Ciências ULisboa

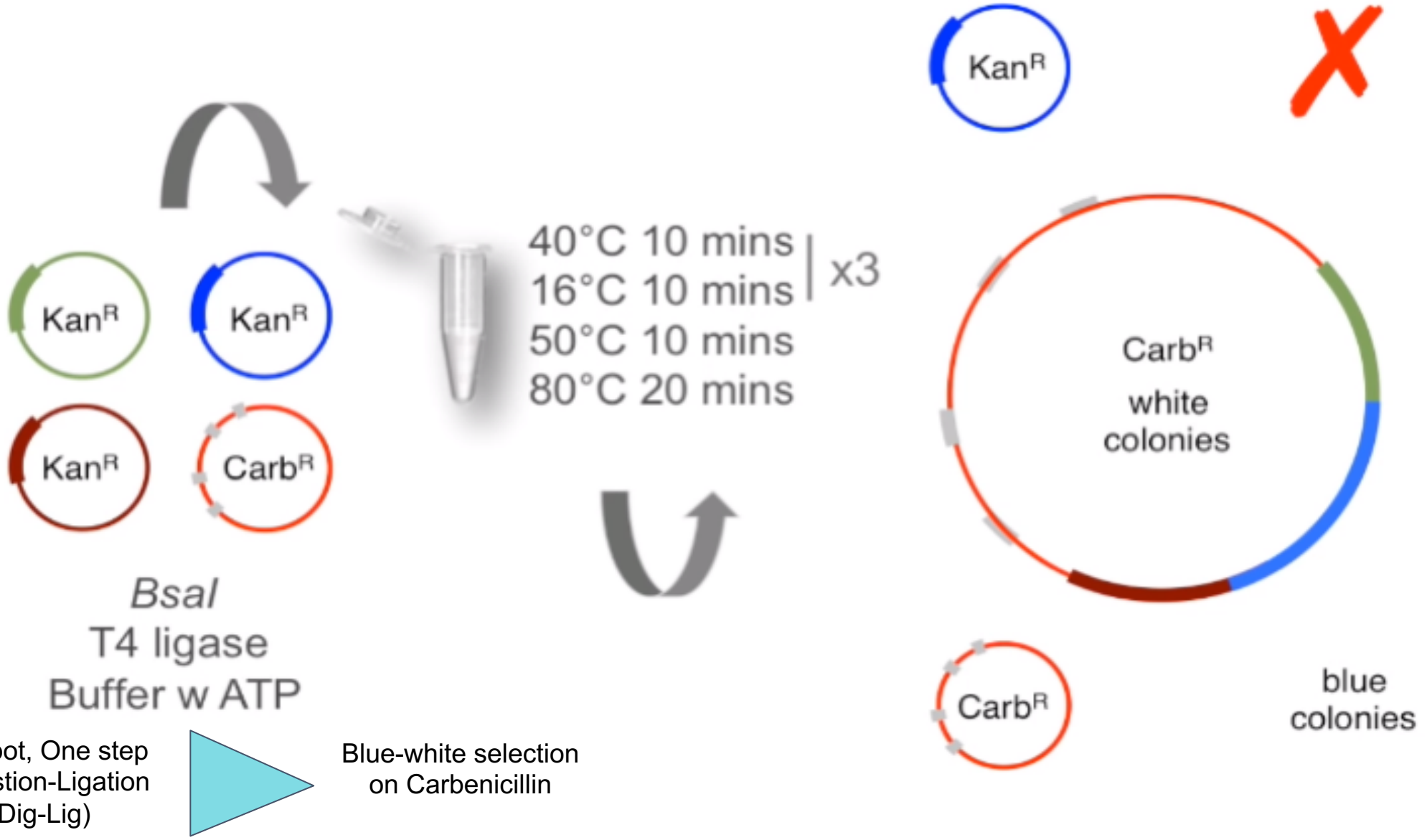
Golden Gate

Enzimas de restrição do tipo IIs

BsaI

BsaI

Luísa Ribeiro, 49294;
Raquel Gonçalo, 49233;
Rita Henriques, 49344;
Sabrina Brito, 49003.



One pot, One step Digestion-Ligation (Dig-Lig)

Blue-white selection on Carbenicillin

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Highly efficient one-step scarless protein tagging by type IIS restriction endonuclease-mediated precision cloning

Zhen Xu*, [1], Yan-Ning Rui [1], Julien Balzeau, Miriam R. Menezes, Airu Niu, John P. Hagan, Dong H. Kim**

Department of Neurosurgery, The University of Texas Health Science Center at Houston, TX, United States

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ABSTRACT

Protein tagging with a wide variety of epitopes and/or fusion partners is used routinely to dissect protein function molecularly. Frequently, the required DNA subcloning is inefficient, especially in cases where multiple constructs are desired for a given protein with unique tags. Additionally, the generated clones have unwanted junction sequences introduced. To add versatile tags into the extracellular domain of the transmembrane protein THSD1, we developed a protein tagging technique that utilizes non-classical type IIS restriction enzymes that recognize non-palindromic DNA sequences and cleave outside of their recognition sites. Our results demonstrate that this method is highly efficient and can precisely fuse any tag into any position of a protein in a scarless manner. Moreover, this method is cost-efficient and adaptable because it uses commercially available type IIS restriction enzymes and is compatible with the traditional cloning system used by many labs. Therefore, precision tagging technology will benefit a number of researchers by providing an alternate method to integrate an array of tags into protein expression constructs.

