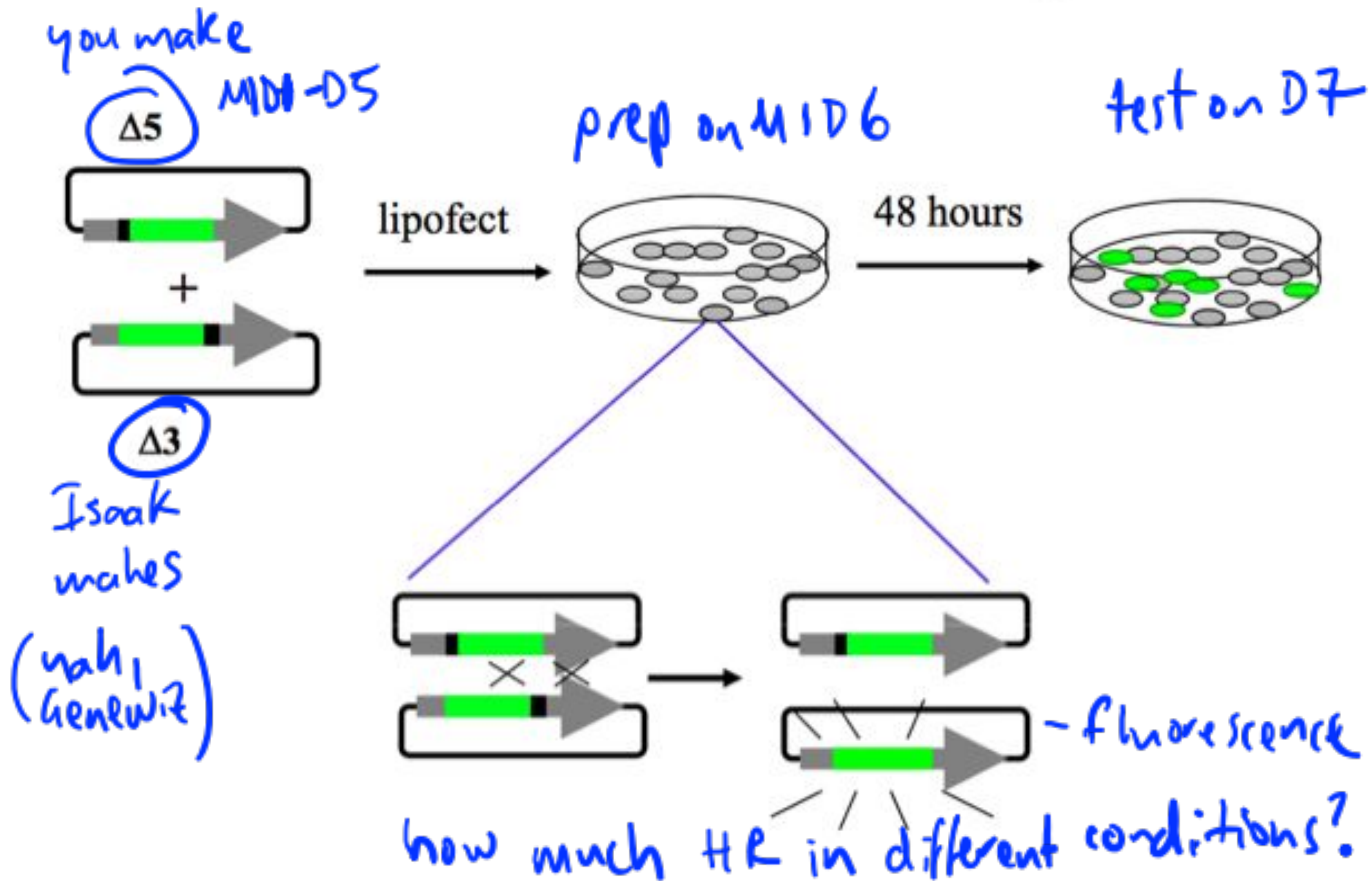
- Announcements
- Pre-lab Lecture
  - ❖ Plasmid review in M1 context
  - ❖ Restriction enzymes intro
  - ❖ PCR recap
  - ❖ Today in lab (M1D1)
- Lab Practical (~40 min)

