

TECHNICAL PROTOCOL
FOR
pCAGGS-FLPe
Enhanced Eukaryotic FLP Expression
Plasmid
(A201, A202)

CONTENTS

1 Eppendorf tubes + manual

1. pCAGGS-FLPe: expression plasmid for FLPe recombinase (0.2 µg/µl, 20 µl)
2. This manual

Store tube at -20°C

Please read

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Short Description:

pCAGGS-FLPe-IRESpuro is an improved vector for expression of an enhanced FLP recombinase in mouse ES cells and oocytes. FLPe shows enhanced thermo stability at 37°C compared to the normal FLP recombinase (see figure 1). After micro-injection of pCAGGS-FLPe into oocytes approximately one-third of heterozygotic mice born show complete FLP recombination (Schaft J. et al., Genesis 31, 6-10 (2001)).

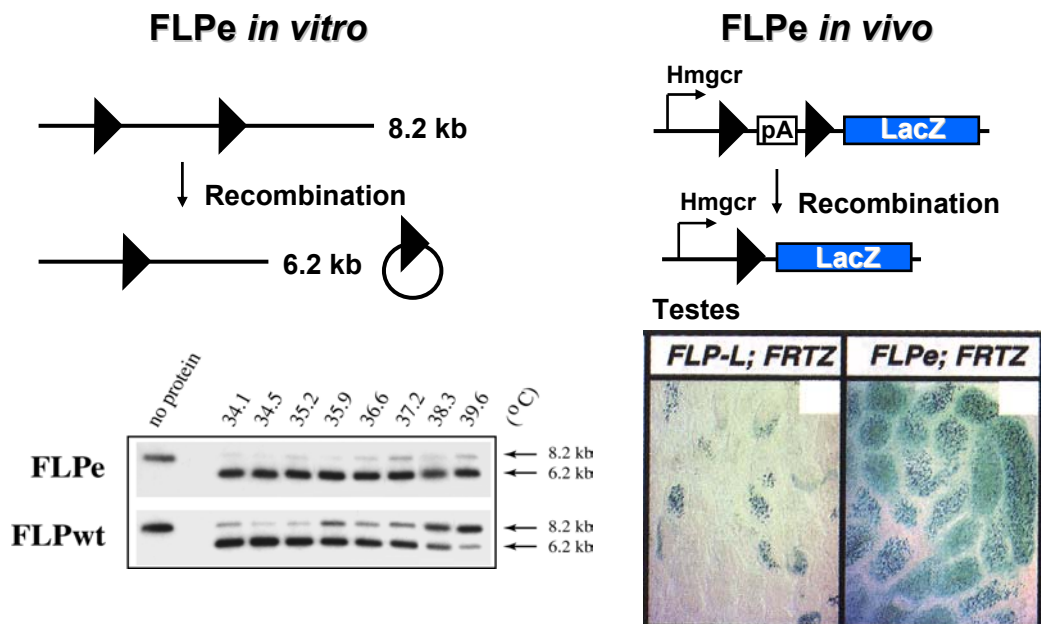


Fig. 1: Comparison of FLPe and FLPwt *in vitro* and *in vivo*. Effect of different temperatures on the recombination efficiency of FLPe in comparison to FLPwt (left part). Treatment with FLPe instead of FLPwt shows a much higher percentage of recombined products when incubated at 37°C. FLPe deleter strains achieve complete excision of the polyA signal in the germ line in comparison to FLP-L strain (for details see Rodriguez et al. 2000).

pCAGGS-FLPe-IRESpuro carries a strong, constitutive expression cassette for an *in vitro*-evolved FLP recombinase (FLPe; Buchholz F., Angrand P.O. and Stewart A.F. Nature Biotechnology 16, 657-662 (1998)) under the control of the chicken- β -actin promoter and an hCMV immediate early enhancer. The use of the chimeric CMV enhancer/ β -actin promoter leads to a ubiquitous expression profile in eukaryotes. The FLPe recombinase carries a minimal, nuclear localization signal from the SV40 larger T antigen and is operatively linked by an IRES to a puromycin resistance gene. Additional elements of the expression cassette are an intron with splice donor and acceptor sites upstream of the FLPe gene and a bovine growth hormone polyadenylation signal downstream of the puromycin resistance gene.

The pCAGGS-FLPe plasmid is appropriate for efficient excision of DNA stretches flanked by FRT sites e.g. for excision of a resistance cassette in a conditional allele in eukaryotic cells. The plasmid carries an ampicillin resistance cassette.

Electroporation in ES cells:

10 μ g of supercoiled pCAGGS-FLPe plasmid were used for a single electroporation of 5×10^6 cells in 500 μ l. Cells were diluted 1000-fold afterwards and aliquots plated without selection. 22 hours later cells were changed to 1 μ g/ml puromycin. Cells were left under puromycin selection for 48 hours and changed afterwards to media without puromycin. (see also: Schaft et al. 2001). Using transient puromycin selection, some 10% of all colonies show recombination (without replating or mosaics).

Literature:

- Brown E.J. and Baltimore D. 2003: Essential and dispensable roles of ATR in cell cycle arrest and genome maintenance. *Genes & Development* 17: 615-628.
- 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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- Morey C. et al. 2004: The region 3' to Xist mediates X chromosome counting and H3 Lys-4 dimethylation within the Xist gene. *The EMBO J.* 23: 594-604.
- Rodriguez C. et al. 2000: High-efficiency deleter mice show that FLPe is an alternative to Cre-*loxP*. *Nature Genetics* 25: 139-140.
- Schaft J. et al. 2001: Efficient FLP Recombination in mouse ES cells and oocytes. *Genesis* 31: 6-10.
- Umana P. et al. 2001: Efficient FLPe recombinase enables scalable production of helper-dependent adenoviral vectors with negligible helper-virus contamination. *Nature Biotechnology* 19: 582-585.
- Yadav et al. 2003: Specific protein methylation defects and gene expression perturbations in coactivator-associated arginine methyltransferase 1-deficient mice. *PNAS* 100: 6464-6468.

Map:

