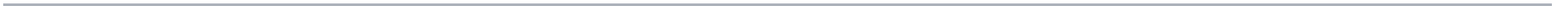


DNA Engineering: M1D2 Lab Talk

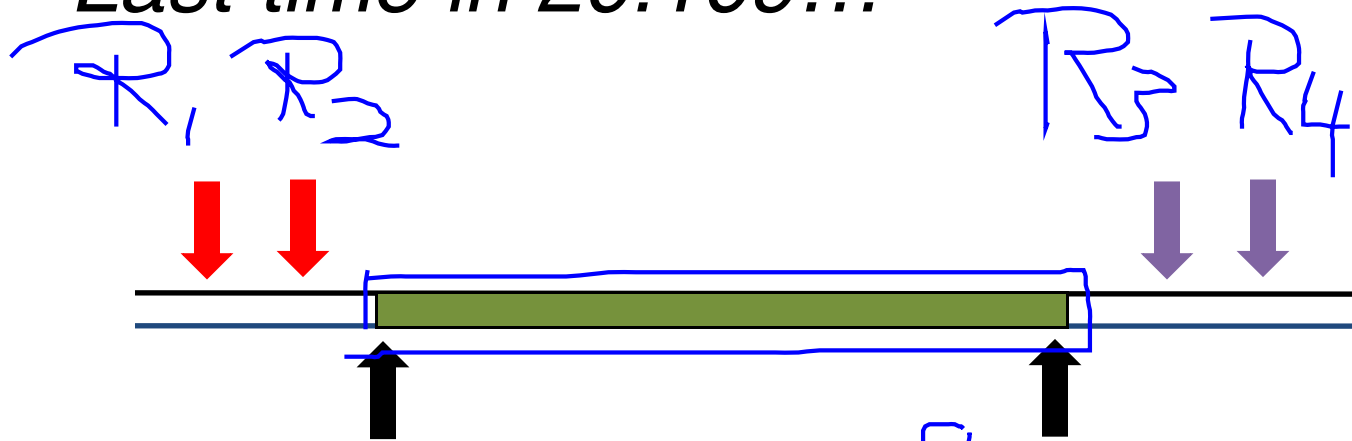
20.109 (F11)

09.15.11

Quick review of lab practical glitches...



“Last time in 20.109...”



