

TECHNICAL PROTOCOL

FOR

**707-FLPe tet<sup>R</sup> / 708-FLPe cm<sup>R</sup>**

expression plasmids

**(A104/A105)**

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## CONTENTS

### Reaction tube and manual

1. 707-FLPe or 708-FLPe expression plasmid encoding for FLPe recombinase (0.2 µg/µl, 20 µl)
2. This manual

### Store tube at -20°C

### Please read

The product listed in this manual is for research purposes only. It is not designed for diagnostic or therapeutic use in humans, animals or plants. The Red<sup>®</sup>/ET<sup>®</sup> recombination technology is the intellectual property of Gene Bridges GmbH.

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## Short Description of 707-FLPe / 708-FLPe

The expression plasmids enable for FLP-mediated site specific recombination. The plasmids confer tetracycline (707-FLPe) or chloramphenicol resistance (708-FLPe) and propagate at 30°C in low copy number. *flp* expression is under control of the thermosensitive *cl578* promoter and takes place at 37 - 42 °C. A temperature upshift from 30°C to 37°C results in a transient FLPe recombinering activity since the expression plasmids are no longer replicated due to their pSC101-based origin and finally get lost. Compared to the wild type FLP protein FLPe has an improved thermo stability and shows enhanced recombinase activity at 37 – 40 °C (Fig. 1).

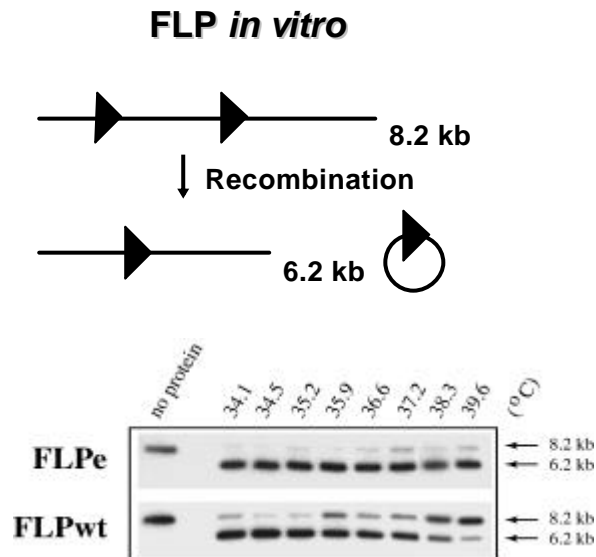


Figure 1: Temperature effect on the *in vitro* recombination efficiency of FLP derivatives (data taken from Rodriguez et al., 2000). At 37.2 - 39.6 °C FLPe treatment results in a significant higher amount of recombined products.

## 706-FLP (wt) versus 707-FLPe recombination

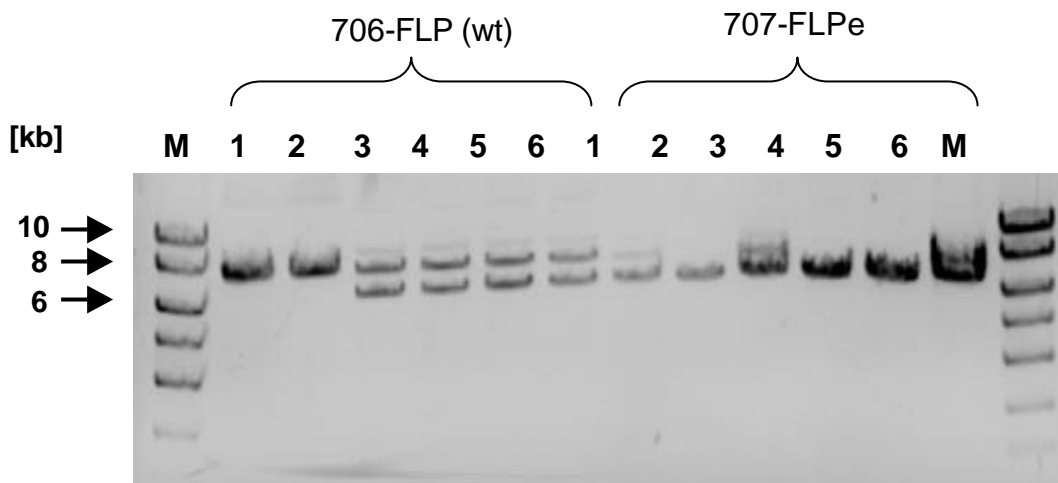


Figure 2: pSVtest (7.3 kb) encoding for a 1.1 kb FRT-flanked fragment was used as targeting plasmid. Upon Flp-recombination at 37 °C, plasmid DNA was isolated from six independent colonies and the migration patterns of *NotI*-treated samples were analyzed by gel electrophoresis. The digestion patterns of Flp-treated plasmids display a strong signal at 6.2 kb, indicating successful removal of most of the FRT-flanked sequences (1.2 kb). Only traces of the parental plasmid (7.3 kb) are visible. In contrast, Flp-treated plasmids show a contrary ratio of recombined products to parental plasmids.

### Note:

The sequence of 707-FLPe and 708-FLPe were compiled from information found in the sequence databases, published literature, and other sources, together with partial sequences obtained by Gene Bridges. The plasmids have not been completely sequenced.

## Protocol I: Site Specific Recombination on Plasmids or BACs

1. Transform the *E.coli* strain, which contains the FRT-flanked DNA fragment, with the expression plasmid (707-/ 708-FLPe).
2. Streak out cells on a LB plate supplemented with 3 µg/ml of tetracycline (selection marker for 707-FLPe) or 15 µg/ml chloramphenicol (selection marker for 708-FLPe) plus the antibiotic(s) required to maintain the target plasmid or BAC in the cells and incubate the plates at 30 °C for approximately 24 hours.
3. Pick several independent colonies and grow them in 1 ml LB medium at 30 °C for 2-3 hours at 1000 rpm.
4. Increase the temperature to 37 °C and incubate the culture overnight. (During incubation at 37 °C, *flpe* is expressed and the FRT sites will recombine; at the same time the expression plasmid gets lost.)
5. Isolate plasmid/BAC DNA and analyse the migration pattern of digested samples by gel electrophoresis.
6. When appropriate, re-transform competent cells with small amounts of plasmid/BAC DNA to obtain clones harboring exclusively recombined replicons.

## Protocol II: Site Specific Recombination on the *E. coli* chromosome

1. Transform the *E. coli* strain, which carries the FRT-flanked DNA fragment, with the expression plasmid (707-/ 708-FLPe).
2. Streak out cells on a LB plate supplemented with 3 µg/ml of tetracycline (selection marker for 707-FLPe) or 15 µg/ml chloramphenicol (selection marker for 708-FLPe) and incubate the plates at 30 °C for approximately 24 hours.
3. Pick several independent colonies and grow them in 1 ml of LB medium at 30 °C for 2-3 hours at 1000 rpm.
4. Increase the temperature to 37°C and incubate the sample overnight. (During incubation at 37°C, *flpe* is expressed and the FRT sites will be recombined; at the same time the expression plasmid gets lost.)
5. Streak out a sample of the culture on LB plate to obtain single colonies. Incubate overnight at 37°C. The next day, analyze twelve single colonies by PCR for the successful removal of the FRT-flanked fragment.

## Maps:

