Molecular Technologies in the (near) Future

Sir John Burn MD
FRCP FRCPE FRCPCH FRCOG
FMedSci
Professor of Clinical Genetics
Genetics lead, NIHR
Chair, British Society for Genetic Medicine
Medical Director, QuantuMDx ltd

UK NEQAS
For leucocyte immunophenotyping
24th June 2013
Sheffield

QuantuMDx

Newcastle University

CCG

	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

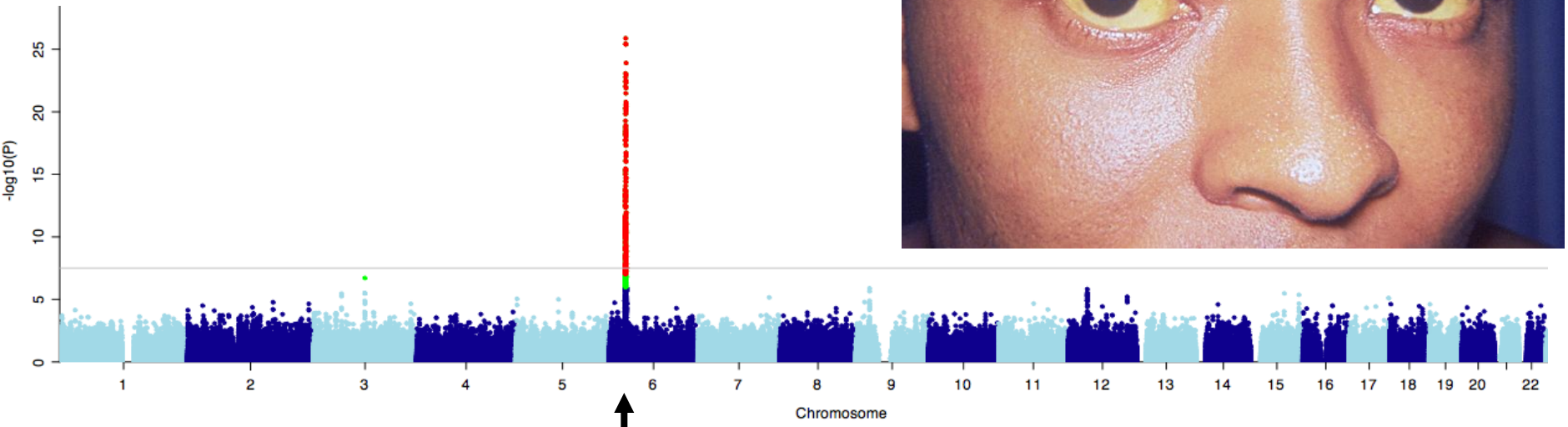
Ankylosing Spondylitis

72/75 AS patients were HLA B27 positive compared to 3/75 controls

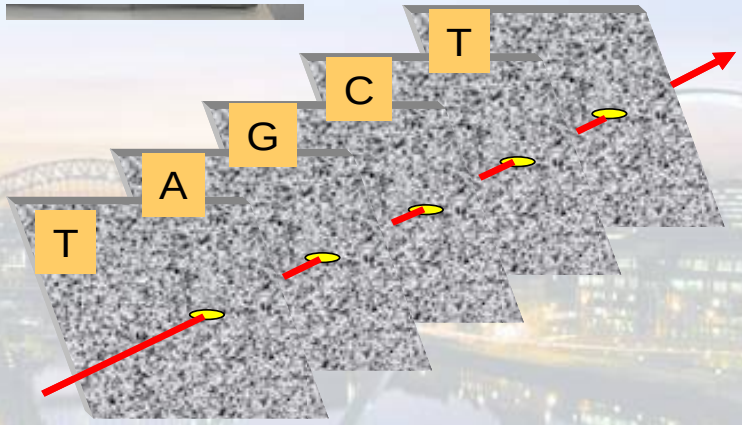
(Brewerton Lancet 1973:904)

Manhattan plot for flucloxacillin GWAS
HLA B*5701 responsible for liver toxicity

Daly A et al Nature Genetics 2009

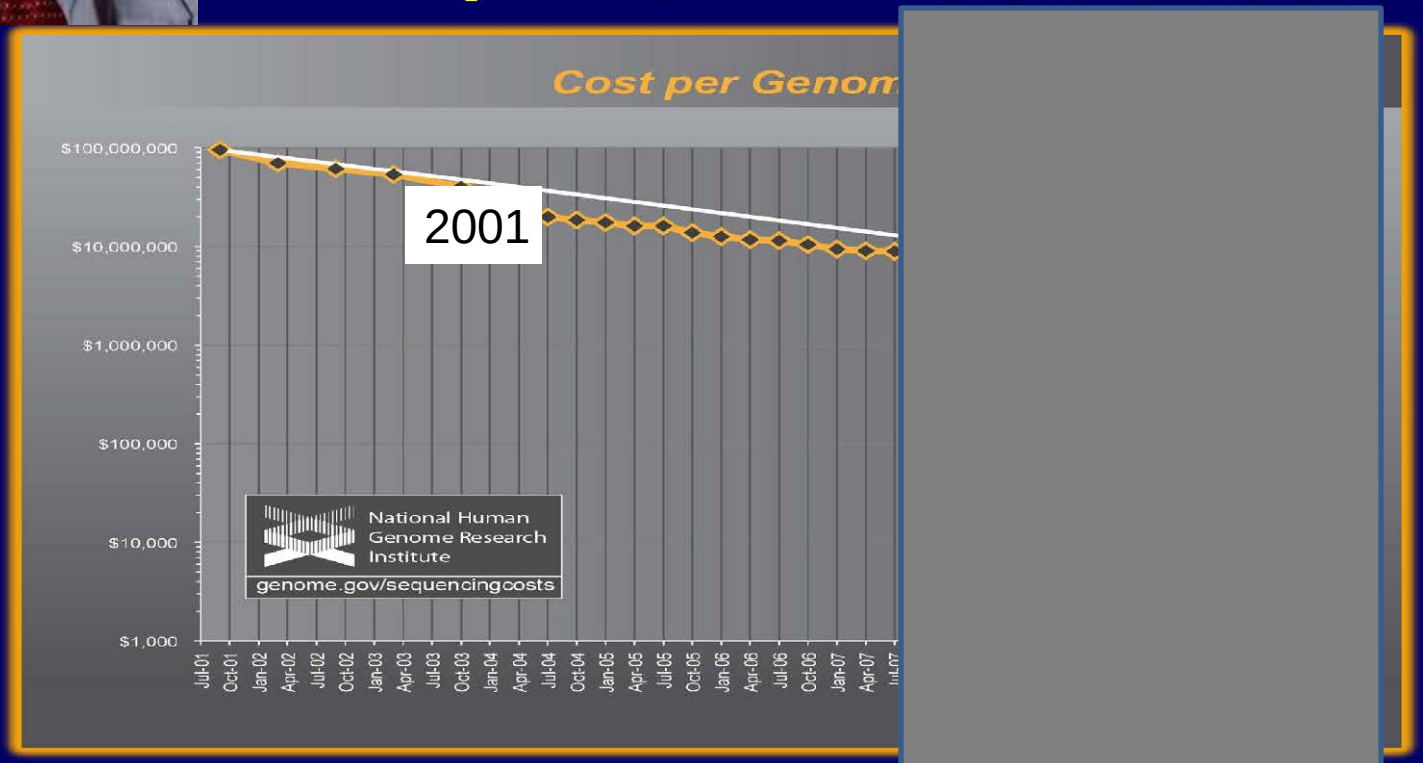


MHC region
Strongest signal with
HCP5 SNP-marker for
B*5701



Roche Genome Sequencer FLX

ber Sequenced Human Genome



Deciphering Developmental Disorders
Govt. funded exome analysis of 12000 families with a multiple malformation

