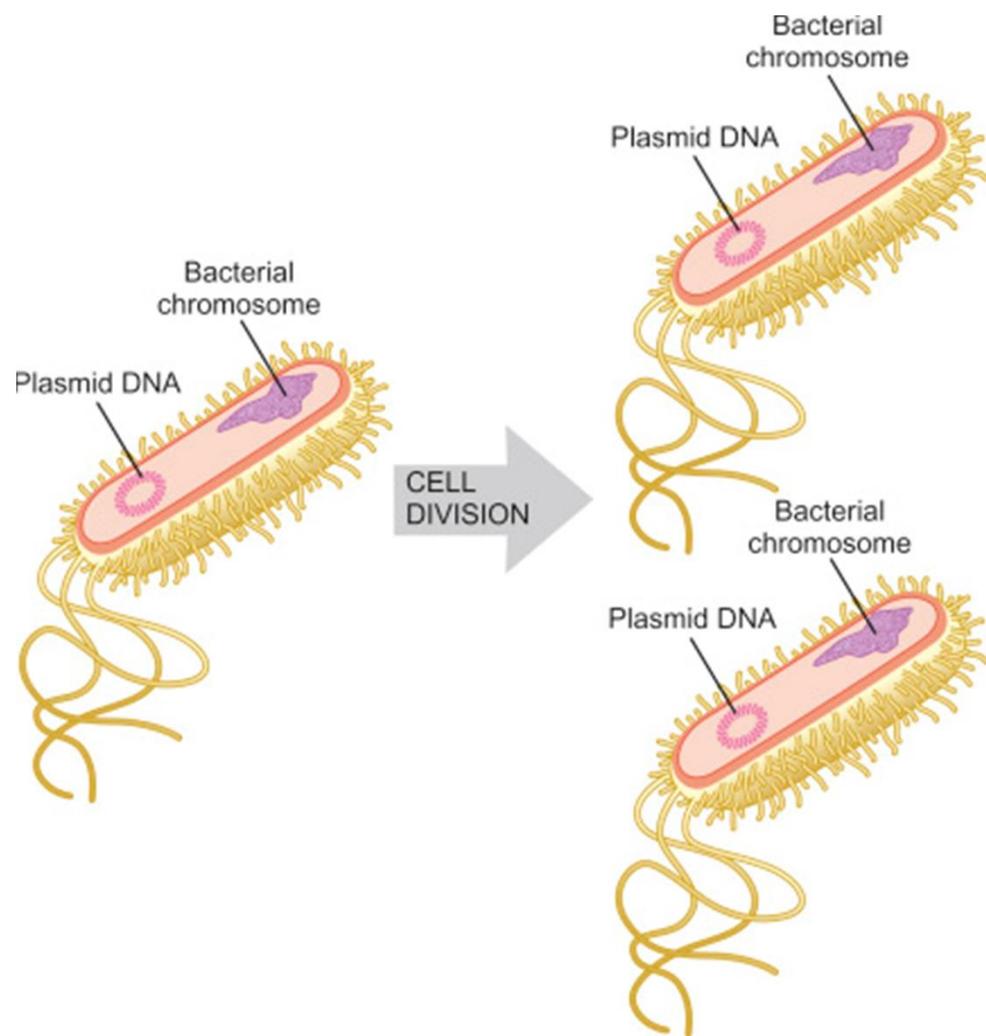
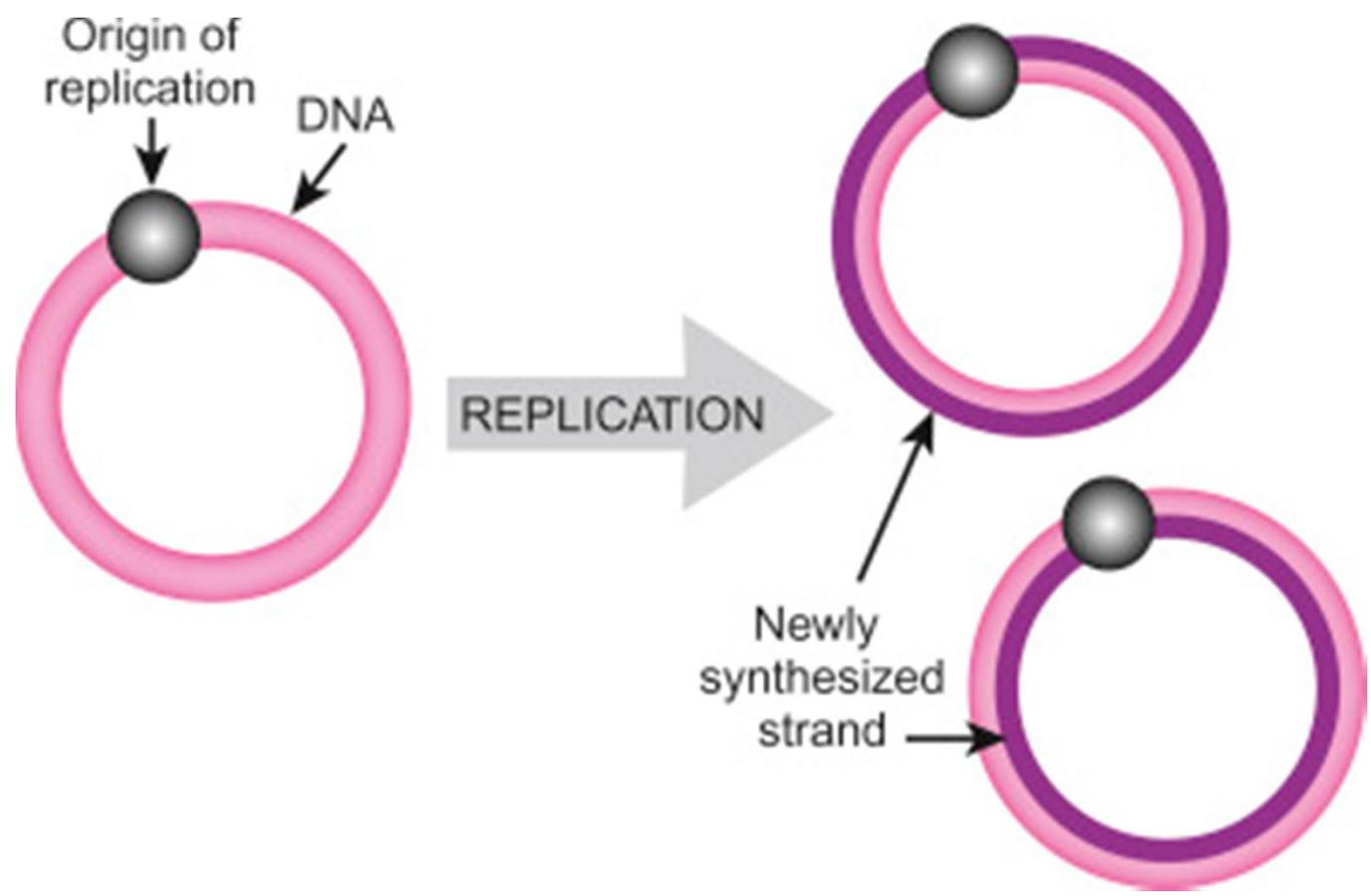
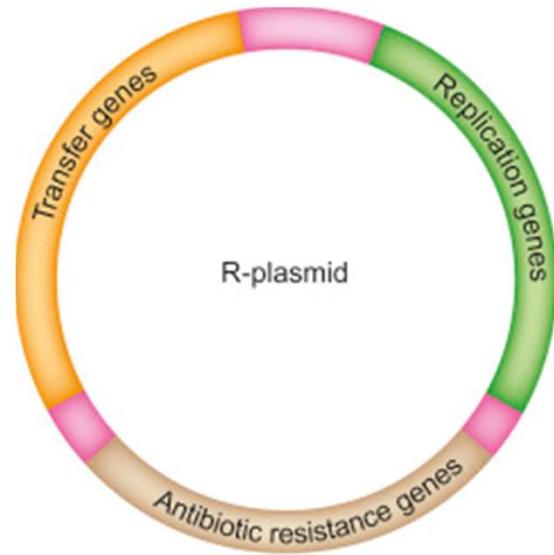


