- Announcements
- Pre-lab Lecture
  - Plasmid Overview
  - Restriction Enzymes Intro
  - PCR Recap
  - Safety + Technical Tips
- Lab Practical (~40 min)

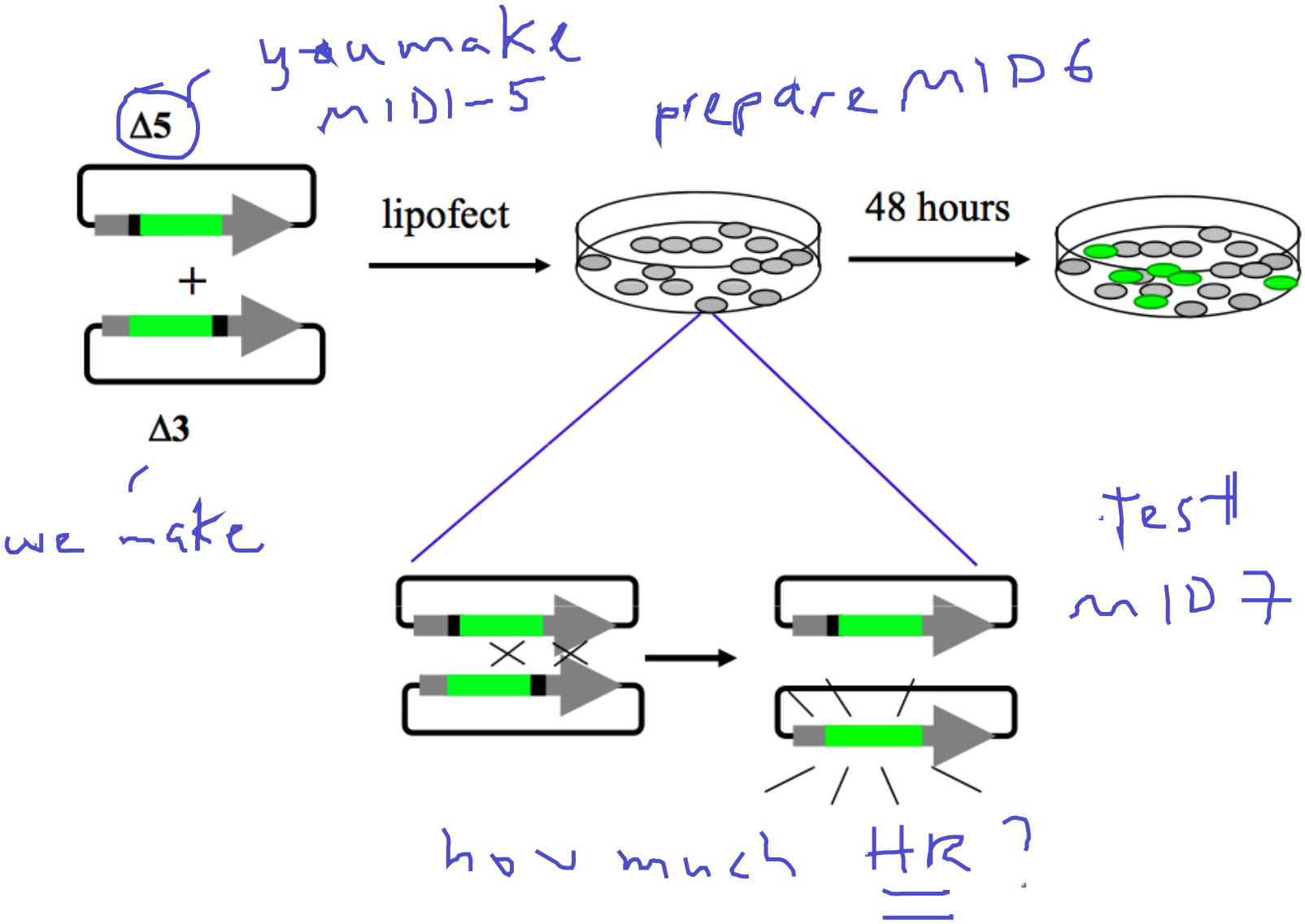
Announcements

- BE seminar series:
  - Thursdays at 4:05 pm in 32-141
  - First seminar is Sept. 15th
  - Full schedule linked from BE website

