

Seqüenciamento (continuação...)

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Embrapa
Recursos Genéticos e Biotecnologia

BRASIL
A NATION FOR ALL
GOVERNO FEDERAL

'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

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Tecnologias de Seqüenciamento disponíveis comercialmente

1. [illumina](#) (Avantome, GAIIx, 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. [Mobiob](#) (Nexus I)
15. [Polonator](#) (MPS by ligation)
16. [Cracker](#) (SMRT on a chip)

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

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Tecnologias de sequenciamento de "próxima" geração (NGS)

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

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)

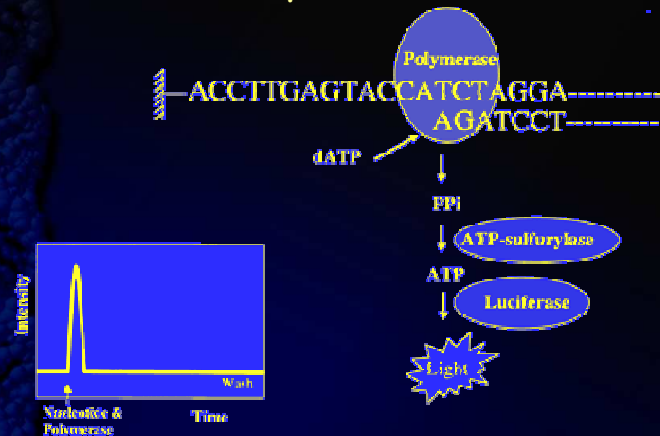
The apparatus (...) is able to sequence 25 million bases, at 99% or better accuracy, in one 4-hour run. (...) shotgun sequencing and *de novo* assembly of the *Mycoplasma genitalium* [580 Mb] genome with 96% coverage at 99.96% accuracy in one run of the machine.

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

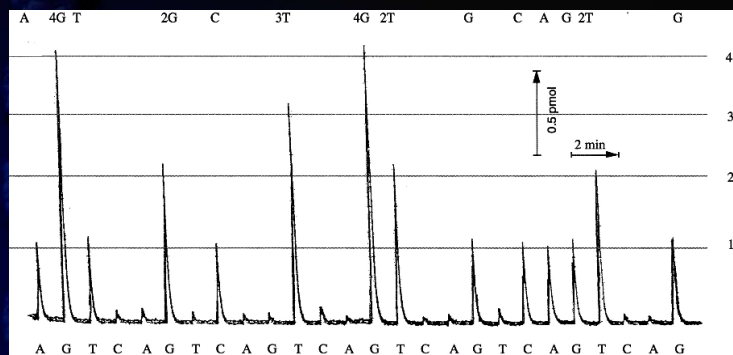
Pirossequenciamento



Pirossequenciamento



Pirossequenciamento

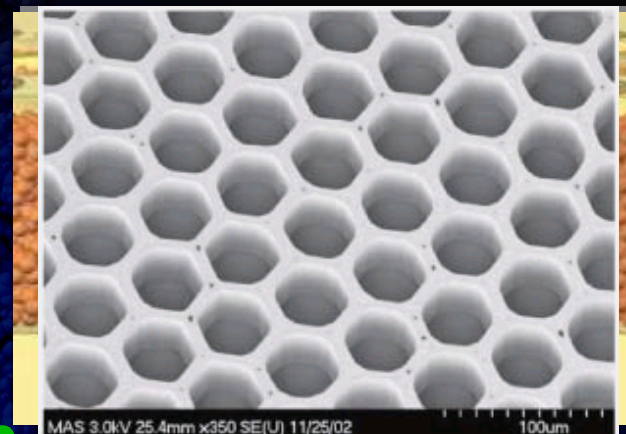


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Pirossequenciamento Genome Sequencer 20 System

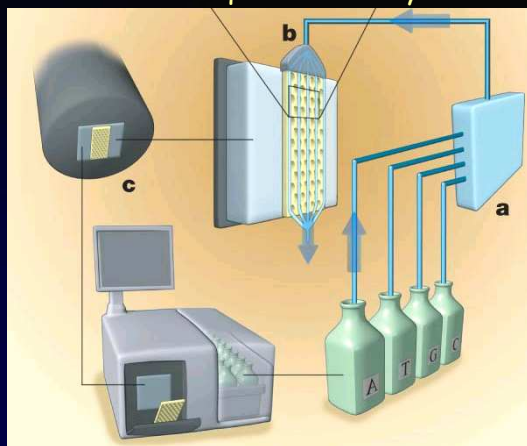


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Pirossequenciamento Genome Sequencer 20 System

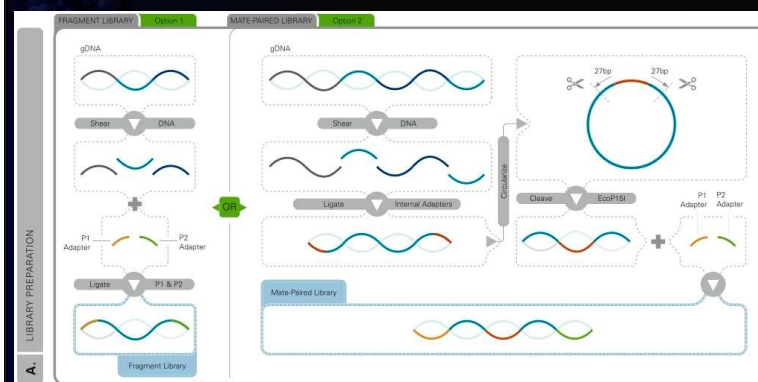


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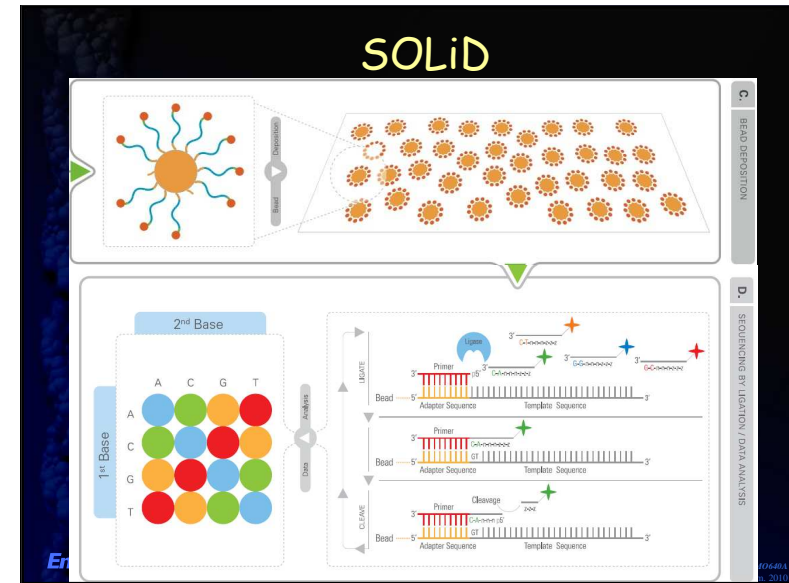
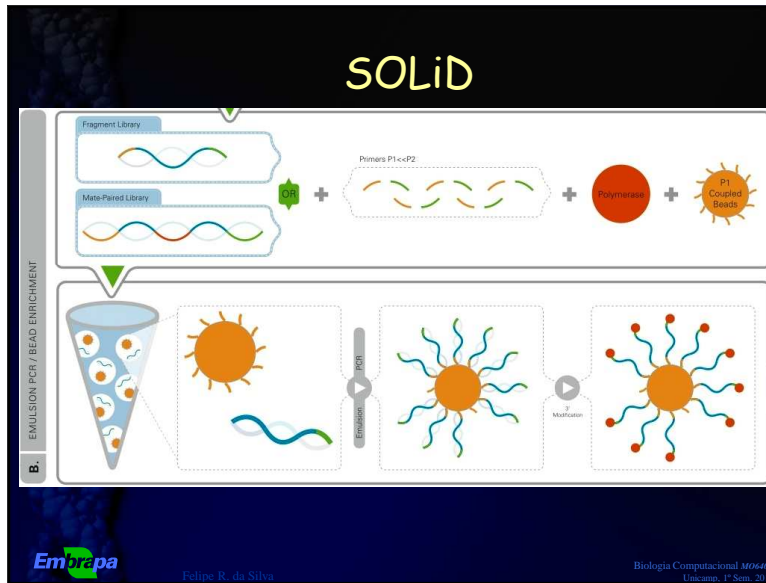
SOLiD



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Oligo

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

CGNNNI II

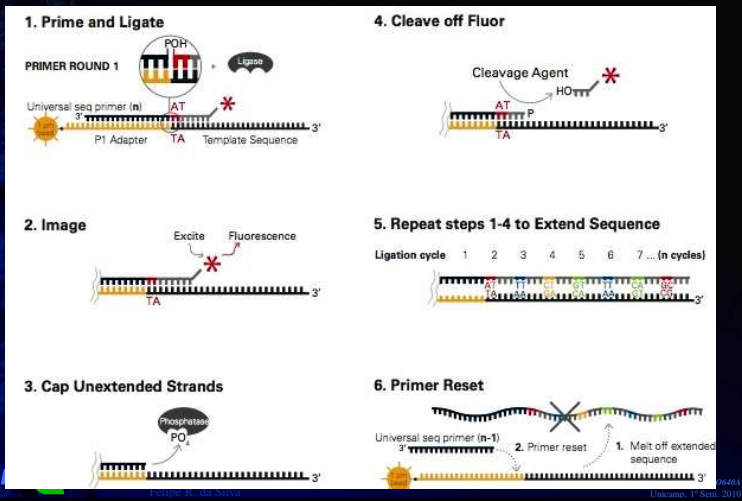
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Procedimento

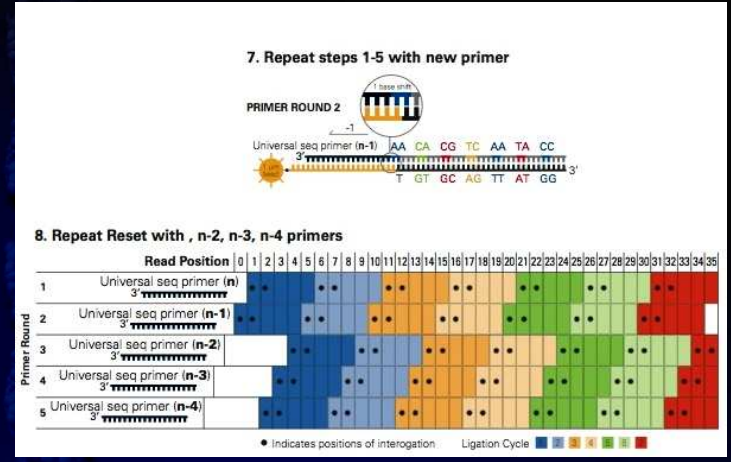
1. Anela *primer* e liga óligo
2. Trata as extremidades
3. Captura a fluorescência
4. Remove o fluoróforo
Deixa um pentanucleotídeo ligado
5. Repete o procedimento (5-7x)
6. Denatura
7. Utiliza um novo *primer*
(comprimento n-1)

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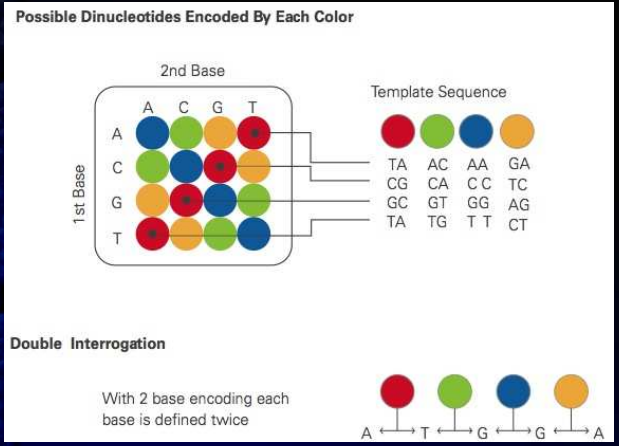
SOLiD



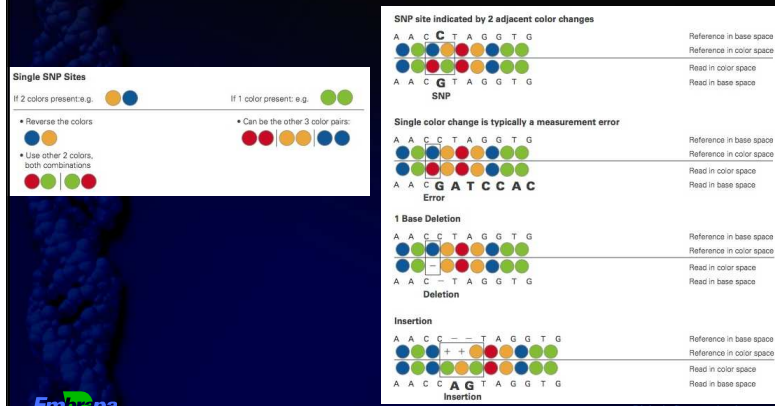
SOLiD



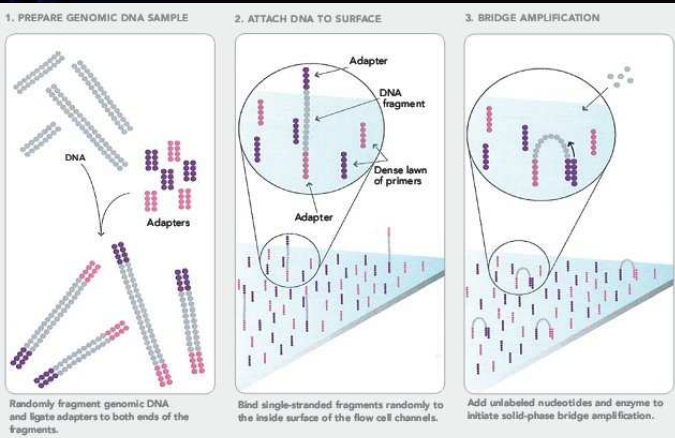
SOLiD



SOLiD



Solexa (Illumina)

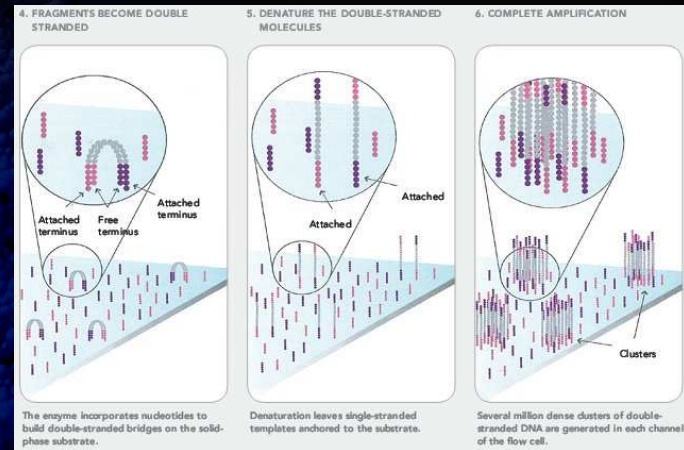


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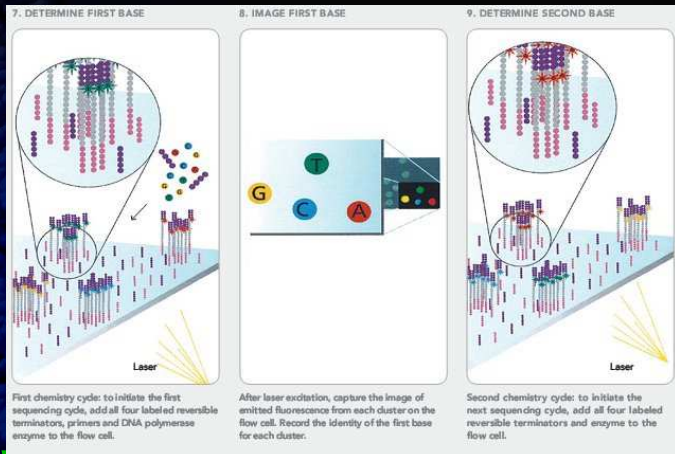


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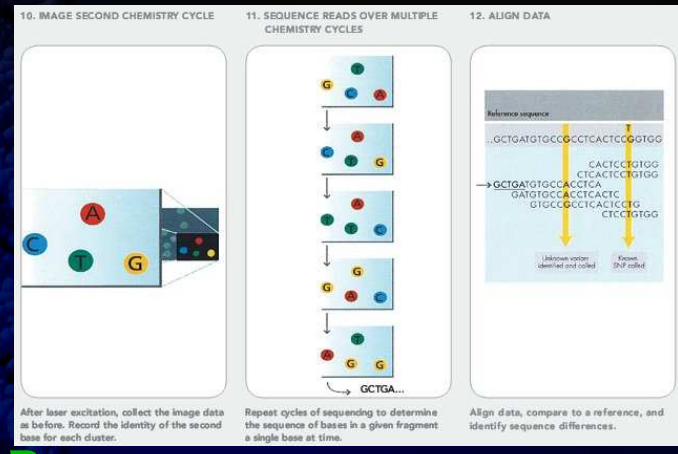


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