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Plasmid vector pBR322 and its special-purpose derivatives — a review

(Recombinant DNA; EK2 multipurpose cloning vehicles; nucleotide sequence)

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SUMMARY

The plasmid pBR322 was one of the first EK2 multipurpose cloning vectors to be designed and constructed (ten years ago) for the efficient cloning and selection of recombinant DNA molecules in *Escherichia coli*. This 4363-bp DNA molecule has been extensively used as a cloning vehicle because of its simplicity and the availability of its nucleotide sequence. The widespread use of pBR322 has prompted numerous studies into its molecular structure and function. These studies revealed two features that detract from the plasmid's effectiveness as a cloning vector: (a) plasmid instability in the absence of selection and, (b) the lack of a direct selection scheme for recombinant DNA molecules. Several vectors based on pBR322 have been constructed to overcome these limitations and to extend the vector's versatility to accomodate special cloning purposes. The objective of this review is to provide a survey of these derivative vectors and to summarize information currently available on pBR322.

INTRODUCTION

Since the early days of molecular cloning, bacterial plasmids have received much attention because of their usefulness as vectors in recombinant DNA experimentation. The design and construction of cloning vectors has now become a complex and highly sophisticated area of study, yielding a vast amount of information and a great number of specialized vectors for investigators to use.

Part of our group has been involved in the development of a series of multipurpose cloning vehicles. One of these vectors, pBR322 (Bolivar et al., 1977b), continues to be the most widely used cloning vehicle

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Abbreviations: aa, amino acid(s); AGPT, amino glycosyl 3′ phosphotransferase gene from Tn*5*; Ap, ampicillin; *ARS*, autonomous replication sequence; βGal, β-galactosidase; *bom*, basis of mobilization; bp, base pair(s); CAT, Cm acetyl-transferase; Cb, carbecillin; Cm, chloramphenicol; Gm, gentamycin; IPTG, isopropyl-β-D-thiogalactopyranoside; kb, 1000 bp; Km, kanamycin; Neo, neomycin; nt, nucleotide(s); *par*, partition locus; *ori*, DNA replication origin; *rop*, repressor of primer; ^{R}, resistance; RBS, ribosome-binding site; SCP, synthetic consensus promoter; Sm, streptomycin; Sp, spectinomycin; Su, sulfonamide; ss, single strand(ed); Tc, tetracycline; Thio, thiostrepton; US, unique sites; Vio, viomycin; XGal, 5-bromo-4-chloro-indoyl-β-D-galactoside.

because of the extensive body of information on its structure and function. Apart from its cloning capabilities, pBR322 has also served as a model system for the study of prokaryotic transcription and replication. The primary objectives of this review are to summarize the extensive knowledge about this vector and to compile information on some of the special purpose vectors that have been derived from pBR322. We hope that the researcher who uses these vectors will find it useful to have this information discussed and summarized in one place.

STRUCTURE AND FUNCTION OF THE pBR322 VECTOR

The construction of pBR322 is outlined in Fig. 1. This vector was constructed using, as its primary constitutents, the origin of replication (*ori*) from a clinical isolate containing the ColE1-like plasmid, pMB1. The Ap^R gene was obtained from the transposon Tn*3* (carried on the plasmid pRSF2124) and the Tc^R gene was taken from pSC101. Although pBR312 and pBR313 (Rodriguez et al., 1977; Bolivar et al., 1977a) have the same *ori* and selective markers, they contained extraneous DNA sequences and restriction enzyme cleavage sites that detracted from their usefulness as vectors. For these reasons, pBR322 was constructed. Several manipulations were used to provide pBR322 with a greater variety of unique restriction sites (for details see Bolivar, 1979). The complete nt sequence of pBR322 (see Fig. 4) was determined by Sutcliffe (1979) and revised by Peden (1983) and Heusterspreute and Davison (1984). Many structural and functional features of pBR322 are also known. A brief summary of such features and their plasmid locations are included in this section. On the functional map

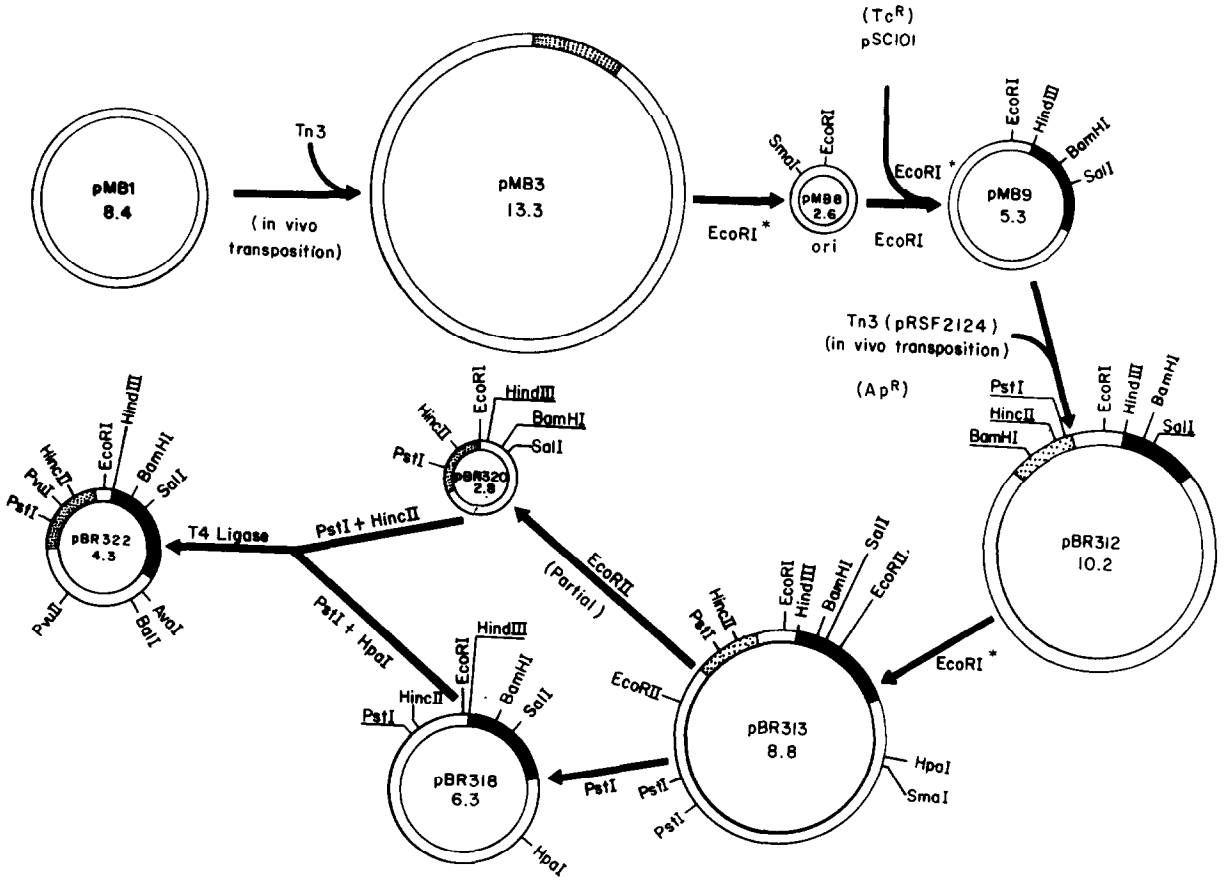

Fig. 1. Construction of pBR322. Stippled box, the complete Ap^R gene. Black box, the complete Tc^R gene. *ori*, origin of replication. The size of the plasmids is given in kb. (Adapted from Rodriguez and Tait, 1983.) Plasmid pBR318 has only a part of the Ap^R gene (not shown).

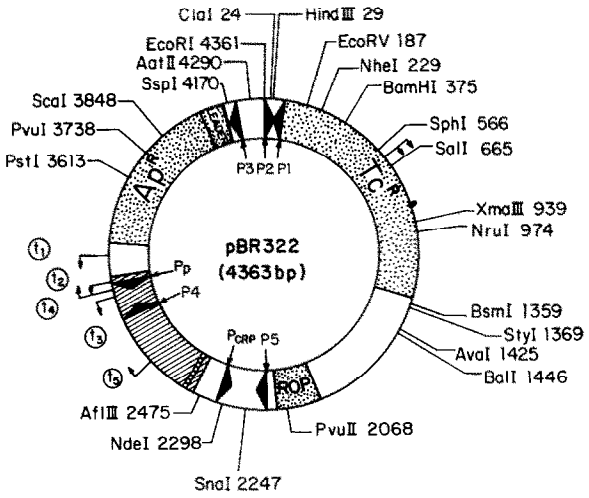

Fig. 2. The pBR322 plasmid. The numbering of the sequence begins at the unique *Eco*RI site. The first T in the palindrome is designated as nt No. 1. Unique restriction sites are noted and the coordinate corresponds to the nt 3′ to the cleavage. Promoters (P1 to P5, P_{CRP} and P_P) are signaled by large arrowheads. Transcription terminators in vivo (t_1 to t_5) are noted by ↑—— and in vitro by ↑↑—— (t_6, above Tc^R in figure), and the arrowheads denote their direction. Hatched box, DNA regions required for replication. Row of small circles designates initiation of DNA replication. Stippled box, structural genes. 'Leader' refers to the β-lactamase leader peptide. For references see Tables I and II, as well as Fig. 4. (The map is drawn to scale.)

(Fig. 2) of pBR322, the salient features are marked to help investigators in the design of future vectors and cloning strategies.

(a) Structural features

There is a good deal of information on the topological aspects of DNA that has been obtained using plasmids. Many of these studies have used pBR-based plasmids to examine the effects of topological changes on DNA conformation. For instance, it has been shown that sites near the *ori* of pBR322, can be rendered progressively more sensitive to S1 nuclease as the superhelicity of the plasmid increases. This property suggests the presence of cruciform structures asociated with palindromic sequences located in this region of the plasmid (Lilley, 1980; Panayotatos and Wells, 1981). Also dependent on the degree of superhelicity, are three potential Z-DNA segments on pBR322 (Table I) (Nordheim et al., 1982; Azorin et al., 1983; Barton and Raphael, 1985). Similarly, ozonolysis-sensitive sites have been located near the S1-sensitive ones (Sawadaishi et al., 1985). It is important to note that because the level of torsional stress due to superhelicity is not known, some of the structures mentioned above may not exist in vivo (Sinden et al., 1983). Contacts between gyrase and pBR322 have also been identified in vitro (Fisher et al., 1981; Kierkegaard and Wang, 1981; Morrison and Cozzarelli, 1981) and in vivo (Lockshon and Morris, 1985). The functional implications of the alteration or deletion of these sites has not been explored. This information is summarized in Table I.

TABLE I

Structural features in pBR322

Site	Position (nt)	Comments	References
S1 hypersensitive, major	3060 ± 10	Defined at natural linking difference	Lilley (1980); Panayotatos and Wells (1981)
S1 hypersensitive, minor	3230 ± 10		
S1 hypersensitive, subminor	3125 ± 10		
Z-DNA stretch	1448–1461	Only at high torsional strain	Nordheim et al. (1982)
	2315–2328		Barton and Raphael (1985)
	3265–3277		
	4254–64		
DNA-gyrase contacts	645–805	Cut at nt 728 and 732	Fisher et al. (1981); Kierkegaard and Wang (1981); Morrison and Cozzarelli (1981)
	991–995	Only cutting sites defined	
	1867–2034	No cuts	
	4148–4308	Cut at nt 4242 and 4246	

TABLE II

Transcriptional signals in pBR322

Promoter	Mapped at position (nt)	Direction of transcription	Function of transcript	Termination: known sites (nt)	Length of resultant transcripts (nt)	Comments on termination	References
P1[a]	80–40	Counterclockwise	β-lactamase (Ap^R)	~3210 (t_1) ~3110 (t_2) ~3070 (t_3)	~1245 ~1345 ~1400	Termination of *bla* is almost complete in these sites. Mapping is approximate.	Stuber and Bujard, (1981); Brosius et al. (1982); von Gabain et al. (1983)
P2	5–45	Clockwise	Tc^R gene	around 650 within Tc^R region (t_6)	630 and others	Termination in this site is observed only in vitro.	Boyer et al. (1977); Rodriguez et al. (1979); Stuber and Bujard (1981).
P3	4225–4190	Counterclockwise	β-lactamase (Ap^R)	~3210 (t_1) ~3110 (t_2) ~3070 (t_3)	~980 ~1080 ~1140	This is the promoter derived from Tn*3*.	Stuber and Bujard (1981); Russell and Bennet (1981); Brosius et al. (1982); von Gabain et al. (1982).
P_P	3123–3098	Counterclockwise	Primer of replication	—	555	555 nt transcript is obtained by RNaseH processing.	Itoh and Tomizawa (1980); Tomizawa et al. (1981).
P4	2970–2995	Clockwise	Replication Inhibitor Transcript	3100 (t_4)	108	Transcription termination is efficient but not complete.	Chain et al. (1979); Itoh and Tomizawa (1980); Tomizawa et al. (1981); Stuber and Bujard (1981).
P5	around 2150	Clockwise	Not known (probably none in pBR322)	around 2700 (t_5)	550	—	Stuber and Bujard (1981).
P_{CRP}	2310–2270	Counterclockwise	Not known (probably none in pBR322)	—	—	—	Queen and Rosenberg (1981).

