M1D2: Clean and Cut DNA

9/12/13

1. Pre-lab discussion

2. Clean-up PCR reaction

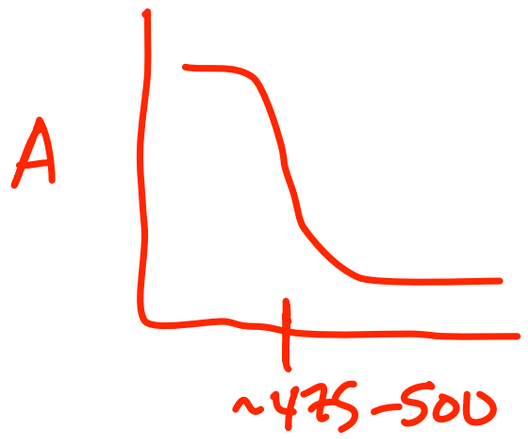
3. Digest vector & PCR rxn ~ ApE

4. 4pm -- visit from Leslie

Note: Practice notebook option -- see wiki!

(REASE GRADE!) 5pm Friday

Lab practical review:



ROYGBIV

* gloves ⇒ benchtop/burn box

0.03% XC → 1% stock

$C_1V_1 = C_2V_2$

$$\frac{(10\ mL)(0.0003)}{0.01} = 0.3\ mL$$

Module 1 major assessments:

5% ① Methods → FNT draft
MID1 - PCR
MID2 - RE dig

15% ② Data Summary — WAC here
- figures + captions
- summary
- abstract

Methods Section Tips

- Divide into sub-sections!

  Put in a logical order -- list primer THEN list PCR reagents, etc

  Start with a overview sentence "EGFP was amplified using polymerase chain reaction (PCR)."

- Methods are clear and concise explanations

  The methods section is not a benchtop 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

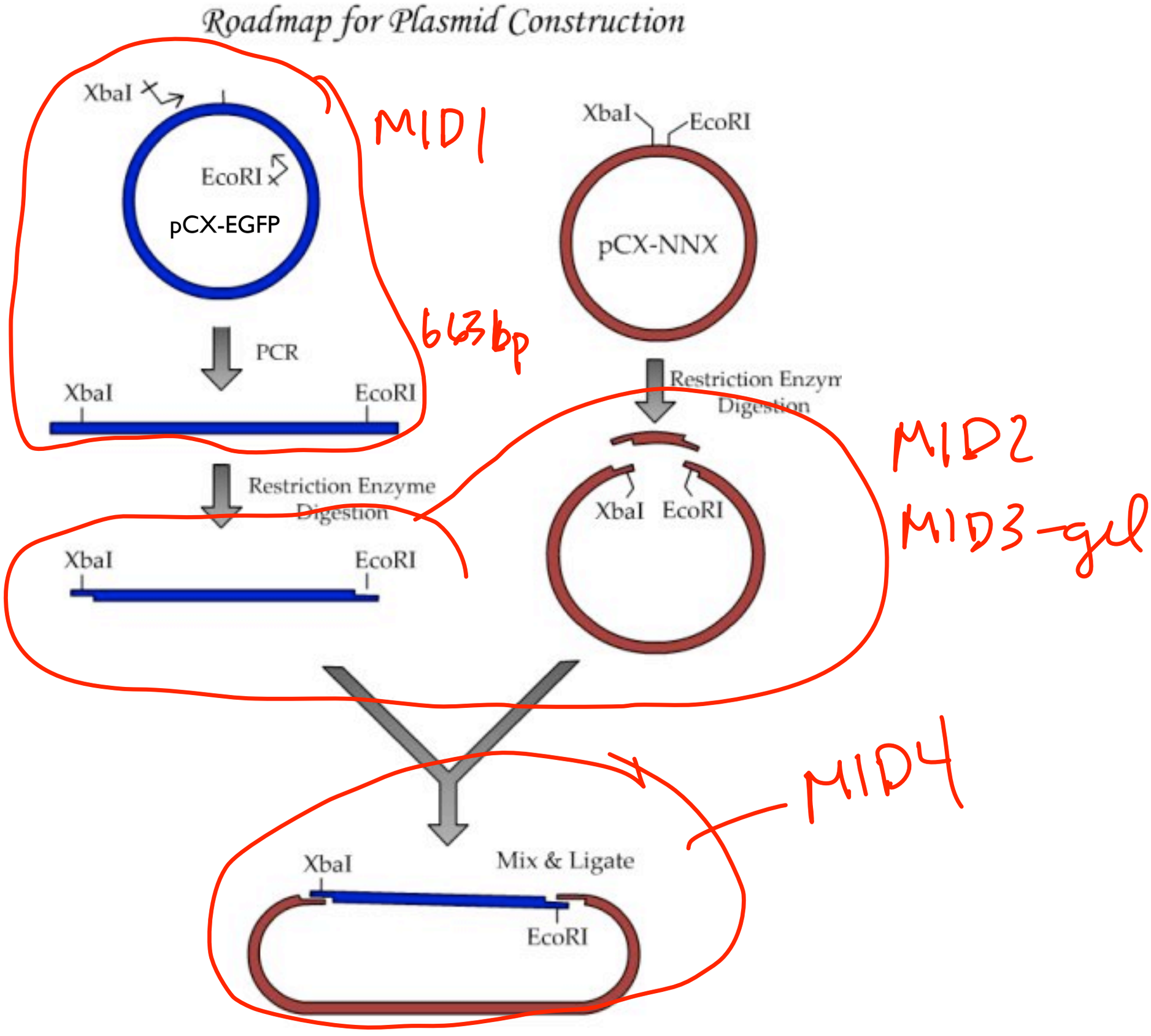
- Think about the most flexible units -- concentration vs volume. (For replication!)

