

FACOLTÀ DI  
SCIENZE MATEMATICHE, FISICHE E NATURALI  
Università degli Studi di Verona

## NGS Technologies for Genomics and Transcriptomics

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Department of Biotechnologies - University of Verona





<http://profs.sci.univr.it/delledonne>

© 2001 Macmillan Magazine Ltd. NATURE VOL. 409 | 25 FEBRUARY 2001 | www.nature.com

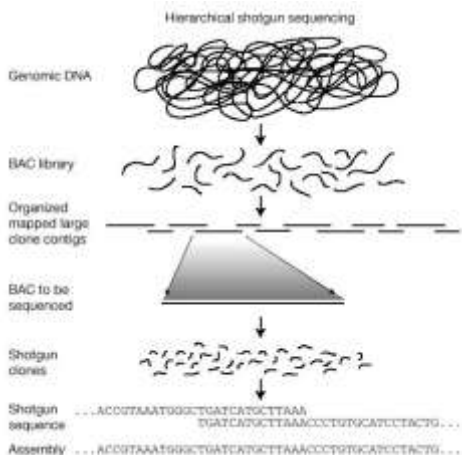
articles

## Initial sequencing and analysis of the human genome

International Human Genome Sequencing Consortium

**13 years** and **\$3 billion** required for the Human Genome Project's reference genome

Hierarchical shotgun sequencing



Genomic DNA

BAC library

Organized mapped large clone contigs

BAC to be sequenced


Shotgun clones

Shotgun sequence

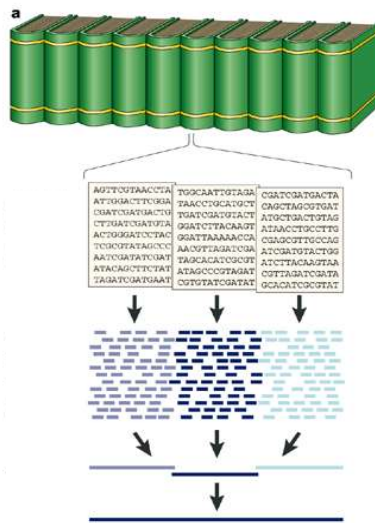
Assembly

...ACCGTAAATGGGCTGATCATGCTTAA...  
...TGATCATGCTTAAACCTGTGATCTACTG...

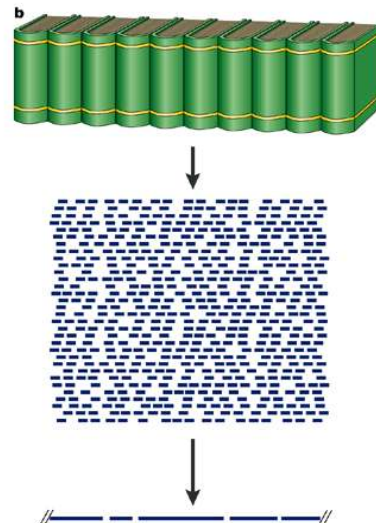
...ACCGTAAATGGGCTGATCATGCTTAAACCTGTGATCTACTG...



## Clone-by-clone shotgun sequencing



## Whole-genome shotgun sequencing

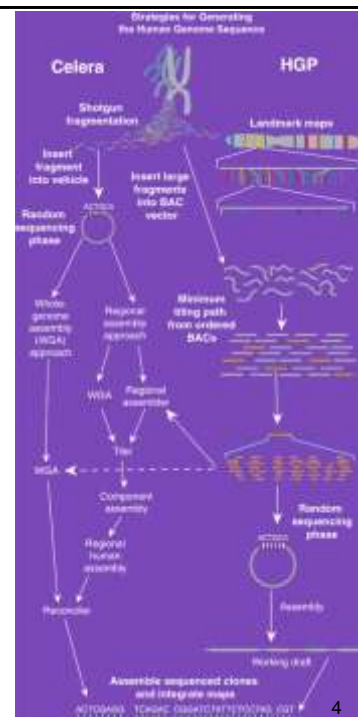


Nature Reviews | Genetics

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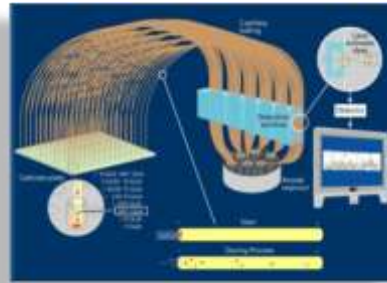
The \$300,000,000 Celera effort was intended to proceed at a faster pace and at a fraction of the cost..



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# Sanger sequencing

(invented in the early 1970s)



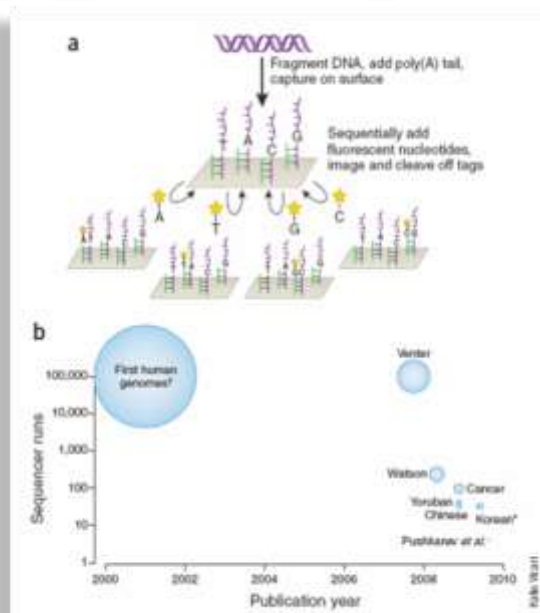
- 96 samples
- 800 nt / sample
- 1.600.000 nt / day

