

TECHNICAL PROTOCOL

FOR

706-Cre

expression plasmid

(version 2.0)

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1 Eppendorf tubes + manual

1. 706-Cre: expression plasmid for Cre recombinase (0.2 µg/µl, 20 µl)
2. This manual

Store tube at -20°C

Please read

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Short Description:

706-Cre plasmid is designed for use in Cre-mediated genomic manipulations. The plasmid has a pSC101 origin which maintains low copy and replicates at 30°C. The plasmids will not propagate and will get lost when incubated at 37°C. The expression of the Cre-recombinase is driven by the thermosensitive promoter cI578 (λ_{PR} promoter). Therefore, the expression of Cre is repressed at 30°C and induced between 37-42°C.

The plasmid carries a tetracycline resistance.

Note:

The sequence of 706-Cre was compiled from information found in sequence databases, published literature, and other sources, together with partial sequences obtained by Genebridges. This vector has not been completely sequenced.

The digestion patterns for BamHI, EcoRI, HindIII, PstI, XbaI and XhoI are indicated

Reference:

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.

Zhang, Y., Buchholz, F., Muyrers, J.P.P. and Stewart, A.F. (1998) “A new logic for DNA engineering using recombination in *Escherichia coli*” *Nature Genetics* 20, 123-128.

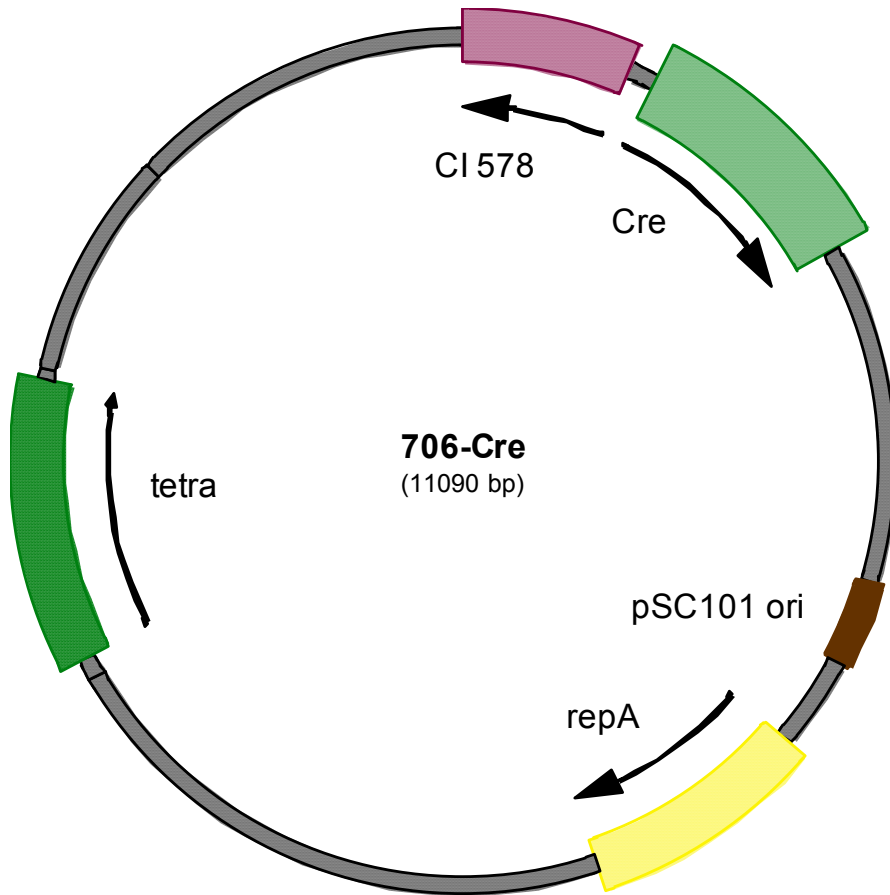
Site Specific Recombination to Remove Selection Marker.

1. 706-Cre plasmid is transformed into an *E.coli* strain, which contains a targeting plasmid carrying a floxed selection marker (e.g. pPGK-neo resistance gene).
2. After transformation (electroporation or heat shock), add 1 ml of LB medium to the tube and incubate at 30°C for 1.5 hr with shaking.
3. Streak out the cells on LB plates containing 5 µg/ml of tetracycline (tet) plus ampicillin (amp; selection marker for the targeting plasmid).
4. Incubate at 30°C for more than 24 hours (since the colonies grow slowly).
5. Pick a single colony and grow the cells in 1 ml of LB medium with 50 µg/ml of amp (resistance of the targeting plasmid) at 30°C for 2-3 hours.
6. Incubate over night at 37°C

(During incubation at 37°C, Cre protein is expressed and the loxP sites recombined, at the same time, 706-Cre plasmid is lost.)
7. Prepare plasmid DNA and digest part of the DNA to check the restriction pattern
8. Re-transform the checked DNA to remove of the unrecombined plasmid.

