Next generation sequencing Applications

The colored slides are modified from Johan den Dunnen.
The rest is modified from Anna Esteve Codina.

Sequencing of the human genome Public Consortium

- Many years of hard work
- BAC based approaches towards WGS
- Amplification in bacterial culture
- More than 20,000 BAC clones
- Each containing about 100kb fragment
- Together provided a tiling path through each human chromosome
- Isolation, select pieces about 2-3 kb
- Subcloned into plasmid vectors, amplification, isolation
- recreate contigs
- Refinement, gap closure, sequence quality improvement
- (less 1 error/ 40,000 bases)

Sanger vs NGS

'Sanger sequencing' has been the only DNA sequencing method for 30 years but…

…hunger for even greater sequencing throughput and more economical sequencing technology…

NGS has the ability to process millions of sequence reads in parallel rather than 96 at a time (1/6 of the cost)

Objections: fidelity, read length, infrastructure cost, handle large volumn of data

Platforms

- Roche/454 FLX: 2004
- Illumina Solexa Genome Analyzer: 2006
- Applied Biosystems SOLiD™ System: 2007
- Helicos Heliscope™ : 2010
- Pacific Biosciencies SMRT: launching 2010

454 vs Solexa

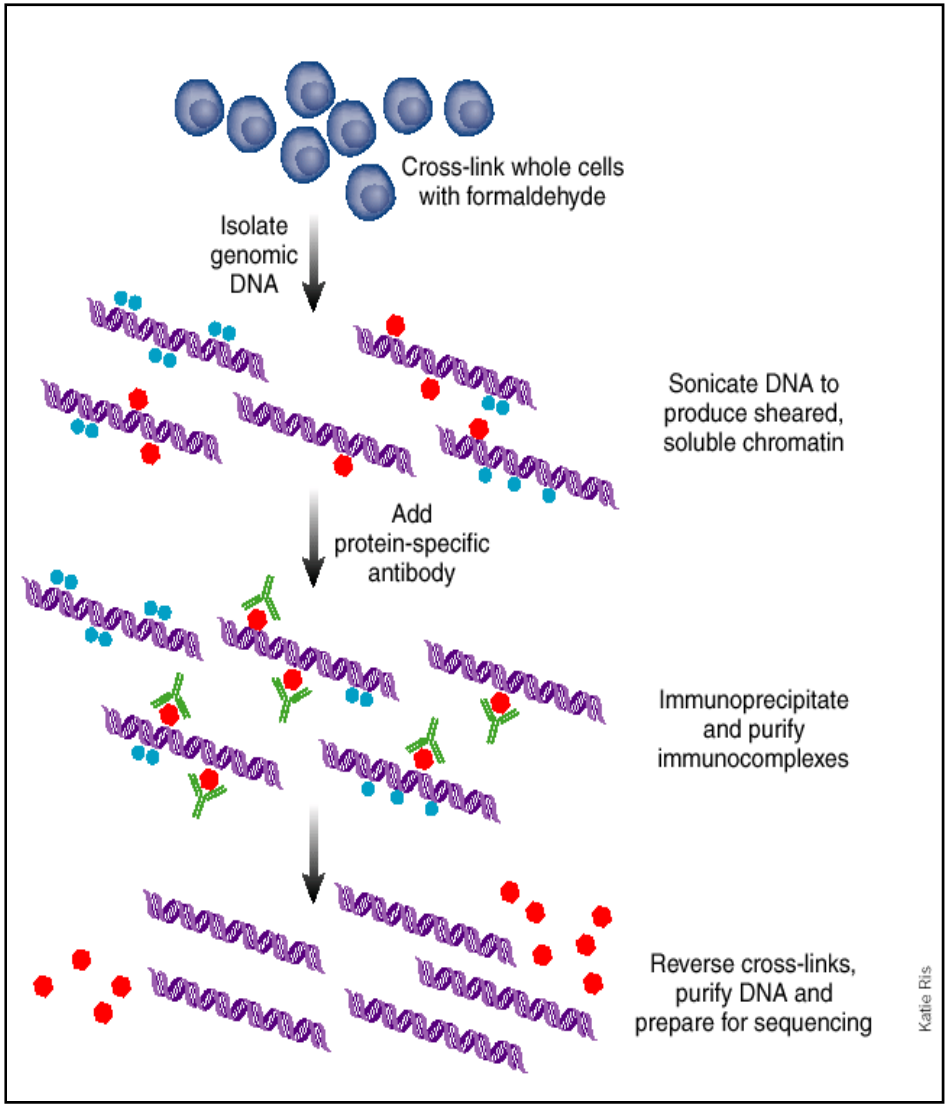
- Homopolymers (AAAAA..)
- Read length: 400 bp
- Number of reads: 400.000
- Per-base cost greater
- Novo assembly, metagenomics
- Read length: 40 bp
- Number of reads: millions
- Per-base cost cheaper
- Ideal for application requiring short reads: ncRNA

Applications

- Ancient DNA
- DNA mixtures from diverse ecosystems, metagenomics
- Resequencing previously published reference strains
- Identification of all mutations in an organism
- Errors in published literature
- Expand the number of available genomes
- Comparative studies
- Deciphering cell's transcripts at sequence level without knowledge of the genome sequence
- Sequencing extremely large genomes, crop plants
- Detection of cancer specific alleles avoiding traditional cloning
- ChIP-seq: interactions protein-DNA
- Epigenomics
- Detecting ncRNA
- Genetic human variation : SNP, CNV (diseases)

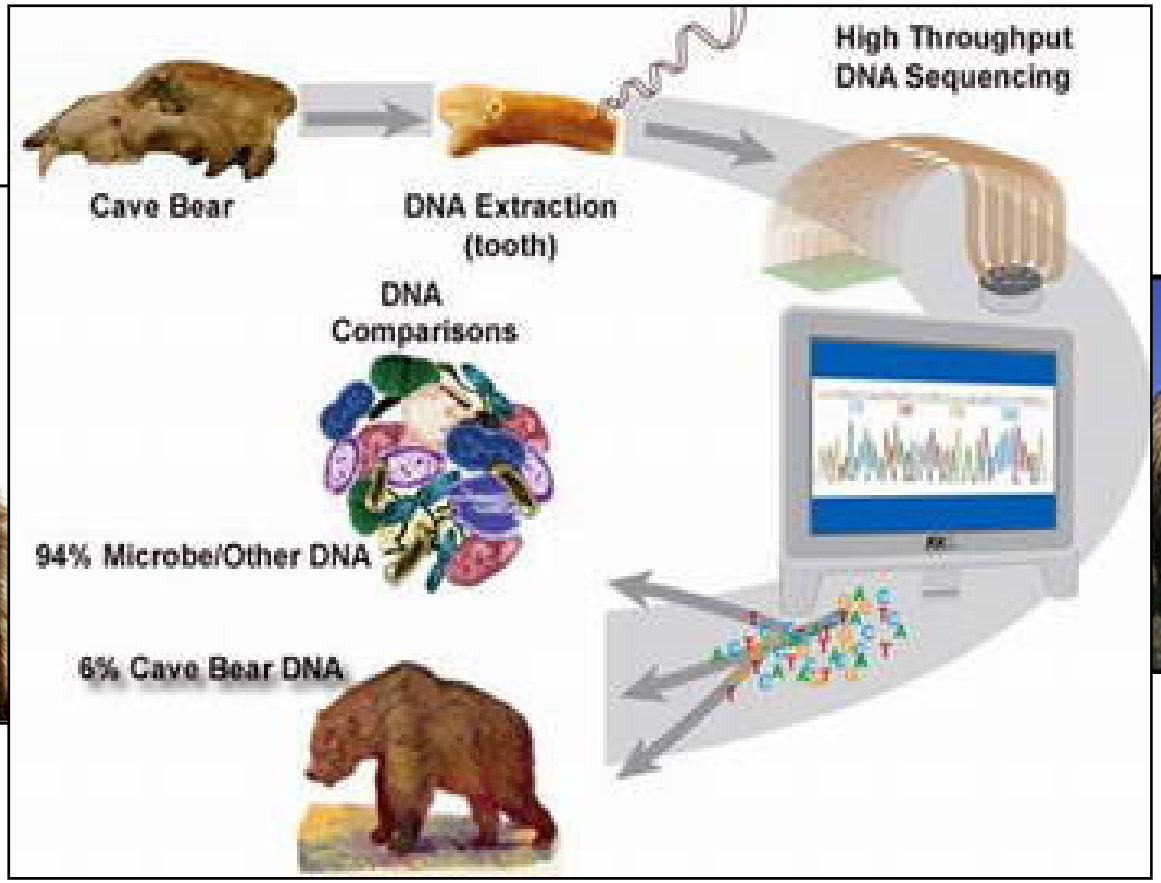
Elucidating DNA-protein interactions through chromoatin immunoprecipitation sequencing

- **Key part in regulating gene expression**
- **Chip: technique to study DNA-protein interaccions**
- **Recently genome-wide ChIP-based studies of DNA-protein interactions**
- **Readout of ChIP-derived DNA sequences onto NGS platforms**
- **Insights into transcription factor/histone binding sites in the human genome**
- **Enhance our understanding of the gene expression in the context of specific environmental stimuli**



Ancient Genomes Resurrected

- **Degraded state of the sample → mitDNA sequencing**
- **Nuclear genomes of ancient remains: cave bear, mommoth, Neanderthal (10^6 bp)**