- Human genome: 3.000.000.000 bp
- Minimum coverage for an accurate analysis: 8X = 24.000.0000.000 nt

$$\frac{24.000.000.000}{1.600.000} = 15.000 \text{ days!}$$



## “No Generation” Sequencing



# New Generation Sequencing!

*GS20 (2005)*

100 bp x 200,000 Reads = 20 Mbp



*GS FLX (2007)*

200 bp x 400,000 Reads = 100 Mbp

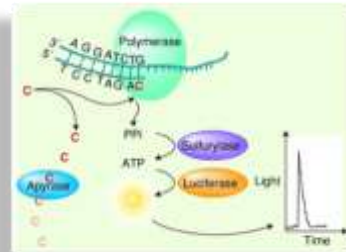
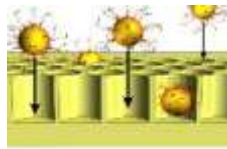


*GS FLX Titanium (2008)*

400 bp x 1 M. Reads = 500 Mbp



454  
SEQUENCING



## 454 "Revolution"

*GS20 (2005)*

100 bp x 200,000 Reads = 20 Mbp



*GS FLX (2007)*

200 bp x 400,000 Reads = 100 Mbp



*GS FLX Titanium (2008)*

400 bp x 1 M. Reads = 500 Mbp

>00405\_2045\_2005

CAGTCTCGTCGTCGTACGATCGTACGTAGCTTCACTTACGTACGCGGCG  
GGGCGCATCTGCGCGCGGATTATATCATCATCATACTCAGCATCGTCC  
ATC

>00343\_3489\_2007

CAGTCTCGTCGTCGTACGATCGTACGTAGCTTCACTTACGTACGCGGCG  
GGGCGCATCTGCGCGCGGATTATATCATCATCATACTCAGCATCGTCC  
ATCGATCGATCGATGATCGACGATCGTAGTCTACGTAGTACGTAGCTAG  
CTTCGATCGATCGTACGTACGTACGTACGTAGTCAGACGTCAGCTACAGT  
CATCTACGTAGCTCTACGTGTCATGCTAGCTATCGATCAGCACTTAT  
GCATC

>01384\_3992\_2008

CAGTCTCGTCGTCGTACGATCGTACGTAGCTTCACTTACGTACGCGGCG  
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CTTCGATCGATCGTACGTACGTACGTACGTAGTCAGACGTCAGCTACAGT  
CATCTACGTAGCTCTACGTGTCATGCTAGCTATCGATCAGCACTTAT  
GCATCCTCGGTATTCGGCGTACGATCGCTGACTGCTCGATTTTCGATCG  
TACGTACCGTCAGCTAGCTAAAAAAGCTGCGCGGTACGATCGTTG  
CATGCGCTACTGCTAGTACGTAGTAGTGTACGTTTATATCGTCGCCA  
AATCTCTTCGGCTTTTCG

nature

Vol 452 | 7 April 2008 | doi:10.1038/nature06888

April 2008

## LETTERS

## The complete genome of an individual by massively parallel DNA sequencing

David A. Wheeler<sup>1,2</sup>, Maithreyan Srinivasan<sup>3,4</sup>, Michael Egholm<sup>5,6</sup>, Yufeng Shen<sup>1,4</sup>, Lei Chen<sup>1</sup>, Amy McGuire<sup>1</sup>, Wei He<sup>1</sup>, Yi-Ju Chen<sup>1</sup>, Vinod Makhiani<sup>1</sup>, G. Thomas Roth<sup>1</sup>, Xavier Gomes<sup>1</sup>, Karrie Tartaro<sup>1</sup>, Faheem Niaz<sup>1</sup>, Cynthia L. Turcotte<sup>1</sup>, Gerard P. Irzyk<sup>1</sup>, James R. Lupski<sup>1,3,4</sup>, Craig Chiu<sup>1,2,4</sup>, Xingzhi Song<sup>1</sup>, Yue Liu<sup>1</sup>, Ye Yuan<sup>1</sup>, Lynne Nazareth<sup>1</sup>, Xiang Qin<sup>1</sup>, Donna M. Muzny<sup>1</sup>, Marcel Margulies<sup>1</sup>, George M. Weinstock<sup>1,2</sup>, Richard A. Gibbs<sup>1,2,4</sup> & Jonathan M. Rothberg<sup>1,2</sup>†