[a] Originally it was a promoter for the Tc^R repressor-protein gene.

(b) Transcriptional signals

Numerous in vitro and in vivo studies have produced a large body of information about the regulatory sequences (promoters) on pBR322 that control transcription of the Tc^R and Ap^R genes and of the genes that code for the RNAs involved in the replication process. These promoters account for approx. 80% of the in vitro transcription from the pBR322 template (Stuber and Bujard, 1981). The remainder of the transcriptional activity comes from two promoters located between the *ori* and the *rop* gene. They serve no apparent purpose within the vector (Queen and Rosenberg, 1981). The transcriptional signals mentioned above are summarized in Table II, and they are also marked on the sequence and in Fig. 2.

As for the Tn*9*-derived Cm^R gene (which codes for the enzyme CAT), present in some derivatives of pBR322, different transcriptional signals are used. For pBR325 and pBR328, the original promoter for the gene is used (see nt position 120 to 140 in Fig. 5) (Alton and Vapnek, 1979; Marcoli et al., 1980). In the case of pBR329, the Cm^R gene is transcribed from promoter P1 (Table II) which produces an RNA molecule that proceeds into the β-lactamase (Ap^R) gene.

(c) Replication, amplification and stability

The *ori* present in pBR322 shows strong homology to several other *ori*'s found in natural isolates. This type of replicon has been termed ColE1-like, for historical reasons. Five plasmids belong to this category; pMB1 (ancestor to pBR series), ColE1, CloDF13, RSF1030 and p15A. Additional information on these plasmids can be found in a series of recent papers on this topic (Davison, 1984; Veltkamp and Stuitje, 1981; Selzer et al., 1983; Cesareni and Banner, 1985).

The initiation of unidirectional replication on these plasmids is primarilly controlled by three genetic elements: (a) a primer RNA molecule, (b) an inhibiting and incompatibility-mediating small RNA molecule and, (c) a small protein with additional inhibitory capabilities. The above gene products are termed RNA primer or RNAII, RNAI, and Rop (repressor of primer), respectively. The interplay of these components determines the frequency of initiation of replication at the *ori* sites, situated at nt positions 2534–2536 (Bolivar et al., 1977c). The RNA precursor molecule (RNAII) forms a hybrid with DNA at the *ori*; this hybrid, in turn, is processed by RNaseH to form a primer for DNA polymerase I. The process is affected by the RNA secondary structure (Tomizawa and Itoh, 1982), which, presumably, is modified by interaction with RNAI. This interaction is required for the additional inhibition by the 63-aa long Rop protein (Cesareni et al., 1984).

Several palindromic sequences have been noticed in the *ori* region, and the role that these play on the replication process is under investigation (Wong and Polisky, 1985; Masukata and Tomizawa, 1986; Gayle III et al., 1986). The most prominent palindromes are shown in Fig. 4.

It has also been shown that pBR322 contains sites that are effectors for replication factor Y (Zipursky and Marians, 1980; Soeller and Marians, 1982). These are located at nt positions 2114–2185 in the leading strand, and at nt 2353–2416 in the lagging strand. Factor Y is a ϕX174 (+)strand-specific DNA-dependent phosphohydrolase which, in conjunction with other *E. coli* replication proteins, is involved in the formation of heterogeneous primers that are elongated by the *E. coli* DNA polymerase III elongation machinery. The role that these sites may have on normal plasmid replication is unclear, since both factor Y sites and the Rop protein gene could be deleted without abolishing replication activity (Soberón et al., 1980; Twigg and Sherrat, 1980).

Transcripts that originate from the *ori* (see the preceding section **b**) may affect the expression of downstream genes, which may mediate the expression of genes that are appropriately oriented. It is also important to consider the effect of any other transcripts that traverse the *ori*. For CloDF13 it has been shown that elimination of the transcriptional terminator just upstream from the promoter for the primer RNA causes an elevation of the copy number (Veltkamp and Stuitje, 1981). In the case of pBR-derived vectors no such mutations have been isolated, but Bujard et al. (1983) have studied the effect that strong transcription, mediated by bacteriophage T5 promoters, has on the replication function of some of such plasmids. They found interference with the replication functions unless a terminator was placed as to transcriptionally isolate the *ori* region.

ColE1-type replicons are amplifiable. Replication

of the plasmid DNA may continue in the absence of protein synthesis, so protein synthesis inhibitors such as Cm and Sp are widely used to obtain large amounts of DNA per cell (Clewell, 1972). For pBR322, the copy number changes from a steady-state number of 18 per chromosome (Covarrubias et al., 1981) to more than 1000 in the presence of Cm.

Plasmid pBR322 multimerizes considerably in RecA$^+$ cells (Bedbrook et al., 1979) and under nutrient limited growth conditions, it is lost in a clearly detectable fashion (Jones et al., 1980). The partition locus (*cer*) is absent from pBR322, so uneven segregation and consequent loss of the vector in the absence of selective pressure is common. pBR322 derivatives that contain the *par* locus have been constructed to overcome this problem (see Table VI and UTILITY VECTORS).

(d) Mobilization

Plasmid pBR322 can be mobilized by a number of conjugative plasmids under certain conditions. In the presence of ColK, pBR322 can be mobilized by R64drd11. For mobilization to occur, a diffusible product from ColK and *bom* (basis of mobilization), a *cis*-acting element, are needed in addition to the conjugative machinery (Young and Poolis, 1978; Twigg and Sherrat, 1980). The first step in the mechanism involves the nicking of the DNA molecule at a site close to *bom* (Young and Poolis, 1978). The relative orientation of the region with respect to the *ori* and the requirement for transcription through *bom*, have been shown to be necessary for mobilization (Finnegan and Sherrat, 1982; Covarrubias et al., 1981).

The nt sequences that comprise *bom* sites in pBR322 and ColE1 are highly conserved and their potential secondary structures are similar to those of CloDF13 (Snijders et al., 1983). The region is indicated around the position of the nick (nt position 2254) in the sequence.

Plasmids pBR327, pBR328 and pBR329 do not contain the *bom* signal and, therefore, are not mobilizable by the system described above (Covarrubias et al., 1981). This fact makes these plasmids safer than their predecesors in terms of biological containment.

THE pBR322 FAMILY OF PLASMIDS

Although pBR322 is a multipurpose cloning vector, several plasmids with improved characteristics have been derived from it. In Fig. 3 a schematic outline of the construction of some of these derivatives is presented.

pBR327 (Soberón et al., 1980) is a deletion derivative of pBR322 lacking a 1089-bp fragment of non-essential DNA. Except for the loss of two unique restriction sites, the plasmid retains all the cloning properties of the parental plasmid; it is not mobilizable and has an elevated steady-state copy number (Covarrubias et al., 1981). Zurita et al. (1984) constructed a derivative of pBR327 in which the *par* locus of plasmid pSC101 was incorporated in order to insure efficient segregation of the plasmid into daughter cells. The nt sequence of *par* and its functions within the vector have been characterized and described by Meacock and Cohen (1980).

Other pBR322 derivatives have been constructed for the purpose of introducing a unique cloning site for the *Eco*RI endonuclease, so that detection of recombinants could be achieved by insertional inactivation of the Cm^R gene. For these constructions, the original *Eco*RI site from pBR322 was removed as shown in Fig. 3. pBR325 (Bolivar, 1978) was the first of the pBR-type, *Eco*RI cloning vectors to be constructed. Prentki et al. (1981) discovered later, using electron microscopy, that this vector forms a snap-back structure due to a 482-bp inverted duplication at the end of the Tc^R gene that originated during the construction of this vector. This duplication was also present in pBR328 (Soberón et al., 1980), a deletion derivative of pBR325 which has unique cloning sites for the restriction enzymes *Pvu*II and *Bal*I. To avoid unwanted recombinational events due to this duplicated region, pBR329 was generated by Covarrubias and Bolivar (1982). In this plasmid, transcription of the Cm^R gene is under the control of the promoter P1 (see Table II). Like pBR322, pBR329 is stable, small in size and easily propagated, and its complete nt sequence is known. It has the additional advantage of unique sites for cloning blunt-ended restriction fragments.

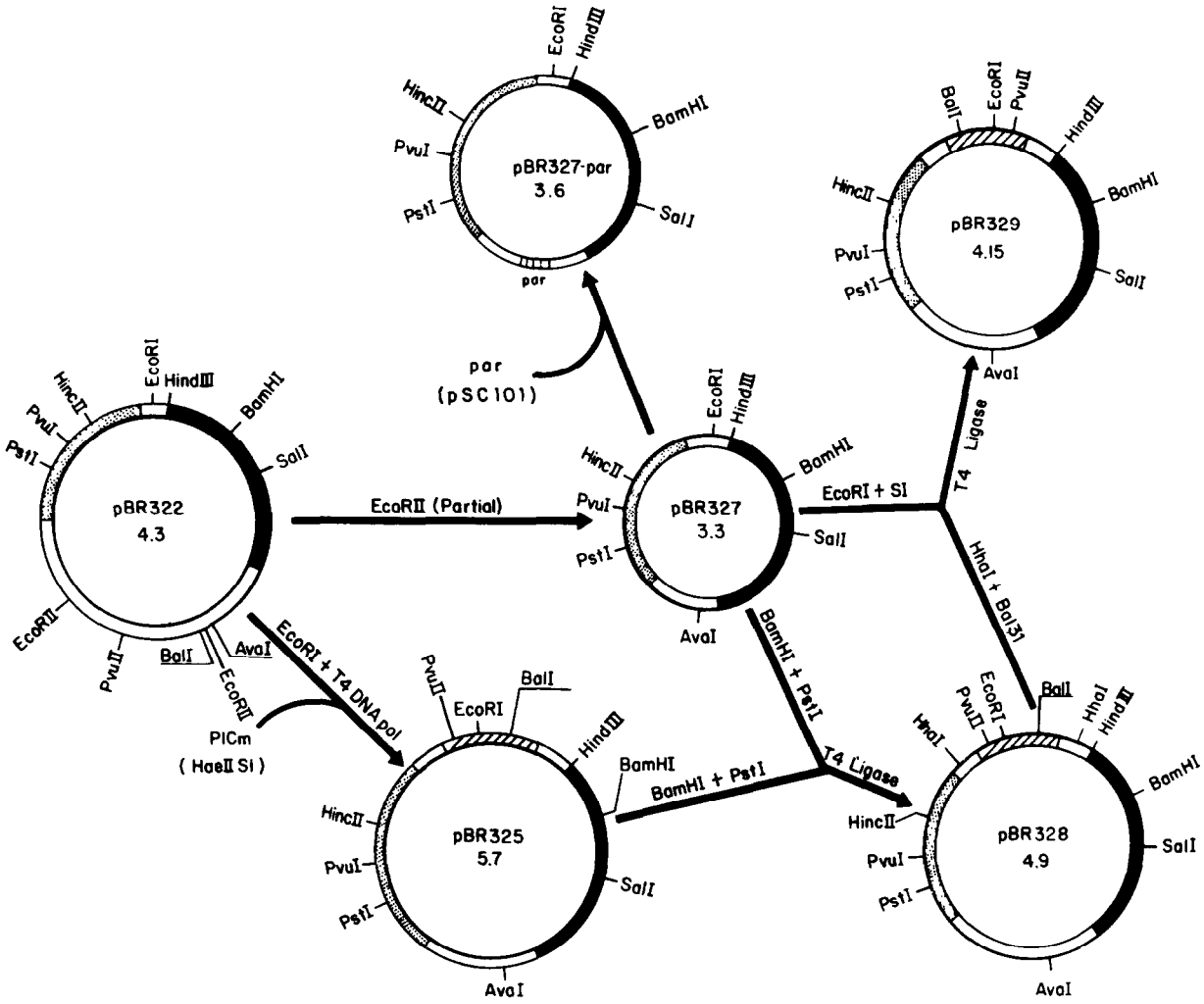

Fig. 3. The family of pBR322-derived vectors. Stippled segment, Ap^R gene. Black segment, Tc^R gene. Hatched segment, Cm^R gene. The size of the plasmid is given in kb. (Adapted from Rodriguez and Tait, 1983.)

COMMENTS ON THE Tc^R, Ap^R and Cm^R DETERMINANTS IN pBR322 AND ITS DERIVATIVES

The two antibiotic resistance determinants present in pBR322, Ap^R and Tc^R are coded from different strands. The 263 aa residues of the enzyme β-lactamase catalyse the hydrolysis of penicillin to penicilloic acids, which possess no antibiotic properties. The first 23 aa of β-lactamase form an hydrophobic leader peptide for the secretion of the active form of the enzyme into the periplasmic space of *E. coli* (Sutcliffe, 1979; Talmadge and Gilbert, 1980). The Tc^R determinant consists of one polypeptide, 399 aa long (Heusterspreute and Davison, 1983; Backman and Boyer, 1983). Its function is to prevent Tc from entering the cell (Franklin, 1967). In SDS-polyacrylamide gels, the Tc^R protein can exhibit anomalous migration rates, and can sometimes be mistaken for the active form of β-lactamase. The expression of both genes in pBR322 and its derivatives is constitutive.

