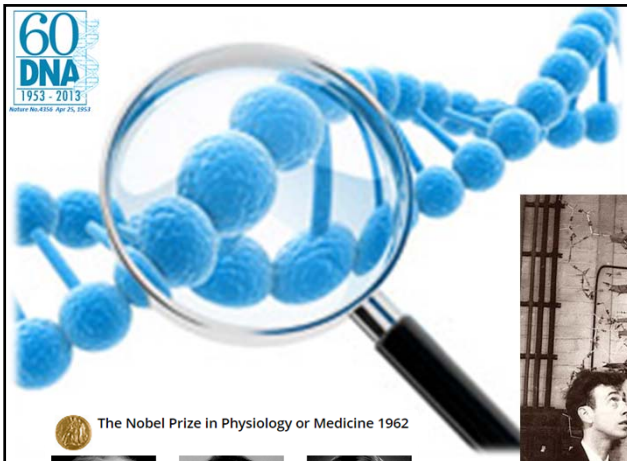
NEW GENERATION SEQUENCING
Technologies and Applications

新世代定序技術 原理與應用 NGS

AGTCCGCGAATACAGGCTCGGT AGTCCGCGAATACAGGCTCGGT

Petrus Tang, Ph.D. (鄧致剛)
Graduate Institute of Basic Medical Sciences
and
Bioinformatics Center, Chang Gung University.
petang@mail.cgu.edu.tw
http://petang.cgu.edu.tw





The Nobel Prize in Physiology or Medicine 1962



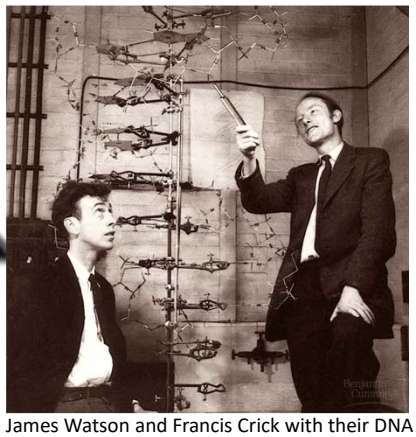
Francis Harry Compton Crick
Prize share: 1/3



James Dewey Watson
Prize share: 1/3

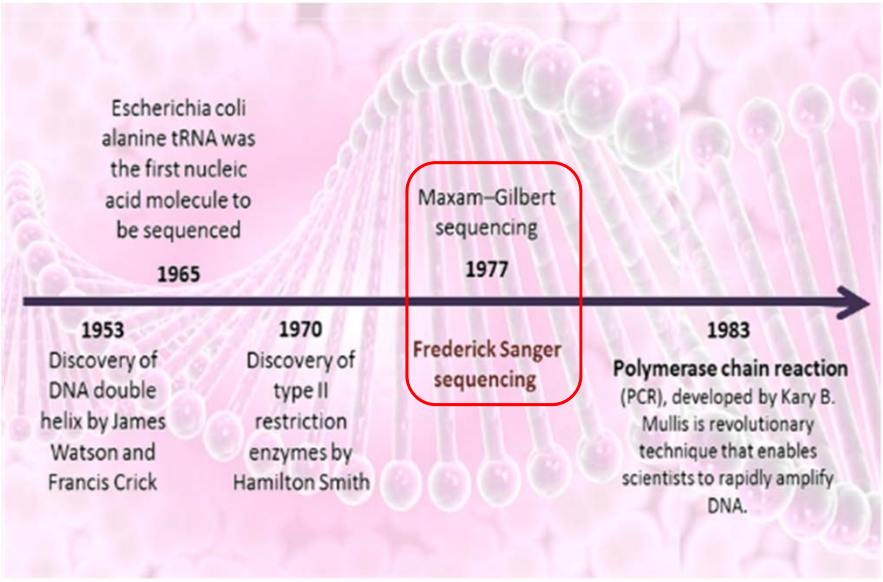


Maurice Hugh Frederick Wilkins
Prize share: 1/3

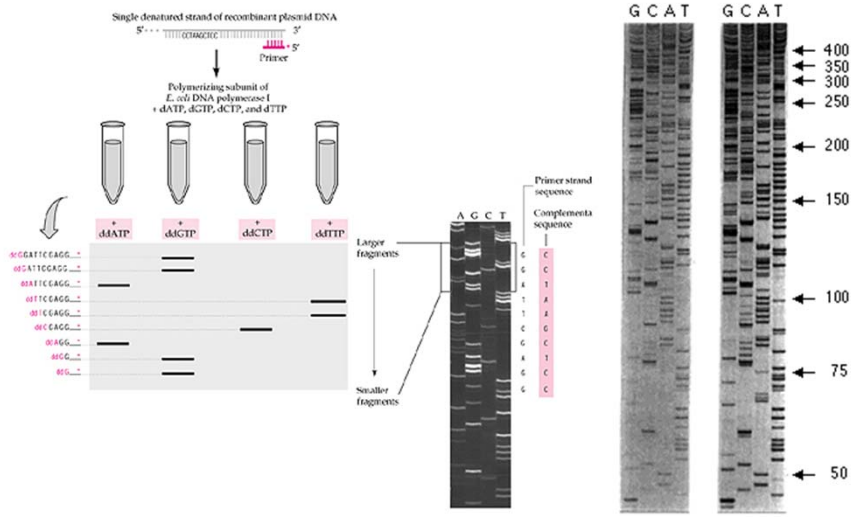


James Watson and Francis Crick with their DNA model at the Cavendish Laboratories in 1953

Do You Read Me?



Sanger Dideoxy Sequencing



Sanger, F. et al. Nature 24, 687–695 (1977).
Sanger, F., Nicklen, S. & Coulson, A.R. Proc. Natl. Acad. Sci. USA 74, 5463–5467 (1977).

The Nobel Prize in Chemistry 1980

"for his fundamental studies of the biochemistry of nucleic acids, with particular regard to recombinant-DNA"

"for their contributions concerning the determination of base sequences in nucleic acids"



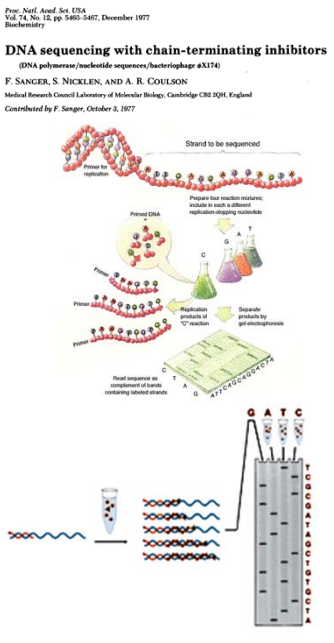
Paul Berg
Recombinant DNA
1/2 of the prize
USA
Stanford University
Stanford, CA, USA



Walter Gilbert
Chemical sequencing
1/4 of the prize
USA
Harvard University,
Biological Laboratories
Cambridge, MA, USA



Frederick Sanger
Chain-termination sequencing
1/4 of the prize
United Kingdom
MRC Laboratory of
Molecular Biology
Cambridge, United



Proc. Natl. Acad. Sci. USA
Vol. 74, No. 12, pp. 5463–5467, December 1977
Biochemistry

DNA sequencing with chain-terminating inhibitors

(DNA polymerase/nucleotide sequences/bacteriophage φX174)

F. SANGER, S. NICKLEN, AND A. R. COULSON

Medical Research Council Laboratory of Molecular Biology, Cambridge CB2 1QH, England

Contributed by F. Sanger, October 3, 1977

February 2001: Completion of the Draft Human Genome

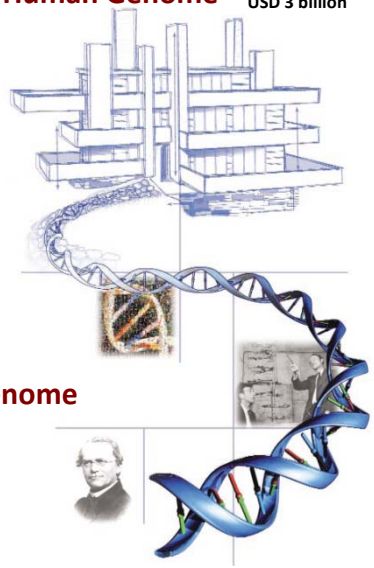
>10 years to finish
USD 3 billion



Science, 16 February 2001
Vol. 291, Pages 1145-1434



Nature, 15 February 2001
Vol. 409, Pages 813-960



April 2003: High-Resolution Human Genome

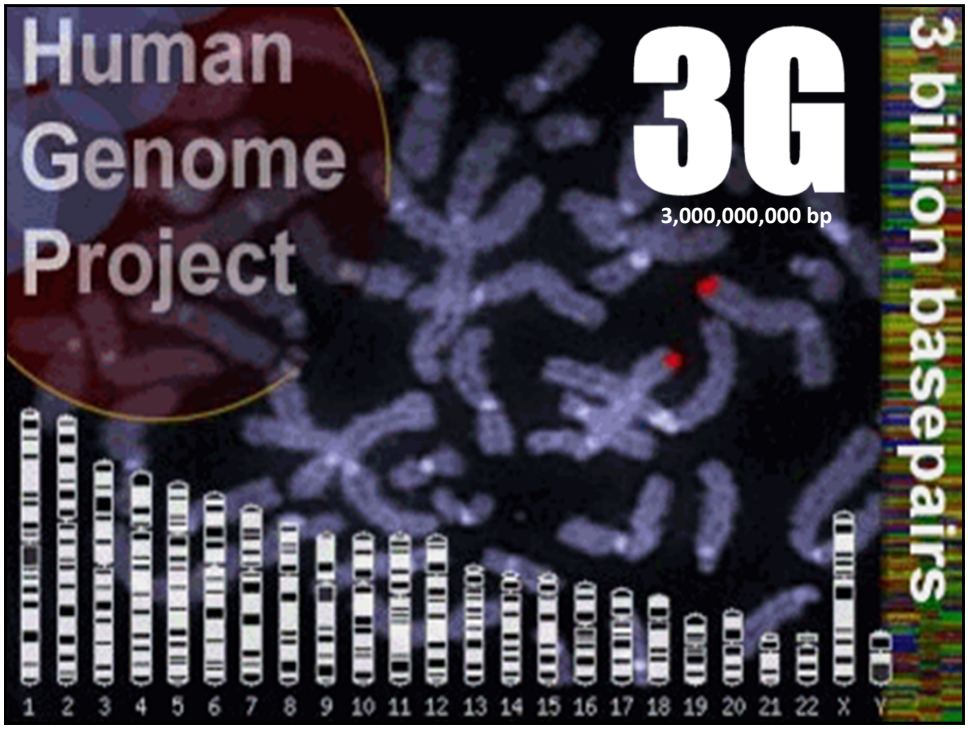
A vision for the future of genomics research

A blueprint for the genomic era.

