Announcements

Quiz

Pre-lab Lecture
  - Genetic control elements
  - Sequencing recap
  - Writing a figure caption
  - Today in Lab (Mod 1 Day 5)
Announcements

• No quiz next time (full day)!
• Methods HW general comments:
  – Cite resources: Nagai and Watcut website
  – Give concentrations, not volumes (when possible)
  – Compositions available in “Reagent List” (Part 4) on each lab protocol
The lac operon

These three genes encode **metabolic enzymes**

![Diagram of the lac operon]

- **lacI**
- **control**
- **lacZ**
- **lacY**
- **lacA**

Encodes a **repressor protein** that binds to the **control area** turning it **OFF**.

In turn, if lactose binds to the **rep. protein**, it is made **inactive**, turning **ON** expression of **Z, Y, A**.
Induction of a chosen protein

**Gene Expression Table**

<table>
<thead>
<tr>
<th>lacI</th>
<th>control</th>
<th>T7RNAP (polymerase)</th>
<th>lacZ</th>
</tr>
</thead>
</table>

**T7RAP** gene is expressed in presence of lactose or analogue.

**bla** promoter is constitutively on.

**T7** is turned on in presence of T7RNAP.
BL21(DE3) bacterial strain

DE3: bacteriophage (virus) used to integrate lac construct into E. coli

pLysS: protein that produces lysozyme, which binds to T7RNAP, reducing "leaky" expression. Retained by chloramphenical selection.
## Sequencing reactions

Dideoxy method: no 3’ OH → can’t elongate
Run 4 rxns: (d)dT, dA, dG, dC and 3 others

<table>
<thead>
<tr>
<th>Reactions</th>
<th>ddT</th>
<th>ddA</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAAATT</td>
<td></td>
<td>TAAATT</td>
</tr>
<tr>
<td>AT*</td>
<td></td>
<td>A*</td>
</tr>
<tr>
<td>ATT*</td>
<td></td>
<td>ATTTA*</td>
</tr>
<tr>
<td>ATTTT*</td>
<td></td>
<td>ATTTA*</td>
</tr>
<tr>
<td>ATTTTT*</td>
<td></td>
<td>ATTTAAA*</td>
</tr>
</tbody>
</table>

* = radioactive or fluorescent label

Gel

<table>
<thead>
<tr>
<th>ddT</th>
<th>ddA</th>
</tr>
</thead>
</table>

A
G
C
T

different fluor.
Figures: Style and Scope

• **Title:** concise, informative → gives overall result/goal. Often similar to Results sub-headings.

• **Caption:** give context for result, from big to small.
  – Introduce what we are looking at.
  – Include just enough methods to understand results.
  – Define all elements (e.g., DNA ladder)
  – Cover primarily facts (results and/or expectations), limiting complex interpretations for Discussion.

• **Aesthetics** simplicity, clarity → at-a-glance labeling
Figure 3. CCL21 impacts naïve T cell proliferation under conditions of rare Ag-specific T-DC encounters. Co-cultures comprising 9% OVA-specific OT-II CD4+ T cells, 81% C57Bl/6 CD4+ T cells, 5% OVA-mDC and 5% iDC with/without CCL21 were analyzed by flow cytometry at 85 h. (A) Sample CFSE histograms are shown for control (left, iDC only) and experimental (right, with OVA-mDC) conditions. (B) OTII cell recovery for all conditions is shown. Ave ± std. dev. for 3 wells per condition. [* indicates bracketed conditions statistically different (p ≤ 0.05)]. (A-B) are from 1 representative of 5 experiments.
Figure Captions: Practice

Fig. N functional title descriptive caption

• Agarose gel title ideas:
  gel analysis to verify (and measure) fragments after SDM-mutagenesis

• Overview sentence topics:
  introduce idea of SDM to IPC

• Supporting detail topics:
  - expected and observed bands sizes
  - what are samples

• Methods to include or not: almost none
Today in Lab: Workflow

Check OD until mid-log (0.6mL samples!)

- IPTG control: ice, eventually spin down

1.5mL

2-3 hours at 37°C

4.5mL (green)

Spin to check pellet colour

1.5mL

Analysis: gel, sequencing

Measure post-growth OD
Pour liquid on top, re-pellet

1.5mL more (3mL total)

Grow rest O/N CRT
Teaching faculty will pellet

3mL
Today in Lab: Samples

• Start with four DE3 samples carrying plasmid
  – WT
  – S101L
  – X#Z candidates 1 and 2
• After gel and sequencing analysis, pick just one X#Z to continue working with
• End of day, “hand in” 6 pellets, or (3 pellets, 3 cultures, and 3 eppendorfs) to teaching faculty