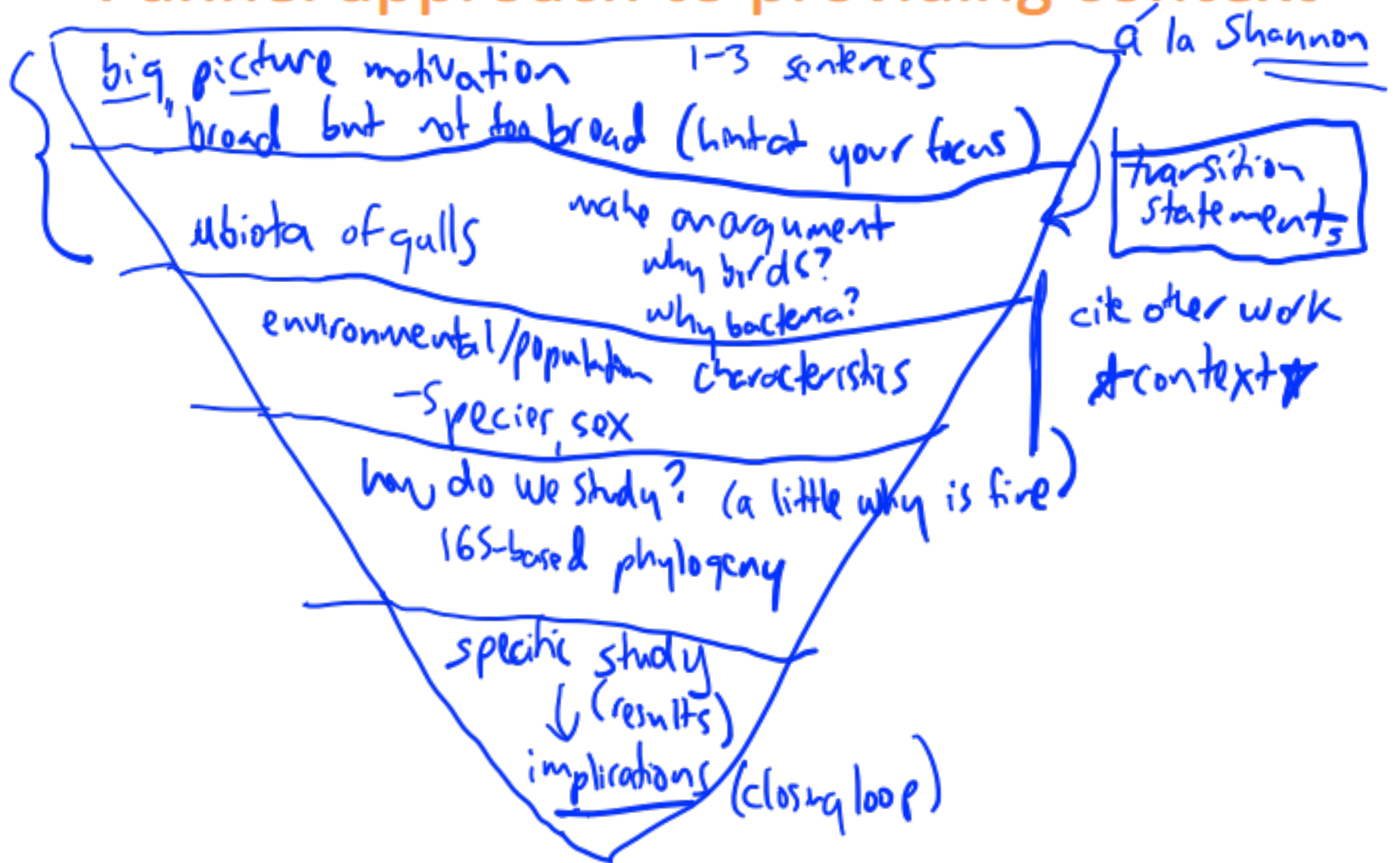


- **Announcements**
- **Pre-lab Lecture**
 - ❖ **Hey remember microsporidia**
 - ❖ **Sequencing expectations**
 - ❖ **Phylogenetics overview**
 - ❖ **Today in Lab: M1D7**

