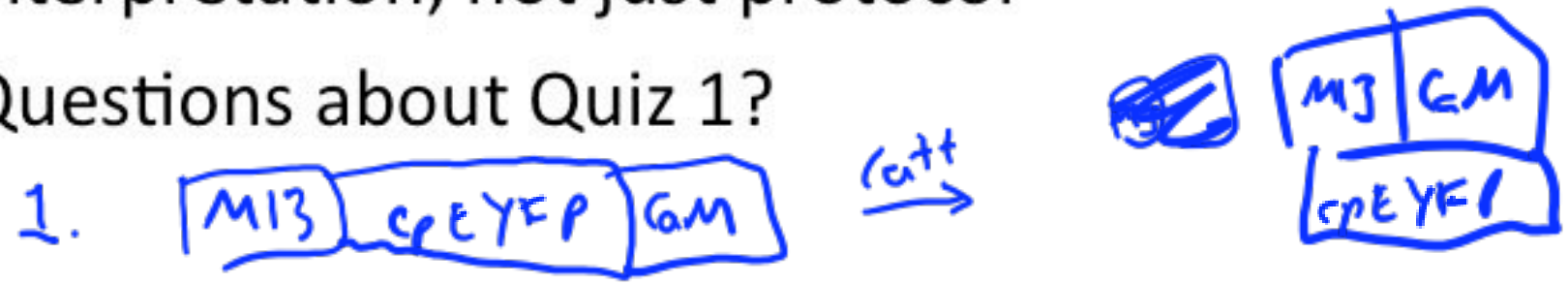
- Announcements
- Quiz
- Pre-lab Lecture
  - ❖ Gel Electrophoresis (cont)
  - ❖ Bacterial Transformation
  - ❖ Adventures in Troubleshooting
  - ❖ Today in Lab: M2D3

Announcements

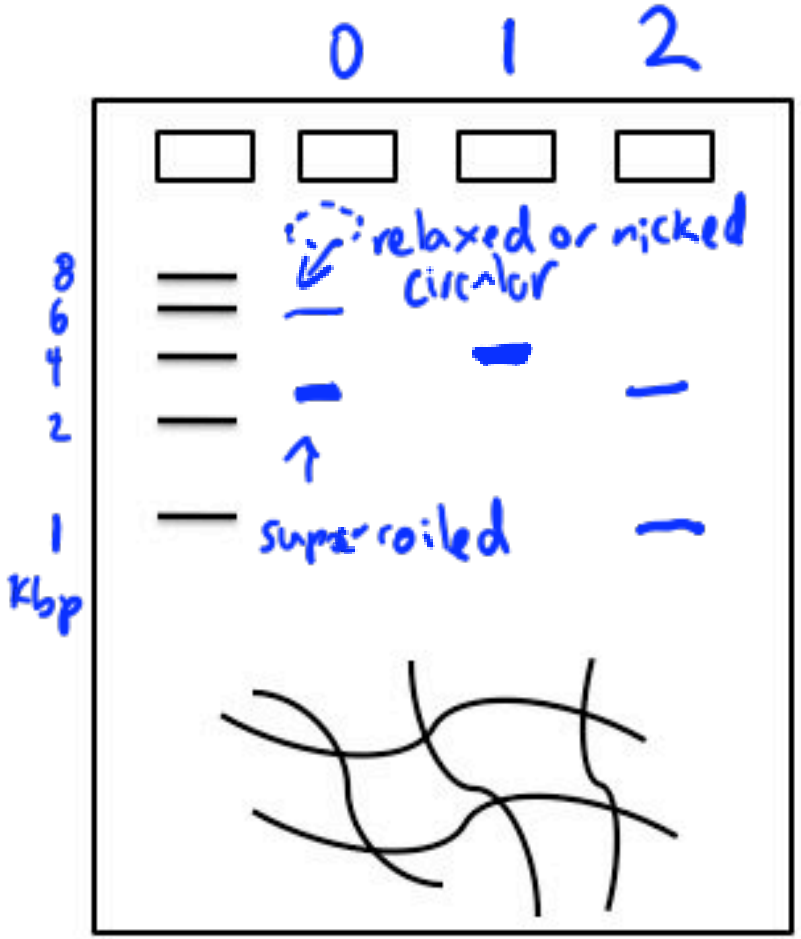
- Lab notebooks: need reasoning and interpretation, not just protocol
- Questions about Quiz 1?



Polymerase error rates

- *Taq* polymerase ~ 1 in 10^5 errors in # bp
  - Standard version has no proofreading capability
    ↓ exonuclease
- *Pfu* polymerase ~ 1 in 10^6
  - Standard version requires longer extension times

DNA EP: Shape-dependence



Plasmid versus linear samples

say, 4Kbp plasmid

linear DNA (1 cut): runs w/ ladder

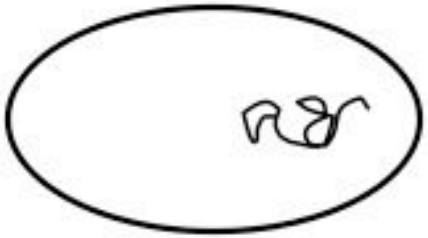
restriction site → 2 cut: sums to 4

uncut plasmid: supercoiled - fast
circular - slow

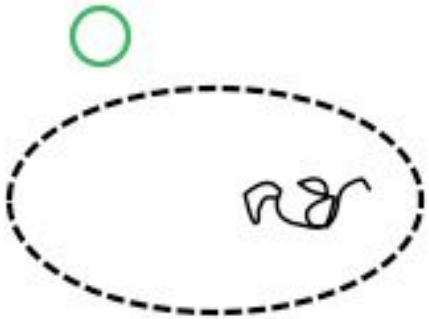
+ high MW dimers, etc.

Remember to wear nitrile gloves.

Bacterial transformation



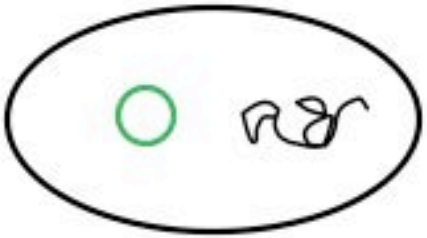
1) chemical treatment



competent cells

(keep cold, don't vortex)

2) heat shock

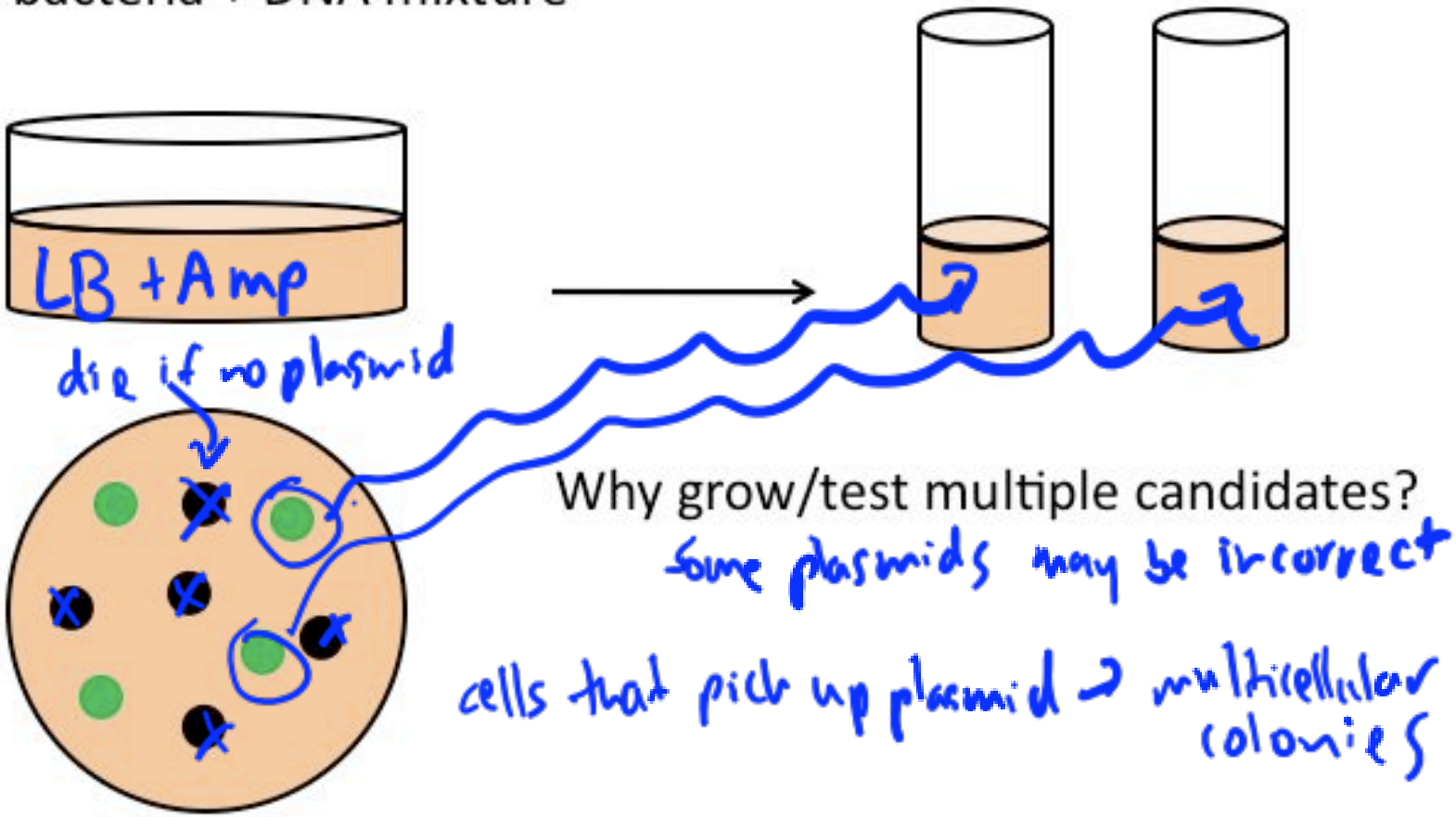


other methods (1-step)

- electroporation
- ballistics

DNA Amplification in Bacteria

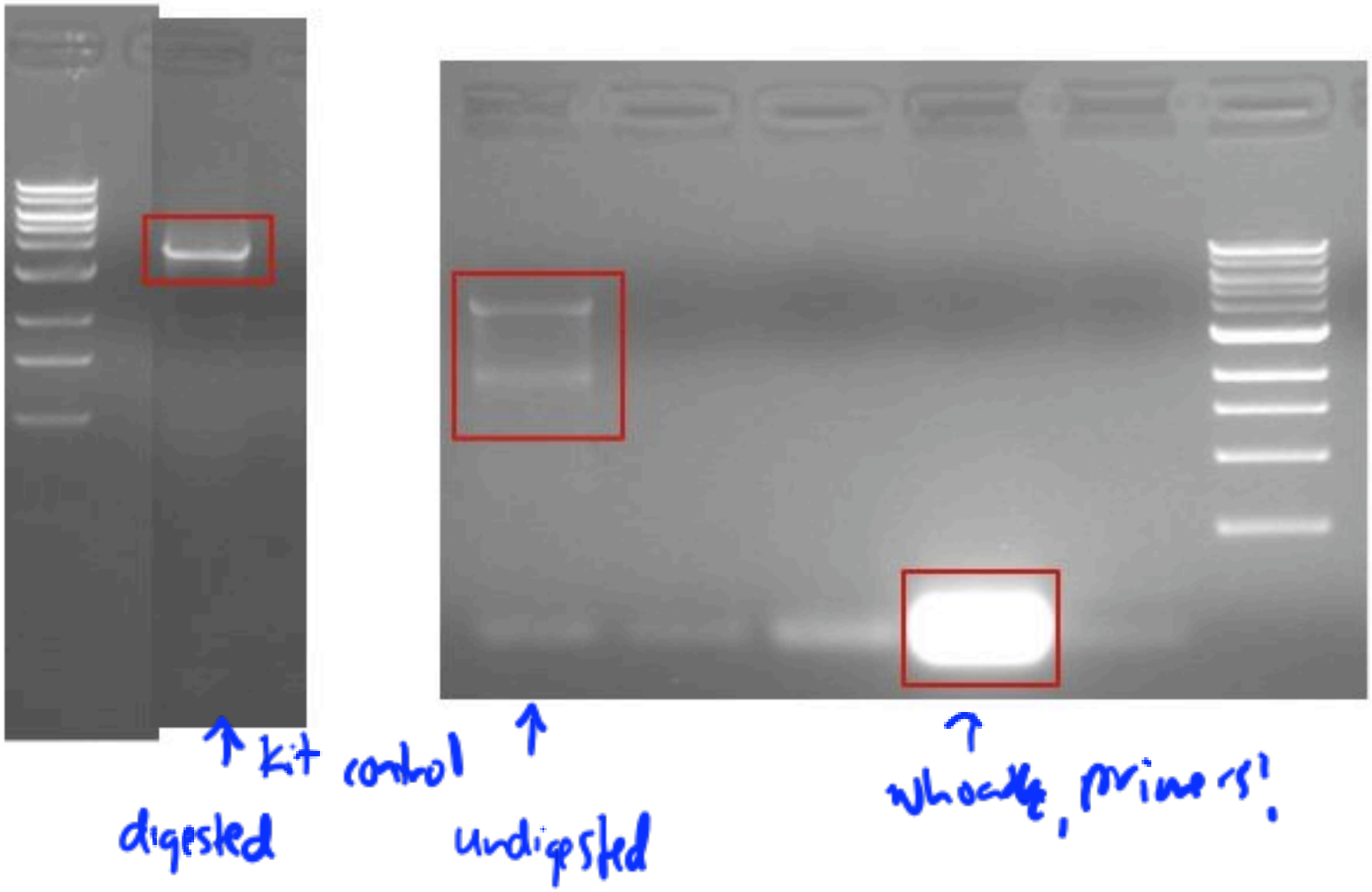
Plate bacteria + DNA mixture



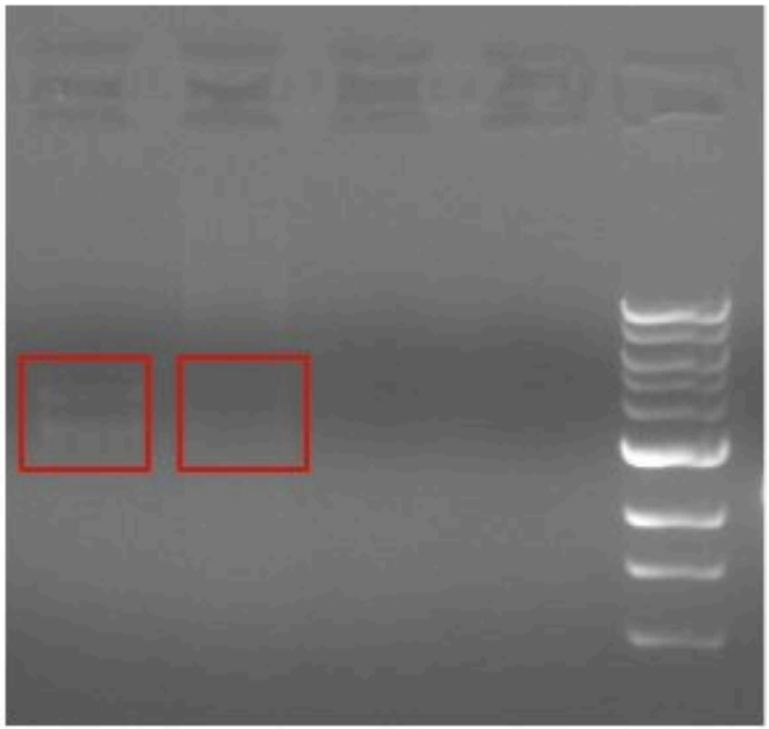
Troubleshooting SDM

- After 2 years of working, suddenly no colonies in teaching sample! What could be wrong?
  - Master Mix problem or changed composition
  - primers — 125 ng
  - thermal cycler conditions, issue
  - template — 5-50 ng ⇒ problem w/ [template] or inhibitory component

SDM: control and class data

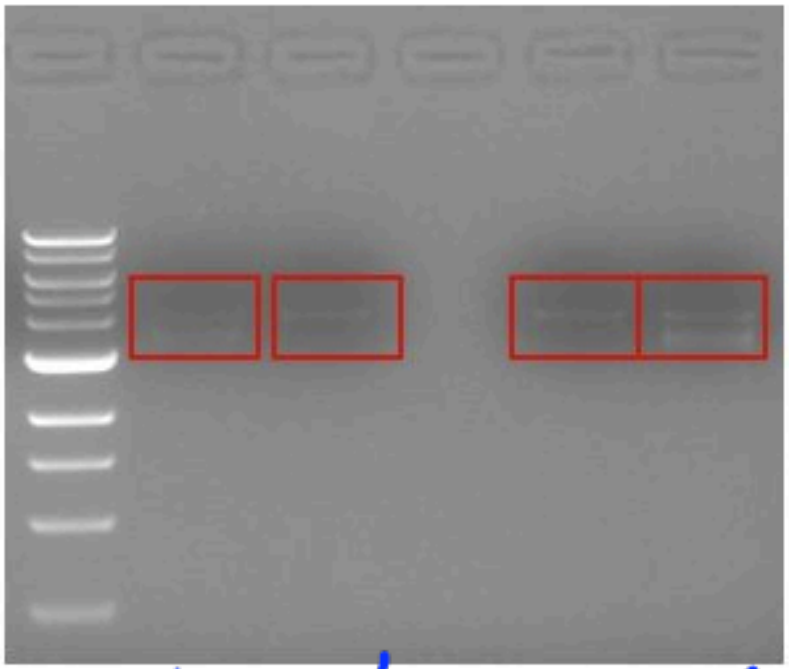


SDM: titration data



1:300 IPC
gave
50-100 colonies

1:100 IPC
sometimes gave
a few colonies



Red

Blue

why would lower [template] be good?
* competing for other reagents (primers, etc.)
* or inhibitor diluted

Today in Lab

- Set up gel: runs 45 min, we will photograph it.
  - Mark your area with coloured tape
- Meanwhile, notebooks/hw/etc.
- Finally, bacterial transformation – be gentle!