2 months and 2.000.000 USD with 454 Life Sciences



Table 3 | SNPs matching HGM mutations causing disease or other phenotypes

HGM accession	Chromosome	Coordinate	HUGO symbol	Gene name	Cytogenetic	Phenotype	Zygosity
CM003589	1	97327178	DFYD	Dihydropyrimidine dehydrogenase	1q22	Dihydropyrimidine dehydrogenase deficiency	Heterozygous
CM950484	1	157442370	FY	Duffy blood-group antigen	1q	Duffy blood group antigen, absent	Homozygous*
CM940304	4	617702	FDBB	Phosphodiesterase 6B, cGMP-specific, testis	4p16.3	Retinitis pigmentosa 40	Heterozygous
CM021718	9	36208221	GNE	UDP-N-acetylglucosamine 2-epimerase	9p	Myopathy, distal, with rimmed vacuoles	Heterozygous
CM990633	30	50348375	BRCC5	Oxidative repair cross-complementing nucleotid repair deficiency, complementation group 5 protein (C580)	10q	Cockayne syndrome	Homozygous†
CM050716	21	76533431	MYO7A	Myosin VIIA	11q13.6	Usher syndrome 1b	Homozygous†
CM950928	12	46822979	PFKM	Phosphofructokinase, muscle	12p13.3	Glycogen storage disease 7	Homozygous*
CM030229	14	20659890	FFGF1	Retinitis pigmentosa GTPase regulator interacting protein 1	14q11	Cone-rod dystrophy	Heterozygous
CM964025	19	18045118	IL13RB2	Interleukin-12 receptor, beta 1	19p13.1	Mycobacterial infection	Heterozygous
CM024138	19	41034441	NPHS1	Nephrin-1, congenital Finnish type	19q	Congenital nephrotic syndrome, Finnish type	Heterozygous
CM910052	22	40420925	ARSA	Arylsulfatase A	22q	Metachromatic leukodystrophy	Heterozygous

\*Coverage at these SNP positions is less than 5. However, both produce benign phenotypes.

†Coverage at these SNP positions is greater than 5. Both would produce severe phenotypes if they were truly homozygous.

apoE unknown

## Now Generation Sequencing

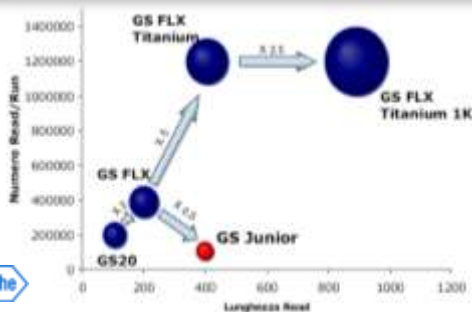
### GS FLX+ System Performance

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



GSFLX

1.100.000 reads (~800 nt)

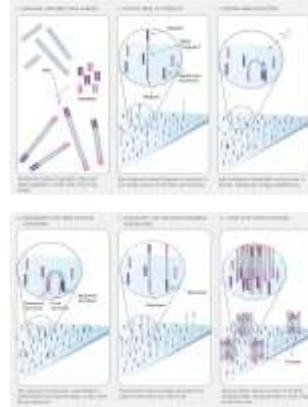


GSJunior

100.000 reads (400-500 nt)



## Genome Analyzer II (x)



**End 2007:** 30 million reads, 33 nucleotides each (1 Gbase)

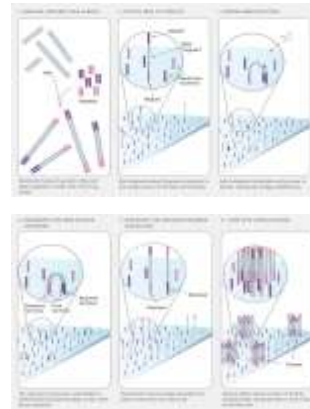
**End 2008:** 50 million reads, 50 nucleotides each (2.5 Gbases)

**2010:** 300 million single reads, 150 nucleotides each  
600 million paired reads, 150 nucleotides each (90 Gbases)



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## HiSeq 2000



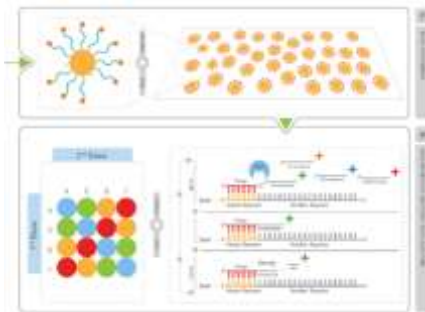
**HIGHEST OUTPUT**  
600 Gb per run

**HIGHEST NUMBER OF READS**  
Three billion (100 or 2 x 100) reads



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## 5500xl SOLiD™ System



<b>Throughput/Run</b>	Microbeads: Up to 100 Gb, or more than 2.8 B reads (paired-end or mate-paired runs) Nanobeads: Up to 300 Gb, or more than 4.8 B reads (paired-end or mate-paired runs)	
<b>Samples/Run<sup>1</sup></b>	Microbeads: • 2 genomes • 24 exomes • 12 transcriptomes	Nanobeads: • 3 genomes • 40 exomes • 20 transcriptomes
<b>Read Length</b>	• 75 bp (fragment) • 75 bp x 25 bp (paired-end) • Up to 60 bp x 60 bp (mate-paired)	
<b>Run Time<sup>2</sup></b>	• 1 day for 25 bp, 1 lane • 7 days for 75 bp x 25 bp or 60 bp x 60 bp, 12 lanes	

**AB Applied Biosystems**

**AB applied biosystems**  
part of Life Technologies

**Complete**  
genomics



Figure 7. Complete Genomics Sequencing Instrument.



The screenshot shows the Complete Genomics website. At the top left is the logo. To the right are links for Home, Information, Blog, Contact, and a search bar. Below the logo is a navigation bar with links: About Us, Services, Analysis Tools, Public Data, Support & Community, News & Events (highlighted in red), and Investor Relations. Below the navigation bar is a red banner with the text: "Home » News & Events » Press Releases » Complete Genomics Reports Fourth Quarter and Fiscal Year 2011 Results". The main content area features a date "Mar 8, 2012" and the headline "Complete Genomics Reports Fourth Quarter and Fiscal Year 2011 Results". The text below the headline states: "MOUNTAIN VIEW, Calif. - Mar. 8, 2012 - Complete Genomics, Inc. (NASDAQ: CGEM), the whole human genome sequencing company, today announced financial results for the fourth quarter and fiscal year ended December 31, 2011." It then lists "Fiscal Year 2011 Results" with bullet points: revenue of \$16.3 million, gross profit of \$5.4 million, and net loss of \$22.5 million. A "More Press Releases" box is on the right.

