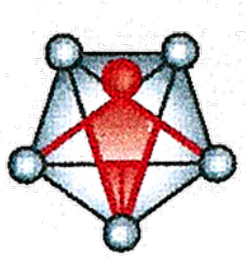
Technology reaching the clinic: an overview

A (forever) changing landscape

Prof dr Gertjan van Ommen

Centre for Medical Systems Biology

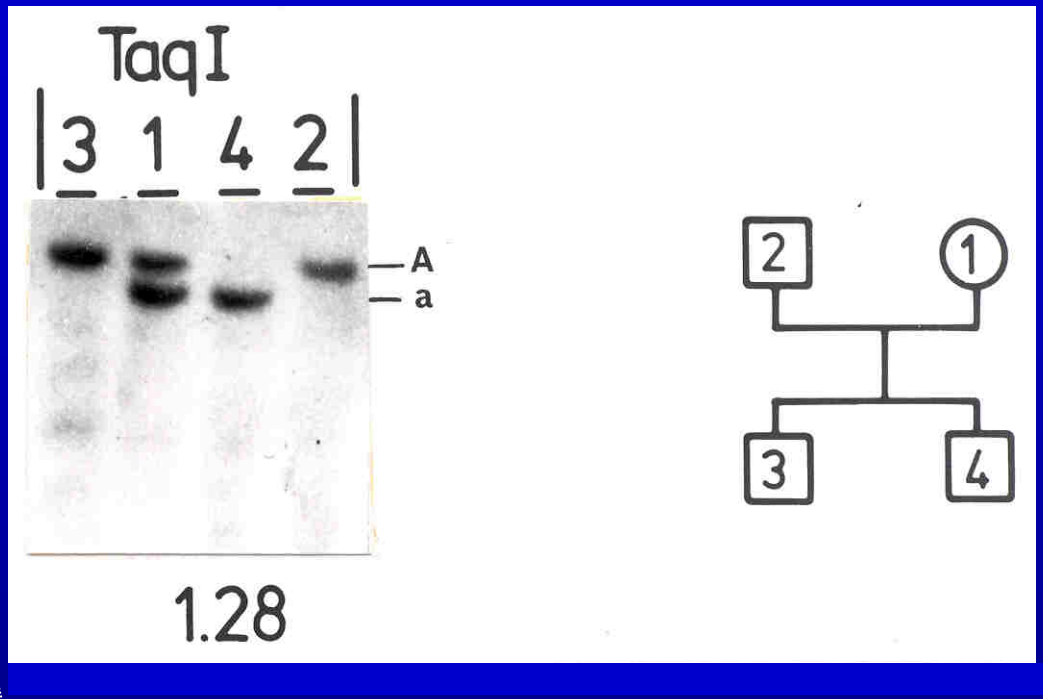


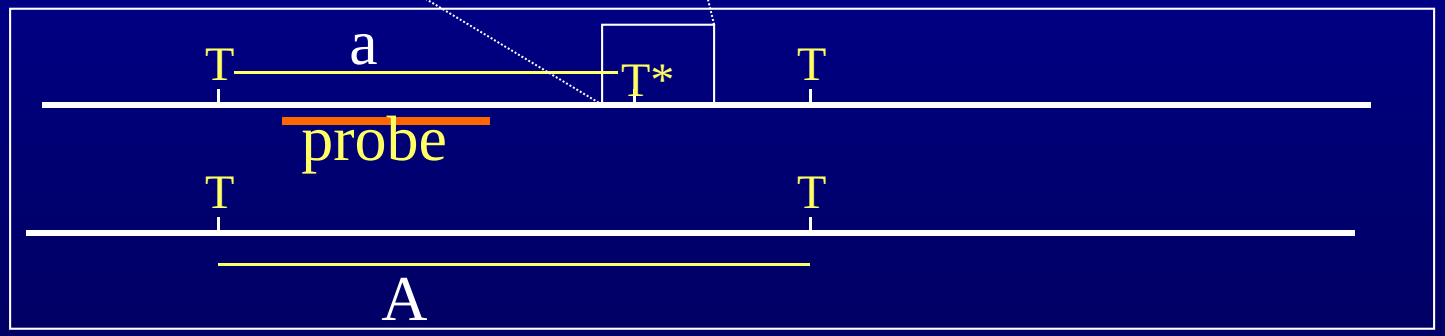
Leiden University Medical Centre

Restriction Fragment Length Polymorphism (RFLP) / 1981

Taq I -Knipplaats

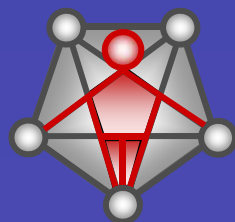
-AACT*CGATGG-
- TTGAGC T ACC-



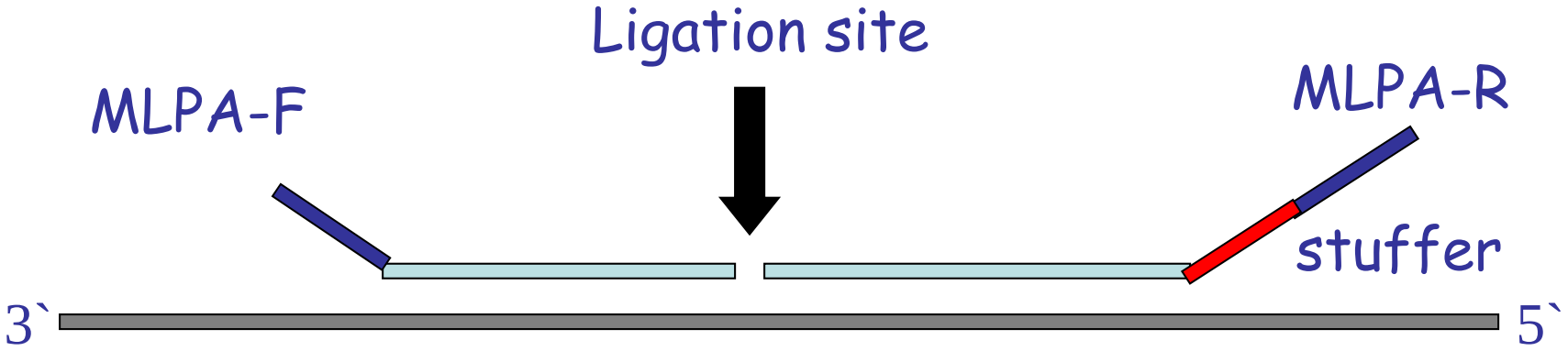


The ‘new kid on the block’: CNV Detecting deletions and duplications

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

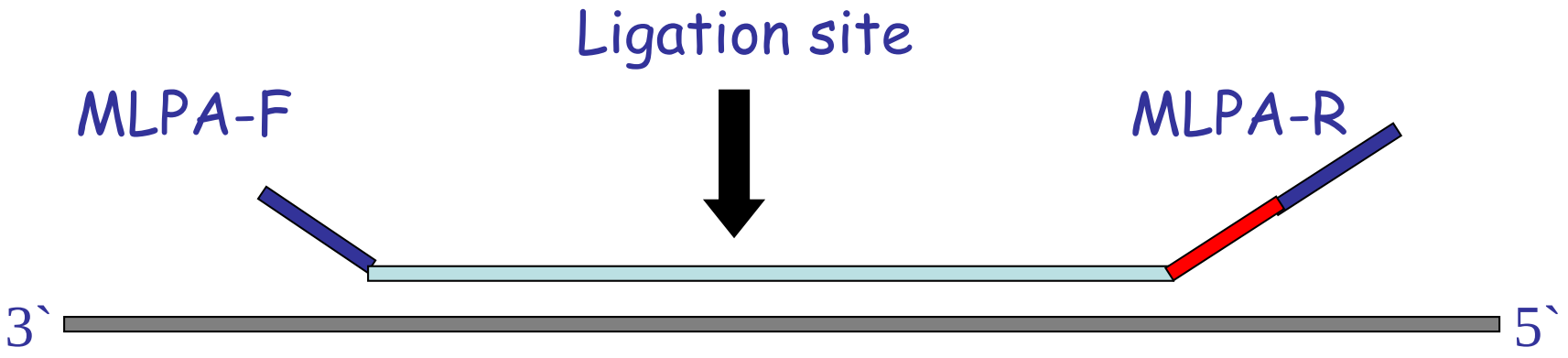


MLPA



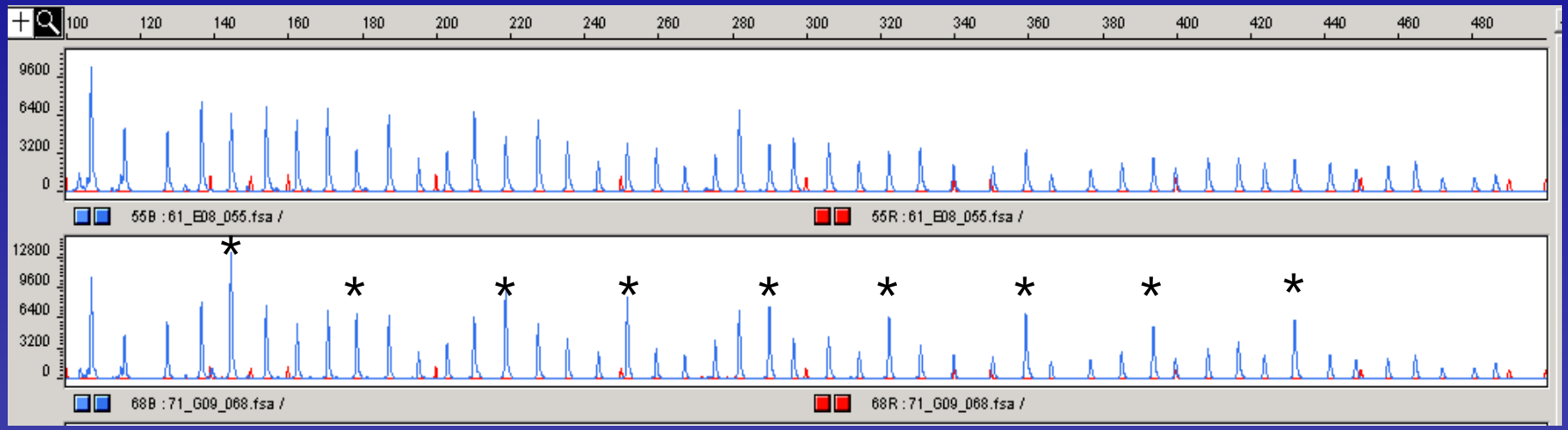
Probes hybridize adjacently on genomic DNA

