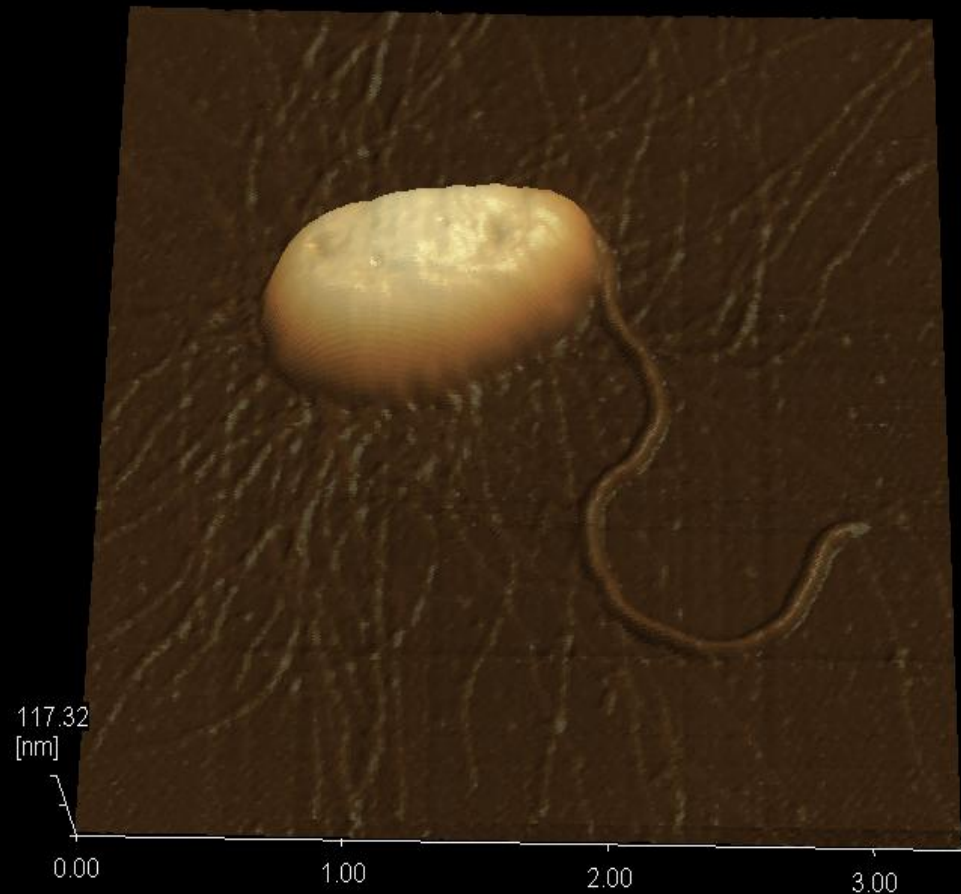


Biogeography, a case for microbes in marine environments

Kazu Kogure
Ocean Research Institute
The University of Tokyo
kogure@ori.u-tokyo.ac.jp



I may be the only one “marine” and “microbiologist” here.



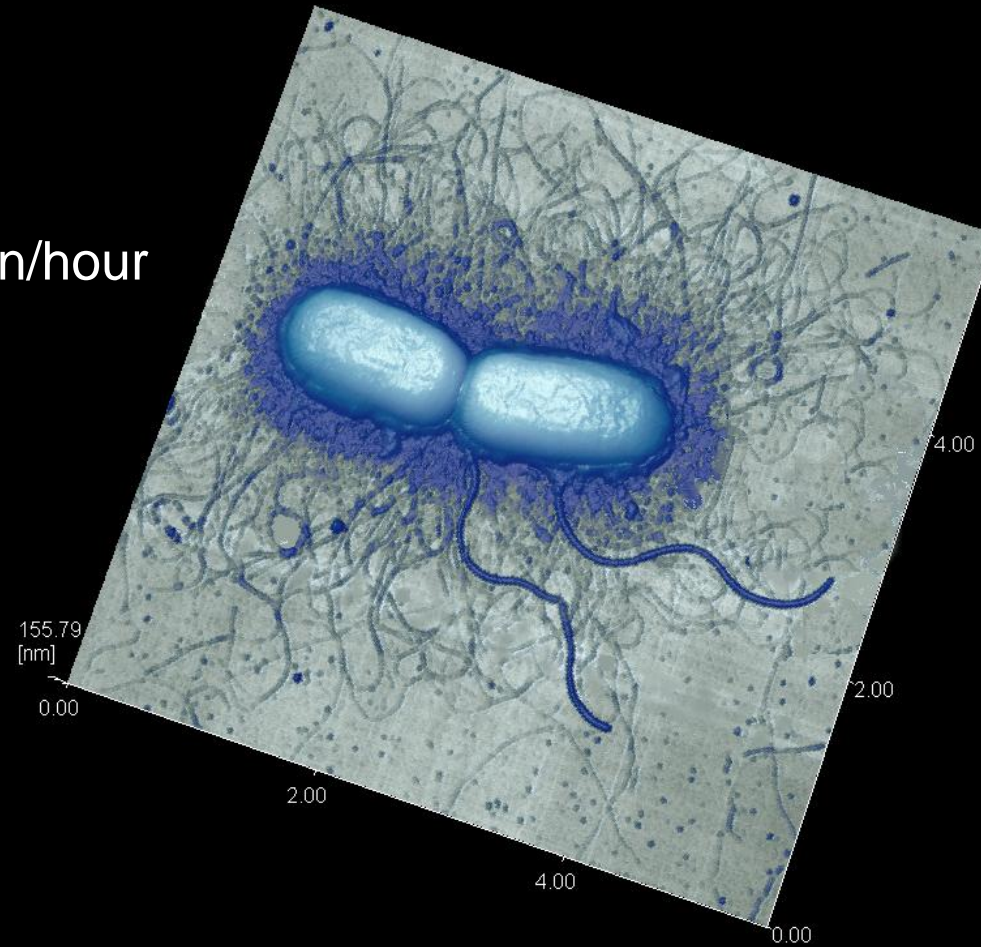
Bacteria:

No sexual reproduction

Small size: c.a. 1-2 μm

High potential growth rate: 1 division/hour

First appeared on this planet



5.63 x 5.63 [μm] Z-Max 155.79[nm]

Bacteria:

No sexual reproduction

Minute size: c.a. 1-2 μm

High potential growth rate: 1 division/hour

First appeared on this planet

We cannot see them.

We cannot culture most of them.

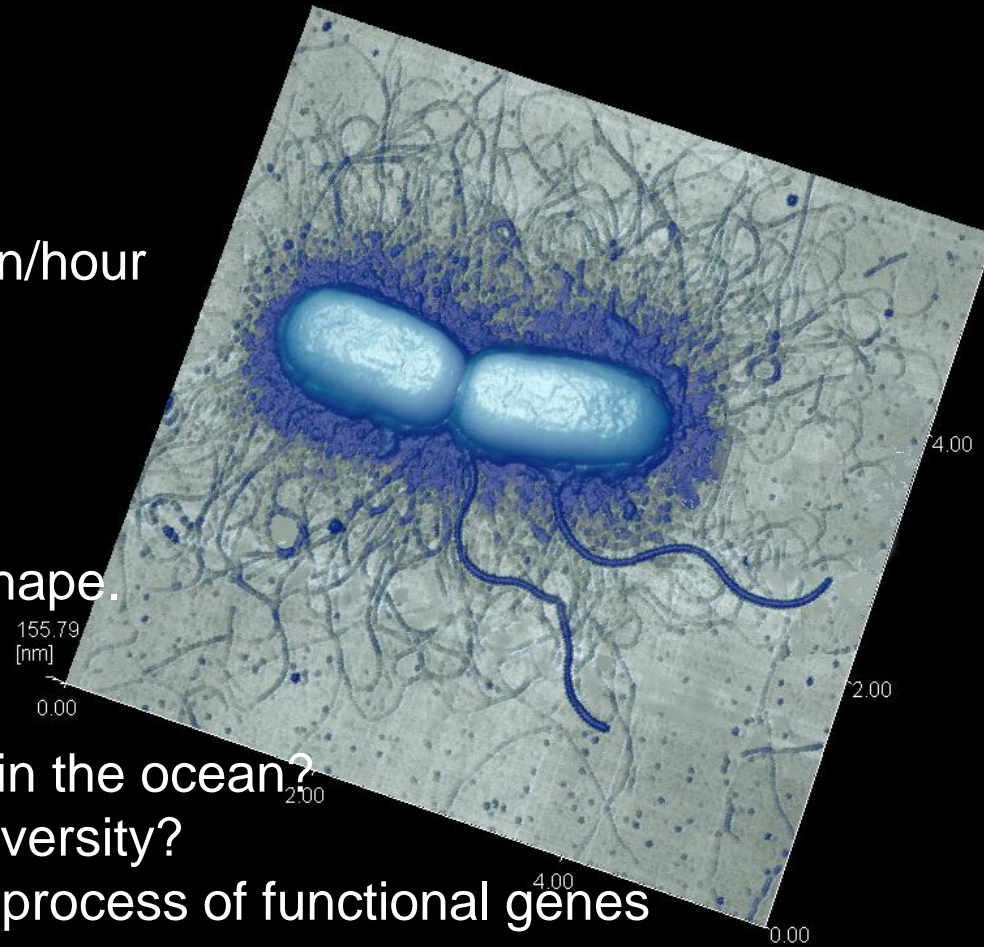
We cannot identify them from the shape.

General view on bacteria

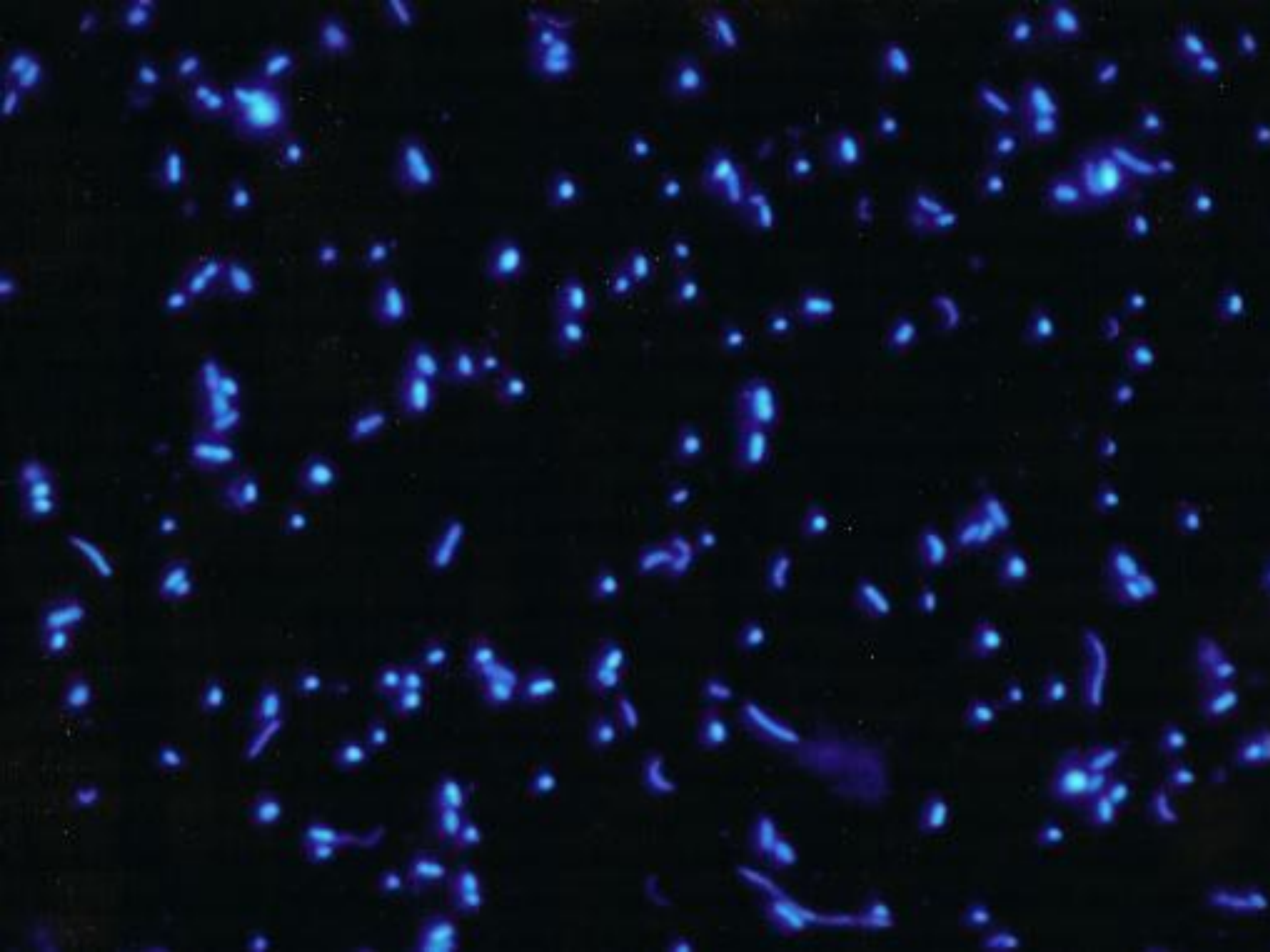
How can we clarify the biodiversity in the ocean?

What is our problem to look at biodiversity?

How can we know the evolutionary process of functional genes

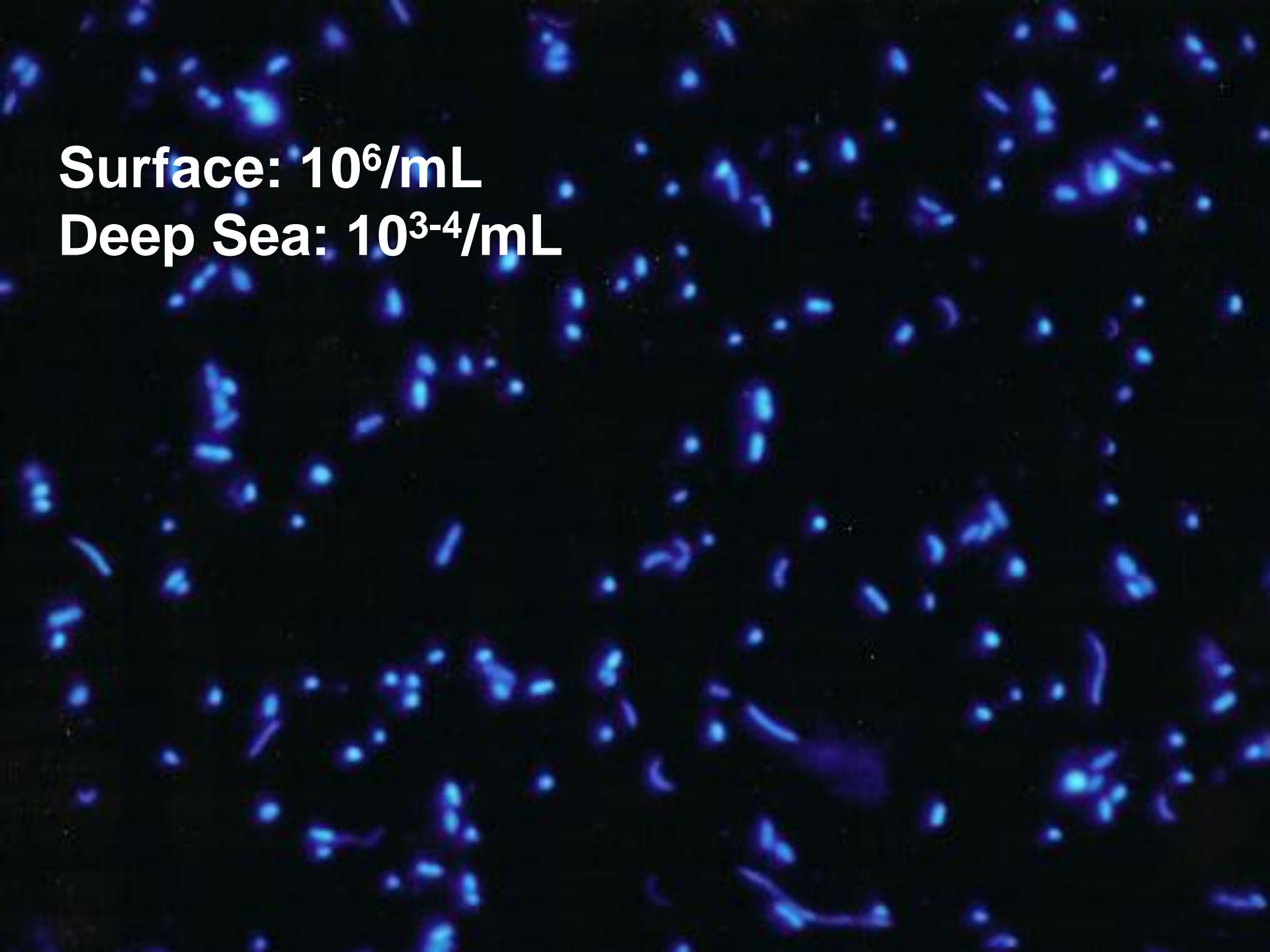


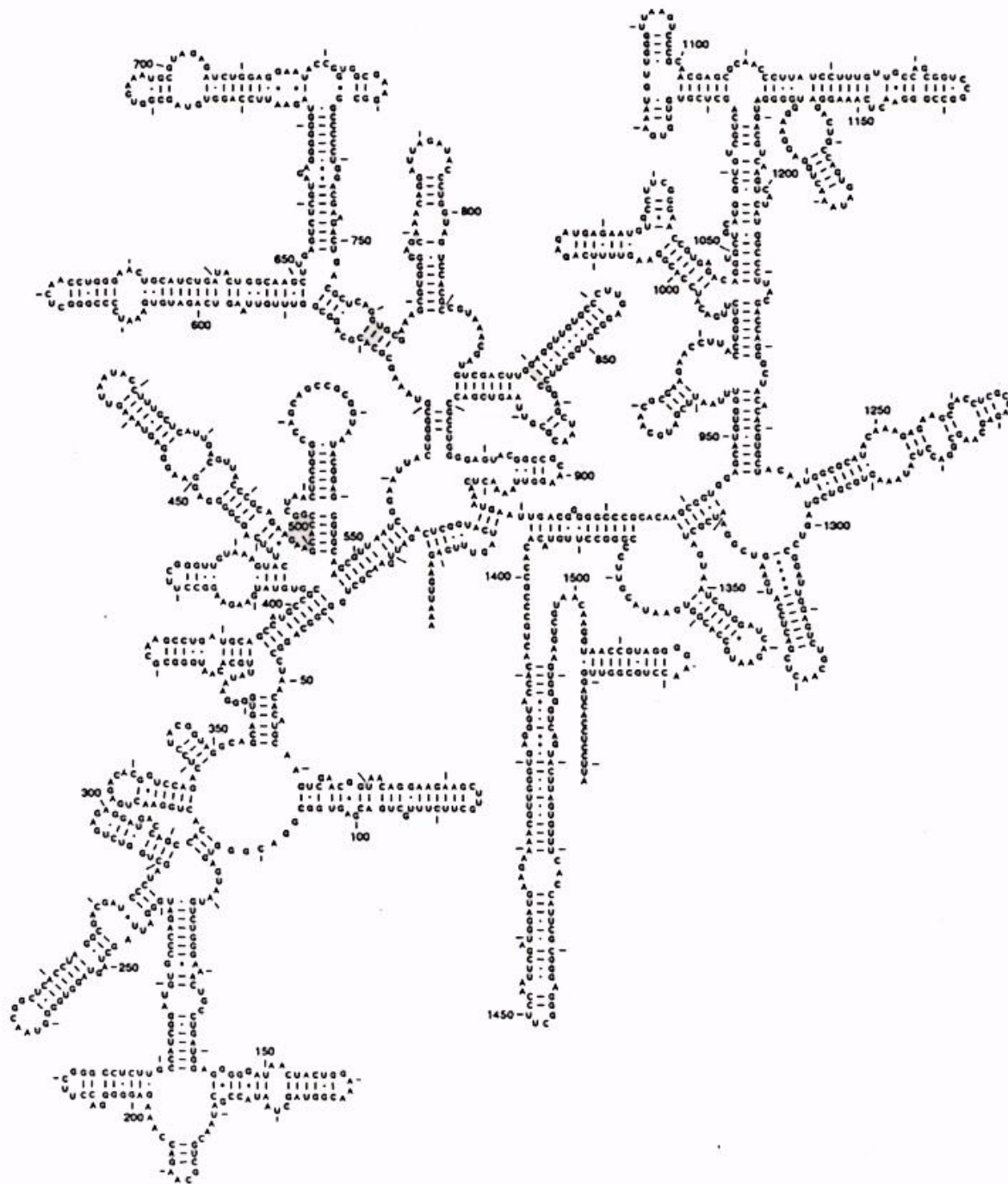
5.63 x 5.63 [μm] Z-Max 155.79[nm]



