M1D2:
Clean and cut DNA

9/17/15

Lab business

1. Visit from BE Communications Instructors
2. Homework due M1D3
3. Quiz on M1D4
   - Material from lectures (M1D1-M1D3): ~60%
   - Material from prelab/wiki (M1D1-M1D3): ~40%
4. Please share laboratory notebooks with Andee (andreakw@mit.edu) and Maxine (jonas_m@mit.edu)

Homework due M1D3

Due M1D3 [edit]

1. Sketch the expected product from the PCR you performed on M1D1.
   - 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*
   - Use the skills you learned from the BE Communications instructors to write a title and caption for your plasmid map figure.
3. In Module 2, you will document your experiments in a written methods section that will be part of a larger report. To help you prepare, as well as give you feedback early on, you will draft portions of the Module 1 methods. For this assignment, write a draft of the Methods concerning 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.

Methods section tips

- Use subsections with descriptive titles
  - Put in logical order
  - Begin with topic sentence
- Use clear and concise full sentences
  - Avoid tables and lists
- Use the most flexible units
  - Write concentration rather than volume

primer name (5' GTAG 3') [ ]

Some methods practice…

EGFP amplified from pCX-EGFP

"A plasmid with template DNA (5ng) 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 prepare and labeled control."

Master Mix (5' prime, Somewhere, USA)

Homework and major assignments

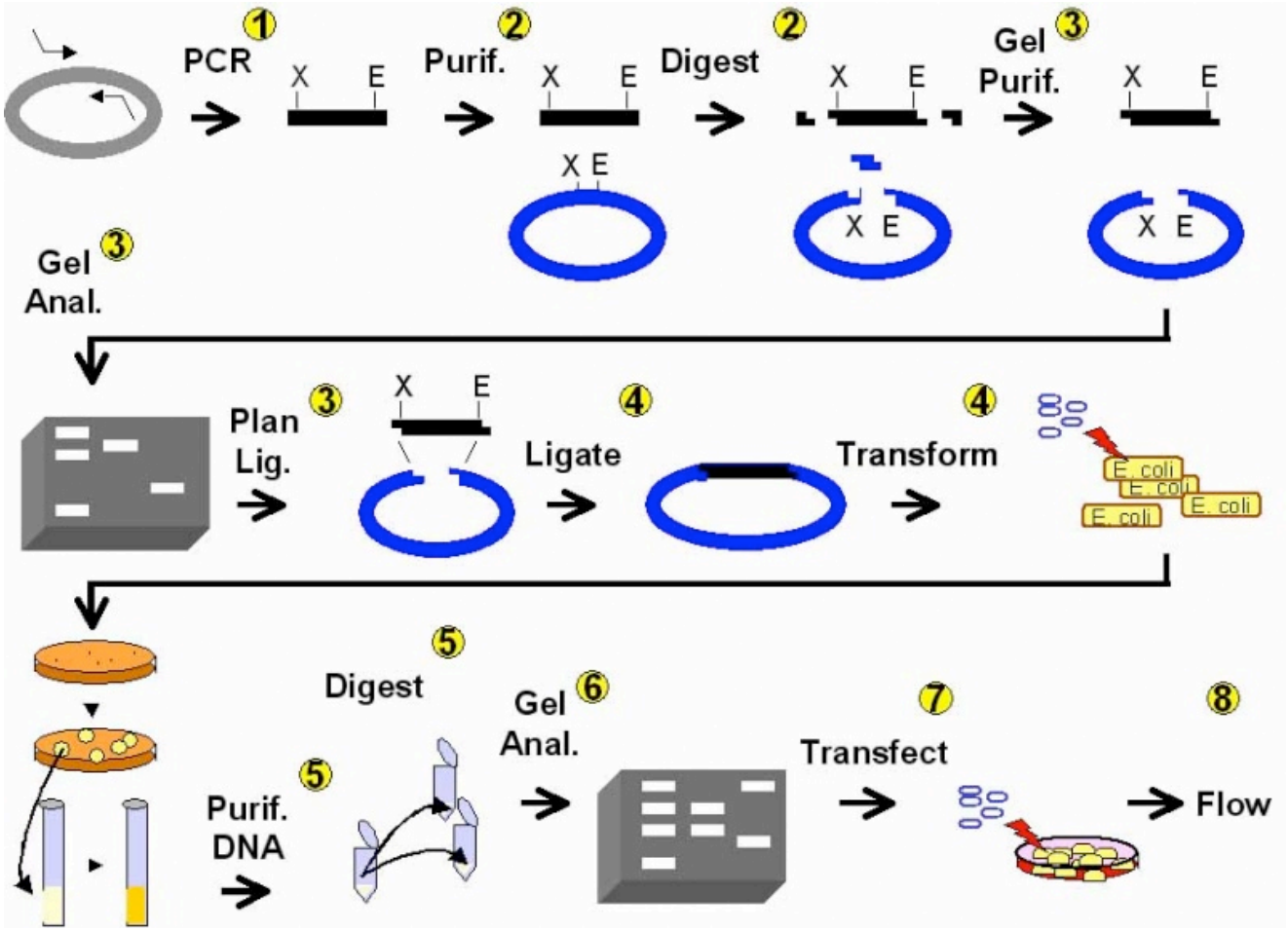
- DNA engineering summary (15%)
  - Title and abstract
  - Background/motivation
  - Results and interpretations (figures with captions)
  - Implications/future work
- DNA engineering mini-presentation (5%)
  - Abbreviated oral overview of summary
  - ‘elevator pitch’ or ‘flask talk’

