

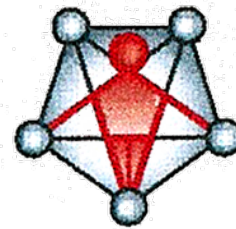
# ***Technology reaching the clinic: an overview***

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**A (forever) changing landscape**

**Prof dr Gertjan van Ommen**

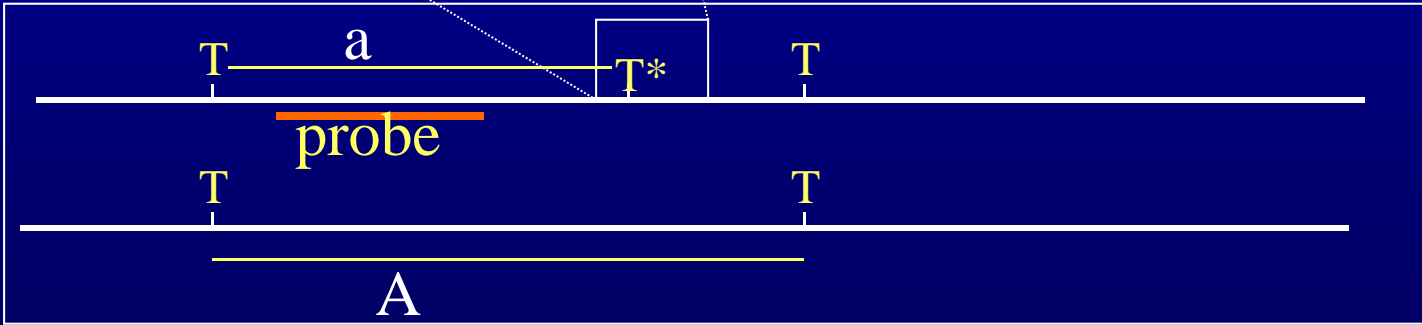
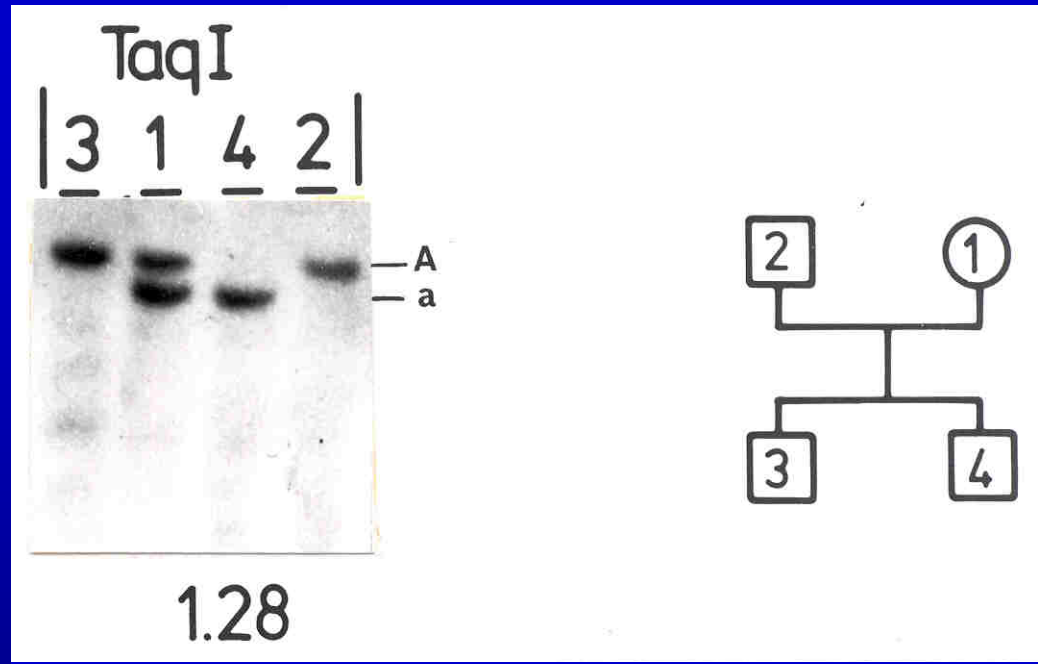
**Centre for Medical Systems Biology**





# Restriction Fragment Length Polymorphism (RFLP) / 1981

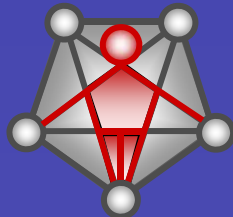
Taq I -Knipplaats



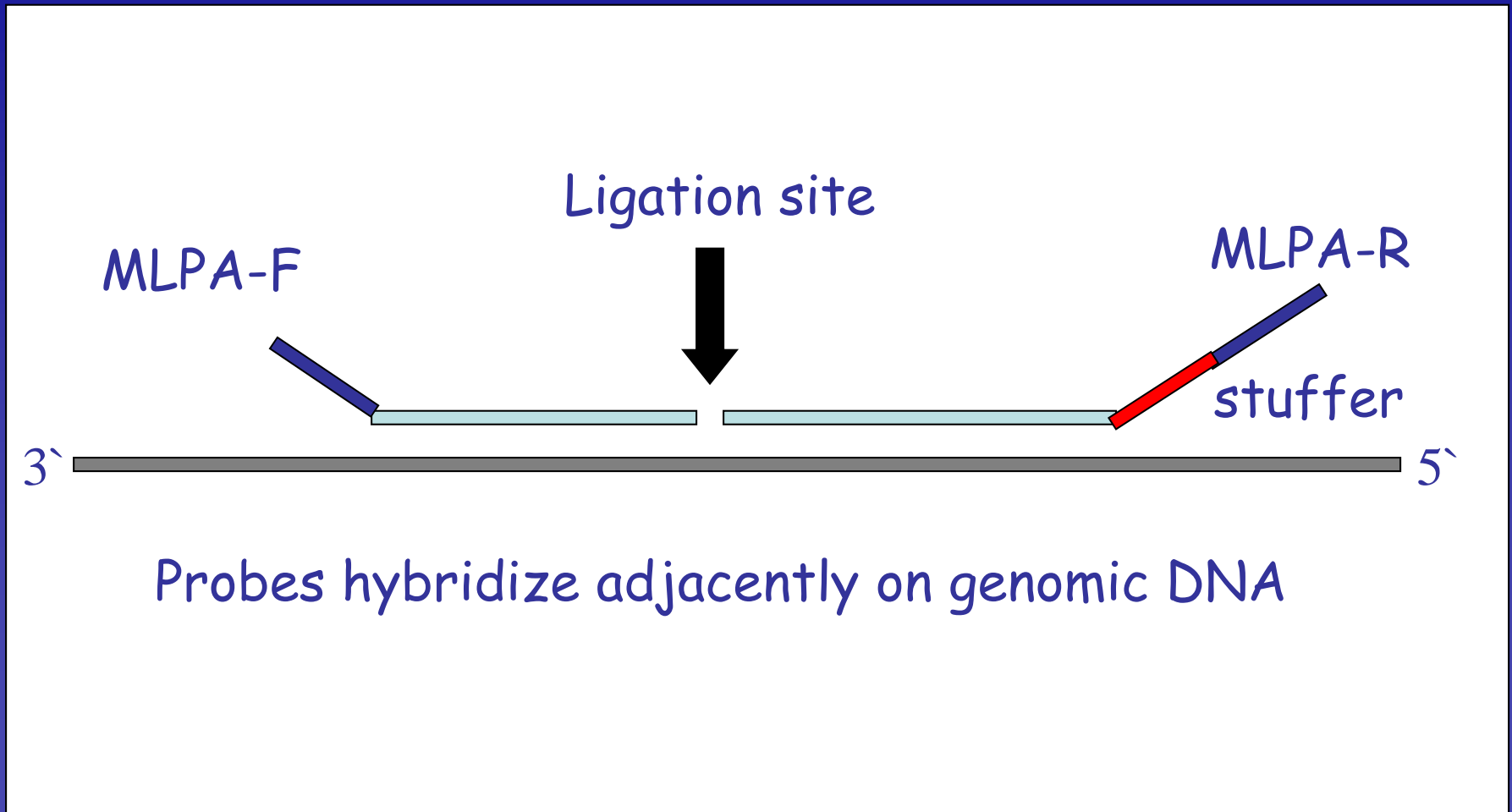
# The 'new kid on the block': CNV

## Detecting deletions and duplications

- Southern blotting
- FISH
- Quantitative PCR
- Genome-wide, array based approaches (BAC, oligo)
- MAPH
- MLPA

