Welcome Back!

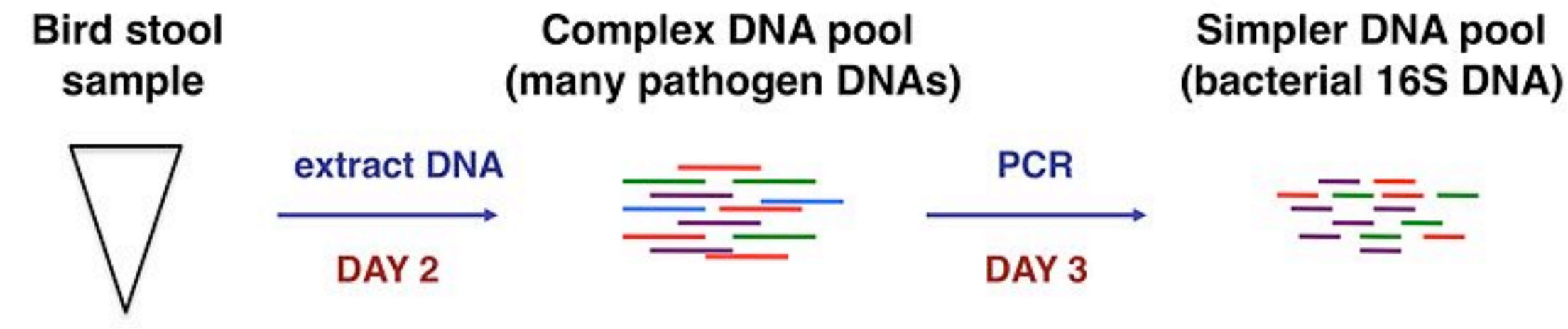
M1D4: DNA Cloning

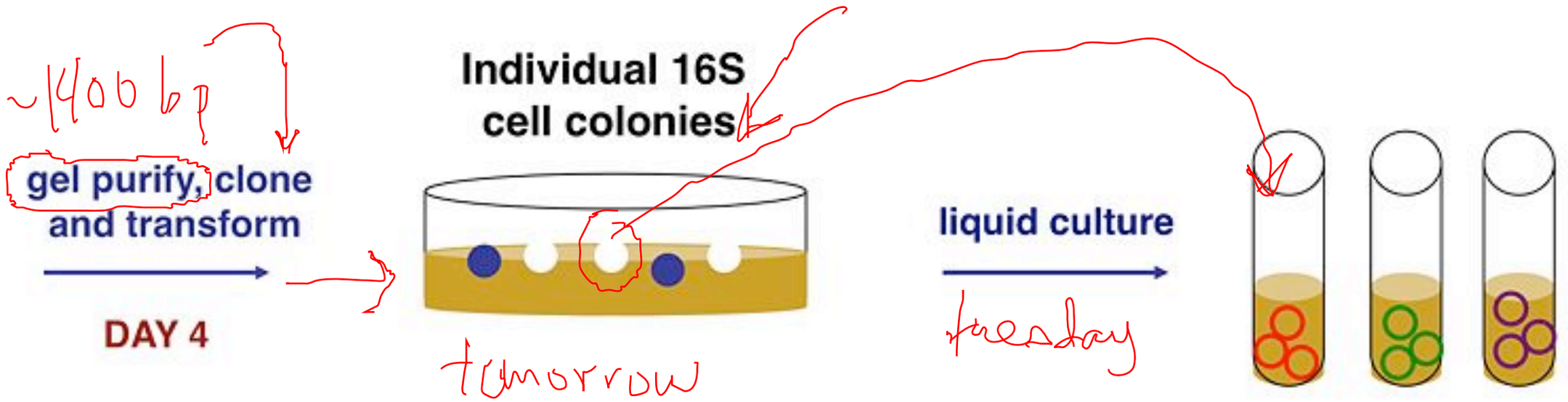
2/22/13

Announcements

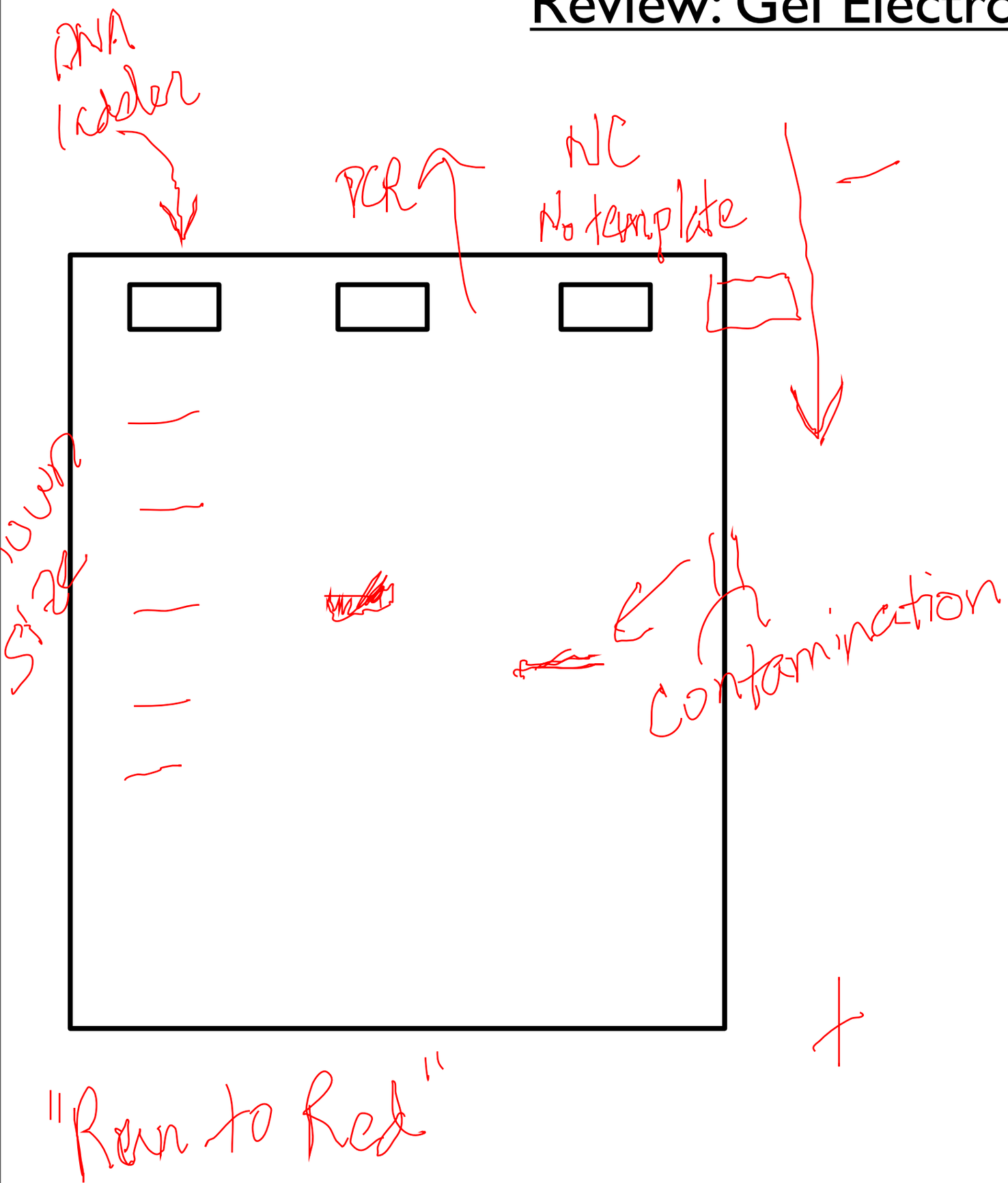
- Hand in primer tables + comments to me. Remember that the other part of the FNT due on Stellar.
- Lab treat next time (M1D5)
- Journal club! Sign up for paper and day (M1D8 is full!)
- First notebook M1D7: D2, 4, 5 collected