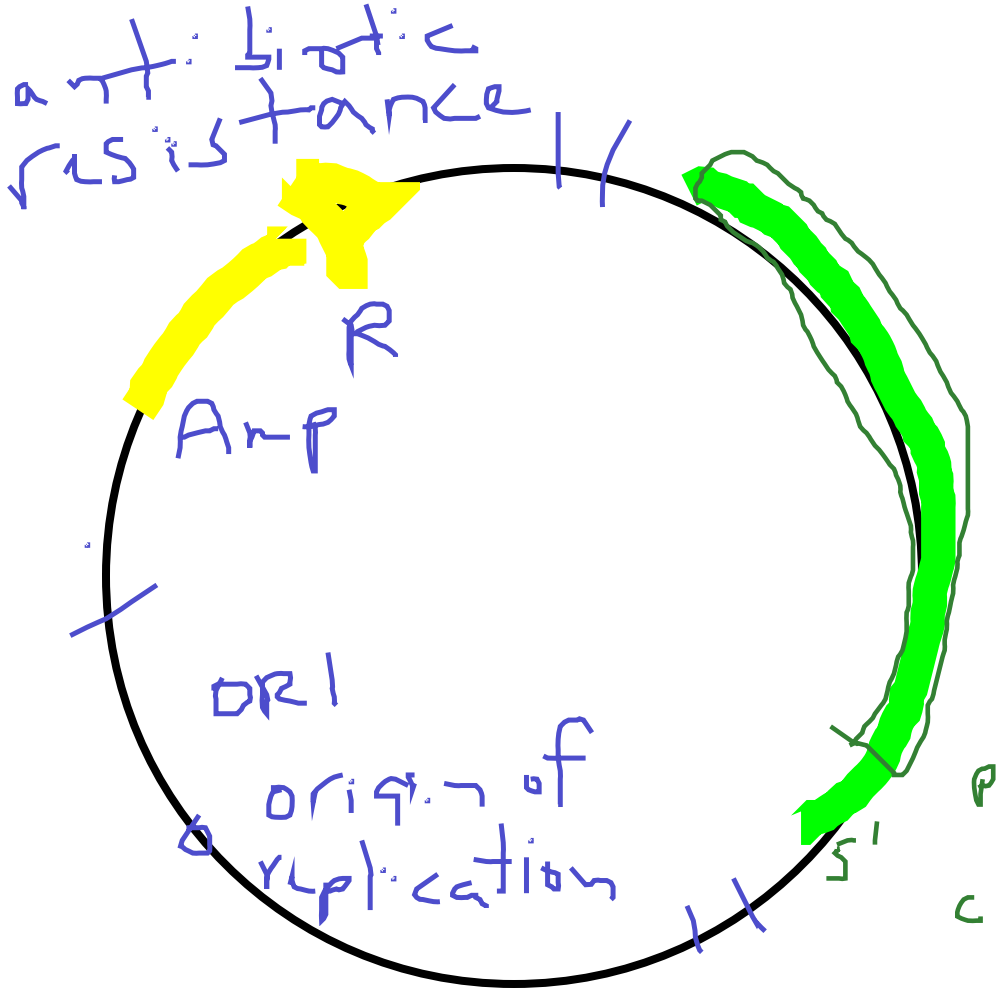
- Introducing… Marcus, your TA for Module 1

blog vs wiki

Module 1 research goal



Plasmid overview: pCX-EGFP



Extrachromodomal, DS, circular DNA. Why?

vector to introduce foreign gene (in cell)

