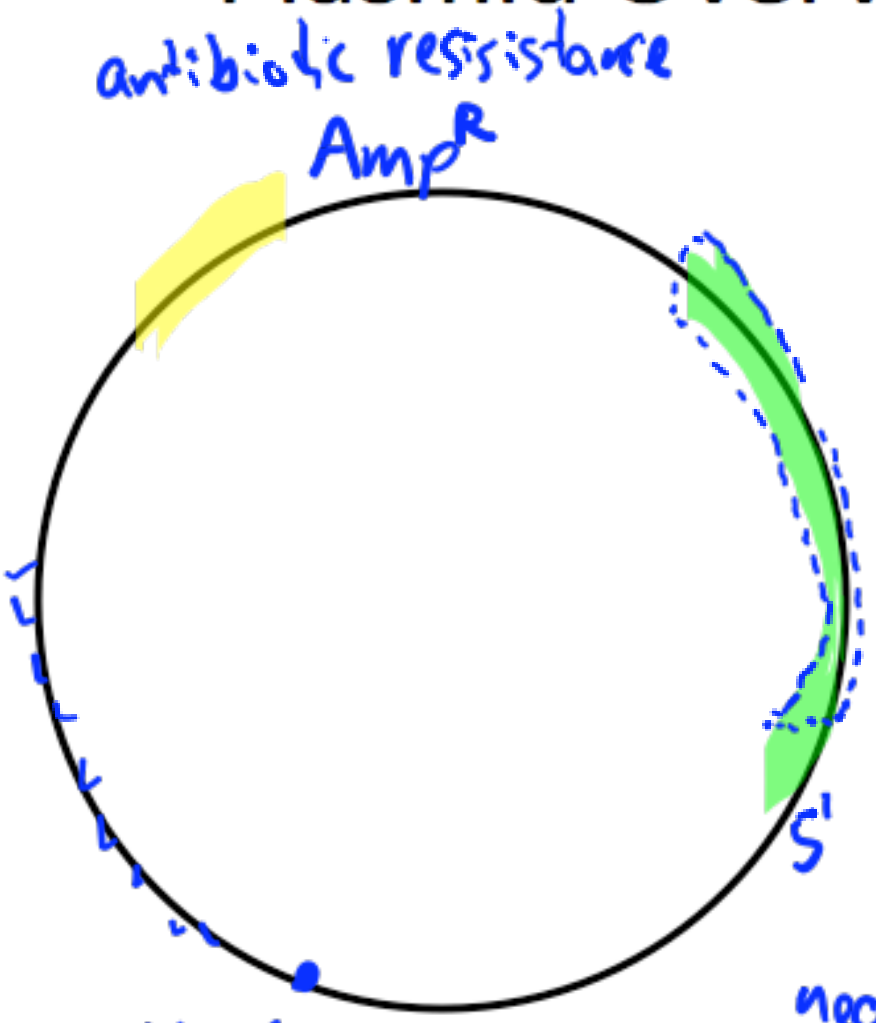
- Announcements
- Pre-lab Lecture
  - Plasmid Overview
  - Restriction Enzymes Intro
  - PCR recap
  - Safety + Technical Tips
- Lab Practical (~45 min)

Announcements

- BE seminar series:
  - Thursdays at 4:05 pm in 32-141
  - First seminar is Sept. 24th
  - Full schedule linked from BE website

