

MID4: Cloning

2/19/15

I. Upcoming office hours:

- Friday, 3-4pm
- No office hours on Monday, will try to respond to email

Announcements

- Please make sure your ligation spreadsheet works!
- Hand in up front in folder:
 1. Primer table + summary paragraph
 2. Schematic diagram
 3. Methods section

Schedule for today:

1. Leslie here to talk about figures (~2pm)
2. PCR purification + set-up gel (~2:30pm)
3. “Pre-lab” lecture while gel is running (~3:00 pm)
4. Djenet will help you image your gel — quick estimates (~3:30)
5. Set-up ligation —> transformations —> plating (~5pm)

FNT Assignment(s)

Next time Part I due on Stellar — Figure of gel + Good caption

Homework

[add intro text](#)

[add topic](#) - [change topic order](#)

[view all submissions](#) - [find submission](#)

General

[edit topic](#) - [delete topic](#) - [add assignment](#)

[M1D4 FNT TR -- Gel Figure](#) [edit](#) - [delete](#)

Due 25 February 2014 1:00 p.m. Posted 20 February 2014 10:11 a.m.

[M1D4 FNT WF -- Gel Figure](#) [edit](#) - [delete](#)

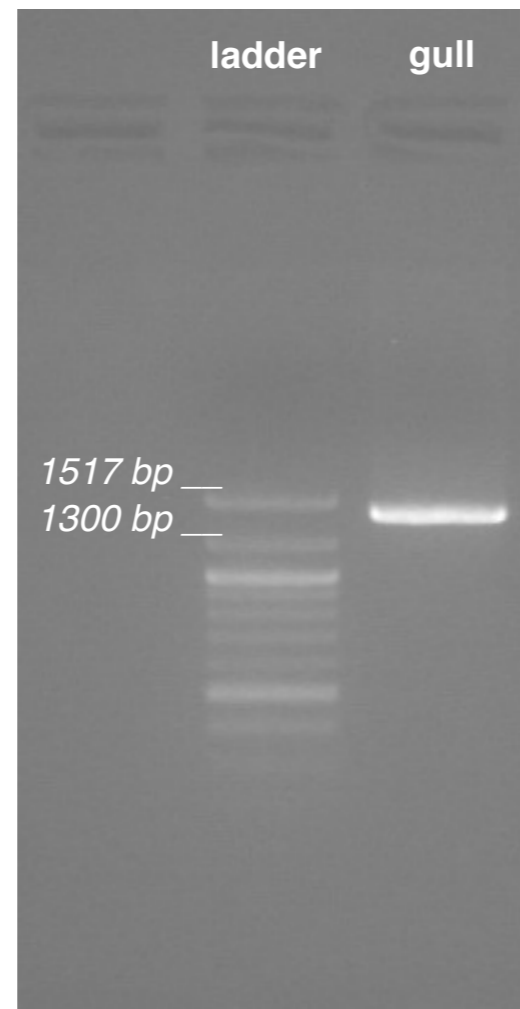
Due 26 February 2014 1:00 p.m. Posted 20 February 2014 10:12 a.m.

Bird Microbial Communities -- MID4 Overview

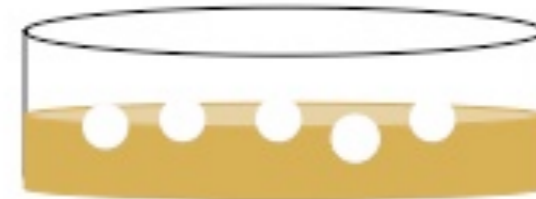
gel purify, clone
and transform



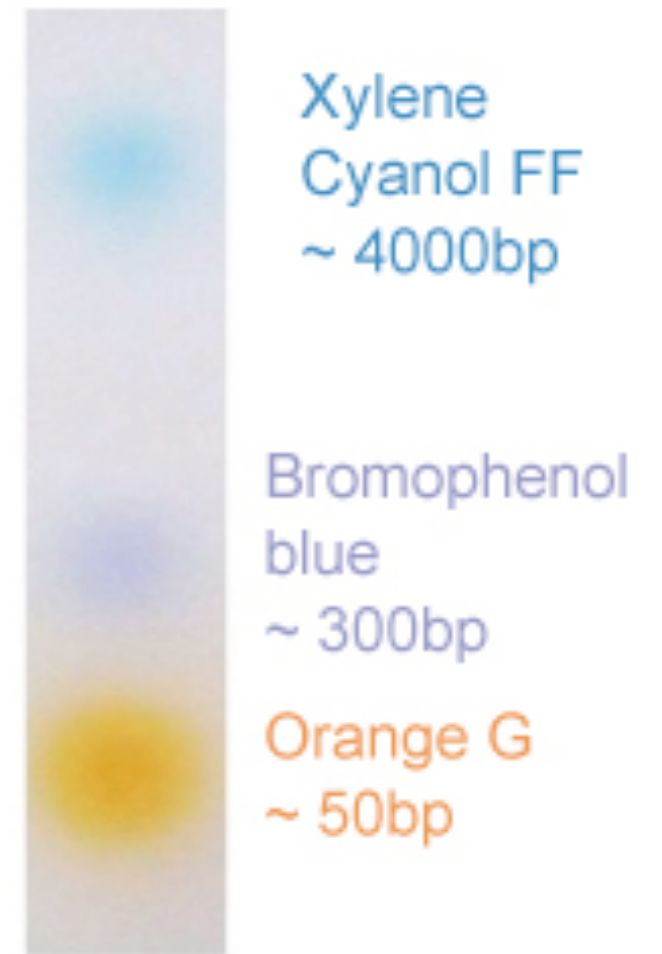
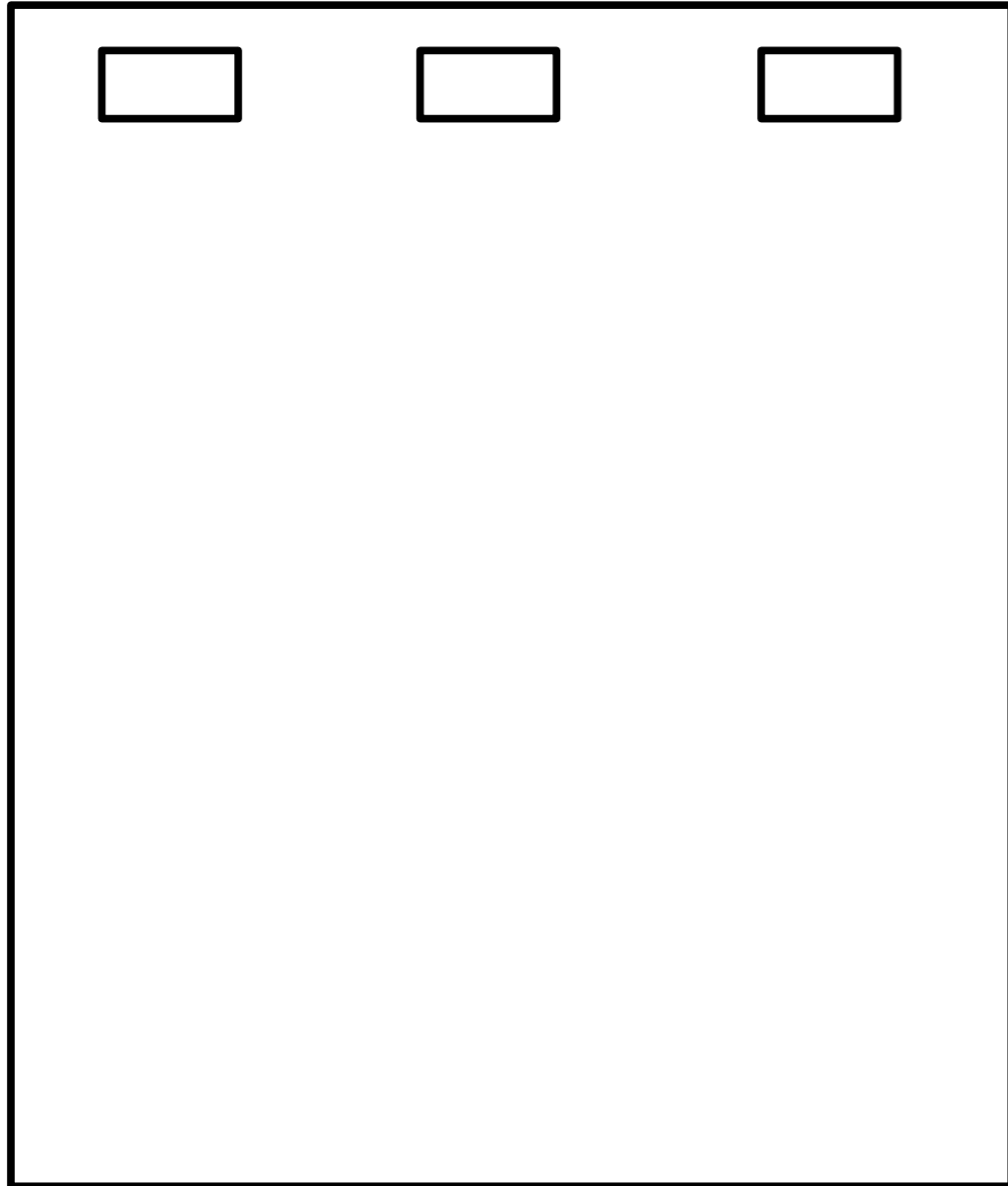
DAY 4



