Molecular Methods in Microbial Ecology

Contact Info: Julie Huber and Nancy Akerman

Schedule: 25 Oct: Introductory Lecture, DNA extraction
Run DNA products on gel

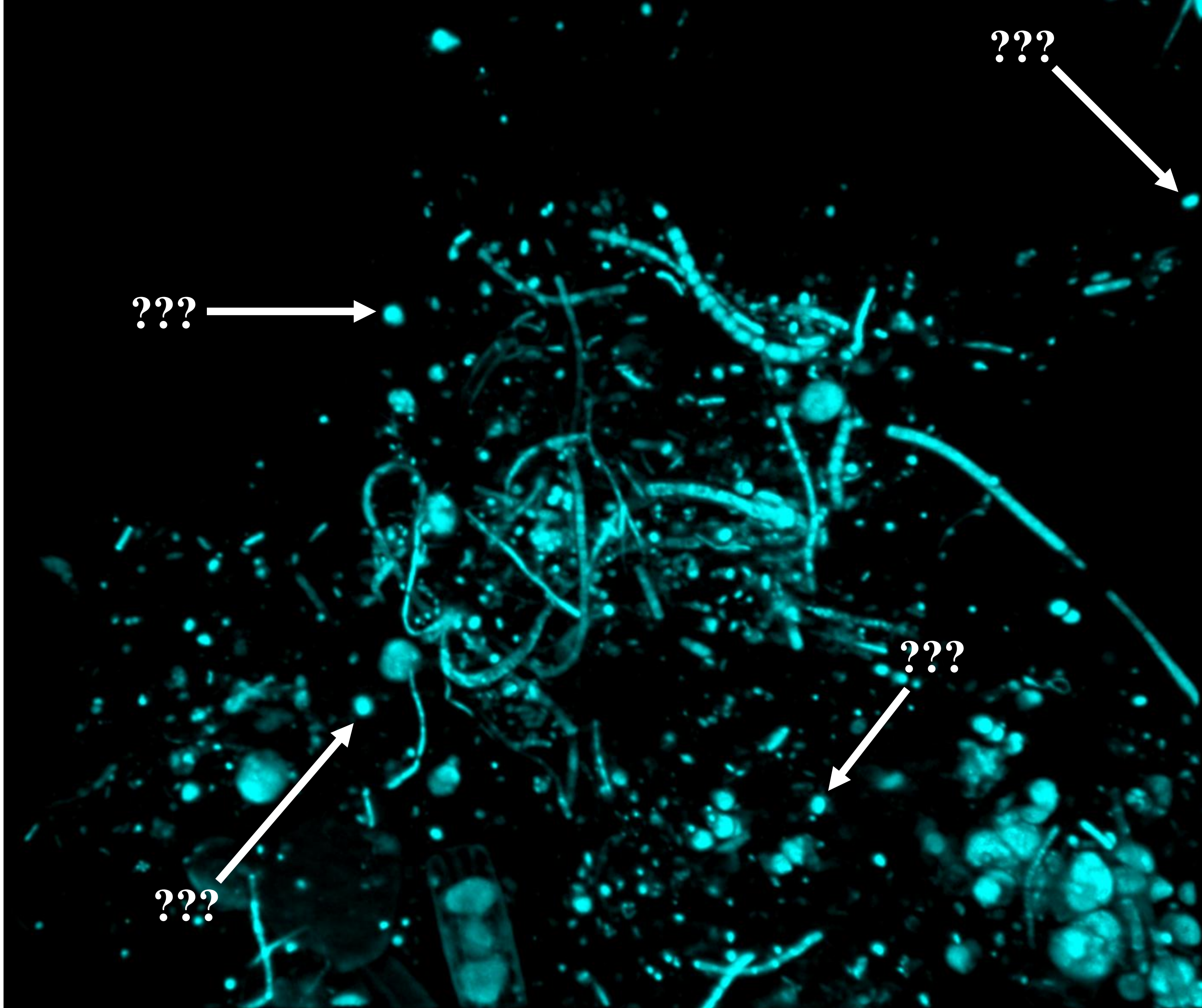
27 Oct: Lecture on PCR
Prepare PCR reactions

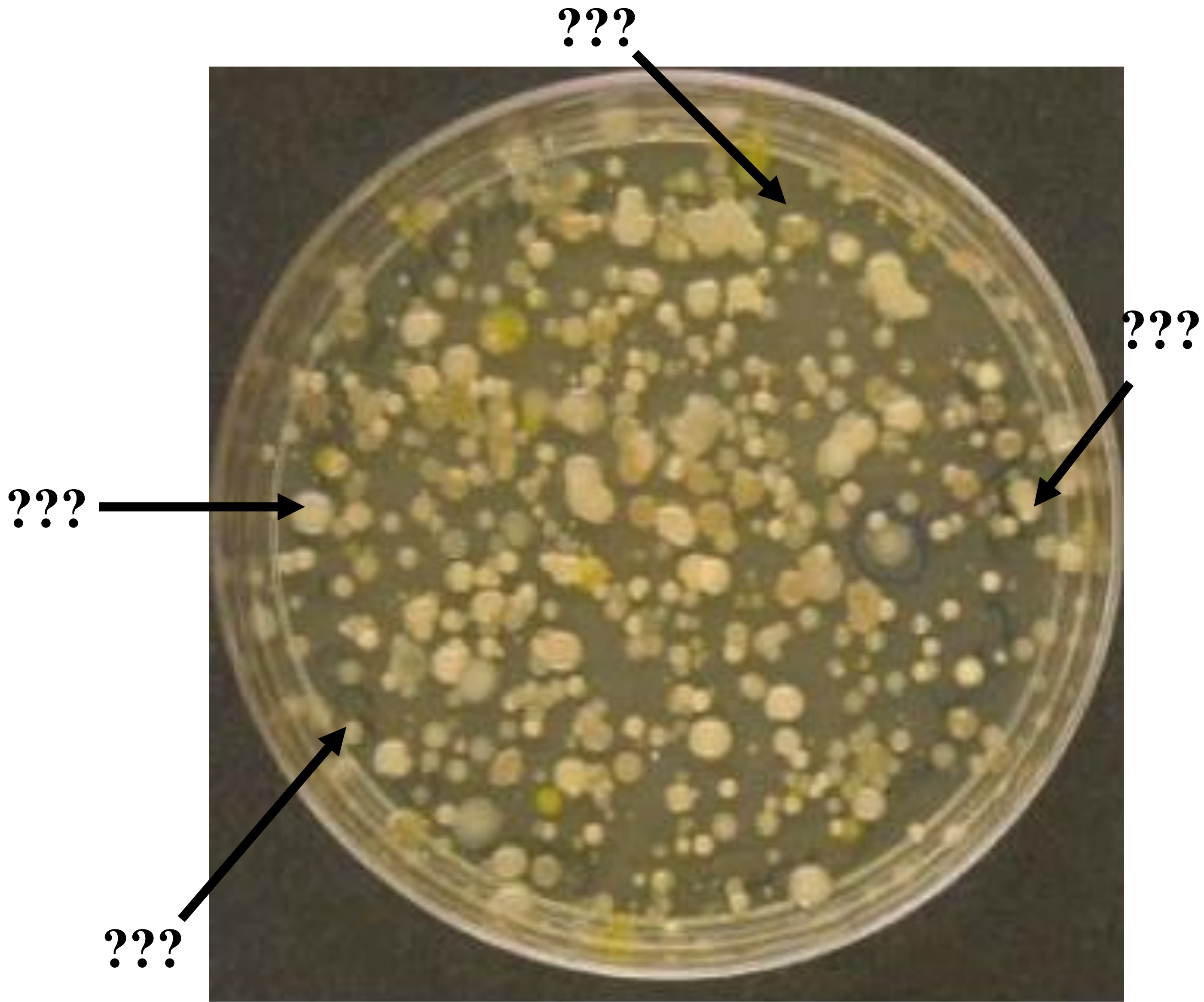
1 Nov: Analyze gels from PCR
Lecture on other molecular methods

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





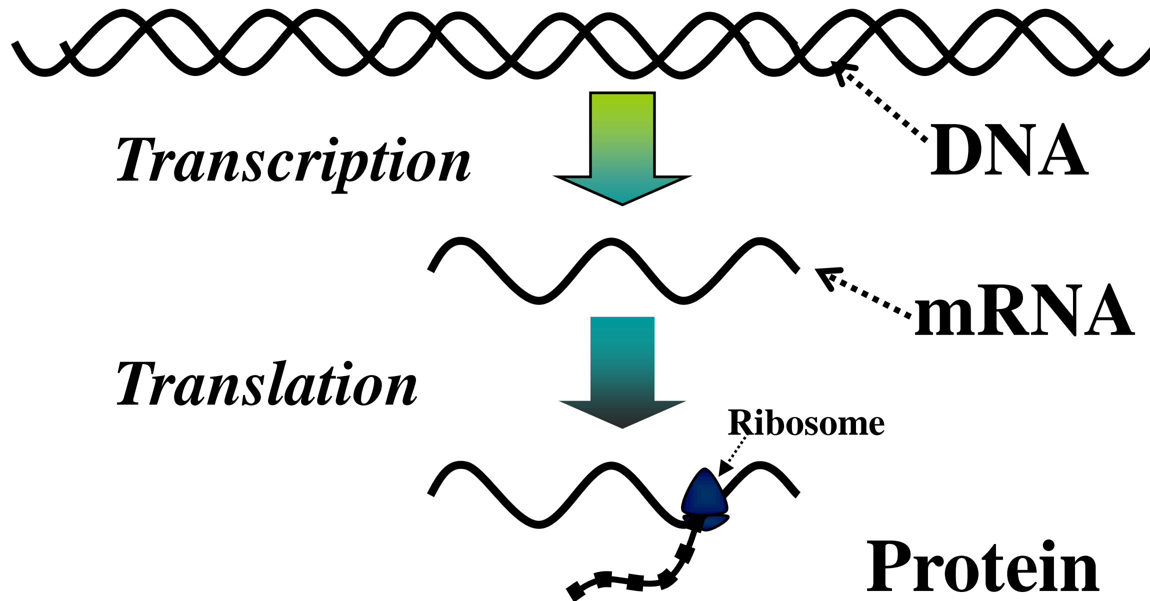
The Challenge for Microbial Ecology

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 Solution: Molecular Biology



- Present in all cells- Bacteria, Archaea and Eukaryotes
- Documents of evolutionary history
- Basis of all molecular biological techniques

Environmental Sample

DNA

What organisms are present?
What genes are present?

