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tetA dual selection protocols

Carolyn N Bayer[1], Ana Gabriela Veiga Sepulchro[1], Maja Rennig[1], Morten Norholm[1]

[1]Technical University of Denmark

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Carolyn N Bayer
Technical University of Denmark

ABSTRACT

Engineering of bacterial genomes is a fundamental craft in contemporary biotechnology. The ability to precisely edit bacterial chromosomes allows for the development of cells with specific phenotypes for metabolic engineering and for creation of minimized genomes. Genetic tools are needed to select for cells that underwent editing, and dual selection markers that enable both positive and negative selection are highly useful. Here we present an optimized and easy-to-use version of the *tetA* dual selection marker and demonstrate how this $tetA^{OPT}$ can be used efficiently to engineer at different stages of the central dogma of molecular biology. On the DNA level, $tetA^{OPT}$ can be used to create scarless knockouts across the *E. coli* genome with an efficiency above 90% whereas recombinant gene integrations can be achieved with approximately 50% efficiency. On the expression level, we show that $tetA^{OPT}$ enables advanced genome engineering of both genetranslation and transcription. Finally, we demonstrate the use of $tetA^{OPT}$ for genome engineering in the industrially relevant probiotic strain *E. coli* Nissle.

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Engineering of bacterial genomes is a fundamental craft in contemporary biotechnology. The ability to precisely edit bacterial chromosomes allows for the development of cells with specific phenotypes for metabolic engineering and for creation of minimized genomes. Genetic tools are needed to select for cells that underwent editing, and dual selection markers that enable both positive and negative selection are highly useful. Here we present an optimized and easy-to-use version of the *tetA* dual selection marker and demonstrate how this $tetA^{OPT}$ can be used efficiently to engineer at different stages of the central dogma of molecular biology. On the DNA level, $tetA^{OPT}$ can be used to create scarless knockouts across the *E. coli* genome with an efficiency above 90% whereas recombinant gene integrations can be achieved with approximately 50% efficiency. On the expression level, we show that $tetA^{OPT}$ enables advanced genome engineering of both genetranslation and transcription. Finally, we demonstrate the use of $tetA^{OPT}$ for genome engineering in the industrially relevant probiotic strain *E. coli* Nissle.

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FILES

Gene knockout strategy
Version 1
by Carolyn N Bayer, Technical University of Denmark

Tuning the expression levels of native genes
Version 1
by Carolyn N Bayer, Technical University of Denmark

Important note on the medium type and plating procedure
Version 1
by Ana Gabriela Veiga Sepulchro

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