The nucleotide sequence of pACYC177

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Submitted November 18, 1987 Accession no. X06402

pACYC177 is a commonly used multicopy cloning vector (1), however, its complete nucleotide sequence has never been reported. The plasmid was constructed by ligating restriction fragments from three different sources: Tn903, Tn3, and p15A each of which has been sequenced (2,3,4). The sequence was completed by using oligonucleotide primers designed to span the junctions between each of the previously sequenced regions. pACYC177 is 3940 bp in length with nucleotide 1 corresponding to the HincII site in the original map (1). The Tn3 region extends from bases 3026 to 756 with the beta-lactamase gene encoded by bases 3698 (ATG) to 615. Part of the Tn3 repressor extends from bases 3025 to 3515, but the promoter region and part of the coding region have been deleted. In addition, one inverted repeat from Tn3 extends from bases 728 to 765. The Tn903 region, bases 1595 to 3025, contains the kanamycin resistance determinant which extends from bases 1922 (ATG) to 2734 flanked by 226 bp inverted repeats. The p15A origin of replication extends from bases 766 to 1595. The underlined sequence was determined while the rest of the sequence was taken from the published sequences of Tn903 (2), Tn3 (3), and p15A (4).

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1     GTTGACGCCG GGCAAGAGCA ACTCGGTCGC CGCATACACT ATTCTCAGAA TGACTTGGTT GAGTACTCAC CAGTCACAGA AAAGCATCTT ACGGATGGCA
101   TGACAGTAAG AGAATTATGC AGTGCTGCCA TAACCATGAG TGATAACACT GCGGCCAACT TACTTCTGAC AACGATCGGA GGACCGAAGG AGCTAACCGC
201   TTTTTTGCAC AACATGGGGG ATCATGTAAC TCGCCTTGAT CGTTGGGAAC CGGAGCTGAA TGAAGCCATA CCAAACGACG AGCGTGACAC CACGATGCCT
301   GCAGCAATGG CAACAACGTT GCGCAAACTA TTAACTGGCG AACTACTTAC TCTAGCTTCC CGGCAACAAT TAATAGACTG GATGGAGGCG GATAAAGTTG
401   CAGGACCACT TCTGCGCTCG GCCCTTCCGG CTGGCTGGTT TATTGCTGAT AAATCTGGAG CCGGTGAGCG TGGGTCTCGC GGTATCATTG CAGCACTGGG
501   GCCAGATGGT AAGCCCTCCC GTATCGTAGT TATCTACACG ACGGGGAGTC AGGCAACTAT GGATGAACGA AATAGACAGA TCGCTGAGAT AGGTGCCTCA
601   CTGATTAAGC ATTGGTAACT GTCAGACCAA GTTTACTCAT ATATACTTTA GATTGATTTA AAACTTCATT TTTAATTTAA AAGGATCTAG GTGAAGATCC
701   TTTTTGATAA TCTCATGACC AAAATCCCTT AACGTGAGTT TTCGTTCCAC TGAGCGTCAG ACCCCTTAAT AAGATGATCT TCTTGAGATC GTTTTGGTCT
801   GCGCGTAATC TCTTGCTCTG AAAACGAAAA AACCGCCTTG CAGGGCGGTT TTTCGAAGGT TCTCTGAGCT ACCAACTCTT TGAACCGAGG TAACTGGCTT
901   GGAGGAGCGC AGTCACCAAA ACTTGTCCTT TCAGTTTAGC CTTAACCGGC GCATGACTTC AAGACTAACT CCTCTAAATC AATTACCAGT GGCTGCTGCC
1001  AGTGGTGCTT TTGCATGTCT TTCCGGGTTG GACTCAAGAC GATAGTTACC GGATAAGGCG CAGCGGTCGG ACTGAACGGG GGGTTCGTGC ATACAGTCCA
1101  GCTTGGAGCG AACTGCCTAC CCGGAACTGA GTGTCAGGCG TGGAATGAGA CAAACGCGGC CATAACAGCG GAATGACACC GGTAAACCGA AAGGCAGGAA
1201  CAGGAGAGCG CACGAGGGAG CCGCCAGGGG AAACGCCTGG TATCTTTATA GTCCTGTCGG GTTTCGCCAC CACTGATTTG AGCGTCAGAT TTCGTGATGC
1301  TTGTCAGGGG GGCGGAGCCT ATGGAAAAAC GGCTTTGCCG CGGCCCTCTC ACTTCCCTGT TAAGTATCTT CCTGGCATCT TCCAGGAAAT CTCCGCCCCG
1401  TTCGTAAGCC ATTTCCGCTC GCCGCAGTCG AACGACCGAG CGTAGCGAGT CAGTGAGCGA GGAAGCGGAA TATATCCTGT ATCACATATT CTGCTGACGC