RNA

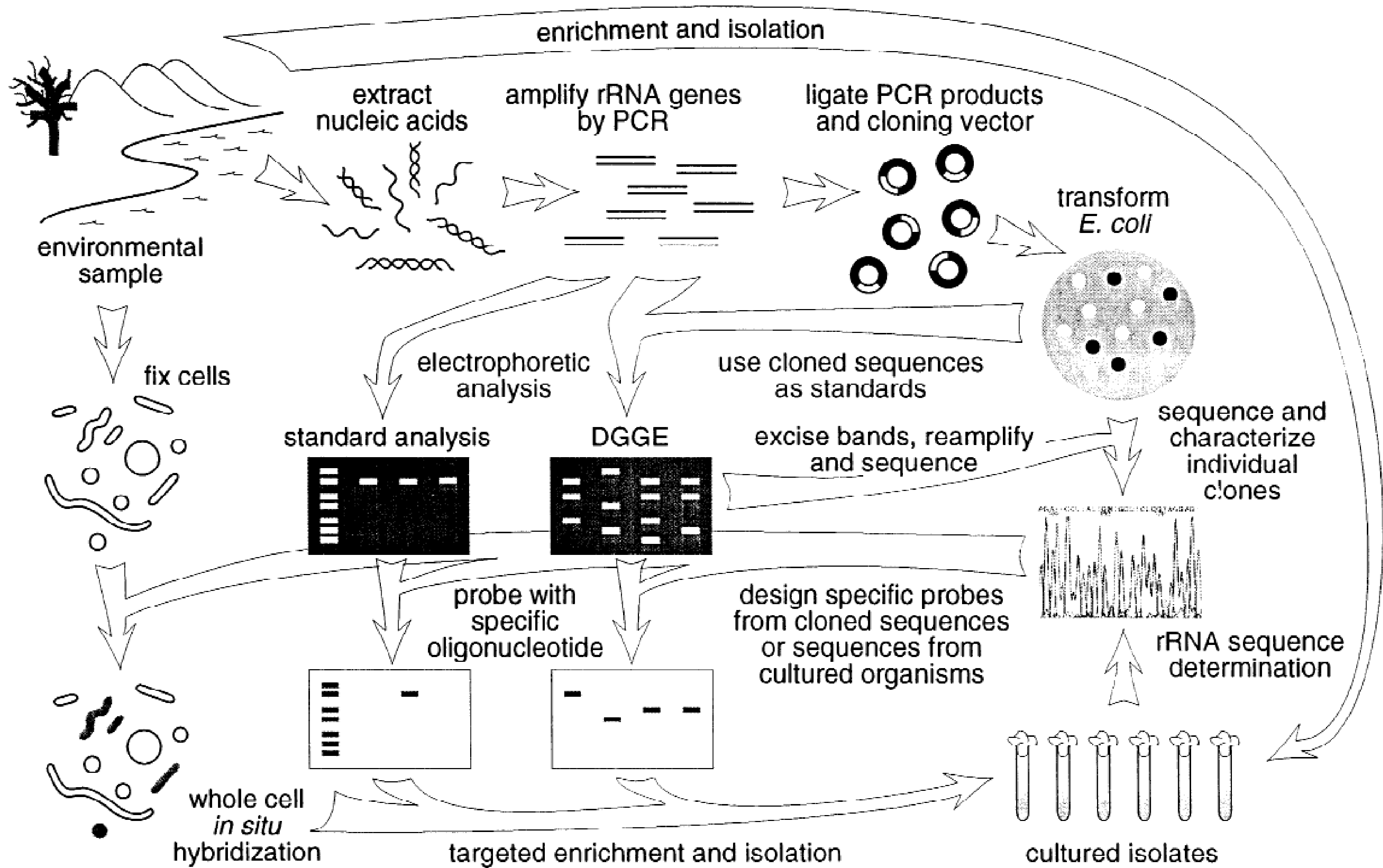
What organisms are active?
What genes are being expressed?

