RAGOUT

Enlarge your contigs

Ragout – a reference-assisted assembly tool for bacterial genomes

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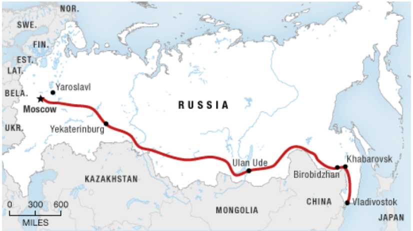
Outline

1. Introduction
2. Quick Overview
3. Algorithm Description
4. Results
5. Further plans

Trans-Siberian Railway

- The longest railroad in the world
- 9248 km
- $\sim$ 15 000 000 railroad ties



A Secret Story

- Once Aliens came:

A Secret Story

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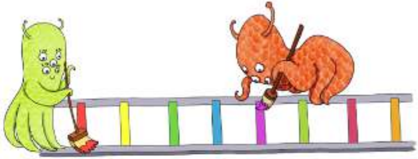
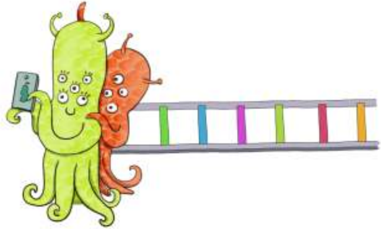
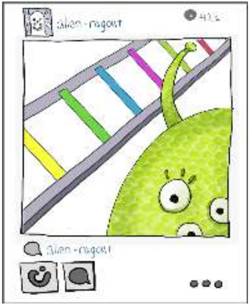
- And they have painted the ties in different colors:



A Secret Story II

- After, they took a lot of pictures:





A Secret Story III

- And after they had been gone, rain has wanished all dyes from the railroad :(

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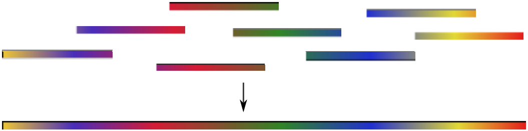
- Can we now reconstruct the original coloring using those pictures?



A Secret Story III

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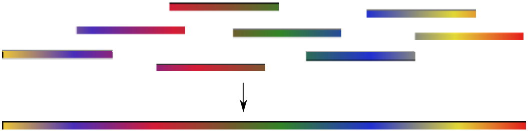
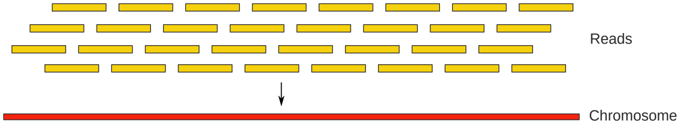


- This is exactly a problem that genome assemblers solve!
  - SPAdes
  - ABySS
  - Velvet
  - SOAPdenovo
  - SGA
  - ...

Genome Assembly

- Join short overlapping reads into chromosomes
- Expectation:



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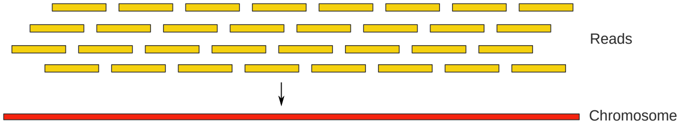
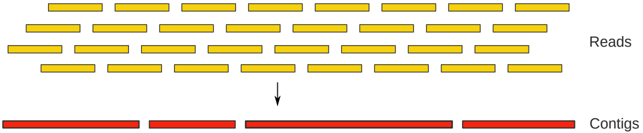
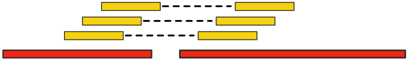


- Reality:



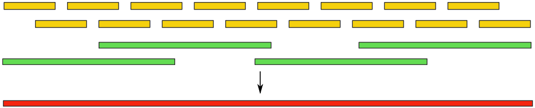
Complete Sequence?

- Jumping libraries:



illumina

- Long reads:

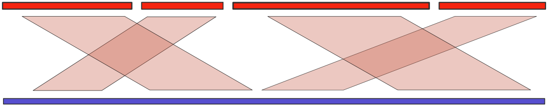


PACIFIC BIOSCIENCES

- Still expensive and not as reliable as short reads
- Is there any alternative?

