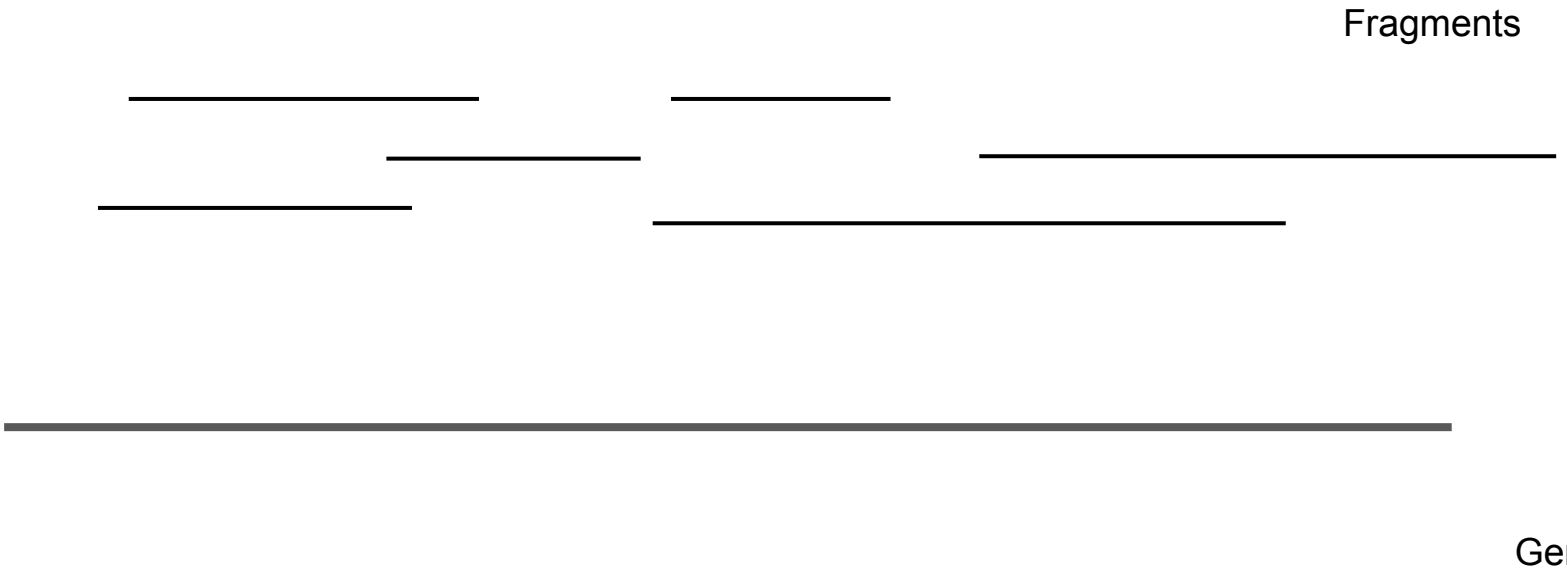
Assembly of 10x Genomics GemCode reads

Anton Bankevich, Ivan Tolstoganov

GemCode barcoded sequencing



- DNA is sheared into long fragments (up to 50 Kb)

GemCode barcoded sequencing

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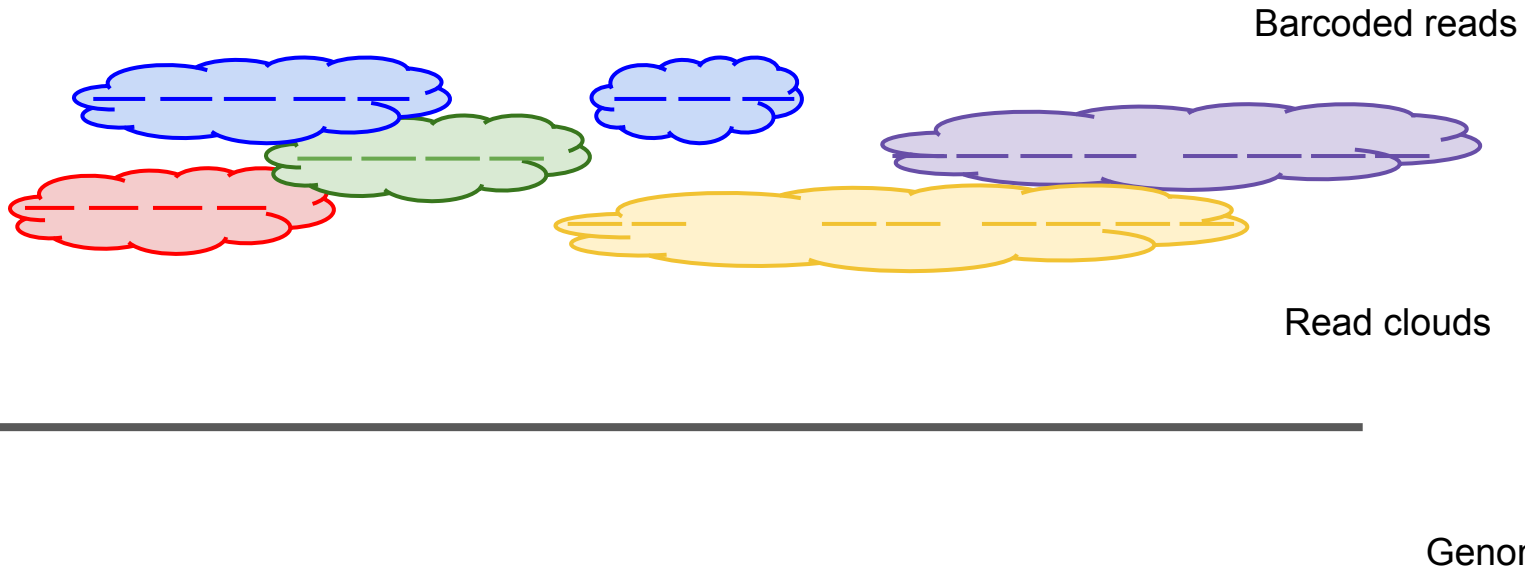
- Fragments are barcoded and sequenced

