A little tour of assembly methods

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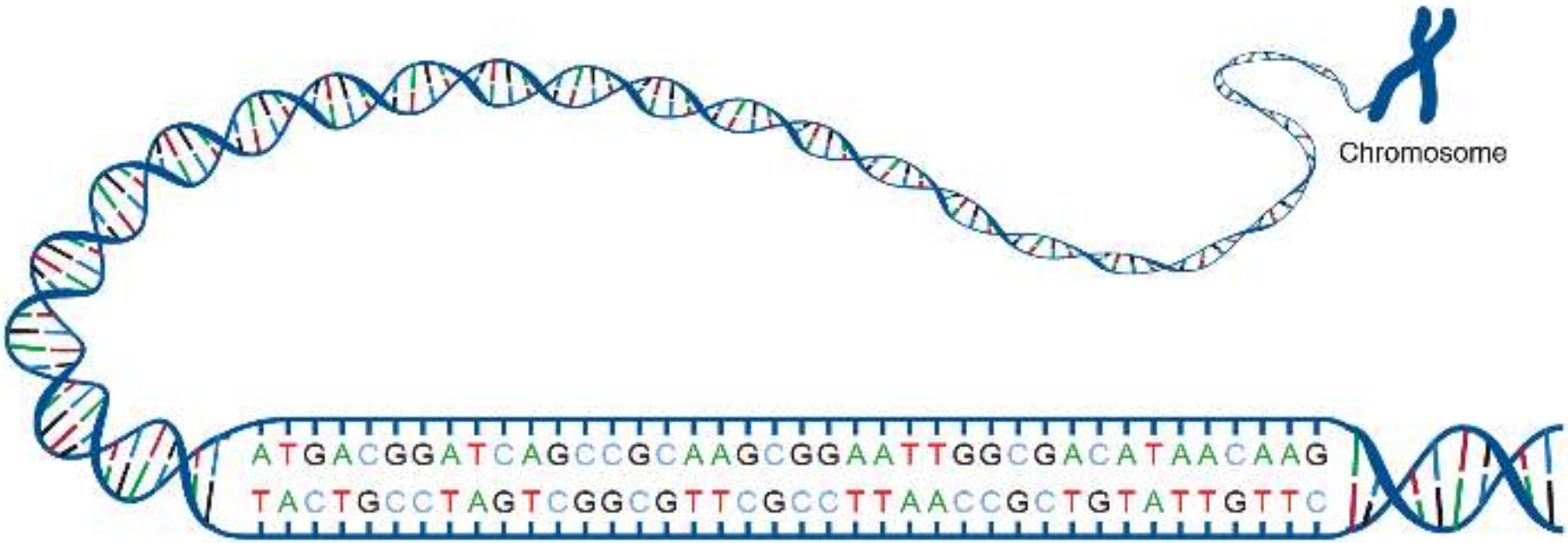
Camille Marchet

Content of this course

- How to reconstruct a genome with sequencing data?
- What are the main challenges?
- Which solutions have been proposed?

genome size: $\sim$ 32 gigabases

- Accessing a genome



From `www.genome.gov/genetics-glossary/acgt`

- Reads are subsequences from the genome

- Reads are **shuffled** subsequences from the genome

- Genome assembly task

- Using read overlaps

- Genome sequencing: coverage

- Genome sequencing: coverage

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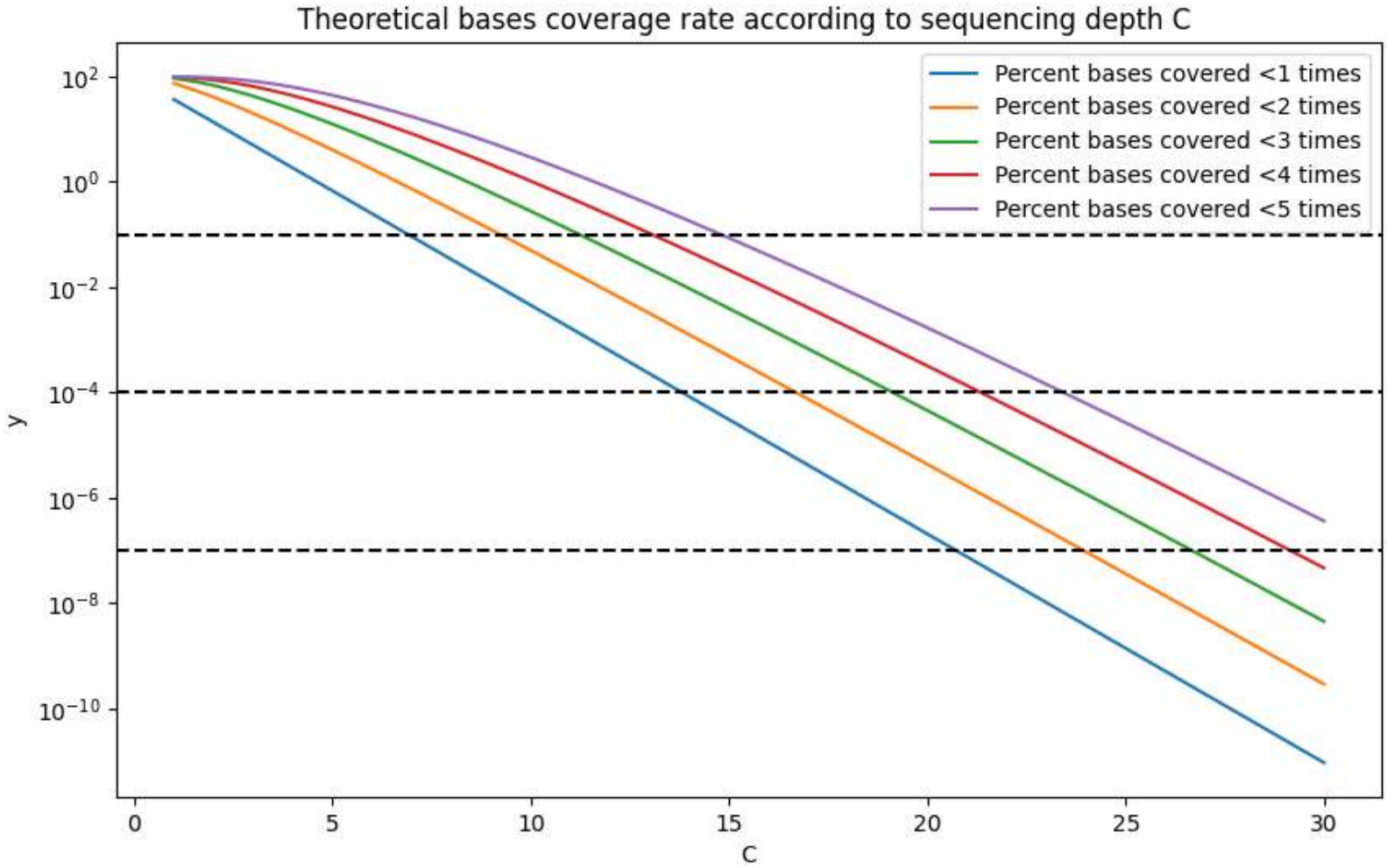
Genome sequencing: coverage



30-100X are often required for assembly projects

- Which overlap?

- Assembly idea number 1: assemble the longest overlaps

- Your time to shine!

- The Greedy solution

- The actual solution

- What happened?

- Do we expect many repeats?

Input interpretation:

$$\left(\frac{4^{31}-1}{4^{31}}\right)^{1/2\left(5\times 10^6\left(5\times 10^6-1\right)\right)}$$

Decimal approximation:

0.99999728949878430238317205542136383671202317193893202410 6...

From en.wikipedia.org/wiki/Birthday_problem

- The burden of assembly: genomic repeats

- 15: 44,994
- 21: 1,169
- 31: 559
- 41: 323
- 51: 225
- 61: 192

Genomic repeats are NOT random events

- Greedy assemblers
  - Simple and efficient scheme
  - Rely on **local** best choice (greedy)
  - May create errors because of local choices when there are repeats

- History: the human genome project while finding a successor to the greedy approach (according to MidJourney)

- Graph representation

- Assembly **idea number 2: consider all overlaps**

- Greedy solution

- One piece solution

- Multiple repeats

- First solution

- Second solution

- Parsimonious solution: do not assemble

Repeats lead to the fragmentation of the assembly

- Missing information also fragments the assembly

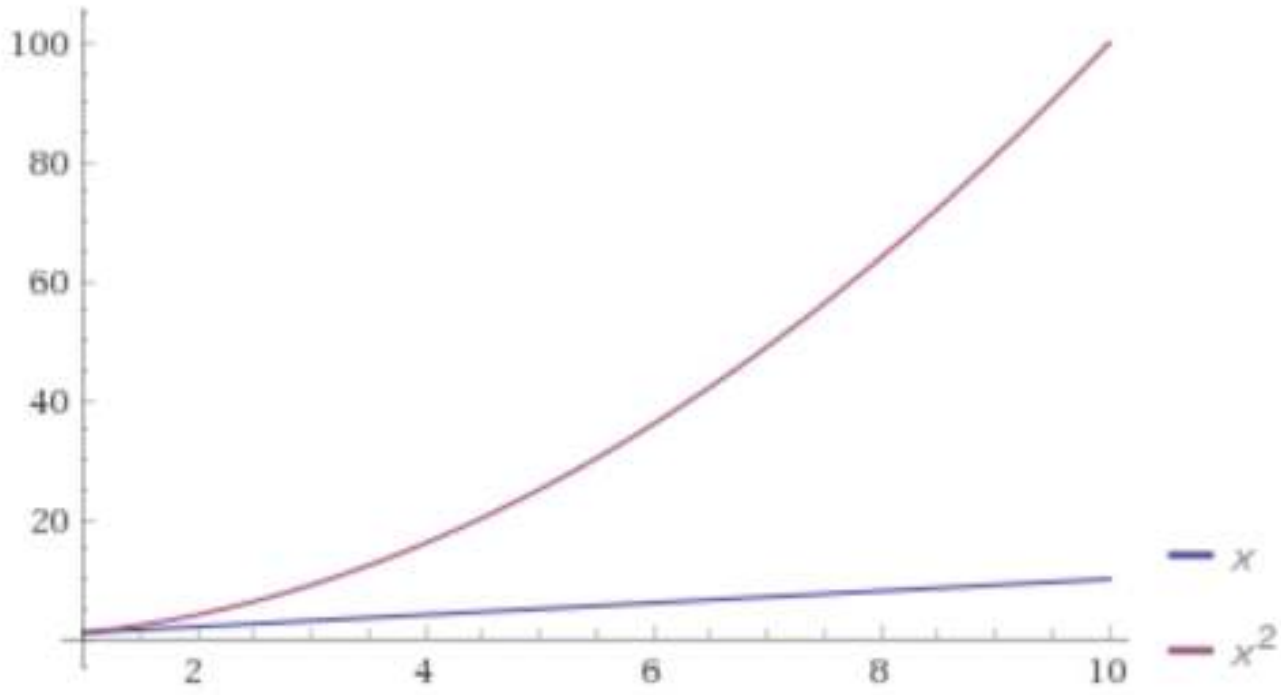
- Assembly **concession number 1: output fragments**

In the real world, assemblers often provide pieces of genomes rather than complete ones

- Overlap graph prerequisite : all overlaps

Overlap graph burden: number of reads

$n(n-1)/2 = \mathcal{O}(n^2)$ possible overlaps for n reads



Linear: 2X data 2X time
Quadratic: 2X data 4X time

Overlap graph burden: number of reads

$n(n-1)/2 = \mathcal{O}(n^2)$ possible overlaps for n reads

# Reads	# Overlaps
1000	499,500
10,000	50 million
100,000	5 billion
1 million	500 billion
10 million	50 trillion...

The overlap computation is not linear

Talking about CPU years on large genomes...

We have to be efficient here and focus on "relevant" overlaps

- Overlap graph simplifications

Overlap graphs in a nutshell

- Graphs of overlaps between the reads
- Can provide a global solution for assembly
- Can be difficult in real cases because it requires a lot of computation (overlaps)

S. cerevisiae, *D. melanogaster*, human could be assembled using overlap graphs approaches (Celera (Myers et al. 2000), SGA (Simpson & Durbin 2011), ...)

- History: the introduction of another graph structure for assembly, according to MidJourney

- Assembly **idea number 3: Focus on genome words**

- Find consecutive genome words in read words

- How to connect read words?

- Reconstitute larger genomic words

- The de Bruijn graph

- de Bruijn graph assembly

- de Bruijn graph time!

Hint: Use 7-mers

- Solution

- de Bruijn graphs abstract redundancy

- de Bruijn graphs only rely on $k-1$ overlaps

- de Bruijn graphs limitation 1: Fixed overlaps

GGACT and ACTTA overlap is only of size 3 !

de Bruijn graphs limitation 2: Repeats

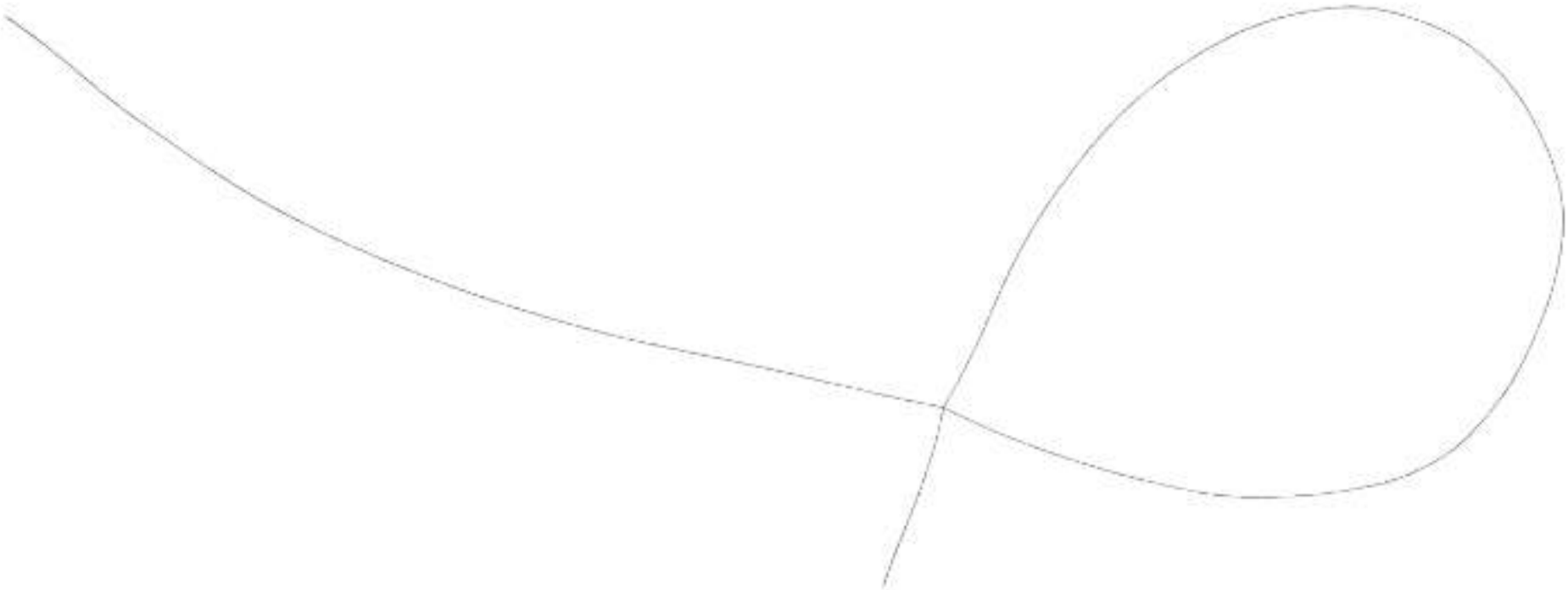
each k-mer appears only once in a de Bruijn graph