Reference-assisted Assembly

- Using a complete genome of another closely-related organism
- Contigs are being aligned on that *reference* genome



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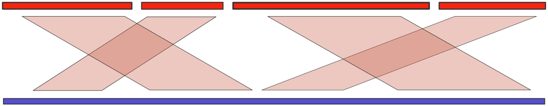

- Structural variations?

Rearrangement Approaches

- Gaul and Blanchette. "Ordering Partially Assembled Genomes Using Gene Arranements", *Springer, 2006*
  - Tries to minimize number of structural variations between two genomes
- Kim et. al. "Reference-assisted Chromosome Assembly", *PNAS, 2013*
  - First attempt to use multipe genomes simultaneously
  - One *reference* and multiple *outgroups*
  - Still heavily rely on that reference
- Both approaches may introduce errors

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  - First attempt to use multipe genomes simultaneously
  - One *reference* and multiple *outgroups*
  - Still heavily rely on that reference
- Both approaches may introduce errors
- So maybe we need multiple references?

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Ragout Recipe

- Ragout – Reference-Assisted Genome Ordering UTility
- Written in Python/C++
- Ingredients:
  - Multiple references (in FASTA format)
  - Contigs/scaffolds from short-read assembly
  - Phylogenetic tree
- Output: scaffolds

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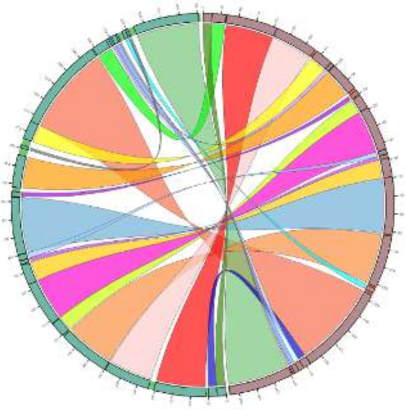
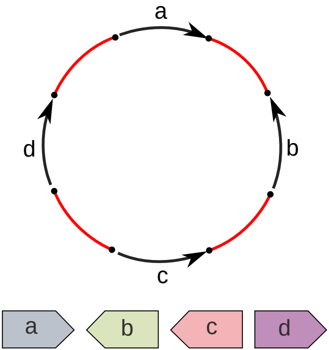
Genome Representation

- Comparing nucleotie by nucleotide is expensive
- Extract conserved segements (synteny blocks)
- Assumption: each block is represented exactly once in each genome



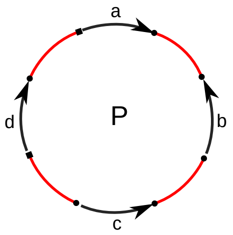
SIBELIA

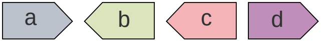
Genome as Synteny Blocks and Adjacencies

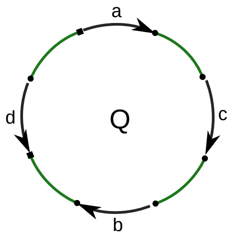


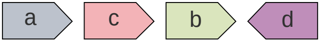
- Chromosome is represented as an aleterating cycle of **directed black** and **undirected red** edges
- **Black** edges correspond to synteny blocks
- **Red** edges connect ends of adjacent synteny blocks

Breakpoint Graphs Are Simple!









