Next generation sequencing

in an academic medical hospital

(Leiden University Medical Center)

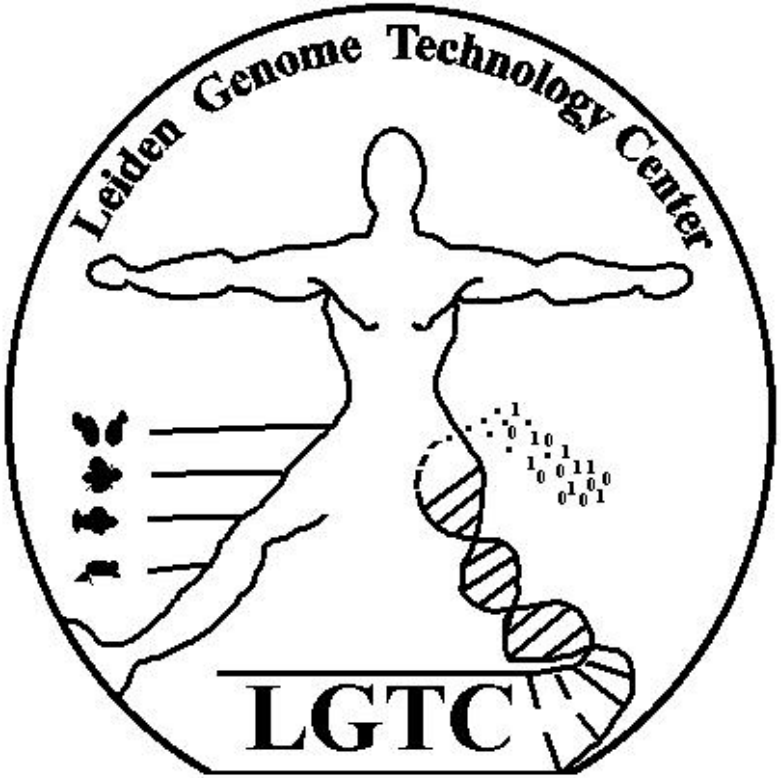
Johan den Dunnen

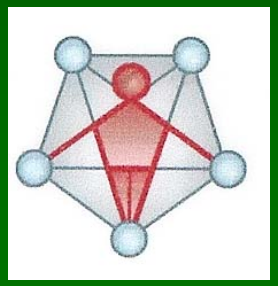

Round of 16
Quarter-finals
Semi-finals
Final
Semi-finals
Quarter-finals
Round of 16
URU
2:1 (1:0)
KOR
USA
1:2 a.e.t.
(1:1, 0:1)
GHA
NED
2:1 (1:0)
SVK
BRA
3:0 (2:0)
CHI
URU
1:1 a.e.t.
(1:1, 0:1)
4:2 PSO
GHA
NED
2:1 (0:1)
BRA
URU
July 6
20.30
NED
W61
[64] 11/07
W62
L61
[63] 10/07
L62
GER
July 7
20.30
ESP
ARG
0:4 (0:1)
GER
PAR
0:1 (0:0)
ESP
ARG
3:1 (2:0)
MEX
GER
4:1 (2:1)
ENG
PAR
0:0 a.e.t.
5:3 PSO
JPN
ESP
1:0 (0:0)
POR

Leiden Genome

Technology Center

http:// www.LGTC.nl

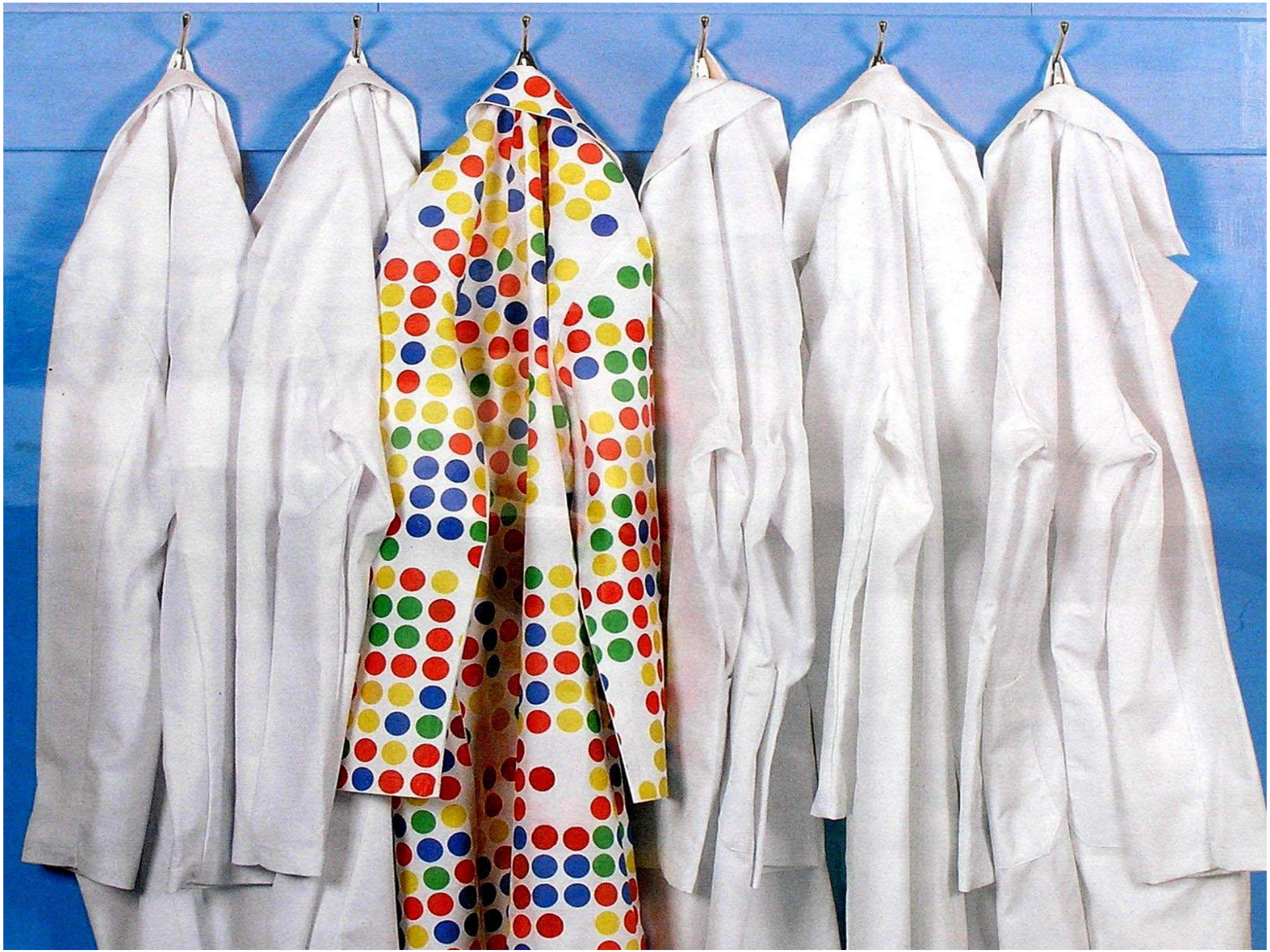




Human and Clinical Genetics

Current projects

- **RNA**
  gene expression profiling, miRNA
- **Chromatine-IP** *(ChIP-seq)*
- **SNP-discovery**
  targeted de novo sequencing
- **genome re-sequencing**
  virus, bacteria, insects, ..., human
- **targeted sequencing**
  candidate gene / gene region
  hybridisation capture
- **diagnostics**
  non-invasive trisomy screening
- **metagenomics**
- **forensics**
  degraded / mixed samples



Subjects today

- **targeted sequencing**

  PCR-based

  AccessArray (Fluidigm)

  hybridisation capture

  FlexSelect

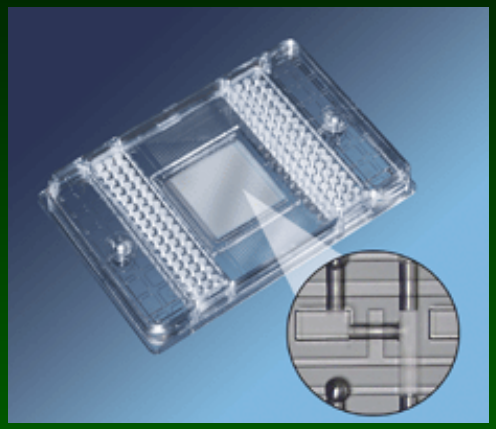
  X-linked disease

- **Helicos**

  single molecule sequencing

Fluidigm system

Real-time PCR



nanoliter PCR

plates

upto 96x96 PCRs

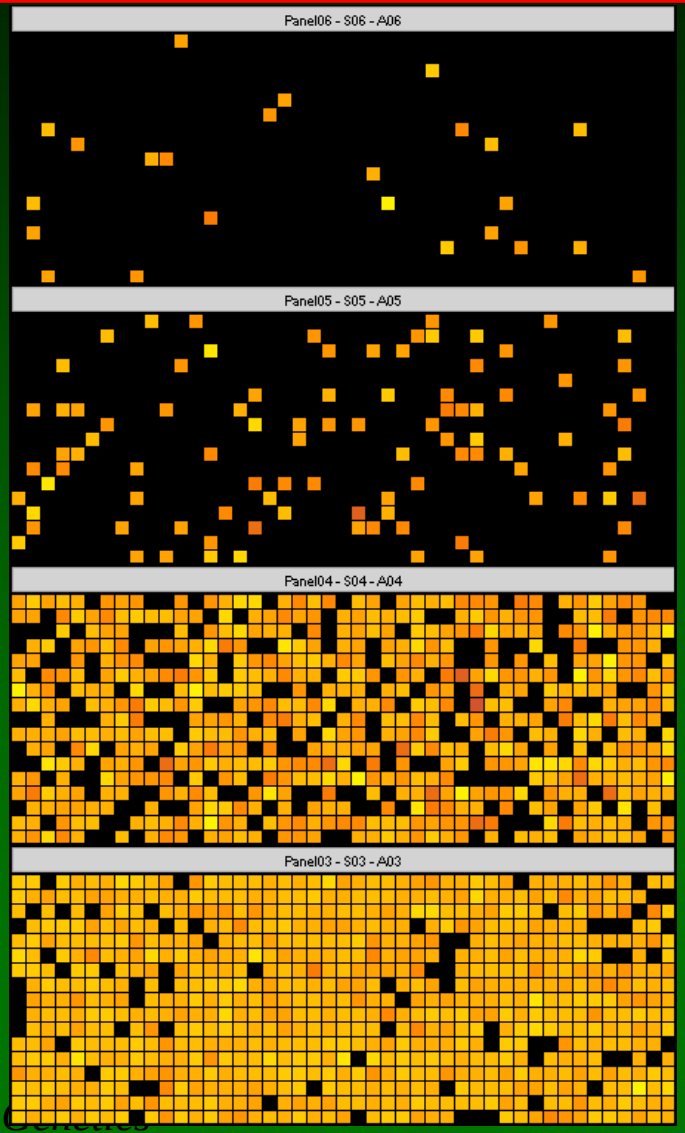
Liquid flow control

Nano-liter PCR

- **normal PCR**
  - *real time measurement, melt curve analysis*
    - *TaqMan SNP typing 9,216 in parallel*
- **measure sample concentration**
  - *digital-PCR (12 samples - 765 wells)*
    - *Illumina and 454*
- **PCR target enrichment**
  - *AccessArray*
  - *48 samples x 48 assays*
    - *real time PCR*
  - *retrieve products in sample well*

> improved equimolar yield…
reduced sequencing cost

Fluidigm digital-PCR



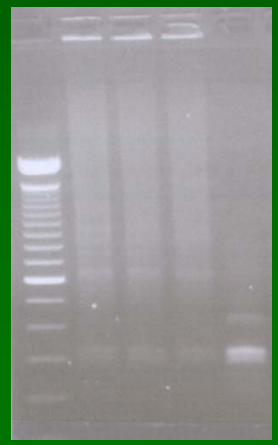
Determine exact DNA concentration before SEQ