33aa
CODON

Stop

R₁ = Xba I

R₂ = Bam HI

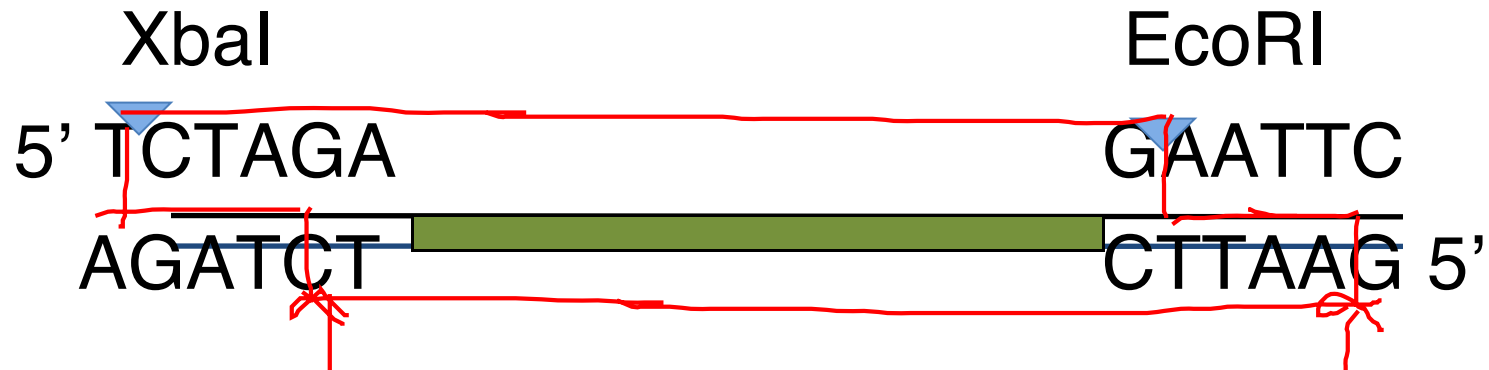
R₃ = Eco RV

R₄ = Eco RI

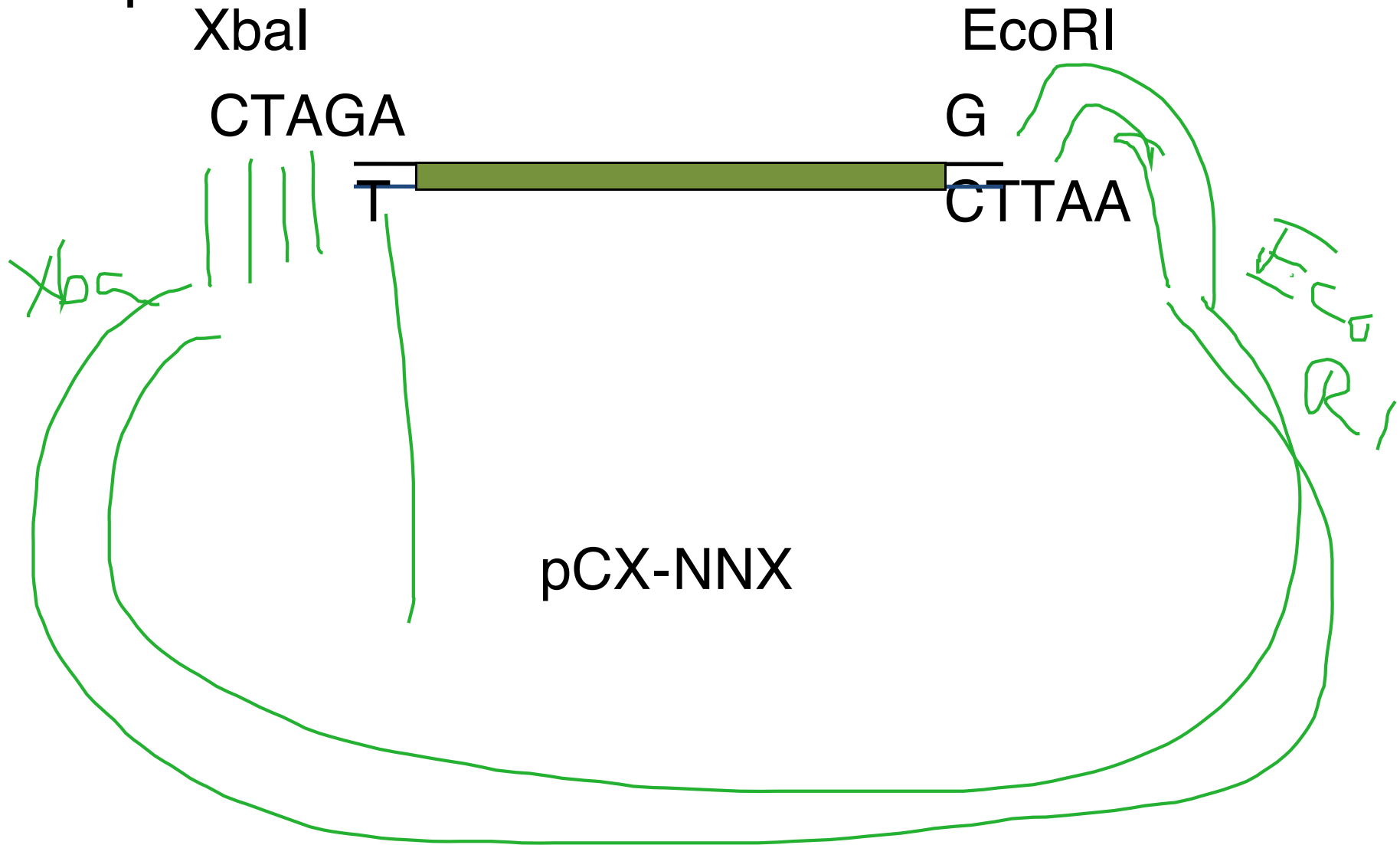
pCX-EGFP

ori/selectable
marker
EGFP/MCS

Digesting your PCR product



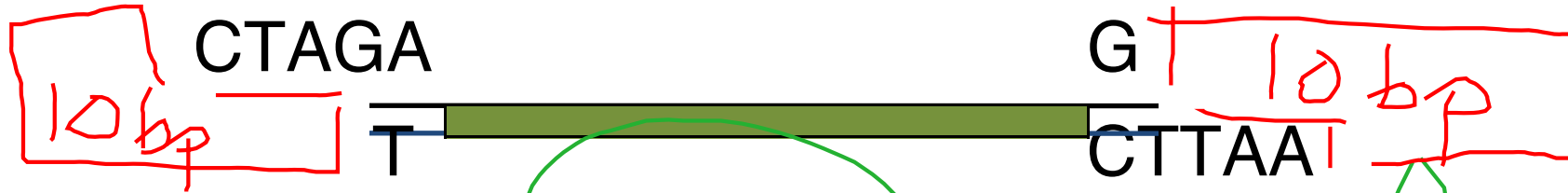
(Sub)Cloning PCR product into new plasmid



(Sub)Cloning PCR product into new plasmid

XbaI

EcoRI



Xba

RI

- Uncut

+

- Xba only

- RI only

- both

“backbone”

1

But first, clean up your PCR product

Why?

1. Dntps still there so need to remove or they fill in the end
2. using taq

PCR product

Change buffer

What about other things
in rxn

↓

But first, clean up your PCR product

How?



Resin

1. Bind DNA to resin on column (= silica)

High salt / low pH

2. Wash in presence of EtOH

DNA stays Bound

3. Elute DNA in small volume

Low salt / pH ↗

Your reactions

Component	Details
DNA (3)	Pcr bkb
Enzyme (4)	Xba R1
10XNEB (2)	# 3
H ₂ O (1)	(10X buffer to 1X)
Temperature	37°

Be careful with stock solutions and order of addition!!

In lab today and next week

R Clean DNA

Digest GO!

I Lecture No lab ☹️

R Run gel, isolate DNA

Writing goals

1. Chatted about difference between writing for a HASS class which seems more visceral and opinion-driven vs writing for a science class, where you are beholden to the data.

Conversation led to the realization that some aspects of scientific writing not interpretive (eg frag that is 700 bp can't be called 700 if it looks like 500 on a gel) but other parts are intentionally persuasive, eg discussion section makes reader believe your analysis.

2. Language of scientific writing has particularities, like pasive or active voice or like verb tenses. Writing science is a lot like learning a new language and so vocabulary needs to be practiced, so expect some mis-steps along the way

3. Writing clearly takes practice too. See "writing readable prose" that is linked to wiki.

4. Knowing what level of detail to include is a challenge, especially in the abstract. How is it possible to make this section informative to the savvy but also interesting to newcomers.

5. Want to learn more about each section of research article (will have linda coming in to do just this during module2. The emphasis in the first module is on data presentation but these skills will carrv over to article in module 2.
