M1D5: DNA Sequencing

2/24/15

Office Hours this week:
Thursday
Leslie, 1-2pm in 16-429C
Noreen, 2-4 pm in 16-429C

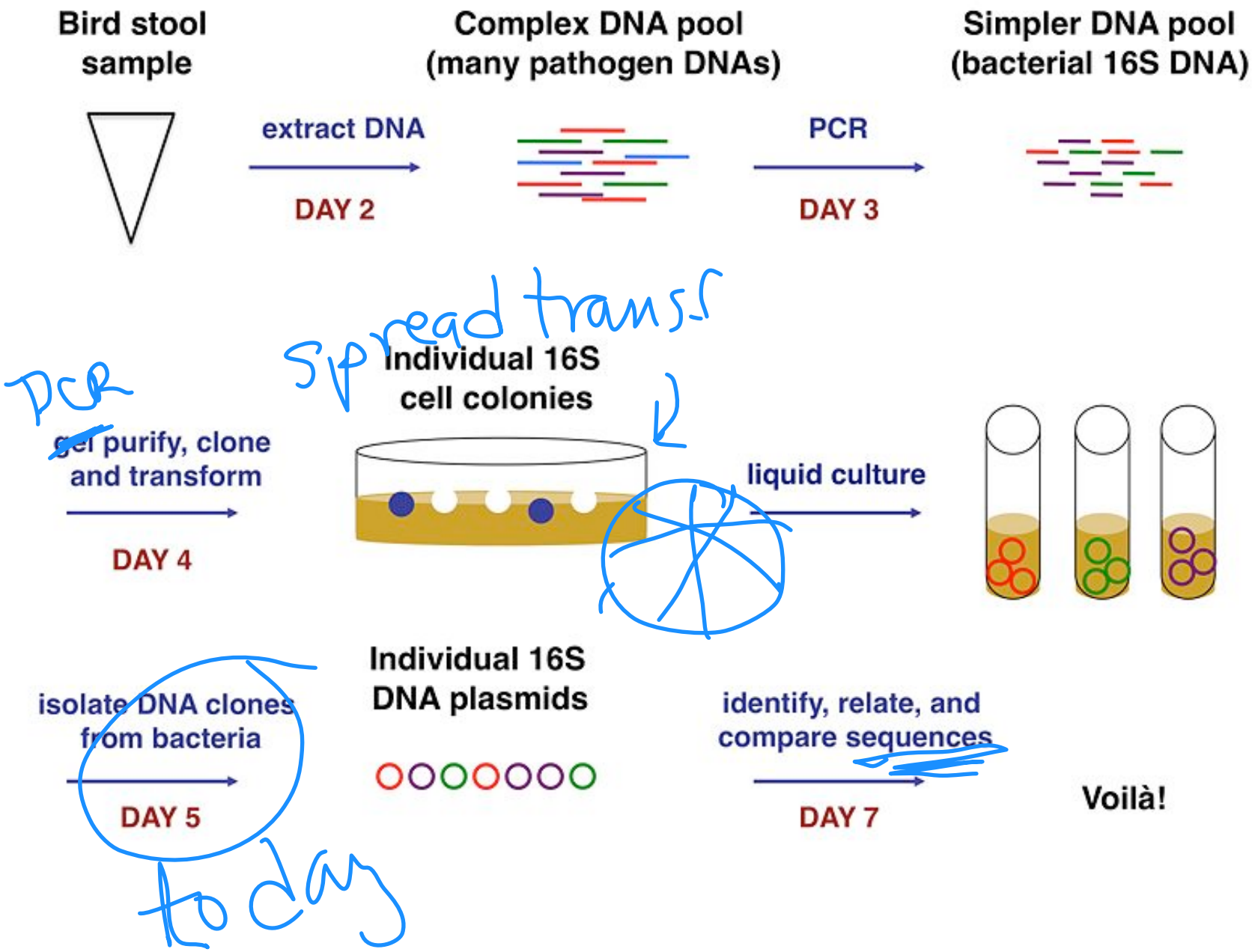
Announcements

- Lab treat today!

