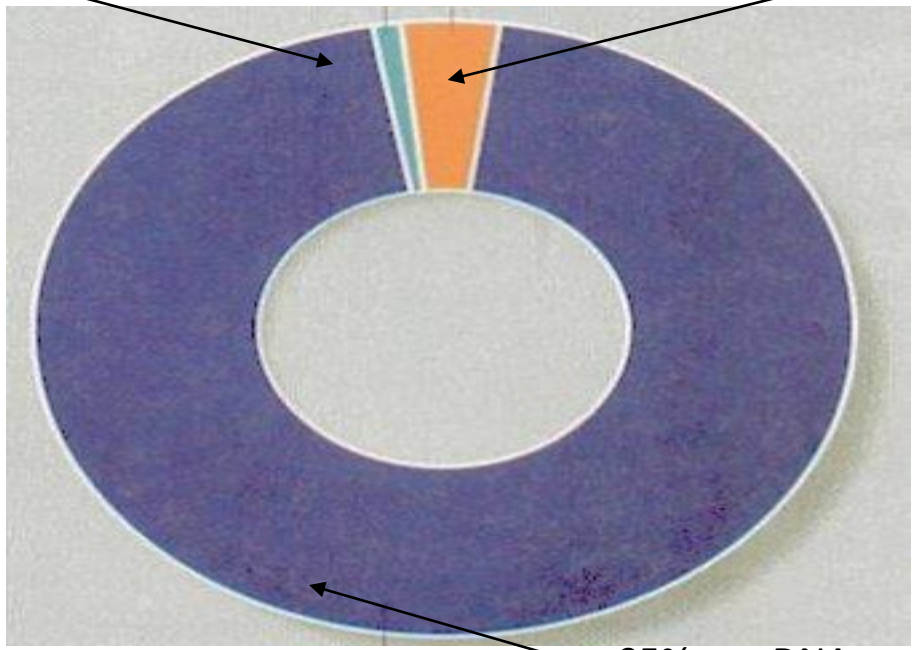
1.2% of DNA codeert voor eiwitten

3.8% geconserveerd in zoogdieren

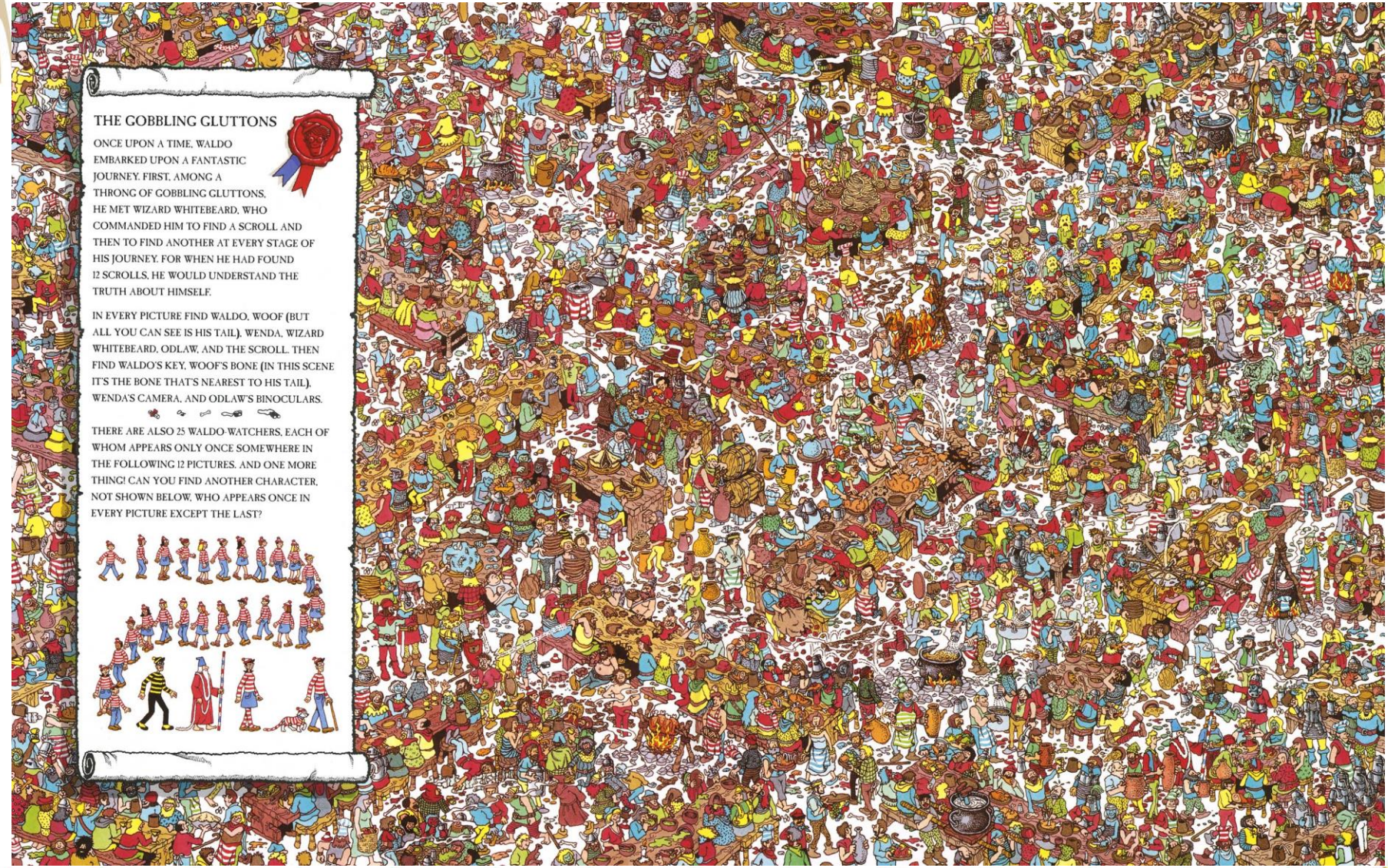
→Exoom
(alle exonen van een genoom)

