- Announcements
- Pre-lab Lecture
  - Crash course on microsporidia
  - Goals+approach for M1 Exp 2
  - Intro to PCR and primer design
  - Today in Lab: M1D2

Announcements

- Brief discussion of orientation day quiz → all quiz dates
- Remember, *Assignments* + *Schedule* = our syllabus → ntbk dates
- Next time: lab quiz!
  - covers M1D1 and M1D2 lab *and* lecture content
  - purpose: continuity and accountability

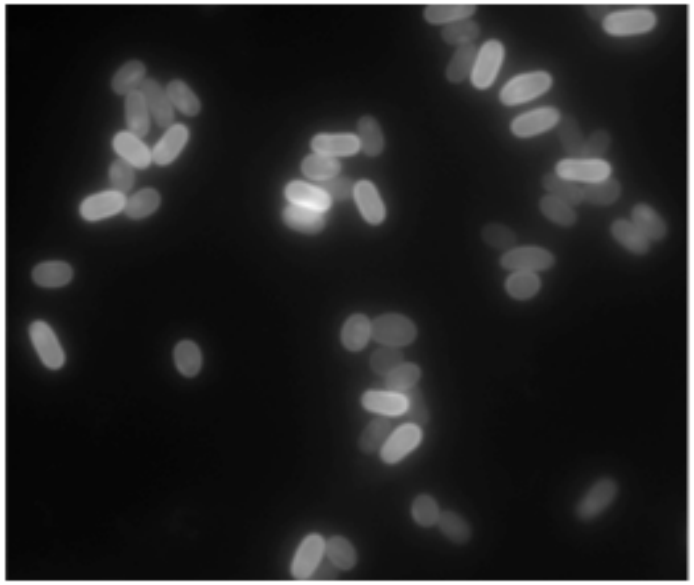
• absorption ≠ reflection

• $C_1V_1 = C_2V_2$ is your friend when answer is not obvious; ditto unit cancellations

one foaming:) = have, (= want

Microsporidia: fascinating bugs

- Puzzle to categorize
  - no mitochondria
  - protist? ancient?
  - nope!
  - resolved by sequencing ~2001
- Highly evolved fungus
  - toward simplicity
  - few genes *and* few non-coding regions for a eukaryote
  - does have mitosomes



