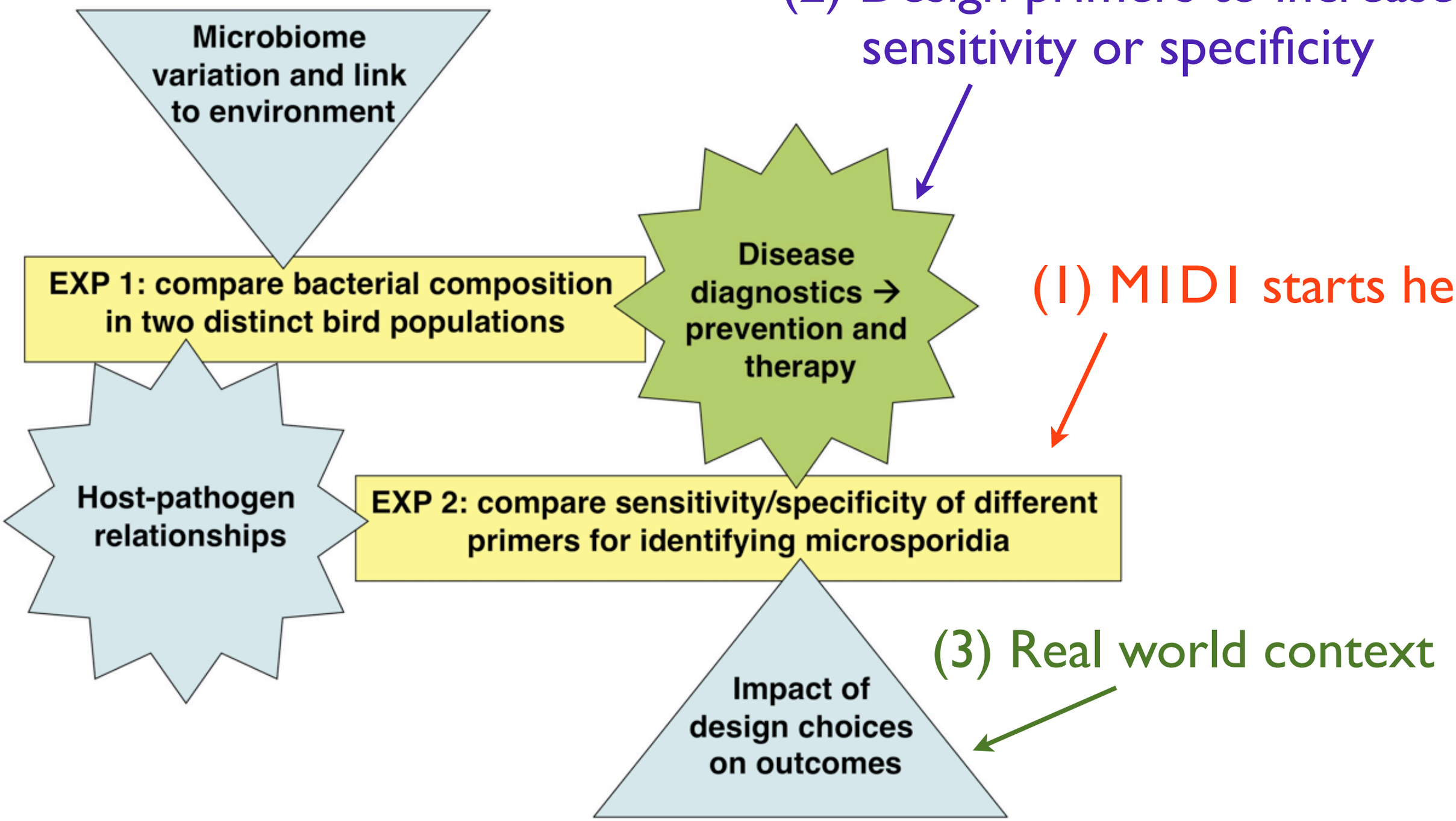
MIDI: Context Setting and Primer Design

2/8/13

Module 1 Overview & Outline for lab today



(1) MIDI Starts Here

Microsporidia

G = germinated spore
U = ungerminated spore
PT = polar tube

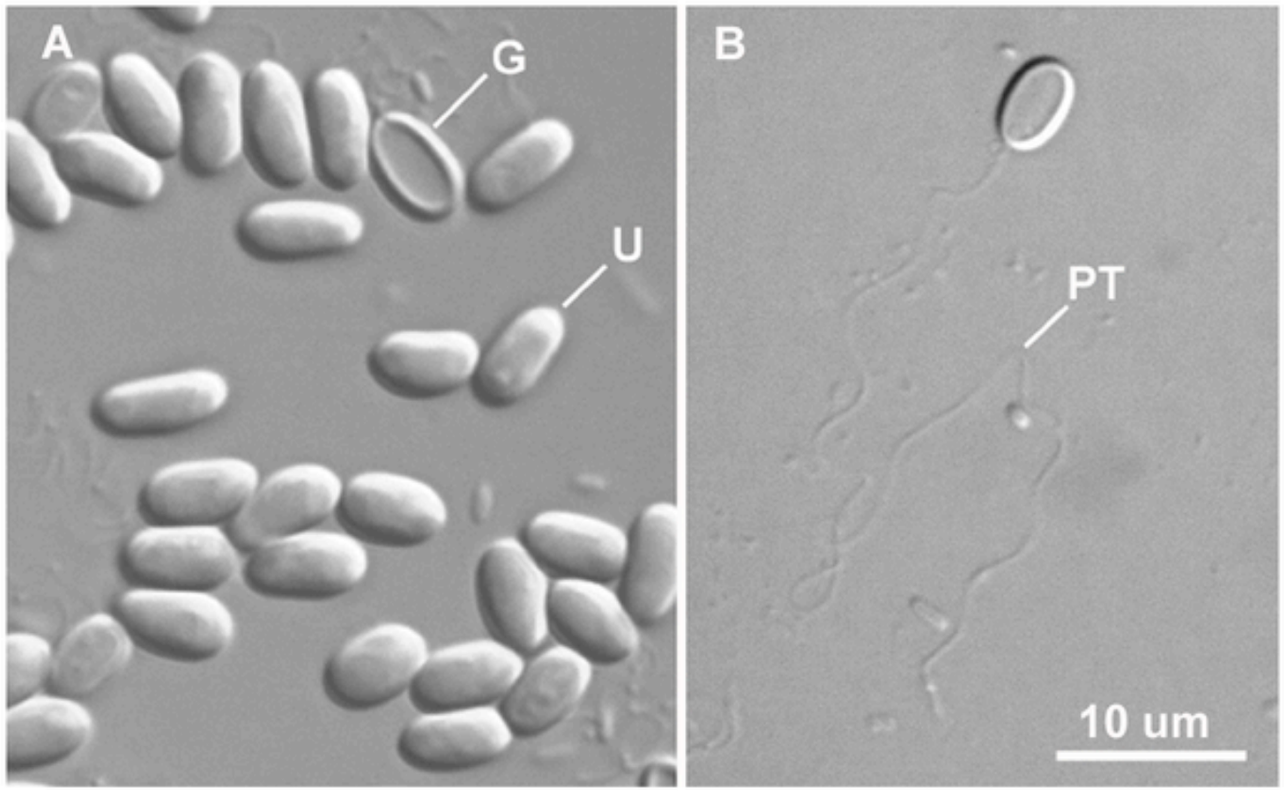


Image from: http://www.plospathogens.org/article/info%3Adoi%2F10.1371%2Fjournal.ppat.1000489

This is a free resource that has great information about microsporidia in a friendly format.

We will work with DNA from two species of microsporidia:
Encephalitozoon hellem (or *E. hellem*)
Vittaforma corneae (or *V. corneae*)

