



**Escola Superior de Agricultura “Luiz de Queiroz” – USP**  
**Disciplina: LGN0232-1 Genética Molecular**



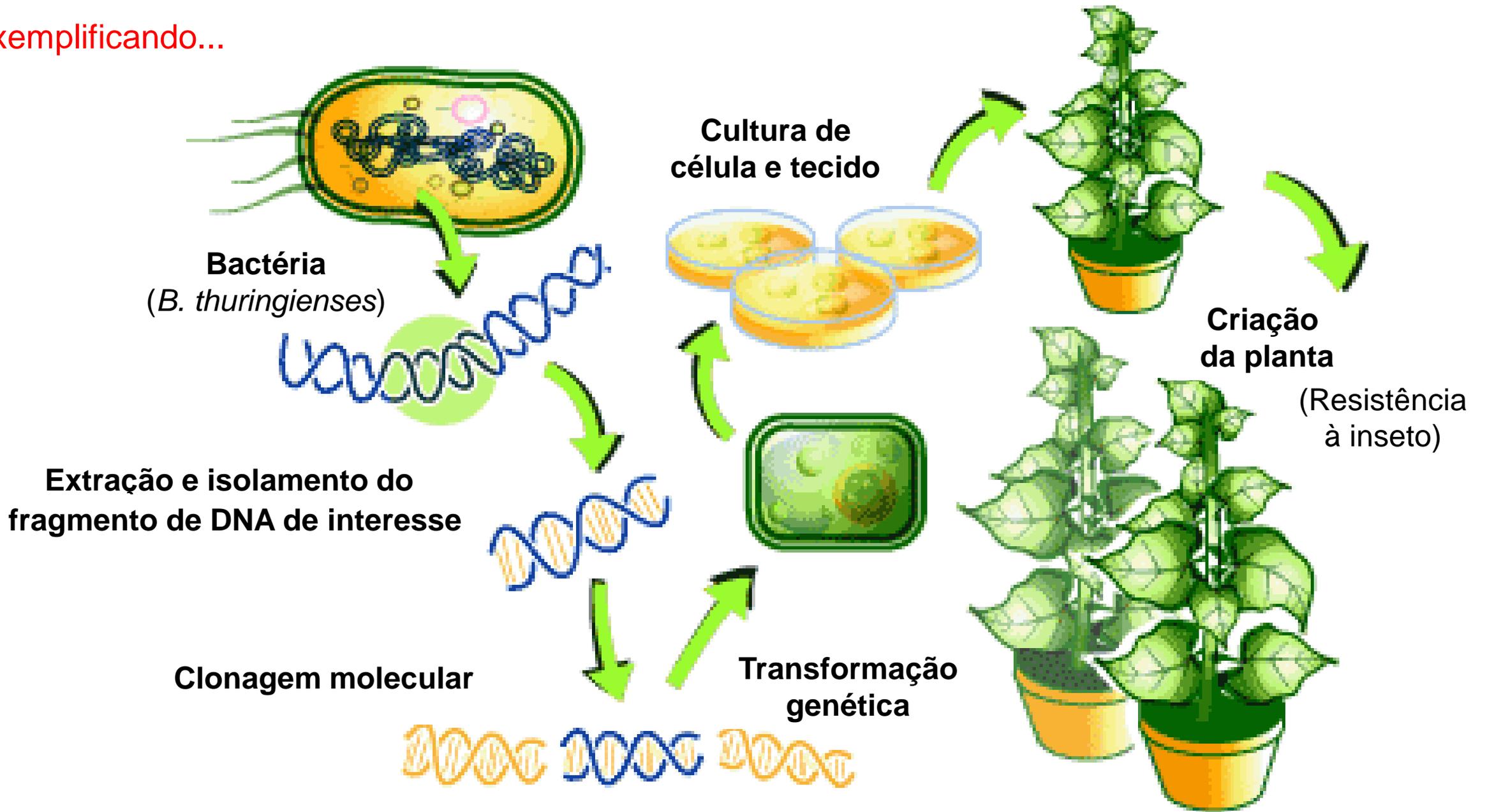
# **AULA PRÁTICA**

**Enzimas de restrição, PCR e eletroforese**

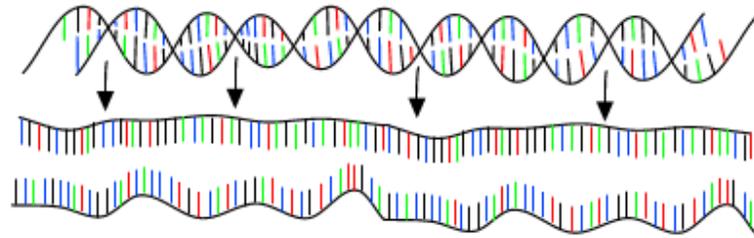
Piracicaba

Setembro/2017

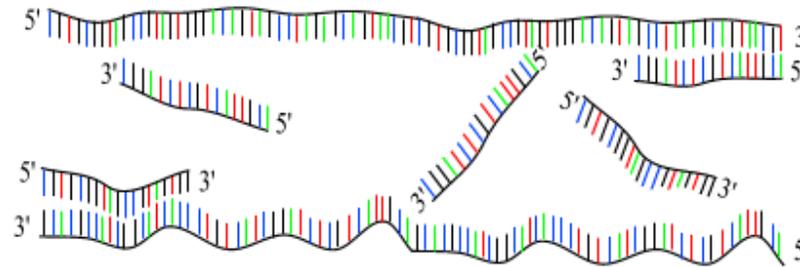
## Exemplificando...



