# Molecular Methods in Microbial Ecology

**Contact Info:** Kristin Gribble, kgribble@mbl.edu

**Schedule:**

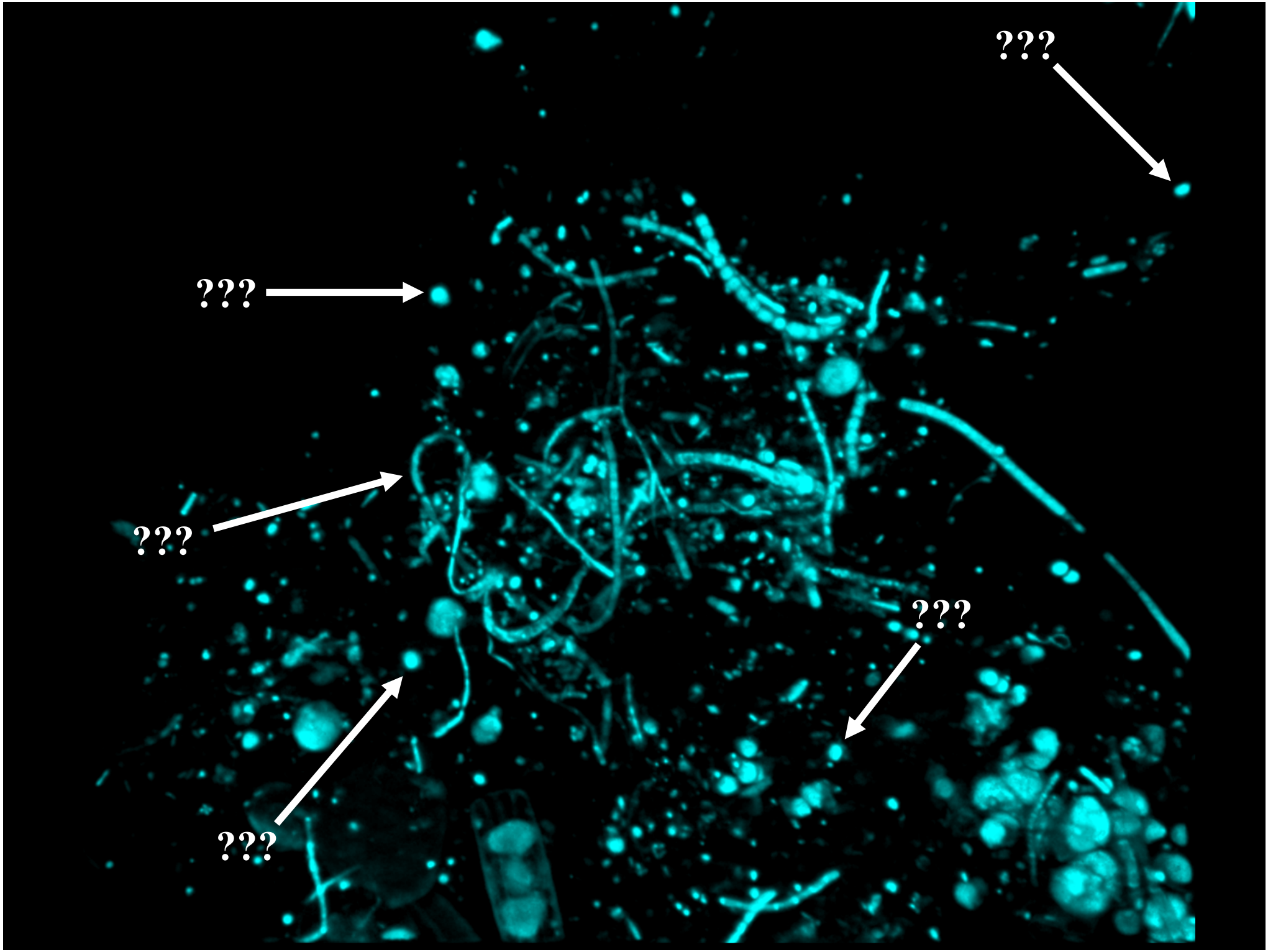
| | | |
|---|---|---|
| Tuesday | 10/26/21 | Introduction,<br>Extraction of DNA from Winogradsky columns<br>Run DNA products on gel |
| Thursday | 10/27/21 | Lecture on PCR,<br>Prepare PCR reactions |
| Tuesday | 11/2/21 | Analyze PCR results, Lecture |

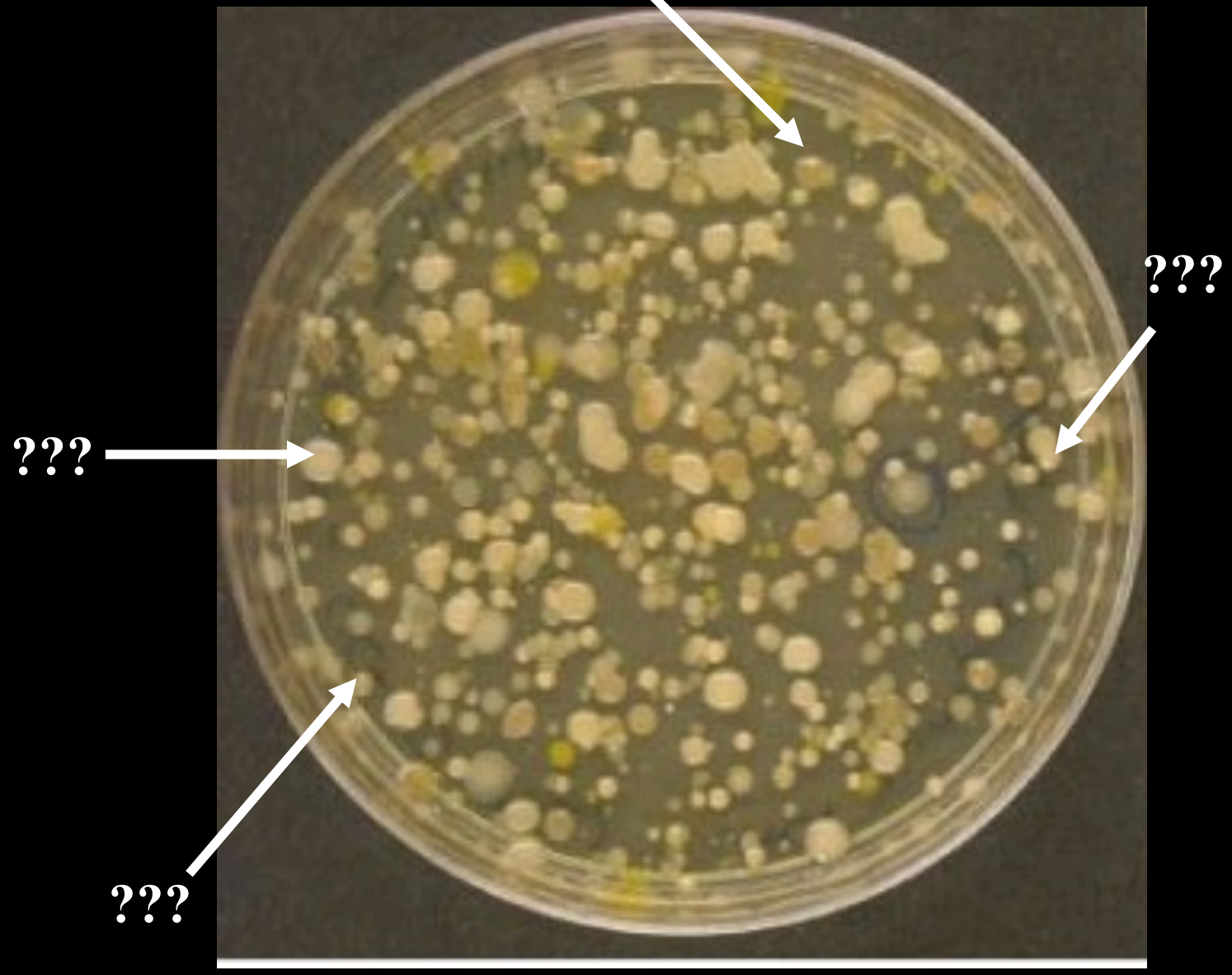
**Readings:** Head *et al*. 1998. Microbial Ecology 35: 1-21.

# Day 1

- Introduction to molecular methods in microbial ecology
- Extract DNA from Winogradsky Columns
- Run DNA on agarose gel


OregonDiscovery.com





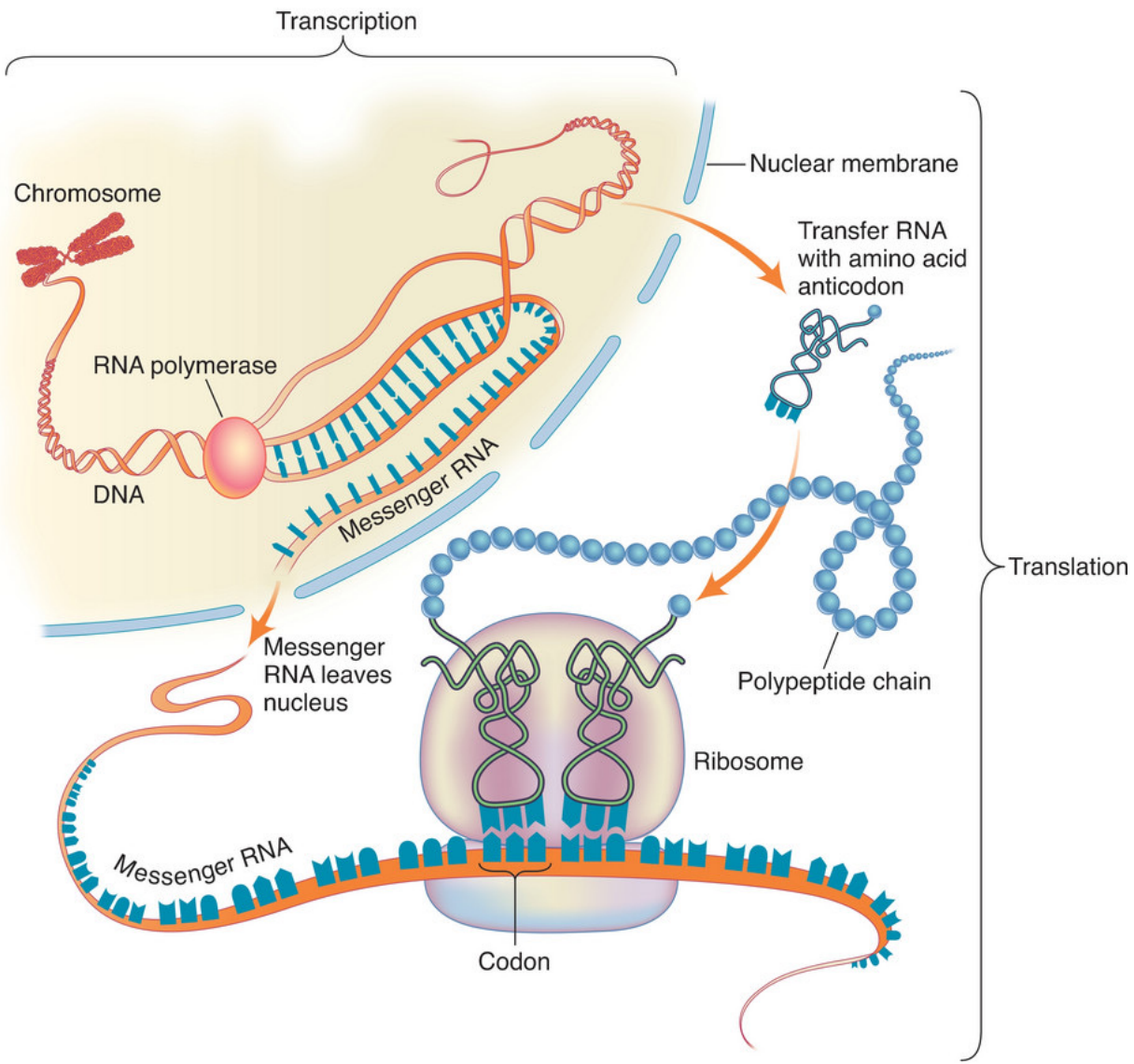
# The Challenge for Microbial Ecology

| Habitat | Culturability (%) |
|---|---|
| Seawater | 0.001-0.1 |
| Freshwater | 0.25 |
| Sediments | 0.25 |
| Soil | 0.3 |