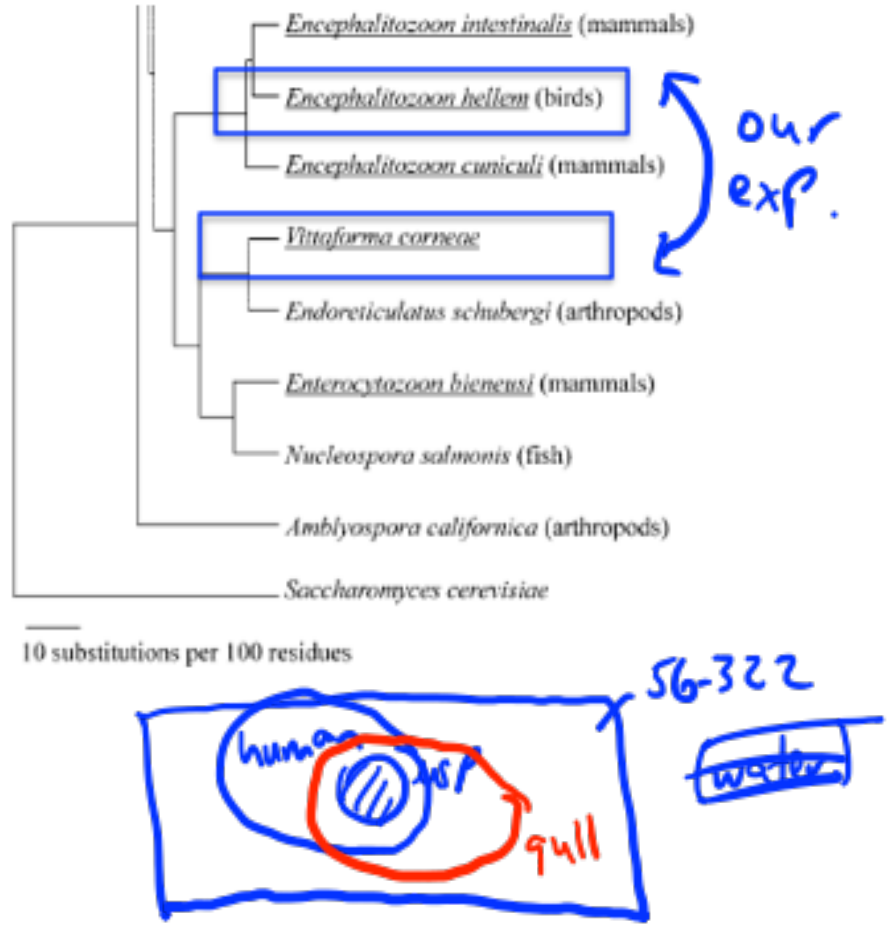
Microsporidia and their hosts

- Spores survive w/out host
- Need host to propagate

  obligate, intracellular
- Opportunistic infections
  - immunocompromised (e.g., HIV and cancer) people ↳ chemo. age
  - travelers

? is water a reservoir?

See wiki and Mathis et al. for full tree.



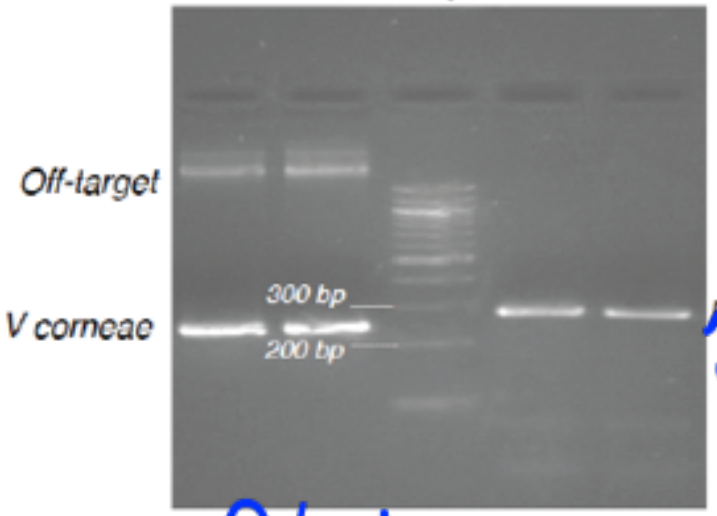
Microsporidia and our Mod 1 goal: diagnostic for microsporidiosis

- Tough to isolate! We'll work with purified DNA.
- Sensitivity means improve detection limit (low [DNA])
- Specificity means distinguish species < E hellem v corneae (exclusion or different size)
- When is each useful? Sens. → first pass, distinguish from other infection (cf flu primers)
  Spec. → Know therapeutic regimens

Mod 1 Exp2 end-point

You design —> We order —> You prep primers (D5) —>
We run PCR —> You analyze via electrophoresis (D7)

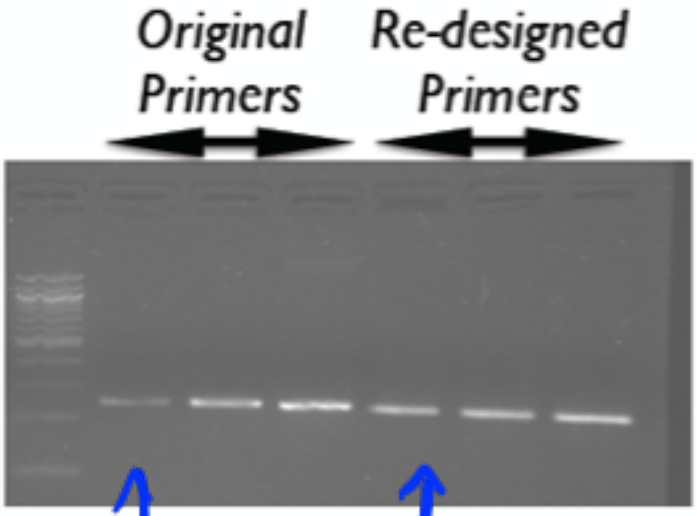
current primer set



@ high conc.

spec.