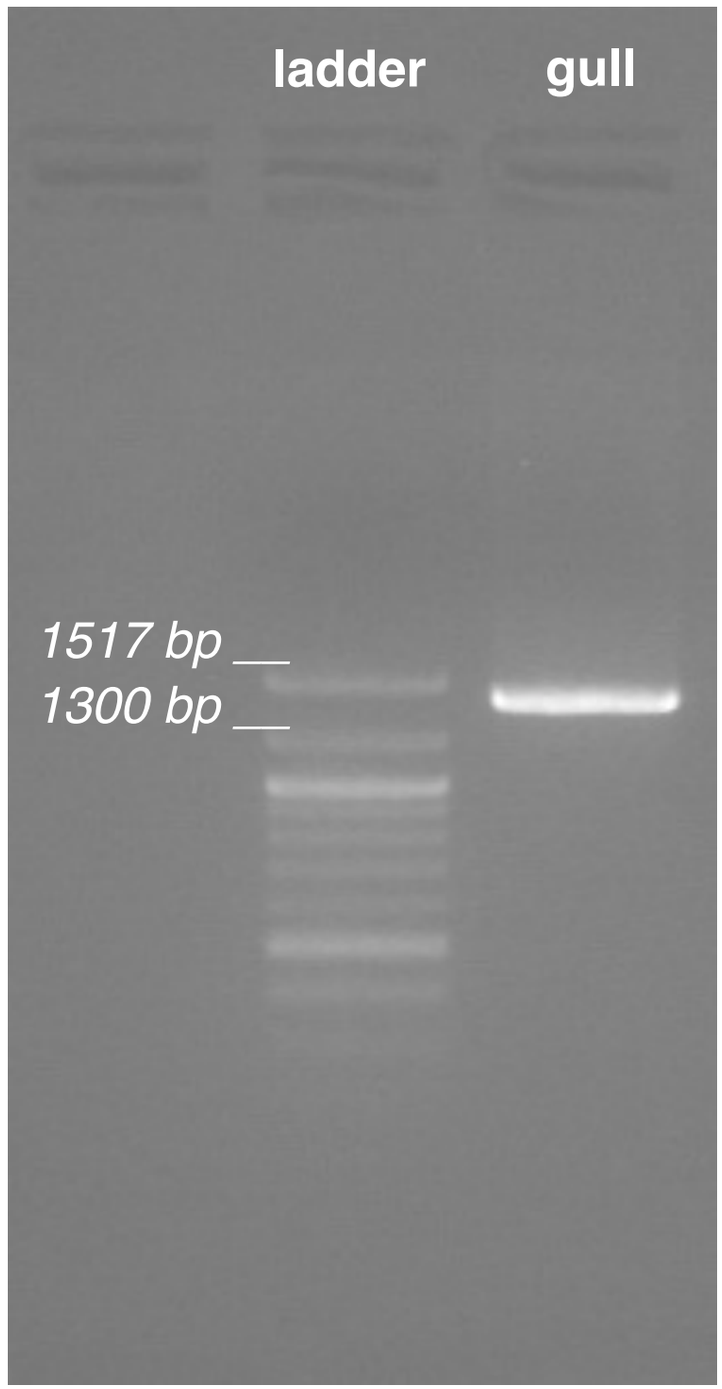
Bird Microbial Communities -- Experimental Overview





