Single cell genomics: A deeper look at life's building blocks

Ramunas Stepanauskas

Bigelow | Laboratory for Ocean Sciences

Art by Glynn Gorick

Acknowledgements

SCGC staff:
Joseph Brown
Julia Brown
Wendy Bellows
Elizabeth Fergusson
Christian Harris
Monica Lluesma
Corianna Mascena
Dashiell Masland
Ben Tupper
Brian Thompson

FACS facility:
Nicole Poulton

SCGC Advisory Board
Rita Colwell
George Garrity
Kostas Konstantinidis
Craig Muir
Ger van den Engh
Tanja Woyke
Robert Young

RS postdocs (current and past):
Eric Becraft
Jane Heywood
Jessica Labonté
Manuel Garcia Martinez
Maria Pachiadaki
Brandon Swan

Key collaborators
Sallie Chisholm
Gerhad Herndl
Arvydas Lubys
Duane Moser
Stefan Sievert
Tullis Onstott
Kathuri Venkateswaran
Tanja Woyke

Bigelow | Laboratory for Ocean Sciences

Open postdoc positions

Scope: Single cell genomics and horizontal gene transfer in the marine microbiome

Application deadline: January 15, 2017

Further information:
https://www.bigelow.org/about/careers.html
rstepanauskas@bigelow.org

Bigelow | Laboratory for Ocean Sciences

Single cell genomics:
A deeper look at life's building blocks

Ramunas Stepanauskas

1. SCG opportunities in microbiology

Coffee break

2. Do we know how much we don't know?

Leg stretcher

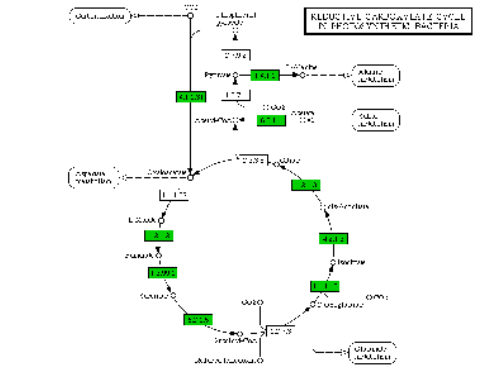
3. Infrastructure and method advances

Art by Glynn Gorick

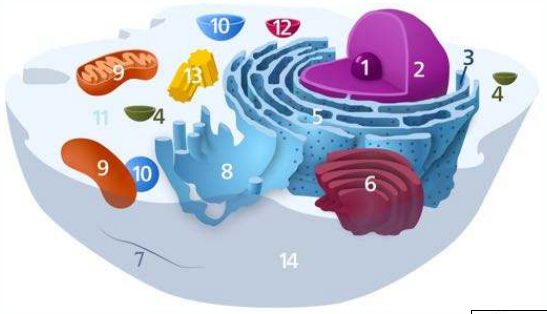
Bigelow | Laboratory for Ocean Sciences

Why single cell genomics?

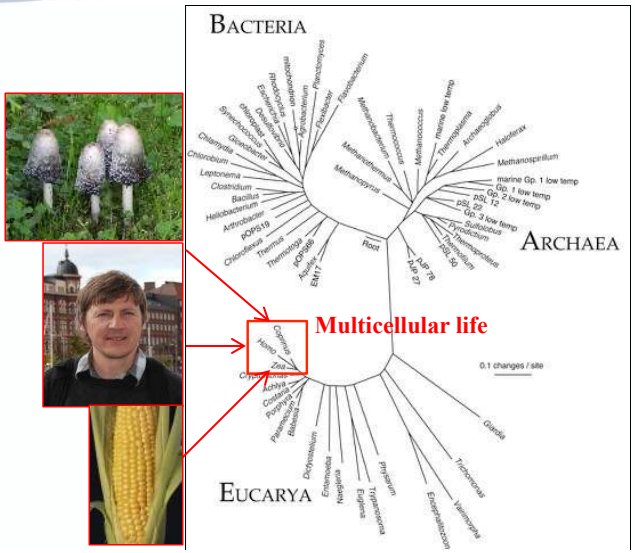
Genomes are blueprints of life



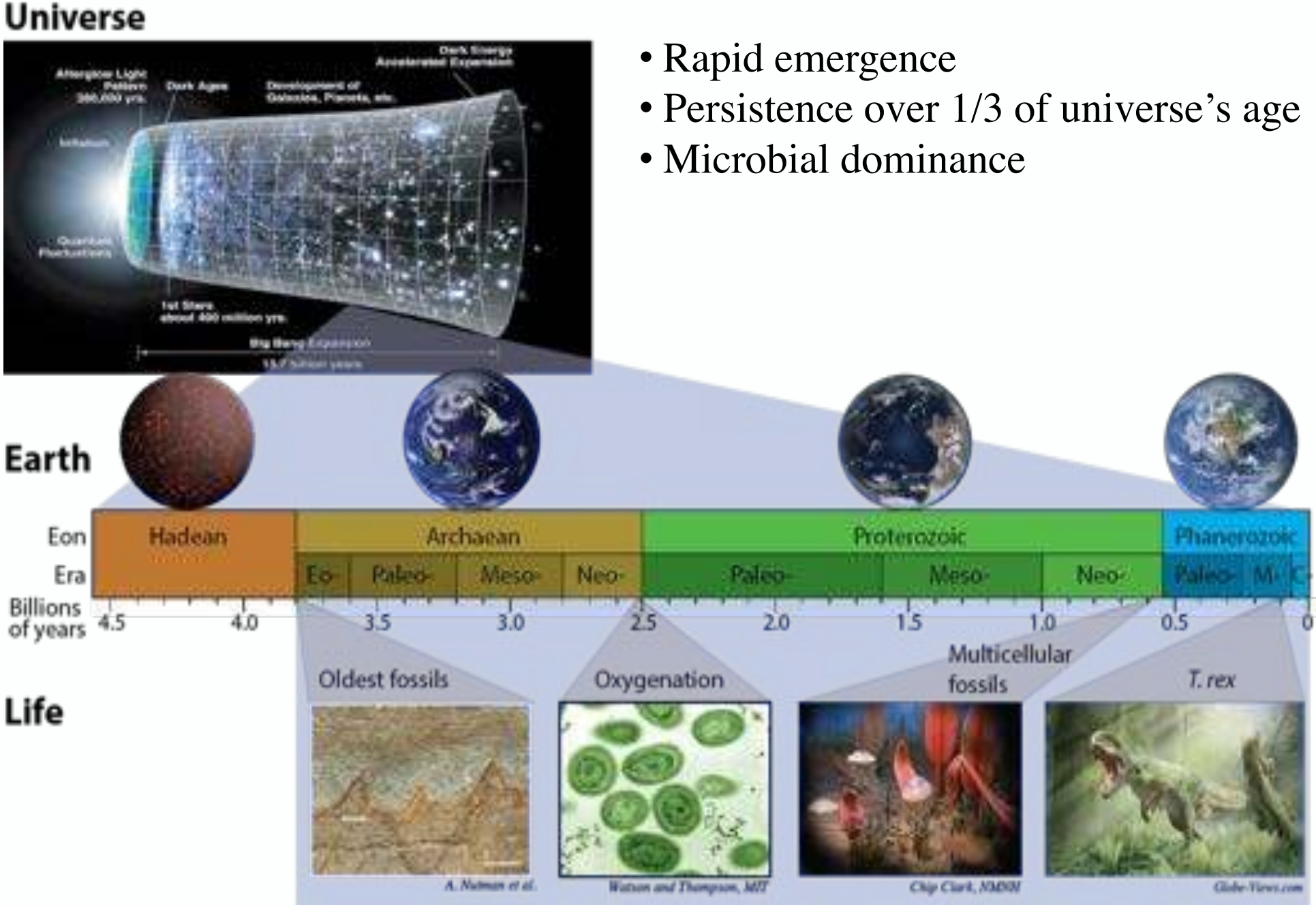
Cells are fundamental units of biology



For most of life on Earth, 1 cell = 1 organism

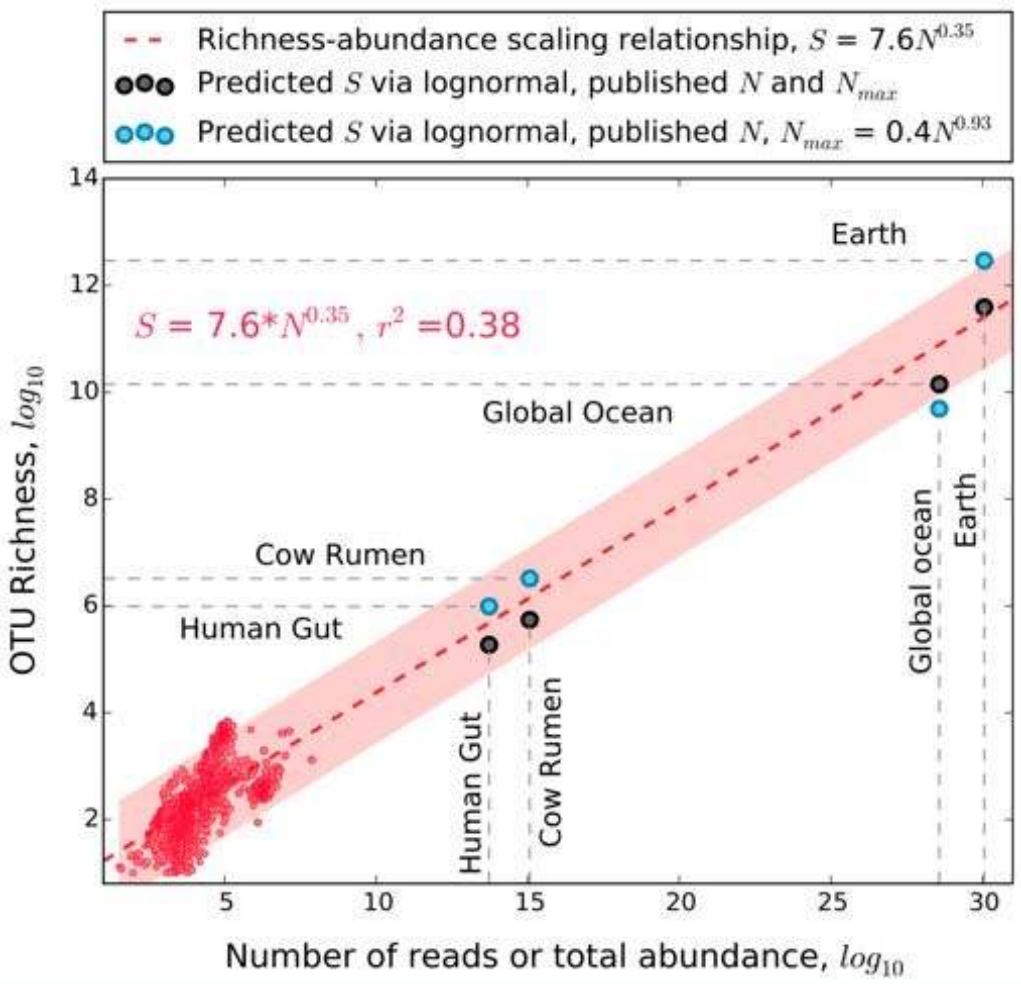


Amazing features of life on Earth



- Rapid emergence
- Persistence over 1/3 of universe's age
- Microbial dominance

Our planet may hold trillions of microbial species…



From: Locey and Lennon 2016

Implications:

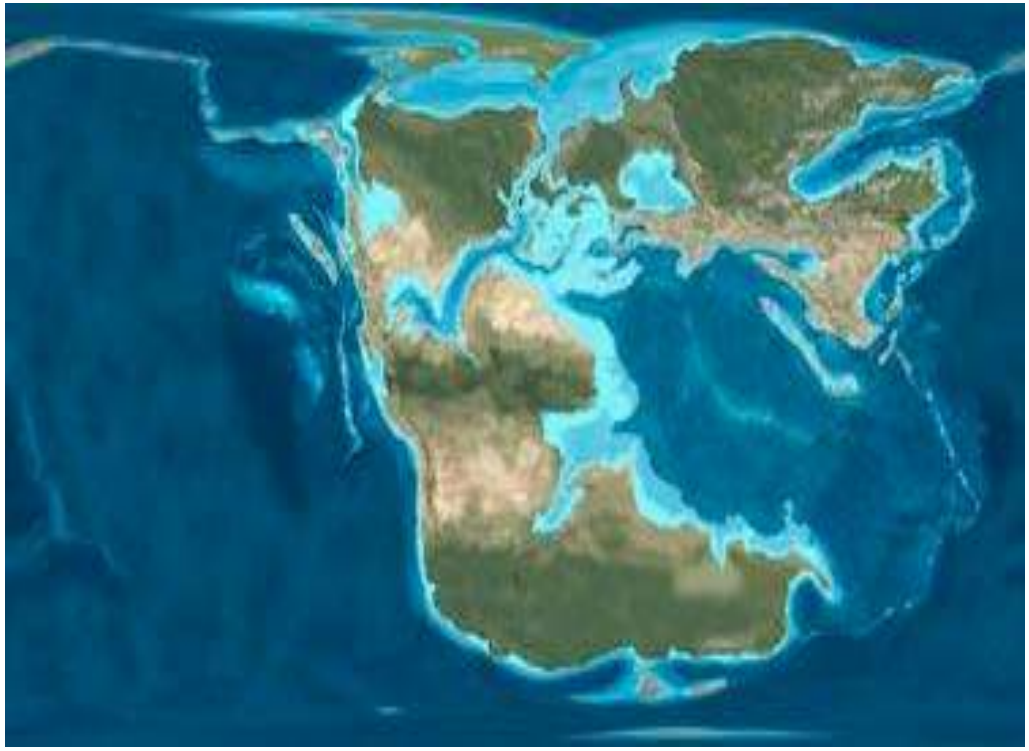
- Uncultured microbes run the planet
- Only a tiny fraction will ever be cultivated
- Molecular technologies have to be pushed further

OTUs are not species!

3% divergence in the 16S rRNA gene takes ~150 million years, so...

...last common ancestor of an average OTU lived in Jurassic!

Don't bacteria evolve faster than mammals?



Images from Wikipedia

Oceans of genetic information

1 mL surface ocean water encodes ~1 TB genetic information

1 g of agricultural soil encodes ~1 PB

The entire planet encodes ~10^{21} PB

<1% of this genetic resource can be accessed by cultivation

Assumptions of metagenomic assemblies

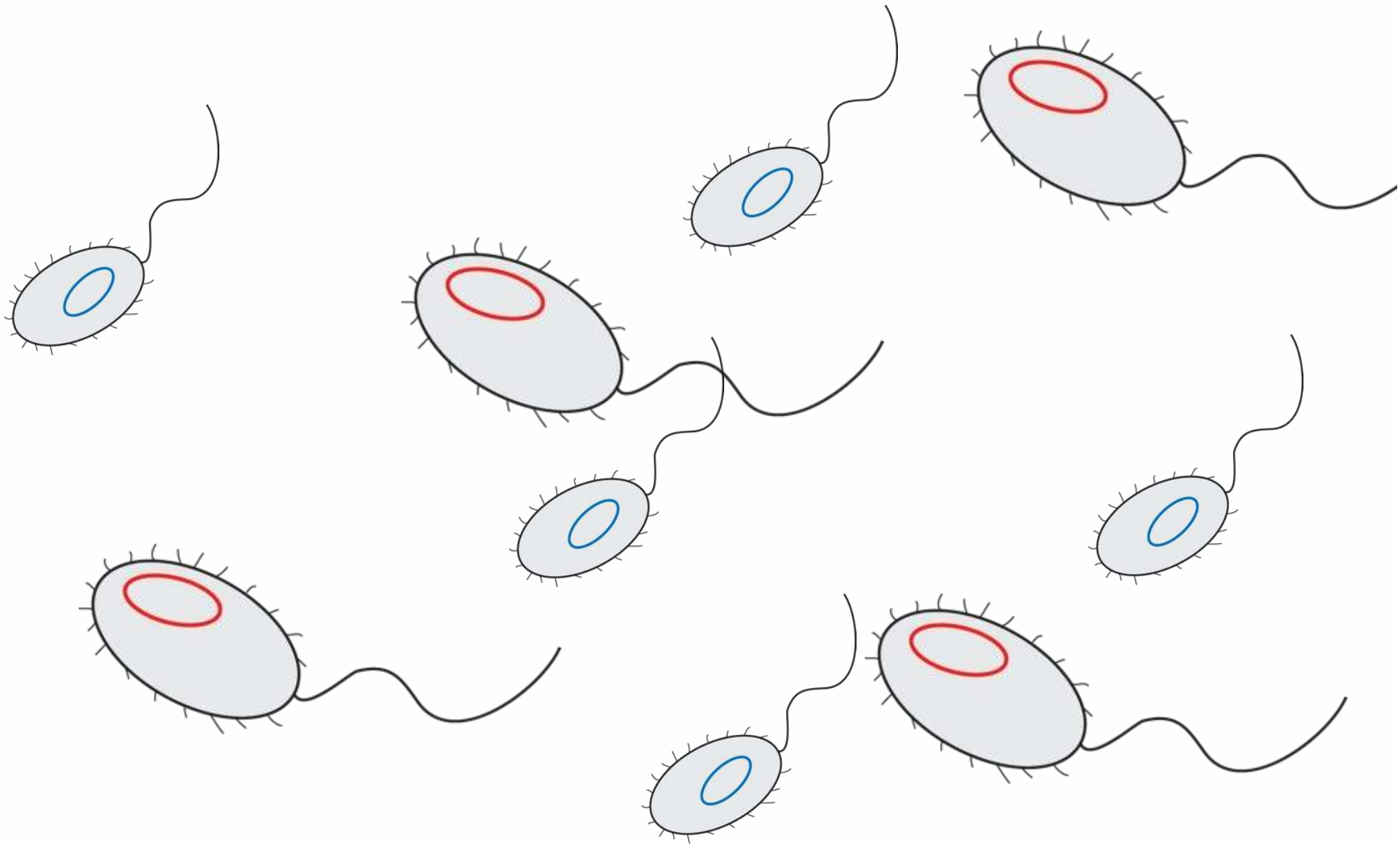
Image from Wikipedia

Microbial communities are composed of clonal populations

Entire genome evolves synchronously

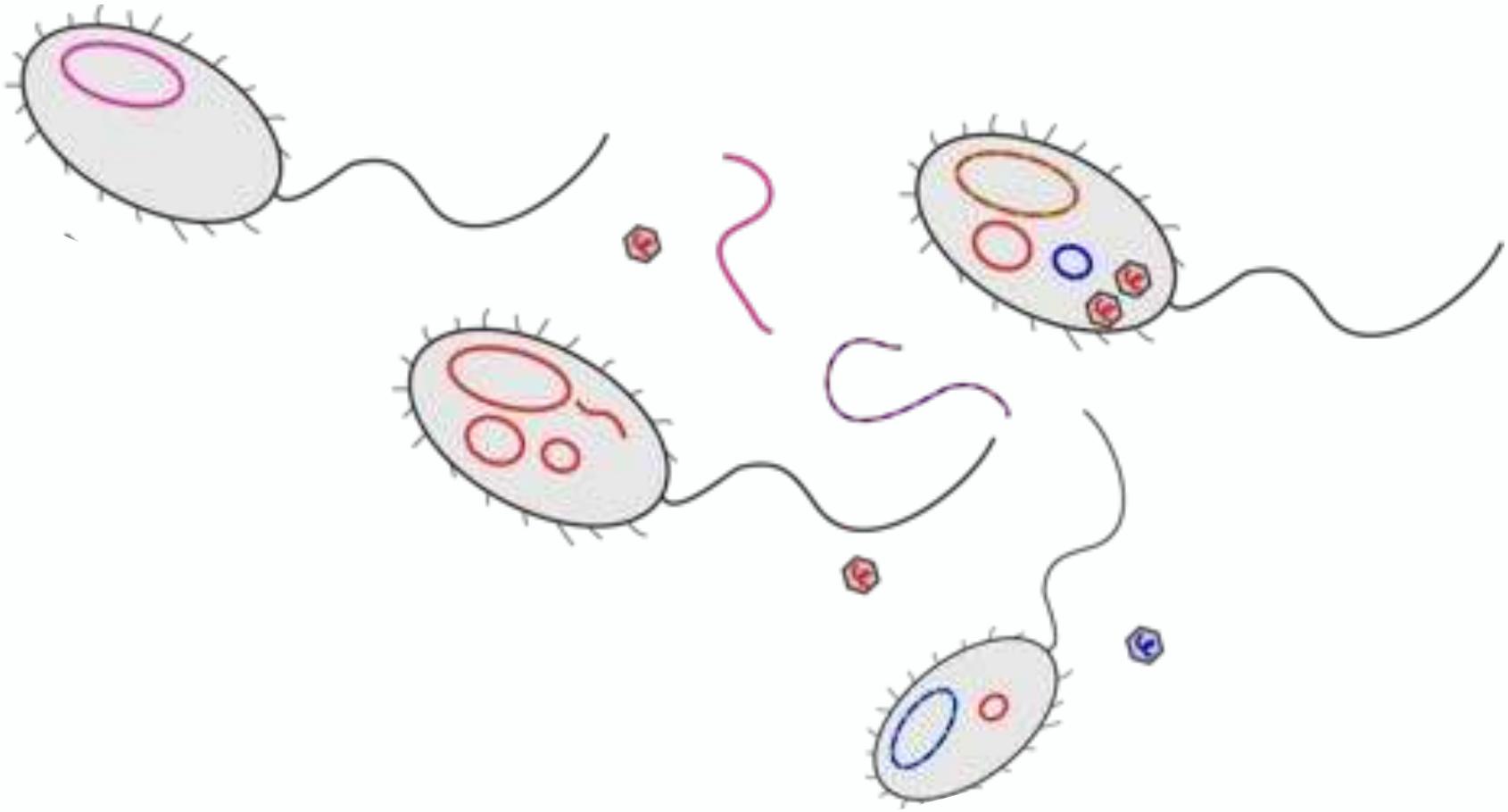
De novo assembly software does not cross-assemble different populations

DNA in a idealized microbial assemblage



Bigelow | Laboratory for Ocean Sciences

Complex DNA distribution in a microbial assemblage



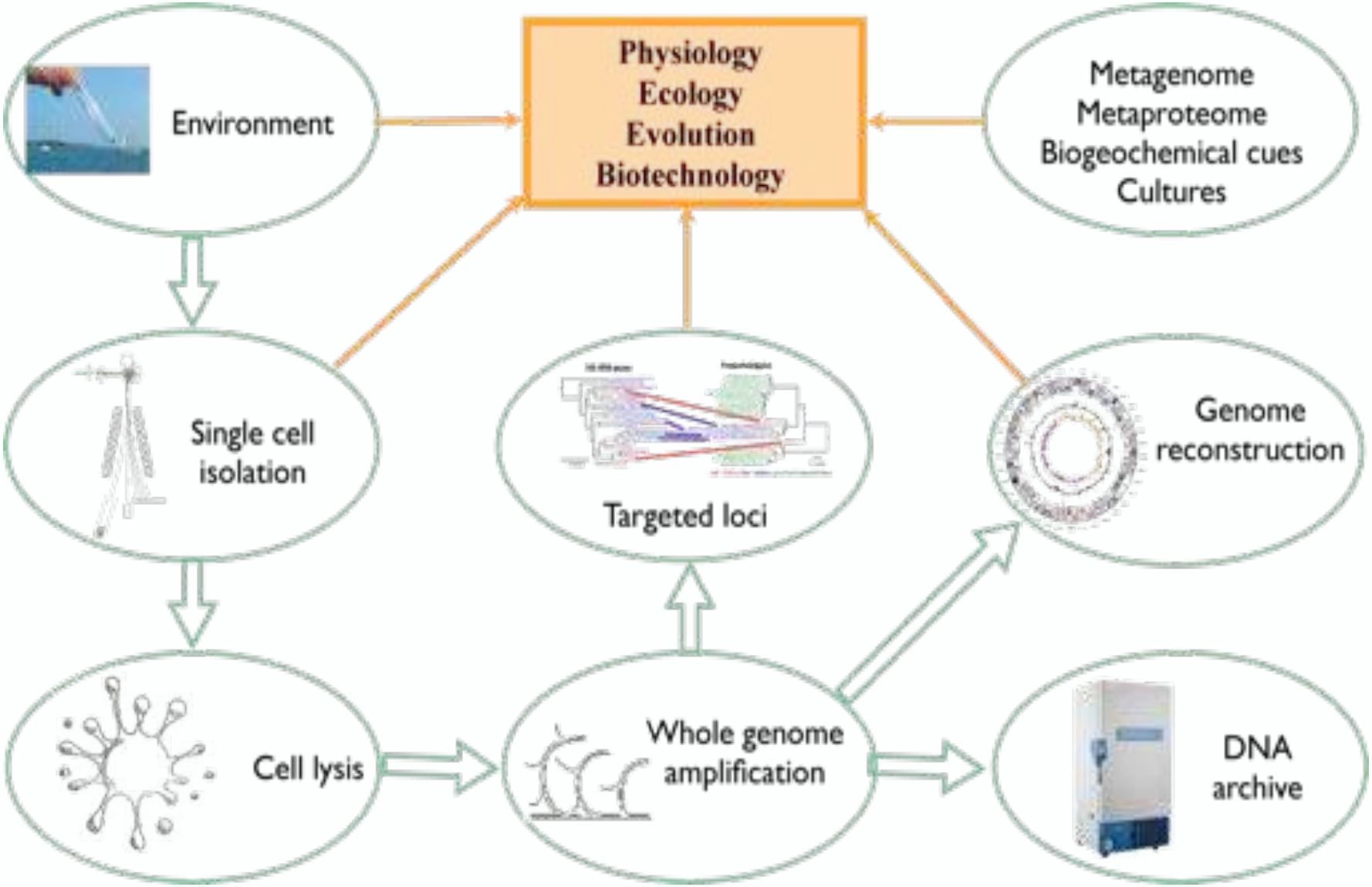
Implications:

Horizontal spread of antibiotic resistance and other traits

Large differences among close relatives

16S and metagenomics provide incomplete information

Microbial Single Cell Genomics Workflow



Milestones of microbial single cell genomics

Pre-2000: Experimenting with PCR-based single cell DNA amplification

- *e.g. Zhang et al. (1992) PNAS 89:5847-5851*

2001-2002: Development of multiple displacement amplification (MDA)

- *Dean et al. (2001) Genome Research 11:1095-1099*
- *Dean et al. (2002) PNAS 99:5261-5266*

2005-2006: Proof-of-concept single cell MDA on cultured microorganisms

- *Raghunathan et al. (2005) AEM 71: 3342-3347*
- *Zhang et al. (2006) Nature Biotech. 24:680-686*

2007-2008: First genomic data from uncultured, single cells

- *Stepanauskas and Sieracki (2007) PNAS 104: 9052-9057*
- *Marcy et al. (2007) PNAS 104:11889-11894*

2009-now: High-throughput facilities; major research discoveries

- *Over 100 publications in microbial ecology, evolution, bioprospecting and human health*

Bigelow Laboratory Single Cell Genomics Center

scgc.*bigelow.org*

SCGC
SINGLE CELL GENOMICS CENTER
Reading nature's genetic tales, one cell at a time

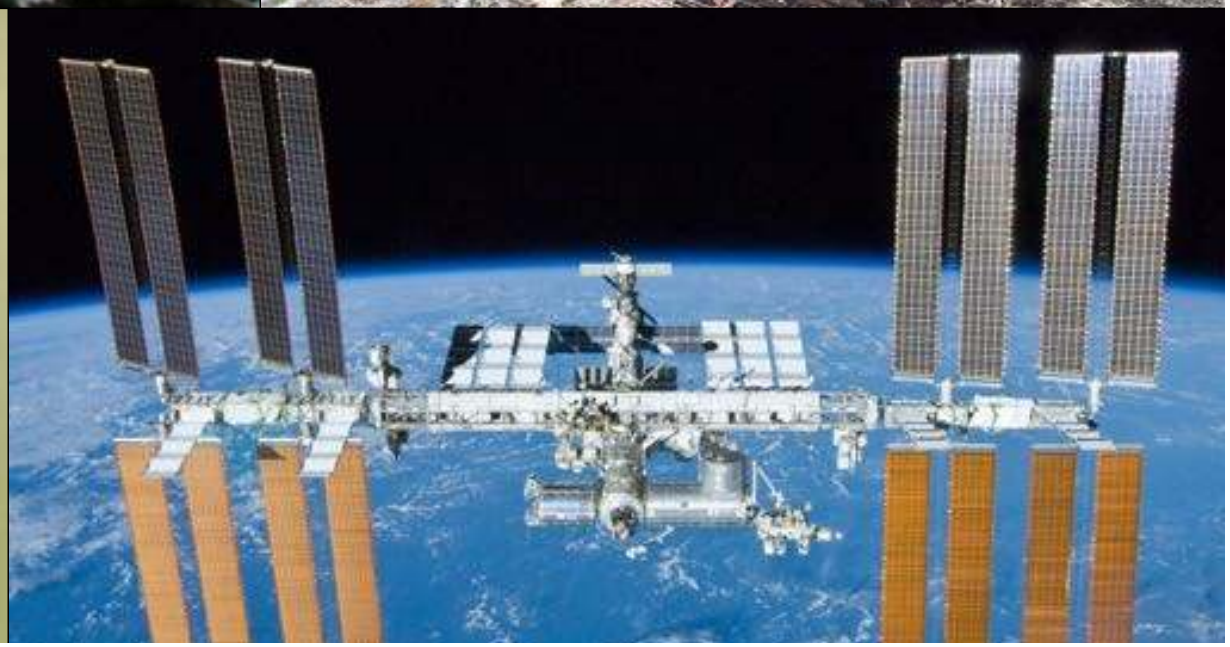
<u>Mission:</u> make single cell genomics accessible to the broad research community; serve as an engine for discoveries in microbial ecology, evolution, bioprospecting and human health.