- de Bruijn graph limitation

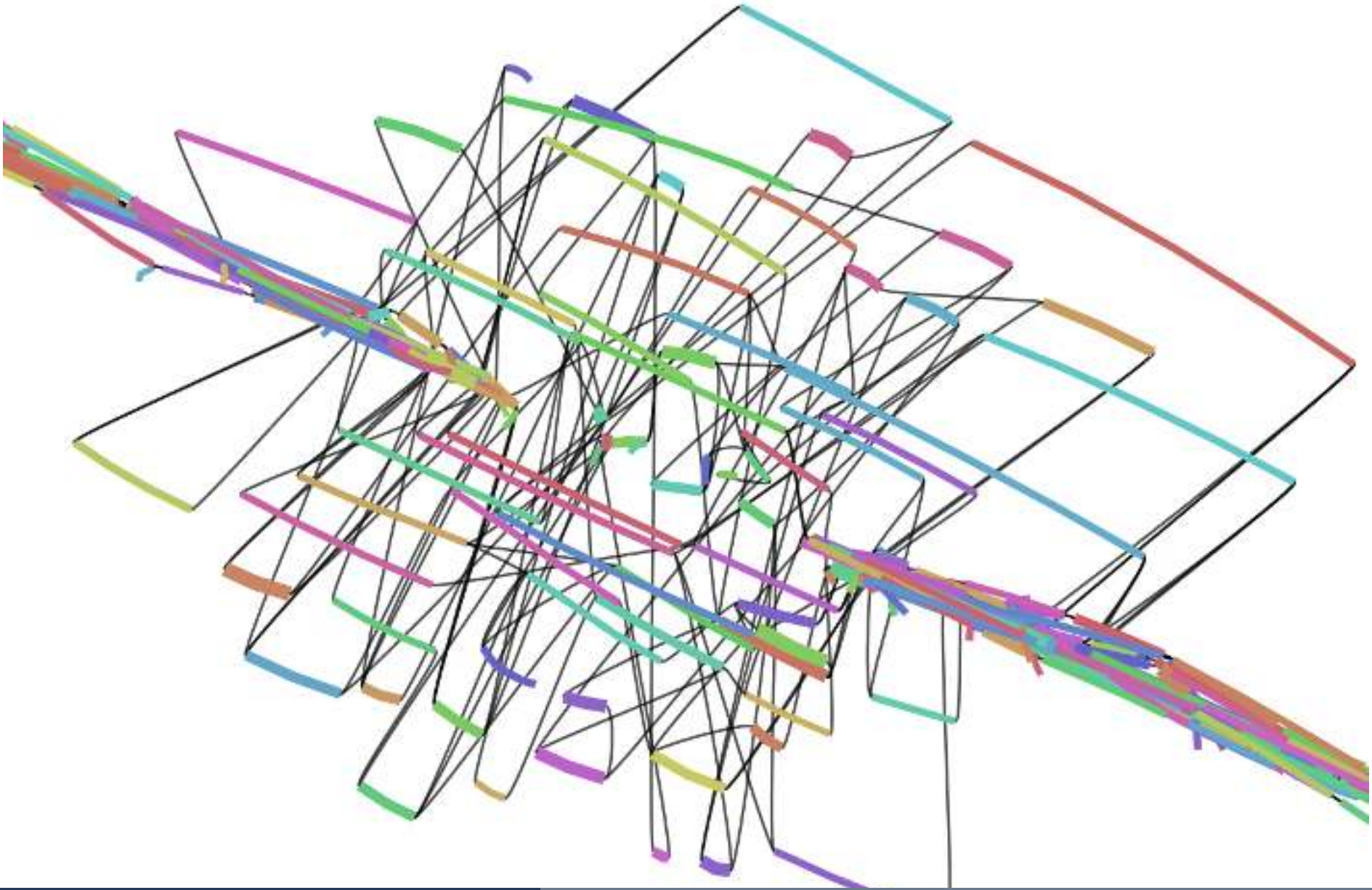
- de Bruijn graph versus overlap graph

- On the representation of de Bruijn graphs

- de Bruijn graph on a real dataset



- de Bruijn graph on a real dataset ZOOMED IN

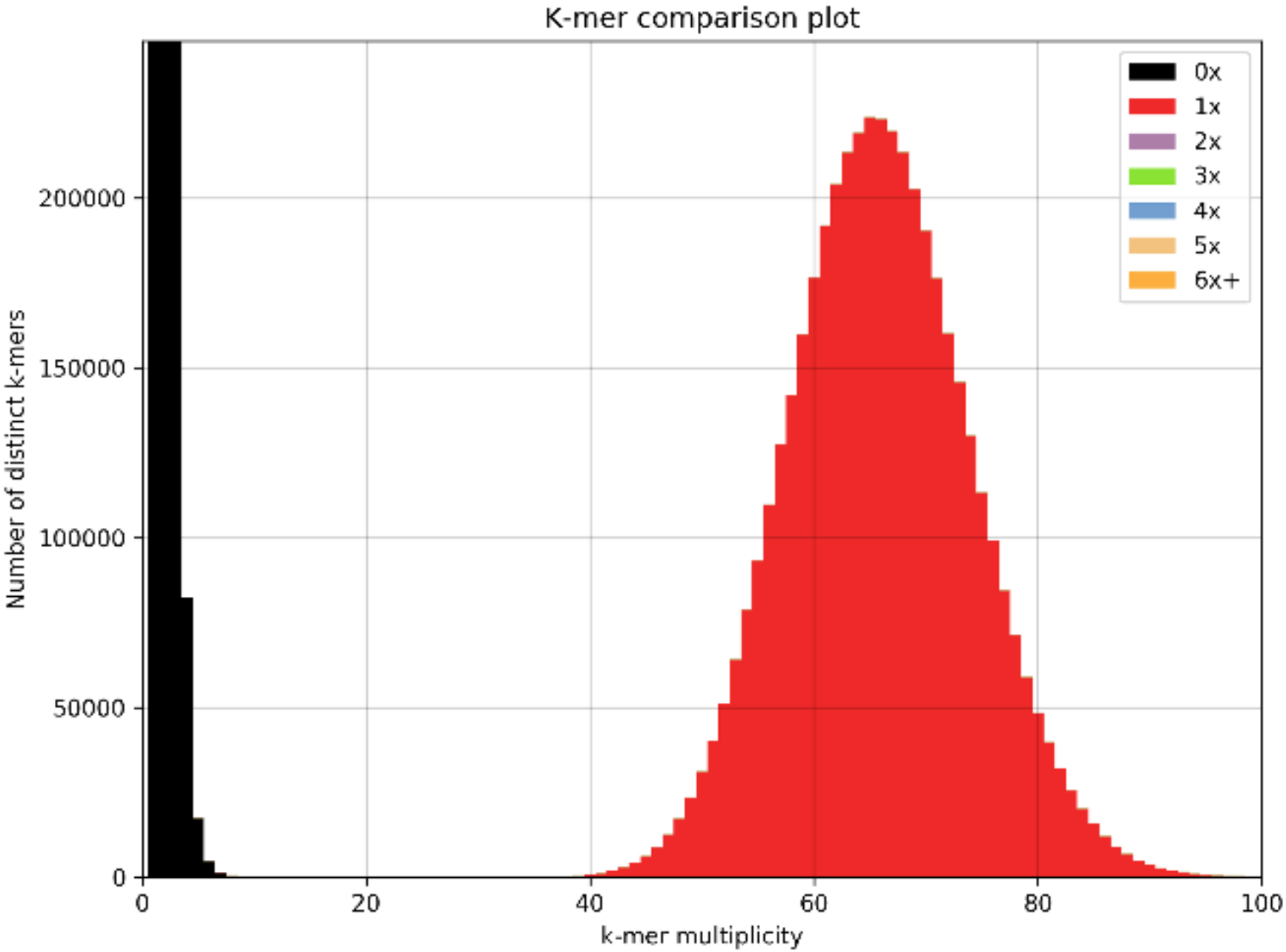


- Sequencing errors

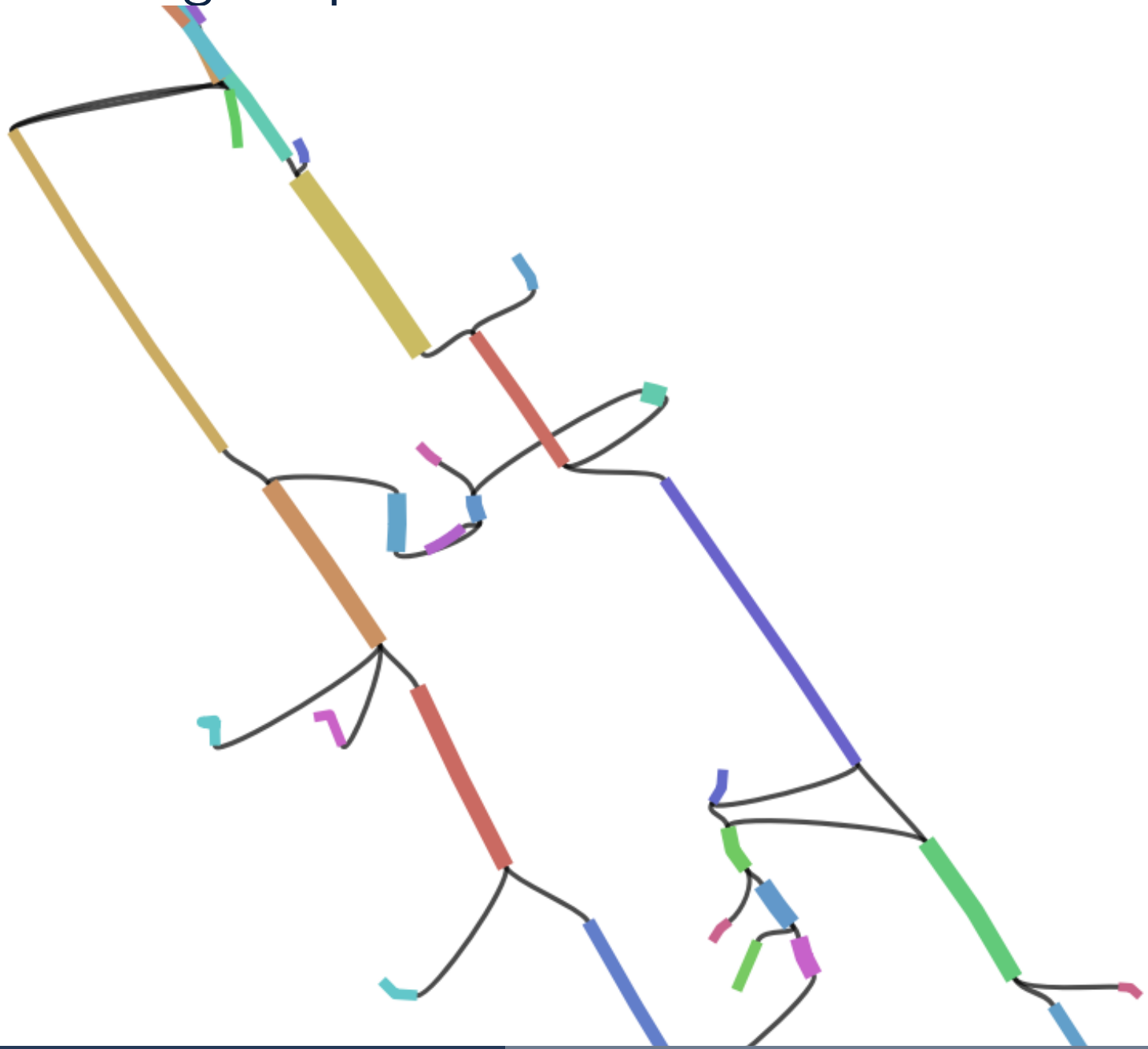
- Erroneous k-mers vs genomic k-mers

Erroneous k-mers are seen less than genomic ones

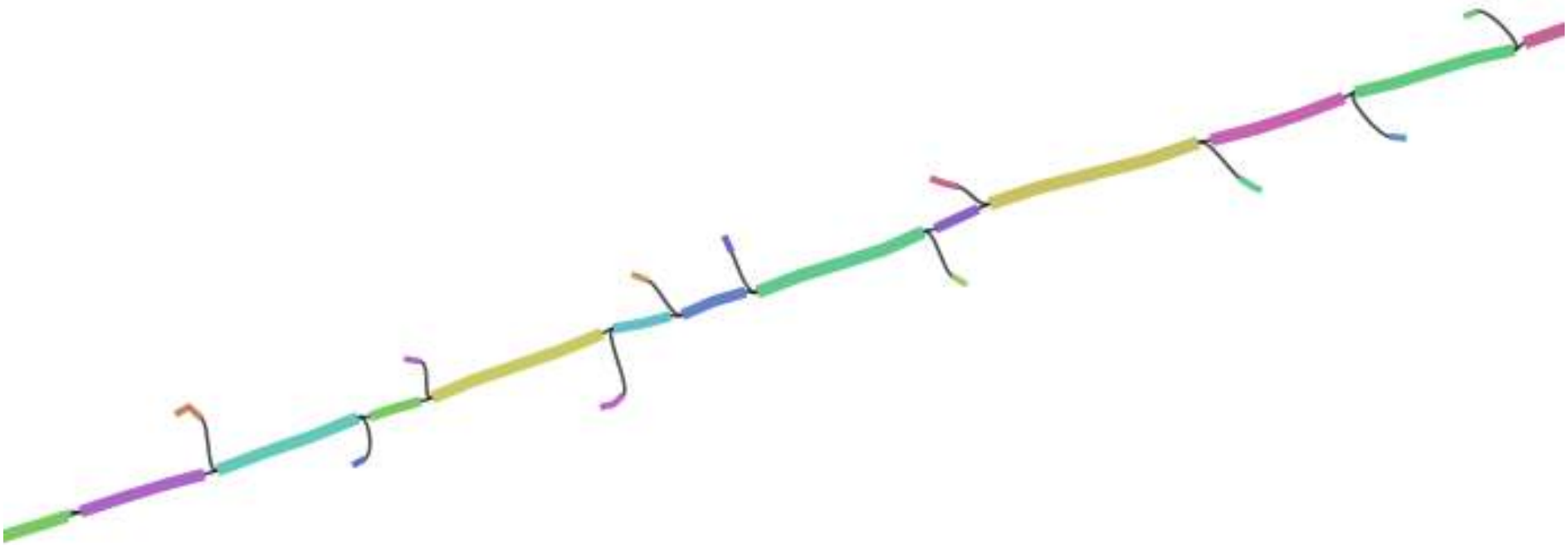
- *K*-mer histogram



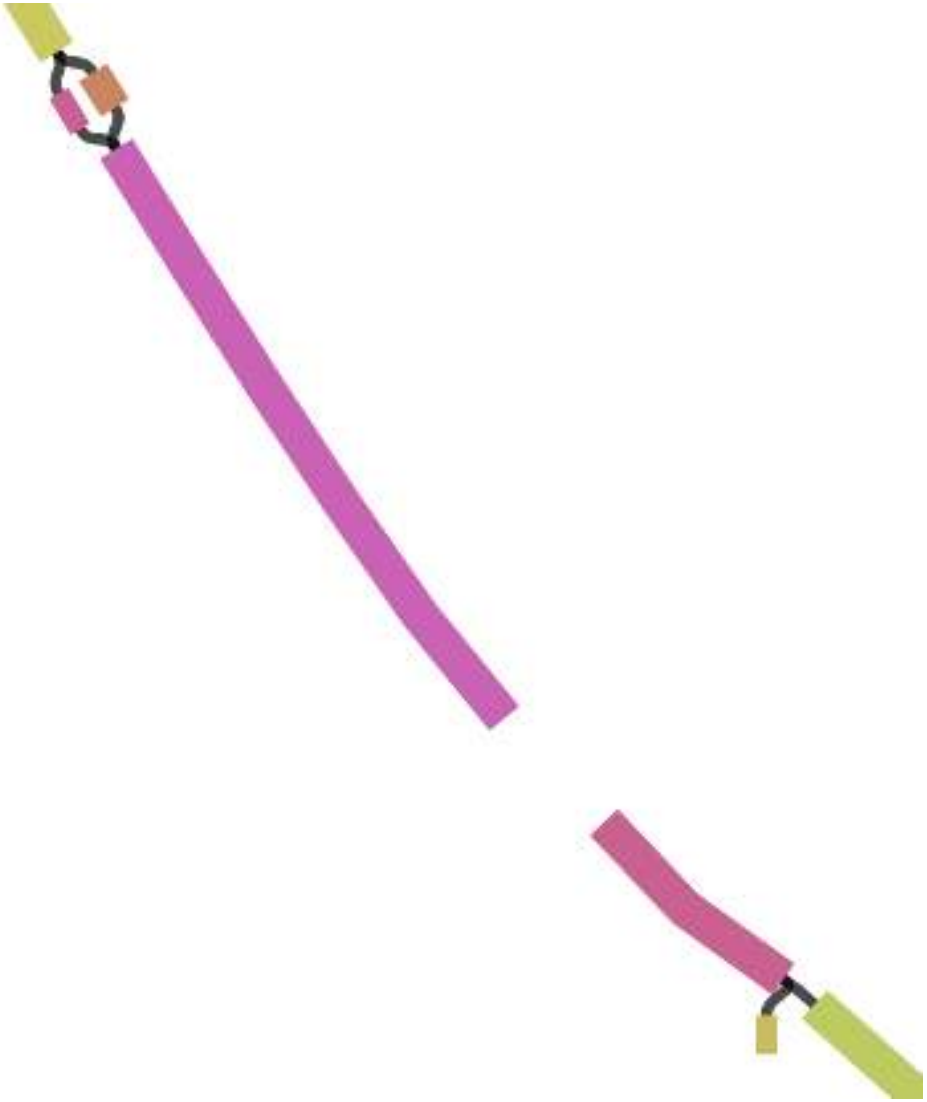
- Removing unique k-mers



- Removing k-mers seen less than 3 times



- Removing k-mers seen less than 4 times



- Errors in de Bruijn graphs

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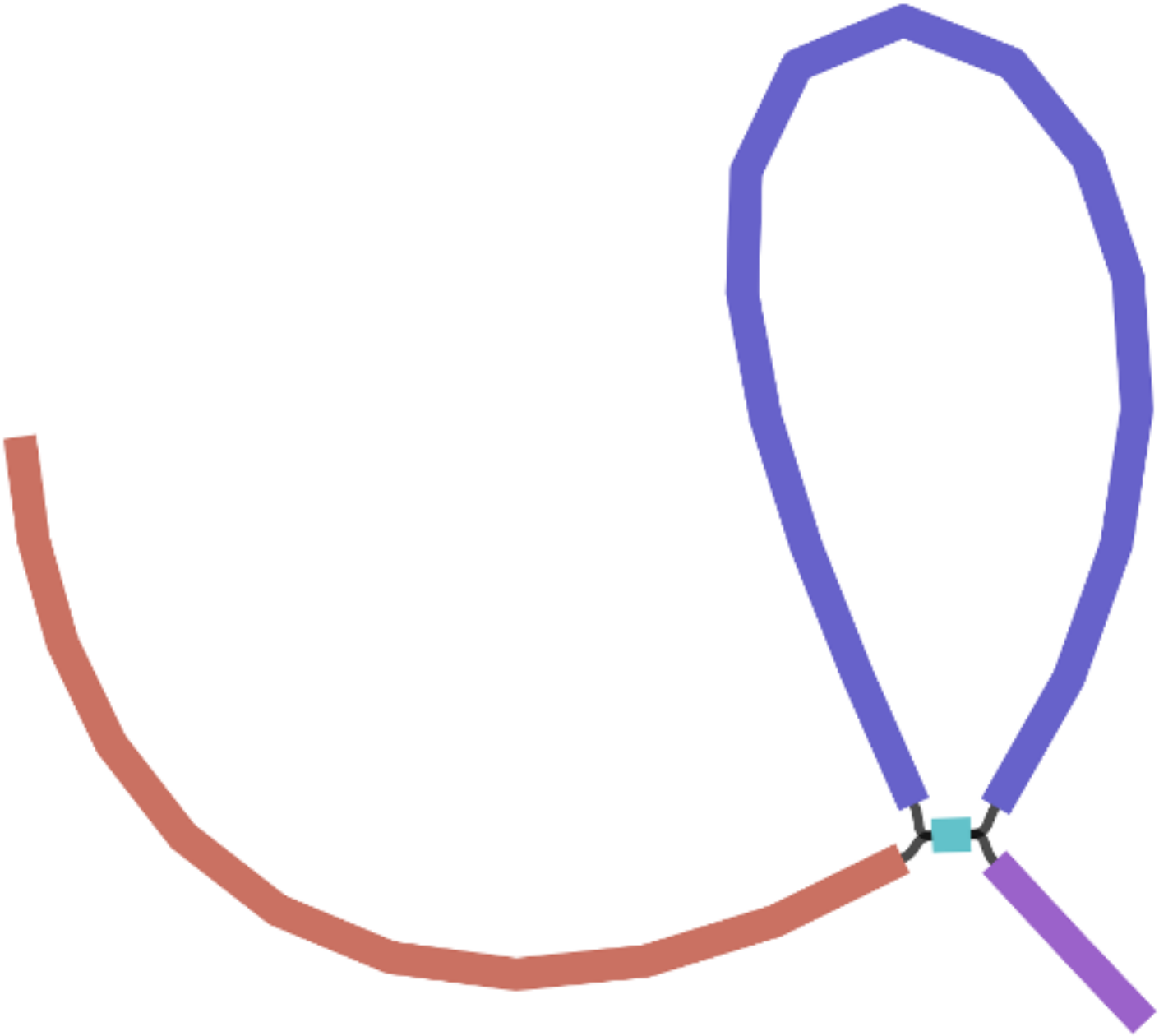
- Errors in de Bruijn graphs

- (Almost assembled phage !)



de Bruijn graphs in a nutshell

- Graph of words of size k, k-1 overlaps
- Collapses identical k-mers
- Very successful, have replaced the overlap graphs with high throughput sequencing data
- Still outputs fragments of the genome

White spruce, 20 gigabases

