



# USING PROXIMITY TO FIX ASSEMBLY

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# Long-Read Sequencing

Web: [phasegenomics.com](http://phasegenomics.com)  
Twitter: @PhaseGenomics



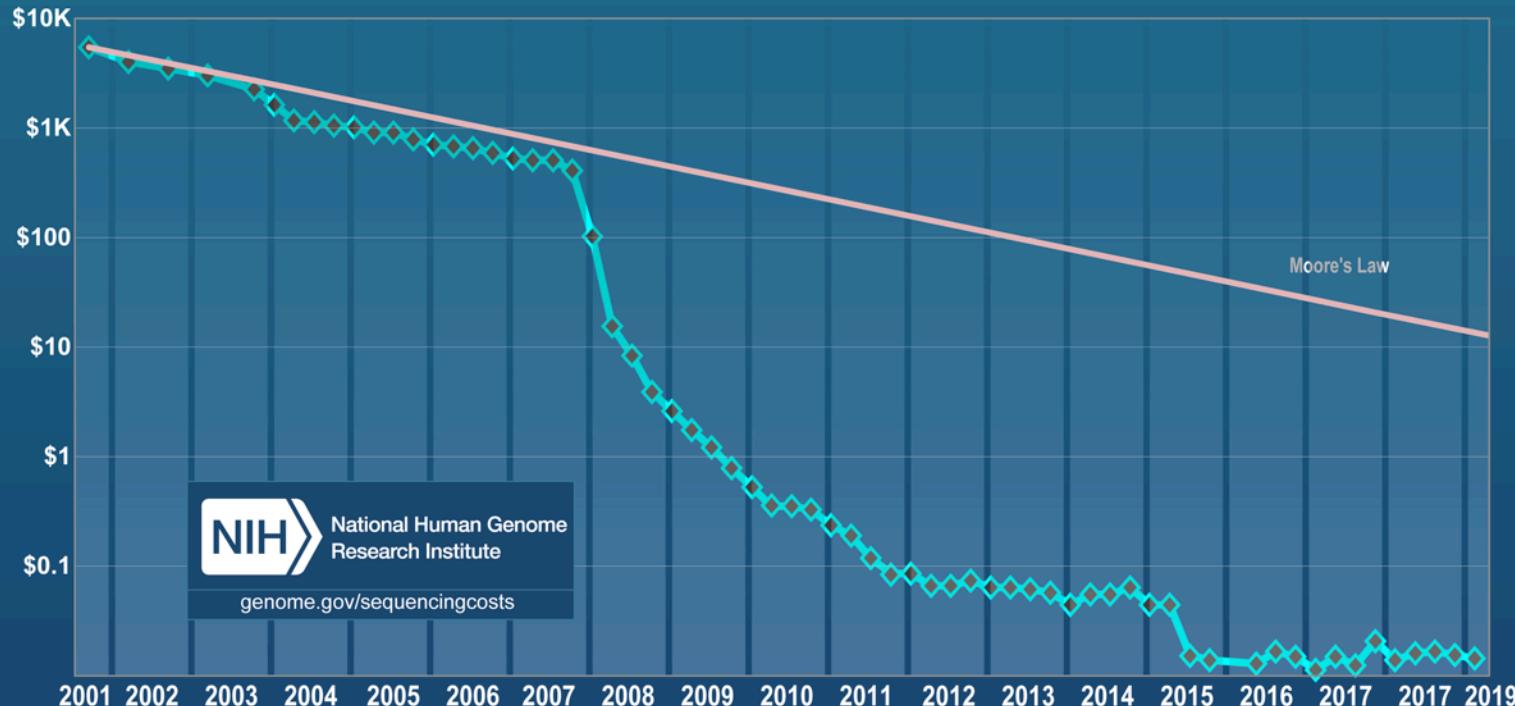
# Long-Range Long-Read Sequencing

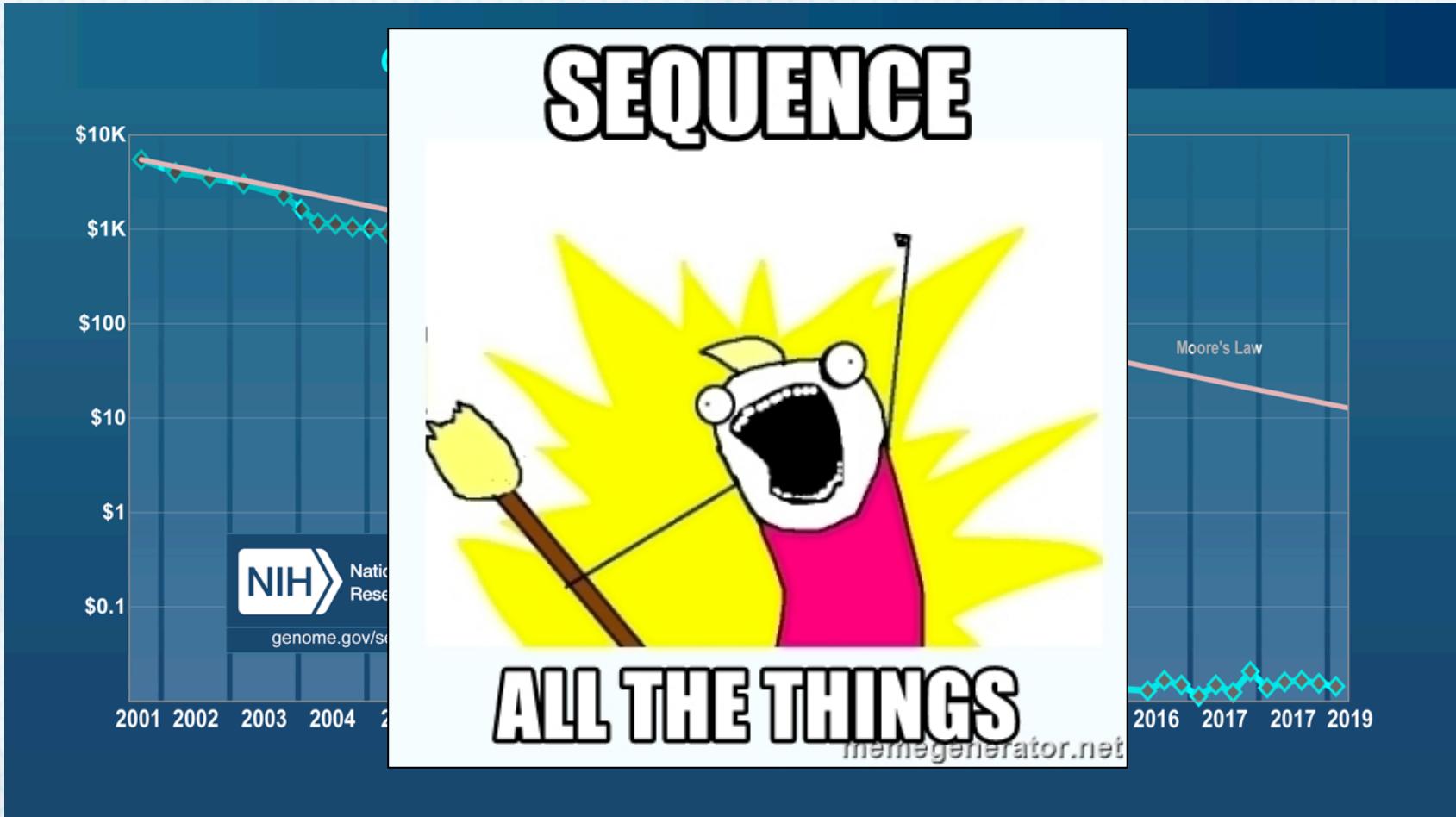
Web: [phasegenomics.com](http://phasegenomics.com)  
Twitter: @PhaseGenomics

If you take away nothing else...

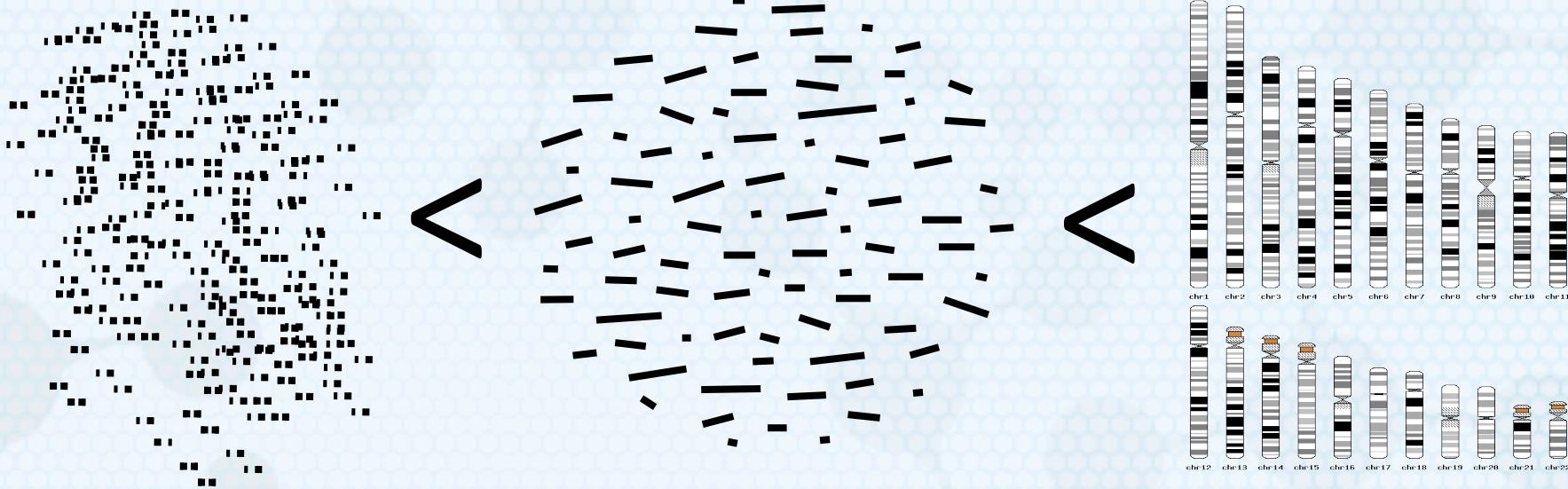
Hi-C data measure the frequency of physical interaction among loci

## *Cost per Raw Megabase of DNA Sequence*

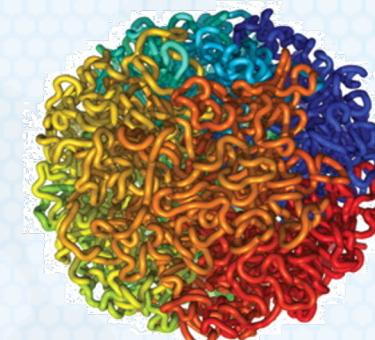
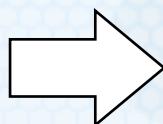
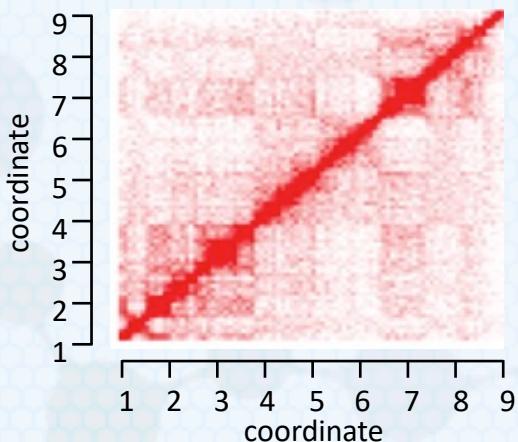
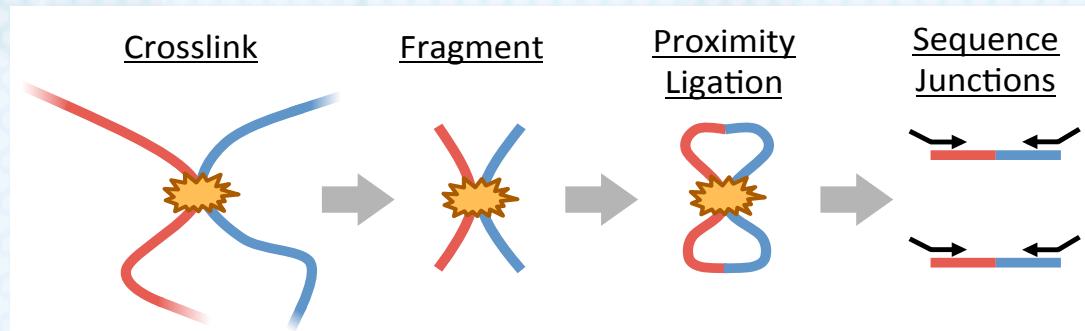