- **First center of its kind, established 2009**
- **Diverse samples: aquatic, soil, organismal microbiomes, etc.**
- **>1,000,000 cells analyzed, representing >70 phyla**
- **60+ publications since 2011, 8 in *Science*, *Nature* & *PNAS***

Locations of SCGC customers

Microbiomes analyzed by SCGC

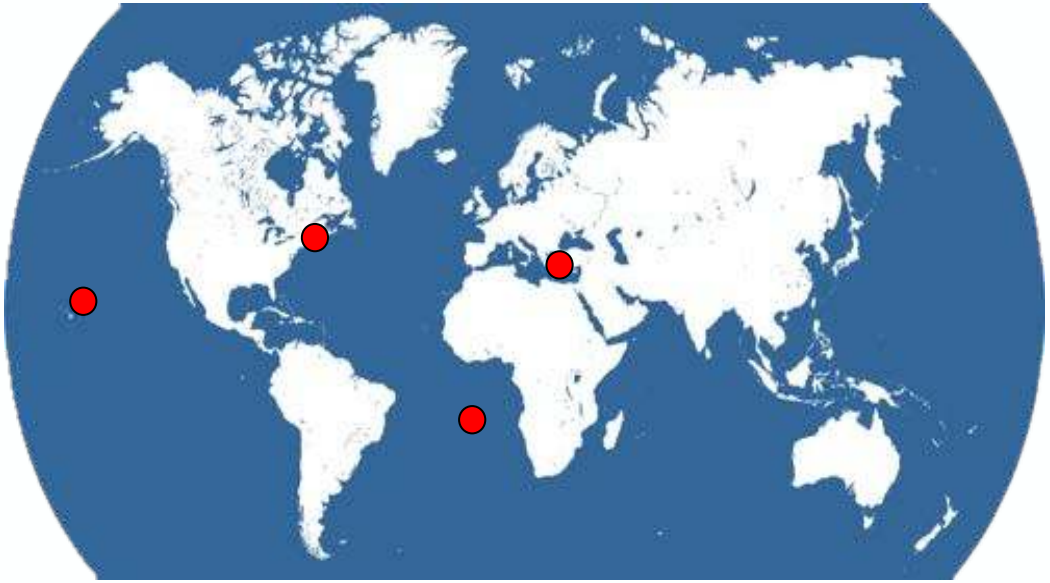
SCGC
SINGLE CELL GENOMICS CENTER
Reading nature's genetic tales, one cell at a time



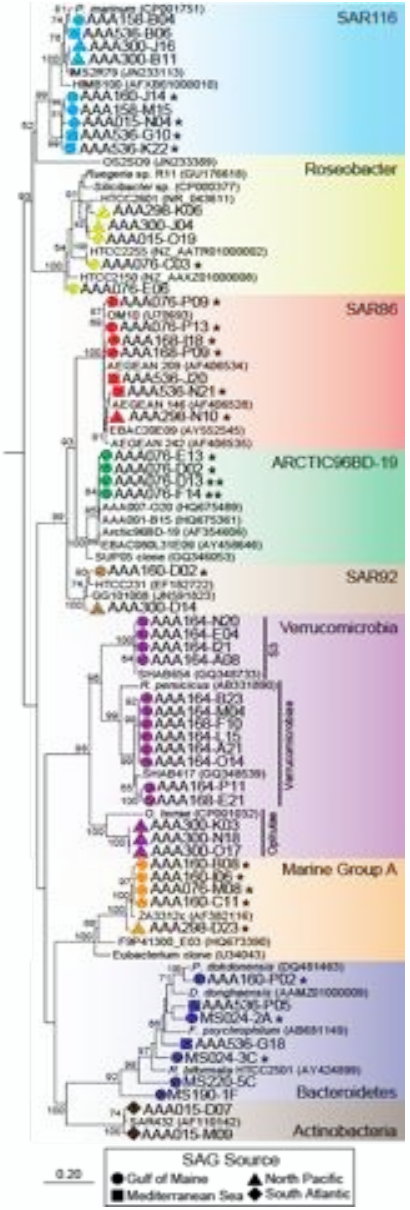
Research example: Surface ocean bacterioplankton

Garcia-Martinez et al. 2012 (PLoS ONE), Swan et al. 2013 (PNAS); Labonté et al. 2015 (ISME J)

Sample collection sites



SAG phylogeny



- **Collected surface samples from the Gulf of Maine, the Mediterranean and the South Atlantic and North Pacific subtropical gyres in 2007-2009**
- **Generated >2,000 single amplified genomes (SAGs) of bacteria & archaea**
- **Genomically sequenced 57 SAGs representing various ubiquitous groups**
- **Used genomic data to analyze metabolism, biogeography and infections**

Bigelow | Laboratory for Ocean Sciences

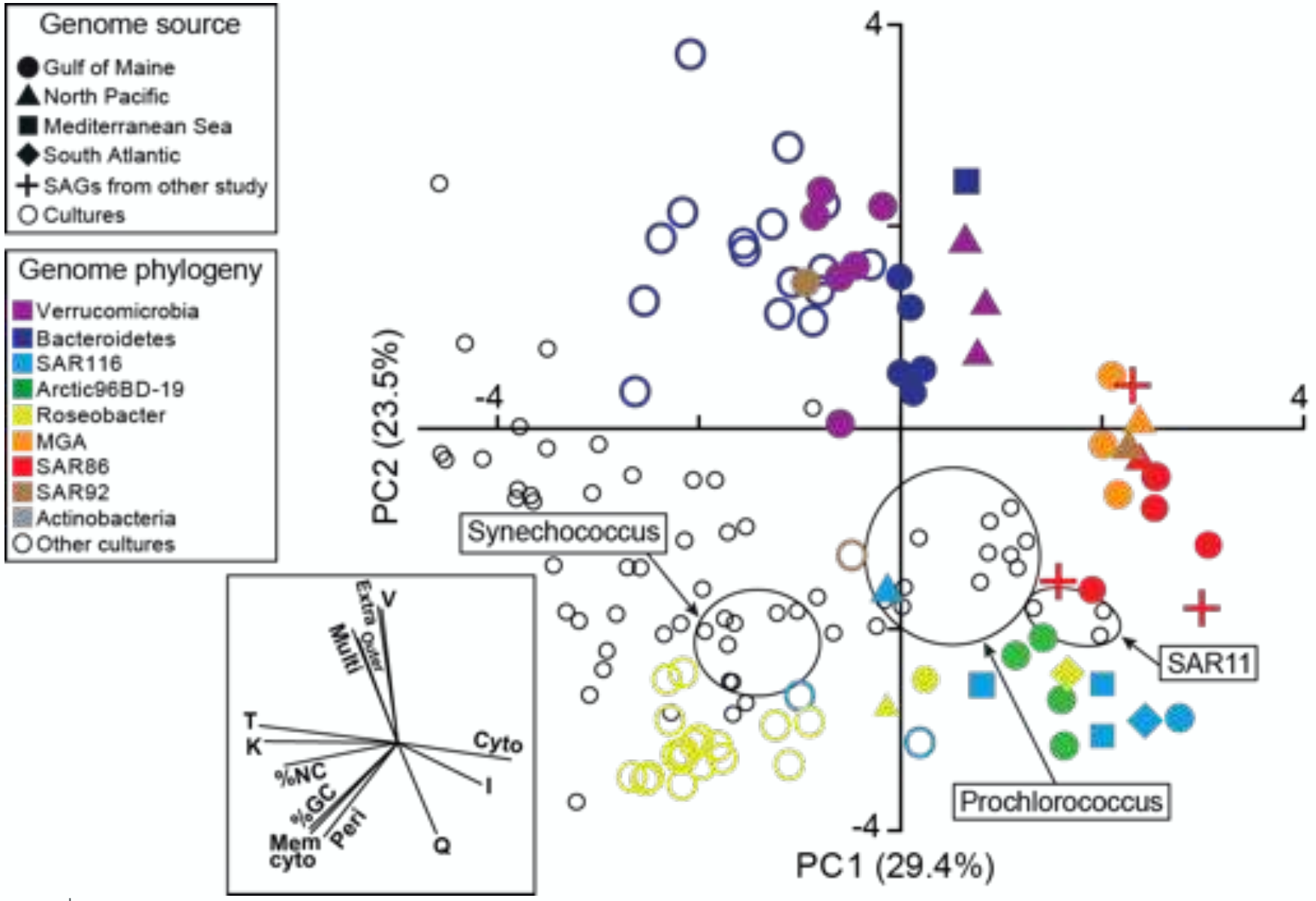
Four tales by 57 marine bacterioplankton cells:

- Metabolism
- Biogeography
- Microevolution
- Interactions

Art by Glynn Gorick

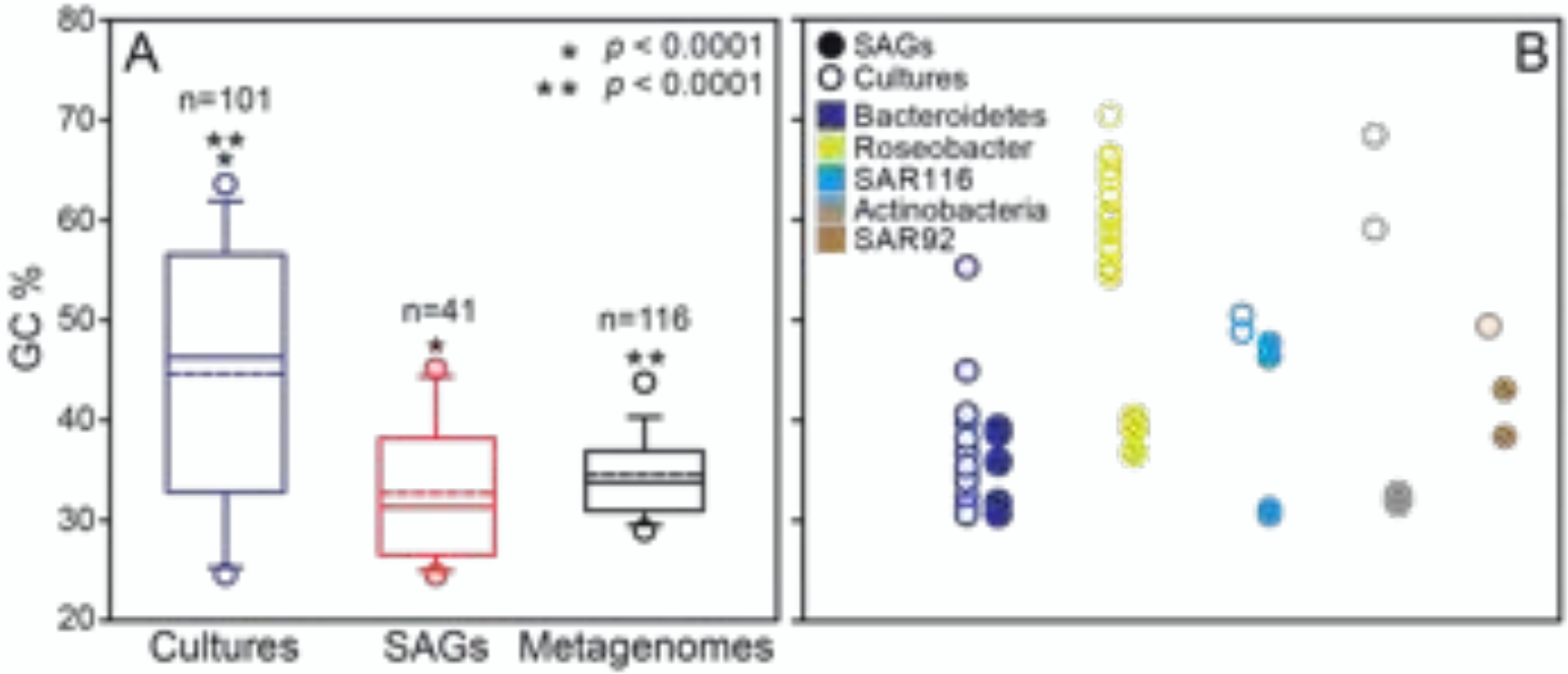
Bigelow | Laboratory for Ocean Sciences

Genomic divergence of cultured and uncultured bacterioplankton



Bigelow | Laboratory for Ocean Sciences

%GC divergence of cultured and uncultured bacterioplankton



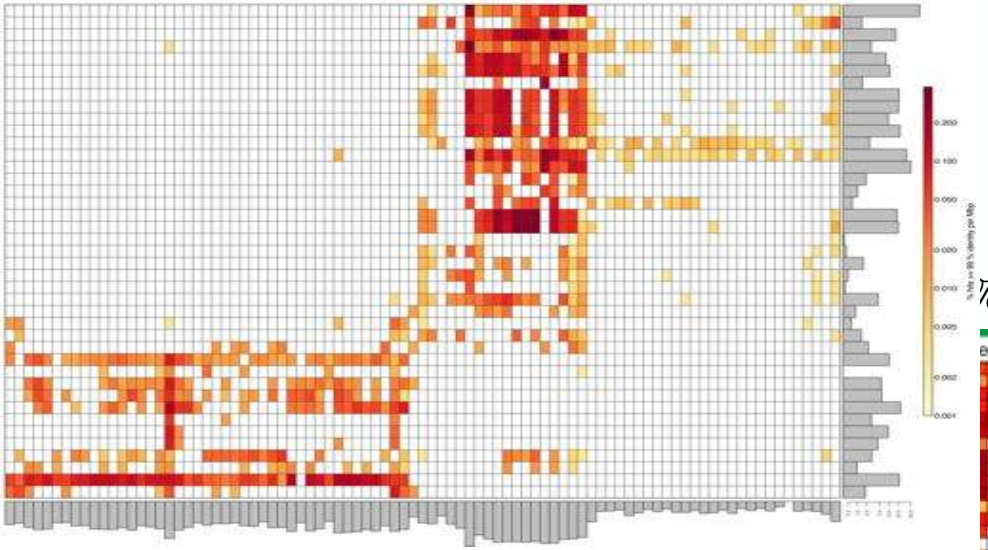
Four tales by 57 marine bacterioplankton cells:

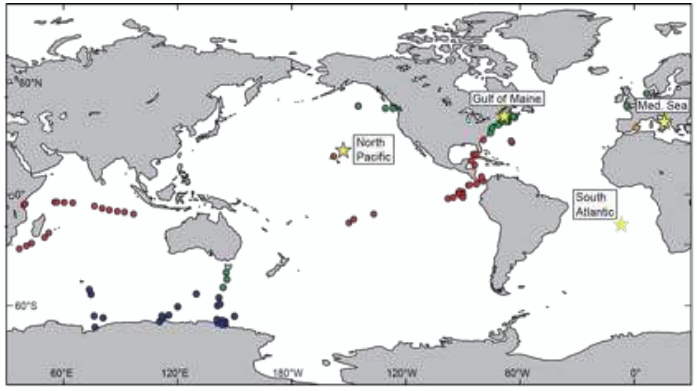
- Metabolism
- Biogeography
- Microevolution
- Interactions

Art by Glynn Gorick

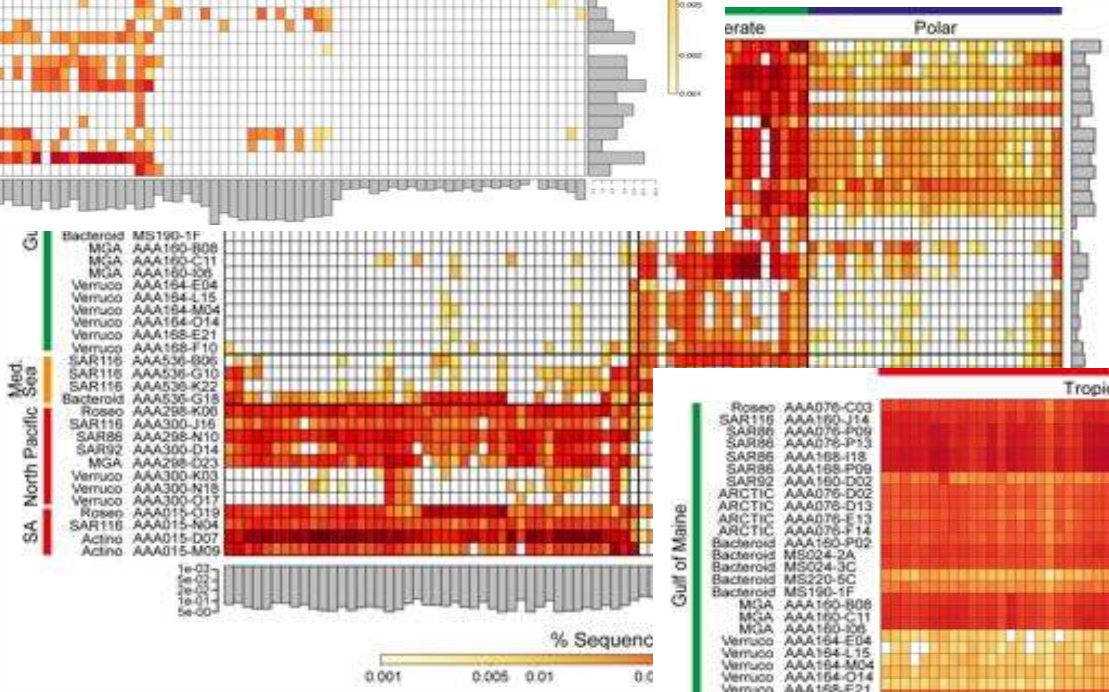
Global biogeography: metagenomic fragment recruitment

99% identity cutoff

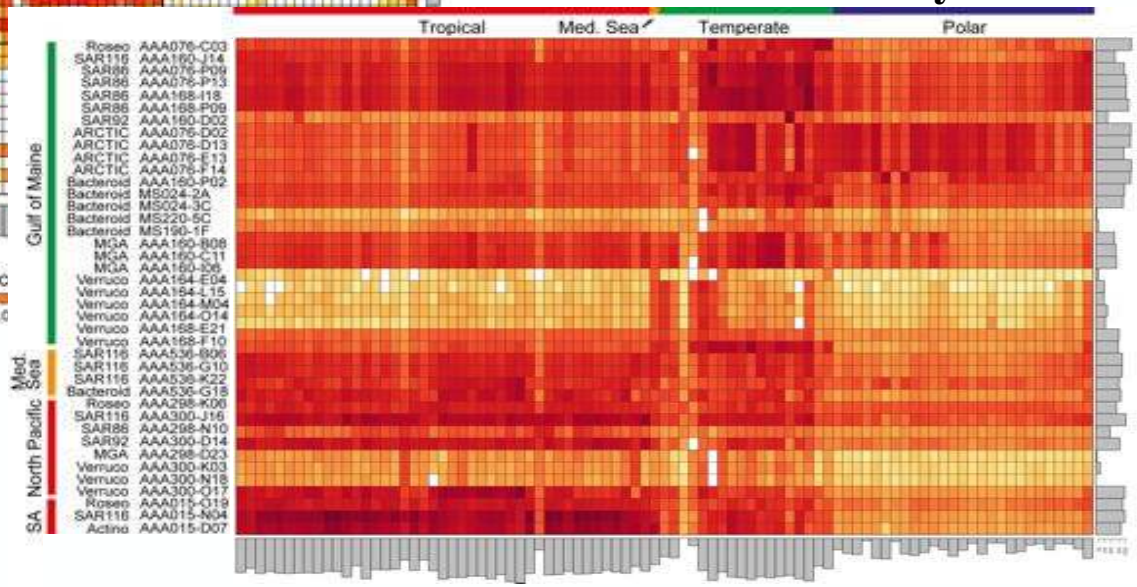




% identity cutoff

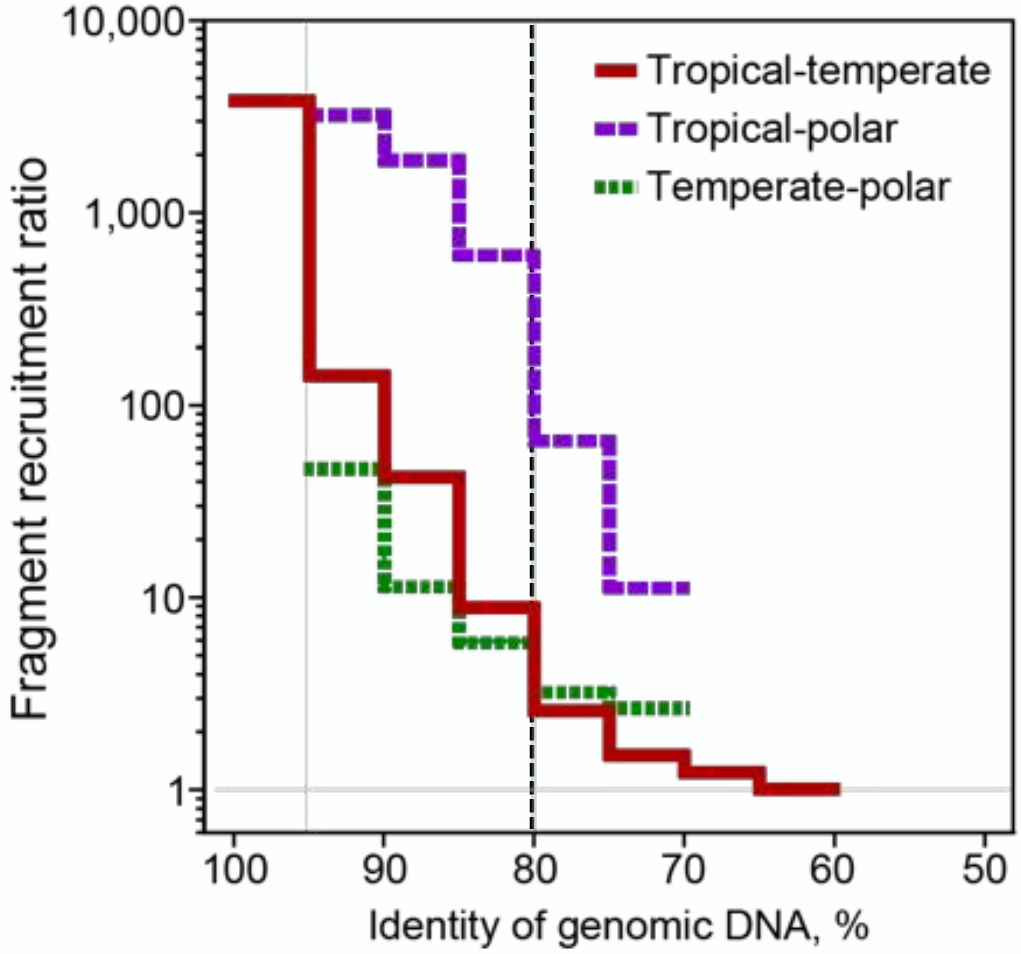


60% identity cutoff

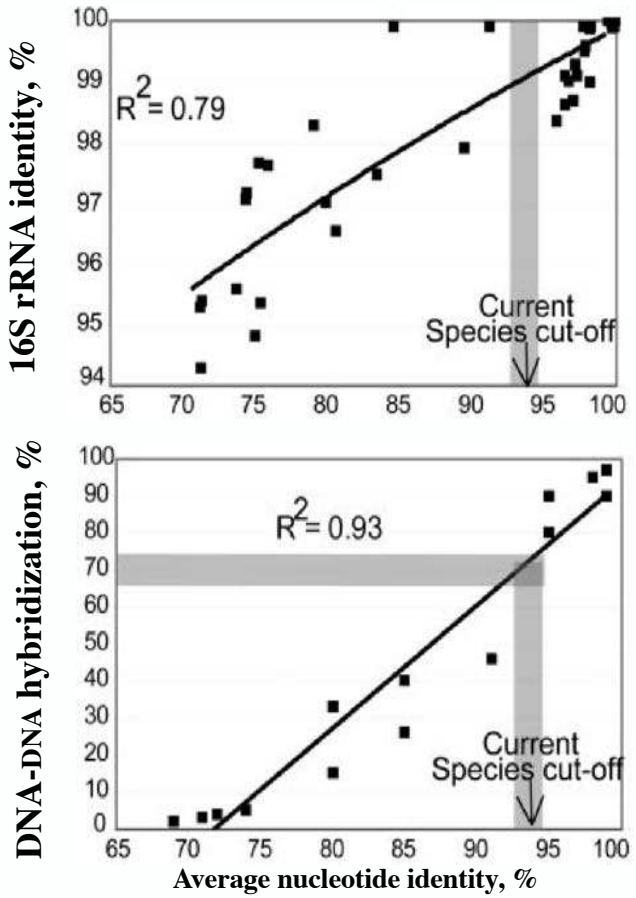


Bigelow | Laboratory for Ocean Sciences

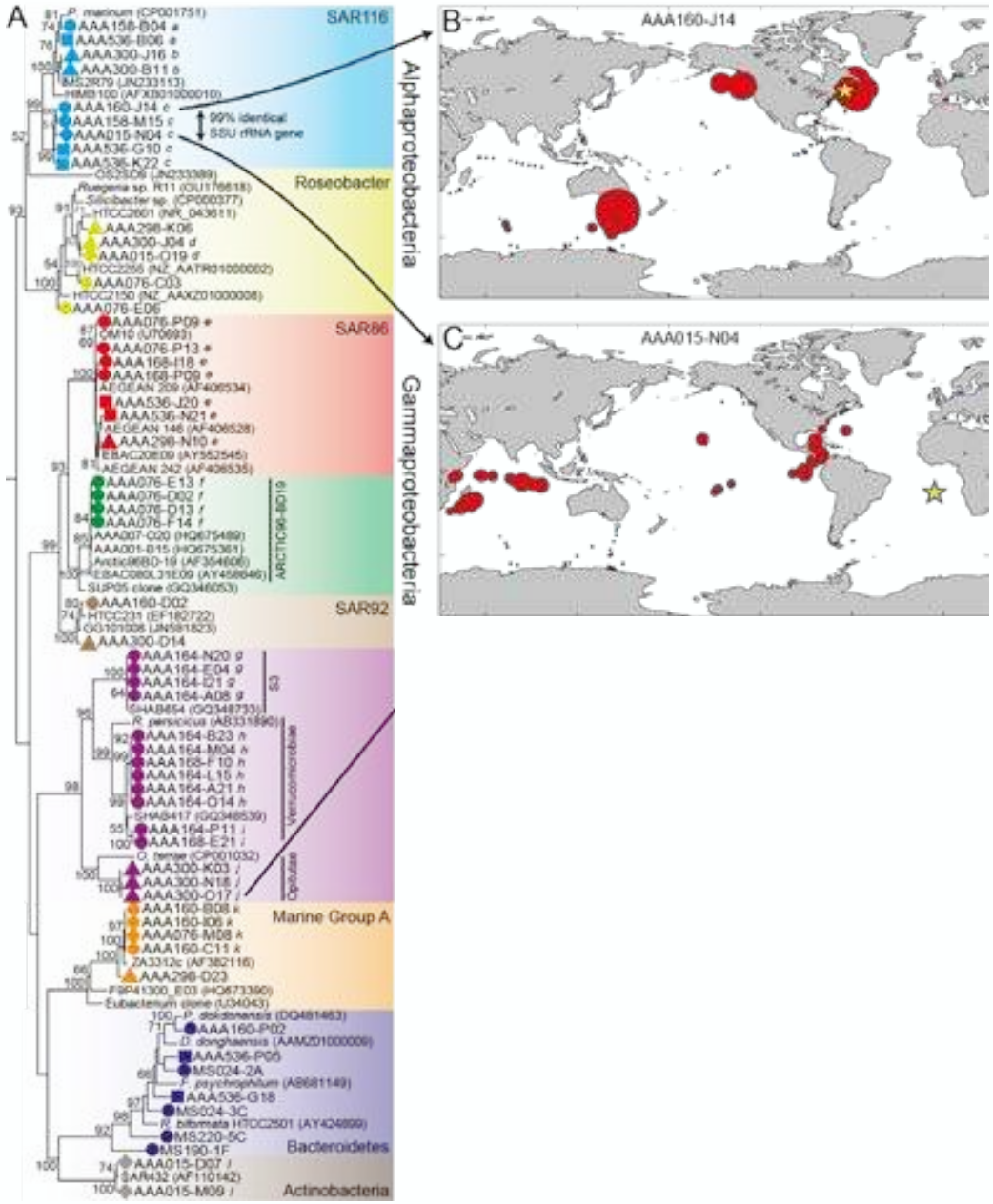
Recruitment ratio at various DNA identity intervals



ANI-16S relationship source:
Konstantinidis and Tiedje 2005, PNAS 102:2567

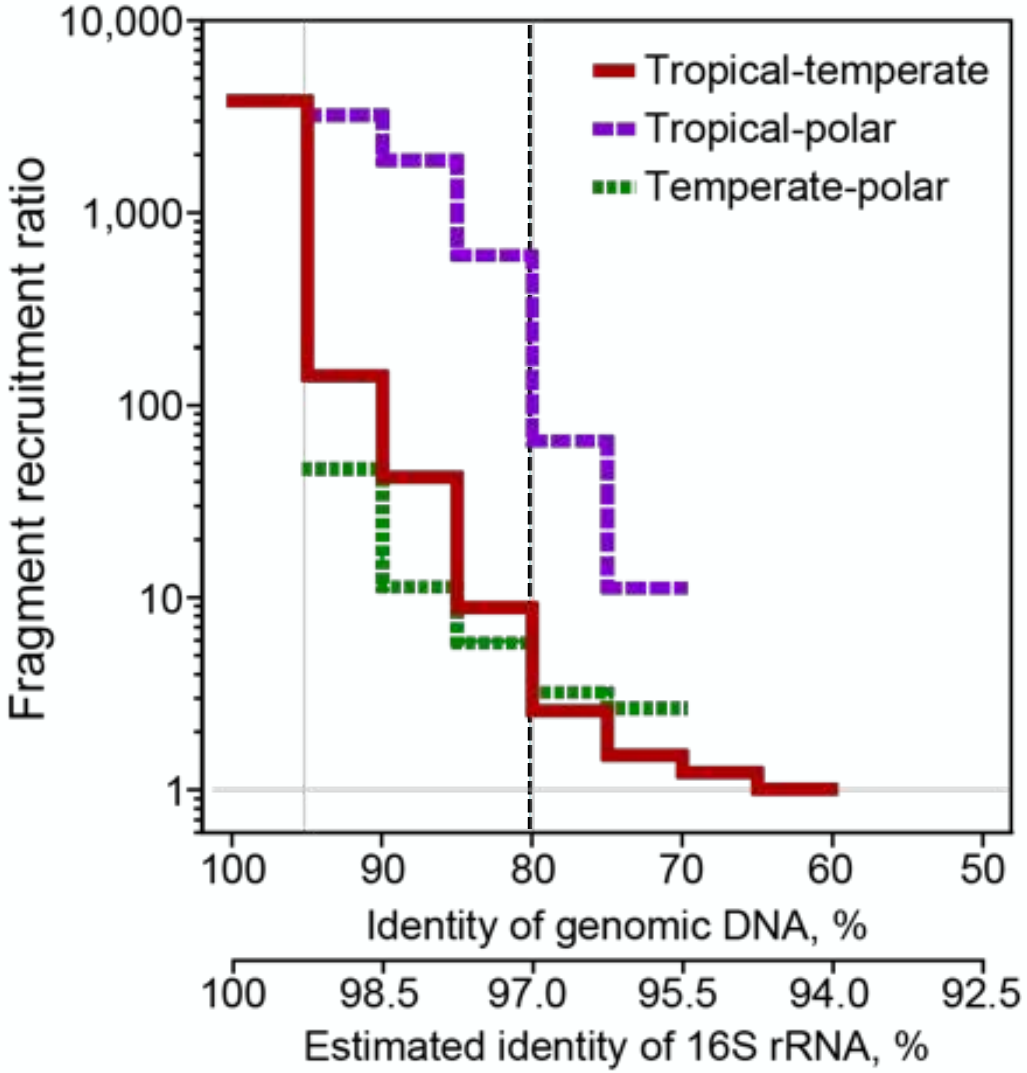


Divergent biogeography of close relatives



Bigelow | Laboratory for Ocean Sciences

Recruitment ratio at various DNA identity intervals



16S rRNA molecular clock:

Ochman and Wilson 1987, J. Mol.Evol. 26:74

Table 1. Events used to calibrate the dates of divergence of bacteria and rates of 16S rRNA evolution

Point	Event	Time (Myr)
A	Diversification of *Cyanobacteria*	>1300
B	Photosynthetic eucaryotes arise	>800
C	Oxygen appears	<2000
D	Oxidative eucaryotes arise	>800
E	Oxygen at high concentration	<800
F	Light organs appear	>50
G	Eyes appear	<500
H	Land plants appear	<400
I	Mammals appear	<150
J	Legumes appear	>100