The gene conferring the Cm^R phenotype in plasmids pBR325, pBR328 and pBR329 was isolated from the phage P1Cm. The enzyme CAT, in the presence of acetyl-S-CoA, catalyzes the formation of OH-acetoxy derivatives of Cm which lack any affinity to their target site, the bacterial ribosomes. Consequently, acetylated Cm does not inhibit the elongation of newly synthesizing polypeptides. The enzyme exists in solution as a tetramer of identical

subunits of 219 aa each. The expression of CAT is mediated by the levels of cAMP, and a five-fold increase in the production of CAT is observed when bacteria are grown using nonglucose carbon sources (Shaw, 1975; Shaw et al., 1979; Harwood, 1971; Le Grice, 1981).

SPECIAL PURPOSE VECTORS CONSTRUCTED FROM THE pBR VECTOR FAMILY

Although the pBR322 family of vectors was originally constructed for general cloning purposes, new derivatives have been designed to fulfill special-purpose cloning needs. The group of pBR-based plasmids consists of an extensive collection of specialized vectors summarized in this section. These vectors have been grouped into four major categories; expression vectors, vectors for the direct selection of recombinant plasmids, vectors for the analysis of transcriptional signals, and general utility vectors, which include improved pBR322 derivatives for multipurpose cloning. In this review, only those vectors constructed for general use have been compiled, while those constructed for the study of a specific gene or DNA fragment have been excluded.

(a) Expression vectors

The construction and use of expression vectors now permits the biosynthesis of virtually any kind of heterologous or foreign protein, in vivo. Since foreign gene expression was first achieved using recombinant DNA techniques, the need for versatile vectors with inducible and efficient regulatory DNA sequences was obvious. Expression of a gene can be achieved in two principal ways: by cloning a DNA cartridge containing the regions necessary for transcription upstream of the gene in question, or by cloning such a gene in an expression vector. The first experiments designed to overproduce foreign gene products involved the cloning of the synthetic genes coding for the hormones somatostatin (Itakura et al., 1977) and the insulin A and B chains (Goeddel et al., 1978) used the first approach. In those experiments the regulatory regions of *lac* and a large *lacZ* DNA fragment were cloned as an *Eco*RI cartridge upstream from the synthetic genes. Today, the second approach is more frequently utilized for the expression of foreign proteins. A variety of expression vectors are now available. These vectors incorporate diverse control signals for efficient transcription and sometimes translation of cloned messages, as well as coding sequences needed to generate hybrid proteins. Expression vectors can be divided into two major categories: vectors for the overproduction of proteins (wild type or fusion), and vectors for the expression of protein fragments.

Vectors for the overexpression of proteins have been grouped in this review according to the way in which the regulation of gene expression is manipulated in the laboratory, and the most commonly used plasmids are summarized in Table III (for a review of eukaryotic proteins expressed in *E. coli* see Harris, 1983).

Group 1: lac *promoter-operator vectors*

This group consists of those vectors that have the *lac* wild-type or *lac*UV5 mutant promoter-operator, as well as those synthetic or chimeric promoters that permit the *lac* operator to control expression. The regulation of transcription can be achieved in strains that overproduce the *lacI*-coded repressor ($lacI^Q$) by the addition of an inducer such as lactose or a synthetic analogue, IPTG. The most widely used vectors employing the *lac* promoter-operator are the pUC-plasmid series. These vectors allow the expression of proteins, provide a positive selection of recombinants and the potential for nt sequencing and site-directed mutagenesis of DNA fragments (also see Tables V and VI) (Vieira and Messing, 1982; Norrander et al., 1983; Hanna et al., 1984; Yanisch-Perron et al., 1985).

Podhajska et al. (1985) constructed some vectors with invertible *lac* and *tac* promoters, by placing these strong promoters in inverse orientation between divergent *att* sites, and mechanically inverting the promoter by a pulse of the Int protein (see also Szybalski et al., 1987).

Group 2: trp-*promoter vectors*

These vectors carry the promoter-operator-leader region of the *trp* operon of *E. coli*. Transcription from this region can be achieved by modulating the concentration of tryptophan in the culture media, as well as by the addition of chemical inducers (3-β-indoleacrylic acid or indol 3-propionic acid). Because complete induction of the system is difficult to achieve in

TABLE III

Expression vectors

Plasmids	Promoter operator	Markers	Cloning sites	Comments	References
Group 1. Expression vectors with promoters under control of the *lac* operator region					
pPCϕ series	*lac*	Ap^R	*Eco*RI	Three vectors. For fusion proteins with βGal. Three reading frames. 4.4 kb.	Charnay et al. (1978)
pLG series	*lac*	Ap^R	*Hin*dIII	Four vectors. For βGal fusions. Easy quantification of products. Promoter can be isolated as a cartridge.	Guarente et al. (1980)
pOP series	*lac*	Ap^R, Tc^R	*Eco*RI	Two vectors. Options for fusion and non-fusion proteins, with or without CAP site.	Fuller (1982)
pMC series pSK series pFR series	*lac*	Ap^R, Cm^R or Km^R	*Bam*HI, *Cla*I, *Pst*I, *Eco*RI, *Sac*I, *Sal*I, *Hin*dIII, *Sma*I, *Xba*I or *Xho*I	Nine vectors. For fusion proteins with βGal C-terminus and three reading frames 9.9–10.1 kb.	Shapira et al. (1983)
pUC8 series pUC9 series	*lac*	Ap^R	*Hin*dIII, *Pst*I, *Eco*RI, *Bam*HI, *Sma*I, *Sal*I	Six vectors, covering the 3 reading frames in both orientations. The XGal color reaction is retained.	Vieira and Messing (1982); Hanna et al. (1984)
pUC18 pUC19	*lac*	Ap^R	Same as pUC8 (+ *Sst*I, *Sph*I, *Xba*I, *Kpn*I)	Two vectors. 2.7 kb.	Norrander et al. (1983)
pIC19 series	*lac*	Ap^R	Same as pUC9 (+ *Bgl*II, *Xho*I, *Nru*I, *Cla*I, *Sac*I, *Eco*RV)	pUC9 derivatives containing a six US polylinker. 2.7 kb.	Marsh et al. (1984)
pIC20 series	*lac*	Ap^R	Same as pUC19 (+ *Bgl*II, *Xho*I, *Nru*I, *Cla*I, *Sac*I, *Eco*RV)	pUC19 derivatives containing a six US polylinker. 2.7 kb.	Marsh et al. (1984)
pICEM19 series	*lac*	Ap^R	Same as pEMBL8 (+ *Bgl*II, *Xho*I, *Nru*I, *Cla*I, *Sac*I, *Eco*RV)	pEMBL8 derivatives containing a six US polylinker. 2.7 kb.	Marsh et al. (1984)
pHG165	*lac*	Ap^R	Same as pUC8	Low-copy-number derivative of pUC8	Stewart et al. (1986)
pWR590	*lac*	Ap^R	*Sac*I, *Eco*RI, *Sma*I, *Hin*dIII, *Bam*HI, *Xba*I	A pUC18 derivative useful for βGal fusions.	Guo et al. (1984)
pIN.II series pIN.III series pIC.III series	*lpp-lac*	Ap^R	*Eco*RI, *Hin*dIII, *Bam*HI	27 vectors. Options for cloning in 3 reading frames, to synthezise fusion, secretable proteins. pIN.III carries *lacI* and pIC.III carries *lacZ*.	Masui et al. (1983) (1984); Nakamura and Inouye (1982)

(Table III, continued)

Plasmids	Promoter operator	Markers	Cloning sites	Comments	References
pNH7a	*lac* *tac*[a]	Ap^R	*Eco*RI, *Bam*HI	Requires host with inducible Int protein.	Podhajska et al. (1985); Szybalski et al. (1987)
pDR540	*tac*[a]	Ap^R	*Bam*HI	*tac* directs the expression of *galK* (from plasmid pKO-1). Promoter can be excised as a 92-bp fragment. 4 kb.	Russell and Bennet (1982)
pTac series	*tac*[a]	Ap^R	*Pvu*II, *Hin*dIII, *Eco*RI	Two vectors. For cloning of genes without RBS. The promoter can be isolated as a cartridge. 2.6–4.6 kb.	Amman et al. (1983)
pKK233-2	*tac*[a]	Ap^R	*Nco*I	Contains *lac* RBS and an ATG for translation initiation.	Amman et al. (1985)
pER series	*rac*	Ap^R	*Eco*RI, *Hin*dIII, *Cla*I	For fusions with βGal.	Boros et al. (1986)
pYEJ001	SCP	Ap^R	various	pBR327 derivative containing two tandem *lac* operators and a *Hin*dIII *cat* cartridge[b].	Rossi et al. (1983)
Group 2. Expression vectors with promoters under control of the *trp* promoter-operator region					
pTrpED series	*trp*	Ap^R, Tc^R	*Hin*dIII, *Sal*I, *Bam*HI, *Eco*RI	Three vectors, for fusion with *trpD*. Plasmids code for *trpE* and carry the attenuator. 6.7–9.8 kb.	Hallewell and Emtage (1980)
pWT series	*trp*	Ap^R, Tc^R	*Hin*dIII, *Sal*I, *Bam*HI	Three vectors, for fusion with *trpE*, allow cloning in 3 reading frames. Plasmids carry the attenuator. 4.8 kb.	Tacon et al. (1980)
pWT series	*trp*	Ap^R, Tc^R	*Hin*dIII, *Sal*I, *Bam*HI	Five vectors without the attenuator sequence. 3.7–3.8 kb.	Tacon et al. (1983)
pEP series pHP series	*trp*	Ap^R, Tc^R	*Hin*dIII, *Bgl*II, *Sst*I	Seven vectors derived from pBR313 or pBR345. Insertional inactivation of the *trp* structural genes. 7.9–18 kb.	Enger-Valk et al. (1980)
pTrpL1	*trp*	Ap^R	*Cla*I	Carries *trpL* RBS. 4.6 kb.	Edman et al. (1981)
pSTP1	*trp*	Ap^R, Tc^R	*Cla*I, *Sal*I, *Taq*I, *Hin*dIII, *Bam*HI	Carries a synthetic *trp* promoter with RBS. 3.72 kb.	Windass et al. (1982)
pDR720	*trp*	Ap^R	*Bam*HI, *Sal*I, *Sma*I	*trp* promoter directs the expression of *galK* (from pKO-1). Promoter can be exised as a 77-bp *Eco*RI fragment. 4 kb.	Russell and Bennett (1982)

pER103	*trp*	Ap^R, Tc^R	*Hin*dIII	Carries a synthetic promoter-operator and RBS. Regulatory region can be isolated as a cartridge.	Dworkin-Rastl et al. (1983)
pKYP series	*trp*	Ap^R, Tc^R	*Hin*dIII, *Sal*I, *Bam*HI, *Cla*I	Four vectors with 2 or 3 tandem *trp* promoters and RBS. Plasmids carry *lpp* transcriptional terminator. The promoter can be isolated as cartridge. 3.7–9.35 kb.	Nishi et al. (1984)
Group 3. Expression vectors with temperature-inducible transcription					
pHUB series	p_L	Km^R, Ap^R	*Eco*RI, *Bam*HI, *Sal*I, or *Hpa*I	Two vectors, with or without λ antiterminator gene *N*. 7.6–6.5 kb.	Bernard et al. (1979)
pLc series pLa series	p_L	Ap^R	*Bal*I, *Eco*RI, *Pst*I, *Bam*HI, *Sal*I, *Acc*I or *Hin*dIII	Five vectors that allow cloning of genes with their own traductional initiation signals, or fusion proteins with MS2pol or *bla*. 2.8–3.8 kb.	Remaut et al. (1981)
pJL6	p_L	Ap^R	*Cla*I, *Hin*dIII, *Bam*HI	Codes for the N-terminus of λ *c*II gene, and carries an efficient traductional initiation signal.	Oppenheim et al. (1982)
pAS1	p_L	Ap^R	*Bam*HI	Allows direct fusion of any coding sequence to the λ *c*II translational initiation signal. 5.8 kb.	Shatzman et al. (1983)
pKC series	p_L	Ap^R	*Bam*HI, *Ava*I	Four vectors containing λ *N* gene.	Rao (1984)
pFCE4	p_L	Ap^R	*Bam*HI	Two vectors containing F1 encapsidation origin, $o_L p_L$, *nut*L, *nut*R, *t*, λ *c*II and RBS. Useful for DNA sequencing.	Lorenzetti et al. (1985)
pEV-vrf series	p_L	Ap^R	*Eco*RI, *Bam*HI, *Cla*I	Three vectors for expression in 3 reading frames. They contain RBS.	Crowl et al. (1985)
pEMBLex2	p_L	Ap^R	*Sal*I, *Eco*RI, *Hin*dIII	Contains RBS of MS2 replicase and *lacZ*. 4.4 kb, pEMBL8 derivative.	Sollazzo et al. (1985)
pANH-1	p_L	Ap^R	*Hpa*I	For nonfusion protein synthesis.	Seth et al. (1986)
pANK-12	p_L	Ap^R	*Kpn*I	For nonfusion protein synthesis	Seth et al. (1986)
pPL2	p_L	Ap^R	*Bam*HI	For nonfusion protein synthesis	Seth et al. (1986)
pCQV2	p_R	Ap^R	*Bam*HI	Contains an ATG codon as a translation start codon.	Queen (1983)
pEMBLex3	p_R	Ap^R	*Bam*HI, *Sal*I, *Pst*I	Contains RBS of λ *cro* and *lacZ*. 4.9 kb. pEMBL8 derivative.	Sollazzo et al. (1985)