Why do we give quizzes?

1. To refresh your memory about the long-term experiment you are performing
2. To provide you with an opportunity to show your technical knowledge decoupled from your communication skills

Attending lecture is

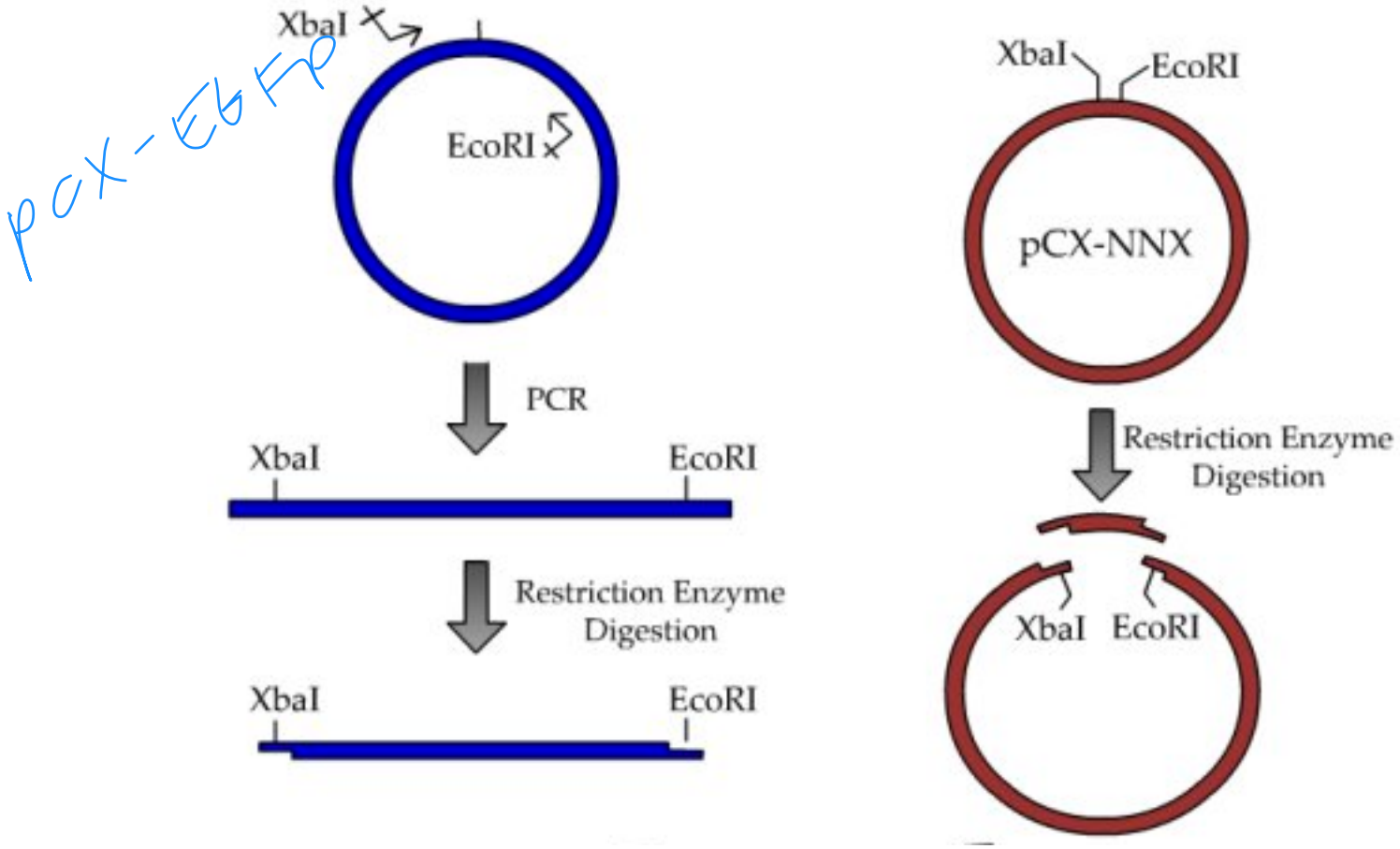
Mod 1 overview



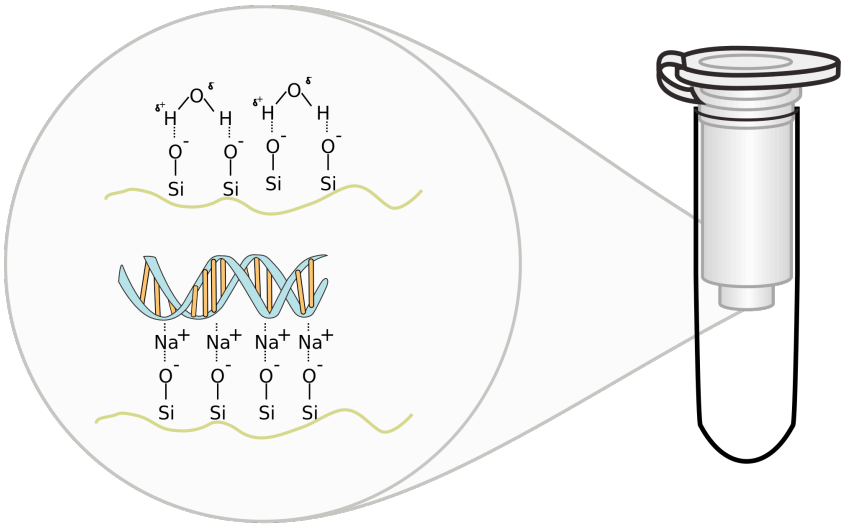
From last time…

Did your primer design meet all of the guidelines?