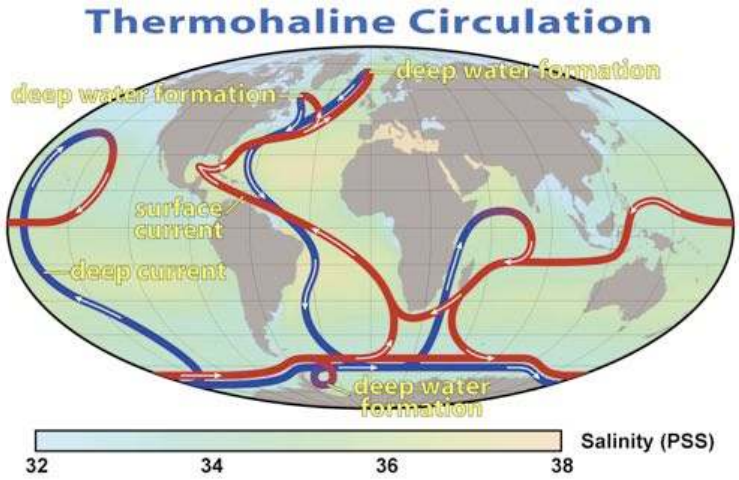
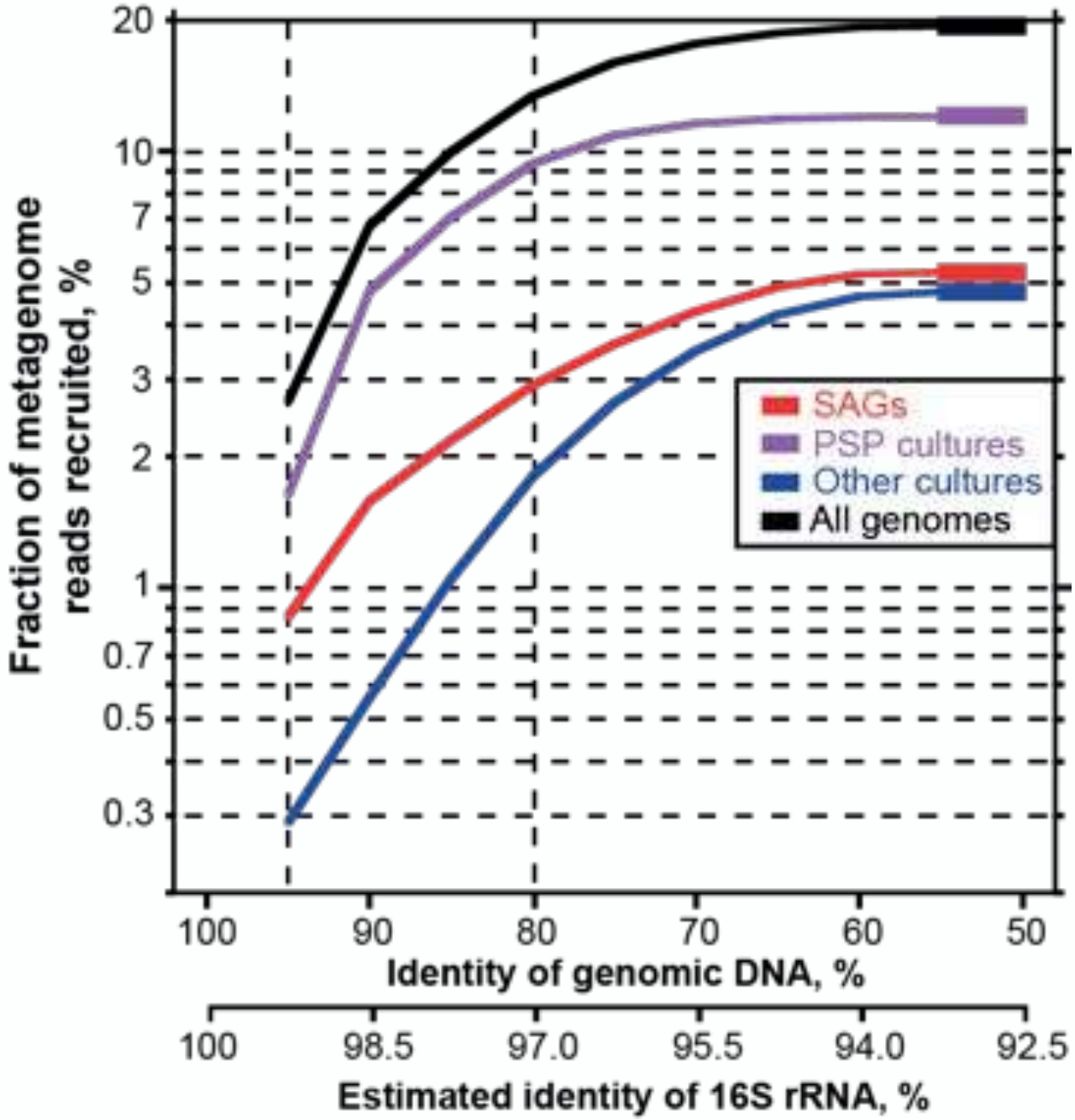
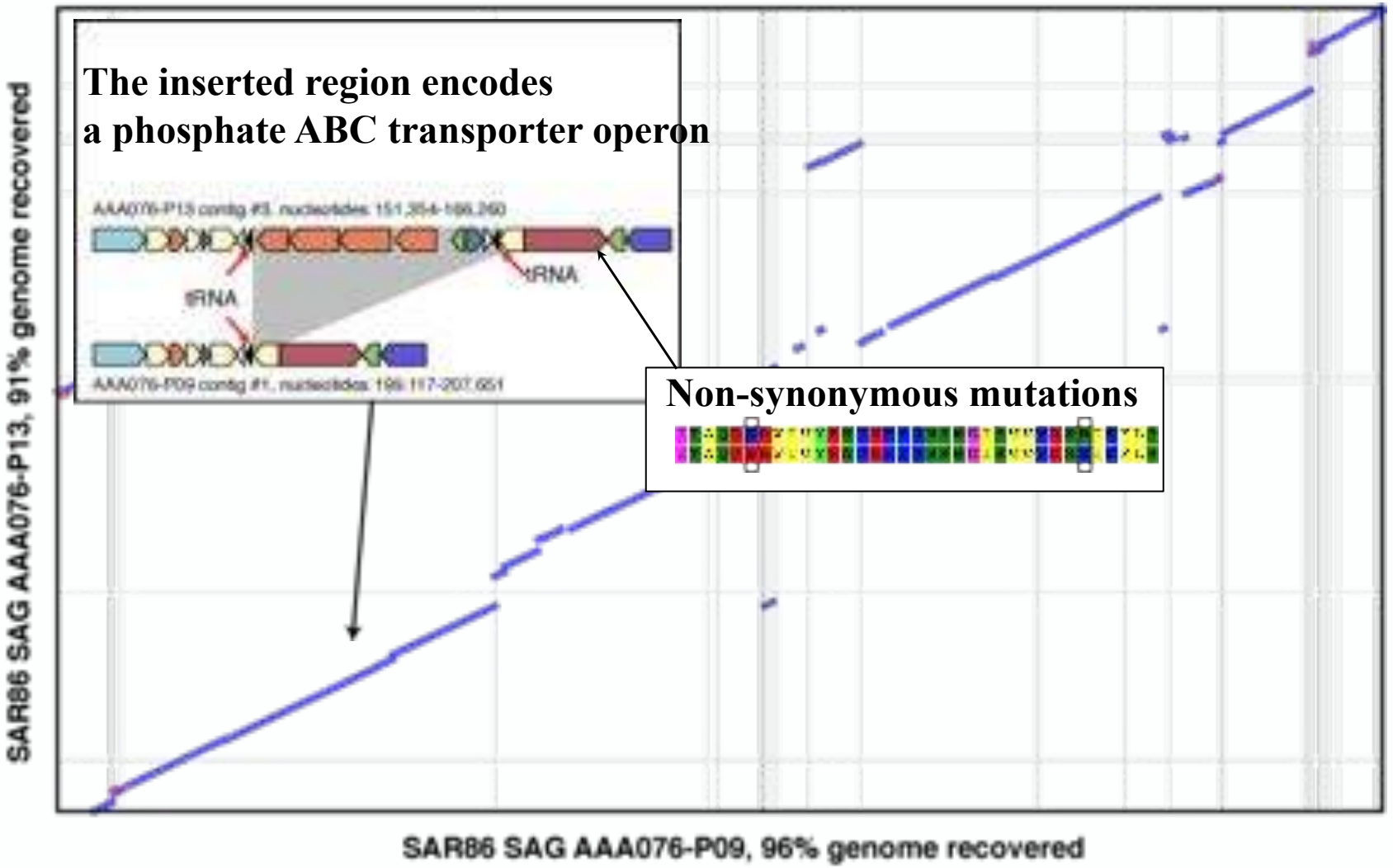


Figure from: Robert Simmon, NASA

We are still only scratching the surface



Genome content differences between two cells of SAR86 from the same drop with 100% identical 16S rRNA



Four tales by 57 marine bacterioplankton cells:

- Metabolism
- Biogeography
- Microevolution
- Interactions

Art by Glynn Gorick

Search for viral DNA in SAGs of bacteria and archaea

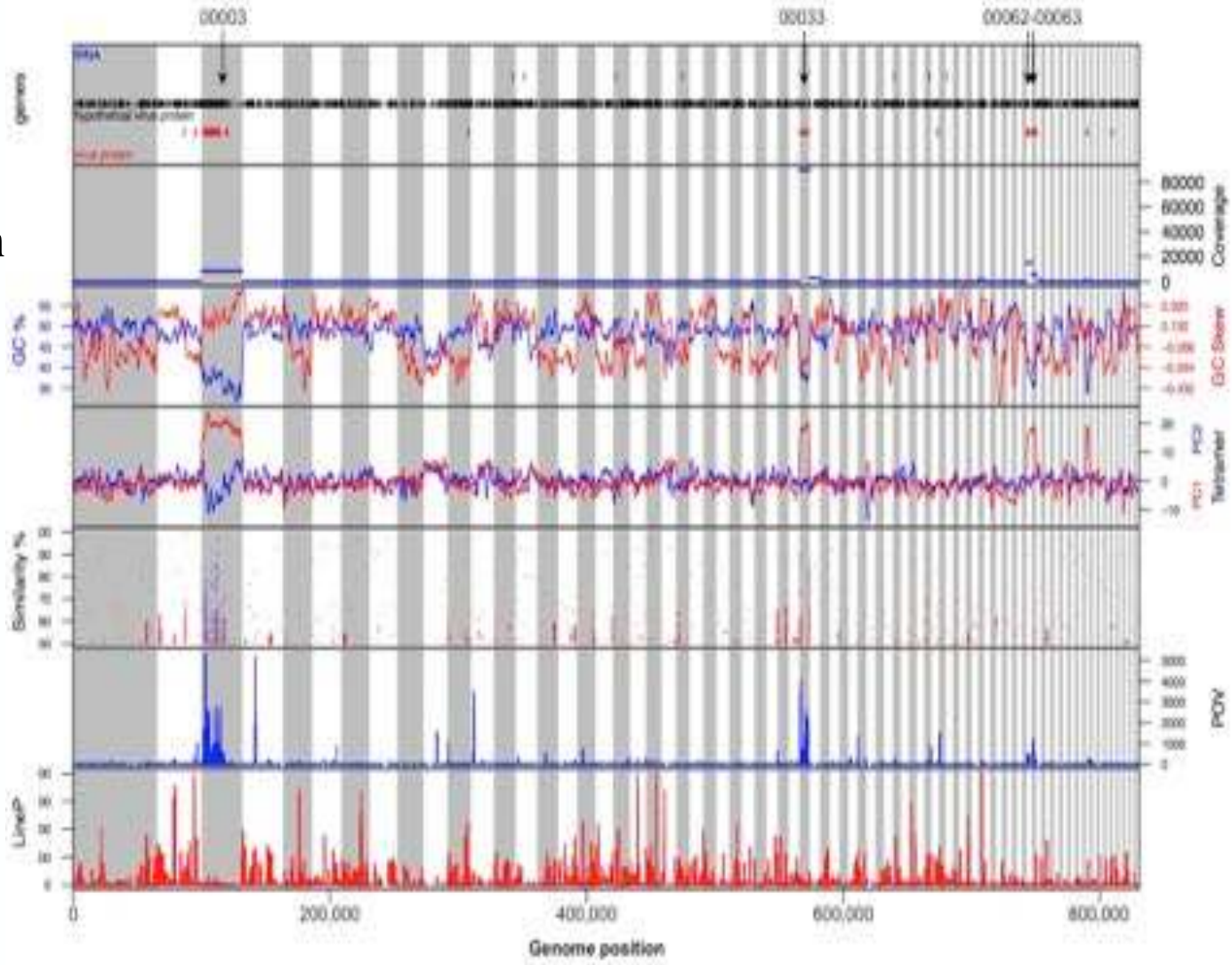
Viral marker genes

Sequence coverage depth

GC % and skew

Tetramer frequency

Recruitment of viral versus bacterial metagenomic reads



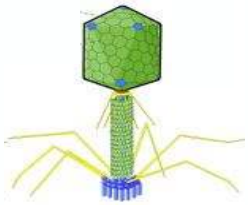
19 out of 57 SAGs (33%) contained viral sequences

10 *Podoviridae* phages

- Marinimicrobia SAR406
- Verrucomicrobia (5)
- Gammaproteobacteria SAR92
- Bacteroidetes
- Roseobacter

5 *Myoviridae* phages

- Verrucomicrobia
- Roseobacter
- Marine Group I crenarchaeon
- Marinimicrobia SAR406
- SAR86



3 *Siphoviridae* phages

- SAR116
- Verrucomicrobia
- Flavobacteria

1 *Phycodnaviridae* virus (likely contaminant)

- Verrucomicrobia

First known viruses of phyla Thaumarchaeota, Marinimicrobia, and Verrucomicrobia

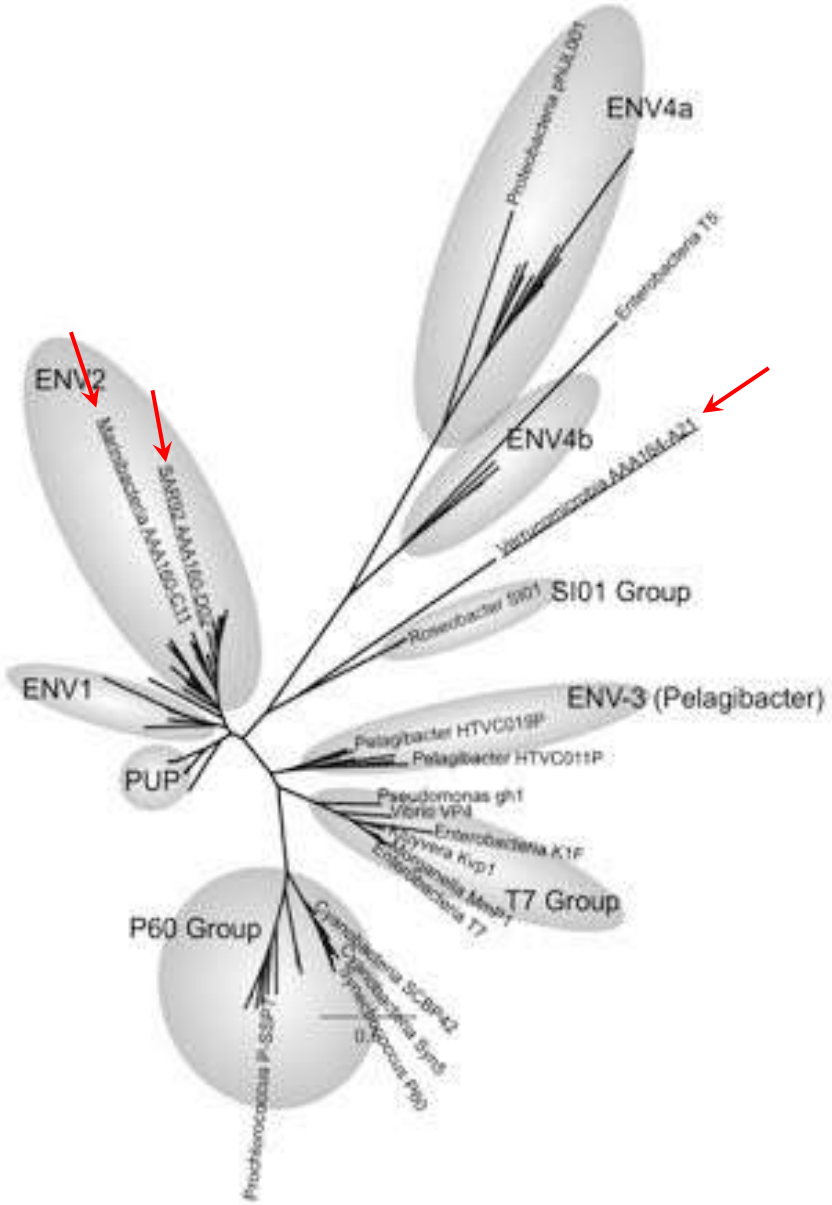
Viruses were also found in SAGs of Alphaproteobacteria, Gammaproteobacteria and Bacteroidetes

Complete genome recovery of 3 phages

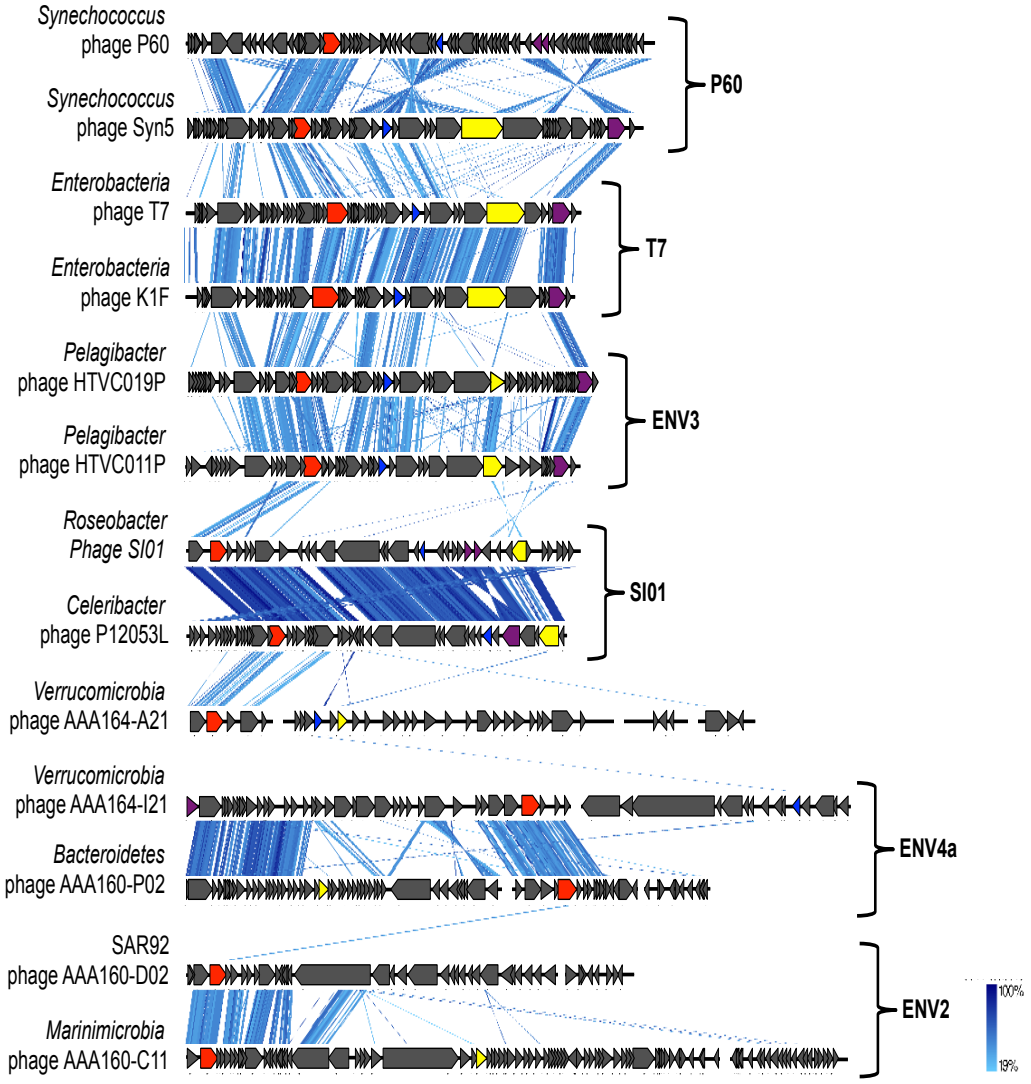
High fragment recruitment of viral metagenomic reads confirmed that most SAG-associated viruses are abundant in the ocean

Podoviridae phages from SAGs

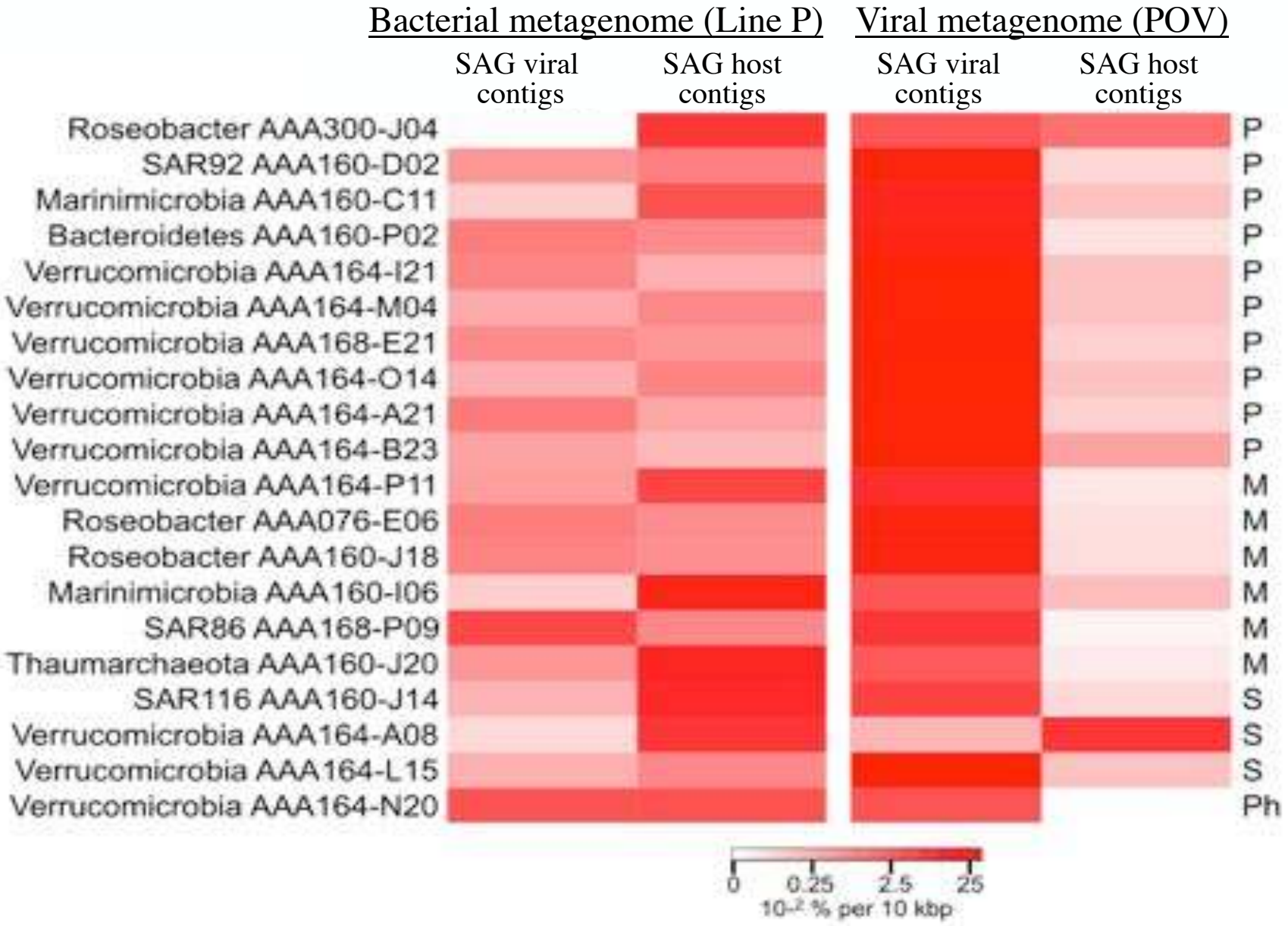
DNA polymerase A phylogeny



Genomic synteny



Metagenomic fragment recruitment from viral and cellular size fractions (70% nucleotide identity cutoff)



