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Brief Notes

A plasmid vector with positive selection and directional cloning based on a conditionally lethal gene *

(Barnase; barstar; polylinker; ribonuclease)

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SUMMARY

A plasmid vector with a multiple cloning site (MCS) for positive selection of cloned inserts in *Escherichia coli* (*Ec*) has been devised, based on the expression plasmid (pMT416) for the bacterial ribonuclease barnase (Barn). The host is protected from the lethal effect of moderate expression of *barn* by expression of the gene *bars*, encoding its inhibitor, barstar (Bars), placed on the same plasmid. Full expression, however, is lethal. Induction is also lethal with the derived plasmid, pMT440, which has the pUC19 MCS inserted into *barn*. Under inducing conditions, transformation by the vector is lethal unless the product of the modified *barn* is inactivated by insertion of cloned DNA fragments into the MCS. Plasmid pMT440 is, therefore, a generally useful selective cloning vector not requiring any special strain of *Ec*.

Several *Ec* plasmid vectors designed for direct selection of cloned inserts have been reported (Ozaki et al., 1980; Dean, 1981; Vernet et al., 1985; Henrich and Plapp, 1986; Bernard et al., 1994; Henrich and Schmidtberger, 1995).

The vector presented here (pMT440; Fig. 1) utilizes the toxic effect of expression in *Ec* of the *barn* (Hartley, 1989) and is based on the *barn* expression plasmid pMT416 (Hartley, 1988). Thanks to the protection provided by the Barn inhibitor Bars, pMT440, like pMT416, may be carried in any *Ec* carrying the *lacI*^{Q} gene but is lethal when the *tac* promoter (De Boer et al., 1983) in front of *barn* is fully induced by IPTG. In strains without *lacI*^{Q}, both plasmids are lethal without induction.

Preliminary experiments proved that pMT416 itself could be used as a positive selection vector. With DNA fragments cloned into its unique *Sma*I site in *barn*, the gene is rendered harmless. The insertion site for the MCS in pMT440 was chosen taking into account that Barn cut at Val^{36} into the separated peptides could reassemble to form an active enzyme (Sancho and Fersht, 1992) and that Val^{36} is on a loop well away form the active site.

pMT440 was tested on a random library of *Sau*3A fragments of *Bacillus amyloliquefaciens* DNA ligated into the *Bam*HI site of the MCS. To minimize cloning of multiple fragments and to make the test more severe, we

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* On request, the authors will supply detailed experimental evidence for the conclusions reached in this Brief Note. The plasmid is available also on request.

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Abbreviations; aa, amino acid(s); Barn, barnase; *barn*, gene (DNA) encoding Barn; Bars, barstar (inhibitor of Barn); *bars*, gene encoding Bars; bp, base pair(s); *Ec*, *Escherichia coli*; IPTG, isopropyl-β-D-thiogalactopyranoside; MCS, multiple cloning site(s); nt, nucleotide(s).

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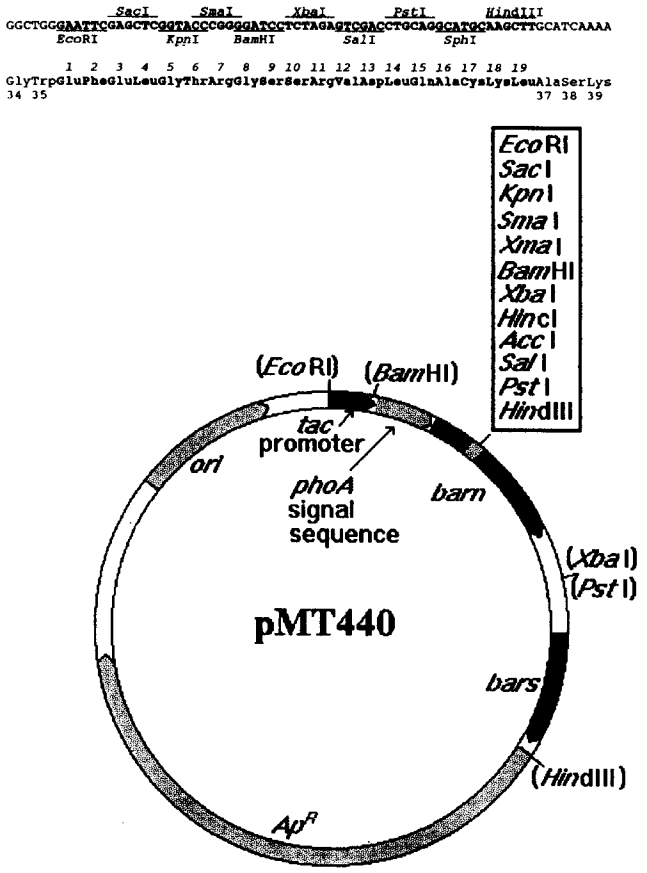

Fig. 1. Positive selection vector pMT440. Restriction sites in parentheses have been removed, making all restriction sites in the MCS (Yanisch-Perron et al., 1985) unique. Above are shown nt and aa sequences of the MCS insert, with junctions.

used a 5–10-fold molar excess of the plasmid. Two library size fractions were cloned, one (*i*) with fragments from roughly 600 to 1200 bp and (*ii*) from 2000 to 4000 bp. Tested by colony hybridization with a ^{32}P-labelled oligodeoxyribonucleotide probe, 97% of the (*i*) clones and 85% of the (*ii*) clones did not contain the *Bam*HI junction of the MCS. Plasmid preparations from 18 (*ii*) clones, negative in the hybridization test, all contained inserts of appropriate size.

pMT440 should be an excellent vector for general purpose cloning. It can be maintained in *lacI*^{Q} strains and, for most cloning purposes, ligation mixtures would be transformed into a *lacI*^{Q}-negative host with no need for the use of IPTG. It may be that the same selection system could be used in a λ phage for the cloning of much larger fragments.

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