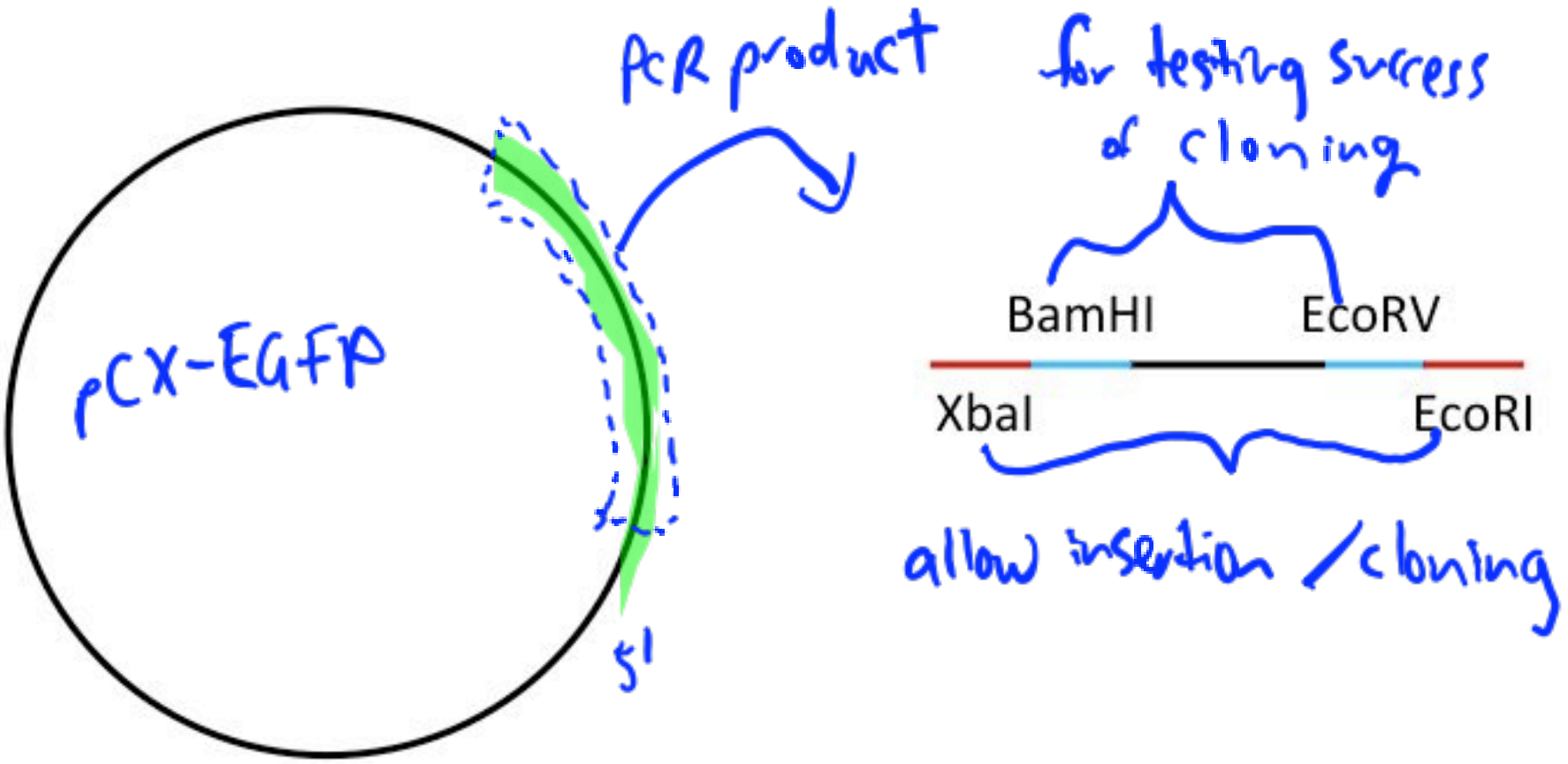
- Announcements, Review Quiz
- Lab Quiz (re: M1D1)
- Pre-lab Lecture
  - Where we are/going
  - More on endonucleases
  - DNA cleanup
  - Safety + Technical Tips

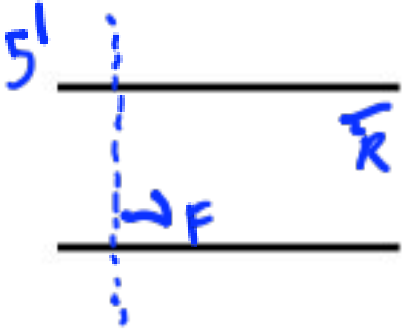
Announcements

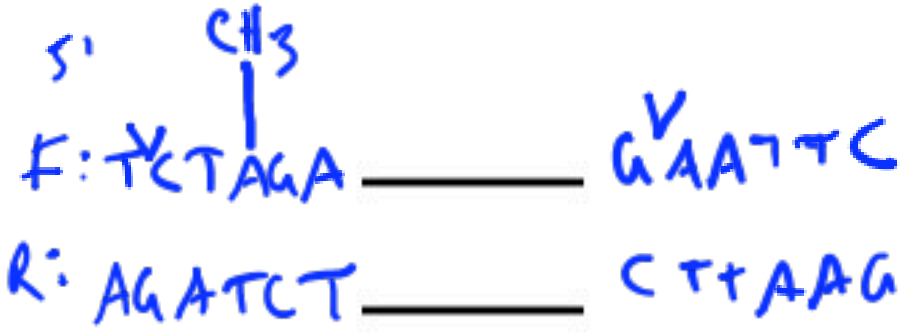
- Protect your classmates – stay home if you're sick
- Neal, Atissa, and Linda from WAC coming at 3:30

Where we are


PCR product
for testing success of cloning
pCX-EGFP
5'
BamHI
EcoRV
XbaI
EcoRI
allow insertion / cloning

Digesting PCR product

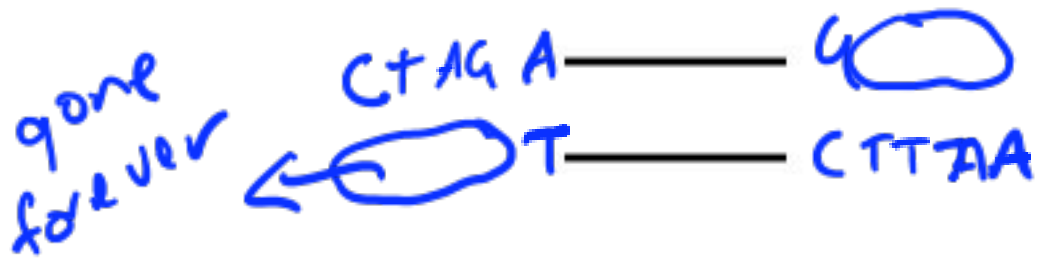




XbaI EcoRI

cut w/ both enzymes

E.coli protected by selective methylation



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 (+ religation)

Can you get multiple inserts?

(and plasmid dimers)

DNA Clean-up

why? get rid of excess reagents & switch buffers

Silica resin column

[qiagen.com]

1. DNA binding → high salt, low pH
   - chaotropic salts disrupt H-bonds, DNA sticks to column

2. keep DNA, wash off rest
   - beads
   - ethanol - precipitate DNA

3. elute DNA → low salt, high pH
   - $\equiv Si/O^-$ DNA ^-O-P
   - e-static repulsion

Today in Lab

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

- There are 4 samples today (2 single digests)

Xba, Eco

- Order of addition for digest

enzyme last