**How do you study something you can't grow in the lab?**

From Amann *et al.* 1995 Microbiological Reviews

# The Central Dogma



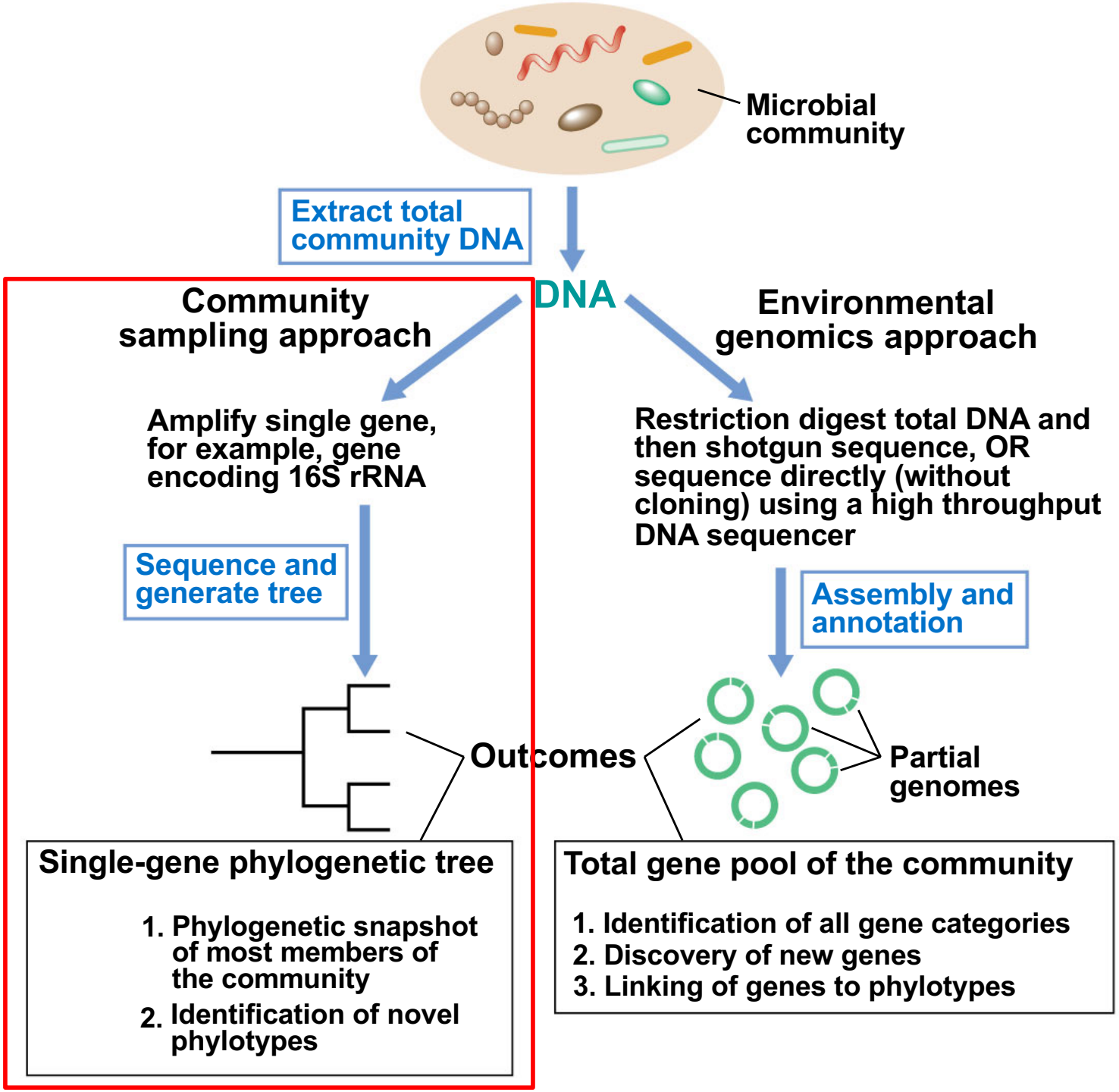

**DNA**

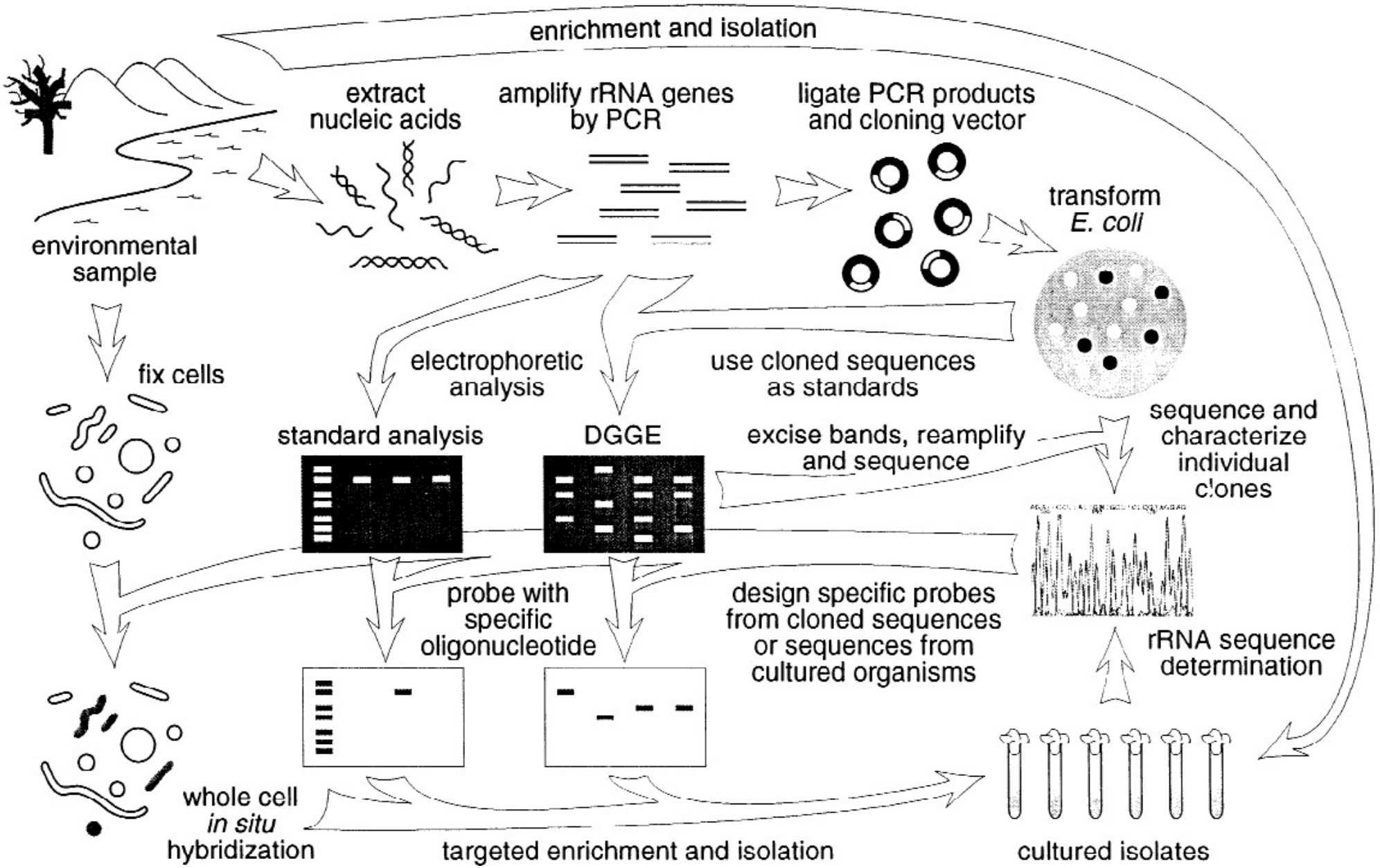
Transcription

**RNA**

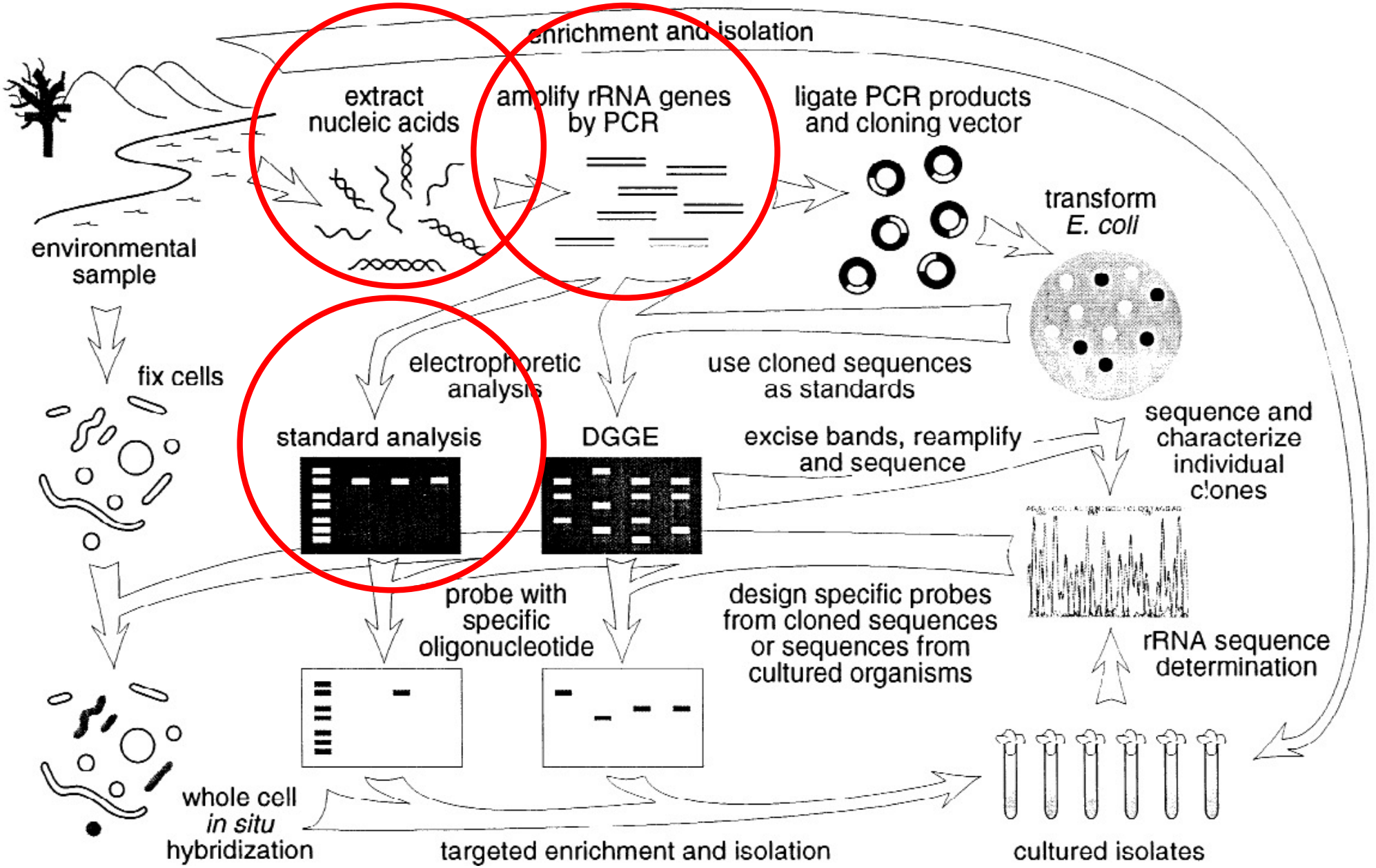
Translation

**Protein**



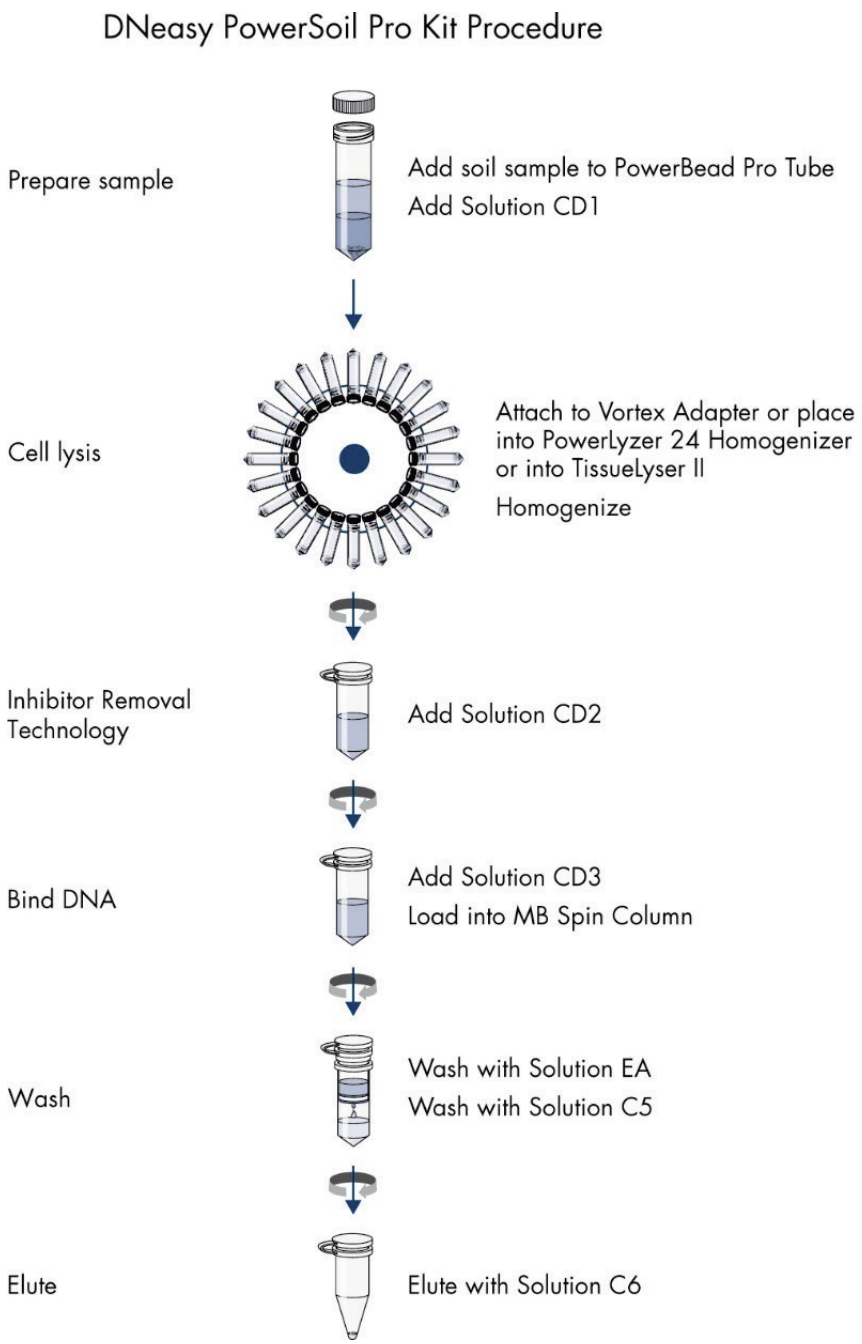



Head *et al*. 1998




Head *et al*. 1998

# DNA Extraction Overview



# DNA Extraction

1. Lyse cell membrane
   a. Chemically → detergent
   b. Physically → bead beating

2. Pellet cell membrane, proteins and other cell parts while DNA stays in solution

3. Remove other inhibitors from DNA

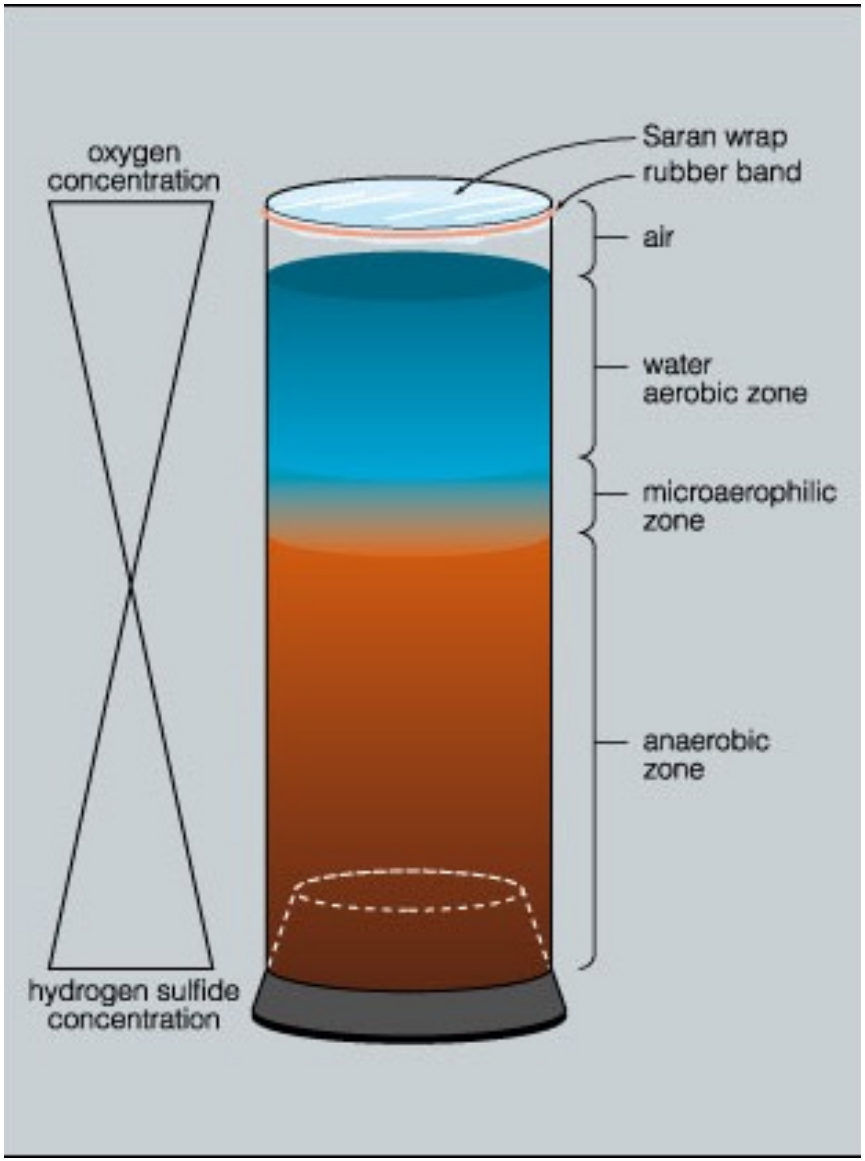
4. Mix DNA with acid and salt → stick to filter

