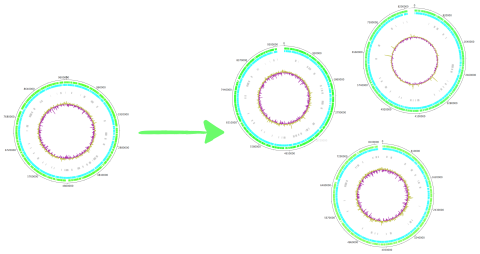
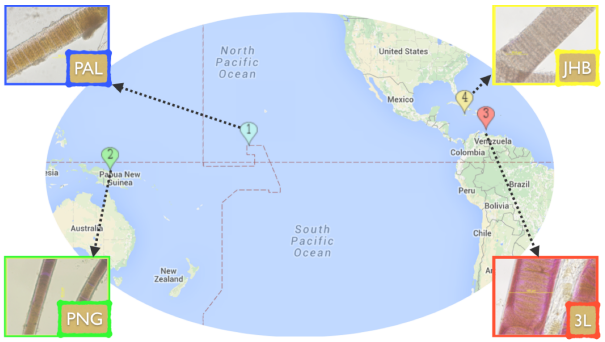
SIO-BUG MEETING

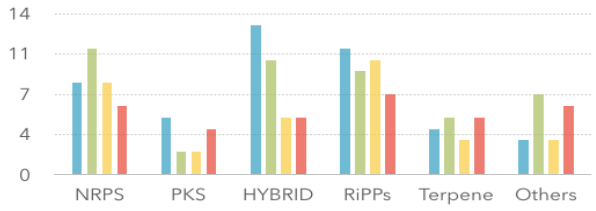
Obtaining genomes from (mini)metagenomes: assembly, binning and scaffolding

TIAGO LEÃO

GERWICK LAB







INTRO => Elevator talk

Importance:

The Nobel Prize in Physiology or Medicine 2015

William C. Campbell | Satoshi Ōmura | Youyou Tu

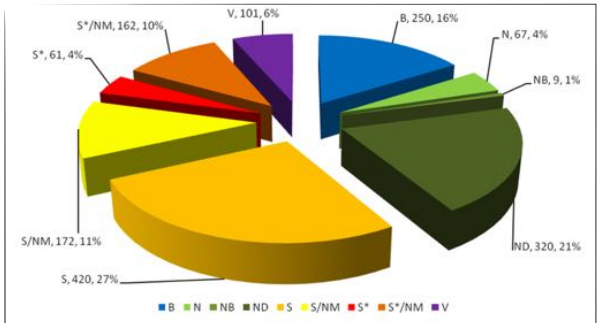
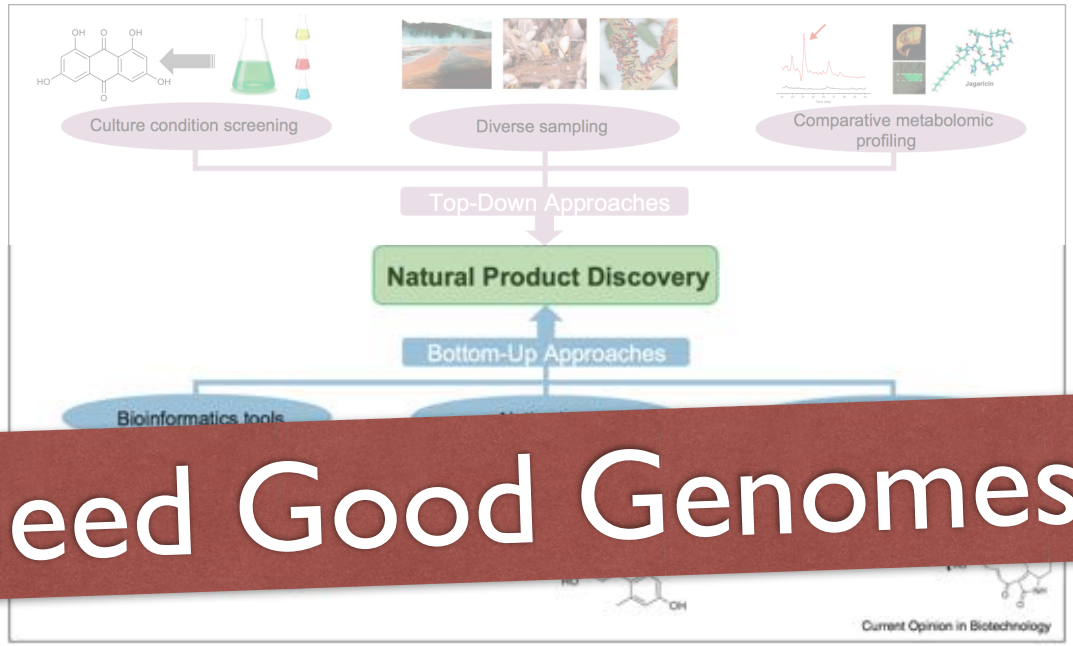
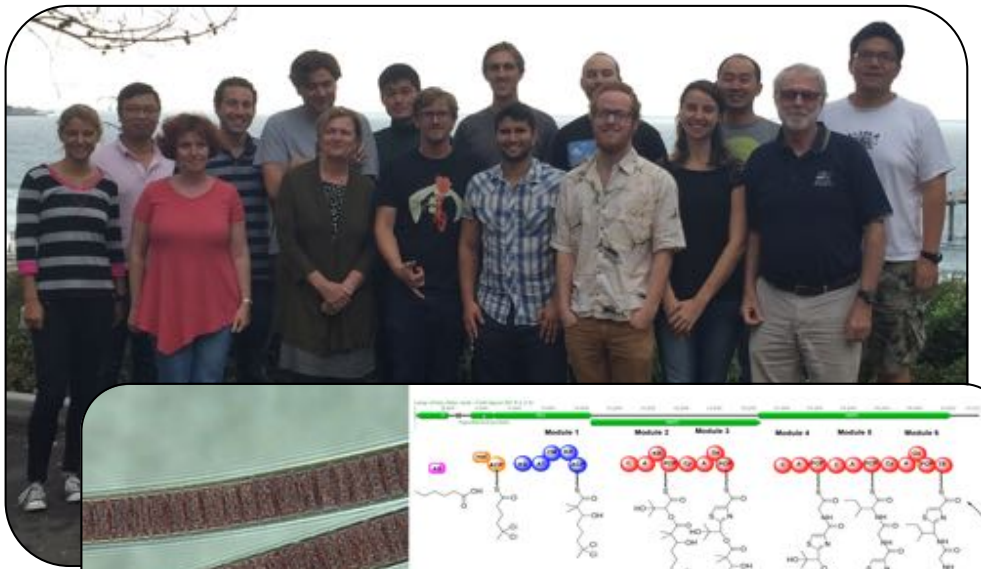


Figure 1. All new approved drugs 1981–2014; $n = 1562$.

Methods:



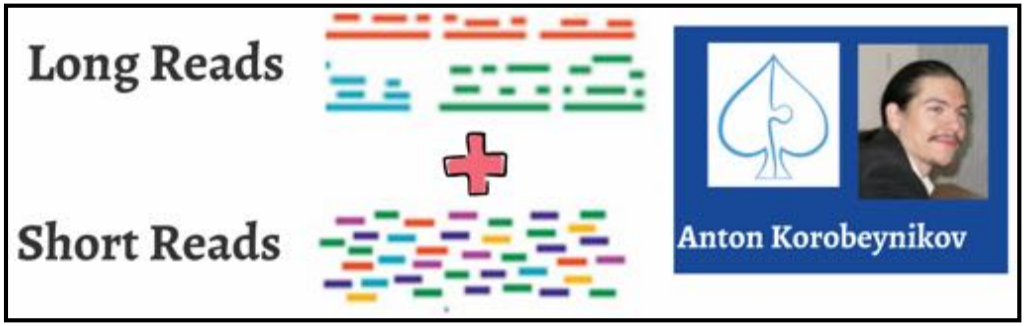
Need Good Genomes!



