

1 LOCUS SYNPBR322 4361 bp DNA circular SYN 18-SEP-1997  
2 DEFINITION Cloning vector pBR322, complete genome.  
3 ACCESSION J01749 K00005 L08654 M10282 M10283 M10286 M10356 M10784 M10785  
4 M10786 M33694 V01119  
5 NID g208958  
6 VERSION J01749.1 GI:208958  
7 KEYWORDS ampicillin resistance; beta-lactamase; cloning vector; drug  
8 resistance protein; origin of replication; plasmid; tetracycline  
9 resistance.  
10 SOURCE Cloning vector pBR322 (tissue library: ATCC 31344, ATCC 37017) DNA;  
11 Plasmid pSC101 DNA; Unclassified DNA; and Transposon Tn3 DNA.  
12 ORGANISM Cloning vector pBR322  
13 artificial sequence; cloning vectors.  
14 REFERENCE 1 (bases 1 to 3; 3259 to 4361)  
15 AUTHORS Sutcliffe,J.G.  
16 TITLE Nucleotide sequence of the ampicillin resistance gene of  
17 Escherichia coli plasmid pBR322  
18 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 75 (8), 3737-3741 (1978)  
19 MEDLINE 79012484  
20 REFERENCE 2 (bases 1 to 4361)  
21 AUTHORS Sutcliffe,J.G.  
22 TITLE Complete nucleotide sequence of the Escherichia coli plasmid pBR322  
23 JOURNAL Cold Spring Harb. Symp. Quant. Biol. 43 Pt 1, 77-90 (1979)  
24 MEDLINE 80002802  
25 REFERENCE 3 (bases 1500 to 2300)  
26 AUTHORS Reed,R.R., Young,R.A., Steitz,J.A., Grindley,N.D. and Guyer,M.S.  
27 TITLE Transposition of the Escherichia coli insertion element gamma  
generates a five-base-pair repeat  
28 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 76 (10), 4882-4886 (1979)  
29 MEDLINE 80056597  
30 REFERENCE 4 (bases 2207 to 2265)  
31 AUTHORS Covarrubias,L., Cervantes,L., Covarrubias,A., Soberon,X.,  
Vichido,I., Blanco,A., Kupersztoch-Portnoy,Y.M. and Bolivar,F.  
32 TITLE Construction and characterization of new cloning vehicles. V.  
33 Mobilization and coding properties of pBR322 and several deletion  
34 derivatives including pBR327 and pBR328  
35 JOURNAL Gene 13 (1), 25-35 (1981)  
36 MEDLINE 81213464  
37 REFERENCE 5 (bases 2000 to 2500)  
38 AUTHORS Marians,K.J., Soeller,W. and Zipursky,S.L.  
39 TITLE Maximal limits of the Escherichia coli replication factor Y  
40 effector site sequences in pBR322 DNA  
41 JOURNAL J. Biol. Chem. 257 (10), 5656-5662 (1982)  
42 MEDLINE 82167416  
43 REFERENCE 6 (bases 1 to 80; 4151 to 4229; 4349 to 4361)  
44 AUTHORS Brosius,J., Cate,R.L. and Perlmutter,A.P.  
45 TITLE Precise location of two promoters for the beta-lactamase gene of  
46 pBR322. S1 mapping of ribonucleic acid isolated from Escherichia  
47 coli or synthesized in vitro  
48 JOURNAL J. Biol. Chem. 257 (15), 9205-9210 (1982)  
49 MEDLINE 82239419  
50 REFERENCE 7 (bases 4241 to 4343)  
51 AUTHORS Van Dyke,M.W., Hertzberg,R.P. and Dervan,P.B.  
52 TITLE Map of distamycin, netropsin, and actinomycin binding sites on  
53 heterogeneous DNA: DNA cleavage-inhibition patterns with  
54 methidiumpropyl-EDTA.Fe(II)  
55 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 79 (18), 5470-5474 (1982)  
56 MEDLINE 83039392  
57 REFERENCE 8 (bases 584 to 709)  
58 AUTHORS Peden,K.W. and Nathans,D.  
59 TITLE Local mutagenesis within deletion loops of DNA heteroduplexes  
60 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 79 (23), 7214-7217 (1982)  
61 MEDLINE 83117649  
62 REFERENCE 9 (bases 373 to 649)  
63 AUTHORS Peden,K.W.  
64 TITLE . . . . .  
65 AUTHORS . . . . .

66 TITLE Revised sequence of the tetracycline-resistance gene of pBR322  
67 JOURNAL Gene 22 (2-3), 277-280 (1983)  
68 MEDLINE 83263146  
69 REFERENCE 10 (bases 132 to 181)  
70 AUTHORS Watabe,H., Iino,T., Kaneko,T., Shibata,T. and Ando,T.  
71 TITLE A new class of site-specific endodeoxyribonucleases. Endo.Sce I  
isolated from a eukaryote, *Saccharomyces cerevisiae*  
72 JOURNAL J. Biol. Chem. 258 (8), 4663-4665 (1983)  
74 MEDLINE 83161053  
75 REFERENCE 11 (bases 368 to 581)  
76 AUTHORS Livneh,Z.  
77 TITLE Directed mutagenesis method for analysis of mutagen specificity:  
application to ultraviolet-induced mutagenesis  
79 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 80 (1), 237-241 (1983)  
80 MEDLINE 83117828  
81 REFERENCE 12 (bases 2627 to 2682; 2781 to 2828)  
82 AUTHORS Mascharak,P.K., Sugiura,Y., Kuwahara,J., Suzuki,T. and Lippard,S.J.  
83 TITLE Alteration and activation of sequence-specific cleavage of DNA by  
84 bleomycin in the presence of the antitumor drug  
85 cis-diamminedichloroplatinum(II)  
86 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 80 (22), 6795-6798 (1983)  
87 MEDLINE 84070716  
88 REFERENCE 13 (bases 4276 to 4336)  
89 AUTHORS Schultz,P.G. and Dervan,P.B.  
90 TITLE Sequence-specific double-strand cleavage of DNA by  
91 penta-N-methylpyrrolecarboxamide-EDTA X Fe(II)  
92 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 80 (22), 6834-6837 (1983)  
93 MEDLINE 84070724  
94 REFERENCE 14 (bases 518 to 528)  
95 AUTHORS Sutcliffe,J.G.  
96 JOURNAL Unpublished (1984)  
97 REFERENCE 15 (bases 2395 to 2495)  
98 AUTHORS Fuller,R.S., Funnell,B.E. and Kornberg,A.  
99 TITLE The dnaA protein complex with the *E. coli* chromosomal replication  
100 origin (oriC) and other DNA sites  
101 JOURNAL Cell 38 (3), 889-900 (1984)  
102 MEDLINE 85024881  
103 REFERENCE 16 (bases 2729 to 2731)  
104 AUTHORS Lathe,R., Kiely,M.P., Skory,S. and Lecocq,J.P.  
105 TITLE Linker tailing: unphosphorylated linker oligonucleotides for  
joining DNA termini  
107 JOURNAL DNA 3 (2), 173-182 (1984)  
108 MEDLINE 84207440  
109 REFERENCE 17 (bases )  
110 AUTHORS Heusterspreute,M. and Davison,J.  
111 TITLE Restriction site bank vectors. II. DNA sequence analysis of plasmid  
112 pJRD158  
113 JOURNAL DNA 3 (3), 259-268 (1984)  
114 MEDLINE 84260952  
115 REFERENCE 18 (bases 2113 to 2186; 2348 to 2415)  
116 AUTHORS Abarzua,P., Soeller,W. and Marians,K.J.  
117 TITLE Mutational analysis of primosome assembly sites. I. Distinct  
118 classes of mutants in the pBR322 *Escherichia coli* factor Y DNA  
119 effector sequences  
120 JOURNAL J. Biol. Chem. 259 (22), 14286-14292 (1984)  
121 MEDLINE 85054885  
122 REFERENCE 19 (bases 2348 to 2415)  
123 AUTHORS Soeller,W., Abarzua,P. and Marians,K.J.  
124 TITLE Mutational analysis of primosome assembly sites. II. Role of  
secondary structure in the formation of active sites  
126 JOURNAL J. Biol. Chem. 259 (22), 14293-14300 (1984)  
127 MEDLINE 85054886  
128 REFERENCE 20 (bases 1 to 4361)  
129 AUTHORS Van Dyke,M.M. and Dervan,P.B.  
130 TITLE Echinomycin binding sites on DNA  
131 JOURNAL Science 225 (4667), 1122-1127 (1984)

