GENE BRIDGES
DNA ENGINEERING SPECIALISTS

TECHNICAL PROTOCOL

FOR

294-Cre/ 294-FLP

E. coli strain

(version 2.0)

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CONTENTS

1 Eppendorf tubes + manual

1. 294-Cre or 294-FLP: Glycerol stock of the *E. coli* strain (500µl, 25% glycerol)
2. This manual

Store tube at -80°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. The Red®/ET® recombination technology is the intellectual property of Gene Bridges GmbH.

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Short Description:

E.coli strains 294-Cre and 294-FLP express either Cre- or FLP-recombinase. Plasmids containing authentic recognition targets for either recombinase (loxP or FRTs) are recombined when propagated in the appropriate strain. 294-Cre and 294-FLP thus provide a simple test for the recombination competence of constructs that are designed for use in Cre- or FLP-mediated genomic manipulations.

In both strains the recombinases are under the control of the λP_R-promoter which limits expression at 23°C so that no FLP, and little Cre recombination is observed in cultures grown at this temperature. Plasmids with recombinase recognition targets are completely recombined at 37°C in the appropriate Cre or FLP strain.

E.coli strains 294-Cre and 294-FLP are based on the MM294 wild-type strain.

Genotype MM294: endA, thiA, hsdR17, supE44

Literature:

- Buchholz F., Angrand P.O. and Stewart A.F. 1996: A simple assay to determine the functionality of Cre or FLP recombination targets in genomic manipulation constructs. Nucleic Acids Research 24, 3118-3119.
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- Reizis B. and Leder P. 2001: The upstream enhancer is necessary and sufficient for the expression of Pre-T cell receptor ά gene in immature T lymphocytes. J. Exp. Med. 194, 979-990.
- Langer S. J., Ghafoori A. P., Byrd M. and Leinwand L. 2002: A genetic screen identifies novel non-compatible loxP sites. Nucleic Acids Research 30, 3067-3077.
- Schnütgen F., Doerflinger N., Calleja C., Wendling O., Chambon P. and Ghselinck N.B. 2003: A direct strategy for monitoring Cre-mediated recombination at the cellular level in the mouse. Nature Biotechnology 21, 562-565
- Seibler J., Zevnik B., Kuter-Luks B., Andreas S., Kern H., Hennek T., Rode A., Heimann C., Faust N., Kauselmann G., Schoor M., Jaenisch R., Rajewsky., Kuhn R. and Schwenk F. 2003: Rapid generation of inducible mouse mutants.
- Zhang X.-M. and Huang J.-D. 2003: Combination of overlapping bacterial artificial chromosomes by a two-step recombinogenic engineering method. Nucleic Acids Research 31, e81.

Technical Protocol

1. Streak 1µl of the cells on an LB agar plate (without antibiotics).
2. Incubate at 30°C overnight.
3. Pick several colonies and grow them in LB medium (without antibiotics). Incubate them at 30°C.
4. Prepare competent cells by any conventional method (electro-competent cells are preferable).
5. Transform the plasmid carrying loxP or FRT sites into 294-Cre or 294-Flp competent cells.
6. After transformation, add 1 ml of LB medium.
7. Incubate at 30°C for 1.5 hours.
8. Plate on the appropriate selection plates and incubate at 30°C for more than 24 hrs.
9. Pick colonies from the plates and isolate plasmid DNA following any of the conventional methods.
10. Re-suspend the DNA pellet in 10µl of 10mM Tris-HCl, pH8.0.
11. Transform 1-2 µl of the plasmid DNA into another *E.coli* strain (DH10B, Top10, DH5α …).
12. Isolate plasmid DNA and check the successful recombination event by digestion.

Since 294-Cre and 294-FLP are both based on the MM294 wild-type *E.coli* strain, the plasmid DNA prepared from them is not of high quality. Therefore, after recombination, the plasmid should be transformed into another strain (e.g. DH10B, Top10, DH5α…).