

Seqüenciamento (continuação...)

Felipe R. da Silva

Embrapa Recursos Genéticos e Biotecnologia



Recursos Genéticos e Biotecnologia



A NATION FOR ALL
BRASIL GOV

'Novas metodologias promissoras'(2001)

- Seqüenciamento por hibridização
 - Khrapko *et al.* (1989). *FEBS Lett.* **256**: 118-122
[http://dx.doi.org/10.1016/0014-5793\(89\)81730-2](http://dx.doi.org/10.1016/0014-5793(89)81730-2)
- Seqüenciamento paralelo de assinaturas baseado em ligação e corte (MPSS)
 - Brenner *et al.* (2000). *Nature Biot.* **18**: 630 - 634
<http://dx.doi.org/10.1038/76469>
- Piroseqüenciamento
 - Ronaghi *et al.* (1996). *Anal. Biochem.* **242**: 84–89.
<http://dx.doi.org/10.1006/abio.1996.0432>

Genome Research **11**: 3–11 (2001). <http://www.genome.org/cgi/content/full/11/1/3>

Advanced Sequencing Technology Awards 2005 (NHGRI)

- Droplet-Based Digital Microfluidic Genome Sequencing
- Single-Molecule DNA Sequencing with Engineered Nanopores
- Electronic Sequencing in Nanopores
- Real-Time DNA Sequencing
- Massively Parallel Cloning and Sequencing of DNA
- Modulating Nucleotide Size in DNA for Detection by Nanopore
- Haplotype Sequencing via Single Molecule Hybridization
- Sequencing a DNA Molecule using a Synthetic Nanopore
- Real-time Multiplex Single-Molecule DNA Sequencing
- Bead-Based Polony Sequencing
- Ultra High Throughput DNA Sequencing System Based on Two-Dimensional Monolith Multi-Capillary Arrays and Nanoliter Reaction Volume
- \$100,000 Genome Using Integrated Microfluidic CE



Felipe R. da Silva

Biologia Computacional M06404
Unicamp, 1º Sem. 2010

Tecnologias de Seqüenciamento disponíveis comercialmente

1. illumina (Avantome, GAIx, HisSeq2000)
2. Roche (454 Genome Sequencer FLX, GS Junior)
3. Life Technologies (SOLID, Single Molecule Sequencing)
4. Helicos (True Single Molecule Sequencing)
5. Pacific Biosciences (Real Time Single Molecule Sequencing)
6. Ion Torrent (Post-Light Sequencing™ technology)
7. Oxford nanopore (Single Molecule Sequencing)
8. IBM nanopore sequencing (Single Molecule Sequencing)
9. Nabsys (nanopore + electronic sequencing)
10. ZS Genetics
11. Visigen
12. Halcyon Molecular
13. Complete Genomics (only human till now)
14. Mobius (Nexus I)
15. Polonator (MPS by ligation)
16. Cracker (SMRT on a chip)

<http://seqanswers.com/forums/showthread.php?t=4229>



Felipe R. da Silva

Biologia Computacional M06404
Unicamp, 1º Sem. 2010

Tecnologias de sequenciamento de "próxima" geração (NGS)

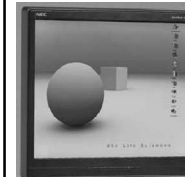
- 454 (Roche)
- SOLiD (Applied Biosystems)
- Solexa (Illumina)



Felipe R. da Silva

Biologia Computacional MO6404
Unicamp, 1º Sem. 2010

Genome Sequencer 20 System



Roche Applied Science
454 Life Sciences

Genome sequencing in microfabricated high-density picolitre reactors.
Nature 437, 376-380
(15 September 2005)



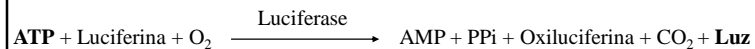
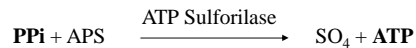
The apparatus (...) is able to sequence 25 million bases, at 99% or better accuracy, in one 4-hour run. (...) shotgun sequencing and *de novo* assembly of the *Mycoplasma genitalium* [580 Mb] genome with 96% coverage at 99.96% accuracy in one run of the machine.



