Biotecnologia

ACH5545 Engenharia Genética

Atividades de Laboratório

2º Semestre 2024

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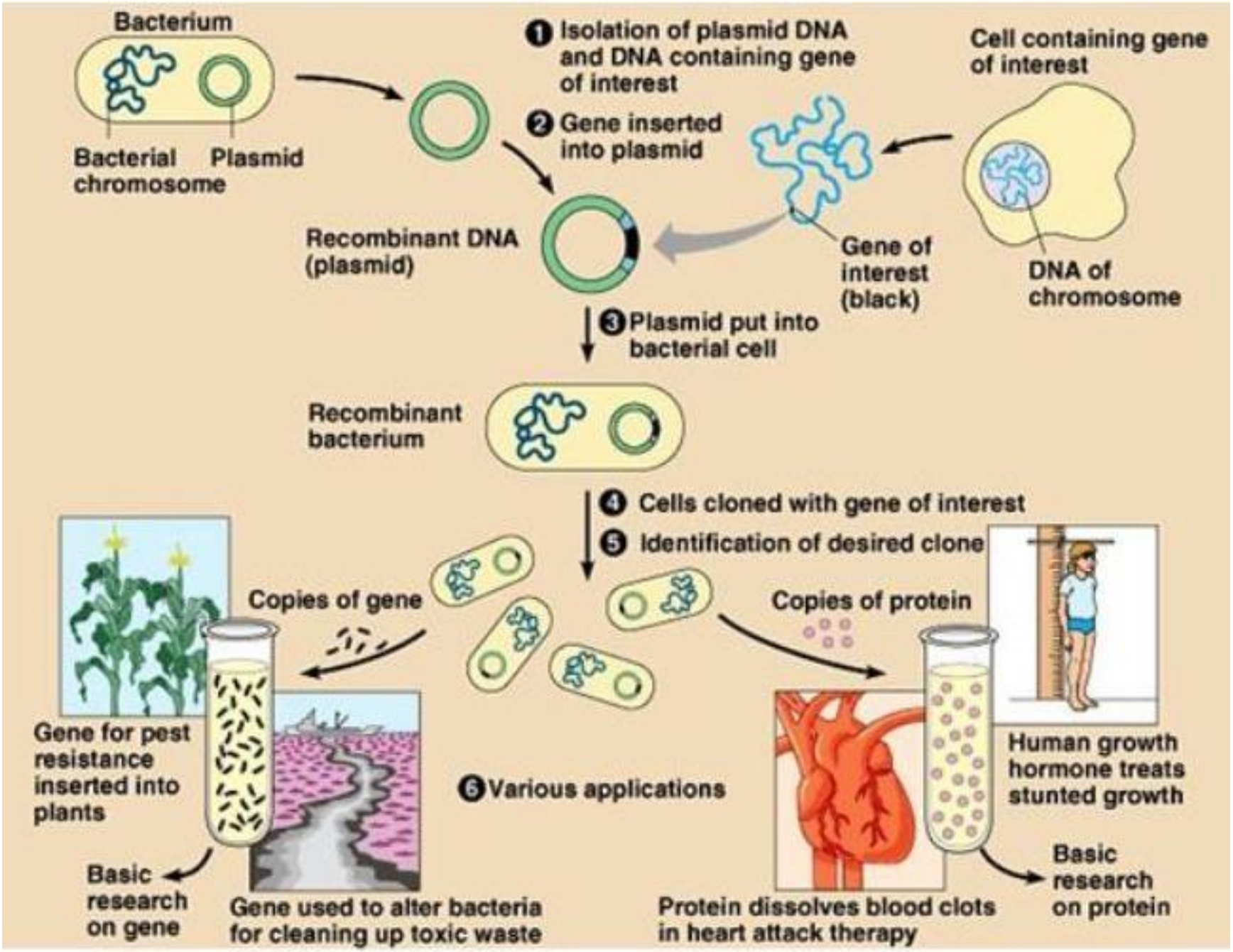
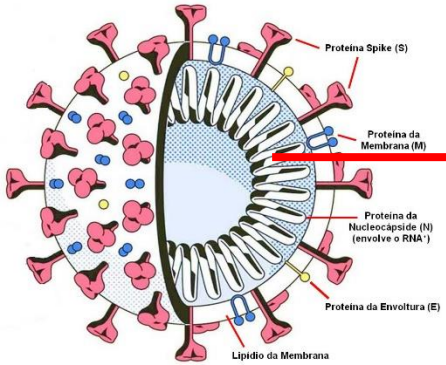
Créditos: 4
Período: Quinta-feira (14h00 -18h00), Laboratório de Biotecnologia – Edifício A2, 1º andar

USP - 2024

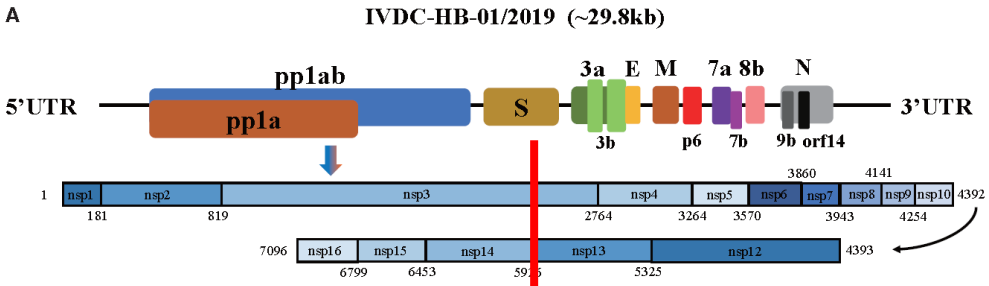
Atividade

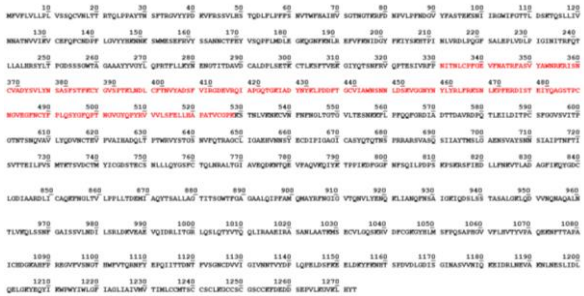
- Seleção de clones recombinantes (Resistência à antibióticos)
- Confirmação de clones positivos (PCR)
- Eletroforese DNA em gel de agarose

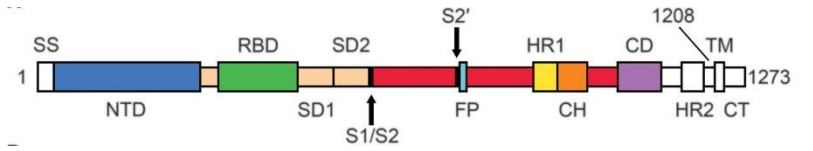

Bacterium
Bacterial chromosome
Plasmid
1 Isolation of plasmid DNA and DNA containing gene of interest
2 Gene inserted into plasmid
Cell containing gene of interest
Recombinant DNA (plasmid)
Gene of interest (black)
DNA of chromosome
3 Plasmid put into bacterial cell
Recombinant bacterium
4 Cells cloned with gene of interest
5 Identification of desired clone
Copies of gene
Copies of protein
Gene for pest resistance inserted into plants
Basic research on gene
Gene used to alter bacteria for cleaning up toxic waste
6 Various applications
Human growth hormone treats stunted growth
Basic research on protein
Protein dissolves blood clots in heart attack therapy