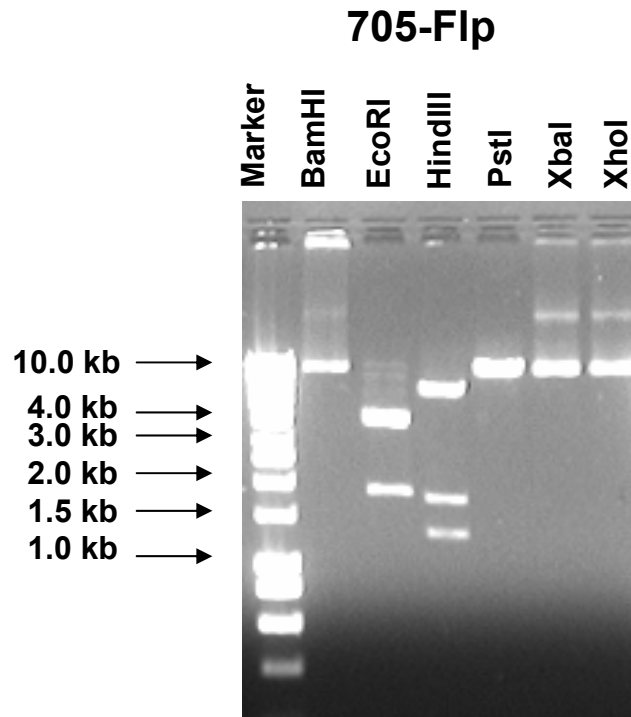
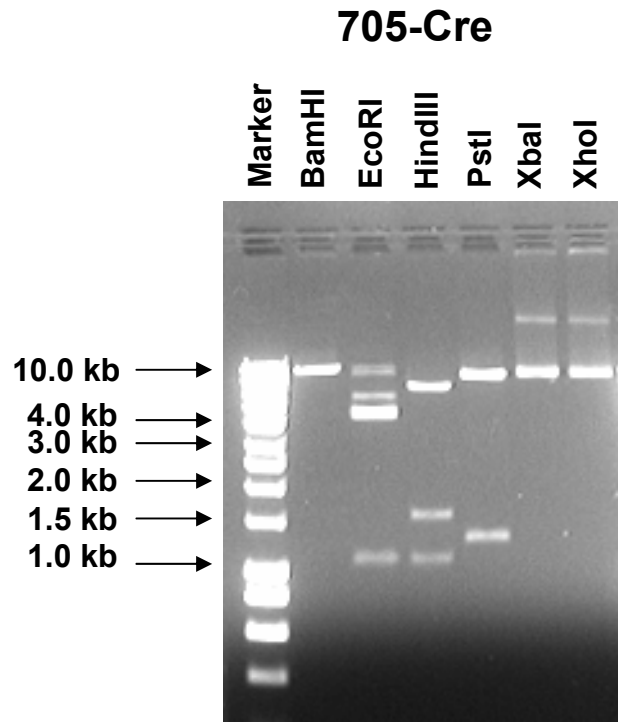
About 95% of floxed fragment will be recombined. Step 8 is therefore important to obtain the pure and recombined plasmid.

Map:



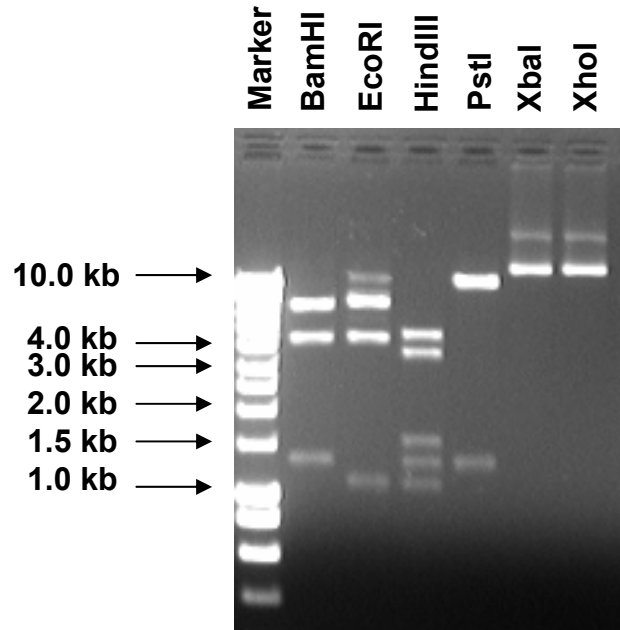
Quality Control Sheet
705-Cre, 706-Cre, 705-Flp and 706-Flp: lot #040405

