System Engineering

20.109(F12) M2D6 lecture 10.30.12

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

<table>
<thead>
<tr>
<th>SELECTION</th>
<th>SCREEN</th>
</tr>
</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></td>
<td>e.g. observable (luciferase or GFP)</td>
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below pH 6.8

above pH 8.0

Genetic Screen: 20.109 (F12)

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

The C-dog

DNA sequence → amino acid sequence of protein

Dideoxy Sequencing: “Sanger” Method

ddNTP: cannot be extended
Automated “one pot” sequencing

Primer: ATTAGACGTCCG
Template: TAATCTGCAGGC
Cocktail: dNTPs

How could a change in protein stability affect β-gal?

<table>
<thead>
<tr>
<th></th>
<th>Autophos rate</th>
<th>Kinasing rate</th>
<th>P-rase rate</th>
<th>Amount OmpR/P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>5 “units”</td>
<td>3 “units”</td>
<td>2 “units”</td>
<td>1</td>
</tr>
</tbody>
</table>

SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Loading dye has glycerol, SDS, reducing agent
Samples boiled before loading

Detection through Western Analysis

SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Summary
Mutant Hunt Strategies
Screens Cobras
Bacterial Photography K’P
Analysis DNA sequencing Protein expression

http://www.genscript.com/product_001/western_application/prp_id/00065/op/detail/Uvrag_Antibody_Analysis.html