



**Ciências
ULisboa**

Faculdade
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da Universidade
de Lisboa

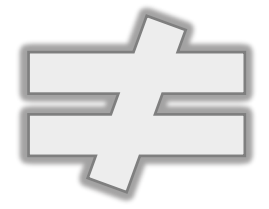
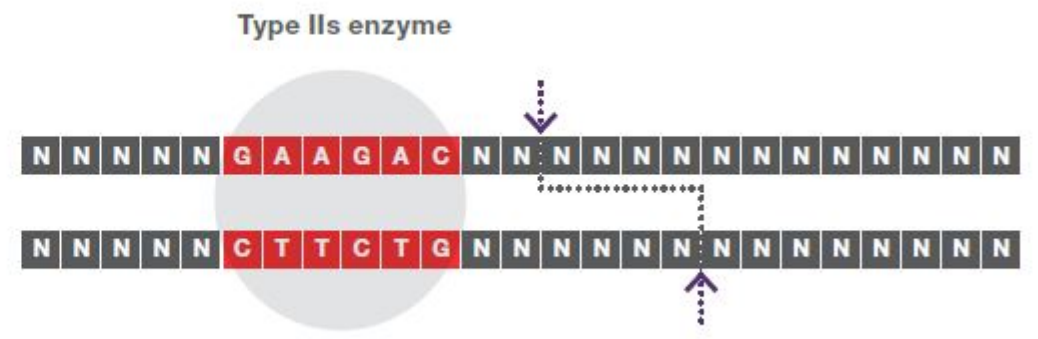
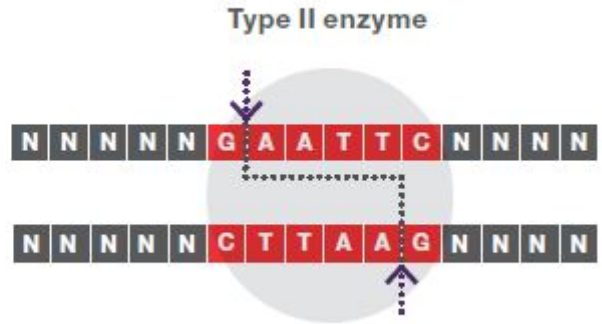
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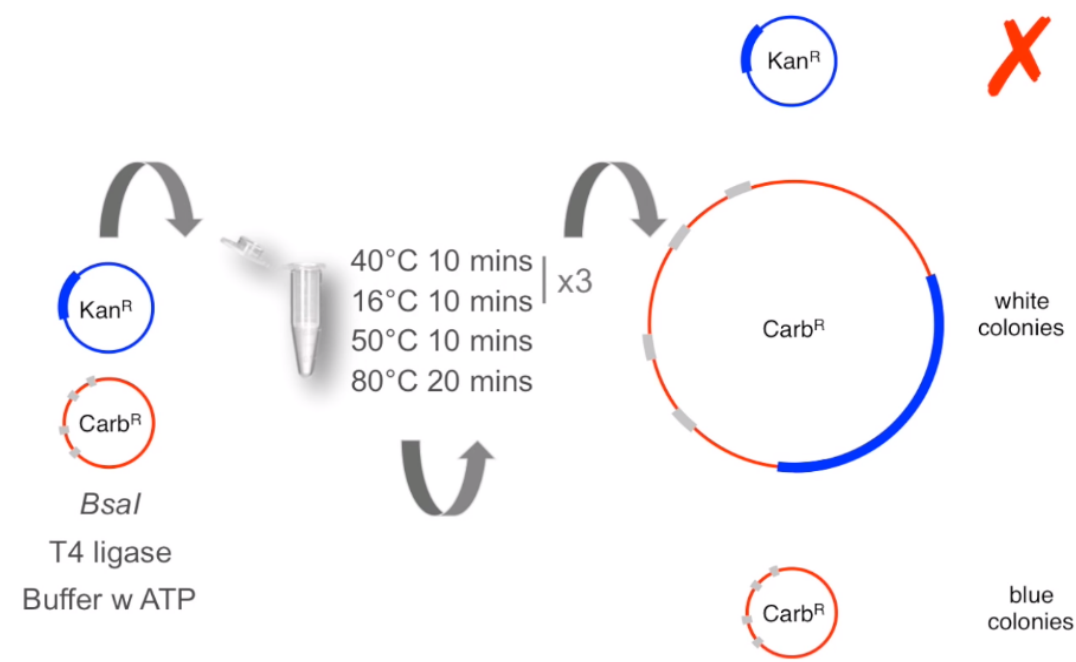
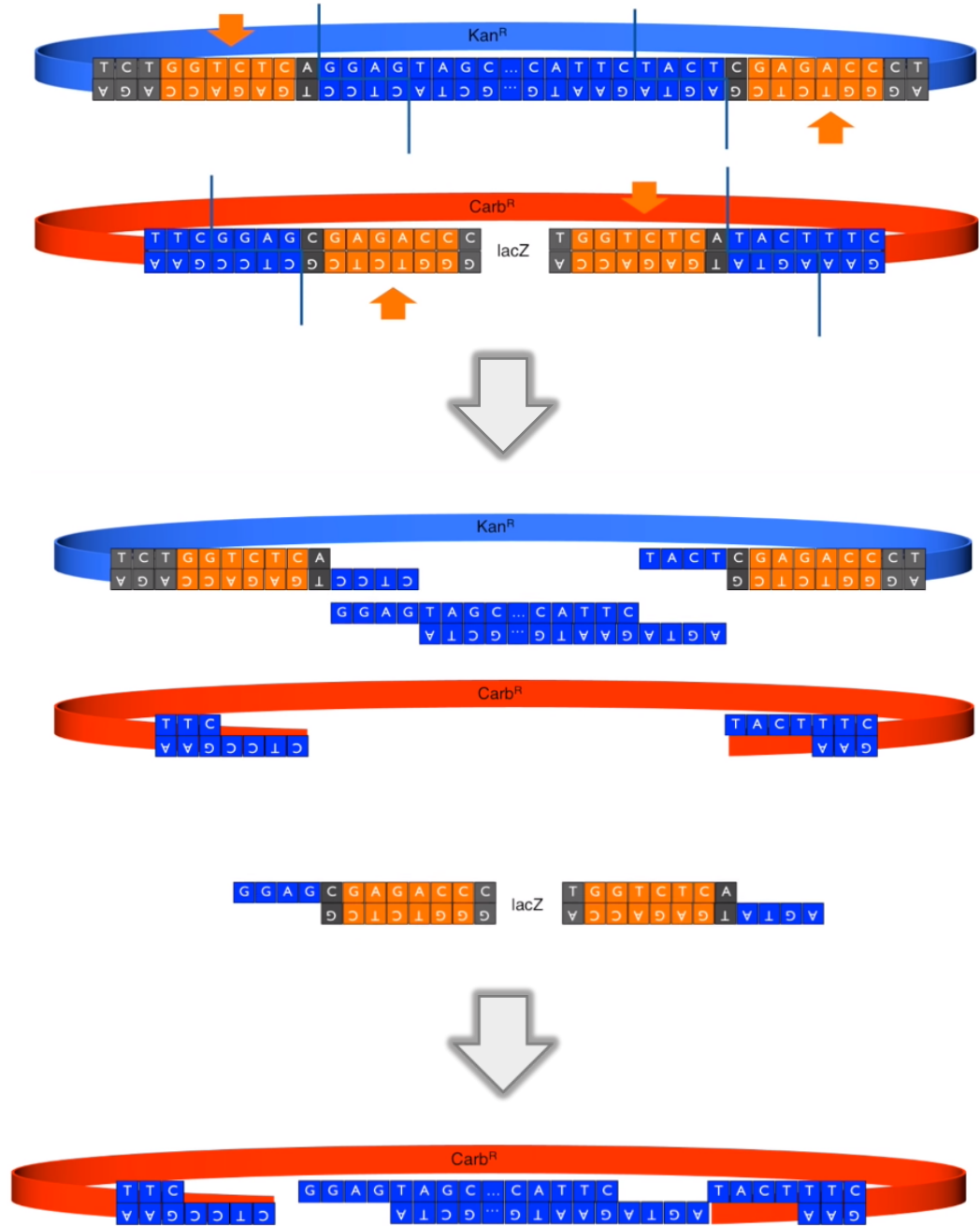
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Golden Gate