(1) MIDI Starts Here

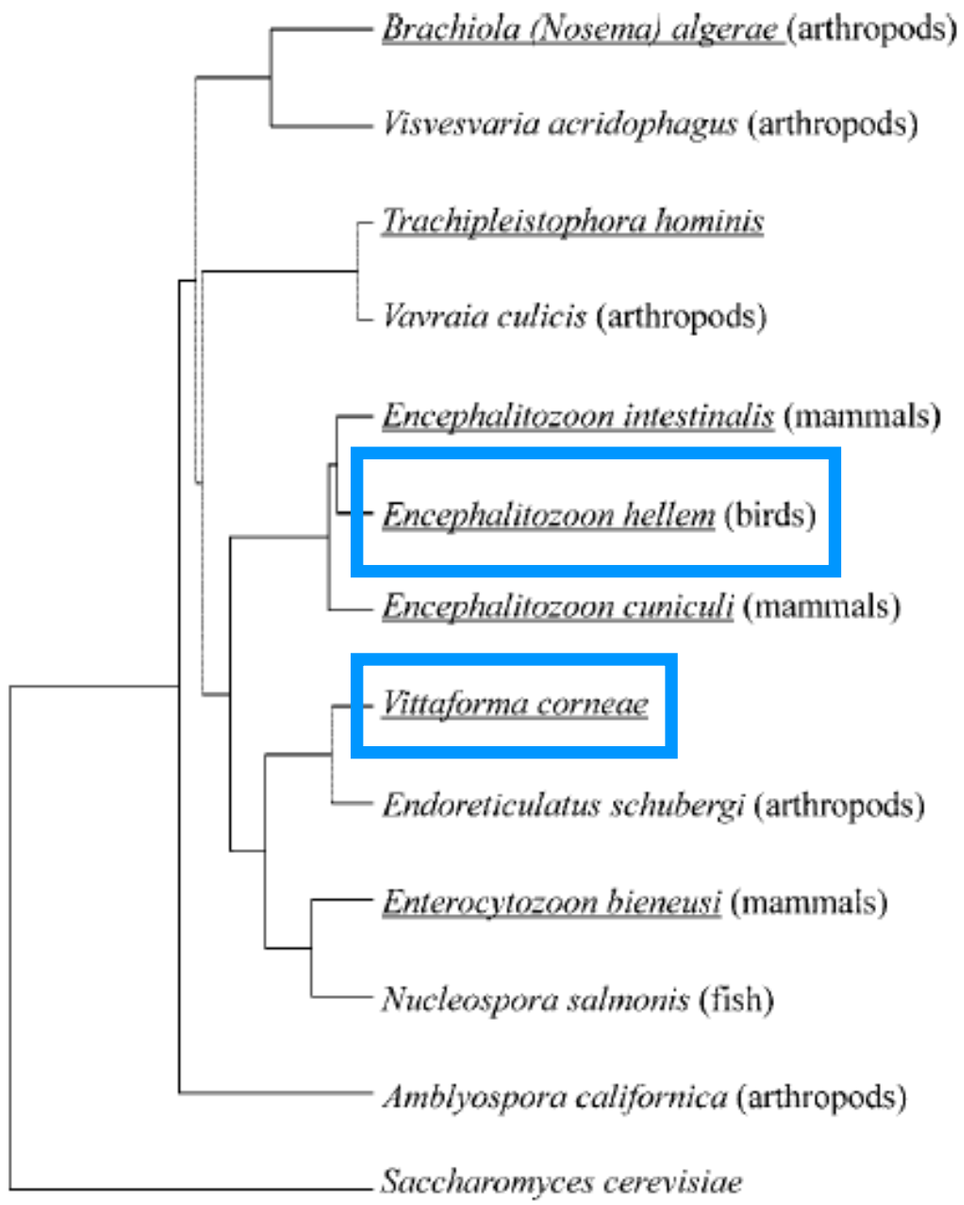


FIG. 2. Dendrogram generated from the small subunit ribosomal RNA (ssrRNA) gene of microsporidian species identified in humans (underlined) and selected other species (Kimura's distance, unweighted pair group method of analysis). Known animal hosts are indicated in brackets; the brewer's yeast *Saccharomyces cerevisiae* serves as an outgroup. No corresponding gene sequences are known for the human-infecting microsporidian species *Pleistophora ronneafiei*, *Trachipleistophora anthropophthera*, *Brachiola* (formerly *Nosema*) *connori*, *B. vesicularum*, *Nosema ocularum*, *Microsporidium ceylonensis*, and *M. africanum* (Table 1).

- μsporidia are most closely related to fungus.
- They have simple genomes that shrunk (!) during evolution. Why?
- μsporidia are obligate intracellular organisms -- meaning they require a host for survival.
- That requirement allowed evolution towards a simple genome!
- What hosts?
  - See tree to the left -- common hosts for μsporidia species are in brackets.
  - Those identified in human infections are underlined.