Recruitment started 2011

wellcome trust sanger institute

NHS

UNIVERSITY OF CAMBRIDGE

MRC Human Genetics Unit

UNIVERSITY OF OXFORD

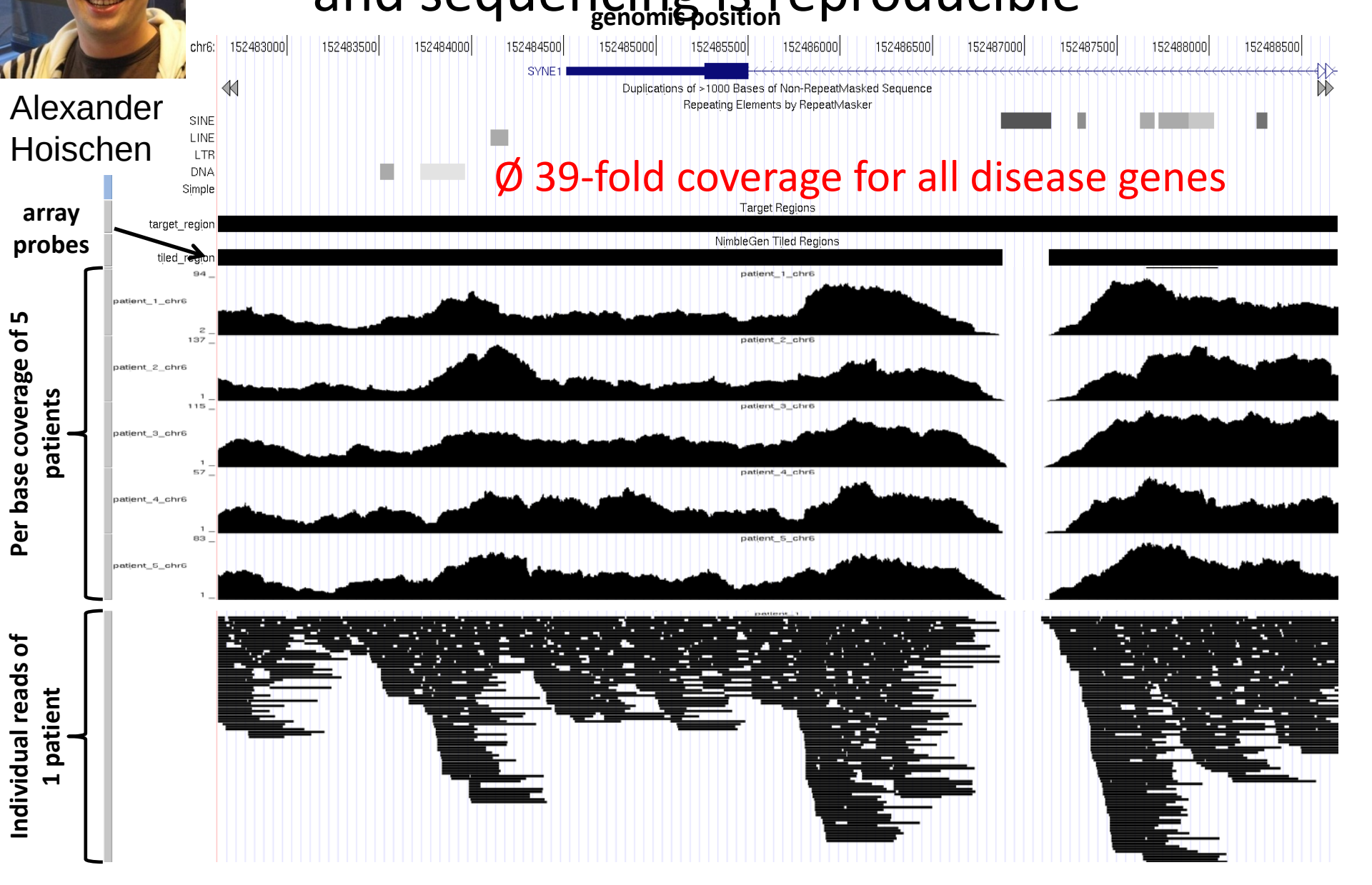
Human Genomics Strategy Group 2011-12

- Government response December 2012
  - 100,000 diagnostic whole genomes
  - £100 million investment
  - NHS Genomics Strategy Board

What should I buy?

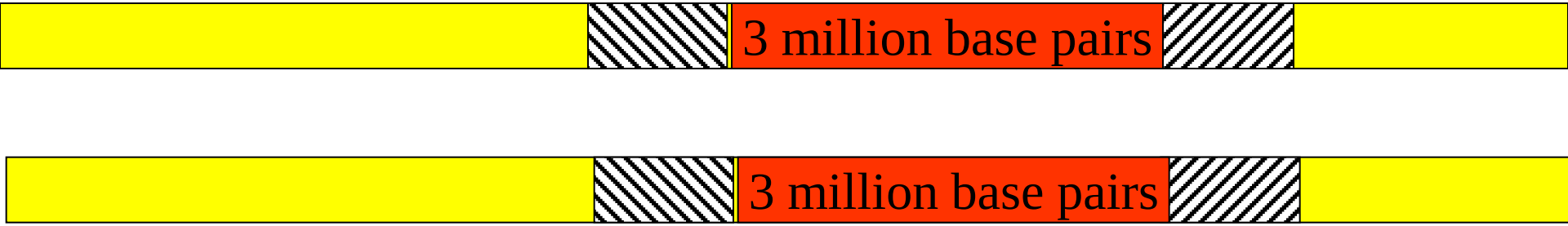
ataxia gene - Enrichment and sequencing is reproducible

Alexander Hoischen



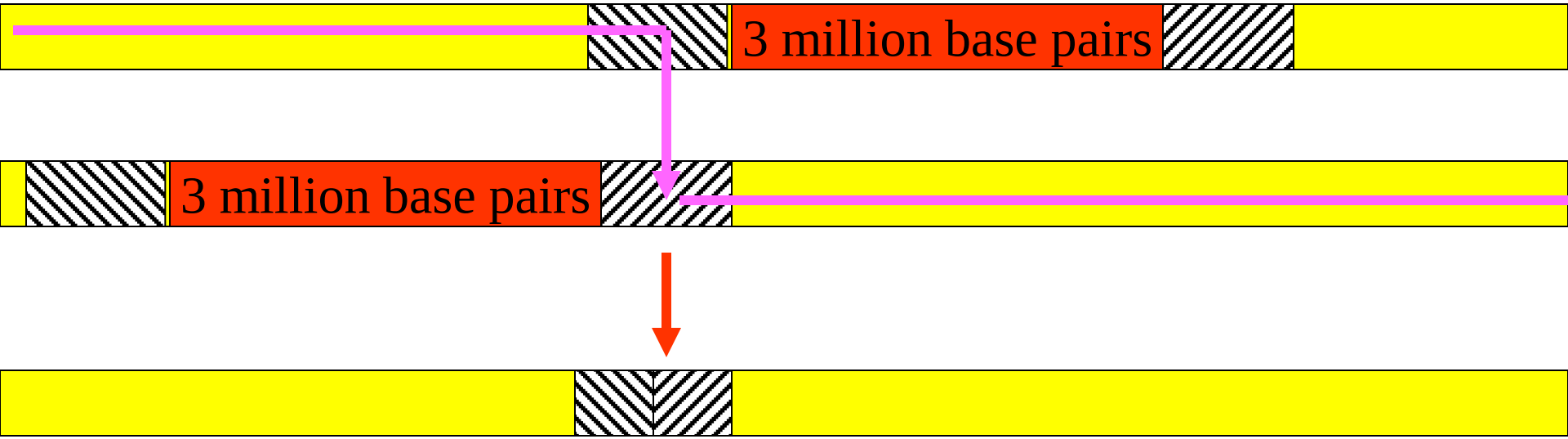
22q11 deletion

- Misalignment and cross over at matching sequences leads to deletion of three million bases and about 30 genes



22q11 deletion: a contiguous gene syndrome

- Misalignment and cross over at matching sequences leads to deletion of three million bases and about 30 genes



– Microsatellites are lengths of short repeated sequences eg

BAT25

tgttttgttttttgattttttttttttttttttttttttgagaacagagca

Instability in tumours indicates breakdown of mismatch repair either due to Lynch syndrome or MLH1 silencing

Slippage in coding microsatellites generates predictable novel peptides

Dr Magnus von Knebel Doeberitz

repeat length

$(A)_{10}$ TGT . AAA . AAA . AAA . ACG . TGC . TGG . CTA . GCT . GA.....

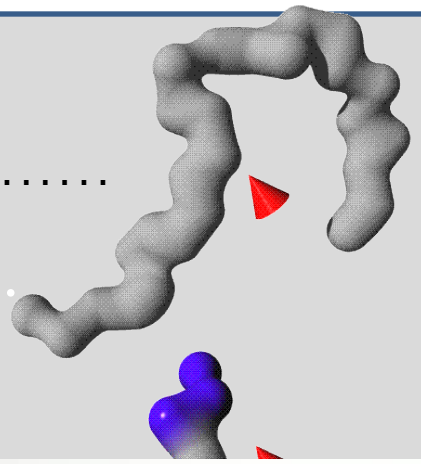
C K K K T C W L A . .

$(A)_9$ TGT . AAA . AAA . AAA . CGT . GCT . GGC . TAG . CTG . A

C K K K R A (

$(A)_8$ TGT . AAA . AAA . AAC . GTG . CTG . G

C K K K V L A



- loss of function
- *generation of cancer specific peptid*

Functional classification of JDW SNPs

SNP Type	Novel SNPs
Missense	2421
Nonsense	5
Synonymous	2261
UTR	7102
Intron	381924

August 2010 China National Highway 110 traffic jam, China, world's worst traffic jam ever….
more than 100 kilometres from August 14 - 26, inc. at least 11 days of total gridlock.