MLPA



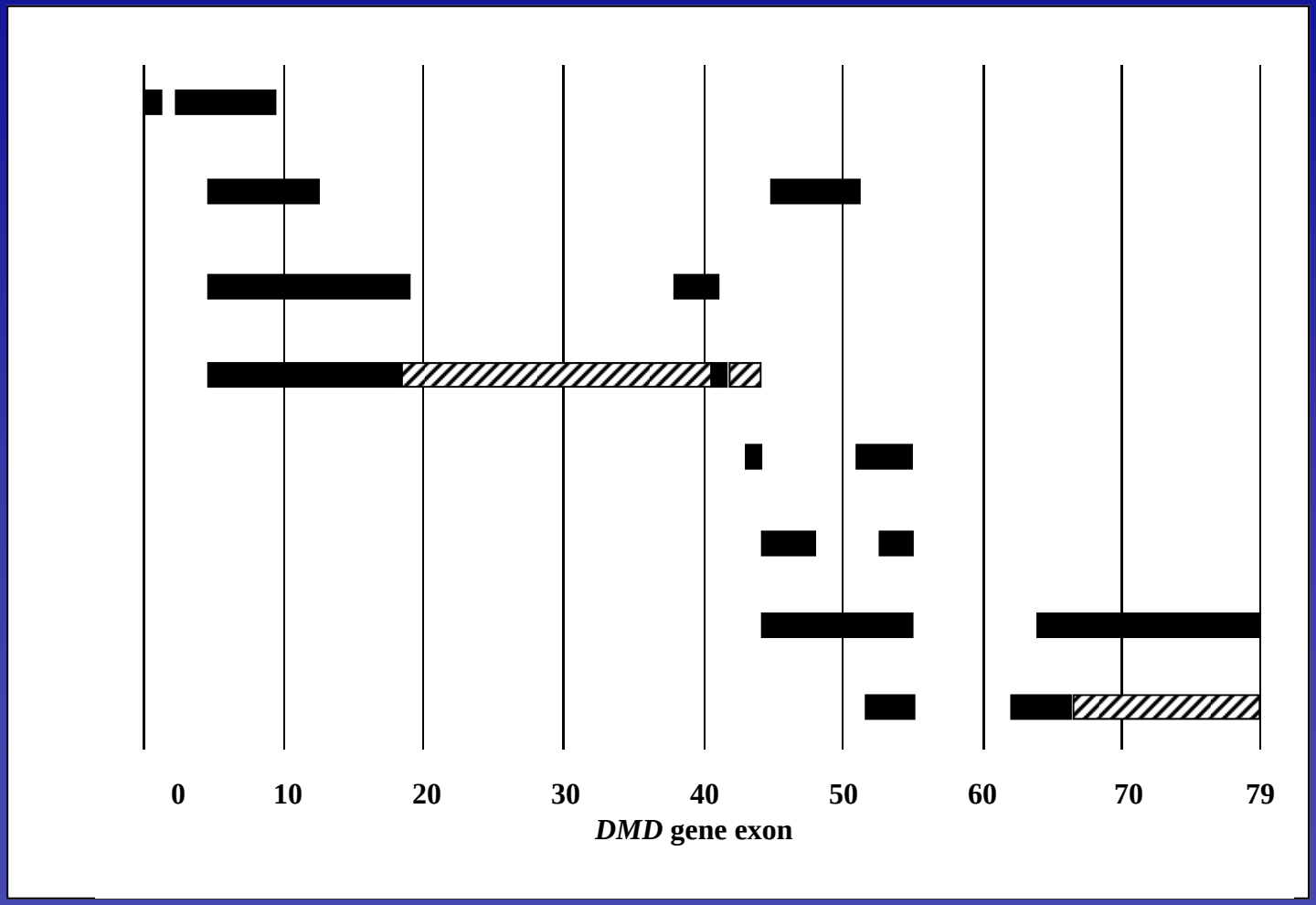
After ligation and PCR amplification a DNA fragment of a defined length is generated

MLPA peaks



Changes in peak height correspond with deletions / duplications

Non-contiguous duplications

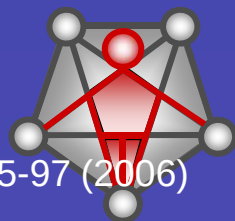


White et al., Cytogenet Gen Res 2006

Large-scale copy number variation

(Large-scale) **C**opy-**N**umber **V**ariation (CNV)

"Gain or loss of several kilobases to several thousands of kilobases of genomic DNA"

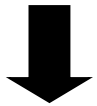


Feuk *et al.* Nat Genet Rev 7: 85-97 (2006)

Screening populations for CNV

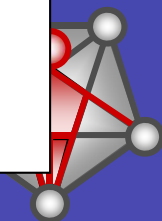
Percentage	No CNV		Moderate CNV		Extensive CNV	
	7q35		5q13.2		17q21.31	
	loss	gain	loss	gain	loss	gain
Pygmy *(n=37)*	0	0	5	5	19	11
Indonesian *(n=88)*	0	0	2	2	24	20
Dutch *(n=84)*	0	0	2	6	28	29
Turkish *(n=64)*	0	0	2	6	23	36
Finnish *(n=36)*	0	0	3	0	31	25
Total *(n=309)*	**0**	**0**	**3**	**4**	**24**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

1p32.3
2q31.1
2q35
7p21.1

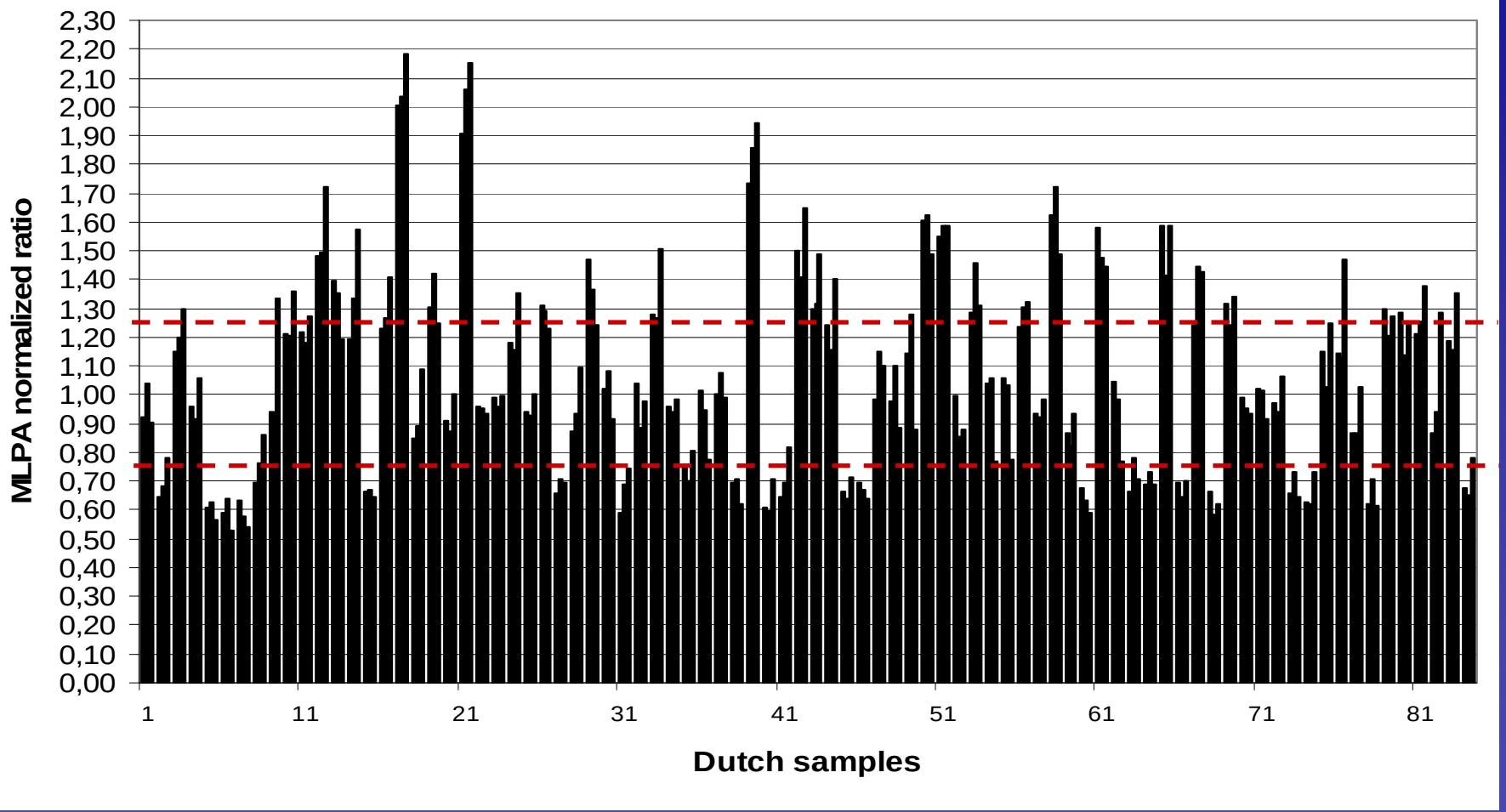


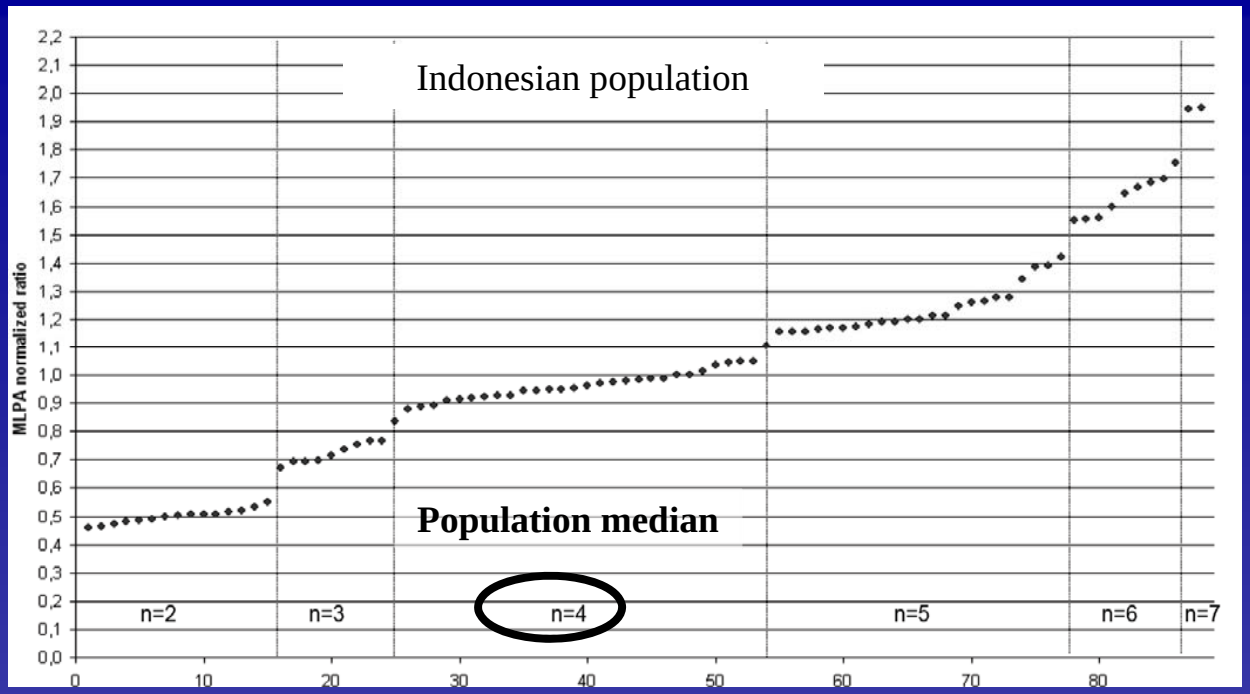
10q11.22
14q11
22q11.21

8p23.1
17q12



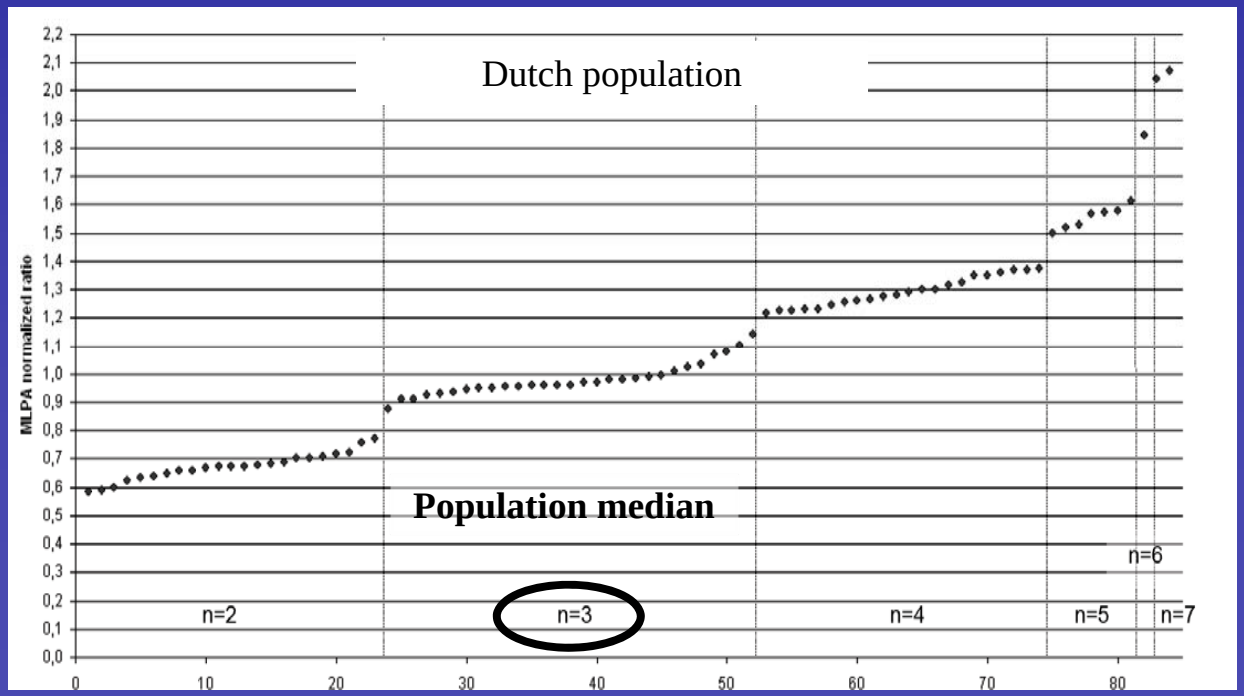
NSF


MLPA normalized ratio
2,30
2,20
2,10
2,00
1,90
1,80
1,70
1,60
1,50
1,40
1,30
1,20
1,10
1,00
0,90
0,80
0,70
0,60
0,50
0,40
0,30
0,20
0,10
0,00
1
11
21
31
41
51
61
71
81
Dutch samples



