Istituto Zooprofilattico Sperimentale
del Lazio e della Toscana *M. Aleandri*

~~Next~~ Generation Sequencing

..o sequenziamento massivo del DNA
..o sequenziamento ad alta resa del DNA

Il termine indica un insieme di tecnologie che, oggi, consente di sequenziare fino a 2-3 miliardi di frammenti di DNA ("Illumina HiSeq XTen") SIMULTANEAMENTE.

	Run Time	Read Length	Reads / Run	Total nucleotides sequenced	Cost / MB
NGS Sequencing (Illumina HiSeqX)	3 days	150nt	2-3 billions	circa 1Tb	0,007$
Capillary Sequencing (ABI3730xl)	20'-3h	400-900nt	96-384	circa 200Kb	2.400$

Cioè:
circa una sessione di prova per 10 genomi umani (circa 1000dollari/genoma, *coverage* 30X)
contro 15000 sessioni di prova per 1 genoma umano (circa 10milioni di dollari/genoma)!

OCTOBER 24, 2005
INNOVATION AWARDS

A Better Idea

By MICHAEL TOTTY | Staff Reporter of THE WALL STREET JOURNAL

Gene sequencing -- the process of unlocking an individual's DNA -- could one day revolutionize medicine, allowing doctors to quickly identify someone's genetic makeup and craft individual treatments for such diseases as cancer and tuberculosis. But first, there has to be a way to speed the cumbersome, slow and expensive sequencing process.

Jonathan Rothberg, founder and chairman of the 454 Life Sciences unit of CuraGen Corp., Branford, Conn., says he found a solution in microelectronics. Just as semiconductor designers were able to squeeze millions of transistors onto a single chip, Mr. Rothberg devised a way to analyze the makeup of millions of DNA strands simultaneously.

The Judges

Journal content

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Letter

James Watson!

Nature **452**, 872-876 (17 April 2008) | doi:10.1038/nature06884; Received 3 December 2007; Accepted 4 March 2008

The complete genome of an individual by massively parallel DNA sequencing

See associated Correspondence: Roche, *Nature* **453**, 281 (May 2008)

David A. Wheeler[1,7], Maithreyan Srinivasan[2,7], Michael Egholm[2,7], Yufeng Shen[1,7], Lei Chen[1], Amy McGuire[3], Wen He[2], Yi-Ju Chen[2], Vinod Makhijani[2], G. Thomas Roth[2], Xavier Gomes[2], Karrie Tartaro[2,8], Faheem Niazi[2], Cynthia L. Turcotte[2], Gerard P. Irzyk[2], James R. Lupski[4,5,6], Craig Chinault[4], Xing-zhi Song[1], Yue Liu[1], Ye Yuan[1], Lynne Nazareth[1], Xiang Qin[1], Donna M. Muzny[1], Marcel Margulies[2], George M. Weinstock[1,4], Richard A. Gibbs[1,4] & Jonathan M. Rothberg[2,8]

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1st generation sequencing

Next (past,2nd, 3rd...) generation sequencing

1977 1986 1995 2002 2005 2007 2011 2012 2013 2014

Sanger Sequencing

ABI 370 Sequencer

ABI 310 Sequencer

ABI 3730xl Sequencer

454 e Solexa Sequencers

Solid Illumina sequencers

Ion Torrent sequencer

Illumina HiSeq X

PacBio

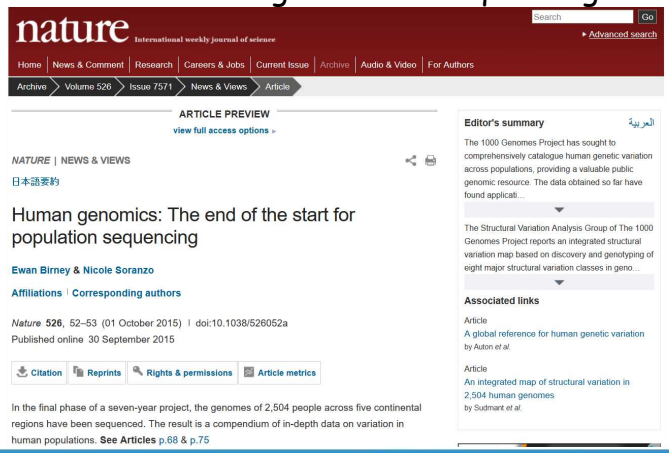
MinIon

«next next» generation sequencing……

1984 Epstein Barr virus 170kb

1995 Haemophilus influenzae 1,83Mb

2003 Human Genome Project 3,5Gb



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In fase di preparazione del campione,
"amplificazione clonale" eseguita su supporto solido:

l'immobilizzazione del DNA "stampo" su supporti come
"beads" o "flow cell" consente di condurre,
parallelamente, milioni/miliardi di reazioni di sequenza

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Sanger sequencing workflow

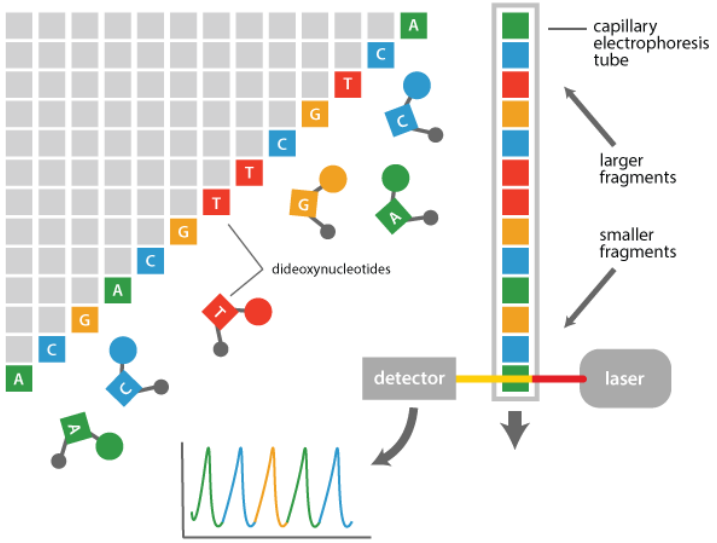
Steps:

one fragment per well

1 Mix DNA with PCR Mastermix[1] and simply dispense In EasySeq Plate

2 PCR amplification

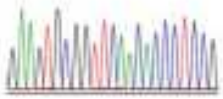
3 Cleanup[2] PCR Product and Cycle Sequence with -21M13 and M13REV primers



