3118–3119 *Nucleic Acids Research, 1996, Vol. 24, No. 15*

A simple assay to determine the functionality of Cre or FLP recombination targets in genomic manipulation constructs

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Received April 12, 1996; Revised and Accepted June 10, 1996

ABSTRACT

We report the construction of two *Escherichia coli* strains (294-Cre and 294-FLP) which express either Cre- or FLP-recombinase. Plasmids containing authentic recognition targets for either recombinase (loxPs or FRTs) are recombined when propagated in the appropriate strain. 294-Cre and 294-FLP thus provide a simple test for the recombination competence of constructs that are designed for use in Cre- or FLP-mediated genomic manipulations.

The site-specific recombinases Cre and FLP are finding increasing usage as tools for genomic engineering of eukaryotic cell lines, animals and plants (for review see 1,2). A range of applications has been demonstrated, including chromosomal translocations and large deletions (3,4), tissue-specific and conditional knockouts (5,6), inducible gene expression and site-specific integration (7,8) and precise removal of selectable markers (9). In general, the recombination target sites are introduced into a plasmid construct to flank the DNA of interest or the selectable marker. In subsequent steps, the construct is transfected into eukaryotic cells and integrated into the genome. Since these subsequent steps often represent a considerable investment of time and labour, it is desirable to verify at an early stage that the introduced recombination target sites are competent for recombination. To address this need, we have constructed the *Escherichia coli* strains 294-Cre and 294-FLP by integrating either Cre- or FLP-recombinases into the *lacZ* locus of strain MM294 [CGSC #6315 (294-Cre: F^-, λ^-, supE44, endA1, thi-1, hsdR17, lacZ::cI857-Cre; 294-FLP: F^-, λ^-, supE44, endA1, thi-1, hsdR17, lacZ::cI857-FLP)] using the gene replacement technique of Hamilton *et al.* (10) (Fig. 1A). This technique has the advantage that no resistance marker stays behind on the *E.coli* genome and therefore 294-Cre and 294-FLP do not carry resistance markers that would prevent the selection for plasmids based on such markers. The recombination competence of Cre or FLP recombination target sites engineered into any plasmid can be simply tested by transformation into the appropriate strain and overnight growth at 37°C. In both strains the recombinases are under the control of the λP_R-promoter which limits expression at 23°C so that no FLP, and little Cre, recombination is observed in cultures grown at this temperature (data not shown). Test plasmids with recombinase recognition targets (Fig. 1C) are completely recombined in all colonies at 37°C in the appropriate Cre or FLP strain. Subsequent isolation of plasmid DNA and restriction digestion will assess the recombination competence of the construct. Examples of this test are shown in Figure 1D.

