M1D2: DNA Extraction

2/13/13

Announcements

- A few notes about the lab practical
- The wiki is your best friend (and will keep you on track): Google calendar
- Lab notebooks & Participation requirements

Sensitivity

Likyhood of detection in + patient
* original vs your new primers
* how low can you go?

Selectivity

Likyhood of being in - sample/patient
your primer detect X, but not Y

From protocol to lab notebook

1. Begin by adding the correct amount of water to a 200 ul PCR tube. Add that amount +1 ul to a second PCR tube.
2. Next add the primers to each reaction. Be sure to change tips between additions.
3. Next add template to the first reaction tube.
4. Finally add PCR Master Mix to each tube, pipetting up and down to mix. Leave your tubes on ice until the entire class

Statement of purpose: Today we will design primers to delete 32 bp from the 5' end of GFP and flank the sequence with new restriction sites. Then we will prepare truncated GFP by PCR as an insert for later cloning.

Design primers for GFP insert (M1D1 Part 1)
See attached Word document.

PCR to make GFP insert (M1D1 Part 2)

Added 27 uL H20 to expt'l, 28 uL H20 to control sample.
Added [1 uL] primer and [20 uL] Master Mix (last) to both samples,
and 1 uL template to expt'l only!
Rxn ready at 3 pm → on ice → thermal cycler started at 4 pm.

Thanks to Agi Stachowiak for this slide!

Lab notebook alternatives

- All include statement of purpose, conclusion, etc.
- Differ in treatment of protocols section

  (1) Cite protocols and write out only unique numbers/conditions

  (2) Write out summary protocol by hand (must include all numbers, but not lab tips, etc.)

  (3) Print out protocol and below/to side of section write out unique numbers/conditions

  (4) Some hybrid of the above that works for you!

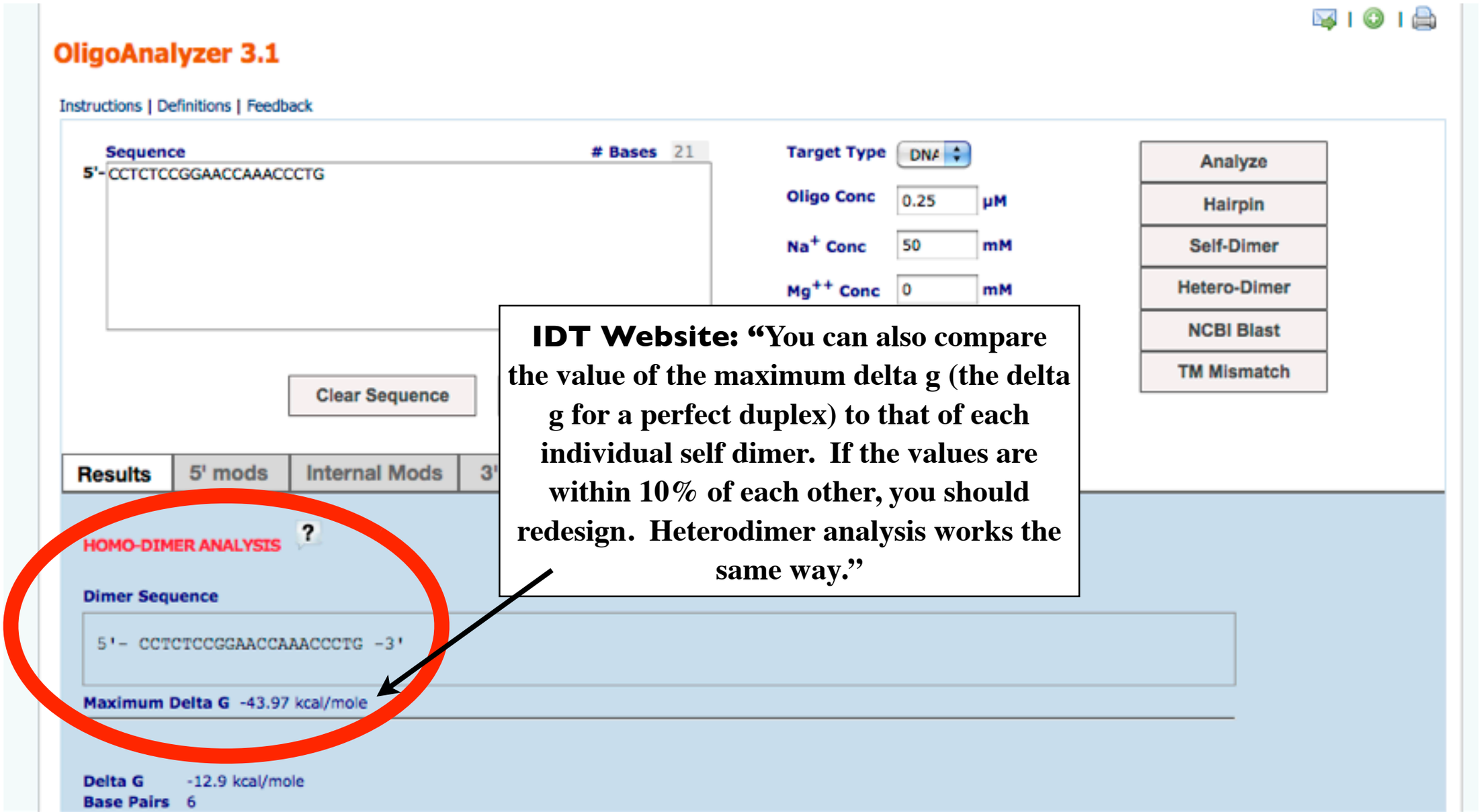
Thanks to Agi Stachowiak for this slide!

Participation and reflection in 20.109

- 1%: our perception of your engagement and contributions
- 2%: four reflections on your own learning
  - journal club self-assessment
  - module 1 report lessons learned
  - module 2 report lessons revisited
  - grab-bag: meeting with peers or instructors; discussion of outside research article
  - *extra-credit reflections*
  - *our hope: make learning gains more concrete*

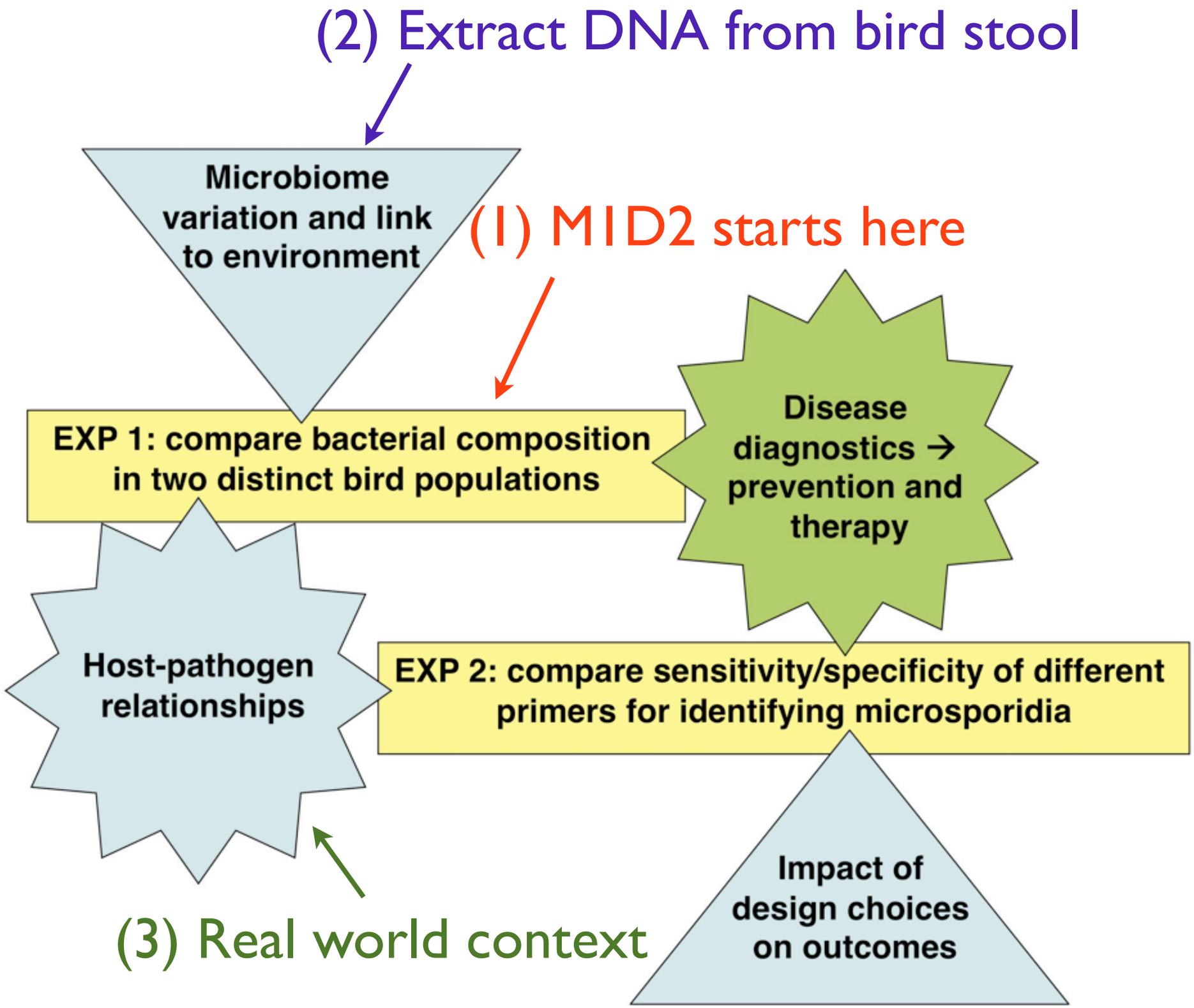
Thanks to Agi for this slide!

One clarification from last time:



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."