- Multiple k assembly

Most de Bruijn graph assemblers can now perform several assemblies with different k-mer sizes to produce an improved "super" assembly

Build DBG with k=5 and k=7 from those reads
AAAATCGATCTC
TCTCATCGAATT

- Multiple k assembly

We are missing GATCTCA and ATCTCAT in the second graph.
But they are present in the first graph!

- Multiple k assembly

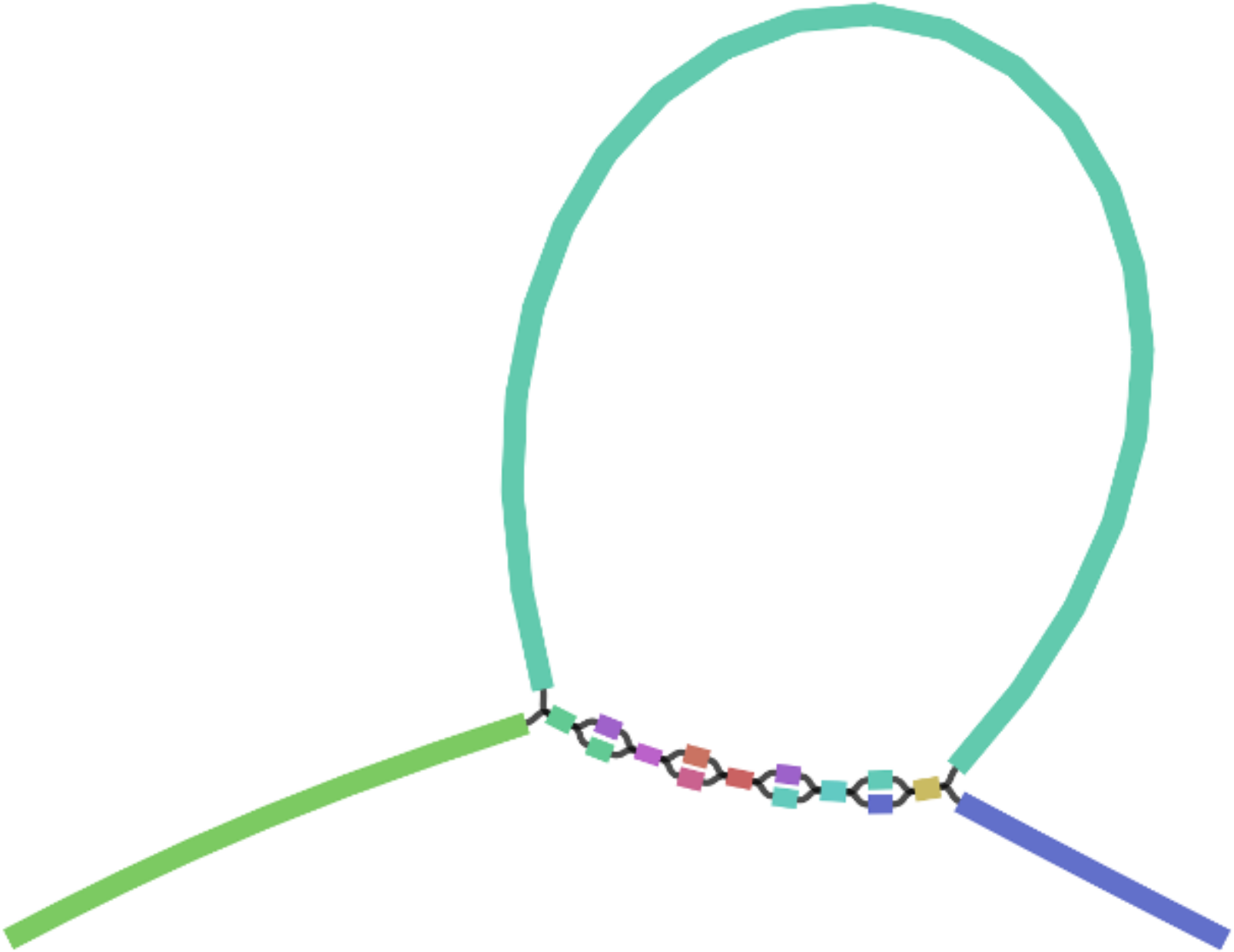
- de Bruijn graph on an eukaryota

- Two or more genomes per individual

- Two or more genomes per individual

- Assembly **concession number 2**: collapse variability

- Paralog genes/repeats



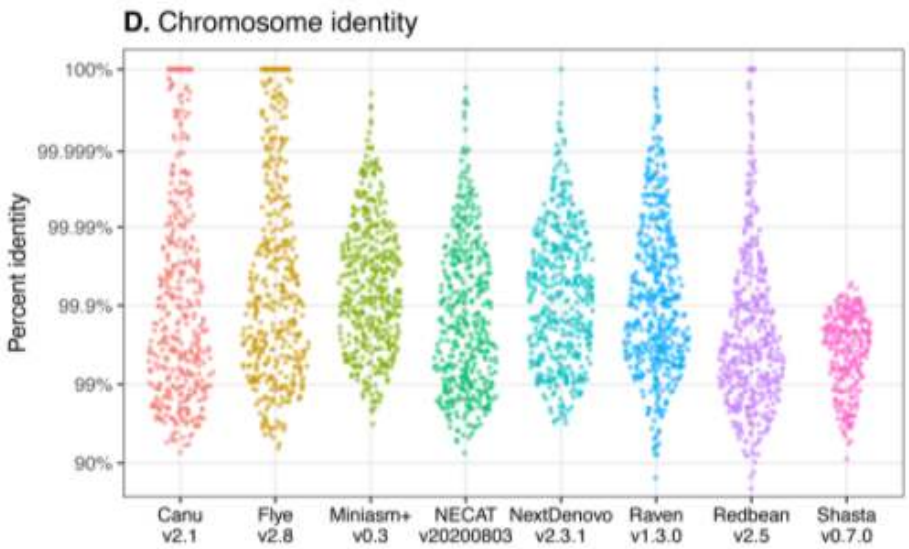
- Paralog genes/repeats in graph

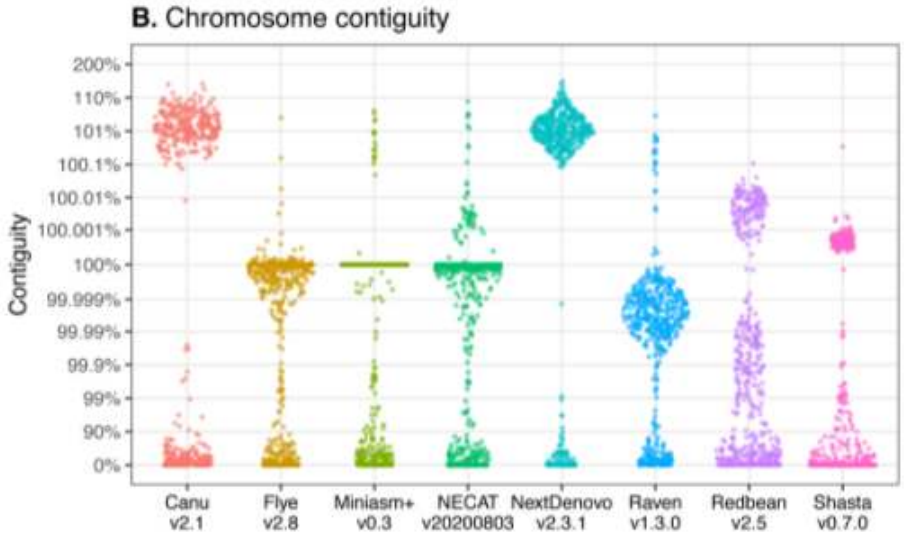
- An assembler is a set of heuristics

- Nodes coverage
- Graph local/global topology
- Reads that can be mapped on nodes
- Estimated coverage/genome size
- ...

An assembly is a model

1. Assemblies contain errors
2. Different tools can produce very similar assemblies
3. A single tool can produce very different assemblies with small changes of parameters(!)