Surface: 10^6 /mL

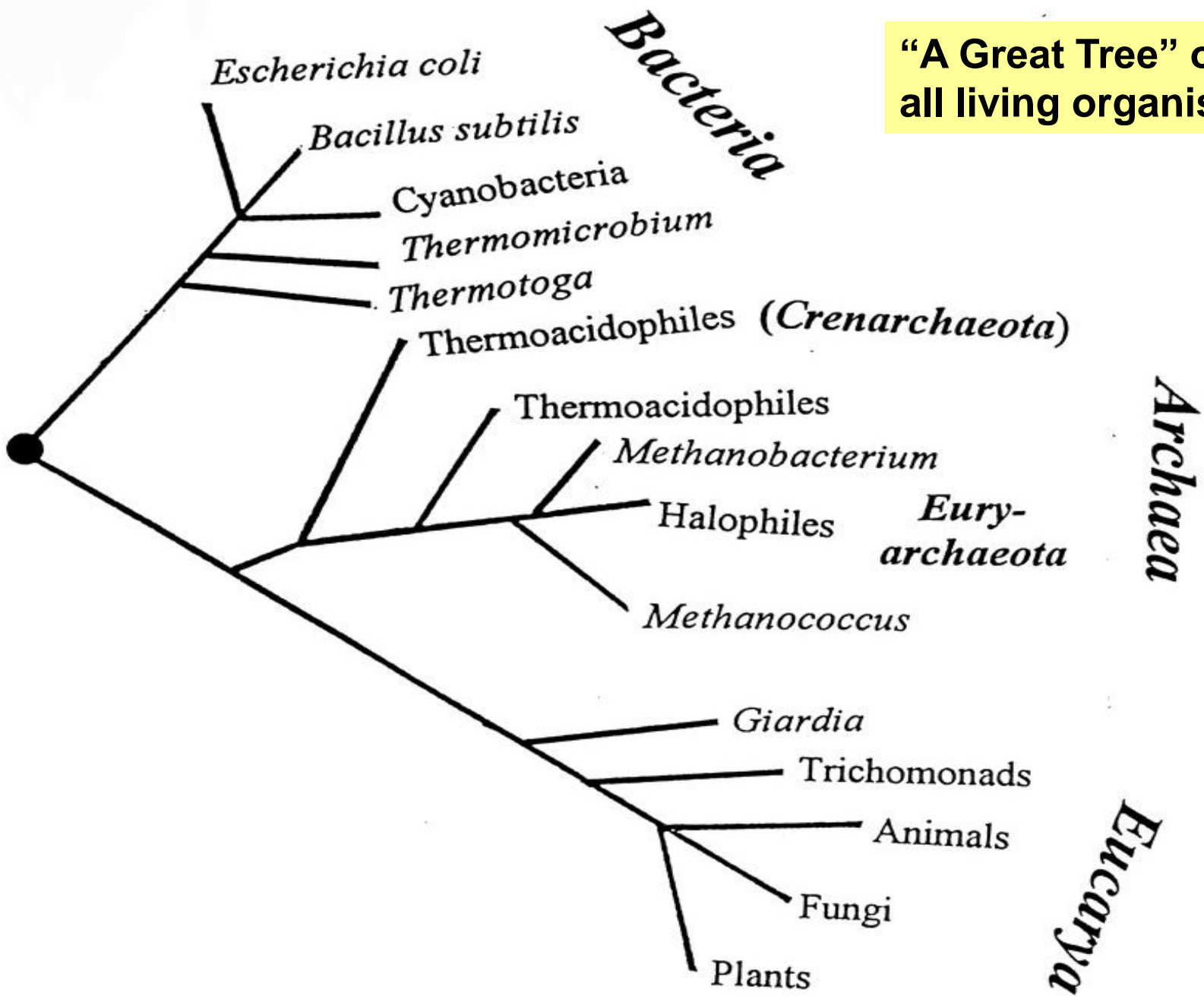
Deep Sea: 10^3 - 10^4 /mL

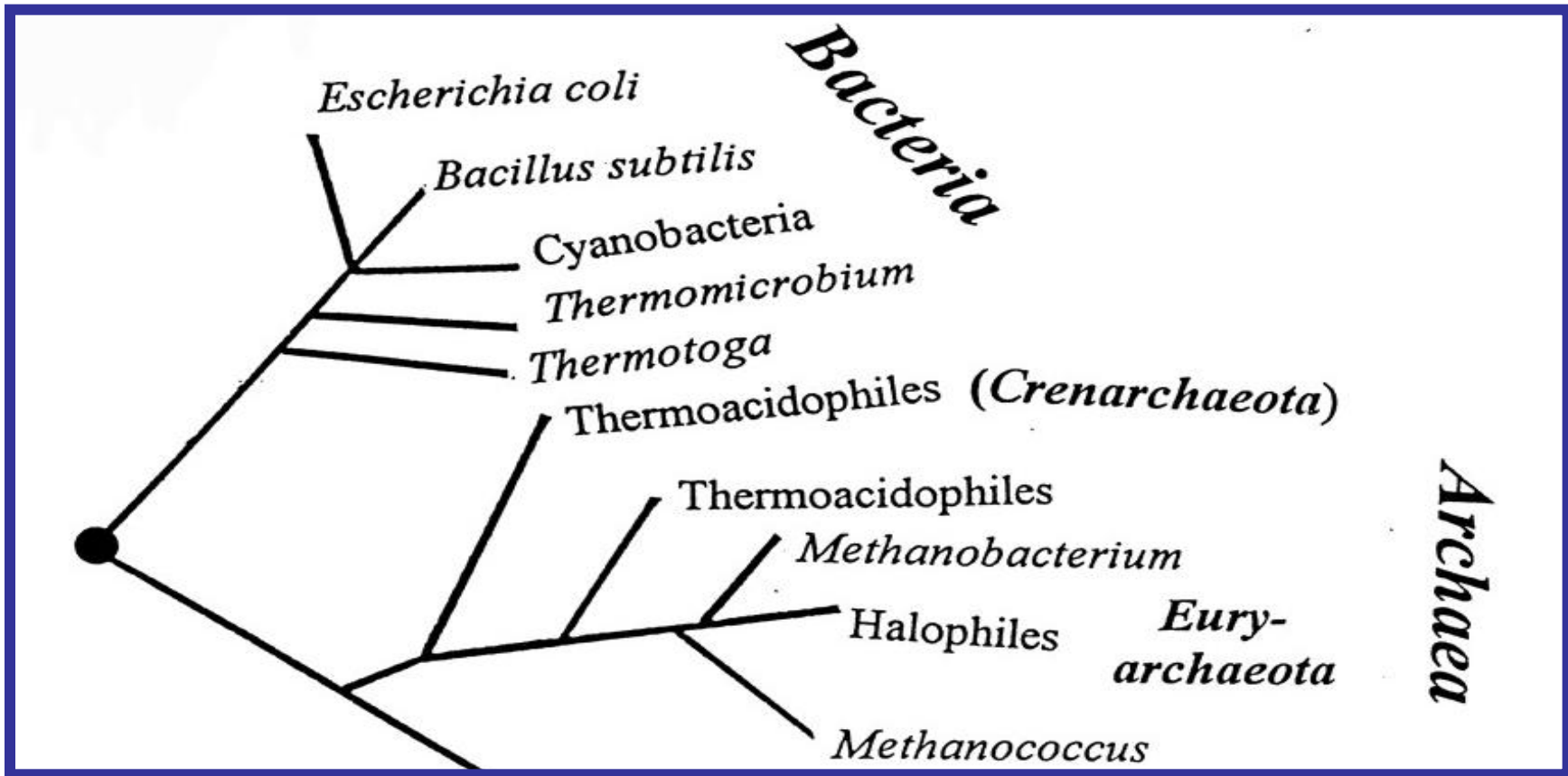




We rely on this particular molecule, 16S rRNA

“A Great Tree” of all living organisms

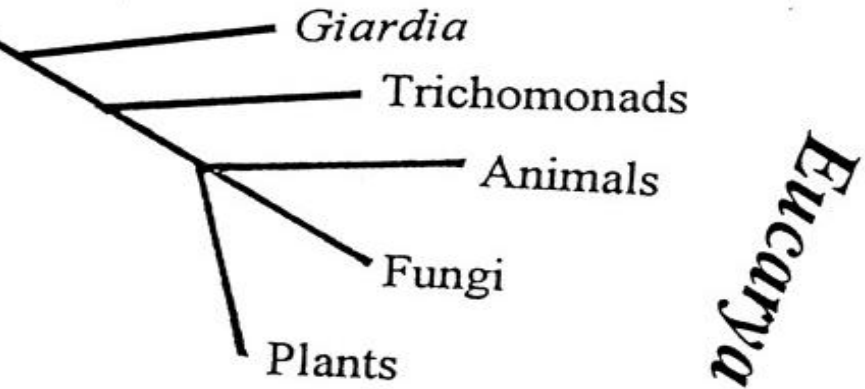




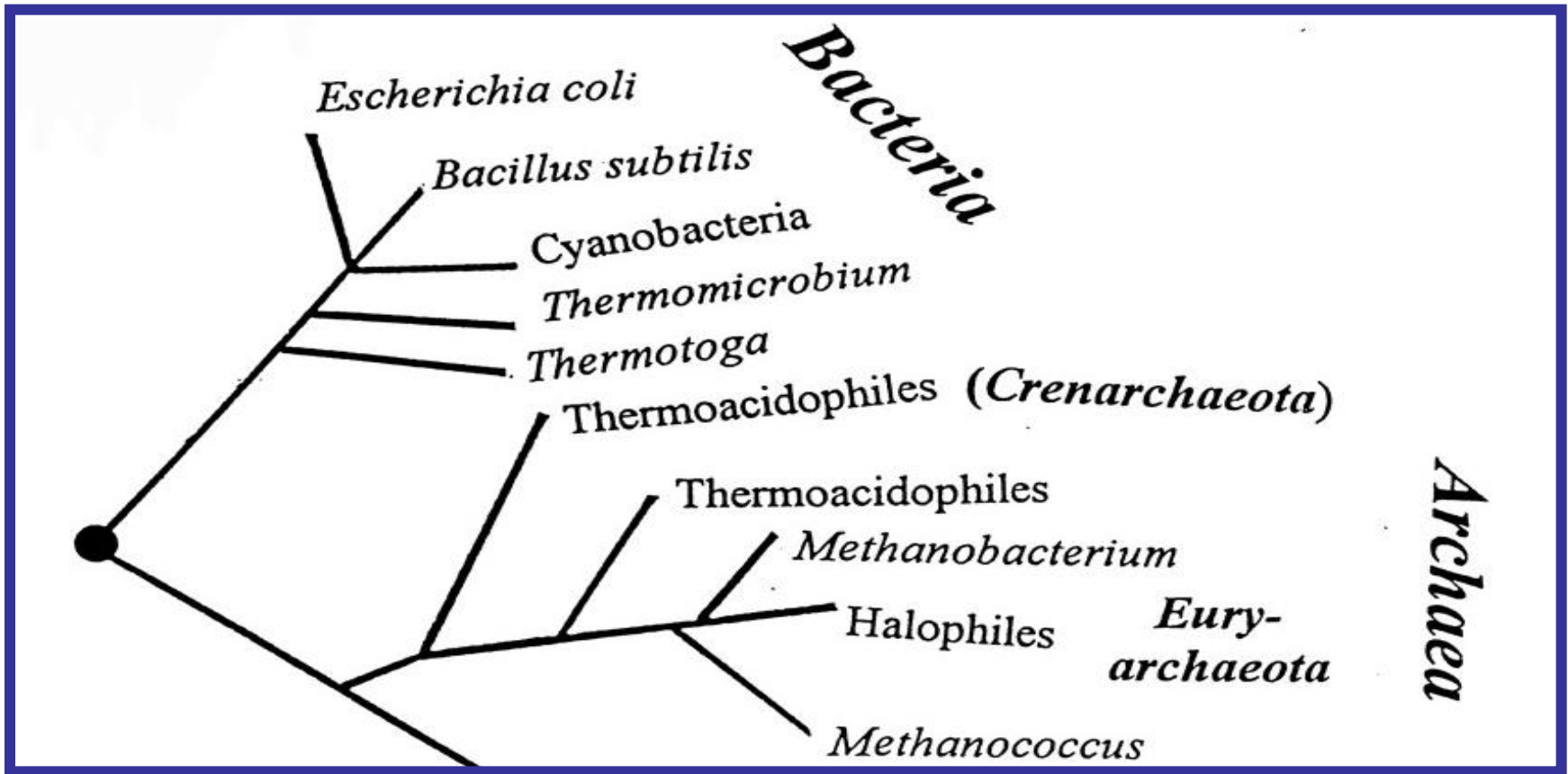
Prokaryotes

Bacteria

Archaea

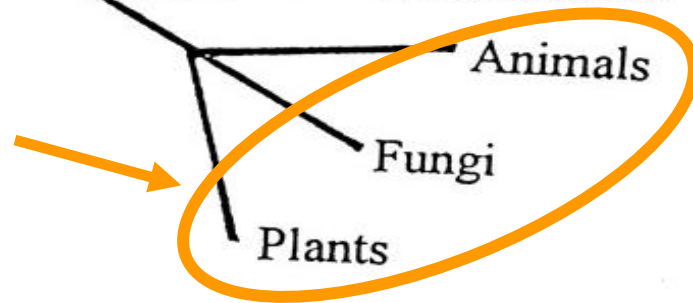


Eucarya



Prokaryotes

**These appeared
“very” recently.**



Bacteria

Archaea

Eucarya

Escherichia coli

Bacillus subtilis

Cyanobacteria

Thermomicrobium

Thermotoga

Thermoacidophiles (*Crenarchaeota*)