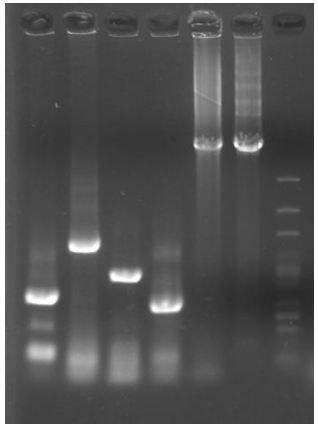
SARS-CoV-2



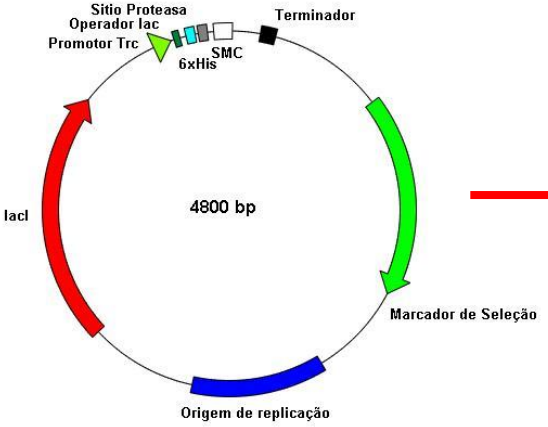


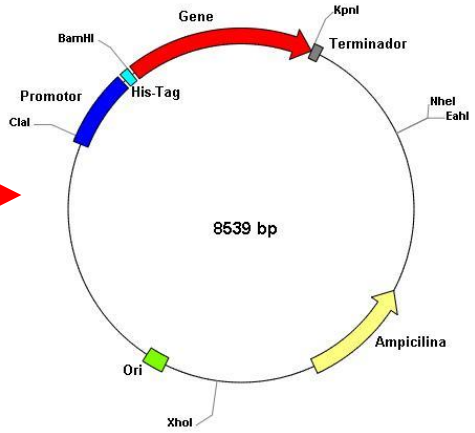


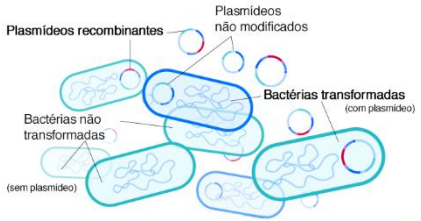
Proteína S

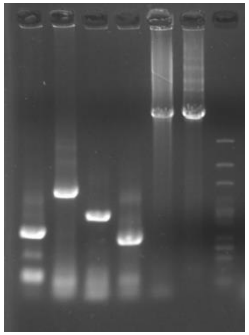


+







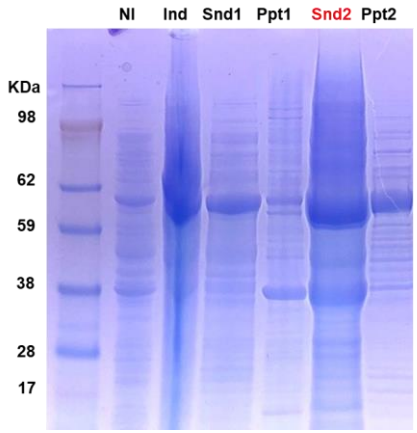


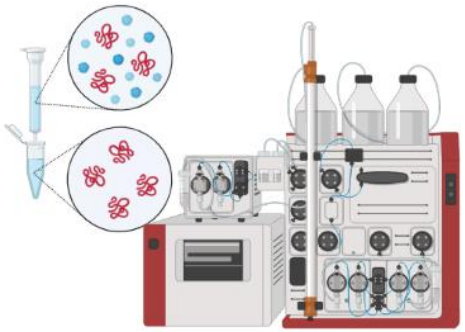
Aplicação

- Gene Terapêutico
- Marcador molecular
- Modificação genética
- Sequenciamento

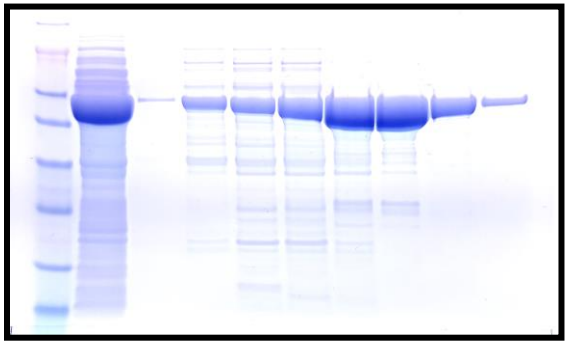
200 mL de meio
LB + amp +
IPTG
37 °C, *overnight*,
150 rmp