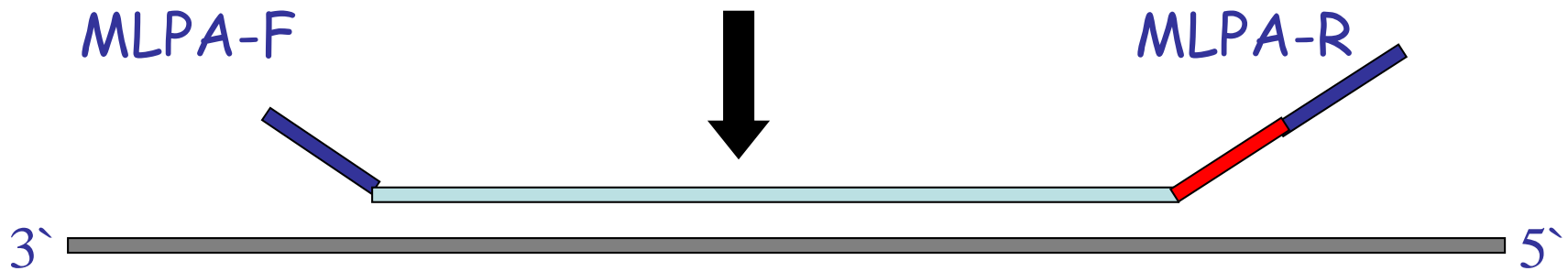


# MLPA



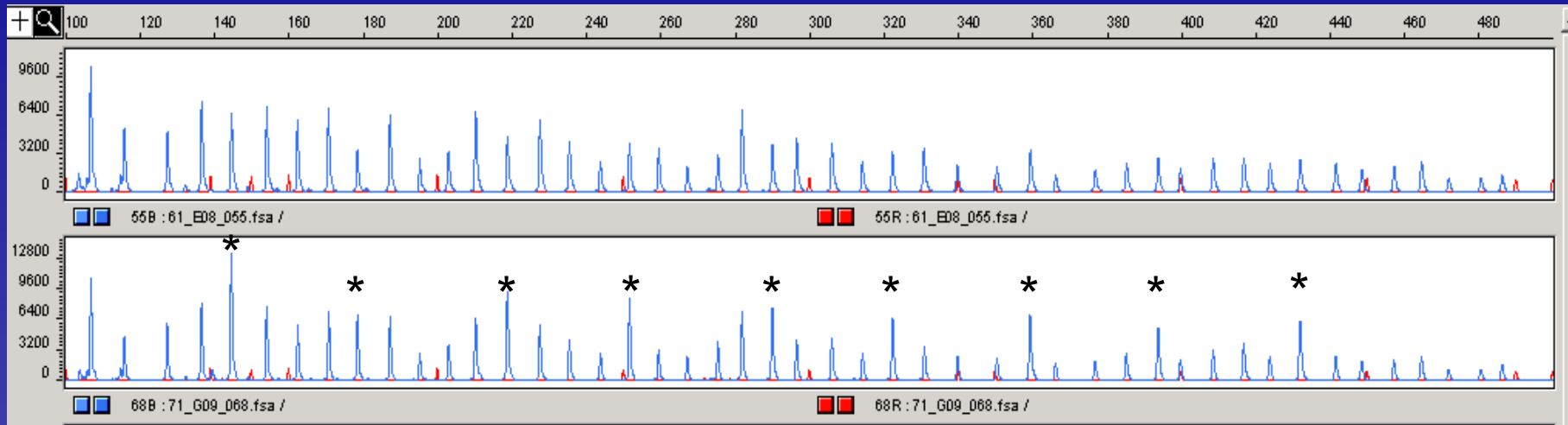
# MLPA

Ligation site



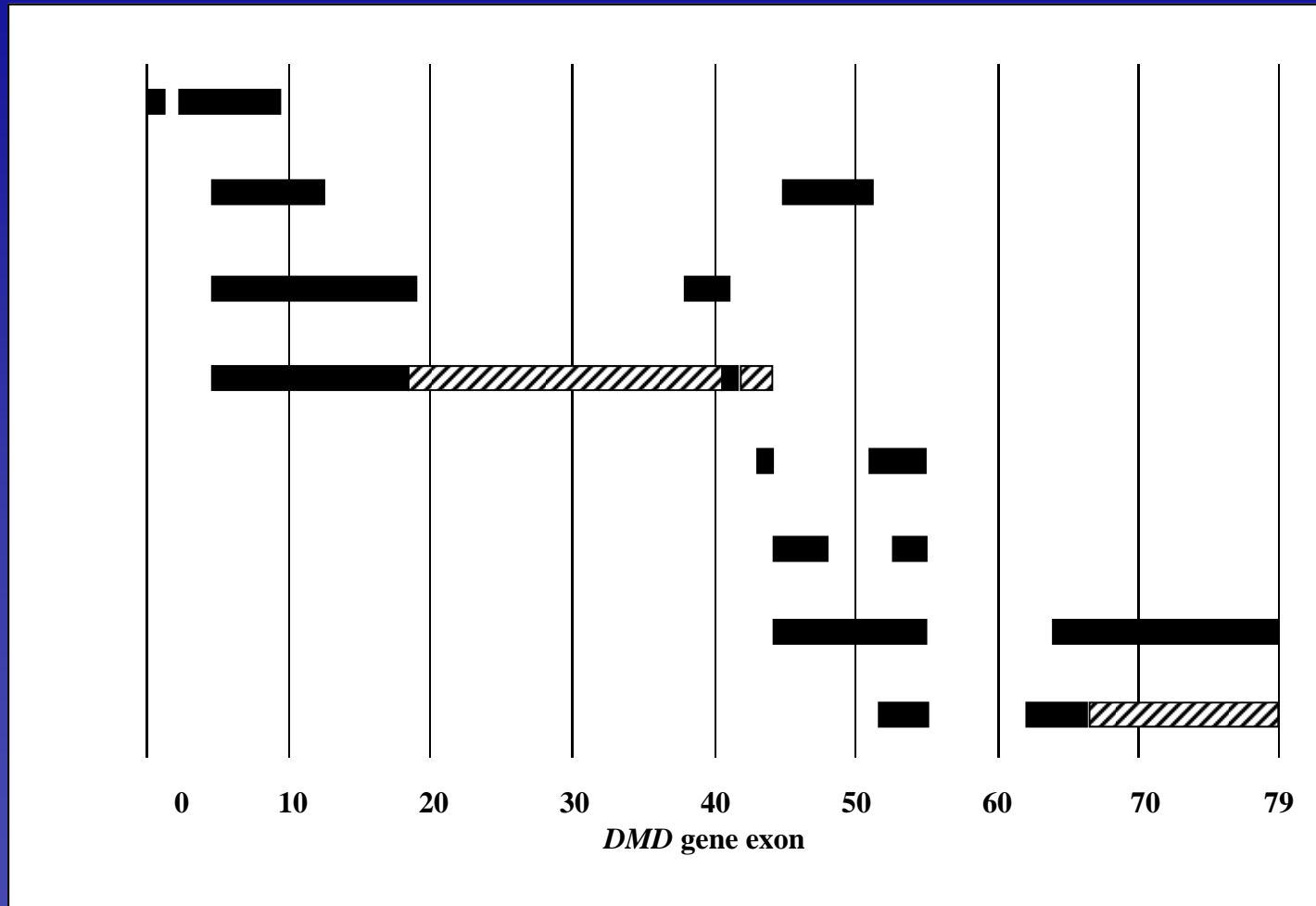
After ligation and PCR amplification a DNA fragment of a defined length is generated

# MLPA peaks



Changes in peak height correspond with deletions / duplications

# Non-contiguous duplications

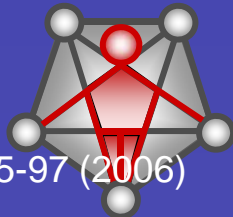




# Large-scale copy number variation

## (Large-scale) Copy-Number Variation (CNV)

“Gain or loss of several kilobases to several thousands of kilobases of genomic DNA”

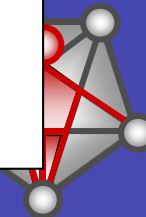


# Screening populations for CNV

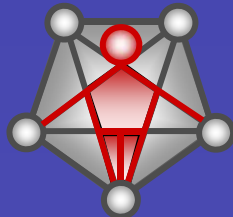
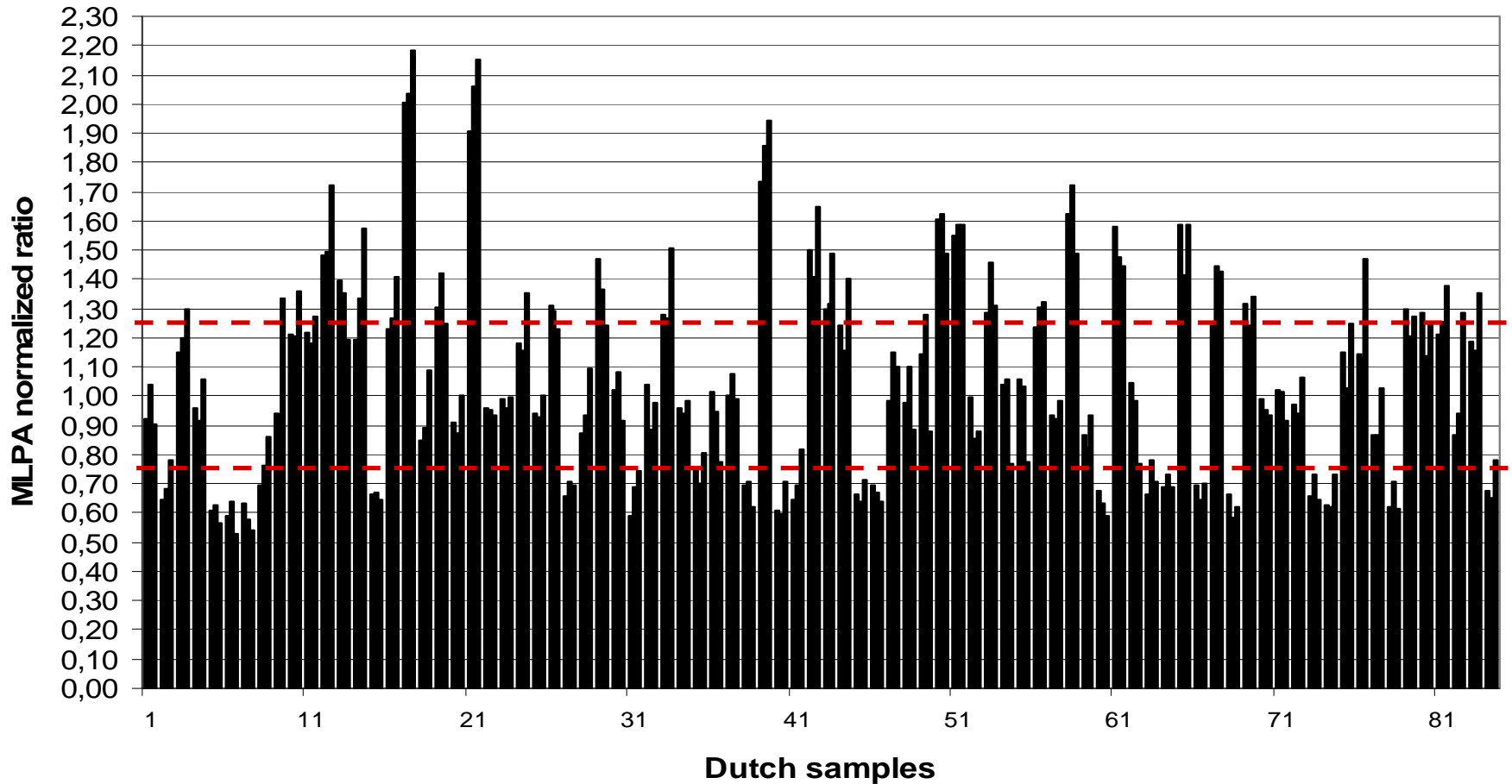
Percentage	<u>No CNV</u>		<u>Moderate CNV</u>		<u>Extensive CNV</u>	
	7q35		5q13.2		17q21.31	
	loss	gain	loss	gain	loss	gain
Pygmy ( <i>n</i> =37)	0	0	5	5	19	11
Indonesian ( <i>n</i> =88)	0	0	2	2	24	20
Dutch ( <i>n</i> =84)	0	0	2	6	28	29
Turkish ( <i>n</i> =64)	0	0	2	6	23	36
Finnish ( <i>n</i> =36)	0	0	3	0	31	25
<b>Total (<i>n</i>=309)</b>	<b>0</b>	<b>0</b>	<b>3</b>	<b>4</b>	<b>24</b>	<b>28</b>