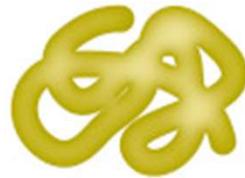
Tecnologia do DNA Recombinante



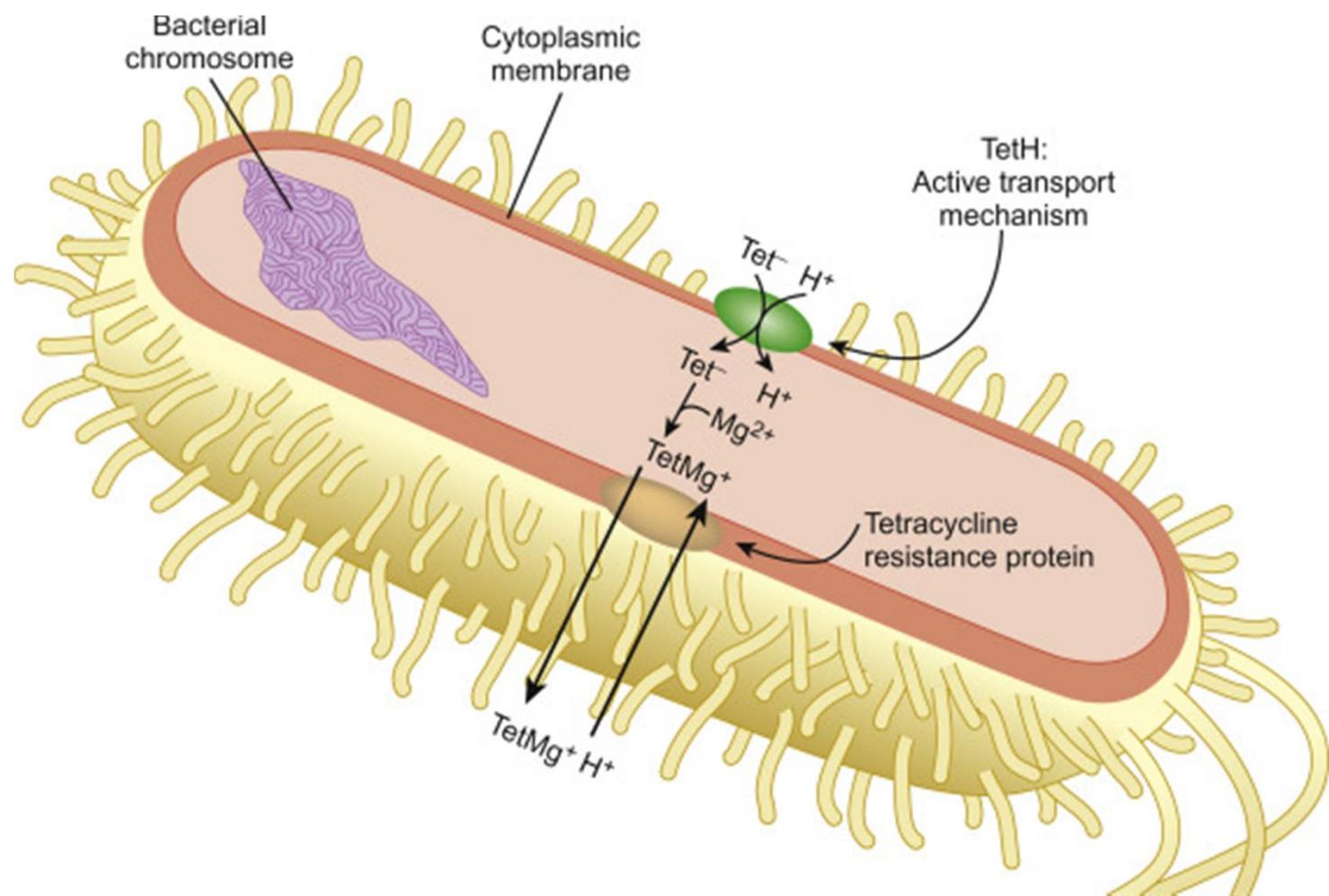


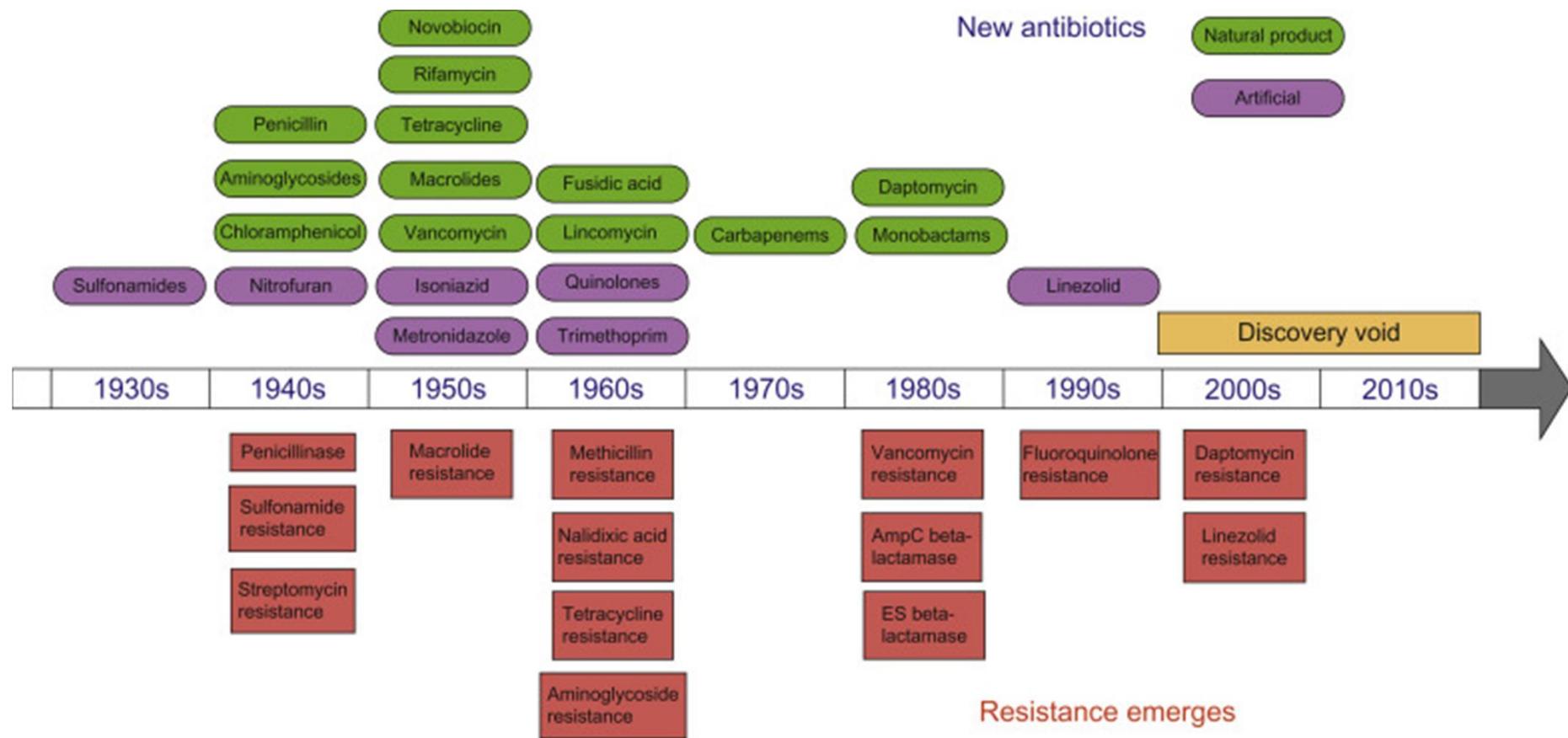


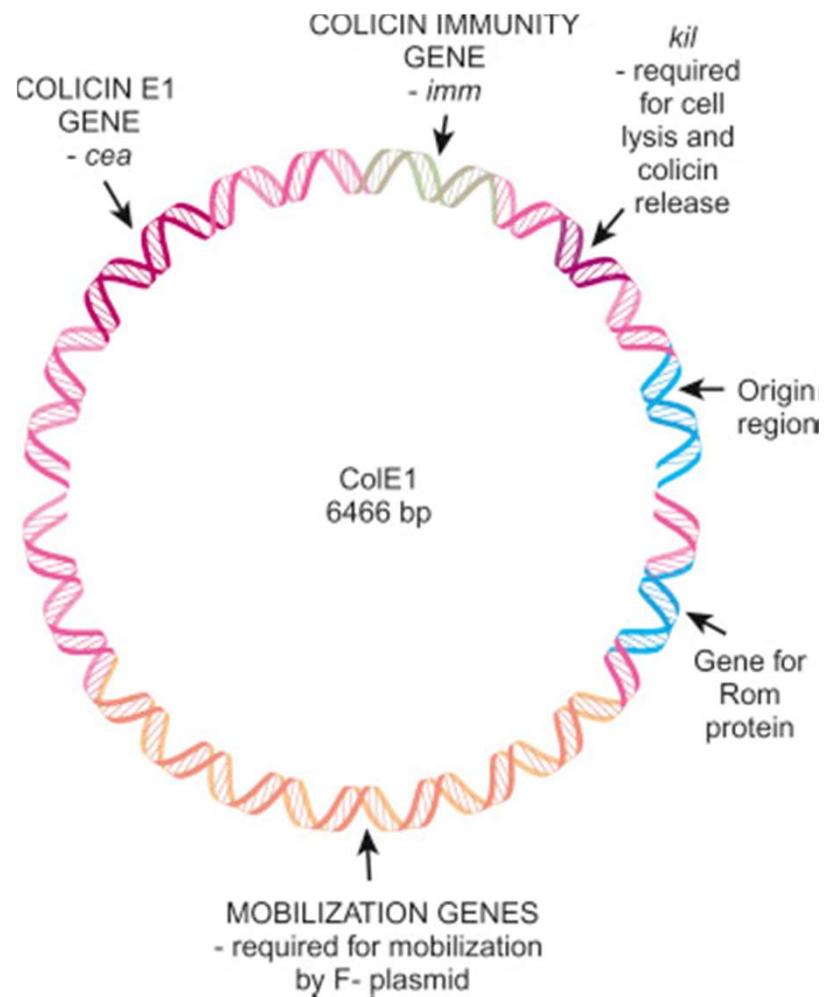
TRANSCRIPTION AND TRANSLATION

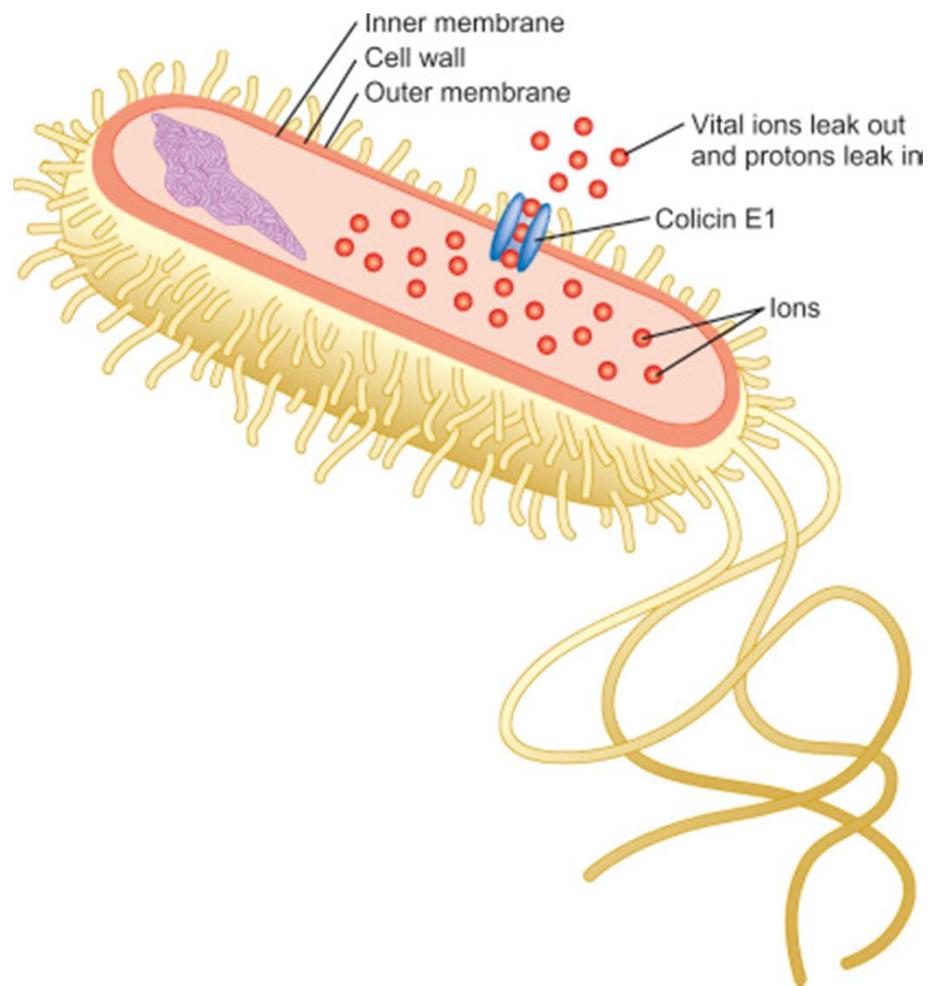


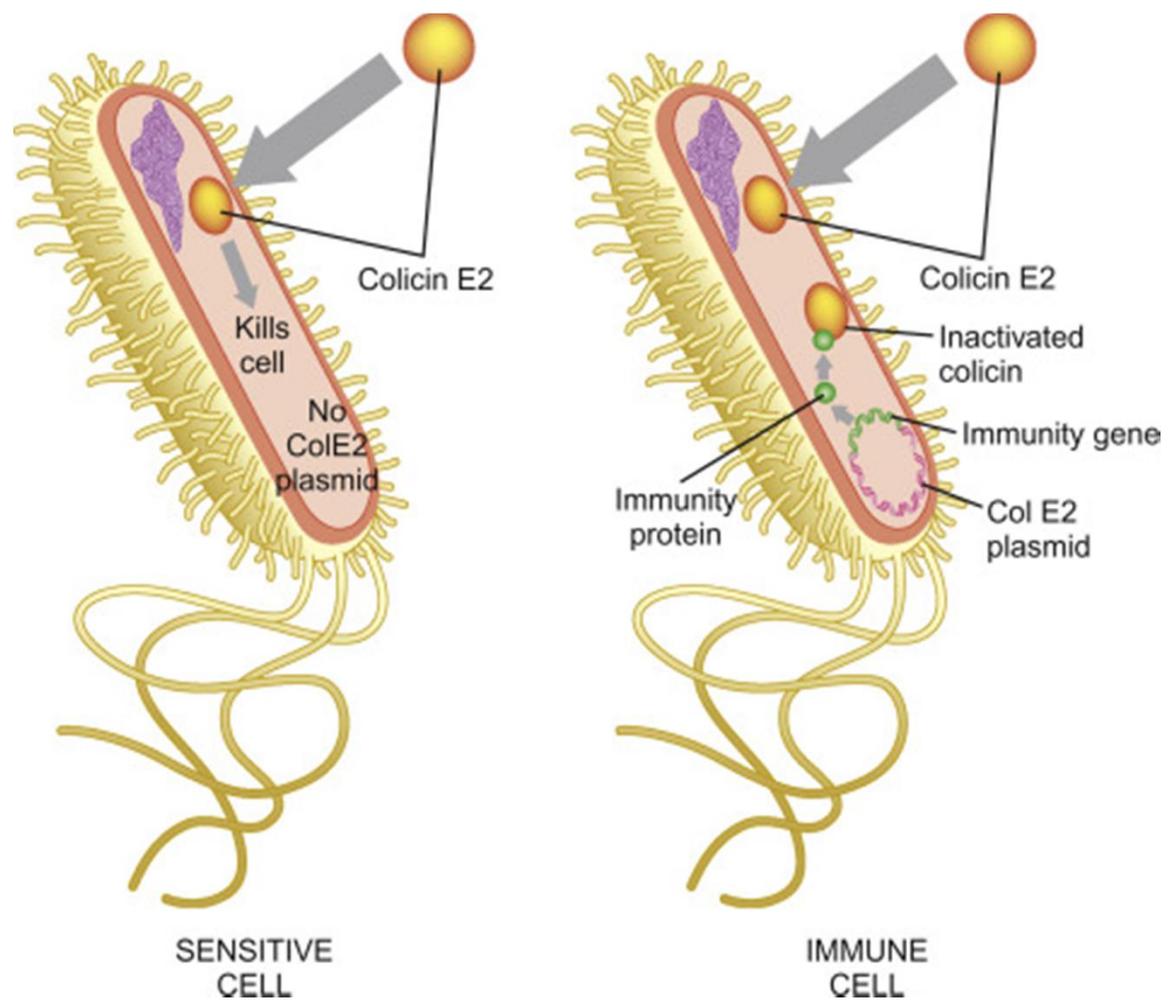
Antibiotic resistance protein



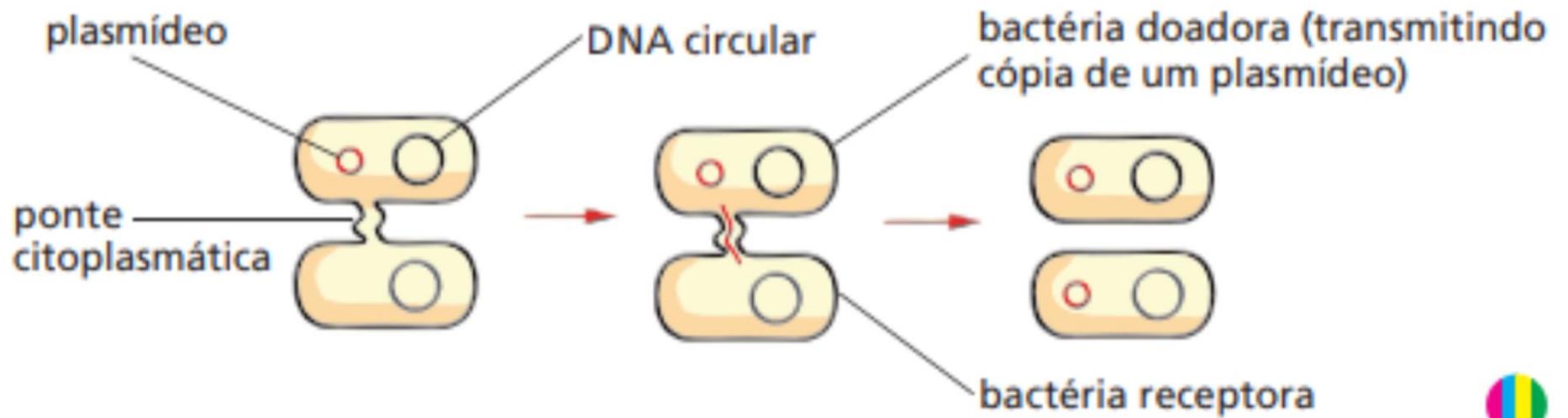




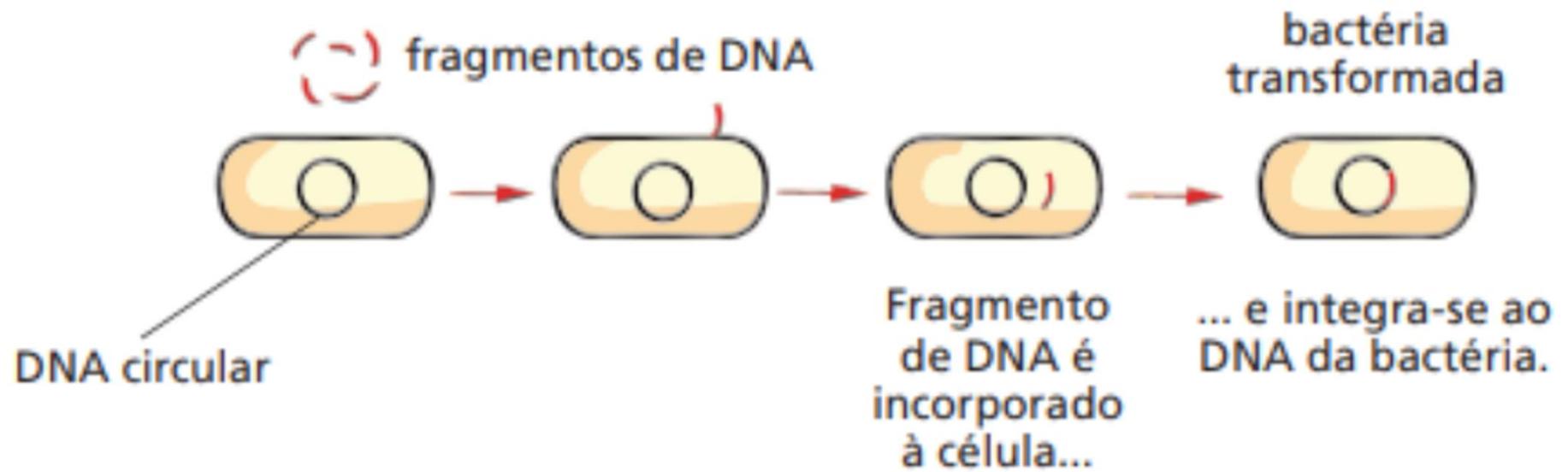




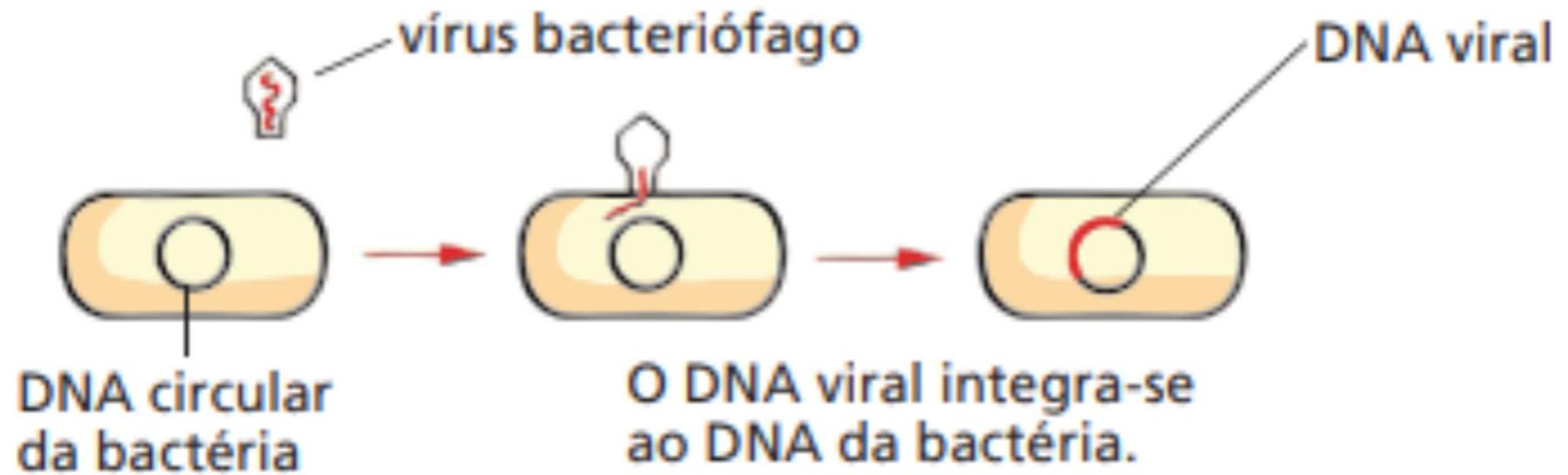
Conjugação



Transformação



Transdução





The Nobel Prize in Physiology or Medicine 1978

Werner Arber, Daniel Nathans, Hamilton O. Smith

The Nobel Prize in Physiology or Medicine 1978

Nobel Prize Award Ceremony

Werner Arber

Daniel Nathans

Hamilton O. Smith



Werner Arber



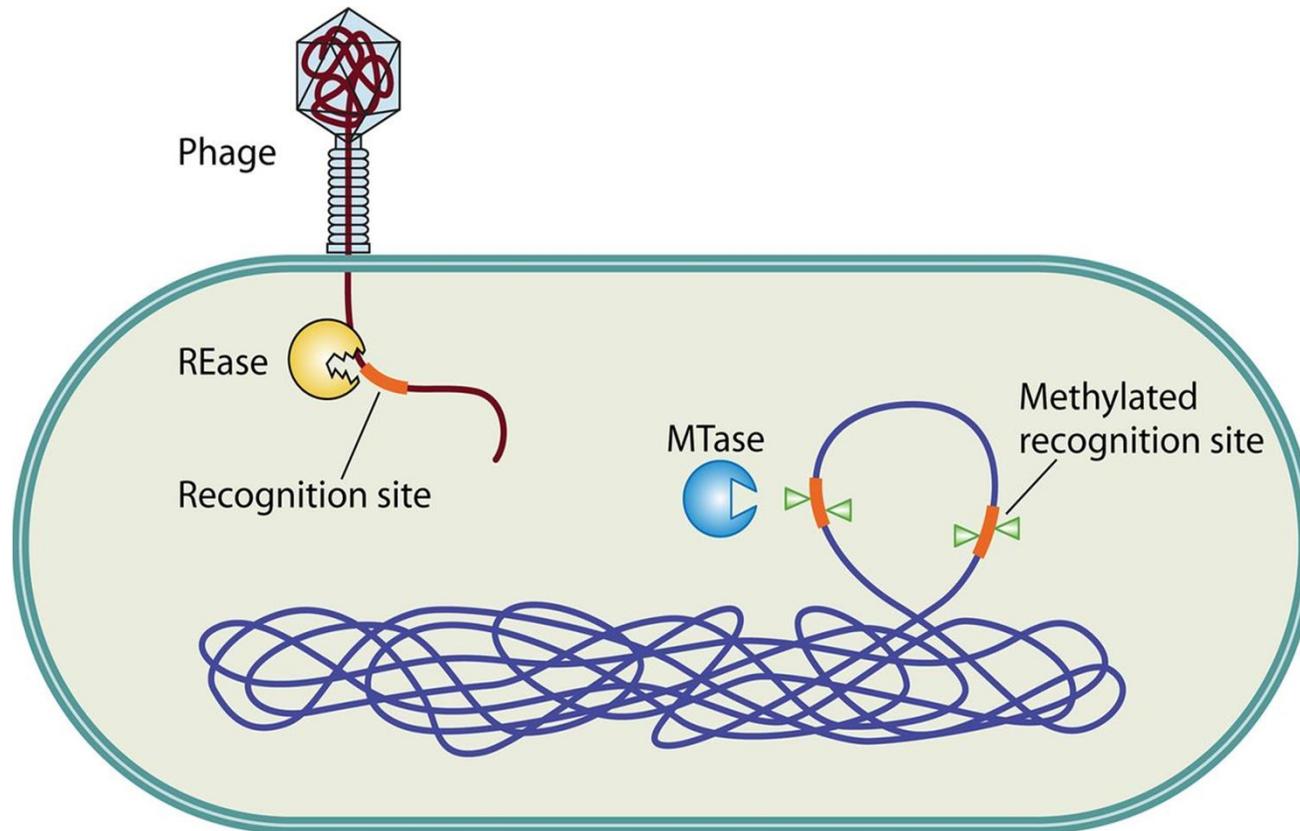
Daniel Nathans



