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.

\[
\begin{align*}
\text{EcoRI} & \quad 50 \text{ bp} \\
\text{StuI} & \quad 50 \text{ bp} \\
\text{SalI} & \quad 50 \text{ bp}
\end{align*}
\]

\( p7013 \) (3000 bp)

\[
\begin{align*}
\text{amp}^R \text{ gene} \\
\text{ori}
\end{align*}
\]

\[
\begin{align*}
\text{EcoRI} & \quad 120 \text{ bp} \\
\text{PvuII} \quad & \quad \text{tet gene} \\
\text{SalI} & \quad 50 \text{ bp}
\end{align*}
\]

\( tet \text{ gene fragment} \)

\[
\begin{align*}
\text{StuI} \text{ cuts this sequence:} & \quad \begin{array}{c}
5' - \text{AGG} | \text{CCT} - 3' \\
3' - \text{TCC} | \text{GGA} - 5'
\end{array} \\
\text{PvuII} \text{ cuts:} & \quad \begin{array}{c}
5' - \text{CAG} | \text{CTG} - 3' \\
3' - \text{GTC} | \text{GAC} - 5'
\end{array}
\end{align*}
\]

\[
\begin{align*}
\text{SalI} \text{ cuts:} & \quad \begin{array}{c}
5' - \text{G} | \text{TCGAC} - 3' \\
3' - \text{CAGCT} | \text{G} - 5'
\end{array} \\
\text{EcoRI} \text{ cuts:} & \quad \begin{array}{c}
5' - \text{G} | \text{AATTC} - 3' \\
3' - \text{CTTAA} | \text{G} - 5'
\end{array}
\end{align*}
\]

a) You digest \( p7013 \) with \( \text{EcoRI} \), 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.

![Diagram showing the digestion pattern of p7013 and the tet gene fragment with EcoRI and SalI.]

You want to produce a plasmid containing genes that confer both ampicillin and tetracycline resistance which can be called \( p7013-\text{AT} \). To accomplish this task, you have available the above four restriction enzymes, \( \text{StuI} \), \( \text{SalI} \), \( \text{PvuII} \), and \( \text{EcoRI} \). 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 \( \text{EcoRI} \), 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 *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:

<table>
<thead>
<tr>
<th>Size Markers</th>
<th>Eco RIDigestion</th>
<th>Sal I Digestion</th>
</tr>
</thead>
<tbody>
<tr>
<td>3000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.