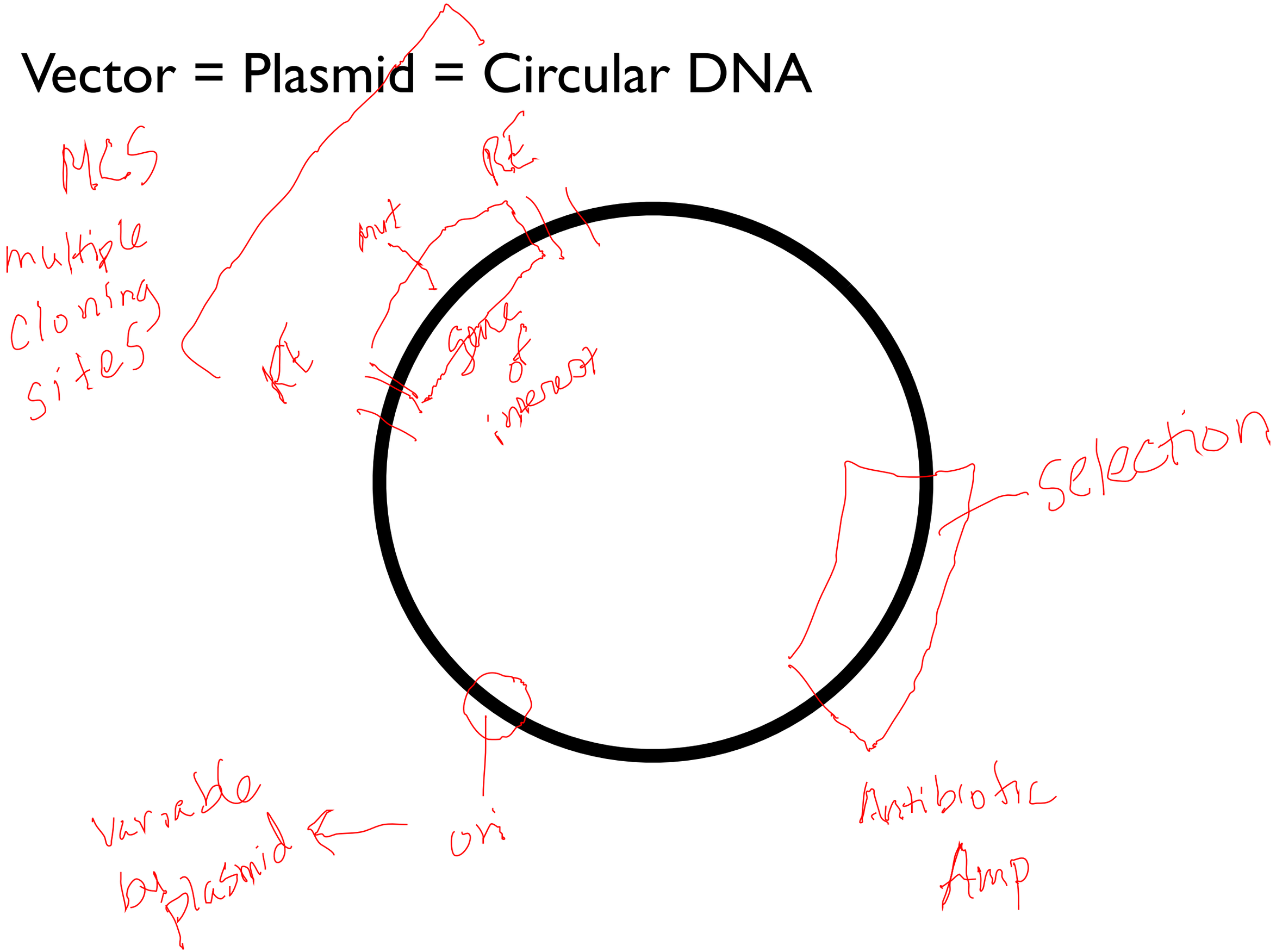
Review: Gel Electrophoresis





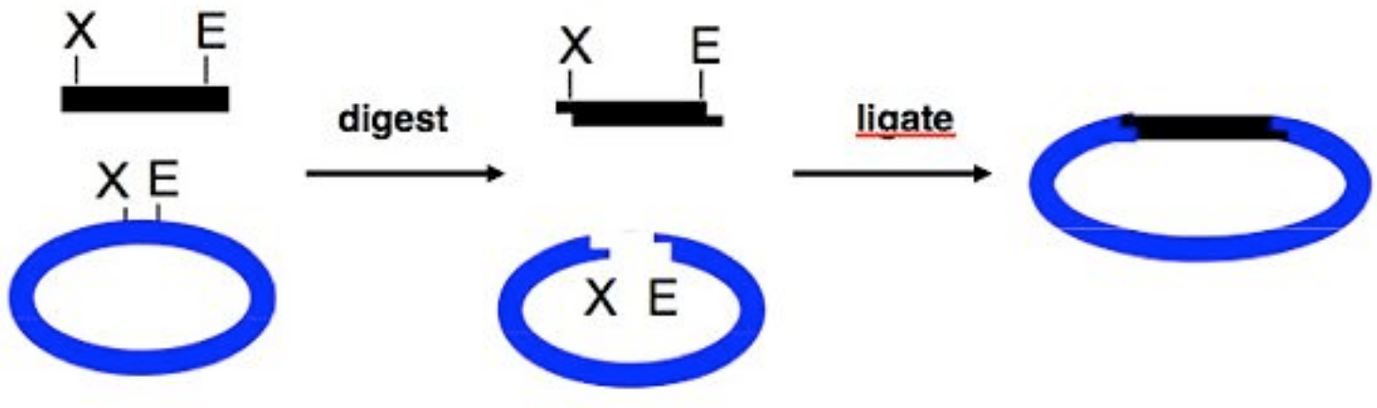
Overview: M1D4 Cloning