Possible sources of viral sequences in SAGs

1. **Lysogeny**

2. **Lytic infection**

3. **Chronic infection**

4. **Non-infectious attachment**

5. **Co-sort of a cell and a free viral particle**

Possible sources of viral sequences in SAGs

1. **Lysogeny**

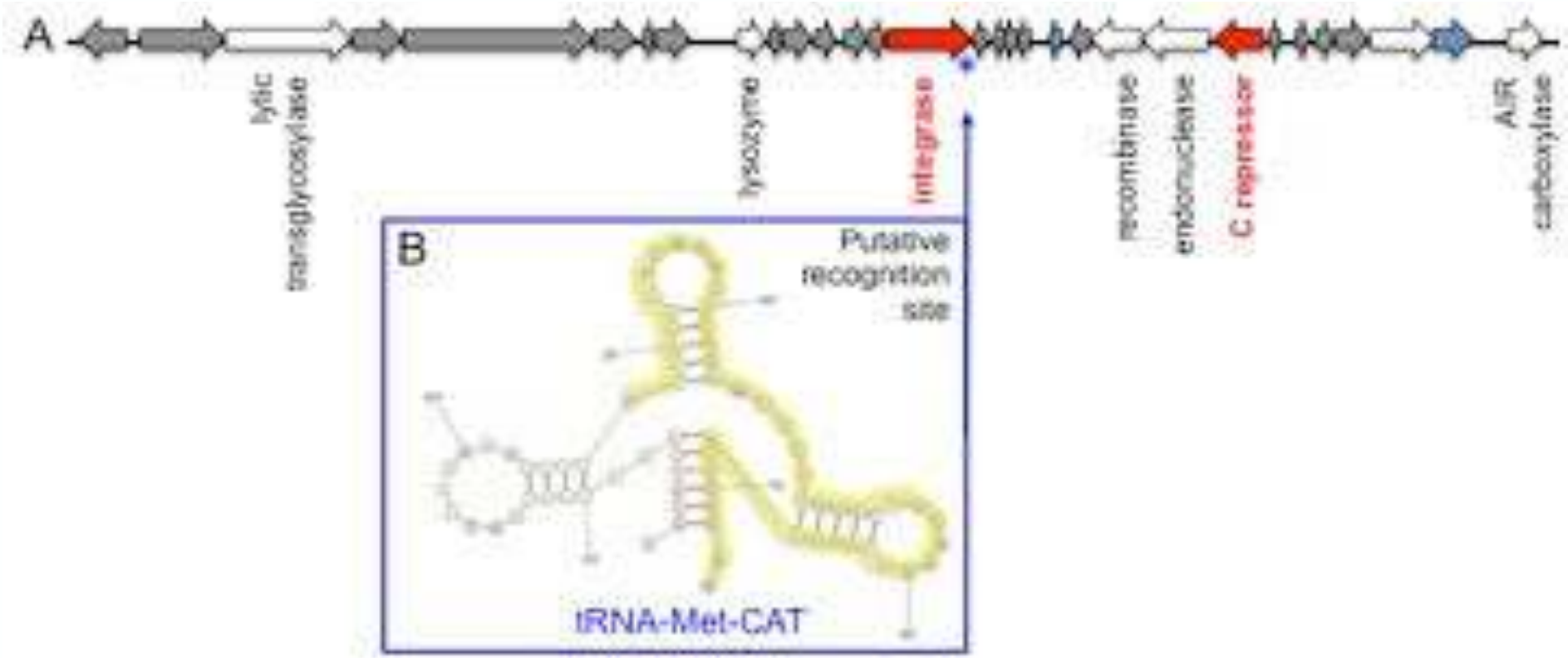
2. Lytic infection

3. Chronic infection

4. Non-infectious attachment

5. Co-sort of a cell and a free viral particle

Potentially lysogenic phage in a SAR116 SAG



Possible sources of viral sequences in SAGs

1 Lysogeny

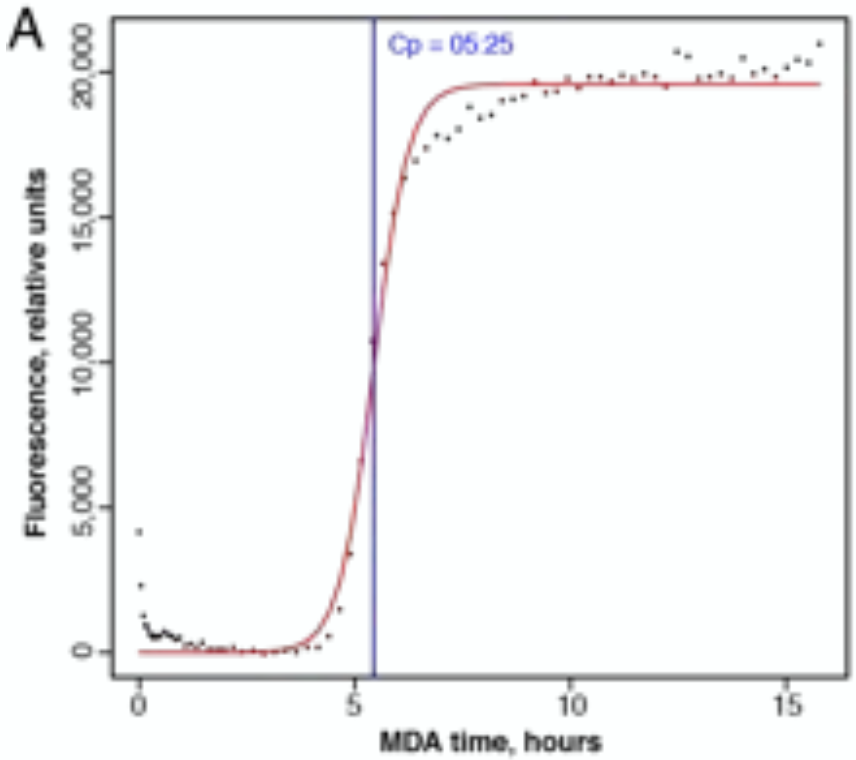
2 **Lytic infection**

3 Chronic infection

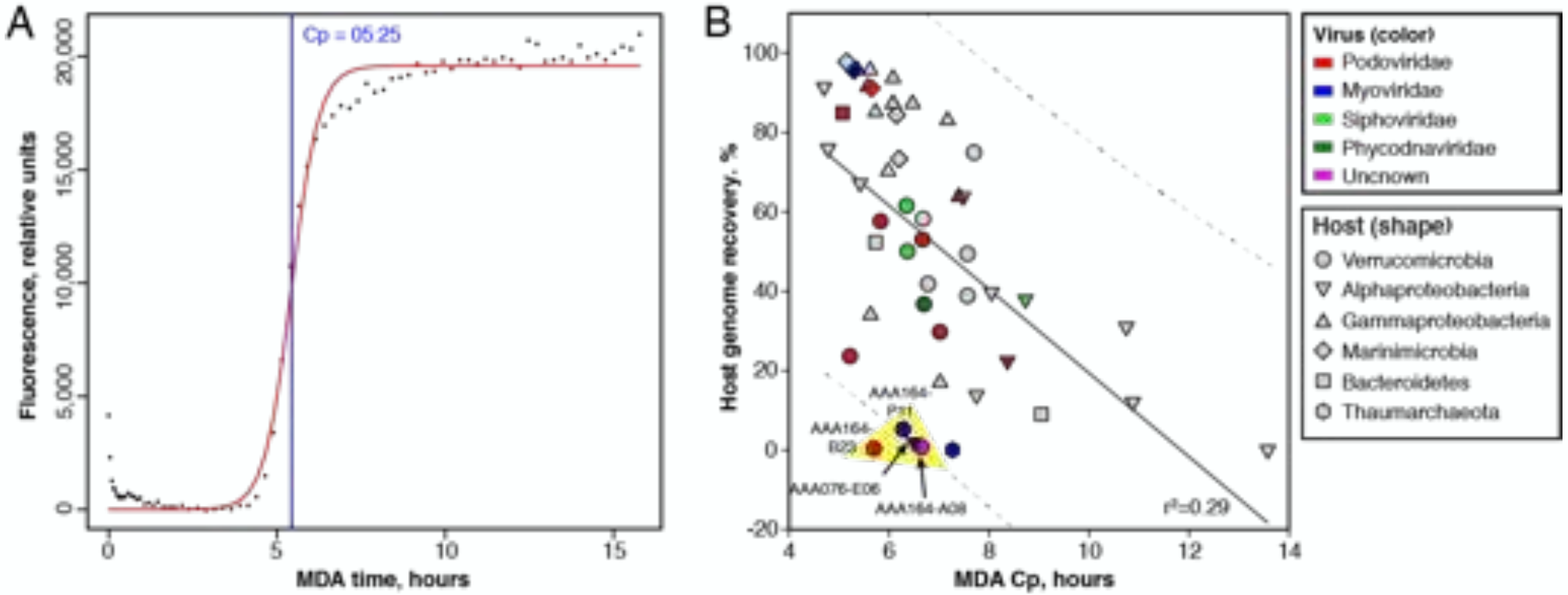
4 Non-infectious attachment

5 Co-sort of a cell and a free viral particle

Correlation between MDA Cp and genome recovery



Correlation between MDA Cp and genome recovery



Possible sources of viral sequences in SAGs

1 Lysogeny

2 Lytic infection

3 **Chronic infection**

4 Non-infectious attachment

5 Co-sort of a cell and a free viral particle

Possible sources of viral sequences in SAGs

1 Lysogeny

2 Lytic infection

3 Chronic infection

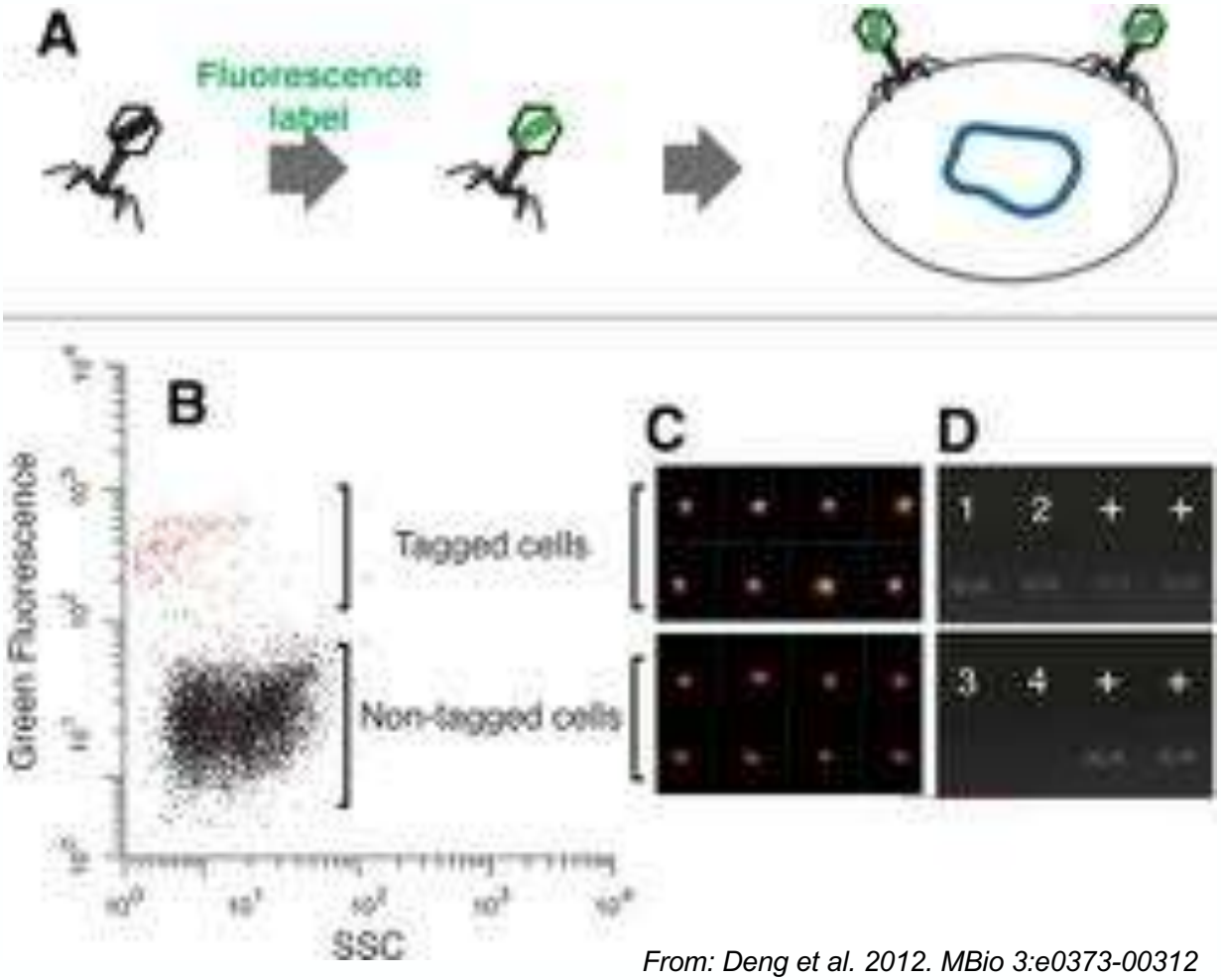
4 **Non-infectious attachment**

5 Co-sort of a cell and a free viral particle

Potential for non-infectious viral attachment to cell

This process is poorly understood

Deng et al. (2012) found that non-specific attachment is rare and that most attachment is infectious

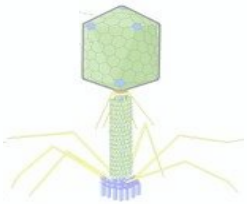


From: Deng et al. 2012. MBio 3:e0373-00312

19 out of 57 SAGs (33%) contained viral sequences

10 *Podoviridae* phages
- Marinimicrobia SAR406
- Verrucomicrobia (5)
- Gammaproteobacteria SAR92
- Bacteroidetes
- Roseobacter

5 *Myoviridae* phages
- Verrucomicrobia
- Roseobacter
- Marine Group I crenarchaeon
- Marinimicrobia SAR406
- SAR86



3 *Siphoviridae* phages
- SAR116
- Verrucomicrobia
- Flavobacteria

1 *Phycodnaviridae* virus
- Verrucomicrobia

First known viruses of Thaumarchaeota, Marinimicrobia, Verrucomicrobia and Gammaproteobacteria clusters SAR86 42 and SAR92

Viruses were also found in SAGs of Alphaproteobacteria and Bacteroidetes

Complete genome recovery of 3 phages

High fragment recruitment of viral metagenomic reads confirmed that most SAG-associated viruses are abundant in the ocean

Possible sources of viral sequences in SAGs

1 Lysogeny

2 Lytic infection

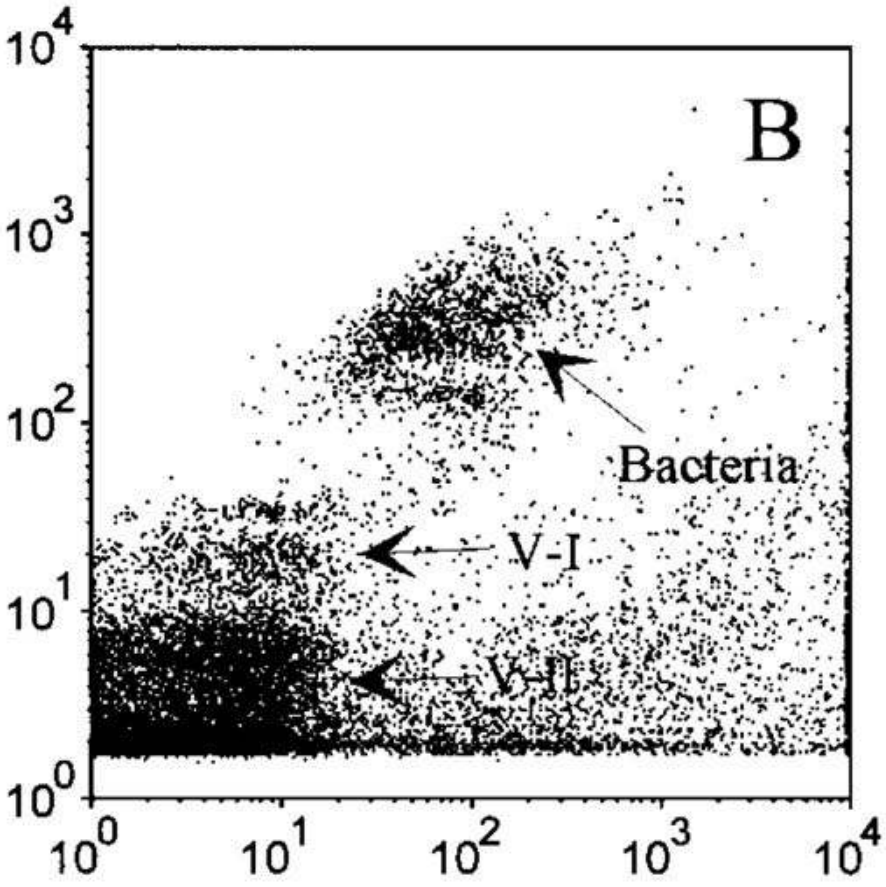
3 Chronic infection

4 Non-infectious attachment

5 **Co-sort of a cell and a free viral particle**

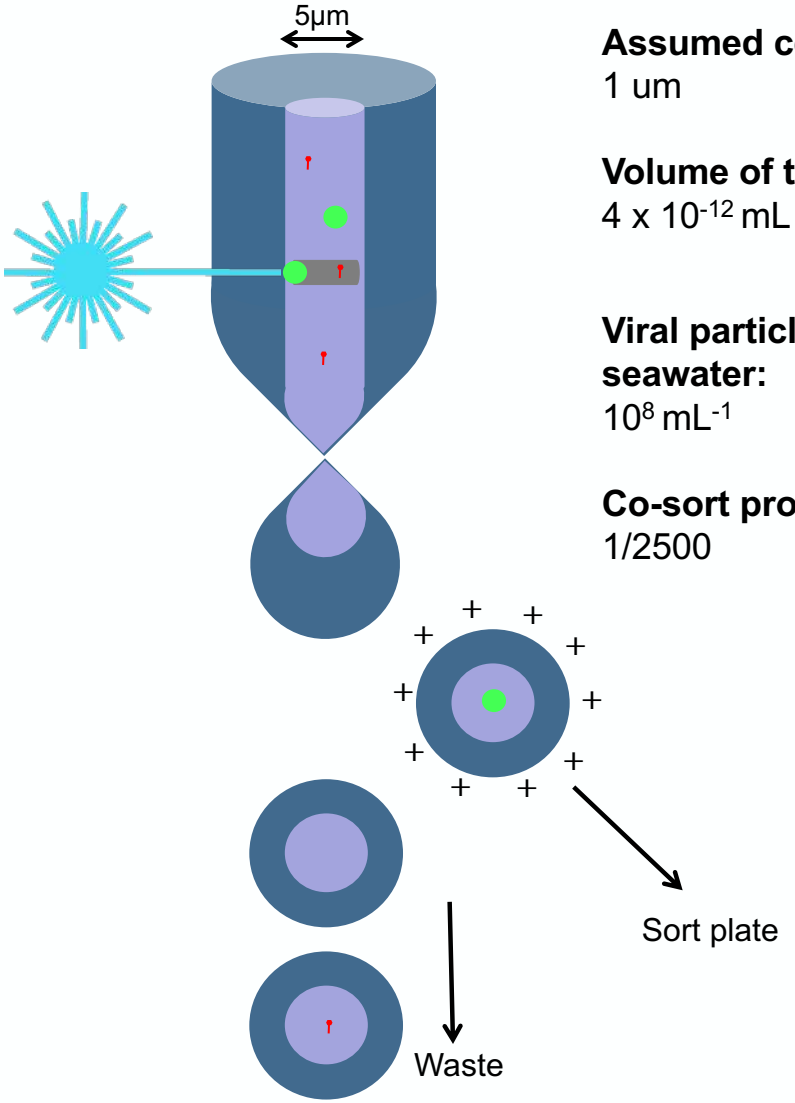
Risk of co-sorting cells free viral particles

Make viral particles visible to the instrument



From Marie et al. 1999

Use single-drop sort mode



Assumed cell diameter:
1 um

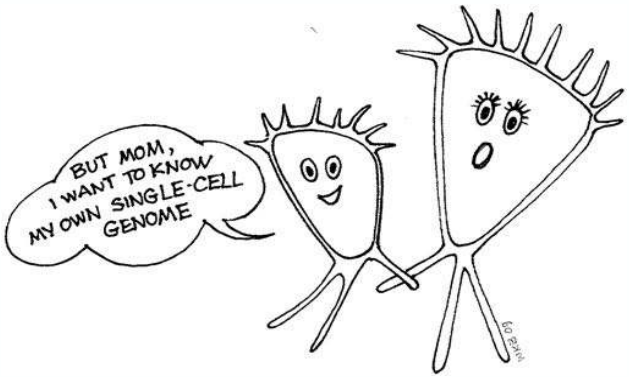
Volume of the shadow:
4×10^{-12} mL

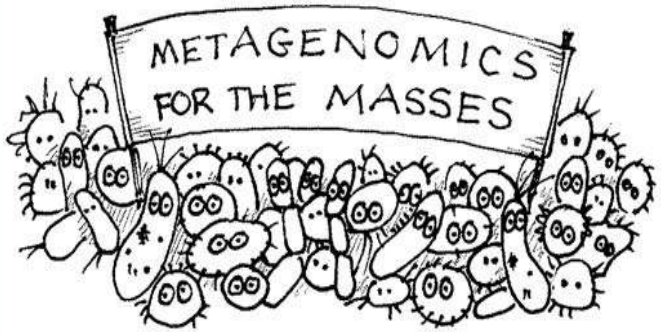
Viral particles in 1:10 dilute seawater:
10^8 mL^{-1}

Co-sort probability:
1/2500

Advantages of single cell genomics

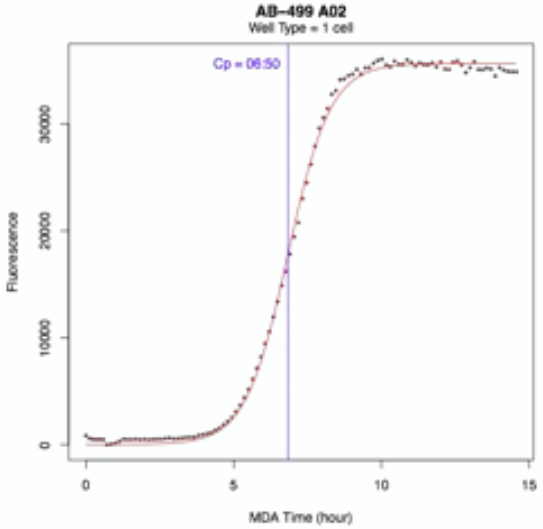
- Independent of cultivation
- Independent of unverified microevolutionary assumptions
- Compatible with high microbial community complexity
- Embraces intracellular genetic complexity
- Requires minimal field sample quantities
- Can be integrated with single cell phenomics





Whole genome amplification kinetics

Individual reaction kinetics

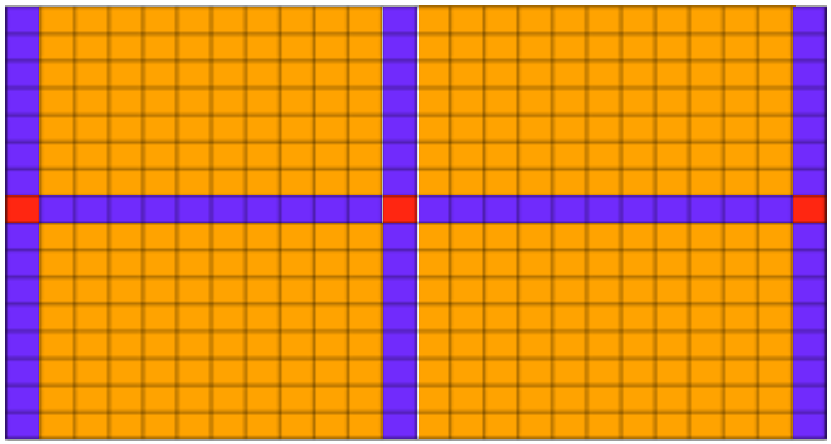


384-well plate layout

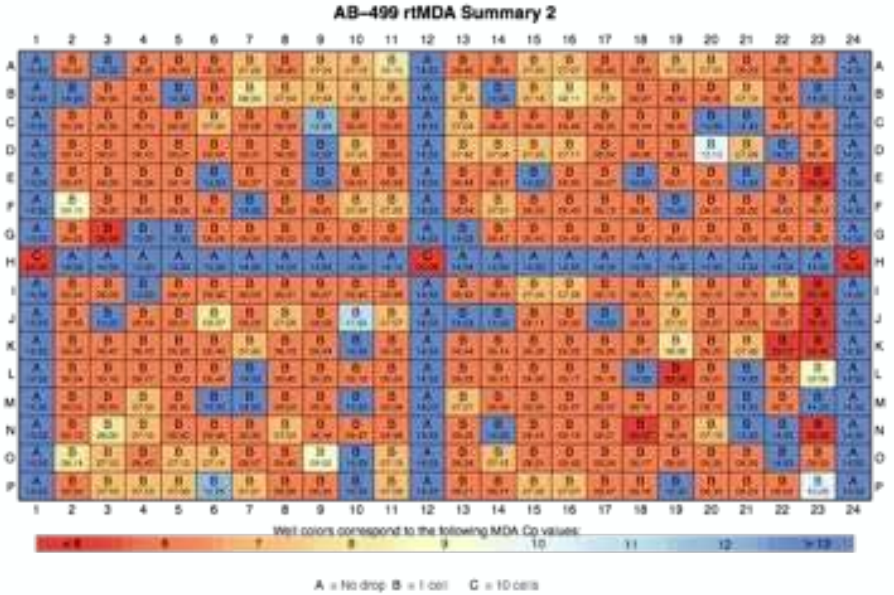
Negative controls: empty wells

Positive controls: 10 cells per well

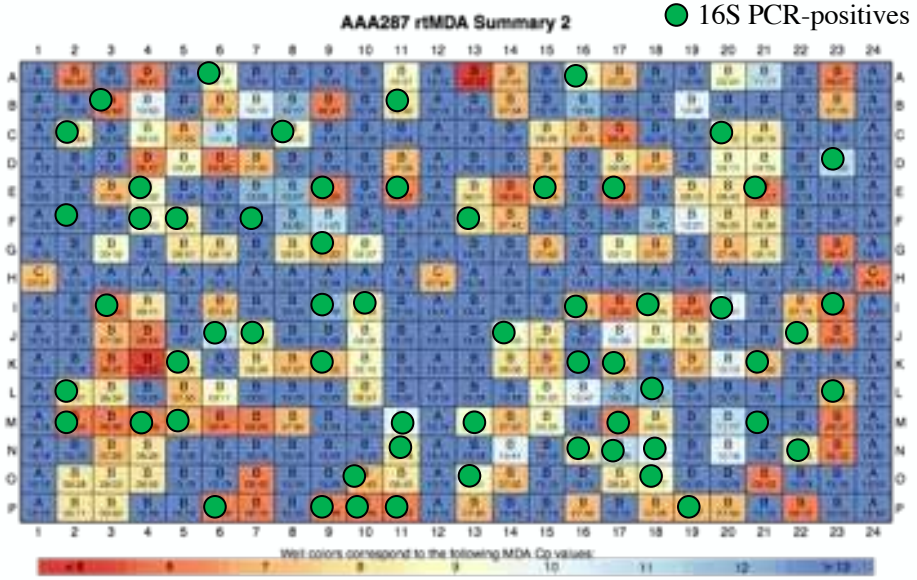
Wells containing single cells



Real data: *Tetraselmis* culture



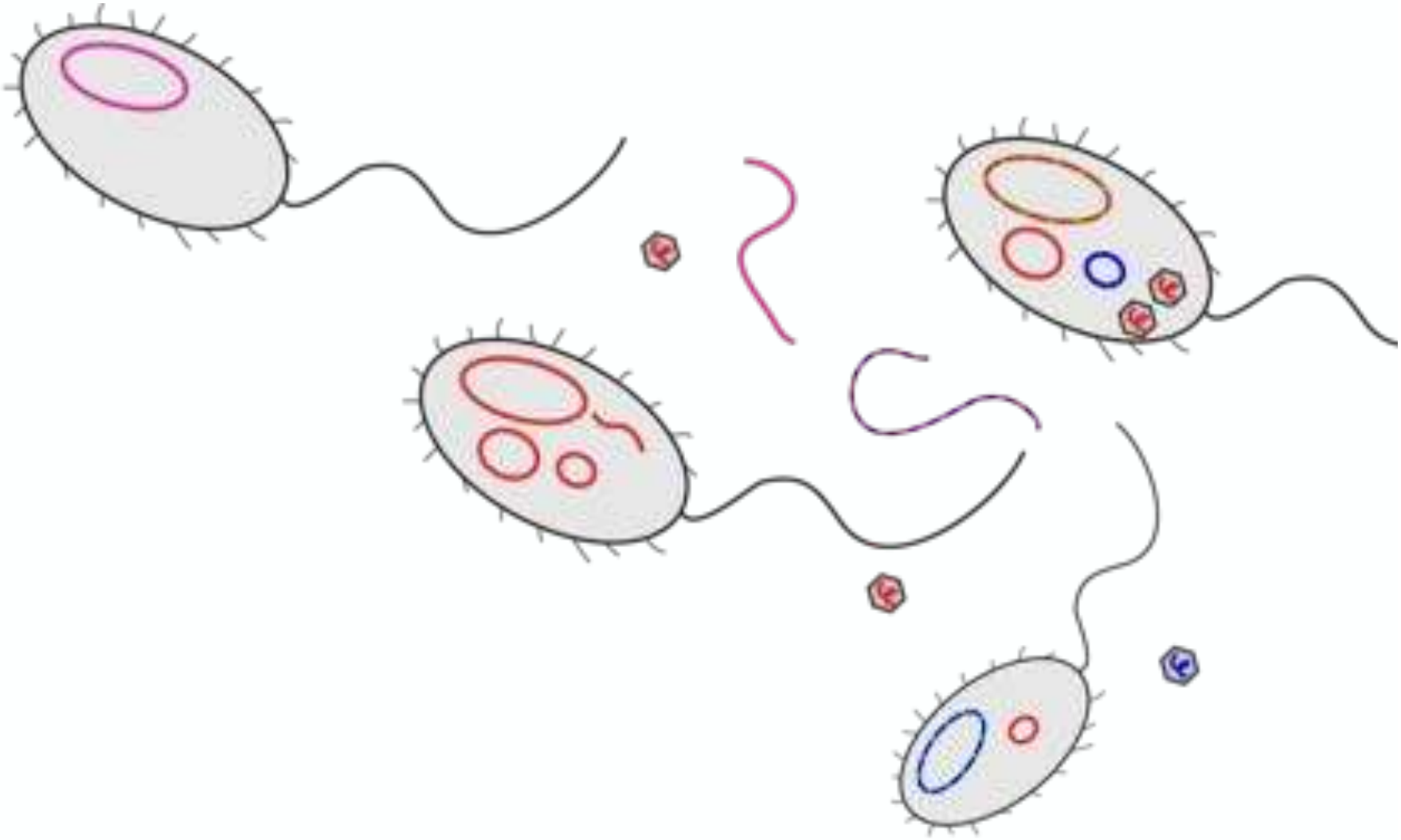
Real data: marine prokaryotes from 3000 m. depth



Propensity to microbial composition biases

Bias source	16S Tags	Shotgun metagenomics	Single cell genomics
Filter type/ sort gate	*Sometimes*	*Sometimes*	*Sometimes*
gDNA extraction	**High**	**High**	**High**
gDNA amplification	*Sometimes*	*Sometimes*	*Sometimes*
Targeted PCR: primer mismatches, inserts, secondary structures, multiple gene copies	**High**	*No*	*Low or No*
DNA sequencing	**High**	**High**	*Low*
Reference databases	*Low*	**High**	*Low*

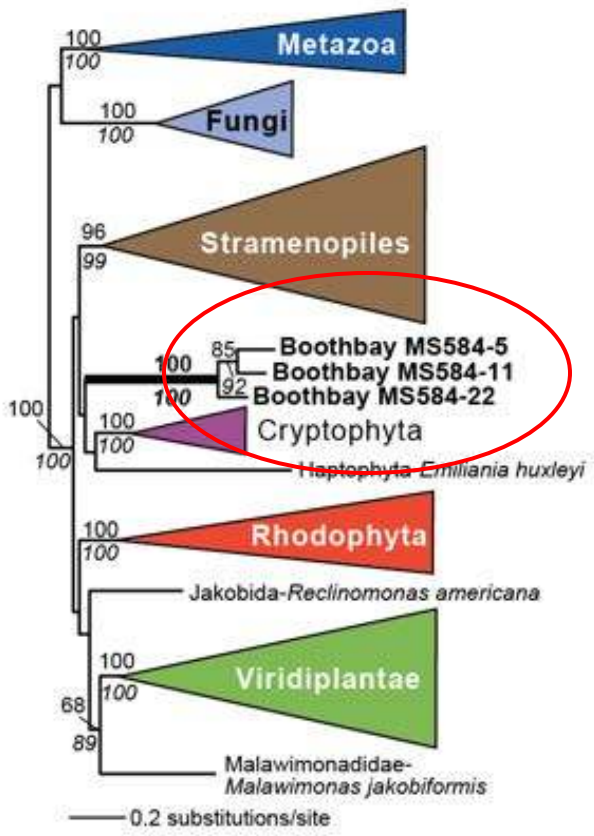
What do the various techniques tell about microbiomes?



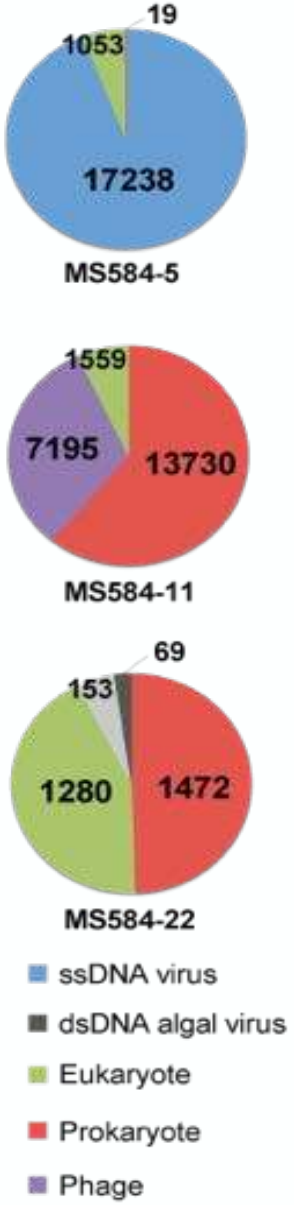
Single cell metagenomics of Picozoa

Yoon et al., *Science 2011*

MLS phylogeny



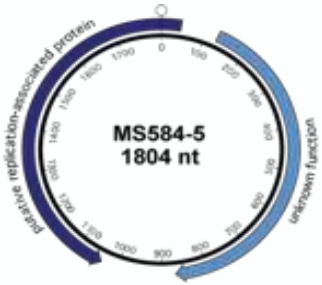
Shotgun read sources

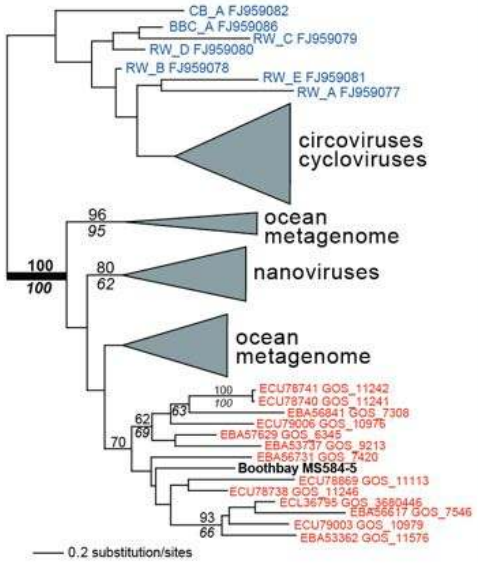


Lead PIs:

HS Yoon (Bigelow) and D Bhattacharya (Rutgers U)

Picozoa-infecting nanovirus





Sequenced three Picobiliphyta SAGs

No evidence for autotrophy

Feed on bacteria and large viruses

Novel, picobiliphyte-infecting nanovirus

OPEN ACCESS Freely available online

PLOS ONE

Picomonas judraskeda Gen. Et Sp. Nov.: The First Identified Member of the Picozoa Phylum Nov., a Widespread Group of Picoeukaryotes, Formerly Known as 'Picobiliphytes'

Ramkumar Seenivasan[1]*, Nicole Sausen[1], Linda K. Medlin[2], Michael Melkonian[1]

1 Department of Botany, Cologne Biocenter, University of Cologne, Cologne, Germany, 2 Marine Biological Association of the UK, The Laboratory, The Citadel, Plymouth, United Kingdom

Take-home message

- We are still only scratching the surface. Current methods provide only partial and biased information about the genomic composition of microbial communities (and multicellular organisms?)

- Reduced reliance on unverified assumptions is one of the key advantages of single cell genomics, as compared to other techniques

Bigelow Laboratory Single Cell Genomics Center

scgc.*bigelow.org*

SCGC
SINGLE CELL GENOMICS CENTER
Reading nature's genetic tales, one cell at a time

<u>Mission:</u> make single cell genomics accessible to the broad research community; serve as an engine for discoveries in microbial ecology, evolution, bioprospecting and human health.

- **First center of its kind, established 2009**
- **Diverse samples: aquatic, soil, organismal microbiomes, etc.**
- **>1,000,000 cells analyzed, representing >70 phyla**
- **60+ publications since 2011, 8 in *Science*, *Nature* & *PNAS***

Locations of SCGC customers

My lab in 2005

- **Rudimentary knowledge of genomics**
- **No sequencing capacity**
- **”Not ideal” buildings**
- **No resources for major technology development or facility setup**

What got SCGC working?

- **Placing research questions first**
- **Team of motivated, smart people**
- **Tons of advice and collaborations**
- **Method development, continuous since 2005, ~$3 M**
- **Laboratory and IT equipment, ~$3 M**
- **Core facility business model and implementation**
- **QC and benchmarking of entire workflow**
- **Proficiency in and integration of technologies:**
  - Fluorescence-activated cell sorting (FACS)
  - Whole genome amplification
  - Robotic liquid handling
  - Cleanroom environment
  - DNA sequencing
  - *De novo* assembly and other bioinformatics
  - Laboratory information management systems (LIMS)

What got SCGC working?

