- Announcements
- Pre-lab Lecture
  - Writing a methods section
  - 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"
  - *Assignments* (scroll down) has notebook guidelines
- First M1 quiz next time! (see wiki)
- *Optional* ungraded notebook submission today

$*C_1V_1 = C_2V_2*$

$0.05 \cdot 10 = 0.5$?

% = g/100mL

"PLEASE GRADE" @ top M1D2 entry by Sat 5pm

Mod 1 major assessments

- Methods section (5%)
  - Done individually, no revision
  - Important building block/skill for Mod 2 report
  - Plasmid construction only
  - Start in today's FNT!
- Abstract and data summary (15%)
  - Done in pairs, with revision
  - More building blocks: abstract, figures/captions, etc.
  - But not yet a full narrative
- starts in next FNT and today's WAC session

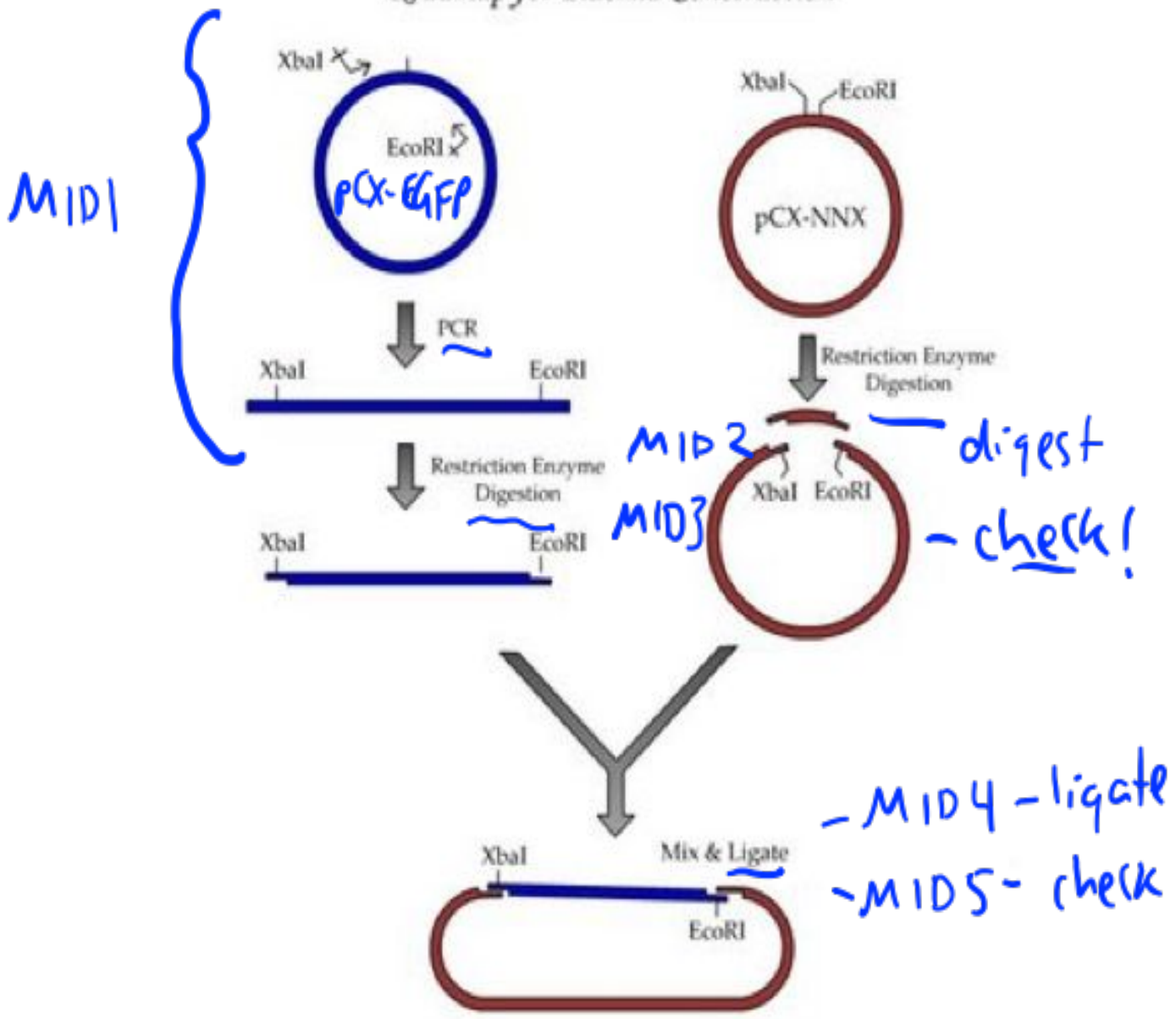
Methods section tips

- Organizing sub-sections in a logical order
  - Often start with an overview/introductory sentence (*what*, not why) → then give step-by-step details
- Methods should be concise and complete not a protocol
  - Space-wise, avoid tables/lists when a sentence will do
  - Sentence-wise, avoid extra words
  - Content-wise, cover what's needed and only that needed to understand and replicate your work
- Concentrations are more useful than volumes; or you can state amounts, plus total volume. "1mL of DNA" useless