Francis S. Collins, Eric D. Green, Alan E. Guttmacher and Mark S. Guyer on behalf of the US National Human Genome Research Institute*

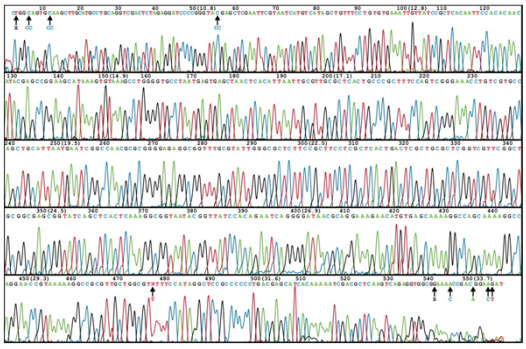
50 Years of DNA: *From Double Helix to Health*
A Celebration of the Genome

Nature, 23 April 2003
Vol. 422, Pages 1-13



ABI 3730 XL DNA Sequencer

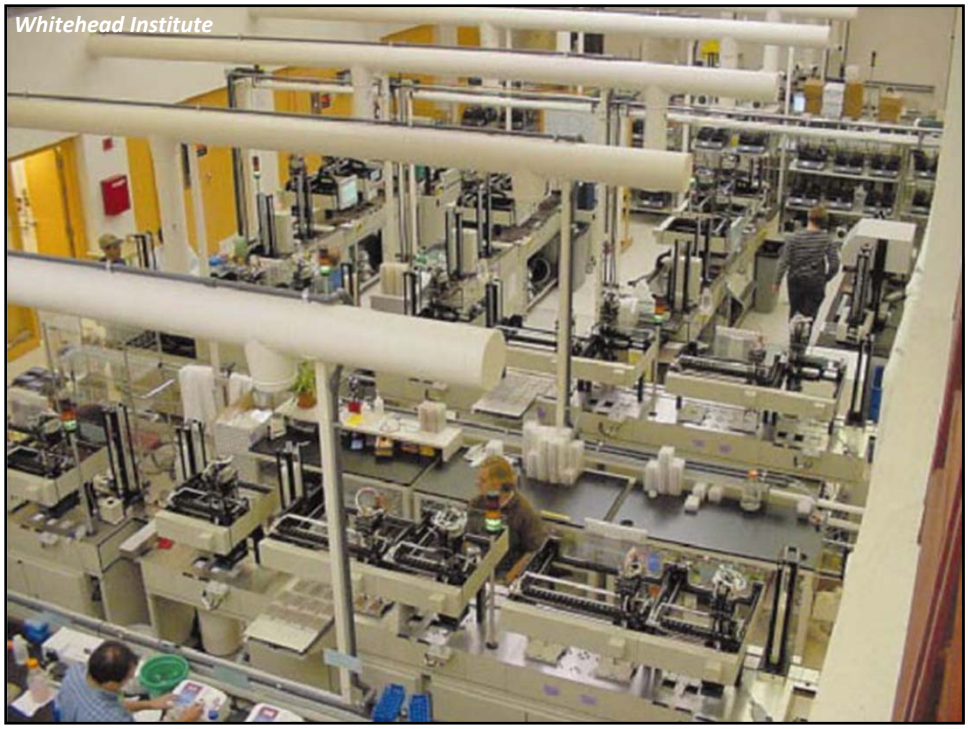




96/384 DNA sequencing in 2 hrs, approximately 600-1000 readable bps per run.

1-4 MB bps/day

A human genome of 3GB need 750 days to finish



273 authors



The Sequence of the Human Genome

J. Craig Venter,[1*] Mark D. Adams,[1] Eugene W. Myers,[1] Peter W. Li,[1] Richard J. Mural,[1] Granger G. Sutton,[1] Hamilton O. Smith,[1] Mark Yandell,[1] Cheryl A. Evans,[1] Robert A. Holt,[1] Jeannine D. Gocayne,[1] Peter Amanatides,[1] Richard M. Ballew,[1] Daniel H. Huson,[1] Jennifer Russo Wortman,[1] Qing Zhang,[1] Chinnappa D. Kodira,[1] Xiangqun H. Zheng,[1] Lin Chen,[1] Marian Skupski,[1] Gangadharan Subramanian,[1] Paul D. Thomas,[1] Jinghui Zhang,[1] George L. Gabor Miklos,[2] Catherine Nelson,[3] Samuel Broder,[3] Andrew G. Clark,[4] Joe Nadeau,[5] Victor A. McKusick,[6] Norton Zinder,[7] Arnold J. Levine,[7] Richard J. Roberts,[8] Mel Simon,[9] Carolyn Slayman,[10] Michael Hunkapiller,[11] Randall Bolanos,[1] Arthur Delcher,[1] Ian Dew,[1] Daniel Fasulo,[1] Michael Flanigan,[1] Liliana Florea,[1] Aaron Halpern,[1] Sridhar Hannenhalli,[1] Saul Kravitz,[1] Samuel Levy,[1] Clark Mobarry,[1] Knut Reinert,[1] Karin Remington,[1] Jane Abu-Threideh,[1] Ellen Beasley,[1] Kendra Biddick,[1] Vivian Bonazzi,[1] Rhonda Brandon,[1] Michele Cargill,[1] Ishwar Chandramouliswaran,[1] Rosane Charlab,[1] Kabir Chaturvedi,[1] Zuoming Deng,[1] Valentina Di Francesco,[1] Patrick Dunn,[1] Karen Eilbeck,[1] Carlos Evangelista,[1] Andrei E. Gabrielian,[1] Weiniu Gan,[1] Wangmao Ge,[1] Fangcheng Gong,[1] Zhiping Gu,[1] Ping Guan,[1] Thomas J. Heiman,[1] Maureen E. Higgins,[1] Rui-Ru Ji,[1] Zhaoxi Ke,[1] Karen A. Ketchum,[1] Zhongwu Lai,[1] Yiding Lei,[1] Zhenya Li,[1] Jiayin Li,[1] Yong Liang,[1] Xiaoying Lin,[1] Fu Lu,[1] Gennady V. Merkulov,[1] Natalia Milshina,[1] Helen M. Moore,[1] Ashwinikumar K Naik,[1] Vaibhav A. Narayan,[1] Beena Neelam,[1] Deborah Nusskern,[1] Douglas B. Rusch,[1] Steven Salzberg,[12] Wei Shao,[1] Bixiong Shue,[1] Jingtao Sun,[1] Zhen Yuan Wang,[1] Aihui Wang,[1] Xin Wang,[1] Jian Wang,[1] Ming-Hui Wei,[1] Ron Wides,[13] Chunlin Xiao,[1] Chunhua Yan,[1] Alison Yao,[1] Jane Ye,[1] Ming Zhan,[1] Weiqing Zhang,[1] Hongyu Zhang,[1] Qi Zhao,[1] Liansheng Zheng,[1] Fei Zhong,[1] Wenyan Zhong,[1] Shiaoping C. Zhu,[1] Shaying Zhao,[12] Dennis Gilbert,[1] Suzanna Baumhueter,[1] Gene Spier,[1] Christine Carter,[1] Anibal Cravchik,[1] Trevor Woodage,[1] Feroze Ali,[1] Huijin An,[1] Aderonke Awe,[1] Danita Baldwin,[1] Holly Baden,[1] Mary Barnstead,[1] Ian Barrow,[1] Karen Beeson,[1] Dana Busam,[1] Amy Carver,[1] Angela Center,[1] Ming Lai Cheng,[1] Liz Curry,[1] Steve Danaher,[1] Lionel Davenport,[1] Raymond Desilets,[1] Susanne Dietz,[1] Kristina Dodson,[1] Lisa Doup,[1] Steven Ferriera,[1] Neha Garg,[1] Andres Gluecksmann,[1] Brit Hart,[1] Jason Haynes,[1] Charles Haynes,[1] Cheryl Heiner,[1] Suzanne Hladun,[1] Damon Hostin,[1] Jarrett Houck,[1] Timothy Howland,[1] Chinyere Ibegwam,[1] Jeffery Johnson,[1] Francis Kalush,[1] Lesley Kline,[1] Shashi Koduru,[1] Amy Love,[1] Felecia Mann,[1] David May,[1] Steven McCawley,[1] Tina McIntosh,[1] Ivy McMullen,[1] Mee Moy,[1] Linda Moy,[1] Brian Murphy,[1] Keith Nelson,[1] Cynthia Pfannkoch,[1] Eric Pratts,[1] Vinita Puri,[1] Hina Qureshi,[1] Matthew Reardon,[1] Robert Rodriguez,[1] Yu-Hui Rogers,[1] Deanna Romblad,[1] Bob Ruhfel,[1] Richard Scott,[1] Cynthia Sitter,[1] Michelle Smallwood,[1] Erin Stewart,[1] Renee Strong,[1] Ellen Suh,[1] Reginald Thomas,[1] Ni Ni Tint,[1] Sukyee Tse,[1] Claire Vech,[1] Gary Wang,[1] Jeremy Wetter,[1] Sherita Williams,[1] Monica Williams,[1] Sandra Windsor,[1] Emily Winn-Deen,[1] Keriallen Wolfe,[1] Jayshree Zaveri,[1] Karena Zaveri,[1] Josep F. Abril,[14] Roderic Guigó,[14] Michael J. Campbell,[1] Kimmen V. Sjolander,[1] Brian Karlak,[1] Anish Kejariwal,[1] Huaiyu Mi,[1] Betty Lazareva,[1] Thomas Hatton,[1] Apurva Narechania,[1] Karen Diemer,[1] Anushya Muruganujan,[1] Nan Guo,[1] Shinji Sato,[1] Vineet Bafna,[1] Sorin Istrail,[1] Ross Lippert,[1] Russell Schwartz,[1] Brian Walenz,[1] Shibu Yooseph,[1] David Allen,[1] Anand Basu,[1] James Baxendale,[1] Louis Blick,[1] Marcelo Caminha,[1] John Carnes-Stine,[1] Parris Caulk,[1] Yan-Hui Chiang,[1] My Coyne,[1] Carl Dahlke,[1] Anne Desattes Mays,[1] Maria Dombroski,[1] Michael Donnelly,[1] Dale Ely,[1] Shiva Esparham,[1] Carl Foster,[1] Harold Gire,[1] Stephen Glanowski,[1] Kenneth Glasser,[1] Anna Glodek,[1] Mark Gorokhov,[1] Ken Graham,[1] Barry Gropman,[1] Michael Harris,[1] Jeremy Heil,[1] Scott Henderson,[1] Jeffrey Hoover,[1] Donald Jennings,[1] Catherine Jordan,[1] James Jordan,[1] John Kasha,[1] Leonid Kagan,[1] Cheryl Kraft,[1] Alexander Lavitsky,[1] Mark Lewis,[1] Xiangjun Liu,[1] John Lopez,[1] Daniel Ma,[1] William Majoros,[1] Joe McDaniel,[1] Sean Murphy,[1] Matthew Newman,[1] Trung Nguyen,[1] Ngoc Nguyen,[1] Marc Nodell,[1] Sue Pan,[1] Jim Peck,[1] Marshall Peterson,[1] William Rowe,[1] Robert Sanders,[1] John Scott,[1] Michael Simpson,[1] Thomas Smith,[1] Arlan Sprague,[1] Timothy Stockwell,[1] Russell Turner,[1] Eli Venter,[1] Mei Wang,[1] Meiyuan Wen,[1] David Wu,[1] Mitchell Wu,[1] Ashley Xia,[1] Ali Zandieh,[1] Xiaohong Zhu[1]

16 FEBRUARY 2001 VOL 291 SCIENCE www.sciencemag.org

254 authors



articles

Initial sequencing and analysis of the human genome

International Human Genome Sequencing Consortium*

Genome Sequencing Centres (Listed in order of total genomic sequence contributed, with a partial list of personnel. A full list of contributors at each centre is available as Supplementary Information.)