From `github.com/rrwick/Long-read-assembler-comparison`

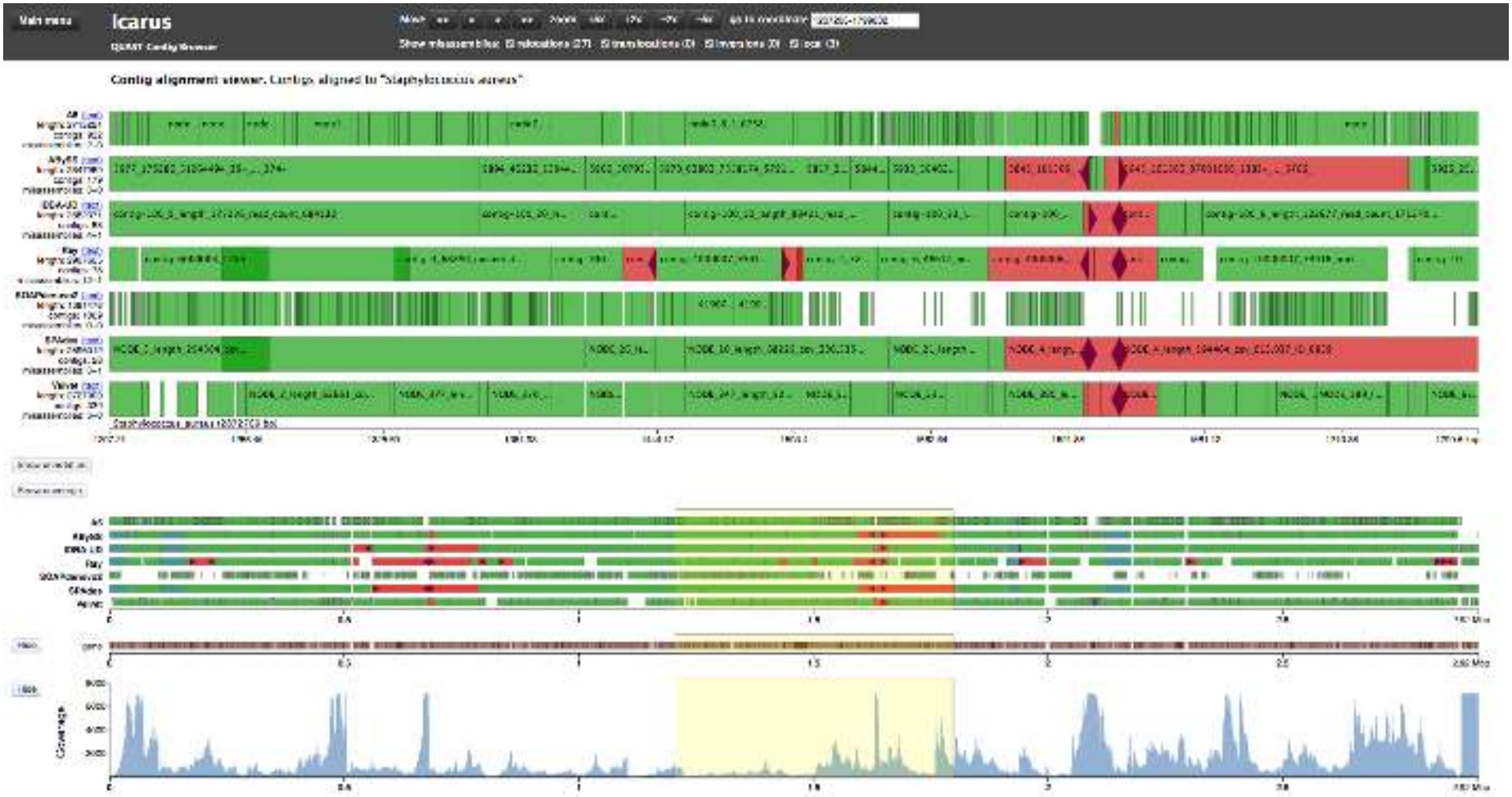
- What do we do post-assembly?

1. Assess its quality
2. Improve it
3. Use it!

Two ways to polish an assembly according to Mid-Journey

Evaluate assembly according to a reference

Contigs can be mapped and compared to a reference/closely related genome



From `quast.bioinf.spbau.ru/manual.html`

Assembly statistics

Alignment-based statistics	ABySS	MEGAHIT	SPAdes	Velvet
Genome fraction (%)	98.661	98.424	98.113	97.997
Duplication ratio	1.043	1	1	1
# genomic features	4525 + 75 part	4511 + 64 part	4489 + 50 part	4486 + 56 part
Largest alignment	248 481	235 933	285 096	264 944
Total aligned length	4 776 214	4 568 317	4 553 809	4 550 150
NGA50	69 801	122 647	133 309	112 446
LGA50	21	14	12	14
Misassemblies				
# misassemblies	4	0	0	4
Misassembled contigs length	231 767	0	0	435 515
Per base quality				
# mismatches per 100 kbp	2.09	2.69	1.03	3.19
# indels per 100 kbp	0.57	1.31	0.29	1.98
# N's per 100 kbp	24.59	0	17.55	94.19
Statistics without reference				
# contigs	176	95	92	90
Largest contig	248 481	235 933	285 196	264 944
Total length	4 777 853	4 571 292	4 557 363	4 552 266
Total length (>= 1000 bp)	4 757 929	4 562 458	4 548 710	4 544 453
Total length (>= 10000 bp)	4 562 801	4 478 614	4 466 223	4 475 223
Total length (>= 50000 bp)	3 248 113	3 833 793	3 812 315	3 817 904
BUSCO completeness				
Complete BUSCO (%)	98.65	98.65	98.65	98.65
Partial BUSCO (%)	0	0	0	0
Predicted genes				
# predicted genes (unique)	3717	3595	3587	3576

- Assembly continuity

N50 can be described as a weighted median statistic such that 50% of the entire assembly is contained in contigs or scaffolds equal to or larger than this value.

- The catsembler

- Assembly continuity

N50

N50 can be described as a weighted median statistic such that 50% of the entire assembly is contained in contigs or scaffolds equal to or larger than this value.

N75

N75 is the same statistic for 75% of the assembly

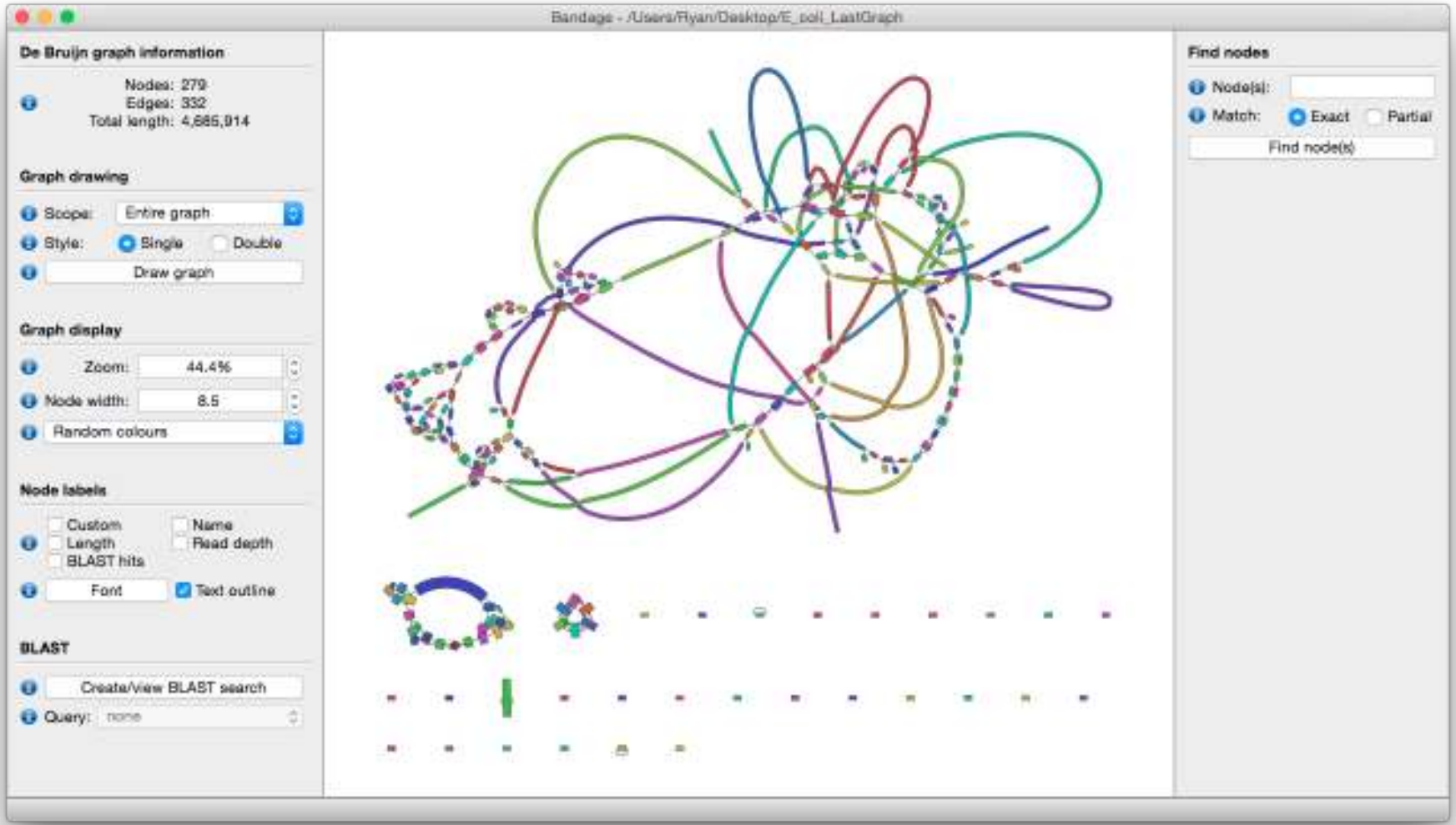
NGA50

Similar to the N50 but only takes into account contigs/scaffolds that can be **aligned** on the reference genome and consider 50% of the **genome size** instead of the assembly size

- Misassemblies

• Visualize assembly

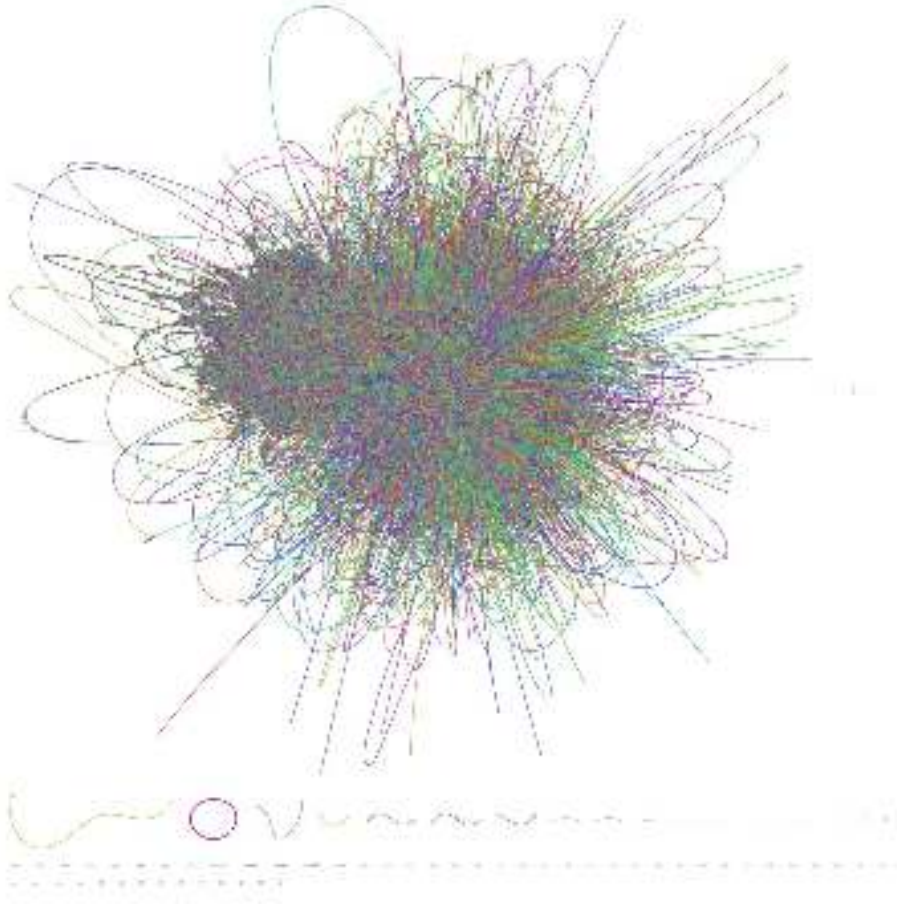
Bandage tool can visualize assembly graphs (GFA)