132 MEDLINE 84300294  
133 REFERENCE 21 (sites)  
134 AUTHORS Pouwels,P.H., Enger-Valk,B.E. and Brammar,W.J.  
135 TITLE Vector I-A-iv-1  
136 JOURNAL (in) Brammar,W.J. (Ed.);  
CLONING VECTORS;  
138 Elsevier Scientific Publishing, Amsterdam (1985)  
139 REFERENCE 22 (bases 1 to 4361)  
140 AUTHORS Watson,N.  
141 TITLE A new revision of the sequence of plasmid pBR322  
142 JOURNAL Gene 70 (2), 399-403 (1988)  
143 MEDLINE 89108024  
144 REFERENCE 23 (sites)  
145 AUTHORS Gilbert,W.  
146 TITLE Obtained from VecBase 3.0  
147 JOURNAL Unpublished (1991)  
148 COMMENT The circular sequence is numbered such that 0 is the middle of the  
unique EcoRI site and the count increases first through the tet  
genes, the pMB1 material, and finally through the Tn3 region.  
Plasmid pBR322 contains ampicillin and tetracycline resistance  
genes. The ampicillin resistance gene (amp-r) is a penicillin  
beta-lactamase. Promoters P1 and P3 are for the beta-lactamase  
gene. P3 is the natural promoter, and P1 is artificially created by  
the ligation of two different DNA fragments to create pBR322. P2 is  
in the same region as P1, but it is on the opposite strand and  
initiates transcription in the direction of the tetracycline  
resistance gene.  
Mutational studies in the primosome assembly sites indicate four  
types of mutations: Class I having no effect on the activities  
elicited by the DNA site and the bases involved are probably  
spacers; Class II requiring higher Mg-2+ concentrations than the  
wild-type to be fully activated as factor Y ATPase effectors; Class  
III co-inactivating both the ATPase effector and DNA replication  
template activity of the site, indicating that they probably  
represent essential contact points between factor Y and the DNA;  
Class IV having a replication template activity intermediate that  
of class III and class II mutant DNAs.  
Specific sites within or near the origins of replication are  
recognized by dnaA protein. Without dnaA binding to the origin of  
replication chromosomal replication is not possible [15]. pBR322  
DNA contains two separate regions on opposite strands and close to  
the origin of replication which, when in single-stranded form, can  
act as effectors for the ATPase activity of E.coli replication  
factor Y [5]. Small fragments of DNA containing these sites when  
cloned in an f1 phage vector act as origins of DNA replication  
allowing the formation of complementary double-stranded DNA in  
rifampicin-resistant, dna[B,G,C]-dependent fashion in vitro [5].  
The biological activity of echinomycin is thought to be related to  
the formation of complexes by intercalating with cellular DNA [20].  
Complete source information:  
Plasmid pBR322 from E.coli [2],[1],[3],[6],[11],[8],[5],[7],[12],  
[13],[10],[9],[14],[18],[19],[15],[20],[16]; pBR322 DNA in pXF3  
[4].  
The following data and their annotation were supplied by Will  
Gilbert under the auspices of the Curator Program.  
CROSSREFERENCE  
#parent  
GenBank(50):pSC101C, GenBank(50):Trn3  
#offspring  
VecBase(3):pBR325, VecBase(3):pBR327,  
VecBase(3):pBR328,  
VecBase(3):pAT153, VecBase(3):pUC7,  
VecBase(3):pJRD158,  
VecBase(3):PiVX, VecBase(3):PiAN7,  
VecBase(3):pSP64,  
VecBase(3):nSP65 VecBase(3):nGFM1

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198      VecBase(3):pGEM2,
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201          VecBase(3):pLBU3, VecBase(3):pTrS3,
202      VecBase(3):pRSVNeo,
203          VecBase(3):pSV2Cat, VecBase(3):M13mp9,
204      VecBase(3):pHC79,
205          VecBase(3):pV34, VecBase(3):pKTH601,
206      VecBase(3):pKTH604,
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208      VecBase(3):YEp24,
209          VecBase(3):YIp5, VecBase(3):YRp17,
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212              VecBase(3):pSP6T3, VecBase(3):pSP6T719,
213      VecBase(3):pT712,
214          VecBase(3):pT713, VecBase(3):pT7T318,
215      VecBase(3):pT7T319,
216          VecBase(3):pT7T3A18, VecBase(3):pT7T3A19,
217      VecBase(3):pEX1,
218          VecBase(3):pEX2, VecBase(3):pEX3,
219      VecBase(3):pCKSP6,
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222          VecBase(3):pKM1,
223              VecBase(3):pKM2, VecBase(3):pMBL1,
224      VecBase(3):pMBL604,
225          VecBase(3):pMC1511, VecBase(3):pMC1871,
226      VecBase(3):pAA37X,
227          VecBase(3):pUR278, VecBase(3):pUR288,
228      VecBase(3):pUR289,
229          VecBase(3):pUR290, VecBase(3):pUR291,
230      VecBase(3):pUR292,
231          VecBase(3):pUR222.
232 FEATURES          Location/Qualifiers
233     source          1..4361
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235             /db_xref="taxon:47470"
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246     promoter        43..49
247             /note="promoter P2 [6]"
248     misc_binding    53..56
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250     misc_binding    67..70
251             /bound_moiety="echinomycin"
252     misc_binding    80..83
253             /bound_moiety="echinomycin"
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258             /codon_start=1
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263             /dh_xref="GT·208959"
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263 /db_xref="GI:208959"
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268 FFIMQLVGQVPAALWVIFGEDRFRWSATMIGLSLAVFGILHALAQAFVTGPATKRFGE
269 KQAIIAAGMAADALGYVLLAFATRGWMAFPIMILLASGGGIGMPALQAMLSRQVDDDHQG
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280 misc_binding 411..414
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285 /citation=[11]
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298 source 1763..3147
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324 /db_xref="GI:456436"
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379 promoter complement(4188..4194)  
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453      3061 ctggtagcggt tgggtttttt gtttgcagc acgagattac gcgcagaaaa aaaggatctc
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459      3421 caatgatacc gcgagacccca cgctcacccgg ctccagattt atcagcaata aaccagccag
460      3481 ccaaaaaaaaaa caaaaaaaaaa aataatcttca caactttatc cacccatccatc caatcttata

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461 3541 attgttgcg ggaagctaga gtaagtagtt cgccagttaa tagtttgcgc aacgttgg  
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465 3781 tggcagcact gcataattctt cttactgtca tgccatccgt aagatgcttt tctgtgactg  
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467 3901 cggcgtcaac acgggataat accgcgccac atagcagaac tttaaaagtg ctcatcattg  
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