Problems: contamination modern humans and coisolation bacterial DNA

De novo genomes

Vol 456|20 November 2008|doi:10.1038/nature07446 nature

Sequencing the nuclear genome of the extinct woolly mammoth

Webb Miller[1], Daniela I. Drautz[1], Aakrosh Ratan[1], Barbara Pusey[1], Ji Qi[1], Arthur M. Lesk[1], Lynn P. Tomsho[1], Michael D. Packard[1], Fangqing Zhao[1], Andrei Sher[2]‡, Alexei Tikhonov[3], Brian Raney[4], Nick Patterson[5], Kerstin Lindblad-Toh[5], Eric S. Lander[5], James R. Knight[6], Gerard P. Irzyk[6], Karin M. Fredrikson[7], Timothy T. Harkins[7], Sharon Sheridan[7], Tom Pringle[8] & Stephan C. Schuster[1]

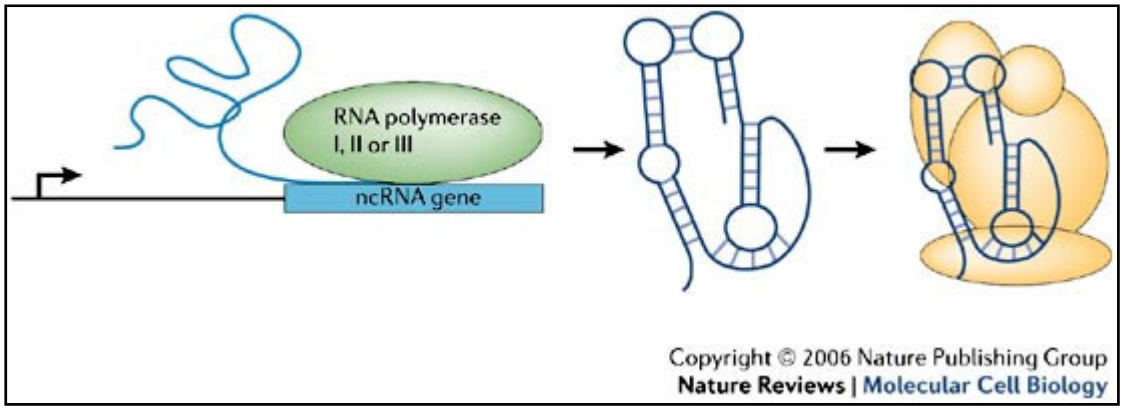
Science **328**, 710 (2010)

A Draft Sequence of the Neandertal Genome

Richard E. Green,[1*†‡] Johannes Krause,[1†§] Adrian W. Briggs,[1†§] Tomislav Maricic,[1†§] Udo Stenzel,[1†§] Martin Kircher,[1†§] Nick Patterson,[2†§] Heng Li,[2†] Weiwei Zhai,[3†‖] Markus Hsi-Yang Fritz,[4†] Nancy F. Hansen,[5†] Eric Y. Durand,[3†] Anna-Sapfo Malaspinas,[3†] Jeffrey D. Jensen,[6†] Tomas Marques-Bonet,[7,13†] Can Alkan,[7†] Kay Prüfer,[1†] Matthias Meyer,[1†] Hernán A. Burbano,[1†] Jeffrey M. Good,[1,8†] Rigo Schultz,[1] Ayinuer Aximu-Petri,[1] Anne Butthof,[1] Barbara Höber,[1] Barbara Höffner,[1] Madlen Siegemund,[1] Antje Weihmann,[1] Chad Nusbaum,[2] Eric S. Lander,[2] Carsten Russ,[2] Nathaniel Novod,[2] Jason Affourtit,[9] Michael Egholm,[9] Christine Verna,[21] Pavao Rudan,[10] Dejana Brajkovic,[11] Željko Kucan,[10] Ivan Gušic,[10] Vladimir B. Doronichev,[12] Liubov V. Golovanova,[12] Carles Lalueza-Fox,[13] Marco de la Rasilla,[14] Javier Fortea,[14¶] Antonio Rosas,[15] Ralf W. Schmitz,[16,17] Philip L. F. Johnson,[18†] Evan E. Eichler,[7†] Daniel Falush,[19†] Ewan Birney,[4†] James C. Mullikin,[5†] Montgomery Slatkin,[3†] Rasmus Nielsen,[3†] Janet Kelso,[1†] Michael Lachmann,[1†] David Reich,[2,20*†] Svante Pääbo[1*†]

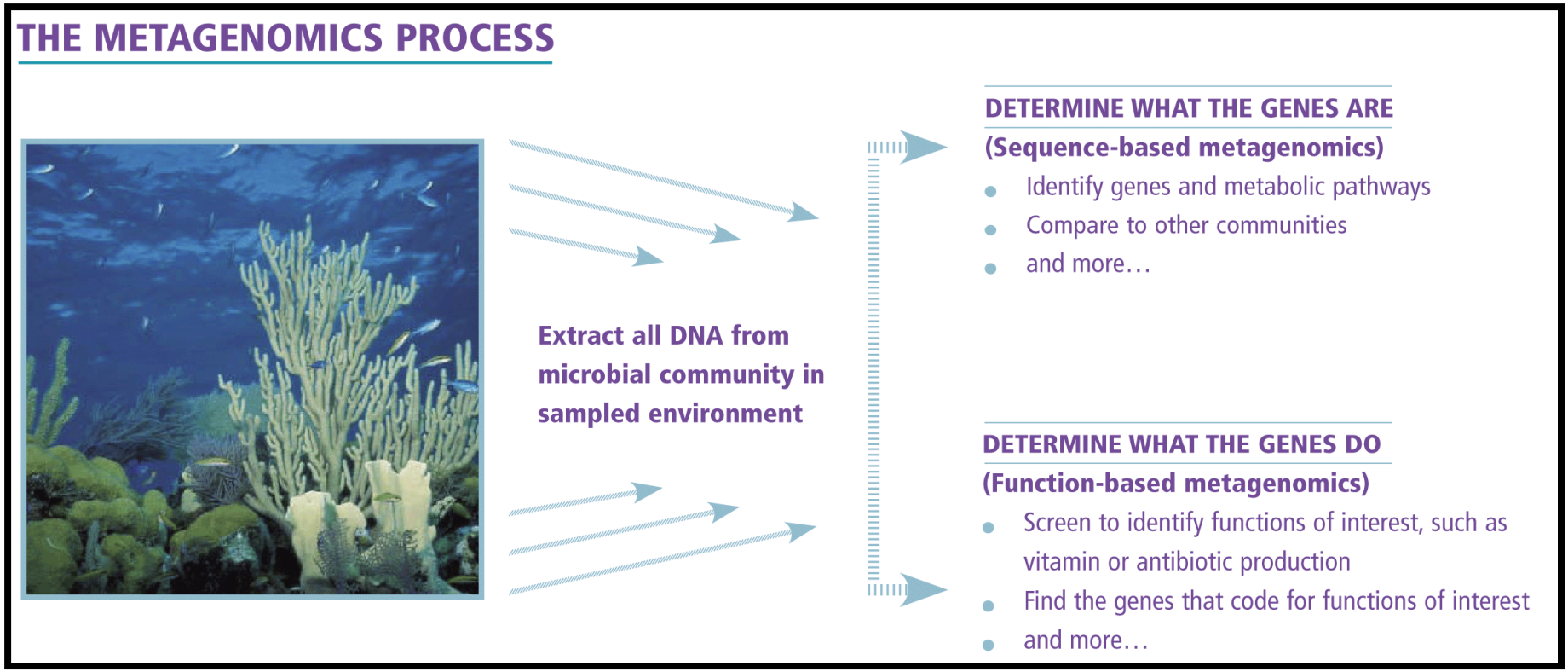
Discovering noncoding RNAs

- ncRNA presence in genome difficult to predict by computational methods with high certainty because the evolutionary diversity
- Detecting expression level changes that correlate with changes in environmental factors, with disease onset and progression, complex disease set or severity
- Enhance the annotation of sequenced genomes (impact of mutations more interpretable)



Metagenomics

- **Characterizing the biodiversity found on Earth**
- **The growing number of sequenced genomes enables us to interpret partial sequences obtained by direct sampling of specific environmental niches.**
- **Examples: ocean, acid mine site, soil, coral reefs, human microbiome which may vary according to the health status of the individual**



Metagenomics$_2$

microbiome
(biological diversity)

994 21 MAY 2010 VOL 328 SCIENCE

A Catalog of Reference Genomes from the Human Microbiome

The Human Microbiome Jumpstart Reference Strains Consortium*†

Cell Host & Microbe Cell Host & Microbe 3, 213–223, April 2008 ©2008 Elsevier Inc.