Read clouds



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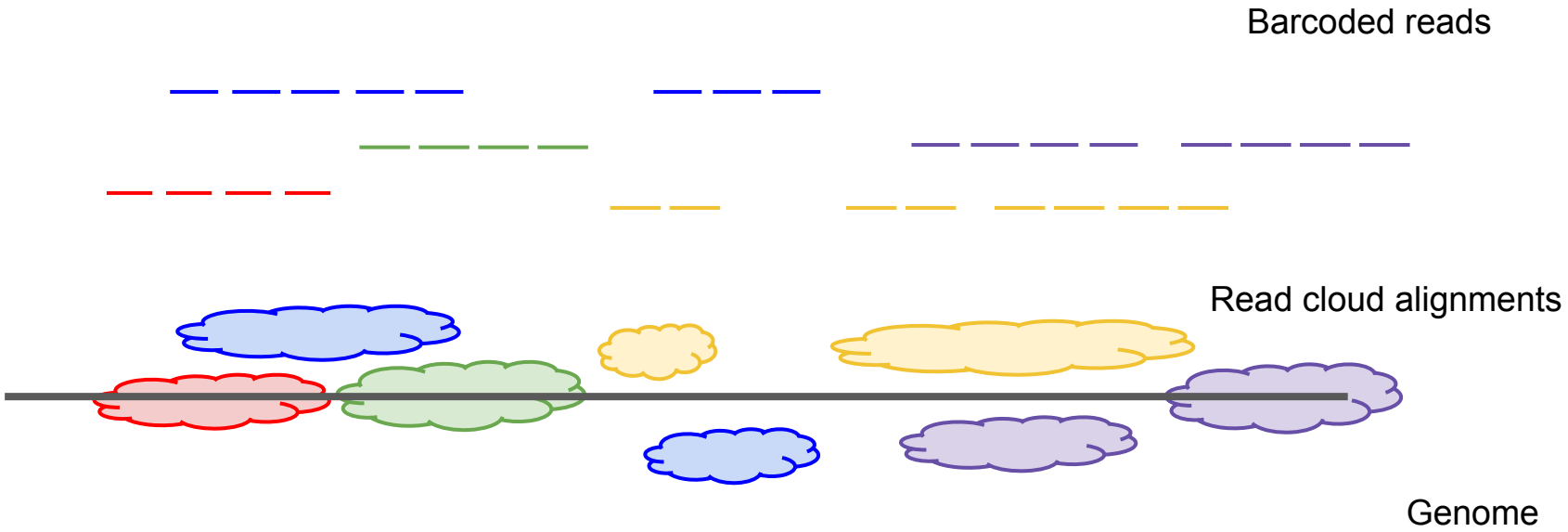
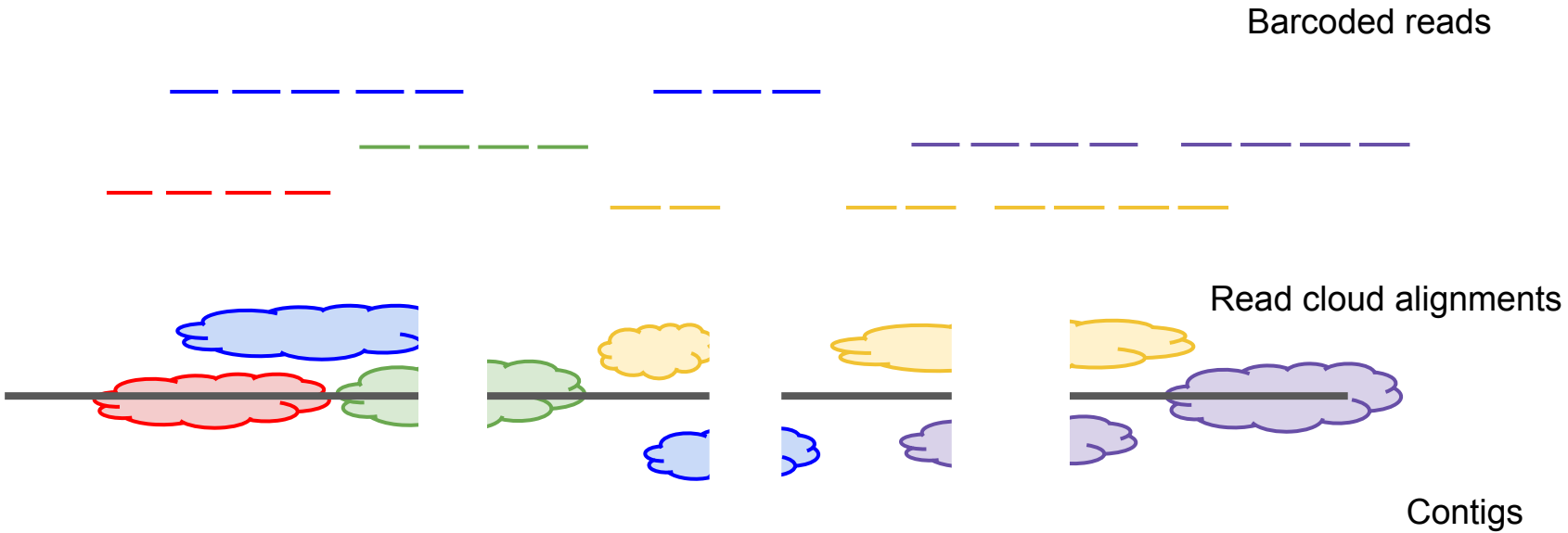
- Fragments are barcoded and sequenced to form read clouds

Read cloud alignment



- DNA is sheared into long fragments (up to 50 Kb)
- Fragments are barcoded and sequenced to form read clouds
- Read clouds can be recovered by alignment