Using REs to build Δ5 EGFP construct



Prep for RE digest



Qiagen kit

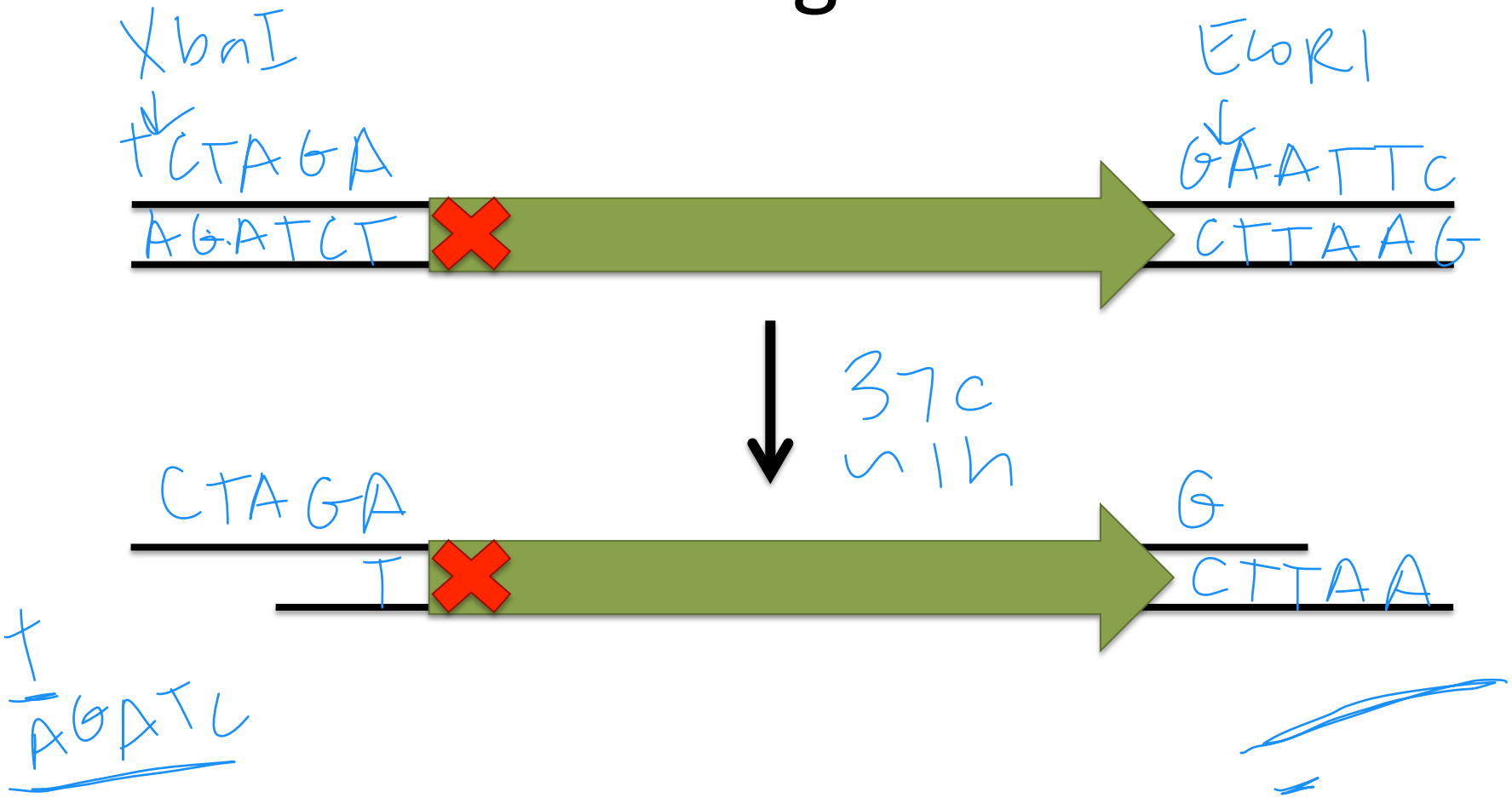
1. butter PB
guanidine HCl
↓pH

2. wash
EtoH

3. elution
TE
pH8
↓salt

Keep track of your DNA!!

RE digests



What about pCX-NNX digest product?

Today

- Clean and digest your PCR product
- Digest pCX-NNX vector
- APE practice

- Networking event
  - Thursday, September 24 at 5:30-7p
  - First floor lobby of the Koch