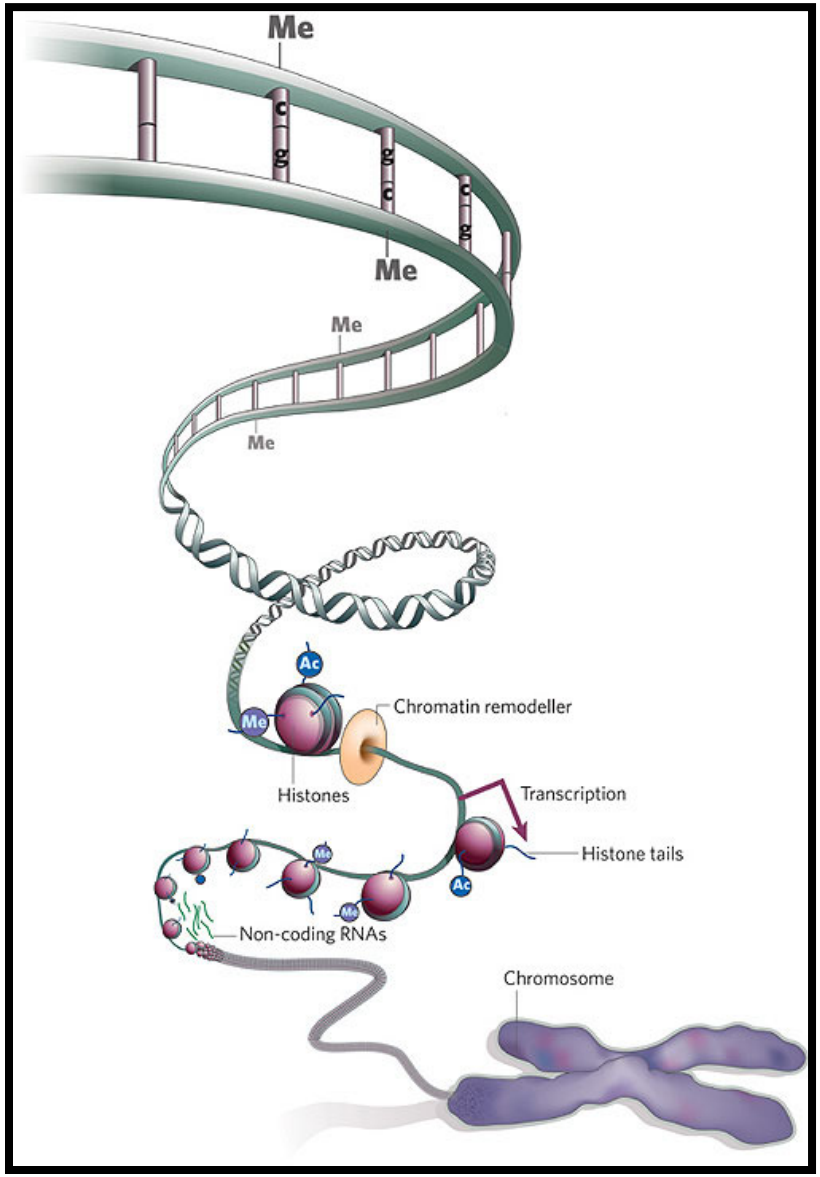
Diet-Induced Obesity Is Linked to Marked but Reversible Alterations in the Mouse Distal Gut Microbiome

Peter J. Turnbaugh,[1] Fredrik Bäckhed,[3] Lucinda Fulton,[2] and Jeffrey I. Gordon[1,*]

Epigenomic variation

- Enable of genome-wide patterns of methylation and how this patterns change through the course of an organism's development.

- Enhanced potential to combine the results of different experiments, correlative analyses of genome-wide methylation, histone binding patterns and gene expression, for example.



More about epigenetics…

- Epigenetics: beyond the sequence. "The major problem, I think, is **chromatin**. What determines whether a given piece of DNA along the chromosome is functioning, since it's covered with the histones? What is happening at the level of methylation and **epigenetics**? You can inherit something beyond the DNA sequence. That's where the real excitement of genetics is now." (James D. Watson). Chromatin is defined as the dynamic complex of DNA and histone proteins that makes up chromosomes.

- Epigenetics is defined as the chemical modification of DNA that affects gene expression but does not involve changes to the underlying DNA sequence. As the emphasis in biology is switching away from genetic sequence and towards the mechanisms by which gene activity is controlled, epigenetics is becoming increasingly popular.

Epigenetic processes are essential for packaging and interpreting the genome, are fundamental to normal development and are increasingly recognized as being involved in human disease. Epigenetic mechanisms include, among other things, histone modification, positioning of histone variants, nucleosome remodelling, DNA methylation, small and non-coding RNAs. (*Nature*, 7 Aug 2008).

Gene expression profiling

Nucleic Acids Research, 2008, 1–12

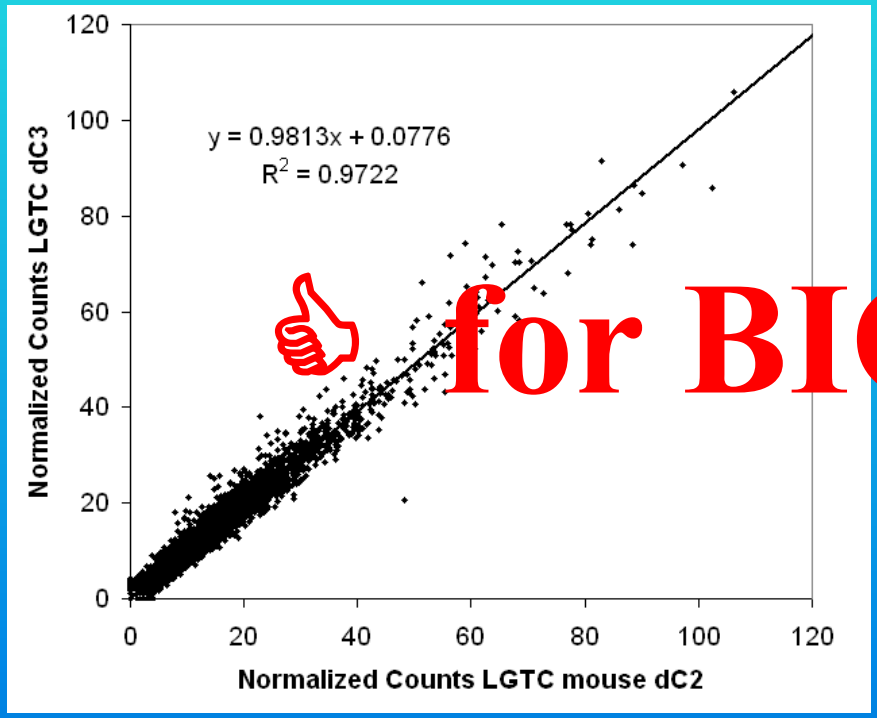
Deep sequencing-based expression analysis shows major advances in robustness, resolution and inter-lab portability over five microarray platforms

Peter A. C. 't Hoen[1,*], Yavuz Ariyurek[1], Helene H. Thygesen[1], Erno Vreugdenhil[2], Rolf H. A. M. Vossen[1], Renée X. de Menezes[1], Judith M. Boer[1], Gert-Jan B. van Ommen[1] and Johan T. den Dunnen[1]

[1]The Center for Human and Clinical Genetics and the Leiden Genome Technology Center, Leiden University Medical Center and [2]The Department of Medical Pharmacology from the Leiden/Amsterdam Center for Drug Research, Leiden, The Netherlands

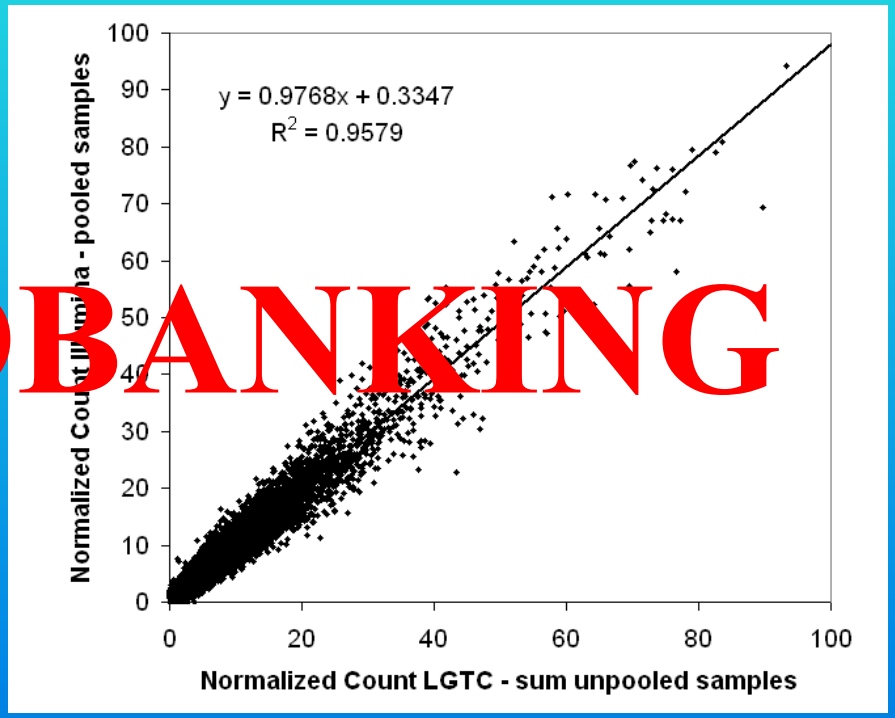
Lab2lab consistency

2 transgenic mice



(biological replicas)

2 different labs



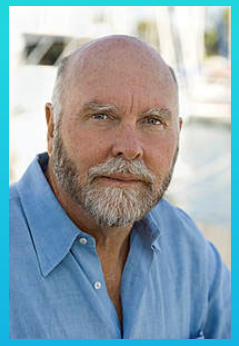
(Illumina <> Leiden)

for BIOBANKING

square root-transformed and scaled data

Human genomes

(Individual genomes)



Craig Venter

James Watson

Marjolein Kriek

<u>ANONYMOUS:</u>
Yoruban male
Yoruban trio
Asiatic genome
Female Cancer


NEWS.com.au | Fox Sports | Newspapers | CareerOne | carsguide
Adelaidenow...
adelaidenow.com.au
May 27, 2008 08:30pm
The Advertiser | Sunday Mail
News Sport Business Money Entertainment Travel Lifes
Homepage Breaking News South Australia National World Techno
Scientists crack women's DNA code
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.