Produção da Proteína de interesse





Purificação

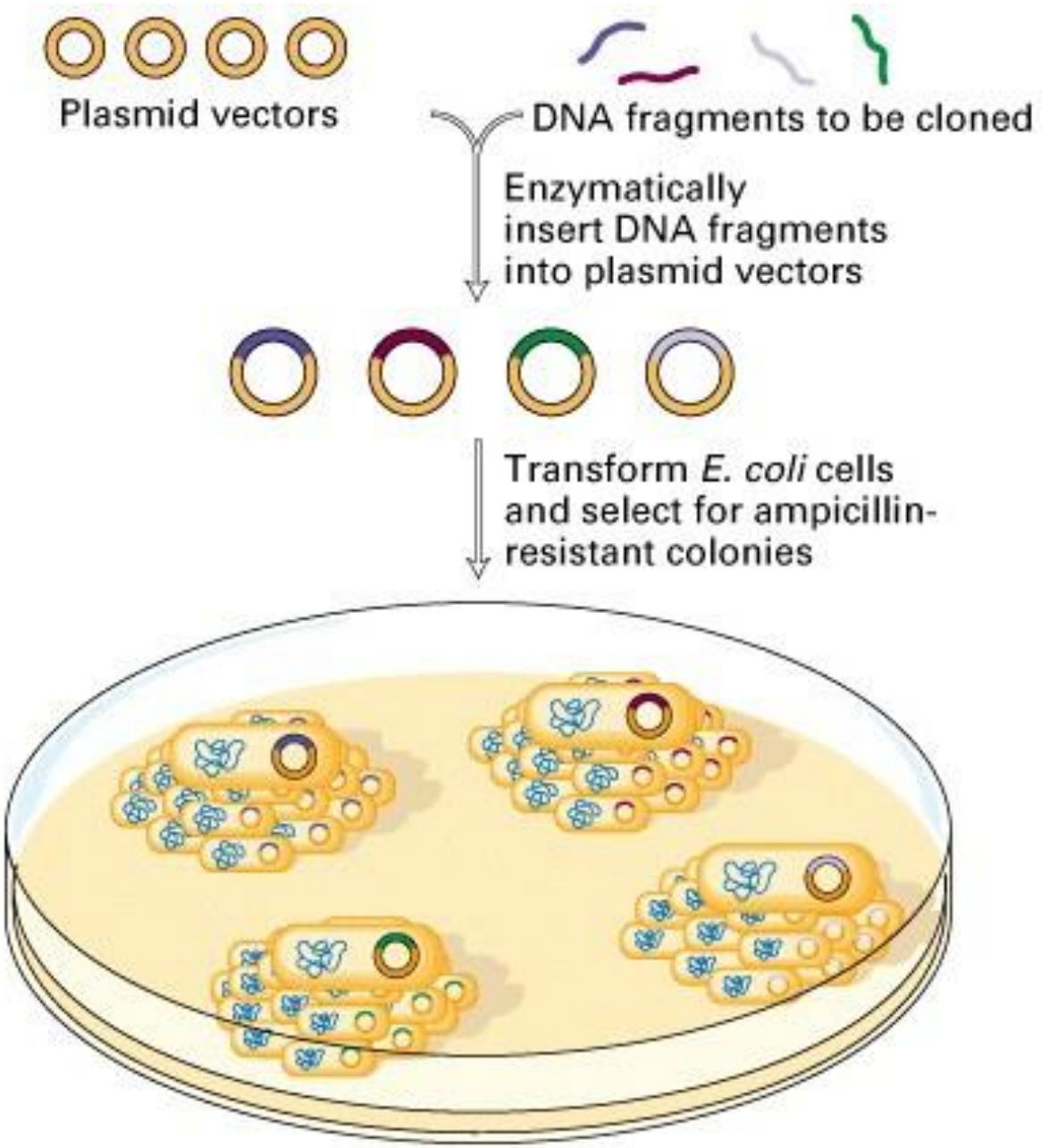


Análise Complementares

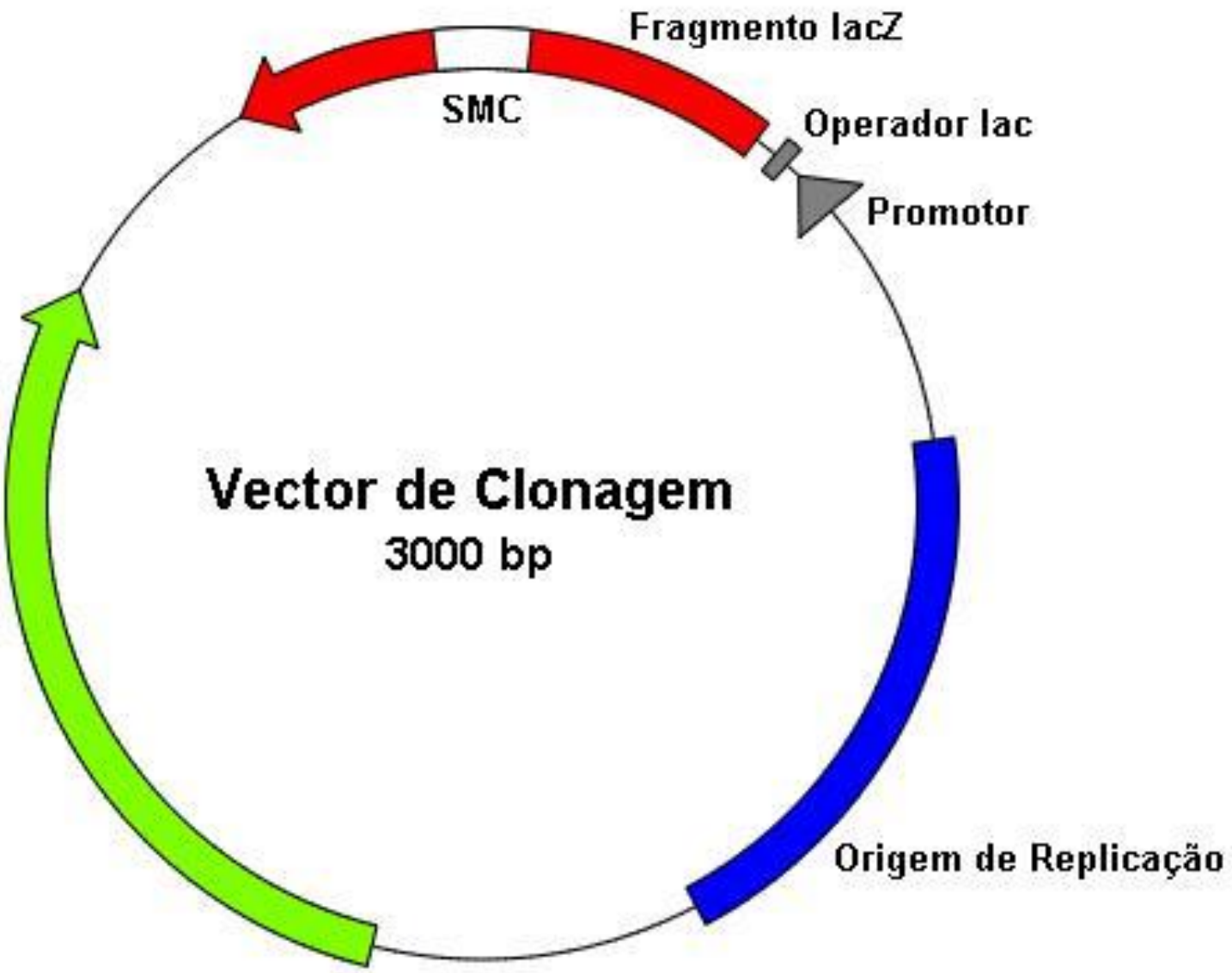
- Sequenciamento
- Estrutura 3D – Cristalografia
- Dicroísmo circular
- Atividade

Aplicação

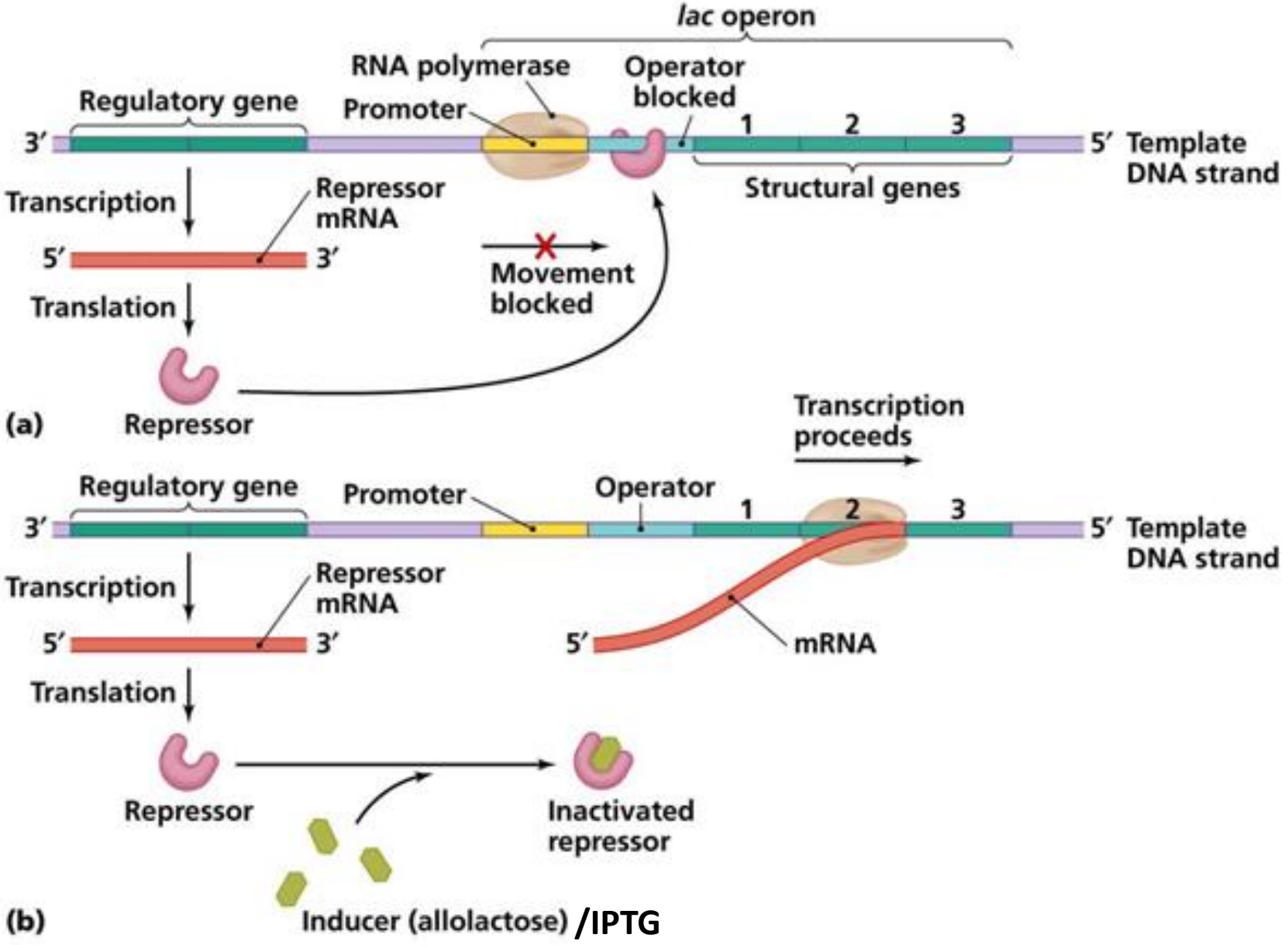
- Proteína terapêutica
- Enzima
- Diagnóstico


Plasmid vectors
DNA fragments to be cloned
Enzymatically
insert DNA fragments
into plasmid vectors
Transform E. coli cells
and select for ampicillin-
resistant colonies

