5530–5531 Nucleic Acids Research, 1993, Vol. 21, No. 23

A shuttle vector system for the rapid detection of recombination in murine cells

Liam J.Fanning and Tommie V.McCarthy
Department of Biochemistry, University College, Cork, Ireland

Received August 20, 1993; Revised and Accepted October 8, 1993

The rearrangement of DNA sequences by site specific and homologous recombination events underlies many aspects of gene regulation and genetic variation (1). Illegimate recombination events also occur and can give rise to altered phenotypes and/or pathological conditions (2). Several DNA rearrangement processes involve joining of non homologous DNA segments accompanined by DNA deletion (3). In this report we describe the construction of a shuttle vector and an appropriate bacterial host which allows rapid detection of intramolecular deletion events mediated by recombination mechanisms in murine cells.

The plasmid pLF498 (fig. 1) was constructed by the sequential removal of the two V(D)J recombination recognition sequences and the structural gene for chloramphenicol acetyltransferase from the vector pJH299 (4), by a stepwise subcloning process after digestion with the restriction enzymes *Sal* I, *Bam* HI and *Sma* I respectively. The transcription terminator *Oop* was removed from the resulting subclone by *Cla* I digestion, and the *supF* gene isolated from pJHR2 (gift from Kathleen Dixon) by *Eco*RI digestion, was gel purified and inserted by blunt-end ligation into the *Cla* I site present on the subclone to yield the final plasmid pLF498. The presence of the early region of polyoma virus and the bacterial origin of replication allows this plasmid to replicate in both murine and *E.coli* cells. The three unique restriction sites *Sal* I, *Sma* I and *Bam* HI facilitate subcloning of sequences to be investigated for recombination activity on either side of the marker *supF* whose presence or absence can be easily detected in *lacZ* (Am) *E.coli* mutants grown on plates containing X-Gal and IPTG (5).

In order to select for the presence or absence of the *supF* and to minimise *E.coli* mediated recombination, an appropriate host strain *E.coli* strain LF302 [*lacZ(Am), recA938, recD1014*] was constructed as follows (6): *E.coli* strain DB1318 (7) [λ^-, *recA938::Tn9–200(cam^R), recD1014, hsdR2, zjj- 202::Tn10*] was cured of tetracycline resistance by the method of Maloy and Nunn (8). The strain LF201 *lacZ125(Am)::Tn10* was constructed by infection of the *E.coli* strain CA274 *lacZ125(Am)* (9) with a phage lysate grown on *E.coli* RS 1071 *zah-281::Tn10* (obtained from B.Bachmann, Yale *E.coli* Stock Center, GCSC 6463) and selection on X-Gal, IPTG and tetracycline. The *E.coli* strain LF201 [*lacZ125(Am)::Tn10*] was used as a suitable donor for the transduction of *lacZ125(Am)::Tn10* into tetracycline sensitive DB1318. This strain was subsequently cured of tetracycline resistance (8) to yield the strain LF302.

The combination of the plasmid pLF498 and the host strain LF302 can be used efficiently for transiently assaying deletion type rearrangement events between chosen sequences flanking the *supF* gene in murine cells. Deletion events removing *supF* can be easily scored by transformation of the host strain following recovery of the plasmid from transfected murine cells and selection on X-Gal, IPTG plates.

Using this shuttle vector system we have carried out preliminary investigation of recombination between class switch sequences. The plasmid substrate pLF114 was constructed by subcloning sequences containing homologous regions from the two murine immunoglobulin class switch regions Sμ and Sγ2b into the *Sal* I and *Bam* HI sites flanking the *supF* gene. The pre-B cell line 300.18 (10) was transfected with the plasmid substrate pLF114 and incubated for 48 hours at 37°C in the appropriate media (4). The plasmid DNA was reisolated, electroporated into

Table 1. Scoring of rearranged plasmid substrates using the *E.coli* strain LF302 and X-Gal, IPTG selection

	Blue cfu	White cfu
Transformation of LF302 with pLF498	505,000	1
Transformation of LF302 with pLF114	265,000	5
Transformation of LF302 with plasmid substrate reisolated from the pre-B cell 300.18.	83,500	105

Electroporation of LF302 was performed using 1 ng of pTZ18R, 100 μl of LF302 at a cell density of $2-4\times10^{10}$ cells/ml and 0.2 cm electroporation cuvettes (11). The electroporation mix was plated onto LB agar supplemented with ampicillin, chloramphenicol, IPTG and X-Gal. The transformation efficency achieved was 1×10^8 cfu/μg of DNA.

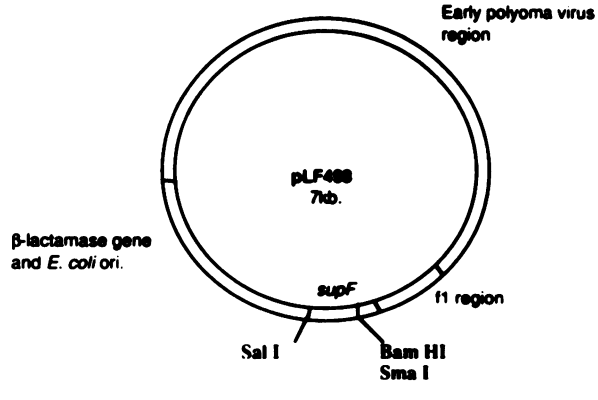

Fig. 1. Schematic diagram of the shuttle vector pLF498.

the *E.coli* strain LF302 which was subsequently plated on ampicillin, IPTG, X-Gal plates. Scoring of blue and white transformants showed that a significant increase in *supF* gene inactivation occurred following passage through the pre-B cells (Table 1). Restriction analysis of the plasmid substrates recovered from white colonies showed that 21% of the rearranged plasmid substrates recovered had undergone a DNA rearrangement event between the switch regions resulting in deletion of the *supF* gene. The remainder of the transforming plasmid molecules had either undergone an illegimate DNA rearrangement event between the switch region Sμ and the polyoma virus DNA (68%) or point mutation inactivation of the *supF* gene (11%).

This system facilitates rapid and sensitive detection of deletional remangement events. The background frequency of mutational inactivation of the *supF* gene ranged from $4-5\times10^{-5}$ cfu in the recombination deficient host strain (Table 1). In addition to its use in rapid detection of intramolecular deletion events this system can also be used for investigating gene mutation events in murine cells since a variety of types of mutations including point mutations inactivate the *supF* gene (5). The use of this vector and the assay system can be easily adapted for use in human cells by replacing the polyoma virus sequences with the appropriate sequences from SV40 which allow replication in human cells.

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