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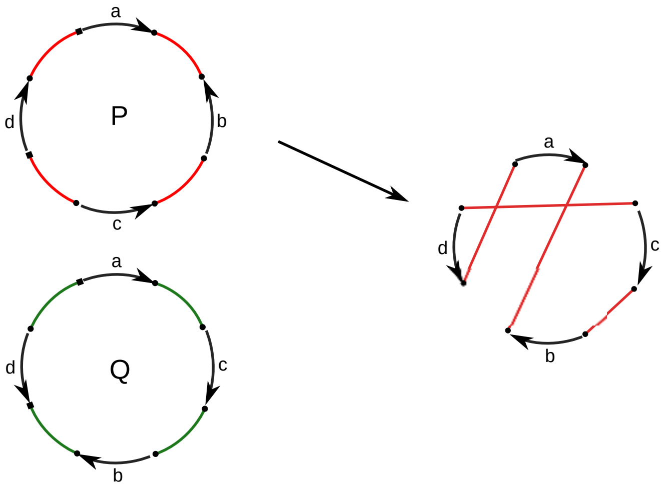

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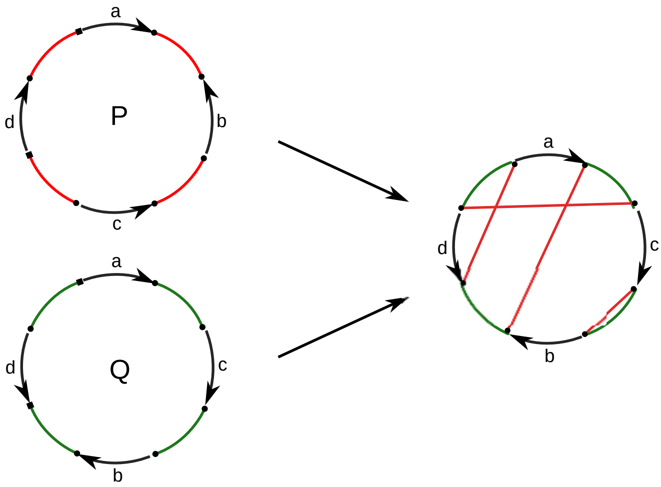

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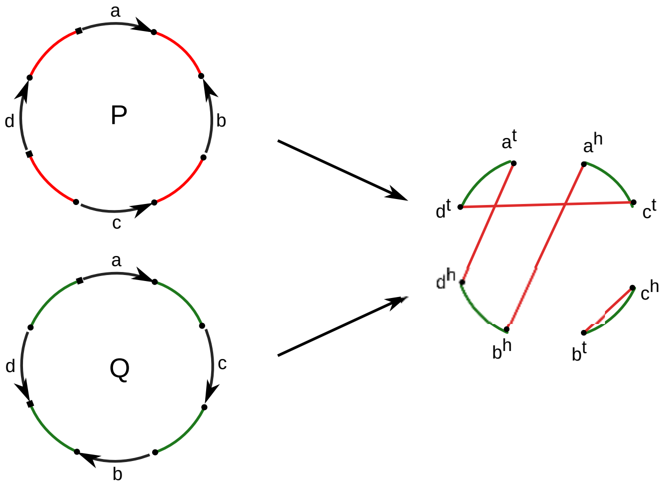

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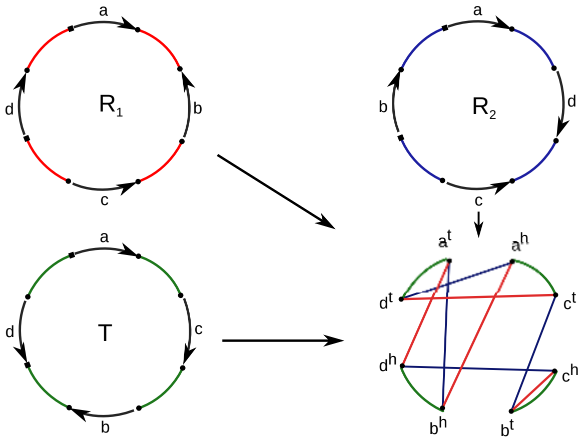

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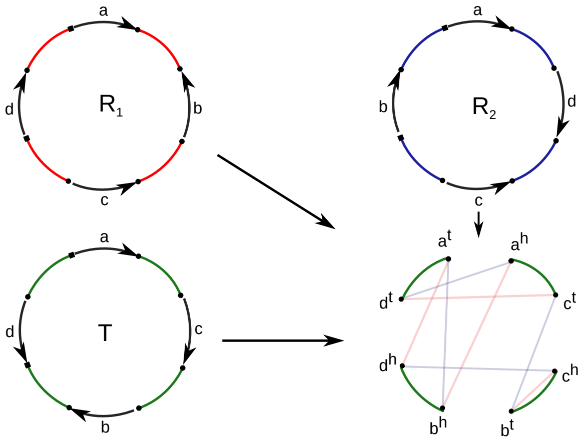

