

Il sequenziamento del DNA: principi e applicazioni presenti e future

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BIOTECNOLOGIE NELLA DIAGNOSTICA VETERINARIA E NEL CONTROLLO DEGLI ALIMENTI

14, 15 Dicembre 2011

Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana,
Sezione di Firenze, S. Martino alla Palma, Via Castelpulci 43, Scandicci (FI)



FINALITA' DELL'INTERVENTO (E DEL CORSO):

fornire ai partecipanti un aggiornamento sulle applicazioni biotecnologiche sviluppate negli ultimi anni presso i laboratori dell'Istituto zooprofilattico Lazio e Toscana negli ambiti della diagnostica veterinaria e del controllo degli alimenti.



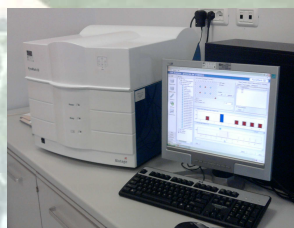
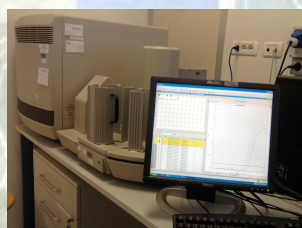
La disponibilità

- di amplificatori e sequenziatori di DNA,
- del *WEB*
- di software e di applicazioni “on line” di bioinformatica,

ha contribuito in modo fondamentale allo sviluppo di nuovi approcci nell'indagine laboratoristica, soprattutto in ambito genetico.

Laboratorio “Analisi Biomolecolare e Genetica”

- un sequenziatore di DNA, monocapillare
- un pirosequenziatore di DNA
- un amplificatore per PCR *Real Time*
- tre *liquid handlers* (stazioni robotiche) e, in fase di acquisizione
- un sequenziatore di DNA, 8 capillari



Nello scegliere lo strumento più idoneo per una determinata caratterizzazione genetica, occorre considerare che:

-Amplificatore per PCR *Real Time*: fornisce informazioni in tempi rapidi

-Sequenziatore (capillare) DNA: fornisce informazioni “assolute” (la sequenza del DNA) ma in tempi più lunghi

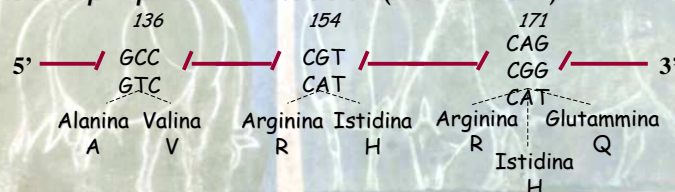
-Pirosequenziatore: fornisce informazioni con qualità e tempi intermedi rispetto alle due precedenti tecnologie

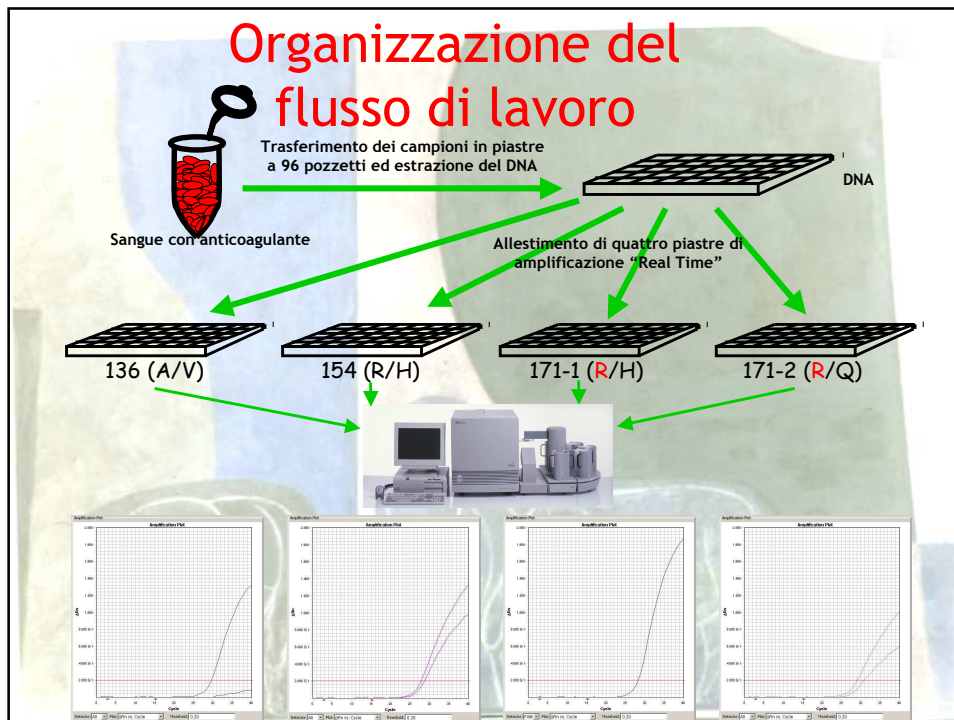
Laboratorio “Analisi Biomolecolare e Genetica”

Esempio 1: attività di caratterizzazione genetica mediante PCR *Real Time*

- Dall'Ottobre 2003 è attivo un piano comunitario per la selezione genetica degli ovini per i fattori di resistenza alle Encefalopatie Spongiformi Trasmissibili (EST).

Gene *prnp*: 771nucleotidi (257 codoni)





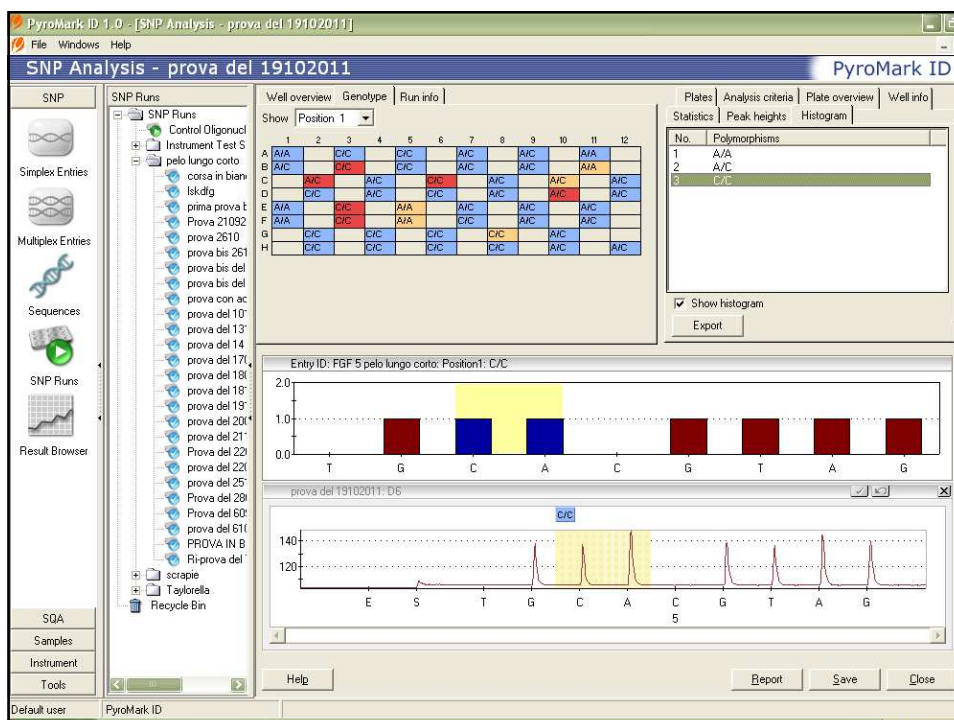
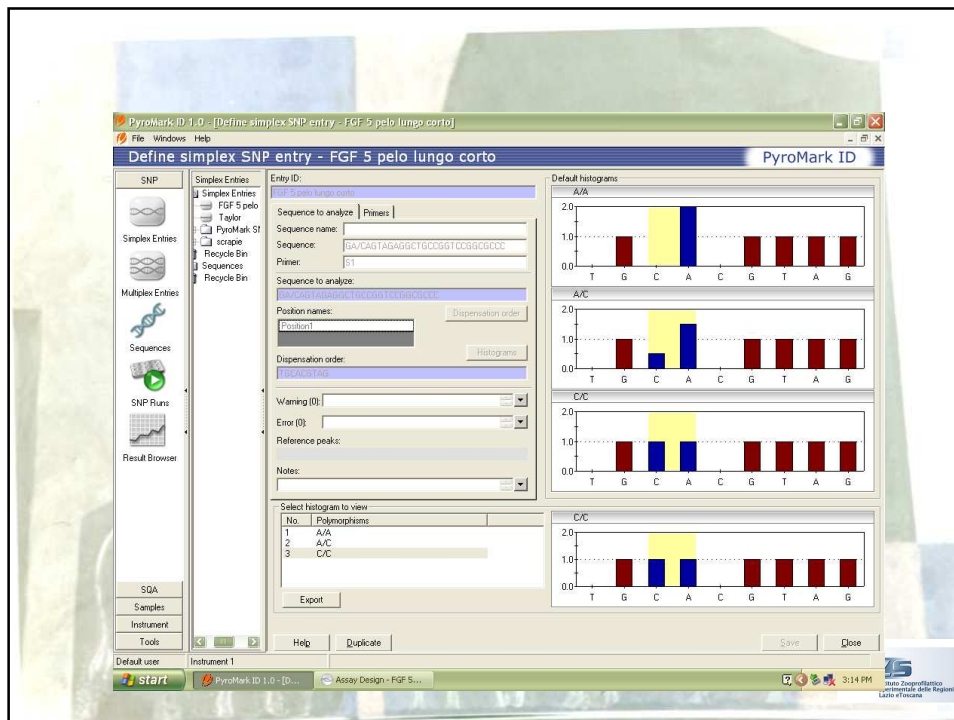
Laboratorio “Analisi Biomolecolare e Genetica”

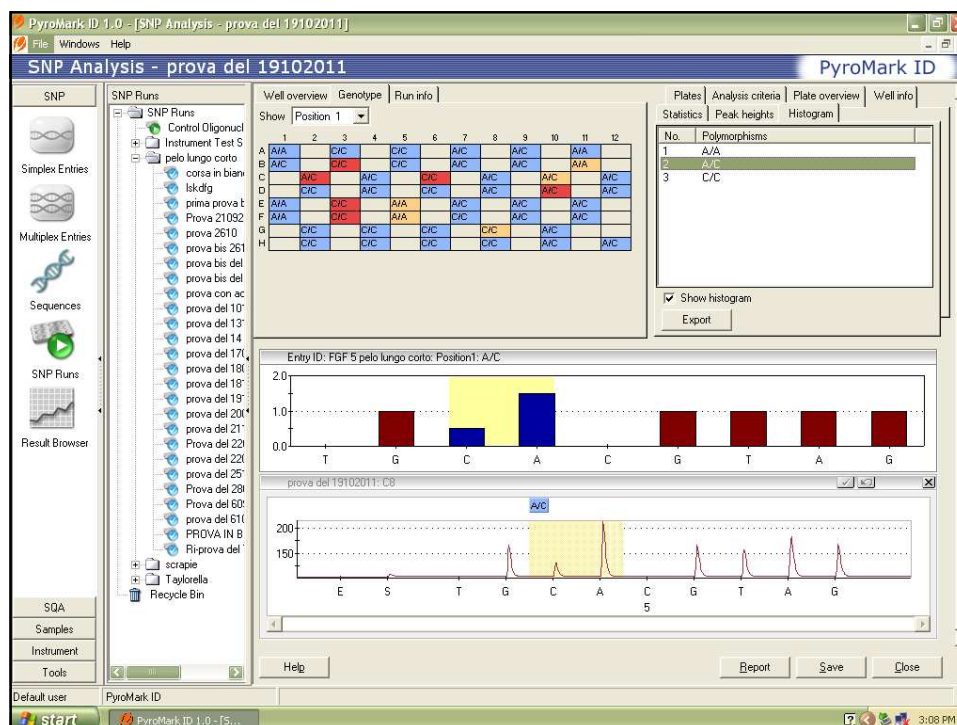
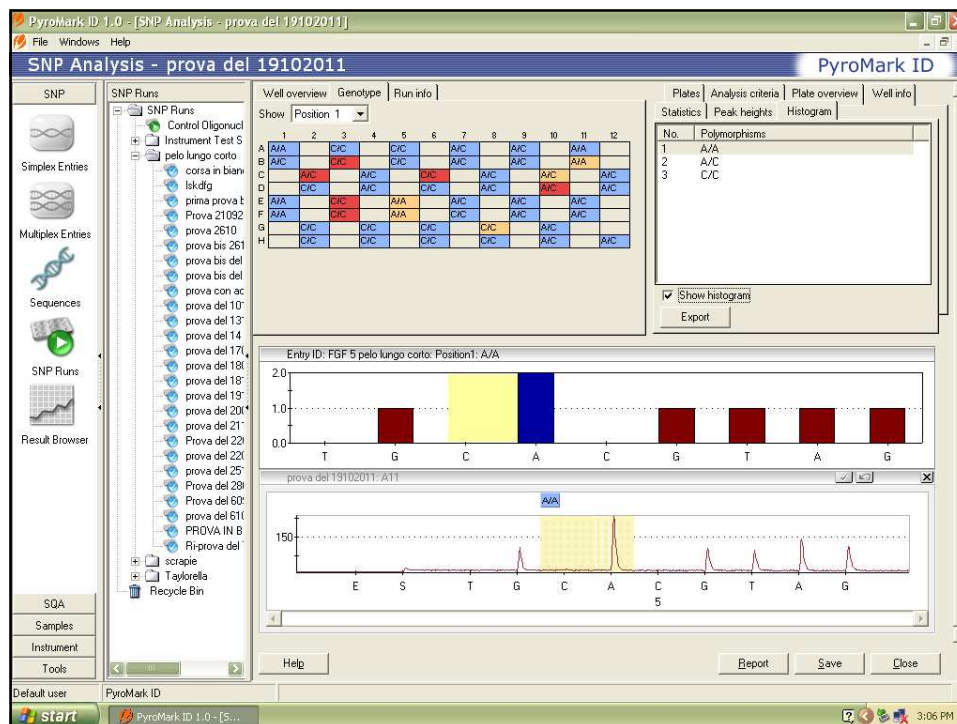
Esempio 2:

attività di caratterizzazione genetica mediante pirosequenziamento

-Nel cane, il fenotipo “pelo lungo” è associato ad una mutazione autosomica recessiva*. Ci è stato chiesto di sviluppare un saggio in grado di evidenziare la presenza di tale mutazione.

**"The long and the short of it: evidence that FGF5 is a major determinant of canine 'hair'-itability" Housley D.J; Venta P.J; Animal genetics 2006 Aug;37(4):309-15





Laboratorio “Analisi Biomolecolare e Genetica”

Esempio 3: attività di caratterizzazione genetica mediante sequenziamento “classico”.

- La “citocromo c-ossidasi subunità I” (COI) è il *marker* genetico d'elezione per l'identificazione biomolecolare di specie.

The screenshot shows the FDA website page for the 'Single Laboratory Validated Method for DNA-Barcoding for the Species Identification of Fish for FDA Regulatory Compliance'. The page is dated September 2011. It includes an index with links to Introduction, SOP Section, SOP in PDF format, and FDA Reference Standard Sequence Library for Seafood Identification. The authors listed are Sara M. Handy and Jonathan R. Deeds, Natalia V. Ivanova and Paul D.N. Hebert, Robert Hanner, Andrea Ormos and Lee A. Weigt, Michelle M. Moore, Rosalee S. Helberg, and Halle F. Yancy. The abstract states: 'A detailed single laboratory validated protocol is provided for DNA sequencing of the cytochrome c oxidase subunit I gene (COI) of seafood tissue samples for the purpose of obtaining unique, species-specific barcodes for species identification. These procedures include tissue sampling, tissue lysis and DNA extraction, Polymerase Chain Reaction (PCR) - COI amplification, PCR product cleanup, PCR product cleanup, Cycle Sequencing Reaction, Sequencing Reaction Cleanup, and Post Sequencing Analysis.' The introduction mentions that seafood is one of the most highly traded commodities in the world and that it is vital to both domestically processed and imported seafood is safe, wholesome, and properly labeled.

BOLD Systems - Mozilla Firefox

File Modifica Visualizza Cronologia Segnalibri Yahoo! Strumenti ?

http://www.boldsystems.org/views/login.php#

Poste Italiane Inaz Communication S... IZSLT - SL RAGES WT2 Nide Nucleotide BLAST: Se... DP Bol Data Portal | Index BOLD Systems - Spec... Barcode of Life Initi... FISHBOL.org: Fish Bar...

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BOLD Systems

BARCODE OF LIFE DATA SYSTEMS v2.5

Advance species identification and discovery through the analysis of short, standardized gene regions

Published Projects | Taxonomy Browser | Request an Account | Identify Specimen | Documentation | Data Release | Citation

The Barcode of Life Data Systems (BOLD) is an online workbench that aids collection, management, analysis, and use of DNA barcodes. It consists of 5 components (MDS, DS, and ECS) that each address the needs of various groups in the barcoding community.

MANAGEMENT & ANALYSIS

BOLD-HAS provides a repository for barcode records coupled with analytical tools. It serves as an online workbench for the DNA barcode community.

Username Password Login

Request a new user account Report your specimen or password

IDENTIFICATION ENGINE

BOLD-ID5 provides a species identification tool that accepts DNA sequences from the barcode region and returns a taxonomic assignment to the species level when possible.

EXTERNAL CONNECTIVITY

BOLD-ECS provides web developers and bioinformaticians the ability to build tools and workflows that can be integrated with the BOLD framework. BOLD-ECS supplies REST services that allows access to public sequence and specimen data. We welcome the addition of new analytical modules.

Ontario Genome Canada Science and Innovation Canada

Ratnasingham S, Hebert PDN (2007) BOLD : The Barcode of Life Data System Molecular Ecology Notes

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BARCODING RECORDS

Formally Described Species With Barcodes 79,151

Total Barcode Records 1,008,388

Source Breakdown

GenBank 108,944

Canadian Centre 829,702

Others 67,454

BOLDSYSTEMS

BOLD 2.5 Release

Version 2.5, unveiled on Nov 11th 2009 at the Third International Barcoding of Life conference in Mexico City, provides new core functionality including support for multiple sequence markers per specimen and more complex workflow. Features include identification services for ITS, mark, and rbcL markers, comparative analysis, web services and a variety of convenience upgrades. A few are highlighted here:

Accumulation curves

Explore diversity of species and sequences by site or higher level taxonomy.

Multi-marker analysis

All analytical tools have been upgraded to support processing and visualization of all registered markers.

Alignment browser

Quickly identify alignment errors and evaluate substitutions through the alignment browser which support visualization of amino translations of coding sequences.

Web Services

A two phase data retrieval service based on Representational State Transfer (REST) is available at services.boldsystems.org to access and retrieve published data on BOLD in text, XML and JSON formats.

BARCODING CAMPAIGNS

BARCODING WEBSITES

Share With Others On: Facebook Twitter YouTube

Copyright 2010 - Biodiversity Institute of Ontario

BOLD Systems - Identification Result - Mozilla Firefox

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http://www.boldsystems.org/views/identifyresult.php?display=99

WorldBTF Inaz Communication S... RAGES WT2 Acced a InfoView IZSLT - SL Nide Nucleotide BLAST: Se... Network Italiano per F... BOLD Systems - Spec... Barcode of Life Initi... DP Bol Data Portal | Index

SEARCH

BOLDSYSTEMS v2.5 | Management & Analysis

Specimen Identification Request

Search Request:

Type: COI SPECIES DATABASE

Search Result:

Identification Summary:

Taxonomic Level	Taxon Assignment	Probability of Placement (%)
phylum	Arthropoda	100
class	Insecta	100
order	Coleoptera	100
family	Nitidulidae	100
genus	Aethina	100
species	Aethina tumida murray	100

A species level match could not be made, the queried specimen is likely to be one of the following:

-Aethina tumida murray

-Aethina tumida

Tree Based Identification

Distance Summary:

Similarity scores of the top 100 matches

TOP 99 Matches:

Phylum	Class	Order	Family	Genus	Species	Specimen Similarity (%)
Arthropoda	Insecta	Coleoptera	Nitidulidae	Aethina	tumidamurray	100
Arthropoda	Insecta	Coleoptera	Nitidulidae	Aethina	tumida	99.24
Arthropoda	Insecta	Coleoptera	Nitidulidae	Aethina	tumida	99.24
Arthropoda	Insecta	Hymenoptera	Fomicidae	Camponotus	MG062	91.11
Arthropoda	Insecta	Hymenoptera	Fomicidae	Camponotus	MG047	90.91
Arthropoda	Insecta	Hymenoptera	Fomicidae	Camponotus	DRQ03	90.91
Arthropoda	Insecta	Lepidoptera	Saturidae	Hylesia	indurata	90.48
Arthropoda	Insecta	Lepidoptera	Praydidae	Prays	nephelomima	90.48
Arthropoda	Insecta	Lepidoptera	Depressariidae	Pentomonta	sp.AN1C4	90.48
Arthropoda	Insecta	Lepidoptera	Lymantriidae	Lymantria	monacha	89.74

Display option: default

Trava: Successivo Precedente Entologia Malscope/minusculo

Alcune identificazioni condotte presso l'IZSLT



EUROPA - Food Safety - Rapid Alert System for Food and Feed (RASFF) - Introduction - Mozilla Firefox

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http://ec.europa.eu/food/rapidalert/index_en.htm

Poste Italiane Inno: Communication S... IZSLT - STL RAGES WT2 Nide Nucleotide BLAST: Se... DP Bol Data Portal | Index BOLD Systems - Spec... Barcode of Life Initiati... FISHBOL.org: Fish Bar...

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cycle sequencing - Cerca con Google EUROPA - Food Safety - Rapid Ale... RASFF-portal RASFF-portal detail

Rapid Alert System for Food and Feed

What's New?

RASFF annual report 2009 pdf

Press release: **RASFF - food safety remains a priority in the EU and beyond**

Frequently Asked Questions: **Rapid Alert System for Food and Feed: role and achievements**

1979-2009 RASFF celebrates its 30th anniversary.

Click **here** to read more about this event.

Press release: **The Rapid Alert System for Food and Feed more efficient than ever on its 30th birthday**

RASFF Portal database

On 16 July 2009, Commissioner for Health **Androulla Vassiliou** opened the RASFF Portal website and its new online searchable database of RASFF notifications. This database replaces the weekly summary of notifications published on this web site. Users of the **RASFF Portal database** can now produce their own tables based on a multitude of criteria.

Welcome to the RASFF portal!

The Rapid Alert System for Food and Feed (RASFF) was put in place to provide food and feed control authorities with an effective tool to exchange information about measures taken responding to serious risks detected in relation to food or feed. This exchange of information helps Member States to act more rapidly and in a coordinated manner in response to a health threat caused by food or feed.

Read more about the legal basis of RASFF

Who are the members of RASFF?

RASFF - Keeping an eye on your food - **Watch the video**

International RASFF conference - 10 conclusions of Robert Madelin

Resources

- Press Releases
- Health & Consumer
- Voice Newsletter
- Publications

International Affairs

- Organisations
- Codex
- OIE
- WTO
- Import Conditions
- Pets and Animal Welfare
- Enlargement
- Agreements
- EU - Russia: SPS Issues

FOOD AND FEED SAFETY

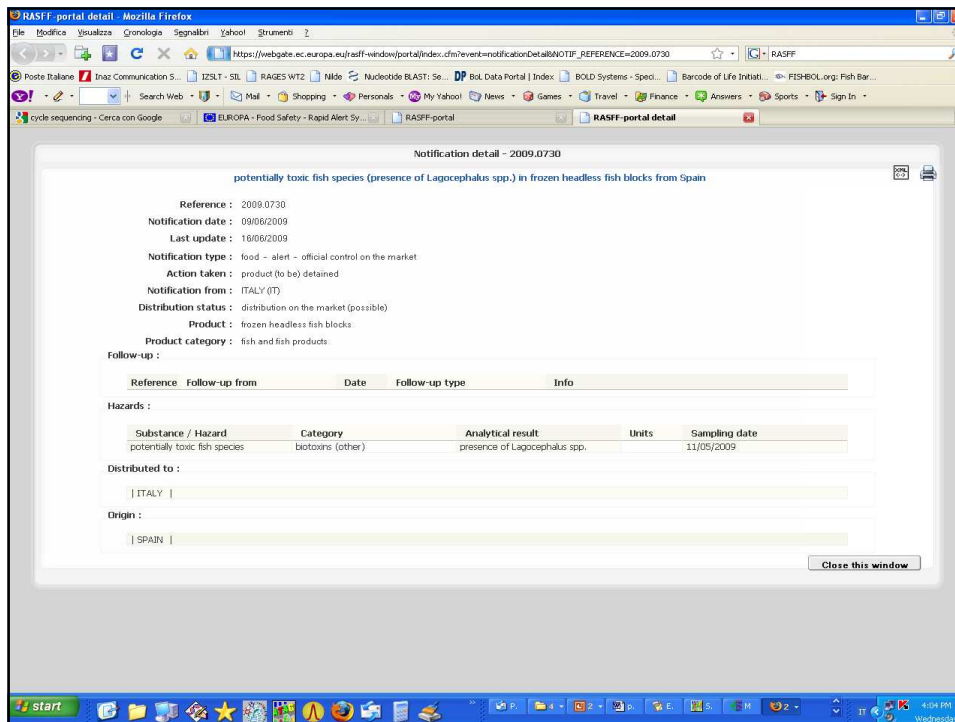
ANIMAL HEALTH AND WELFARE

PLANT HEALTH

FVO INSPECTIONS

start

4:06 PM Wednesday



PUNTI SALIENTI NELLO SVILUPPO DELLE TECNICHE DI 1ª GENERAZIONE PER IL SEQUenziAMENTO DEL DNA

1977: pubblicazione (PNAS: *Proceedings of the National Academy of Sciences*) di due metodi (“terminazione della catena” e “chimico”) per il sequenziamento del DNA

1986: commercializzazione del primo sequenziatore automatico, nella interpretazione dei dati di sequenza, di DNA

1995: commercializzazione del primo sequenziatore automatico, nel caricamento dei campioni e nell’interpretazione dei dati di sequenza, di DNA

-Lo sviluppo dei sequenziatori capillari ha probabilmente raggiunto il culmine.

-Un sequenziatore a 96 capillari è oggi in grado di produrre circa 60.000 basi/ora, al costo di 3 euro/1000 basi.

-Con un sequenziatore 96 capillari occorrerebbero, quindi, circa 6 anni di lavoro (24 ore al giorno) e circa 10 milioni di euro per il sequenziamento di un genoma umano (~3,5Gb)!



THE WALL STREET JOURNAL
Digital Network

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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

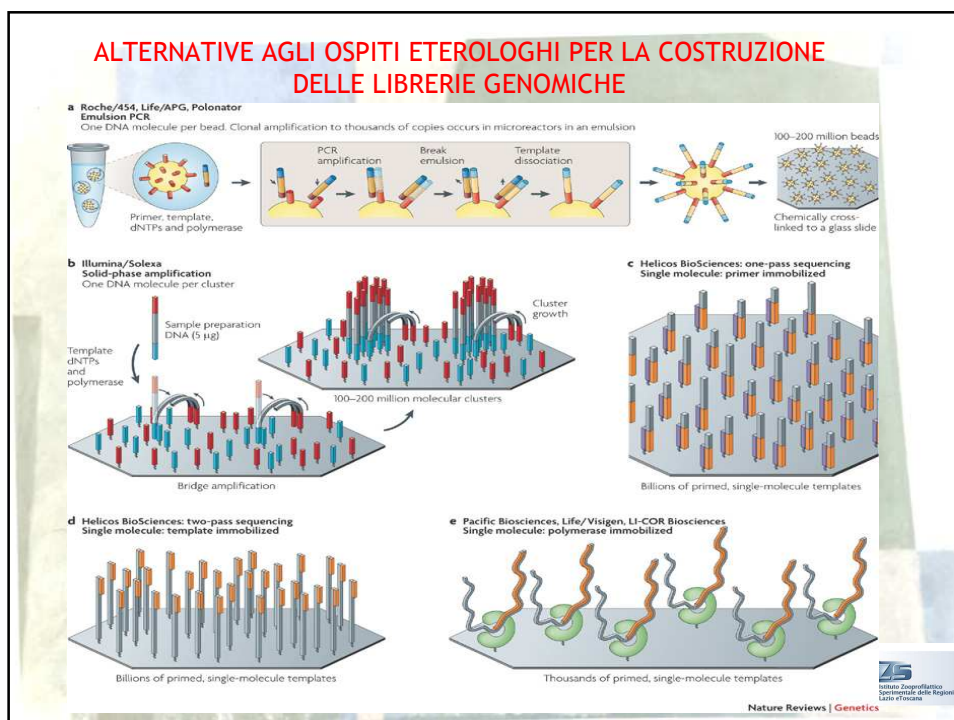
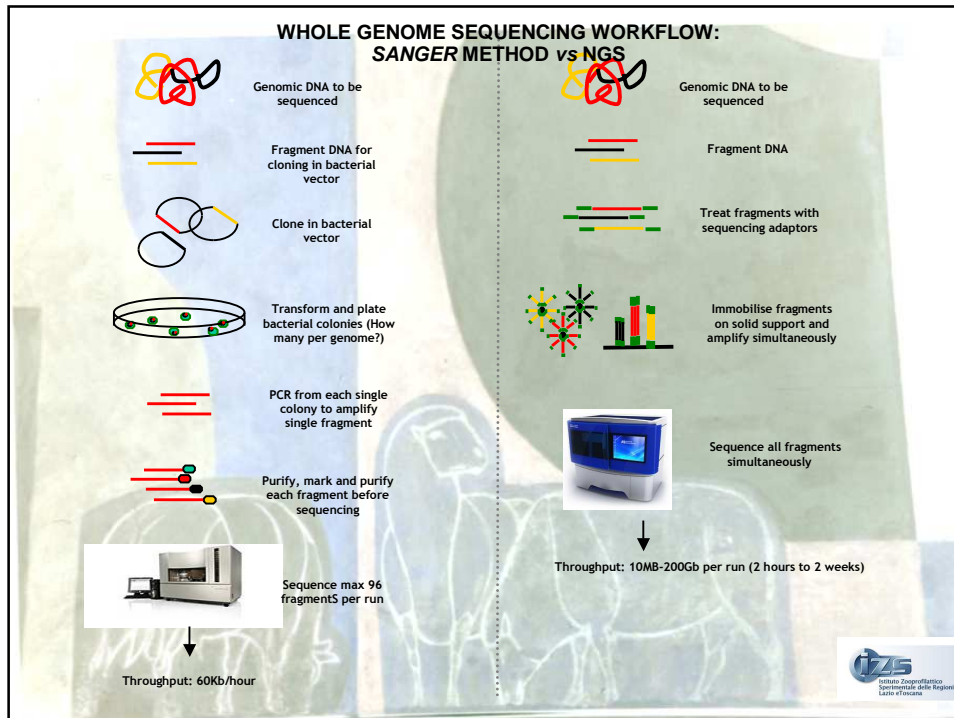
Next Generation Sequencing (NGS)

Le piattaforme NGS consentono di produrre e gestire, in parallelo, milioni di sequenze nucleotidiche.

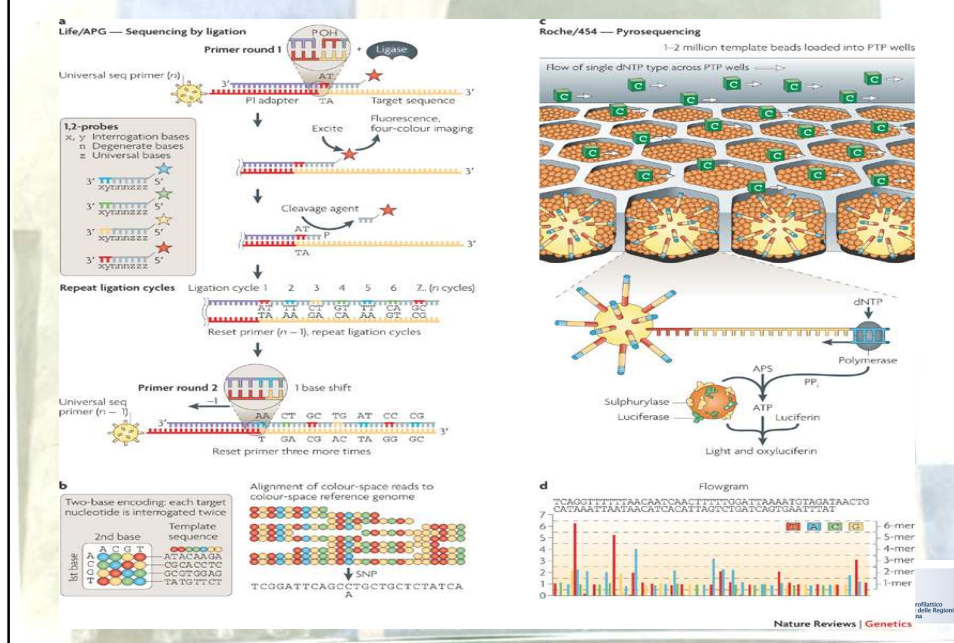
Il loro avvento è stato possibile grazie al superamento:

- 1) degli ospiti eterologhi per la costruzione delle librerie genomiche dei frammenti da sequenziare
- 2) della “chimica di *Sanger*”

- | | |
|------|--|
| 2005 | - Roche 454 (basata su: emPCR e pirosequenziamento) |
| | - Illumina (basata su: bridgePCR e sequenziamento “simil- <i>Sanger</i> ”) |
| | - SOLiD (basata su: emPCR e ibridazione/ligazione) |
| | - PacBio (basata su: rilevamento della SINGOLA MOLECOLA di DNA) |
| 2011 | - IonTorrent (basata su: emPCR e rilevamento variazionepH) |



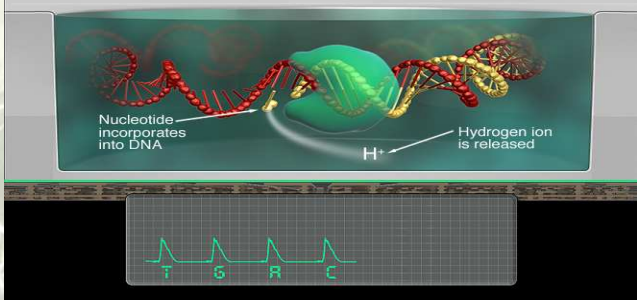
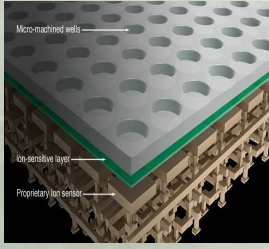
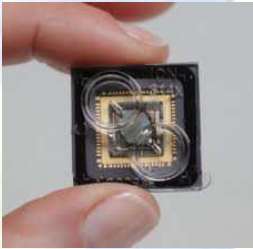
ALTERNATIVE ALLA CHIMICA DI SANGER



Instrument	Purchase Cost	Run time	Millions of Reads/r un	Bases / read	Yield Mb/run	Reagent Cost/Mb	Costo (USD) per 3,5 Gb
3730xl (capillary)	\$376	2 hrs.	0.000096	650	0.06	\$1500	5,25x10 ⁶
Ion Torrent – '314' chip	\$49.5	2 hrs.	0.10	100	>10	<\$50	1,7x10 ⁵
454 GS Jr. Titanium	\$108	10 hrs.	0.10	400	50	\$22	77x10 ³
PacBio RS	\$695	0.5 – 2 hrs.	0.01	860 – 1,100	5-10	\$11-340	
454 FLX Titanium	\$500	10 hrs.	1	400	500	\$12.4	
454 FLX ⁺	\$29.5	18 – 20 hrs.	1	700	900	\$7	
Ion Torrent – '316' chip	\$49.5	2 hrs.	1	>100	>100	<\$7.5	
Ion Torrent – '318' chip	\$49.5	2 hrs.	4-8	>100	>1,000	~\$0.93	
Illumina MiSeq	\$125	26 hrs.	3.4	150+150	1020	\$0.74	
Illumina iScanSQ	\$405	8 days	250	100+100	50,000	\$0.20	
Illumina GAIIx	\$250	14 days	320	150+150	96,000	\$0.12	
SOLID – 4	\$475	12 days	>840	50+35	71,400	<\$0.11	
Illumina HiSeq 2000	\$690	8 days	1000	100+100	200,000	\$0.10	
SOLID – 5500	\$349	8 days	>700	75+35	77,000	<\$0.08	
SOLID – 5500xl	\$595	8 days	>1,410	75+35	155,100	<\$0.07	245



NGS and post light sequencing



Nucleotide incorporates into DNA

H⁺

Hydrogen ion is released

T G R C

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Sperimentale delle Regioni
Lazio e Toscana

Grazie per l'@ttenzione