TECHNICAL PROTOCOL

FOR

Arabinose inducible prokaryotic recombinase expression plasmid

pSC101-BAD-Cre (A301)

pSC101-BAD-Dre (A302)

pSC101-BAD-FLPe (A303)

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CONTENTS

1 Eppendorf tubes + manual

1. recombinase expression plasmid pSC101-BAD-Cre/pSC101-BAD-Dre or pSC101-BAD-FLPe (0.2 µg/µl, 20 µl)

2. This manual

Store tube at -20°C

Please read

The products listed in this manual are for research purposes only. They are not designed for diagnostic or therapeutic use in humans, animals or plants.

MTA

The use of **Dre recombinase is covered by United States Patent Nos. 7,422,889 and 7,915,037 owned by the Stowers Institute for Medical Research, Kansas City, Missouri.** Non-profit and academic institutions have permission to use the plasmid solely for research purpose; for-profit entities require a license from Stowers Institute.

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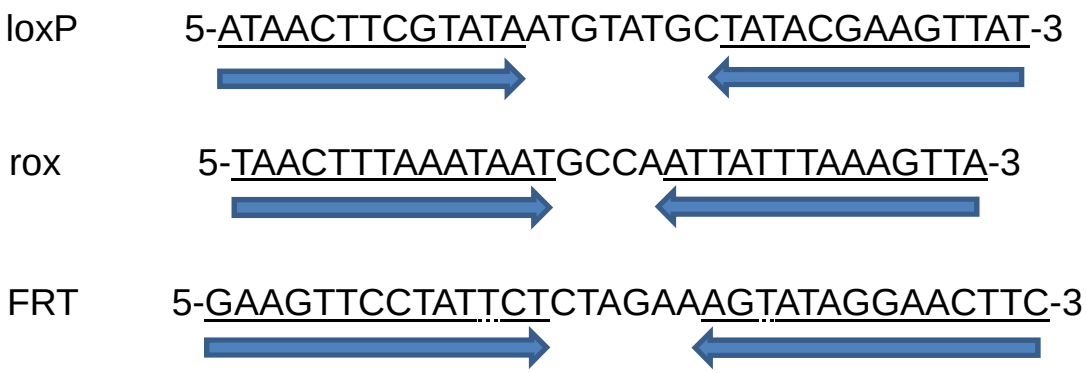
Short Description:

Site-specific recombinases (SSRs) like Cre, Dre or FLPe are valuable tools in functional genomics and have been applied in various organisms. They mediate recombination between target sites of 32-34 base pairs (bp) in length. The target sites, which are called loxP, rox or FRT sites are 13-14 bp palindromes separated by spacers (s. below).



Recognition sites of the site-specific recombinases Cre, Dre and FLP.

Cre recombinase, which was originally isolated from coliphage P1, mediates recombination between two loxP-sites through the spacer regions (e.g. removal of selectable genes). Dre was identified in a systematic search through P1-like phages for a Cre-like enzyme that had diverged sufficiently to recognize a recombination target site (RT) that is distinct from loxP (Sauer and Mc Dermott, 2004).

FLP recombinase was originally isolated from yeast and therefore shows a significantly reduced activity at 37°C due to thermal instability of the protein (Buchholz F. *et al.,* 1996). A screen for thermo-stable mutants resulted in the identification of an enhanced FLP version (FLPe), which exhibits a 4-10 fold higher activity at 37°C (Buchholz F., Angrand P.O. and Stewart A.F. 1998).

The combination of the arabinose inducible BAD promoter and the low-copy pSC101 plasmid backbone provide an excellent on-off regulation of Cre/Dre or FLPe in *E. coli* as proved in a test experiment (Anastassiadis et al. 2009).

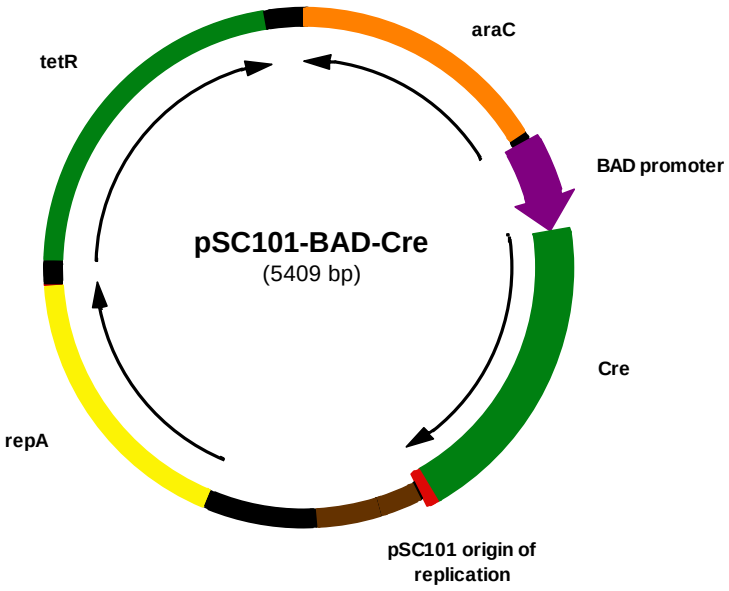
The plasmids carry a tetracyclin resistance gene for selection and are compatible with plasmids based on a ColE1 or p15A origin of replication and an ampicillin or kanamycin resistance marker.

