- Announcements
- Pre-lab Lecture
  - Biosafety refresher -- Damon
  - 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)
- Contributing to 20.109 class blog
- Office hours: Tue 4-5 (16-319) - every wk
  Sun c. 2-4, TBD, some wks

que 8.6

- spec! ↓ trust yourself:) theory Abs ≠ Refl.
- great job w/ safety!
- math: be systematic
  - ✱ % = v/v or w/v ⇝ g/100mL ✱

$C_1V_1 = C_2V_2$

$5\%_0 \cdot V_1 = 0.5\%_0 \cdot 100mL$

1= have already 2= goal/making

side note: efficiency vs. accuracy

Mod 1 major assessments

- Methods section (5%)
  - Important building block/skill for Mod 2 report
  - Plasmid construction only (D1-D5)
  - Start in current FNW, # 3. → due Wed!
- Abstract and data summary (15%)
  - More building blocks: abstract, figures/captions, etc.
  - But not yet a full narrative
- Both assignments done individually

Methods section tips

- Organizing sub-sections
  - Start with an overview/introductory sentence (*what*, not why): "EGFP was amplified using polymerase chain rxn."
  - Put in a logical order: primer design, *then* PCR
- Methods should be concise and complete
  - 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.

Methods section exercise

★ more than one "solution" ★

define sequence amount or concentration

what is it?

- 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?

