TECHNICAL PROTOCOL

FOR

Eukaryotic recombinase expression vector

pCAGGS-FLPo (A203)

pCAGGS-Cre (A204)

pCAGGs-Dre (A205)

Gene Bridges GmbH
Im Neuenheimer Feld 584
69120 Heidelberg, Germany
Tel + 49 (0)6221 13708 11
Fax + 49 (0)6221 13708 29
Email: contact@genebridges.com
www.genebridges.com

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CONTENTS

1 Eppendorf tubes + manual

1. recombinase expression plasmid pCAGGS-FLPo, pCAGGS-Cre or pCAGGS-Dre (0.2 µg/µl, 20 µl)

2. This manual

Store tube at -20℃.

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 **FLPo is covered by United States Patent Application Serial Number 12/307,418 owned by Fred Hutchinson Cancer Research Center ("FHCRC"), Seattle, Washington**. Non-profit and academic institutions have permission to use the plasmid solely for research purpose; for-profit entities require a license from FHCRC.

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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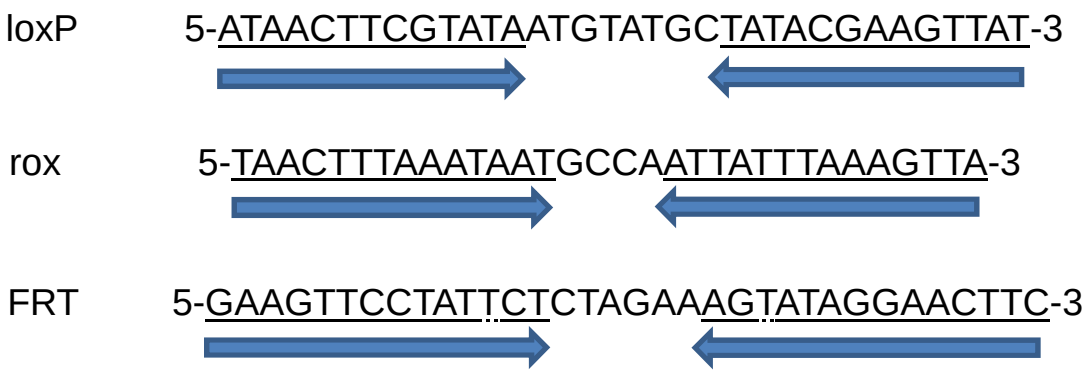
Short Description:

Site-specific recombinases (SSRs) like Cre, FLP or Dre 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, FRT or rox sites are 13-14 bp palindromes separated by spacers (s. below).



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

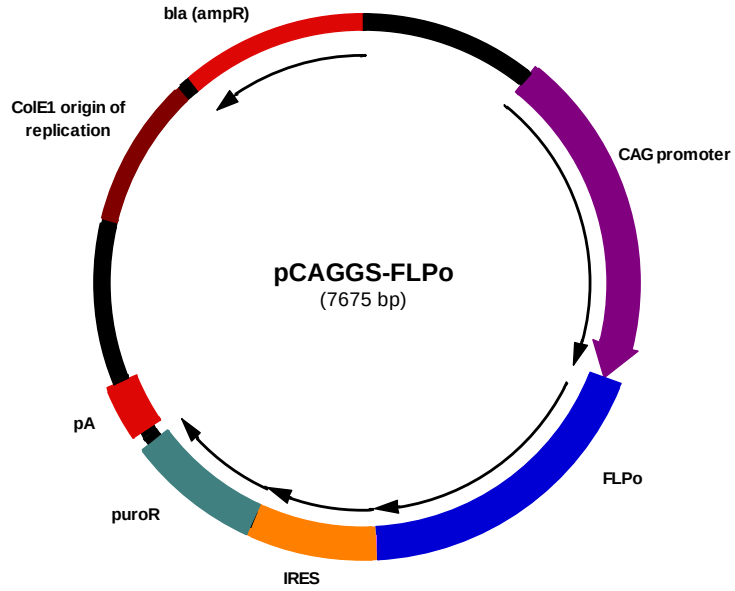
Cre recombinase, which was originally isolated from coliphage P1, mediates recombination between two loxP-sites through the spacer regions. Dre was identified in a systematic search through P1-like phages for a Cre-like enzyme that had diverged sufficiently to recognize a recombination target site (RT) that is distinct from loxP (Sauer and Mc Dermott, 2004). The Dre RT was termed rox. The codon usage of Dre in the plasmid was adapted for the use in mammalian cells (Anastassiadis K. *et al.,* 2009).