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

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

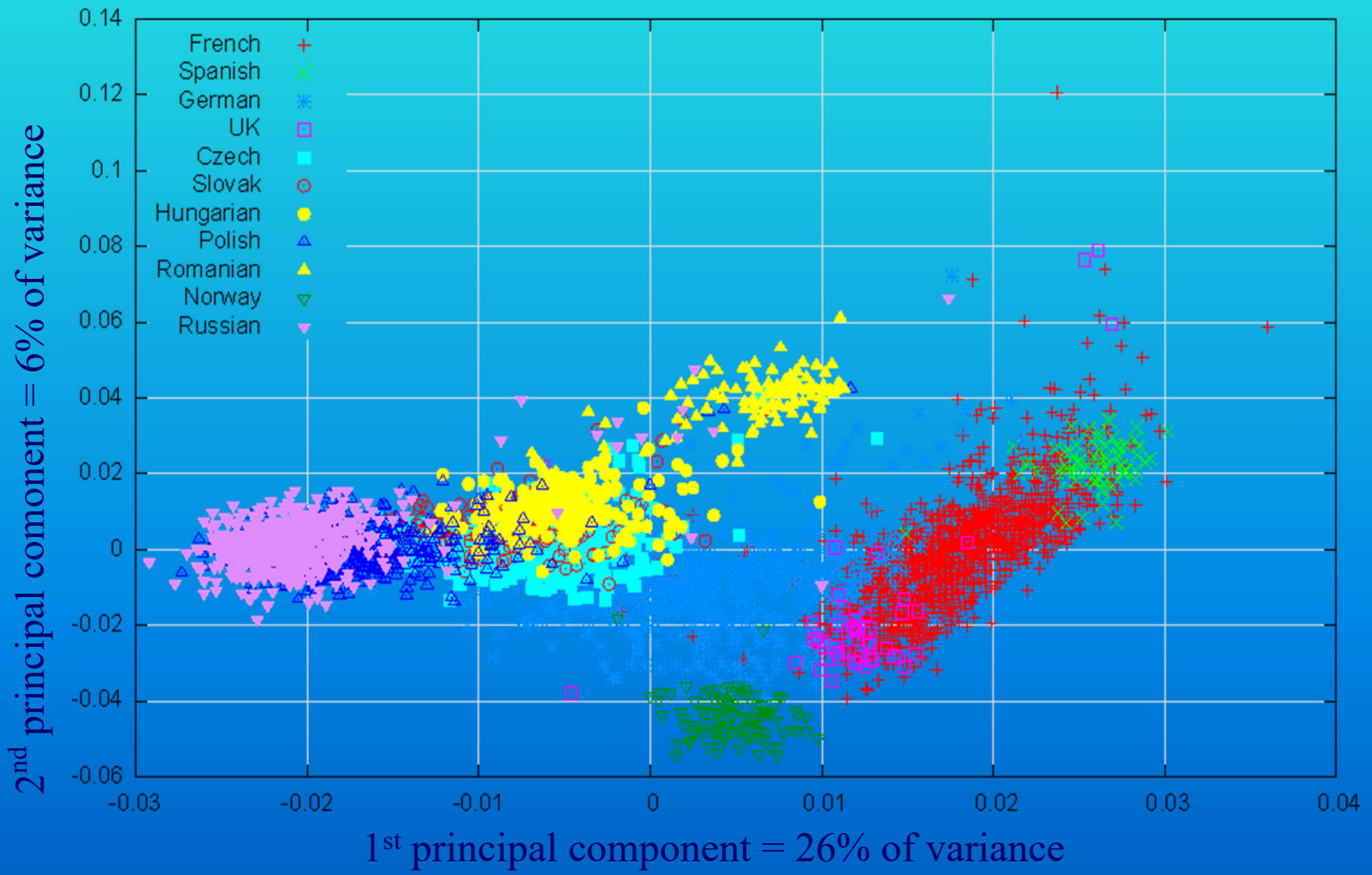
NATIONAL GEOGRAPHIC™ IBM

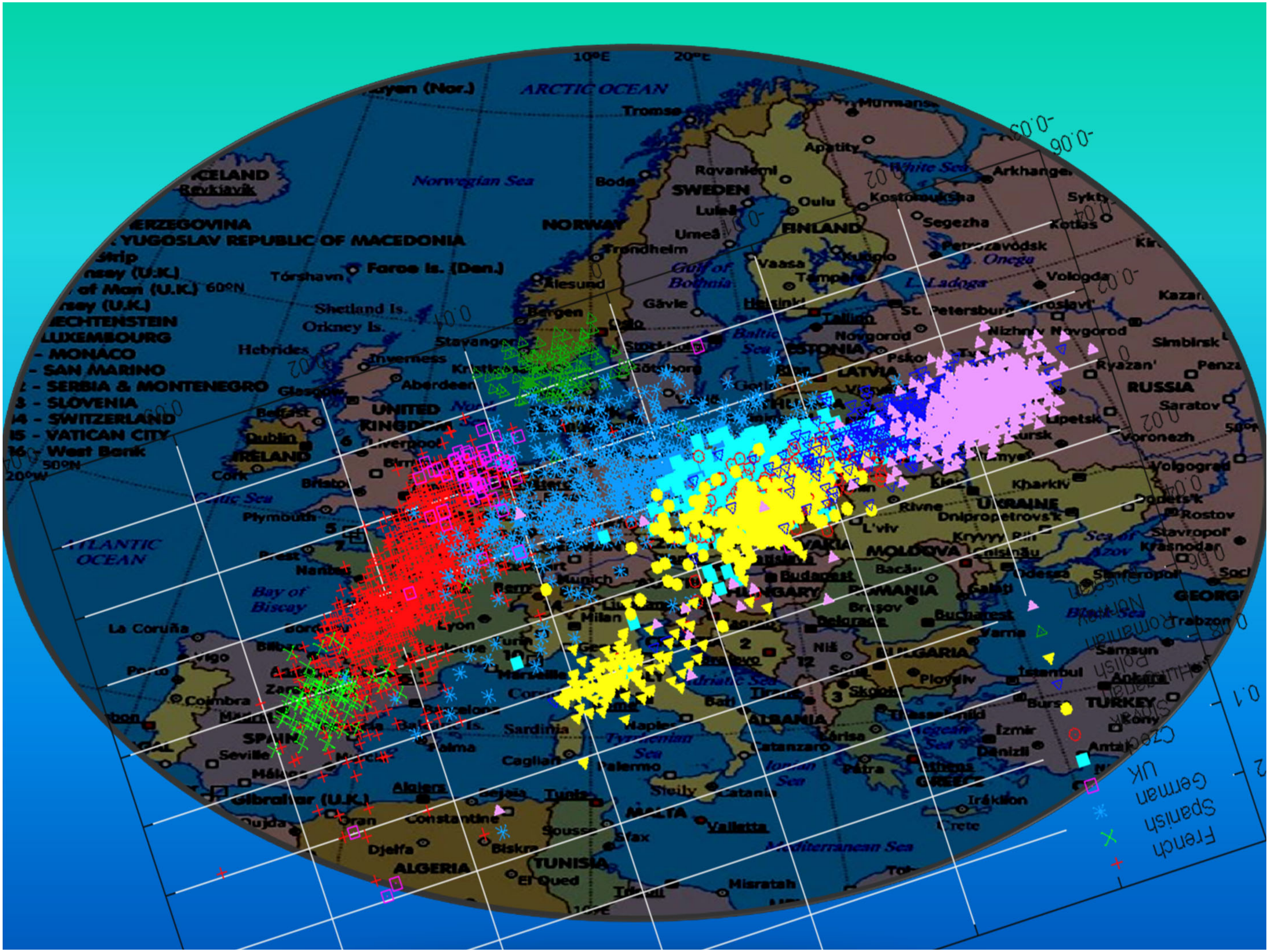
A research partnership of National Geographic and IBM