Target Genes

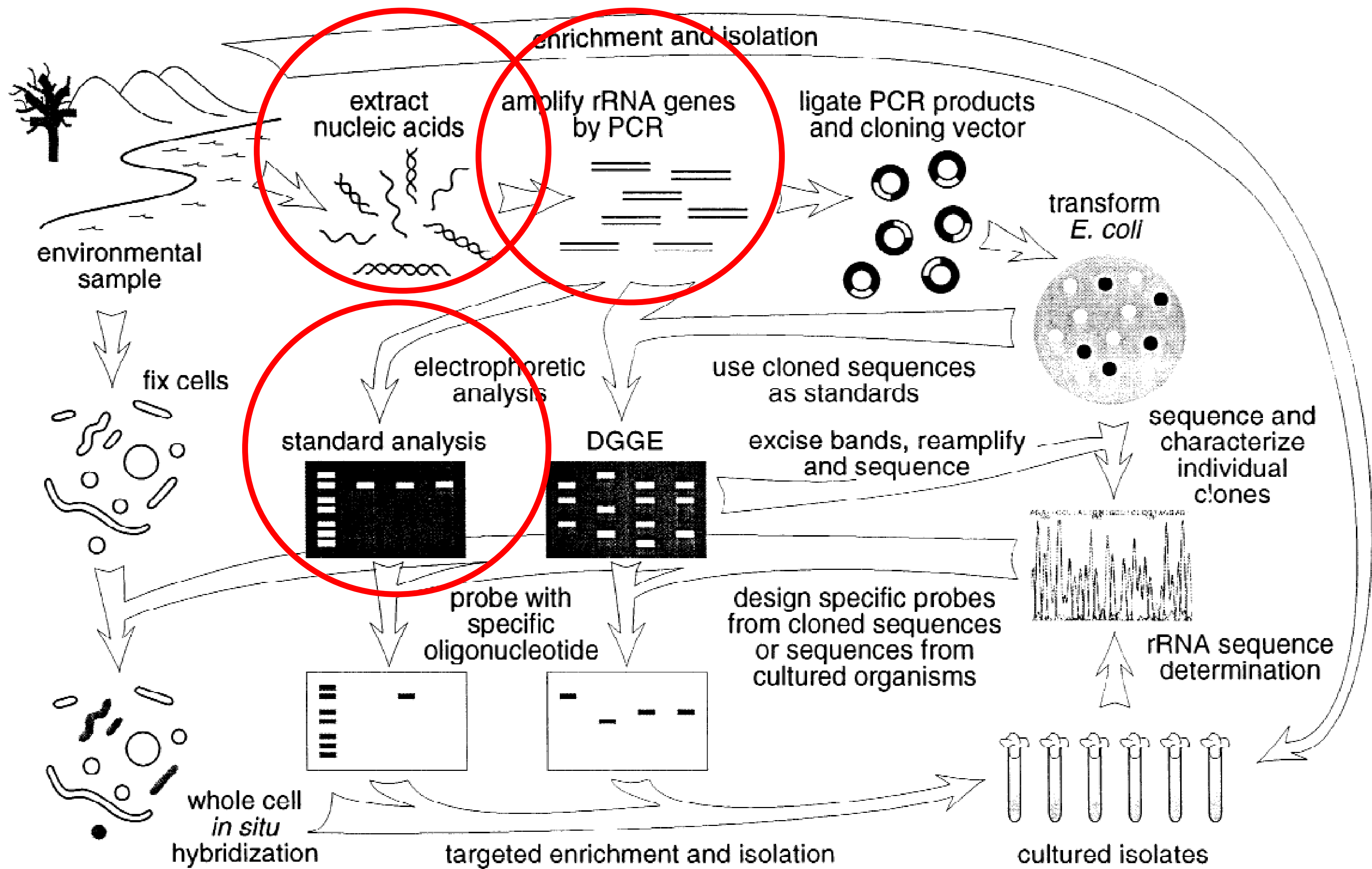
Metagenomics

Microarrays

Proteomics

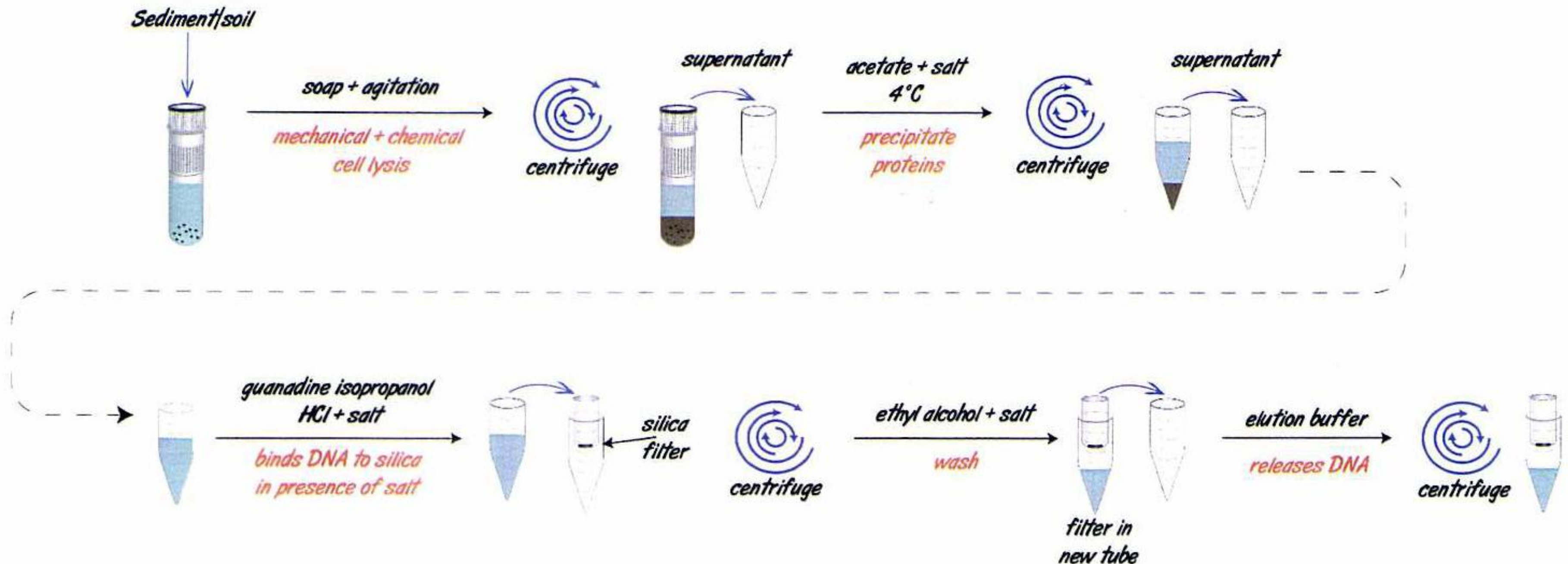


Head *et al*. 1998



Head *et al.* 1998

DNA extraction from Winogradsky Columns

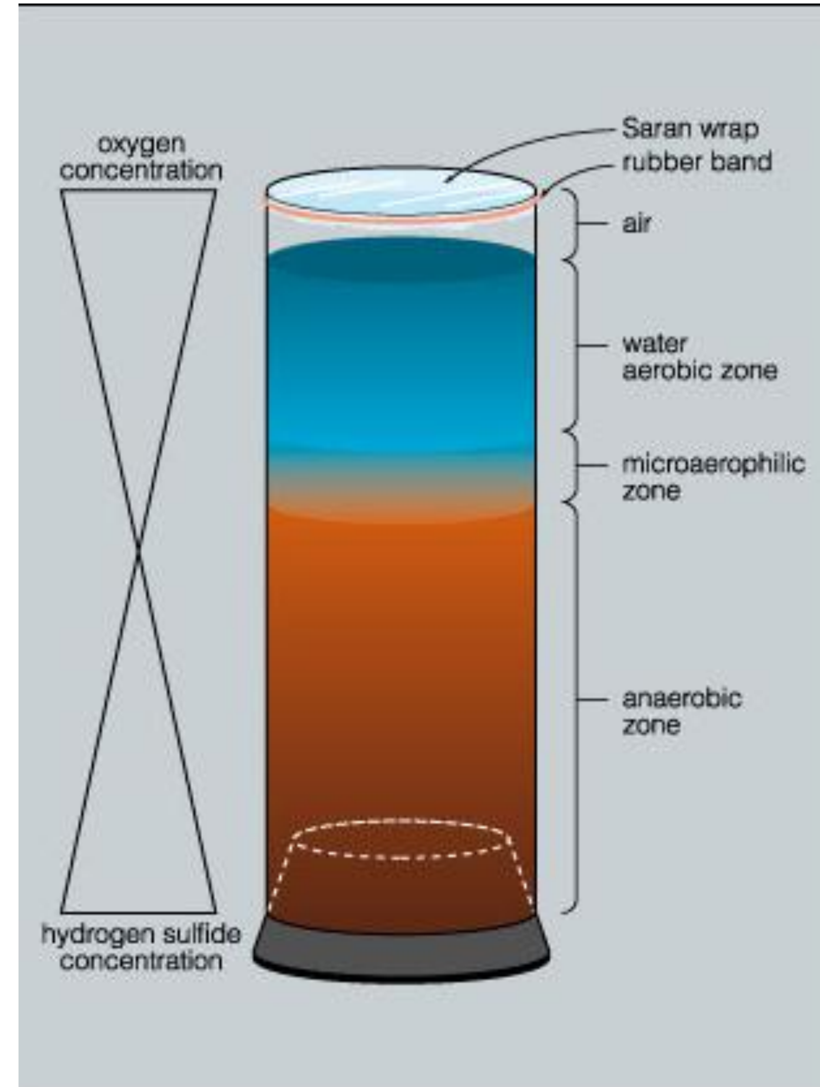


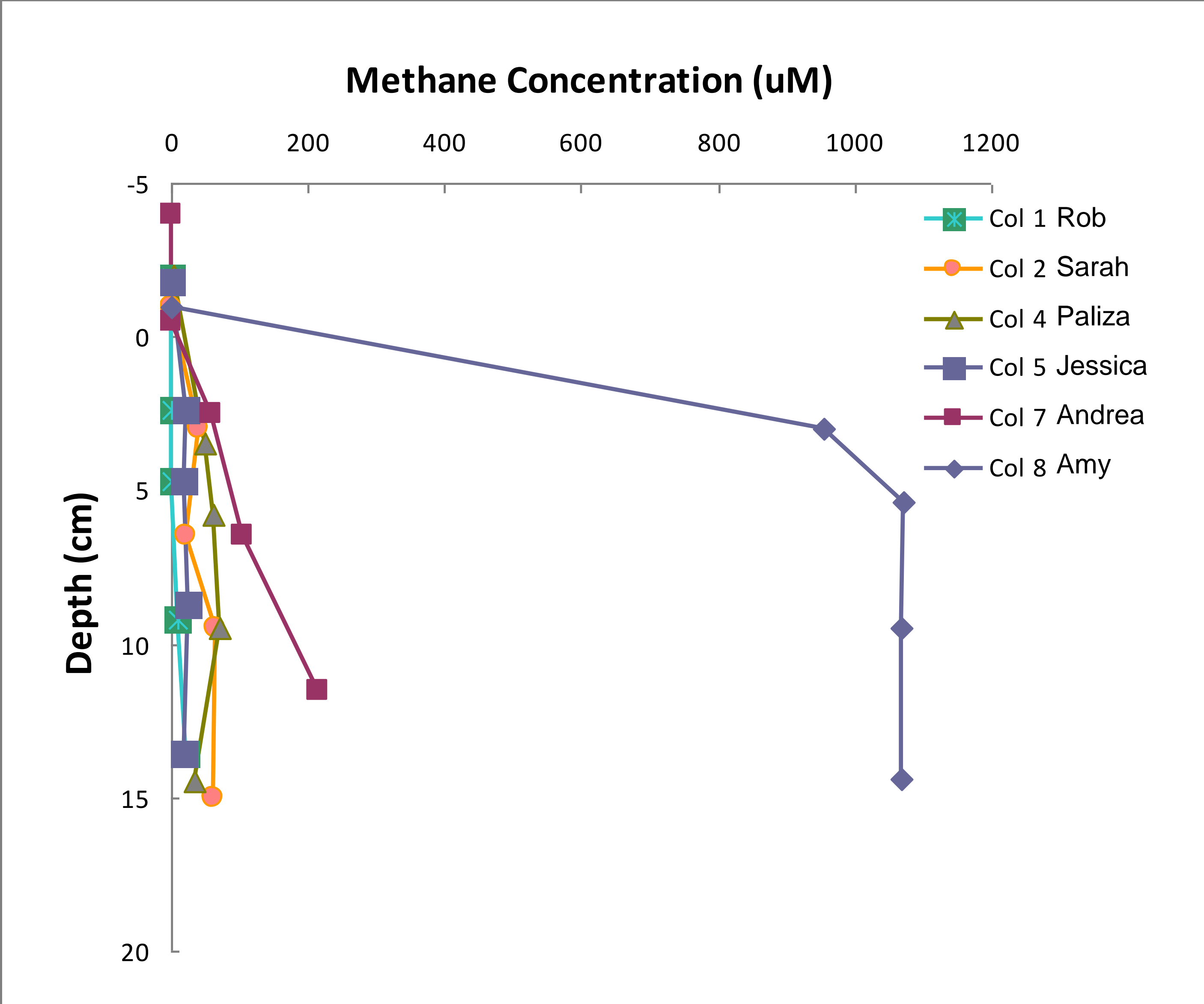
DNA Extraction

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
- *pmo*A Methanotrophs
  - Particulate methane monoxygenase