Lowest value: ~0.50

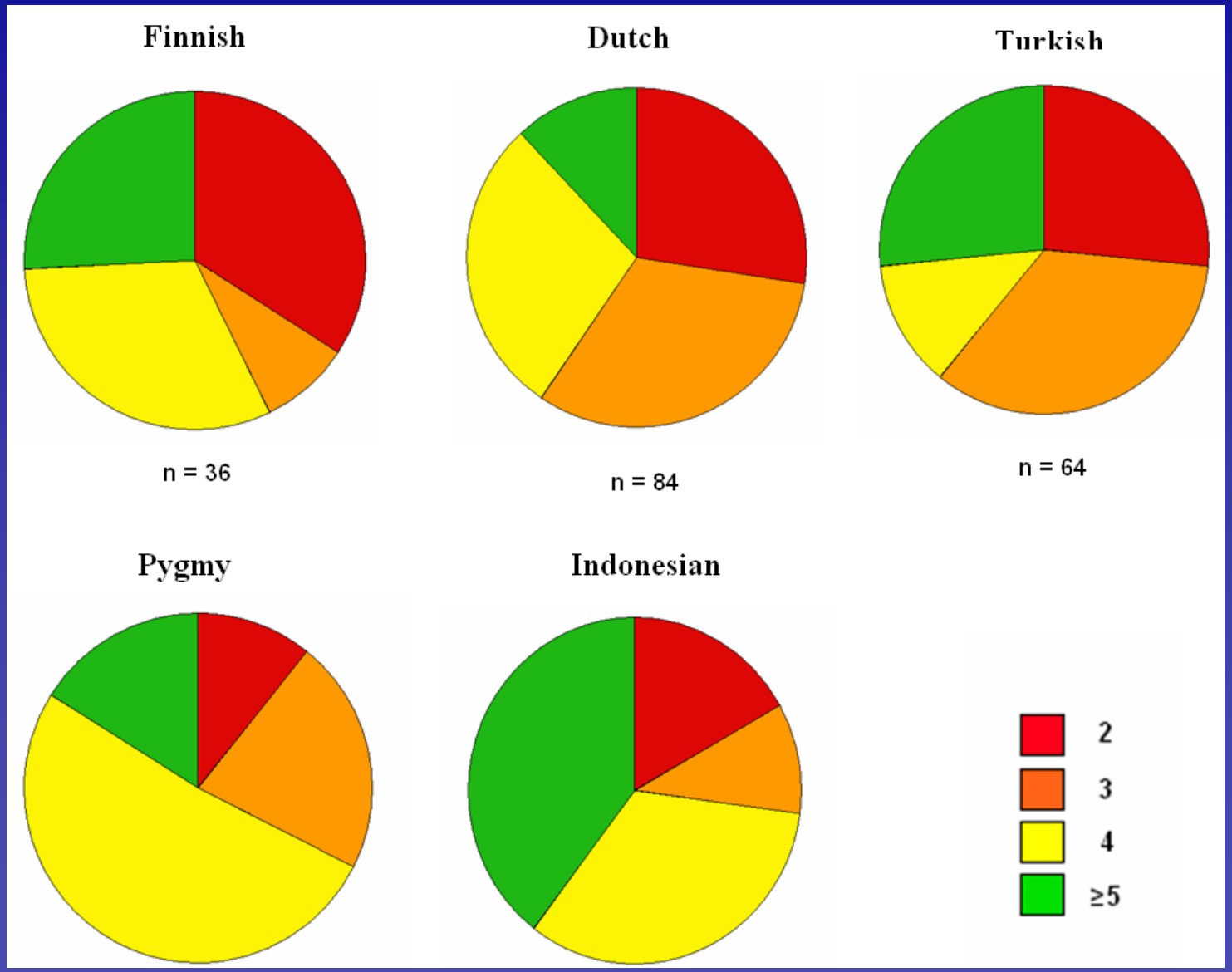
Steps: ~0.25



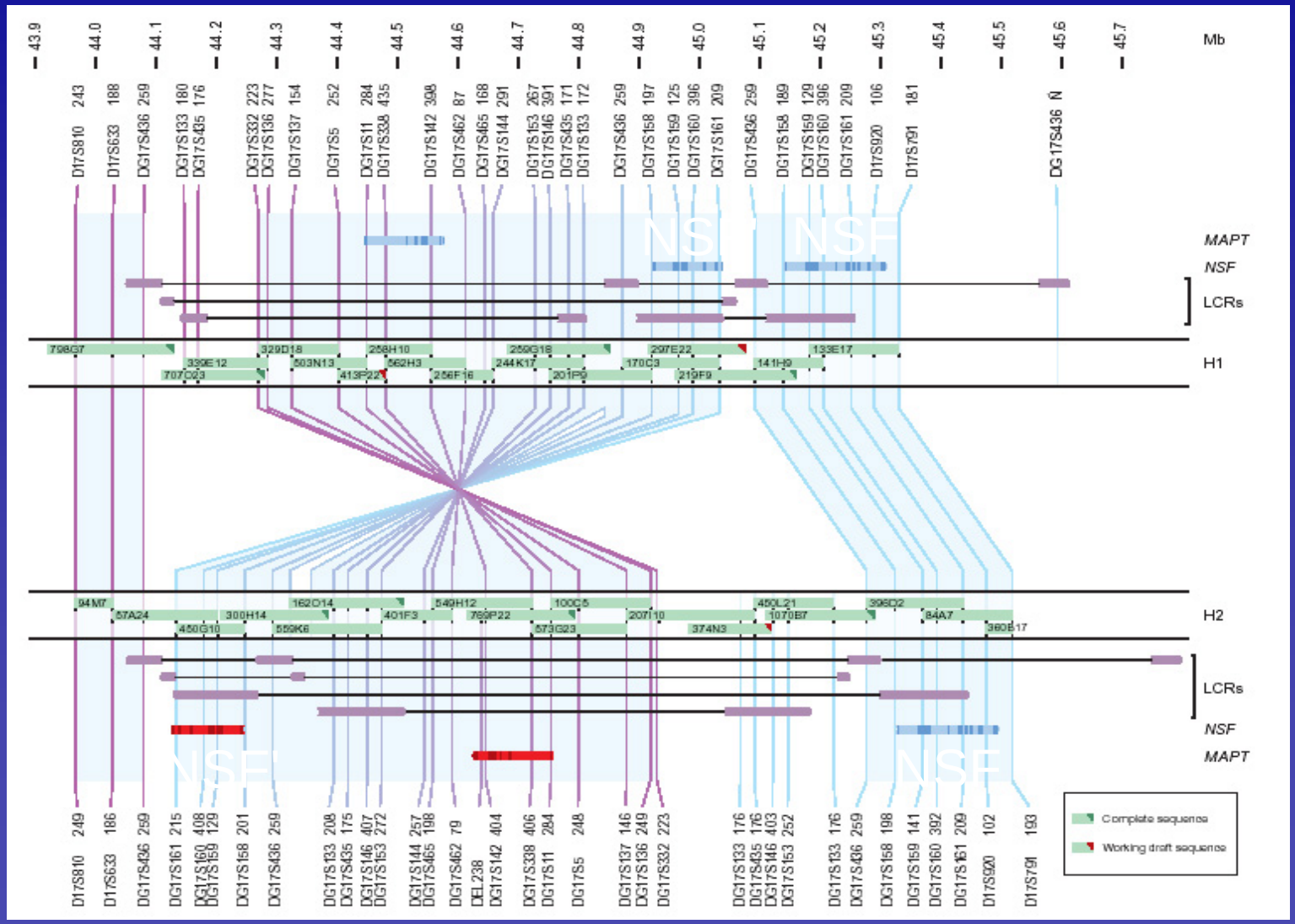
Lowest value: ~0.66

Steps: ~0.33

Population distributions



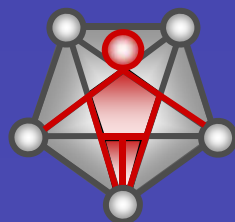
NSF genomic structure



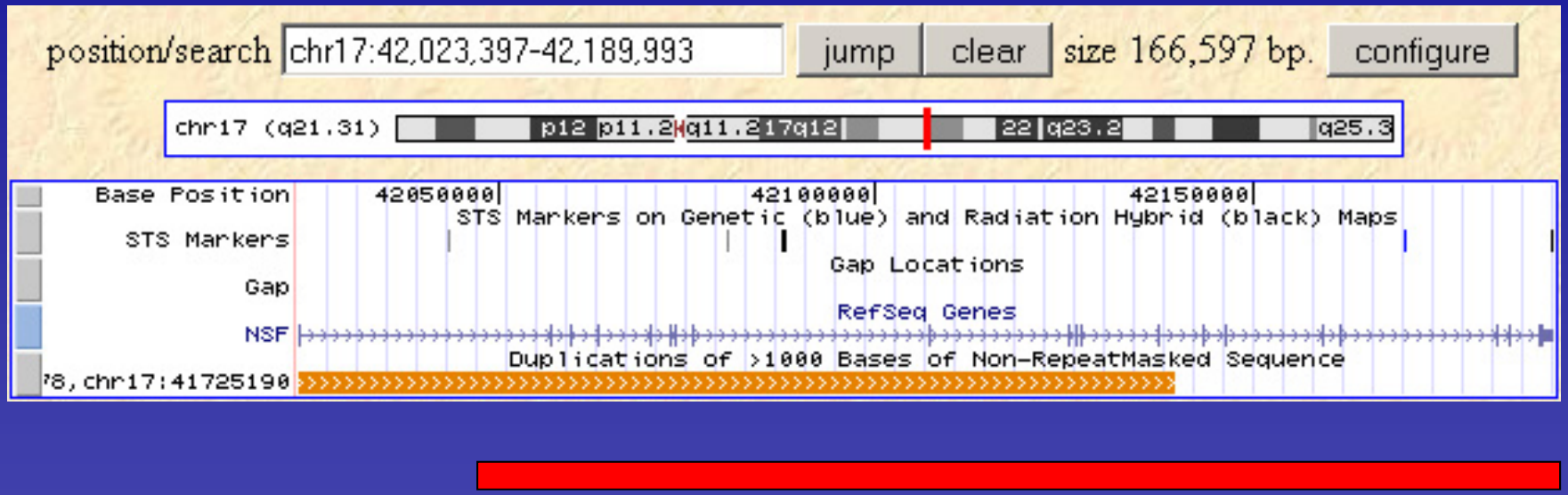
Steffanson et al., *Nature Genetics* 2005

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

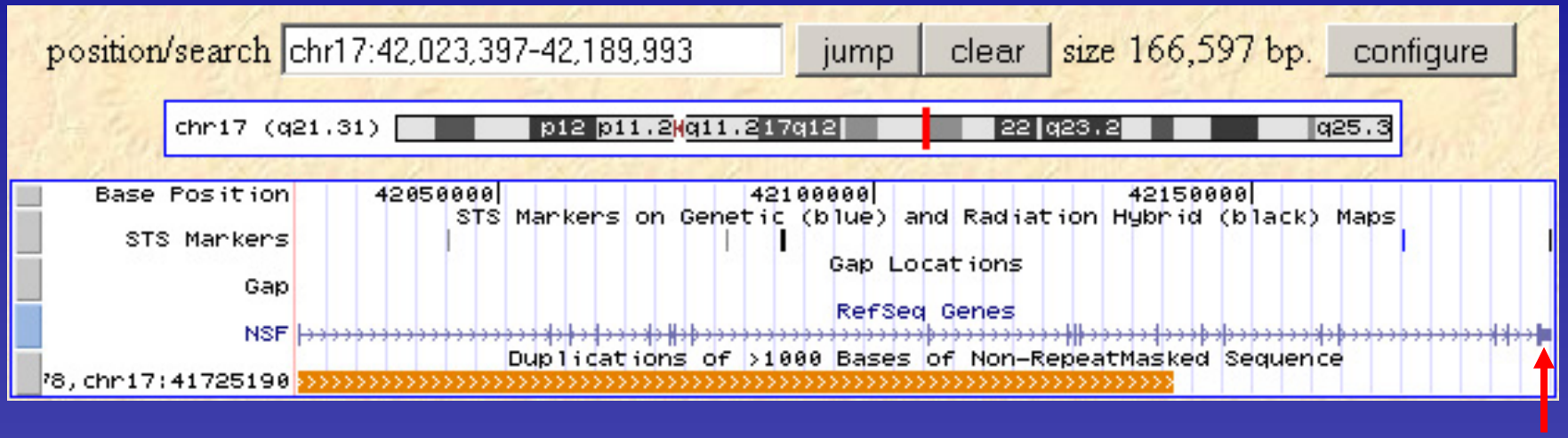


NSF gene



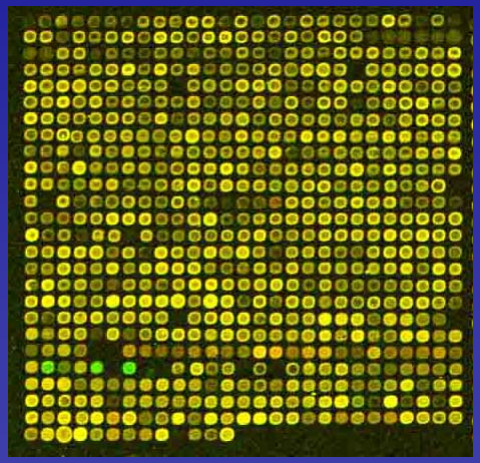
Study that detected difference in expression used cDNA clone, probably covering the majority of the gene (including duplicon)

NSF gene

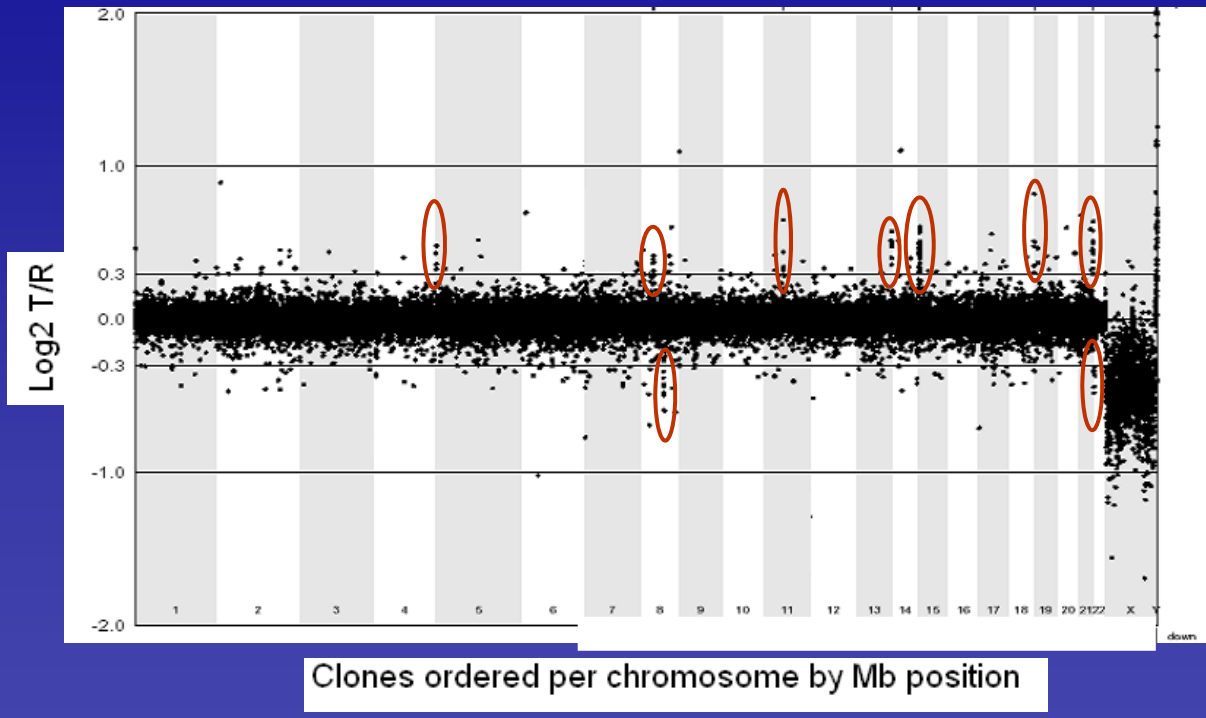


- Study that did not detect a difference in expression used qPCR, with product based in last exon (not in duplicon)

Increasing resolution….



Array CGH



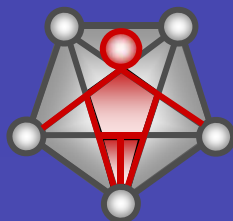
Disease-causing *or* Normal variation?

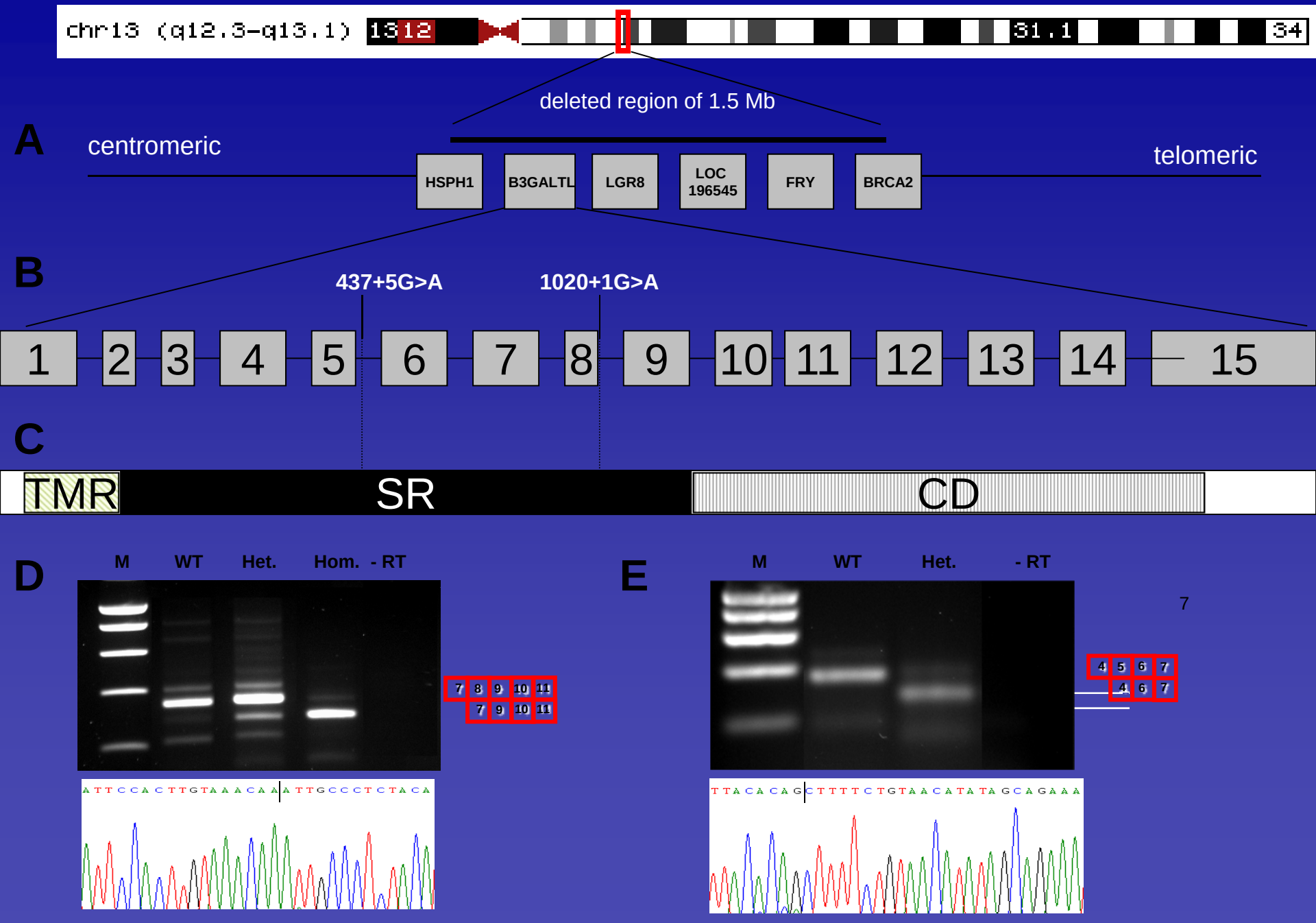
L. Vissers, Nijmegen

Peters' Plus Syndrome

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

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



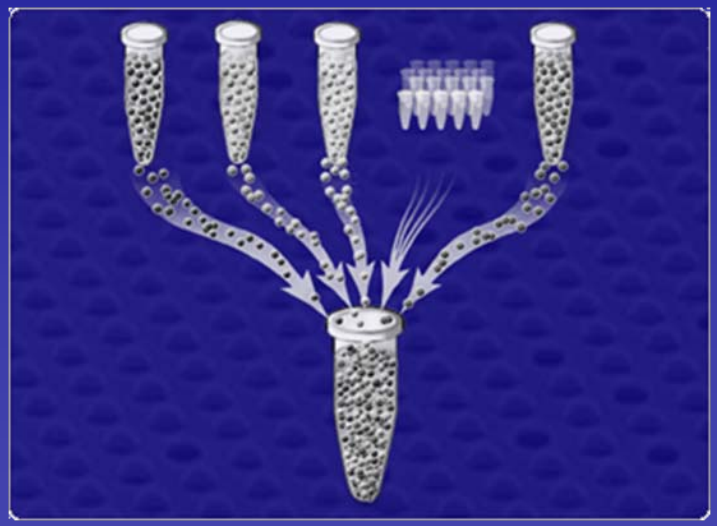


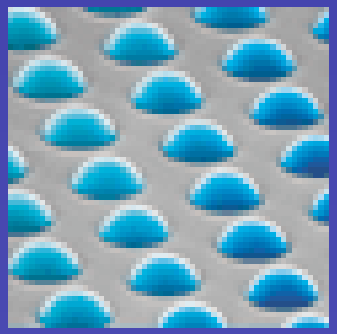
Lesnik Oberstein et al., Am J Hum Genet 2006

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

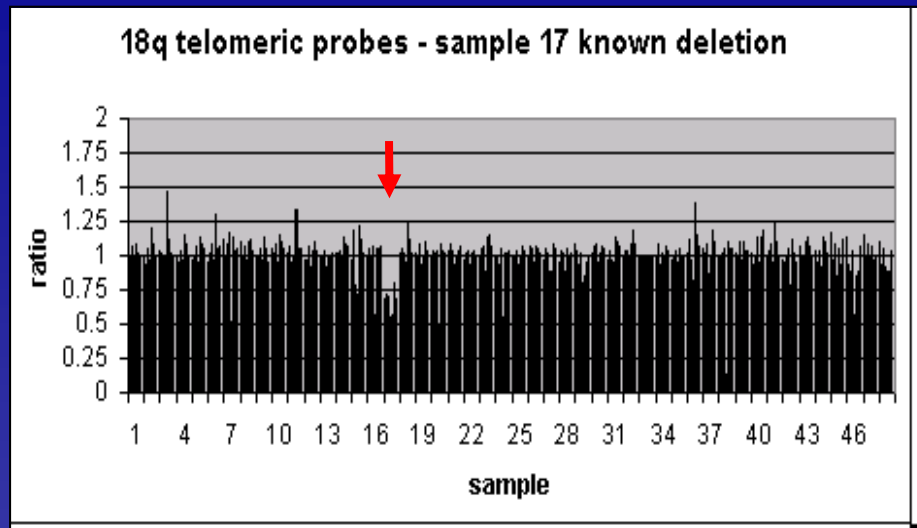
Bead-based SNP-typing

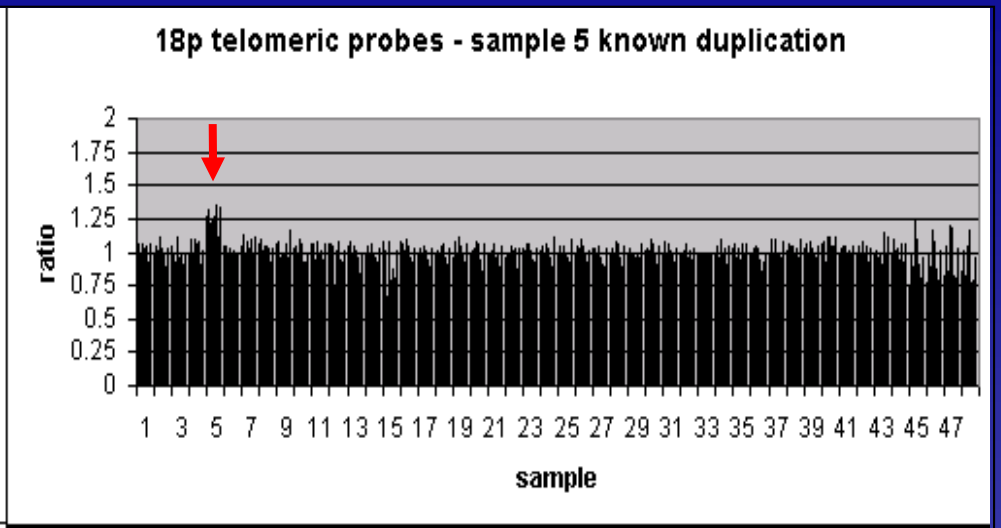
- colour-coded beads
  - one bead = one probe
    - oligonucleotide
- 96-well format
- 1500 probes / well
  - controls + 1350 SNP's
- read-out optic fiber
  - 50,000 beads
    - ~30 beads per SNP