- Each color defines a perfect matching

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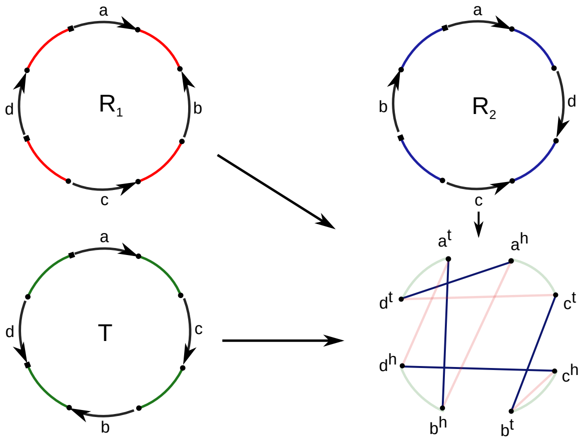

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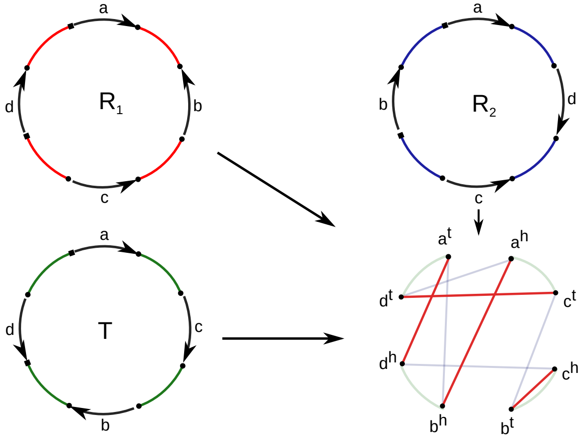

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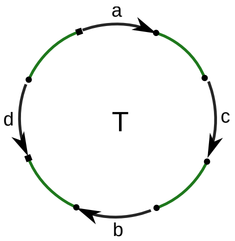
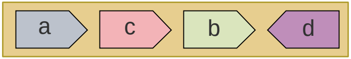
Incomplete Breakpoint Graph





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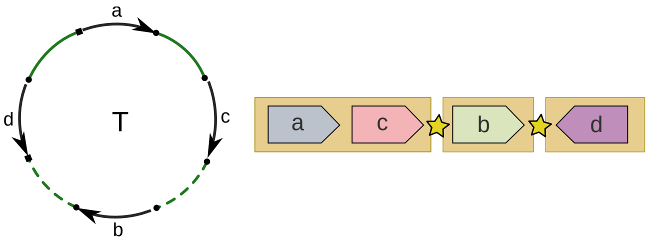
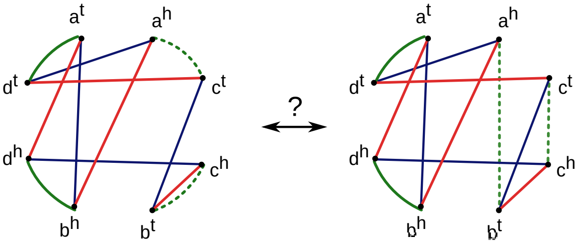


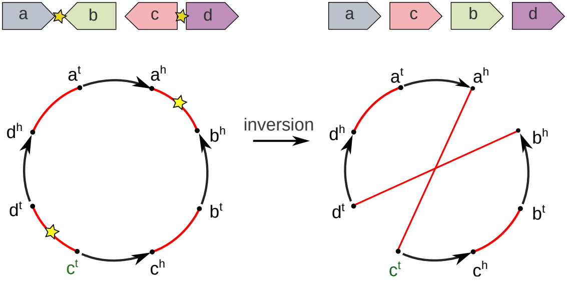
- Some adjacencies are missing

Incomplete Breakpoint Graph II



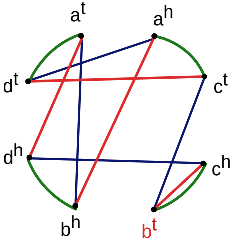
- Find missing edges
- **= Recover perfect matching**
- There are multiple variants of such matching
- How to find the correct one?