Dendogram generated from the ssrRNA gene of microsporidian species

Figure from Mathis et al., *Clinical Microbiology Reviews*, July 2005

(1) MIDI Starts Here

- A couple more things about μsporidia:

1. Humans infected with μsporidia share a common trait -- weak immune systems
   - HIV or other immunodeficiency diseases (lupus, Wegener's, etc)
   - Cancer patients undergoing treatment

2. μsporidia are tricky to isolate -- the isolation has been done for you and you will work with purified DNA (this makes the analysis doable for 109!)

- We will learn a lot more about μsporidia in lecture.

(2) Design primers to increase sensitivity or specificity

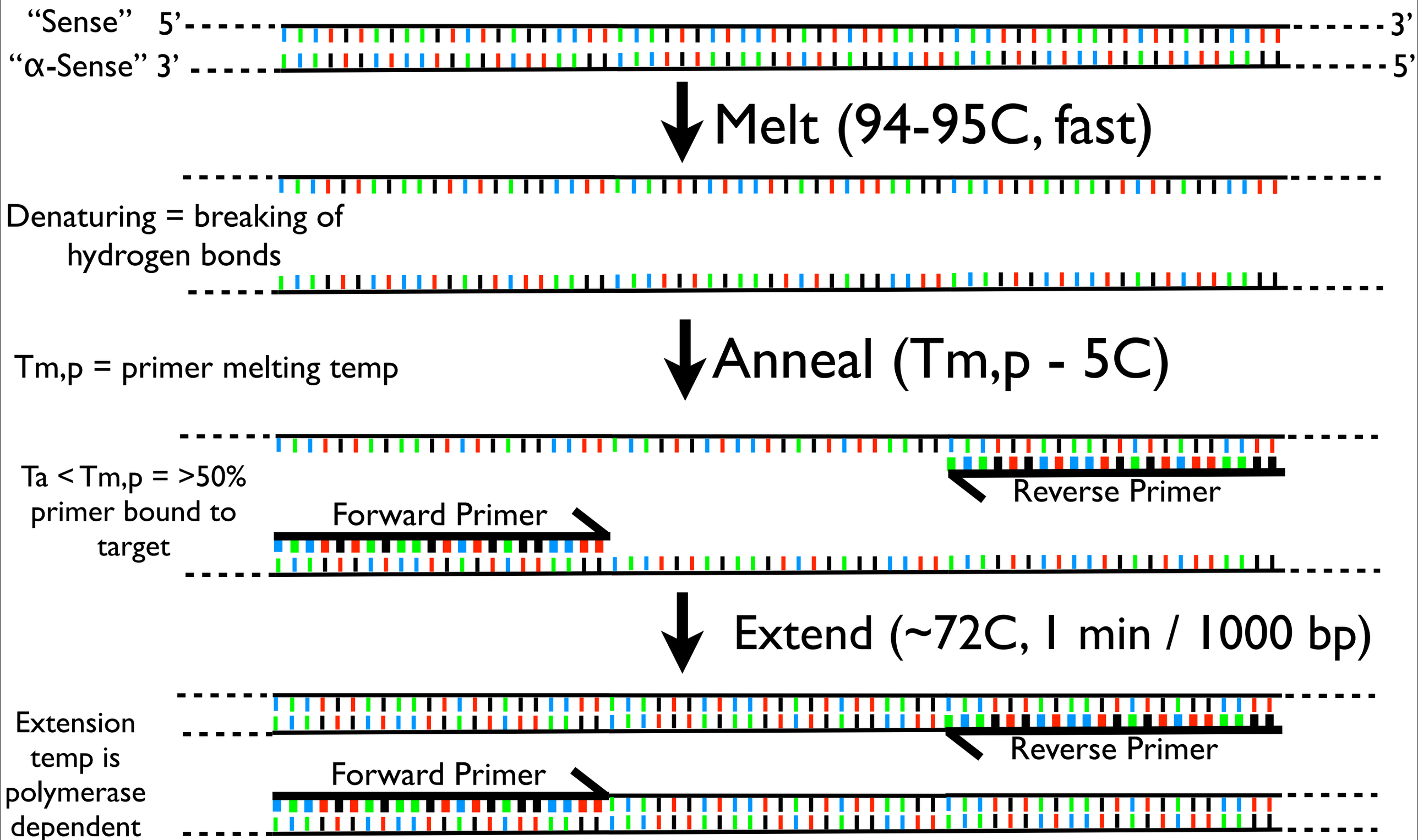
There are two design challenges for this part of Module 1

1. Selectivity
   - ✦ Design PCR primers that can differentiate *E. hellum* and *V. corneae*

2. Sensitivity
   - ✦ Design PCR primers that can improve µsporidia detection.

Four teams per challenge. Sign up on the 'Talk' page of M1D1 -- you can do that now, follow the instructions on the Talk page.

