



TECHNICAL PROTOCOL

FOR

FLPe Expression Plasmids for *E. coli*

707-FLPe tet^R (A104)

708-FLPe cm^R (A105)

709-FLPe ap^R (A106)

710-FLPe km^R (A107)

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1. FLPe expression plasmid encoding FLPe recombinase
(0.2 µg/µL, 20 µL)
2. This manual

Store tube at -20°C

Please read

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Short Description

FLPe expression plasmids 707 to 710 (Table 1) enable FLP-mediated site-directed recombination in *E. coli*, and are propagated at 30°C at a low copy number (10 - 15 copies per cell). The *flpe* gene is driven by the λ R promoter and is under control of the heat labile cl857 repressor. A shift of temperature from 30°C to 37°C results in transient FLPe recombinase expression. All members of Gene Bridges' FLPe collection feature the temperature sensitive pSC101 origin of replication. Therefore plasmid replication is abolished upon incubation at temperatures above 30°C and at sustained periods of cultivation at 37°C the respective *E. coli* cells get cured from such a plasmid by segregational loss. Compared to the wild type FLP derived from *Saccharomyces cerevisiae* the FLPe protein has an improved thermal stability and shows enhanced recombinase activity at 37 - 40°C (BUCHHOLZ F., ANGRAND P.-O. AND STEWART A.F., 1998).

Table 1: FLPe expression plasmids supplied by Gene Bridges¹

Name	Cat. No.	Resistance marker	Recommended drug conc.	Restriction fragments sizes of <i>Hind</i> III digestion [bp]
707-FLPe	A104	Tetracycline	3 µg/mL	3632, 1712, 1312, 125, 121
708-FLPe	A105	Chloramphenicol	15 µg/mL	3632, 1312, 1181, 125, 121
709-FLPe	A106	Ampicillin	50 µg/mL	3632, 1432, 1312, 125, 121
710-FLPe	A107	Kanamycin	15 µg/mL	3632, 1325, 1312, 125, 121

Note:

The sequences of FLPe expression plasmids were compiled from information found in sequence databases, published literature, and other sources, together with partial sequences obtained by Gene Bridges. The plasmids have not been completely sequenced. The diagnostic restriction pattern for *Hind*III digestion is given above.

¹ For ordering information please visit the Gene Bridges webpage on www.genebridges.com.

Protocols for the elimination of FRT-flanked DNA sequences

On the following pages you will find a detailed protocol for the elimination of FRT-flanked DNA sequences in *E. coli* using Gene Bridges' FLPe expression plasmids. The sequence that has to be removed generally contains an antibiotic resistance gene and successful deletion becomes apparent in sensitivity to the respective antibiotic. However, any DNA located between two aligned FRT sites may be removed by a FLP reaction. A variety of FRT-flanked selectable marker genes can be found on the Gene Bridges homepage (www.genebridges.com).

The present protocol provides instructions for the elimination of FRT-flanked DNA sequences that encompass at least one selectable marker gene from episomal DNA (BACs, plasmids, cosmids) or the *E. coli* chromosome.

1. Transform *E. coli* cells that contain a FRT-flanked DNA in conjunction with a selectable marker with an FLPe expression plasmid.
2. After the transformation plate cells on a LB plate supplemented with the appropriate antibiotic for the maintenance of the FLPe expression plasmid (s. Table 1 on page 3). If you modify episomal DNA (BACs, plasmids, cosmids) you also have to add the antibiotic the respective backbone confers resistance to (e.g. Chloramphenicol for a pBACe3.6 based construct or Ampicillin for pBAD24 derivative). No additional antibiotic is needed in the case of a genomic localization of the FRT-flanked DNA. **Important: Omit the antibiotic the flanked resistance gene confers resistance to!** Incubate the plates at 30°C for approximately 24 hours. Due to the low temperature cell growth is slowed down. Thus, you may have to prolong the incubation time to obtain colonies of proper size.
3. Fill 1 mL LB medium supplemented with the antibiotic the backbone of the episomal DNA confers resistance to or LB medium without antibiotics in the case of a genomic modification to a 2 mL reaction tubes and puncture the lid (e.g. by using a cannula). **Important: Omit**

