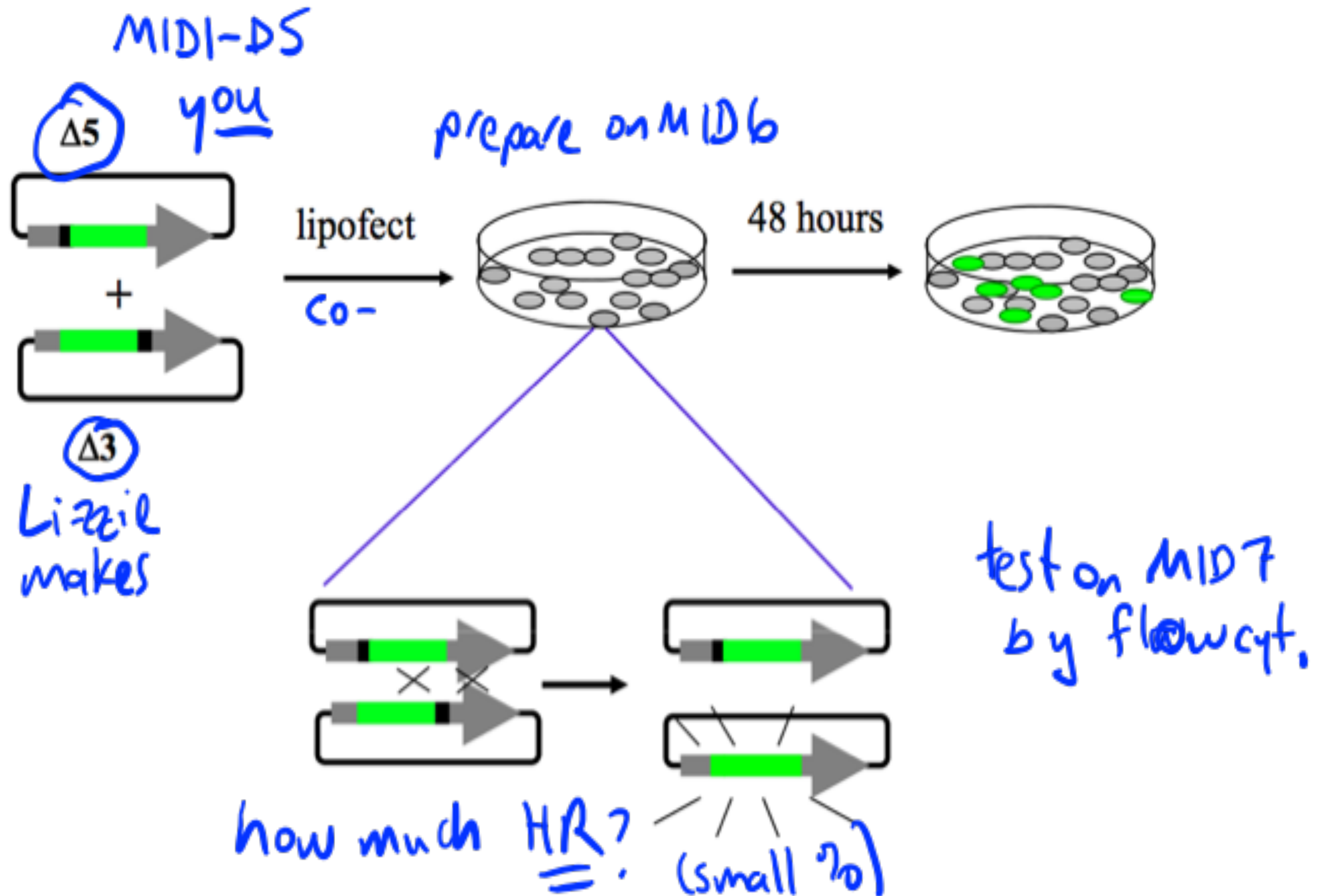
- Announcements
- Pre-lab Lecture
  - Plasmid review in M1 context
  - Restriction enzymes intro
  - PCR recap
  - Today in lab (M1D1)
- Lab Practical (~40 min)

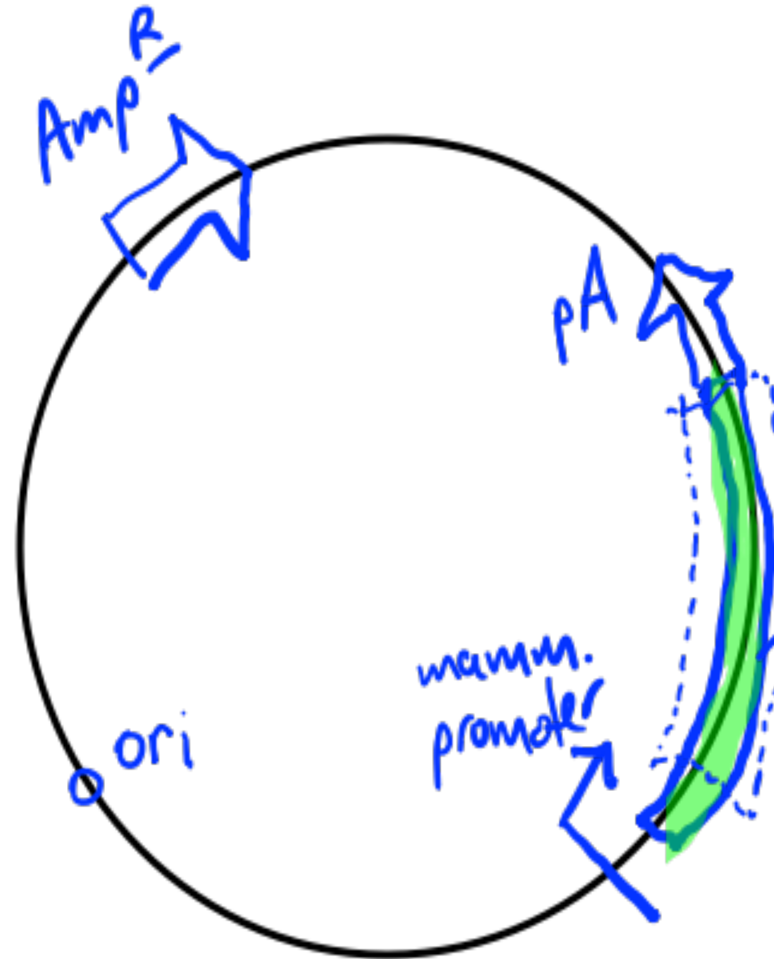
Announcements

- BE seminar series:
  - Thursdays at 4:05 pm in 32-141
  - First seminar is Sept. 19th
  - Full schedule linked from BE website
- CV/Resume workshop hosted by BE Writing Lab: tonight! → see flyer
- Networking event with alumni/industry: 9/19 Koch 5-7 pm → pre-register!
- Introducing… Lizzie, your TA for Module 1

Module 1 research goal



Plasmid overview: pCX-EGFP



______ ds, circular, extrachromosomal DNA

why? vector to introduce foreign gene in cell

EGFP ORF/CDS

PCR to select Δ5 EGFP ......

