Mod 1 Day 5: DNA Sequencing

2/26/2014

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 1:15pm sharp!**
  Presenters – 1:05pm to set up

  Room 16-336

Bird microbial communities: review

Bird cloacal sample

extract DNA

DAY 1

Complex DNA pool (many microbial DNAs)

PCR

DAY 3

Simpler DNA pool (bacterial 16S DNA)

(gel purify); clone and transform

DAY 4

Individual 16S cell colonies

liquid culture

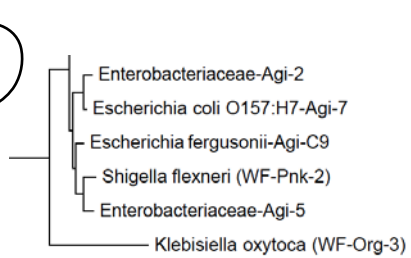
isolate DNA clones from bacteria

DAY 5

Individual 16S DNA plasmids

identify, relate, and compare sequences

DAY 7



ID & Compare

Extracting DNA (miniprep)

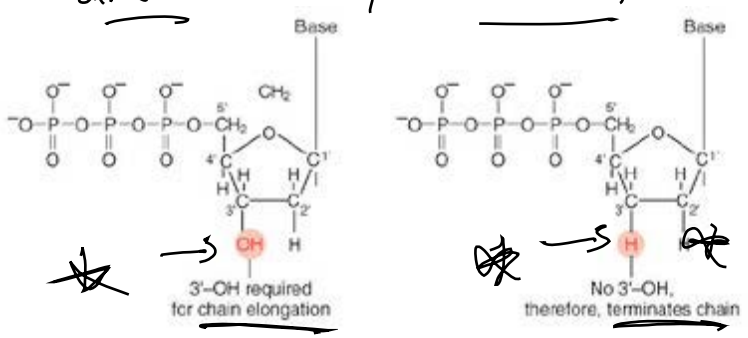
Pellet Cells ↔ SAME EPP + SAME BACT.

Omega Kit

Step	Contains	Purpose
Prepare (resuspend)	Tris EDTA Buffer, glucose	aid lyse, permeate mmb → health & happiness
Lyse	SDS NaOH	→ Solubilizes proteins & lipids → denatures dsDNA → ssDNA
Neutralize pH	Acetic acid KAc	Genomic DNA crashes out, Plasmid DNA renatures, SDS preps.
Transfer to Silica column	KEEP	SUPERNATANT
Wash	Silica column	Extra purification
Elute	EtOH, dry	Precip plasmid

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

ddT TAAATT

- *AT*
- *ATT*
- *ATTT*

ddA TAAATT

- *A*
- *ATTTA*
- *ATTTAA*

Gel

ddT ddA

Large

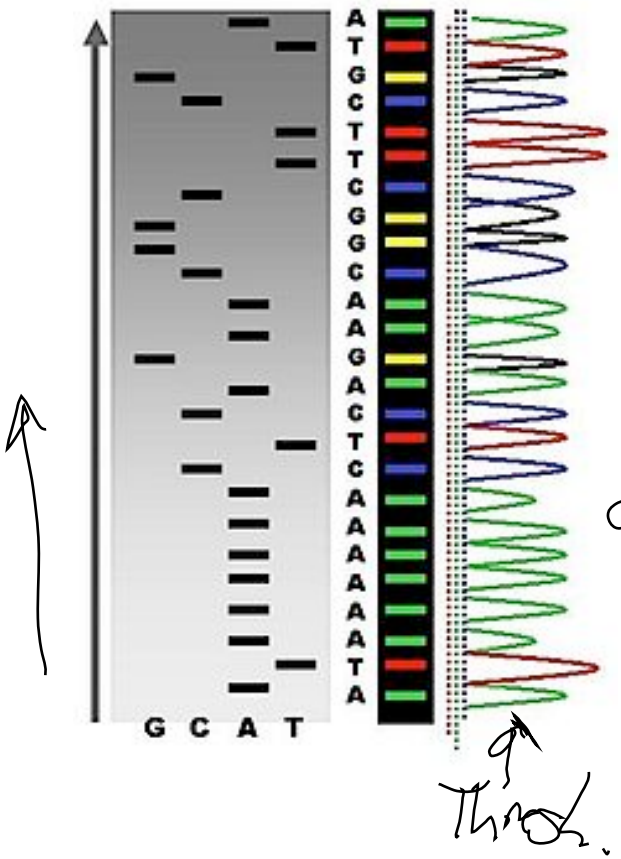
Small

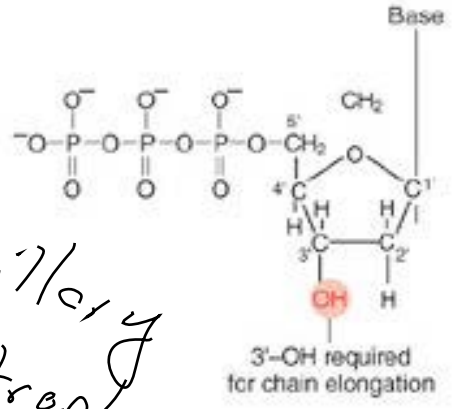
* = radioactive or fluorescent label

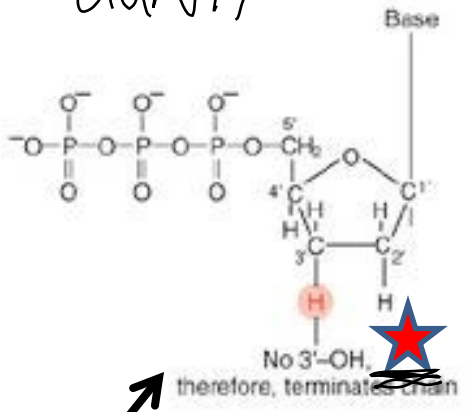
Limitations: 1000 bp max, unreliable at first and last several (~50) bp

Sanger Sequencing overview

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







Fluorophore on chain terminating ddNTP

Today in Lab (M1D5)

- Extract DNA from eight clones each(!)
  - one approach: two staggered shifts
- Measure DNA
  - 260 nm, nucleic acids → concentration
  - 280 nm, proteins → purity ratio
  - Need 3-4 groups to stagger their mini-preps
- Set up 2 sequencing rxns per clone
  - Multichannel pipet for primers
- Count ~~colonies~~
- **SAVE: DNA and agar plates**

Beer's Law

Purity
260 : 280
1.8 : 1