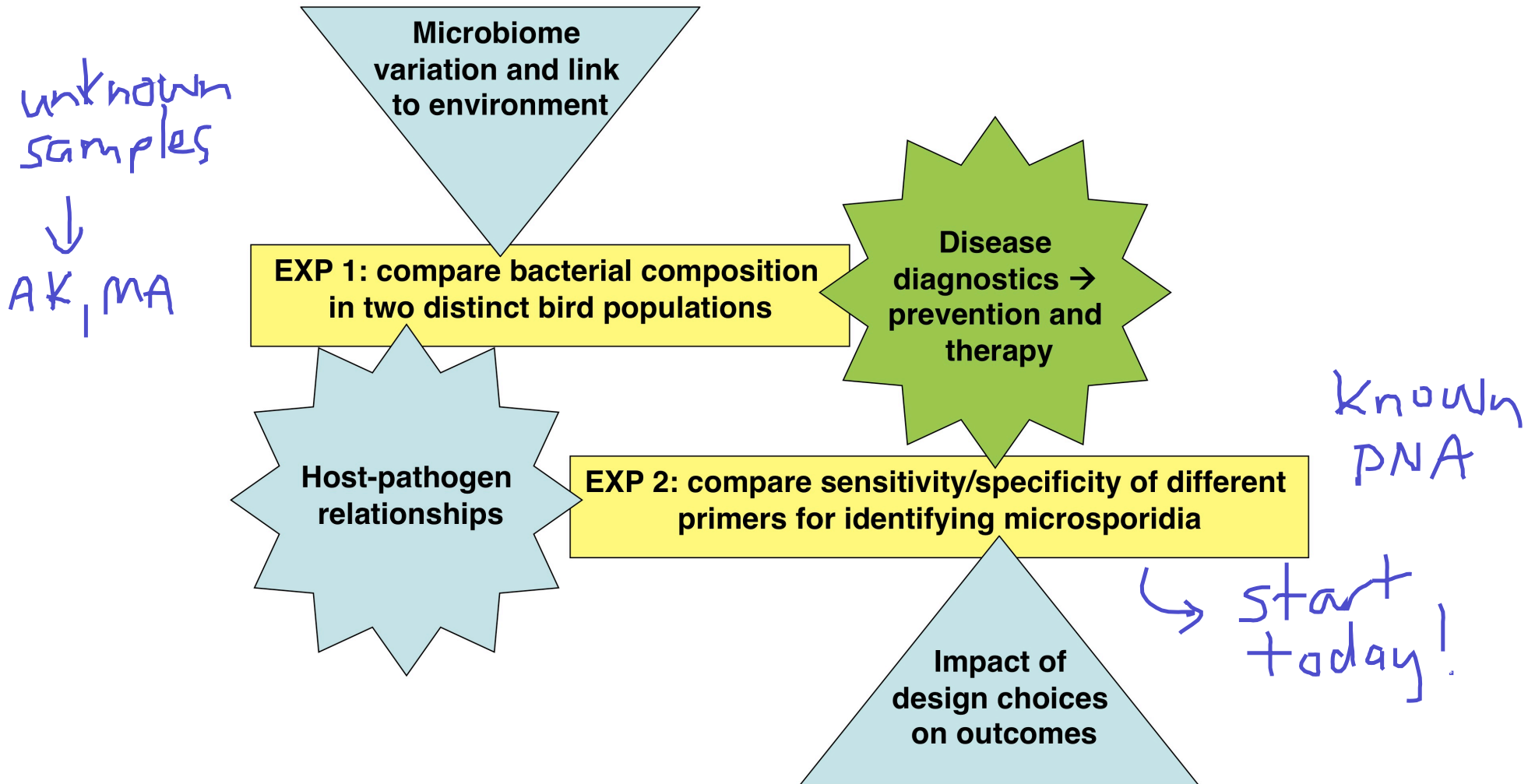


- Announcements
- Pre-lab Lecture
 - ❖ Module 1 overview + μ sporidia
 - ❖ Intro to primers and PCR
 - ❖ Module 1 assignments
 - ❖ Today in Lab: M1D1
- Lab Practical (~40-45 min)

