

Universidade de São Paulo (USP)
Escola de Engenharia de Lorena (EEL)
Engenharia Química

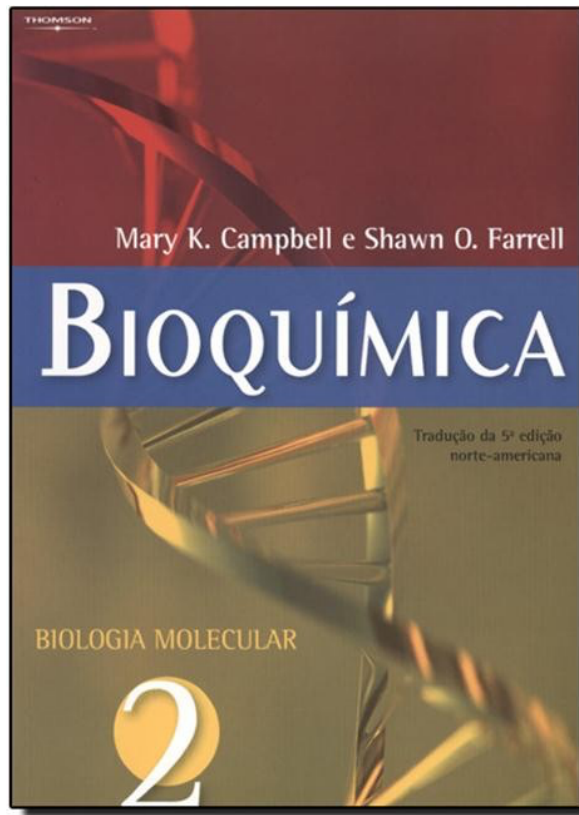


Técnicas e Biotecnologia do Ácido Nucléico

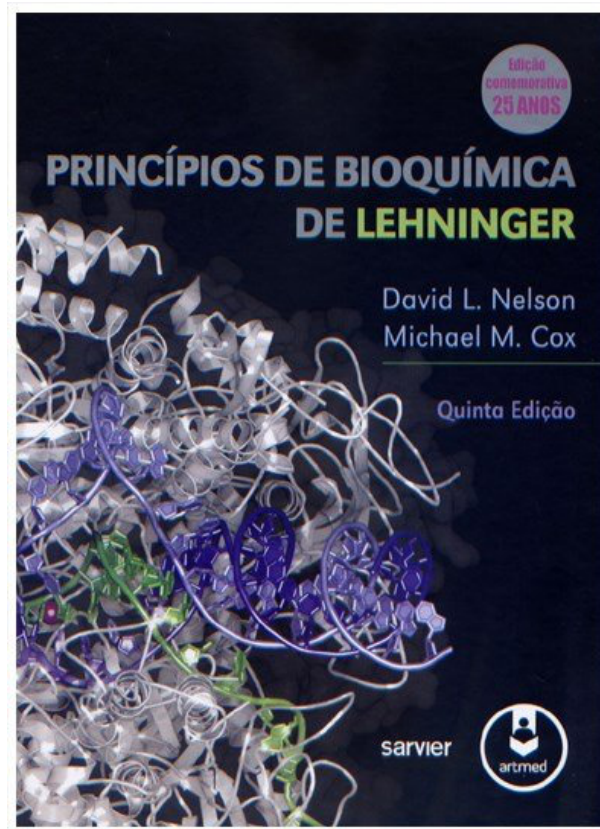
Elisson Romanel

Fontes Bibliográficas

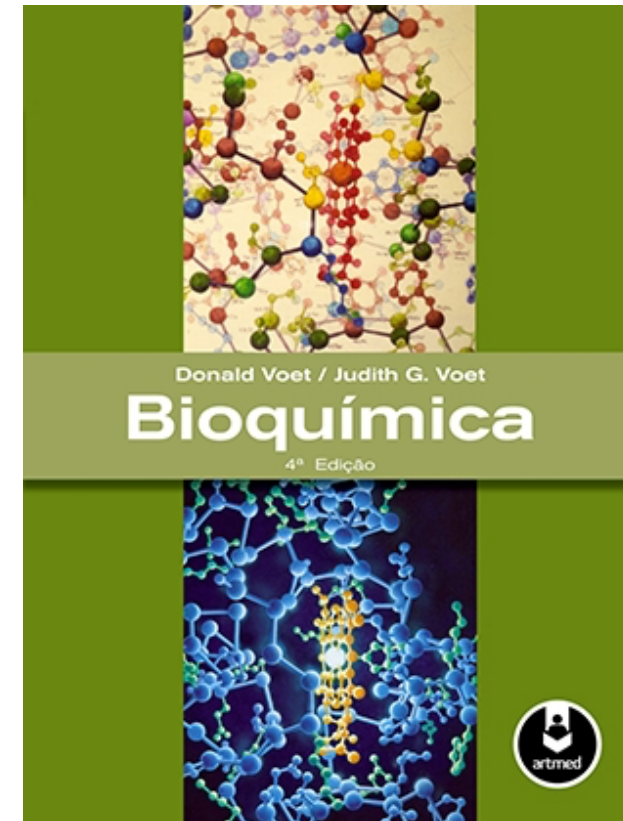
Cap. 13



Cap. 9



Cap. 5



Produtos Aprovados Comercialmente

Produção de insulina *E. coli*

Hormônio do
crescimento humano *E. coli*

Tolerância a herbicida Soja

Resistência a insetos Milho

Resistência a vírus Ameixa



Produtos em Desenvolvimento

Enriquecimento de vitamina Arroz

Vacinas Tabaco

Rápida maturação Salmão



Mosquito transgênico reduz em 81% população de Aedes em bairro de Piracicaba

Empresa diz que inseto diminuiu larvas na região do Cecap, Centro e São Judas; comitiva de cientistas africanos visitou cidade para conhecer projeto.

Melhoria genética vai prolongar efeito do 'Aedes transgênico'

Um melhoramento genético no mosquito modificado vai matar larvas do Aedes fêmea, que pica e transmite doenças, e permitir a sobrevivência de machos. Tecnologia ainda está em testes.



Engenharia Genética

Como tais camundongos foram engenheirados?



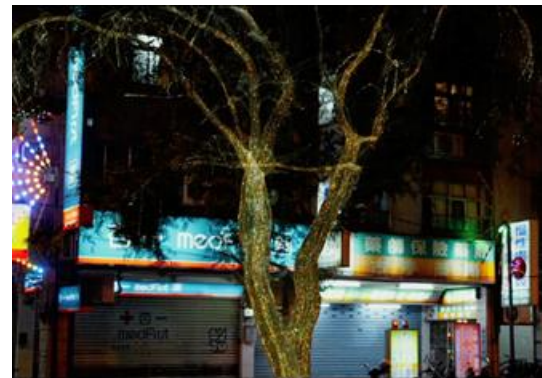
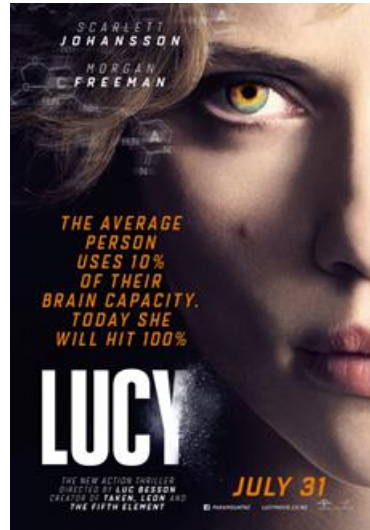
Human 'language gene' makes mice smarter

20:00 15 September 2014 by [Clare Wilson](#)

For similar stories, visit the [Genetics](#) and [Human Evolution](#) Topic Guides

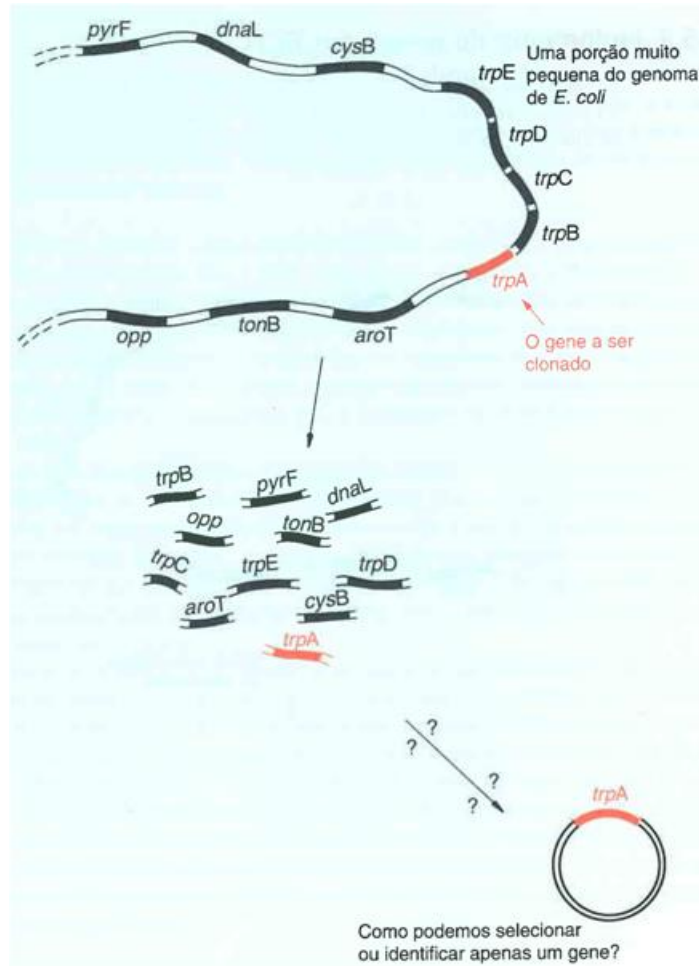
It was named the language gene before we really understood what it did. Now mice given the human version of the *FOXP2* gene are shedding light on how speech evolved in early humans.

Mice with the gene seem to be better at learning to do a task automatically or unconsciously – something we do when we learn a new route to work, for example. The researchers claim that this, in conjunction with other work, suggests the *FOXP2* gene could help us learn to talk as infants by giving us unconscious control over our lips and tongue.

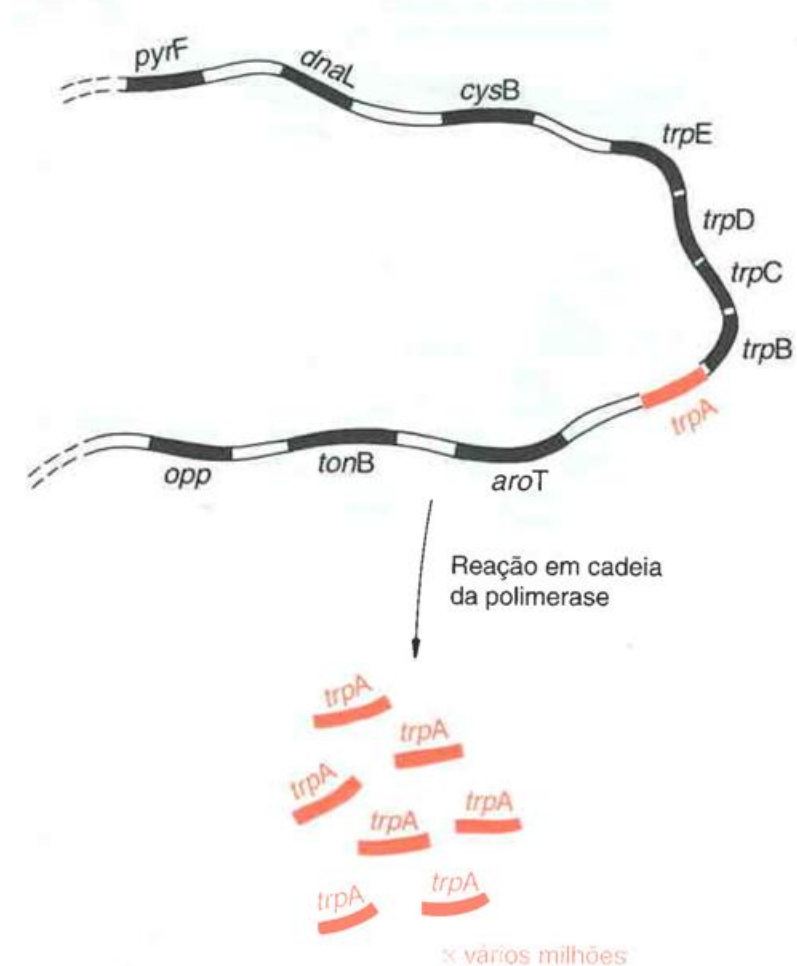


Isolamento de DNA

Isolamento de gene por clonagem:



Isolamento de gene por PCR:



Reação em Cadeia da Polimerase (PCR)

PCR – Reação em cadeia da polimerase (*Polymerase chain reaction*) - 1983



The Nobel Prize in Chemistry 1993
Kary B. Mullis, Michael Smith

The Nobel Prize in Chemistry 1993



Kary B. Mullis



Michael Smith

The Nobel Prize in Chemistry 1993 was awarded "for contributions to the developments of methods within DNA-based chemistry" jointly with one half to Kary B. Mullis "for his invention of the polymerase chain reaction (PCR) method" and with one half to Michael Smith "for his fundamental contributions to the establishment of oligonucleotide-based, site-directed mutagenesis and its development for protein studies".

Olha, há disponibilidade
das sequencias
genômicas na internet

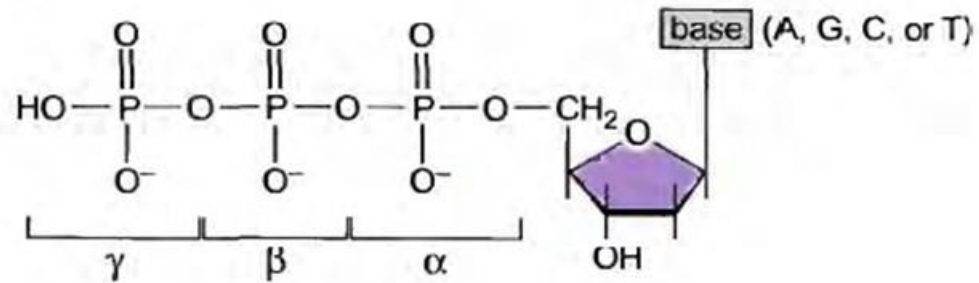


Reação em Cadeia da Polimerase (PCR)

Reação *in vitro*

Componentes da reação:

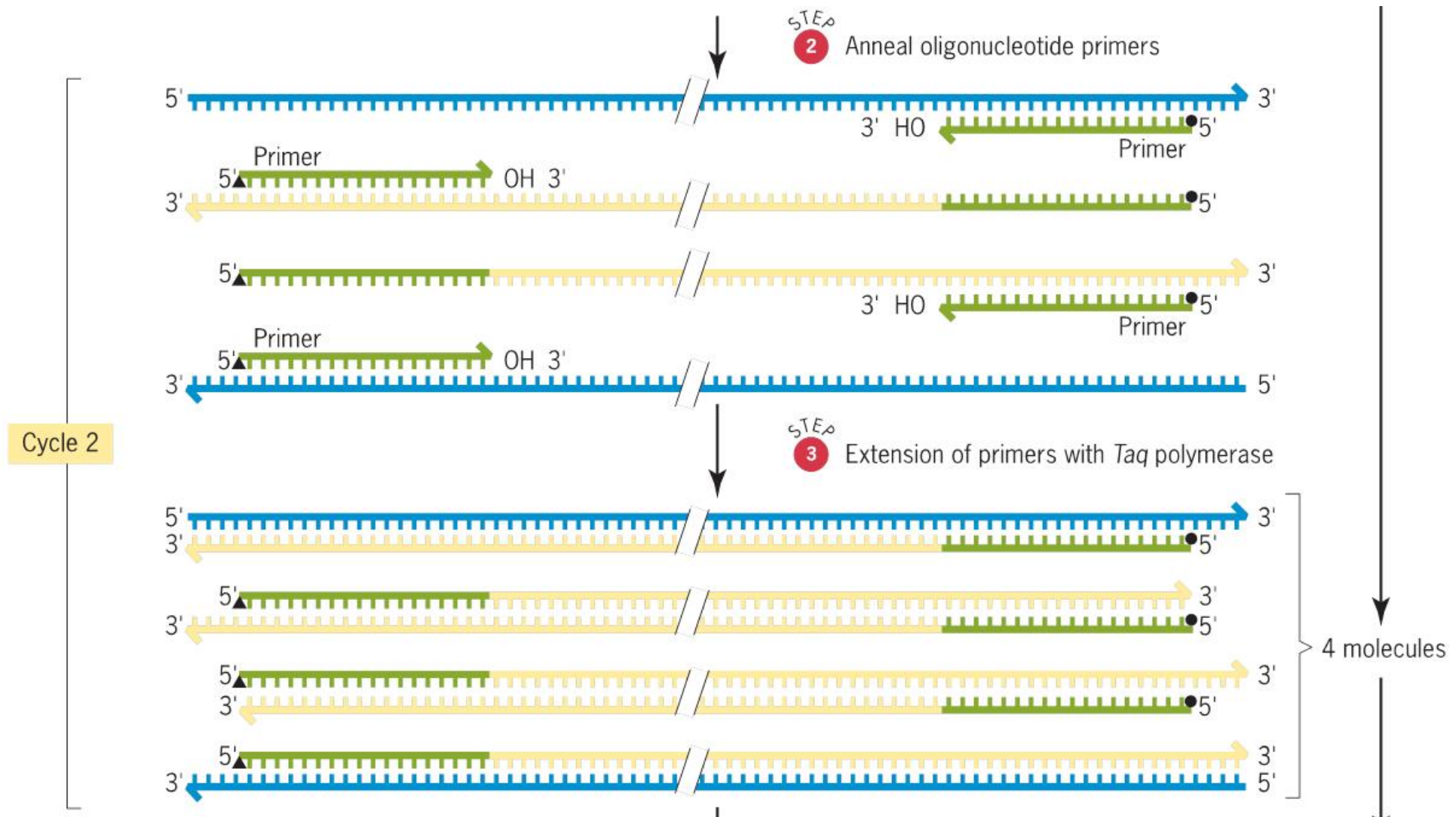
- DNA molde
- Iniciadores (*primer*)
- dNTPs
- DNA polimerase
- Magnésio
- Tampão
- Água



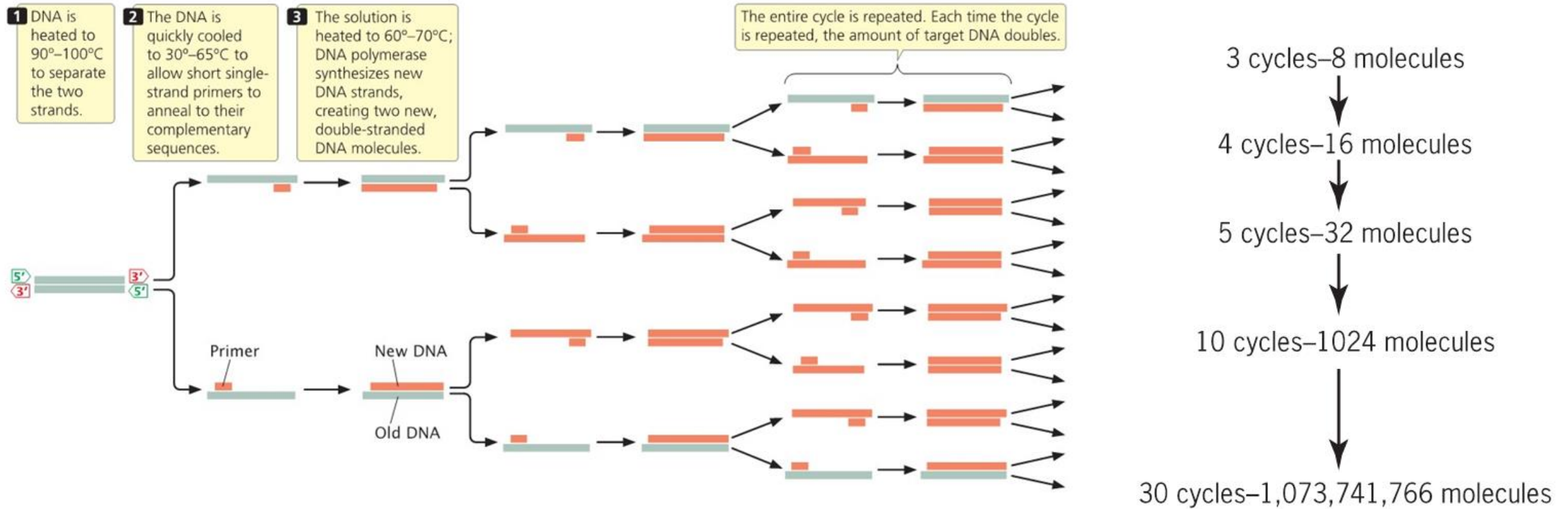
Reação em Cadeia da Polimerase (PCR)



Reação em Cadeia da Polimerase (PCR)



Reação em Cadeia da Polimerase (PCR)



Yellowstone National Park



- *Taq* polymerase (*Thermus **a**quaticus*)

Inovações Tecnológicas

Termociclador



Vídeo

Polymerase Chain Reaction (PCR)

PCR – Polymerase Chain Reaction

Aplicações Biotecnológicas



CAÇOS CRIMINAIS EM QUE A ANÁLISE DE DNA FOI USADA EM 2016

38%
estupros

34%
homicídios

28%
roubos, furtos,
identificação humana e
pessoas desaparecidas

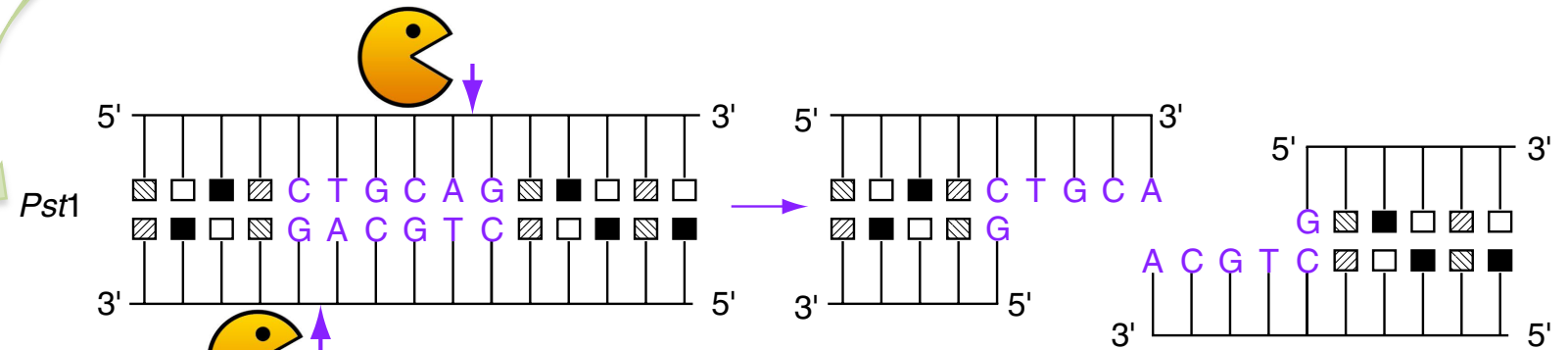
Edição de arte/Agência Brasília



Importância das Enzimas de Restrição



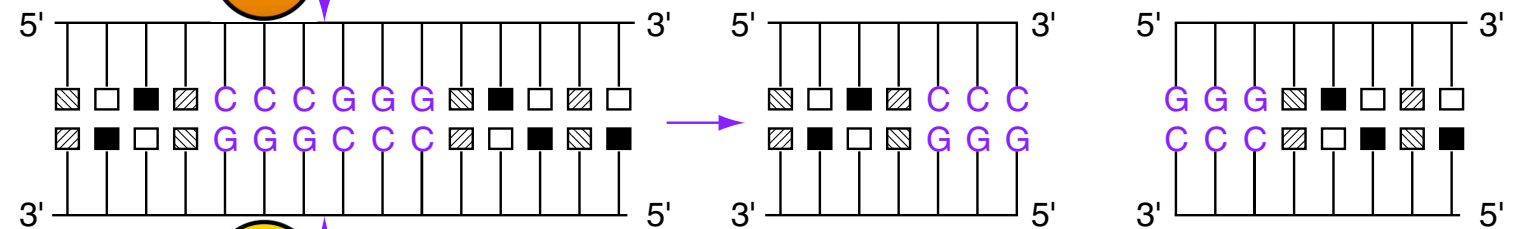
Providencia stuartii



- Pontas adesivas/coesiva

- Hibridização

*Sma*I



Blunt ends

Serratia marcescens

- Pontas cega/reta

Importância das Enzimas de Restrição

TABLE 13.1 Restriction Endonucleases and Their Cleavage Sites

Enzyme*	Recognition and Cleavage Site	Enzyme*	Recognition and Cleavage Site
<i>Bam</i> HI	↓ 5'-GGATCC-3' 3'-CCTAGG-5' ↑	<i>Hpa</i> II	↓ 5'-CCGG-3' 3'-GGCC-5' ↑
<i>Eco</i> RI	↓ 5'-GAATTC-3' 3'-CTTAAG-5' ↑	<i>Not</i> I	↓ 5'-GCGGCCGC-3' 3'-CGCCGGCG-5' ↑
<i>Hae</i> III	↓ 5'-GGCC-3' 3'-CCGG-5' ↑	<i>Pst</i>	↓ 5'-CTGCAG-3' 3'-GACGTC-5' ↑
<i>Hind</i> III	↓ 5'-AAGCTT-3' 3'-TTCGAA-5' ↑		

Palíndromo

Descoberta das Enzimas de Restrição

The Nobel Prize in Physiology or Medicine 1978



Werner Arber
Prize share: 1/3

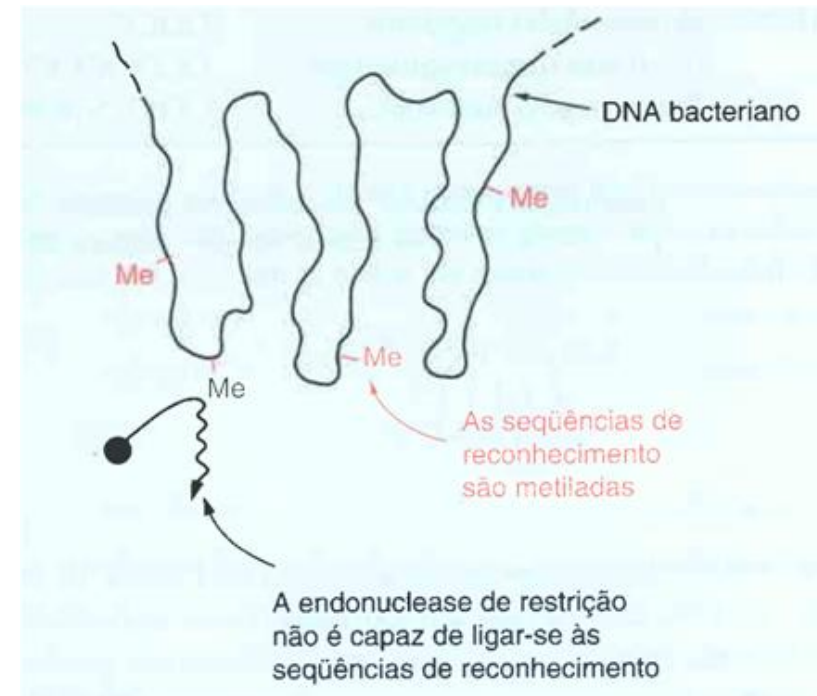
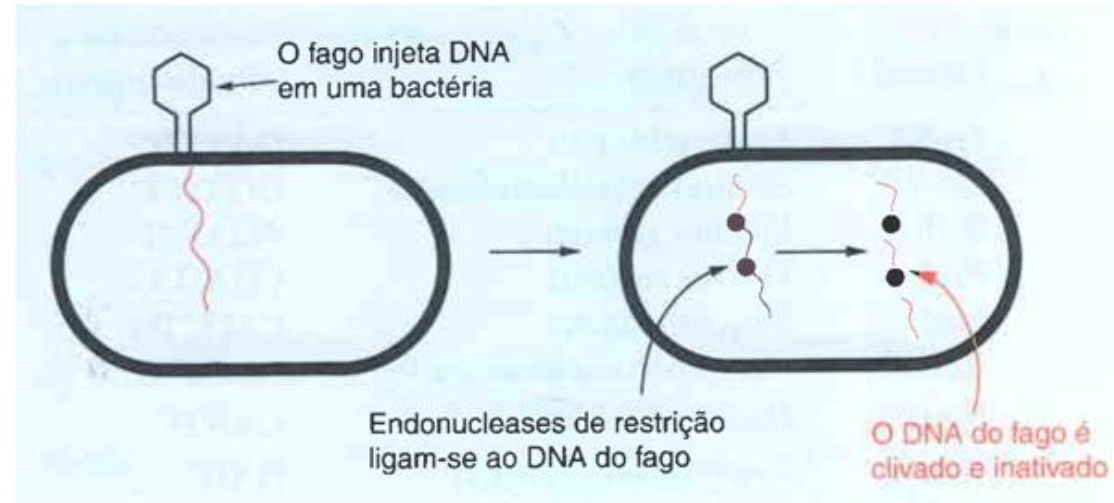


Daniel Nathans
Prize share: 1/3



Hamilton O. Smith
Prize share: 1/3

The Nobel Prize in Physiology or Medicine 1978 was awarded jointly to Werner Arber, Daniel Nathans and Hamilton O. Smith *"for the discovery of restriction enzymes and their application to problems of molecular genetics"*.