Whitehead Institute for Biomedical Research, Center for Genome Research: Eric S. Lander[*†], Lauren M. Linton[†], Bruce Birren[*†], Chad Nusbaum[*†], Michael C. Zody[*†], Jennifer Baldwin[†], Keri Devon[†], Ken Dewar[†], Michael Doyle[†], William FitzHugh[*†], Roel Funke[†], Diane Gage[†], Katrina Harris[†], Andrew Heaford[†], John Howland[†], Lisa Kann[†], Jessica Lehoczky[†], Rosie LeVine[†], Paul McEwan[†], Kevin McKernan[†], James Meldrim[†], Jill P. Mesirov[*†], Cher Miranda[†], William Morris[†], Jerome Naylor[†], Christina Raymond[†], Mark Rosetti[†], Ralph Santos[†], Andrew Sheridan[†], Carrie Sougnez[†], Nicole Stange-Thomann[†], Nikola Stojanovic[†], Aravind Subramanian[†] & Dudley Wyman[†]

The Sanger Centre: Jane Rogers[*], John Sulston[*†], Rachael Ainscough[†], Stephan Beck[†], David Bentley[†], John Burton[†], Christopher Clee[†], Nigel Carter[†], Alan Coulson[†], Rebecca Deadman[†], Panos Deloukas[†], Andrew Dunham[†], Ian Dunham[†], Richard Durbin[*†], Lisa French[†], Darren Grafham[†], Simon Gregory[†], Tim Hubbard[*†], Sean Humphray[†], Adrienne Hunt[†], Matthew Jones[†], Christine Lloyd[†], Amanda McMurray[†], Lucy Matthews[†], Simon Mercer[†], Sarah Milne[†], James C. Mullikin[*†], Andrew Mungall[†], Robert Plumb[†], Mark Ross[†], Ratna Shownkeen[†] & Sarah Sims[†]

Washington University Genome Sequencing Center: Robert H. Waterston[*†], Richard K. Wilson[*†], LaDeana W. Hillier[*†], John D. McPherson[†], Marco A. Marra[†], Elaine R. Mardis[†], Lucinda A. Fulton[†], Asif T. Chinwalla[†], Kymberlie H. Pepin[†], Warren R. Gish[†], Stephanie L. Chissoe[†], Michael C. Wendl[†], Kim D. Delehaunty[†], Tracie L. Miner[†], Andrew Delehaunty[†], Jason B. Kramer[†], Lisa L. Cook[†], Robert S. Fulton[†], Douglas L. Johnson[†], Patrick J. Minx[†] & Sandra W. Clifton[†]

US DOE Joint Genome Institute: Trevor Hawkins[*], Elbert Branscomb[*], Paul Predki[*], Paul Richardson[*], Sarah Wenning[*], Tom Slezak[*], Norman Doggett[*], Jan-Fang Cheng[*], Anne Olsen[*], Susan Lucas[*], Christopher Elkin[*], Edward Uberbacher[*] & Marvin Frazier[*]

Baylor College of Medicine Human Genome Sequencing Center: Richard A. Gibbs[*†], Donna M. Muzny[*†], Steven E. Scherer[*†], John B. Bouck[*†], Erica J. Sodergren[*†], Kim C. Worley[*†], Catherine M. Rives[†], James H. Gorrell[†], Michael L. Metzker[†], Susan L. Naylor[†], Raju S. Kucherlapati[†], David L. Nelson[†] & George M. Weinstock[†]

RIKEN Genomic Sciences Center: Yoshiyuki Sakaki[*], Asao Fujiyama[*], Masahira Hattori[*], Tetsushi Yada[*], Atsushi Toyoda[*], Takehiko Itoh[*], Chiharu Kawagoe[*], Hidemi Watanabe[*], Yasushi Totoki[*] & Todd Taylor[*]

Genoscope and CNRS UMR-8030: Jean Weissenbach[*], Roland Heilig[*†], William Saurin[*†], Francois Artiguenave[*†], Philippe Brottier[*†], Thomas Bruls[*†], Eric Pelletier[*†], Catherine Robert[*†] & Patrick Wincker[*†]

GTC Sequencing Center: Douglas R. Smith[*†], Lynn Doucette-Stamm[*†], Marc Rubenfield[*†], Keith Weinstock[*†], Hong Mei Lee[*†] & JoAnn Dubois[*†]

Department of Genome Analysis, Institute of Molecular Biotechnology: André Rosenthal[*†], Matthias Platzer[*†], Gerald Nyakatura[*†], Stefan Taudien[*†] & Andreas Rump[*†]

Beijing Genomics Institute/Human Genome Center: Huanming Yang[*†], Jun Yu[*†], Jian Wang[*†], Guyang Huang[*†] & Jun Gu[*†]

Multimegabase Sequencing Center, The Institute for Systems Biology: Leroy Hood[*†], Lee Rowen[*†], Anup Madan[*†] & Shizen Qin[*†]

Stanford Genome Technology Center: Ronald W. Davis[*†], Nancy A. Federspiel[*†], A. Pia Abola[*†] & Michael J. Proctor[*†]

Stanford Human Genome Center: Richard M. Myers[*†], Jeremy Schmutz[*†], Mark Dickson[*†], Jane Grimwood[*†] & David R. Cox[*†]

University of Washington Genome Center: Maynard V. Olson[*†], Rajinder Kaul[*†] & Christopher Raymond[*†]

Department of Molecular Biology, Keio University School of Medicine: Nobuyoshi Shimizu[*†], Kazuhiko Kawasaki[*†] & Shinsei Minoshima[*†]

University of Texas Southwestern Medical Center at Dallas: Glen A. Evans[*†]; Maria Athanasiou[*†] & Roger Schultz[*†]

University of Oklahoma's Advanced Center for Genome Technology: Bruce A. Roe[*], Feng Chen[*] & Huaqin Pan[*†]

Max Planck Institute for Molecular Genetics: Juliane Ramser[*†], Hans Lehrach[*†] & Richard Reinhardt[*†]

Cold Spring Harbor Laboratory, Lita Annenberg Hazen Genome Center: W. Richard McCombie[*†], Melissa de la Bastide[*†] & Nobuy Dedhia[*†]

GBF—German Research Centre for Biotechnology: Helmut Blöcker[*†], Klaus Homicher[*†] & Gabriele Nordsiek[*†]

* Genome Analysis Group (listed in alphabetical order, also includes individuals listed under other headings): Richa Agarwala[*†], L. Aravind[*†], Jeffrey A. Bailey[*†], Alex Bateman[*], Serafim Batzoglou[*], Ewan Birney[*†], Peer Bork[*†‡§], Daniel G. Brown[*], Christopher B. Burge[*†], Lorenzo Cerutti[*†], Hsiu-Chuan Chen[*†], Deanna Church[*†‡], Michele Clamp[*], Richard R. Copley[*†], Tobias Doerks[*†‡], Sean R. Eddy[*†], Evan E. Eichler[*†], Terrence S. Furey[*†], James Galagan[*], James G. R. Gilbert[*], Cyrus Harmon[*†], Yoshihide Hayashizaki[*†], David Haussler[*†], Henning Hermjakob[*†], Karsten Hokamp[*†], Wonhee Jang[*†], L. Steven Johnson[*†], Thomas A. Jones[*†], Simon Kasif[*†], Arek Kaspryzk[*†], Scot Kennedy[*†], W. James Kent[*†], Paul Kitts[*†], Eugene V. Koonin[*†], Ian Korf[*], David Kulp[*†], Doron Lancet[*†], Todd M. Lowe[*†], Aoife McLysaght[*†], Tarjei Mikkelsen[*†], John V. Moran[*†], Nicola Mulder[*†], Victor J. Pollara[*], Chris P. Ponting[*§], Greg Schuler[*†], Jörg Schultz[*†], Guy Slater[*†], Arian F. A. Smit[*], Elia Stupka[*†], Joseph Szustakowski[*†], Danielle Thierry-Mieg[*†], Jean Thierry-Mieg[*†], Lukas Wagner[*†], John Wallis[*], Raymond Wheeler[*†], Alan Williams[*†], Yuri I. Wolf[*], Kenneth H. Wolfe[*†], Shiaw-Pyng Yang[*] & Ru-Fang Yeh[*†]

Scientific management: National Human Genome Research Institute, US National Institutes of Health: Francis Collins[*‡], Mark S. Guyer[*‡], Jane Peterson[*‡], Adam Felsenfeld[*‡] & Kris A. Wetterstrand[*‡]; **Office of Science, US Department of Energy:** Aristides Patrinos[*‡]; **The Wellcome Trust:** Michael J. Morgan[*‡]

Tong *et al. Genome Biology* 2010, **11**:R91
http://genomebiology.com/2010/11/9/R91

Genome Biology

RESEARCH Open Access

Sequencing and analysis of an Irish human genome

Pin Tong[1†], James GD Prendergast[2†], Amanda J Lohan[1], Susan M Farrington[2,3], Simon Cronin[4], Nial Friel[5], Dan G Bradley[6], Orla Hardiman[7], Alex Evans[8], James F Wilson[9], Brendan Loftus[1*]

Abstract

Background: Recent studies generating complete human sequences from Asian, African and European subgroups have revealed population-specific variation and disease susceptibility loci. Here, choosing a DNA sample from a population of interest due to its relative geographical isolation and genetic impact on further populations, we extend the above studies through the generation of 11-fold coverage of the first Irish human genome sequence.

Results: Using sequence data from a branch of the European ancestral tree as yet unsequenced, we identify variants that may be specific to this population. Through comparisons with HapMap and previous genetic association studies, we identified novel disease-associated variants, including a novel nonsense variant putatively associated with inflammatory bowel disease. We describe a novel method for improving SNP calling accuracy at low genome coverage using haplotype information. This analysis has implications for future re-sequencing studies and validates the imputation of Irish haplotypes using data from the current Human Genome Diversity Cell Line Panel (HGDP-CEPH). Finally, we identify gene duplication events as constituting significant targets of recent positive selection in the human lineage.

Conclusions: Our findings show that there remains utility in generating whole genome sequences to illustrate both general principles and reveal specific instances of human biology. With increasing access to low cost sequencing we would predict that even armed with the resources of a small research group a number of similar initiatives geared towards answering specific biological questions will emerge.

nature genetics

Whole-genome sequencing and comprehensive variant analysis of a Japanese individual using massively parallel sequencing

Akihiro Fujimoto, Hidewaki Nakagawa, Naoya Hosono, Kaoru Nakano, Tetsuo Abe, Keith A Boroevich, Masao Nagasaki, Rui Yamaguchi, Tetsuo Shibuya, Michiaki Kubo, Satoru Miyano, Yusuke Nakamura & Tatsuhiko Tsunoda

Affiliations | Contributions | Corresponding authors

Nature Genetics **42**, 931–936 (2010) | doi:10.1038/ng.691
Received 18 February 2010 | Accepted 10 September 2010 | Published online 24 October 2010

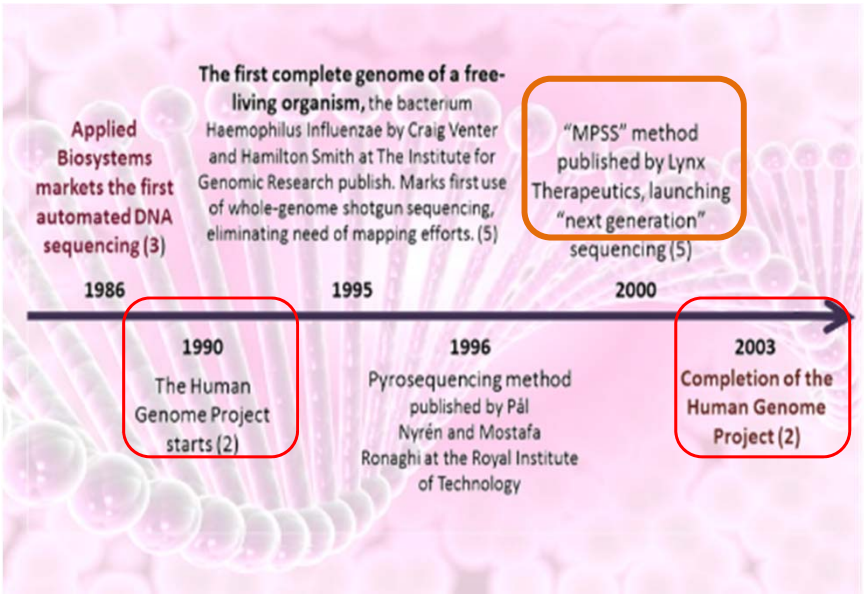
Abstract

Abstract • Author information • Supplementary information

We report the analysis of a Japanese male using high-throughput sequencing to ×40 coverage. More than 99% of the sequence reads were mapped to the reference human genome. Using a Bayesian decision method, we identified 3,132,608 single nucleotide variations (SNVs). Comparison with six previously reported genomes revealed an excess of singleton nonsense and nonsynonymous SNVs, as well as singleton SNVs in conserved non-coding regions. We also identified 5,319 deletions smaller than 10 kb with high accuracy, in addition to copy number variations and rearrangements. *De novo* assembly of the unmapped sequence reads generated around 3 Mb of novel sequence, which showed high similarity to non-reference human genomes and the human herpesvirus 4 genome. Our analysis suggests that considerable variation remains undiscovered in the human genome and that whole-genome sequencing is an invaluable tool for obtaining a complete understanding of human genetic variation.

