M1D4: DNA Cloning

2/20/14

Announcements

- **While you wait: Please (re)read Part 2 of M1D4 protocol**
- Marilee here first to talk Figures/Captions
- Set-up agarose gel *and then have pre-lab lecture*

- Lab treat next time (M1D5)
- Two things about journal club:
  - Remember to sign up for a PAPER (not just day!) — M1D8 is full.
  - M1D6 must choose a paper by end of the day tomorrow — or I will pester you.
- First notebook collection is M1D7 (D4, D5, D7 possible)

FNT Assignment(s)

Volume of insert (from the gel) = $\frac{20\,\mu L\ DNA}{24\ total\ volume}$ = $\frac{125\,ng\ DNA}{16.67\,\mu L\ DNA}$

25 ng bkb	nmol · bp bkb	10 nmol insert	1400 bp · insert	660 ng	µL insert
	660 ng	1 nmol bkb	3500 bp · bkb	$\frac{nmol}{bp \cdot insert}$	7.5 ng

Noted in your lab notebook!

- Adjust molar ratio to achieve < 5µL insert

= 13.3 µL

10.1 ins:bkb molar ratio

Next time Part 2 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.

Part 1 start bkgrd/motivation gull microbiota

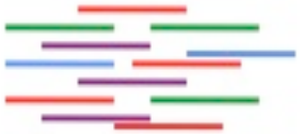
Bird Microbial Communities -- Review of Overview

Bird Cloacal sample

extract DNA

DAY 1

Complex DNA pool (many microbial DNAs)



PCR

DAY 3

Simpler DNA pool (bacterial 16S DNA)

Done!

Today

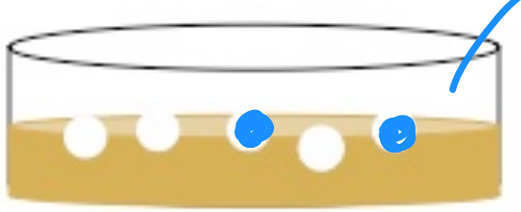
Monday

8 "clones"

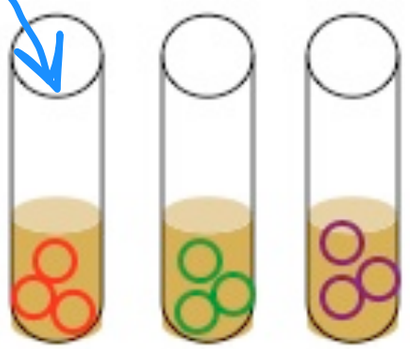
(gel purify); clone and transform

DAY 4

Individual 16S cell colonies



liquid culture



isolate DNA clones from bacteria

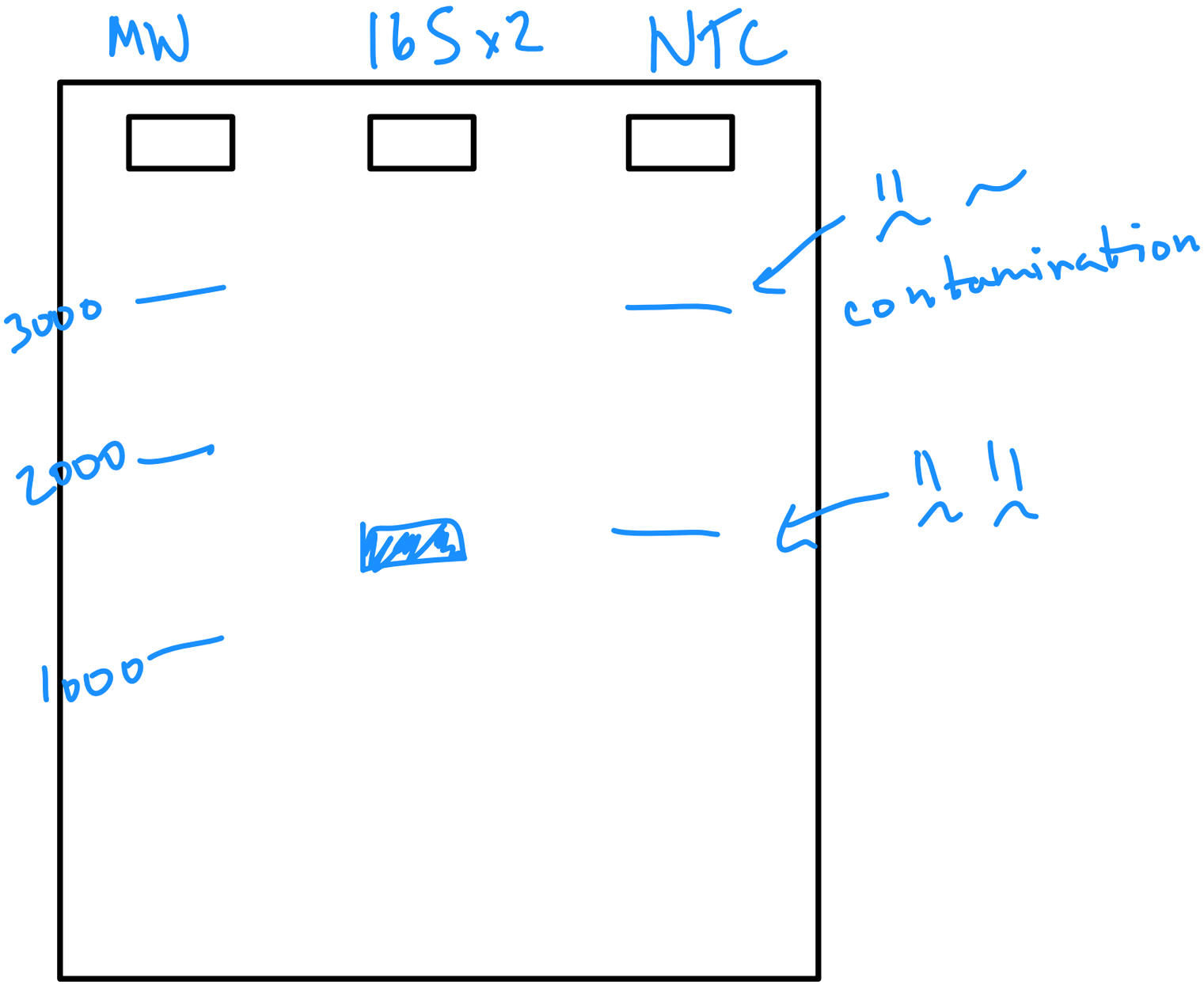
DAY 5

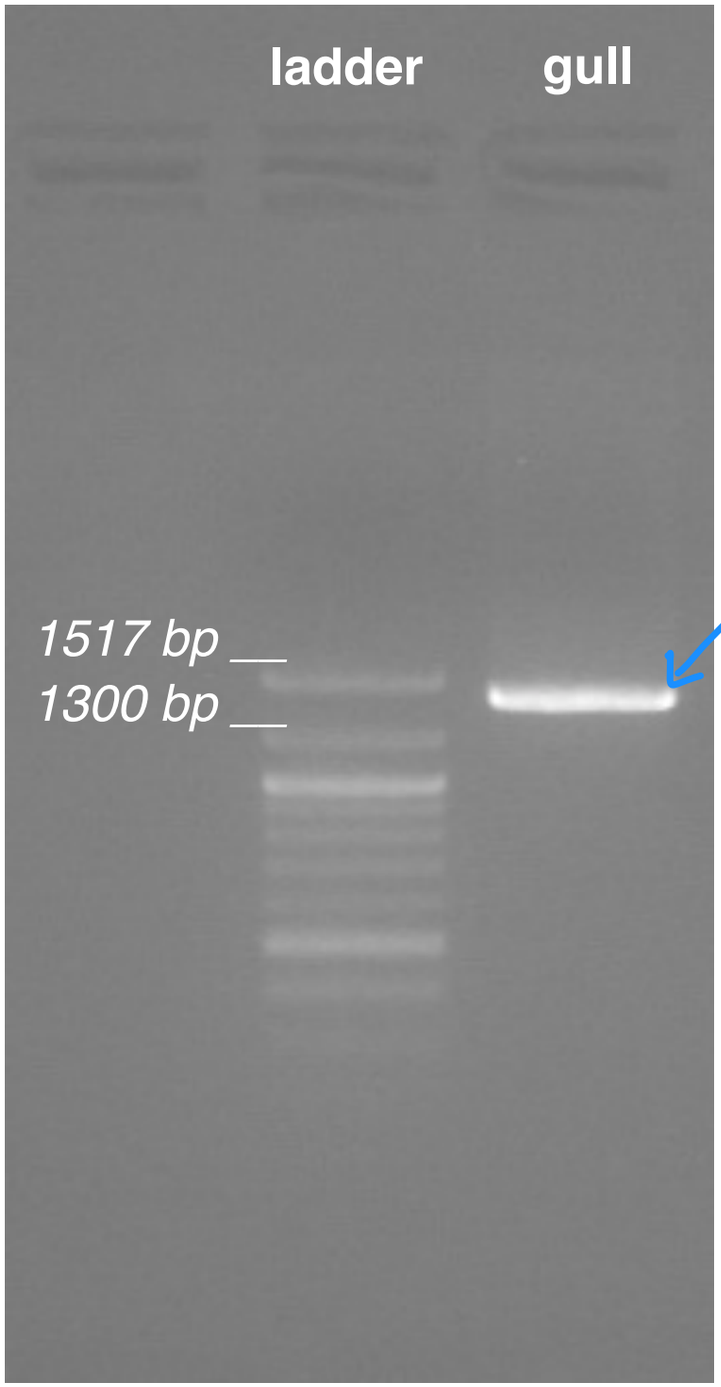
Individual 16S DNA plasmids

identify, relate, and compare sequences

DAY 7

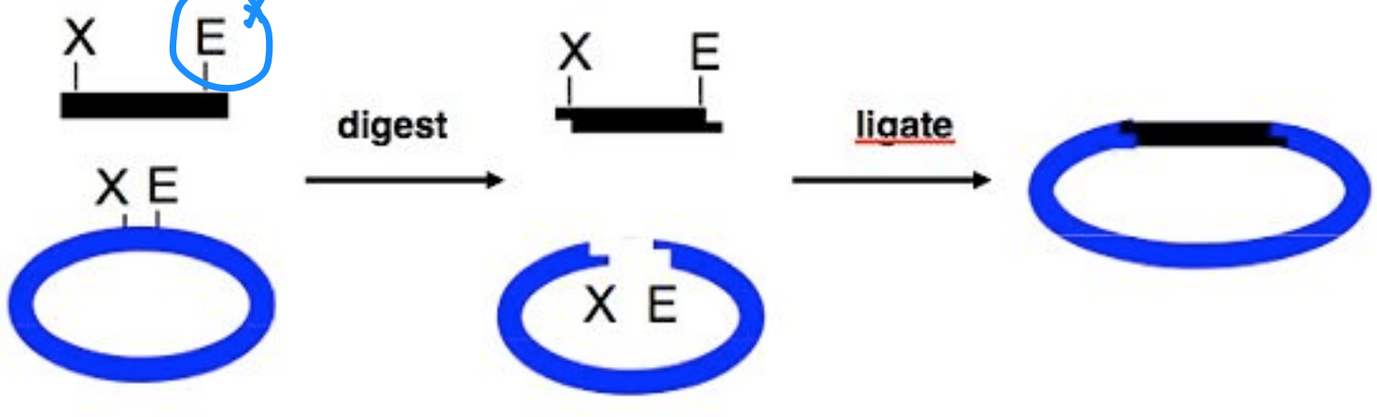
Review: Gel Electrophoresis





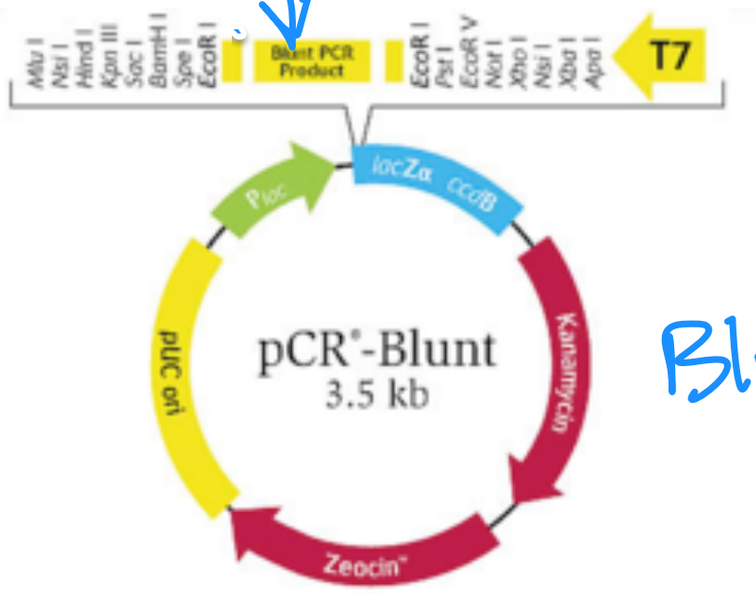
Cloning — review

You may have done this before:



Restriction enzymes → "sticky end"

You can also do it this way:

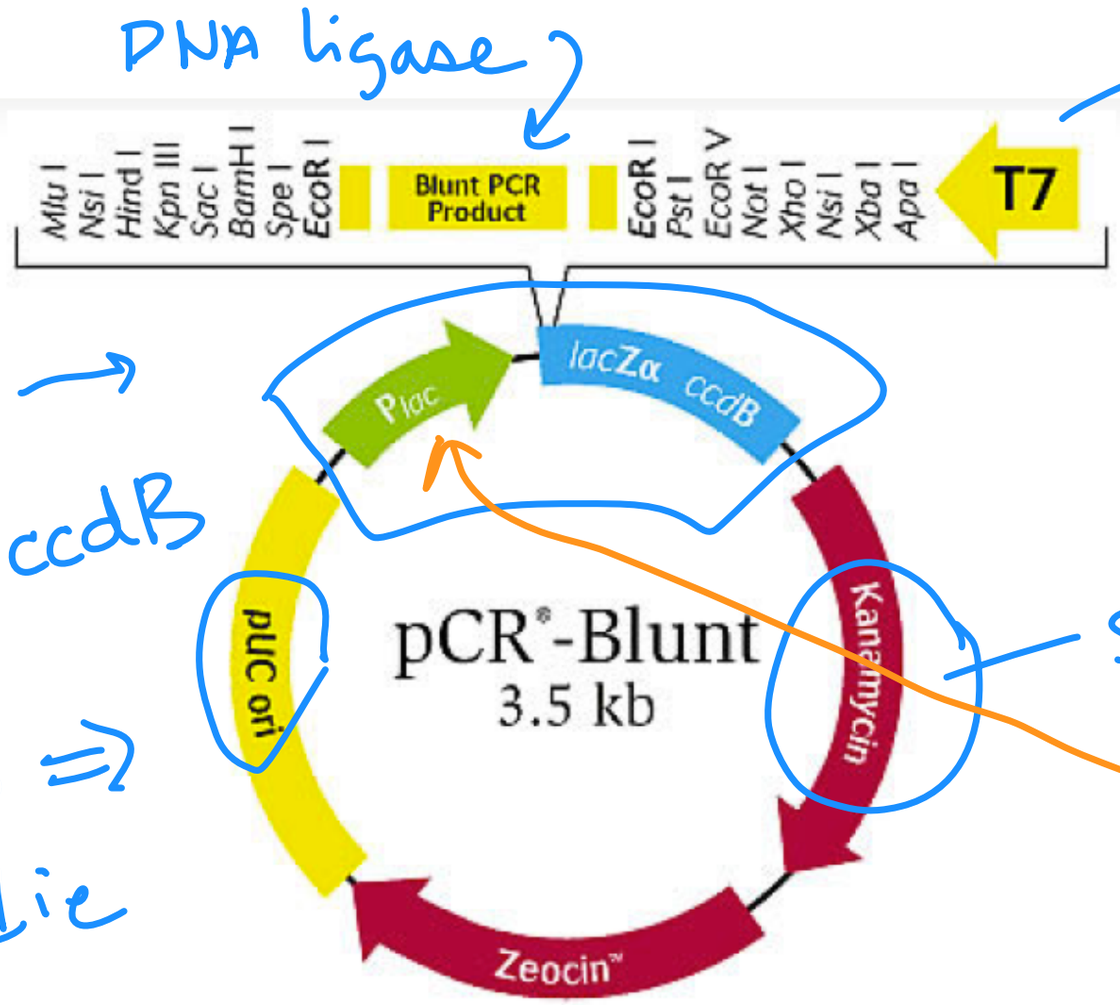


16s DNA ~1400

blunt end → pfu polymerase

Blunt end cloning

Our system & Ligation



pCR®-Blunt Vector

DNA ligase

Multiple cloning site

lac →

lacZ-ccdB

↑ccdB ⇒ cells die

selection

IFF plasmid is circular it will be Kan resistant

IFF we insert our pcr prd ↓ccdB ⇒ cells live

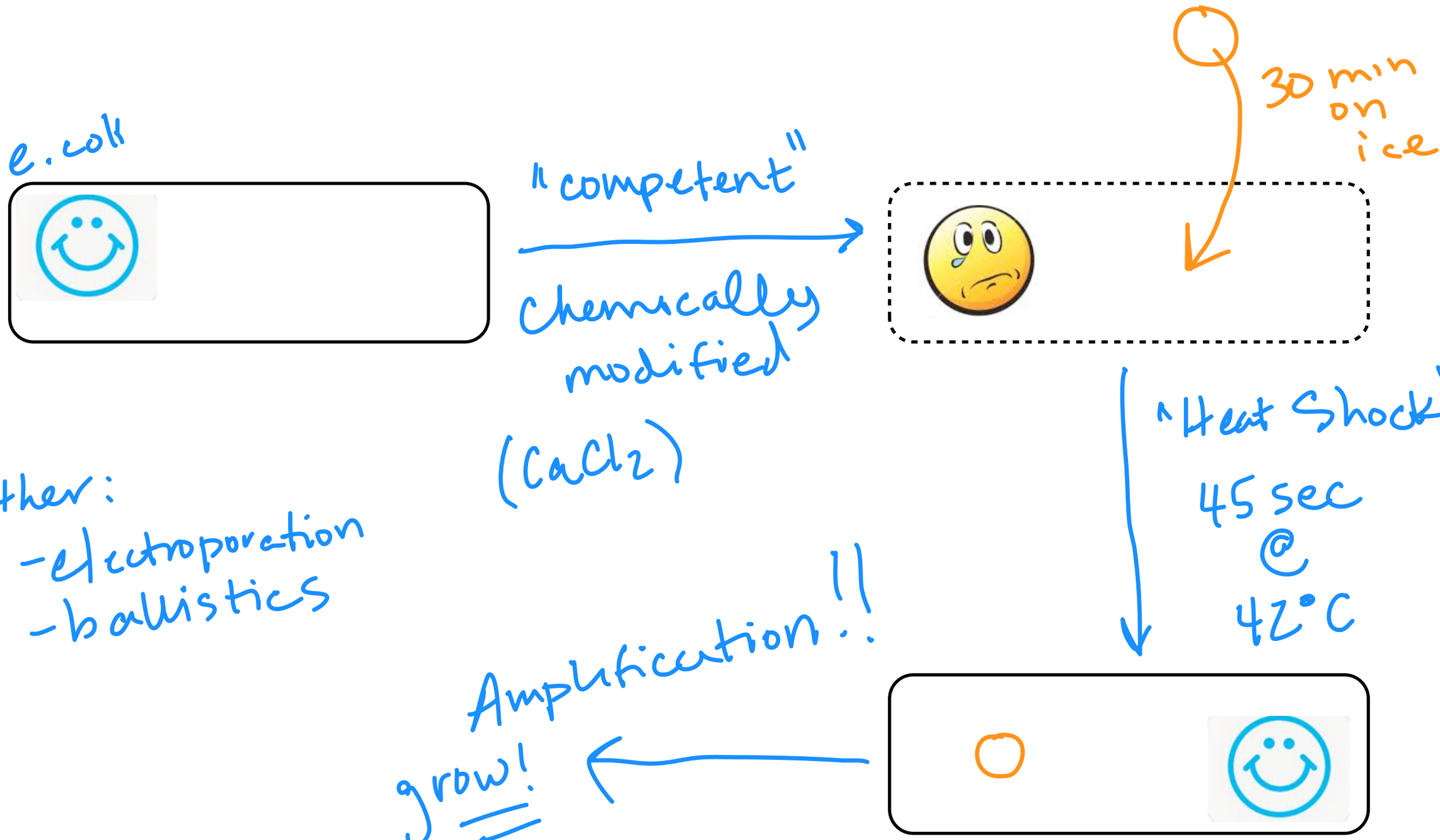
★ usually we need IPTG to turn on the lac promoter

→ our e.coli lack lacI

★ we don't need IPTG

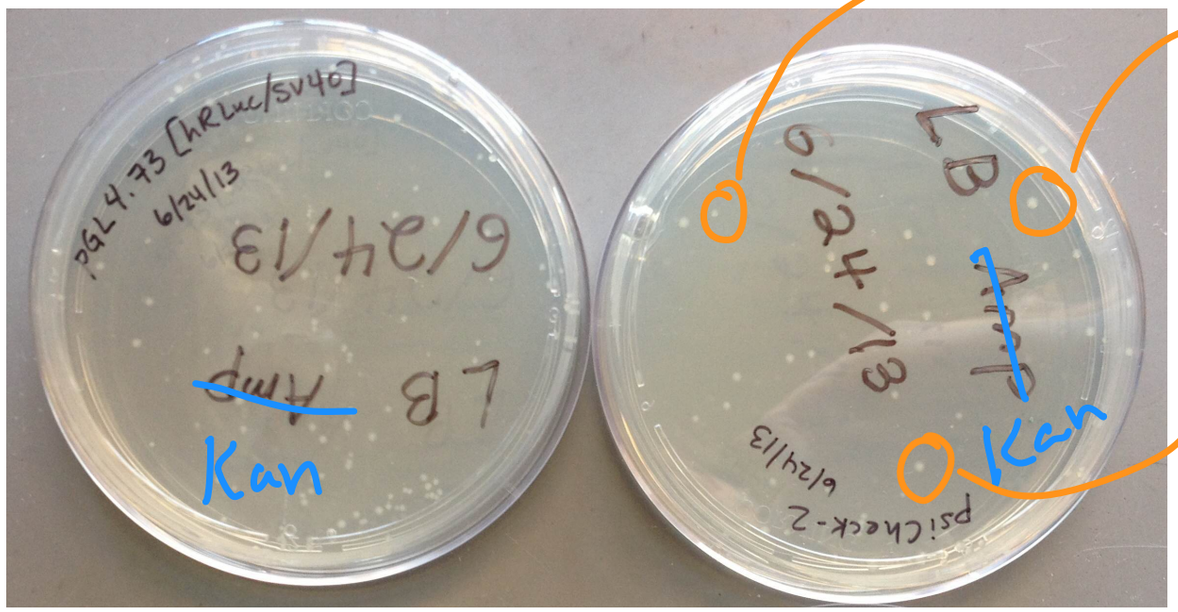
Transformation

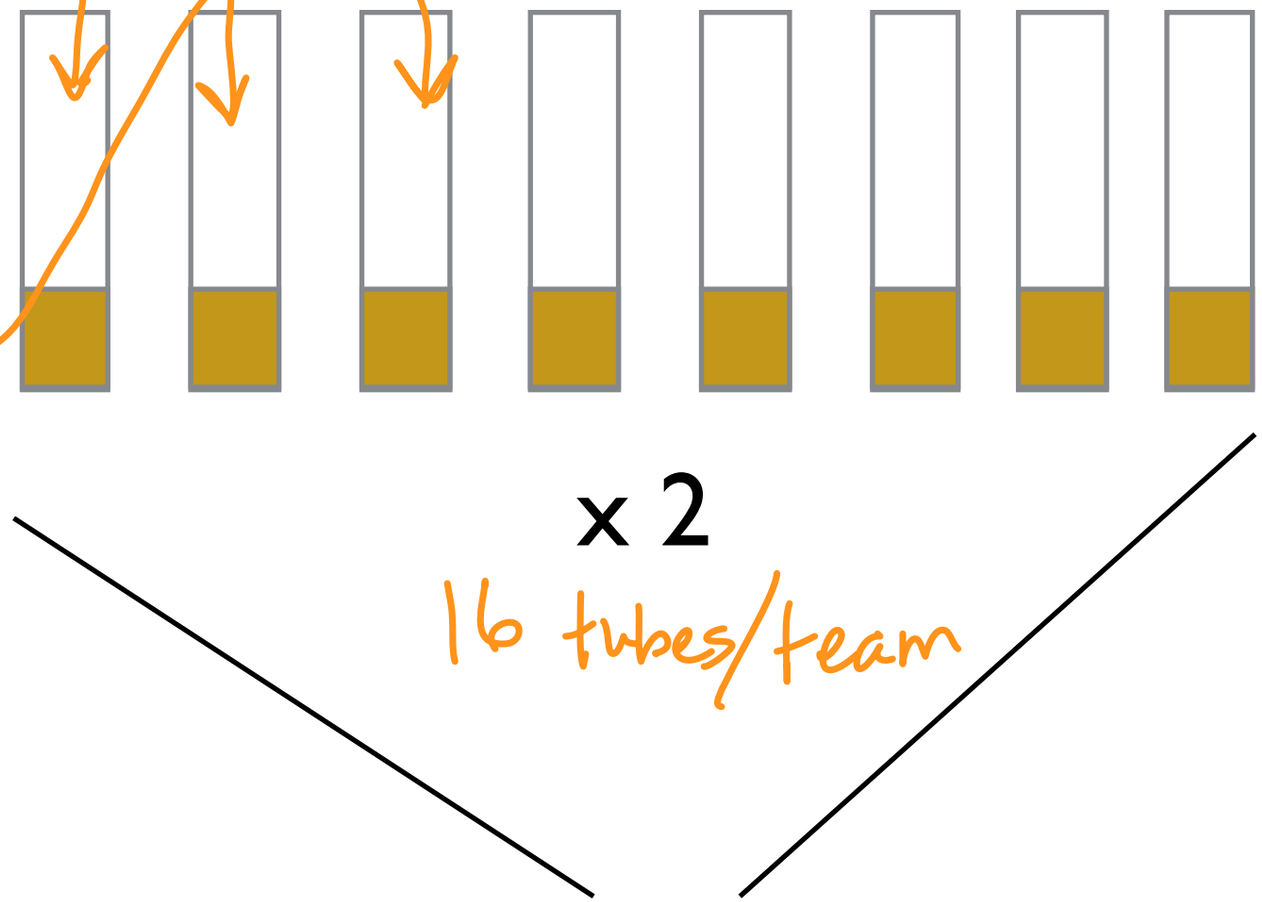
OneShot TOP10



Other:
- electroporation
- ballistics

Plasmid propagation in bacteria





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: Have us help you determine if you need to gel purify.
  - If there is no product — share!
- 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?

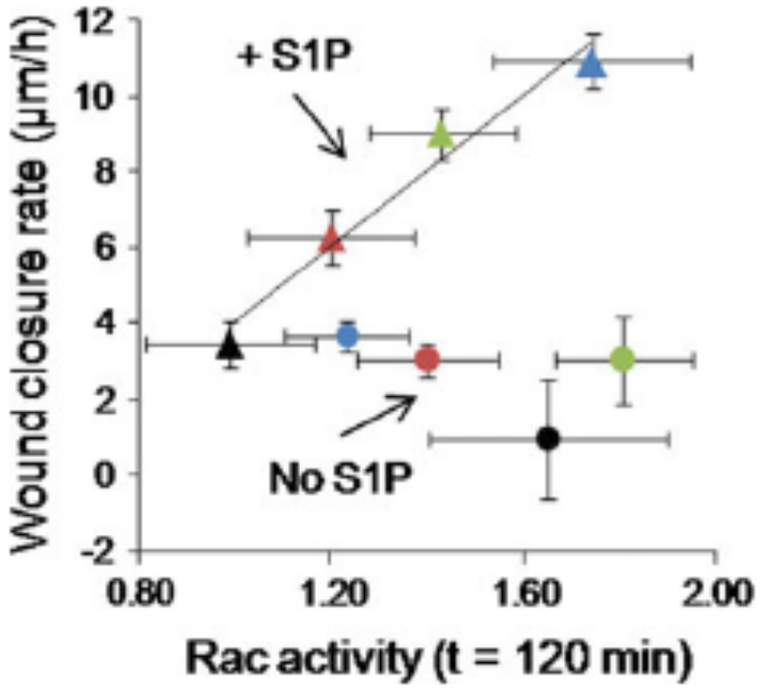


FIGURE 5. Rac activity at 120 min post-stimulation is correlated with migration rate in the presence of S1P. In the absence of S1P (circles), the level of active Rac is not predictive of migration. However, upon the addition of 1 μM S1P (triangles), the Rac-GTP concentration at 120 min post-stimulation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

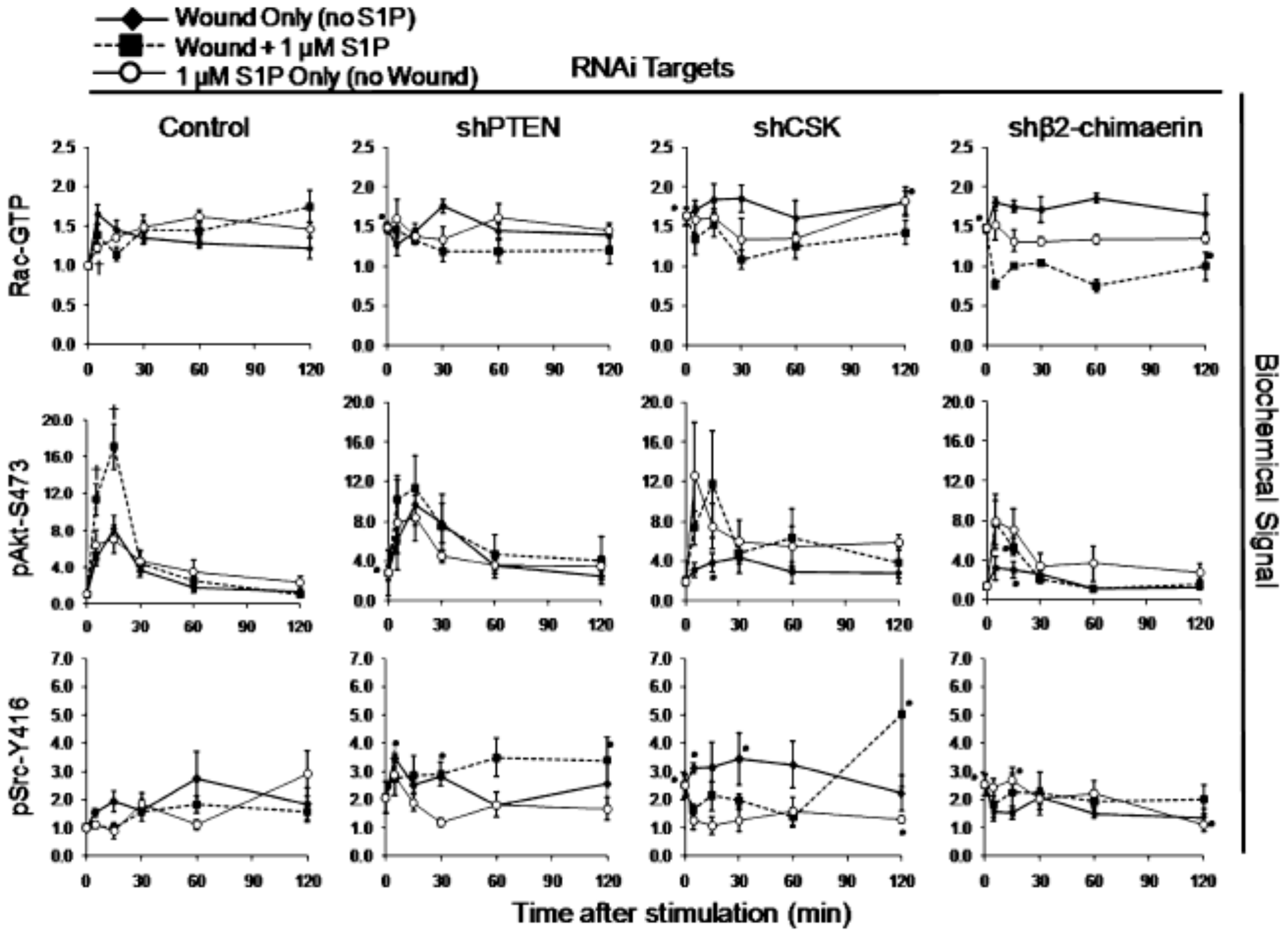


FIGURE 6. Effect of mechanical wounding and S1P stimulation on Rac, Akt, and Src activity. Normalized, time-dependent active protein concentration in control (shLuciferase), PTEN-deficient (shPTEN), CSK-deficient (shCSK), and β2-chimaerin-deficient (shβ2-chimaerin) endothelial cells in response to mechanical wounding (diamonds, solid line), wounding + 1 μM S1P (squares, dotted line), or 1 μM S1P (circles, solid line). Rac-GTP was measured using ELISA, and phospho-specific antibodies were used to detect active levels of Akt and Src by Western blot. Data are presented as mean $\pm$ SEM for at least three independent replicates of responses at 0, 5, 15, 30, 60, and 120 min post-stimulation.