Hamilton O. Smith

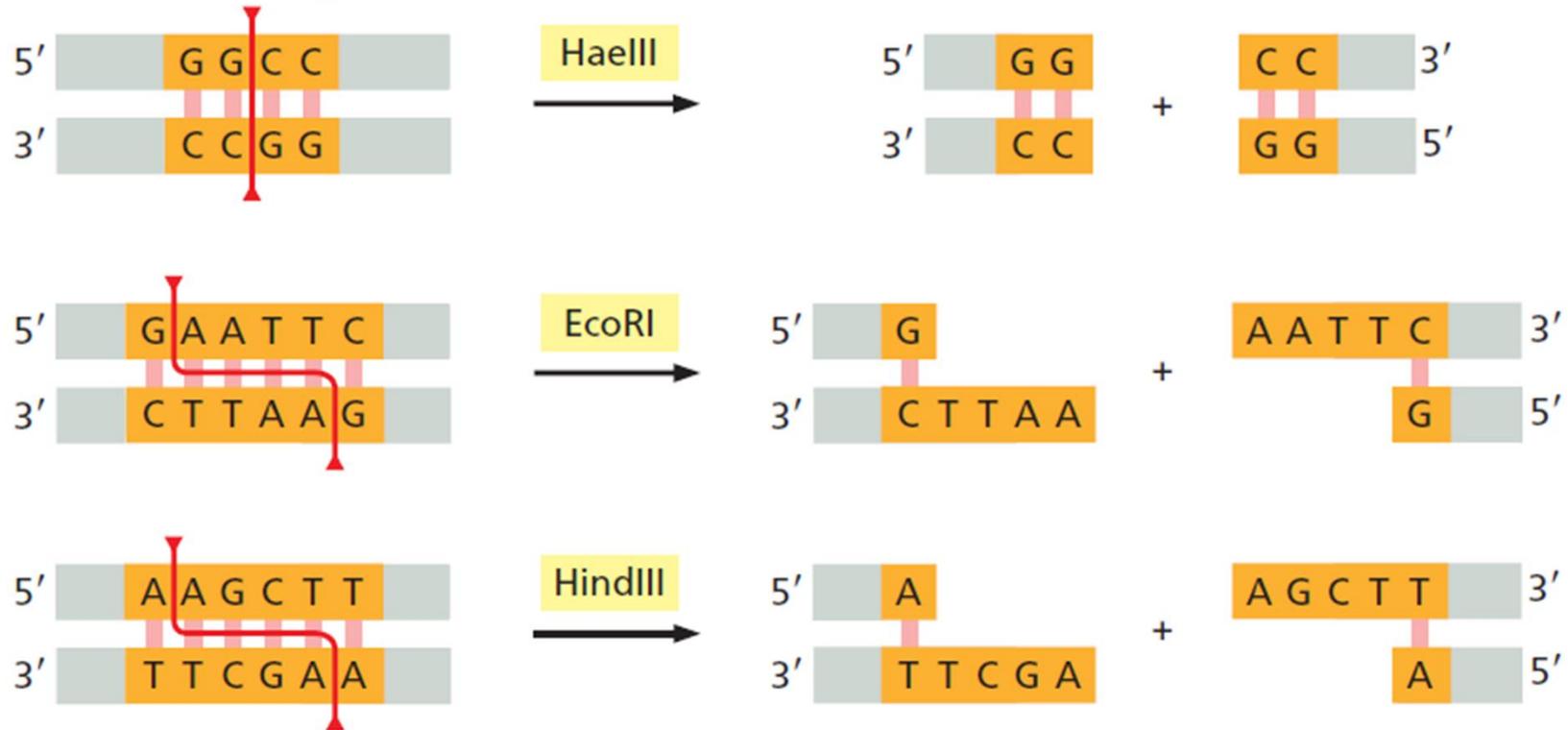
The Nobel Prize in Physiology or Medicine 1978 was awarded jointly to Werner Arber, Daniel Nathans and Hamilton O. Smith *"for the discovery of restriction enzymes and their application to problems of molecular genetics"*.

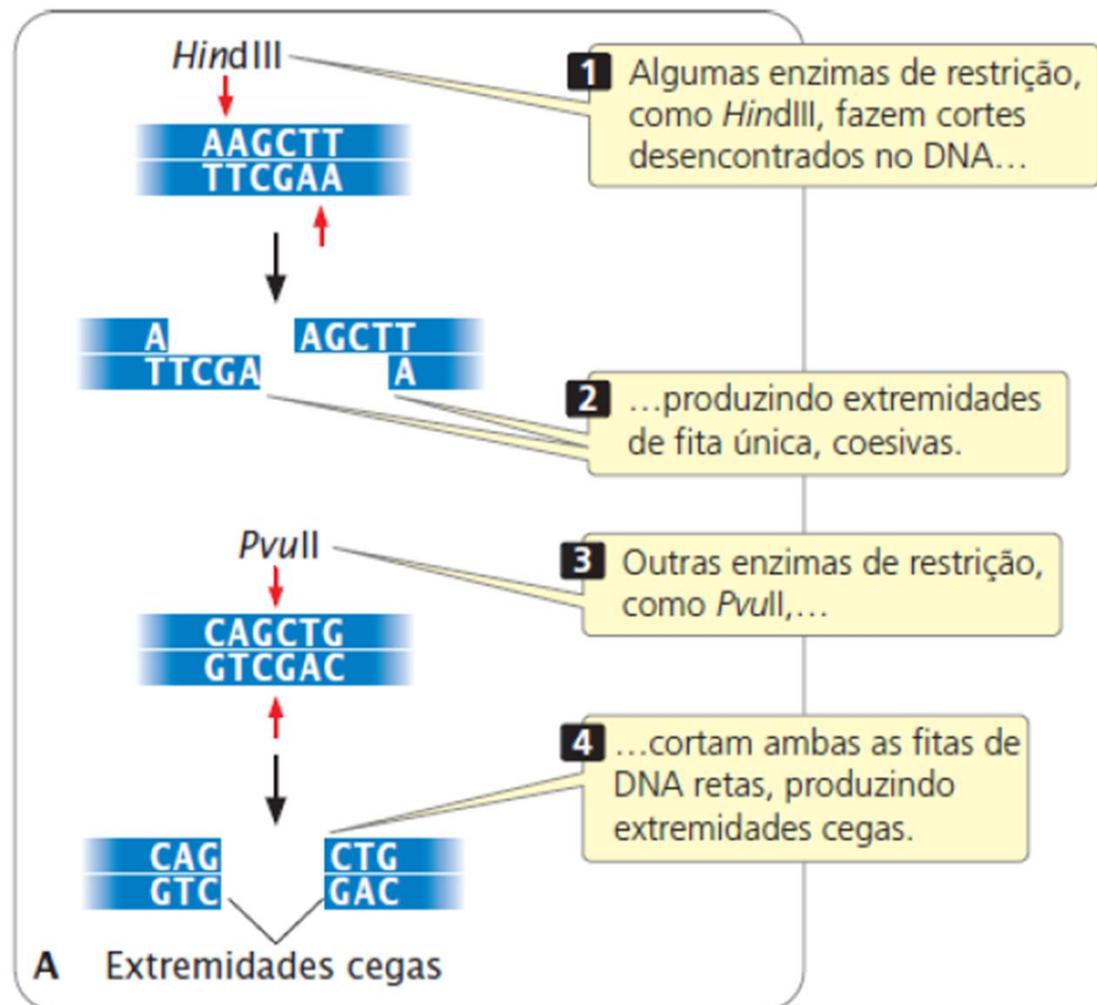
Restriction-modification (R-M) systems as defense mechanisms.

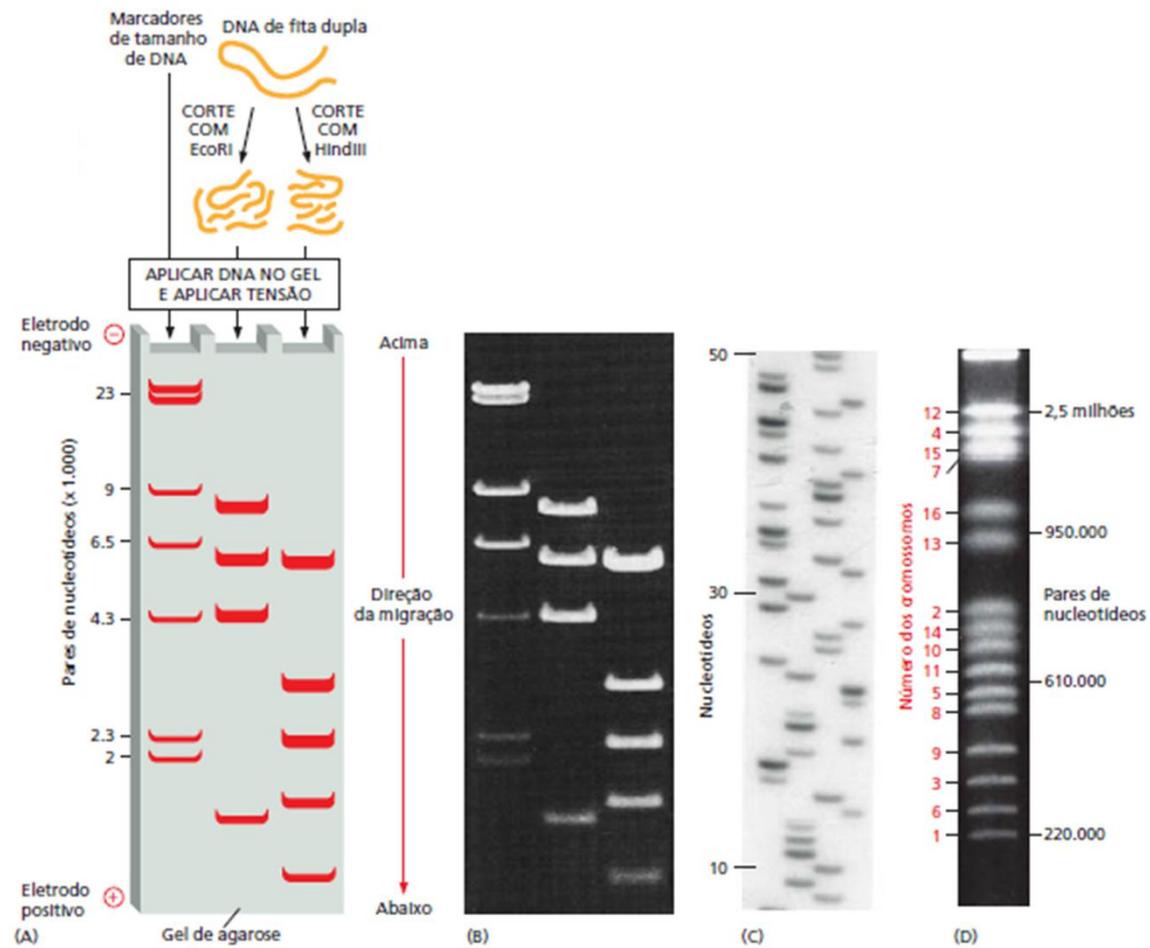


Kommireddy Vasu, and Valakunja Nagaraja
Microbiol. Mol. Biol. Rev. 2013

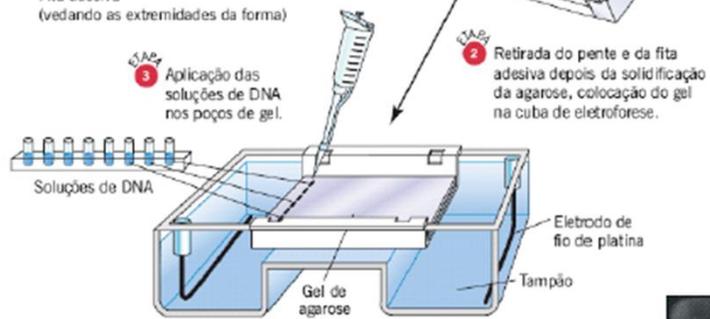
Sítio de clivagem



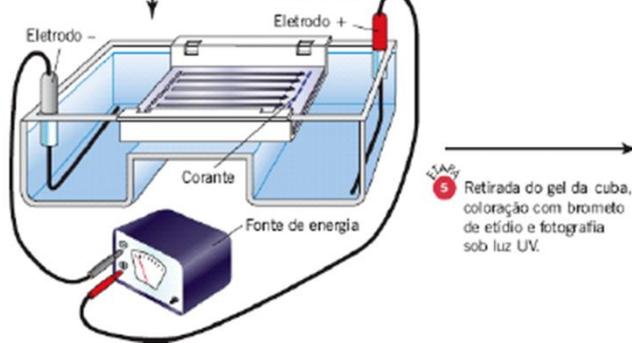




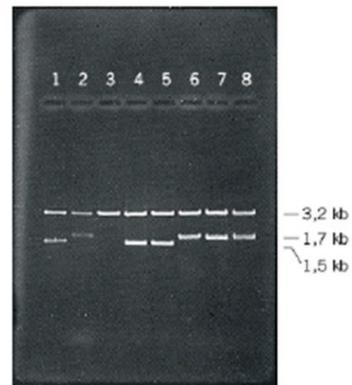
1 Preparo de um gel de agarose semissólido com poços para amostras de DNA.



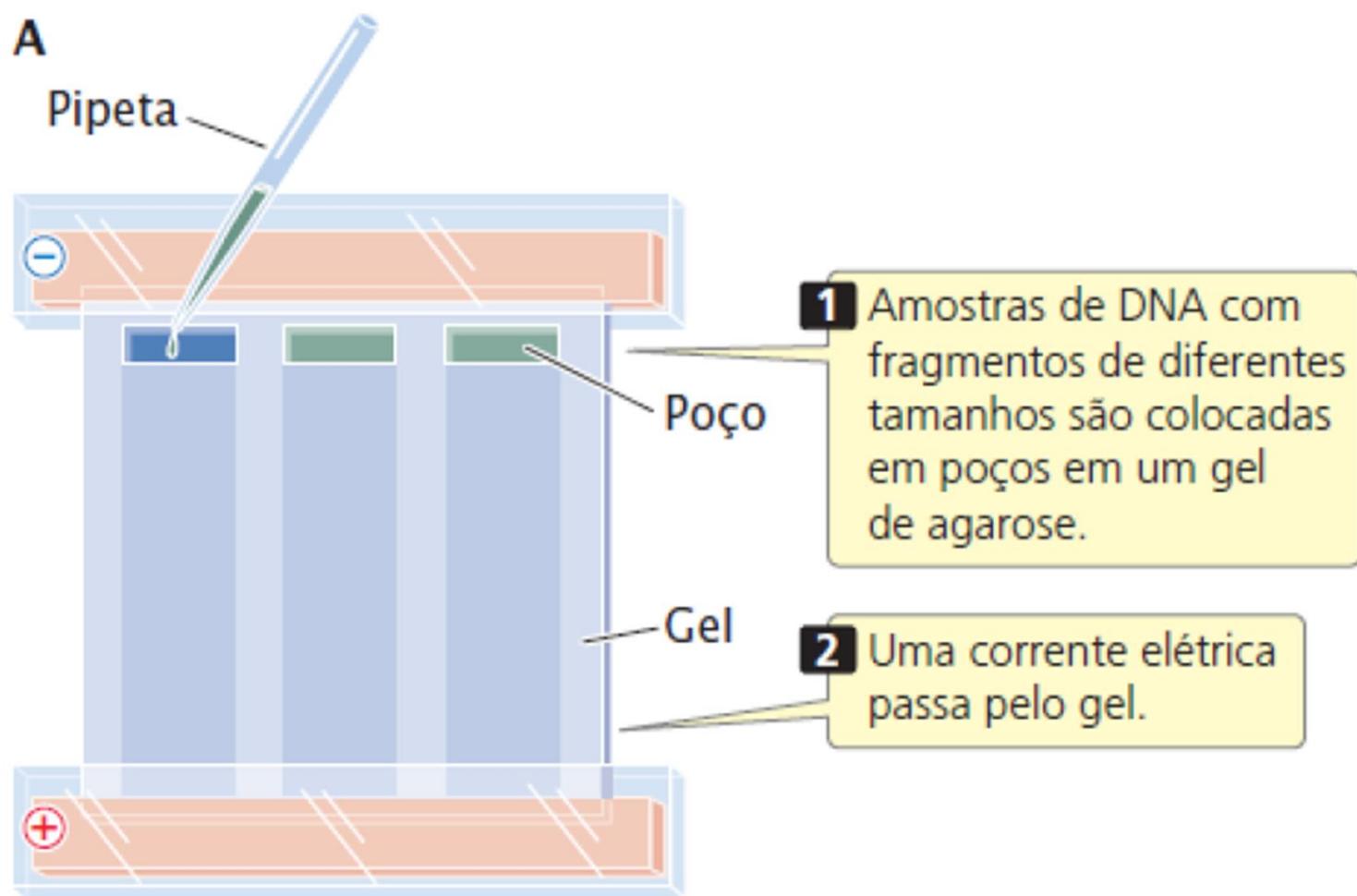
4 Ligação da fonte de alimentação e início da eletroforese.

