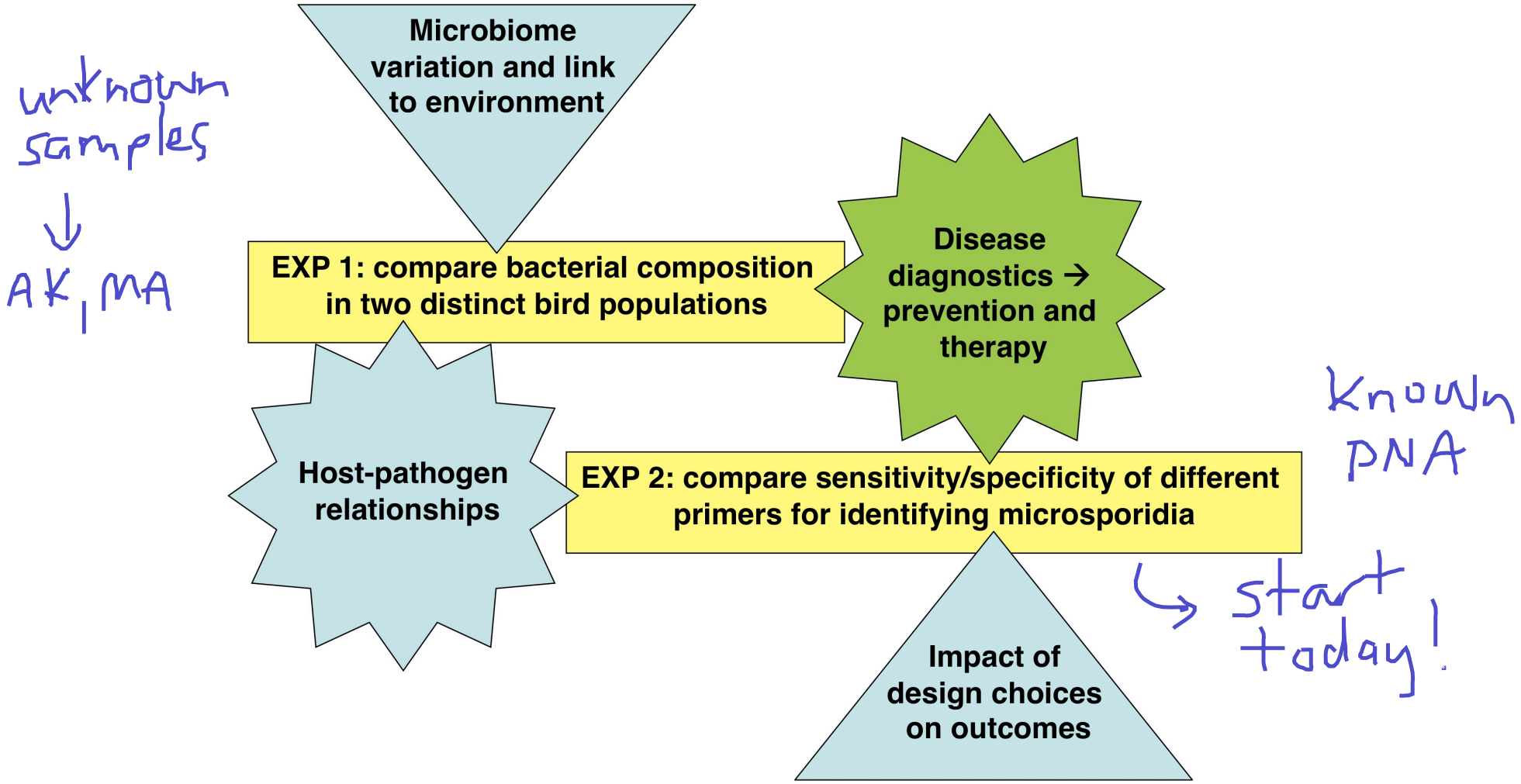
- Announcements
- Pre-lab Lecture
  - ❖ Module 1 overview + µsporidia
  - ❖ Intro to primers and PCR
  - ❖ Module 1 assignments
  - ❖ Today in Lab: M1D1
- Lab Practical (~40-45 min)

