



Ragout – a reference-assisted assembly tool for bacterial genomes

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ISMB 2014, Boston

Outline

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

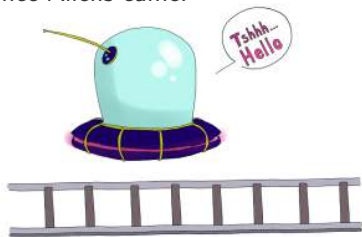
Trans-Siberian Railway

- ☞ The longest railroad in the world
- ☞ 9248 km
- ☞ ~ 15 000 000 railroad ties



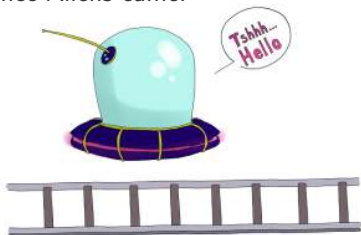
A Secret Story

☛ Once Aliens came:

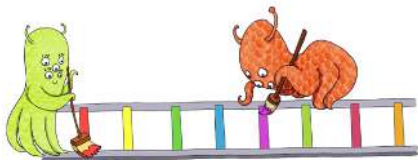


A Secret Story

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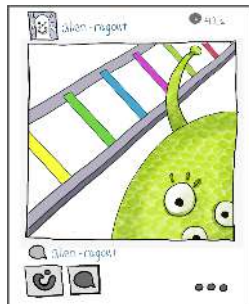
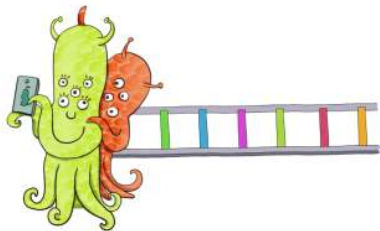


- And they have painted the ties in different colors:



A Secret Story II

☞ After, they took a lot of pictures:

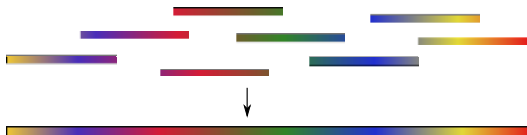


A Secret Story III

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

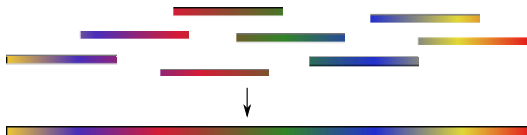
A Secret Story III

- ☹ And after they had been gone, rain has washed all dyes from the railroad :(
- ☹ Can we now reconstruct the original coloring using those pictures?



A Secret Story III

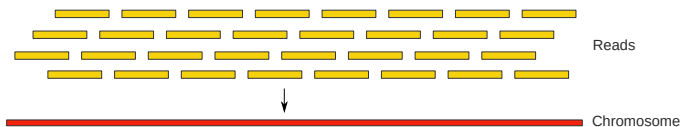
- ☹ And after they had been gone, rain has washed all dyes from the railroad :(
- ☹ Can we now reconstruct the original coloring using those pictures?



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

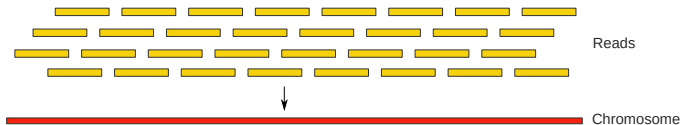
Genome Assembly

- Join short overlapping reads into chromosomes
- Expectation:

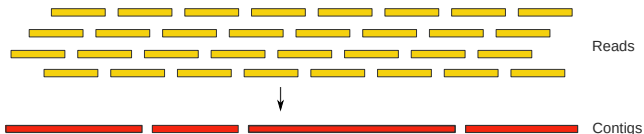


Genome Assembly

- Join short overlapping reads into chromosomes
- Expectation:



- Reality:



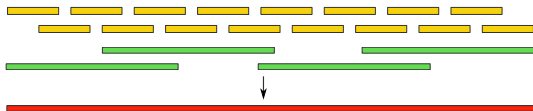
Complete Sequence?

