Molecular Methods in Microbial Ecology

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Schedule:

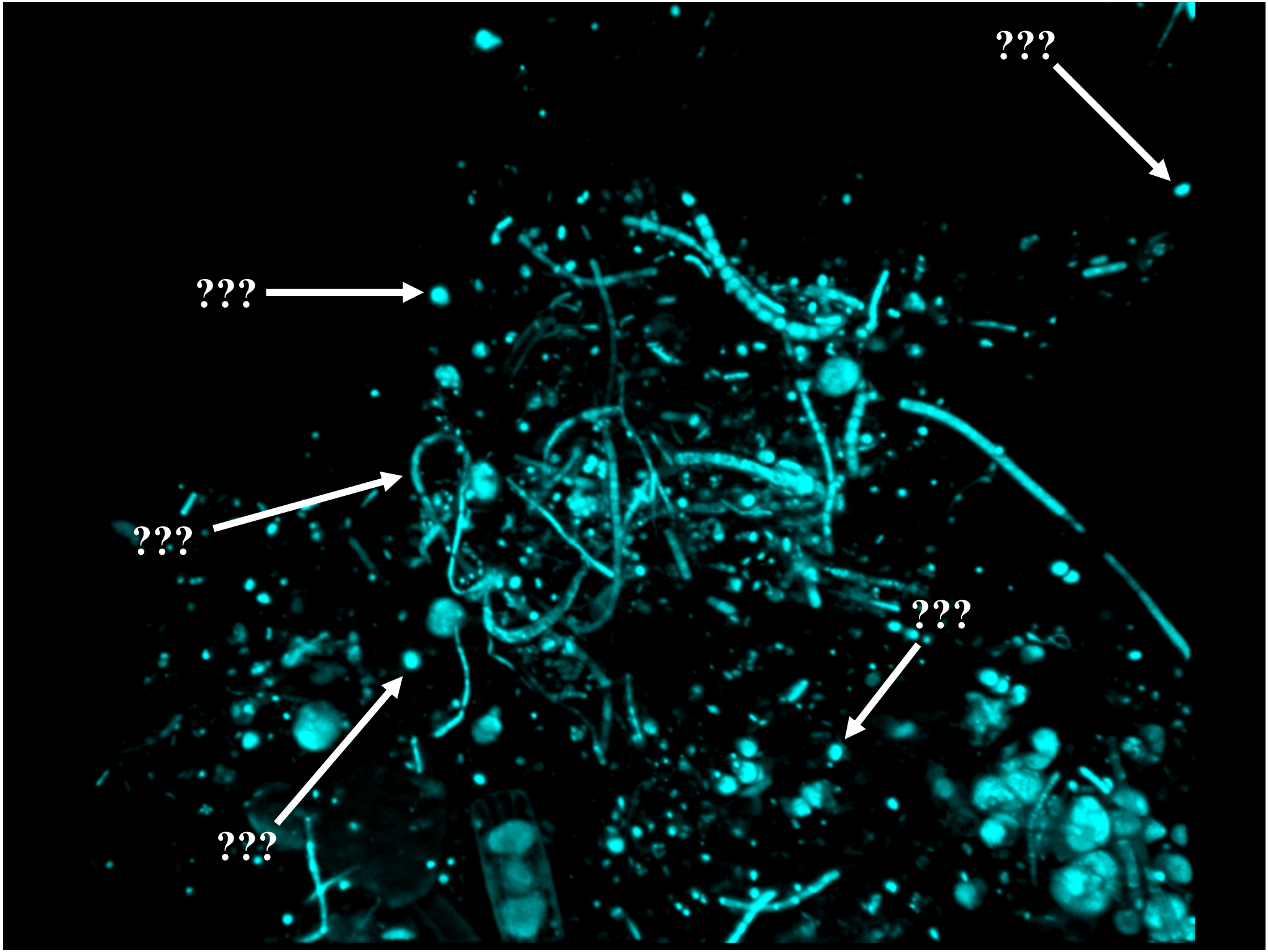
Tuesday	10/23/19	Introduction, Extraction of DNA from Winogradsky columns Run DNA products on gel
Thursday	10/24/19	Lecture on PCR, Prepare PCR reactions
Tuesday	10/29/19	Analyze PCR results, Lecture

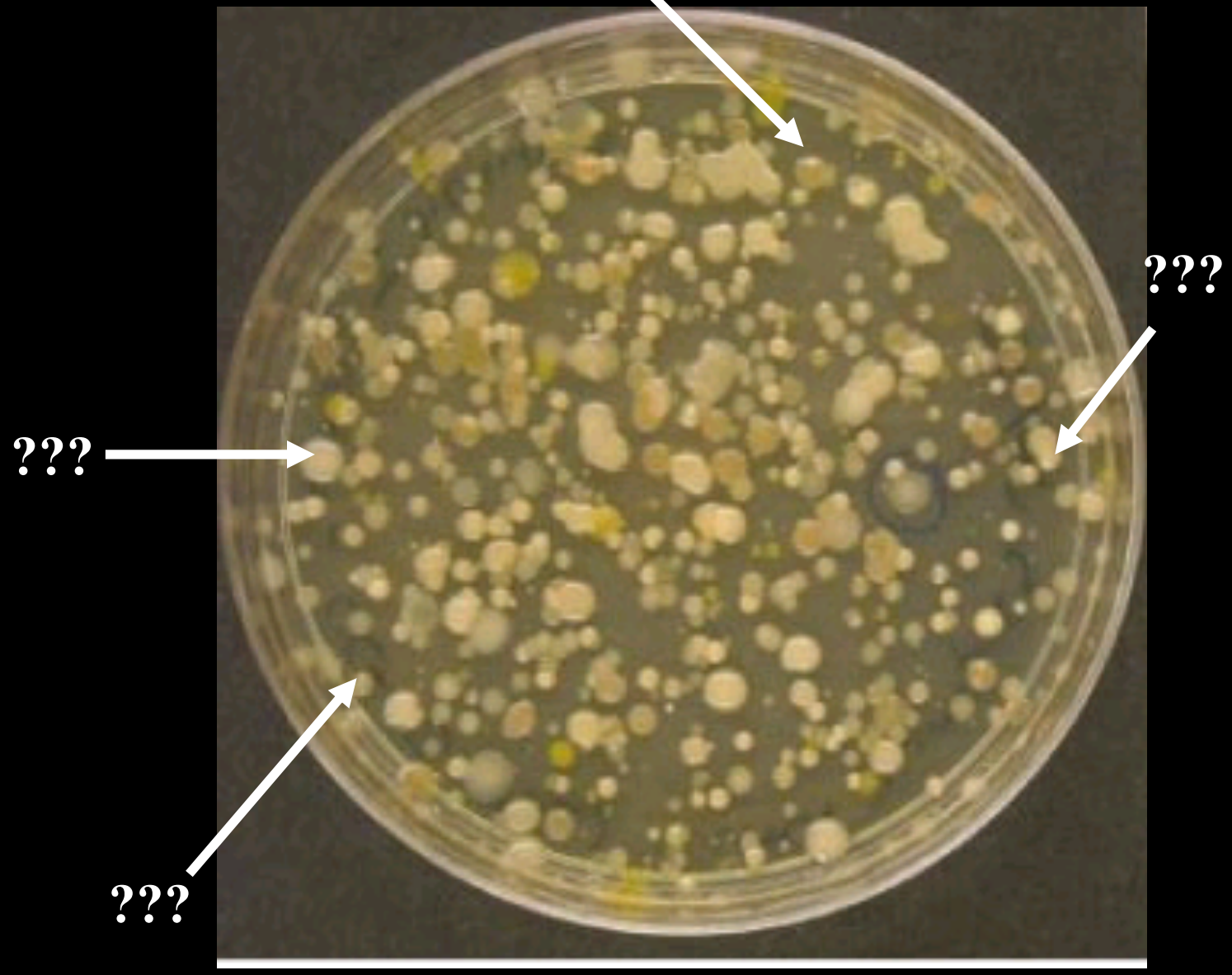
Readings: Head *et al*. 1998. Microbial Ecology 35: 1-21.

