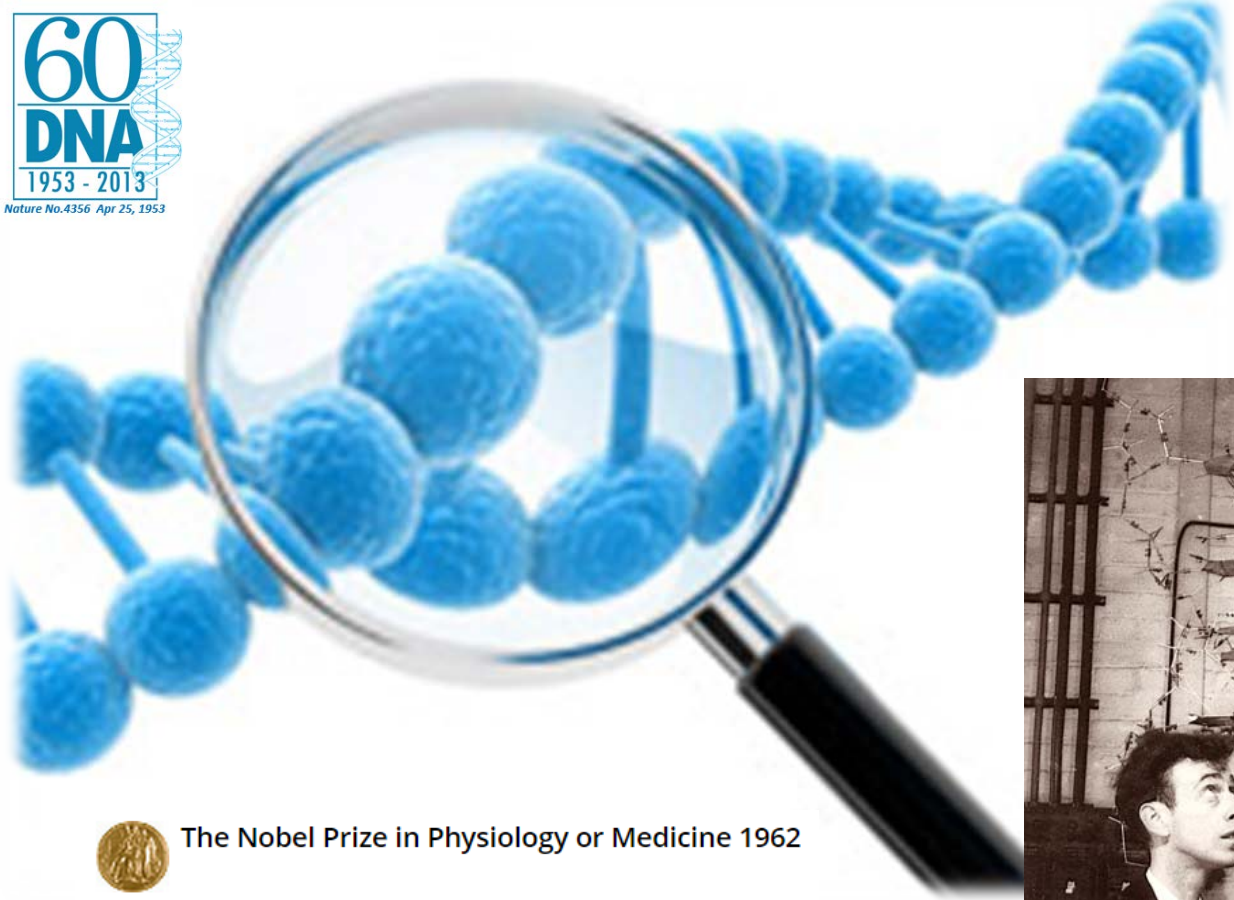
New Generation Sequencing Technologies

NGS



60 DNA 1953 - 2013

Nature No.4356 Apr 25, 1953



The Nobel Prize in Physiology or Medicine 1962



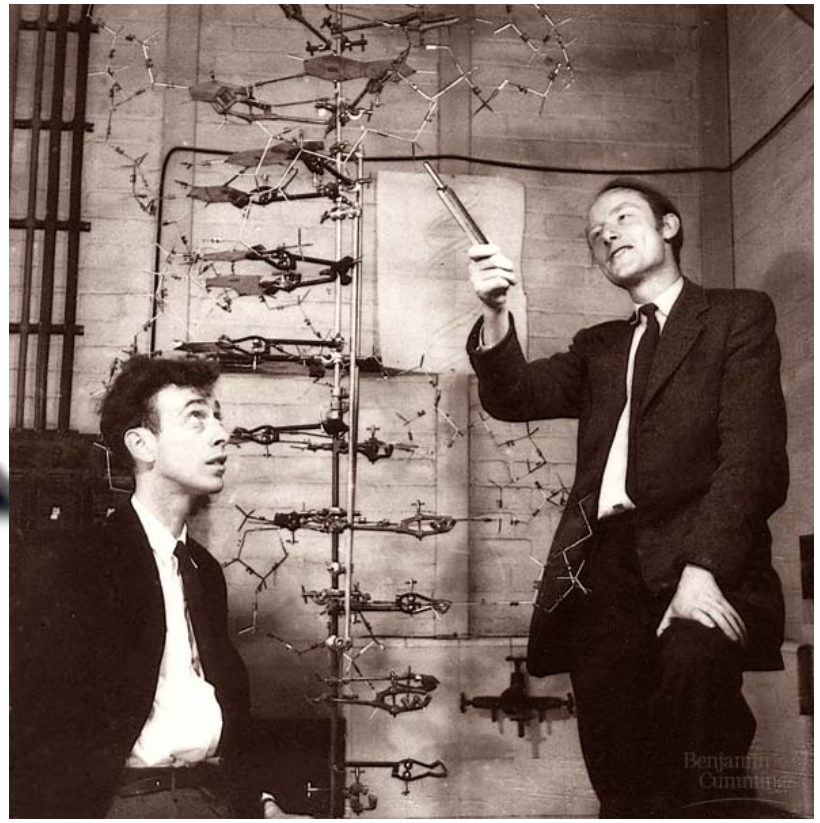
Francis Harry Compton Crick
Prize share: 1/3



James Dewey Watson
Prize share: 1/3

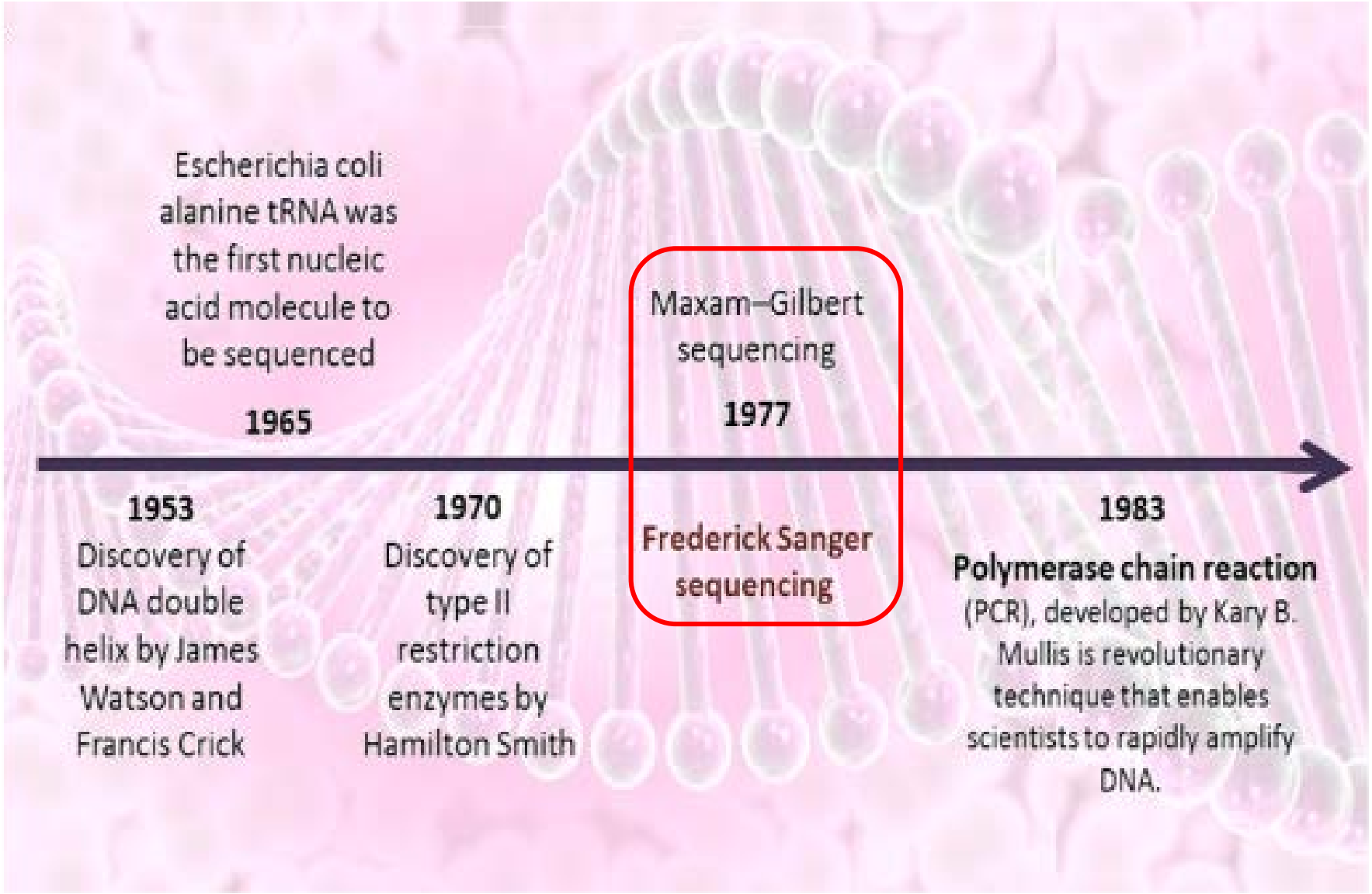


Maurice Hugh Frederick Wilkins
Prize share: 1/3

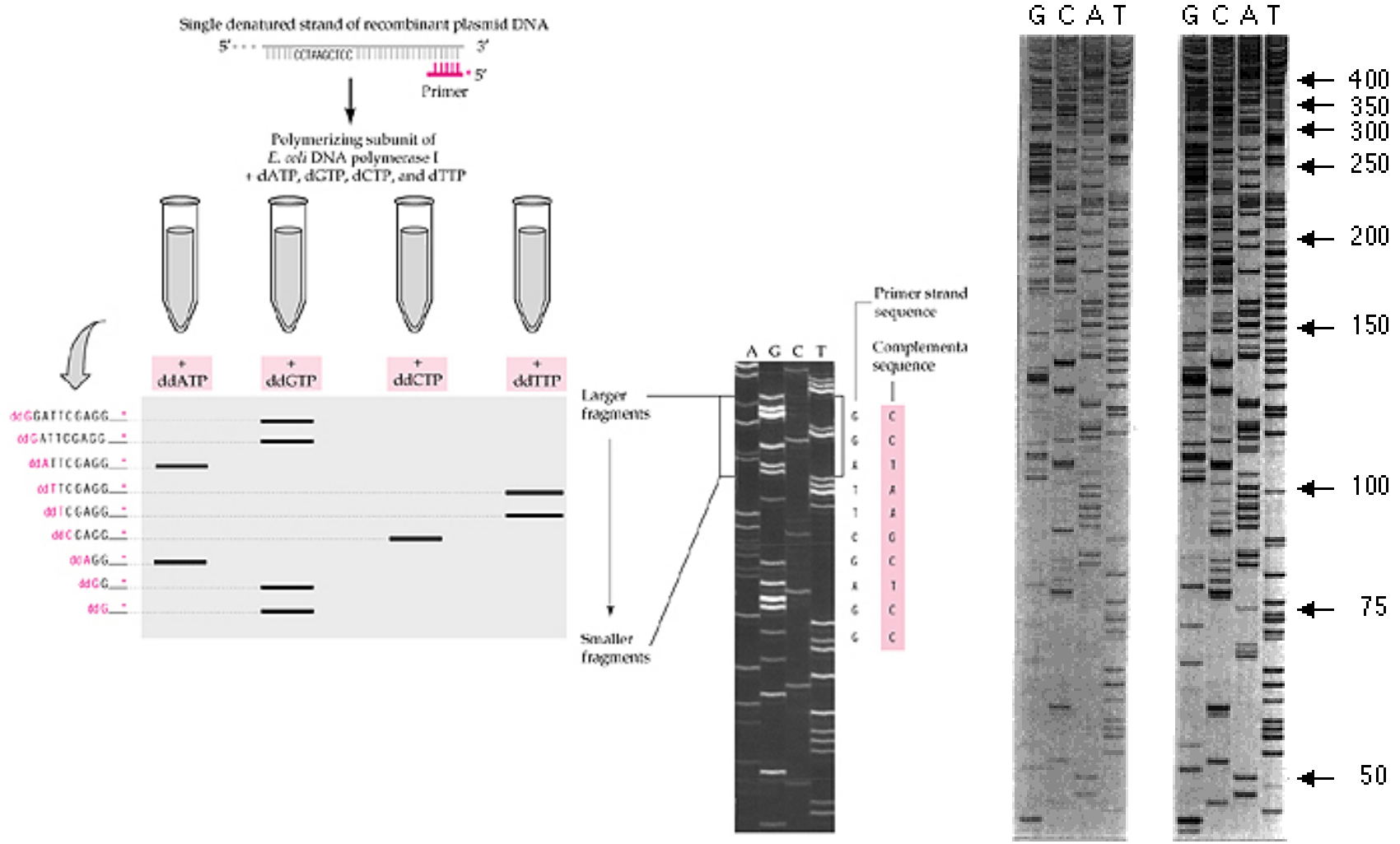


James Watson and Francis Crick with their DNA model at the Cavendish Laboratories in 1953

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

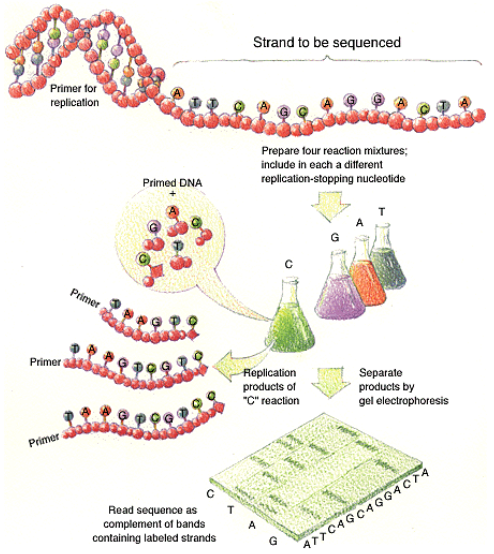
Proc. Natl. Acad. Sci. USA
Vol. 74, No. 12, pp. 5463–5467, December 1977
Biochemistry

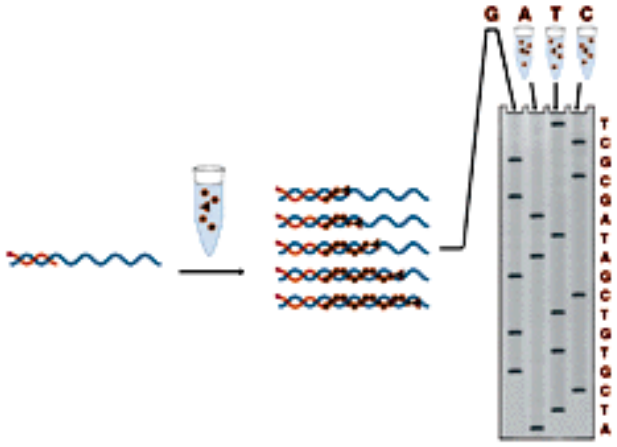
DNA sequencing with chain-terminating inhibitors

(DNA polymerase/nucleotide sequences/bacteriophage φX174)

F. SANGER, S. NICKLEN, AND A. R. COULSON
Medical Research Council Laboratory of Molecular Biology, Cambridge CB2 2QH, England

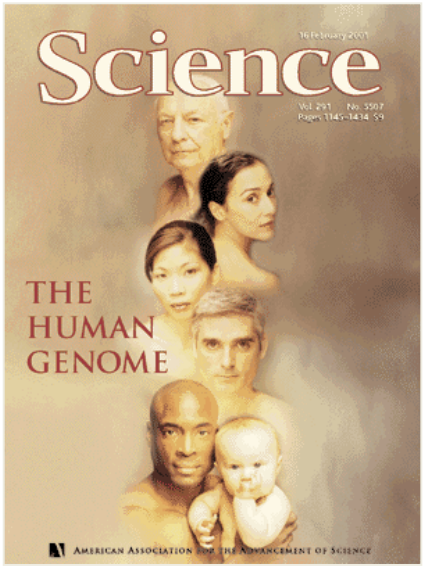
Contributed by F. Sanger, October 3, 1977





February 2001: Completion of the Draft Human Genome

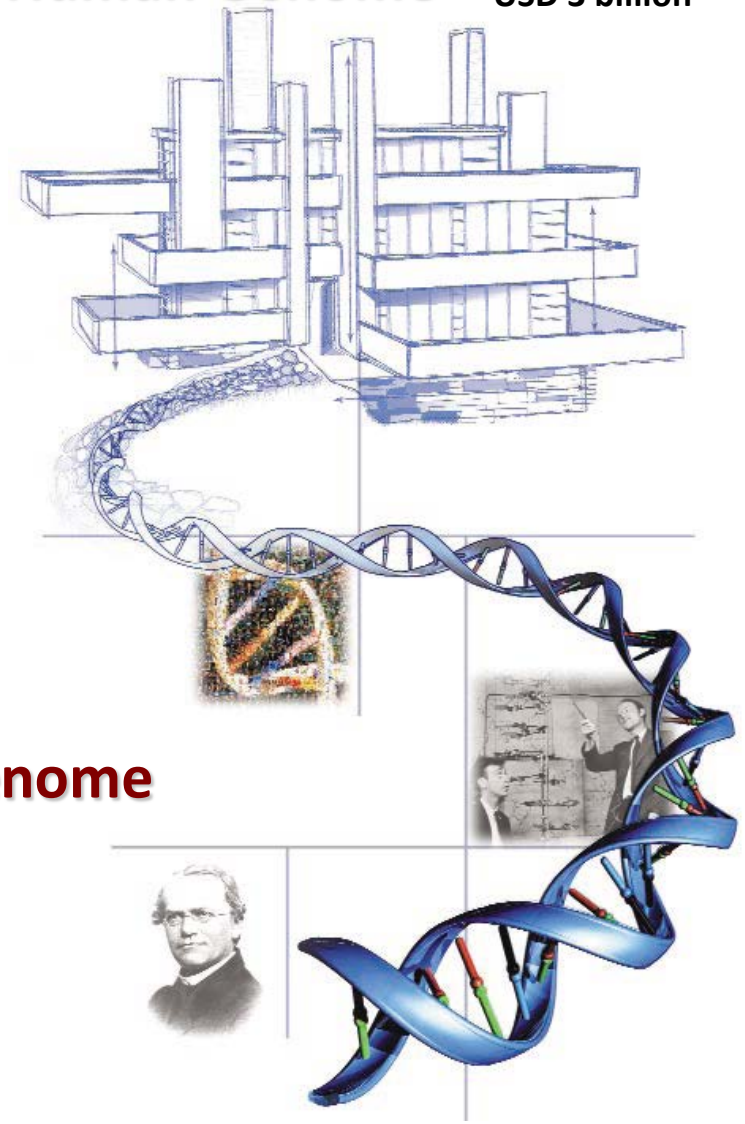
>10 years to finish
USD 3 billion



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

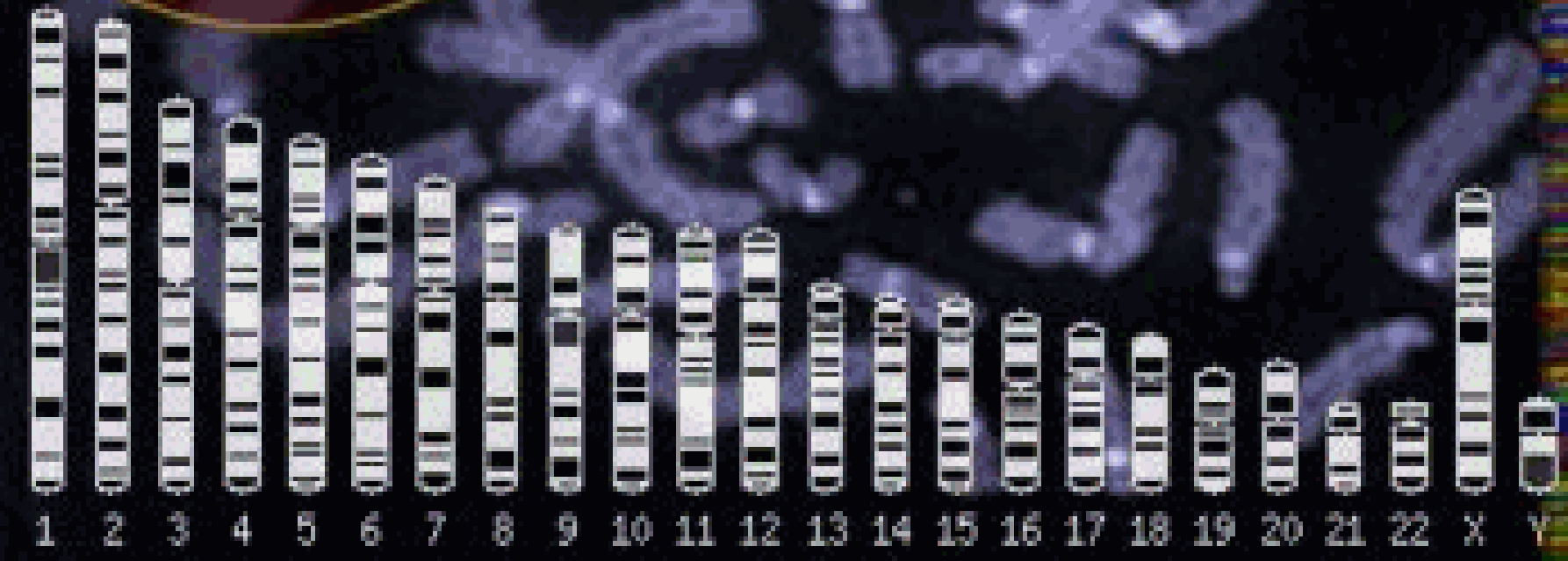
Nature, 23 April 2003
Vol. 422, Pages 1-13

Human Genome Project

3G

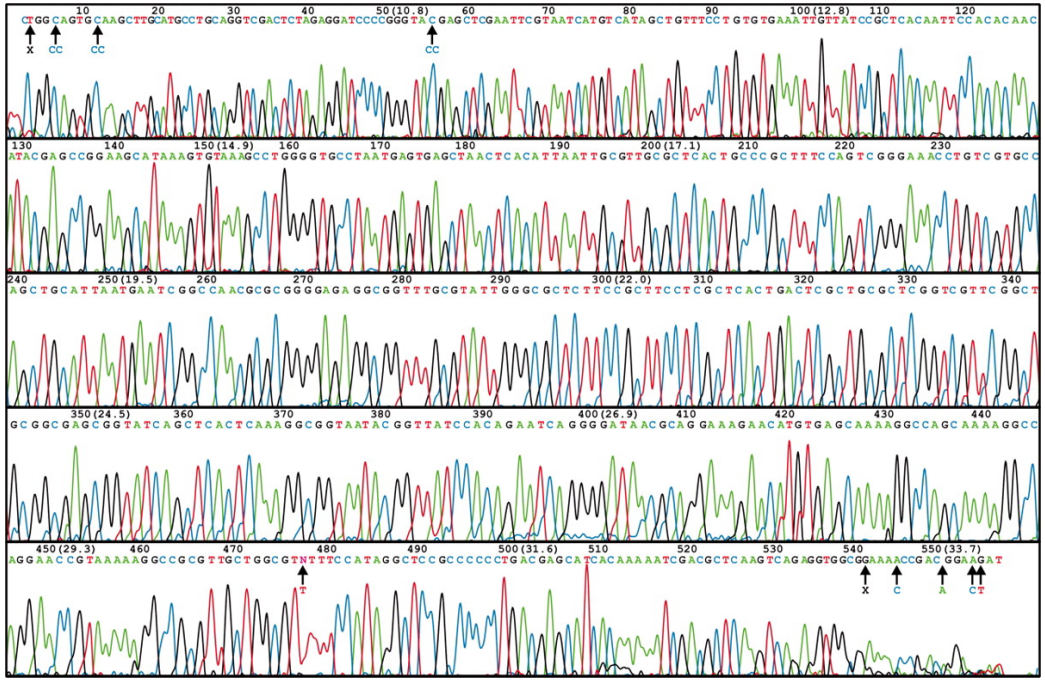
3,000,000,000 bp

3 billion base pairs



ABI 3730 XL DNA Sequencer

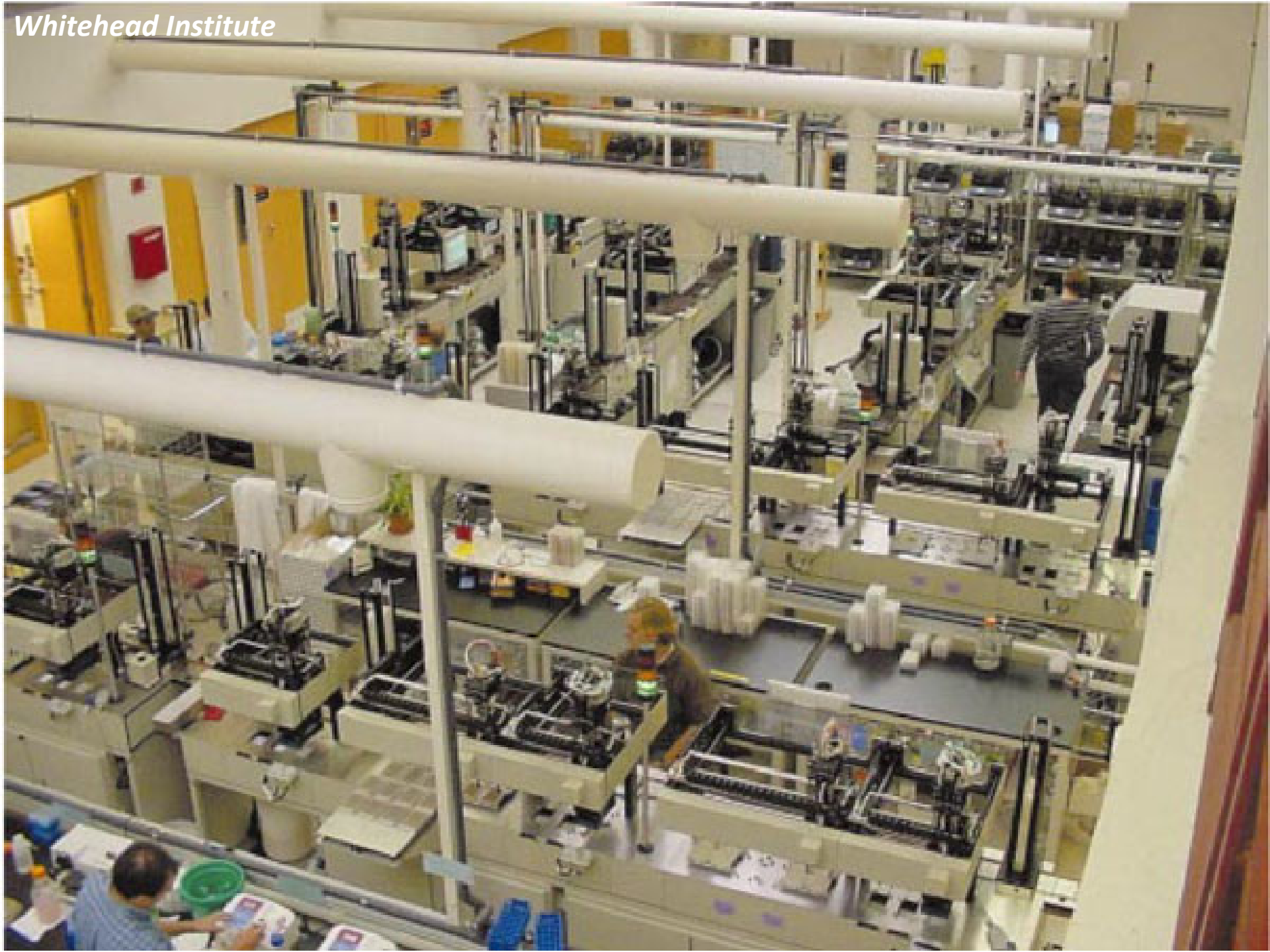




96/384 DNA sequencing in 2 hrs, approximately 600-1000 readable bps per run.