D) Post-Acquisition

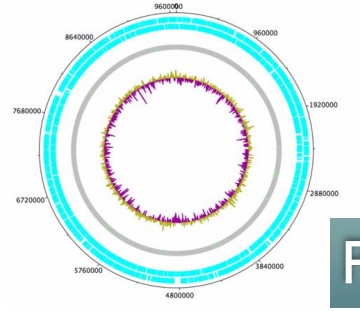
PAL

PacBio Sequence
+ Previous MiSeq



55 Contigs

Binning

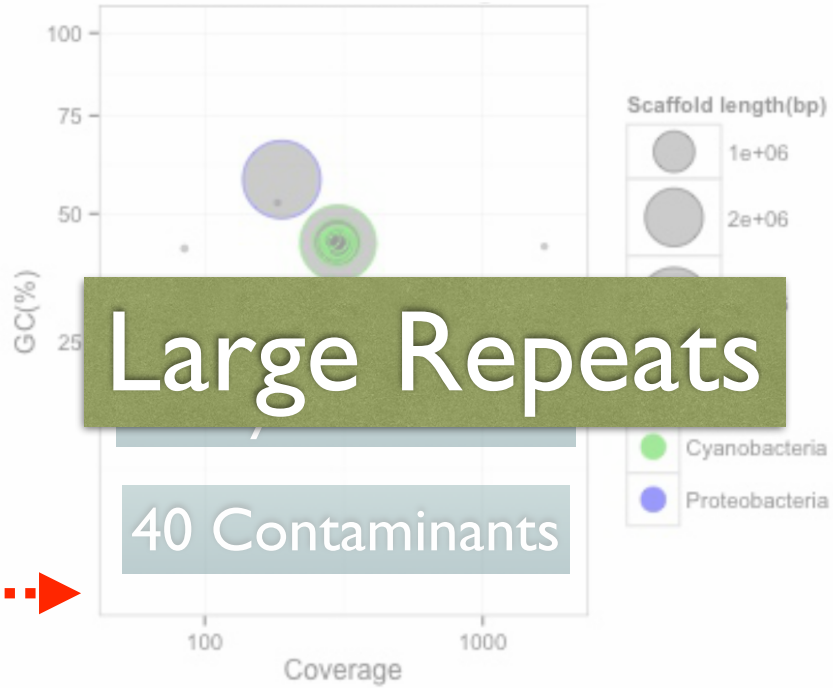


Plasmid (35.5 kb)

Chromosome (9.67 Mb)

Scaffolding w/
Long Reads

SSPACE



Large Repeats

40 Contaminants

A) *De Novo* Assembly

PAL

PacBio Sequence
+ Previous MiSeq

Long Reads

Short Reads

Anton Korobeynikov

55 Contigs

Which sequencing method is the best?

SIMPLEST ANSWER: We'll never know

Depends on: Sample & Goal

- -Complexity
- -Abundance
- -Overall Size
- -Repeats
- -[…]

- -Specific Genome
- -Completness
- (Reference?)
- -Genomic Target
- -[…]

A) *De Novo* Assembly

Many Papers

Which assembler is the best?

Long Read

Short Read

OPEN ACCESS Freely available online

PLoS one

A Practical Comparison of *De Novo* Genome Assembly Software Tools for Next-Generation Sequencing Technologies

Wenyu Zhang, Jiajia Chen, Yang Yang, Yifei Tang, Jing Shang, Bairong Shen*

Center for Systems Biology, Soochow University, Suzhou, Jiangsu, China

BIOINFORMATICS **ORIGINAL PAPER**

Vol. 30 no. 19 2014, pages 2709–2716
doi:10.1093/bioinformatics/btu391

Genome analysis

Advance Access publication June 14, 2014

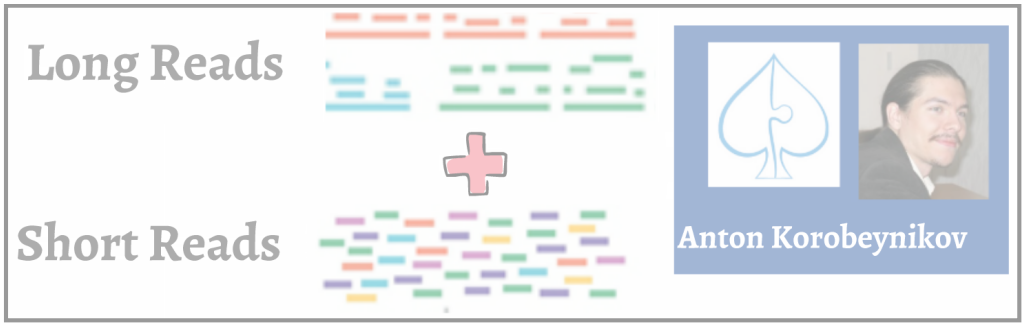
Evaluation and validation of *de novo* and hybrid assembly techniques to derive high-quality genome sequences

Sagar M. Utturkar[1], Dawn M. Klingeman[2], Miriam L. Land[2], Christopher W. Schadt[1,2], Mitchel J. Doktycz[1,2], Dale A. Pelletier[1,2] and Steven D. Brown[1,2,*]

[1]Graduate School of Genome Science and Technology, University of Tennessee, Knoxville, TN 37919, USA and [2]Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA

A) *De Novo* Assembly

PacBio Sequence
+ Previous MiSeq



55 Contigs

Which assembler is the best?