- Jumping libraries:



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- Long reads:

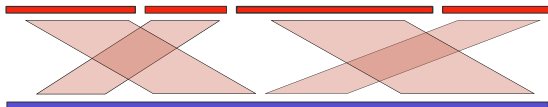


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- Still expensive and not as reliable as short reads
- Is there any alternative?

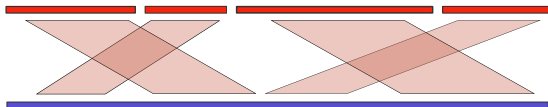
Reference-assisted Assembly

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



Reference-assisted Assembly

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- ☛ Contigs are being aligned on that *reference* genome



- ☛ **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 multiple 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 multiple 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

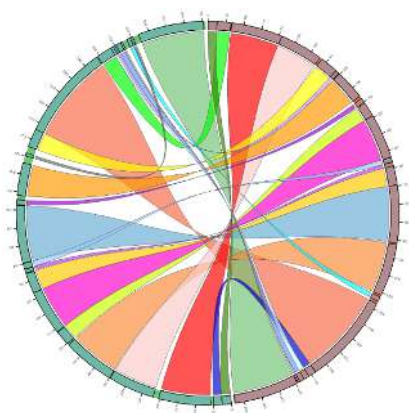
- ☞ Ragout – Reference-Assisted Genome Ordering UTility
- ☞ Written in Python/C++
- ☞ Ingredients:
 - Multiple references (in FASTA format)
 - Contigs/scaffolds from short-read assembly
 - Phylogenetic tree
- ☞ Output: scaffolds

Outline

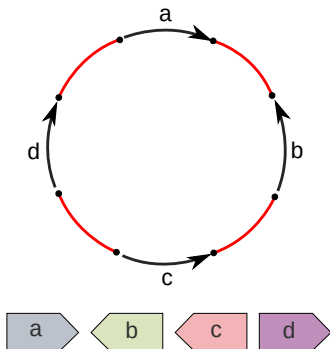
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Genome Representation

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

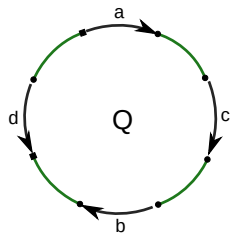
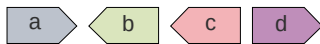
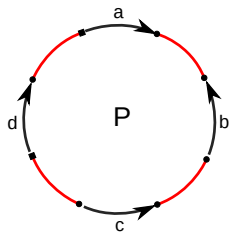


Genome as Synteny Blocks and Adjacencies

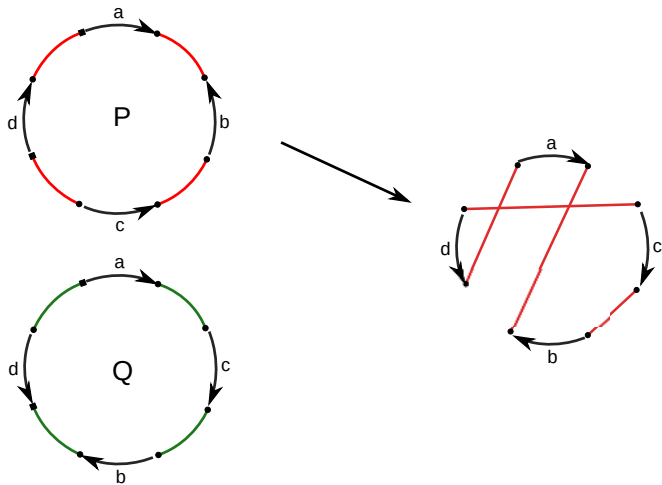


- Chromosome is represented as an alternating cycle of **directed black** and **undirected red** edges
- Black** edges correspond to synteny blocks
- Red** edges connect ends of adjacent synteny blocks

