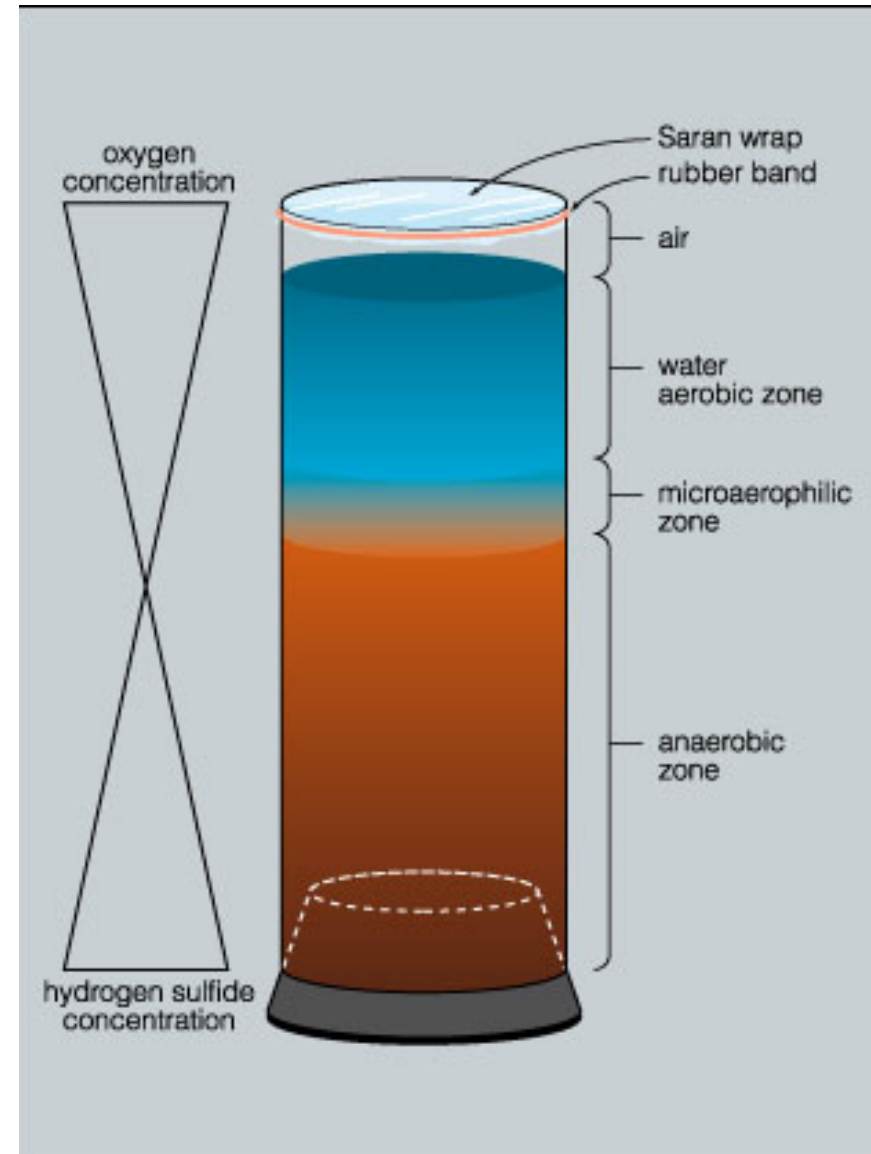


Day 3

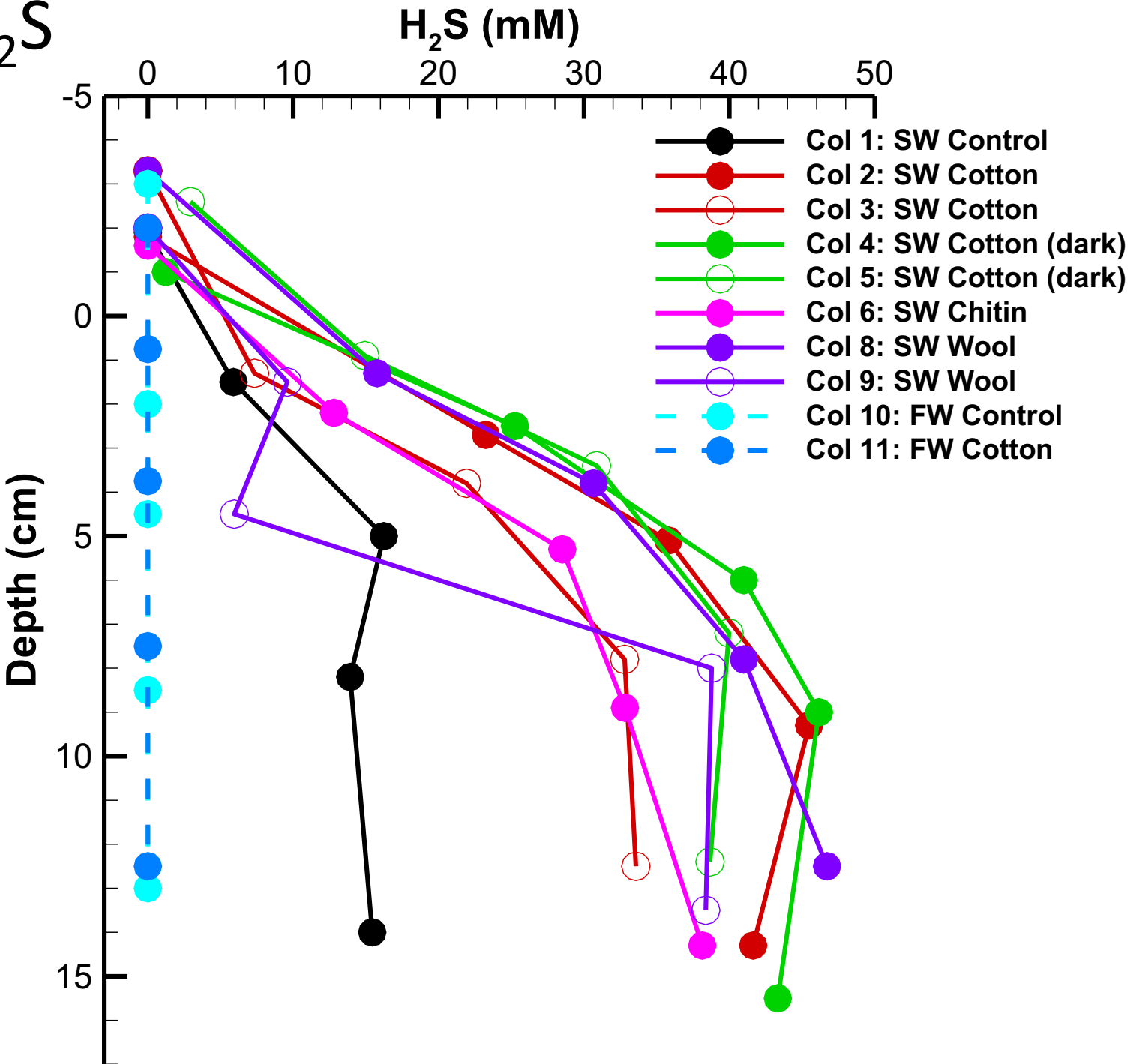
- Examine gels from PCR
- Learn about more molecular methods in microbial ecology

Genes We Targeted

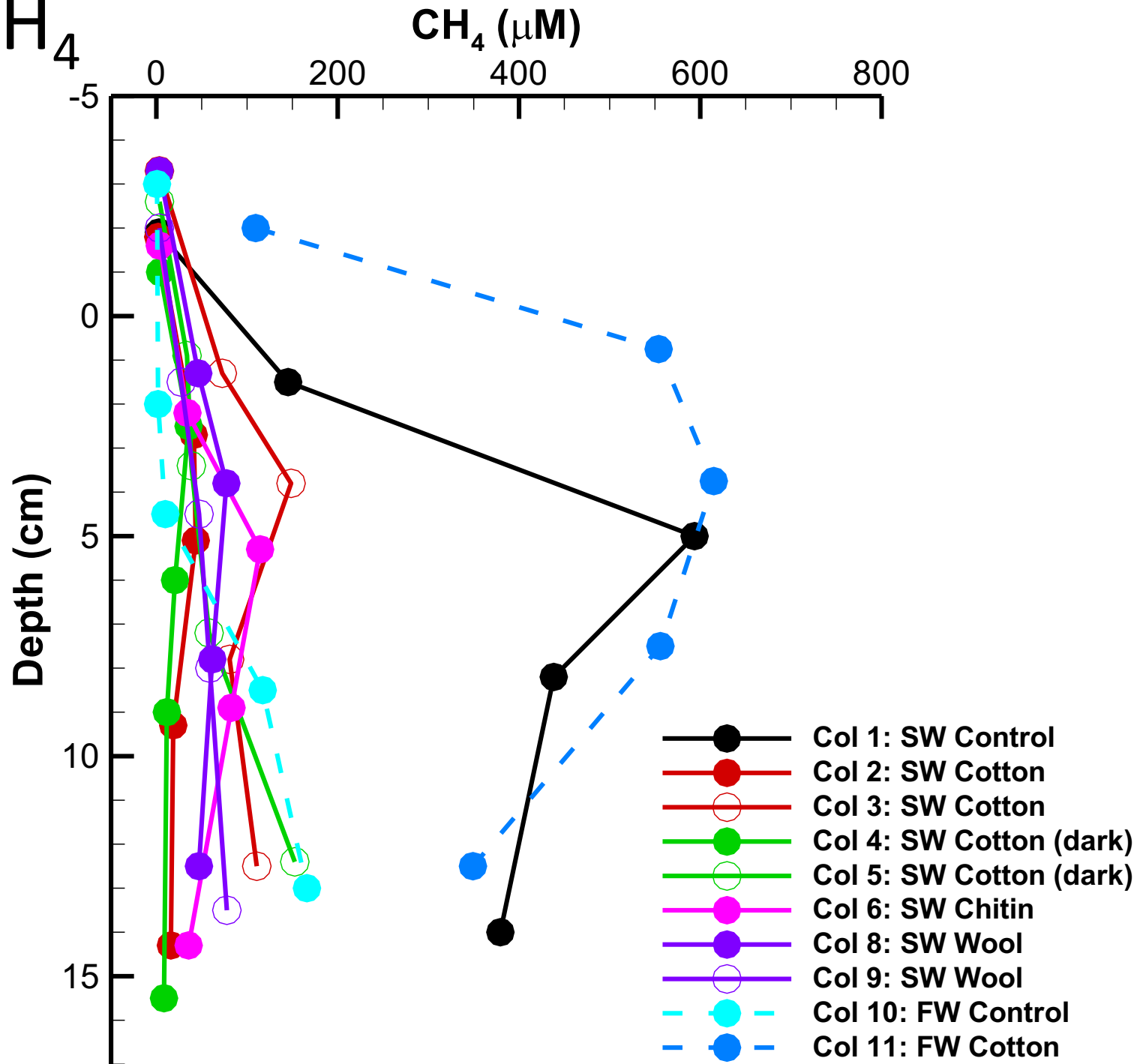
- 1: dsrAB 1800bp
- 2: mcrA 750bp
- 3: Bacteria 1450bp
- 4: Archaea 950bp
- 5: Archaea + 950bp
- 6: Negative control

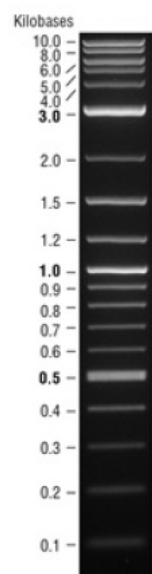
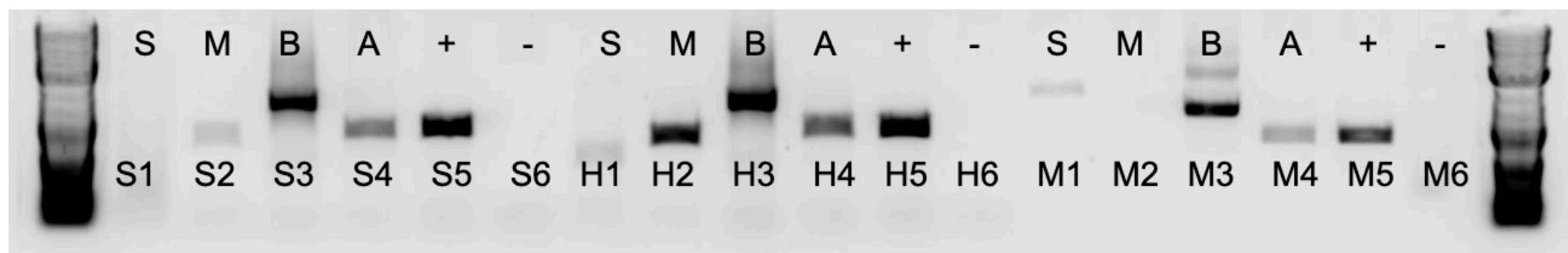
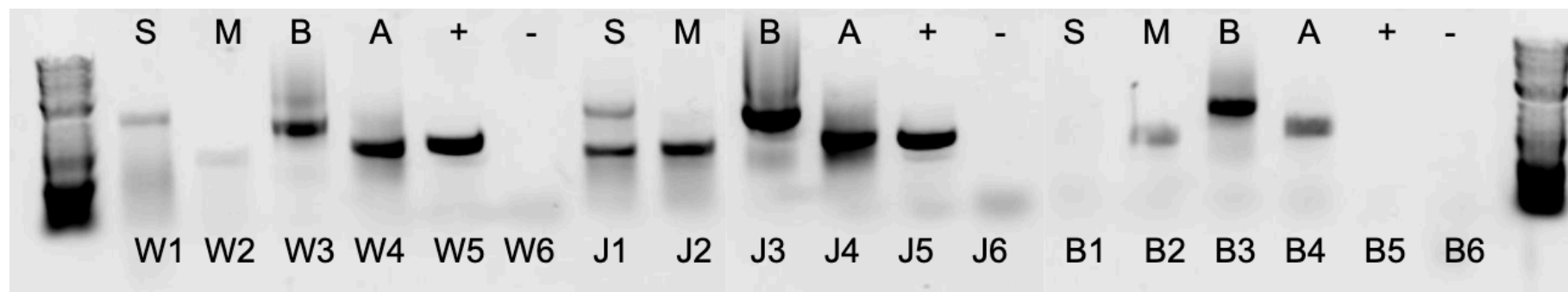


2018 H₂S



2018 CH₄





- 1: dsrAB 1800bp
- 2: mcrA 750bp
- 3: Bacteria 1450bp
- 4: Archaea 950bp
- 5: Archaea + 950bp
- 6: Negative control

1: dsrAB 1800bp

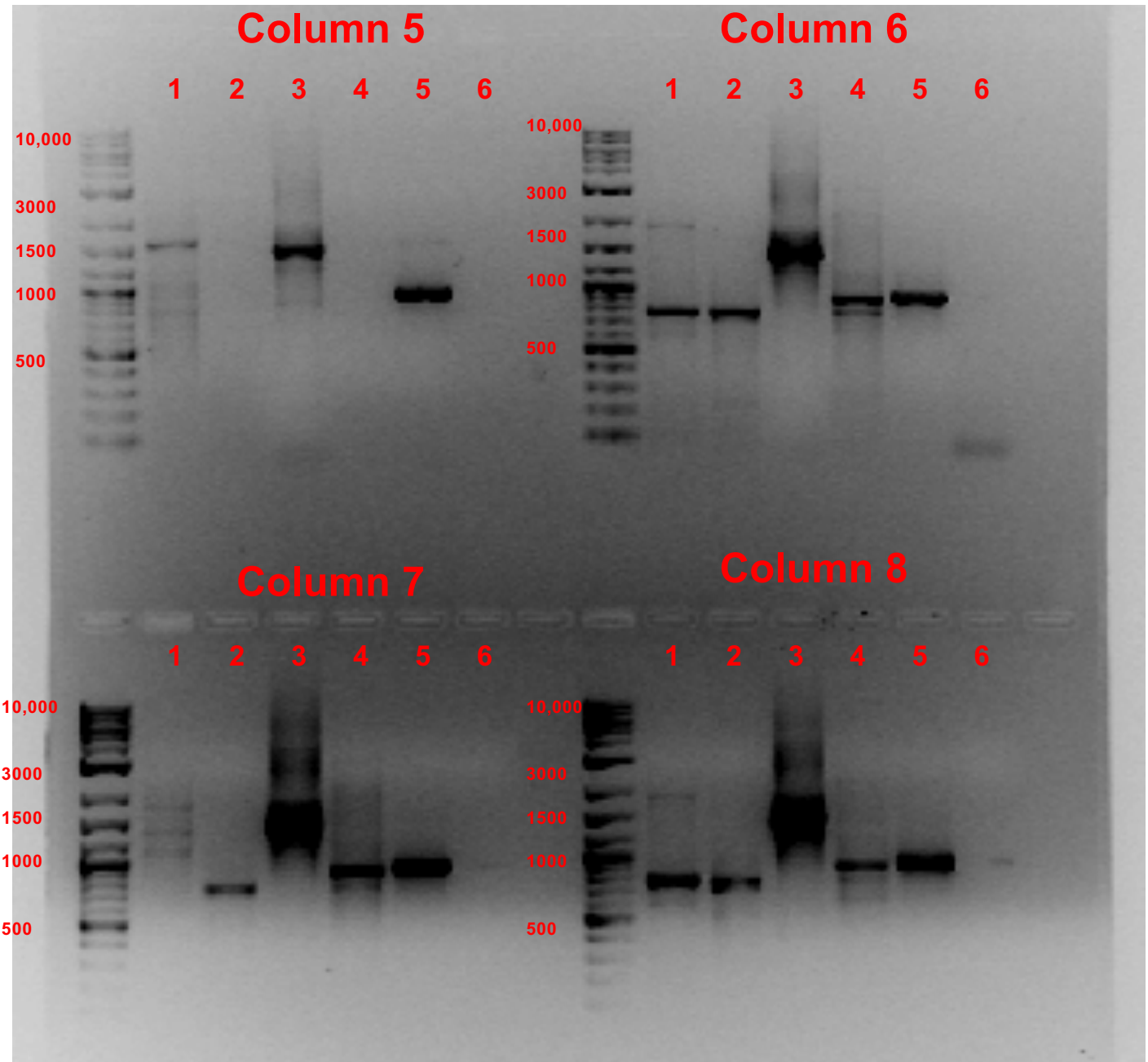
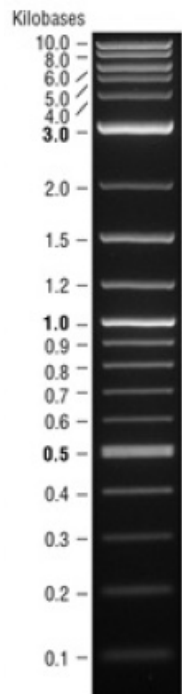
2: mcrA 750bp

3: Bacteria 1450bp

4: Archaea 950bp

5: Archaea + 950bp

6: Negative control



Some Problems with PCR

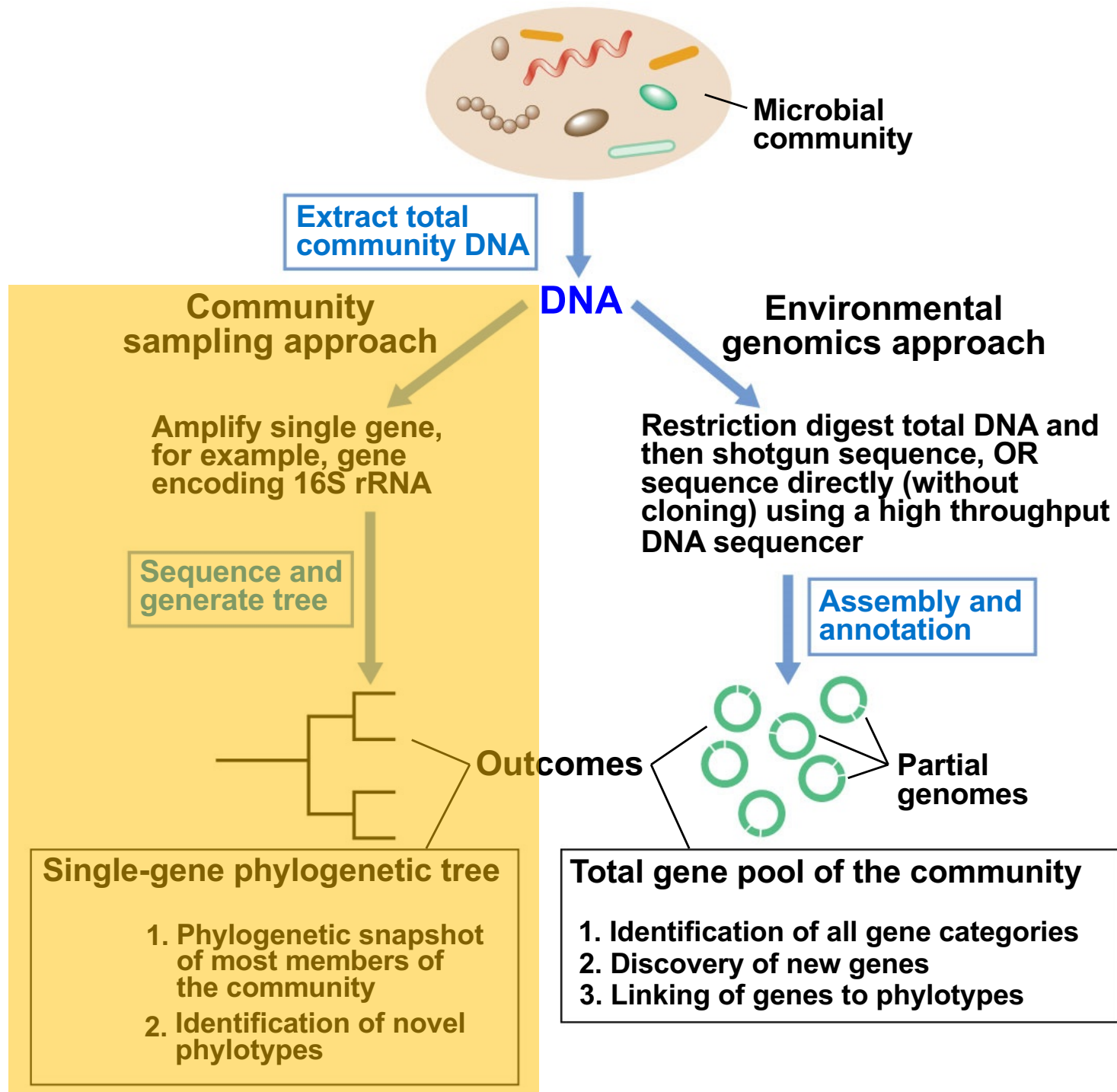
- Inhibitors in template DNA
- Amplification bias
- Gene copy number
- Limited by primer design
- Differential denaturation efficiency
- Chimeric PCR products may form
- Contamination w/ non-target DNA
- Potentially low sensitivity and resolution
- General screw-ups

(Some) Problems with Molecular Methods

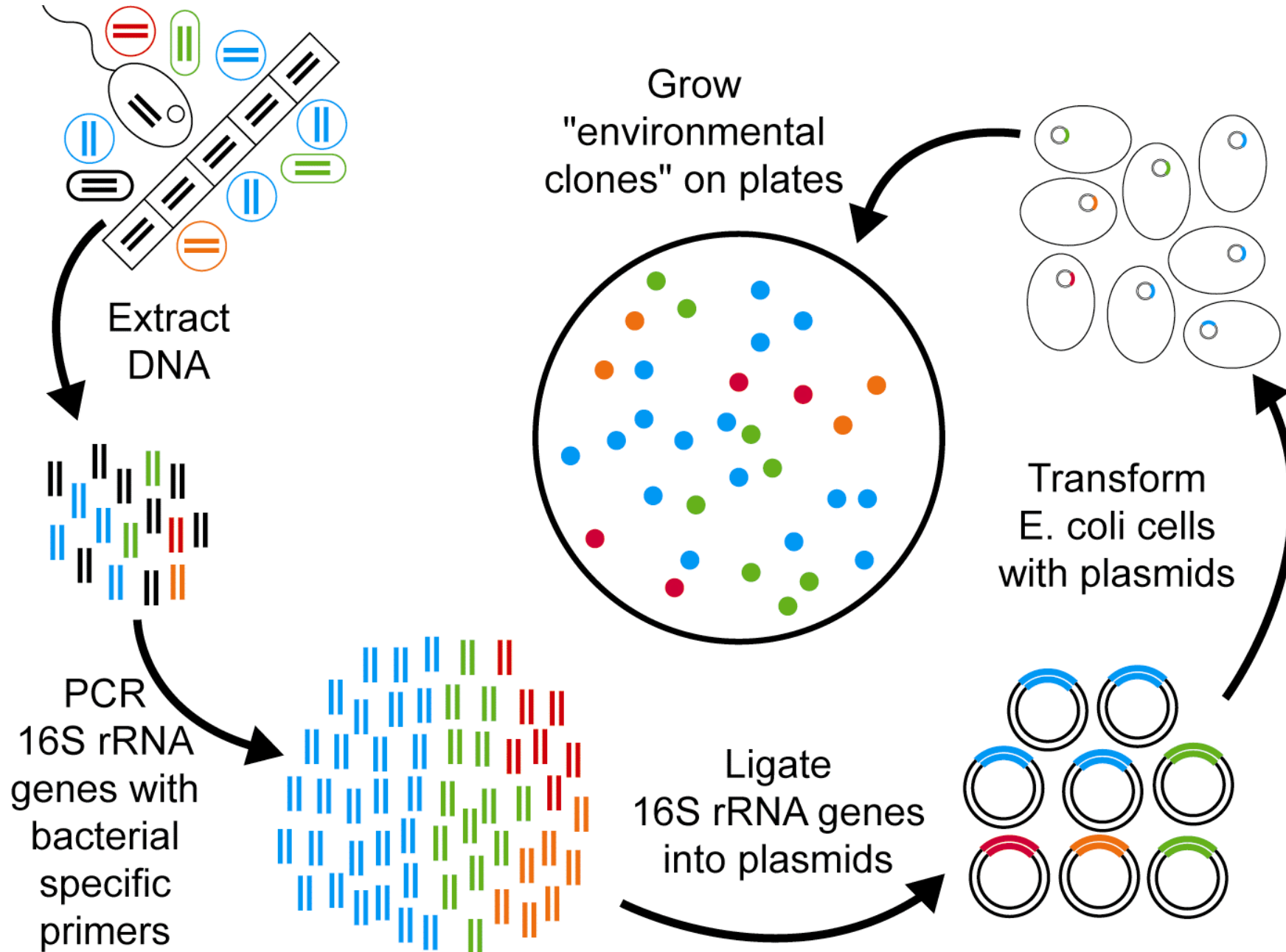
D/RNA extraction	Incomplete sampling
	Resistance to cell lysis
Storage	Enzymatic degradation
PCR	Inhibitors in template DNA
	Amplification bias
	Gene copy number
	Fidelity of PCR
	Differential denaturation efficiency
	Chimeric PCR products
Anytime	Contamination w/ non-target DNA

So you have a positive PCR product: Now what?

- Clone and sequence
- Get “community fingerprint” via T-RFLP, DGGE, etc.
- Design probes for imaging to provide spatial information
- Quantify
- Go straight into sequencing (next generation sequencing)



Traditional Gene Cloning



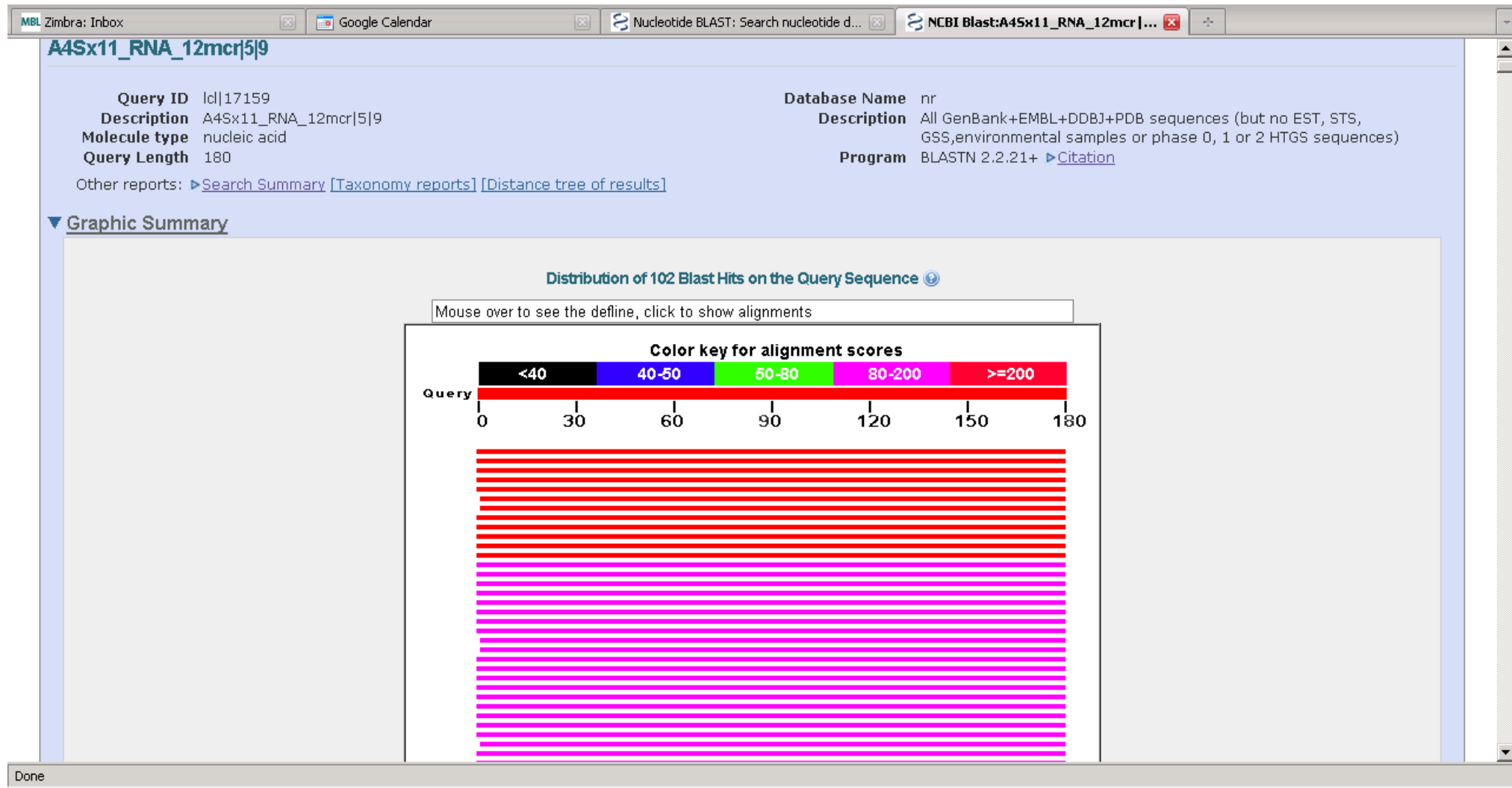
Schematic courtesy of B. Crump

What do you DO with sequences?

- Perform a similarity search
- Align the sequences
- Build a tree and classify
- Reconstruct genomes
- Categorize functions
- Compare organisms/samples
- Design probes and quantify
- Examine expression patterns
- Etc. Etc. Etc.

BLAST

Basic Local Alignment Search Tool



<http://blast.ncbi.nlm.nih.gov/Blast.cgi>

Making Sense of Sequences: Molecular Phylogeny

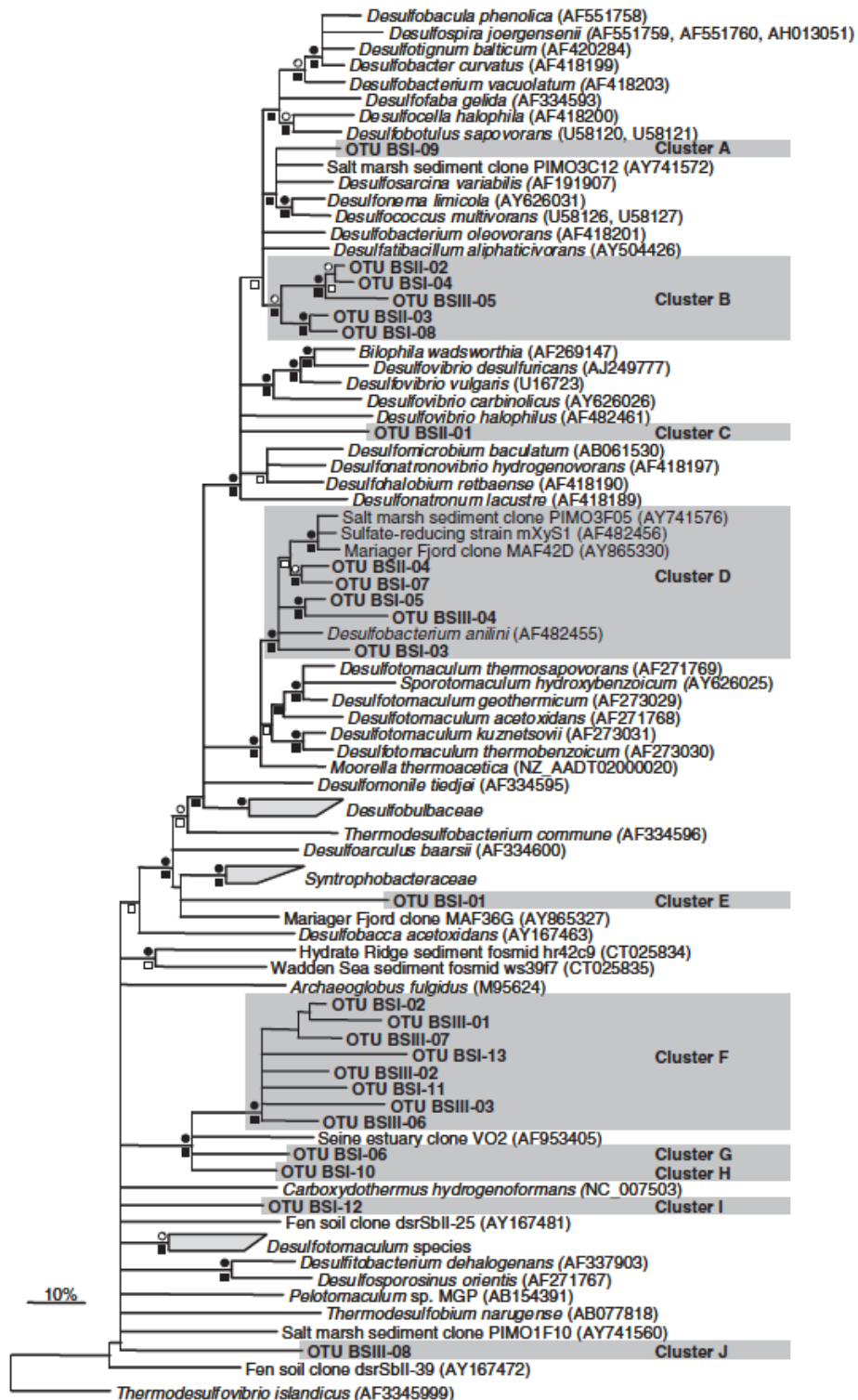
1. Align sequences so that “homologous” residues are juxtaposed.
2. Count the number of differences between pairs of sequences; this is some measure of “evolutionary distance” that separates the organisms.
3. Calculate the “tree,” the relatedness map, that most accurately represents all the pairwise differences.

Cursor positioned at nucleotide 521 of sequence number 1 (Human)

501	1 Human	CC AUGGUGACCA C GGGUGAC GGGGAAUC A GGGUUC GAUUC CGAGAGGGAGCCUGAGAAA C GGCUACCA CAUCC AA GGA-AAGGCAGCAGGC GCG AAAU
501	2 Rabbit	CC AUGGUGACCA C GGGUGAC GGGGAAUC A GGGUUC GAUUC CGAGAGGGAGCCUGAGAAA C GGCUACCA CAUCC AA GGA-AAGGCAGCAGGC GCG AAAU
501	3 Shrimp	CC AUGGUGGCAAC C GGGUAA C GGGGAAUC GGGGUUC GAUUC CGAGAGGGAGCCUGAGAAA C GGCUACCA CAUCC AA GGA-AAGGCAGCAGGC GCG AAAU
501	4 Termite	CC AUGGUGUAA C GGGUAA C GGGGAAUC A GGGUUC GAUUC CGAGAGGGAGCCUGAGAAA C GGCUACCA CAUCC AA GGA-AAGGCAGCAGGC GCG AAAU
501	5 Drosophi	CC AUGGUGGCAAC C GGGUAA C GGGGAAUC A GGGUUC GAUUC CGAGAGGGAGCCUGAGAAA C GGCUACCA CAUCC AA GGA-AAGGCAGCAGGC GCGU AAAU
501	6 Sponge	CC AUGGUGGCAAC C GGGUGA C GGA GAA UUA GGGUUC GAUUC CGAGAGGGAGCCUGAGAG C GGCUACCA CAUCC AA GGA-AAGGCAGCAGGC GCG AAAU
501	7 Mucor	CA AUGCCUA CAA C GGGUAA C GGGGAA UUA GGGUUC GAUUC CGAGAGGGAGCCUGAGAAA C GGCUACCA CAUCC AA GGA-AAGGCAGCAGGC GCG AAAU
501	8 S. pombe	CC AUGGUVUUAA C GGGUAA C GGGGAA UUA GGGUUC GAUUC CGAGAGGGAGCCUGAGAAA C GGCUACCA CAUCC AA GGA-AAGGCAGCAGGC GCG AAAU
501	9 Candida	CC AUGGUVU CAA C GGGUAA C GGGGAA UAA GGGUUC GAUUC CGAGAGGGAGCCUGAGAAA C GGCUACCA CAUCC AA GGA-AAGGCAGCAGGC GCG AAAU
501	10 Pneumocy	CC AUGGUVU C GAG C GGGUAA C GGGGAA UAA GGGUUC GAUUC CGAGAGGGAGCCUGAGAAA C GGCUACCA CAUCC AA GGA-AAGGCAGCAGGC GCG AAAU
501	11 Yeast	CC AUGGUVU CAA C GGGUAA C GGGGAA UAA GGGUUC GAUUC CGAGAGGGAGCCUGAGAAA C GGCUACCA CAUCC AA GGA-AAGGCAGCAGGC GCG AAAU
501	12 Pennicil	CC AUGGUGGCAAC C GGGUAA C GGGGAA UUA GGGUUC GAUUC CGAGAGGGAGCCUGAGAAA C GGCUACCA CAUCC AA GGA-AAGGCAGCAGGC GCG AAAU
501	13 Corn	CC AUGGUGGUGA C GGGUGA C GGA GAA UUA GGGUUC GAUUC CGAGAGGGAGCCUGAGAAA C GGCUACCA CAUCC AA GGA-AAGGCAGCAGGC GCG AAAU
501	14 Rice	CC AUGGUGGUGA C GGGUGA C GGA GAA UUA GGGUUC GAUUC CGAGAGGGAGCCUGAGAAA C GGCUACCA CAUCC AA GGA-AAGGCAGCAGGC GCG AAAU
501	15 Tomato	CC AUGGUGGUGA C GGGUGA C GGA GAA UUA GGGUUC GAUUC CGAGAGGGAGCCUGAGAAA C GGCUACCA CAUCC AA GGA-AAGGCAGCAGGC GCG AAAU
501	16 Volvox	CC AUGGUGGUAAC C GGGUGA C GGA GGA UUA GGGUUC GAUUC CGAGAGGGAGCCUGAGAG UGGCUACCA CAUCC AA GGA-AAGGCAGCAGGC GCG AAAU
501	17 Chlorell	CC AUGGUGGUAAC C GGGUGA C GGA GGA UUA GGGUUC GAUUC CGAGAGGGAGCCUGAGAAA C GGCUACCA CAUCC AA GGA-AAGGCAGCAGGC GCG AAAU
501	18 Porphyra	CC AUGGUGUGA C GGGUAA C GGA CCGUGGGUG C GGAUUC CGAGAGGGAGCCUGAGAG C GGCUACCA CAUCC AA GGA-AAGGCAGCAGGC GCG AAAU
501	19 Gracilar	CC AUGGUGUGA C GGGUAA C GGA CCGUGGGUG C GGAUUC CGAGAGGGAGCCUGAGAG C GGCUACCA CAUCC AA GGA-AAGGCAGCAGGC GCGAA U
501	20 Parameci	CC AUGGCAGUCAC C GGGUAA C GGA GAA UUA GGGUUC GAUUC CGAGAGGGAGCCUGAGAAA C GGCUACCA CAUCC AA GGA-AAGGCAGCAGGC GCGU AAAU
501	21 Tetrahym	CC AUGGCAGUCAC C GGGUAA C GGA GAA UUA GGGUUC GAUUC CGAGAGGGAGCCUGAGAAA C GGCUACCA CAUCC AA GGA-AAGGCAGCAGGC GCGU AAAU
501	22 Dinoflag	CC GUGGCAGUGA C GGGUAA C GGA GAA UUA GGGUUC GAUUC CGAGAGGGAGCCUGAGAAA C GGCUACCA CAUCC AA GGA-AAGGCAGCAGGC GCG AAAU
501	23 Toxoplas	CC GUGGCAGUGA C GGGUAA C GGGGAA UUA GGGUUC GAUUC CGAGAGGGAGCCUGAGAAA C GGCUACCA CAUCC AA GGA-AAGGCAGCAGGC GCG AAAU
501	24 Theileri	CC GGGGCAGCGA C GGGUAA C GGGGAA UUA GGGUUC GAUUC CGAGAGGGAGCCUGAGAAA C GGCUACCA CAUCC AA GGA-AAGGCAGCAGGC GCG AAAU
501	25 Achlya	CC AUGGCAGUAA C GGGUAA C GGGGAA UUA GGGUUC GAUUC CGAGAGGGAGCCUGAGAAA C GGCUACCA CAUCC AA GGA-AAGGCAGCAGGC GCGU AAAU
501	26 Phytopht	CC AUGGCAGUAA C GGGUAA C GGGGAA UUA GGGUUC GAUUC CGAGAGGGAGCCUGAGAAA C GGCUACCA CAUCC AA GGA-AAGGCAGCAGGC GCGU AAAU
501	27 Diatom	CC AUGGCAGUAA C GGGUAA C GGGGAA UUA GGGUUC GAUUC CGAGAGGGAGCCUGAGAG C GGCUACCA CAUCC AA GGA-AAGGCAGCAGGC GCGU AAAU
501	28 Ochromon	CC AUGGCAGUAA C GGGUAA C GGA GAA UUA GGGUUC GAUUC CGAGAGGGAGCCUGAGAAA UGGCUACCA CAUCC AA GGA-AAGGCAGCAGGC GCGU AAAU
501	29 Synura	CC AUGGCAGUAA C GGGUAA C GGA GAA UUA GGGUUC GAUUC CGAGAGGGAGCCUGAGAAA UGGCUACCA CAUCC AA GGA-AAGGCAGCAGGC GCGU AAAU
501	30 Brown Al	CC AUGGCAGUAA C GGGUAA C GGGGAA UUGGGGUUC GAUUC CGAGAGGGAGCCUGAGAAA C GGCUACCA CAUCC AA GGA-AAGGCAGCAGGC GCGU AAAU
501	31 Dictyost	CC AUGGUGUAA C GGGUAA C GGGGAA UUA GGGUUC GAUUC CGAGAGGGAGCCUGAGAAA UGGCUACCA CAUCC AA GGA-AAGGCAGCAGGC GCG AAAU
501	32 Euglena	C AUGGCCUUGA C GGGUAA C GGA GAA UC A GGGUUC GAUUC CGAGAGGGAGCCUGAGAG C GGCUACCA CAUCC AA GGU-GGGCAGCAGGC CCG AAAU
501	33 Trypanos	CC AUGGCCUUGA C GGG-AAGC GGGGGA UUA GGGUUC GAUUC CGAGAGGGAGCCUGAGAAA UAGCUACCA CAUCC AA GGA-GGGCAGCAGGC GCG AAAU
501	34 Leishman	CC AUGGCCUUGA C GGG-AAGC GGGGGA UUA GGGUUC GAUUC CGAGAGGGAGCCUGAGAAA UAGCUACCA CAUCC AA GGA-GGGCAGCAGGC GCG AAAU
501	35 Crithidi	CC AUGGCCUUGA C GGG-AAGC GGGGGA UUA GGGUUC GAUUC CGAGAGGGAGCCUGAGAAA UAGCUACCA CAUCC AA GGA-GGGCAGCAGGC GCG AAAU

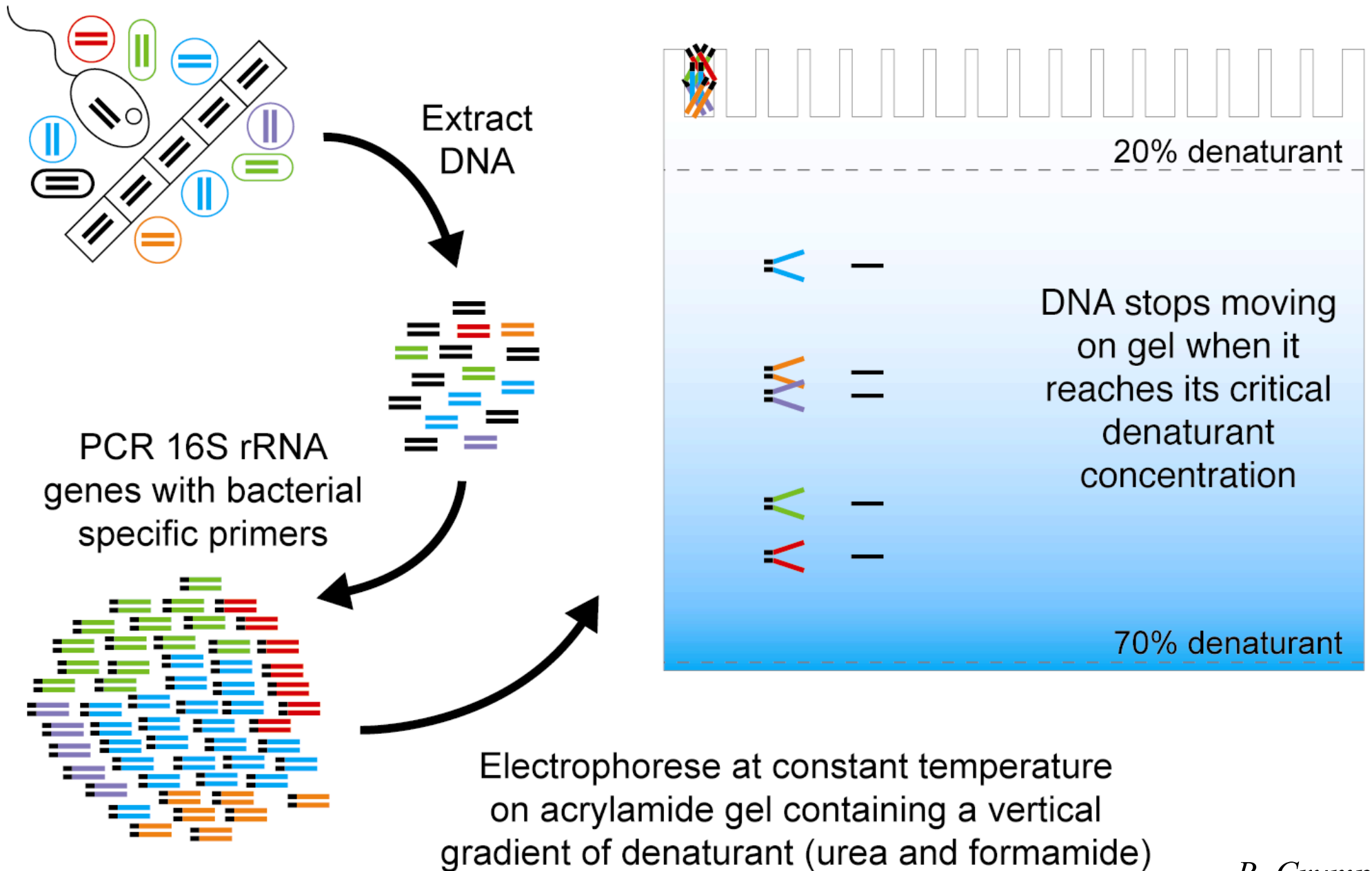
Cursor positioned at nucleotide 1100 of sequence number 35 ()

1050	1	Human	CC G CCCC U UGCC UUGGG CCCCCC UG GAUGCUC UUAAGCU GAGU G U CCCC GGGGCCC GAAGC GUUU A CUUGAA
1050	2	Rabbit	CC G CCCC U UGCC UUGGG CCCCCC UG GAUGCUC UUAAGCU GAGU G U CCCC GGGGCCC GAAGC GUUU A CUUGAA
1050	3	Shrimp	CG GACA AUU CAUUGGA UCGUUCGG SGUGCUC UUAACC GAGU G U CCUG GGUGGCC GAUAC GUUU A CUUGAA
1050	4	Drosophi	AU GUUCGU CCUAUUUAAAAA CCGCAUAGUGCUC UUAACC GAGU G U UAUU GUGGGCC GGUAC UAUU A CUUGAA
1050	5	Sponge	CGC CCUU CCUCUGAAA GCC CCG ACUGCUC UUCACUG GAGU G GUCGGG UAGUU GGGAC GUUU A CUUGAA
1050	6	Mucor	CGC UUUUAU CGAGGC UUUUUUU UGGUUAUGCU A UGAAUAGCUU CGGUU GUUU A UAGUCU UAGCCAG AU GAUUA CCAUGAG
1050	7	S. pombe	GGGG UG GUUAA CCUUUGGCAAAAU A AUCAUGUUCU UUAUU GAGC G UGGUAG GGAAC CAGGAC UUUU A CUUGAA
1050	8	Candida	GAG CCUU CCUUUGGCUAAAC A UUUGCCCU U GU GG U GUUUGG GGAAC CAGGAC UUUU A CUUGAA
1050	9	Pneumocy	GAU CCUC CCUCUGGAUUAAC G GUGCCCU UCGU GGU G UGCCGG AUAGCCAGGGCA UUUU A CUUGAG
1050	10	Yeast	GGG CCUU CCUUUGGCUAAAC U UGAGUCCU U GU GG U C UUGG GGAAC CAGGAC UUUU A CUUGAA
1050	11	Pennicil	GG A CCUU CCUUUGGGAAAC U CAUGGCCU UCAU GGU G UGG GG GGAAC CAGGAC UUUU A CUUGAA
1050	12	Corn	CGA CCUU CUGCCGGC GAUGC GCU CGUGGCC UUAACU GGCC G GGUC G UGCC UCCGG GCCGUU A CUUGAA
1050	13	Rice	CGA CCUU CUGCCGGC GAUGC GCU CGUGGCC UUAACU GGCC G GGUC G UGCC UCCGG GCCGUU A CUUGAA
1050	14	Tomato	CGU CCUU CUGUGGGC GAUGC GCU CGUGGCC UUAUUU GGCC G GGUC G UGCC UCCGG GCGUUA A CUUGAA
1050	15	Volvox	CCA CCUC CUGCCGGGACGG GCU CGUGGGC UUCACU GUAU G GGACUC GGAGUC GCG GAGGUU A CUUGAG
1050	16	Chlorell	CA CCUC UUGCCGGGACGG GCU CGUGGGC UUCACU GUUC G GGACUC GGAGUC GCG GCGUGUUA A CUUGAG
1050	17	Porphyra	UUUUU GUGGAGGGC GG CCGCU UGUGG C UUCACUGUGGC GCGUGUC GCGGCCACC G UUU A CUUGAA
1050	18	Gracilar	CCUUU GUGGAGAGGGG GUGU SGUGG UGC UUGAGUGGCU GCAUGCU GCGGCCACC G UUU A CUUGAA
1050	19	Parameci	C UCGU UACAAUCCC UUU UCGCU UUAGGGUUGC A GUGGG GAGU AGAC AAUUU A CUUGAA
1050	20	Tetrahym	UAUGU UUGCAAAUAAAA UCGCC UUCACUGGUUC G ACUUAG GGAGU AAAA AUUUU A CUUGAA
1050	21	Dinoflag	CUUG ACAUC UUCGU AAAAGA AC GUA UCUGGAC UUCAUU GUGU GGUG CG GUAUUUAGGAC AUUU A CUUGAG
1050	22	Toxoplas	UCUA GCAUC CUUGU GGAUU UCU C AACA UUCAUU GUGU GGAGUUU UUU CAGGAC UUUU A CUUGAG
1050	23	Theileri	UCG GUU UGAUUUUUU UUUUCCGGAU GAUUA CUUGAG
1050	24	Achlya	GGGC CAUUU UUGUGAGGGAU GCUUU UCUGCC AUUCAGUUGGU G GUUGAG UAGAC UUGCAUC GUUU A CUUGAA
1050	25	Phytopht	GAGG CAUUU UUGUGAGGGCU GCUUU UCUGCC AUUAAGUUGGU G GGUUGG UGGG UUGCAUC GUUU A CUUGAA
1050	26	Diatom	UCGC CAUCC UUGGGUGG AA CUG UGUGGC AUUAGGUUGUC G U GCAG GGAUUGCCAU CAUUU A CUUGAA
1050	27	Ochromon	GA AU CAUCC UCGAGAGG AA CAG UCUGUC AUUCAGUUGAU G G GCGU GGAUUCUGUC UUUU A CUUGAG
1050	28	Synura	CGUC CAUCC UCGGGGAG AA CGA UCUGGC AUUAAGUUGUC G G GUGU GGYAUCCUGUC AUUU A CUUGAG
1050	29	Brown Al	CGGCCG CCAUUC UCGGUAG CG UGUU GUGGC AUUAGGUUGUC G G CUUC UUGGCGCCG UCGUUUGCUGGGAA
1050	30	Dictyost	U C UUAUA GUUAGCUUGU AUU A U CUUU G A UAG UG GUUGUUUGGACA UUU A CUUGAG
1050	31	Euglena	ACCCAGCC UCGAGCUG GGUAG UCU ACCUCUGGUCCACCAC C GGAG CCCACCGUCUUG ACACCCUGGA
1050	32	Trypanos	UUUUU A CUUGAC
1050	33	Leishman	UUUUU A CUUGAC
1050	34	Grithidi	UUUUU A CUUGAC



- Found similar novel *dsr* sequences in the sulfate-rich and methane-rich zones
- Different (and already known) *dsr* sequences in SMTZ

Denaturing Gradient Gel Electrophoresis (DGGE)



Microbial Biogeography along an Estuarine Salinity Gradient: Combined Influences of Bacterial Growth and Residence Time

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 and The Ecosystems Center² and The Josephine Bay Paul Center for Comparative Molecular
 Biology and Evolution,³ Marine Biological Laboratory, Woods Hole, Massachusetts*

Received 1 August 2003/Accepted 16 December 2003

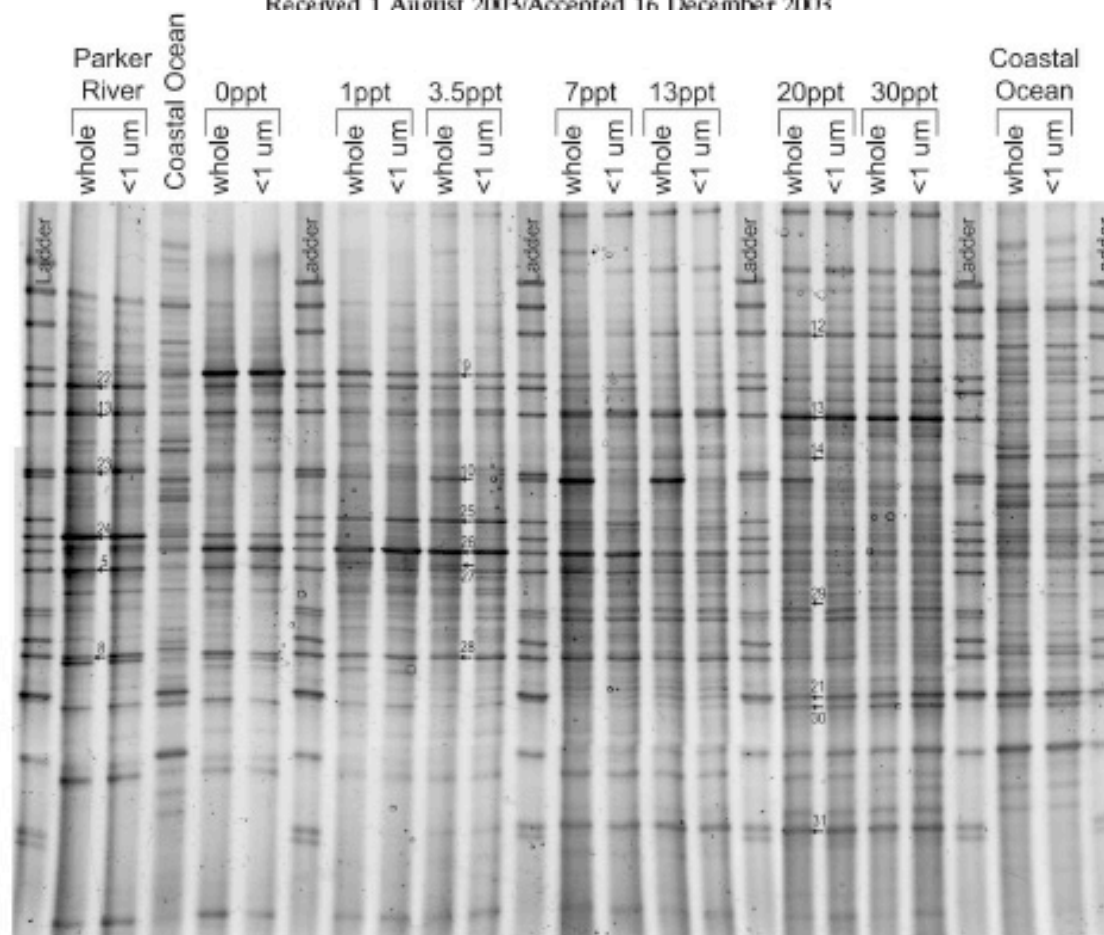
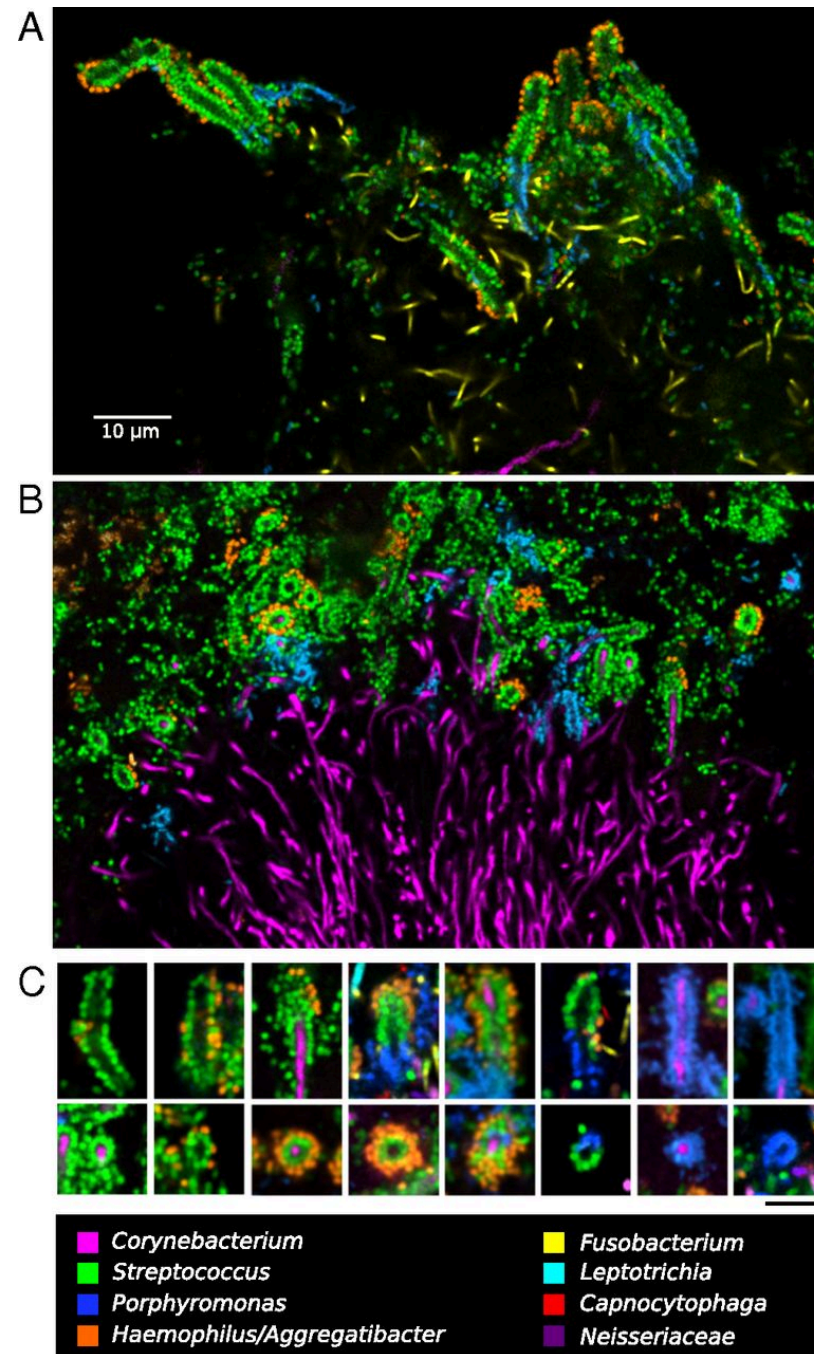
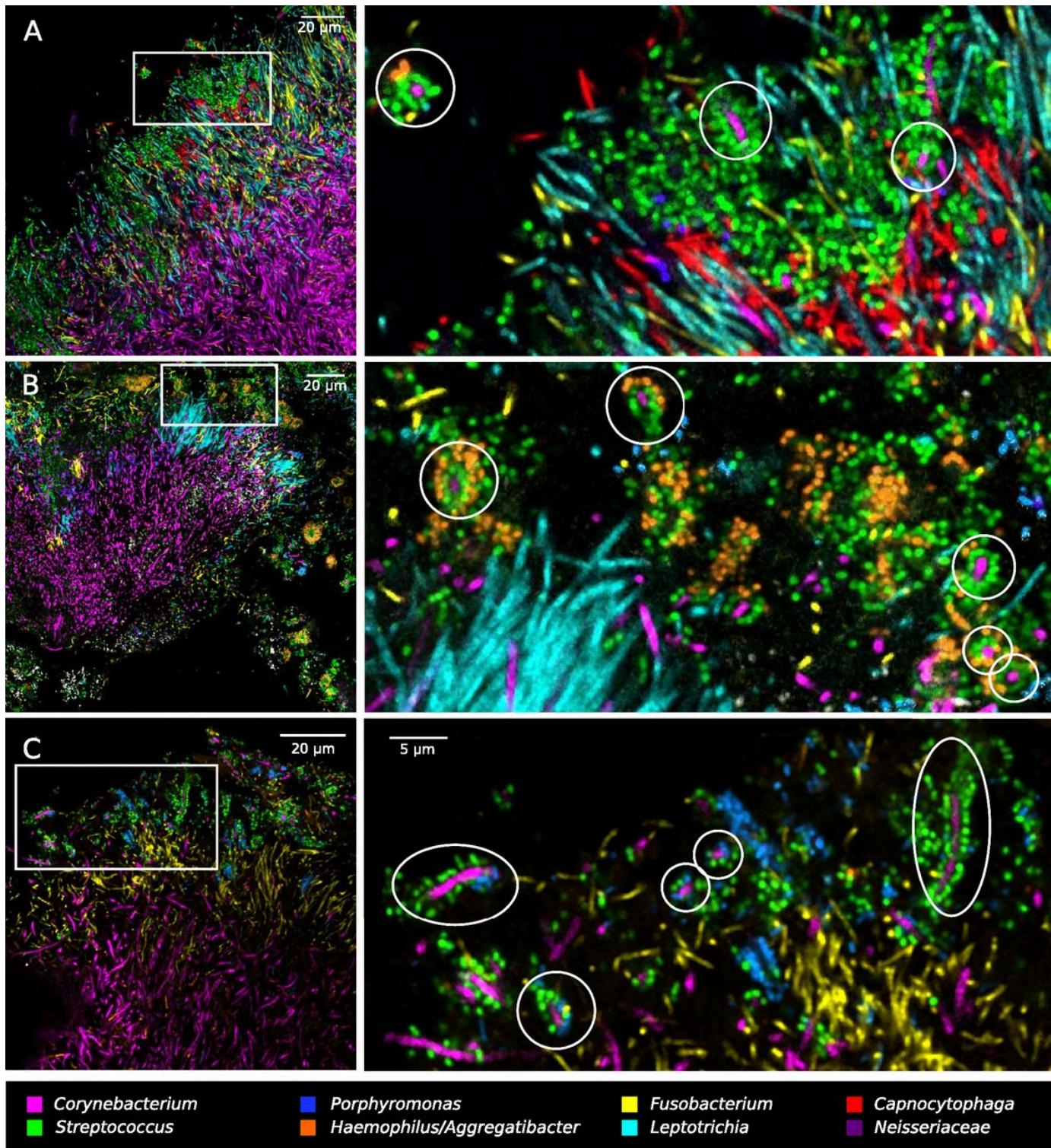


FIG. 4. DGGE gel of PCR-amplified 16S rDNA genes from samples collected along the salinity gradient on 28 September 2000. Bands from which DNA was sequenced are marked and numbered, corresponding to band numbers in Table 3.

rRNA Oligonucleotide Probes => Spatial context





J. Mark Welch et al.
(2016) PNAS

Diversity and abundance of sulfate-reducing microorganisms in the sulfate and methane zones of a marine sediment, Black Sea

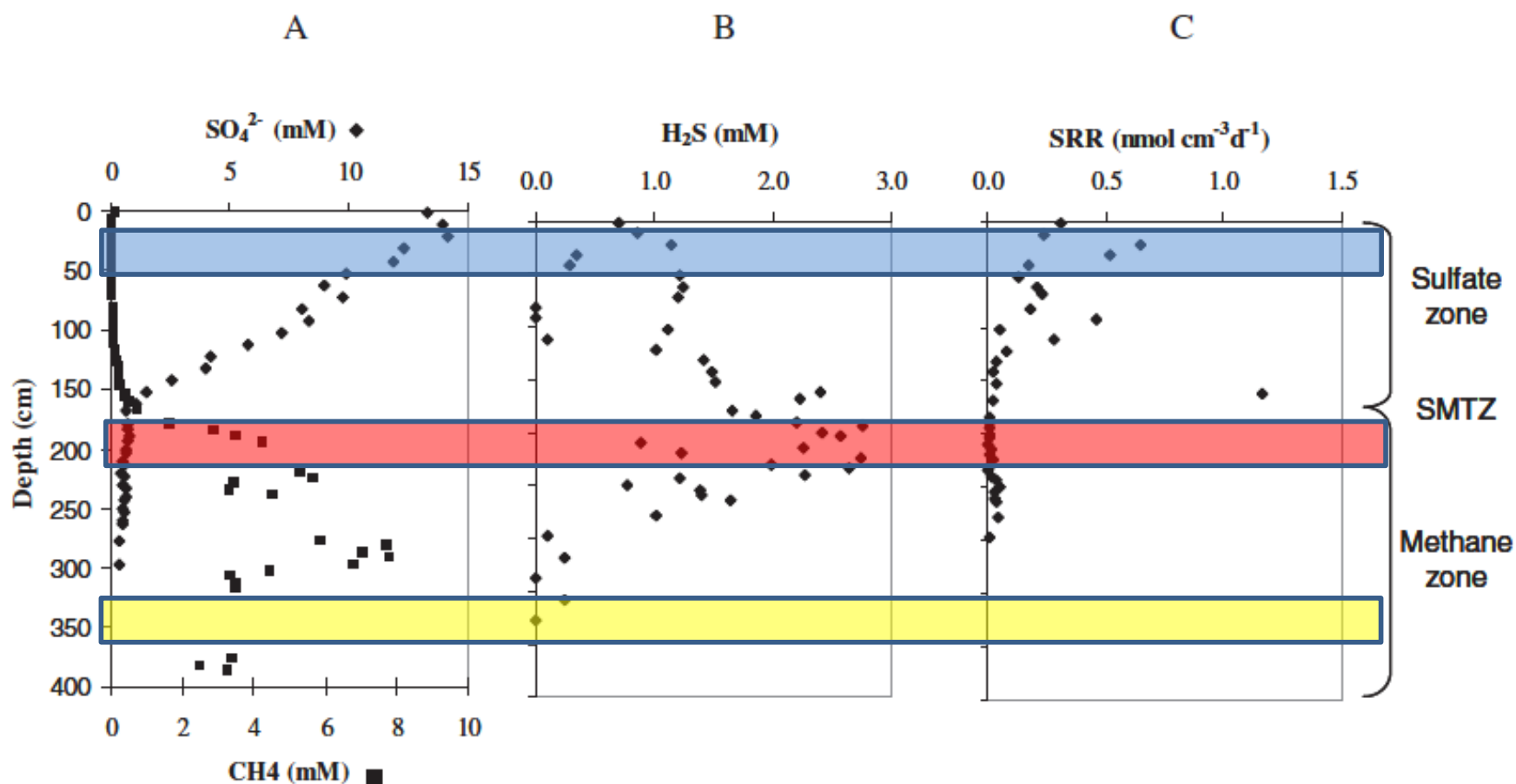


Fig. 2. Biogeochemical zonation and data from the Black Sea sediment core P824-GC.

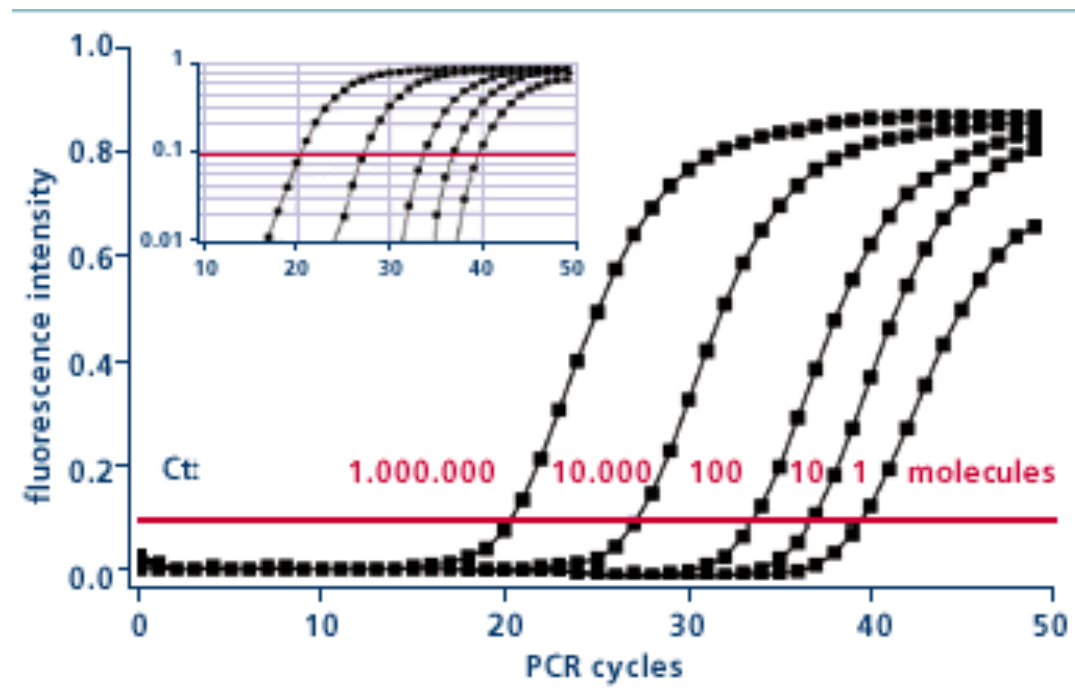
A. Sulfate and methane concentration.

B. Sulfide concentration.

C. Sulfate reduction rate (SRR).

Quantitative PCR (aka qPCR, Real Time PCR)

qPCR monitors the fluorescence emitted during the reactions as an indicator of amplicon production at each PCR cycle (in real time) as opposed to the endpoint detection



Fluorescent dye intercalates into dsDNA

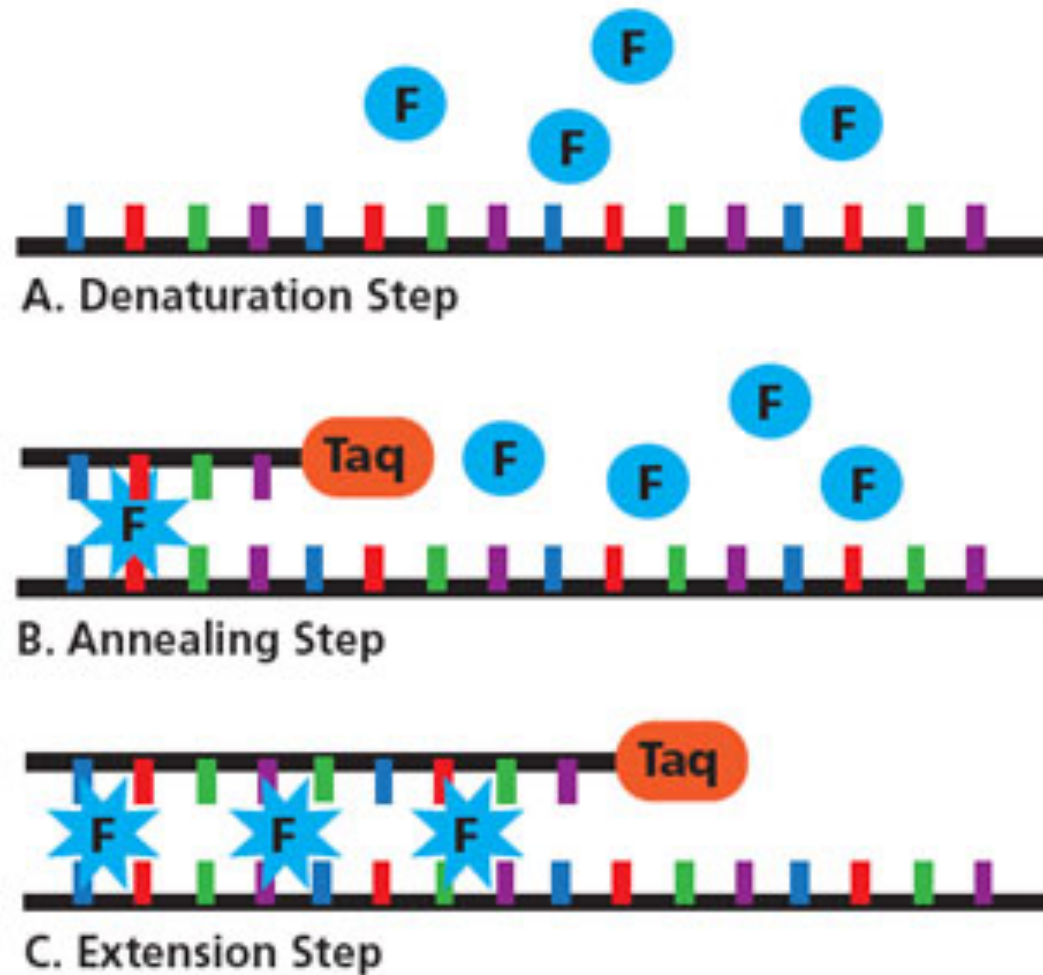
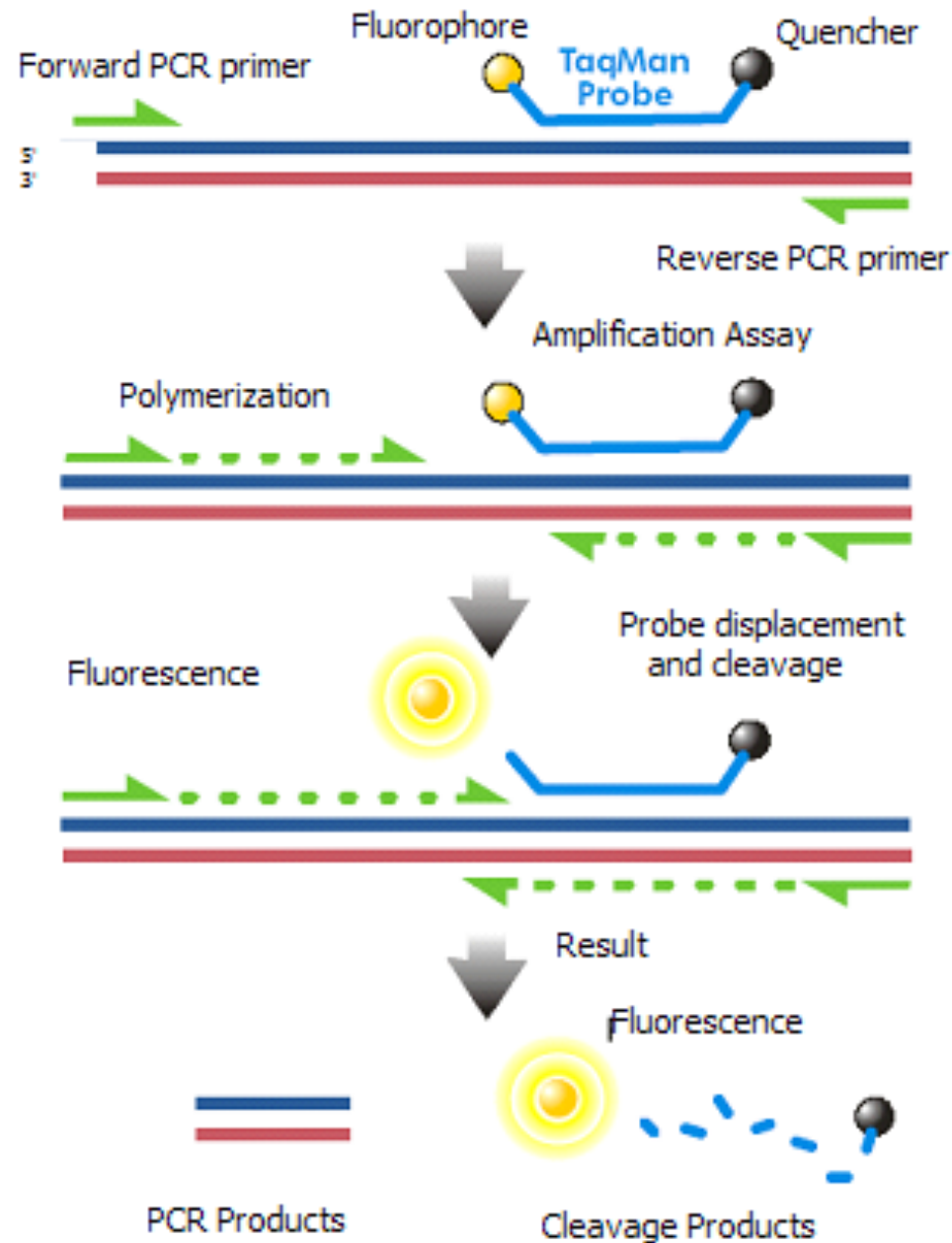


Figure 2: Fluorescent Dyes in qPCR

Probe-based qPCR



Quantitative (Real Time) PCR

- Detection of “amplification-associated fluorescence” at each cycle during PCR
- No gel-based analysis
- Computer-based analysis
- Compare to internal standards
- Must ensure specific binding of probes/dye

Diversity and abundance of sulfate-reducing microorganisms in the sulfate and methane zones of a marine sediment, Black Sea

- Used qPCR to quantify total bacteria (16S rRNA) and total sulfate reducers (*dsr*)

Diversity and abundance of sulfate-reducing microorganisms in the sulfate and methane zones of a marine sediment, Black Sea

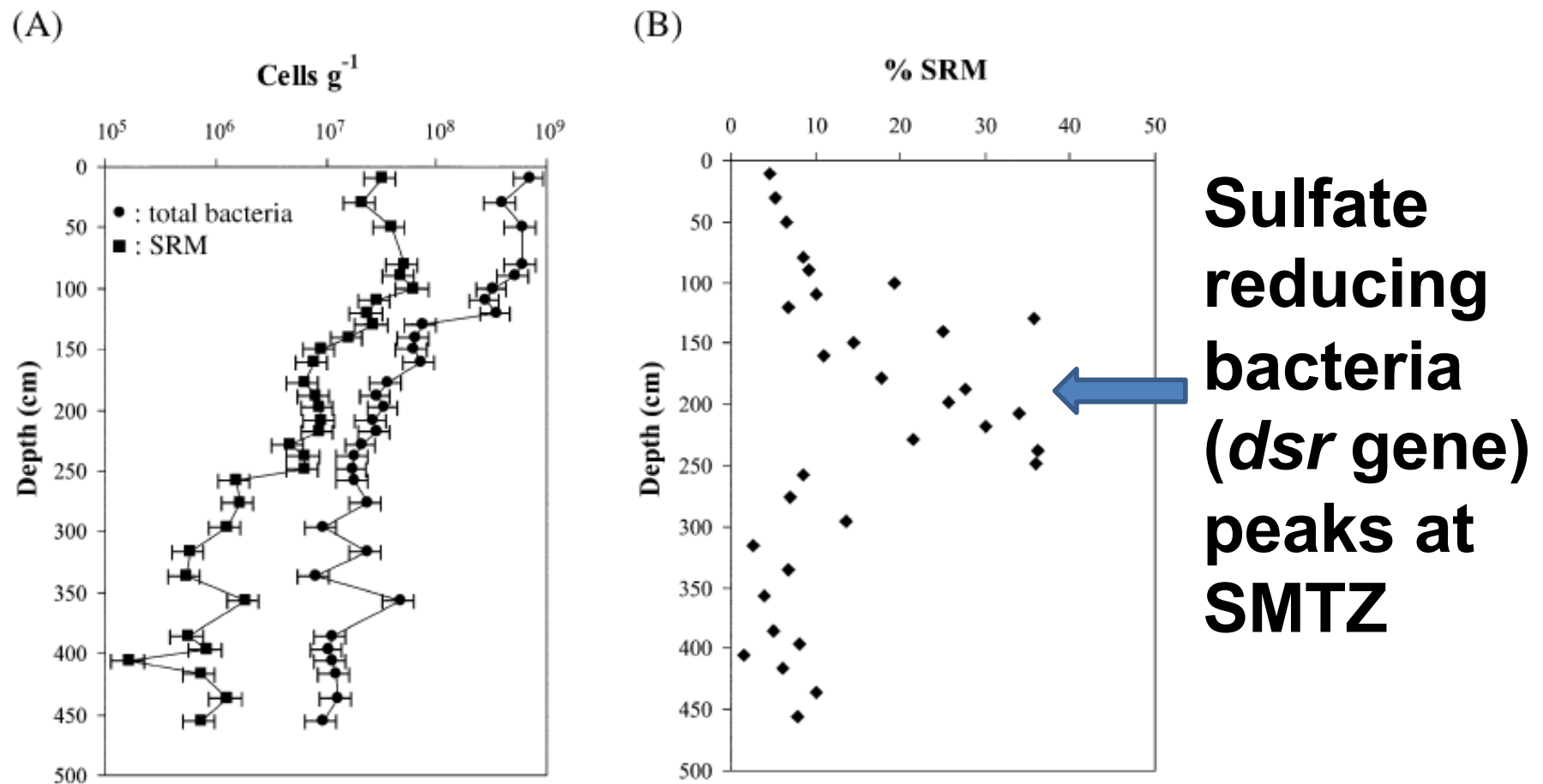


Fig. 3. Abundance of total bacteria and SRM in the Black Sea sediment core P824-GC.

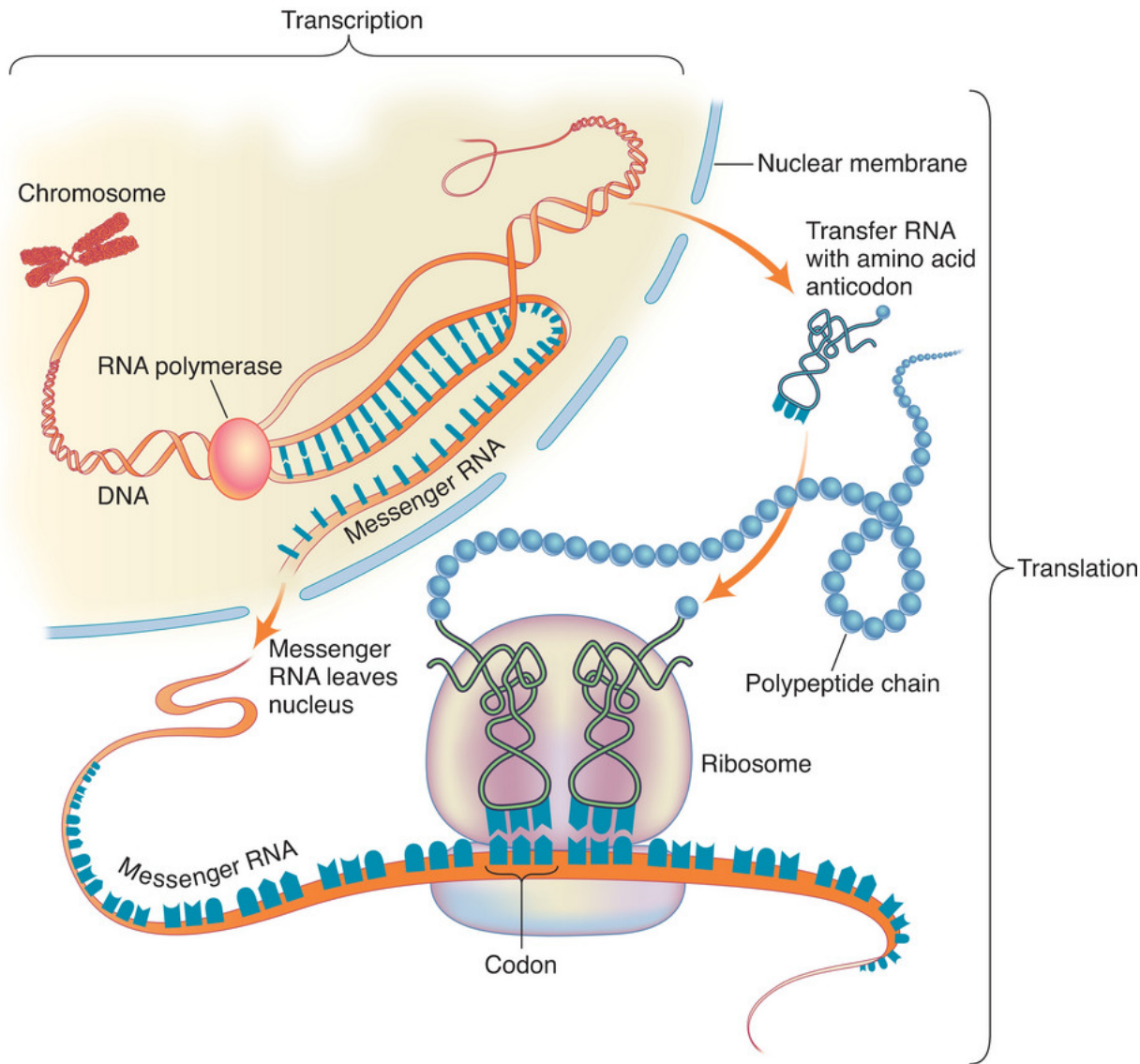
A. Total bacteria and SRM as inferred from real-time PCR data. Values are given as mean \pm standard deviation of triplicates. ● total bacterial cells; ■ sulfate-reducing cells.

B. Depth profile of the relative contribution of SRM to the total bacterial cells as calculated from the data in (A).

Diversity and abundance of sulfate-reducing microorganisms in the sulfate and methane zones of a marine sediment, Black Sea

Future studies will reveal whether these yet unidentified microorganisms with new *dsrAB* variants are **active** in the environment and which life strategies they employ to thrive in low-sulfate habitats that are apparently inhospitable for SRM.

The Central Dogma



DNA

Transcription

RNA

Translation

Protein

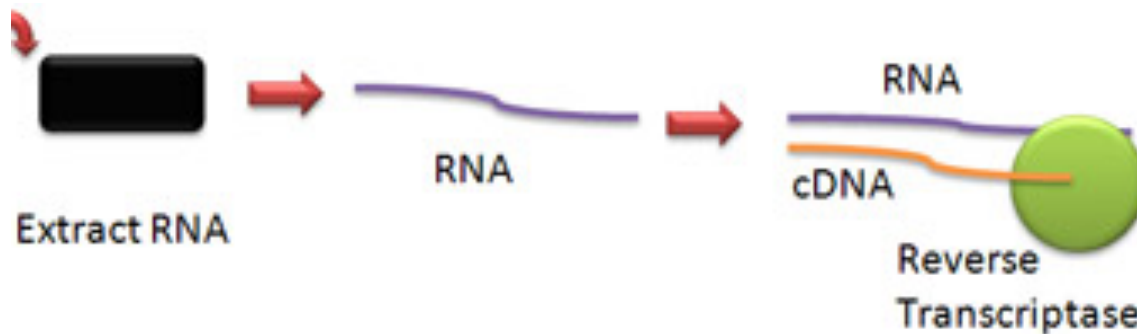
Moving from “who is there?” to “who is active?”

Reverse Transcription PCR (RT-PCR)

- Looks at gene expression in the environment or experimental treatment
- Isolate mRNA
- Reverse transcribe mRNA to produce complementary DNA (cDNA)
- Amplify cDNA by PCR or qPCR

RT-PCR

- RNA + Reverse Transcriptase + dNTPs ➡ cDNA



- cDNA + Primers + Taq + dNTPs ➡ gene of interest
- Who is active? What genes are active?

Diversity and Abundance of Nitrate Reductase Genes (*narG* and *napA*), Nitrite Reductase Genes (*nirS* and *nrfA*), and Their Transcripts in Estuarine Sediments^V



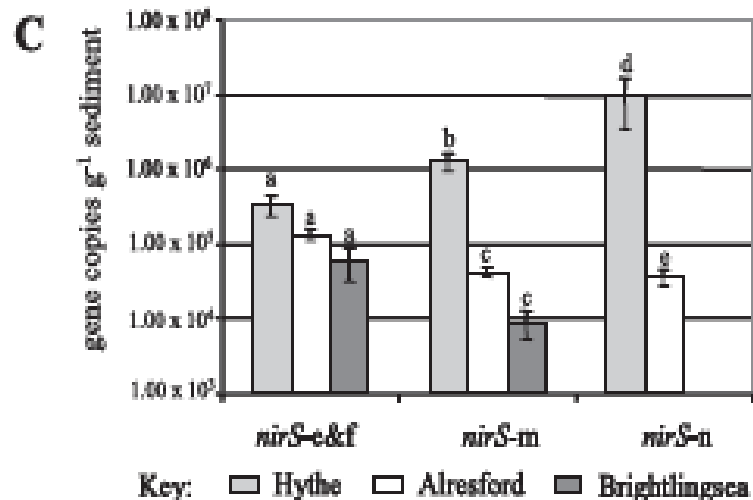
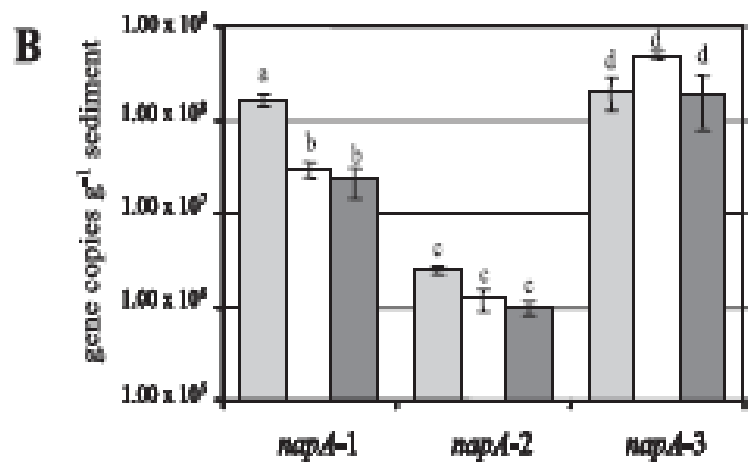
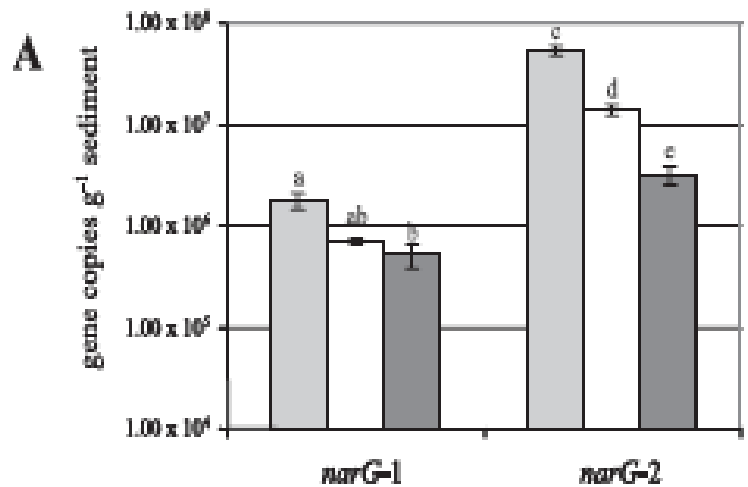
FIG. 1. Map of the Colne estuary, Essex, United Kingdom, showing the locations of the three sampling sites (Hythe, Alresford, and Brightlingsea).

TABLE 1. Primer and probe sets used for Q-(RT)-PCR

Target gene	Phylotype	Amplicon size (bp)	Primer or probe		Q-PCR cycle annealing temp (°C)
			Name ^a	Sequence (5'→3')	
<i>napA</i>	<i>napA</i> -1	111	<i>napA</i> -1F	GTG ATG GAR GAA AAA TTC AA	55
			<i>napA</i> -1R	GAR CCG AAC ATG CCR AC	
			<i>napA</i> -1 (TM-MGB)	AAC ATG ACC TGG AAG	
	<i>napA</i> -2	76	<i>napA</i> -2F	GAA CCK AYG GGY TGT TATG	55
			<i>napA</i> -2R	TGC ATY TCS GCC ATR TT	
			<i>napA</i> -2 (TM-MGB)	CTT TGG GGT TCA A	
	<i>napA</i> -3	130	<i>napA</i> -3F	CCC AAT GCT CGC CAC TG	60
			<i>napA</i> -3R	CAT GTT KGA GCC CCA CAG	
			<i>napA</i> -3 (TM-MGB)	TGG GTT GTT ACG A	
<i>narG</i>	<i>narG</i> -1	69	<i>narG</i> -1F	GAC TTC CGC ATG TCR AC	60
			<i>narG</i> -1R	TTY TCG TAC CAG GTG GC	
			<i>narG</i> -1 (TM-MGB)	TAY TCC GAC ATC GT	
	<i>narG</i> -2	89	<i>narG</i> -2F	CTC GAY CTG GTG GTY GA	55
			<i>narG</i> -2R	TTY TCG TAC CAG GTS GC	
			<i>narG</i> -2 (TM-MGB)	AAC TTC CGC ATG GA	
<i>nrfA</i>	<i>nrfA</i> -2	67	<i>nrfA</i> -2F	CAC GAC AGC AAG ACT GCC G	60
			<i>nrfA</i> -2R	CCG GCA CTT TCG AGC CC	
			<i>nrfA</i> -2 (TM-MGB)	TTG ACC GTC GGC A	
<i>nirS</i>	<i>nirS</i> -e	172	<i>nirS</i> -eF	CAC CCG GAG TTC ATC GTC	60
			<i>nirS</i> -eR	ACC TTG TTG GAC TGG TGG G	
			<i>nirS</i> -e (TM-MGB)	TGC TGG TCA ACT A	
	<i>nirS</i> -m	162	<i>nirS</i> -mF	GGA AAC CTG TTC GTC AAG AC	60
			<i>nirS</i> -mR	CSG ART CCT TGG CGA CGT	
			<i>nirS</i> -m (TM)	TCT GGG CCG ACG CGC CGA TGA AC	
	<i>nirS</i> -n	140	<i>nirS</i> -nF	AAG GAA GTC TGG ATY TC	55
			<i>nirS</i> -nR ^b	CGT TGA ACT TRC CGG T	
			<i>nirS</i> -n (TM-MGB)	ATC CGA AGA TSA	

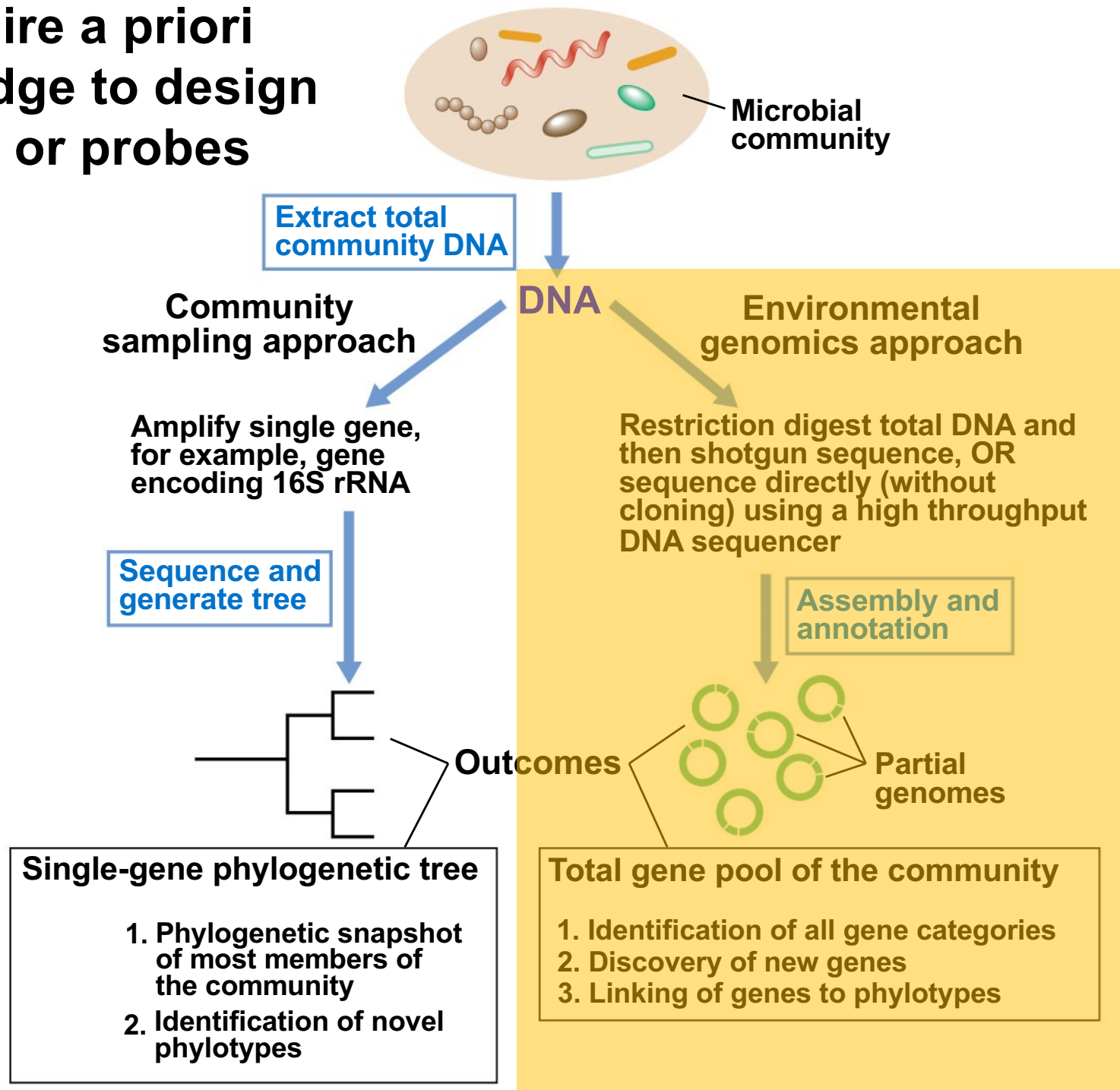
^a For probes: TM-MGB, TaqMan minor groove binding; TM, TaqMan.^b Also known as *nirS6r* (6).

qRT-PCR

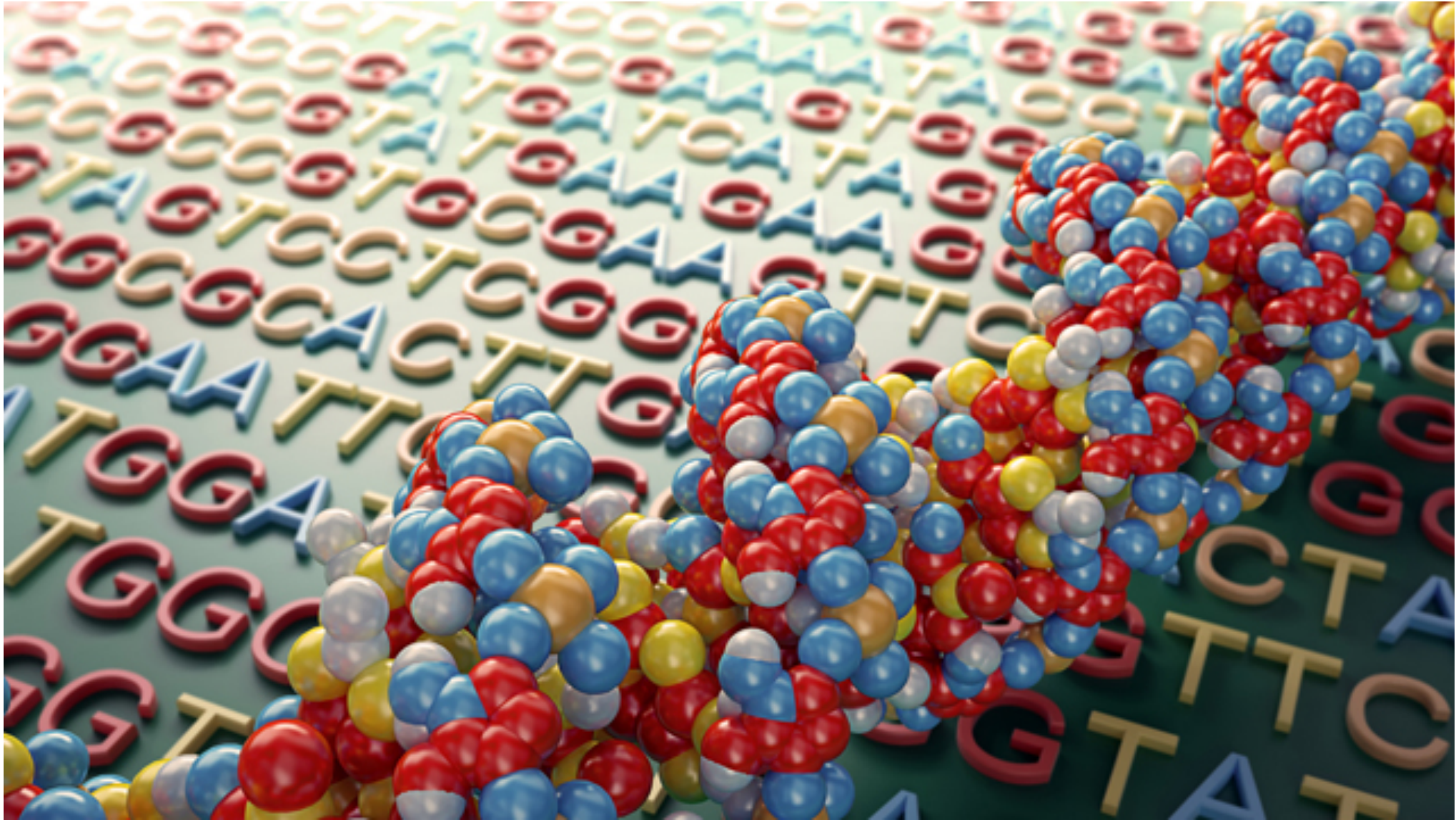


Gene copy and transcript numbers are greatest at the estuary head (Hythe), where the rates of denitrification/DNRA are highest.

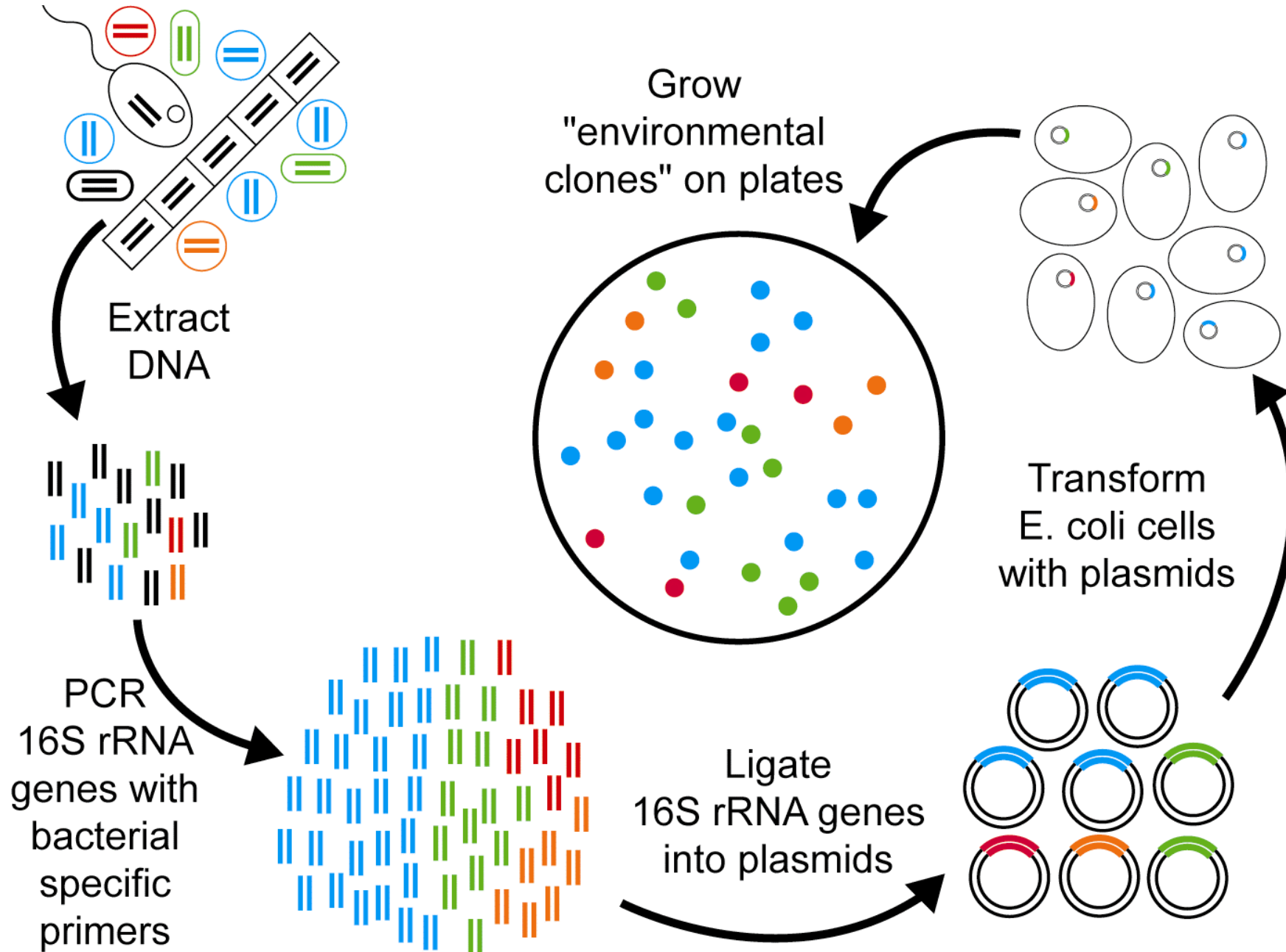
All require a priori knowledge to design primers or probes



Sequencing Revolution

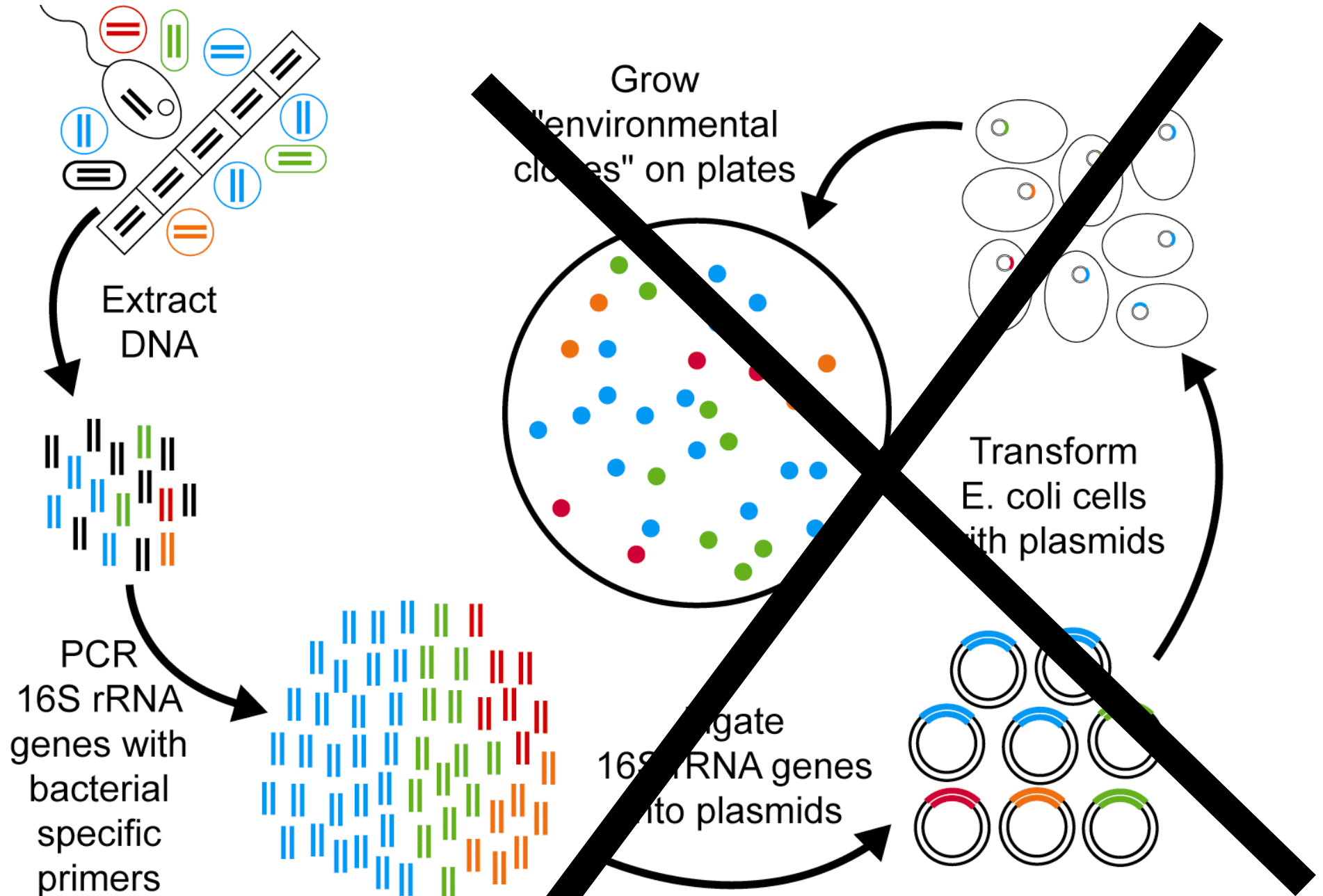


Traditional Gene Cloning

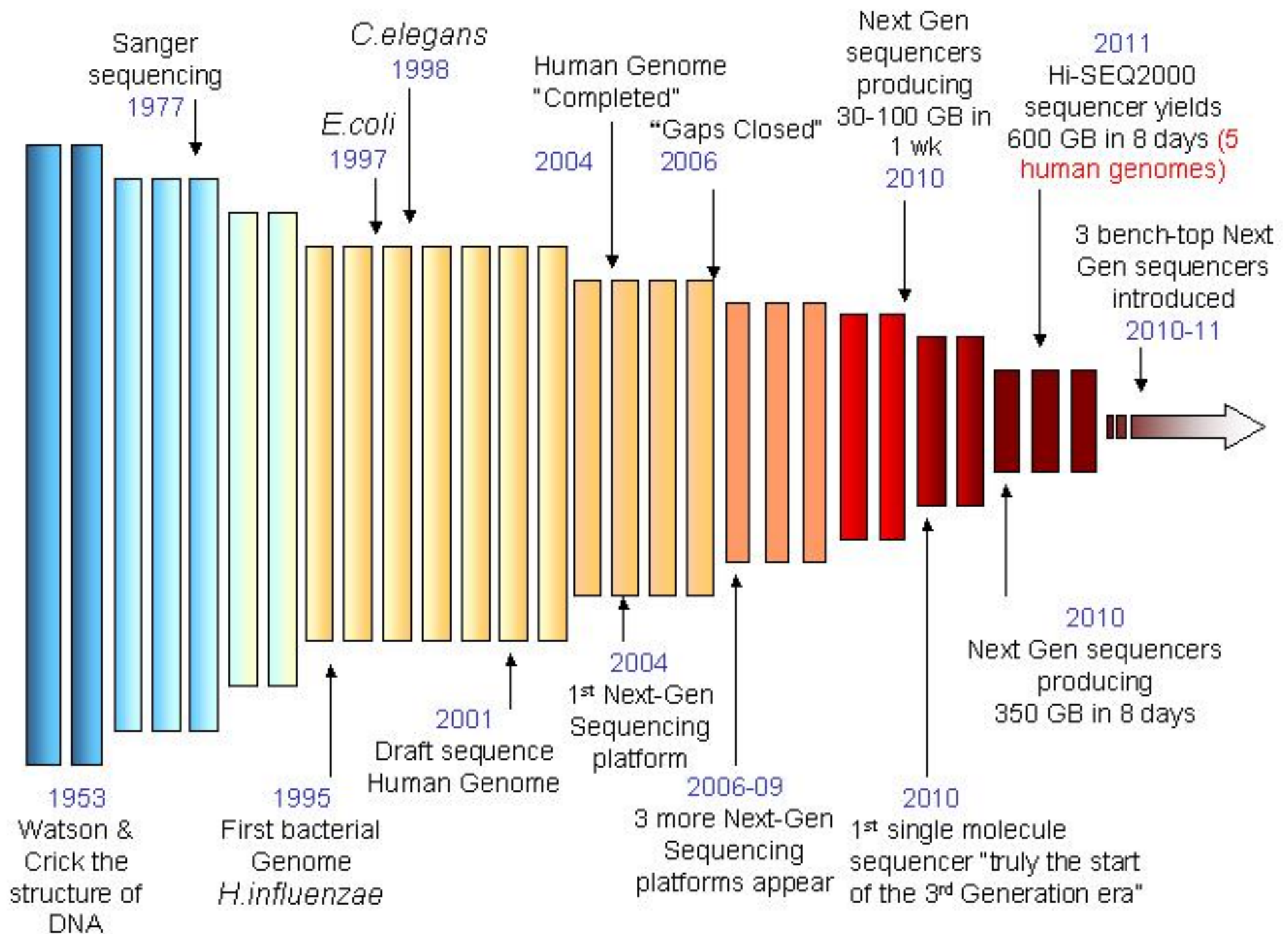


Schematic courtesy of B. Crump

NextGen Approaches



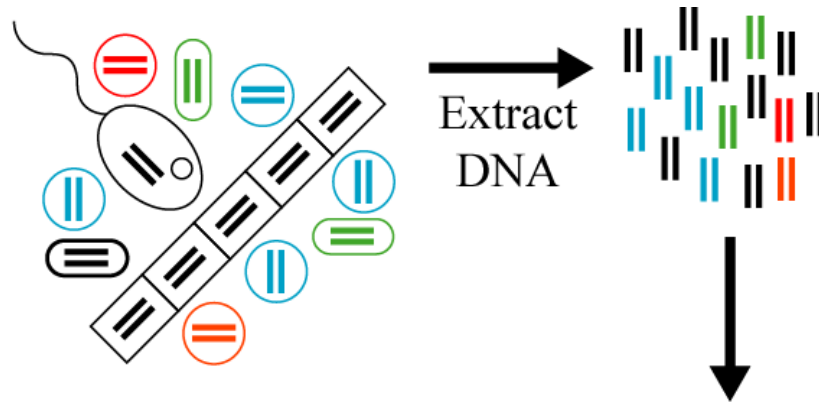
Schematic courtesy of B. Crump



**What is the difference between
“standard” and “next-gen” sequencing?**

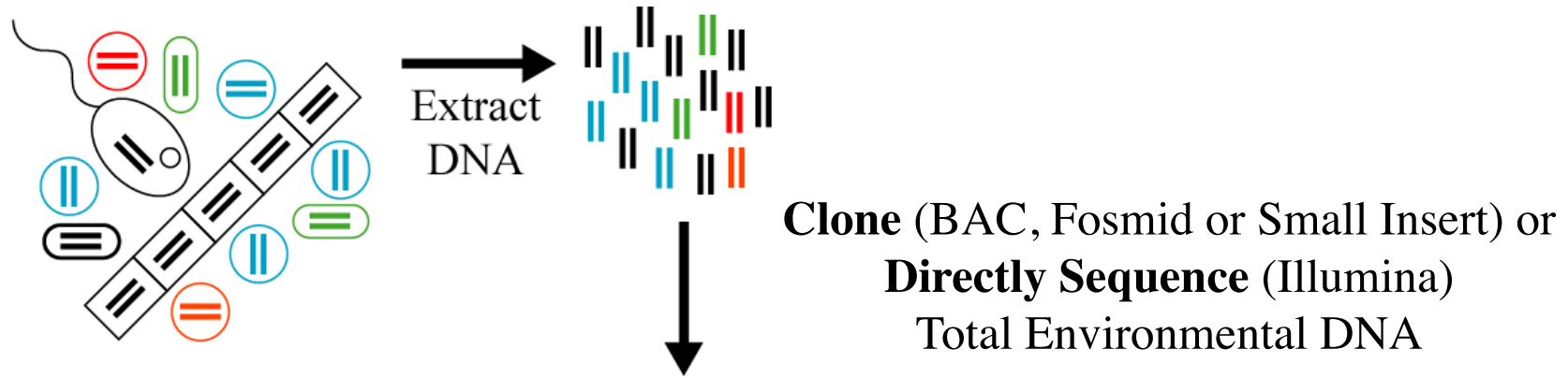


Metagenomics

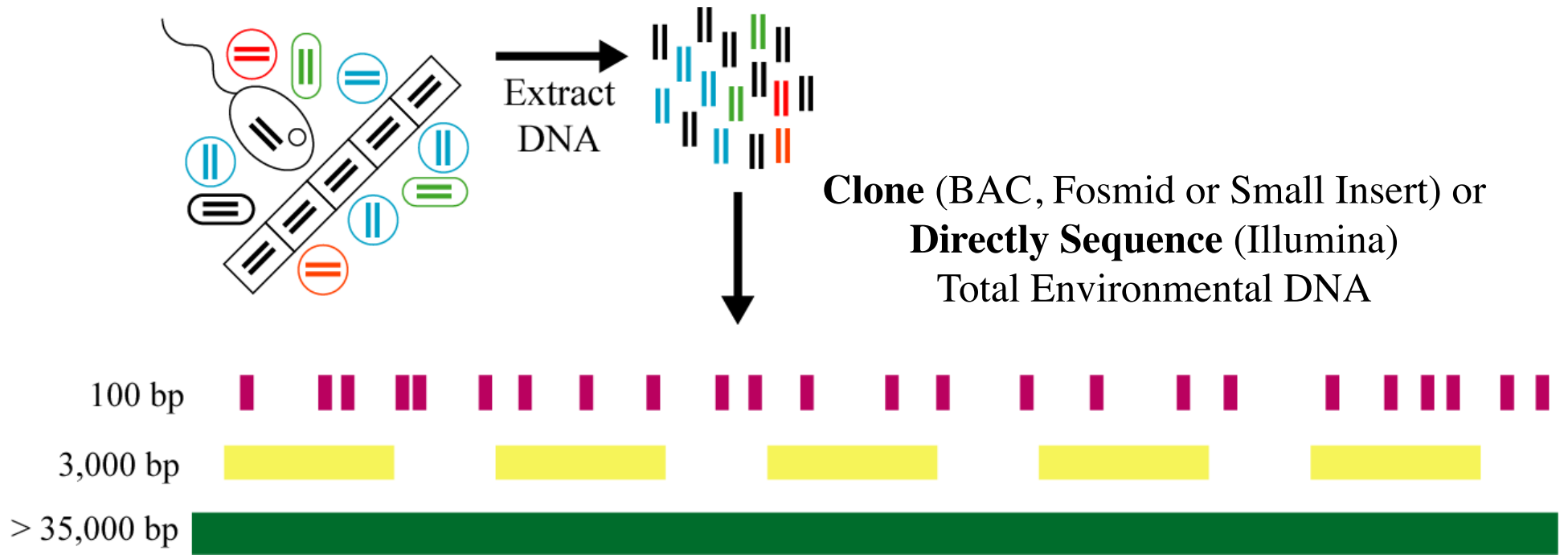


Clone (BAC, Fosmid or Small Insert)
or
Directly Sequence (Illumina, PacBio,
10X, Nanopore, etc)
Total Environmental DNA

Metagenomics



Metagenomics



Access genomes of uncultured microbes:

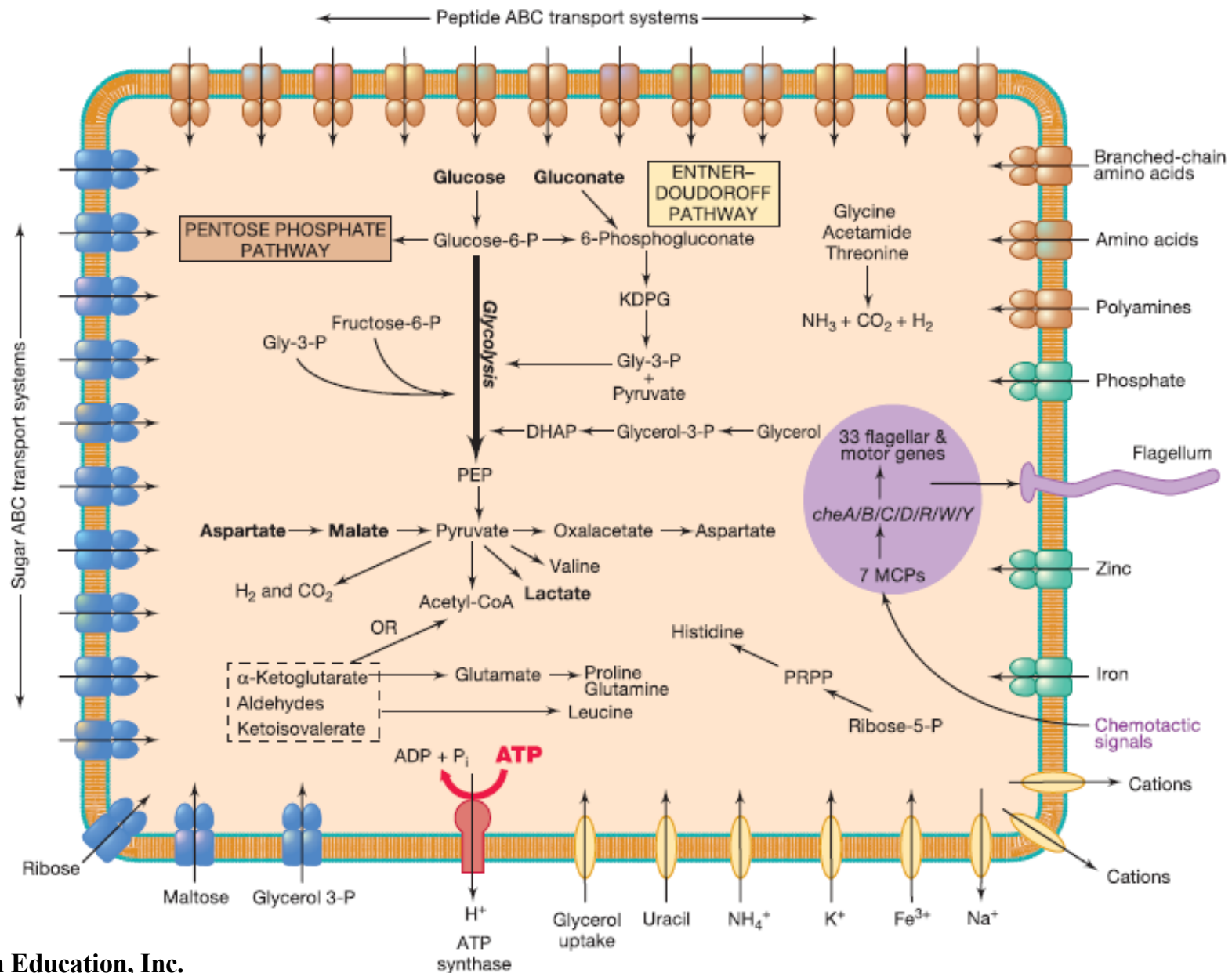
Functional Potential

Metabolic Pathways

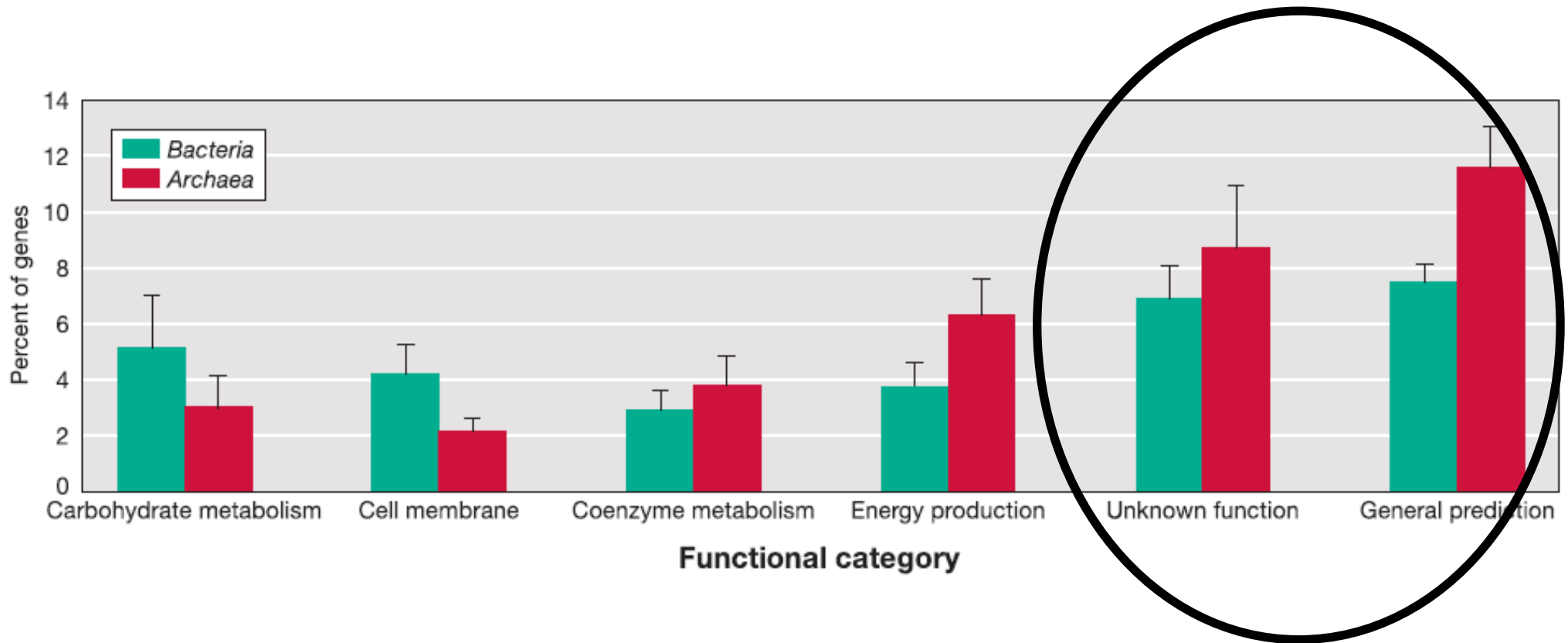
Horizontal Gene Transfer

...

Reconstruct Genomes



Categorize Functions



.....

Proteorhodopsin phototrophy in the ocean

Oded Bèjà^{*†}, Elena N. Spudich^{†‡}, John L. Spudich[‡], Marion Leclerc^{*}
& Edward F. DeLong^{*}

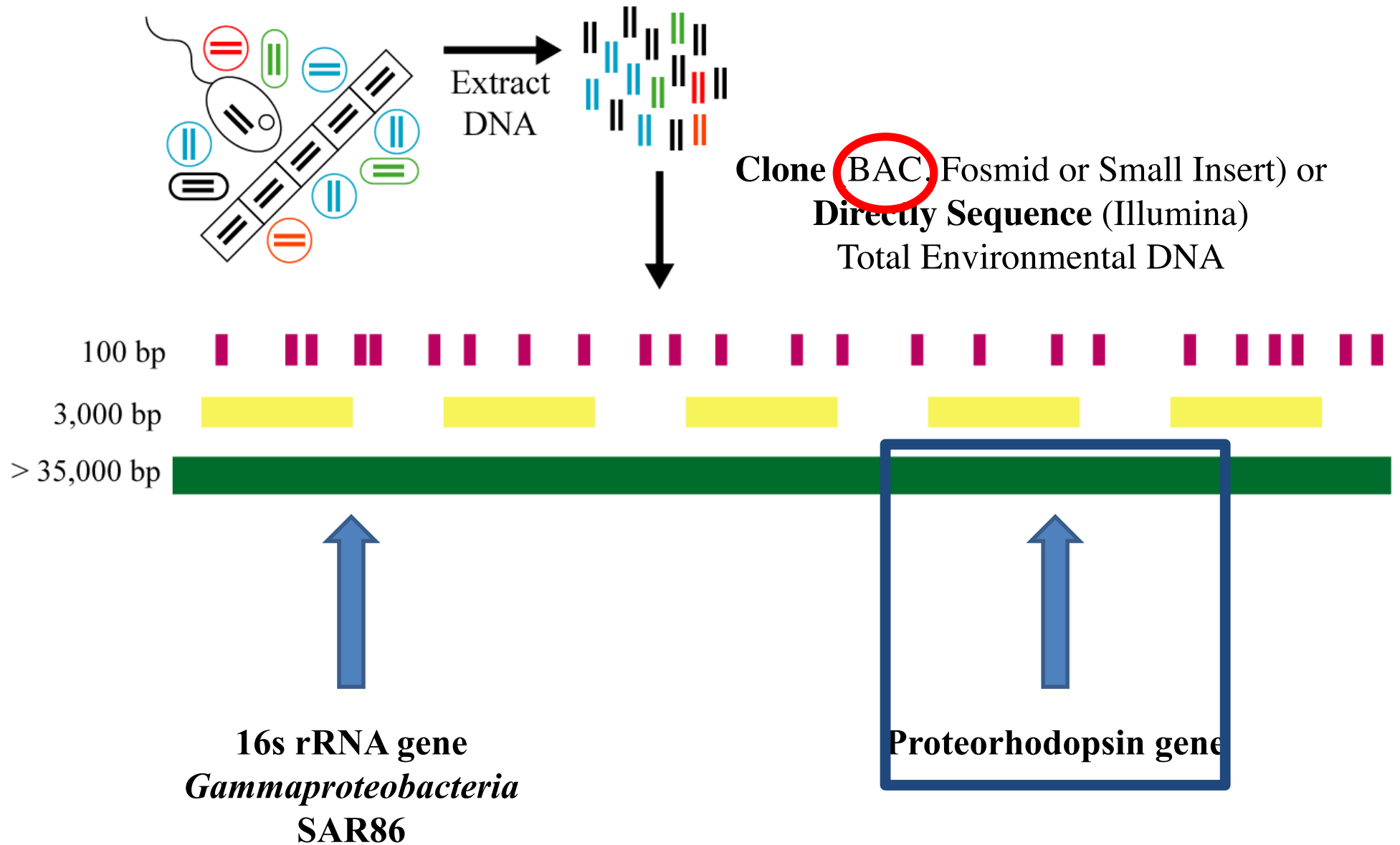
Bacterial Rhodopsin: Evidence for a New Type of Phototrophy in the Sea

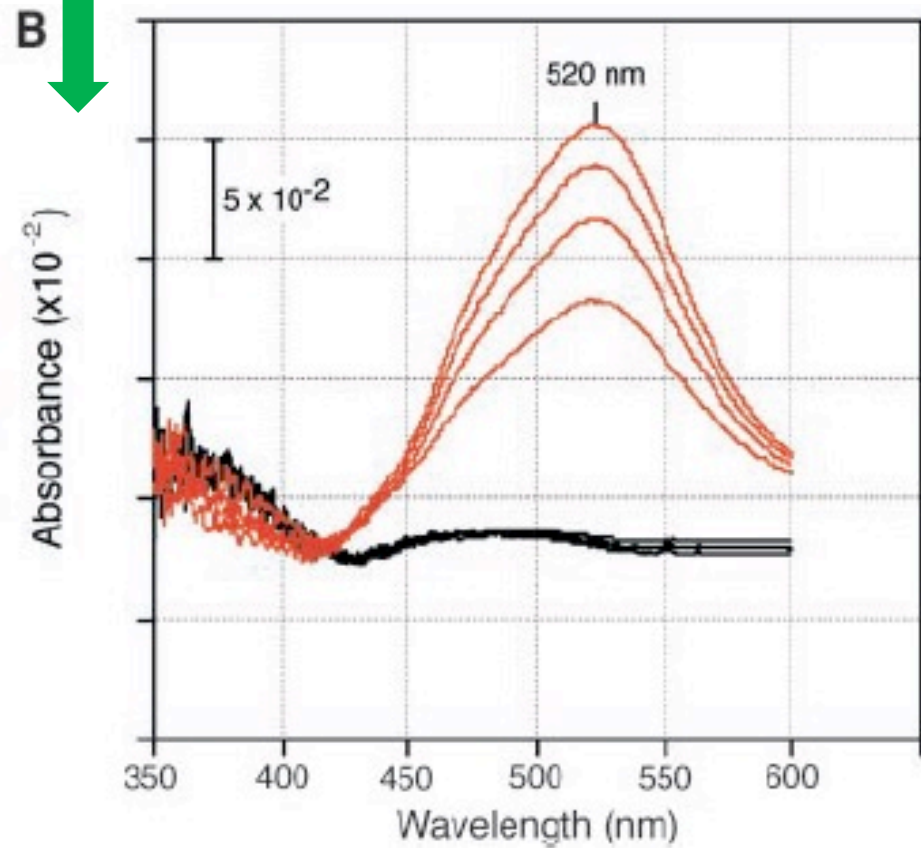
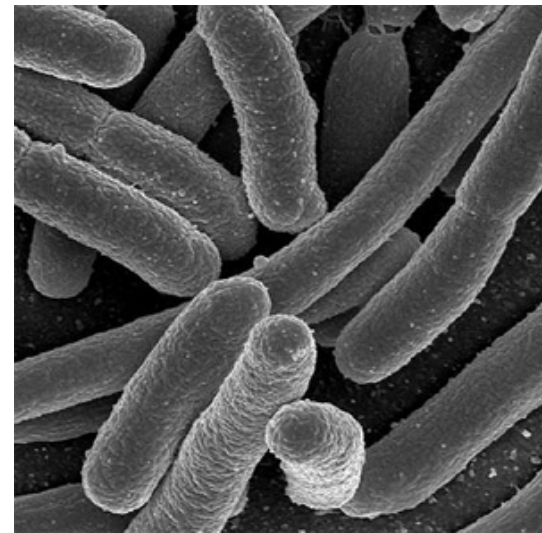
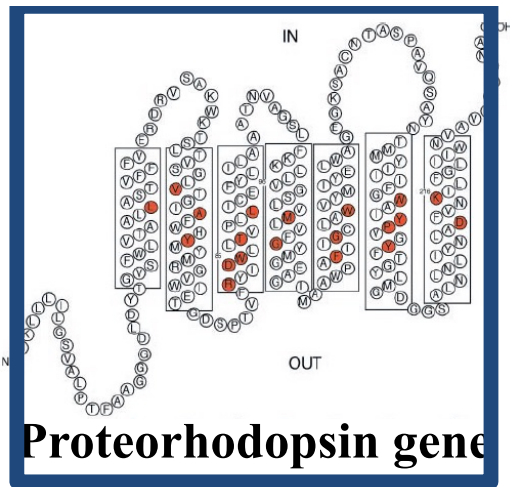
Oded Bèjà,¹ L. Aravind,² Eugene V. Koonin,²
Marcelino T. Suzuki,¹ Andrew Hadd,³ Linh P. Nguyen,³
Stevan B. Jovanovich,³ Christian M. Gates,³ Robert A. Feldman,³
John L. Spudich,⁴ Elena N. Spudich,⁴ Edward F. DeLong^{1*}

Proteorhodopsin genes are distributed among divergent marine bacterial taxa

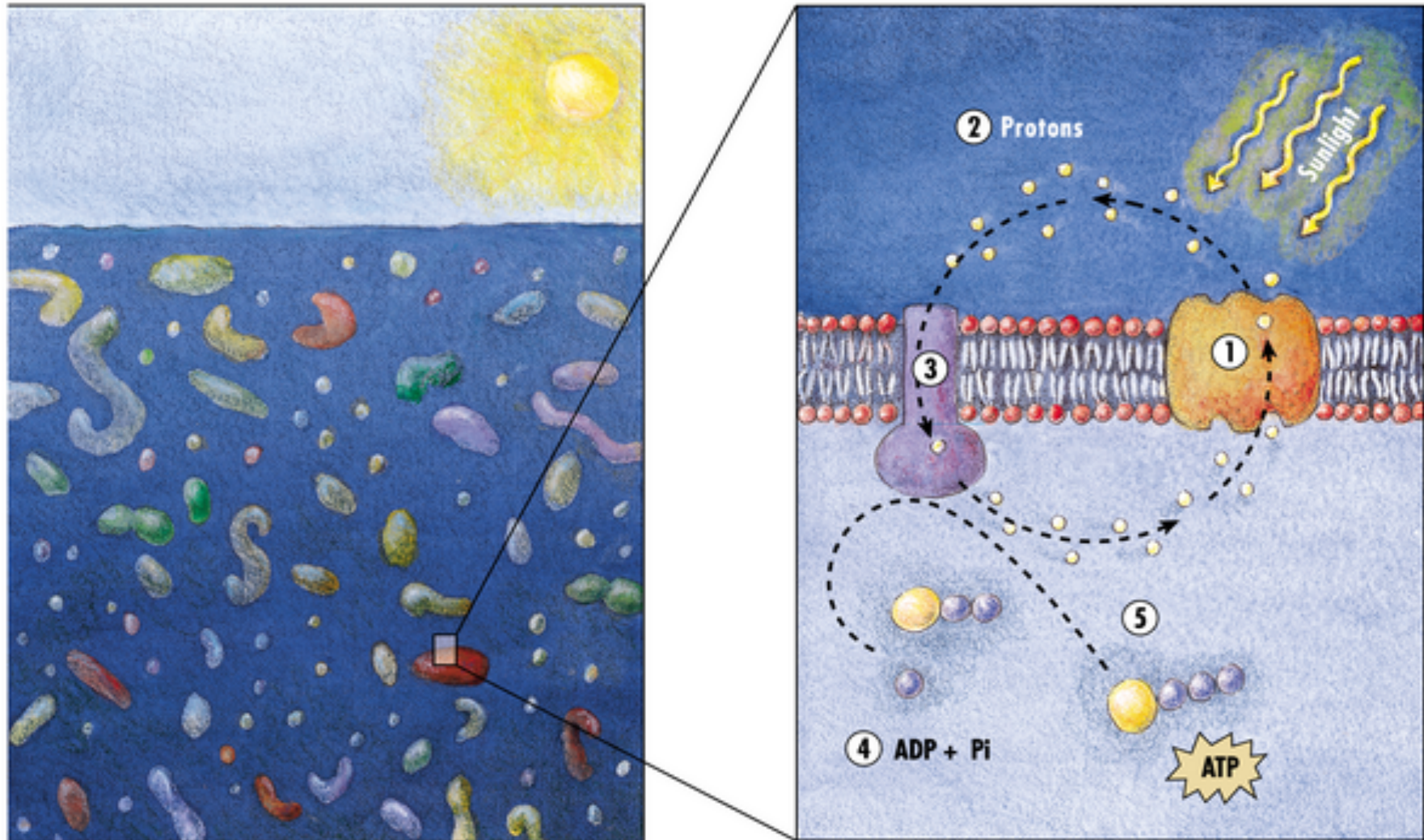
José R. de la Torre^{†‡}, Lynne M. Christianson[†], Oded Bèjà^{†§}, Marcelino T. Suzuki^{†¶}, David M. Karl^{||}, John Heidelberg^{**},
and Edward F. DeLong^{†,††}

Metagenomics





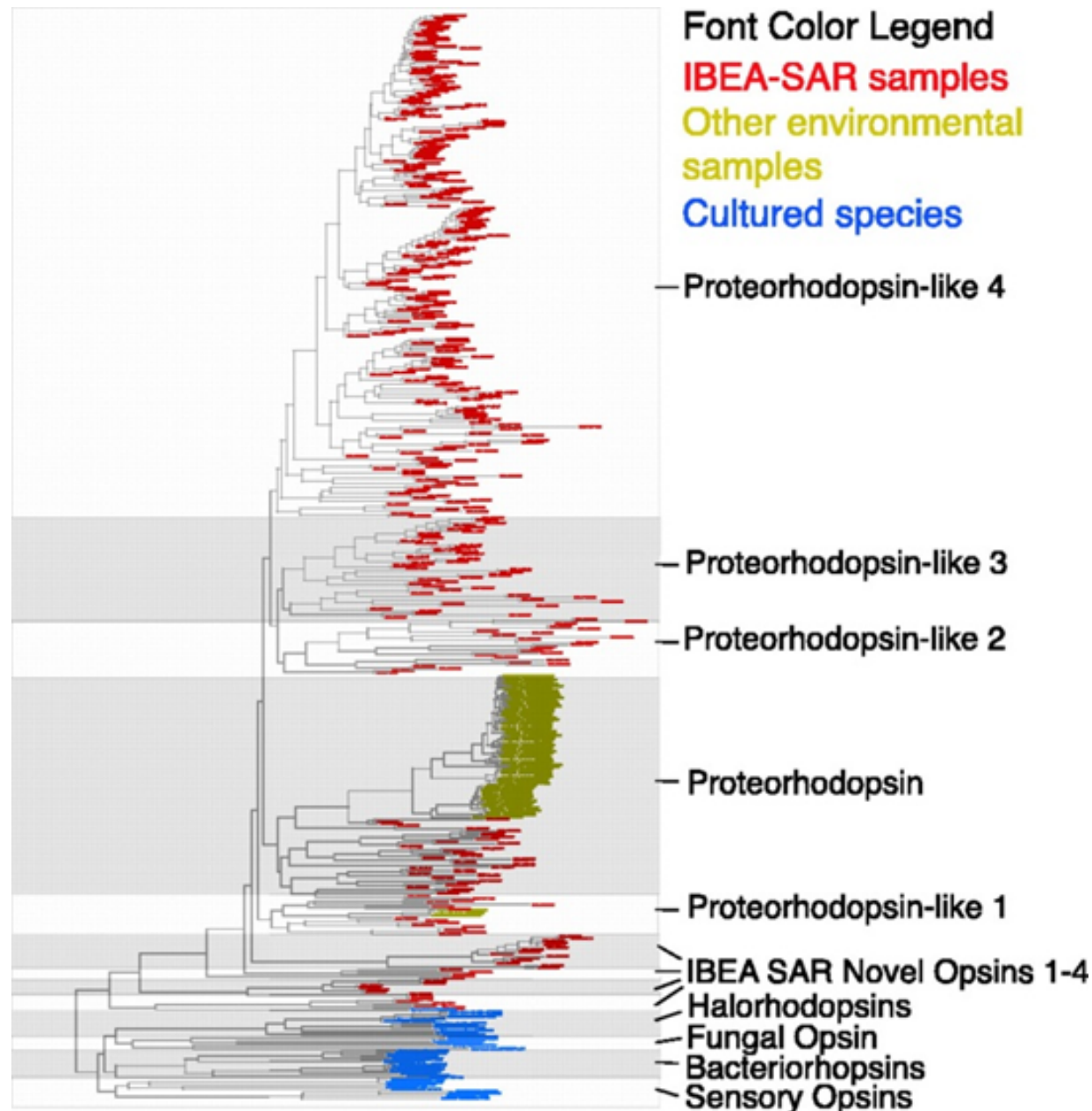
A new way of using sunlight in the surface ocean



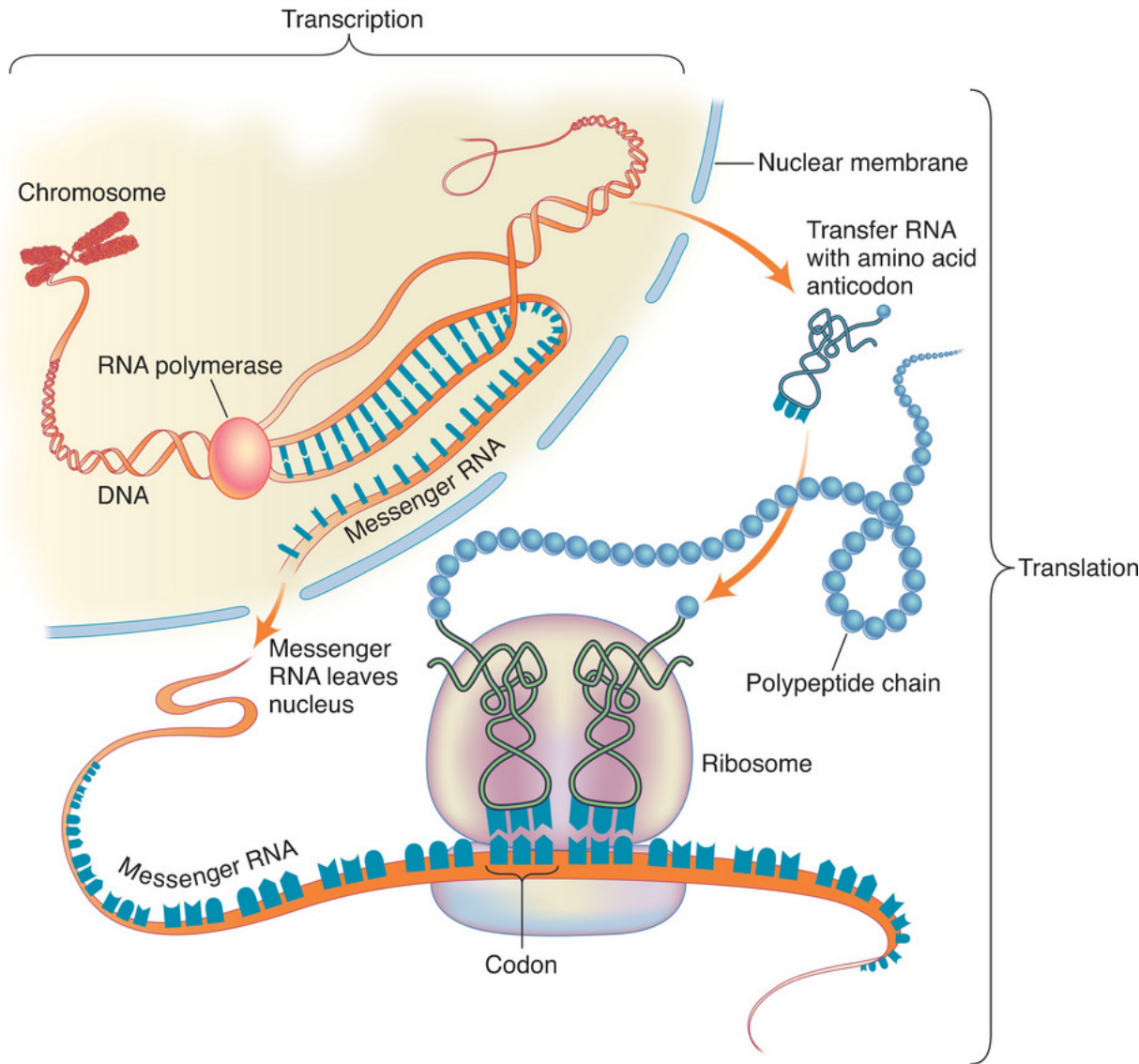
DeLong EF, Béjà O (2010) The Light-Driven Proton Pump Proteorhodopsin Enhances Bacterial Survival during Tough Times. *PLoS Biol* 8(4): e1000359. doi:10.1371/journal.pbio.1000359

<http://www.plosbiology.org/article/info:doi/10.1371/journal.pbio.1000359>

Proteorhodopsins occur in 13%-80% of marine bacteria and archaea in oceanic surface waters



The Central Dogma



DNA

Transcription

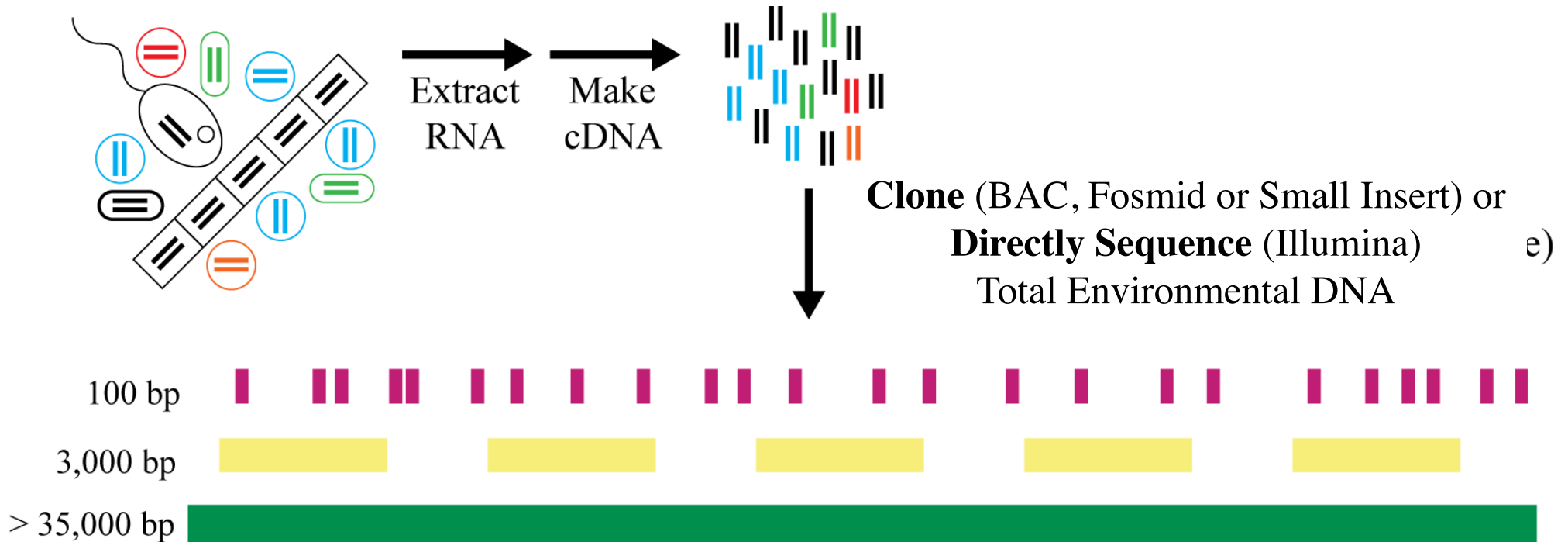
RNA

Translation

Protein

Moving from “who is there?” to “who is active?”

Metatranscriptomics



Access expressed genes of uncultured microbes
Looking at expression of defined genes via PCR
GeoChip-type analyses with RNA
Etc.

Stable Isotope Probing (SIP)

- Links specific metabolic activity to diversity using a stable isotope
- Microorganisms metabolizing stable isotope (e.g., ^{13}C) incorporate it into their DNA/RNA/Lipids
- Characterization of DNA/RNA/Lipids with ^{13}C can then be used to identify the organisms that metabolized the ^{13}C

RNA SIP

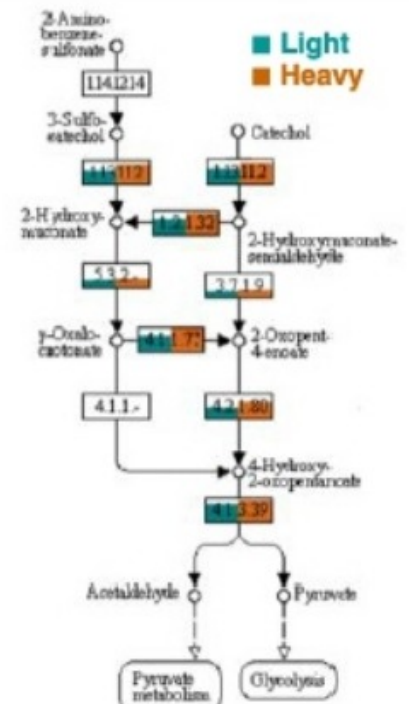
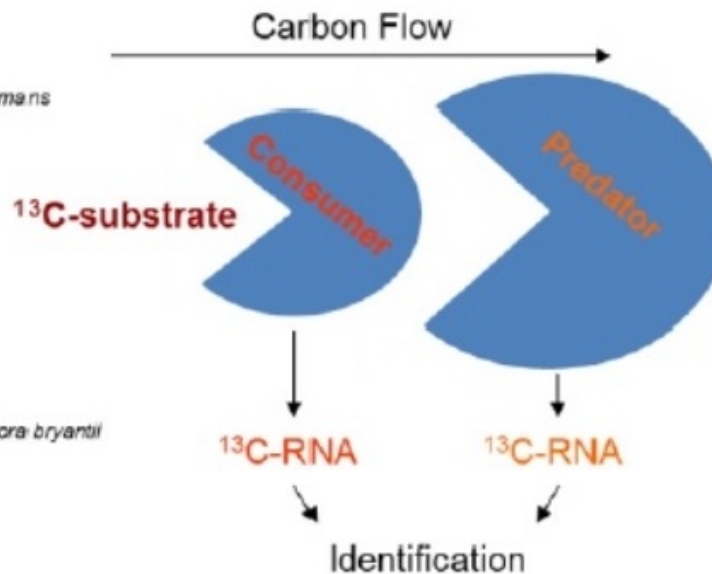
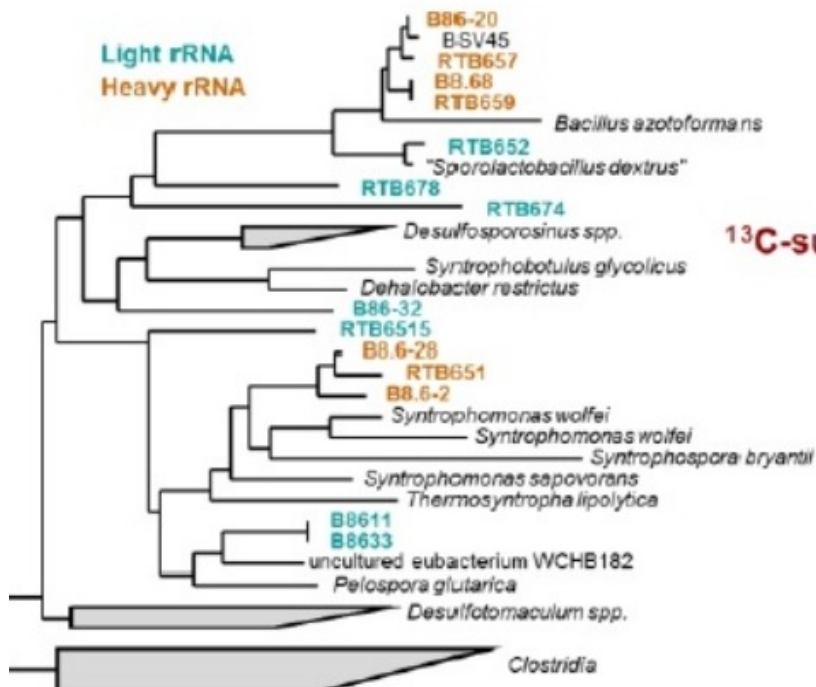
Labelling-based process query
(^{13}C , ^{15}N - or ^{18}O -substrates)

Physical RNA fractionation

Identification of
specifically active
microbes

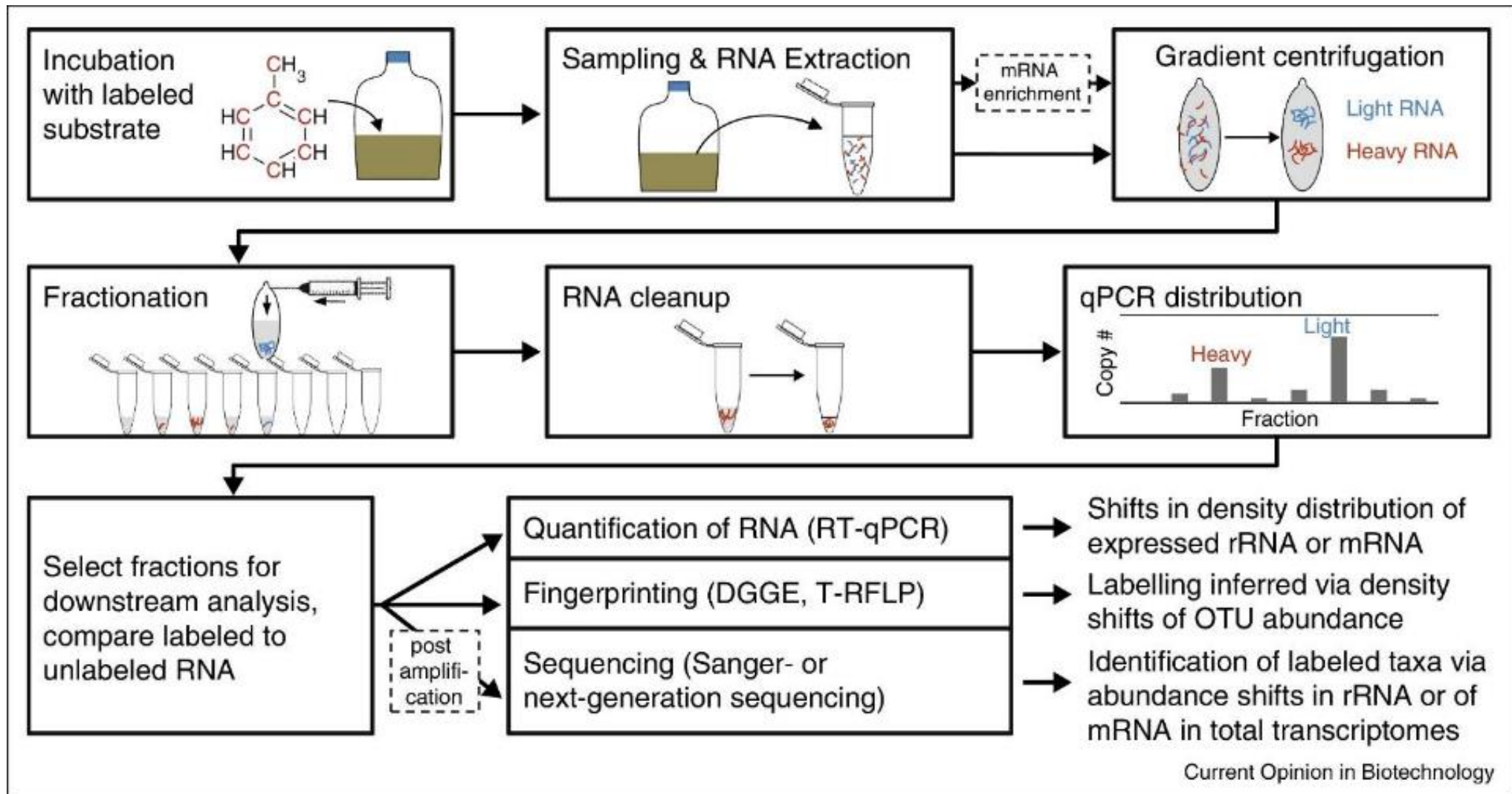
Carbon flow, interactions,
biotechnology

Process-targeted
transcriptomics

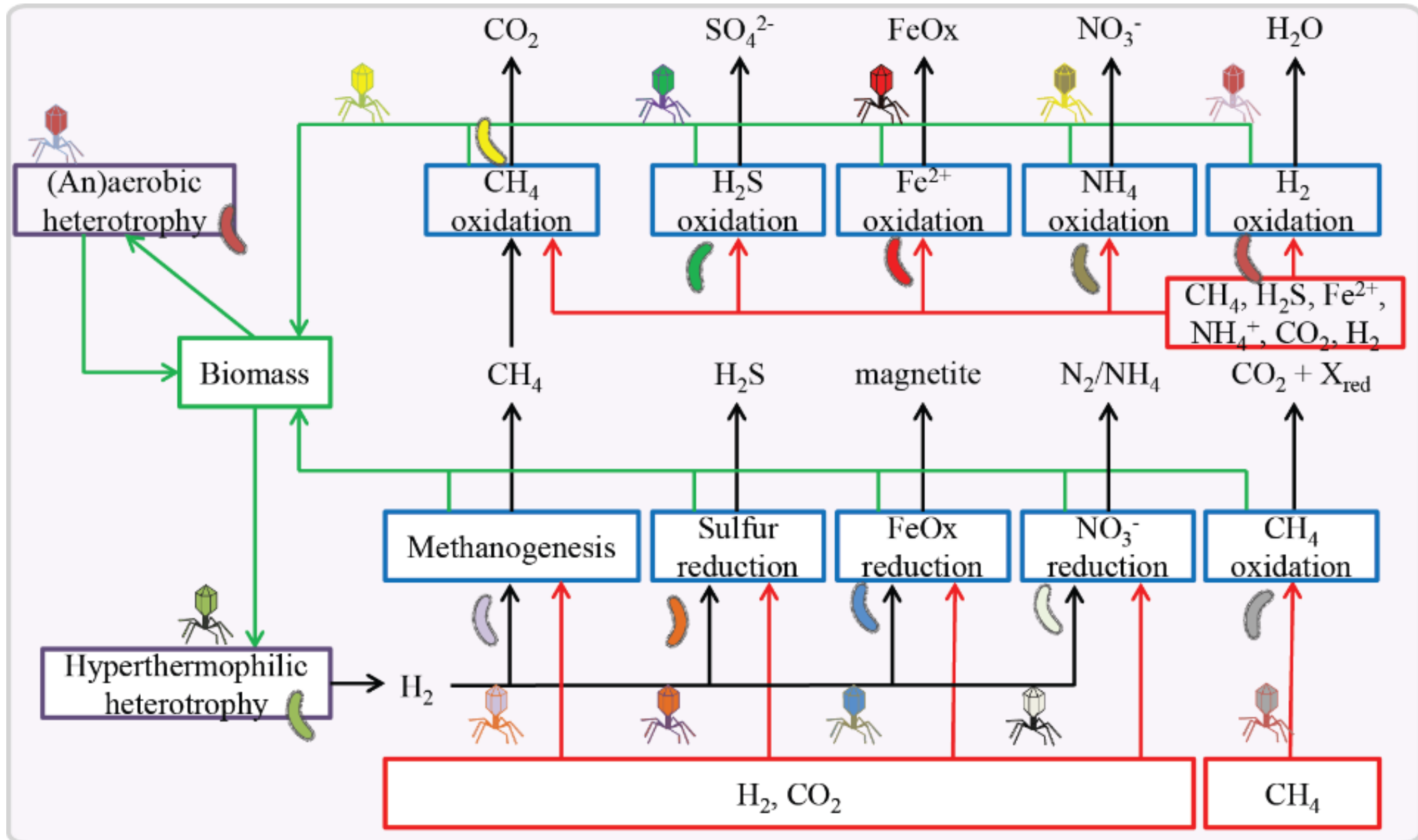


Lueders et al. 2016

RNA SIP

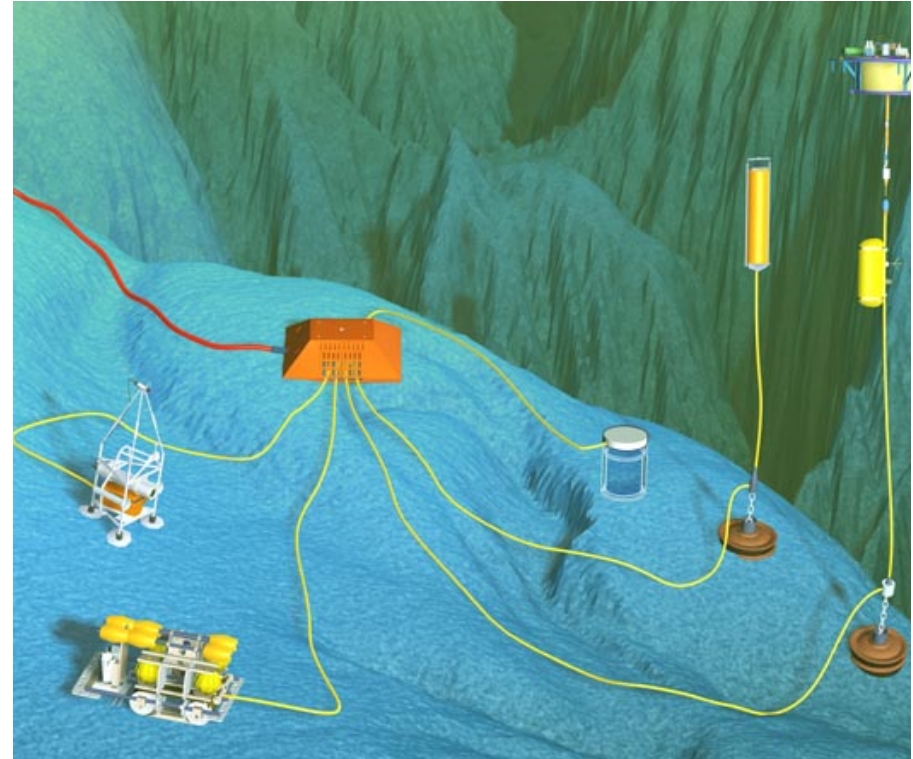


Diverse Metabolisms



And the list goes on...

- Optical tweezers
- Single cell genomics
- Meta-proteomics
- Microarrays
- Flow Cytometry
- Nano-SIMS FISH
- In-situ PCR and FISH
- ...



Cost per Genome

