

# MID7: Phylogenetic Analysis!

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3/5/14

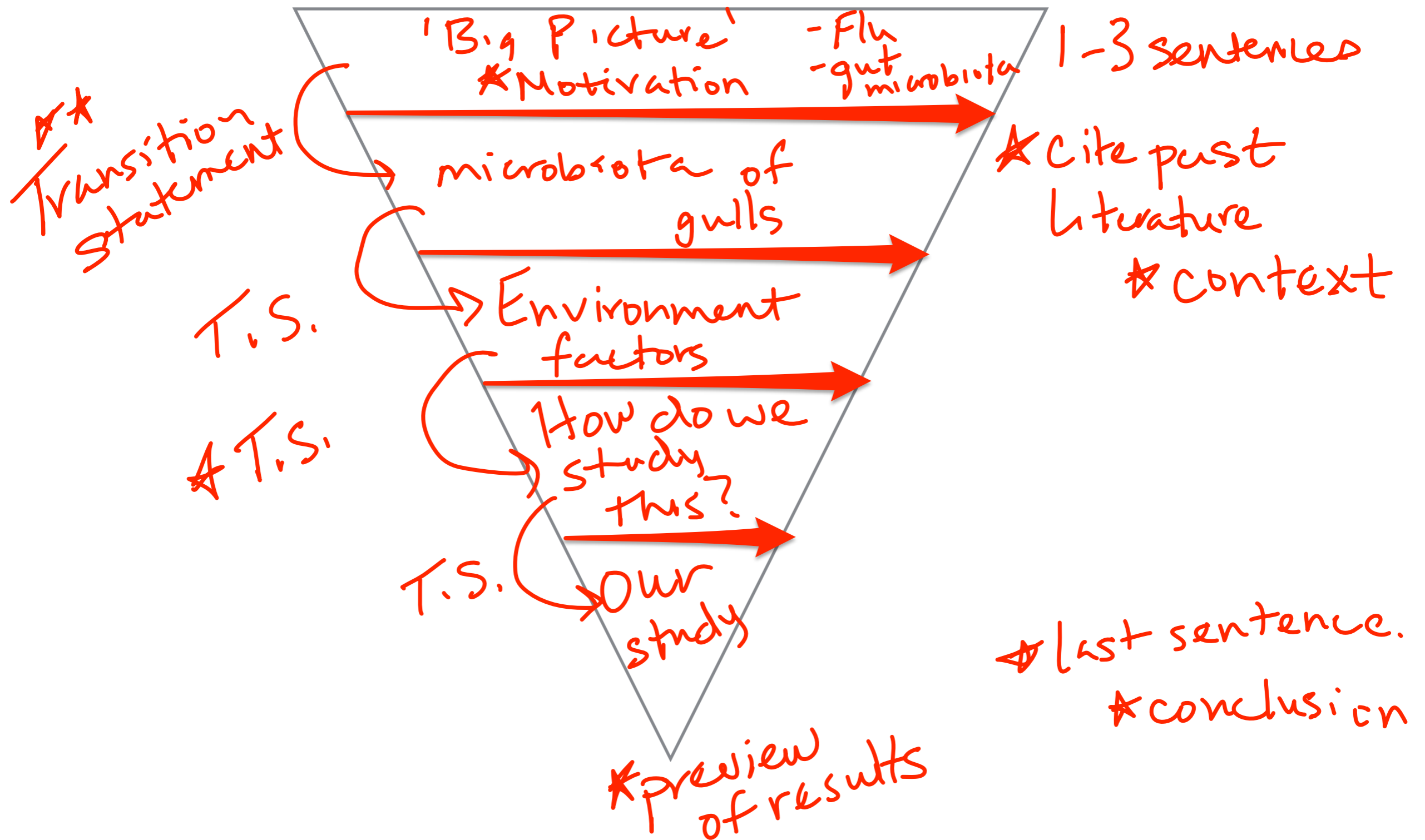
And primer design challenge too!\*\*

# Announcements

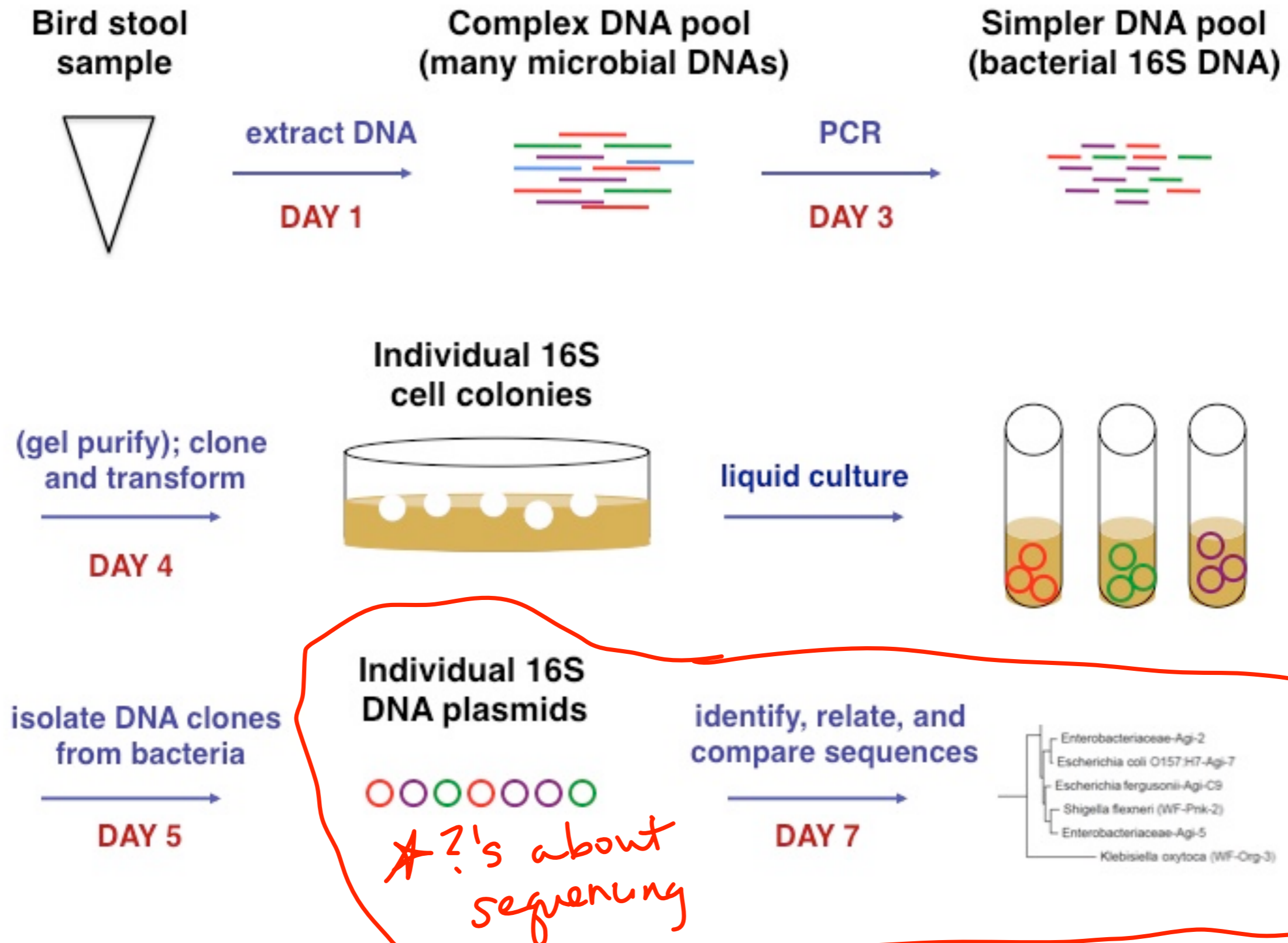
- Lab notebooks due today: MID
  - Journal club next time: Meet in 16-336 at 1:15pm (speakers 1:00pm)
  - Also — lab treat next time at 1:15pm.
  - Discussion of MID5 FNT:
    - figure:** good work! review pre-lab notes as needed.  
*watch the interpretation in your figure caption — specific titles*
    - figure text:** good start. make sure to provide a bit of bkgrd:  
*motivation-complete interpretation-conclusion*  
*don't forget your bird characteristics!*
- If you have questions about comments — please ask!

