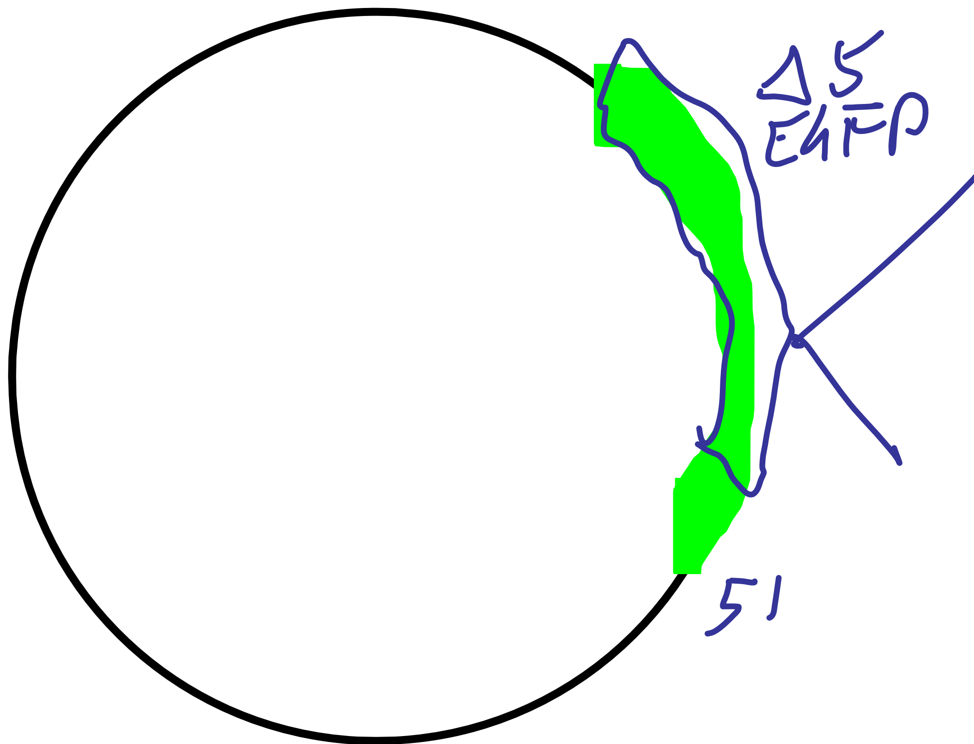
- Announcements, Review Quiz
- Lab Quiz (re: M1D1)
- Pre-lab Lecture
  - Where we are/going
  - More on endonucleases
  - DNA cleanup
  - Safety + Technical Tips

