Now the fun begins!

M1D1: Microbial DNA Extraction

2/6/13

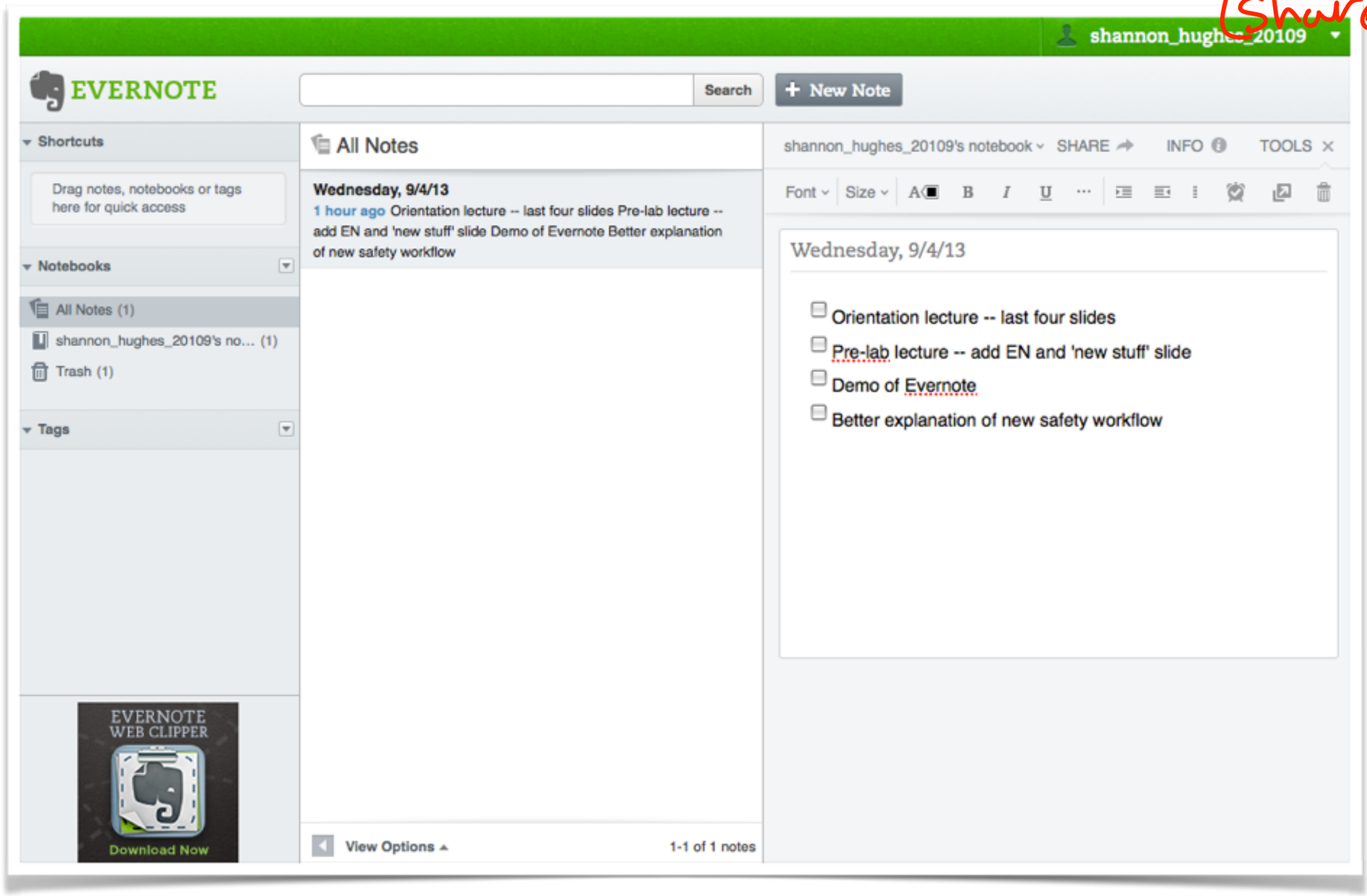
Announcements

- A few notes about the lab practical
- Please sign up for EN and share notebook by end of today

Lab Notebooks
Module Overview
Lab Overview
FNT

Lab Notebooks: Evernote (EN)

- Known issues
  - browser version ≠ stand alone version (share/sync)



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!

Module 1 Conceptual Overview

Model ⇒ 2 independent experiments

start today

Microbiome variation and link to environment

EXP 1: compare bacterial composition in two distinct bird populations

Disease diagnostics → prevention and therapy

"basic" biological engineering

"applied" B.E.