Day 1

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


OregonDiscovery.com





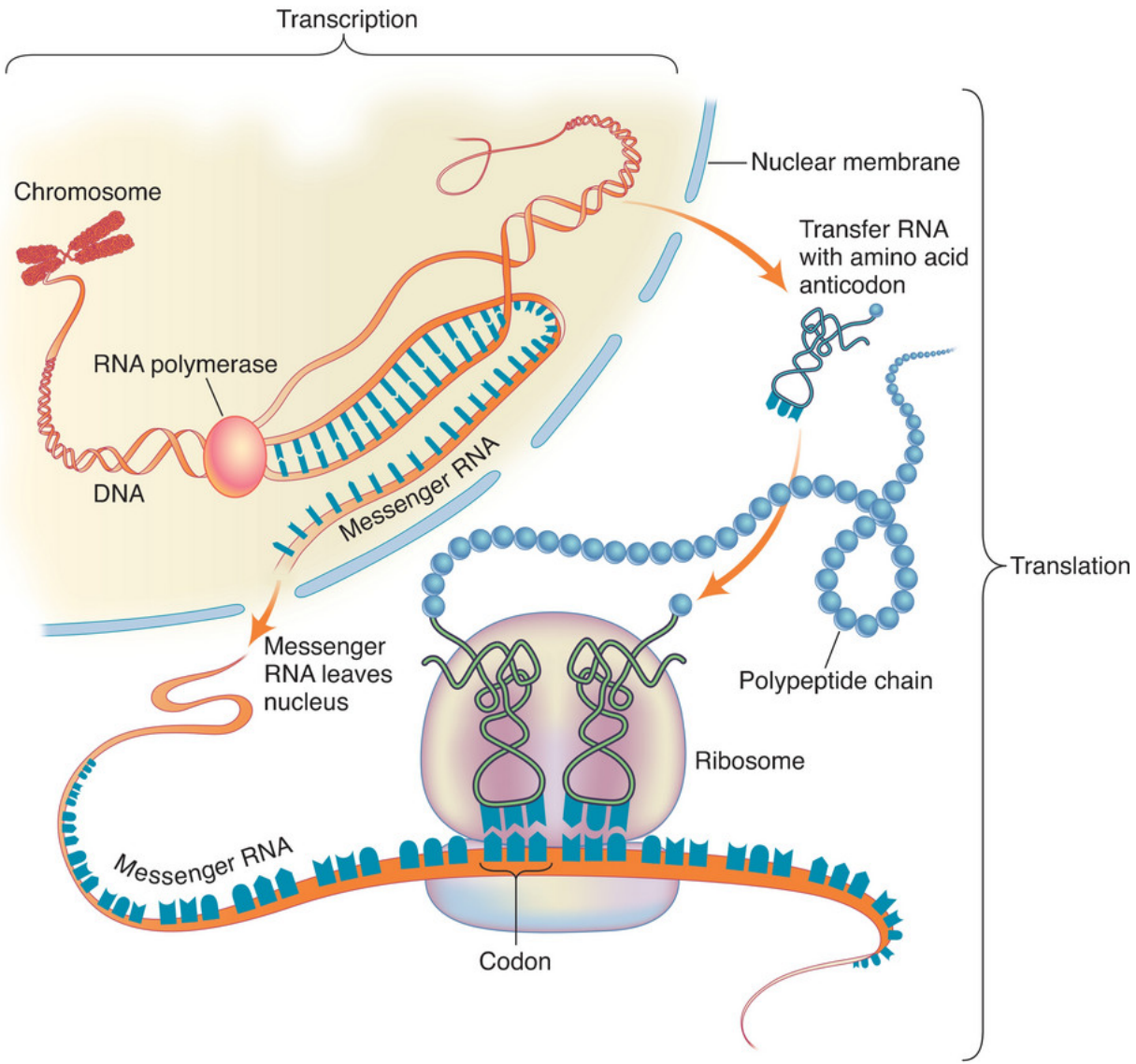
The Challenge for Microbial Ecology

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



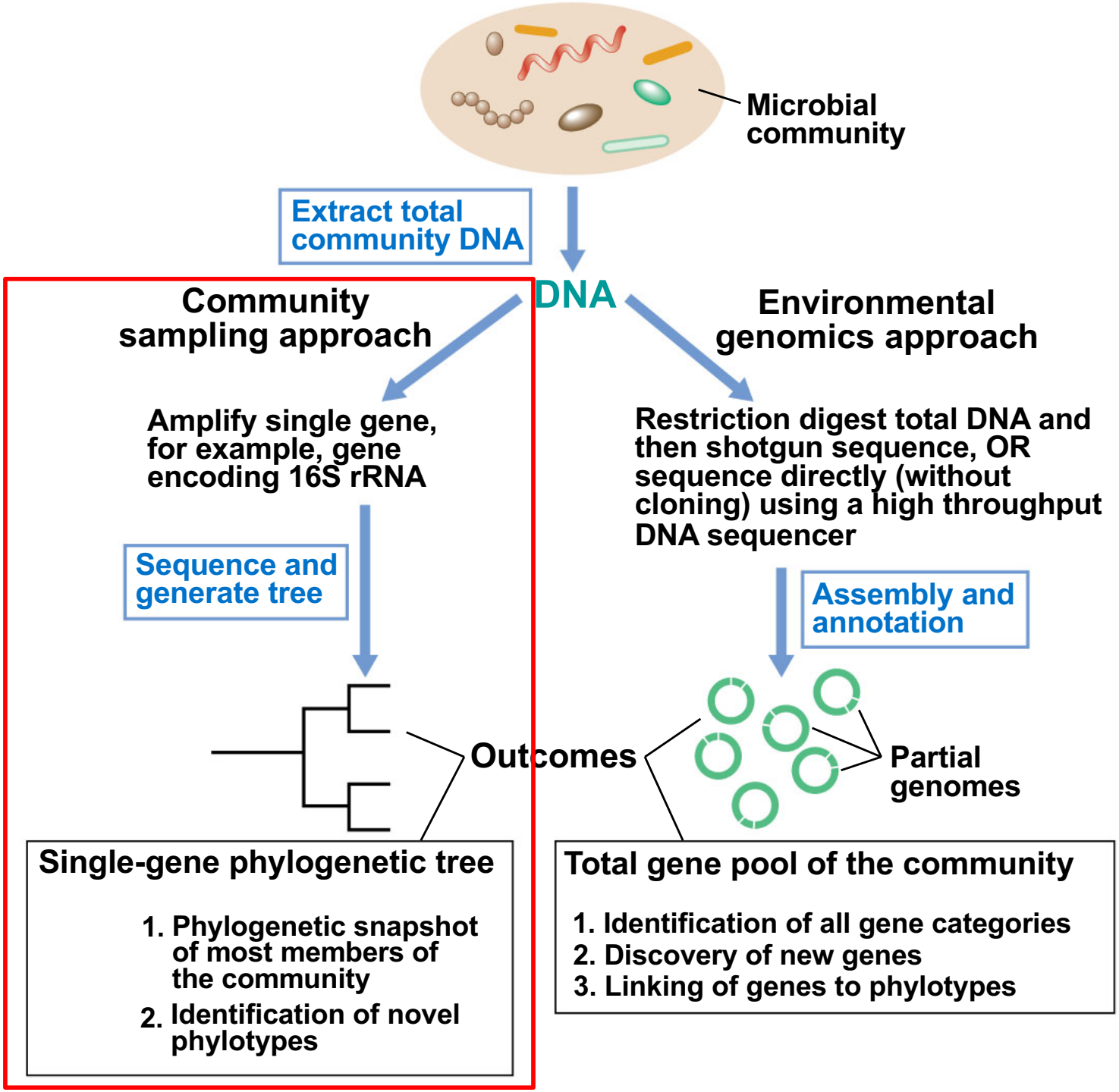
DNA

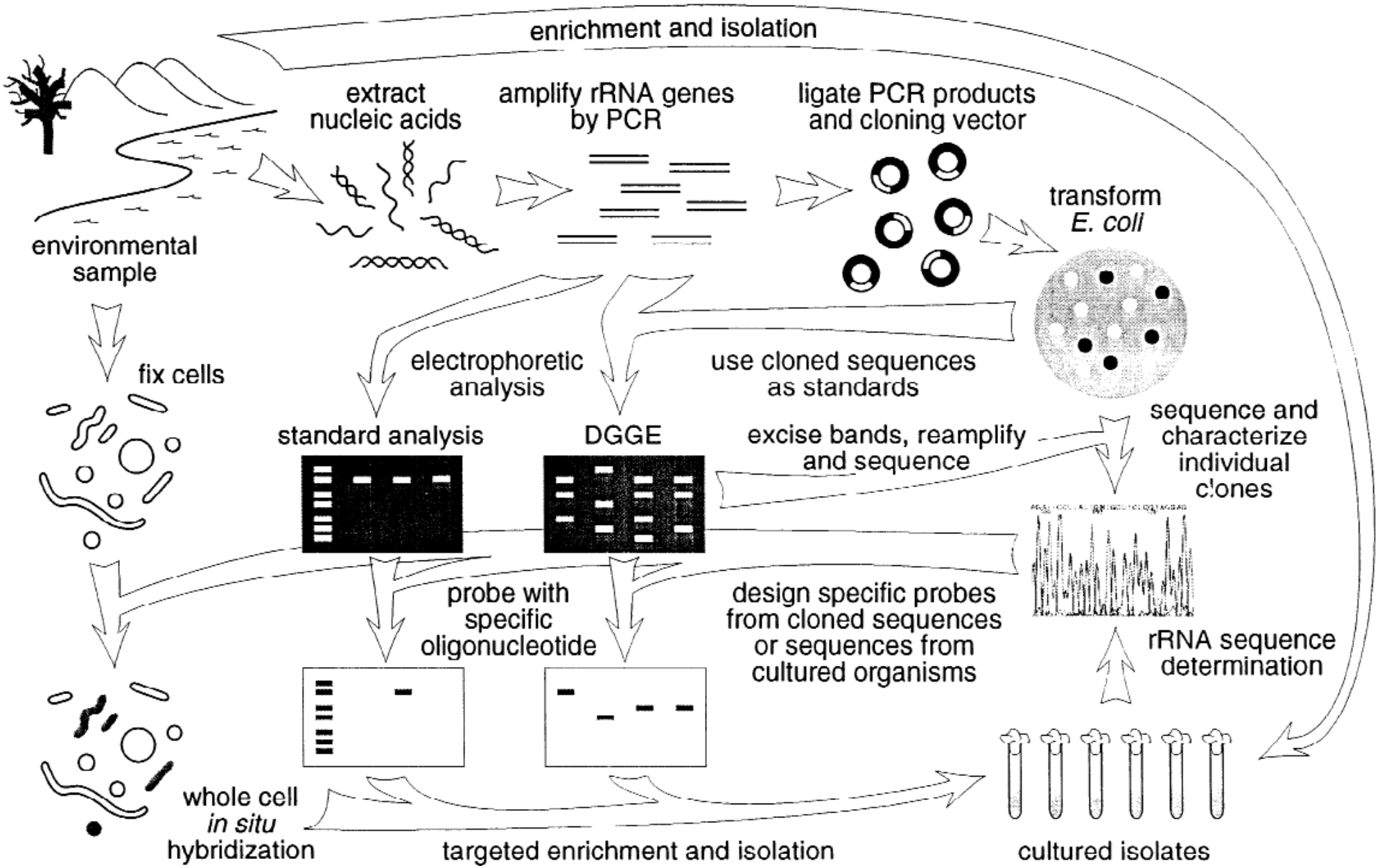
Transcription

RNA

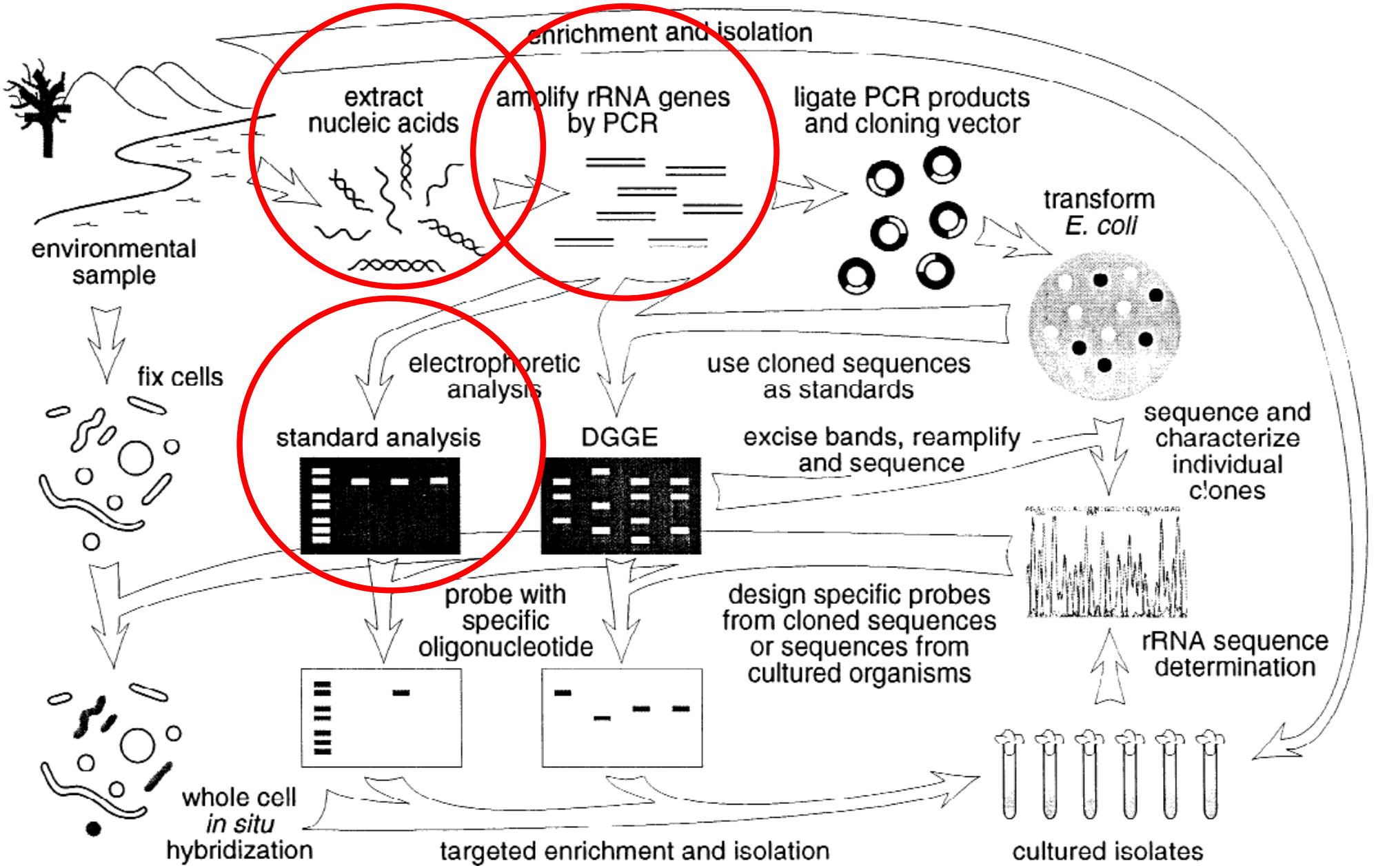
Translation

Protein



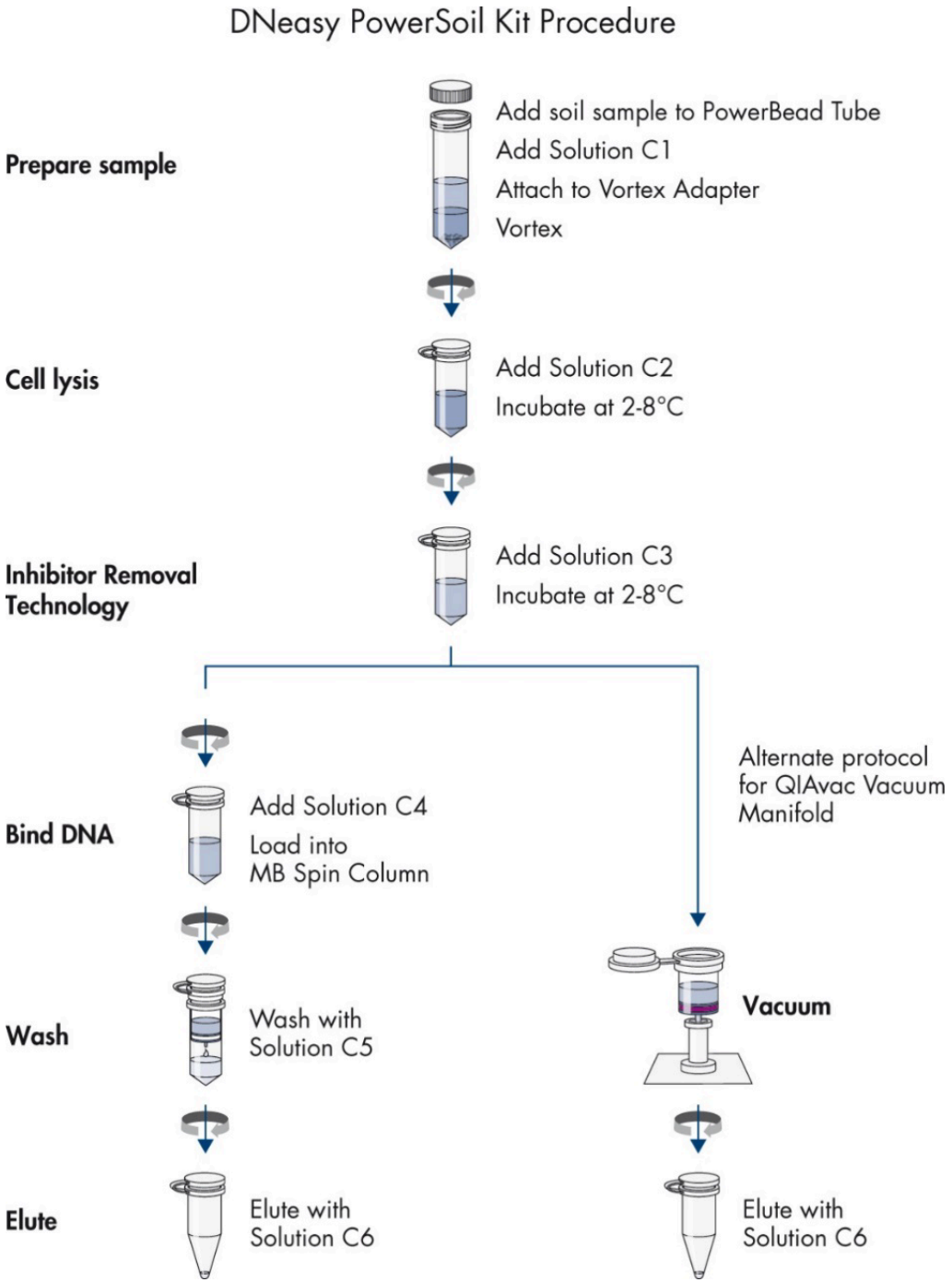


Head *et al*. 1998



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

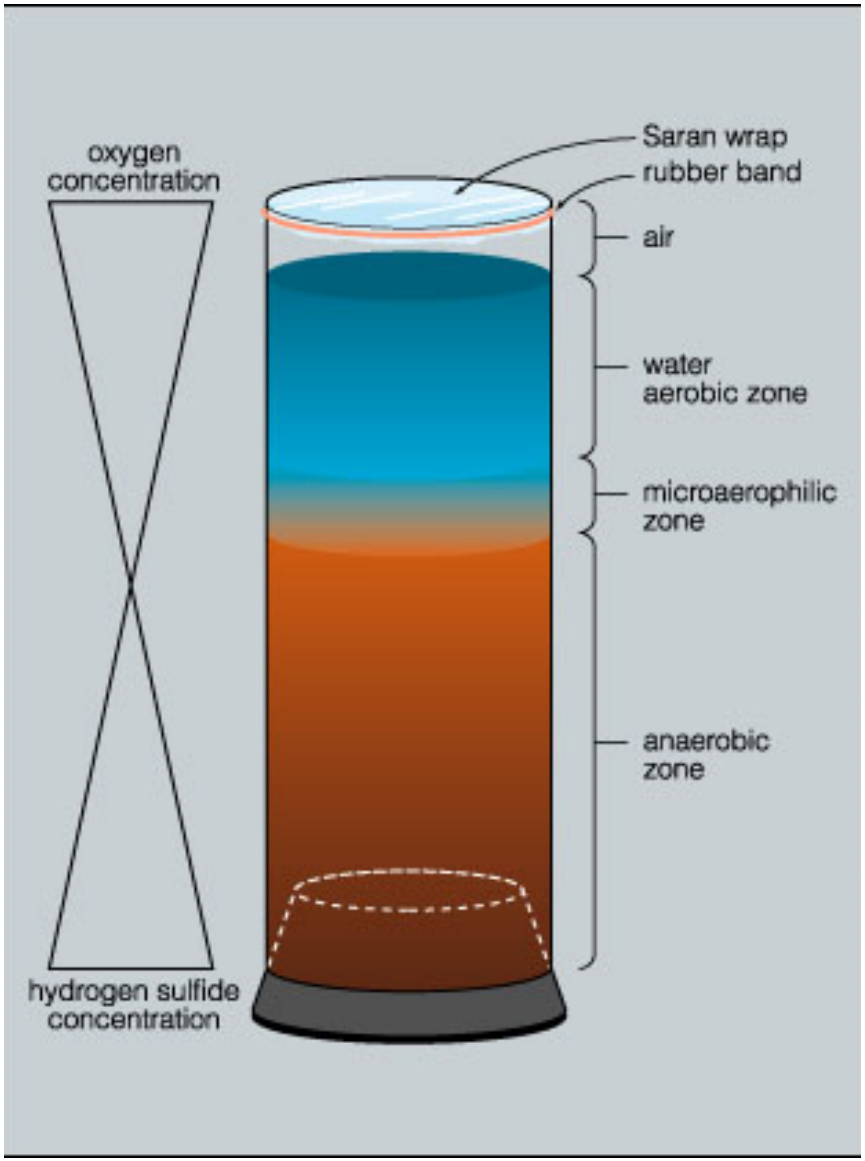
4. Mix DNA with acid and salt → stick to filter

5. Wash filter-bound DNA several times with alcohol

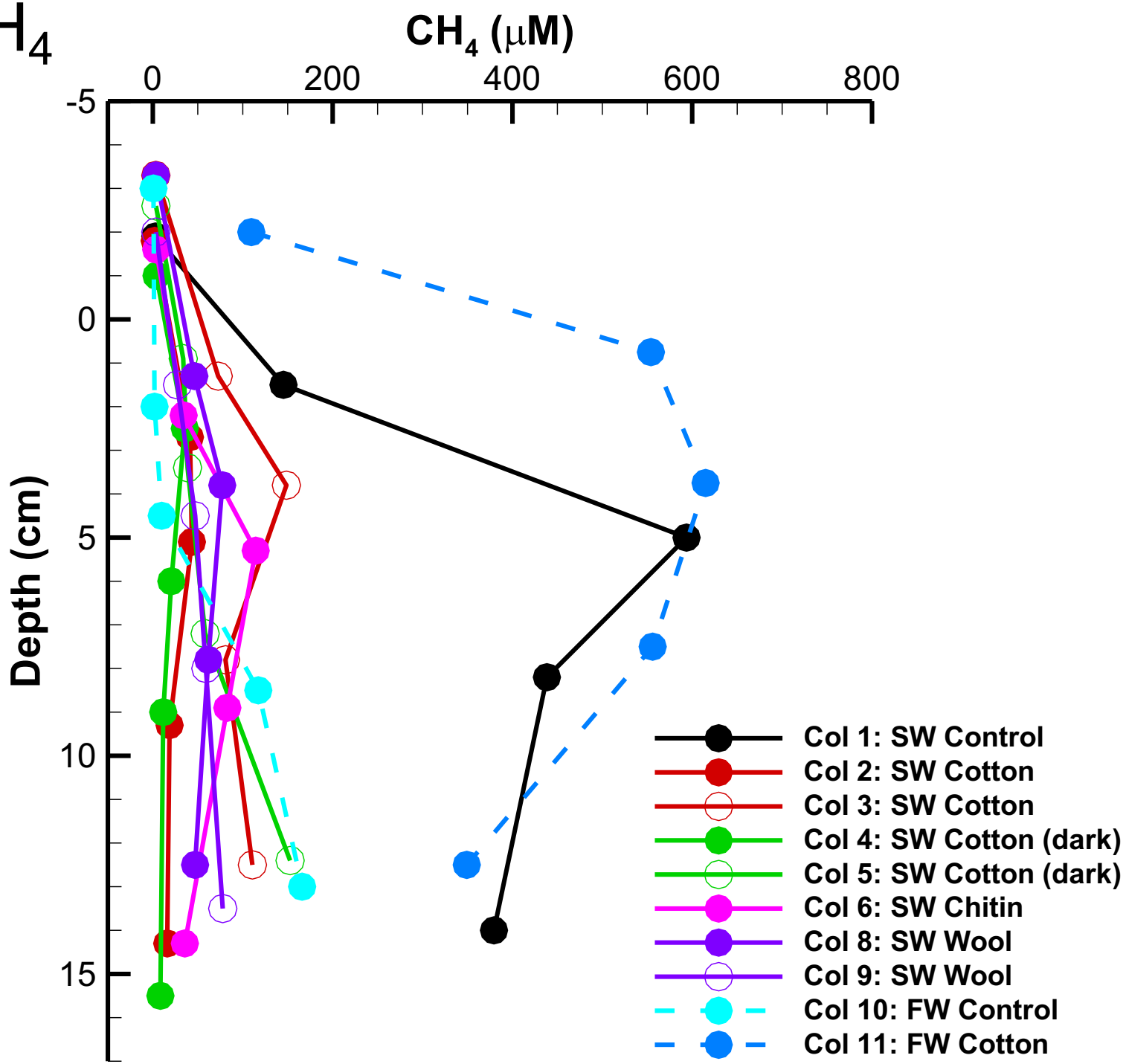
6. Elute DNA off membrane with pH 8, low-salt buffer

Choosing a Depth Horizon

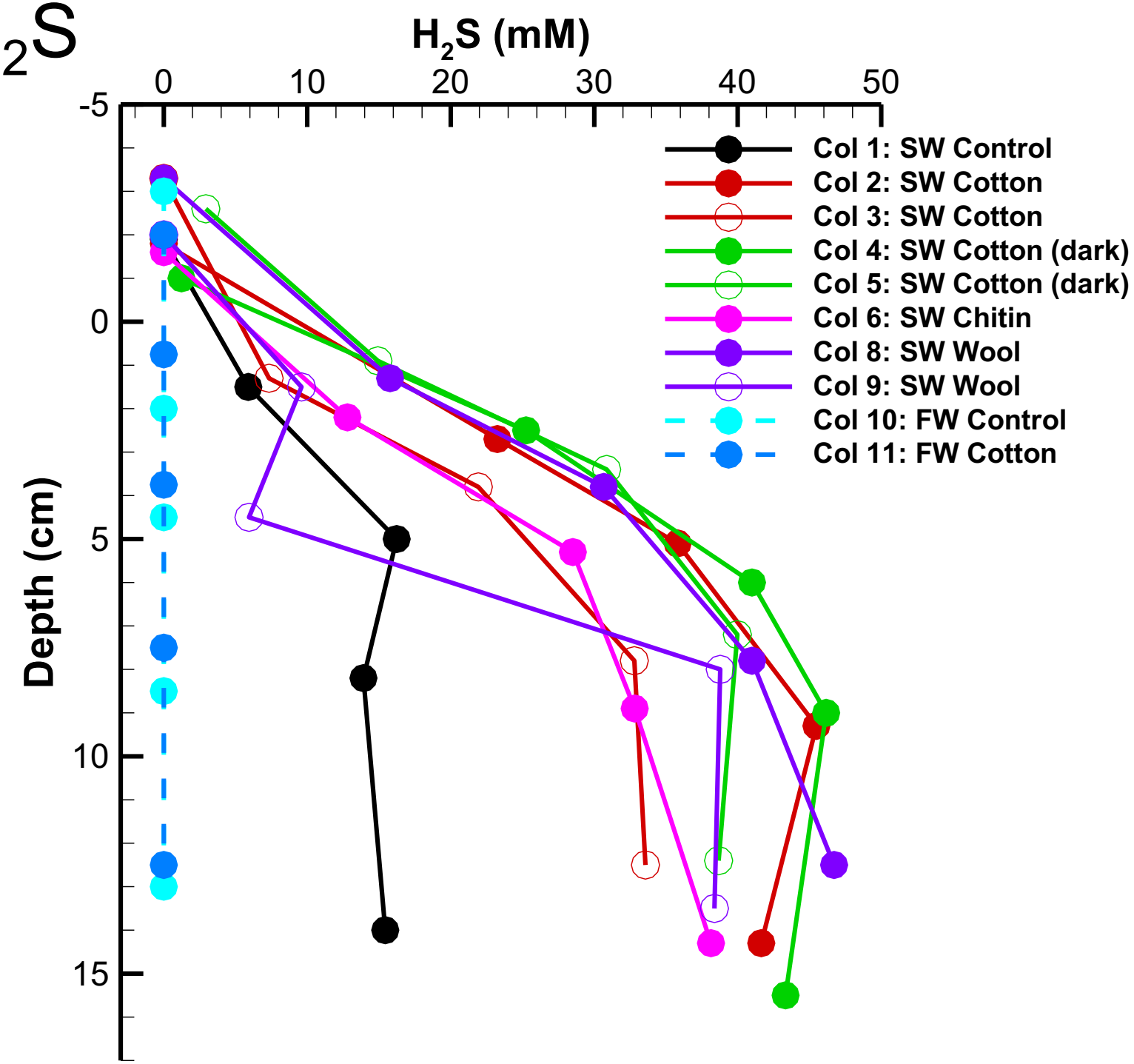
- 16S rRNA Bacteria
- 16S rRNA Archaea
- *mcr*A Methanogens
  - Methyl coenzyme M reductase
- *dsr*B Sulfate reducers
  - Dissimilatory bisulfite reductase



2018 CH_4

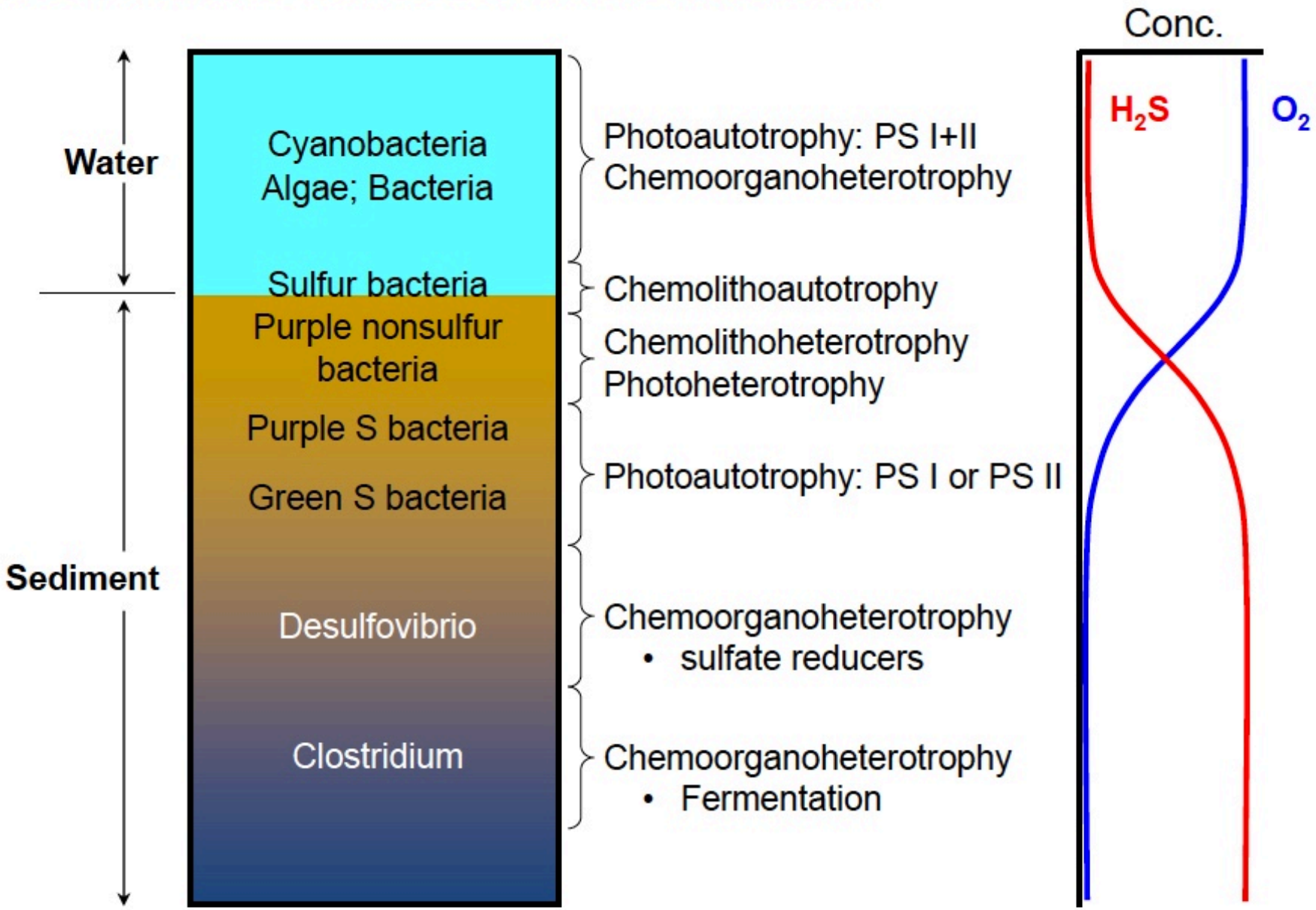

CH4 (μM)
0
200
400
600
800
-5
0
5
10
15
Depth (cm)
Col 1: SW Control
Col 2: SW Cotton
Col 3: SW Cotton
Col 4: SW Cotton (dark)
Col 5: SW Cotton (dark)
Col 6: SW Chitin
Col 8: SW Wool
Col 9: SW Wool
Col 10: FW Control
Col 11: FW Cotton

2018 H_2S

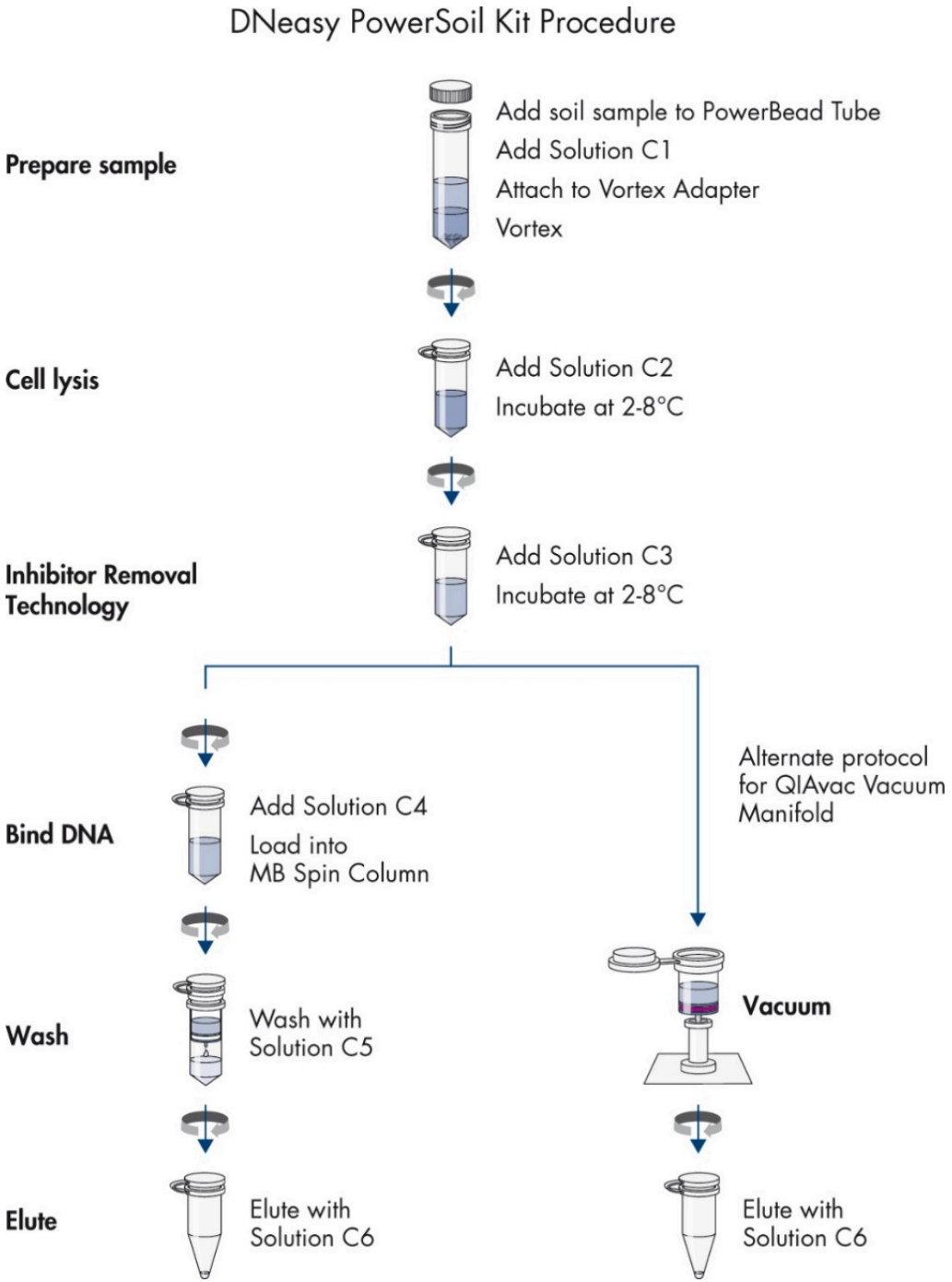

H2S (mM)
0
10
20
30
40
50
-5
0
5
10
15
Depth (cm)
Col 1: SW Control
Col 2: SW Cotton
Col 3: SW Cotton
Col 4: SW Cotton (dark)
Col 5: SW Cotton (dark)
Col 6: SW Chitin
Col 8: SW Wool
Col 9: SW Wool
Col 10: FW Control
Col 11: FW Cotton

Winogradsky Column

Microenvironments generated by chemical gradients.