Felipe R. da Silva

<http://dx.doi.org/10.1038/nature03959>

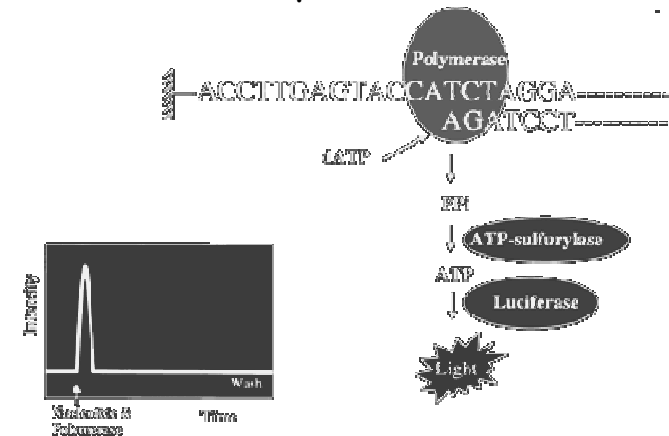
Pirossequenciamento



Felipe R. da Silva

Biologia Computacional MO6404
Unicamp, 1º Sem. 2010

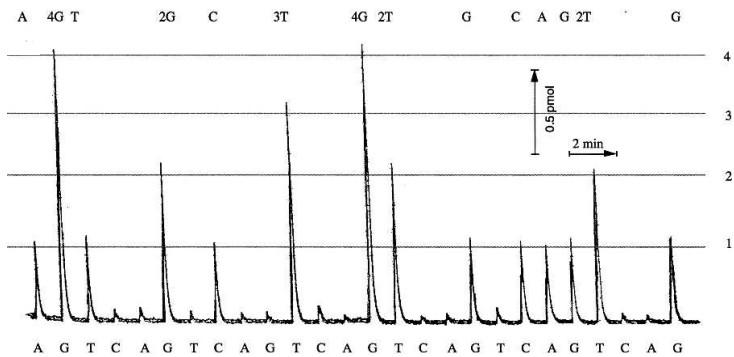
Pirossequenciamento



Felipe R. da Silva

Biologia Computacional MO6404
Unicamp, 1º Sem. 2010

Pirossequenciamento

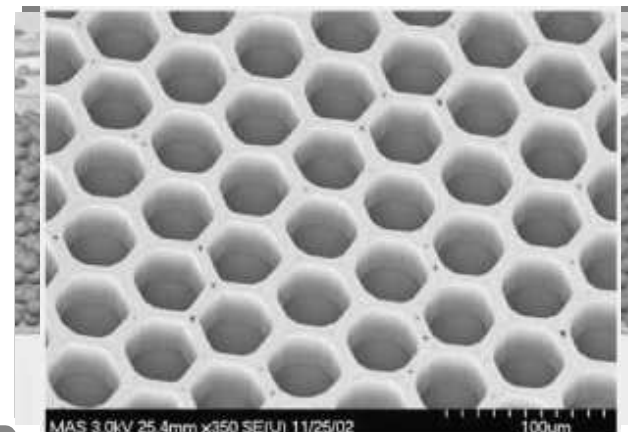


Embrapa

Felipe R. da Silva

Biologia Computacional MO640A
Unicamp, 1º Sem. 2010

Pirossequenciamento Genome Sequencer 20 System

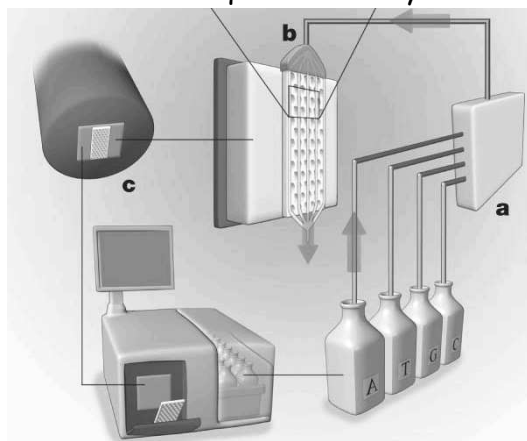


Embrapa

Felipe R. da Silva

Biologia Computacional MO640A
Unicamp, 1º Sem. 2010

Pirossequenciamento Genome Sequencer 20 System

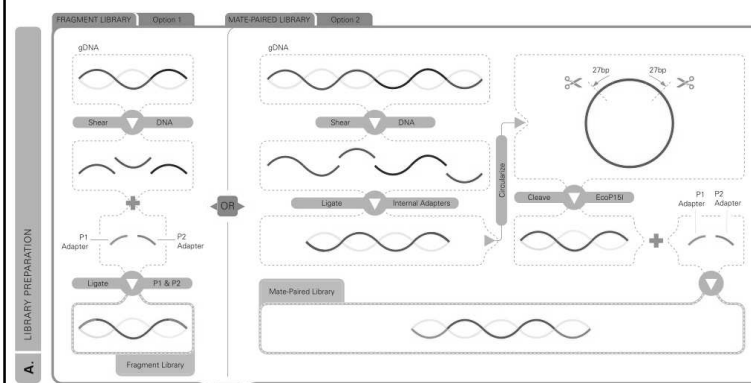


Embrapa

Felipe R. da Silva

Biologia Computacional MO640A
Unicamp, 1º Sem. 2010

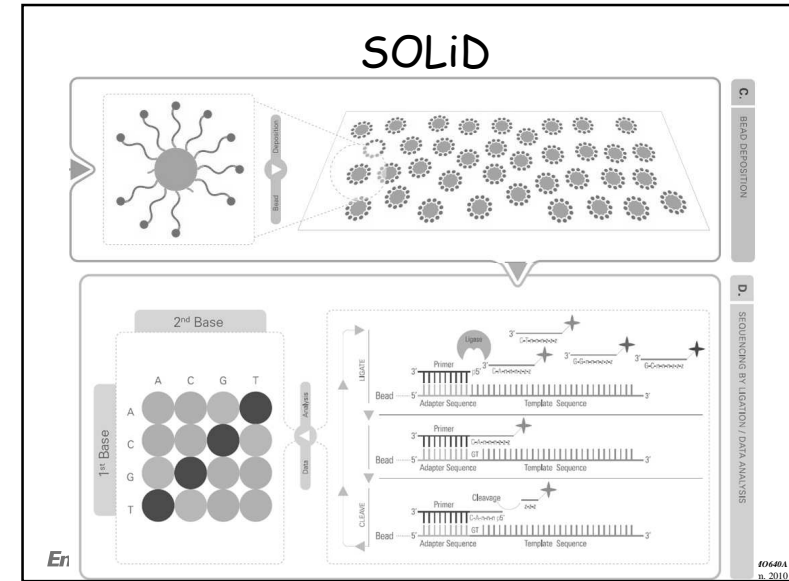
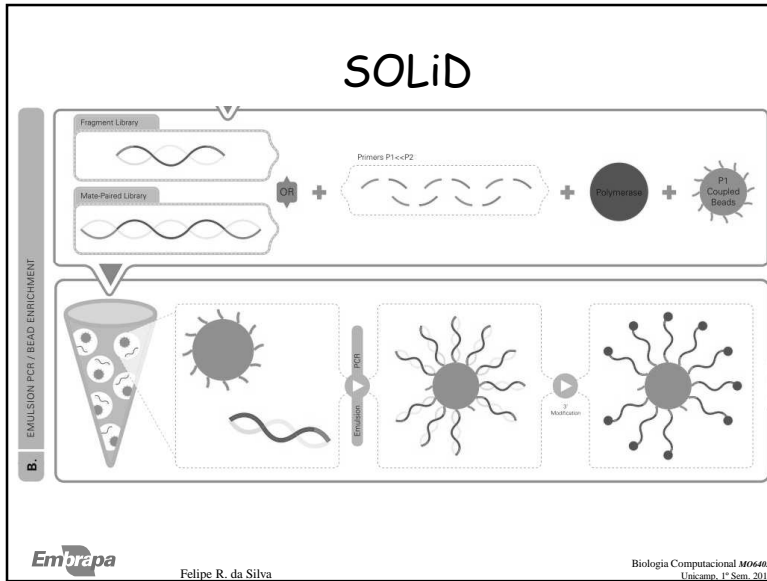
SOLiD



Embrapa

Felipe R. da Silva

Biologia Computacional MO640A
Unicamp, 1º Sem. 2010



Oligo

- 2 primeiros nucleotideos
 - dinucleotideo específico
- nucleotídeos 3-5
 - degenerados
- nucleotídeos 6-8 podem ser inosina.

CGNNNI I I I

A.

Guanine (G)

B.

Cytosine (C)

C.

Adenine (A)

D.

Thymine (T)

140A
Unicamp, 1º Sem. 2010

Embrapa Felipe R. da Silva

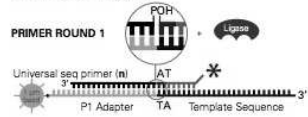
Procedimento

1. Anela *primer* e liga óligo
2. Trata as extremidades
3. Captura a fluorescência
4. Remove o fluoróforo
 - Deixa um pentanucleotideo ligado
5. Repete o procedimento (5-7x)
6. Denatura
7. Utiliza um novo primer
 - (comprimento n-1)

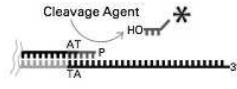
Embrapa Felipe R. da Silva Biologia Computacional MO640A
Unicamp, 1º Sem. 2010

SOLiD

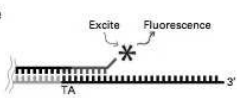
1. Prime and Ligate



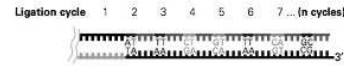
4. Cleave off Fluor



2. Image



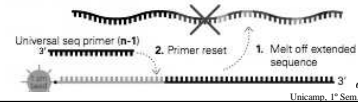
5. Repeat steps 1-4 to Extend Sequence



3. Cap Unextended Strands



6. Primer Reset

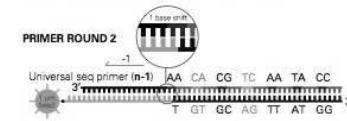


Felipe R. da Silva

06404
Unicamp, 1º Sem. 2010

SOLiD

7. Repeat steps 1-5 with new primer



8. Repeat Reset with n-2, n-3, n-4 primers

Primer Round	Read Position	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35		
1	Universal seq primer (n)																																						
2	Universal seq primer (n-1)																																						
3	Universal seq primer (n-2)																																						
4	Universal seq primer (n-3)																																						
5	Universal seq primer (n-4)																																						

• Indicates positions of interrogation Ligation Cycle

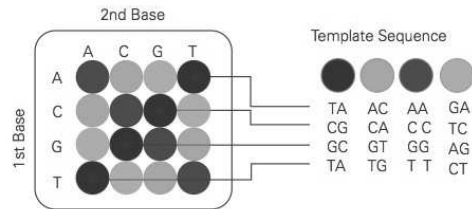
Embrapa

Felipe R. da Silva

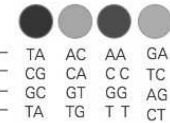
Biologia Computacional MO6404
Unicamp, 1º Sem. 2010

SOLiD

Possible Dinucleotides Encoded By Each Color

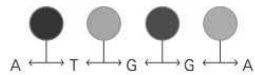


Template Sequence



Double Interrogation

With 2 base encoding each base is defined twice



Embrapa

Felipe R. da Silva

Biologia Computacional MO6404
Unicamp, 1º Sem. 2010

SOLiD

Single SNP Sites



SNP site indicated by 2 adjacent color changes



Single color change is typically a measurement error



1 Base Deletion



Insertion

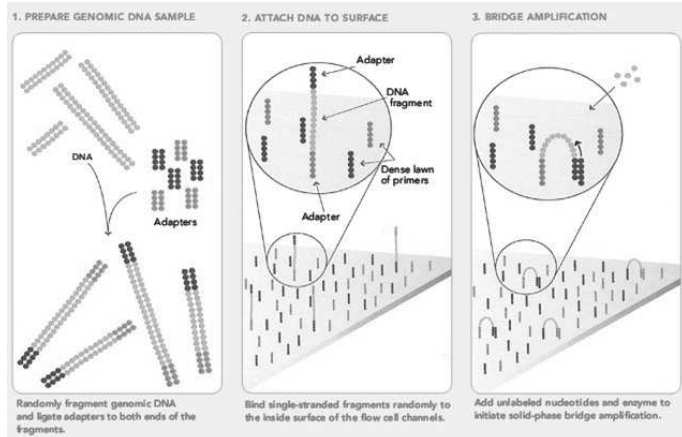


Embrapa

Felipe R. da Silva

Biologia Computacional MO6404
Unicamp, 1º Sem. 2010

Solexa (Illumina)