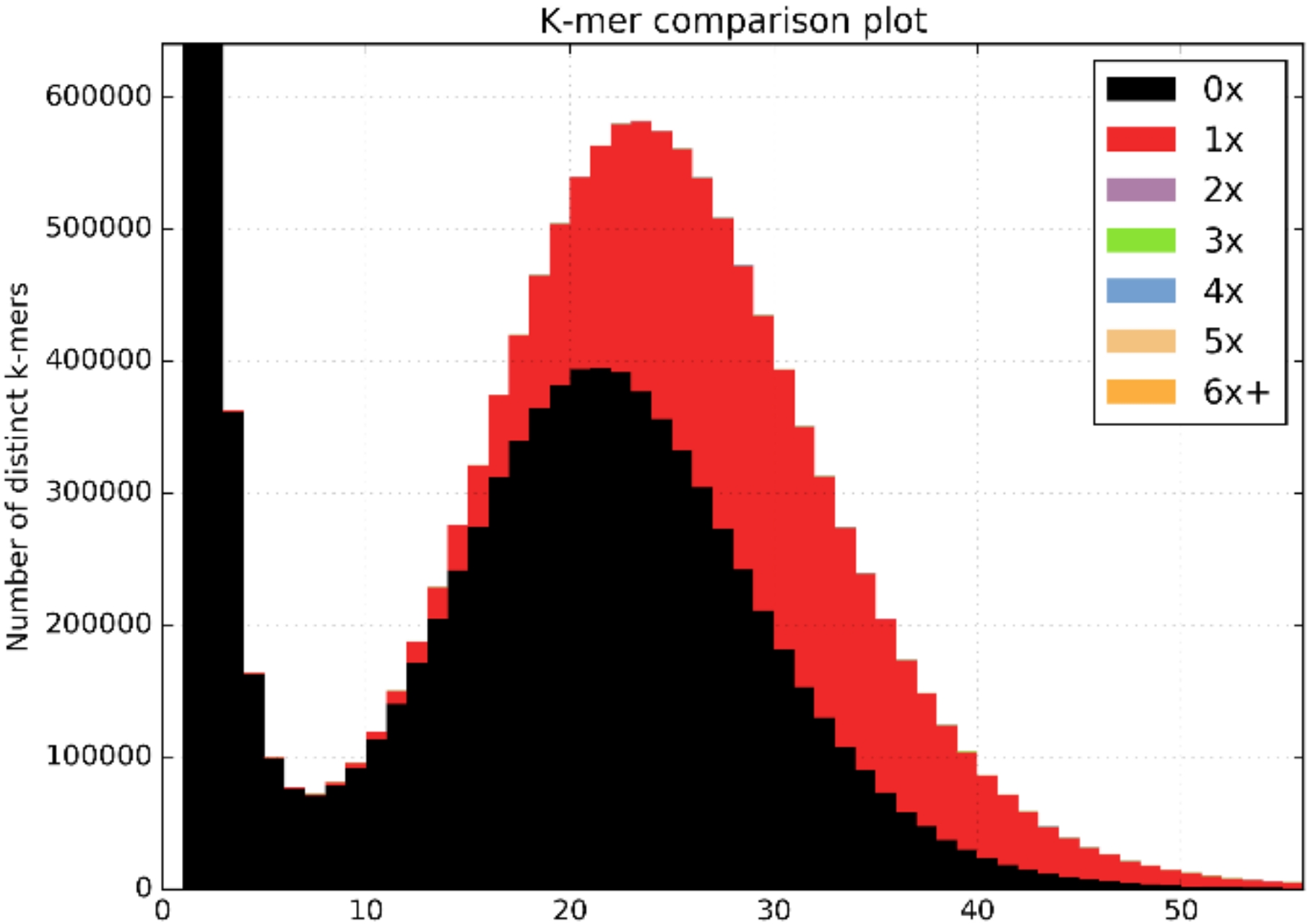
From `rwick.github.io/Bandage`

Visualize assembly

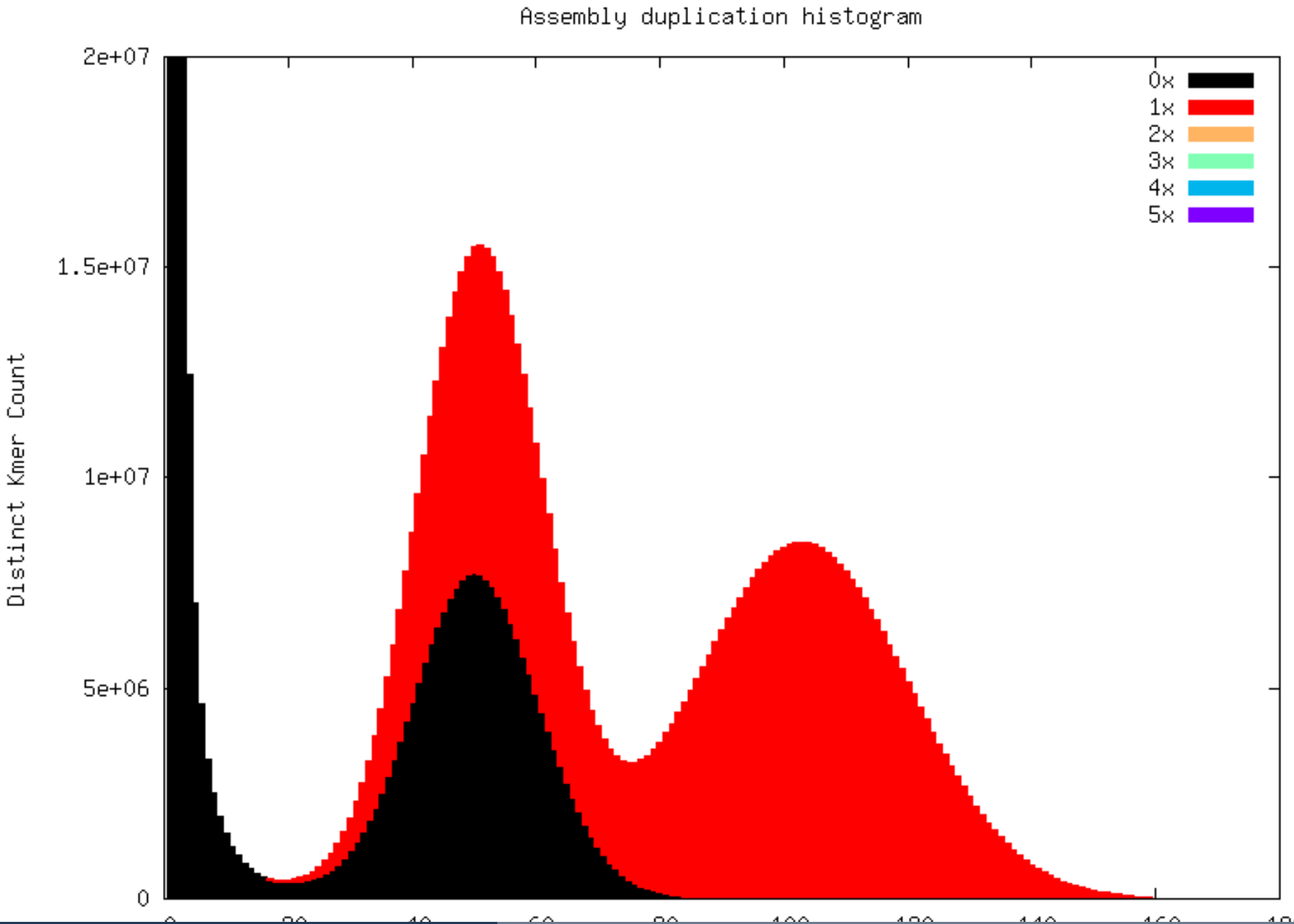
Bandage tool can visualize assembly graphs (GFA)



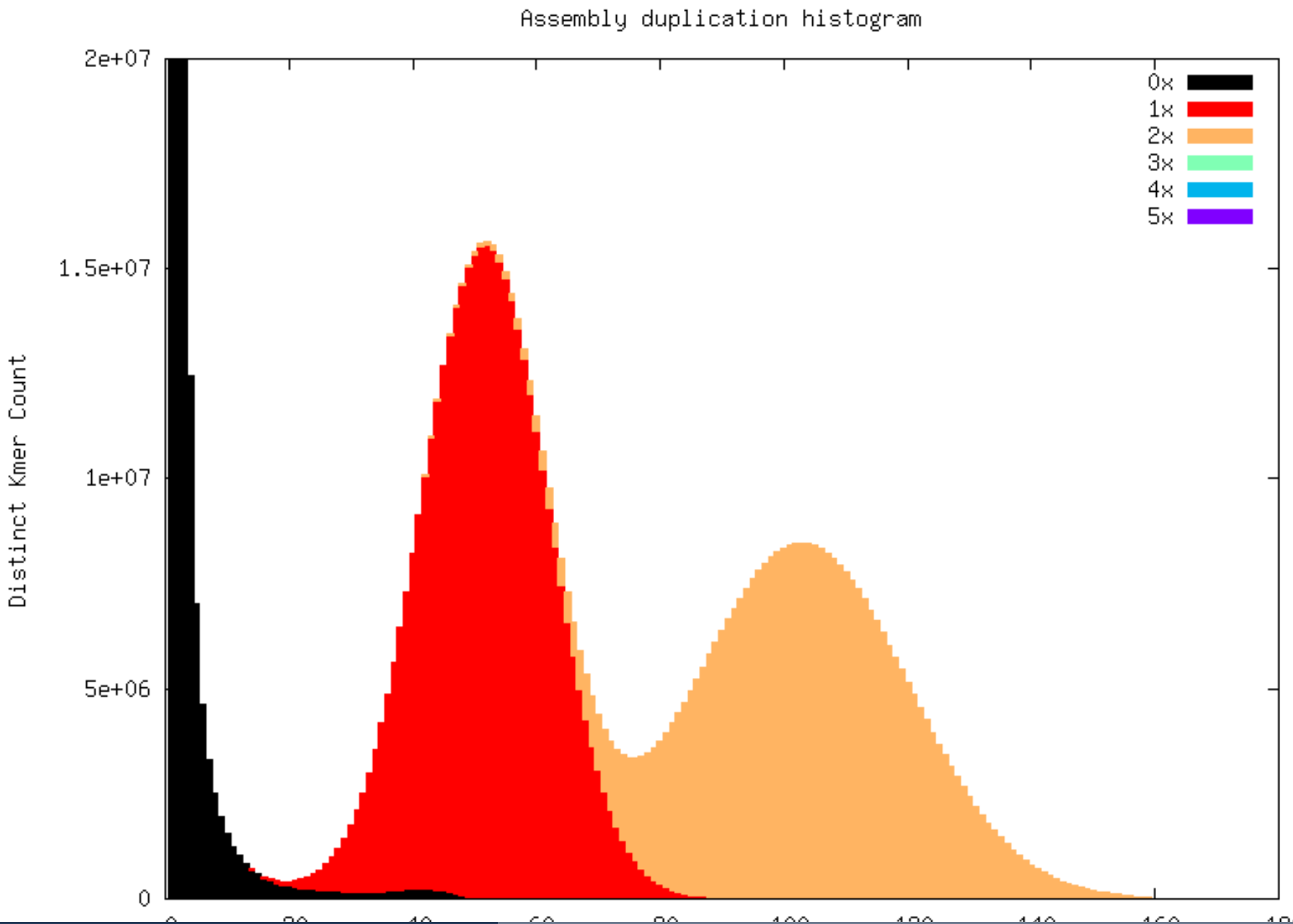
- *K*-mer spectrum visualization with KAT