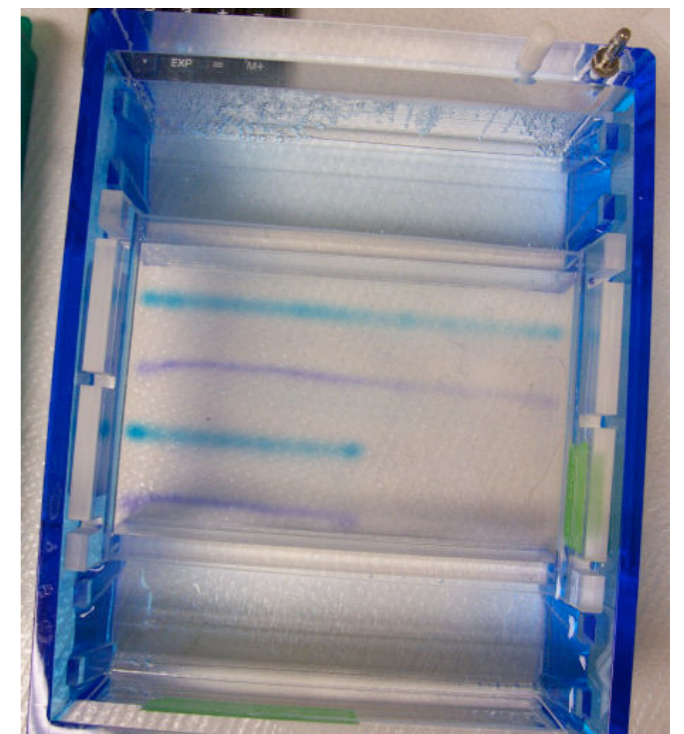
Individual 16S
cell colonies



How do we visualize the DNA?

