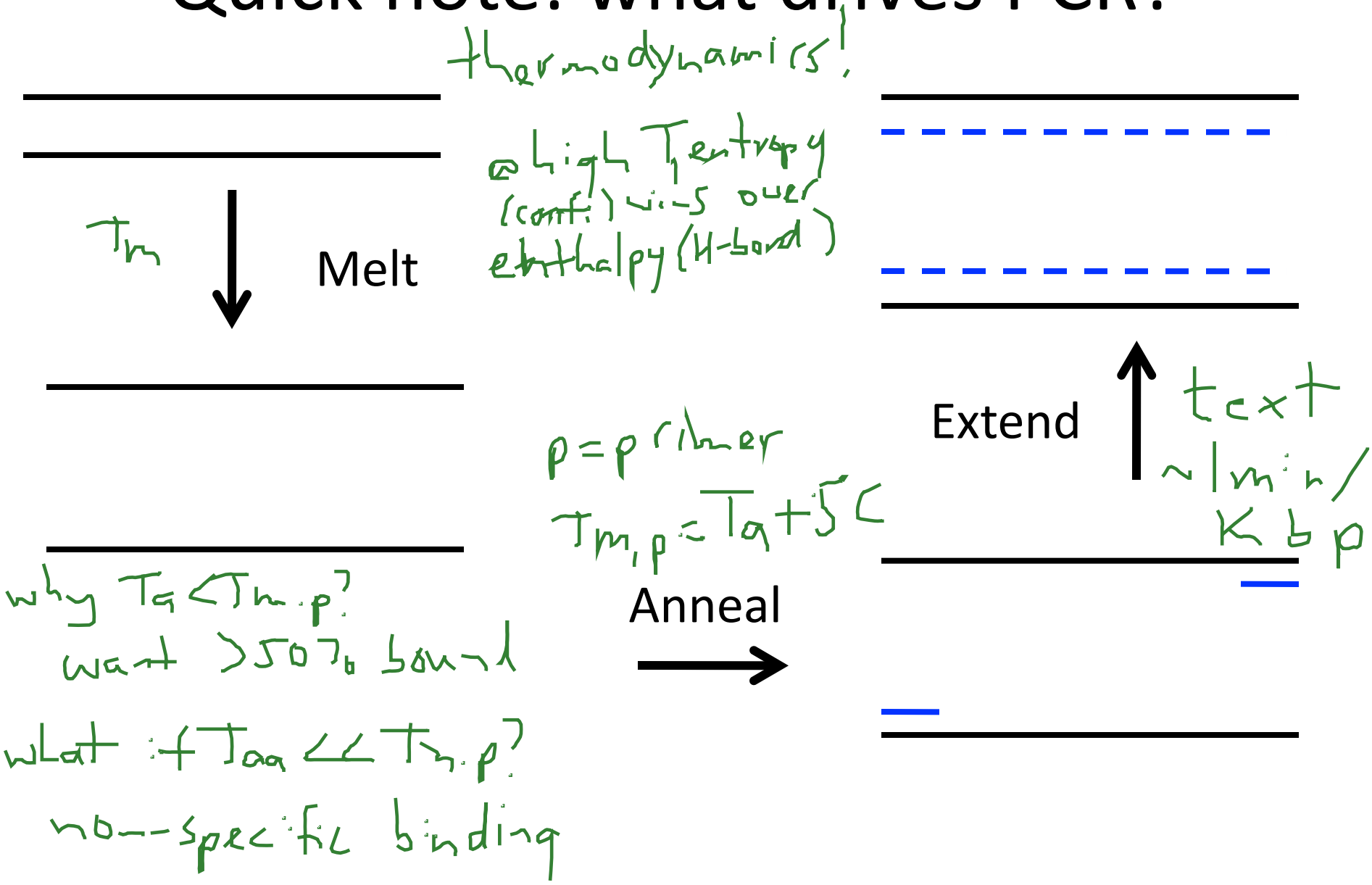
- Announcements
- Lab Quiz
- Pre-lab Lecture
  - 20.110 view of PCR
  - Where we are/going (cloning)
  - DNA cleanup
  - Today in Lab: M1D2

