

Marcadores Moleculares

Marcadores moleculares

DNA Repetitivo

- Microsatelites ou Simple Sequence Repeats (SSRs) – Mono-, di-, tri- and tetra-nucleotdeos repetidos
- Minisatellites or Short Tandem Repeats (STRs) – 5-10 bp repetidos
- Variable Number of Tandem Repeats (VNTRs) – 14-100 bp repetidos

Marcadores Moleculares

Marcadores moleculares

- Genes Nucleares
- Sequencias de Inserção (IS)
- SNPs (Single Nucleotide Polymorphism)

Marcadores Moleculares

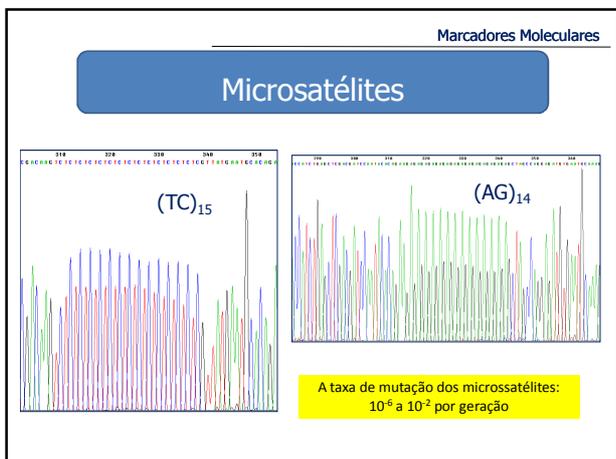
Microsatélites

(CA)_n (AG)_n (AT)_n

CACACACACACACACACACACACACACACACAC

Dinucleotdeos

Amplify flanking and repetitive DNA



Marcadores Moleculares

Microsatelites

SSR Locator: Tool for Simple Sequence Repeat Discovery Integrated with Primer Design and PCR Simulation

Luciano Carlos da Maia,¹ Dario Abel Palmieri,² Valci Queiroz de Souza,¹ Maurício Marini Kopp,¹ Fernando Irajá Félix de Carvalho,¹ and Antonio Costa de Oliveira^{1*}

¹Instituto Genômico and Breeding Laboratory, EMBRAPA, Maracá School of Agronomy, Federal University of Paraíba, Paraíba, RS 58.001-870, Brazil
²Laboratory for Environmental Studies, Catholic University of Salvador, Salvador, BA, 40.220-140, Brazil
 *Instituto, Costa de Oliveira, Email: aco@embrapa.br

Received October 5, 2007; Revised January 29, 2008; Accepted May 20, 2008.

This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Marcadores Moleculares

Minisatélites

atcgtactactagatTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGttagggatcgctagc

Primer 1 → **Hexanucleotídeos**

A TTAGGGTTAGGGTTAGGGTTAGGG

B TTAGGGTTAGGGTTAGGGTTAGGG

C TTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGG ← Primer 2

Marcadores Moleculares

Variable Number of Tandem Repeats

VNTR Repeat Length 17-40 bases

... ACAGGGTGTGGG ...

12

17

Variable Number of Tandem Repeats (VNTRs)

VNTR 19

SE8 P16 A4 P15 C6 D1 P2 I7 I13 G2 I6

1000
800
600
400
200

Marcadores Moleculares

Sequências de Inserção

- São sequências pequenas, em torno de 1Kb
- Possuem genes para transposição nos genomas

Insertion sequence

Genes for transposition

Inverted repeats

Bacterial composite transposon

Inverted Repeats

Genes for transposition

Structural genes

Inverted IS

Marcadores Moleculares

SNP

- Polimorfismos por substituição de um nucleotídeo
- Grande densidade, 12×10^6 loci

a SNPs

SNP SNP SNP

Chromosome 1 A A C A C G C C C A T T C G G G G G T C A G T C A C C G

Chromosome 2 A A C A C G C C C A T T C G A G G T C A G T C A C C G

Chromosome 3 A A C A T G C C C A T T C G G G G T C A G T C A C C G

Chromosome 4 A A C A G C C C A T T C G G G G T C A G T C A C C G

b Haplotypes

Haplotype 1 C T C A A A G T A C G G T T C A G G C A

Haplotype 2 T T G A T T G C C C A A C A G T A A T A

Haplotype 3 C C C G A T C T G T G A T A C T G G T G

Haplotype 4 T C G A T T C C G C G G T T C A G A C A

c Tag SNPs

A G C T

G A C T

A G C T

G A C T

SNP

Caracterização Molecular de Bactérias

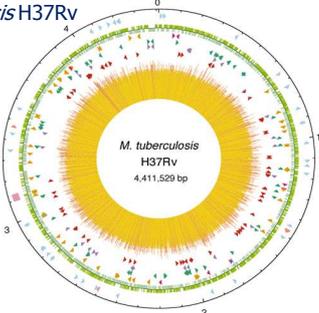
Bactérias

- Regiões PGRS
- DNA repetitivo- SSR, IS, VNTR
- 16S-ITS
- MLST

Caracterização Molecular de Bactérias

Mycobacterium tuberculosis

- *Mycobacterium tuberculosis* H37Rv
- Sequenciada em 1998
- Regiões PGRS
- DNA repetitivo –IS (56)
- SNPs



- **IS6110-RFLP**
- **Spoligotyping**
- **MIRU-VNTR**

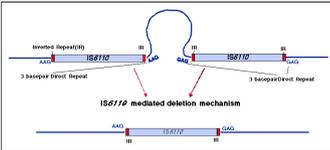
Caracterização Molecular de Bactérias

Mycobacterium tuberculosis

•IS6110-RFLP

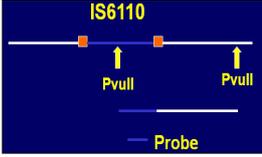
Restriction Fragment Length Polymorphism