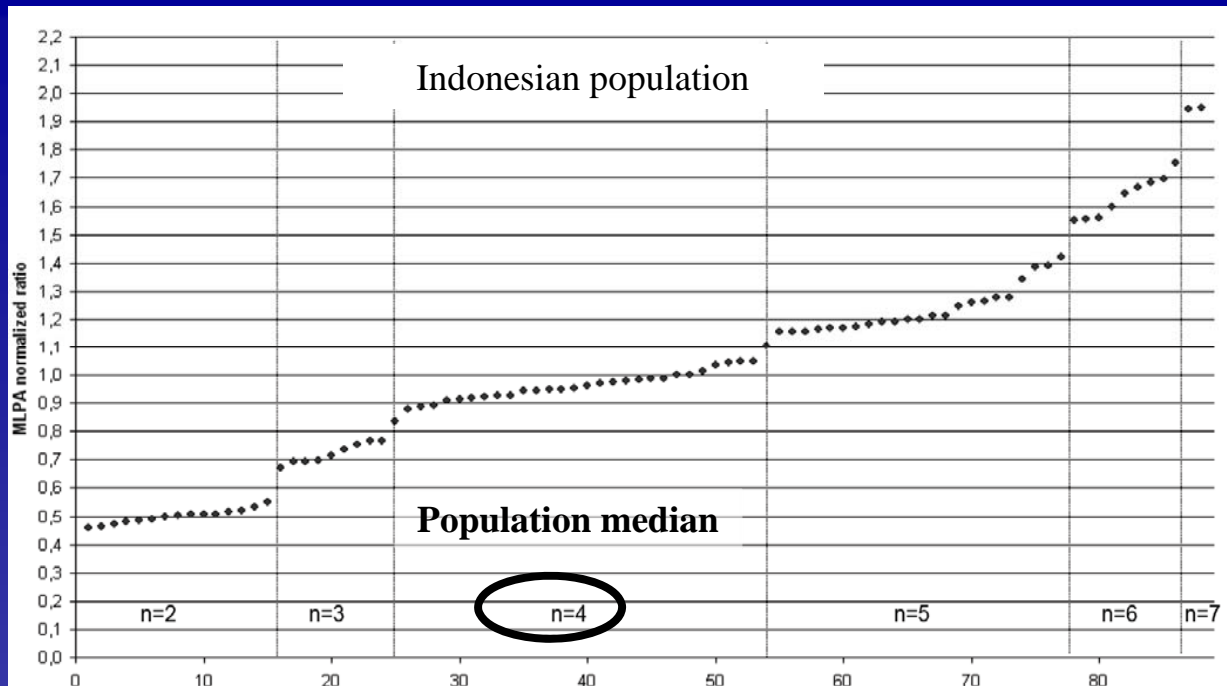
  

<p>↓</p> <p>1p32.3 2q31.1 2q35 7p21.1</p>	<p>↓</p> <p>10q11.22 14q11 22q11.21</p>	<p>↓</p> <p>8p23.1 17q12</p>
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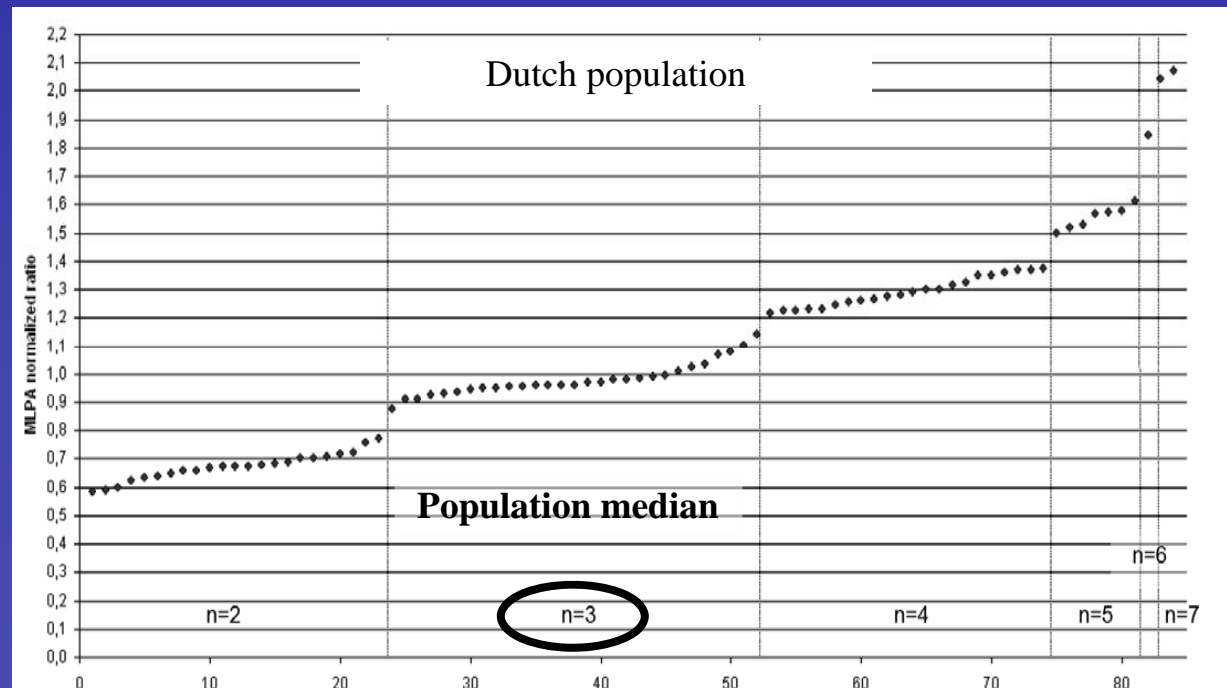
# NSF





Lowest value: ~0.50

Steps: ~0.25



Lowest value: ~0.66

Steps: ~0.33

# Population distributions

**Finnish**



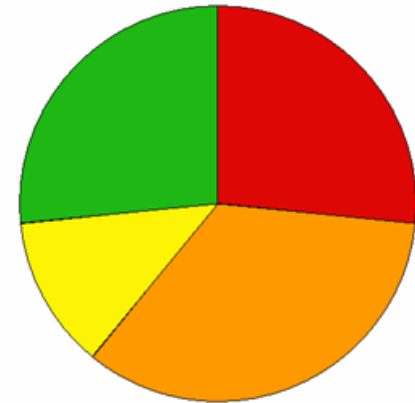
n = 36

**Dutch**



n = 84

**Turkish**

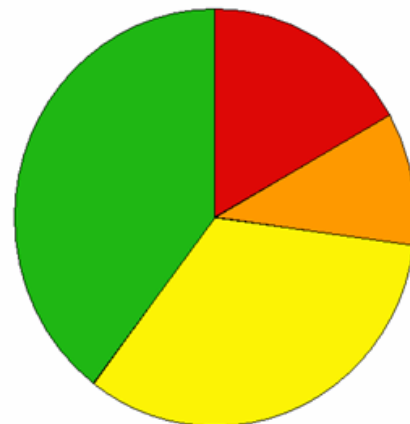


n = 64

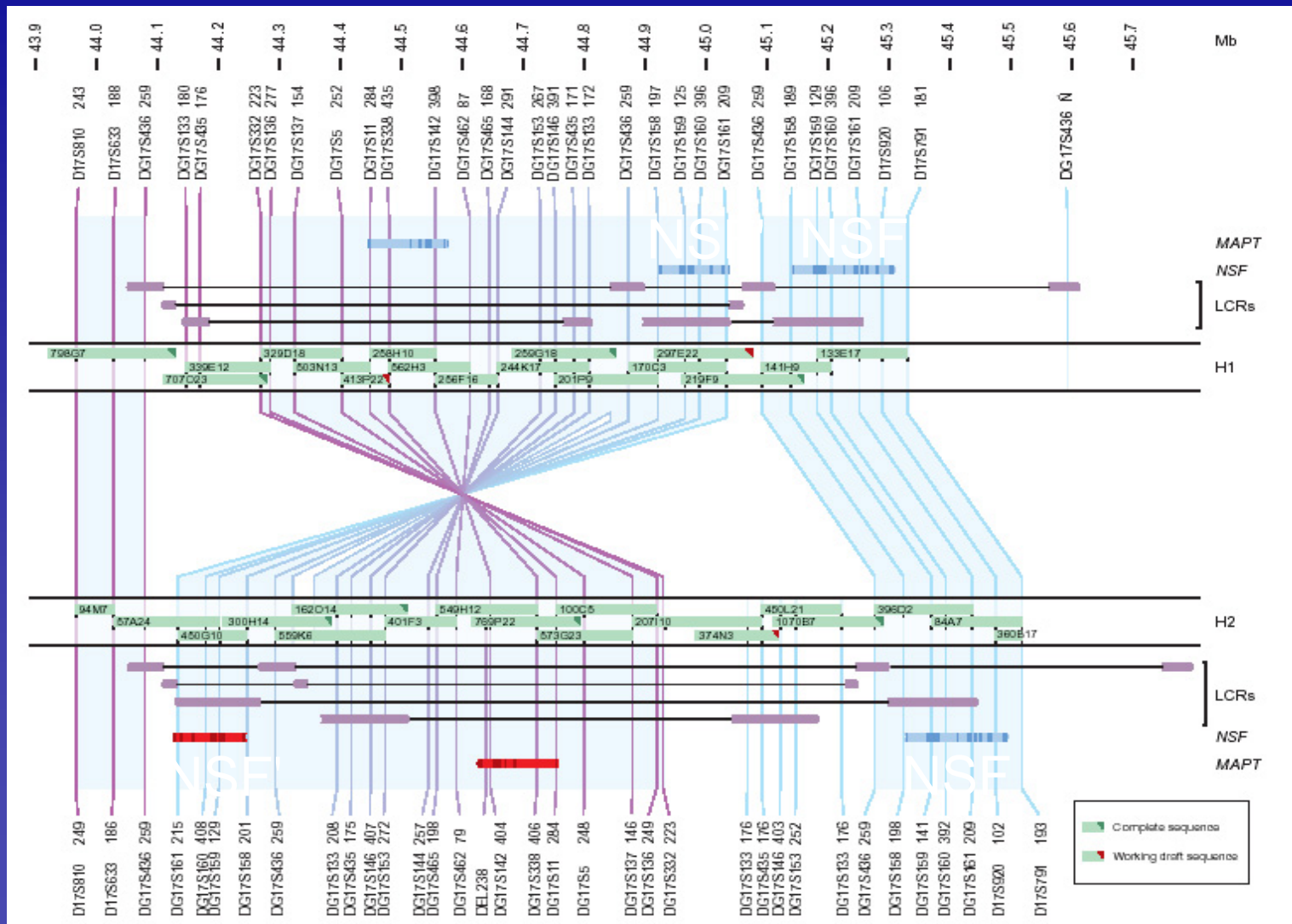
**Pygmy**



**Indonesian**

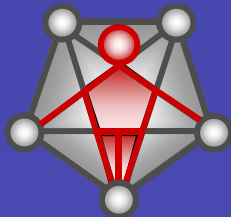


# NSF genomic structure

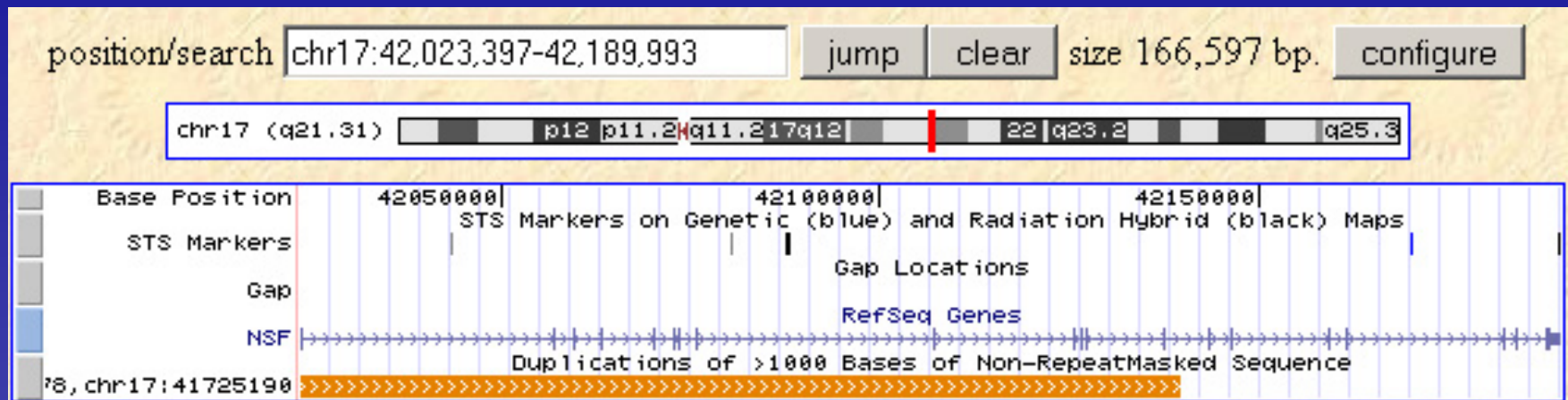


# *NSF* gene

- N-ethylmaleimide sensitive factor
- expressed in neuronal synapses
- was reported to be reduced in expression in prefrontal cortex in schizophrenia patients
- second study found no difference



