

Pre Prelab

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

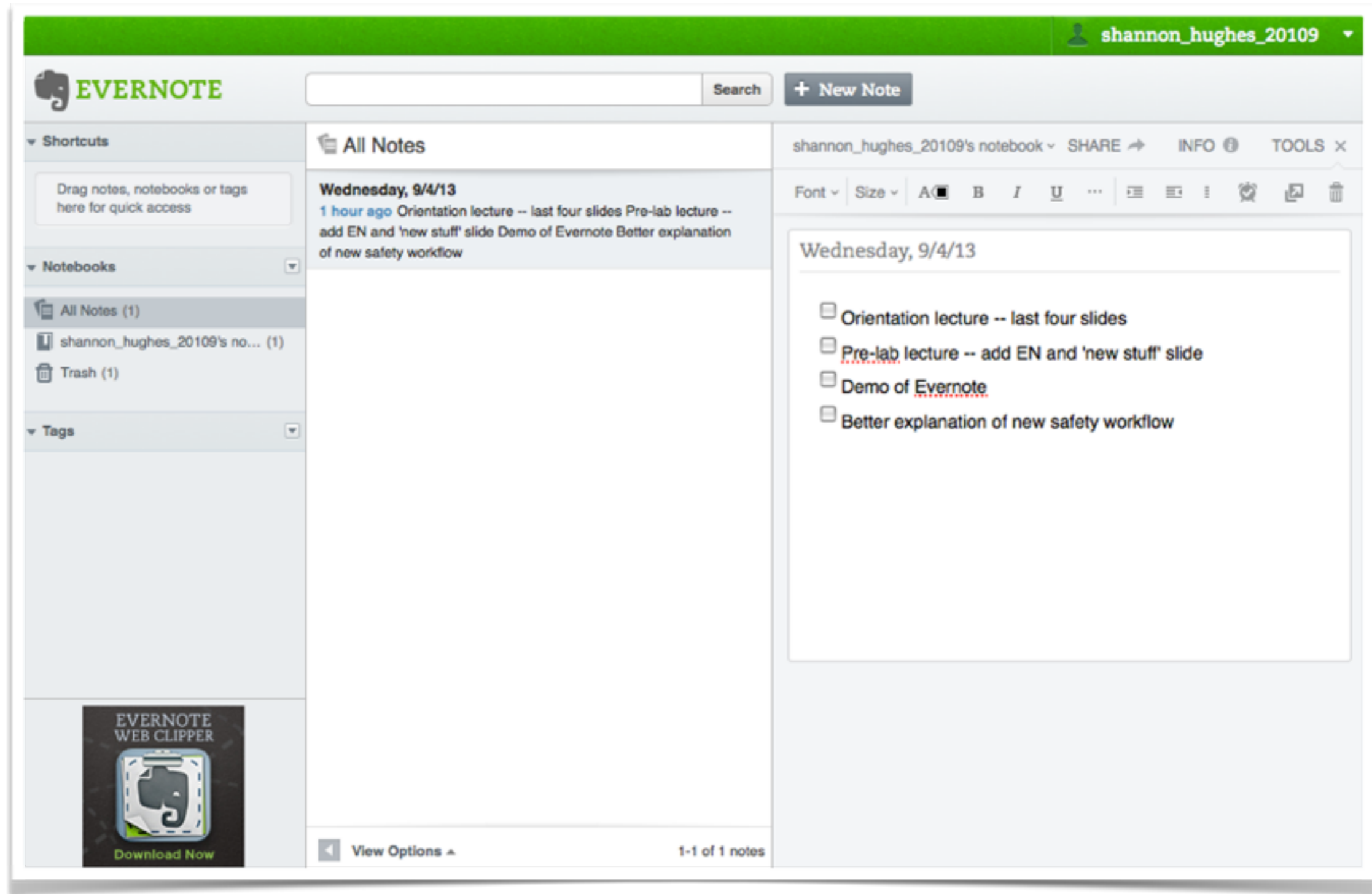
MIDI: Microbial DNA Extraction

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 MIDI protocol for next homework (due Tuesday).