1-4 MB bps/day

A human genome of 3GB need 750 days to finish


Whitehead Institute



273 authors

The Sequence of the Human Genome

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254 authors

articles

Initial sequencing and analysis of the human genome

International Human Genome Sequencing Consortium*

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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

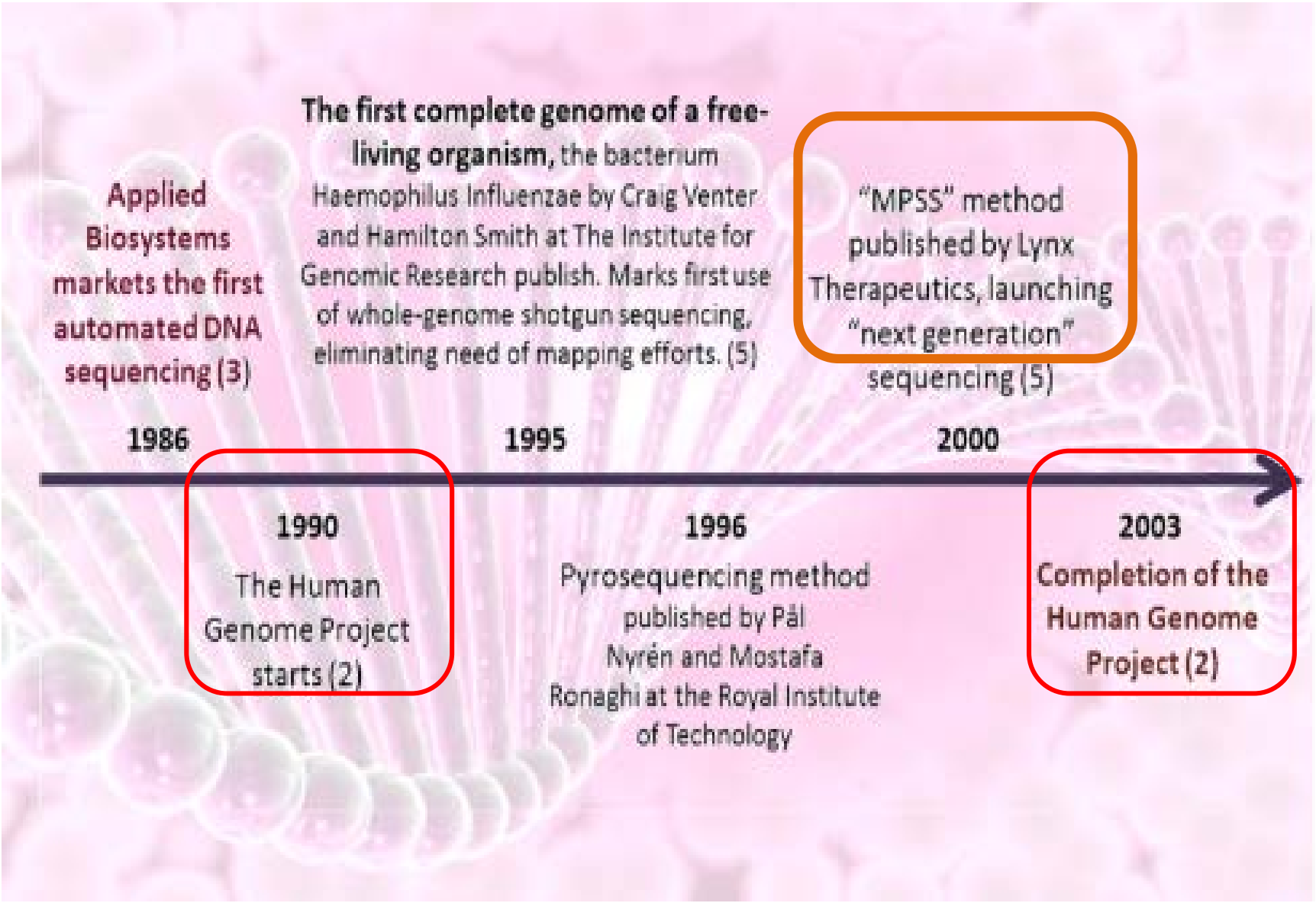
Abstract

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)

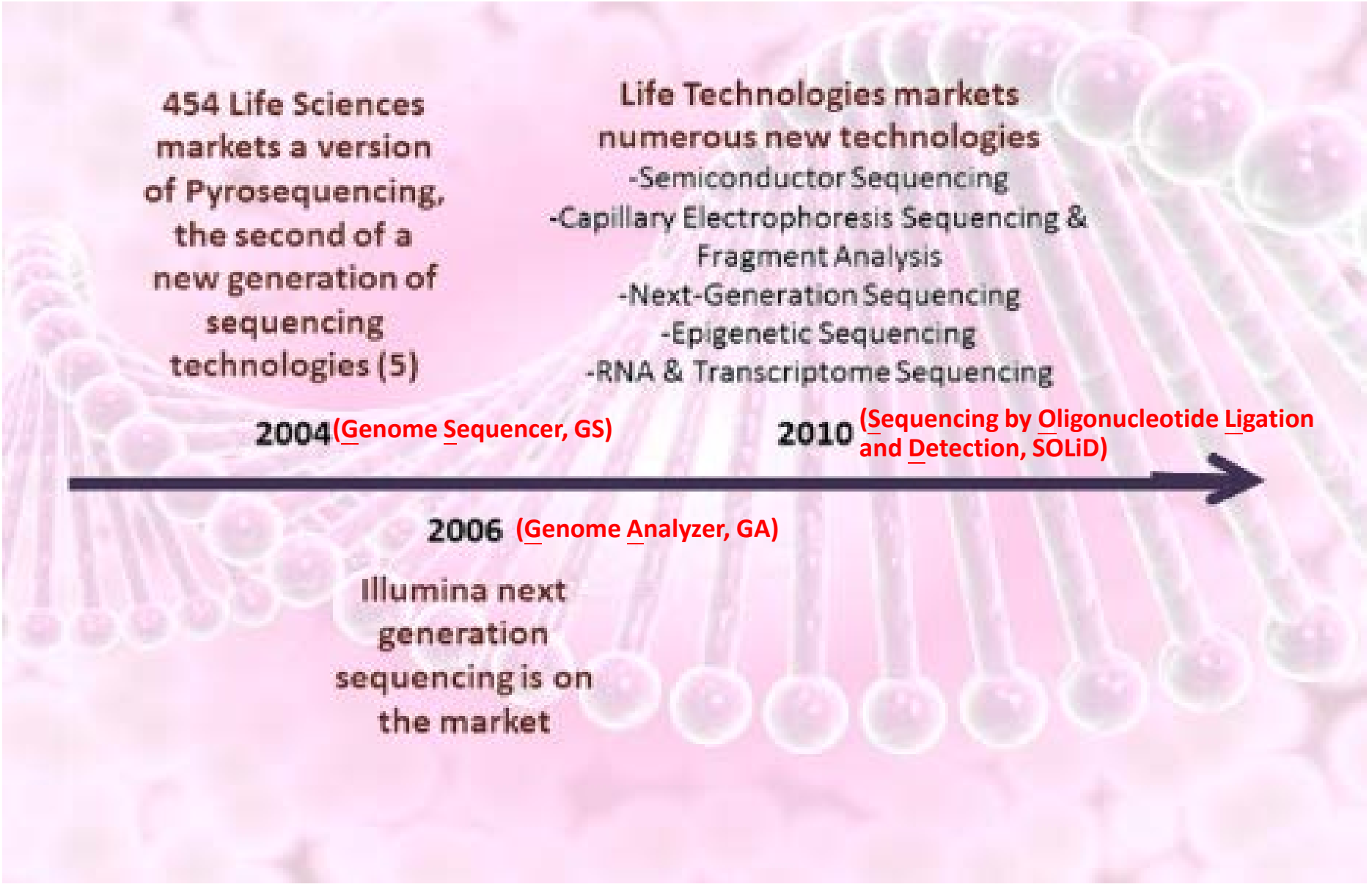


NEXT GENERATION SEQUENCING

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)

life technol applied biosystems



	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)

illumina® 2015 NGS Machines: Hiseq 3000/4000



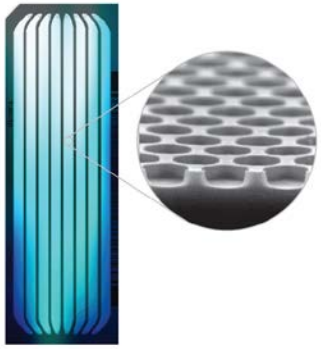


Table 1: Performance Parameters of the HiSeq 3000/4000 Systems.[a]

	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

2014 NGS Machines: Hiseq X



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

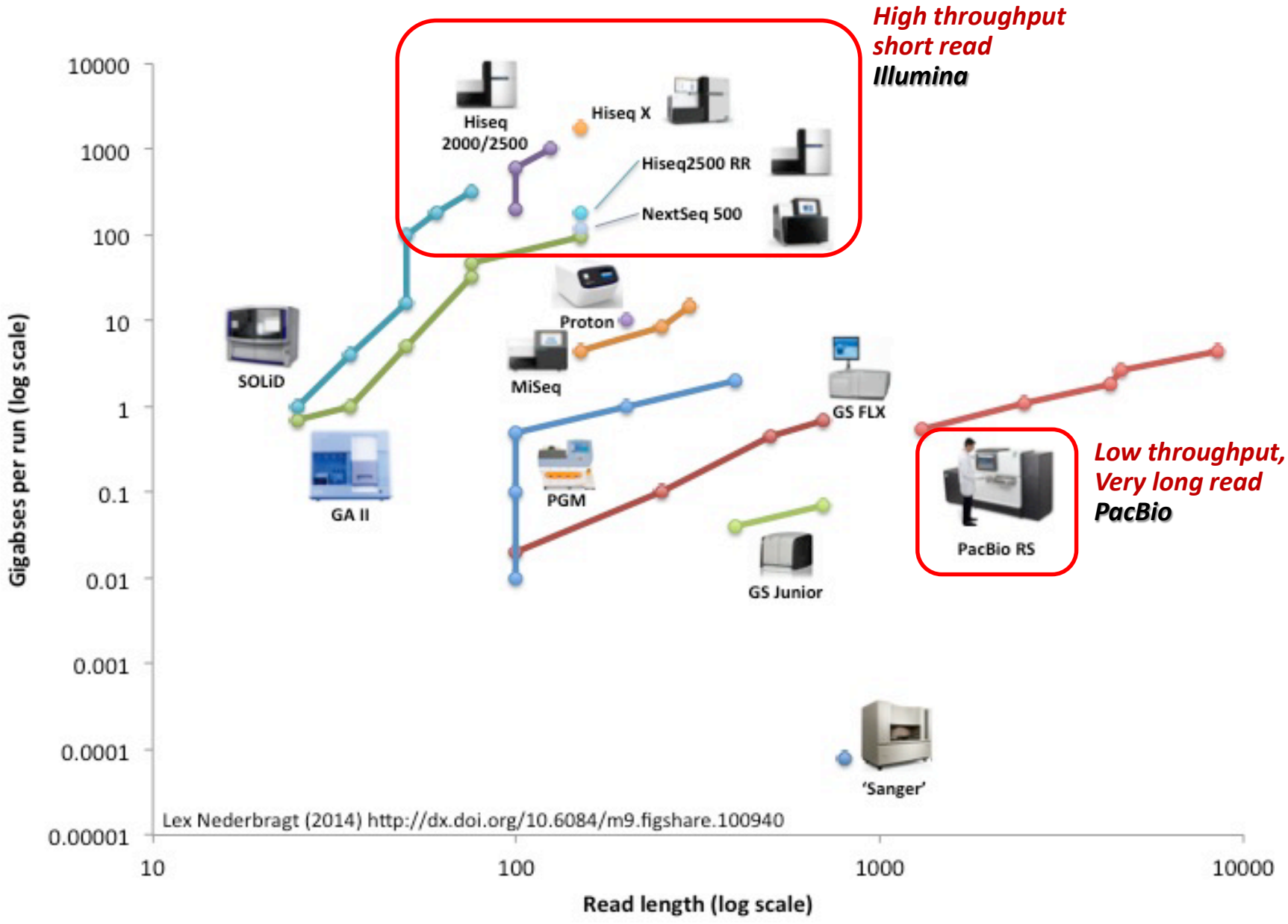
When operating at scale, HiSeq X Ten and HiSeq X Five Systems generate a staggering level of throughput, sequencing thousands of genomes per year.

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

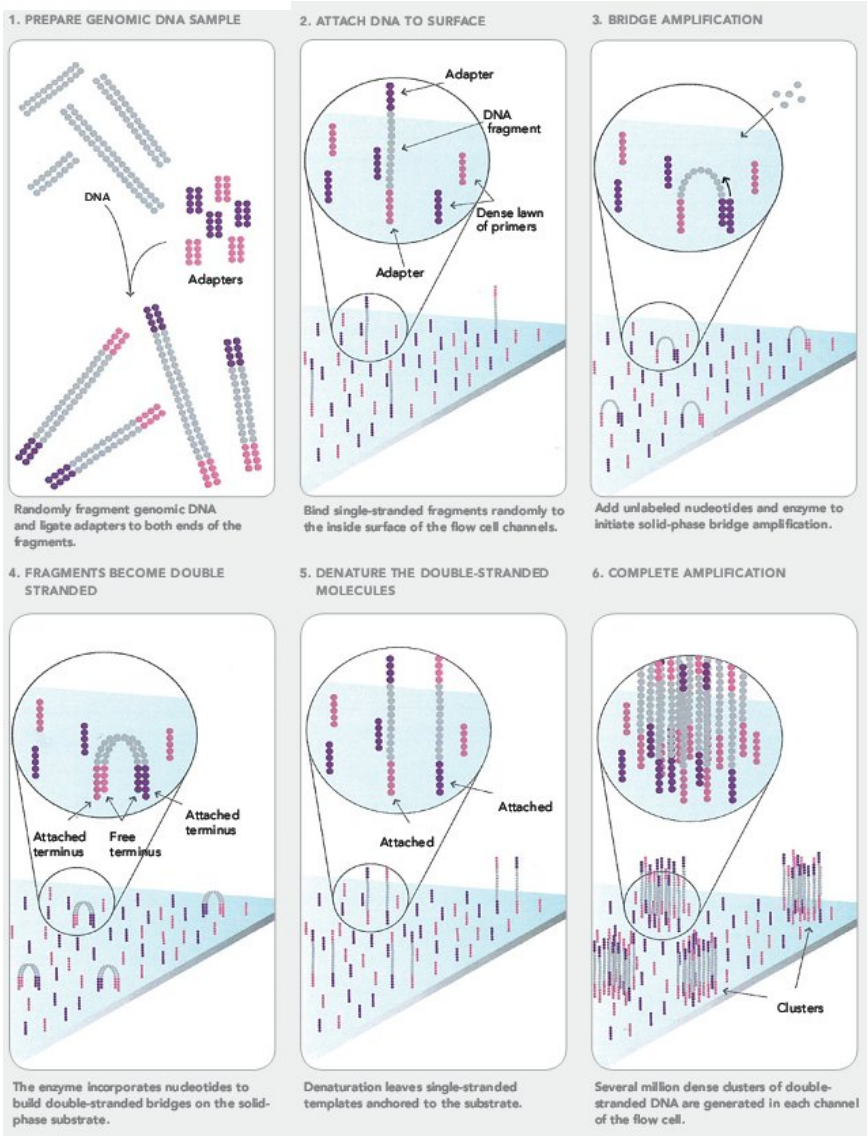
Temple fragmentation (library) ➡ Amplification ➡ Sequencing ➡ Detection

