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Short Communication

Construction and Properties of a New Cloning Vehicle, Allowing Direct Screening for Recombinant Plasmids

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Summary. pUR2, a certified B2(EK2) vector, allows easy isolation of variants containing cloned *Eco*RI-fragments. Bacteria harboring plasmids without inserts make blue colonies on indicator-plates, whilst those harboring recombinant plasmids make white colonies.

Introduction

In most gene cloning experiments replica-plating (Lederberg and Lederberg, 1952) has to be used to detect recombinant plasmids. In this communication I describe the construction of a cloning vehicle allowing screening for recombinant plasmids in one step. This was achieved by combining a reduced pBR322-plasmid (Bolivar et al., 1977) with a fragment containing the *lac*-regulatory region and part of the *Z*-gene.

Bacteria, carrying the regulatory region and the *Z*-gene of the *lac*-operon, can be induced by isopropyl-thiogalactoside (IPTG) to express β-galactosidase. This enzyme hydrolyses the colourless 5-bromo-4-chloro-indolyl-β-D-galactoside (x-gal) to a blue insoluble indigo derivative (Davies and Jacob, 1968). A small deletion in the *Z*-gene (M15) lacking the codons for amino acid residues 11–41 (Langley et al., 1975a) destroys the ability of β-galactosidase to hydrolyze x-gal. Ullmann, Jacob and Monod (1967) reported that the α-peptide (amino acid residues 3–92 of β-galactosidase, Langley et al., 1975b) has donor activity in intracistronic complementation and leads to reactivation of defective M15-β-galactosidase. α-complementation can be demonstrated by blue colonies on indicator-plates containing IPTG and x-gal.

Gronenborn and Messing (1978) have introduced a unique *Eco*RI-site in M13mp1 at a position corresponding to the fifth amino acid of the α-peptide. M13mp1 is a M13-derivative carrying the *lac*-promotor, operator and part of the *Z*-gene coding for α-peptide (Messing et al., 1977). The name of the modified phage is M13mp2; cloning in the *Eco*RI-site of this phage leads often to inactivation of the α-peptide i.e. always when nonsense codons or frameshifts are introduced or created. Bacteria-strains carrying the deletion ΔM15 transformed with such hybrid phages show white colonies (plaques) on x-gal containing indicator-plates. In that way recombinants can be detected easily and the expression of the cloned DNA can be controlled by the *lac*-regulatory region. Because the phage M13mp2 has not yet been certified as a B2 safety vector for cloning experiments I constructed a plasmid with similar advantages for recombinant-screening.

Methods and Results

Cleaving of pBR322 (Bolivar et al., 1977) with *Eco*RI and *Pvu*II results in two fragments, a 2067bp fragment containing the gene coding for tetracycline resistence and a 2294bp fragment containing the ampicillin resistence gene and the origin of replication (Sutcliffe, 1978). The *Eco*RI-site was destroyed during incubation of the cleaved DNA for about one hour with DNA-polymerase I in presence of all four nucleotides. The DNA was then treated with T4-DNA-ligase to close the 2294bp fragment to a circle. *E. coli* strain CSH51 (*Δ(lac, pro) ara, thi, str*A, *φ*80d*lac*$^+$; Miller, 1972) was transformed using a slightly modified method of Mandel and Higa (1970). Plasmids were prepared from ampicillin resistant and tetracycline sensitive bacteria. Plasmid-DNA isolated in that way shows a mobility in agarose gels equivalent to approximately 2300bp. The plasmid, called pUR1, is *Eco*RI resistant and can be cleaved once by *Pst*I and *Hind*II and twice by *Hae*II.

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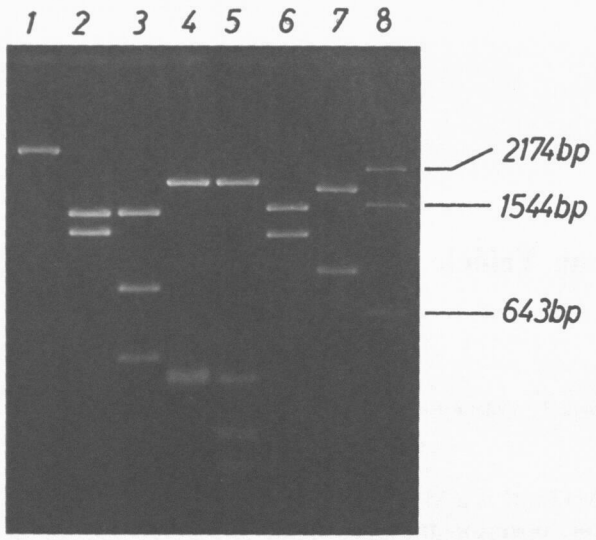

