Day 3

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

Genes We Targeted

1: dsrAB 1800bp

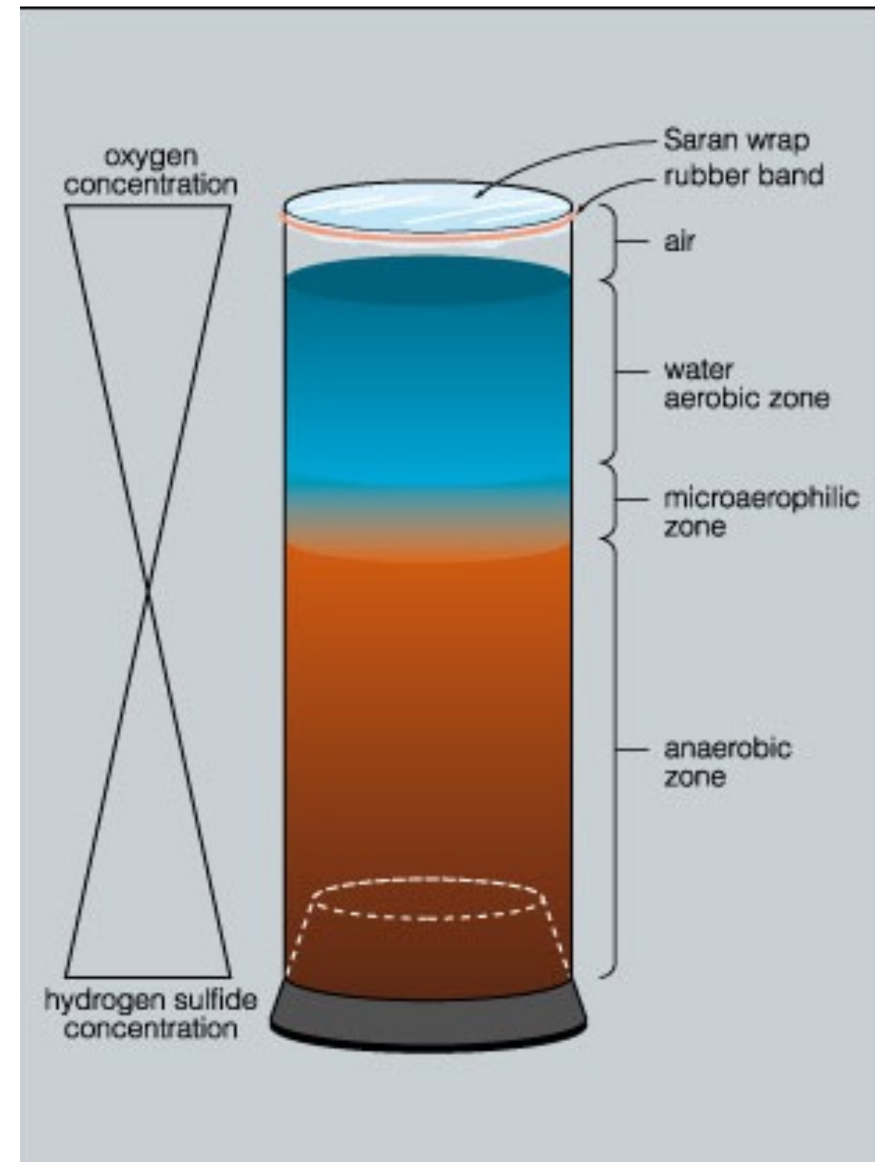
2: mcrA 750bp

3: Bacteria 1450bp

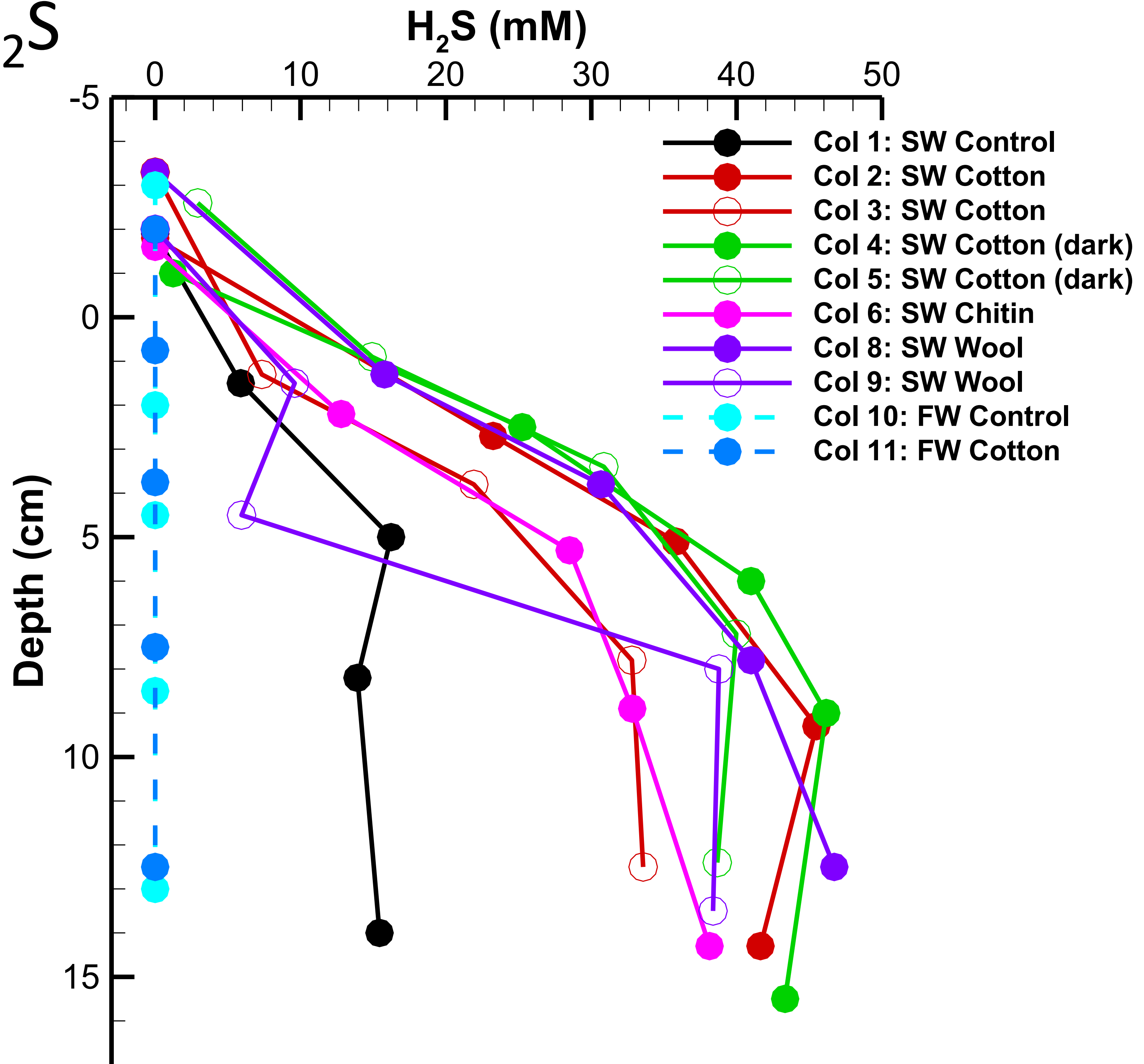
4: Archaea 950bp

5: Archaea + 950bp

6: Negative control



2018 H_2S



2018 CH_4

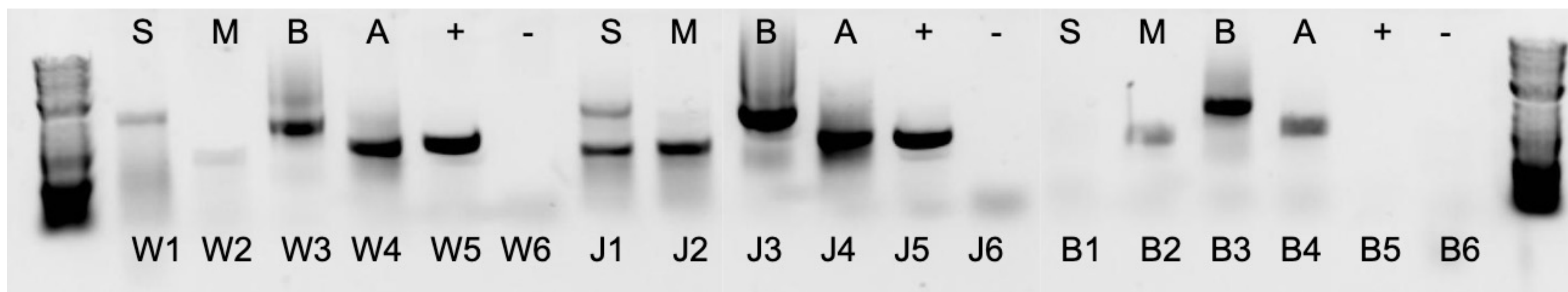
CH_4 (μM)

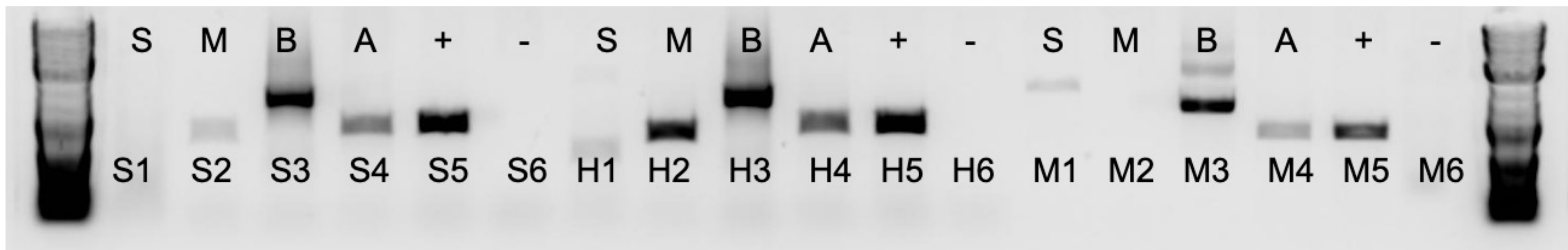
0 200 400 600 800

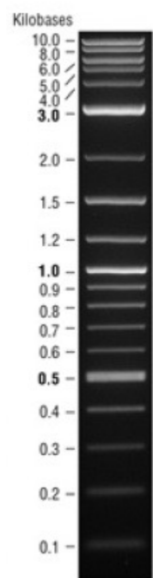
Depth (cm)

-5 0 5 10 15

Col 1: SW Control
Col 2: SW Cotton
Col 3: SW Cotton
Col 4: SW Cotton (dark)
Col 5: SW Cotton (dark)
Col 6: SW Chitin
Col 8: SW Wool
Col 9: SW Wool
Col 10: FW Control
Col 11: FW Cotton







1: dsrAB 1800bp

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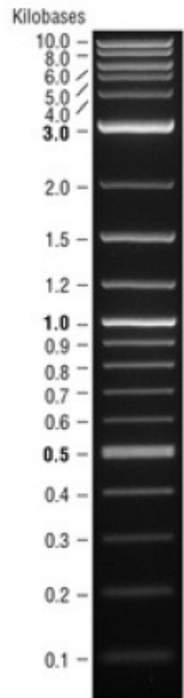


Column 5

1 2 3 4 5 6

10,000
3000
1500
1000
500

Column 6

1 2 3 4 5 6

10,000
3000
1500
1000
500

Column 7

1 2 3 4 5 6

10,000
3000
1500
1000
500

Column 8

1 2 3 4 5 6

10,000
3000
1500
1000
500

Some Problems with PCR

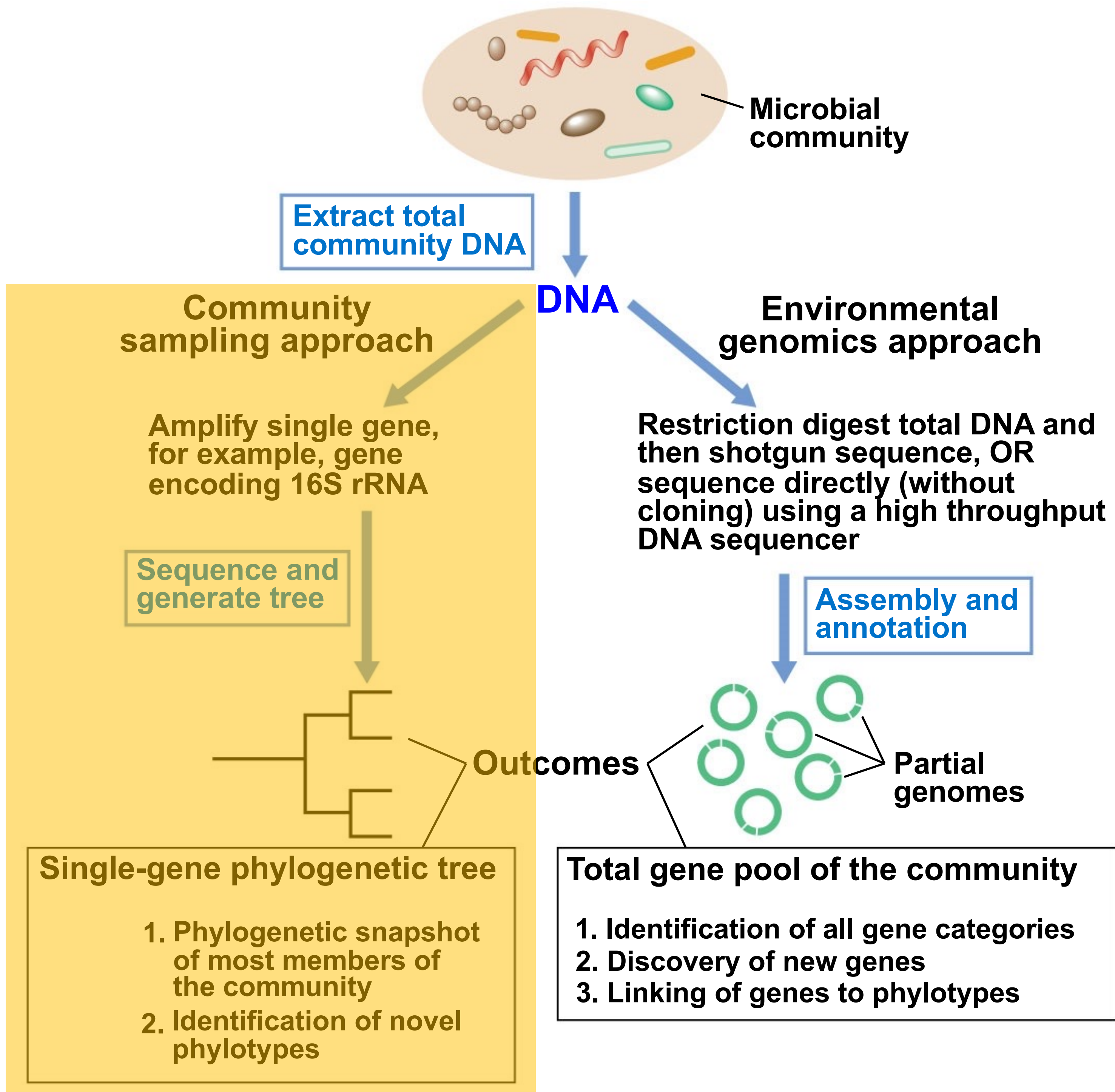
- Inhibitors in template DNA
- Amplification bias
- Gene copy number
- Limited by primer design
- Differential denaturation efficiency
- Chimeric PCR products may form
- Contamination w/ non-target DNA
- Potentially low sensitivity and resolution
- General screw-ups

(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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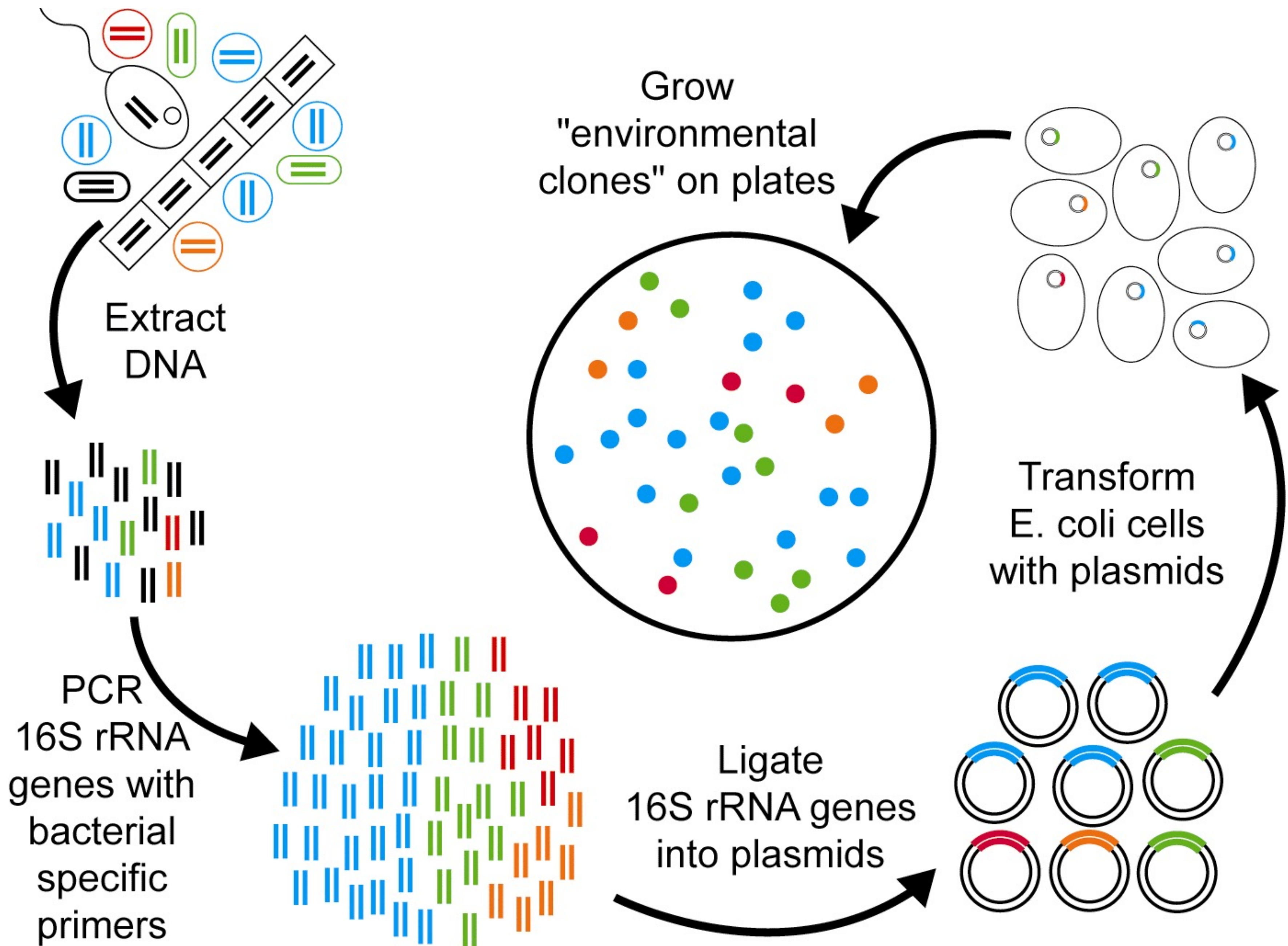
Traditional Gene Cloning