Mar 8, 2012

## Complete Genomics Reports Fourth Quarter and Fiscal Year 2011 Results

MOUNTAIN VIEW, Calif. - Mar. 8, 2012 - Complete Genomics, Inc. (NASDAQ: CGEM), the whole human genome sequencing company, today announced financial results for the fourth quarter and fiscal year ended December 31, 2011.

### Fiscal Year 2011 Results

- We recognized revenue with respect to approximately 3,000 genomes, compared to approximately 600 genomes in 2010.
- Revenue was \$16.3 million, compared to \$5.4 million in 2010.
- Costs and operating expenses were \$22.5 million, compared to \$27.9 million in 2010.
- Net loss was \$22.5 million, compared to \$57.7 million in 2010. The net loss for 2011 has included a \$2.2 million insurance expense resulting from an accounting adjustment to the market value of certain convertible equity securities.

In 2011, we clearly demonstrated the value of our service, signing orders for approximately 6,000 genomes, representing aggregate revenue potential of \$70 million, and delivering data to approximately 100 customers," said Dr. Clifford Hoad, chairman, president and CEO of Complete Genomics. "We entered 2012 with a strong order book of about 5,000 genomes, representing aggregate revenue potential of approximately \$60 million."

More Press Releases  
See additional [press releases](#)

The screenshot shows a Mozilla Firefox browser window. The address bar displays "Complete Genomics and BGI-Shenzhen Announce Definitive Agreement to Merge (iGNOM) - Mozilla Firefox". The page title is "Complete Genomics and BGI-Shenzhen Announce Definitive Agreement to Merge". The date "September 17, 2012" is shown. The headline is "Complete Genomics and BGI-Shenzhen Announce Definitive Agreement to Merge". Below the headline is a sub-headline: "Combination Will Create a Global Innovator in Whole Human Genome Sequencing". The main text of the press release is visible, starting with "MOUNTAIN VIEW, Calif. and SHENZHEN, China, Sept. 17, 2012 (iGNOM Newsroom) - Complete Genomics, Inc. (NASDAQ: CGEM) ('Complete'), an innovative leader in whole human genome sequencing, and BGI-Shenzhen (BGI), a leading international genomics company based in Shenzhen, China, today announced that they have entered into a definitive merger agreement. Through this agreement, a wholly owned U.S. subsidiary of BGI will launch a tender offer to purchase all outstanding shares of common stock of Complete for \$5.15 per share in cash, without interest. This price represents approximately a 54% premium to the \$2.04 closing price per share of Complete common stock on June 4, 2012, the last trading day prior to Complete's announcement that it was considering an evaluation of strategic alternatives to secure the financial resources needed for continued commercialization of its technology." The text continues with details about the merger agreement and the combined company's vision.

Complete Genomics and BGI-Shenzhen Announce Definitive Agreement to Merge (iGNOM) - Mozilla Firefox

Get to a Website

Complete Genomics

September 17, 2012

## Complete Genomics and BGI-Shenzhen Announce Definitive Agreement to Merge

Combination Will Create a Global Innovator in Whole Human Genome Sequencing

MOUNTAIN VIEW, Calif. and SHENZHEN, China, Sept. 17, 2012 (iGNOM Newsroom) - Complete Genomics, Inc. (NASDAQ: CGEM) ("Complete"), an innovative leader in whole human genome sequencing, and BGI-Shenzhen (BGI), a leading international genomics company based in Shenzhen, China, today announced that they have entered into a definitive merger agreement. Through this agreement, a wholly owned U.S. subsidiary of BGI will launch a tender offer to purchase all outstanding shares of common stock of Complete for \$5.15 per share in cash, without interest. This price represents approximately a 54% premium to the \$2.04 closing price per share of Complete common stock on June 4, 2012, the last trading day prior to Complete's announcement that it was considering an evaluation of strategic alternatives to secure the financial resources needed for continued commercialization of its technology.

Complete's board of directors has unanimously recommended that stockholders accept the offer and tender their shares. Based on the number of fully diluted outstanding shares of Complete, the aggregate value of the transaction is approximately \$117.8 million. In addition, Complete and an affiliate of BGI have entered into an agreement pursuant to which Complete will be provided with up to \$33 million in bridge financing for its operations following the signing of the merger agreement.

Complete provides whole human genome sequencing, which is used by research centers to conduct medical research that, in the future, is expected to be used by doctors and hospitals to improve both prevention and treatment of disease. BGI operates international genome sequencing centers, which support genetic research into agriculture, animals and humans, and serve researchers around the world including the United States. The combination of the two companies is expected to bring together complementary scientific and technological expertise and R&D capabilities. Complete will continue to be operated as a separate company with headquarters and operations remaining in Mountain View, California.

