Pre prelab with Jon

1 - a) purine nucleotide
b) pyrimidine

2. Up to 3 methods/assays
Dx Inf. Dz. Sensitive

3. Define Microbial Dz Ecology

M1D1:
Microbial DNA extraction

2/6/15

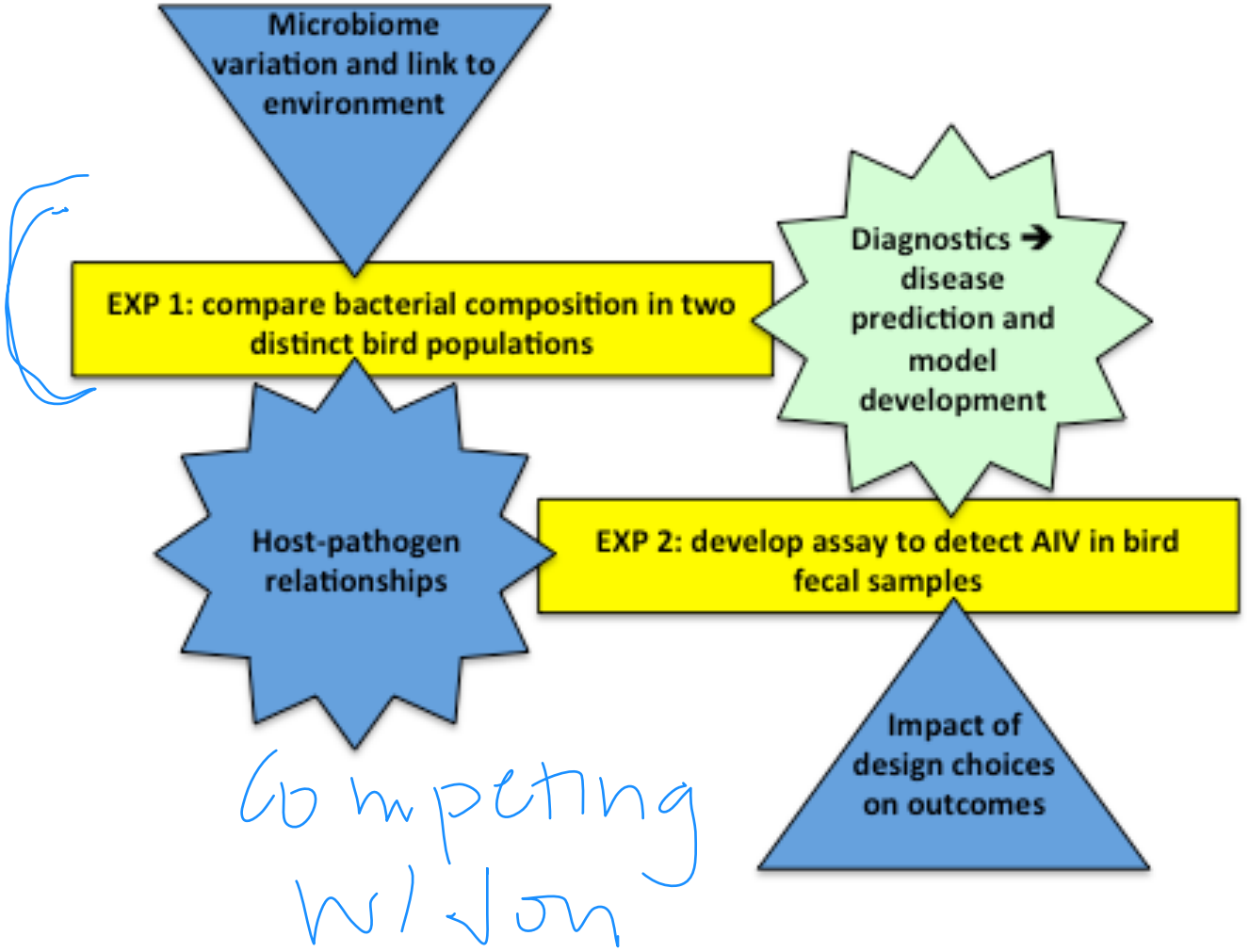
M1 major assignments

- Microbiome data summary (15%)
  - First draft due March 14th at 5:00 pm
  - Revision due April 3rd at 5:00 pm
- Primer design memo (5%)
  - Due March 20th at 5:00 pm
- Journal club presentation (10%)
  - Either M1D6 or M1D9
- Late policy for 20.109…

Lab business

1. Lab treat…
   - Complete on your own…kinda
   - Only one team at a time at stations
2. Homework due M1D1 (today)
   - Be sure to share your Evernote 20.109 notebook
   - See me if you had difficulty completing EHS training
3. Office hours
   - Noreen: M 2-4p, R 2-4p, and by appointment
   - Leslie: M 1-2p, R 1-2p, and by appointment

Module 1 conceptual overview



Bird microbiome project

- Primary research question?