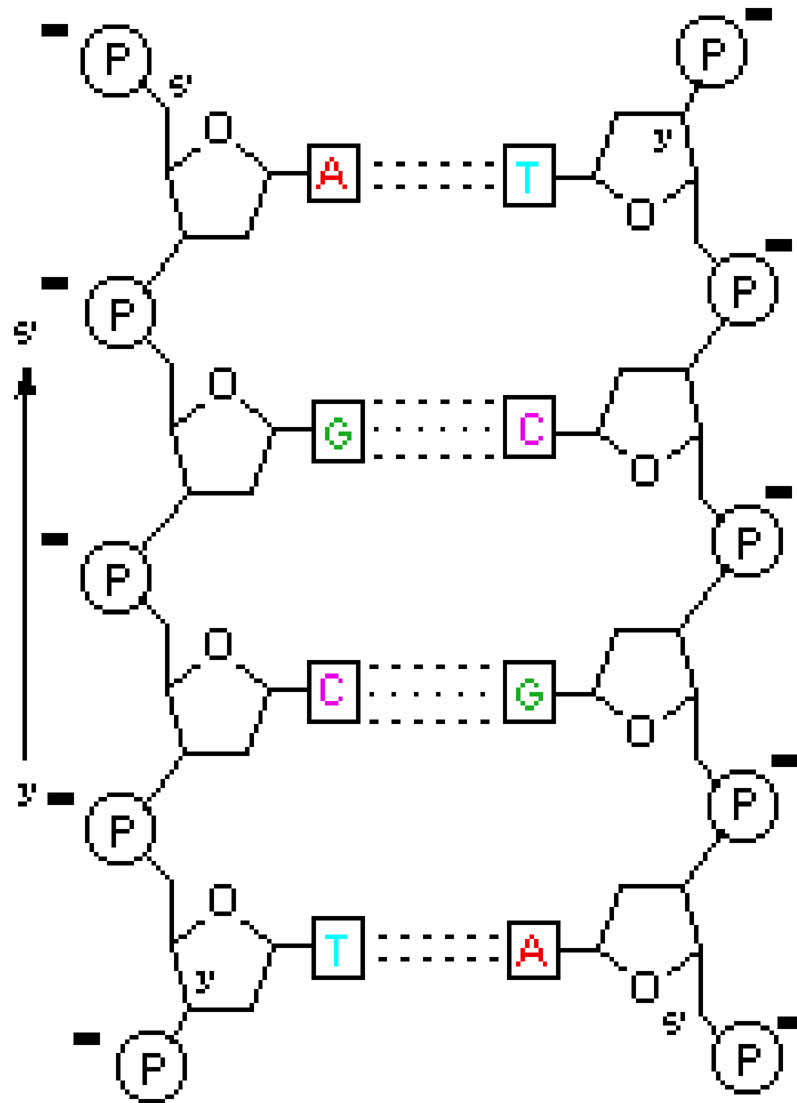
Methane Concentration (uM)
0
200
400
600
800
1000
1200
-5
0
5
10
15
20
Depth (cm)
Col 1 Rob
Col 2 Sarah
Col 4 Paliza
Col 5 Jessica
Col 7 Andrea
Col 8 Amy

Day 1, Part II

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

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'
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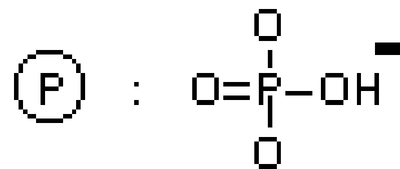
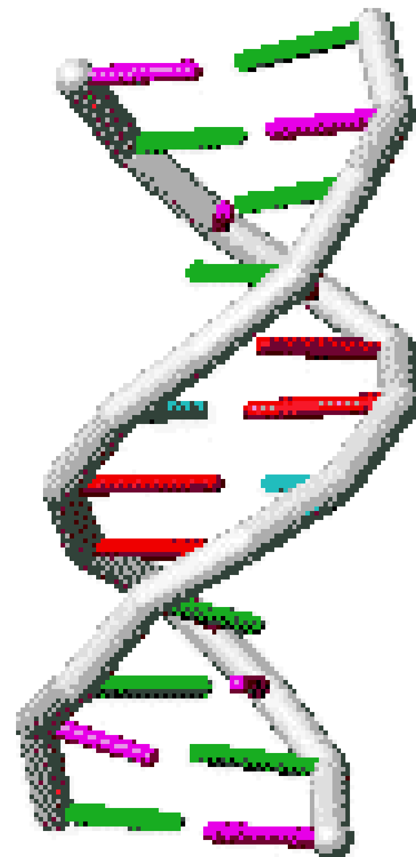
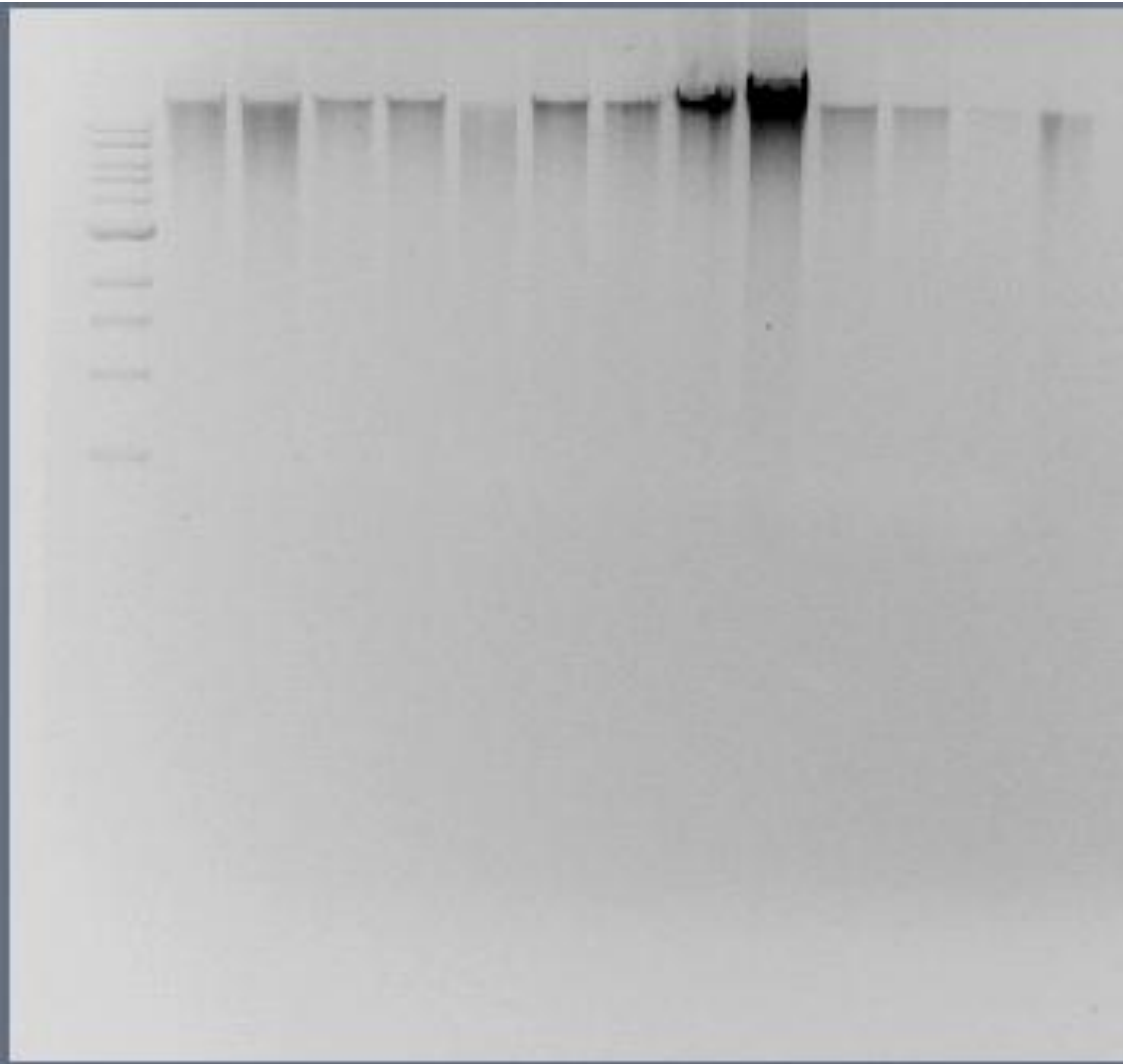

P
:
O
O=P–OH
O



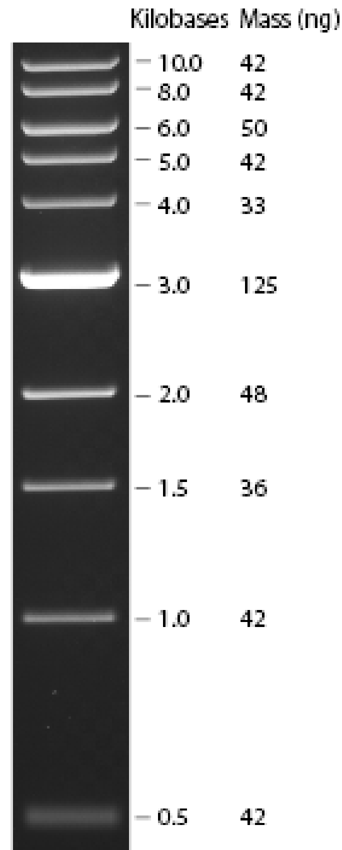
Genomic DNA



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

What to do

- Mix 10 µl of your DNA with 5 µl loading buffer
- Load in well on gel
- I'll load the ladder
- Run it
- Take a picture of it