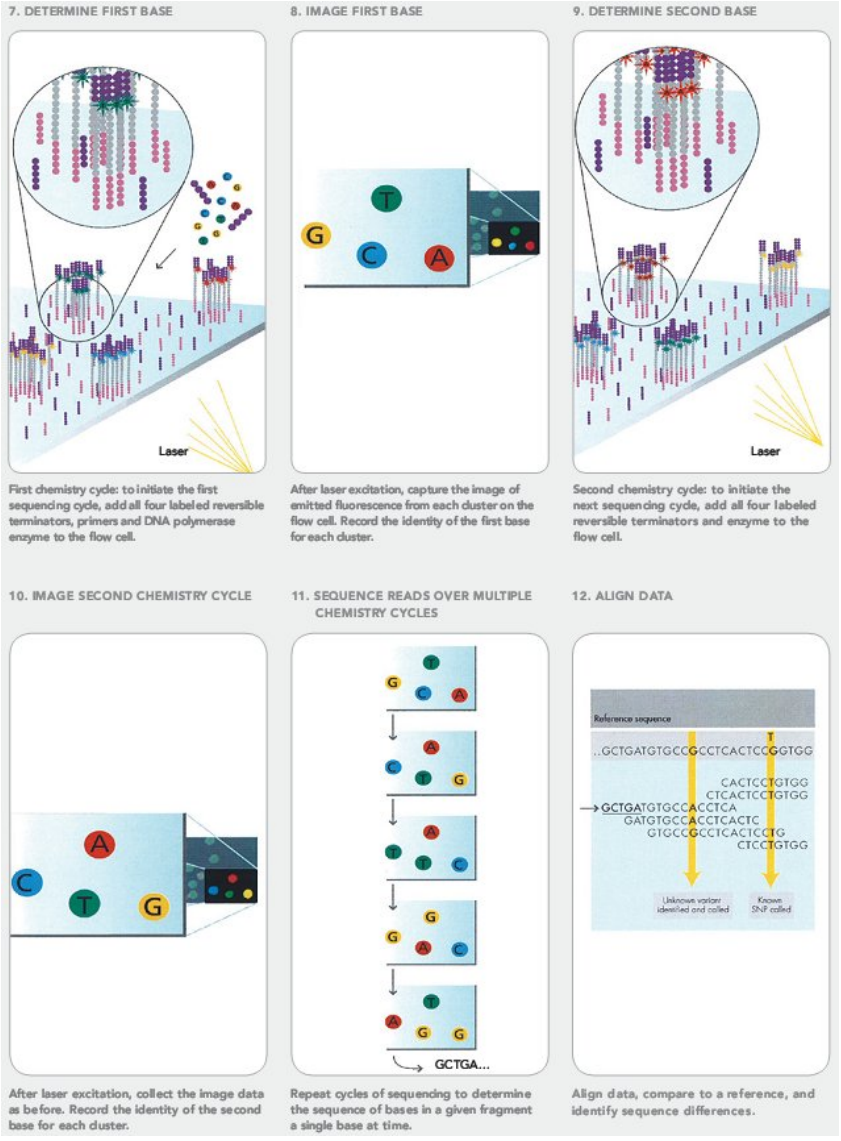
Illumina Sequencing by Synthesis (SBS)