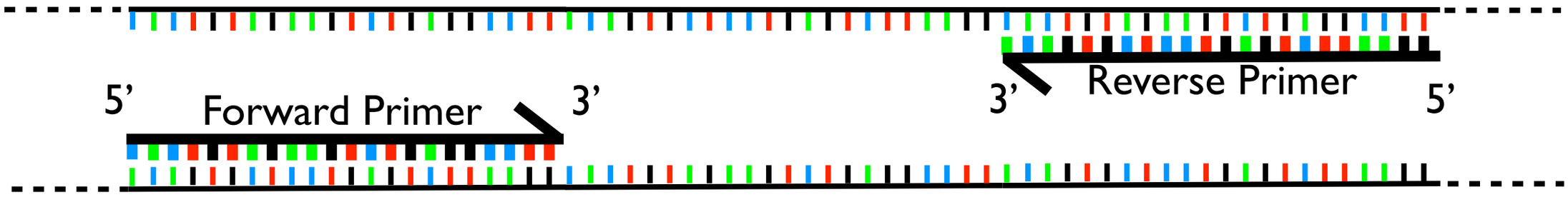
(2) Design primers to increase sensitivity or specificity: A PCR Cycle



(2) Design primers to increase sensitivity or specificity: A PCR Cycle

The first three cycles in a PCR reaction are a bit different from the rest. We will cover this during our 'in-person' pre-lab lecture...

(2) Design primers to increase sensitivity or specificity: Primer Design



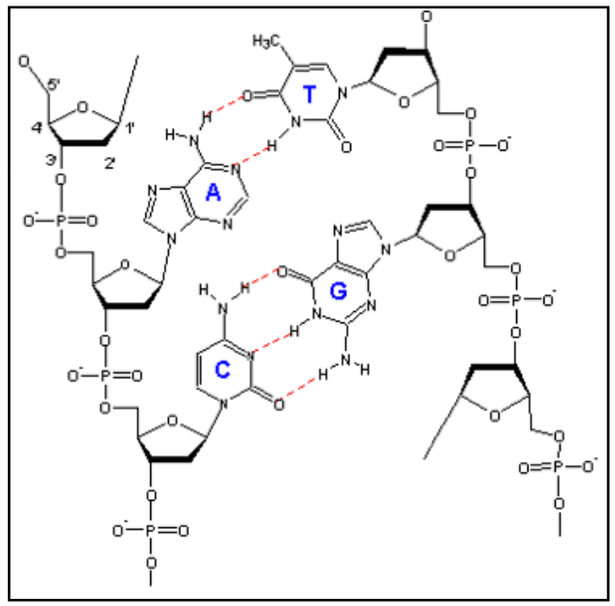
1. The <u>forward primer binds to the α-sense strand</u> (or the 'Template') and 'reads' in an intuitive direction from 5' to 3'. Look at the reverse primer and consider it's orientation.

2. Primer <u>length is important</u> to decrease the chances of off-target binding:
   - Consider that the human genome is ~ 3x10^9 bp. If we designed primers that were only 10 bp long, we might expect to find that 10bp sequence once in every 4^10 ≈ 10^6 bp -- a very risky gamble for off target binding.
   - The optimal primer length is > 16 bp for specificity. Think about why.

3. Primer <u>melting temperature should optimal</u>ly be kept between 55-60 C.
   - Tm,p is the temp ~50% of the primer is double vs. single stranded.
   - The melting temperature will be higher with increased G/C content.