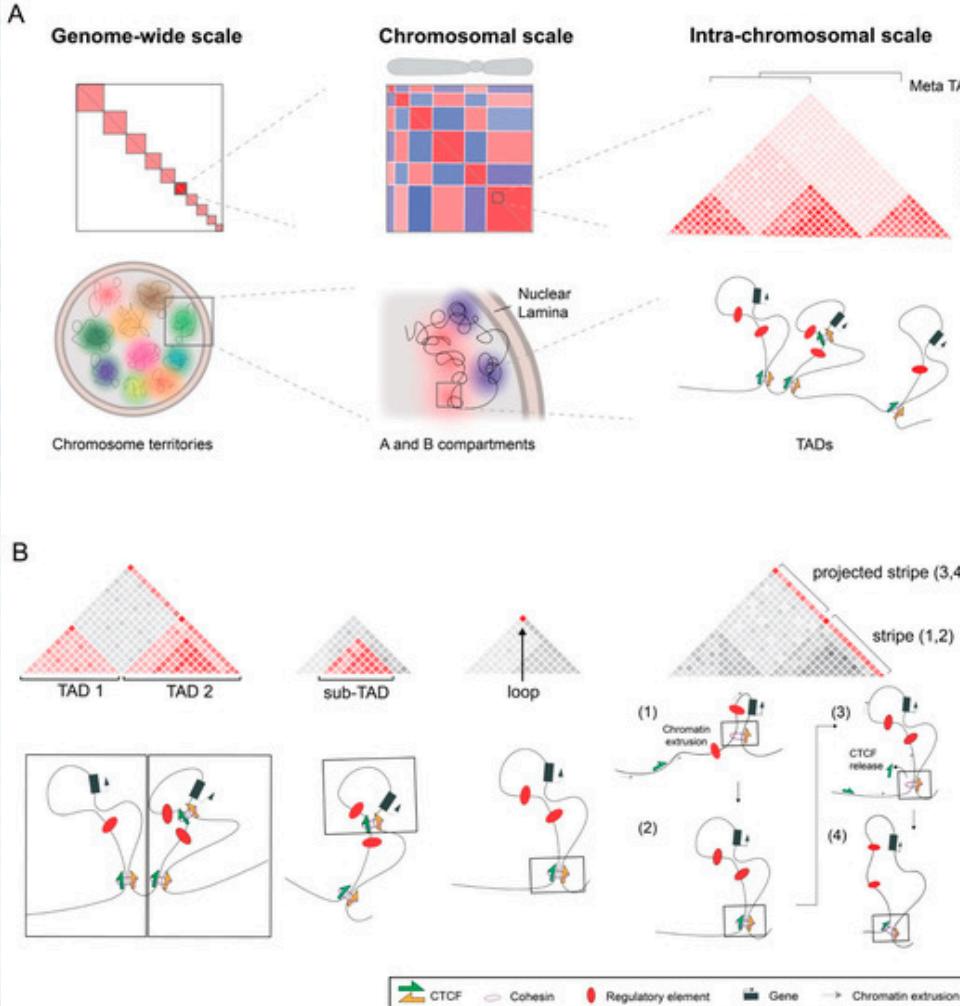
# Reads < Contigs < Genome



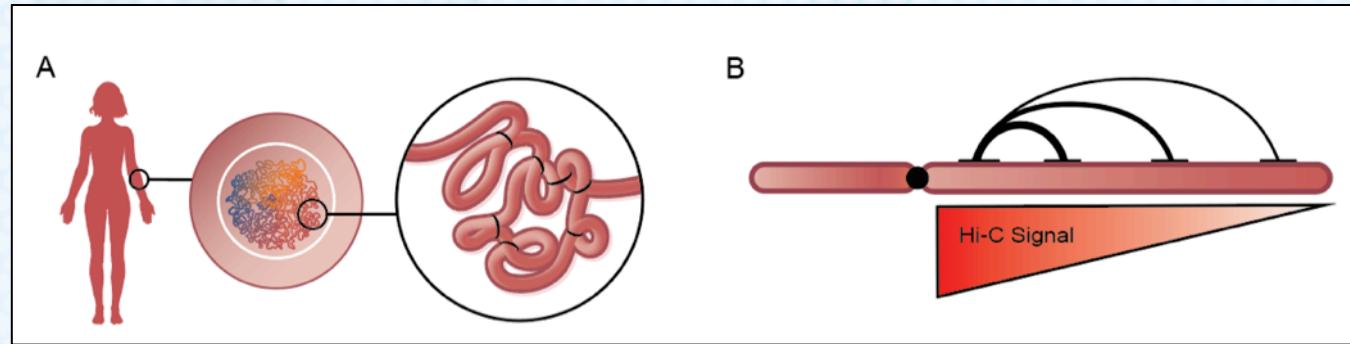
# Proximity Ligation (Hi-C) captures the 3D structure of chromosomes



Lieberman-Aiden, et. al. Science, 2009

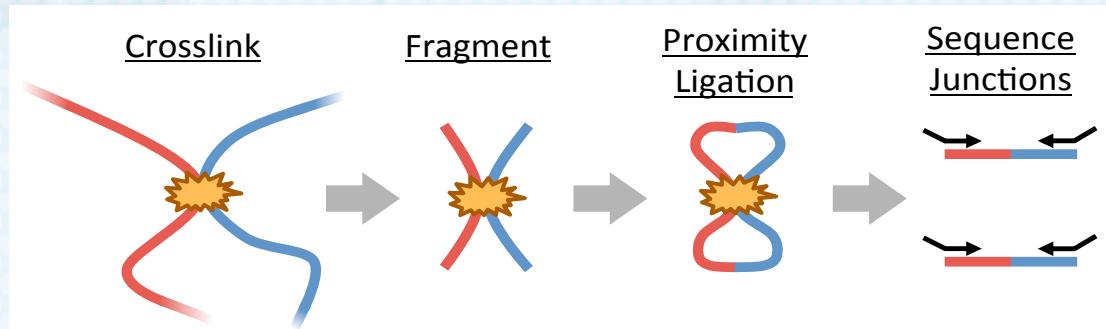


# Proximity Ligation captures ultra-long genomic contiguity



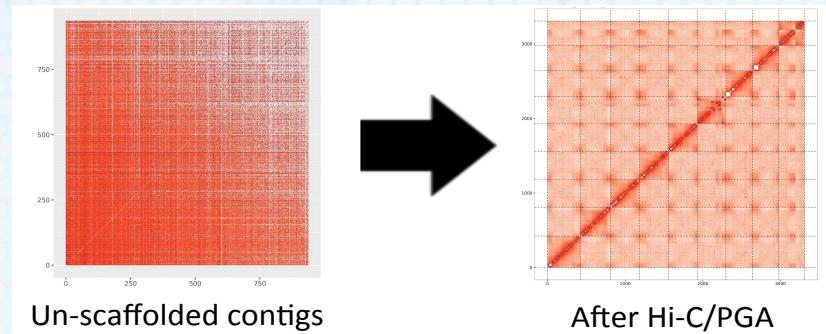
- Proximity in 3D is correlated with genomic distance
- Can be used to:
  - Scaffold and phase a genome of any size
  - Find rearrangements

# Using Hi-C to assemble chromosome-scale genome scaffolds

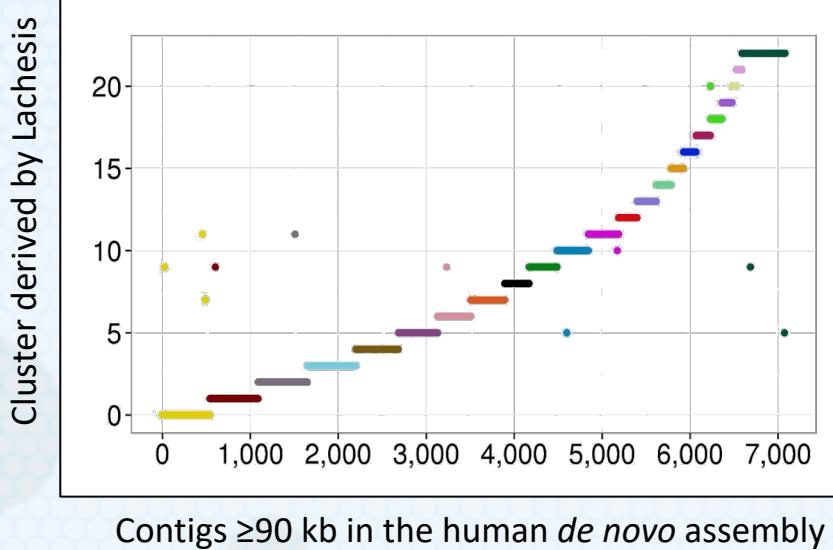


## Proximity-Guided Assembly™ :

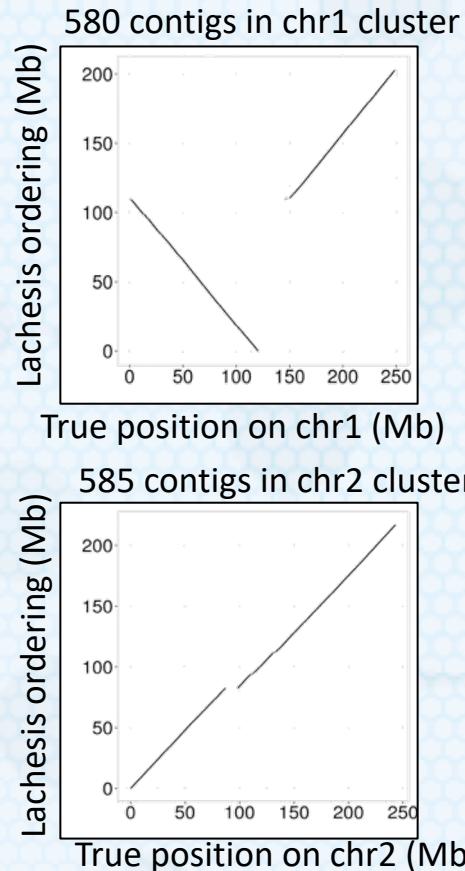
- Clustering contigs into chromosome groups
- Ordering and orienting the contigs in scaffolds



# Assembling the human genome using Hi-C



>99.8% of contigs clustered correctly  
 >97% of contigs ordered correctly



# Hi-C becomes a routine tool in eukaryotic genome assembly



- Human (Burton *et al.* 2013 *Nature Biotech*)
- Goat (Bickhart *et al.* 2017 *Nature Genetics*)
- Stickleback (Peichel *et al.* 2017 *Heredity*)
- Amaranth (Lightfoot *et al.* 2017 *BMC Biology*)
- Firefly (Fallon *et al.* 2017 *BioRxiv*)
- Black raspberry (Jibran *et al.* 2018 *Hort. Res.*)
- Clownfish (Lehmann *et al.* 2018 *BioRxiv*)
- Sugar beet (Funk *et al.* 2018 *Plant J.*)
- Malaria Mosquito (Ghurye *et al.* 2018 *BioRxiv*)
- *Cannabis* (McKernan *et al.* 2018 *OSF*)
- *Cannabis* (Grassa *et al.* 2018 *BioRxiv*)
- *E. festucae* (Winter *et al.* 2018 *PLoS Genetics*)
- Honeybee (Wallberg *et al.* 2018 *BioRxiv*)
- Aphid (Chen *et al.* 2018 *BioRxiv*)
- *T. inflatum* (Olarte, *et al.* 2019 *BMC Genomics*)
- Bee mites (Techer *et al.* 2019 *BioRxiv*)
- ...more from other labs...

Michelle Vierra  
@the\_mviera

Follow

Goat genome is the "greatest of all time" (G.O.A.T...get it?) 😂@PacBio #genomepuns genome.gov/27567880/



SCIENCE

## The Game-Changing Technique That Cracked the Zika-Mosquito Genome

"Hi-C" will make it much easier and cheaper to assemble all of an organism's genetic material from scratch.

ED YONG MAR 29, 2017



MORE STORIES

A Troubling Discovery in the Deepest Ocean Trenches ED YONG

Grieving Parents Are Turning to Posthumous IVF SHIRA RUBIN AND UNDARK

NASA Is Rushing to the Moon MARINA KOREN

The Mystery of



# Time To Get Into It

## Basic Scaffolding Steps

1. Generate contigs/intermediate scaffolds
2. Align Hi-C data
3. QC Hi-C data
4. (optional) Phase contigs with Hi-C
5. Scaffold with Hi-C (one phase at a time)
6. Correct scaffolds (one phase at a time)
7. (optional) Re-phase scaffolds with Hi-C

## Basic Scaffolding Steps

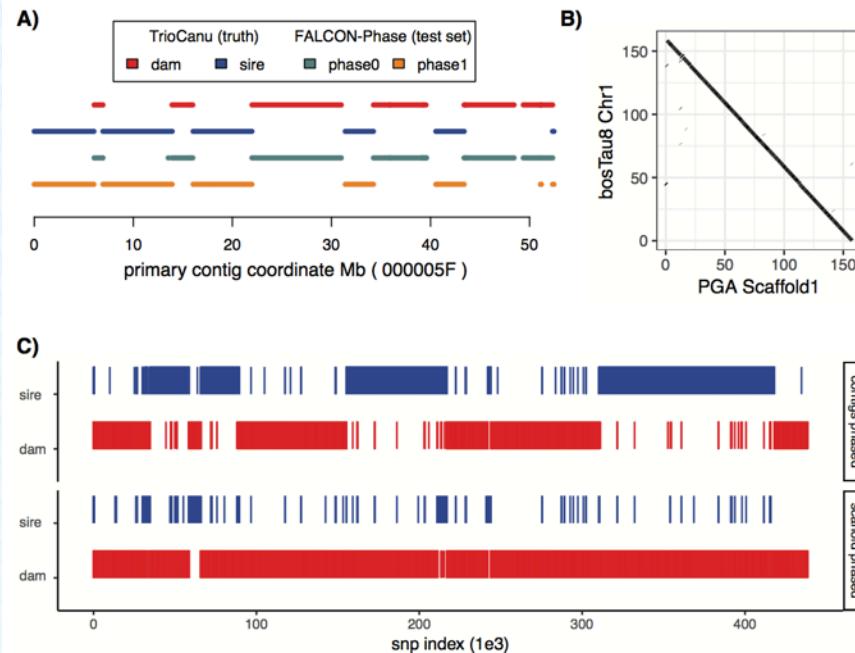
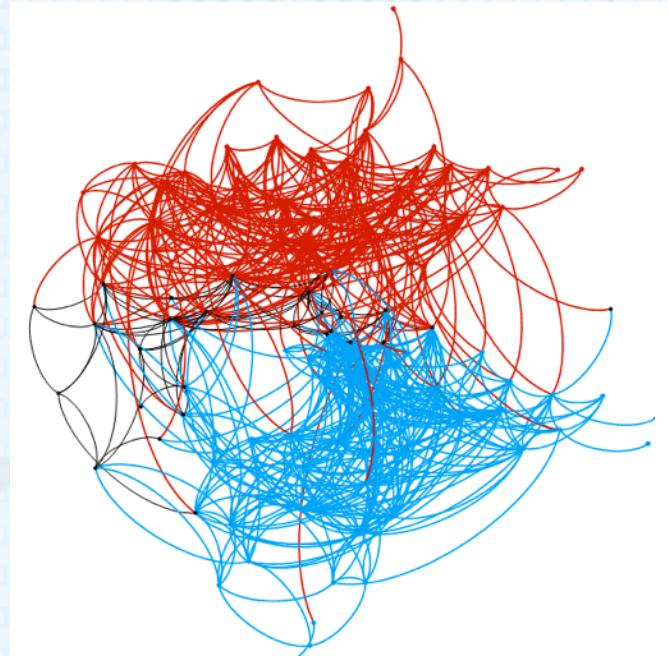
1. Generate contigs/intermediate scaffolds
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6. Correct scaffolds (one phase at a time)
7. (optional) Re-phase scaffolds with Hi-C