plus LOTS of restriction sites

Intro to restriction enzymes

endonucleases
→ cut DNA

palindromic

cut w/EcoRI

"Sticky" ends/
overhangs

Designing PCR primers: topology

Template

coding / sense strand

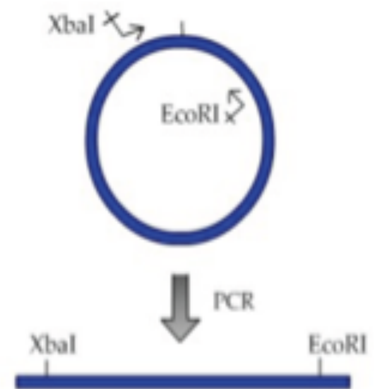
template / α-sense

add a new function
e.g. rest. site
e.g. linker

Primers

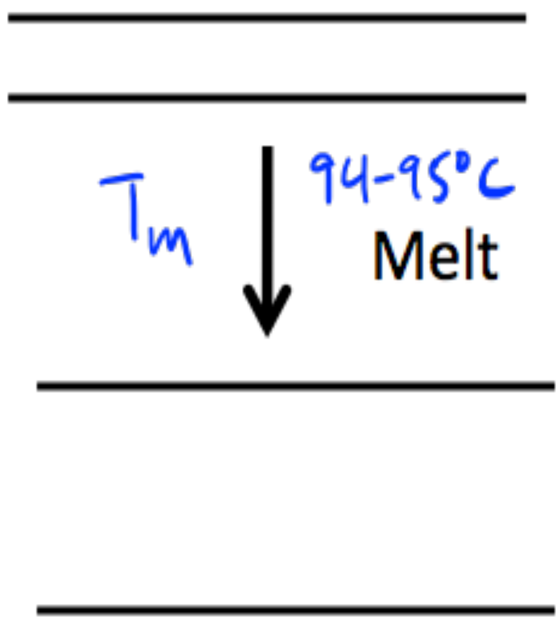
5' → Forward < binds α-sense / reads as sense "easy"

← 5' Reverse – binds sense

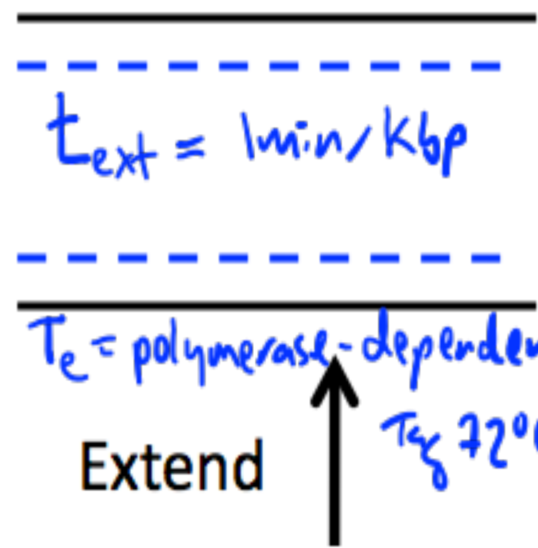


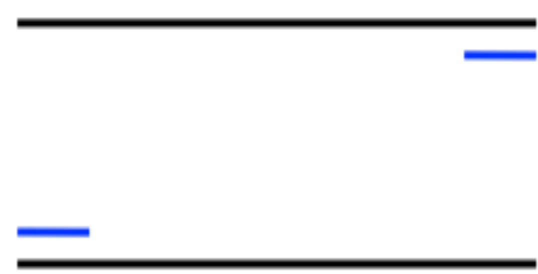
PCR process: three TD-driven steps

(thermodynamics)



@high T
conf.
entropy beats H-bond enthalpy





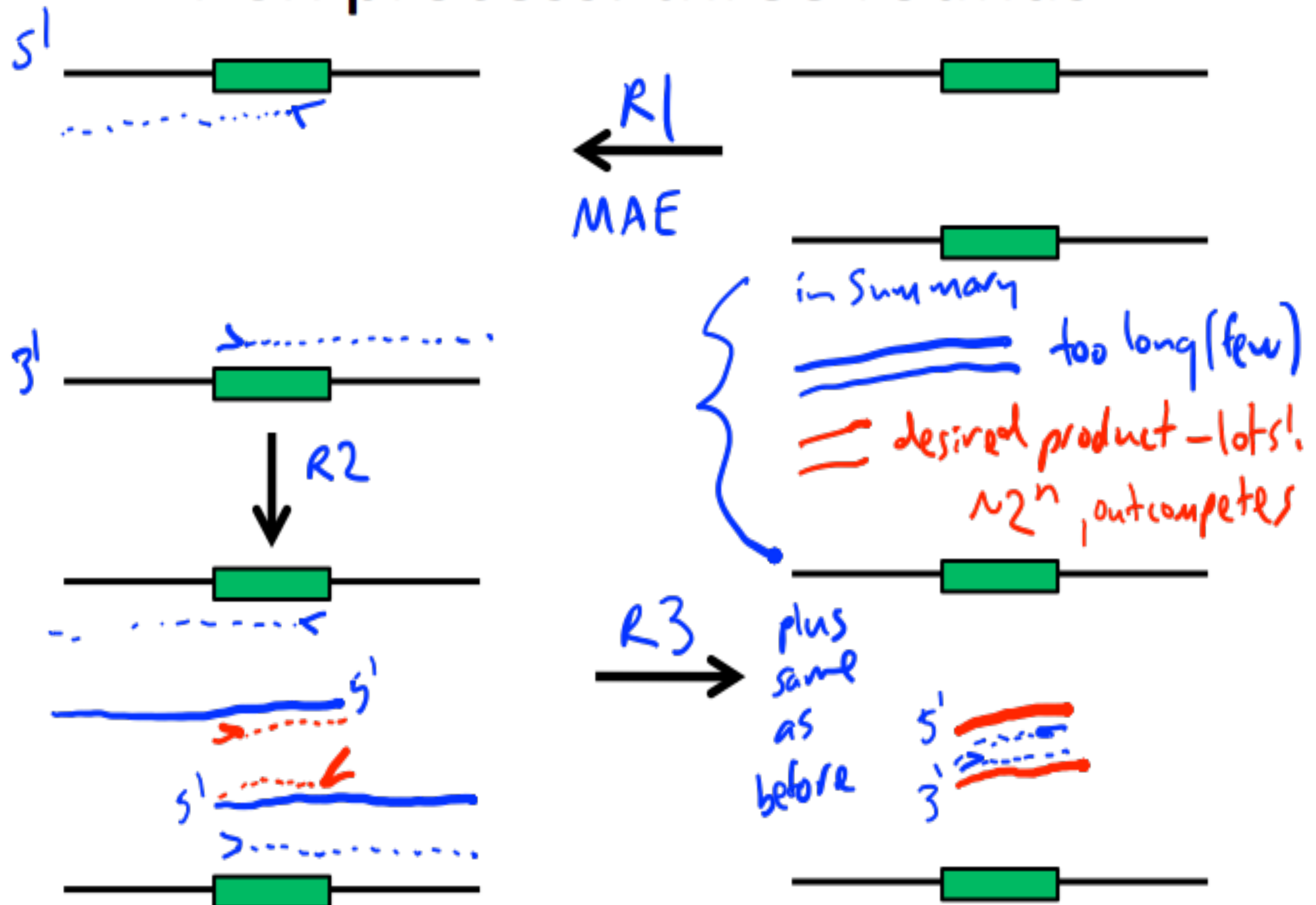
$T_a = T_{m,p} - 5°C$

Anneal

- why $T_a < T_{m,p}$?
  >50% bound
- what if $T_a \ll T_{m,p}$?
  non-specific binding

good target $T_a \sim 50\text{-}60°C$

PCR process: three rounds



PCReaction

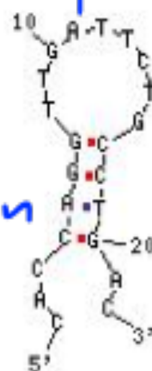
Component	Function
primers	select + initiate DNA
dNTPs	building blocks
template	sequence to copy
polymerase	catalyzes extension
buffer ; Mg^{++}	pH/salt right ; co-factor

Designing PCR primers: properties

- Length: why is 17 bp the magic number?

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

- Melting + annealing temperature efficiency
- G/C content: why is 40-60% best? T_m, and
- Avoid long runs of same/similar base → mispriming
- Secondary structure considerations → poor priming
- Binding considerations (energy; self, other) hairpin



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
- Start notebooks +2 pre-labnotes

+ toddler quote/ science fact: "An ichthyologist is a kind of beetle that FIGHTS your nose... Treat yourself!"

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