Announcements

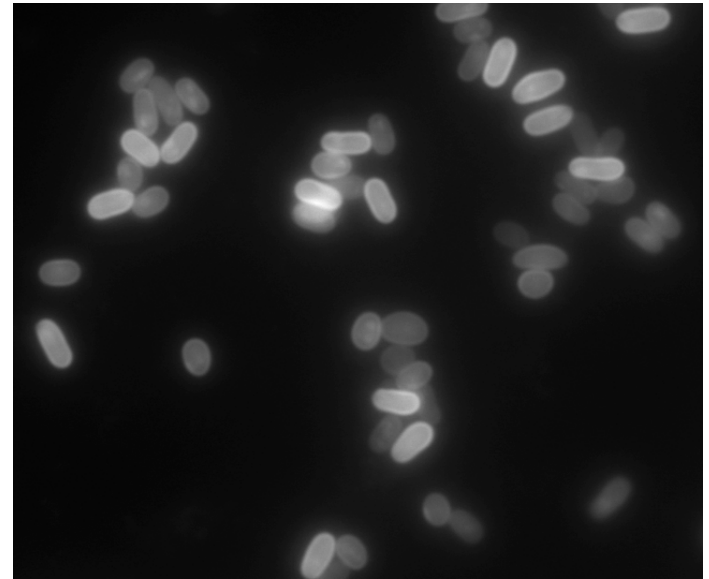
- BE (and other) seminar series:
 - Seminar posters across from BE HQ on 3rd floor
 - Full schedule linked from BE website
 - Part of professional development
- Introducing... Ian, your TA for Module 1
- Different equipment for different volumes/tasks

Module 1 overview



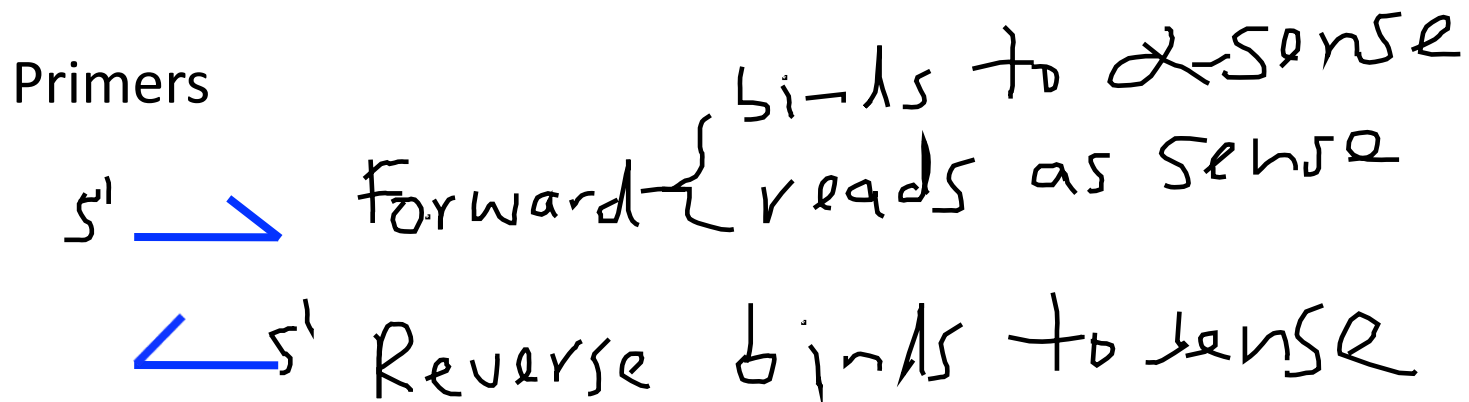
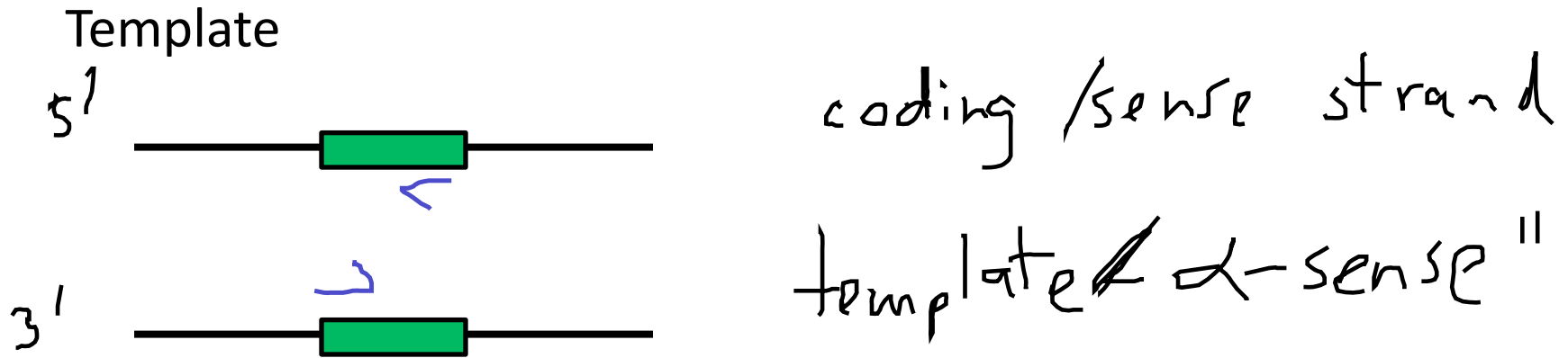
Microsporidia: fascinating bugs