(>180,000 exons)

..bevat 80% van alle mutaties



95% van DNA geen/ onbekende functie


THE GOBBLING GLUTTONS
ONCE UPON A TIME, WALDO EMBARKED UPON A FANTASTIC JOURNEY. FIRST, AMONG A THRONG OF GOBBLING GLUTTONS, HE MET WIZARD WHITEBEARD, WHO COMMANDED HIM TO FIND A SCROLL AND THEN TO FIND ANOTHER AT EVERY STAGE OF HIS JOURNEY. FOR WHEN HE HAD FOUND 12 SCROLLS, HE WOULD UNDERSTAND THE TRUTH ABOUT HIMSELF.
IN EVERY PICTURE FIND WALDO, WOOF (BUT ALL YOU CAN SEE IS HIS TAIL), WENDA, WIZARD WHITEBEARD, ODLAW, AND THE SCROLL. THEN FIND WALDO'S KEY, WOOF'S BONE (IN THIS SCENE IT'S THE BONE THAT'S NEAREST TO HIS TAIL), WENDA'S CAMERA, AND ODLAW'S BINOCULARS.
THERE ARE ALSO 25 WALDO-WATCHERS, EACH OF WHOM APPEARS ONLY ONCE SOMEWHERE IN THE FOLLOWING 12 PICTURES. AND ONE MORE THING! CAN YOU FIND ANOTHER CHARACTER, NOT SHOWN BELOW, WHO APPEARS ONCE IN EVERY PICTURE EXCEPT THE LAST?

Waarom toch Exoom sequencing?

*Flexibel: geen vast genen set

*Alle genen: grotere kans op diagnose (“exoom openen”)