4 Purify Sequencing Reactions

5 Run and Analyse On 31xx, 35xx or 37xx series Genetic Analyzer

6 Data Analysis



one capillary per fragment

[1] In the last two columns (multiplex NTC wells), add only PCR Mastermix with water
[2] When using BD Direct or C-Pure, the PCR cleanup can be eliminated

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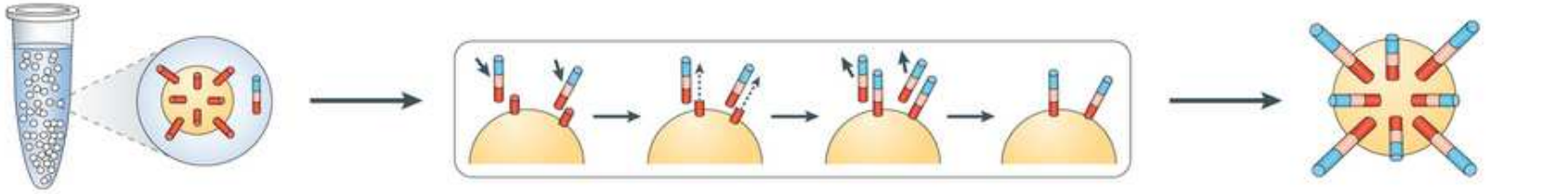
EMULSION PCR

arricchimento della libreria di frammenti da sequenziare

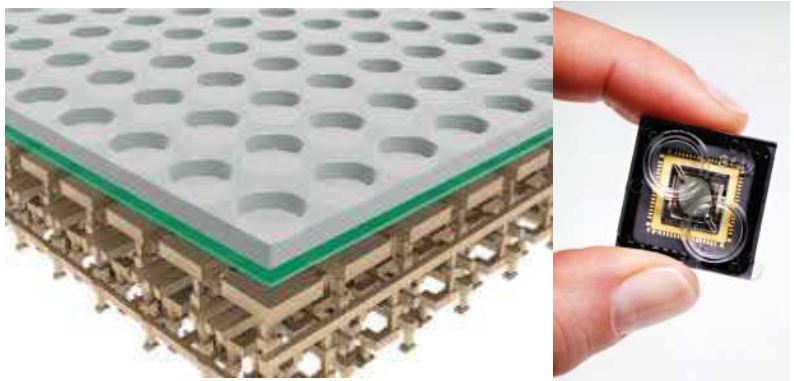
a **Emulsion PCR**
(454 (Roche), SOLiD (Thermo Fisher), GeneReader (Qiagen), Ion Torrent (Thermo Fisher))



Emulsion
Micelle droplets are loaded with primer, template, dNTPs and polymerase

On-bead amplification
Templates hybridize to bead-bound primers and are amplified; after amplification, the complement strand disassociates, leaving bead-bound ssDNA templates

Final product
100–200 million beads with thousands of bound template



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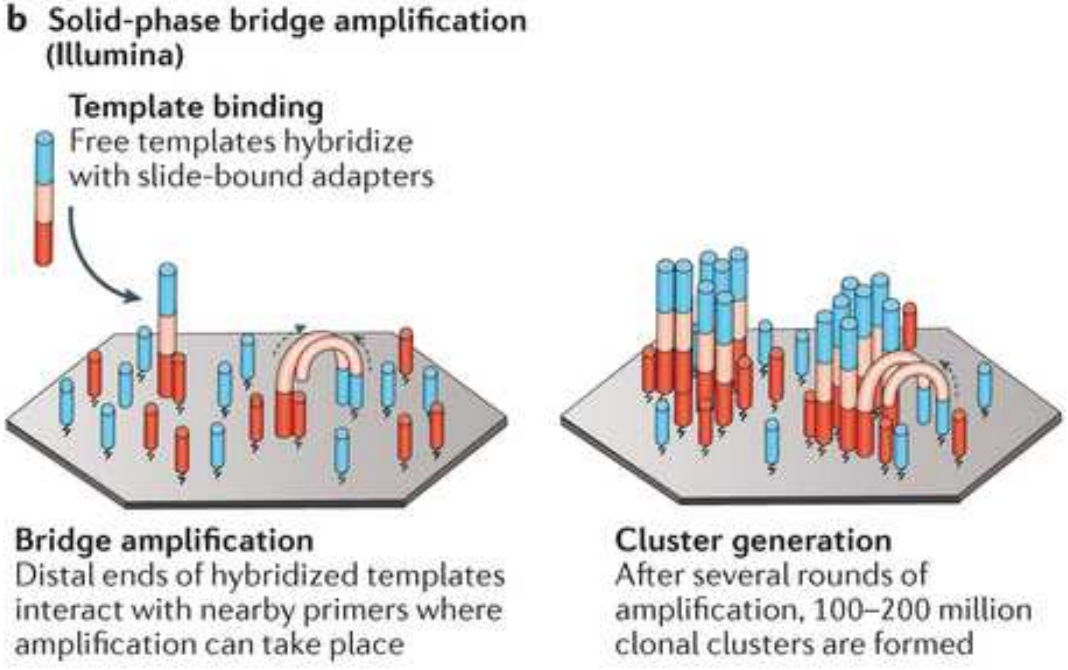
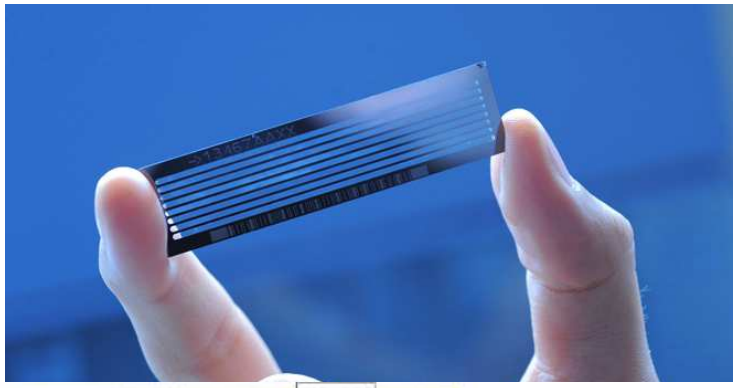
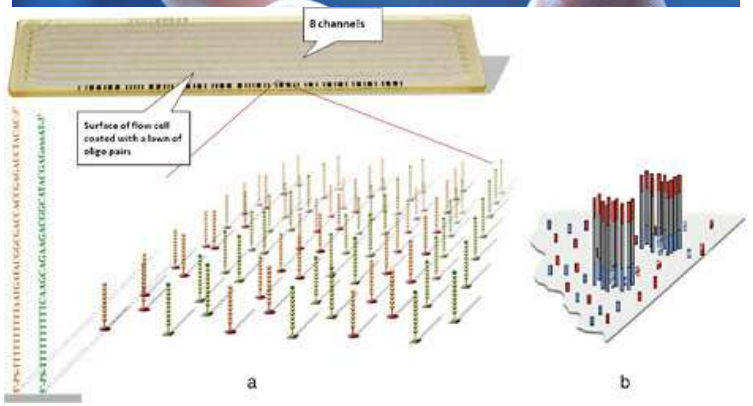
BRIDGE PCR