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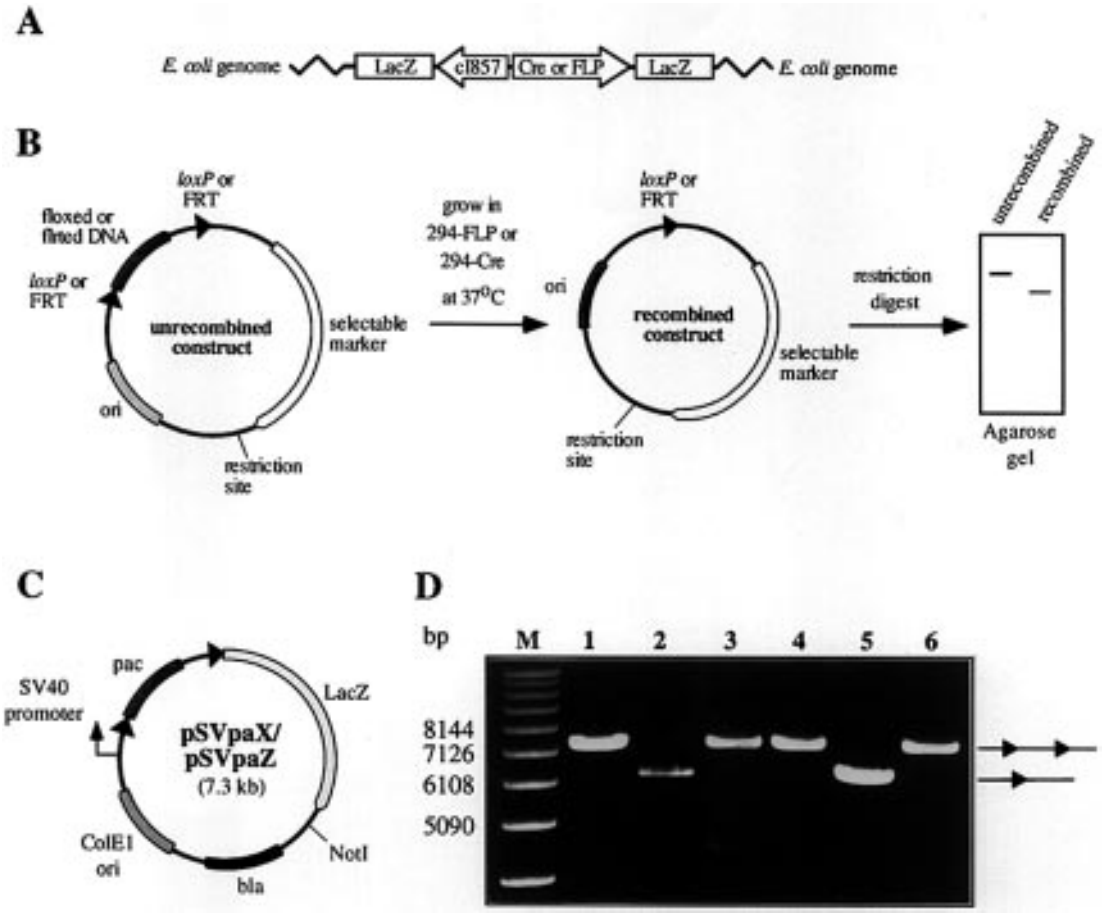

Figure 1. *Escherichia coli* 294-Cre or 294-FLP recombination system. (**A**) Integration locus of either Cre- or FLP-recombinase coding regions to create 294-Cre or 294-FLP strains, respectively. Expression of the recombinase is under the control of the temperature-sensitive cI857 repressor. The Cre gene was obtained from plasmid pcltsCRE (INVITROGEN). The FLP gene was obtained from plasmid pMJ (11), a kind gift from M. Jayaram. (**B**) Scheme to assess the recombination competence of a test plasmid. (**C**) Plasmids pSVpaX1 (Cre-reporter) and pSVpaZ11 (FLP-reporter). Both plasmids are based on pBluescript II SK (Stratagene) and are identical except for their recombinase recognition targets. pSVpaX1 harbours two *loxP* sites spaced by 1.1 kb which includes a puromycin resistance (pac) gene. pSVpaZ11 contains two FRT-sites spaced by the same DNA. The plasmids also include an SV40 early enhancer–promoter region and a lacZ gene. Further details are available on request. (**D**) Recombination competence test of plasmids pSVpaX1 and pSVpaZ11. After overnight growth at 37°C, plasmid DNA was isolated and digested with *Not*I which linearises both plasmids. The digested DNA was then run on a 0.7% agarose gel and stained with ethidium bromide. Recombination is evident as a 1.1 kb reduction in plasmid size and is complete. M: 1 kb ladder (BRL); lane 1: pSVpaZ11 grown in XL1-Blue (Stratagene); lane 2: pSVpaZ11 grown in 294-FLP; lane 3: pSVpaZ11 grown in 294-Cre; lane 4: pSVpaX1 grown in XL1-Blue; lane 5: pSVpaX1 grown in 294-Cre; lane 6: pSVpaX1 grown in 294-FLP.