NATIONALGEOGRAPHIC.COM

Principal component analysis of European populations

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




French
Spanish
German
UK
Czech
Polish
Romanian
Norwegian
ARCTIC OCEAN
Norwegian Sea
ATLANTIC OCEAN
Bay of Biscay
Baltic Sea
Black Sea
Mediterranean Sea
SWEDEN
FINLAND
NORWAY
UNITED KINGDOM
IRELAND
SPAIN
ALGERIA
TUNISIA
HUNGARY
ROMANIA
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TURKEY
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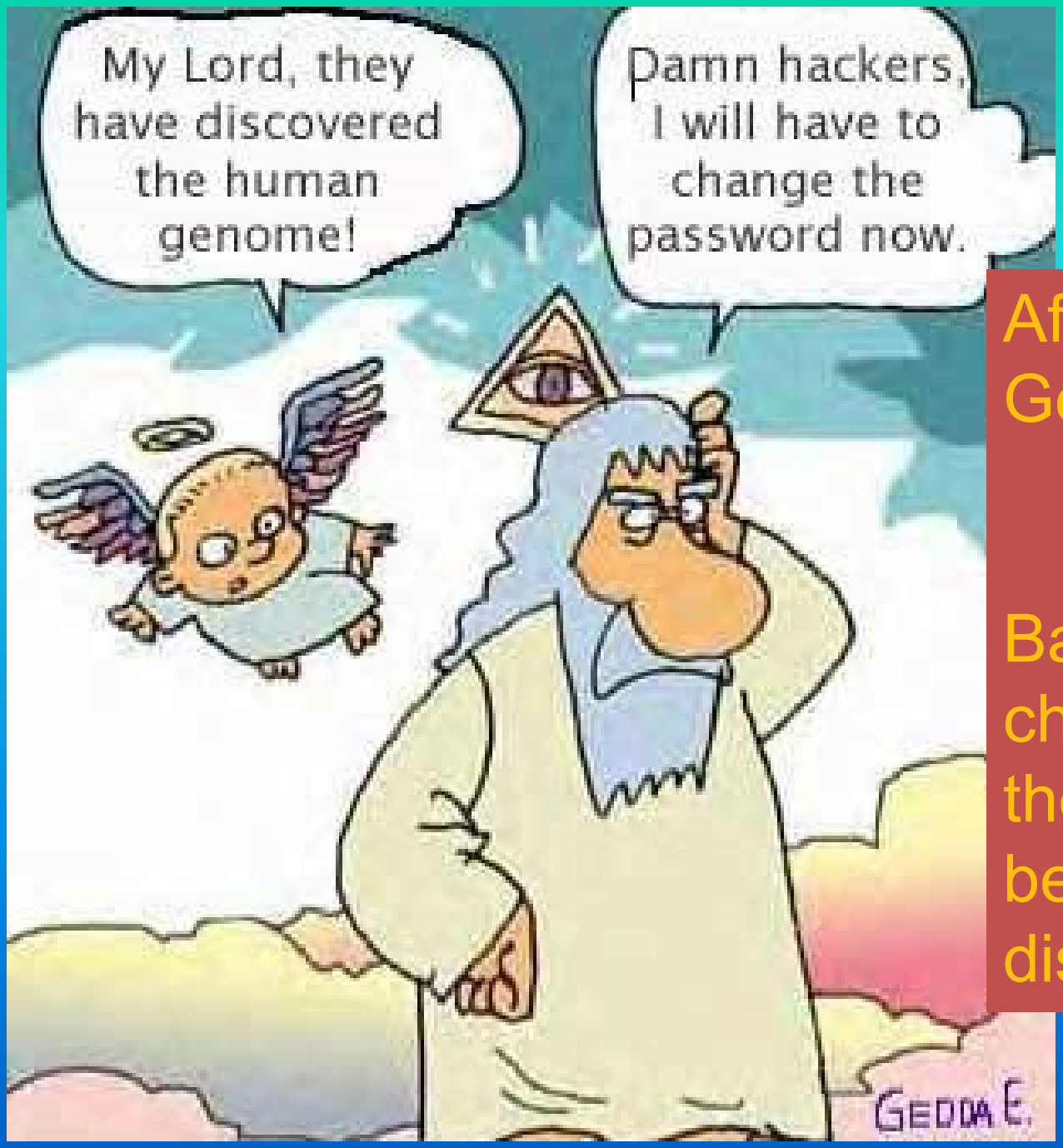
MOLDOVA
RUSSIA
LATVIA
ESTONIA
ALBANIA
GREECE
MALTA



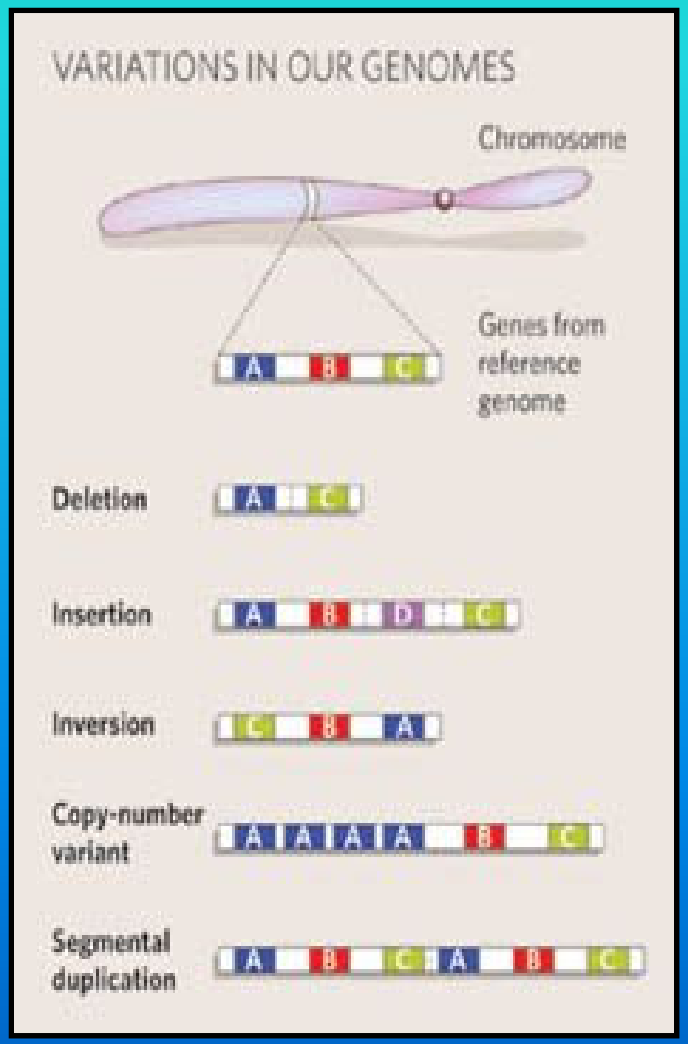
After Human Genome Project

⬇

Basic tools exist to characterize the biology behind human diseases

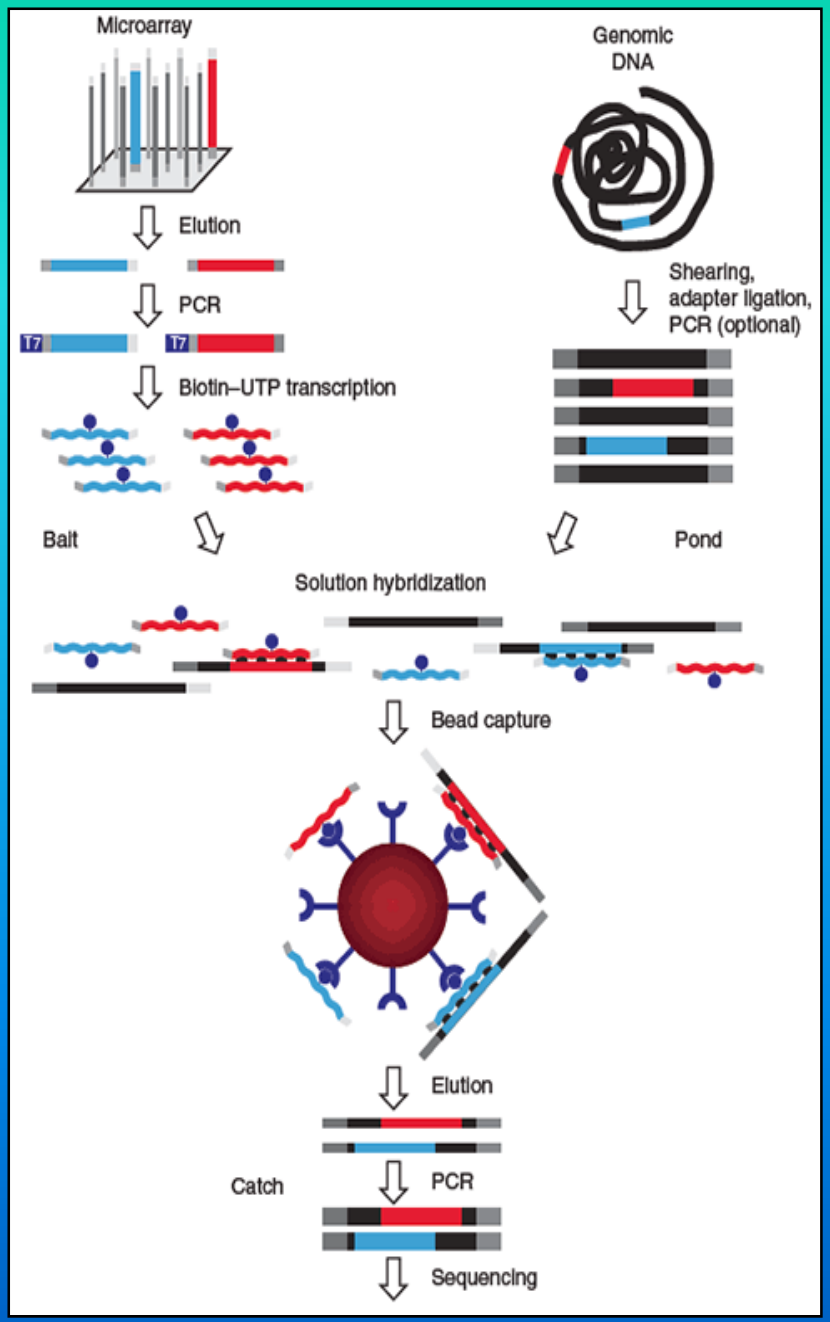
Defining variability in many human genomes

- Common variants have not yet completly explained complex disease genetics →rare alleles also contribute
- Also structural variants, large and small insertions and deletions
- Accelerating biomedical research



Mutation discovery

- Extreme example: multiplexing the amplification of 10 000 human exons using primers from a programmable microarray and sequencing them using NGS.