BGI's CEO Dr. Jiang Jun said, "Complete has developed a proprietary whole human genome sequencing technology that, together with other sequencing platforms owned by BGI, will fit well with our research and business requirements and promote Complete to become an even more successful global innovator. We look forward to growing the business to improve medical research and, where clinical services are provided, support





#### About BGI

BGI was founded in 1999 as the Beijing Genomics Institute. It now has several branches and subsidiaries including: BGI-Shenzhen, a nonprofit research institute; BGI-Hong Kong, a private institute that manages international collaborations and transfers profits to BGI; and BGI-Americas, located in Boston, which just celebrated its one-year anniversary and announced new joint projects with the Broad Institute and the United Kingdom. BGI has about 4,000 employees and the capacity to sequence the equivalent of 1,600 complete human genomes each day.

#### SEC Filing: Helicos Biosciences Provides Financing Update

Filed March 16, 2012



##### Financing Update

As of March 5, 2012, Helicos has approximately \$200,000 in cash and cash equivalents. Helicos will require significant additional capital to conduct its operations.

On February 23, 2012, Helicos entered into a letter agreement amending certain Secured Promissory Notes in the aggregate original principal amount of \$200,000 issued by Helicos to certain investment funds affiliated with **Pinnacle Ventures and Atlas Venture**. The letter agreement extended the maturity date of the Notes to March 31, 2012. Helicos issued the Notes on January 13, 2012 to the Purchasers pursuant to that certain Subordinated Secured Note Purchase Agreement, dated as of November 18, 2010, among Helicos and the Purchasers. The Notes were initially issued with a 12-day maturity in anticipation of closing a large financing, which has not yet occurred. The Notes accrue interest at a rate of 30% per annum. The outstanding principal and any unpaid accrued interest on the Notes is due and payable on the earlier of (i) the Purchasers jointly demanding in writing the payment of the outstanding amounts at any time on or after the Maturity Date or (ii) the occurrence of an event of default. The Company's obligations under the Notes are secured by a security interest in all of the Company's assets, including its intellectual property, that, with regard to all of the Company's assets other than the assets of Helicos, is the subject of the intellectual property litigation against Pacific Biosciences, Illumina and Life Technologies and is junior to the security interest of the Company's outside legal counsel that is representing the Company in the Cases of Action. Helicos used the proceeds from the issuance of the Notes along with available cash to repay all amounts outstanding under the loan and security agreement among the company, certain lenders and General Growth Capital Corporation, as agent, dated as of December 31, 2007, as amended.

Helicos is currently in active discussions with the Purchasers regarding the possibility of additional short-term financing to support Helicos' continued operations. The Company and Purchasers have not yet agreed on such financing and there can be no guarantee that Helicos will obtain such financing or any other source of funding so that Helicos can obtain any additional financing on terms favorable to Helicos or in amounts sufficient to meet Helicos' needs.

If Helicos is unable to repays its obligations, according to its plans and obtain additional financing, Helicos will be forced to cease operations.

##### We Recommend...

Sell on IPO (Risk: Moderate) Buy on Pkgs (Risk: High) Buy on Pkgs (Risk: High)

Spec in R Share (Risk: High)

Spec in R Share (Risk: High)

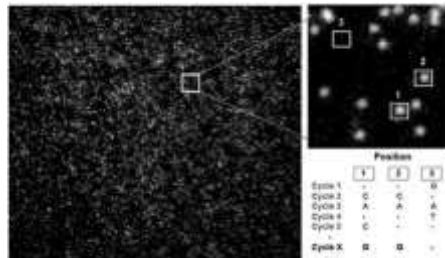
The Vanguard Group (Risk: Moderate) Buy on Pkgs (Risk: High)

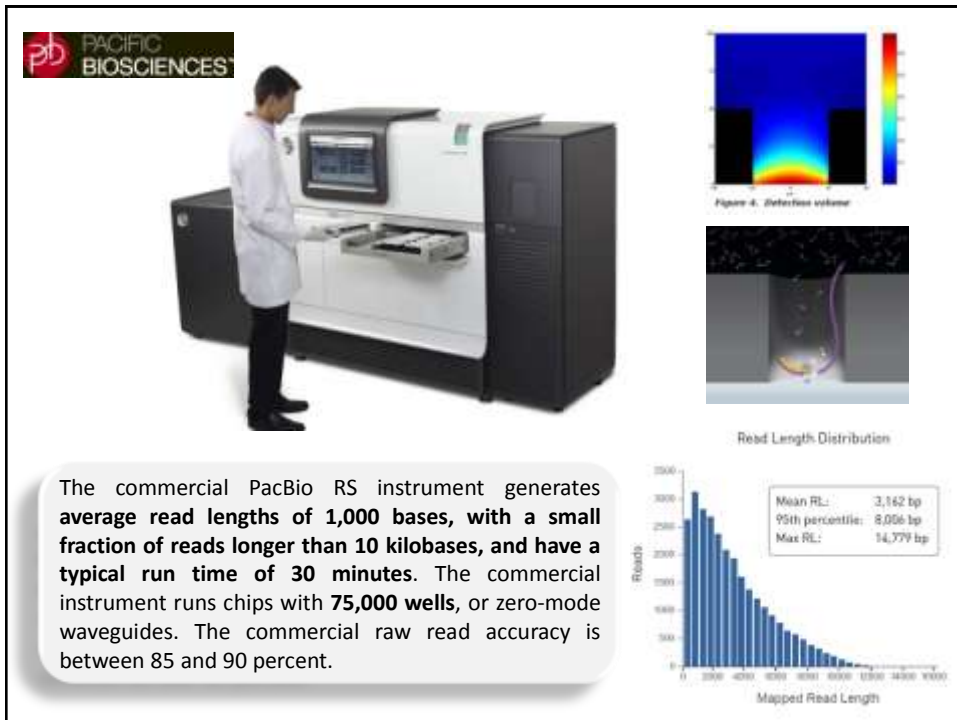
John Hancock (Risk: Moderate) Buy on Pkgs (Risk: High)