- Introducing… Michelle, your TA for Module 1

Plasmid Overview: pCX-EGFP



extrachromosomal, ds circular DNA

why? gene expression in a cell

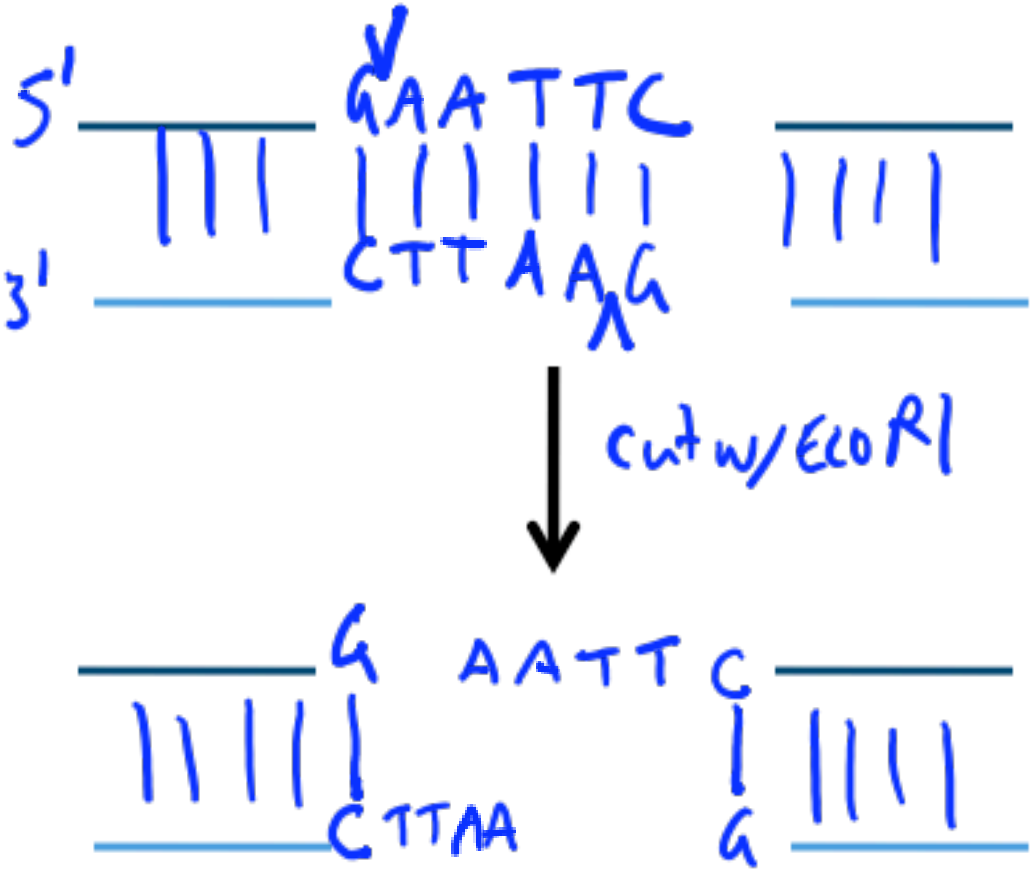
EGFP → ORF, CDS

create ΔEGFP
(later, combine w/ ΔEGFP → recombine)

- ORI - origin of replication (2 on this plasmid)

near ORF → promoter / polyA
introns / enhancer

Intro to Restriction Enzymes



EcoRI endonuclease

↓

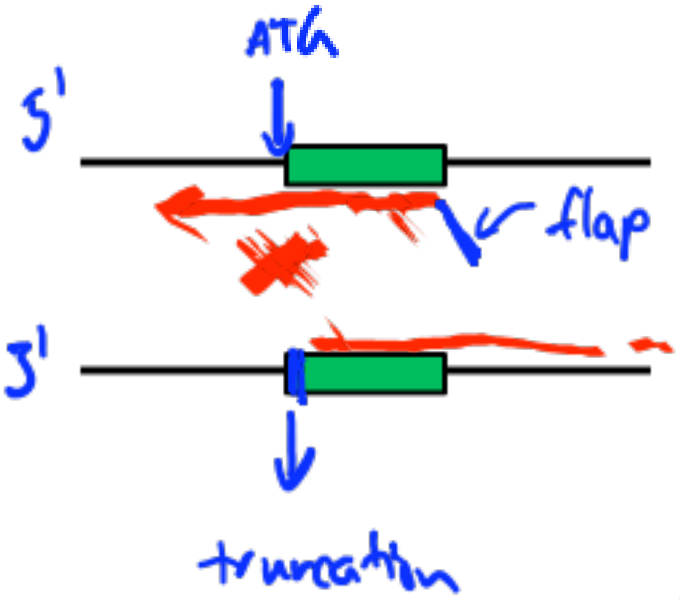
cuts up DNA

palindromic

created sticky ends

next time: how used in cloning

Designing PCR primers

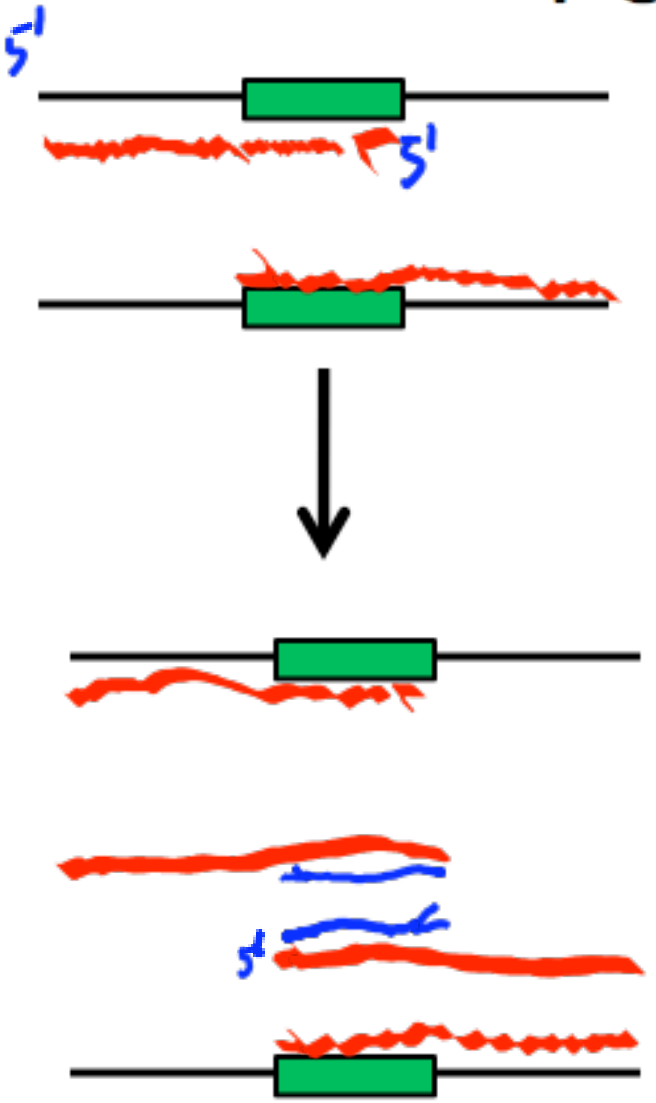


coding strand

primers:

flap: useful for adding rest. sites

PCR Process



MAE

Melt → 95°C

Anneal → 50's-60's

Extend → 72°C

depends on Tm of primer

* Ignore flap, truncation
↳ try on your own

- too long
- template for desired product

PCR Reaction

* no template control → spurious products, contamination

Component	Function
DNA polymerase (Taq)	catalyzes DNA elongation
dNTPs	material for elongation
primers	selection, initiation of new DNA strands
$MgCl_2$, buffers (chem. environment)	provide the right
template	provide desired sequence

cofactor

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