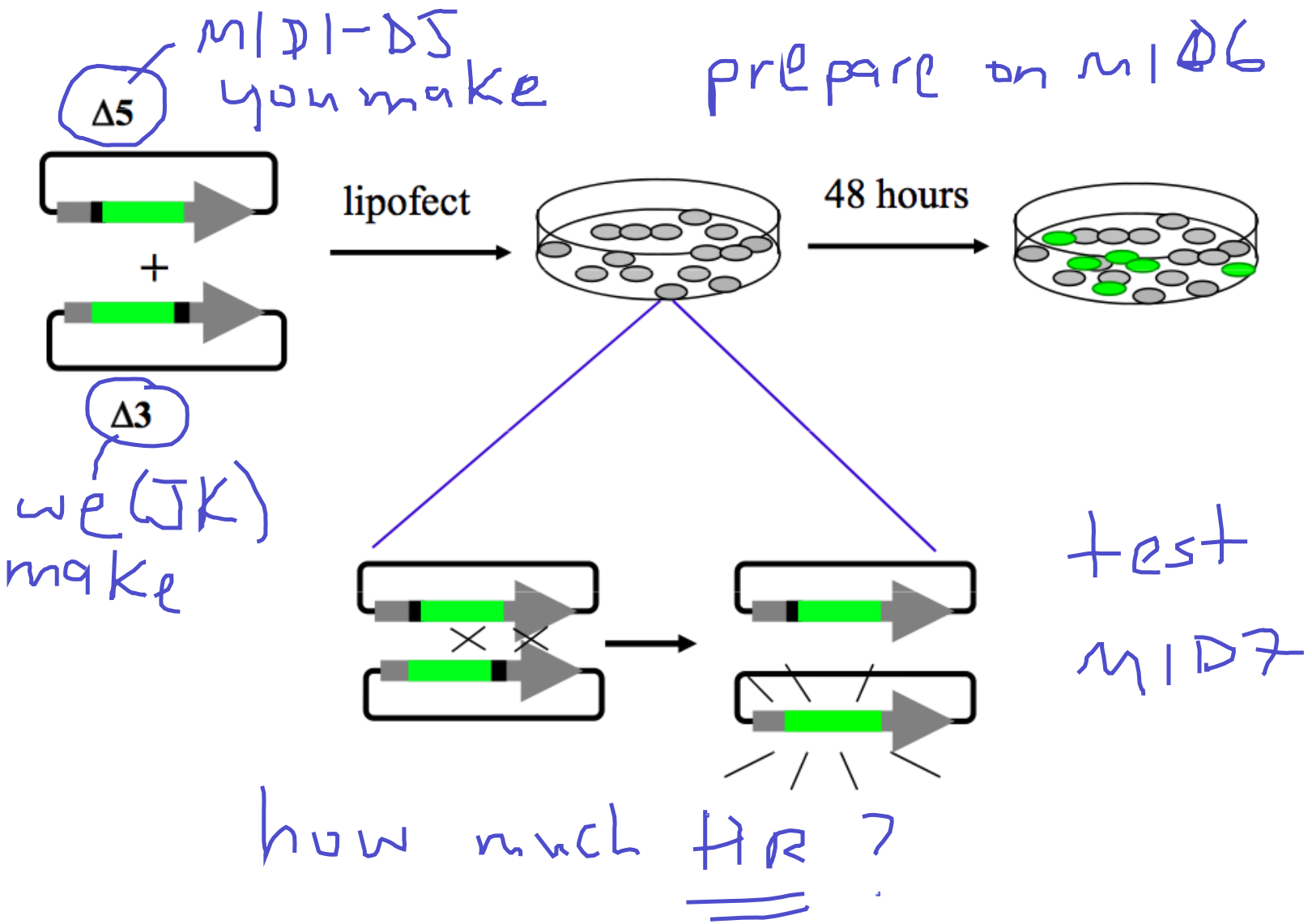
- Announcements
- Pre-lab Lecture
  - Plasmid review
  - Restriction enzymes intro
  - PCR recap
  - Safety + technical tips
- Lab Practical (~40 min)