Next Generation Sequencing Technology

Massively **P**arallel **S**ignature **S**equencing (MPSS)

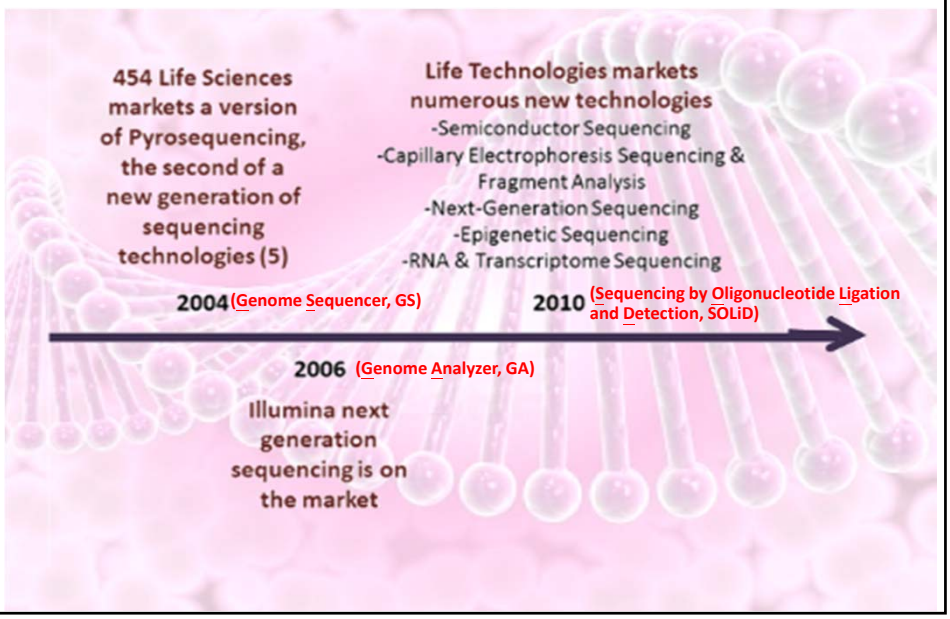


NEXT GENERATION SEQUENCING

NGS

Next Generation Sequencing Technology

Massively **P**arallel **S**ignature **S**equencing (MPSS)



Next Generation Sequencing Technology

Massively **P**arallel **S**ignature **S**equencing (MPSS)

NATURE METHODS | VOL.5 NO.1 | JANUARY 2008





***Nature Methods*' Method of the Year 2007 goes to next-generation sequencing.** **This series of articles showcase how these novel sequencing methods came into their own in 2007 and the incredible impact they promise to have in a variety of research applications. The Methods to Watch feature provide a glimpse and a wish list for future Methods of the Year.**

Roche **454 GS FLX**
http://www.454.com/

illumina **SOLEXA**
http://www.illumina.com/pages.ilmn?ID=250

applied biosystems **SOLID**
http://marketing.appliedbiosystems.com/

Throughput of NGS machines (2007-2009)

Throughput = total number of reads x read length

Vendor:	Roche			Illumina		ABI	
Technology:	454			Solexa		SOLiD	
Platform:	GS 20	FLX	Ti	GA	GA II	1	2
Reads:	500 k	500 k	1 M	28 M	80 M	40 M	115 M
Fragment							
Read length:	100	200	350	35	50	75	25	35
Run time:	6 hr	7 hr	9 hr	3 d	3 d	4 d	6 d	5 d
Yield:	50 Mb	100 Mb	400 Mb	1 Gb	4 Gb	6 Gb	1 Gb	4 Gb
Images:	11 GB	13 GB	27 GB	500 GB	1.1 TB	1.7 TB	1.8 TB	2.5 TB
PA Disk:	3 GB	3 GB	15 GB	175 GB	300 GB	350 GB	300 GB	750 GB
PA CPU:	10 hr	140 hr	220 hr	100 hr	70 hr	100 hr	NA	NA
SRA:	500 MB	1 GB	4 GB	30 GB	50 GB	75 GB	100 GB	140 GB
Paired-end							
Read length:		200		2×35	2×50	2×75	2×25	2×35
Insert:		3.5 kb		200 b	200 b	200 b	3 kb	3 kb
Run time:		7 hr		6 d	6 d	8 d	12 d	10 d
Yield:		100 Mb		2 Gb	8 Gb	11 Gb	2 Gb	8 Gb
Images:		13 GB		1 TB	2.2 TB	3.4 TB	3.6 TB	5 TB
PA Disk:		3 GB		350 GB	500 GB	600 GB	600 GB	1.5 TB
PA CPU:		140 hr		160 hr	120 hr	170 hr	NA	NA
SRA:		1 GB		60 GB	100 GB	150 GB	200 GB	280 GB

High throughput
Short read/run
Illumina
SOLiD

Low throughput-
Long read/run
Roche
(~4Gb/4 days)

Throughput of NGS machines: Roche (2012)

Roche 454 SEQUENCING



GS FLX+ System Performance	
Read Length	Up to 1,000 bp
Mode Read Length	700 bp
Throughput Profile	85% of total bases from reads >500 bp 45% of total bases from reads >700 bp
Typical Throughput	700 Mb
Reads per Run	1,000,000
Consensus Accuracy*	99.997%
Run Time	23 hours



	GS Junior System
Read Length	~400 bp
Throughput Profile	- 85% of total bases from reads >400 bp in length - 45% of total bases from reads >500 bp in length*
Throughput	~35 Mb

Throughput of NGS machines: Roche

Roche 454 SEQUENCING





Roche Shutting Down 454 Sequencing Business

Oct 15, 2013 | a GenomeWeb staff reporter

NEW YORK (GenomeWeb News) – Roche is shuttering its 454 Life Sciences sequencing operations and laying off about 100 employees, the company confirmed today.

The 454 sequencers will be phased out in mid-2016, and the 454 facility in Branford, Conn., will be closed "accordingly," Roche said in a statement e-mailed to *GenomeWeb Daily News*.

Throughput of NGS machines: SOLiD 4 (2013)



	5500 SOLiD™ Sequencer with microbeads	5500xl SOLiD™ Sequencer with microbeads	5500xl SOLiD™ Sequencer with nanobeads
System accuracy[1]	up to 99.99%	up to 99.99%	up to 99.99%
Typical throughput/day[2]	10–15 Gb/day	20–30 Gb/day	30–45 Gb/day
Samples/run[3]	• 1 genome • 12 exomes • 6 transcriptomes	• 2 genomes • 24 exomes • 12 transcriptomes	• 3 genomes • 40 exomes • 20 transcriptomes
Independent lanes	1–6	1–12	1–12
Typical run time	• 1 day for 35 bp (1 lane) • 7 days for 60 bp x 60 bp (6 lanes) • 7 days for 75 bp x 35 bp (6 lanes)	• 1 day for 35 bp (1 lane) • 7 days for 60 bp x 60 bp (12 lanes) • 7 days for 75 bp x 35 bp (12 lanes)	• 1 day for 35 bp (1 lane) • 7 days for 60 bp x 60 bp (12 lanes) • 7 days for 75 bp x 35 bp (12 lanes)
Typical throughput/run[4]	90 Gb	180 Gb	300 Gb
Read length	• MP: 60 bp x 60 bp • PE: 75 bp x 35 bp • Fragment: 75 bp	• MP: 60 bp x 60 bp • PE: 75 bp x 35 bp • Fragment: 75 bp	• MP: 60 bp x 60 bp • PE: 75 bp x 35 bp • Fragment: 75 bp
Multiplexing	96 for RNA and DNA	96 for RNA and DNA	96 for RNA and DNA

In 2014 ABI SOLiD was replaced by the Ion Torrent sequencing platforms (Proton and Personal Genome Machine)

Next Generation Sequencing Technology

Massively **P**arallel **S**ignature **S**equencing (MPSS)

NATURE METHODS | VOL.5 NO.1 | JANUARY 2008





***Nature Methods*' Method of the Year 2007 goes to next-generation sequencing. This series of articles showcase how these novel sequencing methods came into their own in 2007 and the incredible impact they promise to have in a variety of research applications. The Methods to Watch feature provide a glimpse and a wish list for future Methods of the Year.**

~~454 GS FLX~~
http://www.454.com/

SOLEXA
http://www.illumina.com/pages.ilmn?ID=250

~~SOLID~~
http://marketing.appliedbiosystems.com/

illumina Miseq





MISEQ REAGENT KIT V2

READ LENGTH	TOTAL TIME*	OUTPUT
1 × 36 bp	~4 hrs	540-610 Mb
2 × 25 bp	~5.5 hrs	750-850 Mb
2 × 150 bp	~24 hrs	4.5-5.1 Gb
2 × 250 bp	~39 hrs	7.5-8.5 Gb

MISEQ REAGENT KIT V3

READ LENGTH	TOTAL TIME*	OUTPUT
2 × 75 bp	~24 hrs	3.3-3.8 Gb
2 × 300 bp	~65 hrs	13.2-15 Gb

illumina Hiseq 3000/4000



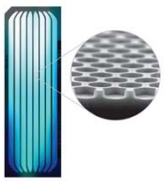


Table 1: Performance Parameters of the HiSeq 3000/4000 Systems.[a]

	HiSeq 3000 System	HiSeq 4000 System
Number of Flow Cells per Run	1	1 or 2
Output[b] 2 × 150 bp 2 × 75 bp 1 × 50 bp	 630–750 Gb 315–375 Gb 105–125 Gb	 1300–1500 Gb 650–750 Gb 215–250 Gb
Clusters Passing Filter (Single Reads)	2.1–2.5 billion	4.3–5 billion
Quality Scores	≥ 75% of bases above Q30 at 2 × 150 bp	≥ 75% of bases above Q30 at 2 × 150 bp
Daily Throughput	> 200 Gb	> 400 Gb
Run Time	< 1–3.5 days	< 1–3.5 days
Human Genomes per Run[c]	up to 6	up to 12
Exomes per Run[d]	up to 90	up to 180
Transcriptomes per Run[e]	up to 50	up to 100

illumina® Hiseq X Ten/Five



Table 1: HiSeq X System Sequencing Capacity.

	HiSeq X Ten System	HiSeq X Five System
Minimum Number of Instruments	10	5
Annual Genome Capacity	> 18,000	> 9000
Price per 30x genome	< $1000	< $1500

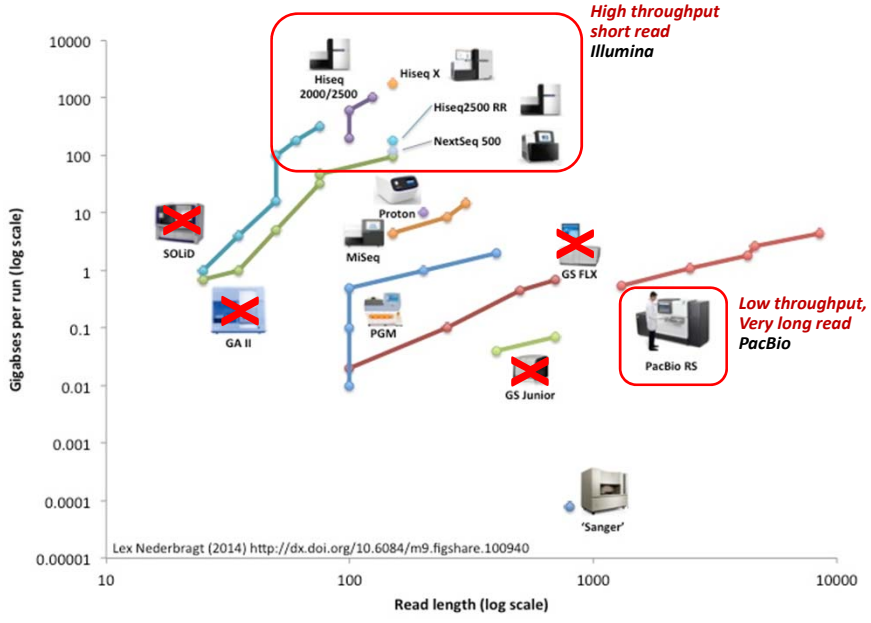
When operating at scale, HiSeq X Ten and HiSeq X Five Systems generate a staggering level of throughput, sequencing thousands of genomes per year.

