Genetic Screen: Generalized

Step 1: Mutagenize gene of interest
Step 2: Put DNA in cells (if not there already)
Step 3: Look for mutant phenotype

Genetic Screen: 20.109 (F12)

Step 1: Mutagenize gene of interest
Step 2: Put DNA in cells
Step 3: Look for mutant phenotype

A long time ago in a galaxy far, far away...

K+P Library Variations: oligo design

Mutagenesis based on Stratagene’s “QuickChange”

Two problems here:
• Mismatch
• DNA nicked

So what will happen if we put this pool of DNA into cells?
Mutagenesis based on Stratagene’s “QuickChange”

- Step 1: Mutagenize gene of interest
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K-P Library Variations: building library

- Starting
- Round 1: Denature, Anneal, Extend
- Round 2

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Ways to move DNA into cells

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“The art and design of genetic screens: E. coli”

<table>
<thead>
<tr>
<th>SELECTION</th>
<th>SCREEN</th>
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</thead>
<tbody>
<tr>
<td>e.g. growth on a particular sugar</td>
<td>e.g. fermentation of a particular sugar</td>
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<tr>
<td>e.g. resistance to a virus or an antibiotic</td>
<td>e.g. chromogenic substrate</td>
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<td>e.g. observable (luciferase or GFP)</td>
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Below pH 6.8

Above pH 8.0
Step 1: Mutagenize gene of interest
Step 2: Put DNA in cells
Step 3: Look for mutant phenotype
Step 4: Study the behavior of the mutant cells

Genetic Screen: 20.109 (F12)

DNA sequence \rightarrow amino acid sequence of protein

Possible mutant “phenotypes”

β-galactosidase Activity (Miller Units)

<table>
<thead>
<tr>
<th>WT</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
<th>M4</th>
<th>M5</th>
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<tbody>
<tr>
<td>dark</td>
<td>light</td>
<td></td>
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</table>

M1: PCB
M2: Cph8 kinase dead
M3: K^P
M4: Cph8 truncation
M5: K^P^*