illumina
Solexa



Solexa Genome Analyzer

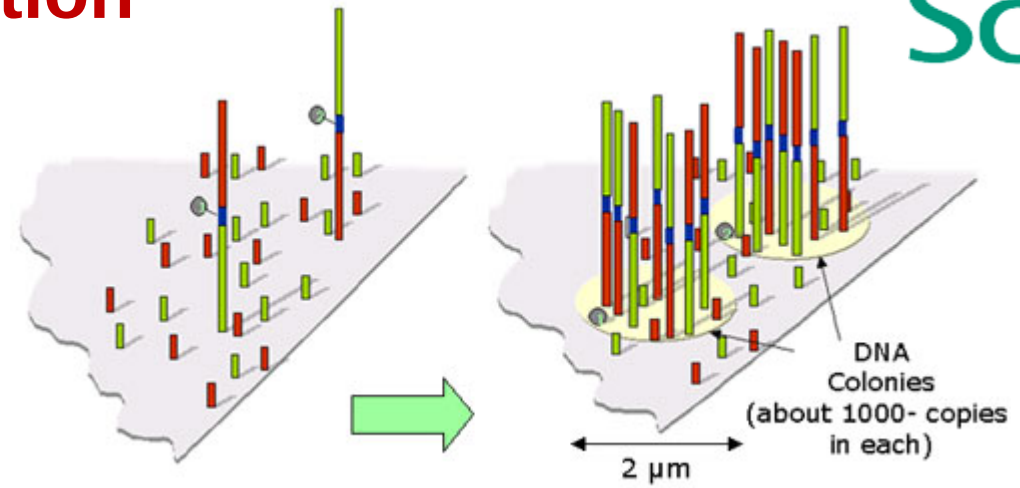




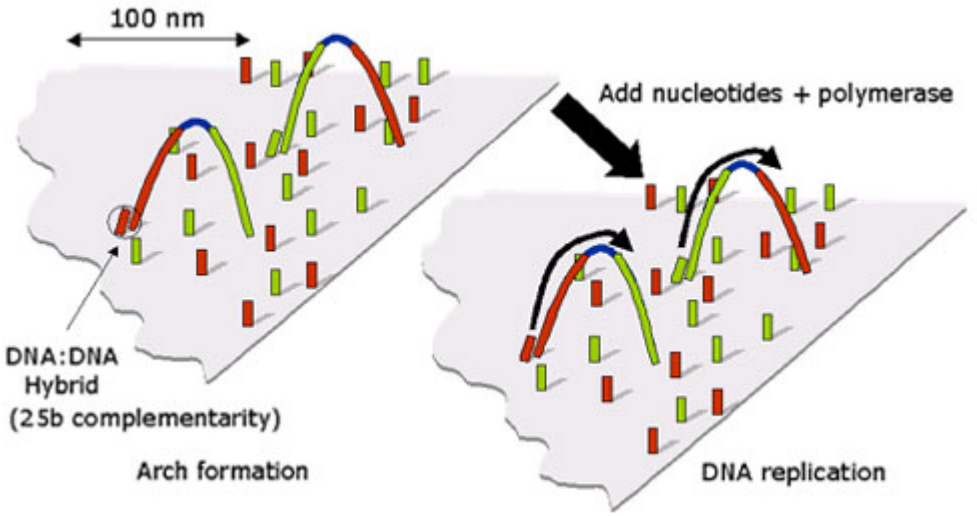
Cluster Generation

Solexa™

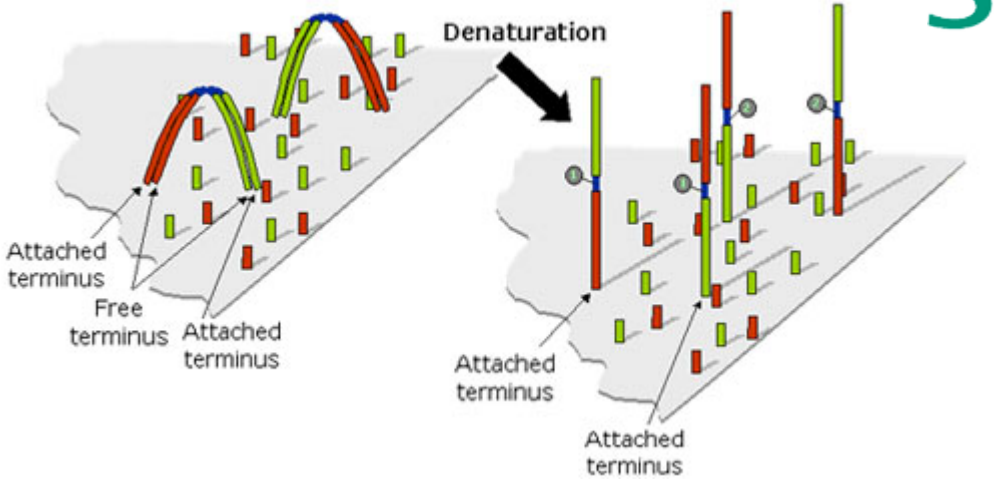
Clusters Array



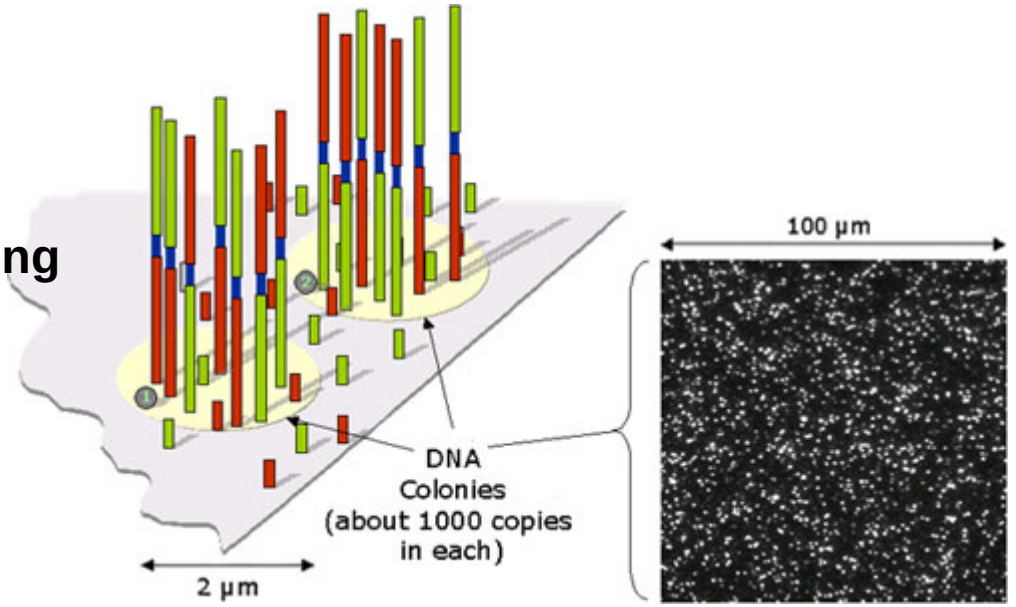
Template Bridging



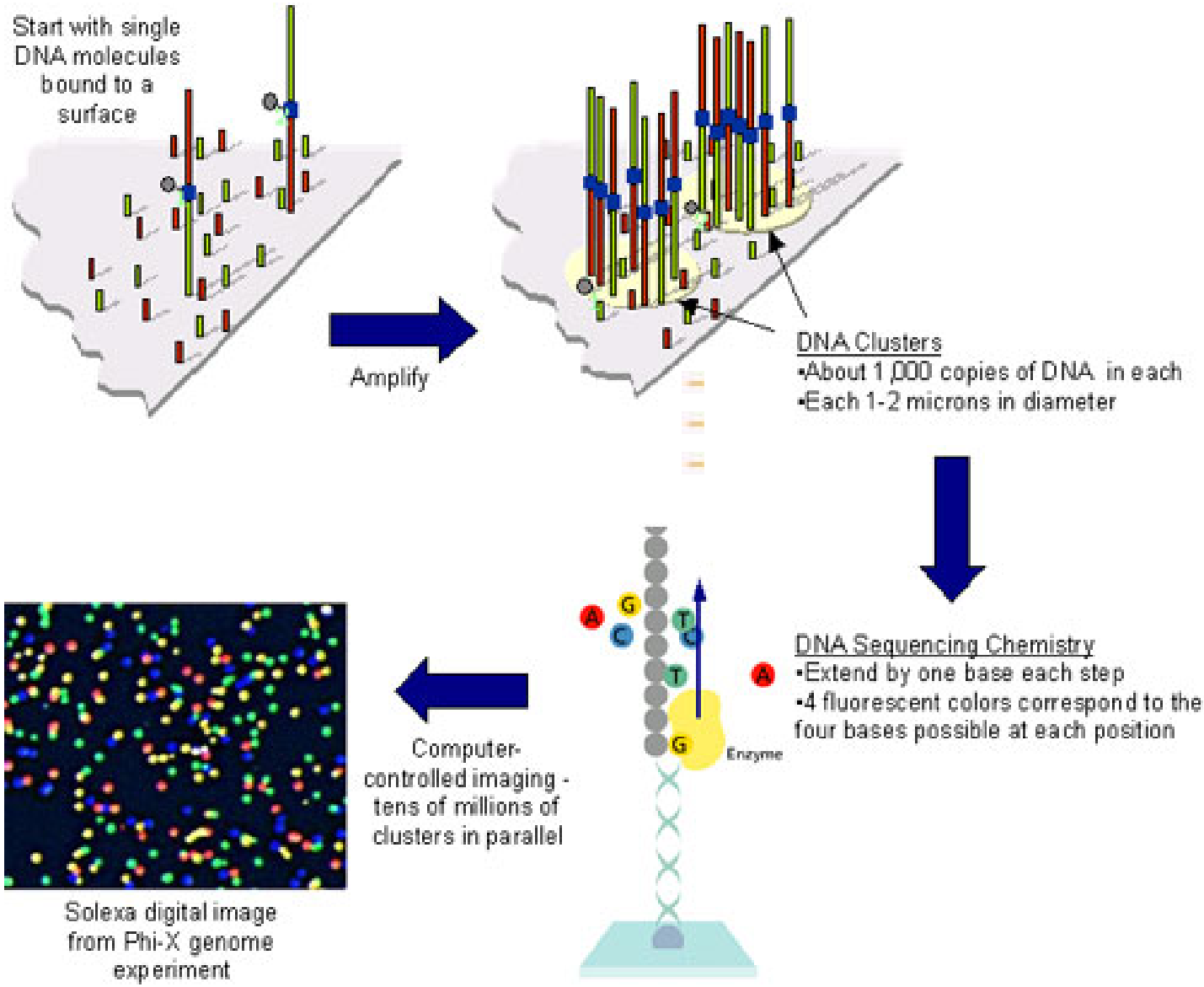
Denaturation



Ready For Sequencing



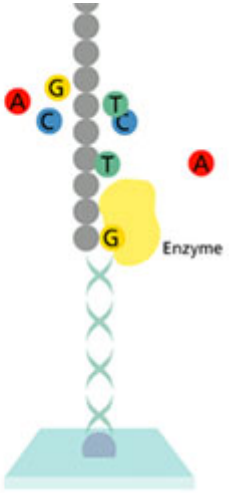
Sequencing-By-Synthesis



Solexa™

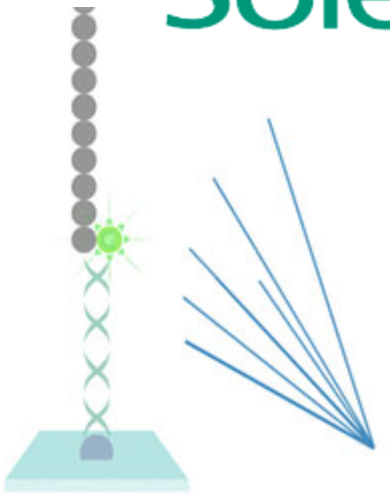
Cycle 1

Incorporation

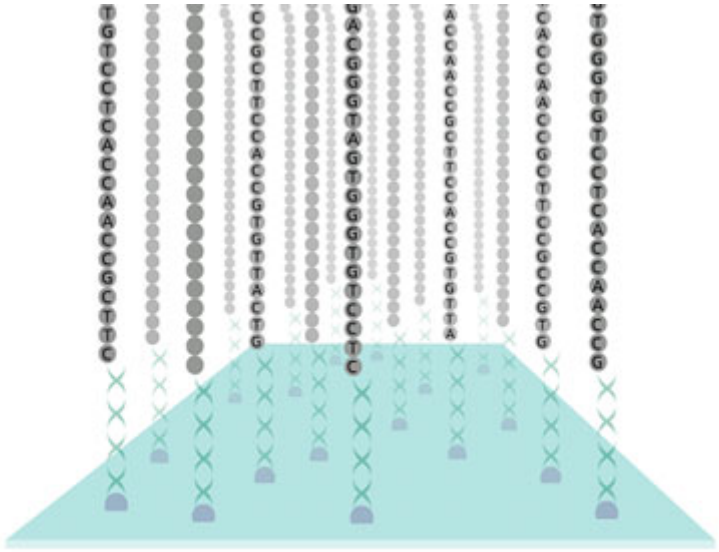


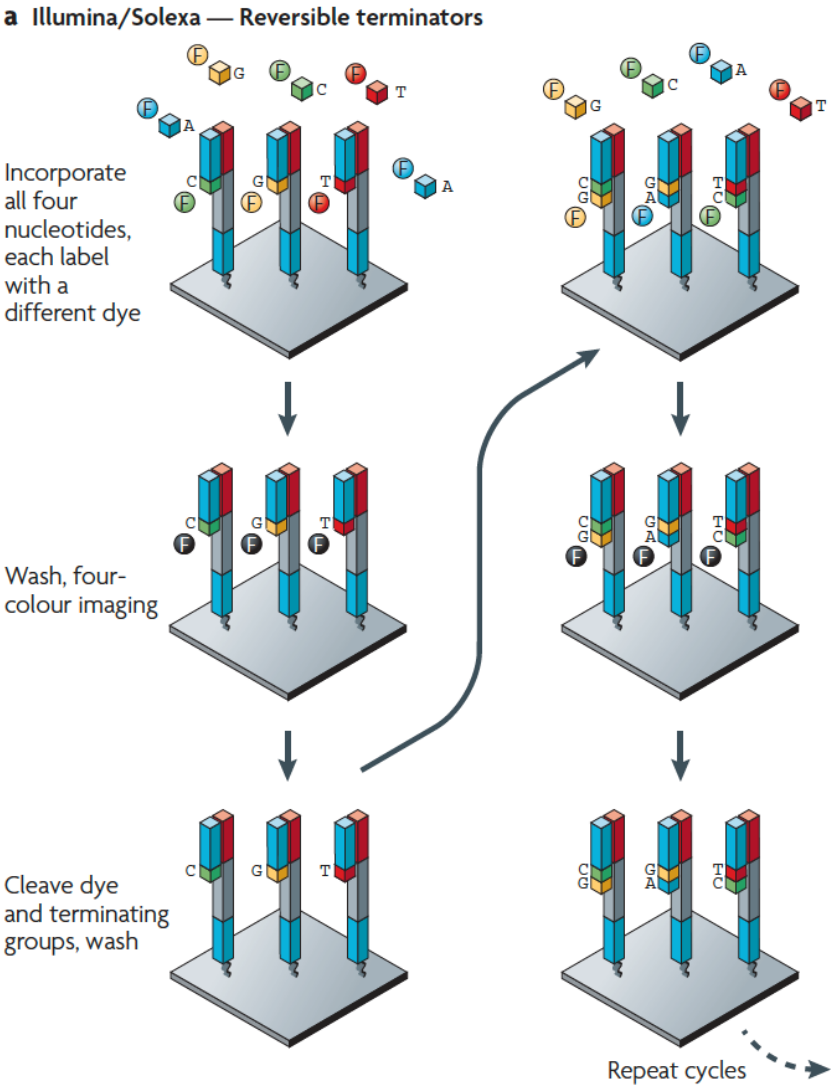
Cycle 1

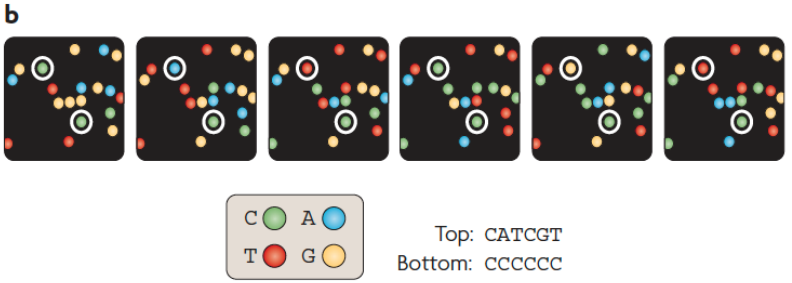
Fluorescent Detection



Sequence Generated At Every Site On The Array

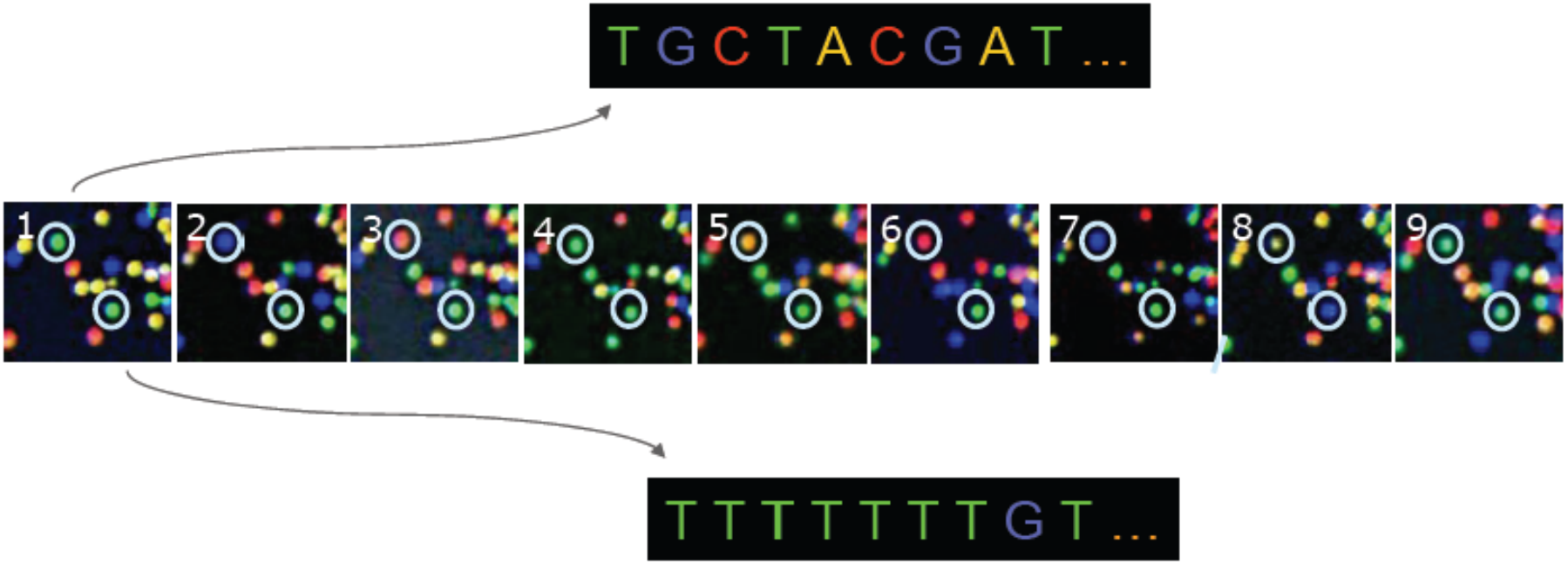






From Debbie Nickerson, Department of Genome Sciences, University of Washington, http://tinyurl.com/6zbzh4

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

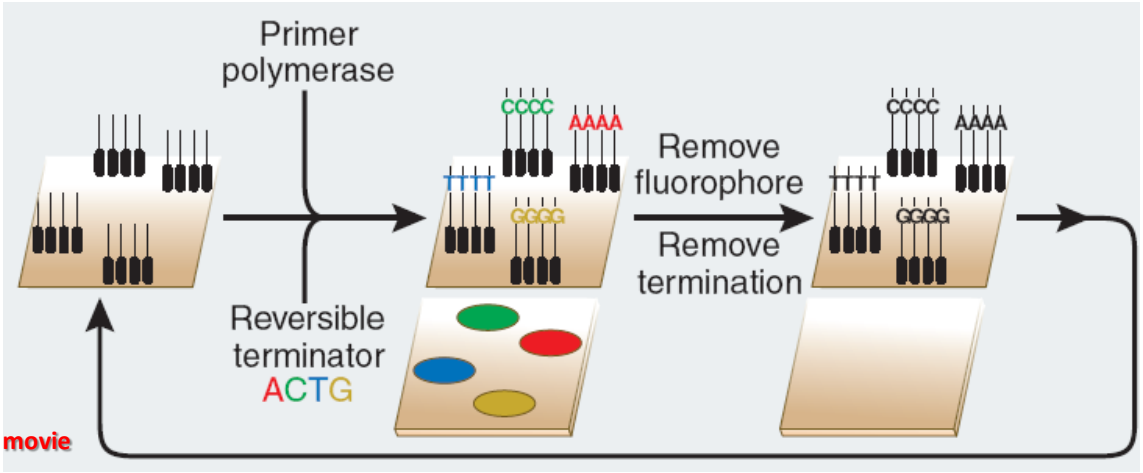
Solexa technology (sequencing-by-synthesis)

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.



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

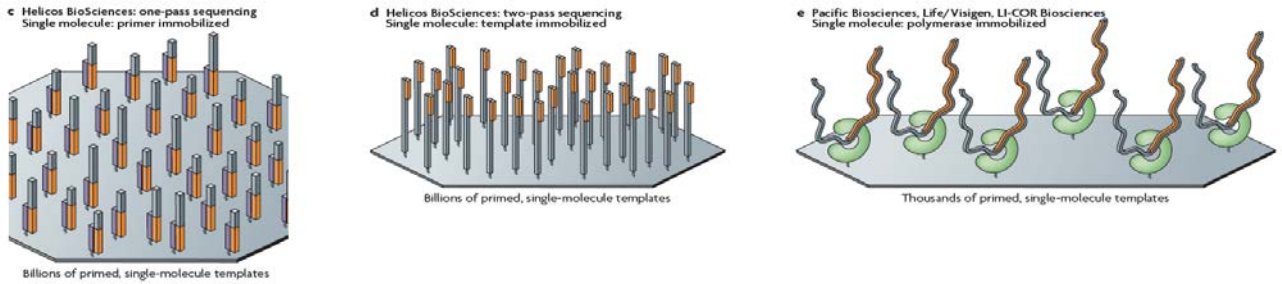
Temple fragmentation (library) ➡ Amplification ➡ Sequencing ➡ Detection

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~~

Pacific Biosciences

~~Helicos Biosciences~~

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

April 11, 2012

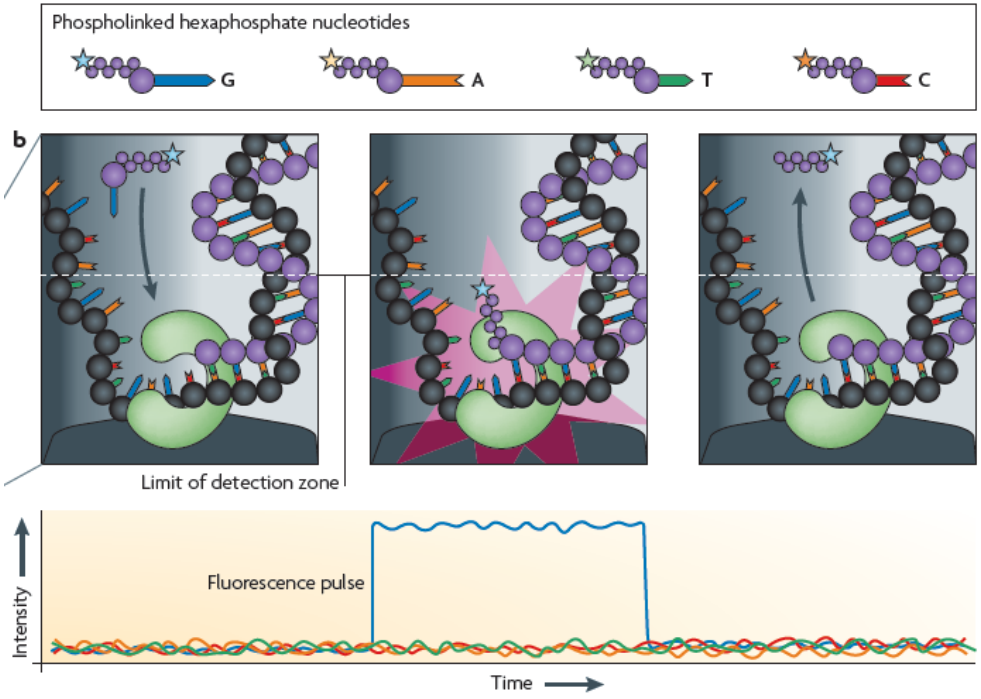
Helicos BioSciences said in a regulatory filing that it has sold certain patent applications covering molecular diagnostic applications of its technology to Sequenom for $1.3 million. The patent applications cover methods for detecting fetal nucleic acids and diagnosing fetal abnormalities. Helicos also 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