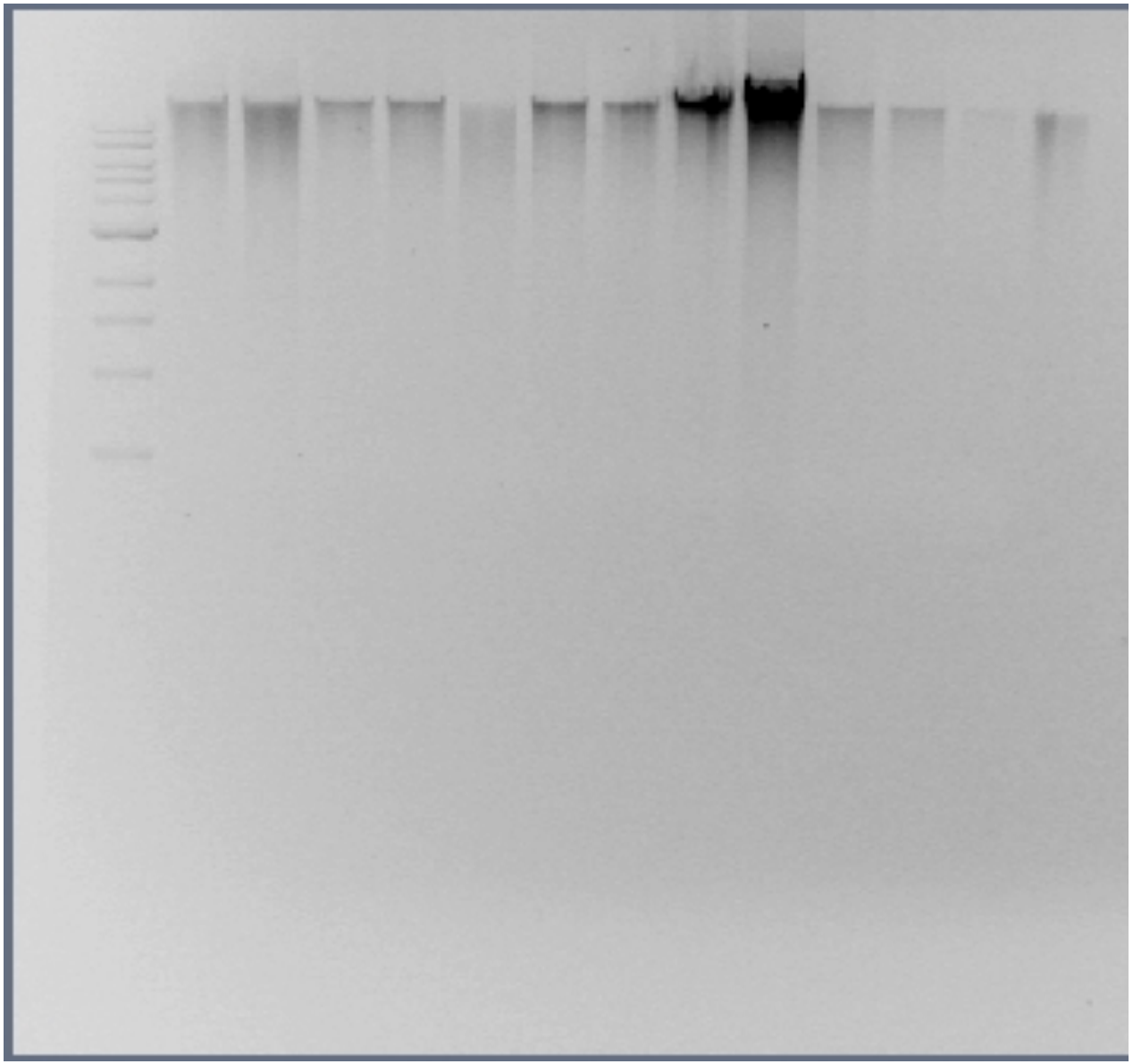


DNA Extraction Overview



Day 1, Part II

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



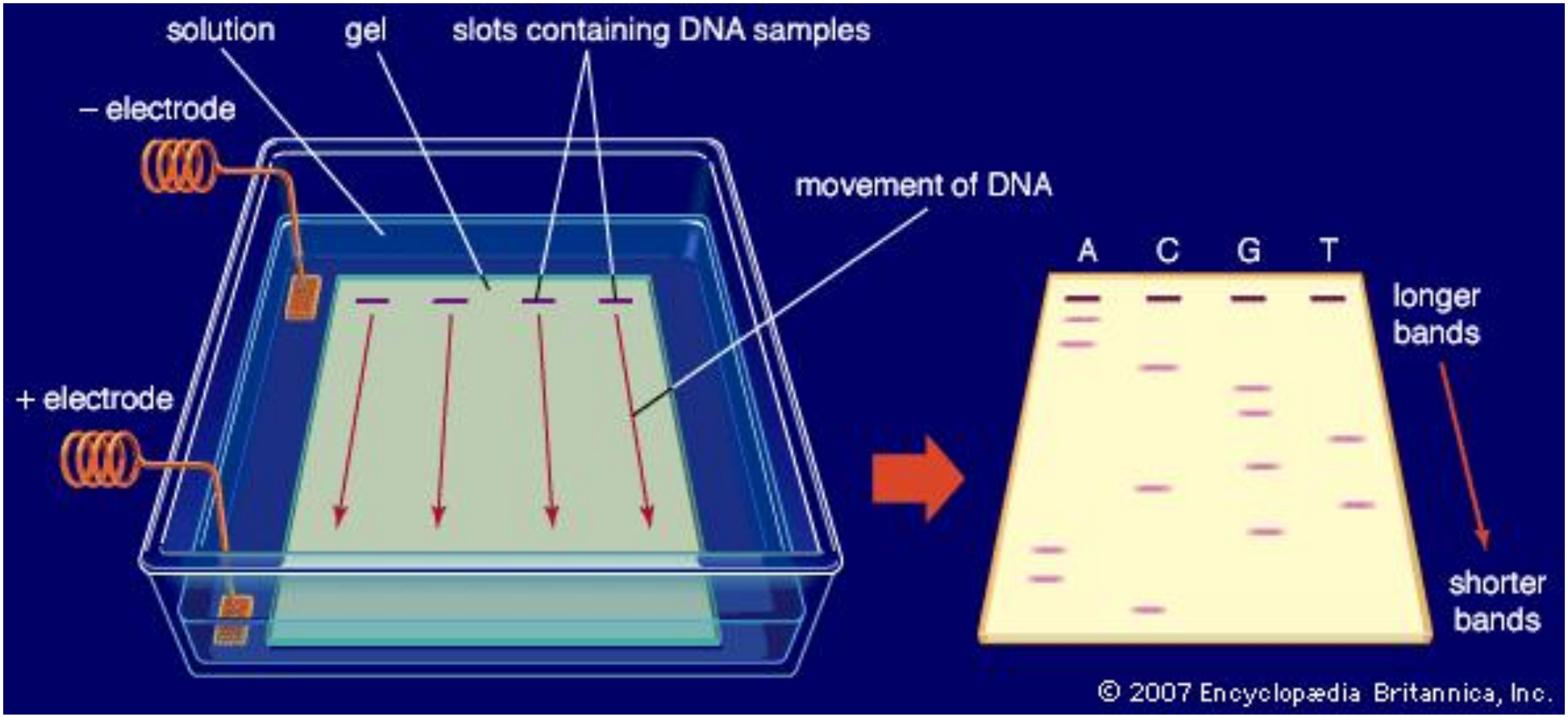
Genomic DNA

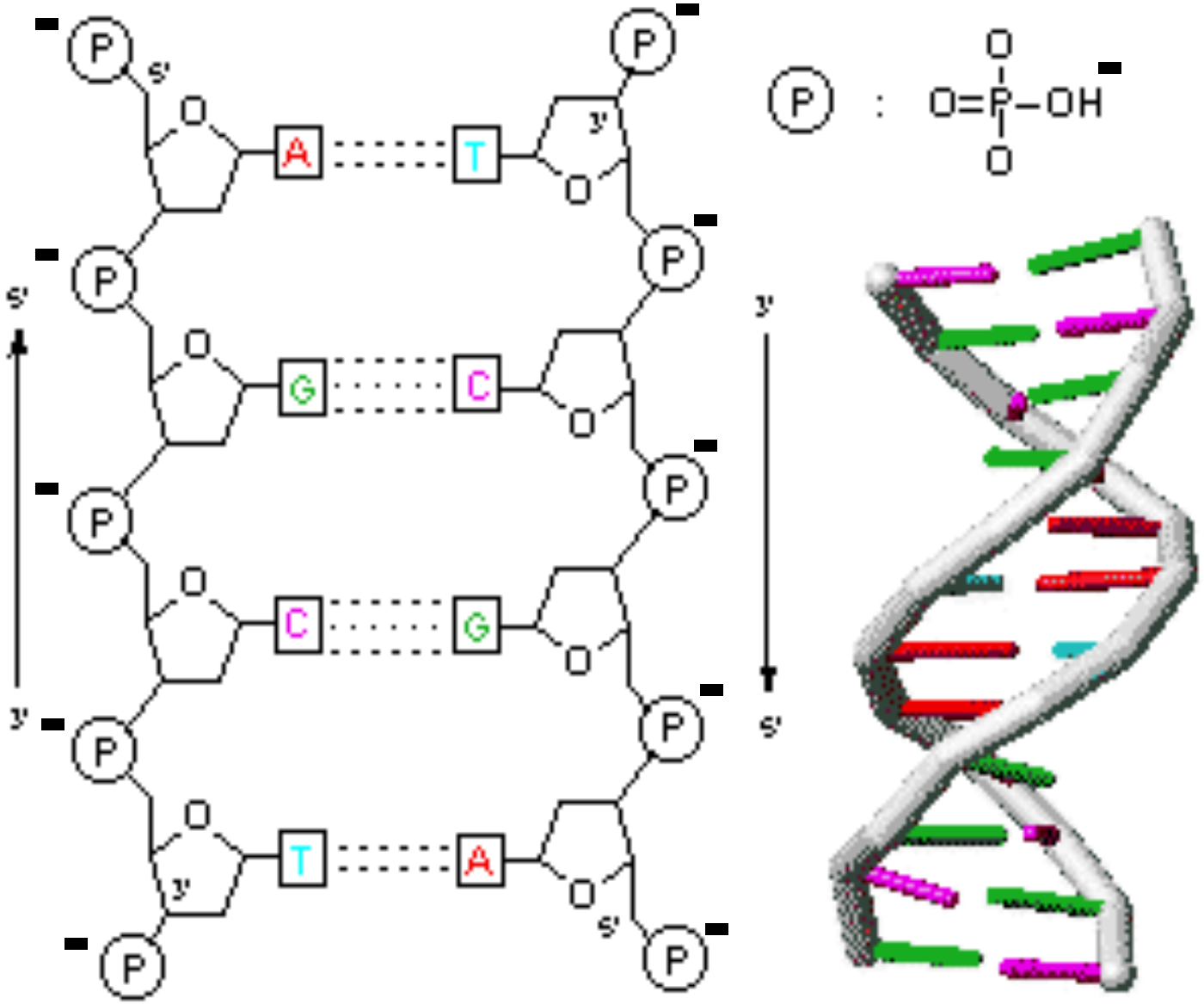
The sum total of all DNA from an organism or a community of organisms

Basics of Gel Electrophoresis

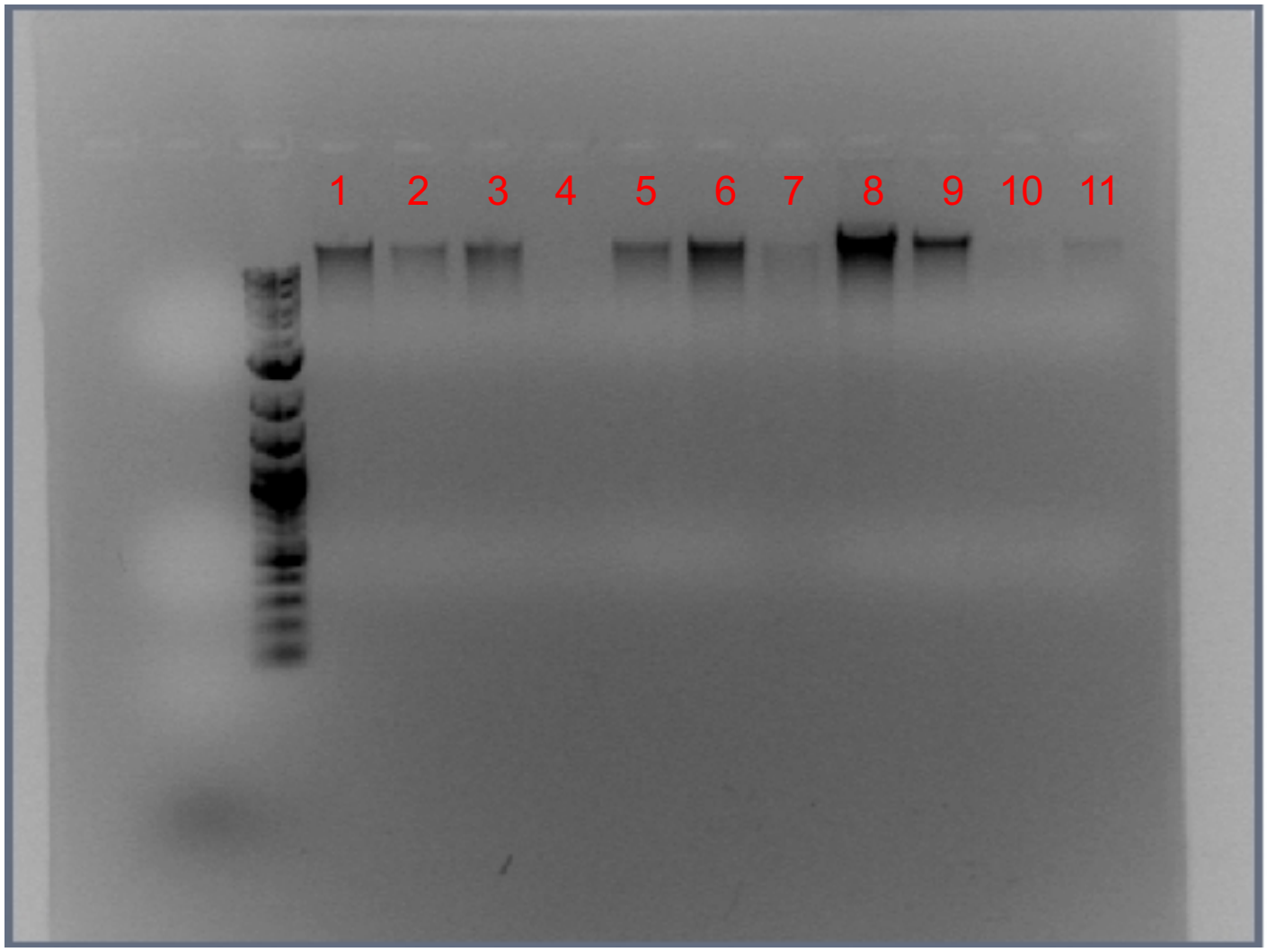
- The gel is a matrix (like jello with holes)
- DNA is negatively charged - will run to positive
- Smaller fragments run faster than larger ones
- Gel contains Ethidium Bromide, which binds to DNA and fluoresces when hit with UV light (WEAR GLOVES!!!)

Gel electrophoresis




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

Gel results . . ..