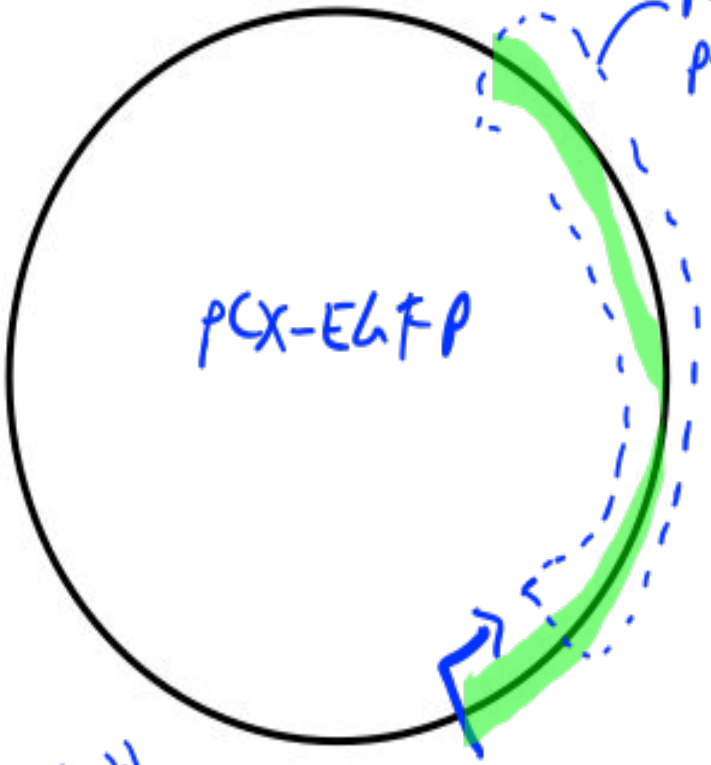
Methods section exercise

- Consider the following passage: "Template DNA (5 ng) and primers were mixed with 20 uL of 2.5X Master Mix in a PCR tube. Water was added to 50 uL. A tube without template was prepared and labeled control. "
  - What information is missing?
  - What information can be cut?

Roadmap for Plasmid Construction



Where we are



fromSKH

About G/C rich templates...

70% G/C ~ Tm ~ 70°C

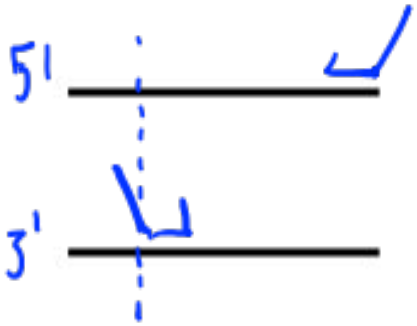
- DMSO, betaine, glycerol
- open structure or shift Tm/a
- "Q soln" - pre-optimized

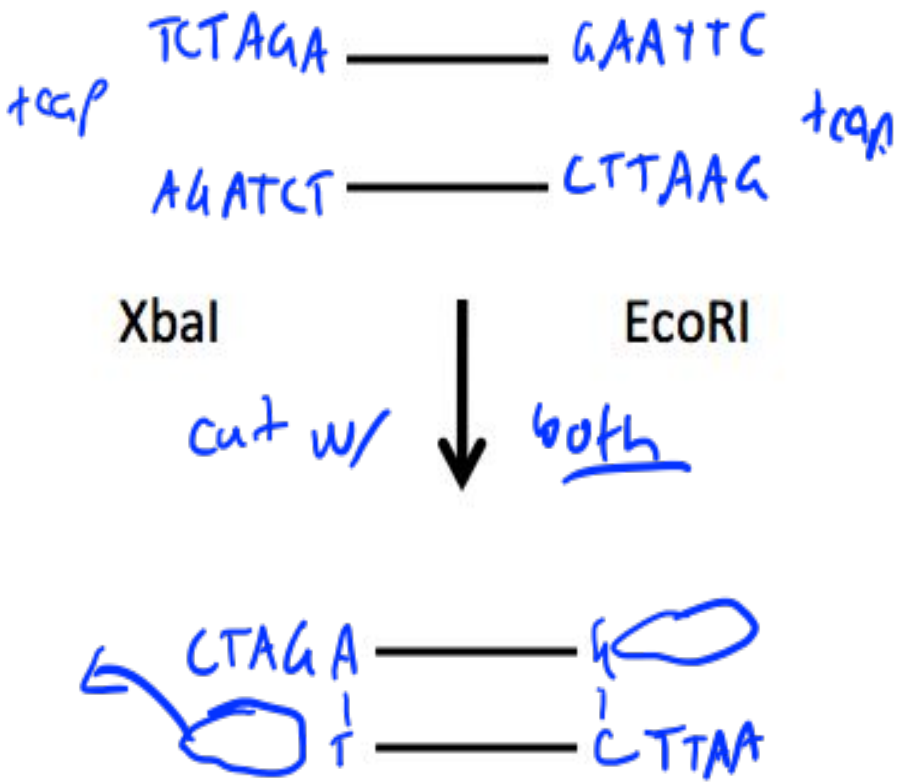
200 M on key elements

to allow cloning/insertion

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

Digesting PCR product





Restriction enzymes for cloning

pCX-NNX

Δ5-EGFP

★ draw and internalize ★

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?

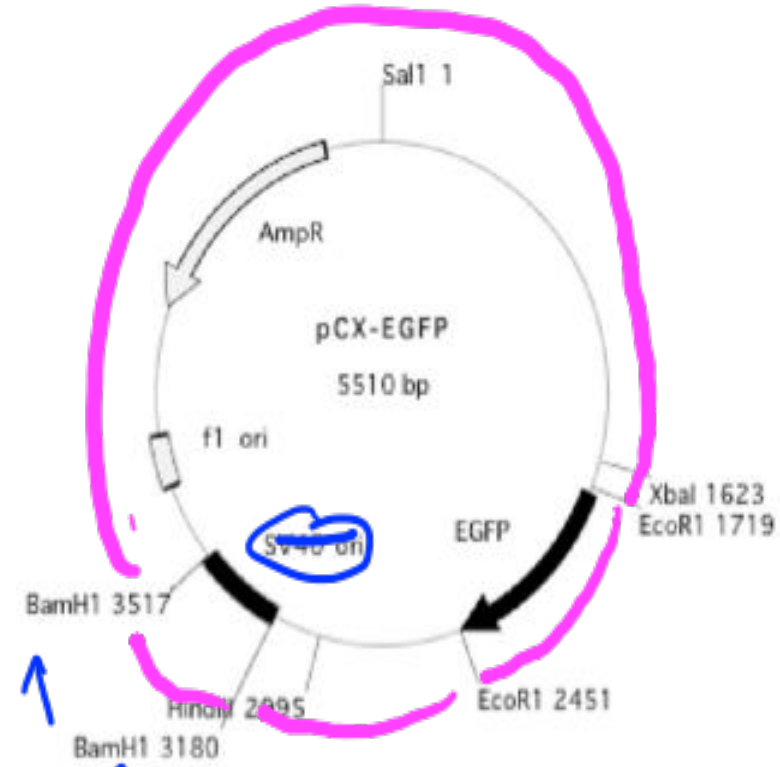
non-directional cloning

Can you get multiple inserts?

Y → odd # (3) plasmid dimers

– X-X – E E – X X – E E –

Restriction enzyme analysis example



not our bkb

Enzyme	# bands	Size bp
EcoRI	2	~700 ~4800
EcoRI + XbaI	3	~100 bp * hard to see ~700 ~4700
EcoRI + BamHI	4	~700] * wide or doublet ~700 ~300 ~3700

salt + EtOH
↳ RH waste
no bleach!!

DNA clean-up

Silica resin column

[qiagen.com]

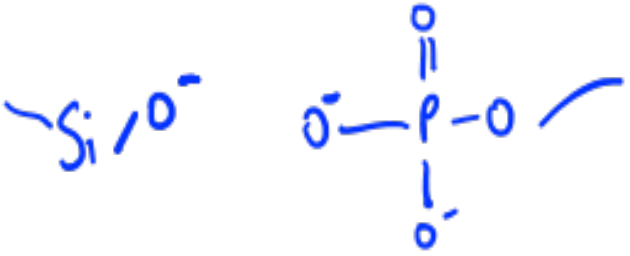
why? 2 reasons

1. bind DNA ↑salt, ↓pH
   chaotropic salt disrupts H-bonds, dehydrates DNA, bridges to silica

2. keep DNA, wash else
   EtOH keeps precipitated

3. elute DNA ↓salt, high pH

repulsion



Today in Lab: M1D2

* your NNX stock use 10mL (not 4) *

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

PCR-dbl NNX-dbl, XbaI, EcoRI-HF (uncut)

engineered

- Order of addition for digest

enzyme last

- WAC session at 3:45 pm
- Note: FNT uses ApE – useful general DNA tool

toddler quote/ Science proposal: "Do you have eyelashes in there? Can I take them?" NOT IRB-approved