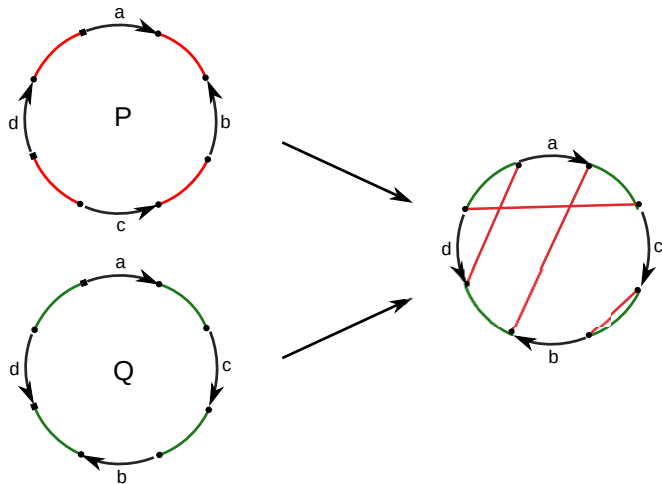
Breakpoint Graphs Are Simple!



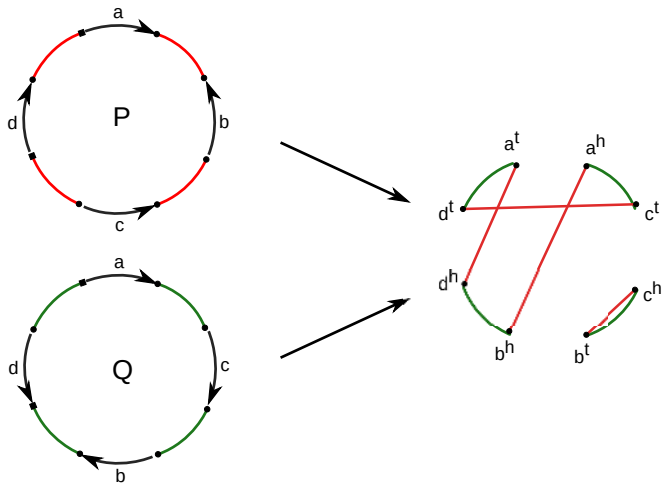
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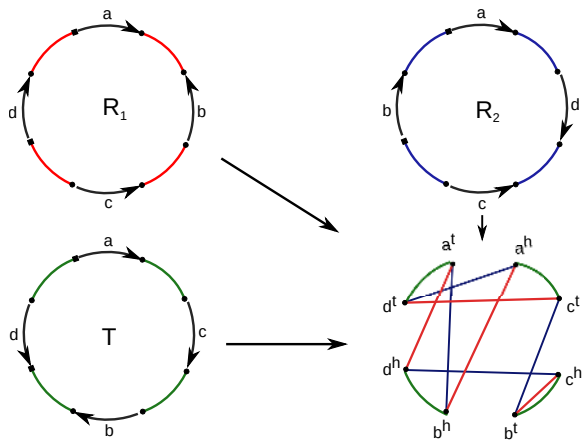
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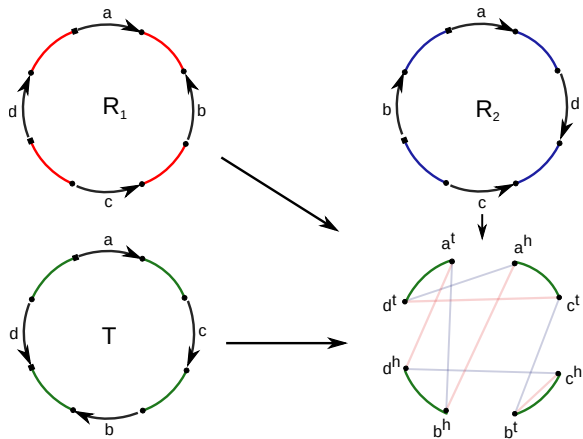
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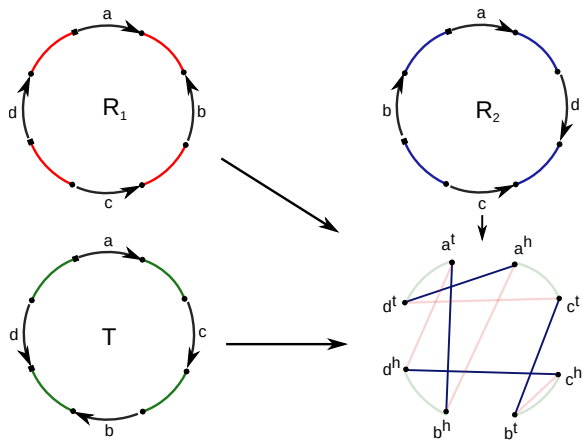