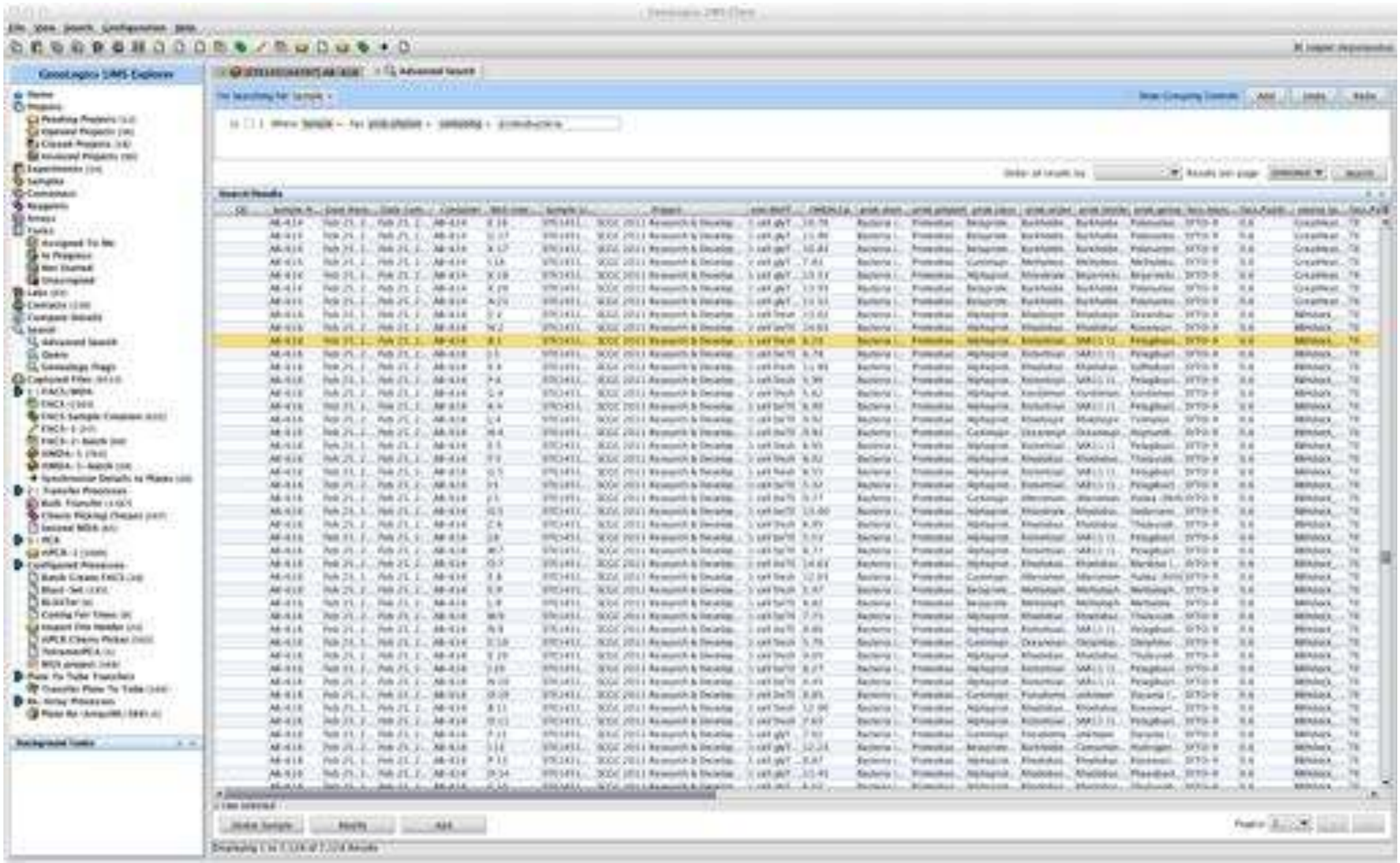
- **Placing research questions first**
- **Motivated, qualified team**
- **A lot of advice and collaborations**
- **Method development, continuous since 2005, ~$3 M**
- **Laboratory and IT equipment, ~$3 M**
- **Core facility business model and implementation**
- **QC and benchmarking of entire workflow**
- **Proficiency in and integration of technologies:**
  - Fluorescence-activated cell sorting (FACS)
  - Whole genome amplification
  - Robotic liquid handling
  - Cleanroom environment
  - DNA sequencing
  - *De novo* assembly and other bioinformatics
  - Laboratory information management systems (LIMS)

Bigelow | Laboratory for Ocean Sciences

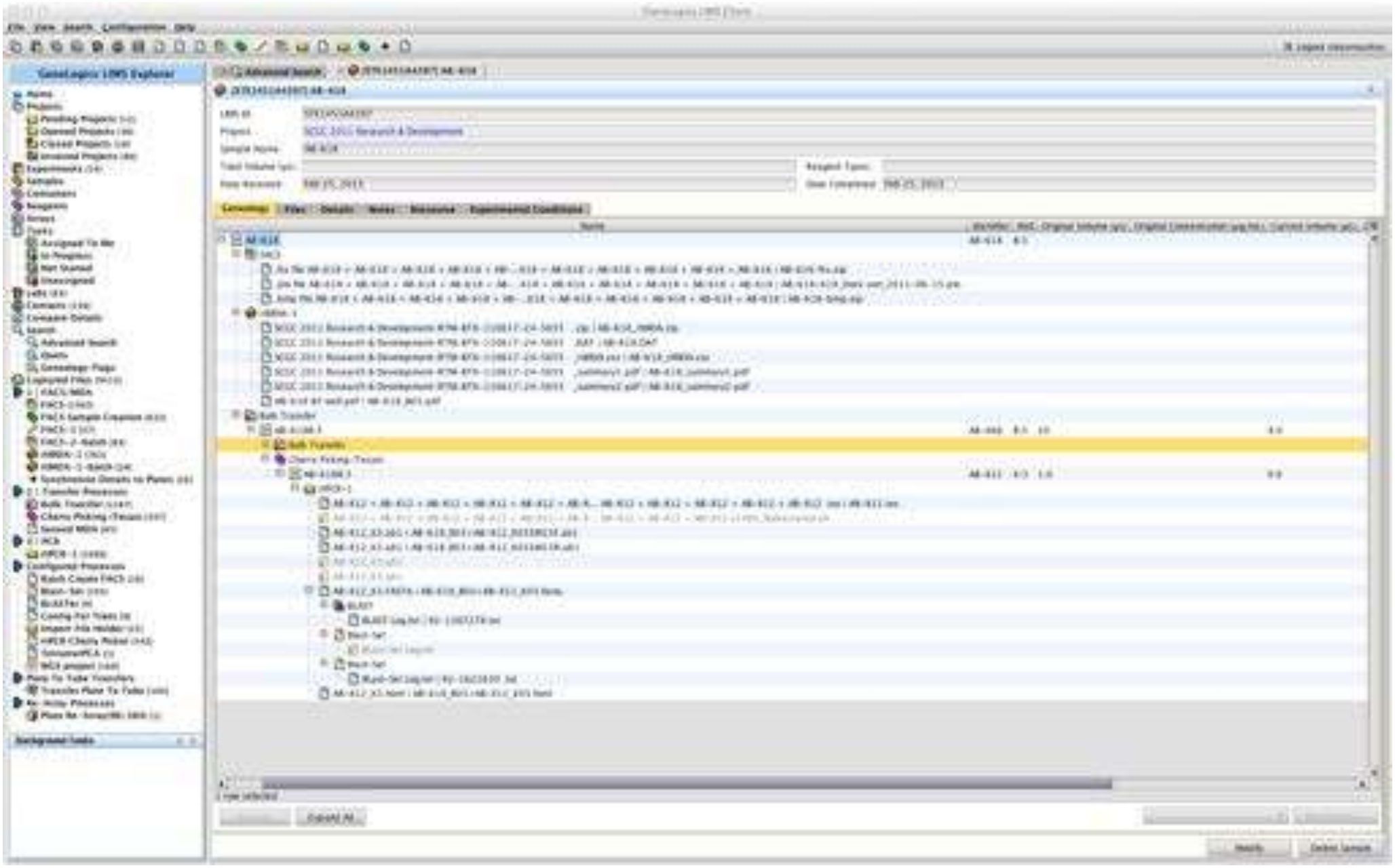
What got SCGC working?

- **Placing research questions first**
- **Motivated, qualified team**
- **A lot of advice and collaborations**
- **Method development, continuous since 2005, ~$3 M**
- **Laboratory and IT equipment, ~$3 M**
- **Core facility business model and implementation**
- **QC and benchmarking of entire workflow**
- **Proficiency in and integration of technologies:**
  - Fluorescence-activated cell sorting (FACS)
  - Whole genome amplification
  - Robotic liquid handling
  - Cleanroom environment
  - DNA sequencing
  - *De novo* assembly and other bioinformatics
  - Laboratory information management systems (LIMS)

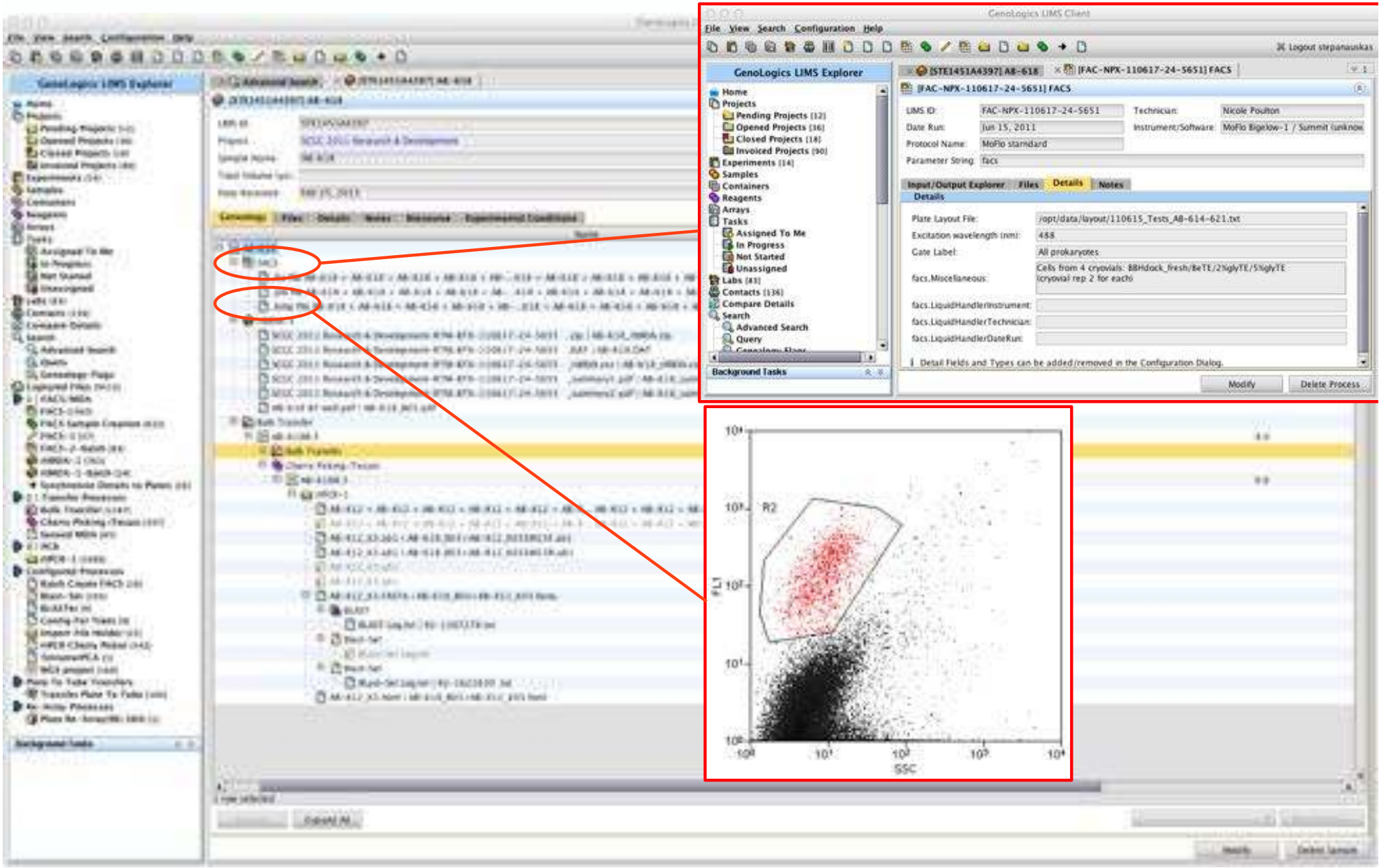
SCGC LIMS – taxonomy search



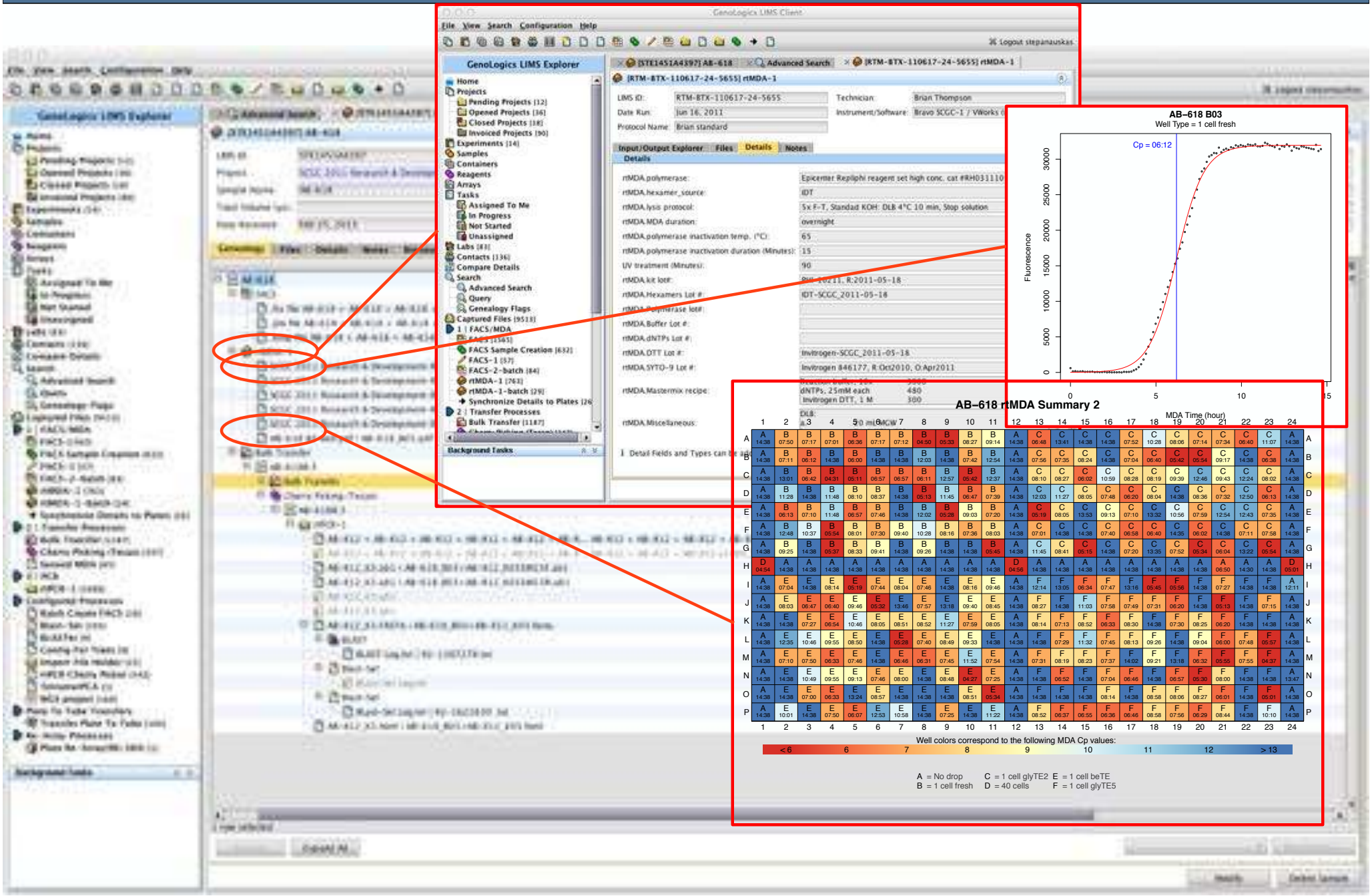
SCGC LIMS – genealogy of one SAG



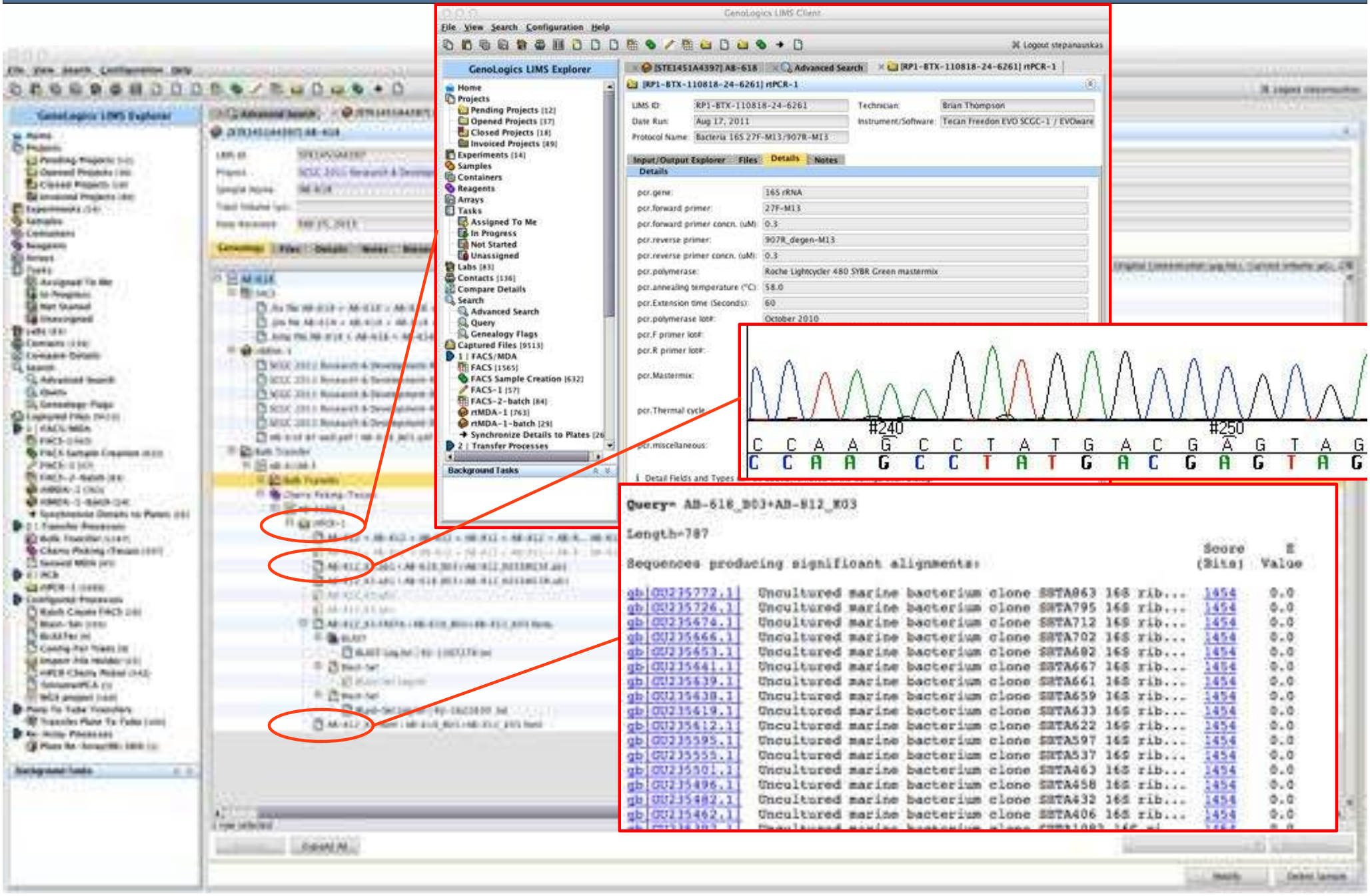
SCGC LIMS – FACS data



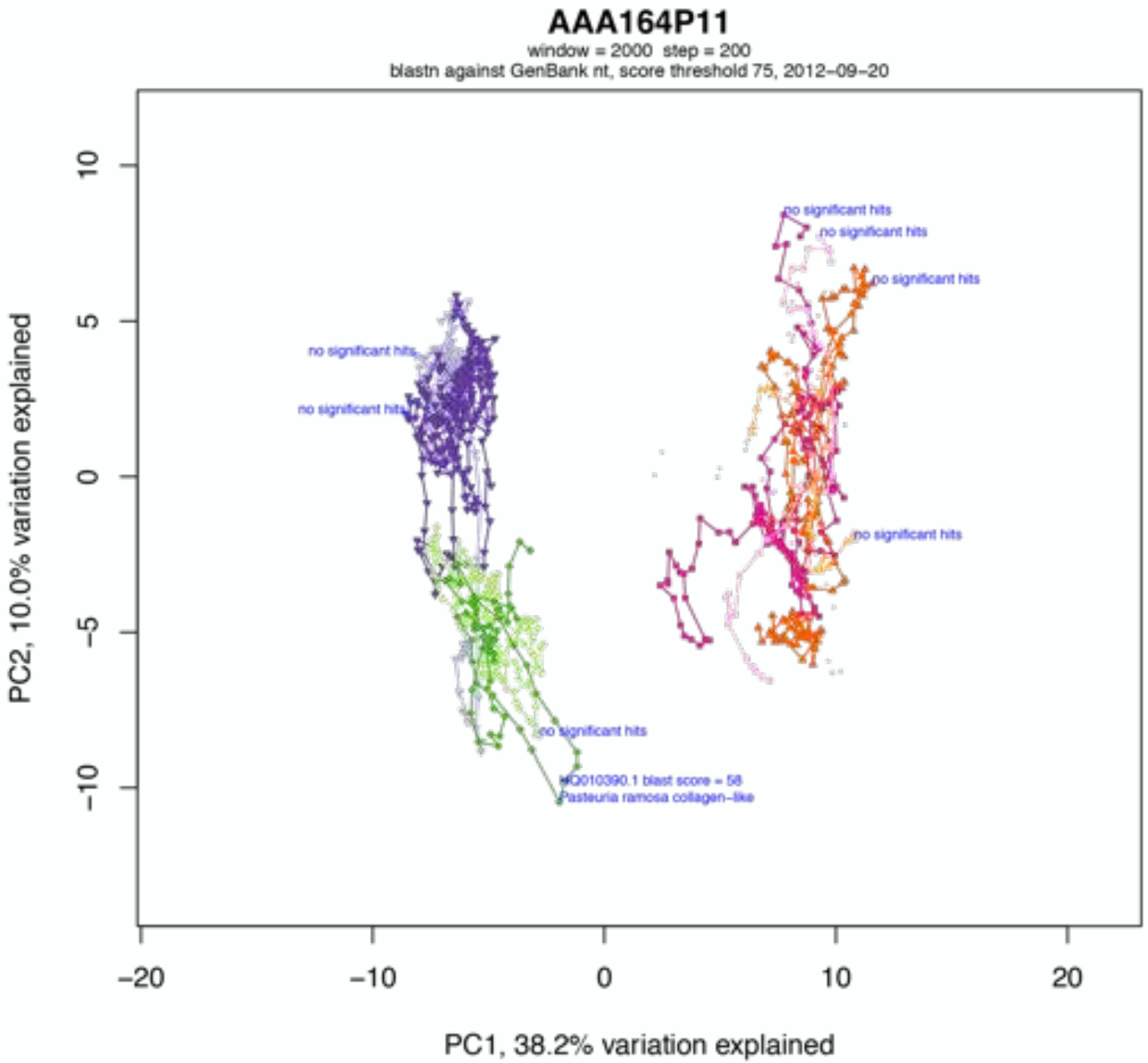
SCGC LIMS – MDA data



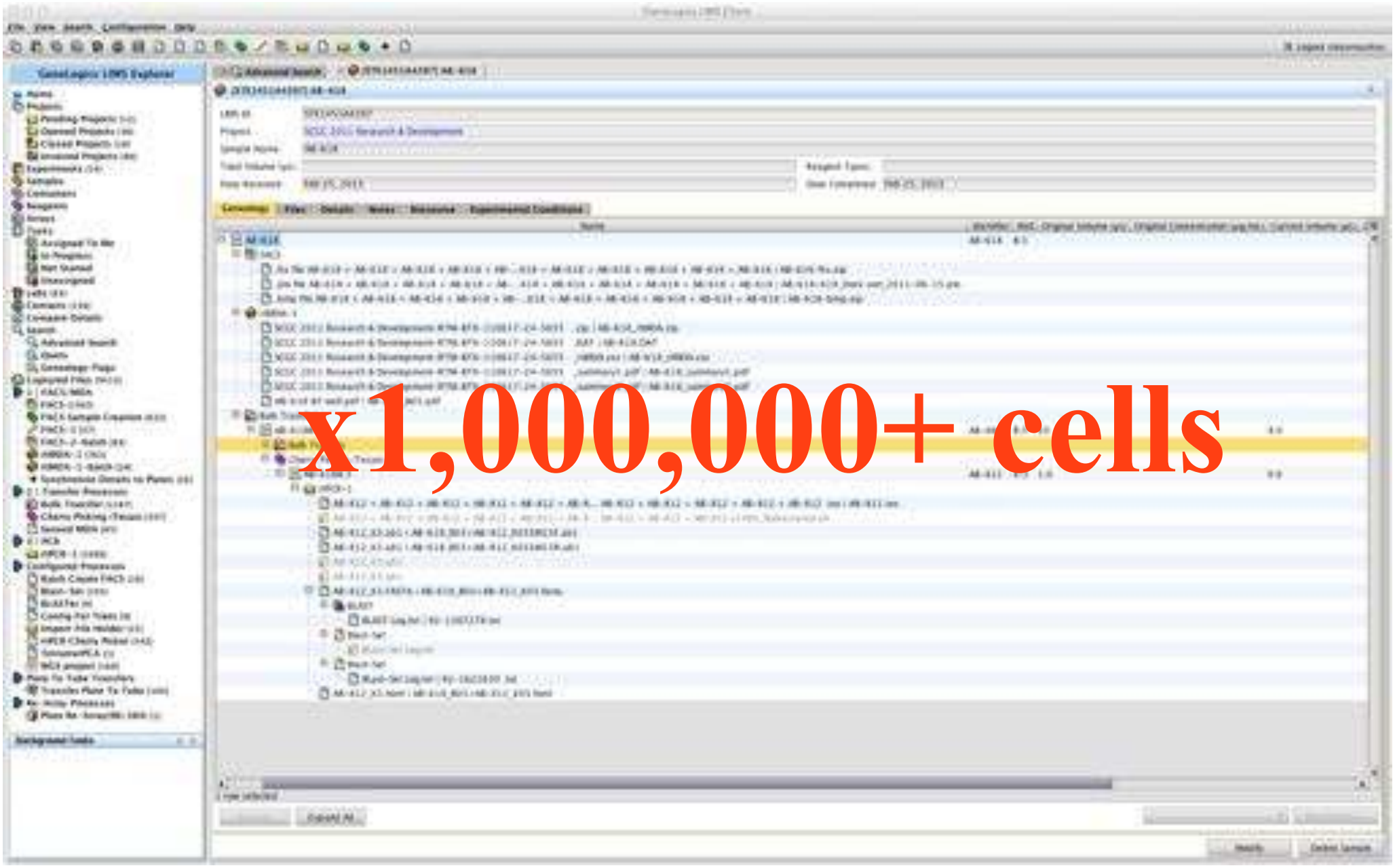
SCGC LIMS – SSU rRNA PCR-sequencing data



SCGC QC: Tetramer PCA



SCGC LIMS



What got SCGC working?

- **Placing research questions first**
- **Motivated, qualified team**
- **A lot of advice and collaborations**
- **Method development, continuous since 2005, ~$3 M**
- **Laboratory and IT equipment, ~$3 M**
- **Core facility business model and implementation**
- **QC and benchmarking of entire workflow**
- **Proficiency in and integration of technologies:**
  - Fluorescence-activated cell sorting (FACS)
  - Whole genome amplification
  - Robotic liquid handling
  - Cleanroom environment
  - DNA sequencing
  - *De novo* assembly and other bioinformatics
  - Laboratory information management systems (LIMS)

Benchmarking SAG assemblies (QUAST, 5 replicates)

Genome characteristics

Organism	Genome size, bp	GC, %
Prochlorooccus marinus	1,751,080	37
Escherichia coli	4,630,707	51
Meiothermus ruber	3,097,457	63

Standard QC metrics

Genome recovery, %	N50	# N's per 100 kbp
78	54,686	0
52	21,687	0
50	15,097	0

Assessment of assembly accuracy

Organism	# unaligned bases	# misassemblies	# local misassemblies	# mismatches per 100 kbp	# indels per 100 kbp
Prochlorooccus marinus	0	2	3	4	1
Escherichia coli	68	16	5	5	1
Meiothermus ruber	28	20	3	5	3

Assumptions:

1. Original assembly is correct.
2. No genetic changes since

Benefits:

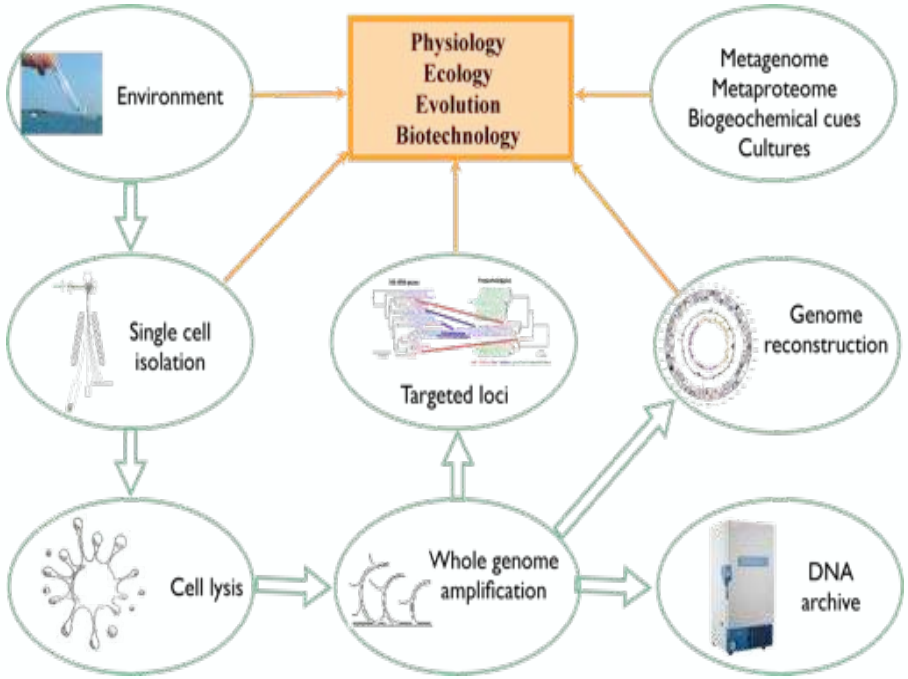
1. Accurate interpretation of results
2. Continued method improvements

Bigelow | Laboratory for Ocean Sciences

Factors that may impair SAG genome recovery

Technical:

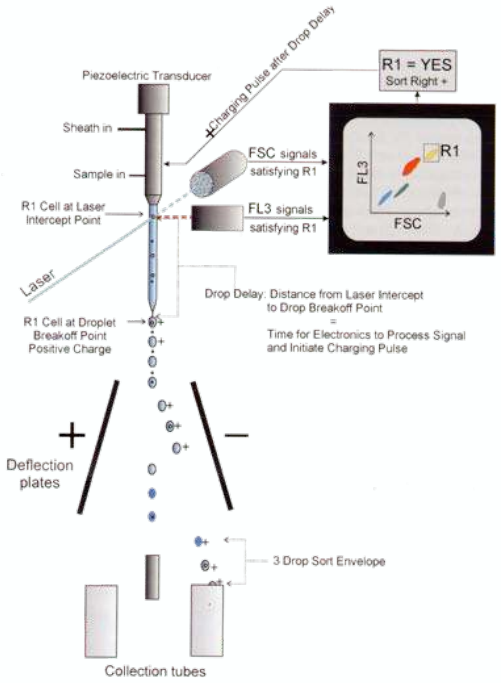
- DNA degradation during storage/shipping
- Failure to deposit a cell into a well
- Failure to lyse the cell
- Failure to denature DNA
- Uneven WGA
- Sequencing artifacts
- Assembly artifacts
- Pipetting error at any lab step
- Computational error



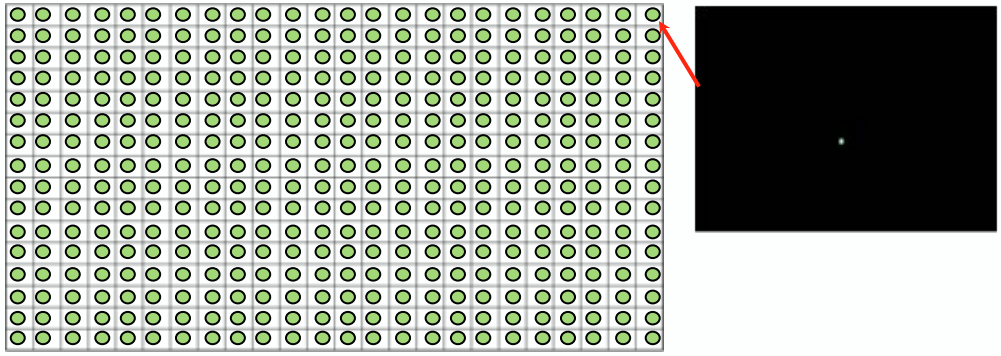
Biological:

- DNA leaked from a mechanically damaged cell
- Cell is in a dormant state
- Host DNA was degraded by a lytic phage
- Polymerase was hijacked by small, circular plasmids
- DNA was fragmented, e.g. due to desiccation
- DNA was bound to proteins and/or other molecules, e.g. due to desiccation
- DNA was protected by intracellular compartmentalization

SCGC QC: Cell sorting



Microscopy of sorted fluorescent beads



<2% wells contain no bead

<0.4% wells contain more than one bead

Contamination prevention:

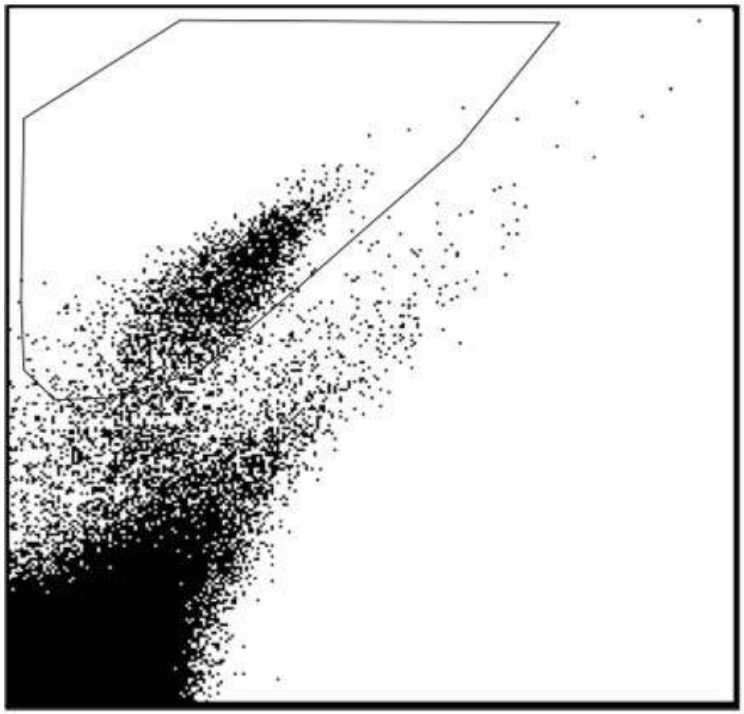
- HEPA-filtered air, cleanroom techniques
- Decontamination of all reagents
- Single-drop sort mode
- Careful drop delay and sort alignment
- Negative controls on each sort plate

Small volumes is the key!