Investment Analysis (Risk: Moderate) Buy on Pkgs (Risk: High)

Investment Analysis (Risk: Moderate) Buy on Pkgs (Risk: High)

Investment Analysis (Risk: Moderate) Buy on Pkgs (Risk: High)

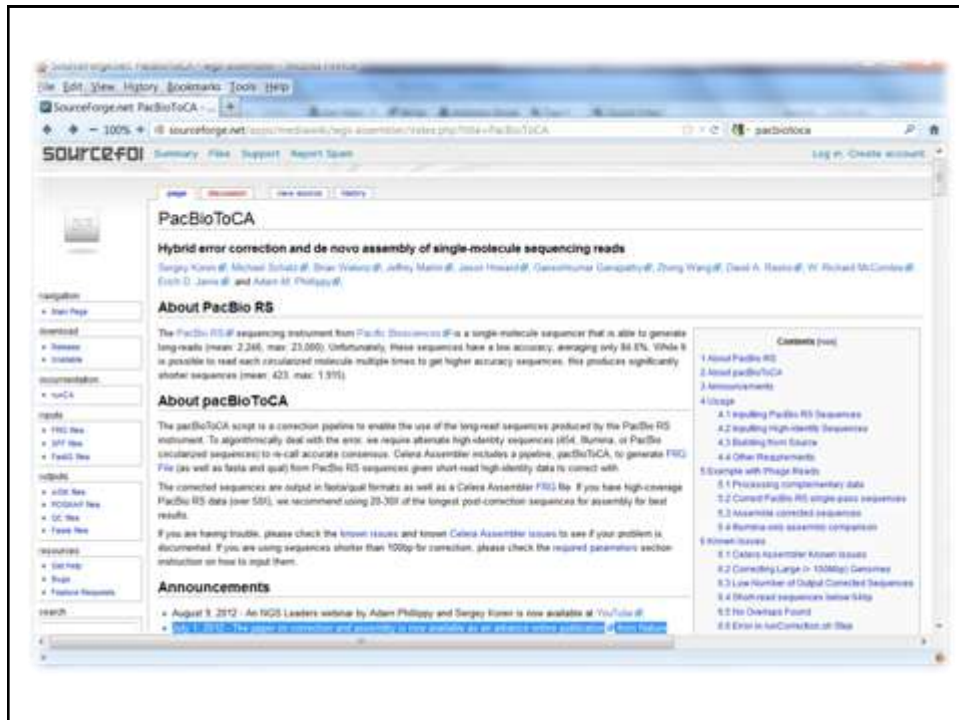




September 27, 2011

With the C2 chemistry, the Expression Analysis team achieved average read lengths of 2,715 base pairs, with a mean maximum read length of 13,091 base pairs. "Our personal best is just over 14,000 bases," Hurban said.

Despite the potential of PacBio's platform to produce very long reads, which would enable better assemblies, adoption of the instrument since its launch in the second quarter of this year has been slower than expected. As a result, **PacBio last week announced that it would have to lay off around 130 employees**



**December, 2010**

**ion torrent**  
by life technologies™

10 Mbp to 1 Gb  
100-400 bp reads  
90-minute run times

**ION TORRENT RESEARCHER**

ION TORRENT RESEARCHER is a next-generation sequencing platform that delivers high-throughput, high-accuracy sequencing data. It is the only platform that delivers high-throughput, high-accuracy sequencing data. It is the only platform that delivers high-throughput, high-accuracy sequencing data.

**THE CHIP IS THE MACHINE™**

ION TORRENT RESEARCHER is a next-generation sequencing platform that delivers high-throughput, high-accuracy sequencing data. It is the only platform that delivers high-throughput, high-accuracy sequencing data. It is the only platform that delivers high-throughput, high-accuracy sequencing data.

**314**

ION TORRENT RESEARCHER

**316**

ION TORRENT RESEARCHER

**218**

ION TORRENT RESEARCHER

## MiSeq

January, 2011



Approximate Run Duration and Output  
Cluster Generation and Sequencing

Read Length	Total Time for Amplification and Sequencing*	Output**
1 x 36 bp	~4 hours	175-245 Mb
2 x 25 bp	~5 hours	250-350 Mb
2 x 100 bp	~19 hours	1.0-1.4 GB
2 x 150 bp	~27 hours	1.5-2.0 GB

\*Includes paired-end read, if applicable.

\*\* Install specifications for MiSeq with an Illumina HiSeq library and cluster densities between 720-800 A/mm<sup>2</sup> that pass filtering. Performance may vary based on certain quality, cluster density, and other experimental factors.

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illumina

## Next Generation Sequencing

### Ion Proton™ Sequencer

The Benchtop Genome Center

January 10th, 2012



- Supports Ion Proton™ I and Proton™ II chips
- €209,800 system list price (sequencer and server)
  - \$164,900 for Ion PGM™ owners
- State-of-the-art electronics to support highest throughput

The Ion Proton™ Sequencer is based on the next generation of semiconductor sequencing technology that made the Ion PGM™ Sequencer the fastest selling sequencer in the world. Now high-throughput chips will enable the Ion Proton™ Sequencer to sequence a human genome with outstanding turn, and single-day workflow, as the Ion PGM™ Sequencer.