I'm happy with SPAdes

Now there is metaSPAdes

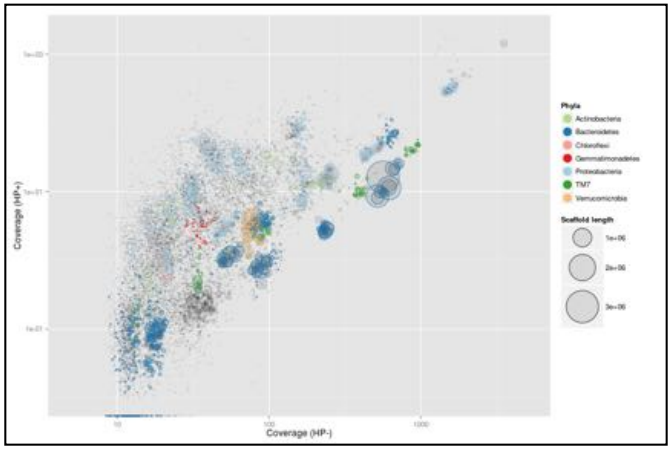
Also tried ~~MIRA~~
and ~~Geneious~~

PS: By experience, try many different k-mers

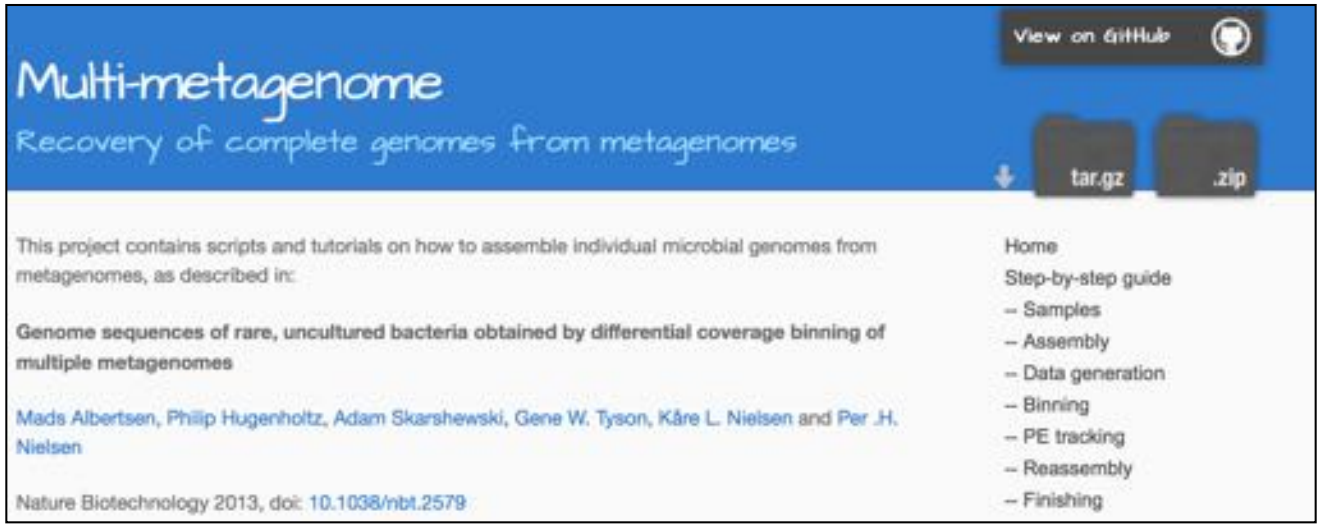
Different k-mer size can assemble better different genomes (from metagenomes)

B) Binning Assembled Contigs

- -GC**
- -Coverage***
- -Tetranucleotide***
- -Taxonomy*

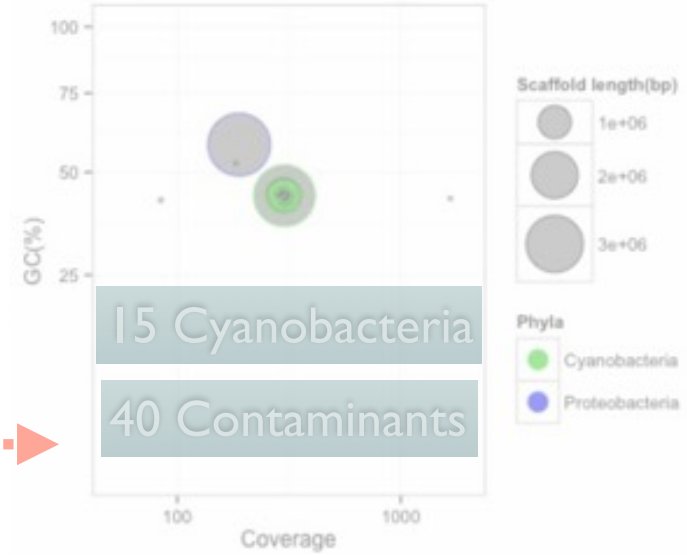


Albertsen *et al.*, 2013

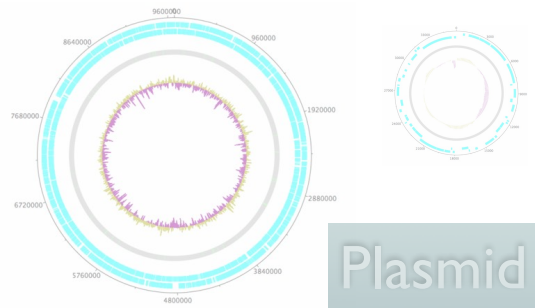


55 Contigs

Binning



C) Scaffolding

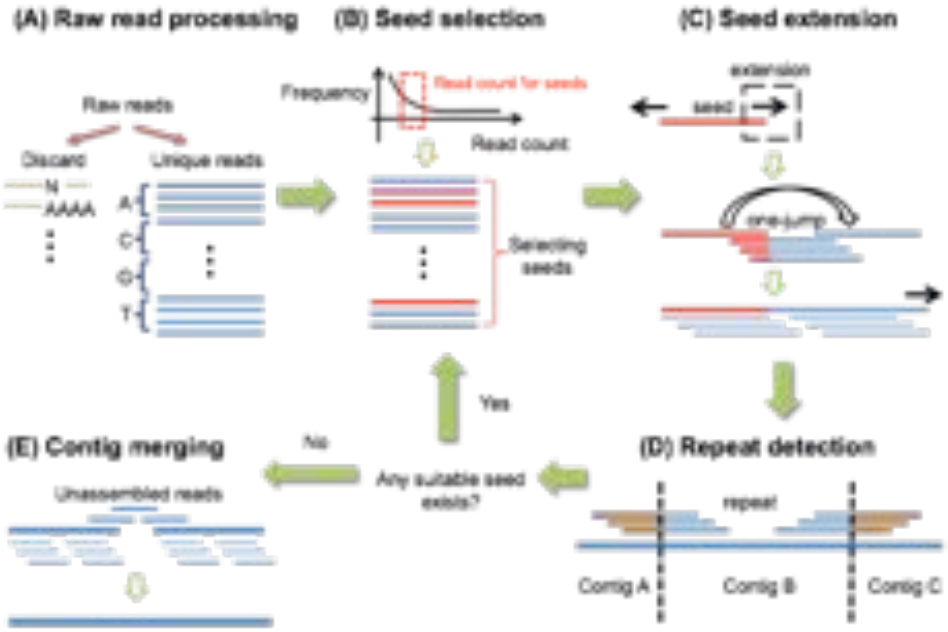


Plasmid (35.5 kb)

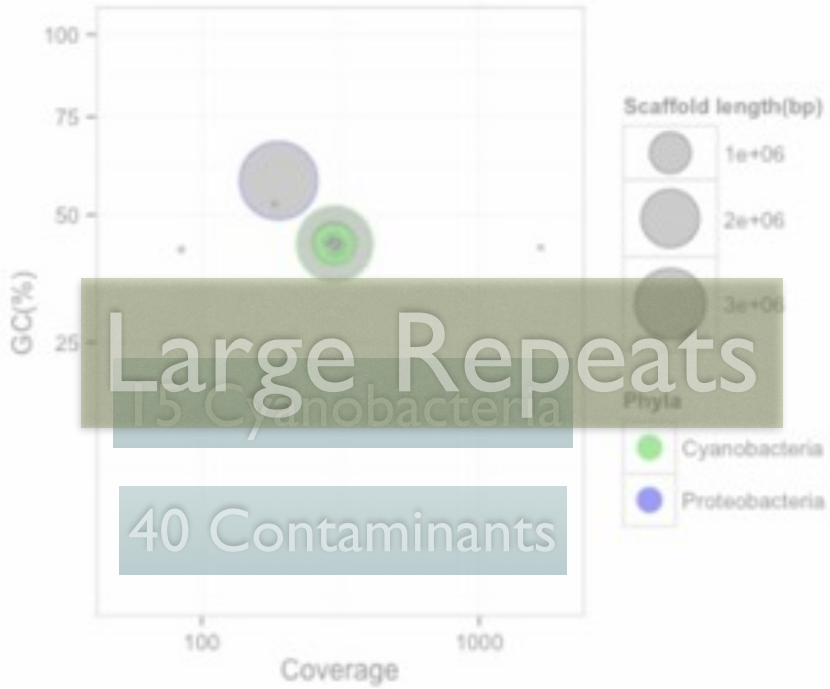
Chromosome (9.67 Mb)

I tried: geneious AlignGraph mauve Multiple Genome Alignment

SSPACE Short (or long)



Scaffolding w/ Long Reads → SSPACE

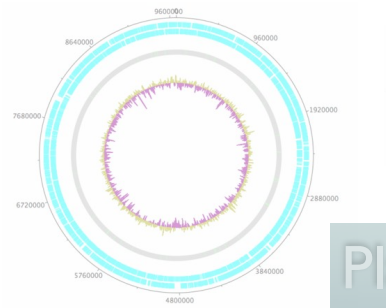


Large Repeats

15 Cyanobacteria

40 Contaminants

C) Scaffolding



Plasmid (35.5 kb)