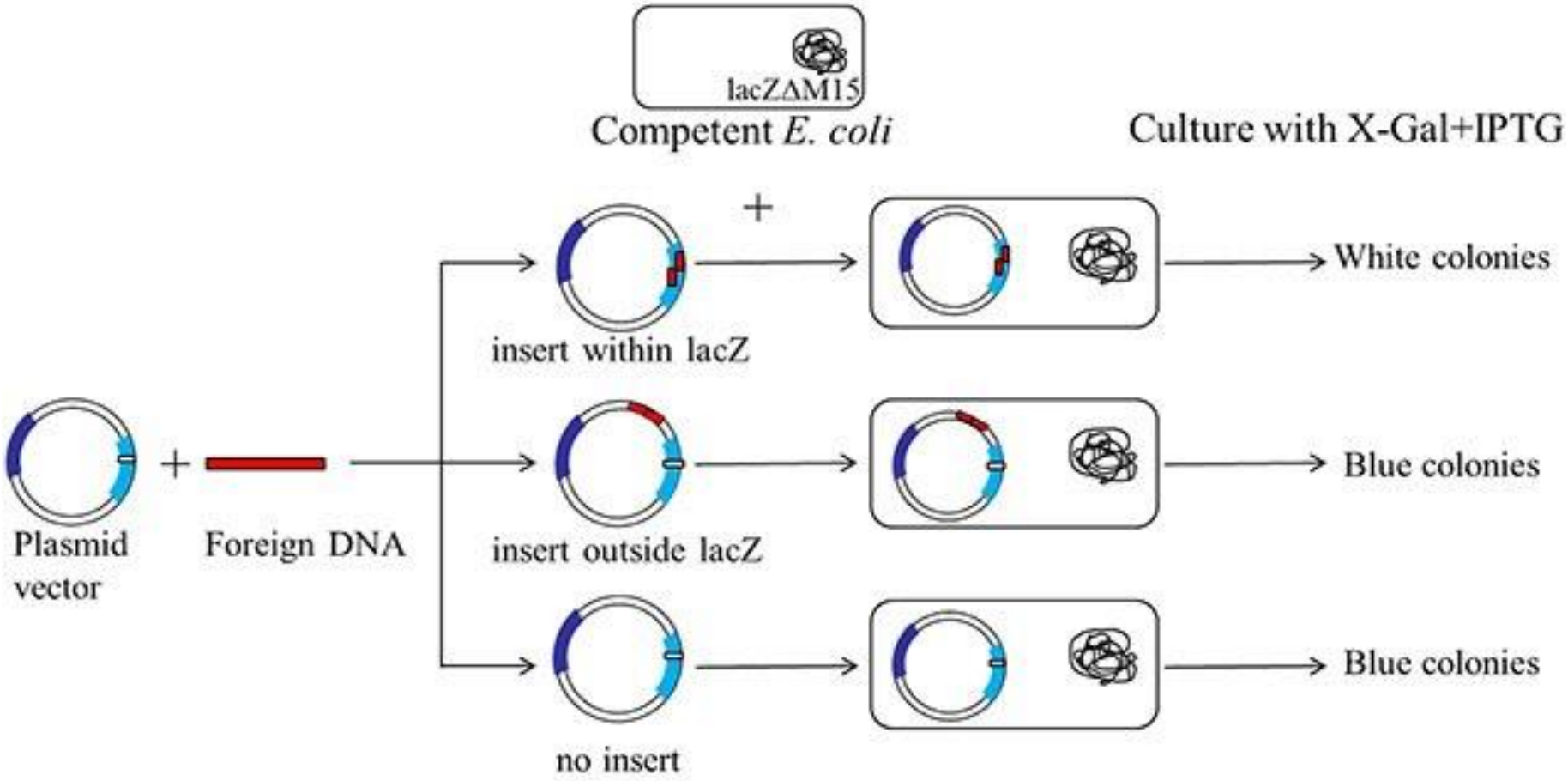
Sistemas de Clonagem/expressão procariota



Operon Lac

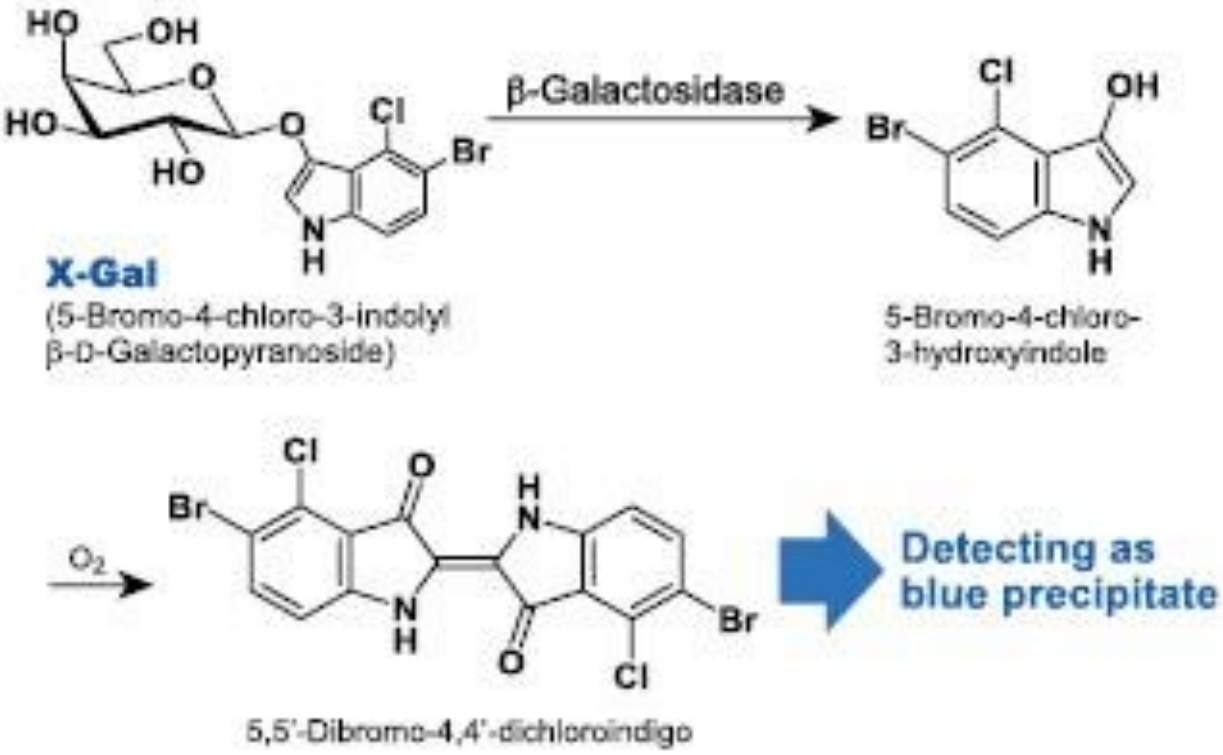


Seleção Branco/Azul

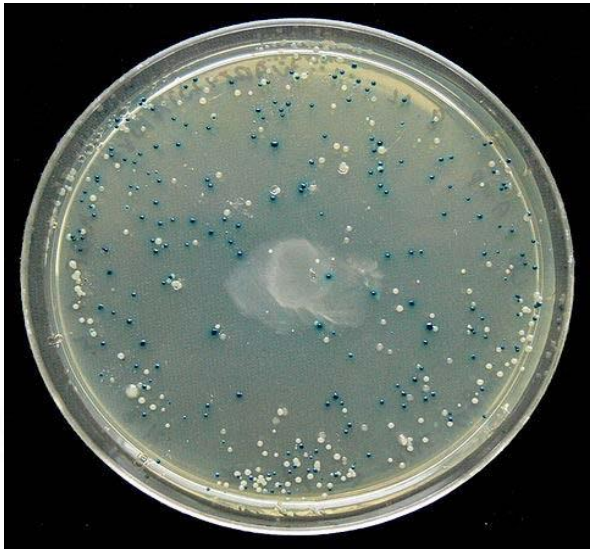


Seleção Branco/Azul

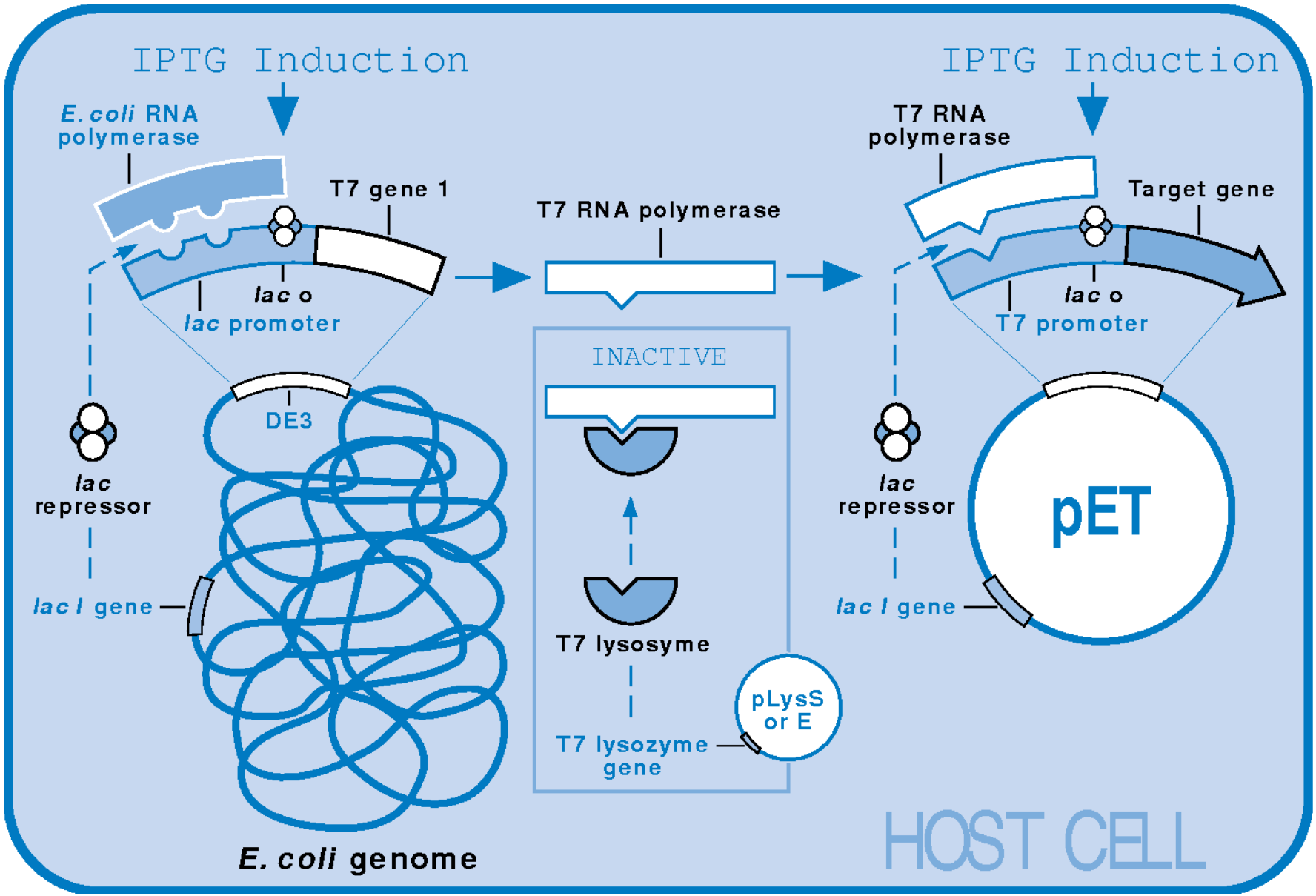
Blue/White Selection of Recombinants: The pGEM®-T and pGEM®-T Easy Vectors are high-copy-number vectors containing T7 and SP6 RNA polymerase promoters flanking a multiple cloning region within the α-peptide coding region of the enzyme β-galactosidase. Insertional inactivation of the α-peptide allows identification of recombinants by blue/white screening on indicator plates.