Lab Notebooks: Evernote (EN)

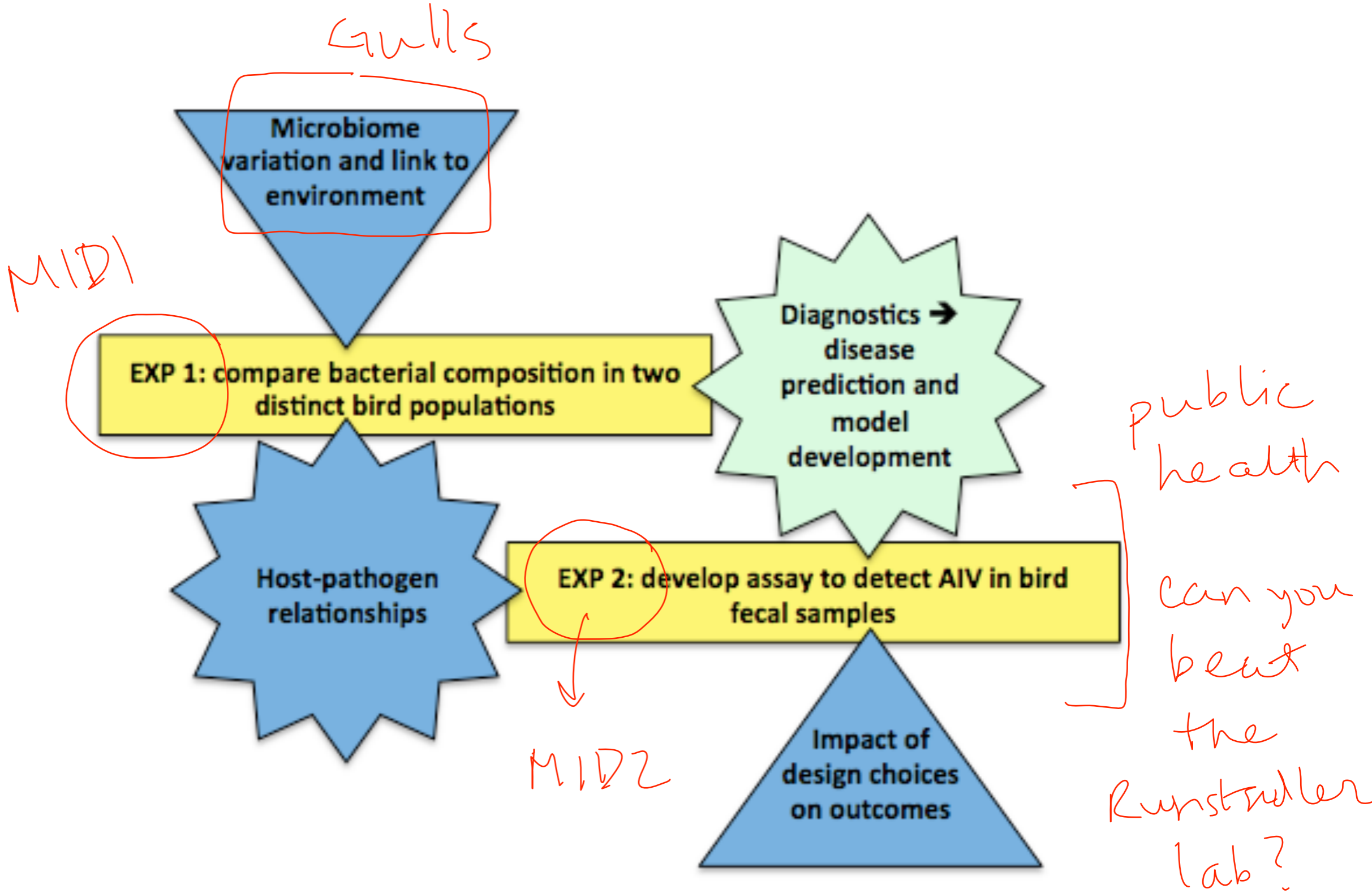


The screenshot displays the Evernote web interface for a user named shannon_hughes_20109. The interface is divided into several sections:

- Header:** Features the Evernote logo, a search bar, and a '+ New Note' button.
- Left Sidebar:** Contains 'Shortcuts' (with a drag-and-drop instruction), 'Notebooks' (listing 'All Notes (1)', 'shannon_hughes_20109's no... (1)', and 'Trash (1)'), and 'Tags'.
- Central Panel:** Shows a list of notes under the heading 'All Notes'. The selected note is dated 'Wednesday, 9/4/13' and is 1 hour old. The note content is: 'Orientation lecture -- last four slides', 'Pre-lab lecture -- add EN and 'new stuff' slide', 'Demo of Evernote', and 'Better explanation of new safety workflow'.
- Right Panel:** Displays the full content of the selected note, including the date 'Wednesday, 9/4/13' and a bulleted list of the same four items. A rich text editor toolbar is visible above the content.
- Bottom:** Includes a 'View Options' button and a '1-1 of 1 notes' indicator.