Bart Sluiter.....49193
Mariana Valente.....49245
Rita Simões.....49278
Sofia Andrêzo.....49343





One pot Digestion-Ligation (Dig-Lig) → Blue-white selection on Carbenicillin



VANTAGENS

Maior probabilidade
interação:
enzima de restrição –
- sequência de
reconhecimento

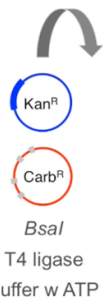


Elevada
eficiência

DESvantagem

Se for necessário
alterar o plasmídeo
de interesse, não
podemos usar a
mesma enzima de
restrição

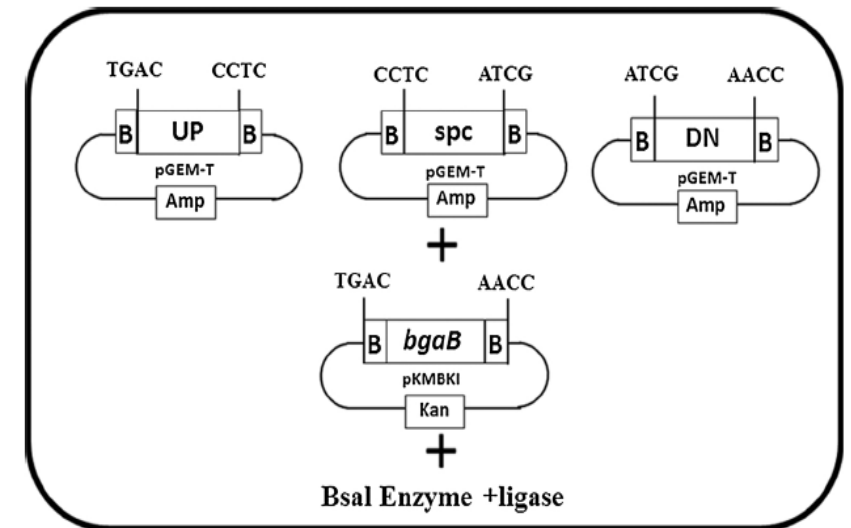
Mesmo tubo
de reação



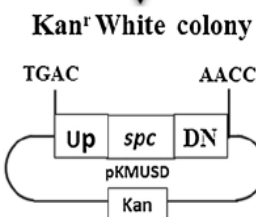
Construction of a high-efficiency cloning system using the Golden Gate method and I-SceI endonuclease for targeted gene replacement in *Bacillus anthracis*

Tiantian Wang, Dongshu Wang, Yufei Lyu, Erling Feng, Li Zhu, Chunjie Liu, Yanchun Wang*, Xiankai Liu*, Hengliang Wang*

State Key Laboratory of Pathogens and Biosecurity, Beijing Institute of Biotechnology, 20 Dongdajie Street, Fengtai District, Beijing 100071, China



Transform *E. coli*
Plate on Kan+X-Gal



Kan^r White colony