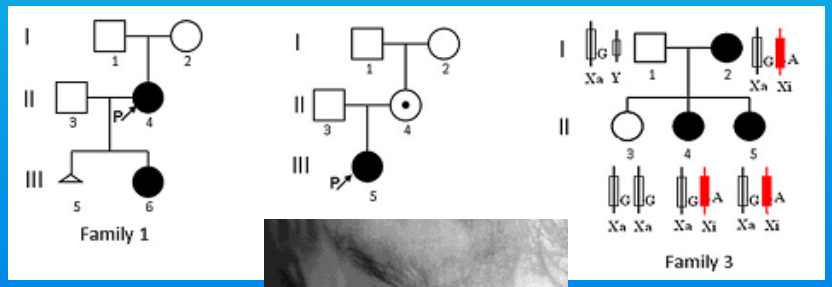
Exome sequencing

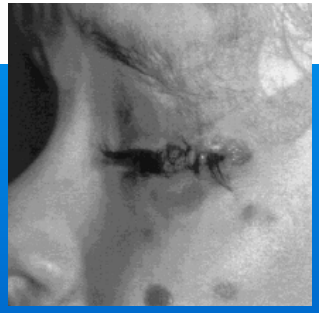
The American Journal of Human Genetics *87*, 146–153, July 9, 2010

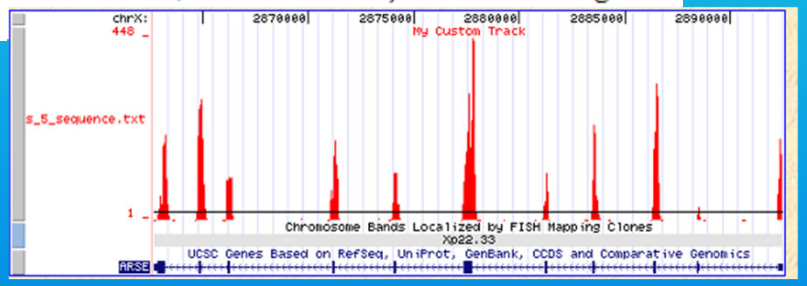
REPORT

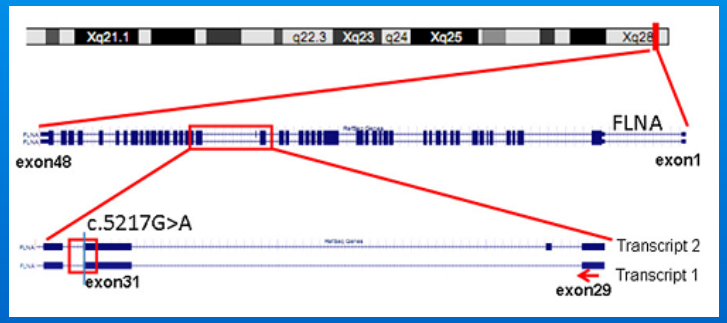
Terminal Osseous Dysplasia Is Caused by a Single Recurrent Mutation in the *FLNA* Gene

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,*]









SEPTEMBER 2010

Direct Measure of the De Novo Mutation Rate in Autism and Schizophrenia CohortsR. Awadalla, J. Gauthier, R.A. Myers, F. Casals, F.F. Hamdan, A.R. Griffing, M. Côté, E. Henrion, D. Spiegelman, J. Tarabeux, A. Piton, Y. Yang, A. Boyko, C. Bustamante, L. Xiong, J.L. Rapoport, A.M. Addington, J.L.E. DeLisi, M.-O. Krebs, R. Joober, B. Millet, É. Fombonne, L. Mottron, M. Zilversmit, J. Keebler, H. Daoud, C. Marineau, M.-H. Roy-Gagnon, M.-P. Dubé, A. Eyre-Walker, P. Drapeau, E.A. Stone, R.G. Lafrenière, and G.A. Rouleau

BOOST: A Fast Approach to Detecting Gene-Gene Interactions in Genome-wide Case-Control StudiesX. Wan, C. Yang, Q. Yang, H. Xue, X. Fan, N.L.S. Tang, and W. Yu

Mutability of Y-Chromosomal Microsatellites: Rates, Characteristics, Molecular Bases, and Forensic ImplicationsK.N. Ballantyne, M. Goedbloed, R. Fang, O. Schaap, G. Lao, A. Wollstein, Y. Choi, K. van Duijn, M. Vermeulen, S. Brauer, R. Decorte, M. Poetsch, N. von Wurmb-Schwark, P. de Knijff, D. Labuda, H. Vézina, H. Knoblauch, R. Lessig, L. Roewer, R. Ploski, T. Dobosz, L. Henke, J. Henke, M.R. Furtado, and M. Kayser

Reports

Recessive Mutations in the Gene Encoding the Tight Junction Protein Occludin Cause Band-like Calcification with Simplified Gyration and PolymicrogyriaM.C. O'Driscoll, S.B. Daly, J.E. Urquhart, G.C.M. Black, D.T. Pilz, K. Brockmann, M. McEntagart, G. Abdel-Salam, M. Zaki, N.I. Wolf, R.L. Ladda, S. Sell, S. D'Arrigo, W. Squier, W.B. Dobyns, J.H. Livingston, and Y.J. Crow

TBC1D24, an ARF6-Interacting Protein, Is Mutated in Familial Infantile Myoclonic EpilepsyA. Falace, F. Filipello, V. La Padula, N. Vanni, F. Madia, D. De Pietri Tonelli, F.A. de Falco, P. Striano, F. Dagna Bricarelli, C. Minetti, F. Benfenati, A. Fassio, and F. Zara

A Focal Epilepsy and Intellectual Disability Syndrome Is Due to a Mutation in *TBC1D24*M.A. Corbett, M. Bahlo, L. Jolly, Z. Afawi, A.E. Gardner, K.L. Oliver, S. Tan, A. Coffey, J.C. Mulley, L.M. Dibbens, W. Simri, A. Shalata, S. Kivity, G.D. Jackson, S.F. Berkovic, and J. Gecz

Nonsense Mutations in *FAM161A* Cause *RP28*-Associated Recessive Retinitis PigmentosaT. Langmann, S.A. Di Gioia, I. Rau, H. Stöhr, N.S. Maksimovic, J.C. Corbo, A.B. Renner, E. Zrenner, G. Kumaramanickavel, M. Karlstetter, Y. Arsenijevic, B.H.F. Weber, A. Gal, and C. Rivolta

Homozygosity Mapping Reveals Null Mutations in *FAM161A* as a Cause of Autosomal-Recessive Retinitis PigmentosaD. Bandah-Rozenfeld, L. Mizrahi-Meissonnier, C. Farhy, A. Obolensky, I. Chowers, J. Pe'er, S. Merin, T. Ben-Yosef, R. Ashery-Padan, E. Banin, and D. Sharon

Mutations in *DHDPSL* Are Responsible For Primary Hyperoxaluria Type IIIR. Belostotsky, E. Seboun, G.H. Idelson, D.S. Milliner, R. Becker-Cohen, C. Rinat, C.G. Monico, S. Feinstein, E. Ben-Shalom, D. Magen, I. Weissman, C. Charon, and Y. Frishberg

A Mutation in *ZNF513*, a Putative Regulator of Photoreceptor Development, Causes Autosomal-Recessive Retinitis PigmentosaL. Li, N. Nakaya, V.R.M. Chavali, Z. Ma, X. Jiao, P.A. Sieving, S. Riazuddin, S.I. Tomarev, R. Ayyagari, S.A. Riazuddin, and J.F. Hejtmancik

Mutations in *ABHD12* Cause the Neurodegenerative Disease PHARC: An Inborn Error of Endocannabinoid MetabolismT. Fiskerstrand, D. H'mida-Ben Brahim, S. Johansson, A. M'zahem, B.I. Haukanes, N. Drouot, J. Zimmermann, A.J. Cole, C. Vedeler, C. Bredrup, M. Assoum, M. Tazir, T. Klockgether, A. Hamri, V.M. Steen, H. Boman, L.A. Bindoff, M. Koenig, and P.M. Knappskog

Exome Sequencing Identifies WDR35 Variants Involved in Sensenbrenner Syndrome Christian Gilissen, Heleen H. Arts, Alexander Hoischen, Liesbeth Spruijt, Dorus A. Mans, Peer Arts, Bart van Lier, Marloes Steehouwer, Jeroen van Reeuwijk, Sarina G. Kant, Ronald Roepman, Nine V.A.M. Knoers, Joris A. Veltman, Han G. Brunner

Dominant Mutations in *RP1L1* Are Responsible for Occult Macular DystrophyM. Akahori, K. Tsunoda, Y. Miyake, Y. Fukuda, H. Ishiura, S. Tsuji, T. Usui, T. atase, M. Nakamura, H. Ohde, T. Itabashi, H. Okamoto, Y. Takada, and T. Iwata

A Locus on Chromosome 1p36 Is Associated with Thyrotropin and Thyroid Function as Identified by Genome-wide Association StudyV. Panicker, S.G. Wilson, J.P. Walsh, J.B. Richards, S.J. Brown, J.P. Beilby, A.P. Bremner, G.L. Surdulescu, E. Qweitin, I. Gillham-Nasenya, N. Soranzo, E.M. Lim, S.J. Fletcher, and T.D. Spector

Protein Tyrosine Phosphatase PTPN14 Is a Regulator of Lymphatic Function and Choanal Development in HumansA.C. Au, P.A. Hernandez, E. Lieber, A.M. Nadroo, Y.-M. Shen, K.A. Kelley, B.D. Gelb, and G.A. Diaz