5. Wash filter-bound DNA several times with alcohol

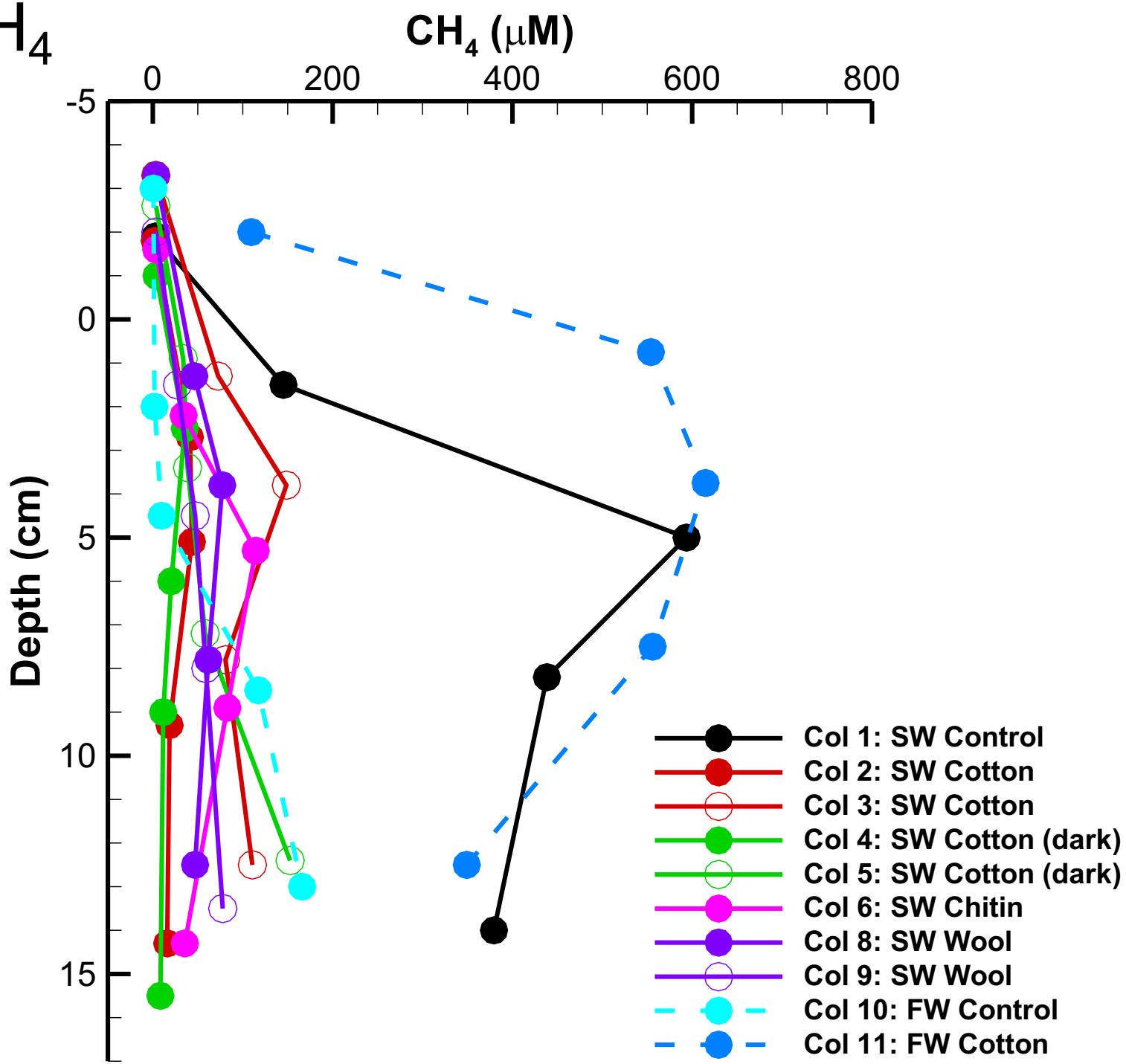
6. Elute DNA off membrane with pH 8, low-salt buffer

# Choosing a Depth Horizon

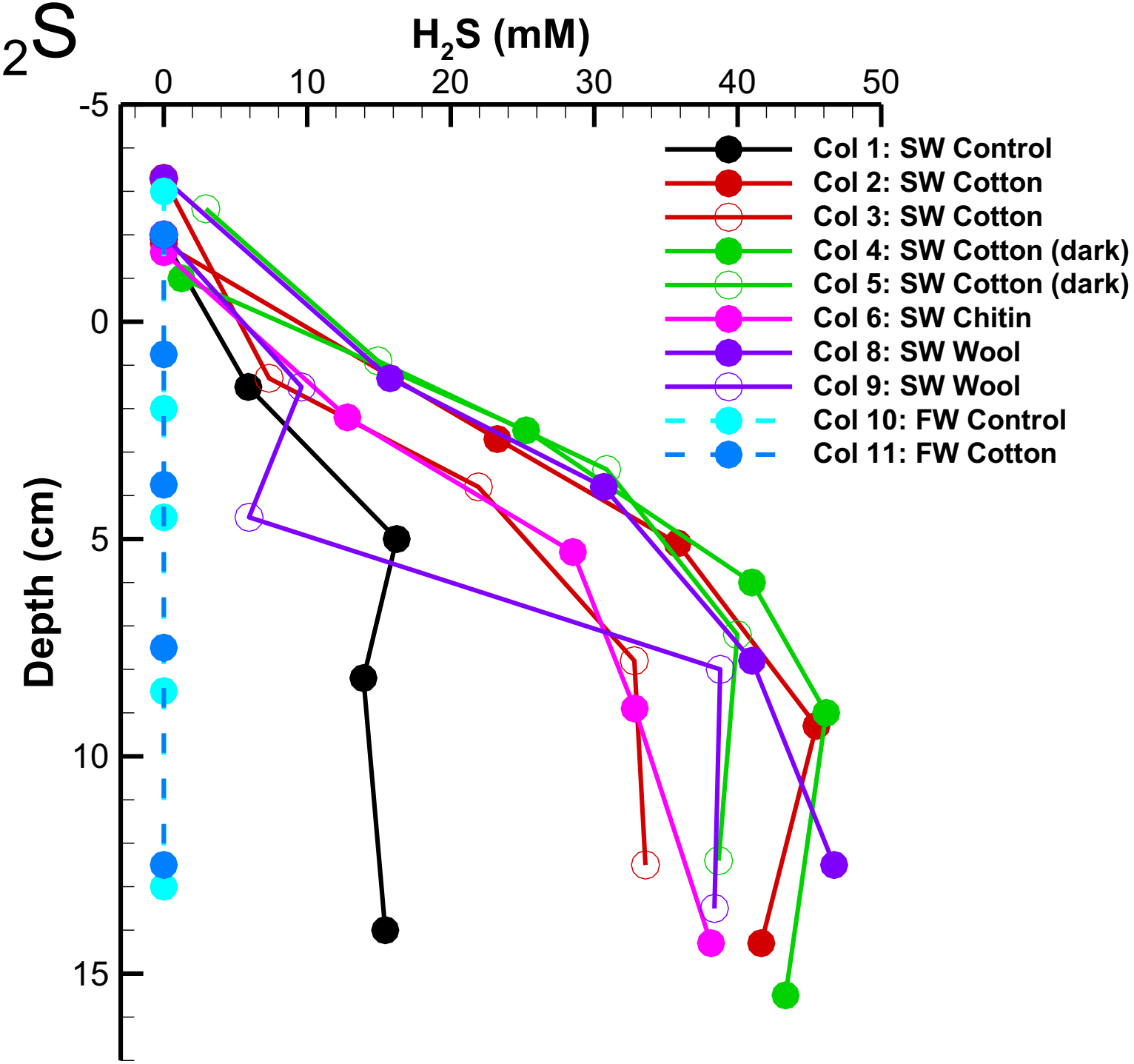
- 16S rRNA Bacteria
- 16S rRNA Archaea
- *mcr*A Methanogens
  - Methyl coenzyme M reductase
- *dsr*B Sulfate reducers
  - Dissimilatory bisulfite reductase



# 2018 $CH_4$

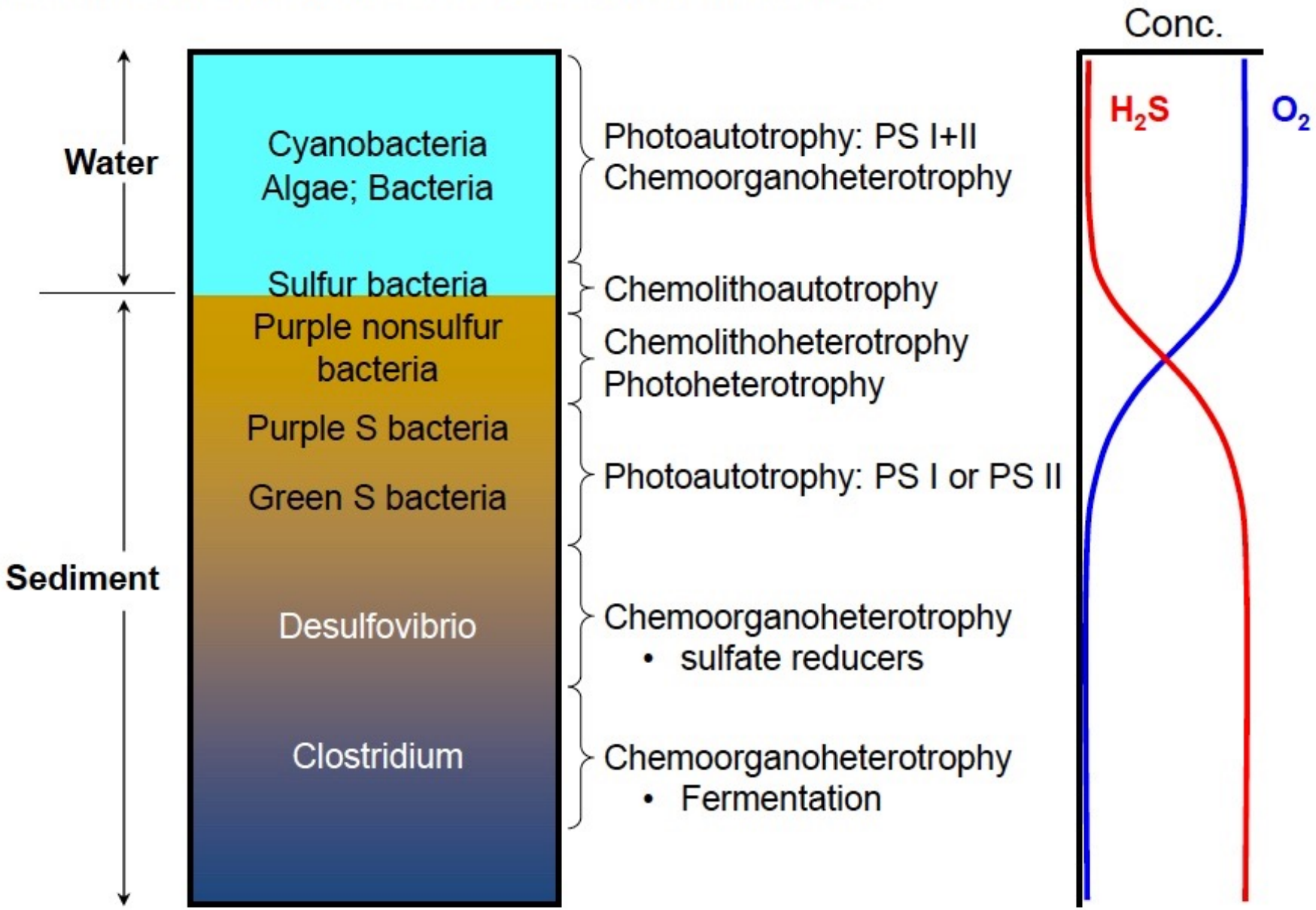

CH4 (μM)
0
200
400
600
800
-5
0
5
10
15
Depth (cm)
Col 1: SW Control
Col 2: SW Cotton
Col 3: SW Cotton
Col 4: SW Cotton (dark)
Col 5: SW Cotton (dark)
Col 6: SW Chitin
Col 8: SW Wool
Col 9: SW Wool
Col 10: FW Control
Col 11: FW Cotton

# 2018 $H_2S$


H2S (mM)
0
10
20
30
40
50
-5
0
5
10
15
Depth (cm)
Col 1: SW Control
Col 2: SW Cotton
Col 3: SW Cotton
Col 4: SW Cotton (dark)
Col 5: SW Cotton (dark)
Col 6: SW Chitin
Col 8: SW Wool
Col 9: SW Wool
Col 10: FW Control
Col 11: FW Cotton

# Winogradsky Column

Microenvironments generated by chemical gradients.