-> kinderen met cardiomyopathie

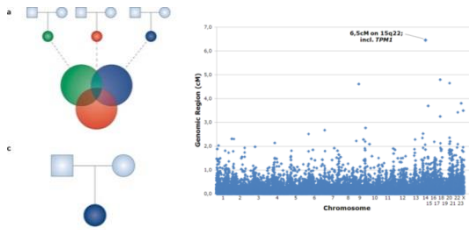
*Zelfde test voor alle ziekten

*Nieuwe genen vinden

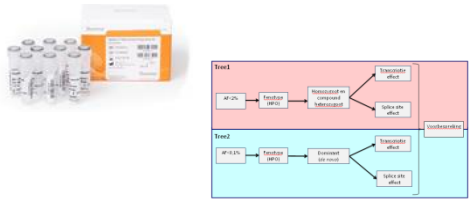
Gene panel based resequencing

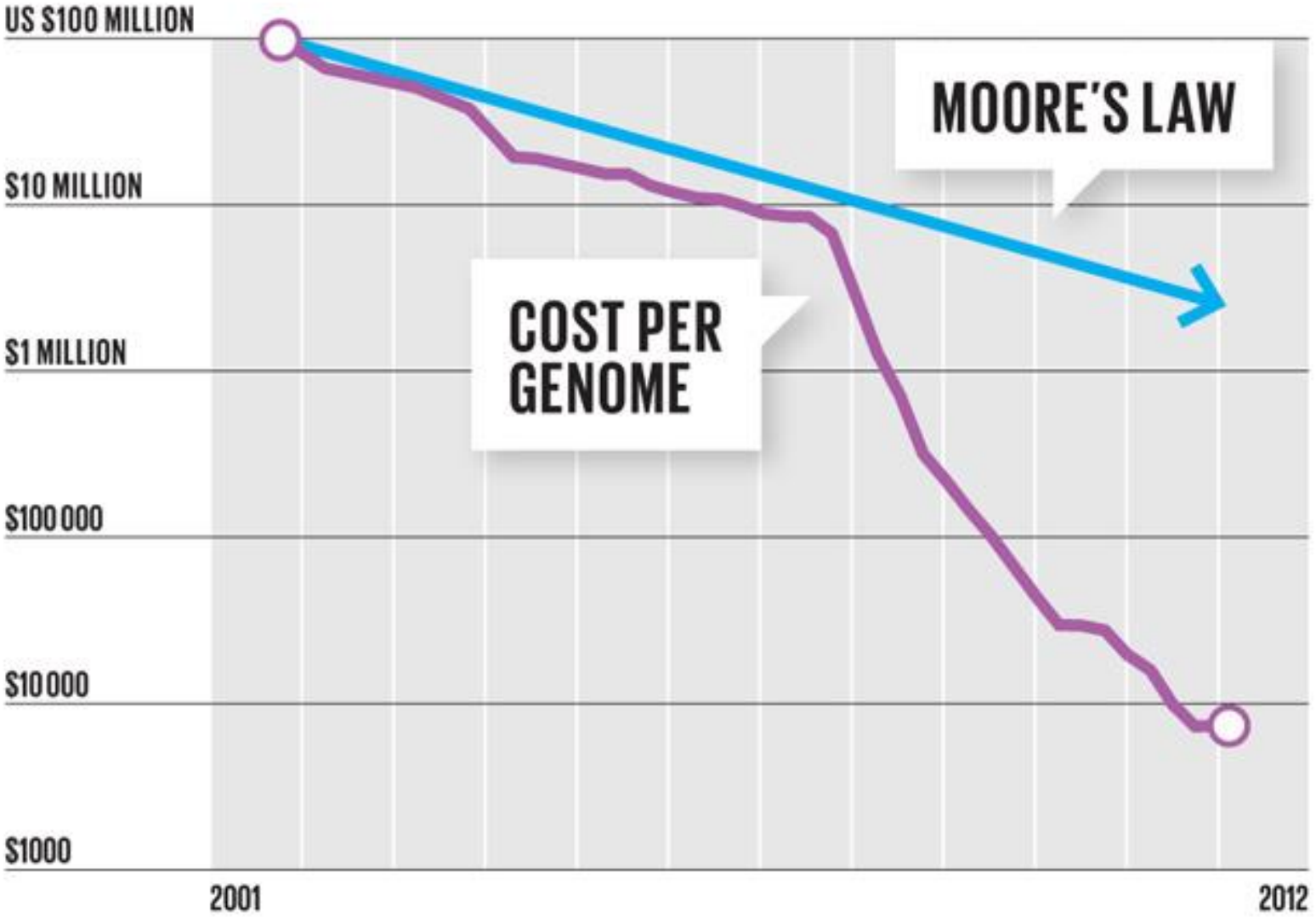
ABCC9, ACTC1, ACTN2, ANKRD1, BAG3, CALR3, CRYAB, SRP3/MLP, DES, DMD, DSC2, DSG2, DSP, EMD, GLA, JPH2, JUP, LAMA4, LAMP2, LMNA, MYBPC3, MYH6, MYH7, MYL2, MYL3, MYPN, MYOZ1, MYOZ2, PKP2, PLN, PRKAG2, PSEN1, PSEN2, RBM20, RYR2, SCN5A, SGCD, TAZ, TBX20, TCAP, TMEM43, TNNC1, TNNI3, TNNT2, TPM1, TTN, VCL, ZASP