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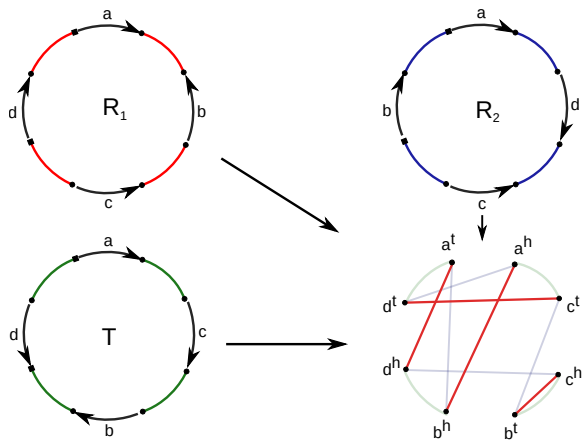
☞ Each color defines a perfect matching

Breakpoint Graphs Are Simple!



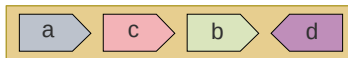
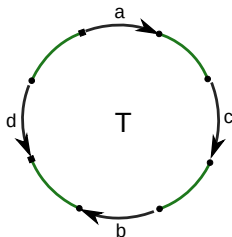
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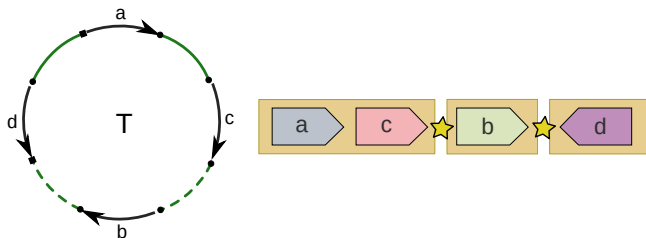


☞ Each color defines a perfect matching

Incomplete Breakpoint Graph

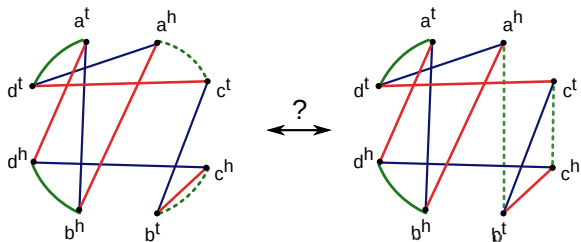


Incomplete Breakpoint Graph



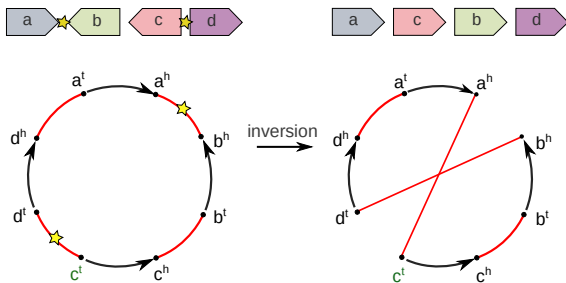
☹ Some adjacencies are missing

Incomplete Breakpoint Graph II



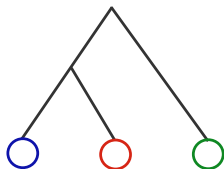
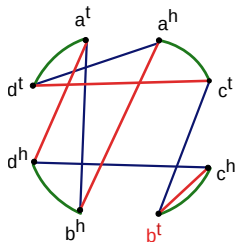
- Find missing edges
- = **Recover perfect matching**
- There are multiple variants of such matching
- How to find the correct one?