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?

Thanks to Agi for this slide!

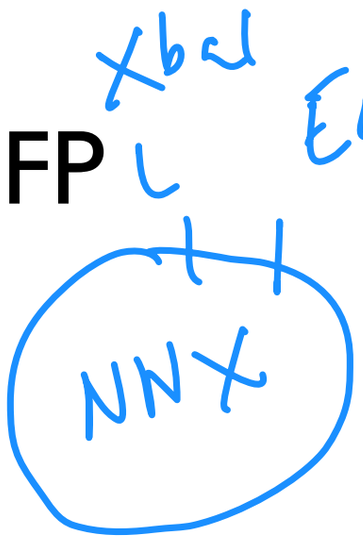
Step 1: Build the system!



Last time:

XbaI & EcoRI – Subcloning tools

pCX-EGFP



BamHI + EcoRV - troubleshooting/ confirming our success

Oh my, so G/C rich -- what can you do to improve your chances?

~70% G/C
T_M = 69.3°C

DMSO – untold

glycerol – NMP: ↓ T_M T_A

First we'll clean up our PCR product:

① PB → ↑salt ↓pH
chaotropic

bind

② wash w/
ethanol

(PE)

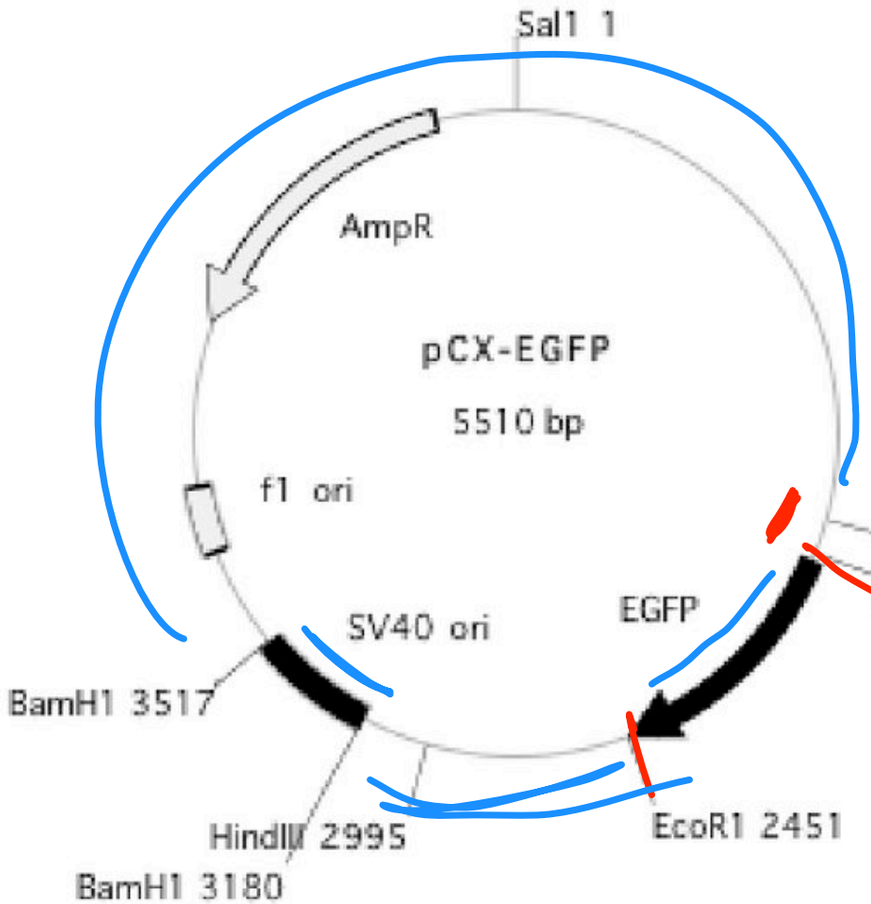
silica membrane

③ Elute
↓salt ~pH
(H_2O) → EB

QIAGEN

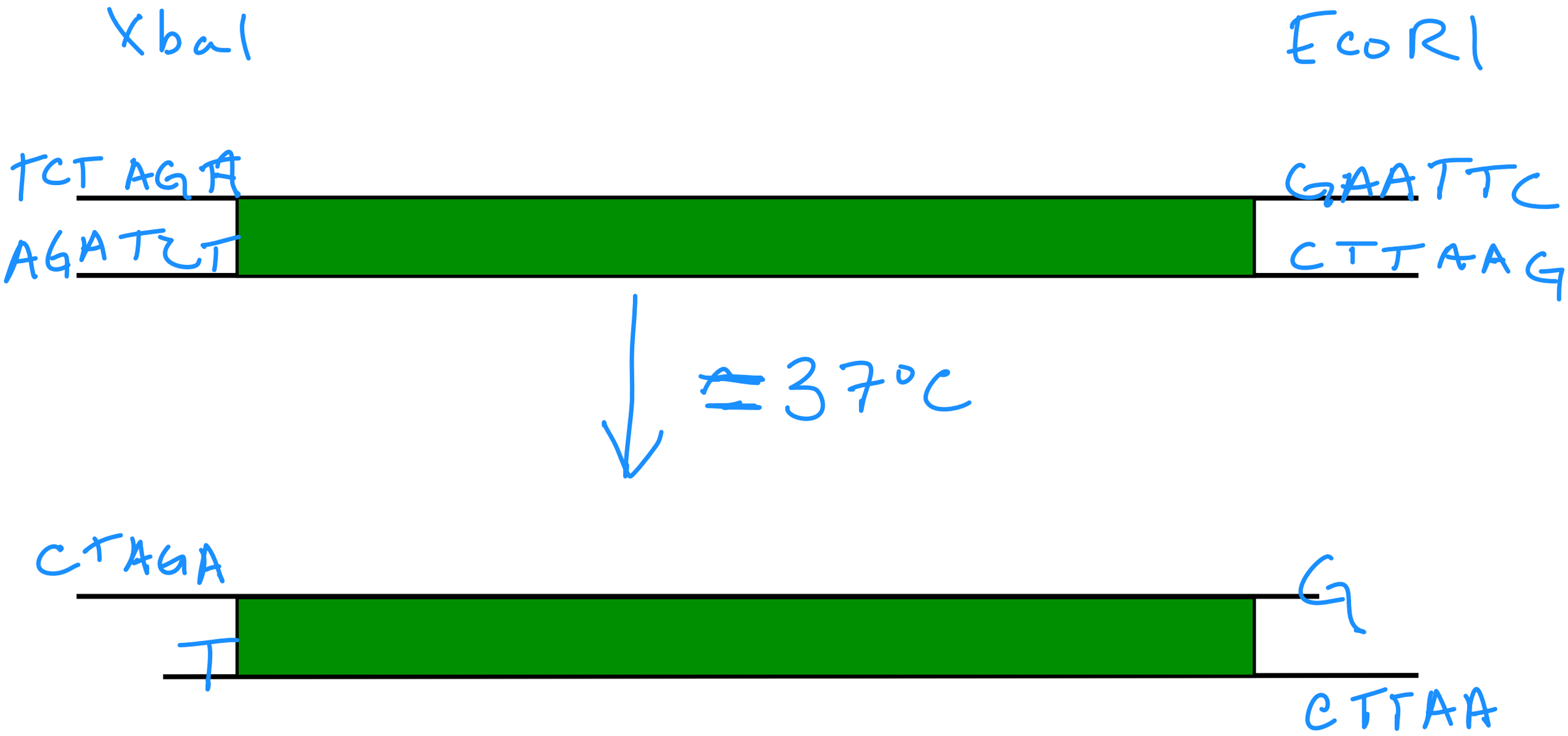
Today we will cut:

Thought experiment:



RE	# of bands	Size
EcoR1	2	~700 ~4800
EcoR1 + XbaI	3	~100 ~700 ~4700
EcoRI + BamHI	4	~700 ~300 ~700 ~3700

Today we will cut:



Total volume of RE digest = 25 µL ☆ Label your tubes ☆

HF

Component	Details	Order
Enzymes	EcoR1 ✓ XbaI ✓	4
DNA	PCR (1) pCX-NNX (3) – single digest – double digest	3
2.5 µL Buffer	CutSmart (10x) ⇒ 1x	2
Water	to 25 µL	1

mix

Rxn temp: 37°C

Total Rxns: 4

Today in the lab:

- PCR purify
- Set-up digests (4)
- ApE tutorial
- WAC visit at 4pm

Next time in the lab:

- Agarose gel visualization
- Purification of DNA
- Longer FNT