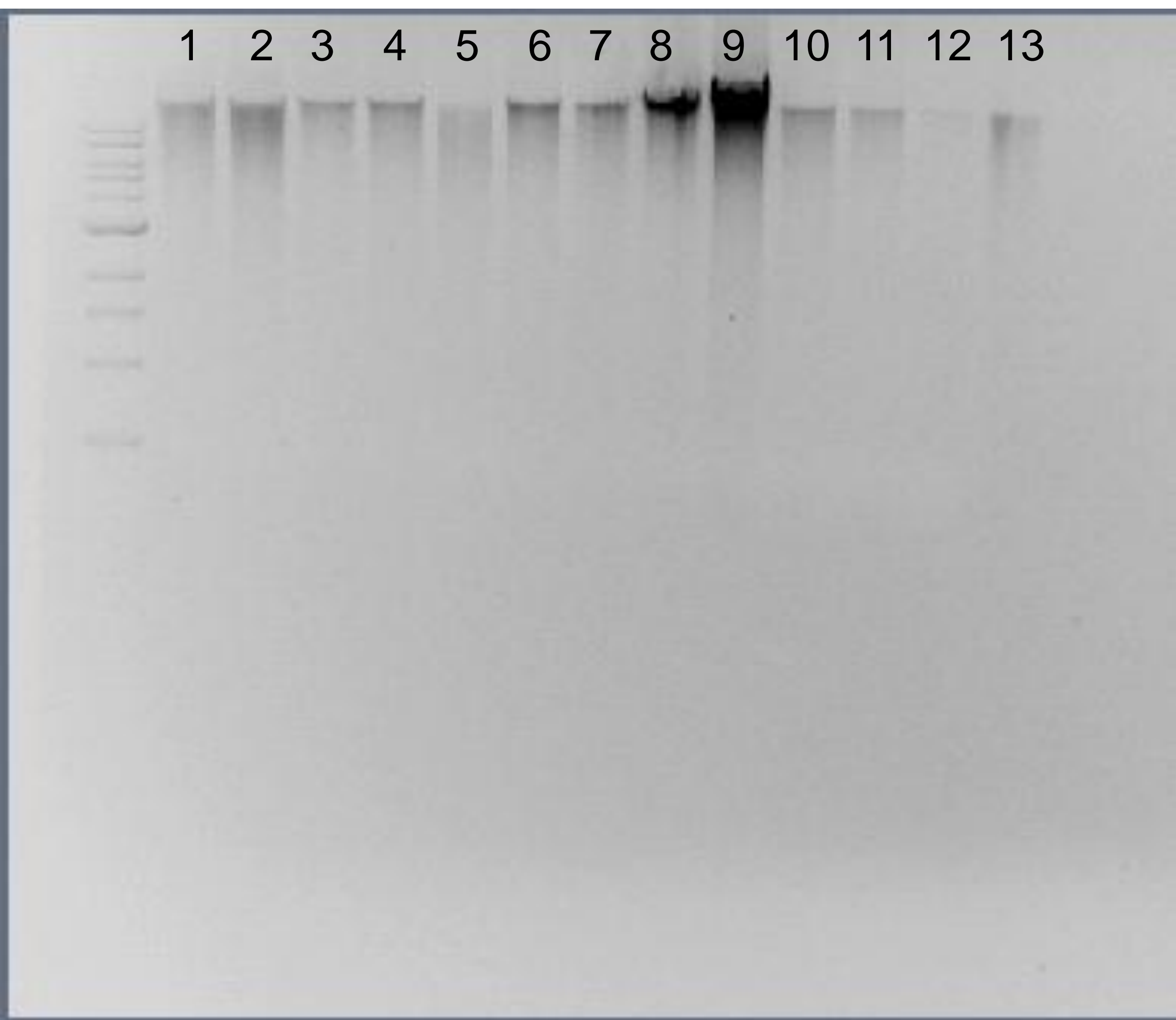


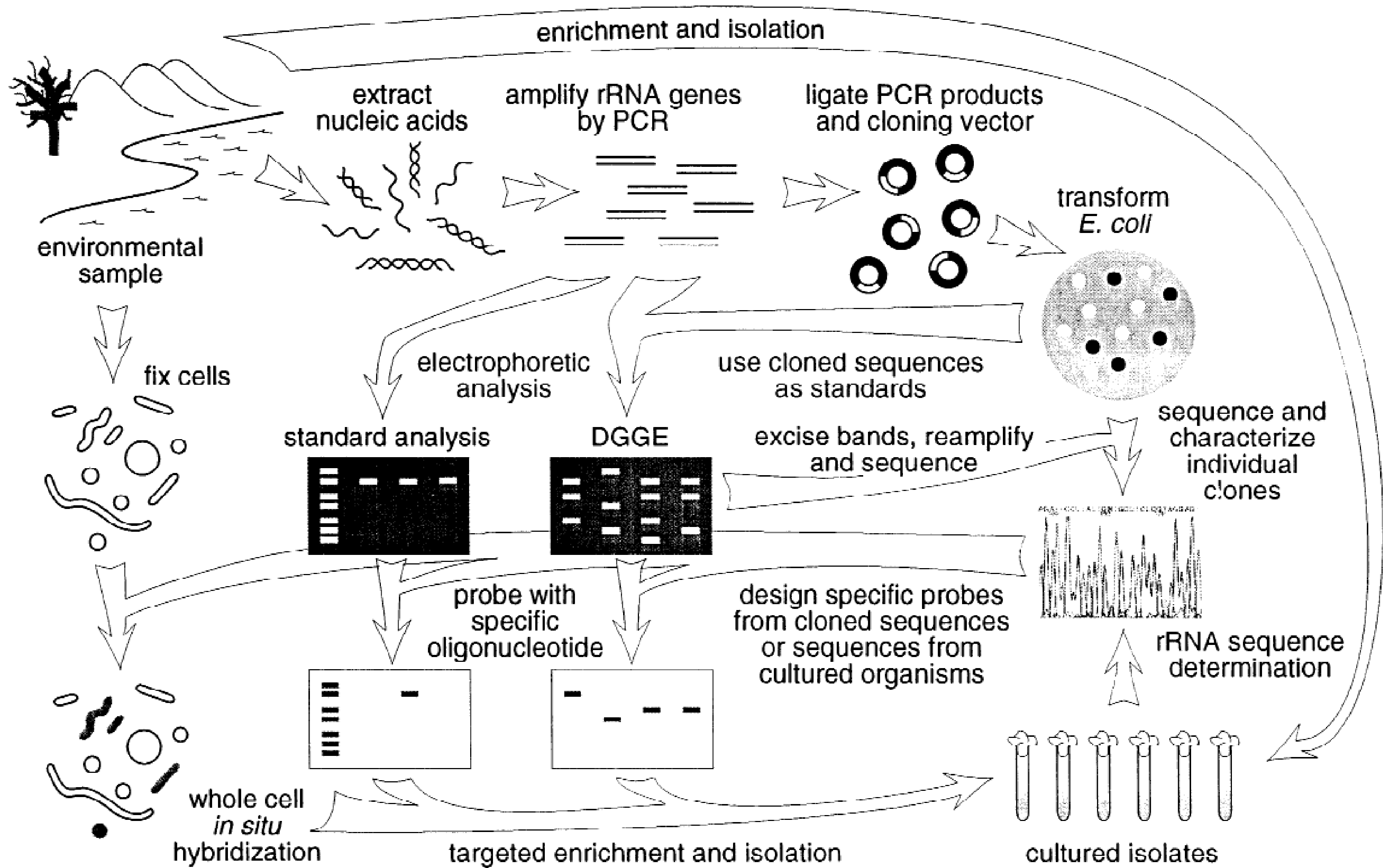
Day 2

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

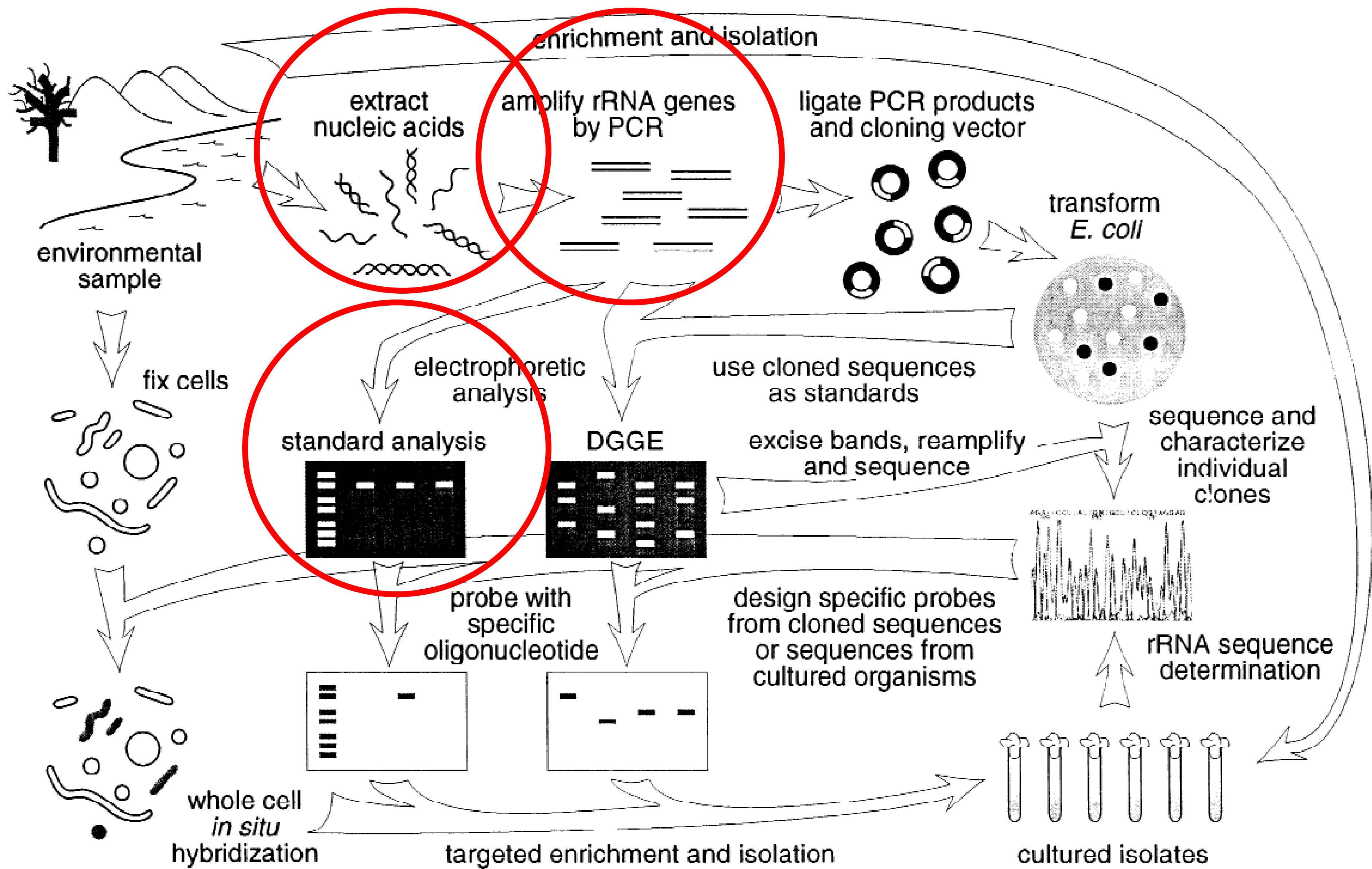
Genomic DNA

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





Head *et al*. 1998



Head *et al*. 1998

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Target Genes

Metagenomics

Microarrays

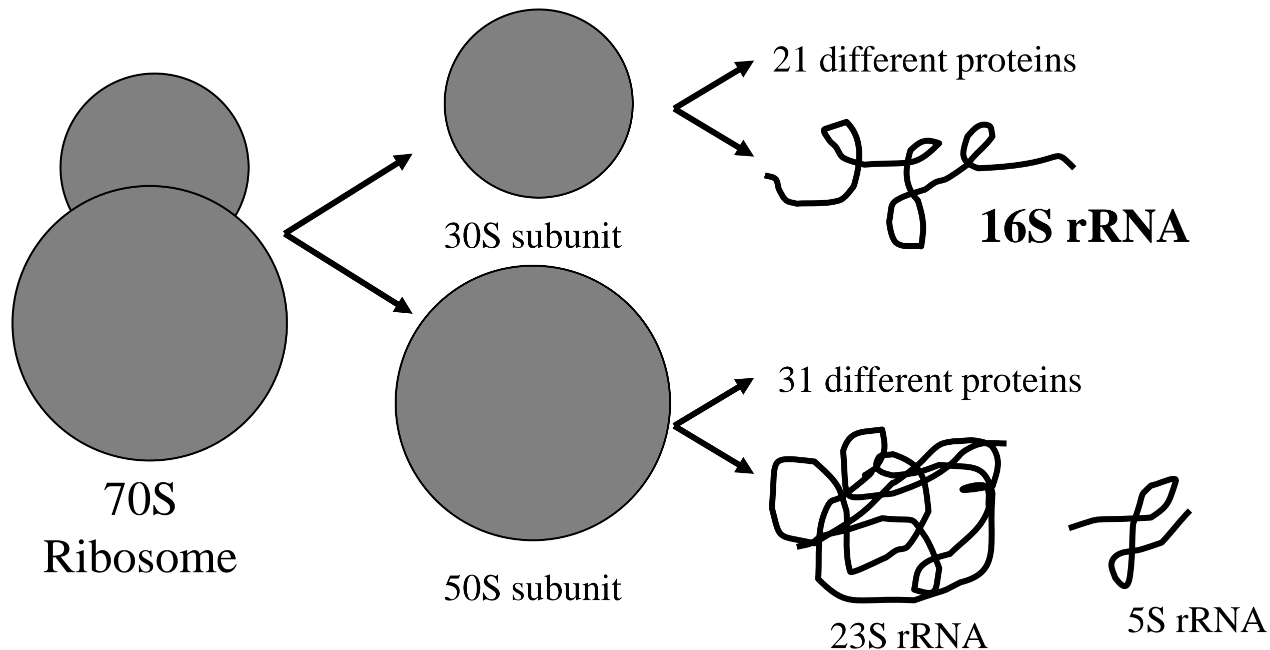
Proteomics