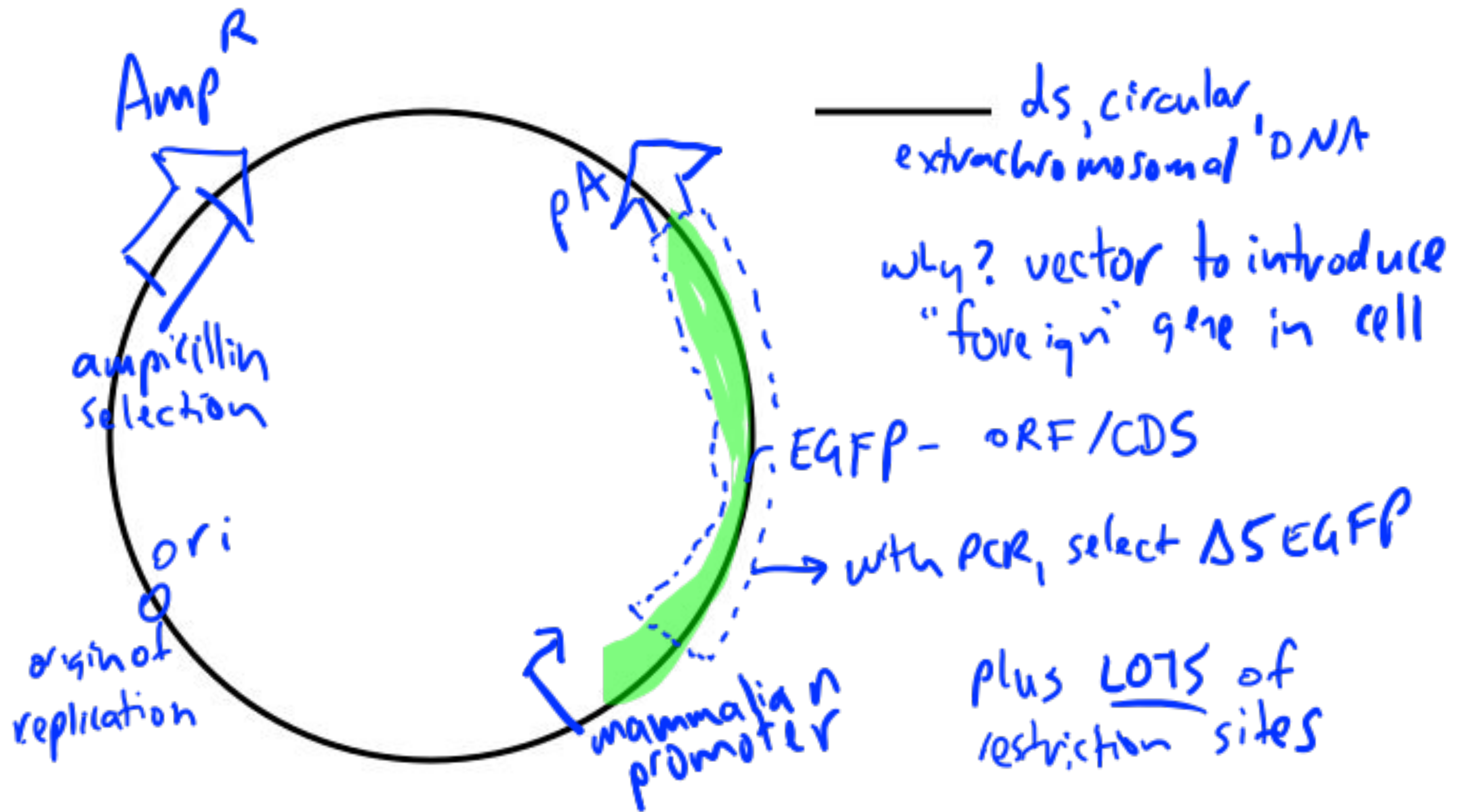
Announcements

- BE seminar series:
  - Thursdays at 4:05 pm in 32-141
  - First seminar is Sept. 18th
  - Full schedule linked from BE website
- CV/Resume workshop hosted by BE Writing Lab:
  - 9/16, 56-614, 7:30 pm
- Networking event with alumni/industry:
  - 9/18, Koch lobby, 5:30-7:30 pm → pre-register!

