# MID5: DNA Sequencing

### 2/24/15

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

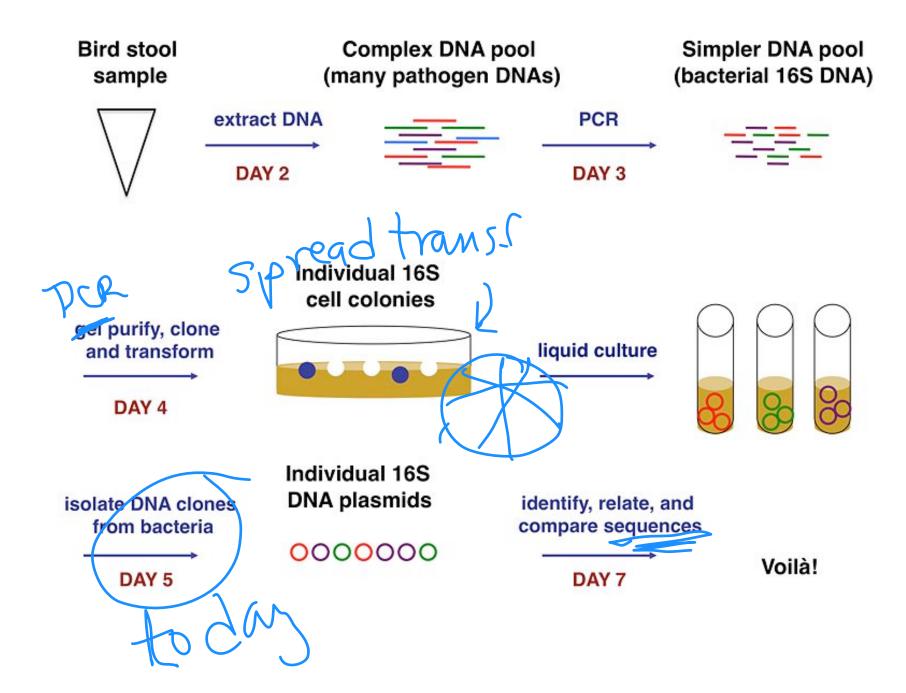
## <u>Announcements</u>

• Lab treat today!



- Journal club next time: Meet in 16-336
  - Presentation order will be determined by upload order on Stellar
  - MID6 presenters at 1:15pm to setup
  - Presentations start at 1:30pm SHARP
- MID3 Homework Noreen will return asap.

### Bird Microbial Communities -- Experimental Overview



**Overview: Plasmid Purification -- Miniprep** 

### Clean it up!

Step	Contents	Purpose	6
Prepare	Tris & EDTA Buffer	2) Cations No	eal
Lyse	SDS NaOHTPH	précip denatures D	nei ) N
Neutralize	Acetic Acid/KAc	VpH refold	
Concentrate	Spin all	plasmid sur	pe
Wash	EtOH, dry	048	
		DNA + CUN	UW

#### **Overview: Sanger Sequencing**

### Four dye labeled dideoxynucleotides added to each reaction 'Chain terminating reaction'

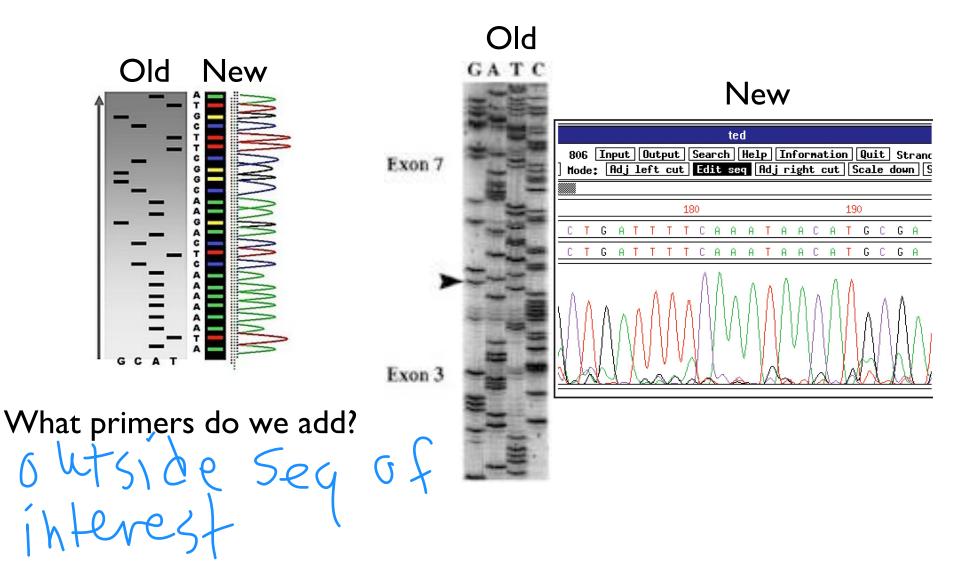
deoxyribonucleoside triphosphate dideoxyribonucleoside triphosphate base base P-0-CH<sub>2.0</sub> -0-CH2 ,0 allows strand prevents strand 31 extension at 3' OH extension at 3' end 3' end normal deoxyribonucleoside small amount of one **dideoxy**ribonucleoside triphosphate precursors (dATP, dCTP, dGTP, and triphosphate (ddATP) **dTTP** rare incorporation of primer dideoxyribonucleoside by DNA for DNA polymerase polymerase blocks further growth 5' of the DNA molecule GCATATGTCA CGTATACAGTCAGGTC 3' 5' single-stranded DNA molecule

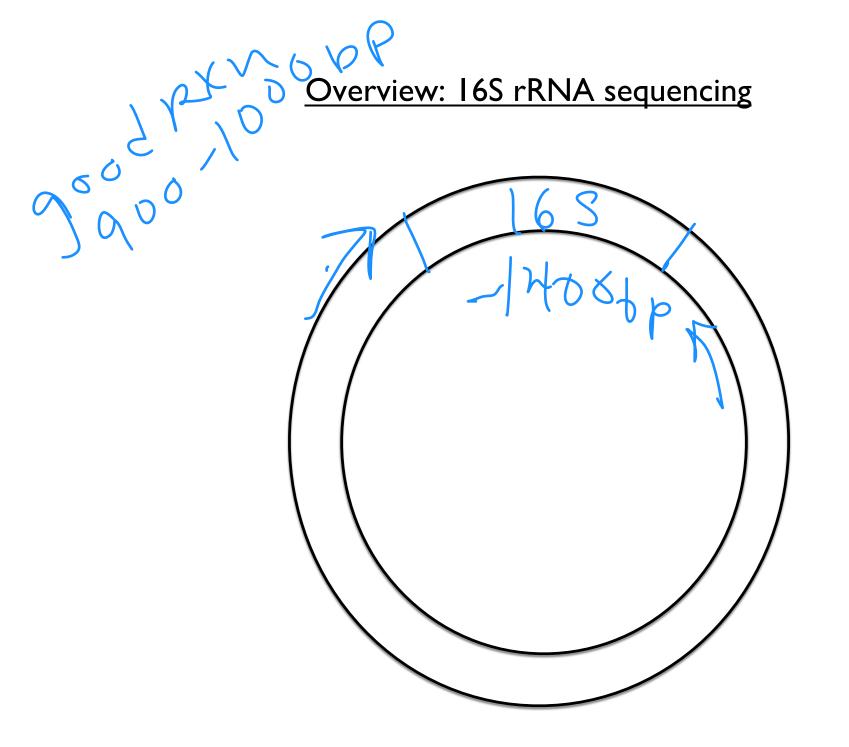
https://www.youtube.com/watch?v=nudG0r9zL2M

to be sequenced

### **Overview: Sanger Sequencing**

Four dye labeled dideoxynucleotides added to each reaction





### 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!!