- Journal club next time: Meet in **16-336**
  - Presentation order will be determined by upload order on Stellar
  - M1D6 presenters at 1:15pm to setup
  - Presentations start at 1:30pm SHARP

- M1D3 Homework — Noreen will return asap.

Bird Microbial Communities -- Experimental Overview



Overview: Plasmid Purification -- Miniprep

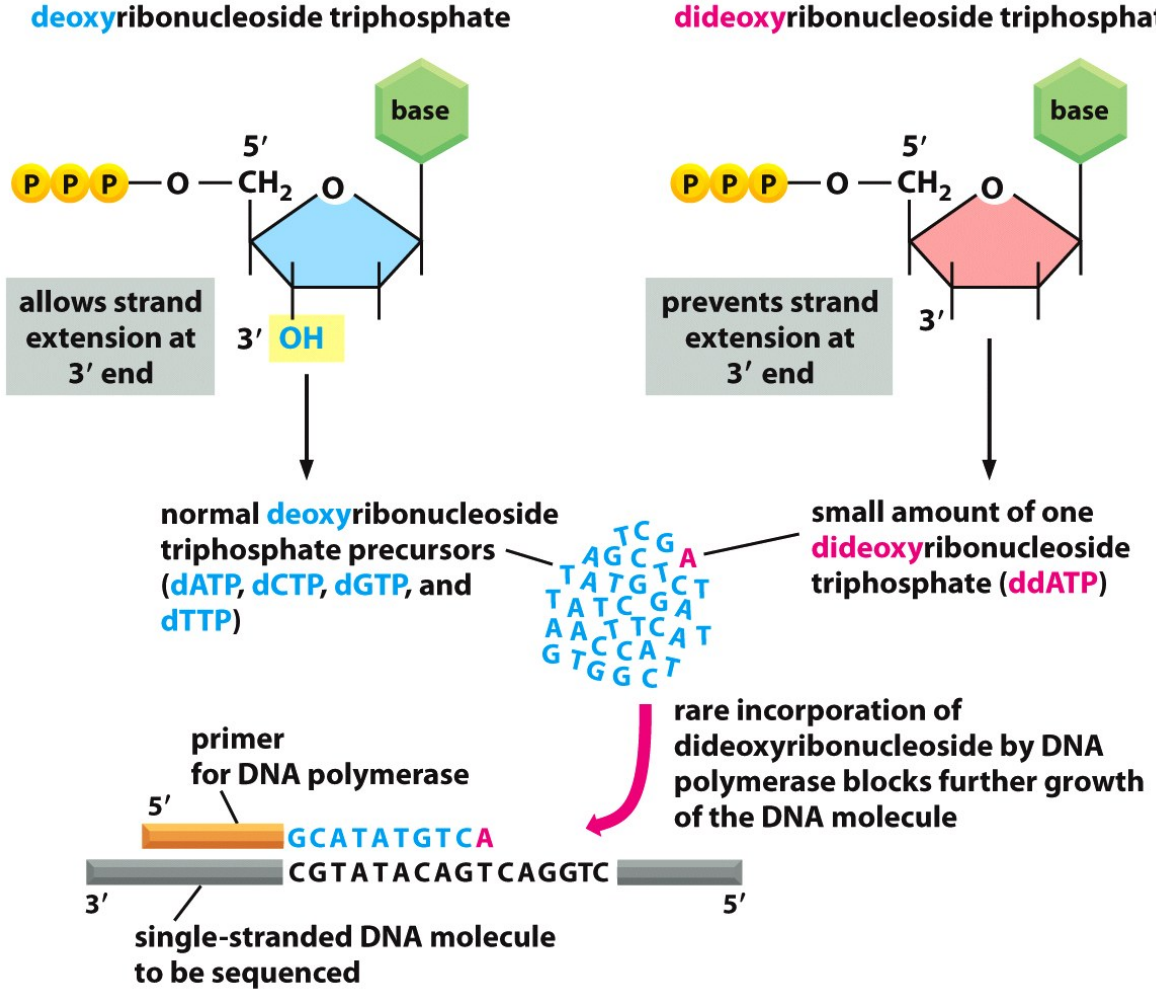
Clean it up!