Fig. 1. Restriction analysis of pUR2 by agarose gel electrophoresis. The agarose concentration in the gel was 2%. Lanes: 1, pUR2 digested with *Eco*RI: 2, digested with *Taq*I; 3, digested with *Taq*I and *Eco*RI; 4, digested with *Hae*II; 5, digested with *Hae*II and *Eco*RI; 6, digested with *Hind*II and *Eco*RI; 7, digested with *Pst*I and *Eco*RI; 8, an *Ava*I, *Pst*I and *Pvu*II digest of pBR322 was used as fragment-size-marker

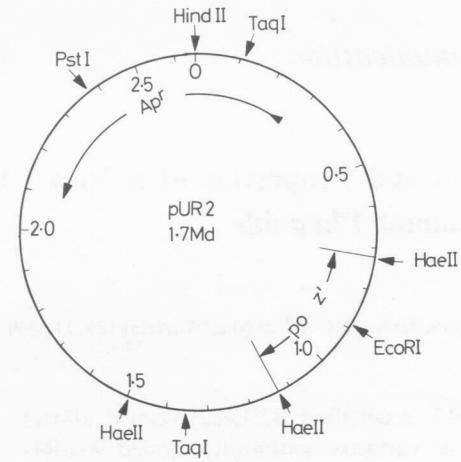

Fig. 2. A circular restriction map of pUR2. The position of restriction sites are drawn on a circular map divided into 0.1 kilobases

In the *lac*-region of M13mp2 (Gronenborn and Messing, 1978) are two *Hae*II-sites, one at the end of the *I*-gene and the other at the position corresponding to amino acid residue 59 in the α-peptide-part of the *Z*-gene. Digestion of M13mp2 with *Hae*II produces this *lac*-fragment (391bp, W. Gilbert, pers. communication) and 5 other fragments of different sizes. To clone the 391 bp fragment in pUR1, M13mp2-DNA was cleaved with *Hae*II. pUR1 was partially digested with *Hae*II and then mixed with the digested M13mp2-DNA. After treatment with T4-DNA-ligase strain CSH51 was transformed with the ligated DNA. Bacteria were plated on ampicillin-x-gal-plates and blue colonies were picked for further analysis. Blue colonies were expected to arise when more than 20 copies of *lac*-operator-containing plasmid titrated out lac-repressor. Initially it was not known whether the first 59 amino acids of β-galactosidase were sufficient for donor activity in the α-complementation-test, therefore a Lac^+-strain was used for the selection. When strain BMH 71−18 (*Δ(lac, pro) thi*, F'*lac* $I^q Z \Delta$M15, pro^+) was transformed by plasmids isolated from blue colonies, all colonies were blue on ampicillin-x-gal-IPTG-plates. DNA-analysis (Fig. 1) indicated that only the 391bp fragment is cloned in pUR1. This shows that the first 59 amino acids of β-galactosidase are sufficient for α-peptide activity in this plasmid. The plasmid is approximately 2700bp large in agarose gels. This new plasmid is called pUR2.

I have cloned different *Eco*RI-fragments in pUR2; recombinant plasmids can be detected in white colonies of BMH 71−18 on ampicillin-x-gal-IPTG-plates. Cloning of some small DNA-fragments (smaller than 100bp) led to light blue colonies. These small inserts may not change the reading frame of the α-peptide and may not code for stop-codons in the same frame.

The mobilisation-frequence of pUR2 was compared to pBR322. Both plasmids have the same mobilisation-frequency (data not shown). As a result pUR2 was certified as a B2 (EK2) vector in West-Germany. For use as a B2 (EK2)-system a suitable host-strain has been constructed (a modified χ 1776 (Curtiss et al., 1977) carrying the lac *Z* deletion *Δ* M15) and this strain has been submitted for certification.

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Note Added in Proof

Variants of pUR2 have been constructed, containing either a *Hind*III- or a *Bam*HI-site in addition to the *Eco*RI-site, allowing the same screening for recombinant plasmids.