  How similar/diff are microbiomes (location/sex)

- Broader impact?

  May these differences contribute to flu susceptibility

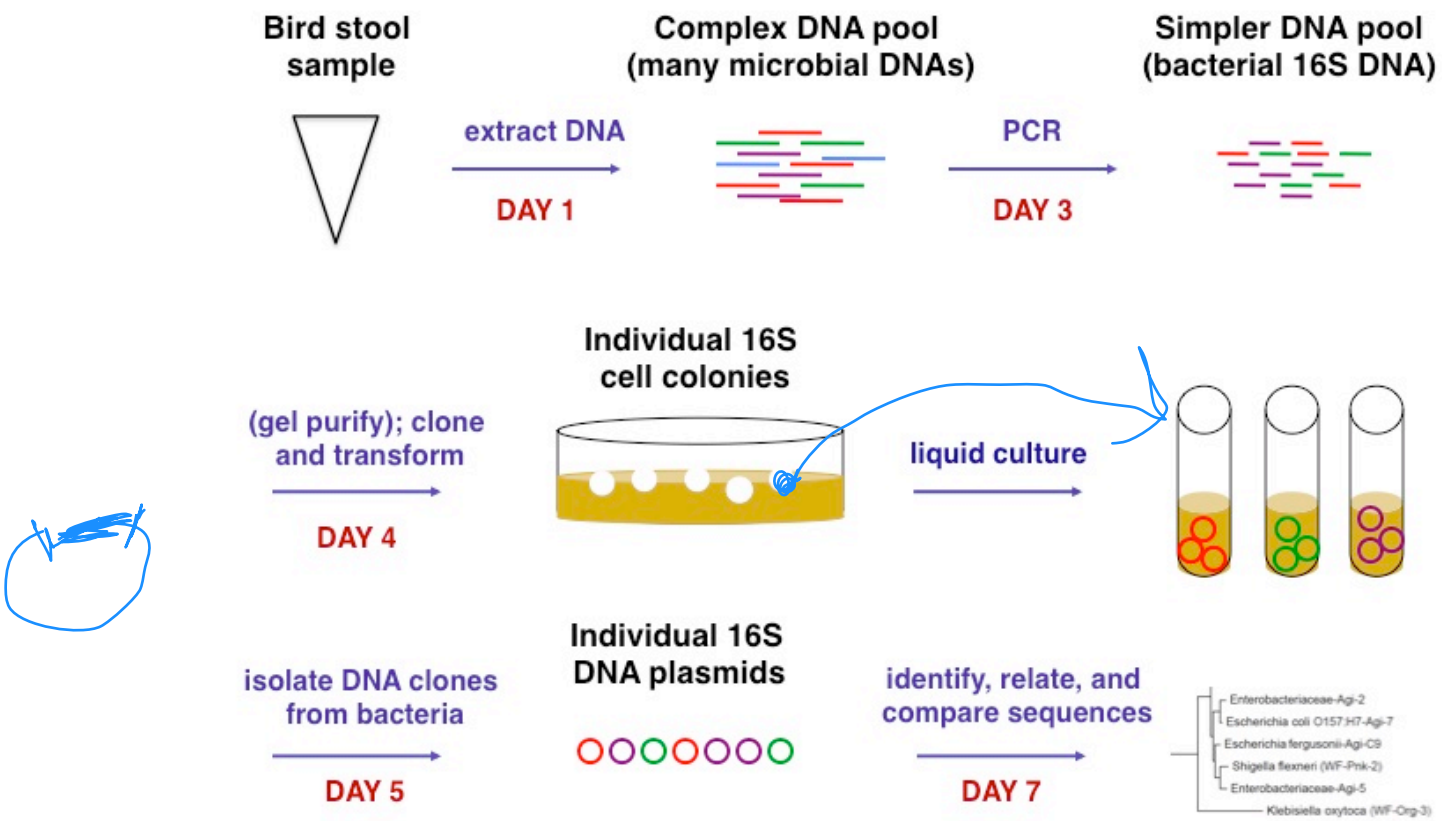
How do we sample bird microbiomes?