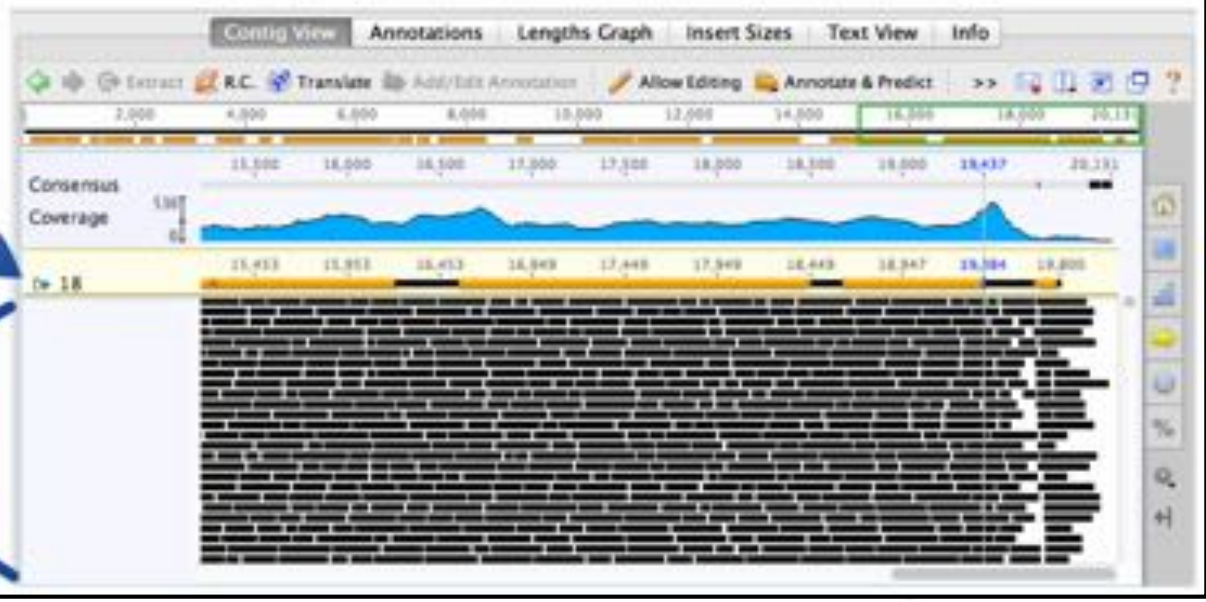
Chromosome (9.67 Mb)

I tried: geneious AlignGraph mauve Multiple Genome Alignment

SSPACE Short (or long)

Scaffolding w/ Long Reads ⇢ SSPACE

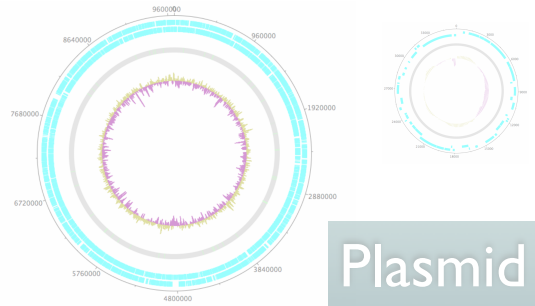
Trimming helps



Contig With ORFs

Paired Reads

C) Scaffolding



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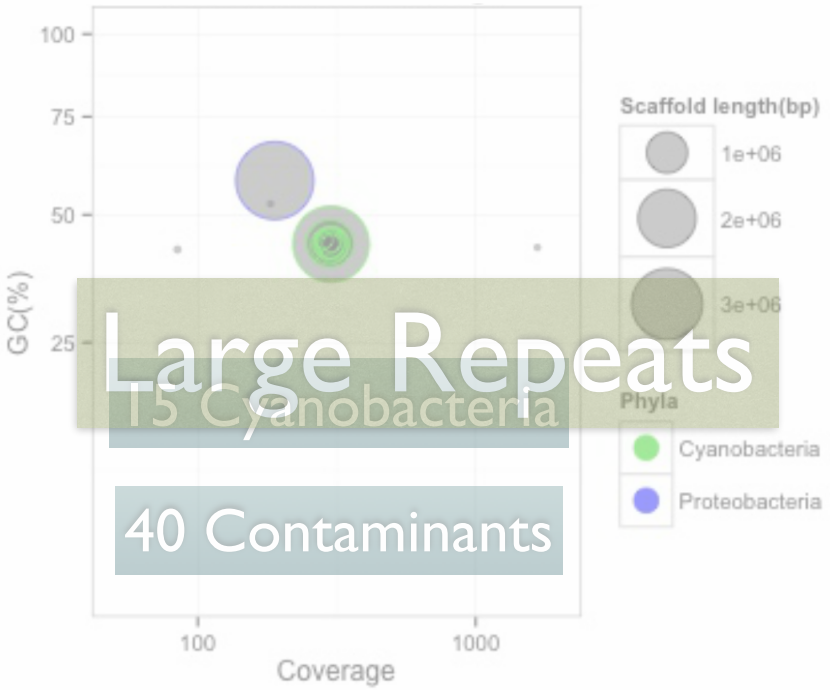
Chromosome (9.67 Mb)

I tried: geneious AlignGraph mauve Multiple Genome Alignment

SSPACE Short (or long)

Scaffolding w/ Long Reads → SSPACE



Large Repeats

15 Cyanobacteria

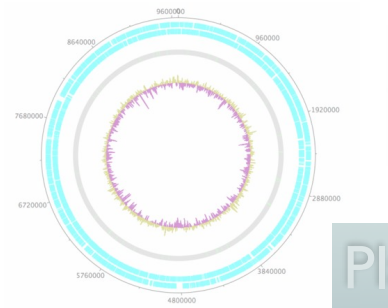
40 Contaminants

C.b) Gap Filling

PBSuite
Software for Long-Rea[illegible]equencing Data from PacBio
Brought to you by: acenglis[illegible]cm

geneious
Good coverage (>60x)
Alignments and Consensus

D) Post-Acquisition



Plasmid (35.5 kb)

Chromosome (9.67 Mb)

How complete is your genome/draft?

i. Select a set of genomes

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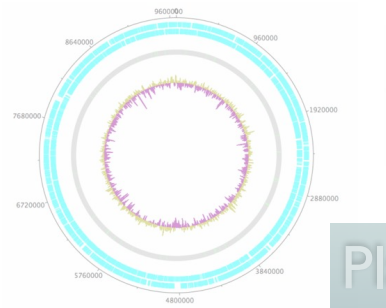
ii. Abundance Profile (COGs and Gene Count)

iii. COGs that are exactly one copy in 95% of all strains (Single Copy Genes)

D) Post-Acquisition

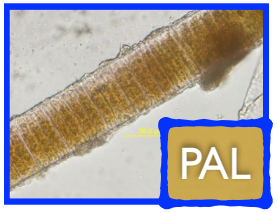


Plasmid (35.5 kb)

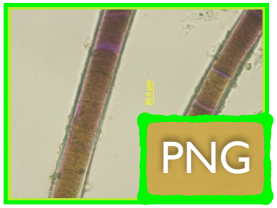
Chromosome (9.67 Mb)

iv. Compare the count of Single Copy Genes

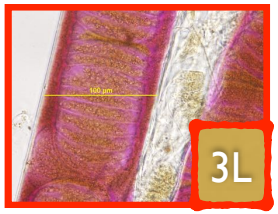
208 Housekeeping Genes from Cyanobacteria



