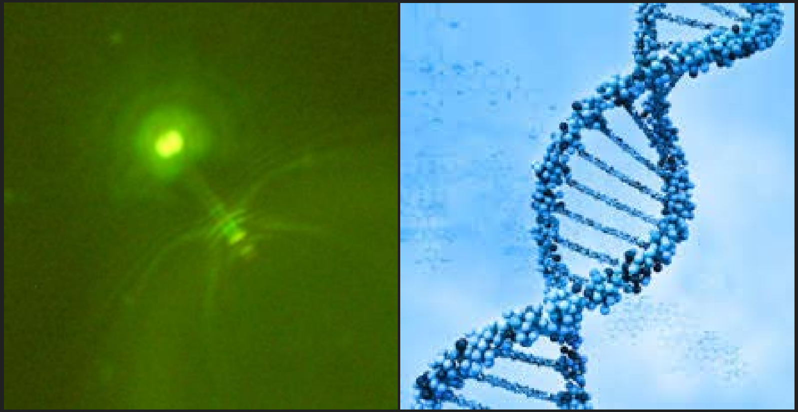
Unveiling the `microbial dark matter´ through Single Cell and Virus Genomics



Manuel Martínez-Garcia
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Òscar Fornas, Francisco Martínez, Mónica Lluesma-Gómez, Josefa Antón Botella, Manuel Martínez-Garcia

Universitat d'Alacant
Universidad de Alicante

Deep Underground Laboratory Integrated
Activity in biology (DULIA-bio)

Index

1. What is `microbial dark matter´ and why is important

What is `microbial dark matter´ and why is important

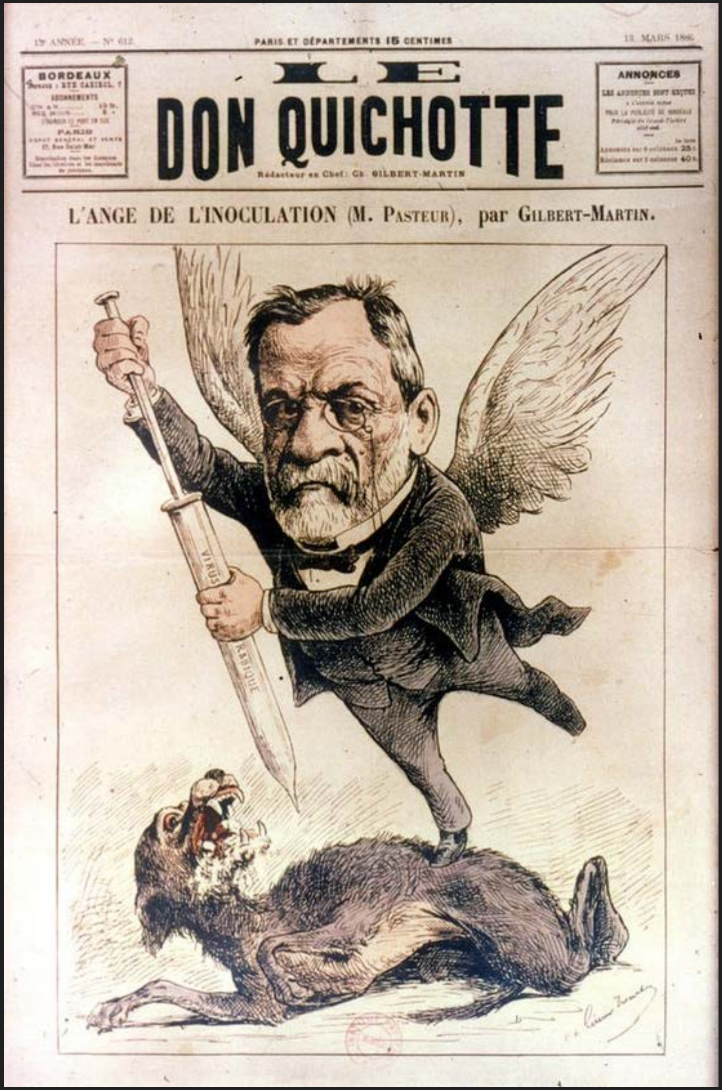
≈80 % of the mass of the universe is made up of material that scientist cannot directly observe=**Dark matter**

From…http://microbialdarkmatter.org/

≈99%

of microbes in nature are

UNKNOWN



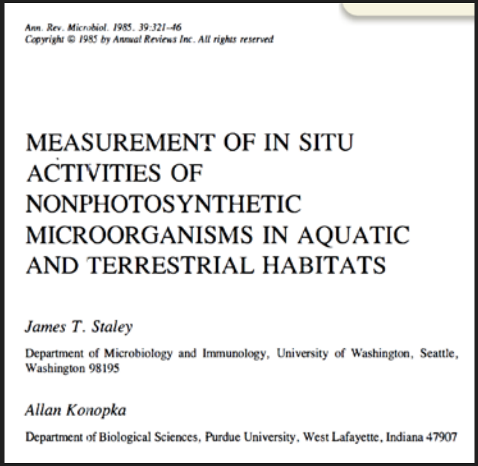
Louis Pasteur

Robert Koch

Culture medium for growing microbes: a brilliant idea in Microbiology (*that…*

…has biased our view of microbes for many years)

GREAT COUNT PLATE ANOMALY, 1985



Ann. Rev. Microbiol. 1985. 39:321–46
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MEASUREMENT OF IN SITU
ACTIVITIES OF
NONPHOTOSYNTHETIC
MICROORGANISMS IN AQUATIC
AND TERRESTRIAL HABITATS

James T. Staley

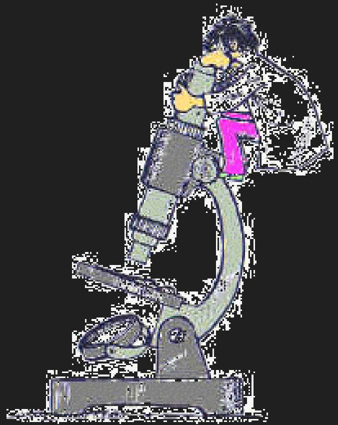
Department of Microbiology and Immunology, University of Washington, Seattle, Washington 98195

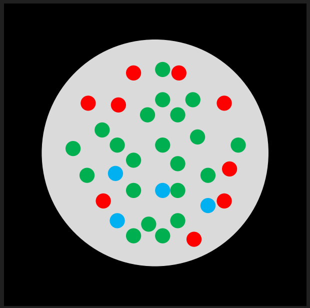
Allan Konopka

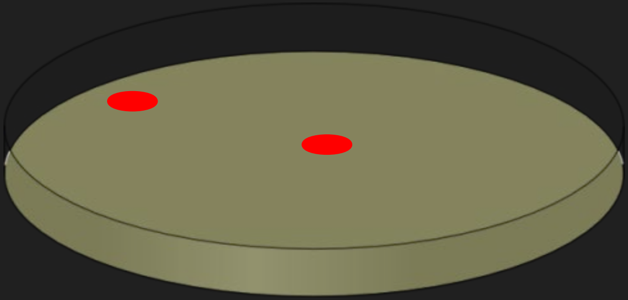
Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907

Direct observation

Culture





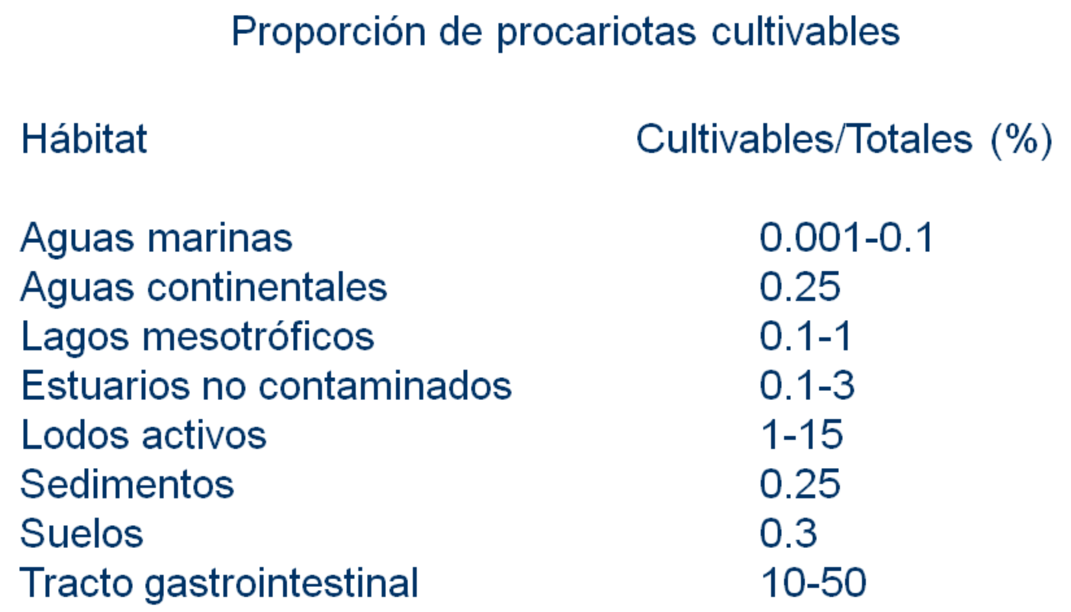


Counting 200 microorganisms in 0,1 ml of sample:
2000 microorganisms/mL in natural sample

Counting 2 CFU in 0,1 ml of sample
20 microorganisms/mL in natural sample

What is the right data?

What is `microbial dark matter´ and why is important

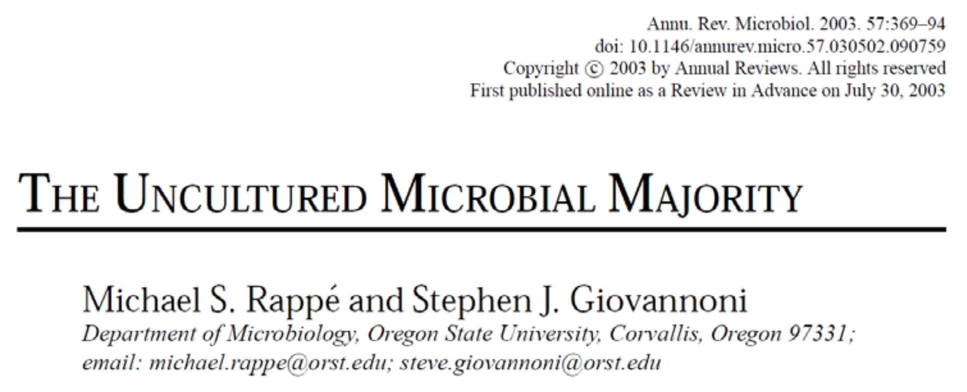


Proporción de procariotas cultivables

Hábitat	Cultivables/Totales (%)
Aguas marinas	0.001-0.1
Aguas continentales	0.25
Lagos mesotróficos	0.1-1
Estuarios no contaminados	0.1-3
Lodos activos	1-15
Sedimentos	0.25
Suelos	0.3
Tracto gastrointestinal	10-50

Amann and Scheiffer, 1985

Unfortunately, nowadays we know that **most microbes cannot be cultured in the lab**



Annu. Rev. Microbiol. 2003. 57:369–94
doi: 10.1146/annurev.micro.57.030502.090759
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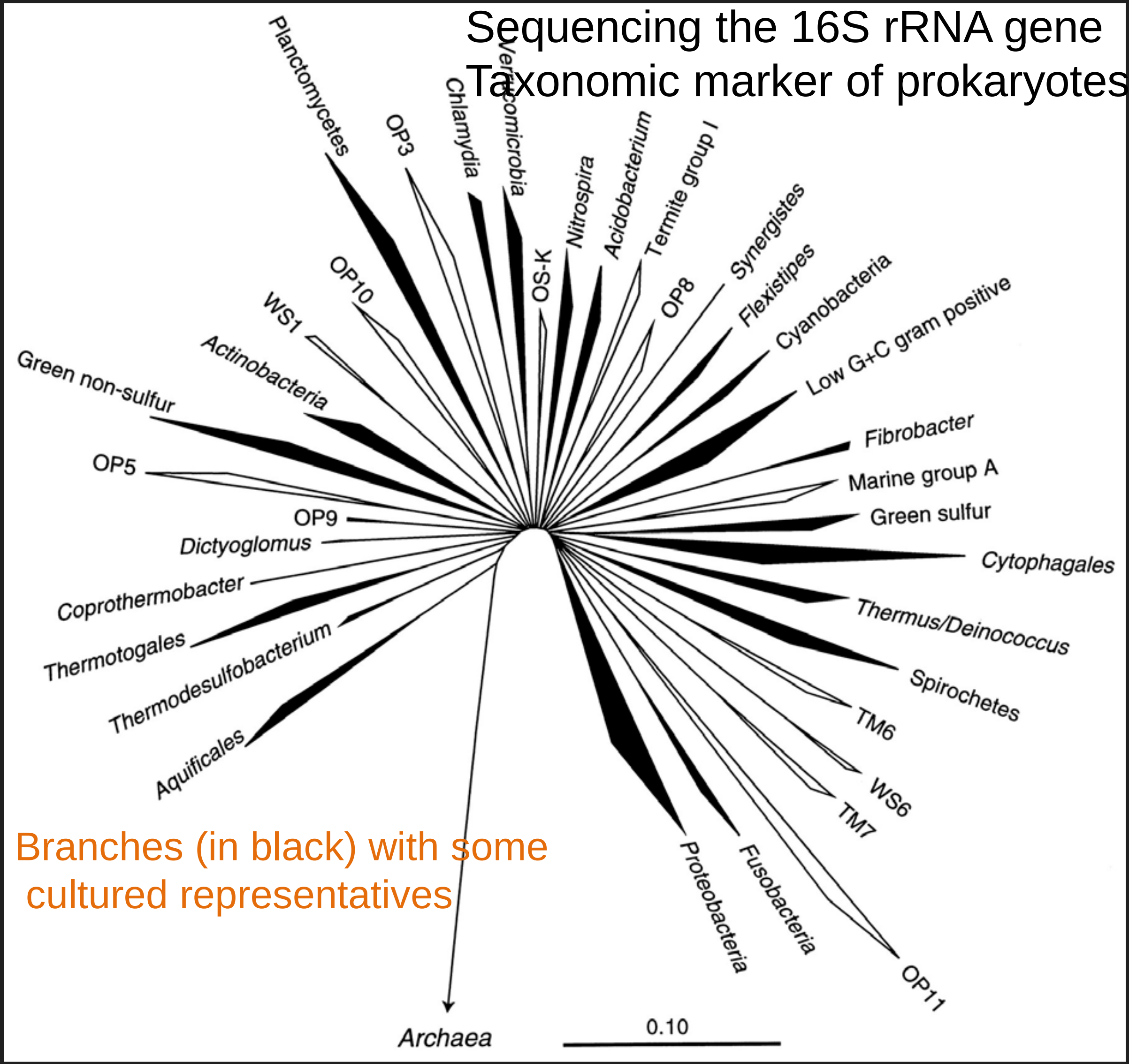
First published online as a Review in Advance on July 30, 2003

THE UNCULTURED MICROBIAL MAJORITY

Michael S. Rappé and Stephen J. Giovannoni
Department of Microbiology, Oregon State University, Corvallis, Oregon 97331;
email: michael.rappe@orst.edu; steve.giovannoni@orst.edu

The Reality
(microbial
dark matter)

Our “reality
with the Culturing view”



Sequencing the 16S rRNA gene
Taxonomic marker of prokaryotes

Branches (in black) with some
cultured representatives

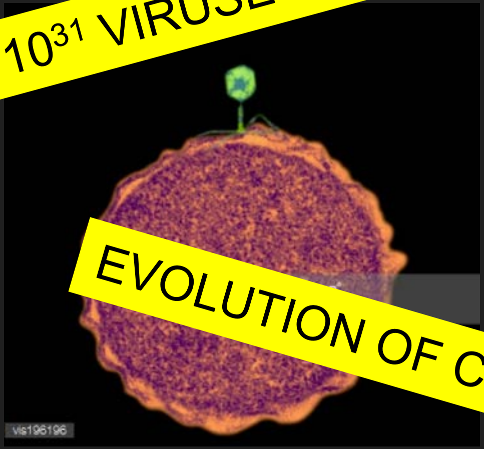
Huge microbial diversity out there to be discovered…

Far from understanding the “whole picture” of bacteria: role, metabolism, physiology, biological interactions, role in diseases, etc…etc…etc…

What about Viruses in nature?. Key players in...