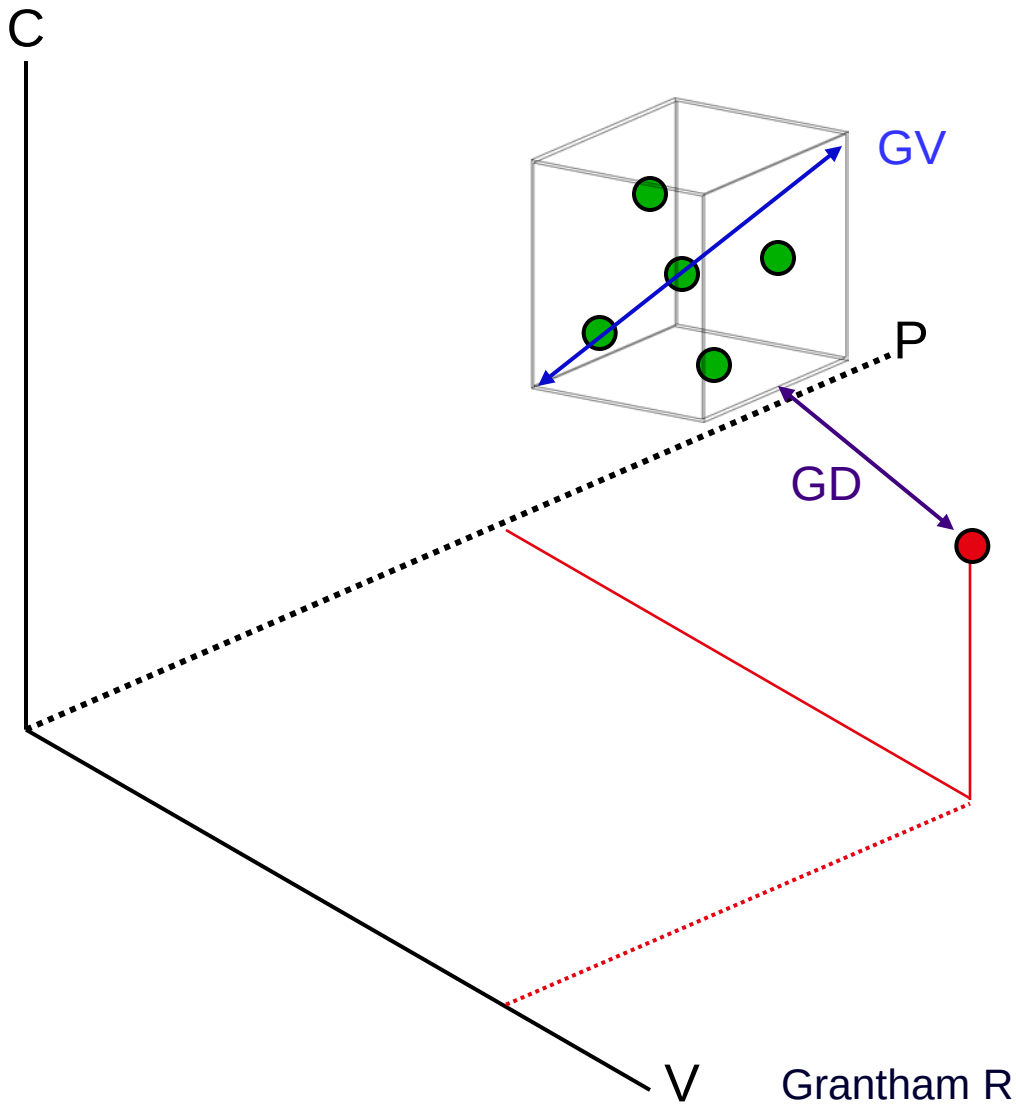
We are heading for the biggest traffic jam in history

BRCA1 Amino-terminal alignment

Human	M	-	D	L	S	A	L	R	V	E	E	V	Q	N	V	I	N	A	M	Q	K	I	L	E	C	P	I	C	L	E	L
Chimp	M	-	D	L	S	A	L	R	V	E	E	V	Q	N	V	I	N	A	M	Q	K	I	L	E	C	P	I	C	L	E	L
Gorilla	M	-	D	L	S	A	L	R	V	E	E	V	Q	N	V	I	N	A	M	Q	K	I	L	E	C	P	I	C	L	E	L
Orangutan	M	-	D	L	S	A	V	R	V	E	E	V	Q	N	V	I	N	A	M	Q	K	I	L	E	C	P	I	C	L	E	L
Rhesus	M	-	D	L	S	A	V	R	V	E	E	V	Q	N	V	I	N	A	M	Q	K	I	L	E	C	P	I	C	L	E	L
Mouse	M	-	D	L	S	A	V	Q	I	Q	E	V	Q	N	V	L	H	A	M	Q	K	I	L	E	C	P	I	C	L	E	L
Dog	M	-	D	L	S	A	D	R	V	E	E	V	Q	N	V	L	N	A	M	Q	K	I	L	E	C	P	I	C	L	E	L
Cow	M	-	D	L	S	A	D	H	V	E	E	V	Q	N	V	L	N	A	M	Q	K	I	L	E	C	P	I	C	L	E	L
Opossum	M	-	D	L	P	T	V	T	I	E	E	V	K	N	V	L	I	G	M	Q	K	I	L	E	C	P	I	C	L	E	L
Chicken	M	-	D	L	S	V	I	A	I	G	D	V	Q	N	V	L	S	A	M	Q	K	N	L	E	C	P	V	C	L	D	V
Frog	M	-	T	C	S	R	M	D	I	E	G	I	C	S	V	I	S	V	M	Q	K	N	L	E	C	P	I	C	L	E	L
Pufferfish	M	-	E	-	-	A	P	T	A	T	D	V	K	K	R	I	S	L	L	W	E	T	L	Q	C	P	I	C	L	D	L
Sea urchin	M	A	E		-	-	V	R	L	K	K	I	T	D	S	I	G	L	M	Q	K	N	L	E	C	S	I	C	L	D	L
Tunicate	M	-	D	-	-	-	-	-	-	N	S	V	L	H	C	L	Q	Q	L	Q	K	V	V	E	C	S	I	C	L	E	T
C elegans	M	A	D	-	-	-	V	A	L	R	-	I	T	E	T	V	A	R	L	Q	K	E	L	K	C	G	I	C	C	S	T
Arabidopsis	M	A	D	-	-	-	-	-	-	-	-	-	T	S	H	L	E	R	M	G	R	E	L	K	C	P	I	C	L	S	L

Courtesy of Sean Tavtigian

Grantham Variation (GV) and Deviation (GD)



Grantham R, *Science* **185**: 862-864. 1974.
Tavtigian et al., *JMG* **43**: 295. 2006.

HVP4, UNESCO Headquarters, Paris, 11-15 June 2012

LOVD

THE HUMAN VARIOME PROJECT

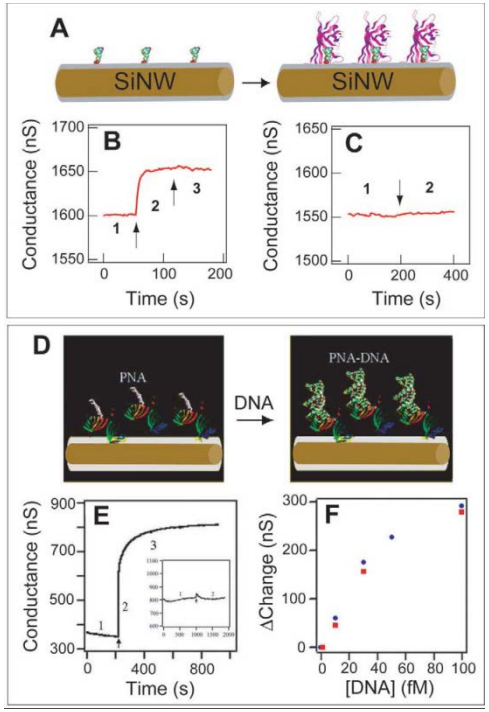
- An online reference database which will provide “free access” to all variants which have been assessed and their functional significance validated
- A background “pool” of all variants detected
- A shared resource which refines and correlates phenotypic features with genotypes
- A combination of rewards and regulation to ensure timely and complete collection of data
- A standard approach to assignment of probability

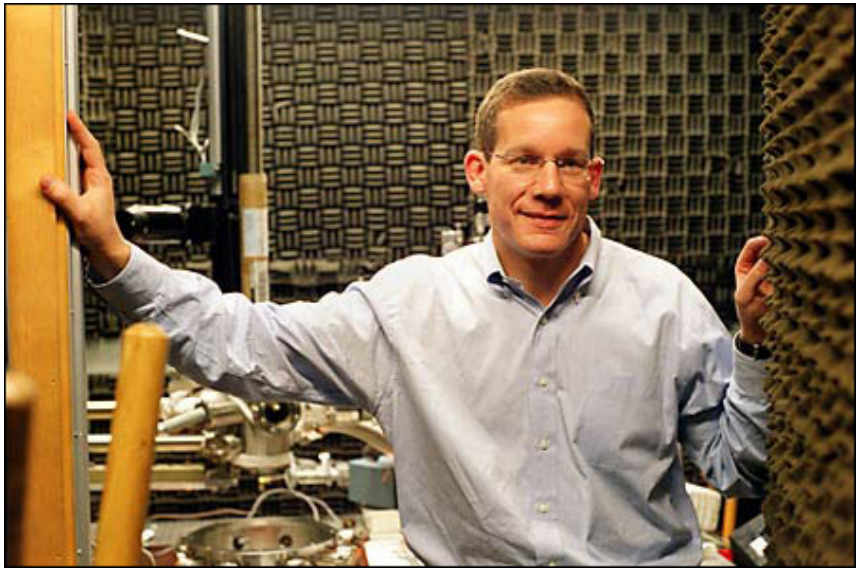
www.thehumanvariomeproject.org

Nat Genet, 2007. **39**(4): p. 433-

2001

Cui, Y., Wei, Q.Q., Park, H.K. & **Lieber, C.M**. Nanowire nanosensors for highly sensitive and selective detection of biological and chemical species. Science 293, 1289–1292 (2001).

