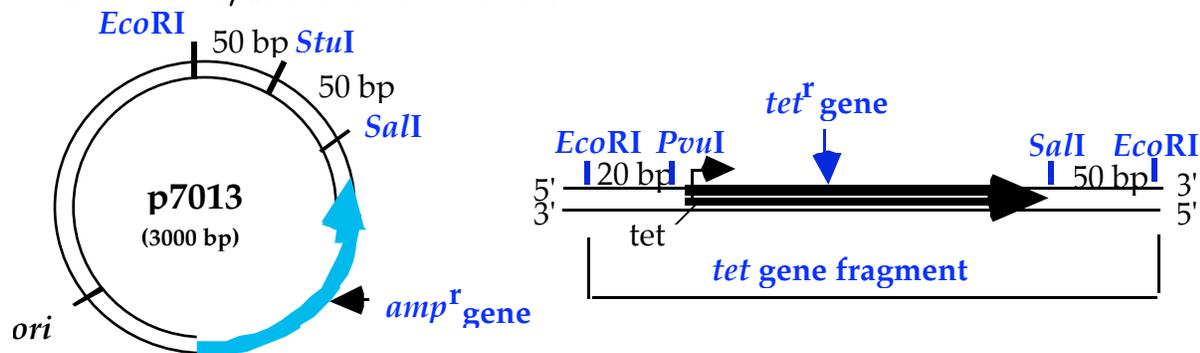


7.013 Section Problem: Recombinant DNA/Cloning

You have been given a purified DNA preparation of *p7013*, a 3000 bp circular plasmid, which contains a bacterial origin of replication (*ori*) and the gene for ampicillin resistance (*amp^r*). In addition, you also have a preparation of a 200 bp linear DNA fragment, isolated from an *EcoRI* restriction digest of some other DNA, which contains the entire gene for tetracycline resistance (*tet^r*). These two DNA molecules with their known restriction enzyme sites are shown below.



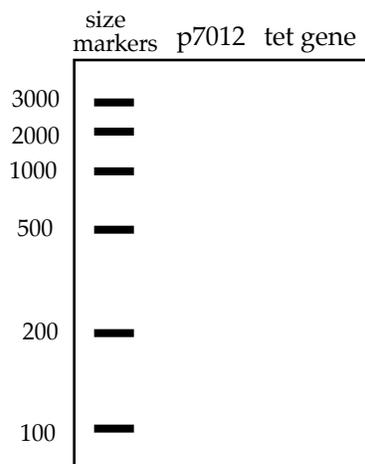
StuI cuts this sequence: 5' -AGG | CCT-3'
3' -TCC | GGA-5'

PvuII cuts: 5' -CAG | CTG-3'
3' -GTC | GAC-5'

SalI cuts: 5' -G | TCGAC-3'
3' -CAGCT | G-5'

EcoRI cuts: 5' -G | AATTC-3'
3' -CTTAA | G-5'

a) You digest *p7013* with *EcoRI*, and separate the *p7013* and the *tet* gene DNA samples by size on an agarose gel. Draw the pattern you would predict on the diagram below.



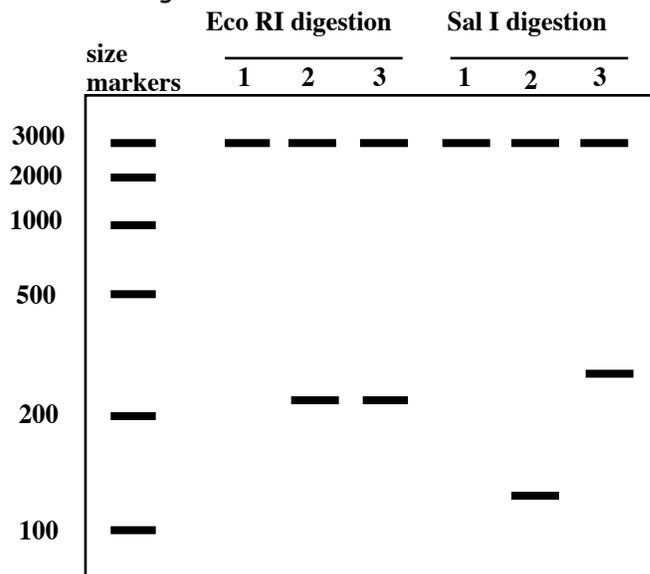
You want to produce a plasmid containing genes that confer both ampicillin and tetracycline resistance which can be called *p7013-AT*. To accomplish this task, you have available the above four restriction enzymes, *StuI*, *SalI*, *PvuII*, and *EcoRI*. The recognition sequences where the enzymes restrict the DNA are shown above.

In order to insert the *tet* gene fragment into the *p7013* plasmid, you decide to cut *p7013* with *EcoRI*, to produce a vector with the same complementary overhanging ends as the *tet* gene fragment. You then mix the two DNAs together in a tube and ligate them with the enzyme DNA ligase. You take your ligation mix and add it to *E. coli* cells* which are then spread on ampicillin-containing plates (solid medium in petri dishes) and grown overnight to isolate bacterial colonies.

*Under special conditions, plasmid DNA can enter *E. coli* cells. The plasmid DNA functions as normal DNA, *i.e.*, genes on the plasmid can be transcribed and translated. *E. coli* cells that have incorporated a plasmid are said to be "transformed".

b) Why is it necessary to grow the cells on plates containing ampicillin?

Each bacterial cell that received a plasmid should grow up into a bacterial colony on a petri dish containing ampicillin medium. When the plasmid DNA from three of these colonies: plasmids 1, 2 and 3, are analyzed by restriction enzyme analysis with *EcoRI* and *SalI*, a distinct pattern is observed for each of the three plasmids. The patterns seen after electrophoretic separation of the DNA fragments on a size separation gel are shown below along with DNA fragment size markers:



c) Make a diagram for each of the plasmid molecules, 1, 2 and 3 based on the restriction patterns shown in the gel above.

d) If the transformation mix (*E. coli* cells + plasmid DNA) had been spread on plates containing both ampicillin and tetracycline, which of the above plasmids would be able to grow?

e) You would like to generate a single product, the p7013-AT with the tet gene in one unique orientation. Use any two of the available restriction enzymes to design such a procedure.