# Announcements

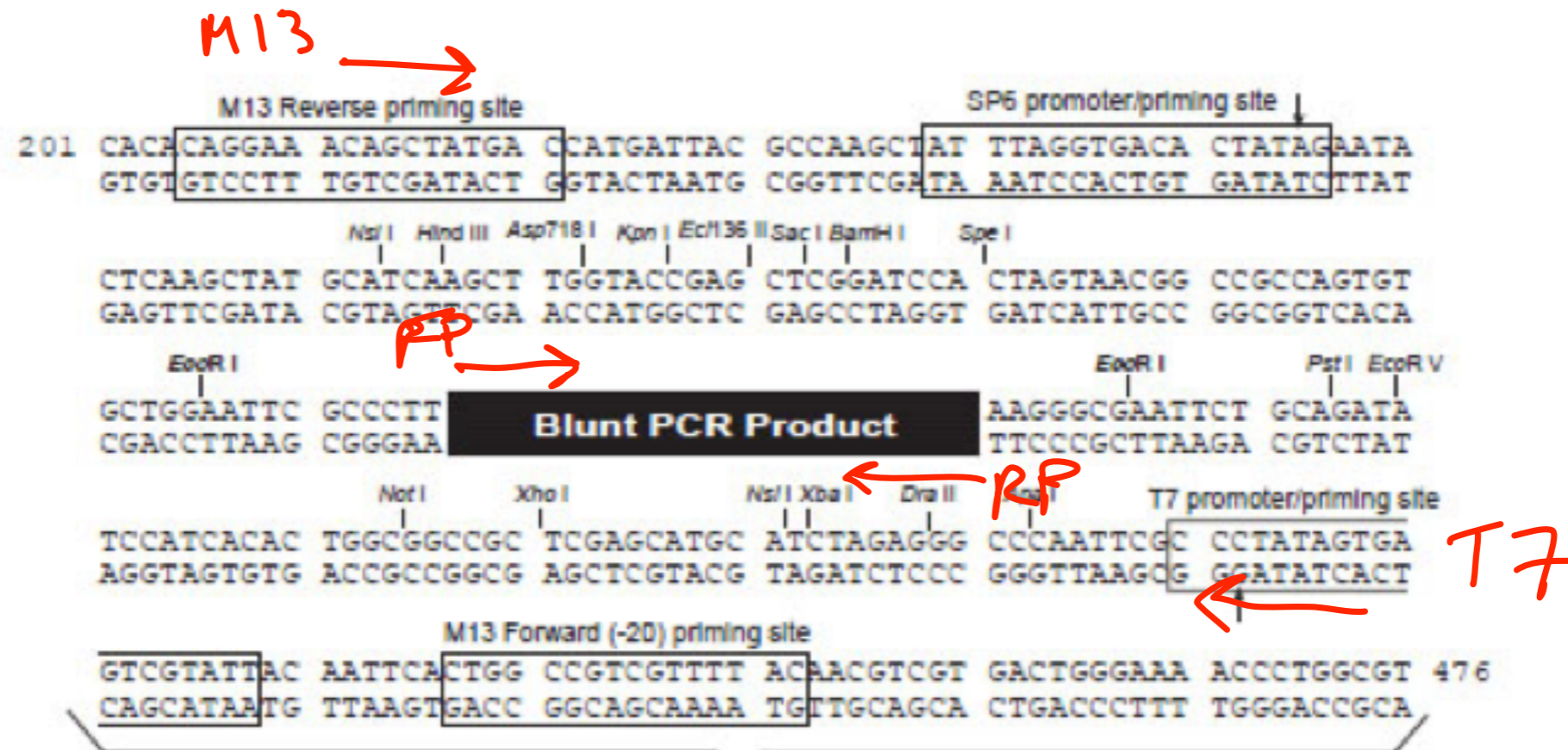
- Discussion of MID5 FNT: Background and Motivation