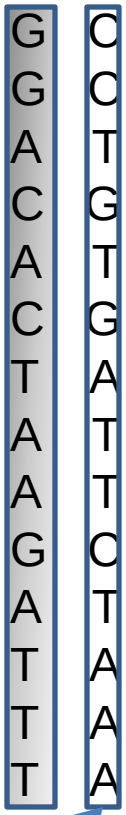




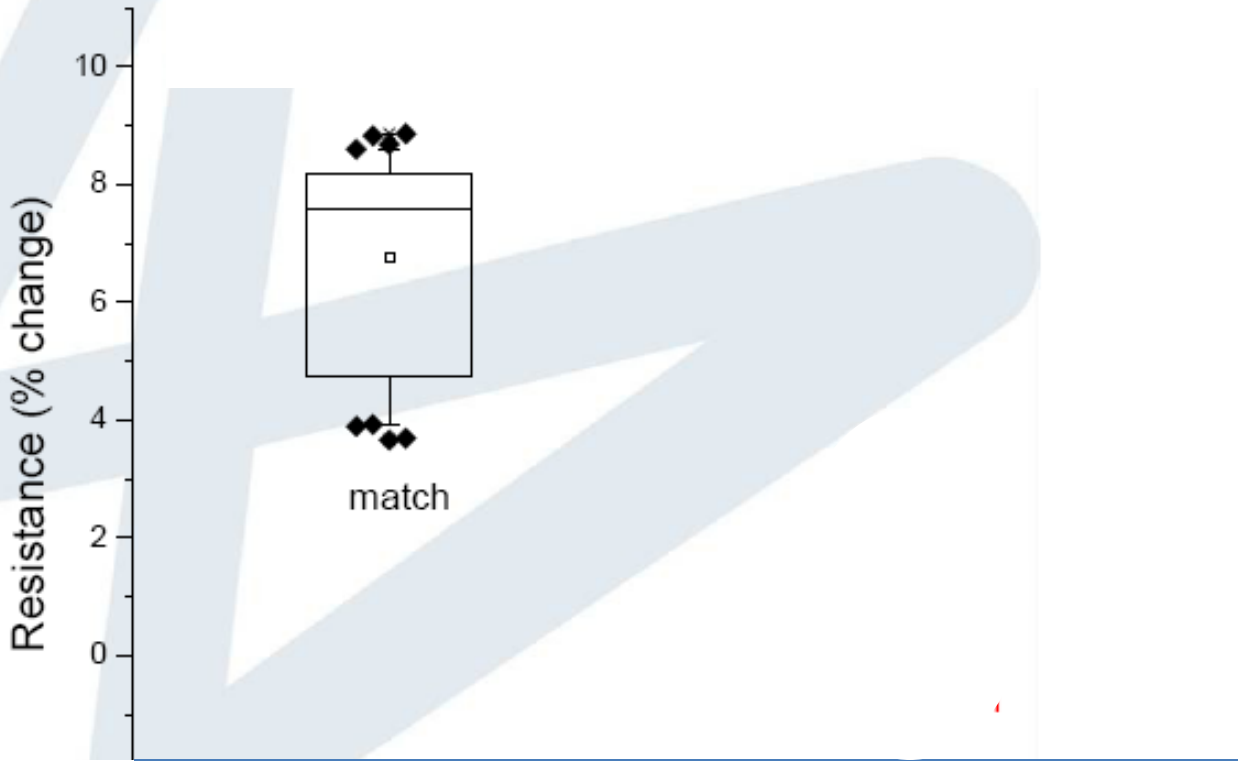
“Devices based on nanowires are emerging as a powerful platform for the direct detection of biological and chemical species, including low concentrations of proteins and viruses.” 1st July 2006 Analytic Chemistry

