EXERCISE 9A - Postlab

1. The DNA sequences that are recognized by restriction enzymes are referred to as palindromic. Explain why.

2. Synthetic plasmids like pUC18 are often designed to have an MCS (multiple cloning site) region, also called a "polylinker" region. This region of the plasmid contains single (unique) recognition sites for several restriction enzymes. For example, pUC18's MCS region contains one recognition site each for the following restriction enzymes: *Eco*RI, *Sac*I, *Kpn*I, *Sma*I, *BamH*I, *Xba*I, *Sal*I, *BspM*I, *Pst*I, *Sph*I *and Hind*III. Why is a plasmid more useful if it has only one site for a particular restriction enzyme, rather than several sites for the same restriction enzyme?

3. The plasmid pUC18 is 2686 base pairs in size. Draw a circular diagram, or map, of pUC18 that shows the location of the features in the table below. **Note:** Number your diagram clockwise.

   - bp 861--Replication origin. (This is the site where DNA copying of the plasmid is initiated.)
   - bp 236-469--lac-Z gene
   - bp 399-450--MCS region (Multiple Cloning Sites-where many restriciton sites have been inserted)
   - bp 396--*Eco*RI site
   - bp 1626-2486--ampicillin resistance gene (for ß-lactamase)

4. Draw another diagram, similar to the one you drew for question 3, that represents the pUC18 plasmid with a segment of phage λ DNA inserted into it.

5. Draw the plasmids you diagrammed for questions 3 and 4 after an *Eco*RI digest. Use the same style or color coding that you used above.

6. The DNA sequence of the pUC18 plasmid is shown below, along with information about its engineering and publication history. This information is typical of the information available on the Internet about vectors, organisms, and DNA sequences used in molecular biology

   Using the information from question 3, locate the six base sequence recognized by *Eco*RI . Underline or highlight this sequence and draw a vertical line where the DNA is cut when digested by *Eco*RI. (Note: The base sequence of only one strand of the double helix is shown, and the sequence should be read from left to right across the page, as in a page of printed text.)

Accessed through GenBank at www.ncbi.nlm.nih.gov:

L09137. Cloning vector pUC18...[gi:20141090]

