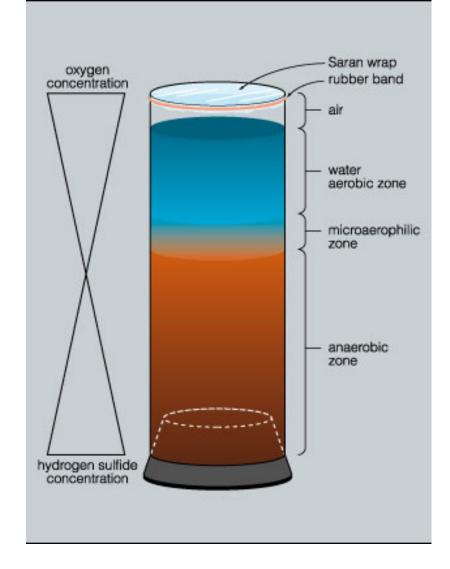
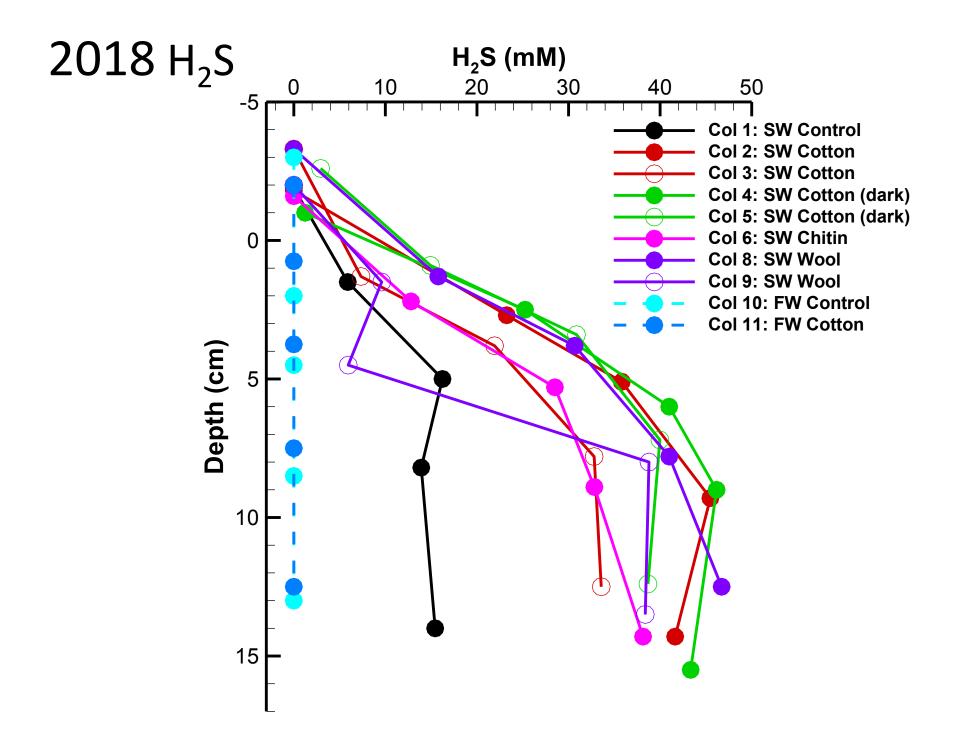
Day 3

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

Genes We Targeted

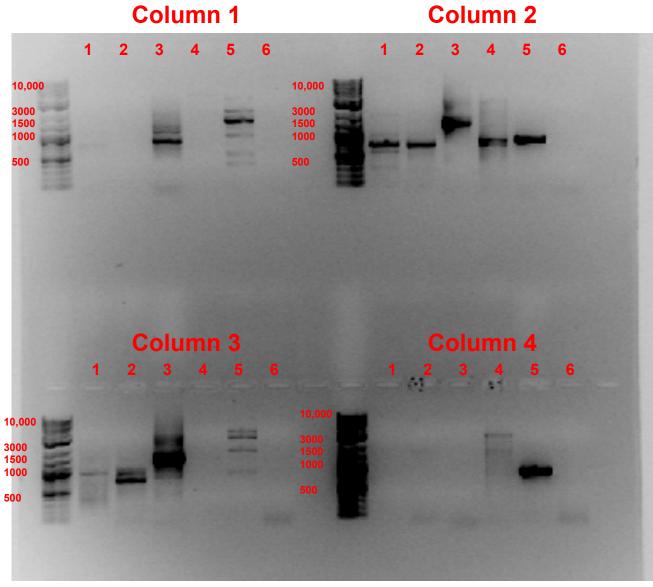
- 1: dsrAB 1800bp
- 2: mcrA 750bp
- 3: Bacteria 1450bp
- 4: Archaea 950bp
- 5: Archaea + 950bp
- 6: Negative control

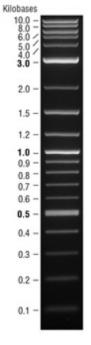




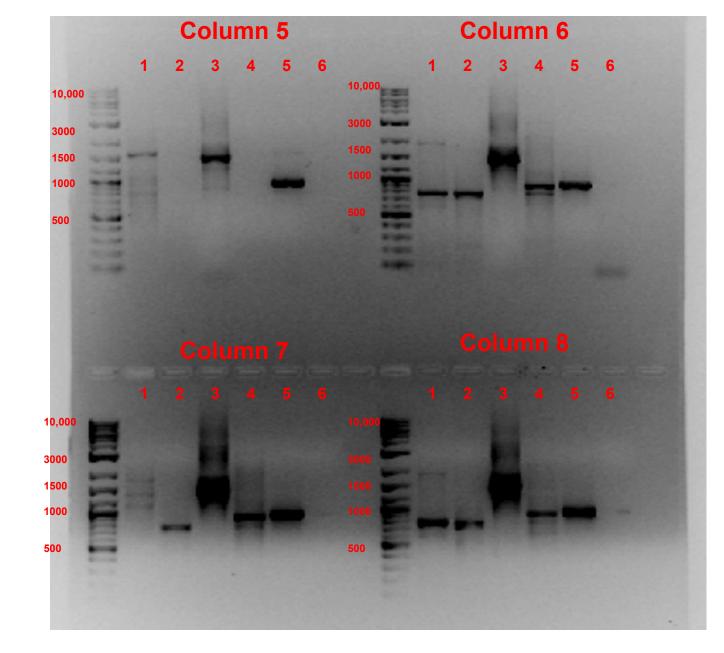
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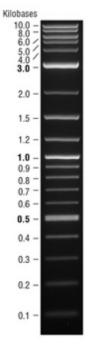
- 2: mcrA 750bp
- 3: Bacteria 1450bp
- 4: Archaea 950bp
- **5:** Archaea + 950bp
- 6: Negative control



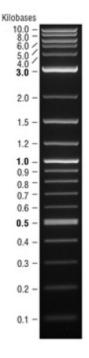


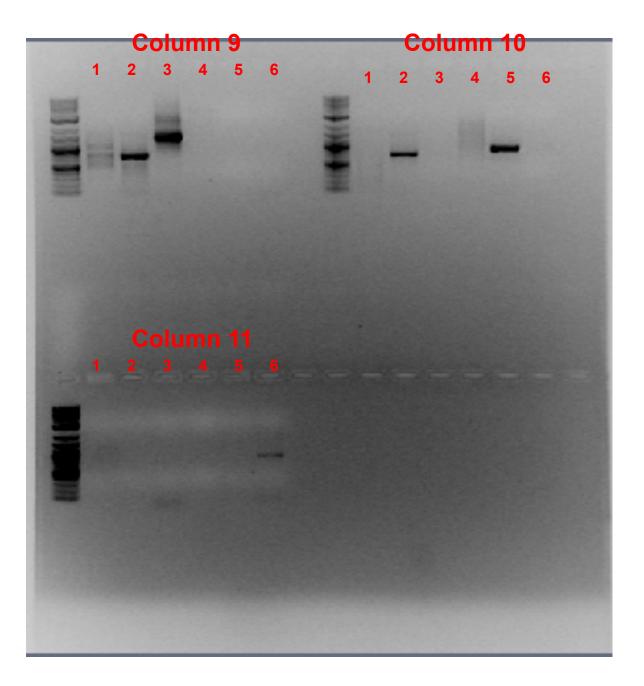
- 1: dsrAB 1800bp
- 2: mcrA 750bp
- 3: Bacteria 1450bp
- 4: Archaea 950bp
- **5:** Archaea + 950bp
- 6: Negative control





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





Some Problems with PCR

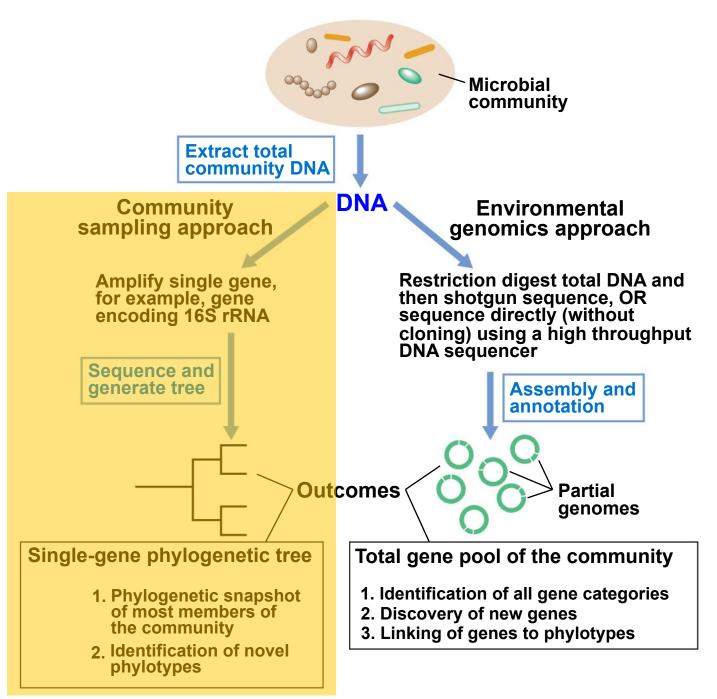
- Inhibitors in template DNA
- Amplification bias
- Gene copy number
- Limited by primer design
- Differential denaturation efficiency
- Chimeric PCR products may form
- Contamination w/ non-target DNA
- Potentially low sensitivity and resolution
- General screw-ups