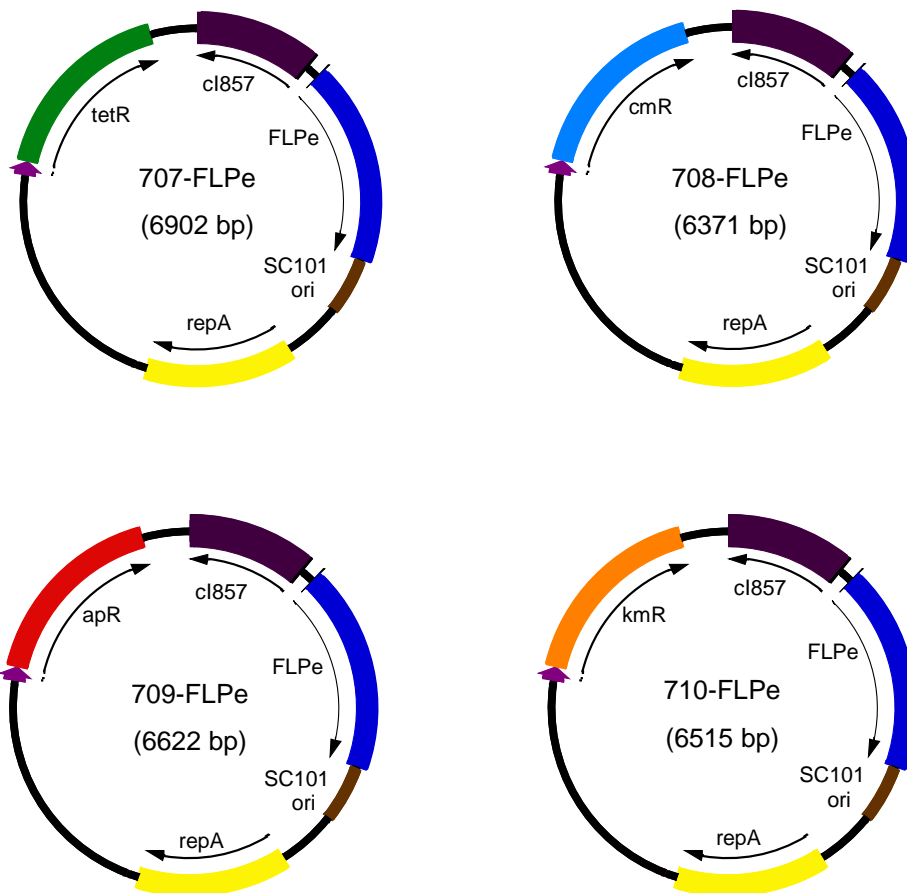
- the antibiotic the flanked resistance gene confers resistance to. Omit the antibiotic the FLPe expression plasmid confers resistance to now, too!** Inoculate up to three such tubes with a single colony obtained in the step 2, each, and incubate at 30°C for 2 - 3 hours at 800 rpm in a bench top thermo mixer (e.g. Thermomixer compact, Eppendorf AG).
4. Increase the temperature to 37°C and incubate the culture for further 2 - 3 hours. During this step expression of *flpe* gene is induced. The FLPe recombinase will subsequently recognise the two aligned FRT sites and all DNA in between (including the selectable marker) gets excised. At the same time segregational loss of the FLPe expression plasmid takes place with high efficiency. However, prolonged cultivation at 37°C (incubation for additional 3 hours or overnight) may enhance the final number of clones without FLPe expression plasmid.
 5. Use an inoculation loop to streak out aliquots of the culture on an LB agar plate to generate single colonies. Use LB plates supplemented with the antibiotic the backbone of the episomal DNA confers resistance to or without antibiotics in the case of a genomic modification and incubate overnight at 37°C.
 6. To identify successfully recombined clones analyze some of the obtained colonies by patching replicates. Therefore, pick a single colony using an inoculation loop and transfer cells **first** to an LB plate supplemented with the antibiotic to which the eliminated marker gene conferred resistance to, and **secondly** (with the same inoculation loop) to an LB plate supplemented with the antibiotic the backbone of the episomal DNA confers resistance to or without antibiotics in the case of a genomic modification.² Repeat this procedure with up to 54 single colonies. Continue analyzing clones from the second plate that were antibiotic sensitive on the first plate, e.g. by PCR or by small scale DNA

² We recommend using the grid pattern on page 9 for the patching of single colonies to two differently supplemented LB agar plates. Place each agar plate on a grid and make diagonal streaks in the numbered squares. **Important:** tick-mark the 6 o'clock position on the back of agar plates (corresponding to the 6 o'clock mark in the grid). This prevents mix up of clone numbering by inadvertent 180° twist of plates.