Stage 1 nanowire diagnostics: Proof of Principle with PNA

PNA



BRCA segment



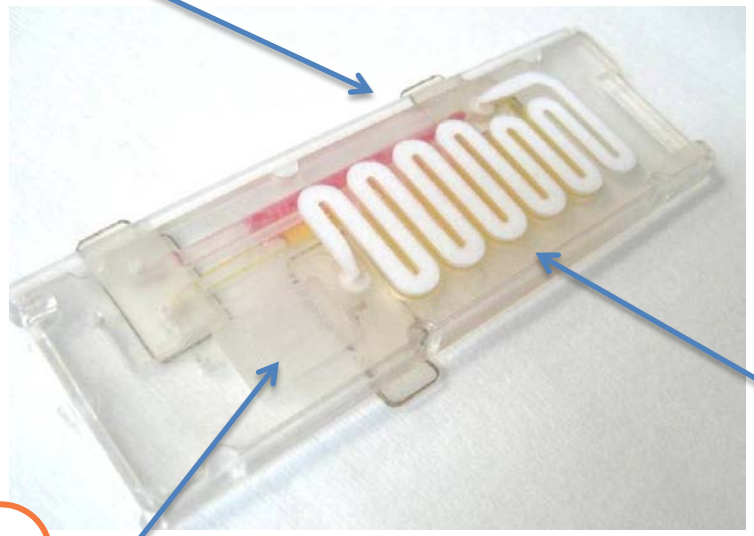
PNA binding to BRCA segment

DNA Extraction/Purification

QuantuMDx

Sample loading channel

Sample in, DNA out

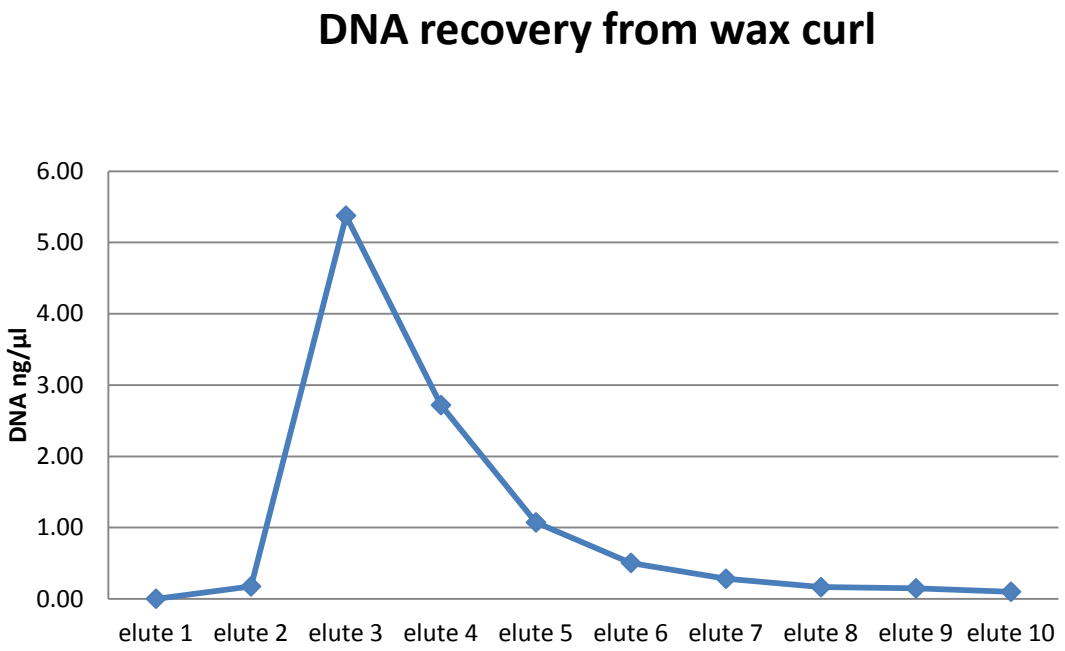


Sample collection well

Sorbent filter in channel

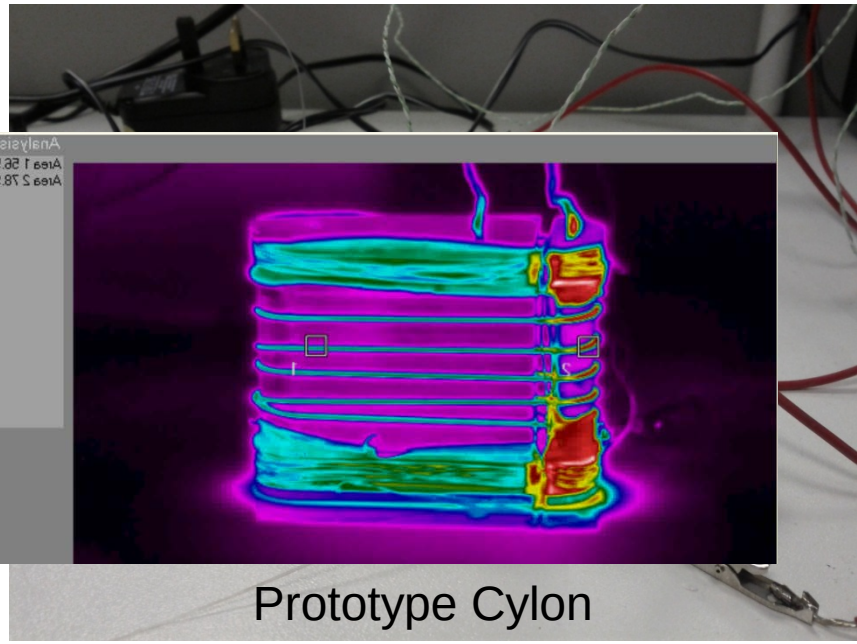
DNA Extraction from wax

- DNA recovery rate of ~56% compared to commercial kit



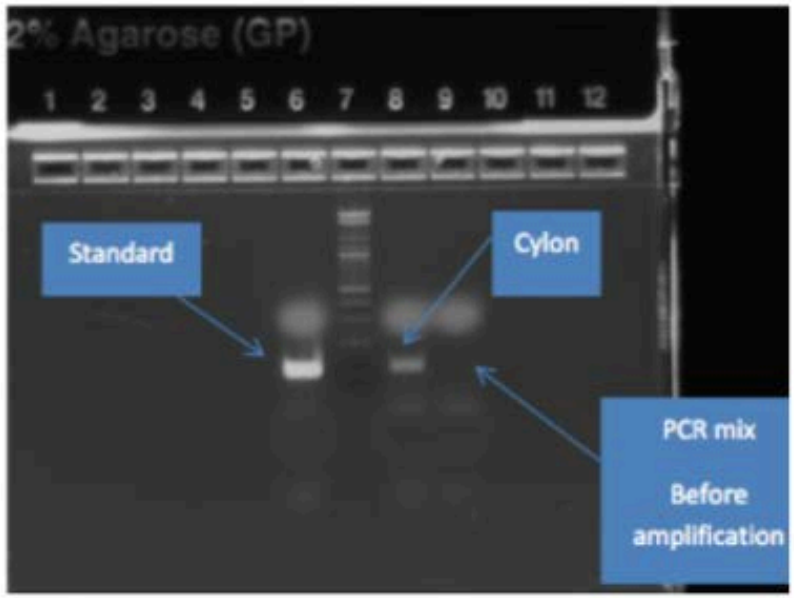
Amplification: Microfluidic thermal PCR Test rig – the Cylon

