TECHNICAL PROTOCOL

FOR

706-Cre

expression plasmid

(A113)

Version 2.1 (01.04.06)

CONTENTS

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 the 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 *Bam*HI, *Eco*RI, *Hin*dIII, *Pst*I, *Xba*I and *Xho*I 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.

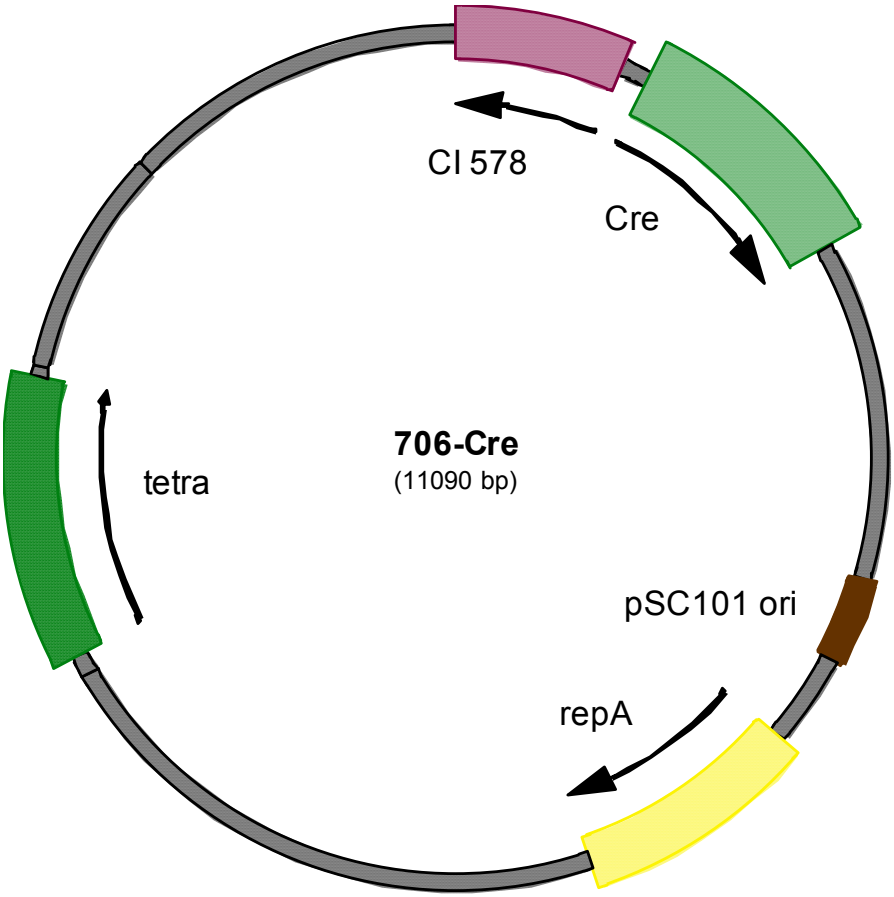
Site Specific Recombination to Remove Selection Marker.

1. 706-Cre plasmid is transformed into an *E.coli* strain, which contains a targeting plasmid carrying a floxed selection marker (e.g. PGK-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 L.B. 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. Switch temperature to 37°C and incubate overnight.

   (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 the unrecombined plasmid.