Horizontal gene trasnfer

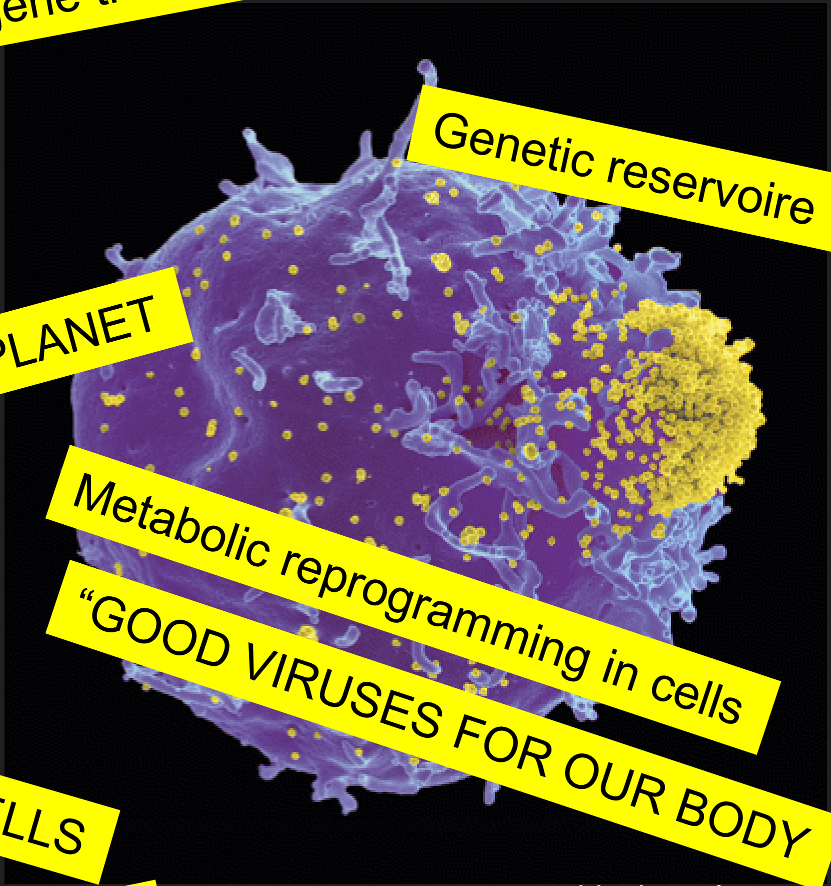
10^{31} VIRUSES IN OUR PLANET



EVOLUTION OF CELLS

RESERVOIRE OF C, P AND N

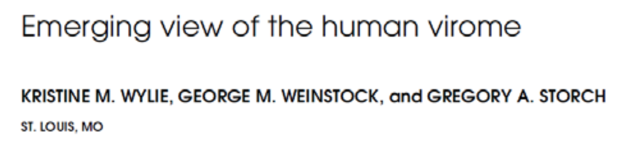
Genetic reservoire



Metabolic reprogramming in cells

“GOOD VIRUSES FOR OUR BODY

Kubinak et al. 2012

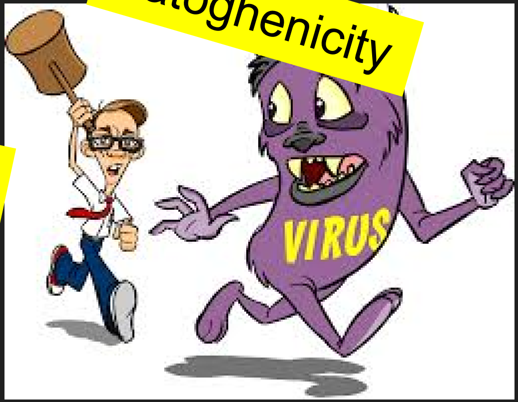


Emerging view of the human virome

KRISTINE M. WYLIE, GEORGE M. WEINSTOCK, and GREGORY A. STORCH

ST. LOUIS, MO

Patoghenicity

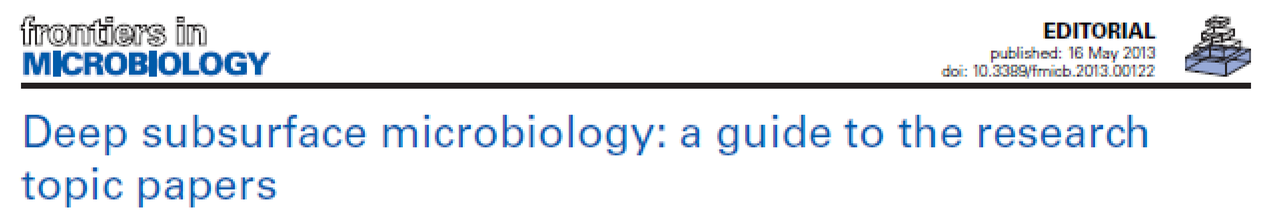


Biogeochemical cycles

VIRUSES SHAPE EVERY CELL OF THIS WORLD

Microbial Diversity and Function

What remain to be answered?



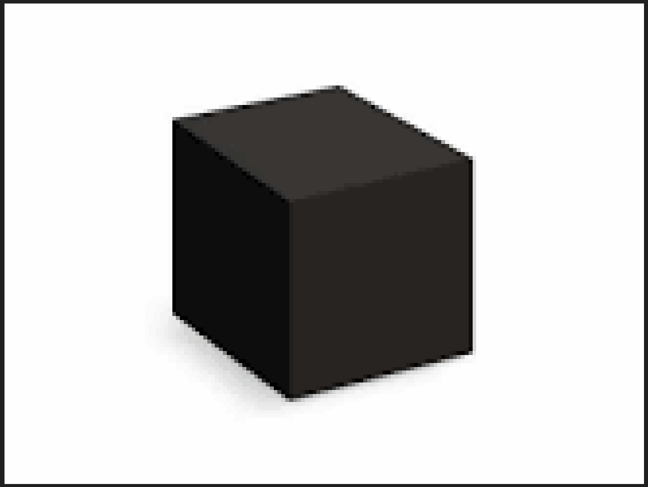
frontiers in
MICROBIOLOGY

EDITORIAL
published: 16 May 2013
doi: 10.3389/fmicb.2013.00122

Deep subsurface microbiology: a guide to the research topic papers

Andreas Teske [1], Jennifer F. Biddle [2], Virginia P. Edgcomb [3] and Axel Schippers [4]*

(almost) EVERYTHING



A few things we know about viruses in deep subsurface

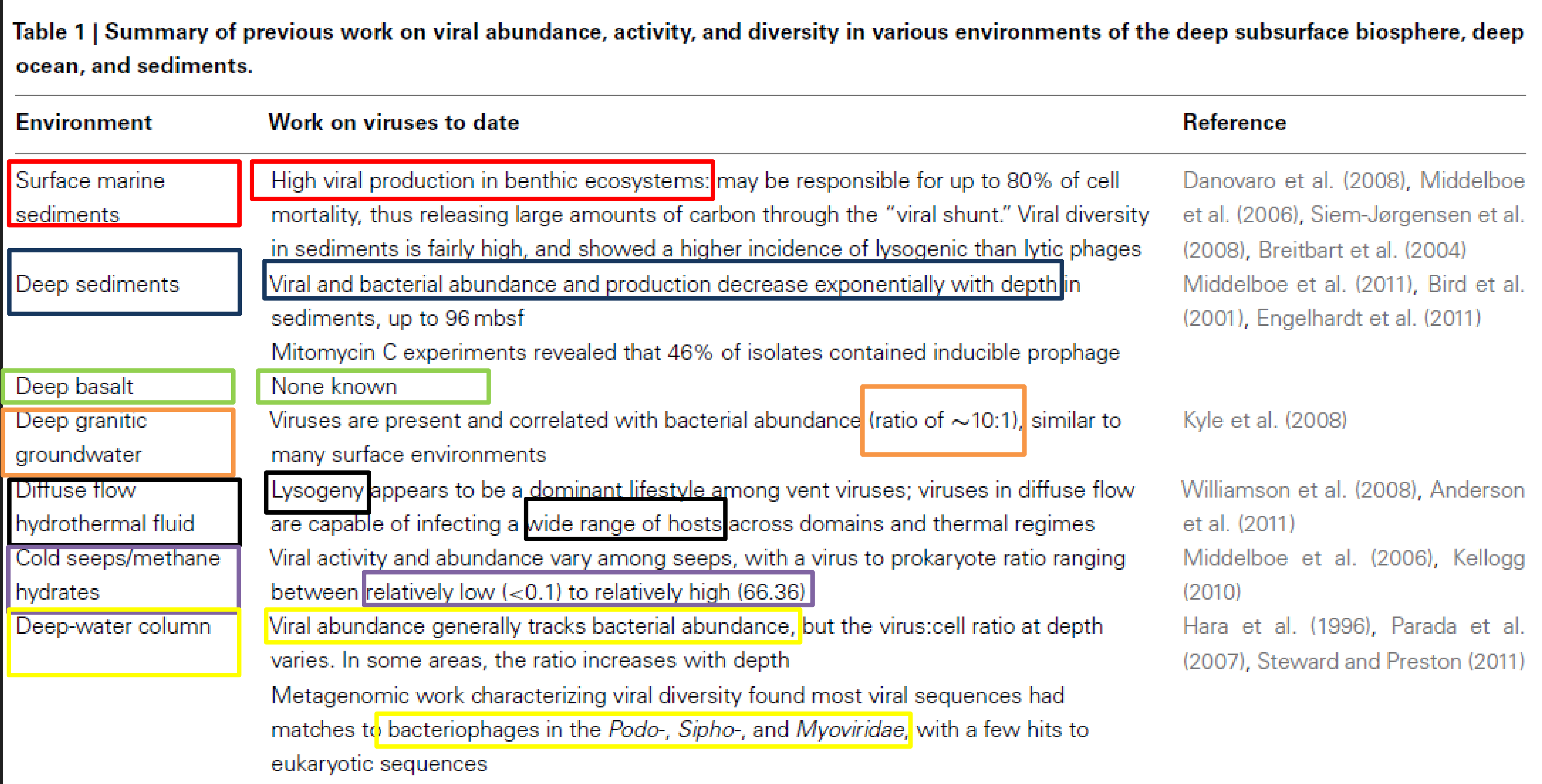
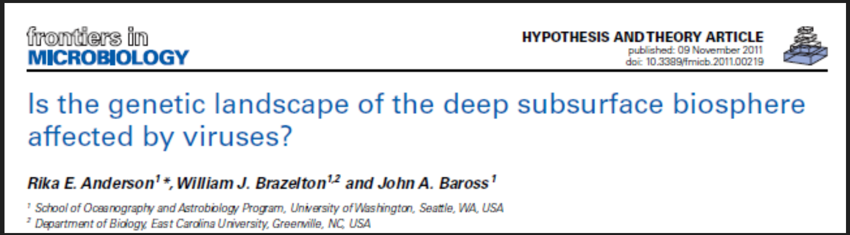


Table 1 | Summary of previous work on viral abundance, activity, and diversity in various environments of the deep subsurface biosphere, deep ocean, and sediments.

Environment	Work on viruses to date	Reference
Surface marine sediments	High viral production in benthic ecosystems: may be responsible for up to 80% of cell mortality, thus releasing large amounts of carbon through the "viral shunt." Viral diversity in sediments is fairly high, and showed a higher incidence of lysogenic than lytic phages	Danovaro et al. (2008), Middelboe et al. (2006), Siem-Jørgensen et al. (2008), Breitbart et al. (2004)
Deep sediments	Viral and bacterial abundance and production decrease exponentially with depth in sediments, up to 96 mbsf Mitomycin C experiments revealed that 46% of isolates contained inducible prophage	Middelboe et al. (2011), Bird et al. (2001), Engelhardt et al. (2011)
Deep basalt	None known	
Deep granitic groundwater	Viruses are present and correlated with bacterial abundance (ratio of ~10:1), similar to many surface environments	Kyle et al. (2008)
Diffuse flow hydrothermal fluid	Lysogeny appears to be a dominant lifestyle among vent viruses; viruses in diffuse flow are capable of infecting a wide range of hosts across domains and thermal regimes	Williamson et al. (2008), Anderson et al. (2011)
Cold seeps/methane hydrates	Viral activity and abundance vary among seeps, with a virus to prokaryote ratio ranging between relatively low (<0.1) to relatively high (66.36)	Middelboe et al. (2006), Kellogg (2010)
Deep-water column	Viral abundance generally tracks bacterial abundance, but the virus:cell ratio at depth varies. In some areas, the ratio increases with depth Metagenomic work characterizing viral diversity found most viral sequences had matches to bacteriophages in the *Podo-*, *Sipho-*, and *Myoviridae*, with a few hits to eukaryotic sequences	Hara et al. (1996), Parada et al. (2007), Steward and Preston (2011)



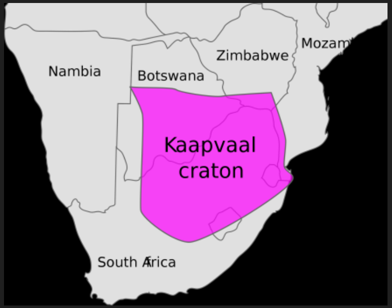
frontiers in
MICROBIOLOGY

HYPOTHESIS AND THEORY ARTICLE
published: 09 November 2011
doi: 10.3389/fmicb.2011.00219

Is the genetic landscape of the deep subsurface biosphere affected by viruses?