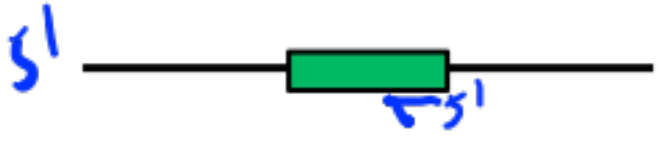
get further apart?



lowest conc.

Designing PCR primers: topology

Template



coding / sense strand

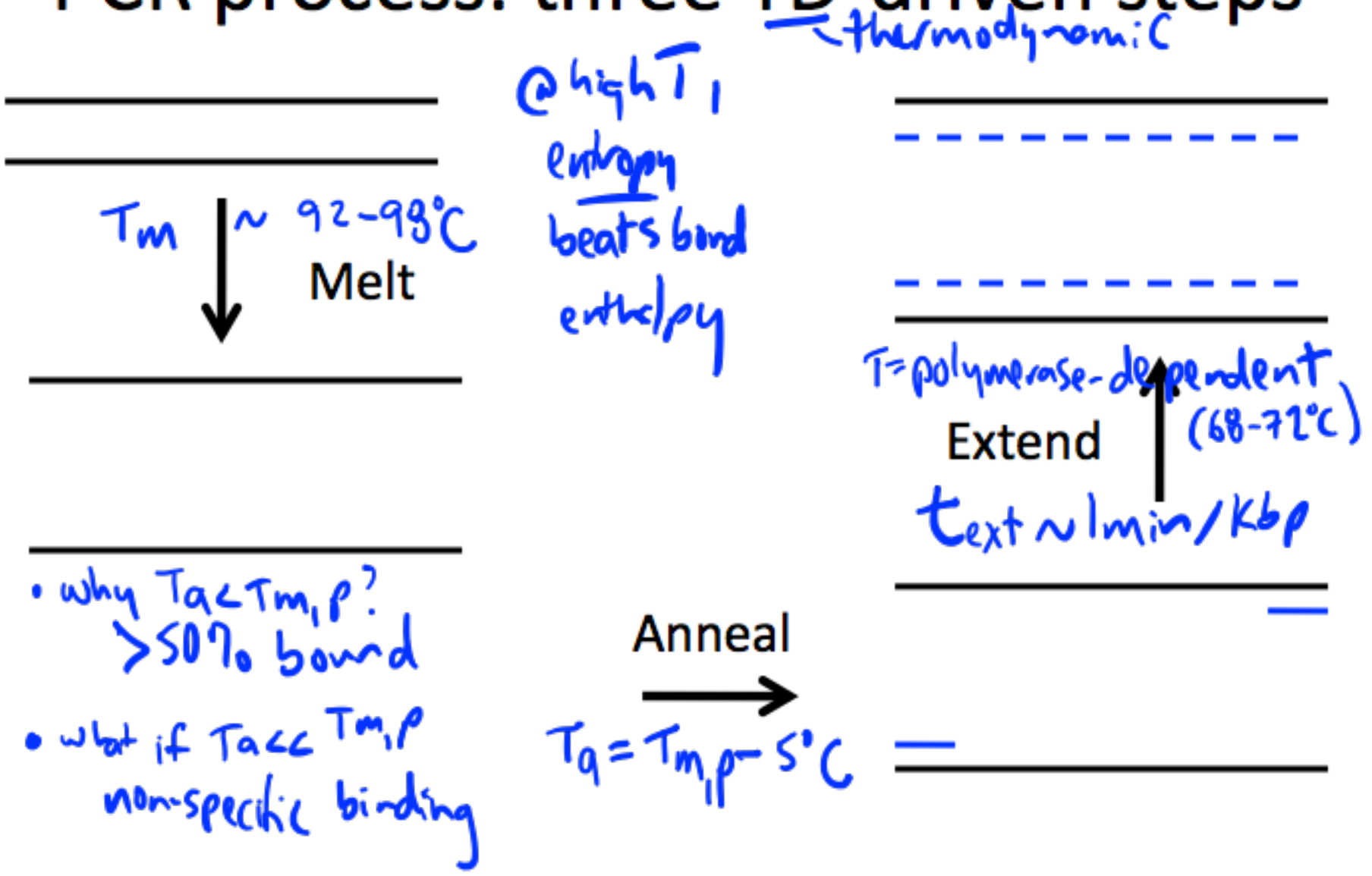
template / α-sense "