Announcements

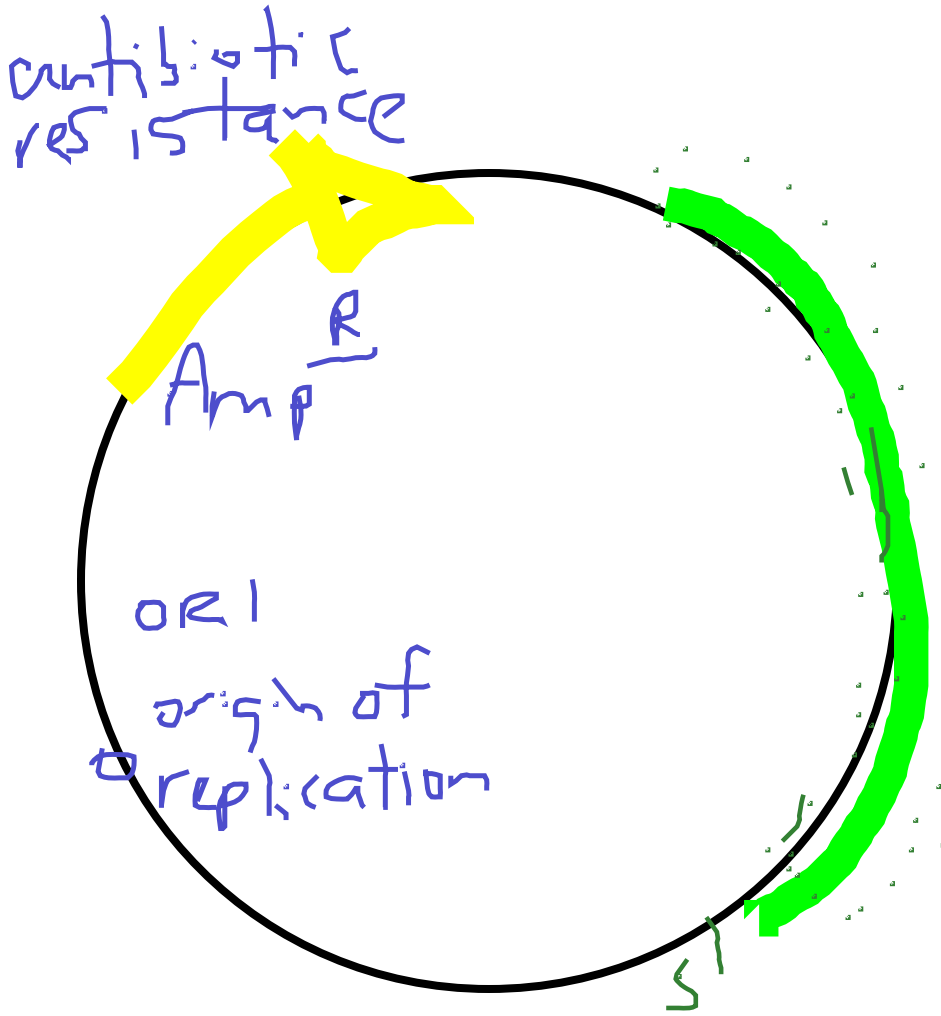
- BE seminar series:
  - Thursdays at 4:05 pm in 32-141
  - First seminar is Sept. 13th
  - Full schedule linked from BE website

- Introducing… Jenny, your TA for Module 1

Module 1 research goal



Plasmid overview: pCX-EGFP



ds circular DNA
extra chromosomal
why? vector to introduce foreign gene (in cell)