- optimal loading

- max. SEQ yield

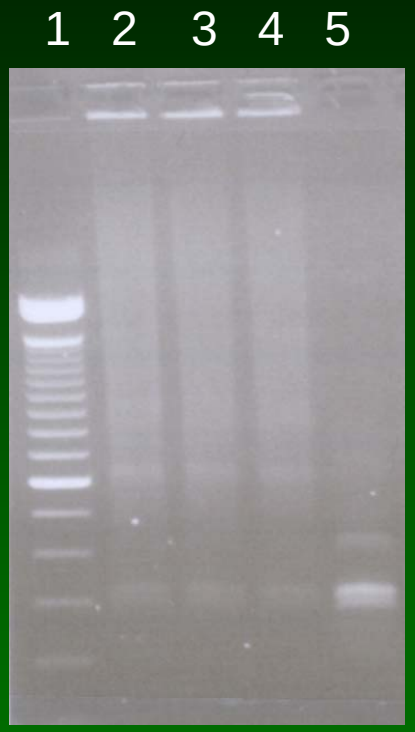
Fluidigm AccessArray

- **target BRCA1/2**
  - ***HRMA PCR set*** ***(48 PCRs)***
  - ***PCR with dye***
    - ***follow qPCR***
      - ***~95% PCR worked immediately***
    - ***perform melt curve analysis***
  - ***retrieve products***
    - ***454 sequencing (500 bp)***
    - ***Illumina sequencing (75 bp)***
  - ***? equal coverage***
  - ***? all variants detected***



AccessArray PCR

- **PCR AccessArray**
  - ***48 x 48***
  - ***phosphorylated-oligo's***
    - ***M13-tails***
- **retrieve products**
  - ***AccessArray***
- **concatenate products**
  - ***add ligase***
- **fragment…**
  - **+ normal sample prep**

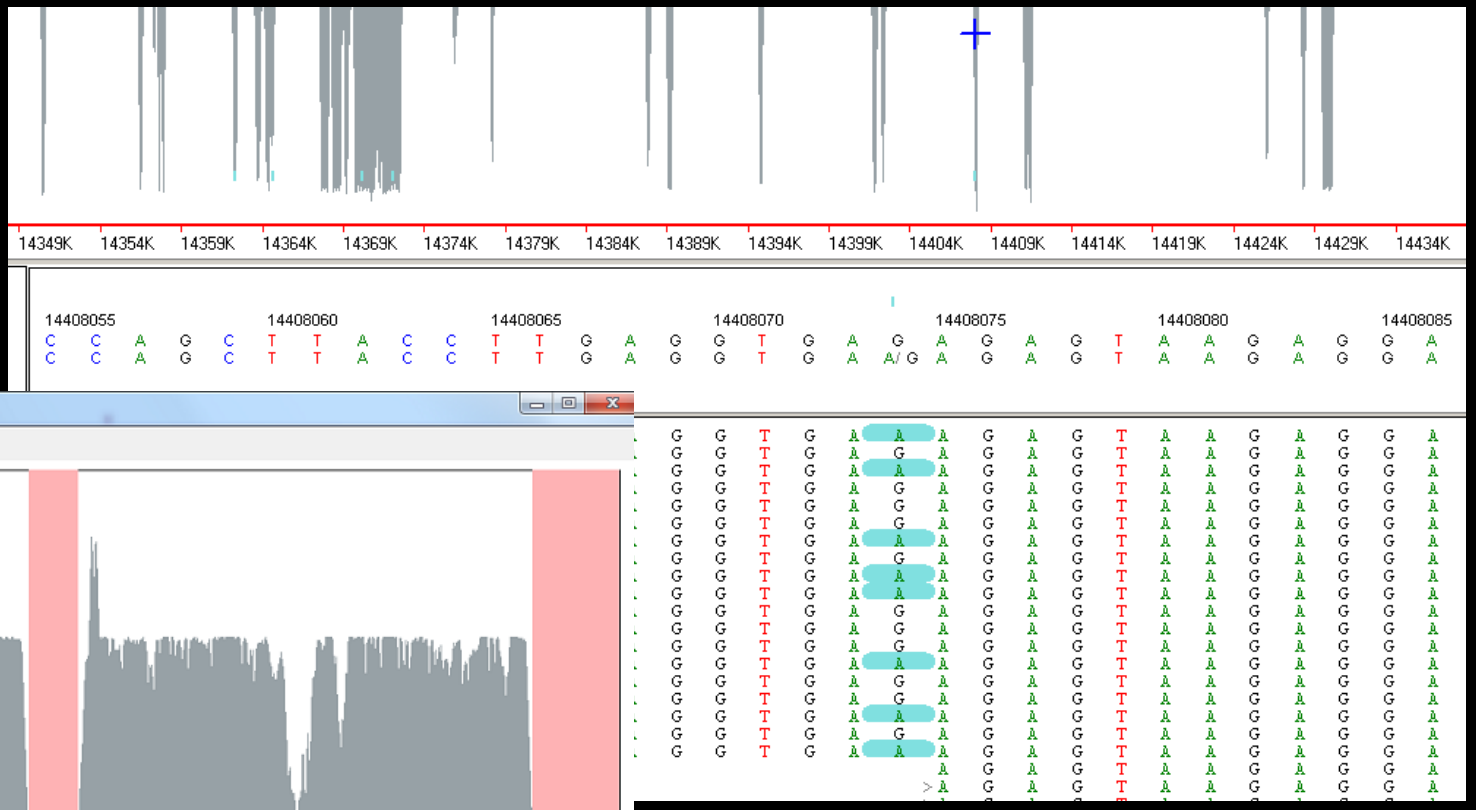


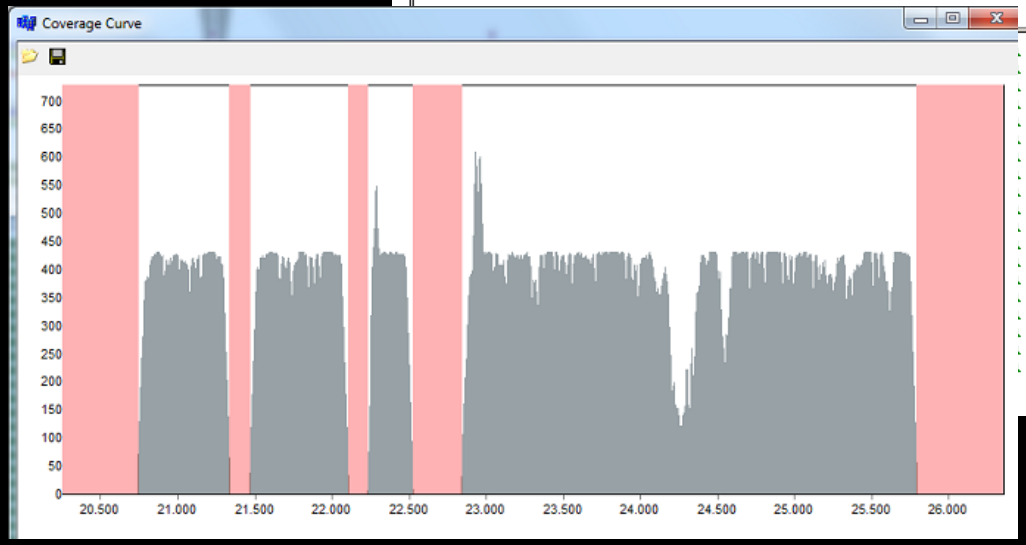
1	= 100 bp ladder
2-4	= 400U ligase
5	= 1U ligase

AccessArray BRCA1

BRCA1

coverage





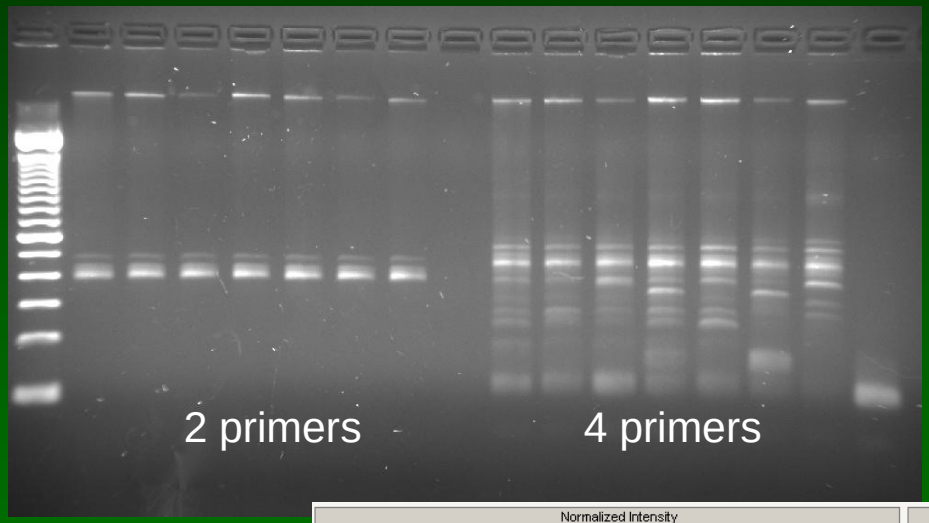
G > A variant

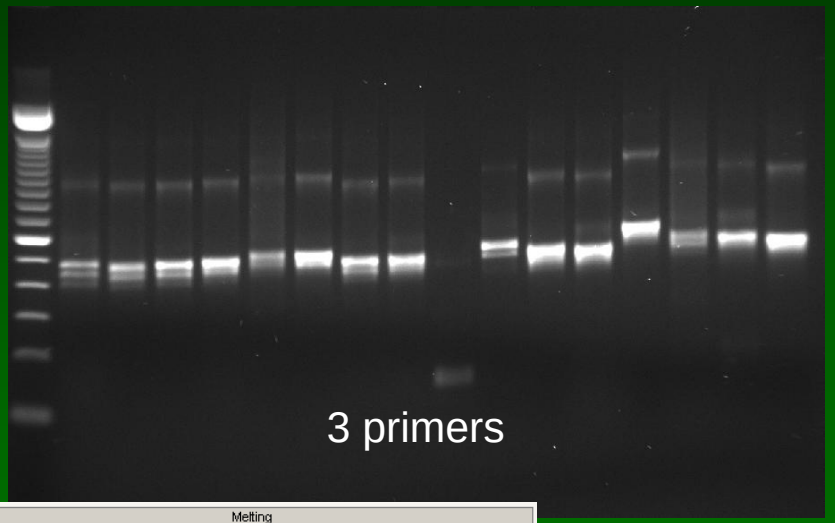
NextGene analysis (SoftGenetics)

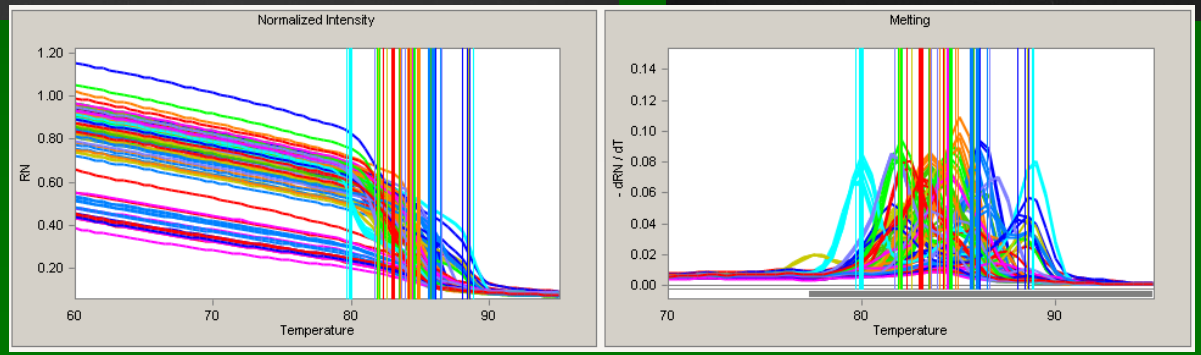
BRCA optimisation

PCR primers:

F	1) CGTATCGCCTCCCTCGCGCCATCAGACGAGTGCGTTGTAAAACGACGGCCAGT	MID-F	***driver***
R	2) CTATGCGCCTTGCCAGCCCGCTCAGACGCTCGACACAGGAAACAGCTATGACC	MID-R	
F	**3) TGTAAAACGACGGCCAGTggacgttgtcattagttctttgg**	**exon specific-F**	***start***
R	**4) CAGGAAACAGCTATGACCtcagcaattacaatagcctaatctt**	**exon specifi-R**	