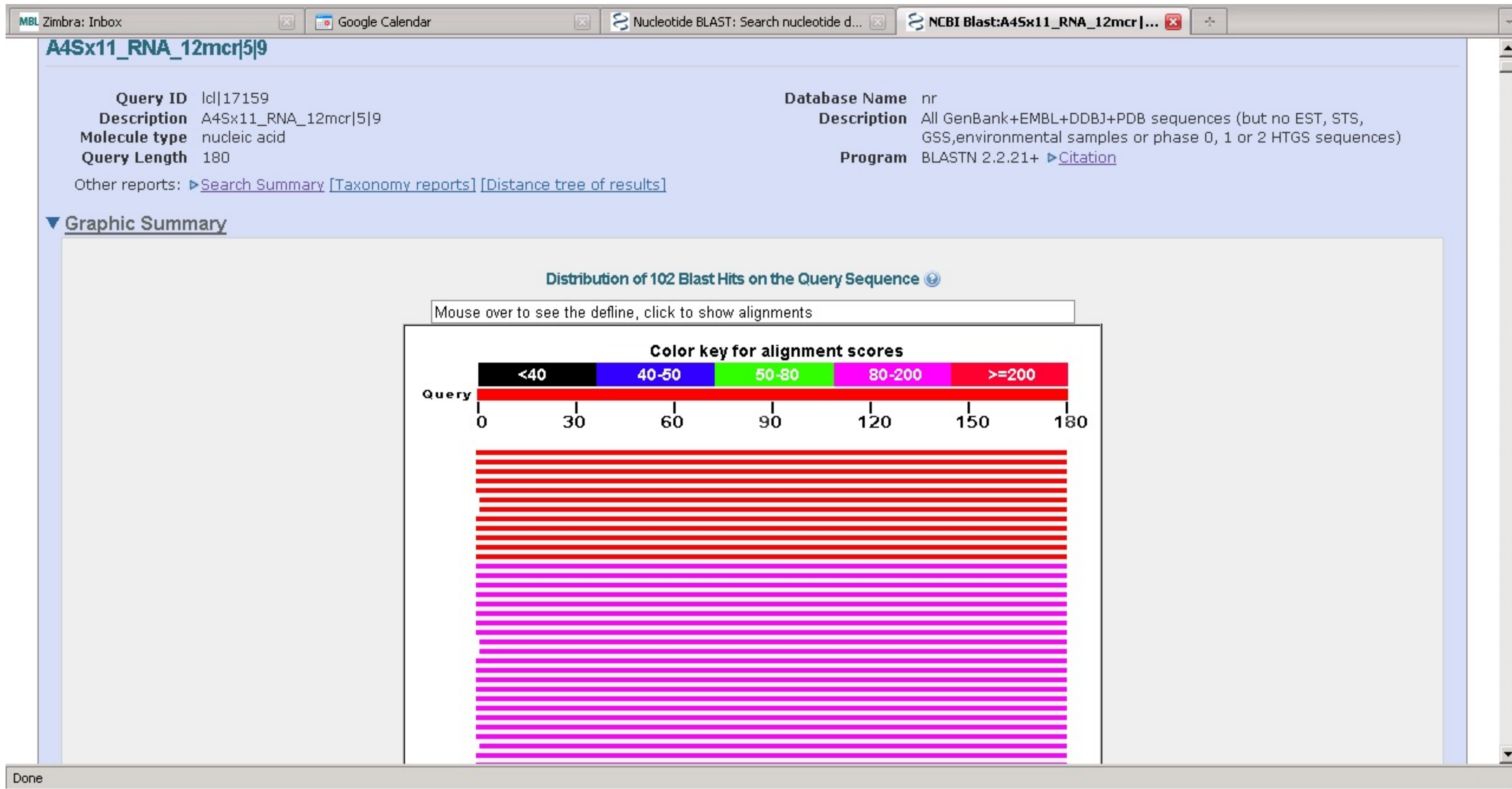


Schematic courtesy of B. Crump

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.

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

```
501    1 Human     CCAUGGUGACCACGGGUGACGGGGAAUCAGGGUUCGAUUCCGGAGAGGGAGCCUGAGAAACGGCUACCACAUCCAAGGA-AGGCAGCAGGCGCGCAAAUU
501    2 Rabbit    CCAUGGUGACCACGGGUGACGGGGAAUCAGGGUUCGAUUCCGGAGAGGGAGCCUGAGAAACGGCUACCACAUCCAAGGA-AGGCAGCAGGCGCGCAAAUU
501    3 Shrimp    CCAUGGUUGCAACGGGUAACGGGGAAUCGGGGUUCGAUUCCGGAGAGGGAGCCUGAGAAACGGCUACCACAUCCAAGGA-AGGCAGCAGGCGCGCAAAUU
501    4 Termite   CCAUGGUUGUAACGGGUAACGGGGAAUCAGGGUUCGAUUCCGGAGAGGGAGCCUGAGAAACGGCUACCACAUCCAAGGA-AGGCAGCAGGCGCGCAAAUU
501    5 Drosophi  CCAUGGUUGCAACGGGUAACGGGGAAUCAGGGUUCGAUUCCGGAGAGGGAGCCUGAGAAACGGCUACCACAUCUAAGGA-AGGCAGCAGGCGCGUAAAUU
501    6 Sponge    CCAUGGUUGCAACGGGUGACGGAGAAUUAGGGUUCGAUUCCGGAGAGGGAGCCUGAGAGACGGCUACCACAUCCAAGGA-AGGCAGCAGGCGCGCAAAUU
501    7 Mucor     CAAUGCCUACAACGGGUAACGGGGAAUUAGGGUUCGAUUCCGGAGAGGGAGCCUGAGAAACGGCUACCACAUCCAAGGA-AGGCAGCAGGCGCGCAAAUU
501    8 S. pombe  CCAUGGUUUUAACGGGUAACGGGGAAUUAGGGUUCGAUUCCGGAGAGGGAGCCUGAGAAACGGCUACCACAUCCAAGGA-AGGCAGCAGGCGCGCAAAUU
501    9 Candida   CCAUGGUUUCAACGGGUAACGGGGAAUAAGGGUUCGAUUCCGGAGAGGGAGCCUGAGAAACGGCUACCACAUCCAAGGA-AGGCAGCAGGCGCGCAAAUU
501   10 Pneumocy  CCAUGGUUUCGACGGGUAACGGGGAAUAAGGGUUCUAUUCCGGAGAGGGAGCCUGAGAAACGGCUACCACAUCCAAGGA-AGGCAGCAGGCGCGCAAAUU
501   11 Yeast     CCAUGGUUUCAACGGGUAACGGGGAAUAAGGGUUCGAUUCCGGAGAGGGAGCCUGAGAAACGGCUACCACAUCCAAGGA-AGGCAGCAGGCGCGCAAAUU
501   12 Pennicil  CCAUGGUGGCAACGGGUAACGGGGAAUUAGGGUUCGAUUCCGGAGAGGGAGCCUGAGAAACGGCUACCACAUCCAAGGA-AGGCAGCAGGCGCGCAAAUU
501   13 Corn      CCAUGGUGGUGACGGGUGACGGAGAAUUAGGGUUCGAUUCCGGAGAGGGAGCCUGAGAAACGGCUACCACAUCCAAGGA-AGGCAGCAGGCGCGCAAAUU
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501   15 Tomato    CCAUGGUGGUGACGGGUGACGGAGAAUUAGGGUUCGAUUCCGGAGAGGGAGCCUGAGAAACGGCUACCACAUCCAAGGA-AGGCAGCAGGCGCGCAAAUU
501   16 Volvox    CCAUGGUGGUAACGGGUGACGGAGGAUUAGGGUUCGAUUCCGGAGAGGGAGCCUGAGAGAUGGCUACCACAUCCAAGGA-AGGCAGCAGGCGCGCAAAUU
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501   18 Porphyra  CCAUGGUUGUGACGGGUAACGGACCGUGGGUGCGGGAUUCCGGAGAGGGAGCCUGAGAGACGGCUACCACAUCCAAGGA-AGGCAGCAGGCGCGCAAAUU
501   19 Gracilar  CCAUGGUUGUGACGGGUAACGGACCGUGGGUGCGGGACUCCGGAGAGGGAGCCUGAGAGACGGCUACCACAUCCAAGGA-AGGCAGCAGGCGCGCAACUU
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501   21 Tetrahym  CCAUGGCAGUCACGGGUAACGGAGAAUUAGGGUUCGAUUCCGGAGAAGGAGCCUGAGAAACGGCUACUACAACUACGGUUCGGCAGCAGGGAAGAAAAUU
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501   27 Diatom    CCAUGGCUUUAACGGGUAACGGGAAAUUAGGGUUUGAUUCCGGAGAGGGAGCCUGAGAGACGGCUACCACAUCCAAGGA-AGGCAGCAGGCGCGUAAAUU
501   28 Ochromon  CCAUGGCAUUAACGGGUAACGGAGAAUUAGGGUUCGAUUCCGGAGAGGGAGCCUGAGAAAUGGCUACCACAUCCAAGGA-AGGCAGCAGGCGCGUAAAUU
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501   30 Brown Al  CCAUGGCUUUAACGGGUAACGGGGAAUUGGGGUUCGAUUCCGGAGAGGGAGCCUGAGAAACGGCUACCACAUCCAAGGA-AGG-AGCAGGCGCGUAAAUU
501   31 Dictyost  CCAUGGUUGUAACGGGUAACGGGGAAUUAGGGUUCGAUUCCGGAGAGGGAGCCUGAGAAAUGGCUACCACUUCUACGGA-AGGCAGCAGGCGCGCAAAUU
501   32 Euglena   CAGUGGCCUUGACGGGUAACGGAGAAUCAGGGUUCGAUUCCGGAGAGGGAGCCUGAGAGACGGCUACCACUACCAAGGU-GGGCAGCAGGCACGCAAAUU
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501   34 Leishman  CCAUGGCGUUGACGGG-AGCGGGGGAUUAGGGUUCGAUUCCGGAGAGGGAGCCUGAGAAAUAGCUACCACUUCUACGGA-GGGCAGCAGGCGCGCAAAUU
501   35 Crithidi  CCAUGGCGUUGACGGG-AGCGGGGGAUUAGGGUUCGAUUCCGGAGAGGGAGCCUGAGAAAUAGCUACCACUUCUACGGA-GGGCAGCAGGCGCGCAAAUU
```

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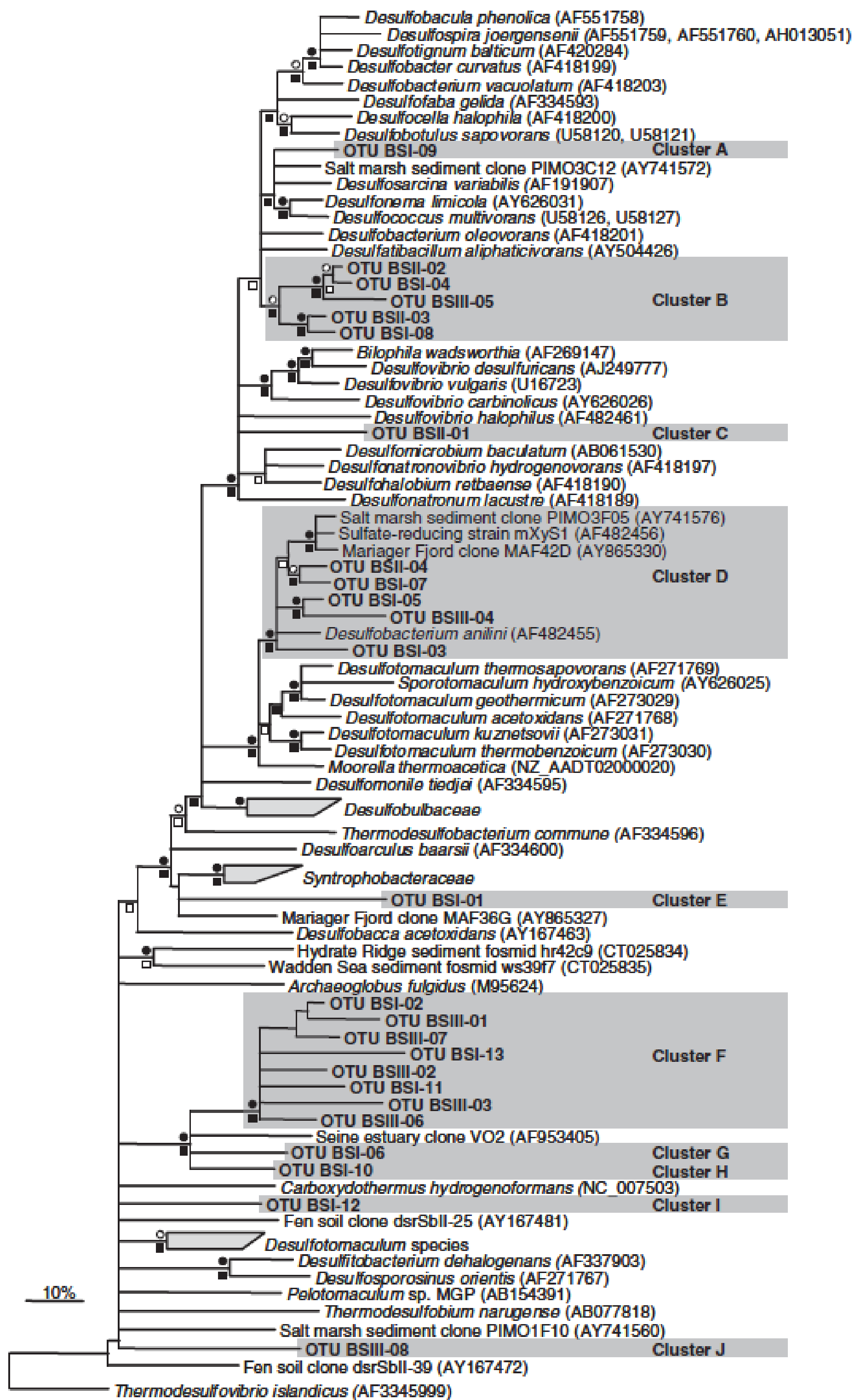
ursor positioned at nucleotide  1100 of sequence number  35 (          )