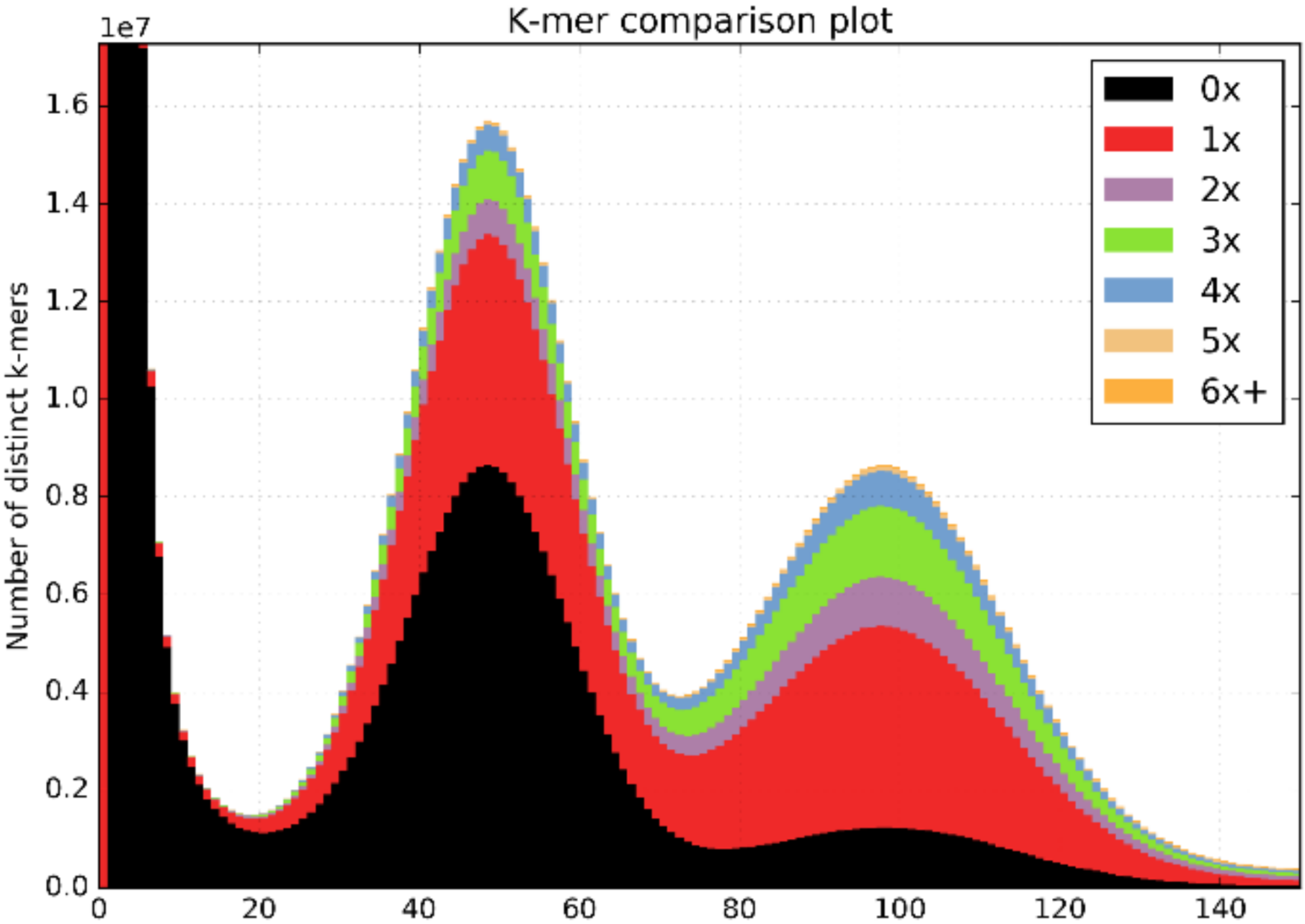
K-mer spectrum visualization with KAT



K-mer spectrum visualization with KAT

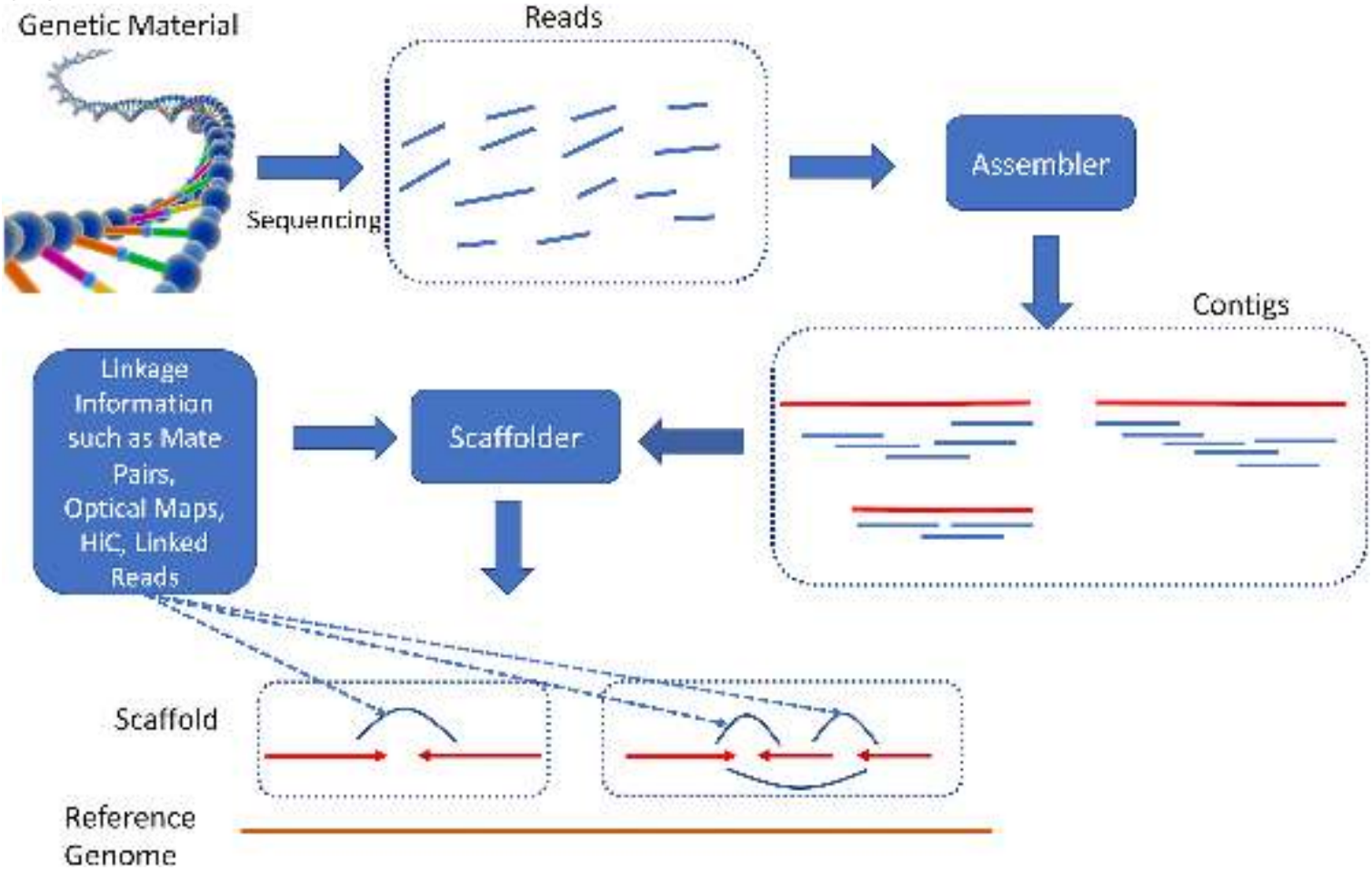


- *K*-mer spectrum visualization with KAT



Scaffolding

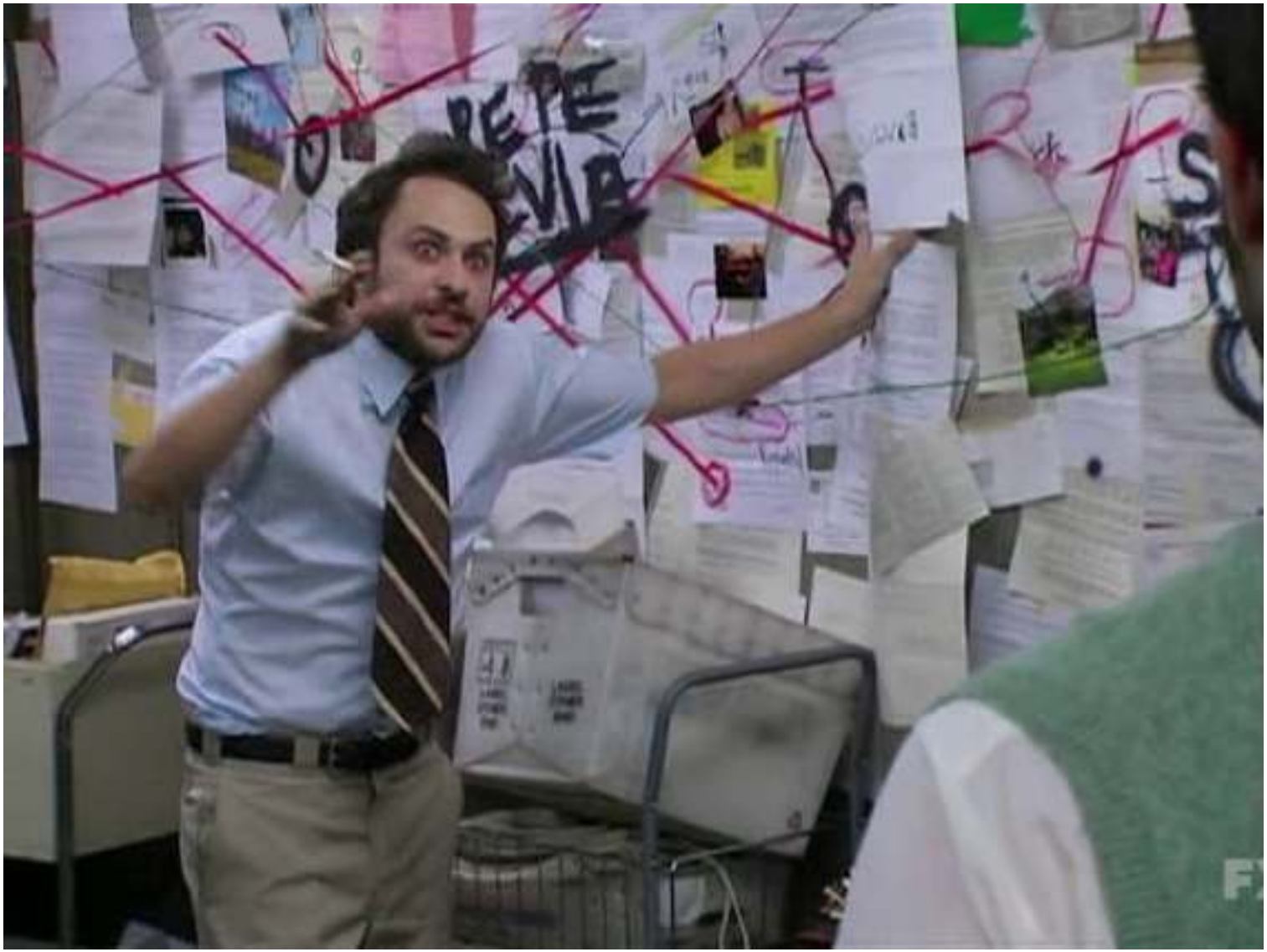
Softwares can improve the assembly continuity by using other kinds of information



From "Modern technologies and algorithms for scaffolding assembled genomes" Plos Computational Biology

The end

. . . of the theoretical part (or is it?)



Intermission

The workshop's team during the annual lungfish genome assembly session according to MidJourney

- Sanger

- Medium reads $\approx 1000bp$
- Very low error rate $\approx 0.01\%$
- Low throughput (up to billion of reads per run)
- Costly ($500/Mb)

No longer used for assembly

Second generation sequencing

NextSeq Series

HiSeq 4000 System

HiSeq X Series‡

NovaSeq 6000 System

- Short reads $\approx 150bp$
- Low error rate $< 1\%$
- High throughput (up to billion of reads per run)
- Cheap ($0.50/Mb)
- GC bias

Which assembly strategy is best suited?

- Short reads $\approx 100bp$
- Low error rate below 1%
- High throughput (up to billions of reads per run)

Based on long reads properties, which assembly solution would you choose and why?

Vote!

- Greedy
- Overlap graph
- de Bruijn graph

Paradigm Breakdown

Does not handle repeats $\times$
Scalable $\checkmark$

Handles repeats smaller than reads size $\checkmark$
Extremely expensive to handle billion reads $\times$

Handle repeat smaller than $k \approx$ reads size $\checkmark$
Scalable $\checkmark$

- State-of-the-art

- SPAdes [Bankevich 2012]
- Megahit [Li 2015]
- IDBA [Peng 2012]

- SGA [Simpson 2012]
- Discovar denovo [Weisenfeld 2014]
- Abyss [Simpson 2009]

Third generation sequencing

- Long reads $\approx 10 - 100kbp$
- High error rate (up to 10%)
- High throughput (up to millions of reads per run)

- Nanopore VS Pacbio

N

- Portable
- Ultra long reads (100kbases, some reads reach the megabase level)
- Mostly deletions

P

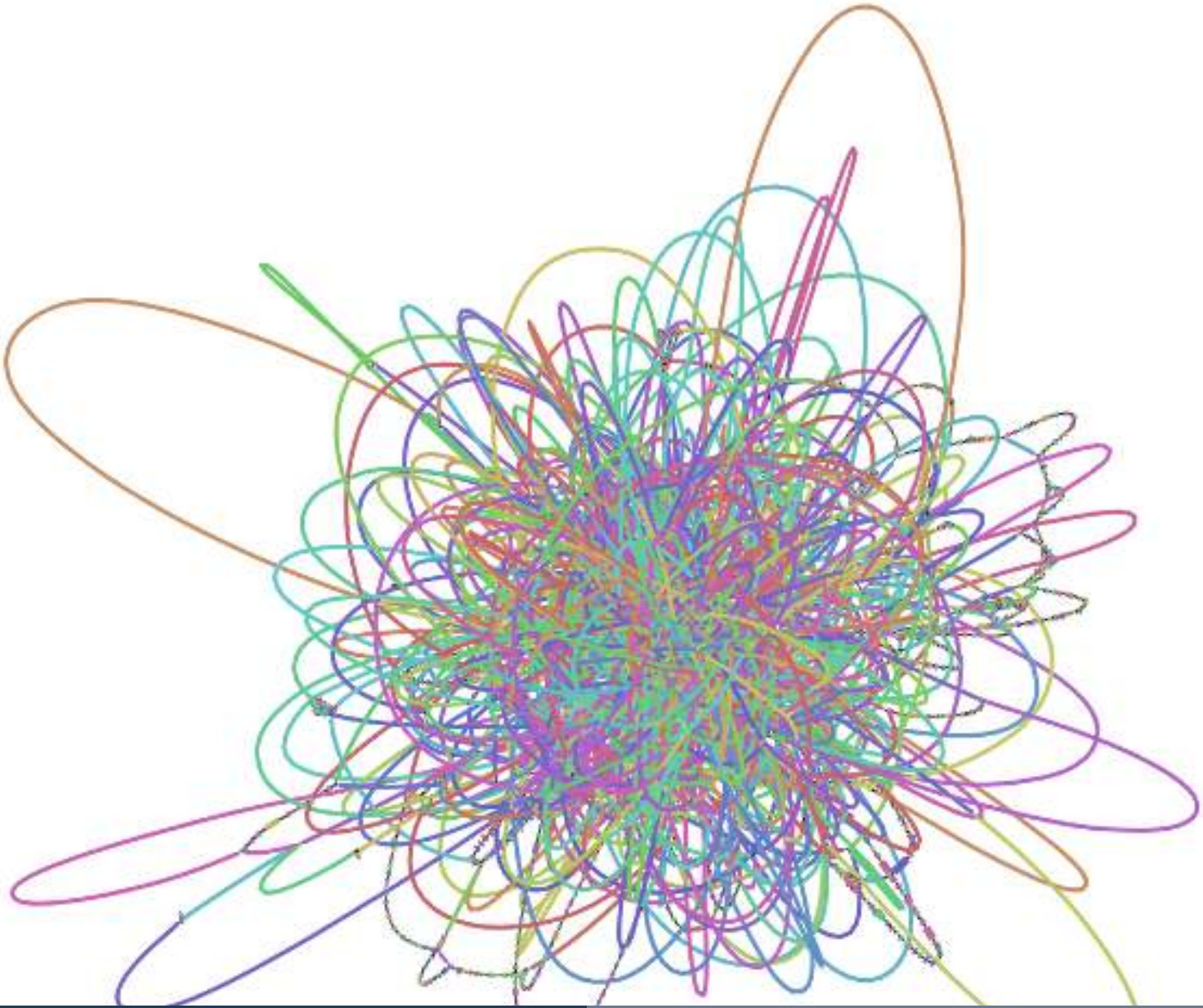
- More mature
- HiFi reads (99.9% identity)
- Mostly insertions

