Day 4 Agenda

- Questions from Day 3
  - transformation
  - PCR
- miniprep AG1111 transformants (to ID correct clones)
- Analysis of PCR
  ara::lacZ + control

Miniprep Protocol

1) spin - collects cells - resuspend in buffer

2) add NaOH/SDS - cells lyse

SDS: ionic detergent:
disrupts membranes
destabilizes hydrophobic interaxns that maintain conformation

NaOH: hi pH denatures macromolecules by changing ionizable groups

- clearing when cells lyse
- more viscous as more molecules soluble
- DON'T VORTEX! **Why not?** chromosome DNA shearing

3) add potassium acetate

low pH neutralizes NaOH
macromolecules try to renature
but bacteria DNA, protein can't
too big - form bonds
nonspecifically + ppt

plasmid - small supercoiled
renatures + stays in soln

4) spin - pellet debris

DNA in sup!

Miniprep - explain name

Purpose? Isolate plasmid DNA from other stuff (bacteria chromosomal DNA, **What!** proteins, etc)

[redacted]

Plasmids - small + supercoiled
bacteria DNA - large, less supercoiled
→ selective ppt of plasmid DNA

5) add Isopropanol - ppt DNA - spin

Ionic (-) DNA soluble in H_2O
but not organic solvents

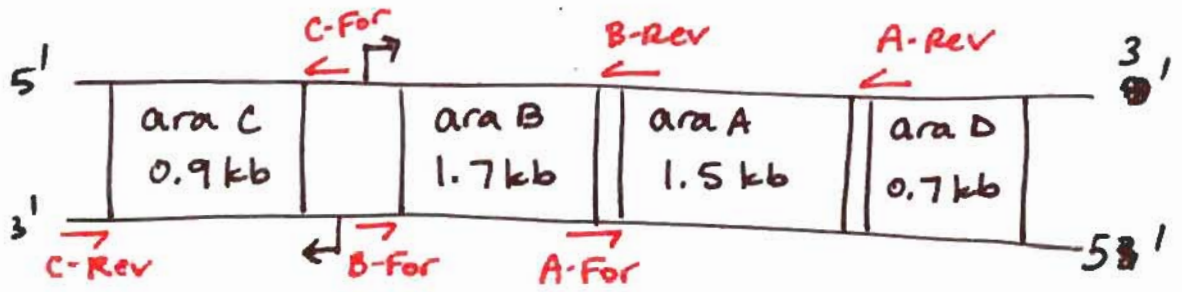
6) wash pellet w/ ~~add~~ 80% EtOH - spin

20% H_2O allows salt in pellet to dissolve
EtOH allows evaporation after pelleting

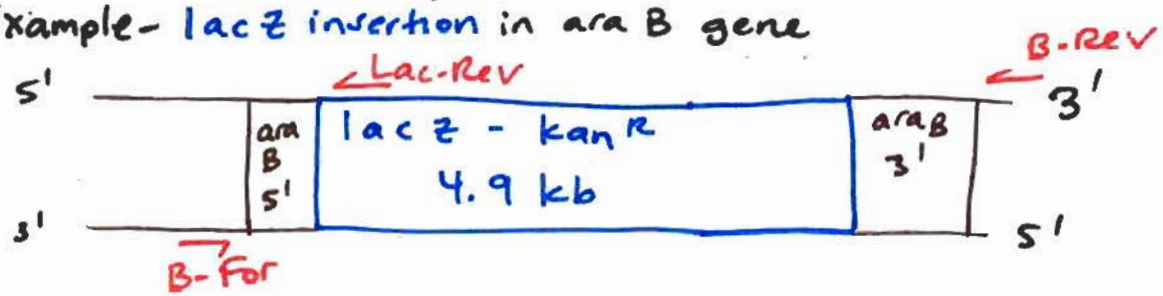
7) resuspend in TE w/ RNAse A

Tris - buffer
EDTA - chelates Mg
RNAse A - degrades RNA

PCR results will tell where transposon inserted during GEN module



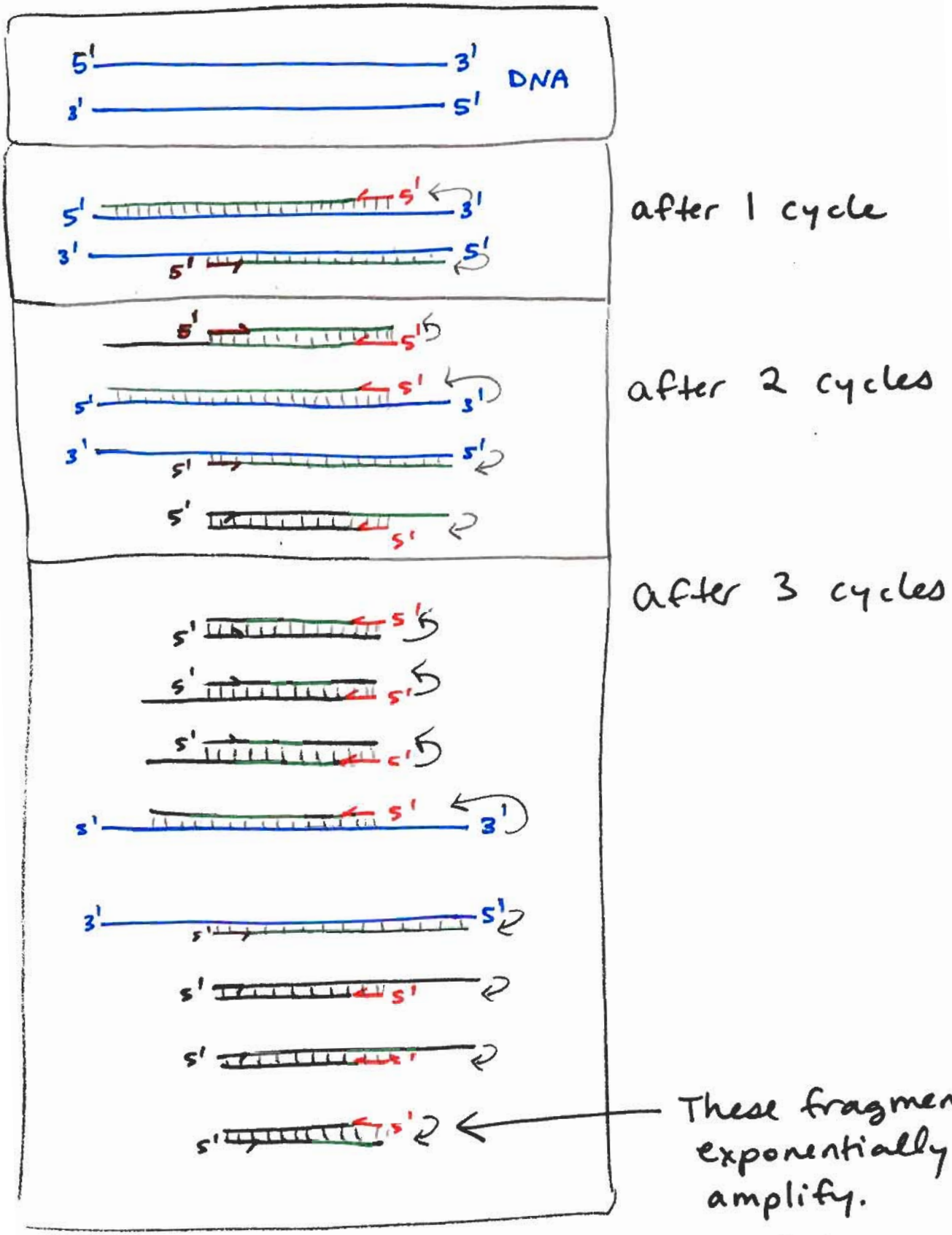
Example- lacZ insertion in ara B gene



B-For + Lac-Rev will give PCR product on gel- after exponential amplification

B-For + B-Rev will <u>not</u> since distance between 2 primers too great- only gets linear amplification

PCR exponential amplification



Each new strand serves as a template in the next cycle.