Primers

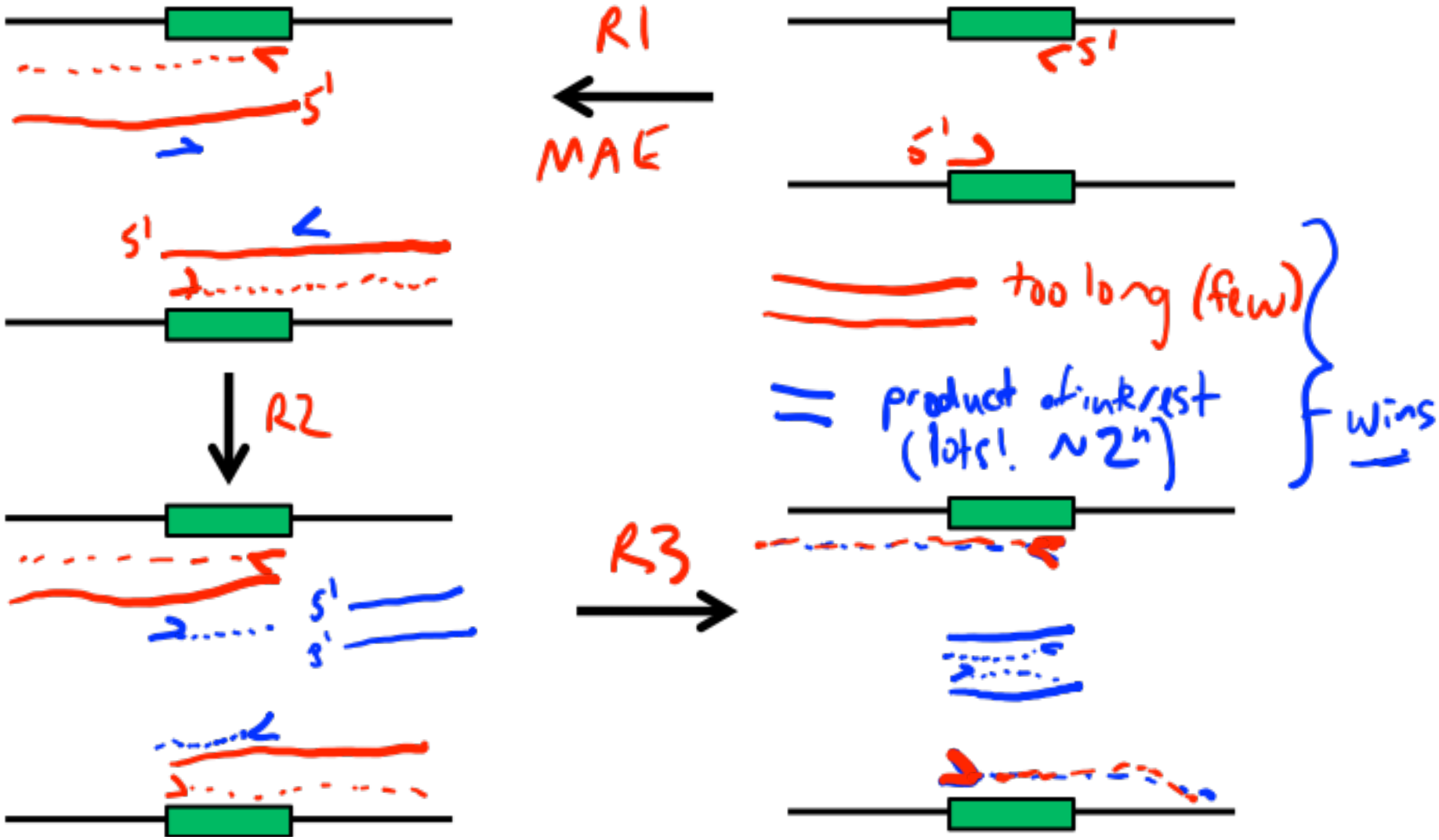
5' → Forward { binds α-sense / reads as sense

