TECHNICAL PROTOCOL

FOR

704-Cre; Amp^{R}

expression plasmid

(A114)

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CONTENTS

1 Eppendorf tubes + manual

1. 704-Cre; Amp^R: 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:

704-Cre; Amp^R 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 an ampicillin resistance.

Note:

704-Cre; Amp^R plasmid was sequenced completely.

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. 704-Cre; Amp^R 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 h with shaking.
3. Streak out the cells on LB plates containing 50 µg/ml of ampicillin (amp) plus 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 containing the antibiotic of the targeting plasmid in a suitable concentration at 30°C for 2-3 hours.
6. Incubate over night at 37°C

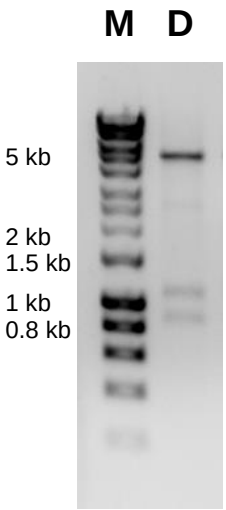
   (During incubation at 37°C, Cre protein is expressed and the loxP sites recombined; at the same time 704-Cre; Amp^R plasmid is lost.)
7. Prepare plasmid DNA and digest part of the DNA to check the restriction pattern.
8. Re-transform the checked DNA to remove of the un-recombined plasmid.

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

Map:

Restriction pattern of plasmid 704-Cre; Amp^R

*Bam*HI digestion:	7095 bp
*Eco*RI digestion:	1105 bp and 5990 bp
*Bam*HI and *Eco*RI digestion:	877 bp, 1105 bp and 5113



M: HyperLadder^{TM} 1 kb, Bioline
D: Digestion of 704-Cre; Amp^R with *Bam*HI and *Eco*RI