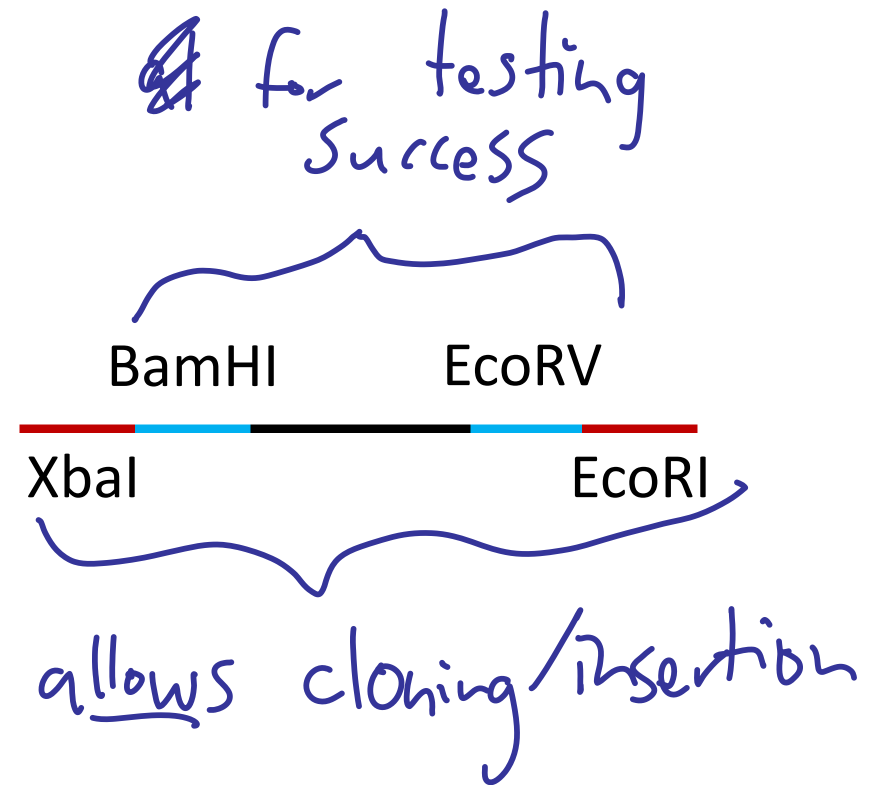
Announcements

- Mya and Atissa from WAC coming at 1:30 pm
- BE UG Board session on Grad School+Fellowships
  - Tue Sept. 16th
  - 7:30 pm, 56-614
  - Dinner!

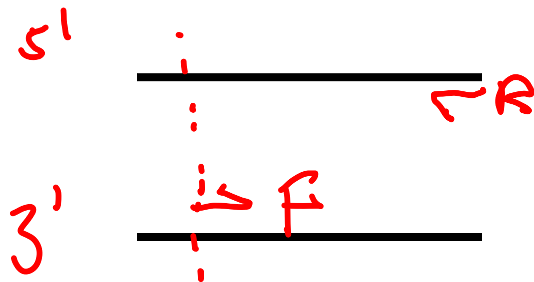
Lab Practical Review (will be erased!)