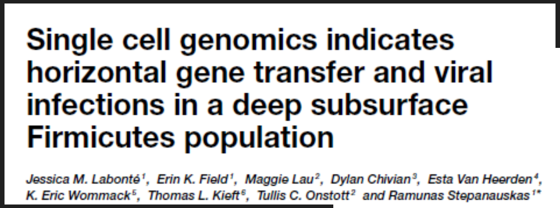
Rika E. Anderson[1], William J. Brazelton[1,2] and John A. Baross[1]*

[1] School of Oceanography and Astrobiology Program, University of Washington, Seattle, WA, USA
[2] Department of Biology, East Carolina University, Greenville, NC, USA



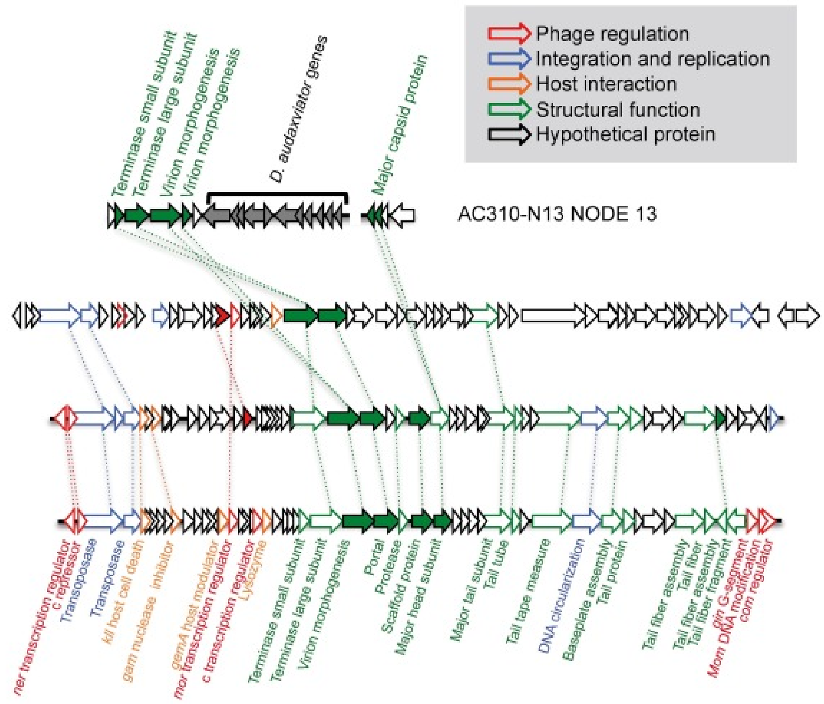
3.15 Km deep fracture water of the Kaapvaal Craton, South Africa

low microbial abundance (8000 cells mL^{-1}),
elevated pH (8–9)
temperature (48–49°C)
high reductive potential (340–370 mV)



Single cell genomics indicates horizontal gene transfer and viral infections in a deep subsurface Firmicutes population

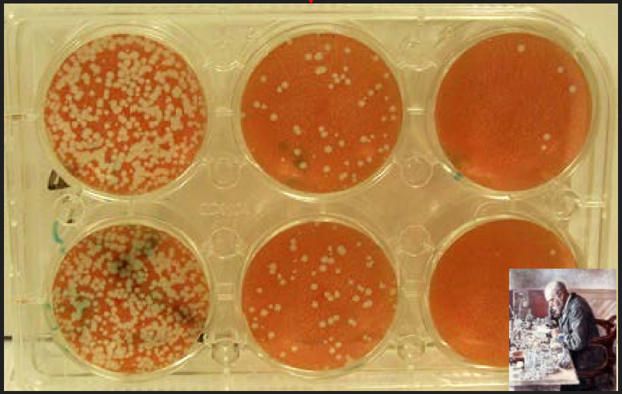
Jessica M. Labonté[1], Erin K. Field[1], Maggie Lau[2], Dylan Chivian[3], Esta Van Heerden[4], K. Eric Wommack[5], Thomas L. Kieft[6], Tullis C. Onstott[2] and Ramunas Stepanauskas[1]*



2. New approaches to unveil the `microbial dark matter´: single cell and virus genomics

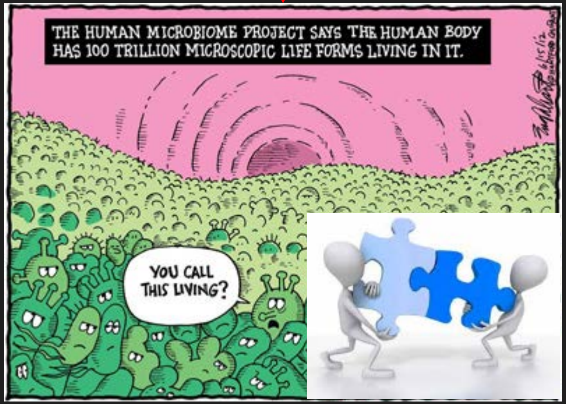
sample

Recovering the genetic information of microbes



CULTURE

99% OF PROKARYOTES DO NOT GROW IN CULTURE



METAGENOMICS

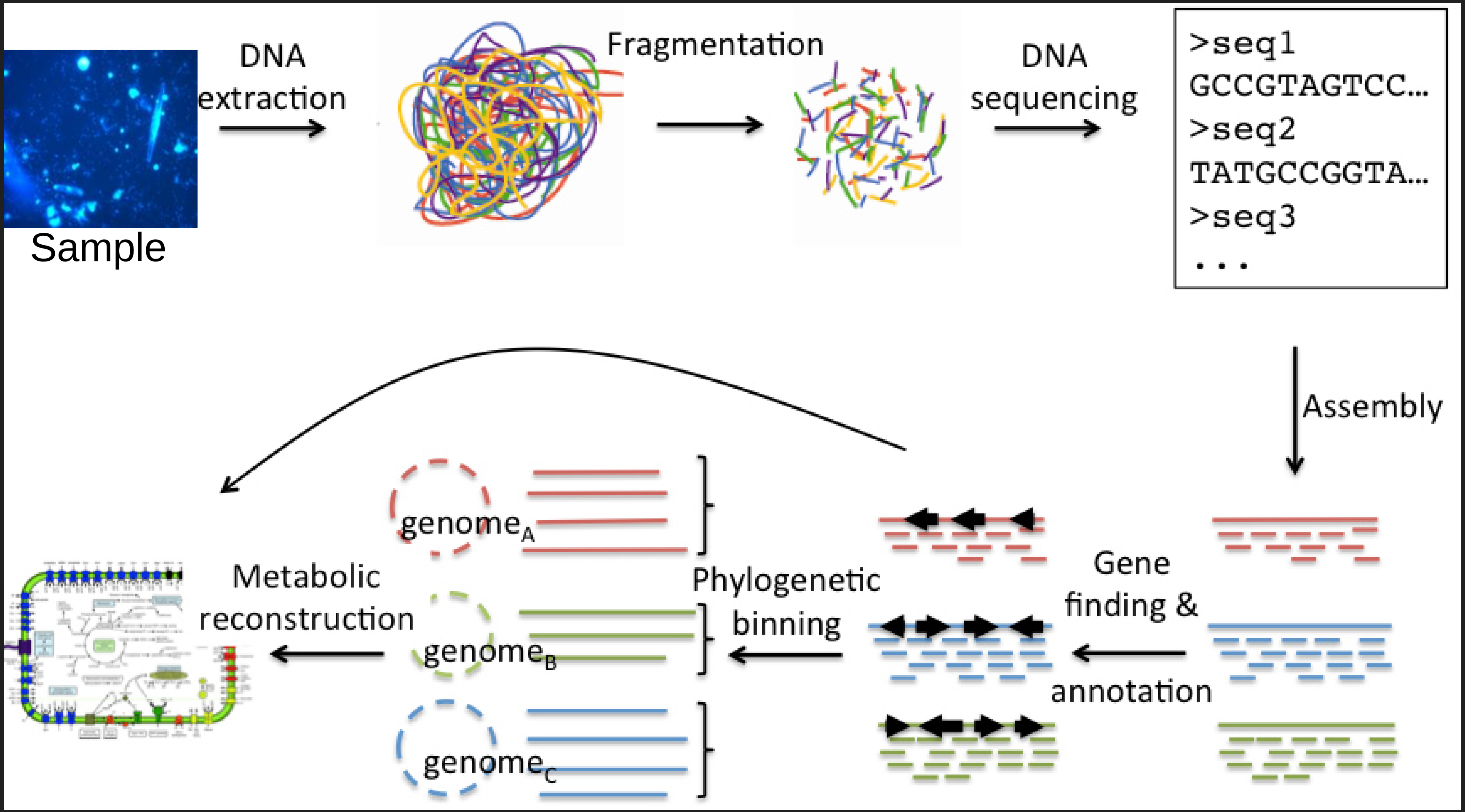
POWERFUL APPROACH TO GET THAT 99% OF UNKNOWN GENETIC INFORMATION

↓

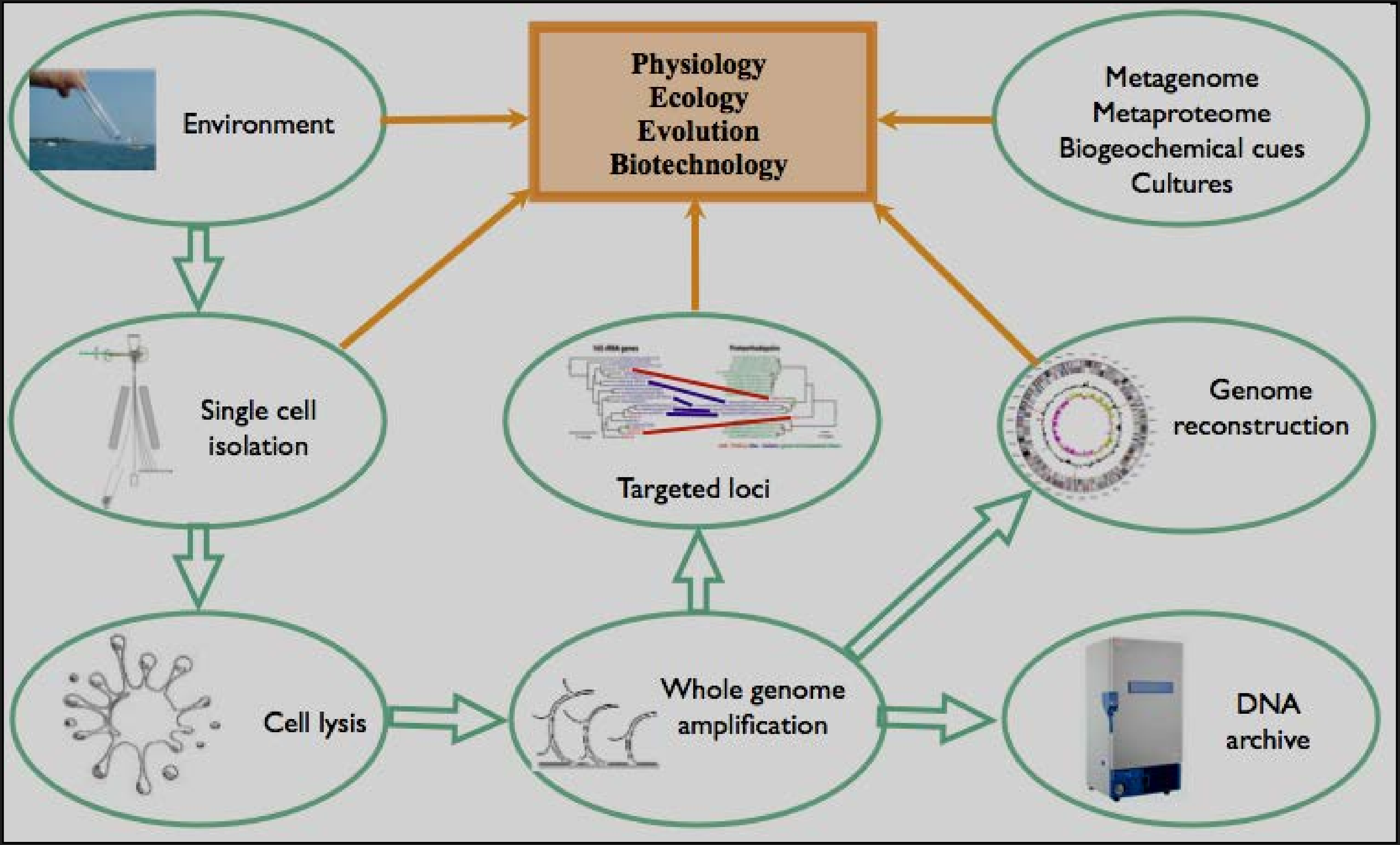
BUT DATA IS LARGELY FRAGMENTED INTO SMALL PIECES AND HAS TO BE RECOMPOSED (GENOME ASSEMBLY…ETC…)

SINGLE CELL AND VIRUS GENOMICS

METAGENOMICS



Single Cell Genomics



What is Single Cell Technologies?: *experimental approaches (Genomics, Proteomics, etc…) applied at the level of single cells, rather than at the level of an entire cell population*

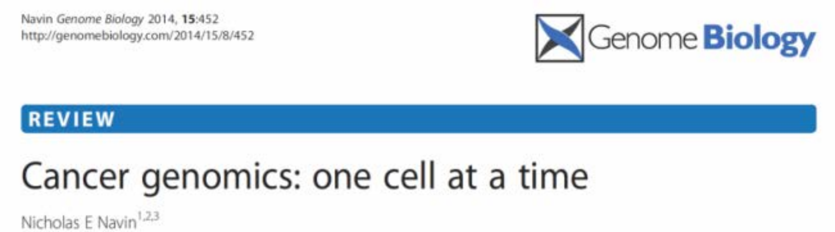
Investigation of clonal diversity in cancer: are all cells genetically identical in a tumour?

LETTER

doi:10.1038/nature09807

Tumour evolution inferred by single-cell sequencing

Nicholas Navin[1,2], Jude Kendall[1], Jennifer Troge[1], Peter Andrews[1], Linda Rodgers[1], Jeanne McIndoo[1], Kerry Cook[1], Asya Stepansky[1], Dan Levy[1], Diane Esposito[1], Lakshmi Muthuswamy[3], Alex Krasnitz[1], W. Richard McCombie[1], James Hicks[1] & Michael Wigler[1]



Navin *Genome Biology* 2014, **15**:452
http://genomebiology.com/2014/15/8/452

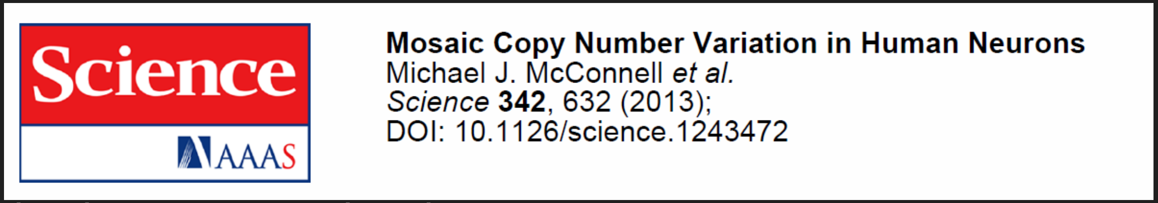
Genome **Biology**

REVIEW

Cancer genomics: one cell at a time

Nicholas E Navin[1,2,3]

Role of genetic mosaicism in the biology of multicellular organisms: chimera



Science
AAAS

Mosaic Copy Number Variation in Human Neurons
Michael J. McConnell *et al.*
Science **342**, 632 (2013);
DOI: 10.1126/science.1243472

Genomic variation in gamete and embryos

Cell

Cell PRESS

Volume 155, Issue 7, 19 December 2013, Pages 1492–1506

Article

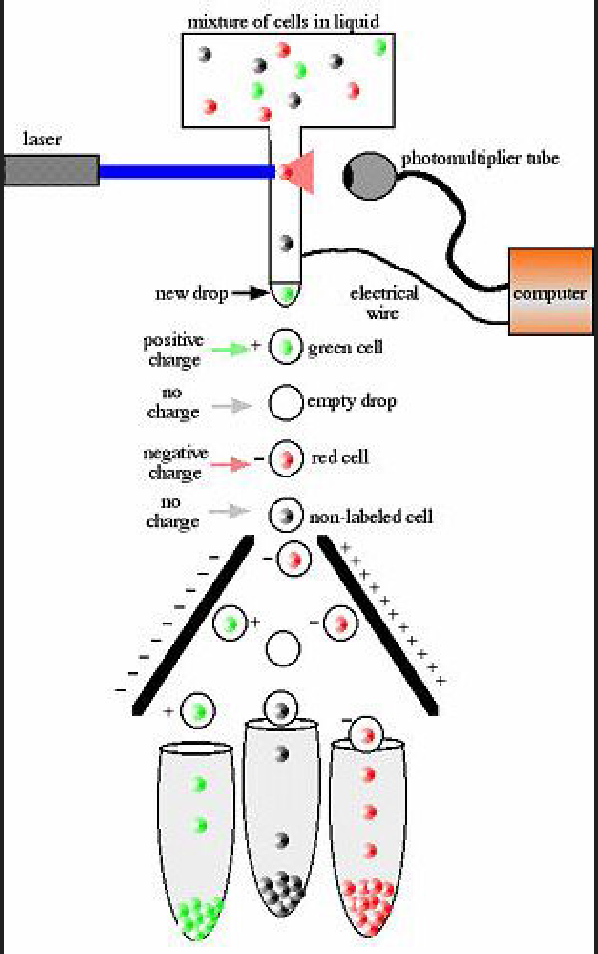
Genome Analyses of Single Human Oocytes

Yu Hou[1, 6], Wei Fan[1, 4, 6], Liying Yan[1, 6], Rong Li[1], Ying Lian[1], Jin Huang[1], Jinsen Li[1], Liya Xu[1], Fuchou Tang[1, 5,] 👤 · ✉, X. Sunney Xie[1, 2,] 👤 · ✉, Jie Qiao[1, 3,] 👤 · ✉

Just some examples!!!!...

Single cell genomics, a bloom in Science in all fields!!!!!

Everything starts with just a single cell




Available online at www.sciencedirect.com

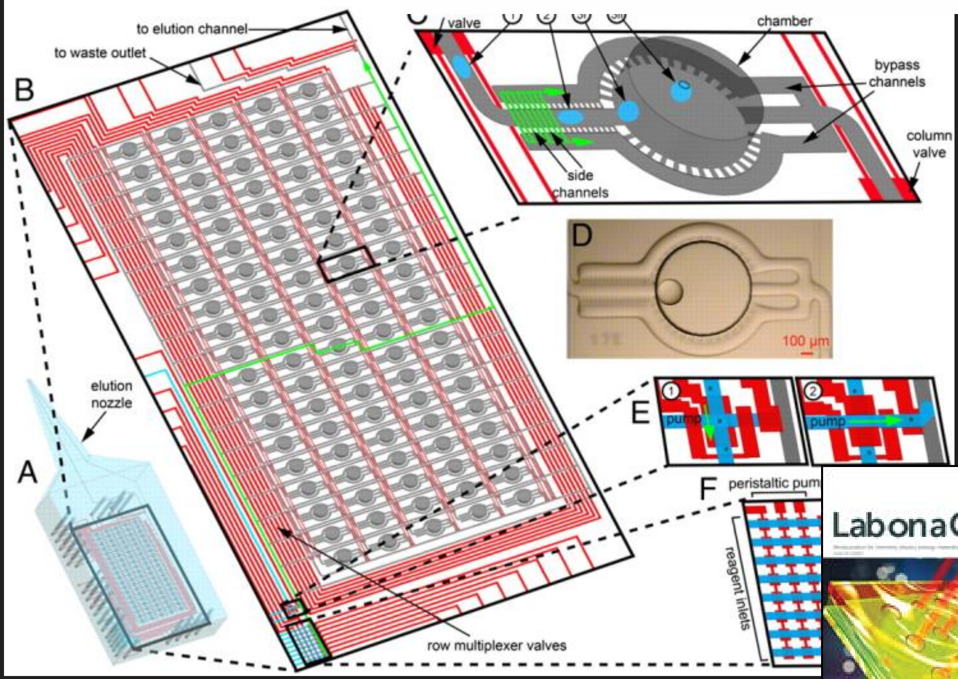
SciVerse ScienceDirect

ELSEVIER

Current Opinion in Biotechnology

Microfluidics for single cell analysis

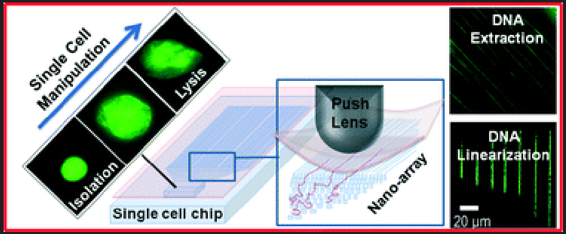
Huabing Yin[1] and Damian Marshall[2]



Leung K et al. PNAS 2012;109:7665-7670

Fluorescence Activated Cell Sorting VS Micro- and Nano-fluidics

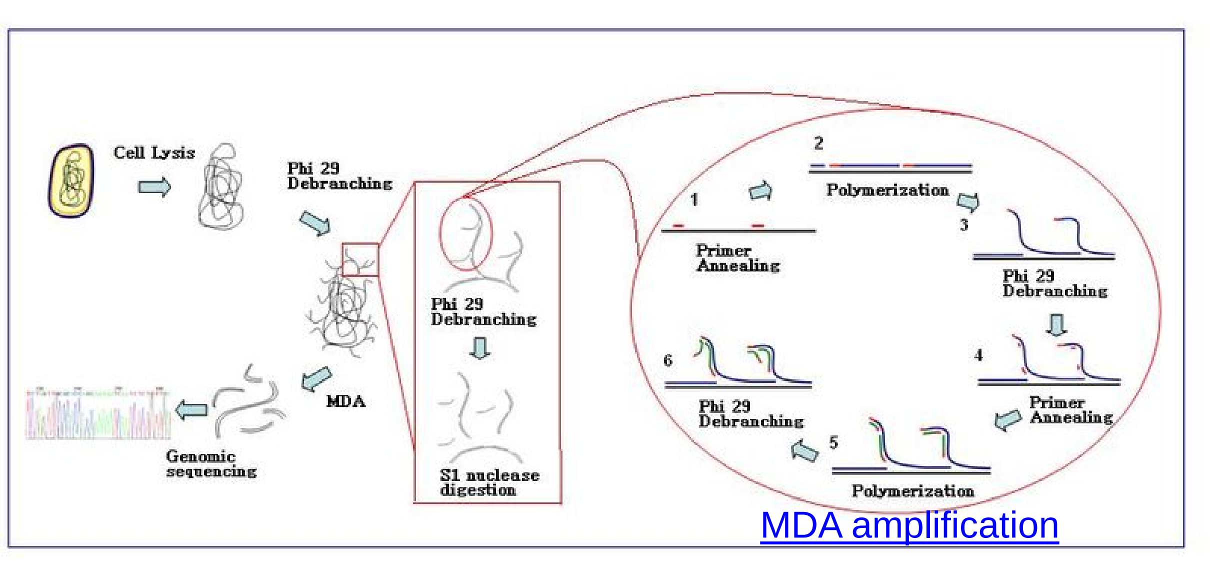
Zhang et al. 2015



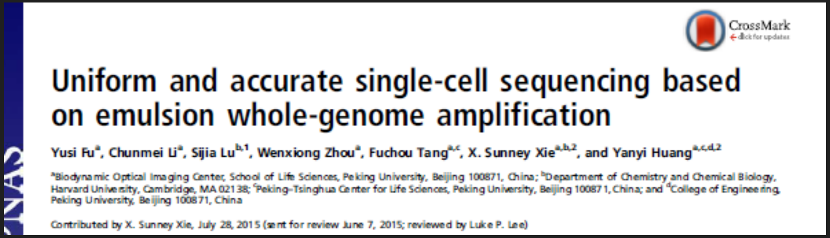
(So far) Whole genome amplification (WGA) is needed

WGA Methods:

- **-MDA (sensiphi)**
- -MDA+PrimPol
- -MIDAS
- **-MALBAC**
- -PicoPLEX
- -DOE-PCR



MDA amplification

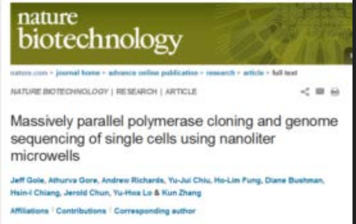


Uniform and accurate single-cell sequencing based on emulsion whole-genome amplification

Yusi Fu[a], Chunmei Li[a], Sijia Lu[b,1], Wenxiong Zhou[a], Fuchou Tang[a,c], X. Sunney Xie[a,b,2], and Yanyi Huang[a,c,d,2]

[a]Biodynamic Optical Imaging Center, School of Life Sciences, Peking University, Beijing 100871, China; [b]Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA 02138; [c]Peking-Tsinghua Center for Life Sciences, Peking University, Beijing 100871, China; and [d]College of Engineering, Peking University, Beijing 100871, China

Contributed by X. Sunney Xie, July 28, 2015 (sent for review June 7, 2015; reviewed by Luke P. Lee)



NATURE BIOTECHNOLOGY | RESEARCH | ARTICLE

Massively parallel polymerase cloning and genome sequencing of single cells using nanoliter microwells

Jeff Gole, Athurva Gore, Andrew Richards, Yu-Jui Chiu, Ho-Lim Fung, Diane Bushman, Hsin-I Chiang, Jerold Chun, Yu-Hwa Lo & Kun Zhang

Affiliations | Contributions | Corresponding author

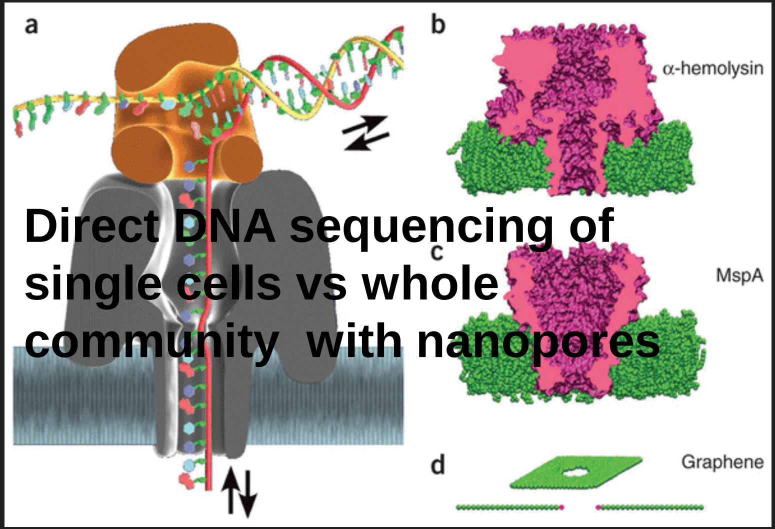
RESEARCH ARTICLE

A Quantitative Comparison of Single-Cell Whole Genome Amplification Methods

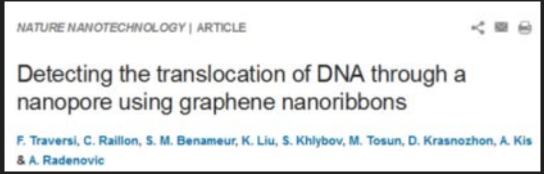
Charles F. A. de Bourcy, Iwijn De Vlaminck, Jad N. Kanbar, Jianbin Wang, Charles Gawad, Stephen R. Quake

Published: August 19, 2014 • DOI: 10.1371/journal.pone.0105585

Removing MDA bias



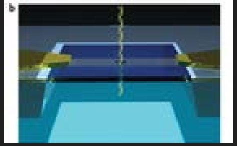
Direct DNA sequencing of single cells vs whole community with nanopores



NATURE NANOTECHNOLOGY | ARTICLE

Detecting the translocation of DNA through a nanopore using graphene nanoribbons

F. Traversi, C. Raillon, S. M. Benameur, K. Liu, S. Khlybov, M. Tosun, D. Krasnozhon, A. Kis & A. Radenovic



Single Cell Genomics in Aquatic Environments

Prevalent genome streamlining and latitudinal divergence of planktonic bacteria in the surface ocean

Brandon K. Swan[a], Ben Tupper[a], Alexander Sczyrba[b], Federico M. Lauro[c], Manuel Martinez-Garcia[a], José M. González[d], Haiwei Luo[e], Jody J. Wright[f], Zachary C. Landry[g], Niels W. Hanson[h], Brian P. Thompson[a], Nicole J. Poulton[a], Patrick Schwientek[i], Silvia G. Acinas[j], Stephen J. Giovannoni[g], Mary Ann Moran[e], Steven J. Hallam[f,h], Ricardo Cavicchioli[c], Tanja Woyke[i], and Ramunas Stepanauskas[a,1]

Single-Cell Genomics Reveals Hundreds of Coexisting Subpopulations in Wild *Prochlorococcus*

Nadav Kashtan,[1]* Sara E. Roggensack,[1] Sébastien Rodrigue,[1,2] Jessie W. Thompson,[1] Steven J. Biller,[1] Allison Coe,[1] Huiming Ding,[1,3] Pekka Marttinen,[4] Rex R. Malmstrom,[5] Roman Stocker,[1] Michael J. Follows,[6] Ramunas Stepanauskas,[7] Sallie W. Chisholm[1,3]*

Original Article

The ISME Journal **8**, 1440-1451 (July 2014) | doi:10.1038/ismej.2013.24

Single-cell enabled comparative genomics of a deep ocean SAR11 bathytype

J Cameron Thrash, Ben Temperton, Brandon K Swan, Zachary C Landry, Tanja Woyke, Edward F DeLong, Ramunas Stepanauskas and Stephan J Giovannoni

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OPEN ACCESS PEER-REVIEWED

RESEARCH ARTICLE

Capturing Single Cell Genomes of Active Polysaccharide Degraders: An Unexpected Contribution of *Verrucomicrobia*

Manuel Martinez-Garcia, David M. Brazel, Brandon K. Swan, Carol Arnosti, Patrick S. G. Chain, Krista G. Reitenga, Gary Xie, Nicole J. Poulton, Monica Lluesma Gomez, Dashiell E. D. Masland, Brian Thompson, Wendy K. Bellows, Kai Ziervogel, Chien-Chi Lo, Sanaa Ahmed, Cheryl D. Gleasner, Chris J. Detter, Ramunas Stepanauskas

