Introductions

- Your name your major, and what drew you to apply to this course?
- Have you had molecular lab experience before? e.g. PCR, DNA extraction, sequencing?
- What about command-line / programming experience?
- What do you hope to get out of *Native Microbiota*?
- What do you think will be the biggest challenge for you in this course?
- Tell us one interesting fact about yourself, such as:
    - Your favourite experience in your degree so far
    - A hobby or pastime that is related to microbiology
    - Your favourite microbe

Lecture 1: *Native Microbiota* origin story, and a brief introduction to metabarcoding

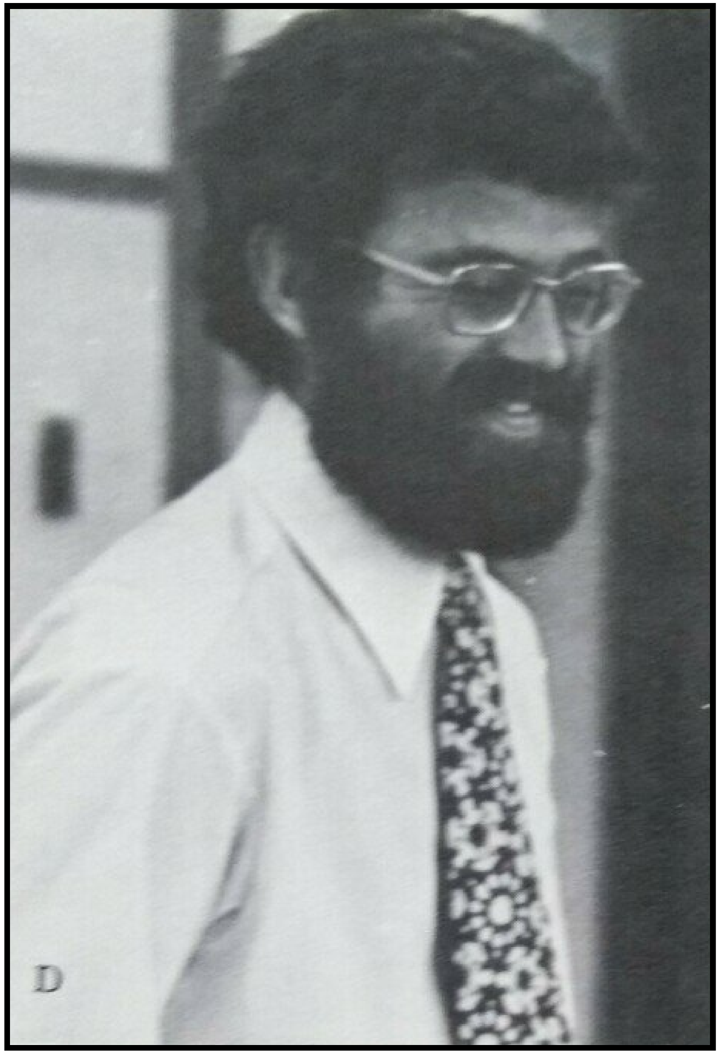
Biology 398, Exploring Native Microbiota

Jan 9th, 2024

Roadmap

1. Dedication, motivation, and my experience
2. Self-directed learning and microbiology
3. How has cheap DNA sequencing revolutionized microbiology and biology in general?
4. Why do we focus on rRNA?
5. Betsey's perspective on microbiology
6. Notes on timing of sampling, analysis, and having backups
7. Syllabus overview

Inspiration, and a dedication...

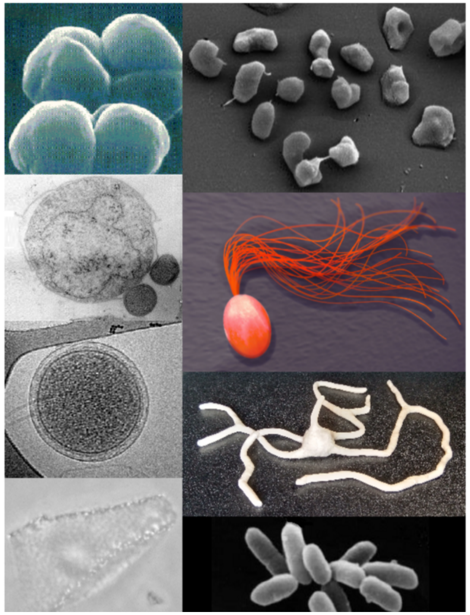


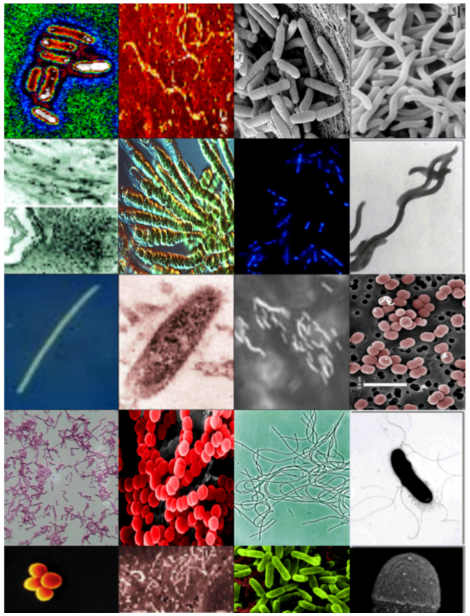
My late undergrad mentor
Dr. Bob Thompson

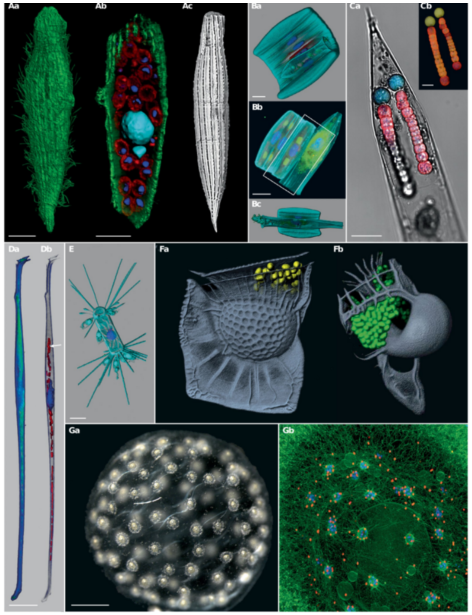
- My all-time favourite course at MtA was called *Native Flora*
- Taught by Bob Thompson, it involved collecting botanical samples *wherever* and *whenever* I wanted!
- We were evaluated on the quality of our herbarium and accuracy of identifications
- *It was so much fun, especially for me as a stubborn, headstrong student who was bored by class...*
- This course is my attempt to replicate some of the same fun, with microbes

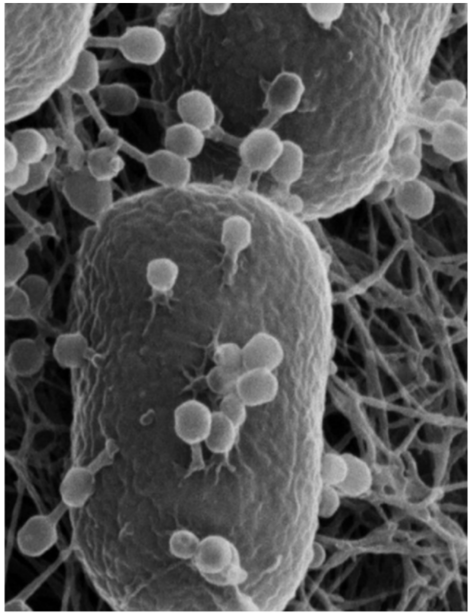
To explore brave new worlds...