Step	Contents	Purpose
Prepare	Tris & EDTA Buffer	
Lyse	SDS NaOH	
Neutralize	Acetic Acid/KAc	
Concentrate	Spin all	
Wash	EtOH, dry	

Overview: Sanger Sequencing

Four dye labeled dideoxynucleotides added to each reaction

'Chain terminating reaction'

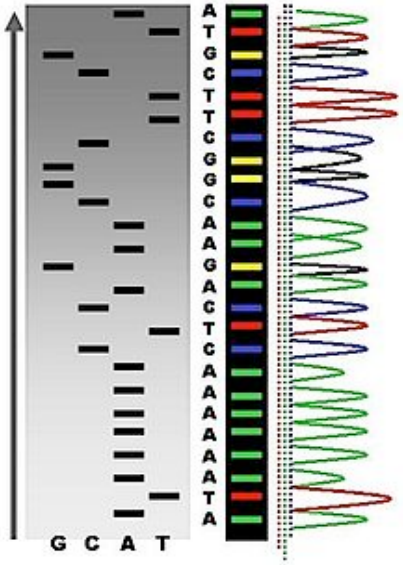


https://www.youtube.com/watch?v=nudG0r9zL2M

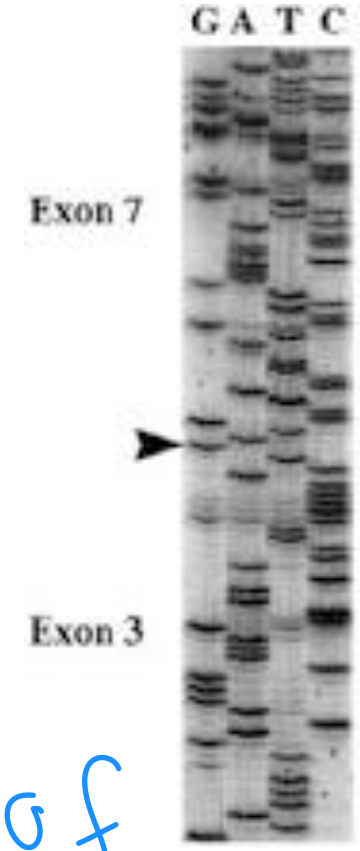
Overview: Sanger Sequencing

Four dye labeled dideoxynucleotides added to each reaction