The Nobel Prize in Physiology or Medicine 1978



Werner Arber
Prize share: 1/3

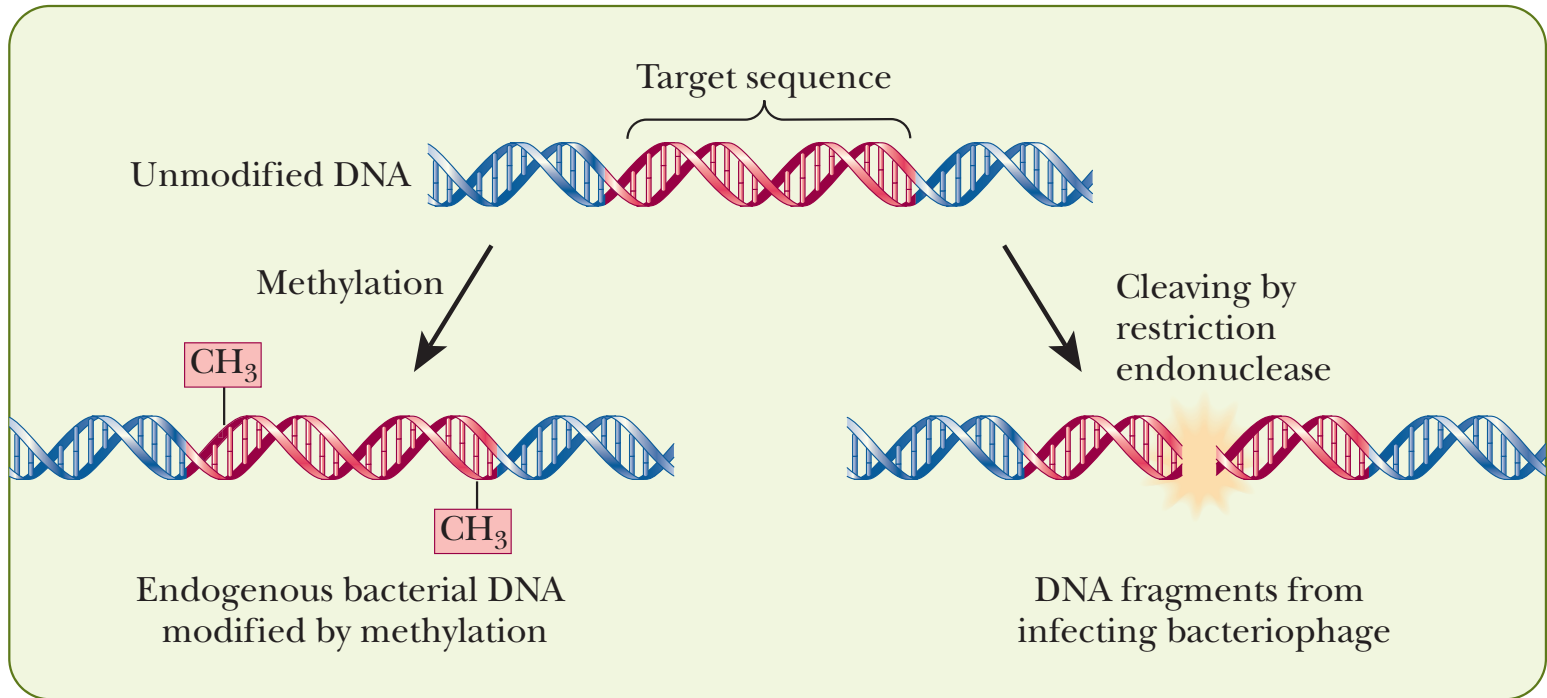


Daniel Nathans
Prize share: 1/3



Hamilton O. Smith
Prize share: 1/3

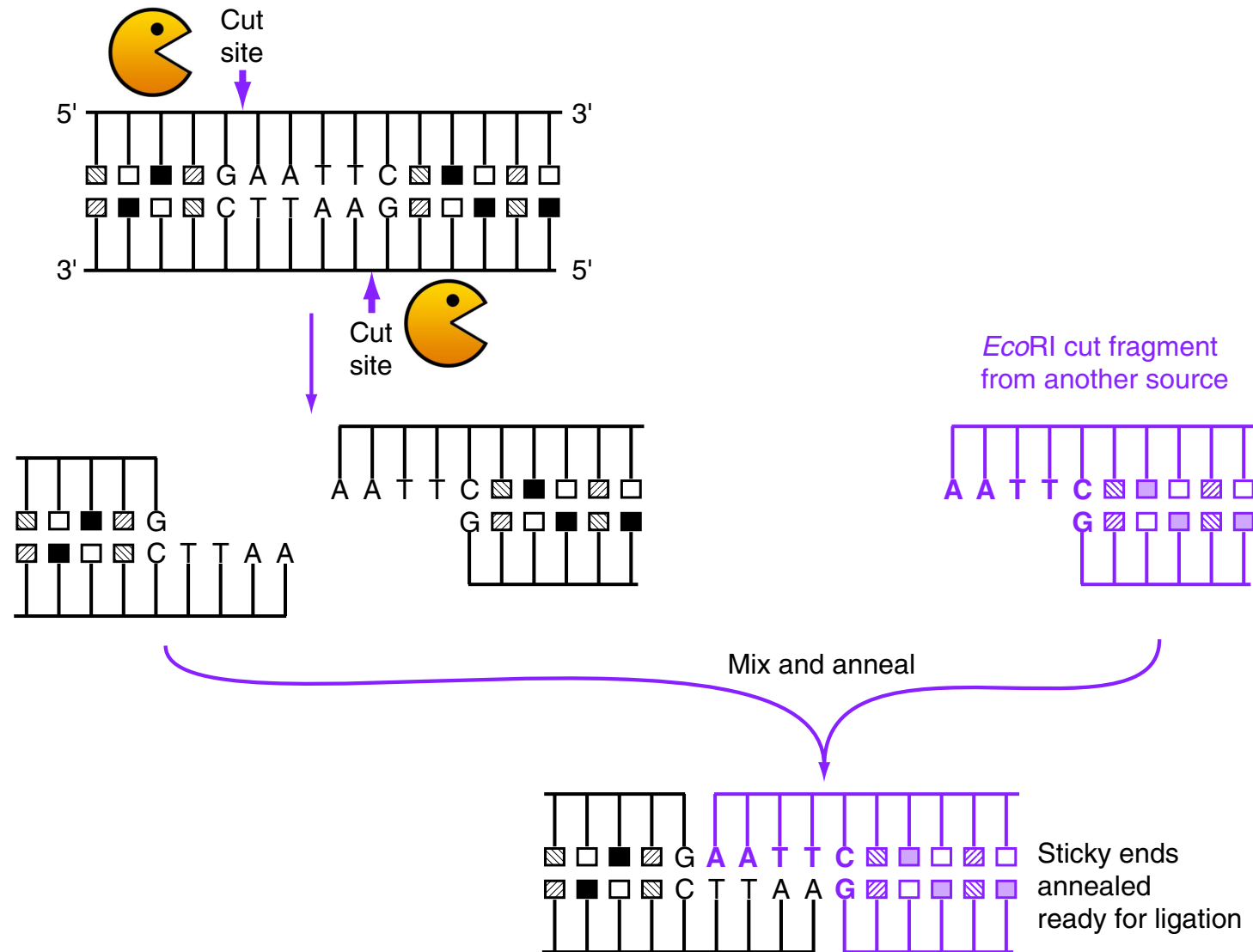
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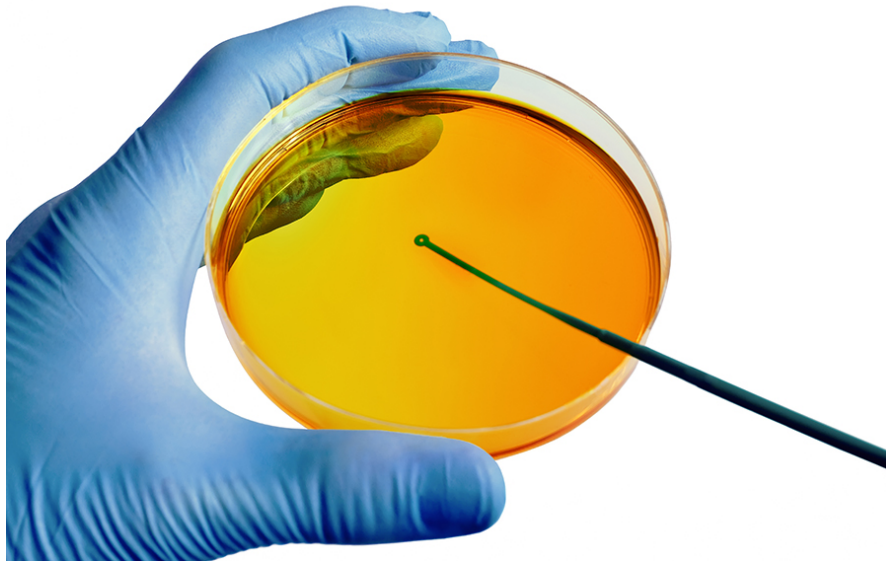
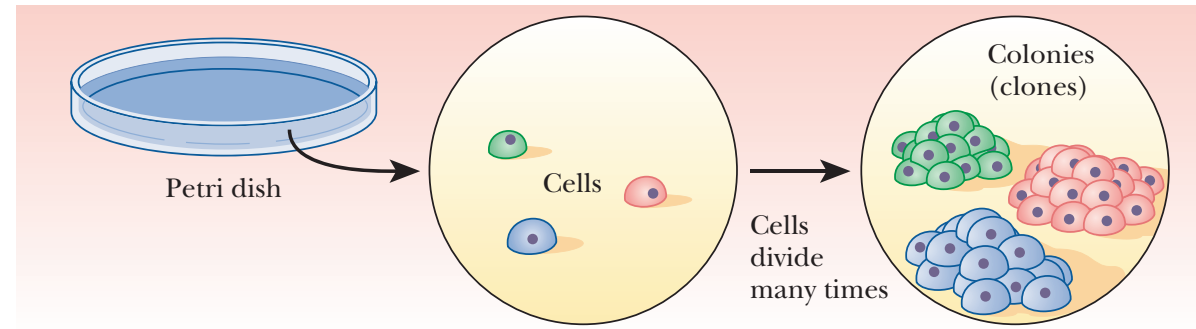
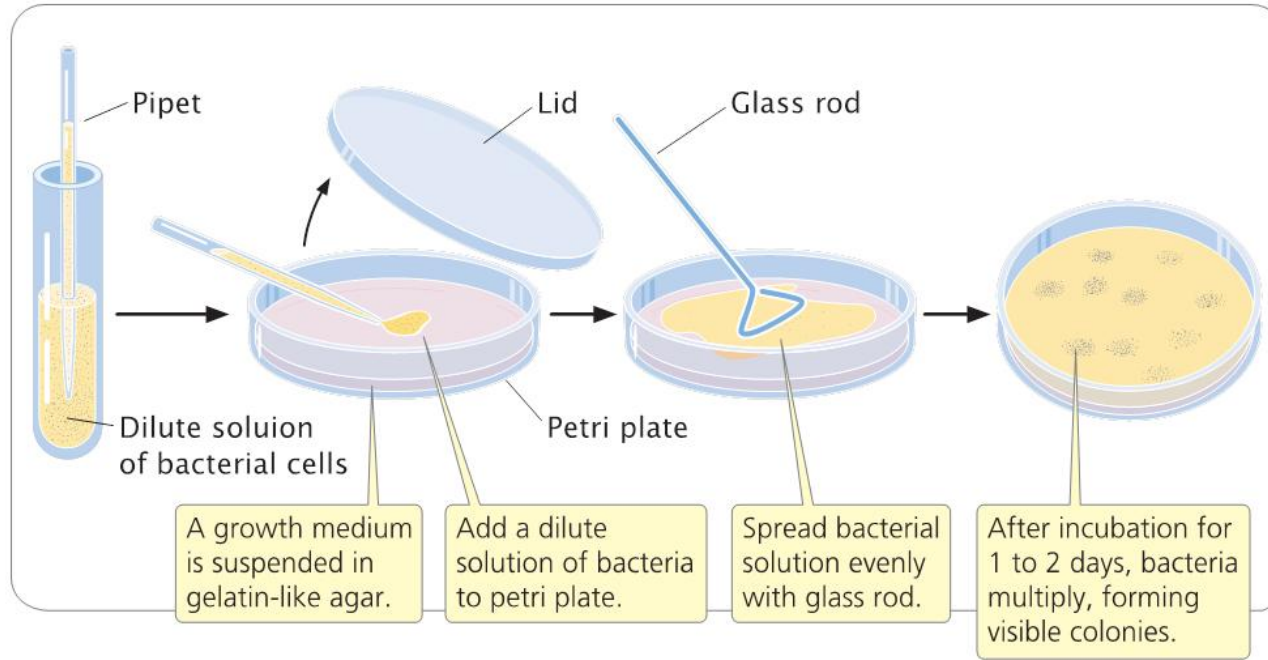
> 800 enzimas de restrição

Construindo DNA recombinante

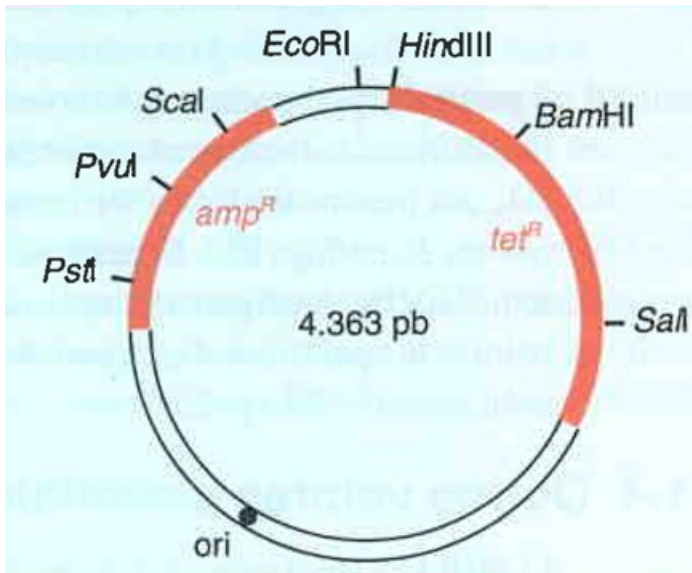
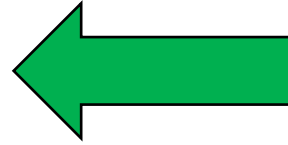
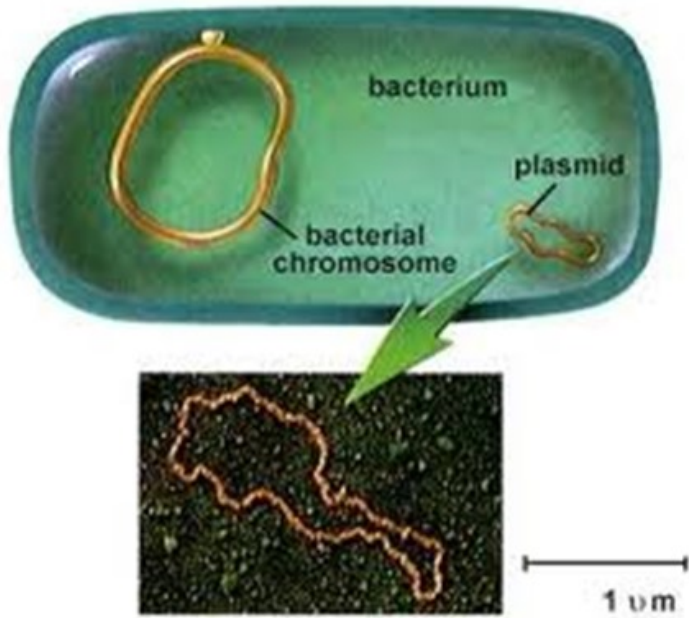
Clivagem de *EcoRI*



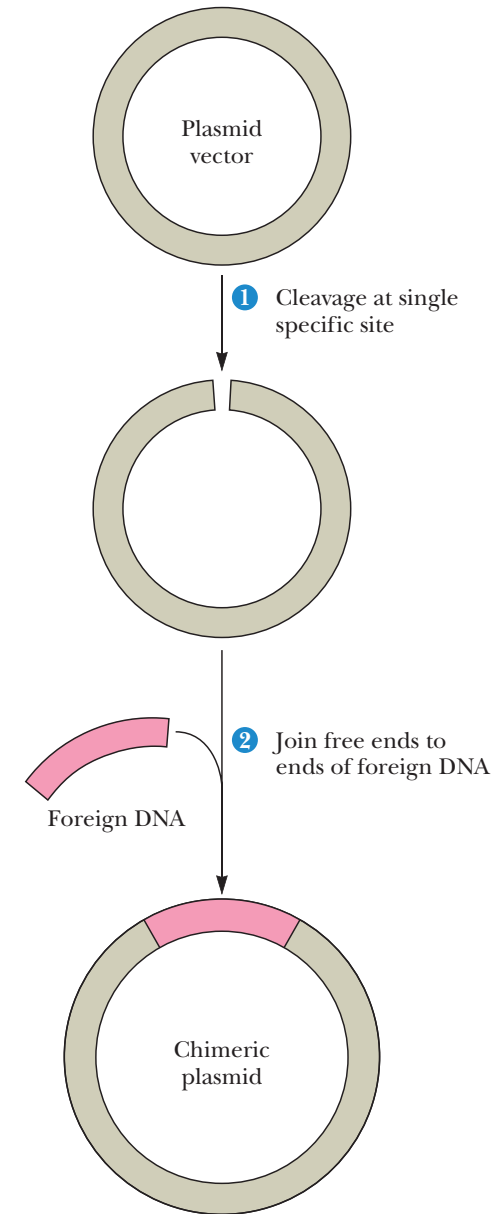
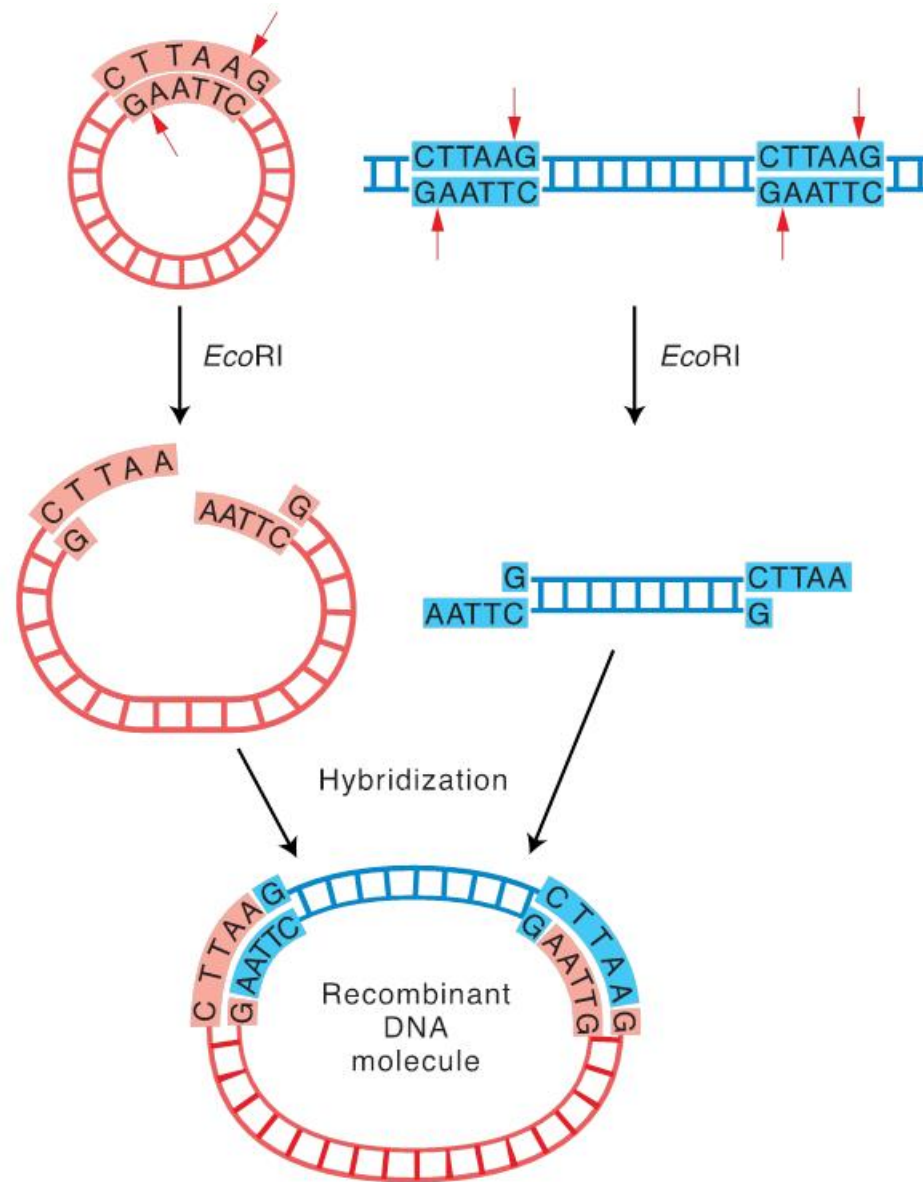
Cultivo de Bactérias



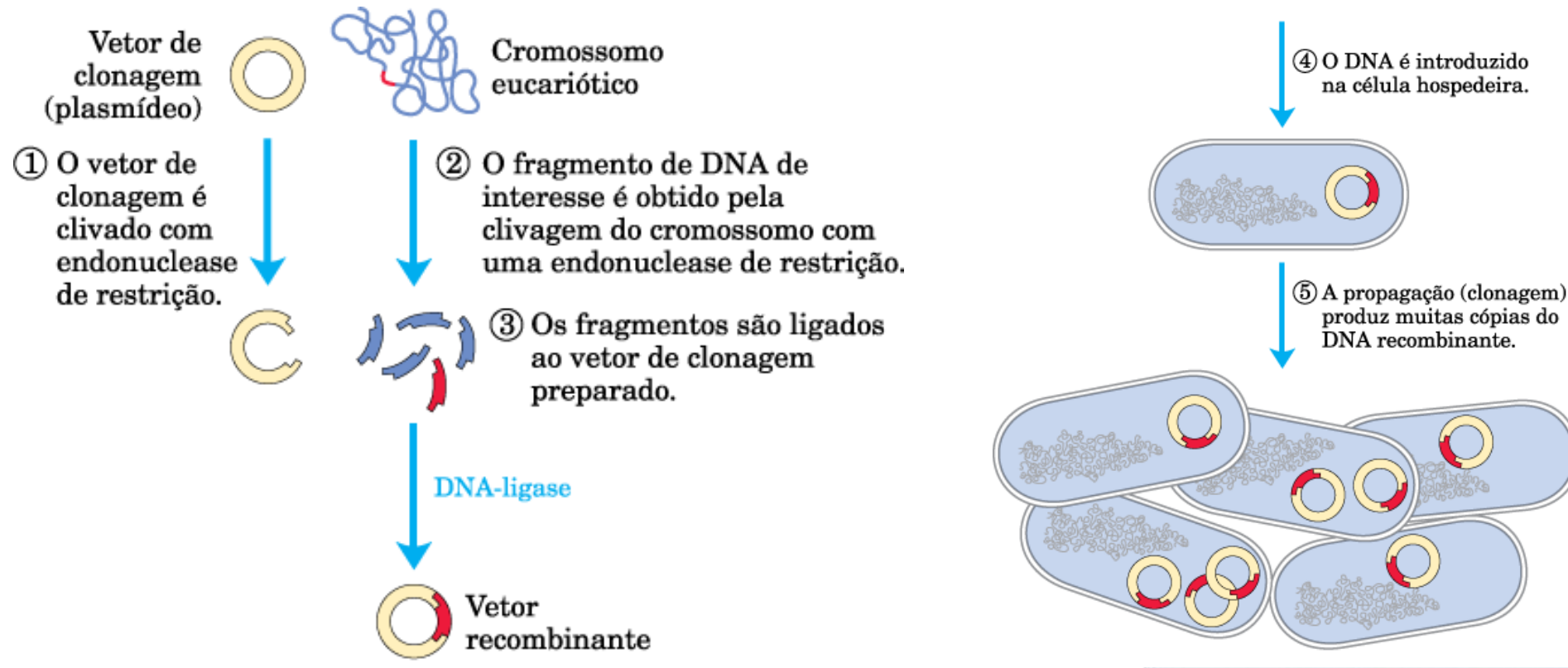
Plasmídeos



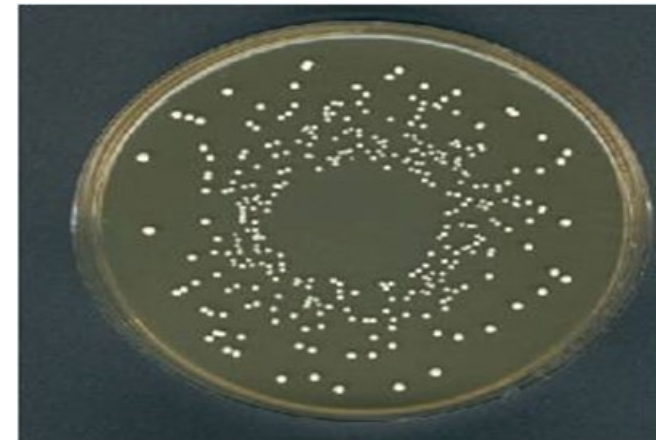
Clonagem



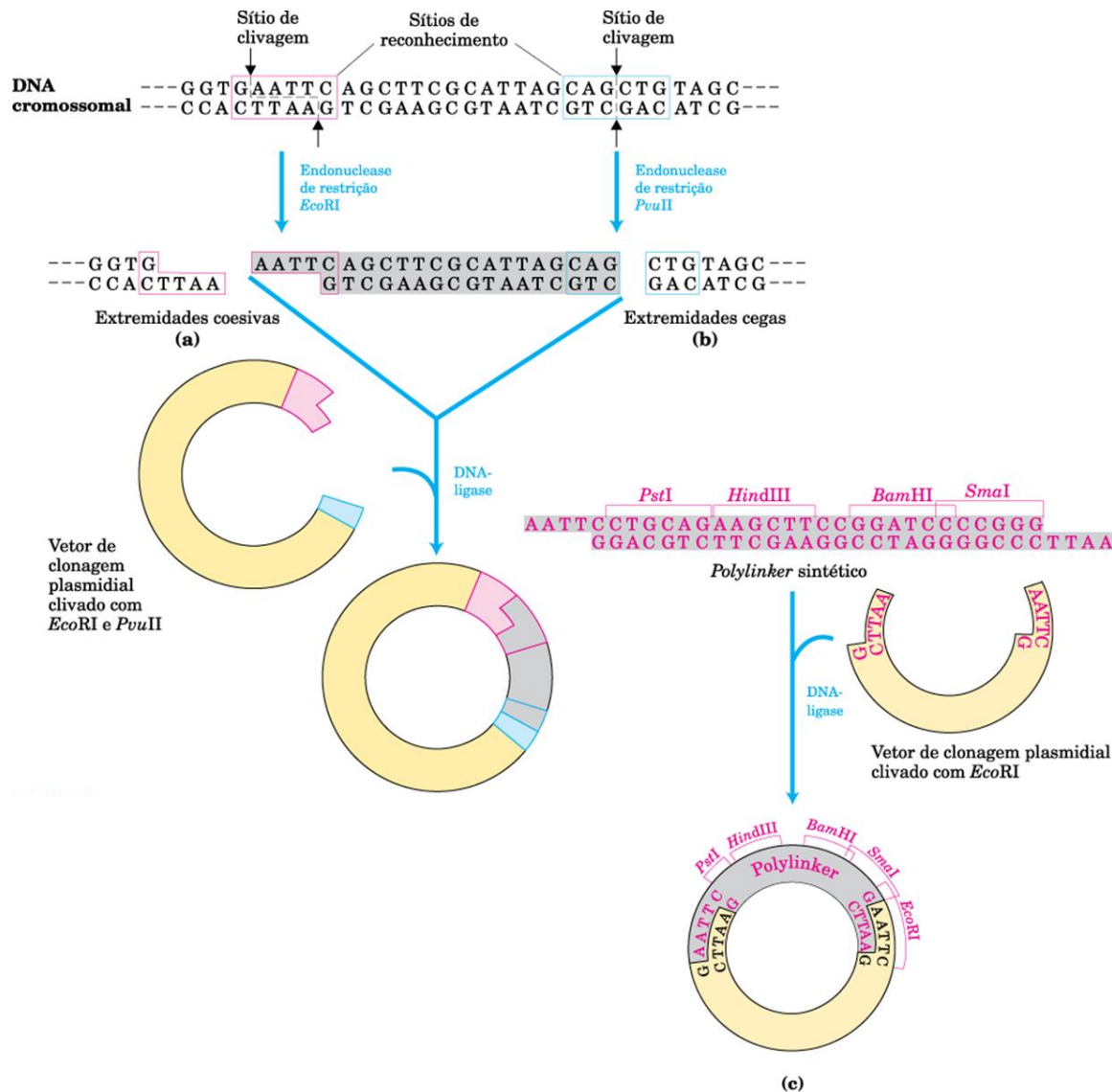
Clonagem



Temos um clone



Clonagem



The Nobel Prize in Chemistry 1980



Paul Berg
Prize share: 1/2



Walter Gilbert
Prize share: 1/4

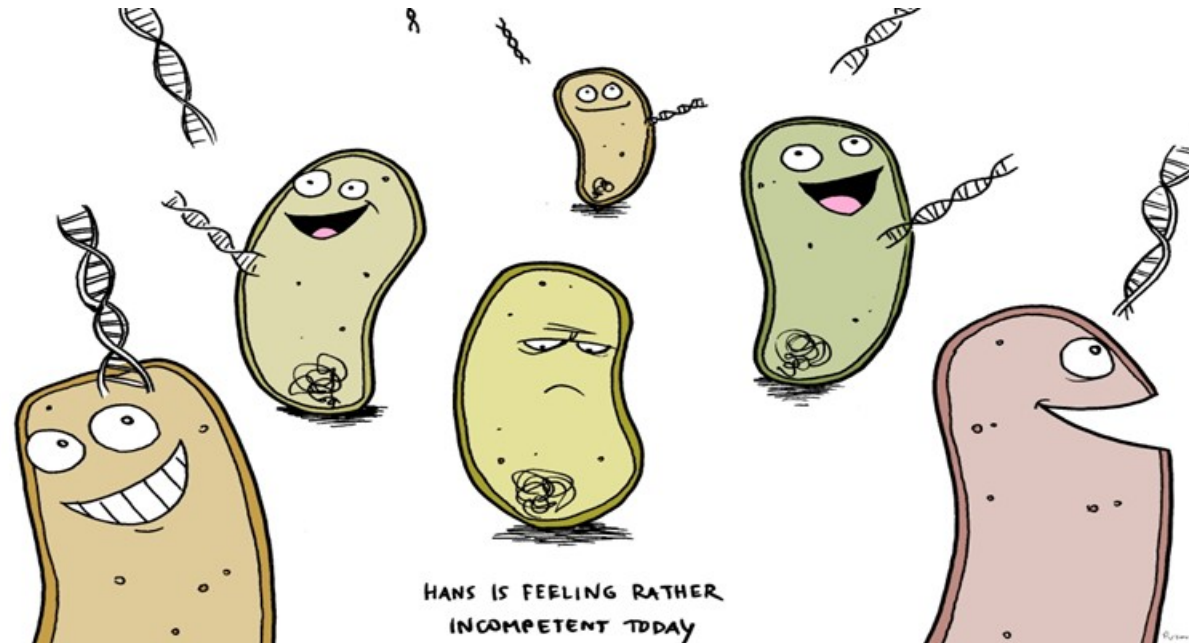
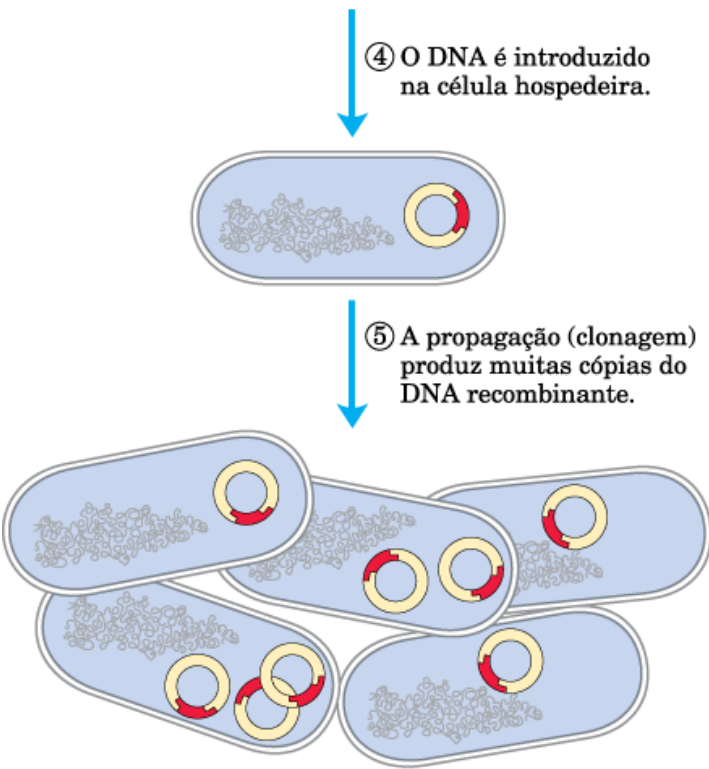


Frederick Sanger
Prize share: 1/4

The Nobel Prize in Chemistry 1980 was divided, one half awarded to Paul Berg "for his fundamental studies of the biochemistry of nucleic acids, with particular regard to recombinant-DNA", the other half jointly to Walter Gilbert and Frederick Sanger "for their contributions concerning the determination of base sequences in nucleic acids".

1971 – Junção de DNA viral símio 40 e DNA bacteriófago λ

Transformação de Bactérias



Em laboratório, poucas espécies conseguem ser transformadas

Incorporam quantidades limitadas de DNA (*E. coli*)

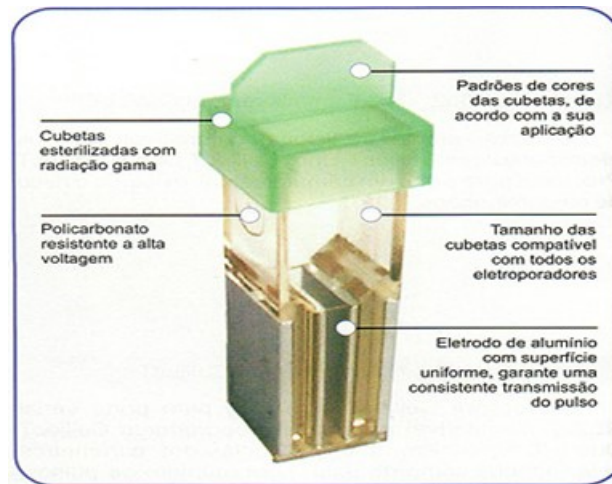
- Eficiência na transformação → Tratamento físico e/ou químico → Competentes

Transformação de Bactérias

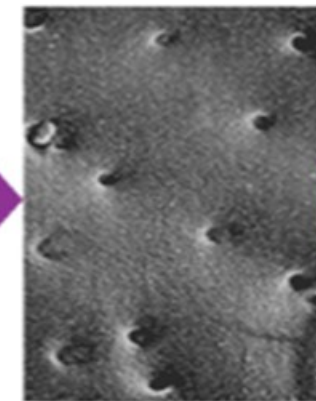
Choque elétrico



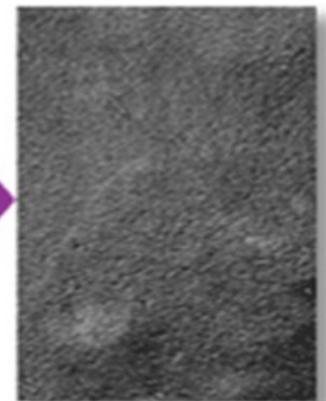
BIO-RAD



Cell membrane
before pulsing

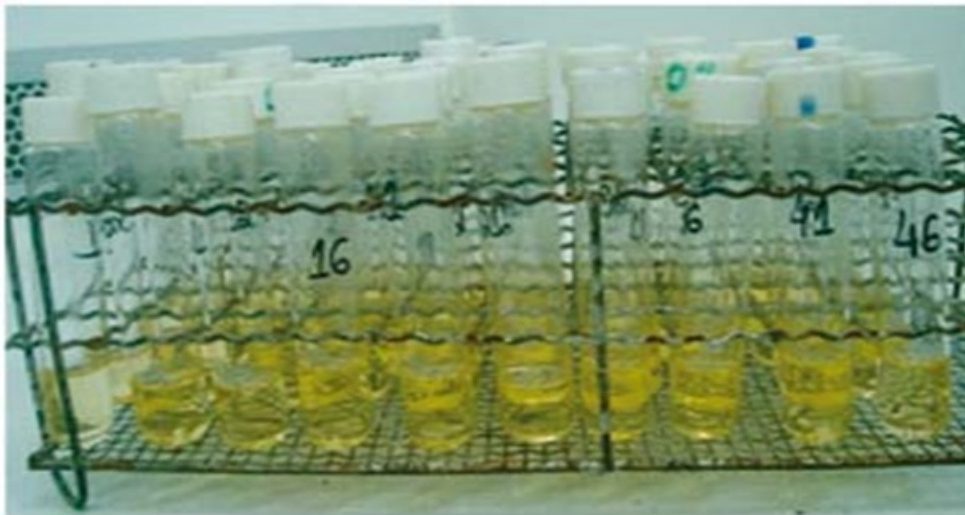
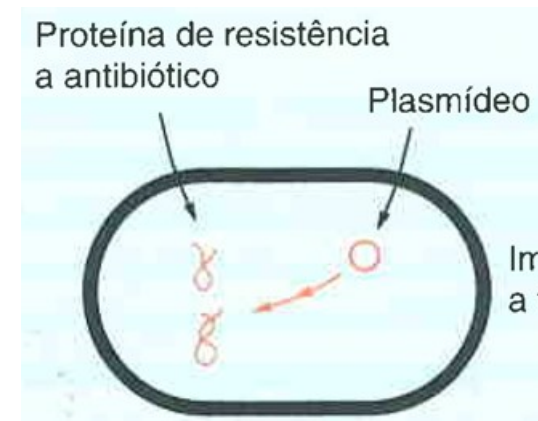


Cell membrane
during pulsing

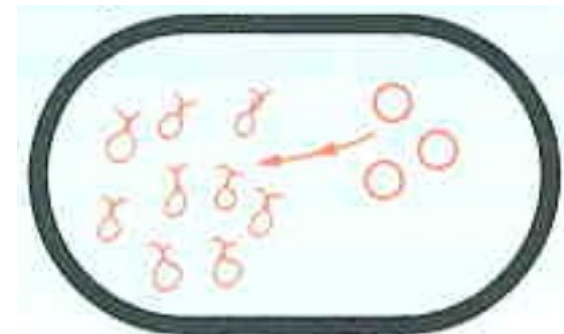


Cell membrane
after pulsing
(cell returns to

Transformação de Bactérias

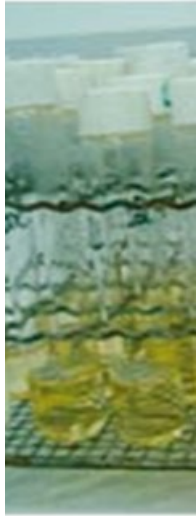


Células incubadas em meio nutritivo



Transformação de Bactérias

Agitador a 37° C

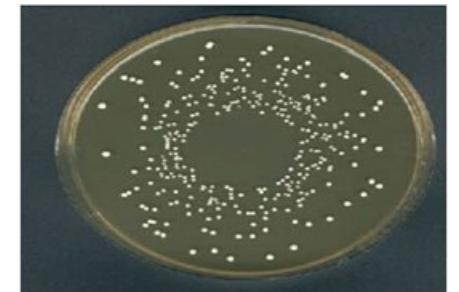
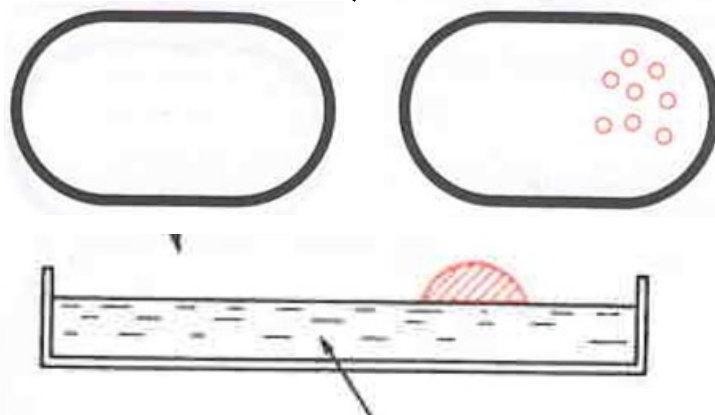
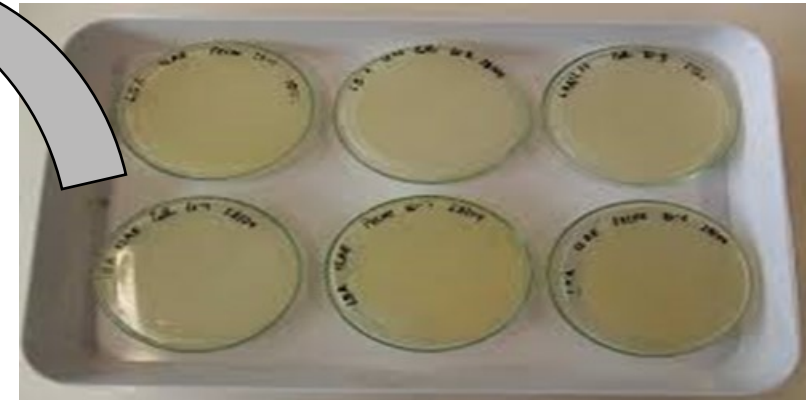
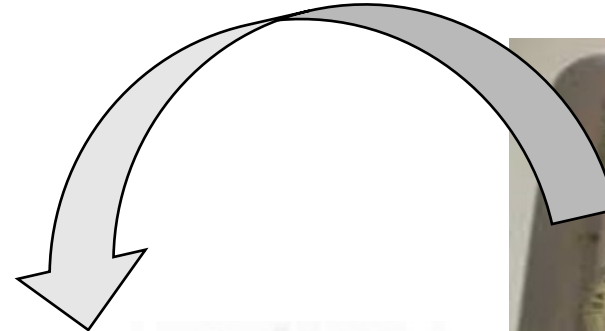


Suspension of bacterial cells



Plaqueamento a 37° C

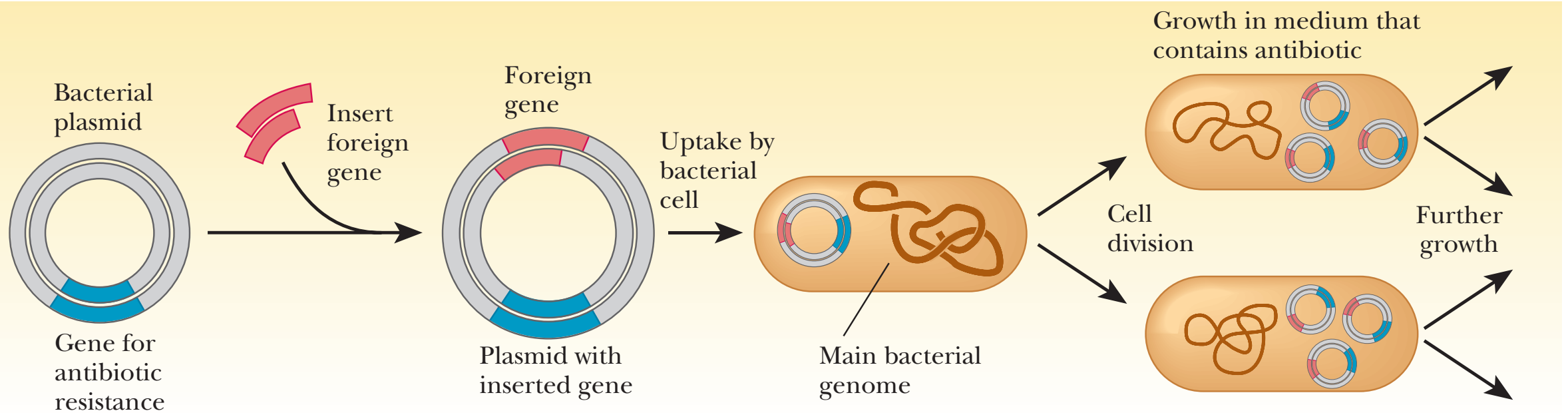
Suspension spread on petri plate with agar gel



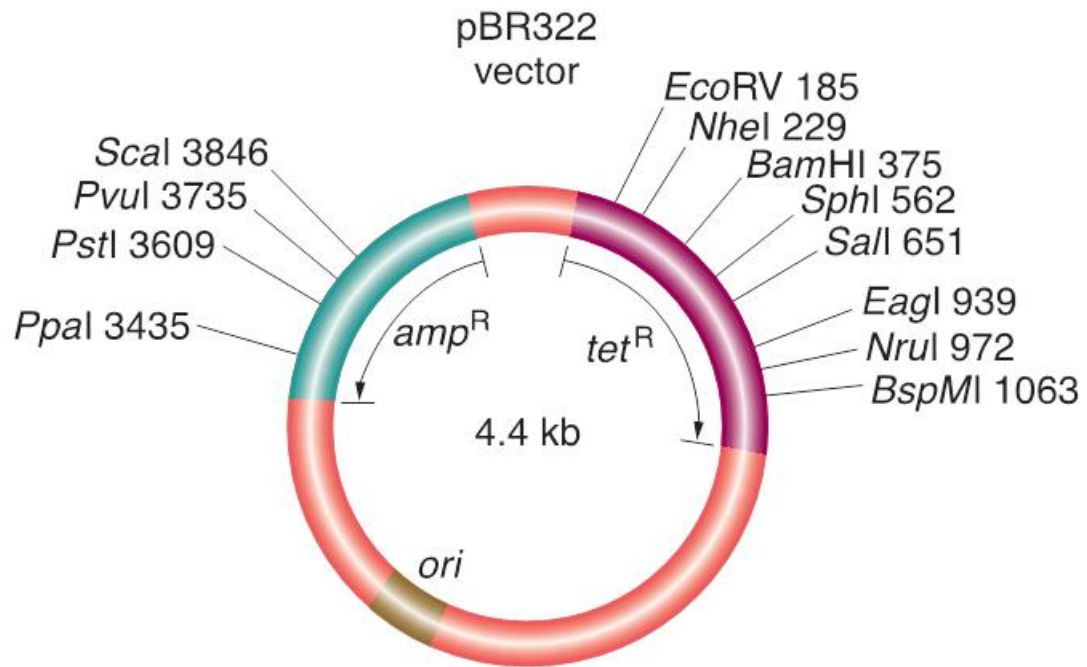
Inovação Biotecnológica



Seleção



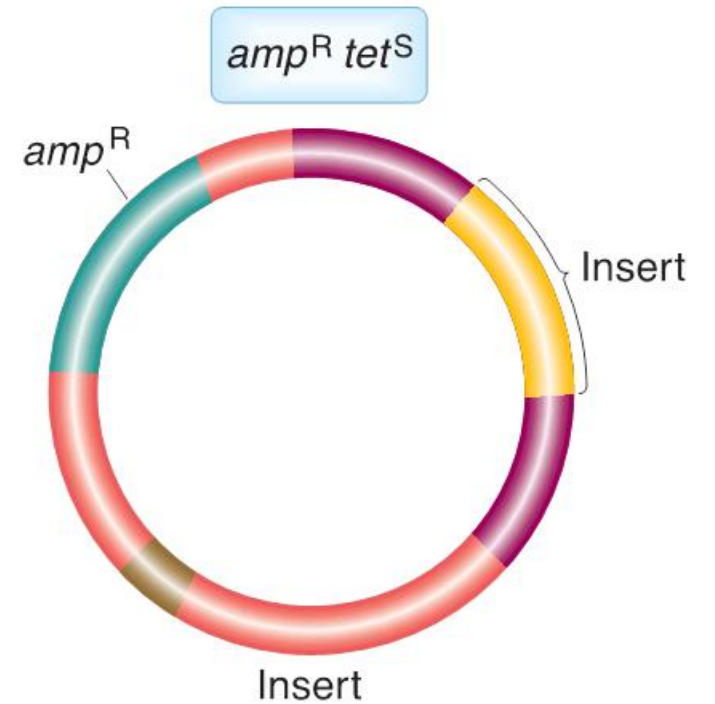
Seleção



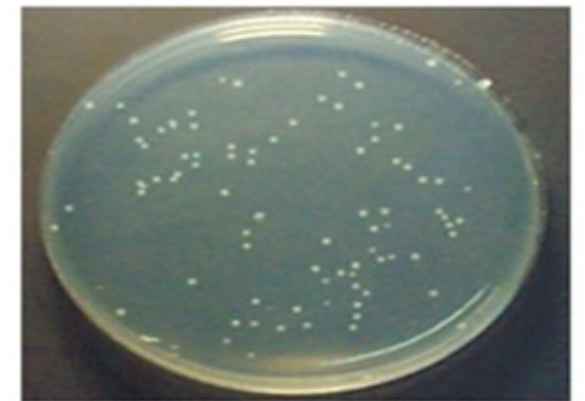
Vamos clonar o fragmento de DNA em *tet^R*

amp^R tet^S

amp⁺ tet

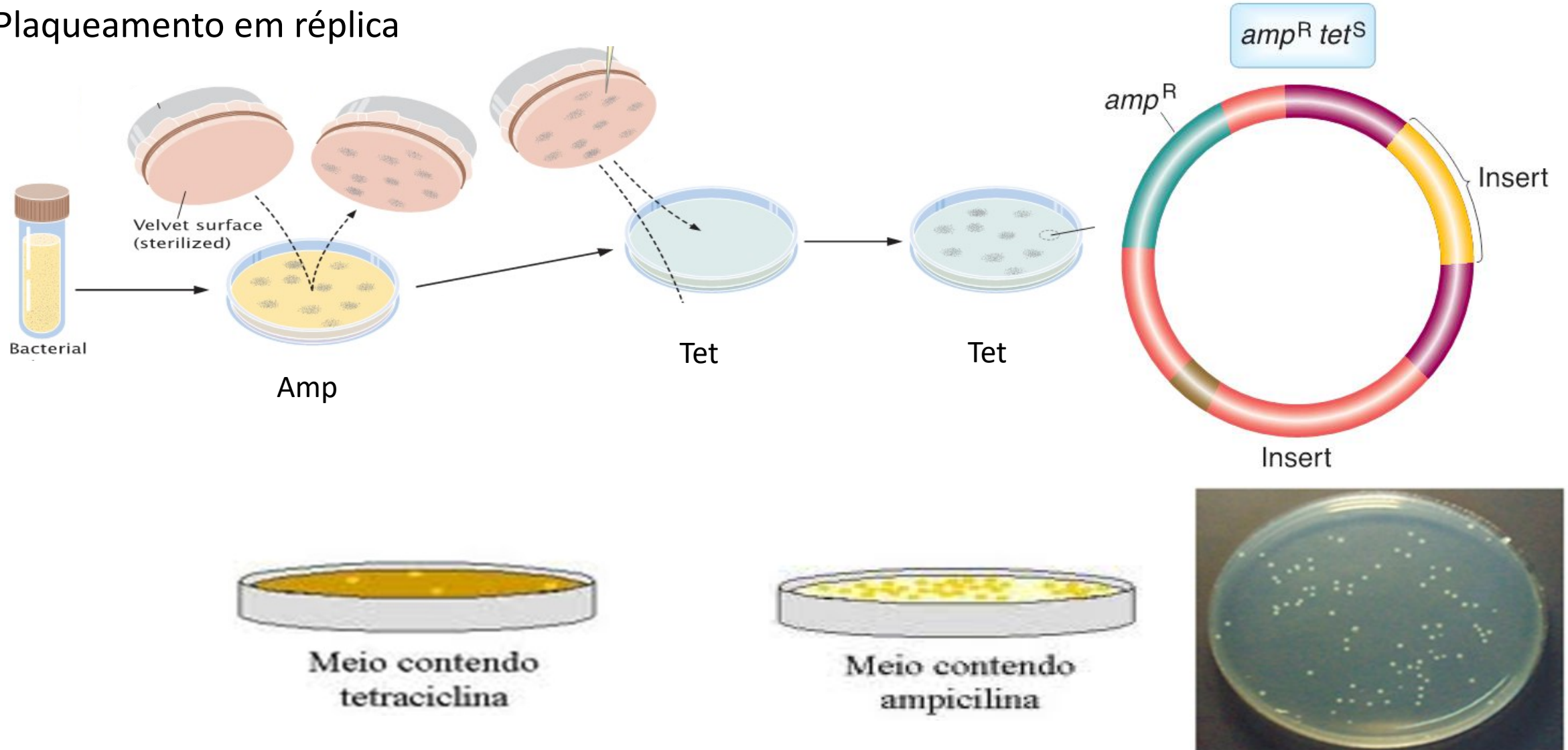


Meio contendo
ampicilina

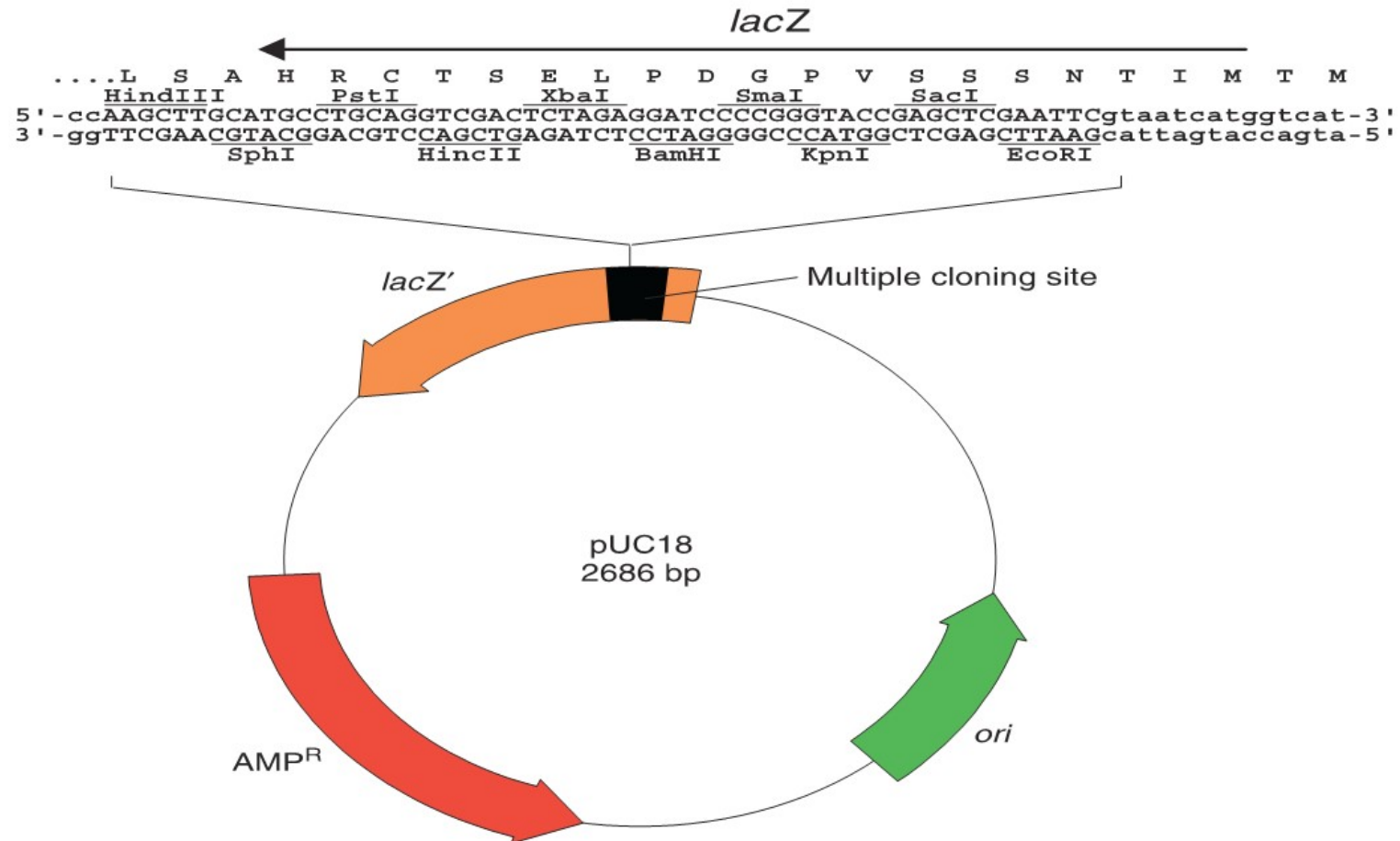


Seleção

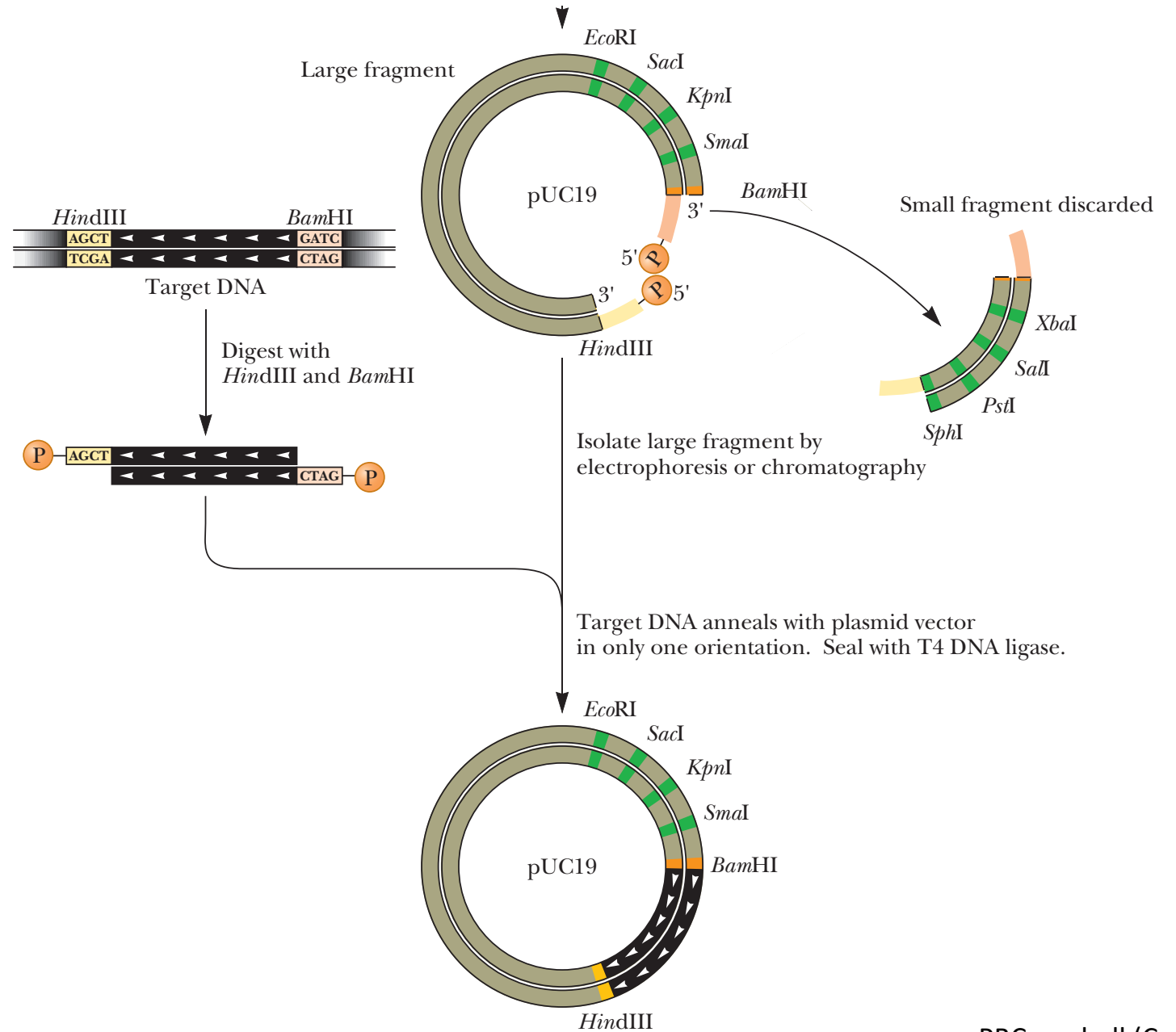
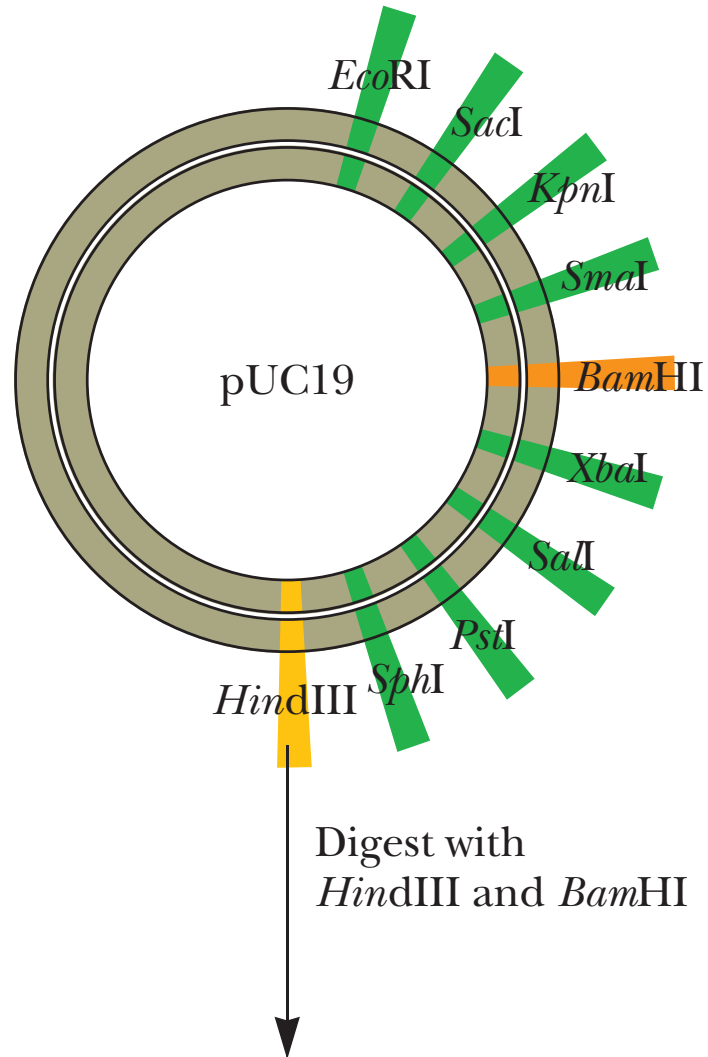
Plaqueamento em réplica



Avanços na clonagem



Sítios múltiplos de clonagem



Marcador de Seleção

lacZ

←

...L S A H R C T S E L P D G P V S S S N T I M T M

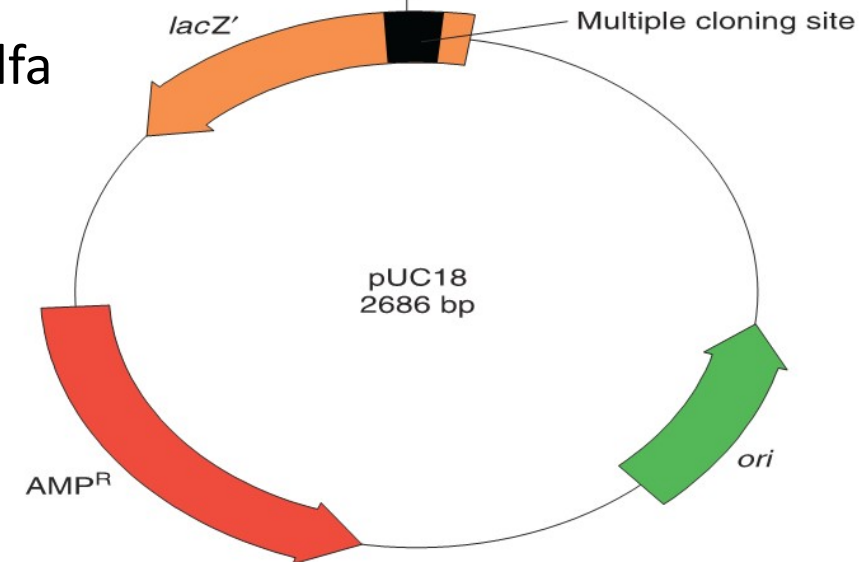
HindIII PstI XbaI SmaI SacI

5'-ccAAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTCgtaatcatgggtcat-3'

3'-ggTTCGAACGTACGGACGTCCAGCTGAGATCTCCTAGGGGCCCATGGCTCGAGCTTAAGcattagtaccagta-5'

SphI HincII BamHI KpnI EcoRI

lacZ' = subunidade alfa



IPTG (isopropiltiogalactosídeo)

X-gal (5-bromo-4-cloro-3-indol- β -D-galactopiranosídeo)

Hidrólise (Produto de cor azul)