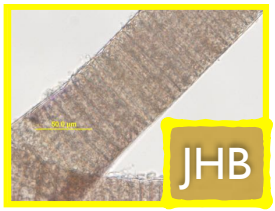
99.52%



98.10%



97.13%



100%

D) Post-Acquisition

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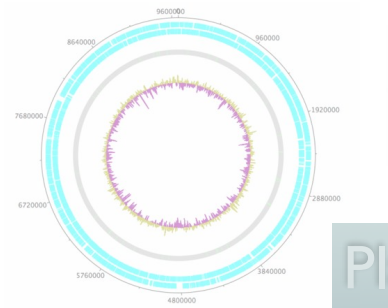
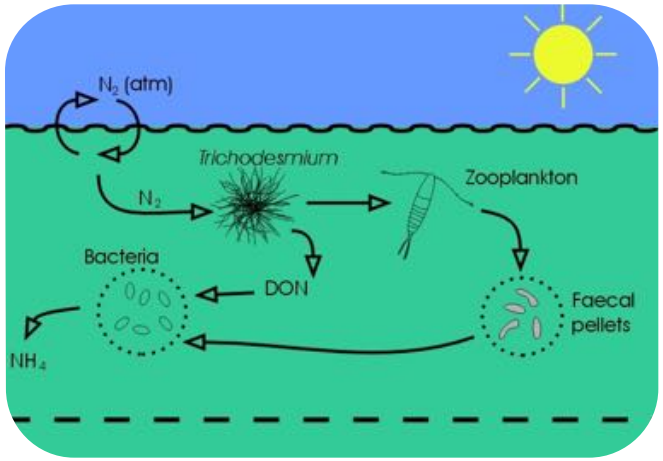


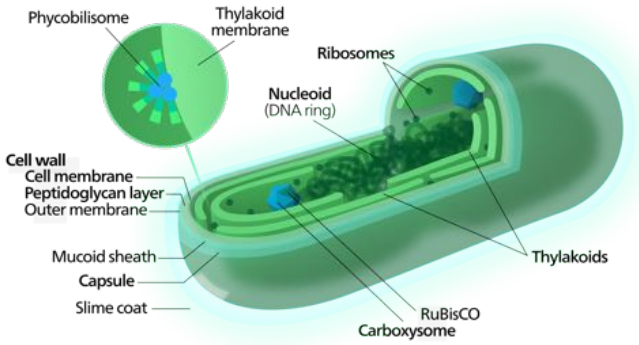
Plasmid (35.5 kb)

Chromosome (9.67 Mb)

-Metabolism and Biosynthesis

Phenotypes/Metabolism from Pathway Assertion	
Metabolism	Prototrophic (L-alanine prototroph) (IMG_PIPELINE; 2015-09-25)
Metabolism	Prototrophic (L-aspartate prototroph) (IMG_PIPELINE; 2015-09-25)
Metabolism	Prototrophic (L-glutamate prototroph) (IMG_PIPELINE; 2015-09-25)
Metabolism	Auxotroph (L-phenylalanine auxotroph) (IMG_PIPELINE; 2015-09-25)
Metabolism	Auxotroph (L-tyrosine auxotroph) (IMG_PIPELINE; 2015-09-25)
Metabolism	Auxotroph (L-tryptophan auxotroph) (IMG_PIPELINE; 2015-09-25)
Metabolism	Auxotroph (L-histidine auxotroph) (IMG_PIPELINE; 2015-09-25)
Metabolism	Prototrophic (Glycine prototroph) (IMG_PIPELINE; 2015-09-25)
Metabolism	Prototrophic (L-asparagine prototroph) (IMG_PIPELINE; 2015-09-25)
Metabolism	Prototrophic (L-glutamine prototroph) (IMG_PIPELINE; 2015-09-25)
Metabolism	Auxotroph (L-isoleucine auxotroph) (IMG_PIPELINE; 2015-09-25)
Metabolism	Auxotroph (L-leucine auxotroph) (IMG_PIPELINE; 2015-09-25)
Metabolism	Auxotroph (L-serine auxotroph) (IMG_PIPELINE; 2015-09-25)
Metabolism	Auxotroph (L-valine auxotroph) (IMG_PIPELINE; 2015-09-25)
Metabolism	(Non-selenocysteine synthesizer) (IMG_PIPELINE; 2015-09-25)
Metabolism	(Non-biotin synthesizer) (IMG_PIPELINE; 2015-09-25)
Metabolism	Auxotroph (Incomplete Coenzyme A biosynthesis) (IMG_PIPELINE; 2015-09-25)

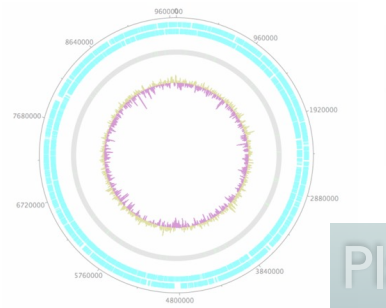




http://www.soes.soton.ac.uk/staff/tt/nf/structure/intro.htm

https://en.wikipedia.org/wiki/Thylakoid

D) Post-Acquisition



Plasmid (35.5 kb)

Chromosome (9.67 Mb)

-COGs Profile and Comparison

		Reference	Draft Genomes				
Category	Category Description	PAL	D-rank	PNG	D-rank	JL	D-rank	JHB
B	Chromatin structure and dynamics	2	0*	2	0*	2	0*	2
C	Energy production and conversion	157	0.03	155	0.05	151	0.02	171
D	Cell cycle control, cell division, chromosome partitioning	43	0.01	40	0.05	35	0.03	39
E	Amino acid transport and metabolism	245	0.05	237	0.03	236	0.02	252
F	Nucleotide transport and metabolism	69	0.01	62	0.01	63	0.03	61
G	Carbohydrate transport and metabolism	141	0.05	155	0.04	137	0.04	153
H	Coenzyme transport and metabolism	210	0.03	206	0.01	194	0.01	210
I	Lipid transport and metabolism	115	0.01	96	0.05	92	0.08	123
J	Translation, ribosomal structure and biogenesis	194	0*	190	0*	188	0*	194
K	Transcription	114	0.03	112	0.09	101	0.08	113
L	Replication, recombination and repair	104	0.09	107	0*	91	0.03	91
M	Cell wall/membrane/envelope biogenesis	296	0.09	285	0.23	293	0.16	326
N	Cell motility	69	0.09	63	0.12	59	0.07	69
O	Posttranslational modification, protein turnover, chaperones	163	0.04	149	0.01	145	0.04	169
P	Inorganic ion transport and metabolism	153	0.04	148	0.01	141	0.02	153
Q	Secondary metabolites biosynthesis, transport and catabolism	171	0.58	118	0.57	112	0.08	161
R	General function prediction only	480	0.02	425	0.04	409	0.03	465
S	Function unknown	211	0.01	203	0.01	200	0.04	226
T	Signal transduction mechanisms	266	0.21	256	0.05	237	0.03	269
U	Intracellular trafficking, secretion, and vesicular transport	62	0.27	39	0.31	42	0.2	49
V	Defense mechanisms	135	0.03	125	0.16	115	0.08	135
W	Extracellular structures	17	0*	16	0*	19	0*	18
X	Mobilome: prophages, transposons	294	0.73	183	0.49	226	0.48	235
B-X	Total	3711		3372		3288		3684