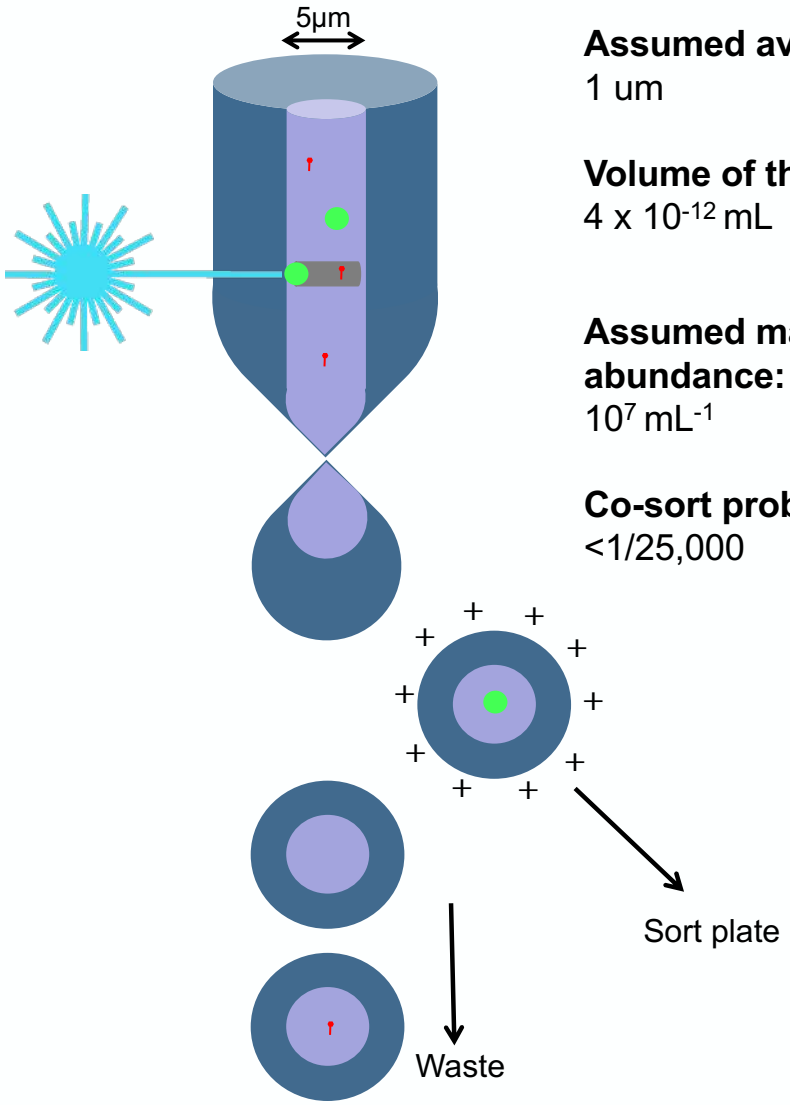
Pressure differential	Ratio of V_s to V_d (R)	Sample stream diameter (µm)	Sample droplet volume, V_s (pL)	ESD of V_s (µm)
0.2	3.13×10^{-3}	5.6	8.7	25.9
0.4	4.98×10^{-3}	7.0	13.9	30.3
0.6	7.93×10^{-3}	8.9	22.1	35.3
0.8	9.71×10^{-3}	9.8	27.0	37.8

Preventing particle co-sort

Maximize detection sensitivity



Use single-drop FACS mode



Assumed average diameter:
1 um

Volume of the shadow:
4×10^{-12} mL

Assumed max particle abundance:
10^{7} mL^{-1}

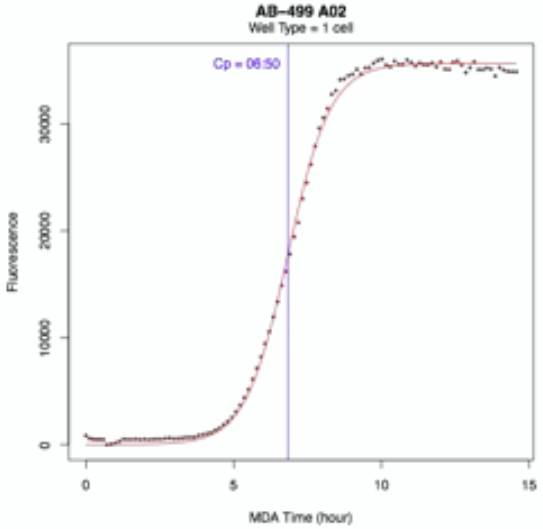
Co-sort probability:
<1/25,000

Generate small droplets

Pressure differential	Sample stream diameter (µm)	Sample droplet volume, V_s (pL)
0.2	5.6	8.7
0.4	7.0	13.9
0.6	8.9	22.1
0.8	9.8	27.0

Whole genome amplification kinetics

Individual reaction kinetics

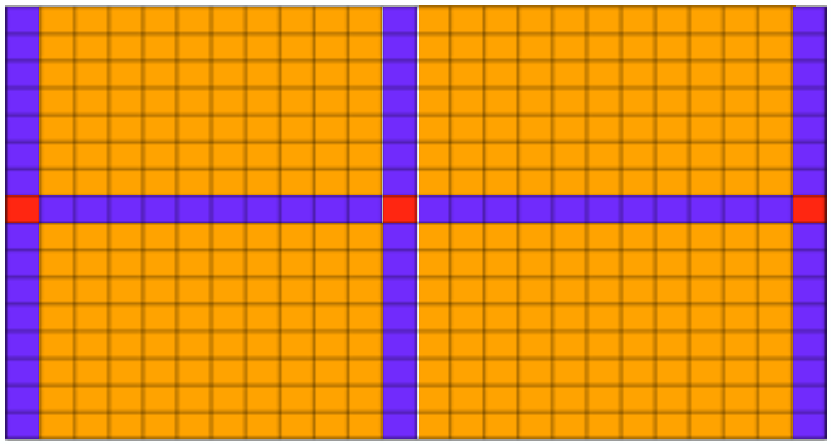


384-well plate layout

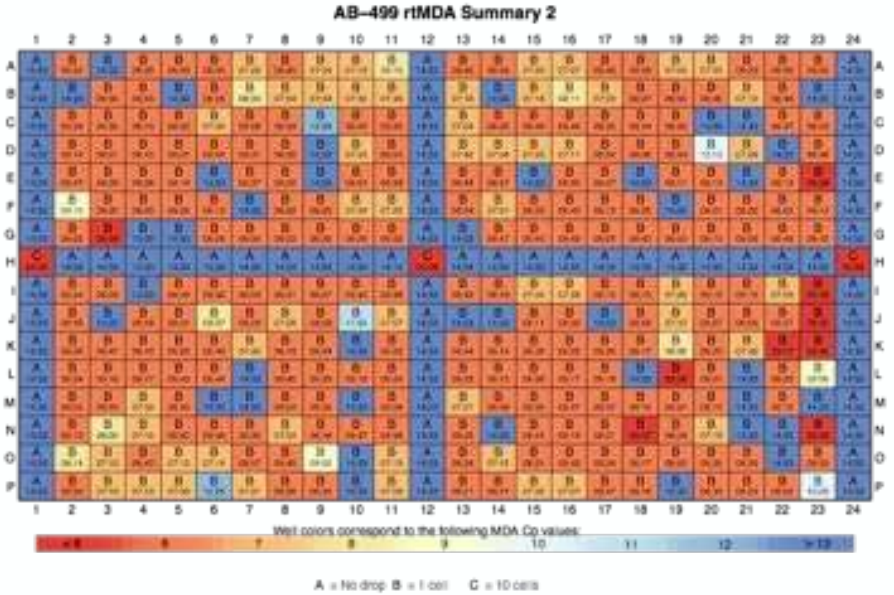
Negative controls: empty wells

Positive controls: 10 cells per well

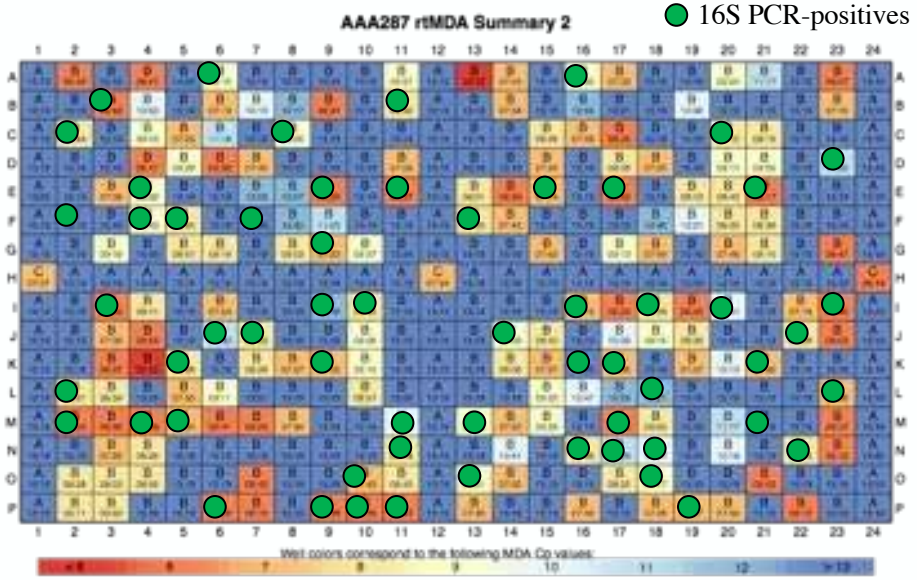
Wells containing single cells



Real data: *Tetraselmis* culture

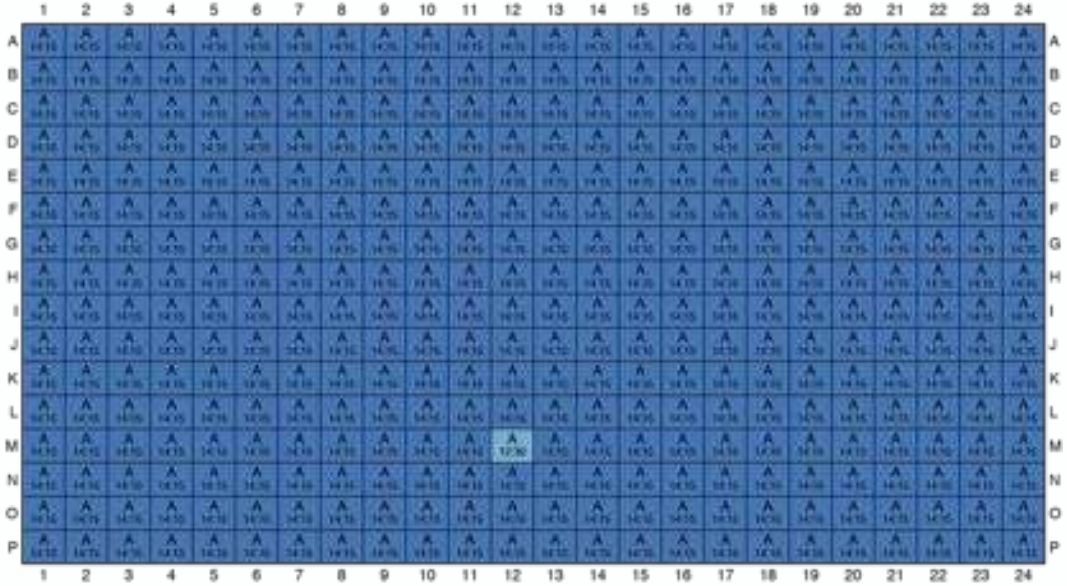


Real data: marine prokaryotes from 3000 m. depth

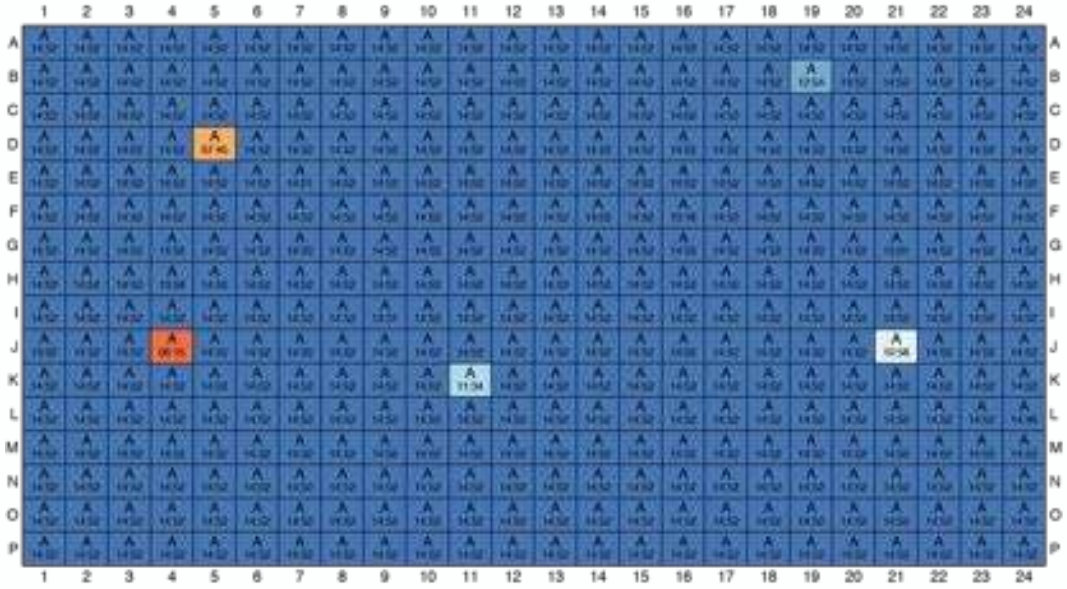


QC of lab consumables

Clean batch of robotic pipetting tips



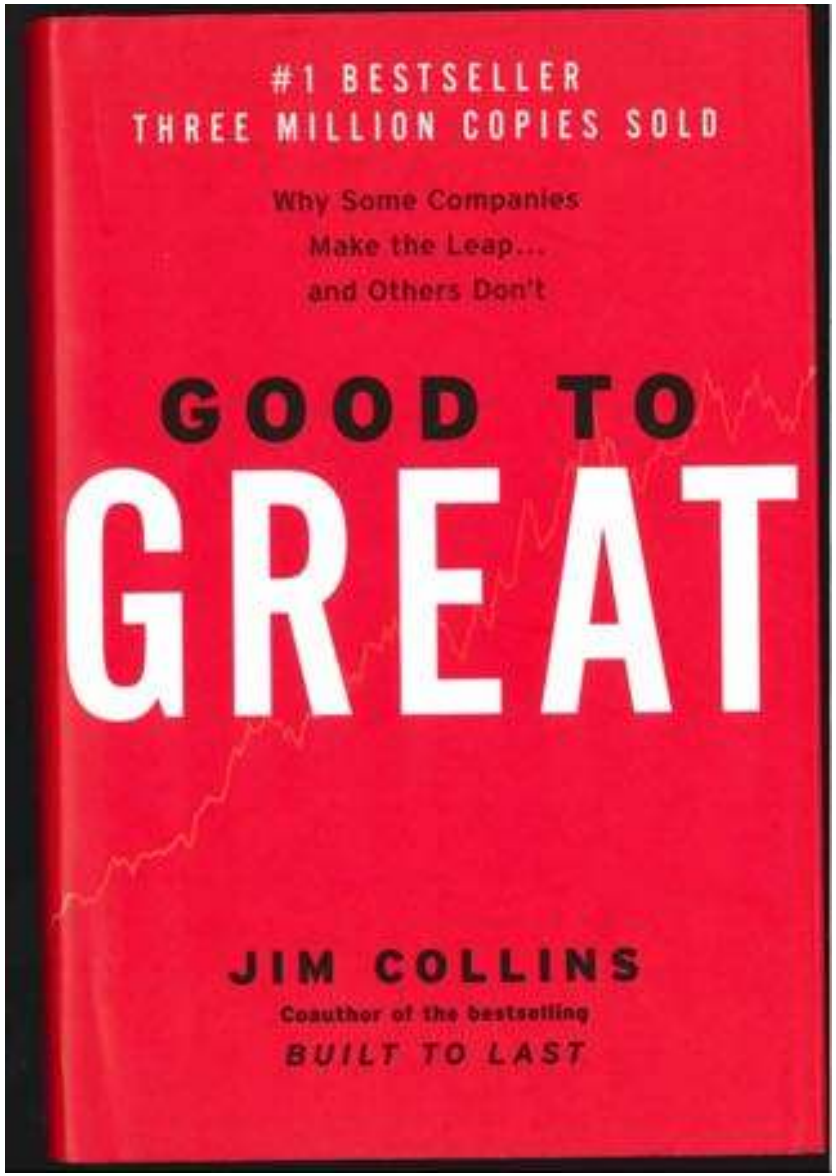
DNA-contaminated batch of tips

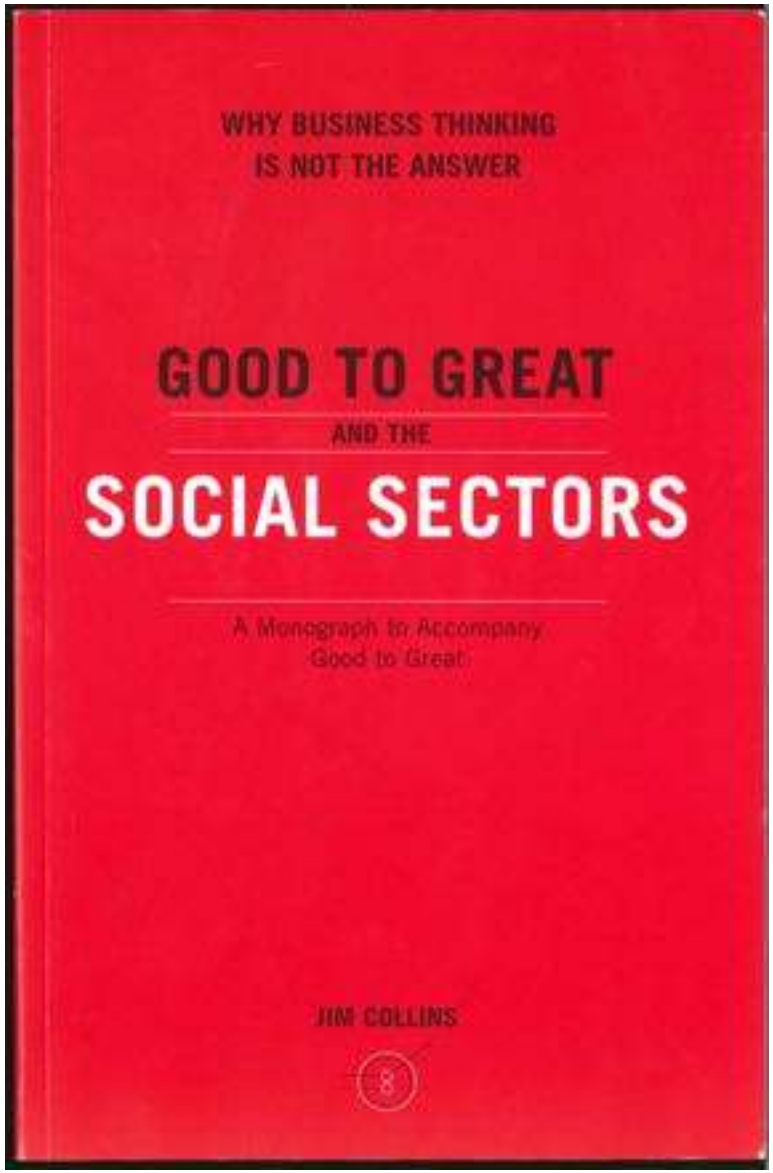


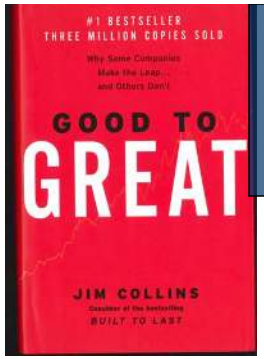
What got SCGC working?

- **Placing research questions first**
- **Motivated, qualified team**
- **A lot of advice and collaborations**
- **Method development, continuous since 2005, ~$3 M**
- **Laboratory and IT equipment, ~$3 M**
- **Core facility business model and implementation**
- **QC and benchmarking of entire workflow**
- **Proficiency in and integration of technologies:**
  - Fluorescence-activated cell sorting (FACS)
  - Whole genome amplification
  - Robotic liquid handling
  - Cleanroom environment
  - DNA sequencing
  - *De novo* assembly and other bioinformatics
  - Laboratory information management systems (LIMS)

Excellent primers in management

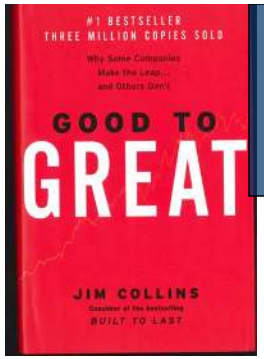




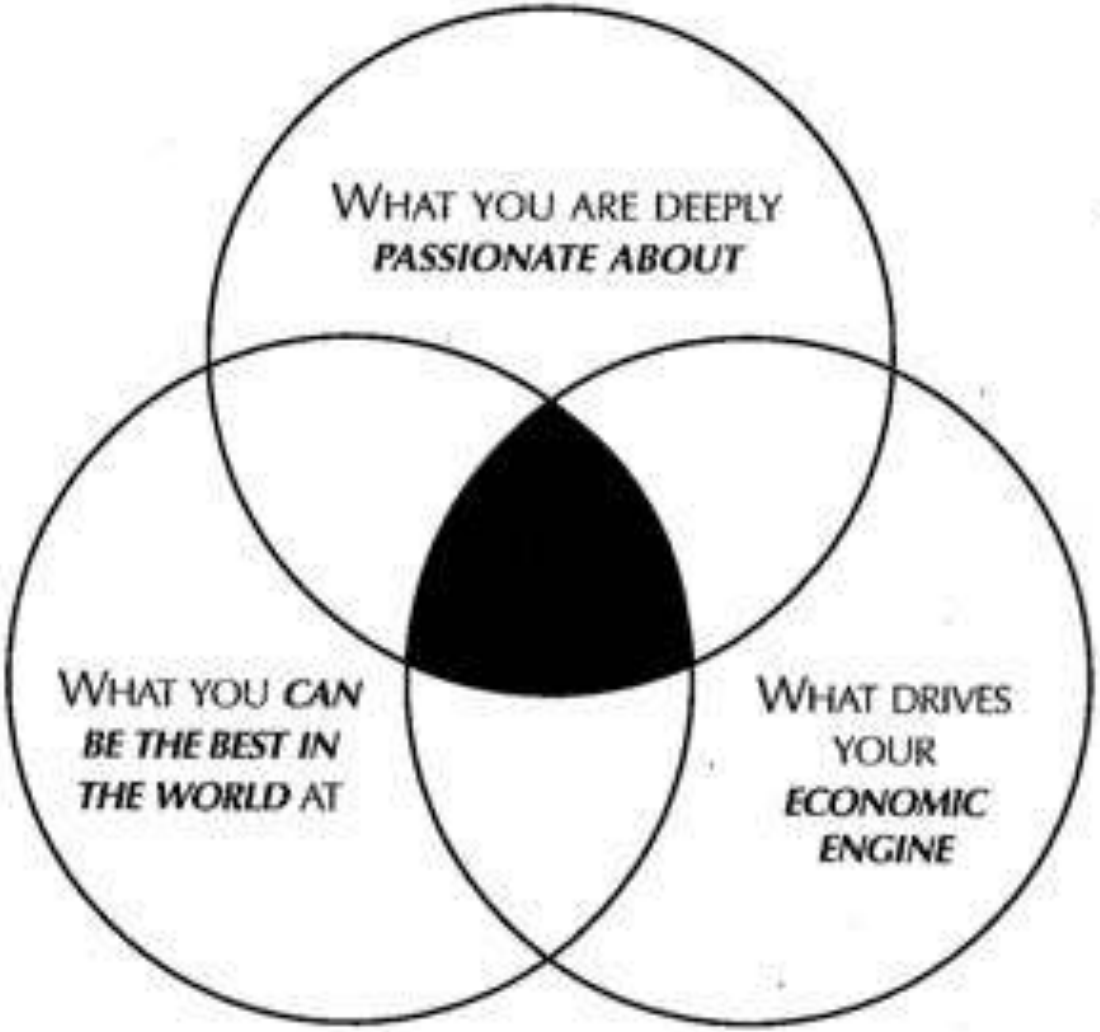


Technology does not come first

When used right, technology becomes an *accelerator* of momentum, not a creator of it. The good-to-great companies never began their transitions with pioneering technology, for the simple reason that you cannot make good use of technology until you know which technologies are relevant. And which are those? Those—and *only* those—that link directly to the three intersecting circles of the Hedgehog Concept.



The Hedgehog Concept

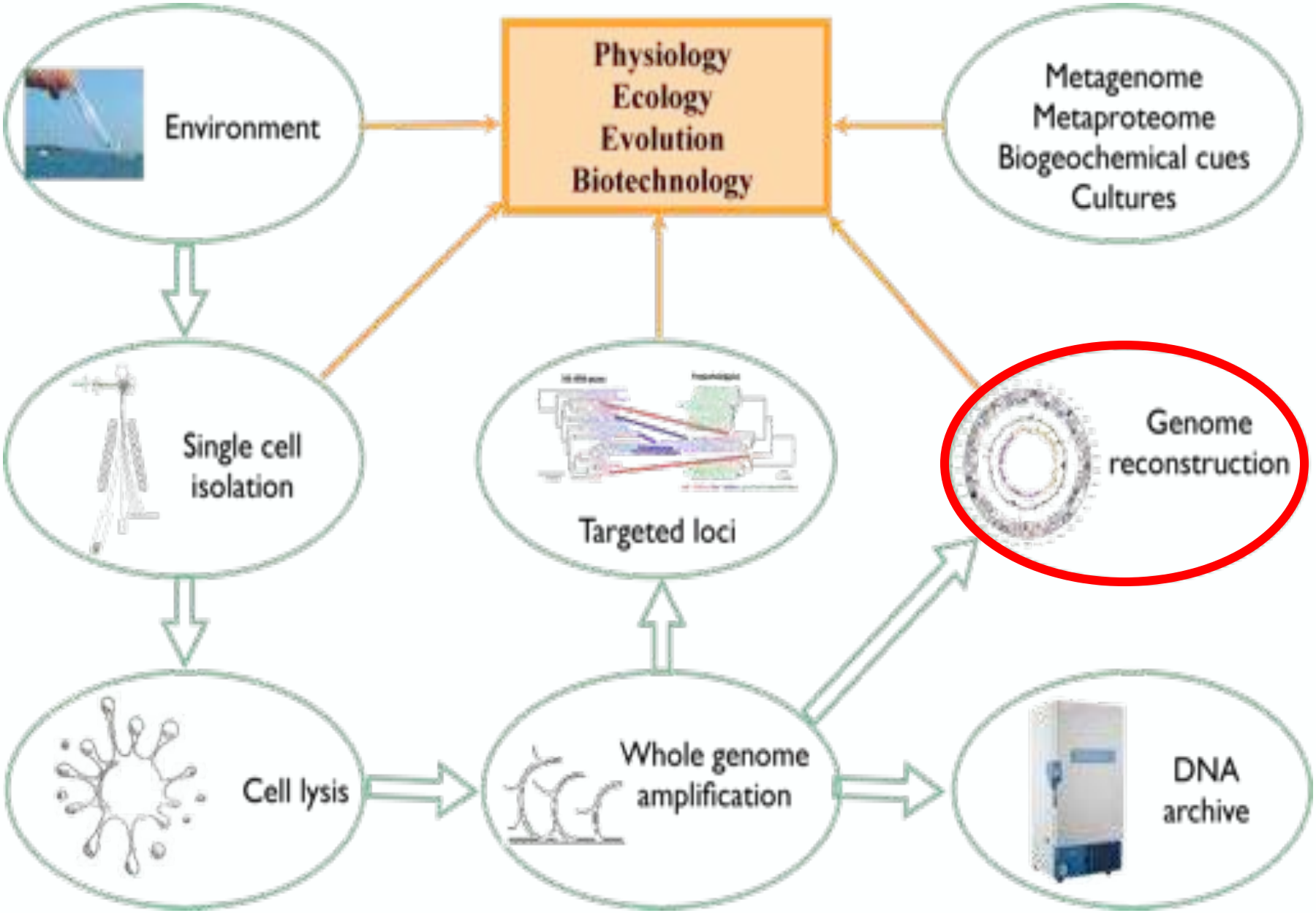


THREE CIRCLES OF THE HEDGEHOG CONCEPT

What got SCGC working?