Table 2: HiSeq X System Performance Parameters.[a]

Output per Run	Dual flow cell: 1.6-1.8 Tb
Single Reads Passing Filter	Dual flow cell: 5.3-6 billion
Supported Read Length	2 × 150 bp
Run Time	< 3 days
Quality	≥ 75% of bases above Q30 at 2 × 150 bp
Supported Library Preparation	TruSeq DNA PCR-Free Library Prep Kit TruSeq Nano DNA Library Prep Kit

a. Specifications based on Illumina PhiX control library at supported cluster densities (1255–1412 K clusters/mm²) on 1 HiSeq X System. Supported library preparation kit includes TruSeq Nano DNA Kit and TruSeq PCR-Free DNA Kit with 350 bp target insert size and HiSeq X Reagent Kit v2. The HiSeq X System was designed, optimized, and licensed for human WGS. Other applications and species are not permitted.

Throughput of NGS machines (2014)



NEXT GENERATION SEQUENCING

NGS

Basis Principles

Temple fragmentation (library) ➧ Amplification ➧ Sequencing ➧ Detection

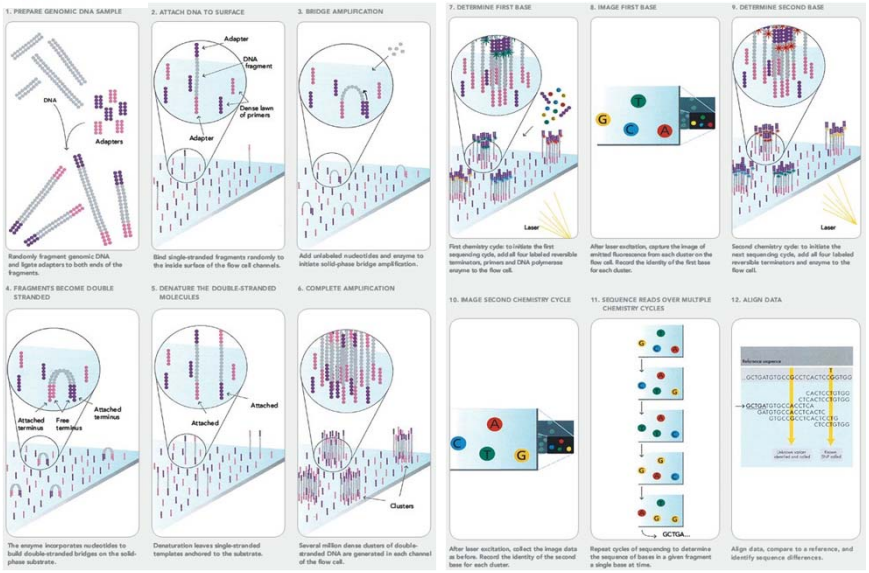
Illumina Sequencing by Synthesis (SBS)

illumina Solexa



illumina

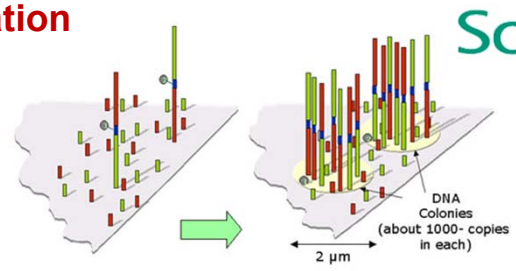
Solexa Genome Analyzer



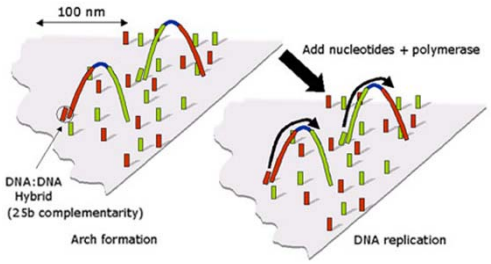
Cluster Generation

Solexa™

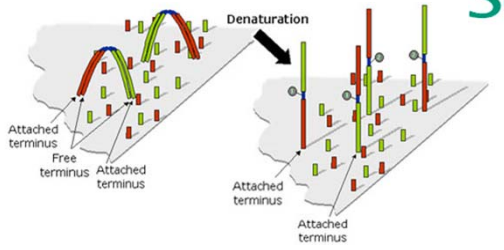
Clusters Array



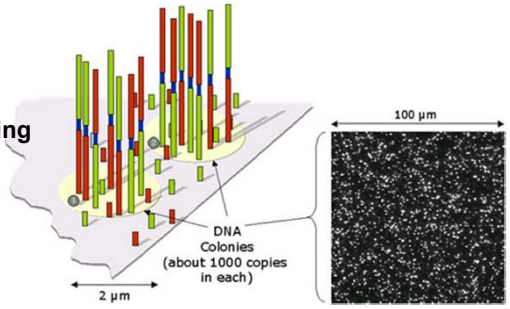
Template Bridging



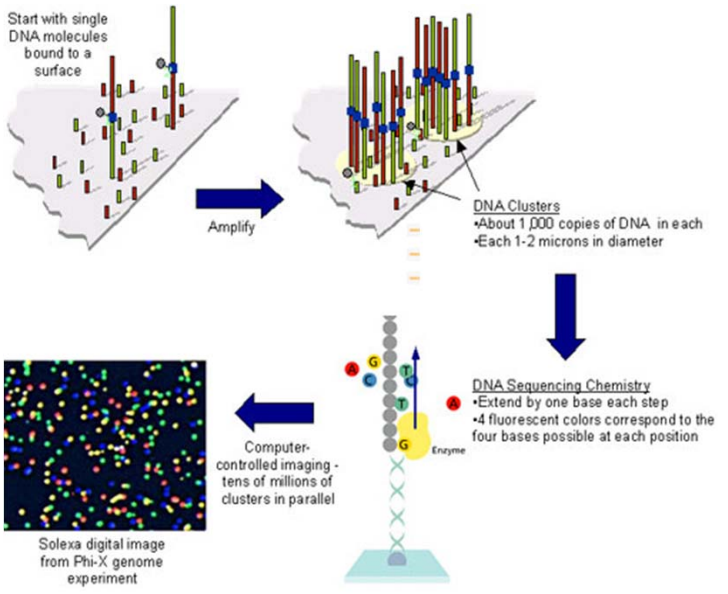
Denaturation



Ready For Sequencing



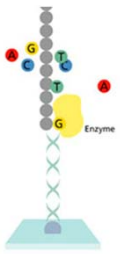
Sequencing-By-Synthesis



Solexa™

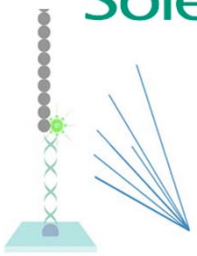
Cycle 1

Incorporation

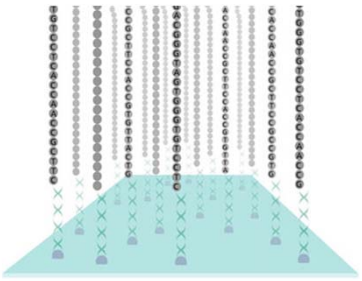


Cycle 1

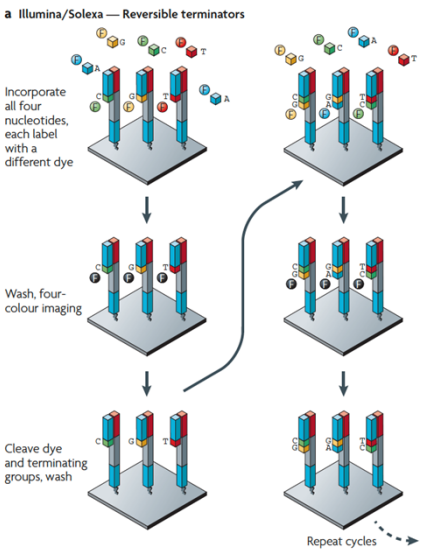
Fluorescent Detection

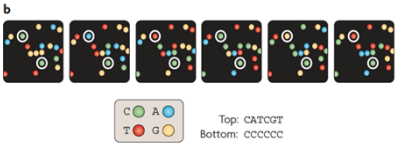


Sequence Generated At Every Site On The Array



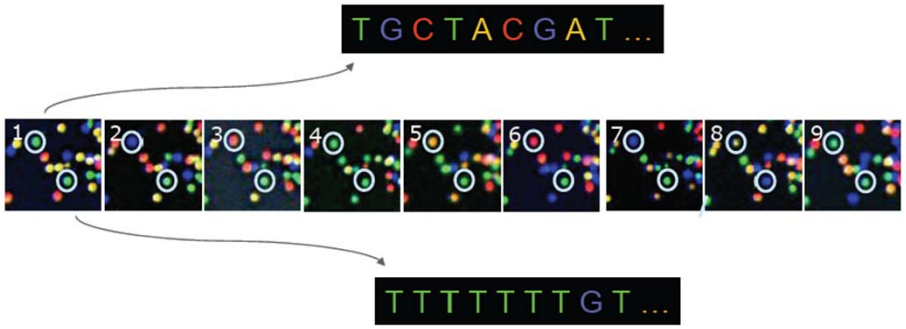
Solexa™





From Debbie Nickerson, Department of Genome Sciences, University of Washington, http://tinyurl.com/6zbzh4

Base calling from raw data



The identity of each base of a cluster is read off from sequential images

From Debbie Nickerson, Department of Genome Sciences, University of Washington, http://tinyurl.com/6zbzh4

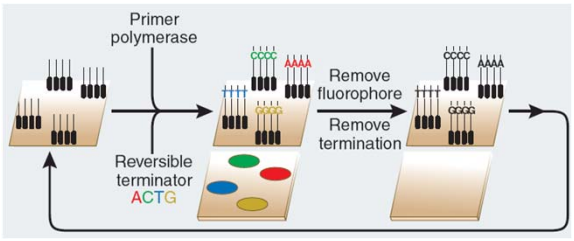
Solexa technology (sequencing-by-synthesis)

Sample preparation.

Fragments of DNA are ligated to end adapters, denatured and bound at one end to a solid surface already coated with a dense layer of the adapters. Each single stranded fragment is immobilized at one end, while its free end 'bends over' and hybridizes to a complementary adapter on the surface, which initiates the synthesis of the complementary strand in the presence of amplification reagents. Multiple cycles of this solid-phase amplification followed by denaturation create clusters of ~1,000 copies of single-stranded DNA molecules distributed randomly on the surface.

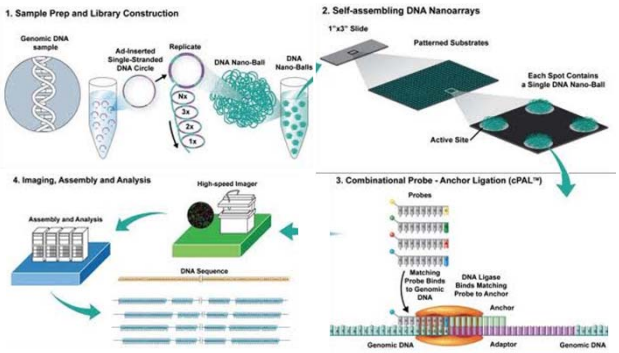
Sequencing with reversible terminators.

Synthesis reagents, added to the flow cell, consist of primers, DNA polymerase and four differently labeled, reversible terminator nucleotides. After incorporation of a nucleotide, which is identified by its color, the 3′ terminator on the base and the fluorophore are removed, and the cycle is repeated for a read length of 30–35 nucleotides.