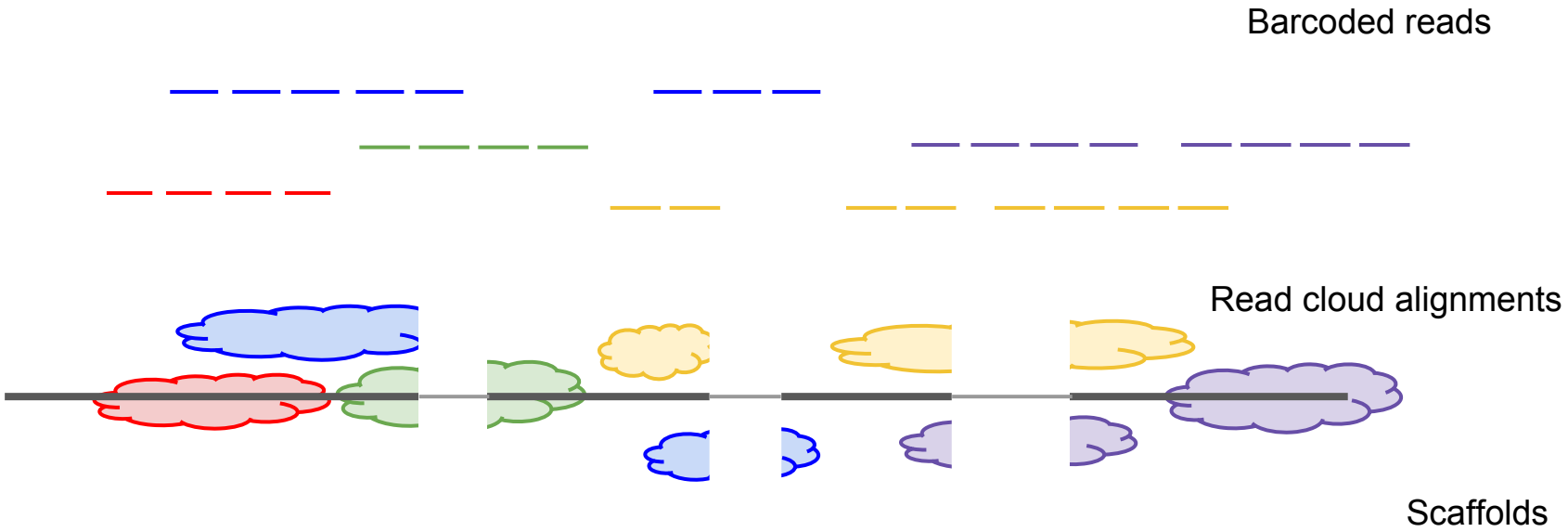
Read cloud scaffolding



- Read clouds can be aligned to contigs

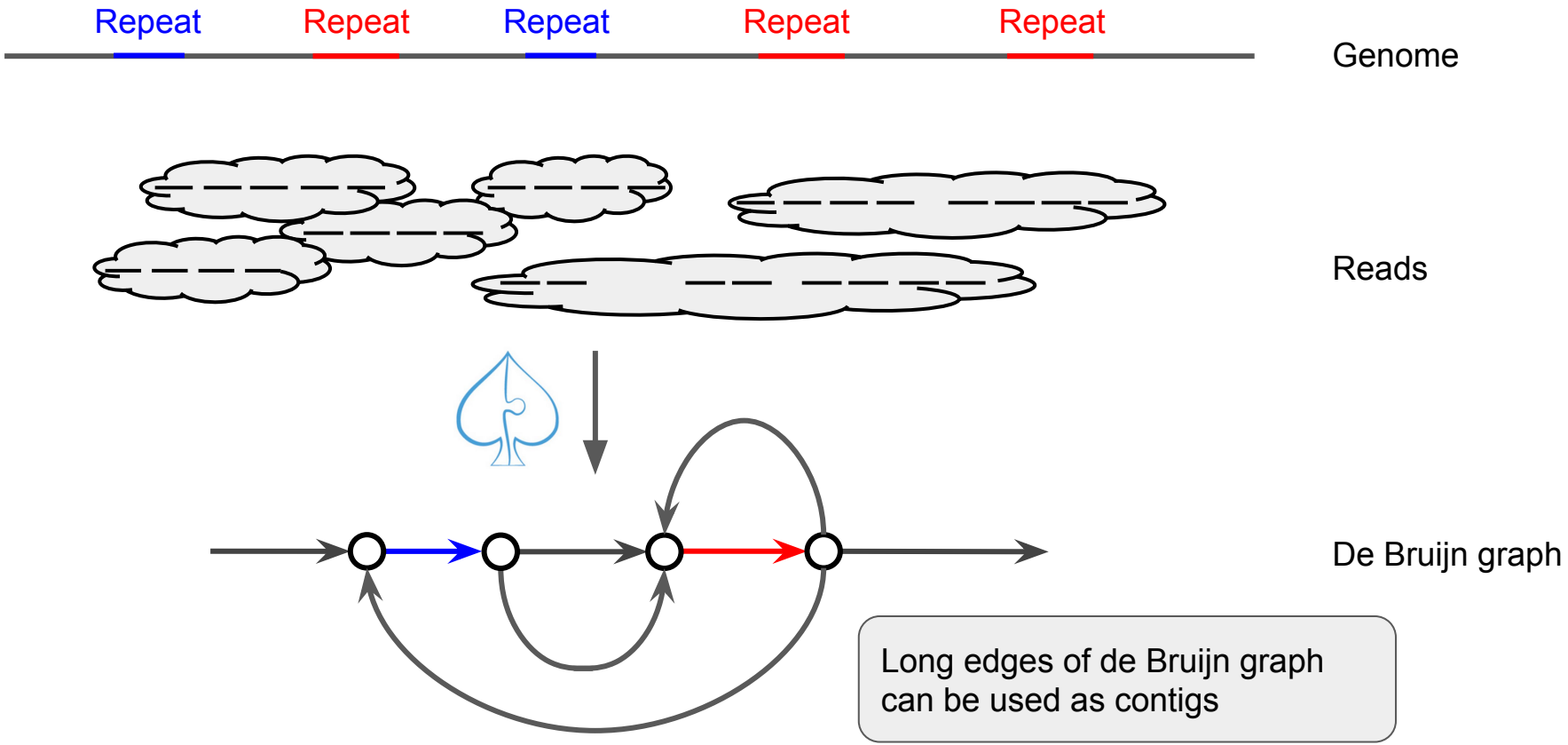
V. Kuleshov et al., Genome assembly from synthetic long read clouds, Bioinformatics (2016)

Read cloud scaffolding

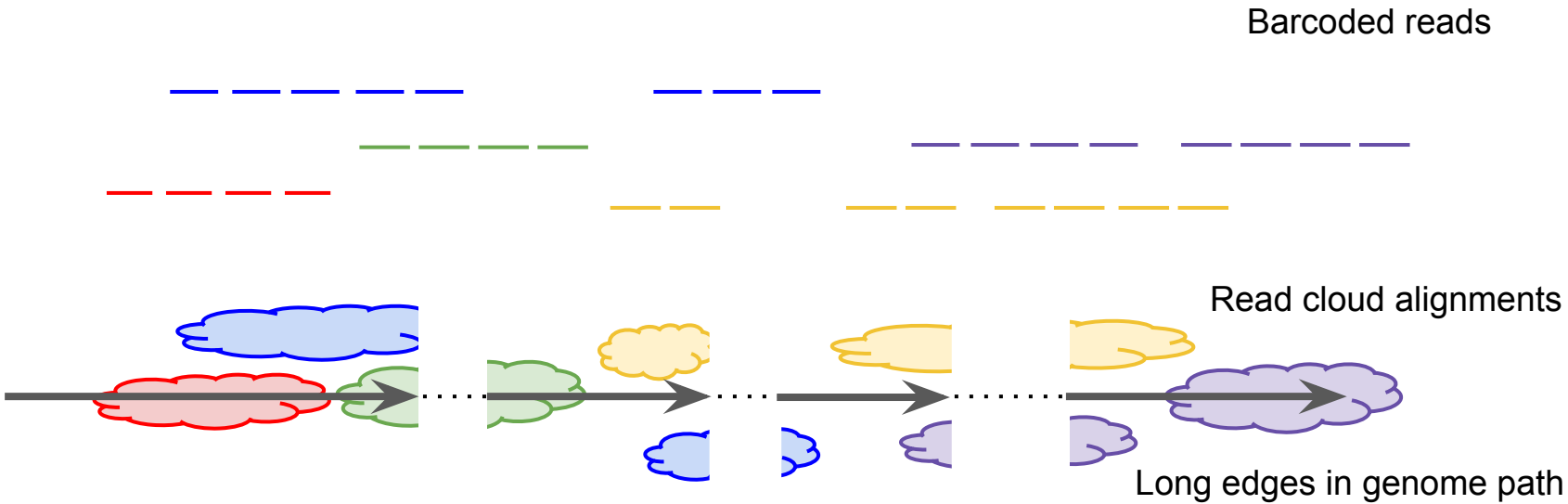


- Read clouds can be aligned to contigs
- Alignment of read clouds can be used for scaffolding