(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		

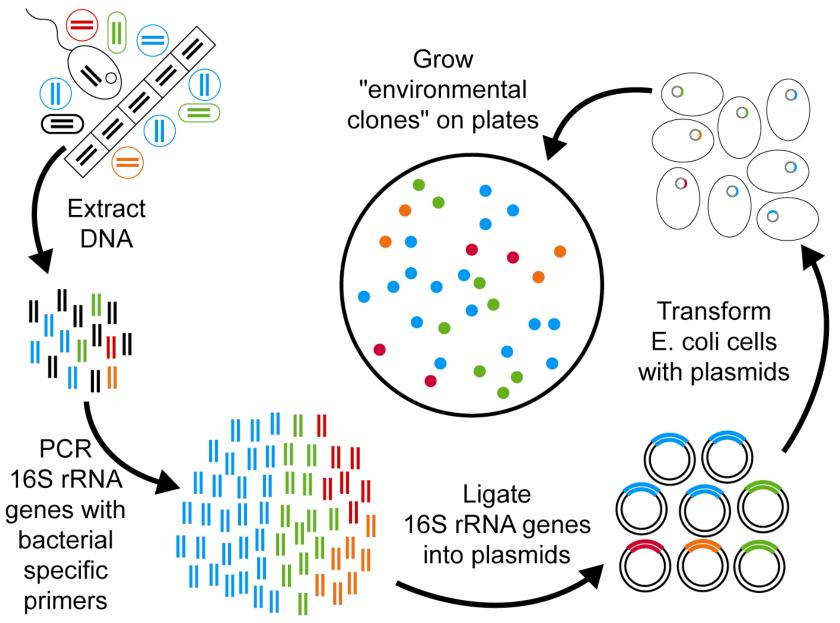
So you have a positive PCR product: Now what?

- 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)



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Traditional Gene Cloning

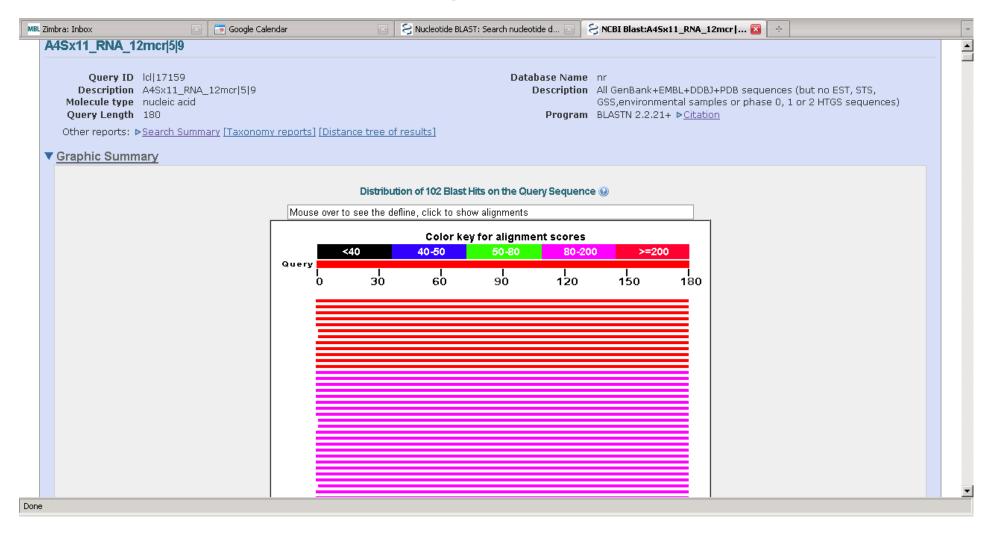


Schematic courtesy of B. Crump

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



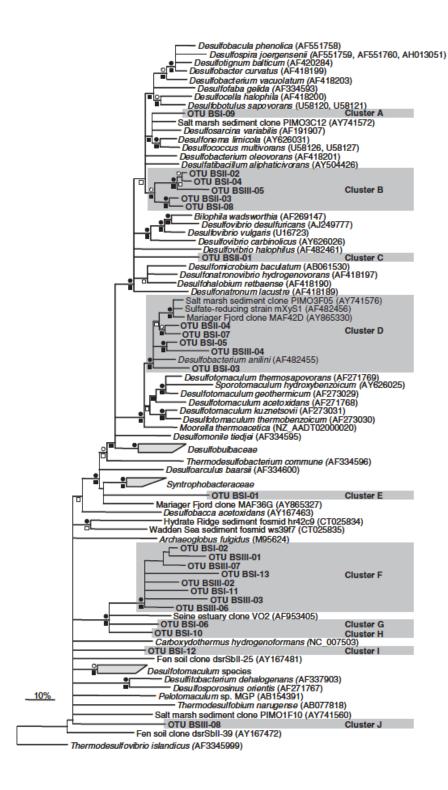
http://blast.ncbi.nlm.nih.gov/Blast.cgi

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.

