Title
The pIC plasmid and phage vectors with versatile cloning sites for recombinant selection by insertional inactivation

Permalink
https://escholarship.org/uc/item/3p06f1vq

Journal
Gene, 32(3)

ISSN
0378-1119

Authors
Marsh, JL
Erfle, M
Wykes, EJ

Publication Date
1984

DOI
10.1016/0378-1119(84)90022-2

License
CC BY 4.0

Peer reviewed
The pIC plasmid and phage vectors with versatile cloning sites for recombinant selection by insertional inactivation

(Recombinant DNA; filamentous bacteriophage M13; polylinkers; β-galactosidase; α-complementation selection; chimeric genomes)

J. Lawrence Marsh, Mary Erfle and Evan J. Wykes *

Developmental Biology Center and Department of Developmental and Cell Biology, University of California, Irvine, CA 92717 Tel. (714) 856-6677, and * Monsanto Corp., 800 N. Lindbergh Blvd., St. Louis, MO 63167 (U.S.A.) Tel. (314) 694-3776

(Received June 25th, 1984)
(Revision received September 13th, 1984)
(Accepted September 20th, 1984)

SUMMARY

The versatility of insertional inactivation of β-galactosidase activity for subcloning and sequencing has been enhanced by combining a chemically synthesized oligonucleotide which specifies nine 6-bp-cutter restriction sites in various configurations with existing polylinkers to create a set of highly versatile cloning sites. These improved polylinkers have been inserted into plasmids (the pICs) for routine cloning of double-stranded DNA, and into chimeric phage/plasmids (the pICEMs) for biological production of single stranded DNA.

INTRODUCTION

The insertional inactivation of β-galactosidase α-complementation is a powerful method for identifying recombinant DNA molecules in plasmids and phage. The ability of the gene fragment to tolerate extensive modification has permitted the engineering of polylinkers in the alpha donor region of the pUC plasmids (Vieira and Messing, 1982) and M13 bacteriophage (Messing and Vieira, 1982). We have extended the versatility of these vectors by combining an oligonucleotide which specifies nine 6-bp-cutter restriction enzyme sites in various configurations with existing polylinkers to construct a set of greatly improved cloning sites. These have been introduced into plasmids and into chimeric phage/plasmids for biological production of single stranded DNA.

MATERIALS AND METHODS

(a) Bacterial strains

The pUC, pIC and pICEM plasmids were grown in the Escherichia coli K-12 strain JM83 (ara, ʌlac-
(b) Procedures

DNA was prepared by the cleared lysate method (Katz et al., 1973), phenol-extracted and loaded on a 250-ml Biogel A-50M, 100-200 mesh column equilibrated with 500 mM NaCl, 20 mM Tris·HCl pH 8 and 1 mM EDTA. Excluded fractions containing plasmid and chromosomal DNA were banded in a CsCl density equilibrium gradient for final purification. Restriction enzyme digests employed a core buffer (6 mM Tris·HCl pH 7.4, 6 mM MgCl₂ and 6 mM mercaptoethanol) with appropriate salt. DNA fragments were purified from gels by soaking the excised bands overnight in GEB buffer (10 mM Tris·HCl pH 8, 500 mM NaCl, 1 mM EDTA) followed by removal of polyacrylamide and ethanol precipitation. Standard procedures were performed as described by Maniatis et al. (1982).

RESULTS AND DISCUSSION

(a) Construction of pIC7 and mIC7

Two complementary 33-mer oligonucleotides were synthesized by the hindered dialkylamino phosphite method on functionalized “long chain alkyl amine” controlled pore glass supports (Pierce Chemical Co.) utilizing diisopropyl phosphoramidites. A 20-min synthesis cycle was used similar to that described by Adams et al. (1983). After deprotection and cleavage from the support, the 33-mers were purified by polyacrylamide gel electrophoresis. After elution from the gel, the fragments were ethanol-precipitated at −70°C and redissolved in deionized water for subsequent annealing and cloning. The sequences of the two 33-mers are 5'-AATTCATC-

GATATCTAGATCTCGAGCTCGCGAA-3' and 5'-AGCTTTTCGCGAGCTCGAGATCTAGATC-

TCGATG-3'. After annealing, the double-stranded oligonucleotide which has EcoRI and HindIII cohesive ends was ligated to EcoRI and HindIII cut pUC8 and M13mp10. Blue colonies or plaques were selected and designated pIC7 and M13mIC7. The structure of the new cloning site in pIC7 was confirmed by restriction analysis and that of M13mIC7 by nucleotide sequence analysis (not shown).

(b) Construction of pIC19R, pIC19H, pIC20R, and pIC20H

The new polylinker of pIC7 was linked to the polylinkers both pUC9 and pUC19 in two orientations thus providing approx. 14 unique restriction enzyme sites in the lacZ gene region. The polylinkers were fused by cutting pIC7 with HindIII and NarI eluting the large fragment bearing the ori, Ap^R and polylinker from an acrylamide gel and ligation to the small NarI-HindIII fragment from pUC9 to give pIC19R or from pUC19 to give pIC20R. The Ap^R, Lac^+ colonies were selected and the structures of pIC19R and pIC20R confirmed by restriction analysis (Fig. 1).