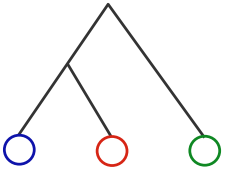
States of Adjacencies



- *State* = adjacent vertex
- *State* of c^t: $d^t \rightarrow a^h$
- Rearrangements change *states* of adjacencies

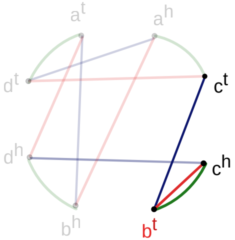
Objective Function

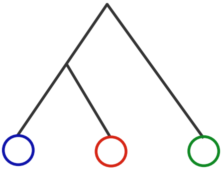




- Choose an arbitrary perfect matching

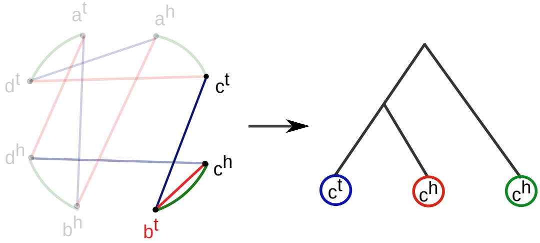
Objective Function





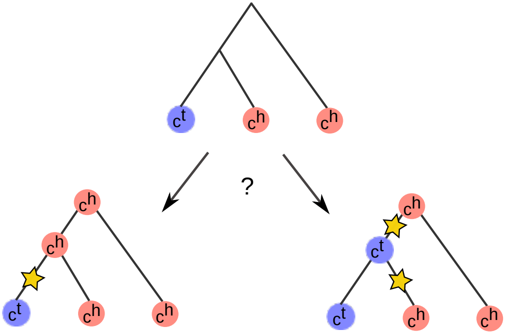
- Choose an arbitrary perfect matching
- Pick a vertex from the graph

Objective Function



- Choose an arbitrary perfect matching
- Pick a vertex from the graph
- Label tree nodes as *states* of chosen vertex in genomes
- The tree represents evolution of breakpoint states

Parsimony Procedure



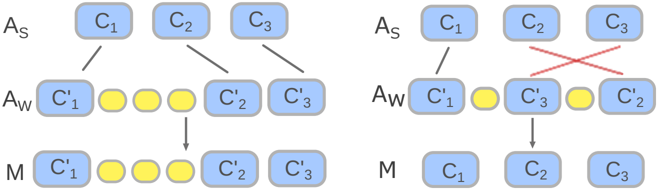
- Find scenario with minimum number of changes
- Associated cost for graph vertex u and tree T:

$$P(u, T) = \sum_{branch\ (i,\ j), i \neq j} W(branchlength)$$

Optimal Contigs Order

- Cost for a complete graph G: $\sum_{u \in G} P(u, T)$
- Want a prefect matching which minimizes this cost
- An efficient solution:
  - Node weight $\rightarrow$ edge weight
  - Find minimum weight perfect matching
  - Blossom algorithm in $\mathrm{O}(n^4)$

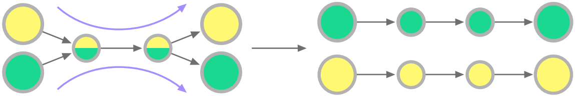
Iterative Assembly



(a) Locally consistent (b) Locally inconsistent

- Solve the dilemma about choice of synteny block size
- Merge scaffolds with different precision into one assembly

Refinement with Assembly Graph



- Incorporate very small/repetitive contigs
- Analogiously to repeat resolution in short-read assembly

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Toy Test – One *E. Coli* Reference