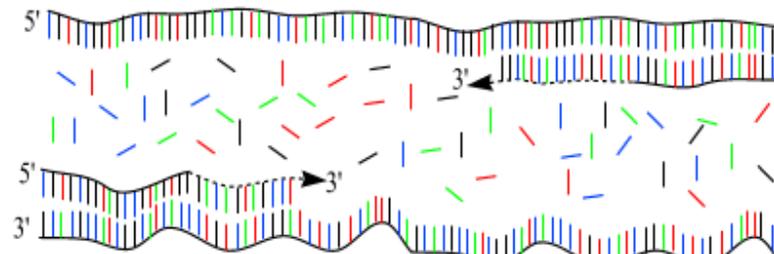
# POLYMERASE CHAIN REACTION - PCR



**Desnaturação**  
94°C



**Anelamento**  
55°C

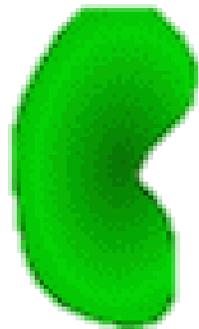


**Extensão**  
72°C

# REAÇÃO DA PCR



DNA molde



DNA polimerase

H<sub>2</sub>O + tampão + Mg<sup>2+</sup>



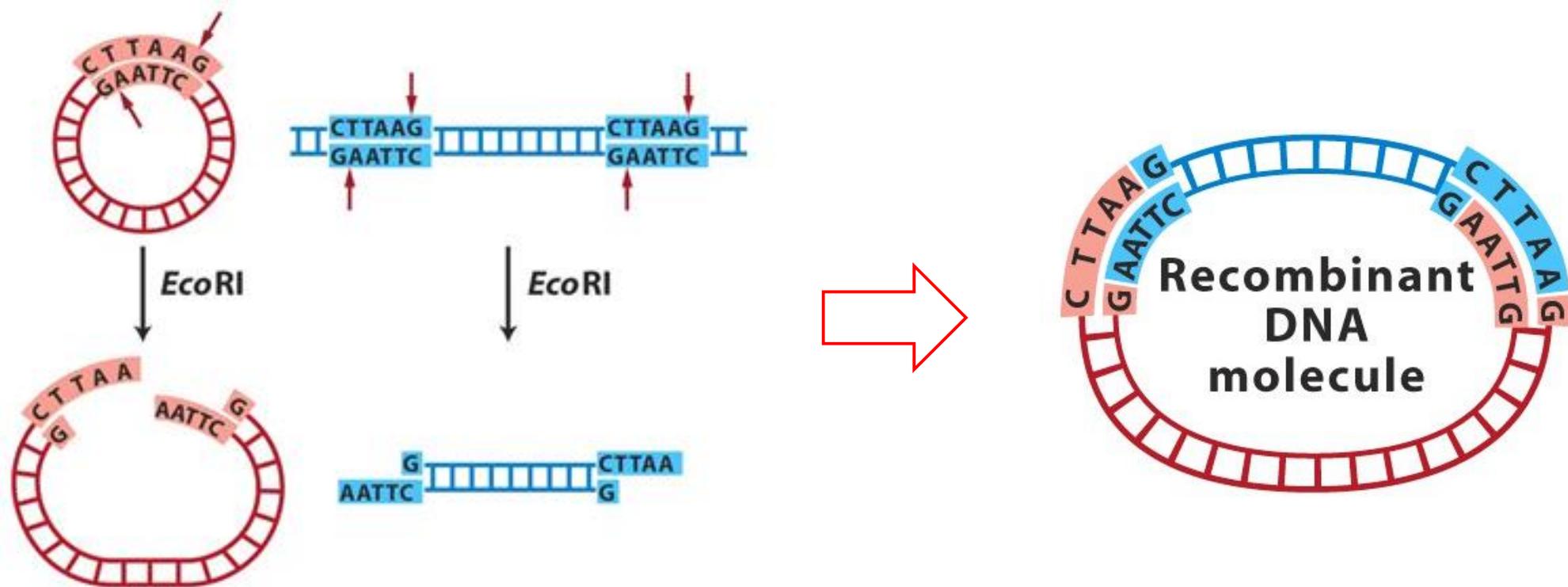
Primers

A T C G

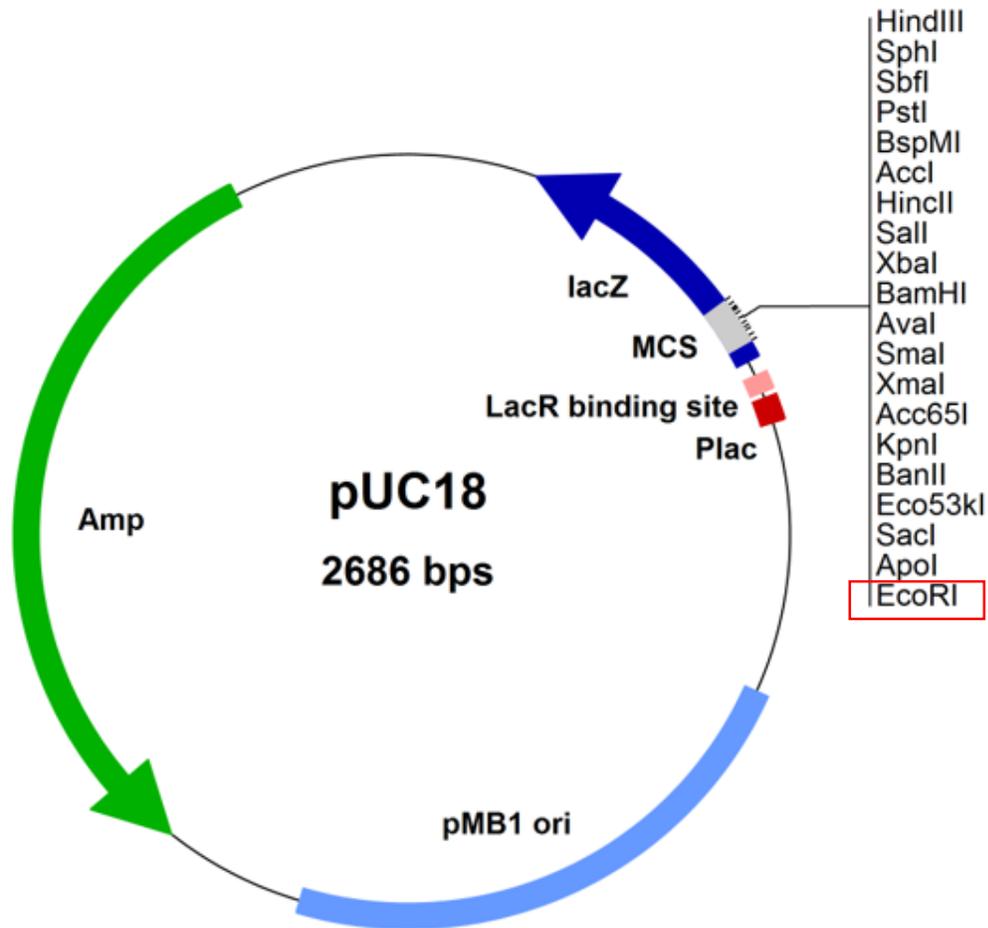
Nucleotídeos



