$\overline{Av}$ - 9.6

M1D2: Clean and Cut DNA

9/11/14

1. Safety training — Damon
2. Pre-lab discussion
3. Clean-up PCR reaction
4. Digest vector & PCR rxn
5. ApE

* Resume Writing
9/16 7:30pm
56-614
BE specific
* Networking Event
9/18 5:30pm
(likely Koch)

FNW: Did you catch it?

For next week (Due M1D3)

[edit]

1. Sketch the expected product from the PCR you performed today.
   - You may work on paper or electronically. Either way, prepare a schematic rather than detailing each base.
   - Clearly indicate the 5' and 3' end of each DNA strand.
   - Be sure to reflect every new feature that you have introduced (e.g., restriction site) or deleted.
   - What is the expected size of the PCR product?
2. Following the directions in Part 4 of M1D2, prepare a plasmid map in ApE of the clone you are trying to create in lab. Print the graphic map with all singly present restriction sites shown.
   - Hint: You may choose to show fewer restriction sites in your Module 1 summary.
   - Using your map, calculate the fragment sizes expected for each double digest below. **Please show your work.**
     - *EcoRV* and *XbaI*
     - *BamHI* and *XhoI*
     - **Note: You should complete problem #2 after the M1D2 lab.**
3. You will document the construction phase of Module 1 in a **formal methods section**. To help you pace your work, as well as give you feedback early on, you will be required to draft and/or outline parts of this assignment in advance. For next week (due M1D3), you should write an early draft of the Methods: on PCR and DNA digestion. Be sure to read the Materials and Methods section guidelines at this link before you begin; doing so may save you some effort.

20.109 Class Blog

More ▾ Next Blog» hughes.shannon.k@gmail.com New Post Design Sig

BE 20.109 Class Blog

Welcome to the 20.109 Class Blog! Our 20.109 Blog is here for MIT's emerging cadre of biological engineers from Course 20. The blog is for your thoughts and work and discoveries in our lab fundamentals class. By capturing your collective experiences in the subject, we hope to learn even more about the work we do -- what's working well and where we need to get better. Please see the first blog post for some important administrative information.

TUESDAY, AUGUST 26, 2014

Overview of 20.109 Class Blog Assignment

OVERVIEW

Two points of your final grade are determined by your blog posts. Each satisfactory post will be worth 0.5 points. After that, each additional post will count as an extra credit FNW (with a maximum of three). That is, 0.5 points will be added to the numerator but not the denominator of your homework grade. With a typical FNW denominator of 60-65 points, you could increase your FNW score by about a quarter of a letter grade if you do all three extra credit assignments.

If a post seems extremely "phoned in," half credit will be given (0.25 points). An extra credit assignment may be used to replace this score. However, no FNW bonus will be given in addition, and the maximum of three such assignments still holds.

DUE DATES AND TOPICS

There are *no specified due dates* for these reflective blog posts. However, there is a date stamp on each one, and you must complete **one per module and a summary post at the end of class**. The end of a module is the day after you turn in your final report. You will receive feedback on your blog posts from your instructors (and anyone

CONTRIBUTORS

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BLOG ARCHIVE

- ▼ 2014 (2)
  - ▼ August (2)
    - Overview of 20.109 Class Blog Assignment

Module 1 major assessments:

① Methods Section *5% total grade

M1D1 - M1D5 (excluding tissue culture)

*construction of plasmid system

② Data Summary & Abstract *15% of your total grade

- figures & captions
- summary (Intro/discussion)
- abstract

*** M1D3 → WRAP Lecture ***

Methods Section Tips

- Divide into sub-sections! Titles that are descriptive.
  - Put in a logical order -- list primer THEN list PCR reagents, etc

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

- Methods are clear and concise explanations

  The methods section is not a benchtop protocol * complete sentences

  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?

forward primer (5' ...... 3')

sequence FP + RP

(2 pM)

