Pre Pre lab

1. Draw 1) purine nucleotide
2) pyrimidine

2. 3 diagnostic tests/methods for infectious Dz order by sensitive

3. Define Microbial Dz Ecology 1-2 sentences

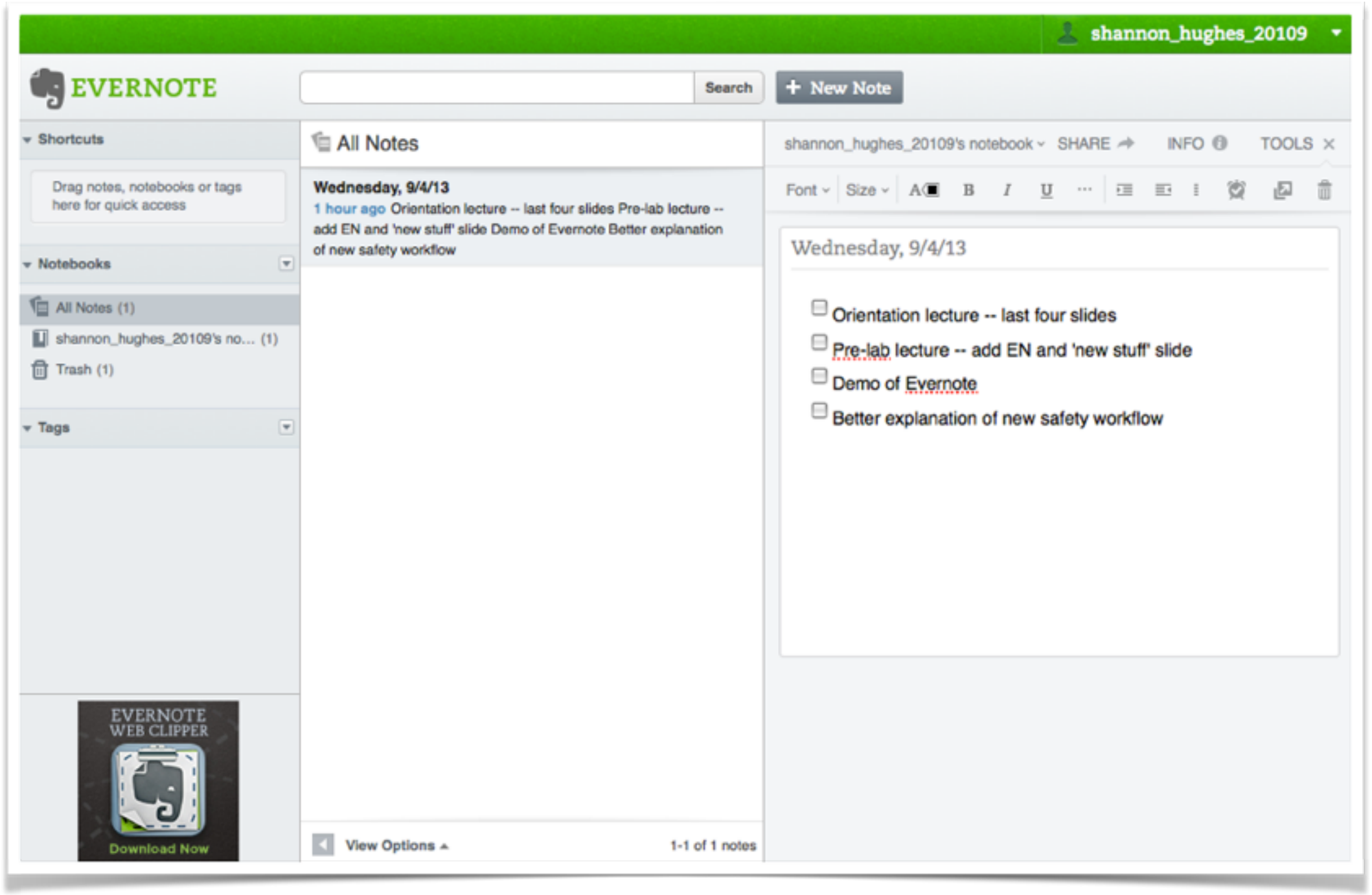
MIDI: Microbial DNA Extraction

2/5/15

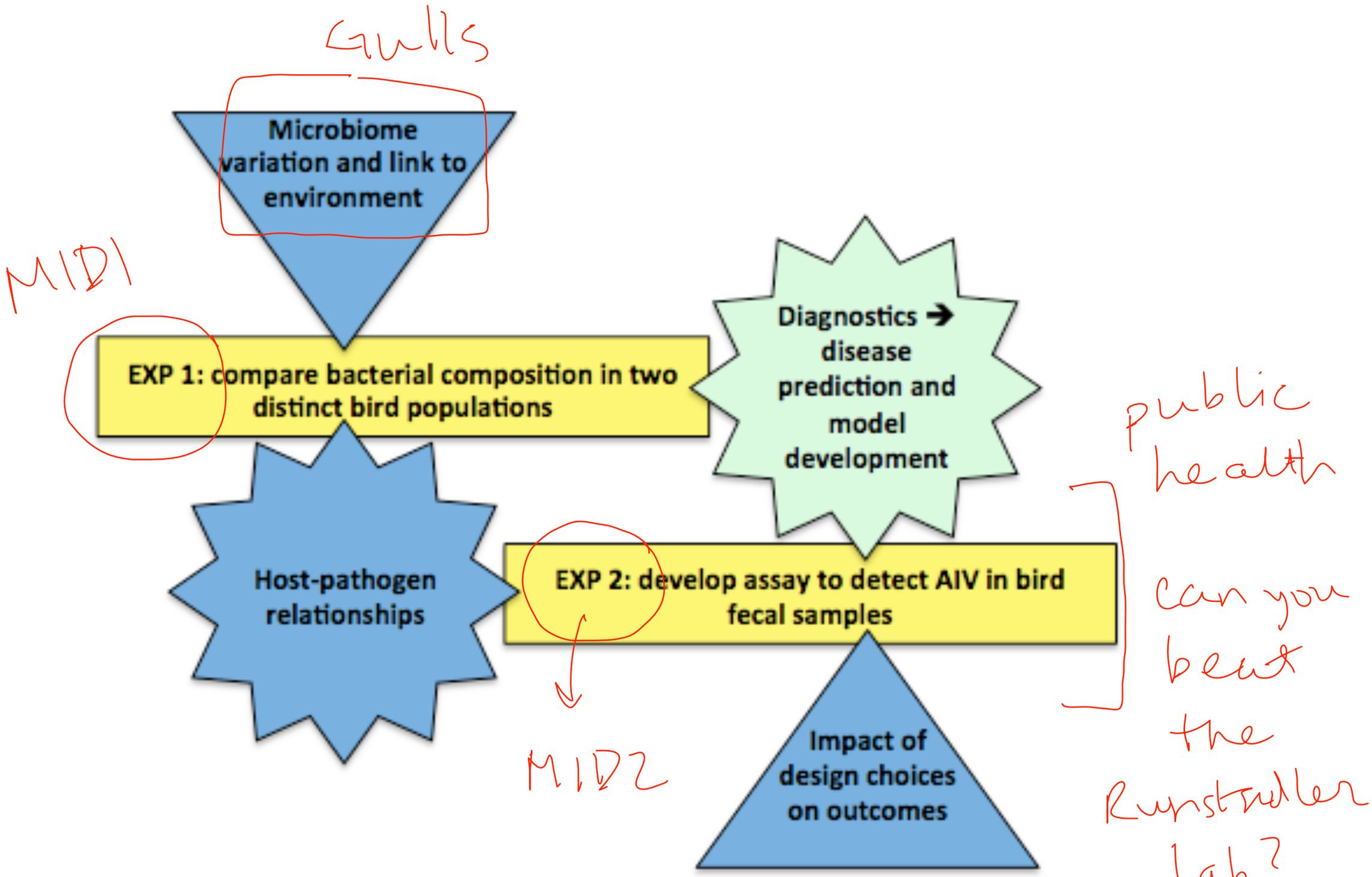
Announcements

1. A few notes about the lab practical
2. Please sign up for EN and share notebook by end of today
3. Please see me if you had trouble with the safety training
4. Sign up for OWW! See bottom of M1D1 protocol for next homework (due Tuesday).