Announcements

- Discussion of previous FNTs
 - D4 schematic: great start! highlight unique elements and big picture
 - D5 figure: good work! review pre-lab ^{2 minutes} notes as needed.
 - D5 data text: good start. even in bullet point form, shoot for *motivation-complete interpretation-conclusion* format.
 - D5 intro text: *next slide*
- Lab notebook due today –
- Half of Thursday's lecture: Leslie on writing effective abstracts
- Journal club next time: meet in **16-336 by 1:05 sharp**
 - first a quiz, then a few minutes to recover, and then 10 (!) talks

Funnel approach to providing context



Preparing for Mod 1 written assignments

- Abstract & data summary re: bird microbiota(15%)
 - Written in pairs
 - Can be revised for up to 1.33 letter grade higher
 - Assignment description was finalized 2/18
- Primer design memo (5%)
 - Written alone
 - Not subject to revision
- Resources
 - Me: extra OH on *Sun 1:30-3 Mon 1-2:30 pm*
 - Jon: happy to chat by appointment
 - WAC, MIT Writing Center, BE Writing Fellows

Microsporidia primer analysis plan

Lane	Sample (21 μ L)	Lane	Sample (21 μ L)
1	Group 1, sample 1	6	V1-PMP2, sample 2
2	Group 1, sample 2	7	V1-PMP2, sample 3
3	Group 1, sample 3	8	Group 2, sample 1
4	DNA ladder 🔗 (load 10 μ L)	9	Group 2, sample 2
5	V1-PMP2, sample 1	10	Group 2, sample 2

Sample preparation: mix by pipetting, take 20 μ L, add 4 μ L loading dye, then load 21 μ L onto gel **with your P20**.

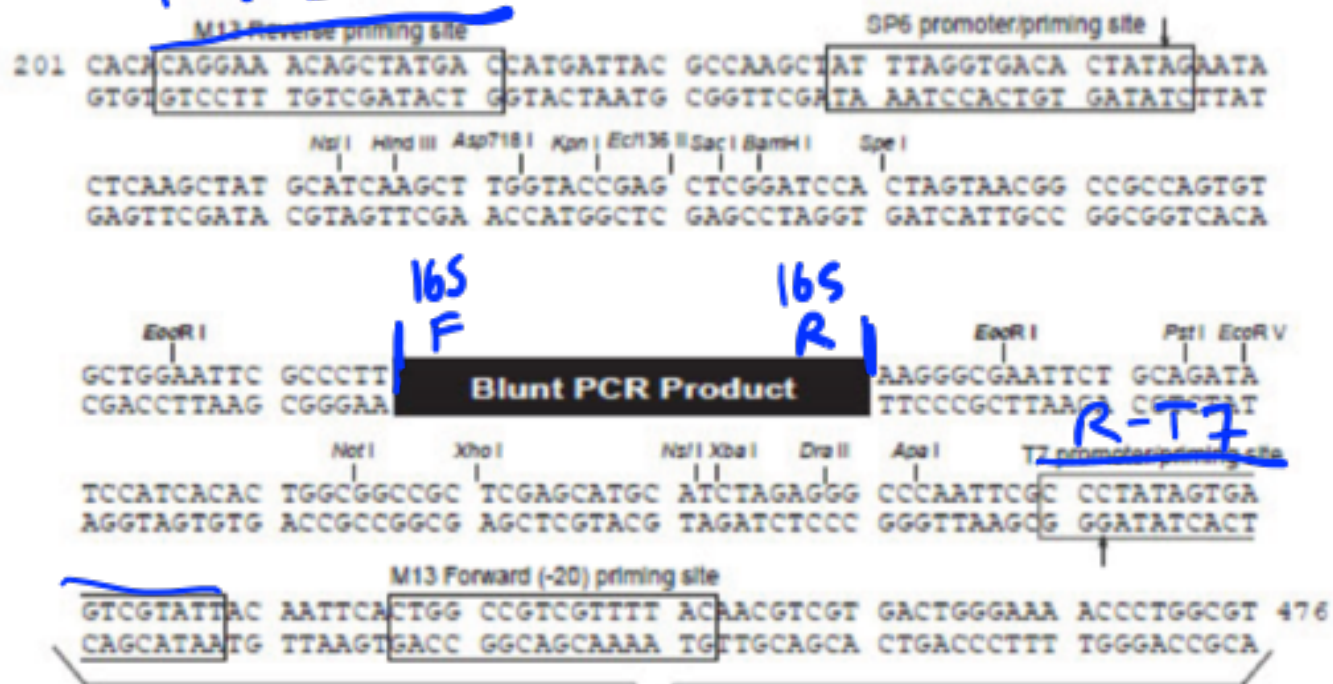
Gel number	Reference samples	Group 1	Group 2
T/R 1	Specificity (VC, EH, mixture)	Orange	Yellow
T/R 2	Specificity (VC, EH, mixture)	Blue	W/F Green
T/R 3	Sensitivity (EH: lo, mid, hi)	Red	Green
T/R 4	Sensitivity (EH: lo, mid, hi)	Pink	Blue
W/F 1	Specificity (VC, EH, mixture)	Blue	Purple
W/F 2	Specificity (VC, EH, mixture)	Silver	White
W/F 3	Sensitivity (EH: lo, mid, hi)	Red	Orange
W/F 4	Sensitivity (EH: lo, mid, hi)	Yellow	Pink