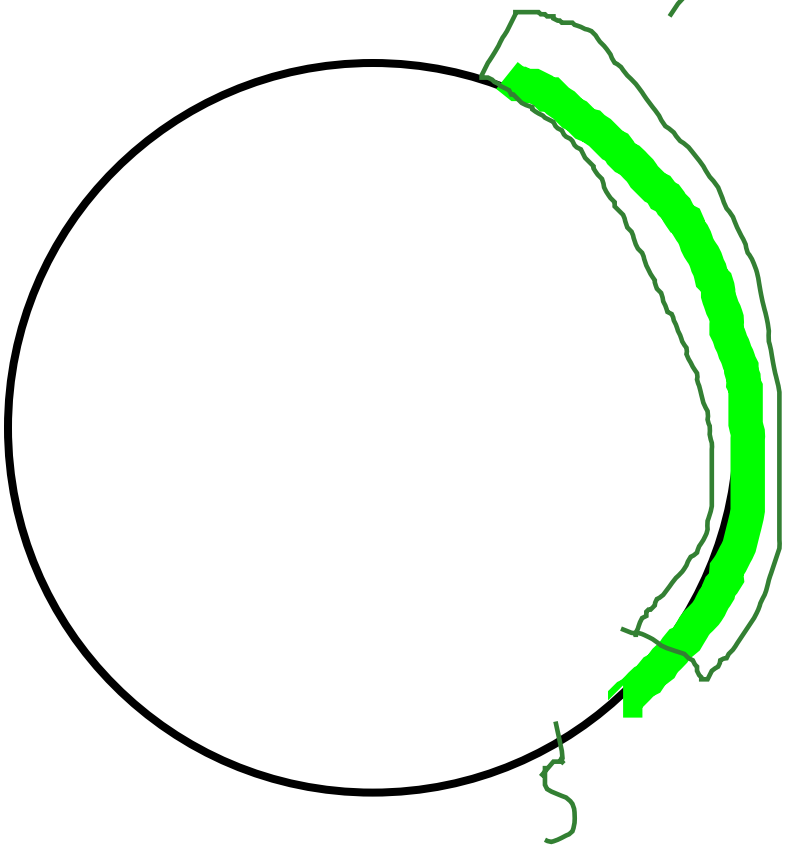
Announcements

- Brief discussion of orientation day quiz
- General reminders
  - *Assignments* + *Schedule* = our syllabus
  - All lecture notes linked from *Schedule* page, "notes"
  - Optional FNTs submitted on Stellar site HW
  - *Lab Basics* has notebook-keeping guidelines
- Be sure to hand in EHS training, make wiki page, hand in questionnaire if you haven't
- Communication choices (written vs oral) due next time, in agreement with your partner ★ reading preview today
- First notebook evaluation also next time

Quick note: what drives PCR?