## 2. Align Hi-C data

- Install:
  - **BWA:** `module load bwa/0.7.17`
  - **SAMtools:** `module load samtools`
  - **samblaster:** `module load samblaster`
- Follow steps at <https://phasegenomics.github.io/2019/09/19/hic-alignment-and-qc.html>
  - FASTA is already indexed (`bwtsw`)
  - Use 16 CPUs in BWA-MEM (allocate that many in SLURM, 16GB RAM and 2-hour time limit)
  - Write BAM to your home directory (or another easy to access location)
  - Data: `/share/workshop/genome_assembly/phase_genomics_2020_data_fungus/`

# More Theory While We Wait

# FALCON-Phase: Combining SMRT and Hi-C data to generate fully phased genome assemblies.



## Chromosome-scale de novo assembly and phasing of a Chinese indigenous pig genome

Yalan Yang, Jinmin Lian, Bingkun Xie, Muya Chen, Yongchao Niu, Qiaowei Li, Yuwen Liu, Guoqiang Yi, Xinhao Fan, Yijie Tang, Jiang Li, Ivan Liachko, Shawn T. Sullivan, Bradley Nelson, Erwei Zuo, Zhonglin Tang  
**doi:** <https://doi.org/10.1101/770958>

This article is a preprint and has not been certified by peer review [what does this mean?].

**Abstract**

Info/History

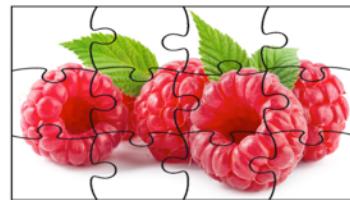
Metrics

 Preview PDF



## KeyGene delivers first-ever fully phased red raspberry genome

October 22, 2019



KeyGene completed the assembly of the red raspberry genome this summer and delivered the final results to the BerryWorld Plus Ltd. berry breeding programme, offering its breeders a unique and practical genome resource. This high-quality reference genome was delivered after Berry World Plus won KeyGene's Genome Insight event in March.

### MORE NEWS

BerryWorld Plus™ wins KeyGene's genome-for-free contest



# Assembling the highest quality and contiguity human genome to date



**Phase Genomics and Pacific Biosciences Assemble the Most Contiguous Human Genome to Date by Combining Long-Reads and Hi-C Data.**

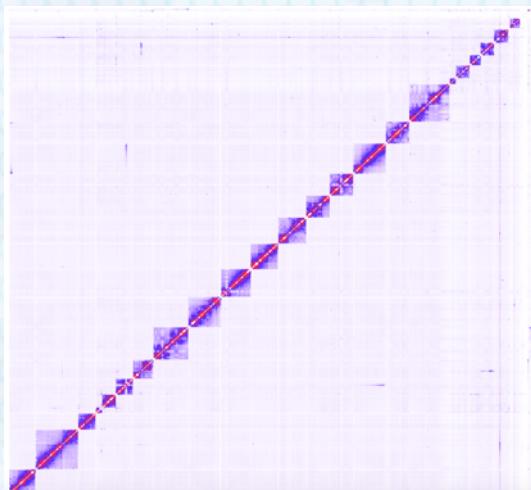
[READ MORE](#)

## Pacific Biosciences Releases Highest-Quality, Most Contiguous Individual Human Genome Assembly to Date

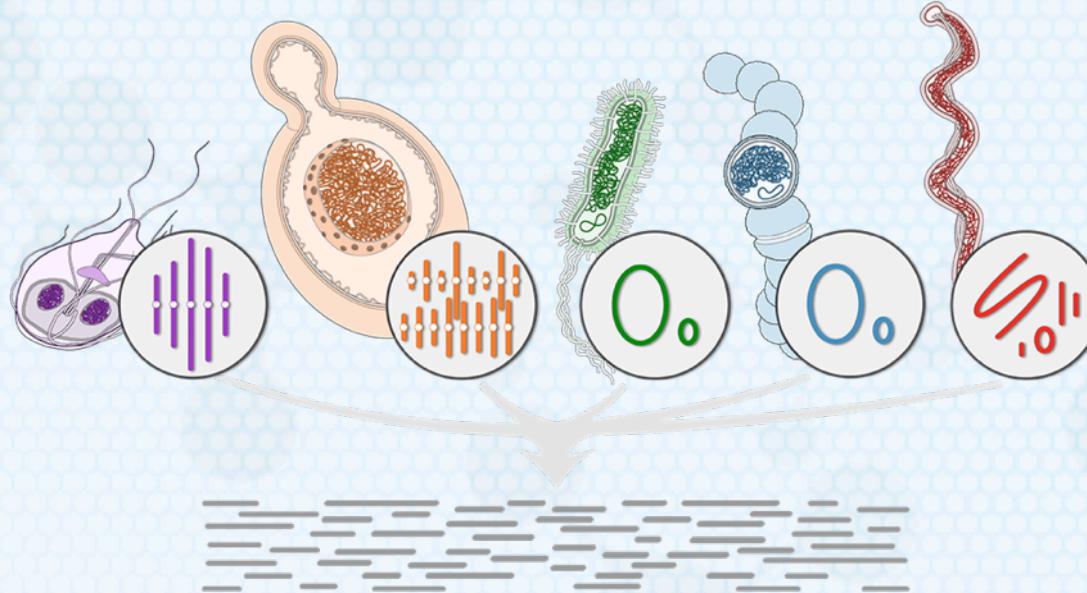
Monday, October 8, 2018

**Diploid Assembly of a Puerto Rican Female Adds a Rich Resource to Population-Specific Reference Genomes**

MENLO PARK, Calif., Oct. 08, 2018 (GLOBE NEWSWIRE) — Pacific Biosciences of California, Inc. (Nasdaq:PACB), the leading provider of high-quality sequencing of genomes, transcriptomes and epigenomes, today announced it has produced the most contiguous diploid human genome assembly of a single individual to date, representing the

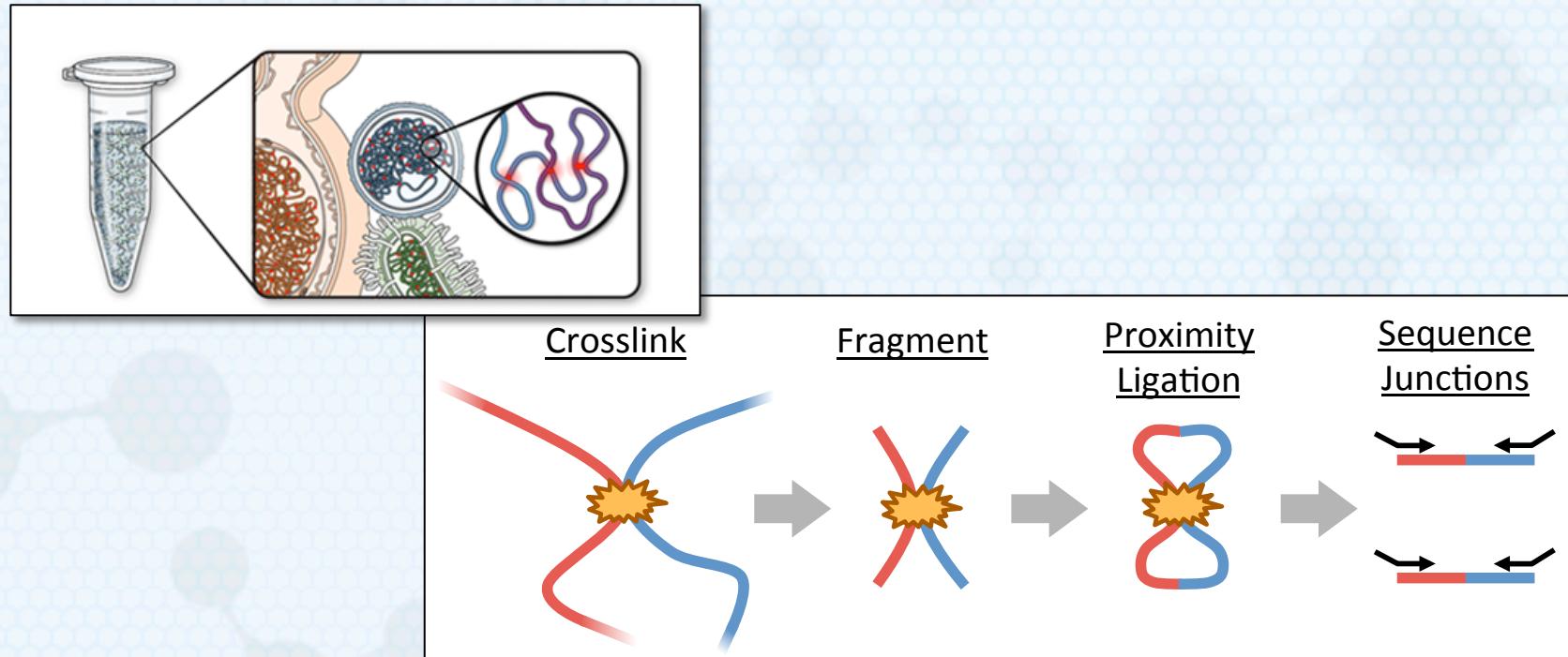


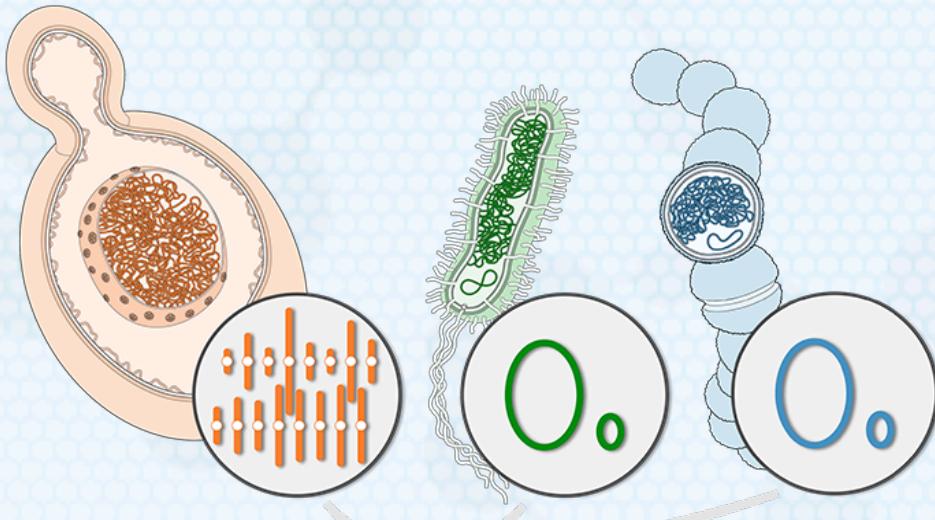
# The problem with shotgun metagenomics



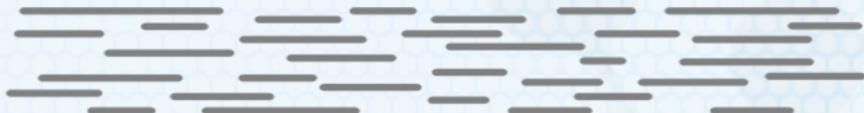
- Cannot tell which sequences belong to which organism
- Binning methods are inaccurate, hard to reproduce
- No way to track plasmids / viruses / antibiotic resistance
- Missing lots of organisms that are not in databases