Announcements

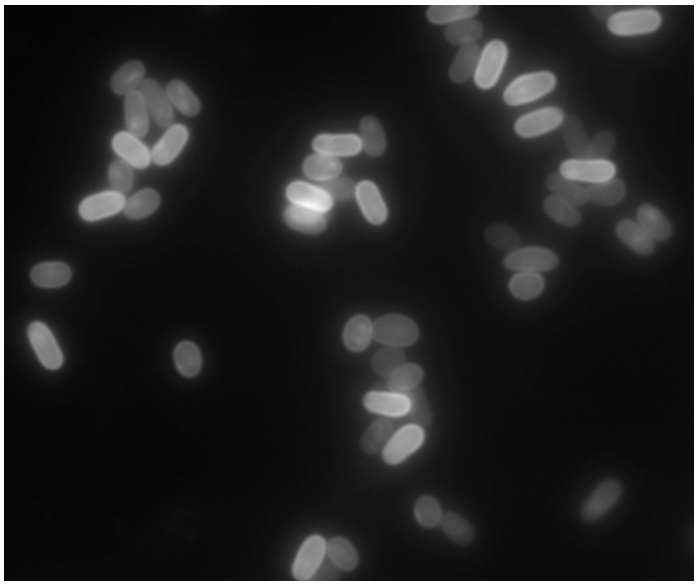
- BE (and other) seminar series:
  - Seminar posters across from BE HQ on 3rd floor
  - Full schedule linked from BE website
  - Part of professional development
- Introducing… Ian, your TA for Module 1
- Different equipment for different volumes/tasks

