Polyclonal Antibody Production

Which protein would we like to have an antibody recognize?

Affinity Chromatography

Affinity Purification

These properties can be exploited to separate individual protein from mixture

Alberts “Essential Cell Biology”
Figure 3. Superior yields and better purity were obtained when using His60 Ni Superflow for purification of recombinant His-tagged protein—as compared to Ni-NTA Superflow resin. Clontech’s pEcoli Linear Expression System was used to express 6xH6-AcGFP1 in E. coli. Equivalent amounts of the same sample were used for comparing His60 Ni Superflow Resin purification (Panel B) with Competitor Q Ni Resin purification (Panel A). Lane M: Molecular weight marker. Lane D5: Original sample. Lane F1: Flowthrough. Lanes W1 and W2: Wash. Lane E1–E4: Eluted 6xH6-AcGFP1 fractions.


Colorimetric Detection

Colorimetric Chemistry

Key

Target protein
Primary antibody
Secondary antibody
Enzyme conjugate
Substrate
Product
Precipitate

http://www.bio-rad.com/

A module in review....

Science? Art? Education? Marketing?

Contagion - Bacteria Billboard

007 meets E. coli

Steganography by Printed Arrays of Microbes (SPAM)

Not all machines are useful

PNAS (2011) 108(40):16510
"I hate to sound elitist," Tom Knight says, "but you hear a lot of people talking in a naive way about doing very complex and difficult things that are challenging projects for the very best scientists and engineers in the world."

There's an elitism around science," Eri Gentry says. "I want to rid American culture of that fear of science by giving the community ways to have fun with it."

A story of ownership and sharing

Abstracted View of Bacterial Photography

<table>
<thead>
<tr>
<th>Photons</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (cells are in the light)</td>
<td>0</td>
</tr>
<tr>
<td>0 (cells are in the dark)</td>
<td>1</td>
</tr>
</tbody>
</table>

β-gal activity reflected with indicator compounds

The 2CS paradigm: in general

\[
1000 \times \frac{(\text{Abs}420 - (1.75 \times \text{Abs}550))}{(t \times \text{Abs}600)}
\]
Preventing cross-talk: phosphatase activity

Assessing Specificity: Phosphotransfer Profiling

Circuitry and Measurement

New tools for reliable engineering of complex biological systems

Physical Composition of Standard Biological Parts

Looking for parts: genetic screening

- histidine kinases exhibit a strong kinetic preference in vitro for their in vivo cognate substrate
- specificity based on molecular recognition

Nature (2005) 438, 441-442
K-P+ Library Variations (in blue)

<table>
<thead>
<tr>
<th>EnvZ</th>
<th>A2397</th>
<th>G2605</th>
<th>V2617</th>
<th>S2422</th>
<th>H2433</th>
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</thead>
<tbody>
<tr>
<td>Cphl = Cphl/EnvZ</td>
<td>ASS3</td>
<td>G954</td>
<td>V595</td>
<td>S555</td>
<td>H557</td>
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<td>GCC</td>
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<td>CAC</td>
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<tr>
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<td>GAC</td>
<td>GTA</td>
<td>AGT</td>
<td>CAC</td>
</tr>
</tbody>
</table>

pos aa  | Val | Ala | Asp | Asn | Gln | Lys | Met | Ser | Thr | Cys | Gly | His | Phe | Pro | Arg |

NOTE: no stop codons should be in mix

Site-Directed Mutagenesis

Starting -> Round 1

Denature

Anneal

Extend

Remove with DpnI digest

Automated “one pot” sequencing

Primer: ATTAGACGTCCG

Template: TAATCTGCAGGC

Cocktail: +dNTPs, +ddNTPs, +polymerase, +buffer

Beyond the C-dog

protein stability?
protein activity?
cross-talk with other systems?

http://publications.nigms.nih.gov/theneugenetics/images/ch1_trans.jpg

Detection through Western Analysis

http://www.genscript.com/product_001/western_application/grp_id/60065/op/detail/UvrAg_Antibody_Analysis.html

Possible mutant “phenotypes”
Observation
7/7 groups got >100 colonies with 20ul EP mix
5/6 groups got no colonies, even with 200 ul EP mix!!

What could be the explanation?
• User error
• Materials
• Scientific

Troubleshooting 101

Electroporation

Screen on Tetrazolium

Some of my hopes...