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LOCUS       SYNPUC19CV              2686 bp    DNA     circular SYN 22-MAY-2002
DEFINITION  Cloning vector pUC18, complete sequence.
ACCESSION   L09137 X02514
VERSION     L09137.2  GI:20141090
SOURCE      Cloning vector pUC18
REFERENCE   1  (bases 1 to 2686)
  AUTHORS   Yanisch-Perron,C., Vieira,J. and Messing,J.
  TITLE     Improved M13 phage cloning vectors and host strains: nucleotide
            sequences of the M13mp18 and pUC19 vectors
  JOURNAL   Gene 33 (1), 103-119 (1985)
  MEDLINE   85180545
   PUBMED   2985470
REFERENCE   2  (bases 1 to 2686)
  AUTHORS   Chambers,S.P., Prior,S.E., Barstow,D.A. and Minton,N.P.
  TITLE     The pMTL nic- cloning vectors. I. Improved pUC polylinker regions
            to facilitate the use of sonicated DNA for nucleotide sequencing
  JOURNAL   Gene 68 (1), 139-149 (1988)
  MEDLINE   89121486
   PUBMED   2851488
REFERENCE   3  (bases 1 to 2686)
  AUTHORS   Gilbert,W.
  TITLE     Obtained from VecBase 3.0
  JOURNAL   Unpublished
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REFERENCE   4  (bases 1 to 2686)
  AUTHORS   Messing,J.
  TITLE     Direct Submission
  JOURNAL   Submitted (27-APR-1993) Department of Biochemistry, University of
            Minnesota, St. Paul, MN 55108, USA
REFERENCE   5  (bases 1 to 2686)
  AUTHORS   Messing,J.
  TITLE     Direct Submission
  JOURNAL   Submitted (11-APR-2002) Rutgers, The State University of New
            Jersey, Waksman Institute of Microbiology, 190 Frelinghuysen Road,
            Piscataway, NJ 08854-8020, USA
  REMARK    Sequence update by submitter
COMMENT     On Apr 11, 2002 this sequence version replaced gi:209213.
            These data and their annotation were supplied to GenBank by Will
            Gilbert under the auspices of the GenBank Currator Program. pUC19c
            - Cloning vector (beta-galactosidase mRNA on complementary strand)
            ENTRY PUC18                    #TYPE DNA CIRCULAR TITLE pUC19c -
            Cloning vector
                         (beta-galactosidase mRNA on complementary strand)
            DATE      03-FEB-1986
               #sequence  16-DEC-1986
            ACCESSION VB0033
            SOURCE    artificial
            COLLECTION  ATCC 37254
            REFERENCE
               #number
               #authors  Norrander J., Kempe T., Messing J.
               #journal Gene (1983) 26: 101-106
            REFERENCE
               #number  1
               #authors  Yanisch-Perron C., Vieira J., Messing J.
               #journal Gene (1985) 33: 103-119
               #comment shows the complete compiled sequence
            REFERENCE
               #number  2
               #authors  Chambers,S.P., et al.
               #journal Gene (1988) 68: 139-149
               #describes mutation at nt1308 and its effect on copy number
            REFERENCE
               #number
               #authors Pouwels P.H., Enger-Valk B.E., Brammar W.J.
               #book    Cloning Vectors, Elsvier 1985 and supplements
               #comment  vector I-A-iv-20
            COMMENT
               This Sequence was obtained 3-MAR-1986 from J. Messing, Waksman
               Institute, NJ on floppy disk.
               Revised 16-DEC-1986 by F. Pfeiffer:
               1062/3 'AT' to 'TA' to match revised sequence of PBR322
               The beta-galactosidase mRNA sequence including the multiple
               cloning site of M13mp19 is on the strand complementary to that
               shown.
            KEYWORDS
            CROSSREFERENCE
               #complement
                 VecBase(3):pUC19
               #prerevised
                 GenBank(50):M11662, EMBL(11):ARPuc19
               #parent
                 VecBase(3):pUC13, VecBase(3):M13mp19, VecSource(3):bGal19
            PARENT
               Features of pUC18 (2686 bp)
                 residue     source
                  1- 137  2074-2210 pBR322
                 138- 237  2252-2351 pBR322
                 238- 395  1461-1304 (c) Lac-Operon
                 396- 452    57-   1 (c) polylinker of M13mp19
                 455- 682  1298-1071 (c) Lac-Operon
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               683-2686  2352-4355 pBR322
              Conflict (cfl) and Mutations (mut):
                   pUC19  source
              mut 1308  A  G  2977   pBR322   linked to increased copy number
              mut 1942  A  G  3611   pBR322
              mut 2243  T  C  3912   pBR322
           FEATURE
              1629-2417 789-1 (c) Ap-R; b-lactamase
           POLYLINKER  HindIII-SphI-PstI-SalI-XbaI-BamHI-SmaI-KpnI-SacI-EcoRI
           SELECTION
              #resistance  Ap
              #indicator beta-galactosidase
           SUMMARY  pUC18    #length 2686   #checksum 4465.
FEATURES             Location/Qualifiers
     source          1..2686
                     /organism="Cloning vector pUC19c"
                     /mol_type="genomic DNA"
                     /db_xref="taxon:174689"
ORIGIN
        1 tcgcgcgttt cggtgatgac ggtgaaaacc tctgacacat gcagctcccg gagacggtca
       61 cagcttgtct gtaagcggat gccgggagca gacaagcccg tcagggcgcg tcagcgggtg
      121 ttggcgggtg tcggggctgg cttaactatg cggcatcaga gcagattgta ctgagagtgc
      181 accatatgcg gtgtgaaata ccgcacagat gcgtaaggag aaaataccgc atcaggcgcc
      241 attcgccatt caggctgcgc aactgttggg aagggcgatc ggtgcgggcc tcttcgctat
      301 tacgccagct ggcgaaaggg ggatgtgctg caaggcgatt aagttgggta acgccagggt
      361 tttcccagtc acgacgttgt aaaacgacgg ccagtgaatt cgagctcggt acccggggat
      421 cctctagagt cgacctgcag gcatgcaagc ttggcgtaat catggtcata gctgtttcct
      481 gtgtgaaatt gttatccgct cacaattcca cacaacatac gagccggaag cataaagtgt
      541 aaagcctggg gtgcctaatg agtgagctaa ctcacattaa ttgcgttgcg ctcactgccc
      601 gctttccagt cgggaaacct gtcgtgccag ctgcattaat gaatcggcca acgcgcgggg
      661 agaggcggtt tgcgtattgg gcgctcttcc gcttcctcgc tcactgactc gctgcgctcg
      721 gtcgttcggc tgcggcgagc ggtatcagct cactcaaagg cggtaatacg gttatccaca
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      841 cgtaaaaagg ccgcgttgct ggcgtttttc cataggctcc gccccctga cgagcatcac
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      961 tttccccctg gaagctccct cgtgcgctct cctgttccga ccctgccgct taccggatac
     1021 ctgtccgcct ttctcccttc gggaagcgtg gcgctttctc atagctcacg ctgtaggtat
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     1141 cccgaccgct gcgccttatc cggtaactat cgtcttgagt ccaacccggt aagacacgac
     1201 ttatcgccac tggcagcagc cactggtaac aggattagca gagcgaggta tgtaggcggt
     1261 gctacagagt tcttgaagtg gtggcctaac tacggctaca ctagaagaac agtatttggt
     1321 atctgcgctc tgctgaagcc agttaccttc ggaaaaagag ttggtagctc ttgatccggc
     1381 aaacaaacca ccgctggtag cggtggtttt tttgtttgca agcagcagat tacgcgcaga
     1441 aaaaaaggat ctcaagaaga tcctttgatc ttttctacgg ggtctgacgc tcagtggaac
     1501 gaaaactcac gttaagggat tttggtcatg agattatcaa aaaggatctt cacctagatc
     1561 cttttaaatt aaaaatgaag ttttaaatca atctaaagta tatatgagta aacttggtct
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     1681 tccatagttg cctgactccc cgtcgtgtag ataactacga tacgggaggg cttaccatct
     1741 ggccccagtg ctgcaatgat accgcgagac ccacgctcac cggctccaga tttatcagca
     1801 ataaaccagc cagccggaag ggccgagcgc agaagtggtc ctgcaacttt atccgcctcc
     1861 atccagtcta ttaattgttg ccgggaagct agagtaagta gttcgccagt taatagtttg
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     1981 tcattcagct ccggttccca acgatcaagg cgagttacat gatcccccat gttgtgcaaa
     2041 aaagcggtta gctccttcgg tcctccgatc gttgtcagaa gtaagttggc cgcagtgtta
     2101 tcactcatgg ttatggcagc actgcataat tctcttactg tcatgccatc cgtaagatgc
     2161 ttttctgtga ctggtgagta ctcaaccaag tcattctgag aatagtgtat gcggcgaccg
     2221 agttgctctt gcccggcgtc aatacgggat aataccgcgc cacatagcag aactttaaaa
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     2521 cagggttatt gtctcatgag cggatacata tttgaatgta tttagaaaaa taaacaaata
     2581 ggggttccgc gcacatttcc ccgaaaagtg ccacctgacg tctaagaaac cattattatc
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//
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