- Announcements
- Lab Practical (~45 min)
- Pre-lab Lecture
  - Design in 20.109
  - Plasmid Overview
  - Restriction Enzymes Intro
  - PCR recap
  - Safety + Technical Tips

Announcements

- BE seminar series:
  - Thursdays at 4:05 pm in 32-141
  - First seminar is Sept. 18th
  - Full schedule linked from BE website

- Introducing… Michelle, your TA for Module 1

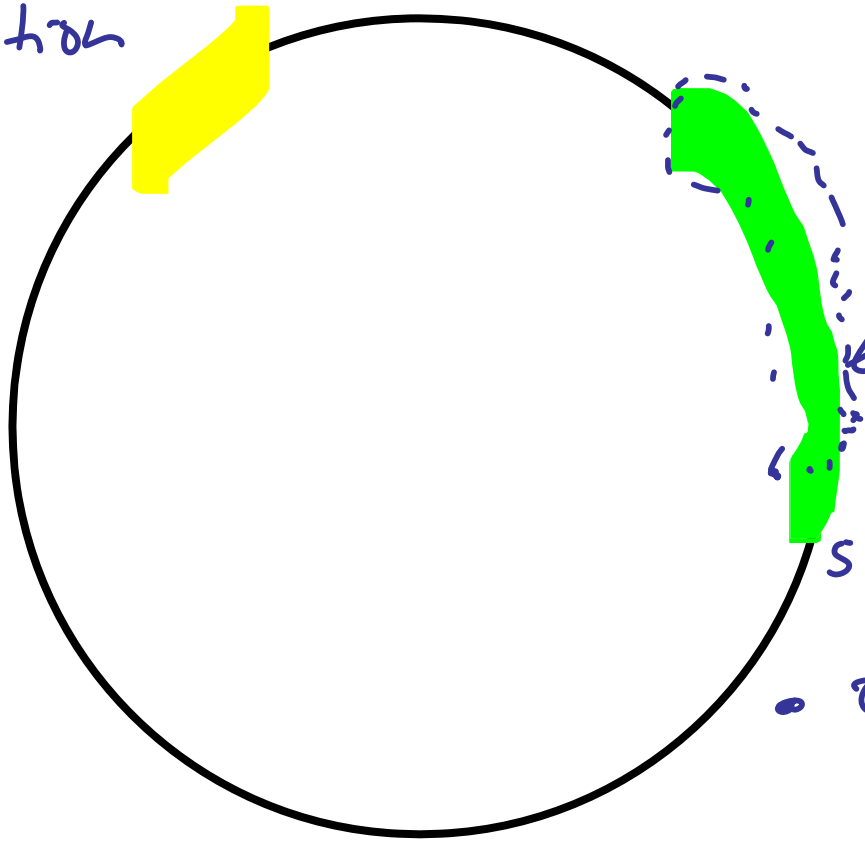
Design in 20.109

Mod 1: primer design (* exp. variation) ⋮ practice

Mod 2: advanced primer desig

od 3: exp. variation

Plasmid Overview: pCX-EGFP



ds, circular DNA
extrachromosomal

why? gene expression in cell

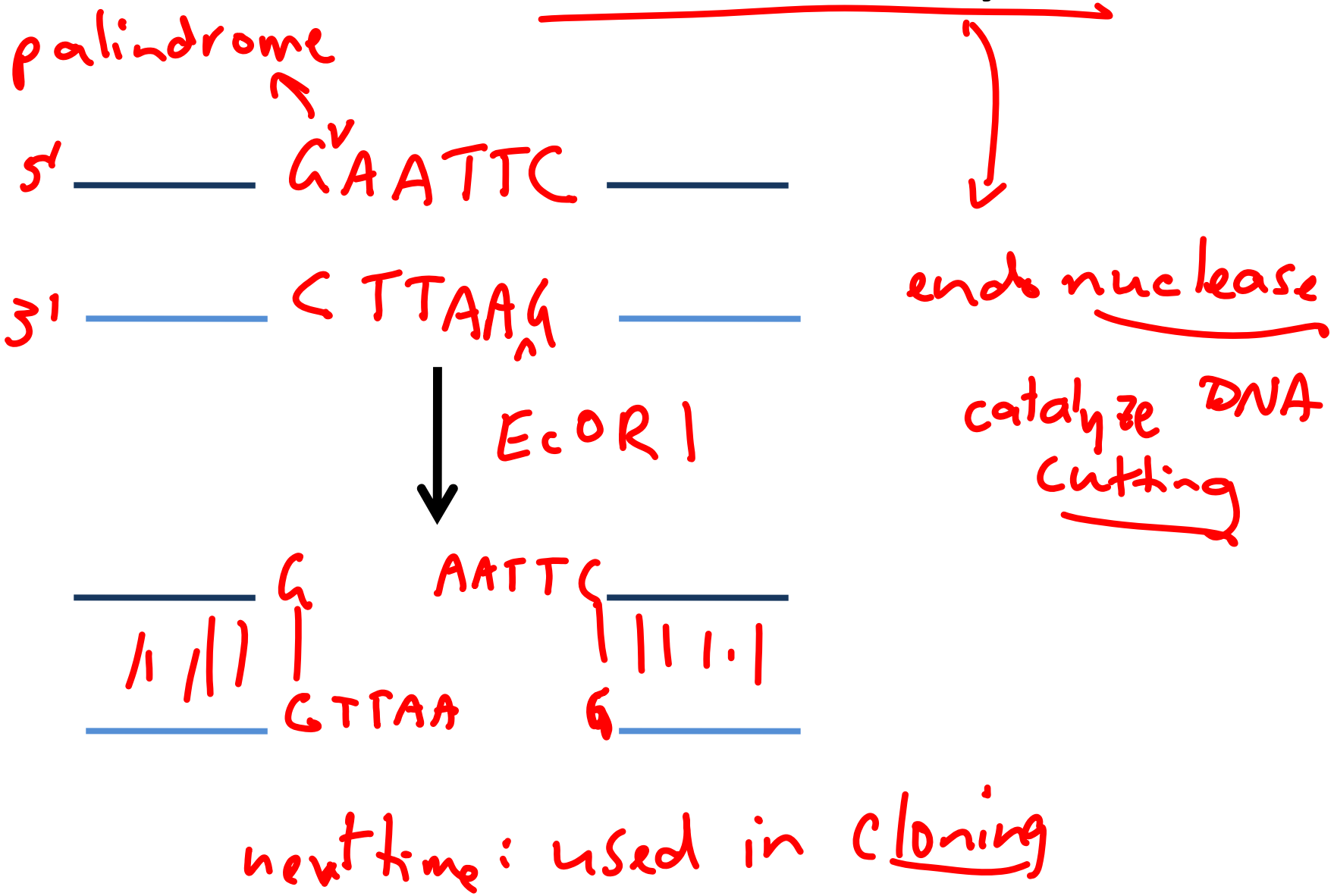
EGFP CDS *
ORF

Δ5 EGFP - today
(+ Δ3 EGFP, recombine)

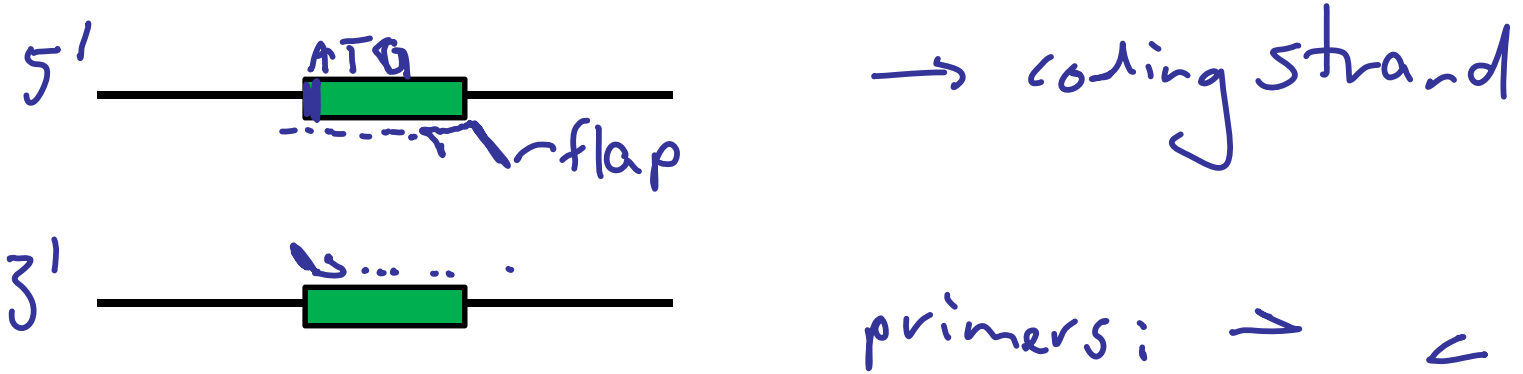
- ORI = replication origin
two - bacterial, mammalian
- pA / promoter / enhancers

* lots of restriction sites!