# DIGESTÃO – ENZIMAS DE RESTRIÇÃO



# DIGESTÃO – ENZIMAS DE RESTRIÇÃO

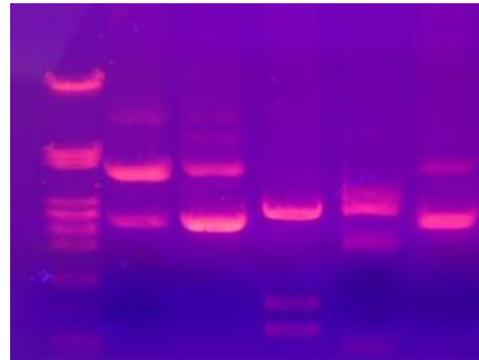
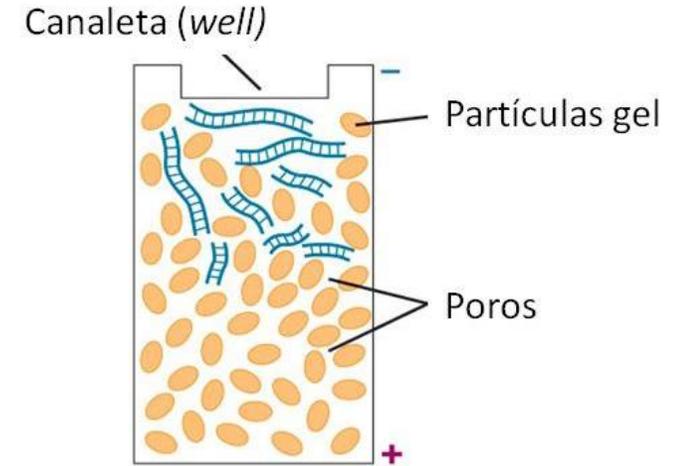
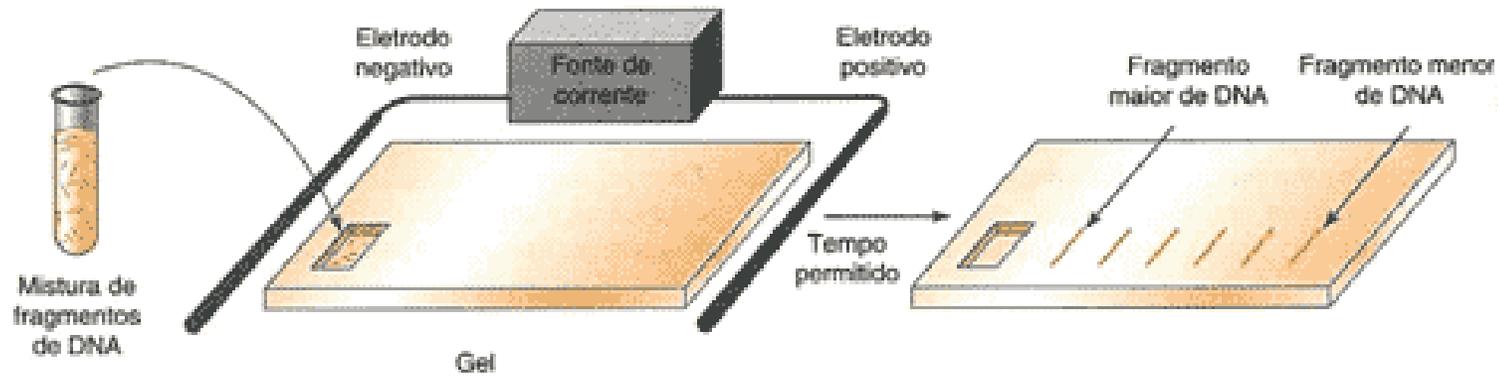