At the bottom left, there is a promotional banner for the 'EVERNOTE WEB CLIPPER' with a 'Download Now' button.

Module I Conceptual Overview



Real World Context -- Bird Microbial Communities

#109# Exp #1:

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 ^Δ microbiome contribute
to flu susceptibility in Gulls?

You will amplify the 16S 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:

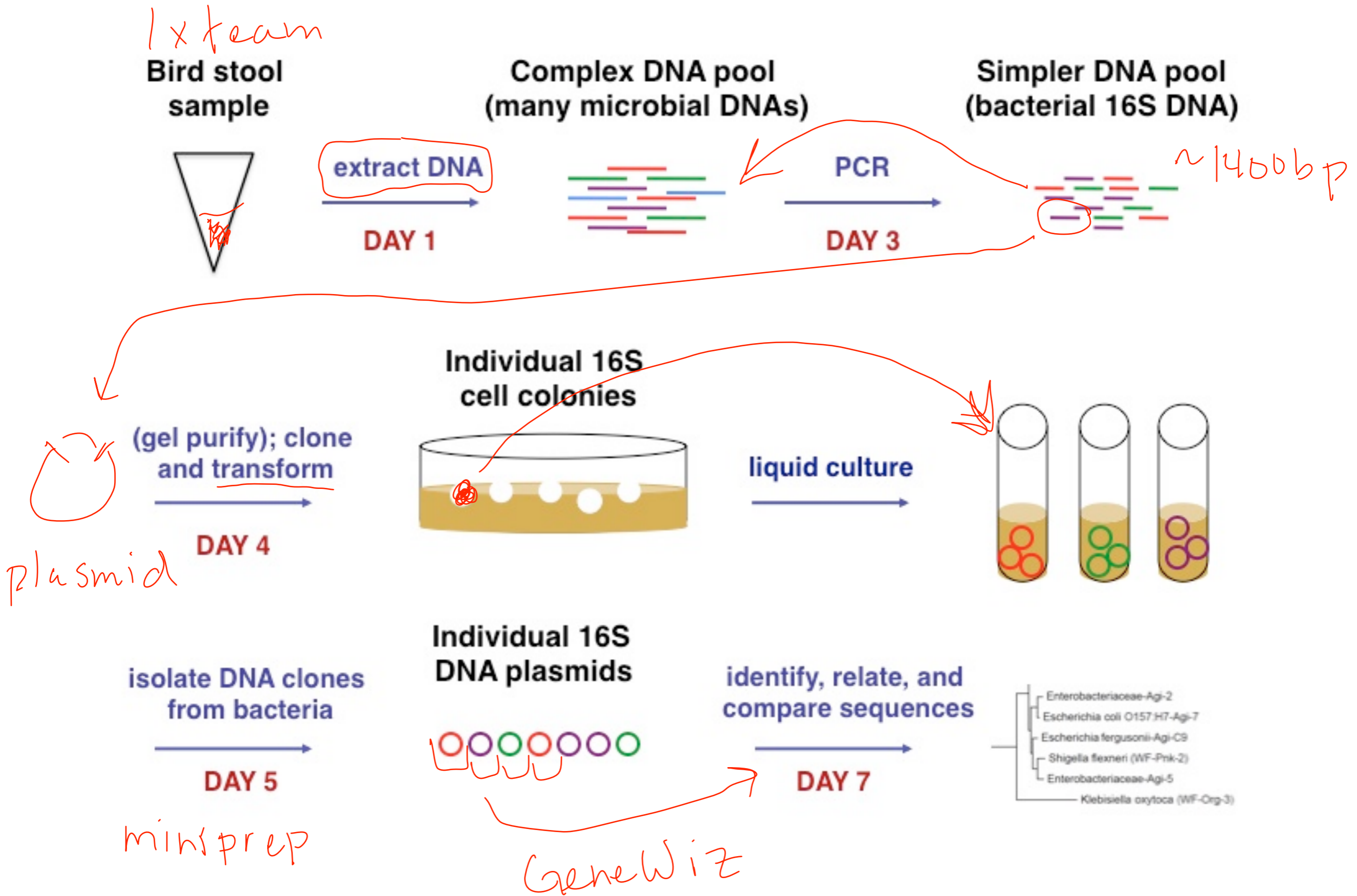


<http://www.wqed.org/birdblog/2010/04/16/anatomy-cloaca-or-vent/>



Undisclosed to preserve bird privacy.