Thermoacidophiles

Methanobacterium

Halophiles

*Eury-
archaeota*

Methanococcus

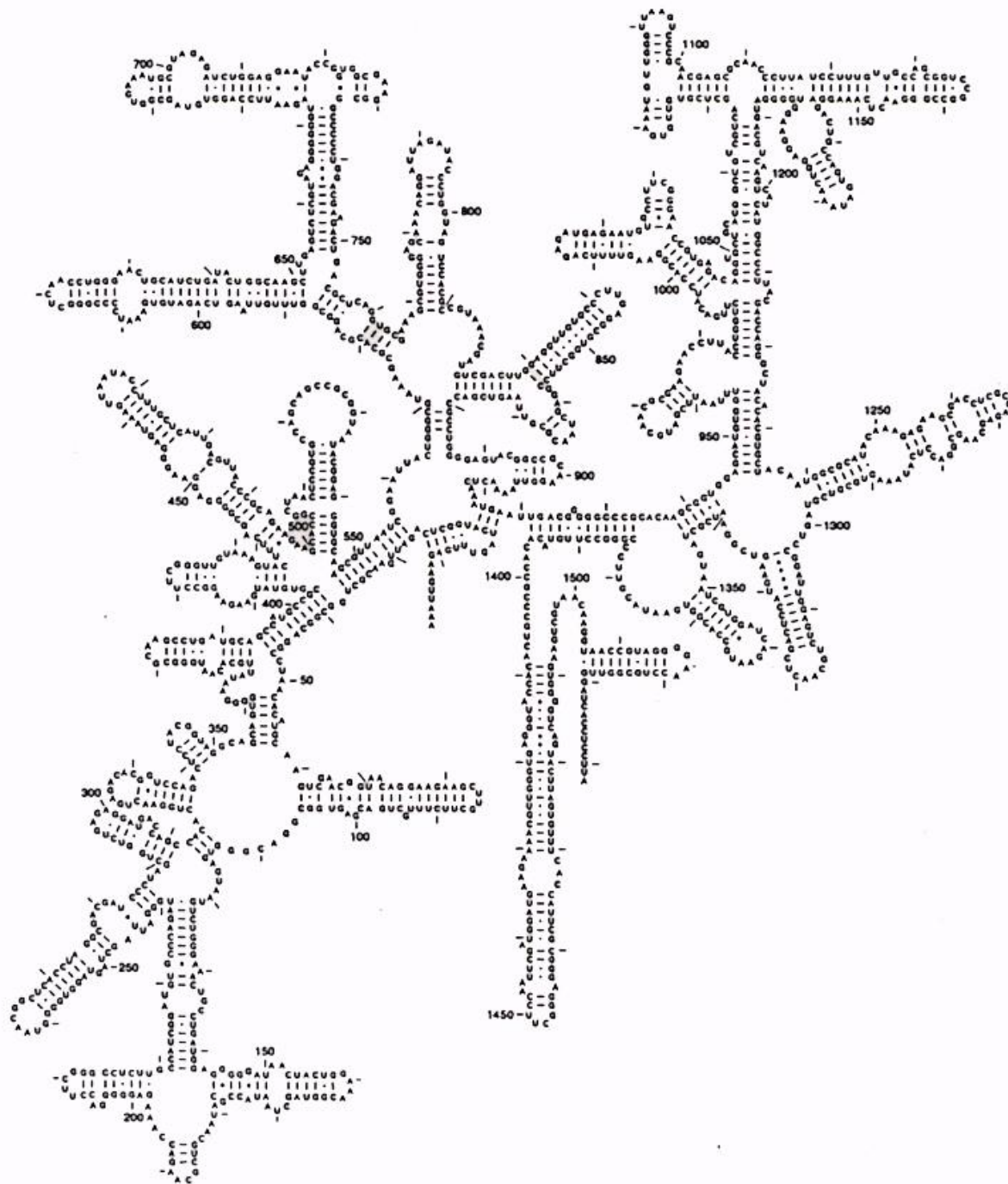
Giardia

Trichomonads

Animals

Fungi

Plants

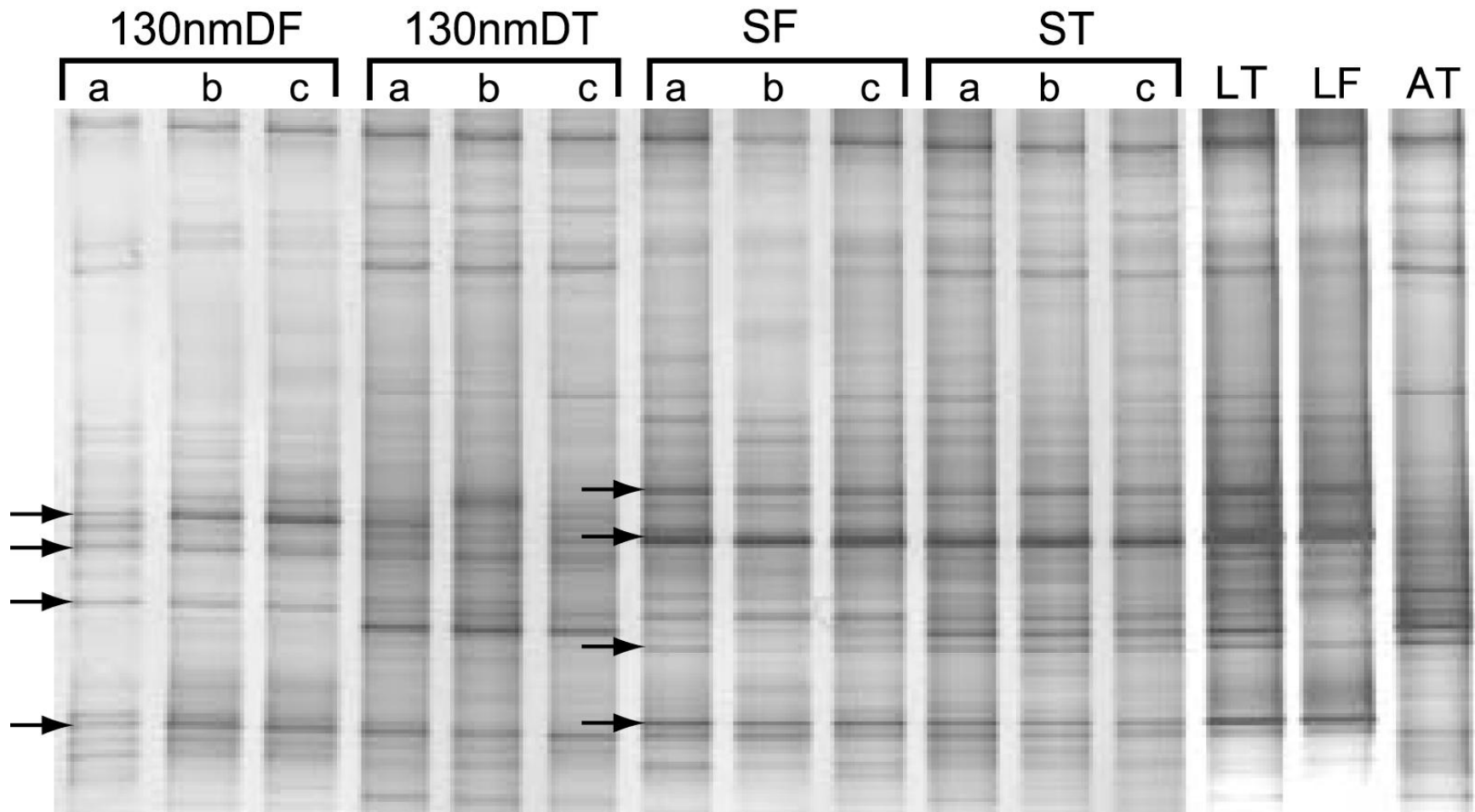


We rely on this particular molecule, 16S rRNA

Once you get the sequence, it tells you it's phylogenetic position and makes it possible to apply other approach, such as FISH.

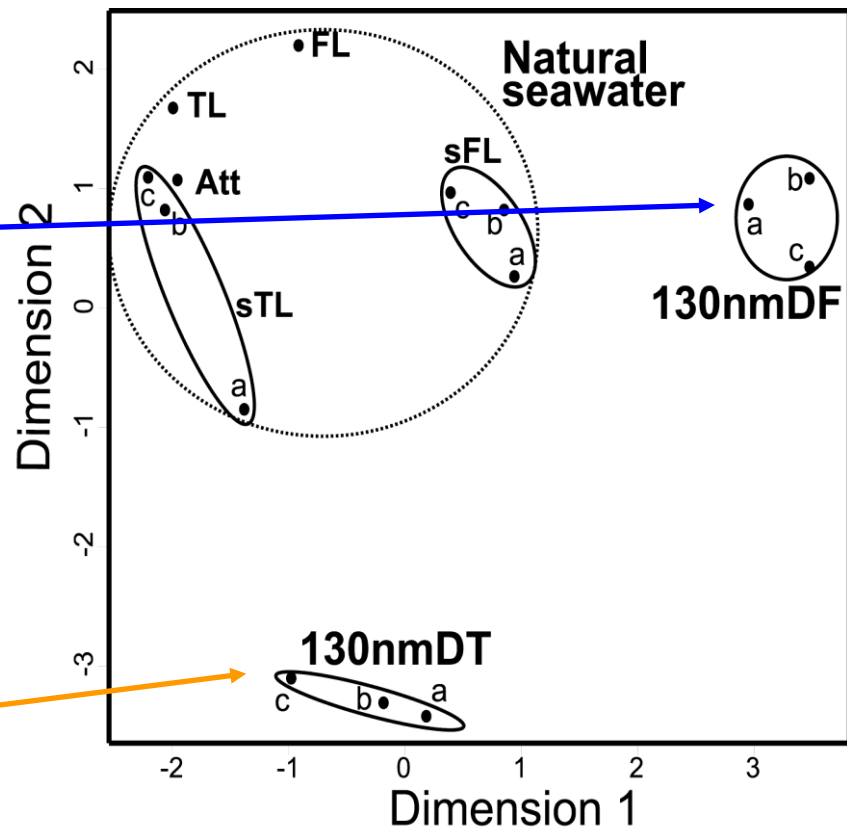
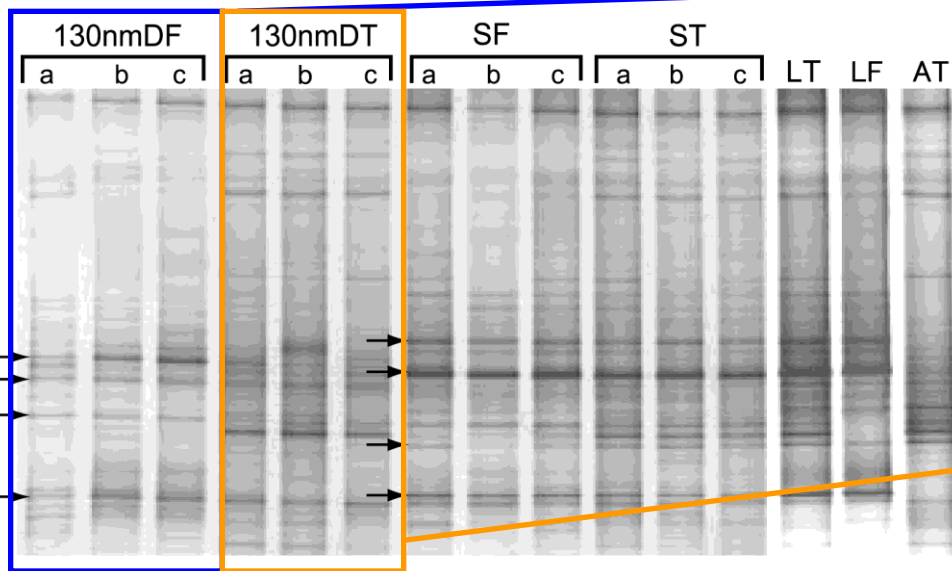
Community composition analyses by DGGE

DGGE: Denaturing Gradient Gel Electrophoresis



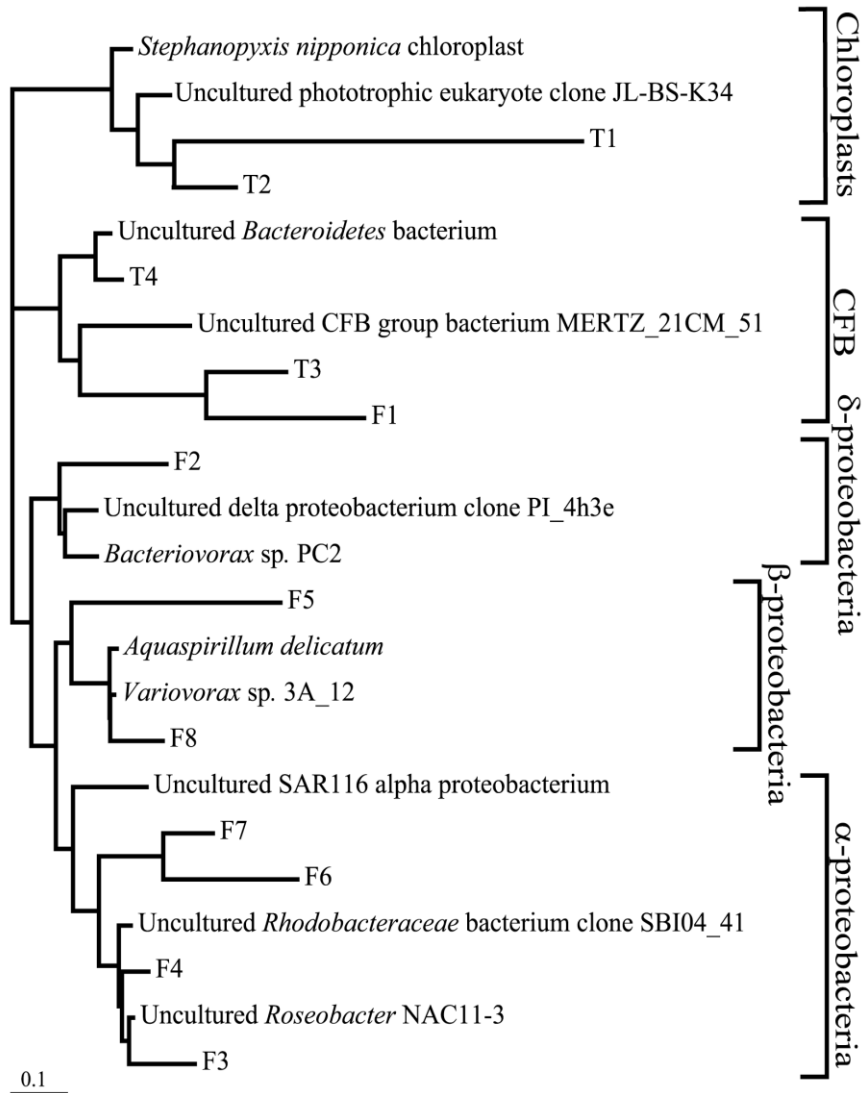
- From 7 different samples, DNA was extracted, partial sequence of 16S rDNA was amplified by PCR, and run on the gel with gradient.
- Each band corresponds to OTU or the species. Banding patterns show community structure. ⇒ MDS
- We can also obtain sequence data for each band.

The similarity of the community structures are visualized by MDS. The distance between any two point shows the degree of similarity.



MDS (Multi Dimensional Scaling)

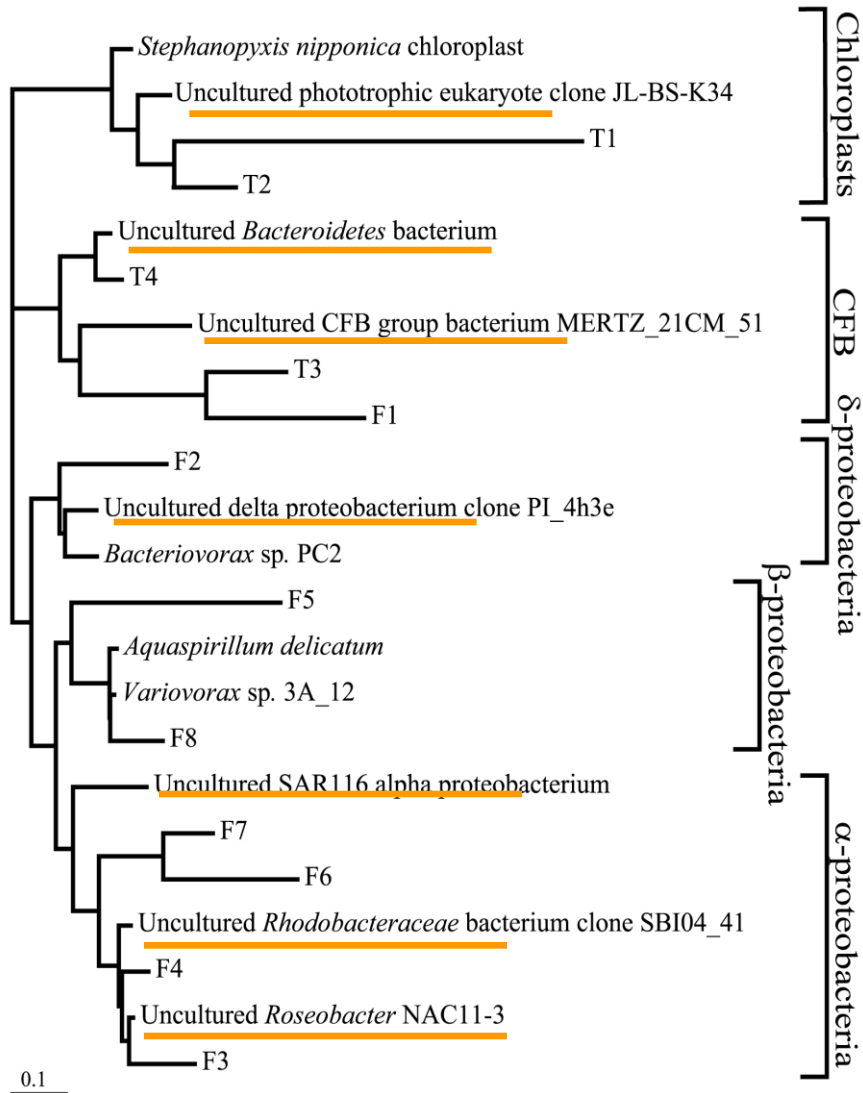
C. Phylogenetic relationship



By this approach,

- Many new sequences that was not listed in database appeared.

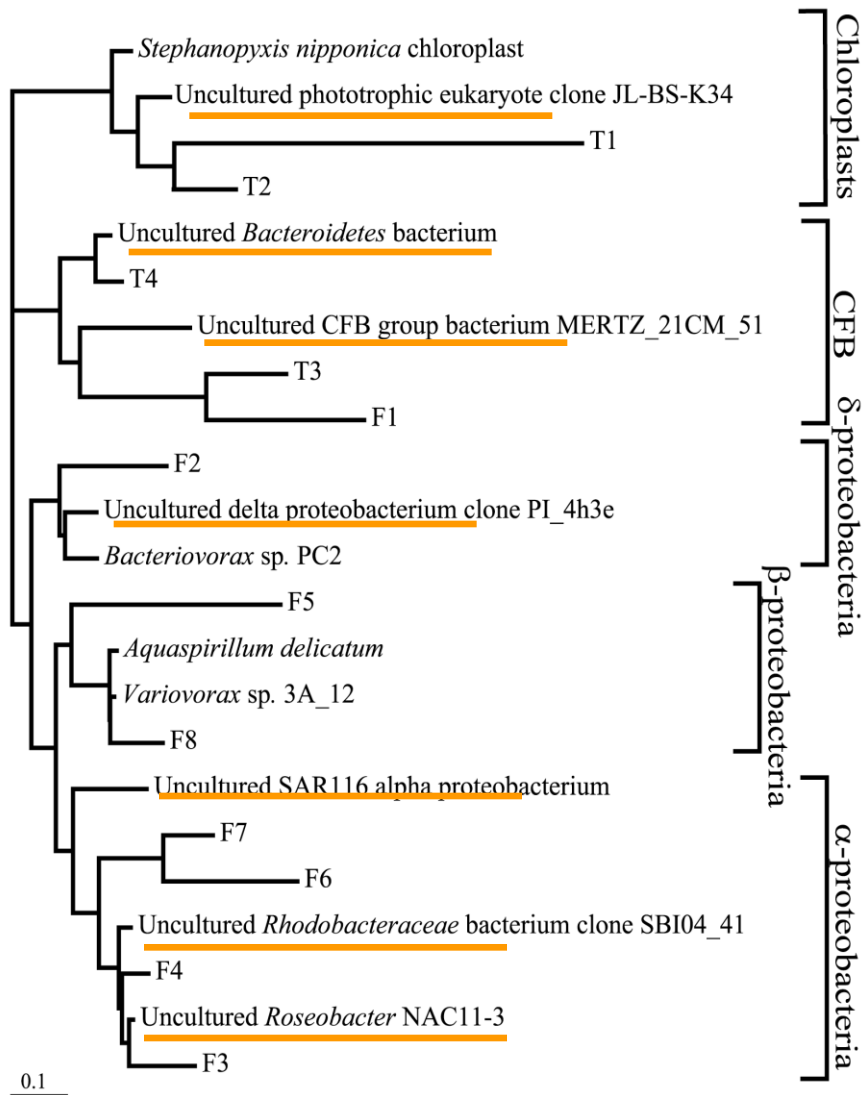
C. Phylogenetic relationship



By this approach,

- Many new sequences that was not listed in database appeared.
- Most of them are not present among cultured bacteria.