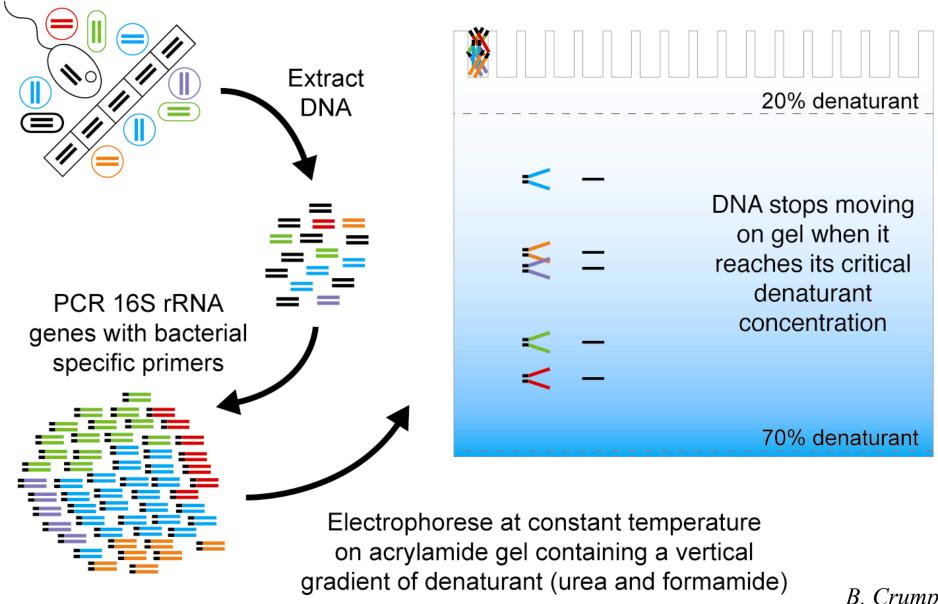
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501	2 Rabbit	CCAUGGUGACCACGGGUGACGGGGAAUCAGGGUUCGAUUCCGGAGAGGGAGCCUGAGAAACGGCUACCACAUCCAGA-AGGCAGGGGGGGGGG	
501	3 Shrimp	CCAUGGUUGCAACGGGUAACGGGGAAUCGGGGUUCGAUUCCGGAGAGGGAGCCUGAGAAACGGCUACCACAUCCAGAAAGGA-AGGCAGGGGGGGGGAAAUU	
501	4 <mark>T</mark> ermite	CCAUGGUUGUAA CGGGUAA CGGGGAAUCAGGGUUCGAUUCCGGAGAGGGAGCCUGAGAAACGGCUACCACAUCCAAGGA-AGGCAGGGGGGGGGG	
501	5 Drosophi	CCAUGGUUGCAA CGGGUAA CGGGGAAUCAGGGUUCGAUUCCGGAGAGGGAGCCUGAGAAACGGCUACCACAUCUAAGGA-AGGCAGGGGGGGGAAAUU	J
501	6 Sponge	CCAUGGUUGCAA CGGGUGACGGAGAAUUAGGGUUCGAUUCCGGAGAGGGAGCCUGAGAGAGGGCUACCACAUCCAAGA-AGGCAGGGGGGGGGG	J
501	7 Mucor	CAAUGCCUACAACGGGUAACGGGGAAUUAGGGUUCGAUUCCGGAGAGGGAGCCUGAGAAACGGCUACCACAUCCAGA-AGGCAGGCAGGGGGGGGGAAAUU	J
501	8 S. pombe	CCAUGGUUUUAA CGGGUAA CGGGGAAUUAGGGUUCGAUUCCGGAGAGGGAGCCUGAGAAACGGCUACCACAUCCAGA—AGGCAGGGGGGGGGG	J
501	9 <mark>C</mark> andida	CCAUGGUUUCAACGGGUAACGGGGAAUAAGGGUUCGAUUCCGGAGAGGGAGCCUGAGAAACGGGUACCACAUAUCCAAGA-AGGCAGGGGGGGGGG	J
501	10 Pneumocy	CCAUGGUUUCGACGGGUAACGGGGAAUAAGGGUUCUAUUCCGGAGAGGGAGCCUGAGAAACGGCUACCACAUAUCCAAGA-AGGCAGGGGGGGGGG	
501	11 Yeast	CCAUGGUUUCAACGGGUAACGGGGAAUAAGGGUUCGAUUCCGGAGAGGGAGCCUGAGAAACGGCUACCACAUAUCCAAGA-AGGCAGGCGGGGGGGAAAUU	J
501	12 Pennicil	CCAUGGUGGCAACGGGUAACGGGGAAUUAGGGUUCGAUUCCGGAGAGGGAGCCUGAGAAACGGGUACCACAUACGA-AGGCAGGGGGGGGGG	J
501	13 <mark>C</mark> orn	CCAUGGUGGUGA GGGUGA GGGAGAAUUAGGGUUGGAUUGGGAGAGGGAGG	J
501	14 Rice	CCAUGGUGGUGA GGGUGA GGGAGAAUUAGGGUUGGAUUCCGGAGAGGGGGGGGGG	J
501	15 <mark>T</mark> omato	CCAUGGUGGUGA GGGUGA GGAGAAUUAGGGUU GAUUCCGGAGAGGGGGGGGGG	J
501	16 Volvox	CCAUGGUGGUAA GGGUGA GGAGGAUUAGGGUUGGAUUCCGGAGAGGGGGGGGGG	J
501	17 <mark>C</mark> hlorell	CCAUGGUGGUAA GGGUGA GGAGGAUUAGGGUUGGAUUCCGGAGAGGGGGGGGGG	J
501	18 Porphyra	CCAUGGUUGUGA GGGUAA GGACCGUGGGGGGGGGGGGG	J
501	19 <mark>G</mark> racilar	CCAUGGUUGUGA GGGUAA GGACCGUGGGGGGGGGGGGG	J
501	20 Parameci	CCAUGGCAGUCACGGGUAACGGAGAAUUAGGGUUCGAUUCCGGAGAGGGGGGGG	J
501	21 <mark>T</mark> etrahym	CCAUGGCAGUCACGGGUAACGGAGAAUUAGGGUUCGAUUCCGGAGAAGGAGCCUGAGAAACGGCUACUACAACUACGGUUCGGCAGGAAGAAAAAUU	J
501	22 Dinoflag	CCGUGGCAAUGACGGGUAACGGAGAAUUAGGGUUUGAUUCCGGAGAGGGAGCCUGAGAAACGGCUACCACAUCUAAGGA-AGGCAGGGGGGGGGG	J
501	23 <mark>T</mark> oxoplas	CCGUGGCAGUGACGGGUAACGGGGAAUUAGGGUUCGAUUCCGGAGAGGGGGGGG	J
501	24 <mark>T</mark> heileri	CCGGGGCAGCGACGGGUAACGGGGAAUUAGGGUUCGAUUCCGGAGAGGGGGGGG	J
501	25 <mark>A</mark> chlya	CCAUGGCGUUAACGGGUAACGGGGAAUUAGGGUUUGAUUCCGGAGAGGGGGGGG	
501	26 Phytopht	CCAUGGCAUUAA CGGGUAA CGGGGAAUUAGGGUUUGAUUCCGGAGAGGGAGCCUUAGAAACGGCUACCACAUCCAAGA—AGGCAGGAGGGGGGUAAAUU	J
501	27 Diatom	CCAUGGCUUUAACGGGUAACGGGAAAUUAGGGUUUGAUUCCGGAGAGGGGGGGCCUGAGAGACGGCUACCACAUCCAAGGA-AGGCAGGGGGGGGGG	J
501	28 Ochromon	CCAUGGCAUUAACGGGUAACGGAGAAUUAGGGUUCGAUUCCGGAGAGGGAGCCUGAGAAAUGGCUACCACAUCCAAGGA-AGGCAGGAGGGGGGGUAAAU	J
501	29 Synura	CCAUGGCUUUAACGGGUAACGGAGAAUUAGGGUUCGAUUCCGGAGAGGGGGGGG	J
501	30 Brown Al	CCAUGGCUUUAACGGGUAACGGGGAAUUGGGGUUCGAUUCCGGAGAGGGGGGGG	J
501	31 Dictyost	CCAUGGUUGUAA CGGGUAA CGGGGAA UUAGGGUUCGAUUCCGGAGAGGGGGGGGGG	J
501	32 Euglena	CAGUGGCCUUGACGGGUAACGGAGAAUCAGGGUUCGAUUCCGGAGAGGGAGCCUGAGAGACGGCUACCACUACCAAGGU-GGGCAGGGAGGGAAGGCACGGAAAUU	J
501	33 T rypanos	CCAUGGCGUUGACGGG-AGCGGGGGGGGGGGGGGGGGGGG	
501	34 Leishman	CCAUGECCUUGACEGE-AGCEGEGEAUUAEGGUUCGAUUCCEGAGAGEGAGCCUGAGAAAUAGCUACCACUUCUACEGA-EGGCAGGAGEGEGEGAAAU	
501	35 <mark>C</mark> rithidi	CCAUGGCGUUGACGGG-AGCGGGGGGUUAGGGUUCGAUUCCGGAGAGGGAGCCUGAGAAAUAGCUACCACUUCUACGGA-GGGCAGGGGGGGGGG	

1050	1 Human	CC-GCCCCU	UGCCUCUCGGCGCCCCCCUCGAUGCUC-UUAGCU-GAGU-G-U-CCCGCGGGGGCCCGAAGC-GUUUACUUUGAA
1050	2 Rabbit	CC- <mark>G</mark> CCCC <mark>U</mark>	<mark>VGCC</mark> VCVCGGCGCCCCCCVCCAVGCVC-UVAGCV-GAGV-G-V-CCCCCCGCGGGGCCCCGAAGC-GUUVACUUVGAA
1050	3 Shrimp	<mark>CG</mark> <mark>GAC</mark> A	<mark>AUU-CAUUGGAUCGUUCGGGGUGCUC-UUAACC-GAGU-G-U-CC</mark> UG-GGUGGCCGAUAC-GUUUACUUUGAA
1050	4 Drosophi	-AUGUUCCU	<mark>CC</mark> UAUUUAAAAACCUGCAUUAGUGCUC-UUAAAC-GAGU-G-U-UAUU-GUGGGCCGGUAC-UAUUACUUUGAA
1050	5 Sponge	CCCCCUU	CCUCUCGAAA-CCC-CCG-ACUCCUC-UUCACUCCAGU-C-CUCCGG-UACUUCGGGAC-CUUUACUUUGAA
1050	6 Mucor	CGCUUUUAU-CGA	AGGCUUUUUUUUUUUUUUGUGUUAUGCU-A-UGAAUAGCUUCGGUU-GUUU-A-UAGUCUCUAGCCAG-AU-GAUUACCAUGAG
1050	7 S. pombe	GGGGUCGUUAA	<mark>CC</mark> UU <mark>CUGGCAAACU-A-CUCAUGUUC</mark> UUUAUU-CAGC-G-UGGUAGGGAACCAGGAC-UUUUACCUUGAA
1050	8 Candida	GAGCCUUU	<mark>CC</mark> UU <mark>CUGGC</mark> UAACC-AUUCGCCCCUUGUGGU-CUUUGGCGAACCAGGAC-UUUUACUUUGAA
1050	9 Pneumocy	GAUCCUUC	<mark>CC</mark> U <mark>CCUGGAUUACC</mark> -GGCUGCCCUUCGCU-GGGU-G-UGCCGGAUAGCCAGGGCAUUUUACUUUGAG
1050	10 Yeast	GGGCCUUU	<mark>CC</mark> UU <mark>CUGGC</mark> U <mark>AACC-U</mark> <mark>UGAGUCC</mark> U <mark>UGU-GGC</mark> <mark>U-C-UUGG</mark> <mark>CGAACCAGGAC-UUUUAC</mark> UUUGAA
1050	11 Pennicil	GGACCUUU	<mark>CC</mark> UU <mark>C</mark> UGGGGAACC-UCAUGGCCUUCACU-GGCU-G-UGG-GGGGAACCAGGAC-UUUUACUGUGAA
1050	12 Corn	CCCUU	<mark>CUGCCGGCGAUGC</mark> GCUCCUGGCC-UUAACU-GGCC-G-GGUC-GUGCCUCCGG-GCCGUUACUUUGAA
1050	13 Rice	CCCUU	<mark>CUGCCGGCGAUGC</mark> GCUCCUGGCC-UUAACU-GGCC-G-GGUUCGUGCCUCCGGCGCCGUUACUUUGAA
1050	14 <mark>T</mark> omato	CCCDCCCDD	<mark>CUGUCGGCGAUGC</mark> GCUCCUGGCC-UUAAUU-GGCC-G-GGUC-GUGCCUCCGGCGCCUGUUACUUUGAA
1050	15 Volvox	CCACCUUC	<mark>CUGCCGGGGACGGGCUCC</mark> UGGGC-UUCACU-GUAU-G-GGACUCGGAGUC-GGCGAGGUUACUUUGAG
1050	16 <mark>C</mark> hlorell	CACCUUG	<mark>UUGCO</mark> GGGGAOGGGOUCOUGGGC-UUCAOU-GUCC-G-GGAOUCGGAGUO-GGOCOUGUUAOUUUGAG
1050	17 Porphyra	<mark>_</mark> UUUU	GUGGAGGGGGGGGGGUUGUGGC-UUGAGUGUGGG-GGGGUGUGGGGGGCGACGGUUUAGUGUGAA
1050	18 <mark>G</mark> racilar	<mark>CC</mark> VVV	<mark>GUGGAGAGGGG-GUGU</mark> GGUGG-UG <mark>C</mark> -UUGAGUG <mark>GGU</mark> -G <mark>CC</mark> AUG <mark>C</mark> UG <mark>CCGCCACC</mark> GUUUA <mark>C</mark> UGUGAA
1050	19 Parameci	CUCCGU	<mark>CUACAAUCCCUUU</mark> <mark>UGCGCU-UUAGGGUUGC</mark> <mark>A-GCUGGG</mark> <mark>CGAGU-AGAC</mark> -AAUUUACCUUGAA
1050	20 <mark>T</mark> etrahym	<mark>UACCU</mark>	<mark>UUGC</mark> AAACUAAAAUCGGCC-UUCACUGGUUC-G-ACUUAGGCAGU-AAAC-AUUUUACUGUGAA
1050	21 Dinoflag	CUUGACAUC	UUCCU-AAAGA-ACGUAUCUGCAC-UUCAUU-GUGU-GGUGCGGUAUUUAGGAC-AUUUACCUUGAG
1050	22 <mark>T</mark> oxoplas	UCUAGCAUC	<mark>CUUCU-GGAUU-UCU-C</mark> CACACUUCAUU-GUGU-GGAGUUUUUU-CCAGGAC-UUUUACUUUGAG
1050	23 <mark>T</mark> heileri	UCGGUU	<mark>0</mark> UUU <mark>CG</mark> GGAU-GAUUACUUGAG
1050	24 <mark>A</mark> chlya		<mark>UUUGUGAGGAU</mark> G <mark>GUUU-UGUGCC</mark> -AUU <mark>GAGUUGGU-G-GUUGAG</mark> U <mark>AGAGUUGGAUGGUUUAG</mark> UGUGAA
1050	25 Phytopht		<mark>uuugugagggu</mark> g <mark>ccuu-ugugcc</mark> -auu <u>aaguuggu-g-gguugg</u> uggg <mark>c</mark> uug <mark>c</mark> au <mark>gguuuac</mark> ugugaa
1050	26 Diatom		UUGGGUGG-AACCUG-UGUGGC-AUUAGGUUGUC-G-U-GCAGGGGAUGCCCAUCAUUUACUGUGAA
1050	27 Ochromon	GAAUCAUCC	<mark>UCGAGAGG-AA</mark> CACG-UCUGUC-AUUCAGUUGAU-G-G-GCGUGGGAUUCUCUCUCUCUCACUGUGAG
1050	28 Synura	C <mark>GUC</mark> CAUCC	UCGCGGAG- <mark>AA</mark> CGCA-UCUGGC-AUUAAGUUGUC-G-G-GUGUGGYAUCCUCGUCAUUUACUGUGAG
1050	29 Brown 🗛	CCCCCCCAUUC	UCGGGUAG-CGUGUU-GCUGGC-AUUAGGUUGUC-G-G-C-UUCGCGGCCG-UCGUUUGCUGGGAA
1050	30 Dictyost	U-CUUAAUA	GUUGAGGUUGU-AUU-A-UGUUUG-A-UAGUGGUUGUUUGGAGAUUUGAGUGUGAG
1050	31 Euglena	ACCCAGCC	UGGAGCUG-GGUAGUCU-ACCUCUGGUCCACCAC-C-GGAGCCCACCGUCUUGG-ACACCCUGGA
1050	32 <mark>T</mark> rypanos		<mark>UUUUAC</mark> UGUGAC
1050	33 Leishman		
1050	34 <mark>C</mark> rithidi		UUUUUA <mark>@</mark> U <mark>G</mark> UGA@