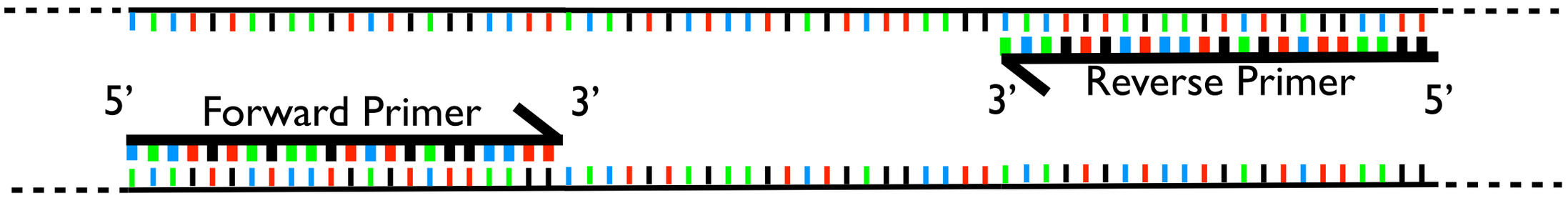
   Why? Look at the diagram of bp hydrogen bonding to the right -- which pair requires more energy to denature? *Also explains why optimal primer design calls for only 40-50% of the bp to be G/C.*

   The Tm,p is kept between 55-60C so that the annealing (hybridization) step is optimally efficient.



Thermodynamics of DNA Duplex, New Mexico State University

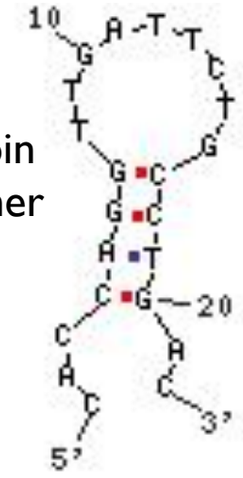
(2) Design primers to increase sensitivity or specificity: Primer Design



4. Avoid long repeats of one type of bp (ex. ATATATA) or one bp individually -- especially TTTT -- remember the polyA tail on pre-mRNA? This can lead to non-specific priming.

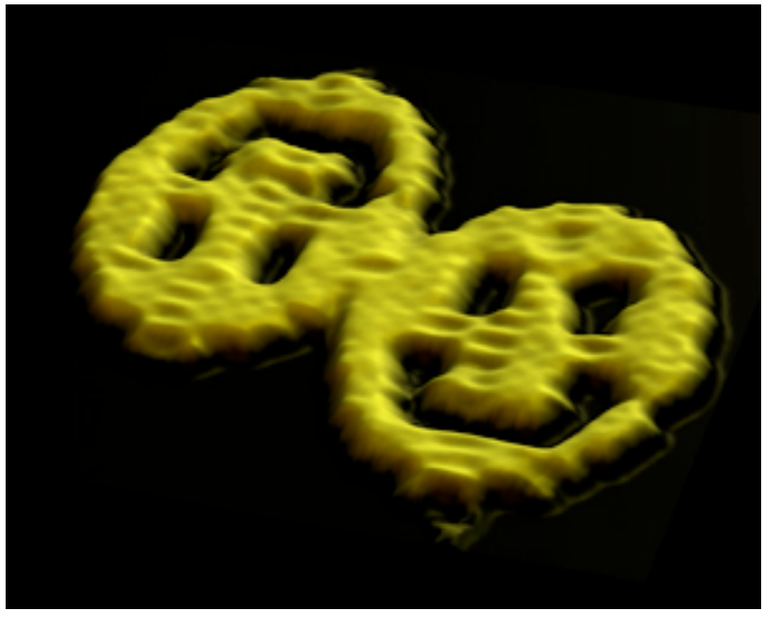
5. Consider secondary structure of your primer.
   - Does the primer have an internal sequence that can bind itself?

   If so, you can end up with a hairpin structure that will prefer (energetically speaking) to bind to itself and not your target sequence.



Making a hairpin with your primer is bad.