The reciprocal constructions involved ligating the small EcoRI-NarI fragment of pIC7 to the large EcoRI-NarI fragment of pUC9 and pUC19, respectively. The structures of the resulting pIC19H and pIC20H vectors are shown in Fig. 1.

(c) Mapping of constructs

The structure of the polylinker in the new vectors was confirmed by cutting each vector with BglII which cuts twice (one cut is 150 bp from the linker site) and again with each of the enzymes specified by the linker region. The digests were analyzed by gel electrophoresis. The observed fragment sizes are those expected from the sequence. For example the ClaI, EcoRV, BglII, XhoI, SacI and NruI sites all map approx. 5 bp apart while the Smal, BamHI, SalI and PstI sites map 7–9 bp apart as indicated by the sequence. Similar mapping performed on each of the constructs confirmed the localization and order of the restriction sites in the linker regions.
Fig. 1. Structure of pIC plasmid vectors. The TaqI site (at 4018 in pBR322) is used as the zero reference point. Other TaqI sites are located between the HaeIII sites (2352 and 2722 on pBR322 map) and in the polylinker. The sequence of the polylinker is shown starting with the ATG codon of the β-galactosidase gene and ending with the natural HaeIII site and the alanine of codon 8 to the right. The conceptual translation of the sequence is presented with the additional amino acids contributed by the inserted polylinker sequences shown in italics. The cloning site of M13mIC7 is the same as that shown for pIC7.

(d) Construction of pICEM19R +; pICEM19R −; pICEM19H +; pICEM19H −

Chimeric phage/plasmids bearing the origin of replication from a single stranded phage in a plasmid combine the advantages of single stranded phage small BglII fragments of pEMBL8 + and 8 − bearing the f1 replications in either orientation (Dente et al., plasmids (e.g., Dotto et al., 1981; Dotto and Horiuchi, 1981). Thus we combined the improved cloning sites of the pIC vectors with the f1 origin of the pEMBL vectors (Dente et al., 1983).

We ligated the large fragment of BglII-cut pIC7 bearing the lacZ gene and part of the ApR gene to the small BglII fragments of pEMBL 8 + and 8 − bearing the f1 replications in either orientation (Dente et al.,
Fig. 2. Structure of pICEM chimeric phage vectors. The reference point is the same as that used by Dente et al. (1983) for the pEMBL vectors and corresponds to the original EcoRI site of pBR322 which has been modified during construction of pUC8. The sequence of the polylinker is shown starting with the ATG codon of the lacZ gene and ending with the natural HaeIII site and the alanine of codon 8 to the right. The conceptual translation of the sequence is presented with the additional amino acids contributed by the inserted polylinker sequences shown in italics.

1983) and the remainder of the ApR gene and selected ApR, Lac+ colonies. Plasmids bearing the polylinker of pIC19R and pIC19H linked to the f1 replicon in both the plus and minus strand orientation were constructed (designated pICEM19R+; pICEM19R--; pICEM19H+ and pICEM19H--). The structures were confirmed by restriction analysis as described above. Confirmation of pICEM19+ and 19R-- is shown in Fig. 3. The presence of a ClaI site in the f1 fragment of the pEMBL vectors compromises the use of this enzyme in these vectors. However, use of smaller ori fragments could avoid unwanted restriction sites in the future.

(e) Modification of the XbaI site

The terminal A of the TCTAGA XbaI site in pIC7 falls within the dam methylase recognition sequence GATC. When grown in a methylase deficient host (GM33, dam-3), the XbaI site shows normal cutting in standard restriction reactions but when grown in JM83, cutting is blocked completely (not shown). Thus hemimethylation of the terminal A blocks cleavage of XbaI (Gruenbaum et al., 1981). The sensitivity of this plasmid to XbaI digestion provides a sensitive test for the state of the dam methylase system in a strain. Additionally, one can mask or unmask this site during complex constructions by the appropriate choice of host bacterium.

(f) Other vectors

The polylinkers described here can now be readily mobilized with either EcoRI or HindIII or a double digest and if desired they can be inserted into the symmetrical EcoRI or HindIII sites of the plC and pICEM vectors to generate other polylinker configurations. We anticipate improvements in the design of the chimeric phage/plasmids to possibly produce
higher yields of chimeric phage and permit more flexibility in vector design. The NarI site of the pIC plasmids provides a convenient site for future insertion of smaller phage ori fragments.

ACKNOWLEDGEMENTS

This work was supported by PHS grants GM28972 and HD16519 to JLM. We are especially indebted to Dr. H.A. Schneiderman for offering the assistance of the Monsanto laboratories for the oligonucleotide synthesis. The expert assistance of P.M. Timmons is gratefully acknowledged.

REFERENCES


Communicated by R.L. Rodriguez.