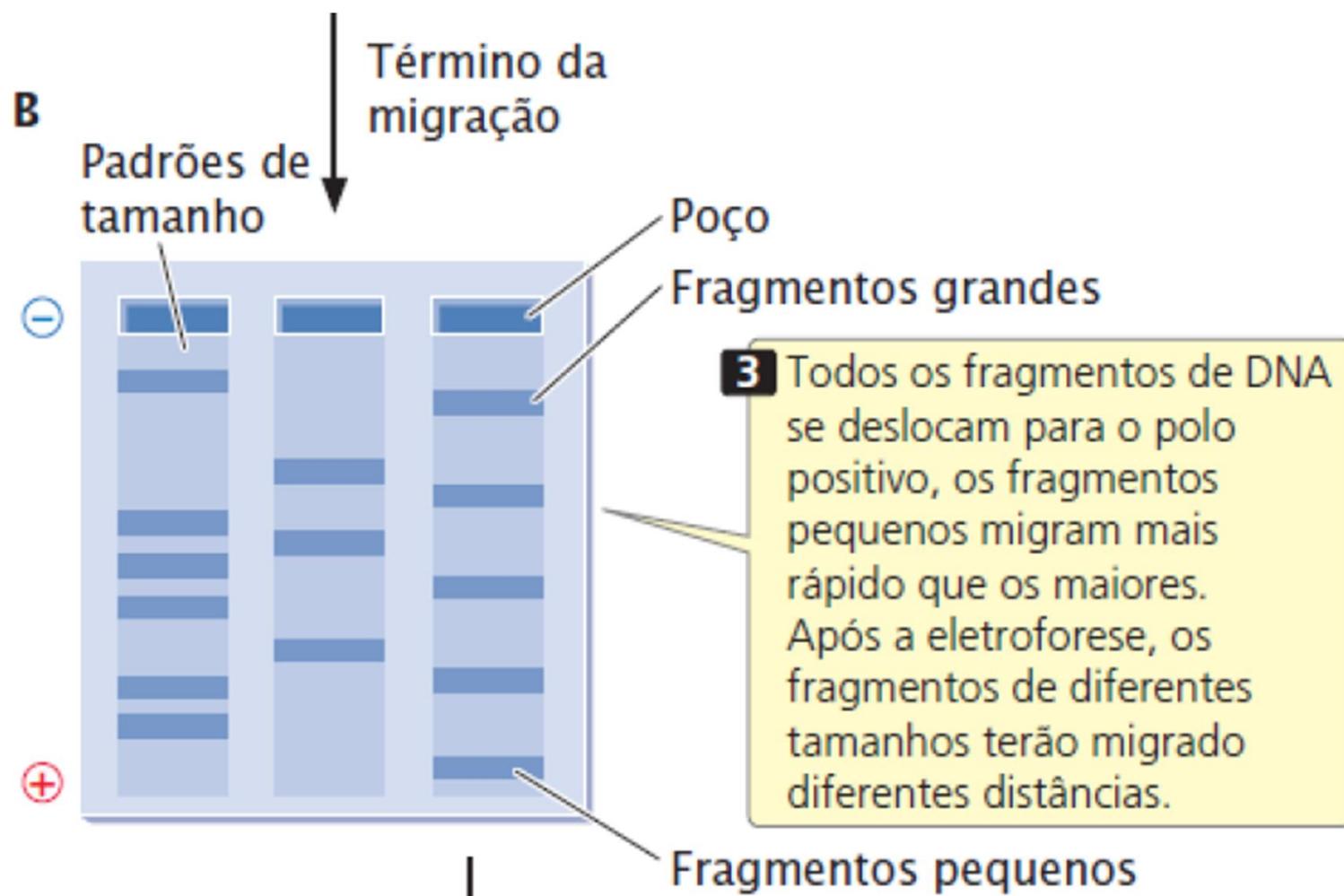


5 Retirada do gel da cuba, coloração com brometo de etídio e fotografia sob luz UV.

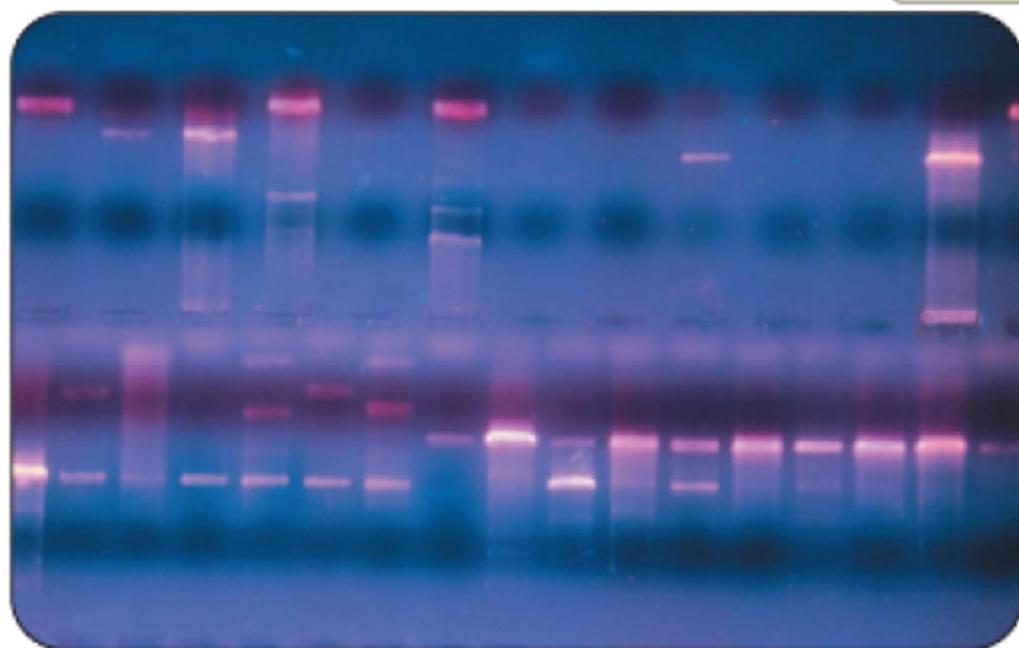


Comissão de P. S. Inútil.



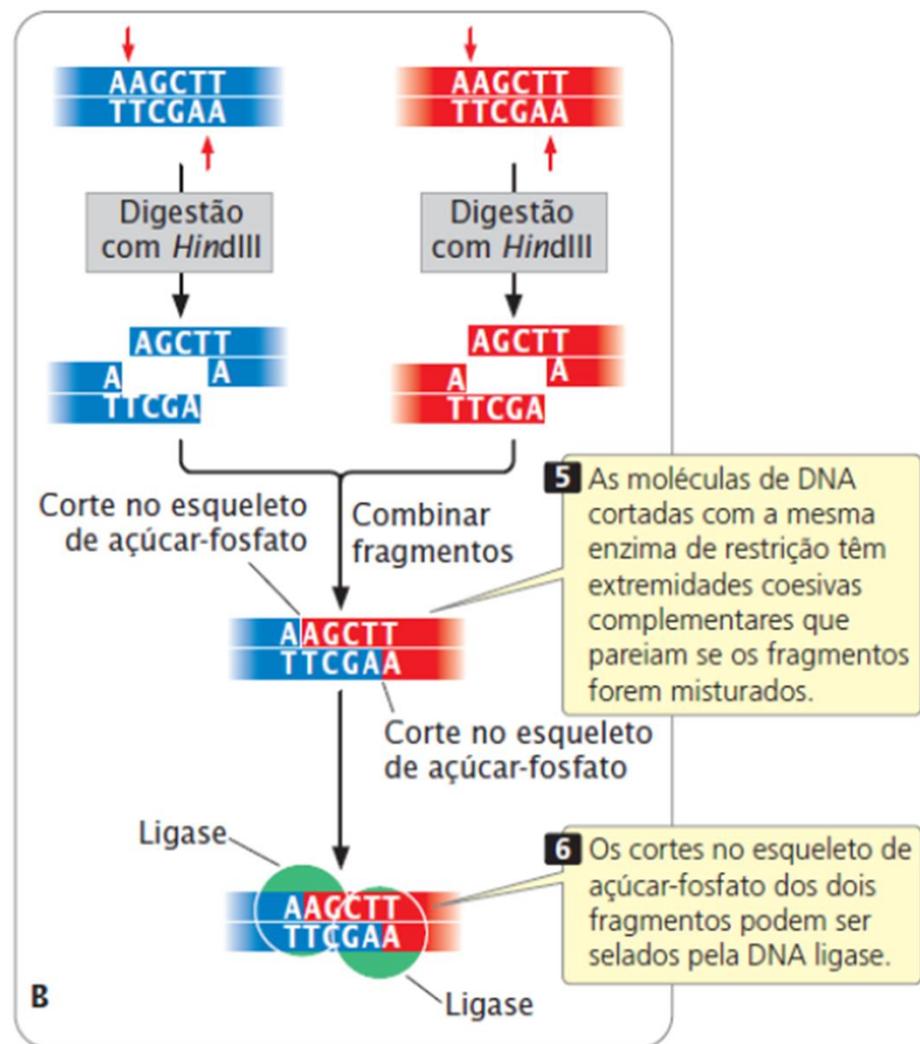


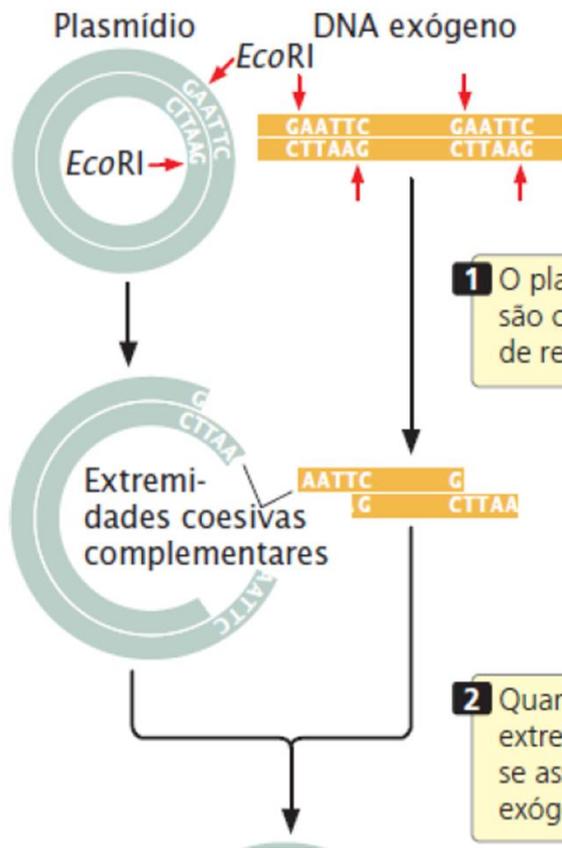
C



4 Um corante específico para ácidos nucleicos é adicionado ao gel.

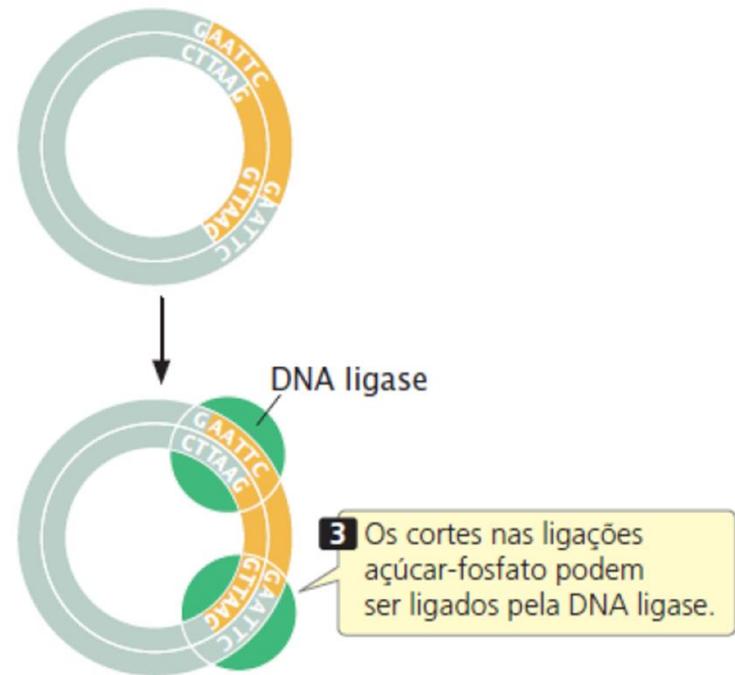
5 Os fragmentos de DNA aparecem como bandas no gel.

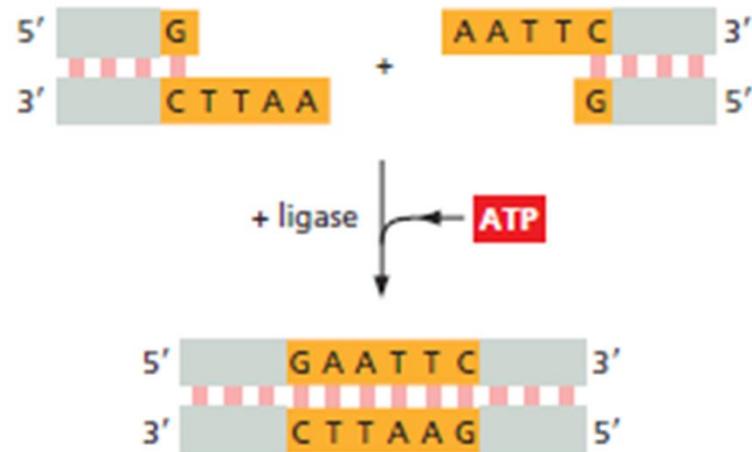




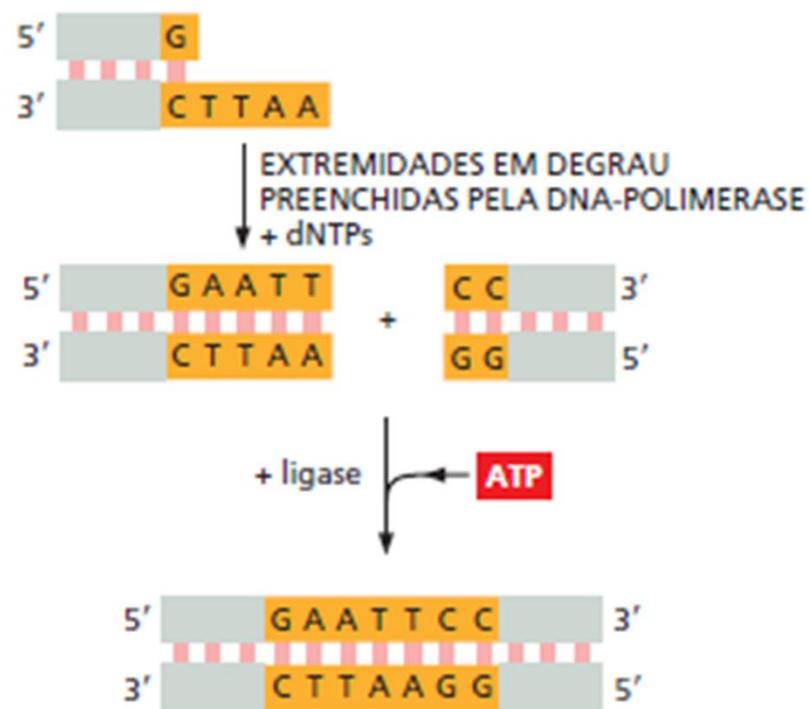
1 O plasmídeo e o DNA exógeno são cortados pela mesma enzima de restrição, neste caso *EcoRI*.

2 Quando misturadas, as extremidades coesivas se associam, unindo o DNA exógeno e o plasmídeo.

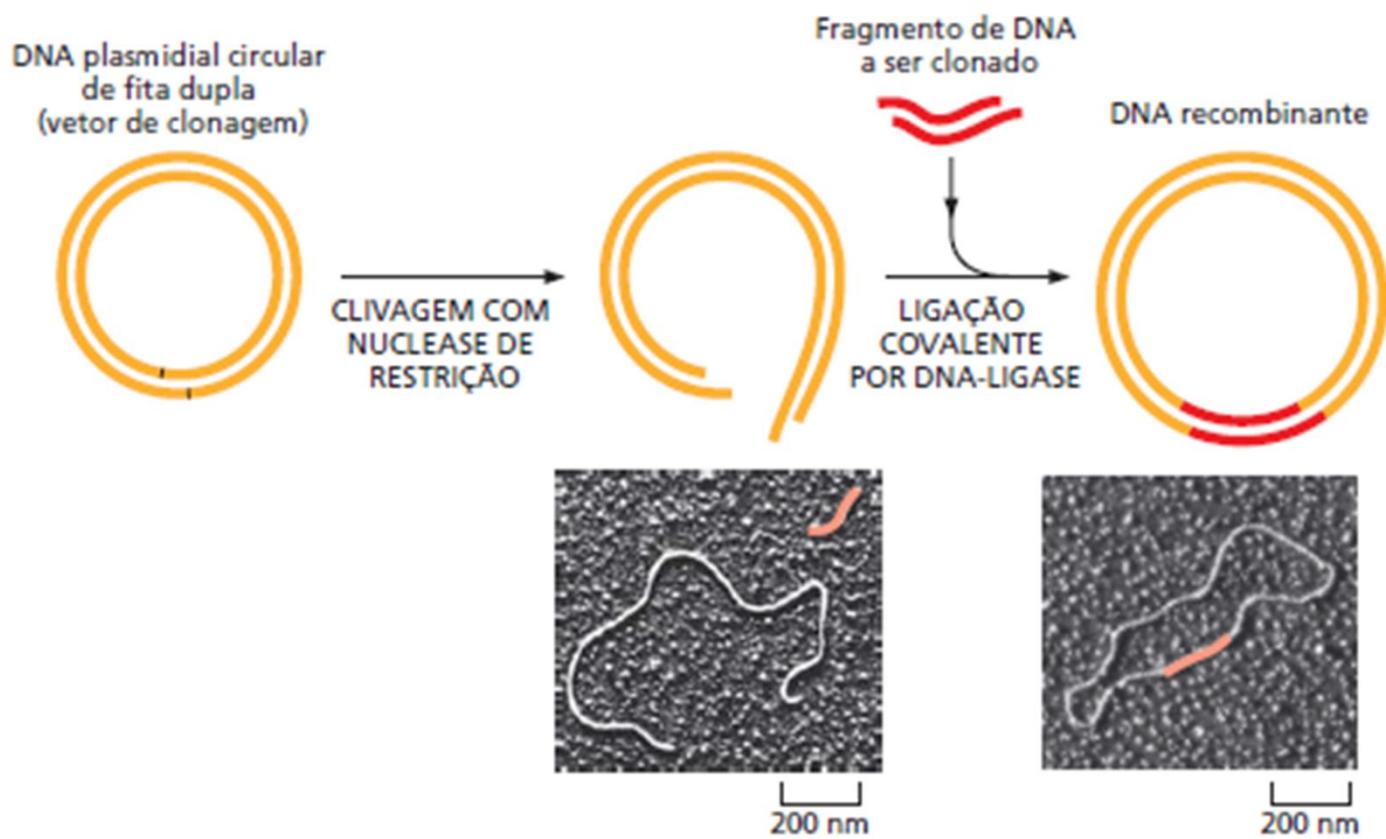




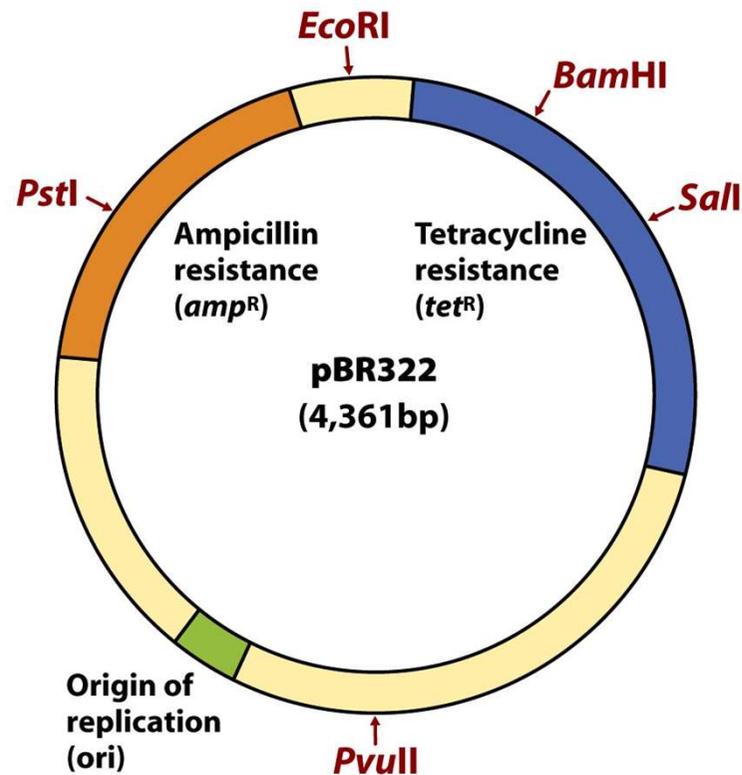
(A) UNIÃO DE DOIS FRAGMENTOS CORTADOS PELA MESMA NUCLEASE DE RESTRIÇÃO



(B) UNIÃO DE DOIS FRAGMENTOS CORTADOS POR NUCLEASE DE RESTRIÇÃO DIFERENTES



Vetor de clonagem plasmidial: pBR322

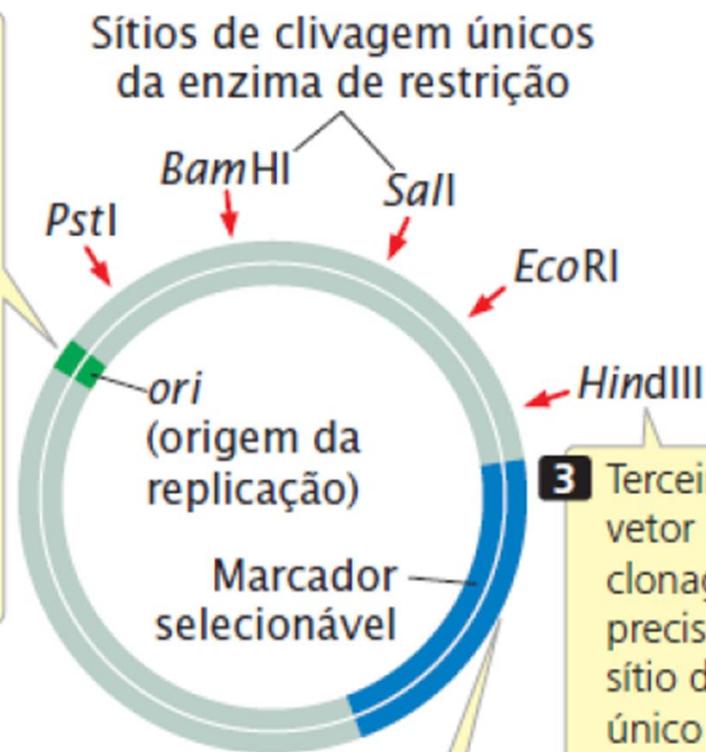


- Pioneiro
- Poucas cópias/célula
- Dois genes de resistência a antibióticos com sítios de restrição para clonagem

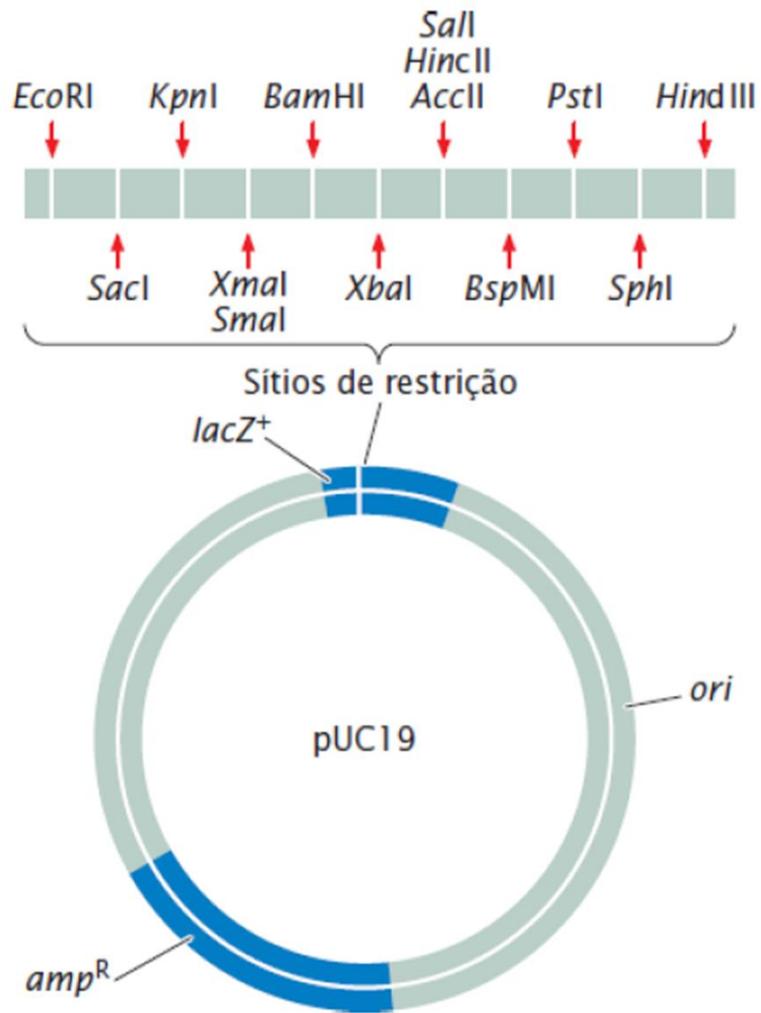
Figure 9-3
Lehninger Principles of Biochemistry, Fifth Edition
© 2008 W.H. Freeman and Company

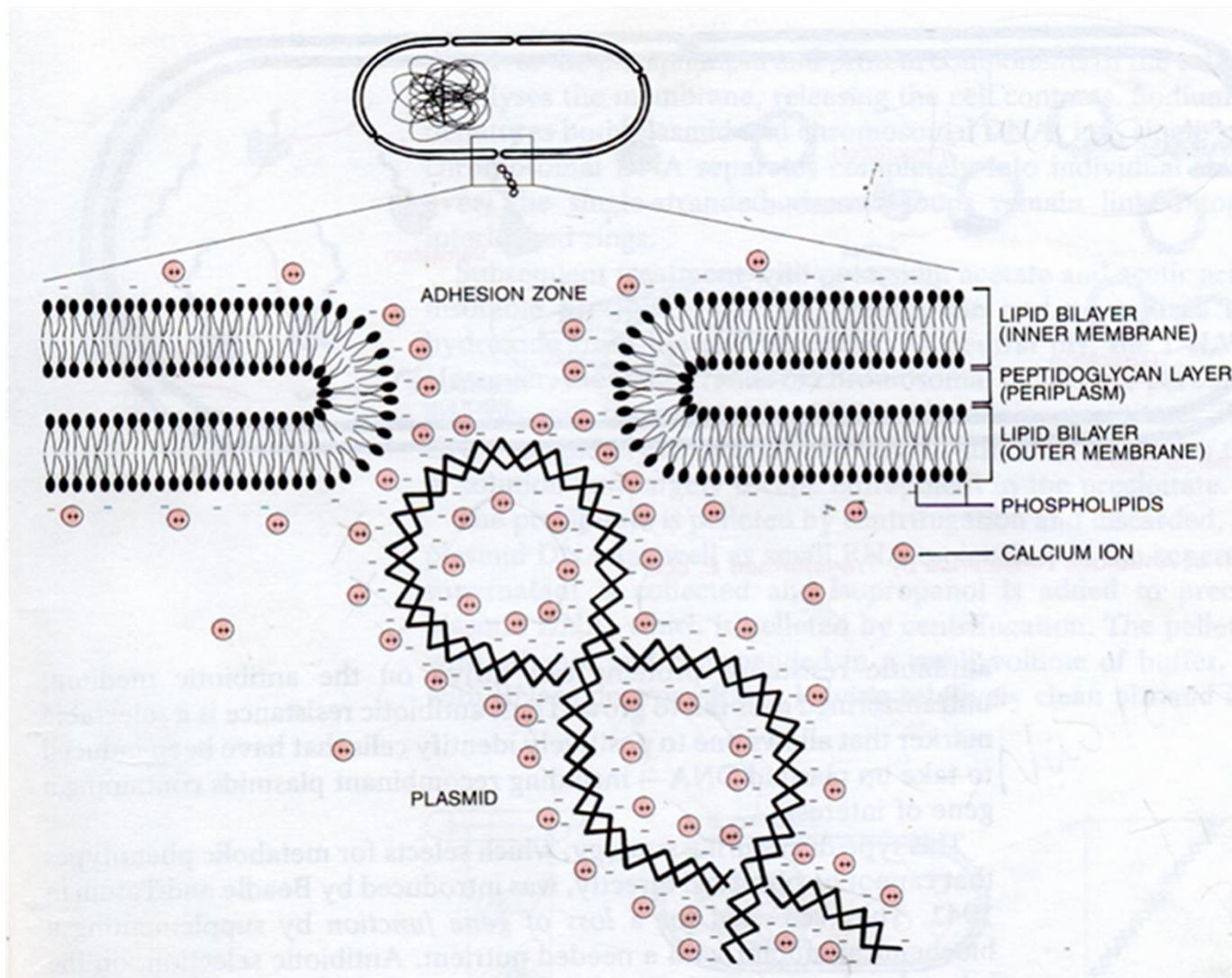
1 Primeiro, um vetor de clonagem precisa conter uma origem de replicação reconhecida na célula hospedeira de modo que seja replicado junto com o DNA que o carrega.

2 Segundo, ele deve carrear marcadores selecionáveis, traços que possibilitam que as células com o vetor sejam selecionadas ou identificadas.



3 Terceiro, um vetor de clonagem precisa de um sítio de clivagem único para cada uma das enzimas de restrição usadas.

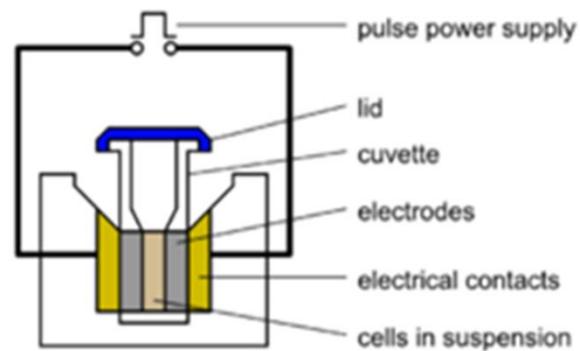
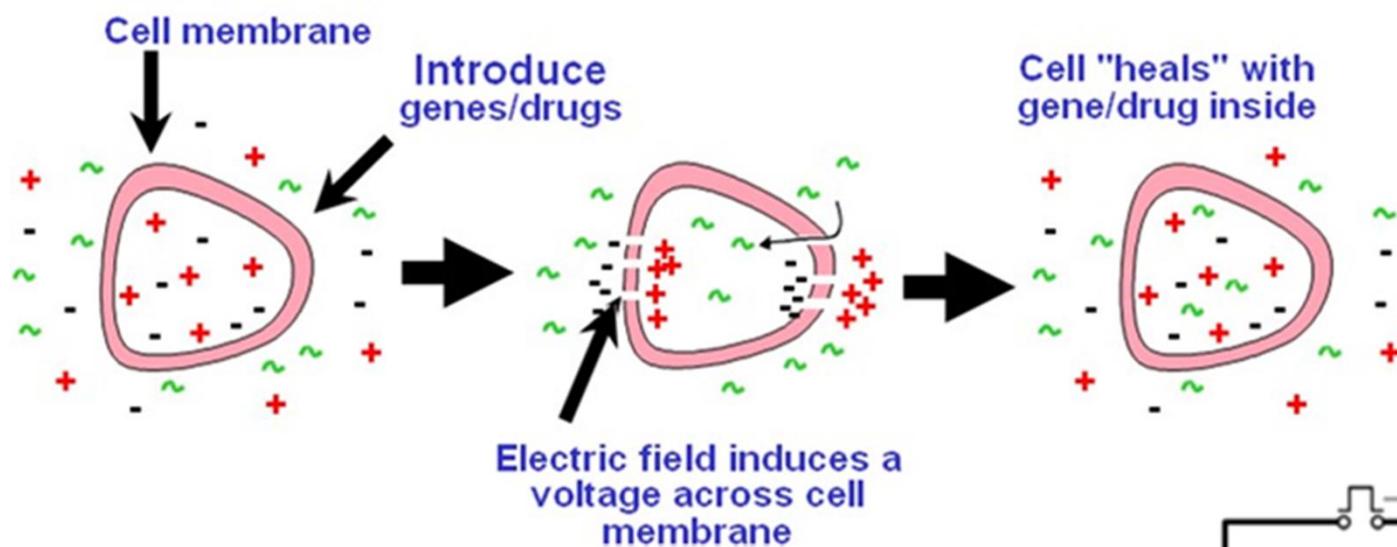