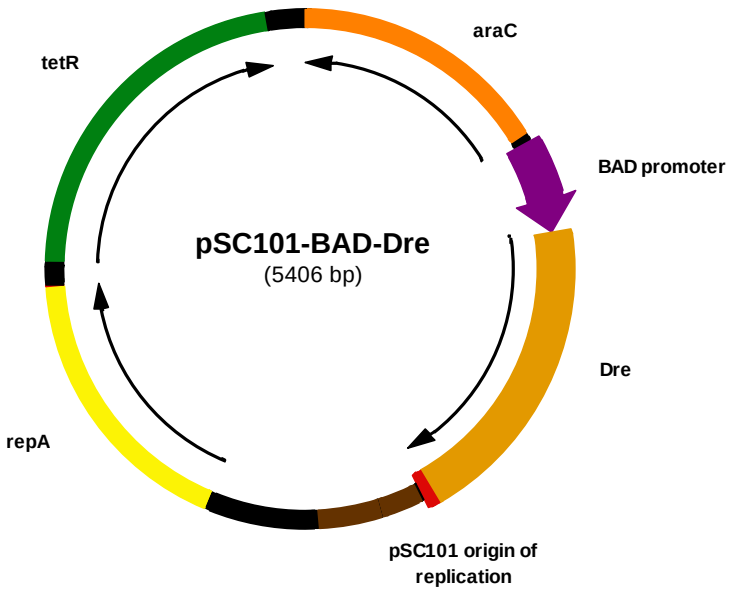
While Cre/loxP and FLPe/FRT are widely used in mouse genetics for conditional mutagenesis with many mouse lines available, another highly efficient system like Dre/rox opens the door for more complex tasks such as a conditional mutagenesis of alternatively spliced exons. Cre/loxP can be used to remove one alternative exon and Dre/rox to remove the other one.

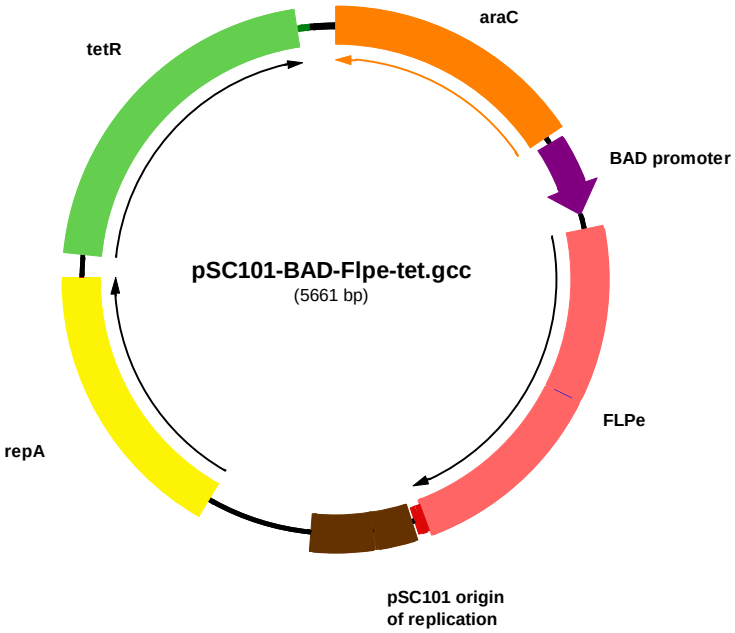
Literature:

- Anastassiadis K., Fu J., Patsch C., Hu S., Weidlich S., Duerschke K., Buchholz F., Edenhofer F. and Stewart A.F. 2009: Dre recombinase, like Cre, is a highly efficient site-specific recombinase in E. coli, mammalian cells and mice. Disease Models & Mechanisms 2: 508 - 515.
- Sauer B. and Mc Dermott J., 2004: DNA recombination with a heterospecific Cre homolog identified from comparison of the *pac-c1* regions of P1-related phages. Nucleic Acids Research 32: 6086 – 6095.

Maps:




araC
tetR
BAD promoter
pSC101-BAD-Dre
(5406 bp)
Dre
repA
pSC101 origin of
replication


araC
tetR
BAD promoter
pSC101-BAD-Flpe-tet.gcc
(5661 bp)
FLPe
repA
pSC101 origin
of replication