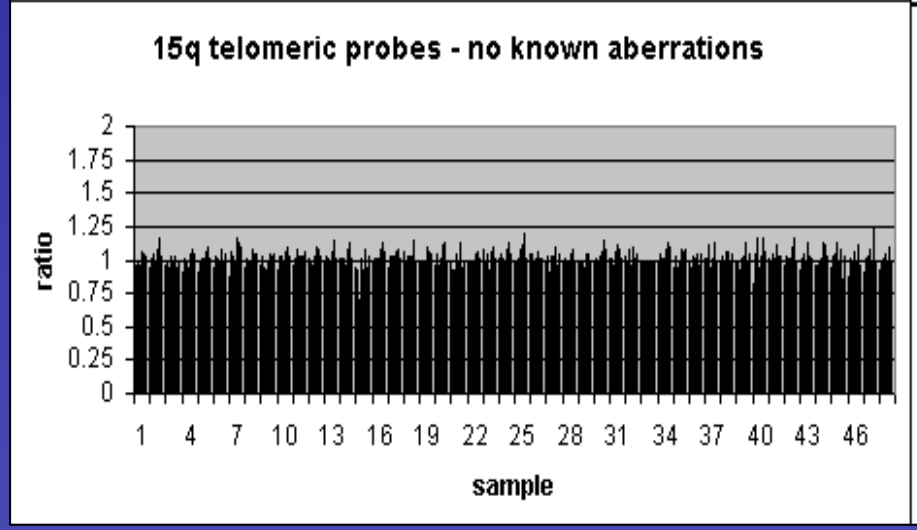


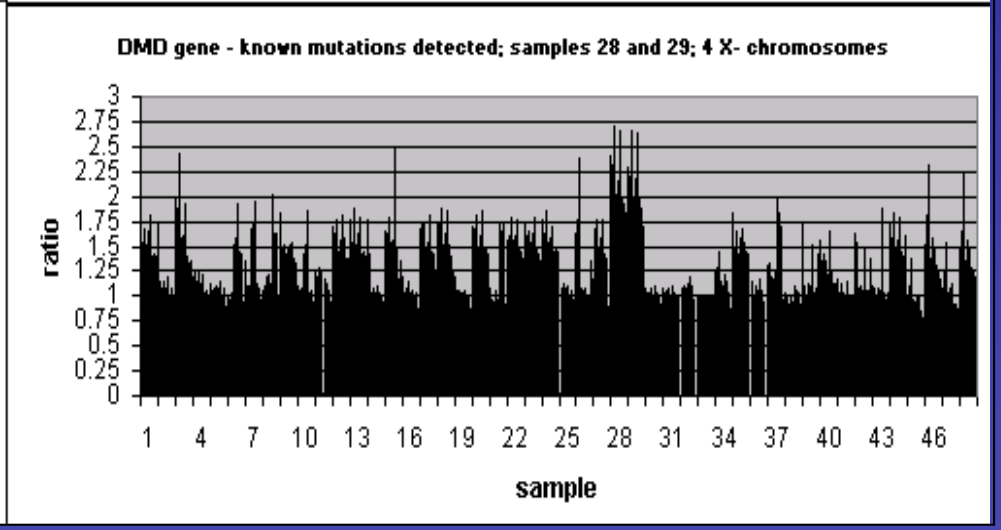


Deletions and Amplifications Detected in Clinical Samples





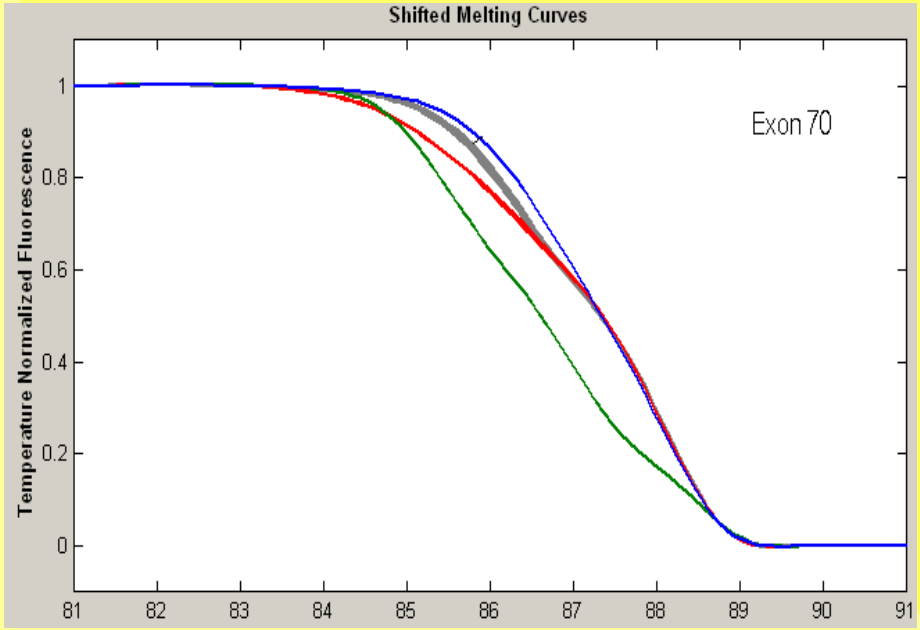


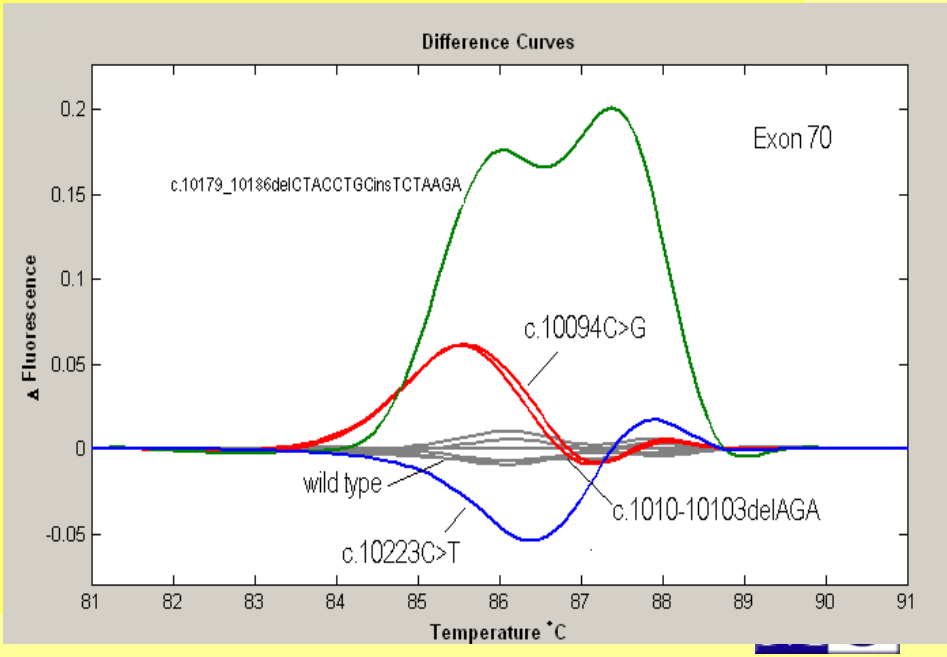


The sequencing revolution

Mutation detection techniques

Methods for scanning	Sensitivity*
➔ Direct sequencing	~100 % **
HR-Melting curve analysis	~> 99 %





HiRes-Melting Curve Analysis (mutation scan)

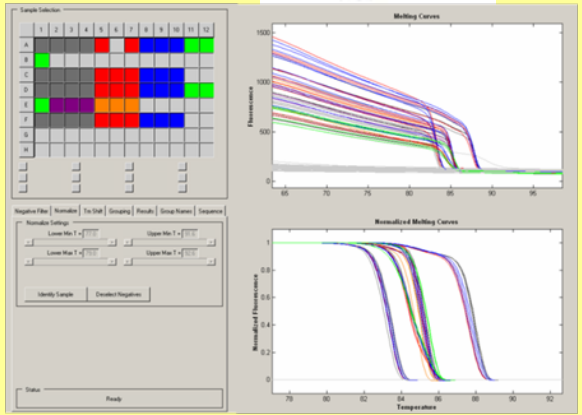
I. PCR amplification of fragments (96 well plate)

I. 1,5 - 2 hours

II. Melt PCR-fragments in Lightscanner

II. 8 minutes

III. Analysis (IT software)



III. 40 min.

Human Mutation, Volume 30, Issue 6 (p 899-909)
Abstract | References | Full Text: PDF (497k) | Supporting Information | Save Article to My Profile

METHODS

Human Mutation
OFFICIAL JOURNAL
HGVS
HUMAN GENOME VARIATION SOCIETY
www.hgvs.org

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,[1]* Chantal D.M. van Paridon,[1] Tom Janssens,[3] Petra Krenkova,[2] Alexandra Stambergova,[2] Milan Macek,[2] Gert Matthijs,[3] and Egbert Bakker[1]

[1]*Center for Human and Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands;* [2]*Institute of Biology and Medical Genetics, Charles University, Prague, Czech Republic;* [3]*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

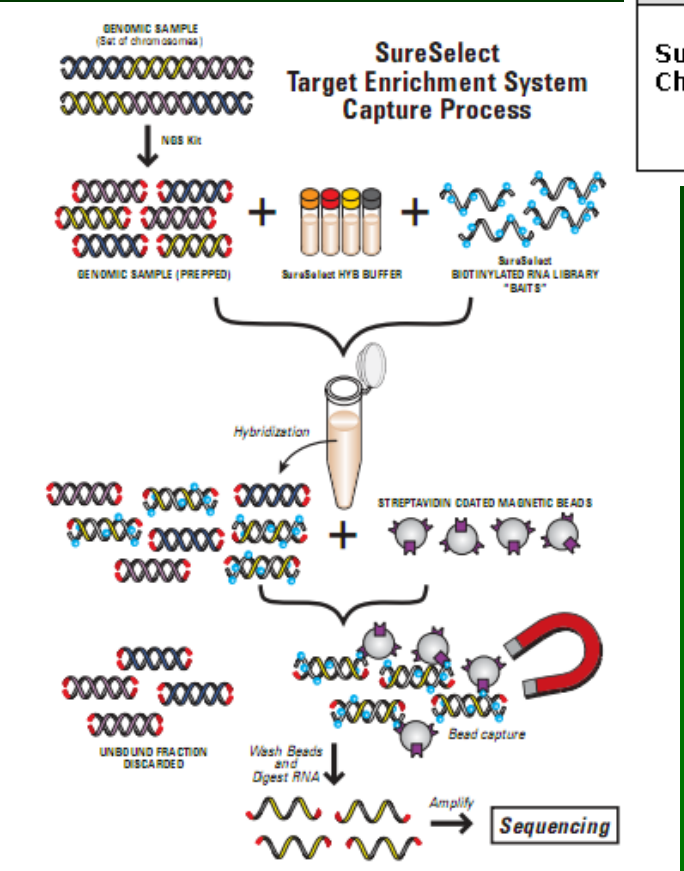
(complexity reduction)

- **chromosome sorting**
- **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	Contact Us



SureSelect

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

ServiceXS

X-exome$_2$

- **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*

Array capture$_{P}$

Mixed tagged samples

custom array

NimbleGen SYSTEMS, INC.

Pool of 2

ACAGTG - 11089031

TGACCA - 4770651

Pool of 5

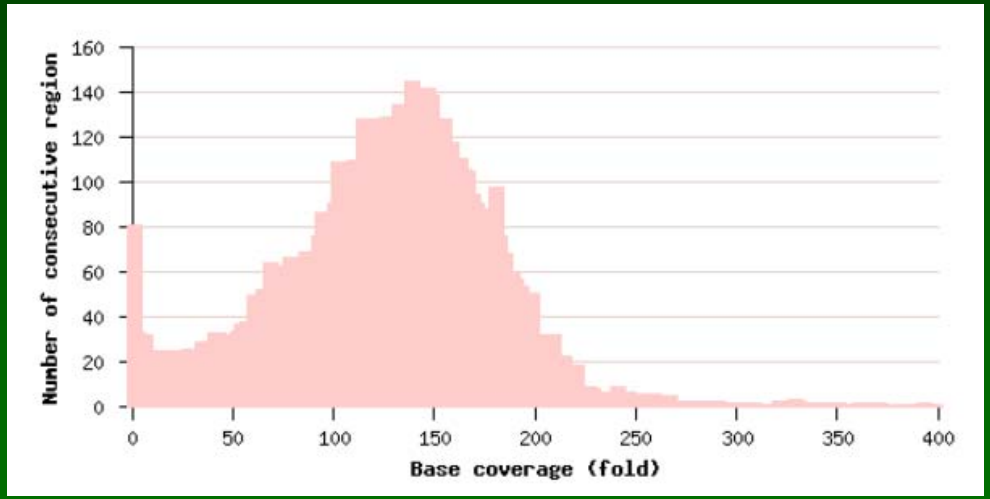
ACAGTG - 4717304

CTTGTA - 3782593

CAGATC - 3022664

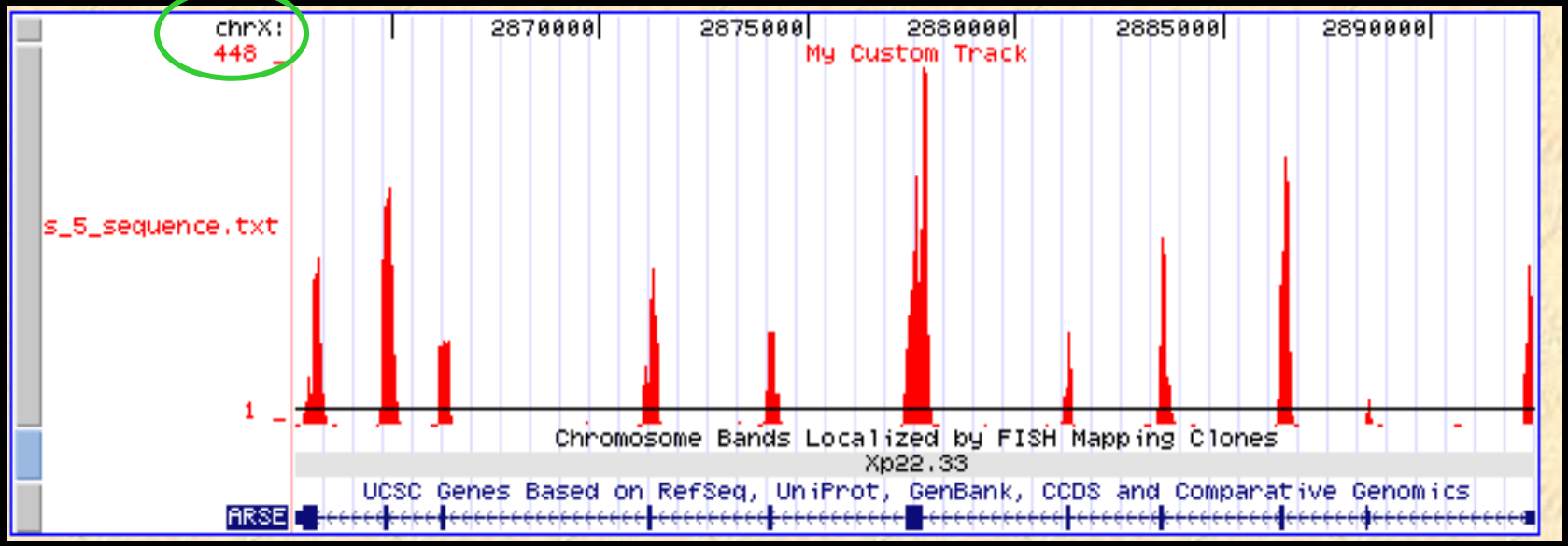
GGCTAC - 2381920

ATCACG - 2376586



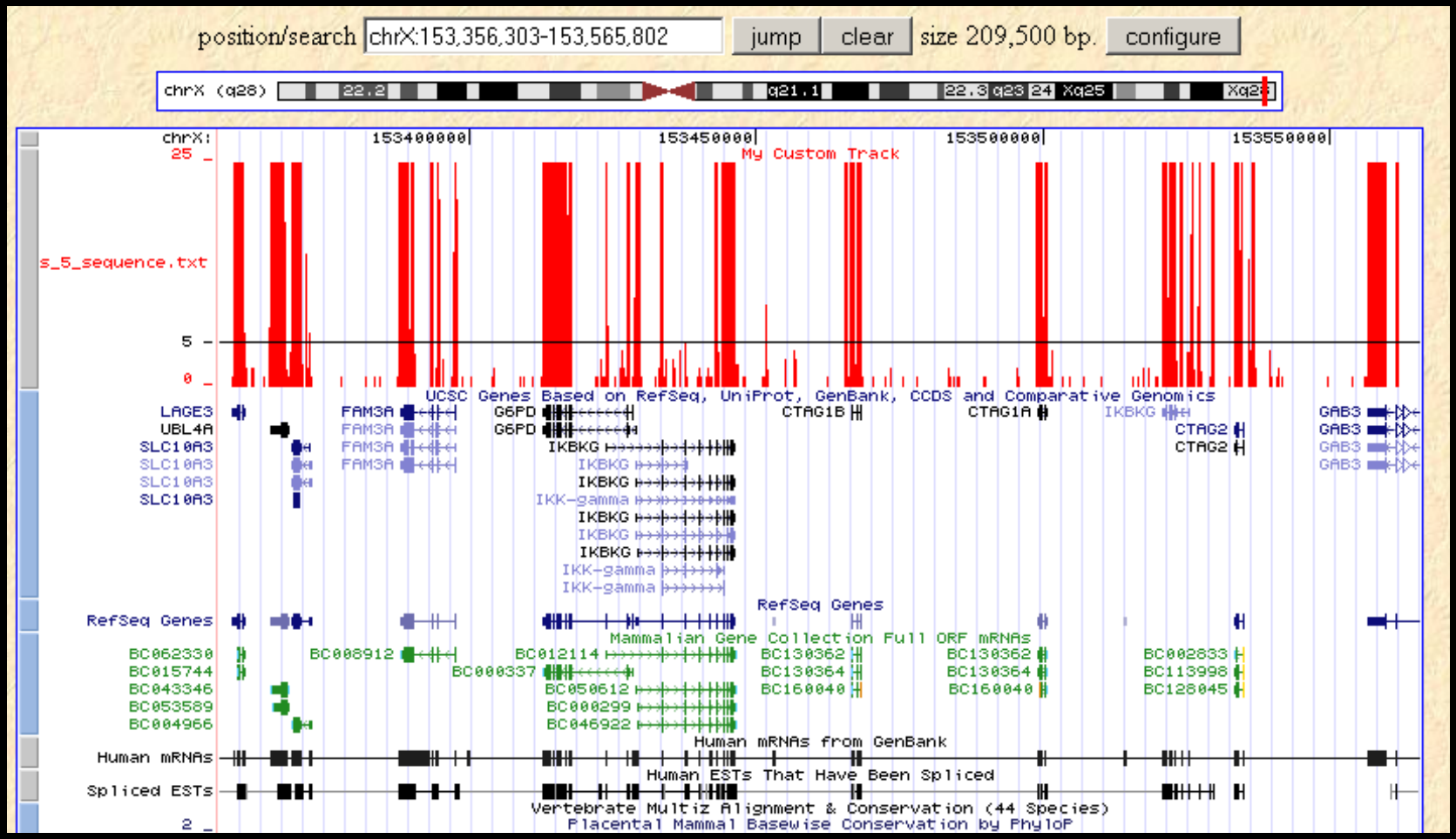
mean coverage 131-fold

X-exome select



ARSE

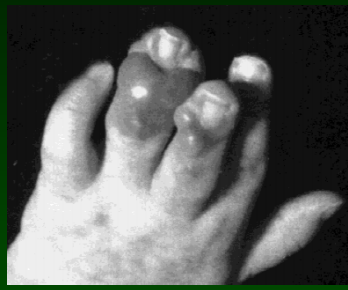
X-exome select



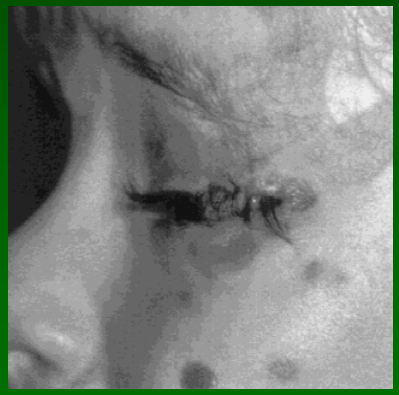
Xq28 region

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_i



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

New Syndrome?

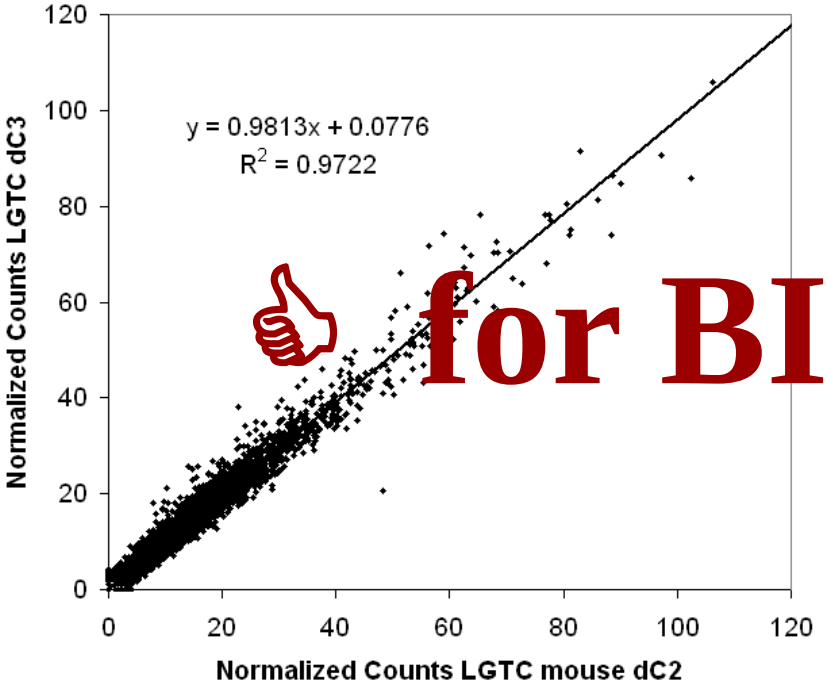
Recurrent Digital Fibroma, Focal Dermal Hypoplasia, and Limb Malformations

Yu Sun et al. AJHG 2010 in press.