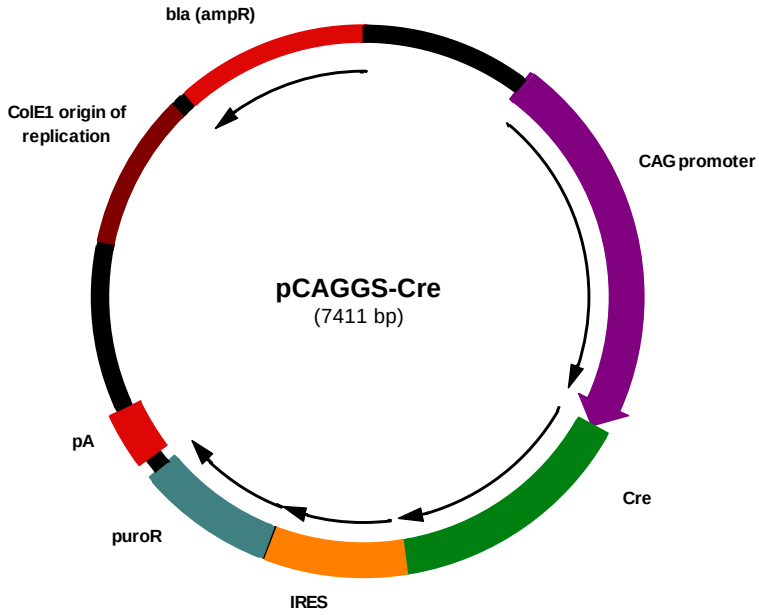
FLP recombinase was originally isolated from yeast and therefore shows a significantly reduced activity at 37℃ due to therm al instability of the protein (Buchholz F. *et al.,* 1996). A screen for thermo-stable mutants resulted in the identification of an enhanced FLP version (FLPe), which exhibits a 4-10 fold higher activity at 37℃ (Buchholz F., Angrand P.O. and Ste wart A.F. 1998). FLPo is a codon-optimized FLP version first described by Christopher Raymond and Philippe Soriano (2007). Its amino acid sequence is identical to that of FLPe but the codon usage was altered to improve expression in mammalian cells. It appears to be at least 10 fold more efficient than FLPe (Kranz A. *et al.,* 2010).

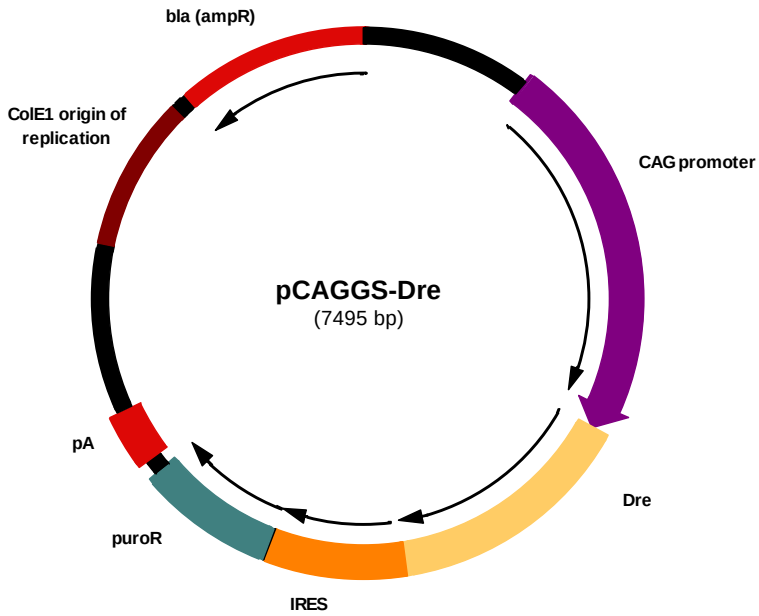
Our pCAGGS expression vectors carry FLPo/ Cre/ Dre under the control of the chicken-ß-actin promoter and an hCMV immediate early enhancer. The use of the chimeric CMV enhancer/ß-actin promoter leads to a ubiquitous expression profile in eukaryotes. The addition of a Sv40 Large T nuclear localization sequence (nls) further improves the performance in mammalian cells (Schaft J. *et al.*, 2001). The recombinases are linked to a puromycin resistance gene by an internal ribosomal entry site (IRES).

The **pCAGGS-FLPo** plasmid allows efficient excision of DNA stretches flanked by **FRT sites**; the **pCAGGS-Cre** plasmid allows excision of DNA stretches flanked by **loxP sites** and the **pCAGGS-Dre** plasmid allows excision of DNA stretches flanked by **rox sites,** such as a resistance cassette in a conditional allele in eukaryotic cells (see Kranz A. *et al.,* 2010 for further details). The plasmids carry a puromycin resistance gene for selection in eukaryotic cells and an ampicillin resistance cassette for selection in *E. coli*.

Maps:


bla (ampR)
ColE1 origin of replication
CAG promoter
pCAGGS-FLPo
(7675 bp)
pA
FLPo
puroR
IRES


bla (ampR)
ColE1 origin of replication
CAG promoter
pCAGGS-Cre
(7411 bp)
pA
Cre
puroR
IRES


bla (ampR)
ColE1 origin of replication
CAG promoter
pCAGGS-Dre
(7495 bp)
pA
Dre
puroR
IRES

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.
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- Buchholz F., Angrand P.O. and Stewart A.F. 1998: Improved properties of FLP recombinase evolved by cycling mutagenesis. Nature Biotechnology 16: 657 – 662.
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- Raymond C.S. and Soriano P. 2007: High-efficiency FLP and ϕC31 site-specific recombination in mammalian cells. PLoS ONE 2(1): e162.
- 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.
- Schaft J. et al. 2001: Efficient FLP Recombination in mouse ES cells and oocytes. Genesis 31: 6-10.