Construction of vectors with the p15a replicon, kanamycin resistance, inducible *lacZα* and pUC18 or pUC19 multiple cloning sites

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Complementation analysis is a powerful technique in the characterization of genes and their products. When studying cloned genes the analysis is facilitated if compatible vectors are available into which restriction fragments can be cloned. Most multicopy cloning vectors currently in use are derivatives of pBR322 (1), with ampicillin (Ap) resistance markers and the ColEl origin of replication. Vectors such as pUC18, pUC19 (2) and the pBluescript vectors (e.g. pKS-) (3) also contain a multiple cloning site (MCS) in a *lacZα* gene fragment for ease of selection of inserts on X-Gal plates, and controlled expression of insert genes using IPTG. We have developed a pair of compatible vectors, with a kanamycin (Km) resistance marker, the p15a origin of replication, and the *lacZα* gene fragment, for use in complementation studies with ColEl-based clones.

The pK18 and pK19 vectors described by Pridmore (4) were used as the basis for the new vectors. These are pUC based vectors with ColEl replicons and a Km resistance gene replacing the Ap resistance gene of the pUC series. The ColEl origin was removed by partial digestion with *NspHI* and the linearized plasmid was isolated, digested with *BstBI* and blunt ended with T4 DNA polymerase and dNTPs. We used pACYC184 (5) as the source for the p15a origin of replication. It was removed as a 685 bp *Sac*II-*Cla*I fragment and treated with T4 DNA polymerase and dNTPs to create a blunt-ended fragment. The

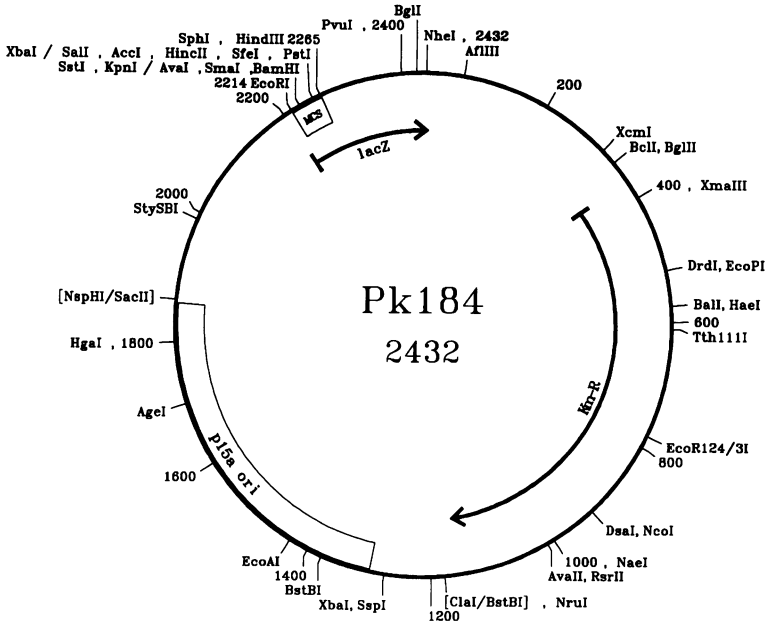

Figure 1: Restriction map of pK184. Arrows mark the position of *lacZα* peptide (lacZ) and kanamycin resistance gene (Km-R). The multiple cloning site (MCS) and origin of replication (p15a ori) are shown as open boxes. Restriction enzymes predicted to cut the plasmid once only (except *XbaI*, two sites) are shown on the outer circle, which is numbered every 200 bp. Restriction enzyme sites eliminated in the construction of the vector are shown in square brackets. Plasmid pK194 has the MCS in the reverse orientation.

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fragment containing the pK18 *lacZα* and Km resistance gene was ligated to the p15a origin fragment and blue, Km-resistant *Escherichia coli* transformants were selected. One transformant named pK184 (2432 bp) consisted of bases 2092–2661/0–1177 of pK18 and bases 1520–836 of pACYC184 (6) (predicted from the sequence of the parental plasmids) (figure 1). Plasmid pK194 (2432 bp) had the MCS in the reverse orientation, and was constructed in the following manner. Plasmid pK184 was digested with *Hind*III and *EcoR*I and treated with *Bal*31 to remove 10–20 bases from either end, to completely eliminate the MCS. The *lacZα* fragment from M13mp19, containing the MCS in the opposite orientation from pK184, was isolated as a 322 bp *Pvu*II fragment. These fragments were mixed, boiled and cooled slowly to room temperature to create a mixture that contained hybrid molecules. T4 DNA polymerase and T4 DNA ligase were used to synthesize closed circular DNA molecules prior to transformation and selection of blue Km-resistant *E. coli* colonies. The presence of the MCS from M13mp19 was verified by double digests with *Nco*I and either *Hind*III or *EcoR*I.

We verified that pK184 was compatible with pKS- (Stratagene, La Jolla, CA) by showing that the plasmids persisted without segregation during 50 generations growth in the absence of selection. The following sites in the MCS's of pK184 and pK194 are unique and usable for cloning: *Hind*III, *Sph*I, *Pst*I, *Hinc*II, *Sal*I, *Acc*I, *BamH*I, *Xma*I, *Sma*I, *Kpn*I, *Sac*I and *EcoR*I. Two *Xba*I sites are present in pK184 and pK194. Derivatives of these plasmids with a unique *Xba*I site in the MCS are being constructed.

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