- Repeats spanning

- Repeats spanning

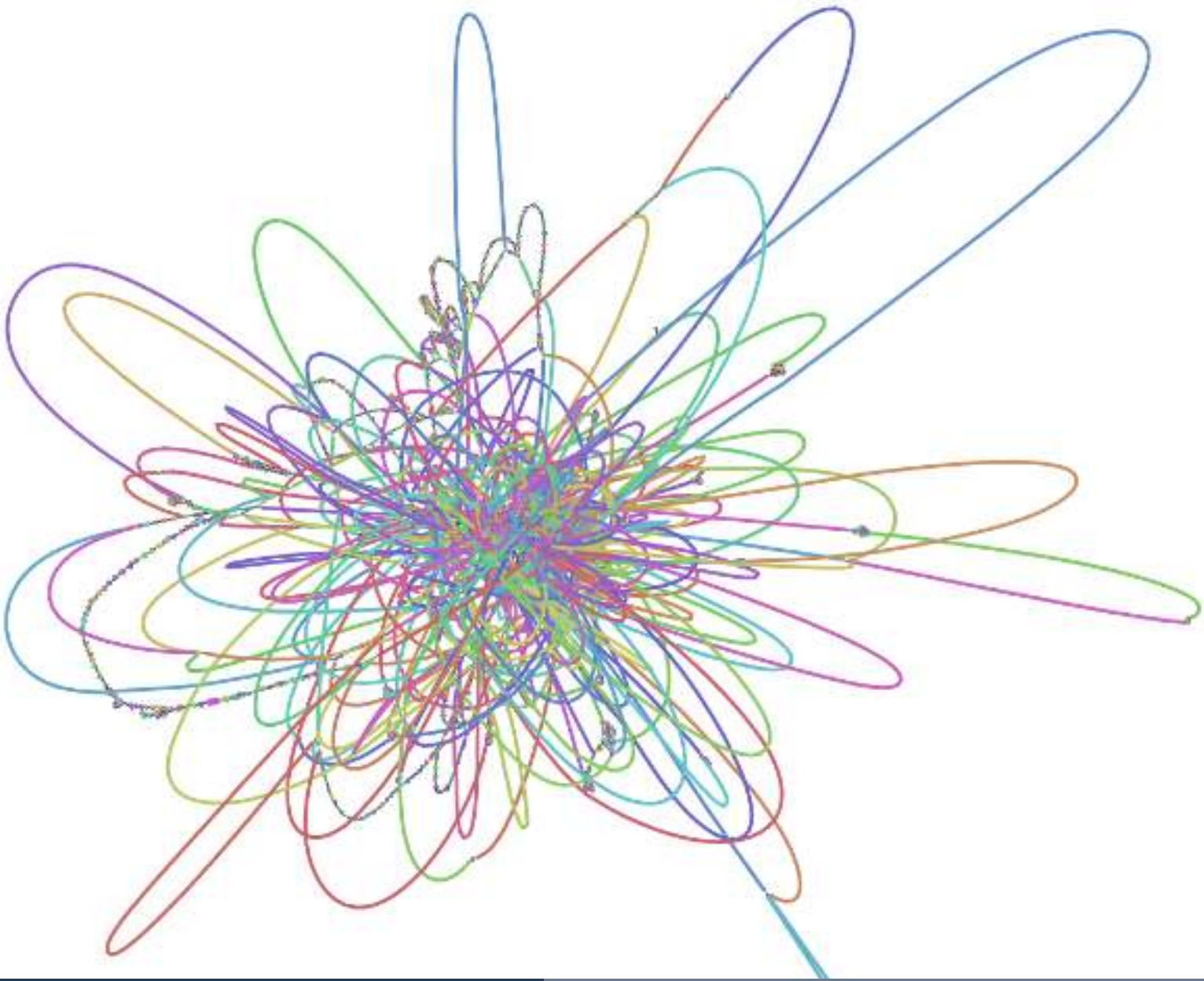
- Read length matters

Read size=21



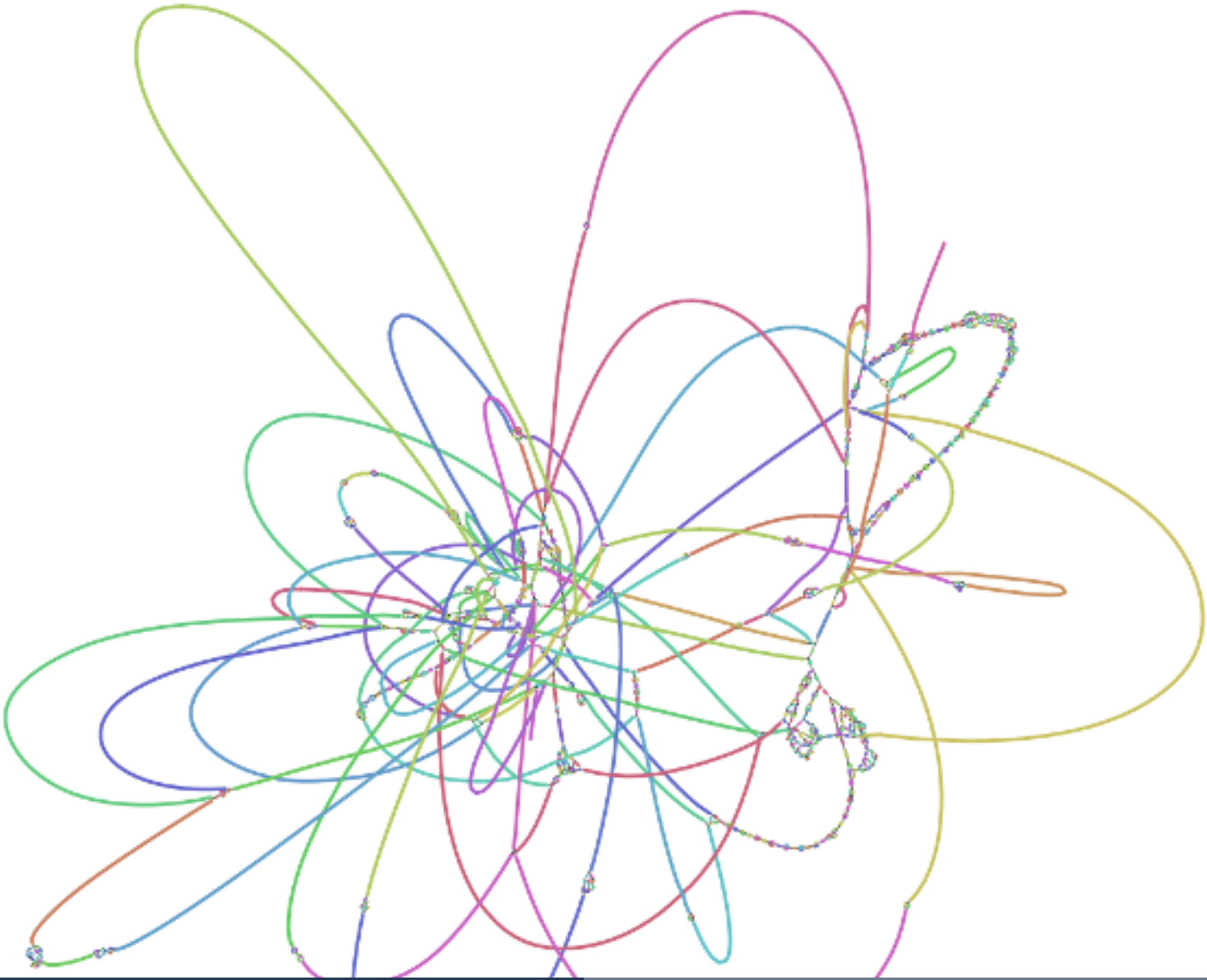
- Read length matters

Read size=31



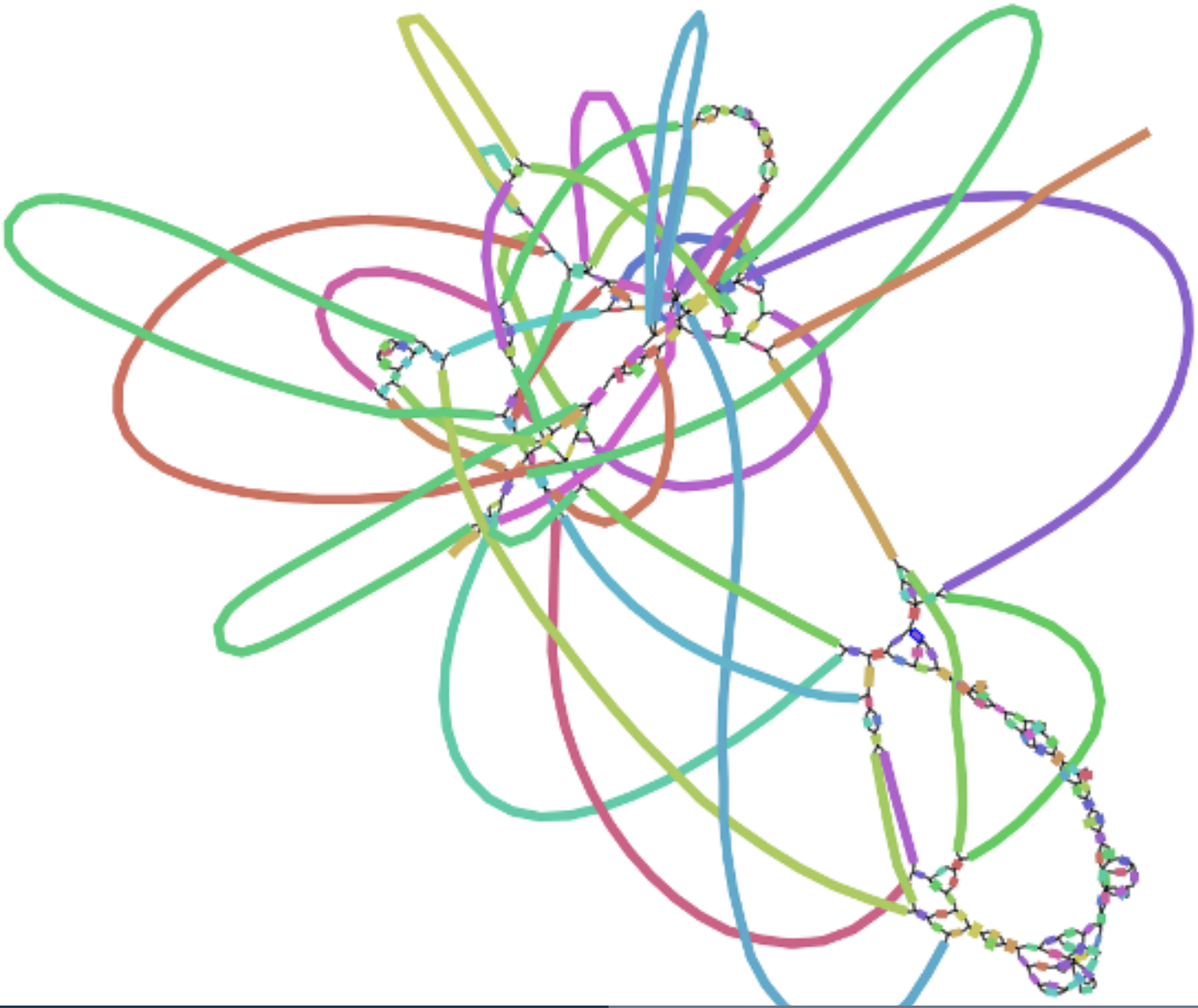
- Read length matters

Read size=63



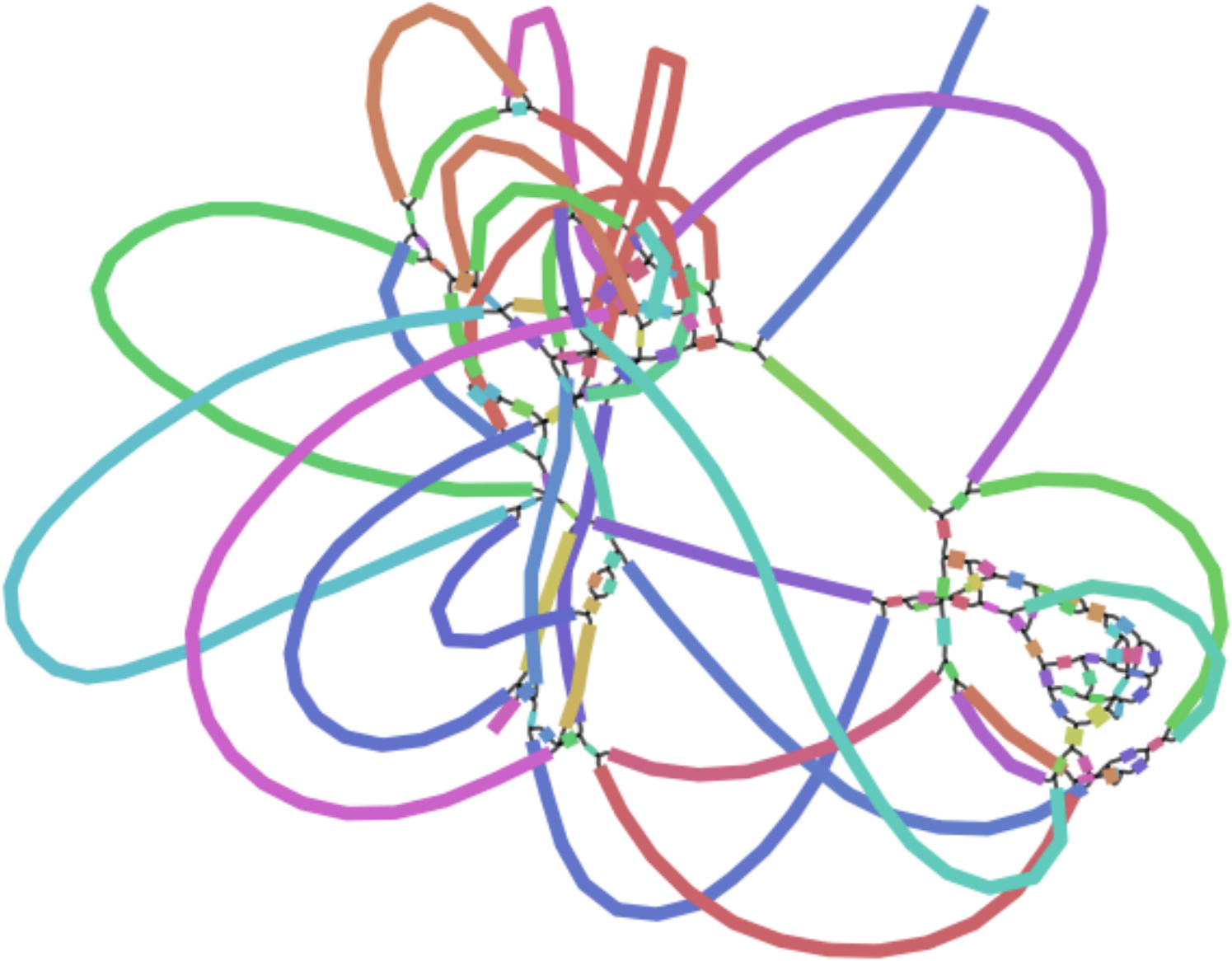
- Read length matters

Read size=255



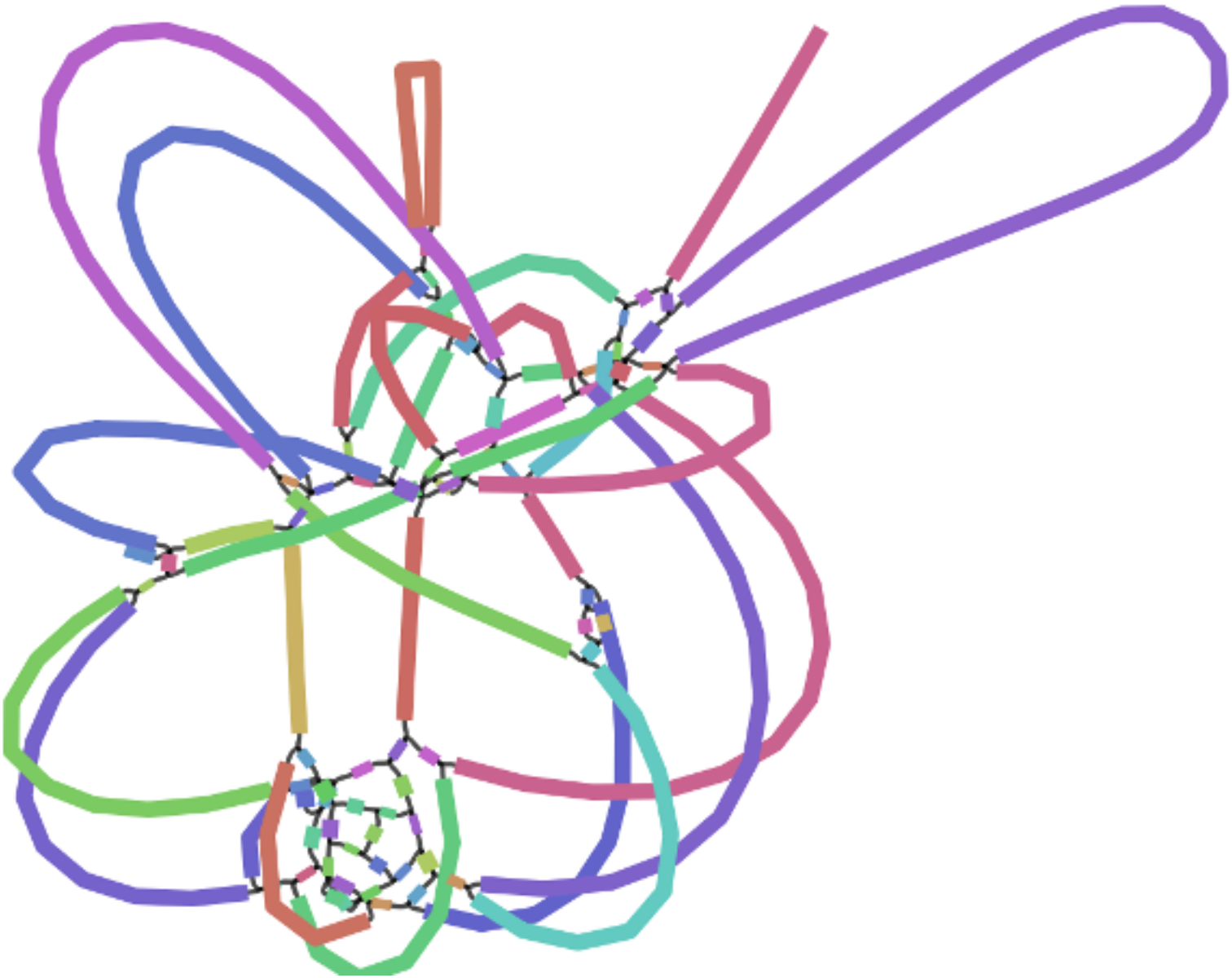
- Read length matters

Read size=500



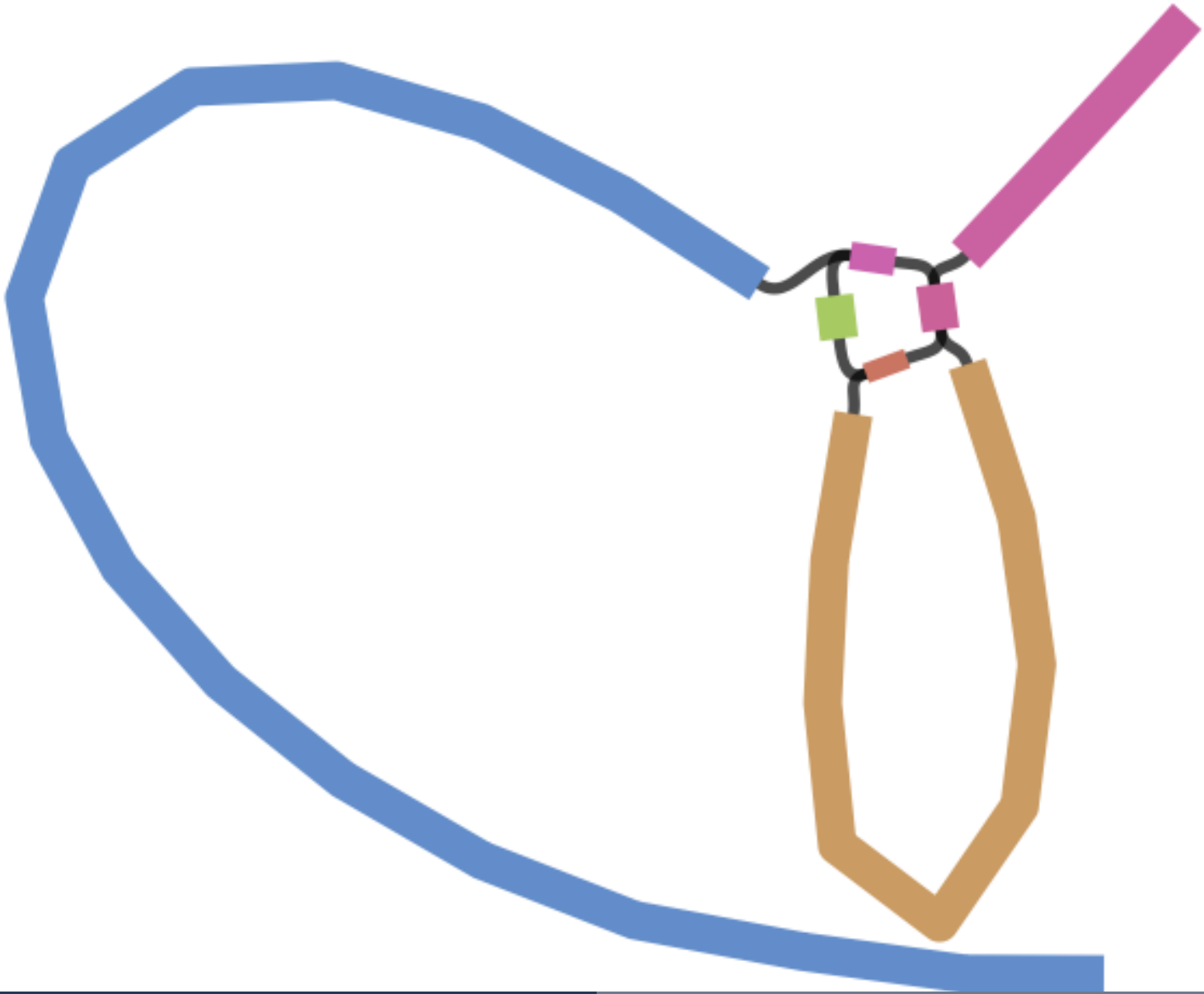
- Read length matters

Read size=1000

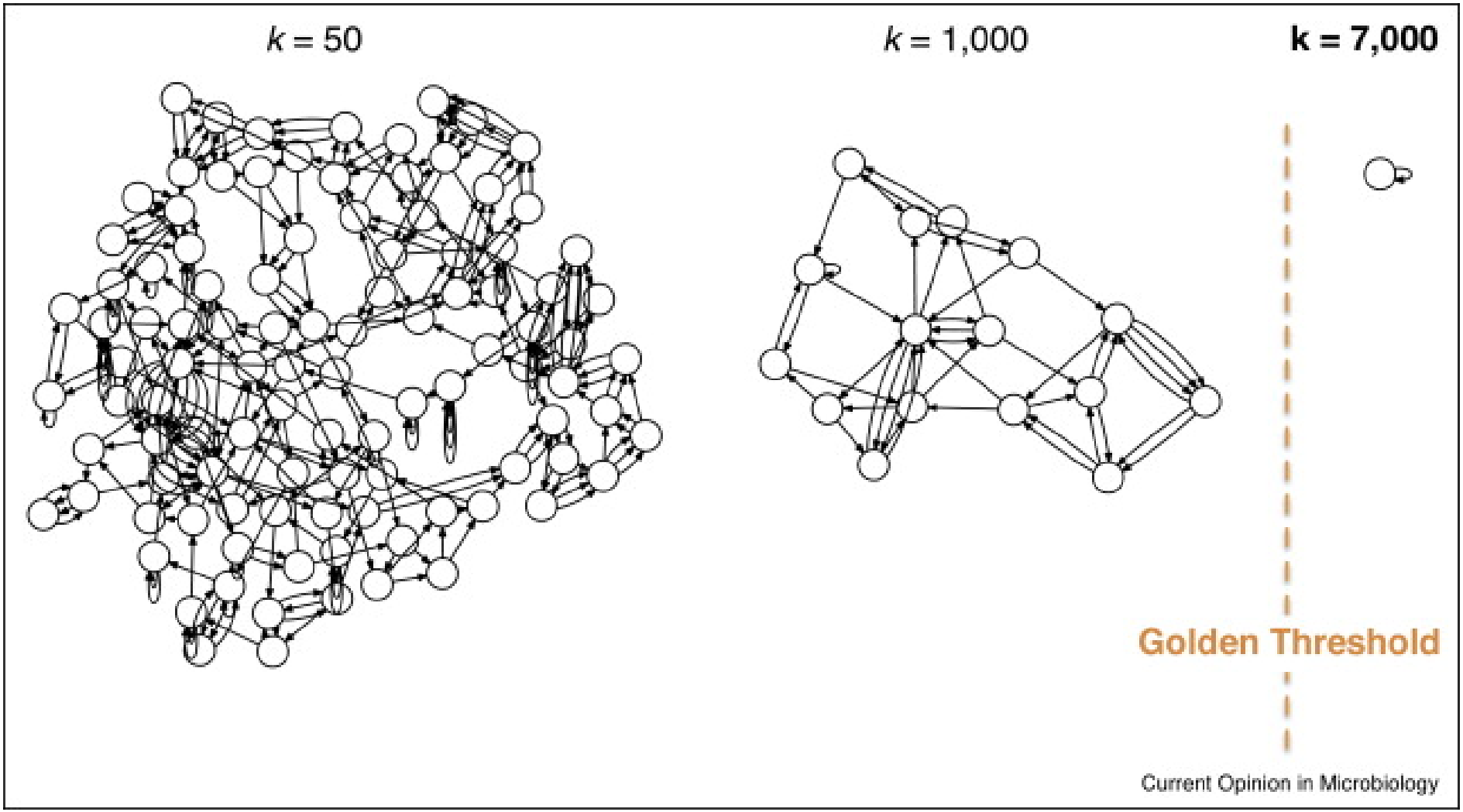


- Read length matters

Read size=2000

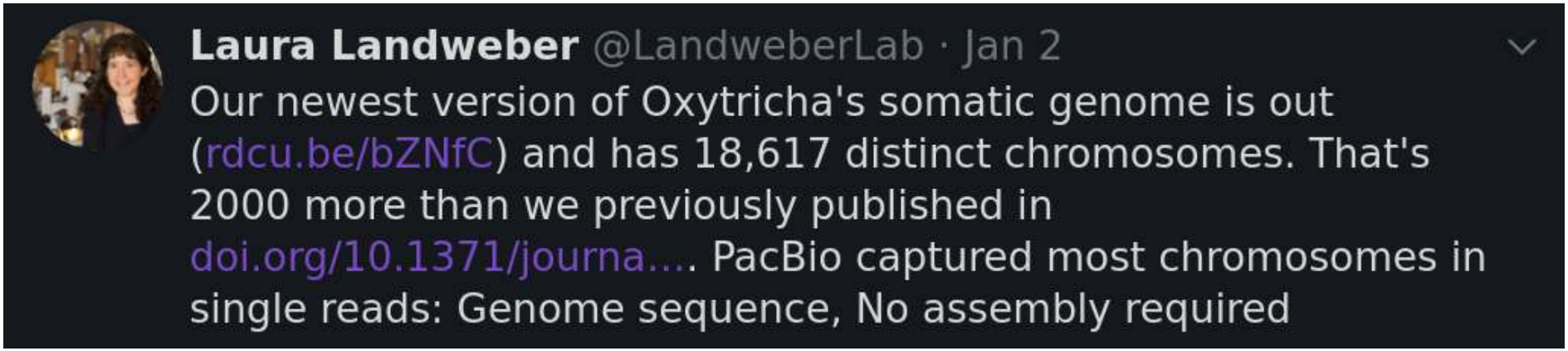


- Great hope for assembly

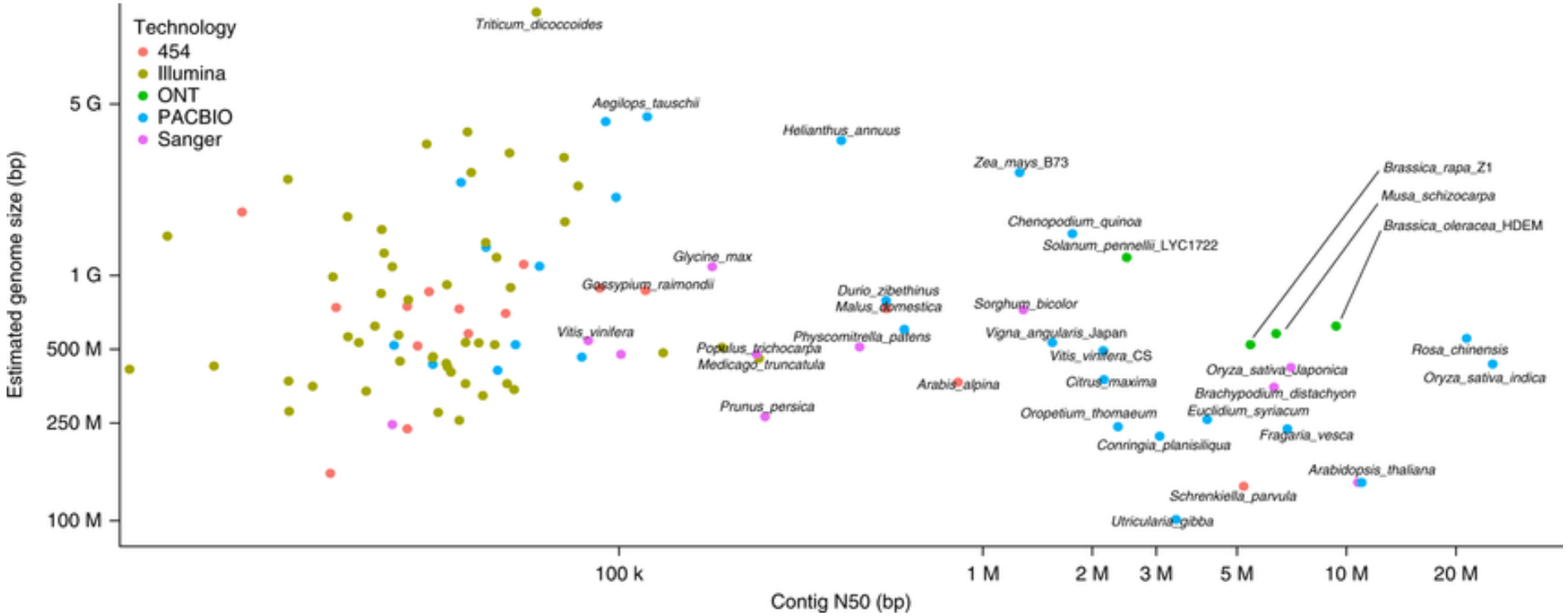


From "One chromosome, one contig: complete microbial genomes from long-read sequencing and assembly" Current Opinion in Microbiology 2015

- Long reads killed the assembly star



- Great hope for assembly



From "Chromosome-scale assemblies of plant genomes using nanopore long reads and optical maps" Nature Plants 2018

Which assembly strategy is best suited?

- Long reads $\approx 10kbp$
- High error rate $\approx 10\%$
- High throughput (up to millions of reads per run)

Based on long reads properties, which assembly solution would you choose and why?

Vote!

- Greedy
- Overlap graph
- de Bruijn graph

- Long reads for assembly: de Bruijn graph?

Most k-mers will contain at least an error and will be useless

- Long reads for assembly: overlap graph?