Module 1 research goal



Plasmid overview: pCX-EGFP



Intro to restriction enzymes

↳ endonucleases
→ cut DNA

palindromic

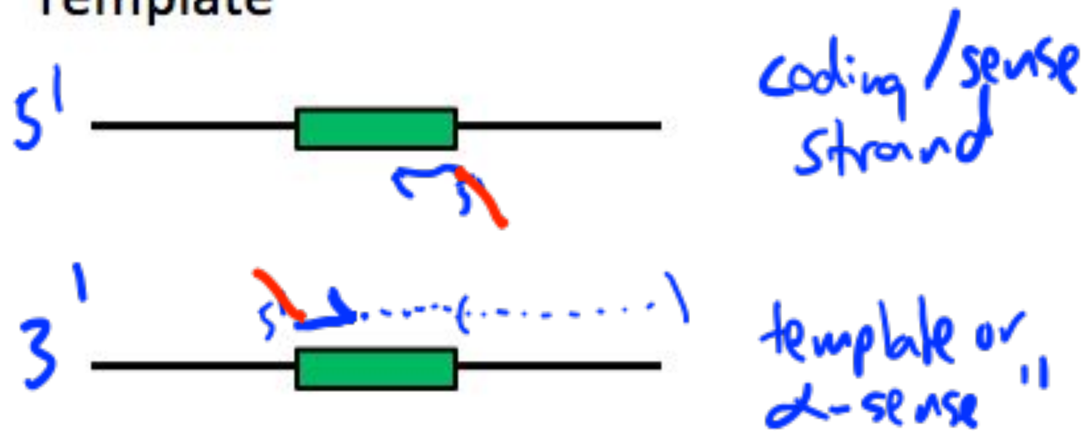
↓ cut w/ EcoRI

"sticky" ends
or
overhangs

Sch. "glue for our project"