Combinatorial Probe-Anchor Ligation (cPAL) sequencing

Complete genomics



Technology description: Drmanac, R et al. (2010) Human Genome Sequencing Using Unchained Base Reads on Self-Assembling DNA Nanoarrays. Science 327, 78-81

Evolution of Sequencing Technology



Sanger dideoxy-sequencing

ABI 3730XL

Massive parallel sequencing

~~Roche 454 FLX~~, ~~Life Technologies SOLiD,~~
Illumina Hiseq, Life Tech Ion Torrent

Bead-based em-PCR and sequencing by ligation

~~Dover Systems' Polonator~~

Massive parallel sequencing and single molecule sequencing

Pacific Biosciences (single-molecule real-time DNA sequencing (SMRT) technology)
~~Helicos (true single-molecule sequencing (tSMS) technology)~~
~~VisiGen Biotechnologies (real-time, single-molecule sequencing fluorescence resonance energy transfer (FRET) technology)~~

Single molecule sequencing and nanopore technology?

Oxford Nanopore Technologies,, Electron Optica, Electronic BioSciences, GenapSys, Genia, GnuBIO, IBM, LaserGen, Lightspeed Genomics, NABsys, NobleGen Biosciences, QuantuMDx, Reveo, Stratos Genomics, Two Pore Guys, ZS Genetics……

NEXT-NEXT-NEXT-

NEW GENERATION SEQUENCING

NGS

Basis Principles

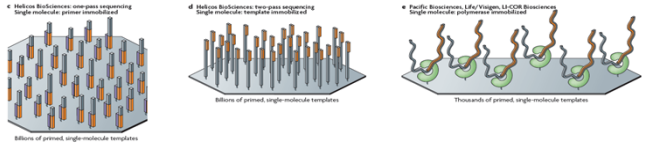
Temple fragmentation (library) ➡ Amplification ➡ Sequencing ➡ Detection

Single Molecule Real-Time Sequencing

Real-time monitoring of PCR activity

Read-out by fluorescence resonance energy transfer between polymerase and nucleotides or

Waveguides allow direct observation of polymerase and fluorescently labeled nucleotides



~~VisGen Biotechnologies~~

~~Helicos Biosciences~~

Pacific Biosciences

勝

Helicos Sells Certain MDx IP to Sequenom for $1.3M, Warns of Potential Bankruptcy

April 11, 2012

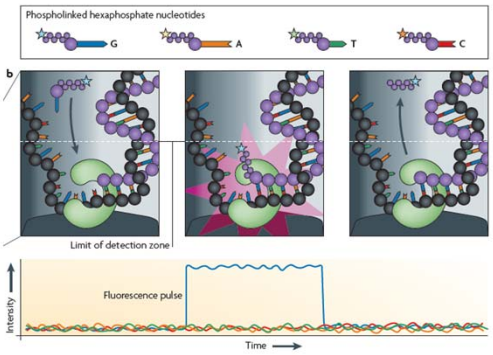
Helicos BioSciences said in a regulatory filing that it has sold certain patent applications covering molecular diagnostic applications of its technology to Sequenom for $1.3 million. The patent applications cover methods for detecting fetal nucleic acids and diagnosing fetal abnormalities. Helicos also warned that if it is "unable to successfully raise additional capital, we may have to cease operations and/or seek bankruptcy protection."

Single Molecule Real-Time (SMRT) Sequencing

www.sciencemag.org SCIENCE VOL 323 2 JANUARY 2009 133

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†



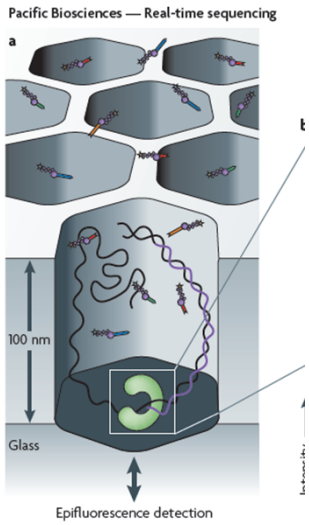
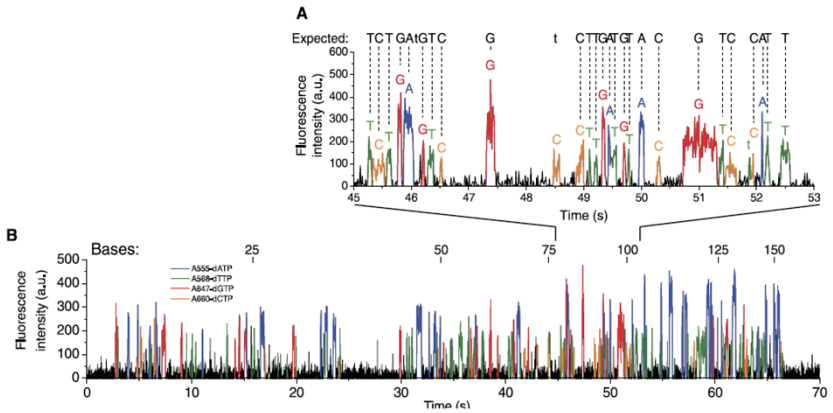




Figure 10. Processive Synthesis with Phospholinked Nucleotides.

Step 1: Fluorescent phospholinked labeled nucleotides are introduced into the ZMW.
Step 2: The base being incorporated is held in the detection volume for tens of milliseconds, producing a bright flash of light.
Step 3: The phosphate chain is cleaved, releasing the attached dye molecule.
Step 4-5: The process repeats.

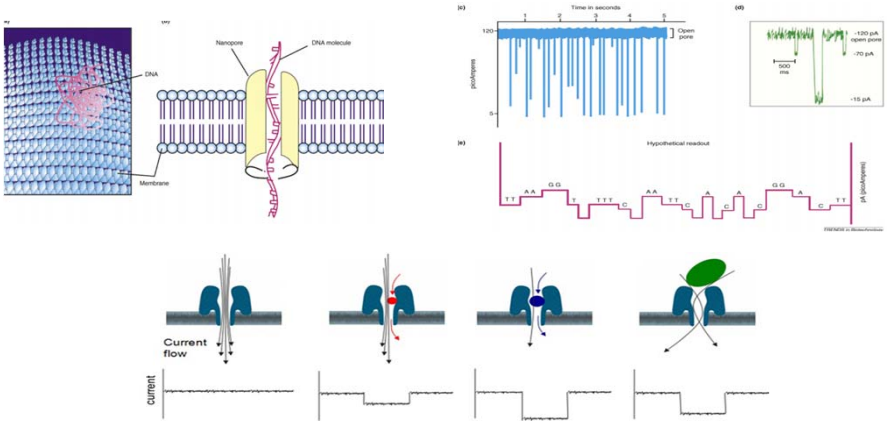


Nanopore Sequencing

Oxford Nanopore

Nucleic acids driven through a nanopore.

Differences in conductance of pore provide readout.



This diagram shows a protein nanopore set in an electrically resistant membrane bilayer. An ionic current is passed through the nanopore by setting a voltage across this membrane.

If an analyte passes through the pore or near its aperture, this event creates a characteristic disruption in current. By measuring that current it is possible to identify the molecule in question. For example, this system can be used to distinguish the four standard DNA bases and G, A, T and C, and also modified bases. It can be used to identify target proteins, small molecules, or to gain rich molecular information for example to distinguish the enantiomers of ibuprofen or molecular binding dynamics.

Bio-IT World

Indispensable Technologies Driving Discovery, Development, and Clinical Trials

Oxford Strikes First in DNA Sequencing Nanopore Wars

By Kevin Davies

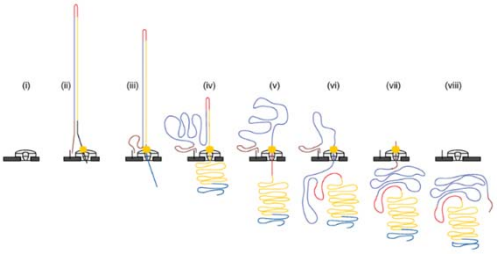
February 17, 2012 | Breaking a near total vow of silence after three years in stealth mode, Oxford Nanopore has offered stunning details of its nanopore next-generation sequencing (NGS) technology to a packed house at the premier genome sequencing conference in Marco Island, Florida.

In a talk at the Advances in Genome Biology and Technology conference this morning, chief technology officer Clive Brown presented details of what the British company calls its "new generation" of single-strand sequencing technology, featuring accurate long reads of single-stranded DNA molecules, including the completion of two viral genomes.

In the second half of this year, the company will commercially release its previously announced GridION instrument, or node, as well as the world's smallest DNA sequencing instrument – a portable cartridge called the MinION that doubles (and works as) a USB drive.



Oxford Nanopore's disruptive MinION USB device



2015
Mean read length: 2 kb
Median read length : 1 kb
Longest single read: 98 kb

The GridION system

Oxford Nanopore's proprietary nanopore-based sensing chemistries are operated on an electronics-based platform, the GridION system. This enables the scaled-up measurements of multiple nanopores and the sensing, processing and analysis of data in real time.

A single instrument, a GridION node, operates with a single-use cartridge that contains the necessary reagents to perform an experiment.



Scalability
A node can be employed as a single desktop instrument, or scaled up in a similar way to computing installations.

As a desktop instrument for the individual researcher, it can write data to the network or a locally attached disc (directly through a USB or through a network) and work in a small lab.

Each node is a network device and multiple nodes can be aggregated together into larger co-operating units or clusters, communicating with each other in a peer-to-peer fashion over the user's network. The system is designed to interface, or even co-locate, with standard or high-performance IT infrastructure. Workflow overheads, and total costs, including IT, scale linearly with the GridION system.

graphic: a single node (left) may be used as a desktop device, or installed in conjunction with other nodes that communicate with each other through a network (centre, right).

A single node as a desktop installation



Nodes may be clustered through a network for larger throughput installations



Each GridION node operates with a single disposable cartridge.



Wellcome Trust Centre for Human Genetics and Genomics plc First to Sequence Multiple Human Genomes Using Hand-Held Nanopore Technology

Demonstrates potential for wide-scale whole-genome sequencing in humans using nanopore approaches

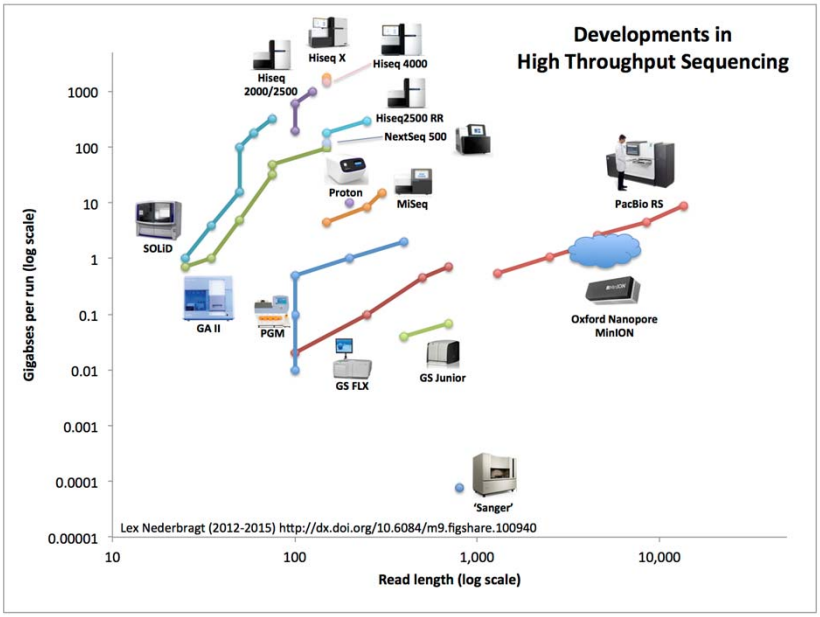
December 01, 2016 07:01 PM Eastern Standard Time

OXFORD, England--(BUSINESS WIRE)--Oxford University's Wellcome Trust Centre for Human Genetics (WTCHG) and the leading genome analytics company Genomics plc today announced the first sequencing and analysis of multiple human genomes using nanopore technology. The announcement, made on Thursday at the Oxford Nanopore Community Meeting in New York City, marks a major breakthrough in sequencing technology in opening up the potential of wide-scale whole-genome sequencing in humans using nanopore approaches.

