







- Announcements
- Pre-lab Lecture
 - ❖ Exp 1 overview
 - ❖ DNA extraction principles
 - ❖ Today in Lab: M1D2

Announcements

- Brief discussion of orientation day quiz
- Remember, *Assignments + Schedule* = our syllabus
- Reflection 4 can be done at *any* time in semester
- Sorry about the Google calendar confusion! Qs?
- Review sensitivity and specificity

	original primers	species-specific primers	sensitive primers	target specificity
<i>E hellem</i>				
<i>V corneae</i>				★ lower concentration

Bird microbial communities: context

- What is our primary research question?

community variation b/w
geo. distant populations

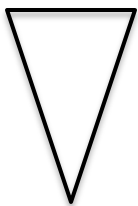
- Broadly, why is it of interest?

bird susceptibility to disease
- zoonotic transfer
- general ecosystem

1.

Bird microbial communities: approach

Bird stool sample



extract DNA

DAY 2

Complex DNA pool
(many pathogen DNAs)



PCR

DAY 3

Simpler DNA pool
(bacterial 16S DNA)

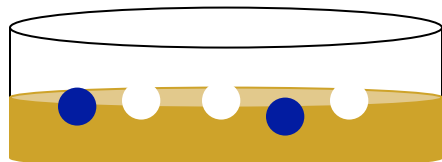


why? conserved - select bacteria
variable - distinguish

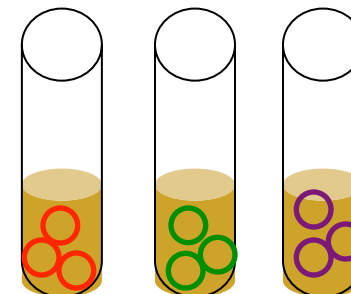
gel purify, clone and transform

DAY 4

Individual 16S cell colonies



liquid culture



isolate DNA clones from bacteria

DAY 5

Individual 16S DNA plasmids



identify, relate, and compare sequences

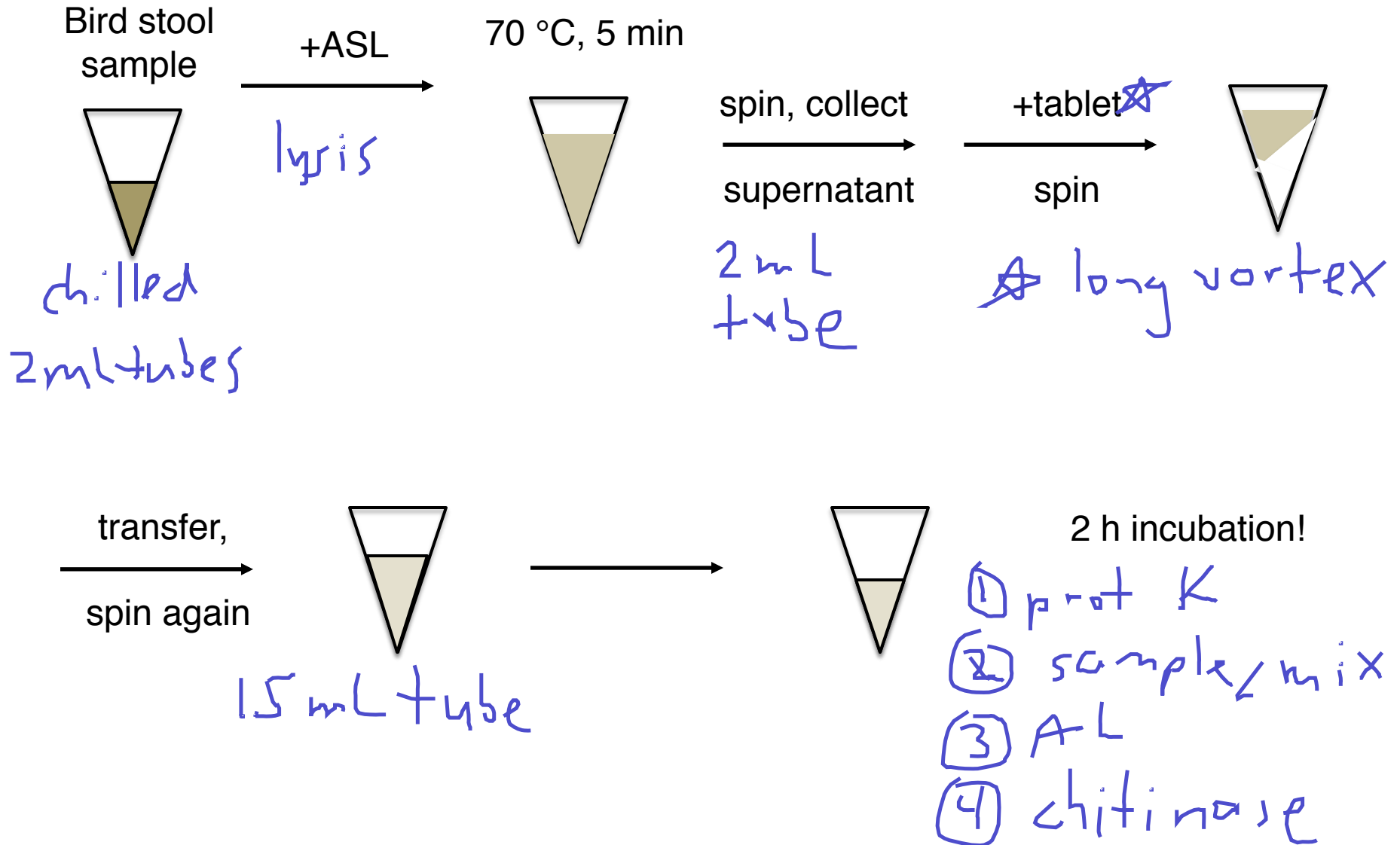
DAY 7

Voilà!

DNA extraction: background

- Stool a complex mixture! Many inhibitors of PCR
 - binders and degraders
- Rid inhibitors
 - proprietary chemicals
 - proteinase K
- Preferential isolation of pathogen DNA
 - high temperature 70°C (human-RT)
- Why chitinase?
 - break open *M. sporidia* ...
someday / maybe

DNA extraction: initial steps



DNA extraction: later steps



Silica resin column

[qiagen.com]

bind DNA — high salt, low pH
 chaotropic salt disrupts
 H-bonds; DNA sticks to column

Keep DNA, wash away, else
 ethanol precipitates DNA

elute DNA — low salt, high pH
 electrostatic repulsion



Today in Lab (M1D2)

- Notebook potentially collected M1D7! Ask Qs.
- **Waste disposal: save all tubes, rinse 2-3 with H₂O wash bottle over marked waste stream in fume hood.**
- DNA extraction through enzymatic incubation
 - Many tube exchanges! May take ~ 45 min
- During 2 h incubation
 - WAC lecture ~ 2:45-3:45 pm
 - Finish primer designs
 - Prepare/label tubes for later steps
- DNA purification on column (~ 30 min)
- FNT: read journal article, prep one slide