De Bruijn graph

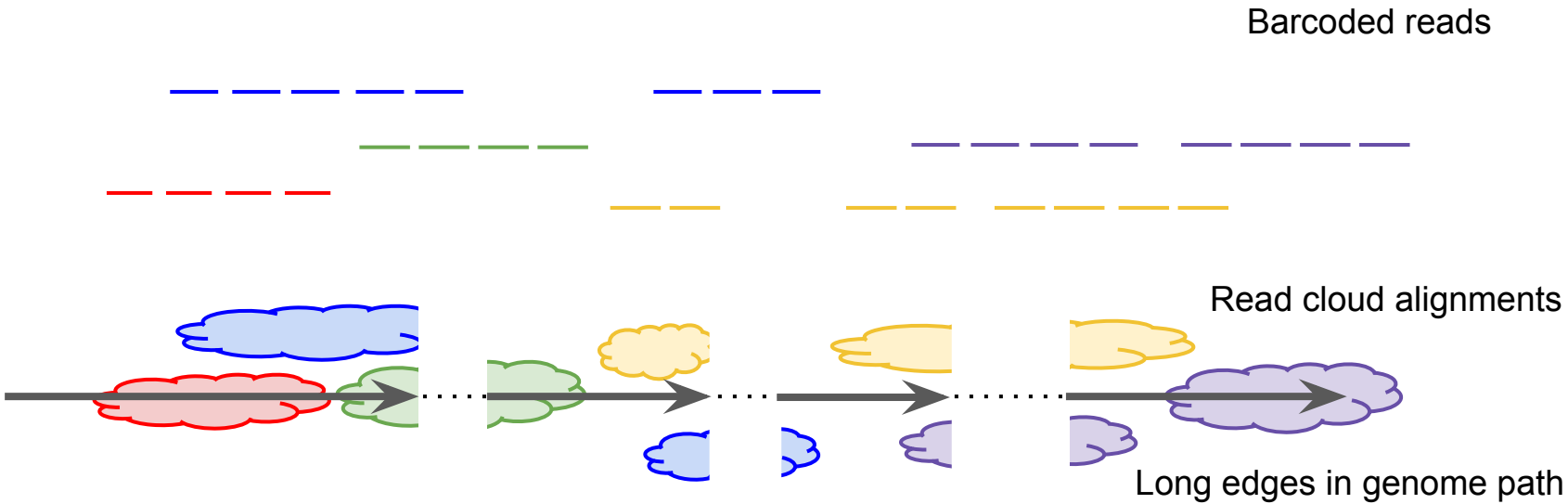


Clouds over de Bruijn graph



- Clouds can be aligned to the long edges of the graph

Read cloud scaffolding of de Bruijn graph

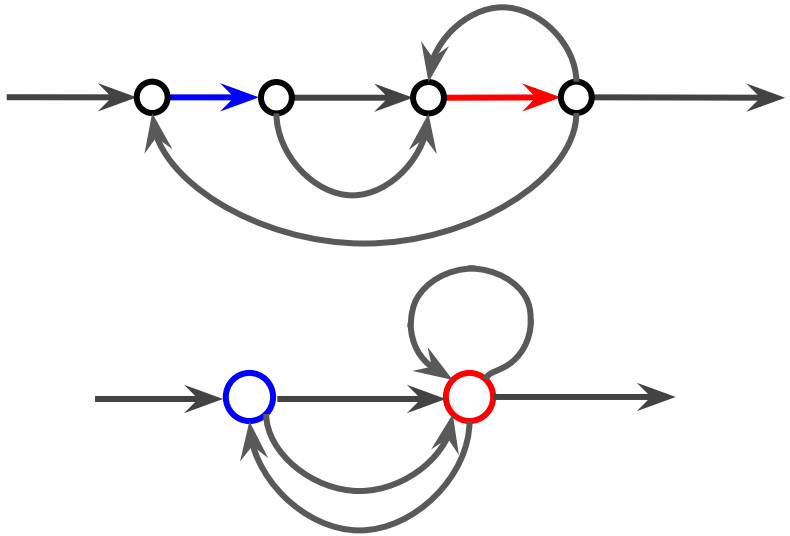


- Clouds can be aligned to the long edges of the graph
- For every edge we will try to find the next long edge in genome path

Contracted de Bruijn graph

- **Vertices:** connected components of short edges of de Bruijn graph
- **Edges:** long edges of de Bruijn graph

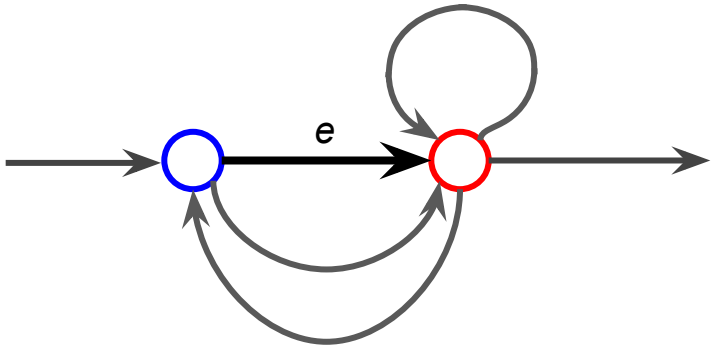
Motivation: finding genome path through complex components of short edges is very difficult. Thus we limit our attention to long edges and connections between them