C. Phylogenetic relationship



By this approach,

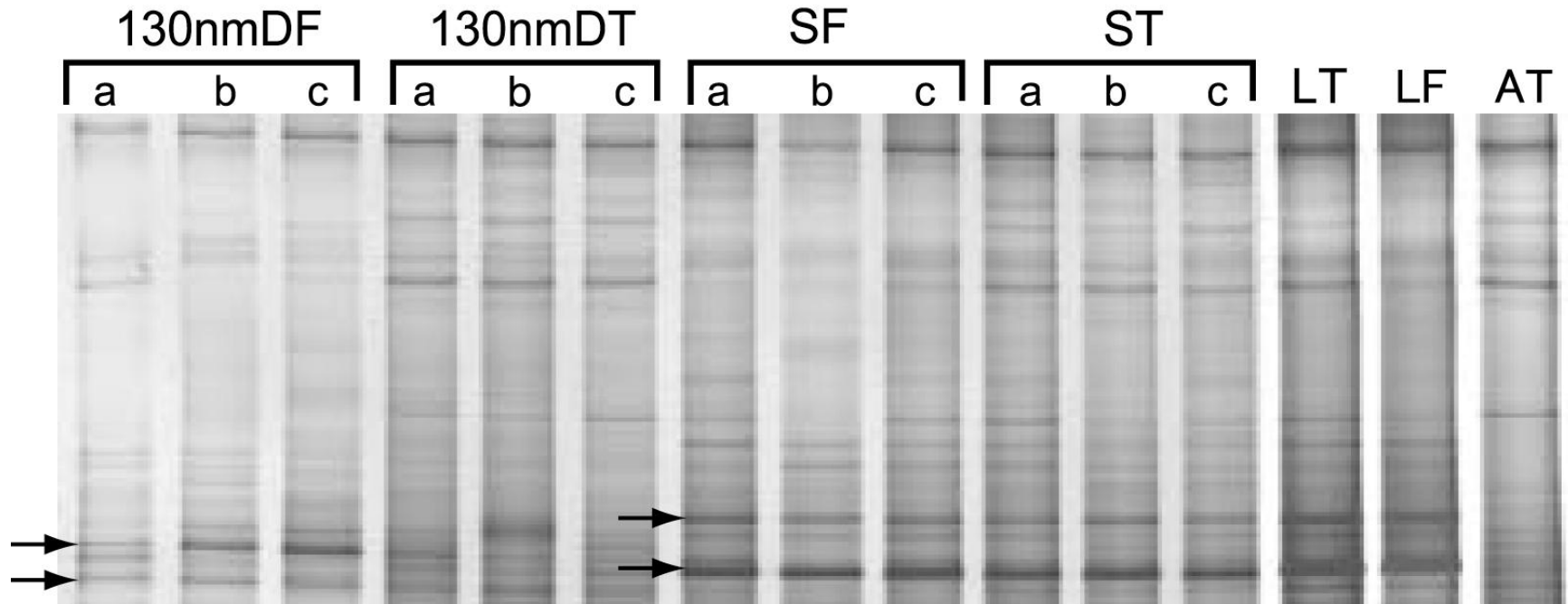
- Many new sequences that was not listed in database appeared.
- Most of them are not present among cultured bacteria.

Most bacteria are not yet cultured

Our knowledge on marine bacterial species and their genetic information is quite limited.

How much do we know?

Do we know more or less all the prokaryotes or not.



The number of bands in each lane are at most 60.

Don't you feel that these numbers are too small, if each band correspond to species. In short, how many bacterial species are there in the ocean?

This technique show us who are present. But more important question is who are active!

How much do we know?

Now do we know more or less all the prokaryotes or not.

The bacterium in question has to make visible band, otherwise ignored.

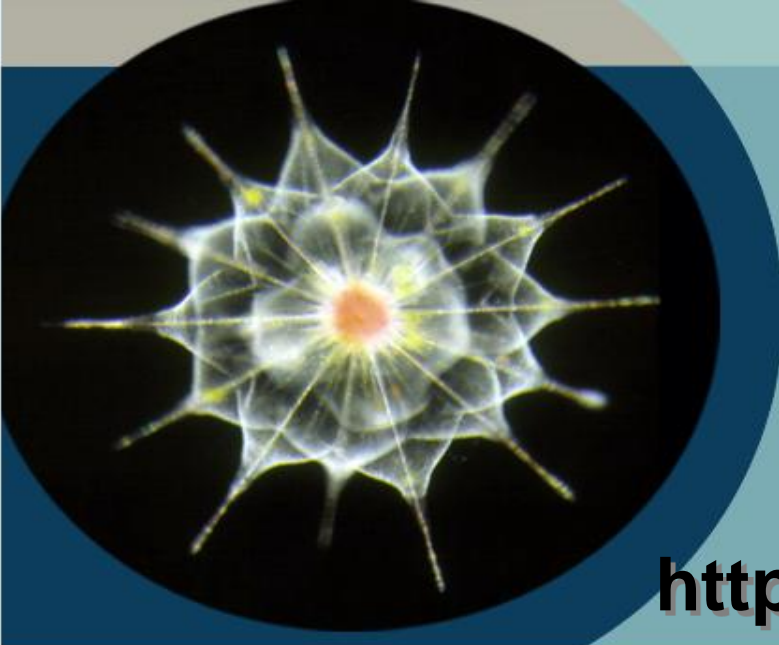
Then:

- "Minorities" are always ignored.
- Those of which DNA extraction is difficult are ignored.
- There are other sources causing biases, such as PCR amplification step and number of *rrn* operon per cell
- If we can sequence huge number of individuals for each sample, we may be able to recover minorities.

Each lane started from one to a few mL of seawater sample.

There should be a million cells there. The number of species are at most 50-60?

This kind of approach may make certain group visible, but others be ignored.



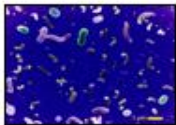
ICoMM

INTERNATIONAL CENSUS OF MARINE MICROBES

The role of the International Census of Marine Microbes (ICoMM) is to promote an agenda and an environment that will accelerate discovery, understanding, and awareness of the global significance of marine microbes.

<http://icomm.mbl.edu>

[HOME](#) [ABOUT ICoMM](#) [WORKING GROUP REPORTS](#) [CONTACT US](#) [FORUM](#) [MICROBIS](#)



In the August issue of PNAS, ICoMM Principal Investigator

Mitchell Sogin and colleagues published a manuscript entitled, "Microbial diversity in the deep sea and the underexplored 'rare biosphere'" Read more about this fascinating ICoMM pilot project [here](#)



The 7th Asia Pacific Marine Biotechnology Conference on

November 2-5th in Kochi, India will feature Marine Microbial Ecology, Marine Biodiversity and Bioinformatics among other ICoMM relevant topics... [Visit Web site](#)

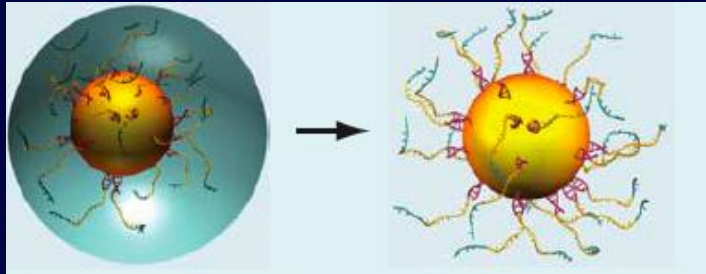
INTERNATIONAL CENSUS OF MARINE MICROBES

A place like no other

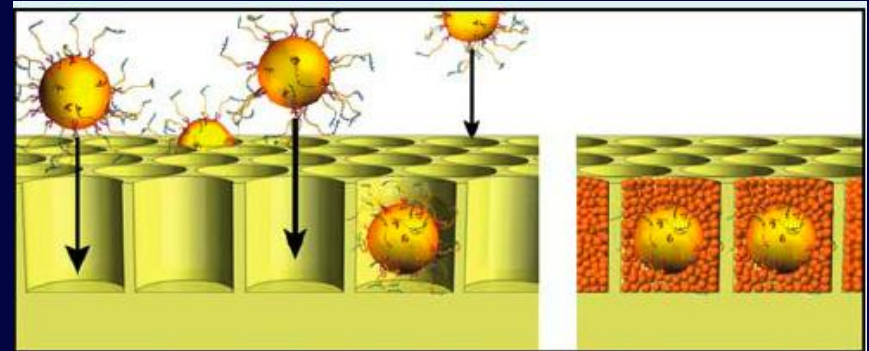
The International Census of Marine Microbes (ICoMM) will facilitate the inventory of marine microbial diversity developing a strategy to (1) catalogue all known diversity of single-cell organisms inclusive of the Bacteria, Archaea, Protista and associated viruses, (2) to explore and discover unknown microbial diversity, and (3) to place that knowledge into appropriate ecological and evolutionary contexts.

To be successful, ICoMM must promote international cooperation and forge linkages with existing and new CoML field projects for collecting samples, contextual information and new technologies. ICoMM recognizes that projects currently underway or completed over the past decade will have an important impact on the census. Participation by principal investigators of current projects in the ICoMM initiative will accelerate progress and ultimately lead to an organized constituency for seeking funding from agencies and foundations. At the same time, ICoMM will engage the broader community of microbiologists with complementary interests in microbial diversity, evolution, biogeography

454 Sequencing Technology - Roche Applied Biosystems



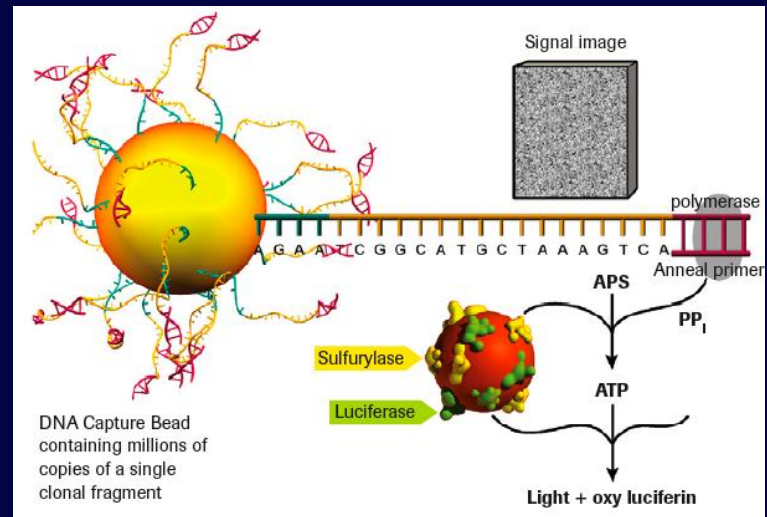
Emulsified PCR Reactions
One template - one bead



PicoTiterPlate Reactions

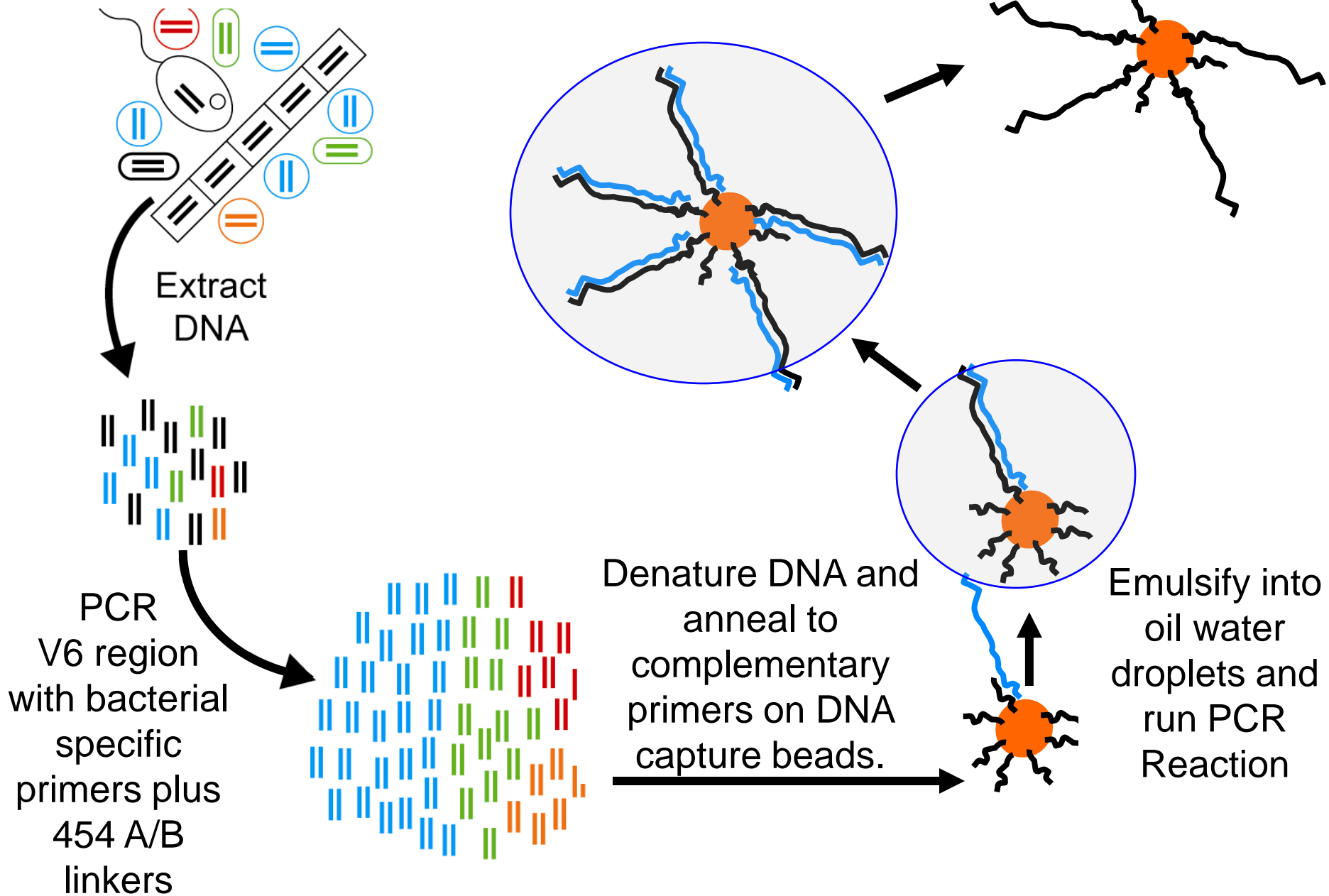
~200,000 reads
80-200 bp in length -
Average = 100 bp