# Bird Microbial Communities -- Experimental Overview



# Insert Orientation

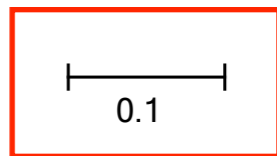
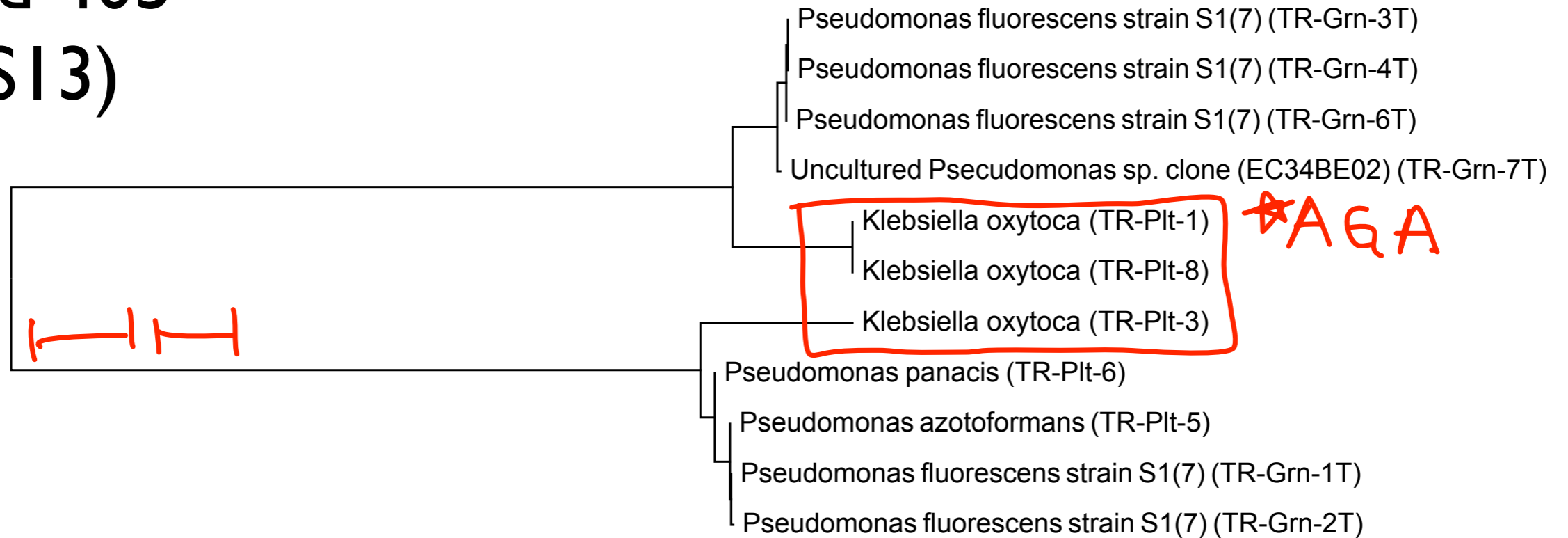


## Today in lab (microbiota experiment):

- Check out your sequencing reactions at [genewiz.com](http://genewiz.com)
- Start with clones that had **TWO successful sequencing reactions** and follow the protocol on the wiki to process the data
- Go through the first sequence together — work out the kinks — then split up the work
- You'll then be a pro — investigate the other reactions — is there enough information? **\*\*Post sequence template files\*\***
- Easiest: coordinate your sequence entry into MEGA with your bird partners.
- Watch for **PINK highlighting**:
  - All sequences *must* start with AGA (5' to 3' orientation)
  - Naming conventions

# Bird Microbial Communities -- Prelim. Analysis

Bird 405  
(S13)



16 bp substitution for every 100 bp  
- 10% rate of "mutation"

# And now...Microsporidia Primer Evaluation

Lane	Sample (X $\mu$ L)	Lane	Sample (Y $\mu$ 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 <a href="#">🔗</a> (load 10 $\mu$ L)	9	Group 2, sample 2
5	V1-PMP2, sample 1	10	Group 2, sample 2

Sample preparation: mix by pipetting, take 20  $\mu$ L, add 4  $\mu$ L loading dye, then **load 21  $\mu$ 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

**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.**



## Today in lab:

1. Load microsporidia gels — specificity teams only!  
*take careful look at PCR sample table and gel lanes!*
2. Bird microbiota analysis:  
*trim sequences*  
*identify closest species*
3. Bird microbiota analysis: with bird partner(s)  
*align sequences for a given gull sample*  
*create a phylogenetic tree — \*\*don't forget WF data\*\**
4. Lots of file posting along the way! Today it is important to be a good collaborator.

**\*\*WAC presentation about Abstract at lecture on Thursday\*\***