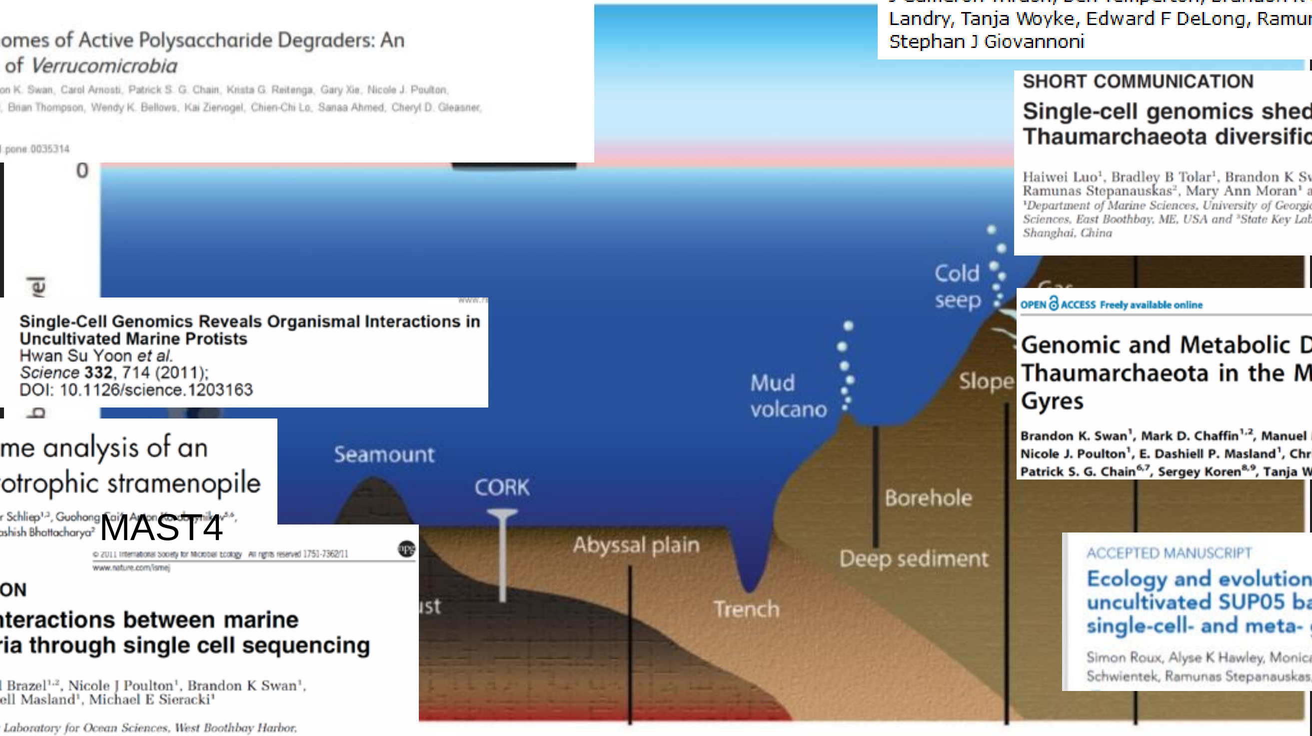
Published: April 20, 2012 • DOI: 10.1371/journal.pone.0035314

SHORT COMMUNICATION

Single-cell genomics shedding light on marine Thaumarchaeota diversification

Haiwei Luo[1], Bradley B Tolar[1], Brandon K Swan[2], Chuanlun L Zhang[1,3], Ramunas Stepanauskas[2], Mary Ann Moran[1] and James T Hollibaugh[1]

[1]*Department of Marine Sciences, University of Georgia, Athens, GA, USA;* [2]*Bigelow Laboratory for Ocean Sciences, East Boothbay, ME, USA and* [3]*State Key Laboratory of Marine Geology, Tongji University, Shanghai, China*



Science AAAS

Single-Cell Genomics Reveals Organismal Interactions in Uncultivated Marine Protists

Hwan Su Yoon *et al.*

Science **332**, 714 (2011);

DOI: 10.1126/science.1203163

OPEN ACCESS Freely available online PLOS ONE

Genomic and Metabolic Diversity of Marine Group I Thaumarchaeota in the Mesopelagic of Two Subtropical Gyres

Brandon K. Swan[1], Mark D. Chaffin[1,2], Manuel Martinez-Garcia[3], Hilary G. Morrison[4], Erin K. Field[1], Nicole J. Poulton[1], E. Dashiell P. Masland[1], Christopher C. Harris[1], Alexander Sczyrba[5], Patrick S. G. Chain[6,7], Sergey Koren[8,9], Tanja Woyke[7], Ramunas Stepanauskas[1]*

Single cell genome analysis of an uncultured heterotrophic stramenopile

Rajat S. Roy[1,2], Dana C. Price[2], Alexander Schliep[1,3], Guohong Cai, Anton [illegible][5,6], Hwan Su Yoon[7], Eun Chan Yang[8] & Debashish Bhattacharya[2]

MAST4

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www.nature.com/ismej

SHORT COMMUNICATION

Unveiling *in situ* interactions between marine protists and bacteria through single cell sequencing

Manuel Martinez-Garcia[1], David Brazel[1,2], Nicole J Poulton[1], Brandon K Swan[1], Monica Lluesma Gomez[1], Dashiell Masland[1], Michael E Sieracki[1] and Ramunas Stepanauskas[1]

[1]*Single Cell Genomics Center, Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, ME, USA and* [2]*Colby College, Waterville, ME, USA*

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eLIFE

ACCEPTED MANUSCRIPT

Ecology and evolution of viruses infecting uncultivated SUP05 bacteria as revealed by single-cell- and meta- genomics

Simon Roux, Alyse K Hawley, Monica Torres Beltran, Melanie Scofield, Patrick Schwientek, Ramunas Stepanauskas, Tanja Woyke, Steven J Hallam, Matthew B Sullivan

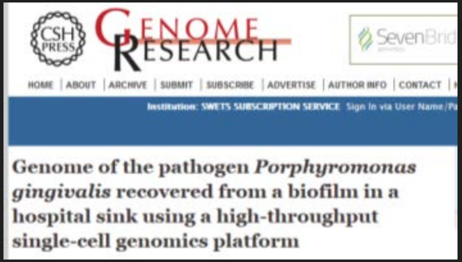
---

Potential for Chemolithoautotrophy Among Ubiquitous Bacteria Lineages in the Dark Ocean

Brandon K. Swan,[1] Manuel Martinez-Garcia,[1] Christina M. Preston,[2] Alexander Sczyrba,[3] Tanja Woyke,[3] Dominique Lamy,[4]* Thomas Reinthaler,[4] Nicole J. Poulton,[1] E. Dashiell P. Masland,[1] Monica Lluesma Gomez,[1] Michael E. Sieracki,[1] Edward F. DeLong,[5] Gerhard J. Herndl,[4] Ramunas Stepanauskas[1]†

Just some examples…

Bacterial Single Cell Genomics in Human


CSH PRESS GENOME RESEARCH

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Genome of the pathogen *Porphyromonas gingivalis* recovered from a biofilm in a hospital sink using a high-throughput single-cell genomics platform


OPEN ACCESS Freely available online PLoS one

The "Most Wanted" Taxa from the Human Microbiome for Whole Genome Sequencing

Anthony A. Fodor[1], Todd Z. DeSantis[2], Kristine M. Wylie[3], Jonathan H. Badger[4], Yuzhen Ye[5], Theresa Hepburn[6], Ping Hu[7], Erica Sodergren[3], Konstantinos Liolios[8], Heather Huot-Creasy[9], Bruce W. Birren[6], Ashlee M. Earl[6]*


McLean and Lasken *Genome Medicine* 2014, **6**:108
http://genomemedicine.com/content/6/11/108

Genome **Medicine**

COMMENT

Single cell genomics of bacterial pathogens: outlook for infectious disease research

Jeffrey S McLean[1,2]* and Roger S Lasken[2]


nature REVIEWS GENETICS

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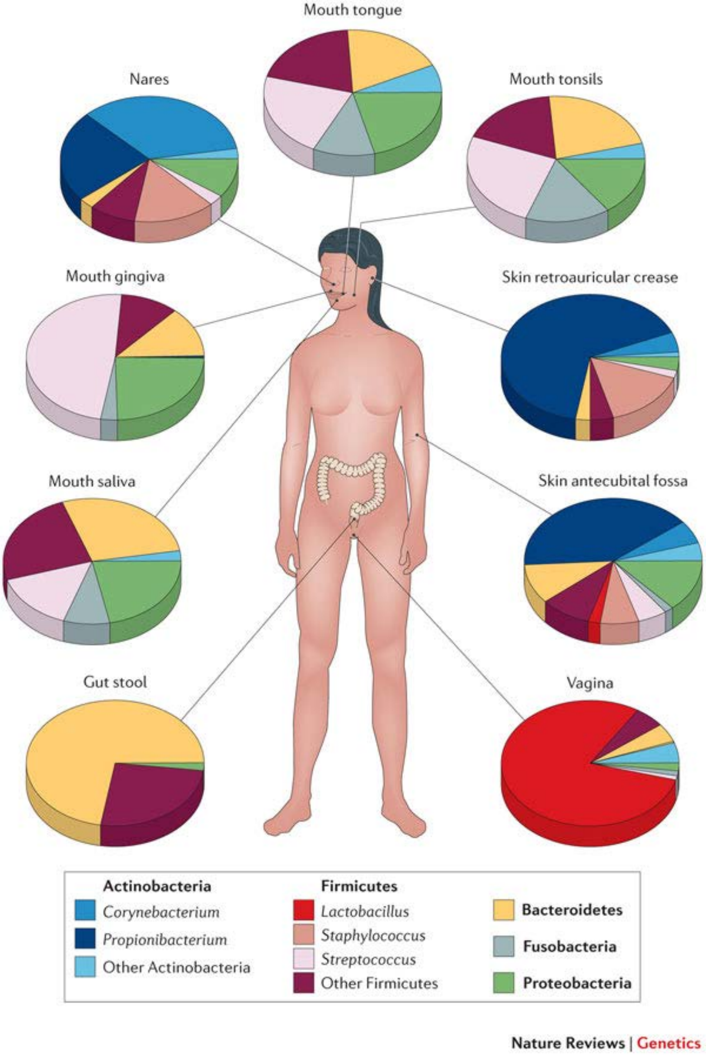
NATURE REVIEWS GENETICS | PROGRESS

ARTICLE SERIES: Applications of next-generation sequencing

Recent advances in genomic DNA sequencing of microbial species from single cells

Roger S. Lasken & Jeffrey S. McLean

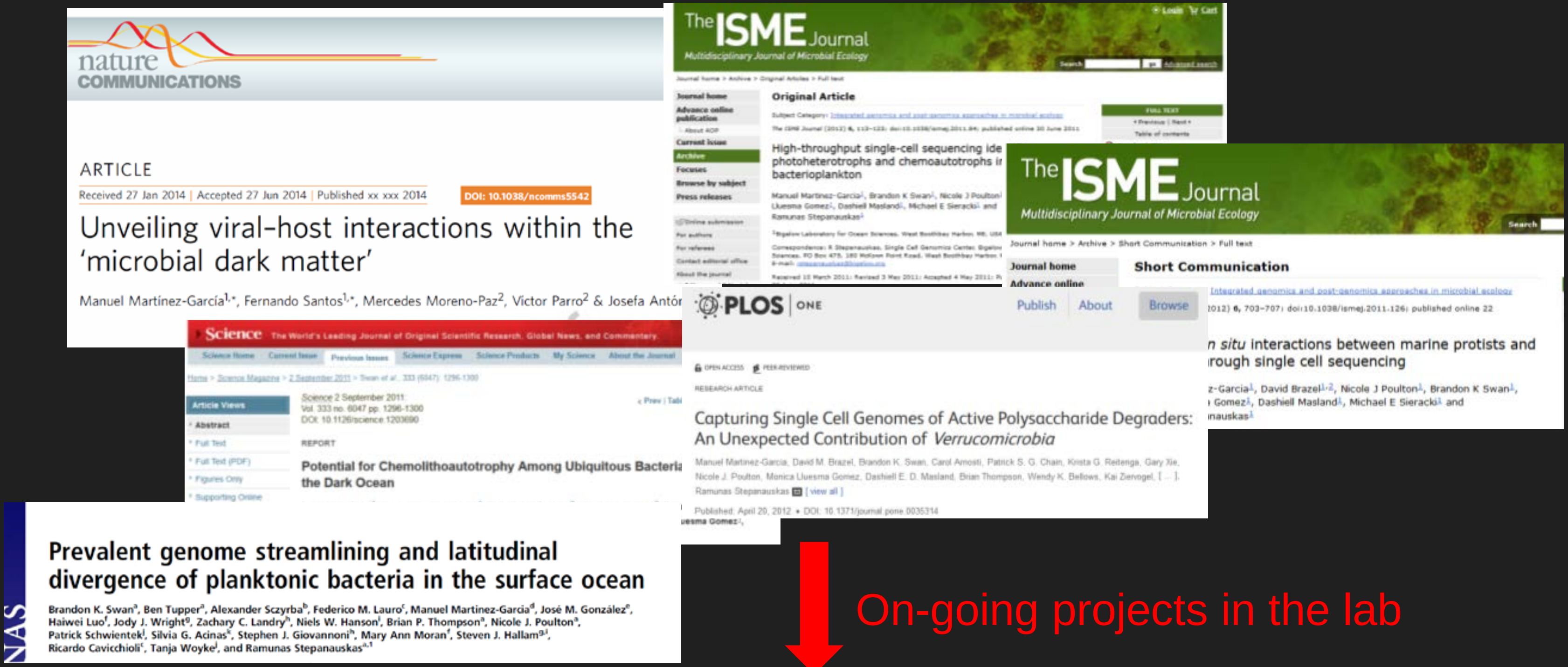
Affiliations | Corresponding author



Nature Reviews | Genetics

45 **uncultured** `bacterial species´, out of 100 most wanted taxa from the Human Microbiome, have been sequenced through Single Cell Genomics

Some of my contributions to Single cell Genomics in Aquatic Environments



nature COMMUNICATIONS

ARTICLE

Received 27 Jan 2014 | Accepted 27 Jun 2014 | Published xx xxx 2014 DOI: 10.1038/ncomms5542

Unveiling viral-host interactions within the 'microbial dark matter'

Manuel Martínez-García[1,*], Fernando Santos[1,*], Mercedes Moreno-Paz[2], Victor Parro[2] & Josefa Antón

The ISME Journal
Multidisciplinary Journal of Microbial Ecology

Original Article

High-throughput single-cell sequencing ide
photoheterotrophs and chemoautotrophs in
bacterioplankton

Manuel Martinez-Garcia[1], Brandon K Swan[1], Nicole J Poulton[1]
Lluesma Gomez[1], Dashiell Masland[1], Michael E Sieracki[1] and
Ramunas Stepanauskas[1]

The ISME Journal
Multidisciplinary Journal of Microbial Ecology

Journal home > Archive > Short Communication > Full text

Short Communication

Integrated genomics and post-genomics approaches in microbial ecology

2012) 6, 703–707; doi:10.1038/ismej.2011.126; published online 22

n situ interactions between marine protists and
rough single cell sequencing

z-Garcia[1], David Brazel[1,2], Nicole J Poulton[1], Brandon K Swan[1],
Gomez[1], Dashiell Masland[1], Michael E Sieracki[1] and
nauskas[1]

Science The World's Leading Journal of Original Scientific Research, Global News, and Commentary.