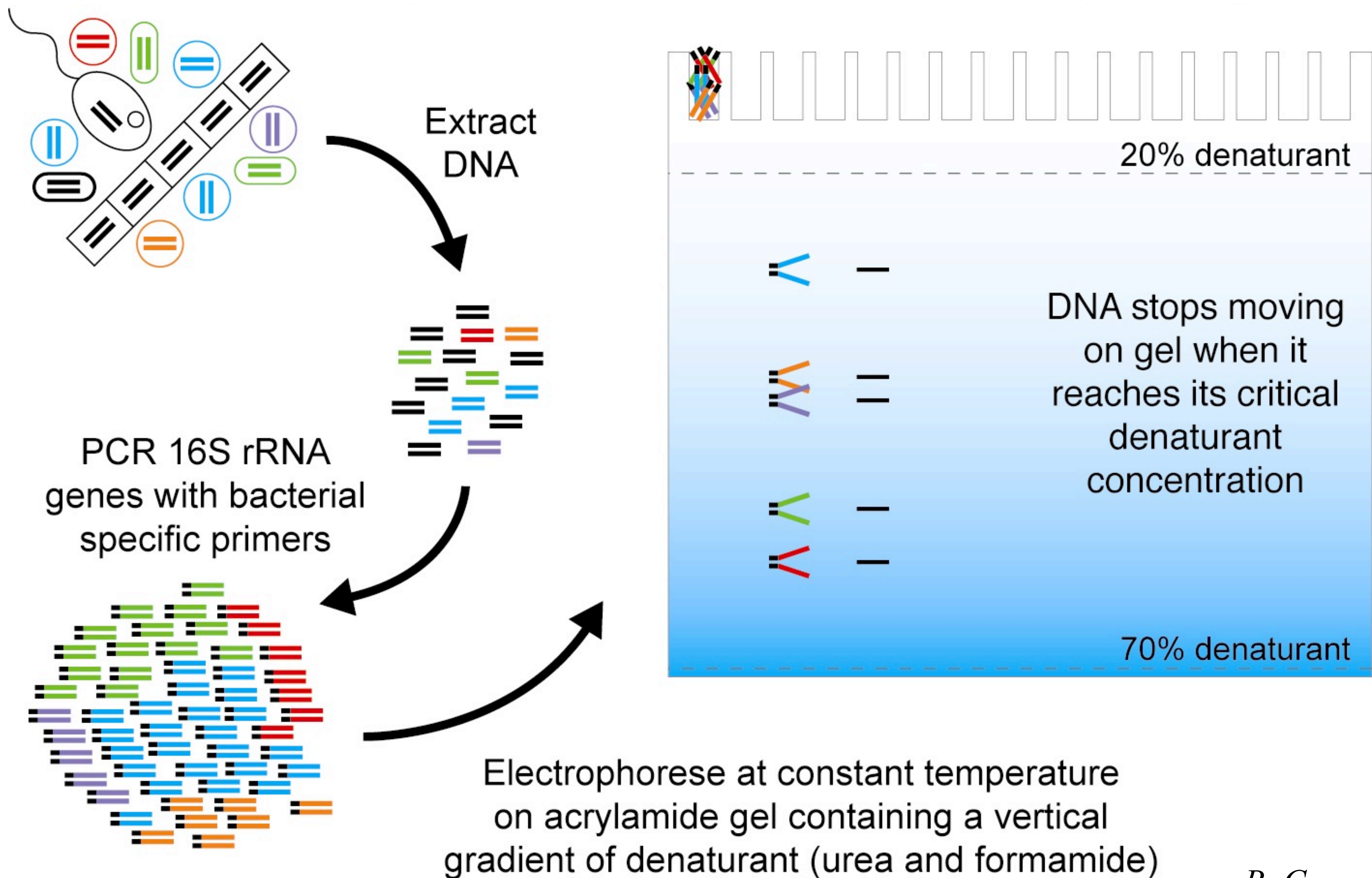
1050    1 Human     CC-G---CCCCU----------UGCCUCUCGG---CGCCCCCUC--GAUGCUC-UUAGCU-GAGU-G-U-CCCGCGGGGCCCGAAGC-GUUUACUUUGAA
1050    2 Rabbit    CC-G---CCCCU----------UGCCUCUCGG---CGCCCCCUC--GAUGCUC-UUAGCU-GAGU-G-U-CCCGCGGGGCCCGAAGC-GUUUACUUUGAA
1050    3 Shrimp    CG------GACA--------AUU-CAUUGGA----UCGUUCGG---GGUGCUC-UUAACC-GAGU-G-U-CCUG-GGUGGCCGAUAC-GUUUACUUUGAA
1050    4 Drosophi  -AU----GUUCCU-------------CCUAUUUAAAAACCUGCAUUAGUGCUC-UUAAAC-GAGU-G-U-UAUU-GUGGGCCGGUAC-UAUUACUUUGAA
1050    5 Sponge    CGC------CCUU-------------CCUCUCGAAA-GCC-CCG--ACUGCUC-UUCACUGCAGU-G-GUCGGG--UAGUUCGGGAC-GUUUACUUUGAA
1050    6 Mucor     CGC------UUUUAU-CGAGGCUUUUUUUCUGGUUAUGCU-A-UGAAUAGCUU--CGGUU-GUUU-A-UAGUCU--CUAGCCAG-AU-GAUUACCAUGAG
1050    7 S. pombe  GGGG-----UCGUUAA----------CCUUCUGGCAAACU-A-CUCAUGUUCU--UUAUU-GAGC-G-UGGUAG--GGAACCAGGAC-UUUUACCUUGAA
1050    8 Candida   GAG------CCUUU------------CCUUCUGGCUAACC-A---UUCGCCCU--U--GU-GG---U-GUUUGG--CGAACCAGGAC-UUUUACUUUGAA
1050    9 Pneumocy  GAU------CCUUC------------CCUCCUGGAUUACC-G---GCUGCCCU--UCGCU-GGGU-G-UGCCGG--AUAGCCAGGGCAUUUUACUUUGAG
1050   10 Yeast     GGG------CCUUU------------CCUUCUGGCUAACC-U---UGAGUCCU--U--GU-GGC--U-C-UUGG--CGAACCAGGAC-UUUUACUUUGAA
1050   11 Pennicil  GGA------CCUUU------------CCUUCUGGGGAACC-U---CAUGGCCU--UCACU-GGCU-G-UGG-GG--GGAACCAGGAC-UUUUACUGUGAA
1050   12 Corn      CGA------CCCUU------------CUGCCGGCGAUGC--GCU--CCUGGCC-UUAACU-GGCC-G-GGUC-G--UGCCUCCGG-GCCGUUACUUUGAA
1050   13 Rice      CGA------CCCUU------------CUGCCGGCGAUGC--GCU--CCUGGCC-UUAACU-GGCC-G-GGUUCG--UGCCUCCGGCGCCGUUACUUUGAA
1050   14 Tomato    CGU------CCCUU------------CUGUCGGCGAUGC--GCU--CCUGGCC-UUAAUU-GGCC-G-GGUC-G--UGCCUCCGGCGCUGUUACUUUGAA
1050   15 Volvox    CCA------CCUUC------------CUGCCGGGGACGG--GCU--CCUGGGC-UUCACU-GUAU-G-GGACUC--GGAGUC-GGCGAGGUUACUUUGAG