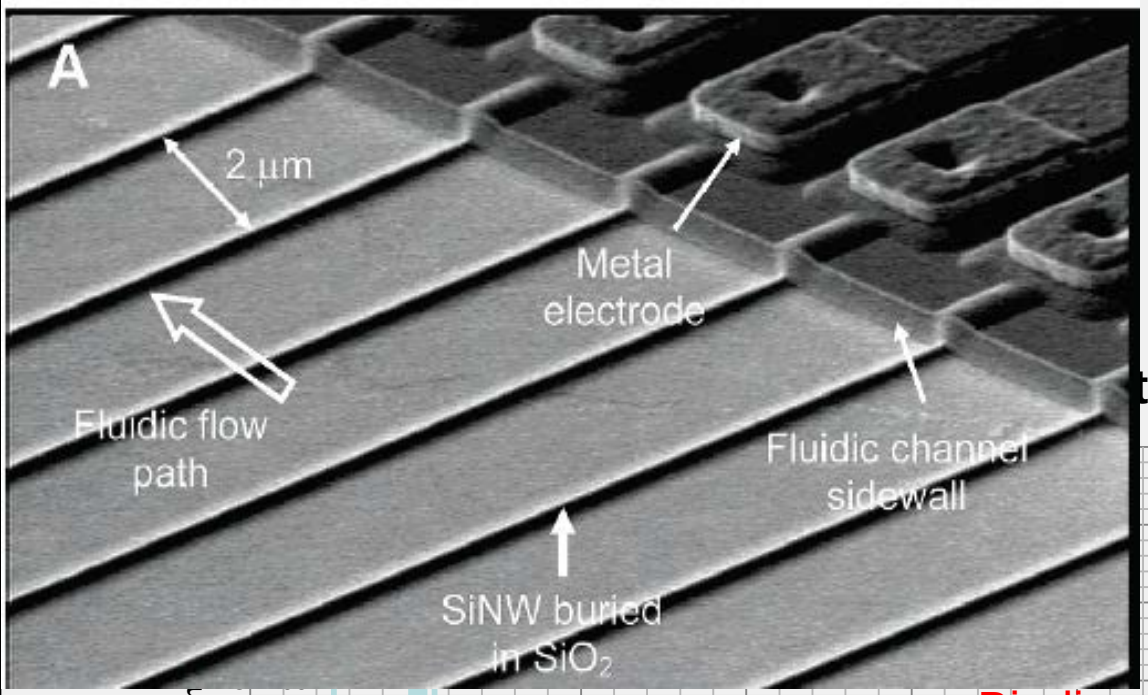
QuantuMDx



Prototype Cylon

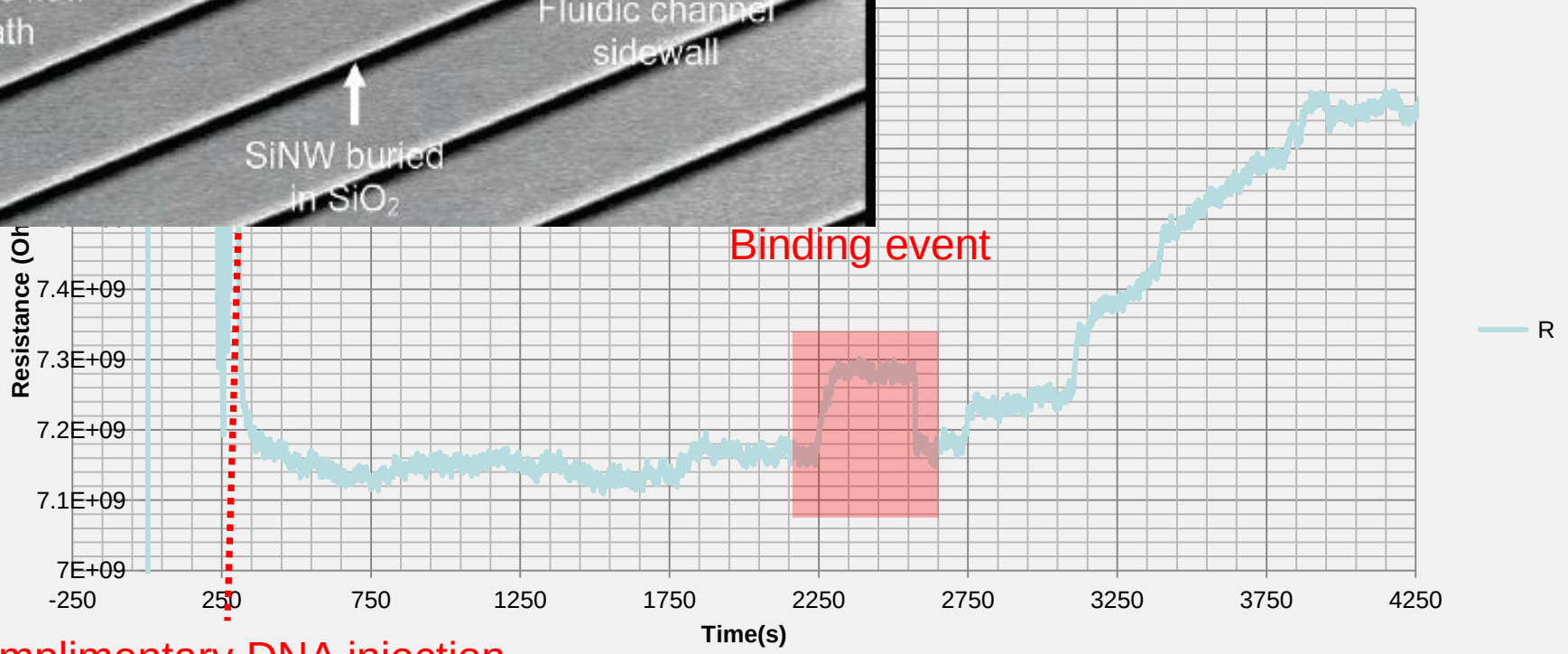
20 cycles of PCR in under 5 minutes





QuantuMD

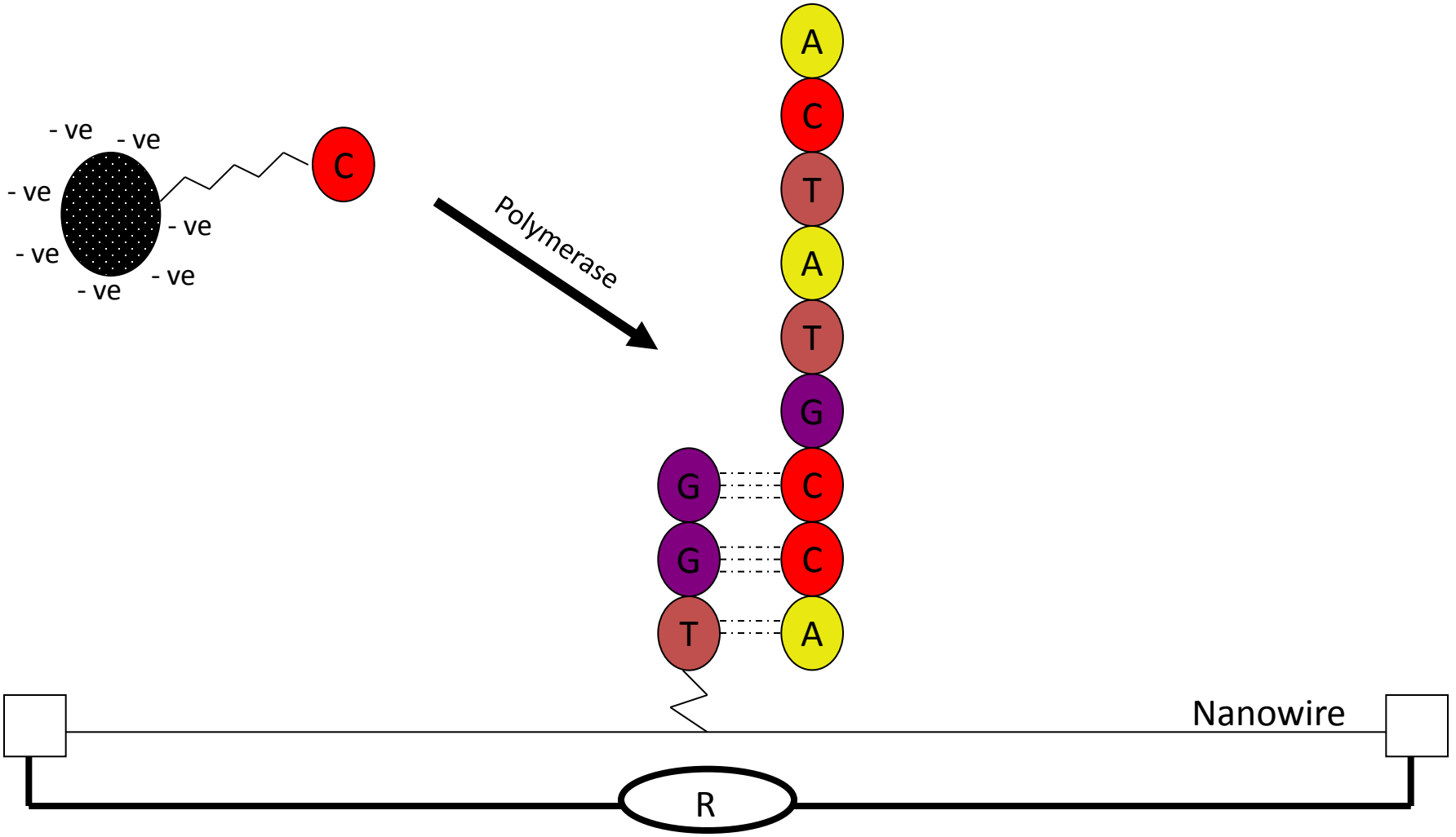
tary (50oC)