# Any sequences that interact by Proximity must have originated from the same cell

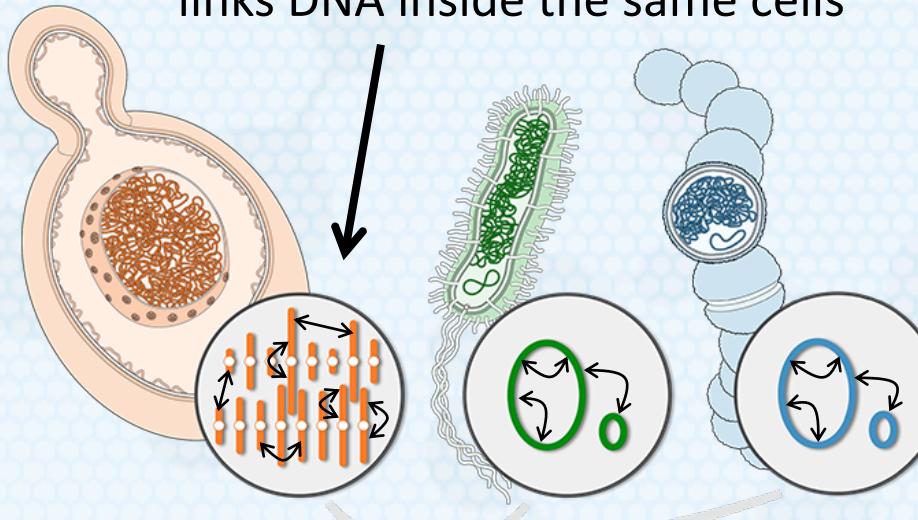




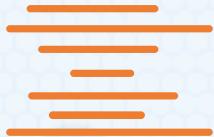
Shotgun sequencing



Proximity ligation chemically links DNA inside the same cells

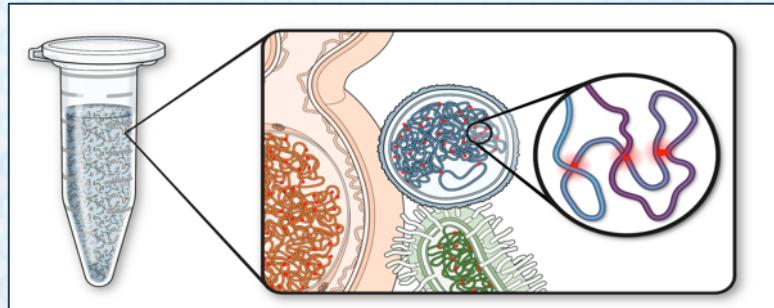


Connects metagenome  
sequences

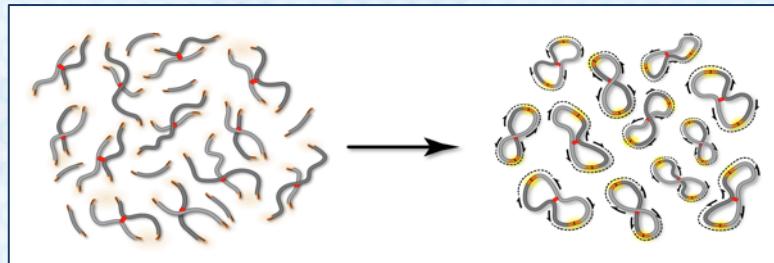


# Proximity-Guided Metagenome Assembly (ProxiMeta™)

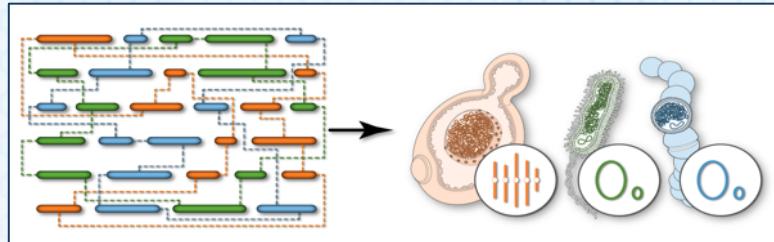
Crosslink intact cells to capture intra-cellular interactions



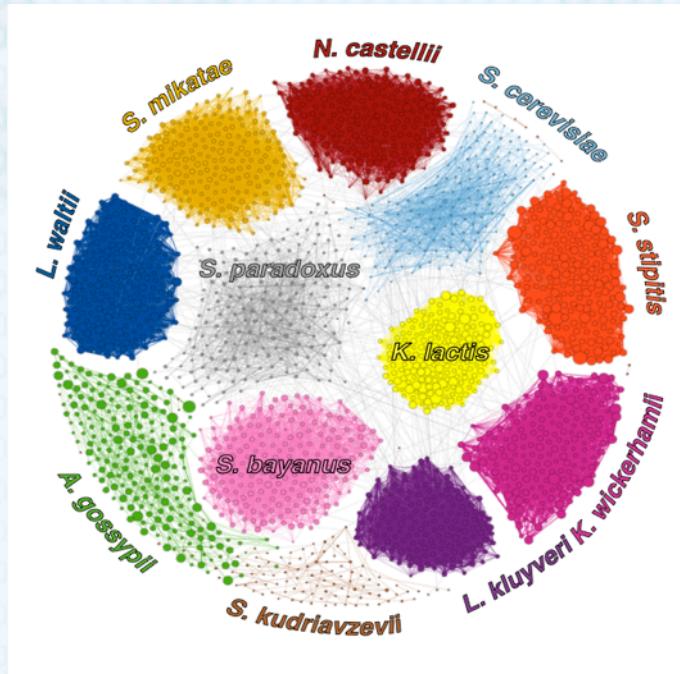
Isolate and sequence crosslinked junctions



Use proximity connections to deconvolute metagenome

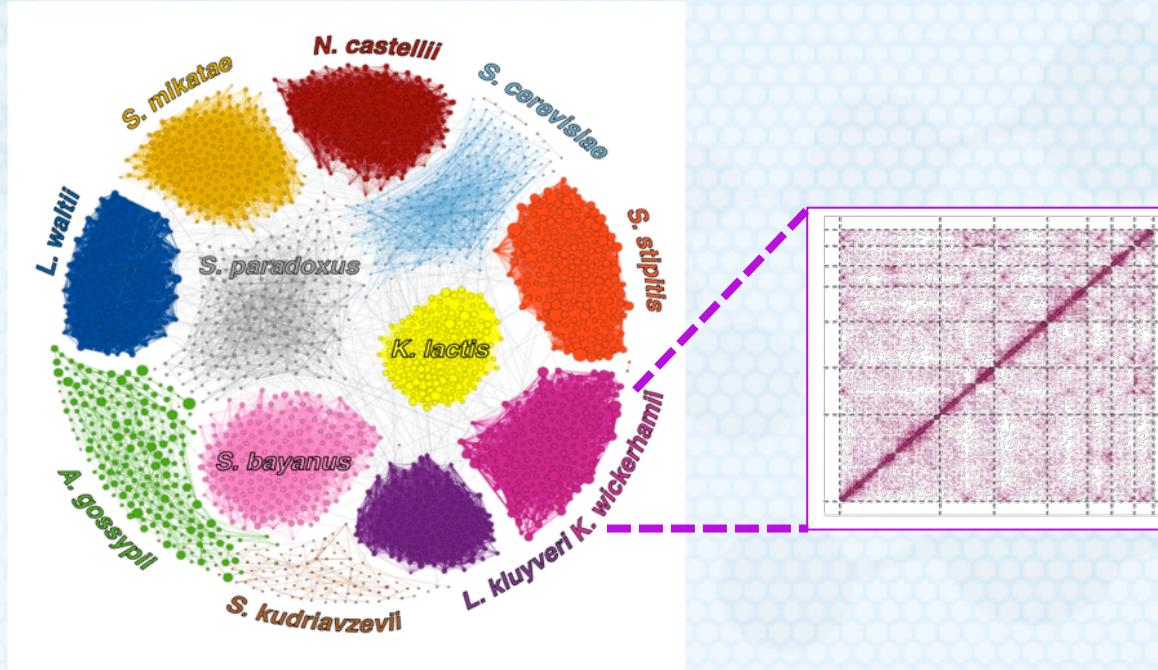


# Reference-quality pro- and eu- karyotic genomes from mixed populations



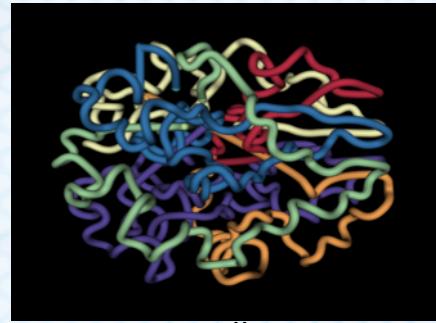
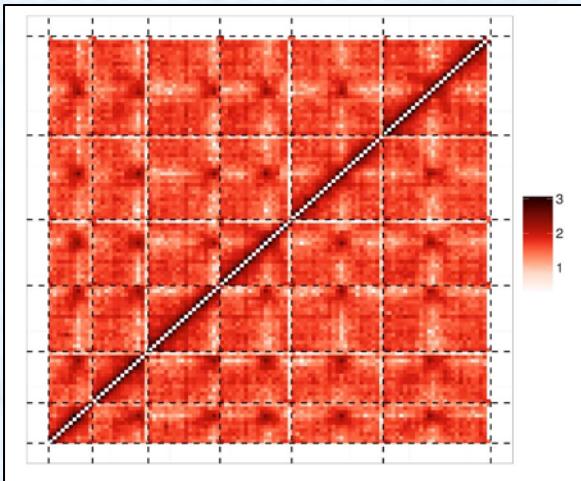
Draft assembly:  
Size = 135.2 Mbp  
Contig N50 = 17.3 Kb  
**Error rate <1%**

# Reference-quality pro- and eu- karyotic genomes from mixed populations

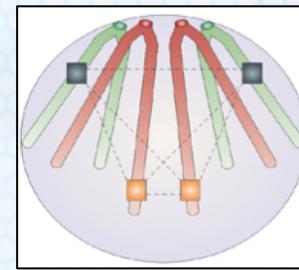
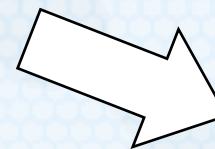


# 3D modeling of genomes directly from mixed populations

*Kluyveromyces lactis*



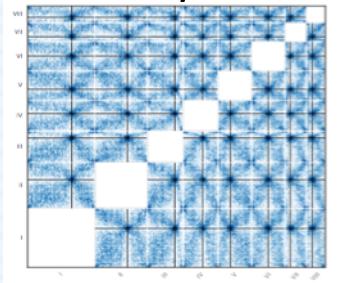
Nelle Varoquaux



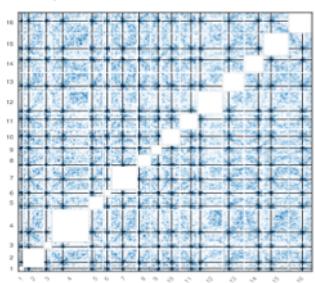
Barzel and Kupiec, 2008

\*Dark spots in the middle of each chromosome are centromeric regions.

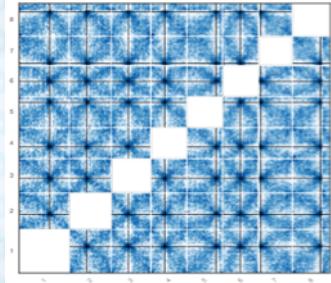
*S.stipitis*



*S.paradoxus*

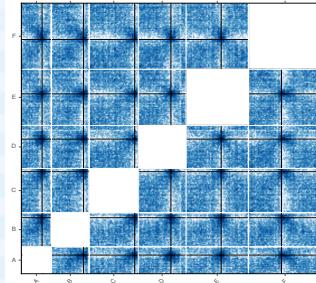


*L.waltii*

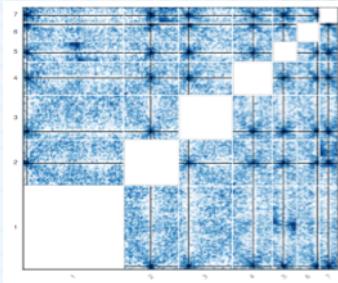


K.lactis PHASE  
NOMICS

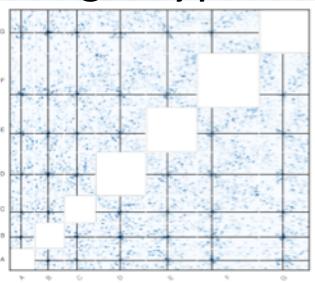
*K.lactis*



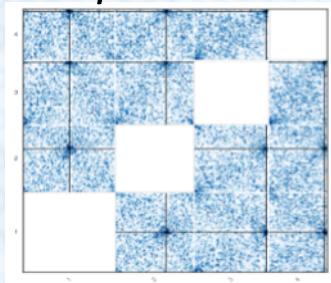
*K.wickerhamii*



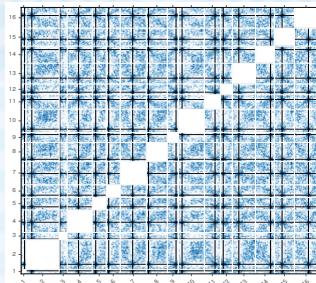
*A.gossypii*



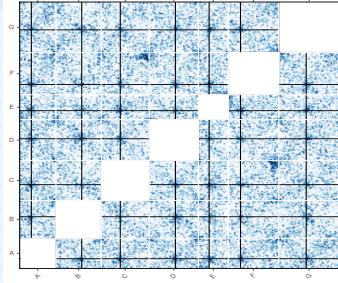
*P.pastoris*



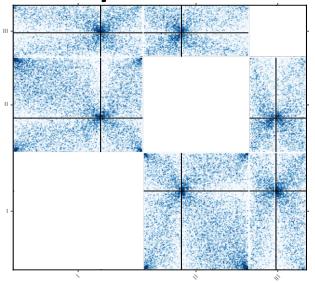
*S.uvarum*



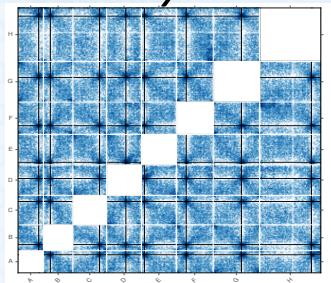
*Z.rouxii*



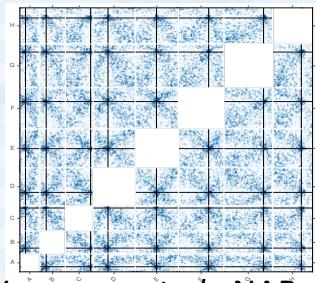
*Sz. pombe*



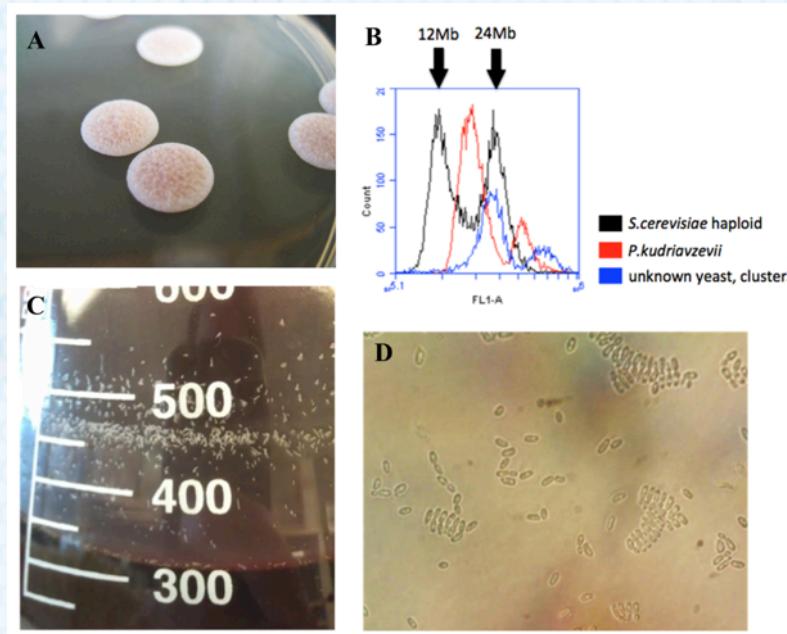
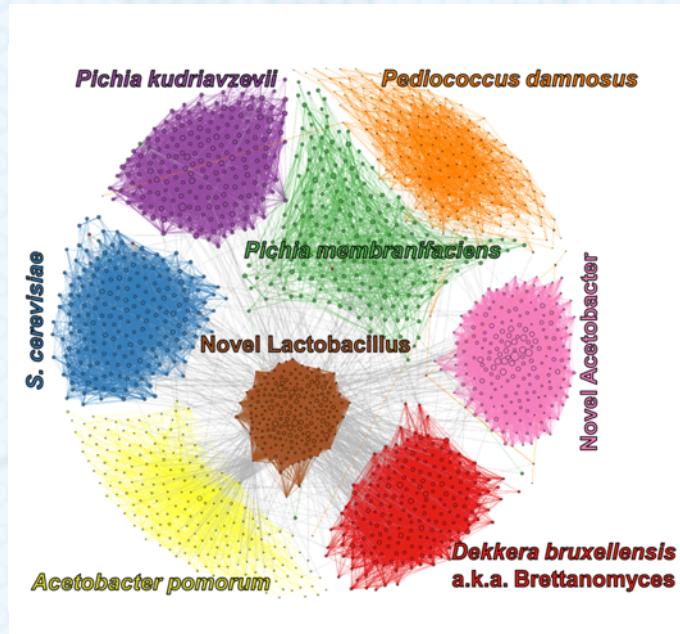
*L.kluyveri*



*L.thermotolerans*



# Assembly of a hybrid yeast from a beer metagenome



# Assembly of a hybrid yeast from a beer metagenome

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Open fermentation vessels at Pilsner Urquell brewery in Pilsen, Czech Republic.  
kgphoto/Alamy Stock Photo

Microbe new to science found in self-fermented beer  
By Aleszu Bajak | Jul. 28, 2017, 1:30 PM

In May 2014, a group of scientists took a field trip to a small brewery in an old warehouse in

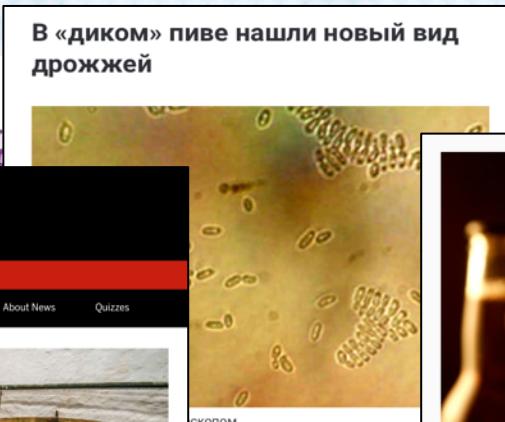
**Pichia kudriavzevii**

**В «диком» пиве нашли новый вид дрожжей**

СКОПОМ  
*bioRxiv:130722*

ские ученые обнаружили в пиве, ферментированном с помощью диких дрожжей, новый вид дрожжей. Отчет о работе доступен на сайте [bioRxiv](#), также о ней пишет *Science*.

**Yeast**  
*ekkera bruxellensis*  
k.a. *Brettanomyces*



**WILDES BIER**

**Neue Mikrobenart in Brauerei entdeckt**

Eigentlich wollten US-Biologen in einer Brauerei bloß das Erbgut exotischer Hefepilze analysieren. Doch in einer Probe entdeckten sie etwas Unerwartetes.

von Robert Gast

**CIENCIA / BEBIDAS ALCOHÓLICAS**

La mayoría de cervezas se fermentan con la ayuda de levaduras como *Saccharomyces cerevisiae*.

**Esta es la cerveza artesana que ha permitido descubrir un nuevo microbio**

Un equipo de la Universidad de Seattle encuentra por casualidad una levadura desconocida hasta la fecha por la comunidad científica.

1 agosto, 2017 - 02:25

EN: CERVEZA MICROBIOLOGÍA Y PARASITOLOGÍA SEATTLE

José Andrés Gómez

**B**

© iStock / Yuri Arcurs (Ausschnitt)

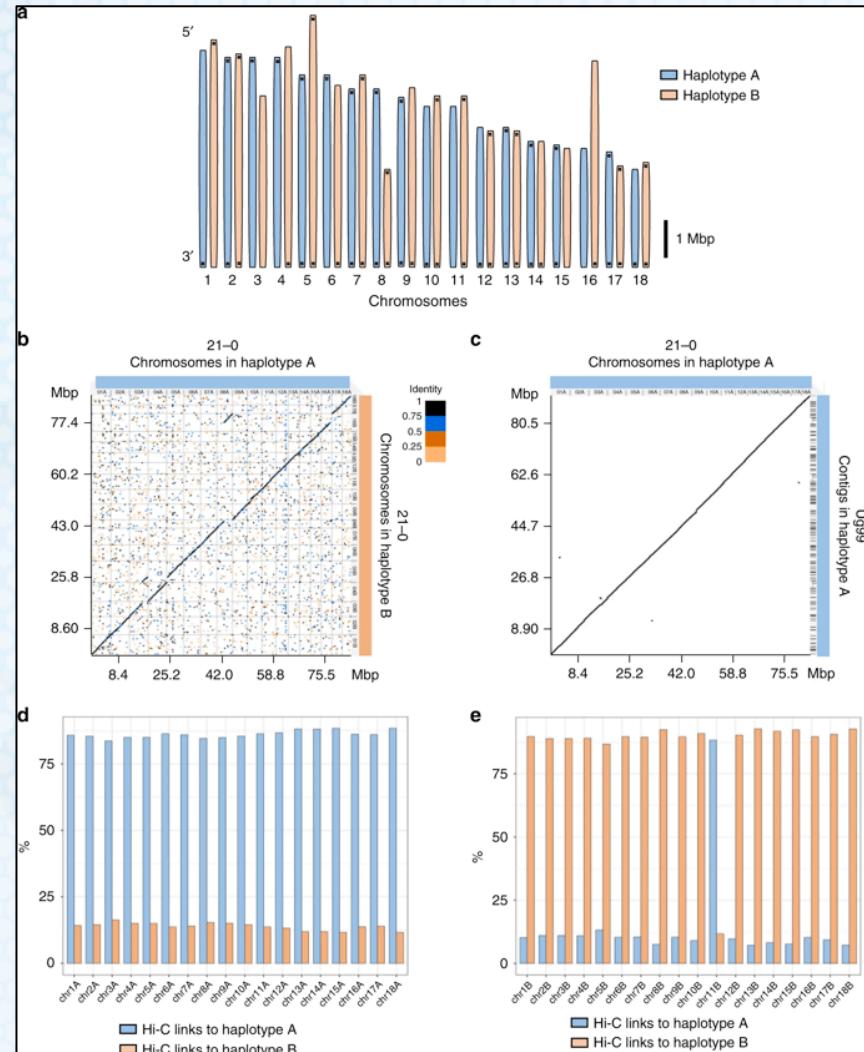
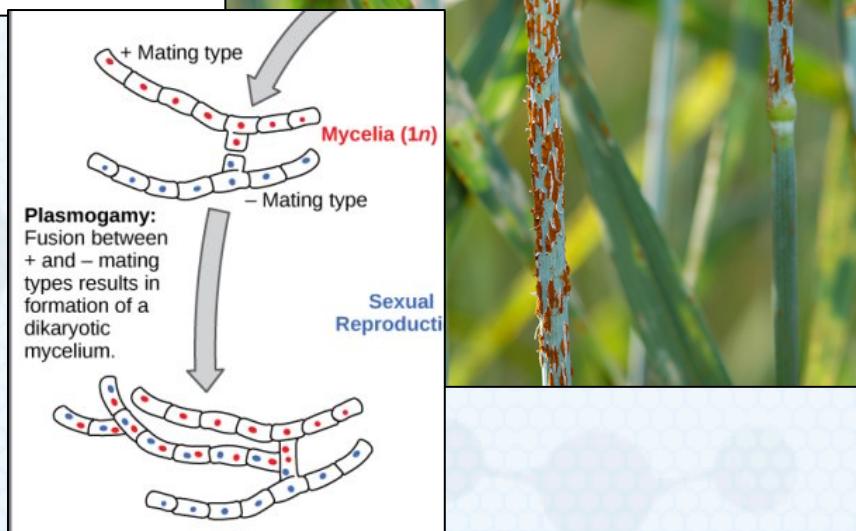
Heil, Burton, Liachko, et al., Yeast, 2017

# Emergence of the Ug99 lineage of the wheat stem rust pathogen through somatic hybridisation

Feng Li, Narayana M. Upadhyaya, Jana Sperschneider, Oadi Matny, Hoa Nguyen-Phuc, Rohit Mago, Castle Raley, Marisa E. Miller, Kevin A. T. Silverstein, Eva Henningsen, Cory D. Hirsch, Botma Visser, Zacharias A. Pretorius, Brian J. Steffenson, Benjamin Schwessinger, Peter N. Dodds & Melania Figueroa

Nature Communications 10, Article number: 5068 (2019) | Cite this article

906 Accesses | 121 Altme



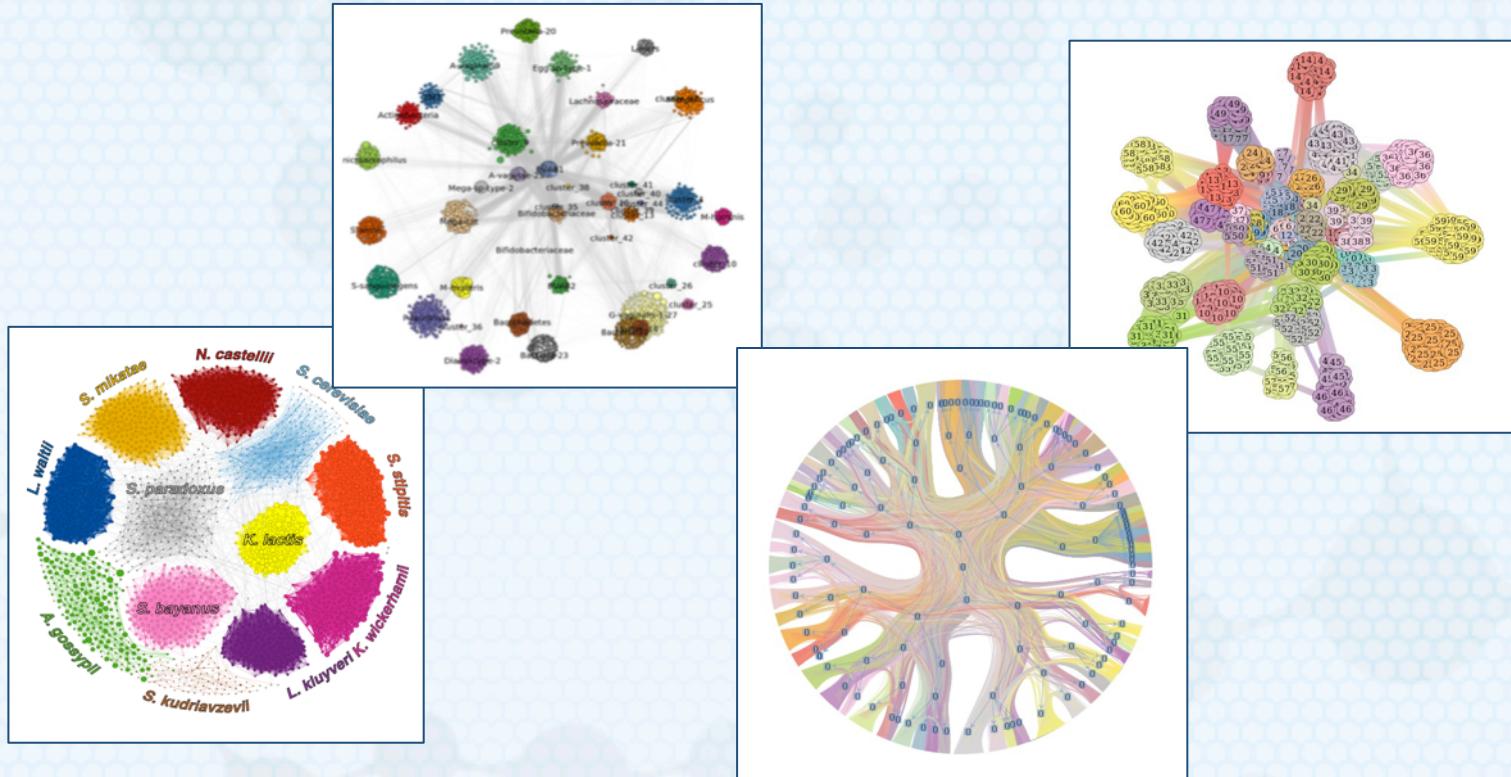
# Scaffolding a contaminated plant sample