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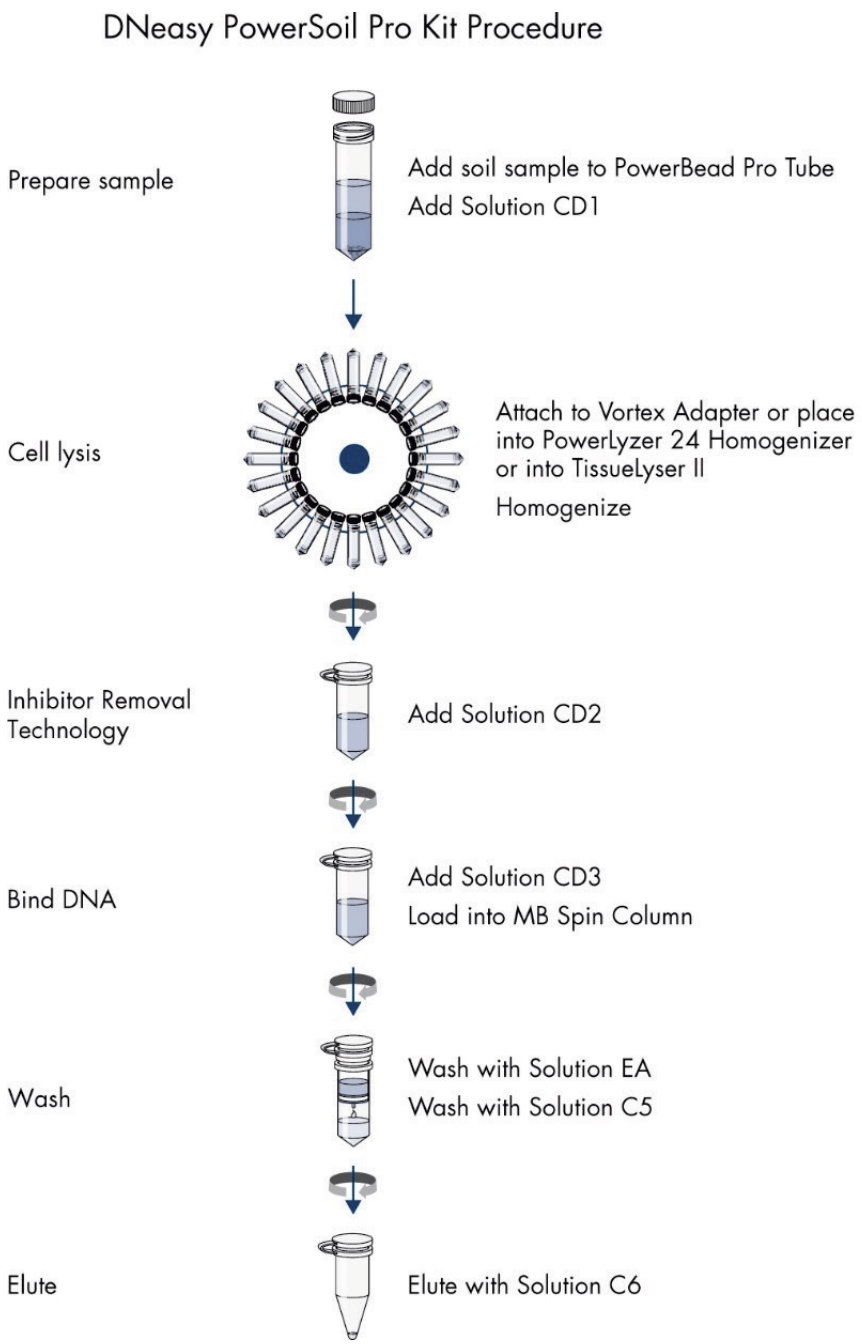
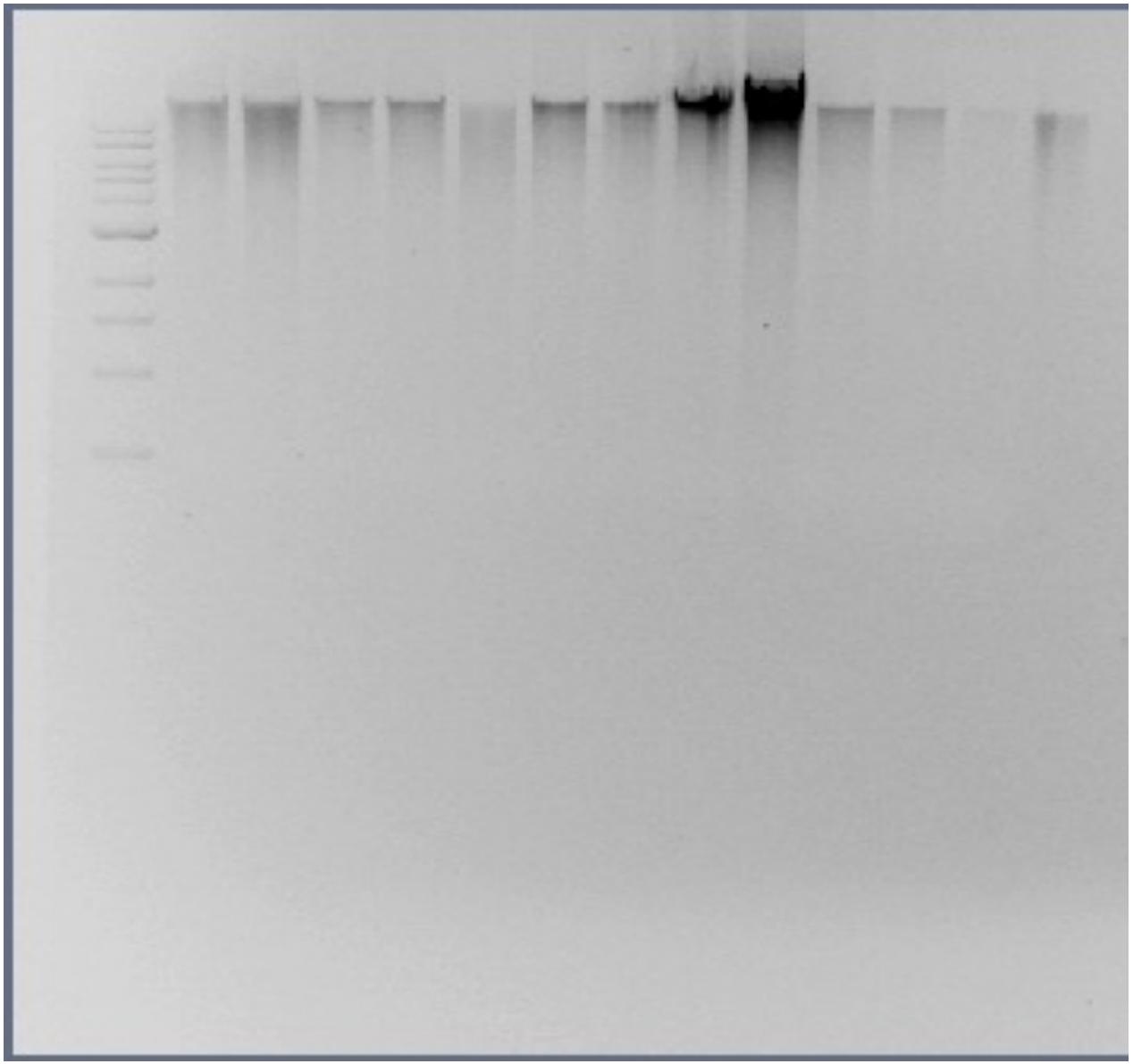


# Day 1, Part II

Run an electrophoresis gel of the DNA products extracted from your columns

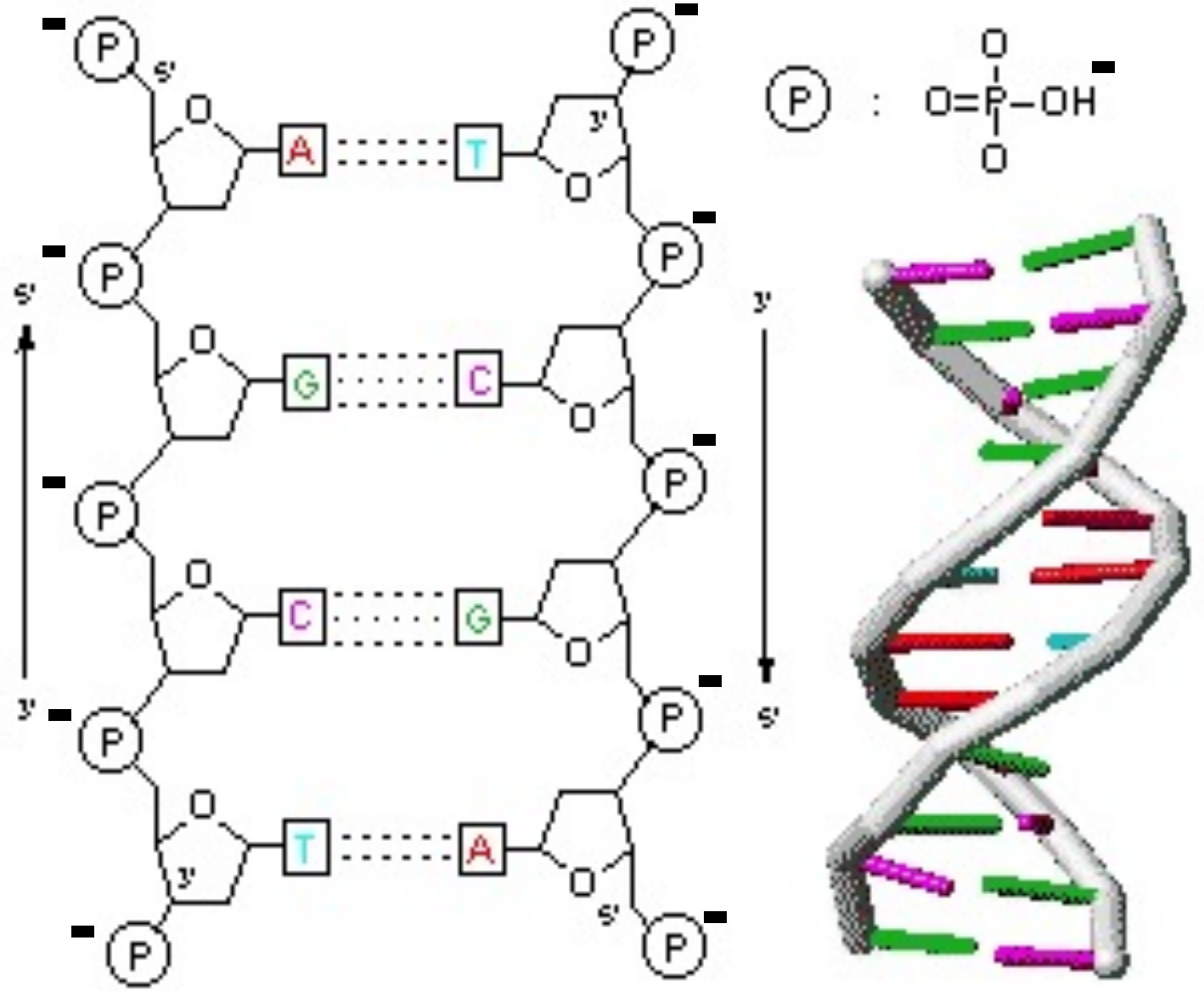


# Genomic DNA

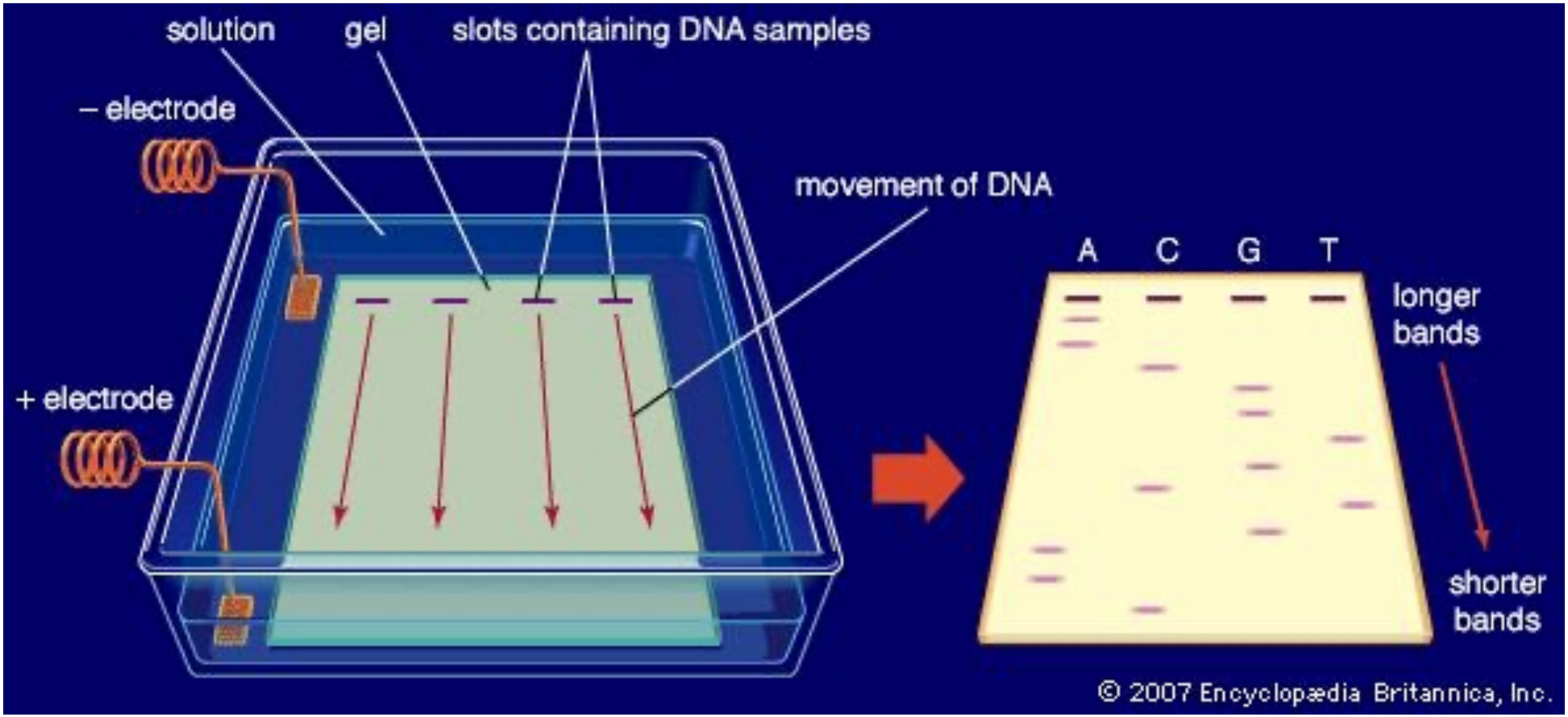
The sum total of all DNA from an organism or a community of organisms

# Basics of Gel Electrophoresis

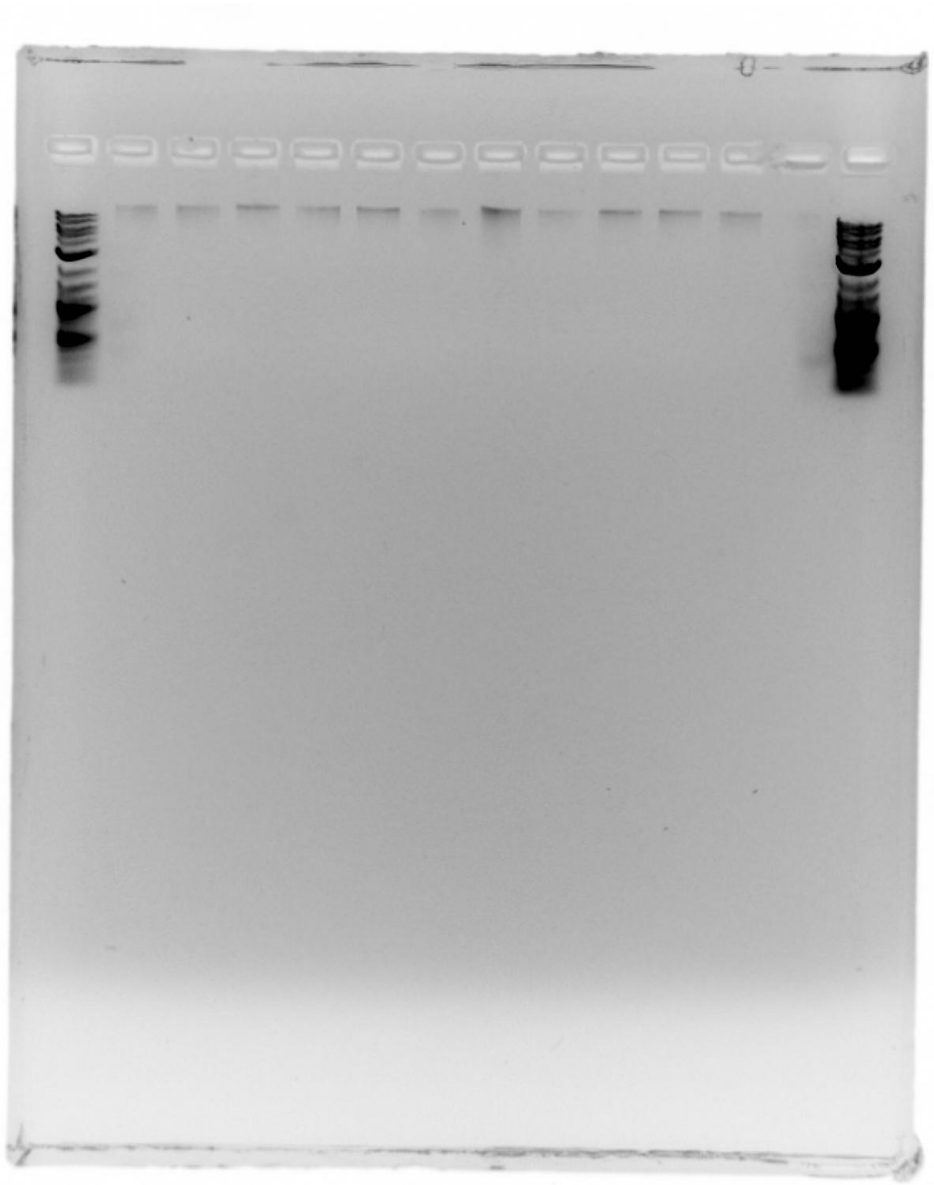
- The gel is a matrix (like jello with holes)
- DNA is negatively charged - will run to positive
- Smaller fragments run faster than larger ones
- Gel contains Ethidium Bromide, which binds to DNA and fluoresces when hit with UV light (WEAR GLOVES!!!)