(Table III, continued)

Plasmids	Promoter operator	Markers	Cloning sites	Comments	References
pEX series	p_R	Ap^R	*Pst*I, *Sal*I, *Bam*HI, *Sma*I, *Eco*RI	ORF DNA expression vectors for fusion proteins with λ Cro and βGal	Stanley et al. (1984)
pCL series	p_R	Ap^R	*Bam*HI	ORF DNA supression vectors for fusion proteins with λ Cro and βGal	Zabeau and Stanley (1982)
Group 4. Expression vectors with constitutive promoters					
pSPA series	*P* protA	Ap^R, Tc^R	*Eco*RI, SmaI, *Sal*I, *Acc*I, *Bam*HI, *Pst*I, or *Hin*cII	Two vectors for fusion with the staphylococcal protein A. 6–7.5 kb.	Uhlen et al. (1983)
pJP1	T5 P25	Ap^R, Tc^R	*Hin*dIII	Contains synthetic early T5 promoter instead of the Tc^R promoter. 4.6 kb.	Rommens et al. (1983)
pIN.I series	p_{LPP}	Ap^R	*Eco*RI, *Hin*dIII, or *Bam*HI	Nine vectors. Options for cloning in any reading frame, to synthezise fusion proteins, secretable proteins. 4.9 kb.	Nakamura and Inouye (1983); Masui et al. (1983)
pXJ002	SCP	Ap^R, Cm^R	*Hpa*II, *Bam*HI, *Sal*I, *Pst*I	pBR325 derivative conferring strong heparin resistance.	Rossi et al. (1983)
pY2	SCP	Ap^R, Tc^R, Cm^R	various	pBR327 derivative carrying a *cat* gene cartridge[b]	Rossi et al. (1983)
pSP series	SP6	Ap^R	*Eco*RI, *Sac*I, *Sma*I, *Bam*HI, *Xba*I, *Sal*I, *Pst*I, *Hin*dIII	Contains the promoter of phage SP6. For ssRNA synthesis.	Melton et al. (1984); Sollozzo et al. (1985)
pORF series	OmpF	Ap^R	*Bgl*II, *Sma*I, *Xma*I, *Ava*I, *Sal*I, *Bam*HI, *Pst*I	ORF DNA expression vectors for fusion proteins with OmpF and βGal.	Weinstock et al. (1983)

[a] The *tac* promoter was originally constructed by De Boer et al. (1982).

[b] Close and Rodriguez (1982).

wild-type cells, Trp$^-$ mutants are generally utilized. A number of vectors carrying different segments of the *trp* operon are available (Enger-Valk et al., 1980), and *trpD* fusion proteins exhibit good stability in *E. coli* (Hallewell and Emtage, 1980).

Group 3: Vectors employing promoter-operators with temperature-sensitive control

This group includes those vectors containing the bacteriophage λ leftward (p_L) and rightward (p_R) promoters. Vectors with such promoter-operators work only in conjunction with λ repressor-coding gene containing the *c*Its857 mutation. Chemical induction with nalidixic acid (or UV induction) of the λ $p_L o_L$ promoter-operator has also been employed (Mott et al., 1985), when the repressor was coded by the cI^+ (ind^+) gene. Remaut et al. (1981) constructed the early set of expression vectors with the λ p_L promoter inserted in pBR322.

Group 4: Vehicles with constitutive promoters

Expression from these promoters cannot be regulated. The most widely used system with constitutive expression is the β-lactamase gene from pBR322. Expression vectors carrying strong, nonregulated promoters may be lethal (T5).

Open reading frame DNA cloning and expression vectors provide a general method for the identification of coding fragments from structural genes when antibodies against the protein are available. Besides being a useful way to screen genomic and cDNA libraries, this has also proven to be an interesting approach for antigen production in *E. coli* (Pilacinski et al., 1984; Weis et al., 1983; Gray et al., 1982; Rüther and Muller-Hill, 1982; Masui et al., 1983; Weinstock et al., 1983; Stanley and Luzio, 1984; Zabeau and Stanley, 1982). These vectors are described in Table III according to the promoter carried on the vector.

(b) Vectors for the identification of regulatory signals

These vectors are useful for the identification of transcriptional signals such as promoters and terminators. Promoter probe vectors have two general components: a transcriptionally silent structural gene lacking the regulatory signal and one or several restriction sites for the insertion of the DNA to be tested. Cloning a promoter in the correct orientation expresses the structural gene, usually an antibiotic resistance protein or an auxotrophy complementation product. The Tc^R gene in pBR322 has been extensively used for this type of vector since the *Hin*dIII site interrupts the Tc^R promoter (Widera et al., 1978; Neve et al., 1979; Rodriguez et al., 1979). A list of such vectors is presented in Table IV, group A. Plasmid pKO-1, which employs the galactokinase gene from *E. coli*, has been extensively used for promoter cloning and analysis of promoter strength (McKenney et al., 1981).

Vectors for the isolation and characterization of transcription terminators are also available, and they are presented in Table IV, group B. These vectors utilize the insertional inactivation of the plasmid marker gene by blocking transcription rather than translation. DNA fragments containing terminator sequences will block expression of the marker gene when inserted in the correct orientation, between the promoter and translation start site of that gene. In some vectors, the absence of the marker gene product can be easily detected as an antibiotic-sensitive phenotype, while in others, the marker gene product is lethal to the host cells. The presence of terminator fragments in these vectors allow for increased cell viability (Brosius, 1984b).

While a number of vectors are available for the cloning and characterization of prokaryotic promoters and terminators, a small number of vectors exist for the study of antiterminators (de Crombrugghe et al., 1979; Drahos and Szybalski, 1981; Drahos et al., 1982; Luk and Szybalski, 1982; Somasekhar and Szybalski, 1983; Peltz et al., 1985) and of ribosome-binding sites (Casadaban et al., 1980; Lin et al., 1985).

(c) Direct selection vectors

Insertional inactivation of a selective marker of pBR322 is an efficient way to screen for recombinant DNA molecules, but it can be time-consuming. For this reason, vectors that allow for the positive selection of recombinant clones have been developed. These vectors can be grouped into two categories:

(1) Plasmids coding for a lethal phenotype

In this case, cells harboring hybrid DNA molecules can survive on special selective media, while

TABLE IV

Vectors for the detection of transcriptional signals

Plasmids	Probe assay	Markers	Cloning sites	Comments	References
(A) Promoter probe vectors					
pBRH series	Tc^R expression	Ap^R colicin $E1^{imm}$	*Eco*RI	Three vectors derived from pBR316. 4.2–7.8 kb.	West et al. (1979)
pPV33	Tc^R expression	Ap^R	*Eco*RI	Carries termination codons in 3 reading frames before Tc^R gene. 4.3 kb.	West and Rodriguez (1982)
pBRS188	Tc^R expression	Ap^R	*Eco*RI	4.3 kb	Rechiniskii et al. (1982)
pJR011	Tc^R expression	Ap^R	*Eco*RI, *Hin*dIII	3.2 kb. pBR327 derivative	Soberón et al. (1982)
pKEN005	Tc^R expression	Ap^R	*Eco*RI	3.2 kb.	Masui et al. (1983)
pKK175-6	Tc^R expression	Ap^R	*Eco*RI, *Hin*dIII, *Sal*I, *Pst*I, *Sma*I, *Bam*HI	Carries ribosomal transcription terminators after Tc^R gene. 4.4 kb.	Brossius (1984a)
pKK231-1	Cm^R expression	Ap^R	*Eco*RI, *Hin*dIII, *Sal*I, *Pst*I, *Sma*I, *Bam*HI	Carries ribosomal transcription terminators after Cm^R gene. 5.1 kb.	Brossius (1984a)
pEP series	*trpA* expression	Ap^R	*Eco*RI, *Hin*dIII, *Hpa*I, *Aos*I, *Sst*I, *Bgl*II	Four vectors derived from pBR313. Strain requirement *trpA* $^-$. 8.2–9.2 kb.	Enger-Valk et al. (1981a)
pKO-1	*galK* expression	Ap^R	*Eco*RI, *Hin*dIII, *Sma*I	Strain requirement: $galE^+T^-K^-$ or $galE^-T^-K^-$. Carries termination codons in 3 reading frames before *galK*, and *gal* leader. 3.9 kb.	McKenney et al. (1981)
pKO-2 pKM-2	*galK* expression	Ap^R	*Eco*RI, *Hin*dIII, *Cla*I, *Sac*I, *Xho*I, *Xba*I	Derivatives of pKO-1. Plasmid pKM-2 is suitable for cloning strong promoters.	de Boer (1984)
pCB series	*lacZ* expression *galK* expression	Ap^R	*Sma*I, *Bam*HI, *Sal*I, *Pst*I, *Xba*I, *Hin*dIII	For the analysis of divergent control regions.	Schneider and Beck (1986)
pMC1403	*lacZ* expression	Ap^R	*Eco*RI, *Sma*I, *Bam*HI	Carries *lacZ,Y* without translation initiation signals.	Casabadan et al. (1980)
pNRC series	*lacZ* expression	Ap^R	*Eco*RI	Derivative of pMC1403 (pBR322). Carries ATG codons in 2 reading frames	Thomas et al. (1982)
pNM series	*lacZ* expression	Ap^R	*Eco*RI, *Hin*dIII, *Bam*HI, *Sal*I, *Acc*I, *Pst*I, *Sma*I	Three vectors. Derivatives of pMC1403. High copy number. Fusion of genes in 3 reading frames.	Minton (1984)

pKM005	*lacZ* expression	Ap^R	*Xba*I	Host strain requirement: *lacZ*$^-$. 9.9 kb.	Masui (1983)
pTL25	*lacZ* expression	Ap^R	*Hin*dIII, *Xba*I, *Xho*I, *Bam*HI	pBR325 derivative. Transcription of *lacZ* is independently translated from the hybrid DNA.	Linn and Ralling (1985)
(B) Terminator probe vectors					
pBdCl	Decrement of Tc^R	Ap^R	*Hin*dIII	5.3 kb.	de Crombrugge et al. (1979)
pEP series	Decrement of Tc^R	*trpE*	*Hin*dIII, *Pst*I, *Kpn*I	Host strain requirement: *trpE*$^-$. Transcription initiates in *trp* promoter; Tc^R is fused to *trpE*, so cloning between these genes scans for terminators. Derivatives of pBR345. 7.2 kb.	Enger-Valk et al. (1981b)
pKG-1800	Avoid *galK* expression	Ap^R	*Hin*dIII, *Sma*I	Host strain requirement: *galE*$^+$ *T*$^-$ *K*$^-$ or *galE*$^-$ *T*$^-$ *K*$^-$. Transcription initiates in *gal* promoter. 4 kb.	McKenney et al. (1981)
pKK series	Avoid insulin overexpression	Ap^R	*Eco*RI	Transcription initiates in the ribosomal RNA promoter. 7 kb.	Brosius (1984b)