(plant genome assembly contains >35% fungal/bacterial contigs)

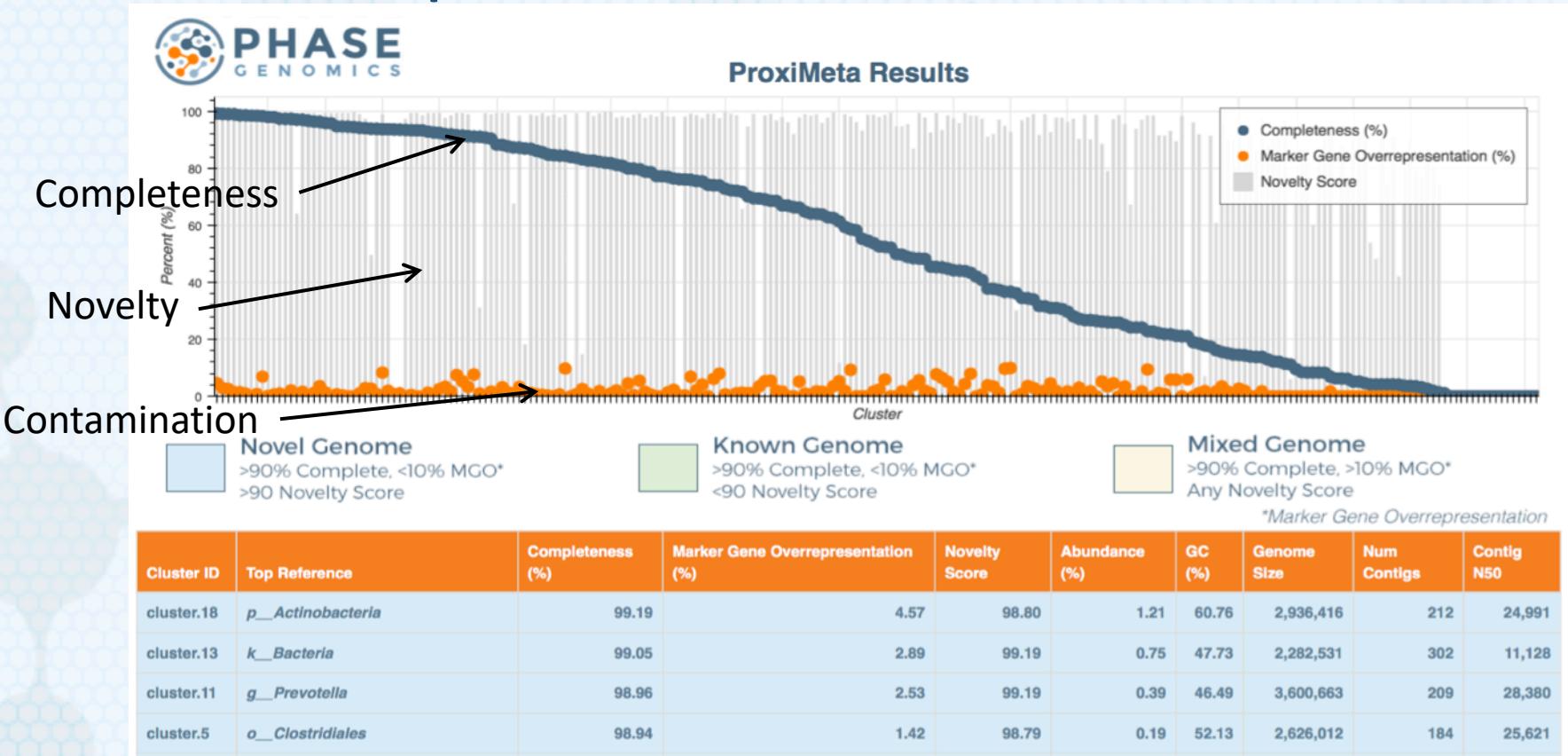
Metric	Original Assembly	+Proximo
Scaffolds	12,787	11
N50	3.8 kb	26.3 Mb
N90	1.0 kb	20.7 Mb
Scaffolded Length	471 Mb	295 Mb
% Scaffolded (len)	N/A	62.8 %
% Plantlike (len)	N/A	99.3 %
Fungal contigs in assembly	1471	4
Bacterial contigs in assembly	1582	0



# Reference-quality genomes from mixed populations



# High numbers of high-quality, novel genomes directly from rumen samples



# High numbers of high-quality, novel genomes directly from rumen samples



Altmetric: 565 [More detail >](#)

Article | OPEN

## Assembly of 913 microbial genomes from metagenomic sequencing of the cow rumen

Robert D. Stewart, Marc D. Auffret, Amanda Warr, Andrew H. Wiser, Maximilian O. Press, Kyle W. Langford, Ivan Liachko, Timothy J. Snelling, Richard J. Dewhurst, Alan W. Walker, Rainer Roehe & Mick Watson

*Nature Communications* 9, Article number: 870 (2018) Received: 26 October 2017 Accepted: 05 February 2018

FOOD FOR THOUGHT

## Mysteries of the Moo-crobiome: Could Tweaking Cow Gut Bugs Improve Beef?

March 6, 2018 · 8:00 AM ET

MENAKA WILHELM



Novelty Score	Any Novelty Score					
	*Marker Gene Overrepresentation					
Novelty Score	Abundance (%)	GC (%)	Genome Size	Num Contigs	Contig N50	
98.80	1.21	60.78	2,936,416	212	24,991	
99.19	0.75	47.73	2,282,531	302	11,128	
99.19	0.39	46.49	3,600,663	209	28,380	
98.79	0.19	52.13	2,626,012	184	25,621	

# Sequencing Lil Bub's magical poop



Collaboration with AnimalBiome

With Holly Ganz, Jennifer Gardy

# Sequencing Lil Bub's magical poop

**Crowdfunded whole-genome sequencing of the celebrity cat Lil BUB identifies causal mutations for her osteopetrosis and polydactyly**

Mike Bridavsky, Heiner Kuhl, Arthur Woodruff, Uwe Kornak, Bernd Timmermann, Norbert Mages,  
 99 Lives Consortium,  Darío G Lupiáñez,  Orsolya Symmons,  Daniel M Ibrahim

**doi:** <https://doi.org/10.1101/556761>

This article is a preprint and has not been peer-reviewed [what does this mean?].



Novel Genome  
 >80% Complete, <10% MGO\*  
 >90 Novelty Score

Cluster ID	Top Reference
cluster.1	<i>o_Clostridiales</i>
cluster.2	Dialister_sp._CAG_486.fna
cluster.3	Parabacteroides_merdeae_CAG_48.fna
cluster.4	<i>c_Epsilonproteobacteria</i>
cluster.5	Clostridium_sp._CAG_299.fna

**Der Da @der\_bri · Feb 23**

Replying to **@LilBUBome**

my favourite coincidence in this project: the mutation is a missing A in a C-A-T sequence. **#youcantmakethisup**

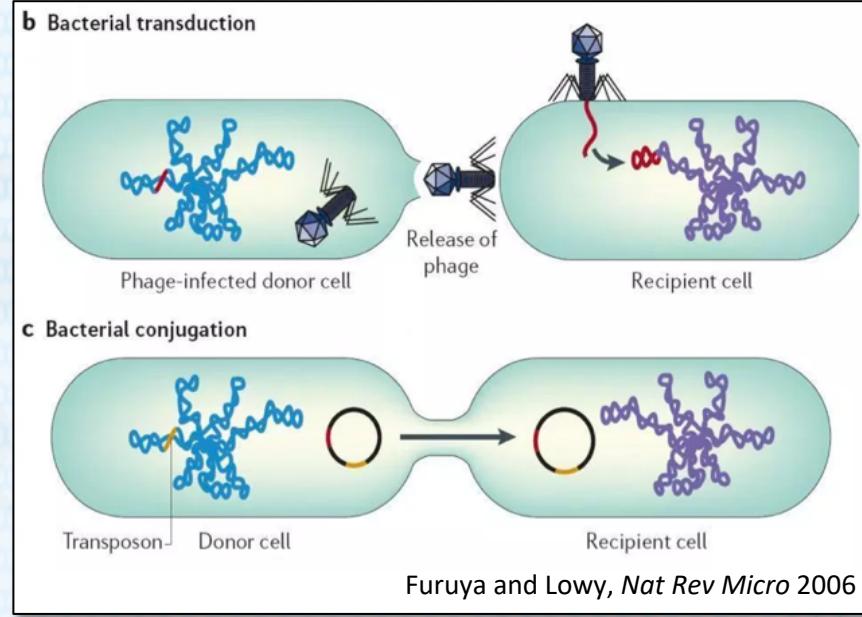


3



# Plasmids/viruses are key players in the microbiome

- Plasmids/Phage transmit AMR (Anti-microbial resistance)
- Plasmids often transmit pathogenic/toxic genes (ex. Anthrax)
- Virtually impossible to connect AMR and mobile elements with host strains using normal NGS
- Need a method that can directly link plasmids/viruses to hosts.



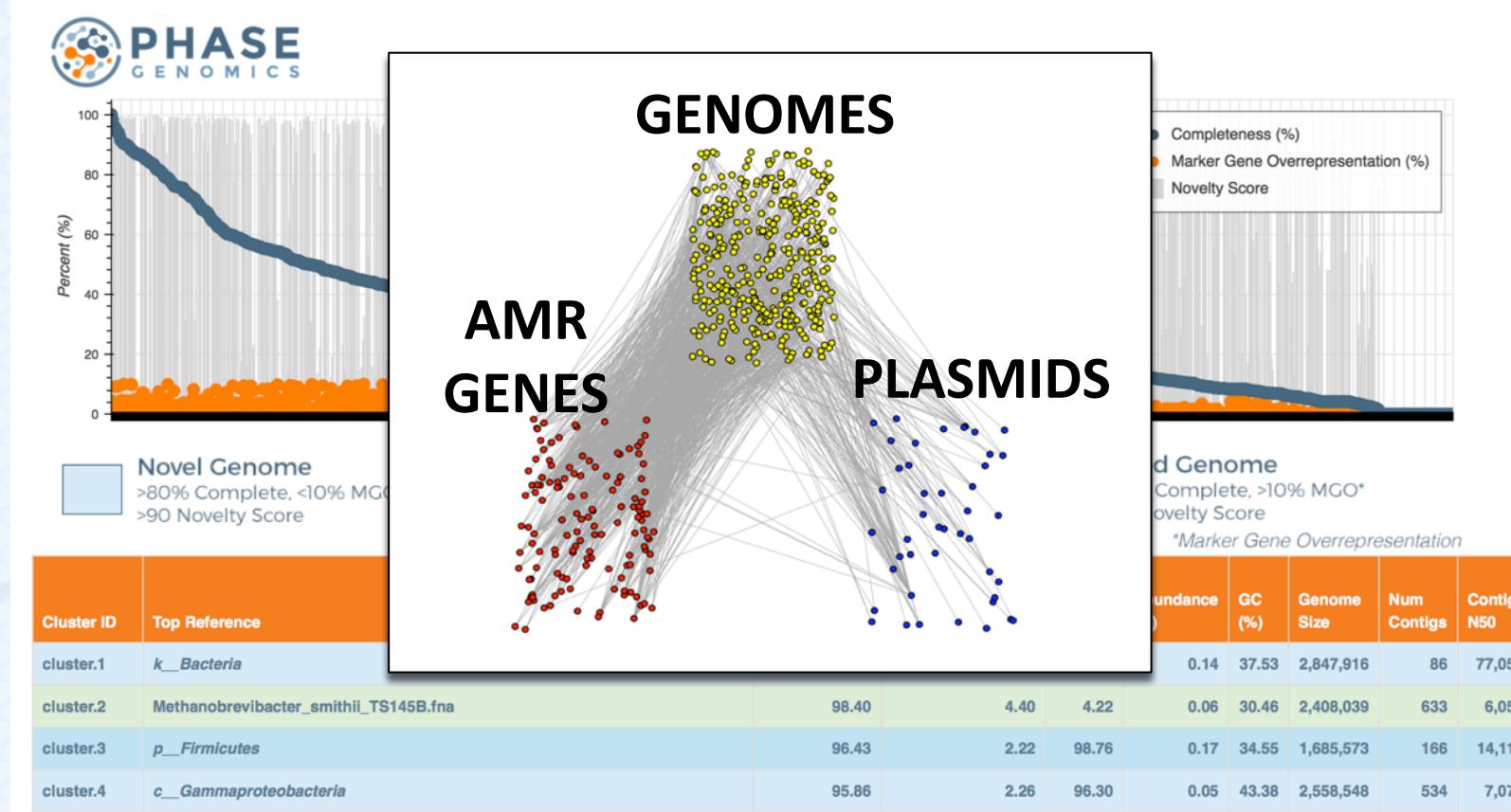
## ABSTRACT

[Go to:](#) 