# DIGESTÃO – ENZIMAS DE RESTRIÇÃO

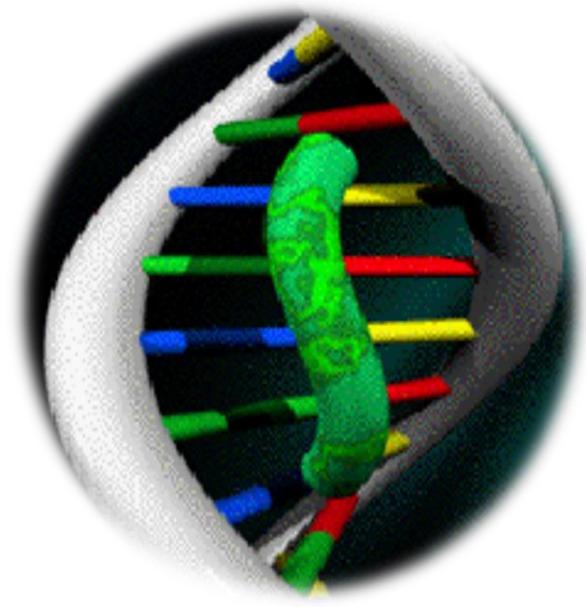
1. DNA MOLDE : plasmídeo / DNA genômico
2. Enzima
3. Tampão
4. Água