arricchimento della libreria di frammenti da sequenziare







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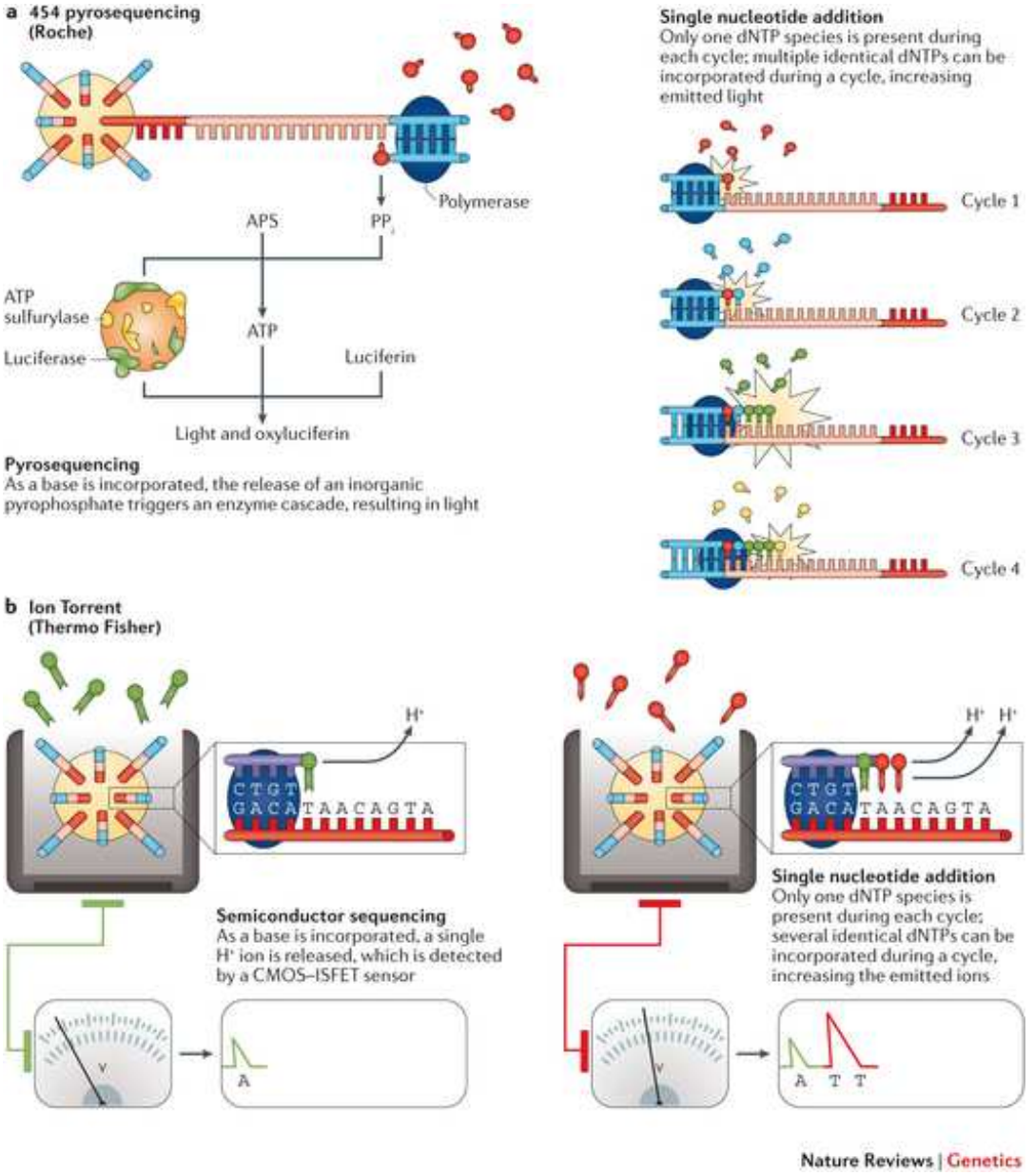
Sequenziamento della libreria mediante aggiunta di singole specie nucleotiche (Single Nucleotide Addition)



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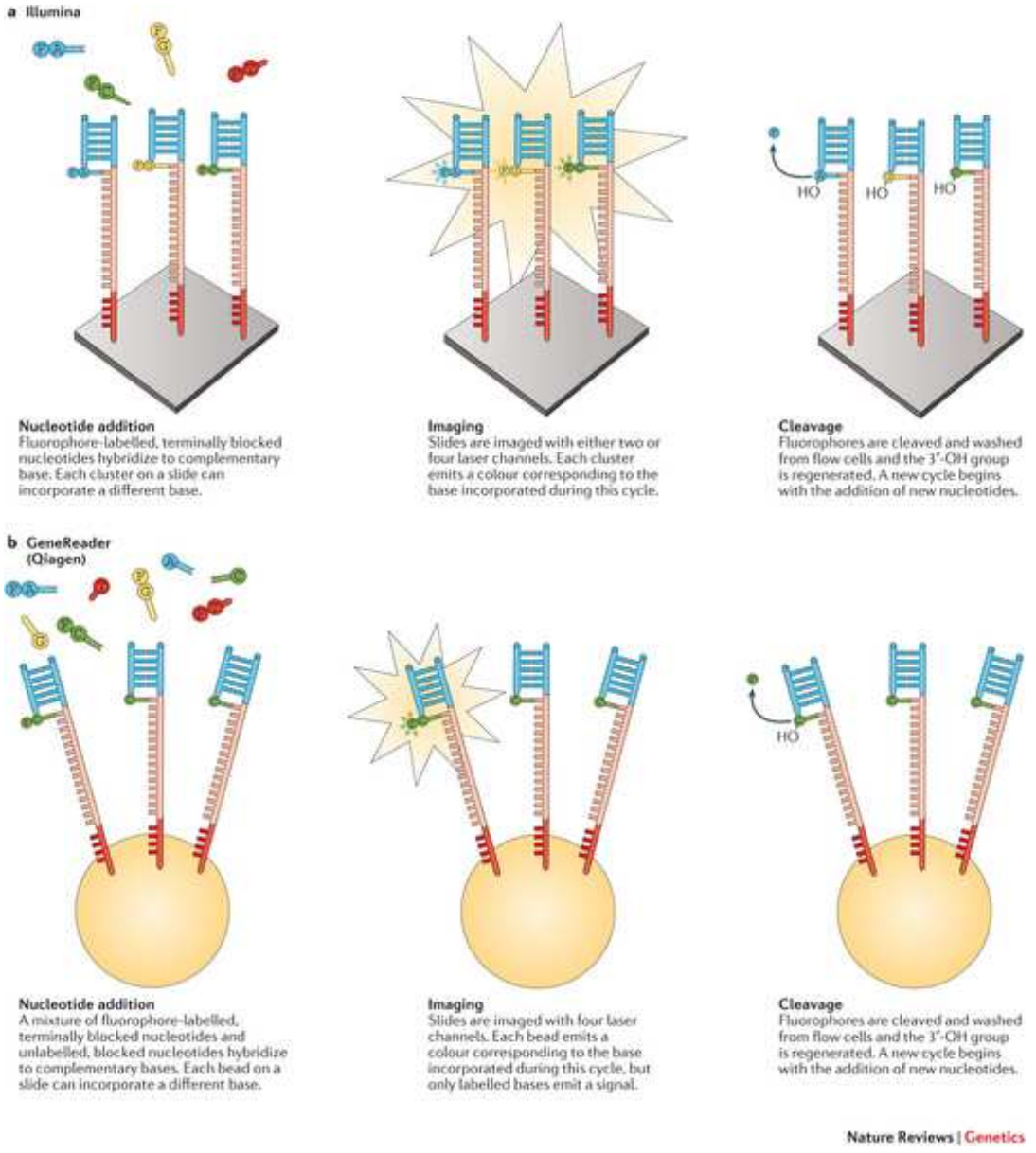
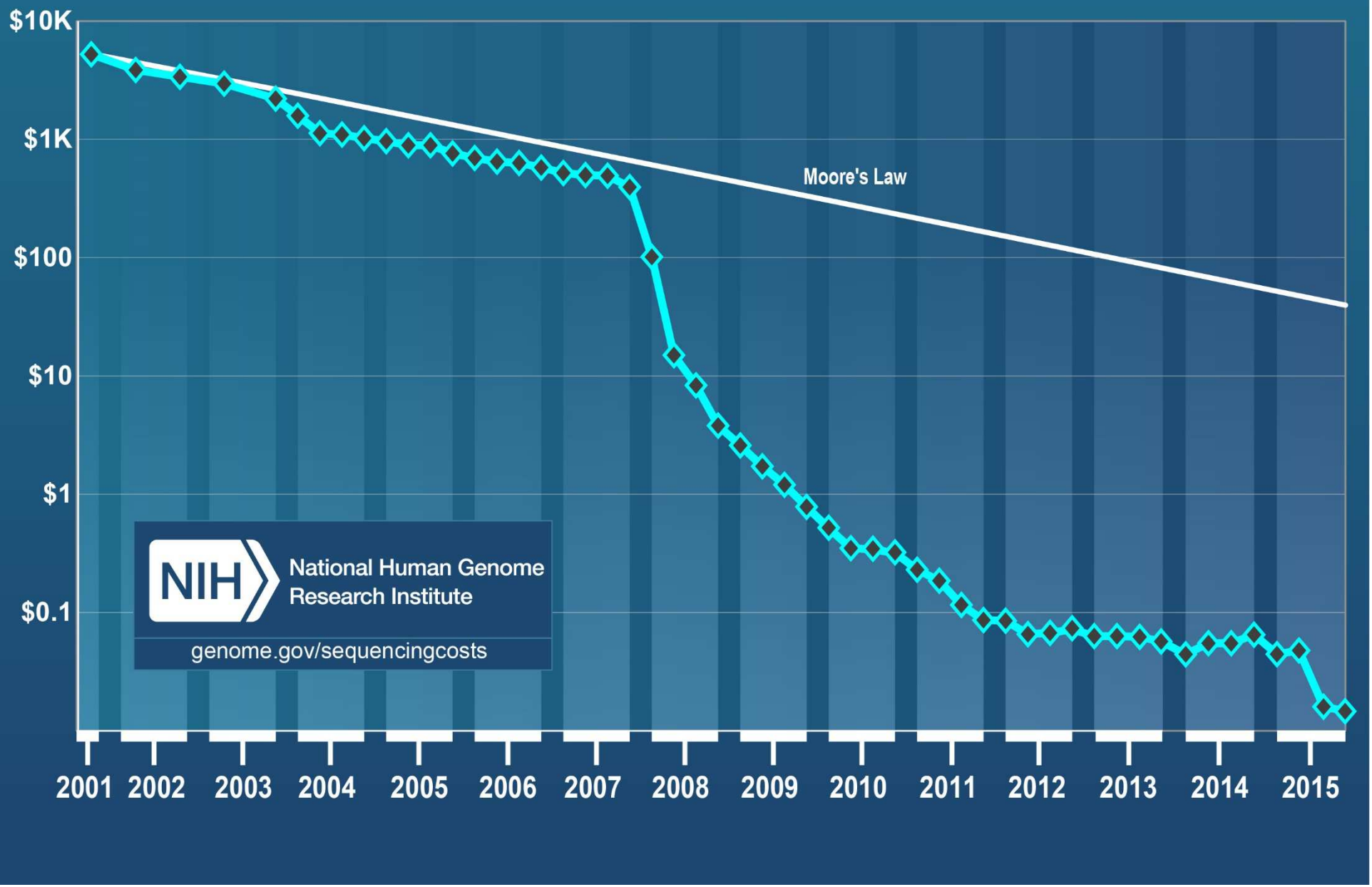
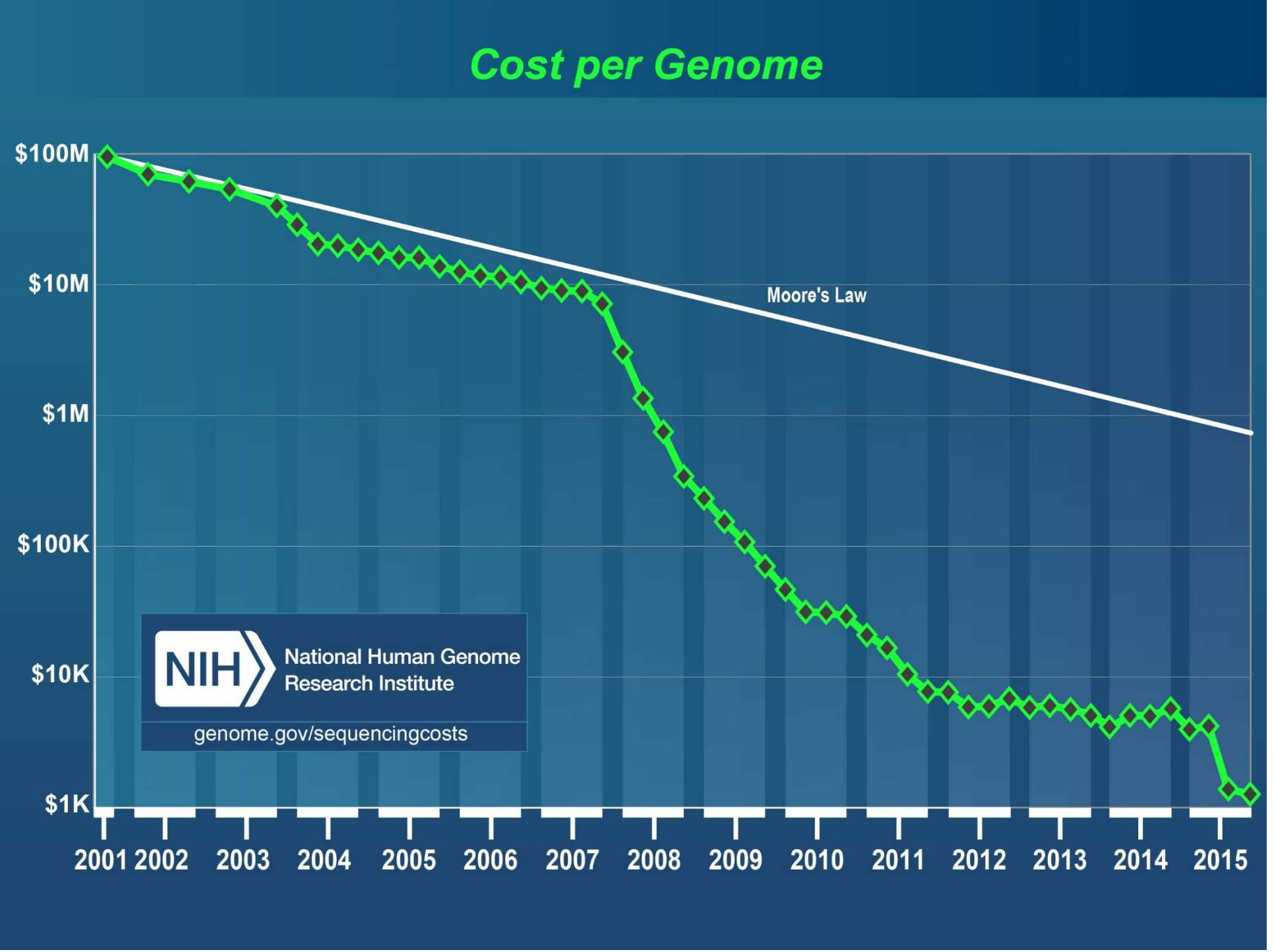
Sequenziamento della libreria mediante aggiunta di nucleotidi "bloccati" (Cyclic Reversible Termination)