In order to cause the disease anthrax, *Bacillus anthracis* requires two plasmids, pX01 and pX02, which carry toxin and capsule genes,

Luna et. al., J Clin Microbiol. 2006 Jul; 44(7): 2367–2377

# Highly complex wastewater community

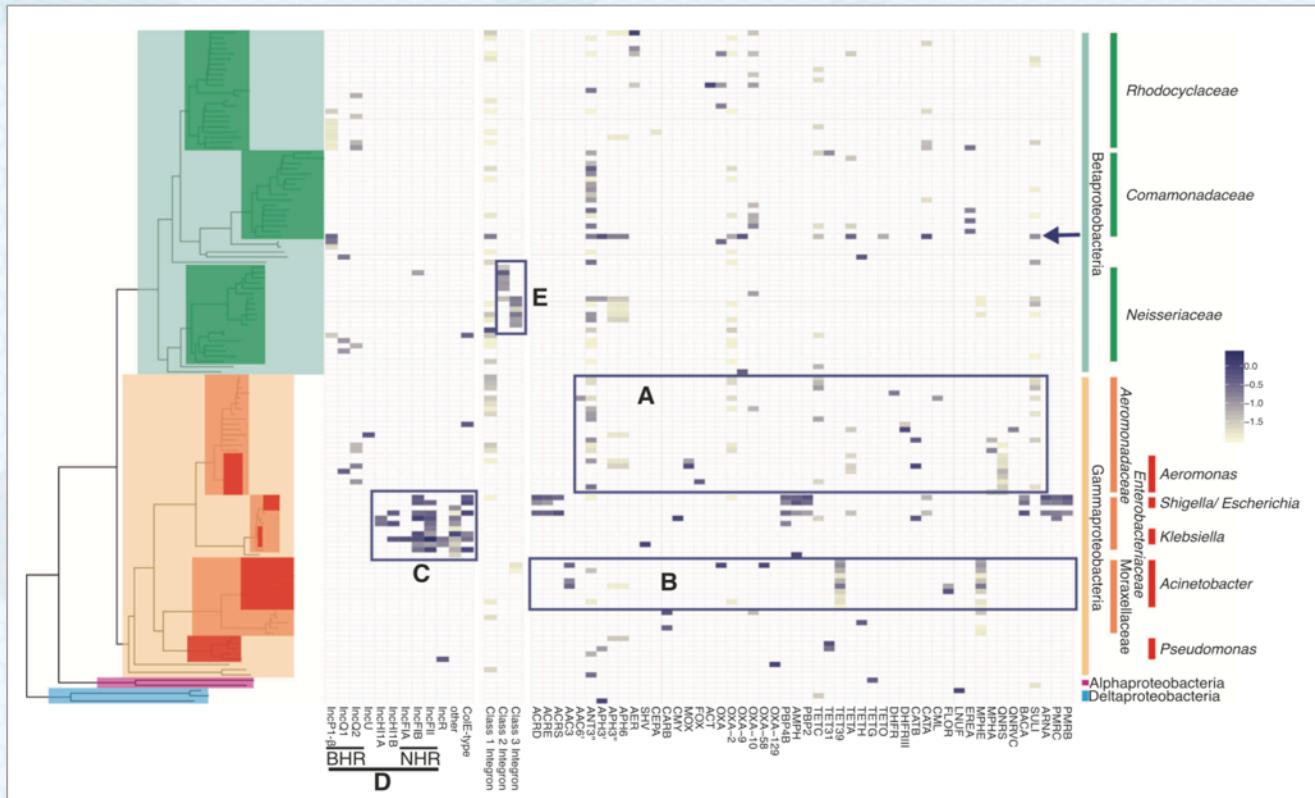


# Linking the ‘Mobilome’ to the Microbiome

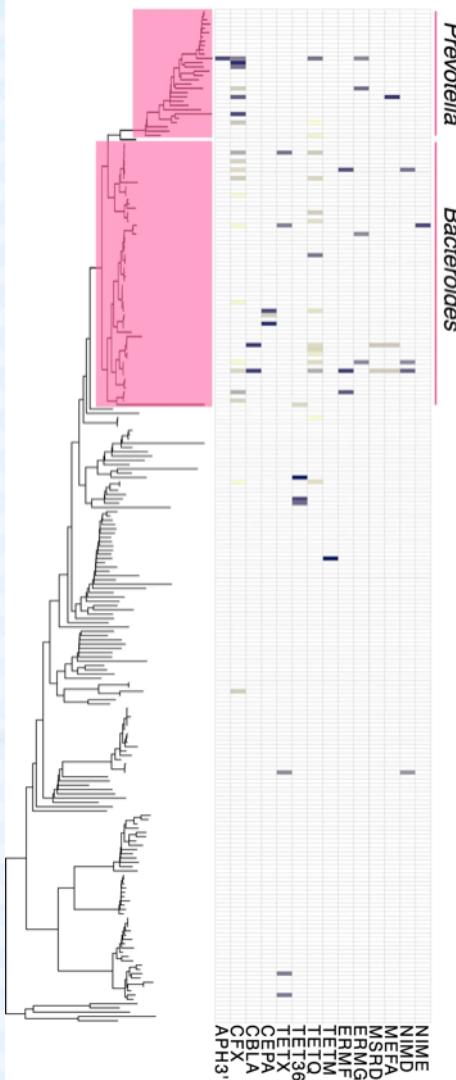


Genomes Plasmids Antibiotic resistance genes

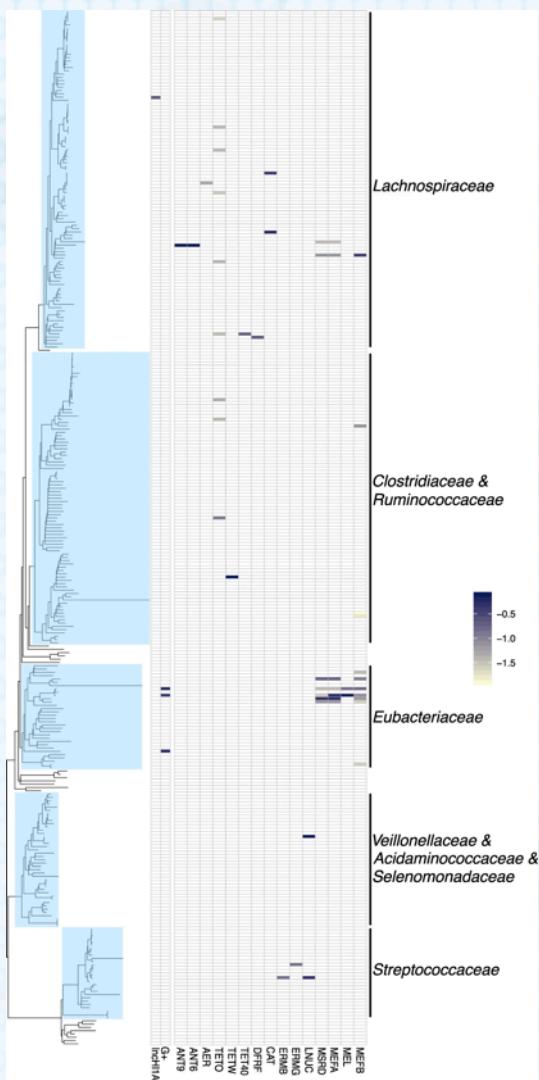
## Proteobacteria



## Bacteroidetes



## Firmicutes



**~1200 PAGs**

**396 host-ARG assn**

**83 plasmid-host assn**

**58 integron-host assn**

# Combining long reads and ProxiMeta in a complex microbiome context



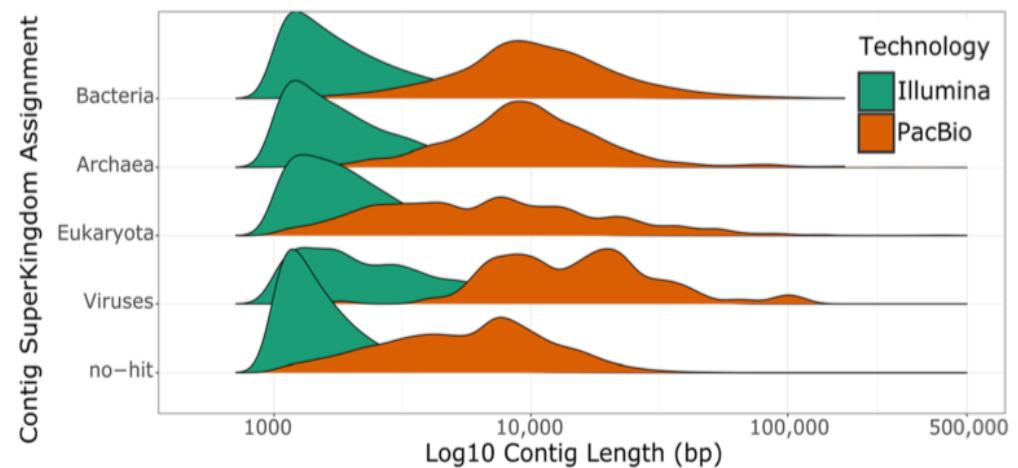
METHOD

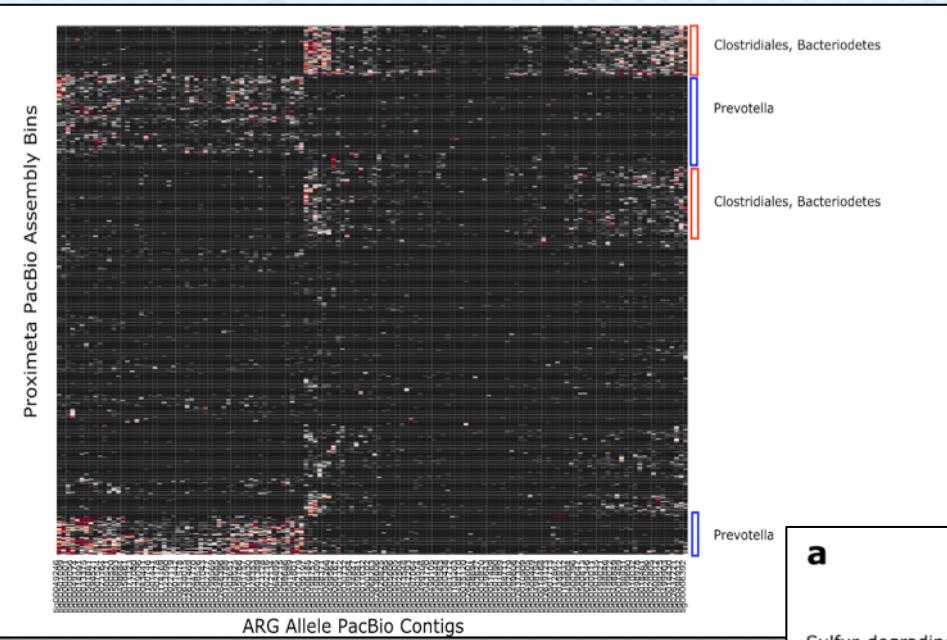
Open Access



Assignment of virus and antimicrobial resistance genes to microbial hosts in a complex microbial community by combined long-read assembly and proximity ligation

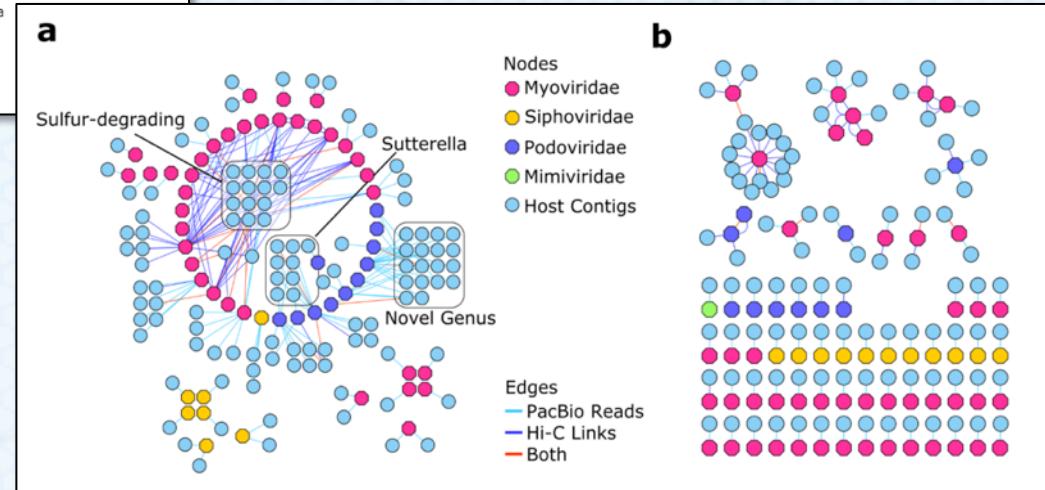
Derek M. Bickhart<sup>1†</sup>, Mick Watson<sup>2†</sup>, Sergey Koren<sup>3†</sup>, Kevin Panke-Buisse<sup>1</sup>, Laura M. Cersosimo<sup>4</sup>, Maximilian O. Press<sup>5</sup>, Curtis P. Van Tassel<sup>6</sup>, Jo Ann S. Van Kessel<sup>7</sup>, Bradd J. Haley<sup>7</sup>, Seon Woo Kim<sup>8</sup>, Garret Suen<sup>9</sup>, Kiranmayee Bakshy<sup>1</sup>, Ivan Liachko<sup>5</sup>, Shawn T. Sullivan<sup>5</sup>, Phillip R. Myer<sup>10</sup>, Jay Ghurye<sup>11</sup>, Paul J. Weimer<sup>1,9</sup>, Adam M. Phillippy<sup>3</sup> and Timothy P. L. Smith<sup>12\*</sup>





## Connecting ARGs and viruses to their hosts

\*188 Novel viruses and host interactions discovered from one rumen sample



# Proximity-Guided Metagenome Assembly

## Genomes, Strains, Mobile Elements

- No culturing
- No binning/de-replication
- No *a priori* information
- No HMW-DNA
- No special machinery

# Using Hi-C Data

# Using Hi-C Data

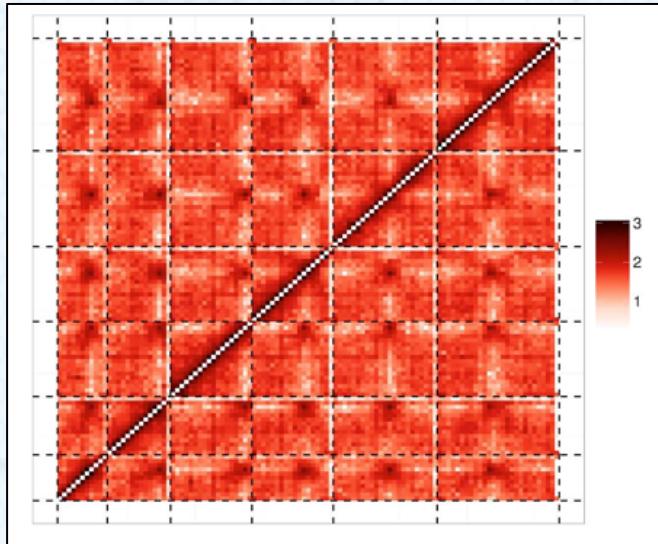


If you take away nothing else...