The microbial world is the "final frontier" in Biology





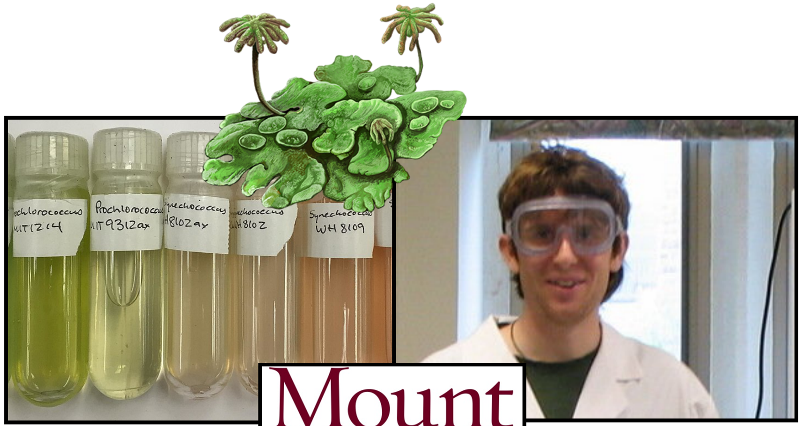




I believe that we learn best when curiosity drives our learning

- You choose the environment, take your own samples - *real samples!*
- You get to sequence 6 samples with metabarcoding - *real data!*
- You will interpret the results yourself, and identify an organism to study further, and present your plan to the class - *real research!*

Who am I and why am I teaching this course?



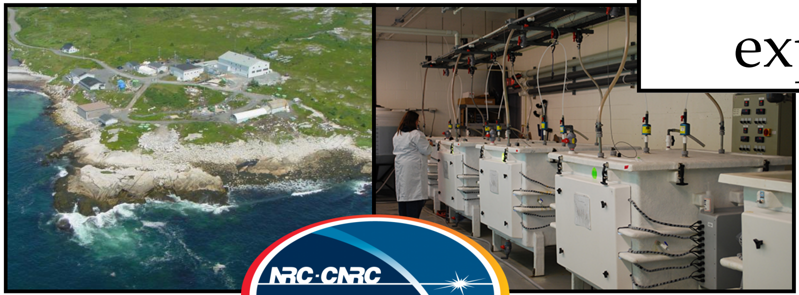
Mount Allison UNIVERSITY

BSc, Biology 2003-2008

MIT WHOI
JOINT PROGRAM IN OCEANOGRAPHY/APPLIED OCEAN SCIENCE & ENGINEERING

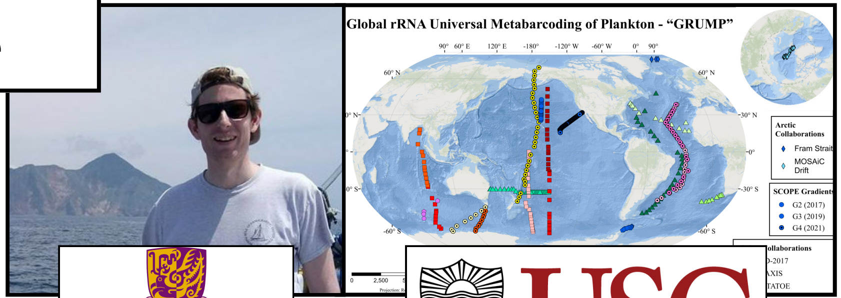
PhD Biological Oceanography, 2011-2016

This course is a synthesis of my scientific / life experience



NRC·CNRC Canada

2009-2011 NRC (Algal Biofuels, Halifax)



香港中文大學
The Chinese University of Hong Kong

USC

Postdocs CUHK, USC, 2017-2023

This is my first time running the course...

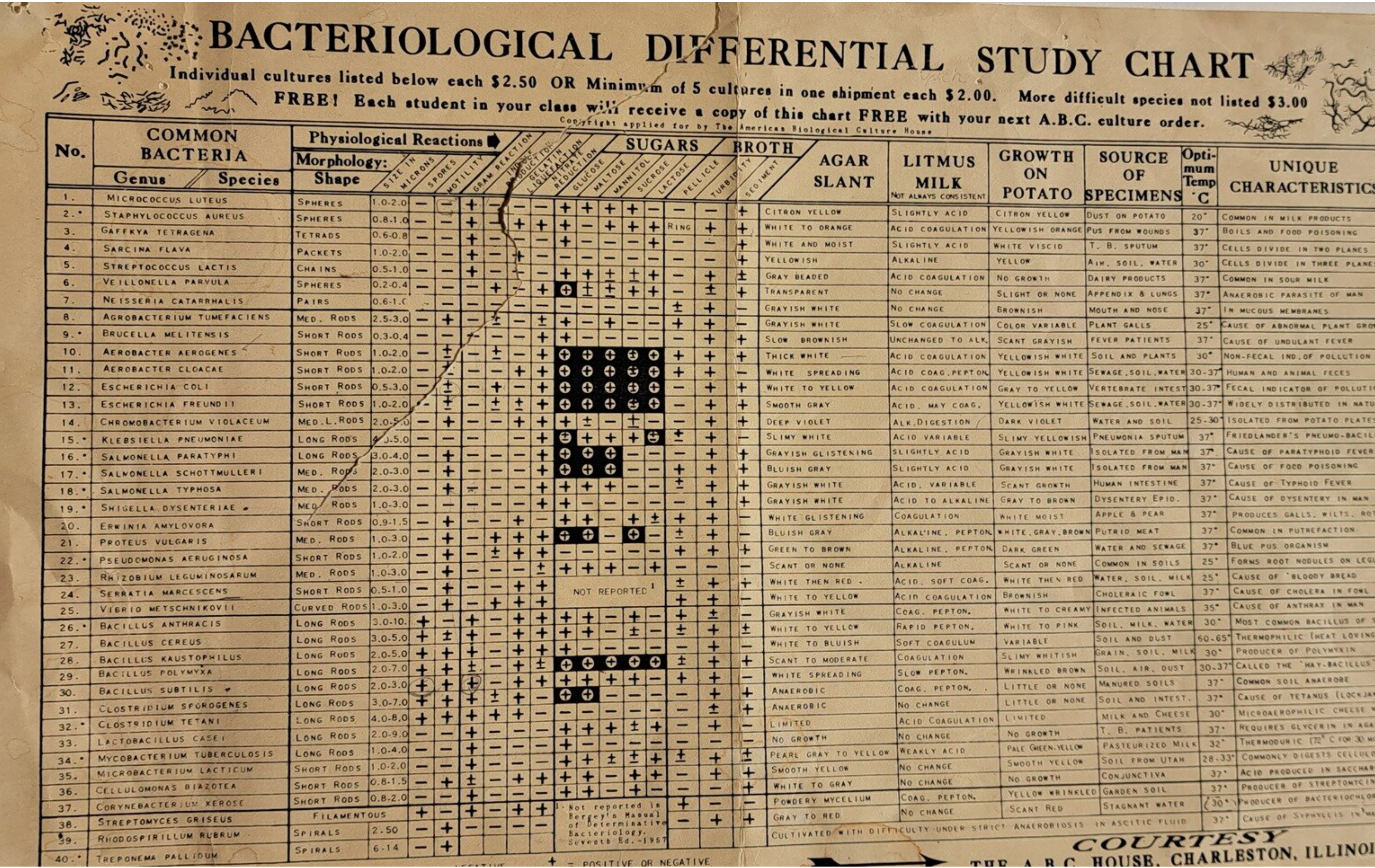
It may feel slightly chaotic at times, but I'm confident we can pull it off together!

Time for some background...

What did microbiology look like 100 years ago, in 1923? This was before:

- *Discovery of the structure of DNA (1953)*
- *Electron microscopes (1930s)*

Classical bacteriology



BACTERIOLOGICAL DIFFERENTIAL STUDY CHART

Individual cultures listed below each $2.50 OR Minimum of 5 cultures in one shipment each $2.00. More difficult species not listed $3.00

FREE! Each student in your class will receive a copy of this chart FREE with your next A.B.C. culture order.

