M1D5: DNA Sequencing

2/24/15

Office Hours this week:

Wednesday 4-5pm in 16-319
Friday 3-4 pm in 16-319

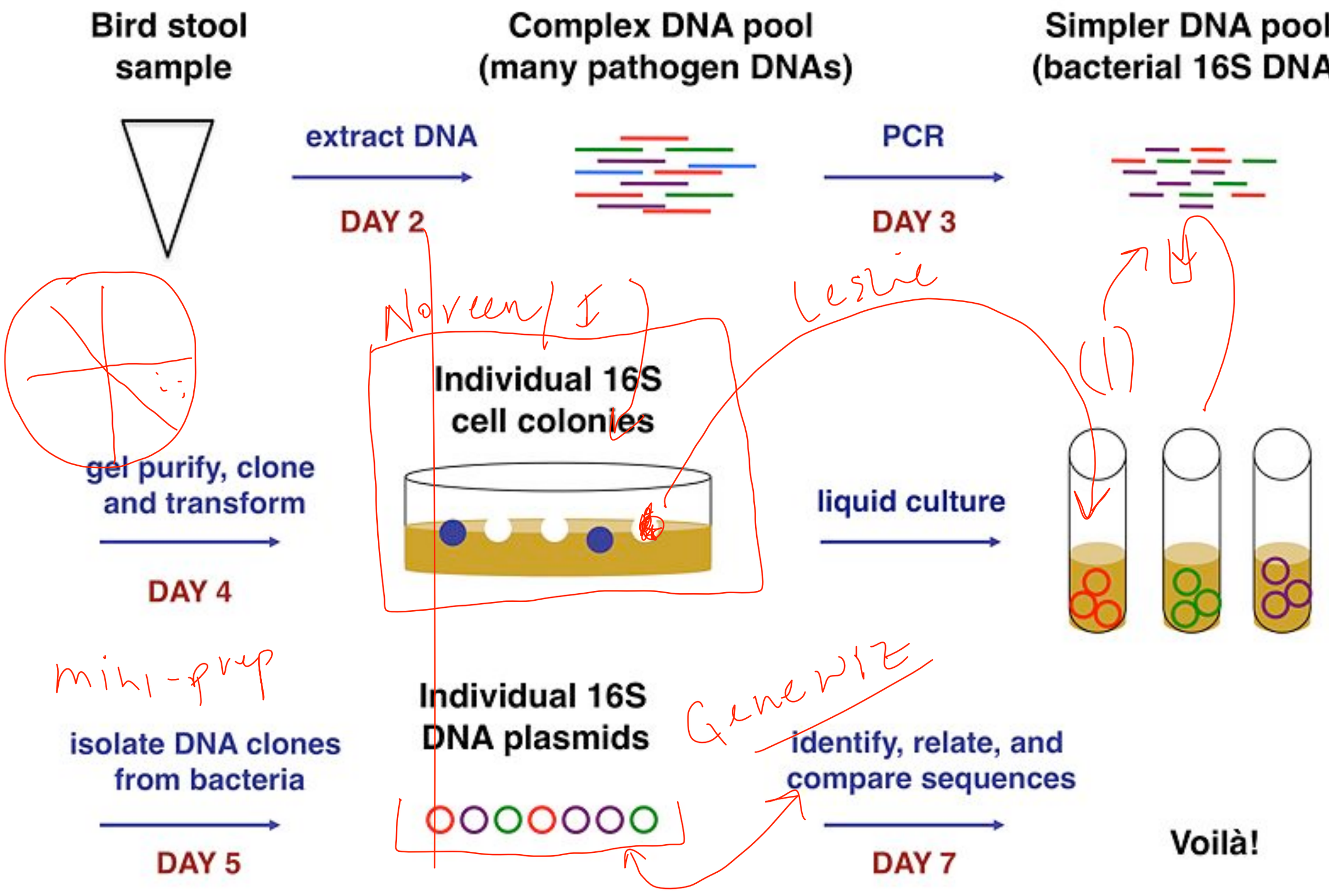
Announcements

- 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 — most will be returned on Thursday.

Bird Microbial Communities -- Experimental Overview



Overview: Plasmid Purification -- Miniprep

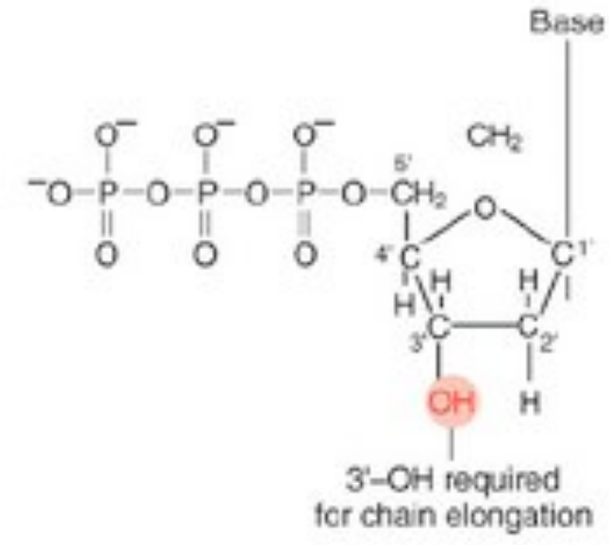
Clean it up!