**INCUBAÇÃO EM BANHO MARIA  
(37°C por 12h)**

# ELETROFORESE



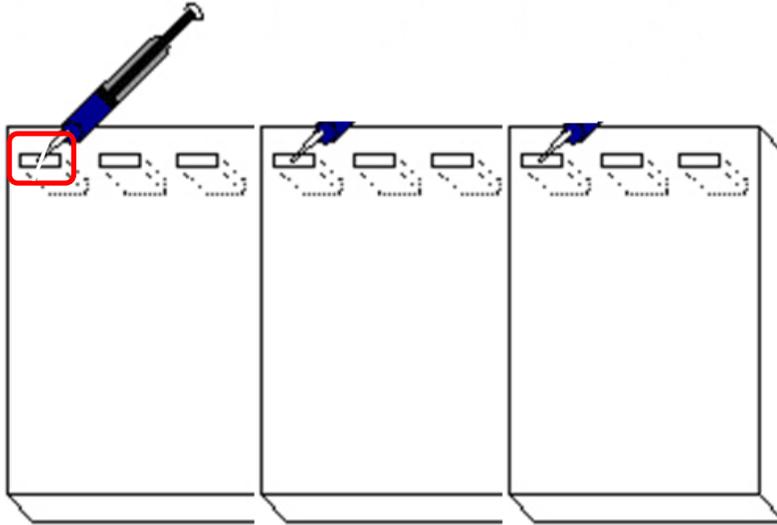
# ELETTROFORESE



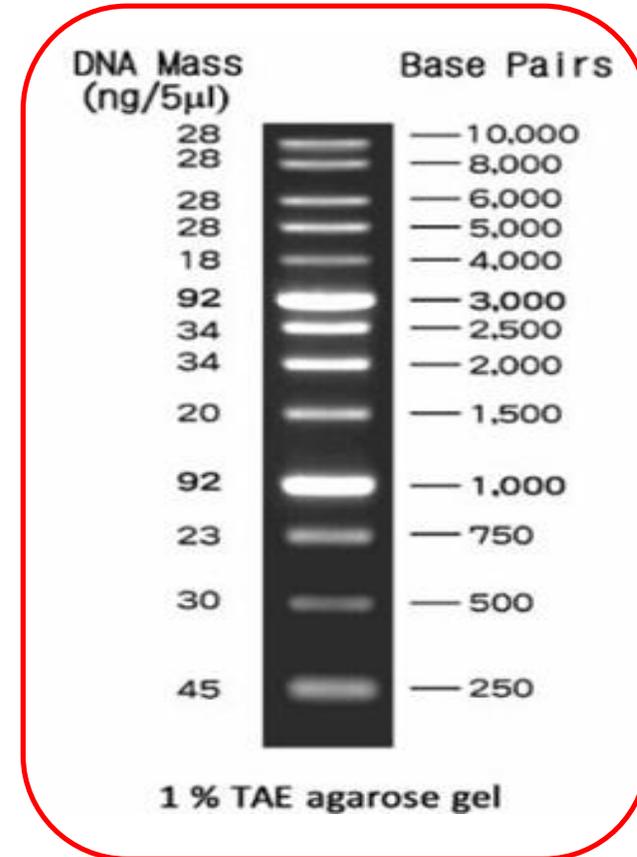
# ELETROFORESE

- Reação de digestão – enzima de restrição *EcoRI*  
PLASMÍDEO pUC18  
DNA genômico
- Reação de PCR – utilizando *primers* específicos  
PLASMÍDEO  
CONTROLE
- PLASMÍDEO E DNA genômico - ÍNTEGROS