Bird Microbial Communities -- Experimental Overview

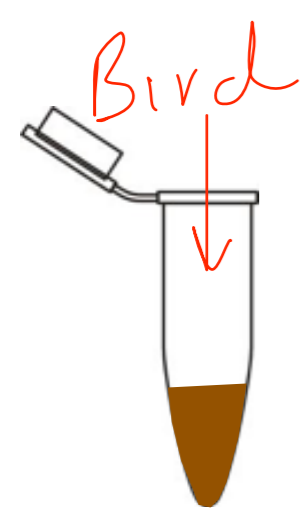


Today: Purify DNA from coacal samples

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

★ Many tube changes ★

DNA Extraction from Stool: Part I

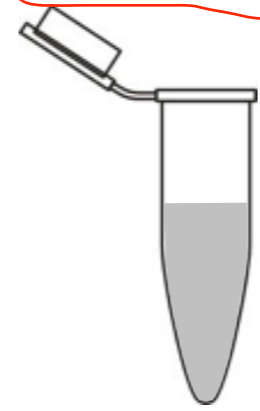


Lysis buffer

+ ASL

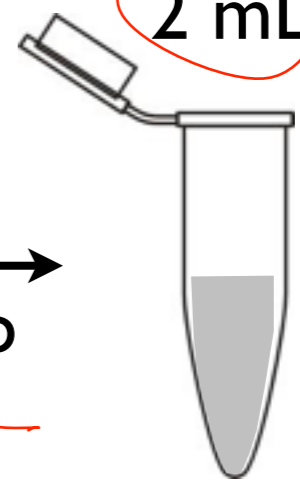
- high salt concentration

70C, 5 min



spin
collect sup

2 mL tube!

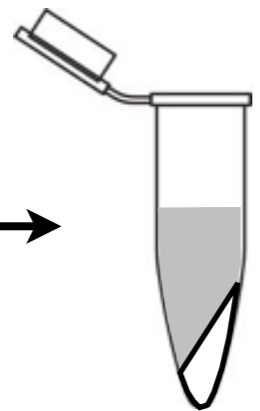


1.4 mL → 2 x 700 μL
★ vortex

Vortex 3 min

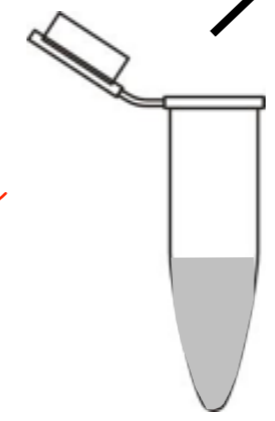
+ tablet

spin



transfer sup

spin



1.5 mL tube!

- (1) Proteinase K
- (2) Buffer AL
- (3) sup
- (4) chitinase

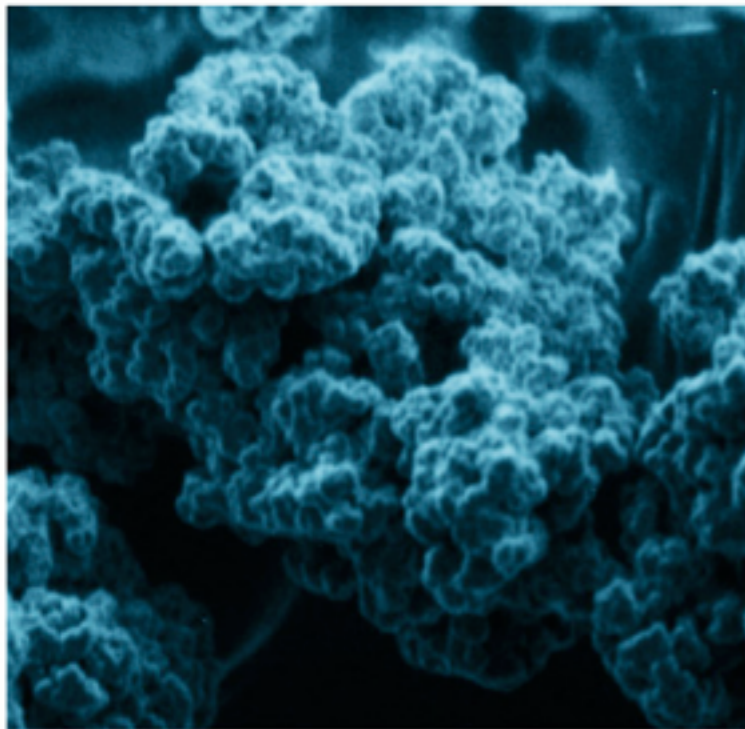
1.5 mL tube!