X-Gal hydrolysis with β-galactosidase.



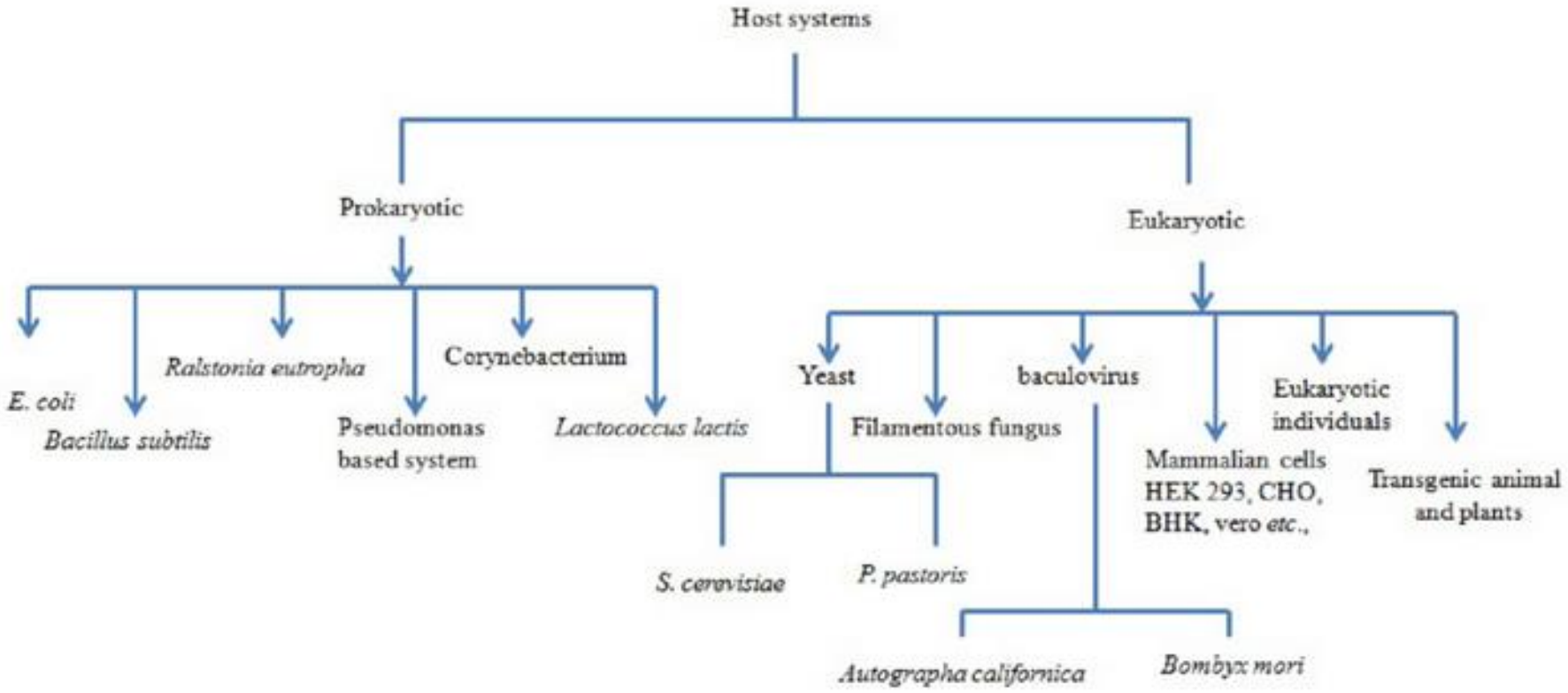
Sistema pET - BL21(DE3)



Cepas *E. coli* para produção de proteínas recombinantes

Feature	Strain	K-12	B	Supplier
Tighter regulation of T7 RNAP	Lemo21(DE3)		+	NEB
	NEB Express LysY		+	NEB
	NEB Iq	+	+	NEB
	BL21(DE3) LysS		+	EMD Millipore/ Thermo Fisher Scientific
	BL21-AI		+	Thermo Fisher Scientific
	Walker Strains			
C41/C43 (DE3)		+	Lucigen	
Codon bias correction	BL21 Codon Plus		+	Agilent Technologies
	Rosetta		+	EMD Millipore
Cytoplasmic disulfide bond formation	SHuffle	+	+	NEB
	Origami	+	+	EMD Millipore
	AD494	+		
	BL21 *trxB*		+	
Co-express Cpn10 and Cpn60 chaperonins from *O. antarctica*	Arctic Express		+	
Tyrosine kinase	TKB1 / TKX1	+	+	
His-tag contamination	NiCo21(DE3)		+	
Endotoxin contamination	ClearColi		+	
mRNA stability	BL21(DE3) Star		+	Thermo Fisher
Seleno-methionine labeling	T7 Express Crystal	+		NEB
	B834		+	EMD Millipore

its activity at the temperature optimum of 30°C.[3] To overcome this obstacle, ArcticExpress competent cells have been engineered for improved protein processing at low temperatures. These cells co-express the cold-adapted chaperonins Cpn10 and Cpn60 from the psychrophilic bacterium, *Oleispira antarctica*. The Cpn10 and Cpn60 chaperonins from *O. antarctica* have 74% and 54% amino acid identity to the *E. coli* GroEL and GroES chaperonins, respectively, and show high protein refolding activities at temperatures of 4–12°C.[3] When expressed in ArcticExpress cells, these chaperonins confer improved protein processing at lower temperatures, potentially increasing the yield of active, soluble recombinant protein.