De Bruijn graph

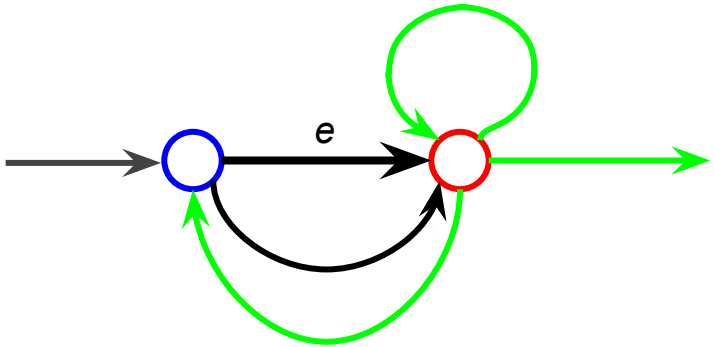
Contracted graph

Scaffolding: next edge candidate selection



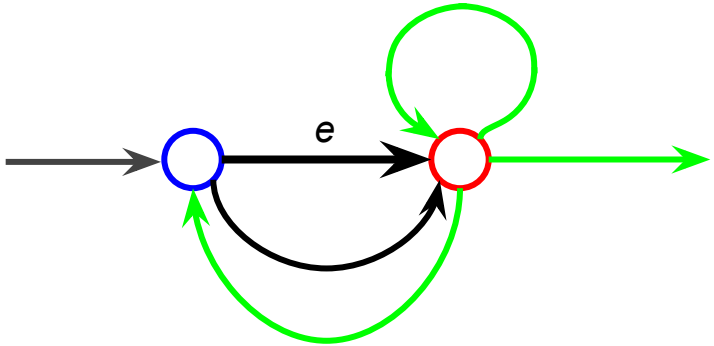
- We want to find the next long edge in the genome path after edge *e*

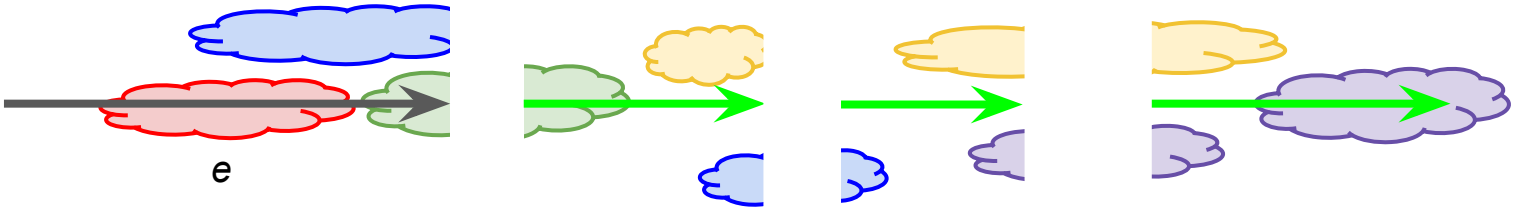
Scaffolding: next edge candidate selection



- We want to find the next long edge in the genome path after edge *e*
- We limit a set of candidates to edges adjacent to *e* in contracted graph

Scaffolding: next edge candidate selection





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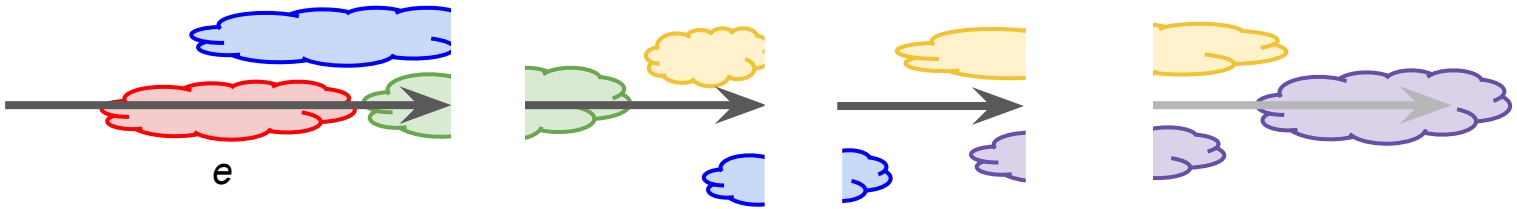
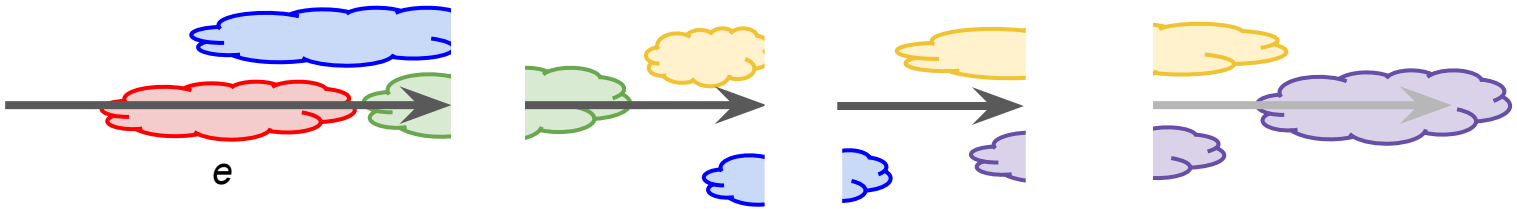
Shared barcodes pruning



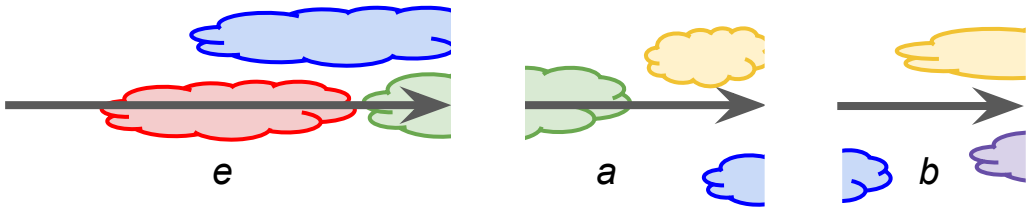
- We discard candidates that share no or very few barcodes with *e*

Shared barcodes pruning

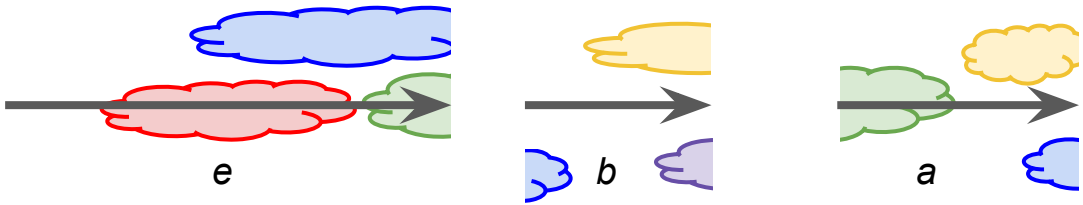


- We discard candidates that share no or very few barcodes with *e*
- Remaining candidates are typically close to *e* in genome path
- Next step is to determine their order