	Ragout	MCM	OSLay
Scaffolds	**1**	**1**	8
Contigs (coverage)	**129 (97.9%)**	77 (97.6%)	80 (96.7%)
Miss-ordered	**0**	**0**	1

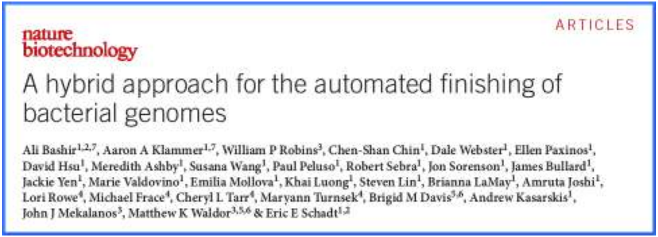
- One *E. Coli* reference without rearrangements
- #Contigs – 156 (98.18%)

Assembly with Rearrangements – Four *H. Pylori* References

#References	Scaffolds	Contigs (cov.)	Miss-ordered
		Ragout	
1	2	91 (97.7%)	6
2	2	**95 (97.8%)**	1
3	**1**	**95 (97.8%)**	1
4	**1**	93 (97.6%)	**0**
		RACA	
2	3	35 (83.6%)	2
3	2	35 (83.6%)	1
4	2	35 (83.8%)	1

- Four *H. Pylori* references with rearrangements
- #Contigs – 183 (98.57%)

Long Reads or ...



ARTICLES

nature biotechnology

A hybrid approach for the automated finishing of bacterial genomes

Ali Bashir[1,2,7], Aaron A Klammer[1,7], William P Robins[3], Chen-Shan Chin[1], Dale Webster[1], Ellen Paxinos[1], David Hsu[1], Meredith Ashby[1], Susana Wang[1], Paul Peluso[1], Robert Sebra[1], Jon Sorenson[1], James Bullard[1], Jackie Yen[1], Marie Valdovino[1], Emilia Mollova[1], Khai Luong[1], Steven Lin[1], Brianna LaMay[1], Amruta Joshi[1], Lori Rowe[4], Michael Frace[4], Cheryl L Tarr[4], Maryann Turnsek[4], Brigid M Davis[3,6], Andrew Kasarskis[1], John J Mekalanos[3], Matthew K Waldor[3,5,6] & Eric E Schadt[1,2]

- 40 bp non-paired Illumina reads
- Roche 454 reads
- PacBio reads

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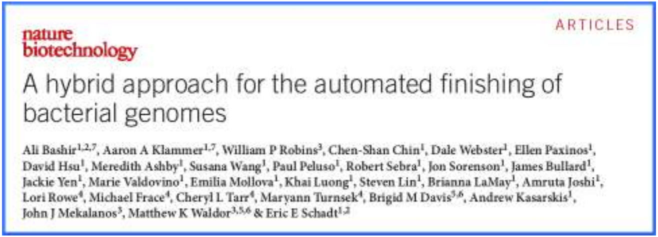

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- 40 bp non-paired Illumina reads
- Roche 454 reads?
- PacBio reads?
- Can we replace long reads with Ragout here?

Long Reads or Reference-assisted Assembly?

#References	Scaffolds	Contigs (cov.)	Miss-ordered
		Ragout	
1	3	**185 (94.8%)**	3
2	**2**	179 (94.7%)	4
3	**2**	174 (94.7%)	**0**
		RACA	
2	6	124 (85.8%)	0
3	3	127 (90.0%)	**0**

- Three *V. Cholerae* references with rearrangements
- #Contigs – 1407 (96.89%)
- Results are shown without refinement (poor assembly quality)

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New results & further plans

- Assembly of *Drosophila yakuba* with three other *Drosophila* species:

Scaffolds	10
Contigs	1538 (94.92%)
Miss-ordered	26
Contigs N50	162 216
Scaffolds N50	30 316 814

- Assembly of multiple mouse lines
- Capturing rearrangements with assembly graph
- Illumina BaseSpace integration

BaseSpace®
Genomics Cloud Computing

Acknowledgements

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http://fenderglass.github.io/Ragout