Host-pathogen relationships

EXP 2: compare sensitivity/specificity of different primers for identifying microsporidia

Impact of design choices on outcomes

MID 2

Real World Context -- Bird Microbial Communities

What is our primary research question?

How does the microbiome (bacteria population) differ/vary w/ environment?

- bird type
- male/female
- location

What are the broader impacts of our research?

What accounts for flu susceptibility?

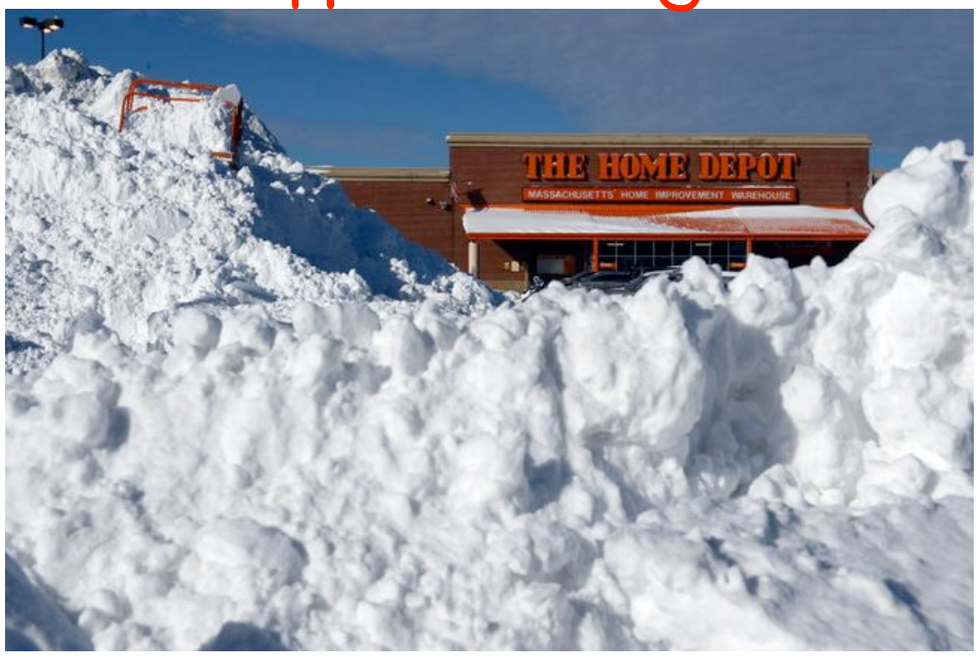
You will amplify the16S rRNA gene to profile the microbiome of New England gulls

two types of gulls → [redacted]-billed

Herring gull

ring

Sources:



South Bay Center parking lot - South Boston
Jan 4, 2014 (Photo by Darren McCollester/Getty Images)

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

Samples:

cloacal swabs

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

"cloacal kiss"

Male/Female

Undisclosed to preserve bird privacy.

Bird Microbial Communities -- Experimental Overview

Bird stool sample

extract DNA

DAY 1

Complex DNA pool (many microbial DNAs)

PCR

DAY 3

Simpler DNA pool (bacterial 16S DNA)

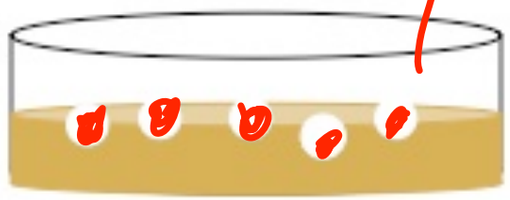
(gel purify); clone and transform

DAY 4

isolate DNA clones from bacteria

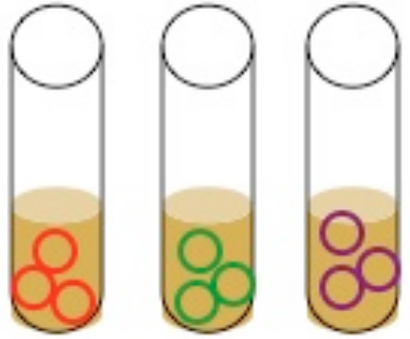
DAY 5

Individual 16S DNA plasmids



Individual 16S cell colonies

liquid culture



identify, relate, and compare sequences

DAY 7

Today: Purify DNA from coacal samples

Both → Red, yellow, green, pink

Different → orange, blue

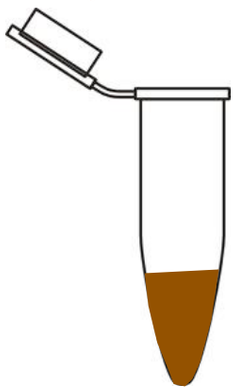
★ cross contamination (minimize) ★
- filter tips
- tube changes

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

Inhibit enzyme activity
- heme - bilirubin
- bile salts
- Humic cpds → degradation products

DNA Extraction from Stool: Part I

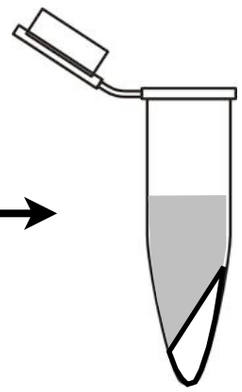
coacal



InhibiTEX

+ tablet

spin



lysis

+ ASL

3min

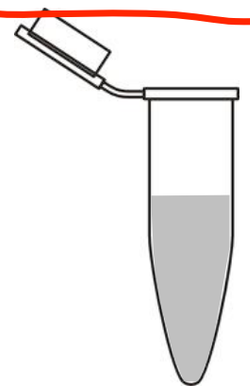
vortex

transfer

spin

Select for microbial DNA

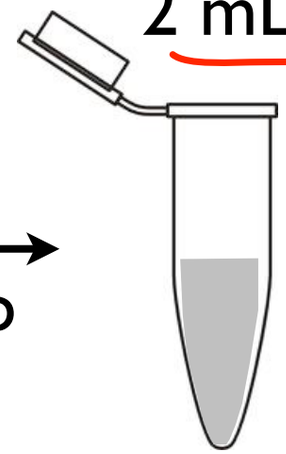
70C, 5 min



spin

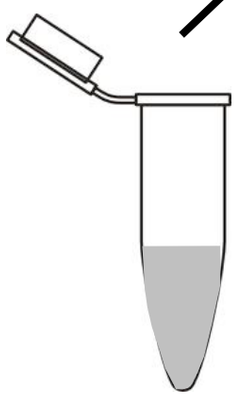
collect sup

2 mL tube!

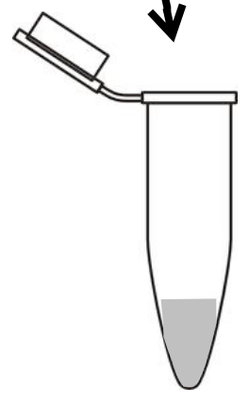


+ Proteinase K

+ chitanase



1.5 mL tube!

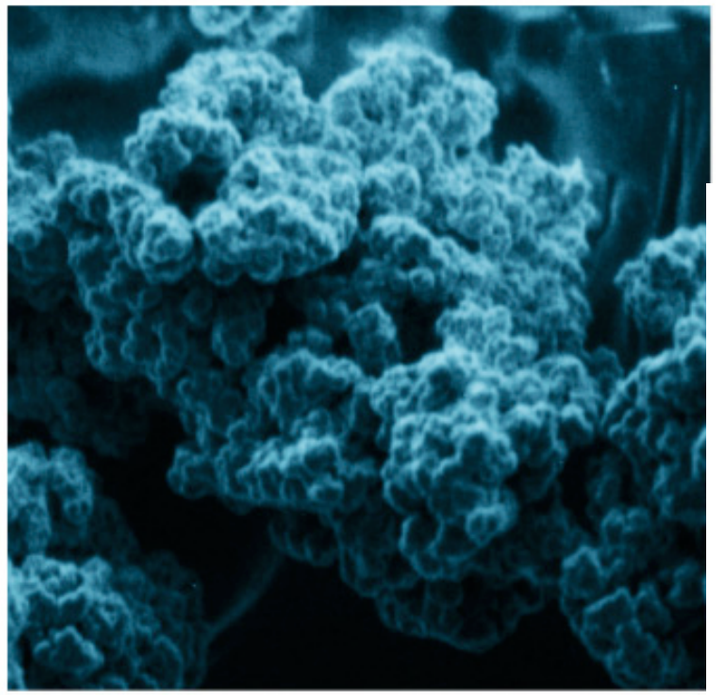


1.5 mL tube!