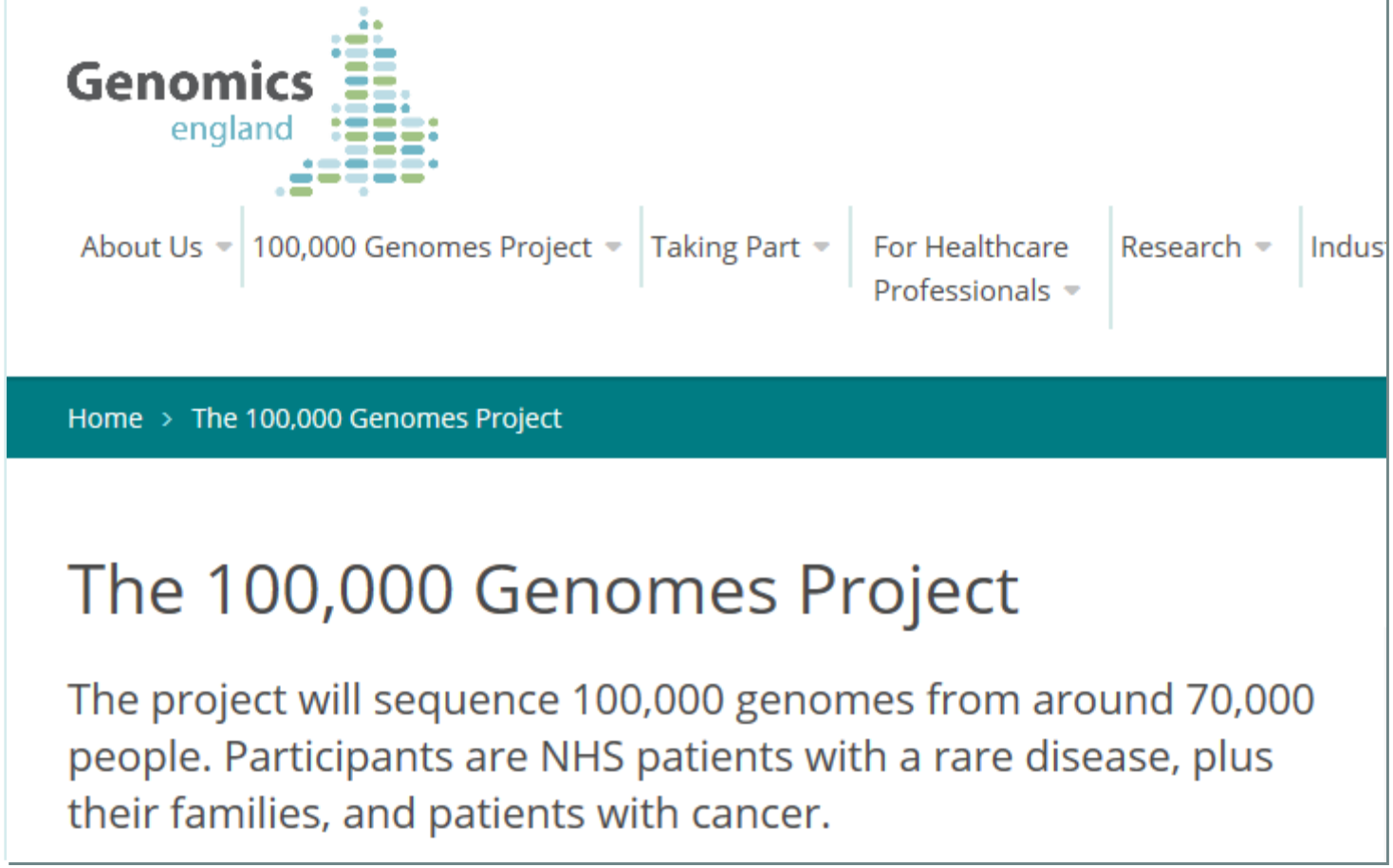
Exome Sequencing



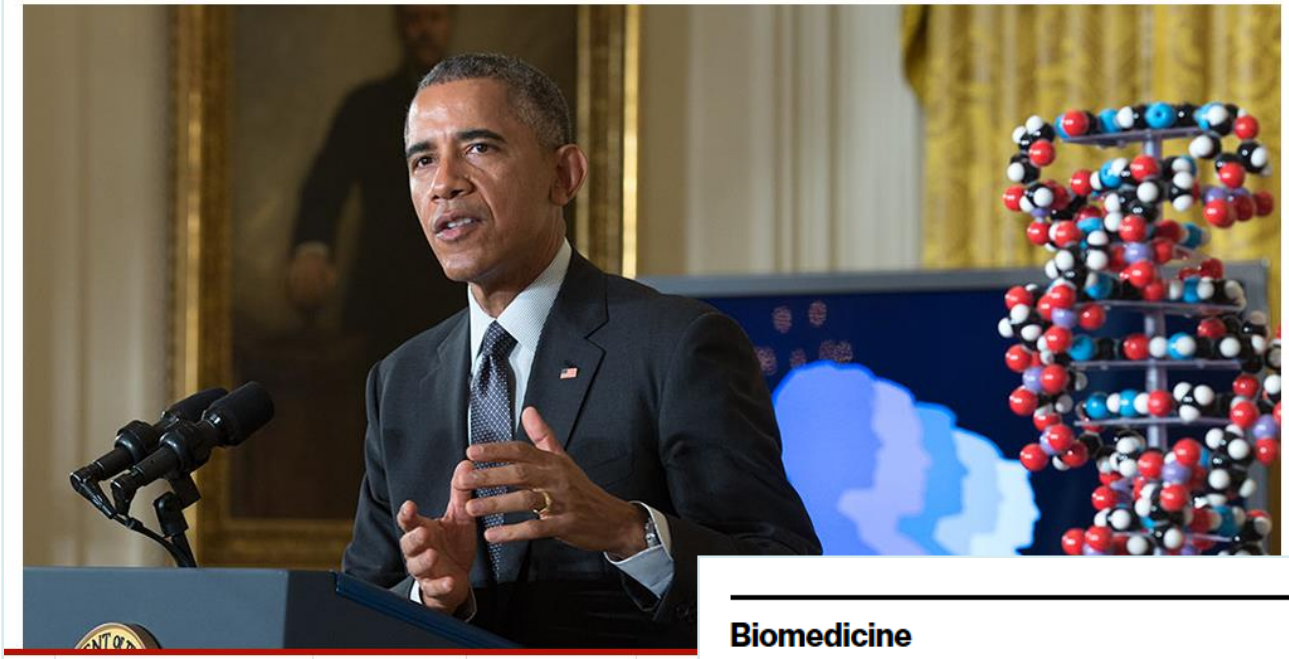
Whole Genome Sequencing




US $100 MILLION
$10 MILLION
$1 MILLION
$100 000
$10 000
$1000
2001
2012
MOORE'S LAW
COST PER GENOME


Genomics england
About Us
100,000 Genomes Project
Taking Part
For Healthcare Professionals
Research
Indus
Home > The 100,000 Genomes Project
The 100,000 Genomes Project
The project will sequence 100,000 genomes from around 70,000 people. Participants are NHS patients with a rare disease, plus their families, and patients with cancer.

THE PRECISION MEDICINE INITIATIVE



Biomedicine

U.S. to Develop DNA Study of One Million People

An Obama initiative seeks to channel a torrent of gene information into treatments for cancer, other diseases.

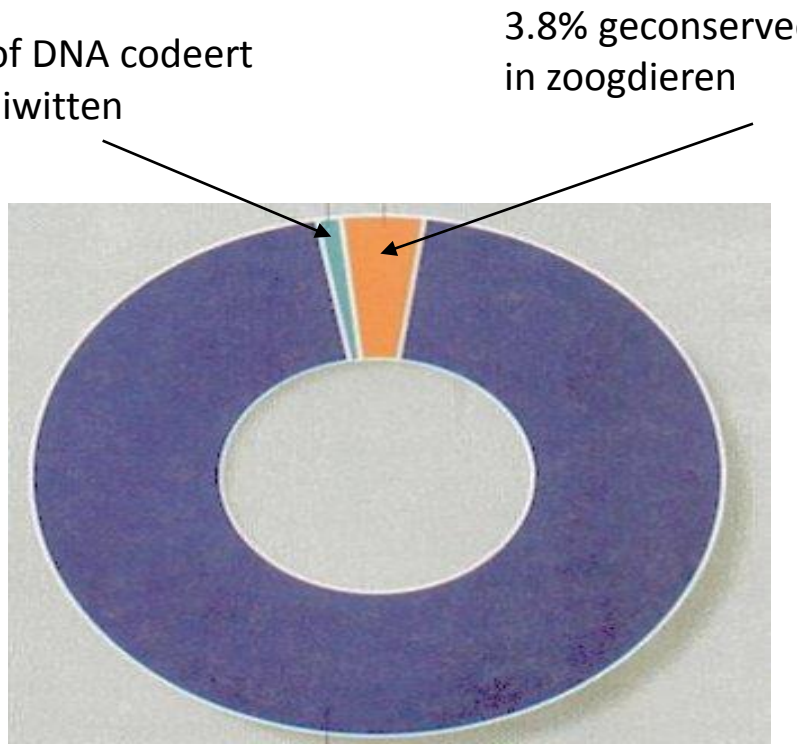
Waarom genoom sequencing?

Sneller

Beter

Meer informatie

Alles beschikbaar



NEWS IN FOCUS

CONRAD BOEHMAN/GETTY

The genomes of ill newborns can be sequenced in less than 24 hours to give clinicians a rapid diagnosis.

GENOMICS

Fast sequencing saves newborns

Rapid analysis of infant genomes is aiding diagnosis and treatment of inexplicably ill babies.

and healthy. Had physicians sent his DNA off for a conventional genomic test, the diagnosis could have taken more than a month — by which time he would probably have died.

The boy is one of 44 sick infants whose genomes Kingsmore's group has sequenced using a process that can provide a diagnosis in as little as 24 hours. In 28 of these cases, the researchers have been able to diagnose the baby's condition. And in about half of these, they have been able to recommend changes in treatment, Kingsmore reported on 19 September at the Genomics of Common Diseases meeting in Potomac, Maryland. On 6 October, his group will kick off a larger project to sequence hundreds of babies' genomes. It will be the first of four newborn-sequencing studies that each received multimillion-dollar grants from the US National Institutes of Health (NIH) in September 2013. The studies will address both the feasibility and the ethics of a process that could soon become standard for inexplicably ill newborns.

Over the next five years, Kingsmore's group will sequence the genomes of 500 sick babies from the Children's Mercy Hospital NICU and compare the infants' clinical outcomes with those of 500 NICU babies who are diagnosed using conventional genetic and metabolic tests. The researchers will assess whether rapid sequencing allows babies to avoid unnecessary tests and unhelpful treatments, and whether it helps parents to make decisions about care when the child is diagnosed as having a fatal disease. Even when an infant does die, Kingsmore says, a genome sequence and diagnosis can provide closure to parents and give more information about the genetic conditions they carry.

Kingsmore calls the rapid sequencing technique a 'factory' approach, in which four or five specialists each perform one step of the process — from the blood draw to the final

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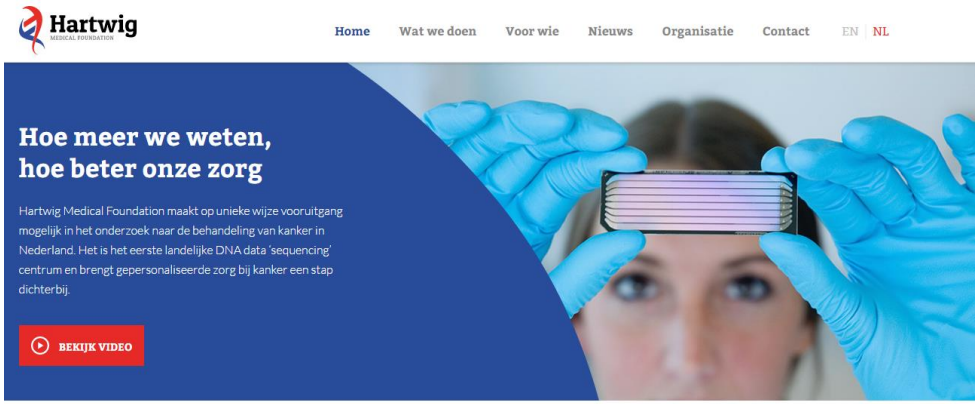
De Hart & Vaatgroep

umcg

STICHTING GENETISCHE HARTSPIERZIEKTE PLN

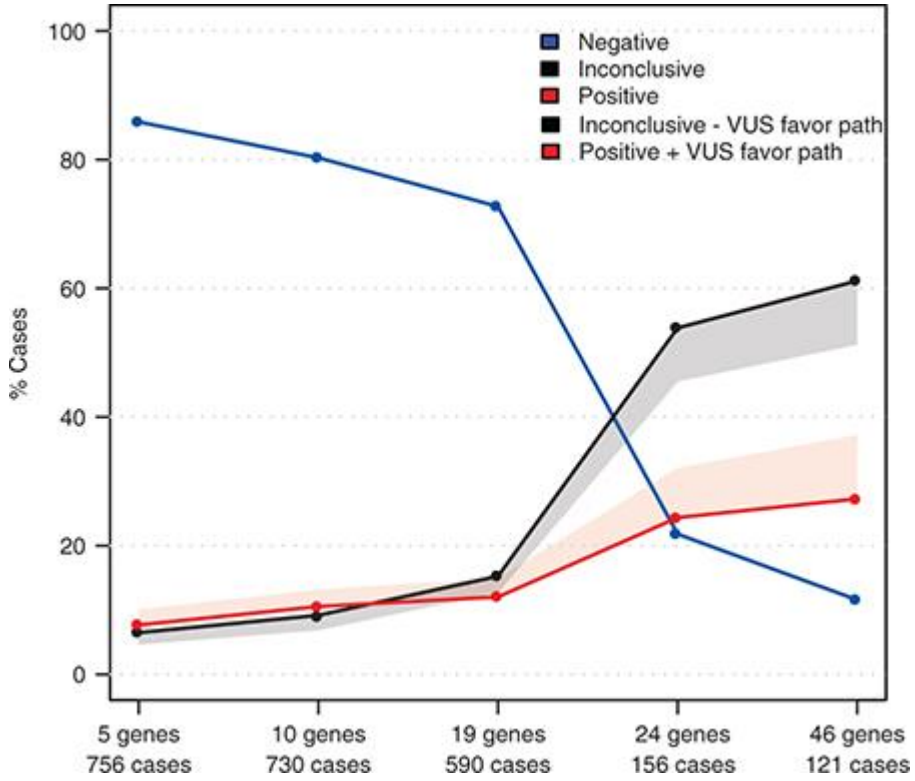
Hartwig
MEDICAL FOUNDATION



18.000
Genomen
Per jaar!

Expertisecentrum cardiogenetica UMCG

Panel: meer genen = meer varianten



Klasse	Omschrijving	
5	Pathogeen	>99%
4	Waarsch. Pathogeen	95-99%
3	Onzeker (VUS, variant of unknown significance)	5-95%
2	Waarsch. Niet pathogeen	0,1-5%
1	Niet pathogeen	< 0,1%

Ongeveer 50% heeft een variant waarvan de betekenis niet duidelijk is

Puch et al. Genetics in Med. 2014

Varianten beter begrijpen:

- Data delen
- Data “cureren”
- RNA sequencen
- Functie testen: CRISP CAS e.a.

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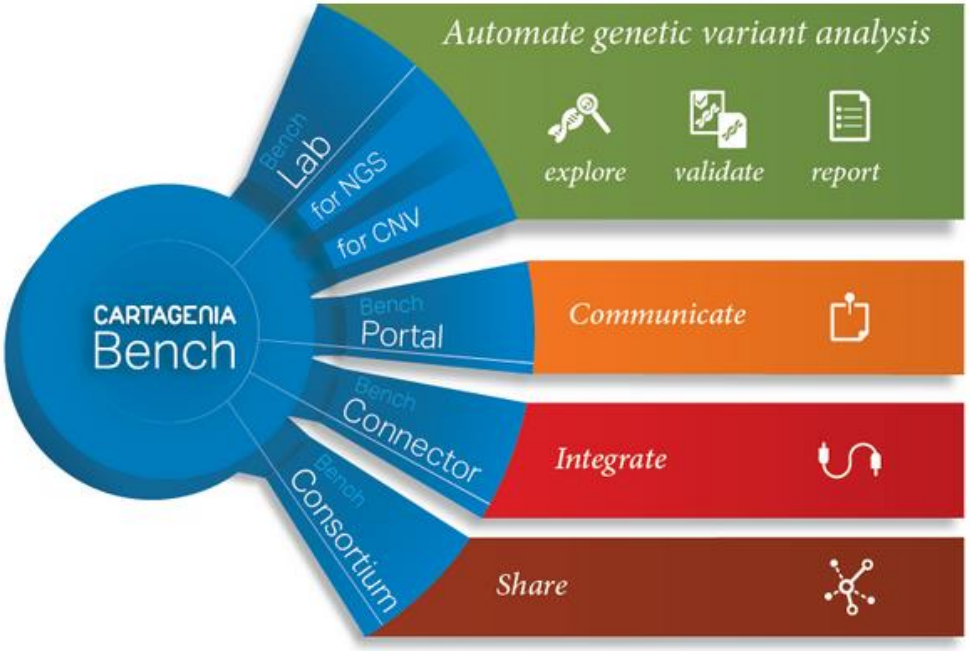
Gen panel gebaseerde analyses:

5 Centers:



Dennis Dooijes (UMCU)
Ronald Lekanne dit Deprez (AMC)
Marjon Slegtenhorst (EMC)
Arthur van de Wijngaard (MUMC)
Jan Jongbloed (UMCG)

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Official journal of the American College of Medical Genetics and Genomics **ORIGINAL RESEARCH ARTICLE** Genetics inMedicine

Open

Reassessment of Mendelian gene pathogenicity using 7,855 cardiomyopathy cases and 60,706 reference samples

Roddy Walsh, BSc, MSc[1,2], Kate L. Thomson, BSc, FRCPath[3,4], James S. Ware, PhD, MRCP[1,2,5], Birgit H. Funke, PhD, FACMG[6,7], Jessica Woodley, BSc[3], Karen J. McGuire, BSc[3], Francesco Mazzarotto, BSc, MSc[1,2], Edward Blair, BMSc, MRCP[8], Anneke Seller, PhD[3], Jenny C. Taylor, PhD[9,10], Eric V. Minikel,MS[11–14], Exome Aggregation Consortium[14], Daniel G. MacArthur,PhD[11,12,14,15], Martin Farrall, FRCPath[4,10], Stuart A. Cook, PhD, MRCPath[2,5,16,17] and Hugh Watkins,MD, PhD[4,10]

Exomen van >60.000 mensen

Varianten beter begrijpen:

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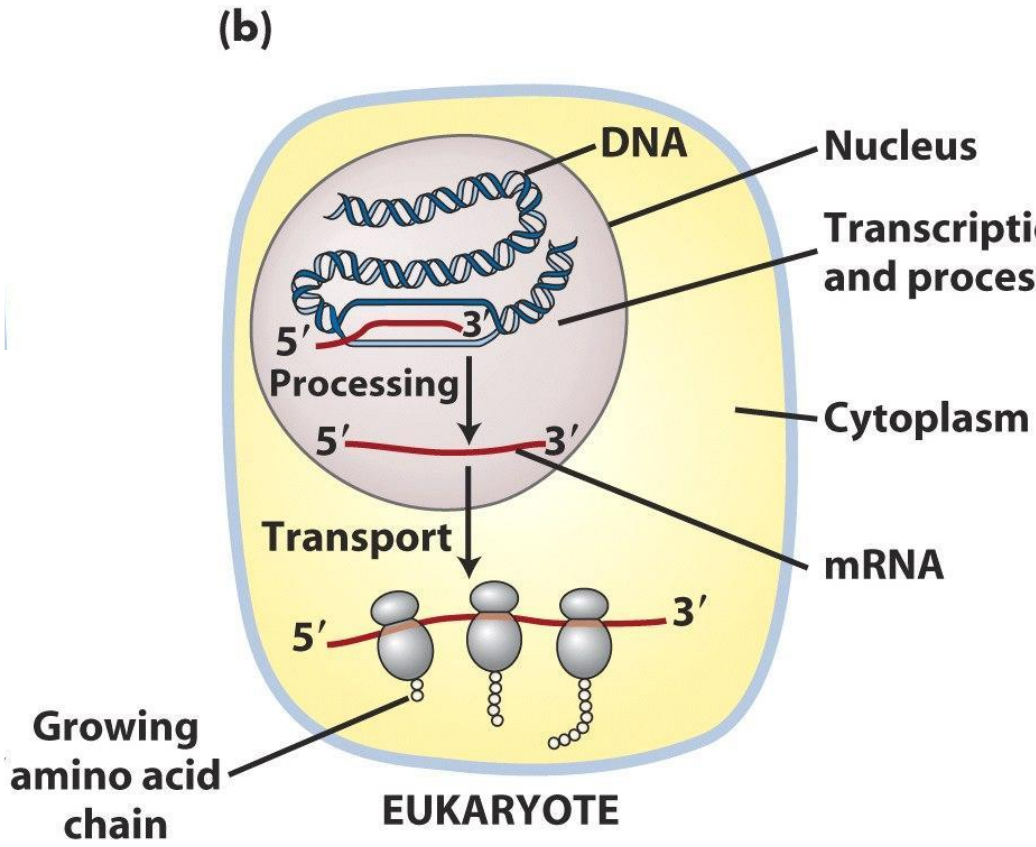
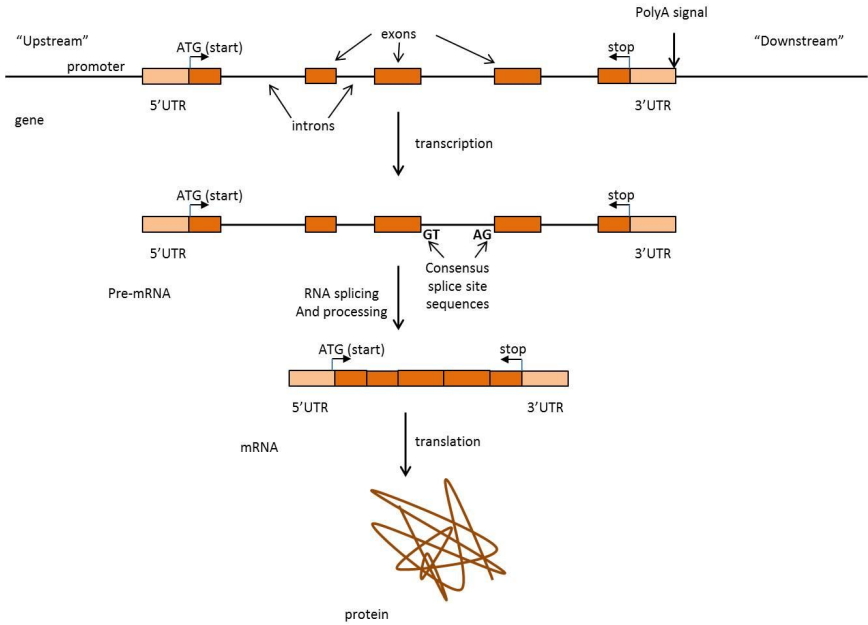