1050   16 Chlorell  CA-------CCUUG------------UUGCCGGGGACGG--GCU--CCUGGGC-UUCACU-GUCC-G-GGACUC--GGAGUC-GGCGCUGUUACUUUGAG
1050   17 Porphyra  ---------CUUUU-----------GUGGAGGGCGG-CGCGU--UGUGG---C-UUCACUGUCGC-GGCGUGUC--GGCGCCACCG---UUUACUGUGAA
1050   18 Gracilar  ---------CCUUU-----------GUGGAGAGGGG-GUGU---GGUGG-UGC-UUGAGUGCGCU-GCCAUGCU--GCCGCCACCG---UUUACUGUGAA
1050   19 Parameci  C--------UCCGU------------CUACAAUCCCUUU--------UGCGCU-UUAGGGUUGC--A-GCUGGG--CGAGU-AGAC-AAUUUACCUUGAA
1050   20 Tetrahym  ---------UACGU------------UUGCAAACUAAAA--------UCGGCC-UUCACUGGUUC-G-ACUUAG--GGAGU-AAAC-AUUUUACUGUGAA
1050   21 Dinoflag  CUUG-----ACAUC-----------UUCCU-AAAGA-AC--GUA--UCUGCAC-UUCAUU-GUGU-GGUG--CG--GUAUUUAGGAC-AUUUACCUUGAG
1050   22 Toxoplas  UCUA-----GCAUC-----------CUUCU-GGAUU-UCU-C-------CACACUUCAUU-GUGU-GGAGUUU---UUU-CCAGGAC-UUUUACUUUGAG
1050   23 Theileri  UCG------GUU-------------------UGAUUUUUU-------------------------------------CUUUCCGGAU-GAUUACUUUGAG
1050   24 Achlya    GGGC-----CAUUU------------UUUGUGAGGAU----GCUUU-UCUGCC-AUUCAGUUGGU-G-GUUGAG--UAGACUUGCAUCGUUUACUGUGAA
1050   25 Phytopht  GAGG-----CAUUU------------UUUGUGAGGCU----GCCUU-UCUGCC-AUUAAGUUGGU-G-GGUUGG--UGGGCUUGCAUCGUUUACUGUGAA
1050   26 Diatom    UCGC-----CAUCC------------UUGGGUGG-AA-----CCUG-UGUGGC-AUUAGGUUGUC-G-U-GCAG--GGGAUGCCCAUCAUUUACUGUGAA
1050   27 Ochromon  GAAU-----CAUCC------------UCGAGAGG-AA-----CACG-UCUGUC-AUUCAGUUGAU-G-G-GCGU--GGGAUUCUCGUCUUUUACUGUGAG
1050   28 Synura    CGUC-----CAUCC------------UCGGGGAG-AA-----CGCA-UCUGGC-AUUAAGUUGUC-G-G-GUGU--GGYAUCCUCGUCAUUUACUGUGAG
1050   29 Brown Al  CGGCCGCUCCAUUC------------UCGGGUAG-CG-----UGUU-GCUGGC-AUUAGGUUGUC-G-G-CUUC--UUCGCGCCG-UCGUUUGCUGGGAA
1050   30 Dictyost  U-C----UUAAUA------------GUUCAGCUUGU-AUU-A-U--CUUU-------G-A-UAG--------UG--CUUGUUUGGACAUUUCACUGUGAG
1050   31 Euglena   ACCCAGCC------------------UCGAGCUG-GGUAG-------UCU-ACCUCUGGUCCACCAC-C-GGAG--CCCACCGUCUUCG-ACACCCUGGA
1050   32 Trypanos  ----------------------------------------------------------------------------------------UUUUACUGUGAC
1050   33 Leishman  ---------------------------------------------------------------------------------------UUUUUACUGUGAC
1050   34 Crithidi  ---------------------------------------------------------------------------------------UUUUUACUGUGAC
```



- 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)



Applied and Environmental Microbiology, Mar. 2004, p. 1494–1505 Vol. 70, No. 3
0099-2240/04/$08.00+0 DOI: 10.1128/AEM.70.3.1494–1505.2004
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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. Hobbie[2]

Horn Point Laboratory, University of Maryland Center for Environmental Science, Cambridge, Maryland,[1] and The Ecosystems Center[2] 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

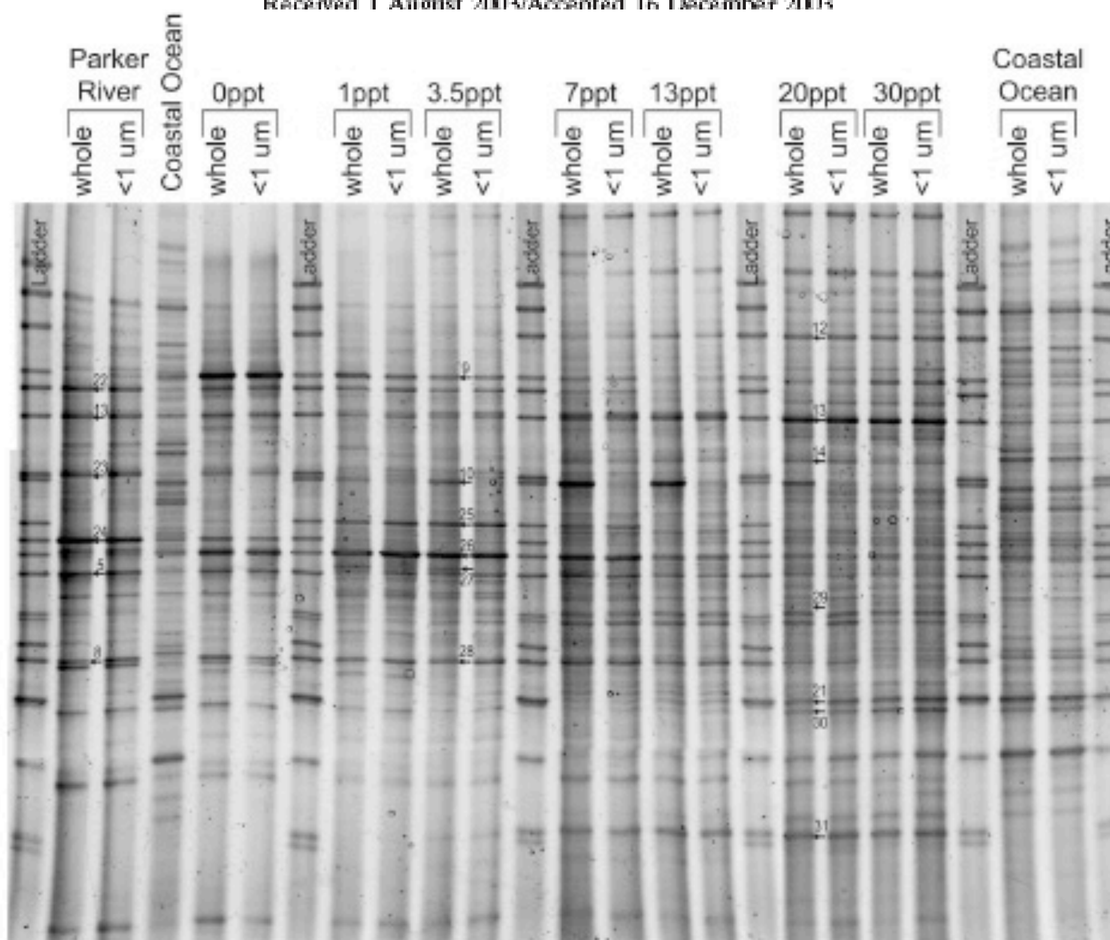
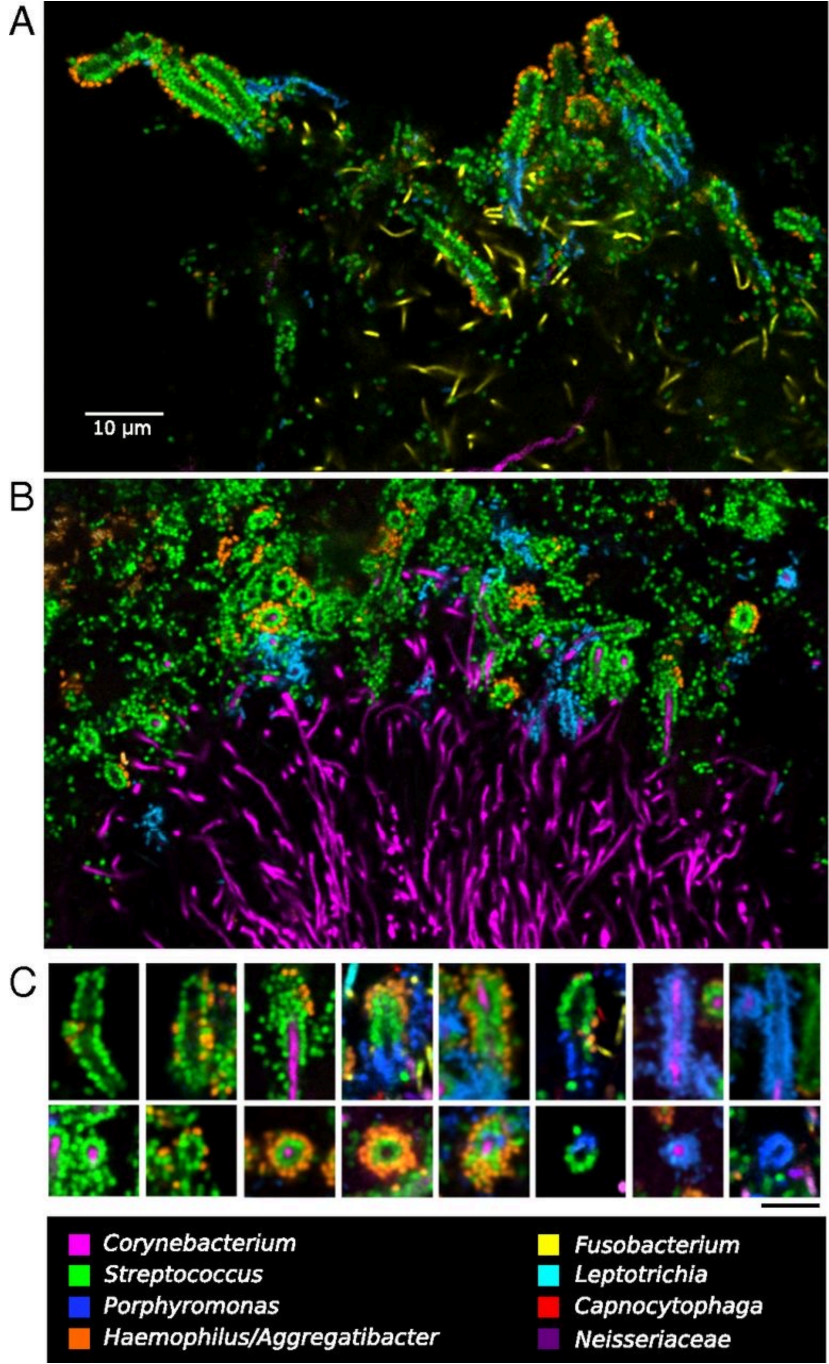
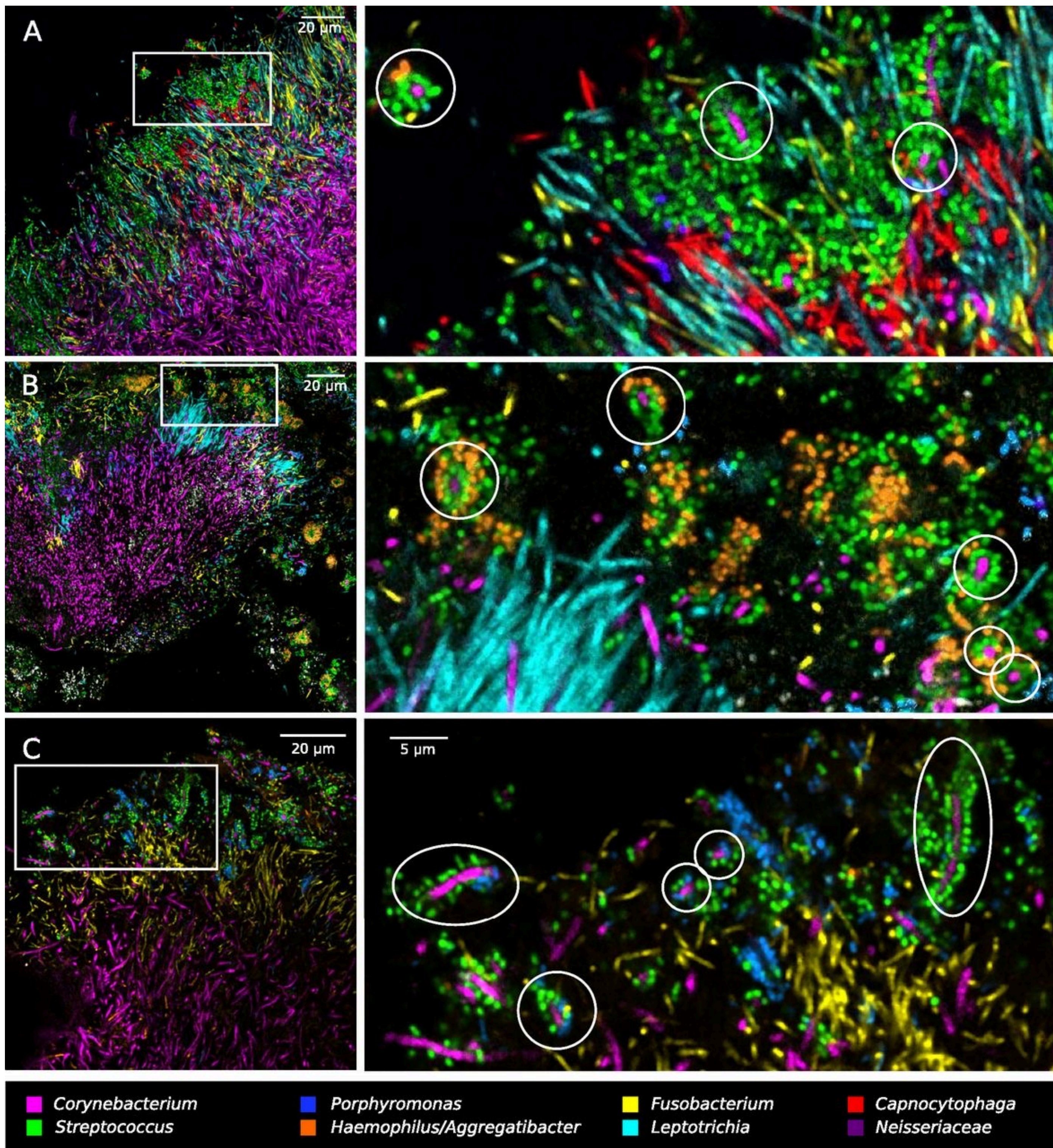


FIG. 4. DGGE gel of PCR-amplified 16S rDNA genes from samples collected along the salinity gradient on 28 September 2000. Bands from which DNA was sequenced are marked and numbered, corresponding to band numbers in Table 3.

rRNA Oligonucleotide Probes => Spatial context



J. Mark Welch et al. (2016) PNAS



J. Mark Welch et al. (2016) PNAS

Environmental Microbiology (2007) 9(1), 131–142 doi:10.1111/j.1462-2920.2006.01122.x

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

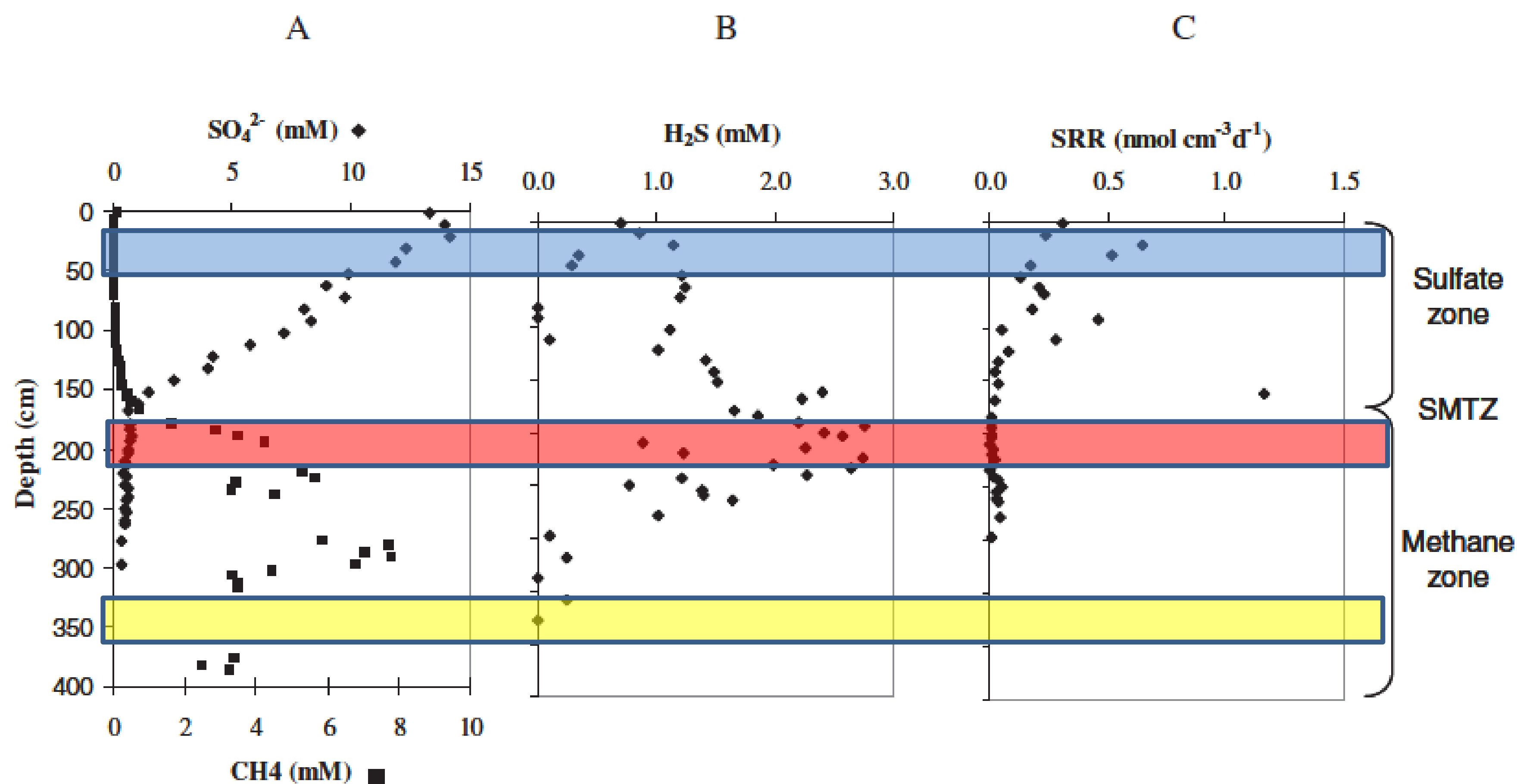
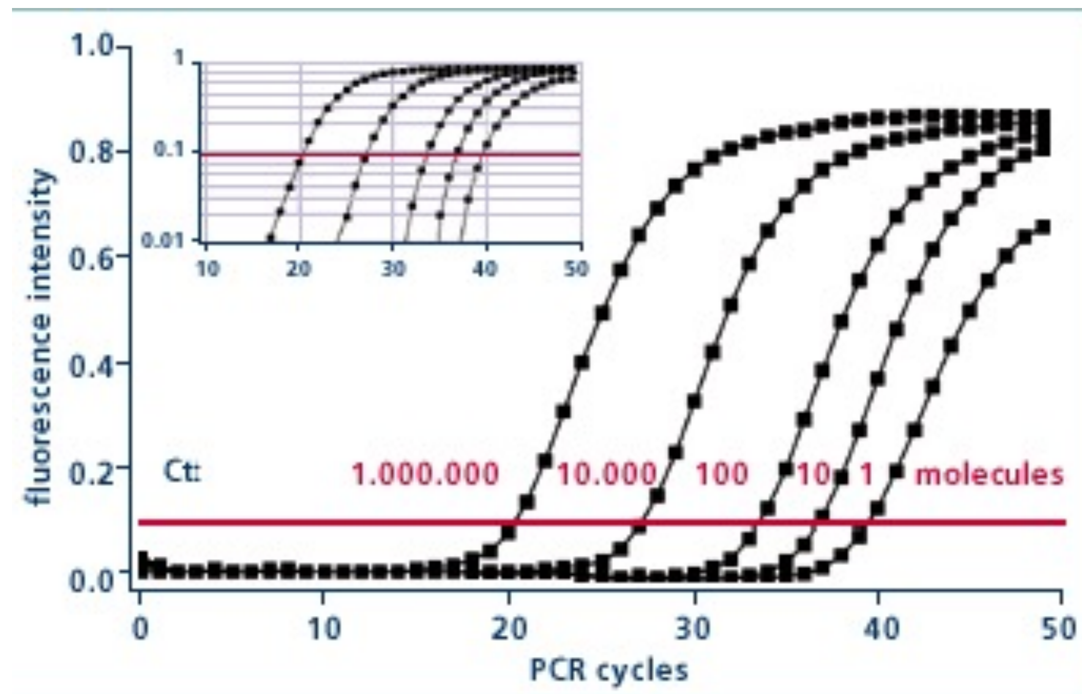


Fig. 2. Biogeochemical zonation and data from the Black Sea sediment core P824-GC.
A. Sulfate and methane concentration.
B. Sulfide concentration.
C. Sulfate reduction rate (SRR).

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

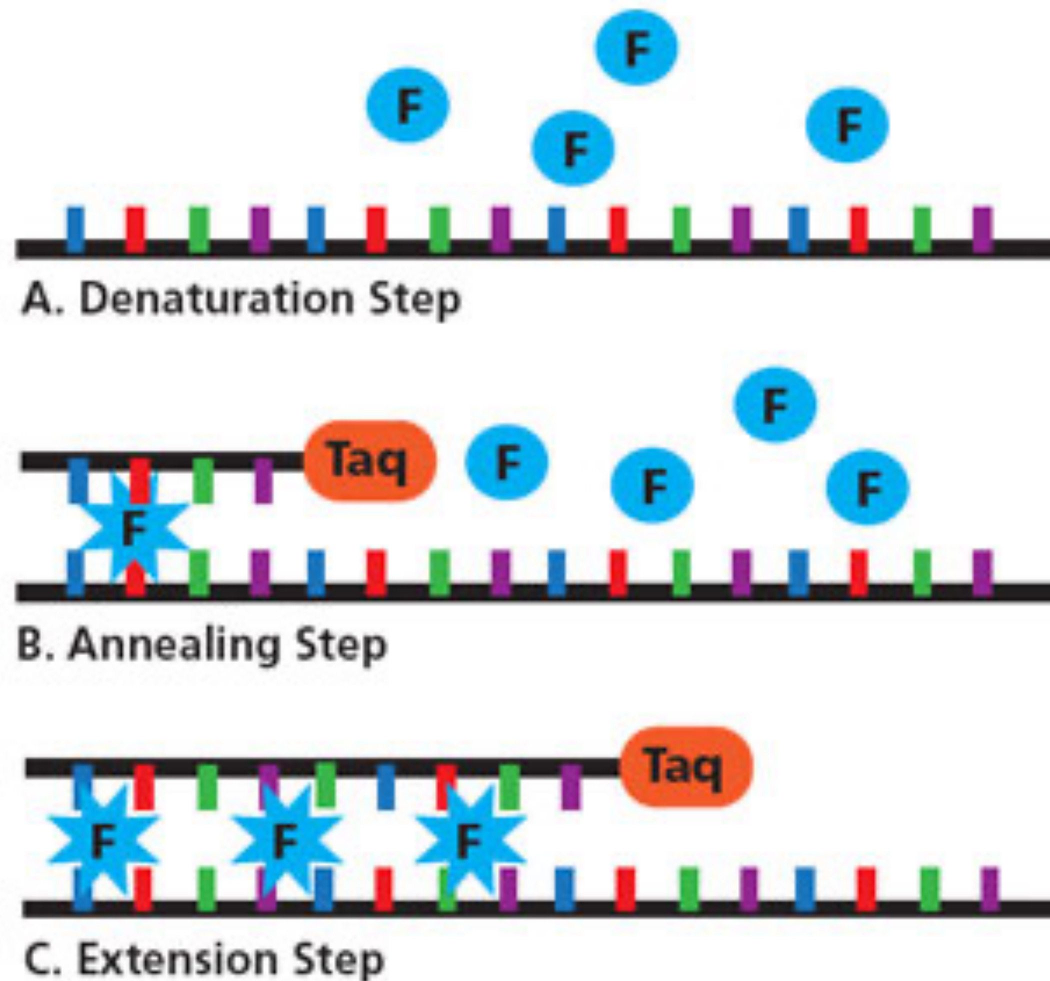
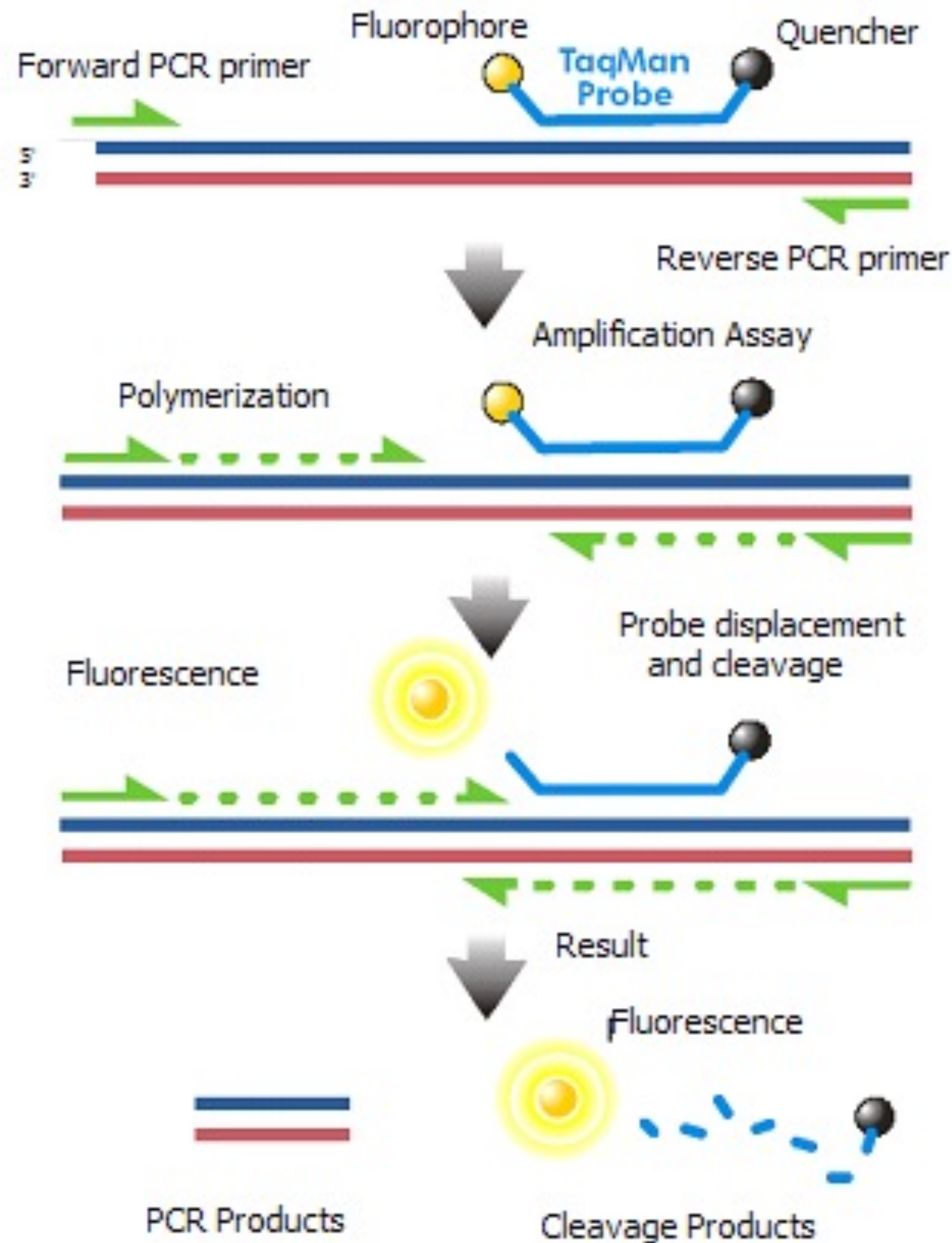


Figure 2: Fluorescent Dyes in qPCR

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

Environmental Microbiology (2007) 9(1), 131–142 doi:10.1111/j.1462-2920.2006.01122.x

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*)

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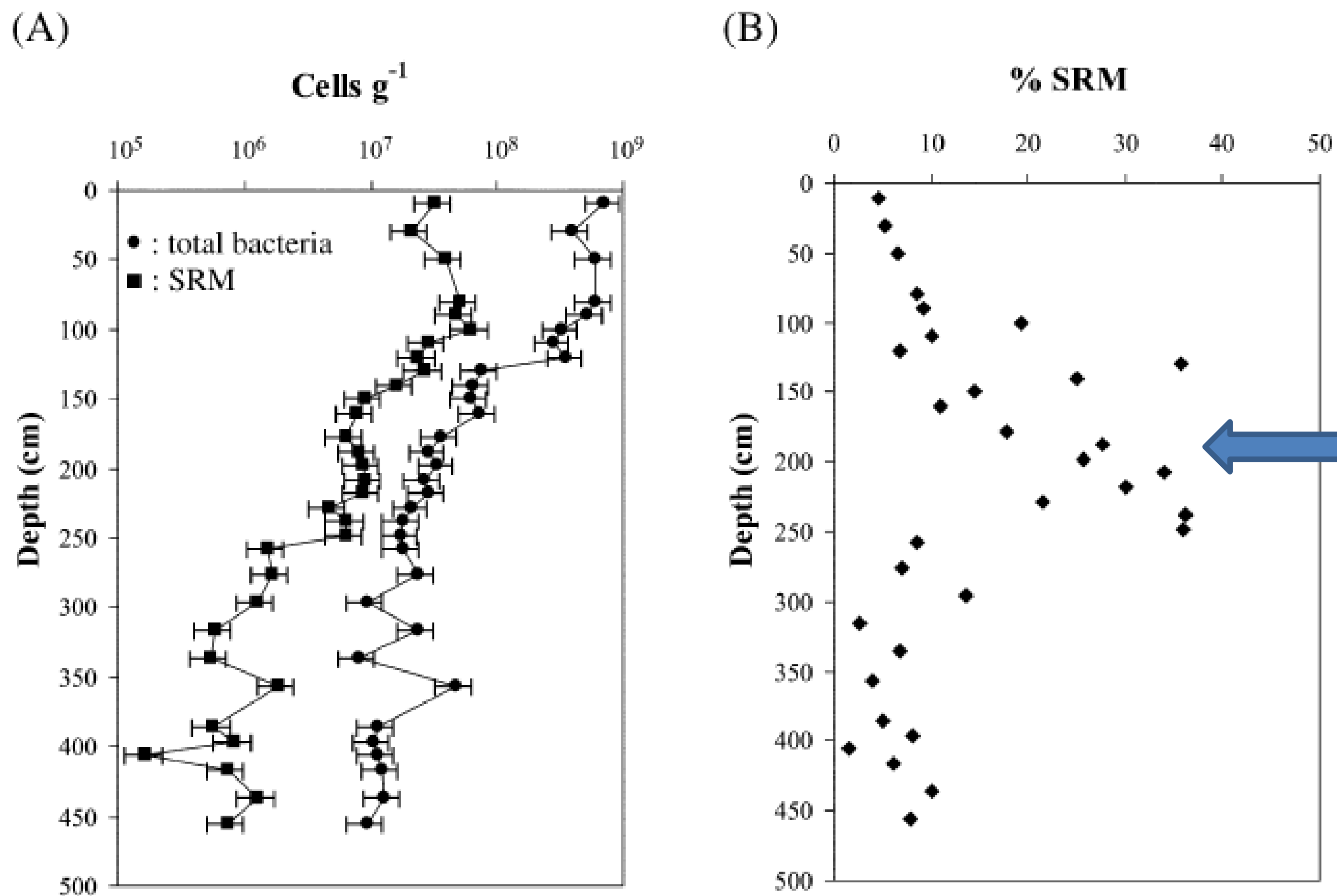
Diversity and abundance of sulfate-reducing microorganisms in the sulfate and methane zones of a marine sediment, Black Sea



Sulfate reducing bacteria (*dsr* gene) peaks at SMTZ

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).

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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

Transcription

Chromosome

Nuclear membrane

RNA polymerase

DNA

Messenger RNA

Transfer RNA with amino acid anticodon

Translation

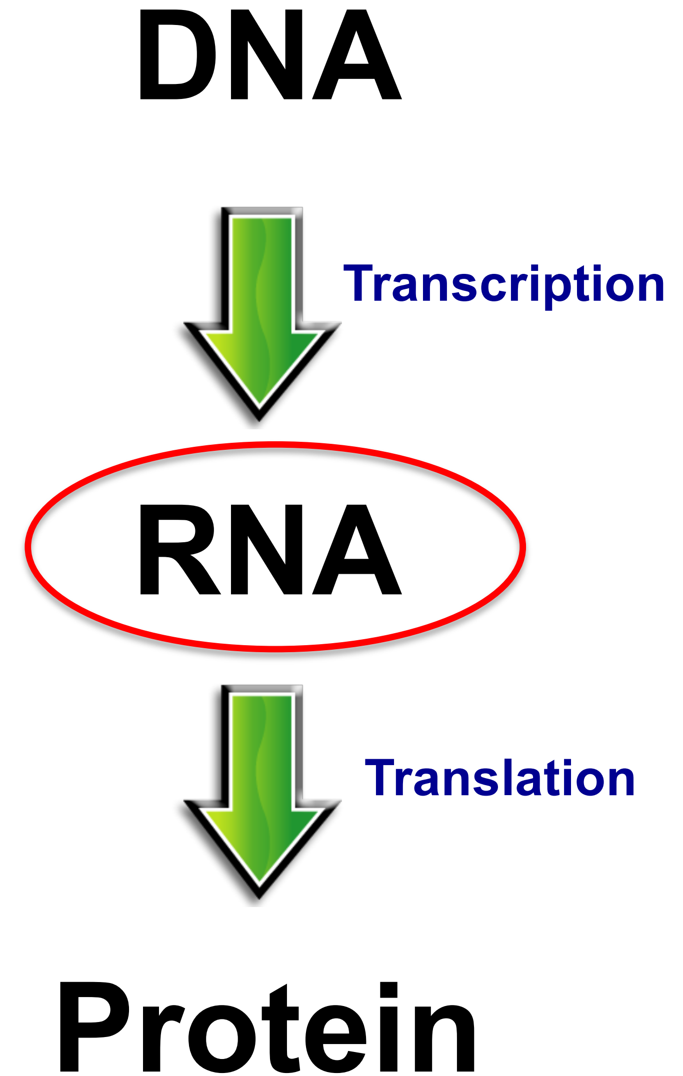
Messenger RNA leaves nucleus

Polypeptide chain

Ribosome

Messenger RNA

Codon



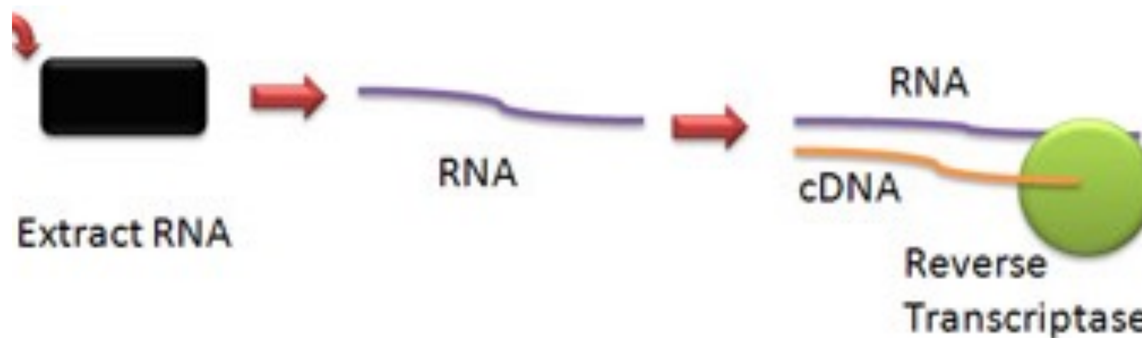
Moving from "who is there?" to "who is active?"

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?

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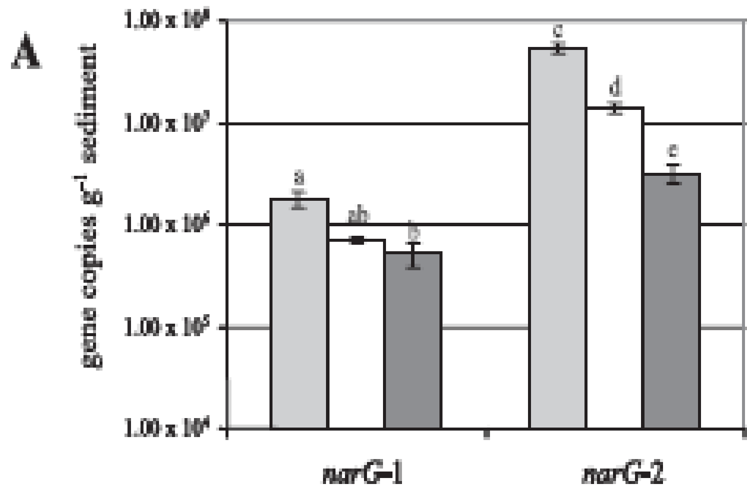
Diversity and Abundance of Nitrate Reductase Genes (*narG* and *napA*), Nitrite Reductase Genes (*nirS* and *nrfA*), and Their Transcripts in Estuarine Sediments[∇]

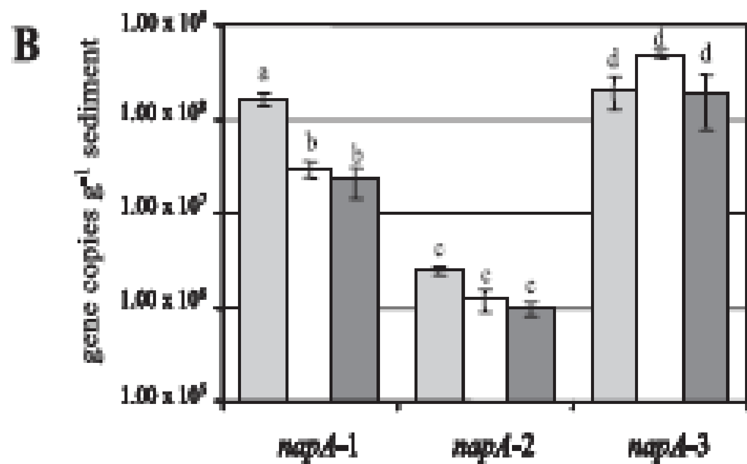
FIG. 1. Map of the Colne estuary, Essex, United Kingdom, showing the locations of the three sampling sites (Hythe, Alresford, and Brightlingsea).

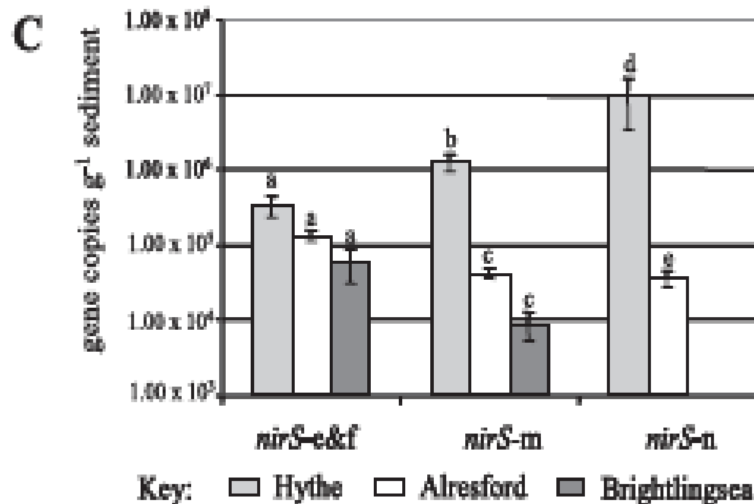
TABLE 1. Primer and probe sets used for Q-(RT)-PCR

Target gene	Phylotype	Amplicon size (bp)	Primer or probe		Q-PCR cycle annealing temp (°C)
			Name[a]	Sequence (5′→3′)	
napA	*napA*-1	111	*napA*-1F	GTY ATG GAR GAA AAA TTC AA	55
			napA-1R	GAR CCG AAC ATG CCR AC	
			napA-1 (TM-MGB)	AAC ATG ACC TGG AAG	
	napA-2	76	*napA*-2F	GAA CCK AYG GGY TGT TATG	55
			napA-2R	TGC ATY TCS GCC ATR TT	
			napA-2 (TM-MGB)	CTT TGG GGT TCA A	
	napA-3	130	*napA*-3F	CCC AAT GCT CGC CAC TG	60
			napA-3R	CAT GTT KGA GCC CCA CAG	
			napA-3 (TM-MGB)	TGG GTT GTT ACG A	
narG	*narG*-1	69	*narG*-1F	GAC TTC CGC ATG TCR AC	60
			narG-1R	TTY TCG TAC CAG GTG GC	
			narG-1 (TM-MGB)	TAY TCC GAC ATC GT	
	narG-2	89	*narG*-2F	CTC GAY CTG GTG GTY GA	55
			narG-2R	TTY TCG TAC CAG GTS GC	
			narG-2 (TM-MGB)	AAC TTC CGC ATG GA	
nrfA	*nrfA*-2	67	*nrfA*-2F	CAC GAC AGC AAG ACT GCC G	60
			nrfA-2R	CCG GCA CTT TCG AGC CC	
			nrfA-2 (TM-MGB)	TTG ACC GTC GGC A	
nirS	*nirS*-e	172	*nirS*-efF	CAC CCG GAG TTC ATC GTC	60
			nirS-efR	ACC TTG TTG GAC TGG TGG G	
			nirS-ef (TM-MGB)	TGC TGG TCA ACT A	
	nirS-m	162	*nirS*-mF	GGA AAC CTG TTC GTC AAG AC	60
			nirS-mR	CSG ART CCT TGG CGA CGT	
			nirS-m (TM)	TCT GGG CCG ACG CGC CGA TGA AC	
	nirS-n	140	*nirS*-nF	AAG GAA GTC TGG ATY TC	55
			nirS-nR[b]	CGT TGA ACT TRC CGG T	
			nirS-n (TM-MGB)	ATC CGA AGA TSA	

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



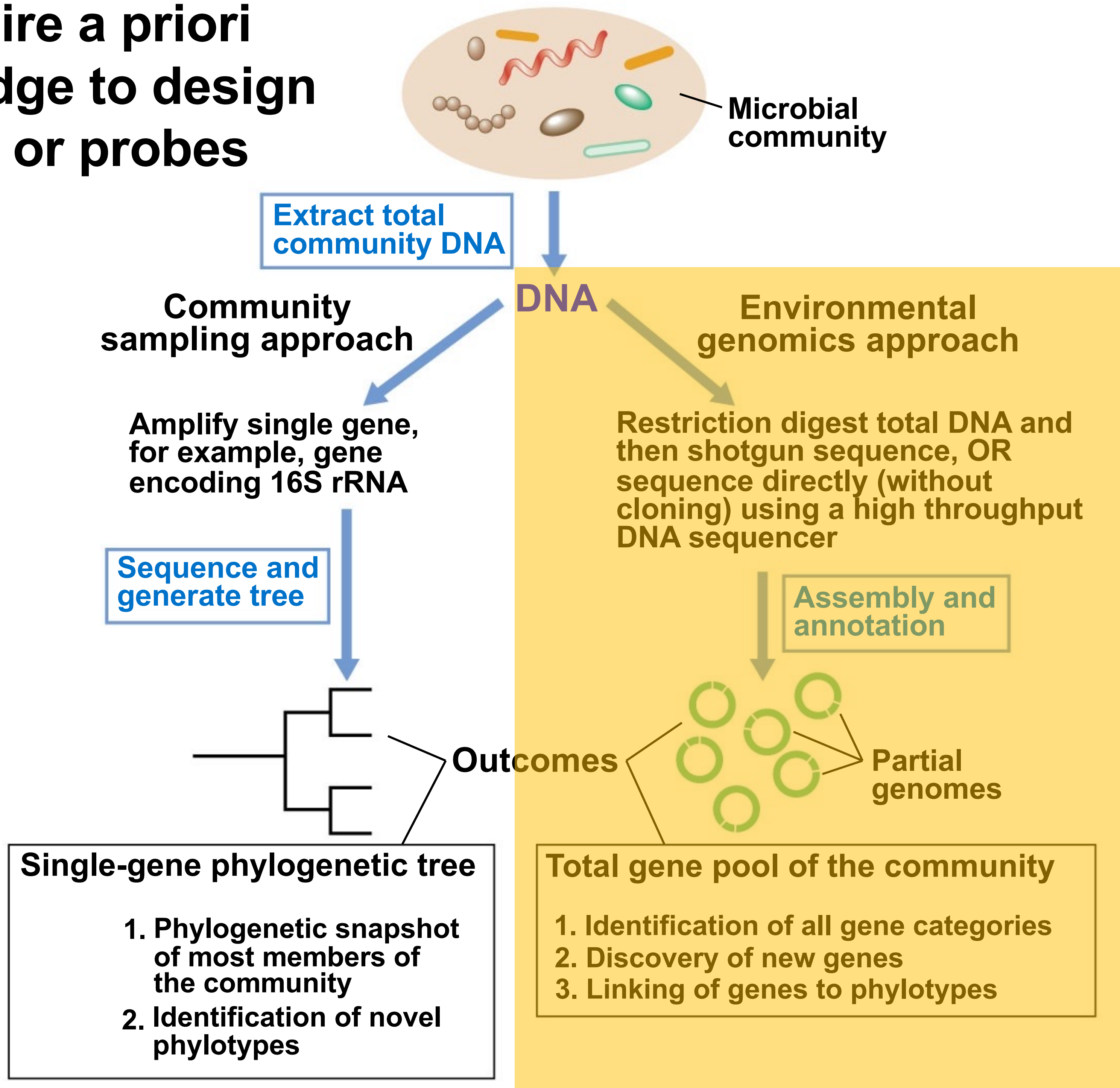




qRT-PCR

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



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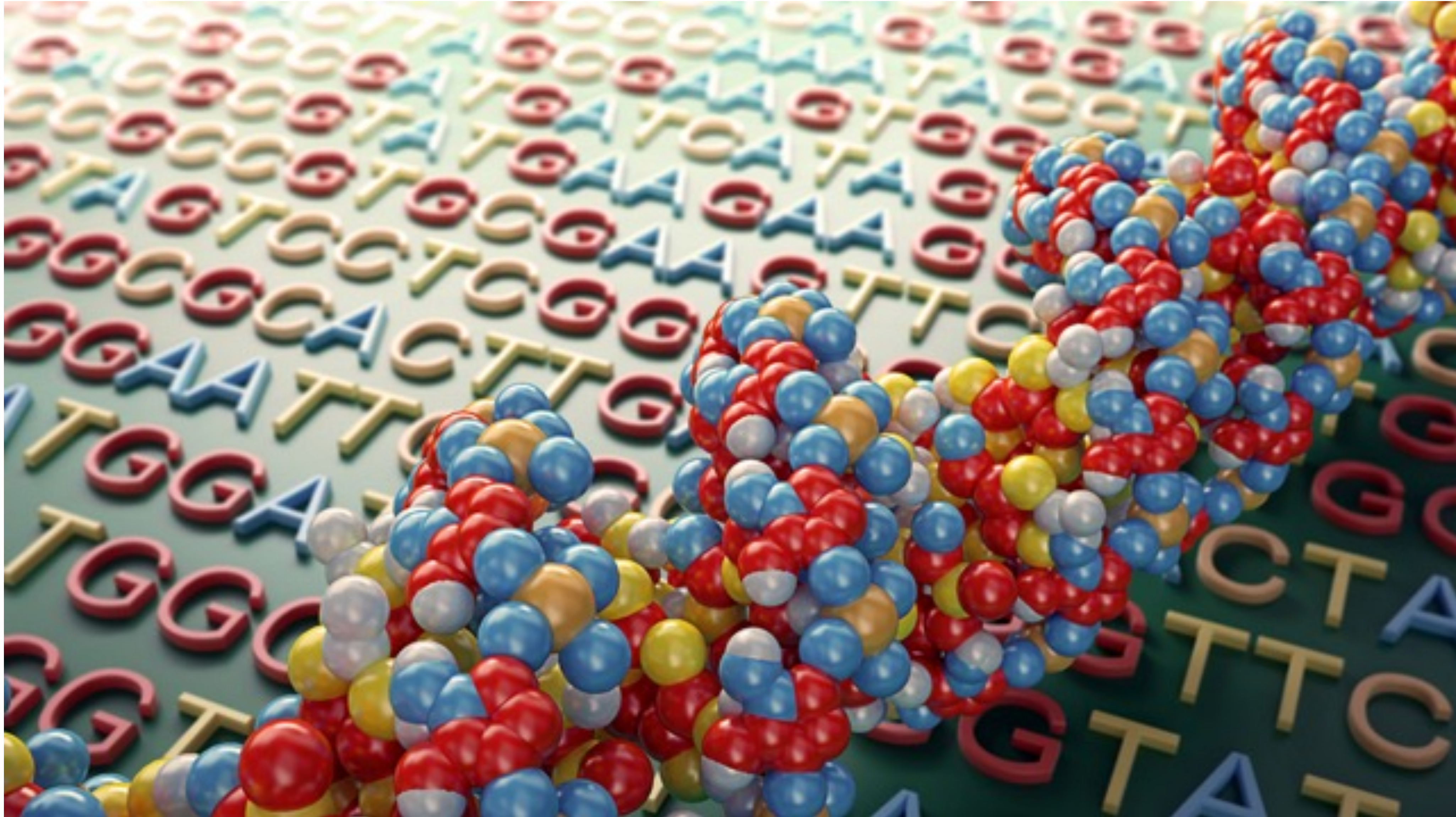
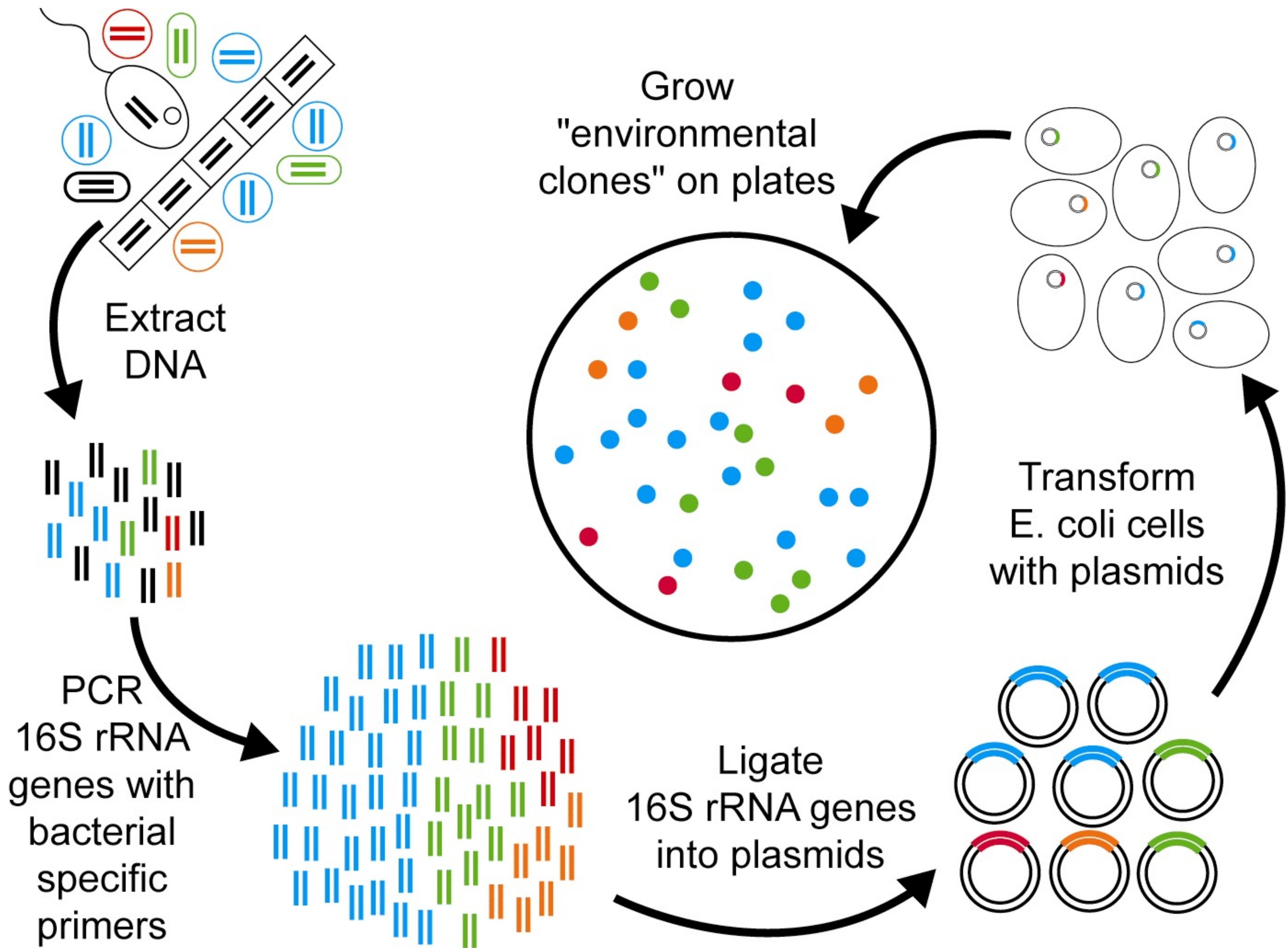
Sequencing Revolution

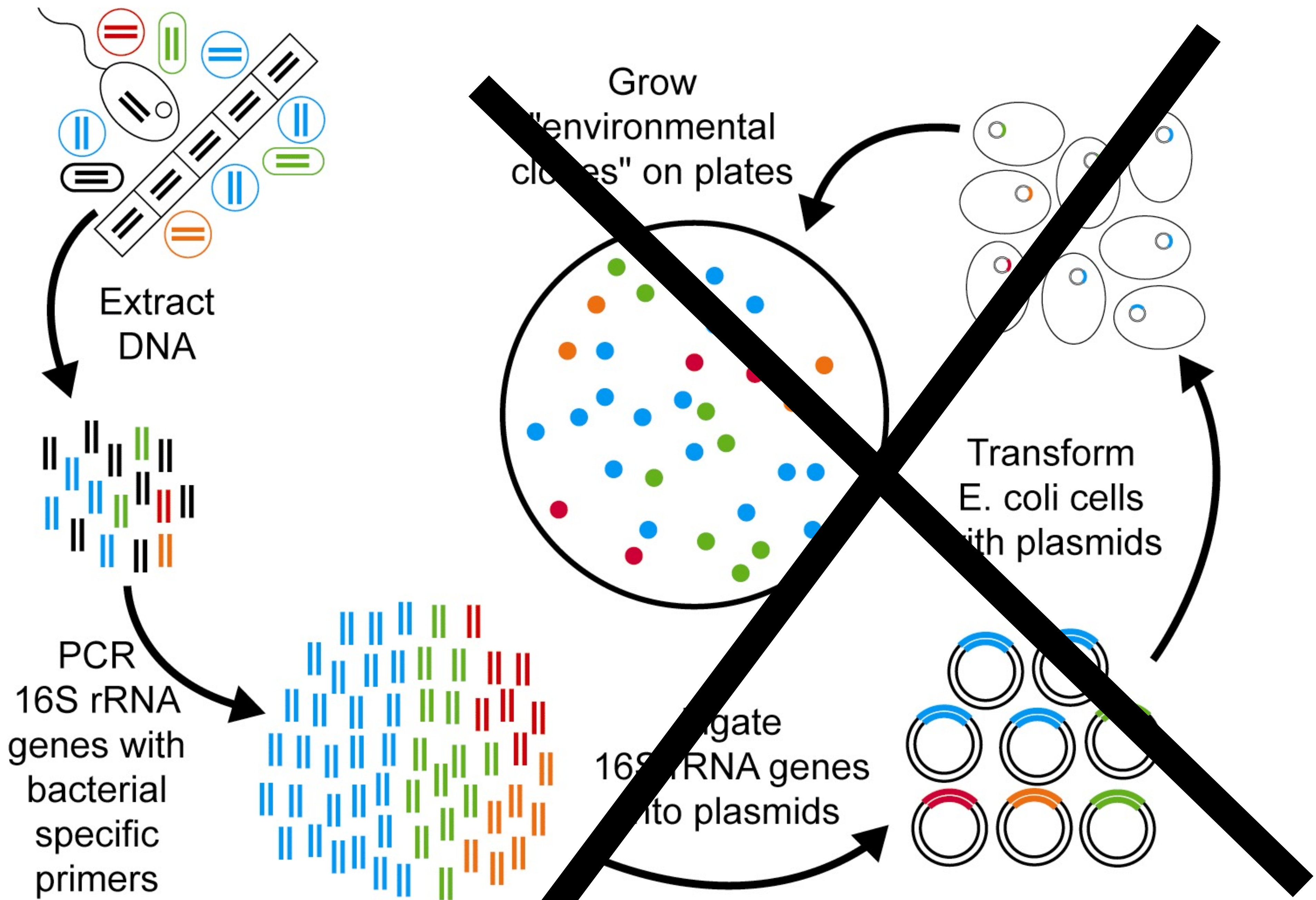


Traditional Gene Cloning

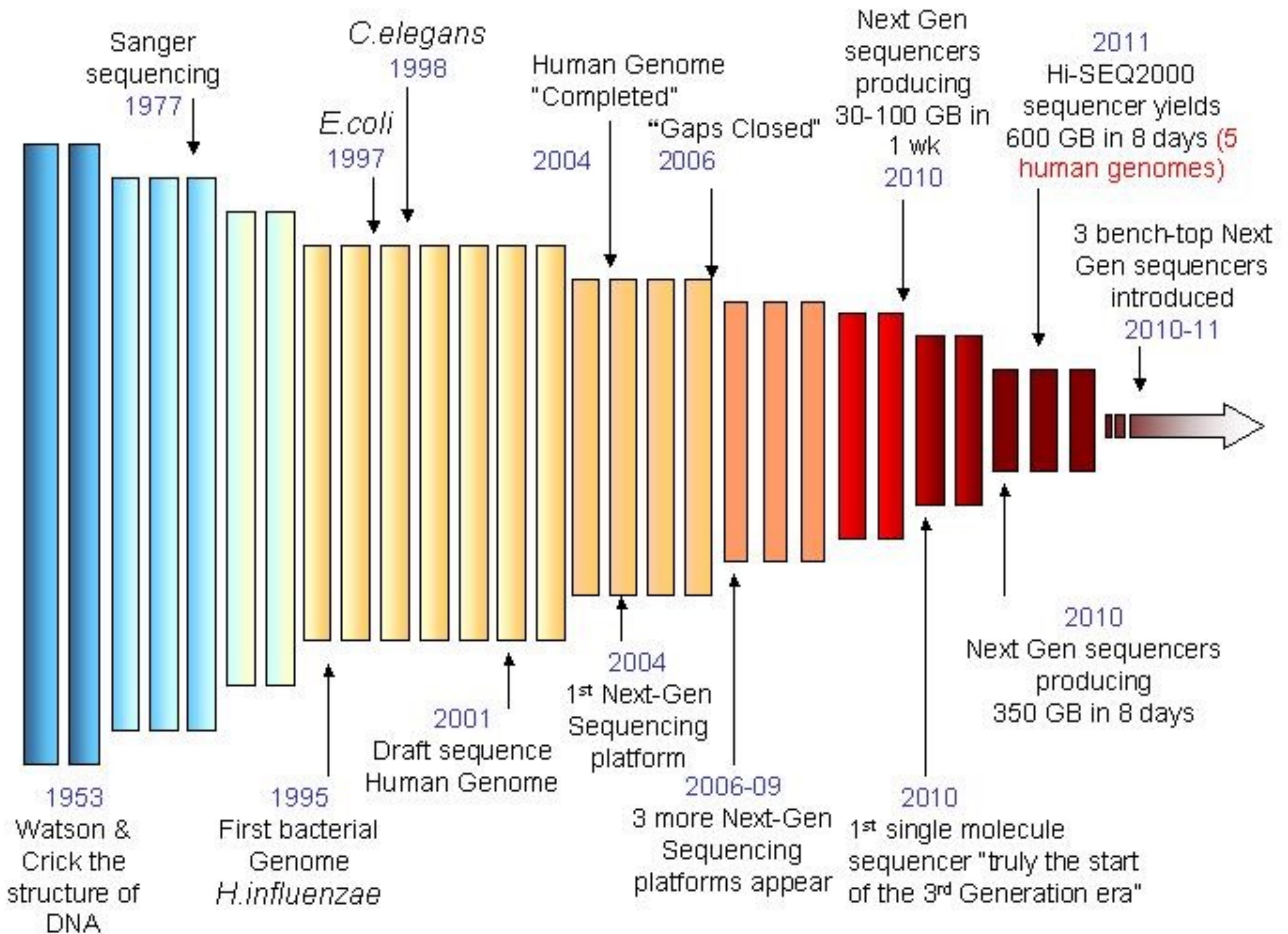


Schematic courtesy of B. Crump

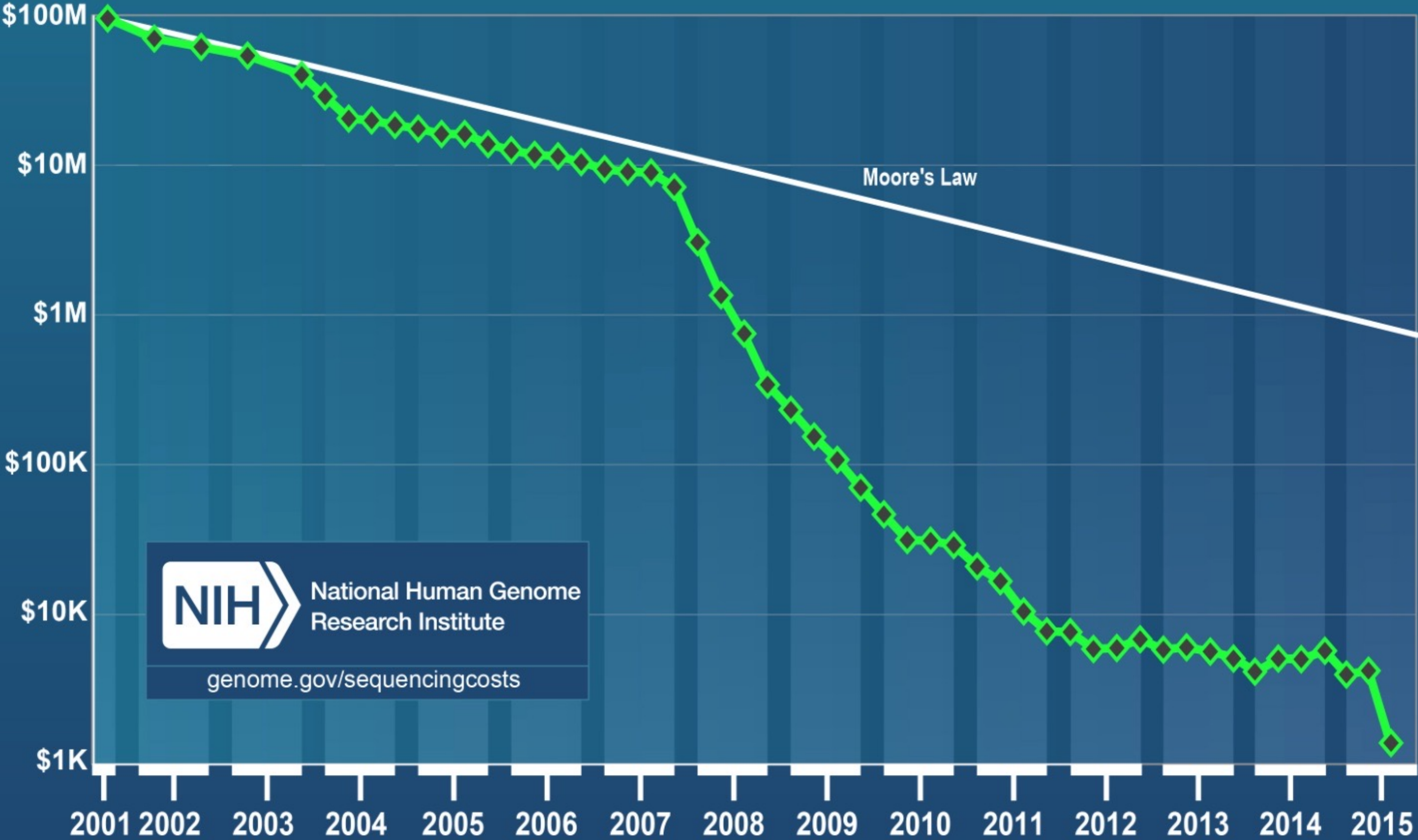
NextGen Approaches



Schematic courtesy of B. Crump

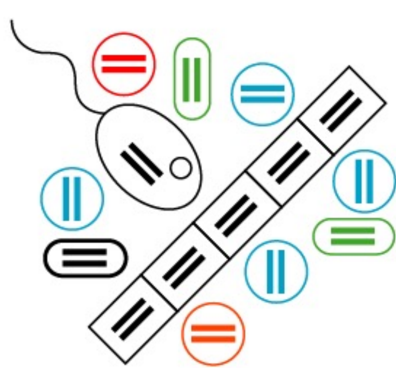


http://www.ipc.nxgenomics.org

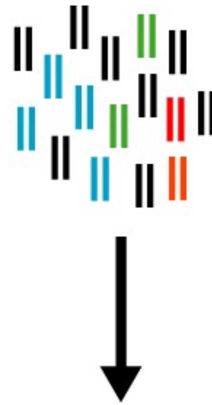

Cost per Genome
$100M
$10M
$1M
$100K
$10K
$1K
Moore's Law
NIH
National Human Genome Research Institute
genome.gov/sequencingcosts
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015

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

Metagenomics

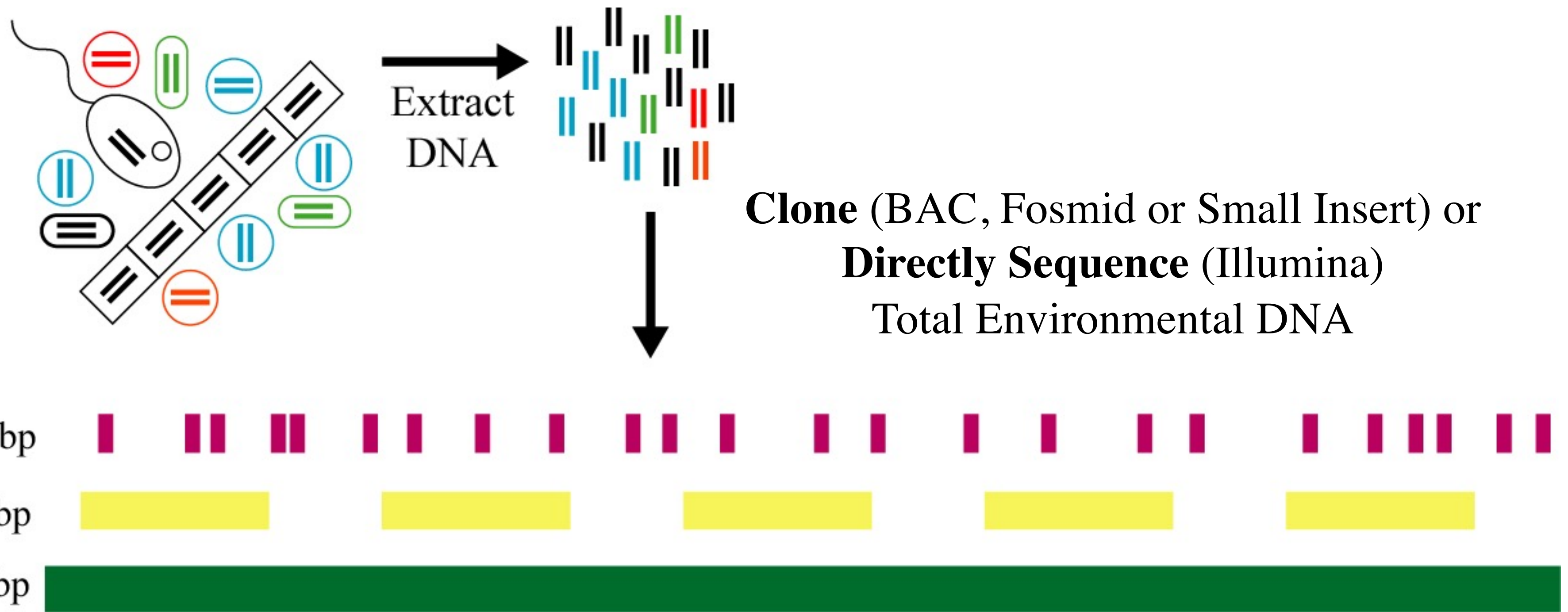


Extract
DNA

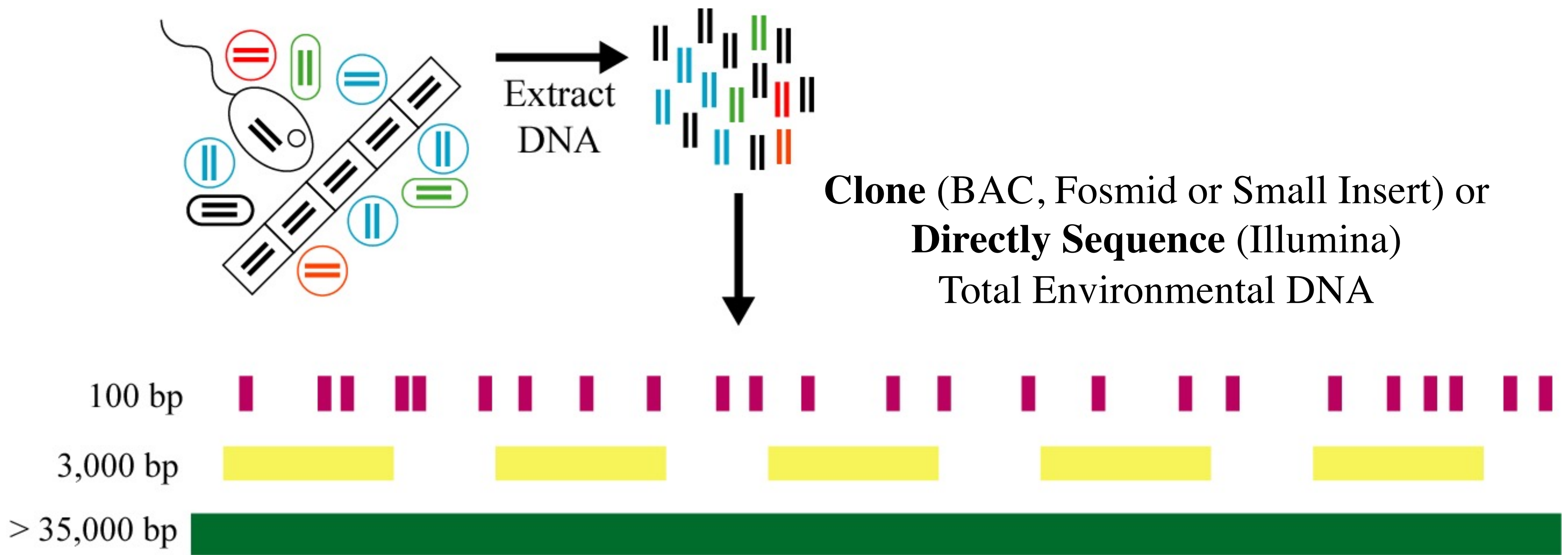


Clone (BAC, Fosmid or Small Insert)
or
Directly Sequence (Illumina, PacBio, 10X, Nanopore, etc)
Total Environmental DNA

Metagenomics



Metagenomics



Access genomes of uncultured microbes:

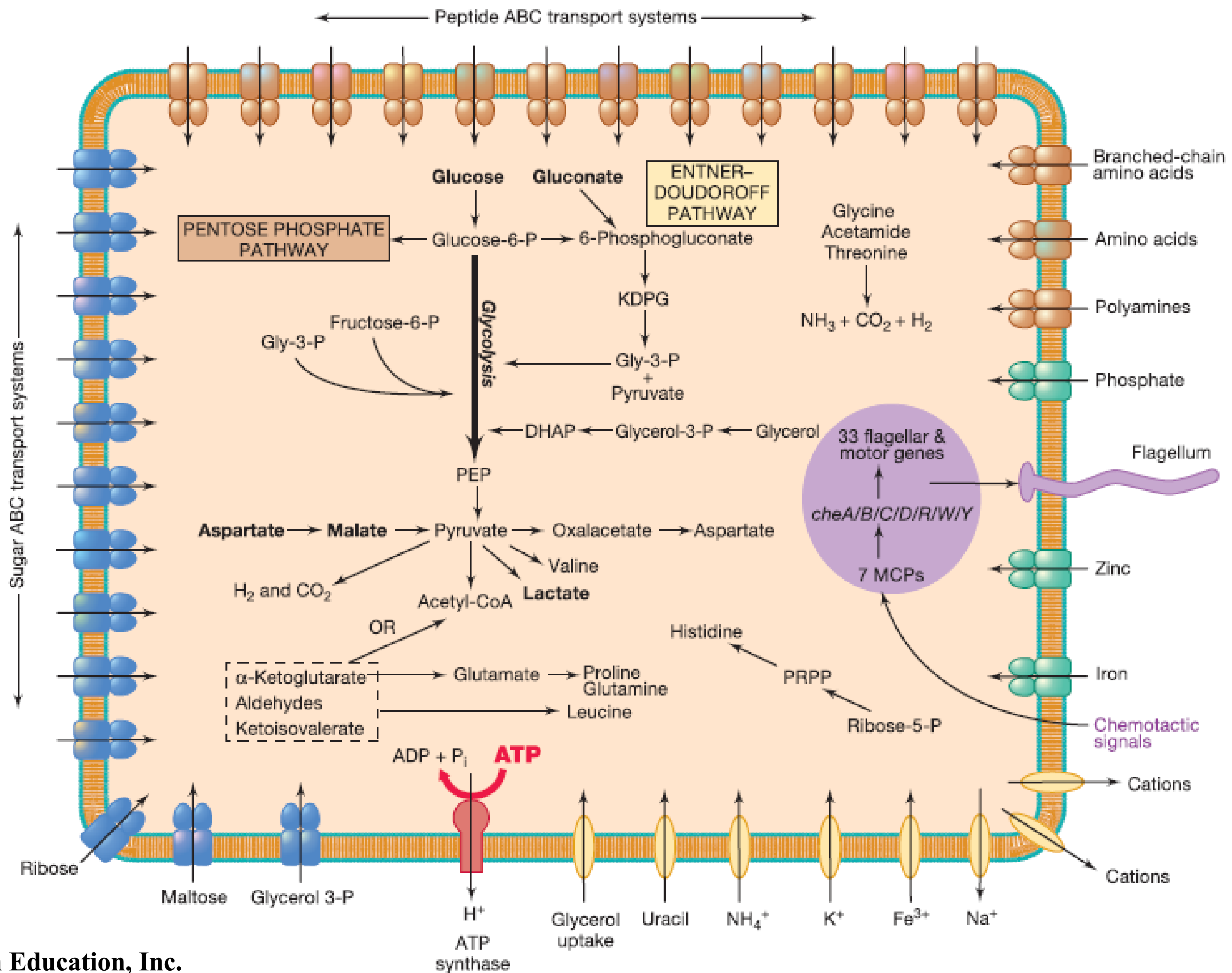
Functional Potential

Metabolic Pathways

Horizontal Gene Transfer

…

Reconstruct Genomes



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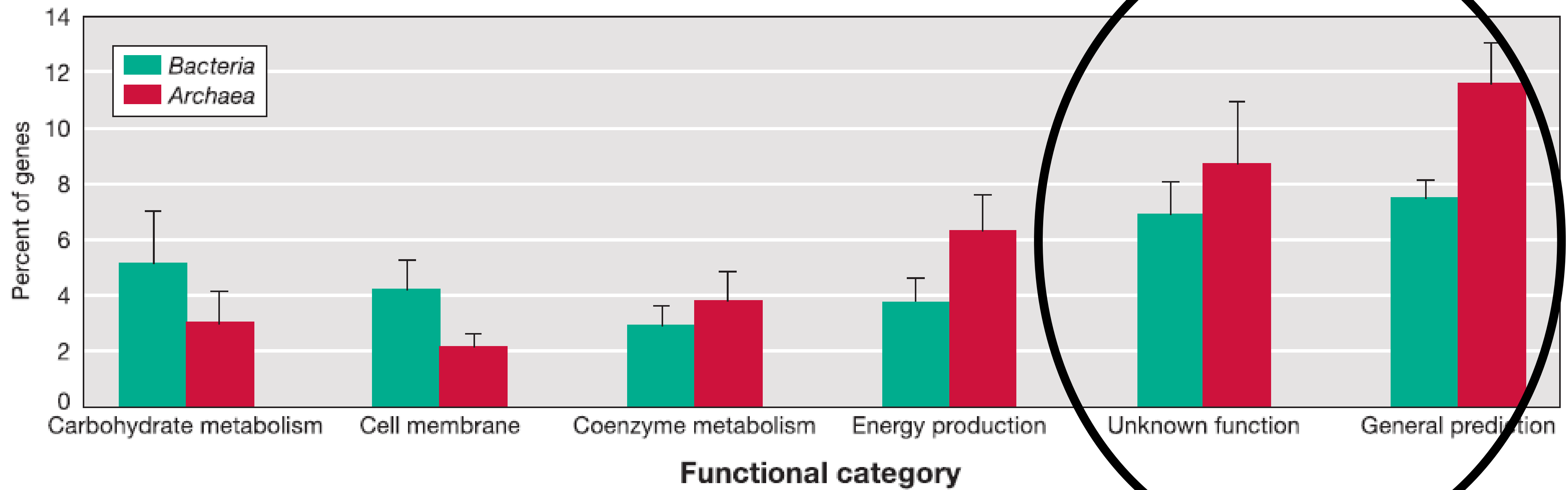
Categorize Functions



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Proteorhodopsin phototrophy in the ocean

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

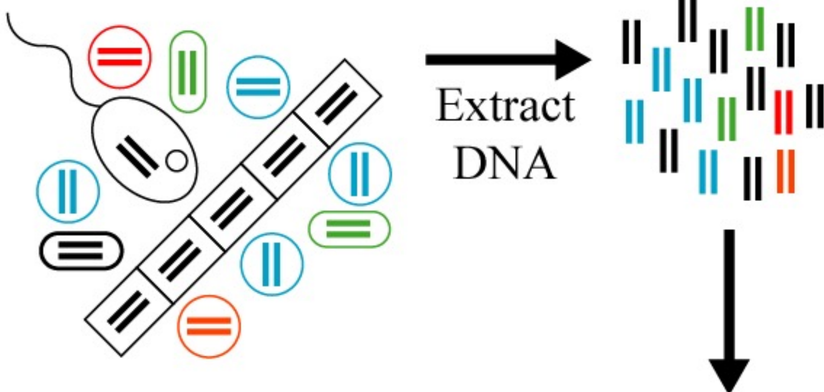
Bacterial Rhodopsin: Evidence for a New Type of Phototrophy in the Sea

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*]

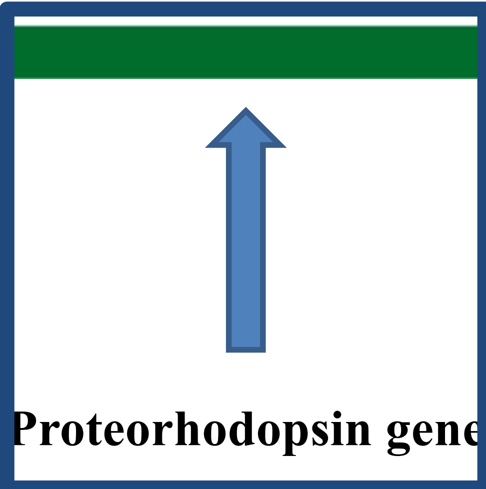
Proteorhodopsin genes are distributed among divergent marine bacterial taxa

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

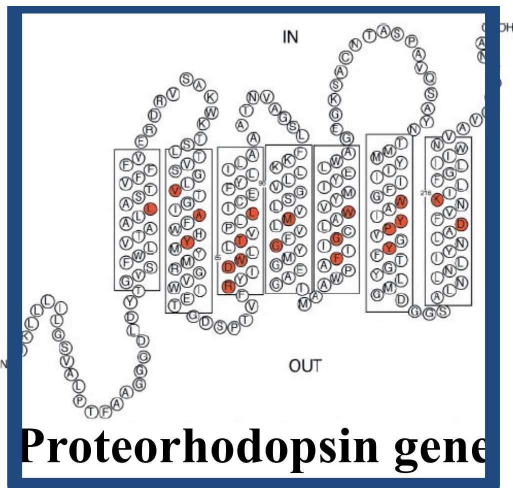
Metagenomics

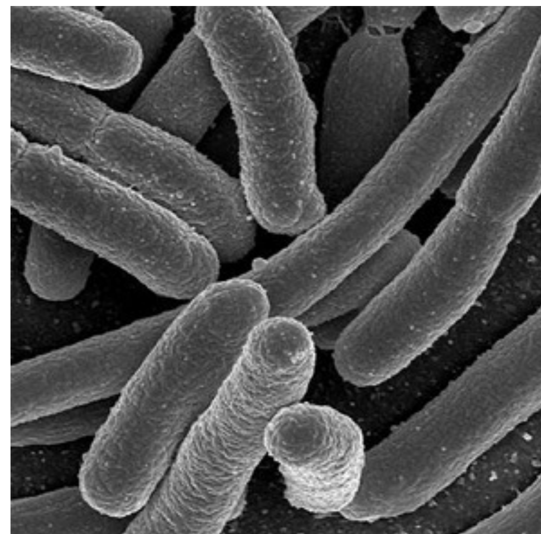


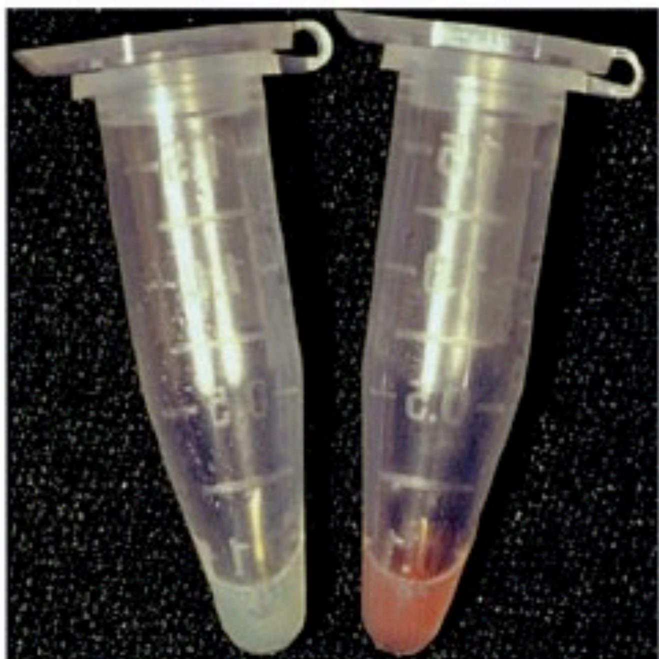
Clone (BAC, Fosmid or Small Insert) or
Directly Sequence (Illumina)
Total Environmental DNA

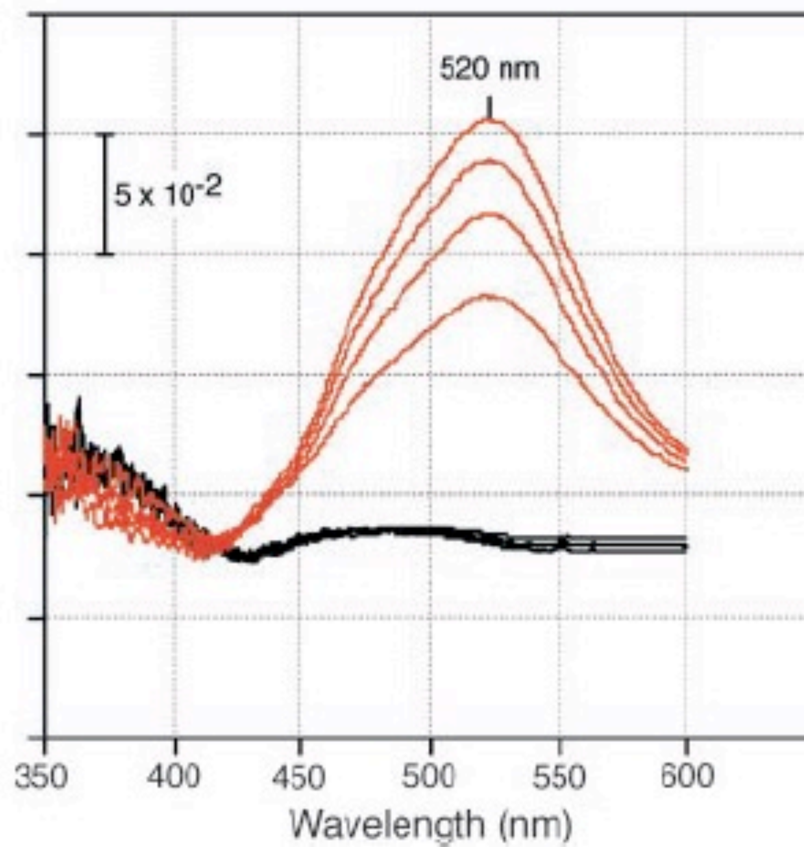


16s rRNA gene
Gammaproteobacteria
SAR86

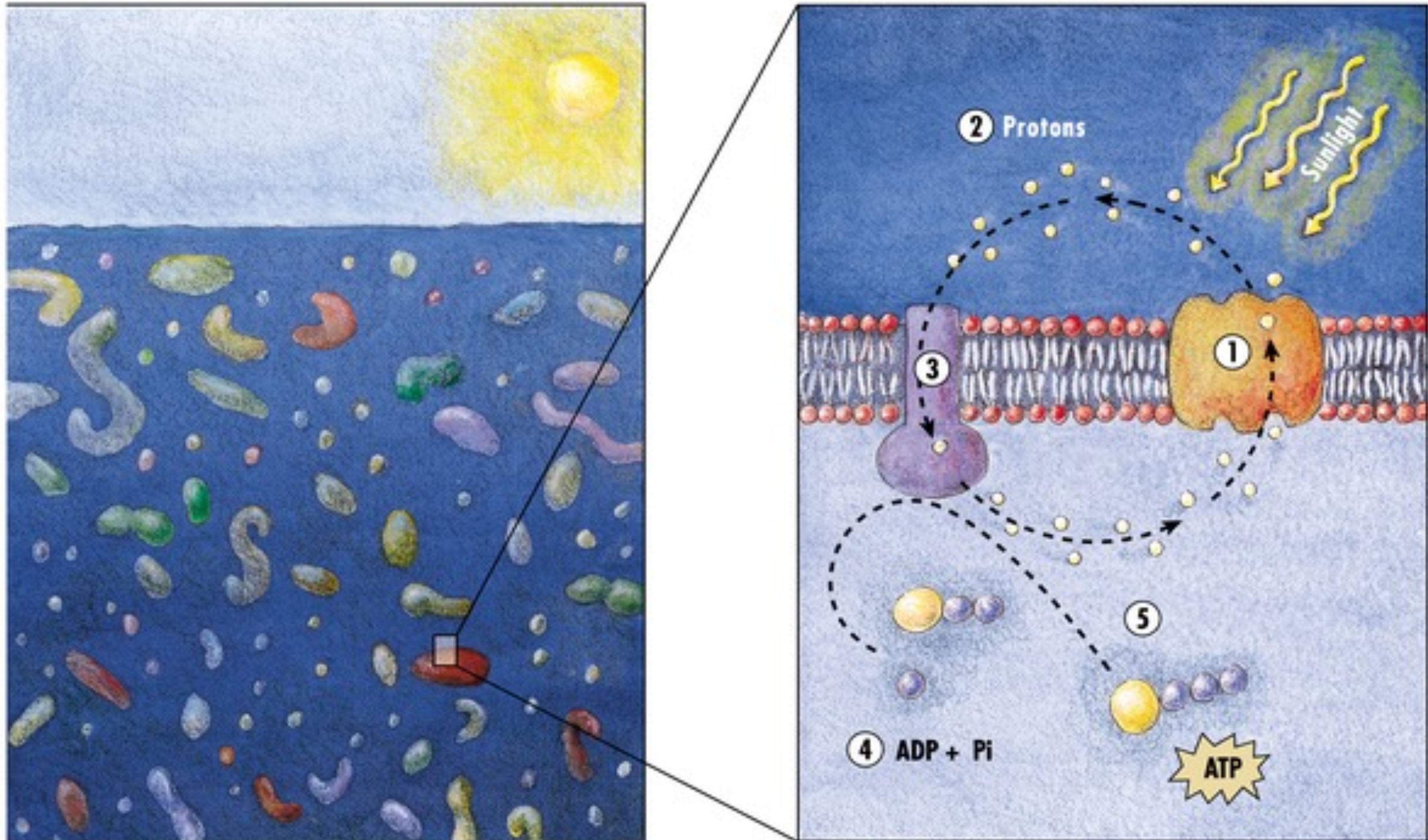

IN
OUT
Proteorhodopsin gene




A


B
520 nm
5 x 10-2
Absorbance (x10-2)
350
400
450
500
550
600
Wavelength (nm)

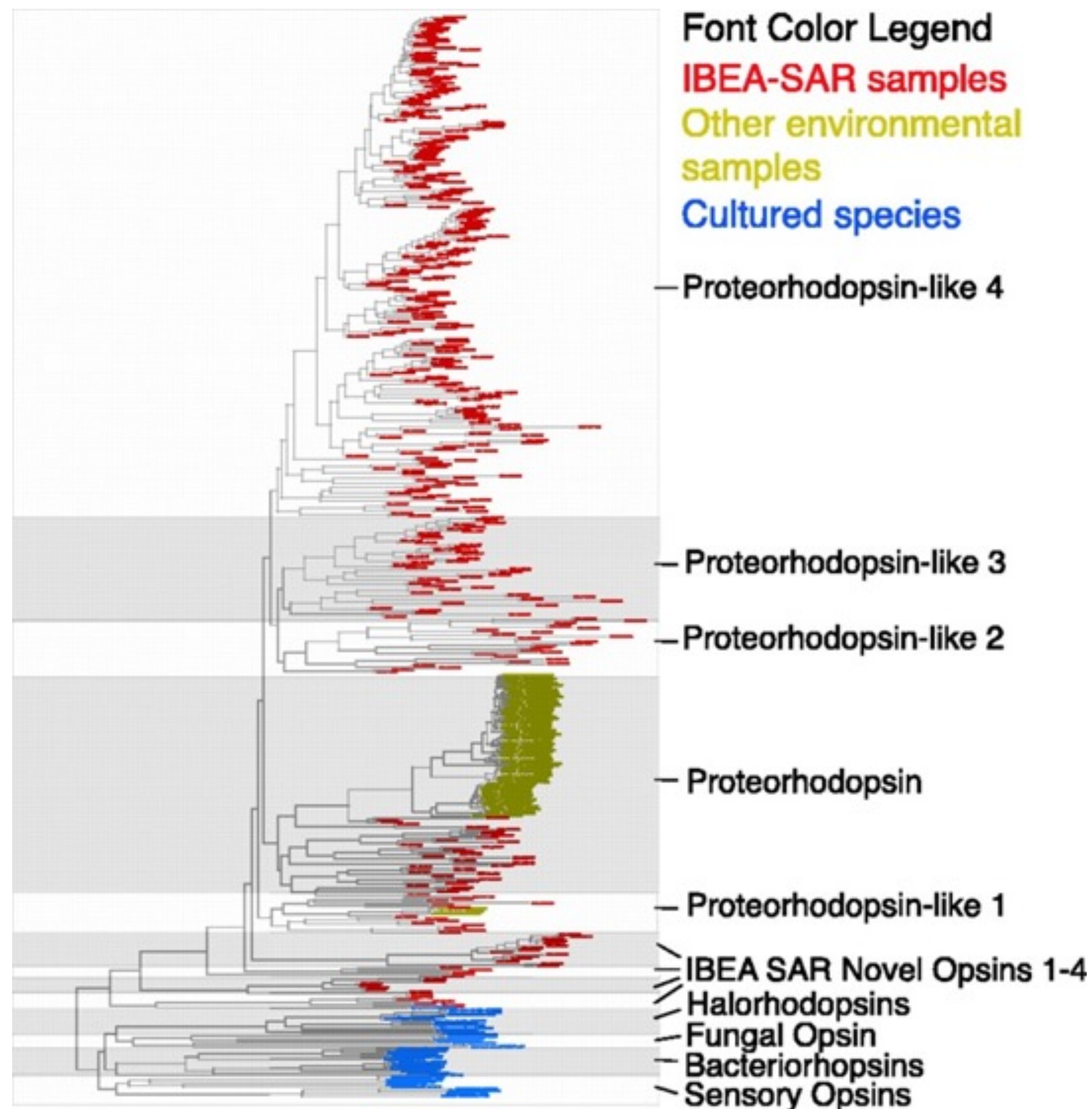
A new way of using sunlight in the surface ocean



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

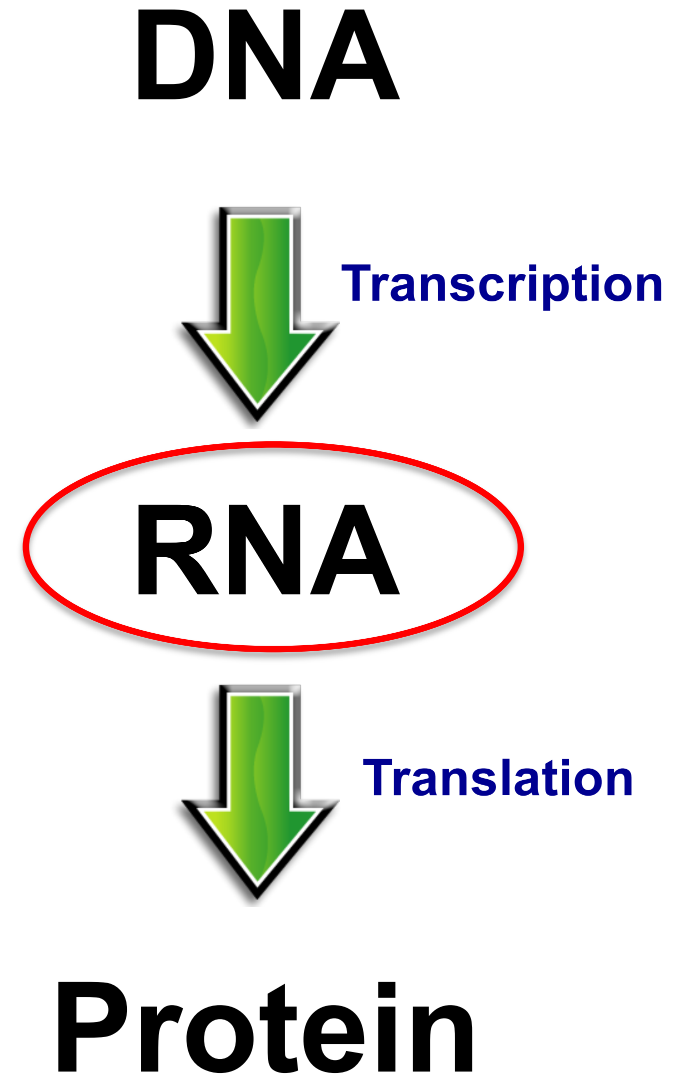
PLoS BIOLOGY

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



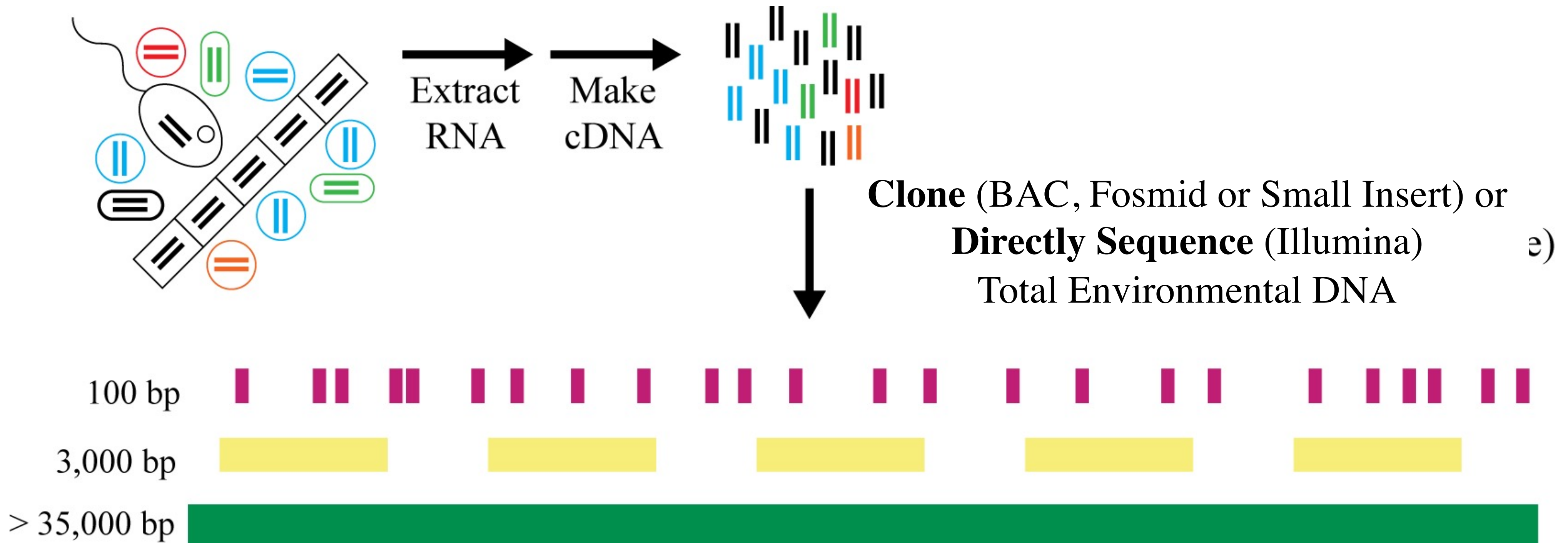
Venter *et al*. 2004

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.

And the list goes on…

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