Construction of a P1 Lysogen of *E. coli*

**STRAINS**

*E. coli* SC109  
See strain checking exercise.  
\( \phi P1 \quad c1-100, \ Tn9 \) Derived by temperature induction of SC191 lysogen.

The *c1* gene codes for the main P1 repressor protein that turns off expression of genes for lytic development. i.e. *c1* expression is necessary for establishment and maintenance of P1 lysogeny. The *c1*-100 allele makes a temperature-sensitive repressor that allows us to control the lytic vs. lysogenic state, simply by switching water baths. The *c1*-100 repressor is functional at temperatures below 37°C. It denatures at higher temperatures leading to entry into lytic development. i.e. E.coli lysogenic for our P1 phage are not viable above 37°C.

Transposon Tn9 carries a gene for chloramphenicol resistance (*cat*) between 2 copies of IS1.

**PROCEDURE**

Inoculate strain SC109 into 2-3 ml of LB medium at 37°C and incubate overnight.

In the AM, subculture to overnight culture 1:50. (Transfer 200 ul into 10 ml sterile LB medium.) Incubate for 1-2 hours at 37°C.

Set up 3 tubes as follows (You can use either microfuge tubes or the small glass culture tubes:

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td>SC109 subculture</td>
<td>500 ul</td>
<td>500 ul</td>
<td>-</td>
</tr>
<tr>
<td>P1 phage suspension</td>
<td>50 ul</td>
<td>-</td>
<td>50 ul</td>
</tr>
<tr>
<td>0.1 M CaCl(_2)</td>
<td>50 ul</td>
<td>50 ul</td>
<td>50 ul</td>
</tr>
<tr>
<td>LB medium</td>
<td>-</td>
<td>50 ul</td>
<td>500 ul</td>
</tr>
</tbody>
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Incubate at 32°C\(^{(2)}\) for 1 hour\(^{(3)}\).

Transfer 10 ul drops from each tube to separate LB + Chloramphenicol + Streptomycin Agar plates and streak for individual colonies in the usual way. Incubate at 32°C.

Re-streak a Str\(^R\), Cam\(^R\) colony on an LB + Chloramphenicol + Streptomycin Agar plate and incubate at 32°C.

\(^{(1)}\) Supplies the divalent cation necessary for P1 adsorption to host cells.

\(^{(2)}\) Permissive temperature for phage repressor protein; necessary for establishment of lysogeny.

\(^{(3)}\) Phage adsorption, DNA injection and expression of the *cat* gene (necessary for chloramphenico-resistance) happen during this incubation.