Data analysis, which has long been a bottleneck for whole-genome sequencing, can also be completed in the same day as a single whole genome. In fact, unlike other systems to have supported A genomes, the Ion Proton™ Sequencer can sequence and analyze 30 genomes for a small fraction of the cost.



Proton I™  
The Ion Proton™ I Chip  
100,000 reads  
100,000 reads



Proton II™  
The Ion Proton™ II Chip  
1,000,000 reads  
1,000,000 reads

January 10th, 2012



MISEQ System Performance Parameters

Read Length	Total Time from Prepped Library through Sequencing <sup>1</sup>	Output
1 x 35 bp	2.2-3.5 hours	440-550 Mb
2 x 25 bp	4.8-6.0 hours	840-830 Mb
2 x 100 bp	14.0-18.0 hours	2.8-3.1 Gb
2 x 150 bp	20.7-24.0 hours	3.7-4.0 Gb
2 x 250 bp <sup>2</sup>	>35 hours	5.0-7.0 Gb
<b>Reads</b>		
12.0-15.0 million clusters passing filter and 24.0-30.0 million paired-end reads		
<b>Performance<sup>3</sup></b>		
>90% bases higher than Q30 at 1 x 25 bp		
>90% bases higher than Q20 at 2 x 25 bp		
>85% bases higher than Q20 at 2 x 100 bp		
>75% bases higher than Q20 at 2 x 150 bp		

<sup>1</sup> Includes paired-end read, if applicable.<sup>2</sup> Performance, output, amplification, and sequencing time for 2 x 250 bp read length depends on instrument upgrade, commercially available in the third quarter of 2012. Customers will be notified of upgrade availability. Upgrade dates are subject to change.<sup>3</sup> The percentage of bases >Q30 is averaged across the entire run, not on a per read or per cycle basis.

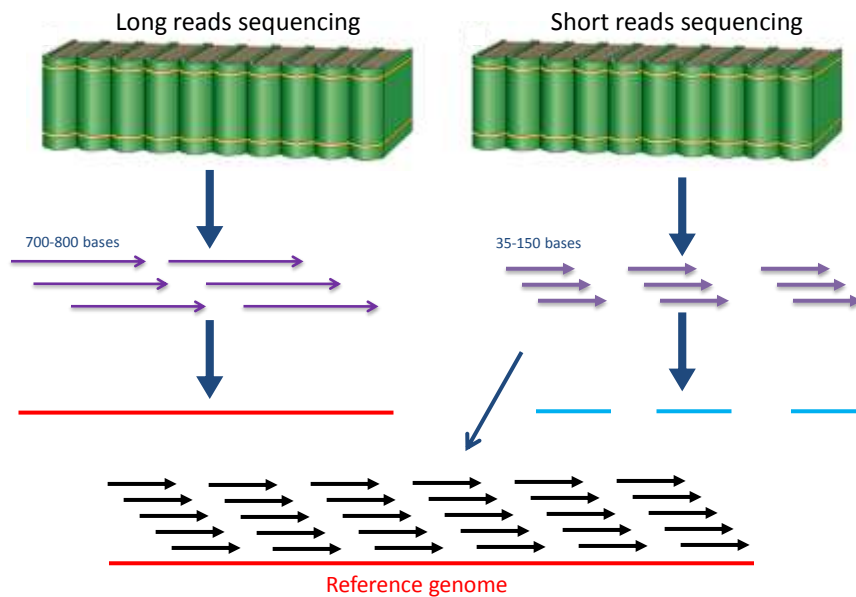
HiSeq 2500/1500

Two run modes. High output or rapid run.

Flexibility to batch process multiple samples with high output in a single run, or get rapid results with fewer samples for time-critical studies.

	HiSeq 2500		HiSeq 1500	
Run Mode	High Output	Rapid Run <sup>1</sup>	High Output	Rapid Run <sup>1</sup>
Output (2 x 100 bp)	600 Gb	120 Gb	300 Gb	60 Gb
Run Time (2 x 100 bp)	~11 days	~27 hours	~5.5 days	~27 hours
Cluster Generation	off	On board	off	On board
Paired-end Reads	6.8M	1.2 M	3.4M	600 M
Single Reads	3.4M	600 M	1.7 M	300 M
Maximum Read Length <sup>2</sup>	2 x 100 bp	2 x 150 bp	2 x 100 bp	2 x 100 bp
Quality Scores <sup>3</sup>	~90% (2 x 50 bp) ~95% (2 x 100 bp)			

## Resequencing vs. de-novo assembling





**Resequencing is good only if the  
reference genome is good**

***Next Next (Next) Generation Sequencing  
Technologies for haplotyping and de-novo  
assembling***

## Biological nanopores

genia

Electronic  
BIOSCIENCES

Oxford  
**NANOPORE**  
Technologies®



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Oxford Nanopore Technologies Ltd is developing a revolutionary technology for direct, electrical detection and analysis of single molecules. The platform is designed to offer substantial benefits in a variety of applications. Our lead application is DNA sequencing, but the platform is also adaptable for protein analysis for diagnostics and drug development and identification of a range of other molecules for security & defence and environmental monitoring. The technology is modular and highly scalable, driven by electronics rather than optics.

Our first generations of DNA sequencing technology, Exonuclease and Strand sequencing, combine a protein nanopore with a processive enzyme, immobilised on a silicon chip. This elegant and scalable system has unique potential to transform the speed and cost of DNA sequencing. We also have collaborative projects in the development of solid state nanopores for further improvements in speed and cost.