Name pCX-EGFP

A No template control was included

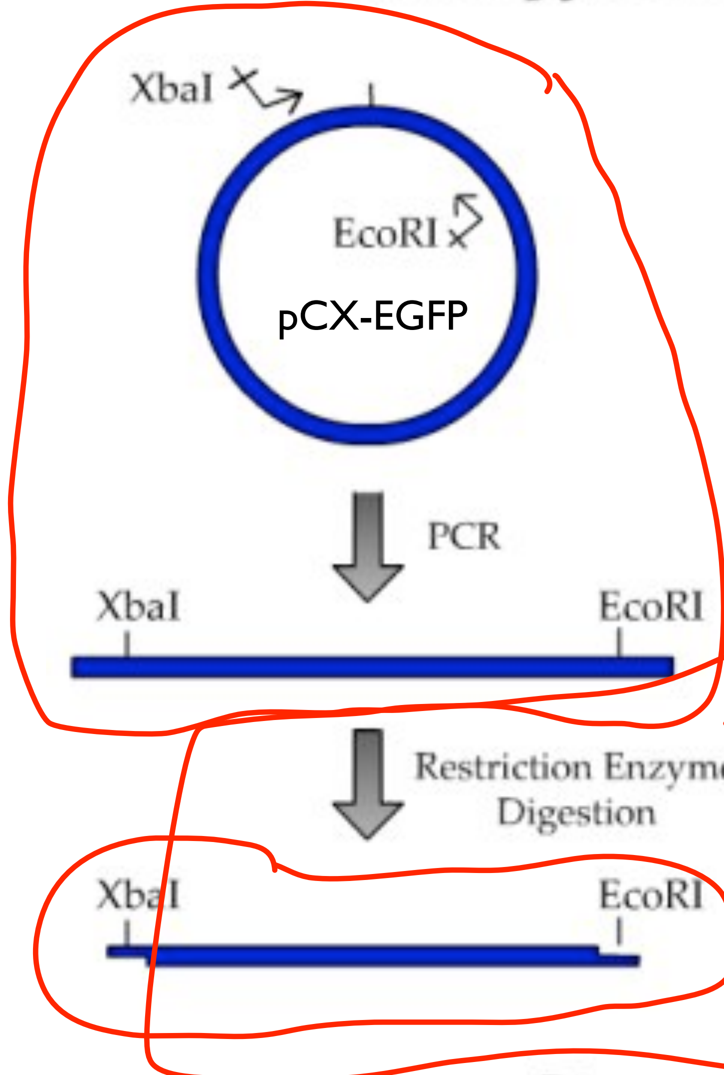
... 1X MasterMix (5Prime Inc, Whereversville, USA)

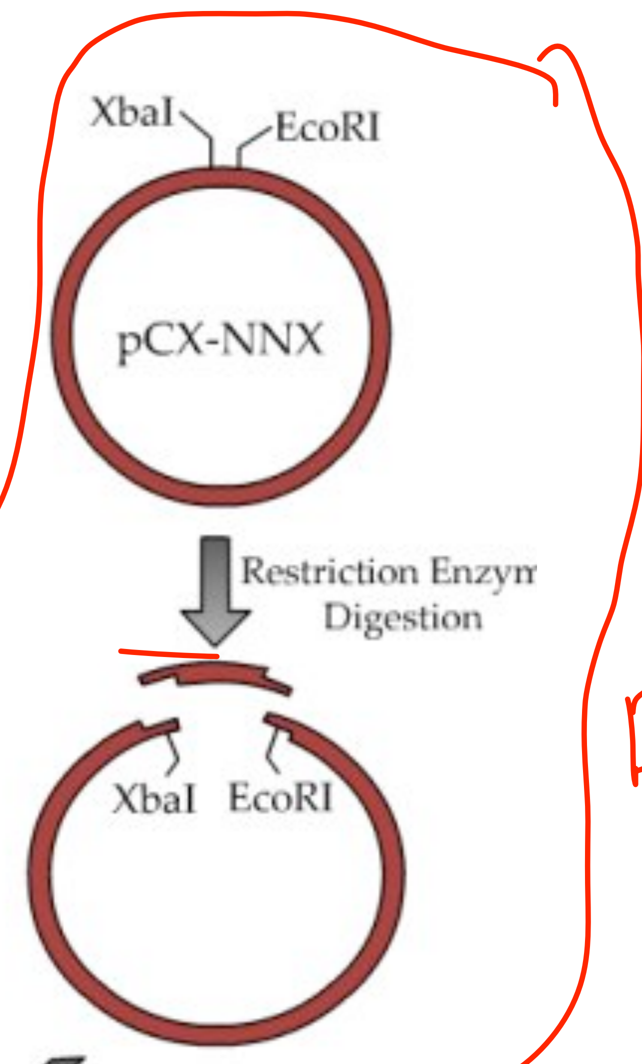
Thanks to Agi for this slide!

Step 1: Build the system!