1.5 hr @ 56°C

DNA Extraction from Stool: Part III



Qiaprep column:
Silica Resin



[Promega.com]

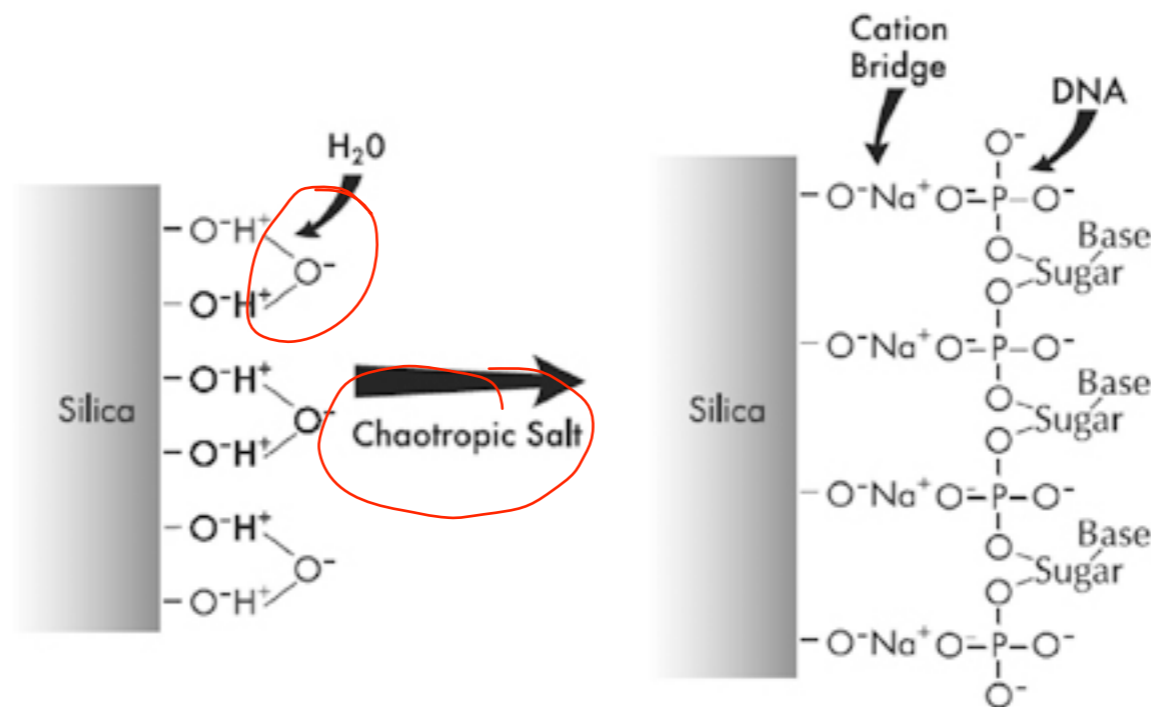
- 1) 200 proof ethanol
- 2) chaotropic salts

← silica

break H₂O bonds
pH < 7.5 ~ 95% DNA

- 3) 70% ethanol

- 4) ↑ pH ~ 8.0 Elute DNA



Stool Lysate



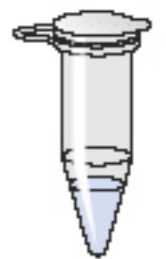
Bind



Wash



Elute



Purified DNA

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, MID3 paper