JULY 2010

Whole Exome Sequencing and Homozygosity Mapping Identify Mutation in the Cell Polarity Protein GPSM2 as the Cause of Nonsyndromic Hearing Loss DFNB82. Tom Walsh, Hashem Shahin, Tal Elkan-Miller, Ming K. Lee, Anne M. Thornton, Wendy Roeb, Amal Abu Rayyan, Suheir Loulus, Karen B. Avraham, Mary-Claire King, Moien Kanaan

Terminal Osseous Dysplasia Is Caused by a Single Recurrent Mutation in the FLNA Gene [Exome Sequencing] Yu Sun, Rowida Almomani, Emmelien Aten, Jacopo Celli, Jaap van der Heijden, Hanka Venselaar, Stephen P. Robertson, Anna Baroncini, Brunella Franco, Lina Basel-Vanagaite, Emiko Horii, Ricardo Drut, Yavuz Ariyurek, Johan T. den Dunnen, Martijn H. Breuning

Bridging common and rare disease

- Common to rare
- Splitting up of fields in rarer subclasses
- More homogeneous subgroups:
  - Smaller, cheaper, shorter trials
  - Longer cost recovery period under patent
- ➔ *Large biobanks needed for recruitment of small subgroups*
- Rare to common
- Good human models for therapy development:
- one knows what one should see when it works - extended use later
- Therapy business models more viable than thought
- Next gen sequencing causes rapid advances, new therapies
- ➔ *Large, well-informed and organized patient constituency*

Exome sequencing

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Sequencing of 50 Human Exomes Reveals Adaptation to High Altitude

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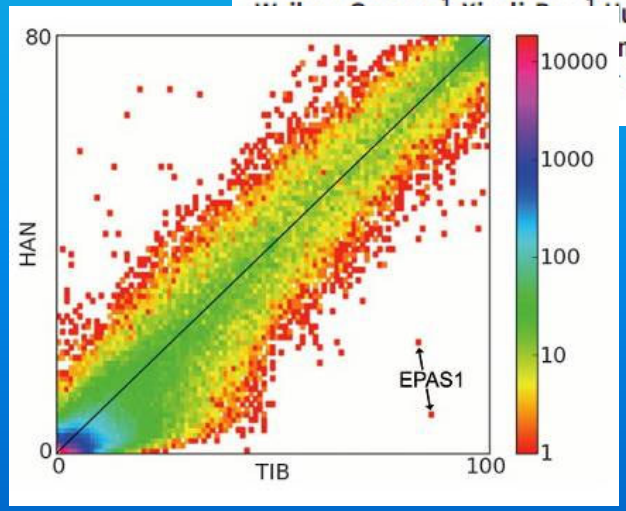
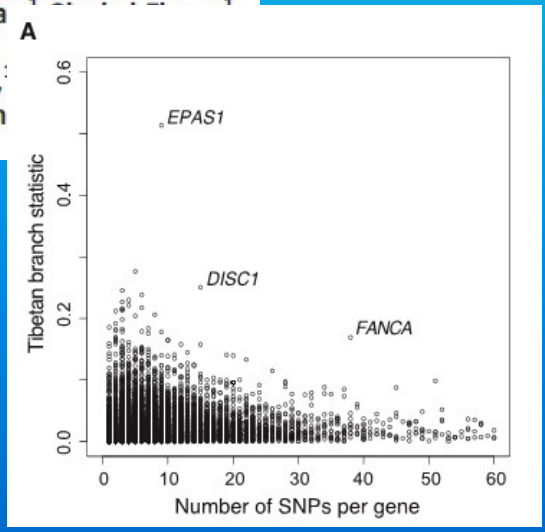
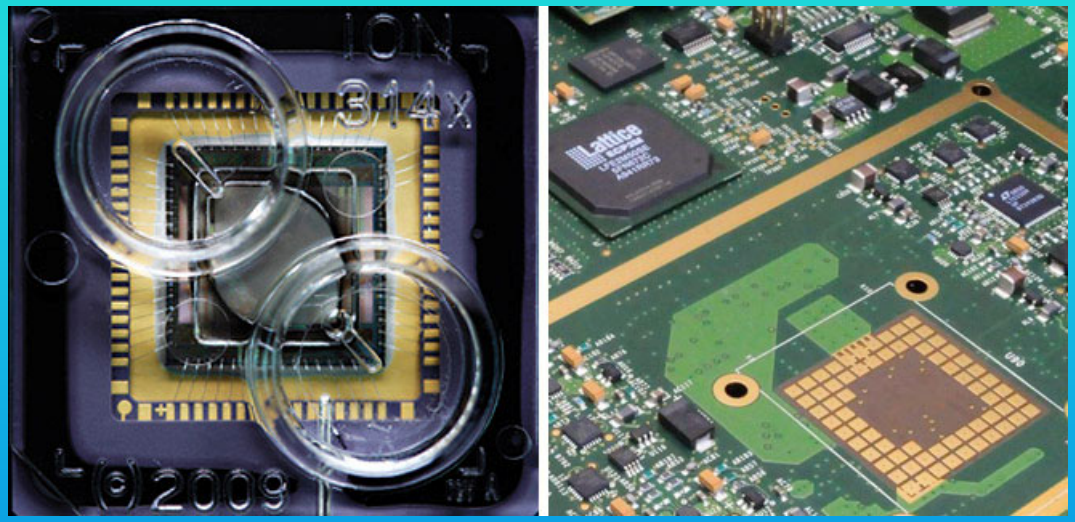
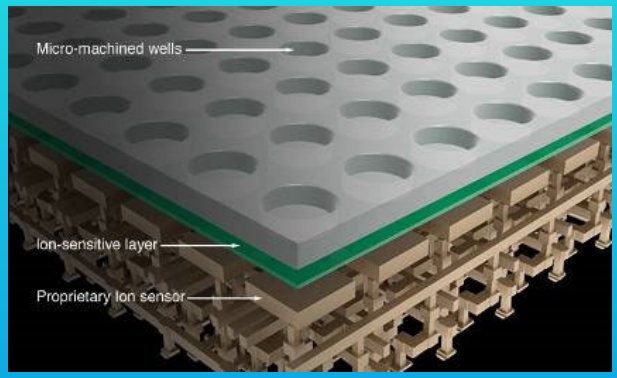
uiqing Liang,[1] Huisong Zheng,[1] Yebo Huang,[1]
n,[1,7] Yingrui Li,[1] Yong Zhang,[1] Xiuqing Zhang,
Rasmus Nielsen,[13,7]† Jun Wang,[1,7]† Jian Wan



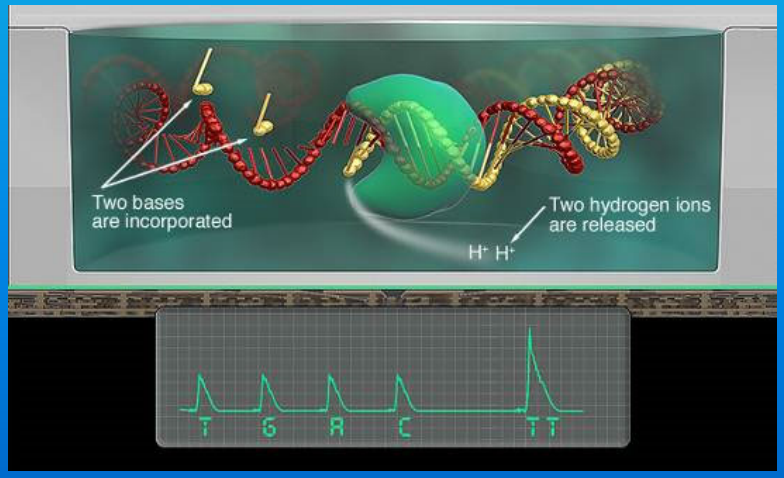


Ion Torrent

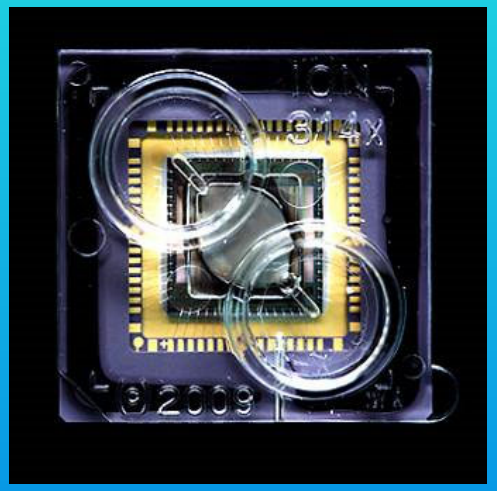




electronic detection protons
1-colour cycle sequencing



Ion Torrent



system € 55,000

seq.cost € 550 / run

What's next ?