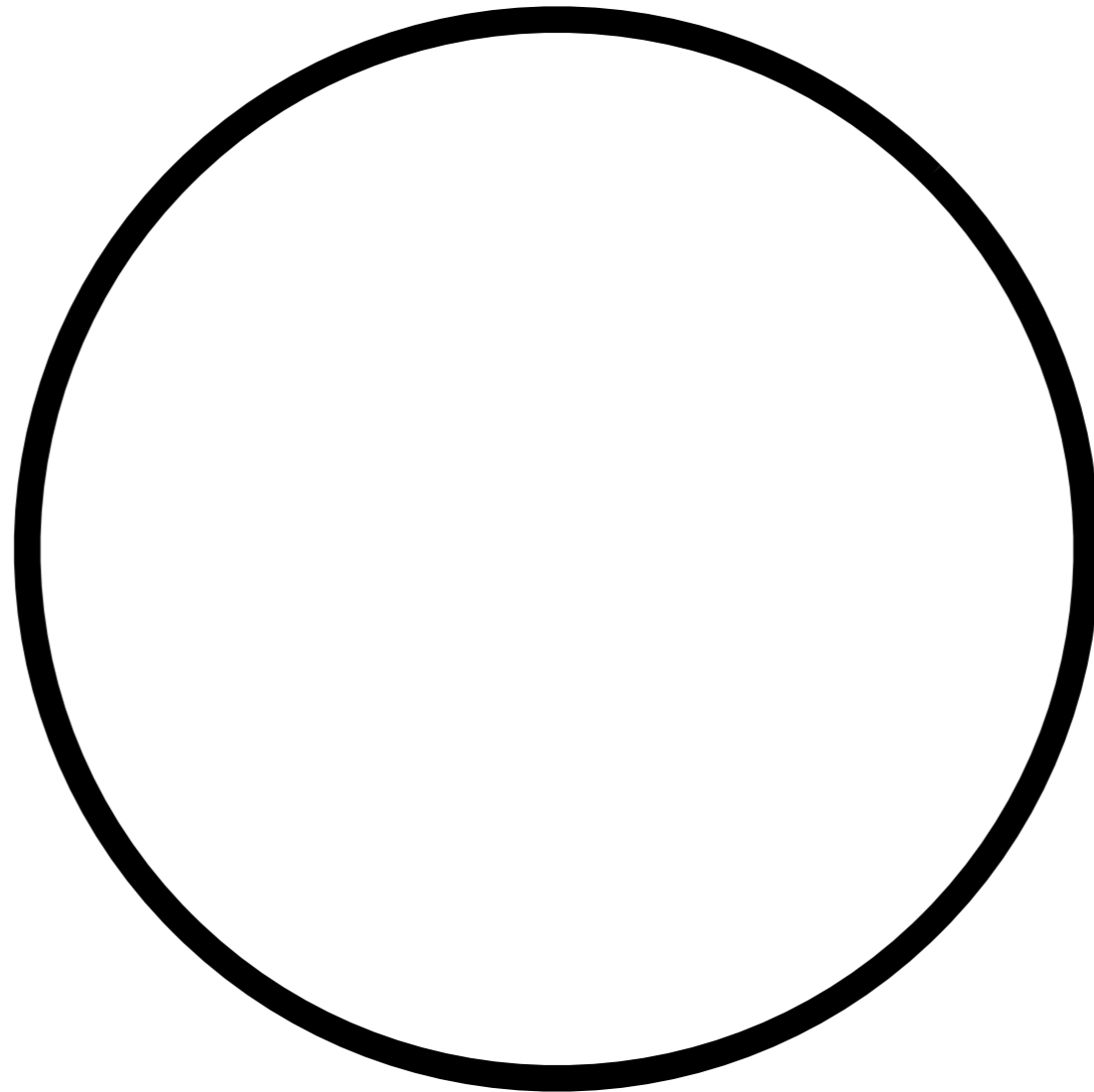


www.base-asia.com



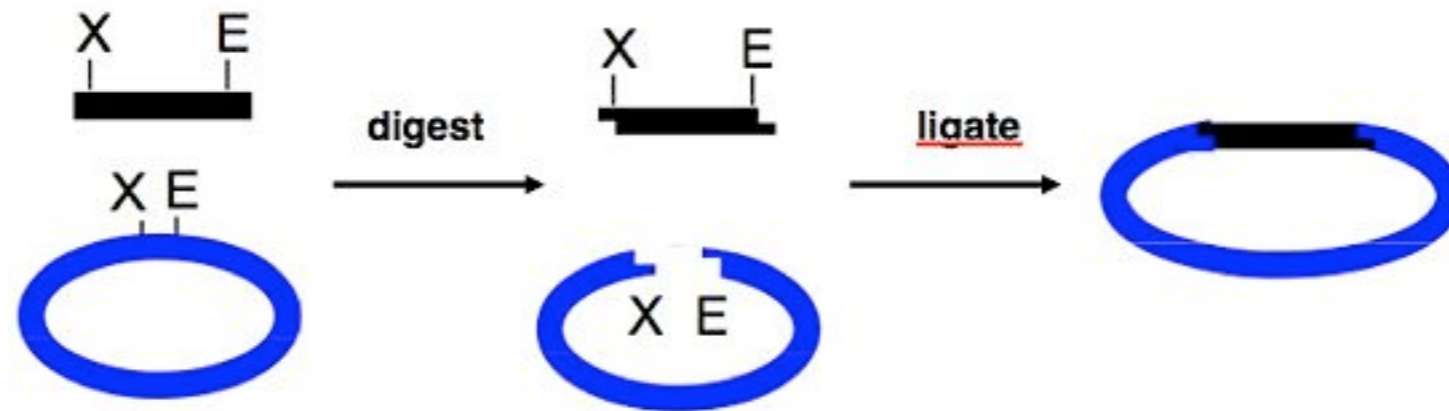
Cloning

Vector = Plasmid = Circular DNA