Vector = Plasmid = Circular DNA



Overview: M1D4 Cloning

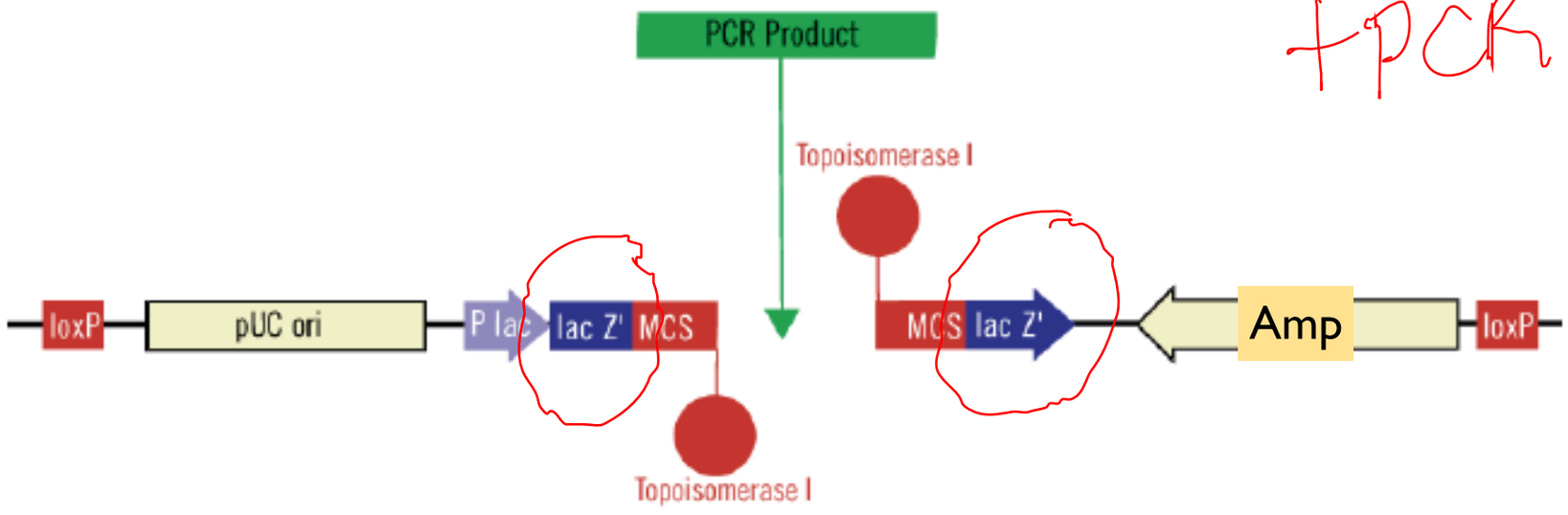
Traditional Method — Mod 2



'Blunt end' Method

No PCR prod inserted = Blue

