Mod 1 Day 5: DNA Sequencing

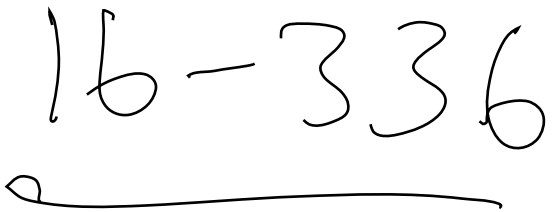
2/25/2014

Lab quiz!

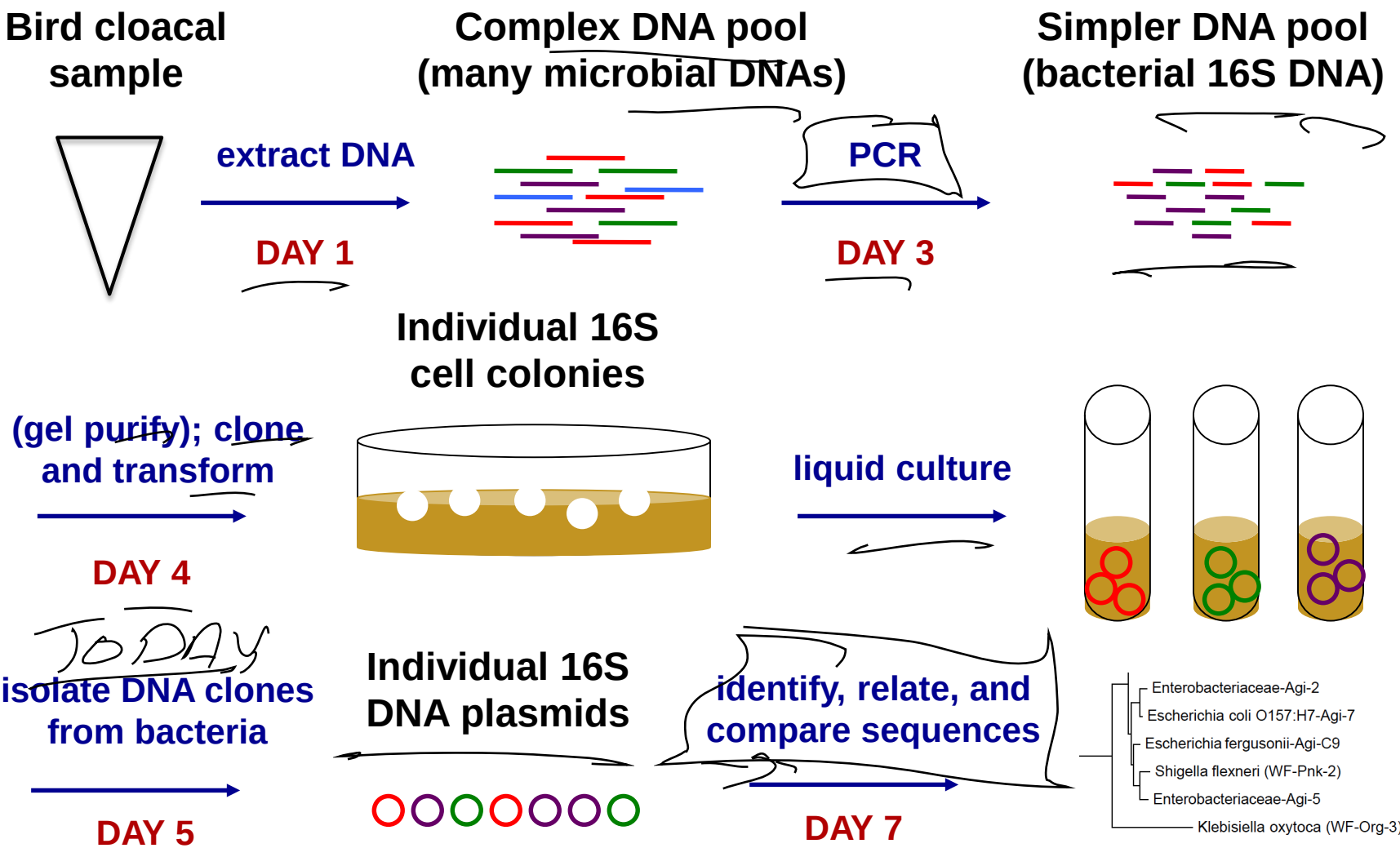
1. **Module overview**
2. **What's next?**
3. **Plasmid DNA isolation**
4. **Sequencing**
5. **Today in lab**

Announcements

- **Lab Quiz**
- **Journal Club: starts at 1:30pm sharp!**
  Presenters – 1:15pm to set up



Bird microbial communities: review

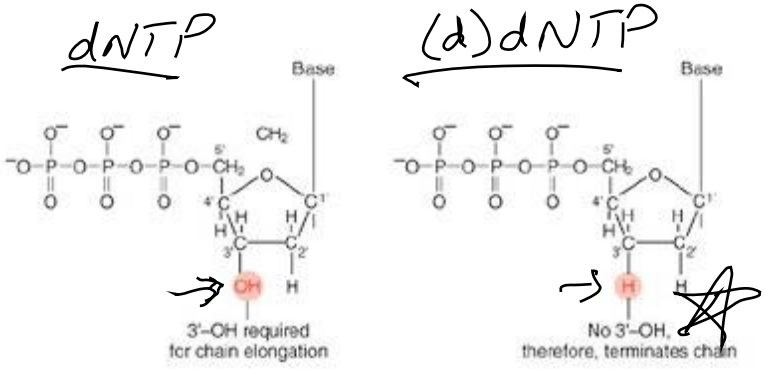


Extracting DNA (miniprep)

Step	Contains	Purpose
Prepare (resuspend)	EDTA, Tris Buffer, glucose	Permeate memb's, prep for lyse. Health + happiness
Lyse	SDS, NaOH	Solubilize lipids + proteins. Denature dsDNA → ssDNA
Neutralize (pH)	Acetic acid, KAc	Genomic DNA crashes out, Plasmid DNA renatures. (SDS precip)
Transfer to Silica column	KEEP	SUPERNATANT
Wash	Silica column	Extra purification
Elute	EtOH, dry	Precip DNA, resuspend in H_2O

Sequencing reactions

Dideoxy method: no 3' OH → can't elongate



Run 4 reactions:

#1: (d)dT, dA, dG, dC
#2: dT, (d)dA, dG, dC
#3: dT, dA, (d)dG, dC
#4: dT, dA, dG, (d)dC

Reactions

#1 TAAATT

AT*
ATT*
ATTT*

TAAATT

A*
ATTTA*
ATTTAA*

Gel

ddT	ddA

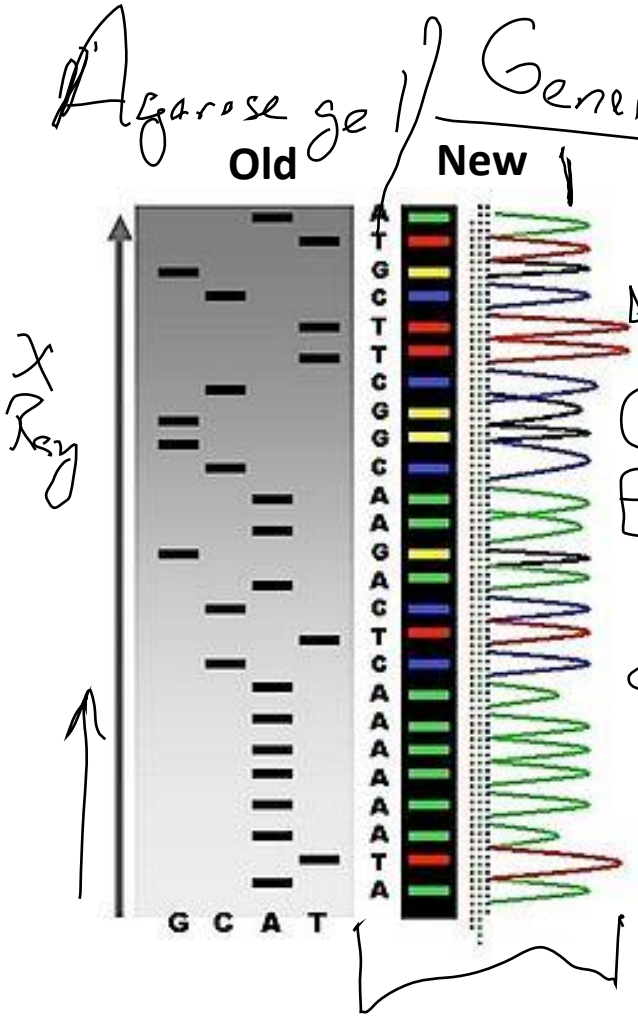
Size: High / Low

* = radioactive or fluorescent label

Limitations: 1000 bp max, unreliable at first and last several (~50) bp

Sanger Sequencing overview

INSTEAD: Four reactions with dye-labelled d(dNTPs)

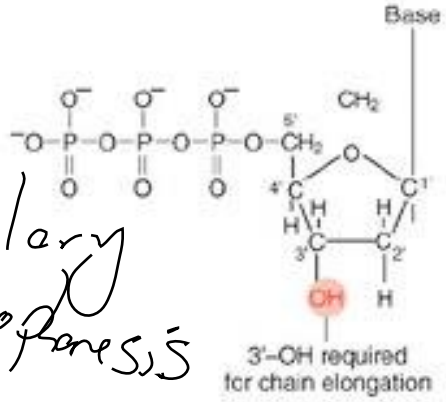


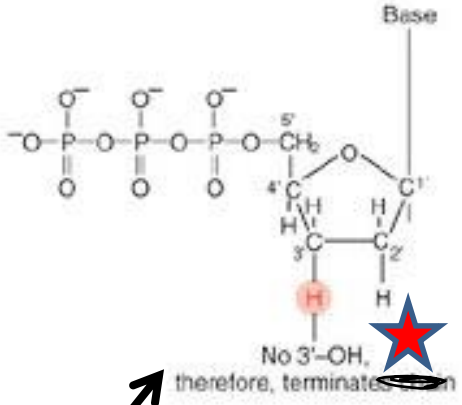
Agarose gel / Genewiz

X Ray

Capillary Electrophoresis + detection Software

A → green T → red





Fluorophore on chain terminating ddNTP

BLAST → Basic Local Alignment Search Tool

Today in Lab (M1D5)

- Extract DNA from eight clones each(!)
  - one approach: two staggered shifts
- Measure DNA Beer's Law
  - 260 nm, nucleic acids → concentration
  - 280 nm, proteins → purity ratio 260:280
- Set up 2 sequencing rxns per clone 1.8:1
  - Multichannel pipet for primers FWD + REV
- Count colonies