Intro to Restriction Enzymes

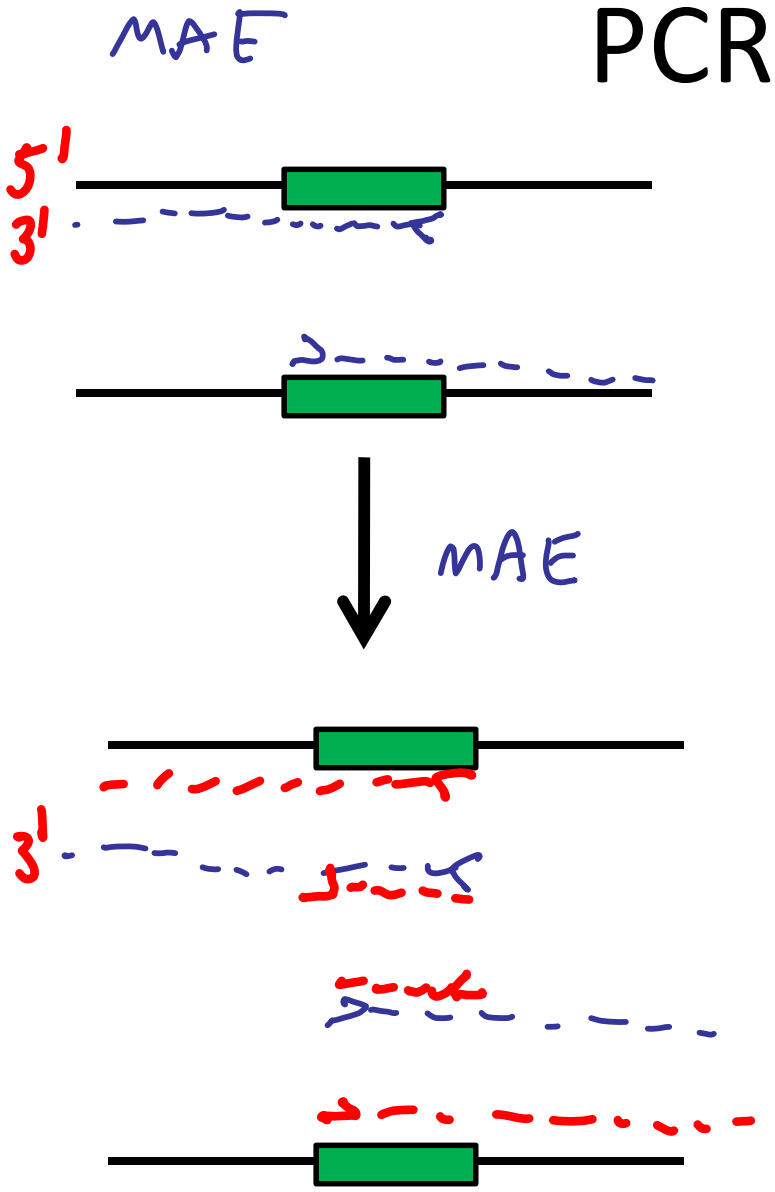


Designing PCR

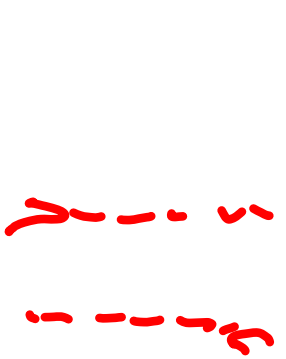


flap: useful for introducing new DNA (e.g., Rest. sites)

PCR Process



process: Melt
Anneal
Extend
ignoring flap truncation



template for desired product

PCR eaction

Component	Function
template	provide desired sequence
dNTPs	material to make DNA copies
DNA polymerase (Taq)	catalyzes extension of DNA
primers	selecting, initiating new DNA
Mg^{++} buffer (co-factor)	provide right chemical environment

Today in Lab

- 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