Plaque Assay for Detecting Lysogeny

This is a qualitative screening of \textit{E. coli} strains for \textit{lysogeny}. The basis of the screening is that, during the growth of a population of lysogenic cells, the prophage in a few cells will spontaneously induce and, following lysis of the host cell, release free phage virions into the culture. The liberated phage will not kill cells of the parent culture because they are lysogenized. The bacteriophage genome present in these cells ("prophage") expresses the gene for a phage repressor protein.

A plaque assay technique is used to detect phage released by the lysogens. When we plate a small number of cells of a lysogenic strain on a lawn of a non-lysogenic inducator strain, some plaques are derived from free phage virions present in the culture due to spontaneous lysis of a small number of lysogenic cells. That's what "lysogenic" means, after all. Other plaques are derived from individual cells of the lysogenic strain. These cells grow into microcolonies that liberate free phage virions due to occasional induction and lysis of cells within the colony. The liberated phage infect cells of the indicator strain and clear the lawn in the vicinity of the lysogenic microcolony. Plaques derived from free phage can be distinguished from those derived from lysogenic microcolonies by examination of the lawn through a dissecting microscope.

\textit{Lysogeny} (n.) refers to an intricate and tenuous relationship between the genomes of \textit{temperate} (adj.) bacteriophages and the genomes of their host cells. \textit{Virulent} bacteriophages are incapable of lysogeny and therefore replicate only via a standard lytic infection process.

\textit{Prophage} (n.) refers to the genome of a temperate bacteriophage while it is stably integrated in a bacterial host cell. The prophage is replicated by the host cell and thus achieves vertical transmission as the host cell population proliferates. Occasionally, the prophage may be \textit{induced}, which means that it re-enters a typical lytic cycle, kills and lyses the host cell, and releases progeny virions into the surroundings. This represents horizontal transmission of the bacteriophage genome. For this reason, a cell carrying a prophage is said to be a \textit{lysogen} (n.) or to be \textit{lysogenic} (adj.).

The jargonistic density tends to distract our attention from more profound questions, namely,

What molecular mechanism is responsible for lysogeny?

What selective forces have driven the evolution of lysogeny and how does lysogeny, in turn, contribute to bacterial evolution?
Procedure

Strains

<table>
<thead>
<tr>
<th>UCSC#</th>
<th>Originator #</th>
<th>CGSG#</th>
<th>Relevant Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC051</td>
<td>C600</td>
<td>λ-</td>
<td>restrictive host</td>
</tr>
<tr>
<td>SC052</td>
<td>W3104</td>
<td>λ+</td>
<td>control</td>
</tr>
<tr>
<td>SC071</td>
<td>MG1655</td>
<td>λ-</td>
<td>control</td>
</tr>
<tr>
<td>SC168</td>
<td>WA803</td>
<td>5611</td>
<td>λ- mcrB1 hsdS3 permissive host</td>
</tr>
</tbody>
</table>

Note that all strains listed in the table are derived from *E. coli* K12.

C600 and WA803 are used as "indicator" or "host" strains for the plaque assay. C600 is just a more or less generic strain of *E. coli* that is not lysogenic for any bacteriophages and does not have any known mutations to resistance to any bacteriophage. WA803 carries mutations (*hsdS* and *mcrB*) that may make it more susceptible to infection by some bacteriophages ("permissive host").

W3104 is a known bacteriophage Lambda lysogen, and will play the role of "positive control" in the assay. MG1655 is the negative control.

You will also test an unknown *E. coli* strain newly isolated from nature.

1. Label 8 small sterile culture tubes and place then into the heating block to pre-warm. (See below.)

   Suggested numbering scheme for tubes and plates:

<table>
<thead>
<tr>
<th>HOST STRAINS</th>
<th>Sample Dilutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC051 (C600) restrictive host</td>
<td>SC052 (W3104) λ+ control</td>
</tr>
<tr>
<td>SC168 (WA803) permissive host</td>
<td>SC071 (MG1655) λ- control</td>
</tr>
<tr>
<td></td>
<td>1R</td>
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<td></td>
<td>1P</td>
</tr>
</tbody>
</table>
2. Make up 16 dilution tubes with 10 ml sterile saline each.

3. Transfer 1 ml of the W3104 culture a sterile microfuge tube and spin at high speed for 5 minutes to pellet the cells. Balance the tubes in the rotor.

4. Make serial dilutions to $10^6$ of:  
   - MG1655 culture
   - W3104 culture
   - W3104 supernatant
   - Unknown culture

   The dilutions can be made in 2 steps. Transfer 10 ul of culture into 10 ml of TMG Buffer ($10^{-3}$ dilution). Vortex the tube and then transfer 10ul to a second 10 ml of TMG (second $10^{-3}$ dilution).

   Mix tubes thoroughly, and change pipette tips for each transfer.

   The TMG diluent contains magnesium. Divalent cations are frequently essential to the stability of bacteriophage, and often facilitate their initial attachment to host cells.

5. Plate each of the four $10^6$ dilutions on BOTH host strains using the overlay method (see below). This means a total of $4 \times 2 = 8$ plates.
Plating a Lawn by the Overlay Method

1. Check that your heating block is 40-45°C.
   
   This is usually near position 5 of the LOW setting on the new style blocks.

2. Put a sufficient number of small sterile tubes in the heating block to pre-warm.

3. Add 0.1 ml culture of “indicator” strain C600 to 4 of the tubes.
   
   Add 0.1 ml culture of “indicator” strain WA803 to the other 4 tubes.

4. Add 0.1 ml of the appropriate sample to each tube.

5. Mix the contents of each tube gently and incubate 5 minutes.

6. Add 3.5 ml melted top agar directly from the 48°C bath to each tube.
   Mix and pour tube contents onto the surface of pre-warmed and labeled agar plates.
   Immediately rock the plates to distribute the agar overlay.
   Allow to solidify at room temperature for at least 5 min.

7. Incubate plates at 37°C overnight.

8. After incubation, count plaques and examine plates with a dissecting microscope.
Assignment

1. The indicator strain WA803 has several mutations (hsdS and mcrB) that may increase its sensitivity to infection by bacteriophages.

Did you see evidence of this?

What are the functions of the hsdS and mcrB gene products, and how can eliminating them make an E. coli strain more sensitive to bacteriophage infection? You can look up the genes in the Coli Genetic Stock Center strain database (http://cgsc.biology.yale.edu/cgsc.html). Use the "Site Query Form" to search for the gene name.

2. Explain in detail the basis of everything you observed on the strain plates. Specifically, decide whether or not there is evidence that your unknown strain is a lysogen.