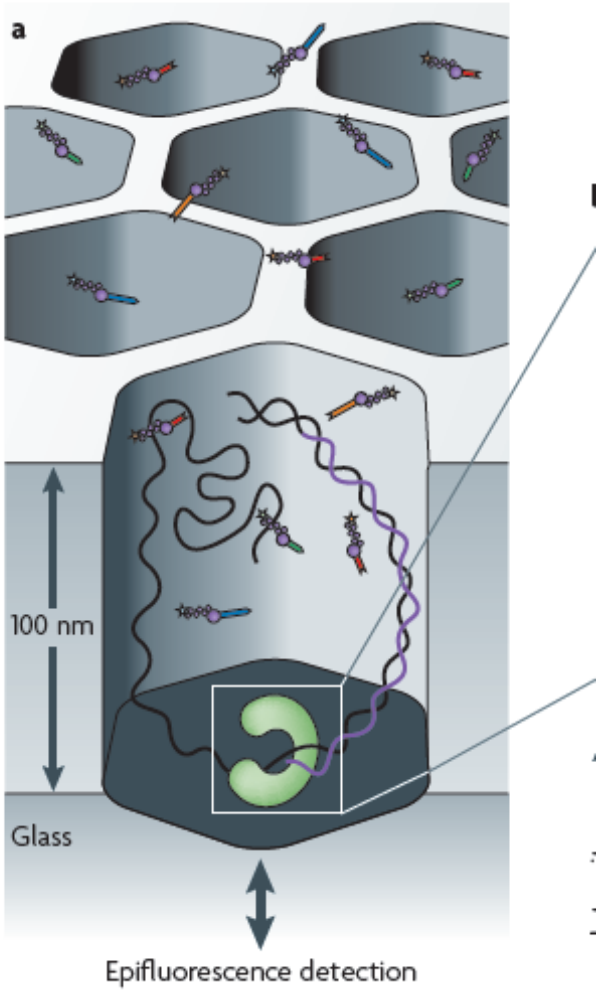
www.sciencemag.org SCIENCE VOL 323 2 JANUARY 2009 133

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†



Pacific Biosciences — Real-time sequencing

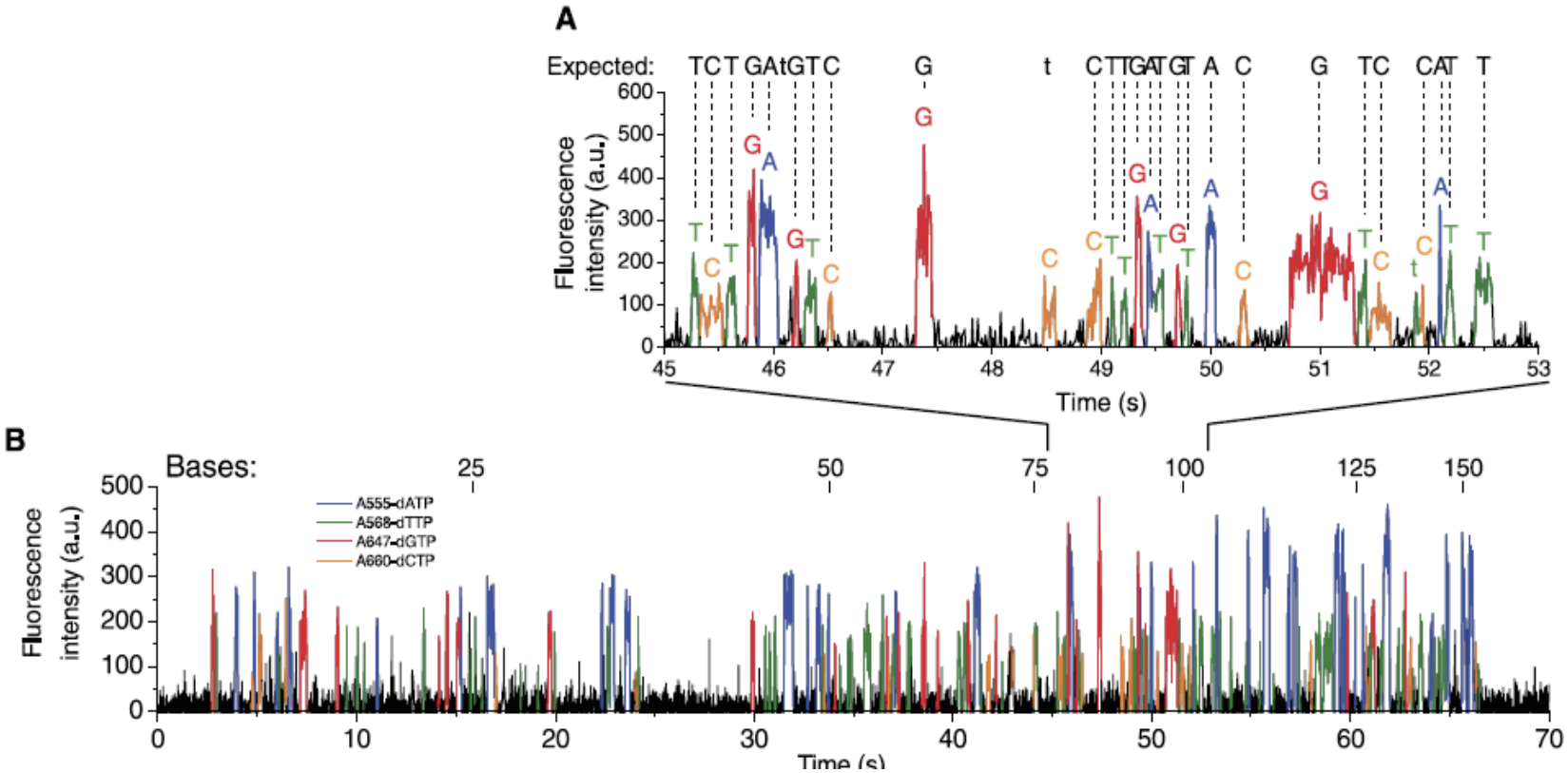


movie



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.



PacBio SMRT Sequencing

Reagents



SMRTbell Template Prep Kit
DNA Polymerase Binding Kit
MagBead Kit
AMPure® PB beads

No amplification required
Automation friendly

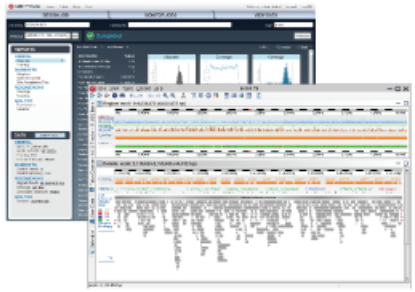
Instrument Run



PacBio RS II with touch screen
RS Remote for run design
SMRT Cells
DNA Sequencing Kit

Sequencing time
30 to 240 min per SMRT Cell

Data Analysis

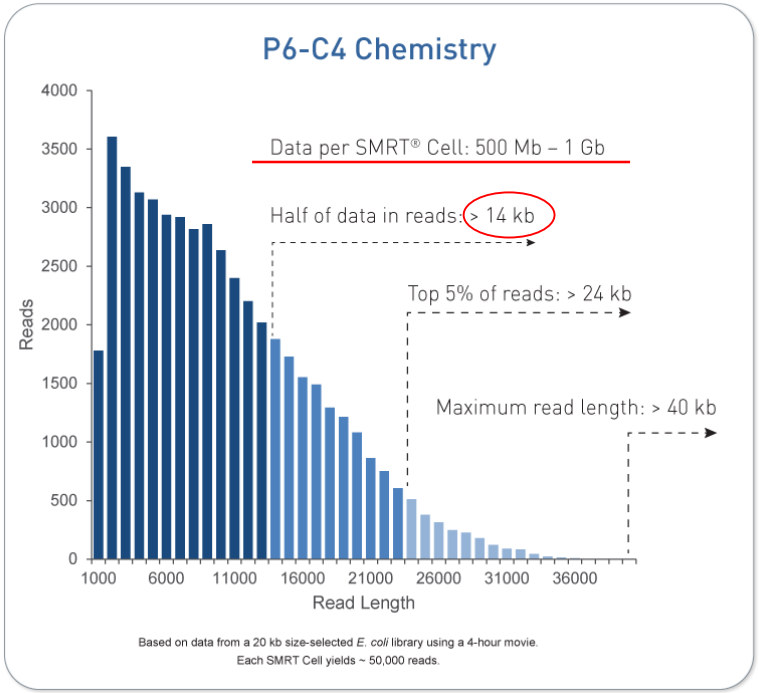


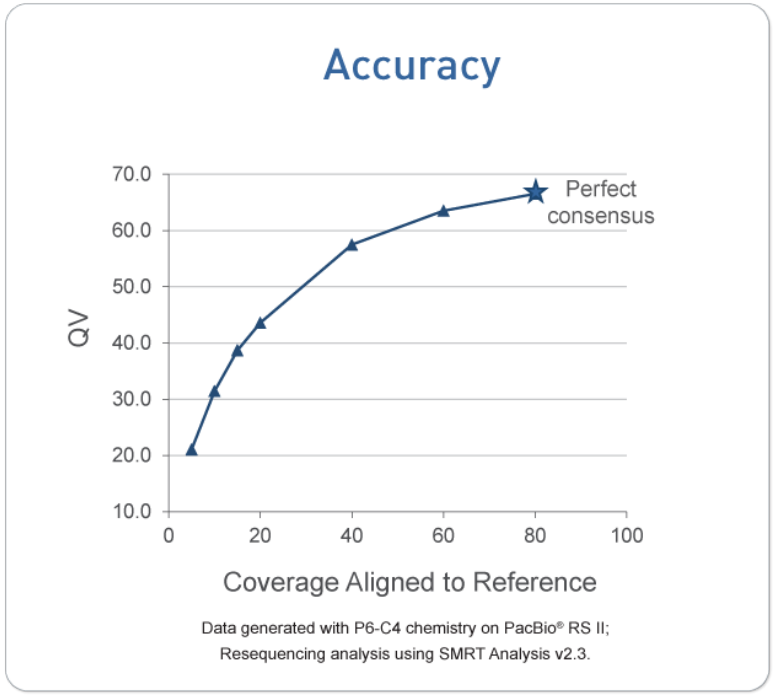
SMRT Analysis
SMRT Portal
SMRT View

Open source
Open standards

From DNA to data in as few as 10 hours

PacBio SMRT Sequencing



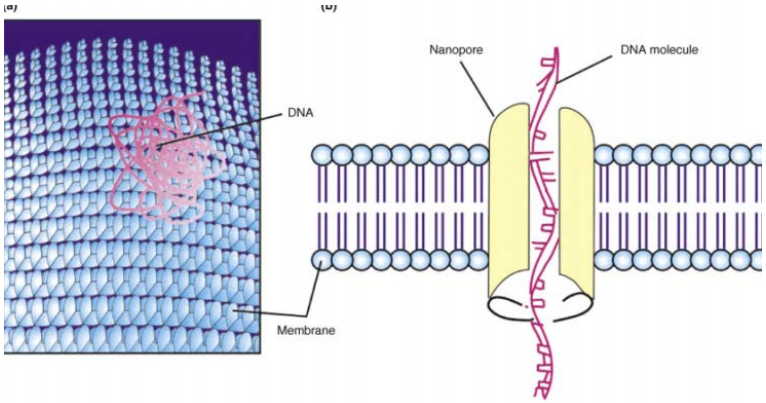


Nanopore Sequencing

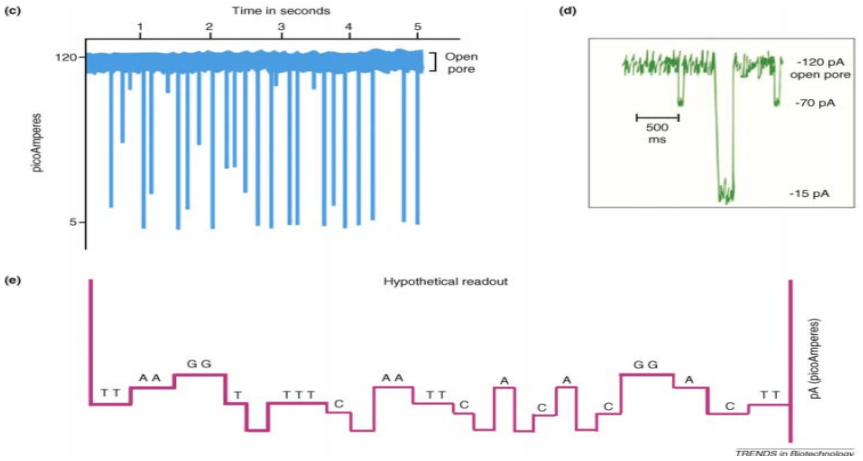
movie

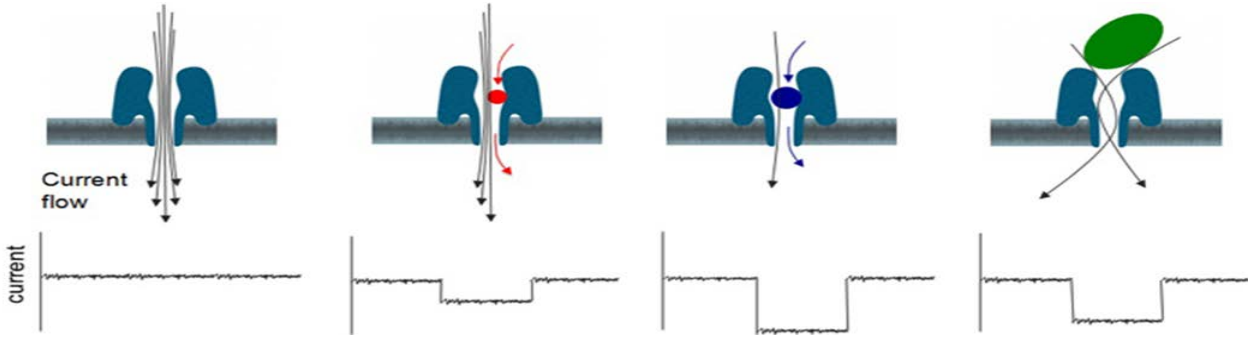
Oxford Nanopore

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.