— 1.5 hr @ 56°C

DNA Extraction from Stool: Part III

Qiaprep column:
Silica Resin

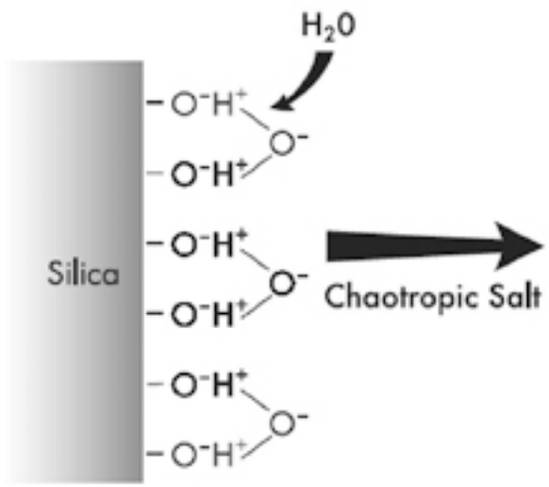


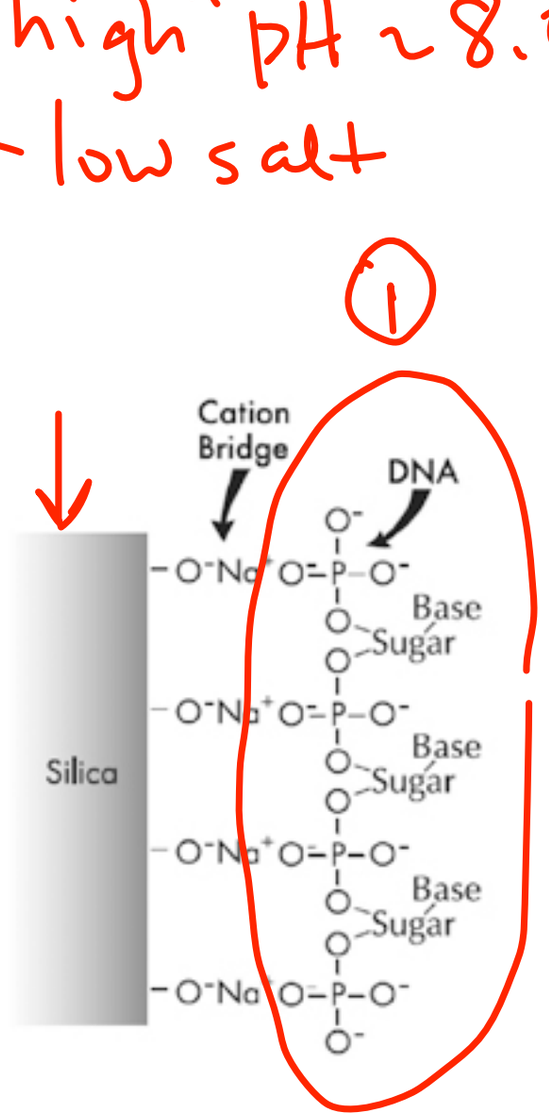
[Promega.com]

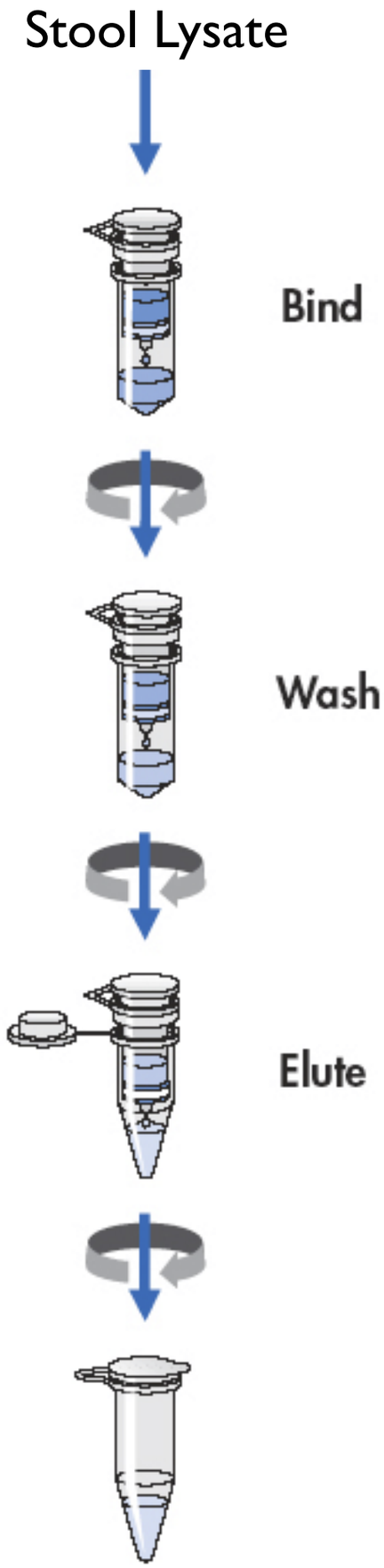
1) chaotropic salt [high]
(broken H+ bonds)

2) bind silica - ethanol

3) elute - "high" pH ~8.0
- 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 (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