← 5' Reverse – binds sense

PCR process: three TD-driven steps



PCR process: three rounds



Designing PCR primers: properties

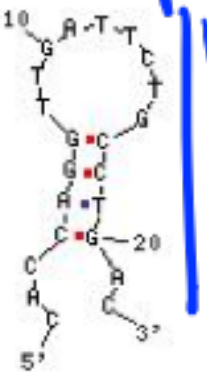
- Length: why is 17 bp the magic number?
  human genome ~ $3 \cdot 10^9$ bp $4^{17} \approx 2 \cdot 10^{10}$ bp
- Melting + annealing temperature ⇒ efficiency T_a ~ 50-60°C
- G/C content: why is 40-60% best?
- Avoid long runs of same/similar base → mis priming
- Secondary structure considerations → poor priming
- Binding considerations (energy; self, other)

G/C clamp @ 3' end → must anneal to extend

primer dimers

F ——→

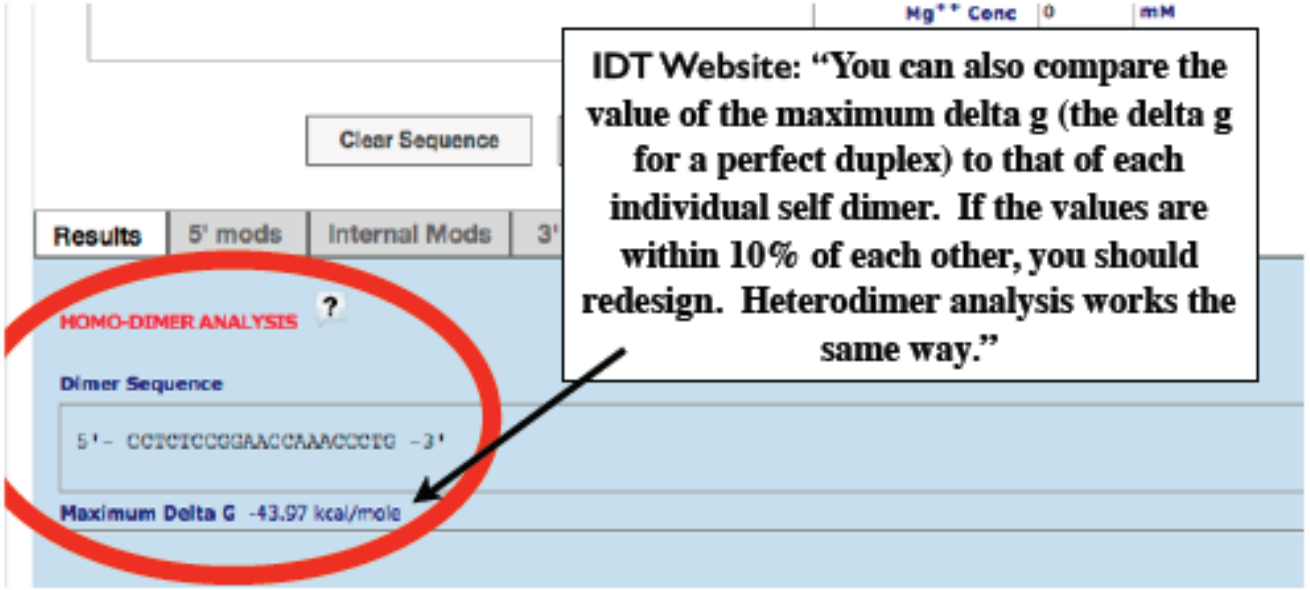
←—— R



hairpin

A few more words about design

- See Shannon's "snow day notes" if you want more detail → slides 16-19
- Also from Shannon --



Today in Lab: M1D2

- Explore existing diagnostic primers for μsporidia
- Design new primers
  - sensitivity *or* specificity challenge (4-5 teams per)
  - sign up on M1D2 "Talk" page
- Notebooks start today!
  - primer table will be used in your M1 memo
- For next time
  - finish reading paper for M1D3 discussion
  - prepare slide pertaining to your assigned figure