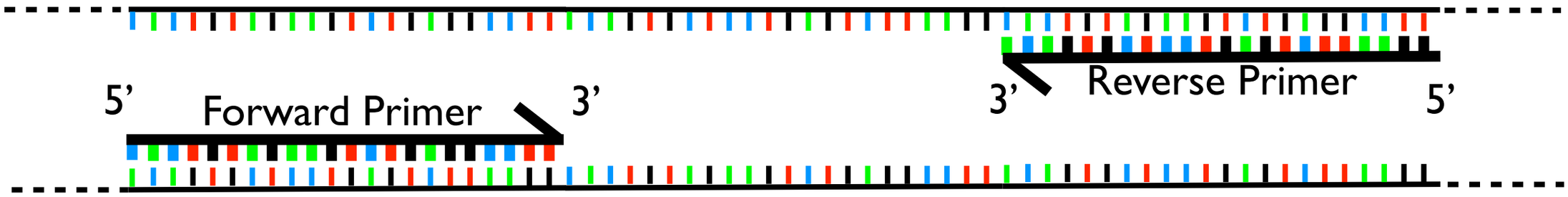
In fact, this behavior of DNA has been harvested to make higher order structures: DNA Origami is an active area of research.



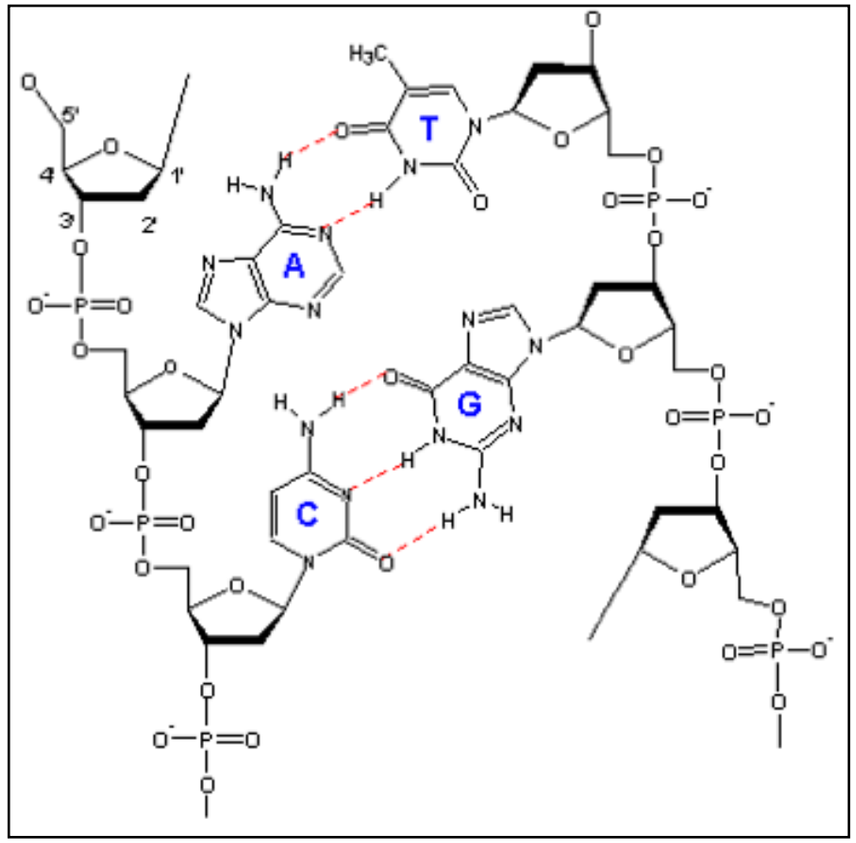
DNA Origami is cool.

Image from: http://www.dna.caltech.edu/~pwkr/

(2) Design primers to increase sensitivity or specificity: Primer Design



6. Tip the deck in your favor: Add a GC clamp to the 3' end if possible.
   - Consider again the image below. G/C binding is more stable and can help to increase efficiency of polymerase binding at the 3' end to promote extension.
   - But don't go overboard! > 5 G/C pairs won't help you.



Thermodynamics of DNA Duplex, New Mexico State University

I have also found this website useful: http://www.premierbiosoft.com/tech_notes/PCR_Primer_Design.html

We can talk more about these details on Monday in the lab.

Mod 1 written assignments

- Lab report re: bacterial communities (15%)
  - Traditional format (intro, methods, etc.)
  - WAC training begins next time
  - Written in pairs
  - 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

Thanks to Agi for this slide!

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

Thanks to Agi for this slide!

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*

Thanks to Agi for this slide!

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

Thanks to Agi for this slide!

Other reminders:

Make sure you are getting emails from 20.109talkwf@gmail.com. If you are not, check your spam folder!!

Any people who spoke with me about classes they need to miss, please send a follow up email so that we can discuss options.