- Estão presentes em posição e número variável no genoma



IS6110 mediated deletion mechanism

Sonda



IS6110

PvuII PvuII

Probe

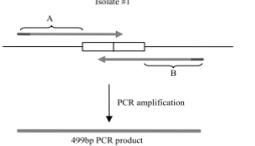
Caracterização Molecular de Bactérias

Mycobacterium tuberculosis

MIRU-VNTR

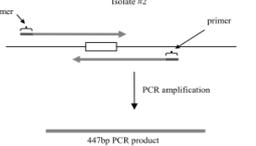
Mycobacterial Interspersed Repetitive-Unit-Variable-Number Tandem-Repeat

Isolate #1



499bp PCR product

Isolate #2



447bp PCR product

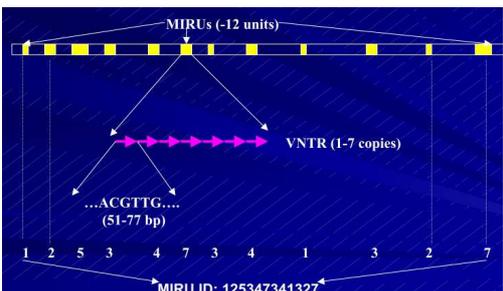


Caracterização Molecular de Bactérias

Mycobacterium tuberculosis

MIRU-VNTR

12 Unidades Repetidas Independentes



MIRUs (-12 units)

VNTR (1-7 copies)

...ACGTTG... (51-77 bp)

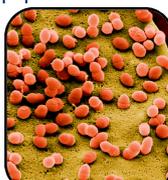
MIRU ID: 125347341327

Caracterização Molecular de Bactérias

Streptococcus pneumoniae

- Um dos agentes causadores da meningite bacteriana
- 90 sorotipos (14,6,18,19,23,49,7,1 e 3)
- Genoma com 2.1Mb
- Alvos 16S-23S-ITS, ply, lyt, psaA, pbp2b, pbp1A
- 5 % do genoma com IS
- SNPs

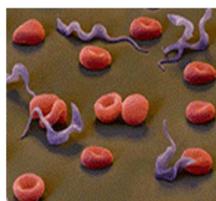
16 S	23 S	5 S
Espaço		
Ribotipagem		



Caracterização Molecular de Parasitas

Trypanosoma cruzi

- Genoma entre 106,4 e 110,7 Mb
- Diplóide – 41 pares de cromossomos
- 50% do genoma DNA repetitivo
 - Retrotransposons
 - Proteínas de superfície (MASP, gp63...)
- DNA do cinetoplasto (kDNA)
- VNTR



Caracterização Molecular de Parasitas

Trypanossoma cruzi

Marcadores Moleculares

- Região 3' do domínio 24Sa do gene do rRNA
- Gene que codifica para a subunidade 2 da oxidase citocromo mitocondrial (COII)
- Microssatélites (SCLE10, SCLE11, MCLF10-locus de repetição CA(n) e TcTAT20, TcAAT8, TcAAAT6)

A.C.J., et al. Acta Trop. (2010)

Caracterização Molecular de Parasitas

Trypanossoma cruzi

SCIENCE VOL 309 15 JULY 2005

RESEARCH ARTICLE

The Genome Sequence of *Trypanosoma cruzi*, Etiologic Agent of Chagas Disease



T. cruzi is diploid, with different-sized homologous chromosome pairs. Its genome size (diploid) has been estimated between 106.4 and 110.7 Mb

Caracterização Molecular de Parasitas

Trypanossoma cruzi

Nomenclatura, 1999

T. cruzi

T. cruzi I

T. cruzi II

Z3 - Miles

ZB - Romanha

Tipo I - Andrade

Grupo 1/2 - Souto

- Z1 - Miles, 1977
- Tipo III - Andrade, 1974
- Linhagem 2 - Souto, 1996
- Grupo 1 - Tibayrenc, 1995
- Ribodemas II/III - Clark & Pung, 1994

- Z2 - Miles, 1977
- ZA - Romanha, 1979
- Tipo II - Andrade, 1974
- Linhagem 1 - Souto, 1996
- Grupo 2 - Tibayrenc, 1995
- Ribodemas I - Clark & Pung, 1994

Caracterização Molecular de Parasitas

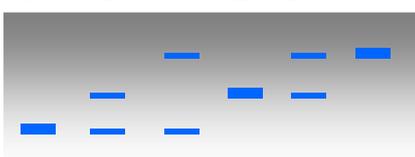
Trypanossoma cruzi

Amplificação de microssatélites (PCR)

alelo A → ●●●●●●●●●● ←

alelo B → ●●●●●●●●●● ←

alelo C → ●●●●●●●●●● ←



Caracterização Molecular de Parasitas em Saúde Pública

Trypanossoma cruzi

24Sa do gene do rRNA

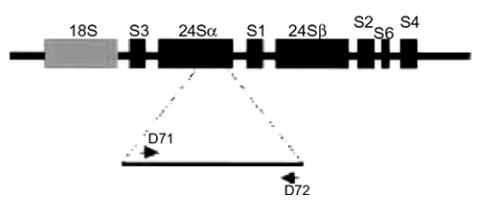


Fig. 2: The ITS 1 separates the coding region of the 18S subunit and the 5.8 S rDNA, and the ITS2 separates 5.8 S rDNA sequences from the 24S rDNA. rDNA typing is performed using primers D71 and D72 that amplifies a dimorphic region at the 3' end of the 24Sa rRNA gene.