Hi-C data measure the frequency of physical interaction among loci

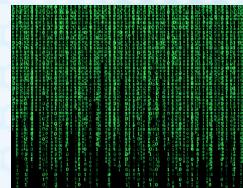
# Using Hi-C Data

Hi-C data are usually represented as a matrix



=

5	9	23	32	33	0	0	0	0	0	0	0
3	28	30	47	0	0	0	0	0	0	0	0
7	1	3	0	0	0	0	0	0	0	0	0
14	21	20	25	27	38	40	0	0	0	0	0
18	29	0	0	0	0	0	0	0	0	0	0
12	13	15	0	0	0	0	0	0	0	0	0
20	26	27	11	25	38	40	0	0	0	0	0
32	33	34	0	0	0	0	0	0	0	0	0
29	18	0	0	0	0	0	0	0	0	0	0
5	9	8	24	0	0	0	0	0	0	0	0
28	16	17	0	0	0	0	0	0	0	0	0
18	29	0	0	0	0	0	0	0	0	0	0
25	26	20	11	0	0	0	0	0	0	0	0
7	1	3	0	0	0	0	0	0	0	0	0
33	32	9	5	23	0	0	0	0	0	0	0
28	16	17	0	0	0	0	0	0	0	0	0



# Using Hi-C Data

Alignment is used to turn Hi-C data into a matrix

- Hi-C reads are paired ends: each pair (typically) maps to loci that were physically close but not necessarily genetically close
- Aligning Hi-C reads to a reference genome counts the number of times loci were physically close
- Think of a Hi-C library as a mate pair library whose insert sizes are infinite, drawn from a probability distribution

## Hi-C matrixes must be normalized

- Normalize for “contig size” (actually restriction site distribution) to transform to frequency
- Normalize for other factors depending on problem
  - Coverage
    - Could mean variation in copy number, abundance, something else

# Using Hi-C Data

Once you have a matrix, do linear algebra,  
graph theory, machine learning, etc.

- Principle component analysis
- Clustering
- Community detection
- Max flow/min cut
- Markov chain Monte Carlo (MCMC)
- Deep learning
- Custom algorithms

# Tools for Hi-C

## Basic Scaffolding Steps

1. Generate contigs/intermediate scaffolds
2. Align Hi-C data
3. QC Hi-C data
4. (optional) Phase contigs with Hi-C
5. Scaffold with Hi-C (one phase at a time)
6. Correct scaffolds (one phase at a time)
7. (optional) Re-phase scaffolds with Hi-C

## 1. Generate contigs/intermediate scaffolds

- Sure, one slide can handle this
- Illumina, PacBio, Nanopore, 10X, BioNano, and more all have roles

## 2. Aligning Hi-C data

- BWA-MEM is the standard, with -5SP options
- In SAMtools, filter read/mate unmapped, not-primary, and secondary alignments (-F 2316)
- Helpful to flag PCR dupes (samblaster is fine, Picard if you want)
- Recommended workflow:  
<https://phasegenomics.github.io/2019/09/19/hic-alignment-and-qc.html>

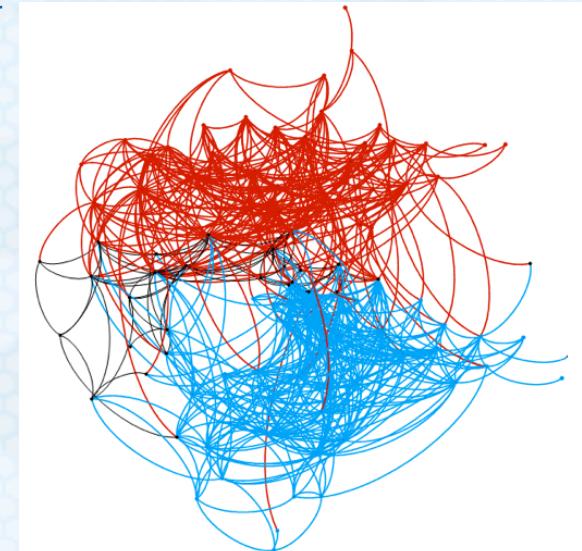
# Tools for Hi-C

## 3. QC'ing Hi-C data

- Several options, all based on trying to detect dependable Hi-C data (e.g., split reads, align to different contigs, align far apart)
  - HiCUP
  - HiC-Pro
  - Juicer
  - hic\_qc
  - Etc.
- Recommended workflow:  
<https://phasegenomics.github.io/2019/09/19/hic-alignment-and-qc.html>

## 4. Phasing contigs with Hi-C data

- FALCON-Phase
- Tool + recommended workflow:  
<https://github.com/phasegenomics/FALCON-Phase>



## 5. Scaffold with Hi-C

- Best open source tools:
  - 3d-dna (Juicer ecosystem from the Aiden lab)
  - SALSA (from the makers of Canu)
- Commercial options
  - Proximo (Phase Genomics)
  - HiRise (Dovetail)

## 6. Correct scaffolds

- The actual key to Hi-C scaffolding – no scaffolder does a perfect job all the time
- Best of breed: Juicebox (Aiden lab)

# Tools for Hi-C

## 6. Correct scaffolds

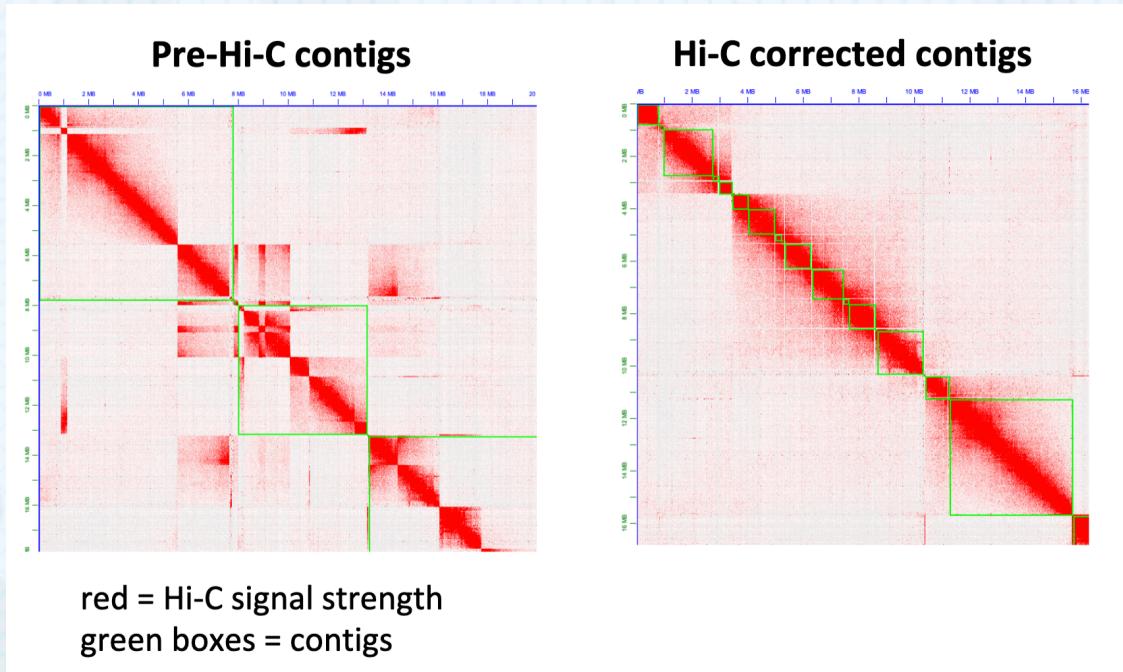
- The actual key to Hi-C scaffolding – no scaffolder does a perfect job all the time
- Best of breed: Juicebox (Aiden lab)  
<https://github.com/aidenlab/Juicebox>
- Helpful auxiliary tools:  
[https://github.com/phasegenomics/juicebox\\_scripts](https://github.com/phasegenomics/juicebox_scripts)

## 7. Re-phase scaffolds with Hi-C

- If you're phasing, run a second FALCON-Phase pass over the scaffolds to detect and correct phasing issues which may have been murky at the contig stage but are now clear in scaffolds

## 6. Correct scaffolds

You'll see stuff like this – correct the contigs too where needed





# Juicebox Demo

# Time To Get Back Into It

## Basic Scaffolding Steps

1. Generate contigs/intermediate scaffolds
2. Align Hi-C data
3. QC Hi-C data
4. (optional) Phase contigs with Hi-C
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## Basic Scaffolding Steps

1. Generate contigs/intermediate scaffolds
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3. QC Hi-C data
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5. Scaffold with Hi-C (one phase at a time)
6. Correct scaffolds (one phase at a time)
7. (optional) Re-phase scaffolds with Hi-C

## 3. QC Hi-C data

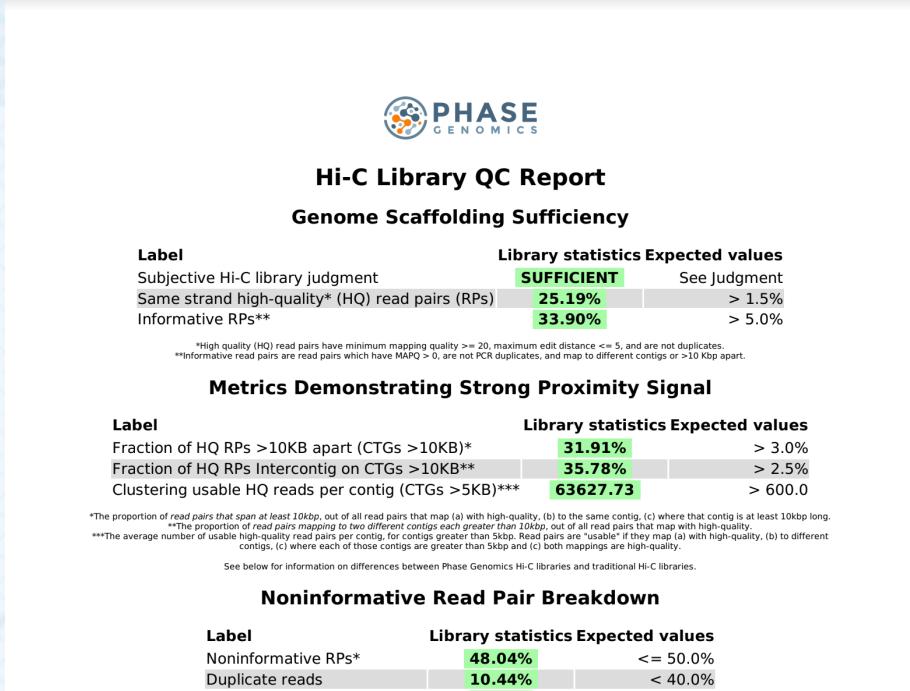
- **Install:** `module load hic_qc`
- **Enter environment:** `source activate hic_qc-1.0`
- Follow steps at  
<https://phasegenomics.github.io/2019/09/19/hic-alignment-and-qc.html>
- Use the alignments you generated in the previous step

# Hands-On



## 3. QC Hi-C data

- Let's talk about the report



## 6. Correct scaffolds

- Install Juicebox: <https://github.com/aidenlab/Juicebox/wiki/Download>
- Load .hic and .assembly files from  
`/share/workshop/genome_assembly/phase_genomics_2020_data_fungus/`
- Fix scaffold issues
- Export new .assembly file
- Use juicebox\_assembly\_converter.py to generate a new FASTA, AGP, BED, and break report for your genome edits
  - Install: `module load juicebox_scripts`
  - Need to run with `python /software/juicebox_scripts/1487f3/lssc0-linu/bin/juicebox_assembly_converter.py`
  - Use `which juicebox_assembly_converter.py` if you can't find it

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