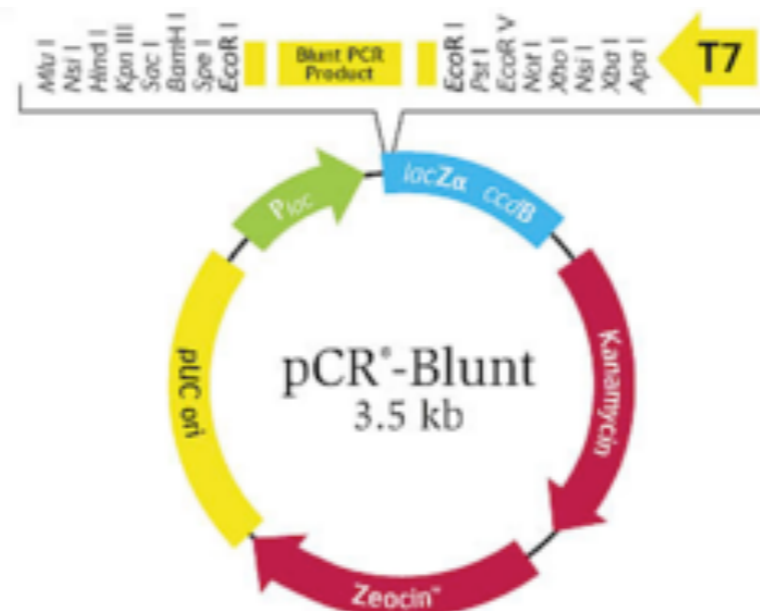


Preview of MID4: Cloning

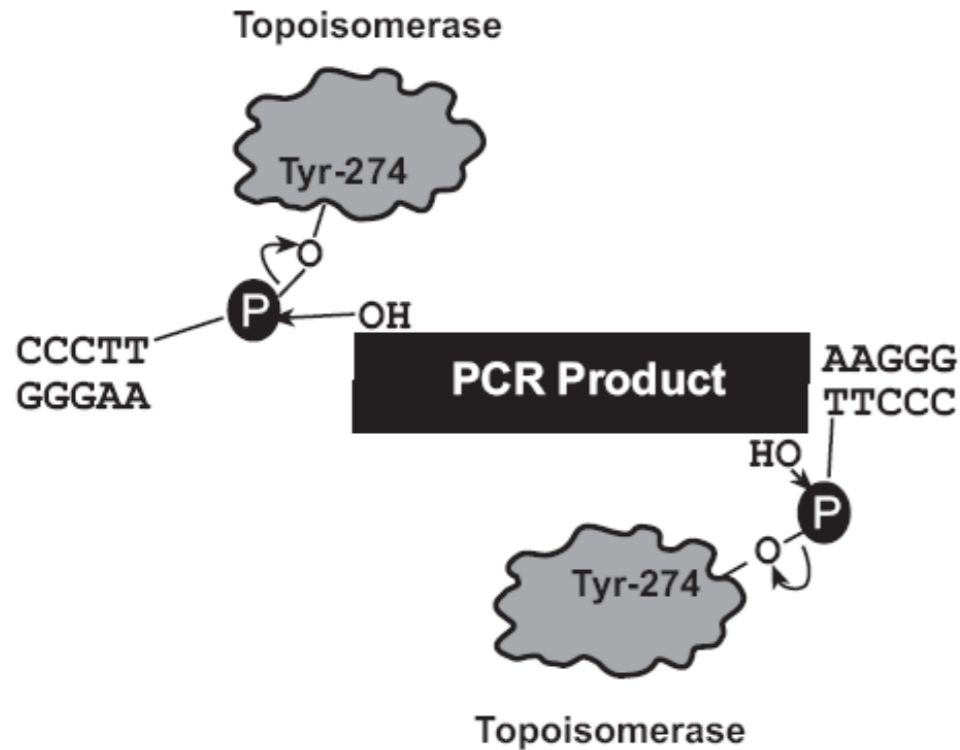
You may have done this before:



You can also do it this way:



Our System:



M13 Reverse priming site

201 CACACAGGAA ACAGCTATGA CCATGATTAC GCCAAGCTAT TTAGGTGACA CTATAGAATA
GTGTGTCCTT TGTCGATACT GGTACTAATG CGGTTTCGATA AATCCACTGT GATATCTTAT

Nsi | *Hind* III | *Asp*718 I | *Kpn* I | *Eco*136 II | *Sac* I | *Bam*HI | *Spe* I

CTCAAGCTAT GCATCAAGCT TGGTACCGAG CTCGGATCCA CTAGTAACGG CCGCCAGTGT
GAGTTCGATA CGTAGTTCGA ACCATGGCTC GAGCCTAGGT GATCATTGCC GCGGTCACA

*Eco*R I

GCTGGAATTC GCCCTT **Blunt PCR Product** AAGGGCGAATTCT GCAGATA
CGACCTTAAG CGGGAA TTCCCGCTTAAGA CGTCTAT

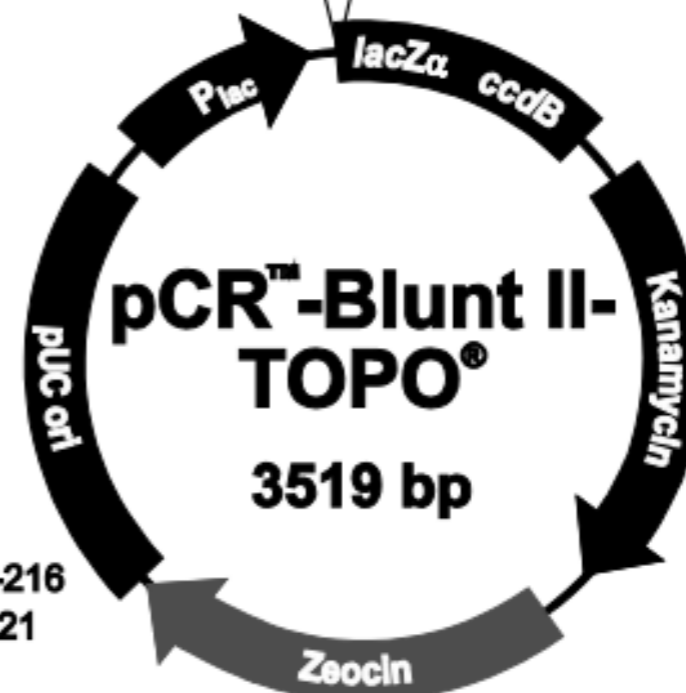
*Eco*R I | *Pst* I | *Eco*R V

Nof I | *Xho* I | *Nsi* I | *Xba* I | *Dra* II | *Apa* I | T7 promoter/priming site

TCCATCACAC TGGCGGCCGC TCGAGCATGC ATCTAGAGGG CCCAATTCGC CCTATAGTGA
AGGTAGTGTG ACCGCCGGCG AGCTCGTACG TAGATCTCCC GGGTTAAGCG GGATATCACT