States of Adjacencies



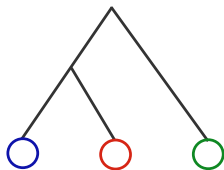
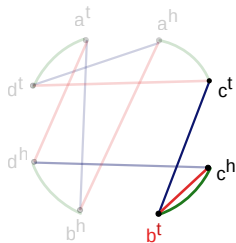
- ☞ *State* = adjacent vertex
- ☞ *State* of c^t : $d^t \rightarrow a^h$
- ☞ Rearrangements change *states* of adjacencies

Objective Function



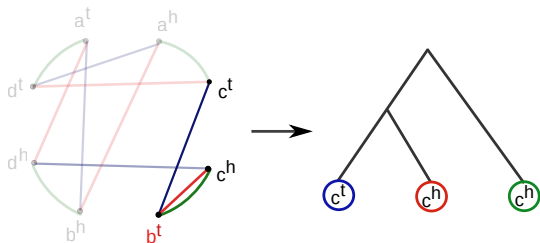
☛ Choose an arbitrary perfect matching

Objective Function



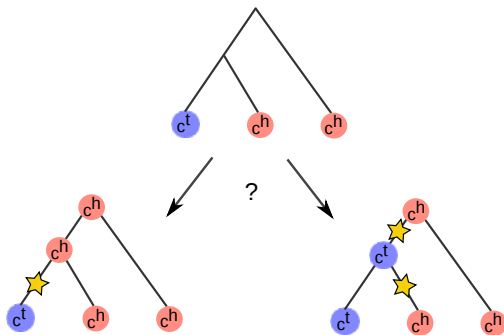
- ☛ Choose an arbitrary perfect matching
- ☛ Pick a vertex from the graph

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



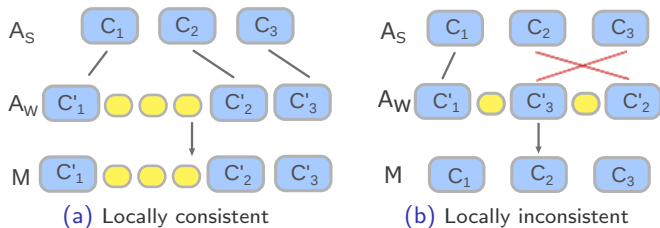
- Find scenario with minimum number of changes
- Associated cost for graph vertex u and tree T :

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

Optimal Contigs Order

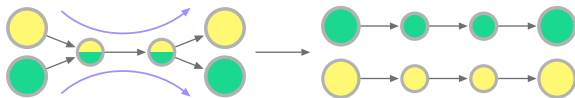
- ☞ Cost for a complete graph G : $\sum_{u \in G} P(u, T)$
- ☞ Want a perfect matching which minimizes this cost
- ☞ An efficient solution:
 - Node weight \rightarrow edge weight
 - Find minimum weight perfect matching
 - Blossom algorithm in $O(n^4)$

Iterative Assembly



- ☛ 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
- Analogously 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%)

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ARTICLES

A hybrid approach for the automated finishing of bacterial genomes

Ali Bashir^{1,2,7}, Aaron A Klammer^{1,7}, William P Robins³, Chen-Shan Chin¹, Dale Webster¹, Ellen Paxinos¹, David Hsu¹, Meredith Ashby¹, Susana Wang¹, Paul Peluso¹, Robert Sebra¹, Jon Sorenson¹, James Bullard¹, Jackie Yen¹, Marie Valdovino¹, Emilia Mollova¹, Khai Luong¹, Steven Lin¹, Brianna LaMay¹, Amruta Joshi¹, Lori Rowe⁴, Michael Frace⁴, Cheryl L Tarr⁴, Maryann Turnsek⁴, Brigid M Davis^{3,6}, Andrew Kasarskis¹, John J Mekalanos⁵, 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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A hybrid approach for the automated finishing of bacterial genomes

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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

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Benedict Paten



Tamara Panesh



Anna Arthuykhova

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<http://fenderglass.github.io/Ragout>