- 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)

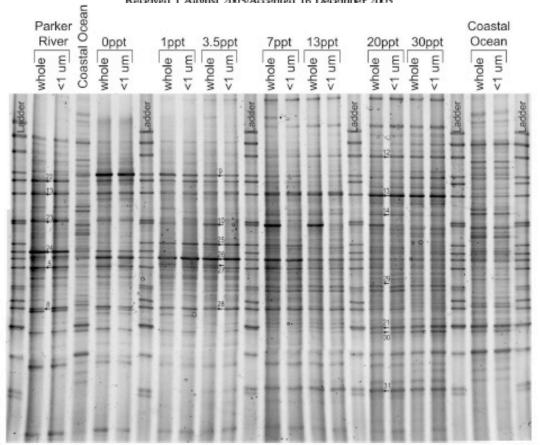


B. Crump

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

Byron C. Crump,1* Charles S. Hopkinson,2 Mitchell L. Sogin,3 and John E. Hobbie2

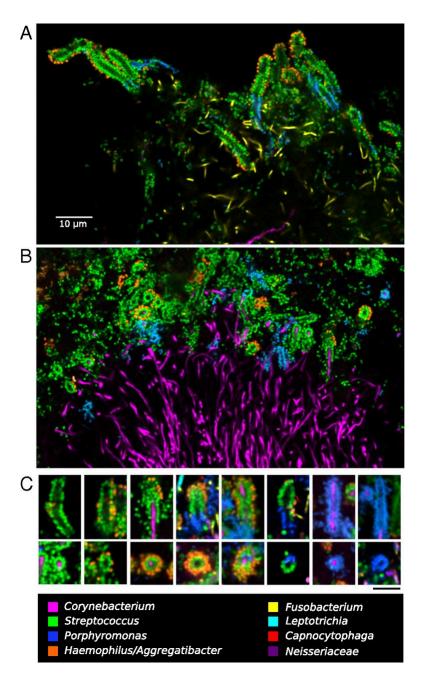
Hom Point Laboratory, University of Maryland Center for Environmental Science, Cambridge, Maryland, 1 and The Ecosystems Center² and The Josephine Bay Paul Center for Comparative Molecular Biology and Evolution,3 Marine Biological Laboratory, Woods Hole, Massachusetts

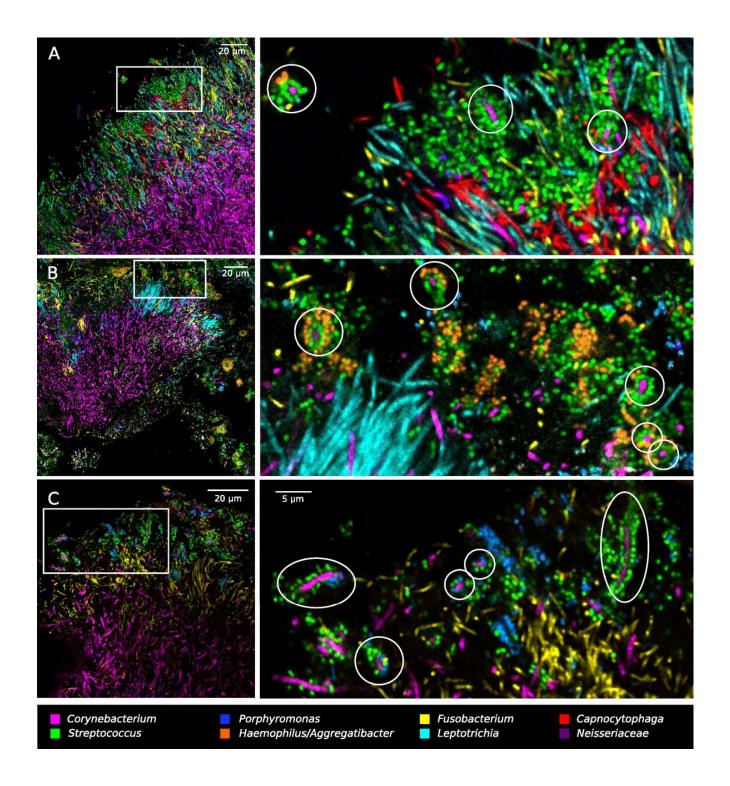


Received 1 August 2003/Accented 16 December 2003

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





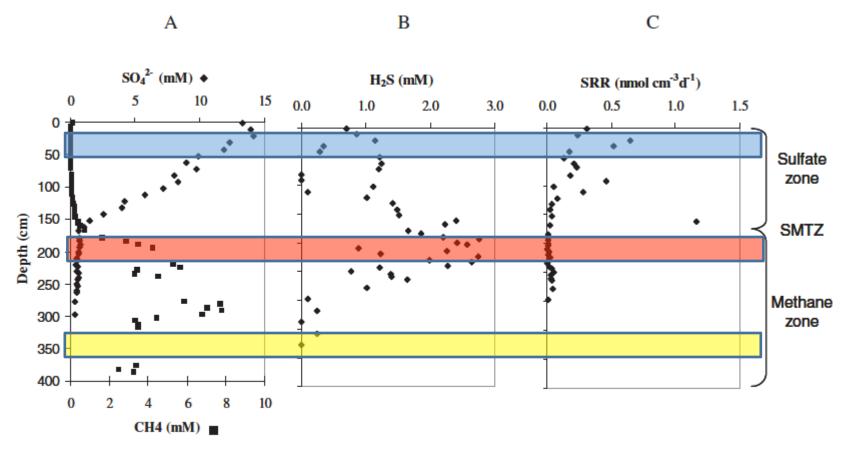
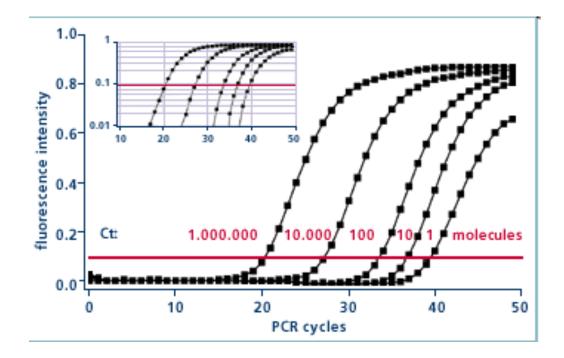


Fig. 2. Biogeochemical zonation and data from the Black Sea sediment core P824-GC.

- 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

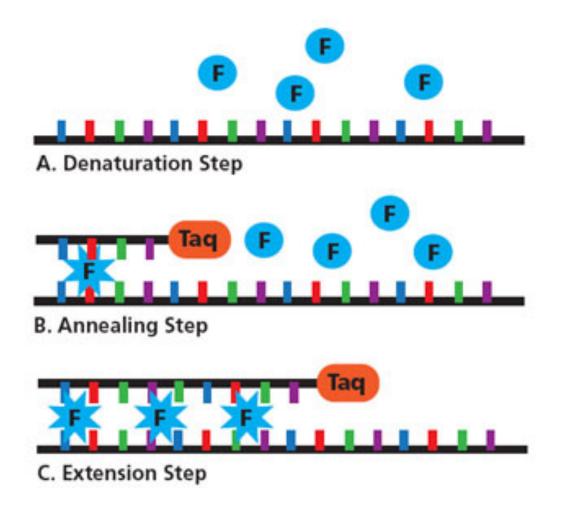
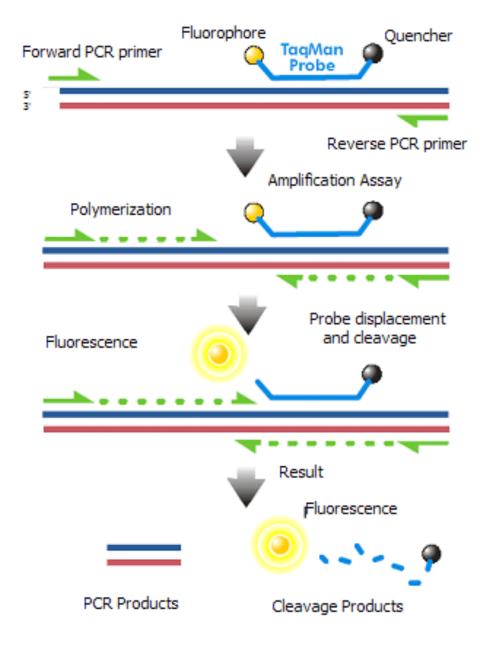


Figure 2: Fluorescent Dyes in qPCR

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

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

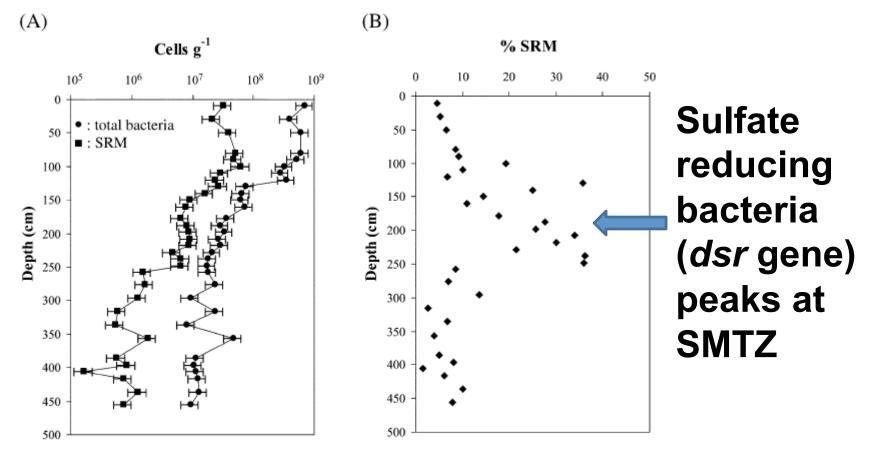


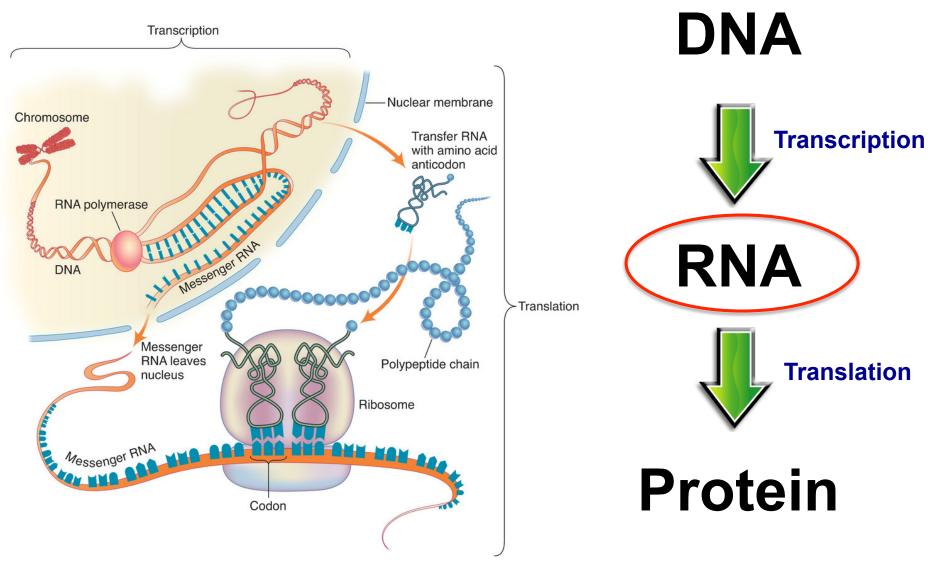
Fig. 3. Abundance of total bacteria and SRM in the Black Sea sediment core P824-GC.

A. Total bacteria and SRM as inferred from real-time PCR data. Values are given as mean ± 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).

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

The Central Dogma



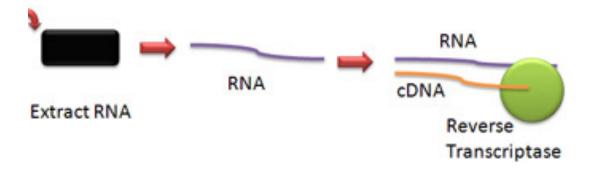
Moving from "who is there?" to "who is active?"

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

RT-PCR

RNA + Reverse Transcriptase + dNTPs > cDNA



- cDNA + Primers + Taq + dNTPs pene 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[⊽]

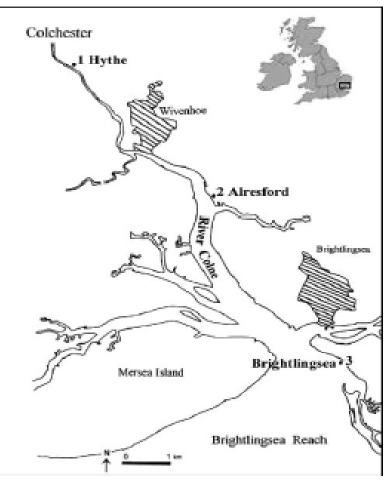
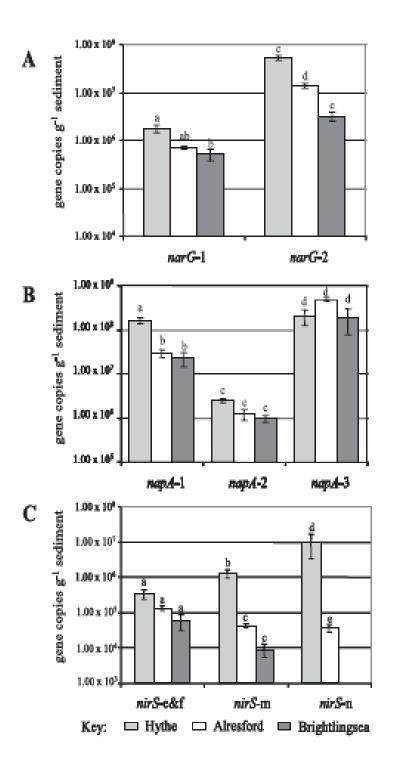


FIG. 1. Map of the Colne estuary, Essex, United Kingdom, showing the locations of the three sampling sites (Hythe, Alresford, and Brightlingsea).

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

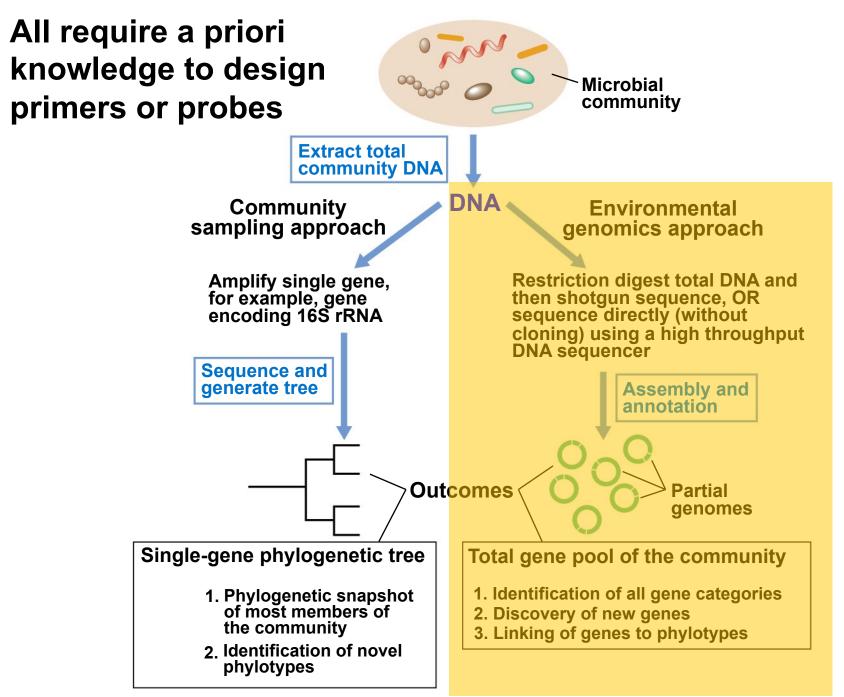
TABLE 1. Primer and probe sets used for Q-(RT)-PCR

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



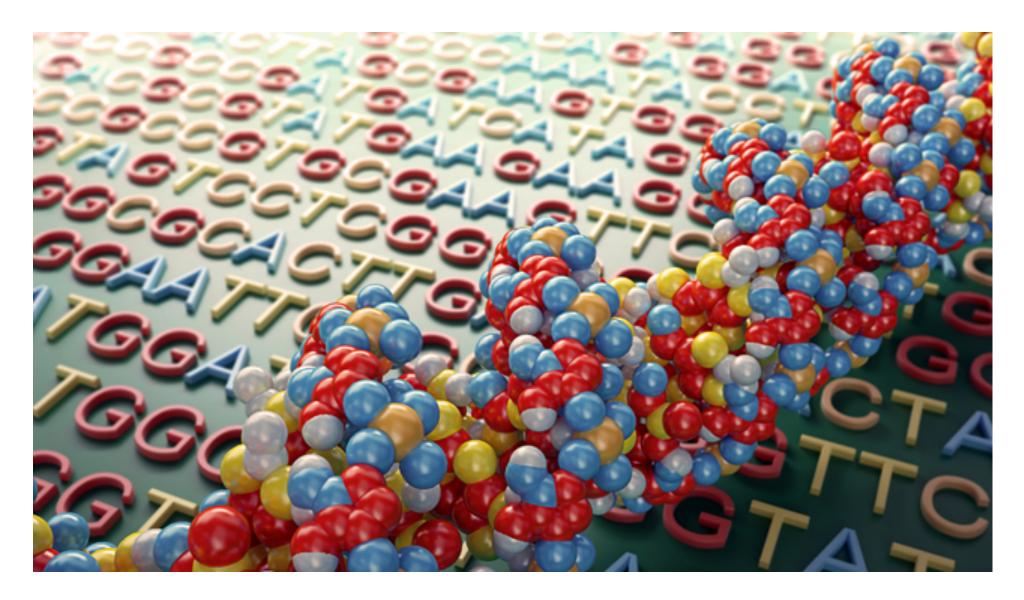
qRT-PCR

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

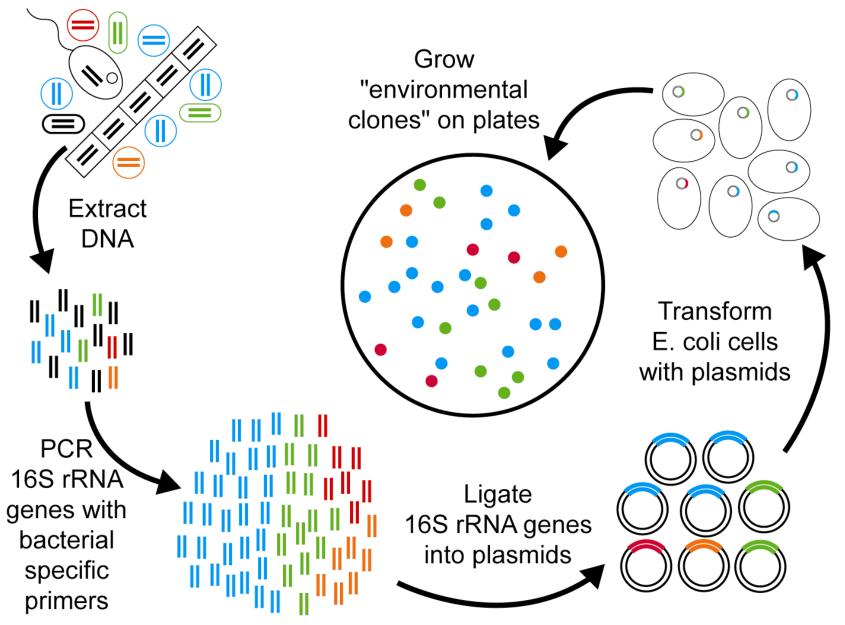


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Sequencing Revolution

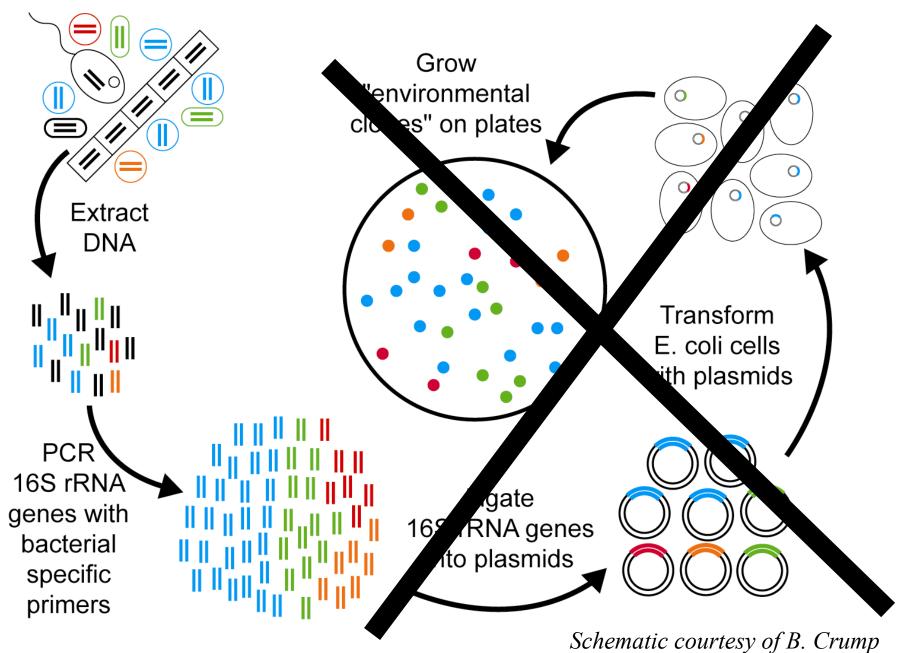


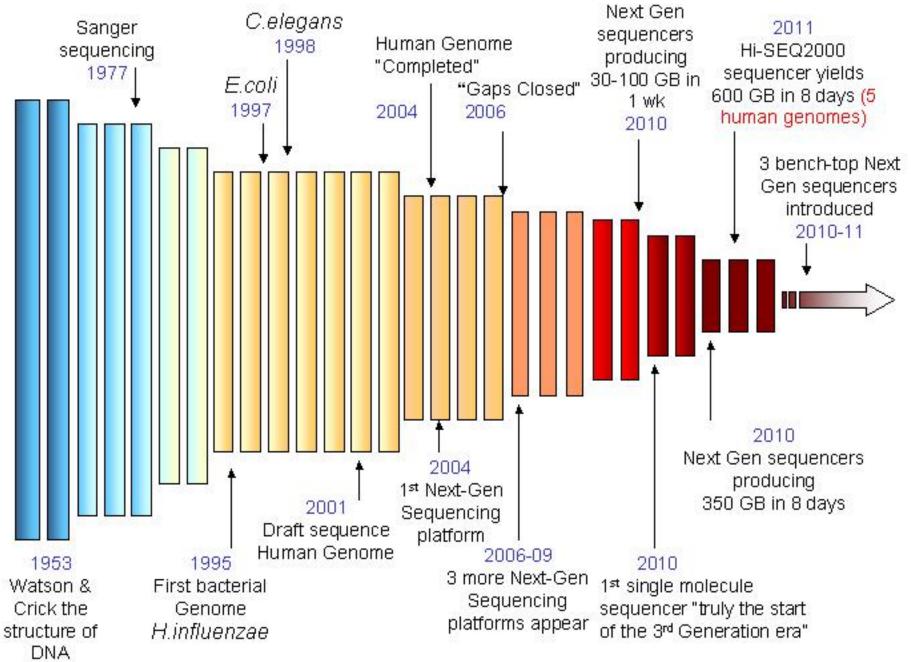
Traditional Gene Cloning



Schematic courtesy of B. Crump

NextGen Approaches

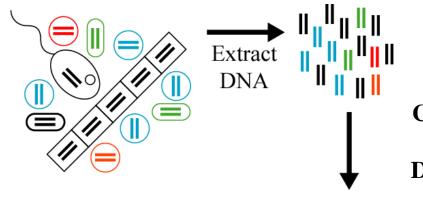




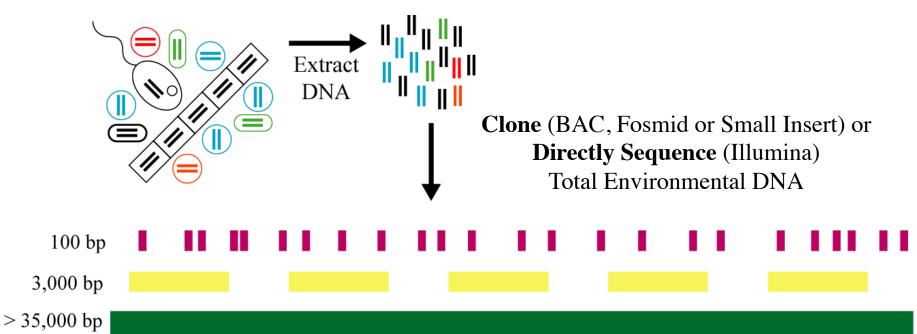
http://www.ipc.nxgenomics.or

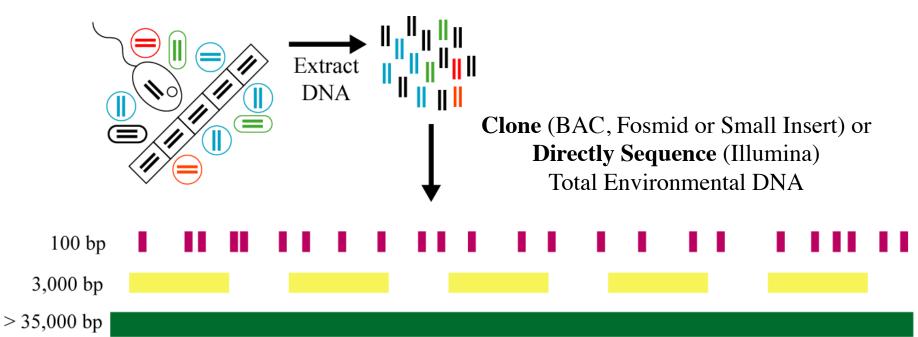
What is the difference between "standard" and "next-gen" sequencing?





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

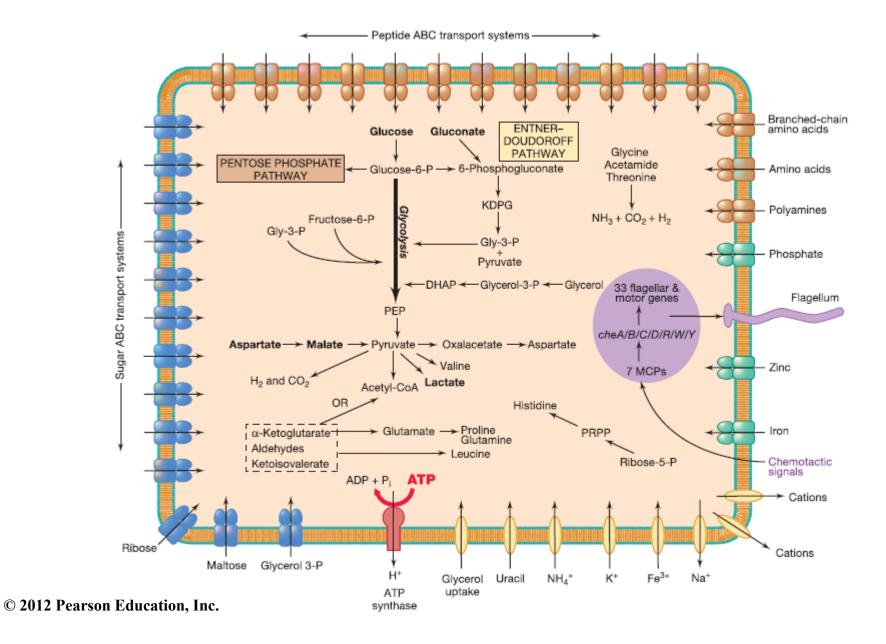




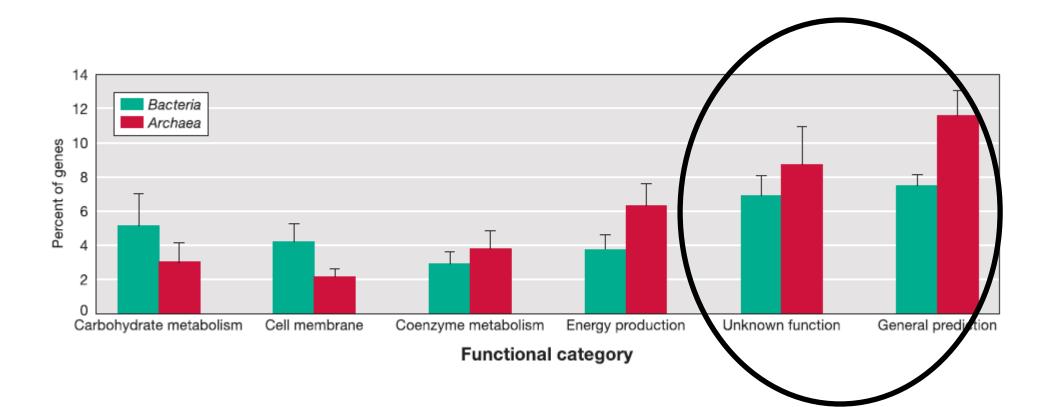
Access genomes of uncultured microbes: Functional Potential Metabolic Pathways Horizontal Gene Transfer

. . .

Reconstruct Genomes



Categorize Functions



Proteorhodopsin phototrophy in the ocean

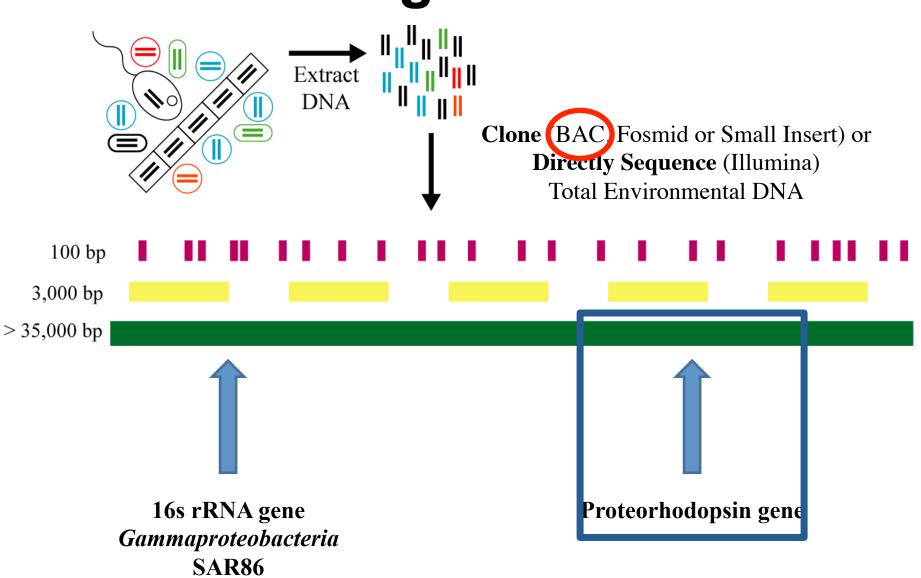
Oded Béjà*†, Elena N. Spudich†‡, John L. Spudich‡, Marion Leclerc* & Edward F. DeLong*

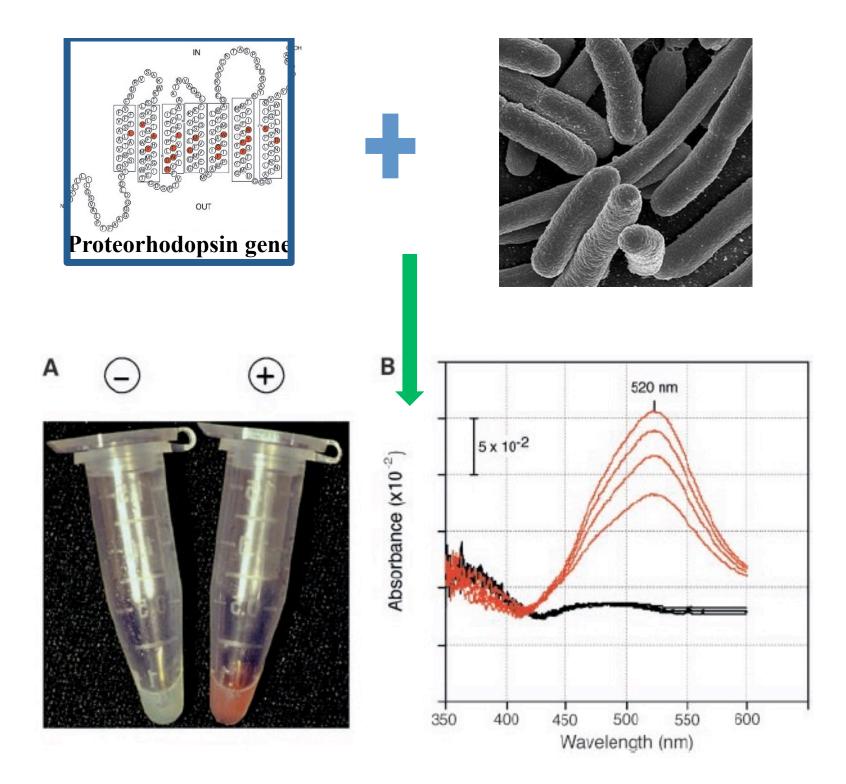
Bacterial Rhodopsin: Evidence for a New Type of Phototrophy in the Sea

Oded Béjà,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*}

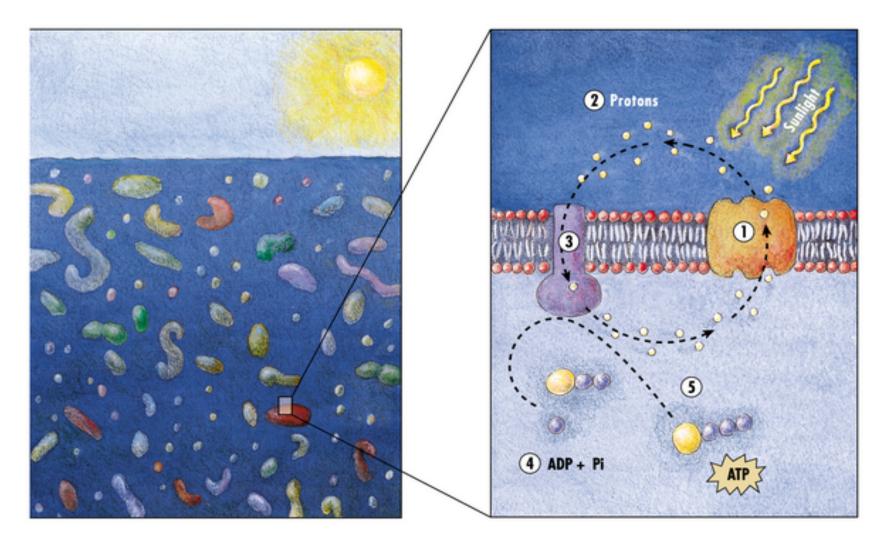
Proteorhodopsin genes are distributed among divergent marine bacterial taxa

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





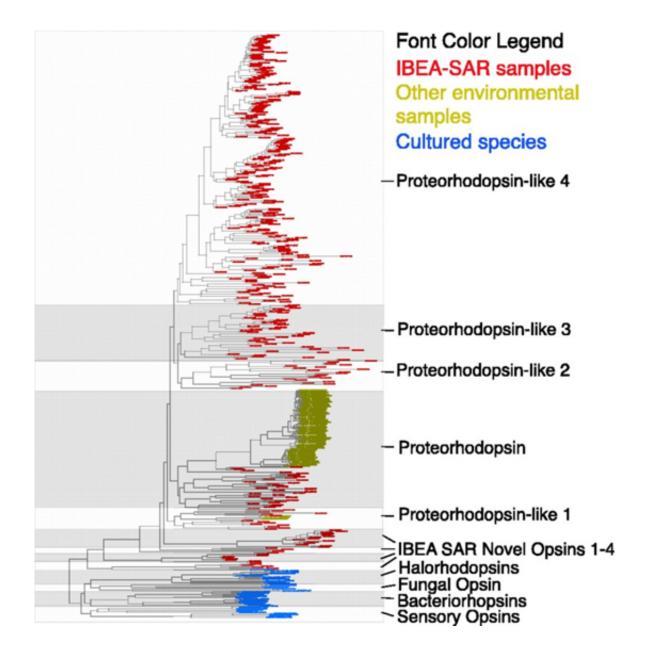
A new way of using sunlight in the surface ocean



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

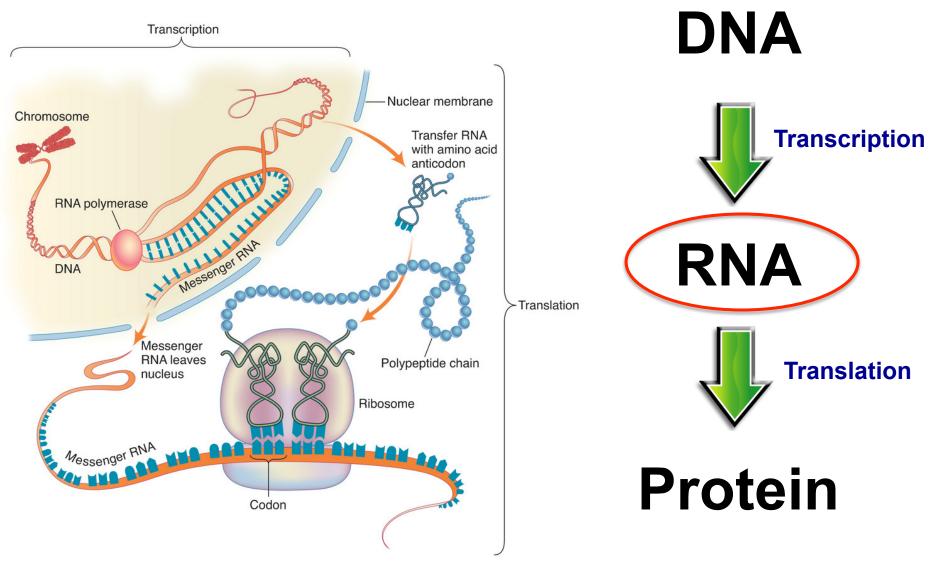


Proteorhodopsins occur in 13%-80% of marine bacteria and archaea in oceanic surface waters



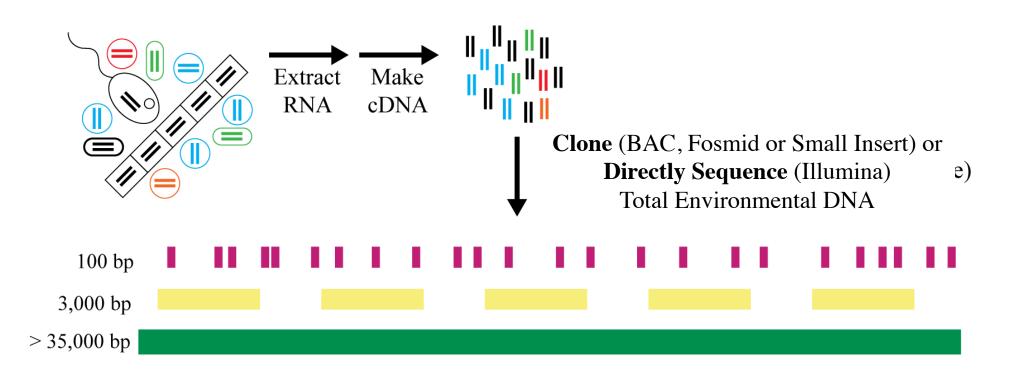
Venter et al. 2004

The Central Dogma



Moving from "who is there?" to "who is active?"

Metatranscriptomics

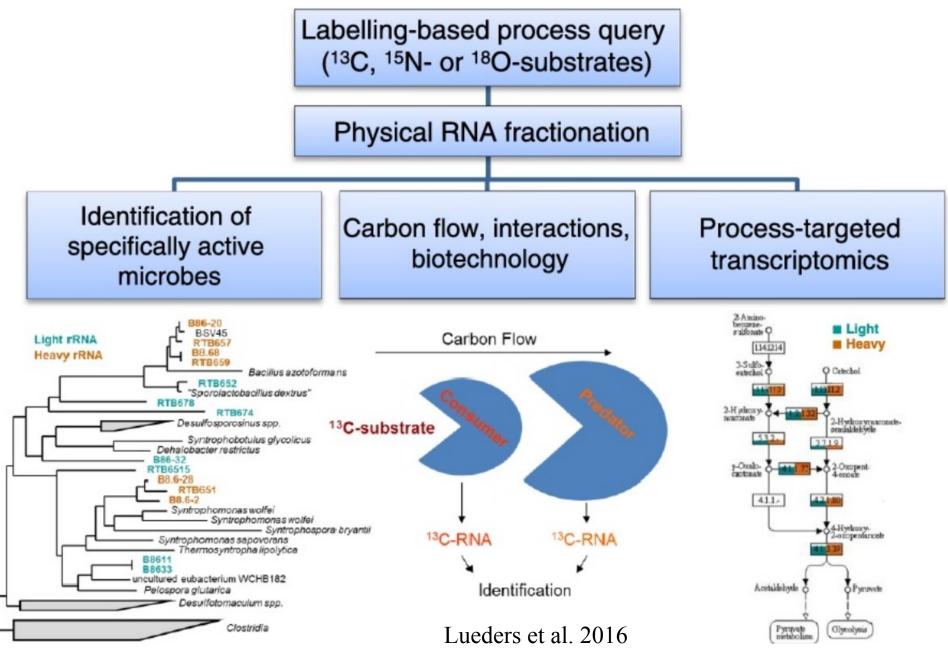


Access expressed genes of uncultured microbes Looking at expression of defined genes via PCR GeoChip-type analyses with RNA Etc.

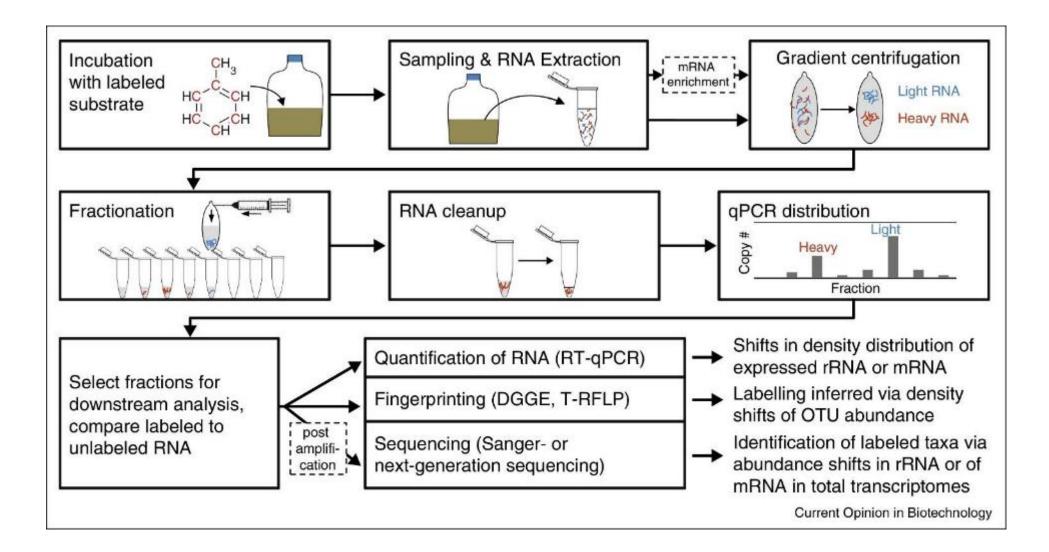
Stable Isotope Probing (SIP)

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

RNA SIP

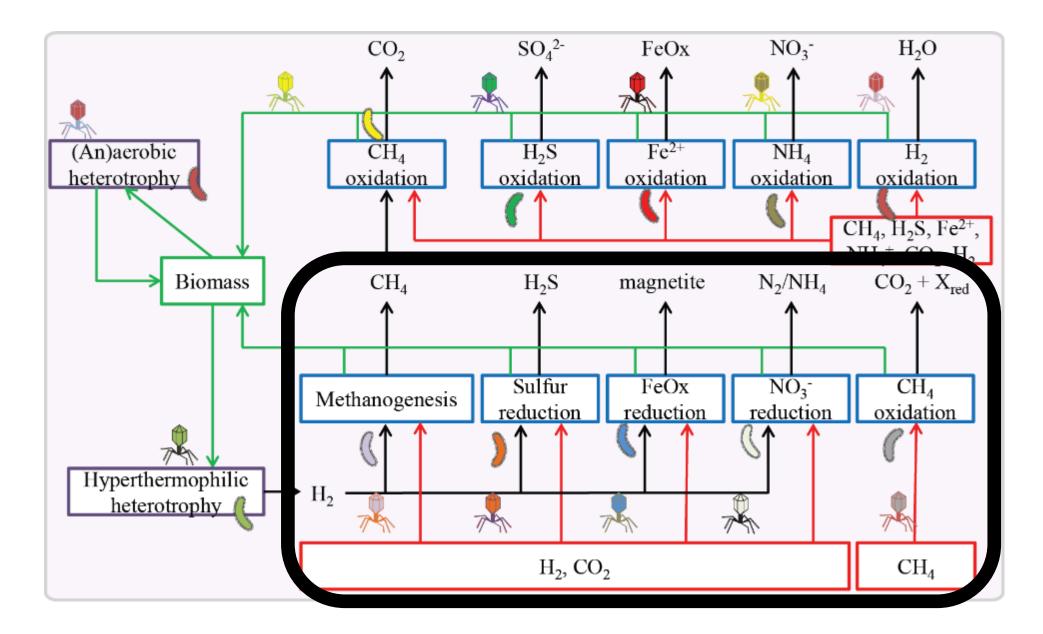


RNA SIP



Lueders et al. 2016

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



