- 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?

1. 

3. 

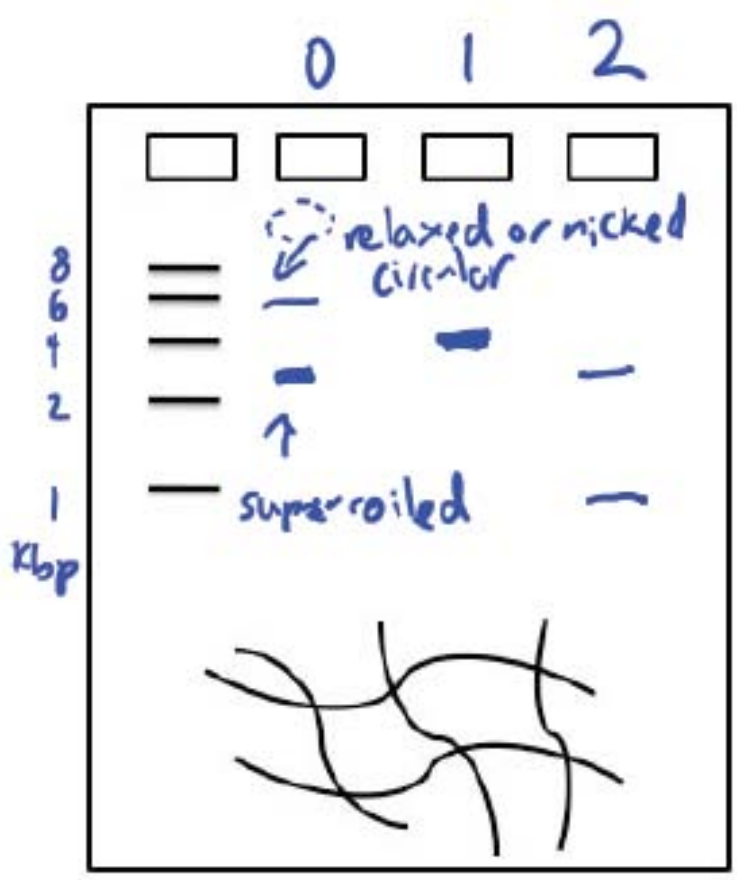
plasmid

primers for SDM (5' ->)
reverse complements

Polymerase error rates

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

DNA EP: Shape-dependence



