Seqüenciamento
(continuação...)

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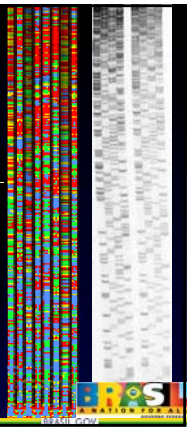


'Novas metodologias promissoras" (2001)

- Seqüenciamento por hibridização
  - Khrapko *et al.* (1989). *FEBS Lett.* **256**: 118-122 http://dx.doi.org/10.1016/0014-5793(89)81730-2
- 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
- Pirosseqüenciamento
  - 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

tecnolgias de sequenciamento de "próxima" geração

- 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

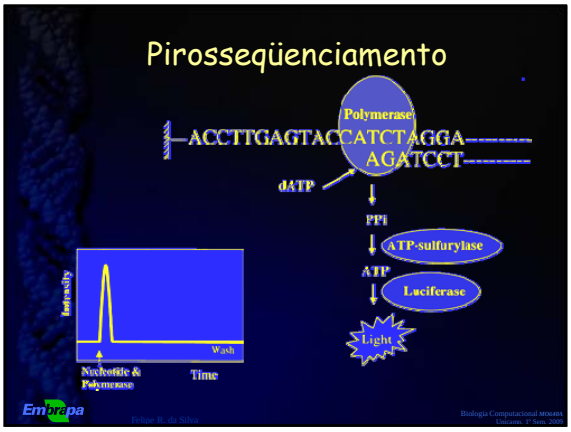
Pirosseqüenciamento

DNA_n + Nucleotídeo $\xrightarrow{\text{Polimerase}}$ DNA_{n+1} + **PPi**

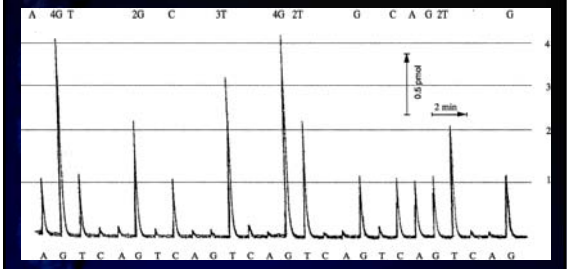
PPi + APS $\xrightarrow{\text{ATP Sulforilase}}$ SO_4 + **ATP**

ATP + Luciferina + O_2 $\xrightarrow{\text{Luciferase}}$ AMP + PPi + Oxiluciferina + CO_2 + **Luz**

Pirosseqüenciamento

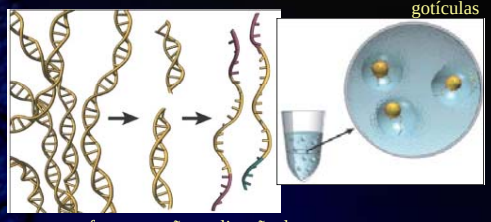


Pirosseqüenciamento



Pirosseqüenciamento
Genome Sequencer 20 System

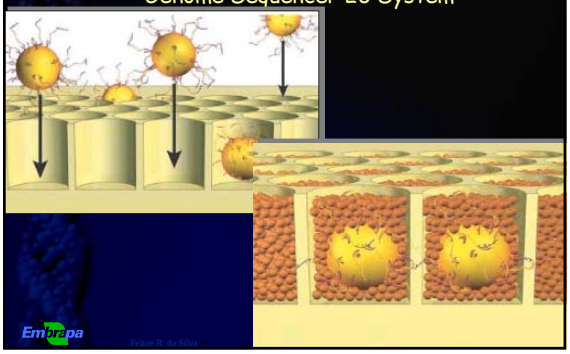
reação de PCR em gotículas



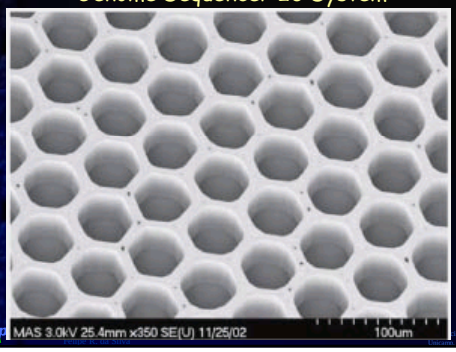
fragmentação

ligação dos adaptadores

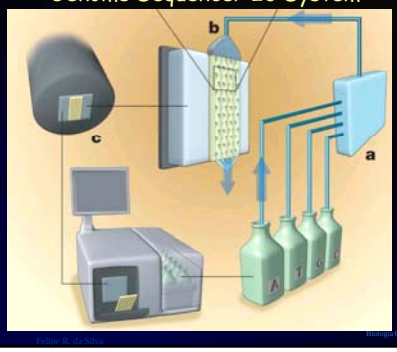
Pirosseqüenciamento
Genome Sequencer 20 System



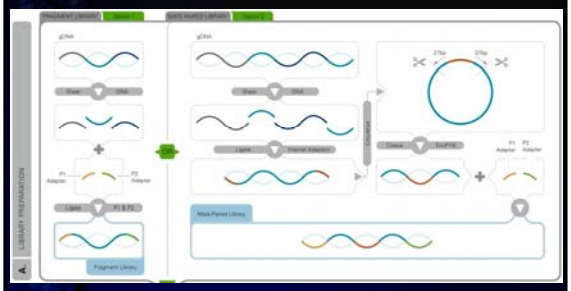
Pirosseqüenciamento
Genome Sequencer 20 System



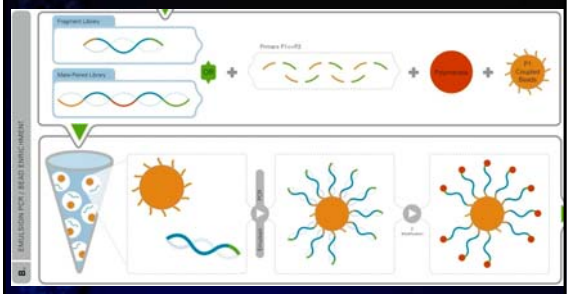
Pirosseqüenciamento
Genome Sequencer 20 System



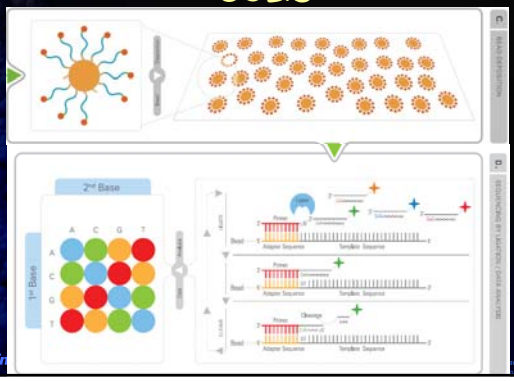
SOLiD



SOLiD



SOLiD



Oligo

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

CGNNNIII

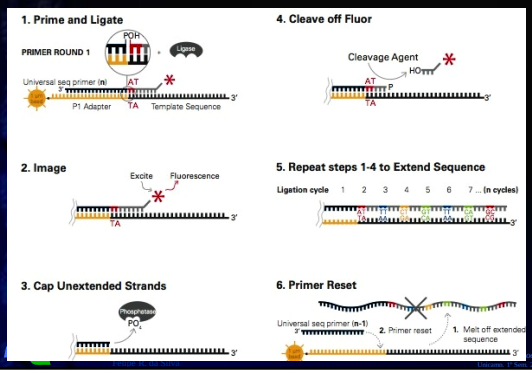
Procedimento

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)

SOLiD



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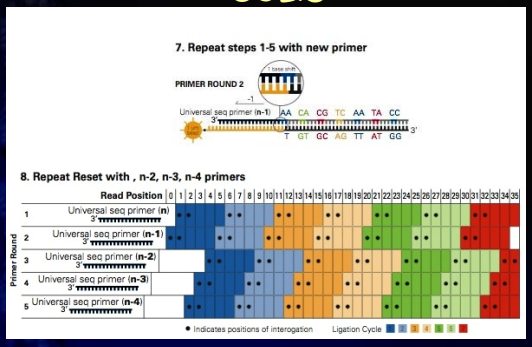
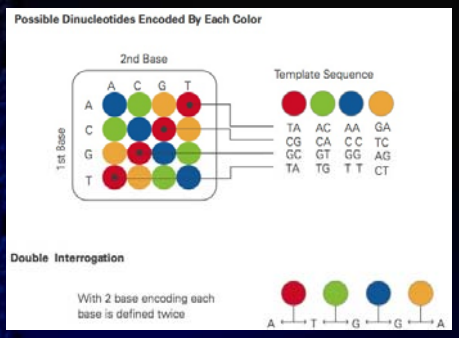


SOLiD



SOLiD