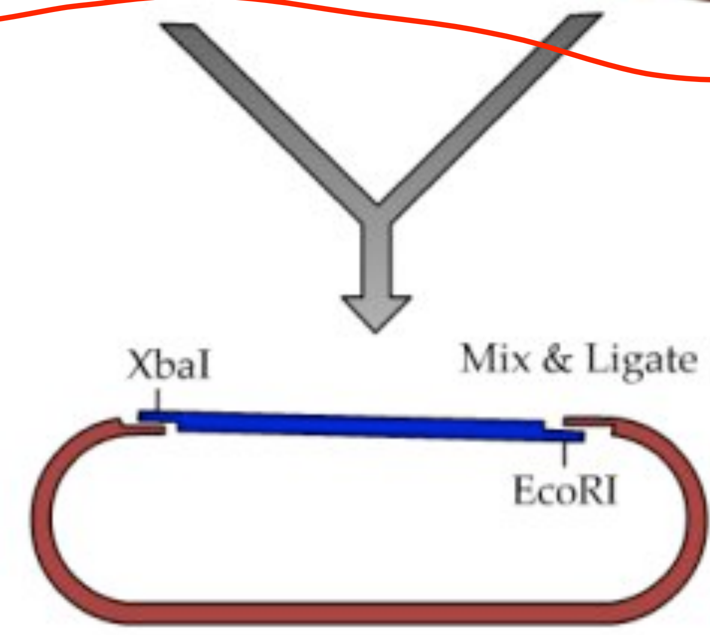
Roadmap for Plasmid Construction

Day 1





Day 2



Last time:

5' 3'

XbaI BamHI EcoRV EcoRI

BamHI + EcoRV = confirm product

XbaI & EcoRI = facilitating sub-cloning

pCX-EGFP

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

$T_m \sim 69$

G/C ~ 68%

DMSO → unfold DNA

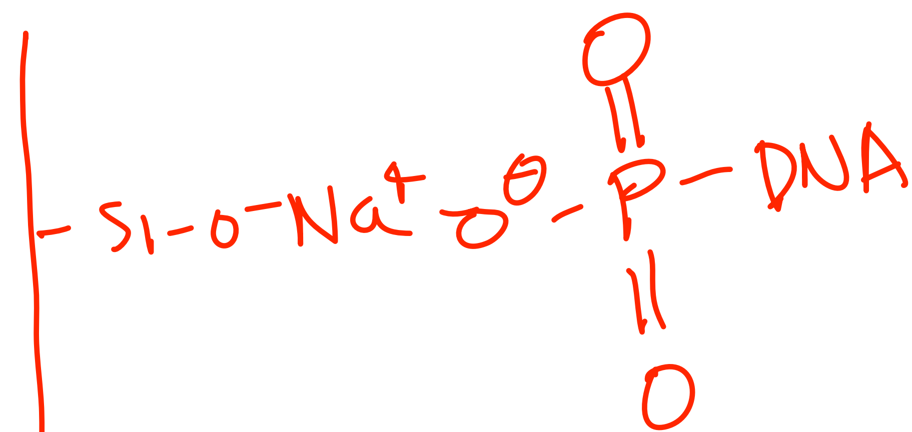
glycerol - NMP

$\downarrow T_m$

First we'll clean up our PCR product:

① Buffer PB } chaotropic salt disrupts H_2O shell
guanidine-HCl } ≠ H-bonds

② ↑salt ↓pH



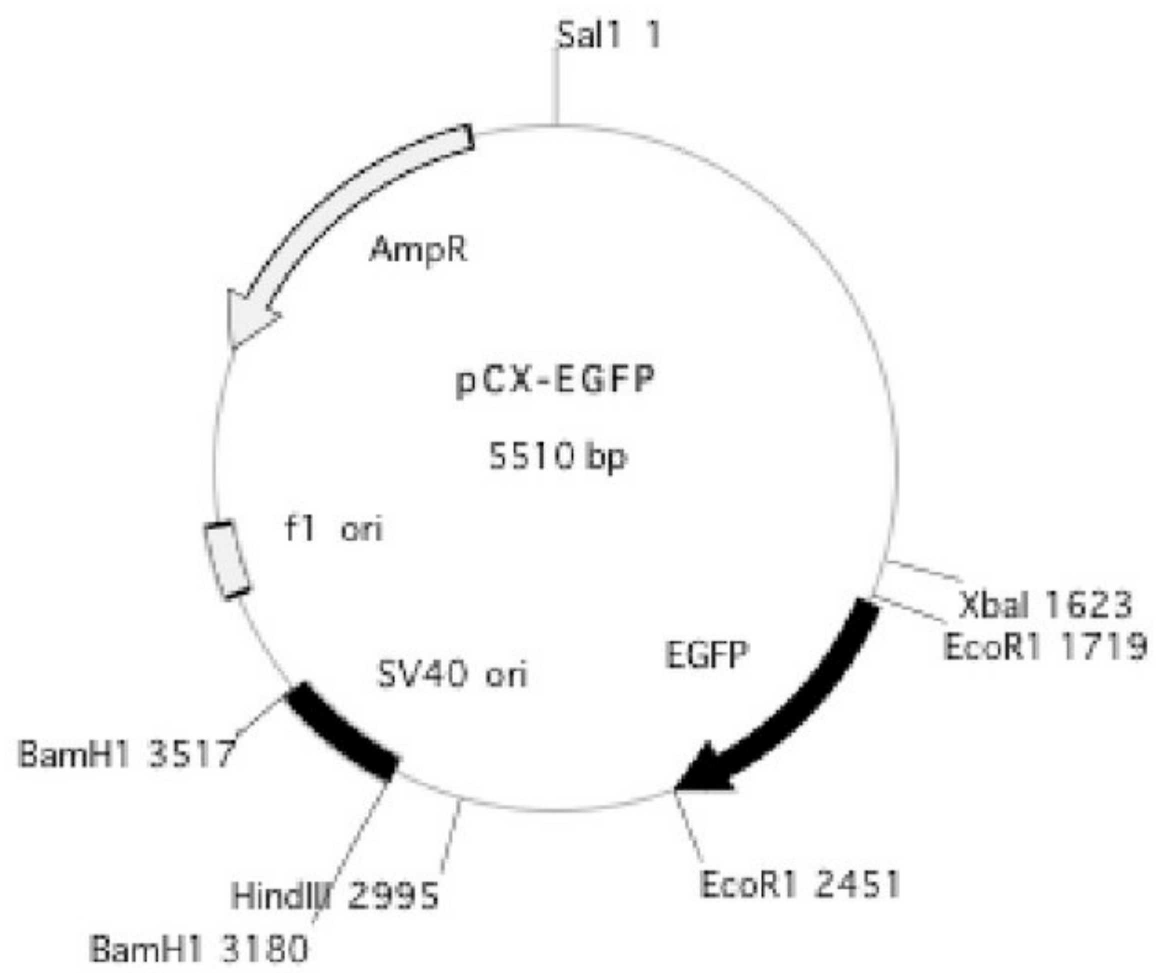
pH < 7.5 ~95%

③ DNA > 40bp bound to column
- wash w/ ethanol

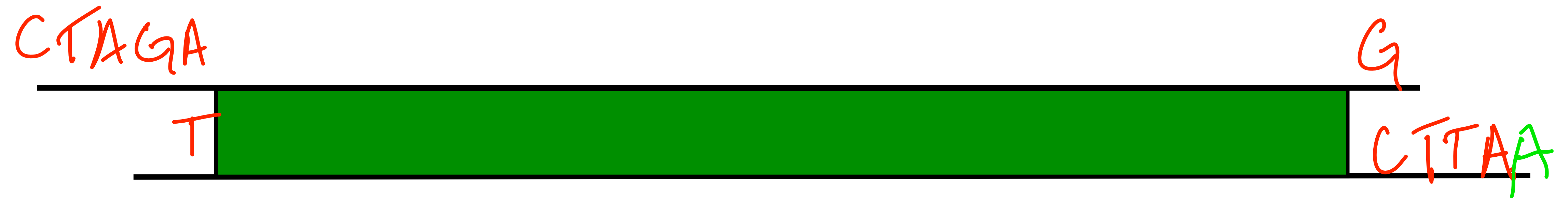
④ Elute → H_2O
→ EB → Tris-HCl pH 8.5

Today we will cut:

Thought experiment:



Today we will cut:



Total volume of RE digest = 25 µL

Component	Details	Order
Enzymes	1) Single → Xba → EcoRI 2) Double Xba + EcoRI	4
DNA	(1) PCR (3) pCX-NNX	3
Buffer	10x → 2.5µL	2
Water	to 25µL	1

Rxn temp: 37°C

Total Rxns: 4

Today in the lab:

- PCR purify
- Set-up digests (4)
- ApE tutorial

Next time in the lab:

- FNW Due
- Agarose gel visualization
- Purification of DNA