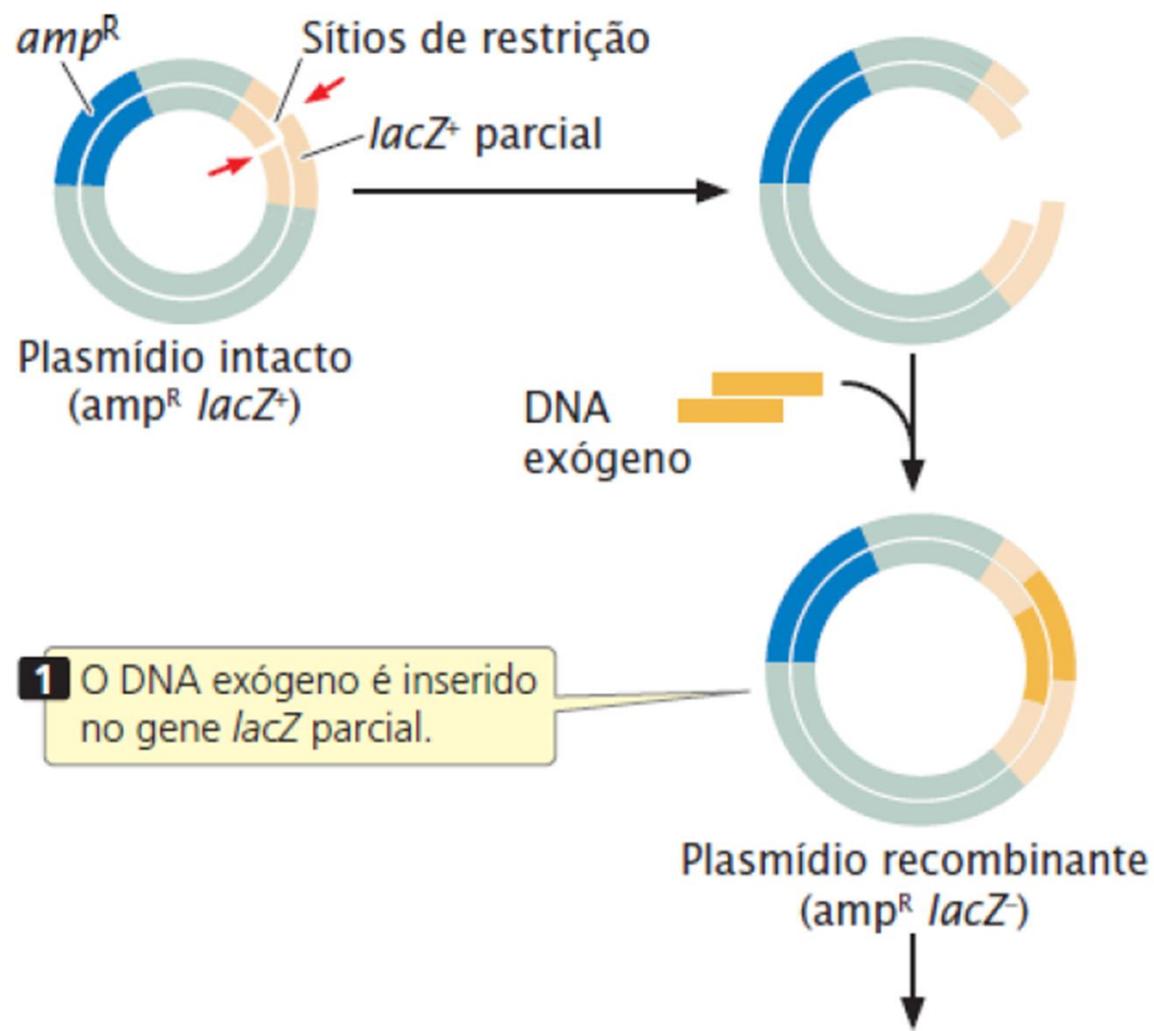


Before Pulse

During E-field

After Pulse





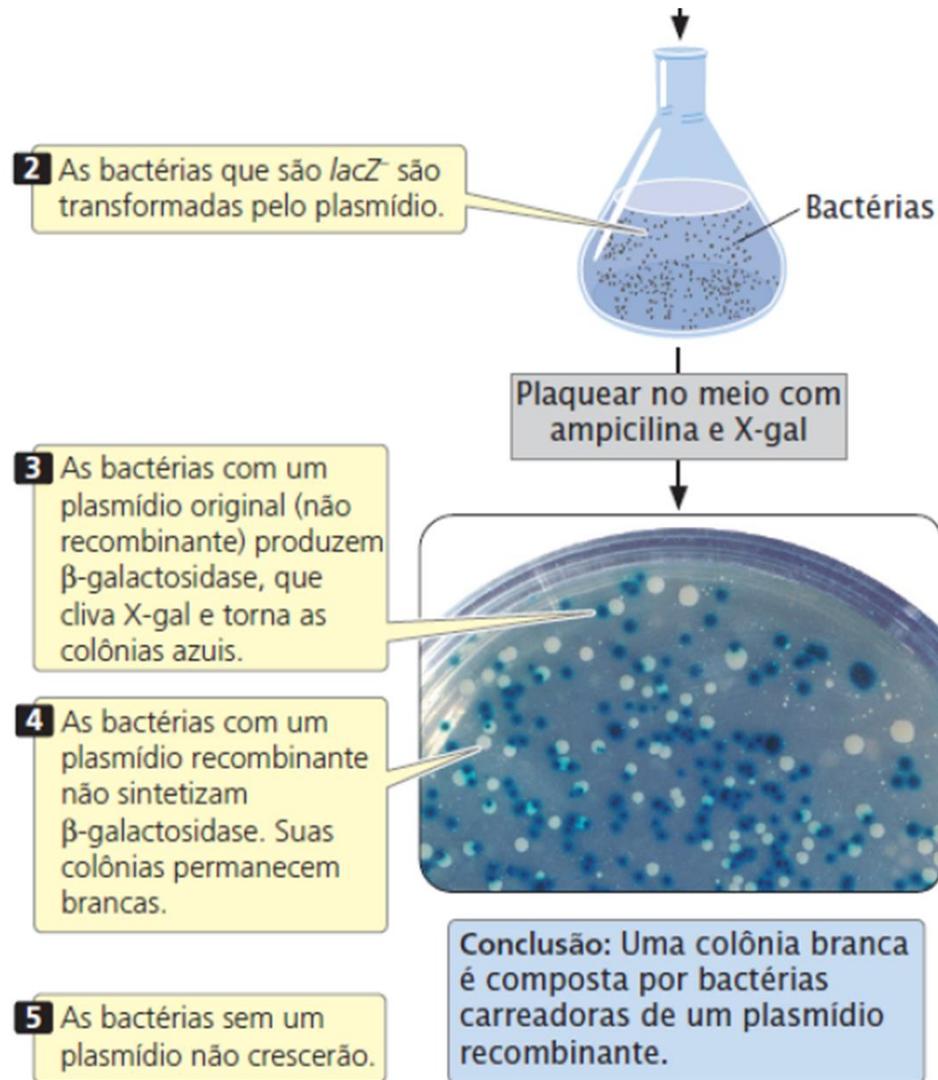
DNA PLASMIDIAL
RECOMBINANTE DE FITA
DUPLA INTRODUZIDO
EM UMA CÉLULA BACTERIANA

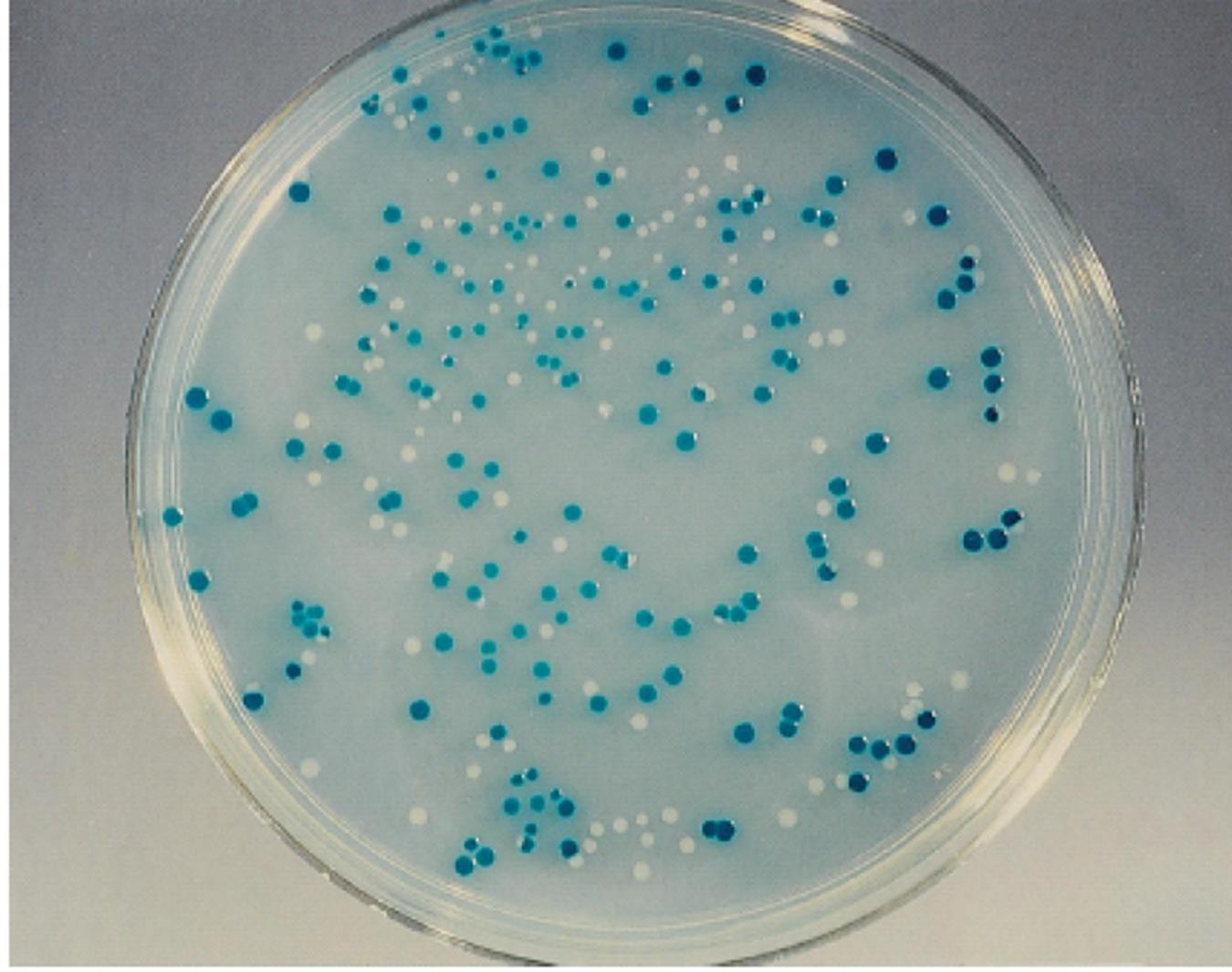


Cultura de células
produz centenas de
milhões de
novas bactérias



Várias cópias do plasmideo
purificado isoladas a partir
de células bacterianas rompidas





Cortesia de S. Kopczak e D. P. Snustad, University of Minnesota.