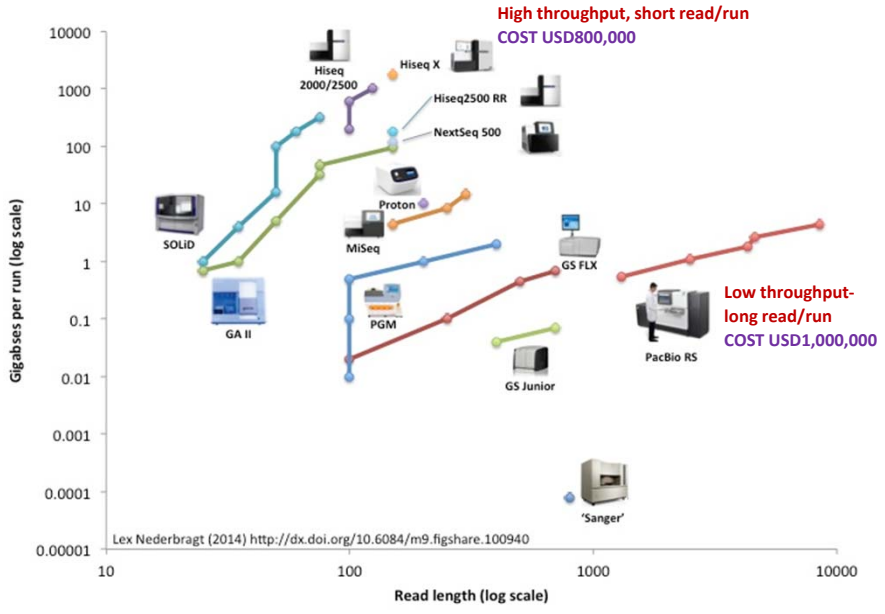
"It is a significant breakthrough to sequence multiple human genomes with nanopore technology. Moving forward, the idea that there will be alternative technologies with new properties in this space will be very appealing to researchers and to clinical geneticists."

Tweet this

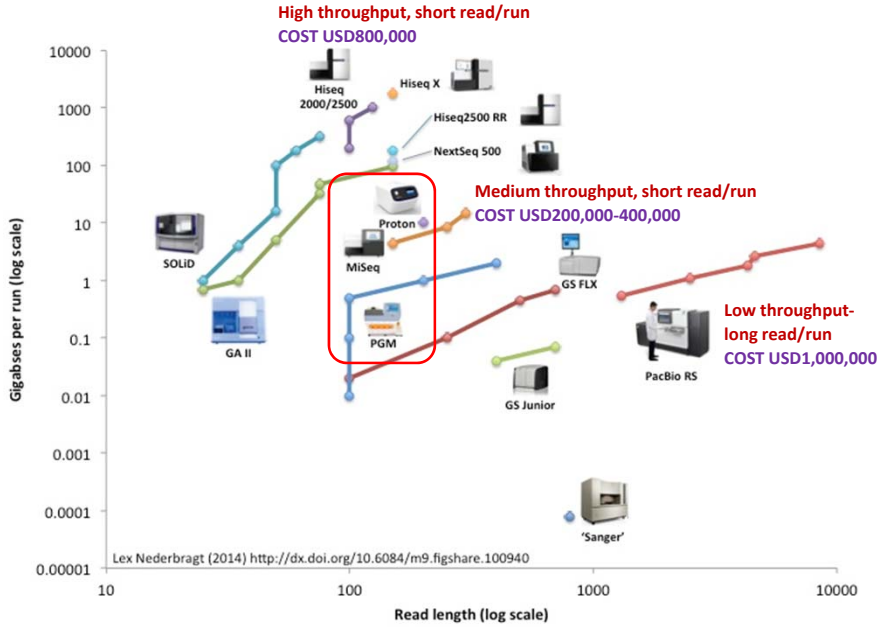
Today's announcement raises the possibility of a major change to both the economics and the science of DNA sequencing. Researchers at the WTCHG and Genomics plc used the MinION sequencer developed by Oxford Nanopore Technologies. The portable MinION is a hand-held device about the size of a Mars bar, and is powered by a laptop computer to which it connects via a USB port. It is available for $1,000 plus the cost of consumables. While nanopore sequencing has been available for two years, it has previously only been practicable to apply it to relatively small genomes or regions of DNA, such as the much smaller genomes of viruses and bacteria. Recent upgrades to the technology have now made it possible for researchers to conduct larger-scale studies such as sequencing complete human genomes by using multiple MinIONs.



Cost of NGS machines (2014)



Cost of NGS machines (2014)



Personal Sequencer

illumina®



Miseq/MiniSeq
Personal Sequencing System

ion torrent
by life technologies™



Ion Torrent/Ion Proton
Personal Genome Machine

illumina® MiSeq Benchtop Sequencer





MISEQ REAGENT KIT V2

READ LENGTH	TOTAL TIME*	OUTPUT
1 × 36 bp	~4 hrs	540-610 Mb
2 × 25 bp	~5.5 hrs	750-850 Mb
2 × 150 bp	~24 hrs	4.5-5.1 Gb
2 × 250 bp	~39 hrs	7.5-8.5 Gb

MISEQ REAGENT KIT V3

READ LENGTH	TOTAL TIME*	OUTPUT
2 × 75 bp	~24 hrs	3.3-3.8 Gb
2 × 300 bp	~65 hrs	13.2-15 Gb

ion torrent by life technologies™



ION PGM™ SEQUENCER

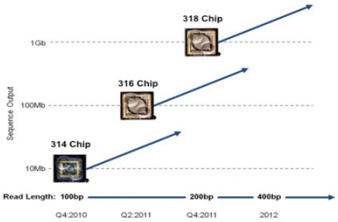
SMALL GENOMES
SETS OF GENES
GENE EXPRESSION
CHIP-SEQ

10 Mb to 1 Gb

100-fold scaling means you can choose the amount of sequencing throughput required for your specific application.



Chip Type	314	316	318
Wells (in millions)	1.2	6.2	11.1
Bases (in Mb)	>10	>100	>1000



90-minute run times

Rapid 100-base sequencing on the Ion 314™ Chip.

35–400* bp reads

If your application requires short-read or long-read sequencing, or single-end, paired-end, or mate-paired sequencing, the Ion PGM™ Sequencer delivers the greatest flexibility.

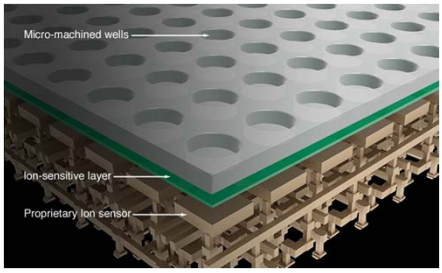


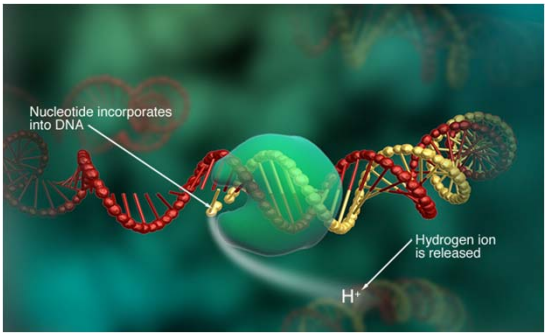
Ion Torrent





314 chip
316 chip





ion torrent
by life technologies

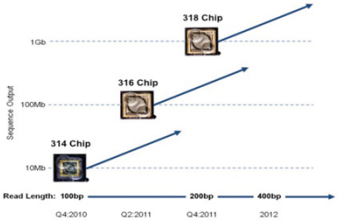


ION PGM™ SEQUENCER
SMALL GENOMES
SETS OF GENES
GENE EXPRESSION
CHIP-SEQ

10 Mb to 1 Gb
100-fold scaling means you can choose the amount of sequencing throughput required for your specific application.



Chip Type	314	316	318
Wells (in millions)	1.2	6.2	11.1
Bases (in Mb)	>10	>100	>1000



90-minute run times
Rapid 100-base sequencing on the Ion 314™ Chip.

35–400* bp reads
If your application requires short-read or long-read sequencing, or single-end, paired-end, or mate-paired sequencing, the Ion PGM™ Sequencer delivers the greatest flexibility.

ROUND 1





Table 1 Insertion/deletion and substitution errors on read level for benchtop NGS platforms

Platform	Sequencing kit	Library	Strain	Date of sequencing	Indels per 100 bp	Indels per read	Substitutions per 100 bp	Substitutions per read
GSJ	GSJ Titanium	Nebulization / AMPure XP	Sakai	June 2012	0.4011	1.8351	0.0543	0.2484
MiSeq	2 × 150-bp PE	Nextera	Sakai	June 2012	0.0009	0.0013	0.0921	0.1318
MiSeq	2 × 250-bp PE	Nextera	Sakai	September 2012	0.0009	0.0018	0.0940	0.2033
PGM	100 bp	Bioruptor / Ion Fragment Library	Sakai	July 2011	0.3520	0.3878	0.0929	0.1024
PGM	200 bp	Ion Xpress Plus Fragment	Sakai	July 2012	0.3955	0.6811	0.0303	0.0521
PGM	300 bp	Ion Xpress Plus Fragment	Sakai	August 2012	0.7054	1.4457	0.0861	0.1765
PGM	400 bp[a]	Ion Xpress Plus Fragment	Sakai	November 2012	0.6722	1.8726	0.0790	0.2202

Error rates were calculated by counting indels and substitutions in the mapping against the EHEC Sakai reference sequence for each uniquely mapped read.
[a]Kit was not officially available during time of study.

ROUND 2

Ion Proton™ Sequencer

The Only Benchtop Genome Center

Human exome sequencing

Using the next generation of semiconductor technology, the Ion Proton™ I Chip will deliver whole-exome sequencing in just a few hours.

"Cost, speed, and accuracy are key elements in the use of DNA sequencing. The technological advances in the new Ion Proton™ instrument promise to be game-changing for both research and clinical applications."

DR. RICHARD LIFTON
YALE SCHOOL OF MEDICINE, USA

ION PROTON™ SEQUENCER

HUMAN GENOMES
HUMAN EXOMES
WHOLE TRANSCRIPTOMES

Human genome sequencing

The Ion Proton™ II Chip will enable fast, affordable, whole-genome sequencing on your benchtop.

2-hour run times

Rapid 100-base sequencing runs on the Ion Proton™ I Chip.



Ion Proton™ System performance specifications* with Ion Proton™ I Chip at commercial launch	
Throughput	Up to 10 Gb (Note: The Ion Proton™ II Chip will be available about six months after the Ion Proton™ I Chip. The Ion Proton™ II Chip will enable sample-to-variant analysis of a human genome in a single day, at up to 20x coverage.)
Read length	Up to 200-base fragment reads
Sequencing run time	As little as 2 hours for 100-base reads

illumina® **Miniseq**



Cluster Generation and Sequencing

MINISEQ SYSTEM HIGH-OUTPUT KIT*		
READ LENGTH	TOTAL TIME†	OUTPUT
2 × 150 bp	~24 hrs	6.6-7.5 Gb
2 × 75 bp	13 hrs	3.3-3.75 Gb
1 × 75 bp	7 hrs	1.65-1.875 Gb

MINISEQ SYSTEM MID-OUTPUT KIT*		
READ LENGTH	TOTAL TIME†	OUTPUT
2 × 150 bp	17 hrs	2.1-2.4 Gb

Reads Passing Filter

MINISEQ SYSTEM HIGH-OUTPUT KIT	
Single Reads	22-25 Million
Paired-End Reads	44-50 Million

MINISEQ SYSTEM MID-OUTPUT KIT	
Single Reads	7-8 Million
Paired-End Reads	14-16 Million

Quality Scores††

MINISEQ SYSTEM HIGH-OUTPUT KIT
> 80% bases higher than Q30 at 2 x 150 bp
> 85% bases higher than Q30 at 2 x 75 bp
> 85% bases higher than Q30 at 1 x 75 bp

MINISEQ SYSTEM MID-OUTPUT KIT
> 80% bases higher than Q30 at 2 x 150 bp

illumina



MiniSeq System
MAX OUTPUT 8 Gb
MAX READ NUMBER 25 million
MAX READ LENGTH 2×150 bp



MiSeq Series
MAX OUTPUT 15 Gb
MAX READ NUMBER 25 million
MAX READ LENGTH 2×300 bp



NextSeq System
MAX OUTPUT 120 Gb
MAX READ NUMBER 400 million
MAX READ LENGTH 2×150 bp



HiSeq Series
MAX OUTPUT 1500 Gb
MAX READ NUMBER 5 billion
MAX READ LENGTH 2×150 bp



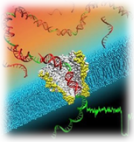
HiSeq X Series
MAX OUTPUT 1800 Gb
MAX READ NUMBER 6 billion
MAX READ LENGTH 2×150 bp

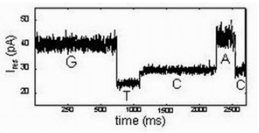
illumina



Product	MiniSeq	MiSeq	NextSeq 500[6]		HiSeq 2500		HiSeq 3000	HiSeq 4000	HiSeq X Five[7]	HiSeq X Ten[7]
Description	Simplicity and accessibility for examining single genes or entire pathways	Speed and simplicity for targeted and small-genome sequencing	Speed and simplicity for everyday genomics		Power and efficiency for large-scale genomics		Maximum throughput and lowest cost for production-scale genomics		Maximum throughput and lowest cost for population- and production-scale human WGS	
Key methods	Targeted DNA and targeted RNA sequencing	Small genome, amplicon, targeted gene panel sequencing	Everyday genome, exome, transcriptome sequencing, and more		Production-scale genome, exome, transcriptome sequencing, and more				Population-scale human WGS	
Run mode	–	–	Mid-output	High-output	Rapid run	High-output	–	–	–	–
Flow cells processed per run	1	1	1	1	1 or 2	1 or 2	1	1 or 2	1 or 2	1 or 2
Output range	0.6–7.5 Gb	0.3–15 Gb	20–39 Gb	30–120 Gb	10–300 Gb	50–1000 Gb	125–750 Gb	125–1500 Gb	900–1800 Gb	900–1800 Gb
Run time	4–24 hours	5–55 hours	15–26 hours	12–30 hours	7–60 hours	< 1–6 days	< 1–3.5 days	< 1–3.5 days	< 3 days	< 3 days
Reads per flow cell[8]	25 million[3]	25 million[3]	130 million	400 million	300 million	2 billion	2.5 billion	2.5 billion	3 billion	3 billion
Maximum read length	2 x 150 bp	2 x 300 bp	2 x 150 bp	2 x 150 bp	2 x 250 bp	2 x 125 bp	2 x 150 bp	2 x 150 bp	2 x 150 bp	2 x 150 bp

Nanopore Sequencing - IBM DNA Transistor







GeneReader



Early-access customers in 2013,
Commercial launch in early 2014.

20 flowcells configuration
20-400M reads per flow cell
12 minutes per base sequencing-by-synthesis
(50-bases ~10 hours)

Droplet-based Sequencing

Microfluidics, the science that deals with the behavior, precise control and manipulation of fluids that are geometrically constrained to a small, typically sub-millimeter scale, emerged in the beginning of the 1980s and is used widely in technologies such as inkjet print heads, DNA chips, lab-on-a-chip technology, micro-propulsion and micro-thermal technologies. GnuBIO has adapted technology developed in Dr. David Weitz laboratory at Harvard. This technology is combined with proprietary molecular biology assays to create a unique next-generation DNA sequencing technology. The science behind this GnuBIO sequencing technology utilizes droplet microfluidics to perform the biochemical reactions for sequencing inside of tiny picoliter -sized aqueous drops. Each droplet acts as a discrete reaction vessel, like a miniature test tube, where the sequencing assay is performed.

The following results are the raw and unfiltered data from the GnuBIO prototype system:

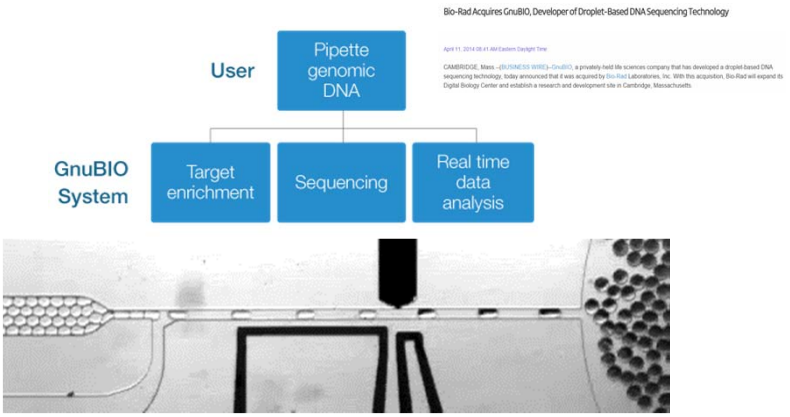
99.993% per base accuracy: This is the true per base accuracy, and is not based on consensus accuracy
100% sequence coverage
100% of the reads were used
Read lengths of 126 base pairs (read lengths of 1Kb can routinely be achieved today)



Droplet-based Sequencing

Microfluidics, the science that deals with the behavior, precise control and manipulation of fluids that are geometrically constrained to a small, typically sub-millimeter scale, emerged in the beginning of the 1980s and is used widely in technologies such as inkjet print heads, DNA chips, lab-on-a-chip technology, micro-propulsion and micro-thermal technologies. GnuBIO has adapted technology developed in Dr. David Weitz laboratory at Harvard. This technology is combined with proprietary molecular biology assays to create a unique next-generation DNA sequencing technology.

The science behind this GnuBIO sequencing technology utilizes droplet microfluidics to perform the biochemical reactions for sequencing inside of tiny picoliter -sized aqueous drops. Each droplet acts as a discrete reaction vessel, like a miniature test tube, where the sequencing assay is performed.



http://allseq.com/knowledgebank

AllSeq NEED SEQUENCING NGS CONFERENCES KNOWLEDGE BANK ABOUT US BLOG SIGN IN search...

Home › Knowledge Bank

The AllSeq Knowledge Bank

The Knowledge Bank is managed by employees at AllSeq. AllSeq matches people's sequencing service needs with labs that have extra capacity on their NGS instruments. See how it works. This totally independent position in the sequencing market has lead to great knowledge about the topic.

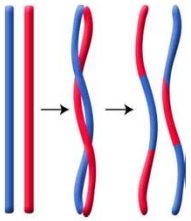
-The AllSeq-team

Knowledge Bank

Sequencing Platforms

Emerging Technologies

Electron Optica
Electronic BioSciences
GenapSys
Genia
GnuBIO
IBM
LaserGen
Lightspeed Genomics
NABsys
NobleGen Biosciences
Oxford Nanopore Technologies
Qiagen
xQuantuMDx
Reveo
Stratos Genomics
Two Pore Guys
ZS Genetics



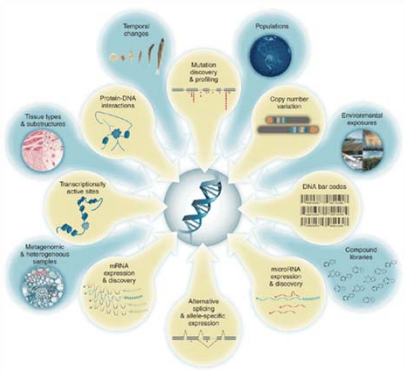
NEXT GENERATION SEQUENCING
Applications & Analysis



Biomarker Discovery
Translational Bioinformatics
NGS
Biological Pathways
Evolution
Bioinformatics
Cancer Genome
Disease
Visualization
Personal Genome
CNVs
SNPs
Next-Gen Sequencing
Variants
ChipSeq
Metagenome
Epigenome
Network Medicine
Genomics
Pharmacogenomics
Network Biology
Semantic Biology
Genome-Phenome-Envirome
Medical Informatics
Drug Repositioning
RNA-Seq
Toxicogenomics
Rare Disease
Bio-data Mining

Applications on Biomedical Sciences







DNA Exome Sequencing
Genome Resequencing
De novo Genome Sequencing
Meta-Genomic Sequencing
Chip-Sequencing

RNA Small RNA Sequencing
Transcriptome Sequencing
De novo Transcriptome Sequencing
Meta-Transcriptome Sequencing

BIOINFORMATICS

Applications on Biomedical Sciences

Category	Examples of applications
Complete genome resequencing	Comprehensive polymorphism and mutation discovery in individual human genomes
Reduced representation sequencing	Large-scale polymorphism discovery
Targeted genomic resequencing	Targeted polymorphism and mutation discovery
Paired end sequencing	Discovery of inherited and acquired structural variation
Metagenomic sequencing	Discovery of infectious and commensal flora
Transcriptome sequencing	Quantification of gene expression and alternative splicing; transcript annotation; discovery of transcribed SNPs or somatic mutations
Small RNA sequencing	microRNA profiling
Sequencing of bisulfite-treated DNA	Determining patterns of cytosine methylation in genomic DNA
Chromatin immunoprecipitation–sequencing (ChIP-Seq)	Genome-wide mapping of protein-DNA interactions
Nuclease fragmentation and sequencing	Nucleosome positioning
Molecular barcoding	Multiplex sequencing of samples from multiple individuals



2016.9.21

癌症連續32年位居台灣10大死因之首，平均每5分18秒就有一位國人罹癌。為此，中央研究院及長庚大學與美國癌症研究所(NCI)於今(2016)年簽署備忘錄，加入美國「癌症登月計畫(National Cancer Moonshot)」，與多國攜手合作，運用嶄新的蛋白基因體學(Proteogenomics)策略進行大規模癌症病人分析，探討疾病發生機制、檢測及治療的線索。

美國副總統拜登(Joe Biden)於19日在「2016社會公益高峰會」發表演說時指出，美國的癌症登月計畫將與台灣、加拿大、中國、德國、瑞士、日本與南韓等國建立合作關係，運用蛋白基因體進行癌症研究及治療。該計畫為美國總統歐巴馬於今年1月時提出，目標是將預計10年才能完成的癌症相關預防、診斷、治療和護理等工作期程縮短一半。

CGU-GIBMS bioinformatics www site

HOME | LECTURE NOTES | MANUALS | SOFTWARES | ON-LINE ANALYSIS | STUDENTS

生物資訊分析 (Bioinformatics) | 高通量定序分析 (HTS Analysis) | BioInfo & Biostat

高通量定序分析

上課時間：每週五上午 08:10~12:00 （08:10~09:00—授課，09:10~12:00—實習）
上課地點：醫學院 9F 生物資訊中心 電腦教室

助教：蔡智宇(分機5690)

進度	課程進度	授課老師	講義下載 L:lecture T:tutorial E:exercise
1	Introduction to New Generation Sequencing (NGS) Technologies	鄧致剛	L
2	Databases and Tools for High Throughput Sequencing Analysis	黃柏榕	L T:FastQC Movie:FastQC T:FASTX T:SRA T:SRA2
3	Small RNA Analysis and Target Identification	黃柏榕	L T:CLC DSAP miRDEEP E
4	Gene Expression Analysis (I)	葉元鳴	
5	Gene Expression Analysis (II)	辛致煒	
6	Transcriptome Functional Profiling-1 (Metacore & DAVID)	鄧致剛/李正陽	
7	Transcriptome Functional Profiling-2 (GSEA & KEGG)	鄧致剛/李季青	
8	MID-TERM EXAMINATION	鄧致剛	
9	Bacteria Genome & Gene Expression Analysis	陳怡原	Lecture
10	Small Genome de novo Assembly	陳亭紋	Lecture Exercise T
11	Genome Annotation & Genome Browser	黃柏榕	
12	Chip Sequencing Analysis	陳亭紋	Lecture+Exercise
13	Metagenome Analysis	葉元鳴	Lecture+Exercise
14	Targeted Resequencing Analysis	黃柏榕	
15	Biological Big Data Visualization & Integration	鄧致剛/李季青	
16	HOLIDAY		
17	Student Presentation	鄧致剛	PDF
18	Final-Examination	鄧致剛	PDF