Cost per Raw Megabase of DNA Sequence




Cost per Genome
$100M
$10M
$1M
$100K
$10K
$1K
Moore's Law
NIH
National Human Genome Research Institute
genome.gov/sequencingcosts
2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014 2015

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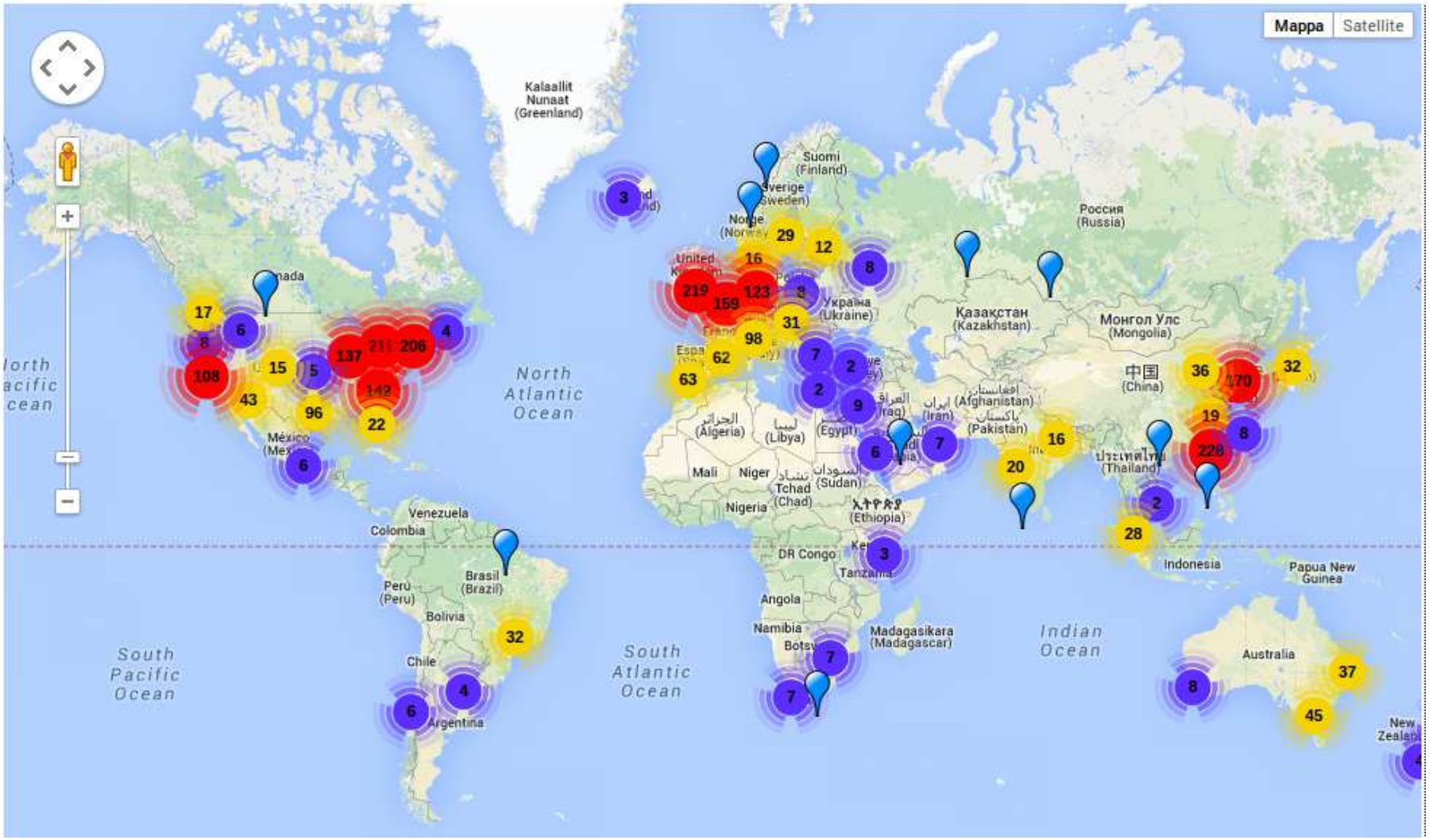
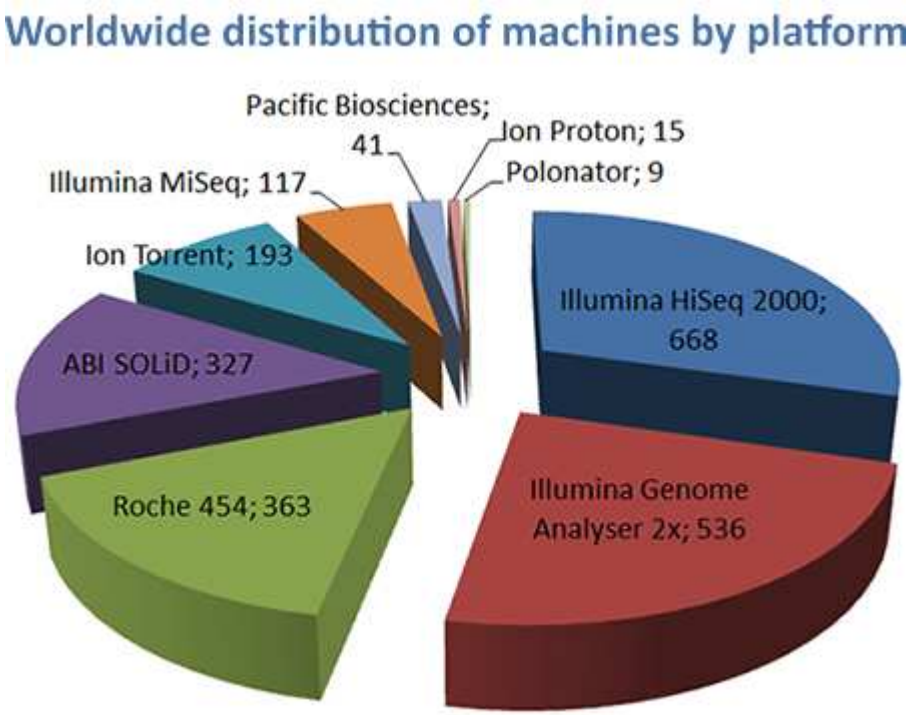
Installato al 2013



Installato al 2013



PERSPECTIVE

Big Data: Astronomical or Genomical?

Zachary D. Stephens[1], Skylar Y. Lee[1], Faraz Faghri[2], Roy H. Campbell[2], Chengxiang Zhai[3], Miles J. Efron[4], Ravishankar Iyer[1], Michael C. Schatz[5]*, Saurabh Sinha[3]*, Gene E. Robinson[6]*

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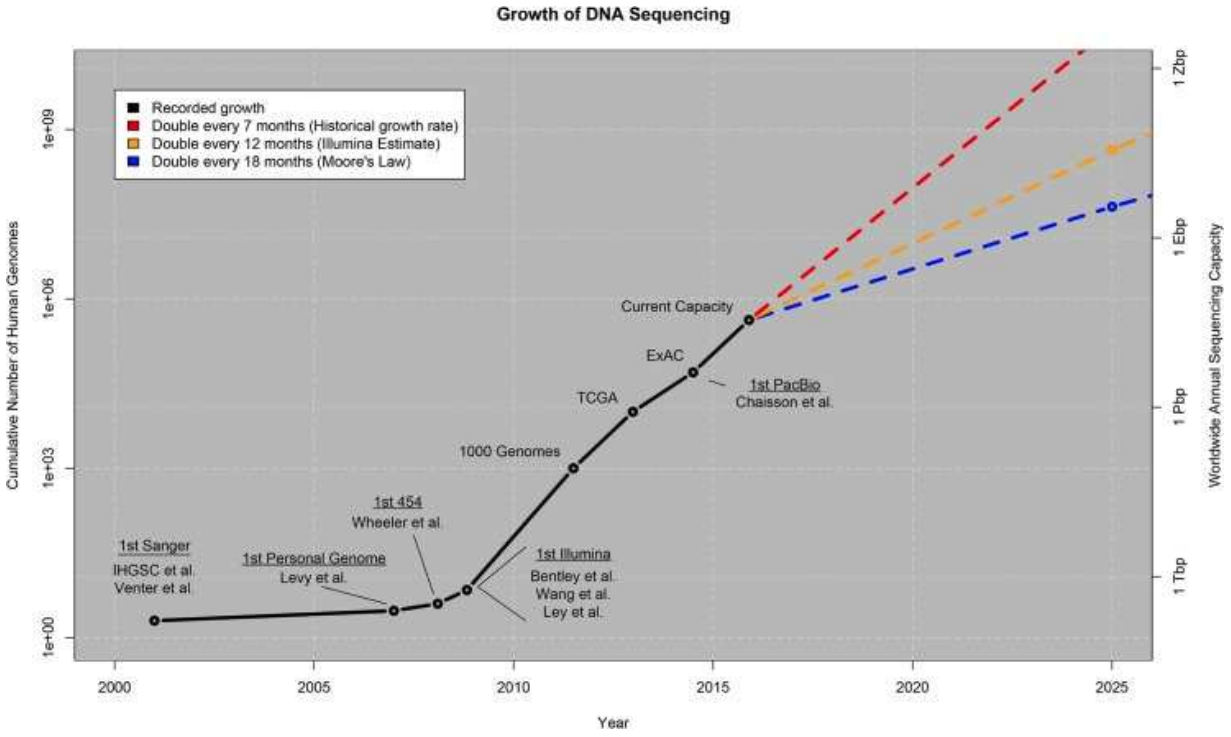
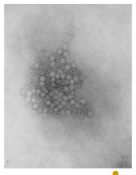
Abstract

Genomics is a Big Data science and is going to get much bigger, very soon, but it is not known whether the needs of genomics will exceed other Big Data domains. Projecting to the year 2025, we compared genomics with three other major generators of Big Data: astronomy, YouTube, and Twitter. Our estimates show that genomics is a "four-headed



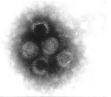


viromica

selezione genetica

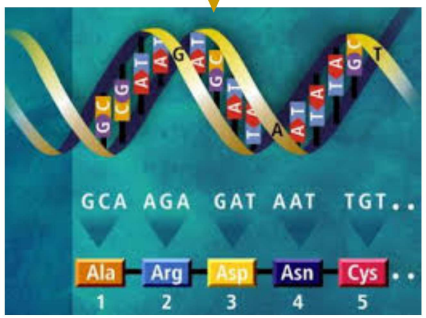
NGS

diagnostica



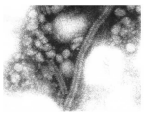
metagenomica

genomica



sicurezza alimentare

igiene allevamenti



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Topics: Gene Sequencing

Roche to close 454 Life Sciences as it reduces gene sequencing focus

October 17, 2013 | By Mark Hollmer

SHARE

Email

Roche ($RHHBY) is taking another step toward reducing its focus on gene sequencing by shutting down 454 Life Sciences, a subsidiary in the space it acquired from Curagen more than 6 years ago for $155 million.

Bio-IT World reported that the move reflects Roche's plan to deemphasize gene sequencing somewhat after failed attempts to invest more in the sector. Last year, for example, Roche made a $6.7 billion hostile takeover bid for gene sequencer Illumina ($ILMN), and the effort ultimately failed.

Roche's purchase of 454 was intended to give it a lead in gene-sequencing technology. And as the article noted, in the beginning 454 was a leader, and became the first to make a next-generation sequencer commercially available several years ago. Roche coveted the company and maintained an exclusive distribution deal with 454 from 2005 until 2007 when it bought 454 outright. But over time, other rivals surged past 454, as companies such as Illumina and Ion Torrent gained an edge with their next-generation sequencing projects, the story pointed out.

TOOLS

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ThermoFisher
SCIENTIFIC
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Life Sciences Solutions
200 Oyster Point Blvd
South San Francisco, CA 94080 USA
www.thermofisher.com

November 9, 2015

Re: SOLiD EZ Bead and 5500 Platforms

Dear Valued Customer,

After careful consideration, we have decided to discontinue the manufacture and sale of our Applied Biosystems ™ 5500 Series Genetic Analyzers as of May 1, 2016. Thereafter, the products listed below will no longer be available for sale.

SKU	Description
4452865	5500 SOLID SEQUENCER
4460730	5500XL SOLID SEQUENCER
4460732	5500XL SOLID SYSTEM
4473730	5500XL W GENETIC ANALYSIS SYSTEM
4481479	Upgrade, 5500XL-W
4460728	5500 SOLID SYSTEM

Thermo Fisher Scientific plans to continue to provide support for SOLiD™ EZ Bead™, 5500, 5500XL, 5500-W, and 5500XL-W users with related consumables, service contracts, technical and applications support until **December 31, 2017**. However, please note that our ability to provide support may be limited by circumstances beyond our reasonable control, such as

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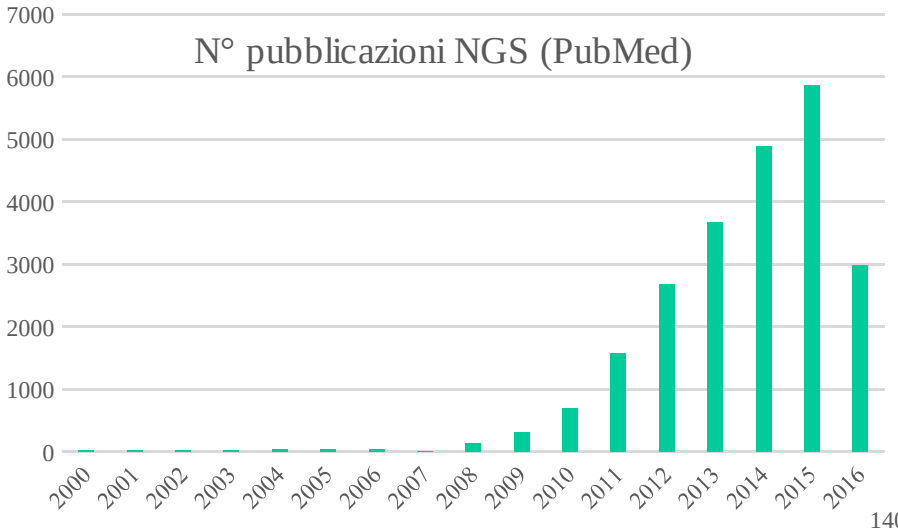

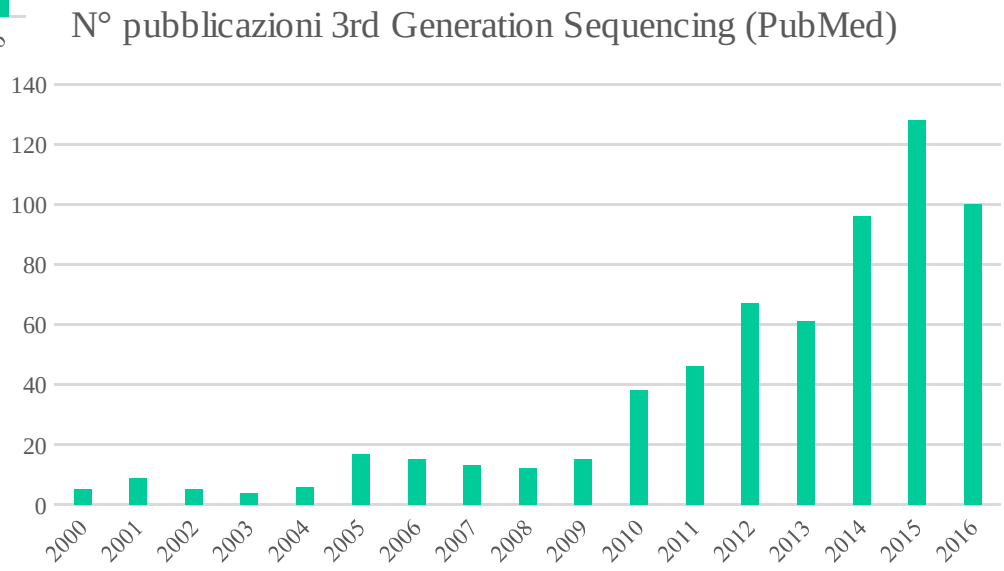

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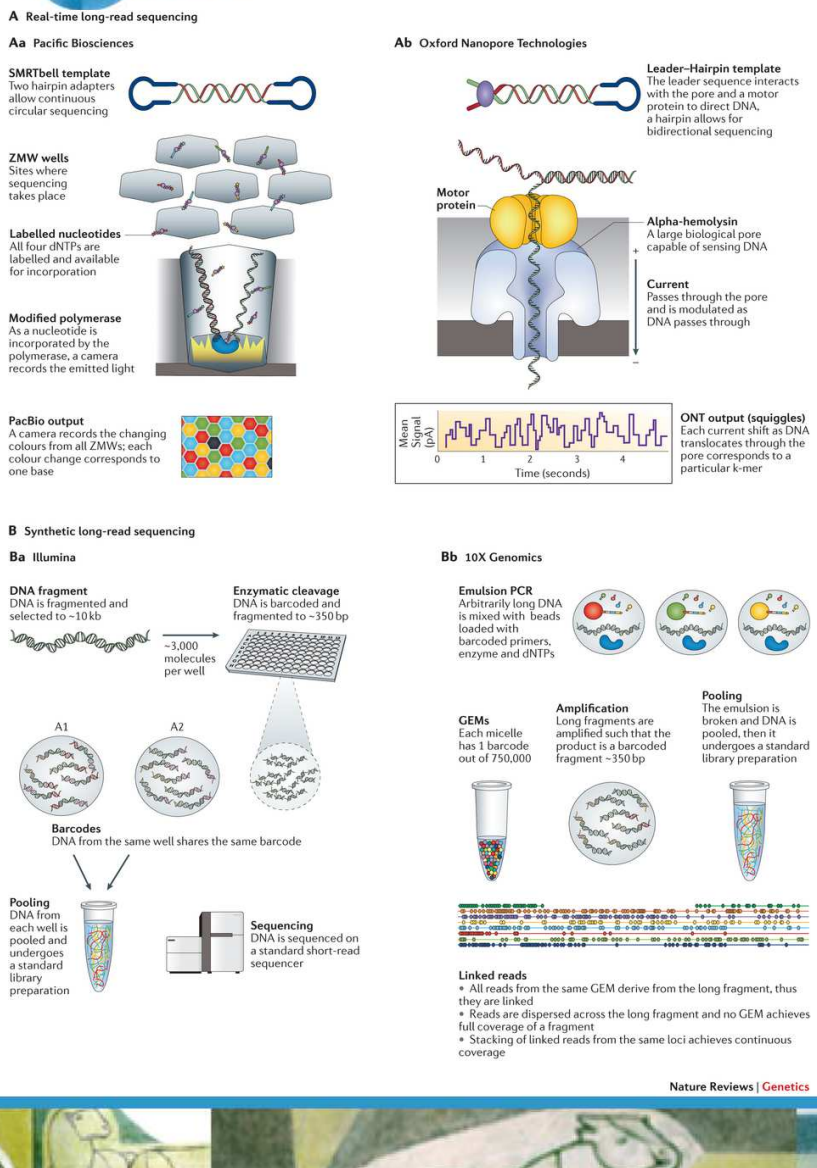

Nature Reviews | Genetics

Systematics and Biodiversity
Volume 14, Issue 1, 2016

Perspective

Third generation sequencing: technology and its potential impact on evolutionary biodiversity research

DOI: 10.1080/14772000.2015.1099575
Christoph Bleidorn[ab]*
pages 1-8

Received: 28 Apr 2015
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Abstract

Next generation sequencing transformed the field of evolutionary biology and high throughput sequencing platforms are routinely used in phylogenomic, population genomic or metagenomic studies. Here I review the recent technical advancements of third generation sequencing instruments, thereby covering nanopore sequencing and single molecule real-time (SMRT) sequencing. The output and error rates are compared with sequencing platforms of the second generation (454 pyrosequencing, Illumina and Ion Torrent). Third generation sequencers produce sequence reads in hitherto unprecedented lengths and will help to strongly increase the quality of genome assemblies. Moreover, the speed of sequencing and ease of sample preparation enables sequencing in the field. Even though the output and error rate of the new generation of sequencer remains to be improved, new possibilities for evolutionary research will open up in the near future by these new techniques.