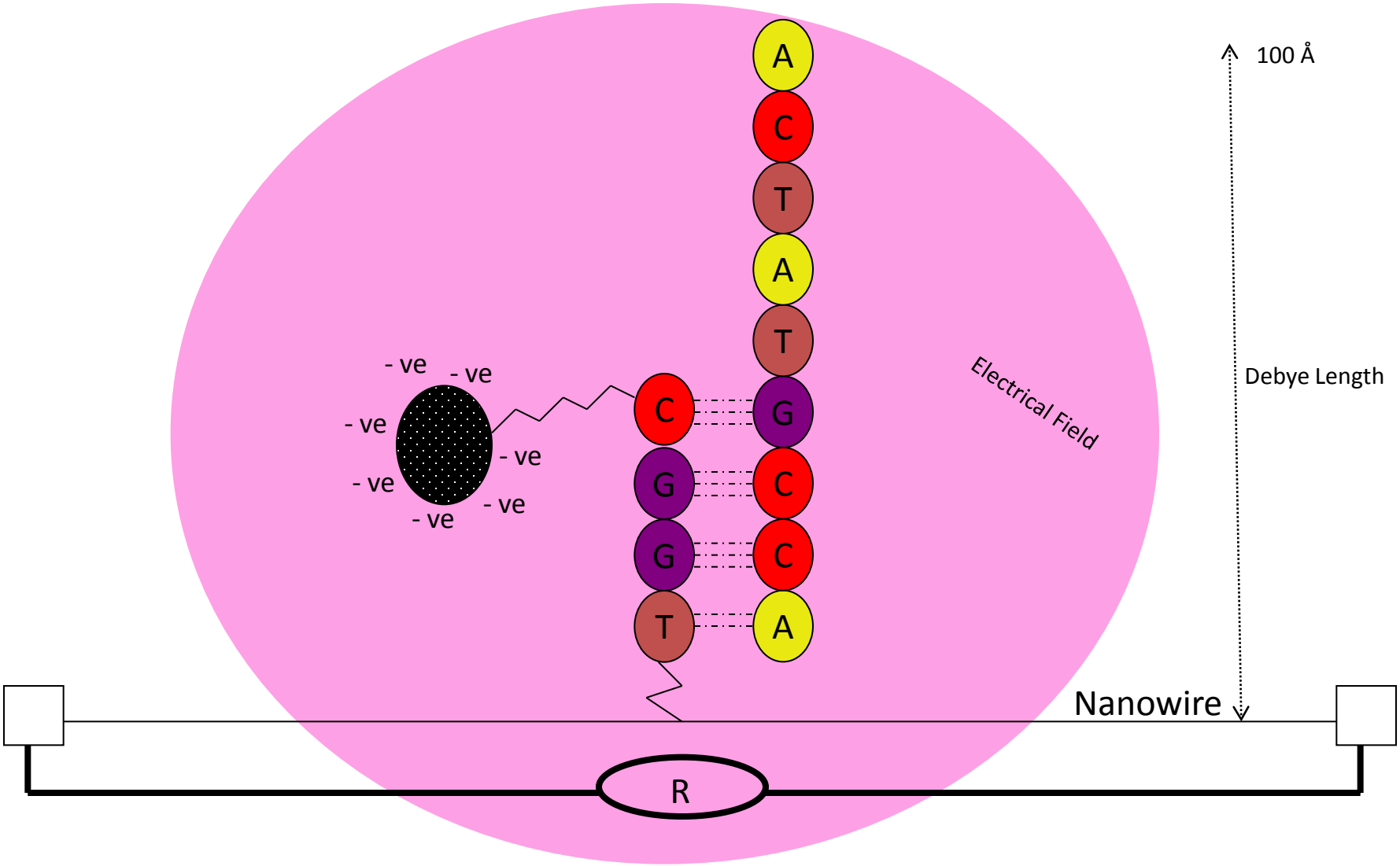
Complimentary DNA injection

Your fingernails grew 30 nanometres while you were reading this slide

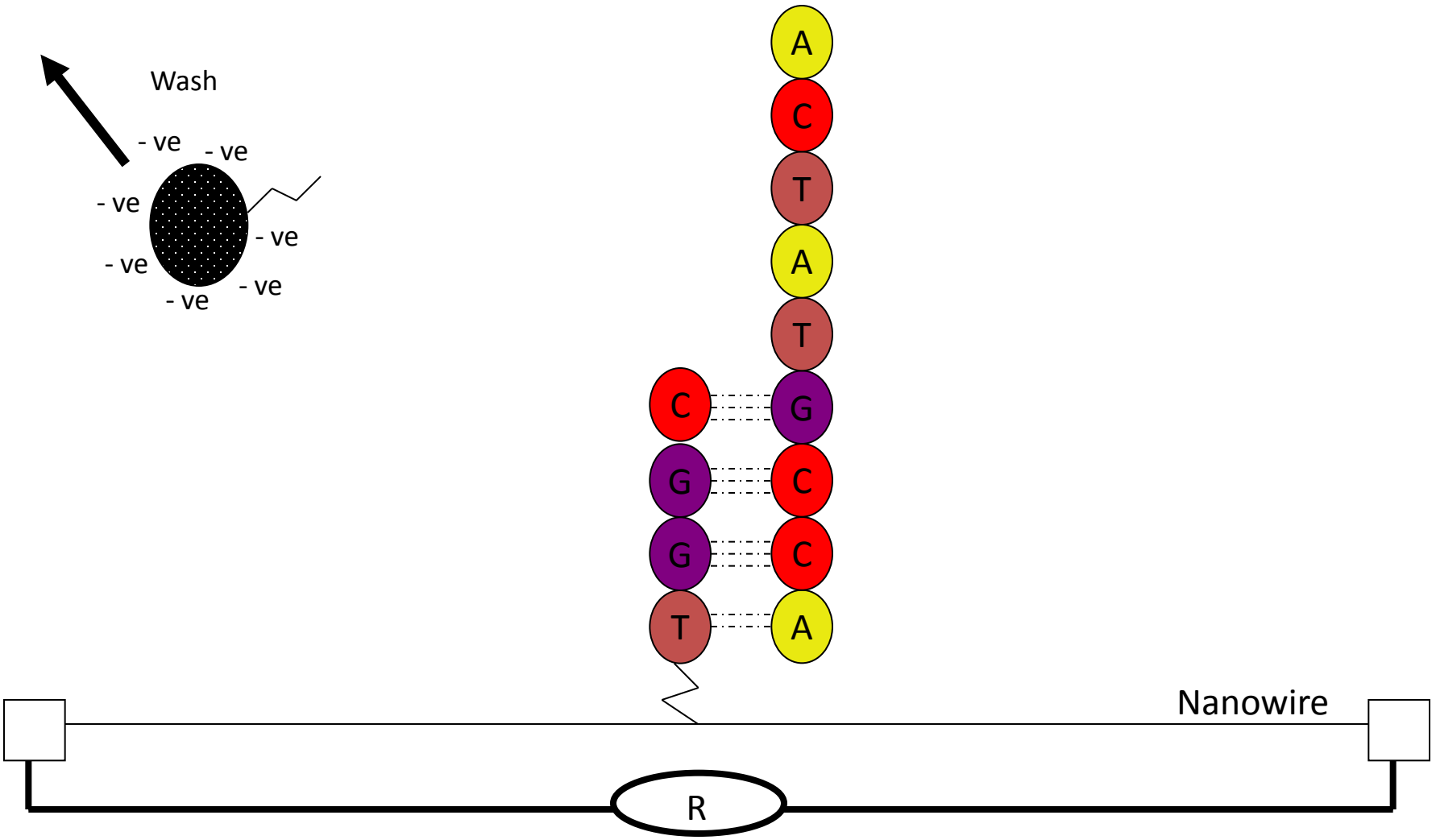
QMDx Sequencing principles



QMDx Sequencing principles



QMDx Sequencing principles



Stage 1 Q Poc: Fluidic breadboard

Keyboard + mouse

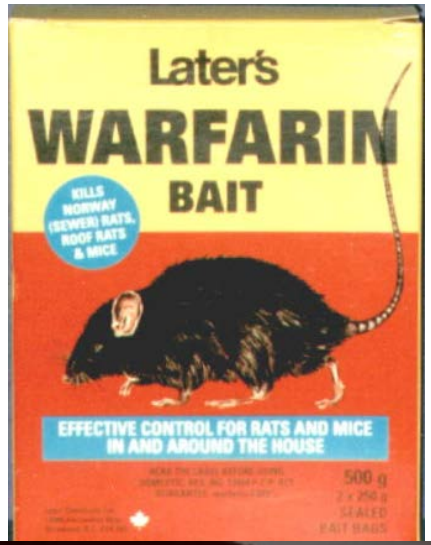
Base 600mm x 600mm

Cy

etachable nowire chip holder

Small is beautiful

Infectious diseases, pharmacogenetics, Molecular pathology



we are developing tumour mismatch repair testing using our nanowire technology

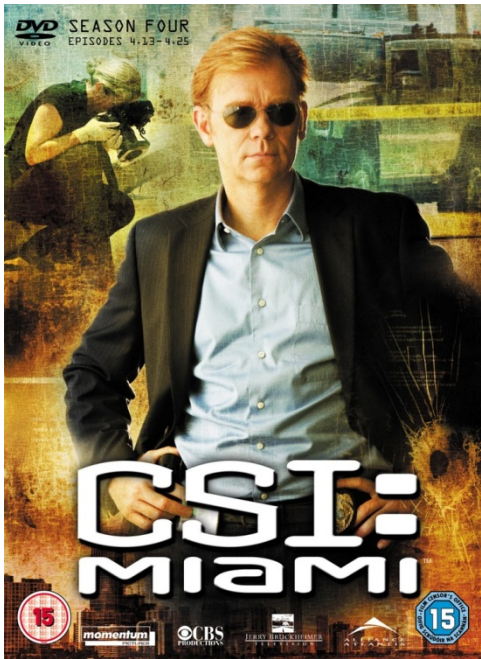
– Microsatellites are lengths of short repeated sequences eg

BAT25

tgttttgttttttgattttttttttttttttttttttttgagaacagagca

Instability indicates breakdown of mismatch repair either due to Lynch syndrome or

MLH1 silencing

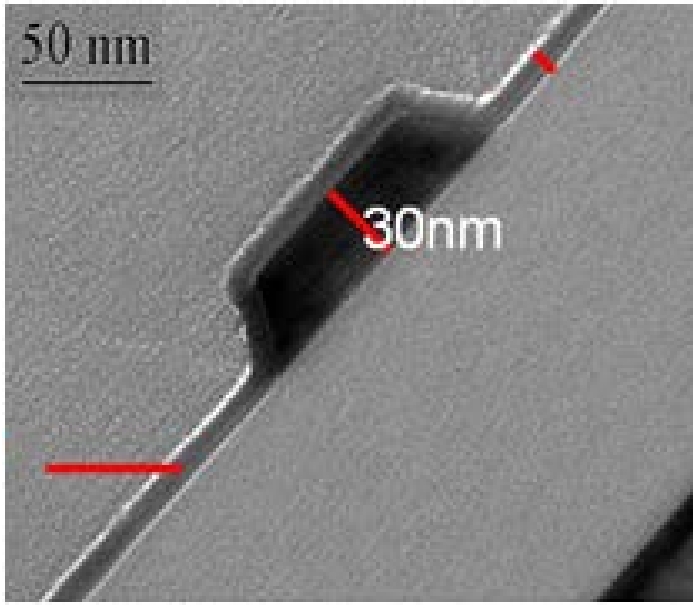


Also Coming soon!

QuantuMD

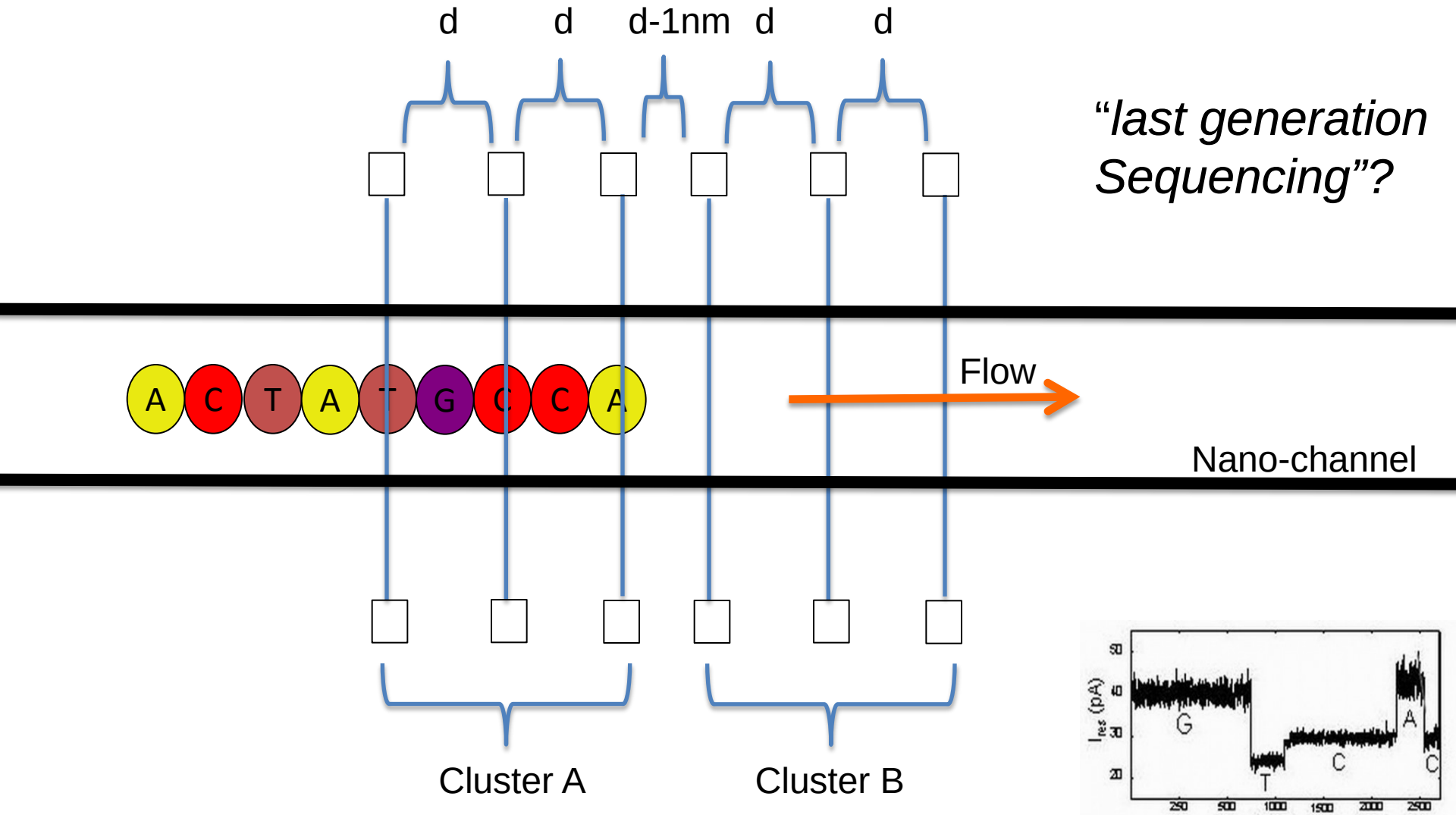
Q Seq Nano-channel Sequencing

Feeding whole DNA molecules into a nanowire-lined channel

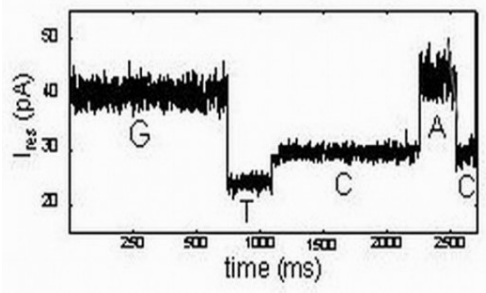


High resolution transmission electron microscopy (TEM) images for cross-sectional profile of nanochannel prepared by focused ion beam milling (FIB).

