

- **Announcements**
- **Pre-lab Lecture**
 - ❖ **Module 1 overview**
 - ❖ **DNA extraction principles**
 - ❖ **Today in Lab: M1D1**

Announcements

- BE (and other) seminar series:
 - Seminar posters across from BE HQ on 3rd floor
 - Full schedule linked from BE website
 - Part of professional development
- Different equipment for different volumes/tasks
- Begin notebooks today! → dates TBA by Mon. share
→ can ask for advice for an alternate date

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 [do xyz task]. Then we will prepare [xyz DNA] by PCR to use as [xyz component] for later cloning.

joys
memory

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

} shimmable

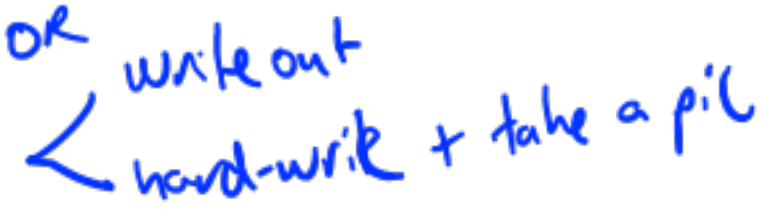
PCR to make GFP insert (M1D1 Part 2)

Copy protocol and fill in exact volumes for #1.

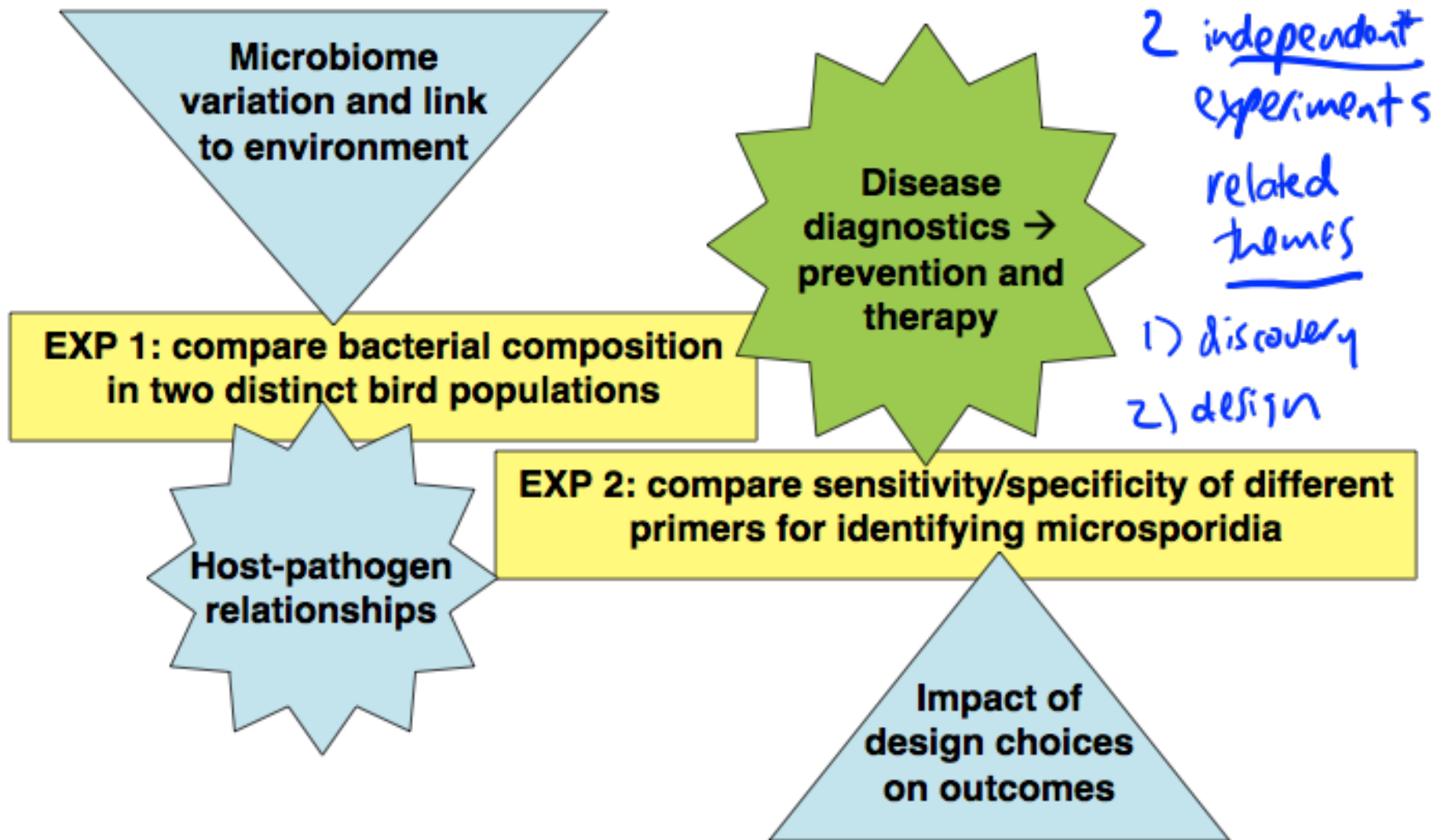
Optionally confirm (say, with checkboxes) key details such as adding Master Mix last, template only to experimental sample.