D) Post-Acquisition

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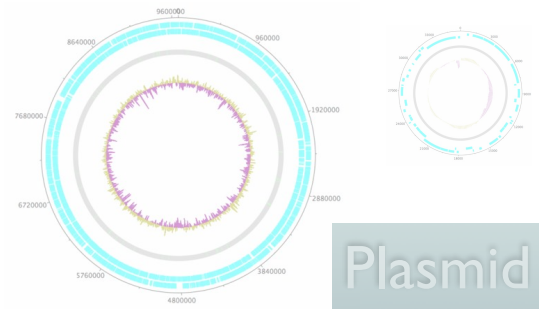
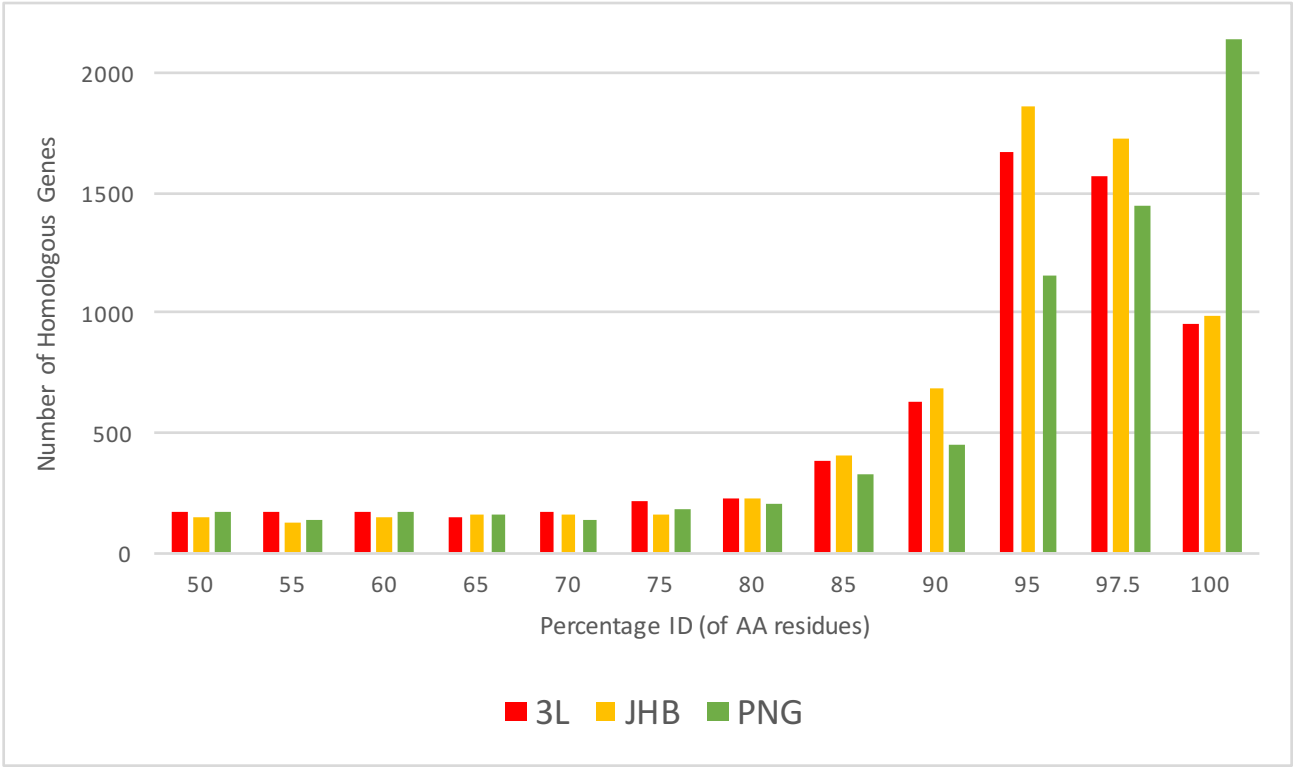


Plasmid (35.5 kb)

Chromosome (9.67 Mb)

-List of Best Homologs Between Genomes



D) Post-Acquisition

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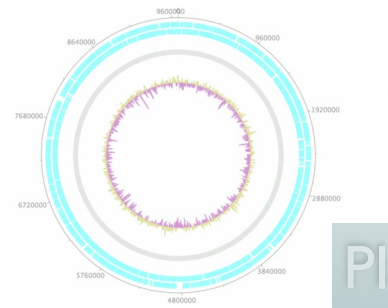
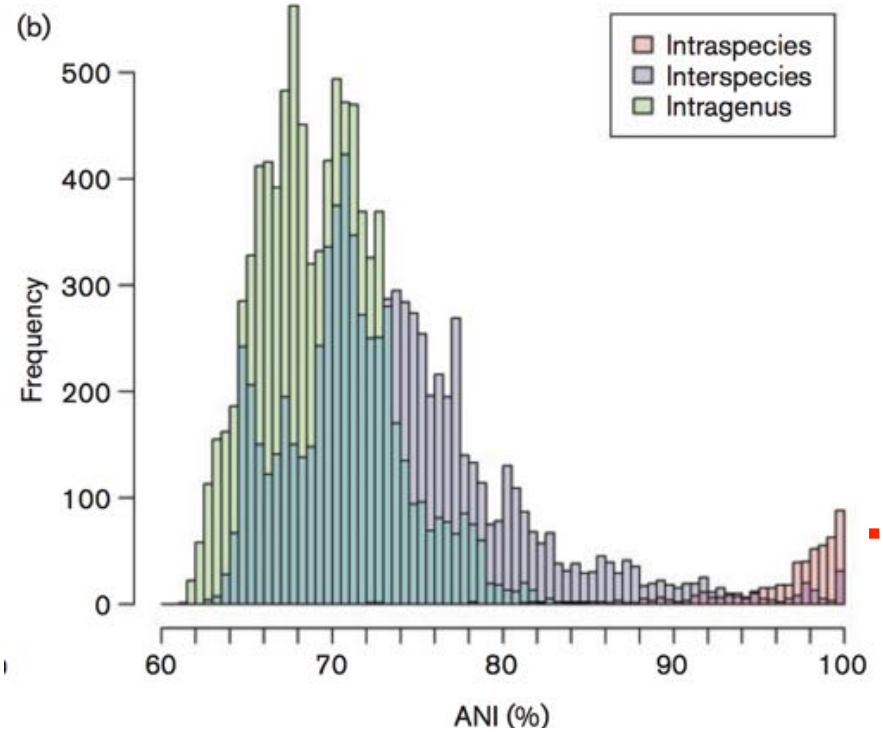


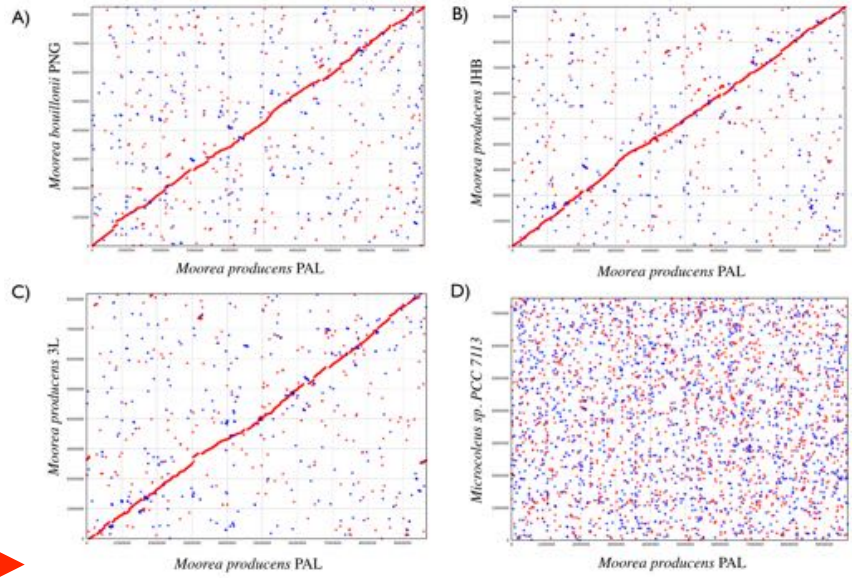
Plasmid (35.5 kb)

Chromosome (9.67 Mb)