M13 Forward (-20) priming site

GTCGTATTAC AATTCACTGG CCGTCGTTTT ACAACGTCGT GACTGGGAAA ACCCTGGCGT 476
CAGCATAATG TTAAGTGACC GGCAGCAAAA TGTTGCAGCA CTGACCCTTT TGGGACCGCA



Comments for pCR™-Blunt II-TOPO®
3519 nucleotides

lac promoter/operator region: bases 95-216

M13 Reverse priming site: bases 205-221

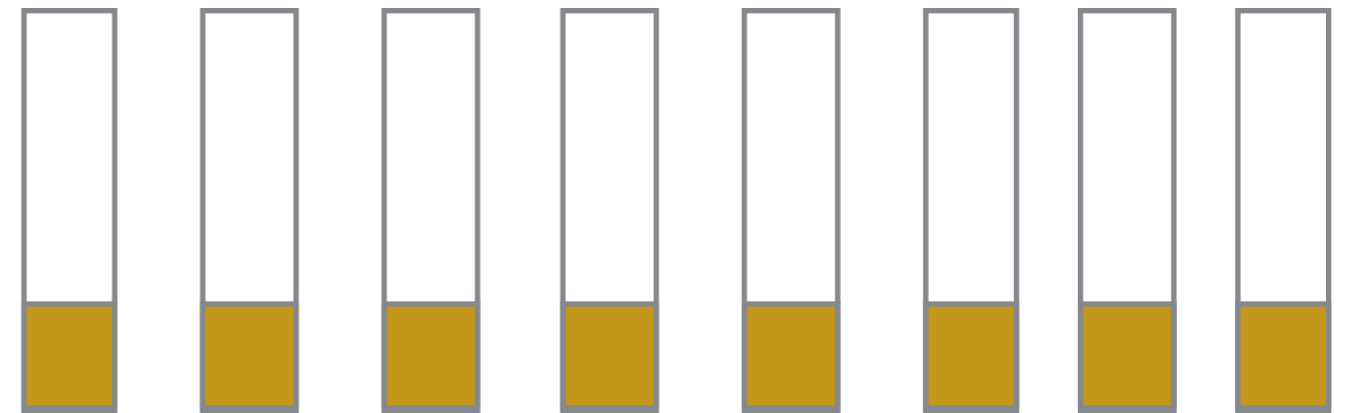
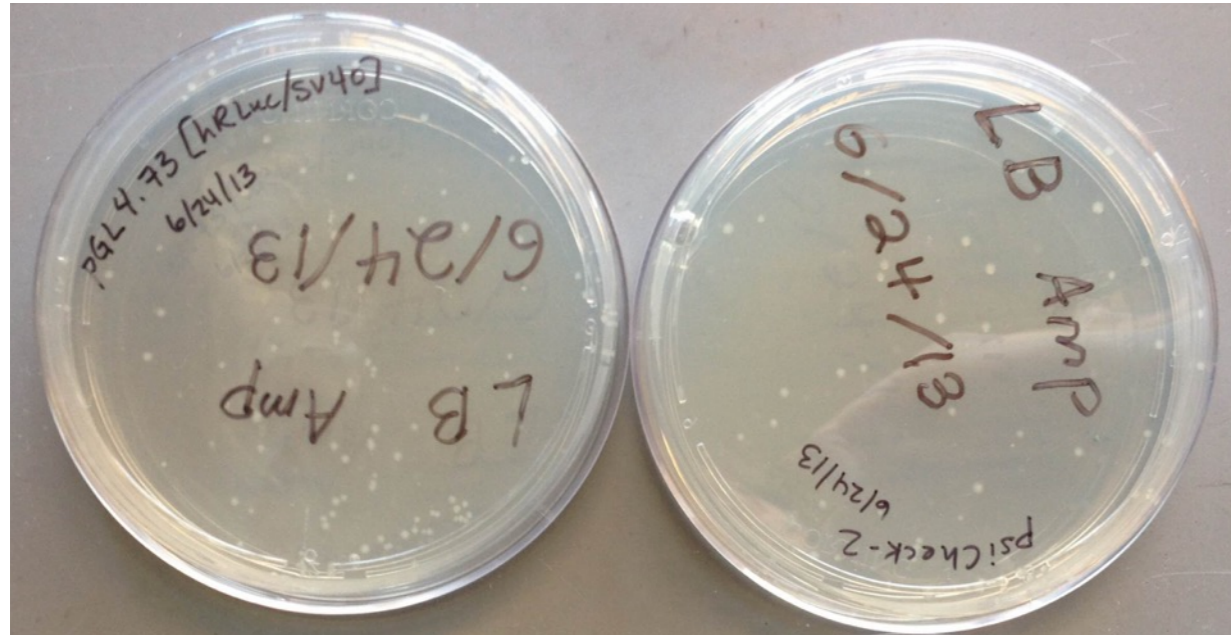
LacZ-alpha ORF: bases 217-576