Add unique notes: Rxn ready at 3 pm → on ice → thermal cycler started at 4.

EN example

- Thanks to Shannon's UROP students!
- Protocols
- Linked Excel calculations 
- Linked images
- For 20.109: front and back matter still *matter*
- Note: Can simultaneously use desktop and web versions, one per partner, on one laptop

Module 1 conceptual overview



Bird microbial communities: context

- What is our primary research question?

How do bird gut microbiota vary
with environment? - species
- sex
(- location)

- What are the broader impacts of our research?

Bird susceptibility to disease
→ impact of microbiota
→ zoonotic transfer

You will amplify the 16S rRNA gene to profile the microbiome of New England gulls

herring gull (M)

ring-billed gulls (M, F)

Sources:



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



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

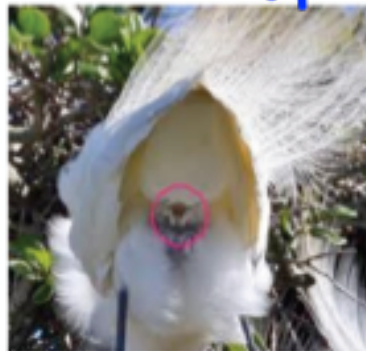
Samples:

from
cloaca

excretion

&

reproduction



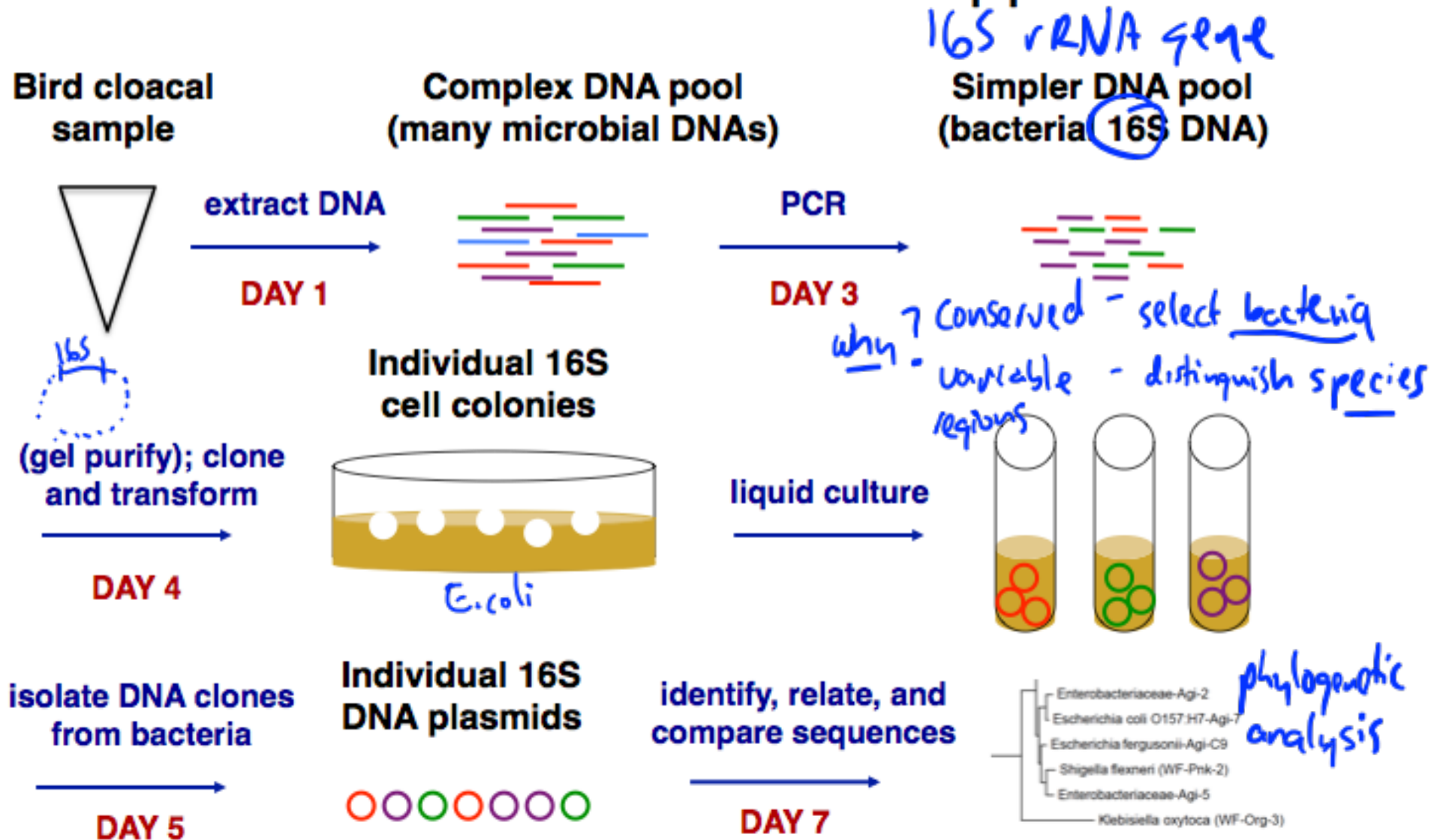
<http://www.wqed.org/birdblog/2010/04/16/anatomy-cloaca-or-vent/>