~ \$0.02/read

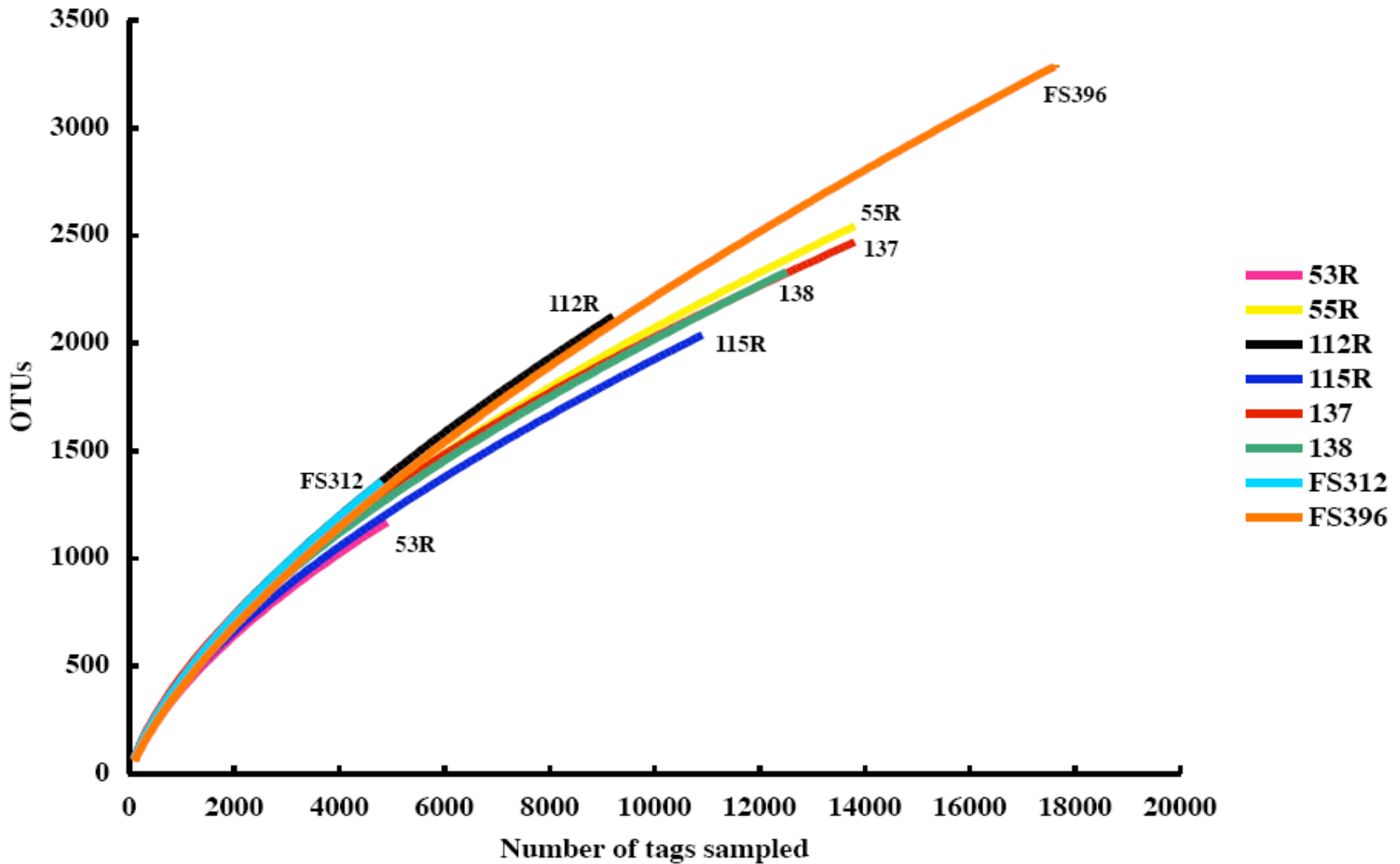


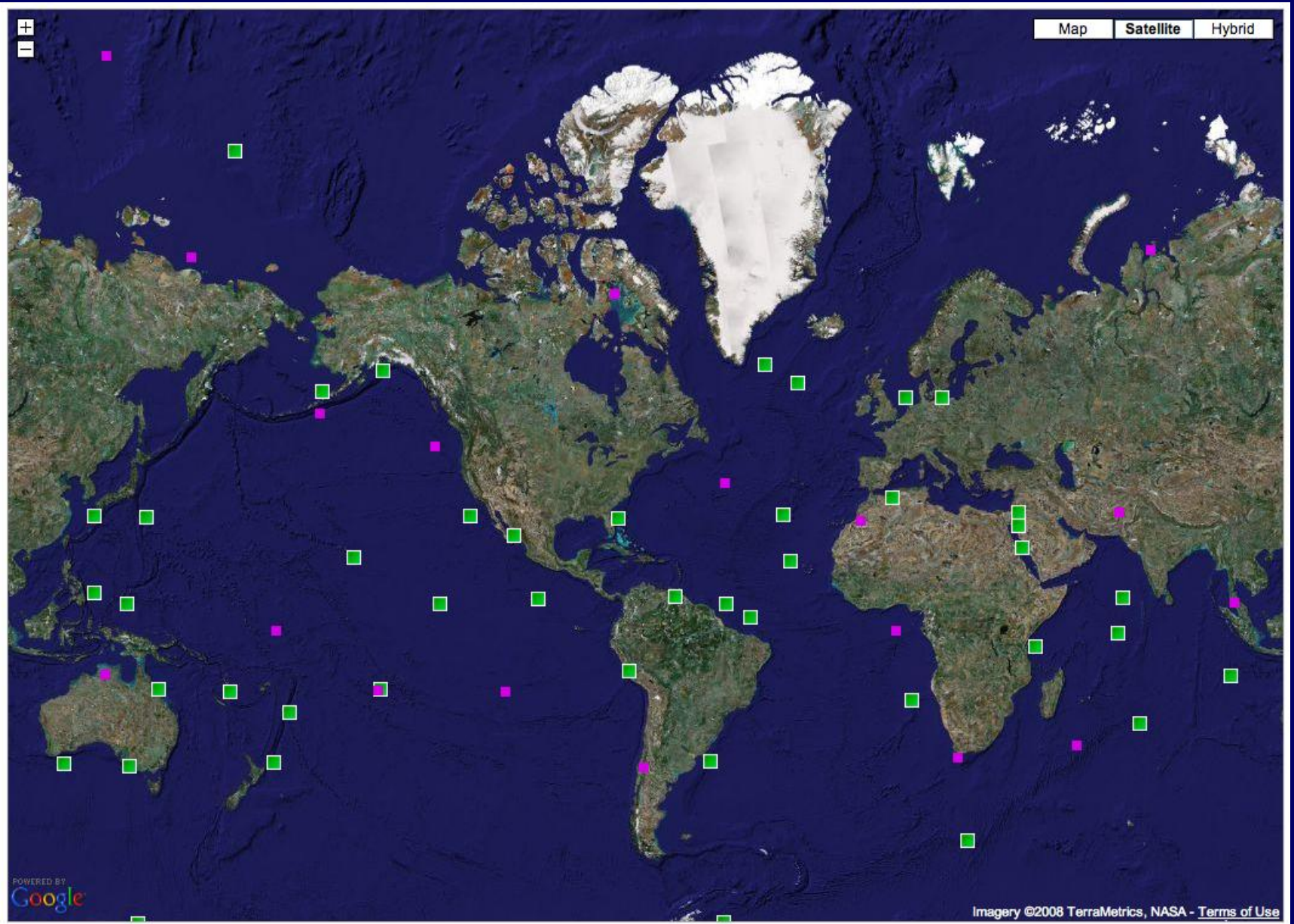
Solid Phase Pyrosequencing

The 454 Approach



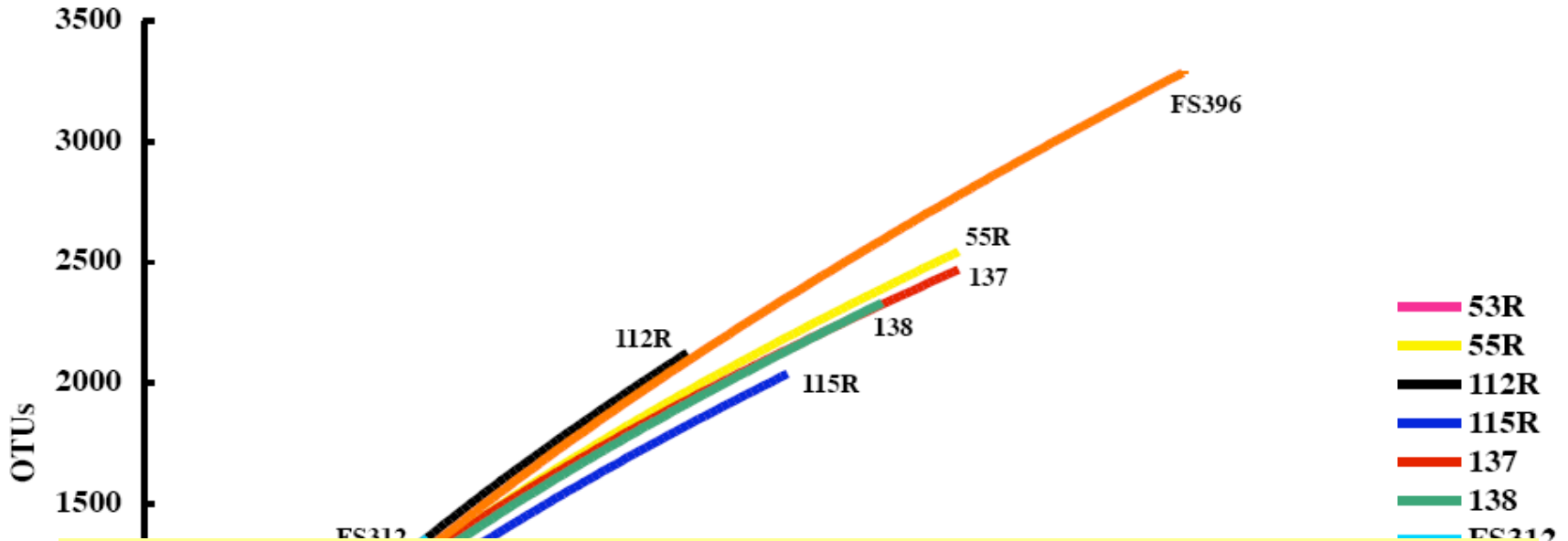
Rarefaction: BLAST OTUs





**Currently, samples covering wide areas are being analyzed.
We may get the results by April 2009.**

Rarefaction: BLAST OTUs

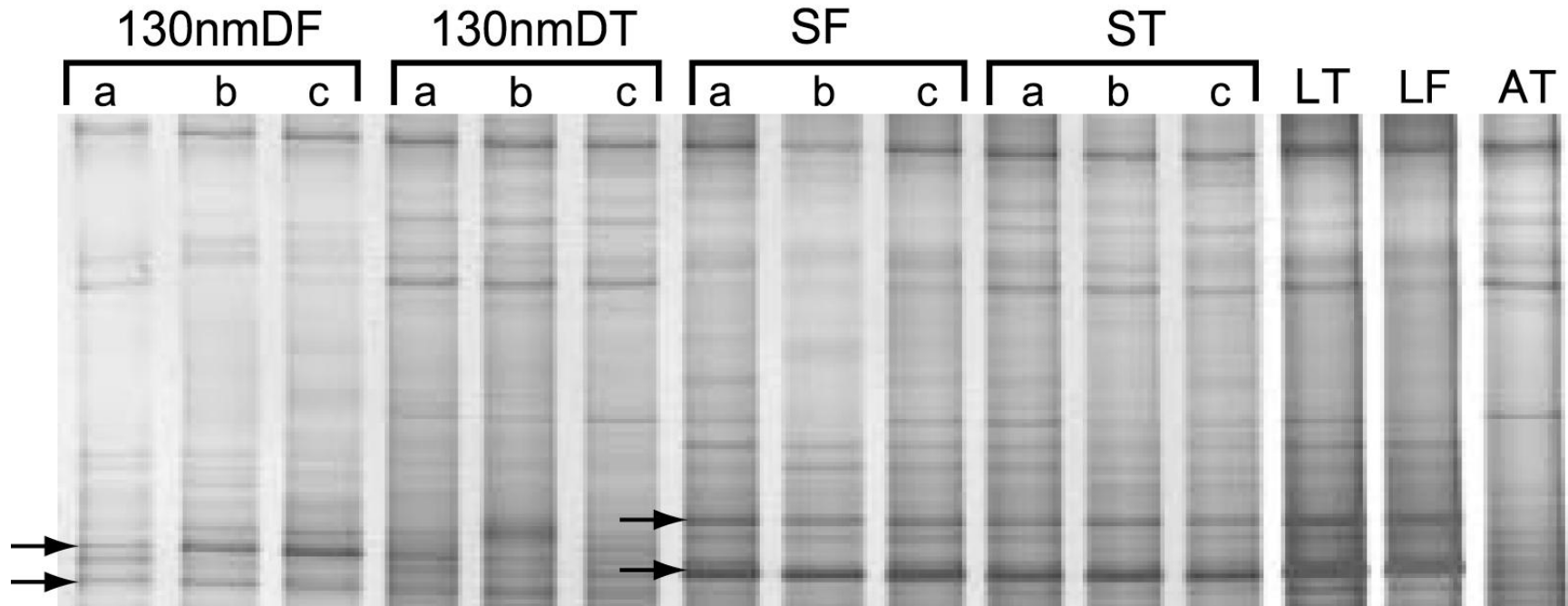


It seems, if you sequence more, more new OTU (corresponding to species) appear.

We still don't know who are in the ocean and how many prokaryotic species are present there.

It is technically not easy to know "Biodiversity" of prokaryotes!

So, are these techniques meaningless?



The number of bands in each lane are at most 60.

Don't you feel that these numbers are too small, if each band correspond to species. In short, how many bacterial species are there in the ocean?

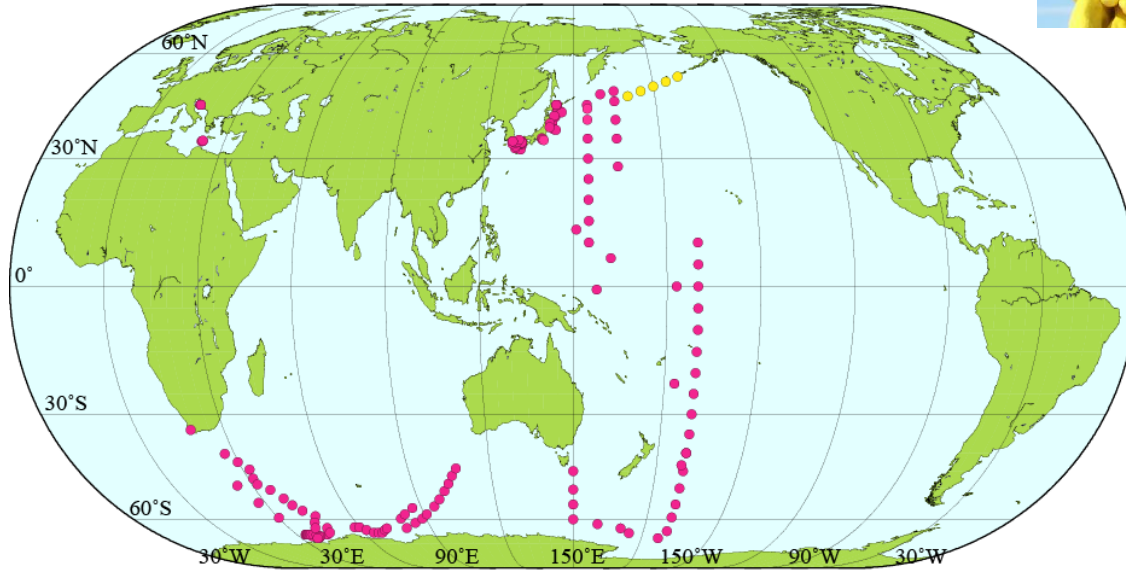
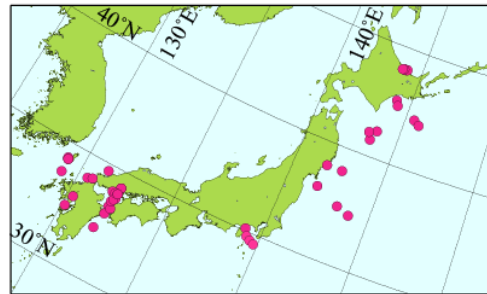
This technique show us who are present. But more important question is who are active!

BrdU approach

TANIGUCHI, Akito

HAMASAKI, Koji

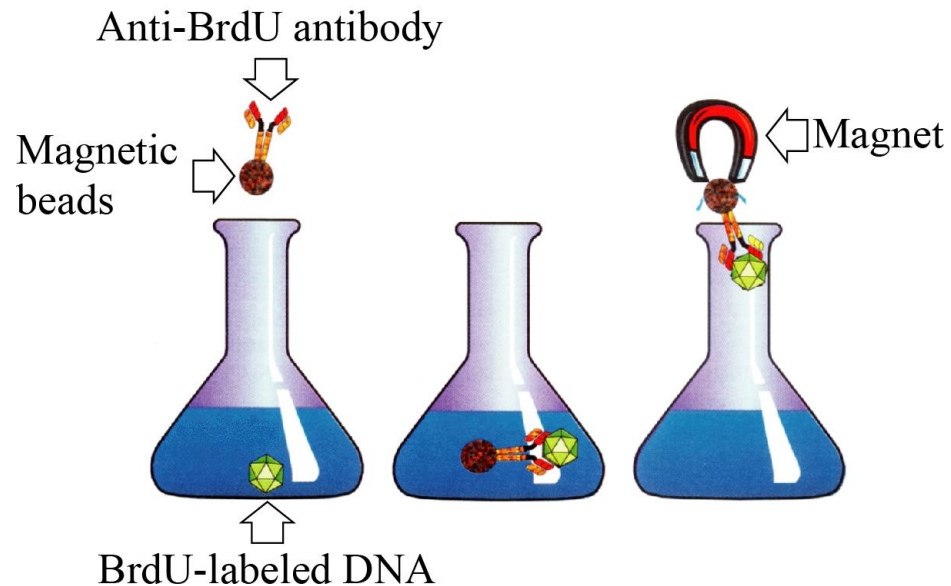
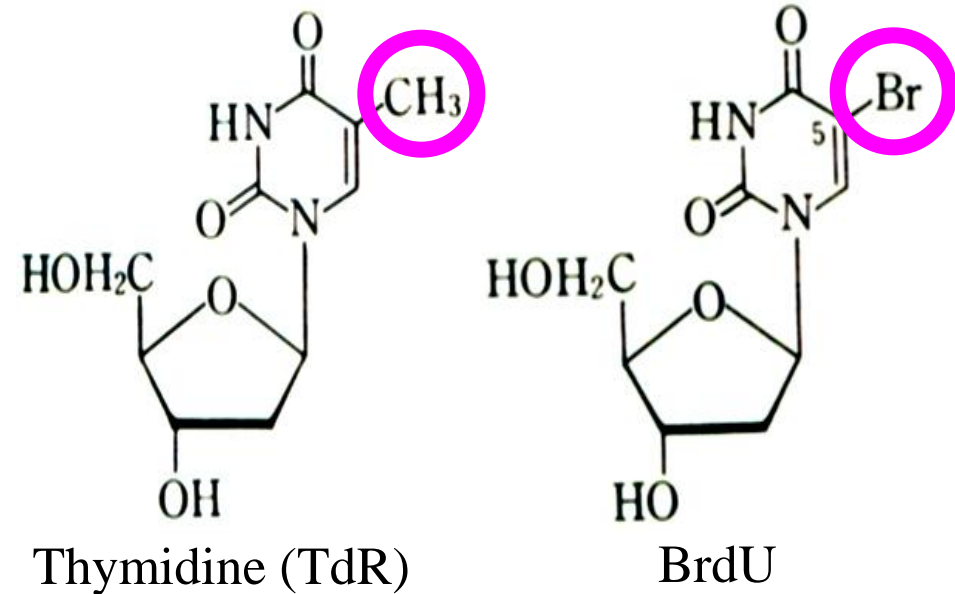
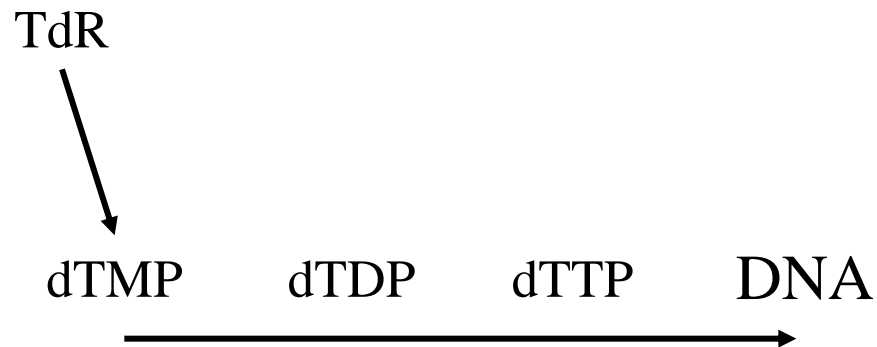
**Ocean Research Institute
The university of Tokyo**



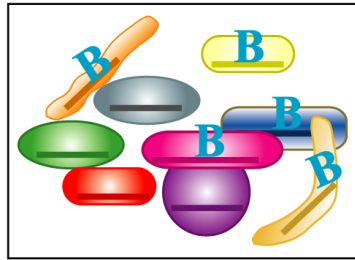
BrdU (2-bromo-5'-deoxyuridine)

Halogenated nucleoside
Thymidine analogue

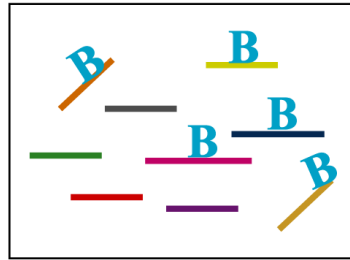
BrdU is incorporated into newly synthesized DNA. The BrdU-labeled DNA is immunocapturable with antibody.