TABLE V

Plasmid vectors for the direct selection of recombinants

Plasmid	Selection	Markers	Cloning sites[a]	Comments	References
Group 1. Plasmids coding for a lethal phenotype					
pTR262	Tc^R	—	*Hin*dIII, *Bcl*I	Inactivation of λ repressor gene. 4.8 kb	Roberts et al. (1980)
pUN121	Tc^R	Ap^R	*Hin*dIII, *Bcl*I, *Eco*RI, *Sma*I, *Xma*I	Inactivation of λ repressor gene. (pTR262 derivative) 4.4 kb	Nilsson et al. (1983)
pNS1	Tc^R	Ap^R	*Stu*I, *Nde*I, *Hpa*I, *Hin*dIII, *Eco*RI, *Asu*I	pBR327 derivative	Nikolnikov et al. (1984)
pNO1523	Sm^R	Ap^R	*Hpa*I, *Sma*I	Requires a *rps*L/(StrA) host strain. Expression is under *Str* promoter. 5.2 kb	Dean (1981)
pHSG664	Sm^R	Ap^R	*Eco*RI, *Hpa*I, *Pvu*II, *Sph*I, *Pst*I, *Sma*I	pNO1523 derivative.	Hashimoto-Gotoh et al. (1986)
pAA3	Galactose^R	Ap^R	*Hin*dIII	Requires *galK*$^-$ strain.	Ahmed (1984)
pHA10	Survival to Kil function of λ	Ap^R	*Sal*I	Expression under p_L. 11.4 kb	Honigman and Oppenheim (1981)
pKL1	Survival to Kil function of λ	Ap^R	*Sal*I	Carries λ *cos* region. Packing into λ capsid. 13.5 kb	Honigman and Oppenheim (1981)
pLV57	Loss of lethal *Eco*RI endonuclease	Ap^R, Cm^R	*Bgl*II, *Hin*dIII, *Pst*I	Lethality at 37°C of nonrecombinants. 6.1 kb	O'Connor and Humphreys (1982)
pSCC31	Loss of lethal *Eco*RI endonuclease	Ap^R	*Bgl*II, *Hin*dIII	Propagation of the vector requires a functional methylase gene. Expression under λ p_L. 5.15 kb.	Cheng and Nordrich (1983)
pVT25	Loss of lethal ColE3 gene	Ap^R	*Cla*I, *Bgl*I	ColE3 promoter may express genes cloned in frame	Vernet et al. (1985)
pLA7	5-fluorouracil + 5-AMP resistance	Ap^R	*Bcl*I	Requires *upp*$^-$, *ush*$^-$ recipients. 4.1 kb	Burns and Beachman (1984)
Group 2. Vectors coding for the βGalα activity					
pUR2	lac$^+$	Ap^R	*Eco*RI	Requires *lacZ*$^-$ host strain.	Rüther et al. (1980)
pUC8 series pUC9 series	lac$^+$	Ap^R	*Pst*I, *Sma*I, *Eco*RI, *Bam*HI, *Sal*I, *Hin*dIII	Requires hosts allowing α-complementation for βGal.	Vieira and Messing (1982); Hanna et al. (1984)
pUC18 pUC19	lac$^+$	Ap^R	Same as pUC8 (+ *Sph*I, *Xba*I, *Kpn*I, *Sst*I)	Requires hosts allowing α-complementation for βGal.	Norrander et al. (1983)

pBS series	lac$^+$	Km^R, Tc^R	Same as pUC8-*Acc*I	Same as pUC8. 3.6-4 kb	Spratt et al. (1986)
pEMBL series	lac$^+$	Ap^R	Same as pUC8	Two vectors containing the intergenic region of phage F1 for encapsidation.	Dente et al. (1983)
pBS +/− series	lac$^+$	Km^R, Tc^R	Same as pEMBL8	Same as pEMBL8	Spratt et al. (1986)
pIC19	lac$^+$	Ap^R	Same as pUC9 (+ *Bgl*II, *Cla*I, *Xho*I, *Nru*I, *Sac*I, *Eco*RV)	Same as pUC plasmids.	Marsh et al. (1984)
pIC20	lac$^+$	Ap^R	Same as pUC19 (+ *Bgl*II, *Cla*I, *Xho*I, *Nru*I, *Sac*I, *Eco*RV)	Same as pUC plasmids.	Marsh et al. (1984)
pICEM19 series	lac$^+$	Ap^R	Same as pEMBL9 (+ *Nru*I, *Bgl*II, *Xho*I, *Cla*I, *Sac*I, *Eco*RV)	Same as pEMBL8	Marsh et al. (1984)
pHG165	lac$^+$	Ap^R	Same as pUC8	Same as pUC8.	Stewart et al. (1986)

[a] Only those sites allowing direct selection of recombinants are noted.

[b] The Lac$^+$ phenotype appears unstable (Hasan et al., 1986).

the non-recombinant cells cannot. A list of these positive selection vectors derived from pBR322 is given in Table V, group A. A brief summary of their most important characteristics is also included (for a review of other positive selection vectors not derived from pBR322 see Burns and Beacham, 1984).

(2) Vectors based on the α-complementation of β-galactosidase activity

Cells containing these plasmids hydrolyze a chromogenic substrate (XGal) to give blue colonies. Cloning onto this fragment coding for the α-donor abolishes the enzymatic activity and the recombinants produce white colonies (Table V, group B). The pUC series and its derivatives (Vieira and Messing, 1982; Norrander et al., 1983; Hanna et al., 1984; Yanisch-Perron et al., 1985), contain a polylinker with multiple restriction sites, thus providing greater cloning possibilities. The main drawback in the use of the pUC vectors is that the only host strains capable of α-complementation can be used with these vectors.

(d) Utility vectors

This section will compile a series of vectors derived from pBR322 for a variety of cloning purposes (Table VI). A few examples of each kind will be noted and their usefulness will be briefly discussed.

(1) Modified pBR322 vectors with additional restriction sites

These vectors were constructed with the aim of making the cloning procedure of a DNA segment straightforward, thus eliminating the need for further manipulation of the fragment to be cloned. They consist of two basic types: vectors with unique sites for the cloning of blunt-ended fragments (Prentki and Krisch, 1982); and vectors with extra sites for the cloning of fragments with 3′-cohesive end fragments (Beckingham, 1980). The plasmid plink322 is a pBR322 derivative with a polylinker that increases the number of useful cloning sites (Seed, referred to in Maniatis et al., 1982). In addition to its 28 unique cloning sites, the plasmid pJRD158 has the added features of high copy number and altered incompatibility due to a mutation in the RNAI region (Davison et al., 1984b; Heusterspreute and Davison, 1984).

(2) Vectors with different and improved selective markers

Some of these vectors encode antibiotic resistance markers such as Km (Rao and Rogers, 1979; Kahn et al., 1979), Neo (Richardson et al., 1982) and Thio (Richardson et al., 1982). In plasmid pDR42 (Herrin et al., 1982) the tryptophan promoter has been inserted in the *Cla*I site of pBR322 to confer higher levels of Tc^R. The increased Tc^R expression permits a better selection of Tc^R transformants on fusaric acid.

(3) Vectors with increased stability

A variety of systems have been designed to minimize plasmid loss due to inefficient segregation in large-scale continuous fermentations. The introduction of partition sequences has been used successfully. The *par* locus from pSC101 (Skogman et al., 1983; Zurita et al., 1984) and *cer* from ColE1 (Summers and Sherrat, 1984; M. Zurita, H. Lomeli, M.E. Munguia and X. Soberón, submitted) are some examples. Other vector systems introduce selective pressure to help maintain recombinant pBR322-based plasmids in the cell (Rosteck and Hershberger, 1983; Skogman and Nilsson, 1984; Miwa et al., 1984).

(4) Derivatives of pBR322 with an altered copy number

As stated earlier, pBR322 is present in an average of 18 copies per cell. The usefulness of high copy number derivatives is clear, first, because no amplification is necessary for higher yields of plasmid DNA, and second, because high gene dosage usually leads to elevated levels of protein synthesis. Deletion derivatives of pBR322 lacking the *rop* gene have been constructed (Soberón et al., 1980; Twigg and Sherrat, 1980; D. Hanahan, in Maniatis et al., 1982). In these plasmids, the copy number per chromosome is about 30 copies. Plasmids carrying mutations in the DNA replication region that affect copy number have also been described (Davison et al., 1983; Boros et al., 1984), as well as plasmids with an inducible copy number (Larsen et al., 1984; Yarranton et al., 1984).

(5) Plasmids for DNA sequencing

These modified pBR322 plasmids simplify nt sequencing for either the enzymatic or the chemical methods. For the Maxam and Gilbert technique,

large quantities of small, highly purified DNA segments are required. Plasmids pUR222 (Rüther et al., 1981), pUR250 (Rüther, 1982), and pHP34 (Prentki and Krish, 1982) have been developed to overcome the difficulties of cloning and recovering the segment to be sequenced, by adding specially designed cloning sites.

For the sequencing procedure described by Sanger et al. (1977), Wallace et al. (1981) have synthesized a set of oligodeoxyribonucleotide primers for the direct sequencing of DNA in pBR322 and some of its derivatives. To improve the efficiency of the sequencing by this method, Hayashi (1980) constructed a pBR322 derivative with poly(dA), poly(dT) tails in the *Pvu*II site to allow strand separation and easy hybridization of primers. Zagursky and Berman (1984) constructed three derivatives inserting the M13 origin and intergenic region in pBR322. Upon infection with M13, the viral origin of replication present in the plasmids permits phage-directed plasmid replication and results in a high yield of ss plasmid DNA in M13-like particles. These ssDNAs are useful as substrates for rapid sequencing by the Sanger's dideoxy method.

Vieira and Messing (1982) have constructed a series of plasmids (pUC) utilizing the multiple cloning site of the M13mp7 system for sequencing of DNA fragments with the universal M13 primers, and a series of improved pUC vectors are now available (Norrander et al., 1983; Hanna et al., 1984); some have been completely sequenced (Yanisch-Perron et al., 1985). Other derivatives of the pUC vectors contain the intragenic region of a filamentous phage such as F1, Ff or 1Ke, so the plasmids are encapsidated as ssDNA upon superinfection with phage. They are useful for the sequencing of long DNA inserts in *E. coli* (Dente et al., 1983; Zinder et al., 1984; Baldari and Cesareni, 1985; Spratt et al., 1986; Peeters et al., 1986).

(6) Hybrid vectors with phage DNA

These vectors include phasmids, cosmids and other chimeras with phage DNA. The cosmid systems are particularly useful for the cloning of large fragments of DNA (>35 kb), which is particularly important for the construction of genomic DNA libraries. Cosmids contain the cohesive ends (*cos*) of bacteriophage λ, and clones may be efficiently packed into λ capsids. Phasmids are λ vectors containing the *ori* of a phage and the *ori* of a plasmid, so they may replicate either as a phage or as a plasmid in the host cell. These vectors do not allow the cloning of large DNA fragments, so their utility is restricted. Phage-pBR322 chimeras are summarized in Table VI.

The vector-host systems constructed by Howard and Gottesman (1983) and Gaitanaris et al. (1986) permit the propagation of the recombinant molecules either in an extrachromosomal manner or as a single copy, integrated in the *E. coli* chromosome at the λ *att* site.

(7) Plasmids for the secretion of proteins

A variety of proteins are exported into the periplasmic space in *E. coli* by virtue of a signal peptide placed at the N-terminal end of the protein. Since β-lactamase is a secreted protein, pBR322 has been used for the construction of hybrid-secretion proteins (Villa-Komaroff et al., 1980). In some cases, like rat insulin, antigen secretion and processing was achieved (Talmadge and Gilbert, 1980). The same was demonstrated for the immunoglobulin light chain (Zemel-Dreasen and Zamir, 1984). However, it has been demonstrated that the presence of the leader peptide is not sufficient to accomplish transport of all proteins through the membranes (Bassford et al., 1979; Ito et al., 1981).

(8) Gene-fusion vectors for the rapid purification of proteins

These vectors will allow fusion of any gene to an easily purified plasmid-encoded gene, thus facilitating the isolation of the hybrid protein. Vectors for general use have been constructed for the biosynthesis of hybrid proteins with β-galactosidase (Germino and Bastia, 1984) and staphylococcal protein A (Uhlen et al., 1983; Nilsson et al., 1985). Other fusion proteins that have been successfully purified include repressor molecules such as those coded by genes *exuR* and *cI* (Mata-Gilsinger and Ritzenthaler, 1983; Flores et al., 1986) and βGal (Rüther and Müller-Hill, 1984). Some vectors have been designed that allow the specific liberation of the peptide of interest by enzymatic cleavage of aa residues (Sassenfeld and Brewer, 1984; Nagai and Thogersen, 1984).

TABLE VI

Utility vectors derived from pBR322

Plasmids	Markers	Reported restriction sites[a]	References
Multipurpose cloning vectors			
pAT153	Ap^R, Tc^R	Same as pBR322 (– *Pvu*II, *Sna*I)	Twigg and Sherrat (1980)
pBR322	Ap^R, Tc^R	*Eco*RI, *Cla*I, *Hin*dIII, *Eco*RV, *Nhe*I, *Bam*HI, *Sph*I, *Sal*I, *Xma*III, *Nru*I, *Bsm*I, *Sty*I, *Ava*I, *Bam*I, *Pvu*II, *Sna*I, *Nde*I, *Afl*III, *Pst*I, *Pvu*I, *Sca*I, *Ssp*I, *Aat*II	Bolivar et al. (1977b)
pBR325	Ap^R, Tc^R, Cm^R	Same as pBR322 (+ *Nco*I, *Mst*I)	Bolivar (1978)
pBR327	Ap^R, Tc^R	Same as pBR322 (– *Bal*I, *Pvu*II)	Soberón et al. (1980)
pBR327-par	Ap^R, Tc^R	Same as pBR327 – *Ava*I	Zurita et al. (1984)
pBR328	Ap^R, Tc^R, Cm^R	Same as pBR325 (+ *Bal*I, *Pvu*II)	Soberón et al. (1980)
pBR329	Ap^R, Tc^R, Cm^R	Same as pBR328 – *Asu*I	Covarrubias and Bolivar (1982)
pDR42	Ap^R, Tc^R	Same as pBR322 – *Cla*I	Herrin et al. (1982)
pFJ164	Ap^R, $Thio^R$	ND	Richardson et al. (1982)
pFJ165	Ap^R, Neo^R	Same as pBR322 + *Kpn*I	Richardson et al. (1982)
pJRD158	Ap^R, Tc^R	Same as pBR327 (+ *Sac*I, *Bss*HII, *Kpn*I, *Bcl*I, *Mlu*I, *Bgl*II, *Asu*II, *Xba*I, *Xho*I) (– *Hin*fIII, *Aat*I, *Ssp*I, *Afl*III, *Nde*I, *Sna*I, *Nhe*I).	Davison et al. (1984) Heusterspreute and Davison (1984)
pKB111	Ap^R	Same as pBR322 + *Kpn*I	Beckingham (1980)
pKC7	Ap^R, Km^R	*Eco*RI, *Hin*dIII, *Bam*HI, *Bgl*III, *Pvu*II, *Bcl*I, *Xho*I, *Cla*I, *Sma*I	Rao and Rogers (1979)
plink322	Ap^R	Same as pBR322 (+ *Xba*I, *Bgl*II) (– *Nhe*I, *Sph*I, *Eco*RV)	Seed B., in Maniatis et al. (1982)
pMK2004	Ap^R, Tc^R, Km^R	*Sma*I, *Eco*RI, *Bam*HI, *Sal*I, *Pst*I, *Xho*I	Kahn et al. (1979)
pXf3	Ap^R, Tc^R	Same as pBR322 (– *Bsm*I, *Sty*I, *Ava*I, *Bal*I, *Pvu*II)	Hanahan, in Maniatis et al. (1982)
Copy number derivatives of pBR322			
pAT153	see above	see above	Twigg and Sherrat (1980)
pBR327	see above	see above	Soberón et al. (1980)
pBR327-par	see above	see above	Zurita et al. (1984)
pKC16	Ap^R	*Bam*HI	Rao and Rogers (1978)
pHG165	Ap^R	Same as pUC8	Stewart et al. (1986)
pMG411	Ap^R	*Nde*I, *Sma*I, *Tth*111I, *Bal*I, *Ava*I, *Bst*EII	Yarranton et al. (1984)
pOU series	Ap^R	ND	Larsen et al. (1984)
Vectors with increased stability			
pBR327-par	Ap^R, Tc^R	Same as pBR327 – *Ava*I	Zurita et al. (1984)
pSGS series	Ap^R, *trpABCDE*	ND	Skogman et al. (1983)