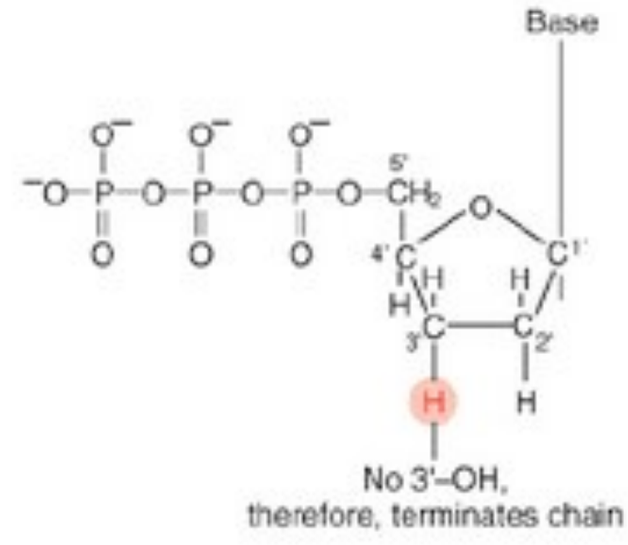
Step	Contents	Purpose
Prepare	Tris & EDTA Buffer	1) resuspend 2) weaken membrane
Lyse	SDS, NaOH	precipitate protein, denature DNA
Neutralize	Acetic Acid/KAc	↓ pH, refold DNA (only plasmid)
Concentrate	Spin all	10 min, max speed; Keep Sup
Wash	EtOH, dry	elute pH 8 water

Overview: Sanger Sequencing

Four dye labeled dideoxynucleotides added to each reaction

'Chain terminating reaction'

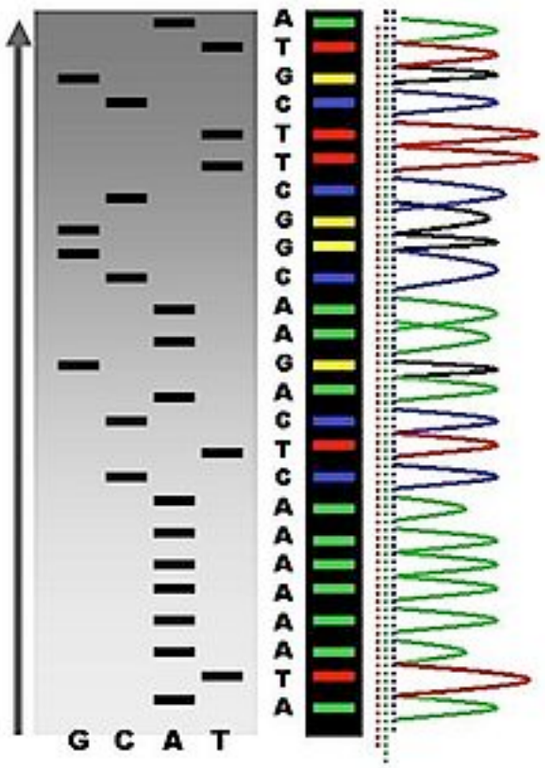


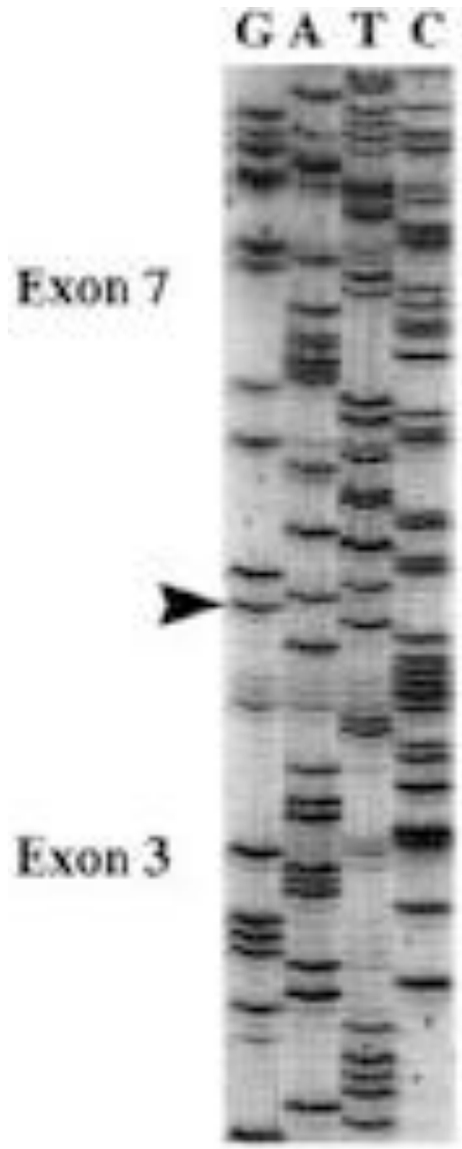


https://www.youtube.com/watch?v=nudG0r9zL2M

Overview: Sanger Sequencing

Four dye labeled dideoxynucleotides added to each reaction





What primers do we add?

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 Tuesday evening!