No.	Common Bacteria (Genus / Species)	Morphology: Shape	Size in Microns	Spores	Motility	Gram Reaction	Indole Production	Gelatin Liquefaction	Nitrate Reduction	Sugars: Glucose	Sugars: Maltose	Sugars: Mannitol	Sugars: Sucrose	Sugars: Lactose	Broth: Pellicle	Broth: Turbidity	Broth: Sediment
1.	Micrococcus luteus	Spheres	1.0-2.0	−	−	+	−	−	−	+	+	+	+	−	−	−	+
2.•	Staphylococcus aureus	Spheres	0.8-1.0	−	−	+	−	+	+	+	−	+	+	+	Ring	+	+
3.	Gaffkya tetragena	Tetrads	0.6-0.8	−	−	+	−	−	−	+	−	−	−	+	−	−	+
4.	Sarcina flava	Packets	1.0-2.0	−	−	+	−	+	−	−	−	−	−	−	−	−	+
5.	Streptococcus lactis	Chains	0.5-1.0	−	−	+	−	−	−	+	+	±	±	+	−	+	±
6.	Veillonella parvula	Spheres	0.2-0.4	−	−	−	+	−	+	⊕	±	±	+	+	−	±	+
7.	Neisseria catarrhalis	Pairs	0.6-1.[illegible]	−	−	−	−	−	−	−	−	−	−	−	±	+	−
8.	Agrobacterium tumefaciens	Med. Rods	2.5-3.0	−	+	−	±	−	±	+	−	+	−	−	+	+	−
9.•	Brucella melitensis	Short Rods	0.3-0.4	−	−	−	−	−	+	+	−	−	−	−	−	+	+
10.	Aerobacter aerogenes	Short Rods	1.0-2.0	−	±	−	±	−	+	⊕	⊕	⊕	⊕	⊕	+	+	+
11.	Aerobacter cloacae	Short Rods	1.0-2.0	−	±	−	−	+	+	⊕	⊕	⊕	⊕	⊕	+	+	−
12.	Escherichia coli	Short Rods	0.5-3.0	−	±	−	+	−	+	⊕	⊕	⊕	⊕	⊕	−	+	+
13.	Escherichia freundii	Short Rods	1.0-2.0	−	±	−	±	±	+	⊕	⊕	⊕	⊕	⊕	−	+	+
14.	Chromobacterium violaceum	Med. L. Rods	2.0-5.0	−	+	−	−	+	+	+	±	−	±	−	−	+	+
15.•	Klebsiella pneumoniae	Long Rods	4.0-5.0	−	−	−		−	+	⊕	+	+	+	⊕	±	+	−
16.•	Salmonella paratyphi	Long Rods	3.0-4.0	−	+	−	−	−	+	⊕	⊕	⊕	−	−	−	+	+
17.•	Salmonella schottmulleri	Med. Rods	2.0-3.0	−	+	−	−	−	+	⊕	⊕	⊕	−	−	+	+	+
18.•	Salmonella typhosa	Med. Rods	2.0-3.0	−	+	−	−	−	+	+	+	+	−	−	±	+	+
19.•	Shigella dysenteriae	Med. Rods	1.0-3.0	−	−	−	−	−	+	+	−	−	−	−	−	+	+
20.	Erwinia amylovora	Short Rods	0.9-1.5	−	+	−	−	+	−	+	+	−	+	±	+	+	−
21.	Proteus vulgaris	Med. Rods	1.0-3.0	−	+	−	+	+	+	⊕	⊕	−	⊕	−	±	+	−
22.•	Pseudomonas aeruginosa	Short Rods	1.0-2.0	−	+	−	±	+	+	−	−	−	−	−	+	+	+
23.	Rhizobium leguminosarum	Med. Rods	1.0-3.0	−	+	−	−	−	±	+	+	+	−	+	−	−	−
24.	Serratia marcescens	Short Rods	0.5-1.0	−	+	−	−	+	+	NOT REPORTED[1]					±	+	+
25.	Vibrio metschnikovii	Curved Rods	1.0-3.0	−	+	−	+	+	+						+	+	−
26.•	Bacillus anthracis	Long Rods	3.0-10.	+	−	+	−	+	+	+	+	−	+	−	+	±	−
27.	Bacillus cereus	Long Rods	3.0-5.0	+	±	+	−	+	+	+	+	−	±	−	±	+	±
28.	Bacillus kaustophilus	Long Rods	2.0-5.0	+	+	+	−	+	+	+	−	−	−	−	−	+	−
29.	Bacillus polymyxa	Long Rods	2.0-7.0	+	+	±	−	+	±	⊕	⊕	⊕	⊕	⊕	±	+	+
30.	Bacillus subtilis	Long Rods	2.0-3.0	+	+	+	−	+	+	+	+	+	+	−	+	+	−
31.	Clostridium sporogenes	Long Rods	3.0-7.0	+	+	+	±	+	−	⊕	⊕	−	−	−	−	+	+
32.•	Clostridium tetani	Long Rods	4.0-8.0	+	+	+	+	+	−	−	−	−	−	−	−	±	+
33.	Lactobacillus casei	Long Rods	2.0-9.0	−	−	+	−	−	−	+	+	+	±	+	−	+	−
34.•	Mycobacterium tuberculosis	Long Rods	1.0-4.0	−	−	+	−	−	−	+	−	−	−	−	−	−	−
35.	Microbacterium lacticum	Short Rods	1.0-2.0	−	−	+	−	−	−	+	+	±	±	+	±	+	±
36.	Cellulomonas biazotea	Short Rods	0.8-1.5	−	+	±	−	+	+	+	+	−	+	+	−	+	+
37.	Corynebacterium xerose	Short Rods	0.8-2.0	−	−	+	−	−	−	+	+	−	+	−	−	−	+
38.	Streptomyces griseus	Filamentous		+	−	+	−	+	+						+	−	−
39.	Rhodospirillum rubrum	Spirals	2-50	−	+	−	−	−	−						−	+	+
40.•	Treponema pallidum	Spirals	6-14	−	+												