The Star of the Show: SSU rRNA

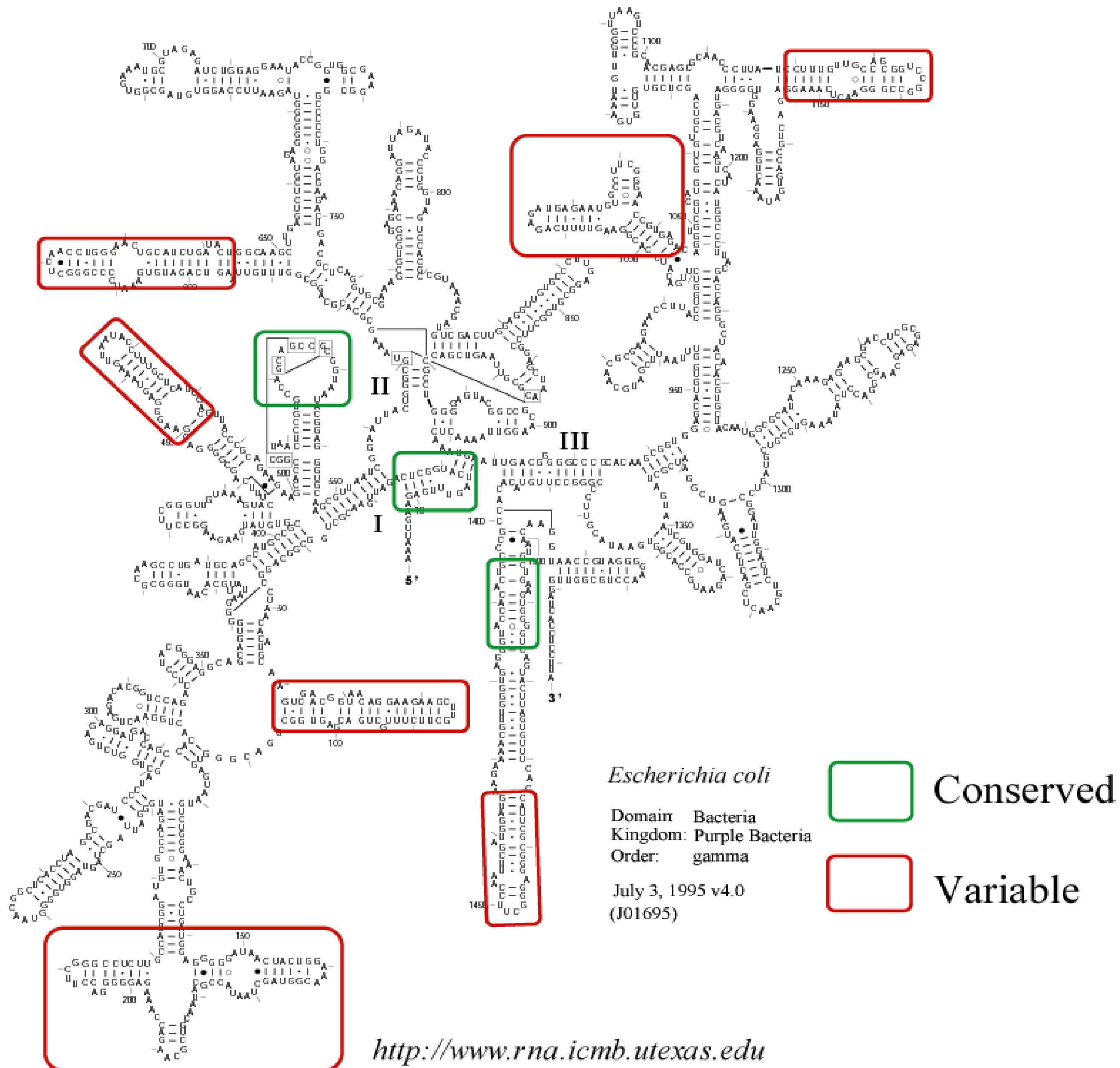
- Everybody has it
- Contains both highly conserved and variable regions
  - -allows making comparisons between different organisms over long periods of time (evolutionary history)
- Not laterally transferred between organisms
- HUGE and growing database

Ribosomes

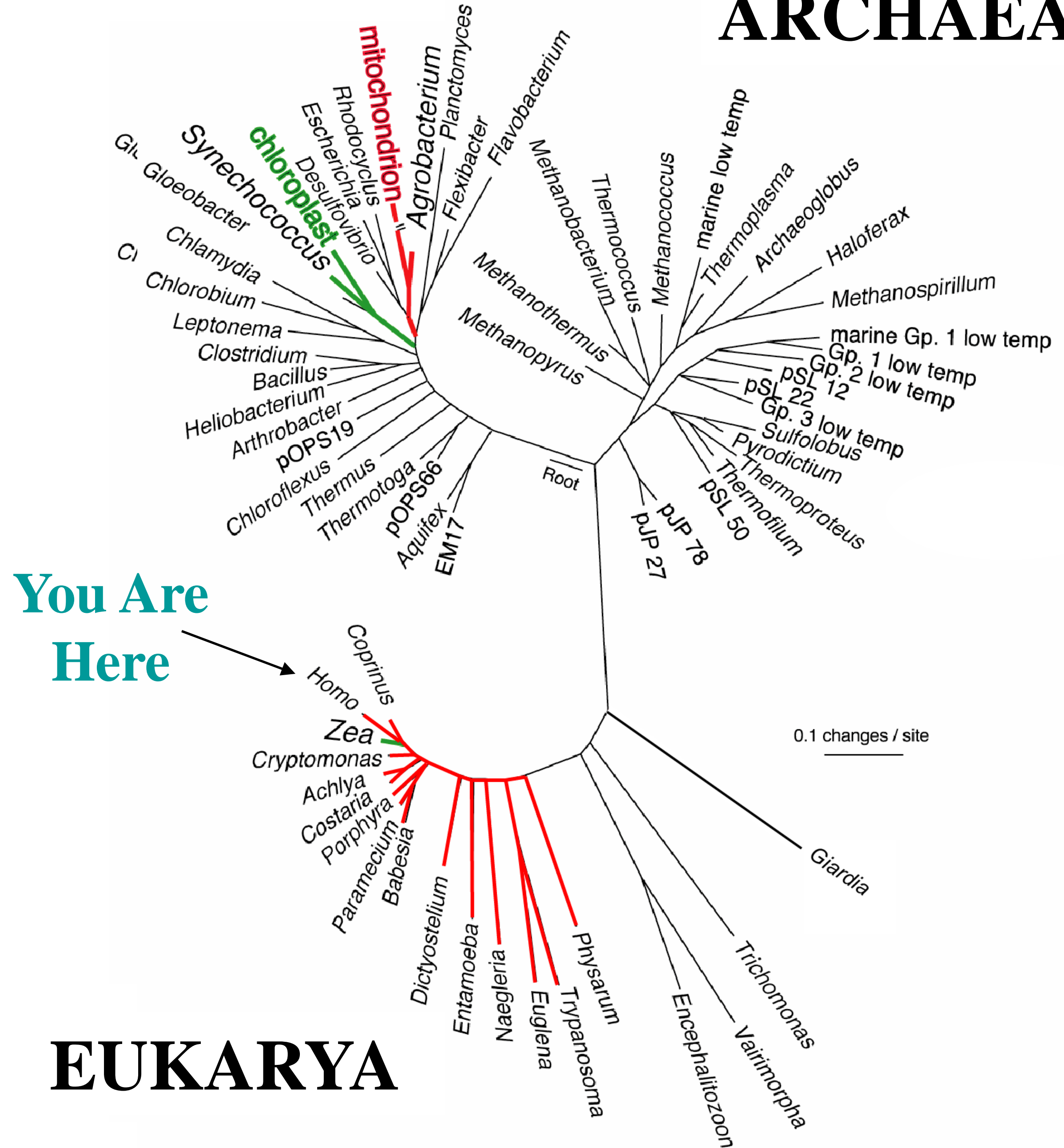
- Make proteins
- rRNA is transcribed from rDNA genes