P
5'
3'
A
T
G
C
C
G
T
A
O
P : O=P–OH
O

# Gel electrophoresis



# Gel results . . ..



**Genomic DNA**

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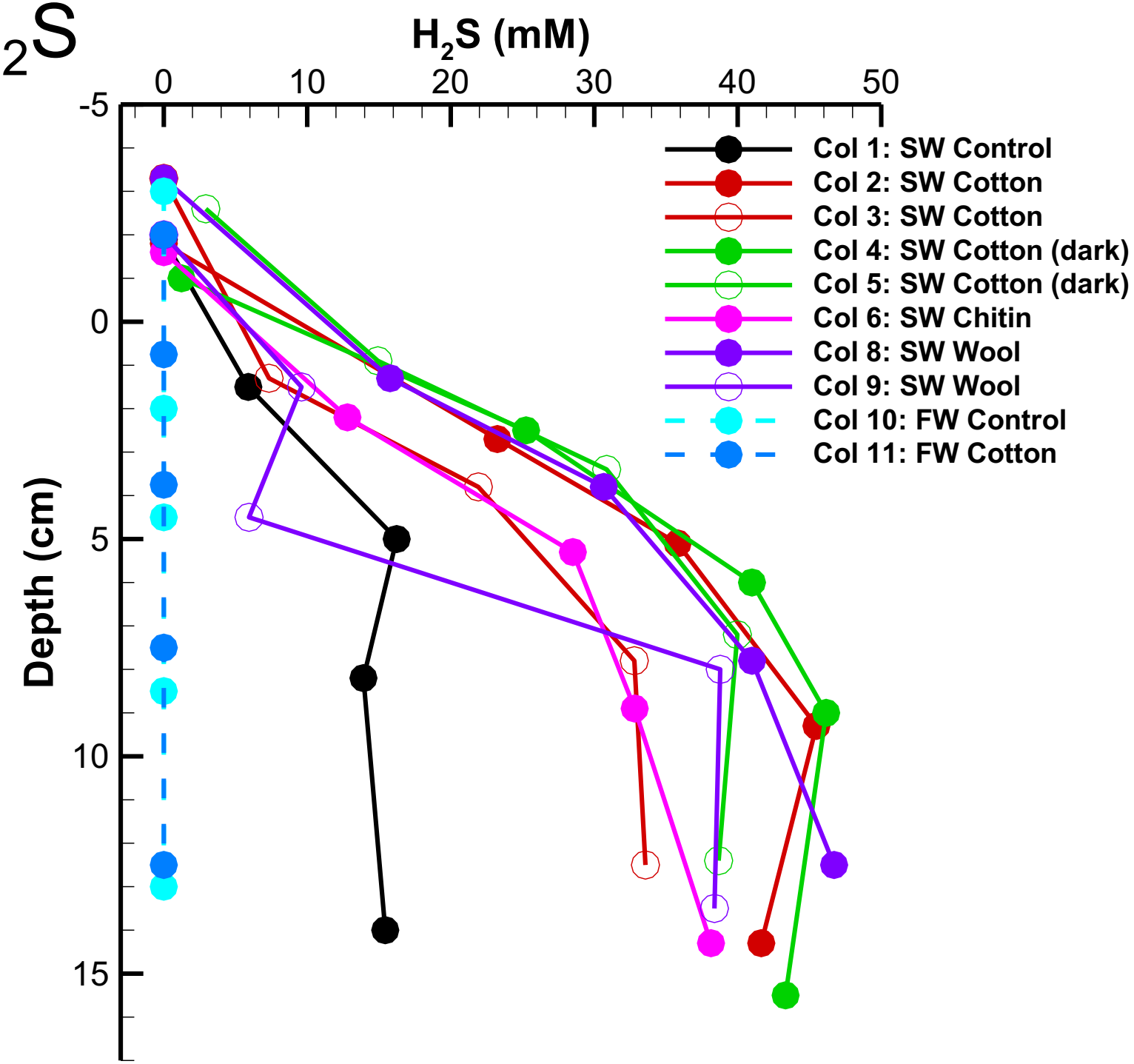
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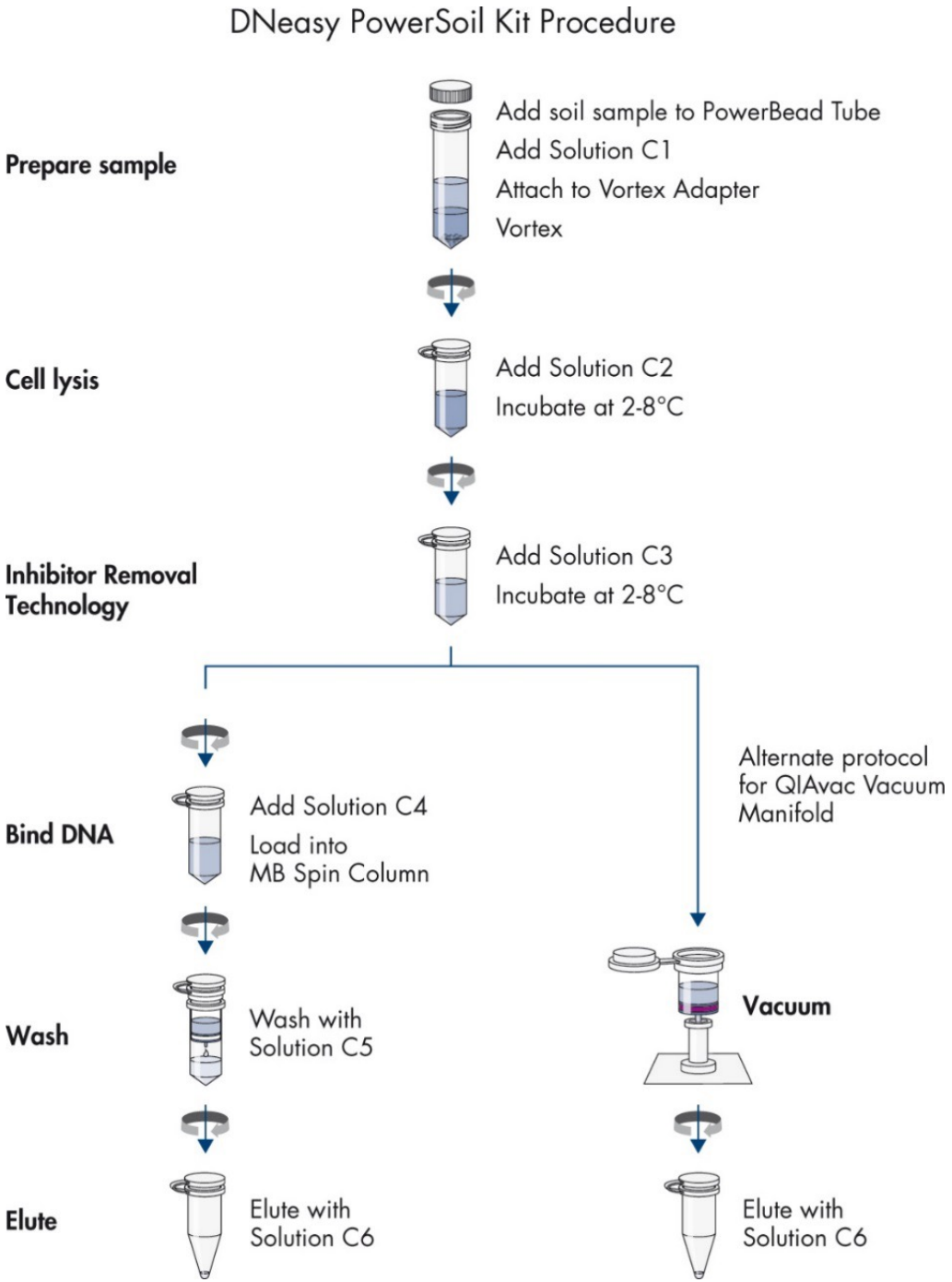
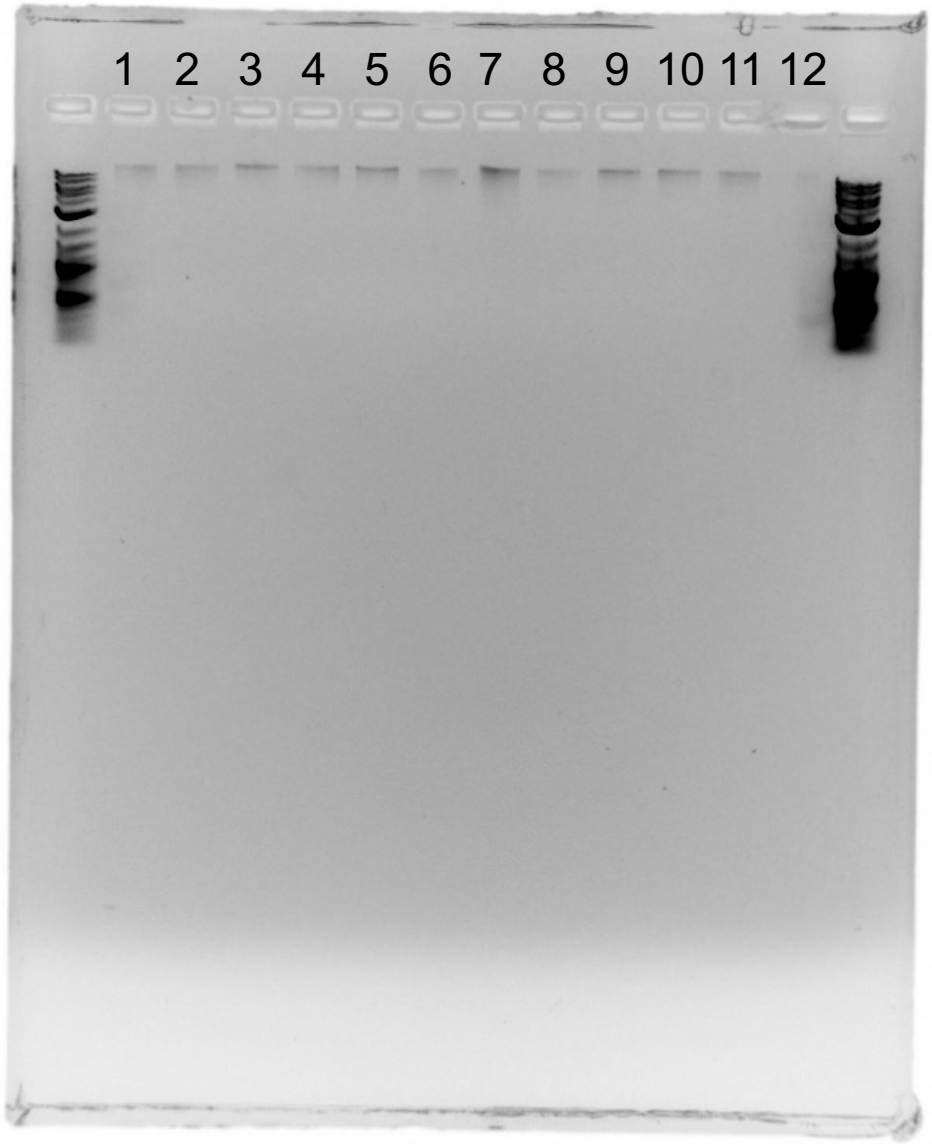
# DNA Extraction Overview



# Day 2

- Basics of PCR
- Set up PCR reactions using the DNA from your extractions and sets of primers targeting genes from specific microbial groups

# DNA extraction results



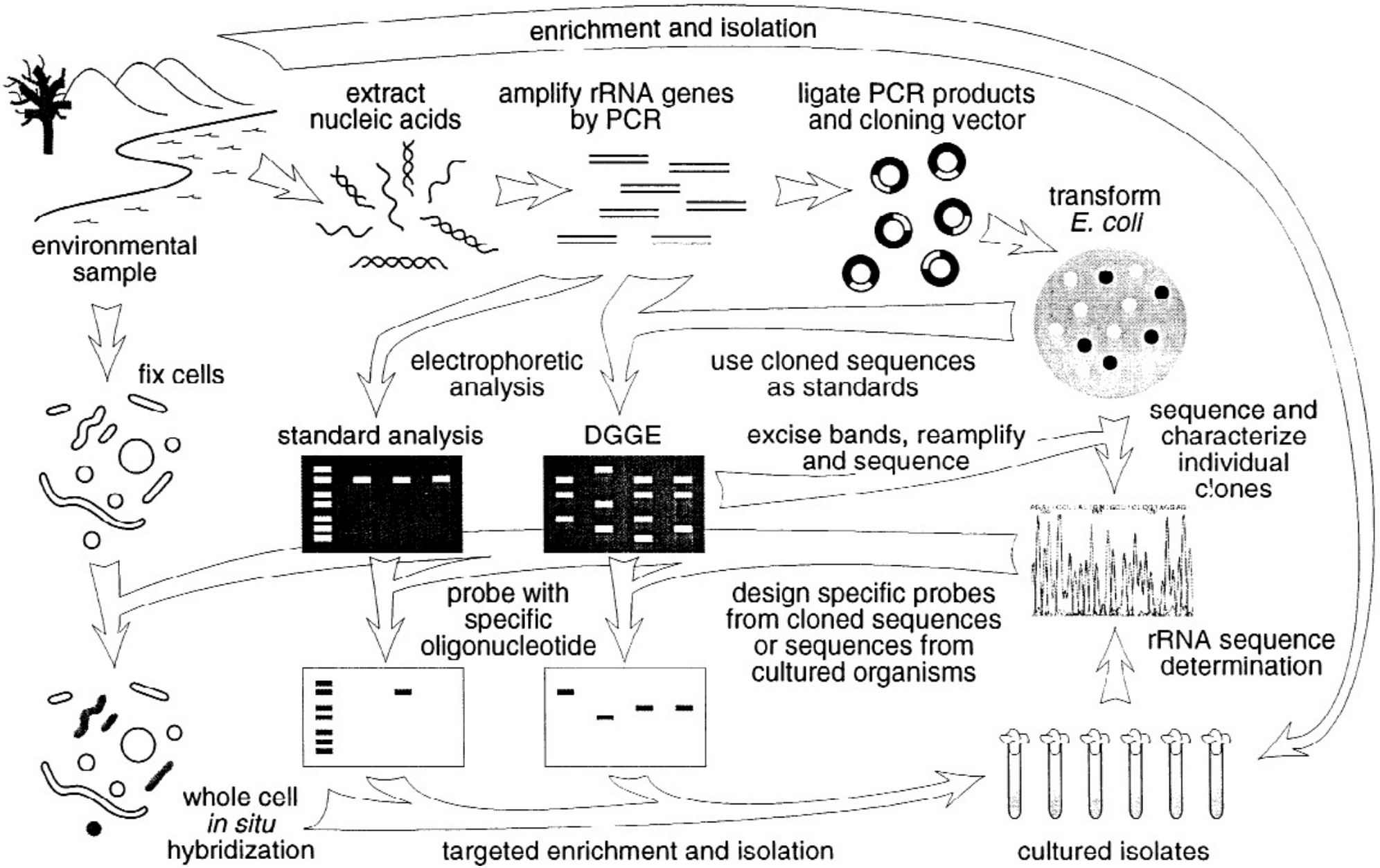

**Genomic DNA**

The sum total of all DNA from an organism or a community of organisms

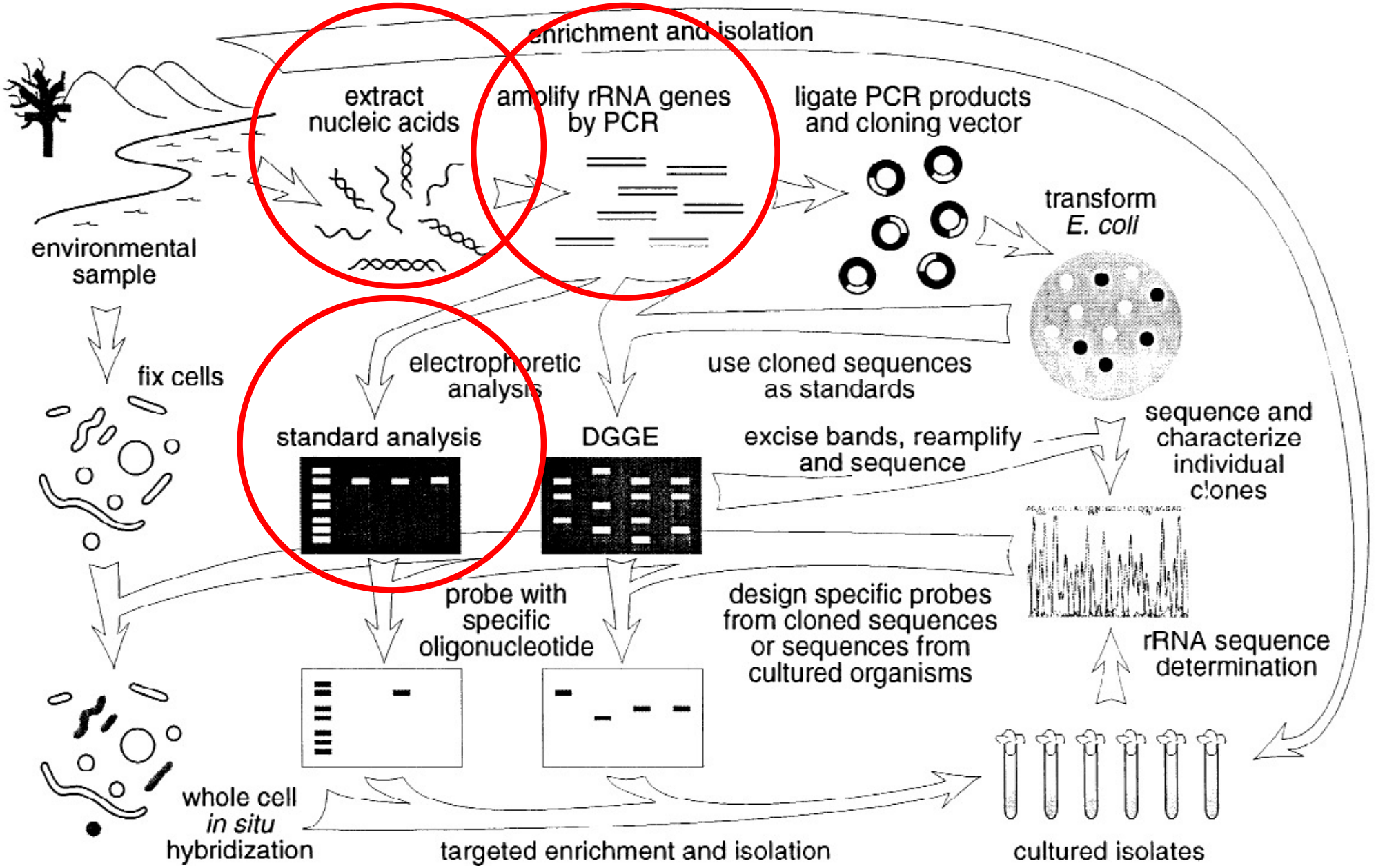
# DNA extraction results (a better gel!)


**Genomic DNA**

The sum total of all DNA from an organism or a community of organisms



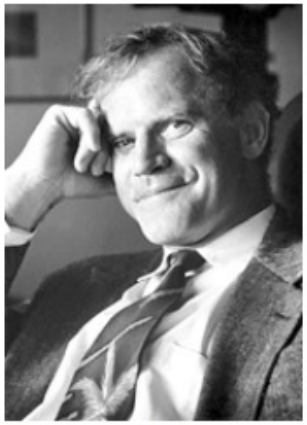

Head *et al*. 1998




Head *et al*. 1998

# Polymerase Chain Reaction (PCR)

## The Nobel Prize in Chemistry 1993



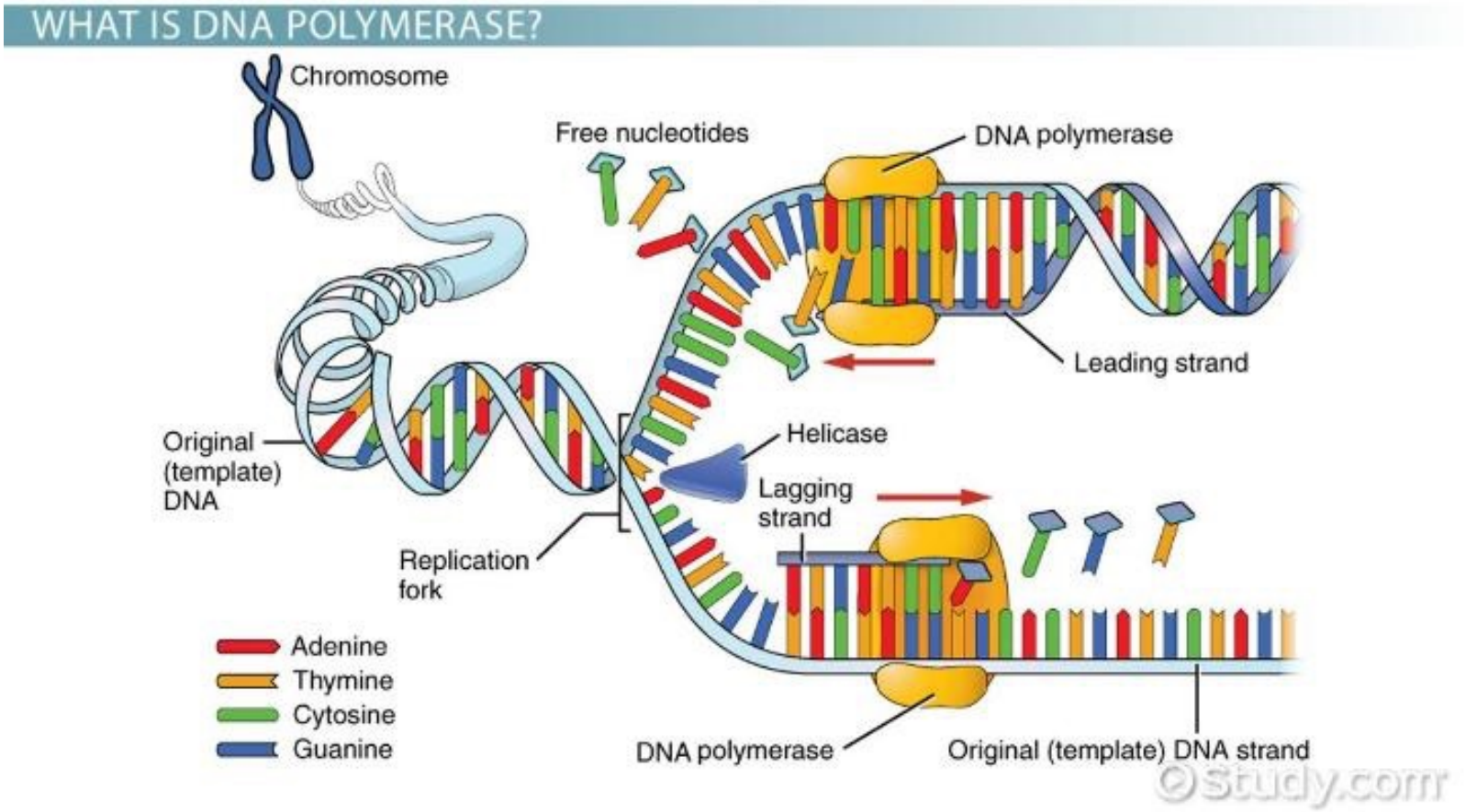
Kary B. Mullis
**Prize share:** 1/2

Michael Smith
**Prize share:** 1/2

The Nobel Prize in Chemistry 1993 was awarded *"for contributions to the developments of methods within DNA-based chemistry"* jointly with one half to Kary B. Mullis *"for his invention of the polymerase chain reaction (PCR) method"* and with one half to Michael Smith *"for his fundamental contributions to the establishment of oligonucleotide-based, site-directed mutagenesis and its development for protein studies"*.

# Polymerase

- An enzyme that catalyzes the synthesis of long chains of polymers or nucleic acids



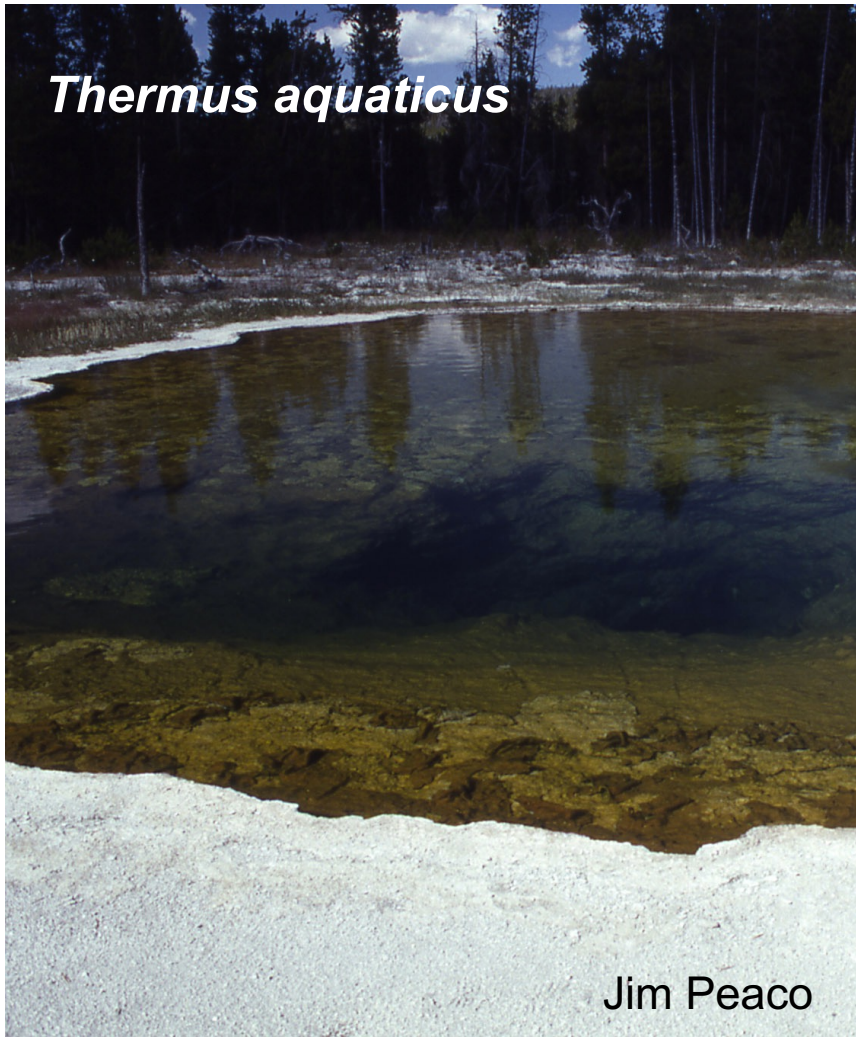
# Polymerase Chain Reaction (PCR)

- Rapid, inexpensive and simple way of making millions of copies of a specific DNA sequence (gene) starting with very few copies

- Does not require the use of isotopes or toxic chemicals

- It involves
  - mixing sample DNA, primers, polymerase, and buffer
  - using cycling of temperatures to facilitate reaction
  - detecting reaction products

# Polymerase Chain Reaction (PCR)




- Takes advantage of properties of Taq DNA polymerase to amplify (make copies of) a selected gene region

- Requirements
  - You must know the sequence flanking the region to be amplified

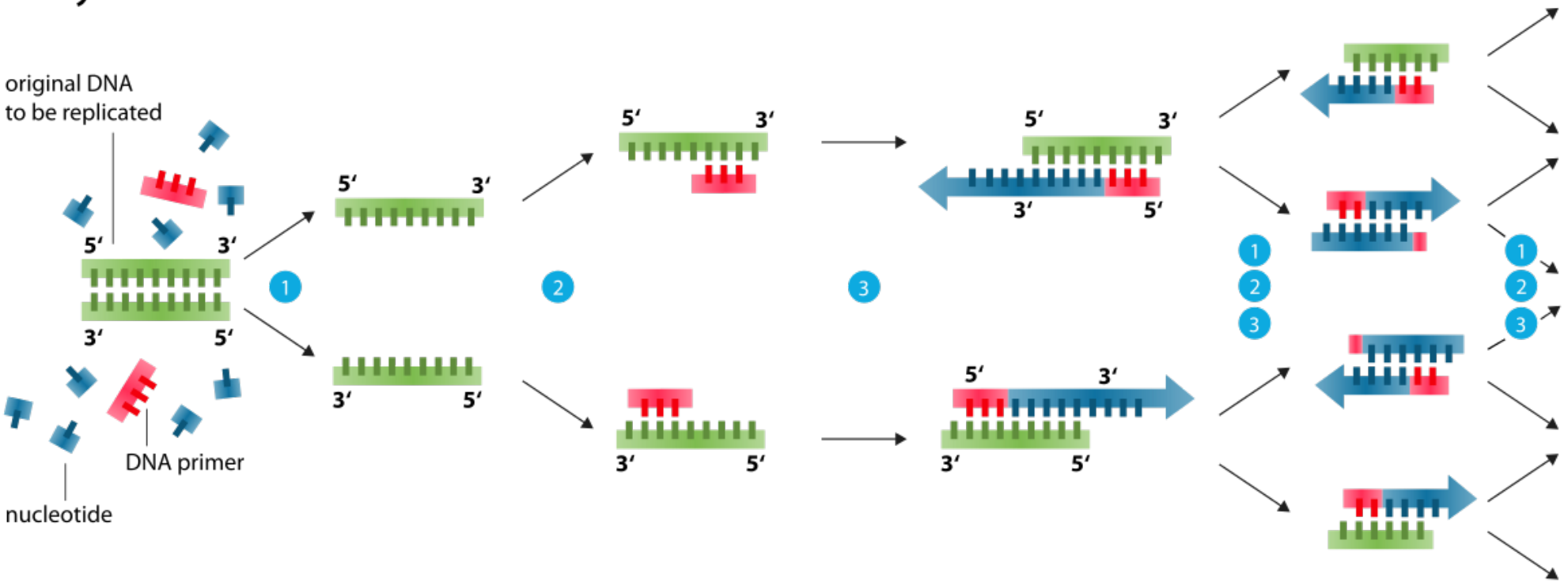
# Every PCR contains:

- A DNA Polymerase (most common, Taq)
- Deoxynucleotide Triphosphates (A, C, T, G)
- Buffer (salt, $MgCl_2$, etc)

2X OneTaq

- A set of primers, one Forward, one Reverse
- Various chemicals to minimize inhibition
- Template DNA

# Polymerase chain reaction - PCR




1. **Denaturation** at 94-96°C
2. **Annealing** at ~68°C
3. **Elongation** at ca. 72 °C

# Typical PCR Profile

| Temperature | Time | Action |
|---|---|---|
| 95ºC | 5 minutes | DNA Taq polymerase activation |
| 35 cycles of:<br>95ºC<br>54ºC<br>72ºC | <br>1 minute<br>1 minute<br>1 minute | <br>DNA denaturization<br>Primer annealing<br>Extension creation |
| 72ºC | 10 minutes | Final extension created |

# Things you can optimize

- Temperature and time to activate Taq polymerase
- Temperature and time to allow primer annealing
- Temperature and time for extension
- Concentration of reagents, especially primers, dNTPs, and $MgCl_2$
- Concentration of template DNA
- Number of replication cycles
- Etc…

# Some Problems with PCR

- Inhibitors in template DNA
- Amplification bias
- Gene copy number
- Limited by primer design
- Differential denaturation efficiency
- Chimeric PCR products may form
- Contamination w/ non-target DNA
- Potentially low sensitivity and resolution
- General screw-ups

# (Some) Problems with Molecular Methods

| | |
|---|---|
| **D/RNA extraction** | Incomplete sampling |
| | Resistance to cell lysis |
| **Storage** | Enzymatic degradation |
| **PCR** | Inhibitors in template DNA |
| | Amplification bias |
| | Gene copy number |
| | Fidelity of PCR |
| | Differential denaturation efficiency |
| | Chimeric PCR products |
| **Anytime** | Contamination w/ non-target DNA |

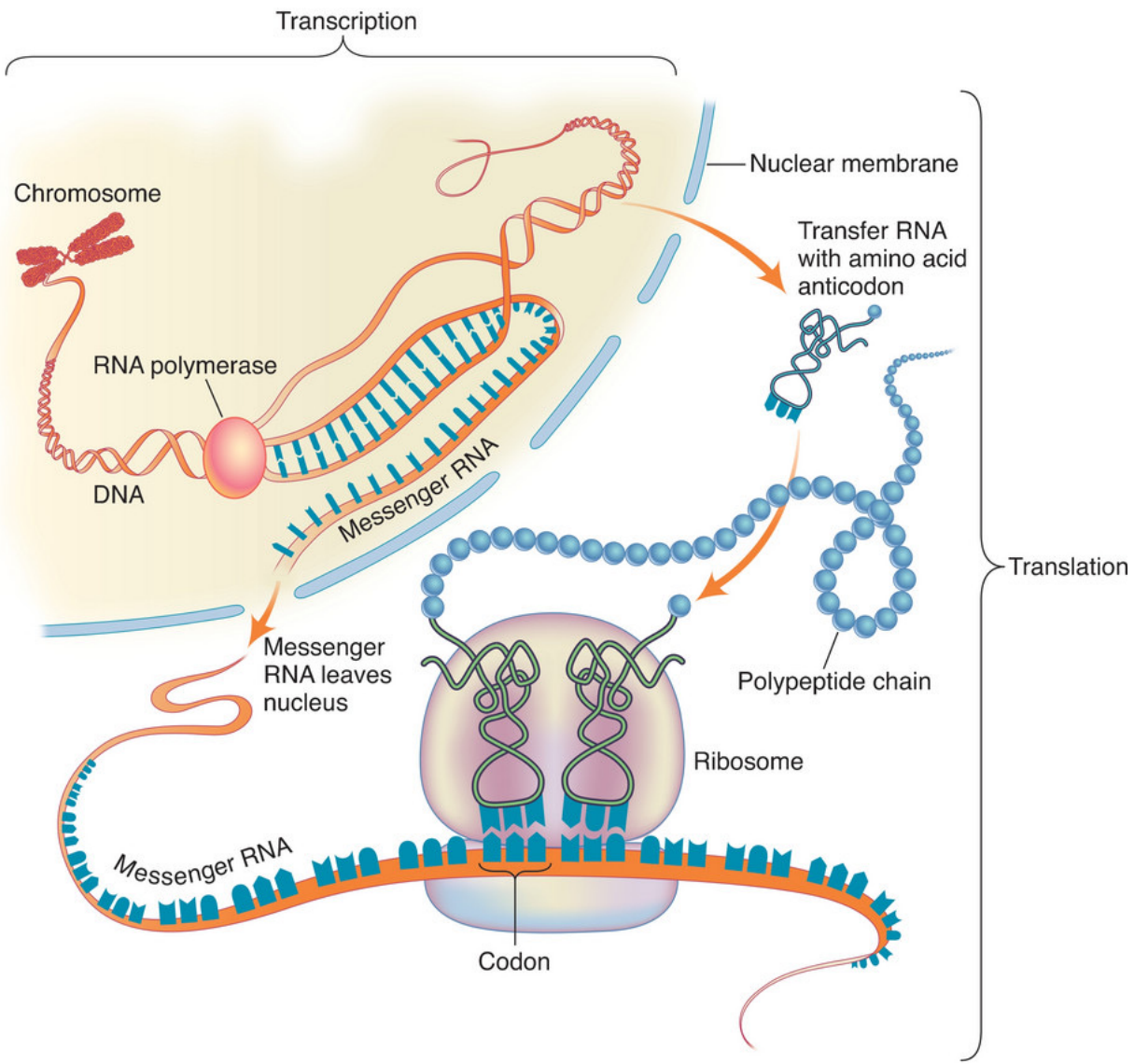
# The Star of the Show: SSU rRNA

- Everybody has it
- Contains both highly conserved and variable regions

  -allows comparisons between different organisms over long periods of time (evolutionary history)