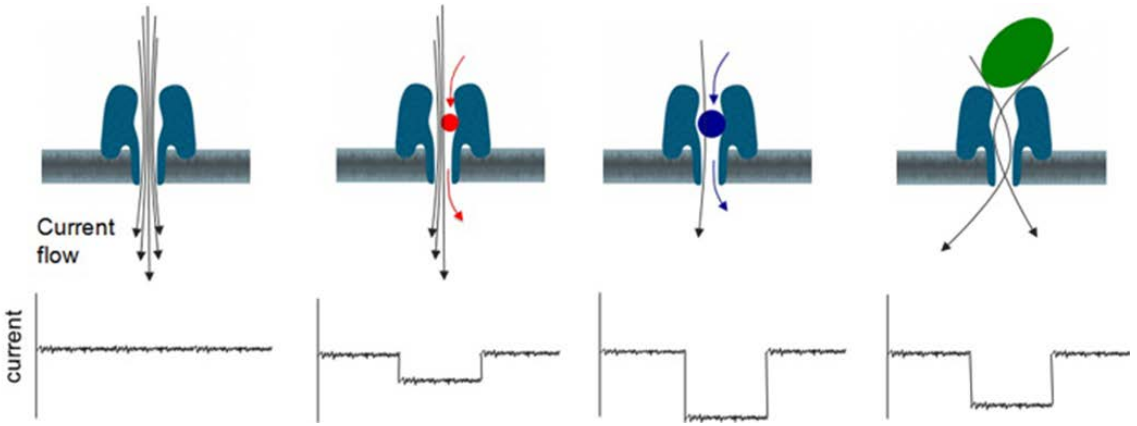
If an analyte passes through the pore or near its aperture, this event creates a characteristic disruption in current. By measuring that current it is possible to identify the molecule in question. For example, this system can be used to distinguish the four standard DNA bases and G, A, T and C, and 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.

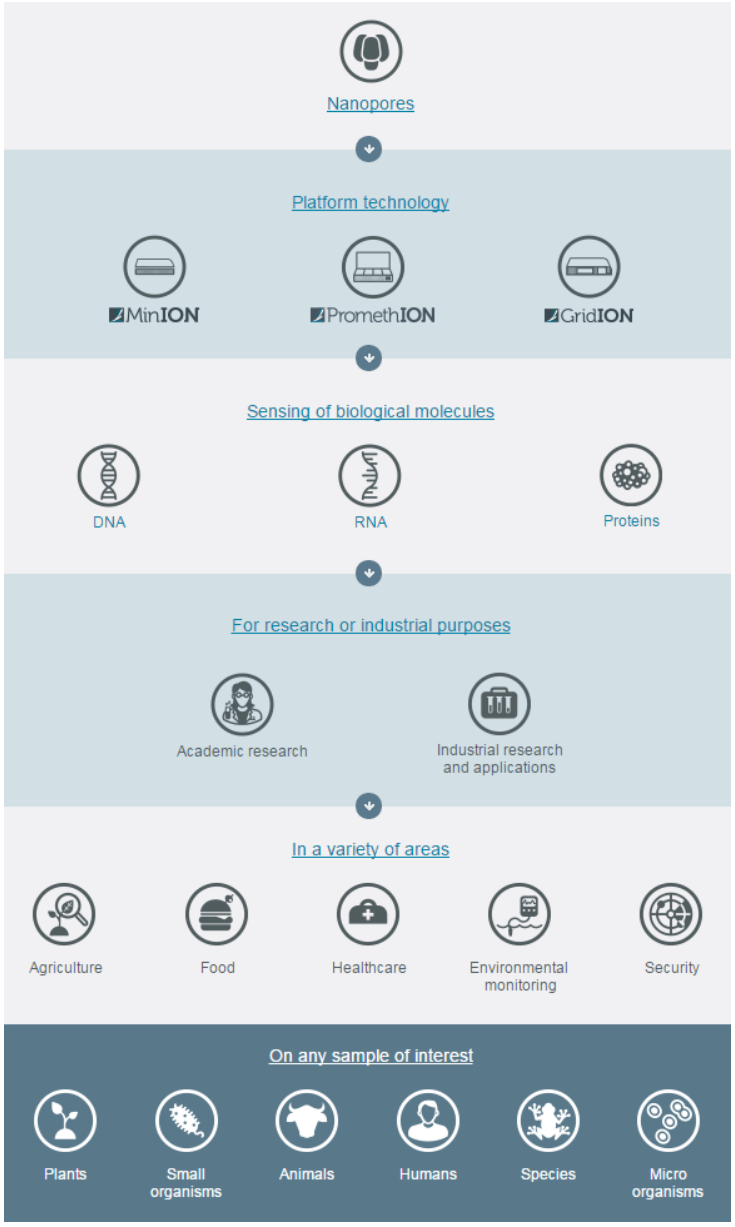
In the second half of this year, the company will commercially release its previously announced GridION instrument, or node, as well as the world's smallest DNA sequencing instrument – a portable cartridge called the MinION that doubles (and works as) a USB drive.



Oxford Nanopore's disruptive MinION USB device

https://nanoporetech.com/

Oxford Nanopore Sequencing Platform



MinION







PromethION

GridION



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).

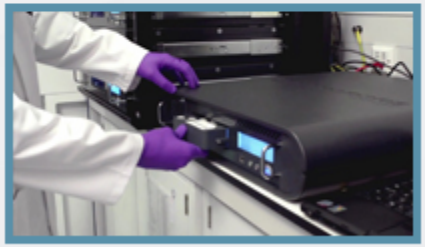
A single node as a desktop installation



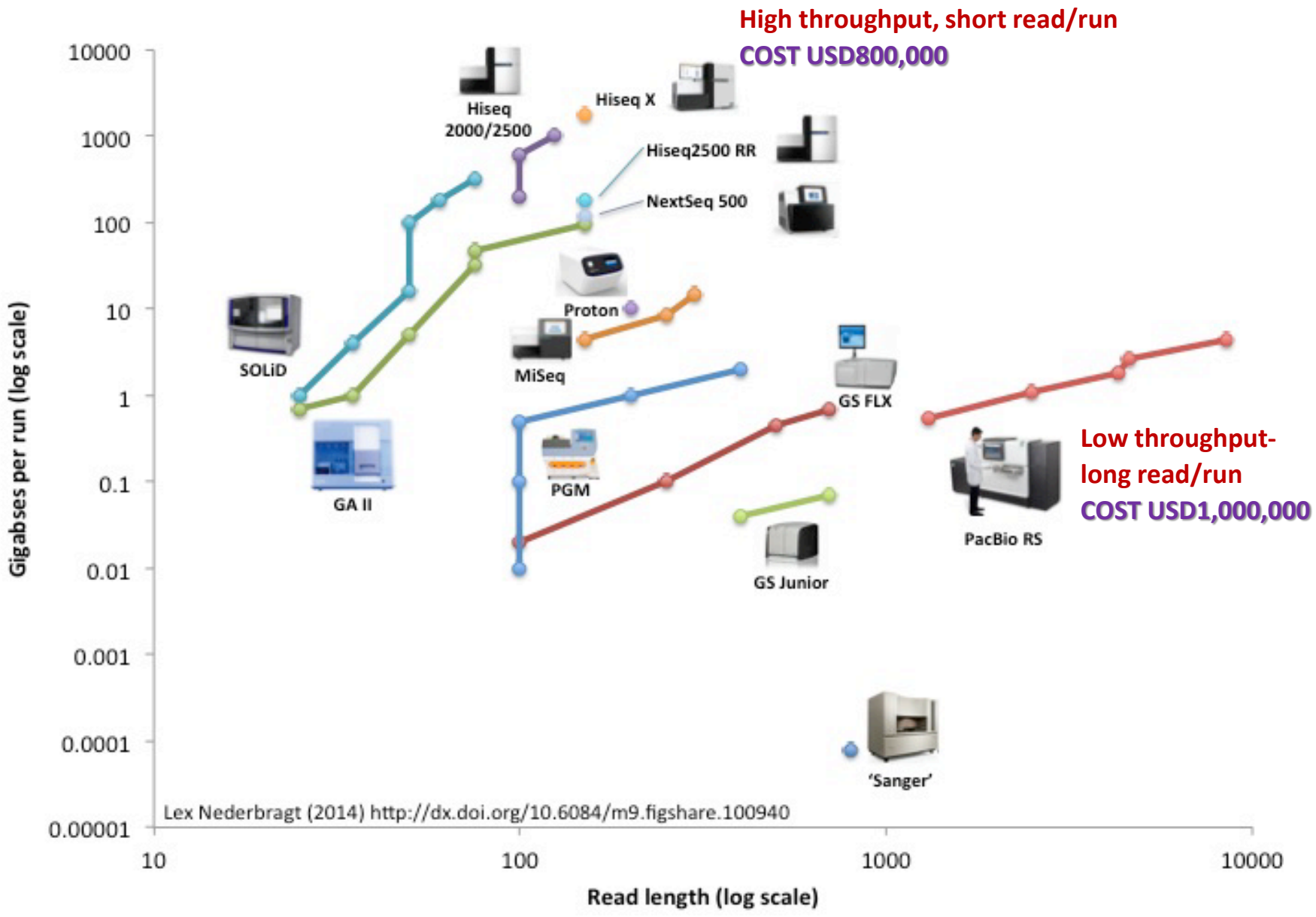
Nodes may be clustered through a network for larger throughput installations



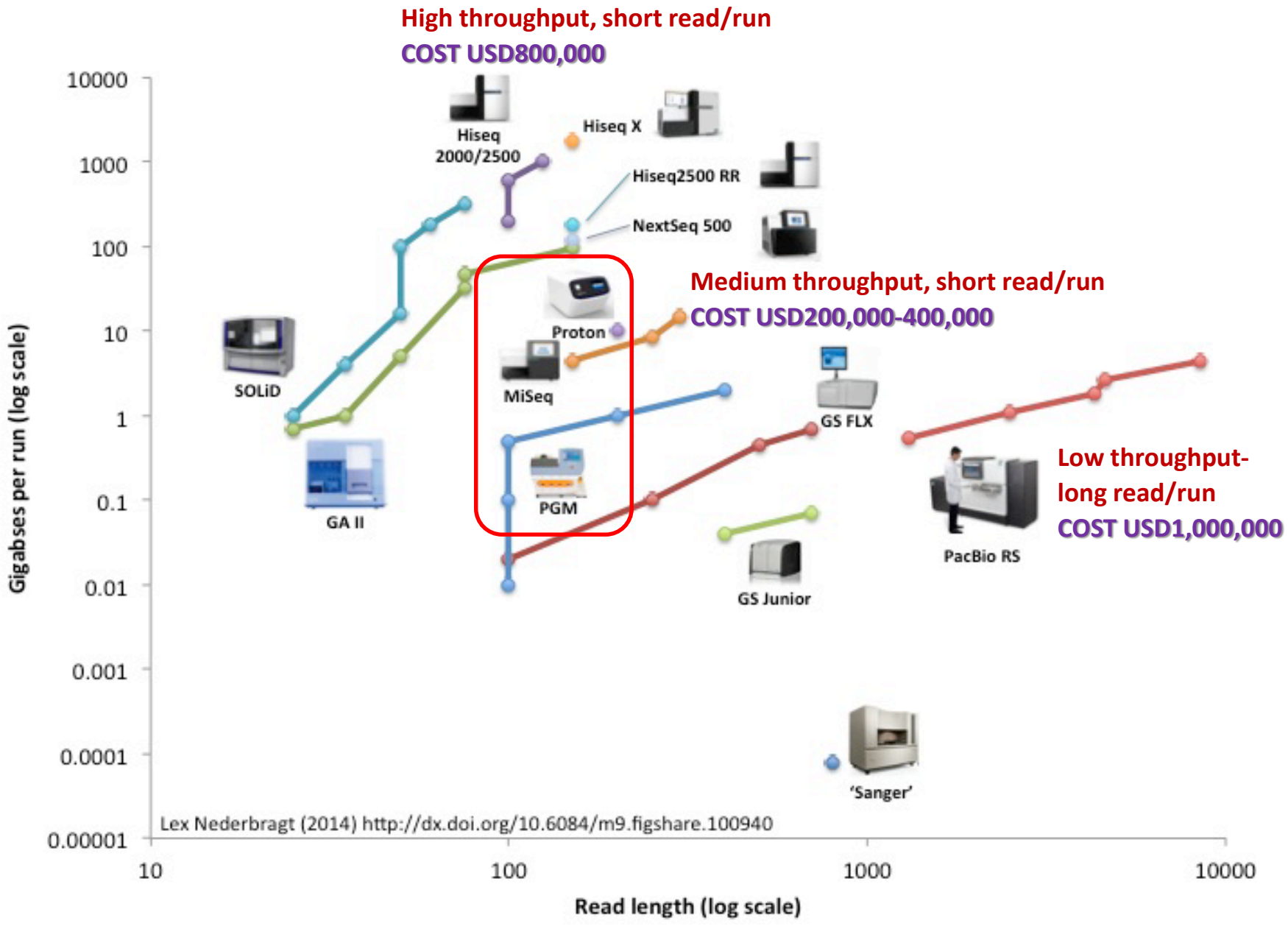
Each GridION node operates with a single dispoable cartridge.