- Highly evolved fungus
 - toward simplicity
 - few genes *and* few non-coding regions for a eukaryote
 - protist? no!
- Opportunistic infections
 - immunocompromised
 - travelers
- Tough to isolate!

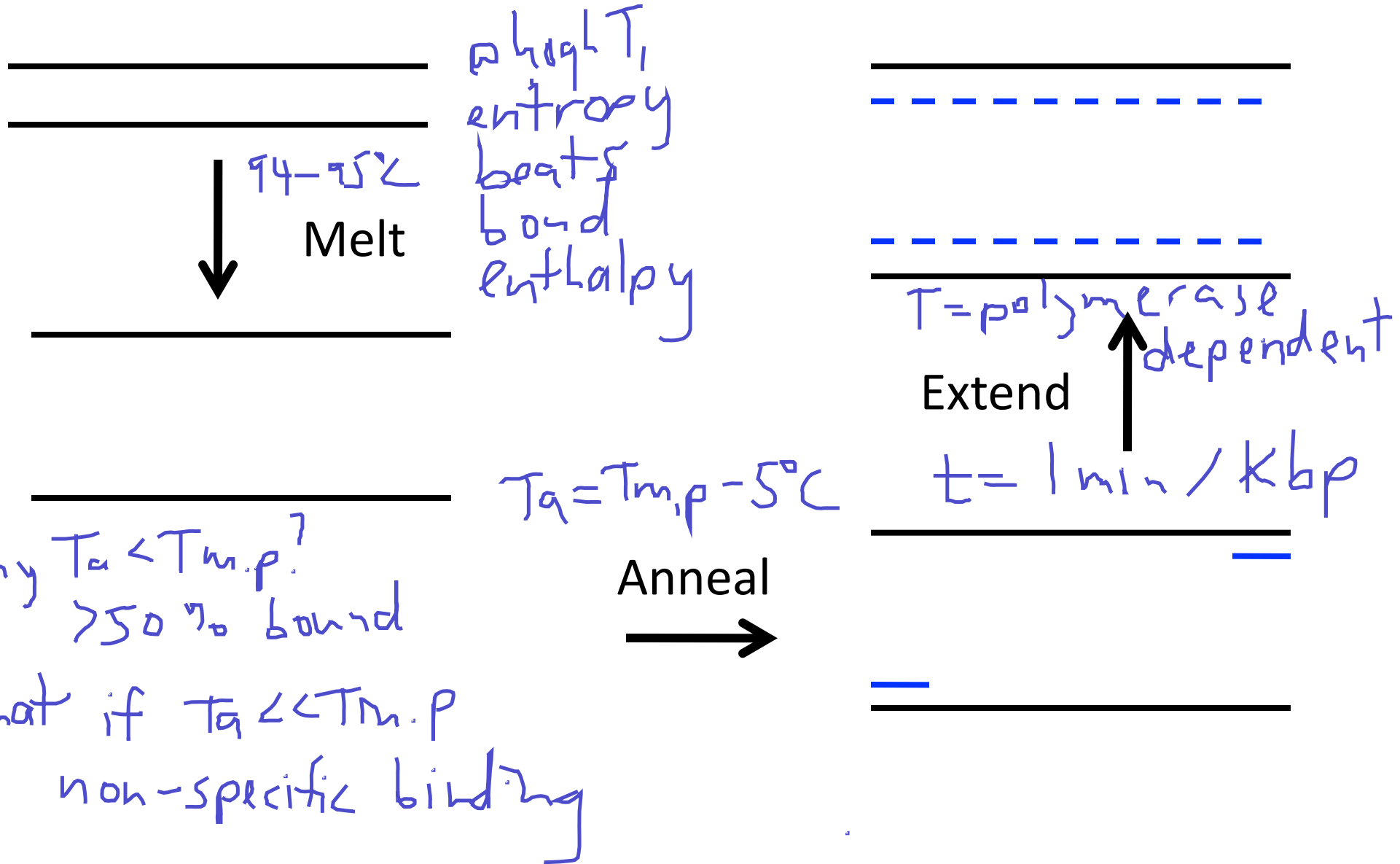


– immunocompromised (HIV, chemotherapy, age)

