M1D5: DNA Sequencing

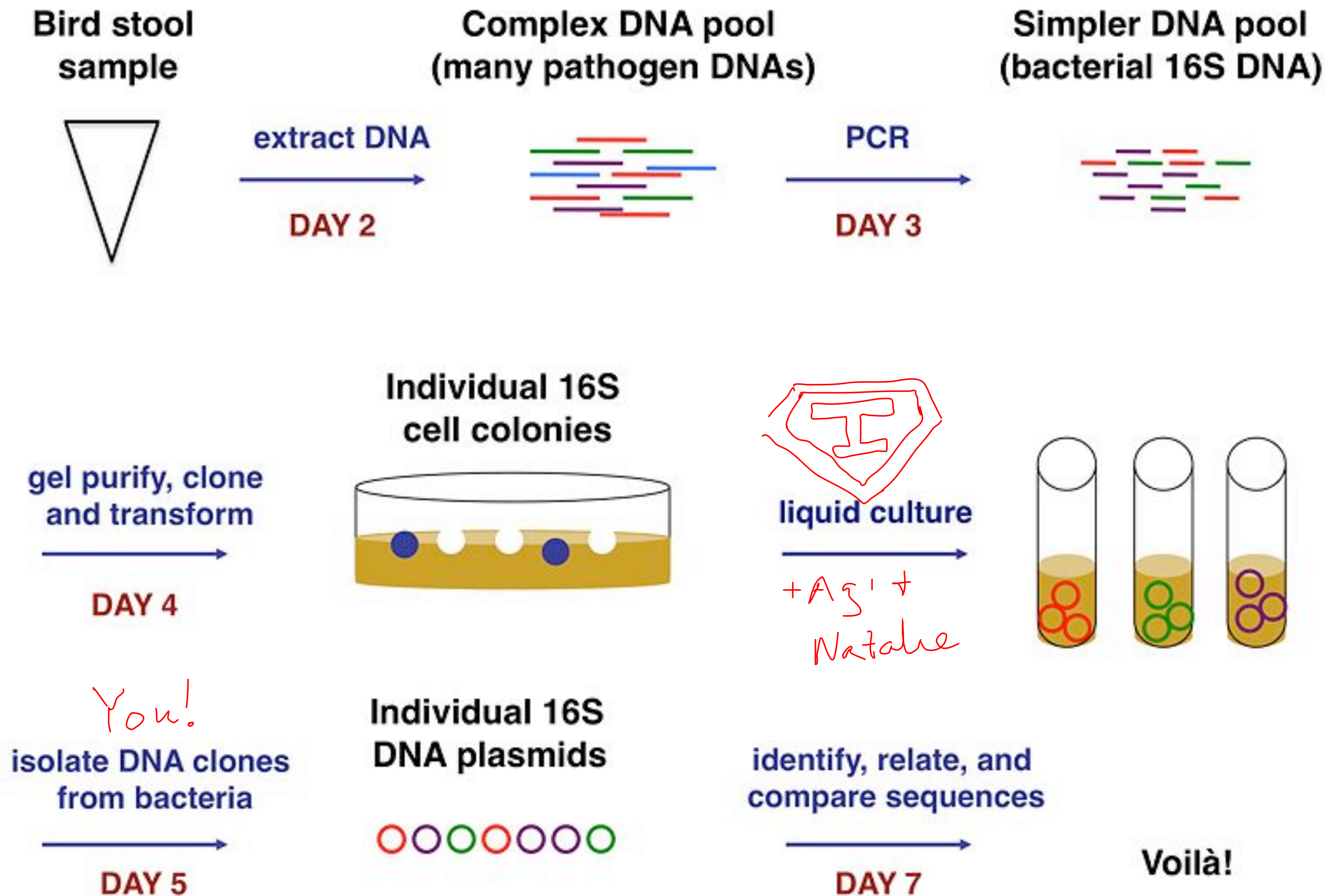
2/27/13

Announcements

The first thing I ate!

- Lab treat today!
- Journal club next time: Meet in **16-336**
- Discussion of M1D3 FNT: Returned via email or handout
  - Methods: Great start! **Concentrations** are key.
  - Methods: Cite the manufacturer's protocol, keep it concise!
  - Introduction: **Motivation** is the key element
  - Introduction: Report NOT about microsporidia
  - Primer design table: Read the wiki about this assignment!
  - *These are good drafts! Office hours: Thursday, 7:30-8:30pm*
- Changes to FNT due today: Results outline due by Thursday midnight if you want to revise due to microsporidia content.
- Changes to lab
  - microsporidia primer analysis delayed to M1D7
  - M1D7 Part 2 FNT is now a bonus assignment – due M1D8

Bird Microbial Communities -- Experimental Overview



Bird Microbial Communities -- What's Left?

Experimental Phase: Isolated bacterial 16S rRNA gene from stool sample from seagulls from MA/AK by cloning technique

Analysis Phase: Identify individual sequences & evaluate phylogenetic diversity

Motivation
- Big Picture: Disease transmission through non-human host (influenza)
- Narrow: Δ microbiome due to geography

Overview: Plasmid Purification -- Miniprep

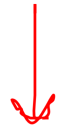
Clean it up!

