XVI ECIB 2012
XVI Encontro Científico do Instituto Biomédico
V Jornada Científica da Biomedicina
IV Workshop de Microbiologia Aplicada

"XVI - "CONTRIBUIÇÃO DA BIOLOGIA MOLECULAR NO ESTUDO DA EPIDEMIOLOGIA, RELAÇÃO PATÓGENO HOSPEDEIRO E TERAPÊUTICA DE DOENÇAS INFECCIOSAS"

Caracterização molecular de bactérias:

ontem, hoje ... Amanhã

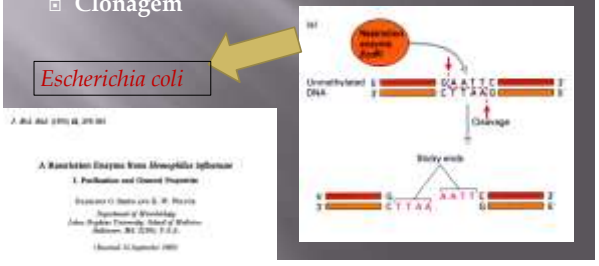
Aloysio de Mello F. Cerqueira
Professor Associado
MIP
19/10/2012

Bactérias e biologia molecular

- Enzimas de restrição
- Plasmídios
- Clonagem

1970

Escherichia coli

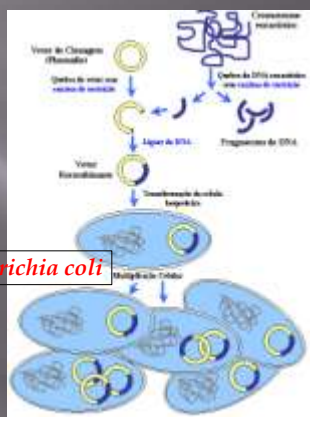


Bactérias e biologia molecular

- Enzimas de restrição
- Plasmídios
- Clonagem

Escherichia coli

Engenharia Genética



Relação bactéria- hospedeiro

- Ensaios fenotípicos
- Ensaios genotípicos
- Diagnóstico
  - Crescimento lento
  - Ausência de crescimento
  - **Confirmação de virulência**
- Genética da resistência antimicrobiana

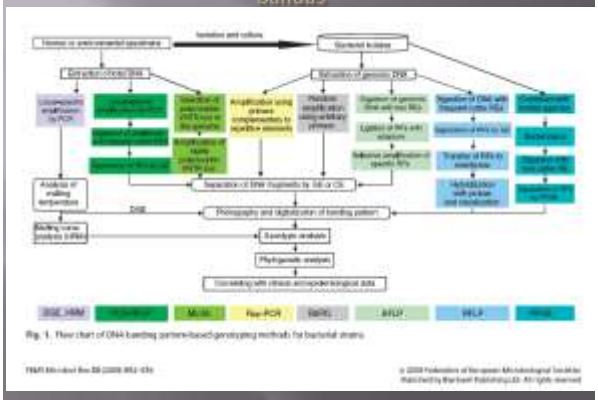
PCR - 1983

Escherichia coli

Métodos Moleculares de Estudo Epidemiológico de Bactérias

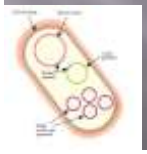
- Genotípicos
  - Análise plasmidial
  - Polimorfismo de fragmentos de restrição (RFLP)
  - " Southern Blot" RFLP (Ex. Ribotipagem)
  - Eletroforese em gel de campo pulsado (PFGE)
  - Técnicas baseadas em PCR
    - PCR- RFLP
    - REP-PCR / ERIC-PCR
    - PCR- RIBOTIPAGEM
    - PCR randômico (AP-PCR / RAPD)
  - Seqüenciamento de DNA

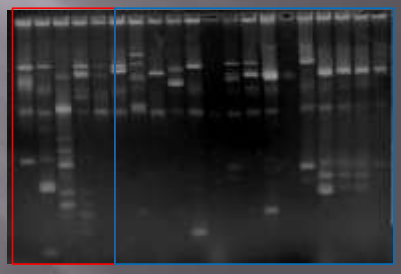
Métodos de genotipagem baseados no padrão de bandas