Edge order pruning



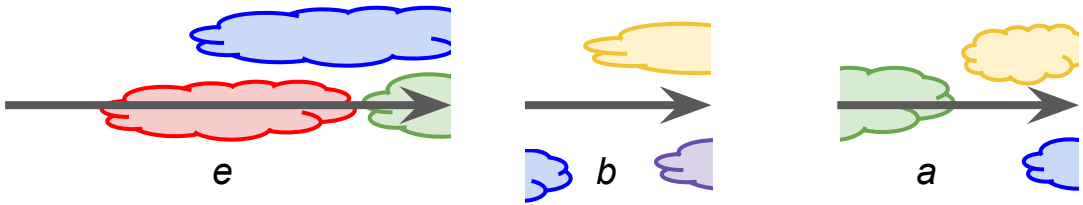
Correct ordering



Incorrect ordering: central edge does not contain green barcode

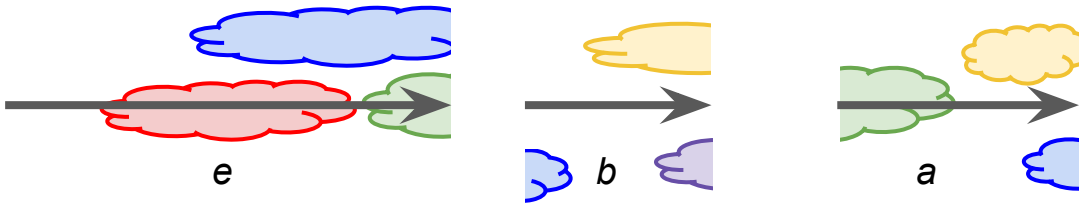
- Next edge should be located between ***e*** and every other candidate
- If edge ***a*** is located between ***e*** and ***b*** then ***a*** should contain every barcode shared by ***e*** and ***b***

Edge order pruning



- It is possible that green barcode may contain long gaps
- Or two green fragments are located near each other in the genome
- We need statistical model to deal with these cases

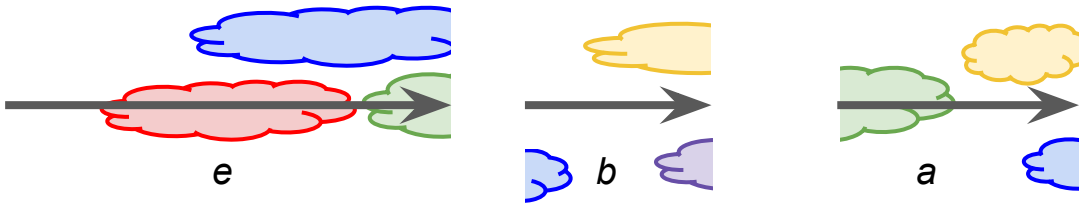
Edge order pruning



We define:

- *Contradiction(***e, a, b***)* : number of barcodes shared by ***e*** and ***a*** and not present on **b**
- *Intersection(***e, b**) : number of barcodes shared by **e** and **b**

Edge order pruning



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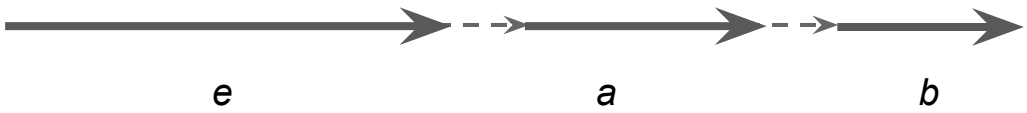
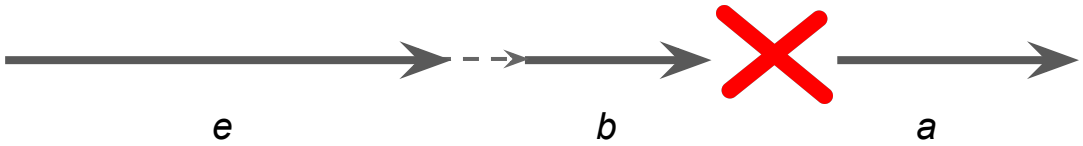
P(*Contradiction, Intersection* | *length(***b***), cov(***e***), cov(***a***))* is estimated using information from long edges

Edge order pruning



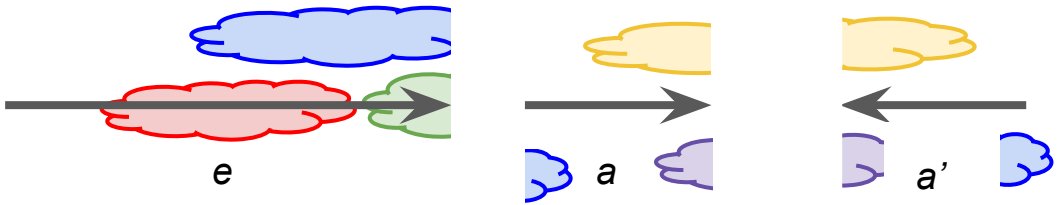
Correct ordering



Incorrect ordering: ***a*** is not reachable from ***b***

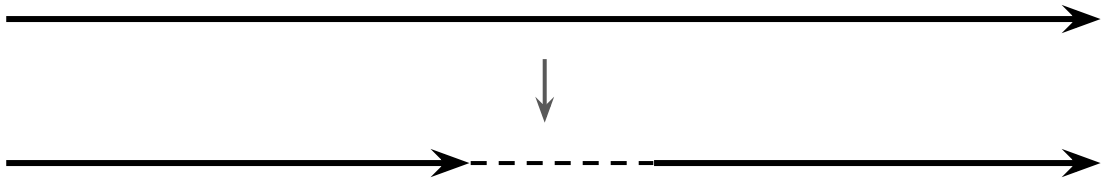
- Next edge could also be detected using graph topology
- If edge ***a*** is located between ***e*** and ***b*** then ***a*** and ***b*** should be adjacent in the contracted graph

Problems: reverse complement edges



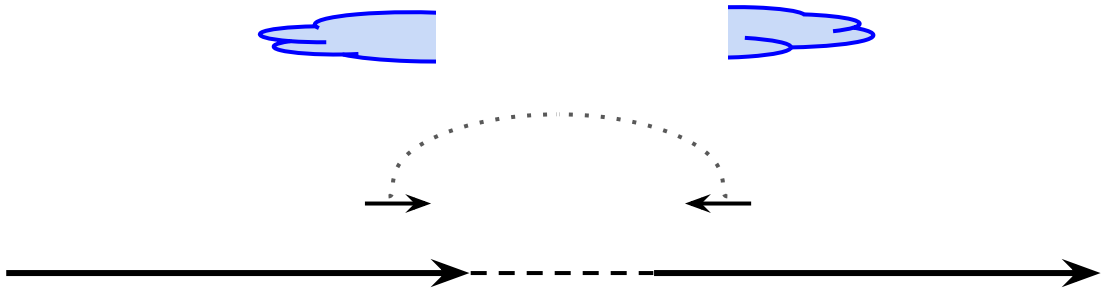
- Reverse complement edges have the same sets of barcodes
- We need specific criterias to find out the right order