Module 1 overview



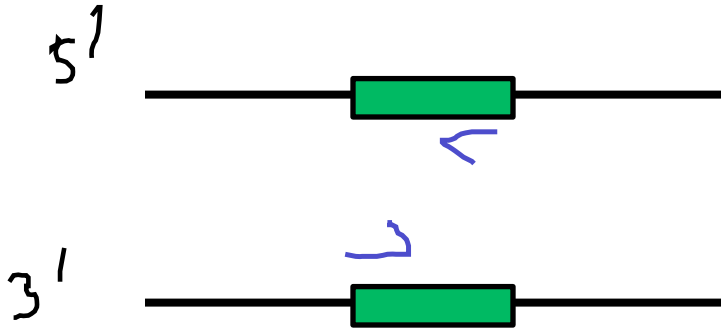
Microsporidia: fascinating bugs

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



Designing PCR primers: topology

Template



coding / sense strand

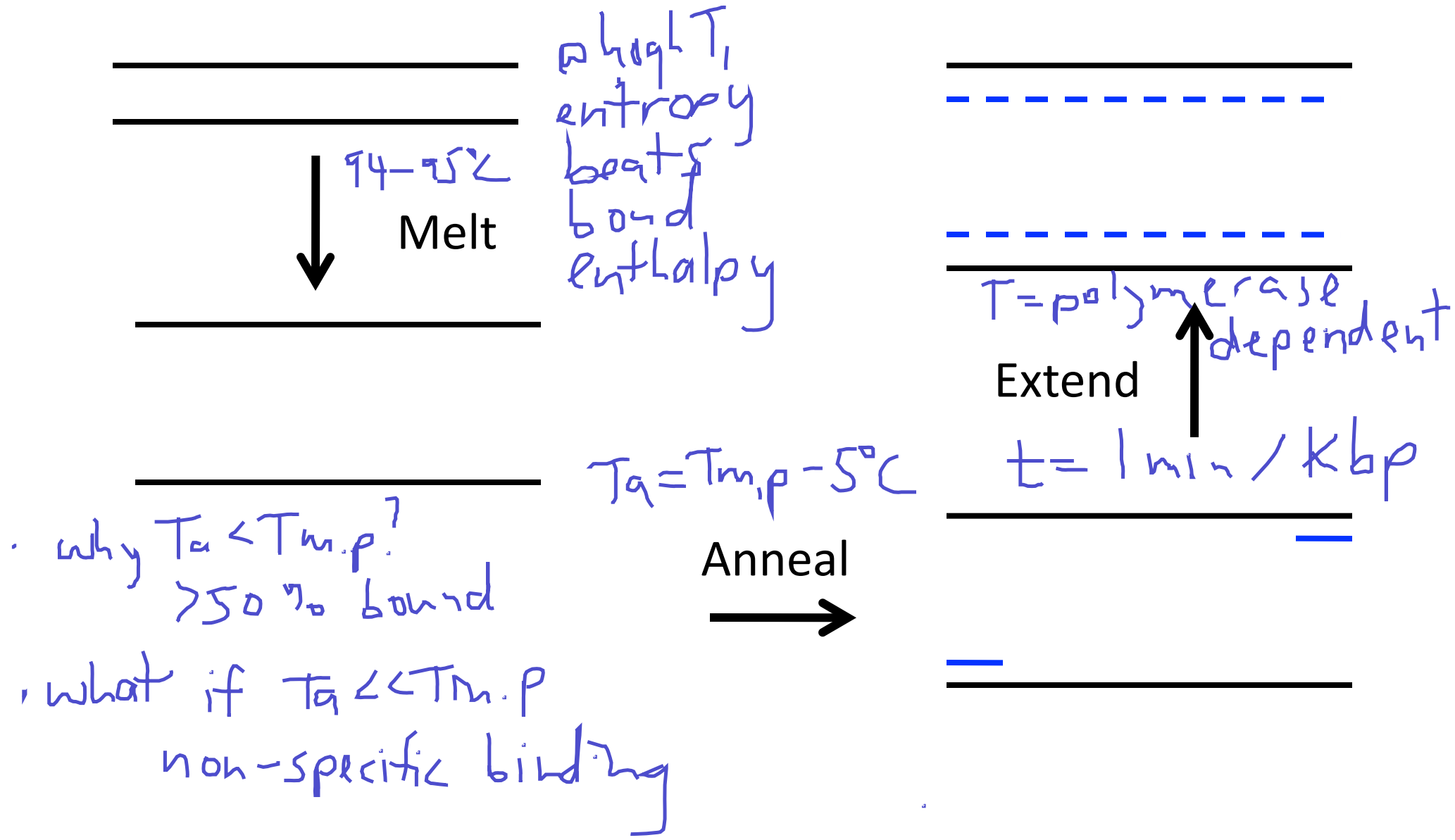
template / α-sense "