Epidemiologia molecular

- Perfil plasmidial aEPEC humanos e caninos

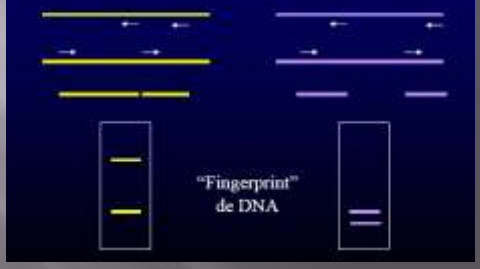




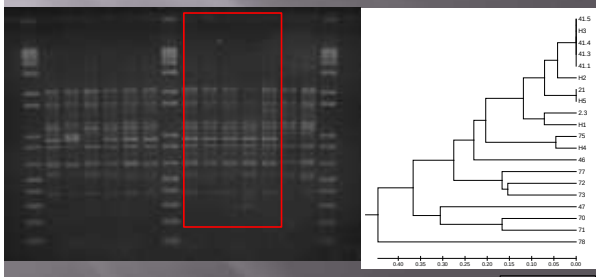
Araes,2010

Randomly Amplified Polymorphic DNA – RAPD

Pequenas quantidades de DNA, dispensa conhecimento prévio de seqüências alvo, único iniciador pequeno (~10 bases) de seqüência inespecífica, baixa estringência.

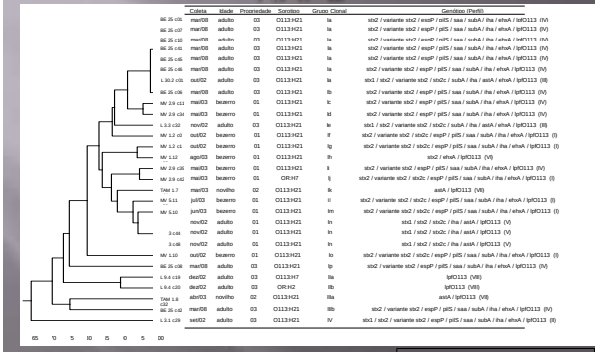


RAPD



Araes,2010

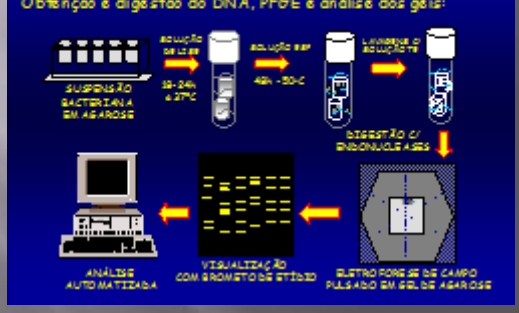
RAPD

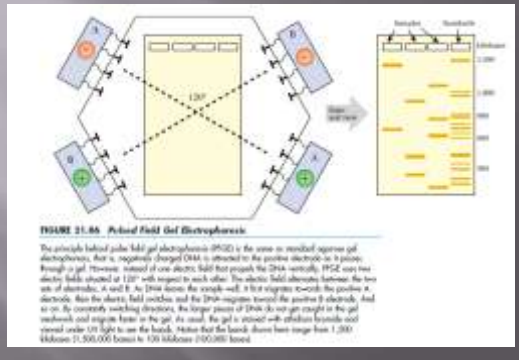


Matheus-Guimarães, 2009

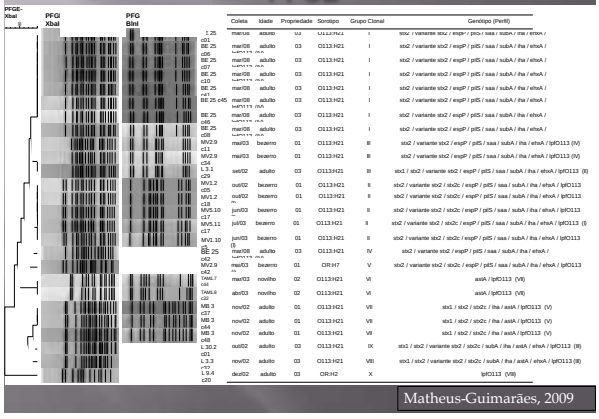
Eletroforese em Gel em Campo Pulsado (Pulsed Field Gel Electrophoresis - PFGE)