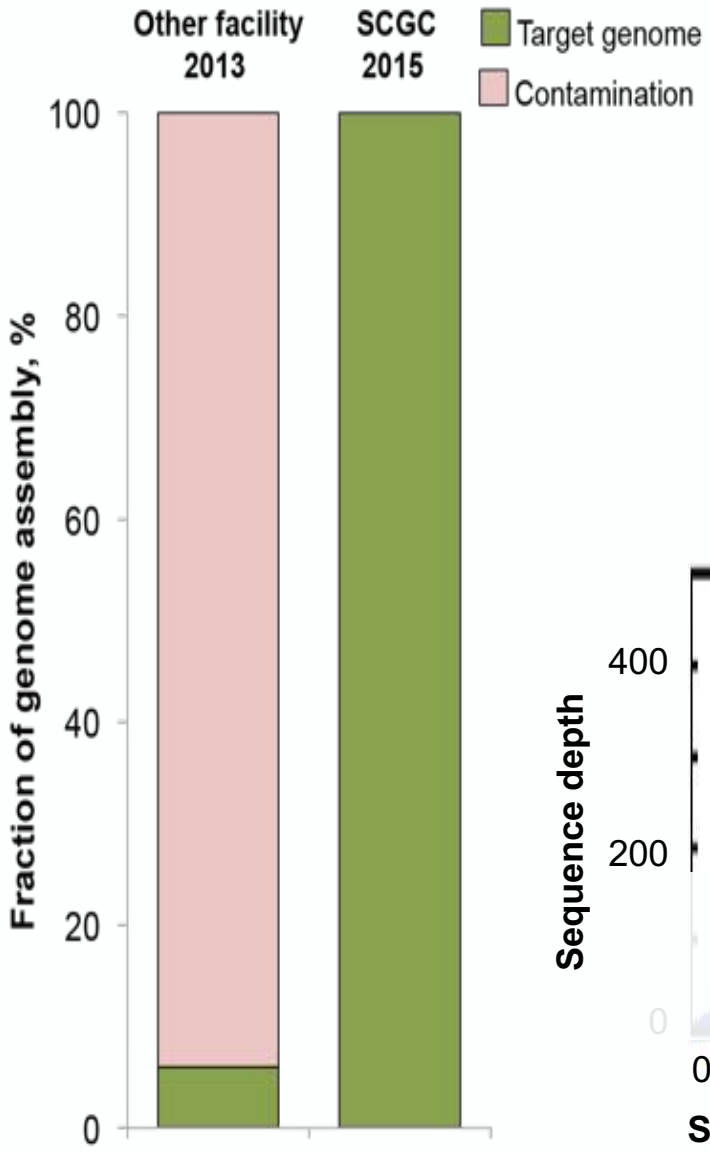
- **Placing research questions first**
- **Motivated, qualified team**
- **A lot of advice and collaborations**
- **Method development, continuous since 2005, ~$3 M**
- **Laboratory and IT equipment, ~$3 M**
- **Core facility business model and implementation**
- **QC and benchmarking of entire workflow**
- **Proficiency in and integration of technologies:**
  - Fluorescence-activated cell sorting (FACS)
  - Whole genome amplification
  - Robotic liquid handling
  - Cleanroom environment
  - DNA sequencing
  - *De novo* assembly and other bioinformatics
  - Laboratory information management systems (LIMS)

Single Cell Genomics Pipeline



SAG cross-contamination during sequencing

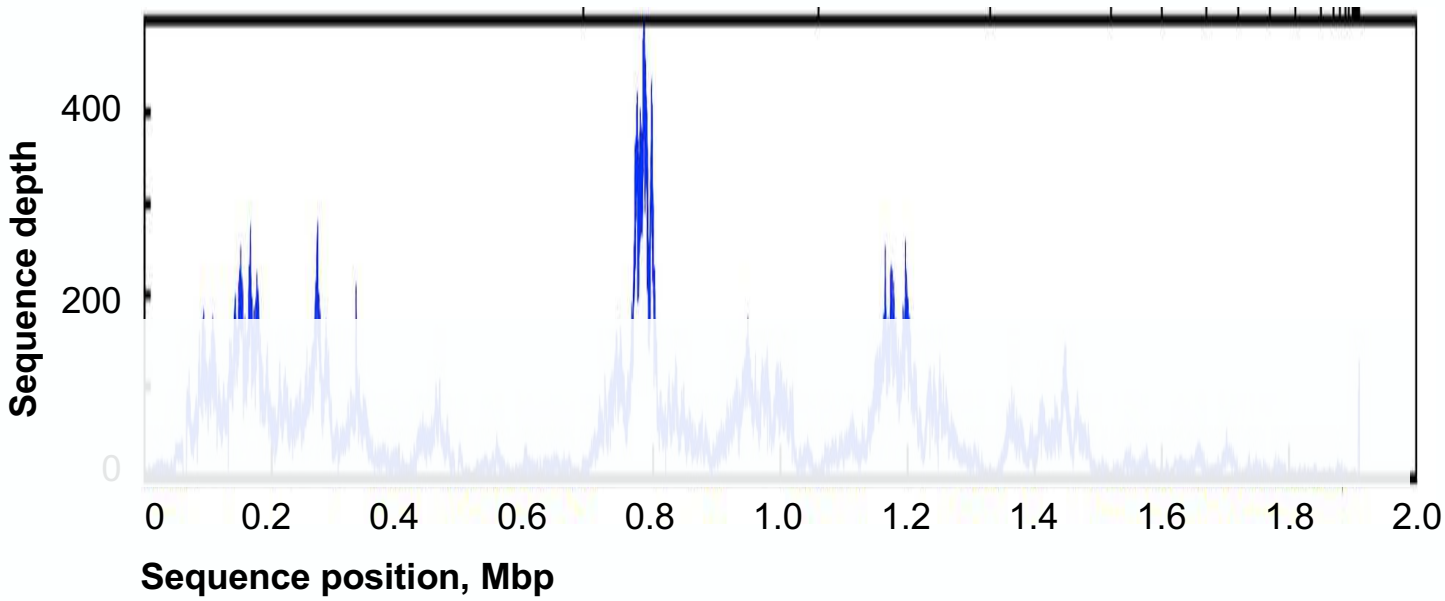
Illumina library cross-contamination
SAG SCGC AC-310-N17 (Firmicutes)



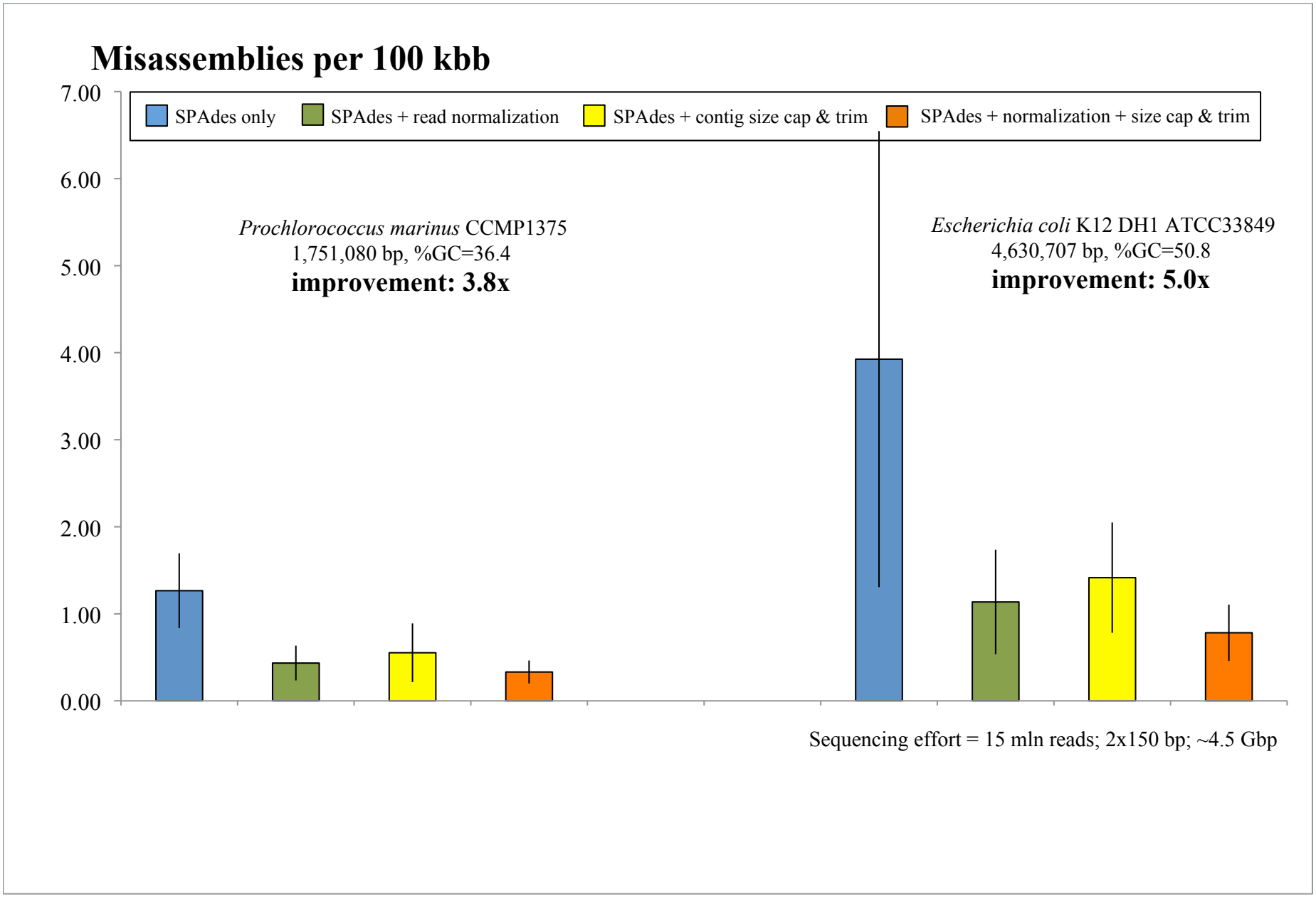
Mechanisms:
- Miss-assignment of reads among multiplexed libraries
- Sample carry-over between runs

Solution:
- Dual barcoding
- Use of NextSeq
- Extra care and validation of each step

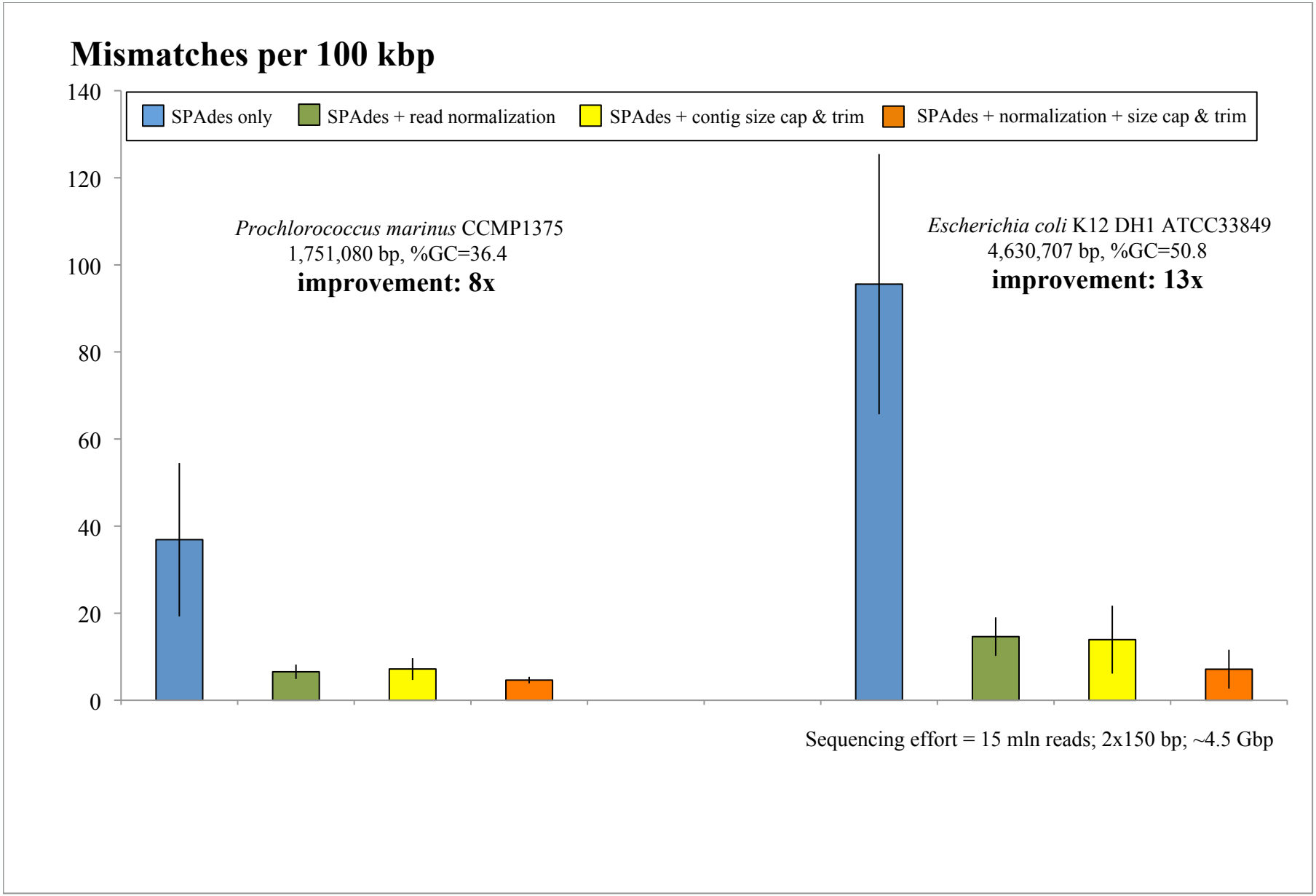
Outcome:
- ~50 SAGs sequenced worldwide in 2013-2014
- ~1k/10k SAGs sequenced at SCGC in 2015-2016