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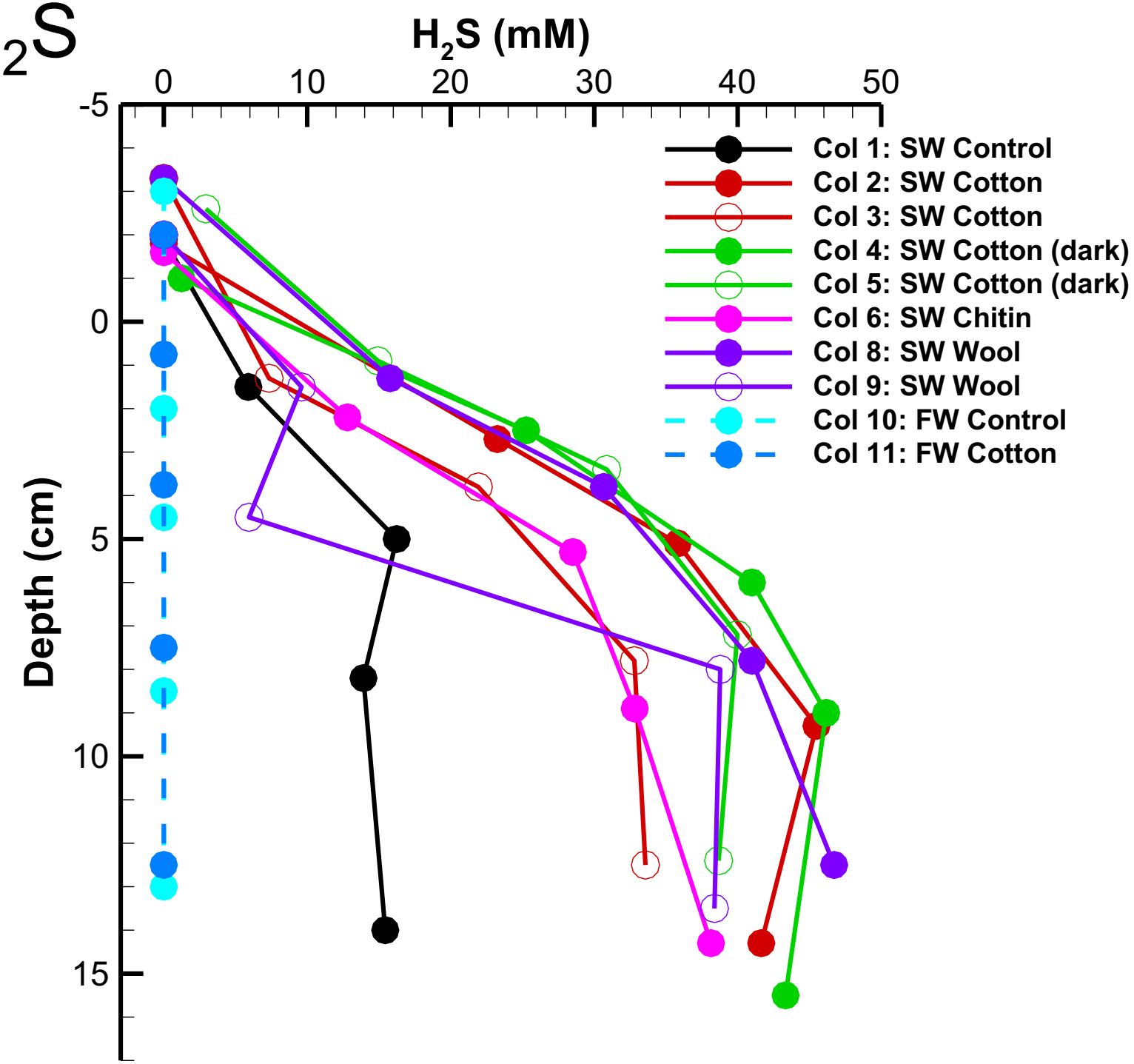
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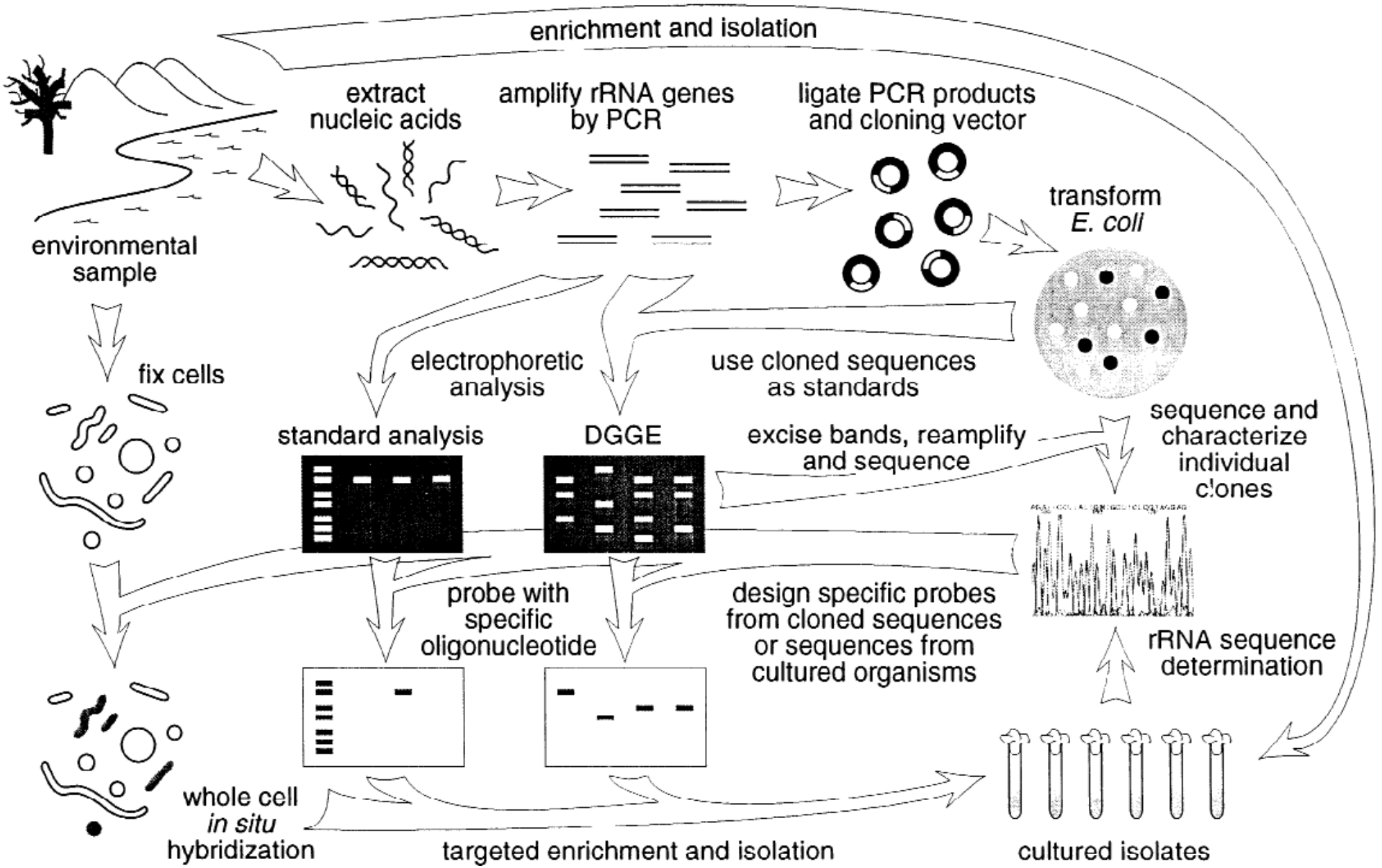
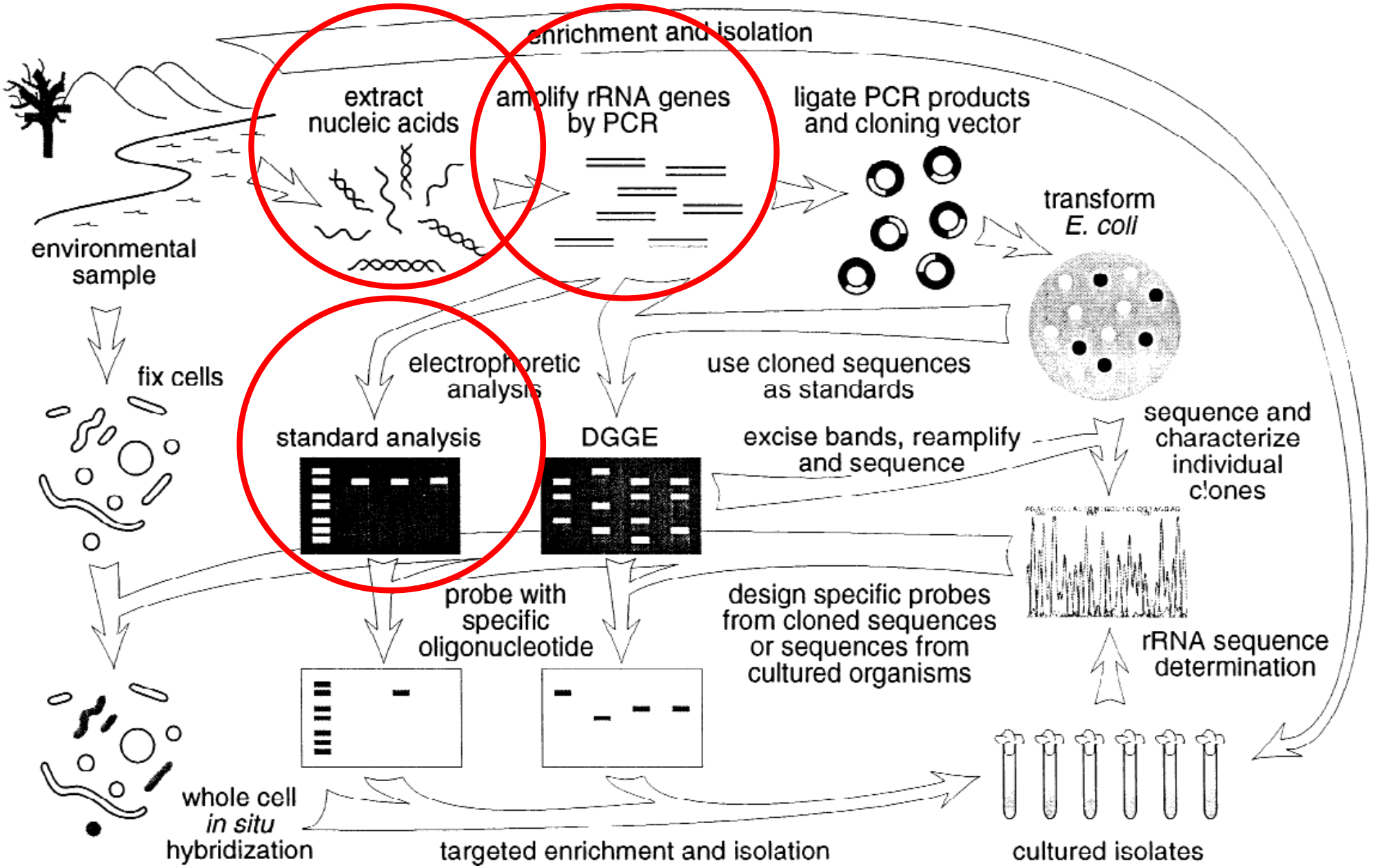
Col 8: SW Wool
Col 9: SW Wool
Col 10: FW Control
Col 11: FW Cotton

Day 2

- Basics of PCR
- Set up PCR reactions using the DNA from your extractions and an assortment of primers



Head *et al*. 1998

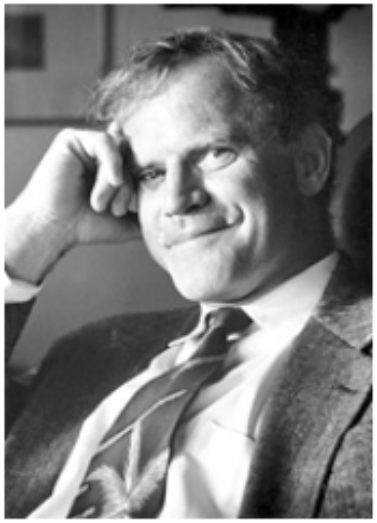


Head *et al*. 1998

Polymerase Chain Reaction (PCR)

- Rapid, inexpensive and simple way of making millions of copies of a gene starting with very few copies
- Does not require the use of isotopes or toxic chemicals
- It involves preparing the sample DNA and a master mix with primers, followed by detecting reaction products

The Nobel Prize in Chemistry 1993



Kary B. Mullis
Prize share: 1/2

Michael Smith
Prize share: 1/2

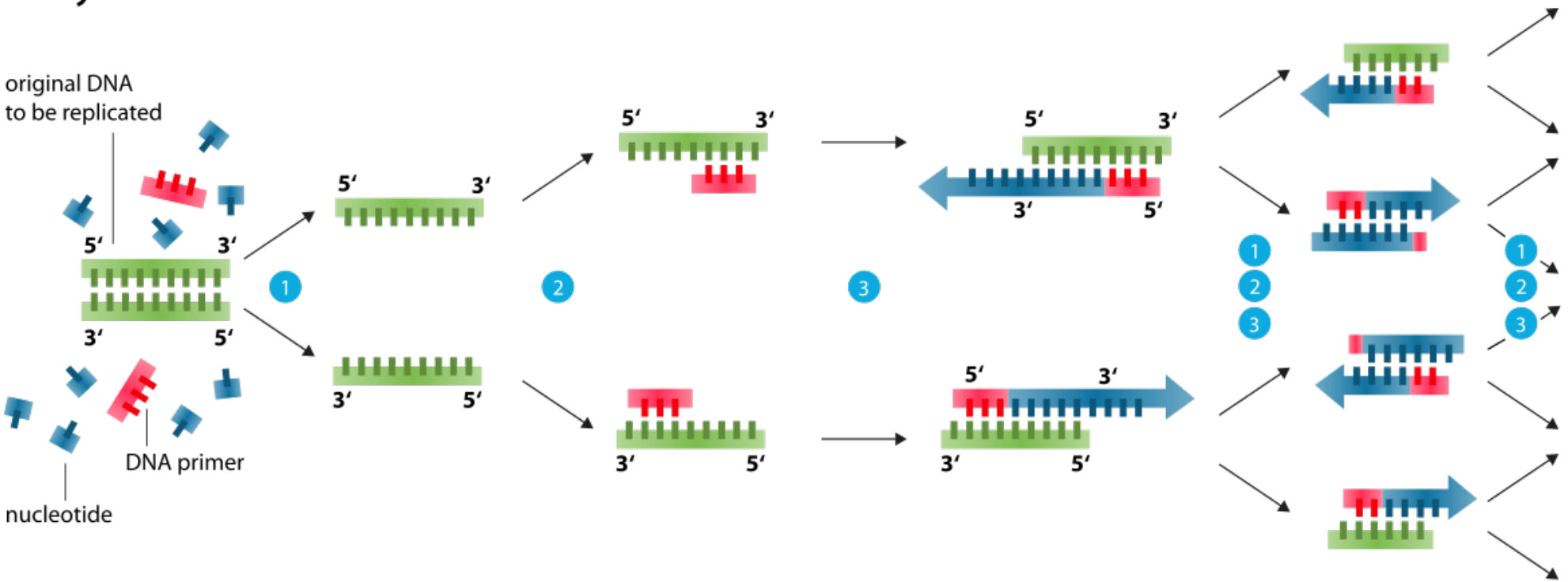
The Nobel Prize in Chemistry 1993 was awarded *"for contributions to the developments of methods within DNA-based chemistry"* jointly with one half to Kary B. Mullis *"for his invention of the polymerase chain reaction (PCR) method"* and with one half to Michael Smith *"for his fundamental contributions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