A Restriction pattern (I)

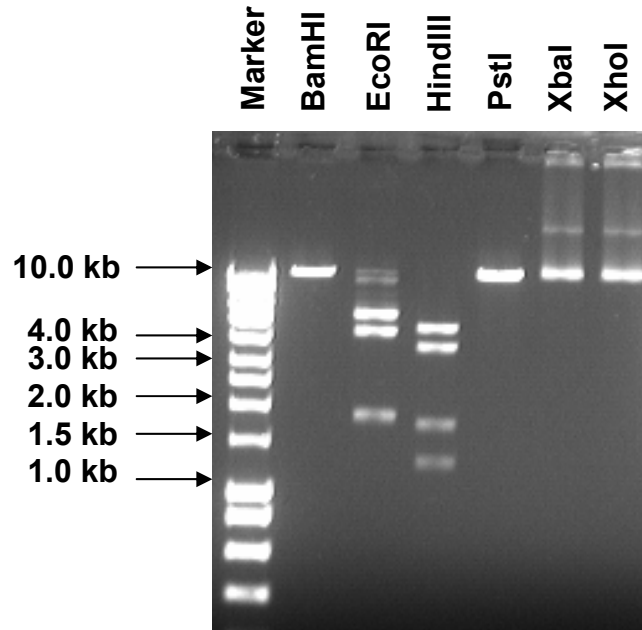


A Restriction pattern (II)

706-Cre



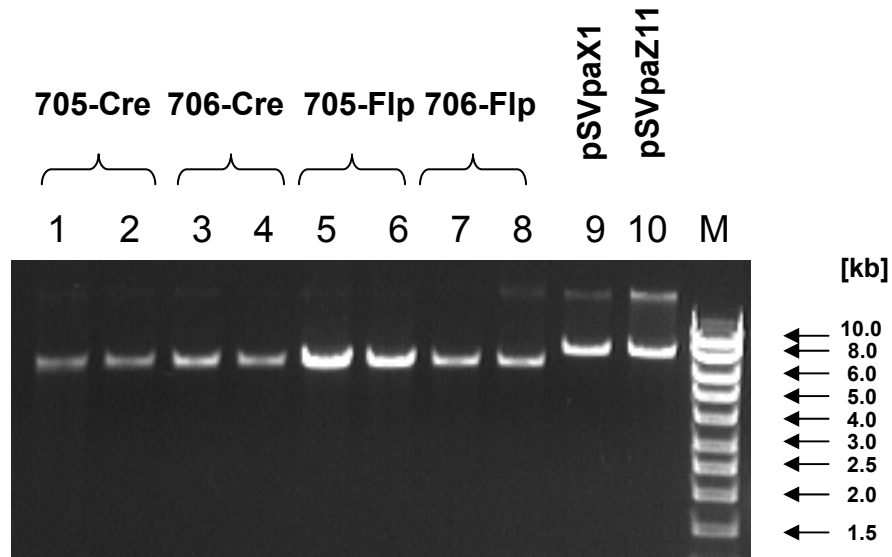
706-Flp



A Restriction pattern (III): fragment size

[bp]	705-Cre	705-Flp	706-Cre	706-Flp
BamHI	9700	undigested	1300, 4200, 5800	1100
EcoRI	1100, 2x 4300	1800, 2x 4200	1000, 4200, 6100	1700, 4000, 5300
HindIII	1100, 1600, 7000	1300, 1800, 7100	1000, 1300, 1600, 3200, 4200	1200, 1600, 3600, 4600
PstI	1300, 8400	10200	1300, 10000	11000
XbaI	undigested	undigested	undigested	undigested
XhoI	undigested	undigested	undigested	undigested

B Functional test:



The functional test was performed as described on page 4 of the manuals.

The plasmids pSVpaZ11 (size 7.3 kb; with a 1.1kb FRT flanked fragment) and pSVpaX1 (size 7.3kb; with a floxed 1.1 kb fragment) were used as targeting plasmids. Miniprep DNA from two colonies was isolated and the targeting plasmids linearized by NotI digestion to check for successful recombination.

705-Cre, 706-Cre: The size of pSVpaX1 shows the successful recombined size of 6.2 kb (lanes 1+2, lanes 3+4, respectively). The negative control pSVpaX1 shows the original 7.3kb band (lane 9).

705-Flp, 706-Flp: The size of pSVpaZ11 shows the successful recombined size of 6.2 kb (lane 5+6, lanes 7+8, respectively). pSVpaZ11 is used as negative control and shows the original size of 7.3 kb (lane 10).