Caracterização Molecular de Parasitas

Trypanossoma cruzi

Isoenzimas

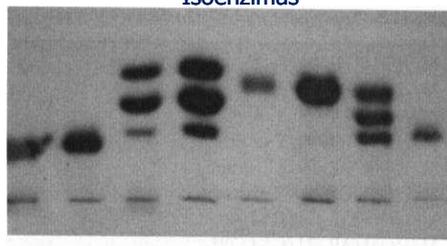


Fig. 15.6 Isoenzyme profiles of *Trypanosoma cruzi* isolates by starch-gel electrophoresis.

Caracterização Molecular de Parasitas

Toxoplasma gondii

- Possui genoma de 78 Mb
- 11 cromossomos
- Famílias de elementos repetitivos
 - ABGTg ou TGR
 - TgIRE
- rRNA
- SAG
- GRA
- SNPs

Caracterização Molecular de Parasitas

Toxoplasma gondii

PCR-RFLP

SAG2 locus SAG2- encoding tachyzoite surface antigen p22

FIG. 1. Design of SAG2 nested PCR analysis. (A) Schematic of the SAG2 locus showing the locations of the primers used for nested PCR amplification of the 5' and 3' ends of the locus and the polymorphic restriction sites used for identification of strain genotypes. The hatched box represents the open reading frame of the SAG2 gene. (B) Sma3AI restriction analysis of the 5' amplification products from type I, II, and III strains. (C) HhaI restriction analysis of the 3' amplification products from type I, II, and III strains. Products were resolved in 1.2% agarose gels stained with ethidium bromide. Molecular weight markers correspond to 6X174 digested with HaeIII.

Howe et al J Clin Microbiol, 1997, p. 1411-1414

Caracterização Molecular de Parasitas

Toxoplasma gondii

PCR-RFLP
GRA6

Fig. 1. PCR-RFLP analysis of GRA6 gene coding region with MseI endonuclease. Lane M is DNA size marker VI (between 2176 and 174 bp). Lanes 1-3 are *Toxoplasma gondii*, type I (RH), type III

A. Fazeeli et al. / International Journal for Parasitology 30 (2000) 637-642

Caracterização Molecular de Parasitas

Toxoplasma gondii

PCR-RFLP e sequenciamento
(5'SAG2, 30-SAG2, SAG3 and GRA6)

Fig. 1. Restriction patterns of PCR products amplified from clinical samples with recombinant *T. gondii* strains. 5'SAG2 (A) and 3'SAG2 (B) amplification products were digested with Sma3AI and HhaI, respectively. The DNA fragments were resolved in 2% agarose gels stained with ethidium bromide. Lane 1, 100-bp ladder (the lowest band shown is 100 bp); lane 2, RH (type I); lane 3, ME49 (type II); lane 4, VEG (type III); lanes 5-7, CS, CSb and CSc (clinical samples). The arrows show the allele combinations.

I.M.R. Ferreira et al. / Exper Paras. 118 (2008) 221-227

Caracterização Molecular de Parasitas

Toxoplasma gondii

PCR-RFLP e sequenciamento

GRA6

```

1  TGTCTCTCTGTTGTA-GAGTATT-TCCT-TCCTTGTGTA-TACTTTTTTCTCTCTCT-CCGAGAACCGCAGCAGTGTATTCATATGAGAGCA 100
111 .....GTC.....C..AA.....C.....E.....E.....C.....C.....C.....
112 GCG.....GCTC.....C.....AA.....C.....C.....T.....T.....T.....T.....
113 A.....GTC.....GTT.....G.....G.....G.....G.....G.....G.....G.....
    
```

SAG3

```

1  GAGAGTACGCTTTCGG-ATCTGGCGCGG-CCGATTTTGTTCAGCTTGATTTCCCTCCCTGATTTCCGGGACATCTCCCTCTCTGATGGCC 100
111 .....GG.....E.....E.....E.....E.....E.....E.....E.....E.....E.....
112 GCG.....GCTC.....C.....AA.....C.....C.....T.....T.....T.....T.....
113 A.....GTC.....GTT.....G.....G.....G.....G.....G.....G.....G.....
    
```

5'SAG2

```

1  GCTTA-CGAC-CGATCTGGATCTCTGTGTGTTTC-CGHTTTCGATCTCGAGACTGATGTGTATTTGGACATCTGGTGGATCACTTCTTC 100
111 .....G.....T.....G.....C.....A.....A.....A.....A.....A.....A.....A.....
112 GCG.....GCTC.....C.....AA.....C.....C.....T.....T.....T.....T.....
113 A.....GTC.....GTT.....G.....G.....G.....G.....G.....G.....G.....
    
```

I.M.R. Ferreira et al. / Exper Paras. 118 (2008) 221-227

Caracterização Molecular de Vírus

Marcadores Moleculares

Ex: O genoma do HIV

HIV-1 GENOME 9749 NUCLEOTIDES

- Três genes estruturais
- Sequências repetitivas regulatórias (LTRs)
- Genes regulatórios (não empacotados no virion)

Caracterização Molecular de Vírus em Saúde Pública

Caracterização Molecular

191 Kpb / 263 genes

Variáveis - Genes não-essenciais - interação com o hospedeiro e virulência

Genes essenciais - polipeptídeos estruturais e enzimas envolvidas no metabolismo da DNA

Caracterização Molecular de Vírus em Saúde Pública

Caracterização Molecular

Alvos

- Genes Estruturais
- Regiões LTR (Long Terminal Repeat)
- ITRs
- SNPs

Caracterização Molecular de Vírus em Saúde Pública

Vírus Hepatite B

- Genoma de 3.2kb
- 4 genes (C, S, P, X)
- 9 Sorotipos: *awy1, awy2, awy3, awy4, ayr, adw2, adw4, adrq-, adrq+*
- 8 genótipos (A-H) No Brasil : A, D e F

Caracterização Molecular de Vírus

Vírus Hepatite B

- Alvos - Genes C e S
- SNPs

Caracterização Molecular de Vírus

Vírus Hepatite B

- Determinação de mutações
- S e C
- PCR e Digestão *AvaI* and *MspI*

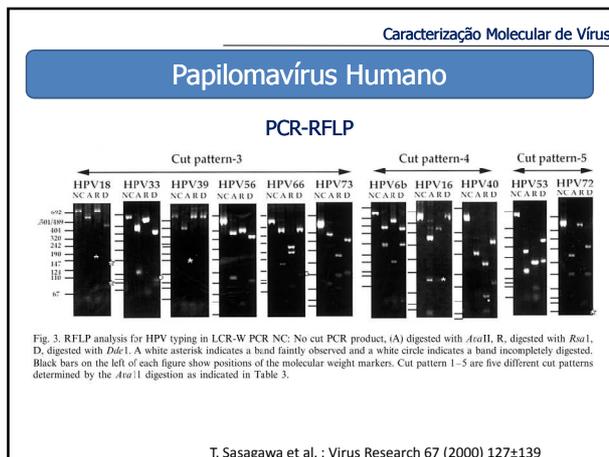
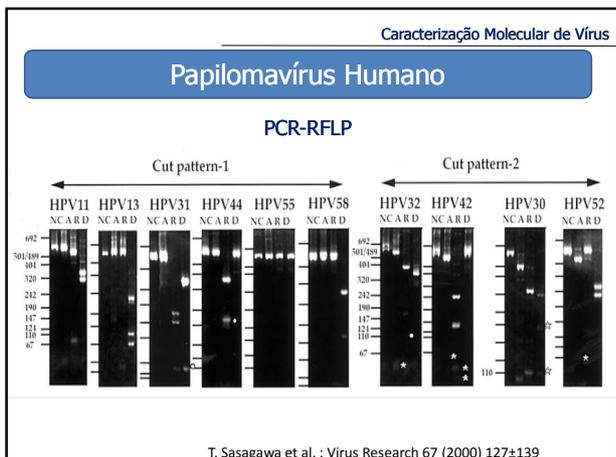
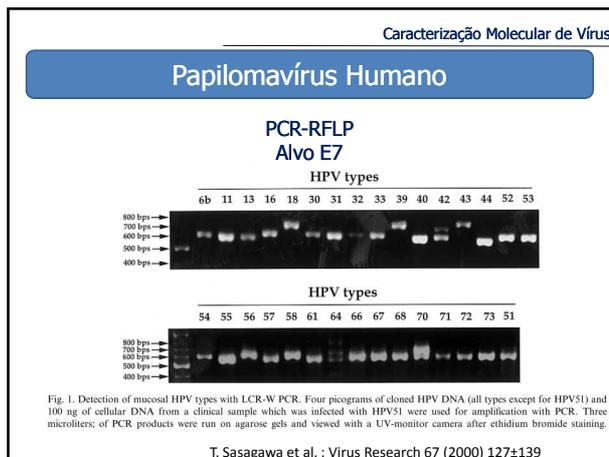
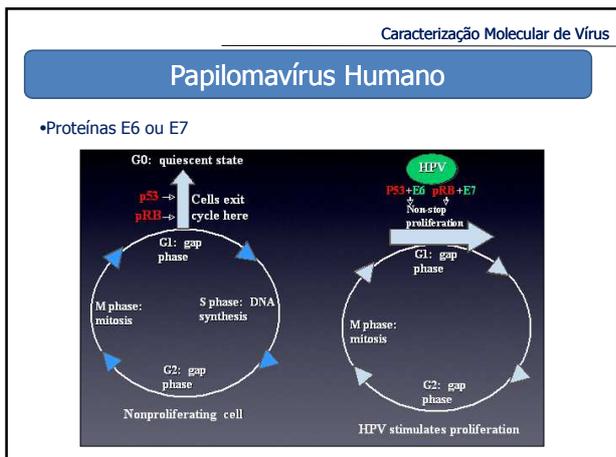
Fig. 2. Gel image of RFLP assay for detection of HBV variants for core region after *AvaI* and *MspI* enzyme digestion. 1 Uncut DNA fragment (552 bp); 2, 9 A1 50-bp ladder (Fermentas); 3, 4 A1 mutants (460, 92 bp); 5 A2 mutants (245, 145, 70, 92 bp); 6-8 A4 mutants (245, 215, 92 bp); 10-12 M1 mutant (429, 123 bp); 11 M2 mutant (136, 293, 123 bp); 12 M3 mutant (552 bp)

M. A. Aslam et al. Arch Virol (2008) 153: 163-170

Caracterização Molecular de Vírus

Papilomavírus Humano

- Família Papillomaviridae
- DNA fita dupla circular
- Genoma 7.9Kb
- Mais de 120 tipos
- Tipo 16 presente em 60-70% dos carcinomas
- Proteínas E6 ou E7
- LR (6, 11, 42, 43, 44)
- HR (16, 18....)



Caracterização Molecular

Técnicas moleculares

RFLP - restriction fragment length polymorphism DNA
 RAPD - random amplified polymorphic DNA
 CAPS - cleaved amplified fragment sequence
 SSR - Simple Sequence Repeat / Microssatélites
 AFLP - amplified fragment length polymorphismic DNA
 SNP - single nucleotide polymorphisms

As diferentes classes de marcadores moleculares podem diferir com respeito a características importantes como:

- abundância genômica
- nível de polimorfismo detectado
- especificidade dos locos
- reprodutibilidade
- labor

- Marcadores Moleculares
- ### Marcadores Moleculares
- RFLP
 - VNTR (minissatélite)
 - RAPD
 - PCR-específico - SSR, ISSR, CAPS
 - MLST
 - AFLP
 - SNPs

Marcadores Moleculares- Histórico

RFLP proposto por Botstein *et al.* (1980)

descrito para humanos

PCR proposto por Mullis & Faloona (1987)

VNTR por Jeffrey (1987)

RAPD por Rafalski *et al.* (1990)

Marcadores Moleculares- Histórico

SSR em plantas por Akkaya *et al.* (1992)

AFLP por Zabeau & Vos (1993)

CAPS por Konieczny & Ausubel (1993)

SNPs em Arabidopsis po Cho *et al.* (1999) -

Marcadores Moleculares- Tipo de Técnica

- Métodos sem uso de PCR
 - RFLP
- Métodos com uso de PCR
 - PCR com primers arbitrários
 - RAPD, AP-PCR, DAF (DNA amplification fingerprint),
 - AFLP;
 - ISSR
 - PCR sítio-específico
 - CAPS, SCAR
 - SSRs (microsatélites)

Marcadores Moleculares- Nº de Cópias

- Seqüência de poucas cópias - codificante
 - RFLP
- Seqüência com cópias repetidas
 - VNTR
 - SSRs (microsatélites)
 - ISSR
- Seqüência com número de cópias indefinido
 - RAPD, AP-PCR, DAF
 - AFLP
 - CAPS, SCAR

Técnicas de Caracterização Molecular



•Baseados em PCR

•Baseado em Hibridização de Ácidos Nucléicos

•Baseado no sequenciamento de Ácidos Nucléicos

Caracterização Molecular baseada em PCR

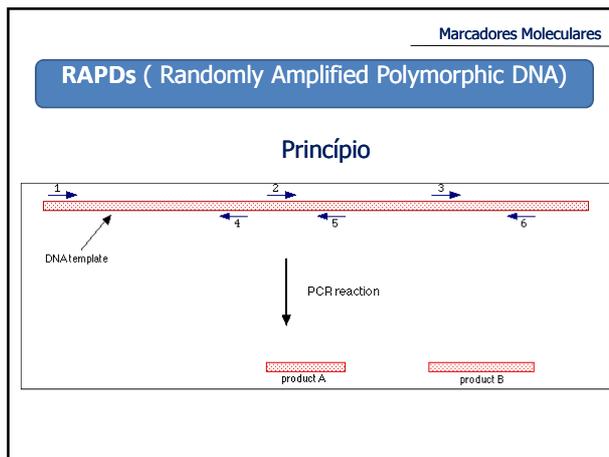
RAPDs (Randomly Amplified Polymorphic DNA)
 ALFP (Amplifid Fragment Length Polymorphisms)
 SSR-PCR (Short Sequence Repeats PCR)
 PCR-RFLP
 MLST- Multi Locus Sequencing Typing

Marcadores Moleculares

RAPDs (Randomly Amplified Polymorphic DNA)

- Utiliza primers pequenos (10bp) para amplificar randomicamente fragmentos de DNA
- Baixa Tm
- Na ha a necessidade de informações sobre o genoma a ser amplificado
- Requer pelo menos 10 primers

5' GGTGCGGGAA 3'
 5' GGTGCGGGAA 3'
 5' GTTCGCTCC 3'
 5' GTAGACCCGT 3'
 5' AAGAGCCCGT 3'



Marcadores Moleculares

RAPDs (Randomly Amplified Polymorphic DNA)

Para cada banda- escure = presença/ ausência da banda

Marcadores Moleculares

RAPDs (Randomly Amplified Polymorphic DNA)

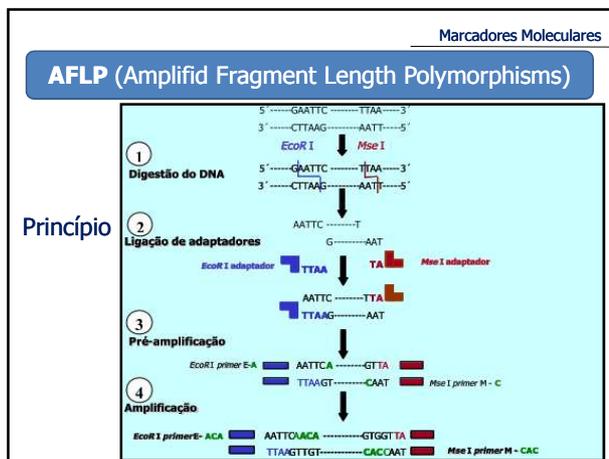
- Marcadores RAPD são anônimos
- Dados binários (presença x ausência)
- Problemas de co-migração
 - mesma banda, mesmo fragmento?
 - uma banda, um fragmento?

Marcadores Moleculares

AFLP (Amplified Fragment Length Polymorphisms)

Procedimento dividido em 4 etapas:

- 1- Digestão do DNA total com enzimas de restrição
- 2- Ligação de adaptadores sitio-especificos
- 3- Pré-PCR de alguns fragmentos
- 4- PCR



Marcadores Moleculares

AFLP (Amplified Fragment Length Polymorphisms)

The diagram illustrates the AFLP process. It shows a DNA fragment with a *MseI* site (TA CCGAGGCTCAT) and an *EcoRI* site (CA CTCTAGCCGATGA). A primer with a 5' end labeled 'Primer + 1' (5'-GTAGACGCGGACG-AMTY-CA) is hybridized to the *MseI* site. An adapter with a 5' end labeled 'Adapter' (5'-GTAGACGCGGACG-AMTY-C) is hybridized to the *EcoRI* site. The resulting structure is labeled 'Fragment'.

Marcadores Moleculares

AFLP (Amplified Fragment Length Polymorphisms)

- Eletroforese em gel de poliacrilamida
- Coloração com prata

The image shows an agarose gel electrophoresis result for AFLP. The gel has 12 lanes labeled I through IV, with sub-lanes 1-3, 4-6, 7-9, and 10-12. Numerous dark bands of varying lengths are visible across the lanes, representing different DNA fragments.

Marcadores Moleculares

PCR-RFLP

PCR – Digestão
CAPS - cleaved amplified fragment sequence

The image shows two agarose gel electrophoresis images. The left image shows a single band for each of four samples, representing the PCR product before digestion. The right image shows multiple bands for each sample after digestion with a restriction enzyme, representing the cleaved amplified fragment sequences (CAPS).

Marcadores Moleculares

MLST- Multi Locus Sequence Typing

Principle

The diagram illustrates the principle of MLST. It shows a DNA fragment of 400 to 500 bp being amplified by PCR. The resulting amplicons are then analyzed for DNA sequence. The diagram shows the 5' and 3' ends of the amplicons and the resulting sequence analysis.

400 a 500 pb

5'-ogttaaattagagggtatogcatogcatogcatatcatctcaagcoaatagcg-3'
3'-gaaatlaattatoo???tgoggtatogc-5'

PCR amplify + DNA sequence analysis

5'-ogttaaattagagggtatogcatogcatogcatatcatctcaagcoaatagcg-3' = allele 1
5'-ogttaaattagagggtatogcatogcatogcatatcatctcaagcoaatagcg-3' = allele 2
5'-ogttaaattagagggtatogcatogcatogcatatcatctcaagcoaatagcg-3' = allele 3
5'-ogttaaattagagggtatogcatogcatogcatatcatctcaagcoaatagcg-3' = allele 4
5'-ogttaaattagagggtatogcatogcatogcatatcatctcaagcoaatagcg-3' = allele 5
5'-ogttaaattagagggtatogcatogcatogcatatcatctcaagcoaatagcg-3' = allele 6

Marcadores Moleculares

MLST- Multi Locus Sequence Typing

The diagram illustrates the principle of MLST. It shows a DNA fragment of 400 to 500 bp being amplified by PCR. The resulting amplicons are then analyzed for DNA sequence. The diagram shows the 5' and 3' ends of the amplicons and the resulting sequence analysis.

olate 1: 1 1 3 1 2 → ST 1
olate 2: 1 3 2 1 1 → ST 2
olate 3: 2 1 1 2 2 → ST 3
etc.

The image is a screenshot of the Leptospira MLST web interface. The page title is 'MLST Multi Locus Sequence Typing'. The interface includes a 'DATA ANALYSIS' section with 'DATABASES' and 'Leptospira spp.' options. The 'DATABASES' section lists various species including *B. burgdorferi*, *B. cereus*, *B. pseudomallei*, *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. jejuni*, *E. coli*, *E. faecalis*, *E. faecium*, *H. influenzae*, *H. pylori*, *Leptospira spp.*, *M. catarrhalis*, *W. meningitidis*, *S. agalactiae*, *S. aureus*, *S. enterica*, *S. seipidermidis*, *S. pneumoniae*, *S. pyogenes*, *S. suis*, and *V. vulnificus*. The 'Leptospira spp.' section includes options for 'Organism Specific Information', 'Concatenate Sequences', 'Download Alleles', 'Download STs', 'Download as Excel', 'Compare profile to refset', 'Draw tree using own MLST data', 'Contact Curator', 'Leptospira Links', 'eBURST', and 'MLST-MAPS'. The 'PROFILE QUERY' section has a dropdown menu labeled 'Please choose...'. The 'LOCUS QUERY' section has a dropdown menu labeled 'Please choose...'. The 'BATCH QUERY' section has a dropdown menu labeled 'Please choose...'. The footer includes the text: 'The Leptospira MLST scheme was developed by Janira Thaipadungpanit, Vanaporn Wuthiekanun, Sharon J. Peacock et al. REF: Click here. This site is hosted at Imperial College and development is funded by the Wellcome Trust.'

Primers used for MLST of *Helicobacter pylori*

Genes
The Helicobacter MLST scheme uses internal fragments of the following eight house-keeping genes:
atpA, efp, mutY, ppa, trpC, ureI, vacA, yphC

Primers
The primer pairs we use for the PCR amplification of internal fragments of these genes are:

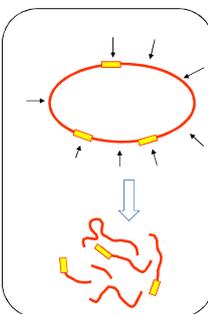
Number	Name	Gene	Sequence
Q_1369	HptrpC1_for	trpC	CAAGCTCCTAGAATCTCTG
Q_1370	HptrpC1_rev	trpC	TAGATGCAGAAAGCGCTCGCCCT
Q_1371	HptrpC5_rev	trpC	CCCAGCTAGAGGATAAAGG
Q_1372	HptrpC7_rev	trpC	TAAAGCCCGACACTTATTTTCGCC
Q_1379	yphC1_for	yphC	GCATATTCCAGGCTCTTTTTTGAC
Q_1382	yphC8_rev	yphC	TTTCTAGCTTTTAAATATC
Q_1493	HPvacA-4	vacA	ATACGCTCCCGCATTTGC
Q_1514	Q2HPvacA-1a	vacA	AGAAAGCGATGATTCACGC
Q_1558	vacA_for2	vacA	CTGCTGTAGGAAGCGCTCC
Q_1559	vacA_rev2	vacA	GCCTGGCCCATCAAAAGAG
Q_1560	yphC_for2	yphC	CAGCGCATTTTTTGAATAAAGC
Q_1561	yphC_rev2	yphC	GGCTTAAAGCGGACCTTTTCG
Q_1562	yphC_for3	yphC	GCCTTAAATCACTAATATTTAATC
Q_1563	yphC_rev3	yphC	CATTAACCTCCCAATGATGC
Q_1564	atpA_for2	atpA	GGACTAGGCTAAAGCGCAGC
Q_1565	atpA_rev2	atpA	CTTGAACCGCAGCCGCCAC
Q_1566	atpA_for3	atpA	GTTCCTTTTGGGATCGCGGT
Q_1567	atpA_rev3	atpA	CCTGATTAAGCAATCCGTTTC
Q_1579	mutY_for2	mutY	GTGGTTATYTYGAACTTTACAC
Q_1580	mutY_rev2	mutY	CAAGCCCAATGACGCTCTTC
Q_1581	mutY_for3	mutY	GGAAATTAAGGGCATTAAGCGC
Q_1582	mutY_rev3	mutY	CTTAAGCGTSTGTYYTCTAGG
Q_1044	efp_for1	efp	GGCAATTTGGTGAAGGAGCTCT
Q_1045	efp_rev1	efp	CTTCACCTTTCAAGATCTC

Marcadores Moleculares

PFGE (Pulsed Field Gel Electrophoresis)

Eletroforese em Campo Pulsado

- DNA e digerido com enzimas de restrição
- Eletroforese




Equipamento

Marcadores Moleculares

PFGE (Pulsed Field Gel Electrophoresis)

Princípio

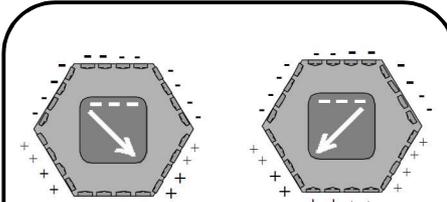
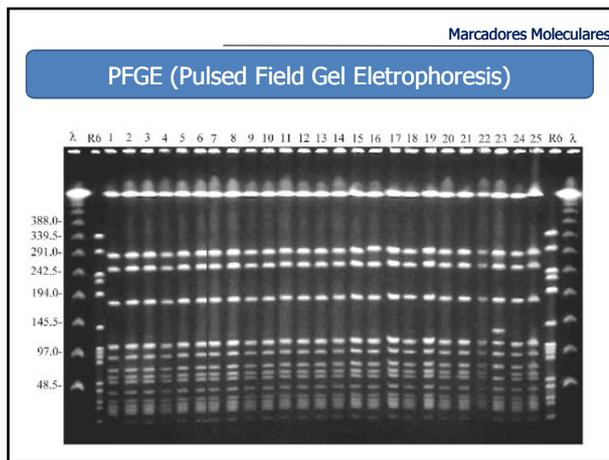


Figura 4: Funcionamento do sistema CHEF. Os campos eléctricos funcionam alternadamente segundo cada uma das diagonais do gel. Como resultado, as moléculas de DNA migram num percurso rectilíneo ao longo do gel.



Marcadores Moleculares

Caracterização Molecular baseada em Hibridização

(RFLP) Restriction Fragment Length Polymorphisms

(RH) Hibridização reversa

Marcadores Moleculares

RFLP

Análise de restrição de fragmentos polimórficos

Procedimento dividido em 4 etapas:

- 1-Digestão com enzimas de restrição
- 2-Eletroforese em gel de agarose
- 3-Transferência para uma membrana de nylon
- 4-Hibridização com sonda de DNA

Marcadores Moleculares

RFLP

(A) unlabelled DNA cut with a restriction nuclease
 labeled DNA of known sizes as size markers
 DNA FRAGMENTS SEPARATED BY AGAROSE GEL ELECTROPHORESIS

(B) stack of paper towels
 agarose gel
 sponge
 alkali solution
 SEPARATED DNA FRAGMENTS BLOTTED ONTO NITROCELLULOSE PAPER

(C) remove nitrocellulose paper with tightly bound DNA
 nitrocellulose paper
 Labeled DNA probe hybridized to separated DNA
 sealed plastic bag
 Labeled DNA probe hybridized to complementary DNA bands visualized by autoradiography

(D) labeled DNA probe in buffer
 Labeled DNA probe hybridized to complementary DNA bands visualized by autoradiography

positions of labeled markers
 labeled bands

Baseado na técnica de Southern blot

Marcadores Moleculares

RFLP

1 DNA
 2 Separate DNA on an Agarose Gel
 3 Transfer or BLOT DNA fragments from GEL to Membrane
 4 Membrane with DNA bands transferred to it
 5 Radiolabeled probe incubated with Membrane
 6 Bound DNA Bands are Exposed on Film

PROBE

Copyright Molecular Station.com

Marcadores Moleculares

RFLP- Resultado

Marcadores Moleculares

Hibridização Reversa

Spoligotyping

PCR- Dra 5'-GGTTTTGGGTCTGACGAC-3'
 Drb 5'-CCGAGAGGGGACGGAAAC-3'

DR spacer DR spacer DR spacer DR spacer DR
 DVR

Marcadores Moleculares

Hibridização Reversa

Spoligotyping -baseado em PCR

Direct Repeated Unique Spacer Unique Spacer Unique Spacer Unique Spacer
 PCR primers
 Example of possible PCR products
 PCR products hybridized to a prefabricated membrane with the sequence of each unique spacer covalently bonded to it in columns.
 Unique Spacer 1 2 3 4 5 6 7 8 9 10 11 12 13 14 etc.
 Indica 11
 Indica 12
 etc.
 Excess PCR product removed, autoradiography film exposed and developed.

