

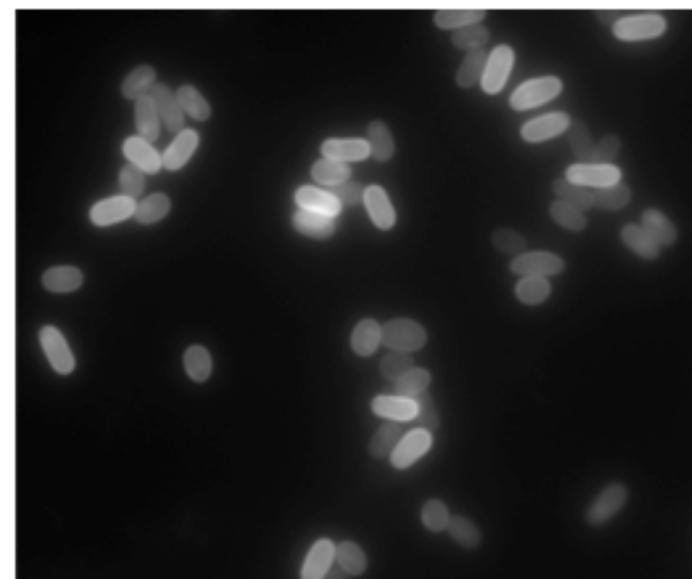
- **Announcements**
- Pre-lab Lecture
 - ❖ Crash course on microsporidia
 - ❖ Goals+approach for M1 Exp 2
 - ❖ Intro to PCR and primer design
 - ❖ Today in Lab: M1D2

Announcements

- Brief discussion of orientation day quiz
→ ntbk dates → all quiz dates
- Remember, *Assignments + Schedule* = our syllabus
- Next time: lab quiz!
 - covers M1D1 and M1D2 lab *and* lecture content
 - purpose: continuity and accountability
 - absorption ≠ reflection
 - $C_1 V_1 = C_2 V_2$ is your friend when answer is not obvious; ditto unit cancellations
one coming: { = 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

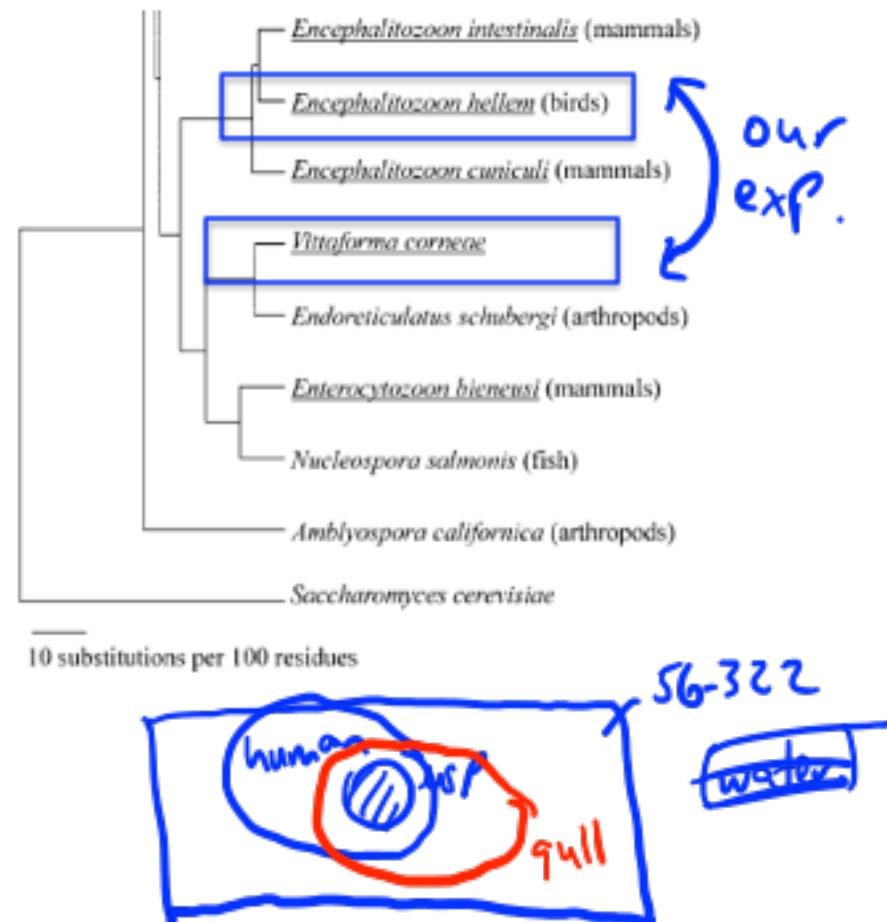


Microsporidia and their hosts

- Spores survive w/out host
- Need host to propagate
obligate, intracellular
- Opportunistic infections
 - immunocompromised (e.g., HIV and cancer) people
 - travelers
 ↳ chemo.
 age

? is water a reservoir?

See wiki and Mathis et al. for full tree.