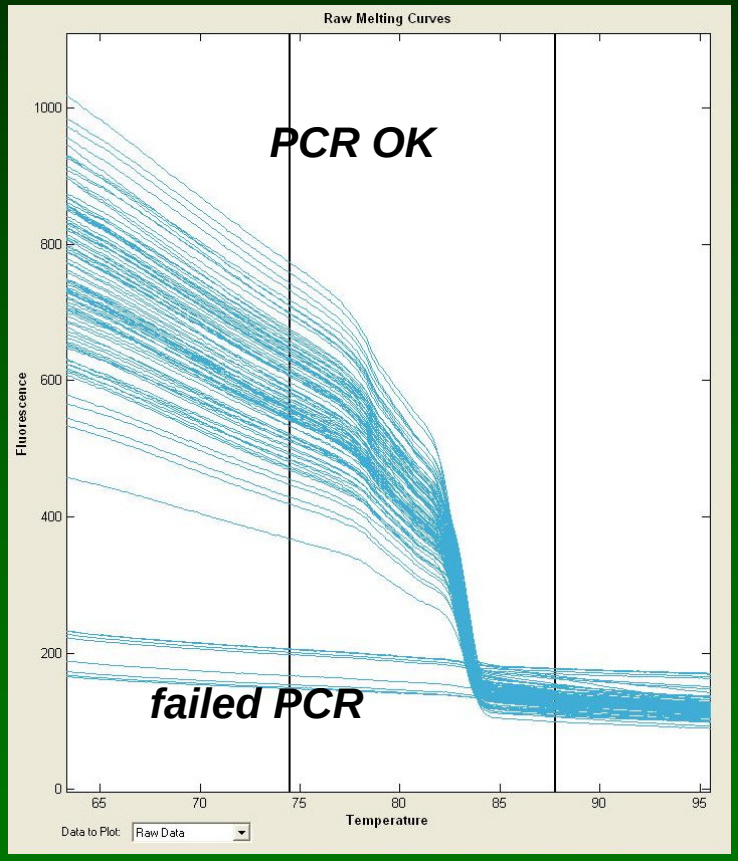


melt curve analysis

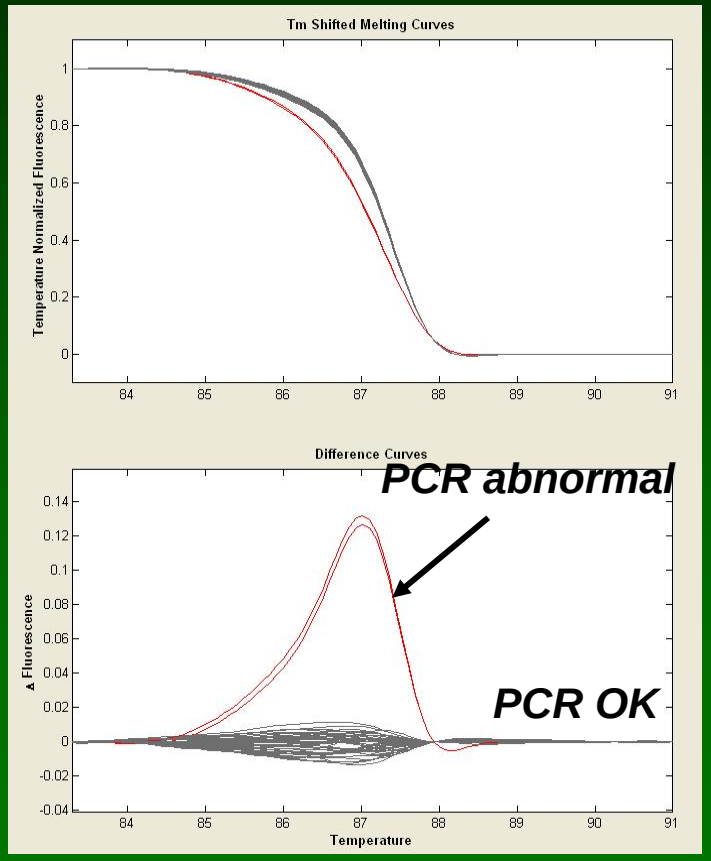
HRMA PCR Q-check

PCR yield

concentration



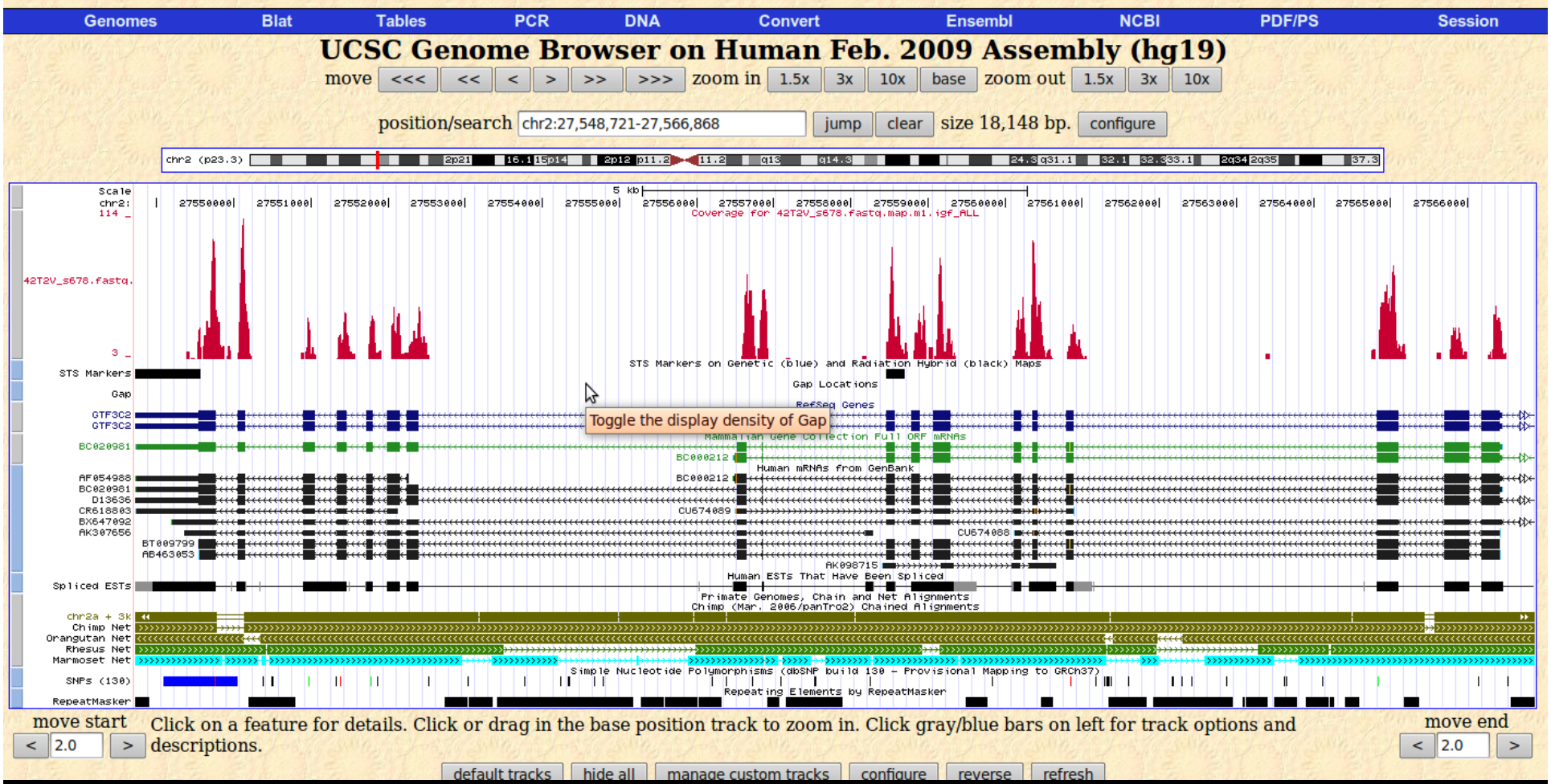
PCR quality



Hybridisation capture

- **many experiments**
  - *on array and in soluiton*
  - *standard, custom, X-exome, exome*
- **lab work**
  - *in solution clear advantages*
    - *can be automated, much easier*
- **region covered**
  - *on array more probes*
    - *no steric hindrance*
  - *in solution capture higher yield*
- **capture both strands**
  - *on array possible*
  - *in solution problematic*

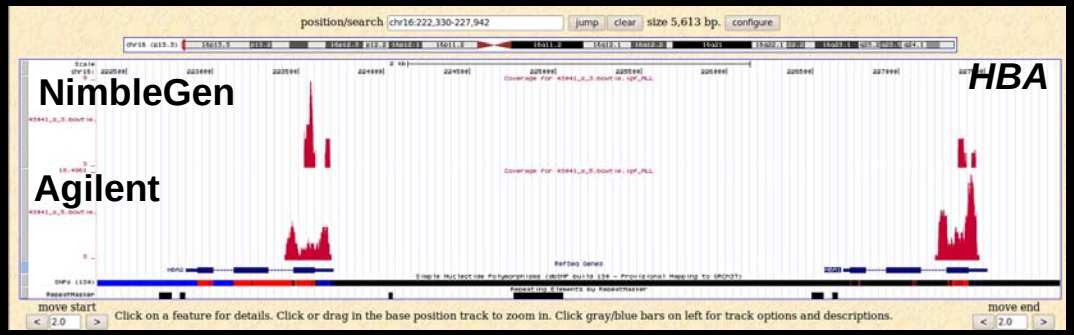
Array capture - exome

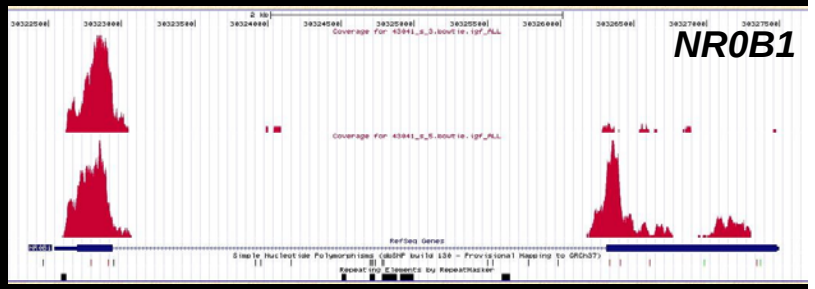


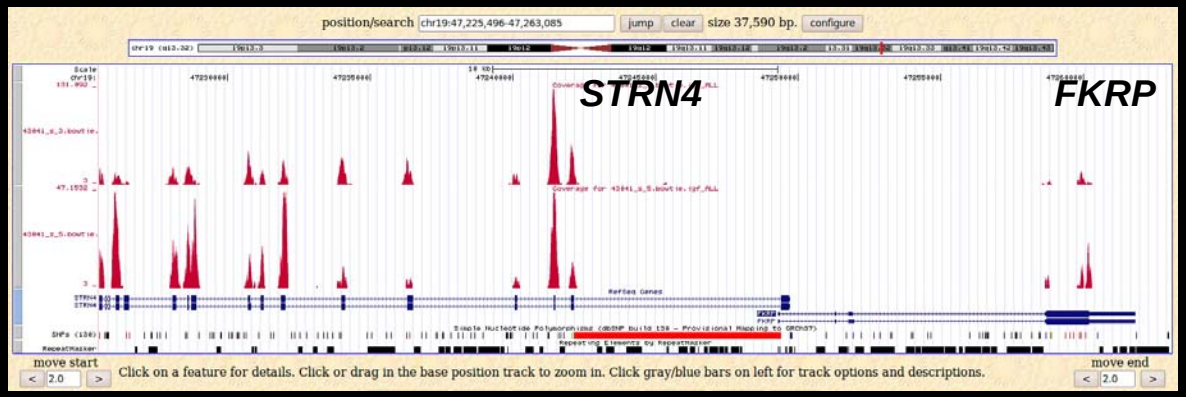
NimbleGen SYSTEMS, INC.