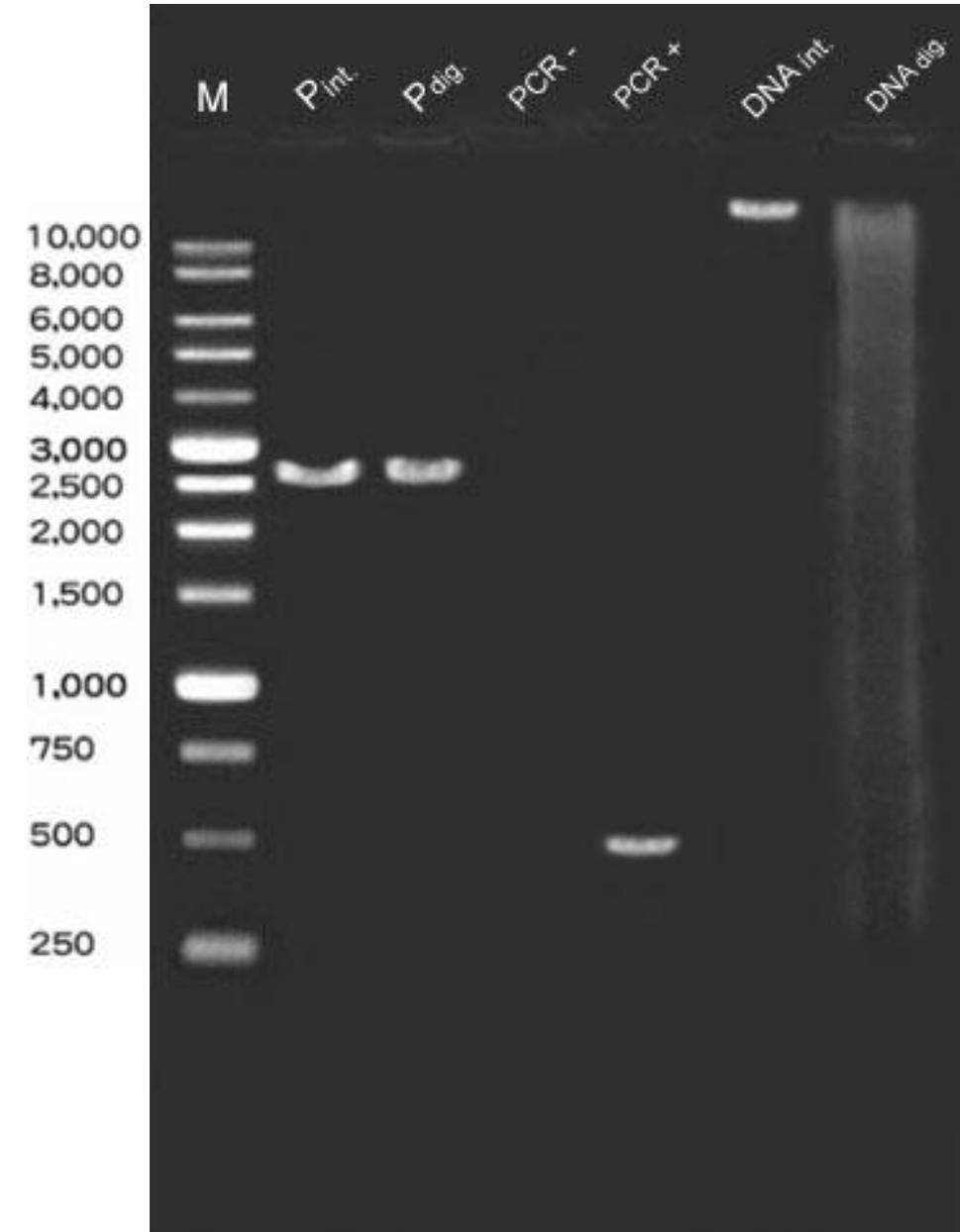
# ELETROFORESE



Pocinho do gel	Amostra
1	Marcador 1Kb
2	Plasmídeo integro
3	Plasmídeo digerido
4	PCR controle
5	PCR inserto
6	DNA genômico
7	DNA genômico digerido



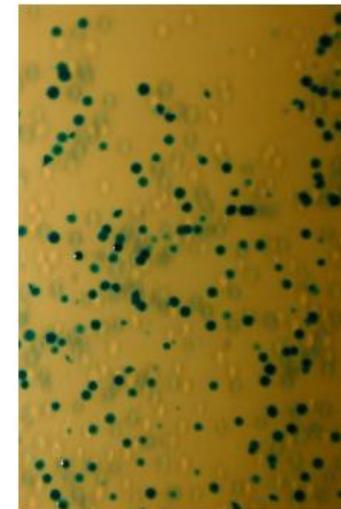
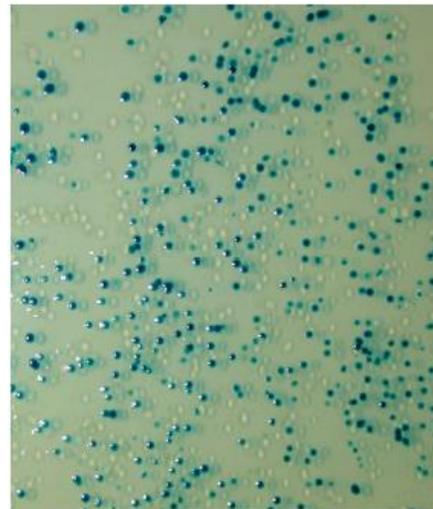
# RESULTADO ELETROFORESE