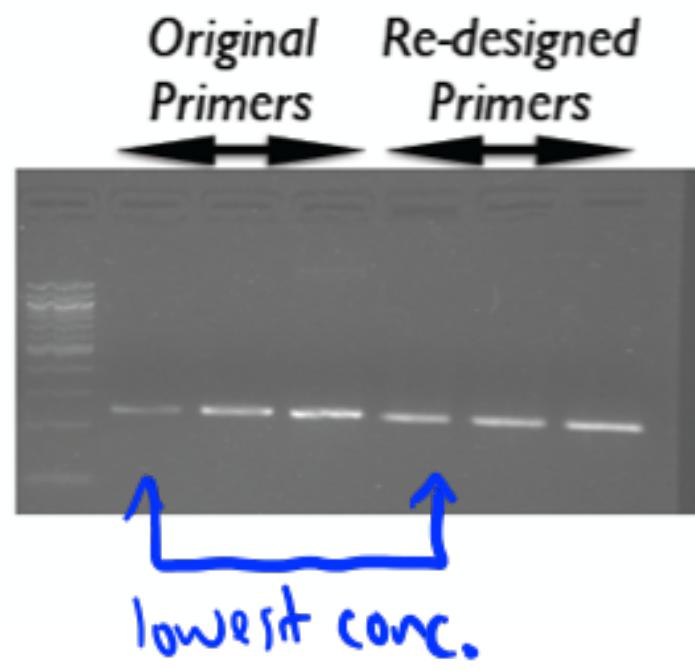
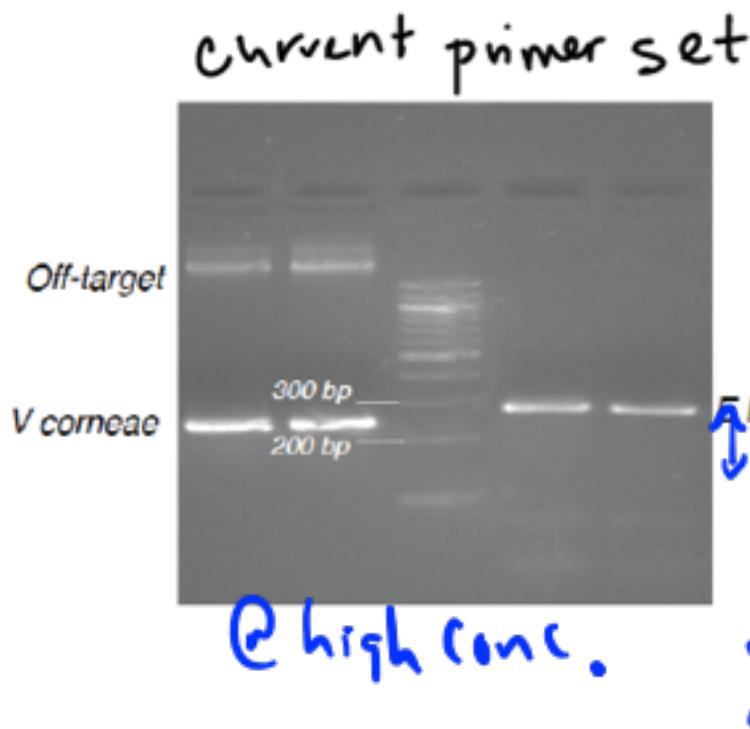


Microsporidia and our Mod 1 goal: diagnostic for microsporidiosis

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

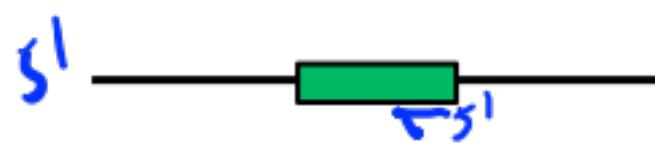
Mod 1^{EXPL} end-point

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



Designing PCR primers: topology

Template



coding/sense strand



template/d-sense "

