M1D7: AIV Detection + Analysis I

3/3/15

Shannon's office hours:

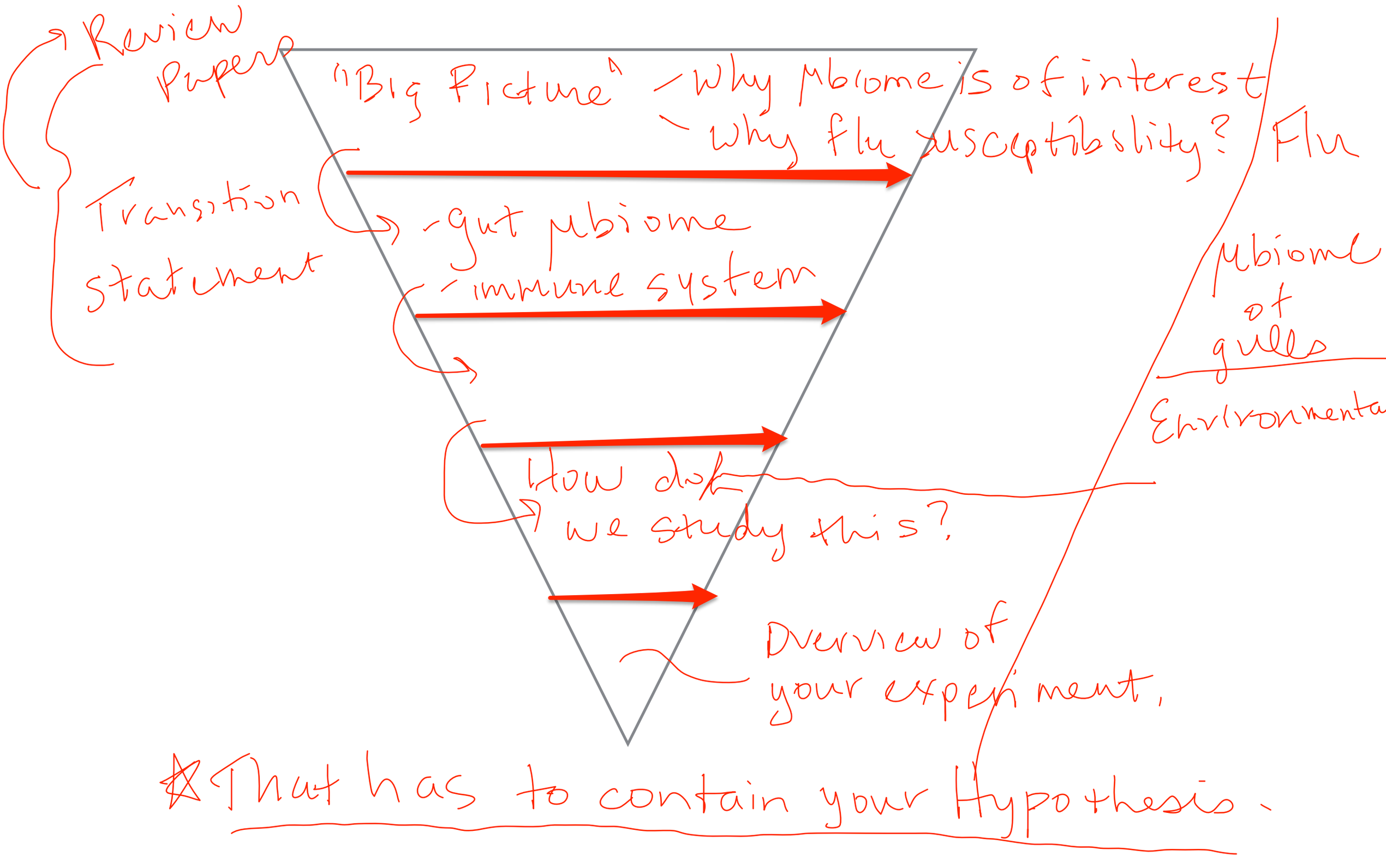
Friday 3-4pm

Sunday 3-5pm in 528 Simmons Hall

Monday 3-4pm

Announcements

- Discussion of homework: Background and Motivation



Announcements

- Journal club next Tuesday: Meet in **16-336 at 1:30pm (speakers 1:15pm)**
- Also — lab treat next time

- What happened since we were in lab last Tuesday:

1) Sequencing reactions were sent to Genewiz
2) Out of 160 sequencing reactions — 120 "successful" reactions
3) Out of 120 successful reactions — only 2 clones contained 16S rRNA gene

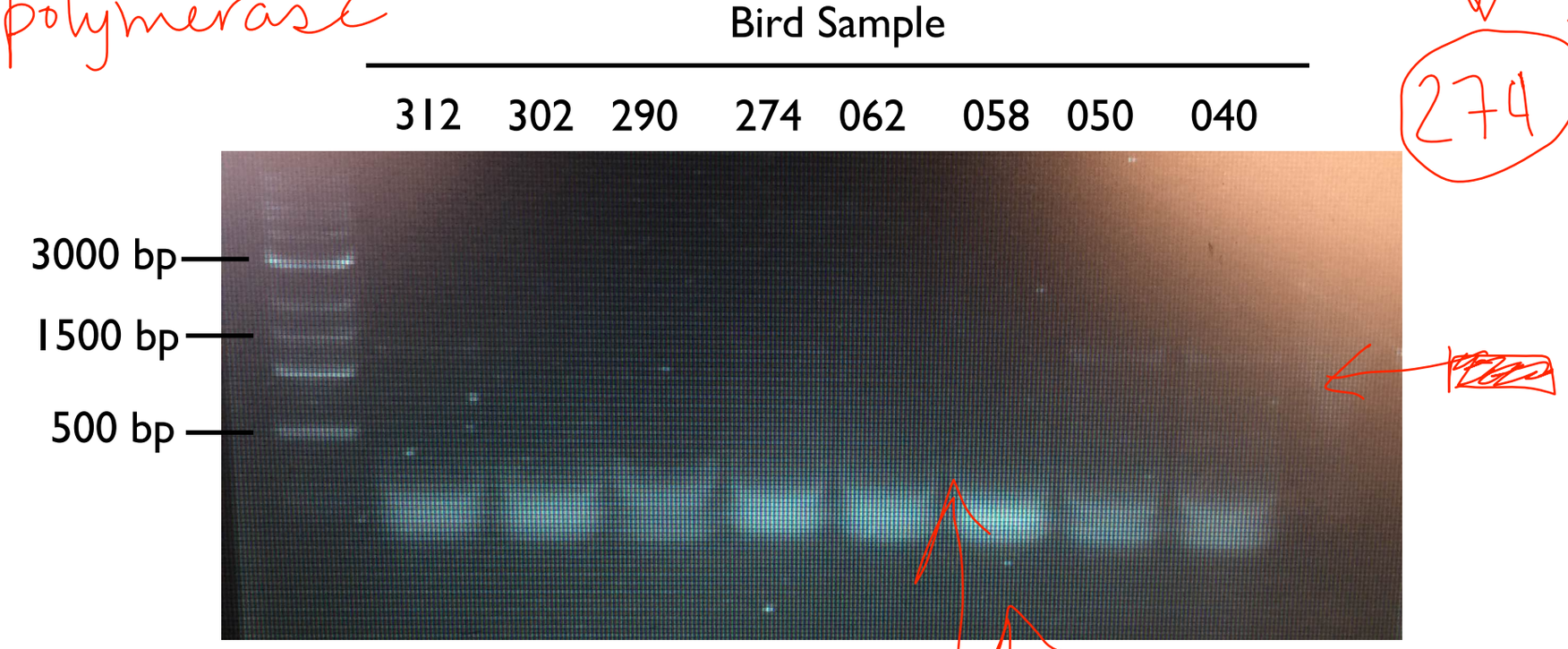
- What did we do? What steps might have gone wrong?

A) PCR
- No specific primers
- primer dimers →
- Template problem

rxn conditions
dNTPs → polymerase

} replaced ALL PCR reagents

Spring 2014



B) Started from swatch w/ frozen bird

- What did we do? What steps might have gone wrong?

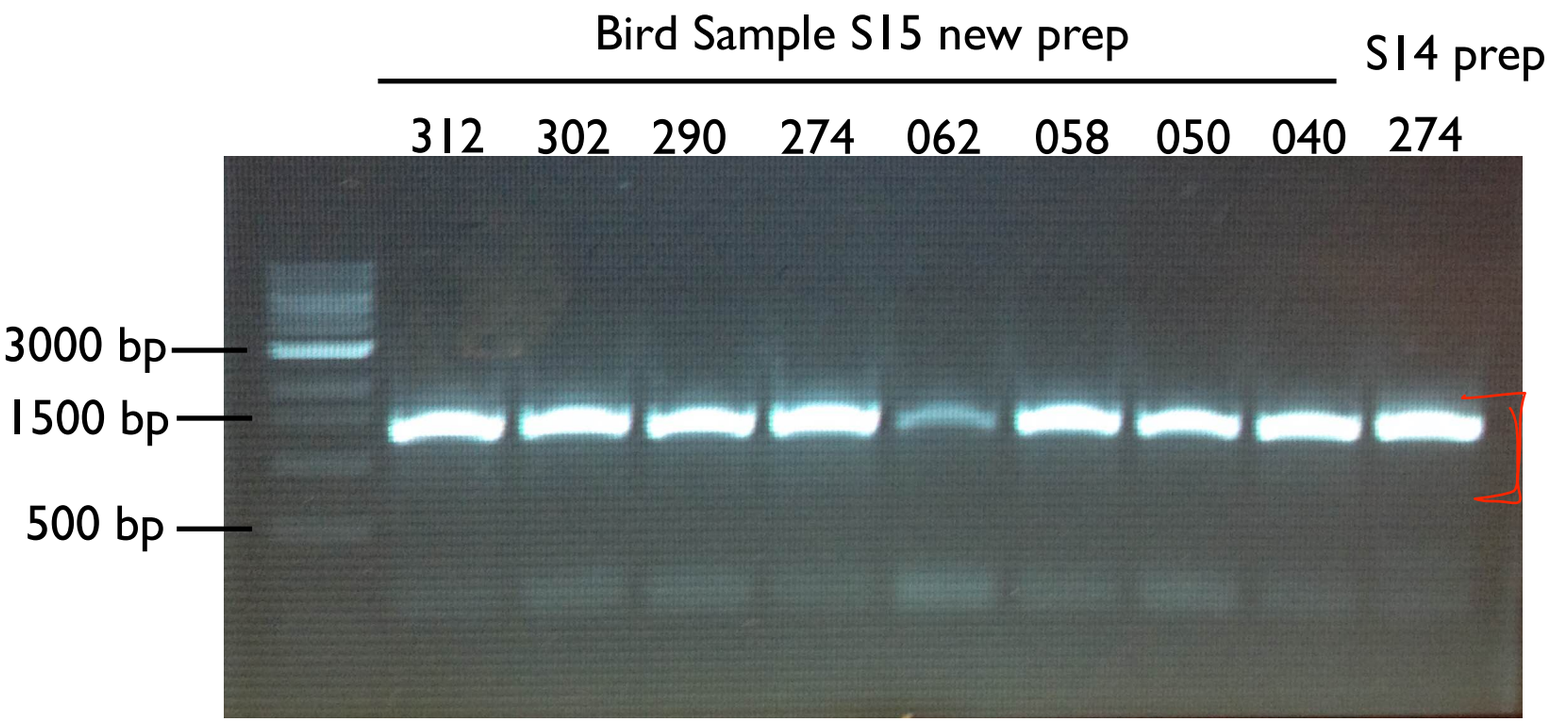
C) Replaced the Qiagen Kit

Short products

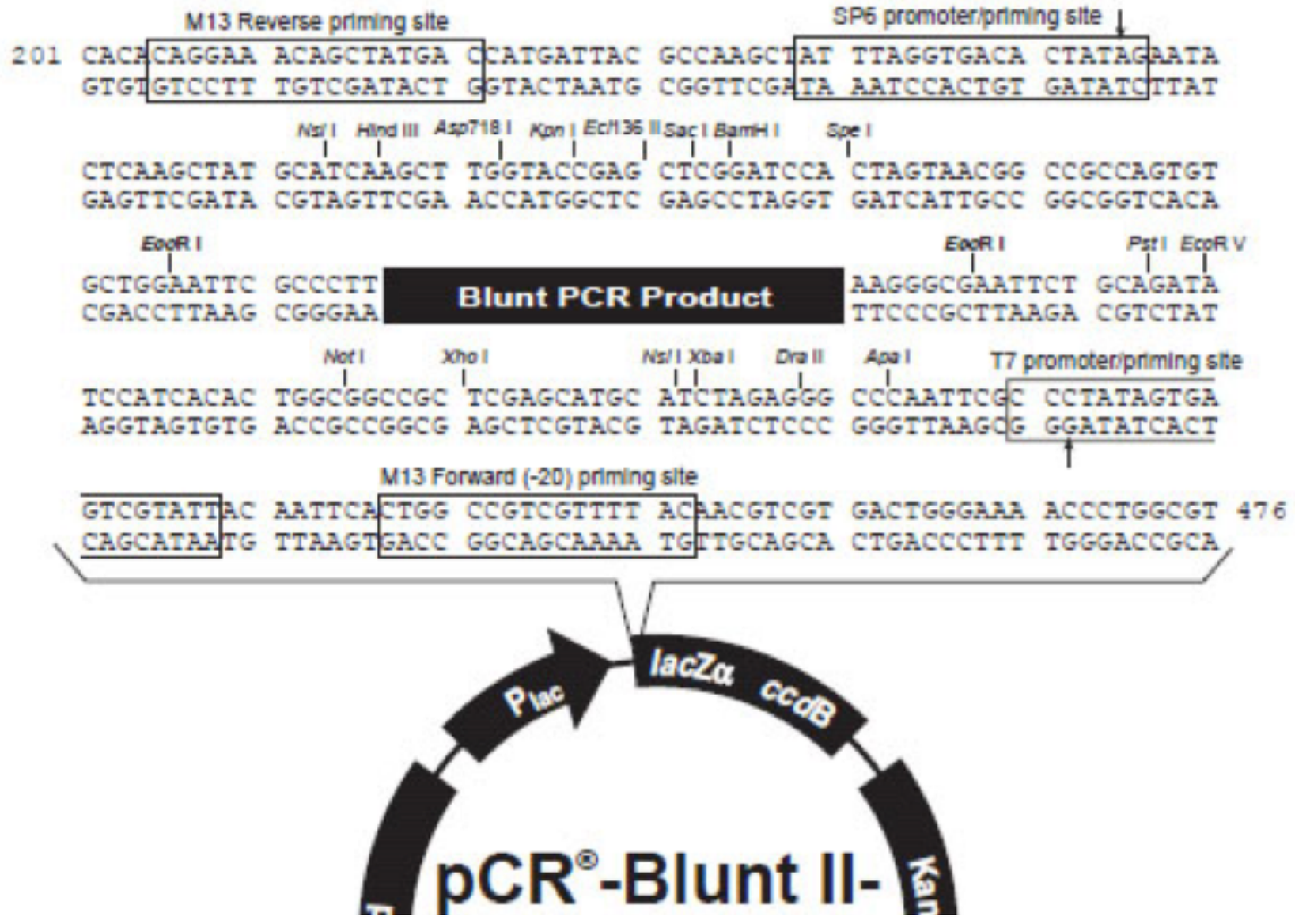
20-120 bp

Fp

Rp

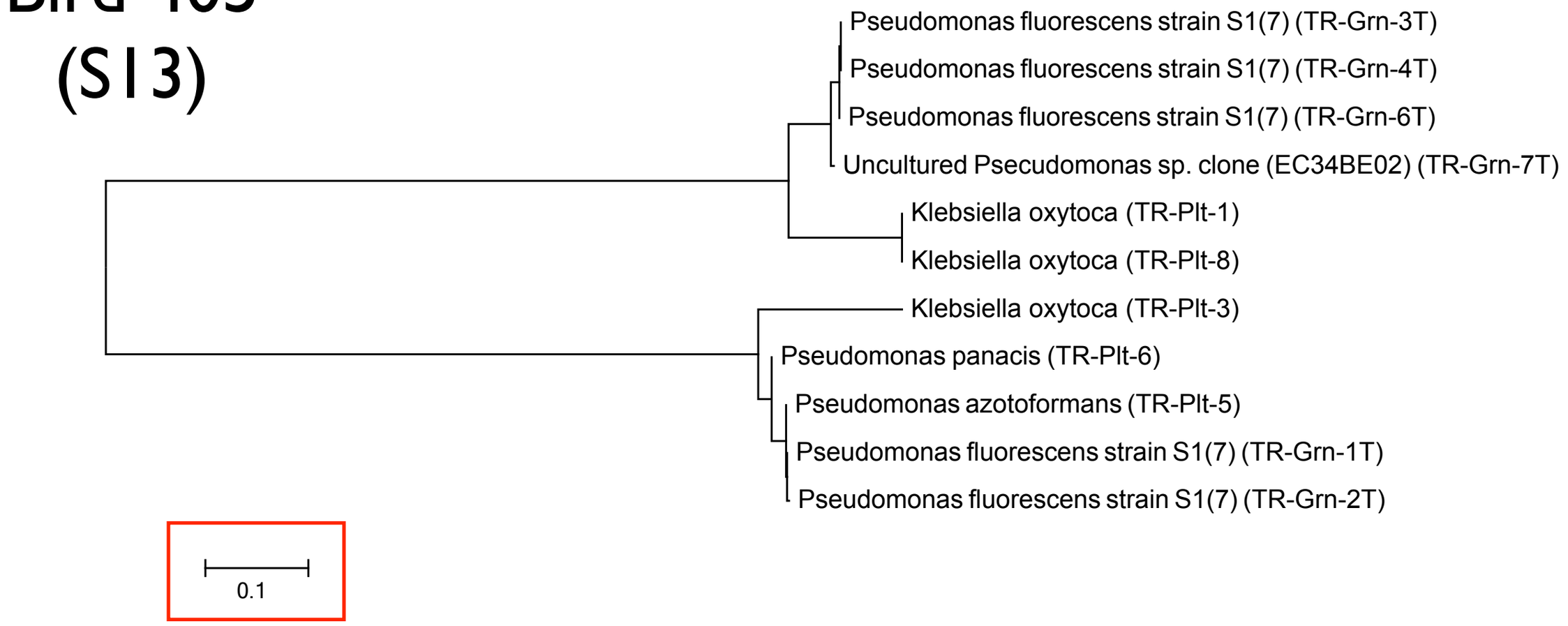


Insert Orientation

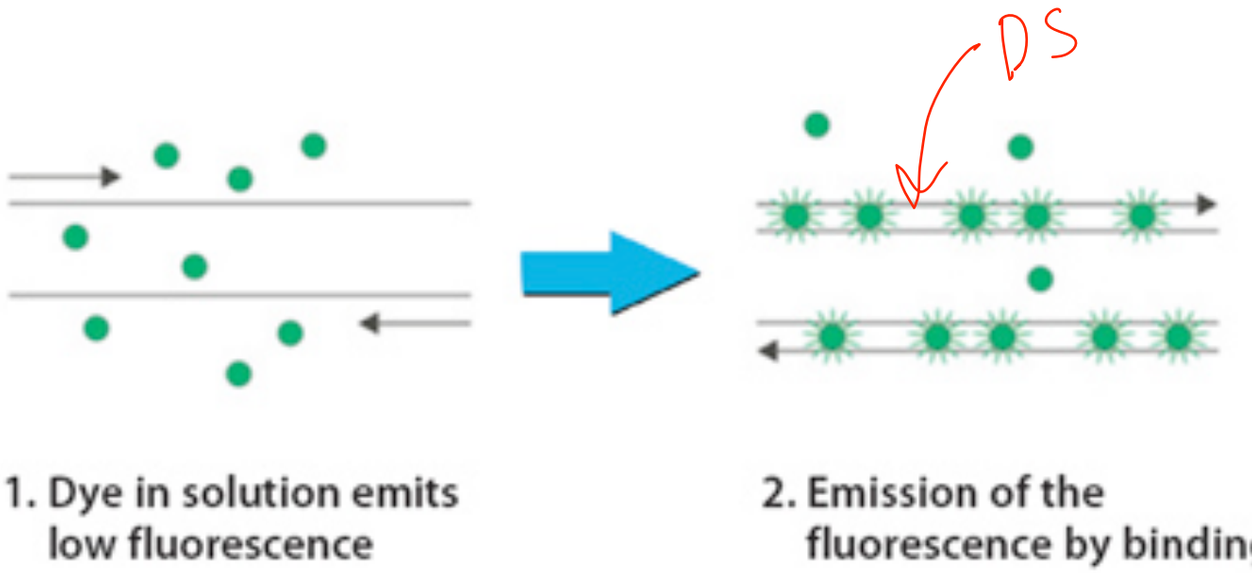


Bird Microbial Communities -- Prelim. Analysis

Bird 405 (S13)



Today in lab (AIV experiment):



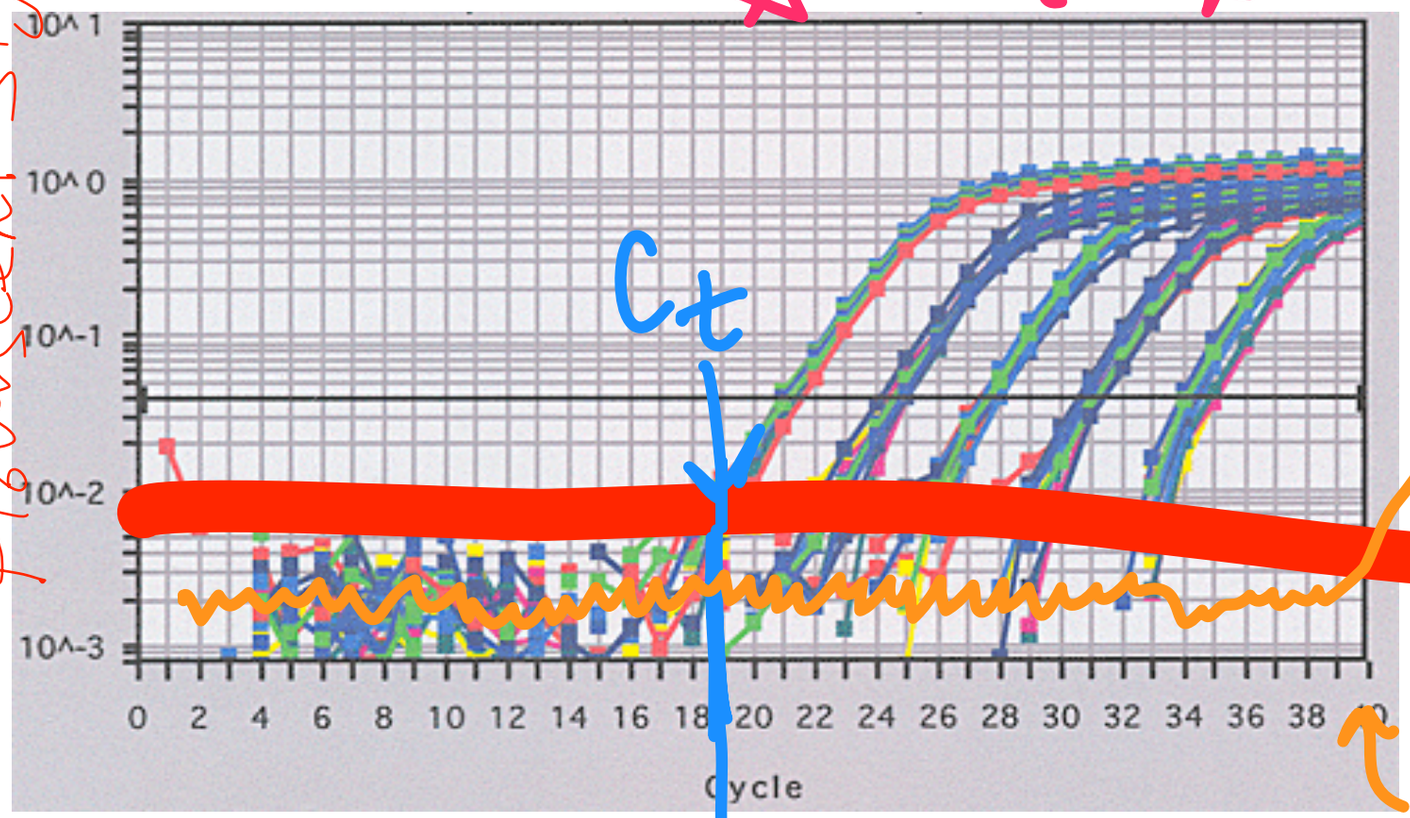
(1) A dye (Sybr Green) is used to detect double stranded DNA product (the product of your PCR reaction!).

(2) There isn't enough Sybr Green in solution to detect, but when the dye is localized within double stranded DNA the signal is brighter — and can be detected.

(3) Therefore, the amount of fluorescent signal is proportional to the amount of PCR product that is formed.

(4) Fluorescence is 'read' once per PCR cycle to quantify the amount of product formed

more product = ↑[target] = ↓Ct



Today in lab (practice analysis):

- Learn to navigate the Genewiz website
- Practice combining sequence and searching BLAST for OTU
- Align example sequences from birds 312, 290, and 274 using MEGA
- Create input files for Fast UniFrac analysis
- Discover how to quantitatively compare gull micro biome

Today in lab (AIV experiment):

- Set-up qPCR reactions
- Bring plate to qPCR machine — 3pm
- Get data — 4:30pm