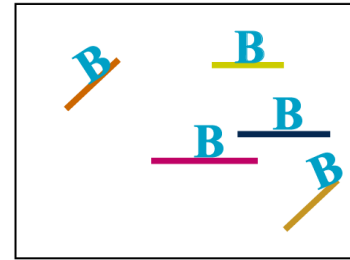
Seawater + BrdU: **B**



BrdU labeling



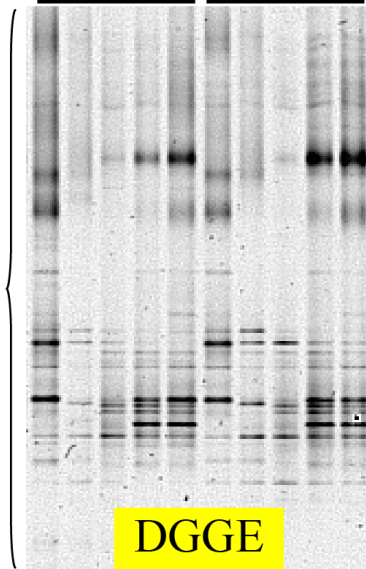
DNA extraction



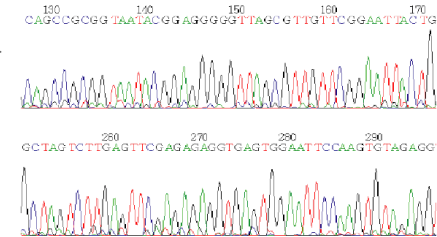
BrdU Immunocapture

PCR amplification (16S rRNA gene)

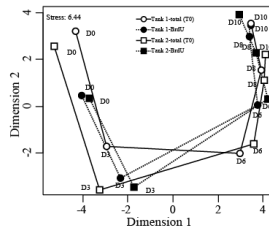
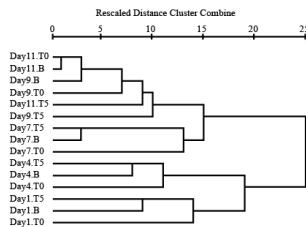
Total AGB



DGGE



Sequencing analysis



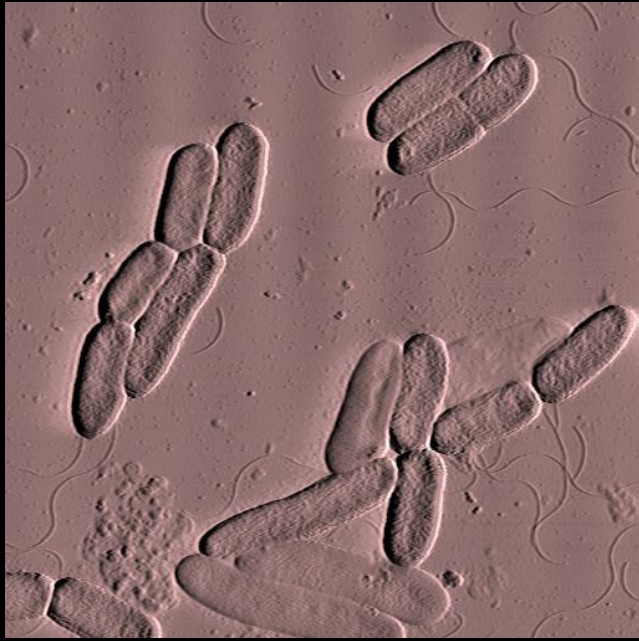
Statistical analysis

Now, which way should we go?

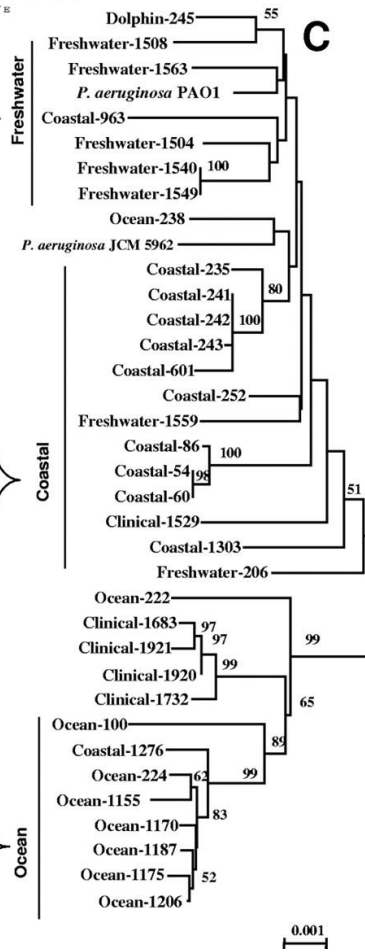
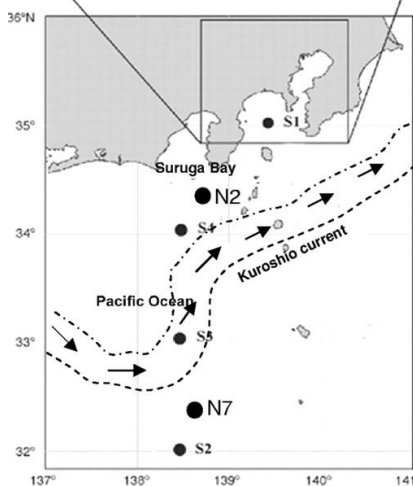
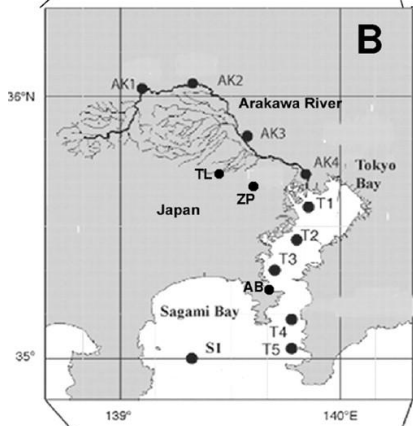
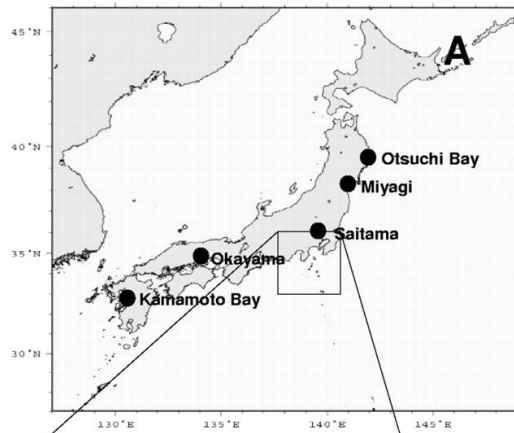
1. Get funding for metagenomic approach with 454 sequencing.
2. Let's look at functions, i.e., clarifying particular function or gene.
 - mRNA analyses (gene expression)
 - SIP (Stable Isotope Probing)
 - Comparative genome analyses of particular microbe
 - Phylogeny of particular genes
⇒ Evolution of functional genes



Pseudomonas aeruginosa



- After *E. coli*, the most thoroughly investigated bacterium.
- Opportunistic pathogen
- Plant and animal pathogen
- Biofilm formation
- Multiple drug resistance
- Mercury resistance
- Denitrification
- Quorum sensing
- Distributed widely except for
marine environments



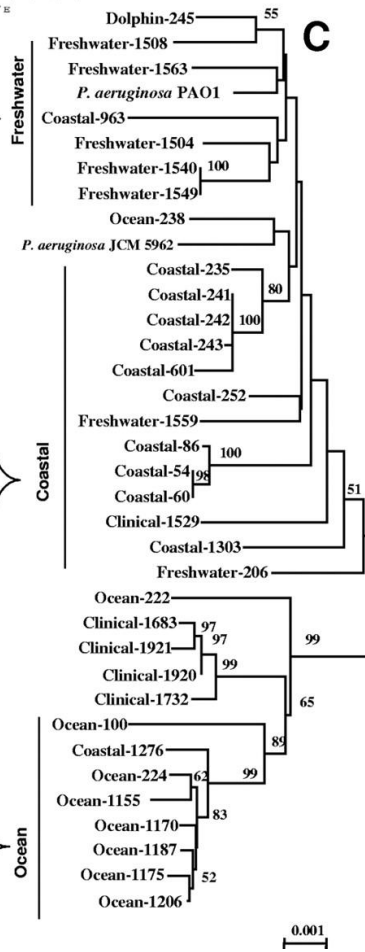
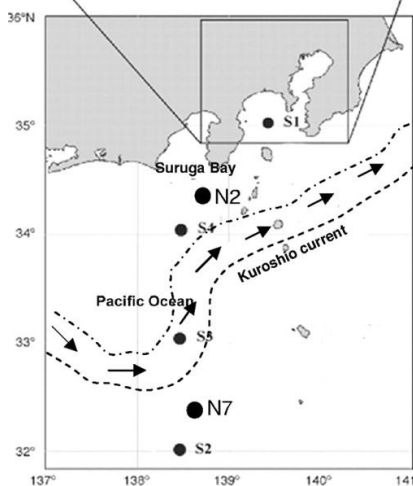
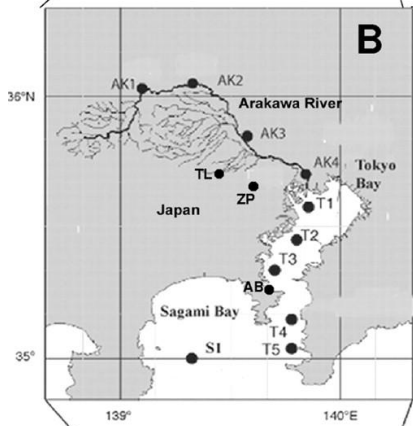
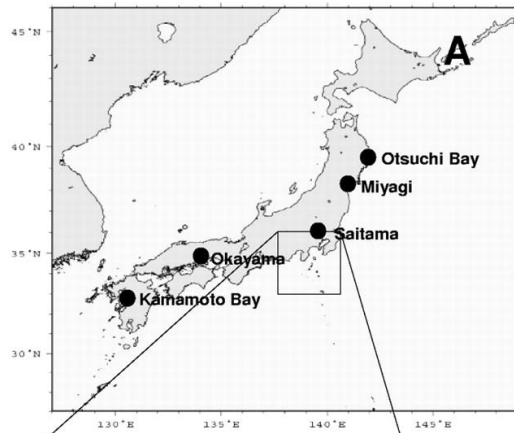
Background:

We have isolates from open ocean
So, this bacterium may be the organism
distributing all over the planet.

Question:

Are marine strains genetically unique?
Are there any biogeographical
distribution pattern?

(Khan et al. AEM 2008)



Background:

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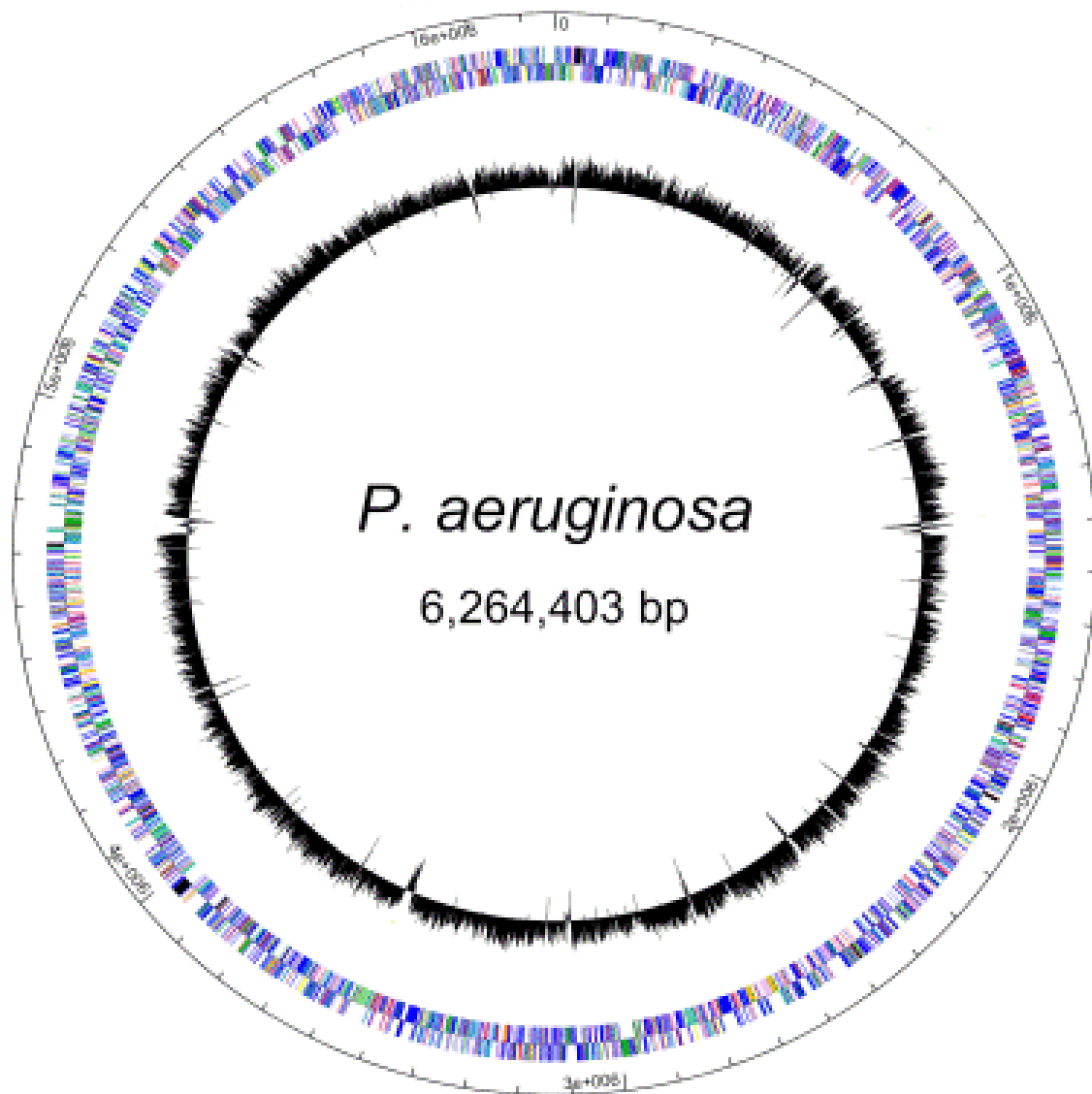
To answer:

Analyses of 7 house-keeping genes
(5,700bp)

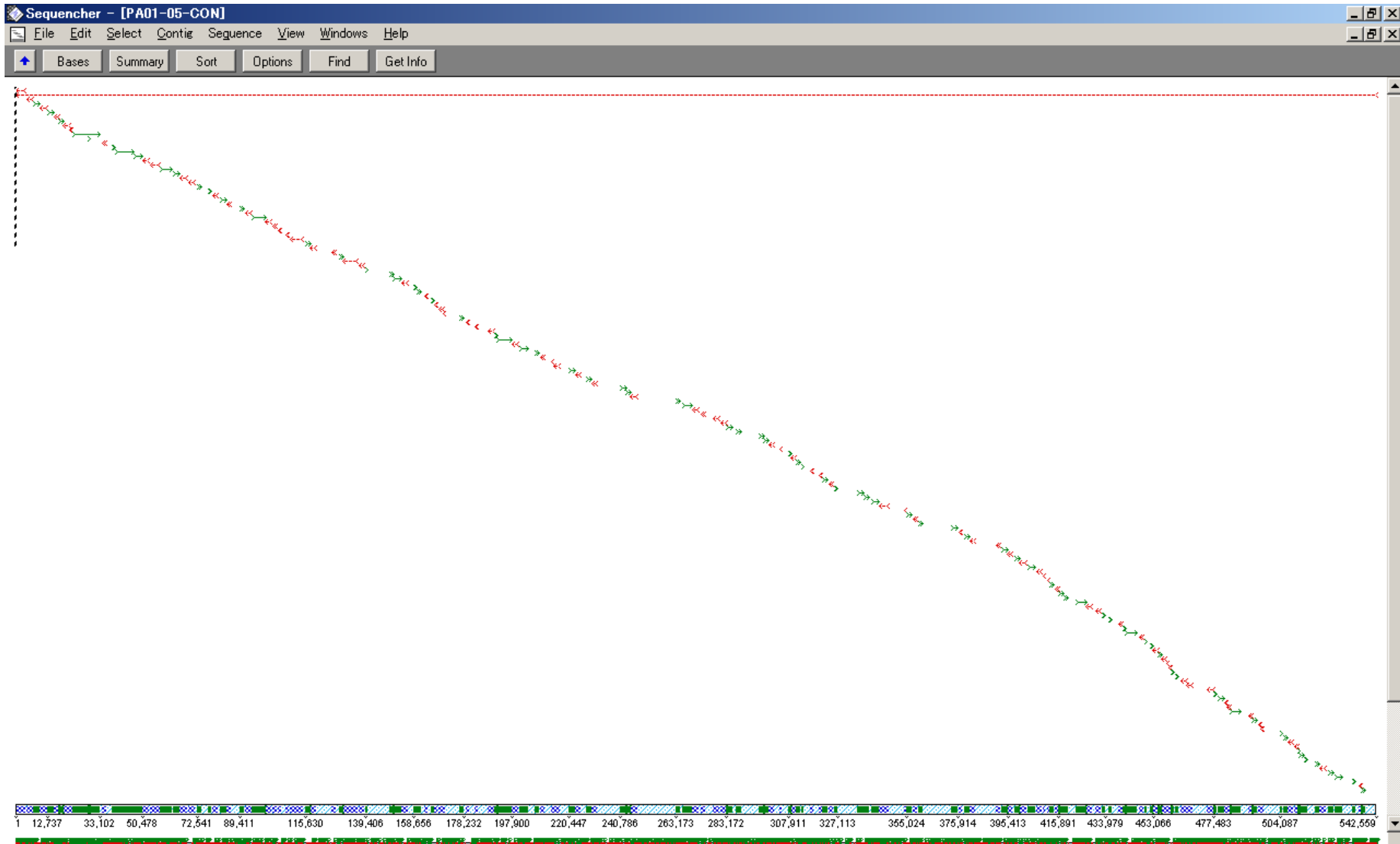
Answer:

Yes, they form a unique cluster
depending on their location

(Khan et al. AEM 2008)



**What kind of gene made it possible for this bacterium to live in the sea?
What makes this bacterium live in any part of the planet?
When did this bacterium go into the sea?**



Genome analyses of our strain 1200 is going on.
Remarkable similarity with PAO1 (genes shared, gene order, sequence)
There are some gaps, which we hope, may be unique to pathogens.











Others:

Accessory functional genes

⇒ factors involved in the geographical distribution

They may be transferred from other person.