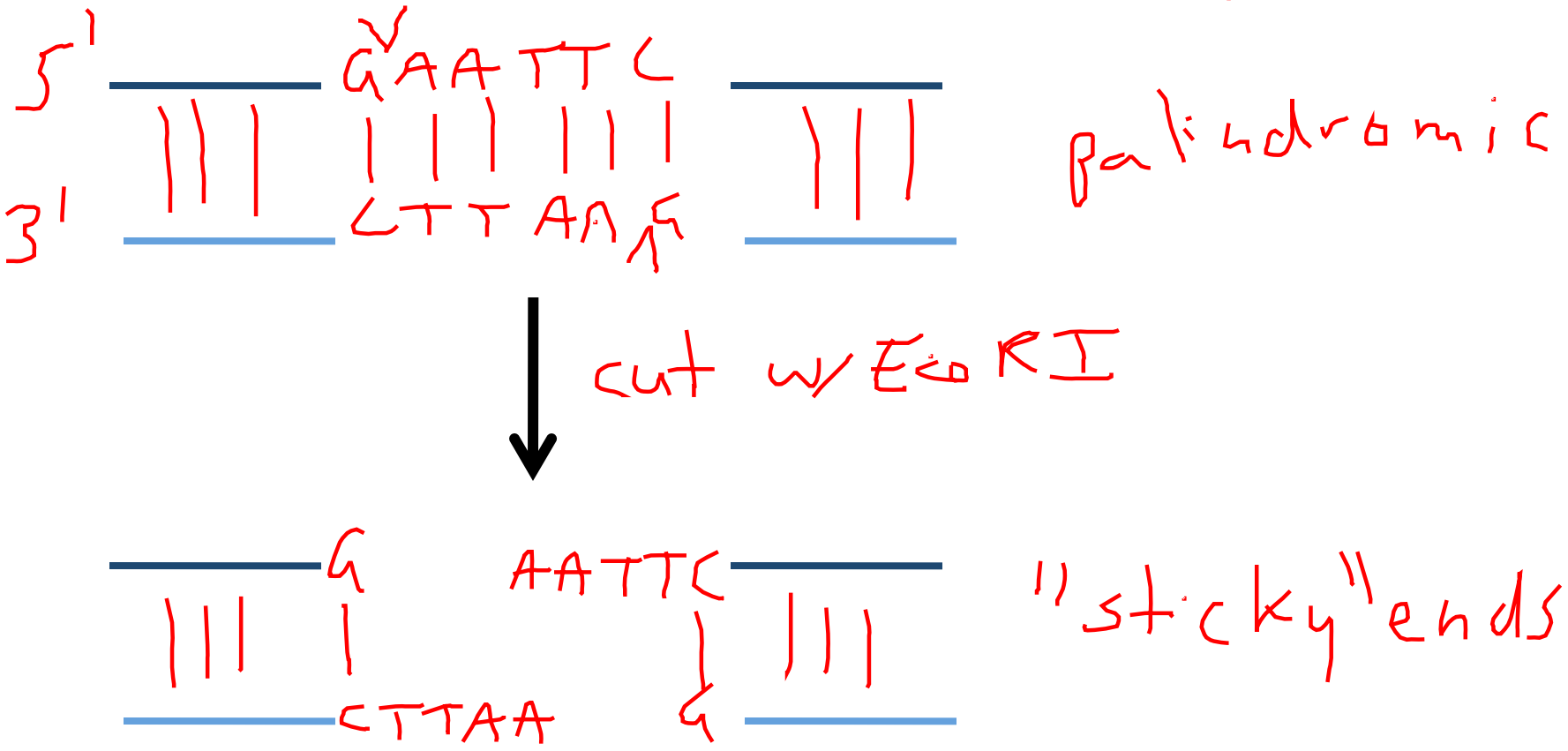
EGFP CDS/ORF
↳ pA

—PCR out ΔS EGFP

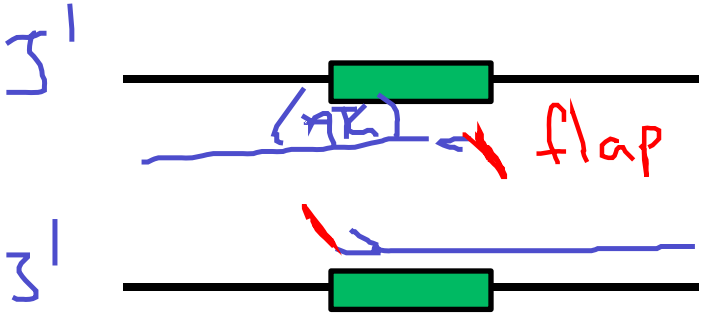
plus LOTS of restriction sites

Intro to restriction enzymes

endonuclease
→ cut DNA



Designing PCR primers



primers

5' → F

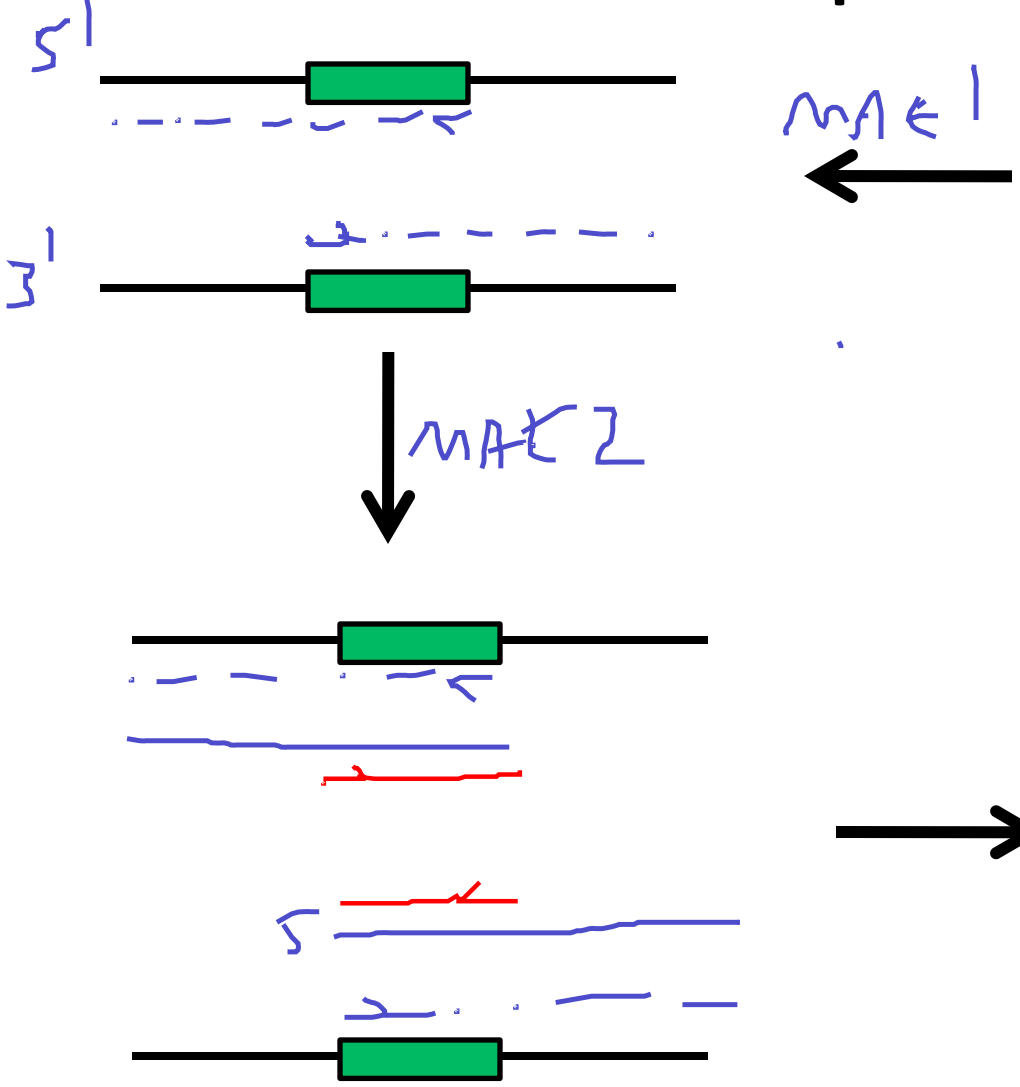
← 5' R

flap: add new function

— ☹ rest. site

— linker

PCR process



Melt 94-95°C

Anneal 50's-60's
↳ more next time!

Extend 72 (Taq)

—— too long

—— template for desired product

PCR eaction

Component	Function
primers	selects + initiates DNA
polymerase	catalyzes extension
template	provides sequence to copy
dNTPs	material for building
buffer; Mg^{++}	pH/salt environment; co-factor

Mod 1 major assessments

- Lab certification (10%)
  - Physical and on-paper components
  - Demonstrate understanding of module's first part: plasmid construction
- Slide summary *or* oral defenses (10%)
  - Long or short slide deck with figures depicting data and written summary of analysis/context
  - Demonstrate understanding of module's first and second parts: recombination experiment analysis

Today in Lab: M1D1

- Goal: design/make Δ5 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

★ notebooks ★