(Table VI, continued)

Plasmids	Markers	Reported restriction sites[a]	References
Vectors for sequencing of DNA			
pBS series	Tc^R, Km^R	Same as pUC8 (see below) – *Acc*I	Spratt et al. (1986)
pBS +/− series	Tc^R, Km^R	Same as pEMBL8 (see below)	Spratt et al. (1986)
pEMBL series	Ap^R	Same as pUC8 (see below)	Dente et al. (1983)
pHP34	Ap^R, Tc^R	Same as pBR322 + *Sma*I	Prentki and Kirsh (1982)
pIC19	Ap^R	Same as pUC9 (see below) (+ *Bgl*II, *Cla*I, *Xho*I, *Nru*I, *Sac*I, *Eco*RV)	Marsh et al. (1984)
pIC20	Ap^R	Same as pUC19 (see below) (+ *Bgl*II, *Cla*I, *Xho*I, *Nru*I, *Sac*I, *Eco*RV)	Marsh et al. (1984)
pICEM19 series	Ap^R	Same as pEMBL8 (+ *Bgl*II, *Cla*I, *Xho*I, *Nru*I, *Sca*I, *Eco*RV)	Marsh et al. (1984)
pKH47	Ap^R, Tc^R	Same as pBR322	Hayashi et al. (1980)
pKUN9	Ap^R	Same as pUC9 (see below)	Peeters et al. (1986)
pPH125	Tc^R, Km^R	*Eco*RI, *Bam*HI, *Eco*RV, *Bal*I, *Pvu*II, *Sal*I, *Sma*I, *Xho*I	Spratt et al. (1986)
pHP126	Tc^R, Km^R	*Eco*RI, *Bam*HI, *Eco*RV, *Sal*I, *Sma*I, *Xho*I	Spratt et al. (1986)
pUC8 series pUC9 series	Ap^R	*Pst*I, *Sma*I, *Eco*RI, *Bam*HI, *Sal*I, *Hin*dIII	Vieira and Messing (1982); Hanna et al. (1985)
pUC18 pUC19	Ap^R	Same as pUC8 (+ *Sph*I, *Xba*I, *Kpn*I, *Sst*I)	Norrander et al. (1983)
pUR222	Ap^R	*Pst*I, *Sal*I, *Acc*I, *Hin*dIII, *Bam*HI, *Eco*RI	Rüther et al. (1981)
pUR250	Ap^R	*Hin*dIII, *Xba*I, *Sal*I, *Acc*I, *Hin*cII, *Bam*HI, *Eco*RI	Rüther (1982)
pZ series	Ap^R, Tc^R	Same as pBR322	Zagursky and Berman (1984)
Vectors for protein secretion			
pIN.III.ompA	Ap^R	*Eco*RI, *Hin*dIII, *Bam*HI	Ghrayeb et al. (1984)
pKT series	Tc^R	*Pst*I	Talmadge and Gilbert (1980)
Vectors for the biosynthesis of easily purifiable proteins			
pJG201	Ap^R	*Bam*HI	Germino and Bastia (1984)
pSPA series	Ap^R, Tc^R	*Pst*I, *Sal*I, *Bam*HI, *Eco*RI	Uhlen et al. (1983)
Phasmids and cosmids			
pDS series	Ap^R	ND	Feiss et al. (1982)
pGNC	Ap^R, HSV TK	*Sal*I, *Bam*HI, *Cla*I	Grosveld et al. (1982)
pHC79	Ap^R, Tc^R	*Eco*RI, *Cla*I, *Bam*HI, *Sal*I, *Eco*I, *Pst*I	Hohn and Collins (1982)
pHEP	Ap^R, HSV TK	*Bam*HI, *Cla*I, *Hin*dIII	Grosveld et al. (1982)
pHomer series	Ap^R	*Sst*I, *Eco*RI, *Hin*dIII, *Sal*I	Chia et al. (1982)
pJB8	Ap^R	*Sal*I, *Hin*dIII	Ish-Horowicz and Burke (1981)

(Table VI, continued)

Plasmids	Markers	Reported restriction sites[a]	References
pMCS	Ap^R, AGPT	*Bam*HI, *Cla*I	Grosveld et al. (1982)
pMF series	Ap^R	ND	Feiss et al. (1982)
MUA-3	Tc^R	*Eco*RI, *Sal*I, *Hin*dIII, *Ava*I, *Bam*HI, *Pvu*II	Meyerowitz et al. (1980)
pλNM::pBR322	Ap^R	ND	Melnikov et al. (1984)
pNNL	Ap^R, ecogpt	*Sal*I, *Bam*HI, *Cla*I	Grosveld et al. (1982)
pOPF	Ap^R, HSV TK	*Bam*HI, *Cla*I	Grosveld et al. (1982)
pP2/pBR322	Ap^R, Tc^R	ND	Nicoletti and Bertant (1983)
pRT	Ap^R, HSV TK	*Bam*HI, *Cla*I, *Hin*dIII	Grosveld et al. (1982)
pSAE	Ap^R, HSV TK	*Bam*HI, *Cla*I	Grosveld et al. (1982)
pTBE	Ap^R	*Cla*I, *Bam*HI, *Sal*I	Grosveld et al. (1982)
pTM	Ap^R, AGPT	*Bam*HI, *Cla*I	Grosveld et al. (1982)

[a] Useful reported cloning sites for the particular function
ND, not determined

TABLE VII

Directory of pBR322 derivatives cited in this review

Vectors are ordered alphabetically, and reference to their positions in the tables is given as well as their classification and original reference.

Plasmid	Type of vector	Table	References
pAA3 series	Direct selection	V	Ahmed (1984)
pANH-1	Expression	III	Seth et al. (1986)
pANK-12	Expression	III	Seth et al. (1986)
pAS1	Expression	III	Shatzman et al. (1983)
pAT153	Multipurpose cloning High copy number	VI	Twigg and Sherrat (1980)
pBdC1	Terminator probe	IV	de Crombrugge et al. (1979)
pBR322	Multipurpose cloning	VI	Bolivar et al. (1977b)
pBR325	Multipurpose cloning	VI	Bolivar (1978)
pBR327	Multipurpose cloning High copy number	VI	Soberón et al. (1980)
pBR327-par	Multipurpose cloning High copy number Increased stability	VI	Zurita et al. (1983)
pBR328	Multipurpose cloning	VI	Soberón et al. (1980)
pBR329	Multipurpose cloning High copy number	VI	Covarrubias and Bolivar (1982)
pBRH series	Promoter probe	IV	West et al. (1980)

(Table VII, continued)

Plasmid	Type of vector	Table	References
pBRS188	Promoter probe	IV	Rechiniskii et al. (1982)
pBS series	Direct selection	V	Spratt et al. (1986)
pBS +/− series	Direct selection	V	Spratt et al. (1986)
pCB series	Promoter probes	IV	Schneider and Beck (1986)
pCQV2	Expression	III	Queen (1983)
pDR42	Multipurpose cloning	VI	Herrin et al. (1982)
pDR540	Expression	III	Russell and Bennet (1982)
pDR720	Expression	III	Russell and Bennet (1982)
pDS series	Cosmid	VI	Feiss et al. (1982)
pEMBL series pEMBLex2 pEMBLex3	Multipurpose cloning DNA sequencing Expression Direct selection Mutagenesis	III V VI	Dente et al., (1983); Sollozzo et al. (1985)
pEP series	Expression	III	Enger-Valk et al. (1980)
pEP series	Promoter probe	IV	Enger-Valk et al. (1981a)
pEP series	Terminator probe	IV	Enger-Valk et al. (1981a)
pER series	Expression	III	Boros et al. (1986)
pER103	Expression	III	Dworkin-Rastl et al. (1983)
pEV-vrf series	Expression	III	Crowl et al. (1985)
pEX series	Expression	III	Stanley and Luzio (1984)
pFCE4	Multipurpose cloning DNA sequencing Expression	III VI	Lorenzetti et al. (1985)
pFJ series	Multipurpose cloning	VI	Richardson et al. (1982)
pFK series	Expression	III	Shapira et al. (1983)
pGNC	Cosmid	VI	Grosveld et al. (1982)
pHA10	Direct selection	V	Honigman and Oppenheim (1981)
pHC series	Multipurpose cloning High copy number	VI	Boros et al. (1984)
pHC79	Cosmid	VI	Hohn and Collins (1980)
pHEP	Cosmid	VI	Grosveld et al. (1982)
pHG165	Direct selection	V	Stewart et al. (1986)
pHK413	Expression	III	Pilacinski et al. (1984)
pHomer series	Cosmid	VI	Chia et al. (1982)
pHP series	Expression	III	Enger-Valk et al. (1980)
pHP34	DNA sequencing	VI	Prentki and Kirsch (1982)
pHSG664	Direct selection	V	Hashimoto-Gotoh et al. (1986)

(Table VII, continued)

Plasmid	Type of vector	Table	References
pHUB series	Expression	III	Bernard et al. (1979)
pIC series	Multipurpose Expression DNA sequencing Direct selection	III V VI	Marsh et al. (1984)
pICEM series	Multipurpose Expression DNA sequencing Direct selection	III V VI	Marsh et al. (1984)
pIC.III series	Expression	III	Masui et al. (1983)
pIN series	Expression	III	Masui et al. (1983)
pINIII-ompA	Protein secretion	VI	Ghrayeb et al. (1984); Inouye and Inouye (1985)
pJB8	Cosmid	VI	Ish-Horowicz and Burke (1981)
pJG201	Protein purification	VI	Germino and Bastia (1984)
pJL6	Expression	III	Oppenheim et al. (1983)
pJP1	Expression	III	Rommens et al. (1983)
pJR011	Promoter probe	IV	Soberón et al. (1982)
pJRD158	Multipurpose cloning High copy number	VI	Davison et al. (1984) Heusterspreute and Davison (1984)
pJS413	Expression	III	Weis et al. (1982)
pKB111	Multipurpose cloning	VI	Beckingham (1980)
pKC7	Multipurpose cloning	VI	Rao and Rogers (1979)
pKC16	Multipurpose cloning Inducible copy number	VI	Rao and Rogers (1978)
pKC series	Expression	III	Rao (1984)
pKEN005	Promoter probe	IV	Masui et al. (1983)
pKG-1800	Terminator probe	IV	McKenney et al. (1981)
pKH47	DNA sequencing	VI	Hayashi et al. (1980)
pKK series	Terminator probe	IV	Brosius (1983)
pKK175-6	Promoter probe	IV	Brosius (1984a)
pKK231-1	Promoter probe	IV	Brossius (1984a)
pKK233-2	Expression	III	Amman et al. (1985)
pKL1	Direct selection	V	Honigman and Oppenheim (1981)
pKM005	Promoter probe	IV	Masui et al. (1983)
pKM-2	Promoter probe	IV	De Boer (1984)
pKO-1	Promoter probe	IV	McKenney et al. (1981)
pKO-2	Promoter probe	IV	De Boer (1984)
pKT series	Protein secretion	VI	Talmadge and Gilbert (1980)

(Table VII, continued)