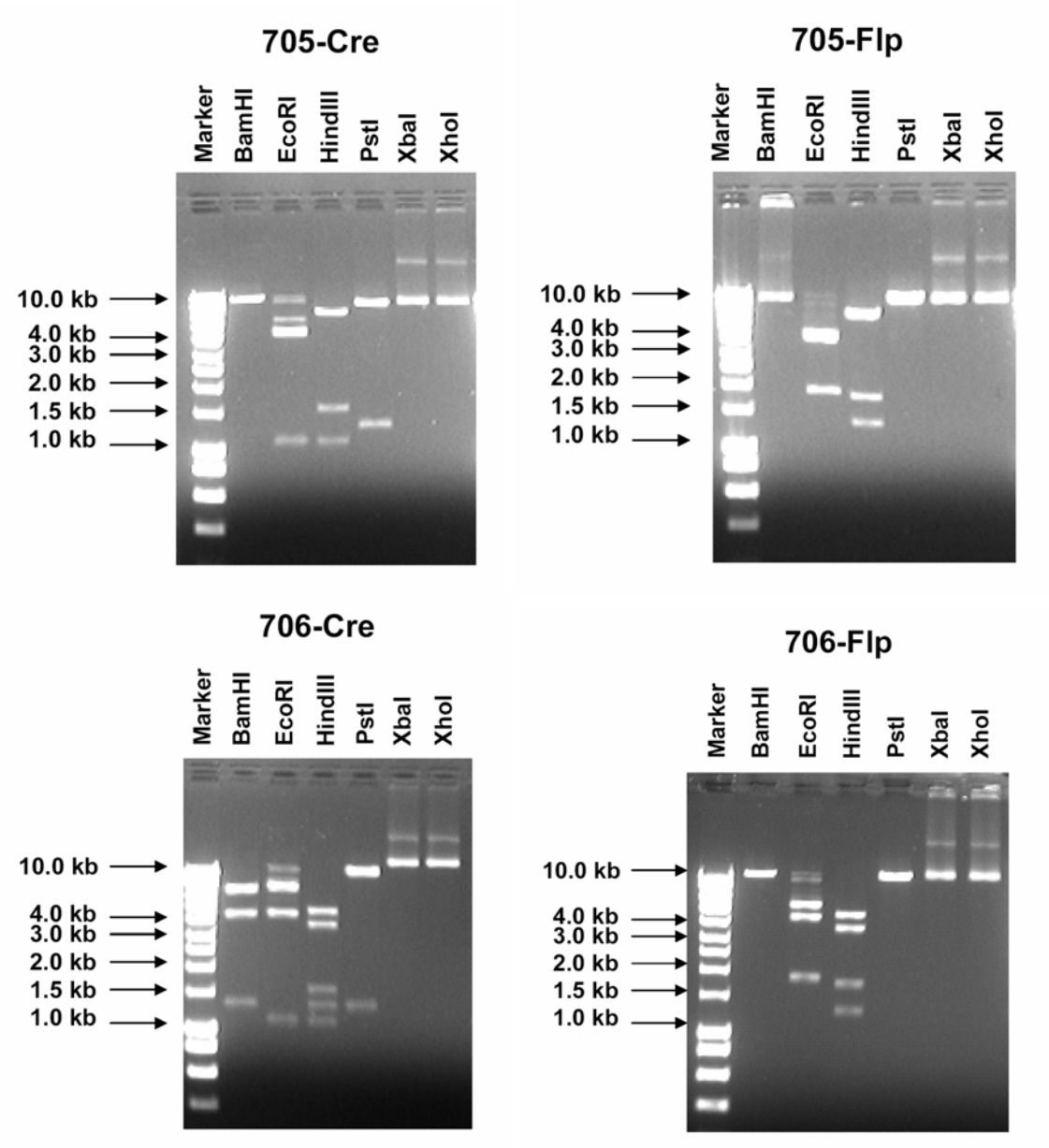
   About 95% of floxed fragment will be recombined. Step 8 is therefore important to obtain the pure and recombined plasmid.

Map:

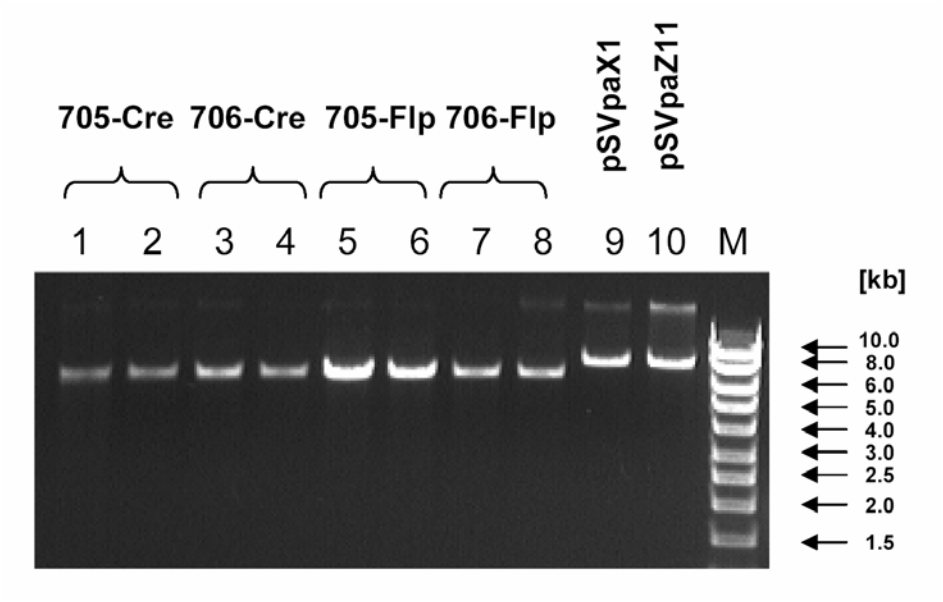


Restriction pattern plasmid 705-Cre, 705-Flp, 706-Cre and 706-Flp

[bp]	705-Cre	705-Flp	706-Cre	706-Flp
*Bam*HI	9700	undigested	1300 4200 5800	11000
*Eco*RI	1100 4300 (2x)	1800 4300 (2x)	1000 4200 6100	1700 4000 5300
*Hin*dIII	1100 1600 7000	1300 1800 7100	1000 1300 1600 3200 4200	1200 1600 3600 4600
*Pst*I	1300 8400	10200	1300 10000	11000
*Xba*I	undigested	undigested	undigested	undigested
*Xho*I	undigested	undigested	undigested	undigested



Functional test 705-Cre, 705-Flp, 706-Cre and 706-Flp



The functional test was performed as described on page 4 of the manual. The plasmids pSVpaZ11 (size 7.3 kb; with a 1.1 kb 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 *Not*I 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).