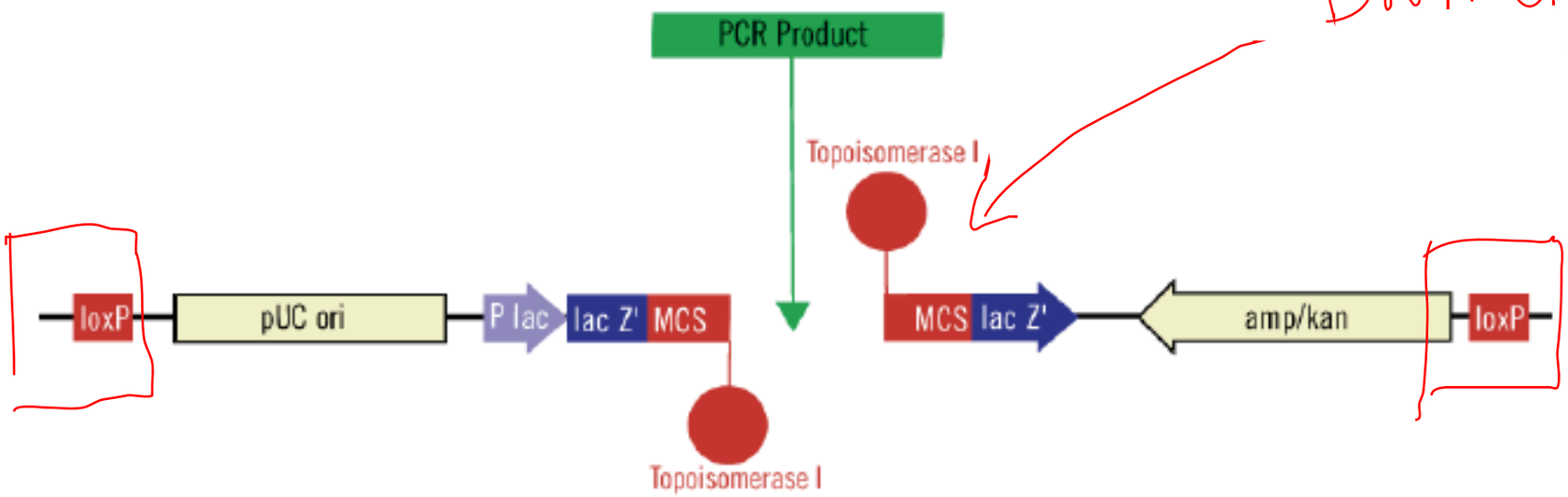
+pCR = White



Overview: M1D4 Cloning

DNA ligase

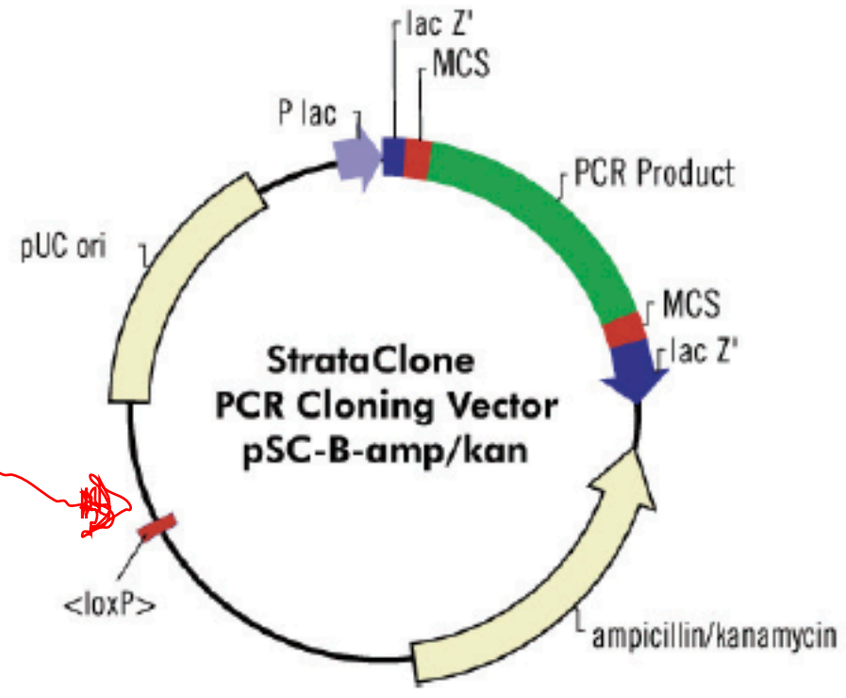
"ligation"