- Not laterally transferred between organisms
- HUGE and growing database

# The Central Dogma




**DNA**

Transcription

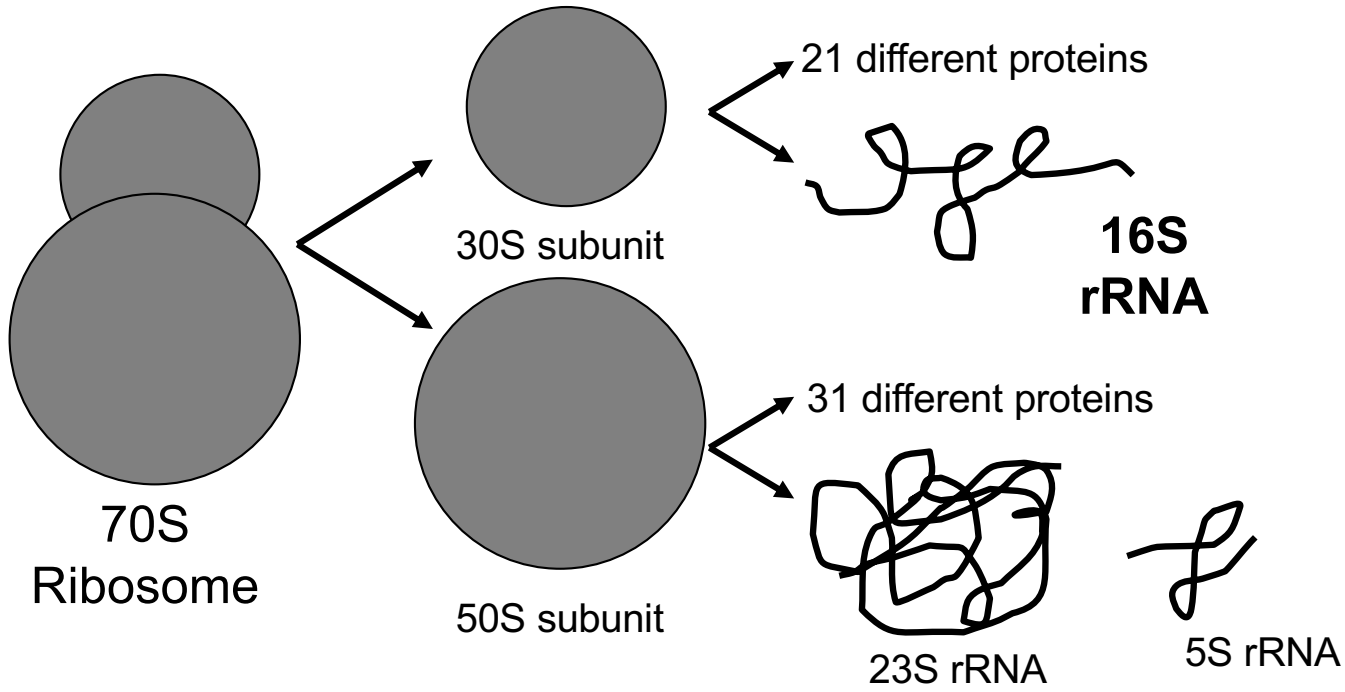
**RNA**

Translation

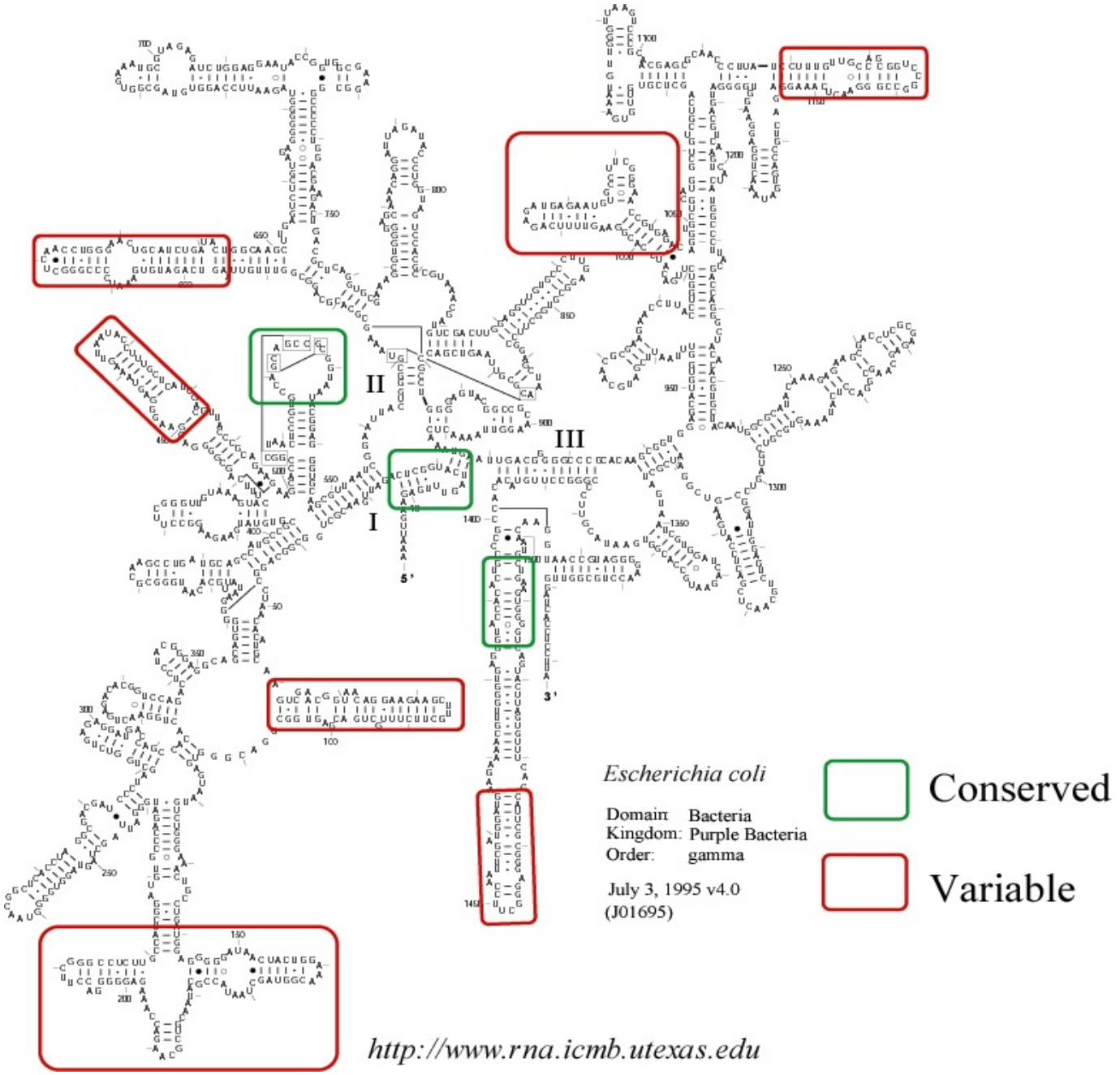
**Protein**

# Ribosomes

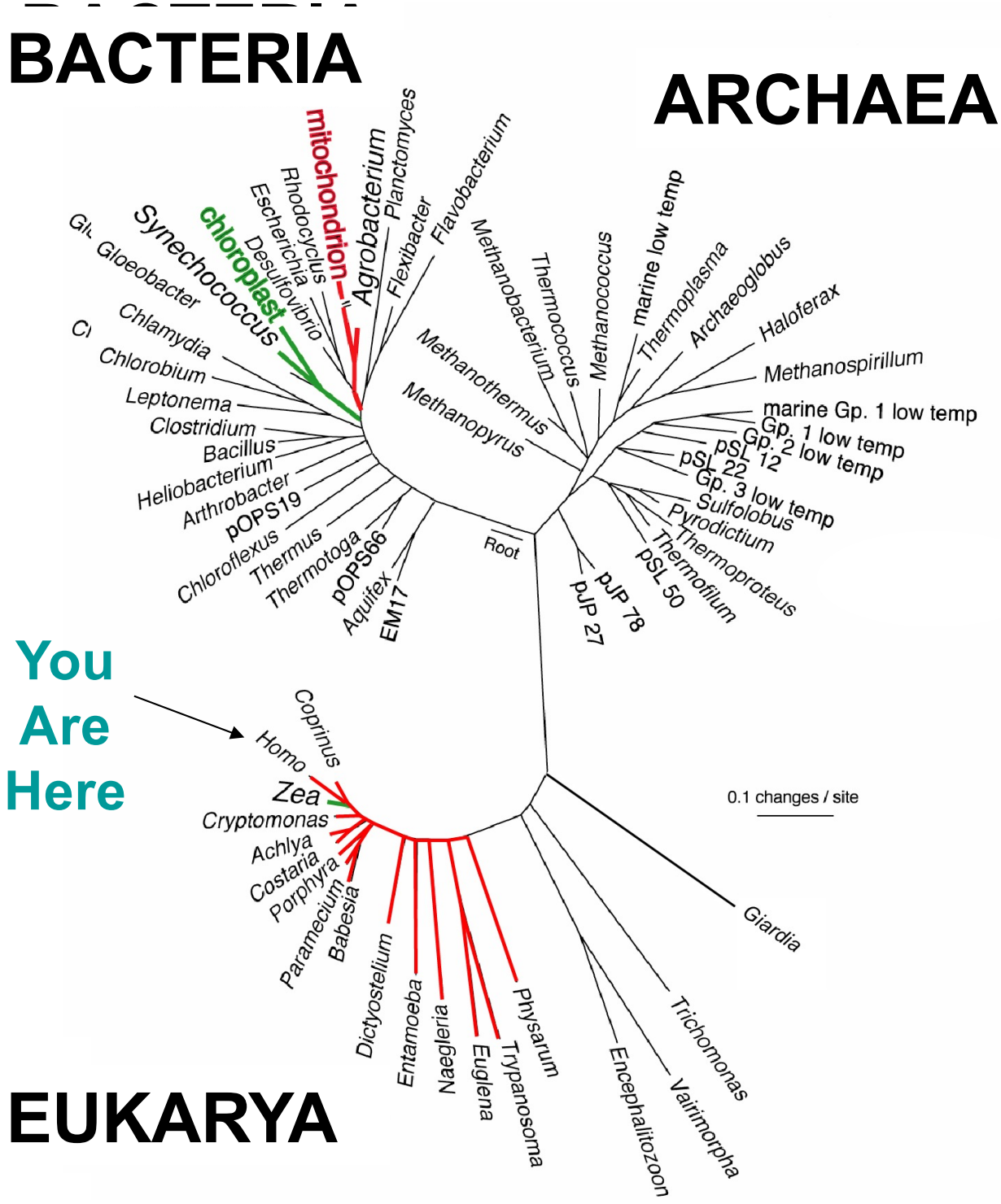
- Make proteins
- rRNA is transcribed from rDNA genes



# SSU rRNA


I
II
III
5'
3'
Escherichia coli
Domain: Bacteria
Kingdom: Purple Bacteria
Order: gamma
July 3, 1995 v4.0
(J01695)
Conserved
Variable
http://www.rna.icmb.utexas.edu

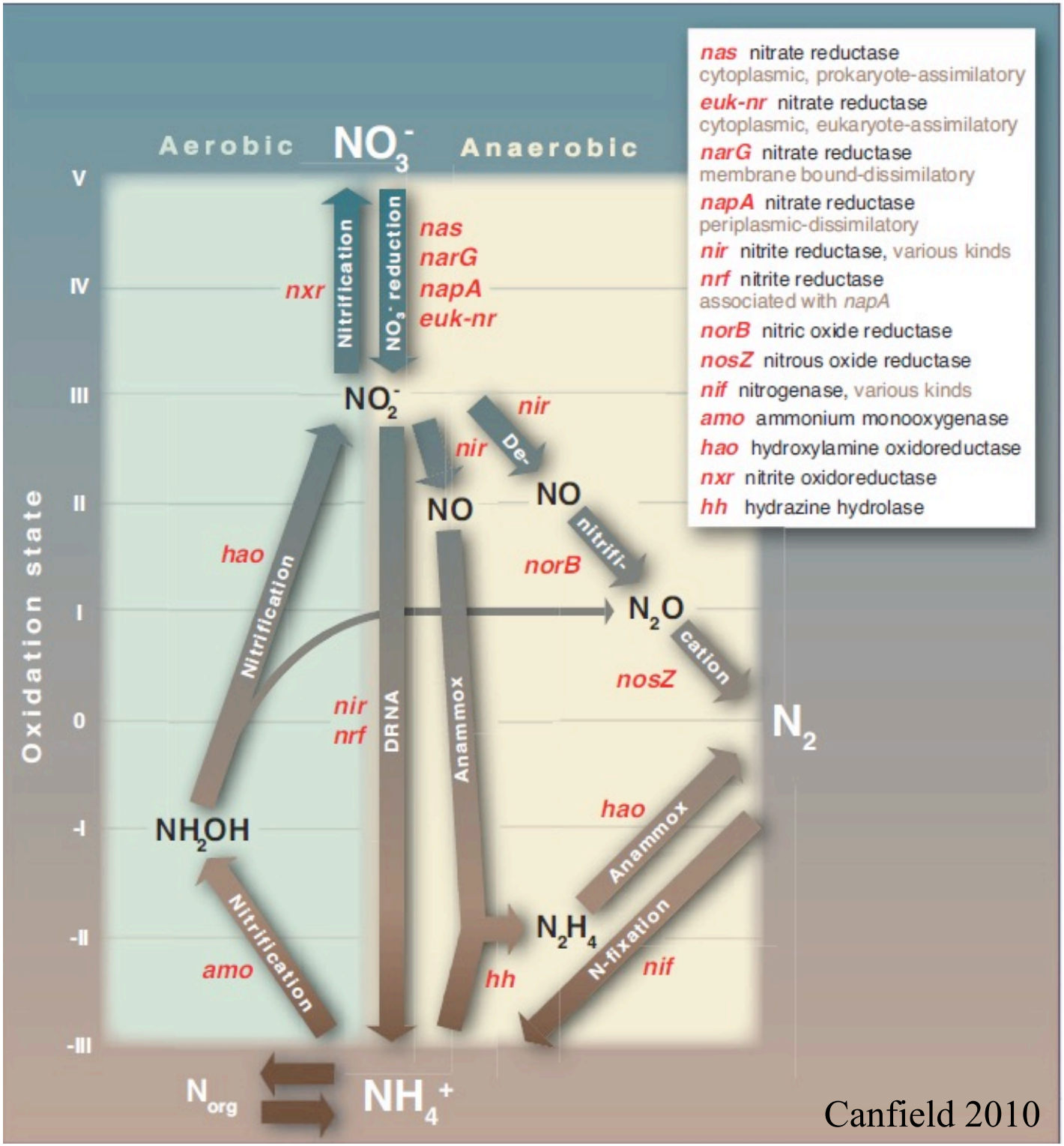
# Universal Tree of Life




*Modified from Norman Pace*

# Beyond 16S rRNA:

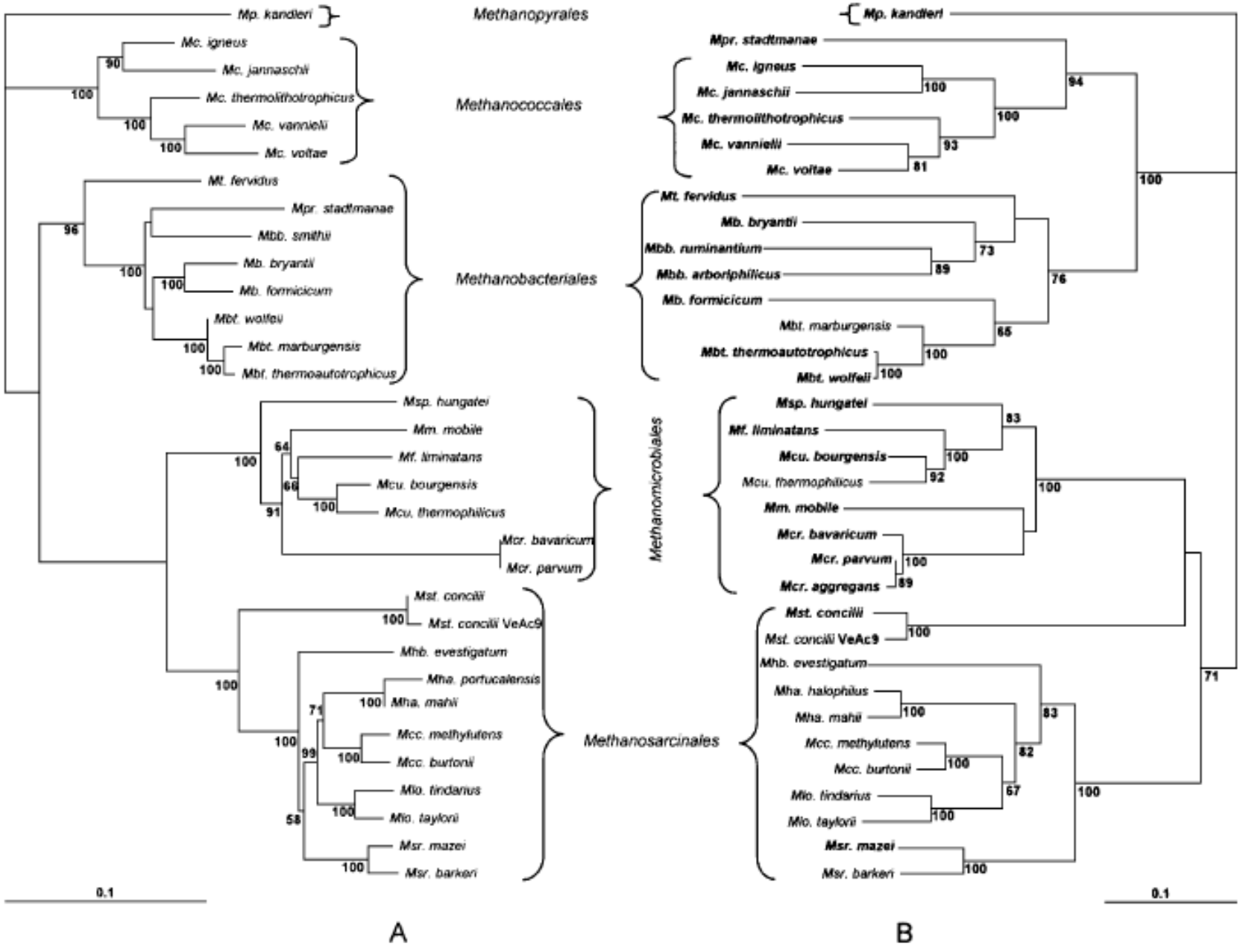
## What is the functional potential of microbes?




Canfield 2010

# 16S rDNA

# mcrA




Luton *et al*. 2002

# Our 4 Targets

- 16S rRNA Bacteria
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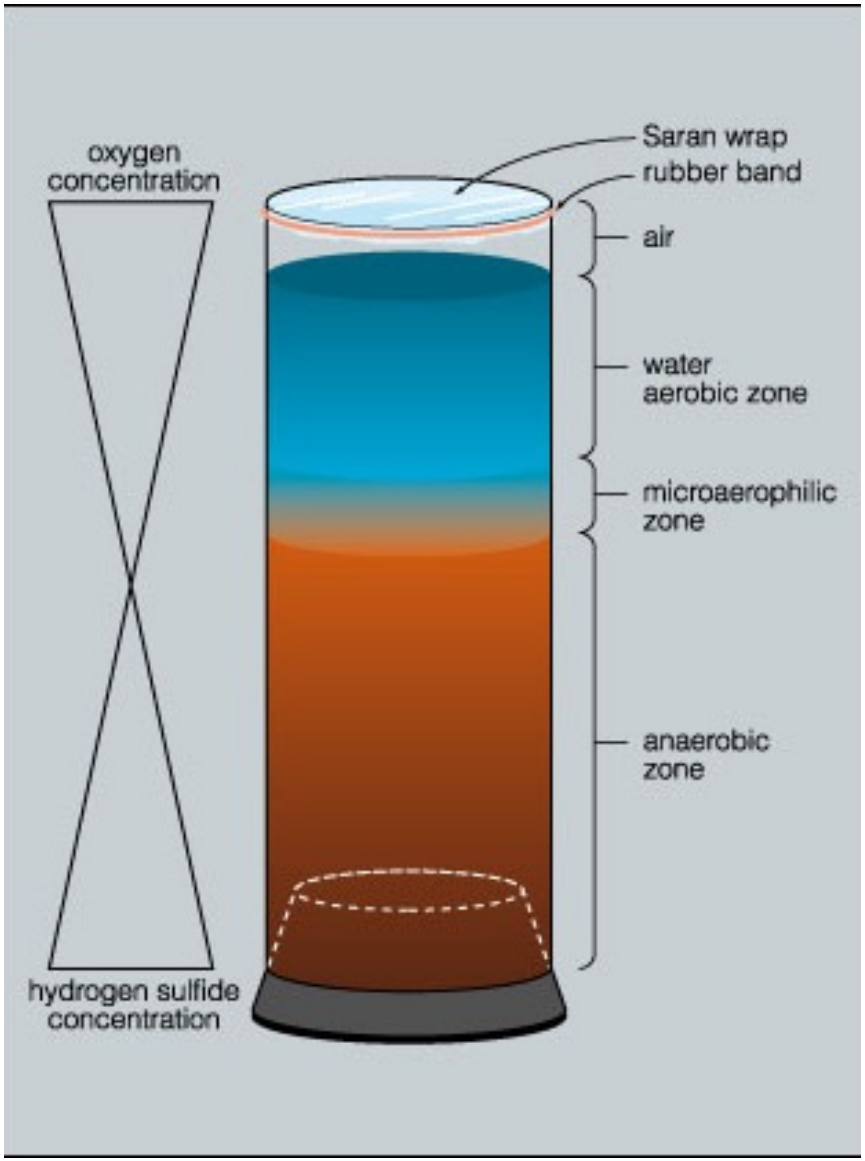
  - Methyl coenzyme M reductase
- *dsr*B Sulfate reducers
  - Dissimilatory bisulfite reductase



# What To Do: Make Master Mix

| Reagent | 1X (25 µl rxn) | X 7 |
|---|---|---|
| Water | 5.5 µl | |
| OneTaq 2X Master Mix | 12.5 µl | |
| 0.4% BSA | 4 µl | |
| Total | 22 µl | |

# What To Do:
# Set up PCR mix with DNA and specific primers

| Tube | Master mix | Target | Template | Vol | F primer | Vol | R primer | Vol |
|---|---|---|---|---|---|---|---|---|
| | µl | | | µl | | µl | | µl |
| 1 | 22 | Sulfate reducers | Sediment DNA | 1 | dsr1F | 1 | dsr4R | 1 |
| 2 | 22 | Methanogens | Sediment DNA | 1 | ME1 | 1 | ME2 | 1 |
| 3 | 22 | Bacteria | Sediment DNA | 1 | 8F | 1 | 1492R | 1 |
| 4 | 22 | Archaea | Sediment DNA | 1 | 21F | 1 | 958R | 1 |
| 5 | 22 | Archaea | + control | 1 | 21F | 1 | 958R | 1 |
| 6 | 22 | Water | - control (water) | 1 | 21F | 1 | 958R | 1 |

# Some Problems with PCR

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- Amplification bias
- Gene copy number
- Limited by primer design
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