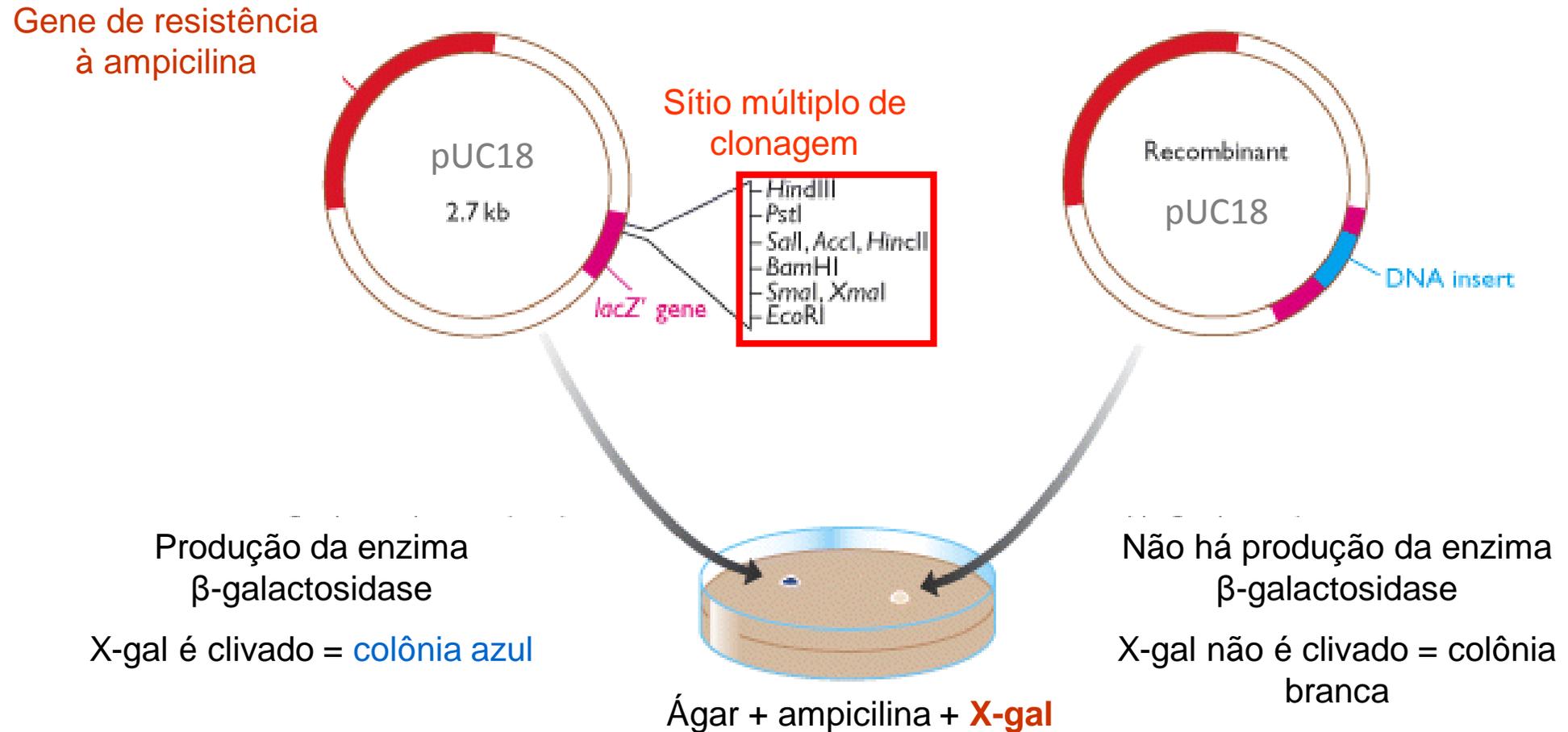
# Relembrando...



Quais colônias nos interessam e por quê?



# SELEÇÃO DE CLONES RECOMBINANTES



**Gene *lacZ* = codifica a enzima  $\beta$ -galactosidase**