Primers

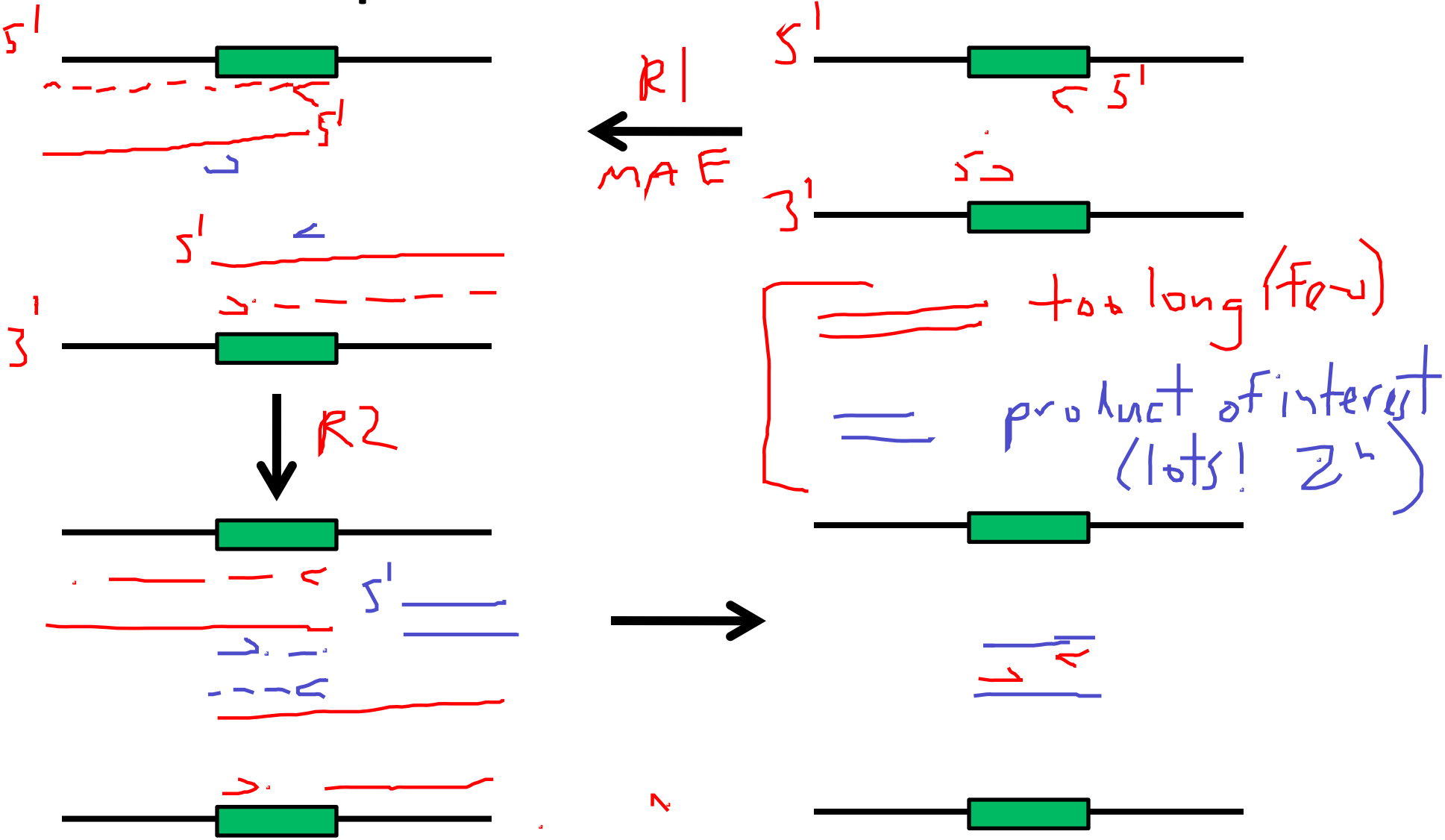
5' → Forward { binds to α-sense / reads as sense

← 5' Reverse binds to sense

PCR process: three TD-driven steps



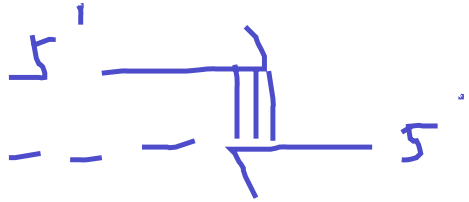
PCR process: three rounds

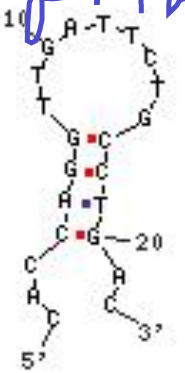


Designing PCR primers: properties

- Length: why is 17 bp the magic number?
  human genome ~3·10⁹ bp $4^{17} \sim 7 \cdot 10^{10}$ bp
- Melting + annealing temperature ⇒ efficiency
  $t_a \sim 55-60\,^{\circ}C$
- G/C content: why is 40-60% best?
- Avoid long runs of same/similar base → mispriming
- Secondary structure considerations → poor priming
- Binding considerations (energy; self, other)

GC clamp
(3' end)





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 µsporidia
- 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