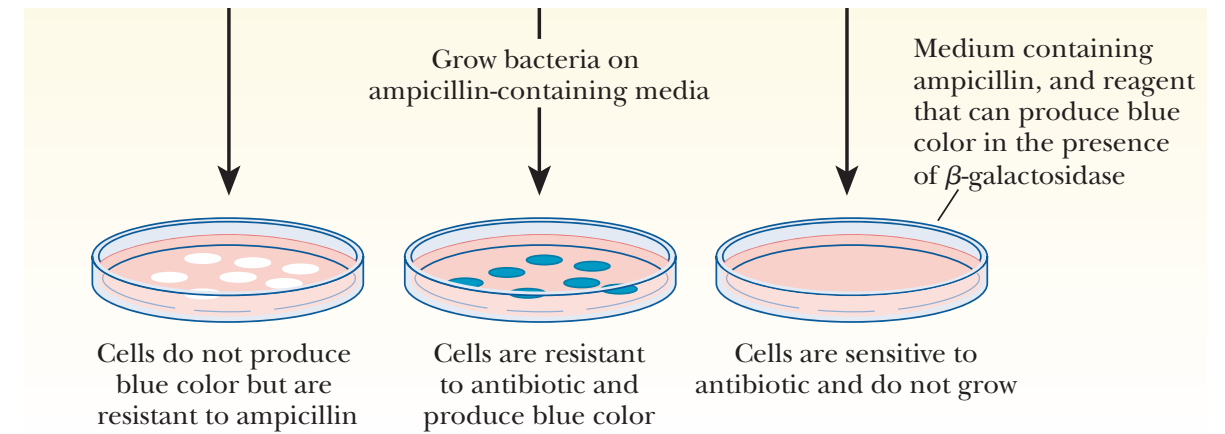
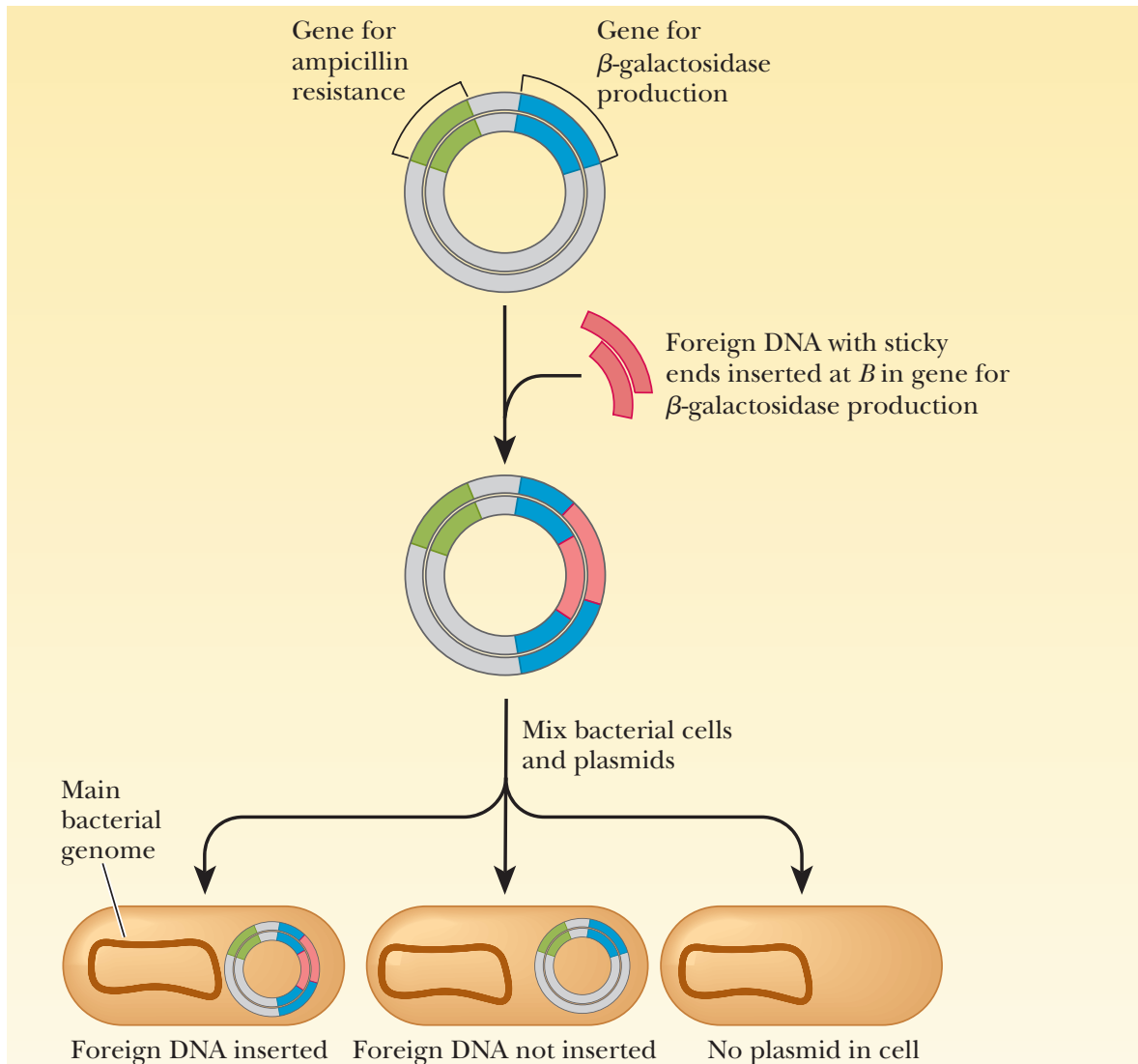
Indutor

β -Galactosidase

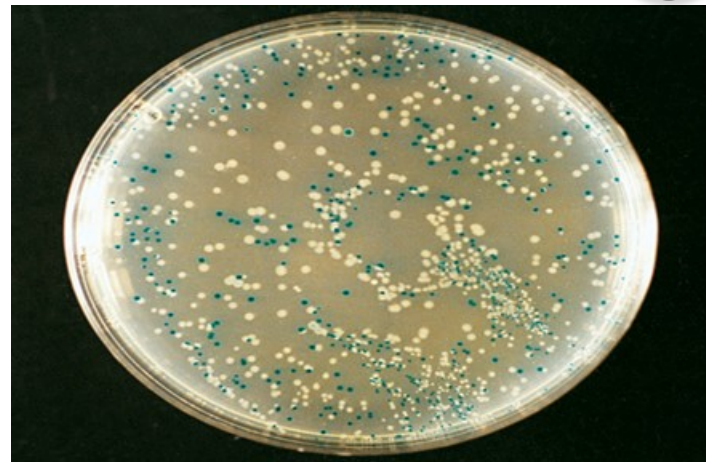
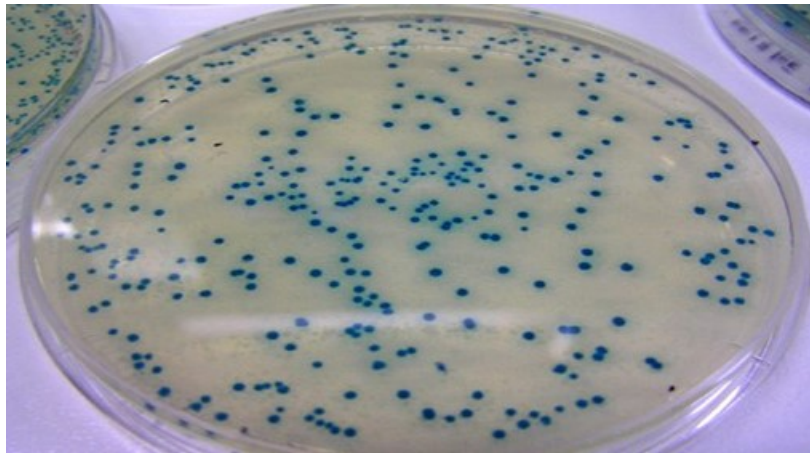
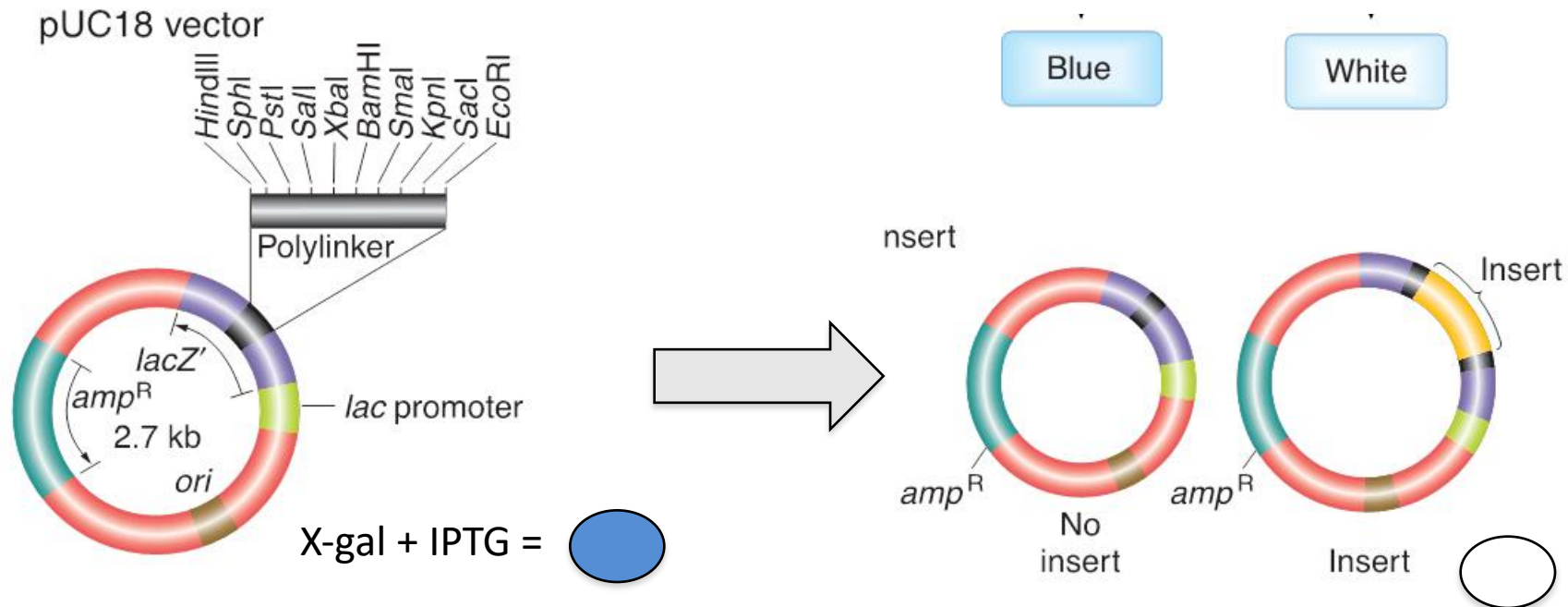
lactose

Glicose
Galactose

Seleção



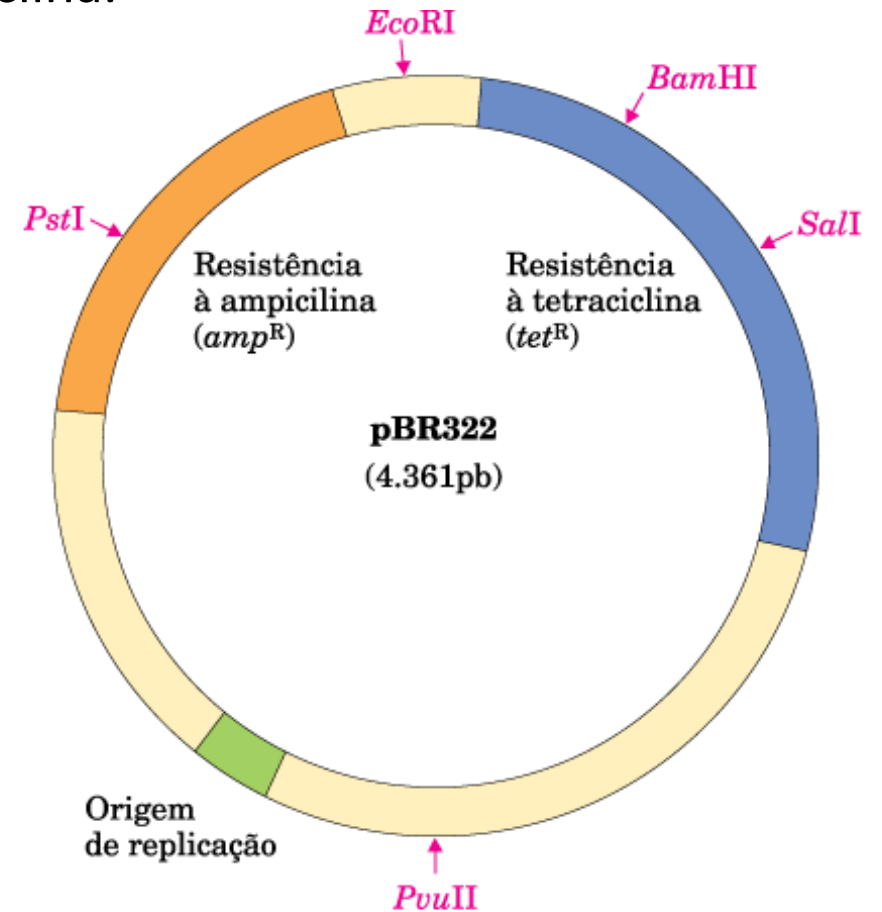
Seleção



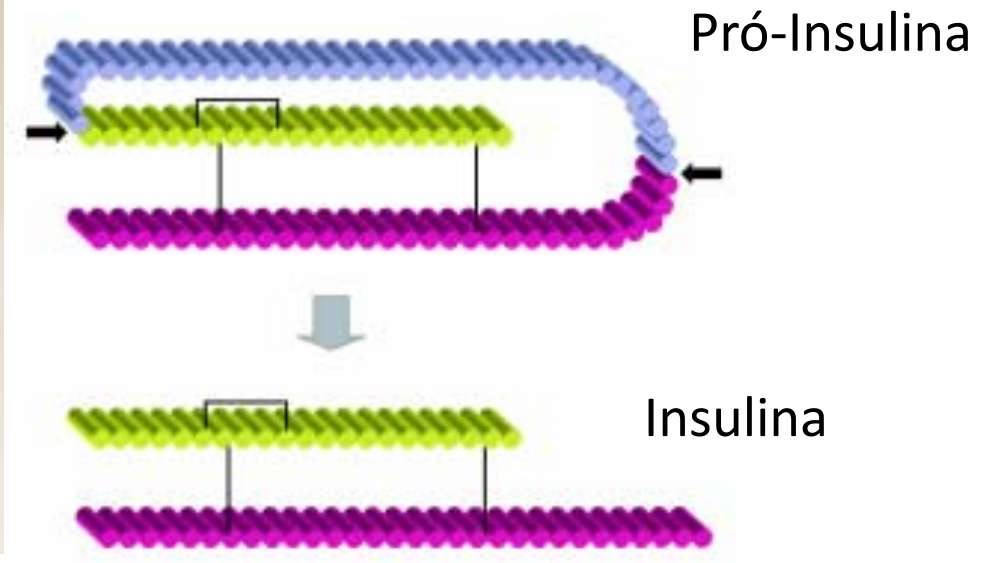
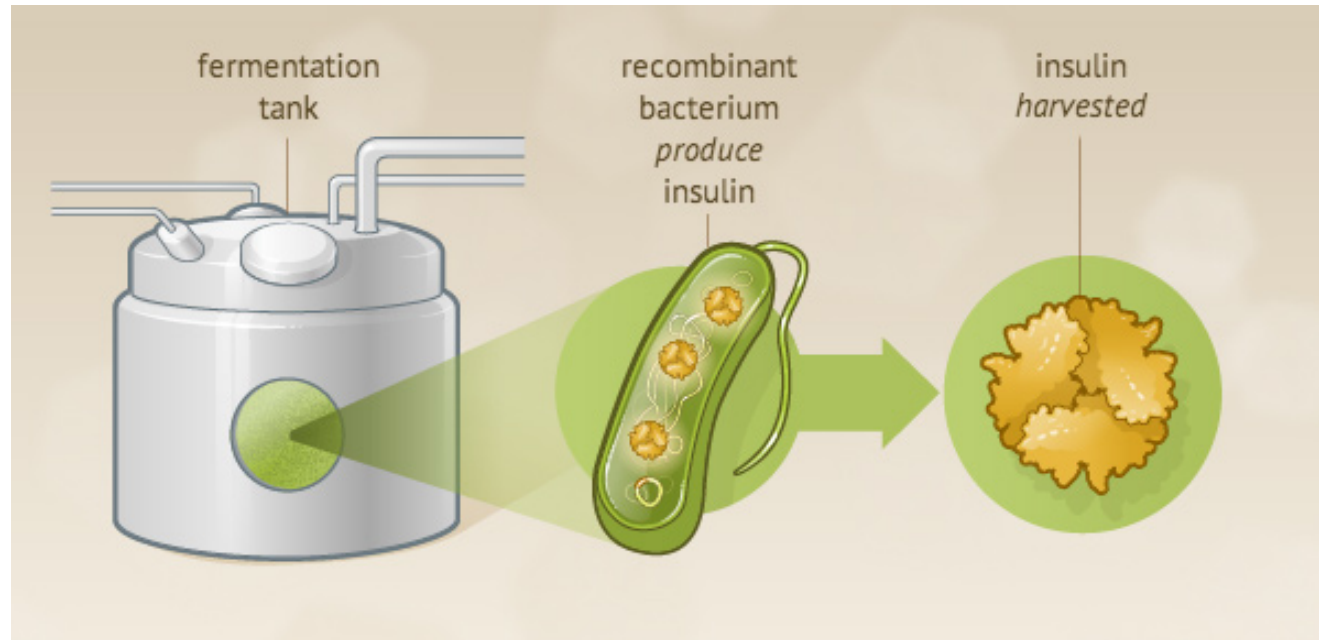
Exercício

O vetor de clonagem plasmídeo pBR322 é clivado com a endonuclease de restrição *Pst*I. Um fragmento de DNA isolado de um genoma eucariótico (também obtido por clivagem com *Pst*I) é adicionado ao vetor preparado e ligado. A mistura de DNAs ligados é então usada para transformar bactérias, e as bactérias contendo os plasmídeos são selecionadas pelo crescimento em presença de tetraciclina.

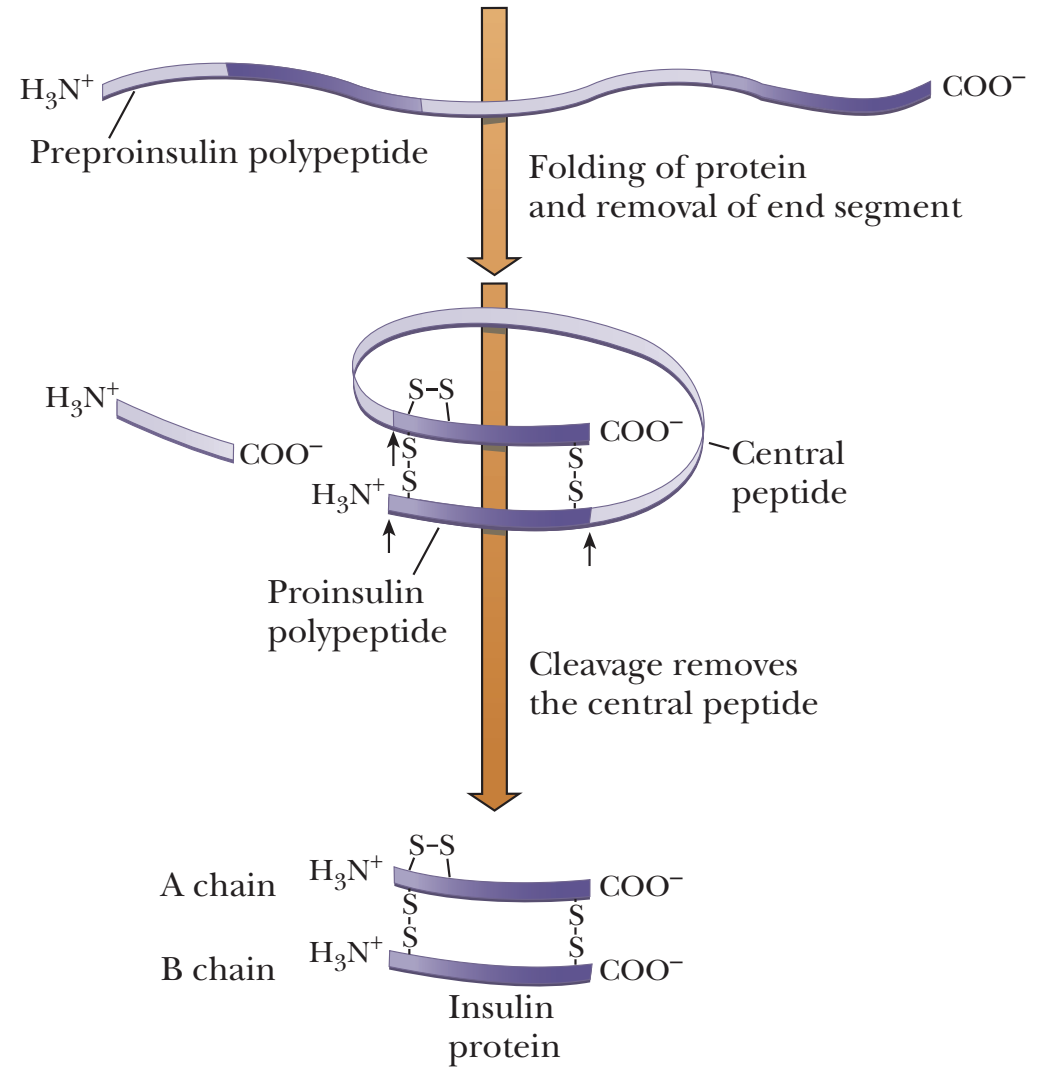
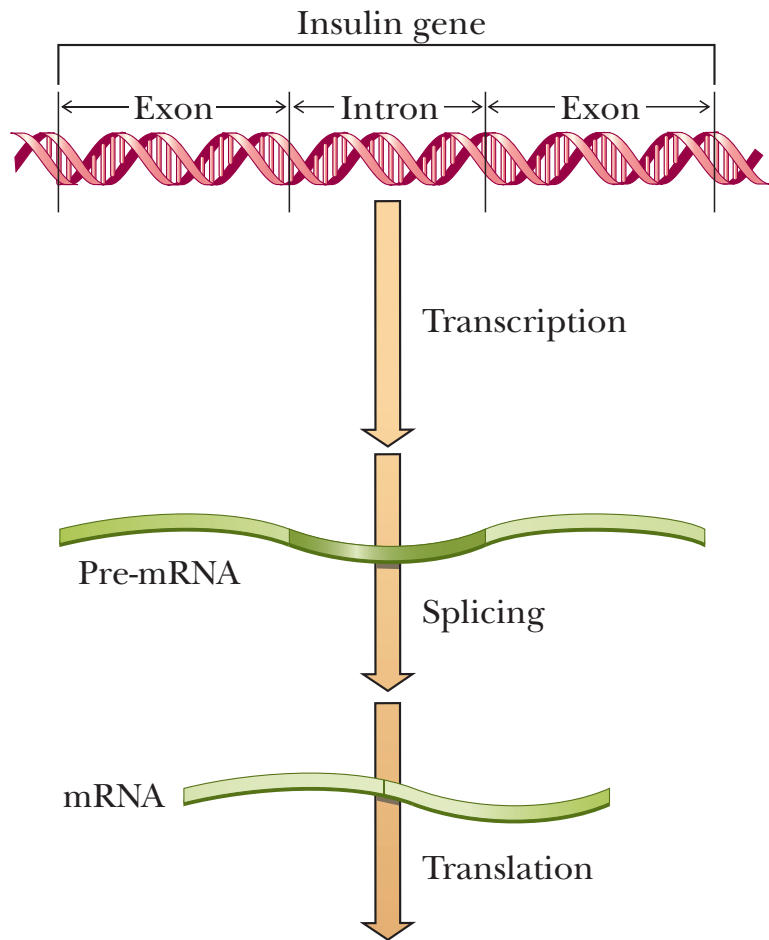
- 1) Que tipo de plasmídeos poderão ser encontrados entre as bactérias transformadas que são resistentes à tetraciclina?



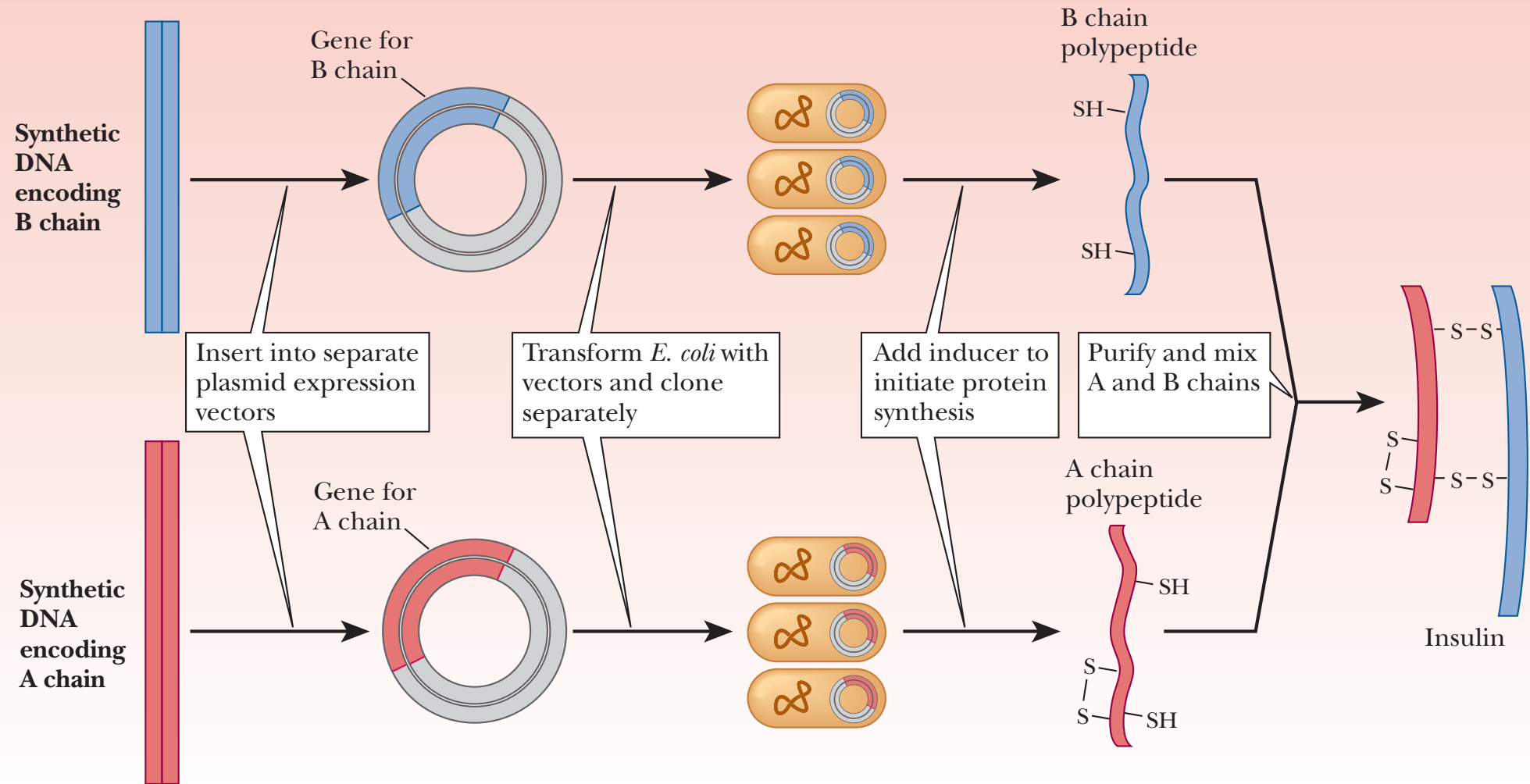
Bactérias como Biofábricas de Proteínas



Bactérias como Biofábricas de Proteínas



Bactérias como Biofábricas de Proteínas



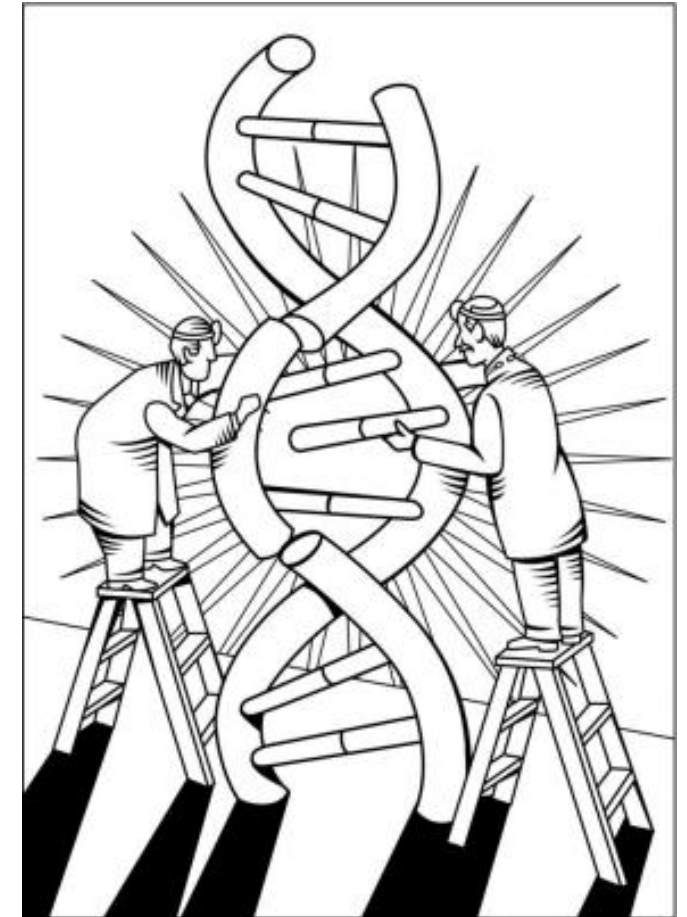
Engenharia Genética



Revolucionando a fermentação

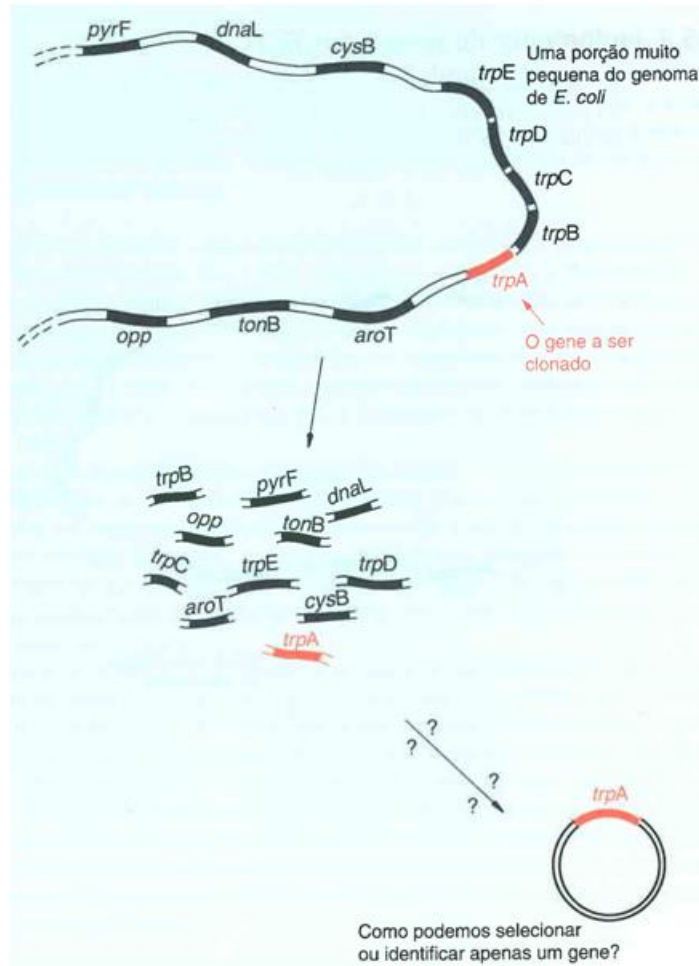


They ordered a synthetic version of the gene that had been built letter-by-letter by a machine. They plugged it into yeast and it worked.



Isolamento de DNA

Isolamento de gene por clonagem:



Isolamento de gene por PCR:

