

Seqüenciamento (continuação...)

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Embrapa Recursos Genéticos e Biotecnologia



Embrapa

'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 de "próxima" geração

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

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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>



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Piroseqüenciamento

$$\text{DNA}_n + \text{Nucleotídeo} \xrightarrow{\text{Polimerase}} \text{DNA}_{n+1} + \text{PPi}$$

$$\text{PPi} + \text{APS} \xrightarrow{\text{ATP Sulfonilase}} \text{SO}_4 + \text{ATP}$$

$$\text{ATP} + \text{Luciferina} + \text{O}_2 \xrightarrow{\text{Luciferase}} \text{AMP} + \text{PPi} + \text{Oxiluciferina} + \text{CO}_2 + \text{Luz}$$

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Pirossequenciamento

ACCTTGAGTACCATCTAGGA
AGATCCT

Polymerase

dATP

ATP-sulfurylase

Luciferase

Light

Intensity

Nucleotido de Polimerase

Time

Wash

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Biologia Computacional Matemática

Volume 17, Maio, 2008

Pirossequenciamento

A 4G T 20 C 3T 4G 2T G C A G 2T G

Signal

0.5 signal

2 min

A G T C A G T C A G T C A G T C A G T C A G

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Biologia Computacional Matemática

Volume 17, Maio, 2008

Pirossequenciamento Genome Sequencer 20 System

reação de PCR em gotículas

fragmentação

ligação dos adaptadores

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Volume 17, Maio, 2008

Pirossequenciamento Genome Sequencer 20 System

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Volume 17, Maio, 2008

Pirossequenciamento Genome Sequencer 20 System

MAS 3.0kV 25.4mm x350 SE(U) 11/25/02

100µm

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Volume 17, Maio, 2008

Pirossequenciamento Genome Sequencer 20 System

a

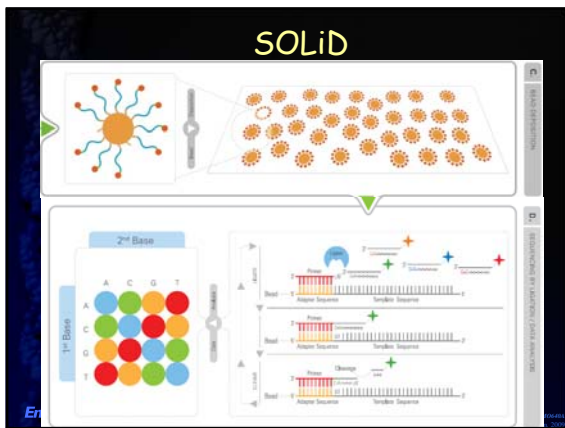
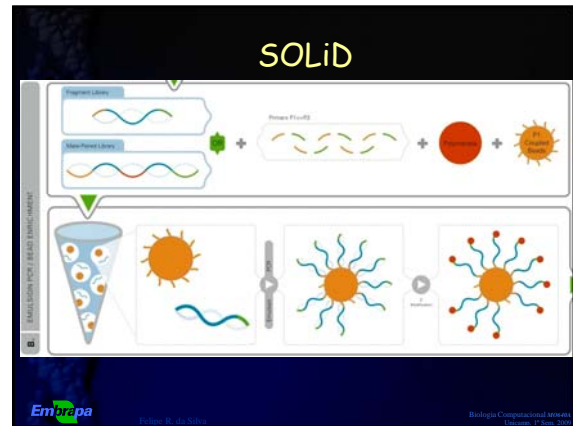
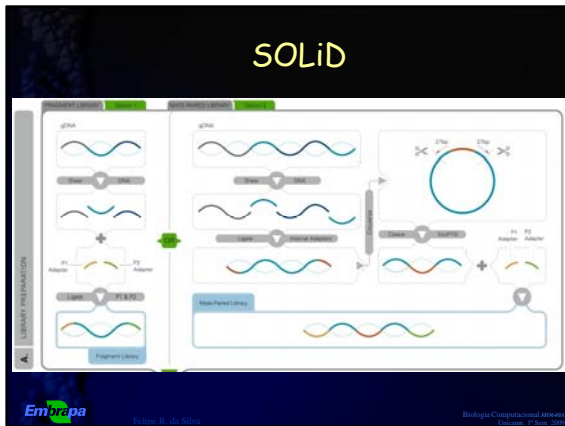
b

c

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Biologia Computacional Matemática

Volume 17, Maio, 2008



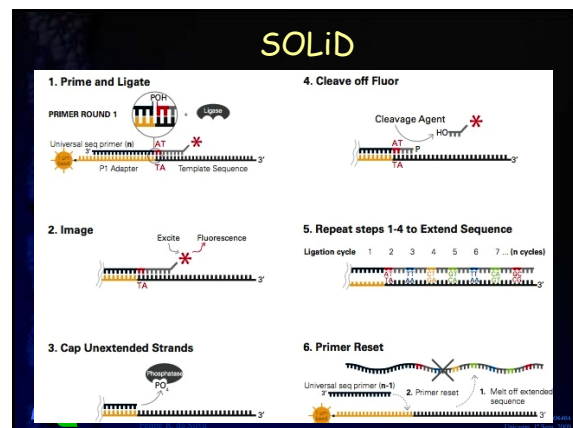
Oligo

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

CGNNNI I I

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- ## Procedimento
1. Anela *primer* e liga oligo
 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)
- Embrapa** | Felipe W. de Sá | Biologia Computacional | Instituto de Genética | Universidade Federal de Viçosa | 2009



SOLiD

7. Repeat steps 1-5 with new primer

PRIMER ROUND 2

Universal seq primer (n-1) 3'-T GT GC AG TT AT GG-5'

5'-AA CA CG TC AA TA CC-3'

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-1)																																								
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: ■ ■ ■ ■

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SOLiD

Possible Dinucleotides Encoded By Each Color

2nd Base

1st Base

Template Sequence

● TA ● AC ● AA ● GA
● CG ● CA ● CC ● TC
● GC ● GT ● GG ● AG
● TA ● TG ● TT ● CT

Double Interrogation

With 2 base encoding each base is defined twice

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SOLiD

Single SNP Sites

- 2 colors present e.g. ● ●
- 3 colors present e.g. ● ● ●
- 4 colors present e.g. ● ● ● ●
- 1 color after 2 colors, e.g. ● ● ●

SNP sites indicated by 2 adjacent color changes

A A C T A G T E
● ● ● ● ● ● ● ●
 A A C T A G T E
● ● ● ● ● ● ● ●

Single color change is typically a measurement error

A A C T A G T E
● ● ● ● ● ● ● ●
 A A C T A G T E
● ● ● ● ● ● ● ●

1 Base Deletion

A A C T A G T E
● ● ● ● ● ● ● ●
 A A C T A G T E
● ● ● ● ● ● ● ●

Deletion

A A C T A G T E
● ● ● ● ● ● ● ●
 A A C T A G T E
● ● ● ● ● ● ● ●

Insertion

A A C T A G T E
● ● ● ● ● ● ● ●
 A A C T A G T E
● ● ● ● ● ● ● ●

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