Different host systems available for the production of recombinant Proteins

Table 1: Merits and demerits of different host systems for expression of recombinant proteins

Host system	Merits	Demerits
Escherichia coli	Easy Quick Economical Rapid growth rate Capacity for continuous fermentation	Does not possess necessary machinery for removing introns from transcripts Foreign gene might contain sequences that act as termination signals resulting in premature termination and loss of gene expression Codon bias Lack of post translational modifications Glycosylation is extremely uncommon in bacteria Production of proteins in the insoluble form or in the form of inclusion bodies Degradation of proteins Accumulation of endotoxins
Bacillus subtilis	Does not produce LPS/endotoxins Can be transformed readily with many bacteriophages and plasmids Capable of secreting functional extracellular proteins directly into the culture medium	Production of extracellular proteases which can recognize and degrade heterologous proteins Instability of plasmids Reduced or non expression of the protein of interest
Yeast system	Rapid growth in low cost medium Appropriate post-translational modifications Safety of the system is guaranteed No endotoxins production	Hyperglycosylation of proteins Codon bias Inefficient in secreting the proteins into growth medium leading to intracellular retention
Filamentous fungus	High-level of expression	Complex Lack of knowledge on physiology
Baculovirus / Insect system	High level of expression Appropriate posttranslational modifications Safe for vertebrates Excellent tool for recombinant glycoprotein production	Continuous expression not possible More demanding culture conditions
Mammalian cells / system	Proper protein folding Appropriate post-translational modifications and product assembly Proper glycosylation	High cost Complicated technology Potential contamination with animal viruses
Transgenic plants	Easy scaling up at low cost Proteins can be localized to different organs at different growth stages High yield	Expression levels are target dependent Functional assays yet to be developed
Transgenic animals	Proper protein folding Appropriate post-translational modifications and product assembly Proper glycosylation	Relatively longer production period Low yield Higher costs

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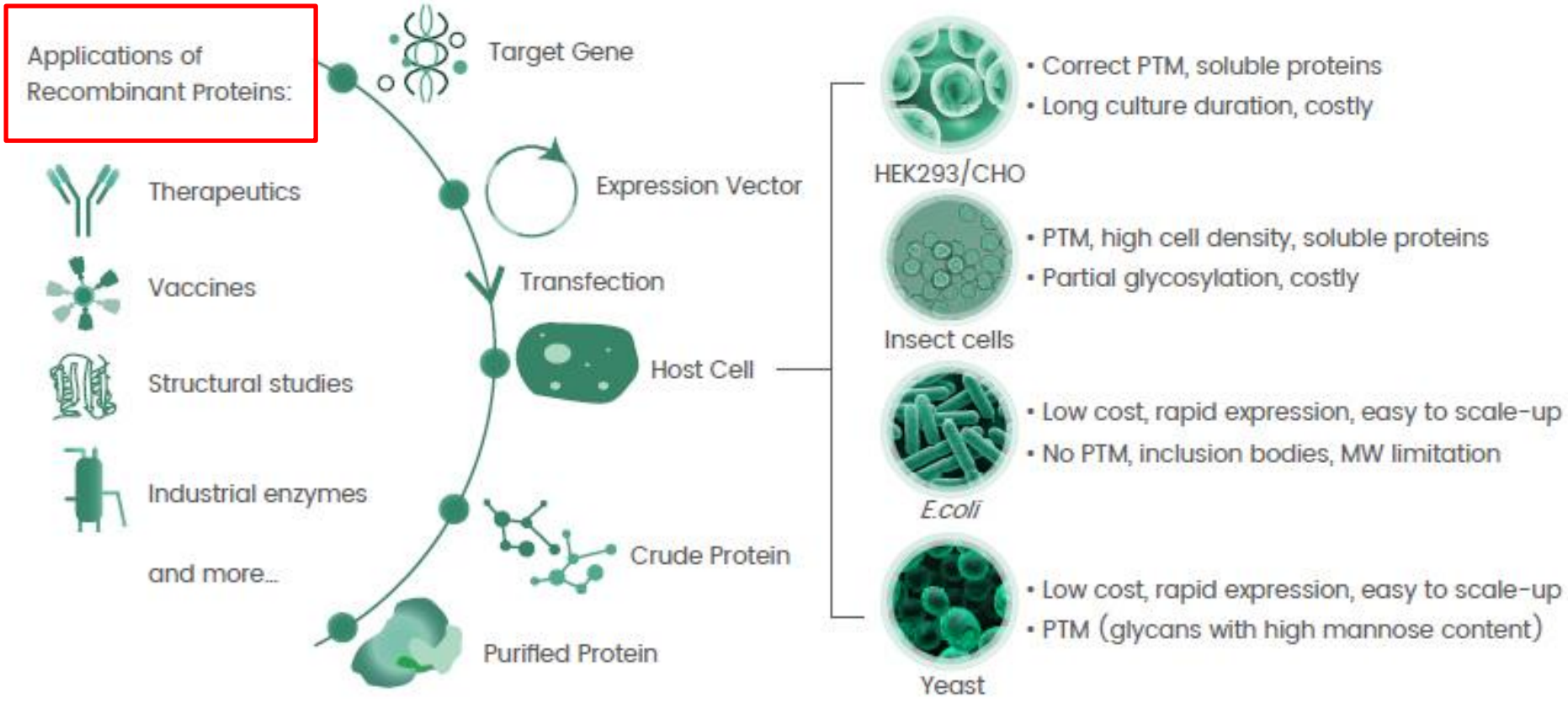
Vetores para produção de proteínas recombinantes

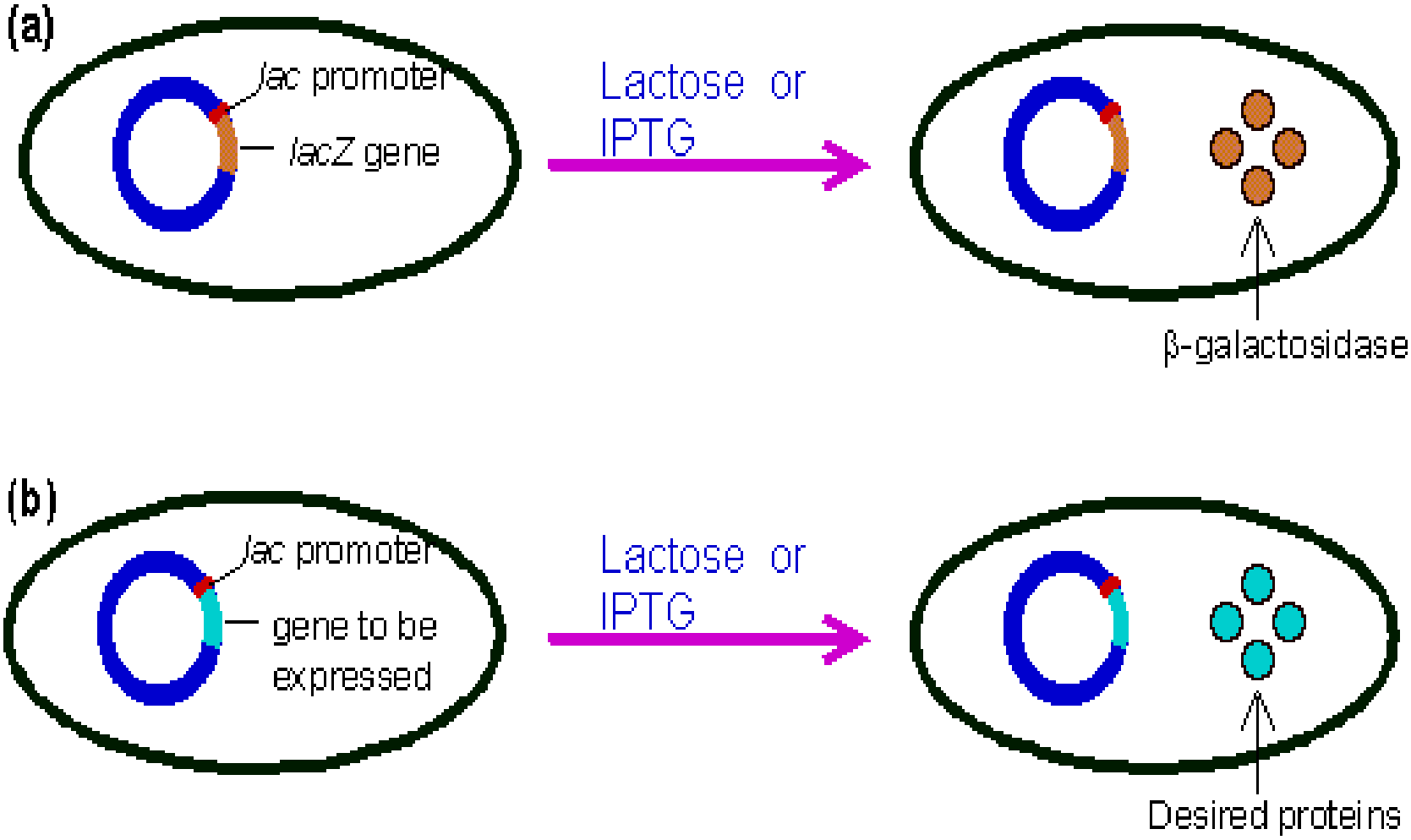
Expression Host	*E.coli*	Yeast	Insect	Mammalian
Category	Prokaryote	Eukaryote	Eukaryote	Eukaryote
Culture density	High	High	High	Medium
Culture duration	Short (1~2 days)	Short (1~2 days)	Medium (2~4 days)	Long (5~7 days)
Protein folding	Limited	Yes	Yes	Yes
PTM	None	Glycosylation	Glycosylation, phosphorylation...	Glycosylation, phosphorylation...
Suitable proteins	Proteins with low MW	Secreted, intracellular	Secreted, intracellular	Secreted
Cost	Low	Low	High	High
Example Cell Lines	BL21(DE3), Rosetta...	pichia pastoris	sf9, sf21, High-five	HEK293, CHO
Note	Inclusion bodies	High mannose glycan	Low MW glycan	



