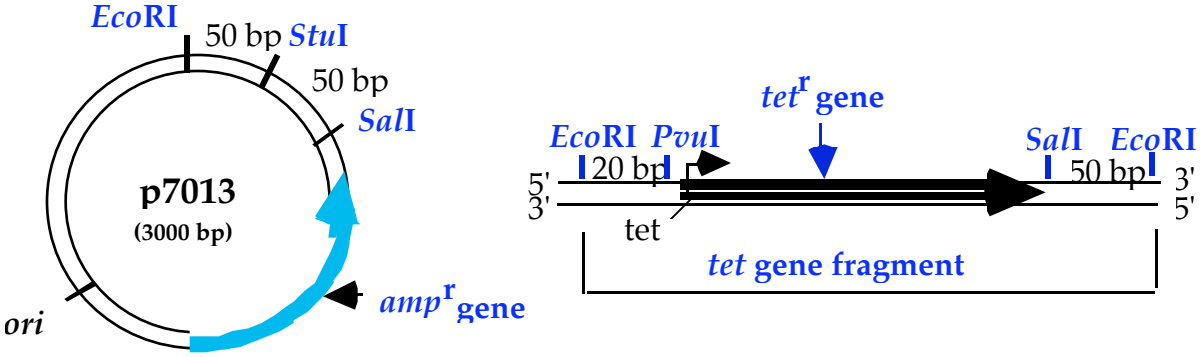
Section Problem: Recombinant DNA/Cloning

You have been given a purified DNA preparation of *p*7013, 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.



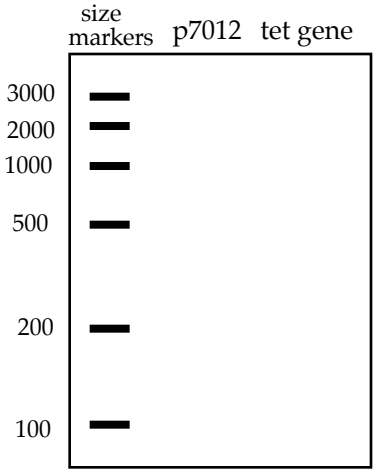
*Stu*I cuts this sequence: 5'–AGG|CCT–3' / 3'–TCC|GGA–5'

*Pvu*II cuts: 5'–CAG|CTG–3' / 3'–GTC|GAC–5'

*Sal*I cuts: 5'–G|TCGAC–3' / 3'–CAGCT|G–5'

*Eco*RI cuts: 5'–G|AATTC–3' / 3'–CTTAA|G–5'

a) You digest *p*7013 with *Eco*RI, and separate the *p*7013 and the *tet* gene DNA samples by size on an agarose gel. Draw the pattern you would predict on the diagram below.



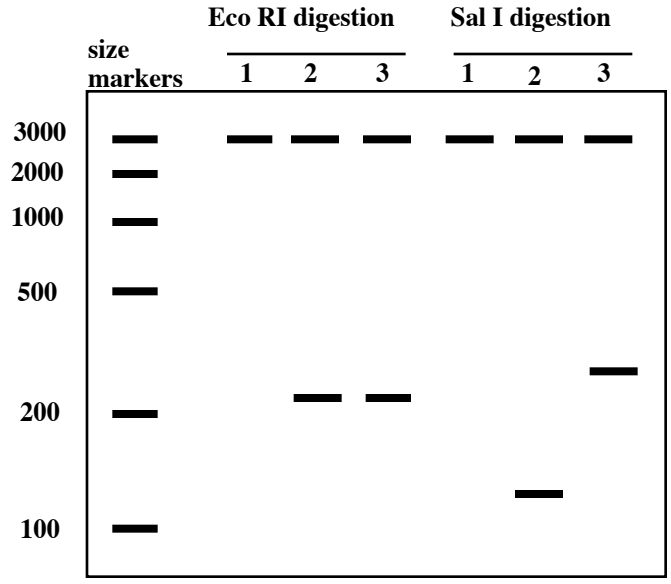
You want to produce a plasmid containing genes that confer both ampicillin and tetracycline resistance which can be called *p*7013-AT. To accomplish this task, you have available the above four restriction enzymes, *Stu*I, *Sal*I, *Pvu*II, and *Eco*RI. The recognition sequences where the enzymes restrict the DNA are shown above.

In order to insert the *tet* gene fragment into the *p*7013 plasmid, you decide to cut *p*7013 with *Eco*RI, 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 *Eco*RI and *Sal*I, 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.