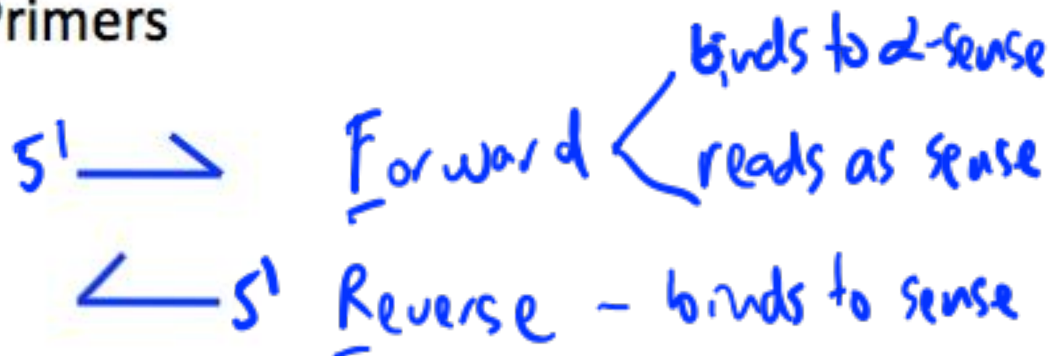
Designing PCR primers: topology

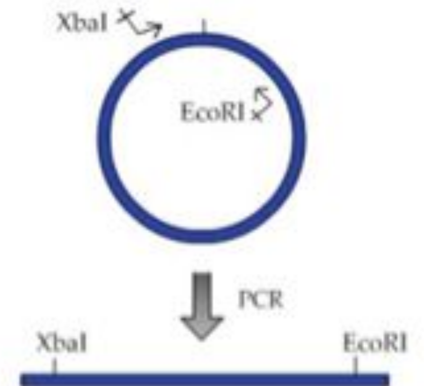
Template



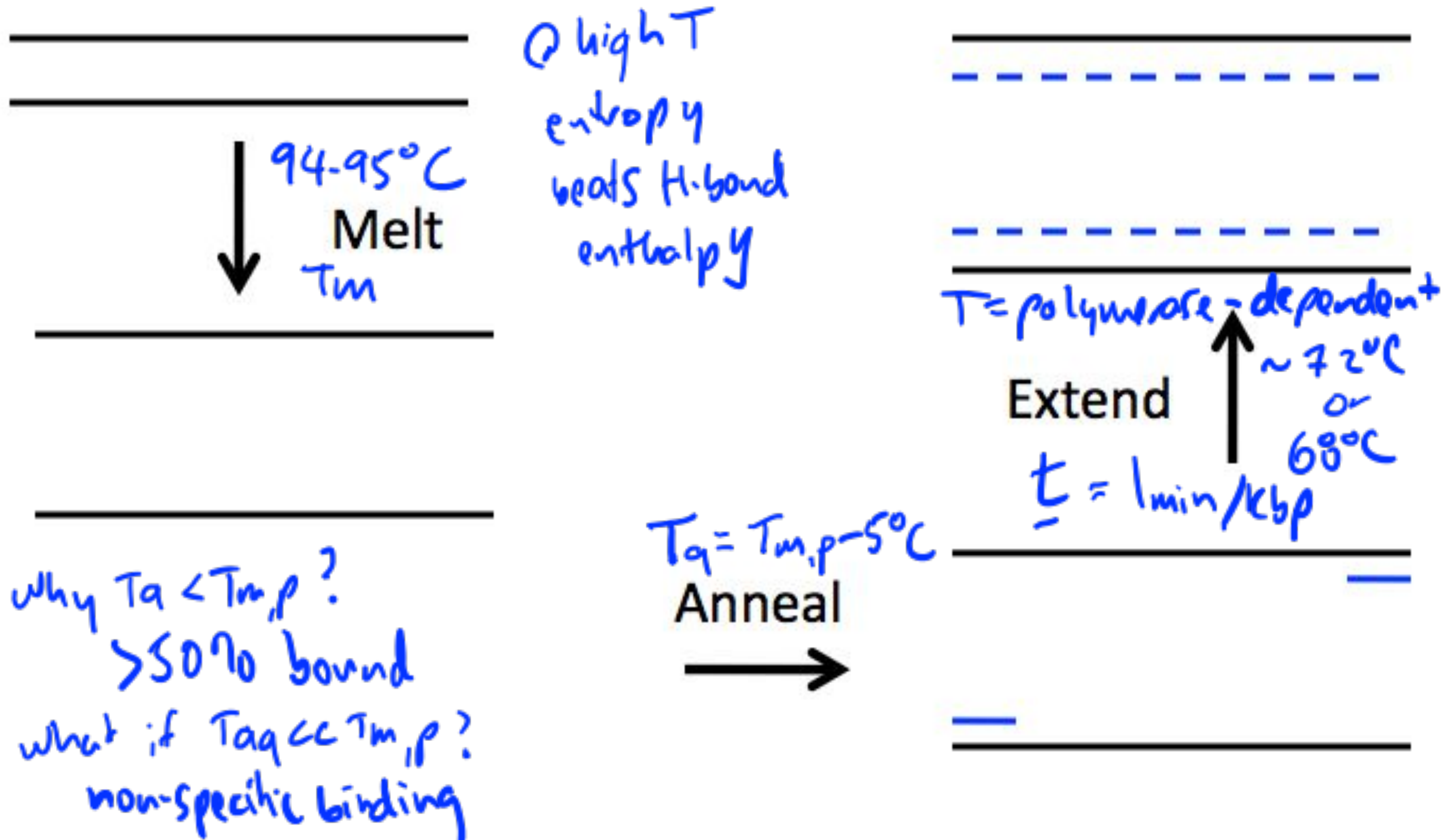
"flap"
add new function
e.g. linker
e.g. restriction site