Plasmid	Type of vector	Table	References
pKYP series	Expression	III	Nishi et al. (1984)
pLa series	Expression	III	Remaut et al. (1981)
pLA7	Direct selection	V	Burns and Beachman (1981)
pLc series	Expression	III	Remaut et al. (1981)
pLG series	Expression	III	Guarente et al. (1980)
plink322	Multipurpose cloning	VI	Seed, B. in Maniatis et al. (1982)
pLV57	Direct selection	V	O'Connor and Humphreys (1982)
pMC series	Expression	III	Shapira et al. (1983)
pMC1403	Promoter-translation initiation probe	IV	Casadaban et al. (1980)
pMCS	Cosmid	VI	Grosveld et al. (1982)
pMF series	Cosmid	VI	Feiss et al. (1982)
pMG411	High copy number	VI	Yarranton et al. (1984)
pMG105	Expression	III	Pilacinski et al. (1984)
pMK2004	Multipurpose cloning	VI	Kahn et al. (1979)
pMR series	Expression	III	Gray et al. (1982)
MUA-3	Cosmid	VI	Meyerowitz et al. (1980)
pNH7a	Expression	III	Podhajska et al. (1985)
pλNM::pBR322	Phasmid	VI	Melnikov et al. (1984)
pNNL	Cosmid	VI	Grosveld et al. (1982)
pNO1523	Direct selection	V	Dean (1981)
pNRC series	Promoter probe	IV	Thompson et al. (1982)
pNS1	Direct selection	V	Nikolnikov et al. (1984)
pOP series	Expression	III	Fuller (1982)
pOPF	Cosmid	VI	Grosveld et al. (1982)
pOU series	High copy number	VI	Larsen et al. (1984)
pORF series	Expression	III	Weinstock et al. (1983)
pP2/pBR322	Phasmid	VI	Nicoletti and Bertant (1983)
pPCϕ series	Expression	III	Charnay et al. (1978)
pPH series	Multipurpose cloning Expression Direct selection DNA sequencing	III V VI	Spratt et al. (1986)
pPL2	Expression	III	Seth et al. (1986)
pPR series	Plasmid retention	VI	Rosteck and Hershberger (1982)
pPV33	Promoter probe	IV	West and Rodriguez (1982)
pRT	Cosmid	VI	Grosveld et al. (1982)

(Table VII, continued)

Plasmid	Type of vector	Table	References
pSAE	Cosmid	VI	Grosveld et al. (1982)
pSCC31	Direct selection	V	Cheng and Nordrich (1983)
pSGS series	Multipurpose cloning Increased stability	VI	Skogman et al. (1983)
pSGS21	Plasmid retention	VI	Skogman and Nilsson (1984)
pSK series	Expression	III	Shapira et al. (1983)
pSP series	Multipurpose cloning Expression	III VI	Melton et al. (1984); Sollazzo et al. (1985)
pSPA series	Expression Protein purification	III	Uhlen et al. (1983)
pSSC31	Direct selection	V	Cheng and Nordrich (1983)
pSTP1	Expression	III	Windass et al. (1982)
pTac series	Expression	III	Amman et al. (1983)
pTBE	Cosmid	VI	Grosveld et al. (1982)
pTCF	Cosmid	VI	Grosveld et al. (1982)
pTL25	Promoter probe	IV	Linn and Ralling (1985)
pTM	Cosmid	VI	Grosveld et al. (1982)
pTR262	Direct selection	V	Roberts et al. (1980)
pTrpED series	Expression	III	Hallewell and Emtage (1980)
pTrpL1	Expression	III	Edman et al. (1981)
pUC series	Multipurpose cloning Expression Direct selection DNA sequencing	III V VI	Vieira and Messing (1982); Norrander et al. (1983); Hanna et al. (1984); Yanisch-Perron et al. (1985)
pUN121	Direct selection	V	Nilsson et al. (1983)
pUR2	Multipurpose cloning	VI	Rüther et al. (1980)
pUR222	DNA sequencing	VI	Rüther et al. (1981)
pUR250	DNA sequencing Direct selection	VI V	Rüther (1982)
pUR278	Protein secretion	VI	Rüther and Müller-Hill (1983)
pVT25	Direct selection	V	Verent et al. (1983)
pWR590	Expression	III	Guo et al. (1984)
pWR77	DNA sequencing	VI	White and Rosbash (1979)
pWT series	Expression	III	Tacon et al. (1980)
pXf3	Multipurpose cloning	VI	Hanahan, in Maniatis (1982)
pXJ002	Expression	III	Rossi et al. (1983)
pY2	Expression	III	Rossi et al. (1983)
pYEJ001	Expression	III	Rossi et al. (1983)
pZ series	DNA sequencing	VI	Zagursky and Berman (1984)

(9) Shuttle vectors

The host range of the pBR322 replicon (ColE1) seems limited to *E. coli* and other few organisms (e.g., *Serratia marcescens*; Reid et al., 1982). To fully utilize the cloning advantages of pBR322 and its derivatives, a series of plasmids, termed 'shuttle vectors', have been developed. Because they contain two different origins of replication, these vectors are capable of replication in *E. coli* and other hosts. Since these vectors contain many of the cloning features already described, they have not been included in this review.

FUTURE TRENDS

The abundance and variety of cloning vectors that now exist serve as powerful tools for DNA research and the development of recombinant DNA techniques. Plasmids and phages of different origins have been used as vehicles for the introduction of foreign DNA into different hosts and its expression. Small, well-characterized plasmids have been used preferentially as vectors for *E. coli*, and although a variety of plasmid vehicles have evolved during the last decade, only a few general cloning vectors are currently in use. pBR322 is one of them.

Experience has now demonstrated that the possibilities for vector improvement are nearly endless. The trend over the past eight years has been away from the contruction of multipurpose vectors, and towards the construction of vectors for specialized uses. Examples of this trend include the construction of shuttle vectors, phage/plasmid hybrids, and vectors to quantitate the effects of regulatory DNA sequences. This trend is expected to continue as the study of gene structure and function is extended to other organisms and gene systems.