(b)
DNA
Nucleus
Transcription and processing
5′
3′
Processing
5′
3′
Cytoplasm
Transport
mRNA
5′
3′
Growing amino acid chain
EUKARYOTE


"Upstream"
promoter
ATG (start)
exons
stop
PolyA signal
"Downstream"
gene
5′UTR
introns
3′UTR
transcription
ATG (start)
stop
5′UTR
GT
AG
3′UTR
Pre-mRNA
RNA splicing And processing
Consensus splice site sequences
ATG (start)
stop
5′UTR
3′UTR
mRNA
translation
protein

Varianten beter begrijpen:

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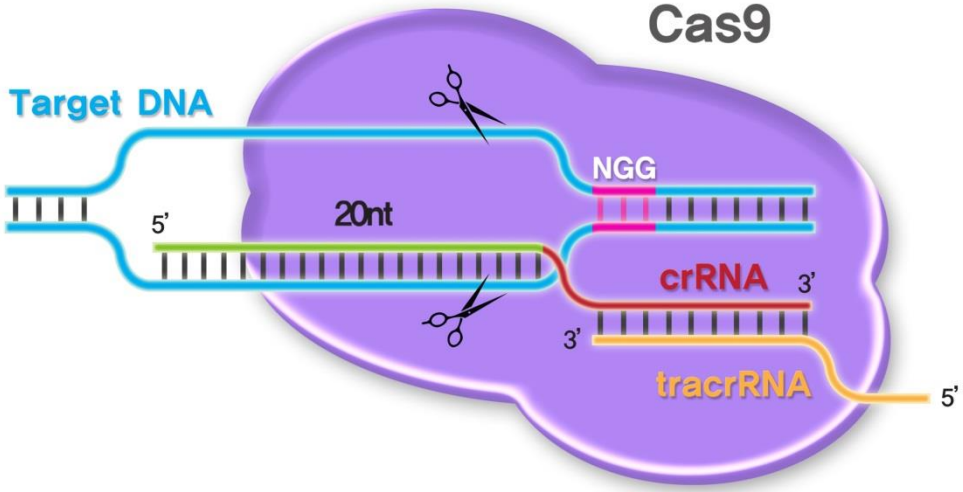
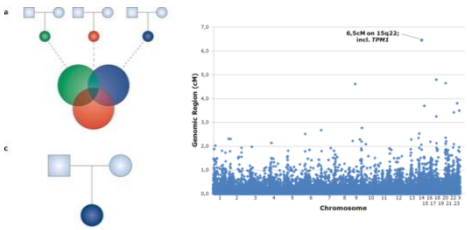


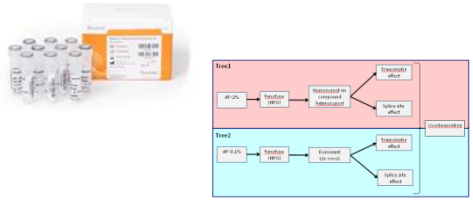
Gene panel based resequencing

ABCC9, ACTC1, ACTN2, ANKRD1, BAG3, CALR3, CRYAB, SRP3/MLP, DES, DMD, DSC2, DSG2, DSP, EMD, GLA, JPH2, JUP, LAMA4, LAMP2, LMNA, MYBPC3, MYH6, MYH7, MYL2, MYL3, MYPN, MYOZ1, MYOZ2, PKP2, PLN, PRKAG2, PSEN1, PSEN2, RBM20, RYR2, SCN5A, SGCD, TAZ, TBX20, TCAP, TMEM43, TNNC1, TNNI3, TNNT2, TPM1, TTN, VCL, ZASP

Exome Sequencing



Whole Genome Sequencing



Vragen?