Plasmid versus linear samples

say, 4 Kbp plasmid
linear DNA runs with ladder

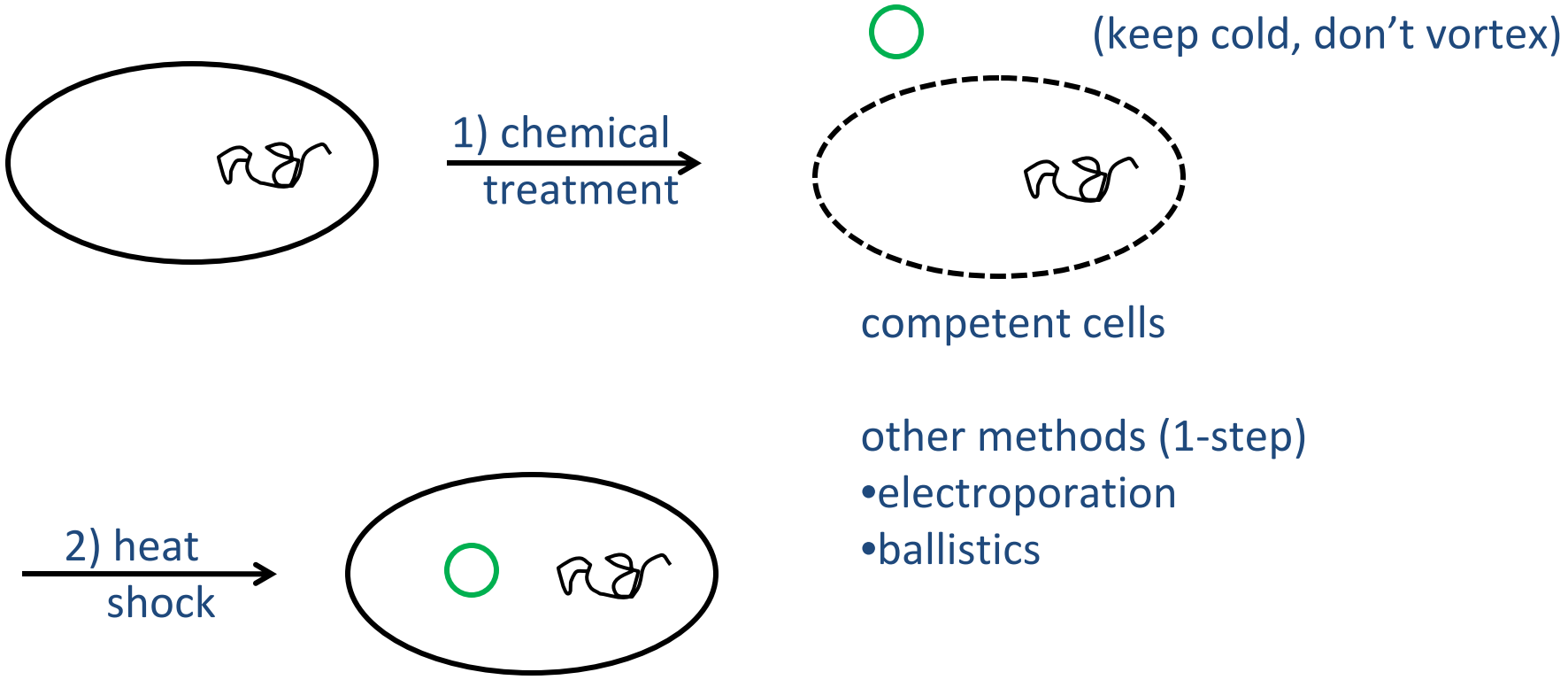
restriction site → 2-cut: sums to 4

uncut plasmid: supercoiled – fast
circular – slow

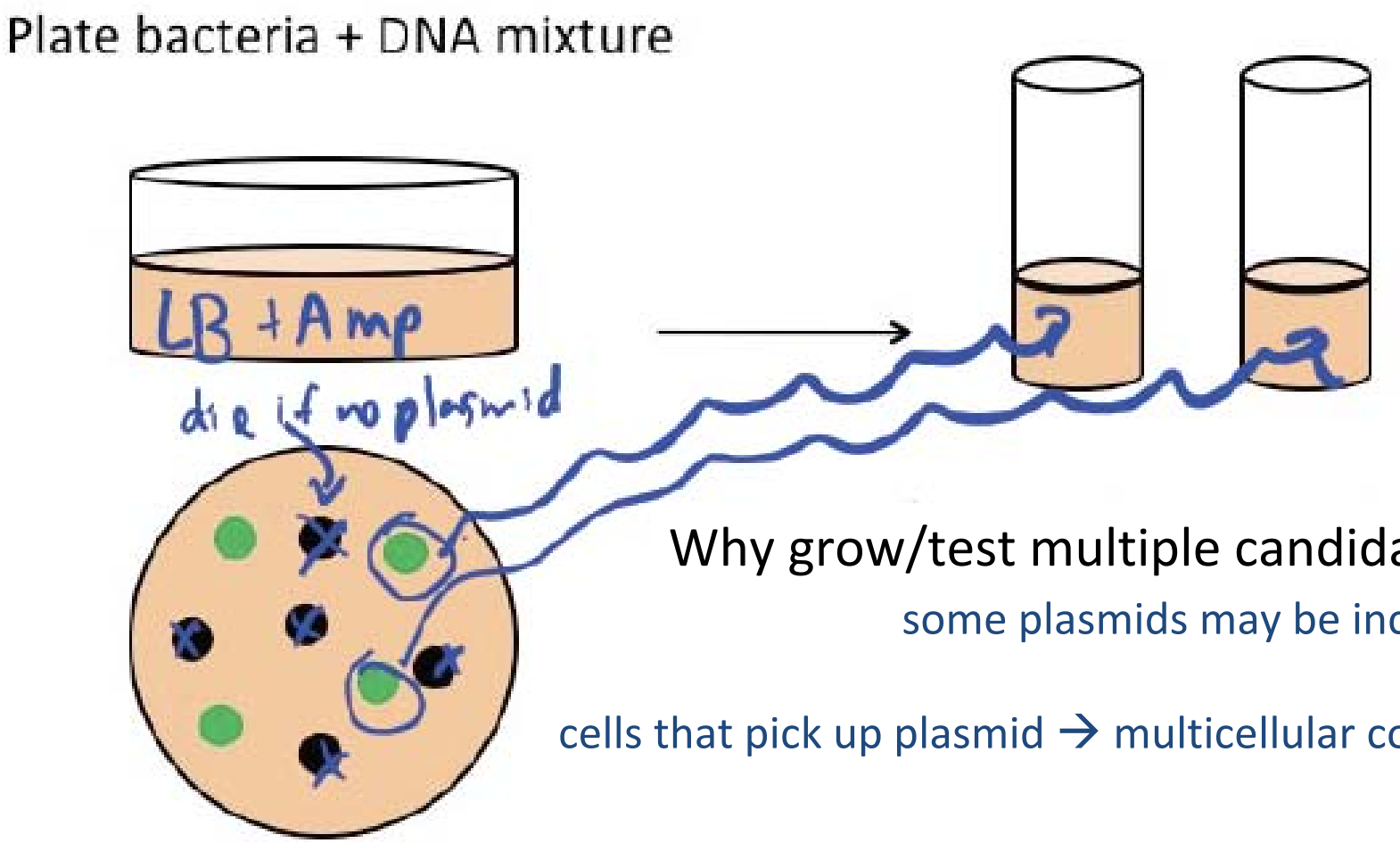
+ high MW dimers, etc.

Remember to wear nitrile gloves.

Bacterial transformation



DNA Amplification in Bacteria



Why grow/test multiple candidates?

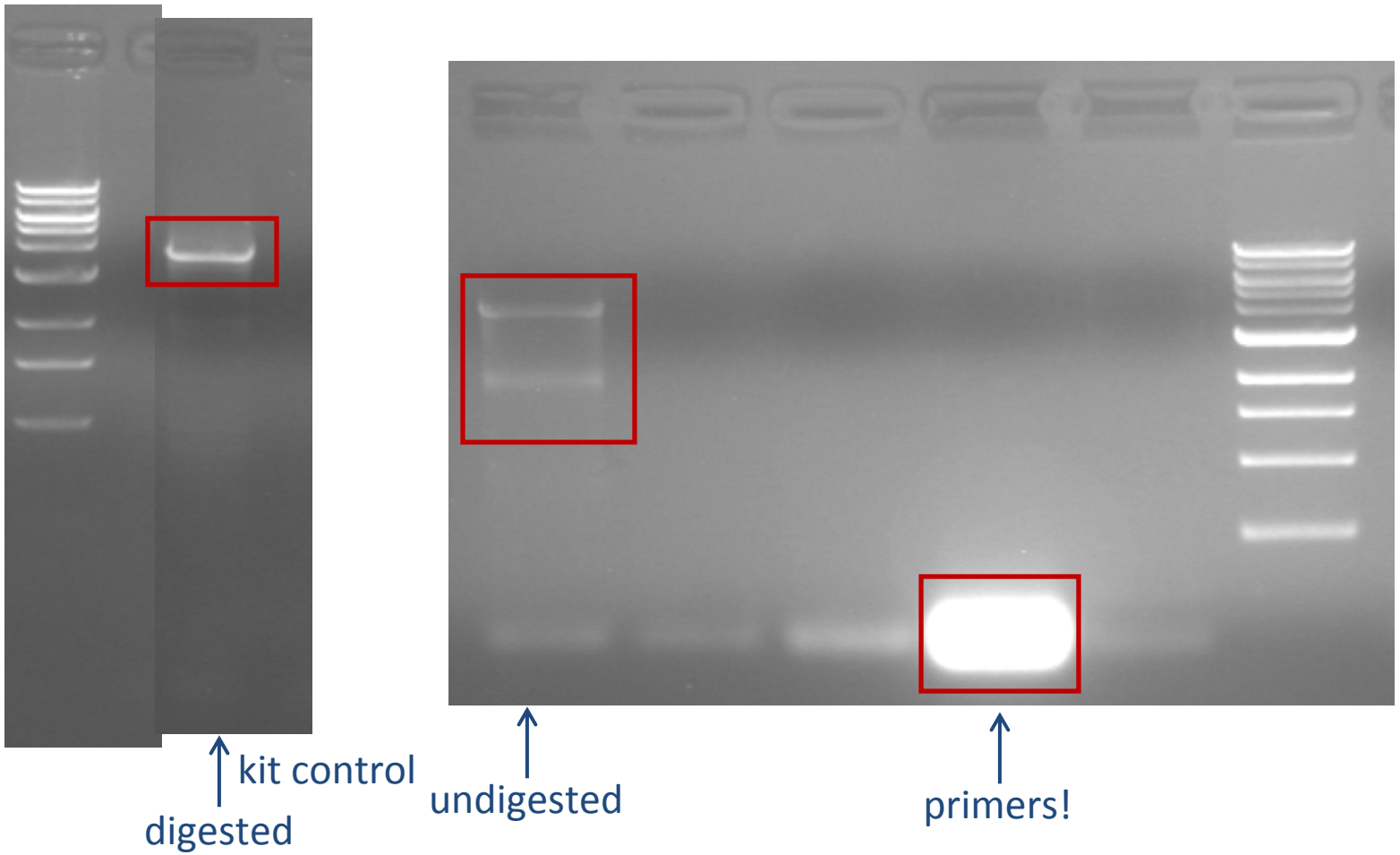
some plasmids may be incorrect

cells that pick up plasmid → multicellular colonies

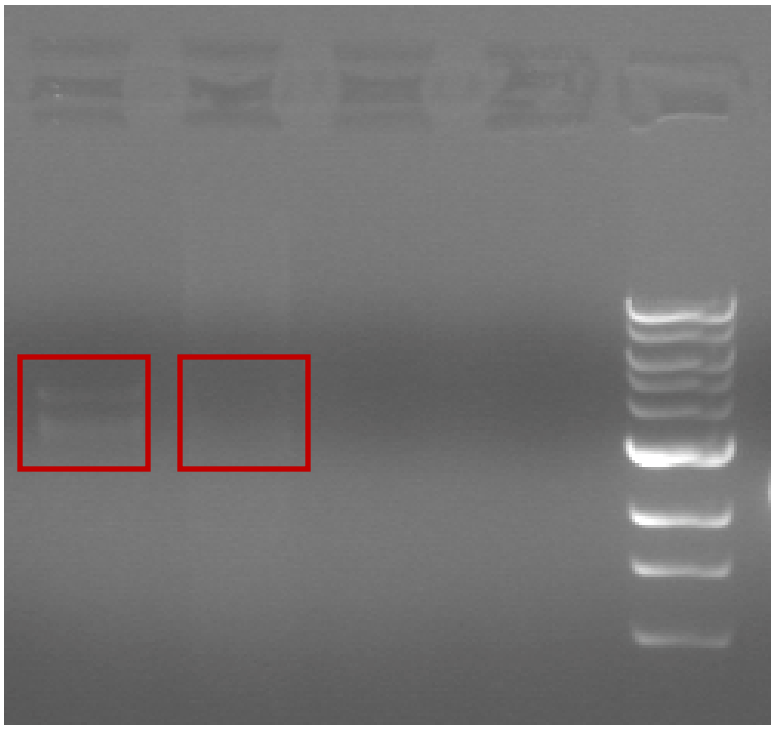
Troubleshooting SDM

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

SDM: control and class data

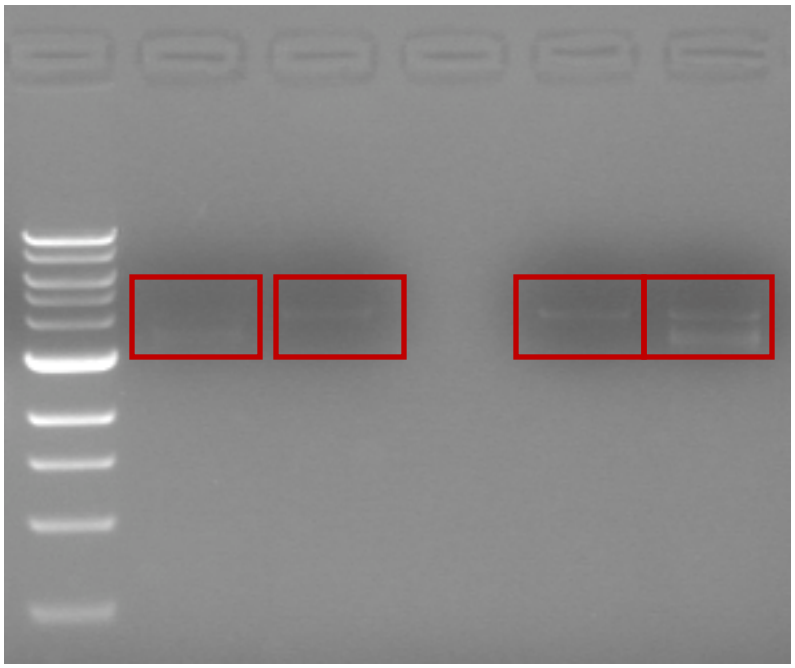


SDM: titration data



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!

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