Cost of NGS machines (2014)



Cost of NGS machines (2014)



Personal Sequencer

illumina®



MiSeq
Personal Sequencing System

ion torrent
by life technologies™



Ion Torrent
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

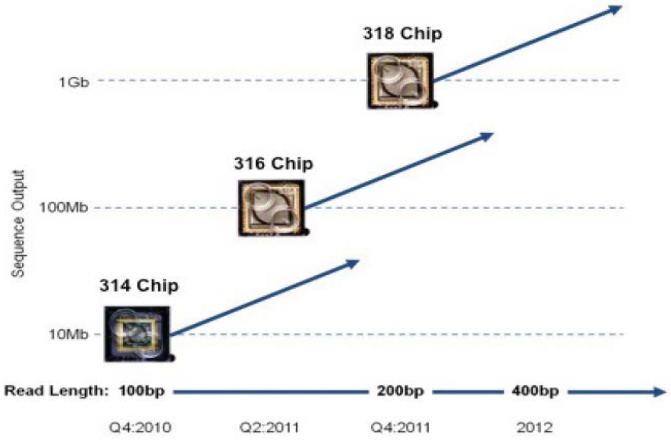
READ LENGTH	TOTAL TIME*	OUTPUT
2 × 75 bp	~24 hrs	3.3-3.8 Gb
2 × 300 bp	~65 hrs	13.2-15 Gb

ion torrent

by life technologies™



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



10 Mb to 1 Gb

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

ION PGM™ SEQUENCER

SMALL GENOMES
SETS OF GENES
GENE EXPRESSION
ChIP-SEQ



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
by life technologies™
ion torrent
by life technologies™
ion torrent
by life technologies™

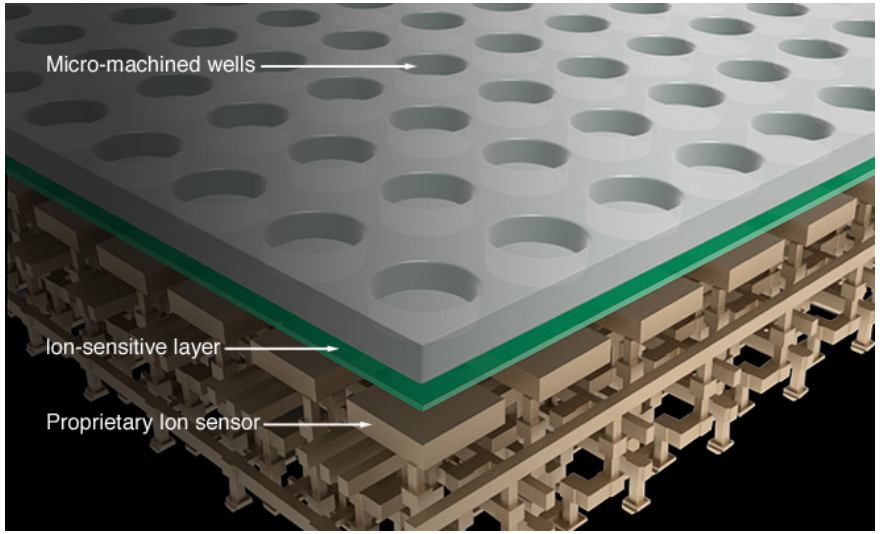
Ion Torrent

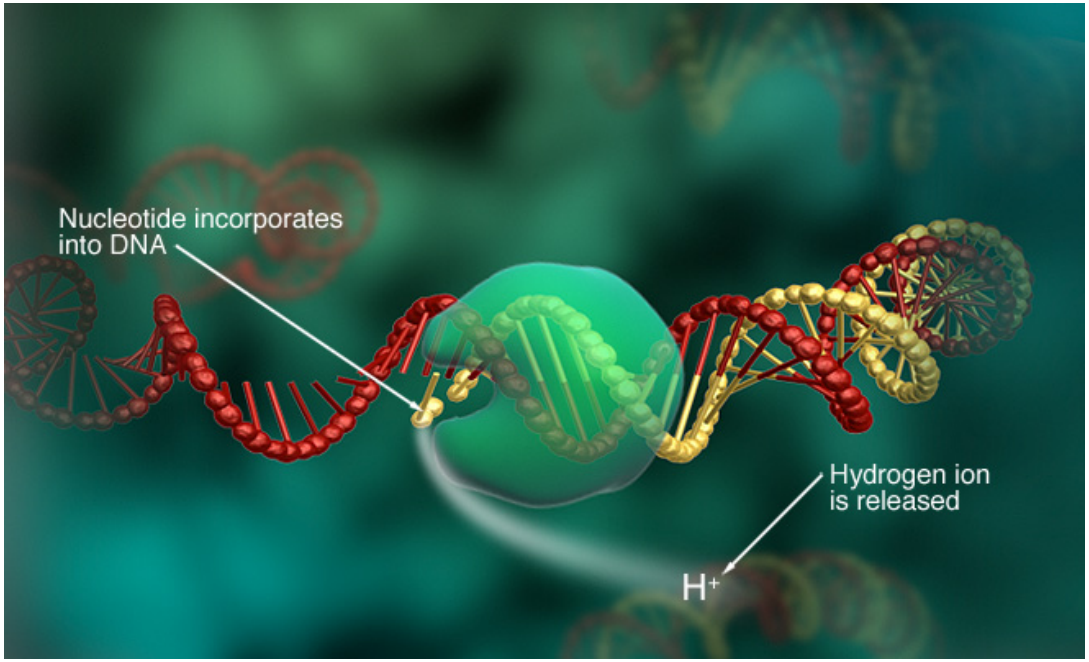
movie





314 chip
316 chip



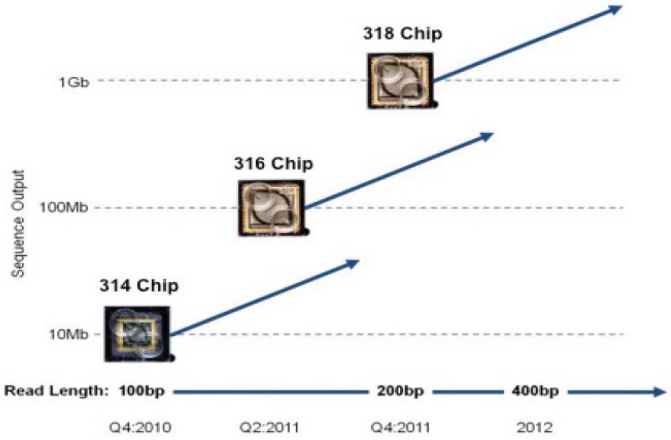


ion torrent

by life technologies™



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



10 Mb to 1 Gb

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

ION PGM™ SEQUENCER

SMALL GENOMES
SETS OF GENES
GENE EXPRESSION
ChIP-SEQ



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

"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

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.

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.

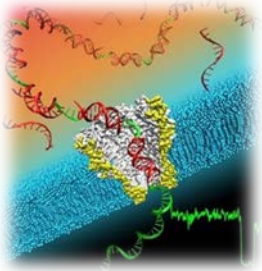
Ion Proton™ System performance specifications* with Ion Proton™ I Chip at commercial launch	
Throughput	Up to 10 Gb (Note: The Ion Proton™ II Chip will be available about six months after the Ion Proton™ I Chip. The Ion Proton™ II Chip will enable sample-to-variant analysis of a human genome in a single 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

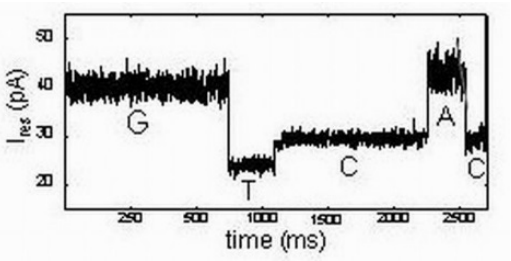
NEXT-NEXT-NEXT-NEXT-NEXT

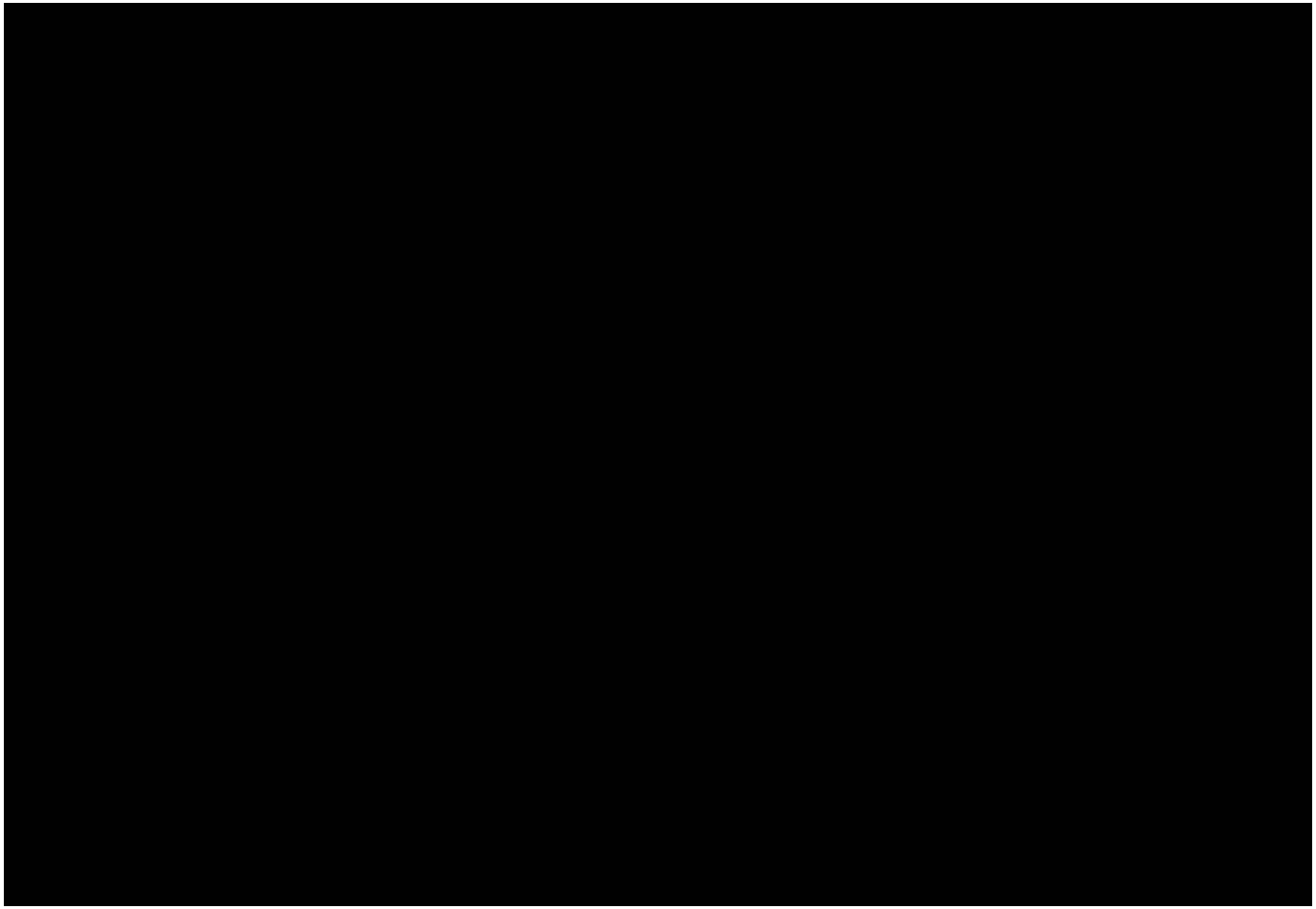
NEW GENERATION SEQUENCING

NGS

Nanopore Sequencing - IBM DNA Transistor







GeneReader



Early access customers in 2012

esis

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.



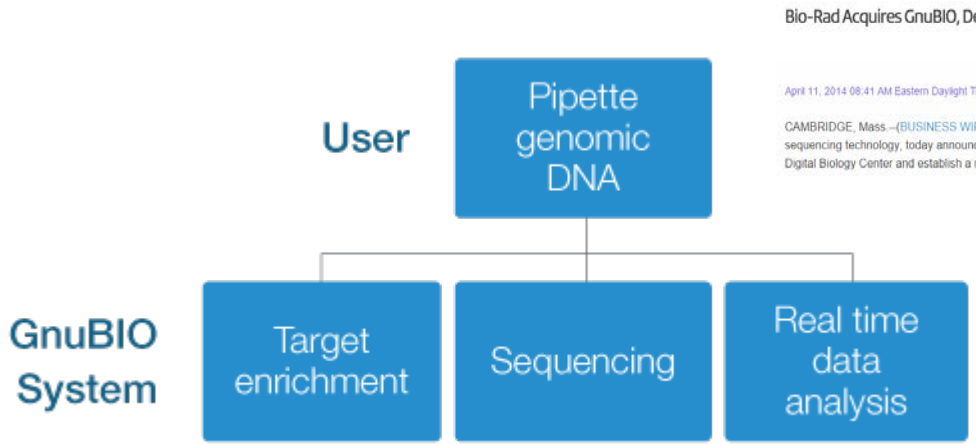
The following results are the raw and unfiltered data from the GnuBIO prototype system:

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

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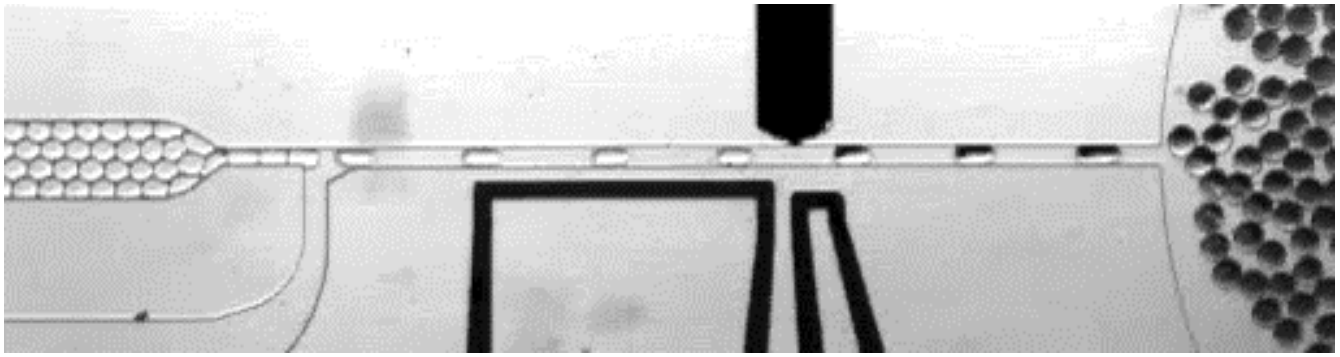
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Bio-Rad Acquires GnuBIO, Developer of Droplet-Based DNA Sequencing Technology

April 11, 2014 08:41 AM Eastern Daylight Time

CAMBRIDGE, Mass.--(BUSINESS WIRE)--GnuBIO, a privately-held life sciences company that has developed a droplet-based DNA sequencing technology, today announced that it was acquired by Bio-Rad Laboratories, Inc. With this acquisition, Bio-Rad will expand its Digital Biology Center and establish a research and development site in Cambridge, Massachusetts.



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.

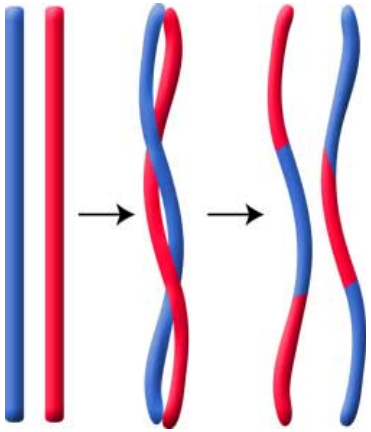
-The AllSeq-team

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
Visualization
Disease
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