SSU rRNA



Universal Tree of Life



Modified from Norman Pace

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

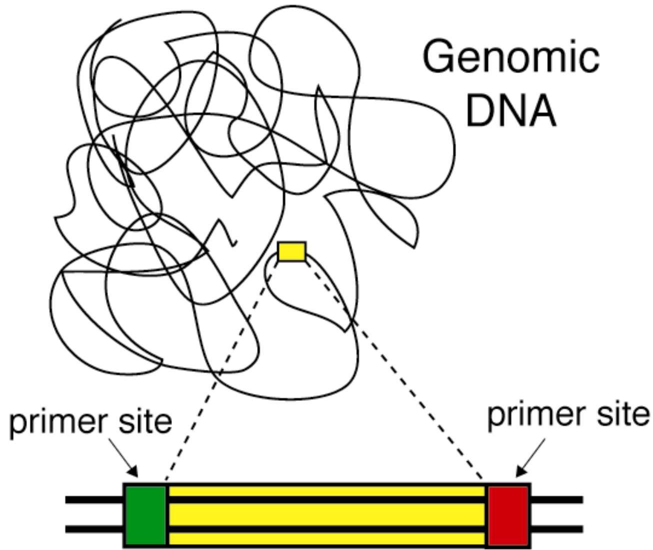
Every PCR contains:

- A DNA Polymerase (most common, Taq)
- Deoxynucleotide Triphosphates (A, C, T, G)
- Buffer (salt, $MgCl_2$, etc)
- A set of primers, one Forward, one Reverse
- Template DNA

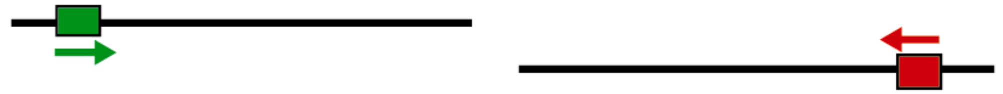
Typical PCR Profile

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

Polymerase Chain Reaction (PCR)

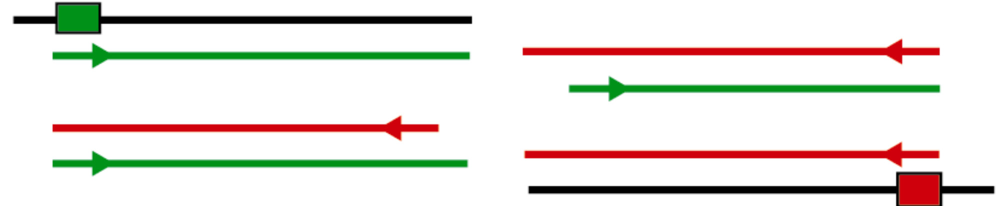


Denature DNA (1 double strand to 2 single strands) and attach primers

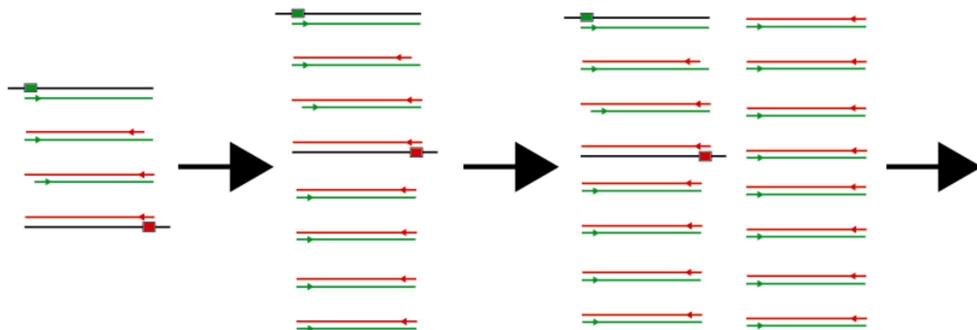


Enzyme (DNA Polymerase) creates complementary strands from free nucleotides

Repeat



Geometric increase in PCR product



30 cycles of PCR creates
2^{30} copies of the original template
> 1,000,000,000 copies

Slide courtesy of Byron Crump

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…

Beyond 16S

- Identical 16S ≠ Identical Function
- Target functional genes

```
                 *        260         *        280         *        300         *        320
species 1  TCAAAGATTAAGC-CATGCATGTCAAAGT--ACAAGCCCCATTA-AG--GTGA-AACCGCGAATGGCTCATTAAA--TCA
species 2  TCAAAGATTAAGC-CATGCATGTCTAAGT--ACAATCCTCTTGA-GG--GAGA-AACTGCGAAAGGCTCATTAAA--TCA
species 3  TCAAAGATTAAGC-CATGCATGTCCAAGT--ACAAGCCTCACTA-AG--GTGA-AACCGCGAATGGCTCATTAAA--TCA
species 4  TCAAAGATTAAGC-CATGCATGTCTAAGT--ACAGNCCG-ATCT-AAG-GCGA-AACCGCGAATGGCTCATTAAA--TCA
species 5  TCAAAGATTAAGC-CATGCATGTCTAAGT--ACAGGCCG-ATCT-AAG-GCGA-AACCGCGAATGGCTCATTAAA--TCA
species 6  TCAAAGATTAAGC-CATGCATGTCTAAGT--ACAGGCCG-AACT-AAG-GCGA-AACCGCGAATGGCTCATTAAA--TCA
species 7  TCGTTGTCTCGTTGCCTGC-TGTCTAAGT--ACAAGCCG-ATTC-AAG-GCGA-AACCGCGAATGGCTCATTAAA--TCA
species 8  TCAAAGATTAAGC-CATGCATGTCTAAGT--ACAAGCCG-ATTT-AAG-GCGA-AACCGCGAATGGCTCATTAAA--TCA
species 9  TCAAAGATTAAGC-CATGCATGTGTAAGT--ACAAGCCG-ATGT-AAG-GTGA-AACCGCGAATGGCTCATTAAA--TCA
species 10 TCAAAGATTAAGC-CATGCATGTCTNNGT--ACA---CCTCTG--GG--GCGA-AACCGCGAATGGCTCAATAAAA-TCA
species 11 TCAAAGATTAAGC-CATGCATGTCTAAGT--ACAAGCGCTATG--CG--GCGA-AACCGCGAATGGCTCATTAAA--TCA
species 12 TCAAAGATTAAGC-CATGCATGTCTAAGT--ACAAGCCGCTAGA-CG--GCGA-AACCGCGAATGGCTCAATAAA--TCA
species 13 TCAAAGATTAAGC-CATGCAGGTCTAAGT--ATAAGCCGAAATA-AA--GTGA-GACCGCGAATGGCTCATTACA--TCA
species 14 TCAAAGATTAAGC-CATGCAGGTCTAAGT--ACGAGCCGAAATA-AAT-GTGA-GACCGCGAATGGCTCATTACA--TCA
species 15 TCAAAGATTAAGC-CATGCAGGTCTAAGT--ACATGCTGTTATA-TATGGTAA-GACTGCGAACGGCTCATTACA--TCG
species 16 TCAAAGATTAAGC CATGCATGTCTAAGT  ACACACCAAATTA AG  GTGA AACCGCGAATGGCTCATTAAA  TCA
species 17 TCAAAGATTAAGC-CATGCATGTCTAAGT--ACAAAGCCTACAA-GG--CTGA-AACCGCGAATGGCTCATTAAA--TCA
species 18 TCAAAGATTAAGC-CATGCATGTCTAAGT--ACATGCCGCATTA-AG--GCGA-AACCGCGAATGGCTCATTAAA--TCA
species 19 TCAAAGATTAAGC-CATGCATGTCTAAGT--ACATGCCGAAATA-AG--GTGA-AACCGCGAATGGCTCATTATC---CA
species 20 TCAGAGATTAAGC-CATGCATGTCTAAGT--ACAGACCTTCATA-CG--GTGA-AACCGCGAATGGCTCATTAAA--TCA
species 21 TCAAAGATTAAGC-CATGCATGTCTAAGA--TCA-AGCTCGTCT-CG--CGGACAACTGCGGATGGCTCATTAAA--TCA
species 22 TTAAAGATTAAGC-CATGCANGTATCAGT--ACAAGCCTCACTN-AG--GTGA-AACCGCGAATGGCTCATTAAA--TCA
species 23 TCAAAGATTAAGCCAACTCATGTCTAAGA--TCATGCCGAAACCAAG--GCGA-AACCGCGAATGGCTCATTAAA--TCA
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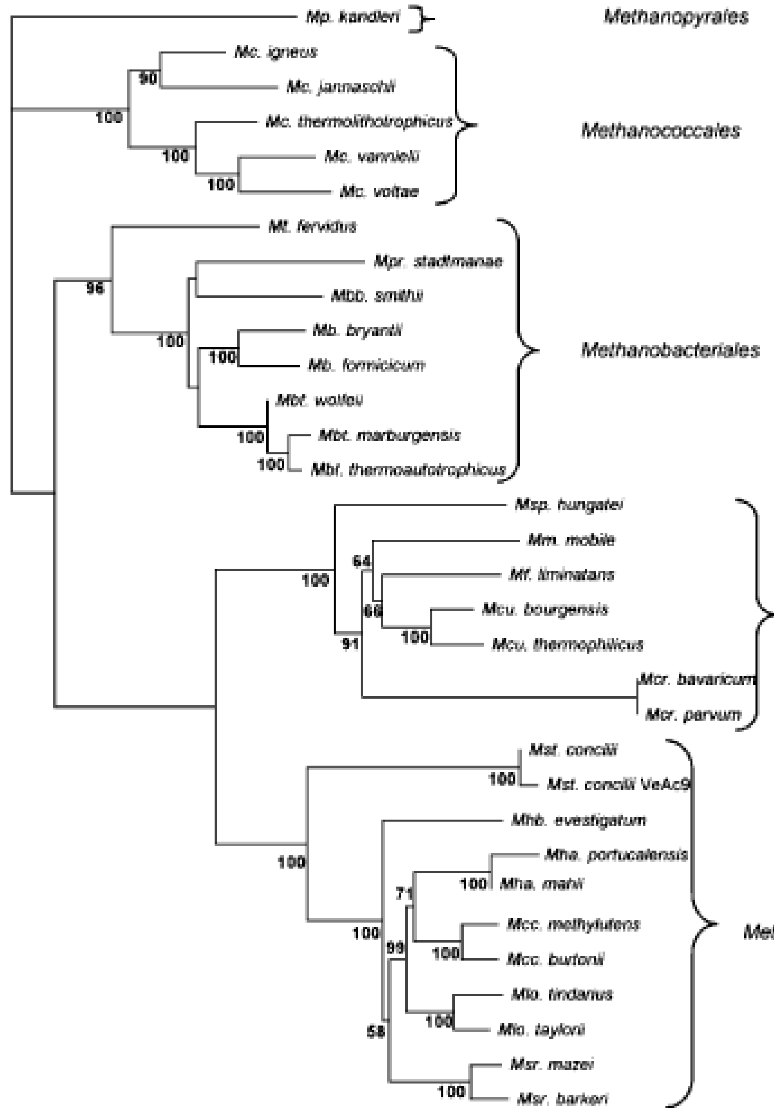
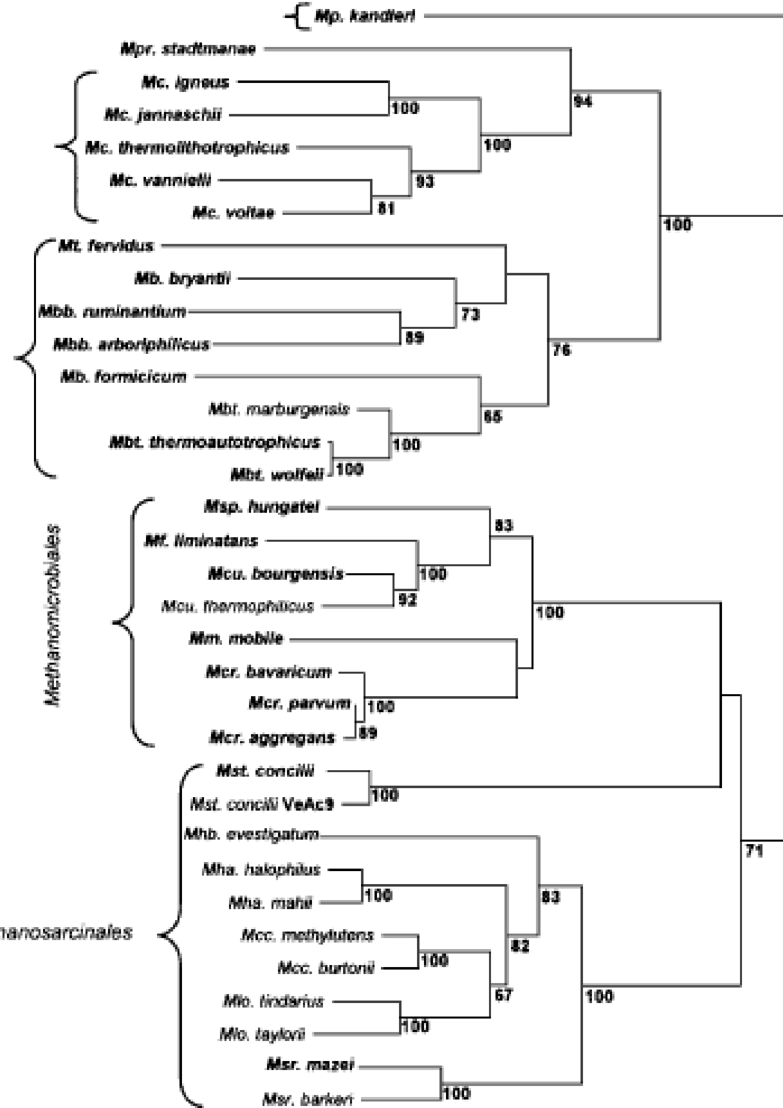
(Andy Vierstraete 1999)

16S rDNA

mcrA

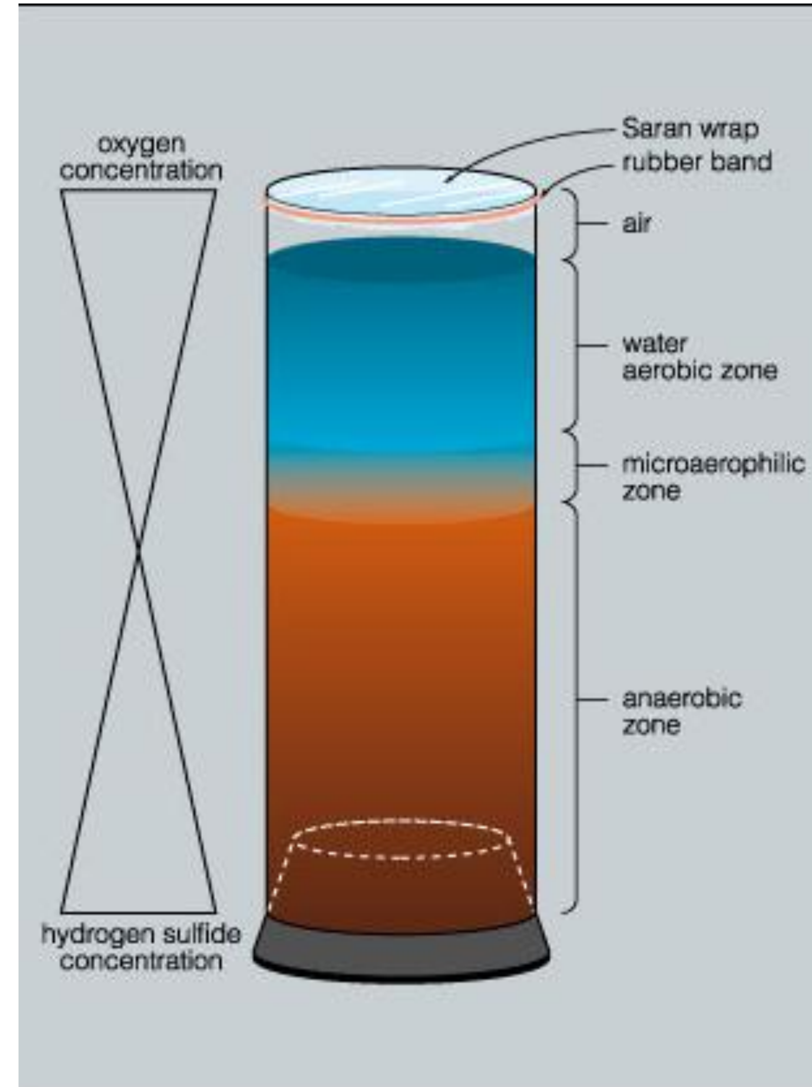




Luton *et al*. 2002

Primers We Are Using

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



Reagent	Volume (µl) per reaction	# of reactions	final volume
Sterile H_20	26.8		
5X PCR buffer	10		
dNTPs (10mM)	1		
Taq polymerase (5 Units/µl)	0.2		

Tube	Master mix	Target	Template	Vol	F primer	Vol	R primer	Vol
	µl			µl		µl		µl
1	38	Sulfate reducers	Column DNA	2	dsr1F	5	dsr4R	5
2	38	Methanogens	Column DNA	2	ME1	5	ME2	5
3	38	Methanotrophs	Column DNA	2	pa189F	5	pa682R	5
4	38	Bacteria	Column DNA	2	8F	5	1492R	5
5	38	Archaea	Column DNA	2	21F	5	958R	5
6	38	Archaea	+ control *M. jannaschii*	2	21F	5	958R	5
7	38	Nothing	- control (water)	2	21F	5	958R	5