TECHNICAL PROTOCOL

FOR

707-FLPe tet^R/ 708-FLPe cm^R

expression plasmids

(A104/A105)

Version 1.0 (01.01.09)

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

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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 themosensitive cI578 promoter and takes place at 37 - 42 °C. A temperature upshift from 30°C to 37°C results in a transient FLPe recombineering 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).

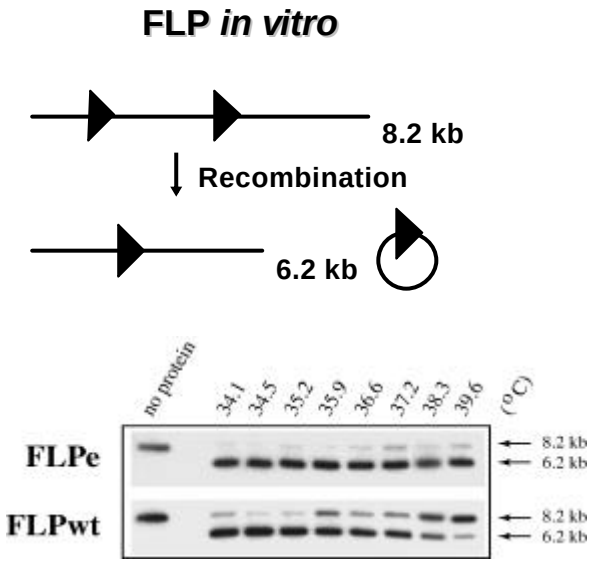


Figure 1: Temperature effect on the *in vitro* recombination efficiency of FLP derivatives (data taken from Rodriguez et al., 2000). At 37.2 - 39.6 °C FLPe treatment results in a significant higher amount of recombined products.

706-FLP (wt) versus 707-FLPe recombination

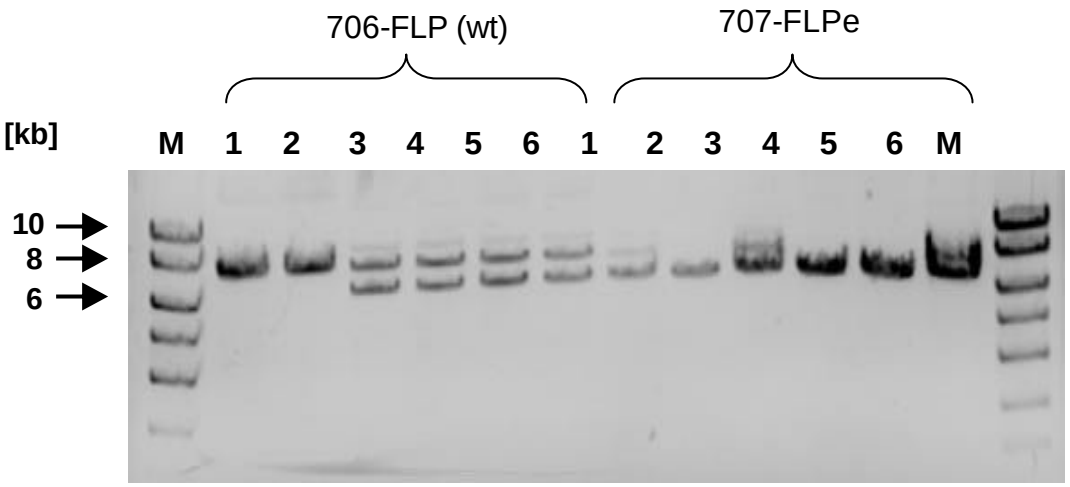


Figure 2: pSVtest (7.3 kb) encoding for a 1.1 kb FRT-flanked fragment was used as targeting plasmid. Upon Flp-recombination at 37 °C, plasmid DNA was isolated from six independent colonies and the migration patterns of *Not*I-treated samples were analyzed by gel electrophoresis. The digestion patterns of Flpe-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.

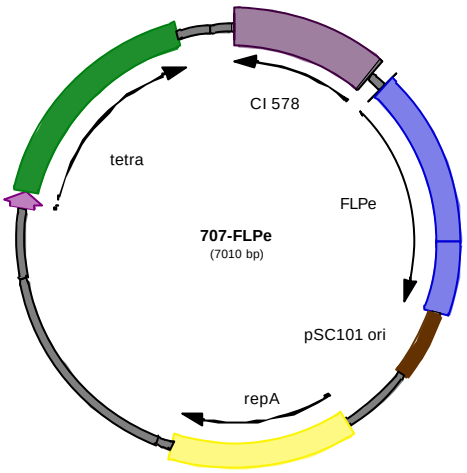
Protocol I: Site Specific Recombination on Plasmids or BACs

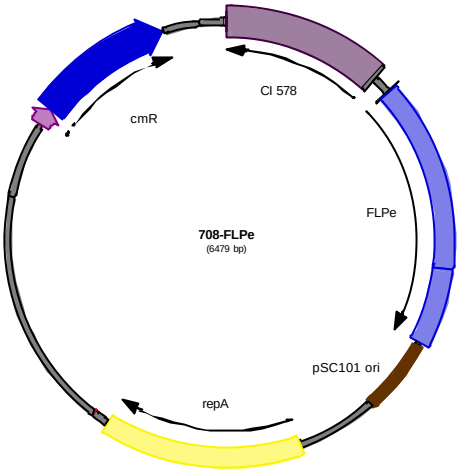
1. Transform the *E.coli* strain, which contains the FRT-flanked DNA fragment, with the expression plasmid (707-/ 708-FLPe).
2. Streak out cells on a LB plate supplemented with 3 µg/ml of tetracycline (selection marker for 707-FLPe) or 15 µg/ml chloramphenicol (selection marker for 708-FLPe) plus the 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:





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