manufacturer

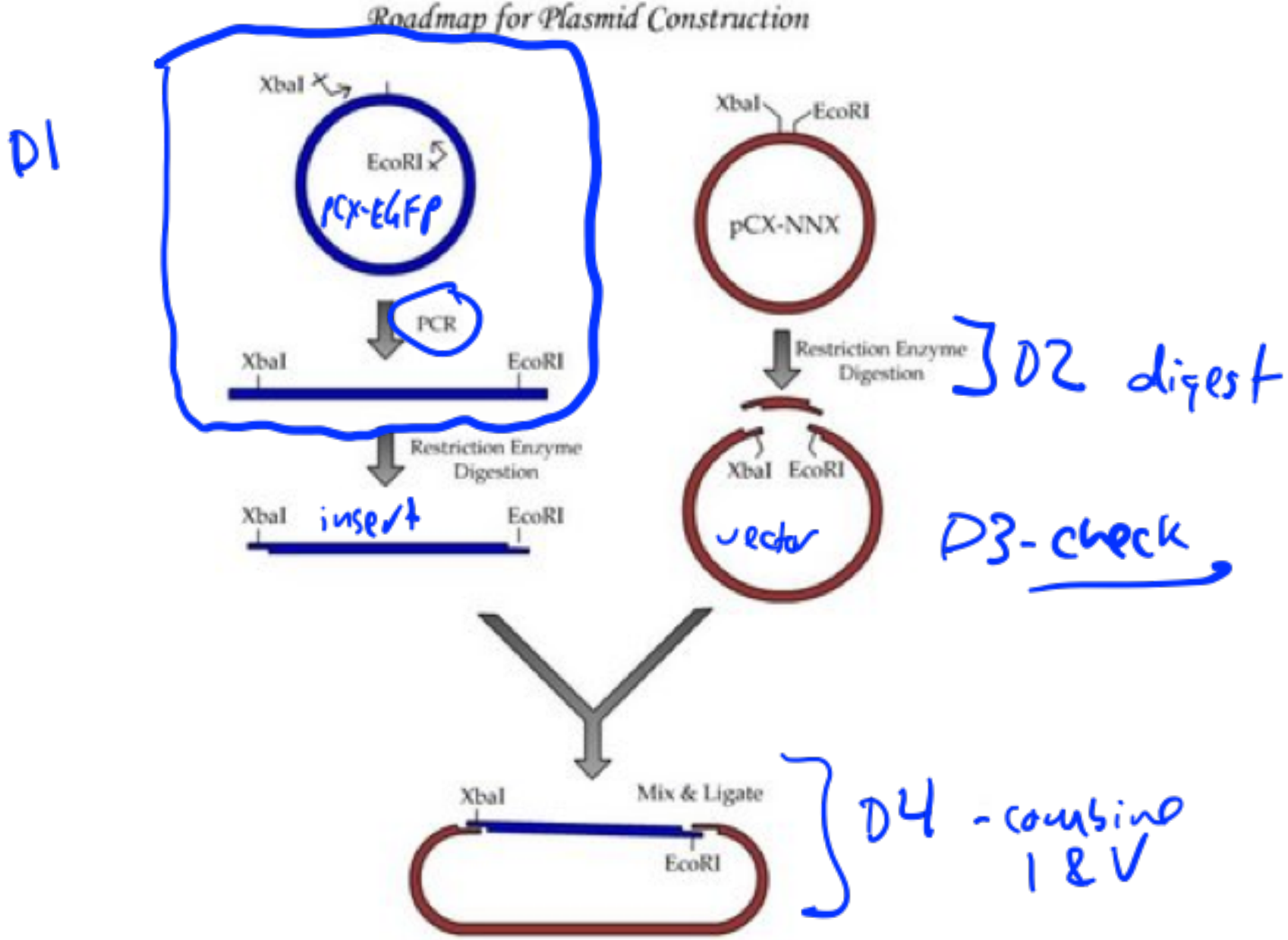
wordy

people can assume 50/2.5=20 is redundant

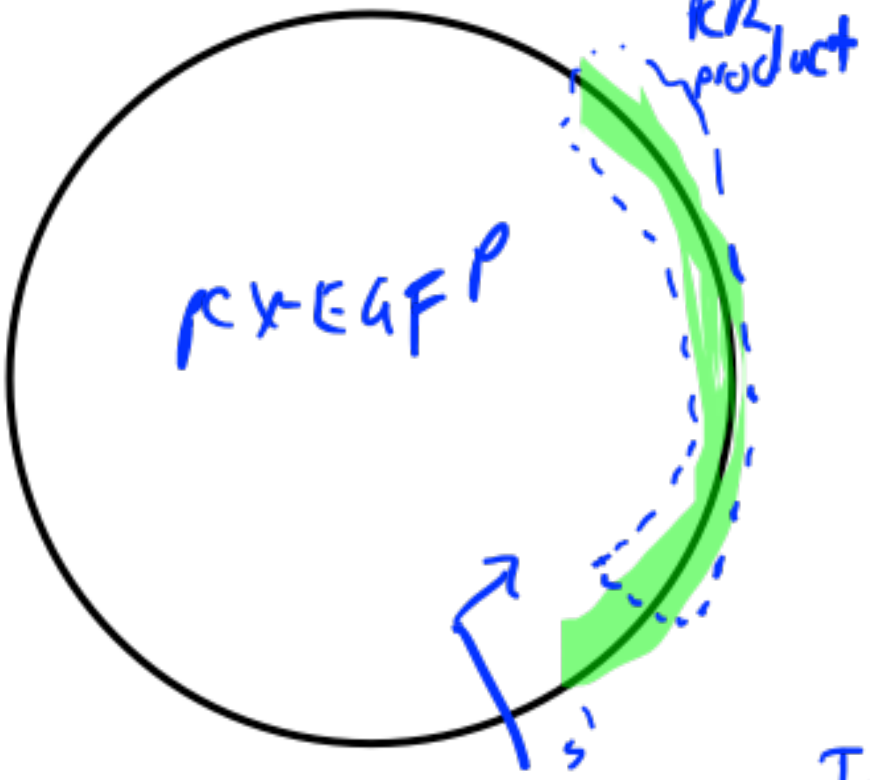
prepared/diluted in Master Mix (spring, city/state)

one option: "A [NTC] was also prepared."

ideally even less


Roadmap for Plasmid Construction
D1
XbaI
EcoRI
pCX-EGFP
PCR
XbaI
EcoRI
Restriction Enzyme Digestion
XbaI
insert
EcoRI
XbaI
EcoRI
pCX-NNX
Restriction Enzyme Digestion
D2 digest
XbaI
EcoRI
vector
D3- check
XbaI
Mix & Ligate
EcoRI
D4 - combine I & V

Where we are



200 nt

to allow cloning/insertion (X, E)

BamHI EcoRV

XbaI ΔEGFP EcoRI

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

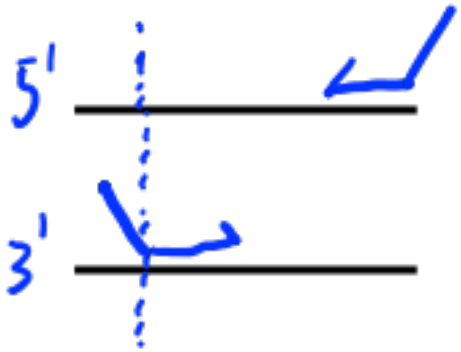
About G/C rich templates...

Tm ~ 70°C

G/C ~ 70%

additives that ↓Tm or have structural changes improve amplification

Digesting PCR product

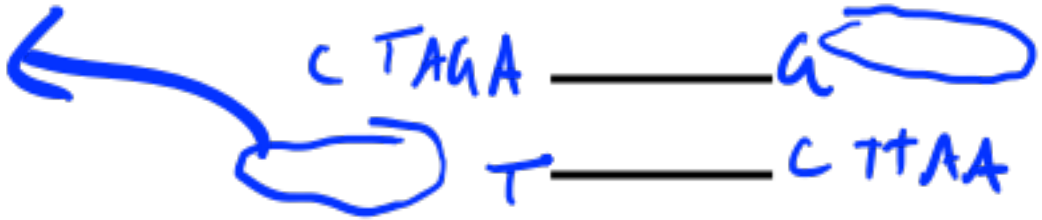


tcap

TCTAGA ——— GAATTC

AGATCT ——— CTTAAG tcap

XbaI EcoRI



CTAGA ——— G

T ——— CTTAA

after clean-up, gone forever

Restriction enzymes for cloning

PCR product not to scale!

pCX-NNX

Δ5-EGFP

★ draw and internalize ★

② What if design primers with EcoRI on 5' and XbaI on 3' end of insert?

reversed, non-coding

① If design w/EcoRI on 5' *and* 3' ends?

non-directional

③ Can you get multiple inserts?

Yes, odd # only

-XX-EE-XX-EE-

DNA clean-up

why?

- residual dNTPs, sticky fragments
- charge buffer

steps

beads

Silica resin column

1. bind DNA
   ↑salt ↓pH
   chaotropic salts disrupts non-covalent bonds w/H2O; dehydrates + bridges DNA
2. keep DNA, wash else
   EtOH keeps DNA precipitated
3. elute DNA
   ↓salt, ↑pH $-Si-O^-$ ⇑ $-O-P-$
   electrostatic repulsion

[qiagen.com]

Today in Lab: M1D2

- Careful with enzyme stocks!
  - Keep cold; don't contaminate
- Qiagen kit: collect as **chemical waste**
- There are 4 samples today (2 single digests)

  PCR-dbl NNX- dbl and singles
- Order of addition for digest

  enzyme last

- Note: FNW uses ApE – useful general DNA tool