- Cloaca used to excrete both urine and feces
- Benefits of cloacal samples
  - Know characteristics of bird
  - For example?

    sex species health location age
  - Why might this information be useful?

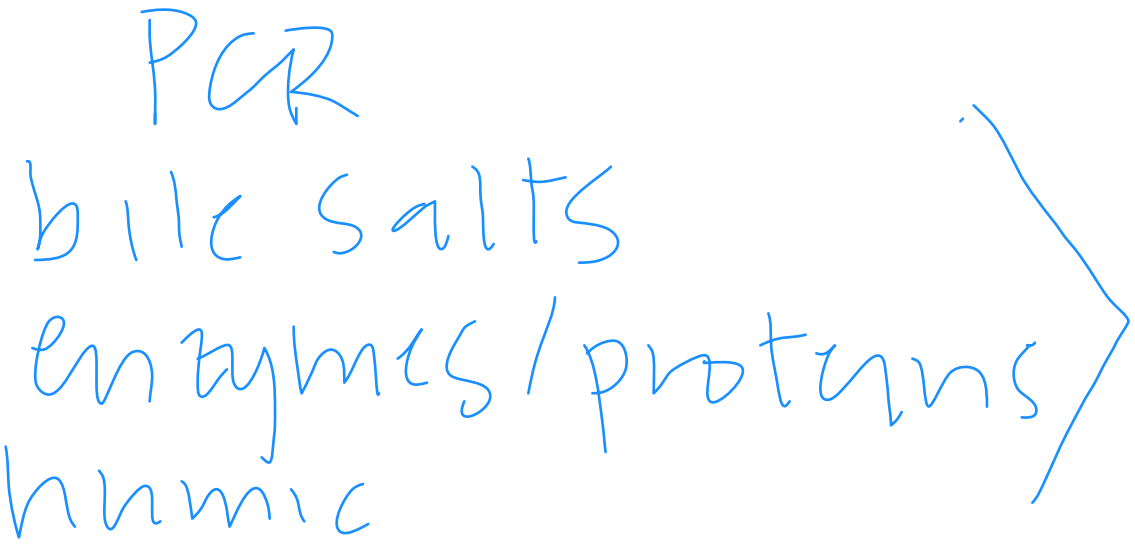
- How do we get cloacal samples from birds?