Transform StrataClone competent cells expressing Cre recombinase

1) Blue/white

2) growth of AmpR colonies



Overview: Transformation

Transform StrataClone competent cells expressing Cre recombinase

Chemically

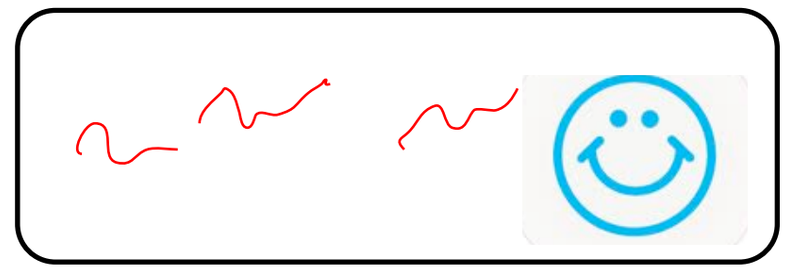
Very gentle

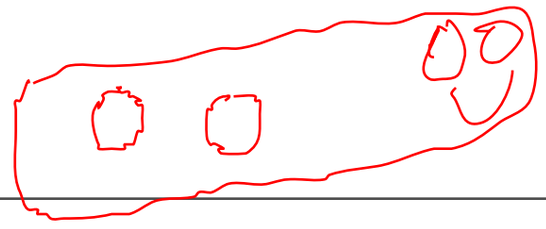
"competent"

heat stock

42°C 45sec

en.wikipedia.org





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 (M1D4)

- Gels w/PCR products run 45 min. Meanwhile,
  - 15 min on figure caption best practices
  - prepare for next steps etc.
- Gel purify IFF multiple products; share if no product.
- Surprise! You each get to do a ligation. No re-pairing.
  - filter tips for prepping ligation reaction
- During 1 hr incubation
  - transformation demo (X-gal prep)
  - prepare tubes for liquid O/N culture
  - prepare primer stocks for μsporidia PCR

Thanks to Agi for this slide

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?

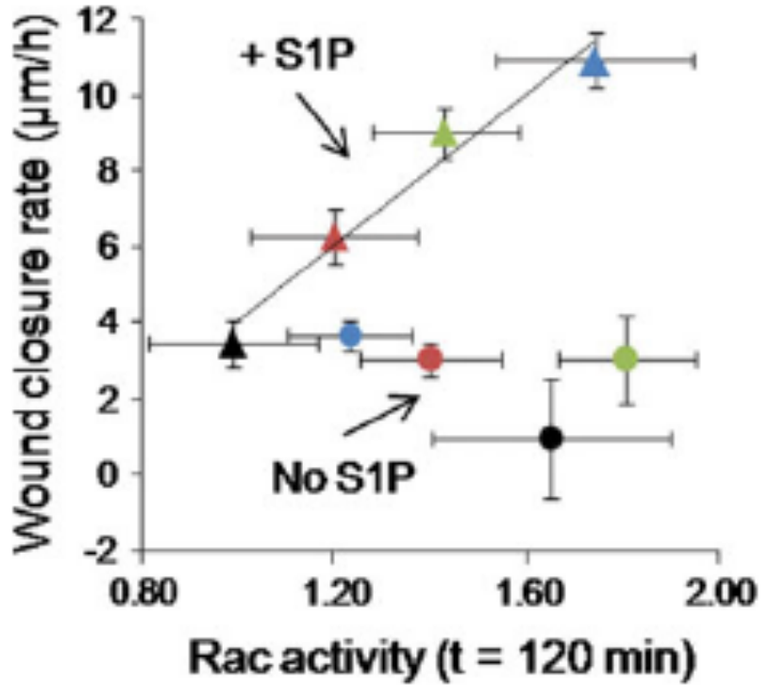


FIGURE 5. Rac activity at 120 min post-stimulation is correlated with migration rate in the presence of S1P. In the absence of S1P (circles), the level of active Rac is not predictive of migration. However, upon the addition of 1 μM S1P (triangles), the Rac-GTP concentration at 120 min post-stimulation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

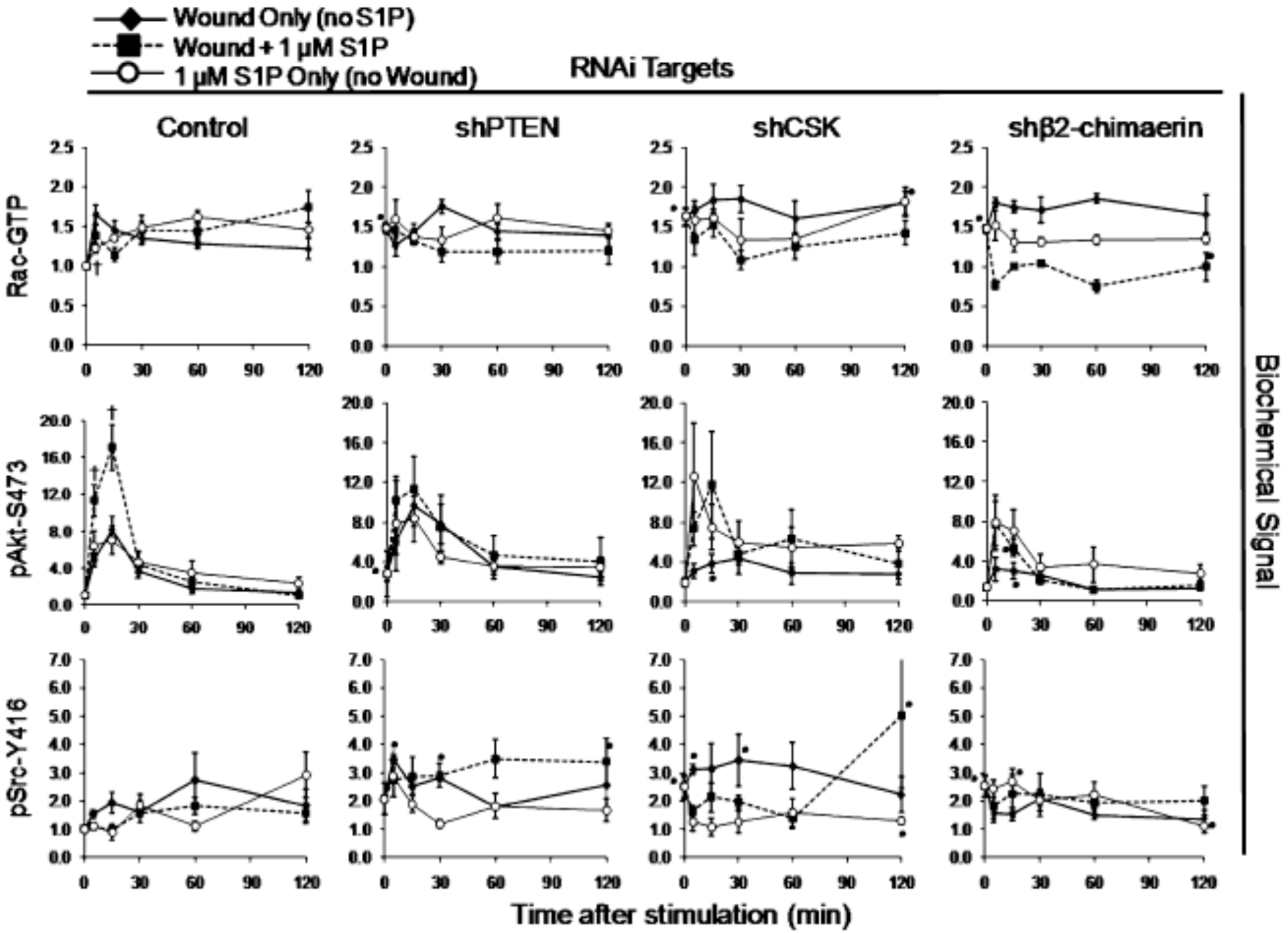


FIGURE 6. Effect of mechanical wounding and S1P stimulation on Rac, Akt, and Src activity. Normalized, time-dependent active protein concentration in control (shLuciferase), PTEN-deficient (shPTEN), CSK-deficient (shCSK), and β2-chimaerin-deficient (shβ2-chimaerin) endothelial cells in response to mechanical 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 ± SEM for at least three independent replicates of responses at 0, 5, 15, 30, 60, and 120 min post-stimulation.