EGFP: CDS/ORF

create ΔEGFP

plus LOTS of restriction sites

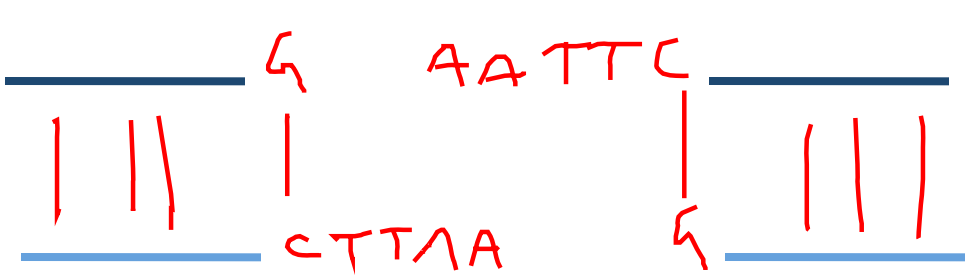
Intro to restriction enzymes

endonuclease

cut DNA

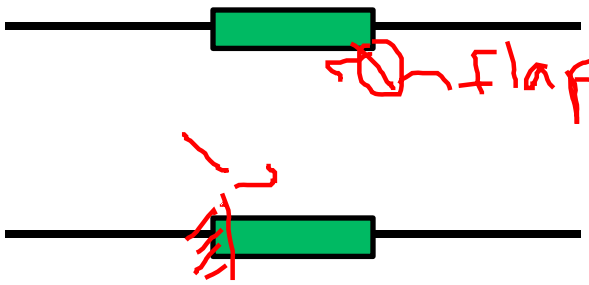
palindromic

cut w/ EcoRI



"sticky" ends

Designing PCR primers

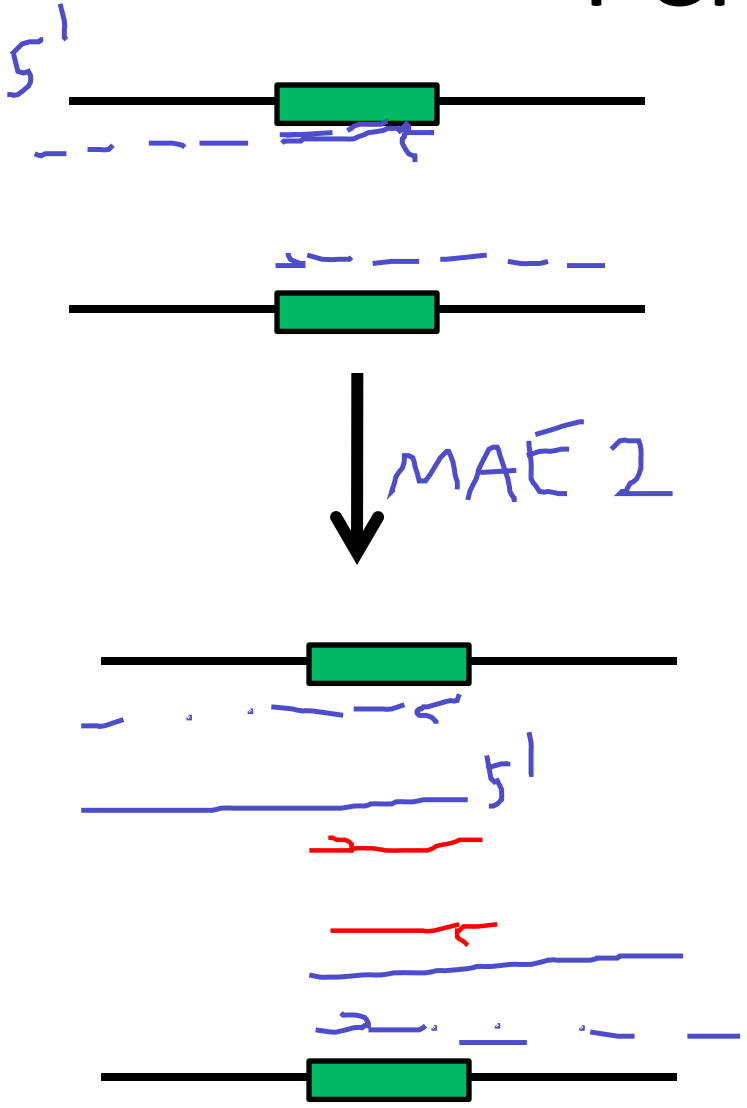


primers 5' → F R ← 5'

flap: add functional sequence

- linker
- rest. site

PCR process



Melt ~95°C

Anneal ~50'). 60's

Extend 72°C

— too long

— template for desired product

PCR eaction

Component	Function
dNTPs	material to extend w/
polymerase	catalyzes extension
template	provides sequence to copy
primers	initiate and select/ Specify DNA
Mg^{++}; buffer	co-factor; right chem. environment

Mod 1 major assessments

- Lab practical (10%)
  - Physical and on-paper components
  - Demonstrate understanding of module's first part: plasmid construction
- Slide summary (10%)
  - Figures depicting data
  - Brief written summary: analysis and context
  - Demonstrate understanding of module's first and second parts: recombination experiment analysis

Today in Lab: M1D1

- Goal: make ΔS GFP insert + also run control
- Keep PCR tubes cold!
- Write small *directly* on the PCR tubes – do not put sticky labels in the PCR machine.
- Safety and disposal for today's experiment