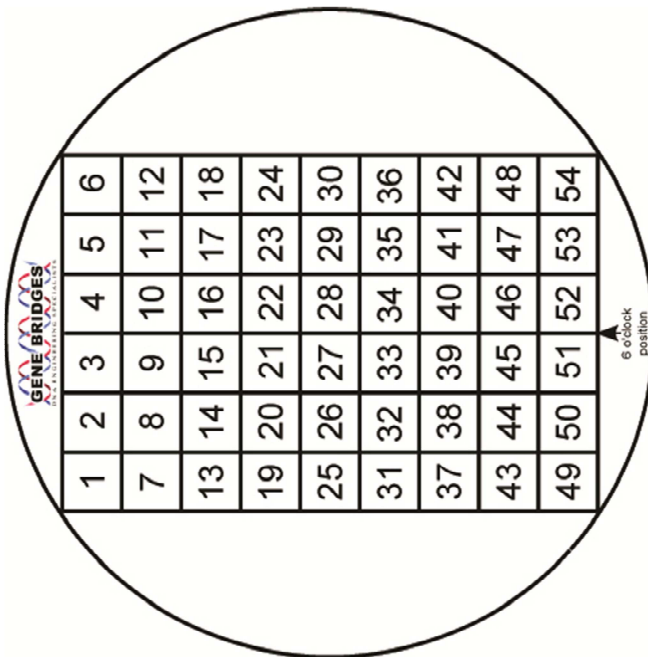
preparation and subsequent analysis of the banding pattern of digested samples by agarose gel electrophoresis.

Additional information for plasmid modification: After the FLPe-modification of plasmids (especially high-copy number plasmids) sometimes no antibiotic sensitive clones can be identified on the first plate. This may be due to a mixed plasmid population (successfully flipped and non-flipped plasmids) in a single *E. coli* cell. In such case we recommend to make small scale plasmid DNA preparation anyway. A subsequent restriction digestion and comparison of the results to the expected banding pattern will allow for a direct review and identification of DNA preparations that contain recombined plasmids. Retransform an aliquot of such plasmid DNA to separate recombined and non-recombined plasmid copies as appropriate and repeat single clone analyses.

Plasmid maps of FLPe expression plasmids:

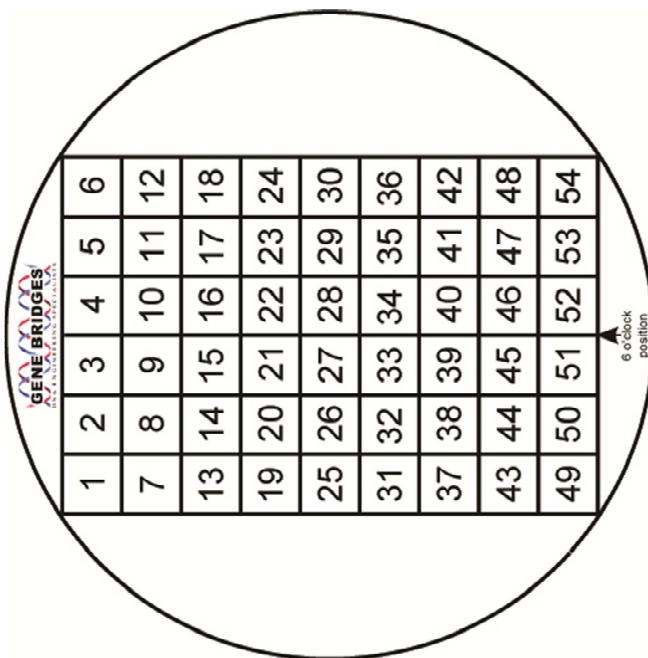


Patching grid (master)



A circular diagram representing a patching grid. Inside the circle is a 10x6 grid of numbered cells. The cells are arranged in 5 rows and 6 columns. The numbers in each row are: Row 1: 1, 2, 3, 4, 5, 6; Row 2: 7, 8, 9, 10, 11, 12; Row 3: 13, 14, 15, 16, 17, 18; Row 4: 19, 20, 21, 22, 23, 24; Row 5: 25, 26, 27, 28, 29, 30; Row 6: 31, 32, 33, 34, 35, 36; Row 7: 37, 38, 39, 40, 41, 42; Row 8: 43, 44, 45, 46, 47, 48; Row 9: 49, 50, 51, 52, 53, 54. To the left of the grid is the logo for 'GENE BRIDGES' with a DNA double helix icon. To the right of the grid, an arrow points to the 5th column, labeled '6 o'clock position'.

1	2	3	4	5	6
7	8	9	10	11	12
13	14	15	16	17	18
19	20	21	22	23	24
25	26	27	28	29	30
31	32	33	34	35	36
37	38	39	40	41	42
43	44	45	46	47	48
49	50	51	52	53	54



A circular diagram representing a patching grid, identical to the one above. It features a 10x6 grid of numbered cells (1-54) arranged in 5 rows and 6 columns. The logo 'GENE BRIDGES' is on the left, and an arrow points to the 5th column labeled '6 o'clock position' on the right.

1	2	3	4	5	6
7	8	9	10	11	12
13	14	15	16	17	18
19	20	21	22	23	24
25	26	27	28	29	30
31	32	33	34	35	36
37	38	39	40	41	42
43	44	45	46	47	48
49	50	51	52	53	54