CENTRE FOR
Medical Systems Biology

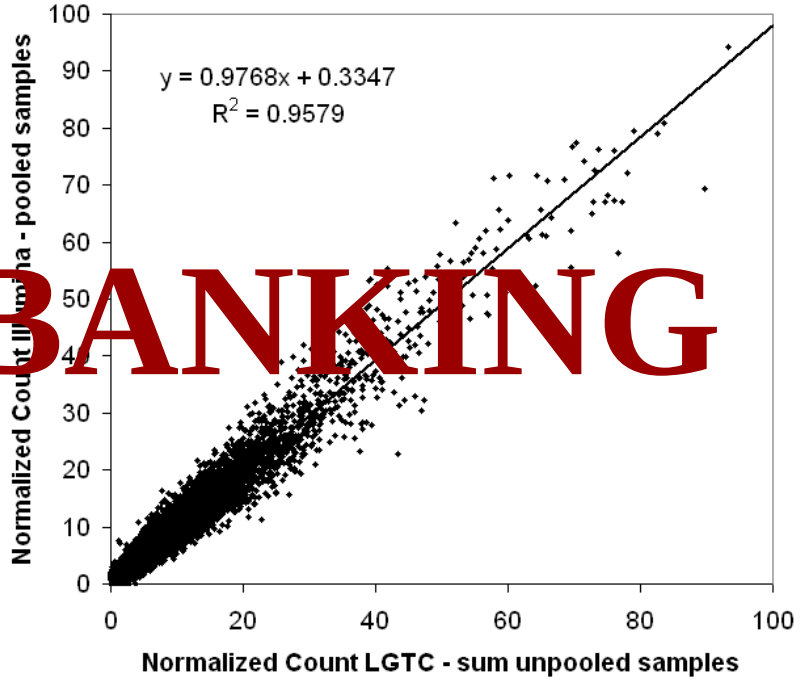
Lab2lab consistency

2 transgenic mice



(biological replicas)

2 different labs

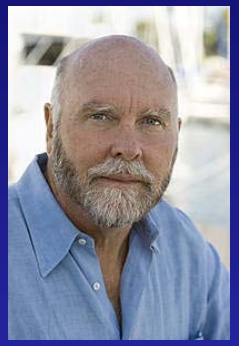


(Illumina <> Leiden)

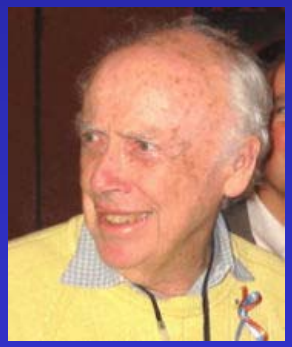
👍 **for BIOBANKING**

Human genomes

(Individual genomes)



Craig Venter



James Watson

Marjolein Kriek

<u>ANONYMOUS:</u>
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**

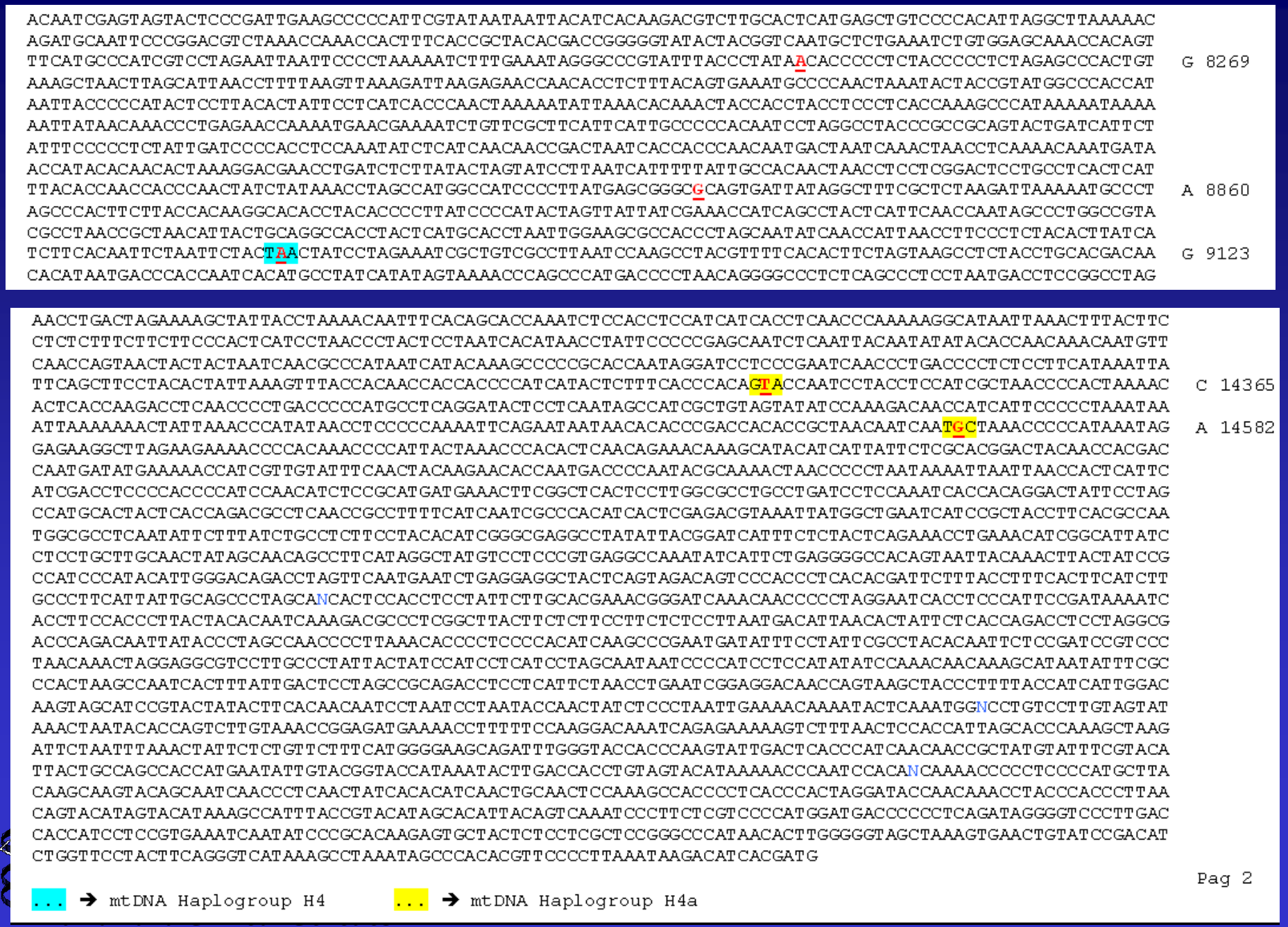
technically	***- no problem***
computationally	***- at our limits***
analytically	***- not (yet) possible***
	- as expected

- **>> to be applied in patients**
  - ***resolve cause genetic disease***

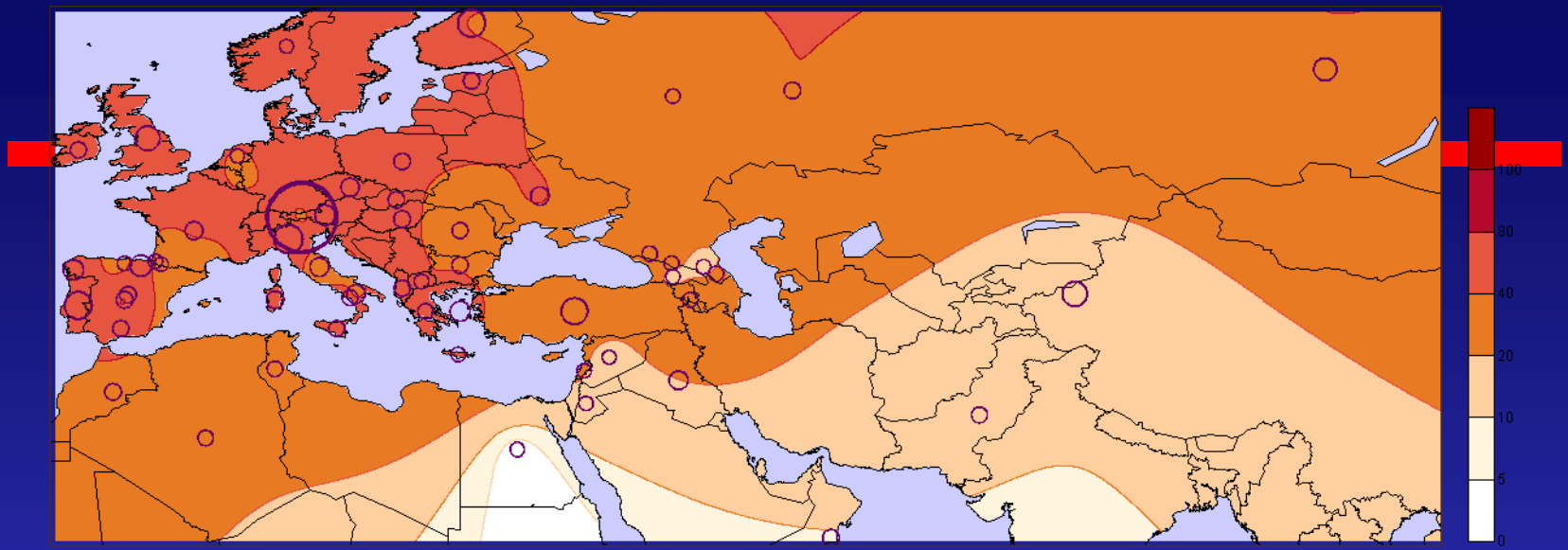
Analysis

draw DNA-based conclusions

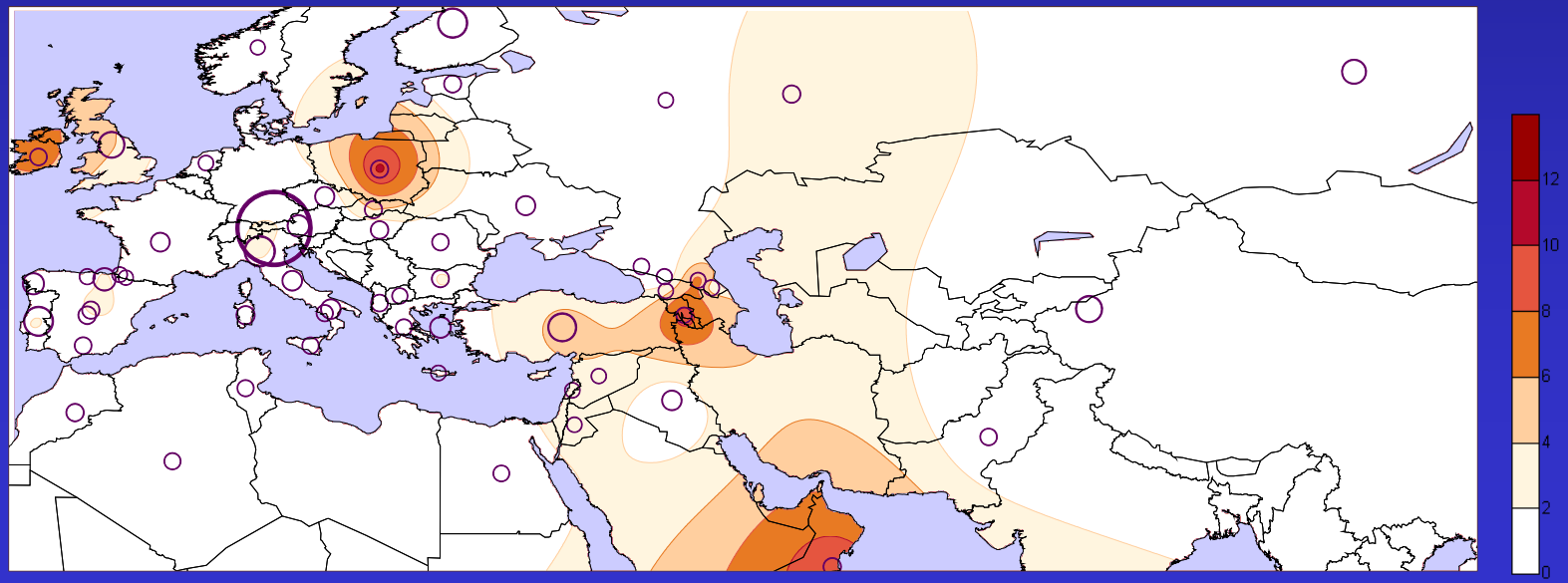
1. ***a female (no Y-chromosome sequences)***


G 8269
A 8860
G 9123
C 14365
A 14582
Pag 2
... → mtDNA Haplogroup H4
... → mtDNA Haplogroup H4a

Distribution of mtDNA Haplogroup H



Distribution of mtDNA Haplogroup H4



(source of data www.genebase.com)

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

Google marjolein kriek Zoeken Geavanceerd zoeken

Doorzoek: 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** second

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

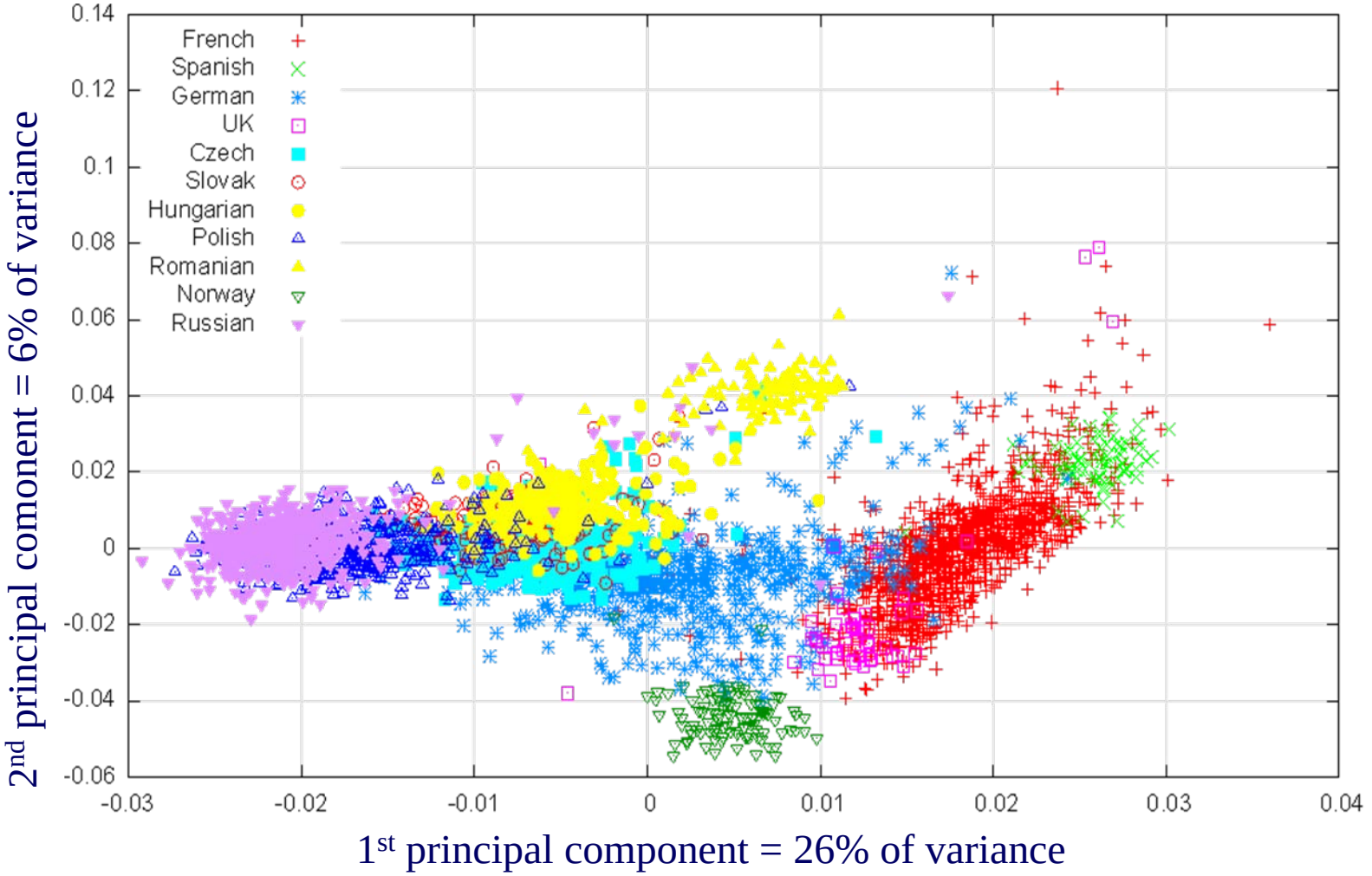
Marjolein Kriek - NextBio - [Vertaal deze pagina]
Search Term: **Marjolein Kriek** (author). Print page. Author. See also "M **Kriek**" · Author · Related Content · Studies · Clinical Trials · Literature · News **...**
www.nextbio.com/b/.../**Marjolein**%20**Kriek** - In cache - Vergelijkbaar

Goooooooooogle ▶
1 2 3 4 5 6 7 8 9 10 **Volgende**

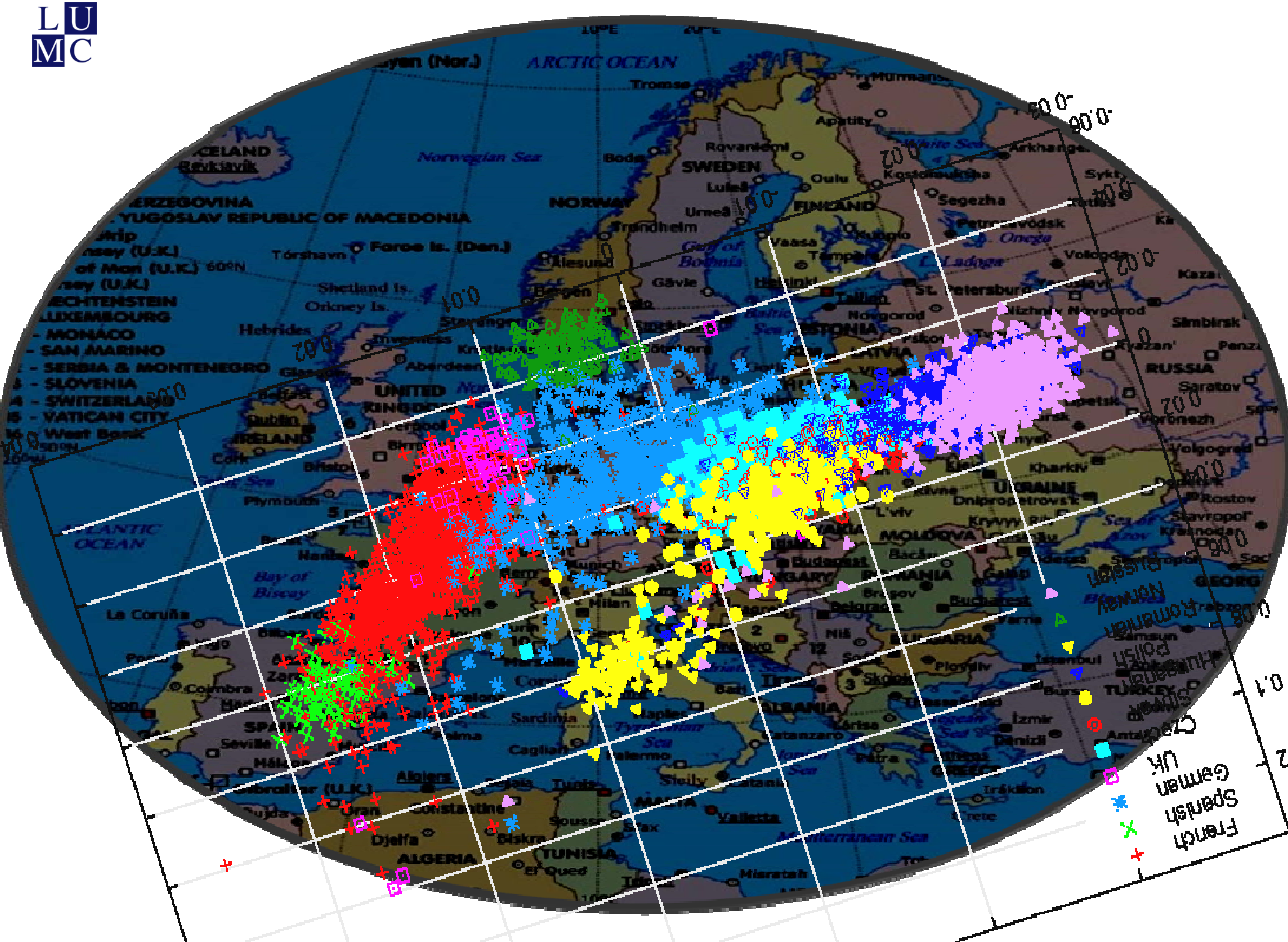
LUMC

Principal component analysis of European populations

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



LUMC


French
Spanish
German
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Czech
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Romanian
Norway
Russian
ARCTIC OCEAN
Norwegian Sea
SWEDEN
NORWAY
FINLAND
UNITED KINGDOM
IRELAND
ESTONIA
LATVIA
RUSSIA
UKRAINE
MOLDOVA
ROMANIA
HUNGARY
BULGARIA
ALBANIA
TURKEY
SPAIN
ALGERIA
TUNISIA
MALTA
ATLANTIC OCEAN
Bay of Biscay
Mediterranean Sea
Tyrrhenian Sea

SELECT AN ERA TO EXPLORE

ATLAS OF THE HUMAN JOURNEY

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.

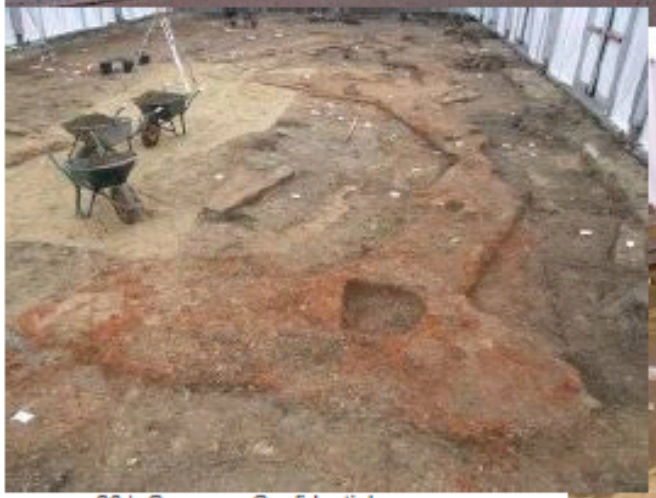
GO TO: GENETIC MARKERS + JOURNEY HIGHLIGHTS +

THE WAITT FAMILY FOUNDATION.
Global field science supported by the Waitt Family Foundation

NATIONAL GEOGRAPHIC™ IBM
A research partnership of National Geographic and IBM

Excavation Site: Various Levels

LEIDS UNIVERSITAIR MEDISCH CENTRUM



Removing Teeth for DNA Research

LUMC
LEIDS UNIVERSITAIR MEDISCH CENTRUM

Helicos
BioSciences Corporation

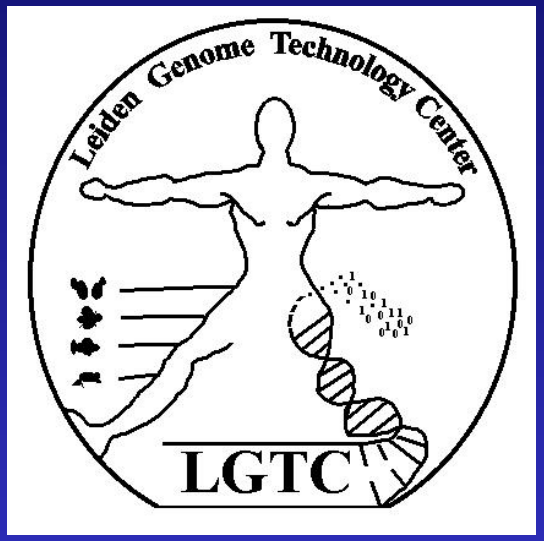
Leiden Genomic DNA Samples

Sequence Yields Obtained from Next-Gen Platform

Sample Number	Extract Volume (µl)	Concentration (ng/µl)	% 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%

Helicos

Acknowledgements

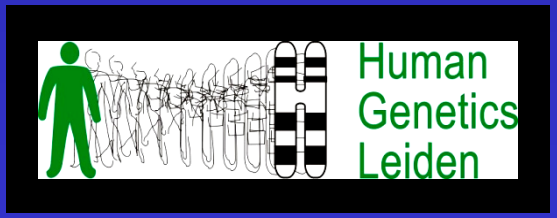


Yavuz Ariyurek

Astrid Out
Rowida Al-Momani
Yu Sun

Jaap vd Heijden
Michiel van Galen
Matthew Hestand

Johan den Dunnen
Bert Bakker - LDGA




87 THST