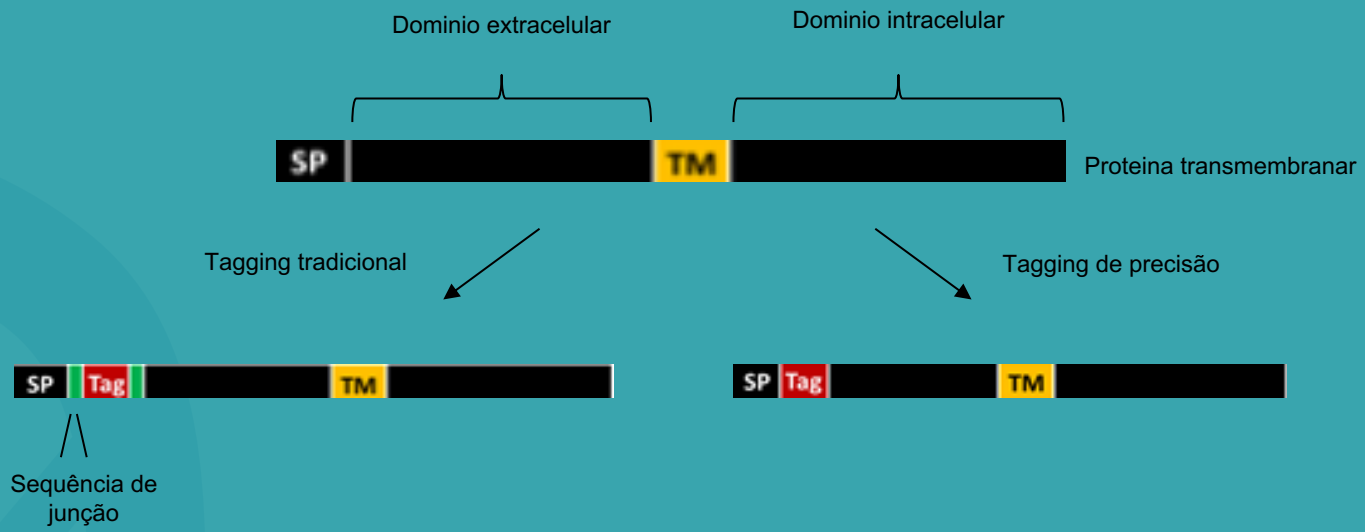
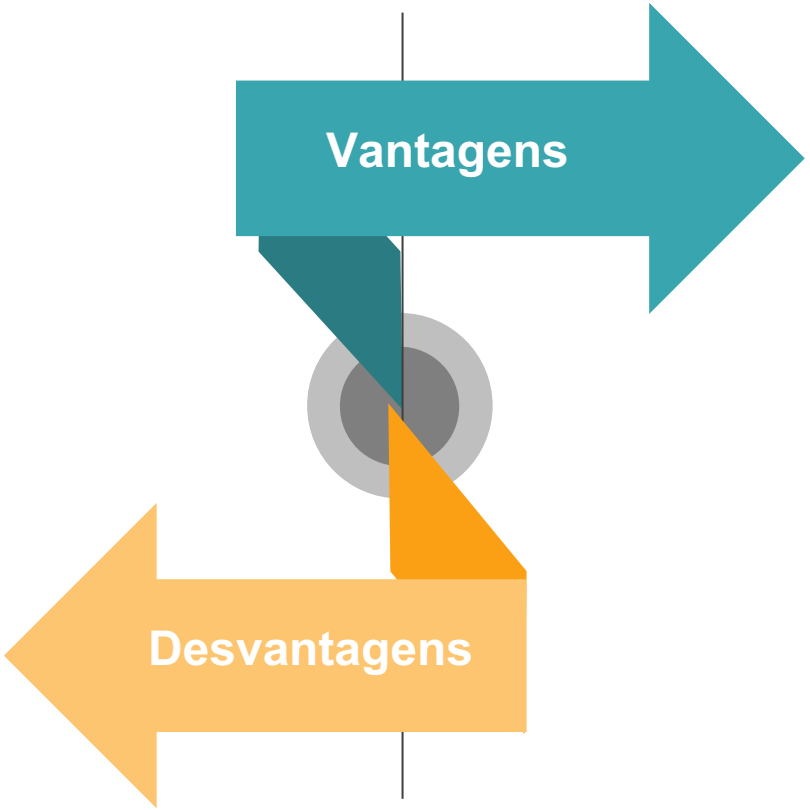


Fig. 1. Um clone sem cicatriz de clonagem gerado por marcação de precisão.
A proteína transmembranar do tipo I é mostrada como exemplo. A metade N-terminal da proteína está localizada fora da membrana citoplasmática (domínio extracelular) depois do peptídeo sinal (SP), a metade C-terminal encontra-se dentro da célula (domínio intracelular). TM identifica a sequência da proteína que ocorre dentro da membrana e é destacada pela caixa amarela. Com tagging tradicional, o tag (caixa vermelha) é inserido entre o SP e o domínio extracelular com duas seqüências de junção indesejadas (caixa verde). Em contraste, o tagging de precisão elimina as sequências indesejadas de junção e gera um clone sem cicatriz.

Golden Gate



- Um tubo → Um passo;
- É irreversível;
- Eficácia próxima de 100%;
- Baixo custo.

- Percentagem de guanosinas e citosina reduzidas nos terminais do fragmento;
- Controlo dificil.