DNA Engineering:
M1D5 Lab Talk

20.109 (F12)
09.27.12
<table>
<thead>
<tr>
<th>Team Color</th>
<th>5 ng pCX-EGFP</th>
<th>bkb+ insert, no ligase</th>
<th>bkb + ligase</th>
<th>bkb+ insert, + ligase*</th>
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*list each plate
Interpreting ligation results

- Did the positive control work for many groups?
- No ligase results?
- Bkb + ligase tells you?
- What variations may be giving differences seen for experimental samples?

\[ \text{bkb : insert} \]

(incubation time, salt in ligation tells recovery not equal)
From cells on a plate to a tube of DNA....
Isolating DNA from bacteria: DNA Miniprep

- Harvest cells + aspirate media
- Resuspend them
- Lyse them + raise pH
- Restore pH
- Concentrate + wash
Diagnostic Digests: XbaI and RI?

- insert  + insert  >1 insert
Diagnostic Digests: BamHI and XhoI?

Also consider: compatible buffers, reaction temperatures, results if one enzyme doesn’t cut
Also today: TC for fun….

Proliferation of fibroblasts

Split/passage with trypsin

MES

Monocellular layer of fibroblasts

1:100

1:400
Figuring out how many cells you have

Count cells in 4 + 4 corners

\[
\text{Avg} \times 10,000 \text{ cells/ml}
\]
Mod 1: what's left?

MID1: V. lab
Lcr5 cont. pt II
+ lipofed

MID2: FACS

10.11.12

[signature]
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