https://www.sinobiological.com/news/recombinant-protein-expression

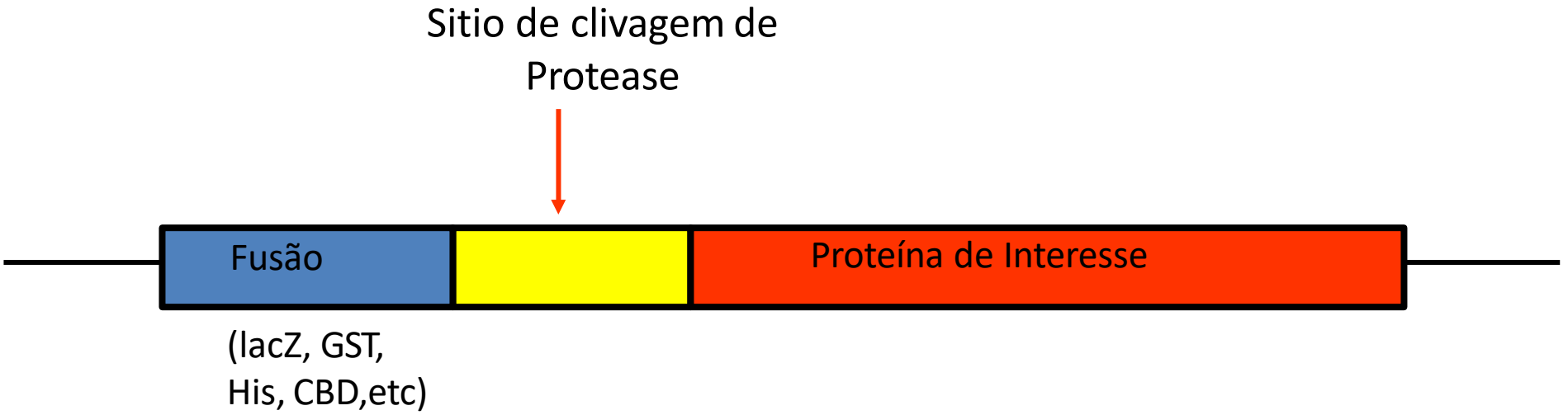
Expressão da Proteína de interesse

O vetor de expressão é empregado para transformar Bactérias competentes (*E. coli* deficientes em Proteasas). Após cultura é Testada a expressão

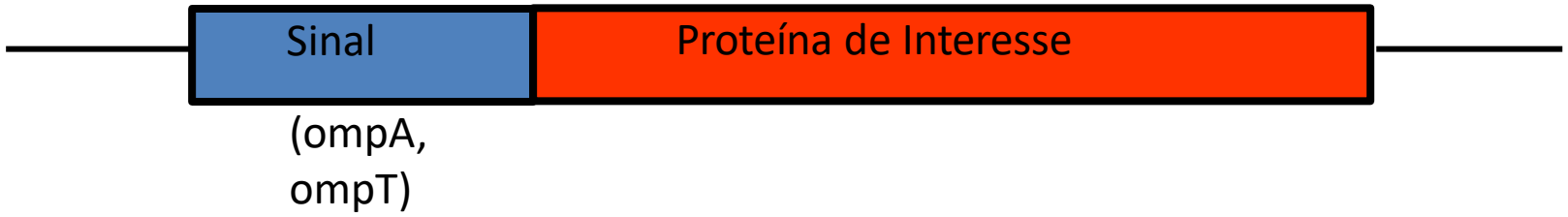


A)Expressão da proteína de interesse

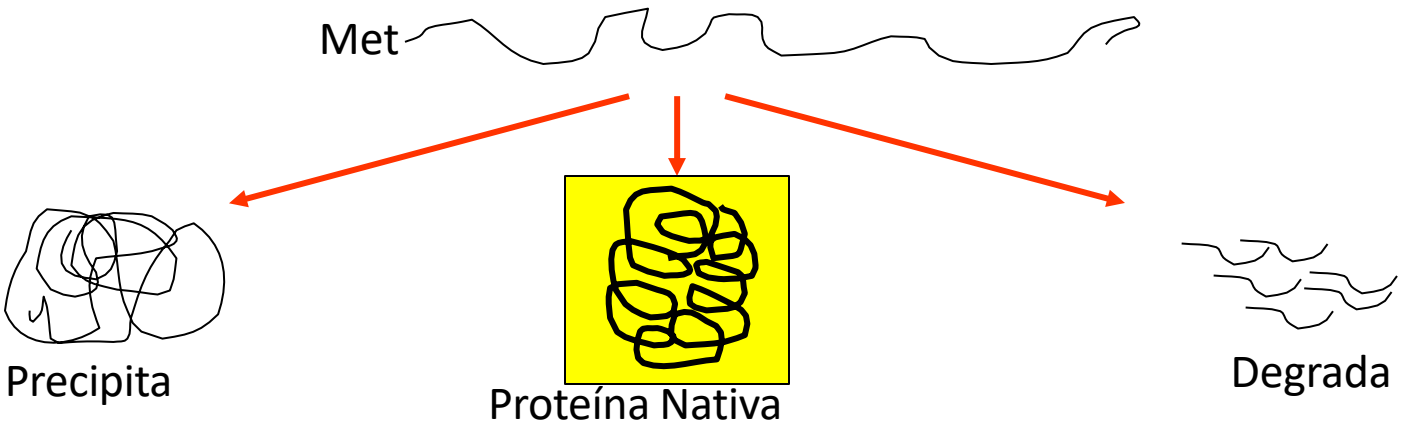
B)Expressão da proteína de interesse fusionada com outra Proteína



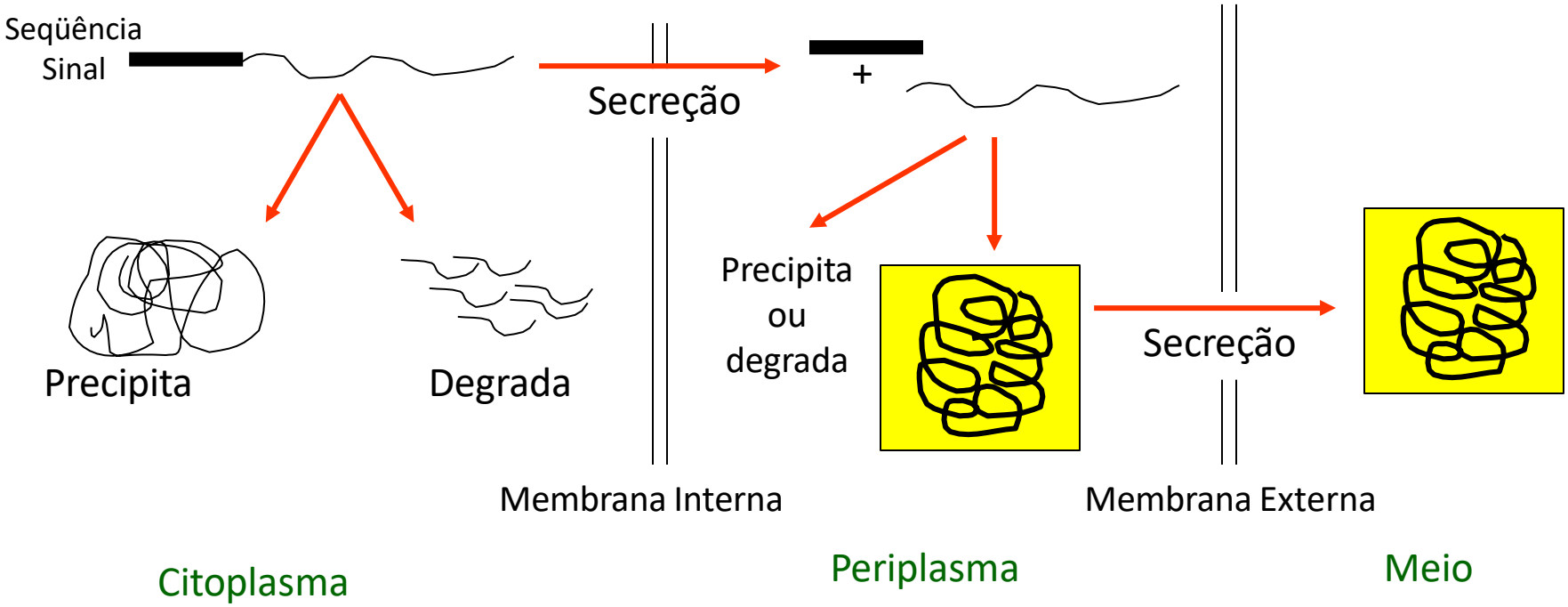
C)Expressão da proteína de interesse com sinal para secreção