Designing PCR primers: topology

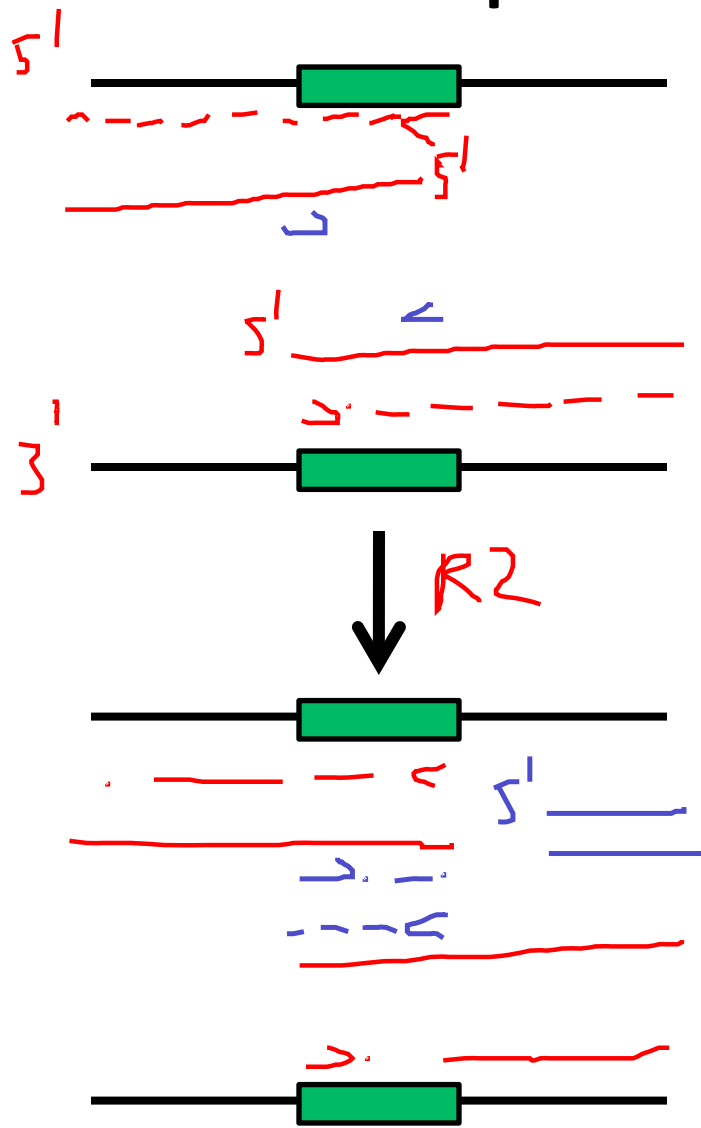


PCR process: three TD-driven steps

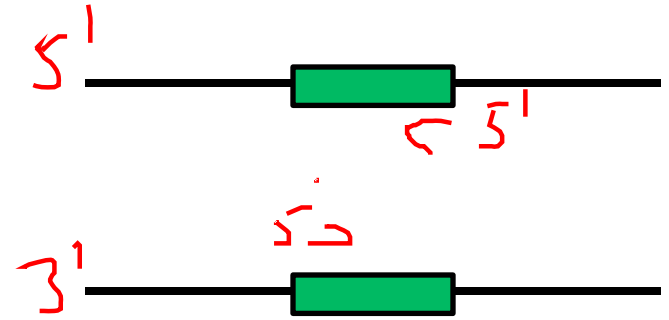


- why $T_a < T_{m,p}$?
 >50% bound
- what if $T_a \ll T_{m,p}$?
 non-specific binding

PCR process: three rounds

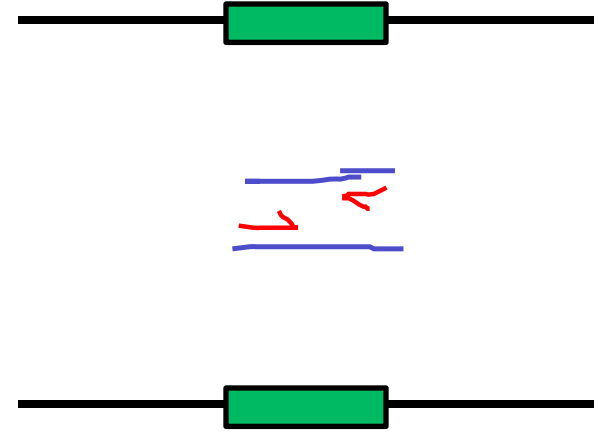


R1
MAE



too long (few)
= product of interest
(lots! 2^n)

→



Designing PCR primers: properties

- Length: why is 17 bp the magic number?

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

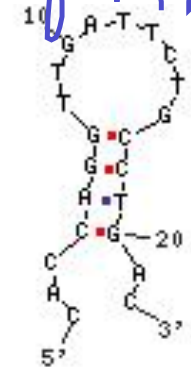
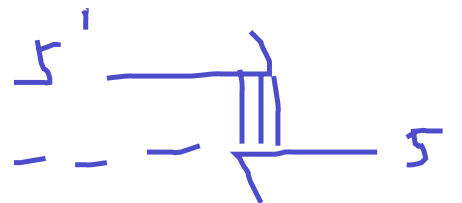
- Melting + annealing temperature \Rightarrow efficiency
 $t_a \sim 55-60^\circ\text{C}$
- G/C content: why is 40-60% best?

- Avoid long runs of same/similar base \rightarrow mispriming

- Secondary structure considerations \rightarrow poor priming

- Binding considerations (energy; self, other)

GC clamp
(3' end tip)



Mod 1 written assignments

- Lab report re: bacterial communities (15%)
 - Traditional format (intro, methods, etc.)
 - WAC training begins next time
 - Written in pairs *HW individually*
 - Can be revised for up to 1.33 letter grade higher
- Primer design summary (5%)
 - Short text and table summarizing design strategy
 - Short text and figure summarizing result
 - Written alone
 - Not subject to revision

Mod 1 oral assignment

- Journal club (10%)
 - Purpose: summarize a recent research article
 - Sign up for Day 6 (Feb 28/Mar 1) or Day 8 (Mar 7/8)
 - Paper list available ?next week?
- Preparation
 - WAC training will be on Day 3 (Feb 14/15)
 - Will also practice discussing an article in-class on M1D3: start reading the paper this weekend
- Presentations will be videotaped, reviewed

Participation and reflection in 20.109

- 1%: our perception of your engagement and contributions
- 2%: four reflections on your own learning
 - journal club self-assessment
 - module 1 report lessons learned
 - module 2 report lessons revisited
 - grab-bag: meeting with peers or instructors; discussion of outside research article
 - *extra-credit reflections*
 - *our hope: make learning gains more concrete*

Today in Lab: M1D1

- Complete lab practical
- Explore existing diagnostic primers for μ sporidia
- Design new primers
 - sensitivity *or* specificity challenge
 - sign up on M1D1 “Talk” page
- Notebooks start today!
 - primer table will be used in your M1 design summary
- For next time
 - keep exploring wiki... and add to it
 - start reading paper for M1D3 discussion