Problems: candidate search



- DBG is not perfect due to coverage breaks and simplification errors
- Often we can not reach any candidates using DBG edges

Problems: coverage breaks



- DBG is not perfect due to coverage breaks and simplification errors
- Often we can not reach any candidates using DBG edges
- We can use paired end and read cloud information to traverse coverage breaks

Validation: metagenomics

Simple metagenomic dataset which comprised of 10 isolates

Complete reference genomes unavailable, instead:

- draft assembly for every isolate
- 23 validated ribosomal operons

One of the draft assemblies was discarded since it was not covered by metagenomic assemblies. 9 remaining draft assemblies were provided as “references” to metaQUAST

We compared our resuts with Athena[1], read cloud assembler aimed at metagenomic data

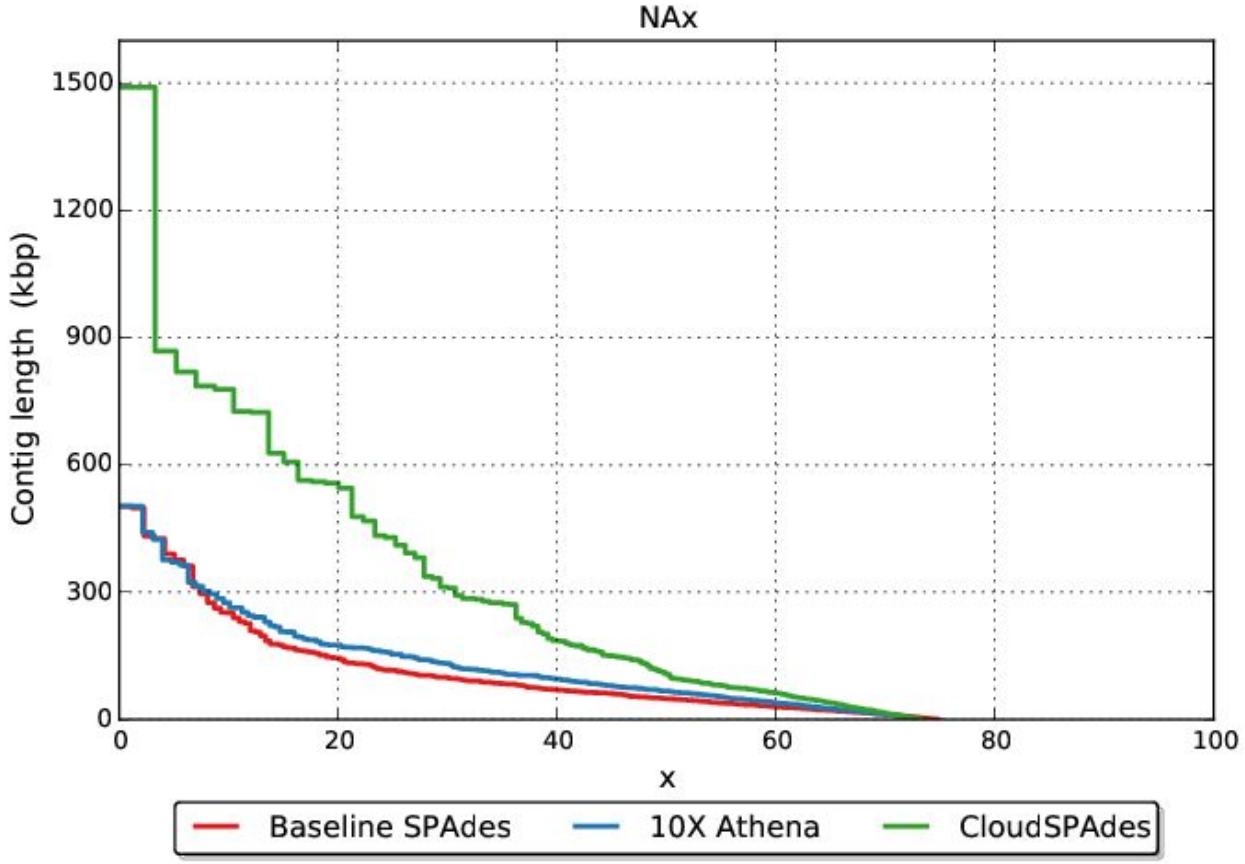
Bishara *et al* (2017). De novo assembly of microbial genomes from human gut metagenomes using barcoded short read sequences. *bioRxiv*.