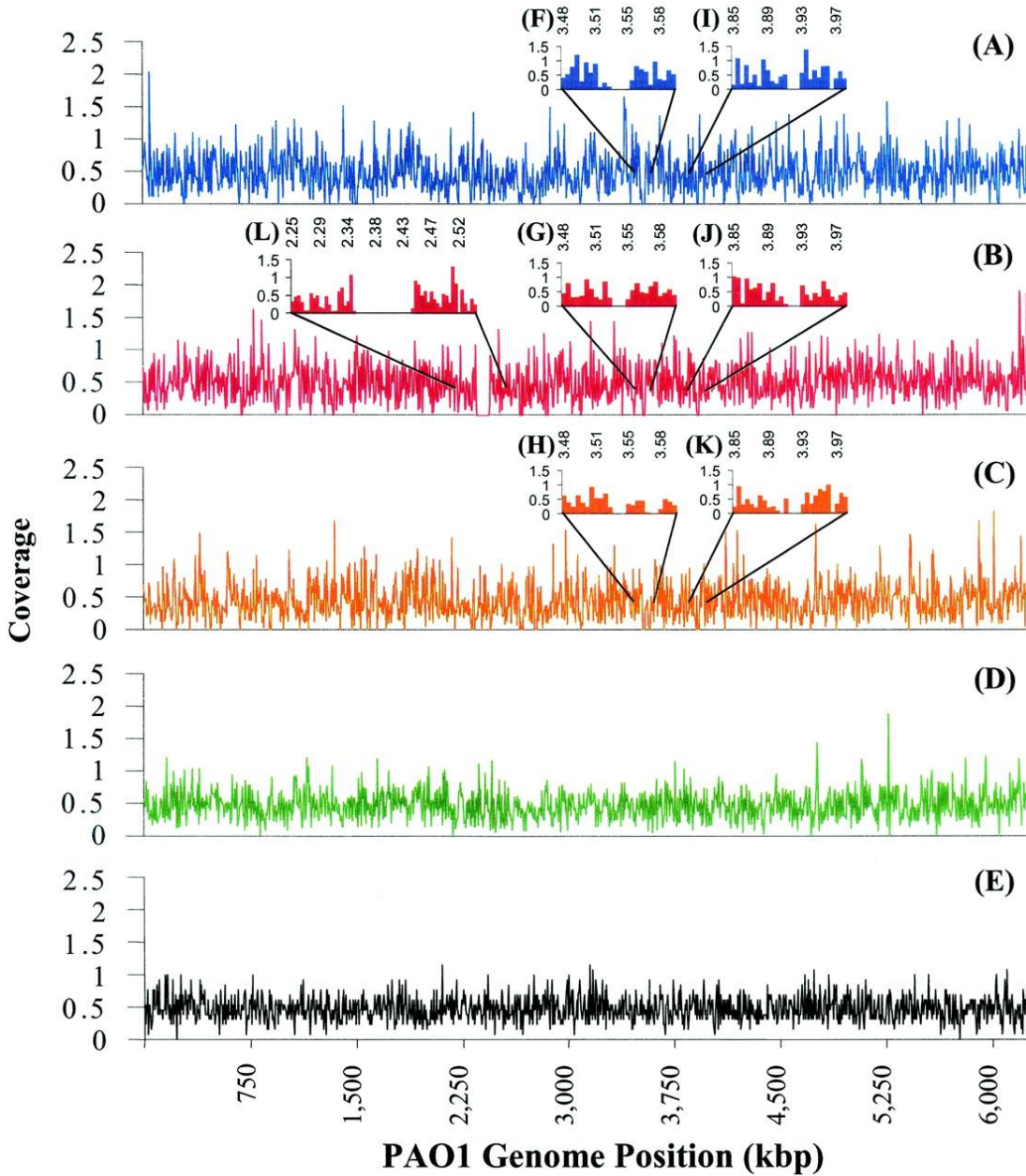
⇒ Horizontal gene transfer

She may somehow modify the cloth after receiving.

⇒ Gene diversification

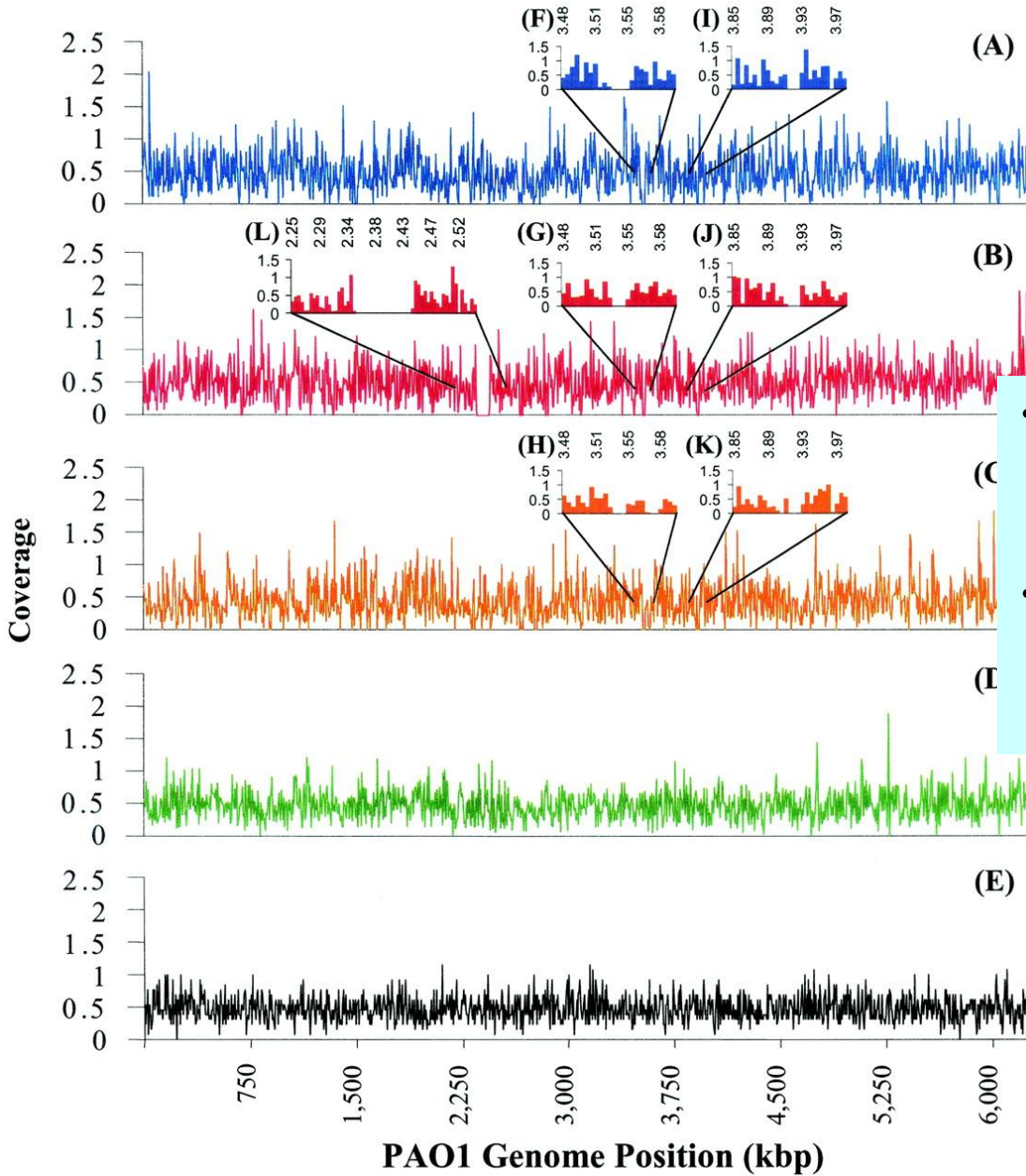
The origin of each style may be traceable.

⇒ Origin of the gene may be seen in phylogenetic tree



**Such insertions of new
“cloth gene” are confirmed
In *P. aeruginosa* genome.**

**Spencer et al. 2003
Whole-genome sequence
variation among multiple
isolates of *Pseudomonas
aeruginosa*.
J. Bacteriol. 185: 1316-1325**



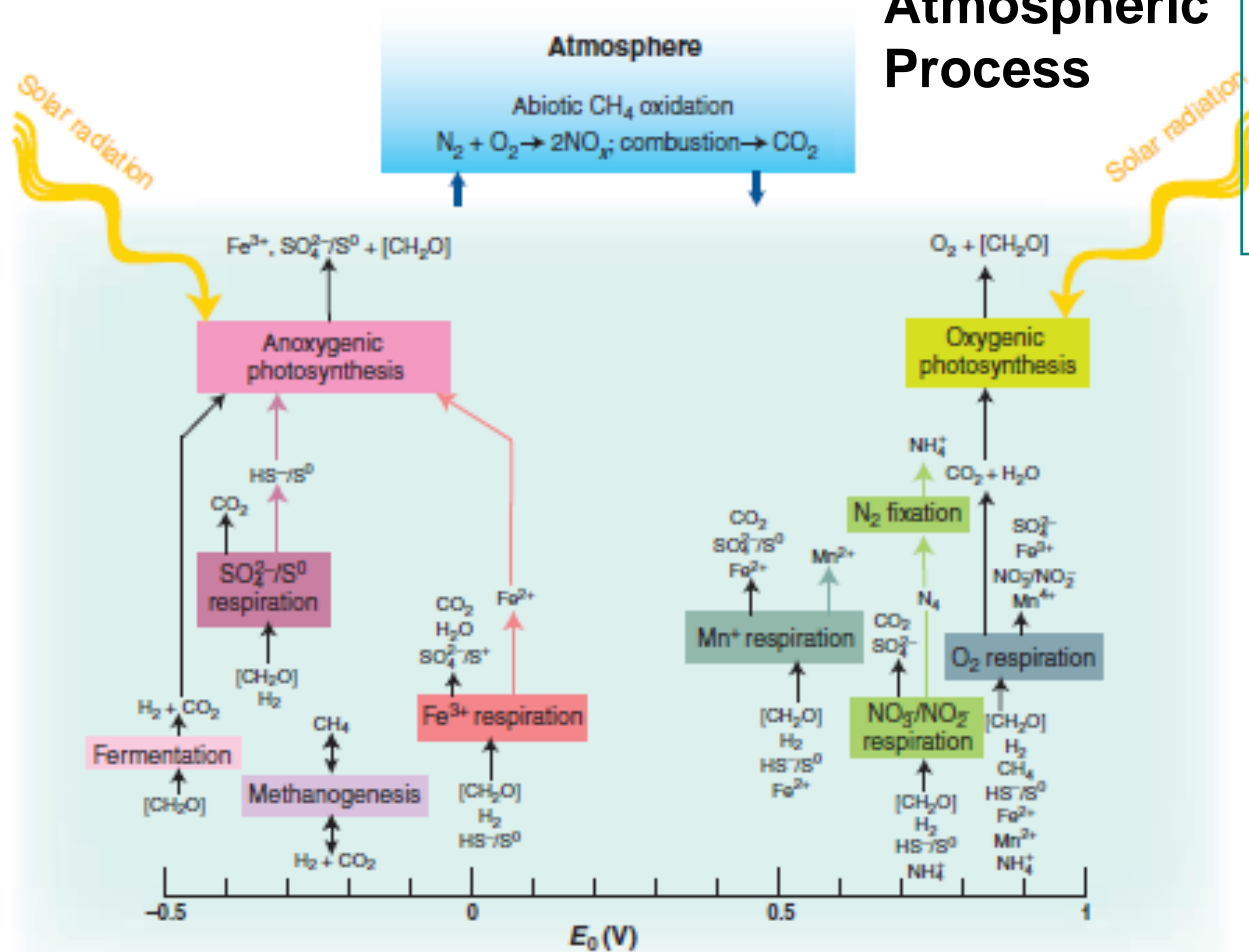
Such insertions of new “cloth gene” are confirmed In *P. aeruginosa* genome.

- The evolution of functional genes is quite interesting to look at.
- It should be related to material cycles on the planet.

Spencer et al. 2003
 Whole-genome sequence variation among multiple isolates of *Pseudomonas aeruginosa*.
 J. Bacteriol. 185: 1316-1325

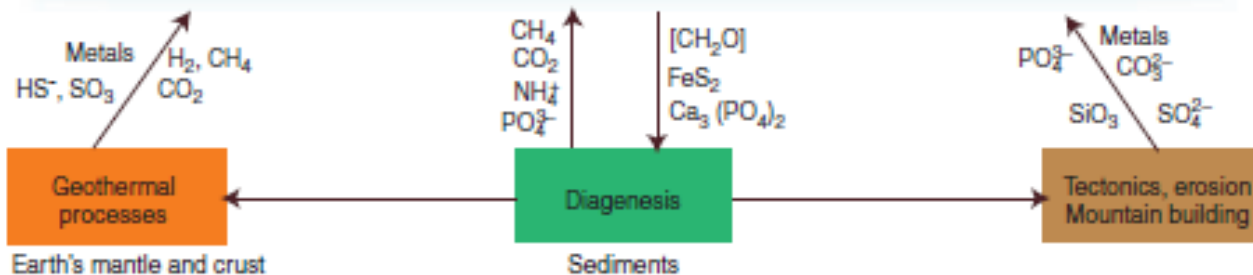
Atmospheric Process

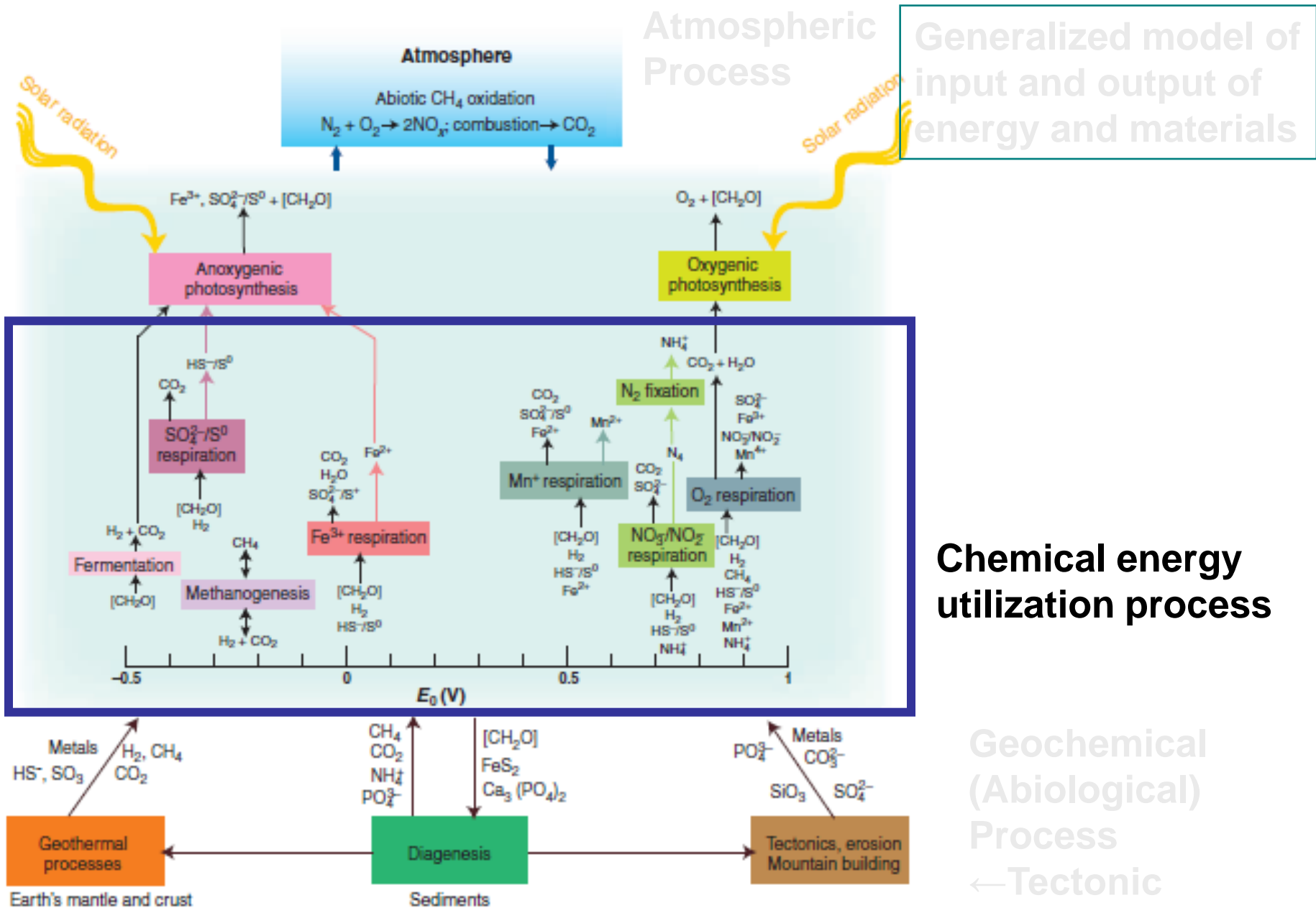
Generalized model of input and output of energy and materials (C, N, H, O, P, S)