Obtenção e digestão do DNA, PFGE e análise dos géis:





PFGE



Matheus-Guimarães, 2009

Métodos de genotipagem baseados no sequenciamento de DNA

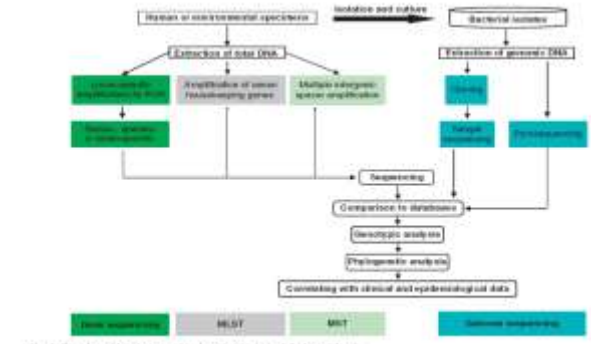
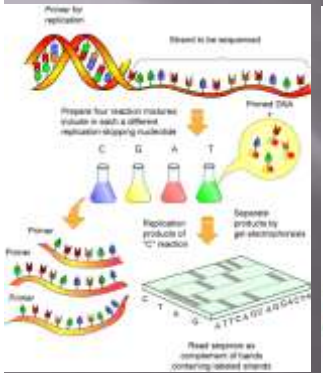
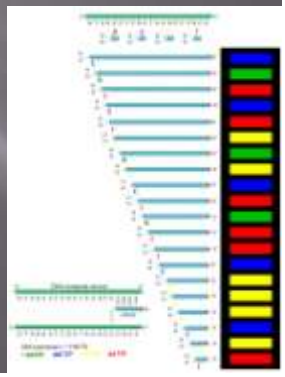
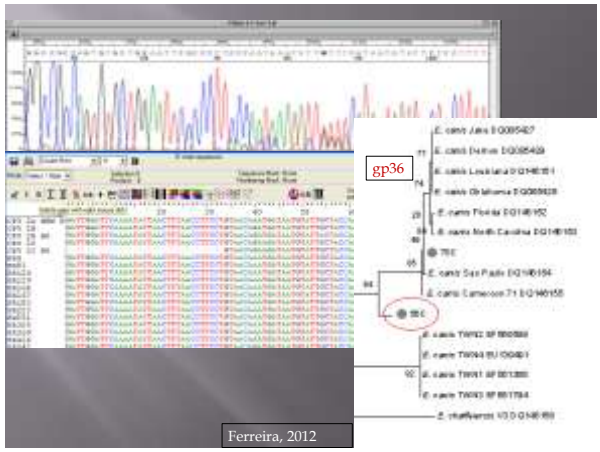


Fig. 2. Flow chart of DNA sequence-based genotyping methods for bacterial strains.

SEQUENCIAMENTO DE DNA

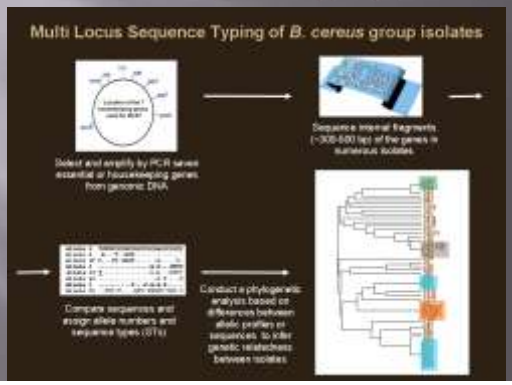






Ferreira, 2012

Multilocus Sequence Typing (MLST)