1501  ACCGGTGCAG CCTTTTTTCT CCTGCCACAT GAAGCACTTC ACTGACACCC TCATCAGTGC CAACATAGTA AGCCAGTATA CACTCCGCTA GCGCTGAGGT
1601  CTGCCTCGTG AAGAAGGTGT TGCTGACTCA TACCAGGCCT GAATCGCCCC ATCATCCAGC CAGAAAGTGA GGGAGCCACG GTTGATGAGA GCTTTGTTGT
1701  AGGTGGACCA GTTGGTGATT TTGAACTTTT GCTTTGCCAC GGAACGGTCT GCGTTGTCGG GAAGATGCGT GATCTGATCC TTCAACTCAG CAAAAGTTCG
1801  ATTTATTCAA CAAAGCCACG TTGTGTCTCA AAATCTCTGA TGTTACATTG CACAAGATAA AAATATATCA TCATGAACAA TAAAACTGTC TGCTTACATA
1901  AACAGTAATA CAAGGGGTGT TATGAGCCAT ATTCAACGGG AAACGTCTTG CTCGAGGCCG CGATTAAATT CCAACATGGA TGCTGATTTA TATGGGTATA
2001  AATGGGCTCG CGATAATGTC GGGCAATCAG GTGCGACAAT CTATCGATTG TATGGGAAGC CCGATGCGCC AGAGTTGTTT CTGAAACATG GCAAAGGTAG
2101  CGTTGCCAAT GATGTTACAG ATGAGATGGT CAGACTAAAC TGGCTGACGG AATTTATGCC TCTTCCGACC ATCAAGCATT TTATCCGTAC TCCTGATGAT
2201  GCATGGTTAC TCACCACTGC GATCCCCGGG AAAACAGCAT TCCAGGTATT AGAAGAATAT CCTGATTCAG GTGAAAATAT TGTTGATGCG CTGGCAGTGT
2301  TCCTGCGCCG GTTGCATTCG ATTCCTGTTT GTAATTGTCC TTTTAACAGC GATCGCGTAT TTCGTCTCGC TCAGGCGCAA TCACGAATGA ATAACGGTTT
2401  GGTTGATGCG AGTGATTTTG ATGACGAGCG TAATGGCTGG CCTGTTGAAC AAGTCTGGAA AGAAATGCAT AAGCTTTTGC CATTCTCACC GGATTCAGTC
2501  GTCACTCATG GTGATTTCTC ACTTGATAAC CTTATTTTTG ACGAGGGGAA ATTAATAGGT TGTATTGATG TTGGACGAGT CGGAATCGCA GACCGATACC
2601  AGGATCTTGC CATCCTATGG AACTGCCTCG GTGAGTTTTC TCCTTCATTA CAGAAACGGC TTTTTCAAAA ATATGGTATT GATAATCCTG ATATGAATAA
2701  ATTGCAGTTT CATTTGATGC TCGATGAGTT TTTCTAATCA GAATTGGTTA ATTGGTTGTA ACACTGGCAG AGCATTACGC TGACTTGACG GGACGGCGGC
2801  TTTGTTGAAT AAATCGAACT TTTGCTGAGT TGAAGGATCA GATCACGCAT CTTCCCGACA ACGCAGACCG TTCCGTGGCA AAGCAAAAGT TCAAAATCAC
2901  CAACTGGTCC ACCTACAACA AAGCTCTCAT CAACCGTGGC TCCCTCACTT TCTGGCTGGA TGATGGGGCG ATTCAGGCCT GGTATGAGTC AGCAACACCT
3001  TCTTCACGAG GCAGACCTCA GCGCTCAAAG ATGCAGGGGT AAAAGCTAAC CGCATCTTTA CCGACAAGGC ATCCGGCAGT TCAACAGATC GGGAAGGGCT
3101  GGATTTGCTG AGGATGAAGG TGGAGGAAGG TGATGTCATT CTGGTGAAGA AGCTCGACCG TCTTGGCCGC GACACCGCCG ACATGATCCA ACTGATAAAA
3201  GAGTTTGATG CTCAGGGTGT AGCGGTTCGG TTTATTGACG ACGGGATCAG TACCGACGGT GATATGGGGC AAATGGTGGT CACCATCCTG TCGGCTGTGG
3301  CACAGGCTGA ACGCCGGAGG ATCCTAGAGC GCACGAATGA GGGCCGACAG GAAGCAAAGC TGAAAGGAAT CAAATTTGGC CGCAGGCGTA CCGTGGACAG
3401  GAACGTCGTG CTGACGCTTC ATCAGAAGGG CACTGGTGCA ACGGAAATTG CTCATCAGCT CAGTATTGCC CGCTCCACGG TTTATAAAAT TCTTGAAGAC
3501  GAAAGGGCCT CGTGATACGC CTATTTTTAT AGGTTAATGT CATGATAATA ATGGTTTCTT AGACGTCAGG TGGCACTTTT CGGGGAAATG TGCGCGGAAC
3601  CCCTATTTGT TTATTTTTCT AAATACATTC AAATATGTAT CCGCTCATGA GACAATAACC CTGATAAATG CTTCAATAAT ATTGAAAAAG GAAGAGTATG
3701  AGTATTCAAC ATTTCCGTGT CGCCCTTATT CCCTTTTTTG CGGCATTTTG CCTTCCTGTT TTTGCTCACC CAGAAACGCT GGTGAAAGTA AAAGATGCTG
3801  AAGATCAGTT GGGTGCACGA GTGGGTTACA TCGAACTGGA TCTCAACAGC GGTAAGATCC TTGAGAGTTT TCGCCCCGAA GAACGTTTTC CAATGATGAG
3901  CACTTTTAAA GTTCTGCTAT GTGGCGCGGT ATTATCCCGT 3940
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REFERENCES

1. Chang, A.C.Y. and Cohen, S.N. (1978) J. Bacteriol. 134, 1141-1156.
2. Oka, A. *et al.* (1981) J. Mol. Biol. 147, 217-226.
3. Heffron, F. *et al.* (1979) Cell 18, 1153-1163.
4. Selzer, G. *et al.* (1983) Cell 32, 119-129.