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Development of a *pyrF*-based counterselectable system for targeted gene deletion in *Streptomyces rimosus*

Key words: **Counterselectable system, *pyrF*, 5-fluoroorotic acid, Gene deletion, *Streptomyces rimosus***

Research Summary

This paper mainly focused on the core components of development of a *pyrF*-based counterselectable system for targeted gene deletion in the following aspects:

- Identification of orotidine-5`-phosphate decarboxylase gene *pyrF in Streptomyces.*
- Constitution and principle of the *pyrF*-based screening system
- Targeted gene deletion in *S. rimosus* with *pyrF*-based gene knockout systems

Innovation points

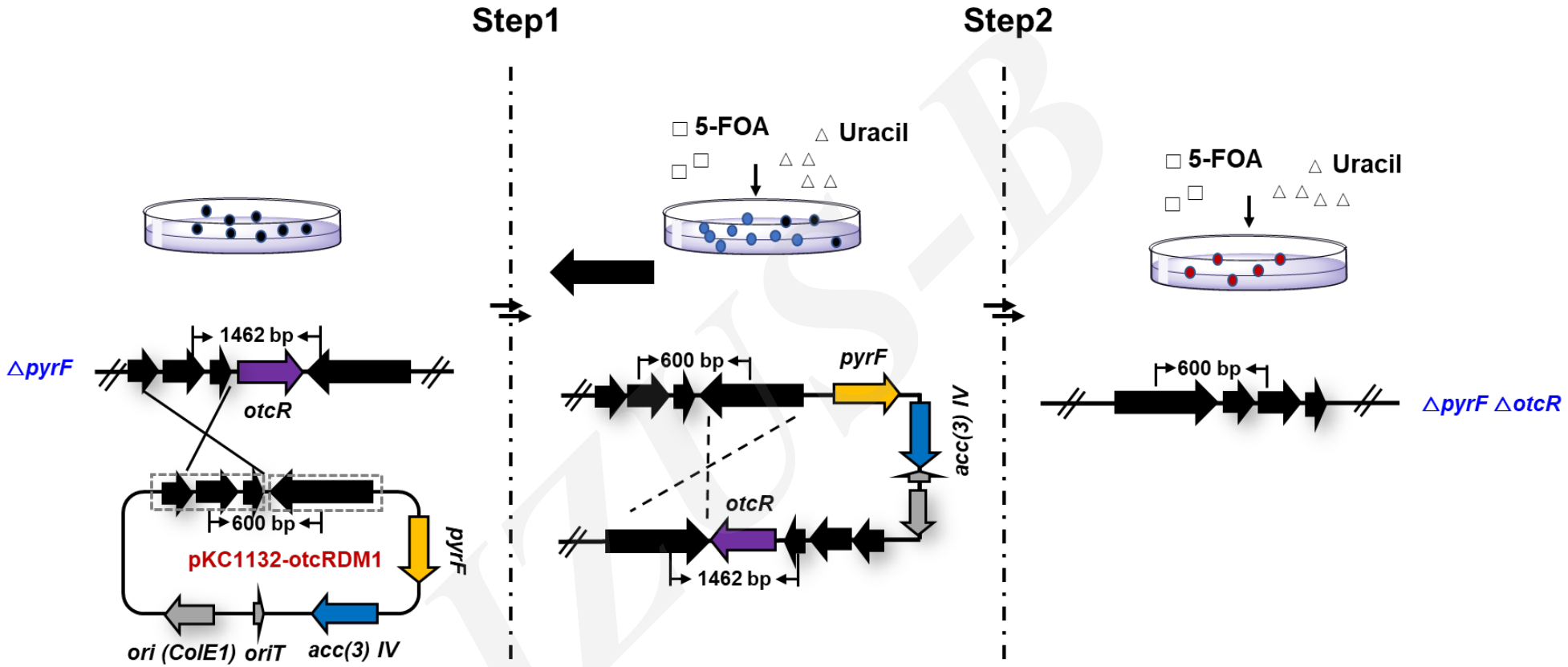
● Alignment analysis revealed that only one copy of the putative orotidine 5’-phosphate decarboxylase gene *pyrF* exists on the genome of *Streptomyces*.

●Deletion of *pyrF* conferred 5-fluoroorotic acid (5-FOA) resistance in the mutant lacking *pyrF* (Δ*pyrF*).

● This *pyrF*-based counterselectable system was very effective and enabled us to obtain the target gene knockout mutants by analyzing a small number of clones, and the time to obtain the desired mutants was greatly shortened.

● The advantage of this system is that the *pyrF* marker does not remain in the genome of Δ*pyrF* , so additional deletion and/or insertion using the same vector system is possible.

Innovation points



Schematic diagram depicting the steps for *otcR* deletion using the *pyrF*-based counterselectable system