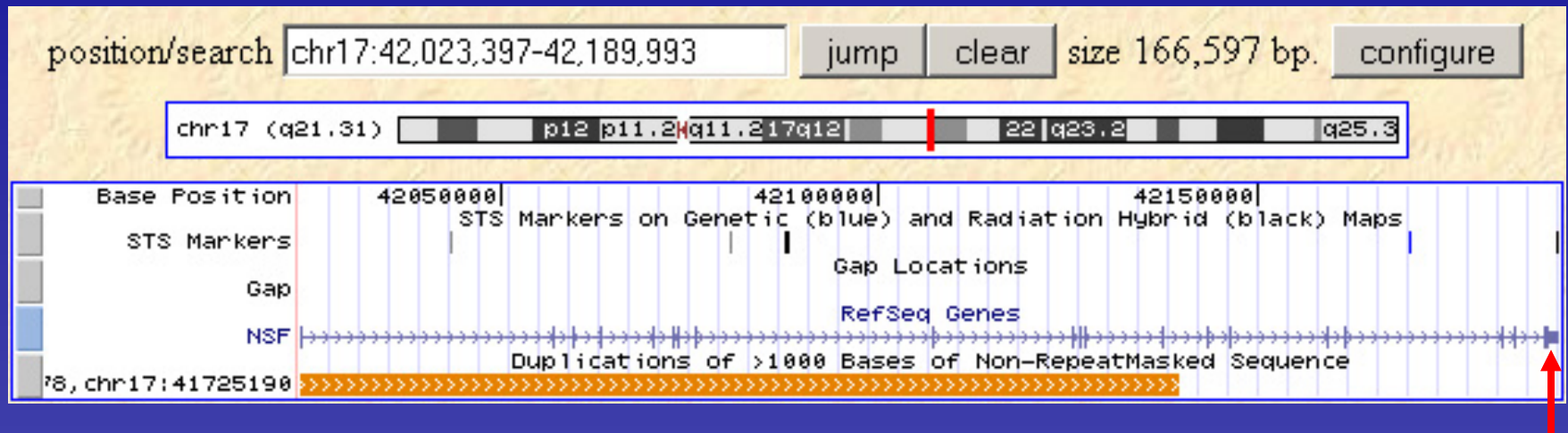
# NSF gene



Study that detected difference in expression used cDNA clone, probably covering the majority of the gene (including duplicon)

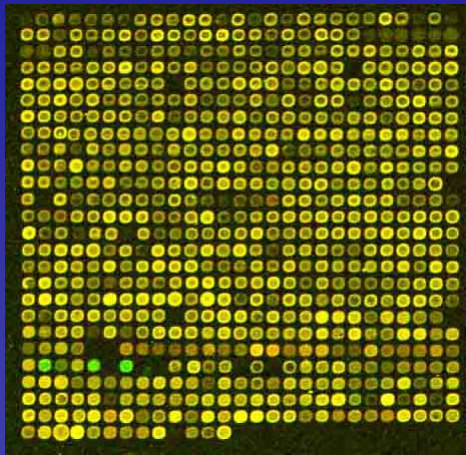


# NSF gene

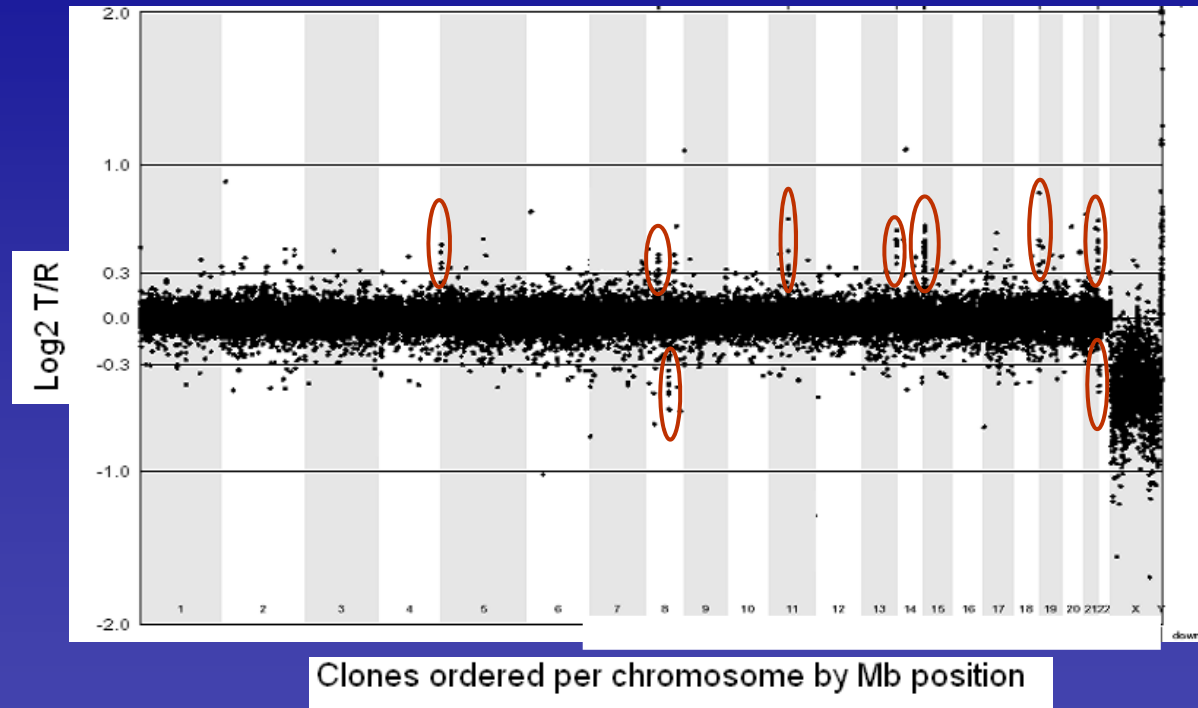


- Study that did not detect a difference in expression used qPCR, with product based in last exon (not in duplicon)

# Increasing resolution....



Array CGH

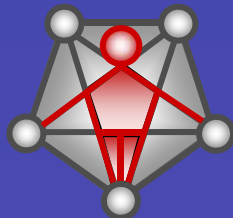


Disease-causing *or* Normal variation?

# Peters' Plus Syndrome

- Characterised by
  - Eye abnormalities
  - Developmental delay
  - Disproportionate short stature

Analysed 6 patients ( including two brothers) by 1 Mb array-CGH



chr13 (q12.3-q13.1) 13 12 31.1 34

deleted region of 1.5 Mb

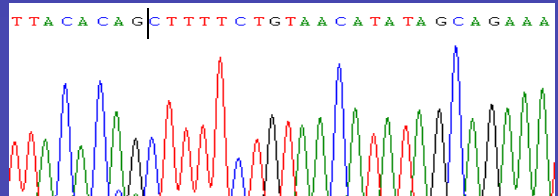
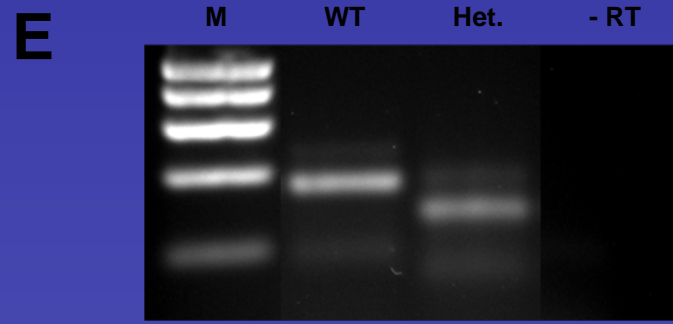
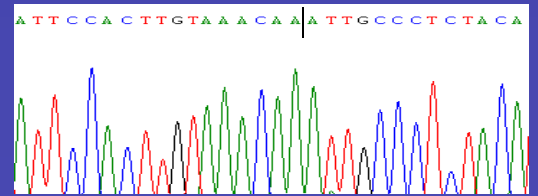
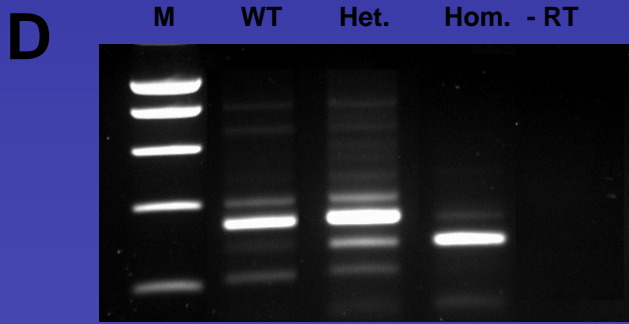
centromeric

telomeric



437+5G>A

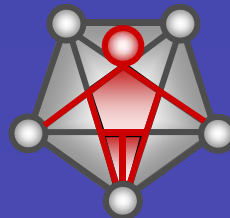
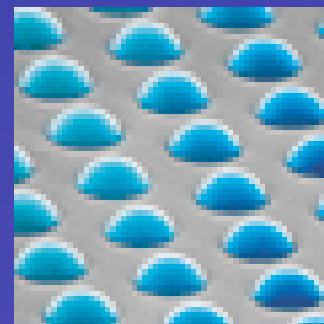
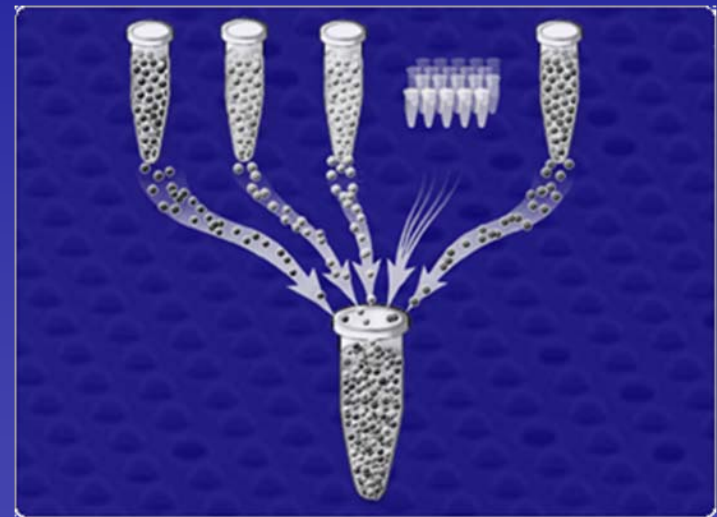
1020+1G>A



Looking for an approach that has the sensitivity of MLPA, but can provide genome-wide coverage

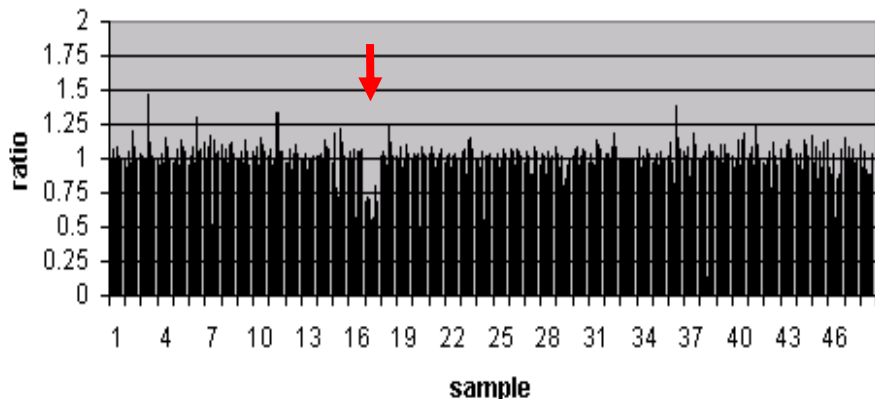
# Bead-based SNP-typing

- colour-coded beads
  - one bead = one probe
  - oligonucleotide
- 96-well format
- 1500 probes / well
  - controls + 1350 SNP's
- read-out optic fiber
  - 50,000 beads
  - ~30 beads per SNP