SP6 promoter priming site: bases 239-256

Transformation



Plasmid propagation in bacteria



x 2

Some safety notes for today:

- Use **nitrile gloves** when handling DNA gels and all equipment used for gels.
- Gels and gel-contaminated papers are disposed of in solid chemical waste.
- Wear **amber glasses (blue light) or face shields (UV)** when cutting DNA bands out of a gel.

Today in Lab

- Part 2B: If there is no product — share or ask us!
- Part 3: Pay attention to pipetting order of ligation reaction!
- Part 3: Be gentle — your cells will thank you!
 - During 60 min incubation — transformation demo
- Part 4: You get a break — just label your tubes with team color, we'll do the rest!

Creating Figures: Good, Bad, Ugly

- Title: Concise, informative, summarizes goal/result
- Caption: what did you do with a little motivation
 - Define all elements
 - Stick to the facts! (examples)
- Figure: the meat of it
 - Need to be accessible
 - Can you figure it out in one glance?
 - How do you display a lot of data at one time?

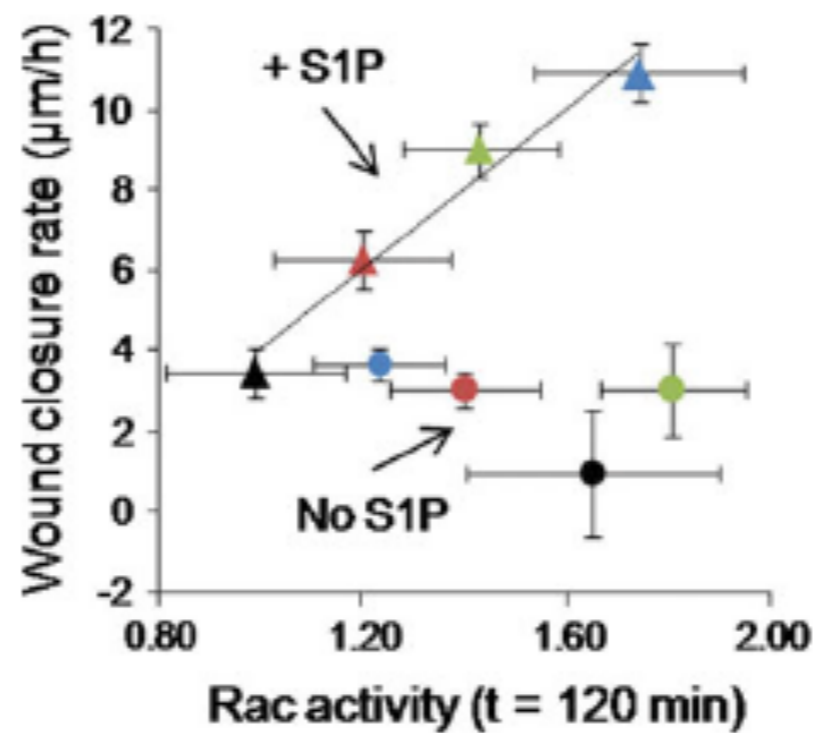


FIGURE 5. Rac activity at 120 min post-stimulation is correlated with migration rate in the presence of S1P. In the absence of S1P (circles), the level of active Rac is not predictive of migration. However, upon the addition of 1 μ M S1P (triangles), the Rac-GTP concentration at 120 min post-stimulation highly correlated with migration rate ($r = 0.96$).