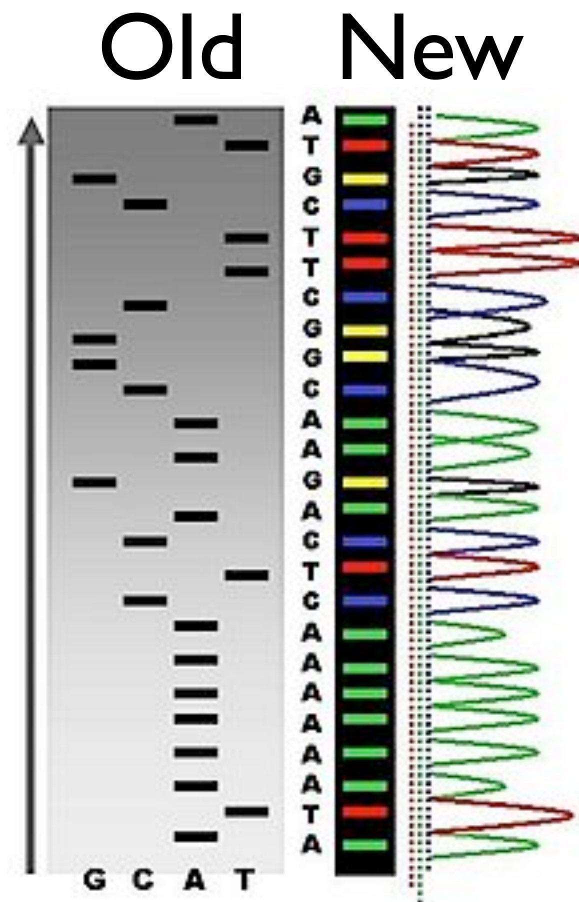
Step	Contents	Purpose
Prepare	EDTA Buffer, glucose	perm cell membrane keep happy
Lyse	SDS NaOH	solubilize lipids + proteins denature
Neutralize	Acetic Acid/KAc	renature plasmid precip SDS
Transfer	KEEP SUPERNATANT	
Wash	A) Column B) EtOH, dry	similar to DNA extraction Concentrate the plasmid

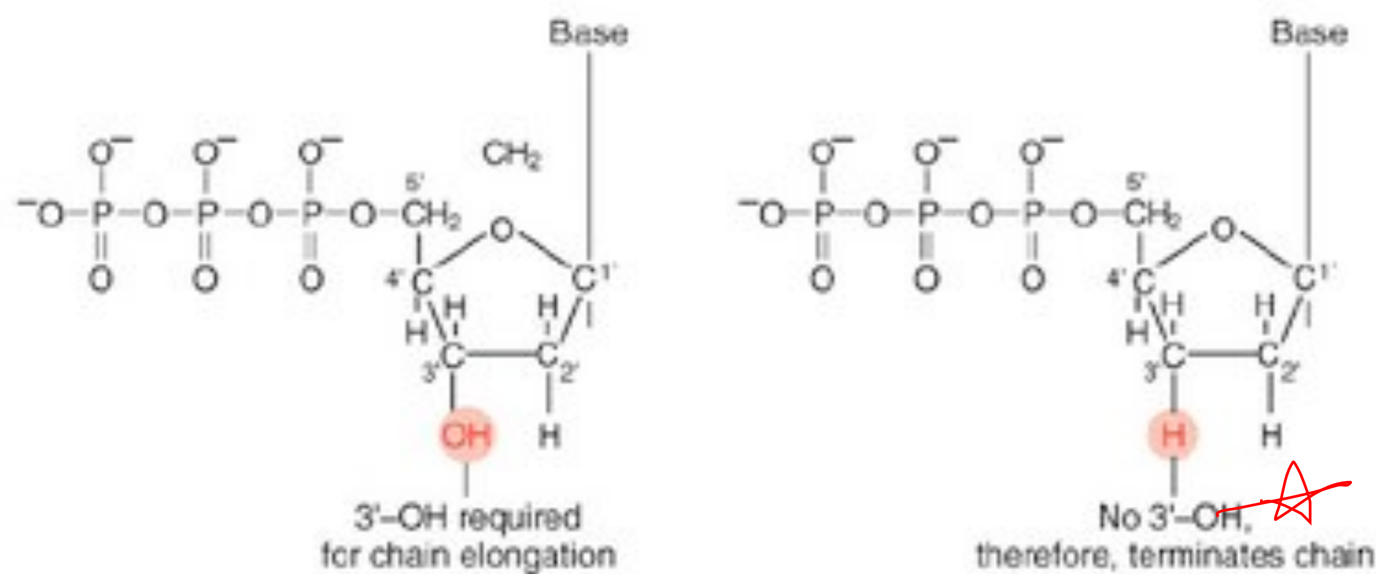
Overview: Sanger Sequencing

Four Rxns with labeled d(dNTPs)!!

~~Four dye labeled dideoxynucleotides added to each reaction~~







'Chain terminating reaction'

What primers do we add? Primers that are outside of your desired sequence

Today in lab:

- Extract DNA from 8 (!) clones
  - may choose to do this in shifts
- Measure DNA concentration
  - 260 nm all nucleic acids for concentration
  - 280 nm, proteins for purity
- Set up duplicate sequencing reactions for each clone
  - use the multichannel pipet for primer addition
- Count colonies!
- Have a most wonderful Wednesday evening!