Undisclosed to preserve bird privacy.

Thanks to Shannon H for this slide!

Bird microbial communities: approach



Step 1: DNA extraction -- background

- Stool a complex mixture! Many inhibitors of PCR
 - binders and degraders e.g., bile salts or nucleases
- Rid inhibitors
 - proprietary chemicals
 - proteinase K
- Preferential isolation of microbial DNA
 - high temperature 70°C (human-RT)
- Minimize cross-contamination:
 - sterile filter tips ; tube exchanges – label

DNA extraction: initial steps

BL21 lay coats

Cloacal sample



chilled 2 mL tubes

+ASL

vortex

lysis

70 °C, 5 min



spin, collect

supernatant

fresh 2 mL tube

InhibiTEX +tablet

spin

★ long vortex



transfer,

spin again



1.5 mL tube

1.5 mL tube



incubate:

- 1) Prot. K
 - 2) sample - mix
 - 3) AL - mix
 - 4) chitinase - vortex
- 1.5h @ 56°C

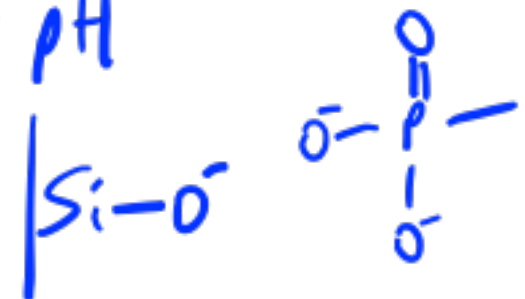
DNA extraction: later steps



Silica resin
column

[qiagen.com]

- 1) bind DNA - \uparrow salt, \downarrow pH
chaotropic salts disrupt H-bonds
DNA sticks to column (cation-bridge - see sk 4)
- 2) keep DNA wash away rest
ethanol precipitates DNA (w/ (+) ion)
 \downarrow charge screening
- 3) elute DNA - \downarrow salt, \uparrow pH
electrostatic repulsion



Today in Lab (M1D1)

- Notebook potentially collected M1D7! Ask Qs.
- **Waste disposal: save all tubes, rinse 2-3x w/H₂O bottle over marked waste stream in fume hood.**
- DNA extraction through enzymatic incubation
 - Many tube exchanges! May take ~ 45 min
- During 1.5 h incubation
 - Lab practical (45-50 min)
 - Prepare/label tubes for later steps
- DNA purification on column (~ 30 min)
- FNT: wiki page, start reading M1D3 paper