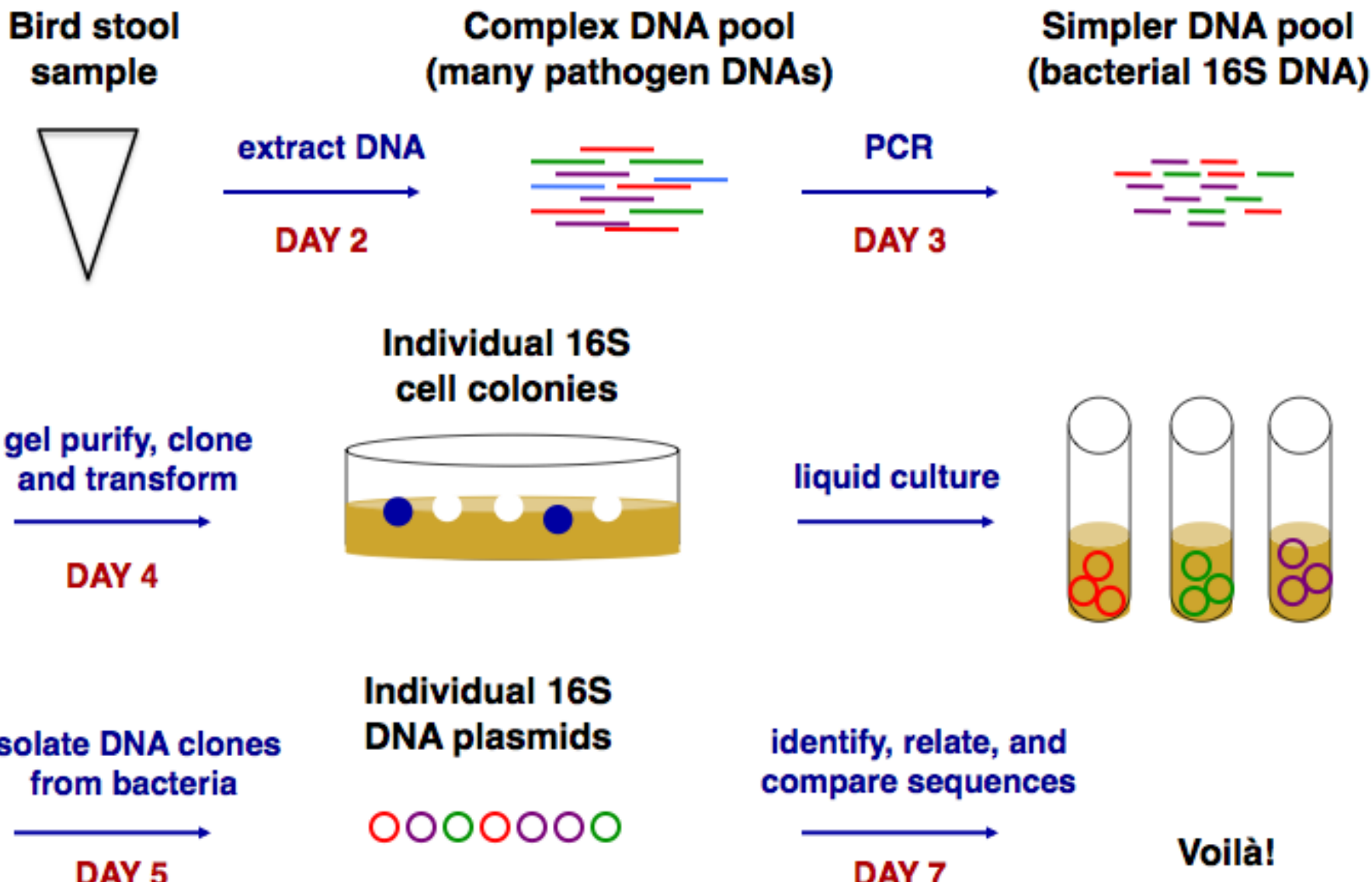
- Announcements
- Lab Quiz
- Pre-lab Lecture
  - Where we are/going
  - Plasmid DNA isolation
  - Sequencing
  - Today in Lab: M1D5

Announcements

- Consider emails we send as being part of our class textbook… as is the wiki of course! (*Assignments* tab is key)
- Discussion of previous FNT: methods and intro
  - methods: great start! keep working to tell *final* concentrations
  - introduction: *motivation* is key element
  - introduction: bird microbiomes and microsporidia primer studies are intellectually separate and are addressed in different assignments
  - primer table/text to be returned next time
- Changes to lab
  - microsporidia primer analysis delayed to M1D7
  - M1D7 Part 2 FNT now bonus assignment due M1D8
- Journal club next time: meet in **16-336**

Bird microbial communities: approach



Where are we/going?

- Construction phase:
  isolate individual bacterial 16S DNA from a pool by cloning into a vector
- Evaluation phase:
  identify individual sequences and evaluate relationships; do phylo. trees differ for MA vs. AK gulls?

Extracting DNA (miniprep)

Step	Contains	Purpose
Prepare	EDTA Buffer, glucose	→ weaken cell envelope → otherwise stable
Lyse	SDS NaOH	→ solubilize lipids, proteins → ds ⇒ ss DNA
Neutralize	Acetic acid/KAc	pH to neutral; precipitate SDS genomic DNA crashes out plasmid renatures
Transfer	N/A	*supernatant* isolate plasmid
Wash, collect	A) Column B) EtOH, dry	extra purification precipitate DNA, resuspend

remove interferents w/ vortex

Sequencing reactions

Dideoxy method: no 3' OH → can't elongate
Run 4 rxns: (d)dT, dA, dG, dC and 3 others

different fluorophore
ddG green
ddA blue

primer →

upstream

Reactions

ddT

TAAATT
AT*
ATT*
ATT*

ddA

TAAATT
A*
ATTTA*
ATTTAA*

Gel

ddT	ddA
	6bp
	16bp

* = radioactive or fluorescent label

Limitations: 1000 bp reads, unreliable at first and last several bp

Today in Lab (M1D5)

- Extract DNA from eight clones each(!)
  - one approach: two staggered shifts
- Measure DNA ★spot-check Beach, share blanks★
  - 260 nm, nucleic acids → concentration
  - 280 nm, proteins → purity ratio
- Set up 2 sequencing rxns per clone
  - use multichannel pipet for primers
- Count colonies
- Brief optional survey 4:40-5 pm if everyone done