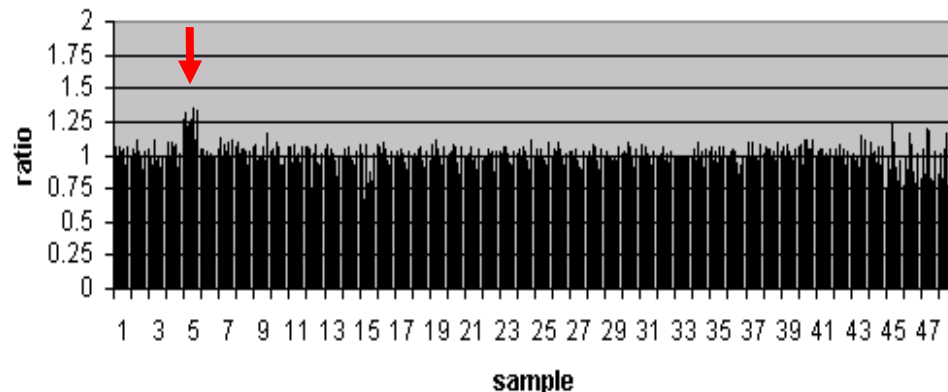


# Deletions and Amplifications Detected in Clinical Samples

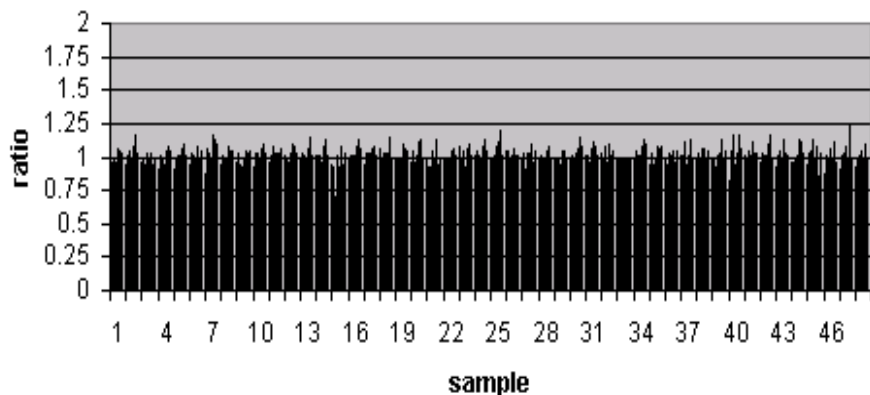
18q telomeric probes - sample 17 known deletion



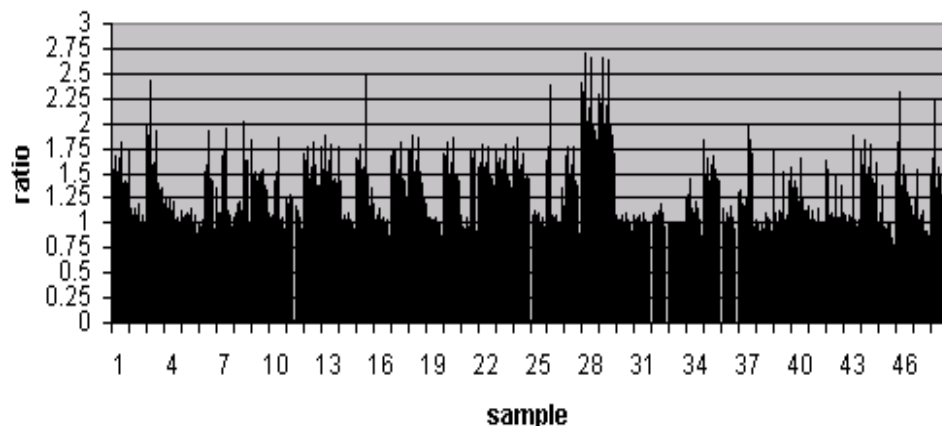
18p telomeric probes - sample 5 known duplication



15q telomeric probes - no known aberrations



DMD gene - known mutations detected; samples 28 and 29; 4 X- chromosomes



# The sequencing revolution



# Mutation detection techniques

Methods for scanning

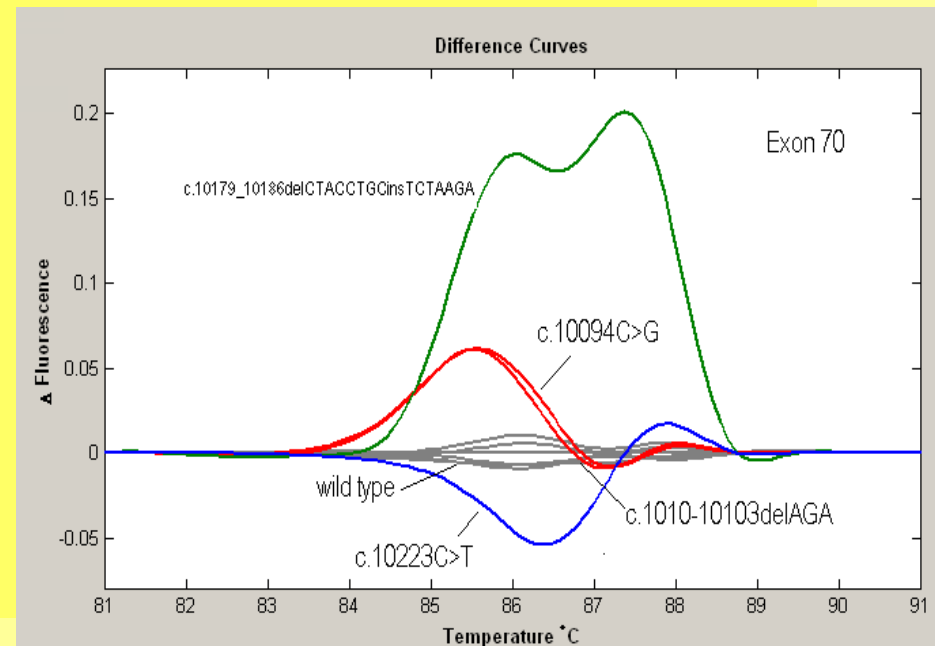
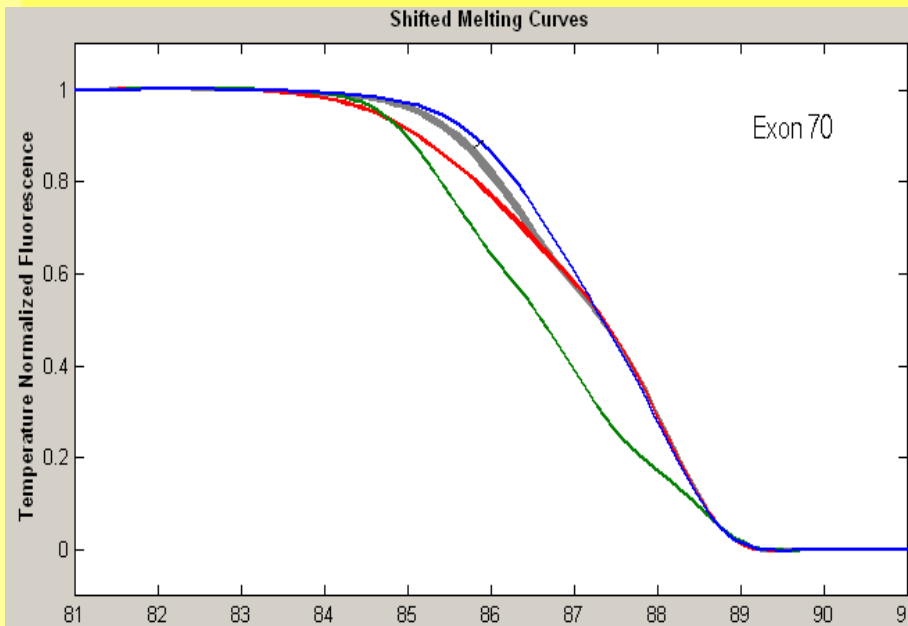
Sensitivity\*

➔ Direct sequencing

~100 % \*\*

HR-Melting curve analysis

~> 99 %



I. PCR amplification of fragments (96 well plate)



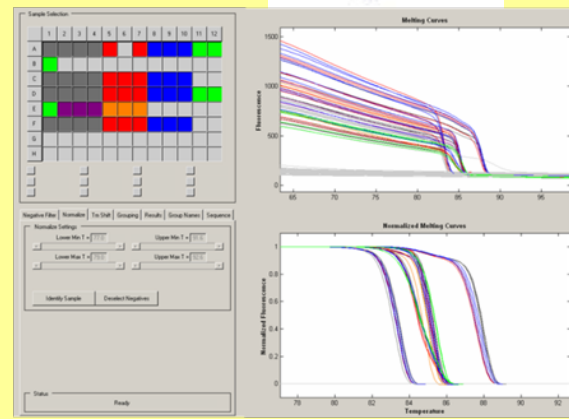
I. 1,5 - 2 hours

II. Melt PCR-fragments in Lightscanner

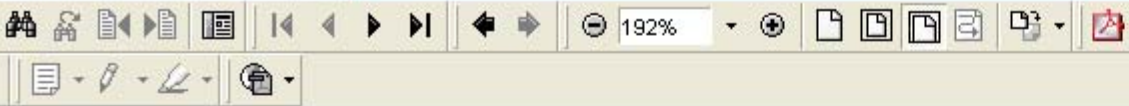


II. 8 minutes

III. Analysis (IT software)



III. 40 min.



## METHODS

Human Mutation

OFFICIAL JOURNAL



# Diagnostic Guidelines for High-Resolution Melting Curve (HRM) Analysis: An Interlaboratory Validation of *BRCA1* Mutation Scanning Using the 96-Well LightScanner™

Nienke van der Stoep,<sup>1\*</sup> Chantal D.M. van Paridon,<sup>1</sup> Tom Janssens,<sup>3</sup> Petra Krenkova,<sup>2</sup> Alexandra Stambergova,<sup>2</sup> Milan Macek,<sup>2</sup> Gert Matthijs,<sup>3</sup> and Egbert Bakker<sup>1</sup>

<sup>1</sup>Center for Human and Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands; <sup>2</sup>Institute of Biology and Medical Genetics, Charles University, Prague, Czech Republic; <sup>3</sup>Center for Human Genetics, University of Leuven, Leuven, Belgium

172 known variants and 197 controls

40 HRM primer pairs for *BRCA1*.

20 pairs tested in 3 labs: (reproducibility, inter-laboratory variability, robustness)

Validation : blind set of 28 samples,

→ 100% sensitivity (no false negatives)

→ 98% Specificity

General guidelines for HRM set up



Solexa

1G GENETIC ANALYZER

# Targeted sequencing

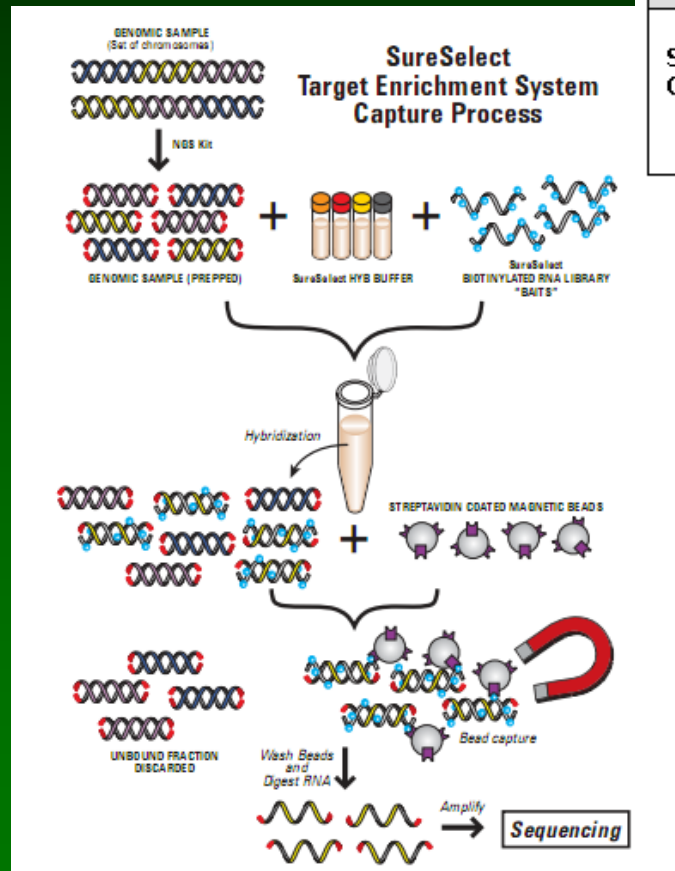
---

- **chromosome sorting** (*complexity reduction*)
- **gel separation**  
*Pulsed-Field Gel-electrophoresis*
- **megabase regions**  
*long-range PCR*  
*1 Mb > 100 x 10Kb fragments*  
*normal PCR (multiplex)*  
*RainDance, Fluidigm*
- **smaller regions**  
*normal PCR (multiplex)*  
*pool samples (+/- sequence tag)*
- **hybridisation capture**  
*on array, in solution*  
*NimbleGen, Agilent*

# In-solution capture



Agilent's SureSelect Target Enrichment System



Product Name	Product Number	Pricing & Availability
SureSelect Human X Chromosome Exome Kit	G4459A	<a href="#">Contact Us</a>

tested X-chromosome whole-exome assay in collab. with



# X-exome<sub>2</sub>

- **X-chromosome**

*85% exons*

*excl. pseudo-autosomal, Y-homology*

- **probes**

*43,073 of 120 nt*

*7663 regions*

*one strand only*

- **capture & sequence**

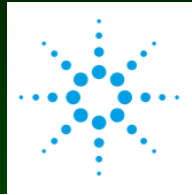
*paired-end 2x50 nt reads*

*8.2 M QC filtered, 6.0 M map uniquely (74%)*

*probes span 3.05 M*

*covered 3.02 M (99%)*

*234 / 7663 regions show gap*



Agilent's SureSelect Target  
Enrichment System

# Array capture<sub>p</sub>

*Mixed tagged samples*

*custom array*



## Pool of 2

ACAGTG - 11089031

TGACCA - 4770651

## Pool of 5

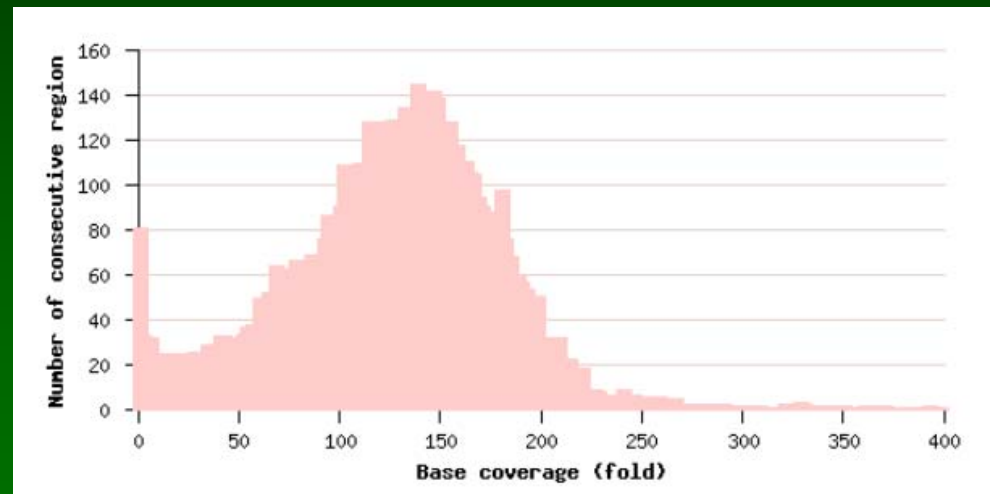
ACAGTG - 4717304

CTTGTA - 3782593

CAGATC - 3022664

GGCTAC - 2381920

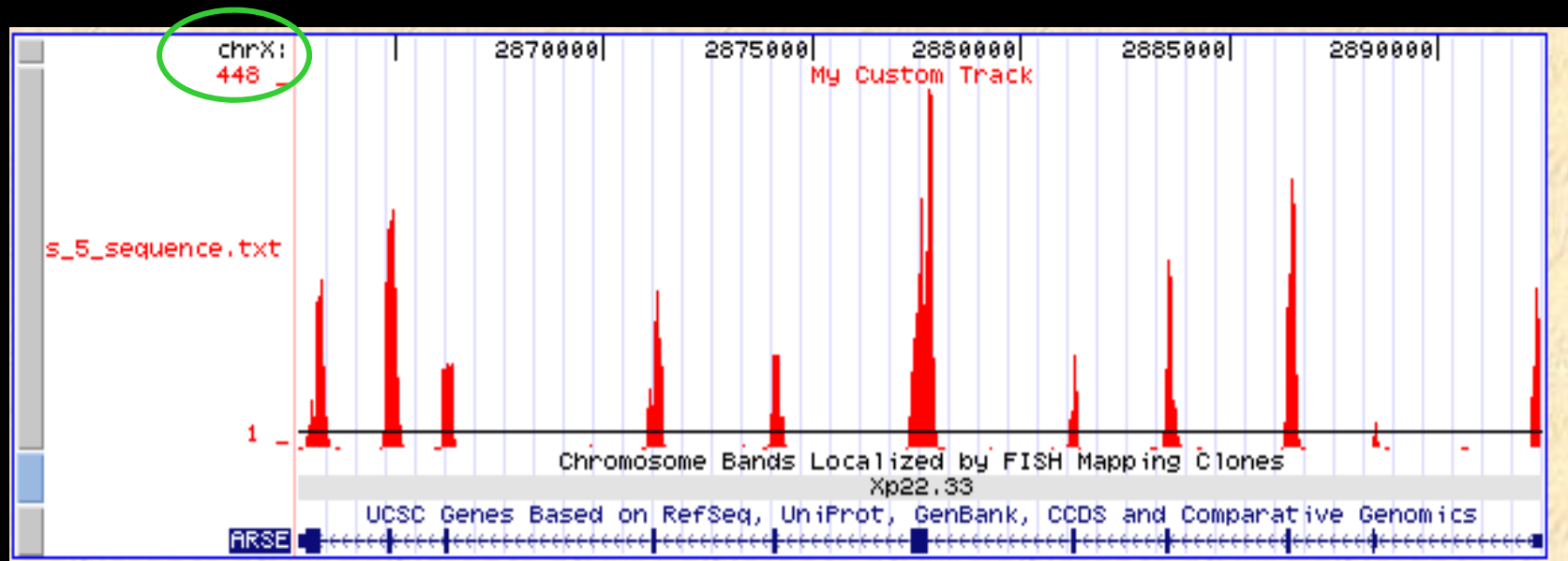
ATCACG - 2376586



*mean coverage 131-fold*



# X-exome select



ARSE





# X-linked

- **Terminal Osseous Dysplasia**  
*pigmentary anomalies skin*  
*skeletal abnormalities limbs*  
*recurring digital fibromatosis childhood*
- **X-linked** (*Xq25-ter*)  
*dominant*  
*male lethal*  
*female skewed X<sub>i</sub>*



American Journal of Medical Genetics 94:91-101 (2000)

*New Syndrome?*

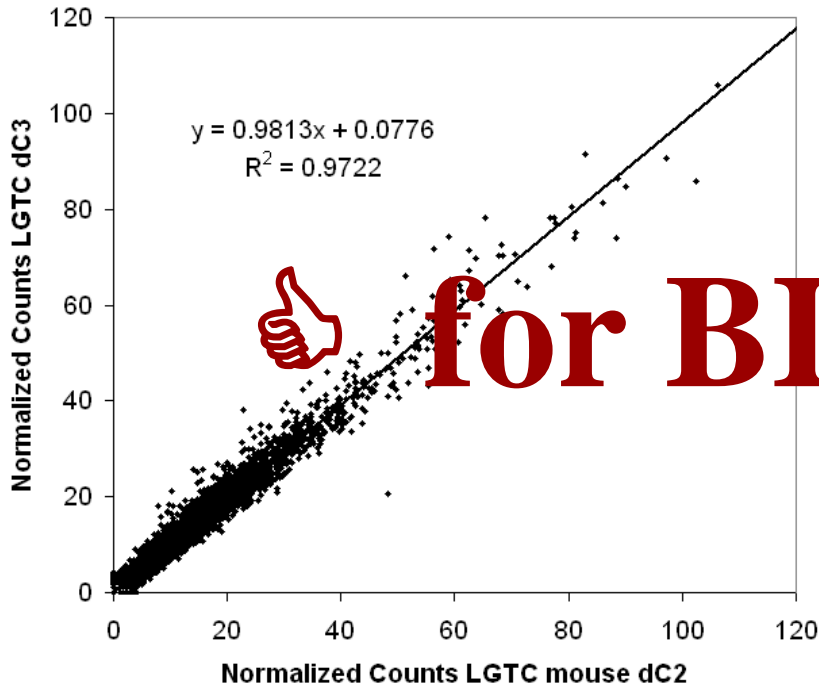
**Recurrent Digital Fibroma, Focal Dermal Hypoplasia, and Limb Malformations**

Yu Sun et al. AJHG 2010 in press.



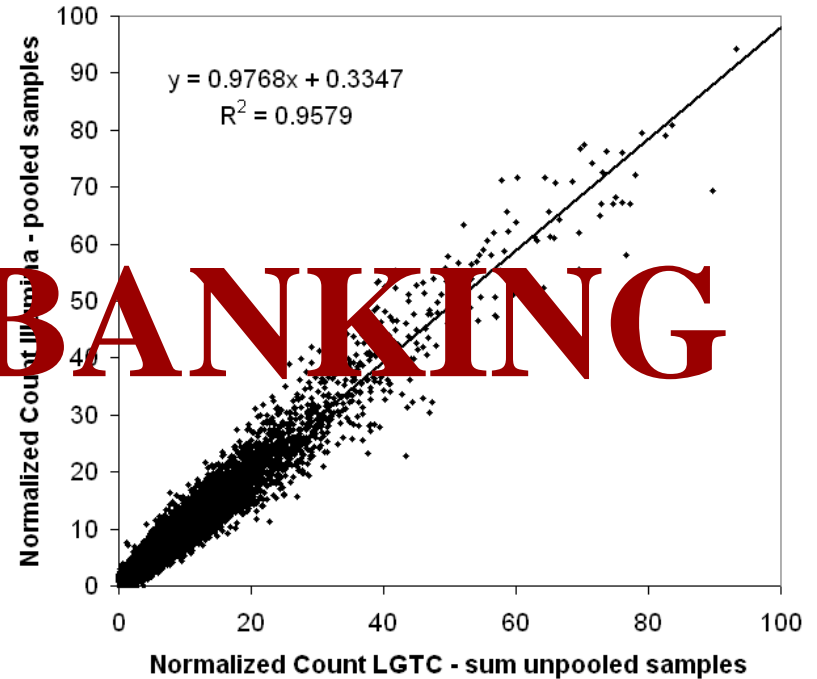
# Lab2lab consistency

## 2 transgenic mice



( *biological replicas* )

## 2 different labs

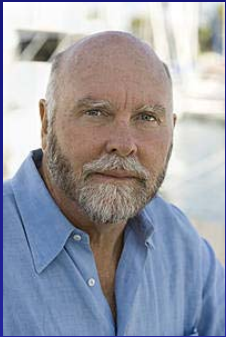


( *Illumina <> Leiden* )

**for BIOBANKING**

# Human genomes

( Individual genomes )



*Craig Venter*



*James Watson*



*Marjolein Kriek*

ANONYMOUS:  
*Yoruban male*  
*Yoruban trio*  
*Asiatic genome*  
*Female Cancer*

# A human genome ...

- **why us?**

*show it is possible*

*technical, computational, analytical*

*to learn*

*technology, data floods, analysis*

*attractive project to tackle*

- **why her ?**

*clinical geneticist*

*X-chromosome less variable*

*look at more, not fewer*

- **results**

*technically* - *no problem*

*computationally* - *at our limits*

*analytically* - *not (yet) possible*

- *as expected*

- **>> to be applied in patients**

*resolve cause genetic disease*



# *Analysis*

---



*draw DNA-based conclusions*

- 1. a female (no Y-chromosome sequences)*



ACAATCGAGTAGTACTCCCGATTGAAGCCCCCATTCGTATAATAATTACATCACAAGACGTCTTGCACTCATGAGCTGTCCCCACATTAGGCTTAAAAAC  
 AGATGCAATTTCCCGGACGTCTAAACCAAACCACTTTCACCCGCTACACGACCGGGGTATACTACGGTCAATGCTCTGAAATCTGTGGAGCAAACCACAGT G 8269  
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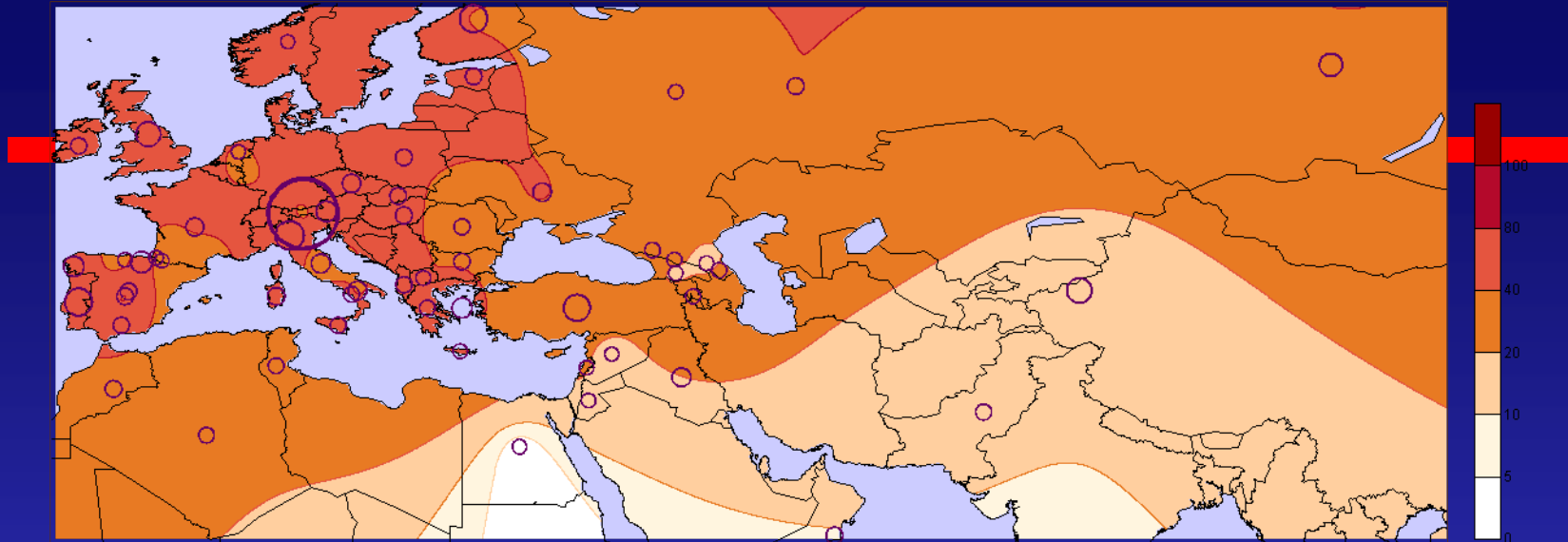
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 CAACCAGTAACTACTACTAATCAACGCCATAATCATACAAAGCCCCCGCACCAATAGGATCCTCCCGAATCAACCCCTGACCCCTCTCCTTCATAAATTA  
 TTCAGCTTCTACACTATTAAAGTTTACCACAACCACCACCCCATCATACTCTTTACCCACAGTAAACCAATCCTACCTCCATCGCTAACCCCACTAAAAC C 14365  
 ACTCACCAAGACCTCAACCCCTGACCCCATGCTCAGGATACTCCTCAATAGCCATCGCTGTAGTATATCCAAAGACAACCATCATTCCCCCTAAATAA  
 ATTAAAAAACTATTAAACCCATATAACCTCCCCAAAATTCAGAATAATAACACACCCCGACCACCCGCTAACCAATCAATGCTAAACCCCAATAAATAG A 14582  
 GAGAAGGCTTAGAAGAAAACCCACAAACCCCATTACTAAACCCACACTCAACAGAAACAAAGCATAACATCATTAATCTCGCACGGACTACAACCACGAC  
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 TGCGCCTCAATATTTCTTATCTGCTCTTCTTACACATCGGGCGAGGCTATATTACGGATCATTTCTCTACTCAGAAACCTGAAACATCGGCATTATC  
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 TTAAGTGCAGCCACCATGAATATTGTACGGTACCATAAATACTTGACCACCTGTAGTACATAAAAACCAATCCACANCAAAAACCCCTCCCCTGCTTA  
 CAAGCAAGTACAGCAATCAACCCCTCAACTATCACACATCAACTGCAACTCAAAGCCACCCCTCACCCACTAGGATACCAACAACCTACCCACCCCTAA  
 CAGTACATAGTACATAAAGCCATTTACCGTACATAGCACATACAGTCAAATCCCTTCTCGTCCCCTGGATGACCCCTCAGATAGGGGTCCCTTGAC  
 CACCATCCTCCGTGAAATCAATATCCGCACAAGAGTGCTACTCTCCTCGCTCCGGGCCATAAAGCTGGGGGTAGCTAAAGTGAAGTGTATCCGACAT  
 CTGGTTCCTACTTCAGGGTCATAAAGCCTAAATAGCCACACGTTCCCCTTAAATAAGACATCAGCATG

... → mtDNA Haplogroup H4

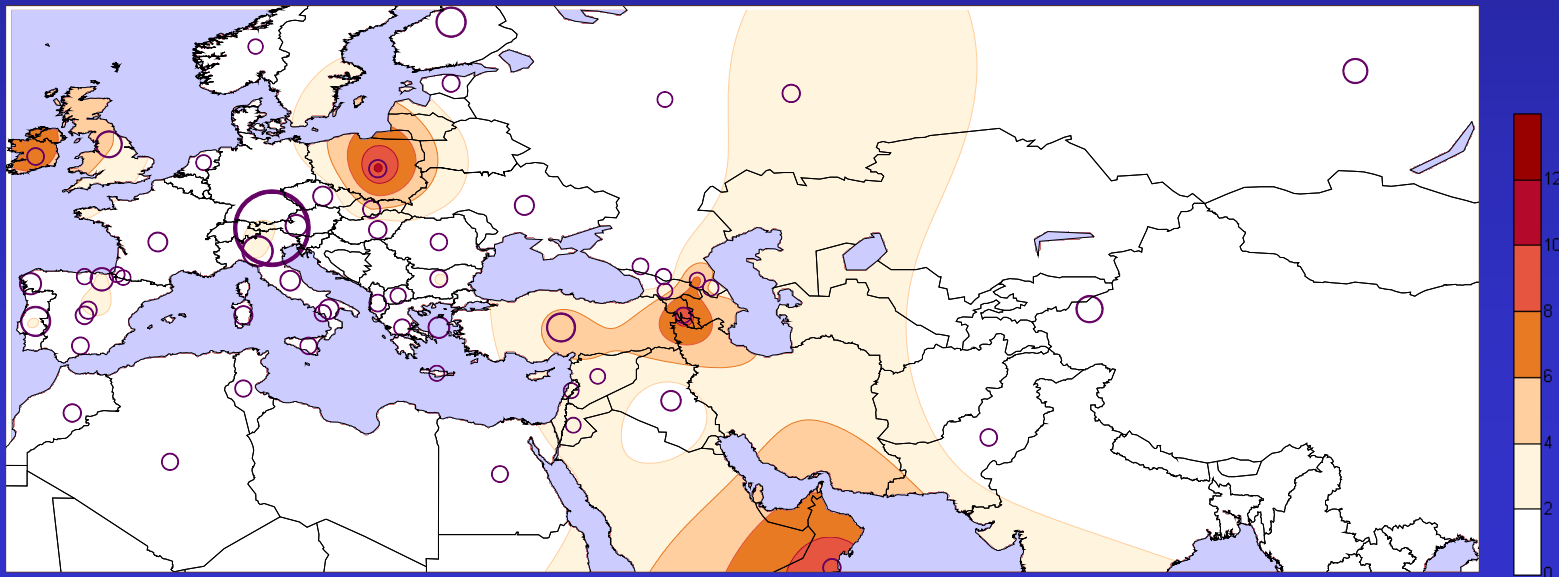
... → mtDNA Haplogroup H4a



## Distribution of mtDNA Haplogroup H



## Distribution of mtDNA Haplogroup H4



## Scientists crack women's DNA code

**FINALLY**, men may be able to understand women, it seems. Dutch scientists said they have mapped the full genetic sequence of an individual woman's DNA for the first time.

Researchers at Leiden University Medical Centre said they had sequenced the genome of one of their researchers, geneticist Marjolein Kriek, and plan to publish it after review.

# Publieks perceptie...



*here the defective  
gene for parking  
a car backwards*



marjolein kriek

Zoeken

[Geavanceerd zoeken](#)

Doelzoek:  het internet  pagina's in het Nederlands  pagina's uit Nederland

Het internet

Resultaten **1 - 10** van circa **131.000** voor **marjolein kriek** (0,31 seconden)

[472 resultaten opgeslagen op uw computer](#) - [Verbergen](#) - [Info](#)



[Illumina Barcelona GJvO.ppt](#) - sequence technology) **Marjolein Kriek** PhD, clinical  
[GJvO\\_Kgenome.ppt](#) - company (sequence technology) **Marjolein Kriek** PhD, clinical

[Eerste genenkaart vrouw ontcijferd - Binnenland - de Volkskrant](#)

27 mei 2008 ... Het dna is van **Marjolein Kriek** (34), klinisch geneticus in opleiding aan het LUMC. Zij is de derde mens van wie het complete erfelijk ...

[www.volkskrant.nl/.../Eerste\\_genenkaart\\_vrouw\\_ontcijferd](#) - [In cache](#) - [Vergelijkbaar](#)

[Noorderlicht Radio en TV: Noorderlicht nieuws](#)

28 mei 2008 ... **Marjolein Kriek** vertelt in Noorderlicht Radio (27 mei 2008) hoe het is om je ... **Marjolein Kriek** is te gast bij Noorderlicht Nieuws. ...

[noorderlicht.vpro.nl/afleveringen/39422270/](#) - [In cache](#) - [Vergelijkbaar](#)

[Dr. Marjolein Kriek, First Woman to Have Her DNA Sequence Determined](#) - [ [Vertaal deze pagina](#) ]

31 Dec 2008 ... Celebrating two firsts in May, Dutch clinical geneticist Dr. **Marjolein Kriek**, 34, of Leiden University Medical Centre (LUMC) in the ...

[www.findingdulcinea.com/.../marjolein-kriek.html](#) - [In cache](#) - [Vergelijkbaar](#)

[First female genome is sequenced – Dr Marjolein Kriek! : Genetics ...](#) - [ [Vertaal deze pagina](#) ]

The DNA is that of Dutch scientist Dr **Marjolein Kriek**, a clinical geneticist at LUMC. "If anyone could properly consider the ramifications of knowing his or ...

[www.blisstree.com/.../first-female-genome-is-sequenced-dr-marjolein-kriek/](#) - [In cache](#) - [Vergelijkbaar](#)

[EMM News Explorer: Marjolein Kriek](#)

World news clustered, updated every day. Explore the news, following stories by time, place or person.

[emm.newsexplorer.eu/NewsExplorer/entities/nl/870829.html](#) - [Vergelijkbaar](#)

[Marjolein Kriek - NextBio](#) - [ [Vertaal deze pagina](#) ]

Search Term: **Marjolein Kriek** (author). Print page. Author. See also "M Kriek" · Author · Related Content · Studies · Clinical Trials · Literature · News ...

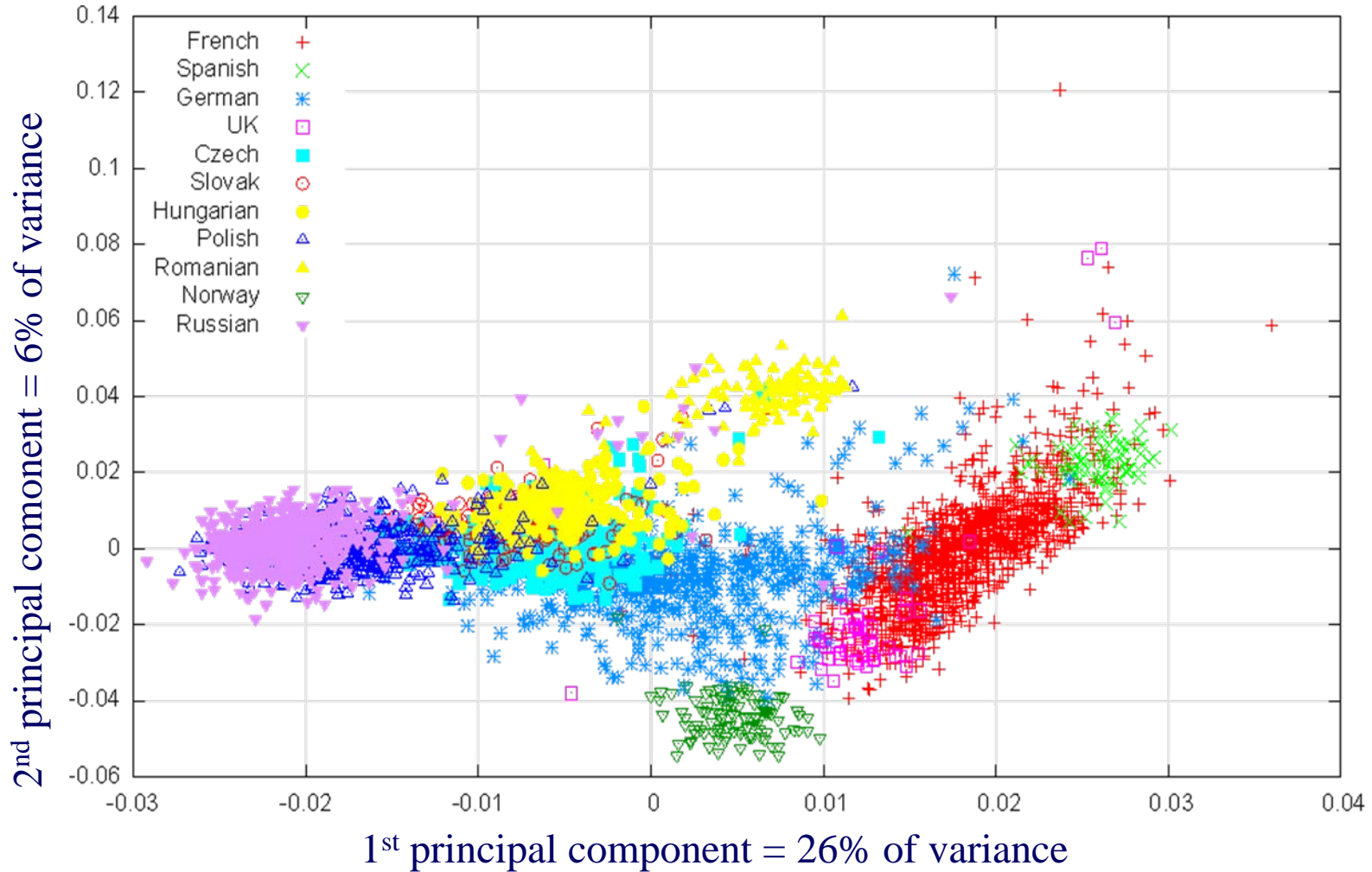
[www.nextbio.com/b/.../Marjolein%20Kriek](#) - [In cache](#) - [Vergelijkbaar](#)

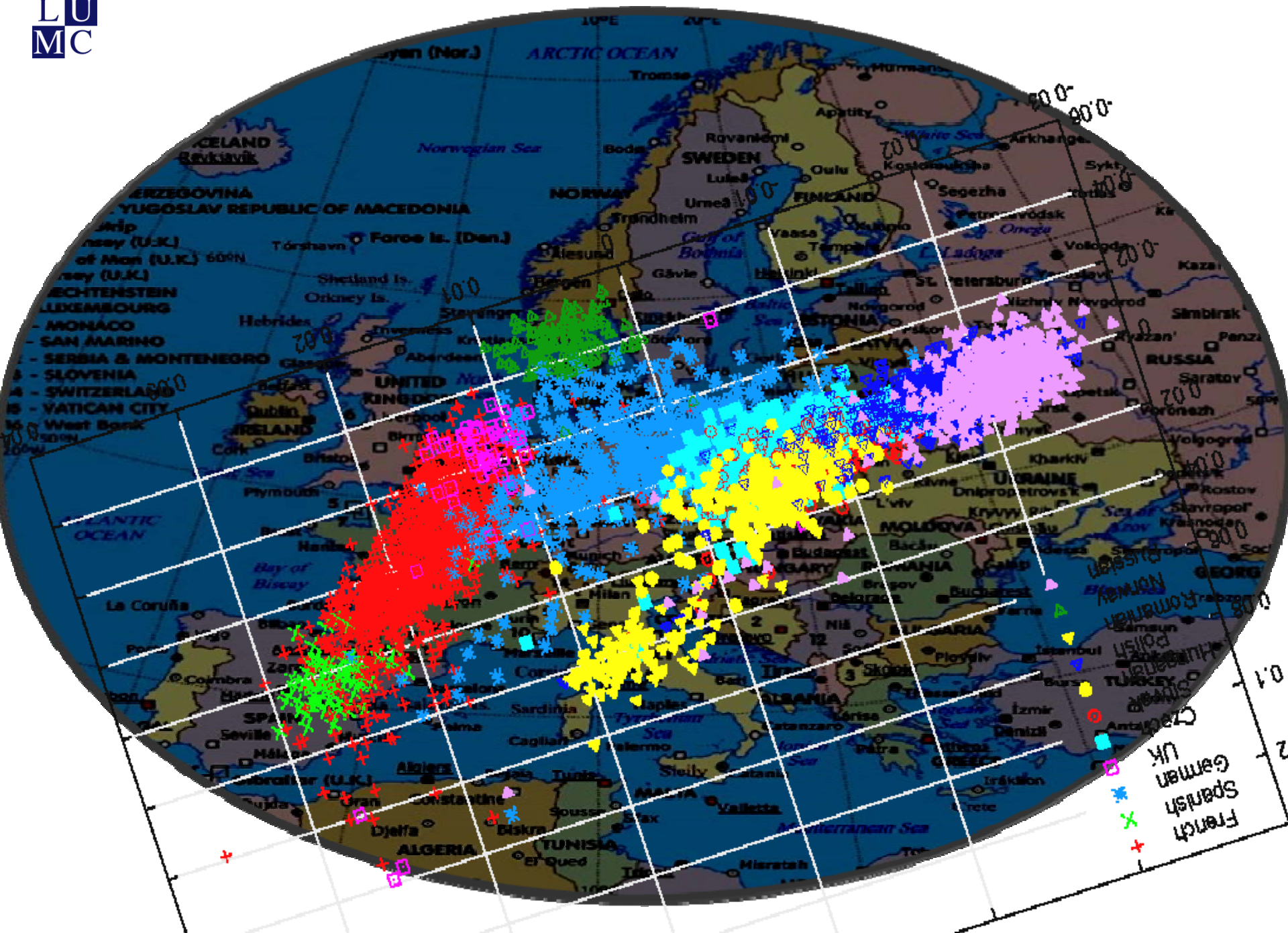
Goooooooooooooogle ▶

1 2 3 4 5 6 7 8 9 10 [Volgende](#)

# Principal component analysis of European populations

Simon Heath et al. (2008) *EJHG* 16, 1413 – 1429





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When humans first ventured out of Africa some 60,000 years ago, they left genetic footprints still visible today. By mapping the appearance and frequency of genetic markers in modern peoples, we create a picture of when and where ancient humans moved around the world. These great migrations eventually led the descendants of a small group of Africans to occupy even the farthest reaches of the Earth.



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# Excavation Site: Various Levels





# Removing Teeth for DNA Research

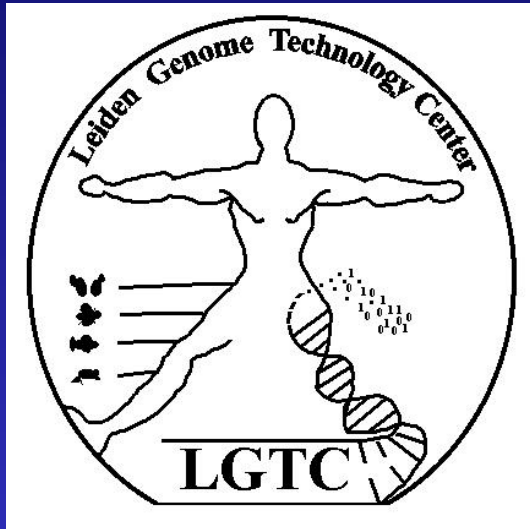


# Leiden Genomic DNA Samples

## Sequence Yields Obtained from Next-Gen Platform

Sample Number	Extract Volume ( $\mu$ l)	Concentration (ng/ $\mu$ l)	% Reads of Human Origin
1	~ 20	26.7	1%
2	~ 20	68.4	Less than 1%
3	~ 20	43.3	Less than 1%
4	~ 20	39.4	Less than 1%
5	~ 20	34.0	6.6%

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