Polymerase 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

Polymerase chain reaction - PCR



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

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

Typical PCR Profile

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

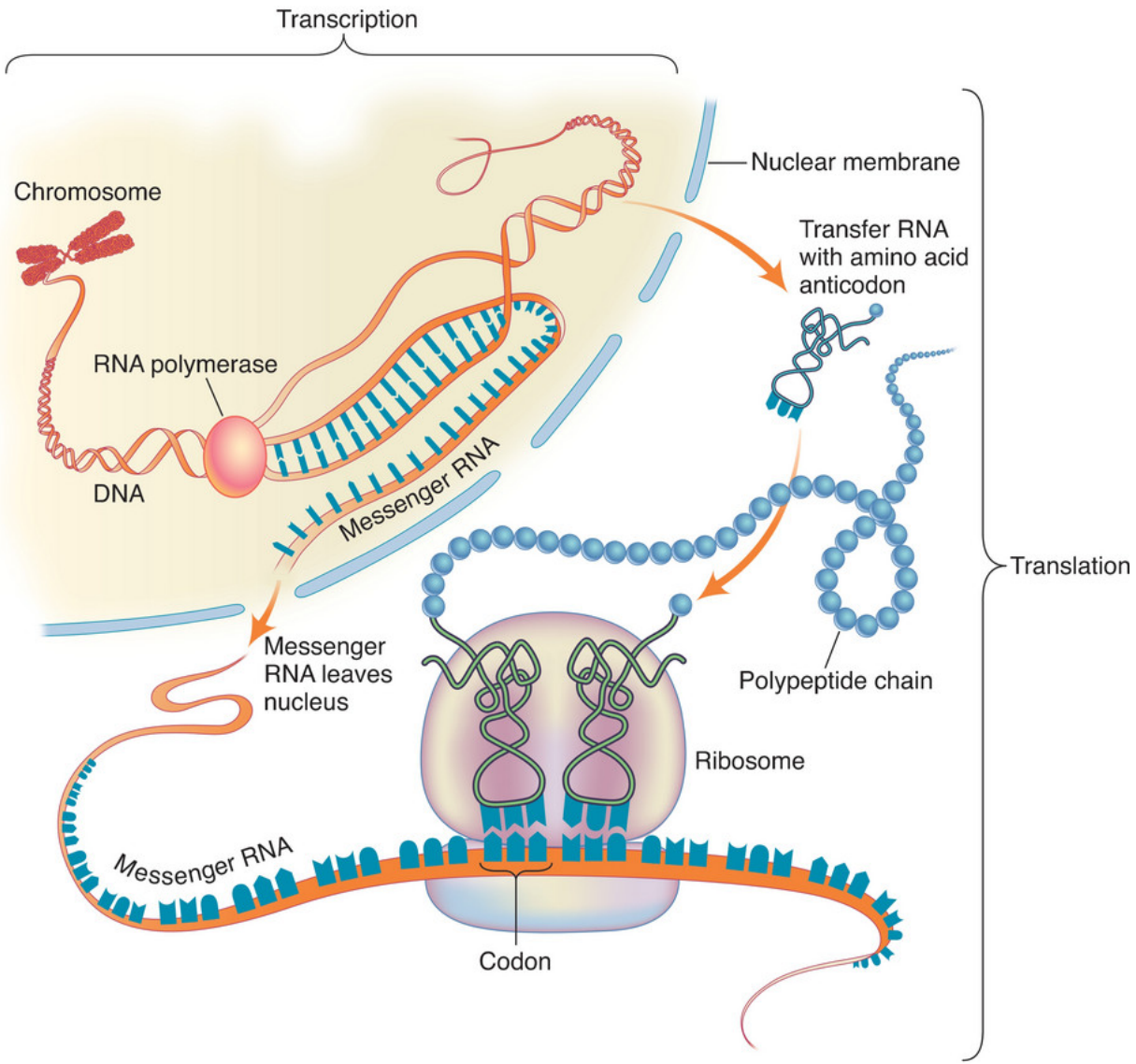
Things you can optimize

- 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…

The Star of the Show: SSU rRNA

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

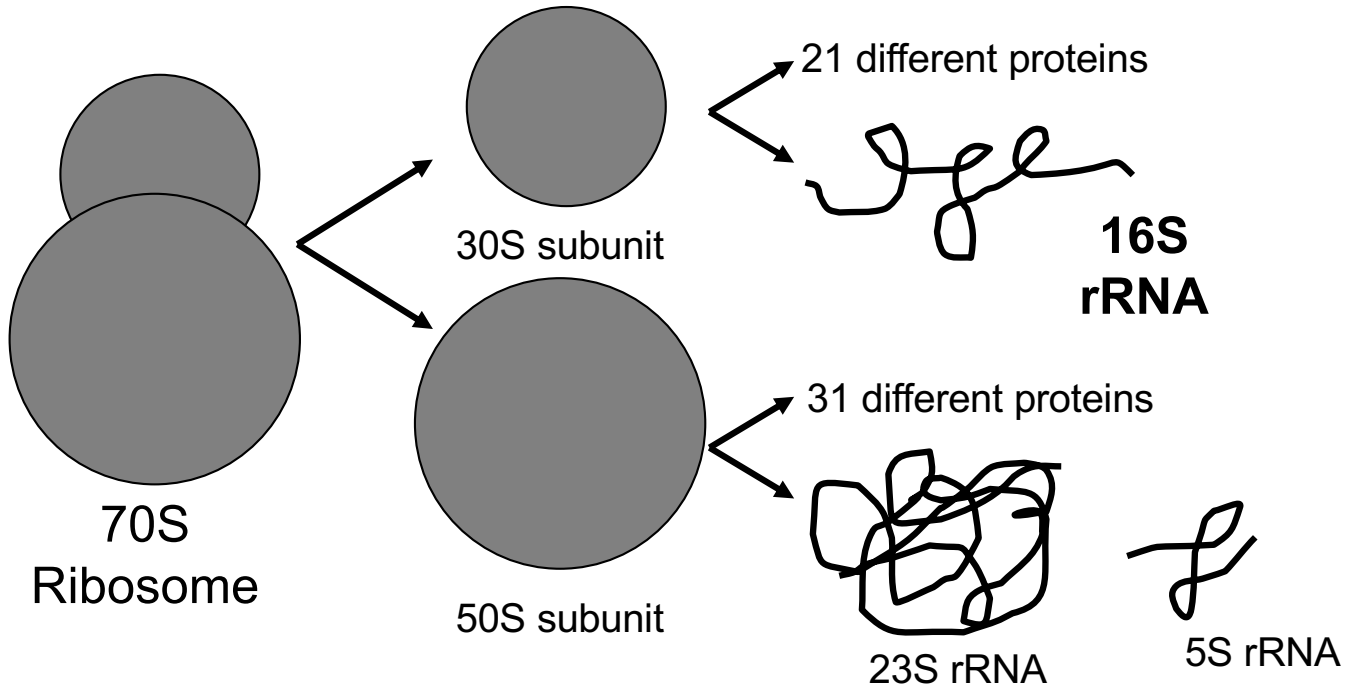
RNA

Translation

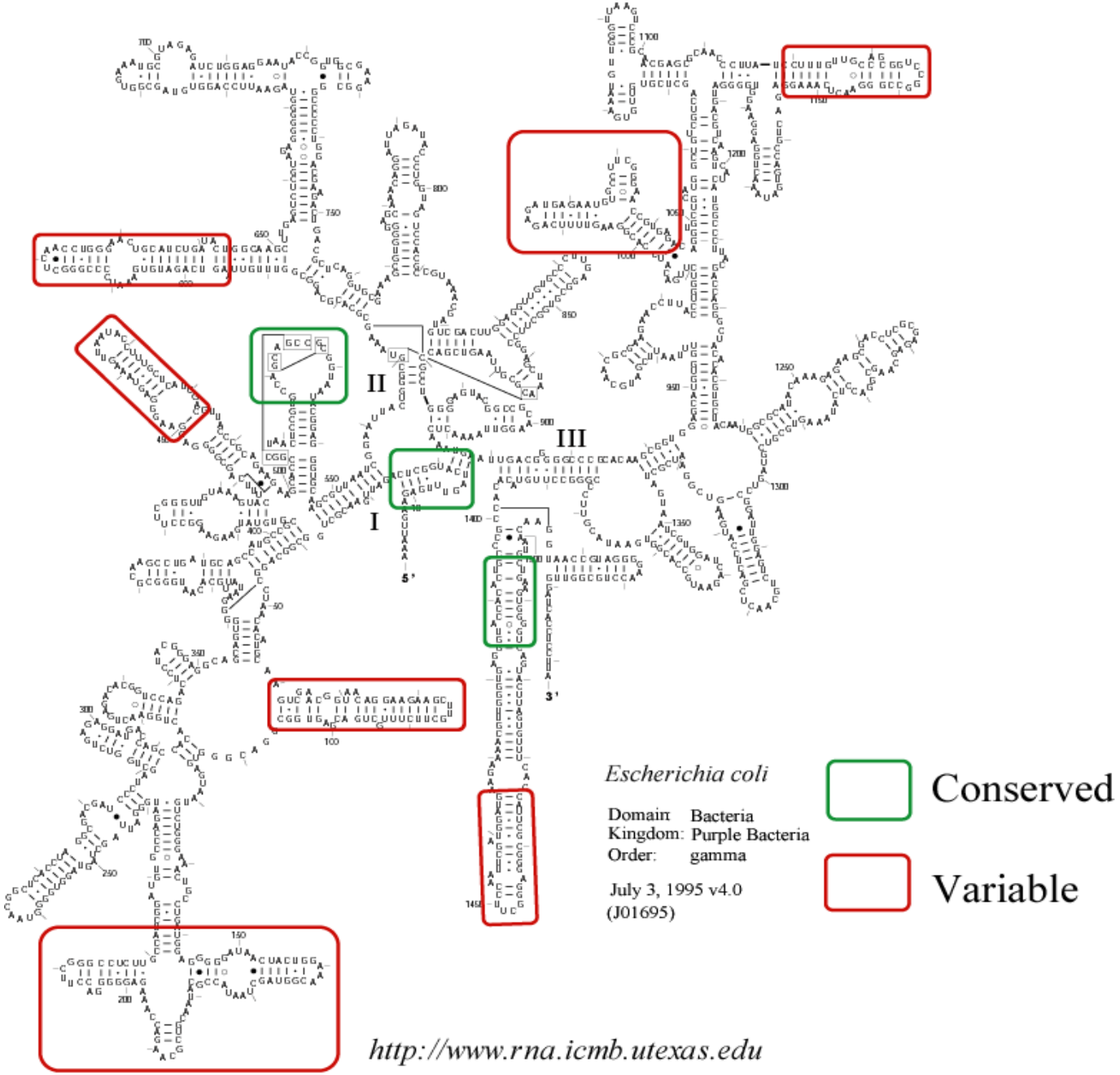
Protein

Ribosomes

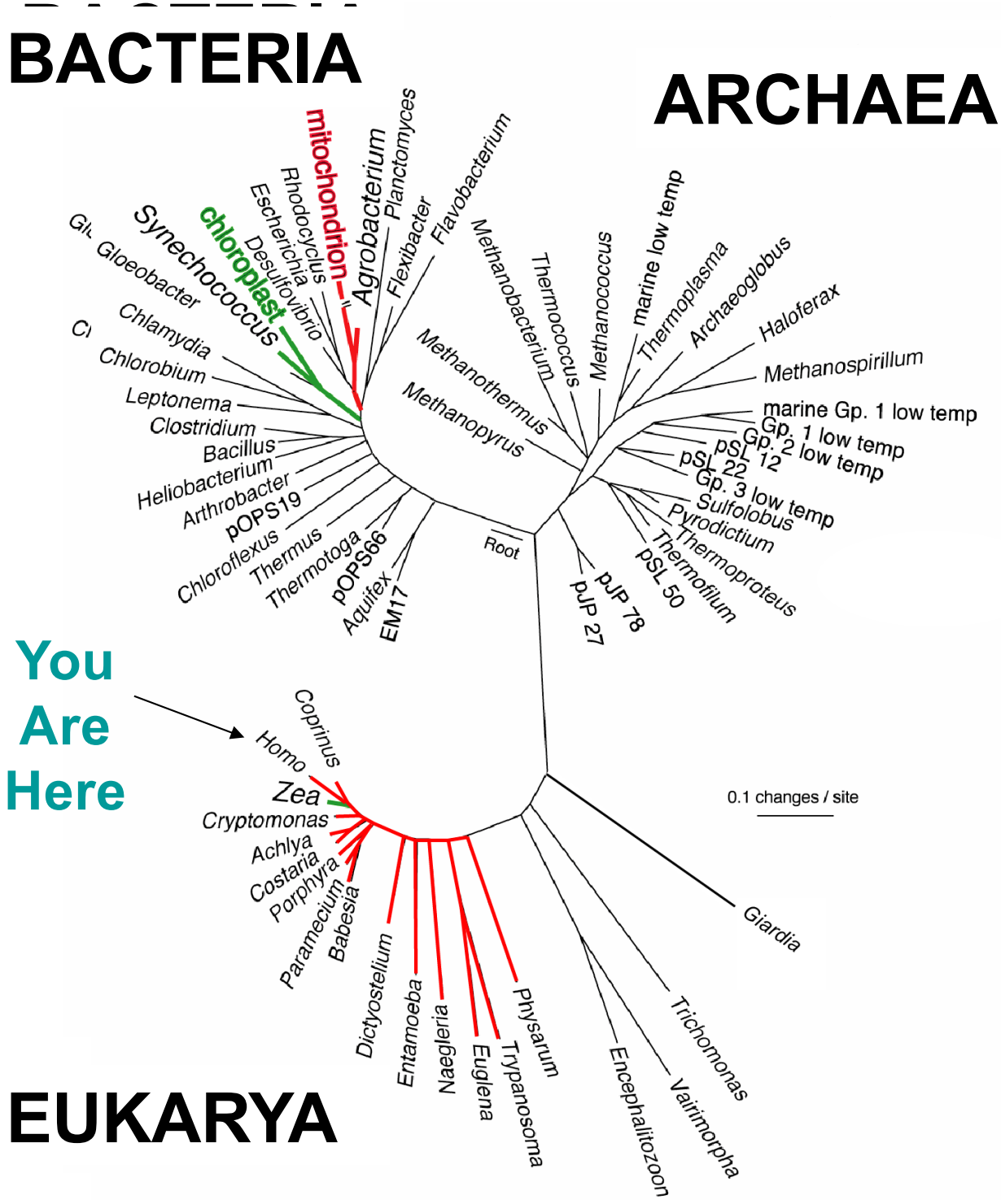
- Make proteins
- rRNA is transcribed from rDNA genes