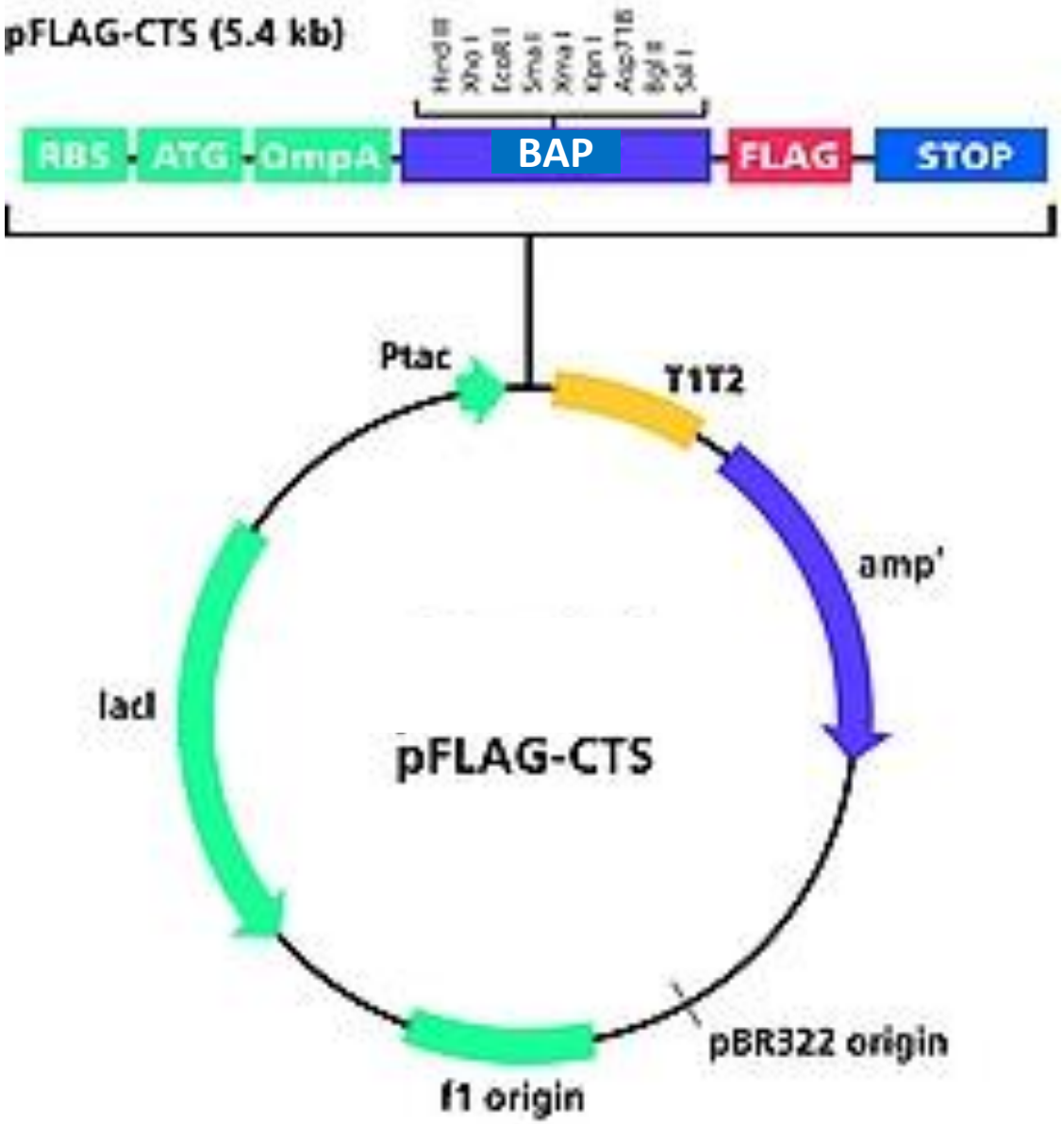
A)Proteínas Citoplasmáticas



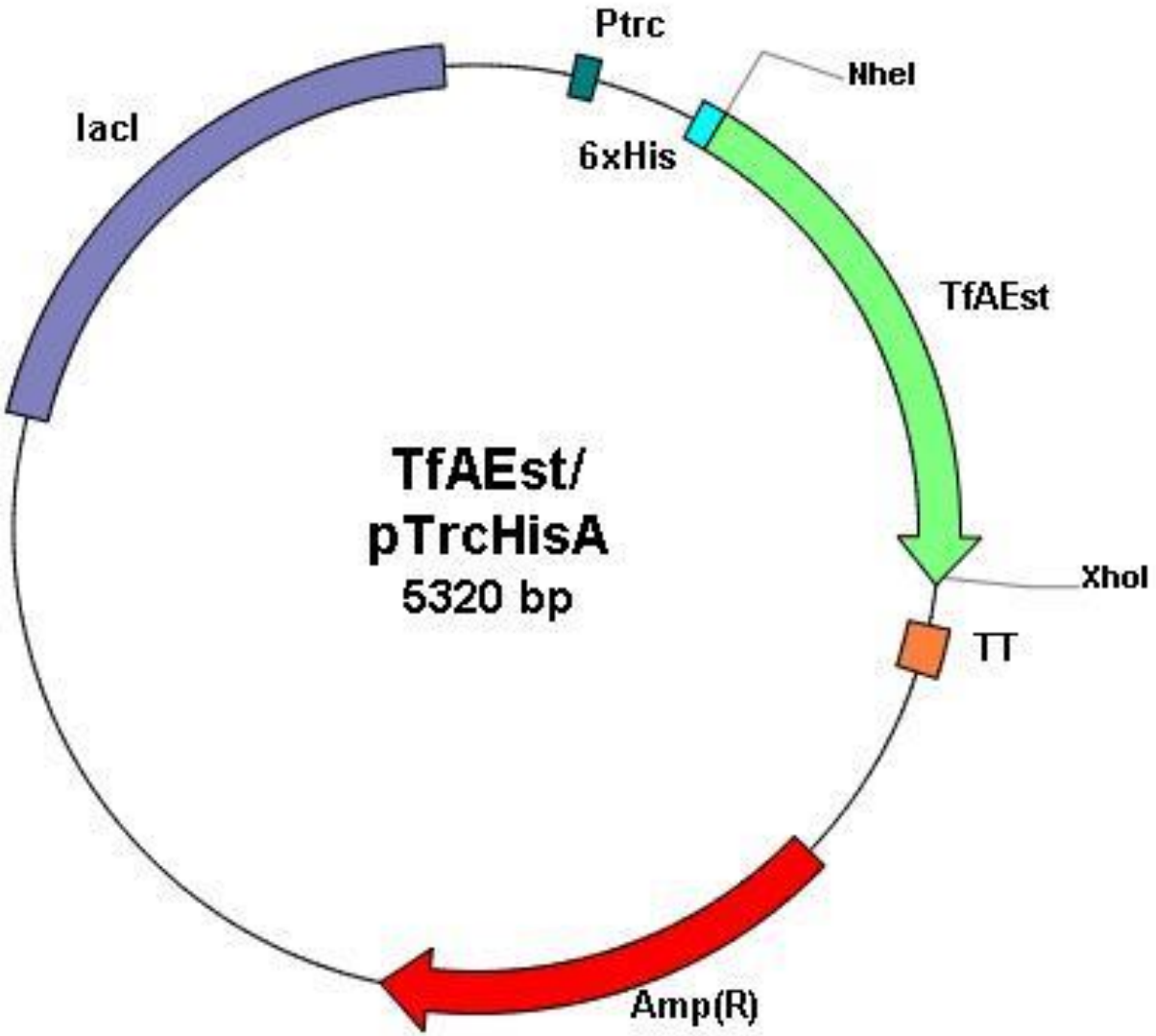
B)Secreção de Proteínas



Enzima Fosfatase Alcalina Bacteriana (1338 pb; 49,5 kDa)



Enzima Acetil Esterase (969 pb; 35 kDa)



Obrigado

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