DNA de fita dupla humano

CLIVAGEM COM
NUCLEASES DE
RESTRICÇÃO



Milhões de fragmentos
de DNA genômico

FRAGMENTOS DE
DNA INSERIDOS
NOS PLASMÍDEOS

FRAGMENTOS DE
DNA INSERIDOS
NOS PLASMÍDEOS

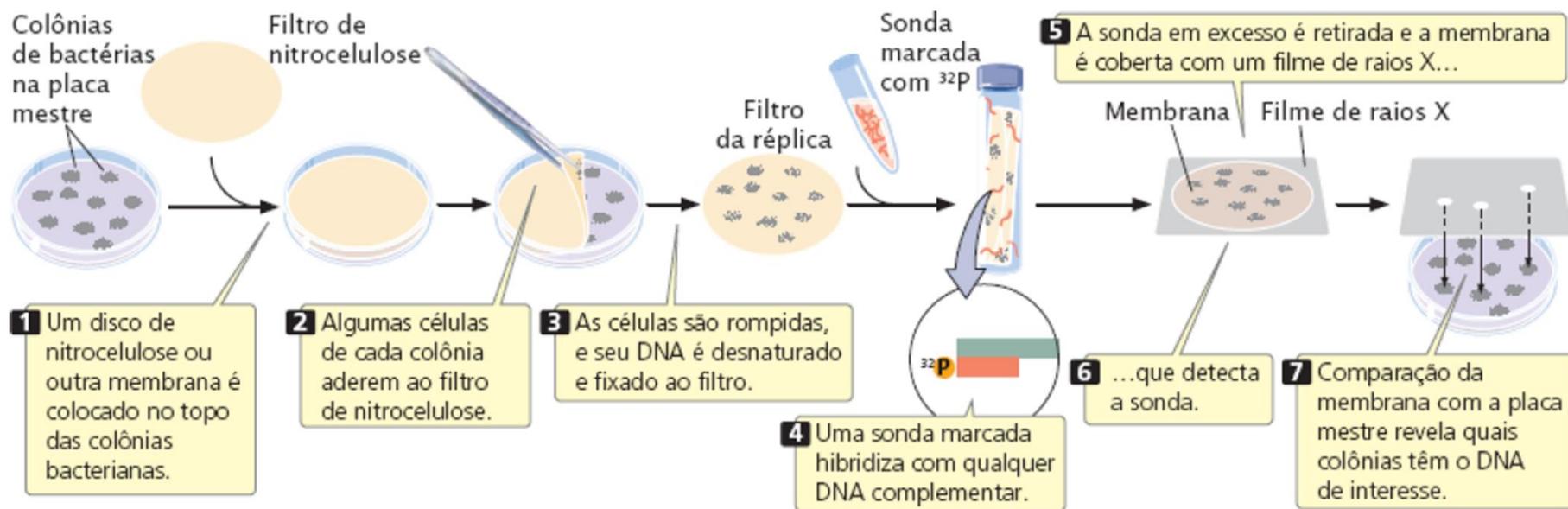


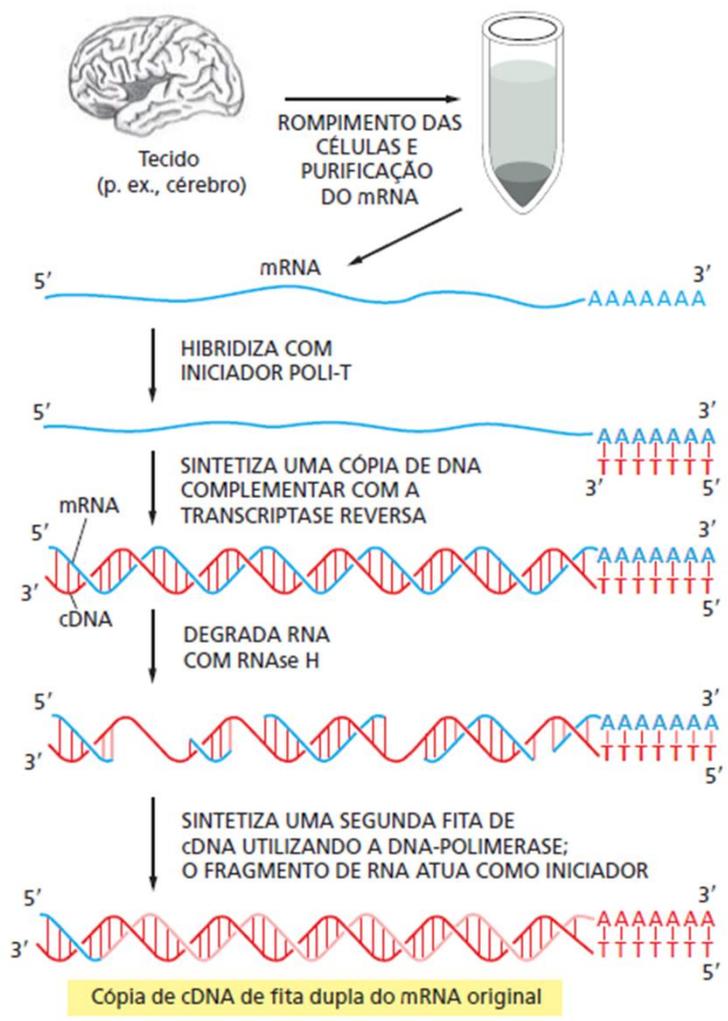
Moléculas de
DNA recombinante

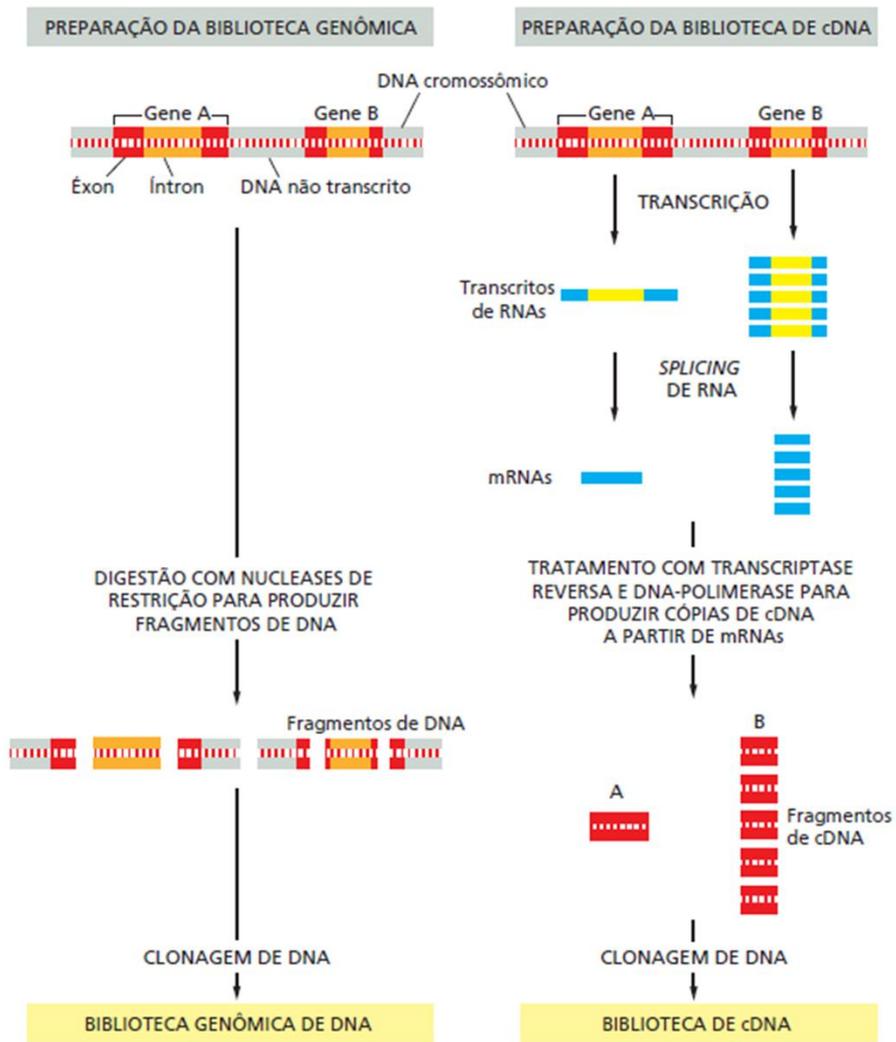
INTRODUÇÃO DOS
PLASMÍDEOS EM
BACTÉRIAS

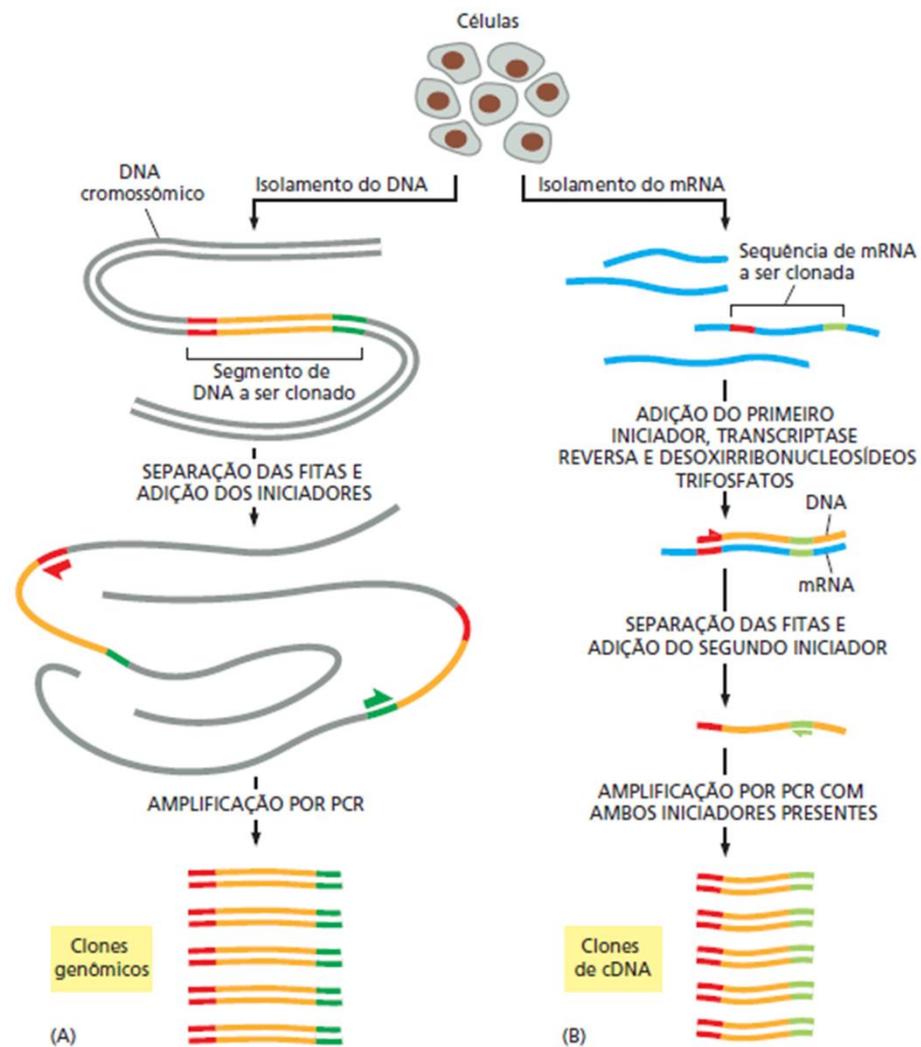


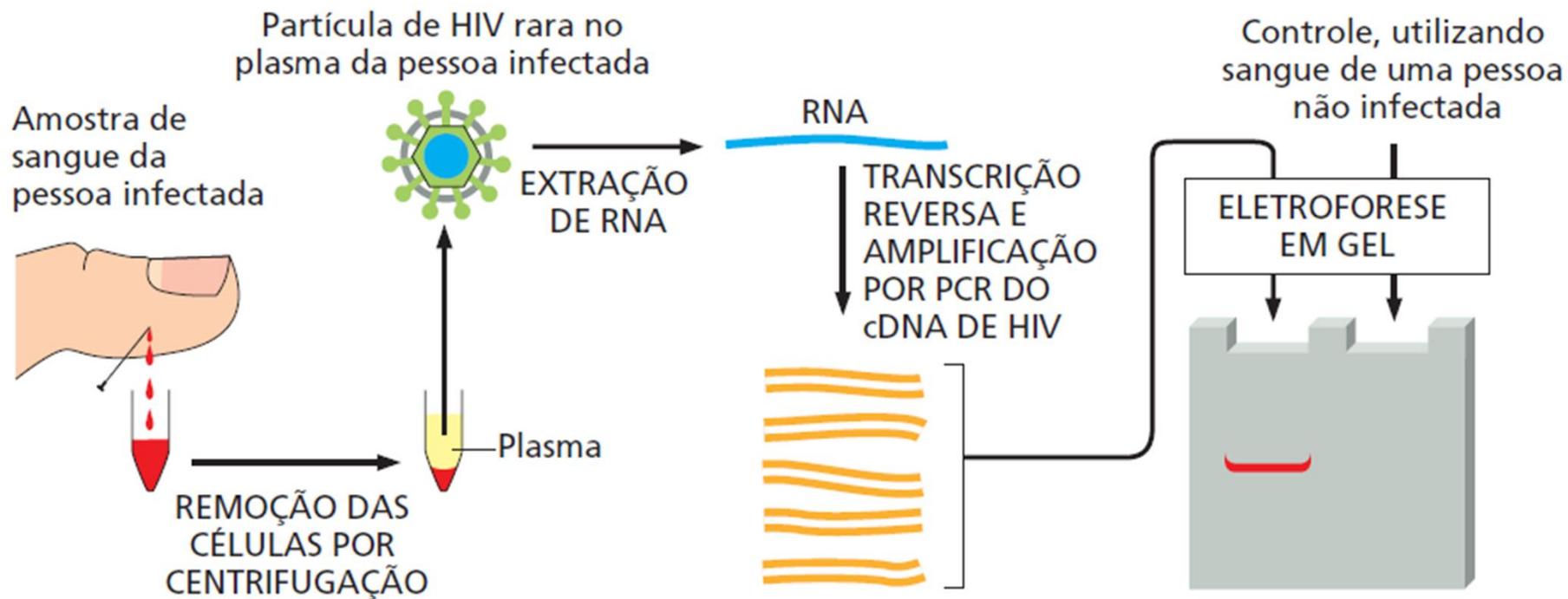
Biblioteca genômica de DNA humano

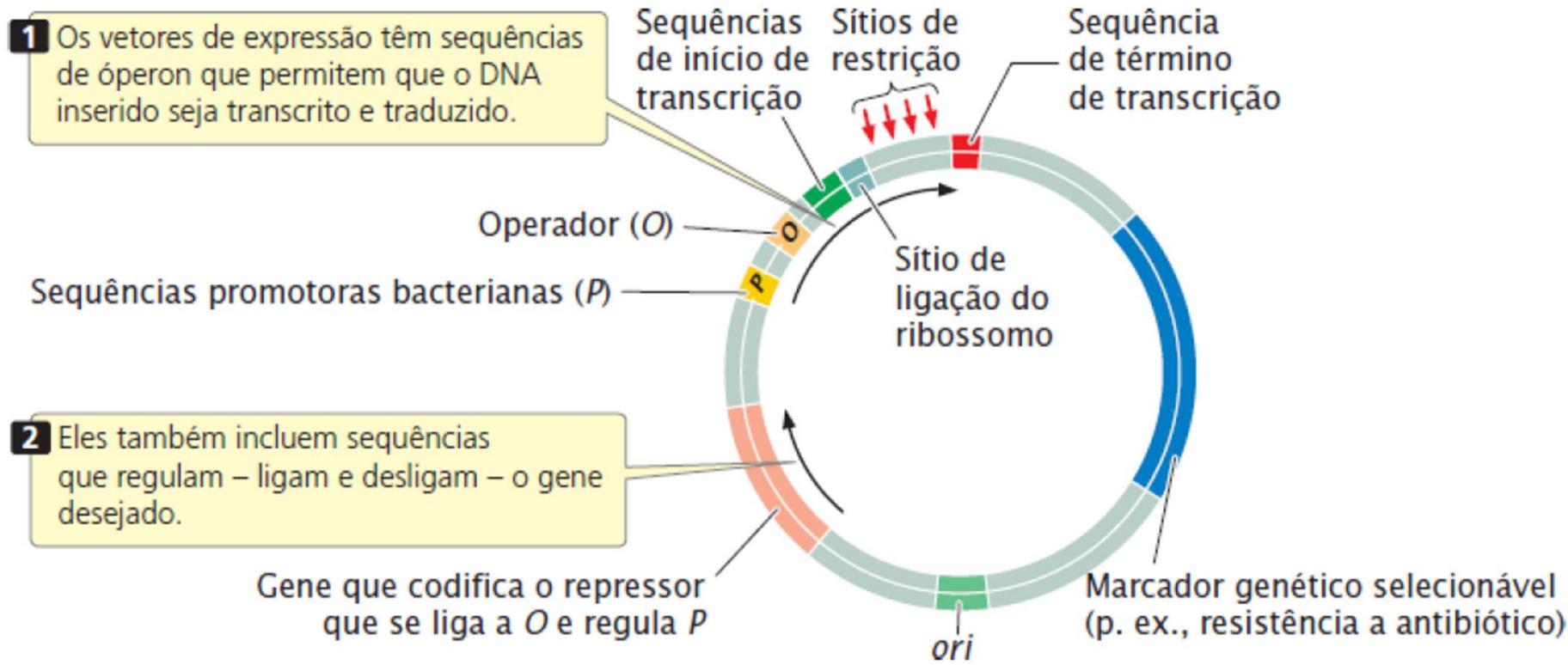








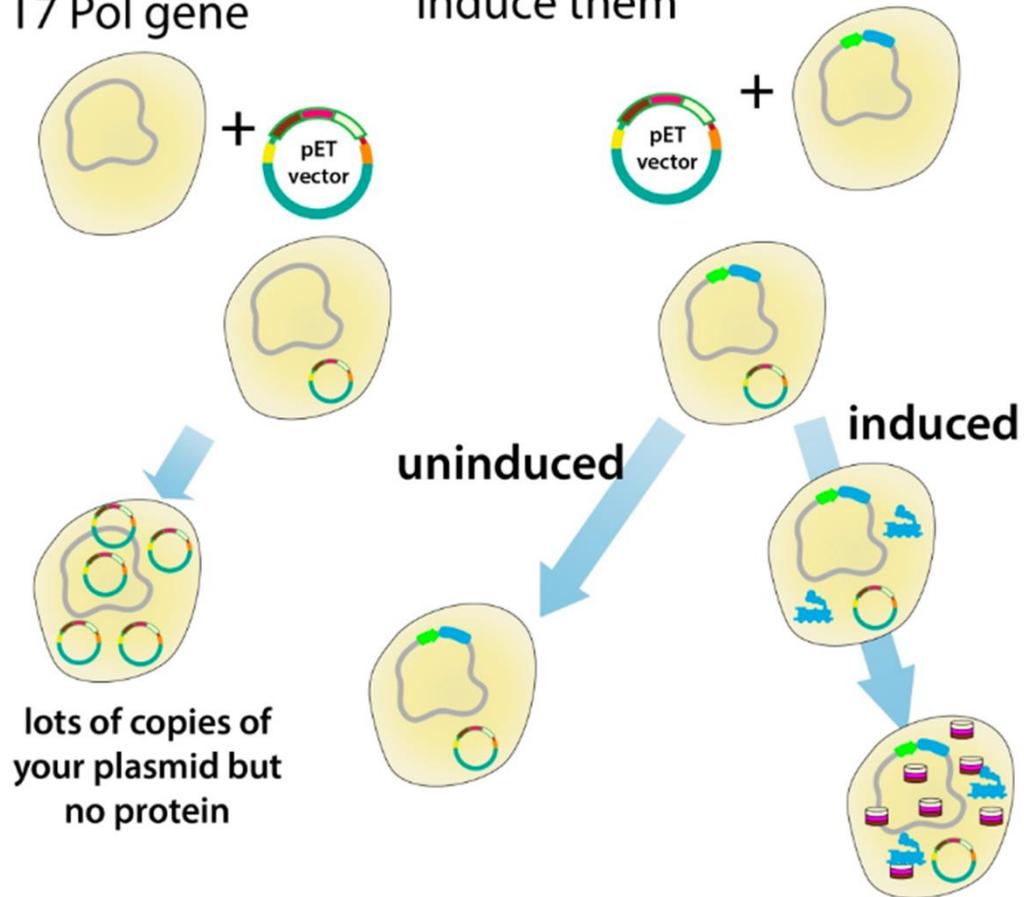


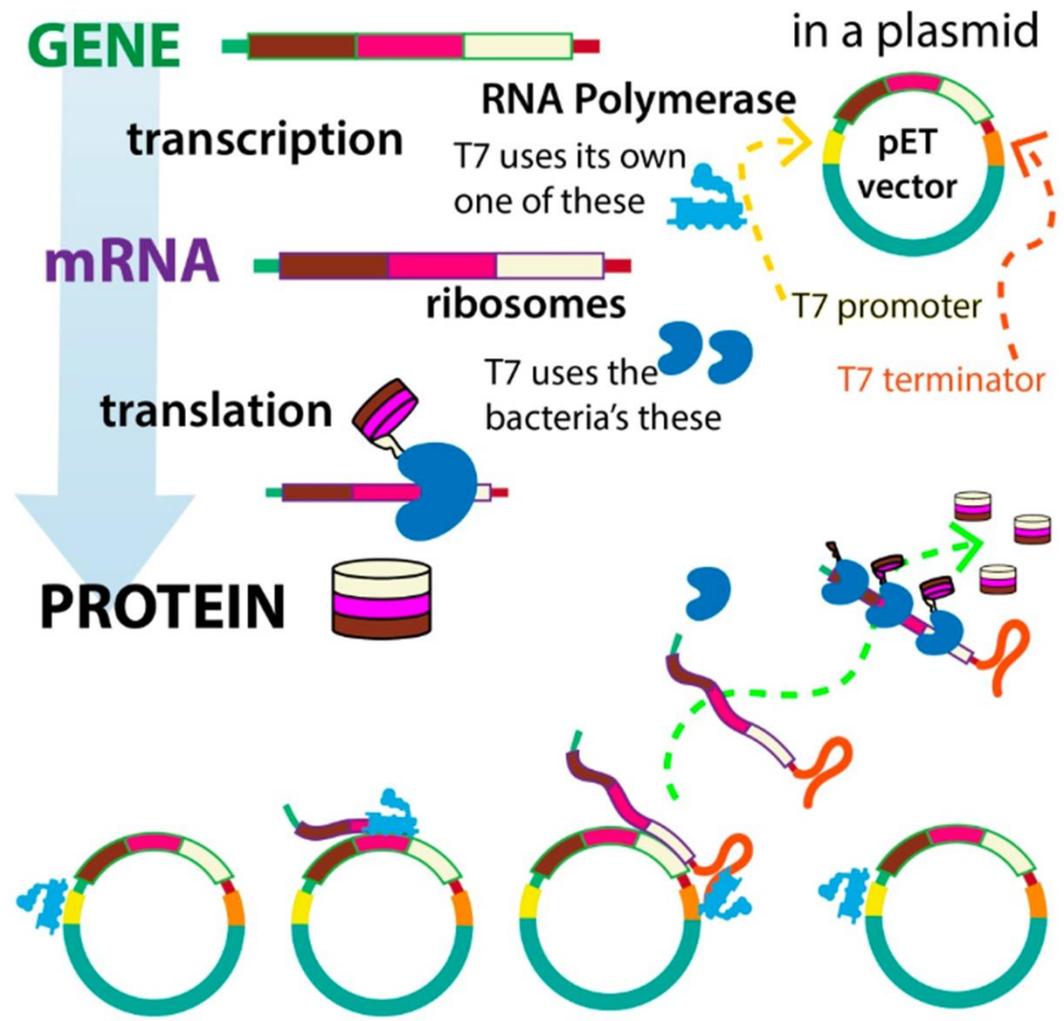


Típico vetor de expressão de E. coli

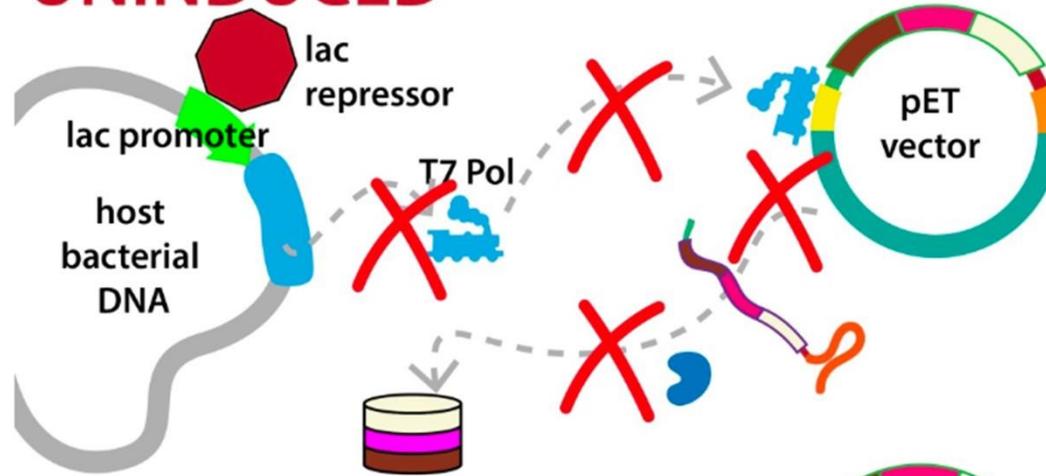
cloning cells
don't have the
T7 Pol gene