1. Not reported in Bergey's Manual of Determinative Bacteriology, Seventh Ed. - 1957

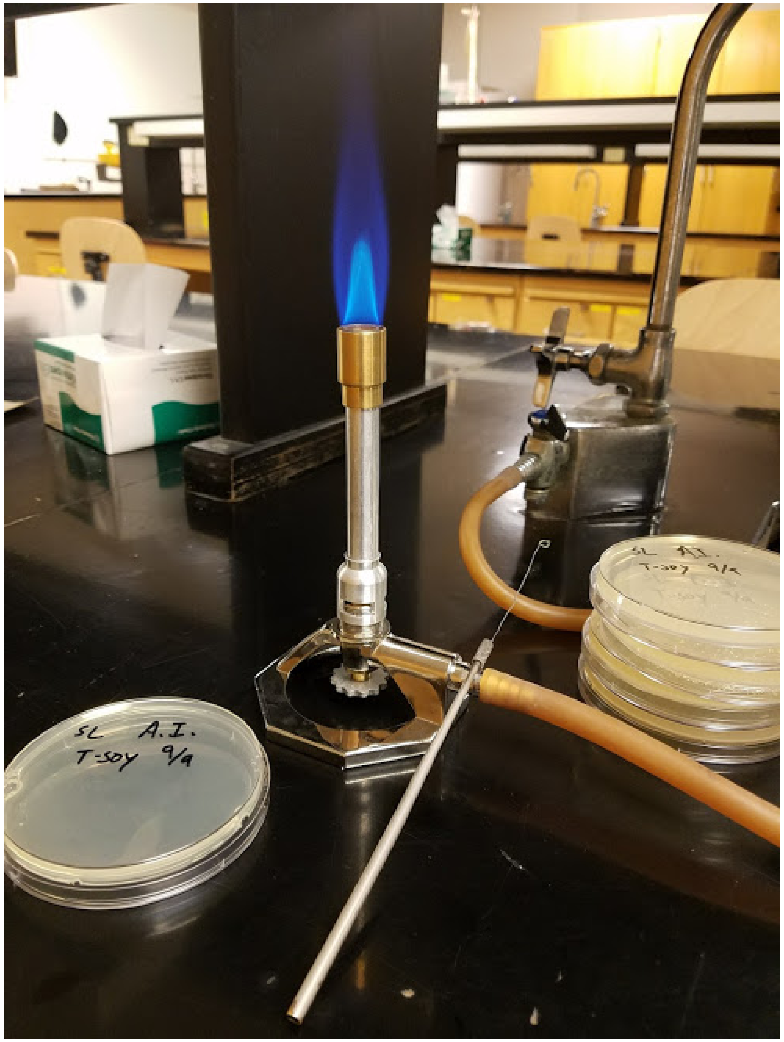
No.	Agar Slant	Litmus Milk (Not always consistent)	Growth on Potato	Source of Specimens	Optimum Temp °C	Unique Characteristics
1.	Citron yellow	Slightly acid	Citron yellow	Dust on potato	20°	Common in milk products
2.•	White to orange	Acid coagulation	Yellowish orange	Pus from wounds	37°	Boils and food poisoning
3.	White and moist	Slightly acid	White viscid	T. B. sputum	37°	Cells divide in two planes
4.	Yellowish	Alkaline	Yellow	Air, soil, water	30°	Cells divide in three planes
5.	Gray beaded	Acid coagulation	No growth	Dairy products	37°	Common in sour milk
6.	Transparent	No change	Slight or none	Appendix & lungs	37°	Anaerobic parasite of man
7.	Grayish white	No change	Brownish	Mouth and nose	37°	In mucous membranes
8.	Grayish white	Slow coagulation	Color variable	Plant galls	25°	Cause of abnormal plant gro
9.•	Slow brownish	Unchanged to alk.	Scant grayish	Fever patients	37°	Cause of undulant fever
10.	Thick white	Acid coagulation	Yellowish white	Soil and plants	30°	Non-fecal ind. of pollution
11.	White spreading	Acid coag. pepton.	Yellowish white	Sewage, soil, water	30-37°	Human and animal feces
12.	White to yellow	Acid coagulation	Gray to yellow	Vertebrate intest	30-37°	Fecal indicator of polluti
13.	Smooth gray	Acid, may coag.	Yellowish white	Sewage, soil, water	30-37°	Widely distributed in natu
14.	Deep violet	Alk. digestion	Dark violet	Water and soil	25-30°	Isolated from potato plates
15.•	Slimy white	Acid variable	Slimy yellowish	Pneumonia sputum	37°	Friedlander's pneumo-bacil
16.•	Grayish glistening	Slightly acid	Grayish white	Isolated from man	37°	Cause of paratyphoid fever
17.•	Bluish gray	Slightly acid	Grayish white	Isolated from man	37°	Cause of food poisoning
18.•	Grayish white	Acid, variable	Scant growth	Human intestine	37°	Cause of Typhoid Fever
19.•	Grayish white	Acid to alkaline	Gray to brown	Dysentery Epid.	37°	Cause of dysentery in man
20.	White glistening	Coagulation	White moist	Apple & pear	37°	Produces galls, wilts, rot
21.	Bluish gray	Alkaline, pepton.	White, gray, brown	Putrid meat	37°	Common in putrefaction
22.•	Green to brown	Alkaline, pepton.	Dark green	Water and sewage	37°	Blue pus organism
23.	Scant or none	Alkaline	Scant or none	Common in soils	25°	Forms root nodules on legu
24.	White then red	Acid, soft coag.	White then red	Water, soil, milk	25°	Cause of 'bloody bread
25.	White to yellow	Acid coagulation	Brownish	Choleraic fowl	37°	Cause of cholera in fowl
26.•	Grayish white	Coag. pepton.	White to creamy	Infected animals	35°	Cause of anthrax in man
27.	White to yellow	Rapid pepton.	White to pink	Soil, milk, water	30°	Most common bacillus of s
28.	White to bluish	Soft coagulum	Variable	Soil and dust	60-65°	Thermophilic (heat loving
29.	Scant to moderate	Coagulation	Slimy whitish	Grain, soil, milk	30°	Producer of Polymyxin
30.	White spreading	Slow pepton.	Wrinkled brown	Soil, air, dust	30-37°	Called the 'hay-bacillus'
31.	Anaerobic	Coag. pepton.	Little or none	Manured soils	37°	Common soil anaerobe
32.•	Anaerobic	No change	Little or none	Soil and intest.	37°	Cause of tetanus (lockja
33.	Limited	Acid Coagulation	Limited	Milk and Cheese	30°	Microaerophilic cheese
34.•	No growth	No change	No growth	T. B. patients	37°	Requires glycerin in aga
35.	Pearl gray to yellow	Weakly acid	Pale Green-yellow	Pasteurized Milk	32°	Thermoduric (72° C for 30 mi
36.	Smooth yellow	No change	Smooth yellow	Soil from Utah	28-33°	Commonly digests cellulo
37.	White to gray	No change	No growth	Conjunctiva	37°	Acid produced in sacchar
38.	Powdery mycelium	Coag. pepton.	Yellow wrinkled	Garden soil	37°	Producer of streptomycin
39.	Gray to red	No change	Scant Red	Stagnant water	(30°)	Producer of bacteriochlor
40.•	Cultivated with difficulty under strict anaerobiosis in ascitic fluid				37°	Cause of Syphilis in ma

NEGATIVE ± = POSITIVE OR NEGATIVE

COURTESY

THE A.B.C. HOUSE, CHARLESTON, ILLINOI

Classical Microbiology: Cultivation-*dependent*



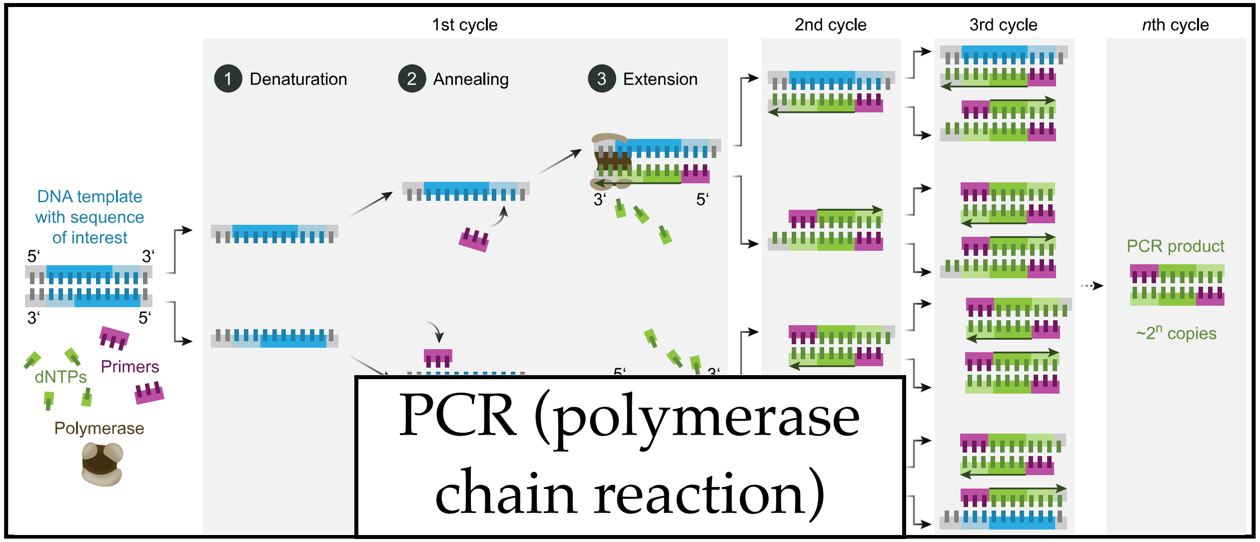
Classical Microbiology: Cultivation-*dependent*

What are the advantages and limitations of cultivation-based approaches?

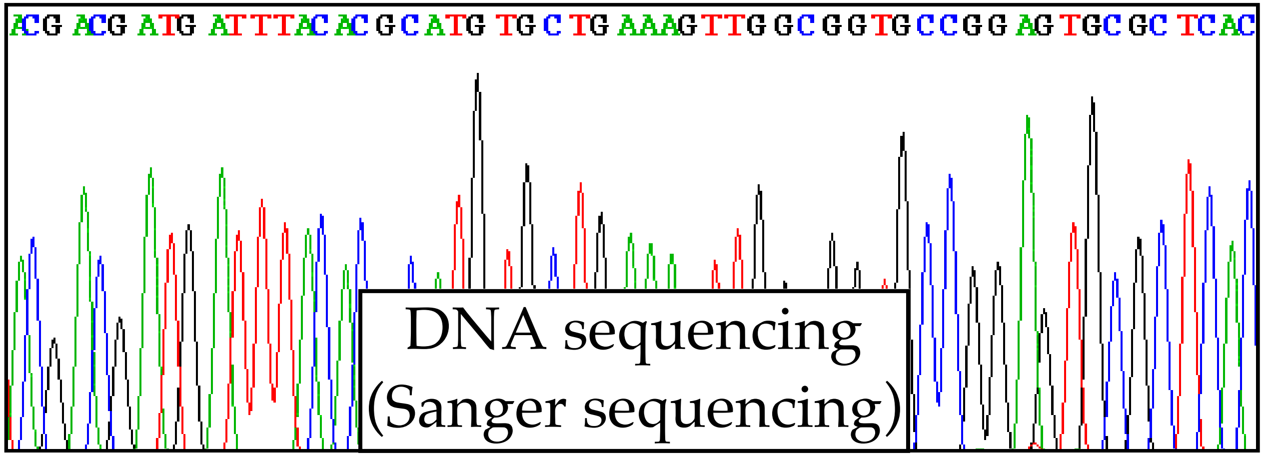
Seagrass microbes: journal.pone.0236135

Modern Microbiology: Cultivation-*independent*

These 2 technologies were the keys to unlock the molecular age (*Hint: one is something we heard a lot about during COVID lockdowns…)*?

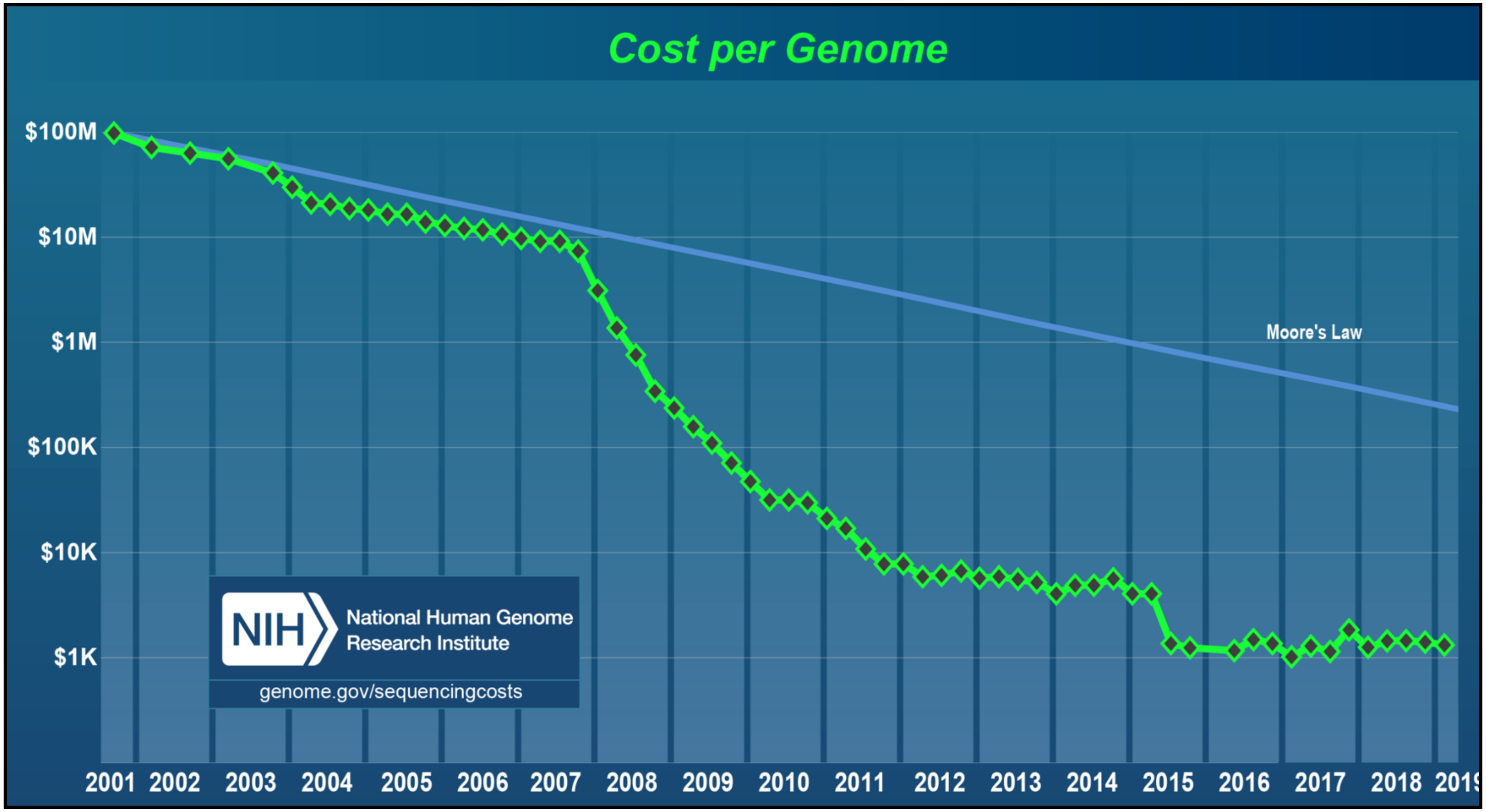


PCR (polymerase chain reaction)



DNA sequencing (Sanger sequencing)

Modern Microbiology: The age of '*omics*

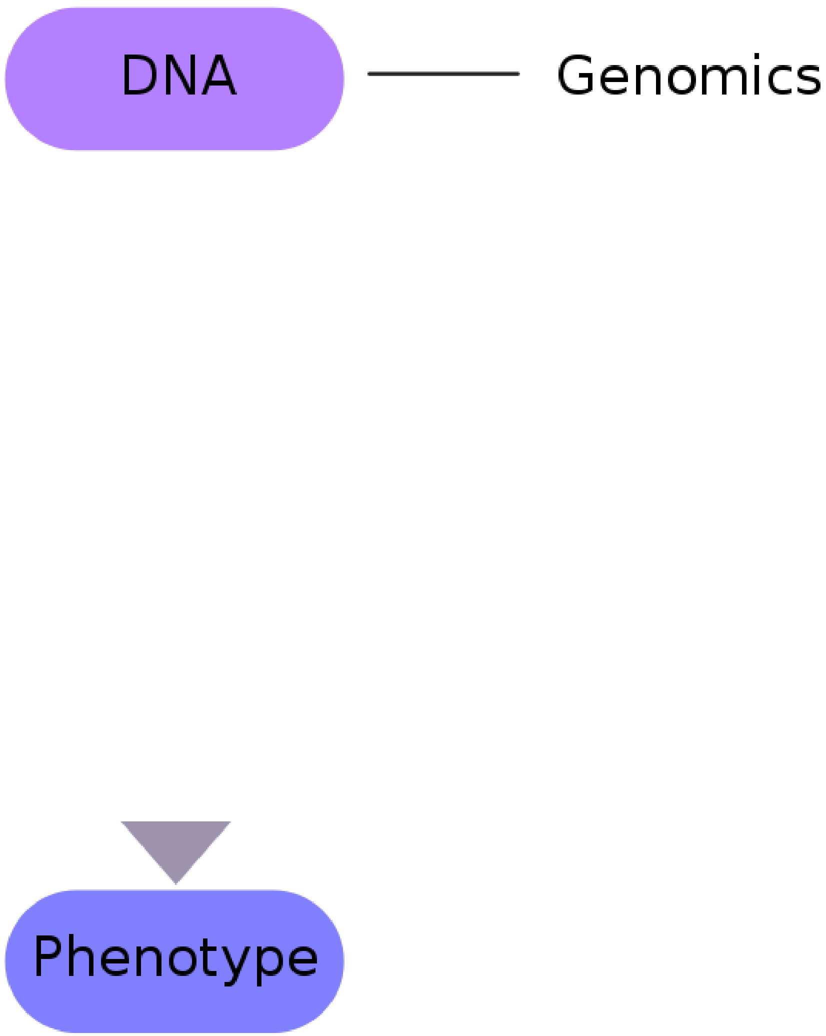


Early sequencing

"Next Generation Sequencing" (NGS)

"3rd generation" sequencing

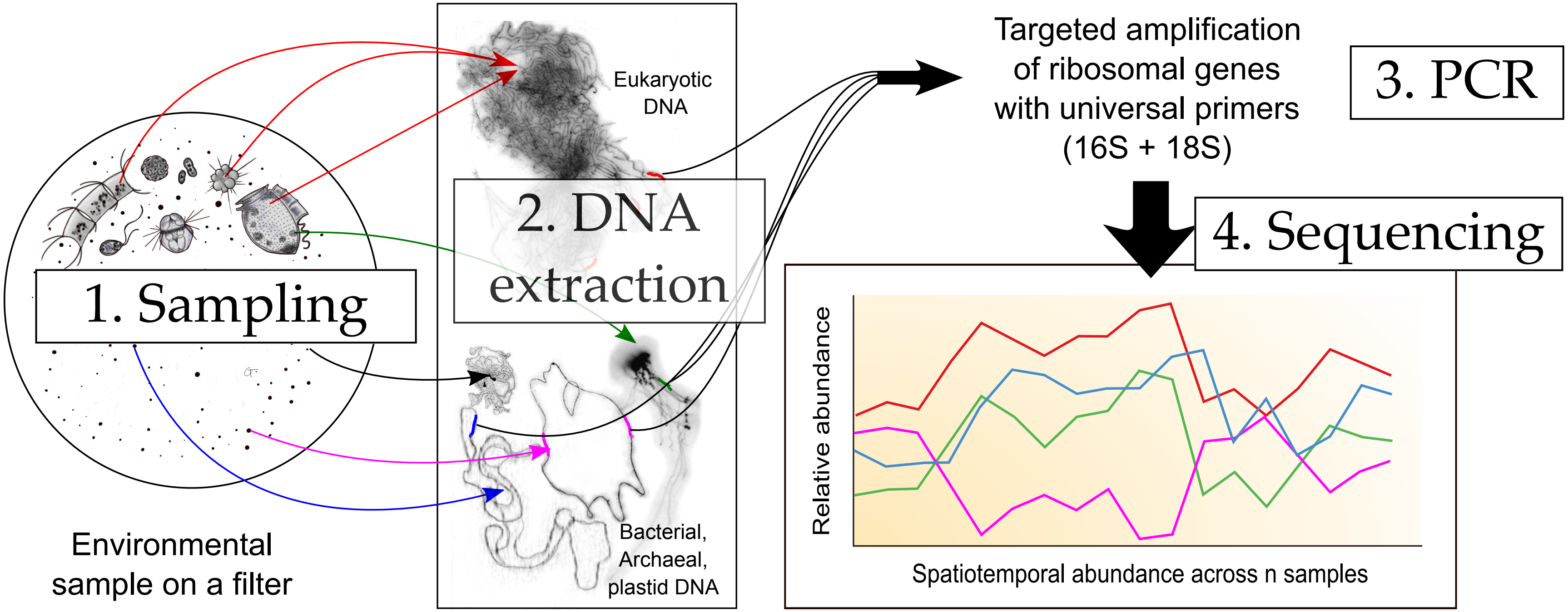
Modern Microbiology: The age of '*omics*



Add a "meta" if you're working with communities... e.g. "metagenomics", "metatranscriptomics"

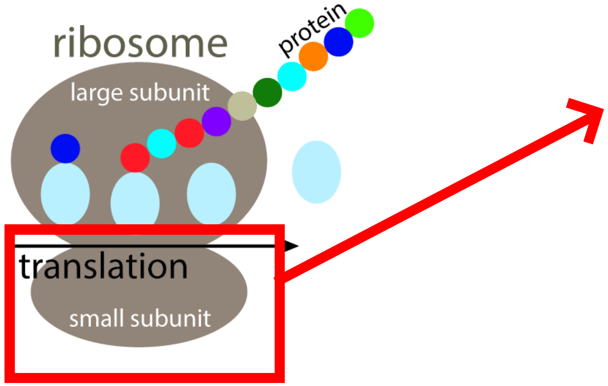
What is metabarcoding?

- ***Metabarcoding*** measures abundance of microbial life across space / time by targeting rRNA "barcodes"
- PCR + sequencing of rRNA from environmental samples
- Allows a "census" of ecosystems ***without cultivation***

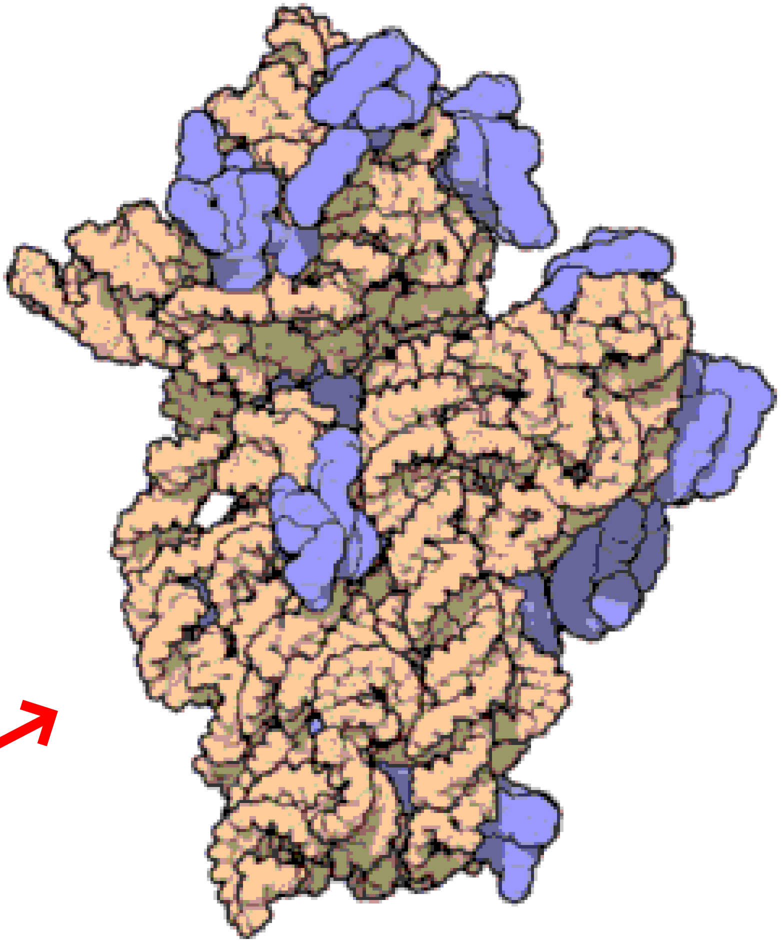


Microbe art: @claudia_traboni

Why use rRNA?



SSU* rRNA

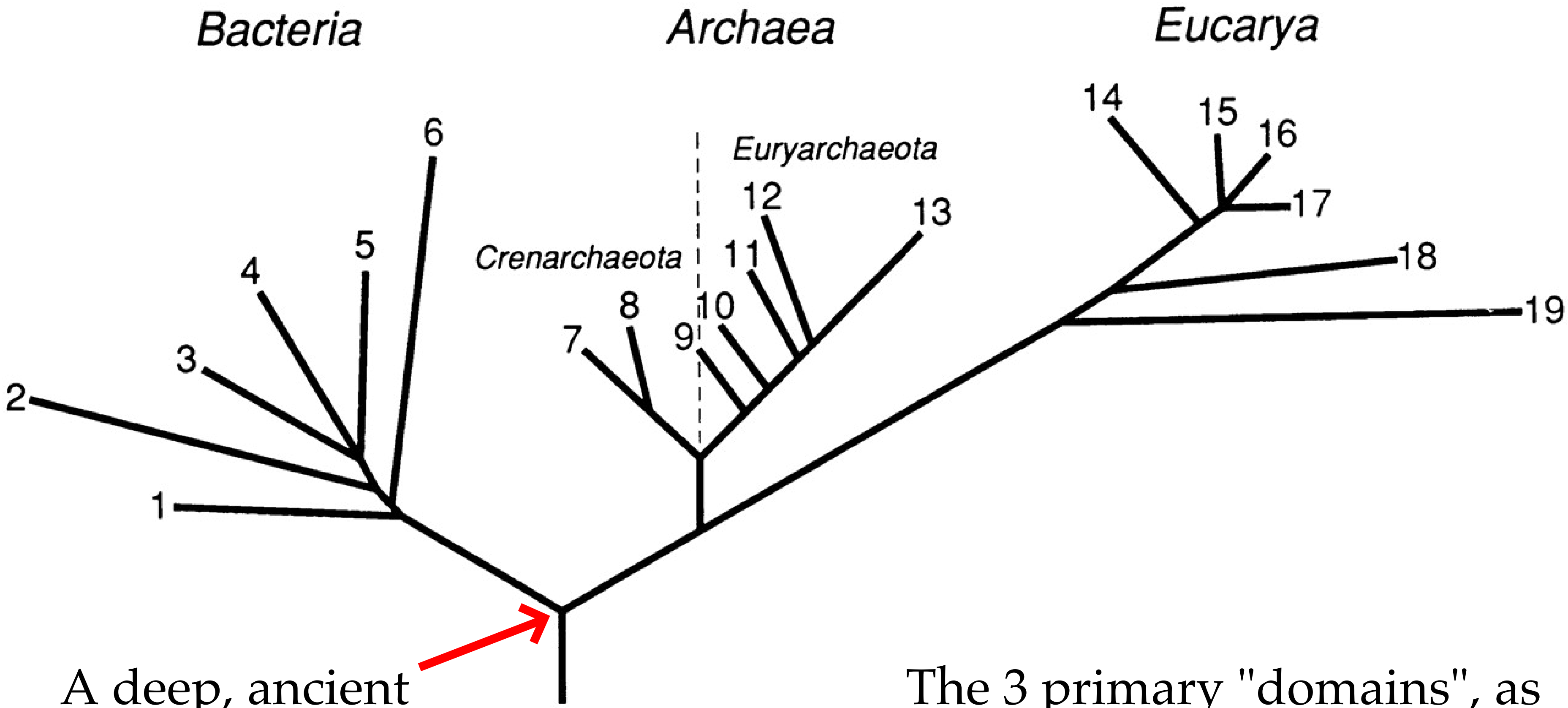


What is ribosomal RNA (rRNA)?

Discussion question: why would rRNA be a good choice for phylogeny / barcoding? What are the advantages? Think about function, structure, distribution...

*SSU=Small Subunit

rRNA → Discovery of *Archaea*



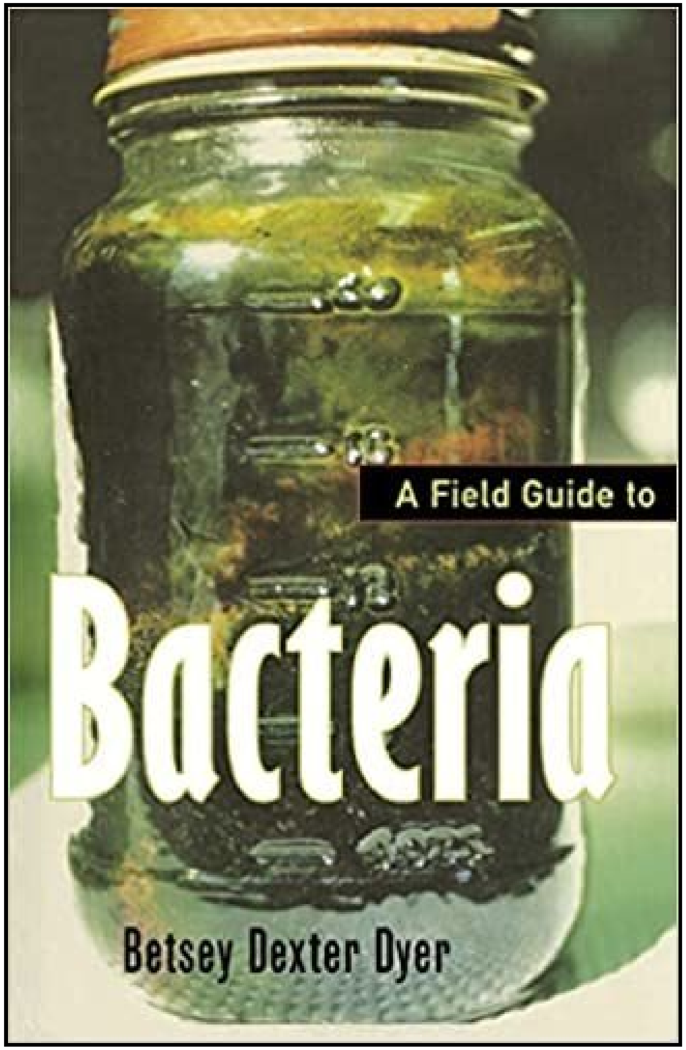
A deep, ancient divergence

The 3 primary "domains", as determined from rRNA

This was discovered before DNA sequencing (1977) and PCR (1983) came into wide use!

What can we do with this tool?

Betsey's perspective...



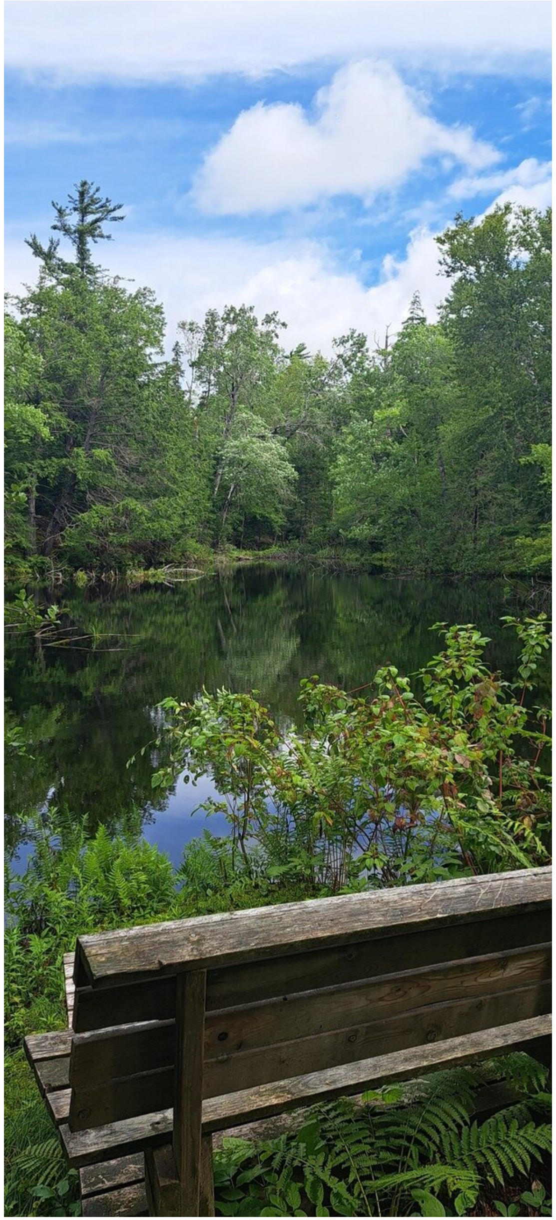
Although their individual cells are invisible, microbial life gives macroscopic hints to its existence:

- Smells
- Colours
- Physical and chemical parameters
  - Fresh vs. saline
  - Oxic vs. anoxic
  - Acidic vs. alkaline

So many local environments to explore

Salt marsh, Jimtown beach

So many local environments to explore



Freshwater on Contemplative Trail

So many local environments to explore

An estuary near Boy Scout beach

Your task in this course

- Take samples of environmental microorganisms from an environment of your choice
- Extract DNA from these samples, amplify SSU rRNA “metabarcodes”, and interpret data
- Choose an organism of interest (OOI) from your environmental sample and present a plan for:
  - Cultivation of your OOI using a media recipe suited to environmental conditions ***OR***
  - Characterizing your OOI's metabolism using fluorescence in situ hybridization with isotope-labelling experiments ***OR***
  - Characterizing the organism's genomic potential using one of the many modern 'omics-enabled techniques ***OR***
  - Another topic chosen in consultation with the course instructor, based on course material or your own research

An ambitious timeline

We need to work quickly to collect samples, extract DNA, and amplify them so we have actual data to play with!

- Weeks 1 and 2 = sampling preparation, field trip (weather permitting)
- Week 3 = self-directed sampling
- Weeks 4, 5, 6 = DNA extraction, PCR
- Week 7 = winter break spillover
- All of us need to succeed in order to send samples for sequencing!

Evaluations

Date	Type of Evaluation	Weight
Throughout semester	Class participation	10%
February 26th	Environmental sampling report	20%
March 28th	DNA microbial community analysis report & OOI proposal**	40%
End of semester	Laboratory record-keeping, findability of data / freezer samples	10%
End of semester	Final class presentation	20%

Class participation

The lab

Weekly schedule

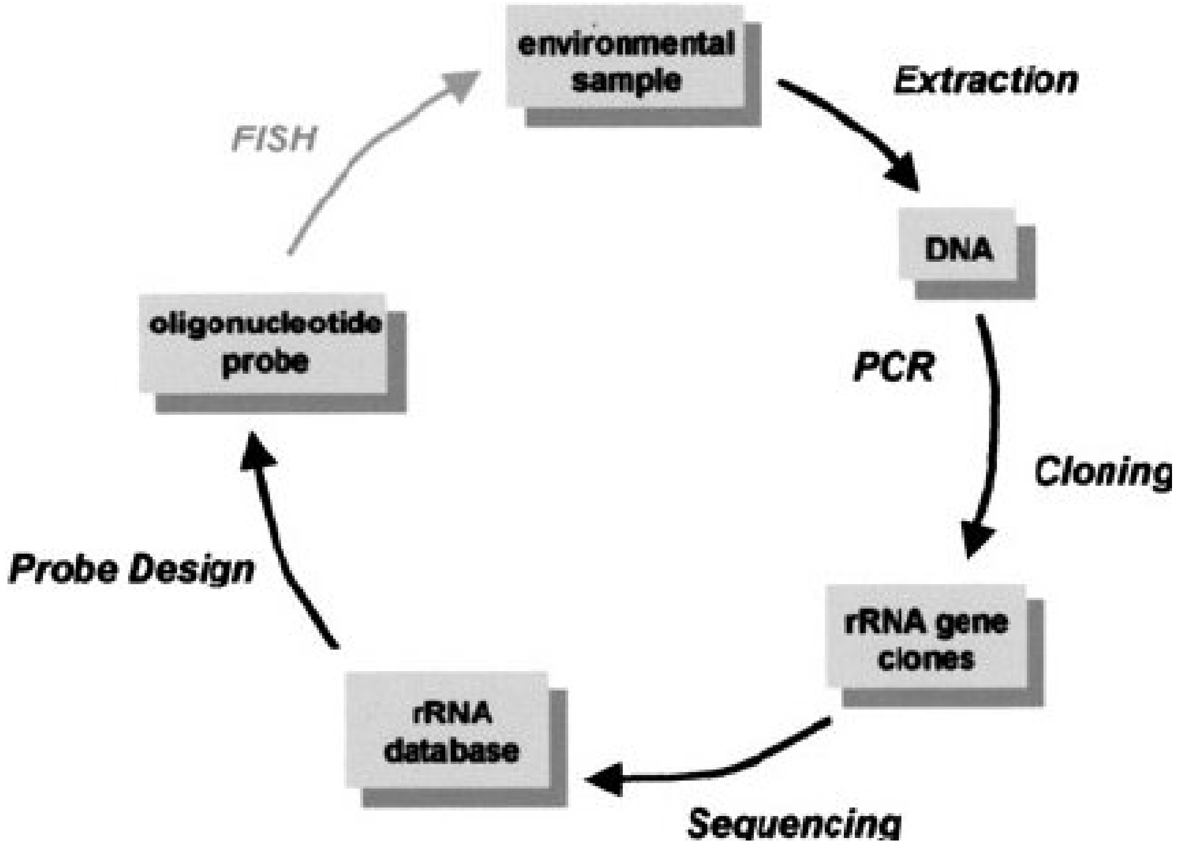
Dr. McNichol, Winter Semester 2024

Time / period	Monday	Tuesday	Wednesday	Thursday	Friday
8:30-9:30		Bio398 (JBB 236)			
9:30-10:30		Bio398 office hours			Bio398 (JBB 236)
10:30-11:30			Bio398 (JBB 236)		
11:30-12:30		Bio115 (SCHW 110)	Dept seminar slot	Bio398 office hours	
12:30-1:30	Dept seminar slot				Bio115 (SCHW 110)
1:30-2:30		Bio115 office hours	Bio115 (SCHW 110)	Dept seminar slot	Bio115 office hours
2:30-3:30			Bio115 office hours	Bio398 Laboratory (JBB 408)	
3:30-4:30					
4:30-5:30					

Supplemental lab session?

Field safety

Next classes



Amann *et al.*, 1995

Next Class: Full-cycle rRNA analysis, and an introduction to FISH

Lab: This Thursday - environmental sampling preparation

Notes: Will post readings for Friday later today; will be asking for