Exonuclease DNA sequencing



DNA strand sequencing



Solid-state nanopore DNA sequencing



Protein analysis



Small Molecules



Polymers

**NEWS**



Robot Nanotechnology paper demonstrates enzyme controlled movement of solute through nanopore  
27 September 2010  
[Read more...](#)



Oxford Nanopore strengthens collaboration with University of Oxford  
14 September 2010  
[Read more...](#)



Oxford Nanopore congratulates Professor Rosa Ghadiri and collaborators on \$6.5 million NSERC grant  
14 September 2010  
[Read more...](#)

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<http://www.nanoporetech.com/sequences>

## Oxford Nanopore megaton announcement: "Why do you need a machine?" – exclusive interview for this blog!

By Nick Loman February 17, 2012

Sometimes this genome blogging lark really pays off. Yesterday I had a preview of the big announcement at AGBT, and 20 minutes to speak with Clive Brown (Chief Technical Officer) and Zoe McDougall (Director of Applications). I couldn't tell anyone what they said until the embargo was lifted at 5pm today!

I asked as many questions as I could without knowing the contents of the AGBT talk. I probably should have asked a bunch more. I do remember saying "wow" quite a lot.

First, go and read the press release!

Good, made possible...  
...in contact with the pore are detected through electrochemistry...  
...molecules (lambda phage) – no theoretical read length limit...  
...sample preparation

### Two products announced:

- MinION – USB disposable sequencer for ~ \$500 has 512 nanopores – target 150mb/hour
- MinION can run at 120-1000 bases/minute per pore for up to 6 hours
- GridION – two versions of rack-mountable sequencer with 2000 nanopores (2nd half 2012), 8000 nanopores (2013)
- GridIONS can be racked in parallel, so could do a whole human genome in 15 minutes
- Each GridION can do "tens of gigabases" over 24 hours
- Both machines commercially available 2nd half 2012
- Sequencing can be paused, sample recovered, replaced, started again
- Accuracy is 96%, errors are deletions, error profile will improve through software





The screenshot shows the Genia website. At the top is a navigation bar with links: "About Us", "Technology", "Careers", and "Contact". Below the navigation bar is a large banner image featuring a blue molecular structure. Overlaid on the banner is the text "Reaching the \$1000 \$100 genome." Below the banner, a paragraph states: "Genia's integrated circuits enable massively parallel single-molecule DNA sequencing." At the bottom, a smaller text box explains: "Genia's mission is to verify Moore's Law with biotechnology to make genetic information universally available. Our scalable nanoscale-based platform allows for single-molecule, electrical real-time analysis without the need for enzymes, complicated optics, labels, amplification, or fluorics. By developing a true integrated circuit on standard semiconductor process technology, Genia brings Moore's Law to the biological world to revolutionize the world of DNA sequencing."

**Current Status:** alpha testing (beta testing to start by end of 2012)

**Projected commercial availability:** 2013

## Sequencing the Human Genome

Human Genome Project

James Watson

Personal genome



1988 – 2001  
13 years  
3.000.000.000 dollars

2007  
2 months  
2.000.000 dollars

2010  
7 days  
10.000 dollars



### PacBio Developing Chip Technology for Highly Multiplexed 'V2' Sequencers; Launch Planned for 2014

February 22, 2013

By Julia Kanou

As it prepares to roll out its first single-molecule real-time sequencing instrument to early-access customers this summer, Pacific Biosciences is already working on a new, highly multiplexed version of its technology that integrates sequencing readouts, optical detection, and signal processing into a microchip. In Sequence has learned.

PacBio believes that its "version 2" or V2 technology "is going to completely replace any second-gen [sequencing] device," according to CEO Hugh Martin, whereas the current version 1 will likely be used in conjunction with high-throughput short-read instruments, for example in genome assemblies.

V2 will enable the company to multiplex several million zero-mode waveguide reaction wells and to use polymerase enzymes that synthesize DNA at a speed of up to 50 bases per second, resulting in a throughput of more than 100 megabases per second.

By using a 2D microfluidics-based system, V2 will enable the company to multiplex several million zero-mode waveguide reaction wells and to use polymerase enzymes that synthesize DNA at a speed of up to 50 bases per second, resulting in a throughput of more than 100 megabases per second.

While the high-throughput system will be geared at data labs and genome centers, the lower-throughput instrument — which will possibly be portable — will be aimed at small clinical labs "or even doctors' offices" and is designed to be "a delivery vehicle for some of the applications developed on V1 around diagnostics," Martin said.

Prior to that, in 2011 and 2012, the company plans to improve the performance of its "version 1" system by increasing the read length, polymerase speed, and number of productive DNAs, lowering the cost per base, upgrading software, and adding new applications such as methylation sequencing, direct RNA sequencing, and protein translation analysis. None of these improvements will require hardware changes, according to Martin.

The first commercial V1 system, scheduled for release later this year (see related story this issue), will likely be used mostly in research. While "you are not going to see that in a doctor's office," Martin said, "you will see large pharma companies or diagnostic companies developing applications that are going to be deployed."







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**Center for Functional Genomics**

Grid-ION-Proton ?


Illumina HiSeq 1000

PowerEdge™ R900

- 4 Intel Xeon® 7450 6-core
- 128 Gb RAM

Cluster HP DL380 G7

- 6 Xeon® 5645 6-core
- 144 Gb RAM



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