Module 1 Overview & Outline for lab today



(3) Real World Context -- Bird Microbial Communities

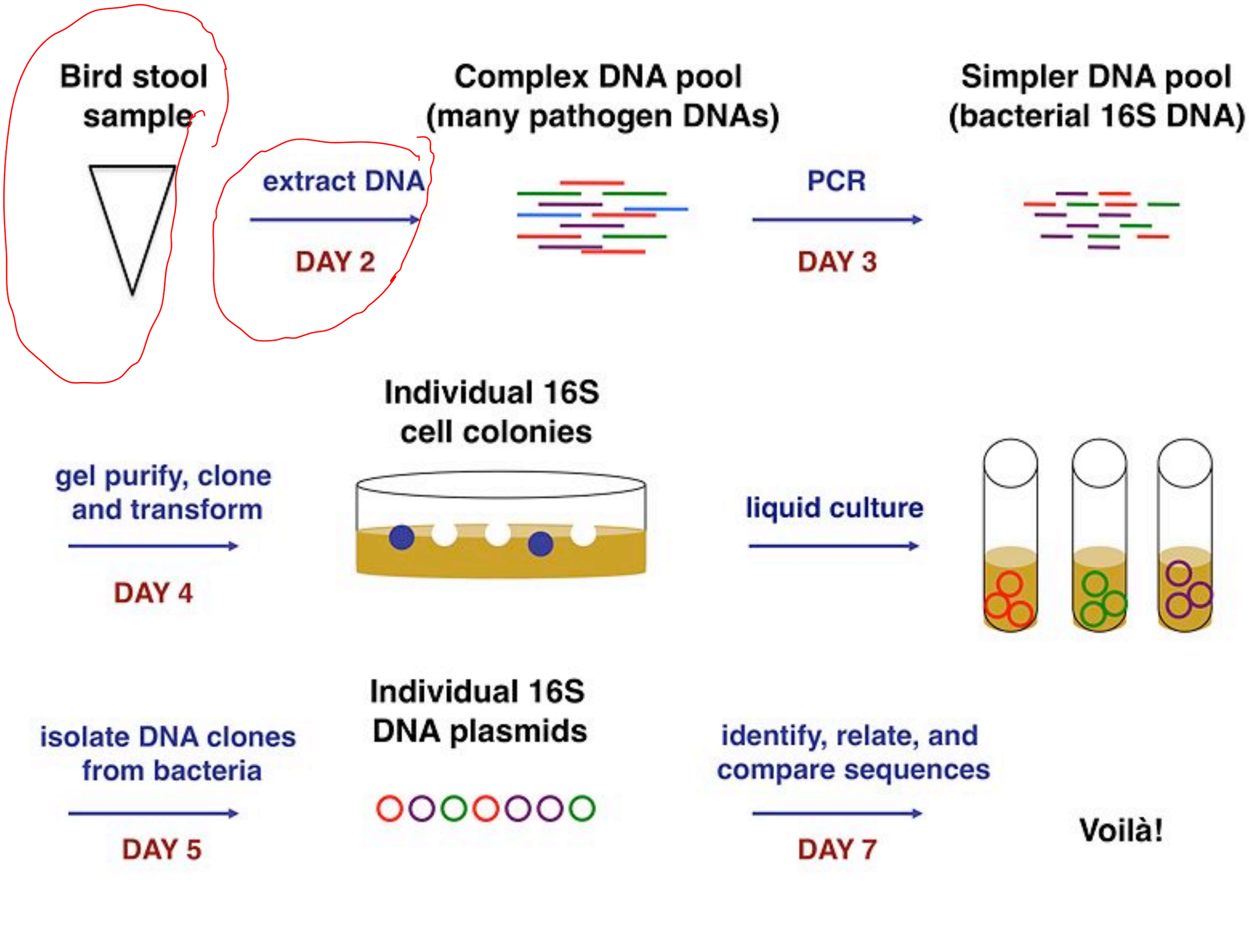
What is our primary research question?

How is the microbiome dependent on geography?

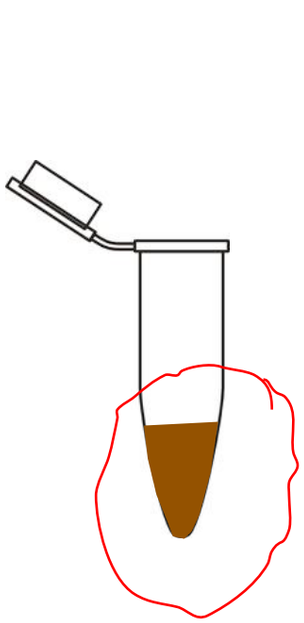
What are the broader impacts of our research?

- Zoonotic transfer
- susceptibility of disease
- general 7's ecosystem

Bird Microbial Communities -- Experimental Overview



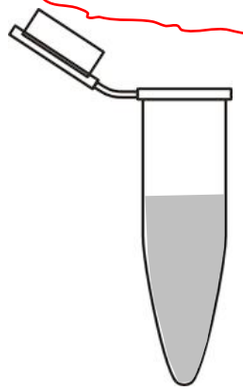
DNA Extraction from Stool: Part I



+ ASL

Vortex ~1 min

70C, 5 min



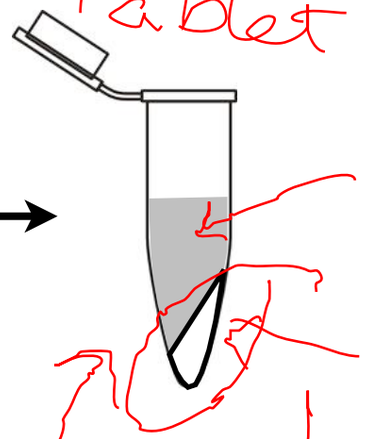
spin

collect sup

+ tablet

spin

Inhibitor tablet



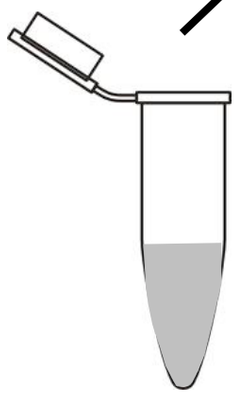
2mL

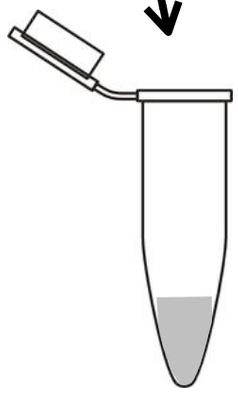
bad

(2)

transfer

spin





(1) Proteinase K

(3) AL

(4) chitinase

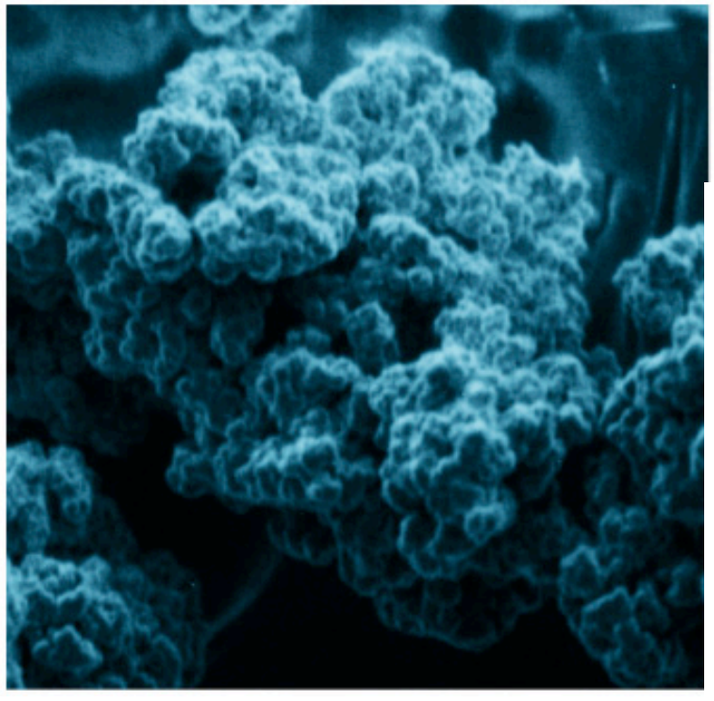
2 hr

DNA Extraction from Stool: Part II

(1) chaotropic salt
break H-bonds

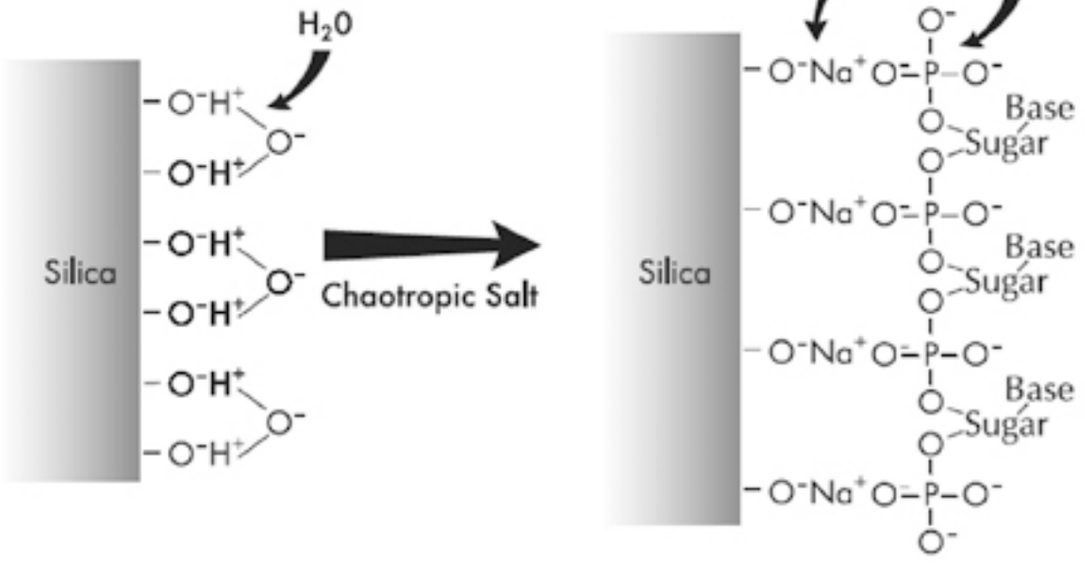
Qiaprep column:
Silica Resin

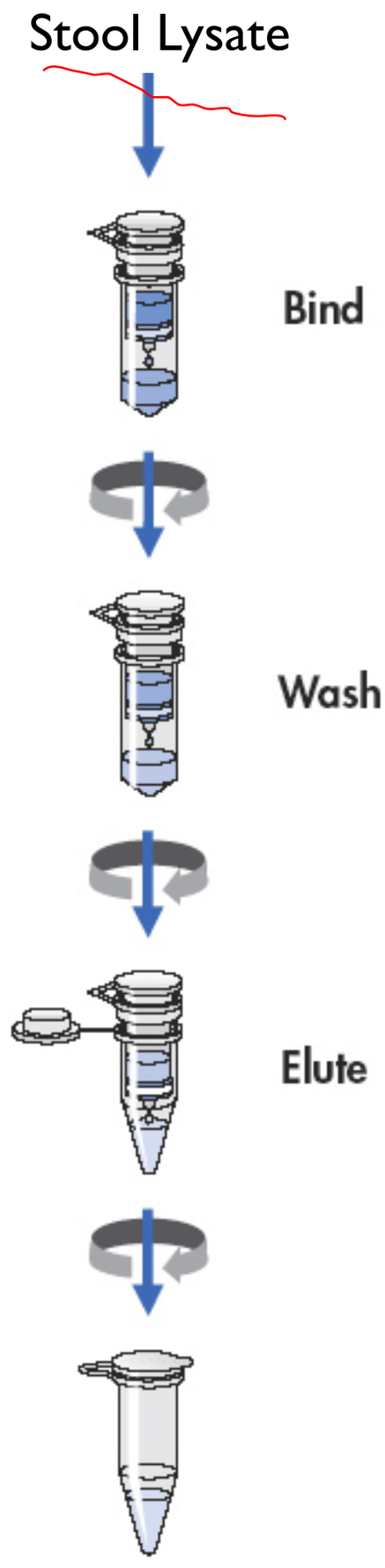
(2) wash - ethanol



[Promega.com]

(3) elute - high pH ~8
low salt





[Modified from http://bioenergy.asu.edu/photosyn/courses/bio_343/lab/Experiment-II.html]

Today in Lab:

Waste disposal: save all tubes, rinse 2-3x with water wash bottle over marked waste stream in fume hood.

Step 1: Stool lysis through adding enzymes

- Keep track of tube changes ~45 min

Step 2: 2 hr incubation

- WAC lecture ~2:45-3:45pm
- Finish primer designs
- Prepare tubes for later steps

Step 3: DNA purification using silica resin column

FNT: Journal article + one slide