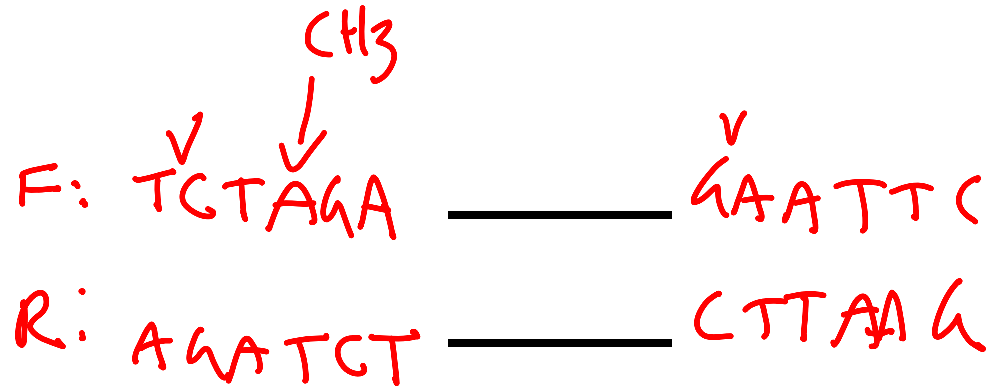
Where we are





Digesting PCR product

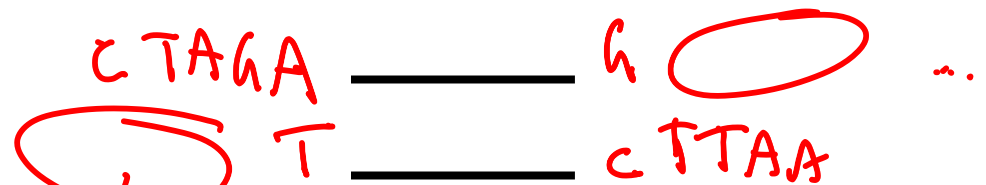




XbaI

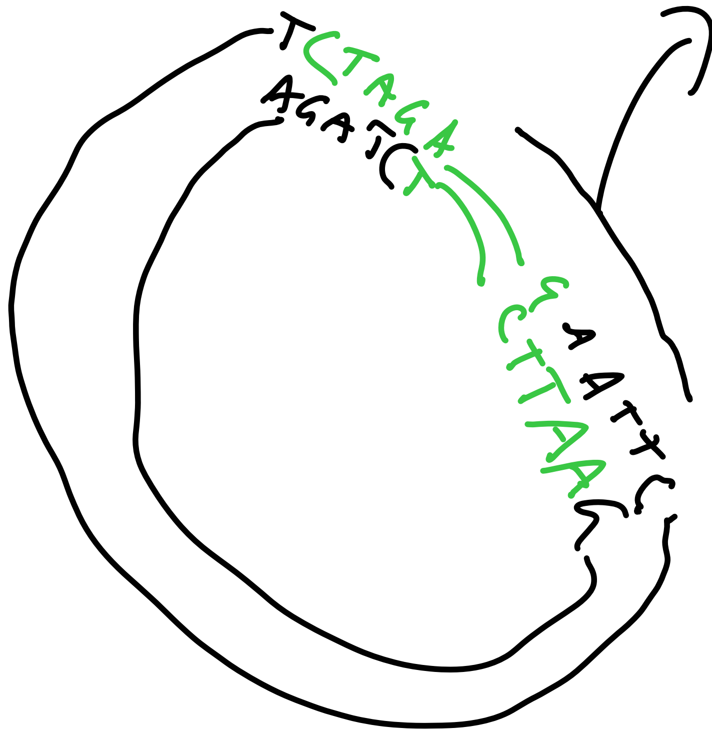
EcoRI

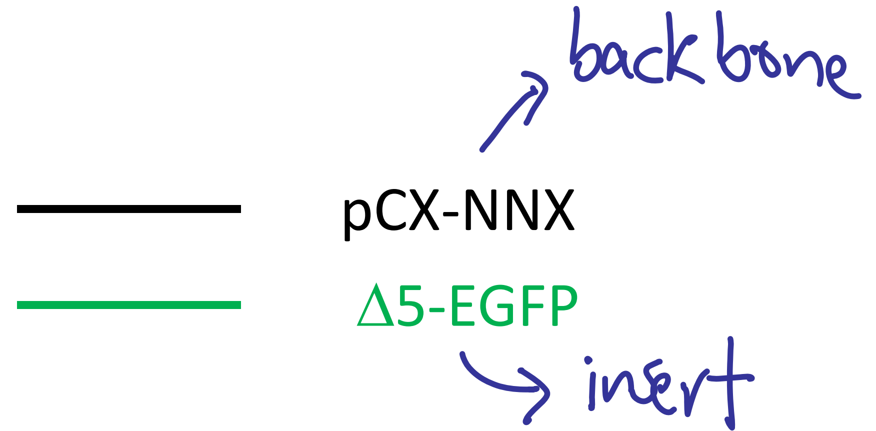
cut w/ both enzymes



gone forever (if other DNA is in use, it's lost)

Restriction Enzymes for Cloning





What if EcoRI is 5’ and XbaI is 3’ on insert? reversed, non-coding product

What if EcoRI on 5’ *and* 3’ ends? non-directional cloning (+ 5Kb re-ligates)

Can you get multiple inserts?

-X X-E-EX-X-E E- 3 inserts

plasmid dimers (odd #)

DNA Clean-up

why: get rid of PCR reactants, switch buffer

1. DNA binding → high salt, low pH

   * chaotropic salts → disrupt DNA/H_2O H-bonds

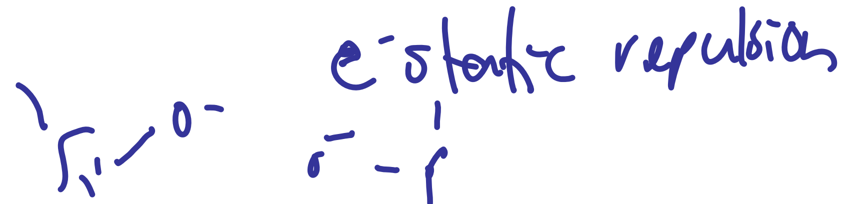
Silica resin column

→ beads

2. Keep DNA, lose other reactants

   ethanol – precipitates DNA

3. elute DNA → low salt, high pH ↓



Today in Lab

- Careful with enzyme stocks!
  - Keep cold; don't contaminate
- There are 4 samples today (2 single digests)
- Order of addition for digest

* R.E.' proteins