Experimental overview

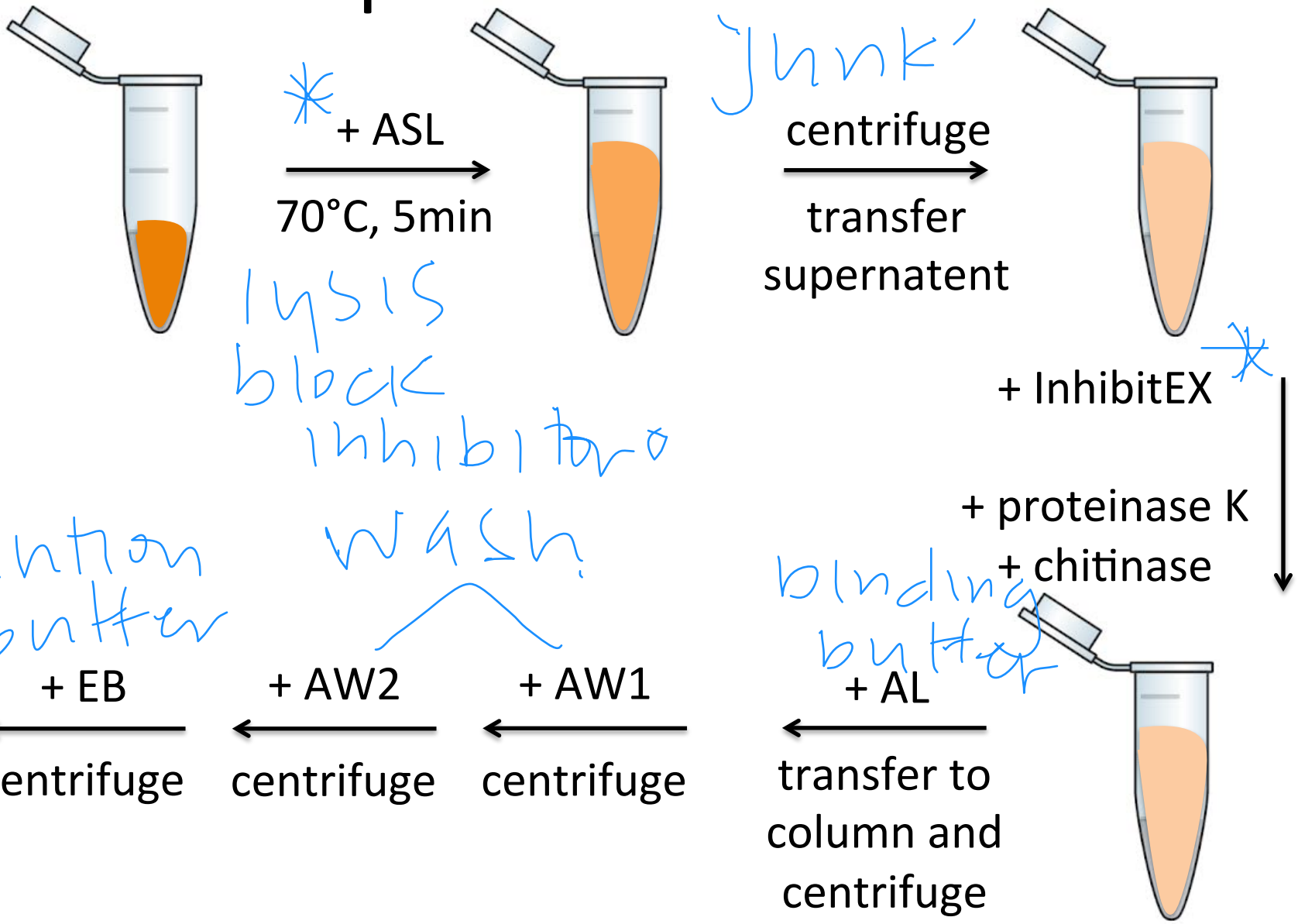


DNA purification

- Why do we need to purify the DNA?

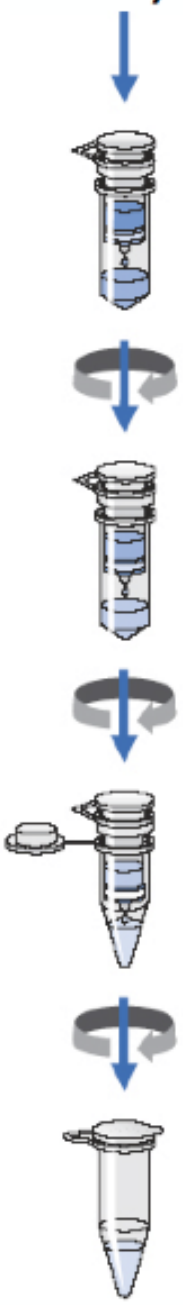


DNA purification overview



DNA binding columns

Stool Lysate



Purified DNA

Bind

charge | size

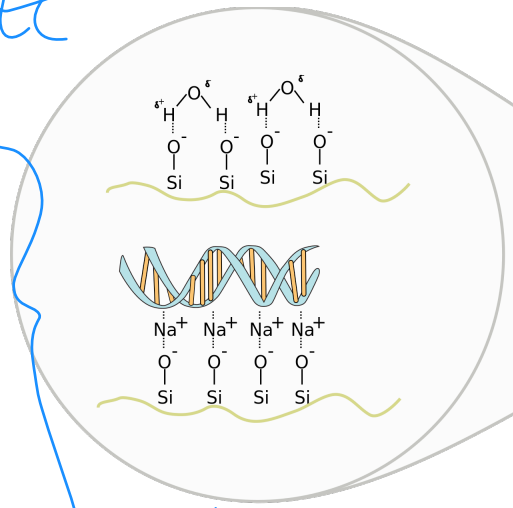
chaotropic salts

Wash

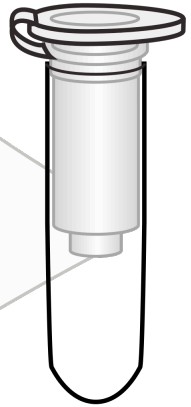
ethanol

Elute

↑ pH 8 low salt



pH 7.5



Important procedural notes

- Keep track of your DNA
- Label your tubes
- Use filtered pipet tips and autoclaved tubes
- Team up for centrifuge spins
- Collect all column waste in conical tube
- Save all used tubes
  - Rinse 2-3x with water into the waste collection stream in fume hood (remember your PPE!)

Today

1. DNA purification up to 56°C, 1.5 hr incubation
2. During incubation…
   - Complete lab practical *treat*
   - Prepare tubes for later steps
3. Finish DNA purification procedure
4. Homework due M2D2
   - Sign-up for OWW and create your people page
   - Take a peak at the journal we will discuss M1D3