- **single molecule sequencing**
  - *–Helicos*
    - *–short reads*
  - *–Pacific Biosciences*
    - *–long reads*
- **new features**
  - *–longer reads*
  - *–faster*
  - *–no label*
  - *–cheaper*
  - *–real-time imaging*

Advantages SMS

(single molecule sequencing)

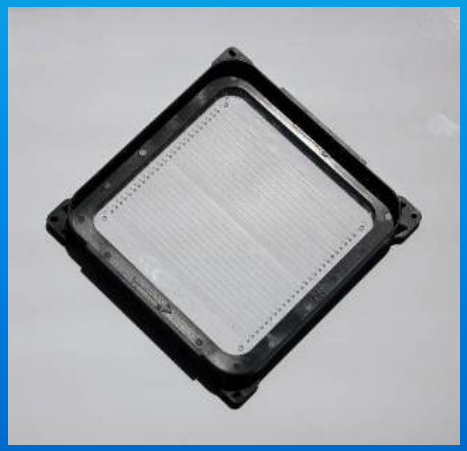
- **saves work** *(= time & cost)*
  - ***'no' sample preparation***
- **no amplification (PCR)**
  - ***no PCR errors*** ***(< sequence error)***
  - ***fewer contamination issues***
  - ***analyse everything*** ***(unPCR-able /unclonable)***
  - ***analysis low quality DNA***
- **absolute quantification**

Helicos

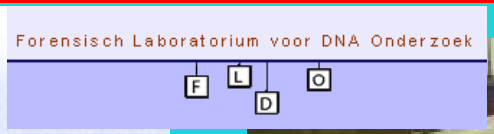
- **HeliScope**

single molecule sequencing

1 Gb / hour



Primary burials



Yield human DNA

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%

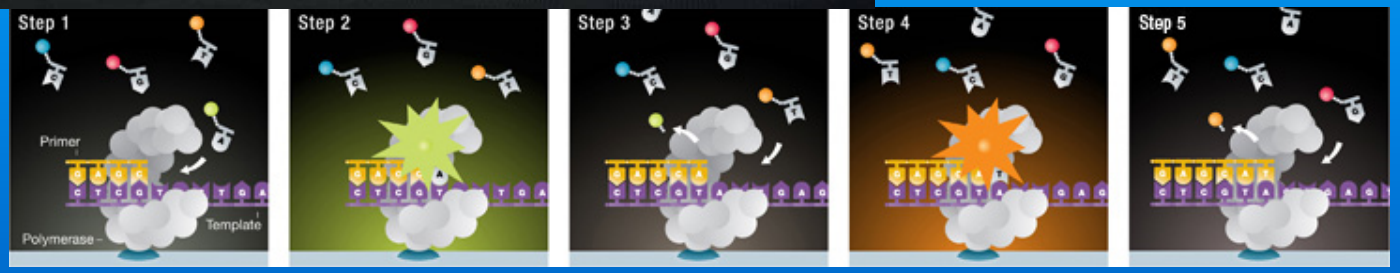
determine gender

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

Applications

- **archeology**
  - *–ancient samples*
  - *– more data then expected*
- **forensics**
  - *–mixed samples*
  - *–low quality / damaged DNA*
- **archived material**
  - *–FFPE*
  - *–museum*
  - *–...*

Pacific Biosciences



Pacific Biosciences

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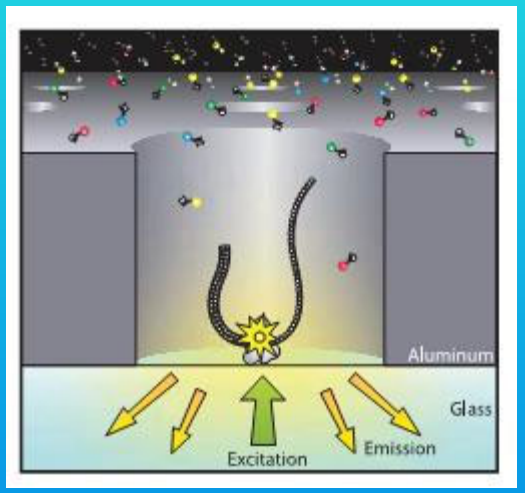
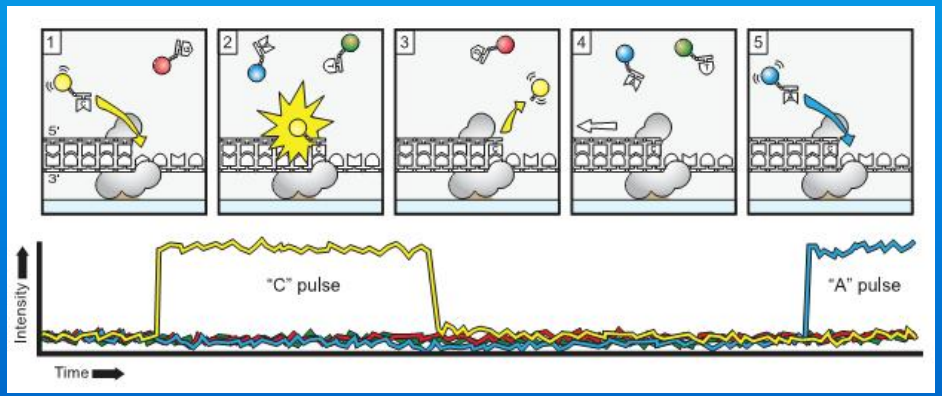
Real-Time DNA Sequencing from Single Polymerase Molecules

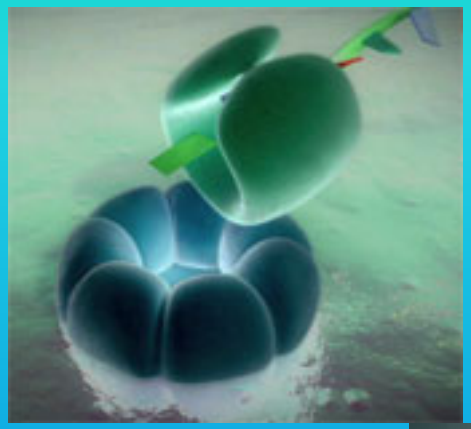
John Eid,* Adrian Fehr,* Jeremy Gray,* Khai Luong,* John Lyle,* Geoff Otto,* Paul Peluso,* David Rank,* Primo Baybayan, Brad Bettman, Arkadiusz Bibillo, Keith Bjornson, Bidhan Chaudhuri, Frederick Christians, Ronald Cicero, Sonya Clark, Ravindra Dalal, Alex deWinter, John Dixon, Mathieu Foquet, Alfred Gaertner, Paul Hardenbol, Cheryl Heiner, Kevin Hester, David Holden, Gregory Kearns, Xiangxu Kong, Ronald Kuse, Yves Lacroix, Steven Lin, Paul Lundquist, Congcong Ma, Patrick Marks, Mark Maxham, Devon Murphy, Insil Park, Thang Pham, Michael Phillips, Joy Roy, Robert Sebra, Gene Shen, Jon Sorenson, Austin Tomaney, Kevin Travers, Mark Trulson, John Vieceli, Jeffrey Wegener, Dawn Wu, Alicia Yang, Denis Zaccarin, Peter Zhao, Frank Zhong, Jonas Korlach,† Stephen Turner†

PACIFIC BIOSCIENCES

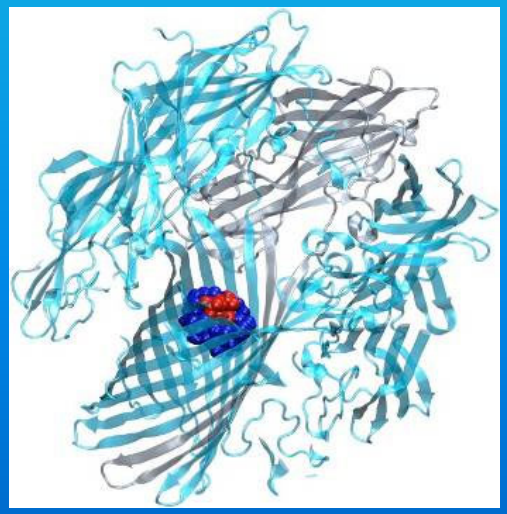


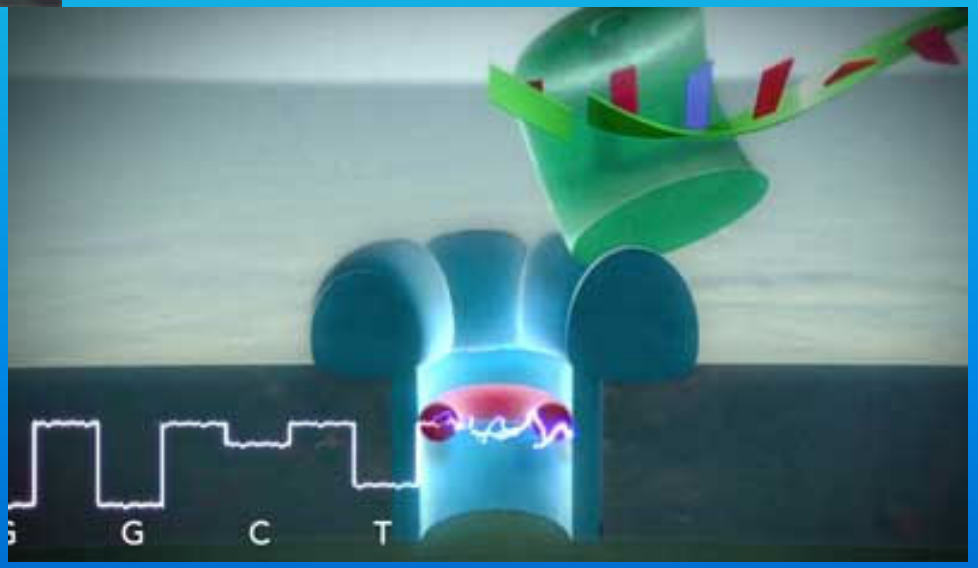


Oxford Nanopore$_3$



no labels





Performance

A human genome sequence

- **2000**
  - *€ 1,000,000,000* *in ~10 years*
- **2008**
  - *€ 50 - 100,000* *in ~4 months*
- **2010**
  - *€ 5 - 10,000* *in ~2 weeks*
- *…2015*
  - *€ 1,000* *in ~1 day*
- *…2020*
  - *€ 10* *in ~1 hour to minutes*