Drive intact DNA molecules
Past nanowire clusters sensitised to A,C,G or T



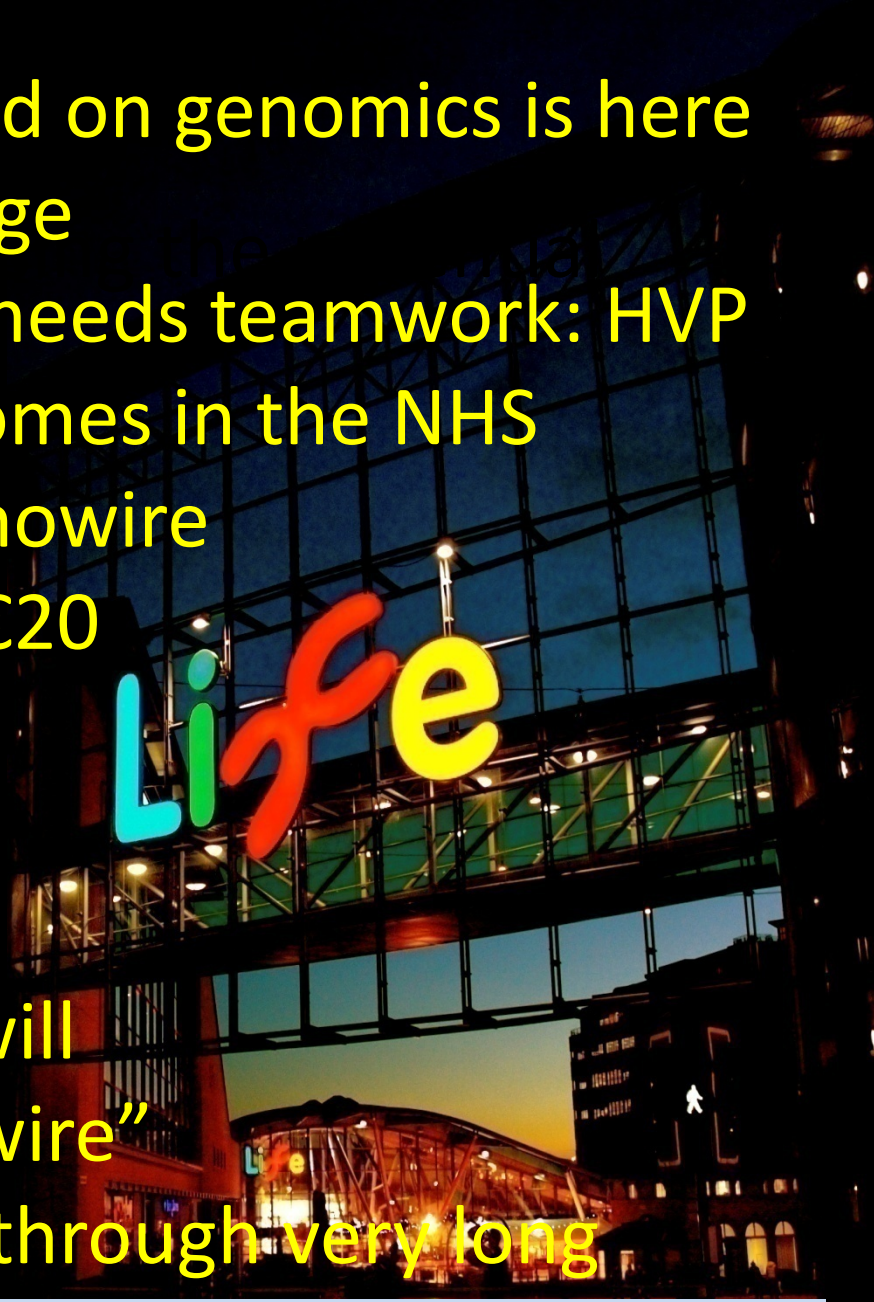
"last generation Sequencing"?



QuantuMD

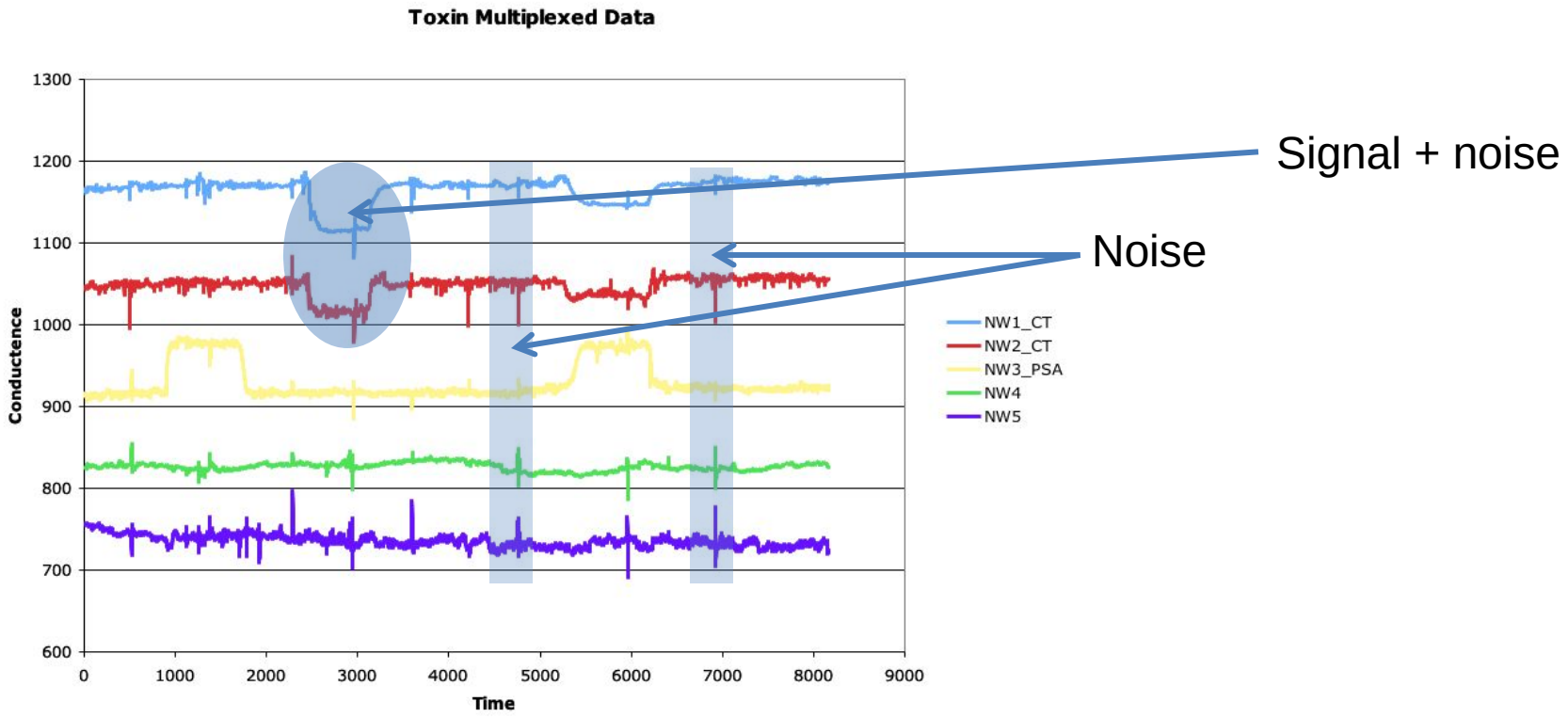
Elaine
Warburton

- Personalised medicine based on genomics is here
- Repeats/CNVs are a challenge
- Creating useful knowledge needs teamwork: HVP
- UK will generate 100k genomes in the NHS
- QuantuMDx will deliver nanowire genotyping <20 minutes <€20 at point of care
- We have lysis,extraction amplification and signal
- supercharged nucleotides will allow sequencing “on the wire”
- Nanochannels should read through very long stretches T2T?



www.quantumdx.com

HVP needs input
Curators can be a little possessive

Multi-site recording

- Multi-site recording gives easy access to the discrimination of noise events