M1D9-J11

M1 Abstract + Data Summary @end of next week

M1D7: AIV Detection + Analysis 1

3/4/15

Office Hours this week:

Thursday (3/5), Monday (3/9) in 16-429C

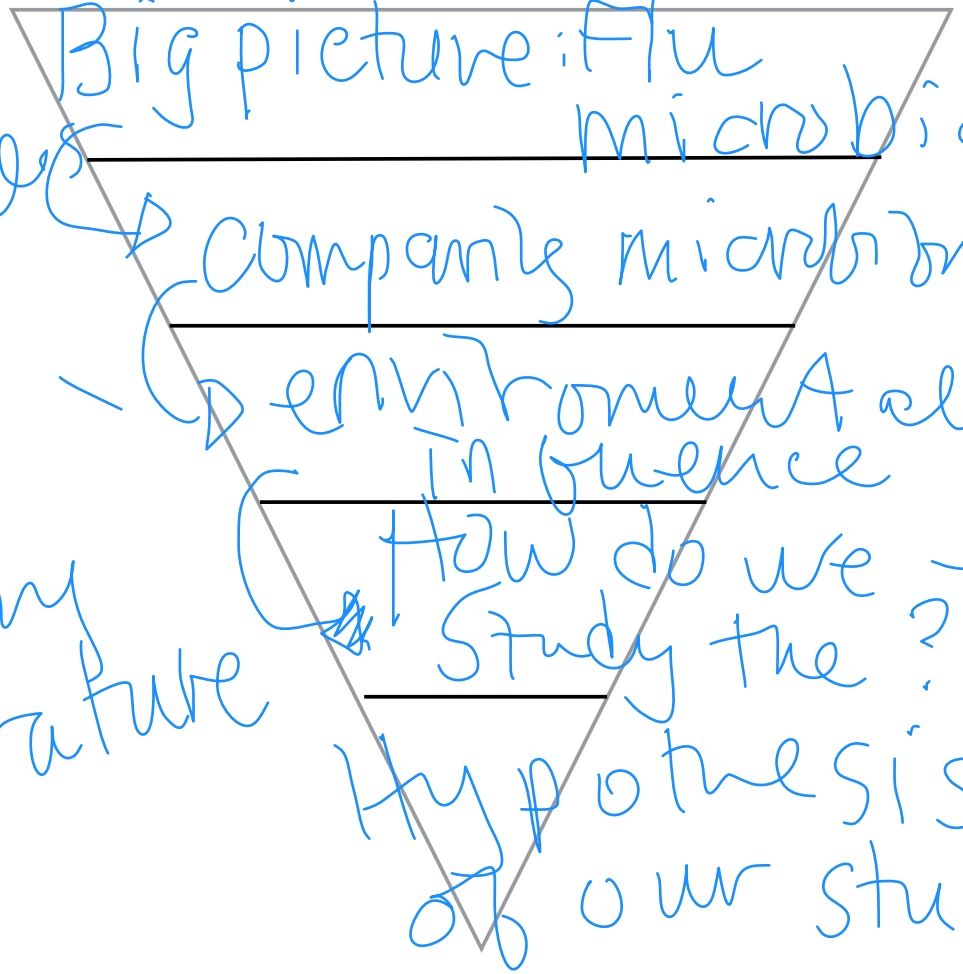
Leslie, 1-2pm

Noreen, 2-4 pm

Email us for OH by appt.

Announcements

- Discussion of homework: Background and Motivation



2 pages

cite references

gov website review articles

1-3

primary literature

analysis 16S

Announcements

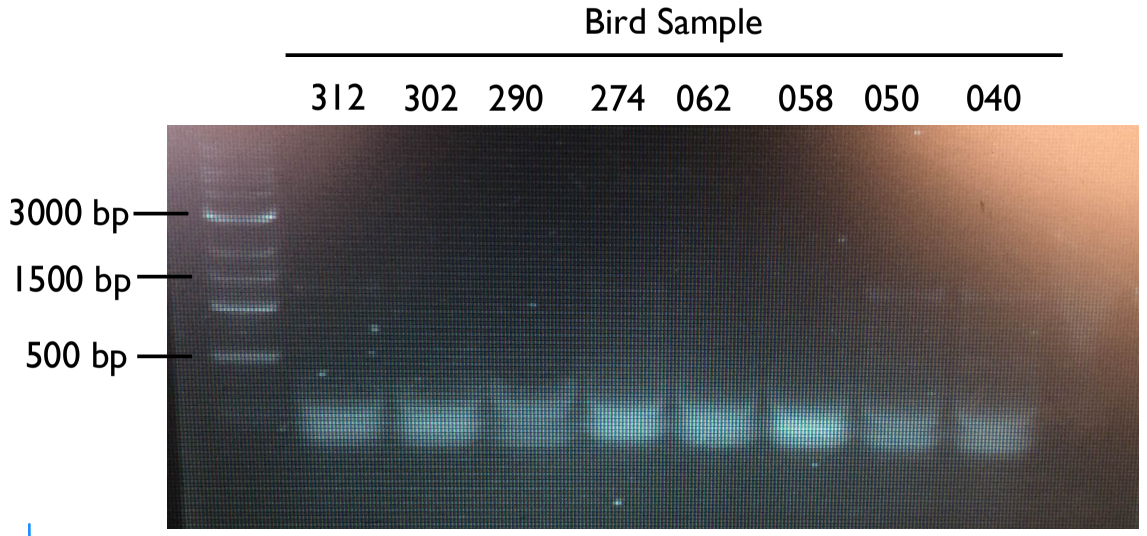
- Journal club next ~~Tuesday~~ Wed: Meet in **16-336 at ~~1:30pm~~ 1:00pm (speakers 1:15pm)**
- Also — lab treat next time

- What happened since we were in lab last ~~Tuesday~~ Wed:

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: primers / reagents bad
+ PCR settings
+ template
then specific priming

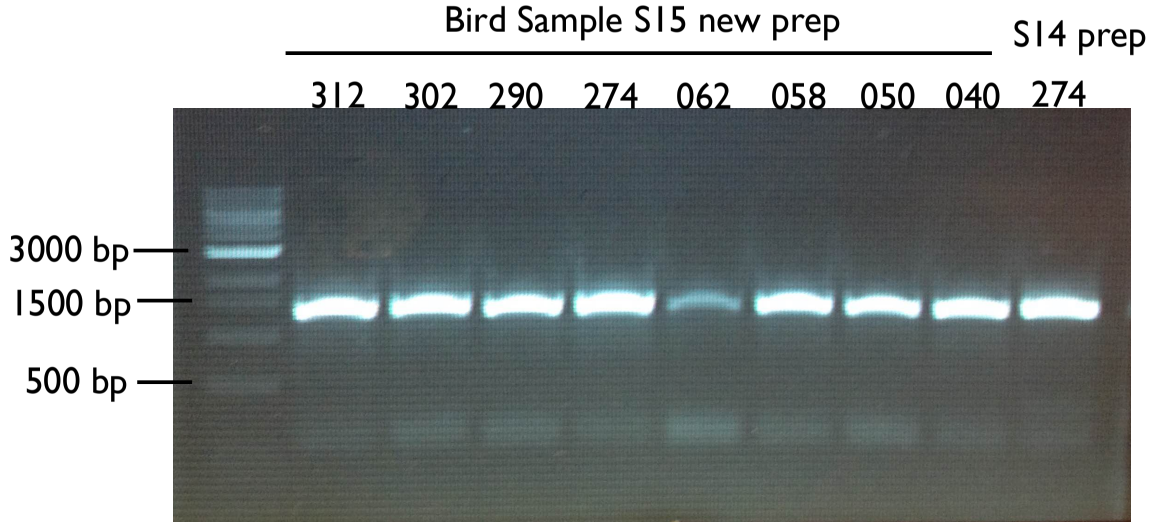


274

B) Qiagen kit → new template
~20-200 ng

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

c) Replace Qiagen kit

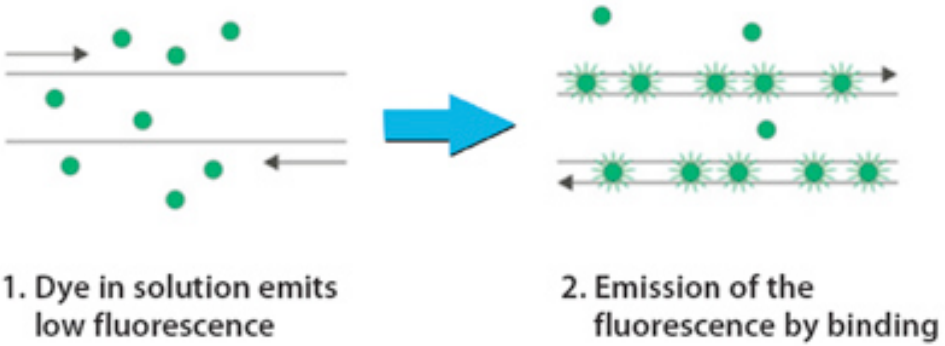


gel pure over pcr purification

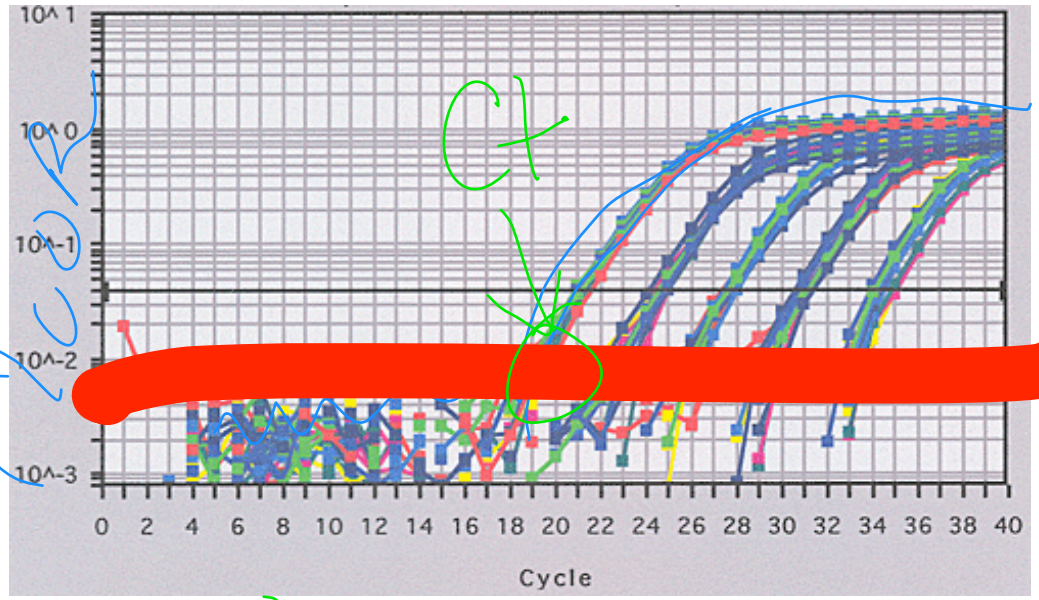
RTPCR

detect fluo

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



Ct

fluor

↑ signal
↓ LOD
↓ noise

Signal earlier
n = cycle

good amplification = low Ct

↑ [target]
↓ Ct value

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