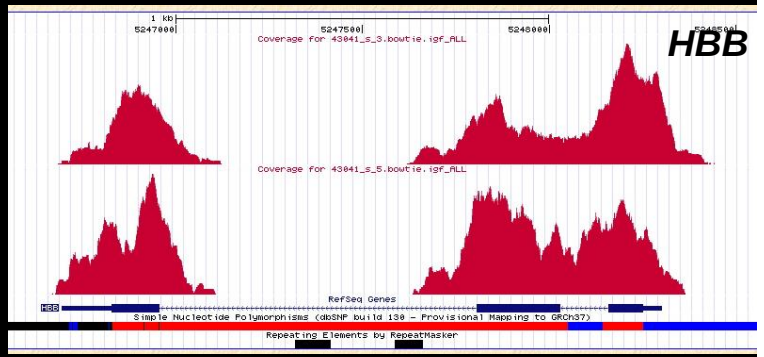
Full exome capture

Full exome capture



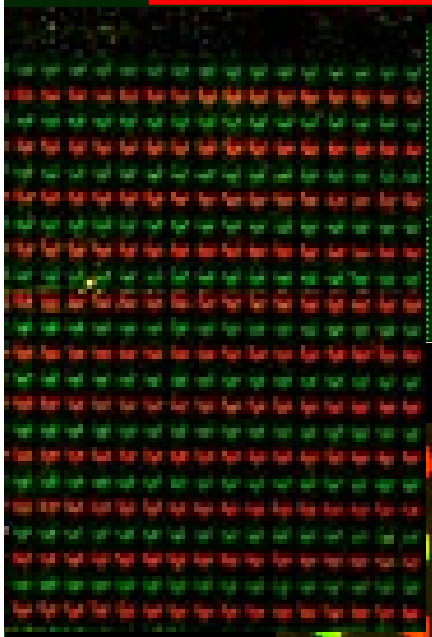


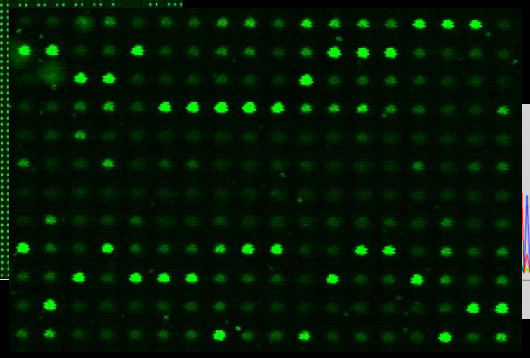


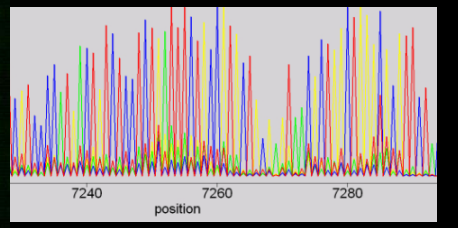


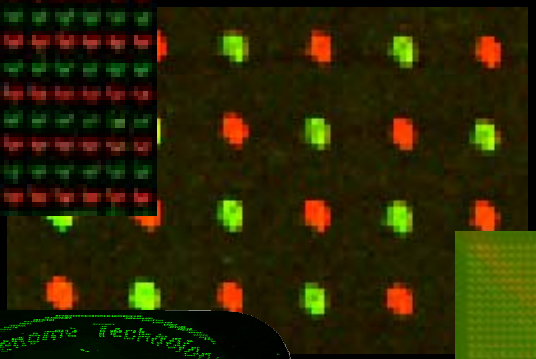

Genome Analyzer
illumina

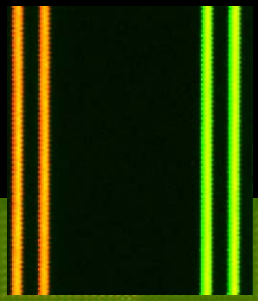
FlexGen arrays

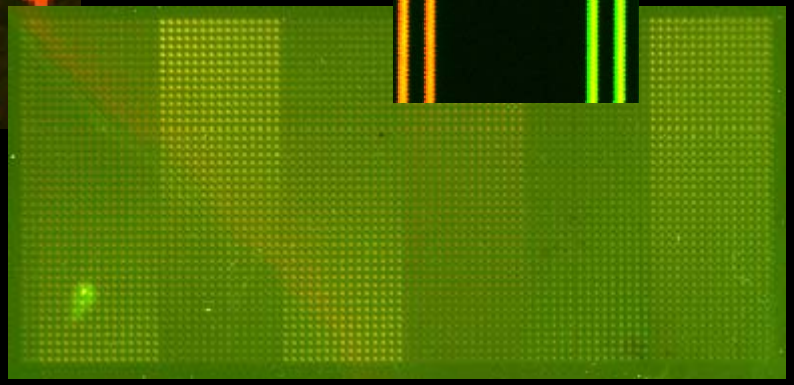


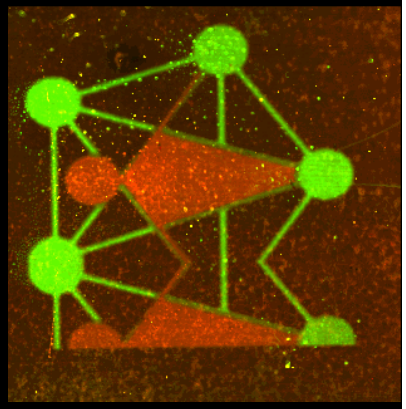












Home brew - design

array oligo synthesis

↓

chemical cleavage

↓

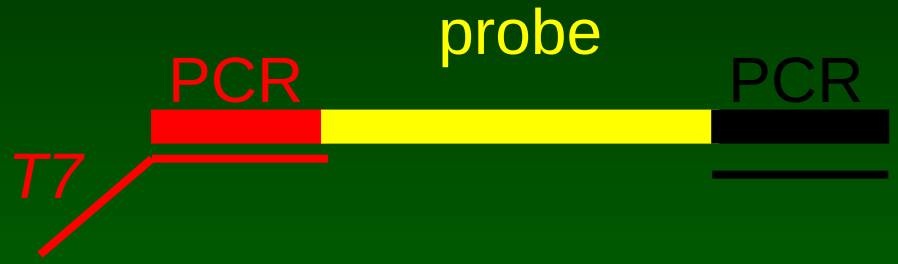
amplifcation

↓

+ T7 RNA polymerase (+biotin)

↓

capture

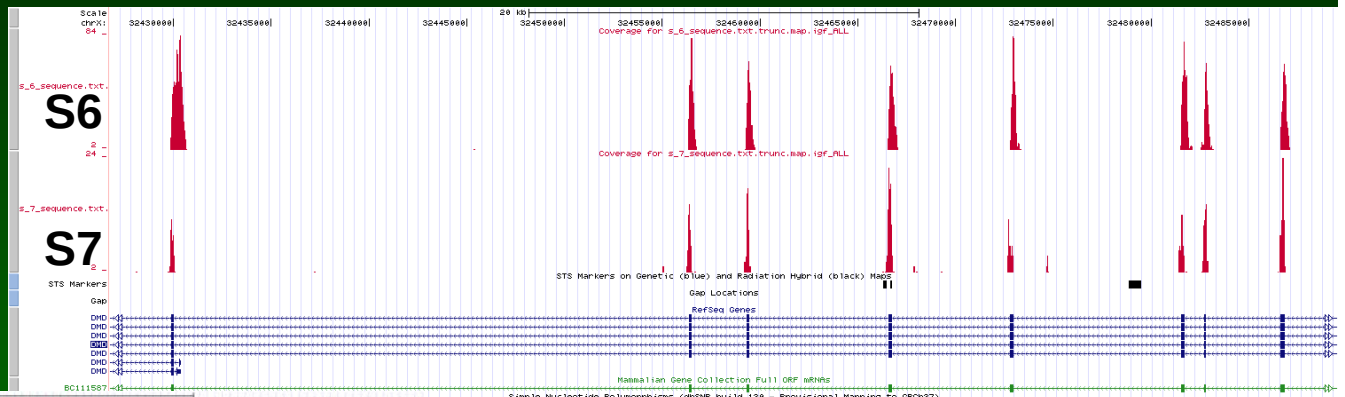


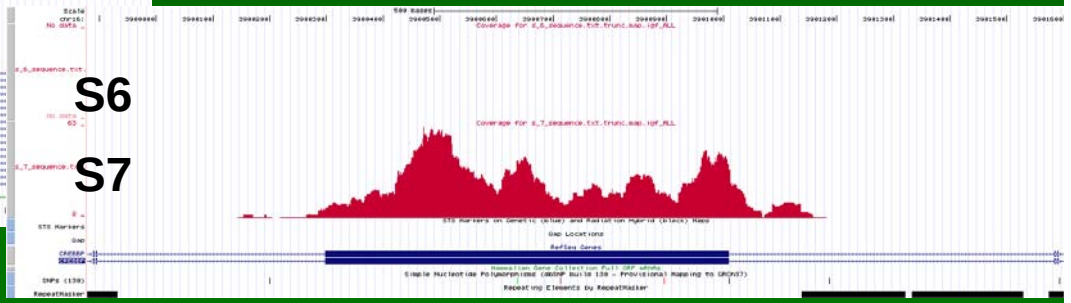
FlexGen

Home brew - DMD

S6 **X-exome SureSelect**
S7 **custom HomeSelect**

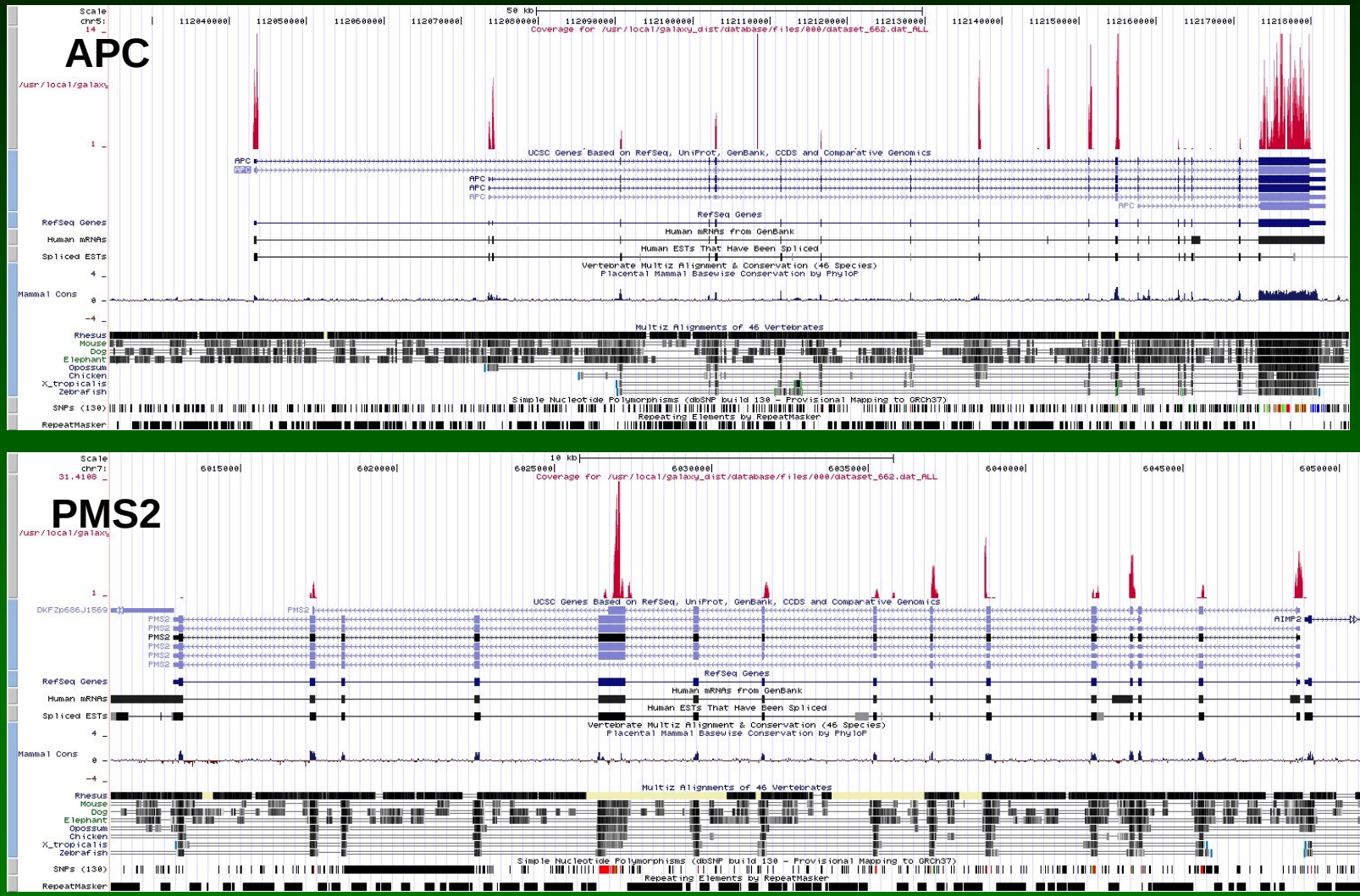
DMD-gene (Xp21.2)





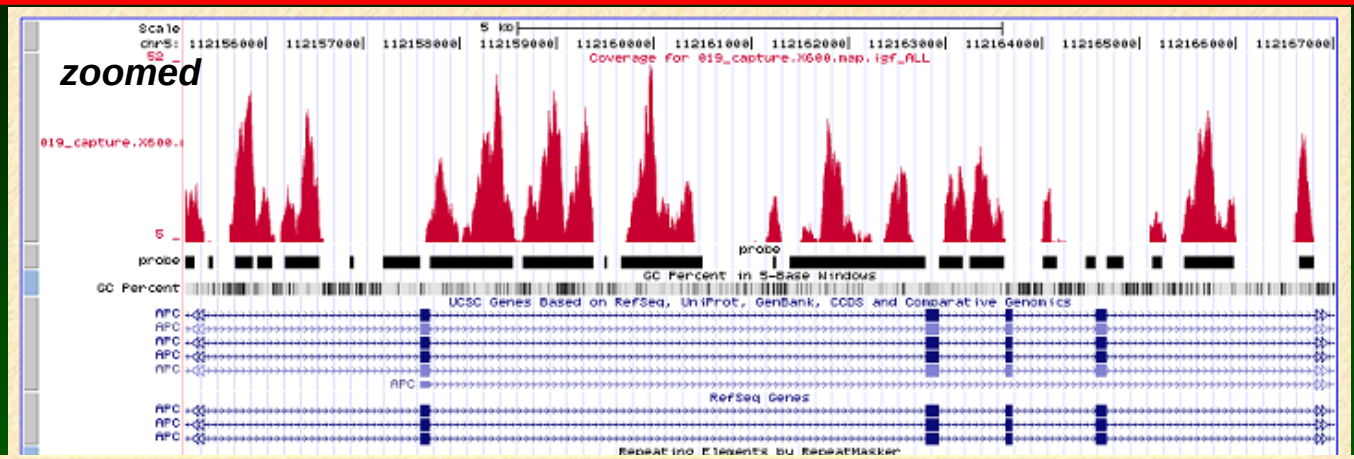
CREBBP (chr.16p)

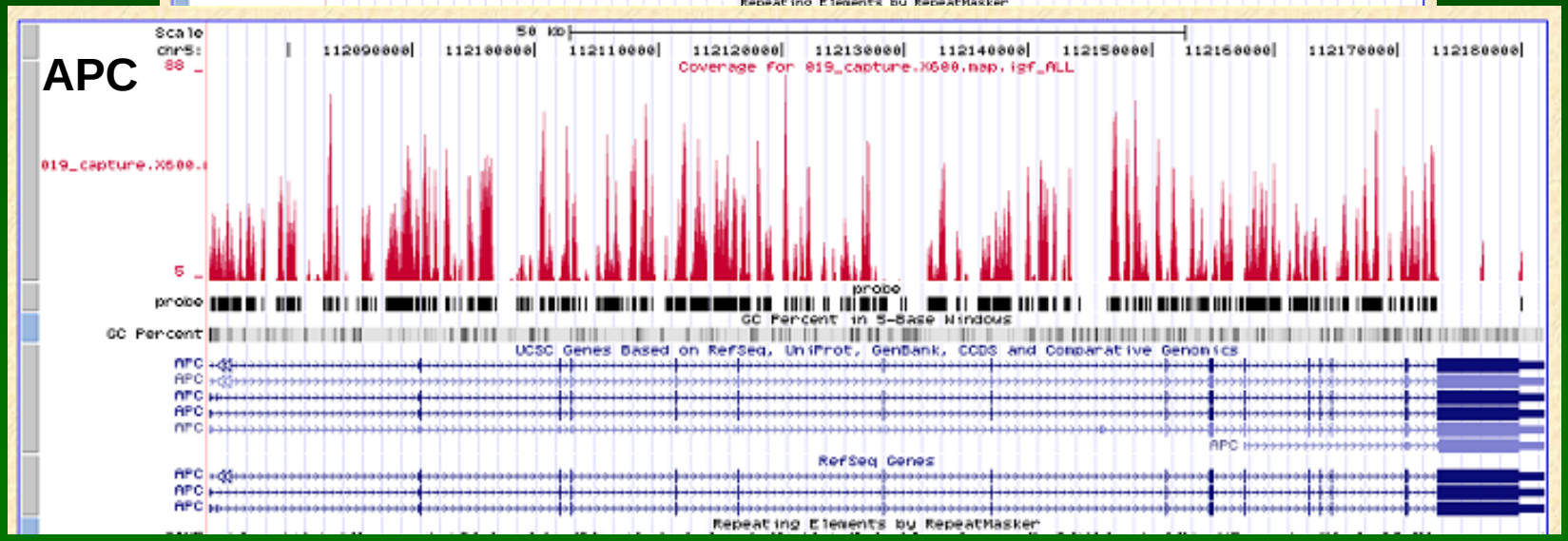
Home brew - MMR



Home brew – MMR

introns added





Advantages

- **fast**
  1 day
- **flexible**
  redesign, add to existing
- **cheap**
  no minimal amount
  one array > indefinite resource

designed array to capture MMR genes

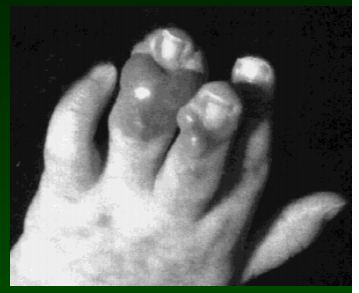
Associated Press

Netherlands shocks Brazil 2-1

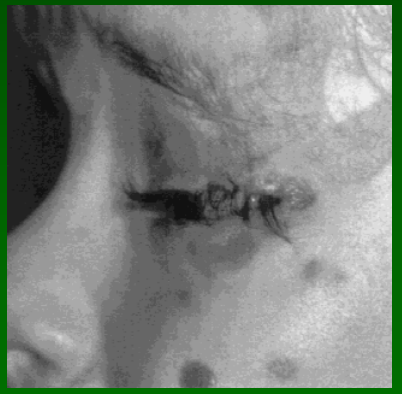
By ANDREW DAMPF (AP) – 2 days ago

PORT ELIZABETH, South Africa — Don't call the Dutch underachievers anymore.

X-linked TOD



- **Terminal Osseous Dysplasia**
  - ***pigmentary anomalies skin***
  - ***skeletal abnormalities limbs***
  - ***recurring digital fibromatosis childhood***



- **X-linked** *(Xq25-ter)*
  - ***dominant***
    - ***male lethal***
    - ***female skewed X_i***

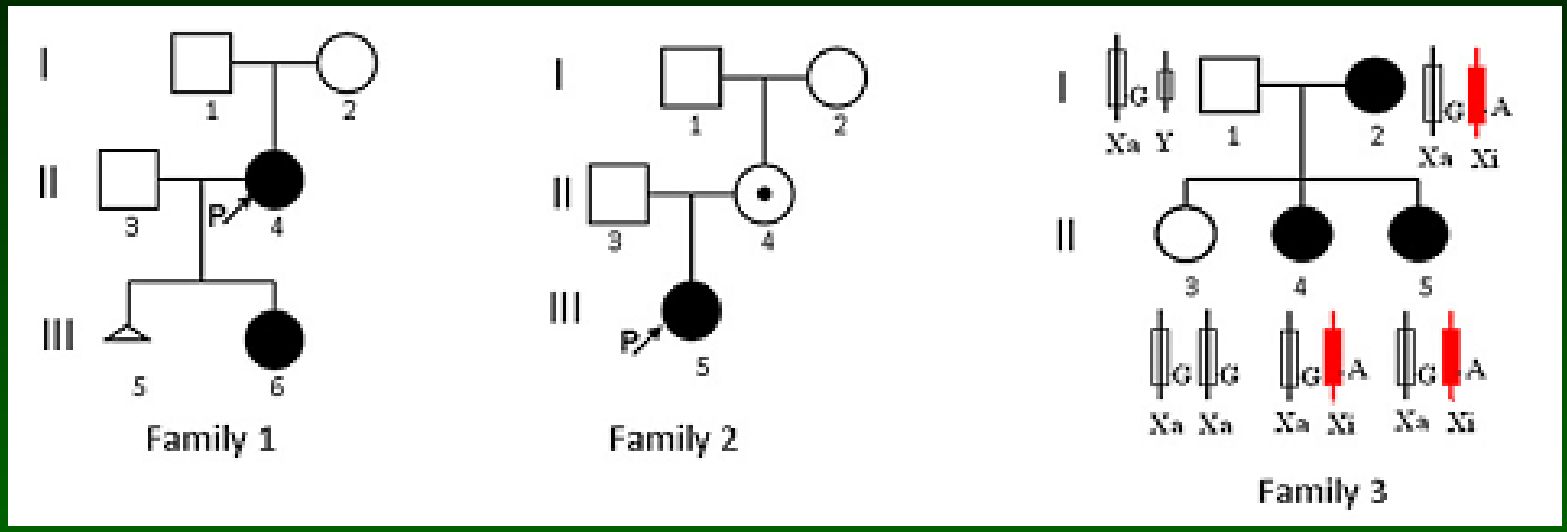
American Journal of Medical Genetics 94:91–101 (2000)

New Syndrome?

Recurrent Digital Fibroma, Focal Dermal Hypoplasia, and Limb Malformations

M.H. Breuning,[1]* A.P. Oranje,[2] R.A.Th.M. Langemeijer,[3] S.E.R. Hovius,[4] A.F.M. Diepstraten,[5] J.C. den Hollander,[6] N. Baumgartner,[7] J.R. Dwek,[8] A. Sommer,[9] and H. Toriello[7]

X-linked TOD



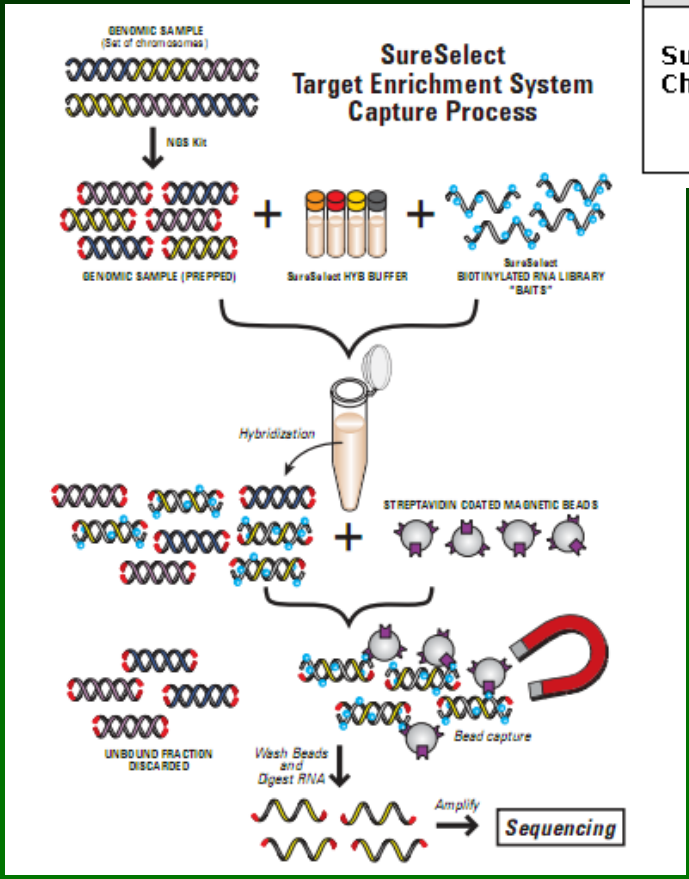
3 families (NL, Japan, Argentina)

+ 3 sporadic cases

In-solution capture

Agilent's SureSelect Target Enrichment System

SureSelect



Product Name	Product Number	Pricing & Availability
SureSelect Human X Chromosome Exome Kit	G4459A	Contact Us

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

ServiceXS

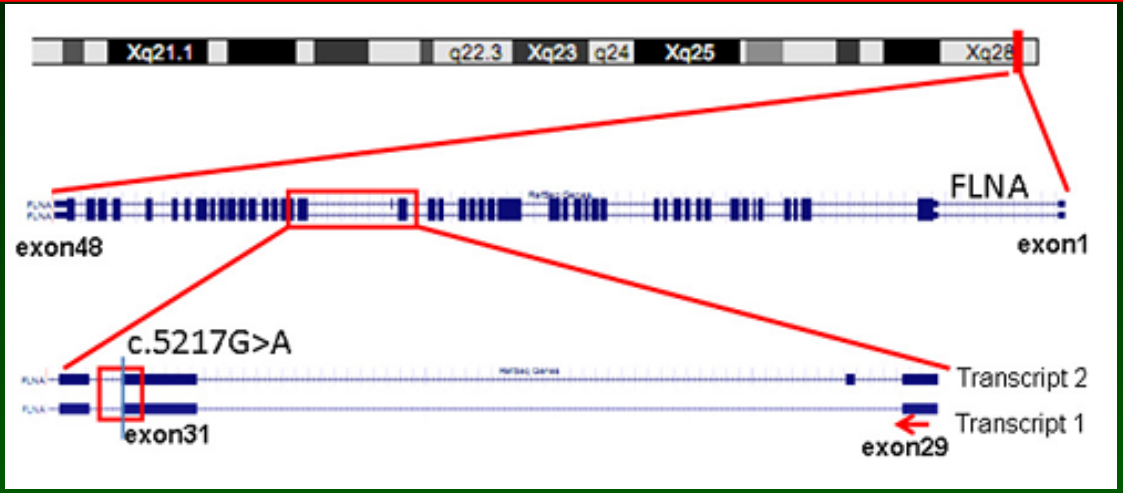
one strand capture

X-exome capture

	Exp.1	Exp.2	Common
exon coding	771	720	**351**
exon UTR	406	631	**257**
splice site	56	89	**25**
intron	793	1853	**452**
intergenic	1085	1716	**425**

(sub-optimal data analysis !)

TOD X-exome

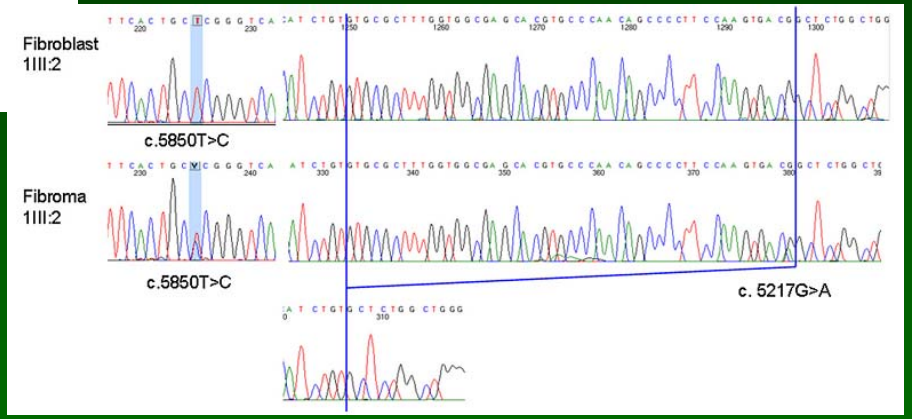


- **cultured cells / blood**
  - ***only normal allele expressed***
    - ***100% X_i***

TOD X-exome

fibromas

RNA



- **archived fibroma tissue**

  (15 year old)

  2 alleles expressed

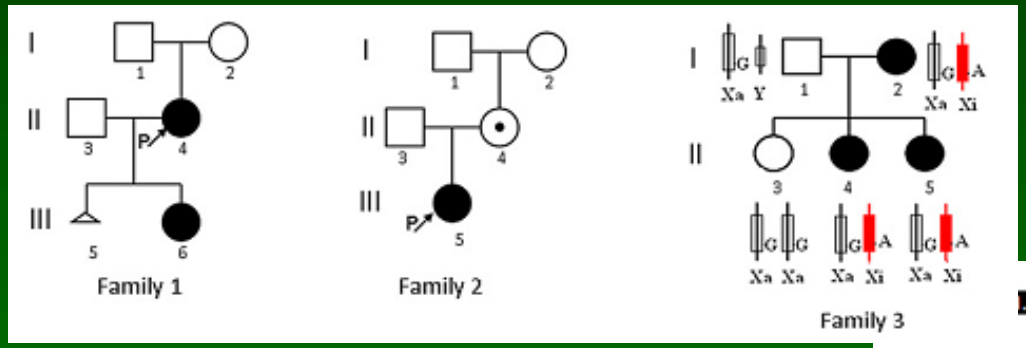
  activated cryptic exonic splice site

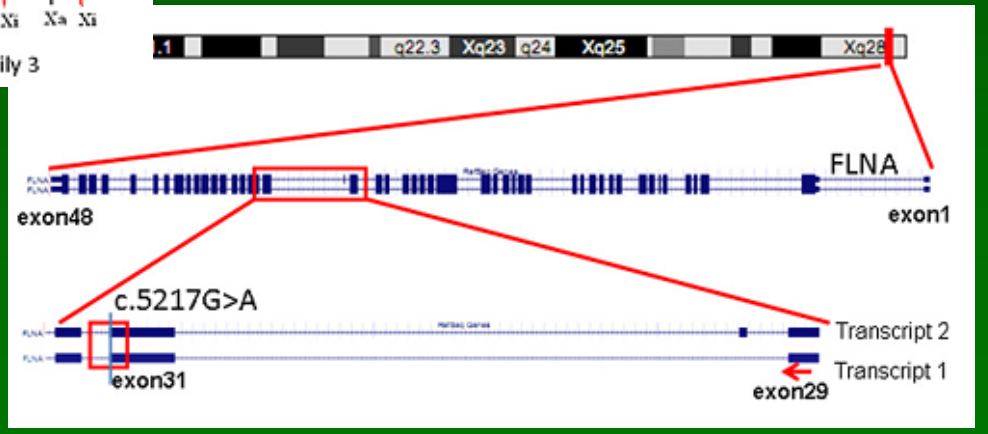
The American Journal of Human Genetics *87*, 1–8, July 9, 2010

REPORT

Terminal Osseous Dysplasia Is Caused by a Single Recurrent Mutation in the *FLNA* Gene

Yu Sun,[1,11] Rowida Almomani,[1,11] Emmelien Aten,[1] Jacopo Celli,[1] Jaap van der Heijden,[1] Hanka Venselaar,[2] Stephen P. Robertson,[3] Anna Baroncini,[4] Brunella Franco,[5,6] Lina Basel-Vanagaite,[7] Emiko Horii,[8] Ricardo Drut,[9] Yavuz Ariyurek,[1,10] Johan T. den Dunnen,[1,10] and Martijn H. Breuning[1,*]

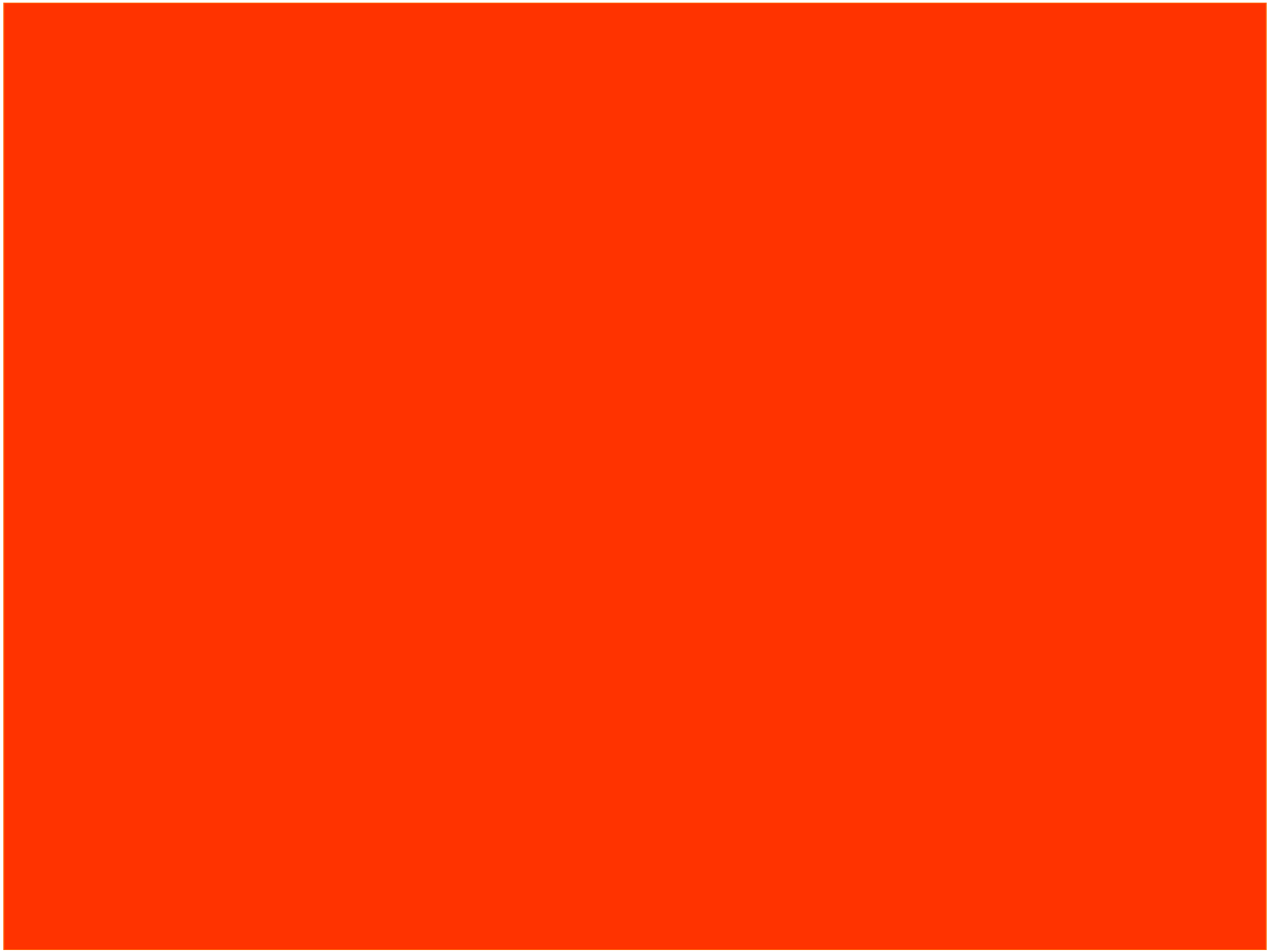




X-linked TOD

- **FLNA gene a surprise ?**
  - ***first FLNA mutations published***
  - ***the obvious candidate for TOD***
    - ***phenotypic overlap***
- **2003 gene analysed**
  - ***send to expert > nothing reported***
  - ***X-exome c.5217G>A > ?; was detected !***
- **2010 paper**
  - ***FLNA gene analysed, no variants found...***

We need to share & report ALL variants, immediately



Helicos

single molecule sequencing

(SMS)

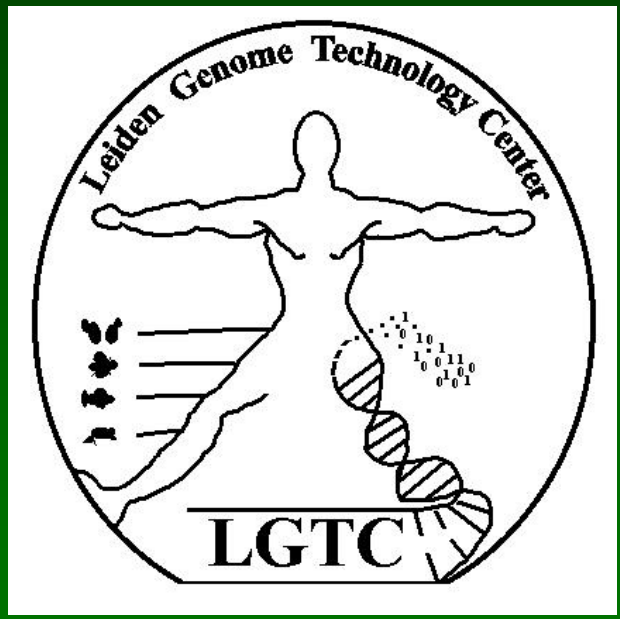
Acknowledgements

Eveline Altena
Peter de Knijff

Forensisch Laboratorium voor DNA Onderzoek

Helicos BioSciences Corporation

Michiel van Galen
Matt Hestand



Gert-Jan van Ommen
Johan den Dunnen

Advantages tSMS™

- **saves work** *(= time & cost)*
  - ***'no' sample preparation***
- **no amplification (PCR)**
  - ***fewer contamination issues***
  - ***absolute quantitation***
  - ***analyse everything*** ***(unPCR-able /unclonable)***
  - ***no PCR errors*** ***(< sequence error)***
  - ***analysis low quality DNA***
    - ***forensics, ancient, archived***
    - ***sample prep enriches contaminants***
- **sequence RNA directly**

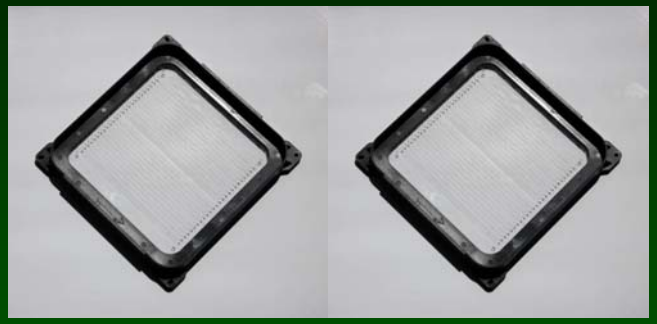
Helicos

sequencer

Helicos BioSciences Corporation

computer

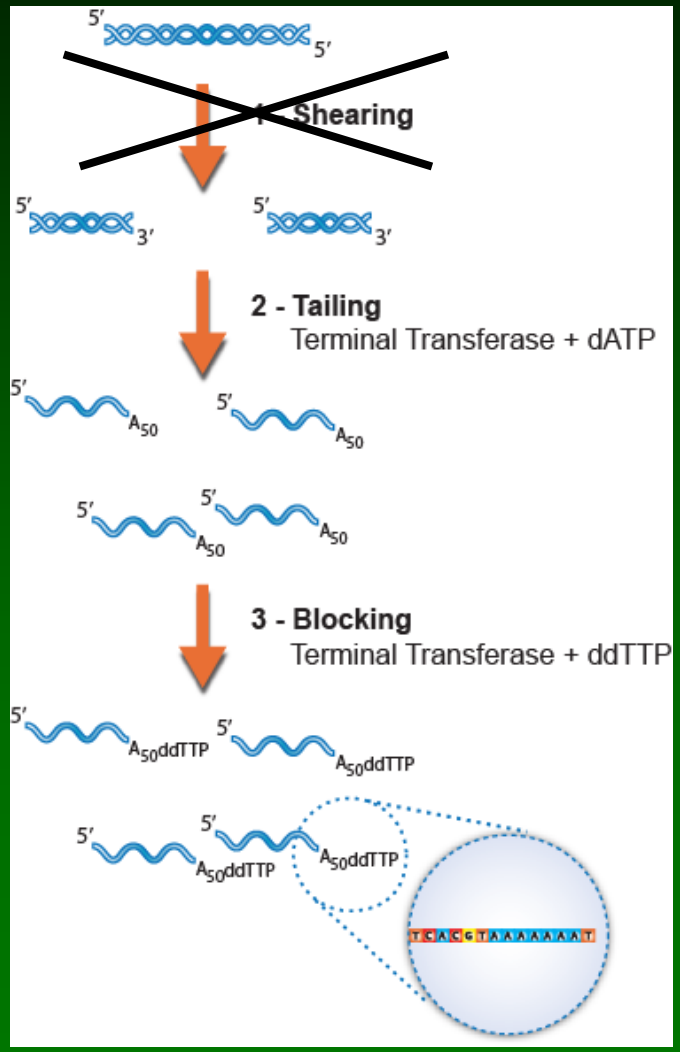
Helicos

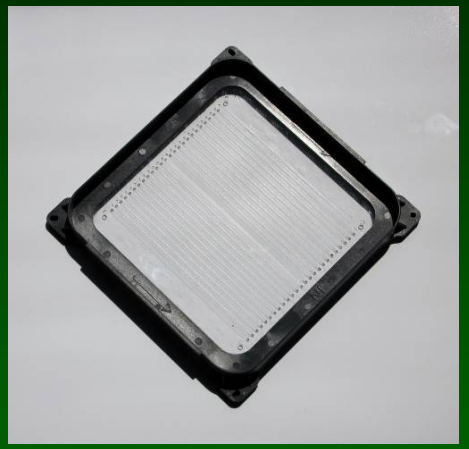


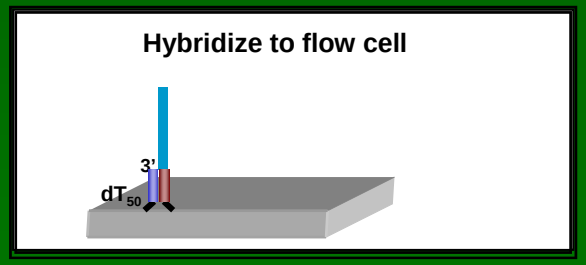
2 flow cells

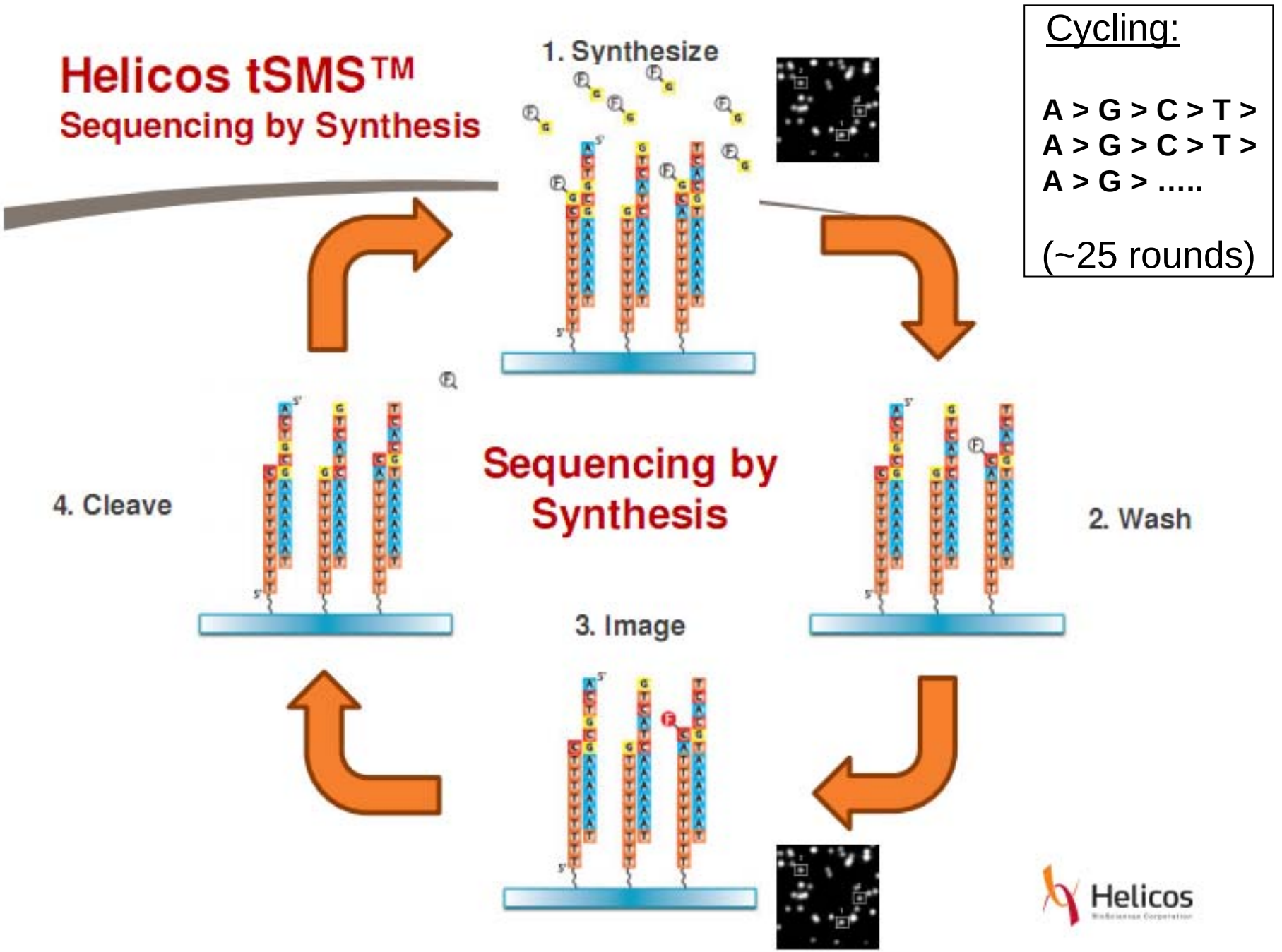
25 channels each

Methodology

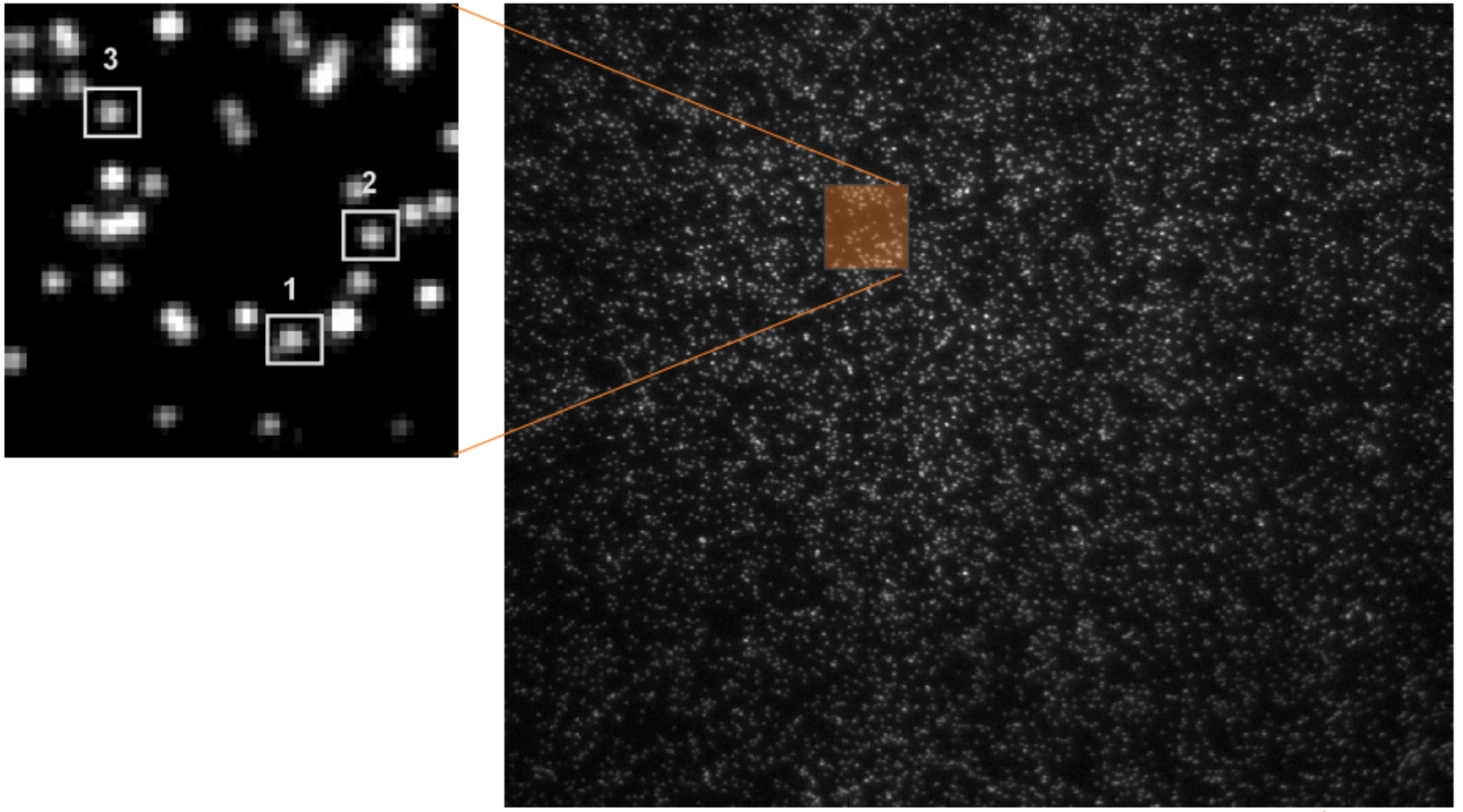







Helicos tSMS™
Sequencing by Synthesis
1. Synthesize
2. Wash
3. Image
4. Cleave
Sequencing by Synthesis
Cycling:
A > G > C > T >
A > G > C > T >
A > G > .....
(~25 rounds)
Helicos

Methodology

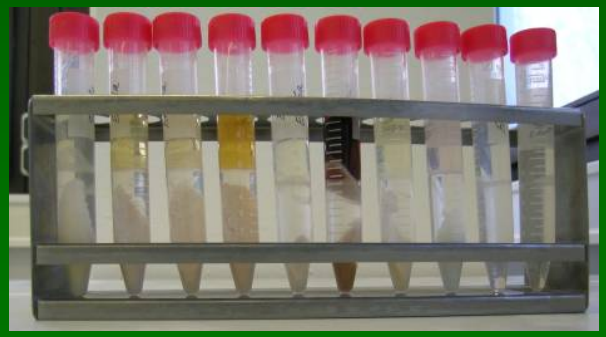


Primary burials

From burial to teeth

Forensisch Laboratorium voor DNA Onderzoek

F L D O



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Mitochondrial DNA

Forensisch Laboratorium voor DNA Onderzoek

Helicos BioSciences Corporation

Reference Sequence in bold
One channel each above and below
forward reads black, reverse blue

Human DNA (NGS)

Forensisch Laboratorium voor DNA Onderzoek

Helicos BioSciences Corporation

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%

up to 30%
…
…
…
up to 40%

determine gender

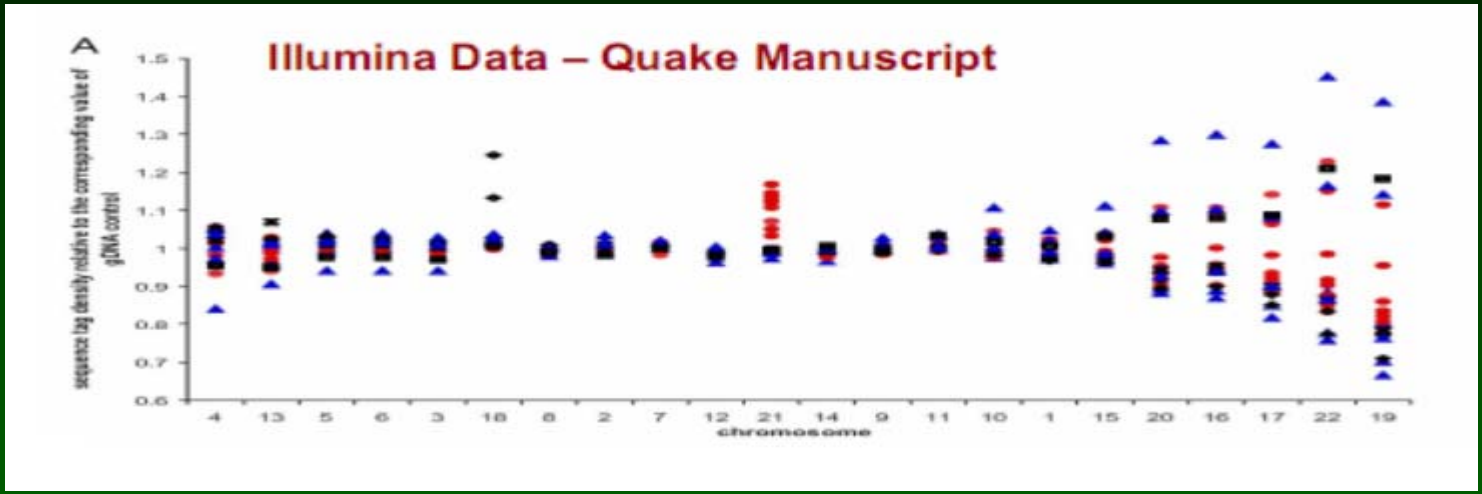
bone structure	***3/5***
PCR	***3/5***
Helicos	***5/5***

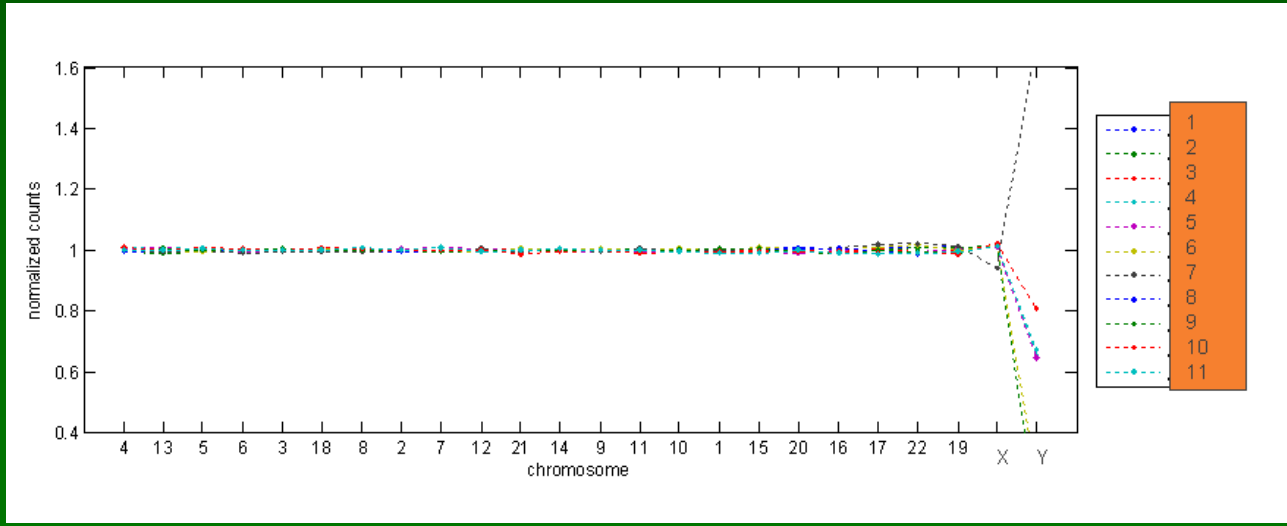
Summary Helicos

- **more human DNA**
  - ***excluded contamination researchers***
    - ***variants in mtDNA***
  - ***mark of DNA-damage***
    - ***"variants" in one strand only***
- **many new possibilities**
  - ***more data from these samples***
    - ***complete mtDNA***
    - ***…capture regions of interest***
    - ***…complete genome sequence***
  - ***archeology in general***
    - ***extinct organisms***
  - ***analysis "bad quality" DNA***
    - ***forensics, archived***

low cost
load 50 samples

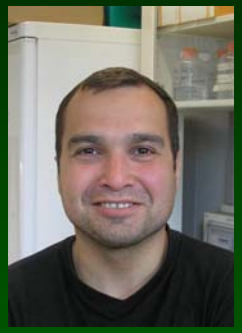
Maternal plasma

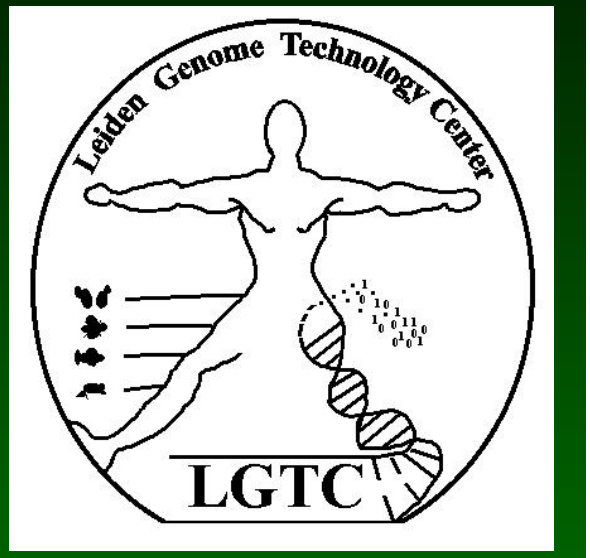


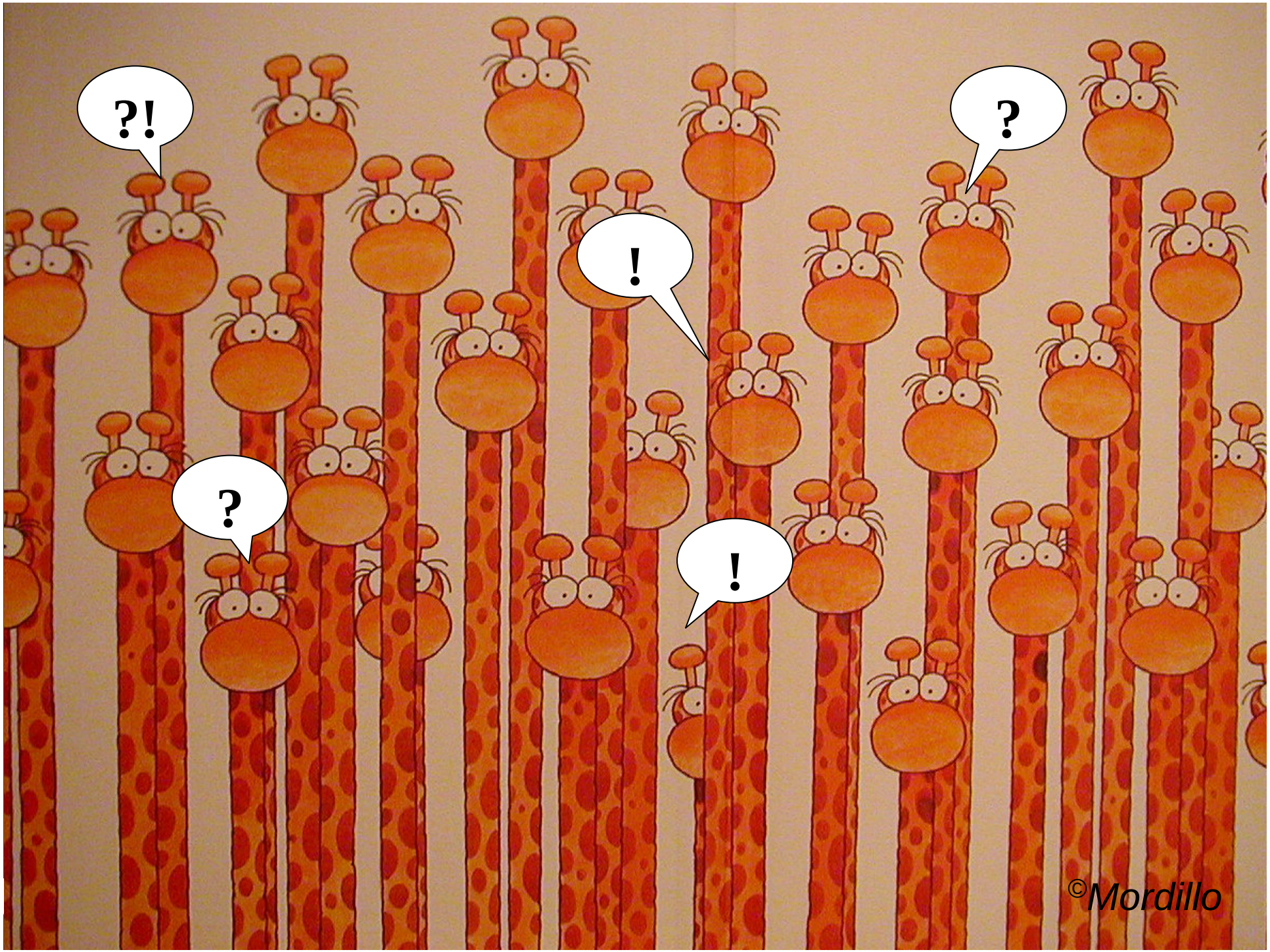


LGTC crew

- **lab manager**
  - *Sophie Greve*
- **technical support**
  - *Yavuz Ariyurek*
  - *Rolf Vossen*
  - *Paul Murphy*
  - *Rudy Pijpers*
  - *Arnoud Schmitz*
  - *Eleonora de Klerk*
- **computer support**
  - *Michiel van Galen*
  - *Matt Hestand*
  - *Bradley ten Broeke*
  - *(Michel Villerius)*



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