http://www.pasteur.fr/recherche/genopole/PF8/mlst/primers_Ecoli.html

Métodos de genotipagem baseados em hibridização de DNA

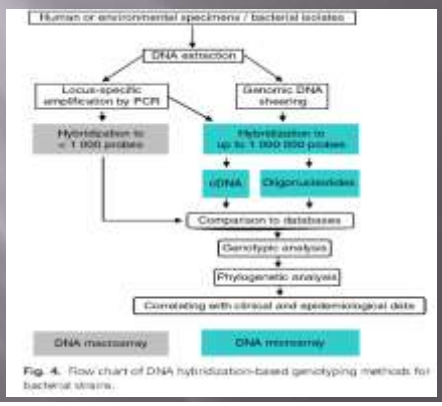
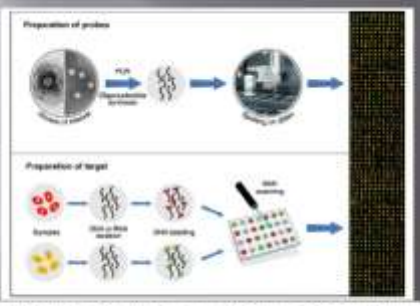
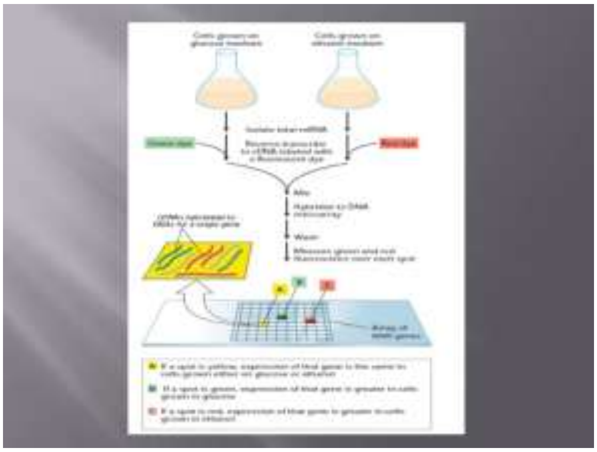
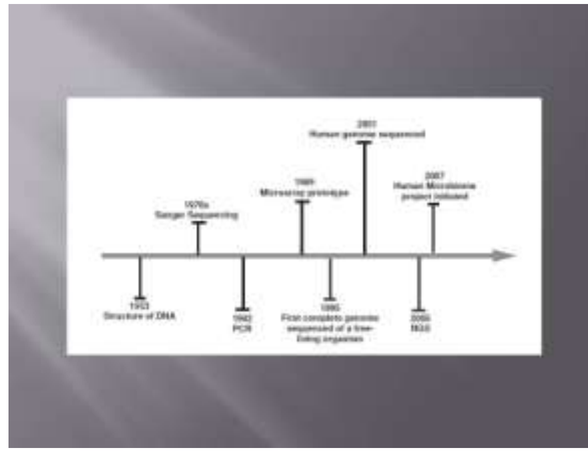


Fig. 4. Flow chart of DNA hybridization-based genotyping methods for bacterial strains.

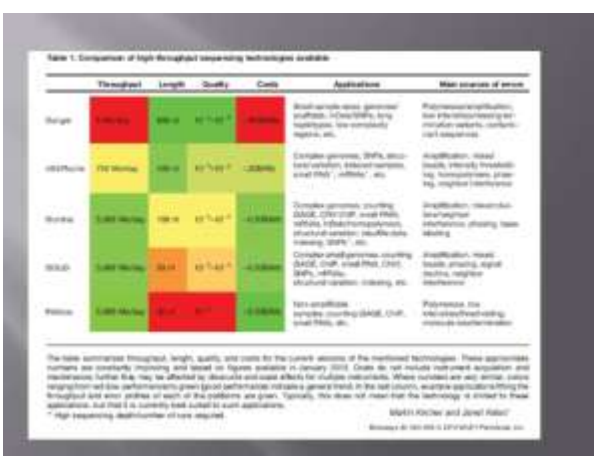
Microarray







Human genome sequenced
Microarray prototype
2007
Human Microbiome project initiated
1970s
Sanger Sequencing
1953
Structure of DNA
PCR
1995
First complete genome sequenced of a free-living organism
NGS



Infection, Genetics and Evolution
Molecular methods for pathogen and microbial community detection and characterization: Current and potential application in diagnostic microbiology
ARTICLE
Structure, function and diversity of the healthy human microbiome

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