today

Note: Due to a miscalculation of how much polymerase we had left, only the specificity gels will be run today. The teaching faculty will run and post the sensitivity gels by Friday mid-day.

Sequencing primer topology

F-M13

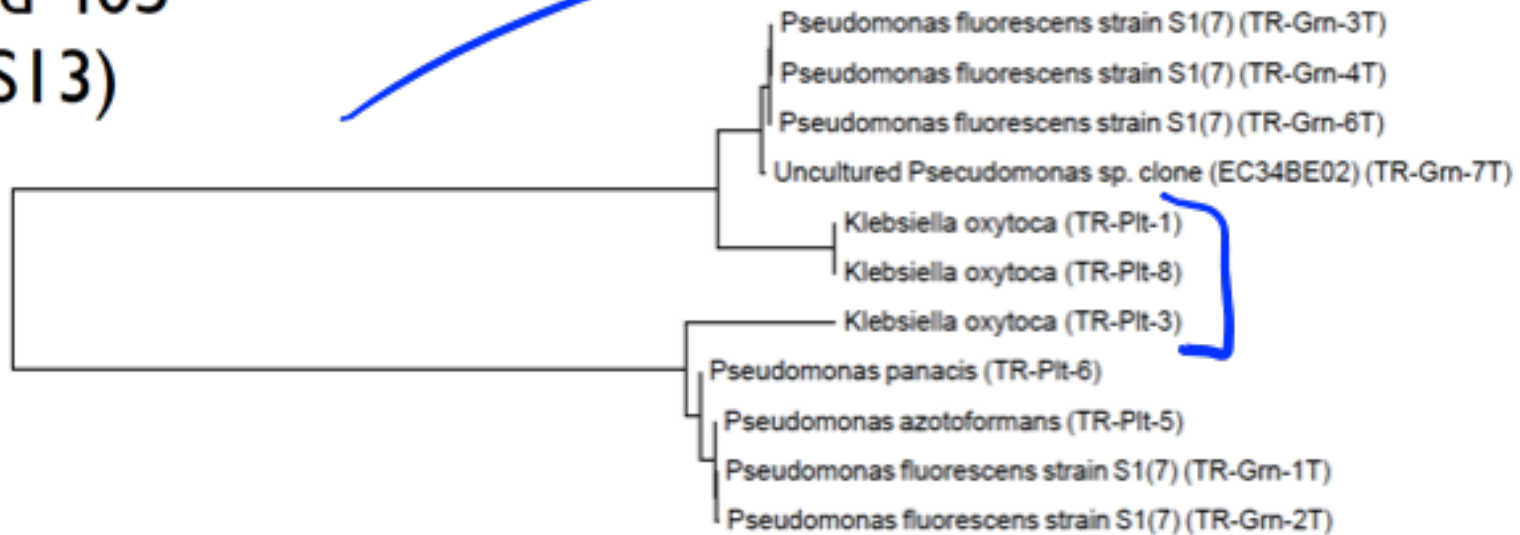


Phylogenetics analysis of microbiota

Bird 405
(S13)

inferences

terminal nodes = data (SEQ)



0.1

↑ 10 substitutions per every 100 bp

Today in Lab (M1D7)

- Load microsporidia gels (specificity teams)
- Bird microbiota analysis: alone
 - trim and orient sequences
 - identify closest species
 - try one with your partner first!
 - start with clones having **two** successful reactions
- Bird microbiota analysis: with ### partners
 - align sequences for a given gull sample
 - create a phylogenetic tree
- Lots of file posting along the way!