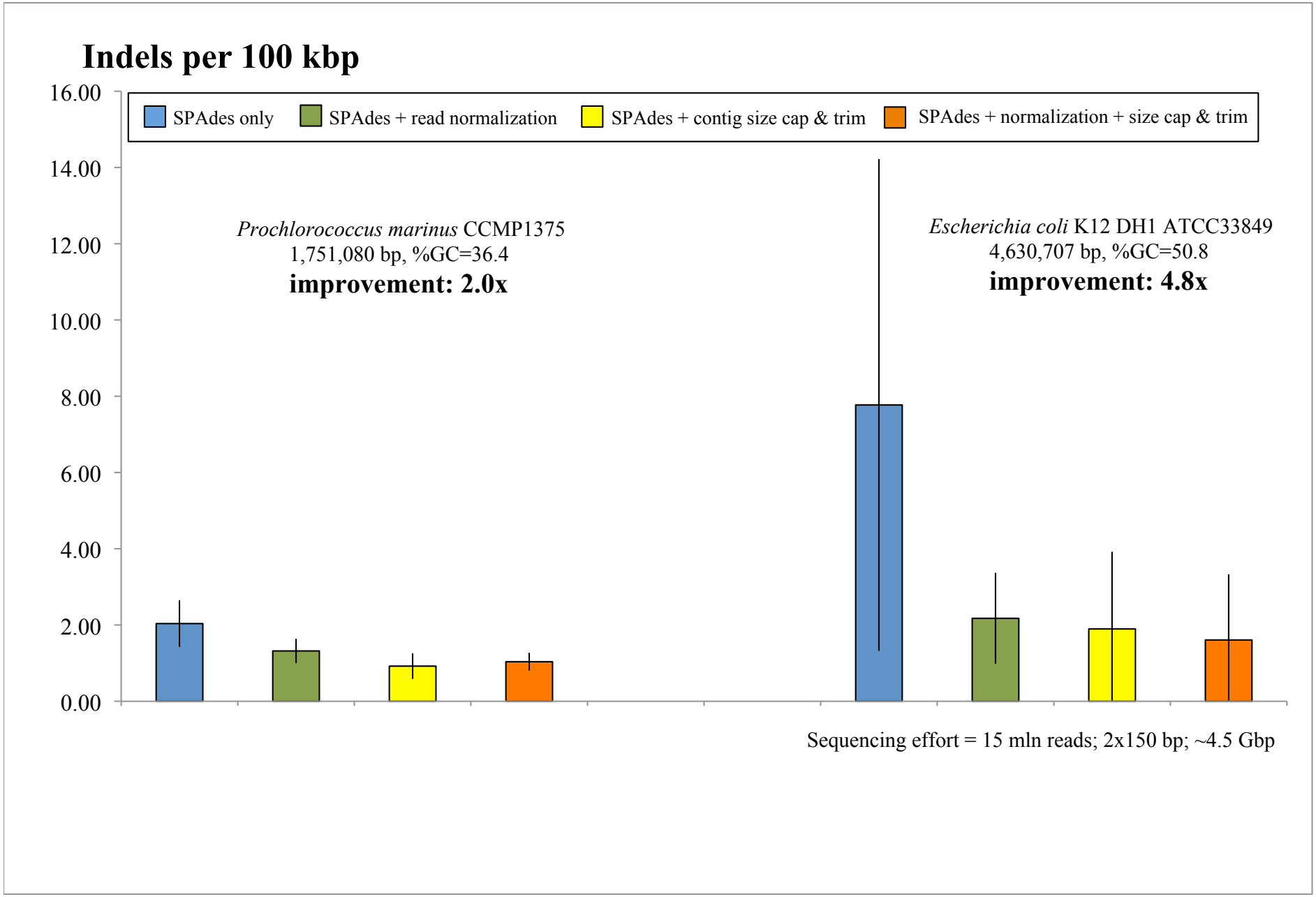
Improved SAG *de novo* assembly



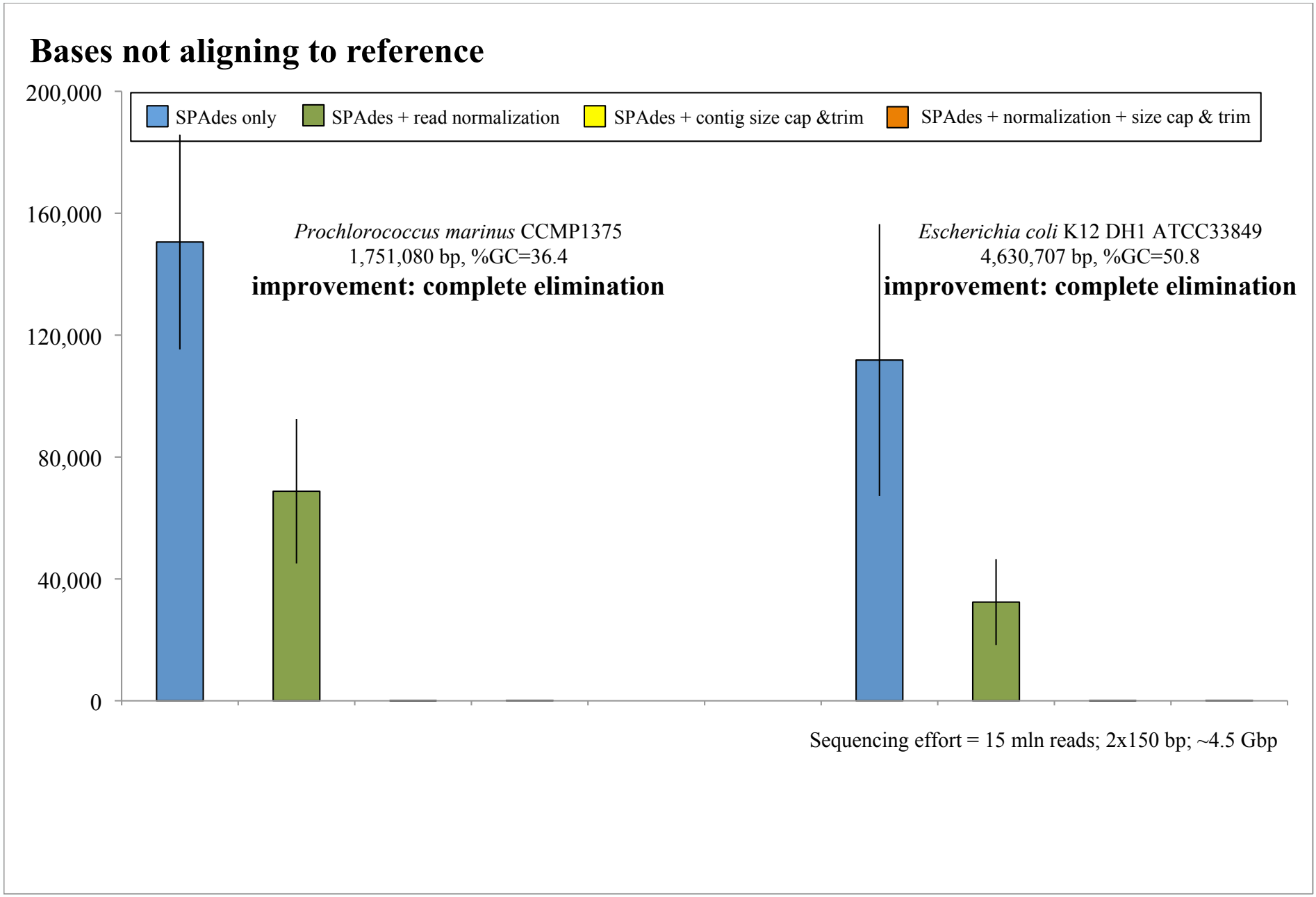
Improved SAG *de novo* assembly



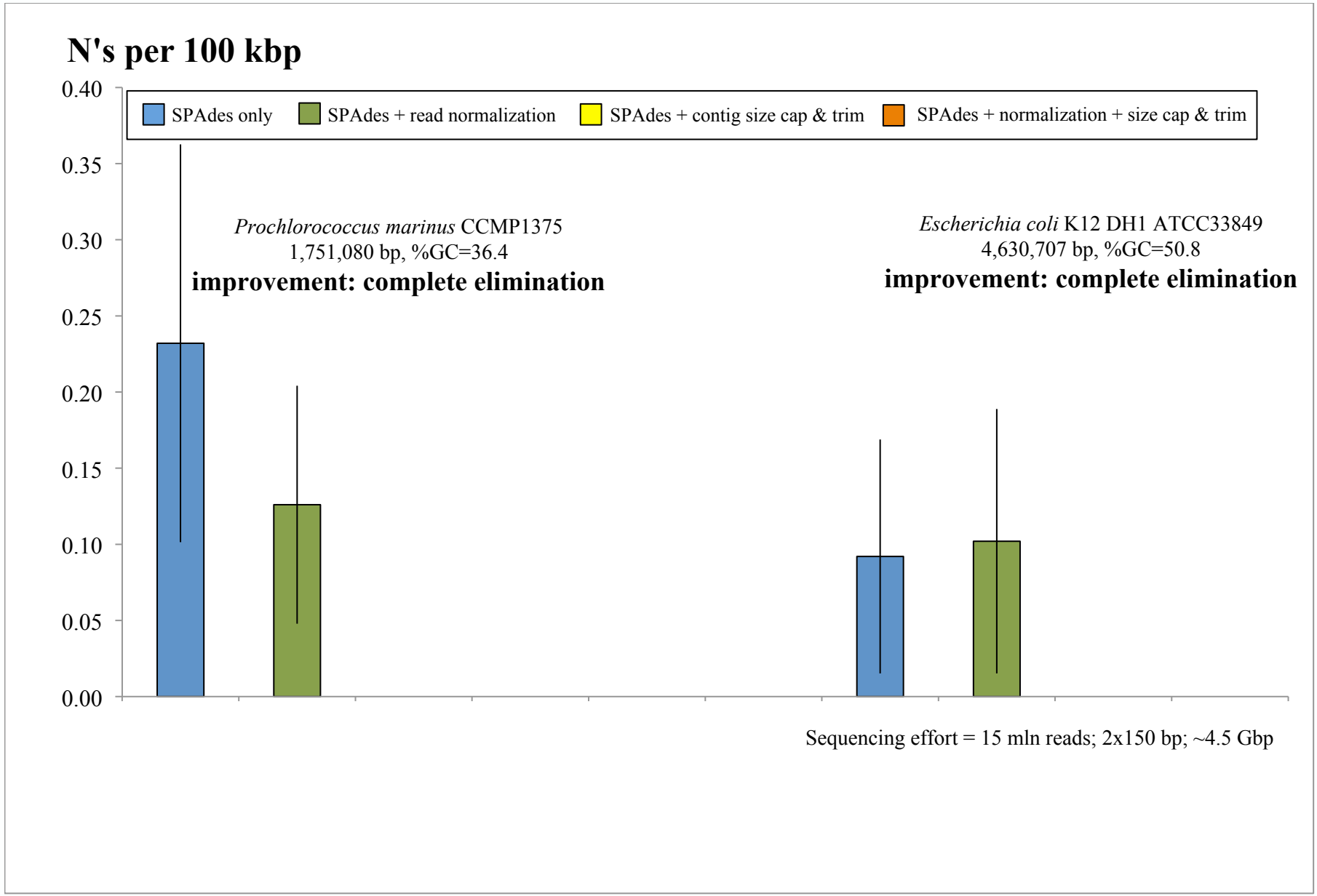
Improved SAG *de novo* assembly



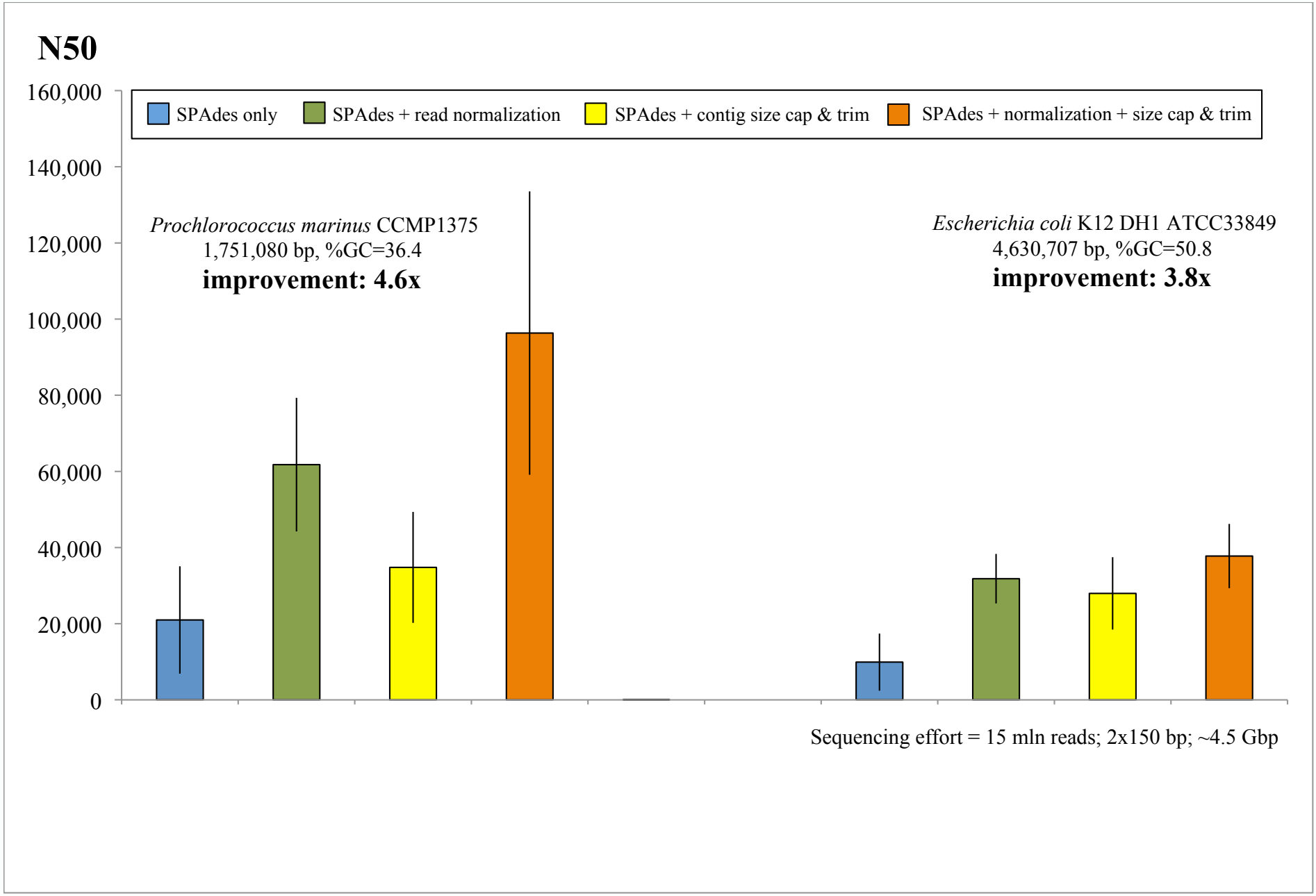
Improved SAG *de novo* assembly



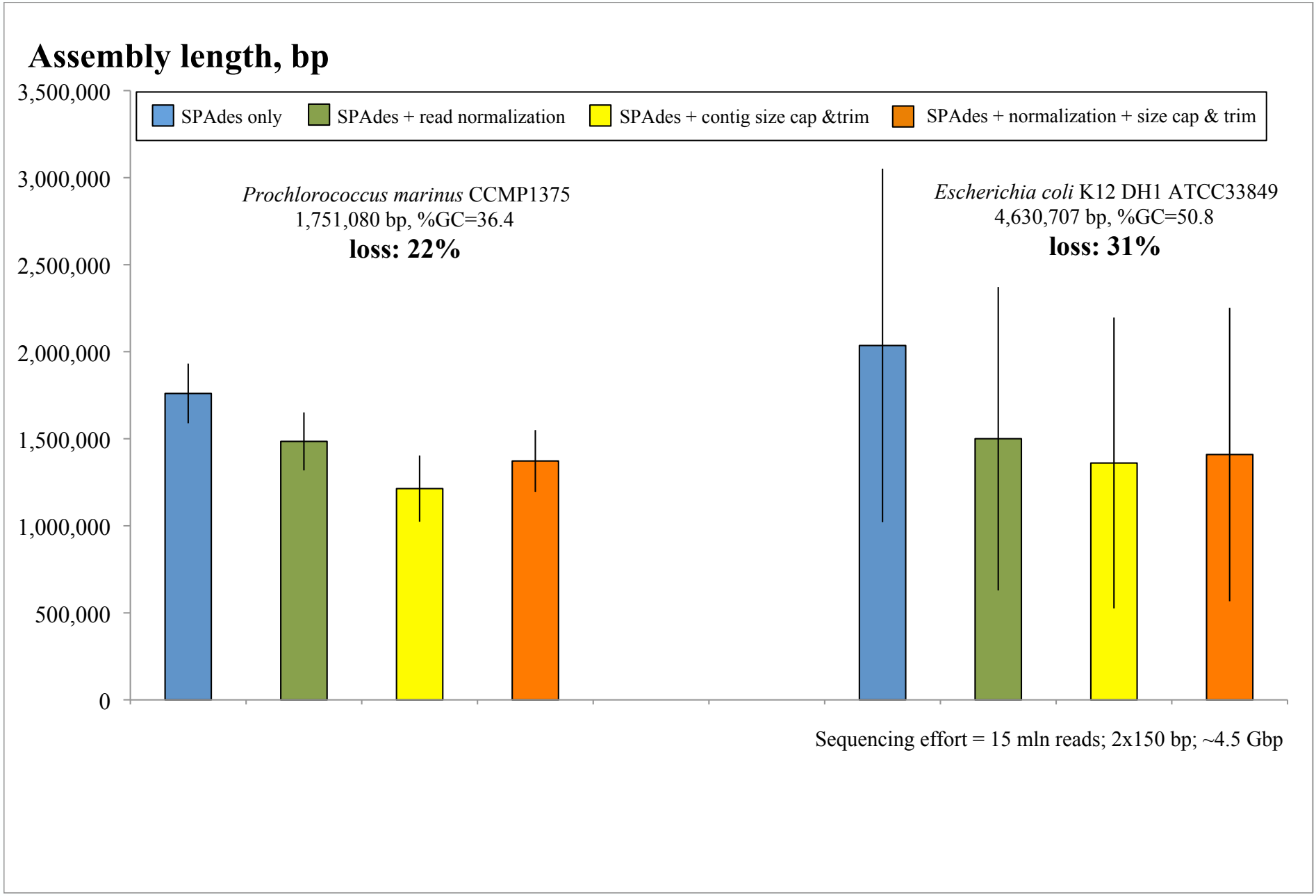
Improved SAG *de novo* assembly



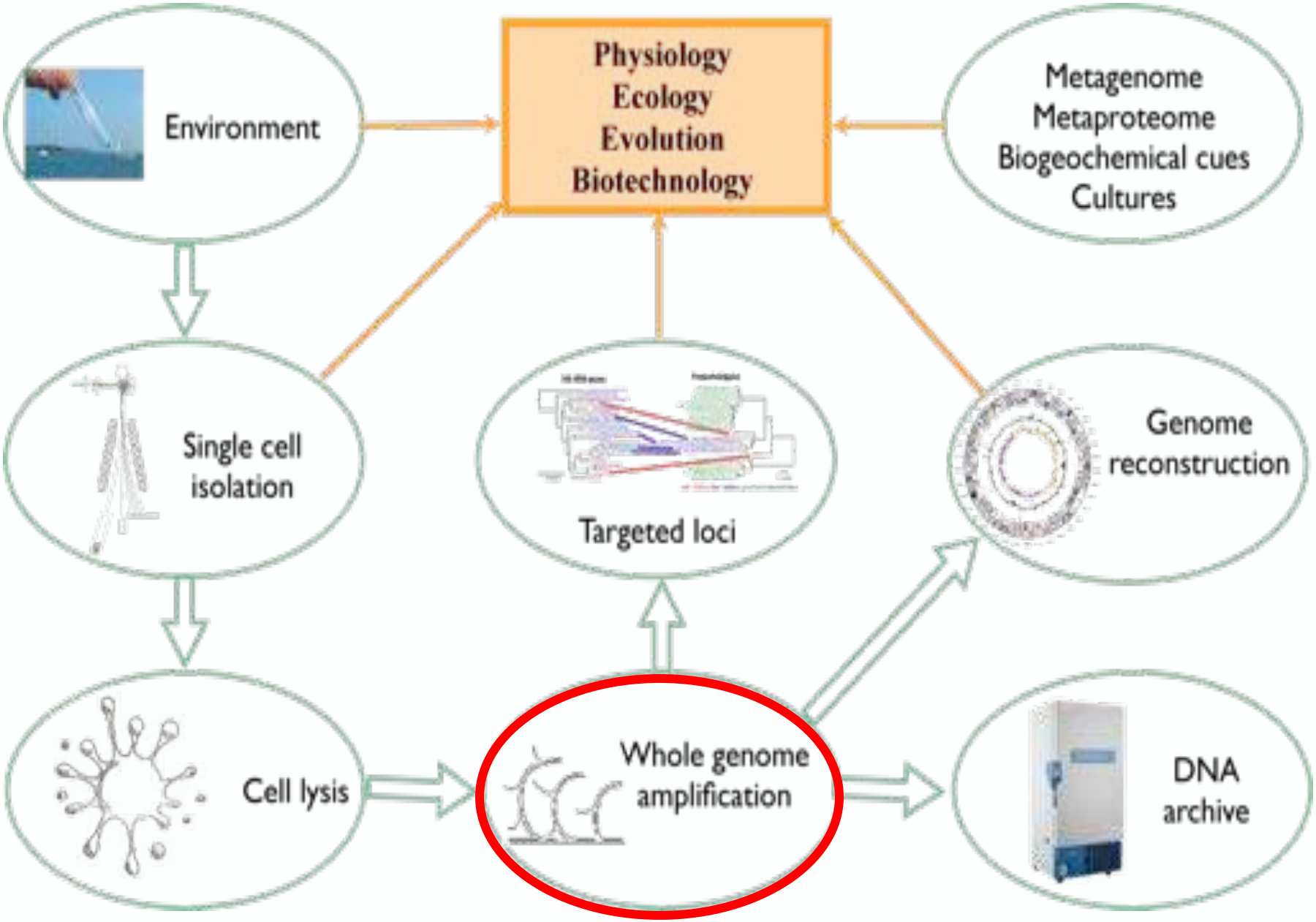
Improved SAG *de novo* assembly



Improved SAG *de novo* assembly

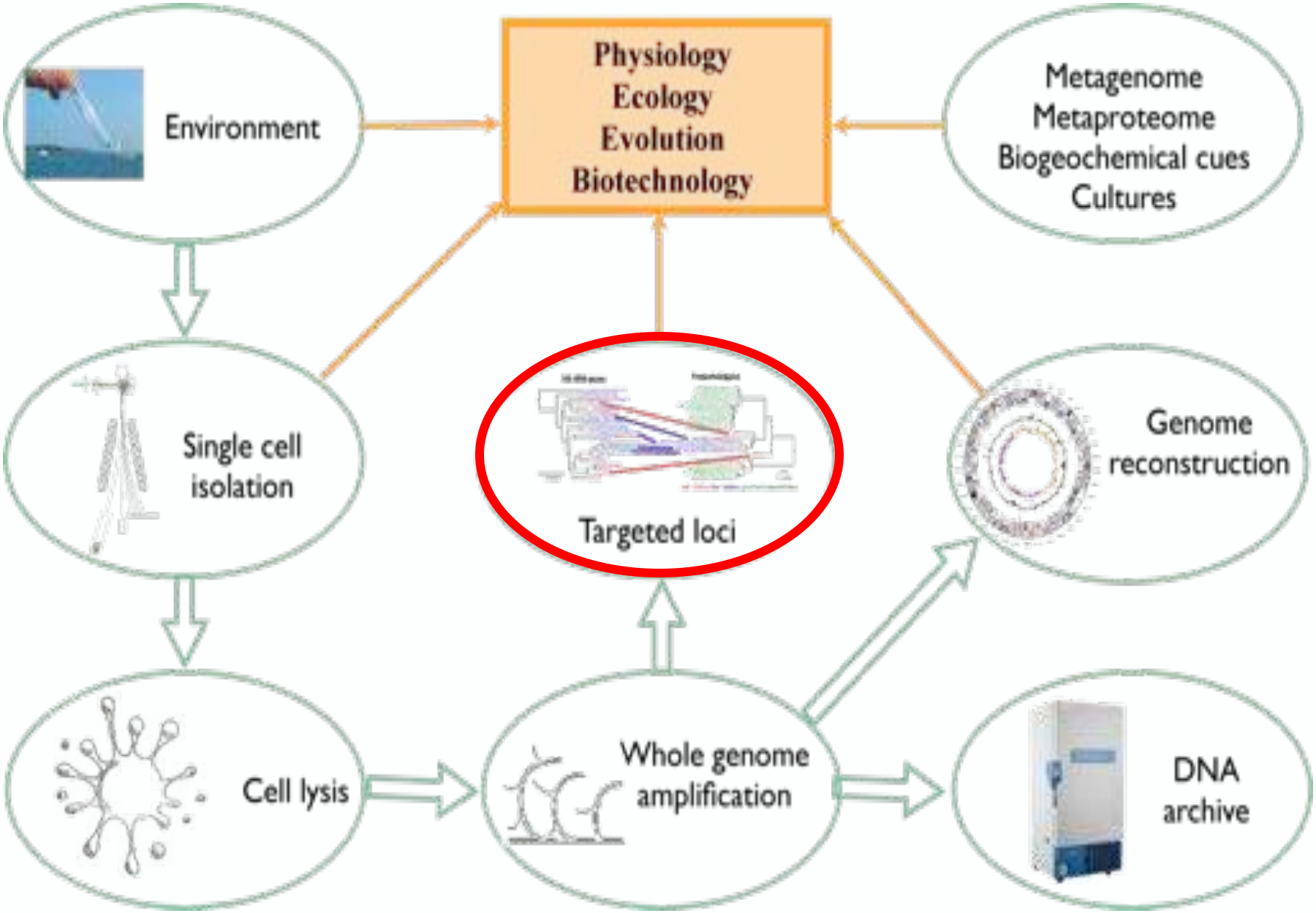


Single Cell Genomics Pipeline

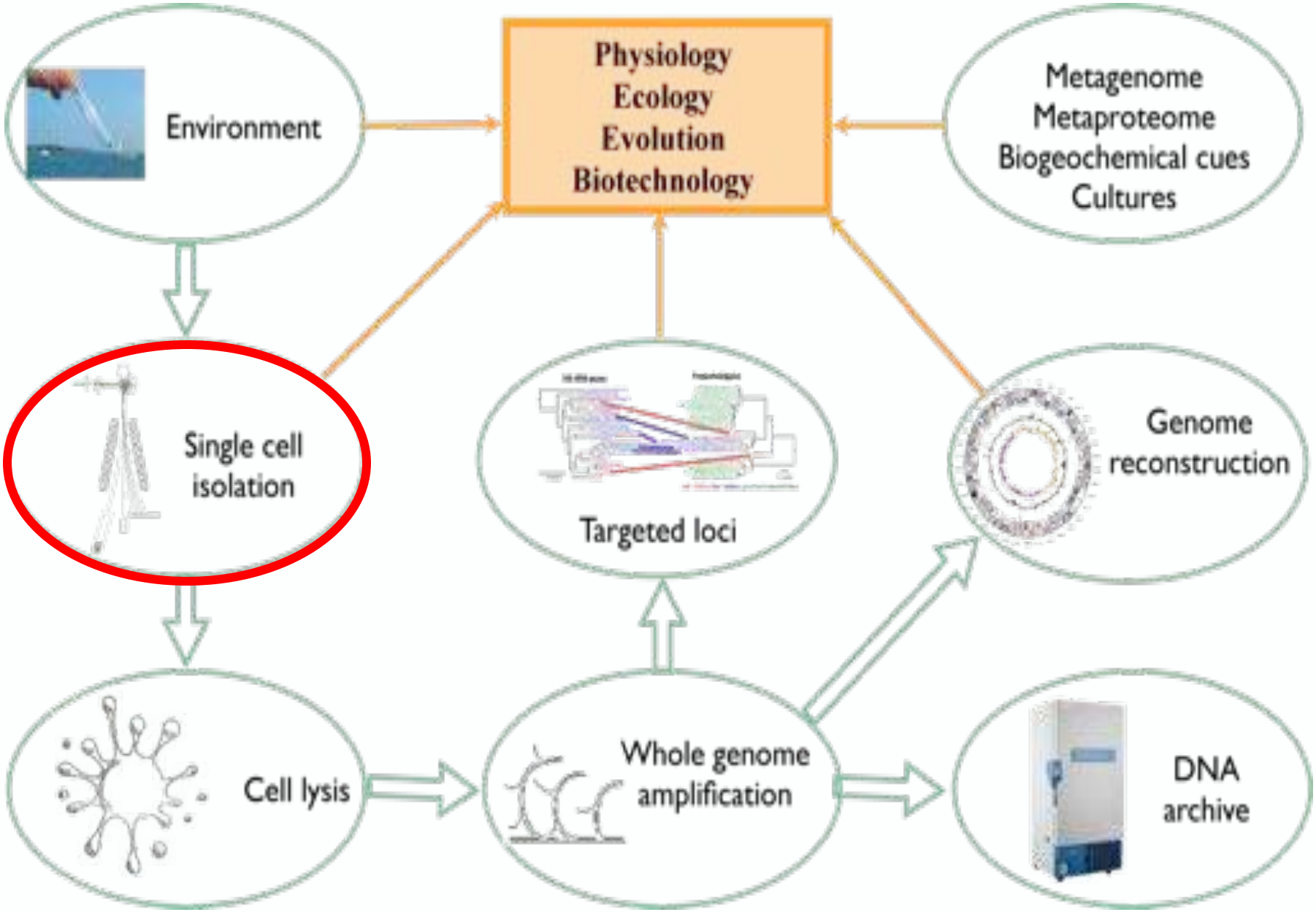


Bigelow | Laboratory for Ocean Sciences

Single Cell Genomics Pipeline

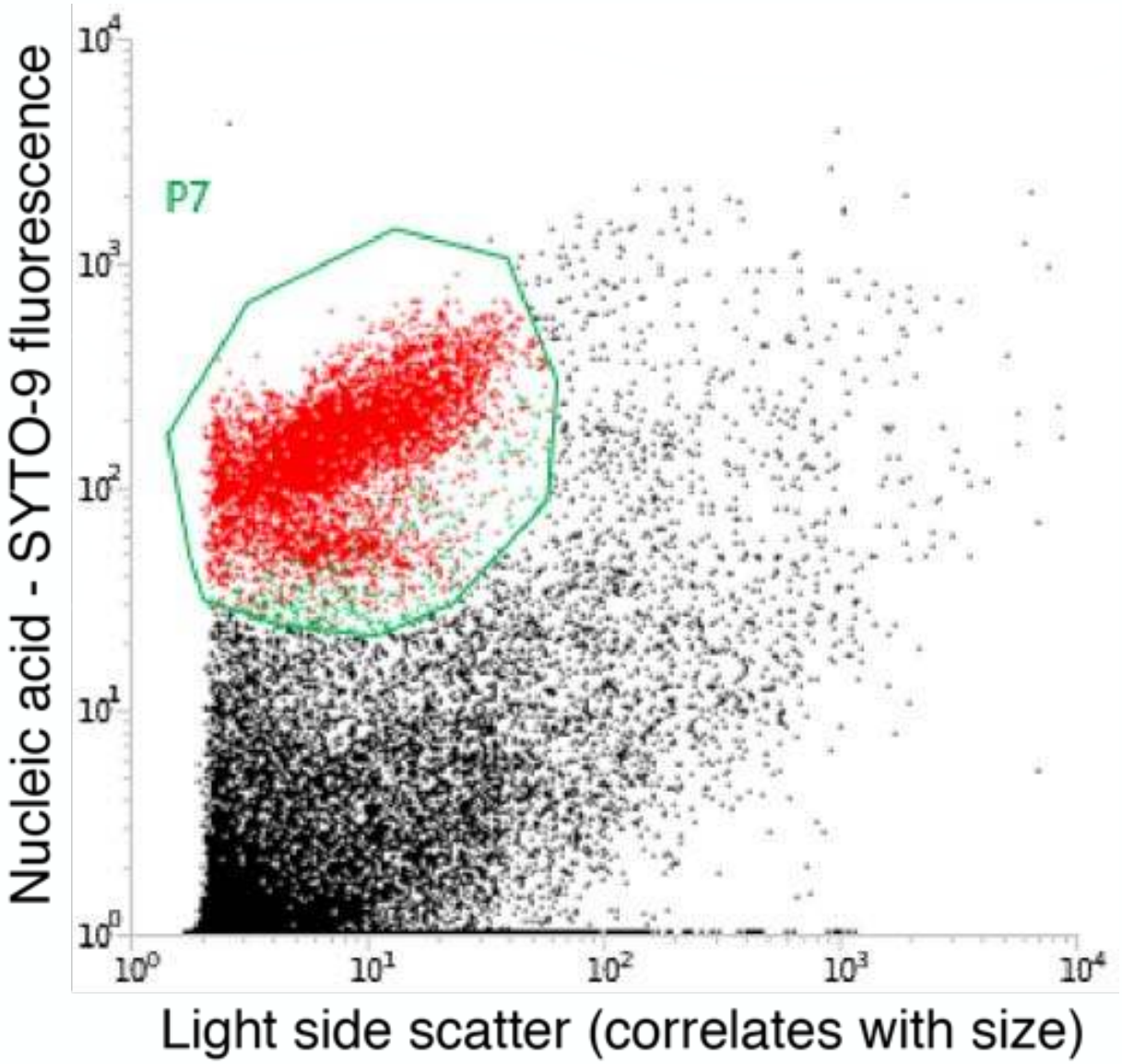


Single Cell Genomics Pipeline



Traditional fluorescence-activated cell sorting

Gulf of Maine surface microbial community

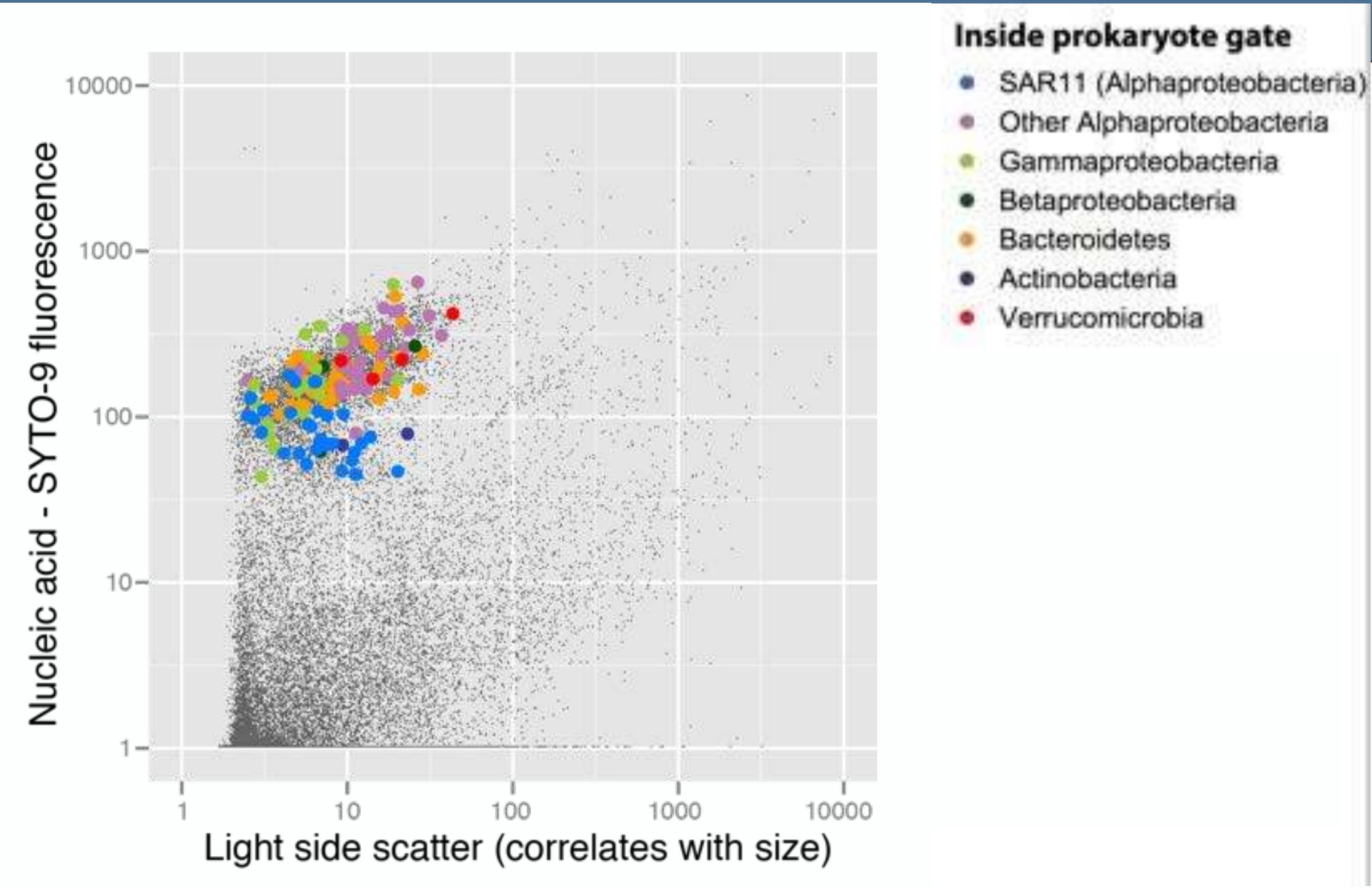


Inside prokaryote gate

- SAR11 (Alphaproteobacteria)
- Other Alphaproteobacteria
- Gammaproteobacteria
- Betaproteobacteria
- Bacteroidetes
- Actinobacteria
- Verrucomicrobia

Index cell sorting integration with single cell genomics

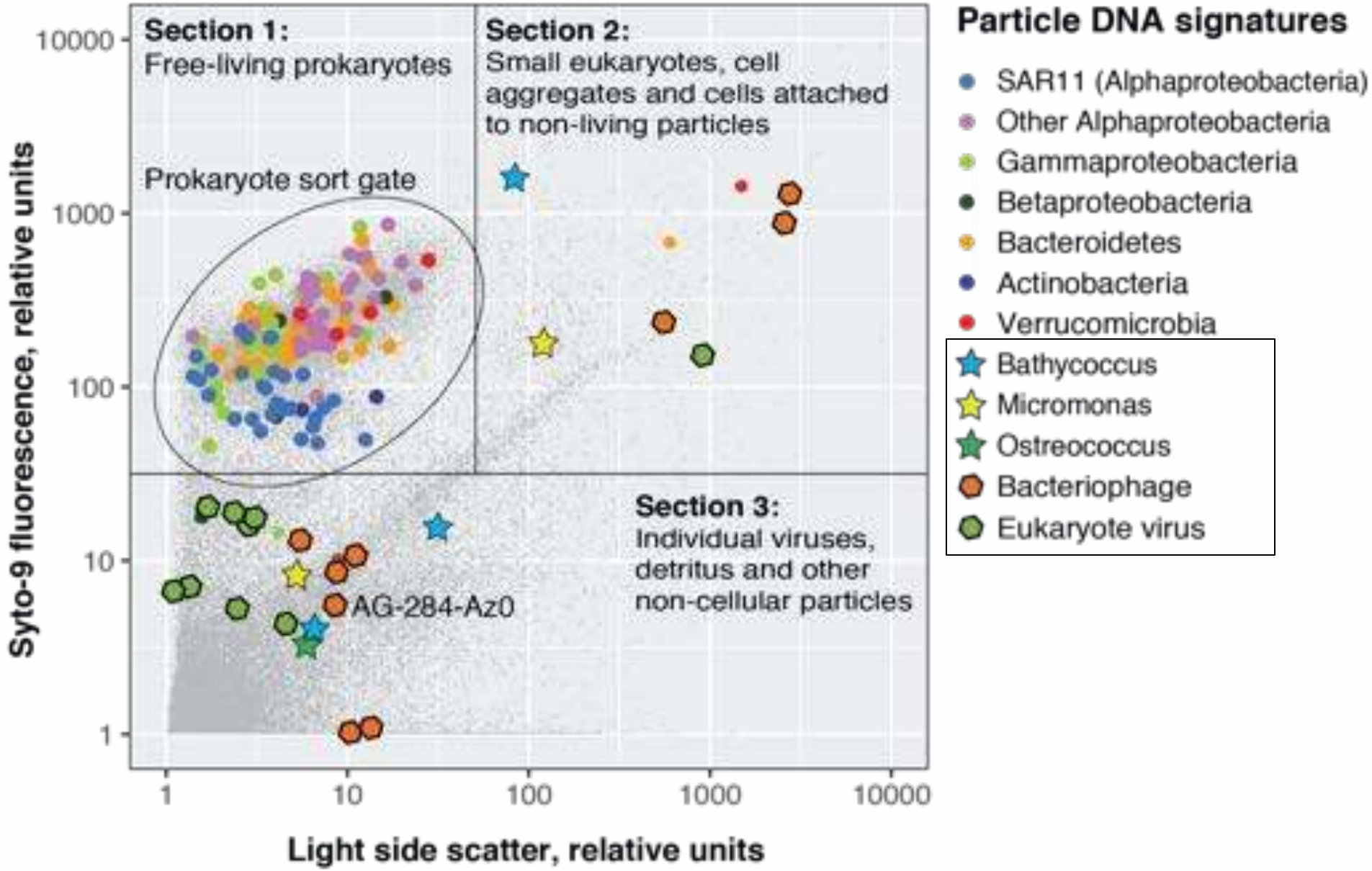
Gulf of Maine surface microbial community



Bigelow | Laboratory for Ocean Sciences

Genomics of individual extracellular particles

Gulf of Maine surface microbial community

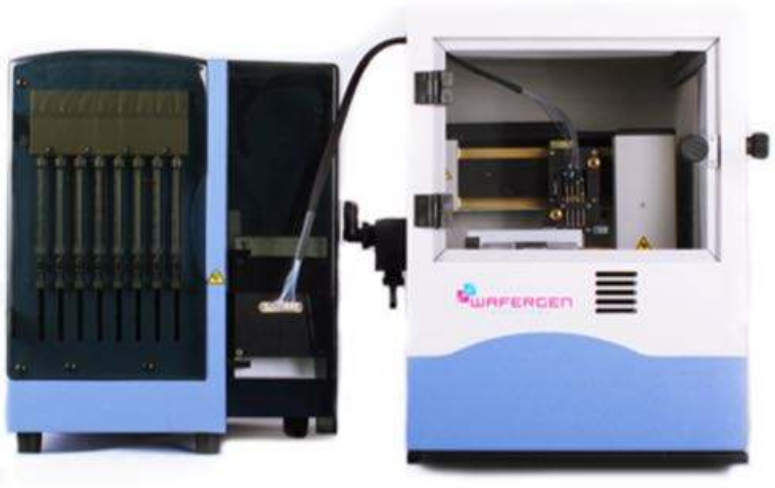


Bigelow | Laboratory for Ocean Sciences

Commercialized systems for human single cell transcriptomics

C1 (Fluidigm)

ICELL8 (Wafergen)

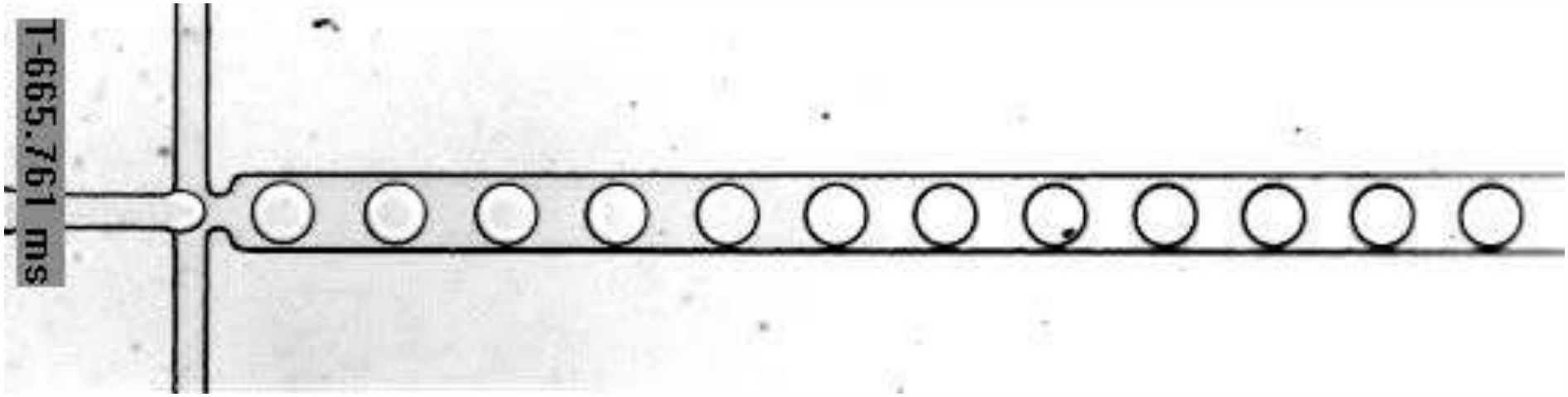


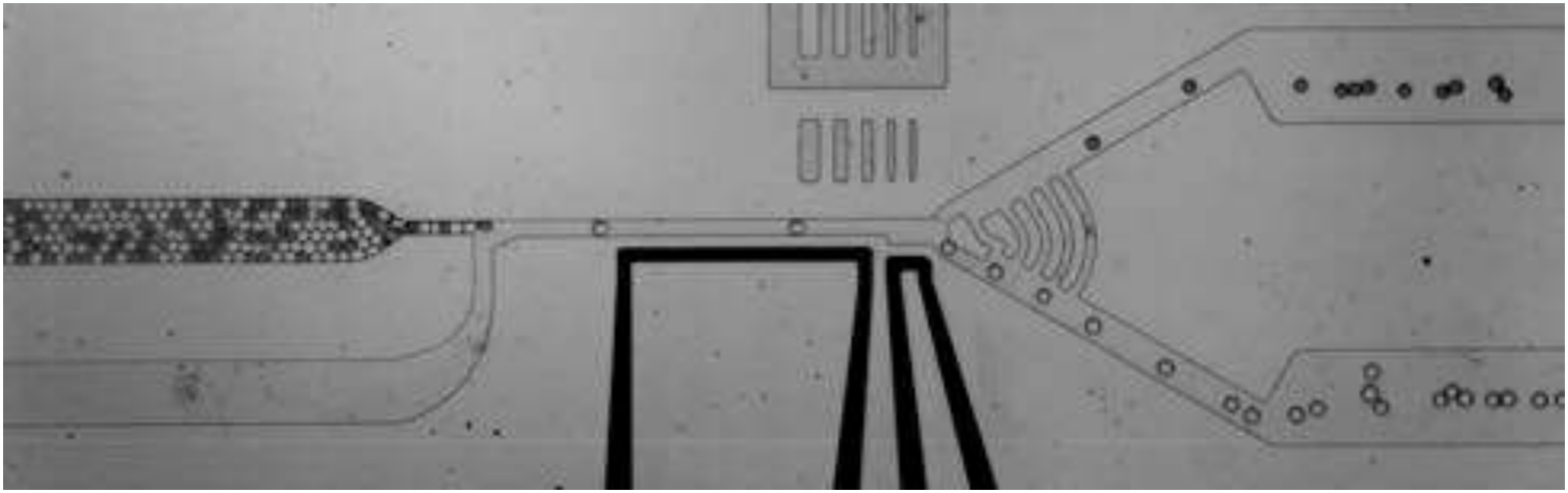
Chromium (10x)

ddSEQ (BIO-RAD & Illumina)

RNA –Seq System (Dolomite Bio)

Droplet microfluidics: Future of single cell genomics?





Video courtesy Linas Mazutis

Take-home message

- Microbial single cell genomics currently requires major investment into specialized facilities

- Methods are improving rapidly for enhanced scalability, genome recovery and integration with single cell phenomics