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

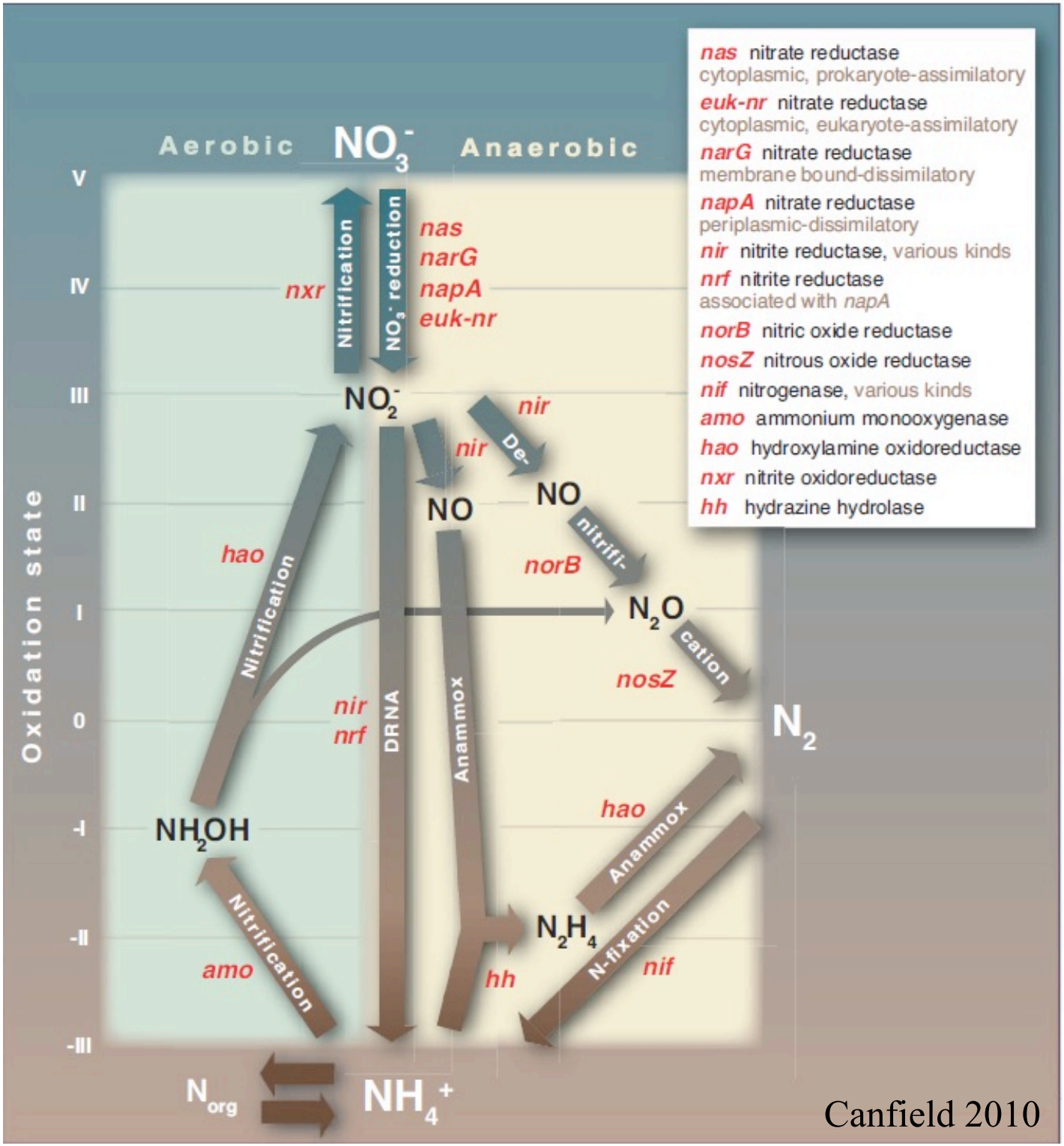
Universal Tree of Life



Modified from Norman Pace

Beyond 16S rRNA:

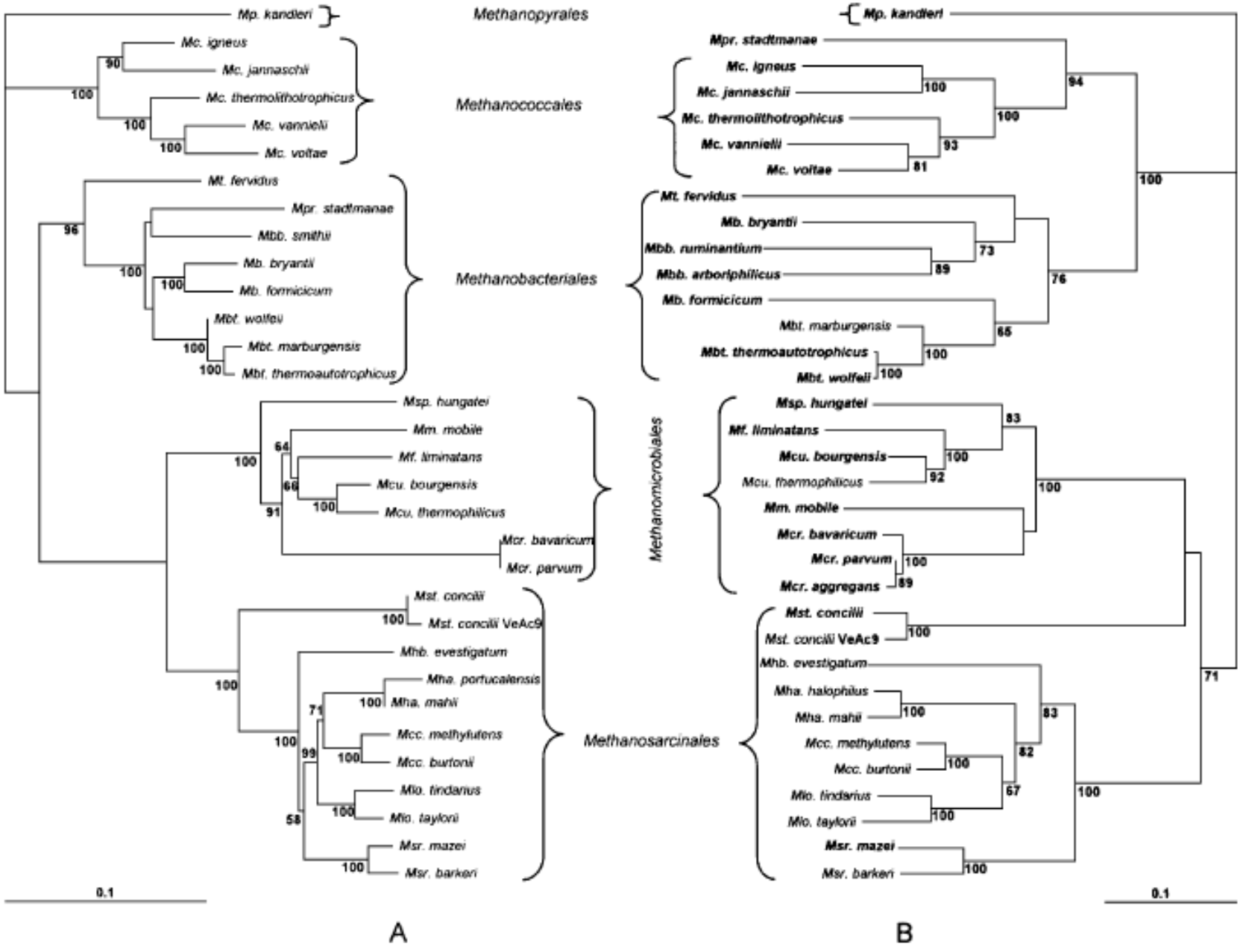
What is the functional potential of microbes?



Canfield 2010

16S rDNA

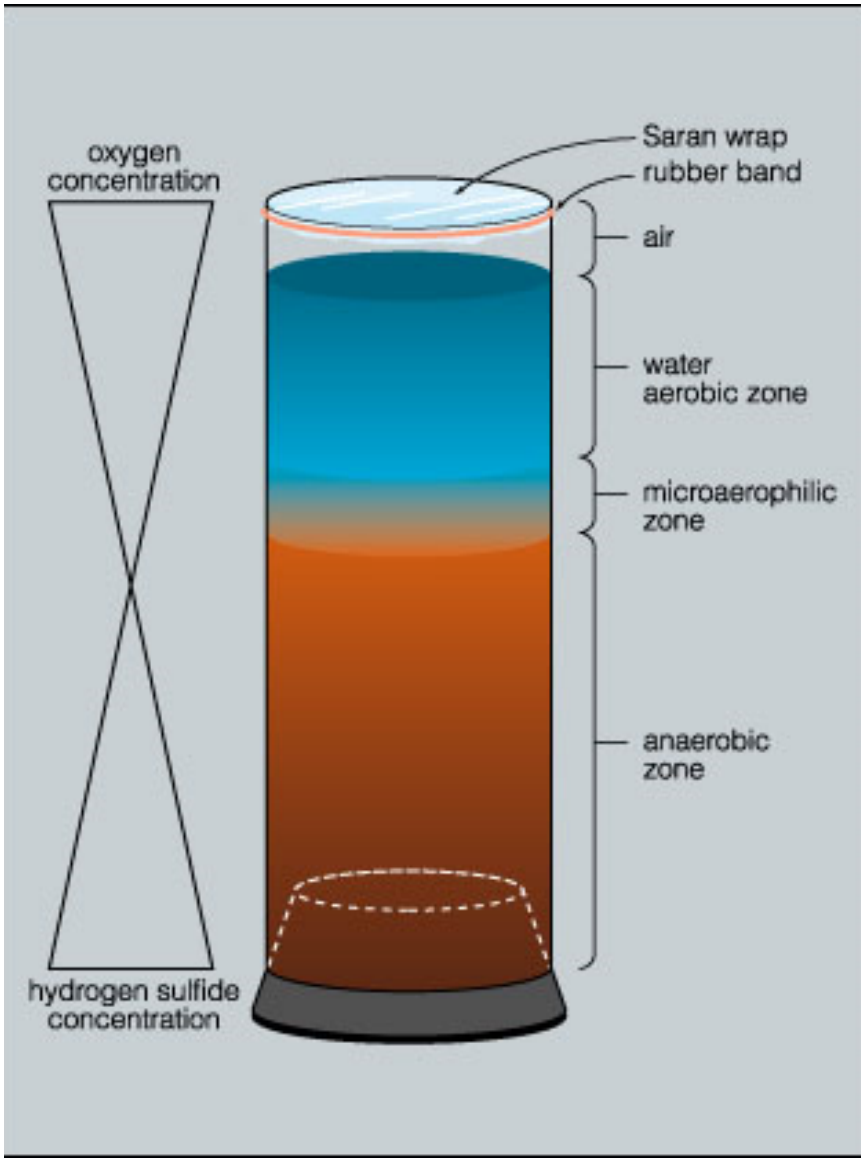
mcrA



Luton *et al*. 2002

Our 4 Targets

- 16S rRNA Bacteria
- 16S rRNA Archaea
- *mcr*A Methanogens
  - 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