expression cells do, but they
don't make T7 Pol until you
induce them

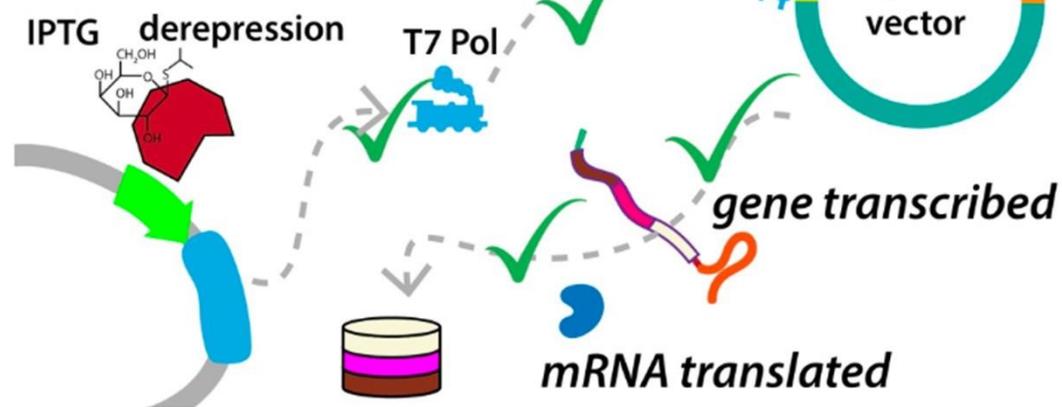


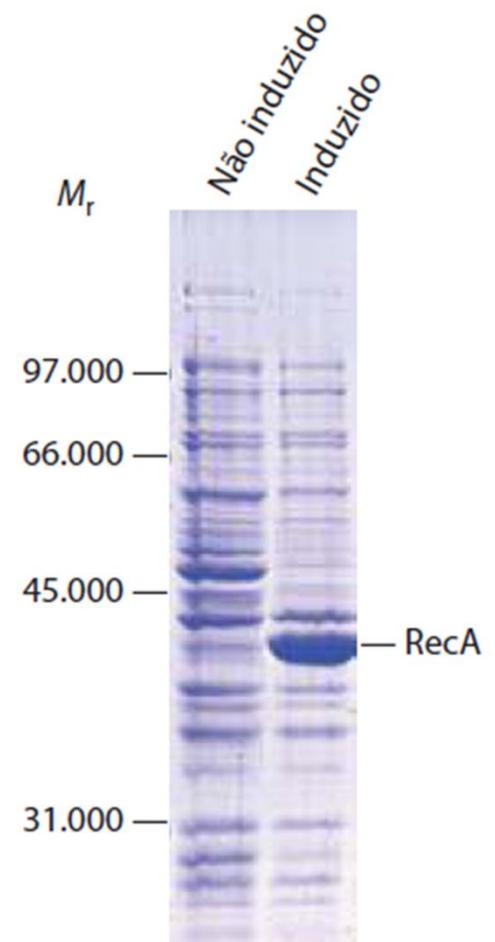
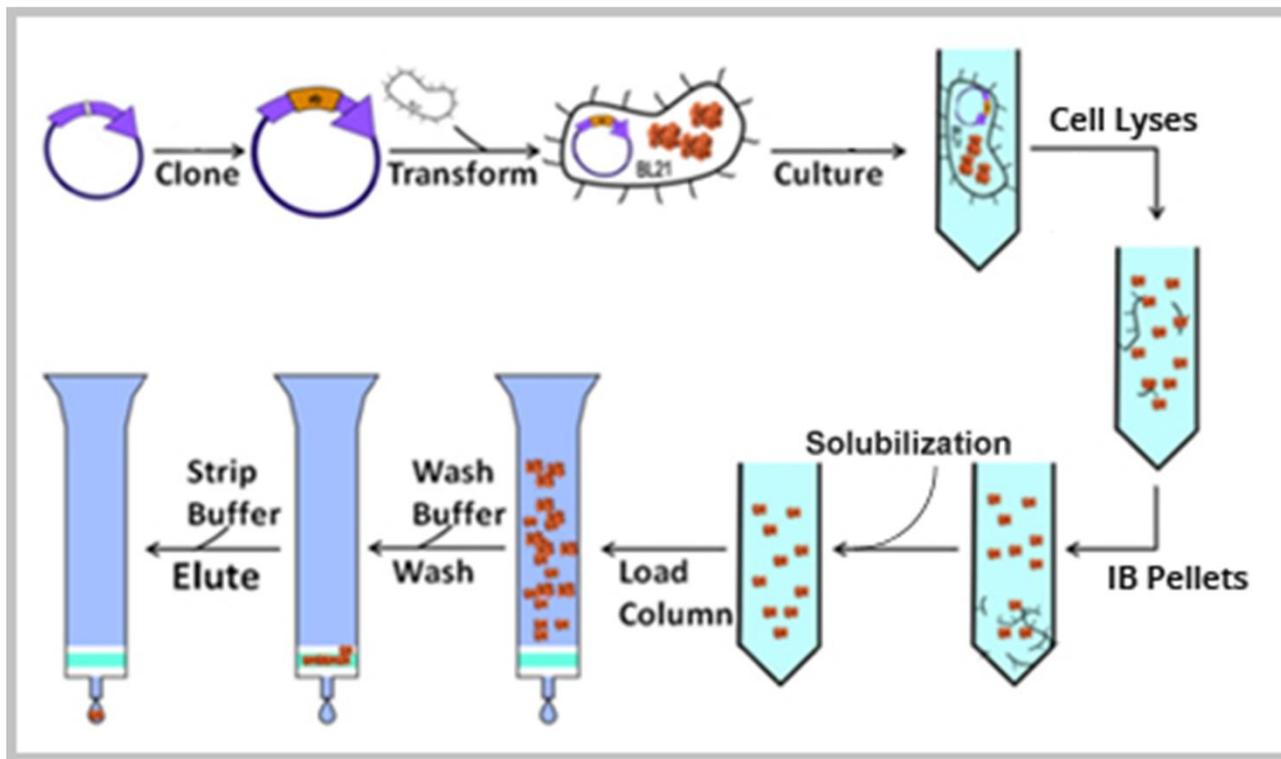


UNINDUCED



INDUCED



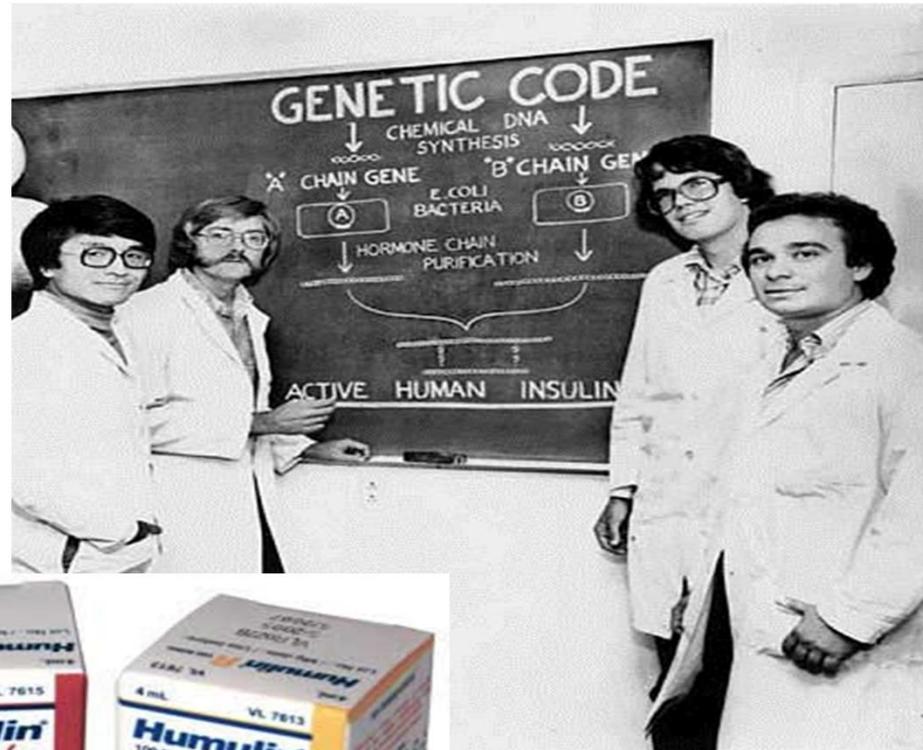


1978

Insulina humana recombinante é obtida pela Genentech

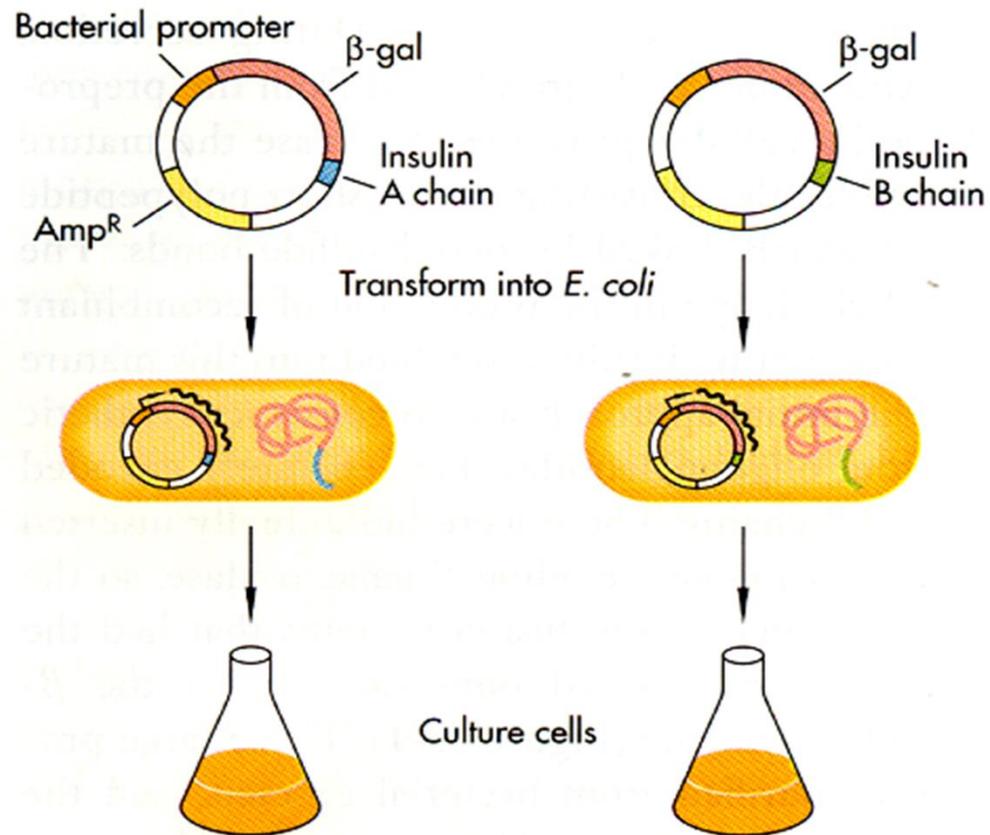
1982

Primeiro produto terapêutico obtido pela tecnologia do DNA recombinante a ser comercializado (Genentech licenciou a patente para Eli Lilly Company).



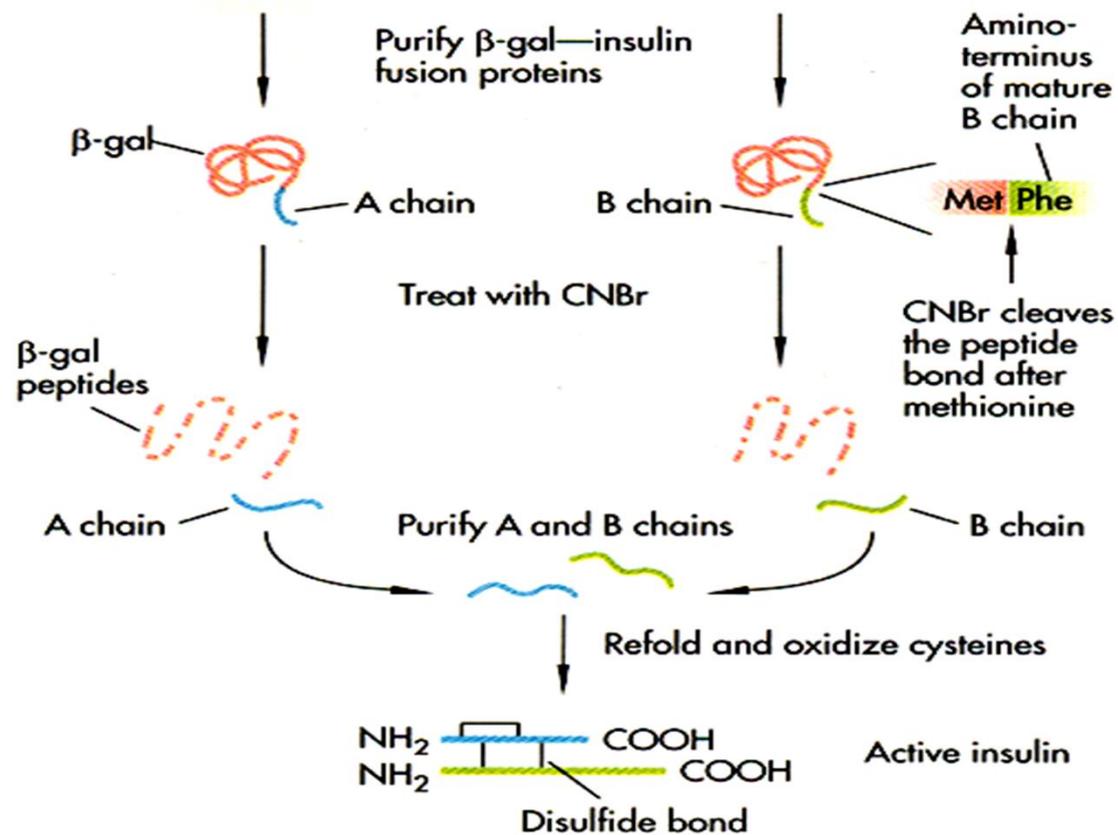
Estratégia para obtenção de insulina recombinante: produção das cadeias A e B separadamente

Segmentos de DNA das cadeias A e B obtidos por síntese química



Genentech, 1978

Estratégia para obtenção de insulina recombinante: produção das cadeias A e B separadamente



Biofármacos recombinantes aprovados na década de 1980

	Produto	Hospedeiro	Indicação Terapêutica	Ano
Humulin	insulina	<i>E. coli</i>	Diabetes	1982
Protropin	rh-GH	<i>E. coli</i>	hGH deficiency	1985
Roferon A	interferon alfa-2a	<i>E. coli</i>	Hairy cell leukaemia	1986
IntronA	interferon alfa-2b	<i>E. coli</i>	Cancer, genital warts and hepatitis	1986
Recombivax	Vacina hepatite B	<i>S. cerevisiae</i>	Hepatitis B	1986
Orthoclone OKT3	anticorpo monoclonal murino contra CD3 de células T	Hybridoma cell line	Reversal of acute kidney and transplant rejection	1986
Humatrope	Somatropina	<i>E. coli</i>	hGH deficiency	1987
Activase	tissue plasminogen activator	CHO	Acute myocardial infarction	1987
Epogen	Hormone_like erythropoietin	CHO	Anaemia	1989

Adaptado de Microbial Cell Factories (2016)15:33

Table 4 Biosimilar products that had gained marketing authorization within the European Union and/or the United States by July 2018

Product type	Biosimilar (trade name)	Year (and region) approved	Reference product	Drug (active ingredient) manufacturer
Somatropin-based				
Human growth hormone-based	Omnitrope	2006 (EU)	Genotropin	Sandoz (Kundl, Austria)
	Valtropin	2006 (EU) Withdrawn 2012	Humatrope	LG Life Sciences (Jeonbuk-do, Republic of Korea)
Epoetin-based				
Epoetin-based	Binocrit	2007 (EU)	Eprex/Erypo	Rentschler (Laupheim, Germany) & Lek (Menges, Slovenia)
	Epoetin alfa hexal	2007 (EU)	Eprex/Erypo	Rentschler & Lek
	Abseamed	2007 (EU)	Eprex/Erypo	Rentschler & Lek
	Retacrit	2018 (US) 2007 (EU)	Eprex/Erypo (EU) Epogen/Procrit (US)	Norbitec (Uetersen, Germany) Norbitec (Uetersen, Germany)
	Silapo	2007 (EU)	Eprex/Erypo	Norbitec
Filgrastim-based				
G-CSF-based	Ratiograstim	2008 (EU)	Neupogen	Sicor (Vilnius, Lithuania)
	Filgrastim ratiopharm	2008 (EU) Withdrawn 2011	Neupogen	Sicor
	Biograstim	2008 (EU) Withdrawn 2015	Neupogen	Sicor
	Tevagrastim	2008 (EU)	Neupogen	Sicor
	Zarxio (US)	2015 (US)	Neupogen	Sandoz (Kundl, Austria)
	Zarzio (EU)	2009 (EU)	Neupogen	Sandoz (Kundl, Austria)
	Filgrastim hexal	2009 (EU)	Neupogen	Sandoz (Kundl, Austria)
	Nivestym (US)	2018 (US)	Neupogen	Hospira (Pfizer) (Zagreb, Croatia)
	Nivestim (EU)	2010 (EU)	Neupogen	Hospira (Pfizer) (Zagreb, Croatia)
	Grastofil	2013 (EU)	Neupogen	Intas Biopharmaceuticals (Gujarat, India)
Accofil	2014 (EU)	Neupogen	Intas Biopharmaceuticals	
Pegfilgrastim-based	Fulphila	2018 (US)	Neulasta	Mylan (Zurich)
Follicle-stimulating hormone-based				
Follicle-stimulating hormone-based	Ovaleap	2013 (EU)	Gonal F	Merckle Biotec (Ulm, Germany)
	Bemfola	2014 (EU)	Gonal F	Polymun Scientific Immunobiologische Forschung (Klosterneuburg, Austria)
Insulin-based				
Insulin glargine-based	Abasaglar	2014 (EU)	Lantus	Lilly del Caribe (Carolina, Puerto Rico, USA) Eli Lilly (Indianapolis)
	Lusduna	2017 (EU) 2017 (US), tentative	Lantus	Merck Sharp & Dohme (Elkton, VA, USA)
	Semglee	2018 (EU)	Lantus	Biocon Nusajaya (Johor, Malaysia)
Insulin lispro-based	Insulin lispro Sanofi	2017 (EU)	Humalog	Sanofi-Aventis (Frankfurt)