-Average Nucleotide Identity



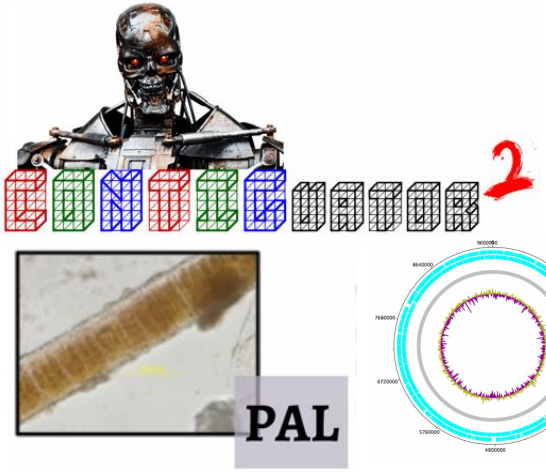
-Synteny Plots



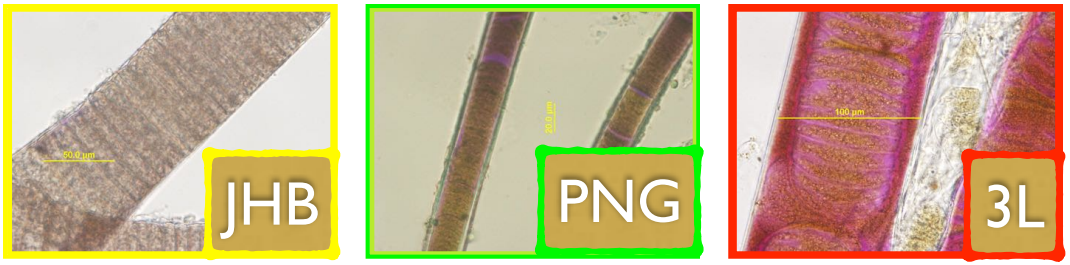
Find the Best Reference Genome

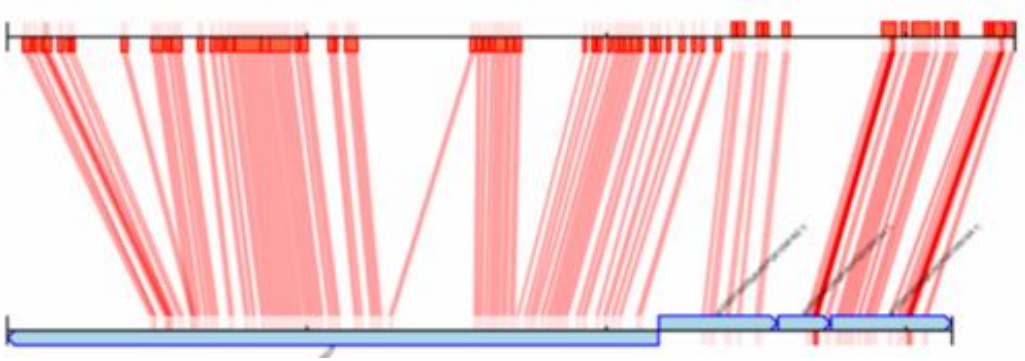
D) Post-Acquisition

How to use my new genome?



Reference Assembly

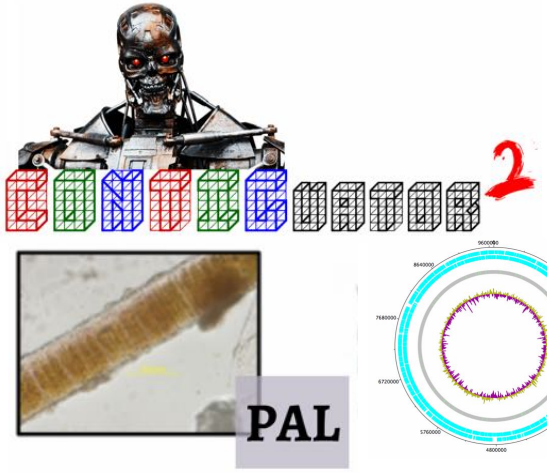




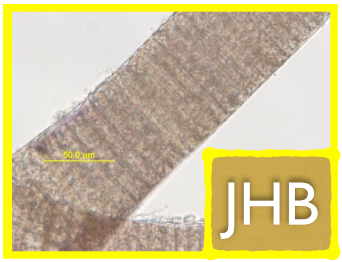
Be careful on your comparisons after reference assembly
Make sure to do not discard **important(?)** unmapped contigs

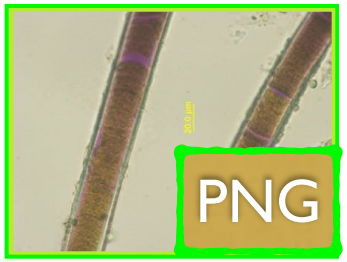
D) Post-Acquisition

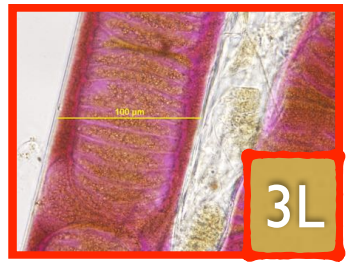
How to use my new genome?



Reference Assembly





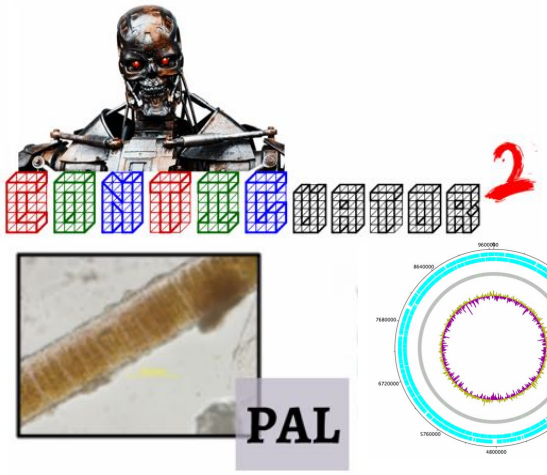


Genome	Sequencing Method	Avg. Read Lengths	Current Size	# Contigs	GC Content	N50
M. producens PAL	**Illumina MiSeq + PacBio**	**300 bp PE + 10 kb**	**9.67 Mb**	**1**	**43.53%**	—
M. producens JHB	Illumina HiSeq	100bp PE	9.64 Mb	2435	43.65%	18,820
M. bouillonii PNG	Illumina HiSeq + PacBio	100bp PE	8.61 Mb	913	43.67%	135,461
M. producens 3L	Sanger + 454 (MDA)	600-800bp	8.38 Mb	287	43.68%	66,028

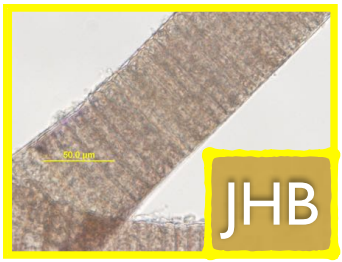
Before

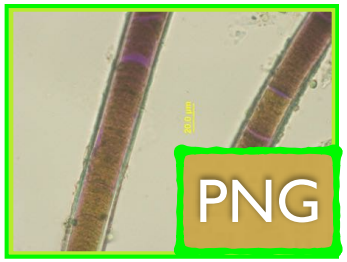
D) Post-Acquisition

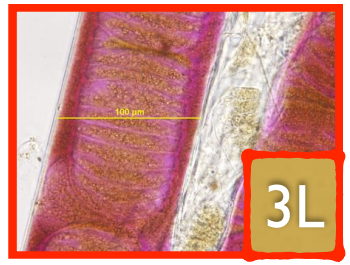
How to use my new genome?



Reference Assembly





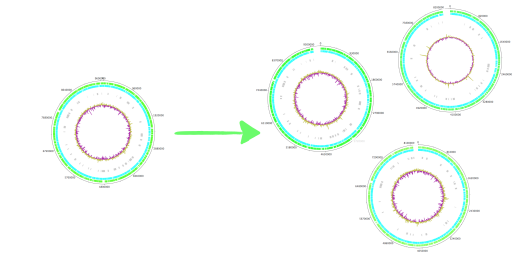


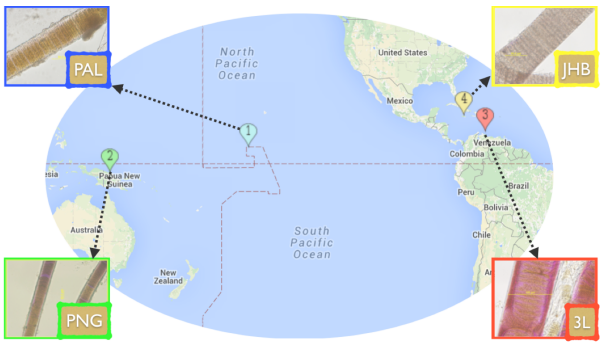
Genome	Sequencing Method	Unmapped Contigs	Scaffold Size	# Contigs	GC Content	N50
M. producens PAL	Illumina MiSeq + PacBio	1 (plasmid)	9.67 Mb	1	43.52%	—
M. producens JHB	Illumina HiSeq	2 (plasmid[s])	9.35 Mb	205	43.67%	—
M. bouillonii PNG	Illumina HiSeq + PacBio	12 (56 kb)	8.23 Mb	291	43.63%	8,262,658
M. producens 3L	Sanger + 454 (MDA)	78 (199 kb)	8.15 Mb	204	43.68%	8,171,464

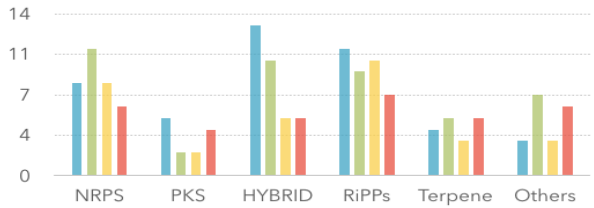
After

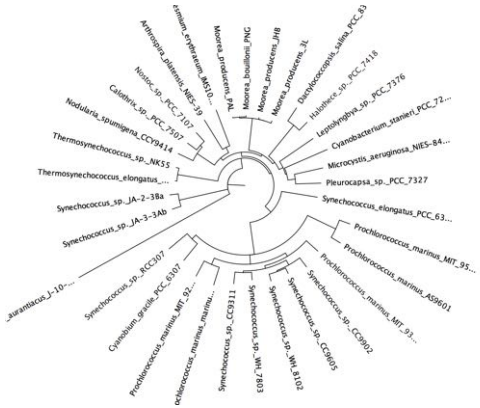
Genome Comparison

First complete genome of a natural product rich filamentous marine cyanobacterium gives insights into a gap in the tree of life: the genus *Moorea* is metabolically and genetically distinct from all known cyanobacteria



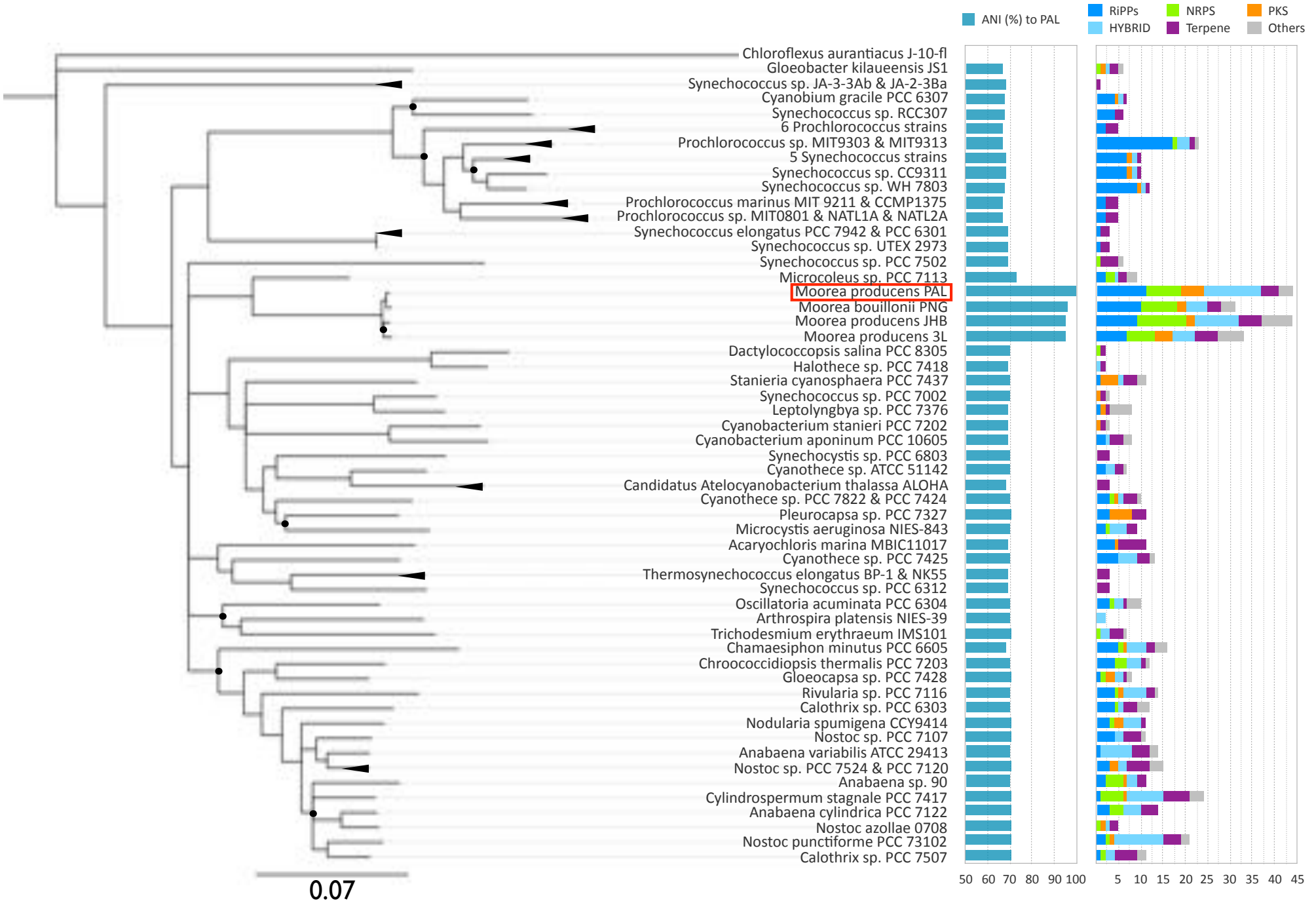






Leao TF, Korobeynikov A, Monroe E, Podell S, Allen E, Gerwick WH, Gerwick L. Under progress. Proposed Journal: PNAS

SOME DATA => Genomes Comparison => Moorea are different from all known cyanobacteria



Dotted bootstraps are higher than 50 and lower than 80, concatenated branches represent multiple sequences with bootstrap 100

Thanks to:

All the Gerwick Lab
(former and current)

Dr William Gerwick
Dr Lena Gerwick
Dr Evgenia Glukhov
Nathan Moss

SCIENCE WITHOUT BORDERS

CAPES

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Dr Sheila Podell
Allen Lab (SIO)

Dr Luke Thompson
Knight Lab (UCSD)

Dr Tristan Carland
Former Gerwick Lab
Illumina®

Uma pequena mensagem: