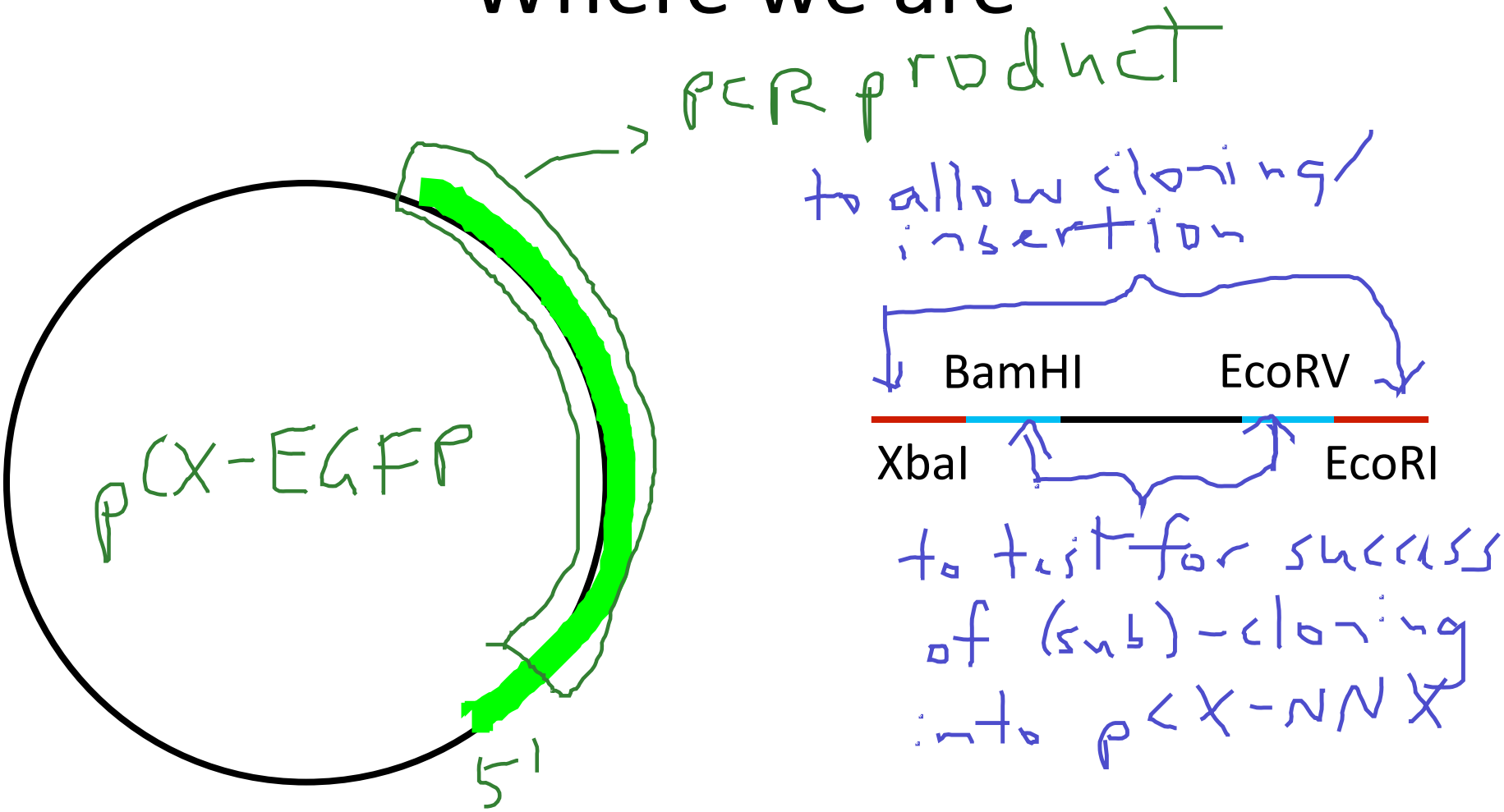
- Announcements, Review Quiz
- Lab Quiz (re: M1D1)
- Pre-lab Lecture
  - Where We Are/Going (Cloning)
  - DNA Cleanup
  - Today in Lab: M1D2
  - Writing Goals Discussion

Lab Practical Review (will be erased!)

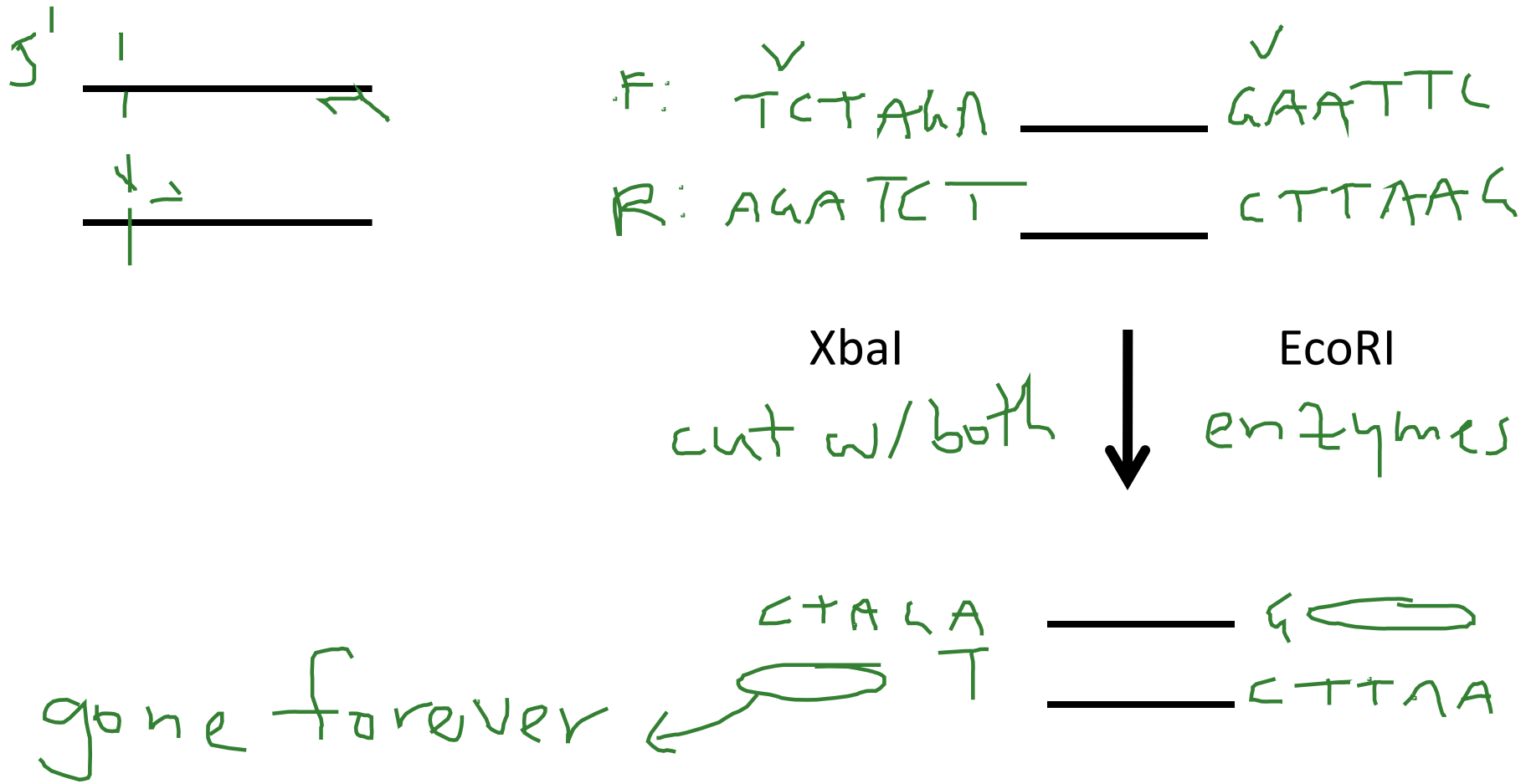
Announcements

- There **is** lecture next Tue, but no lab Wed
- Be sure to hand in EHS training, make wiki page, hand in questionnaire if you haven't
- Reminder: all lecture notes linked from *Schedule* page in rightmost column

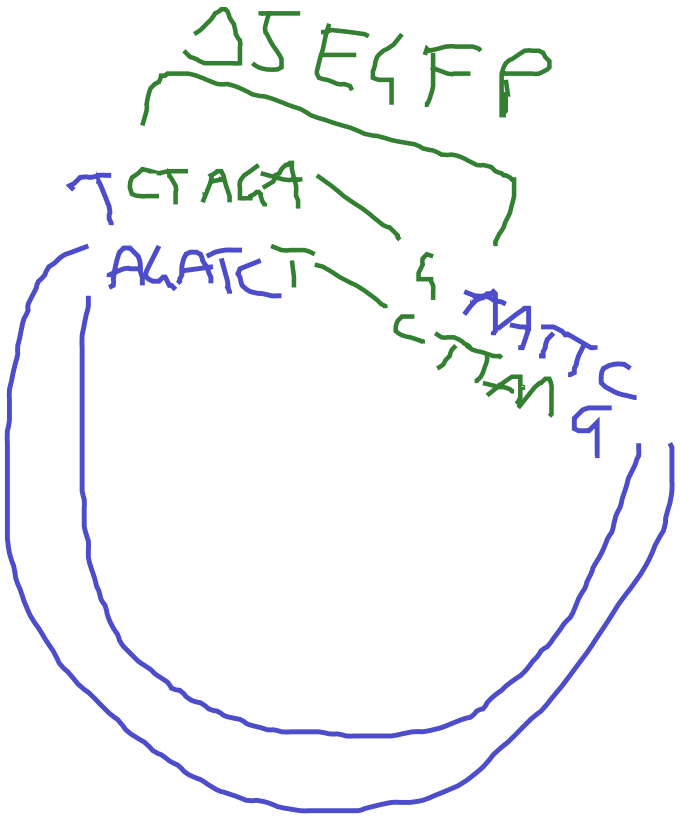
Where we are


PCR product
to allow cloning/ insertion
pCX-EGFP
5'
BamHI
EcoRV
XbaI
EcoRI
to test for success of (sub)-cloning into pCX-NNX

Digesting PCR product



Restriction enzymes for cloning



pCX-NNX

Δ5-EGFP

What if EcoRI is 5' and XbaI is 3' on insert?

reversed, non-coding

What if EcoRI on 5' *and* 3' ends?

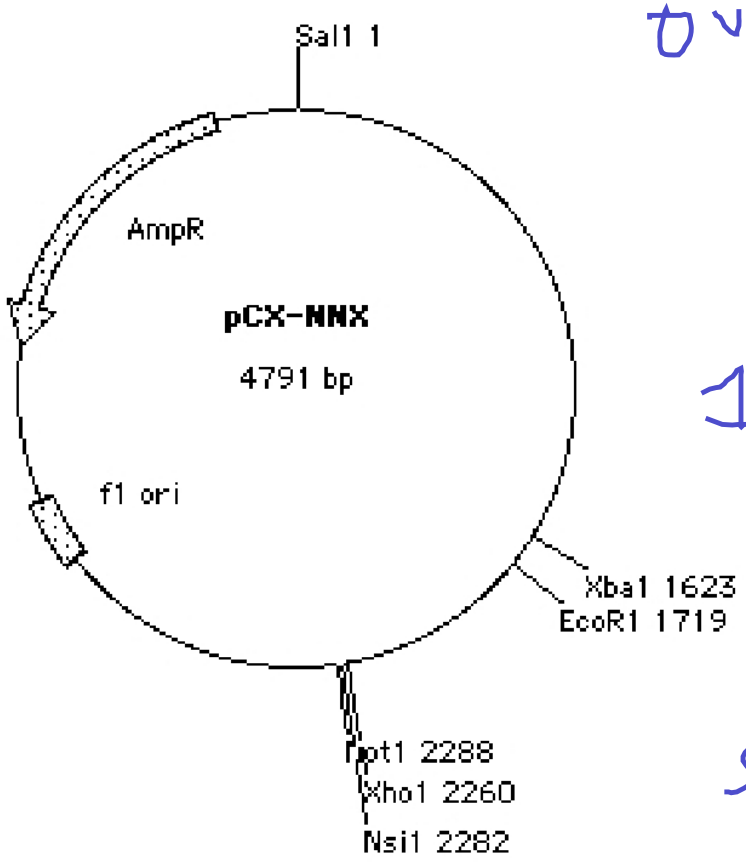
non-directional cloning

Can you get multiple inserts?

XX-E-EX-X-EE odd # only! ←Y also plasmid dimers

Restriction enzyme digest controls

- How many times (0, 1, 2) was DNA cut?



0 vs. 1
- both 4791 bp
- circular vs. linear
  - look different

1 vs. 2
- 4791 vs. 4695 bp
  - can't distinguish

single-cut controls

DNA clean-up

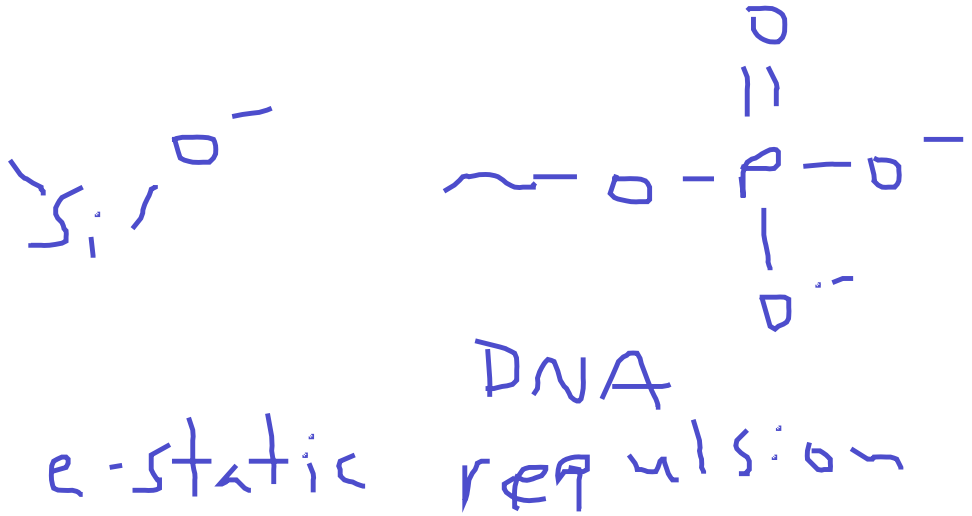
Why? Switch buffers; rid excess reagents

beads

Silica resin column

[qiagen.com]

1. Bind DNA: high salt, low pH
   Chaotropic salts disrupt H-bonds
   DNA sticks to column
2. Keep DNA, wash off rest
   Ethanol precipitates DNA
3. Elute DNA: low salt, high pH



Today in Lab: M1D2

- Careful with enzyme stocks!
  - Keep cold; don't contaminate

- There are 4 samples today (2 single digests)

X or E

- Order of addition for digest

enzyme last

short day! but figt . . .

Writing goals discussion (summary)

Experience reading papers informs writing goals:

Niche research papers can be very dry, difficult to understand, or even boring. Authors need to find a way to draw the general reader in.

Readers are likely to skip to the results section. This part (text and visuals) should be exceedingly clear. In this class, expect to learn which parts of a paper are most important, how so, and how to make them most readable.

There seem to be two categories of papers: good and really bad. The good papers are more enjoyable to read, and somehow make even complex ideas seem simple. So unclear writing cannot be excused by subject complexity.

Readers may particularly enjoy the introduction section. This part gives the chance to see the big picture (e.g., disease) rather than focus on the details (e.g., a single pathway of interest). In this class, expect to learn how to motivate one's work.

Criticizing a paper (e.g., what other controls should have been done?) is much harder than summarizing one. Former mostly beyond the scope of this class.