Marcadores Moleculares

Hibridização Reversa

- Atualmente 94 diferentes espaçadores foram identificados
- 43 espaçadores (35 a 41 pb) são utilizados para a diferenciação de MCT

M. tuberculosis H37Rv
M. bovis
M. africanum 25420
 Patient A
 Patient B
 Patient C
 Patient D
 Patient E
 Spacer no. 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

M. tuberculosis H37Rv
M. bovis
M. africanum 25420
 Patient A
 Patient B
 Patient C
 Patient D
 Patient E
 Spacer no. 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43

Marcadores Moleculares

SNPs

1) primer extension –PCR

- UM PRIMER DEVE TER SEU ÚLTIMO NUCLEOTÍDEO 3' SOBRE O NUCLEOTÍDEO ALVO
- Durante a polimerização de um fragmento (*amplicon*) de DNA pela *DNA Polimerase*, ela necessita de que o último nucleotídeo da posição 3' onde o *primer* esta ligado, tenha uma conformação ABRUPTA

GENOTIPO 1

GENOTIPO 2

Marcadores Moleculares

SNPs

2) PCR e Clivagem enzimática

A) CONJUNTO DE PRIMERS QUE AMPLIFICAM UMA REGIÃO ALVO

B) A REGIÃO ALVO (DENTRO DO AMPLICON) É CORTADA POR UM ENZIMA

Restriction enzyme

Sequence recognized, cleavage

Sequence not recognized, no cleavage

Gel electrophoresis

Marcadores Moleculares

SNPs

2) PCR e Clivagem enzimática

GENOTIPO 1

GENOTIPO 2

Marcadores Moleculares

SNPs

2) PCR e Clivagem enzimática

GENOTIPO 1

3' TAGCATCCCGAAGCATCCGGAATAACTTAA-----GCTAATTACGTTAAGGCCTTTTAAAGGCCTCTCTC 5'

5' ATCGTAGGGCTTTCGTAGGCCTTATTG-----AATTCGATTAATGCAATTCGGAAAAATTCGGAGAGAG 3'

3' TAGCATCCCGAAGCATCCGGAATAACTTAA

5' ATCGTAGGGCTTTCGTAGGCCTTATTG

EcoRI

5' ...G/ AATTC... 3'

3' ...CTTAA G... 5'

GENOTIPO 2

3' TAGCATCCCGAAGCATCCGGAATAAATTAAGCTAATTACGTTAAGGCCTTTTAAAGGCCTCTCTC 5'

5' ATCGTAGGGCTTTCGTAGGCCTTATTTAATTCGATTAATGCAATTCGGAAAAATTCGGAGAGAG 3'

Marcadores Moleculares

SNPs

2) PCR e Clivagem enzimática

GENOTIPO 1 GENOTIPO 2

Marcadores Moleculares

SNPs

3) Hibridização

a) Vários locos e vários genes

EX: Microarray de 25-mers (genes)

Microarray Genoma "painel de SNPs"

b) Poucos locos, poucos genes ou gene específico

EX: sondas *TAQ MAN*

Marcadores Moleculares

SNPs

3) Hibridização

a) Vários locos e vários genes
EX: Microarray de 25-mers (genes) e Microarray Genoma "painel de SNPs"

Fluorescence analysis

Marcadores Moleculares

SNPs

3) Hibridização

b) Poucos locos, poucos genes ou gene específico
EX: sondas TAQ MAN

Fluorescence analysis

Marcadores Moleculares

SNPs

Resultado

Marcadores Moleculares

SNPs

Opinion TRENDS in Microbiology Vol.11 No.3 March 2003 115

Enterobacterial adhesins and the case for studying SNPs in bacteria

Scott J. Weissman¹, Steve L. Moseley², Daniel E. Dykhuizen³ and Evgeni V. Sokurenko²

¹Division of Infectious Disease, Children's Hospital and Regional Medical Center, 4800 Sand Point Way NE, Seattle, WA 98105, USA
²Department of Microbiology, Box 357242, University of Washington, Seattle, WA 98195-7242, USA
³Department of Ecology and Evolution, State University of New York, Stony Brook, NY 11794, USA

Single-nucleotide polymorphisms (SNPs) in structural genes can have a dramatic effect on the biology of whole organisms, from bacteria and viruses to mammals. Here, we underscore the importance of SNPs in bacterial genes that contribute to the ability of pathogens to cause disease. SNPs that confer an adaptive advantage for bacterial pathogens have been discovered in the genes encoding the FimH and Dr adhesins of *Escherichia coli* and, most recently, *Salmonella enterica* sv. Typhimurium FimH.

are almost ubiquitously present in both commensal and pathogenic strains. They mediate bacterial binding to a great variety of host cells and mucosal surfaces and have been shown to be important for virulence [6]. Because of their uniform distribution, type 1 fimbriae do not fit with the definition of virulence factors as determinants selectively associated with pathogenic bacterial lineages. However, pathogenic association has been shown for certain functional variants of FimH, the type 1 fimbrial adhesin. In particular, FimH alleles expressed by many unpathogenic *E. coli* exhibit higher affinity for mono-

Caracterização Molecular

Análises dos Resultados

- Análise dos dados obtidos pela caracterização molecular
- Agrupamento dos perfis moleculares similares-Dendrograma
- Análises filogenéticas

Caracterização Molecular

Utilização

Caracterização Molecular

- Conhecer a distribuição de sorotipos e a diversidade genética
- Conhecer a distribuição de cepas Resistentes aos antimicrobianos
- Identificação de surtos
- Determinar a dinâmica da transmissão em áreas geográficas
- Identificar fatores de risco para a ocorrência das doenças infecciosas

Utilização

Caracterização Molecular

- Identificar determinantes de patogênese
- Rotas de transmissão
- Reatividade sorológica
- Resposta a terapias anti-virais, anti-bacterinas ou anti-parasitarias
- Utilizar os dados em Estudos epidemiológicos