Primers

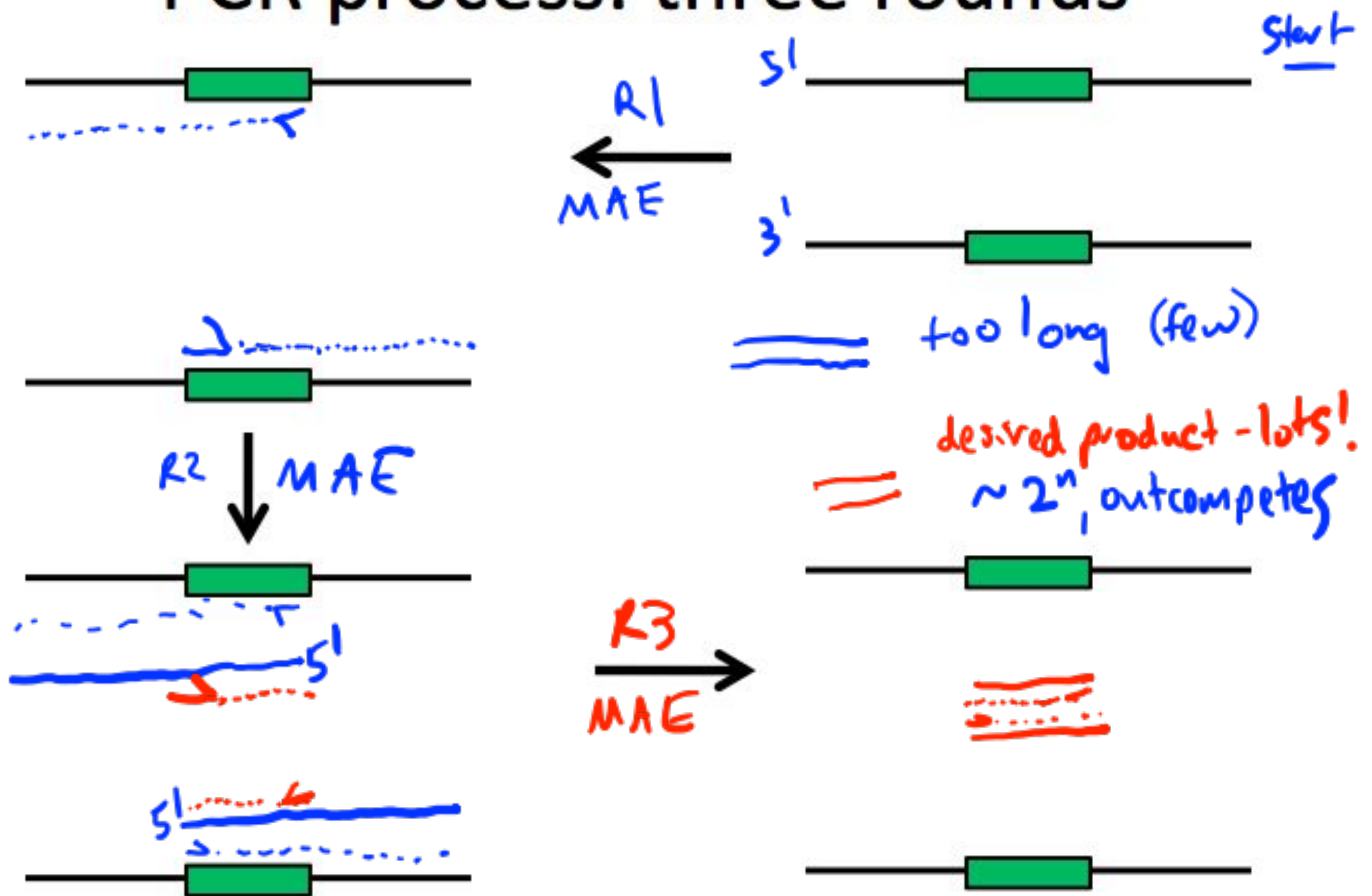




PCR process: three TD-driven steps



PCR process: three rounds



PCReaction

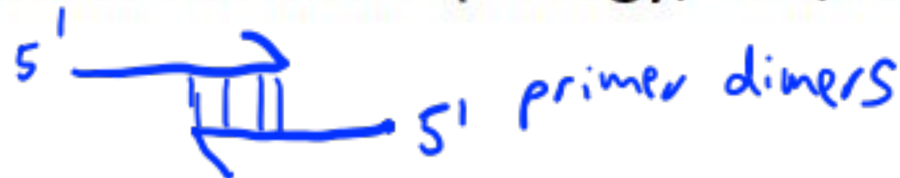
Component	Function
dNTPs	building blocks
polymerase	catalyzes extension
primers	select + initiate DNA strand
template	sequence to copy
buffer; Mg^{++}	pH/salt right enviro.; co-factor for enzyme

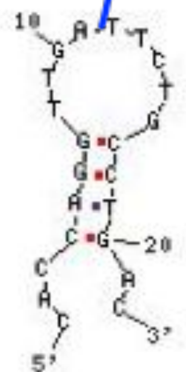
Designing PCR primers: properties

- Length: why is 17 bp the magic number?

  human genome ~ $3 \cdot 10^9$ bp $4^{17} \approx 2 \cdot 10^{10}$ bp

- Melting + annealing temperature efficient $T_a \sim 55\text{-}60°C$
- G/C content: why is 40-60% best? right T_m, p and
- Avoid long runs of same/similar base → mispriming
- Secondary structure considerations → poor priming
- Binding considerations (energy; self, other)





Today in Lab: M1D1

- Goal: design/make Δ5 insert
  also run control (NTC)
- Keep PCR tubes cold!
- Write small *directly* on the PCR tubes – do not put sticky labels in the PCR machine.
- Start notebooks

A few Evernote instructions/observations

- You may bring and use your own laptop.
- You can have one notebook open on the web-hosted version and one on the desktop.
- Work together to copy & modify protocols from the wiki -- you may work off one notebook for this.
- You **must write your own** front/back matter (statement of purpose & interpretation/conclusion).

Slide content from Shannon H

From protocol to lab notebook

1. Begin by adding the correct amount of water to a 200 ul PCR tube. Add that amount +1 ul to a second PCR tube.
2. Next add the primers to each reaction. Be sure to change tips between additions.
3. Next add template to the first reaction tube.
4. Finally add PCR Master Mix to each tube, pipetting up and down to mix. Leave your tubes on ice until the entire class

Statement of purpose: Today we will design primers to [do xyz task]. Then we will prepare [xyz DNA] by PCR to use as [xyz component] for later cloning.

Design primers for xxx (M1D1 Part 1)
See attached Word document.

+see Evernote example

PCR to make xxx (M1D1 Part 2)

Copy protocol and fill in exact volumes for #1.
Optionally confirm (say, with checkboxes) key details such as adding Master Mix last, template only to experimental sample.
Add unique notes: Rxn ready at 3 pm → on ice → thermal cycler started at 4.