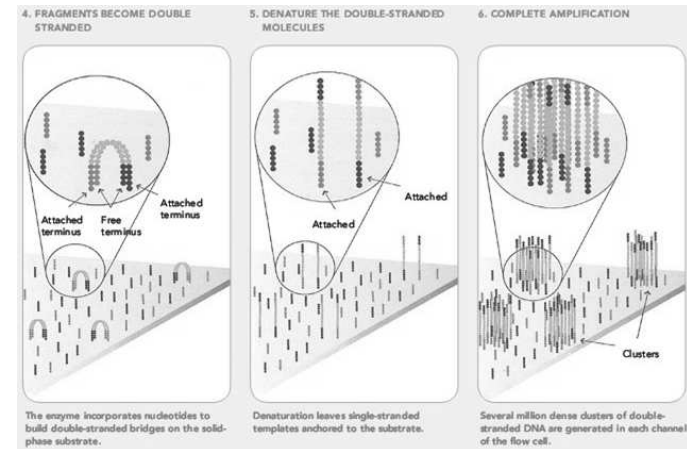


Embrapa

Felipe R. da Silva

Biologia Computacional MO640A
Unicamp, 1º Sem. 2010

Solexa (Illumina)

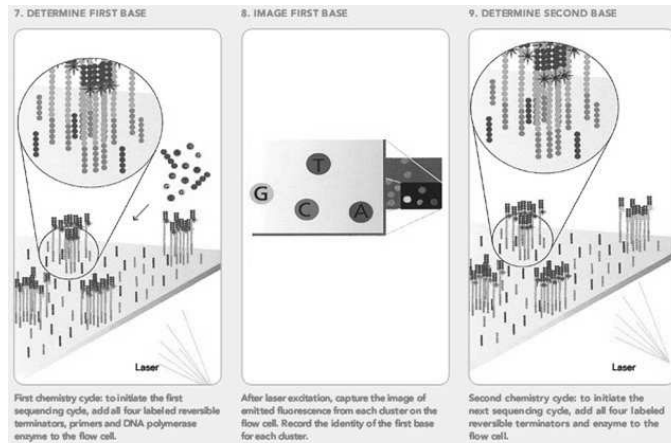


Embrapa

Felipe R. da Silva

Biologia Computacional MO640A
Unicamp, 1º Sem. 2010

Solexa (Illumina)

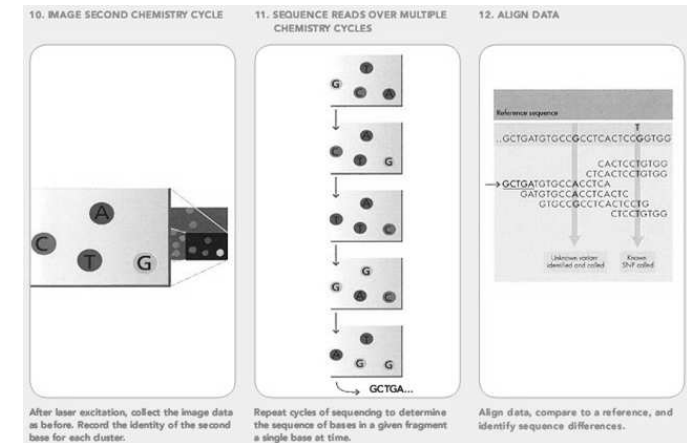


Embrapa

Felipe R. da Silva

Biologia Computacional MO640A
Unicamp, 1º Sem. 2010

Solexa (Illumina)



Embrapa

Felipe R. da Silva

Biologia Computacional MO640A
Unicamp, 1º Sem. 2010