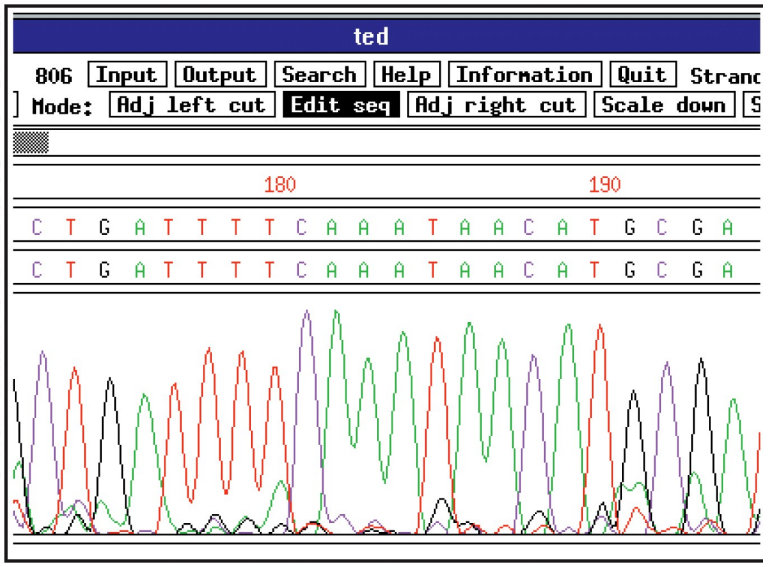
Old New



Old



New

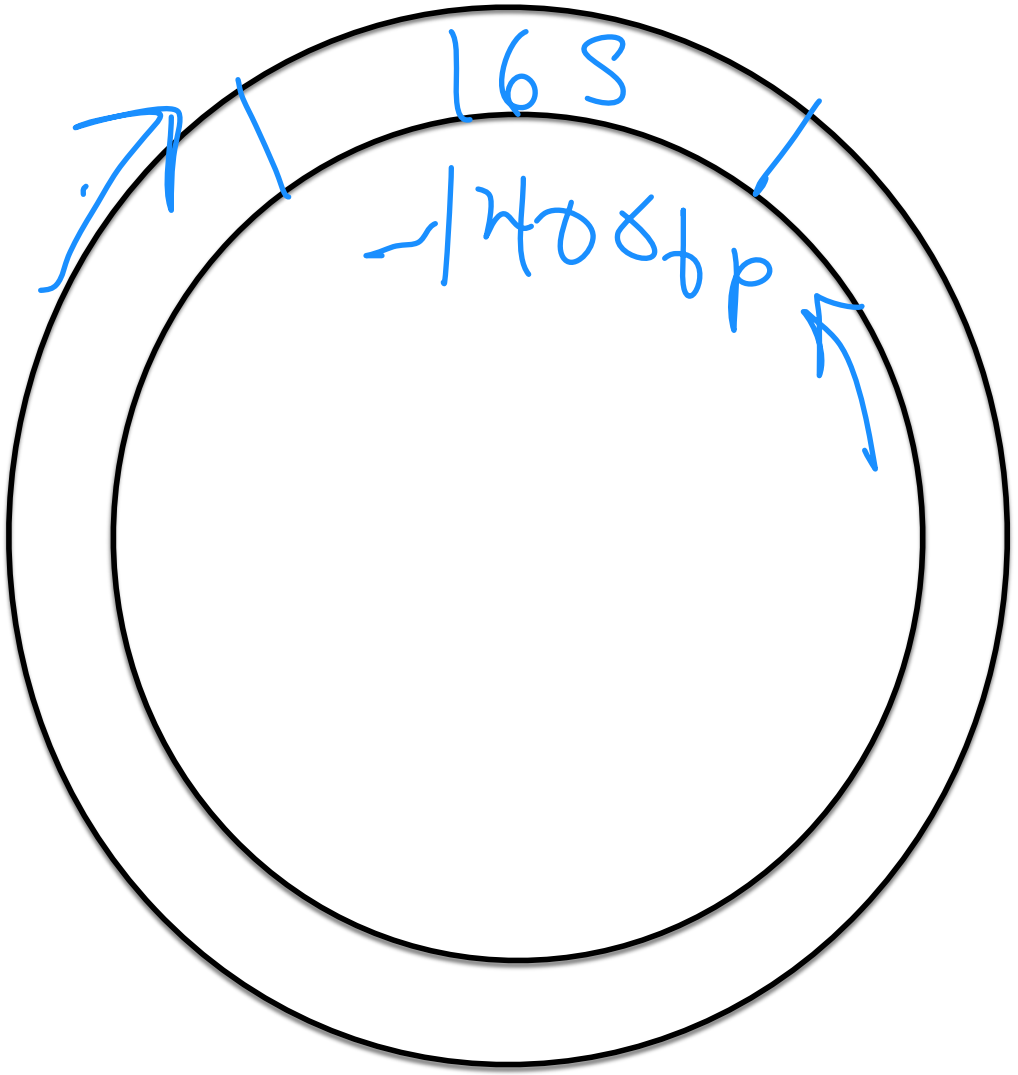


What primers do we add?

outside seq of interest

good rxn 900-1000bp

Overview: 16S rRNA sequencing



Today in lab:

- Extract DNA from 8 (!) clones ***LABEL TUBES***
  - 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 (why?)
- Set up qPCR reactions using your AIV sequencing primers.
- Count colonies!
- Have a most wonderful Wednesday evening! It's above 0°F!!