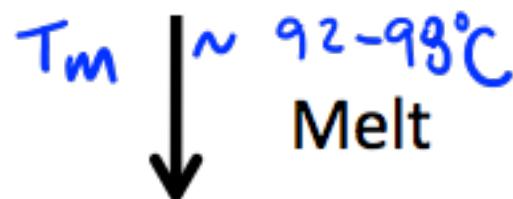
Primers

5' → Forward { binds d-sense
reads as sense

← 5' Reverse - binds sense

PCR process: three TD-driven steps

thermodynamic



@ high T_1
entropy
beats bond
enthalpy

$T =$ polymerase-dependent
Extend $(68-72^\circ\text{C})$

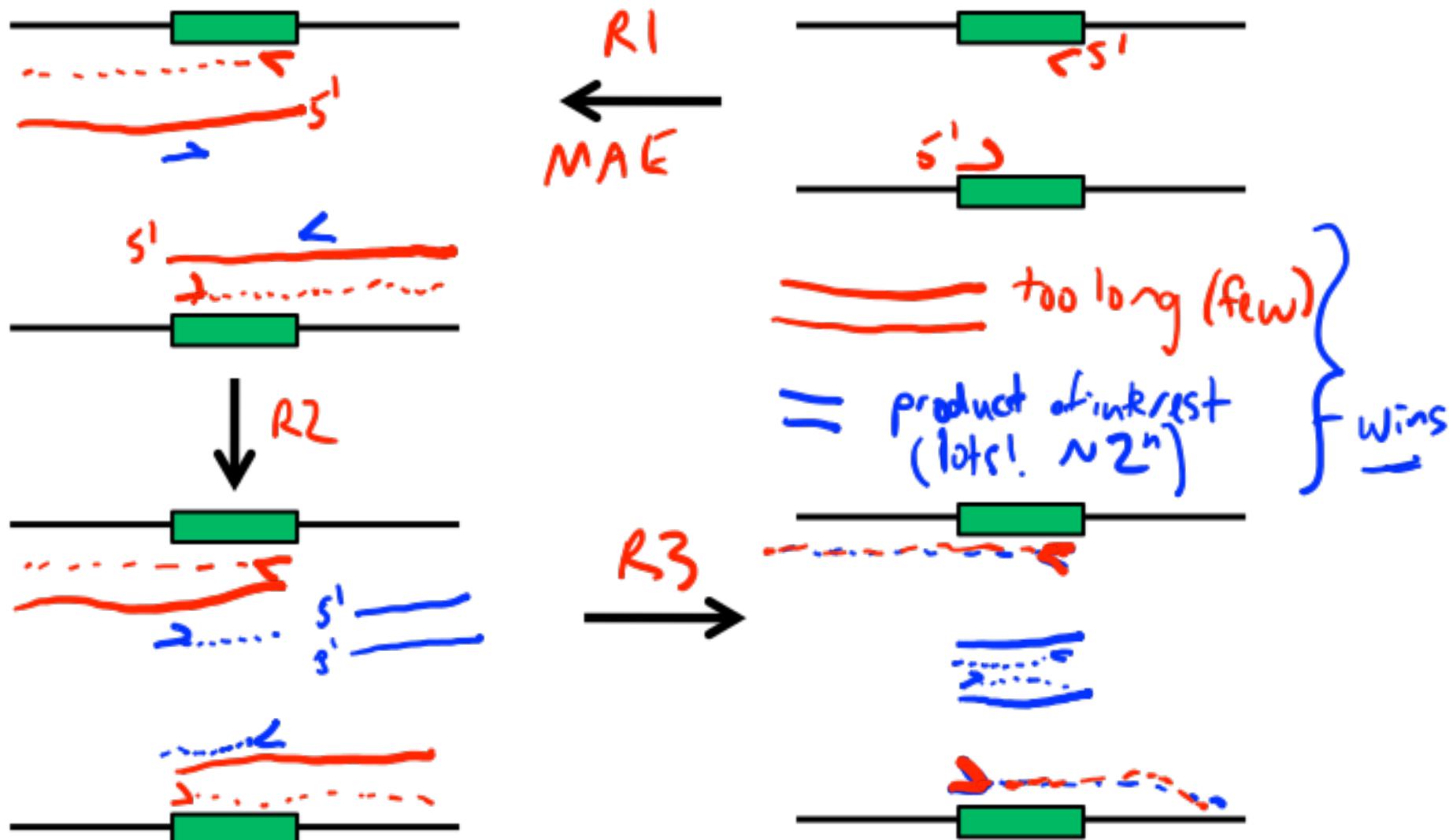
$t_{ext} \sim 1\text{ min}/\text{kbp}$

Anneal

$\xrightarrow{T_a = T_m, p - 5^\circ\text{C}}$

- why $T_a < T_m, p$?
 $> 50\%$ bound
- what if $T_a \gg T_m, p$?
non-specific binding

PCR process: three rounds



Designing PCR primers: properties

- Length: why is 17 bp the magic number?

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

- Melting + annealing temperature \rightarrow efficiency $T_a \sim 50-60^\circ\text{C}$
- 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)

G/C Clamp
at 3' end - must
anneal to extend

primer dimers




A few more words about design

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

The screenshot shows a web-based sequence analysis tool. At the top, there are tabs for 'Results', '5' mods', 'Internal Mods', and '3'. Below these, a red circle highlights the 'HOMO-DIMER ANALYSIS' section. This section includes a 'Dimer Sequence' input field containing the sequence 5' - CCTCTCCGGAAACCAACCCCG - 3' and a text area below it stating 'Maximum Delta G -43.97 kcal/mole'. To the right of this section is a callout box containing the following text:

IDT Website: "You can also compare the value of the maximum delta g (the delta g for a perfect duplex) to that of each individual self dimer. If the values are within 10% of each other, you should redesign. Heterodimer analysis works the same way."

Today in Lab: M1D2

- Explore existing diagnostic primers for μ sporidia
- 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