Science 2 September 2011:
Vol. 333 no. 6047 pp. 1296-1300
DOI: 10.1126/science.1203690

REPORT

Potential for Chemolithoautotrophy Among Ubiquitous Bacteria
the Dark Ocean

PLOS ONE

RESEARCH ARTICLE

Capturing Single Cell Genomes of Active Polysaccharide Degraders:
An Unexpected Contribution of *Verrucomicrobia*

Manuel Martinez-Garcia, David M. Brazel, Brandon K. Swan, Carol Arnosti, Patrick S. G. Chain, Krista G. Reitenga, Gary Xie, Nicole J. Poulton, Monica Lluesma Gomez, Dashiell E. D. Masland, Brian Thompson, Wendy K. Bellows, Kai Ziervogel, [...], Ramunas Stepanauskas [view all]

Published: April 20, 2012 • DOI: 10.1371/journal.pone.0035314

PNAS

Prevalent genome streamlining and latitudinal divergence of planktonic bacteria in the surface ocean

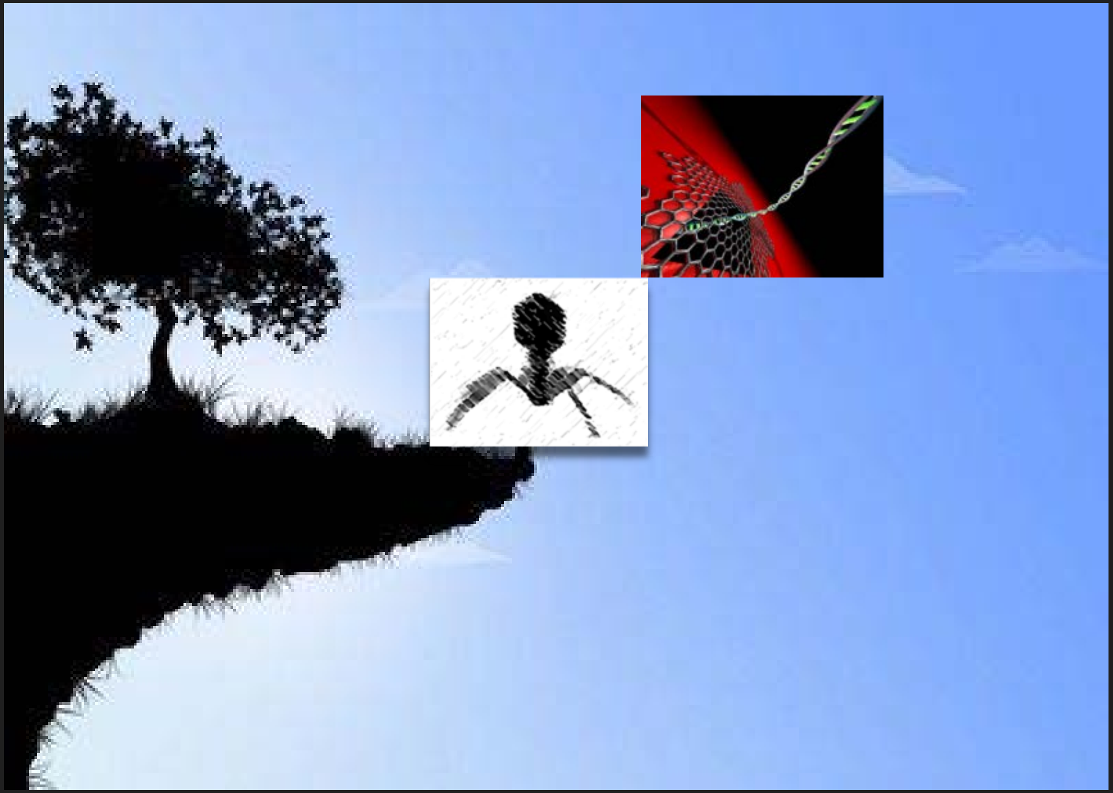
Brandon K. Swan[a], Ben Tupper[a], Alexander Sczyrba[b], Federico M. Lauro[c], Manuel Martinez-Garcia[d], José M. González[e], Haiwei Luo[f], Jody J. Wright[g], Zachary C. Landry[h], Niels W. Hanson[i], Brian P. Thompson[a], Nicole J. Poulton[a], Patrick Schwientek[j], Silvia G. Acinas[k], Stephen J. Giovannoni[h], Mary Ann Moran[f], Steven J. Hallam[g,i], Ricardo Cavicchioli[c], Tanja Woyke[j], and Ramunas Stepanauskas[a,1]

On-going projects in the lab

3. Single Virus Genomics: deciphering the genome of new, uncultured viruses

Can we unveil the genome from just a single virus particle separated from biological samples?

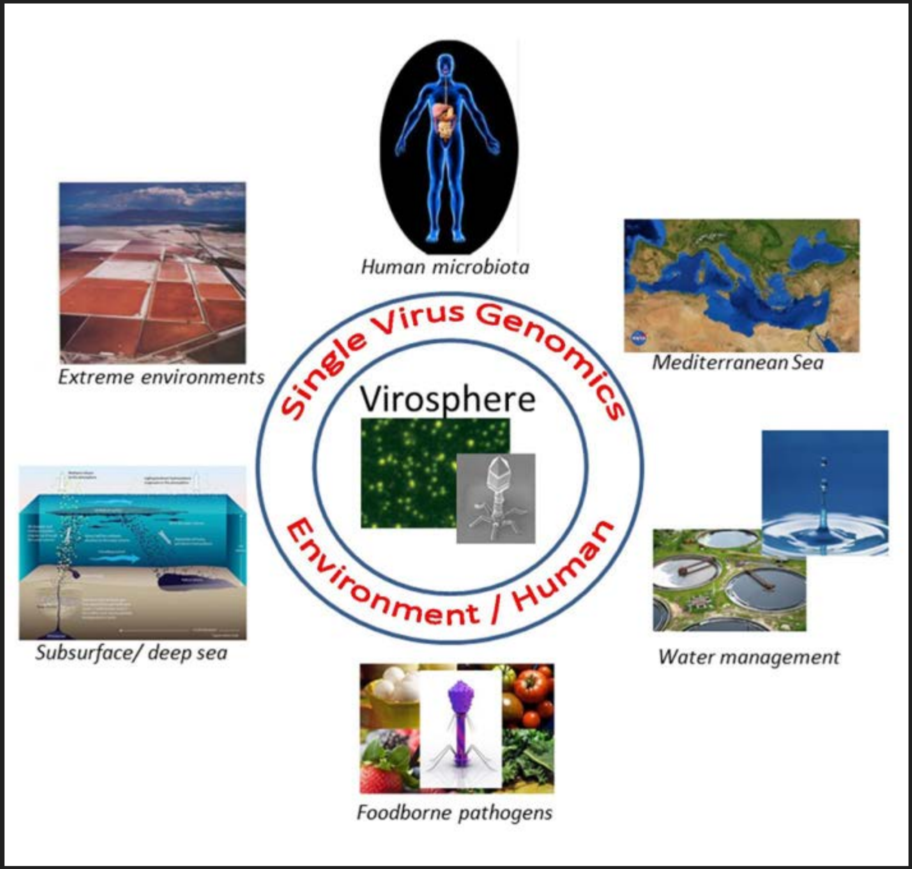
3. Deciphering the genome of new, uncultured viruses: Single Virus Genomics

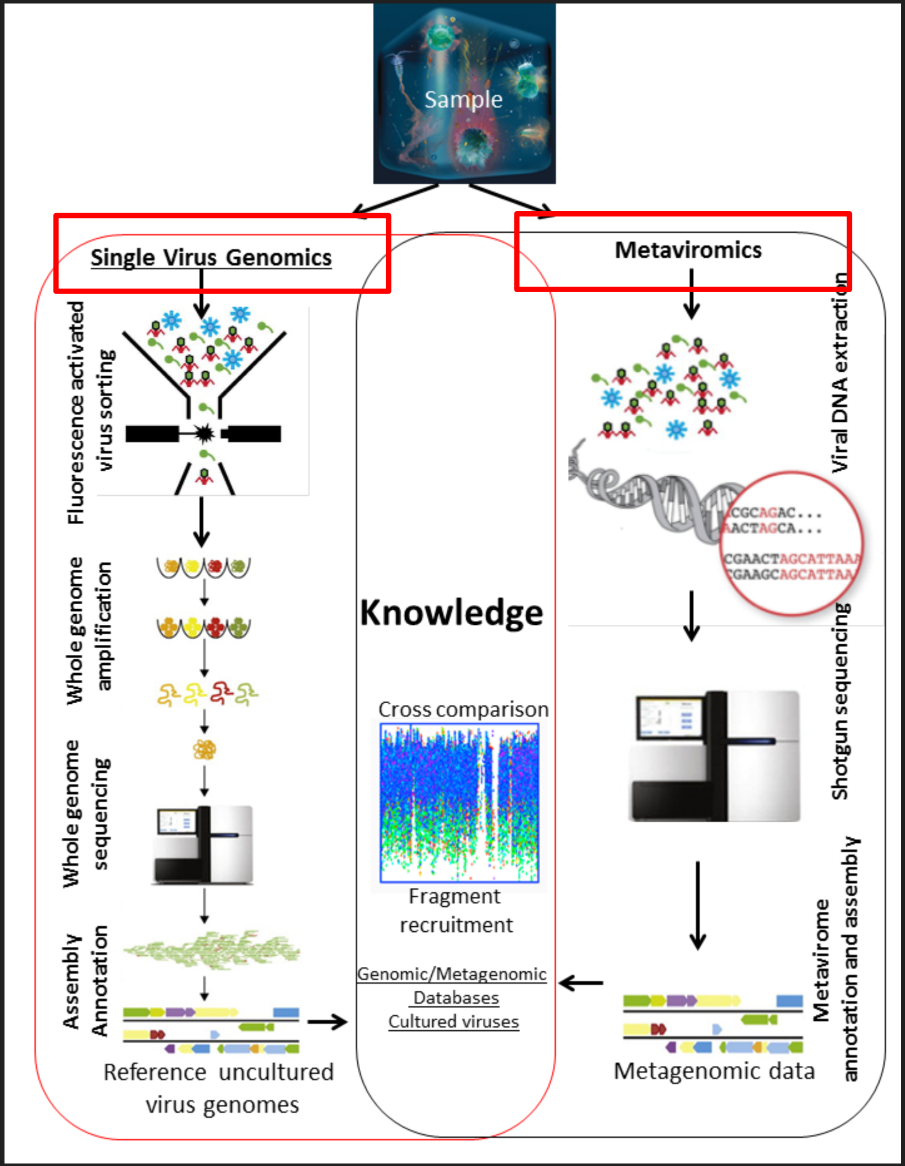


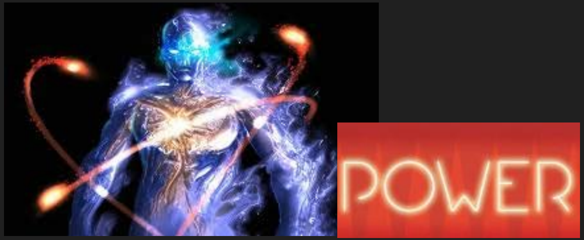
NGS of genetic material extracted from a single viral particle directly sorted out from the sample without the need of culturing the host nor the virus

Rationale of Single Virus Genomics (SVG) The most widespread and abundant viral populations in nature lack cultured representative!! (Brum et al., 2015; Science)
<u>SVG is a new way to deliver reference genomes of these predominant viruses</u>

Single virus Genomics







=Metagenomics+Single Cell Genomics

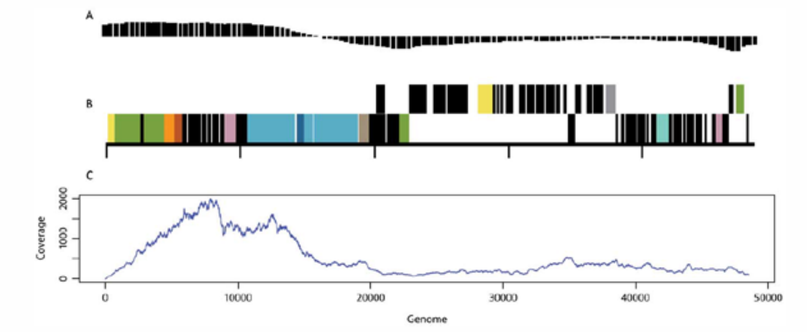
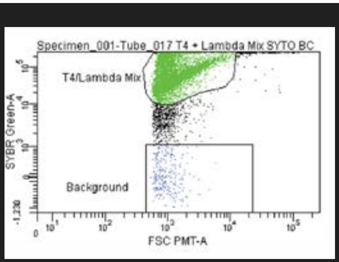


Figure 5. Lambda genome attributes and coverage. A) GC plot with bars indicating %GC above or below the average of 49%, B) Genome map of lambda (adapted from http://img.jgi.doe.gov), and C) Reference mapping of SVG reads to phage lambda, x-axis is genome position, y-axis is %coverage.
doi:10.1371/journal.pone.0017722.g005


OPEN ACCESS Freely available online PLoS one

Single Virus Genomics: A New Tool for Virus Discovery

Lisa Zeigler Allen[1,2], Thomas Ishoey[1], Mark A. Novotny[1], Jeffrey S. McLean[1], Roger S. Lasken[1], Shannon J. Williamson[1*]



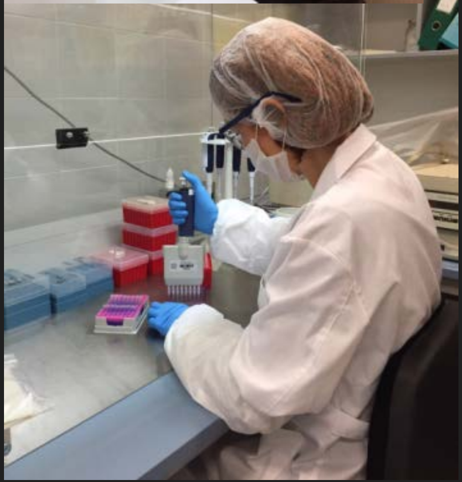
AIMS OF OUR PROJECT…

1
- Set up and optimization of the multidisciplinary technology to perform SVG in natural biological samples

2
- **NGS of uncultured reference viruses from aqutic and human (micro-) environments by SVG**

Blanes Microbiological Observatory (oligotrophic)
Playa de la Barceloneta

Francisco Martinez (PhD student)



Mónica Lluesma (Assistant Researcher)

Influx (BD)
"State of the art"

me…

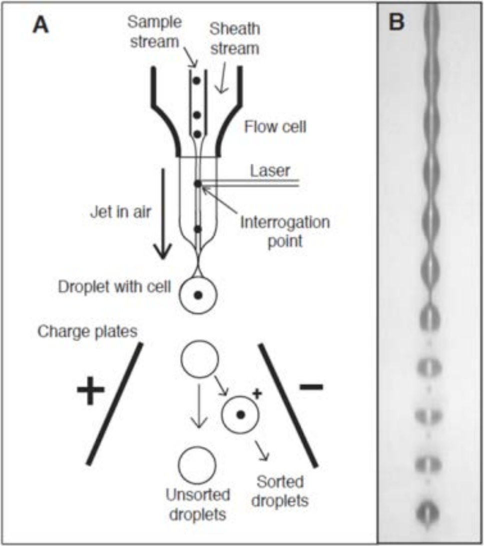
Dr. Oscar Fornas

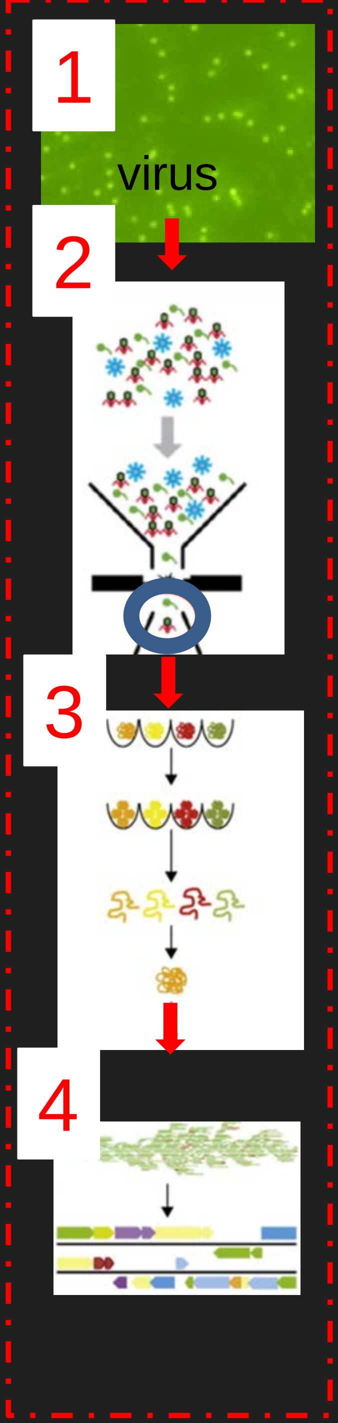
Mª Jose de la Cruz

Universitat d'Alacant
Universidad de Alicante

Barcelona
Biomedical
Research
Park

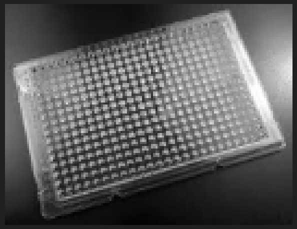
upf. Universitat Pompeu Fabra Barcelona





1. SYBRGold staining

2. Sorting of single viruses by flow cytometry



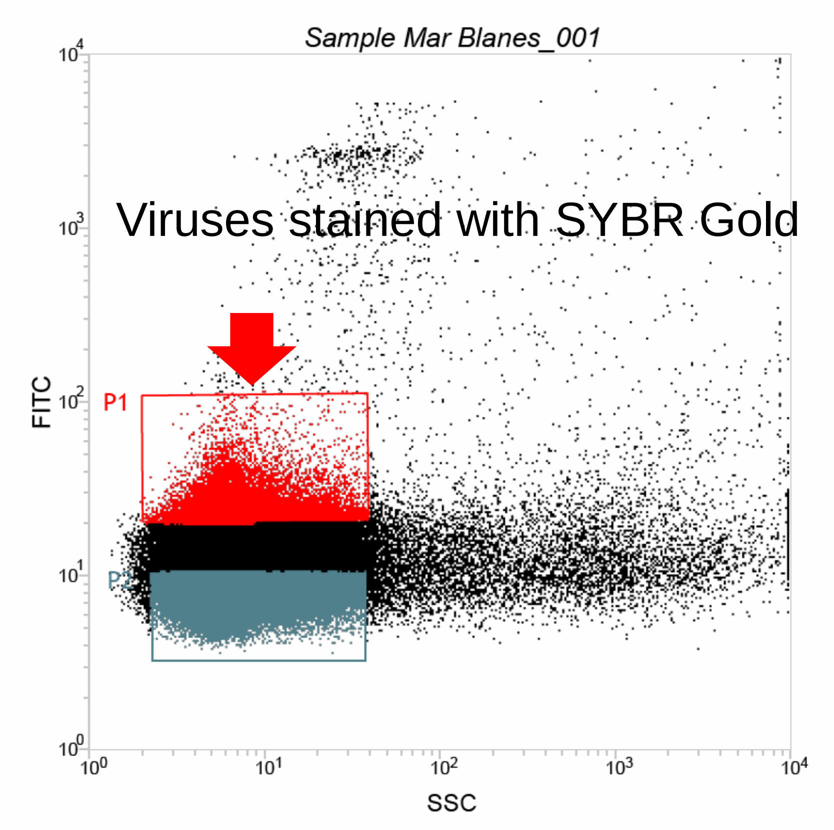
1 virus in each well

3. “*Lysis*” of the capsid and whole genome amplification

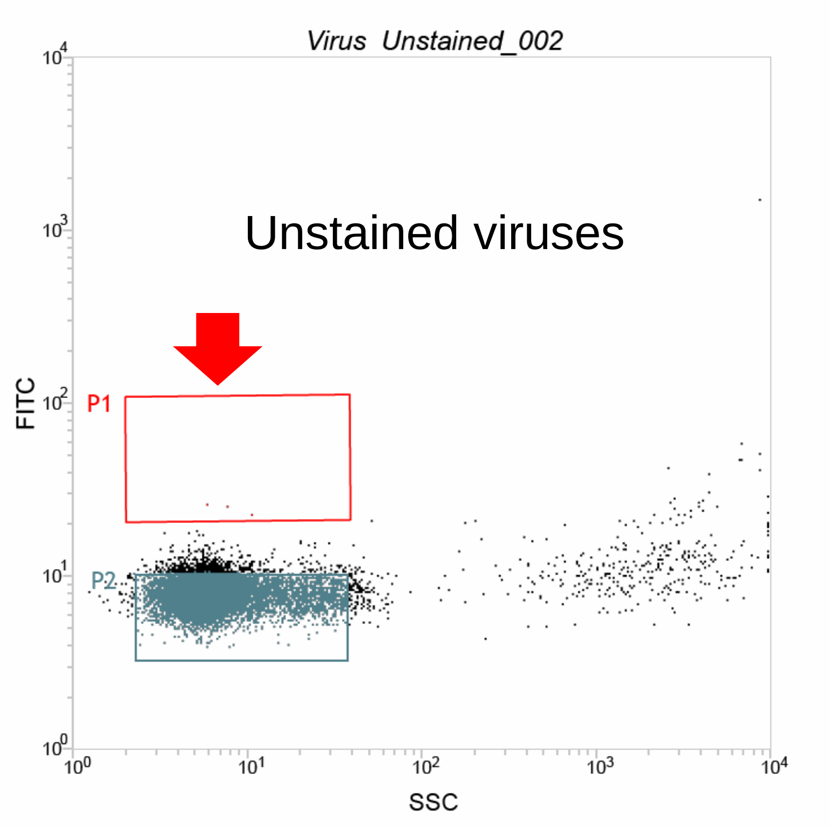
4. Pre-*screening* of viral genes and NGS of viral genomes

Modificado de Allen et al. 2011

Sorting of single viruses by flow cytometry



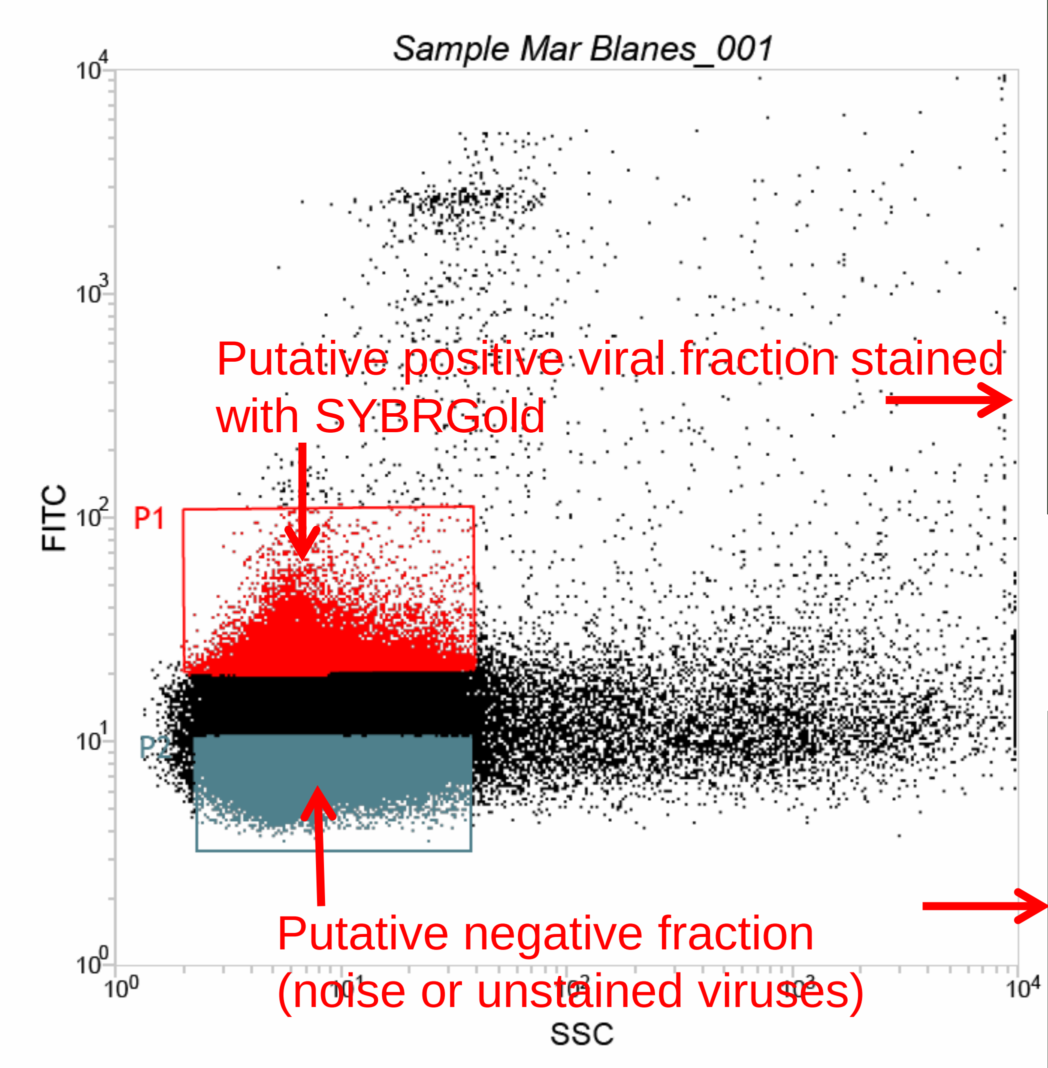
Viral sample (0.22 um pre-filtered)



Blank

Fluorescence microscopy of viral sample before entering in the Influx

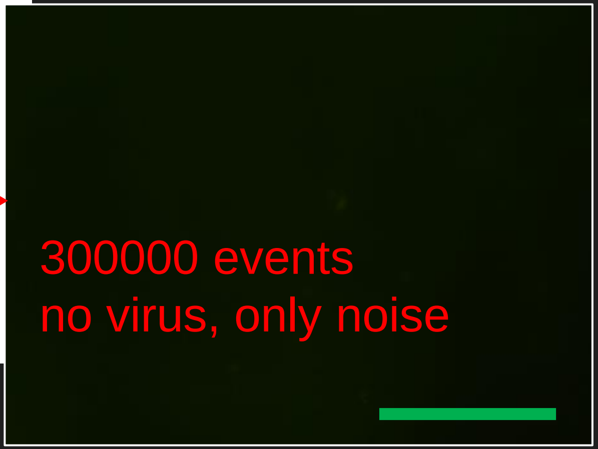
Super-Resolution and confocal Microscopy of sorted viruses



Putative positive viral fraction stained with SYBRGold

Putative negative fraction (noise or unstained viruses)

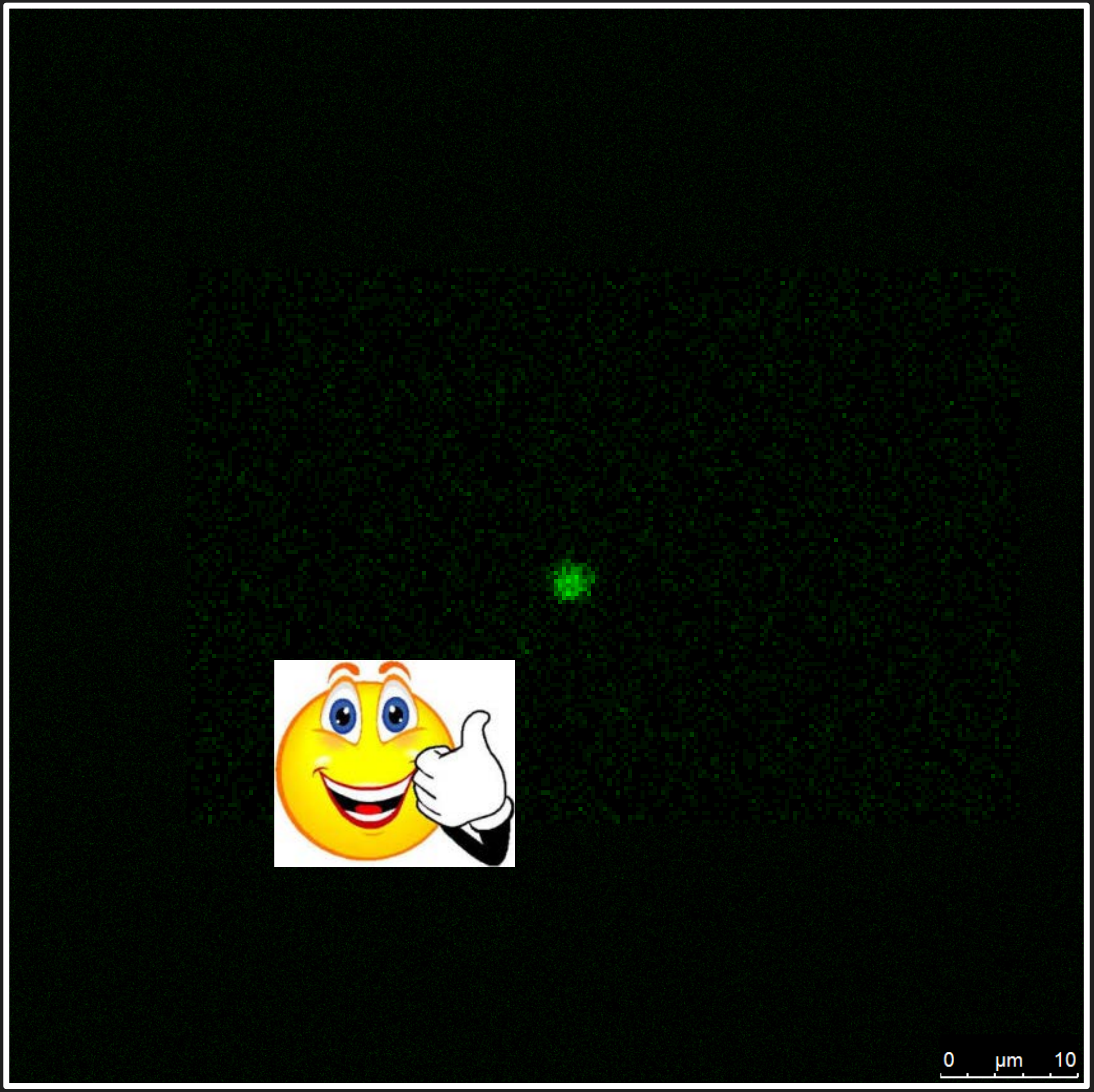
500000 sorted viruses



300000 events
no virus, only noise

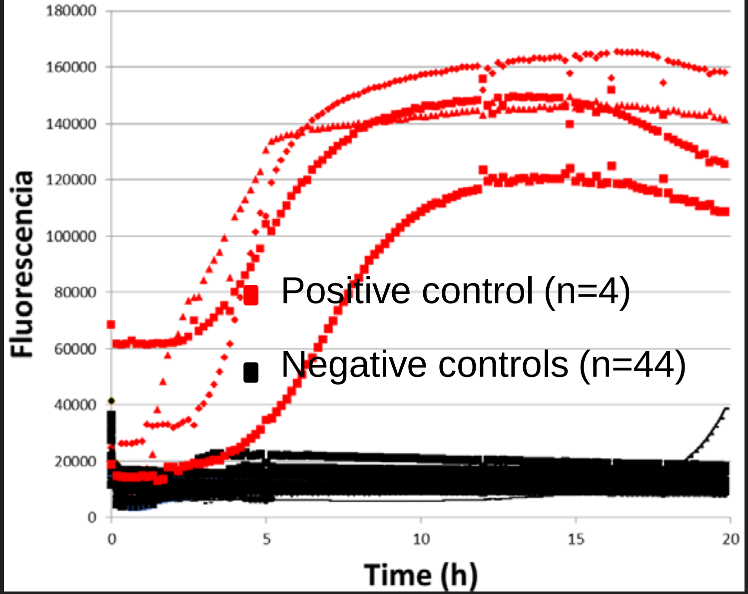
Sorting of a single virus: STED Microscopy

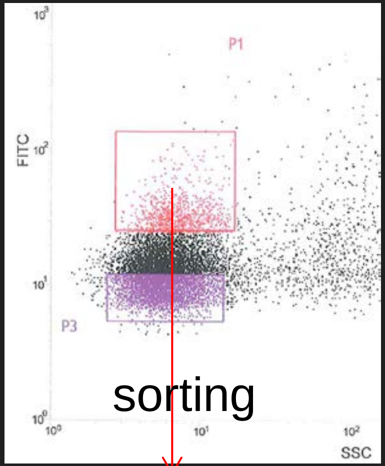
Leica TCS SP5 STED (CRG, Barcelona)

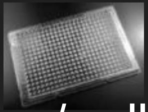


QC that sortig of single viruses is feasible and reliable

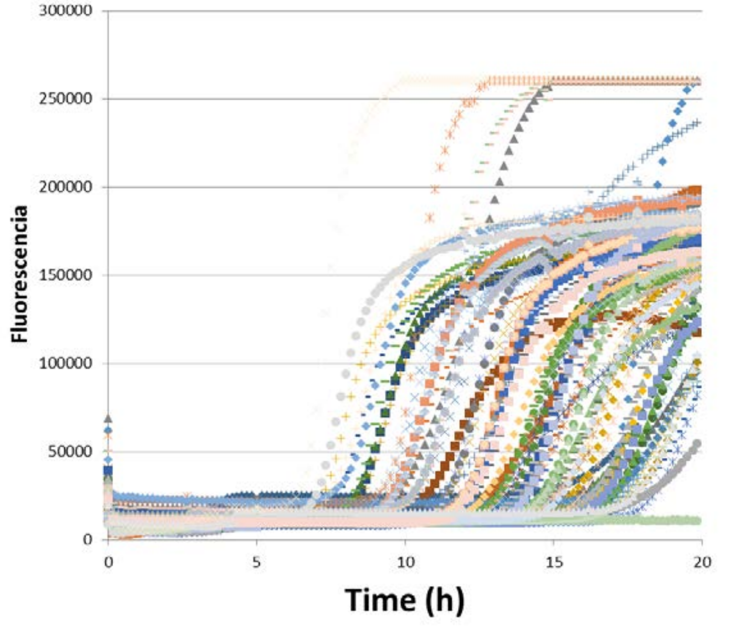
Real time amplification of genetic material from single viruses (real time MDA)





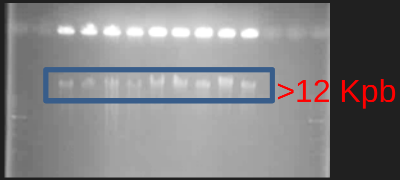


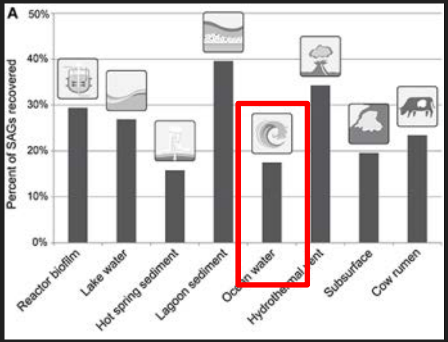
1 virus/well



≈15-55% single viruses were whole genome amplified

No contamination with bacteria
(no amplification for gen rRNA 16S)

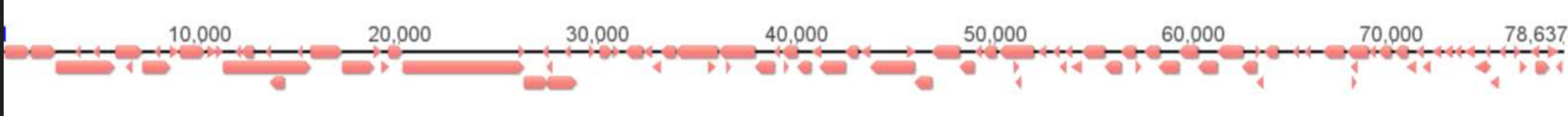




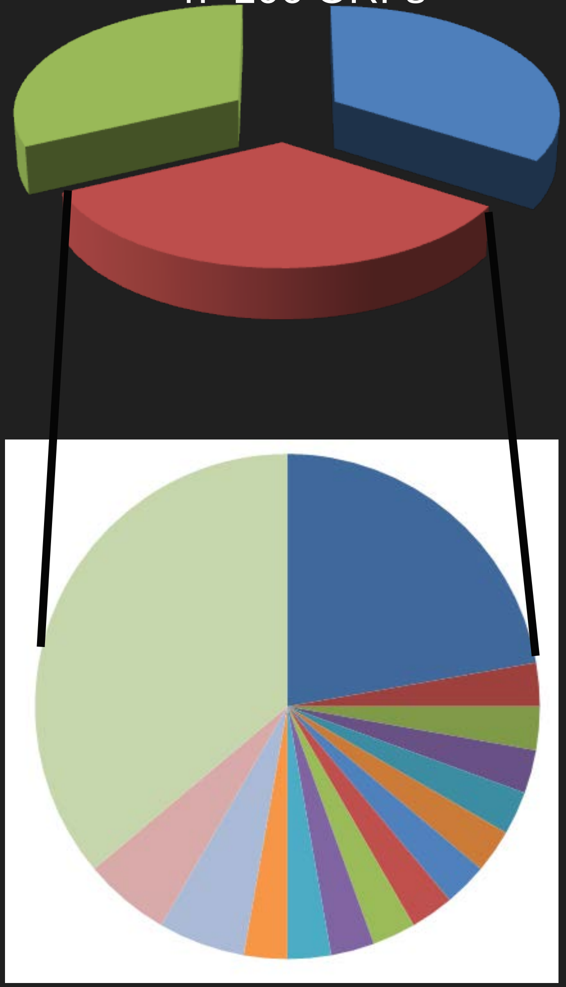
Clingenpeel et al. 2015

15 new viral genomes
- Genome size 11-78 Kbp (in the typical range size for marine viruses)
- GC 35.5%

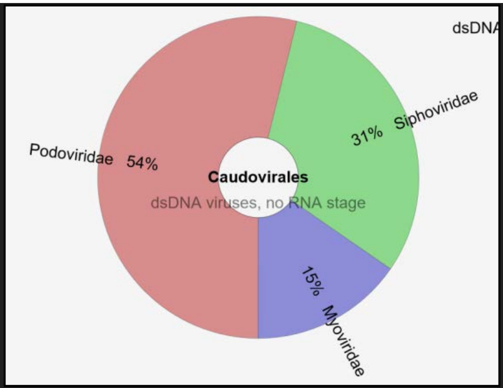
Virus 17_C23 (78637 bp) Closest virus in Genbank: an uncultured Mediterranean phage



n=106 ORFs



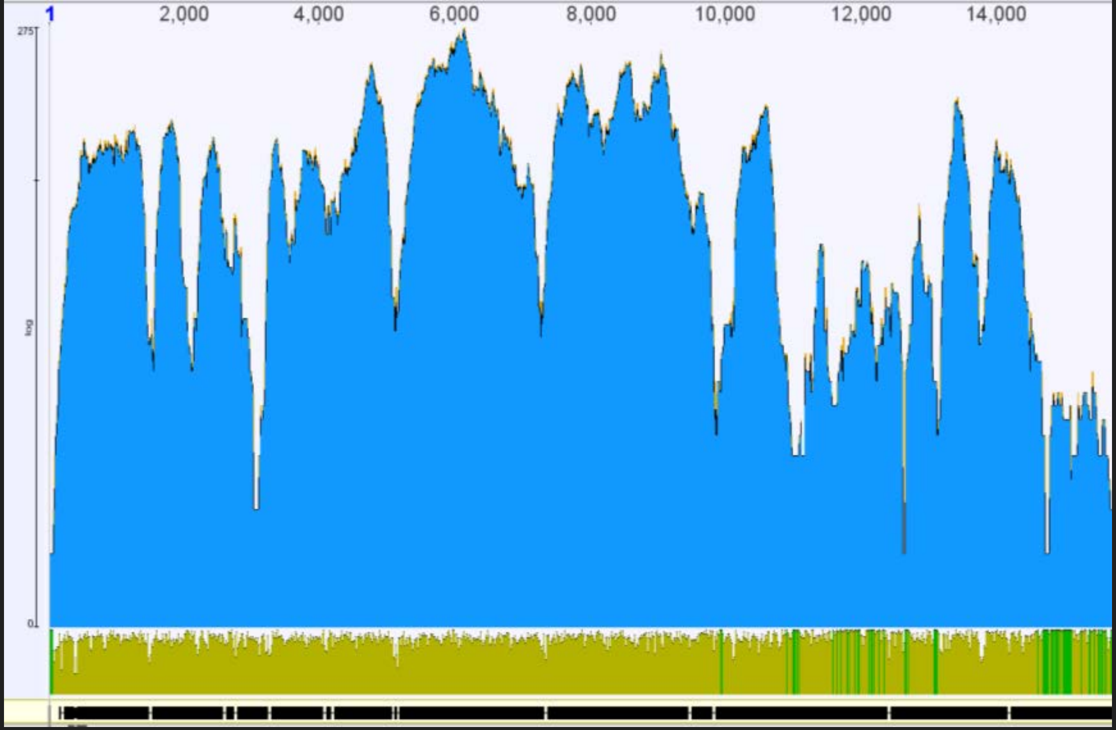
- 🟦 No hit in Genbank database "nr"
- 🟥 Proteins with known function
- 🟩 Hypothetical conserved viral proteins



- 🟦 tail phage, portal protein and structural proteins [Vibrio phages phi 3 pVp-1]
- 🟥 DNA primase Salmonella phage Shivani
- 🟩 DNA polymerase Escherichia phage vB_EcoS_FFH1
- 🟪 DNA ligase Acidaminococcus sp. CAG:917
- 🟦 DNA endonuclease V Synechococcus phage Syn19
- 🟧 UV damage repair endonuclease [Cronobacter sakazakii]
- 🟦 type III restriction protein res subunit [Desulfovibrio frigidus]
- 🟥 putative resolvase [Bacillus phage Bp8p-C]
- 🟩 putative replicative DNA helicase [Vibrio phage pVp-1]
- 🟪 putative recombination endonuclease subunit D12 [Vibrio phage phi 3]
- 🟦 dTDP-glucose 4,6-dehydrataseuncultured marine virus
- 🟧 nucleases [Salmonella phage Shivani]
- 🟦 peptidase [uncultured Mediterranean phage uvMED]
- 🟥 glycosyl transferase and acyl transferase
- 🟩 other proteins

Best BLAST hit
(Percentage identity mean= 41%)

Metagenomic comparison



Virus 37_L15 (16820 bp) Closest virus in Genbank: Uncultured marine virus isolate CBSM-298

Viral genomes recovered by single virus genomics are present in the metagenome

Conclusiones

1. Technology for recovering the genetic information from uncultured single viruses has been set up and can be applied virtually to any kind of biological sample

2. This approach opens a new way to explore the genetic information of viruses with Potential application in Public Health (new emergent viruses) and
Environmental Sciences, such us Deep Subsurface. NO NEED OF LITERS OF WATER

3. 400 new uncultured single viruses have been obtained by this approach bypassing culturing biases

4. So far, a total of 15 new, uncultured viruses have been sequenced

5. Genome annotation confirms that the obtained WGA material is indeed from viruses

Thanks to:

F.E.D.E.R.

Ministerio de Economia y Competitividad
Proyectos CGL2013-40564-R and SAF2013-49267-EXP

GOBIERNO DE ESPAÑA
MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

Toda la gente de nuestro Grupo de Ecología Microbiana Molecular de la UA,
en especial Monica Lluesma, Francisco Martinez y Mª Jose de la Cruz

Universitat d'Alacant
Universidad de Alicante

Financiación Privada: Becton Dickinson BD

Illumina, since it has reimbursed my travel expenses related to this event

Dr. Joaquin Martínez Martínez. Senior Scientist. Bigelow Laboratory, Maine (USA)

BIGELOW LABORATORY FOR OCEAN SCIENCES

Erika Ramírez and Eva Julià. Core Facility. Flow Cytometry. PRBB (Barcelona)

prbb Parc Recerca Biomèdica Barcelona

Silvia Acinas, Pep Gasol y Clara Cardelus. ICM-CSIC

ICM Instituto de Ciencias del Mar