Reference assembly validation

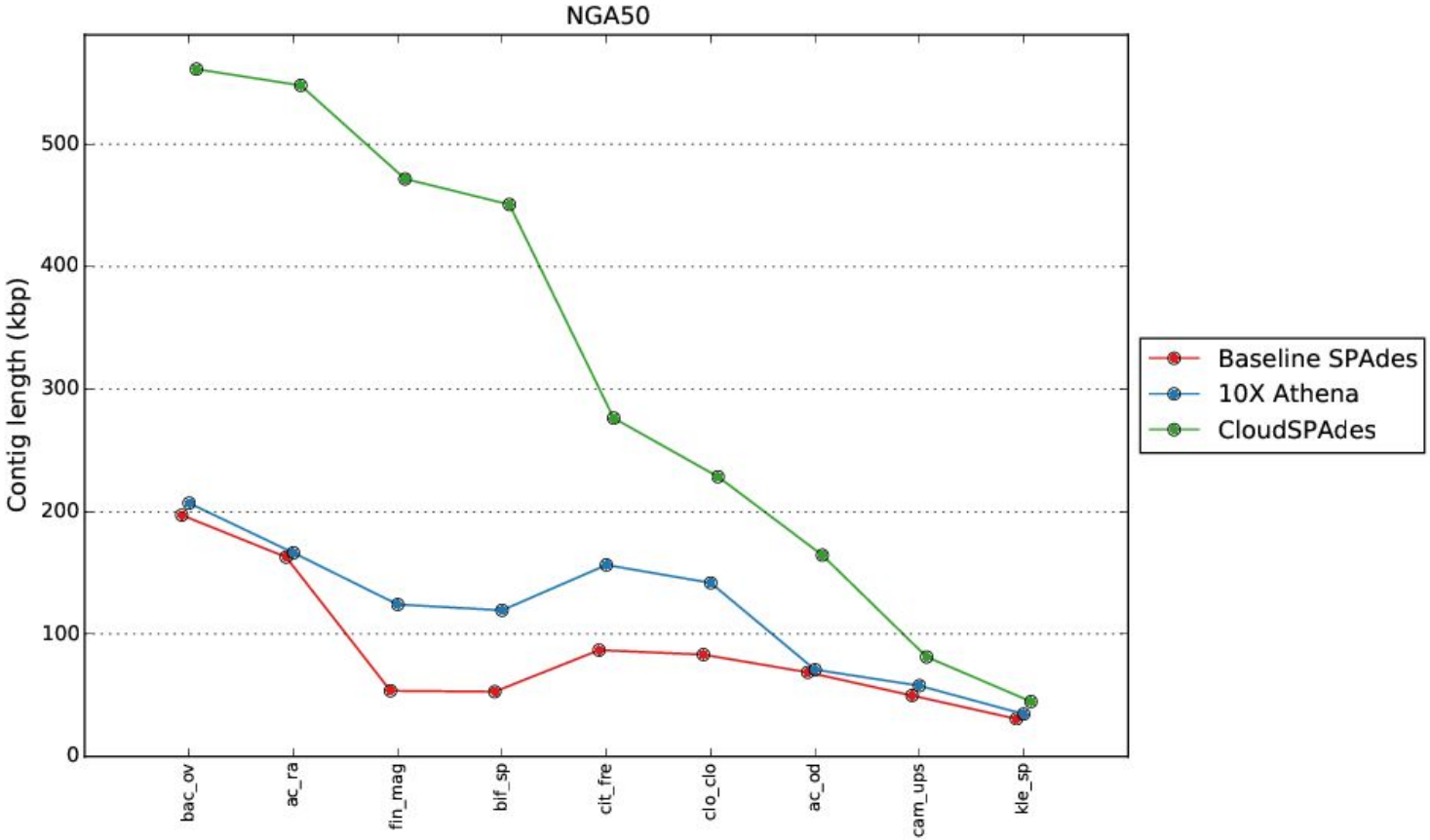
Assembly	Baseline SPAdes	10X Athena	CloudSPAdes
# contigs	4242	3700	3682
Largest contig	618498	506140	**1639058**
Total length	44596618	**47352283**	45423756
Reference length	34324828	34324828	34324828
N50	83644	114821	**271124**
# misassemblies	**16**	22	39
# N's per 100 kbp	27.79	**0.00**	997.58
Largest alignment	503176	503352	**1490986**
NA50	49220	67118	**108061**

cloudSPAdes: SPAdes assembly with read cloud repeat resolution module

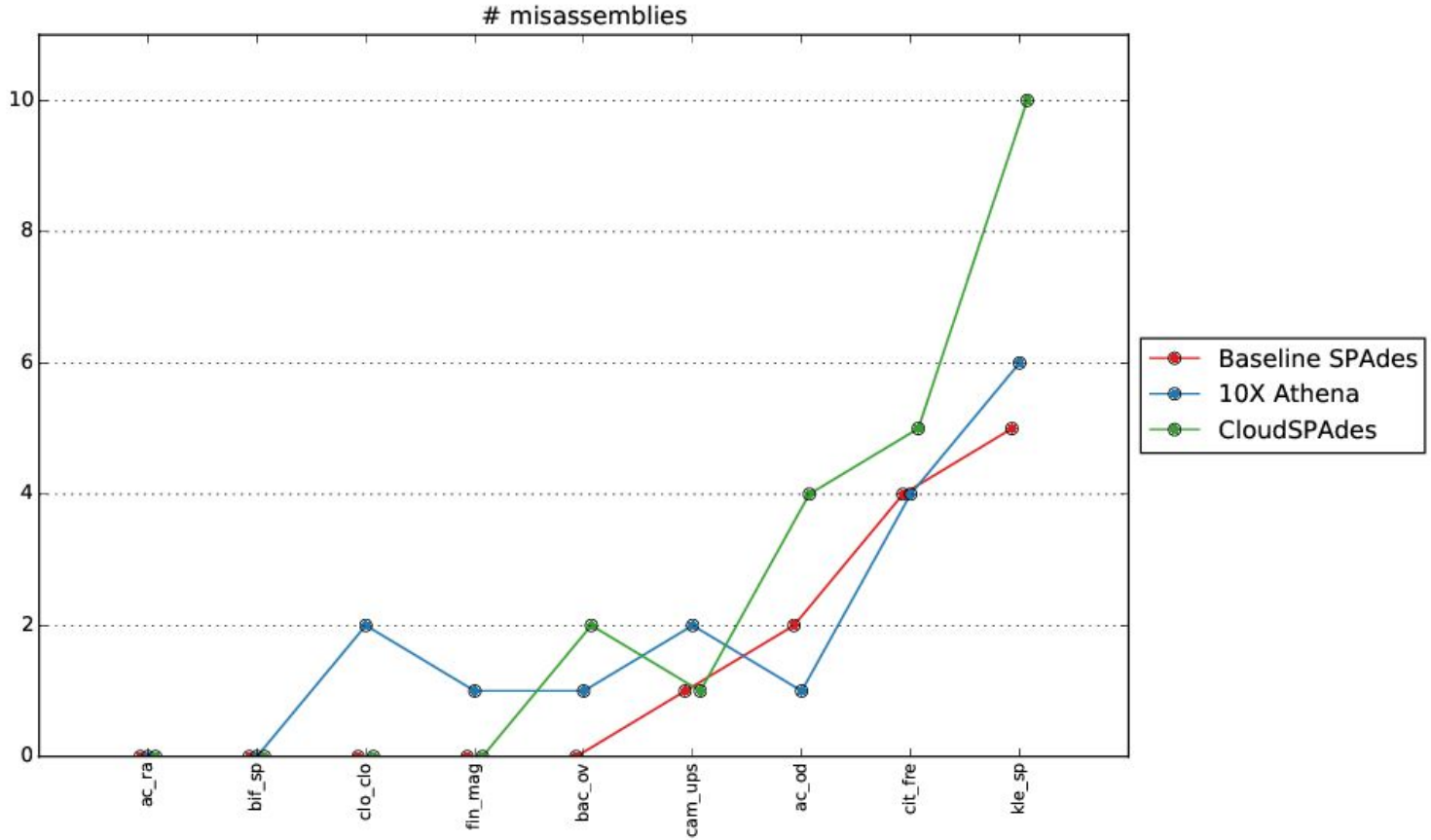
NAx plot


NAx
Contig length (kbp)
1500
1200
900
600
300
0
0
20
40
60
80
100
x
Baseline SPAdes
10X Athena
CloudSPAdes

NGA50 per reference



Misassemblies



Summary

Advantages of cloudSPAdes assembly:

- Longer contigs

Disadvantages:

- More misassemblies
- Large fraction of N’s

cloudSPAdes reconstructed 20 out of 23 operons

Thank you

Questions?