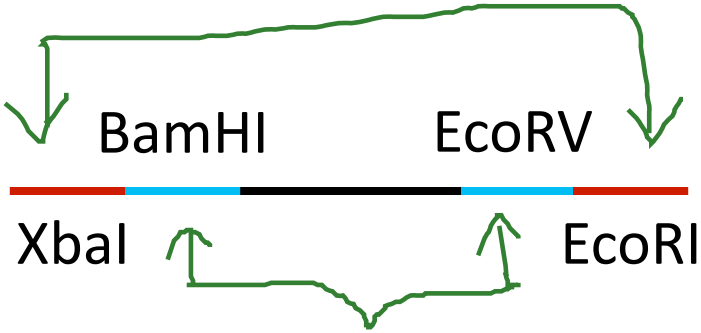


Where we are



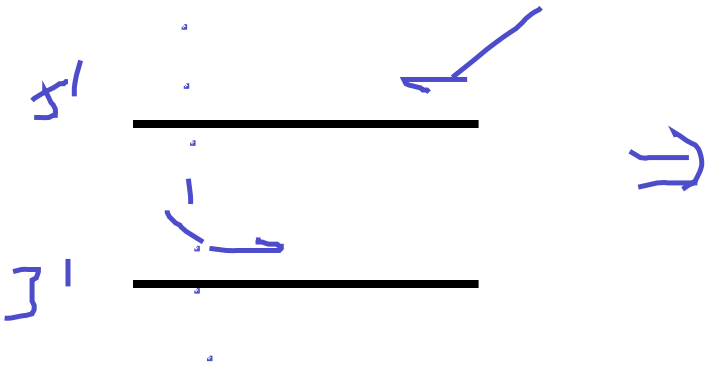
PCR product

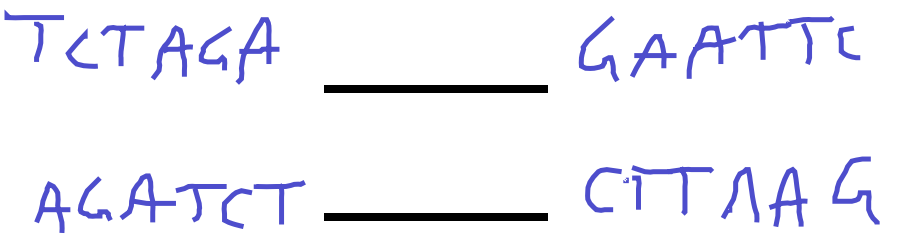
to allow cloning/insertion



to test for success of (sub)-cloning into pcX-NNX

Digesting PCR product

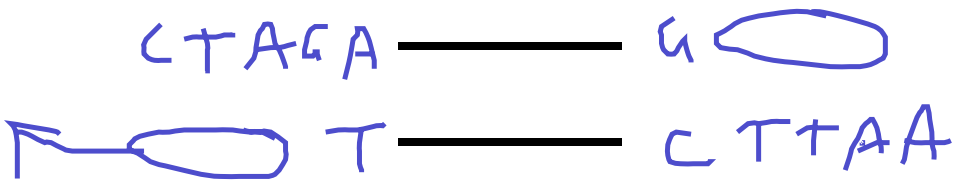




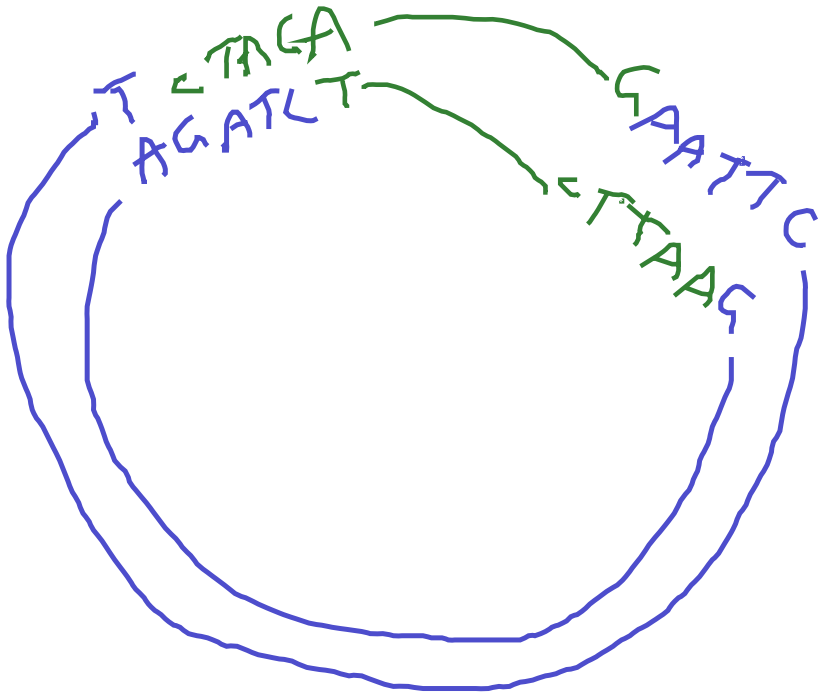
XbaI EcoRI

Cut w/ both X, E

gone forever (after clean)



Restriction enzymes for cloning



pCX-NNX

Δ5-EGFP

What if design primers with EcoRI on 5' and XbaI on 3' end of insert?

reversed, non-coding

If design w/EcoRI on 5' *and* 3' ends?

non-directional

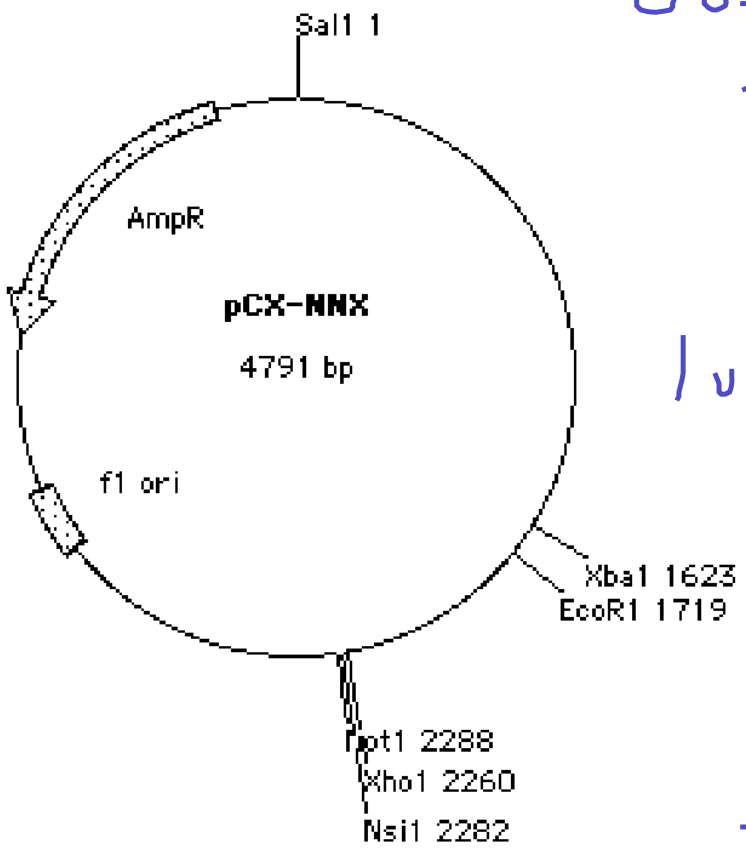
Can you get multiple inserts?

also plasmid dimers

-X XE EX XE- odd # ← Y

Restriction enzyme digest controls

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



Ovs. 1
— both 4791 bp
— circular vs. linear
★ look different

Ivs. 2
— 4791 vs. 4695 (and ~100) bp
★ can't distinguish

use salient controls
for enzyme activity

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 away rest
   ethanol precipitates DNA

3. Elute DNA: low salt, high pH
   electrostatic repulsion

Today in Lab: M1D2

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

enzyme last

short day! :)