Announcements, Review HW

Lab Quiz

Pre-lab Lecture
- DNA Extraction (Miniprep)
- Diagnostic Gel Review
- Intro to Tissue Culture
- Figure Captions (or at OH)
- Today in Lab: M1D5
Results of pre-lab interaction survey

• Mostly #3 (hate talking in class), couple of straight up #1 (too easy) and #2 (too hard)

• Options/thoughts:
  – If the questions are fine, I could call on people instead of wait for volunteers... can be awkward though!
  – Alternatively you could talk in groups of four and then have one person speak for the group.
  – I will ask for others to speak up if only 1-2 people are talking; you don’t need to self-censor.
  – Hearing things put another way (from a peer) can be very helpful to peoples’ learning.
  – Explaining things yourself, out loud, is one of the best ways for you to learn!
Couple of HW notes

• PCR update
  – universal ORF finding
  – *minimum* primer length
  – reaction and cycling rules of thumb

• Figures/captions
  – need better labeling and full captions
  – will discuss in pre-lab (if time) or Monday OH
Announcements

• Lab practical next time! HW returned Monday.
• Please post your colony counts in talk page table before leaving – we’ll discuss them next time
• Vacuum aspirators contain bleach for biohazardous waste (i.e., cells)
  – after bleach treatment, these go down the sink
• Chemical waste and sink-safe chemicals (w/out cells) should not be aspirated
  – the former is a safety risk, the latter just a hassle
## Extracting DNA from XL1-Blue

<table>
<thead>
<tr>
<th>Step</th>
<th>Contains</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prepare</td>
<td>EDTA Buffer, glucose</td>
<td>weakens cell envelope, otherwise stable</td>
</tr>
<tr>
<td>Lyse</td>
<td>SDS NaOH</td>
<td>disrupt soluble lipid membrane proteins, denature ds to ss DNA</td>
</tr>
<tr>
<td>Neutralize</td>
<td>Acetic acid/KAc</td>
<td>neutralize pH, precipitate SDS, renature DNA, DNA &quot;crashes&quot;</td>
</tr>
<tr>
<td>Transfer</td>
<td>N/A</td>
<td>keep supernatant</td>
</tr>
<tr>
<td>Wash, collect</td>
<td>A) EtOH</td>
<td>precipitates</td>
</tr>
<tr>
<td></td>
<td>B) dry, water</td>
<td>EtOH interferes</td>
</tr>
</tbody>
</table>
## Diagnostic DNA Gels

### Choosing restriction sites for digest

<table>
<thead>
<tr>
<th>Kbp</th>
<th>BKB</th>
<th>+INS</th>
<th>+3 INS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>~4.7</td>
<td></td>
<td></td>
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</tr>
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<td>~0.65</td>
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<td>~1.2</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>~9.6</td>
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</tr>
</tbody>
</table>

- XbaI
- EcoRI
- BamHI
- XhoI
Practice Tissue Culture (TC)

- MES = murine embryonic stem cells
- Adherent cells
- Add trypsin to remove from dish
- Re-plate at lower density → “passage” cells
- Practice counting
- More about mammalian cells next time

Image from http://www.stemcellresources.org/library_images.html
Figures: Style and Scope

• Title: concise, informative, tells overall goal/result
• Caption: gives context for result from big → small
  – Introduce what we are looking at
  – Include just enough methods to understand result
  – Define all elements (e.g., DNA ladder)
  – Cover primarily facts, not interpretation
    e.g., observed and/or expected sizes
• Aesthetics: simplicity, clarity → at-a-glance labeling (e.g., some ladder band sizes)
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.
Today in Lab

• Miniprep three Δ5-EGFP candidates, and bacteria transformed with pCX-NNX
  – tip: orient tubes in centrifuge
  – pCX-NNX = control for your technique
• Count and post colony #s
• TC practice session (half of class at a time)
  – don’t need notebook, just a piece of scrap paper
• Set up digests
  – we will add loading dye if lab runs late
  – tip: make reaction cocktail → efficiency