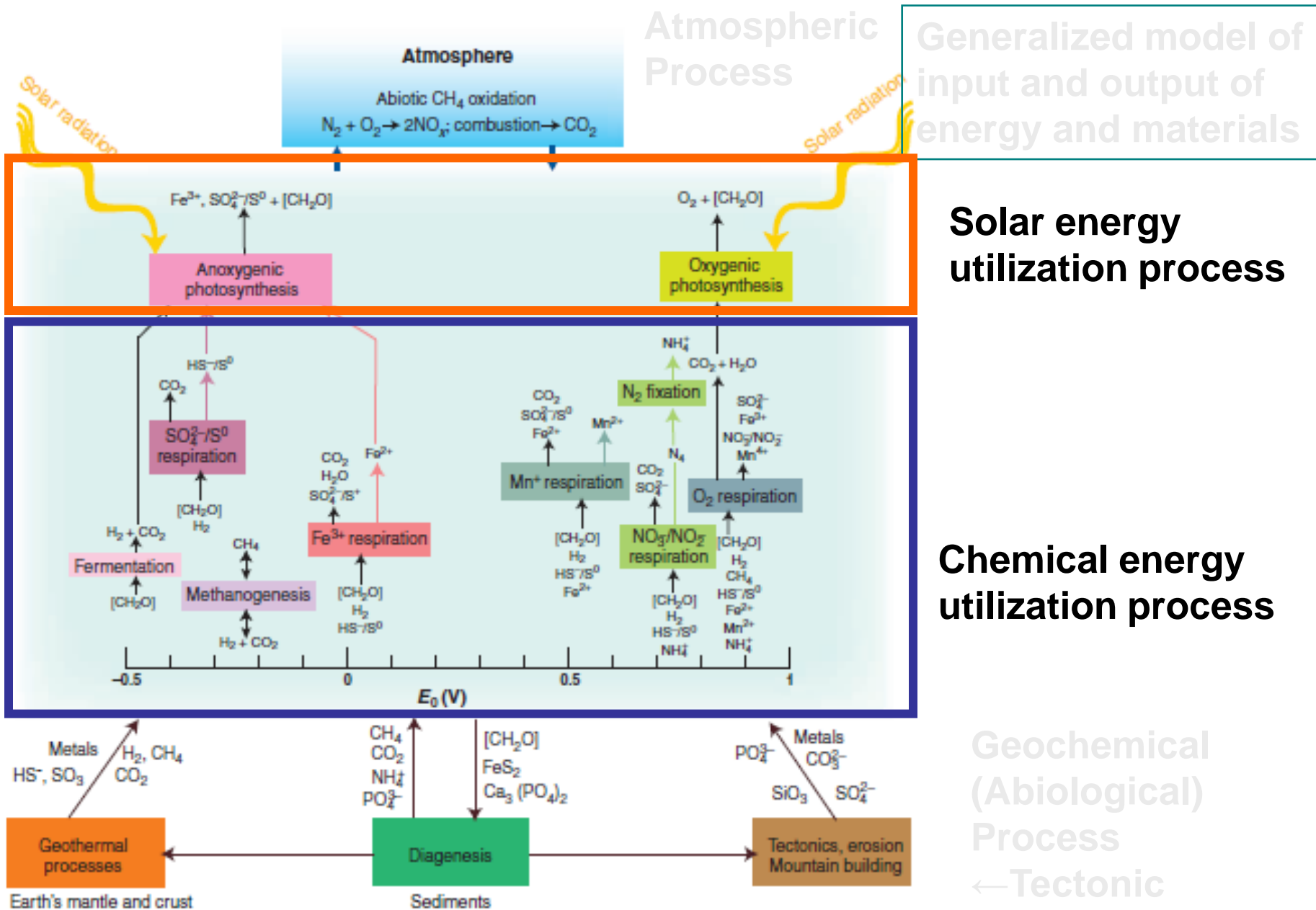


Biological Process

Geochemical (Abiological) Process
← Tectonic







Generalized model of input and output of energy and materials

Solar energy utilization process

Chemical energy utilization process

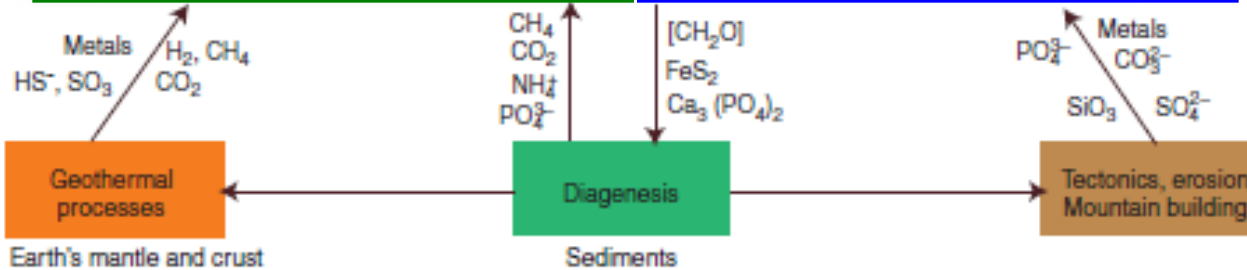
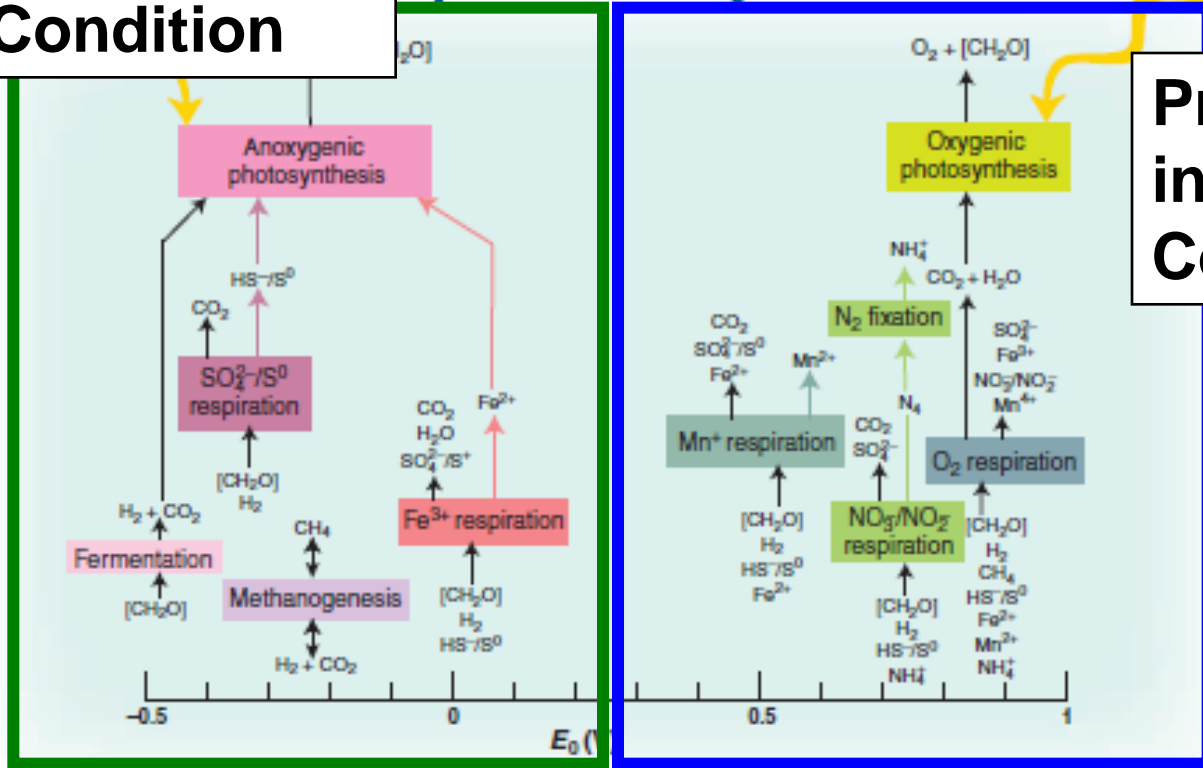
Geochemical (Abiological) Process
 ← Tectonic

Processes in Anaerobic Condition

Atmospheric Process

Generalized model of input and output of energy and materials

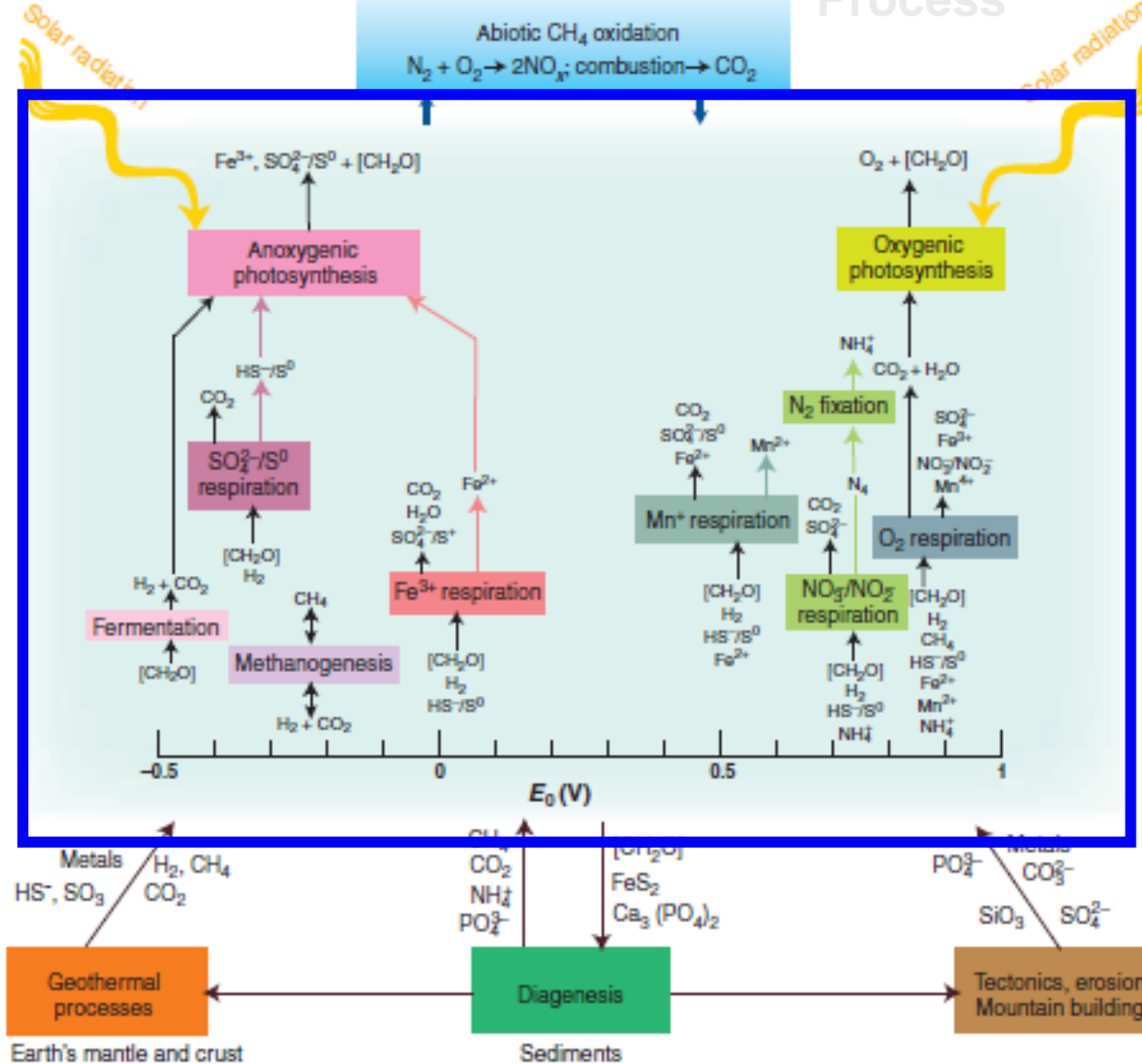
Processes in Aerobic Condition



Geochemical (Abiological) Process
← Tectonic

Atmospheric Process

Generalized model of input and output of energy and materials

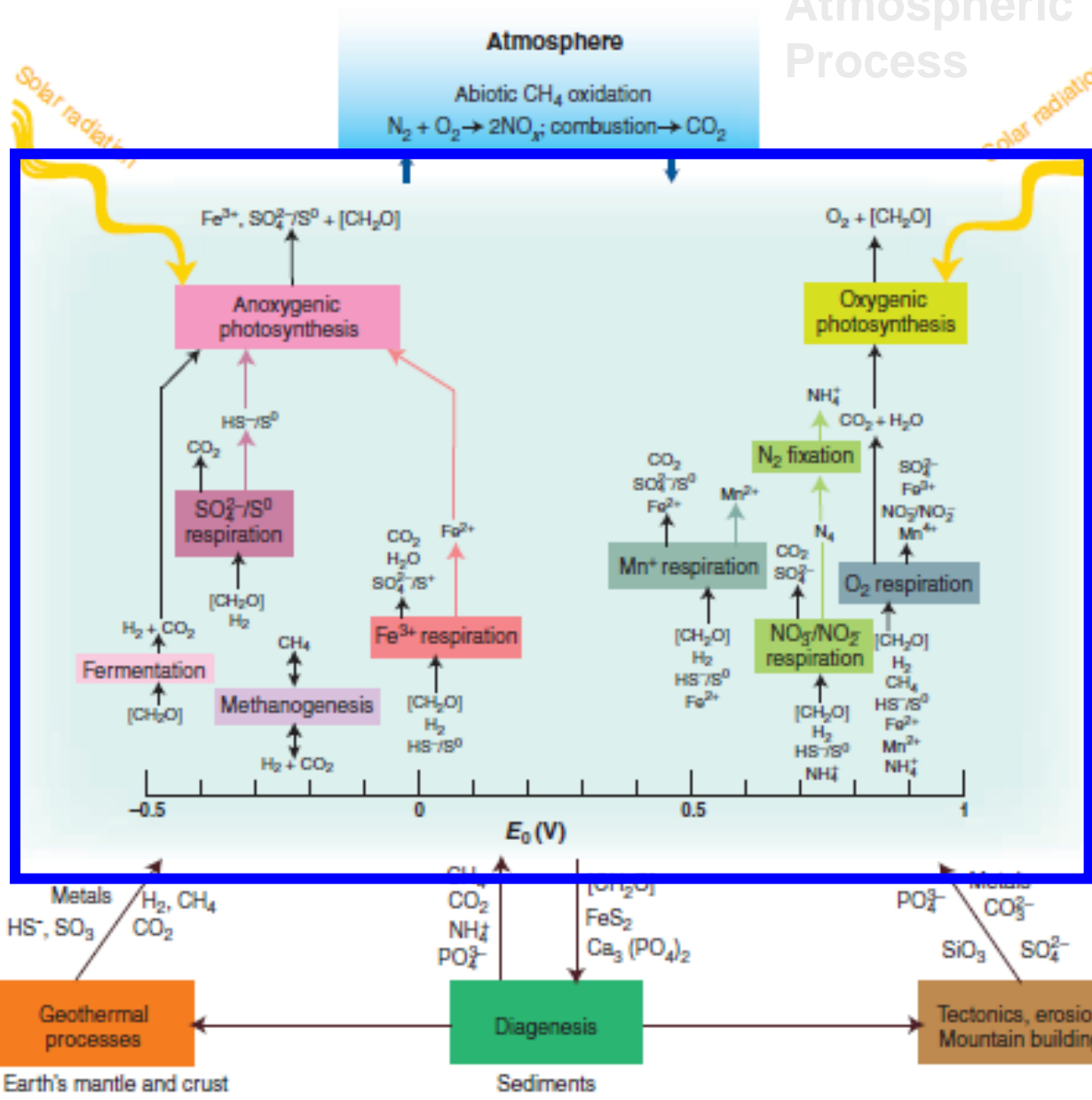


Before plants and animals appeared, all were done by prokaryotes

Geochemical (Abiological) Process
← Tectonic

Atmospheric Process

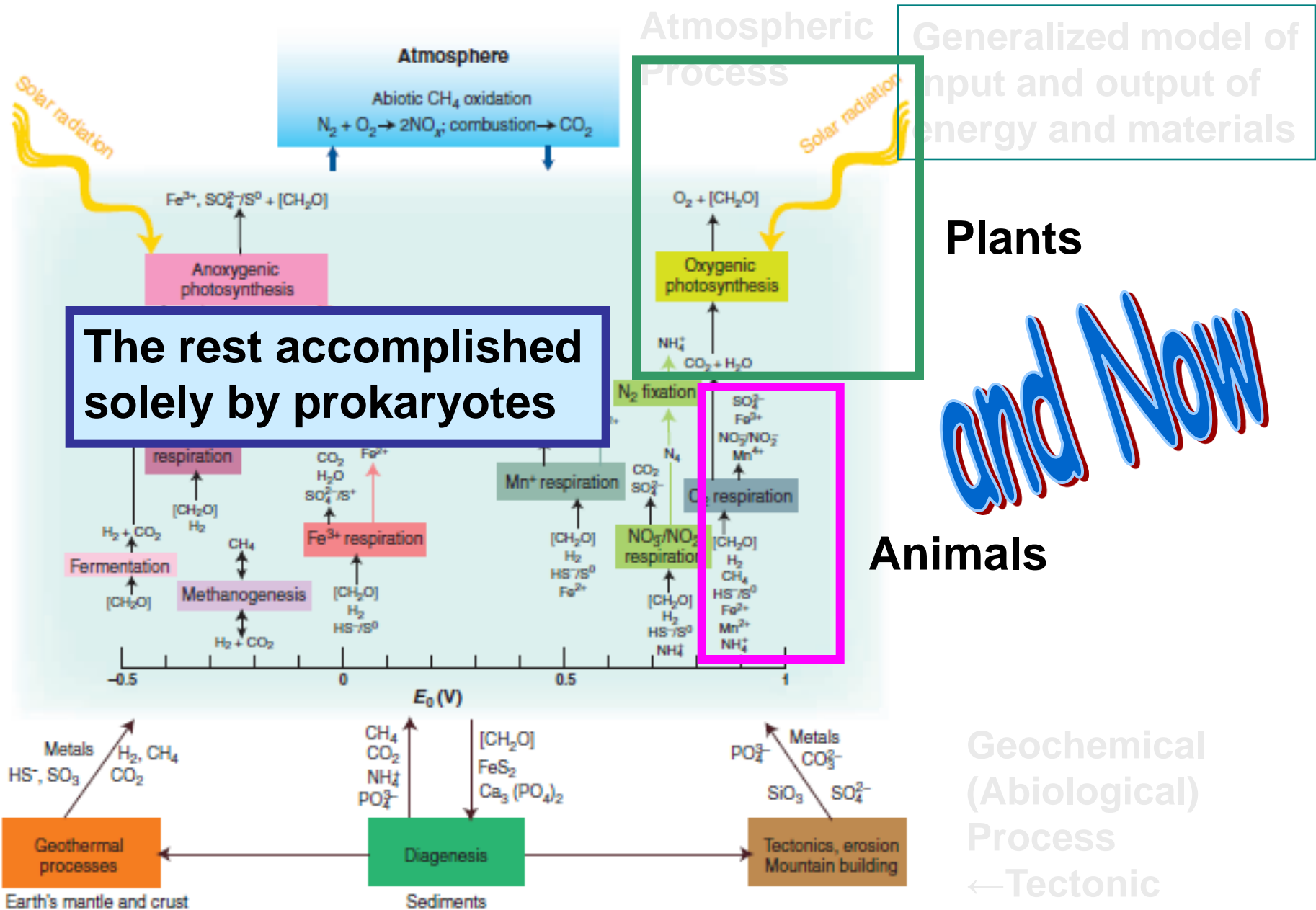
Generalized model of input and output of energy and materials



Before plants and animals appeared, all were done by prokaryotes

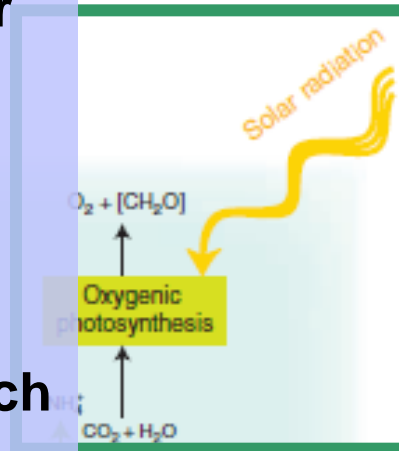
⇒ We guess, more or less all the genes involved had been “invented” at early stages of evolution.

(Abiological) Process
← Tectonic



1. Microbes had established major element cycle systems before plants and animals appeared
2. Core sets of genes responsible for element cycles had evolved at early stage of the history and probably have not changed much since then.
3. Gene transfer among prokaryotes are playing important roles in evolution of prokaryotes
4. New comer (=eukaryotes) take only a part of total metabolic process on the planet.

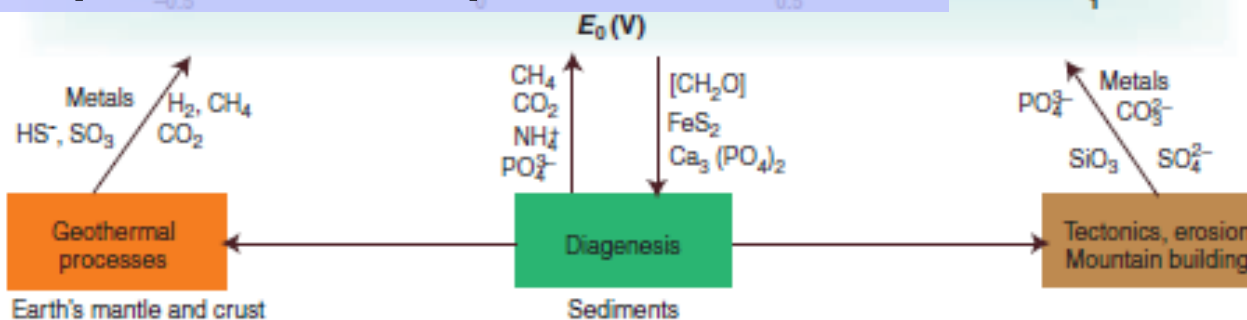
Generalized model of input and output of energy and materials



Plants

and Now

Animals



Conclusion:

- We still have technical problem to clarify bacterial biodiversity in nature.
- By applying new molecular techniques, we continue to find new groups that have never been recovered by culture techniques.
- There should be many more unknown bacteria that are still waiting to be described.
- The genetic approach should clarify the function and evolution of each bacterial group



Thank you