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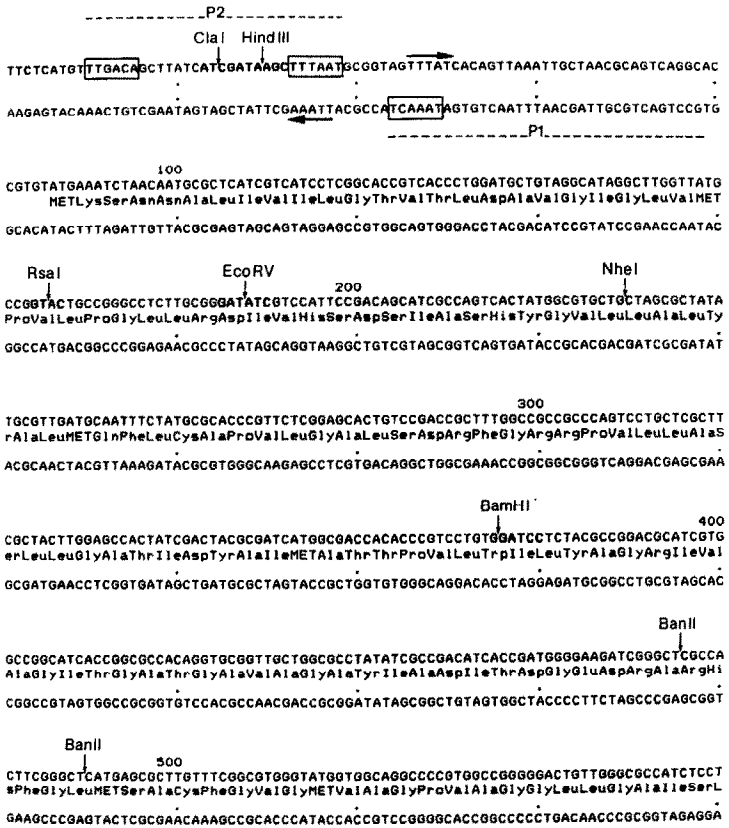
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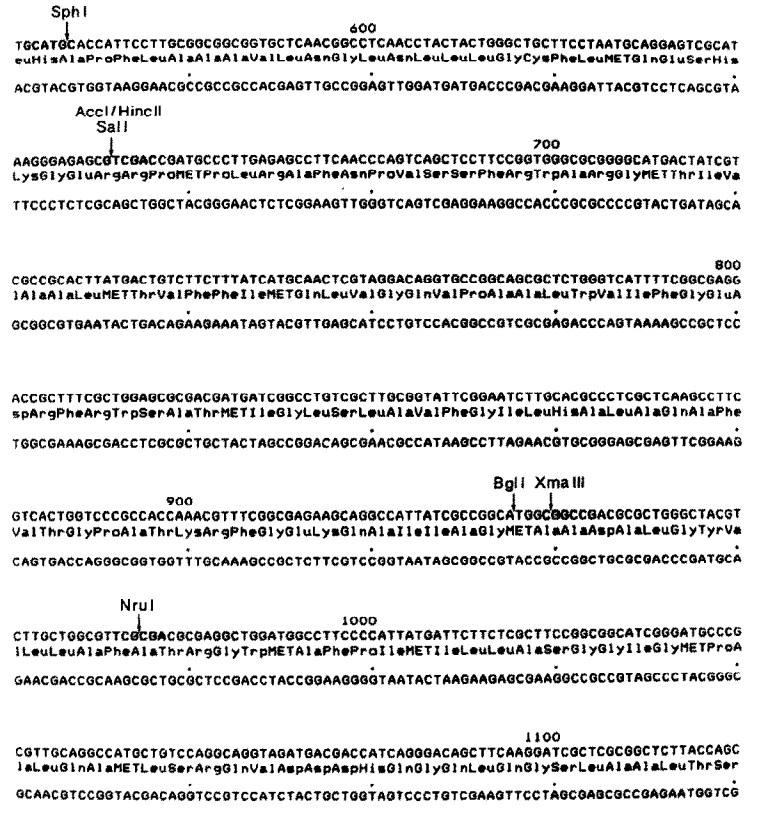
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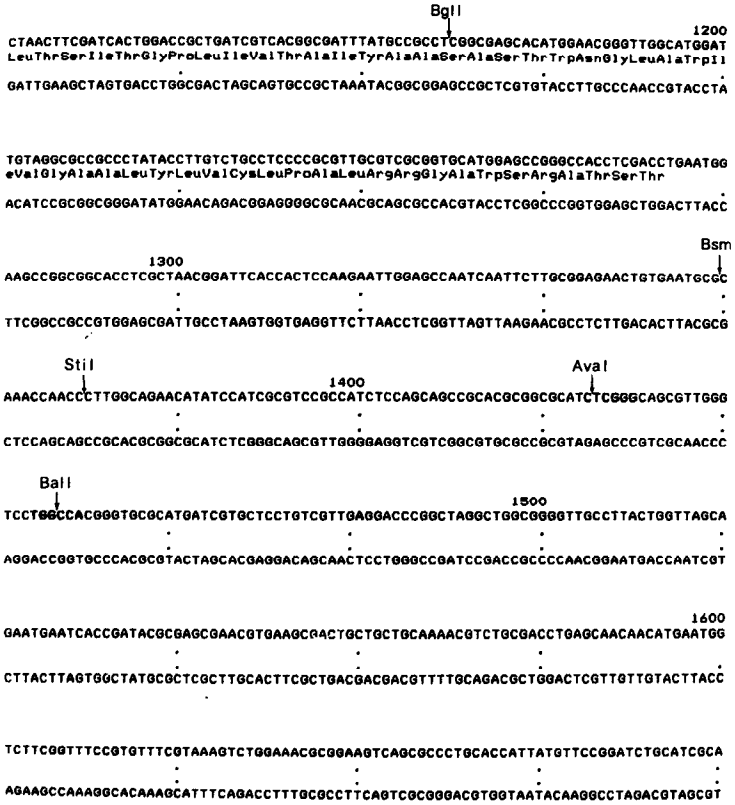
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StiI
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BalI
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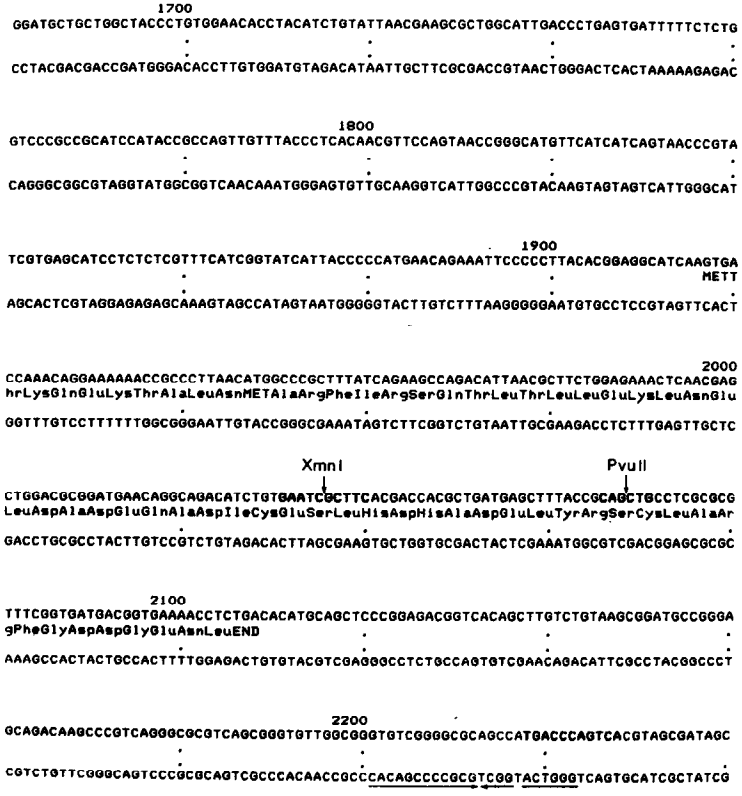
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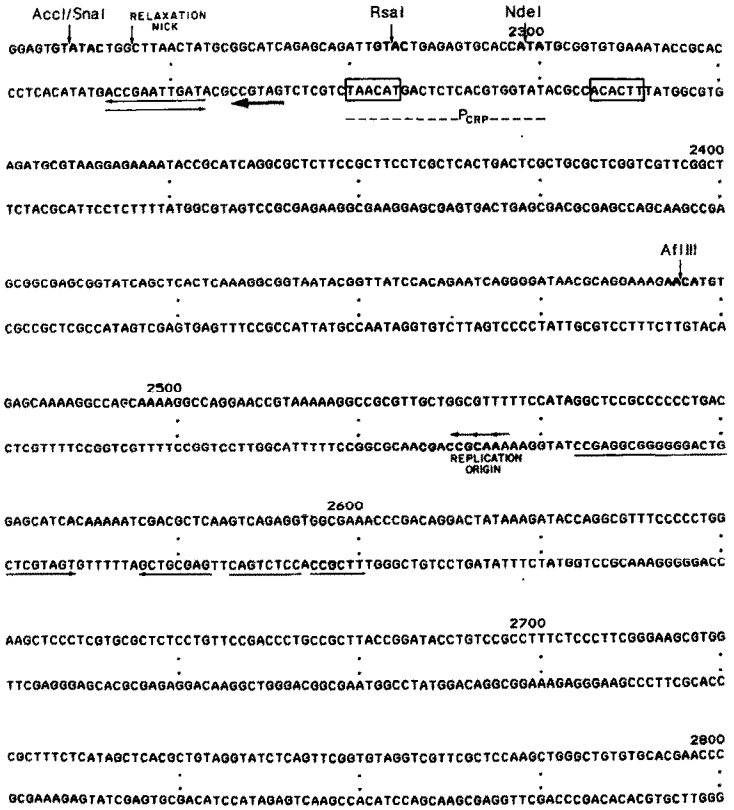
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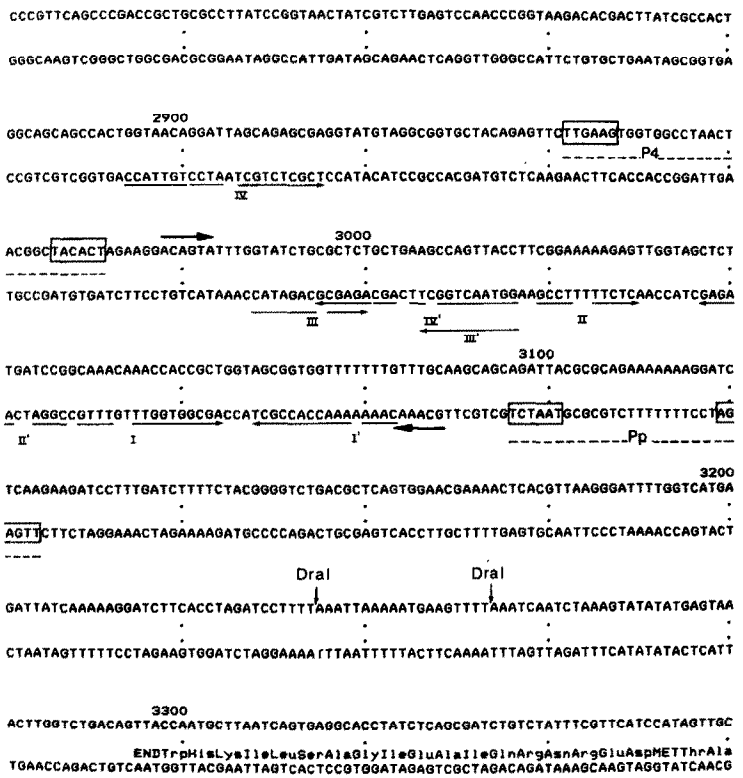
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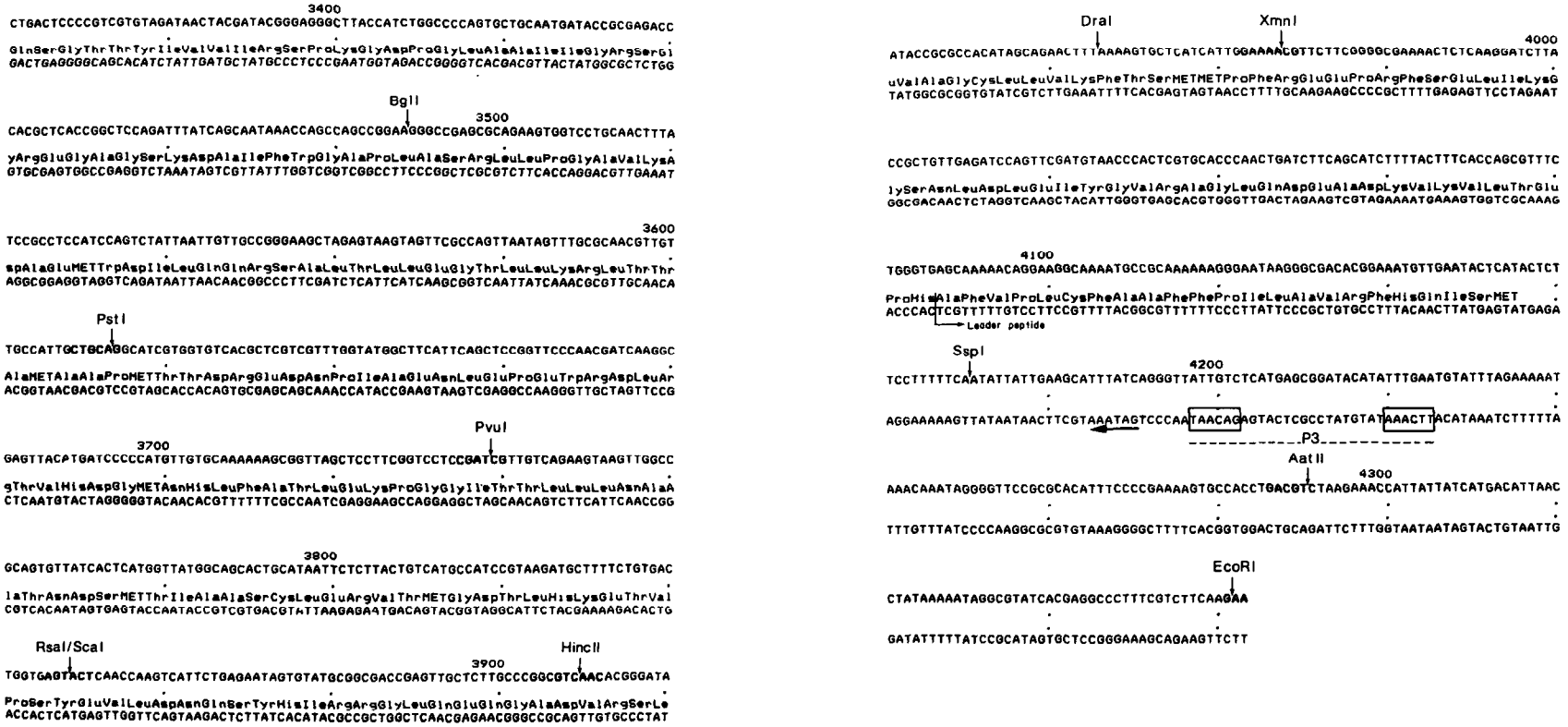

Fig. 4. Nucleotide sequence of pBR322. The symbols and letters that appear throughout the sequence are according to the following conventions: (*a*) *Restriction sites*. Those that are present three or fewer times in the sequence are indicated, with an arrow marking the cleavage site (on the 5′ → 3′ strand only). (*b*) *Structural genes*. Structural genes are indicated by the protein sequence written close to its coding strand. The map shown in Fig. 2 can be used as reference for identification of the proteins whose sequence is shown here. (*c*) *Promoters*. P within a dashed line, where the symbol following P specifies number or name, according to Table II. Boxes indicate the proposed −10 and −35 conserved hexamers. Each arrow signals the transcription start point, next to the tail end of each arrow. (*d*) *Origin of replication (ori)*. The symbol →→ starts at the first base that is found as DNA, attached to the RNA primer, during the initiation of replication. (*e*) *Relaxation nick*. Cleavage site for the mobilization function. Horizontal lines highlight regions of dyad symmetry which have been proposed to be relevant to some function of the plasmid. In the region starting at about nt 3000, the symmetrical segments relevant to the regulation of replication are identified by roman numerals according to standard nomenclature. (For transcriptional terminator location see Fig. 2.)

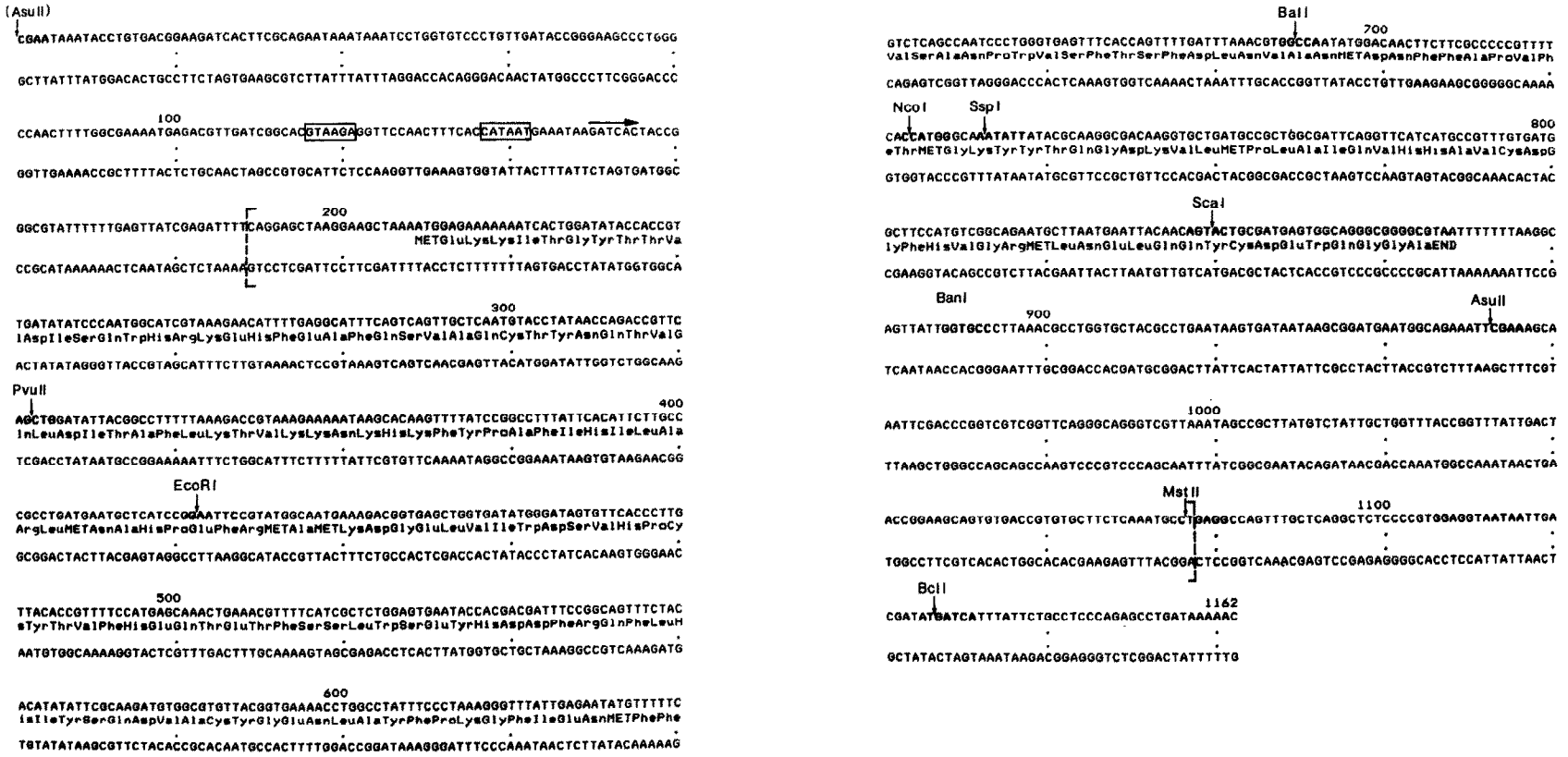

Fig. 5. Nucleotide sequence of the DNA fragment containing the *cat* (Cm^R) gene present in pBR325 (Marcoli, 1979; Alton and Vapnek, 1979; Prentki et al., 1981). This segment was inserted into pBR322 unique *Eco*RI site (Bolivar, 1978). The symbols are as in Fig. 4. The fragments contained in pBR329 are indicated by dashed vertical brackets.

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