Lab Notebooks: Evernote (EN)



Module 1 Conceptual Overview



Real World Context -- Bird Microbial Communities

What is our primary research question?

How does the gut microbiome vary between gulls of different sex and location?

What are the broader impacts of our research?

Could the microbiome contribute to flu susceptibility in Gulls?

You will amplify the16S rRNA gene to profile the microbiome of New England gulls

Sources:

"Scallop Pile"

Giant pile of scallop shells. Gulls love 'em. Gloucester MA — Pictures from Wendy

Carson Beach

Carson Beach, South Boston (1940): John Sanroma - Boston Public Library Flickr Stream

Samples:

http://www.wqed.org/birdblog/2010/04/16/anatomy-cloaca-or-vent/

Undisclosed to preserve bird privacy.

Bird Microbial Communities -- Experimental Overview

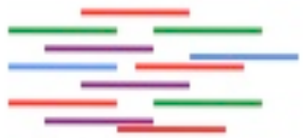
1 x team

Bird stool sample

extract DNA

DAY 1

Complex DNA pool (many microbial DNAs)



PCR

DAY 3

Simpler DNA pool (bacterial 16S DNA)

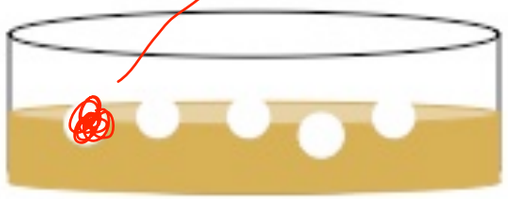
~1400bp

(gel purify); clone and transform

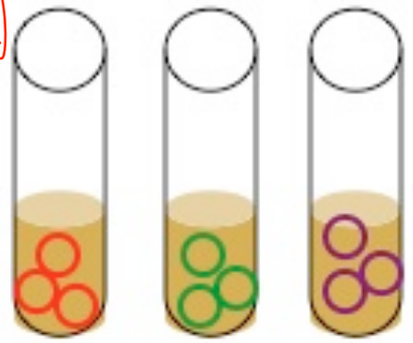
DAY 4

plasmid

Individual 16S cell colonies



liquid culture



isolate DNA clones from bacteria

DAY 5

miniprep

Individual 16S DNA plasmids

GeneWiz

identify, relate, and compare sequences

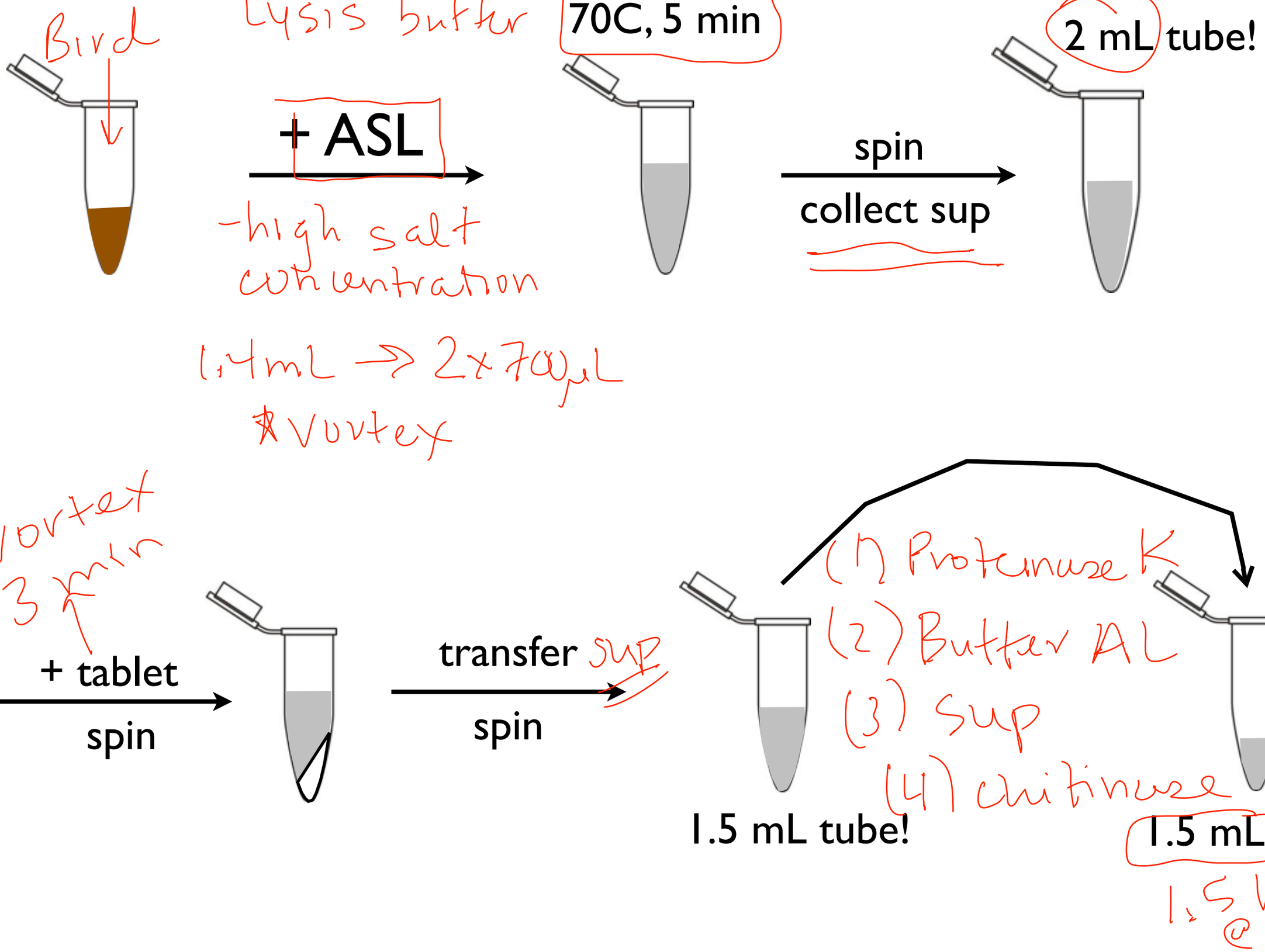
DAY 7

Today: Purify DNA from coacal samples

Inhibitors of Polymerase Chain Reaction (PCR) in coacal samples:

DNA Extraction from Stool: Part I

* Many tube changes *

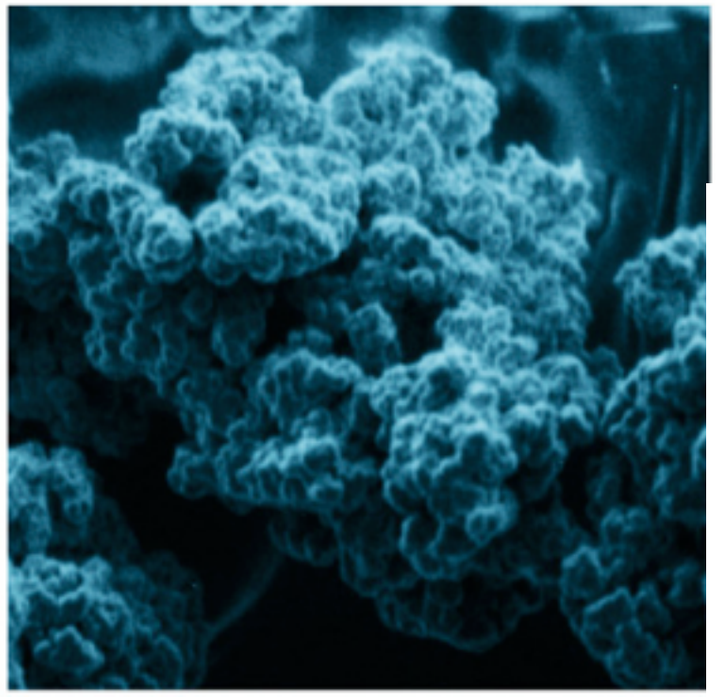


DNA Extraction from Stool: Part III

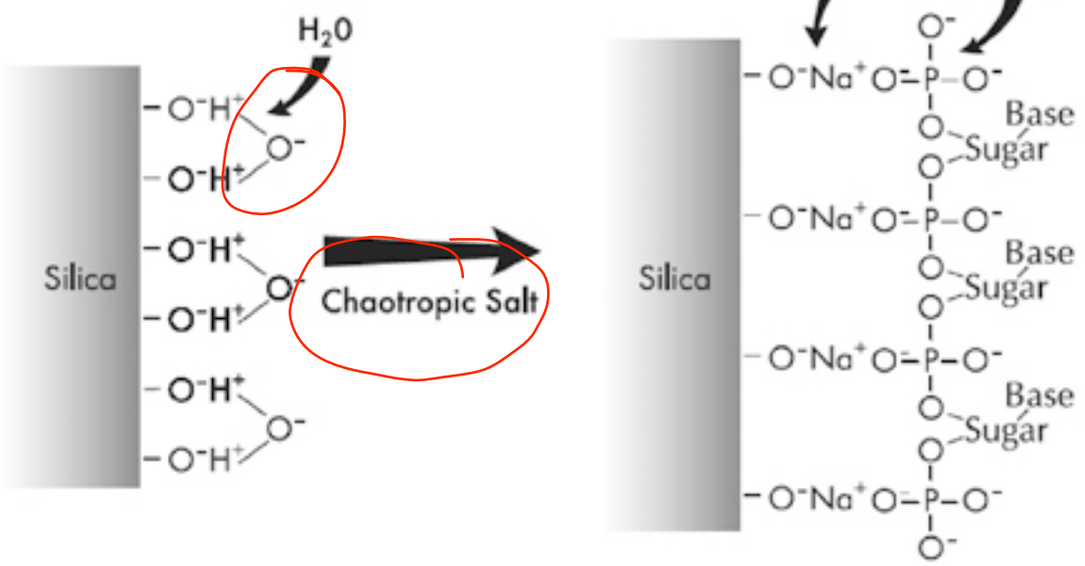
← silica

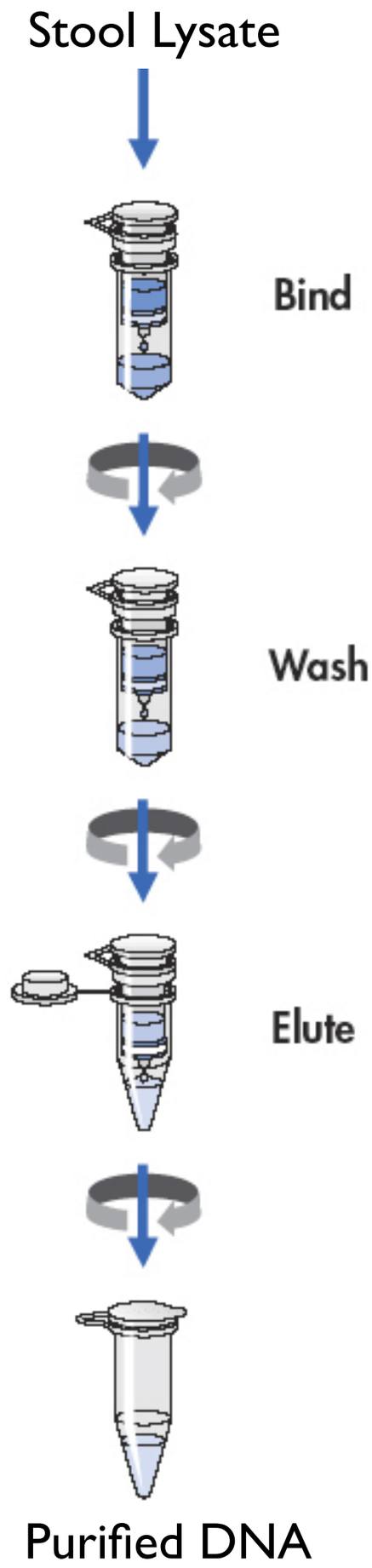
1) 200 proof ethanol
2) chaotropic salts
break H+ bonds
pH < 7.5 ~95% DNA
3) 70% ethanol
4) ↑ pH ~8.0 Elute DNA

Qiaprep column: Silica Resin



[Promega.com]





[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 (safety glasses!).

Step 1: Stool lysis through adding enzymes

- Keep track of tube changes ~45 min
- Use filtered pipette tips — share.

Step 2: 1.5 hr incubation

- *Lab practical*
- Prepare tubes for later steps

Step 3: DNA purification using silica resin column

FNT: wiki page, M1D3 paper