Supposed to be super expensive!

- Longer reads, better overlaps
  - Less reads for the same coverage
  - Larger overlaps

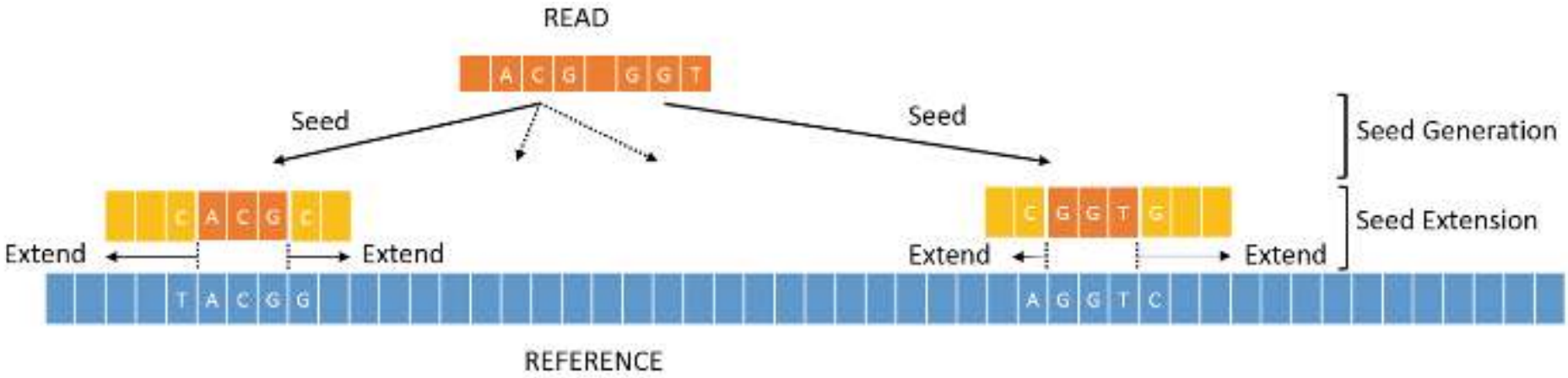
5Mb bacteria example with 100X coverage

- 5 million 100bp reads
- 99 bp average overlap

- 50,000 10kbp reads
- 9,900 bp average overlap

- 5,000 100kbp reads
- 99,000 bp average overlap

- Are large overlaps hard to compute?



Aligning very long and highly erroneous regions is expected to be expensive, as alignment is quadratic $\approx \mathcal{O}(n^2)$!

- "Anchor chaining" in overlap graph

- For long reads: typically Minimap2's [Li 2018] job
- "Anchor chaining": find common chains of anchors (k-mers) in the same order in 2 sequences (can be **linear** in practice in most cases)

- Long reads for assembly: overlap graphs

- Sequencing errors

High rate of insertions and deletions rendered genome annotation nearly impossible

- Using coverage to remove noise: Consensus

- Exercise: Perform a consensus

```
RS1: ACTTCGAACGT
RS2: TCGATCGTTT
RS3: GATCAGTTTAG
RS4: TCATTTCGTA
RS5: GTTTCGTCCG
REF: ACTCGAATGTTTTTCCTACG
```

- Exercise: Perform a consensus - solution

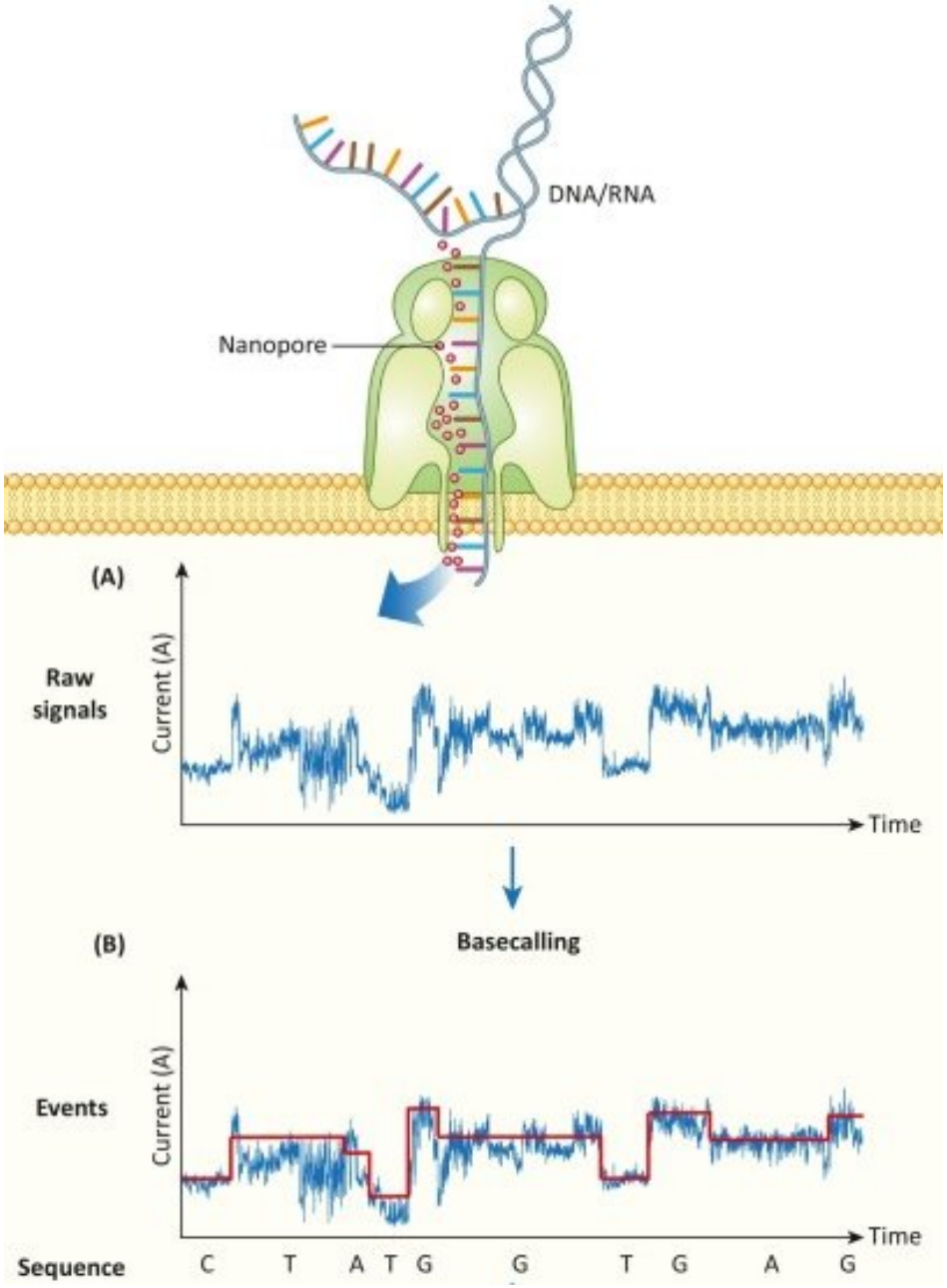
```
RS1: ACTTCGA-AC-GT---------
RS2: --T-CGA-TC-GTTT-------
RS3: -----GA-TCAGTTT-AG----
RS4: ---------TC-ATTT-CGTA--
RS5: ------------GTTT-CGTCCG
REF: ACT-CGAAT--GTTTTCCTACG
CON: ACT-CGAATC-GTTT-CGTACG
```

- Consensus during assembly

- Consensus after assembly: polishing

- Consensus after assembly: polishing

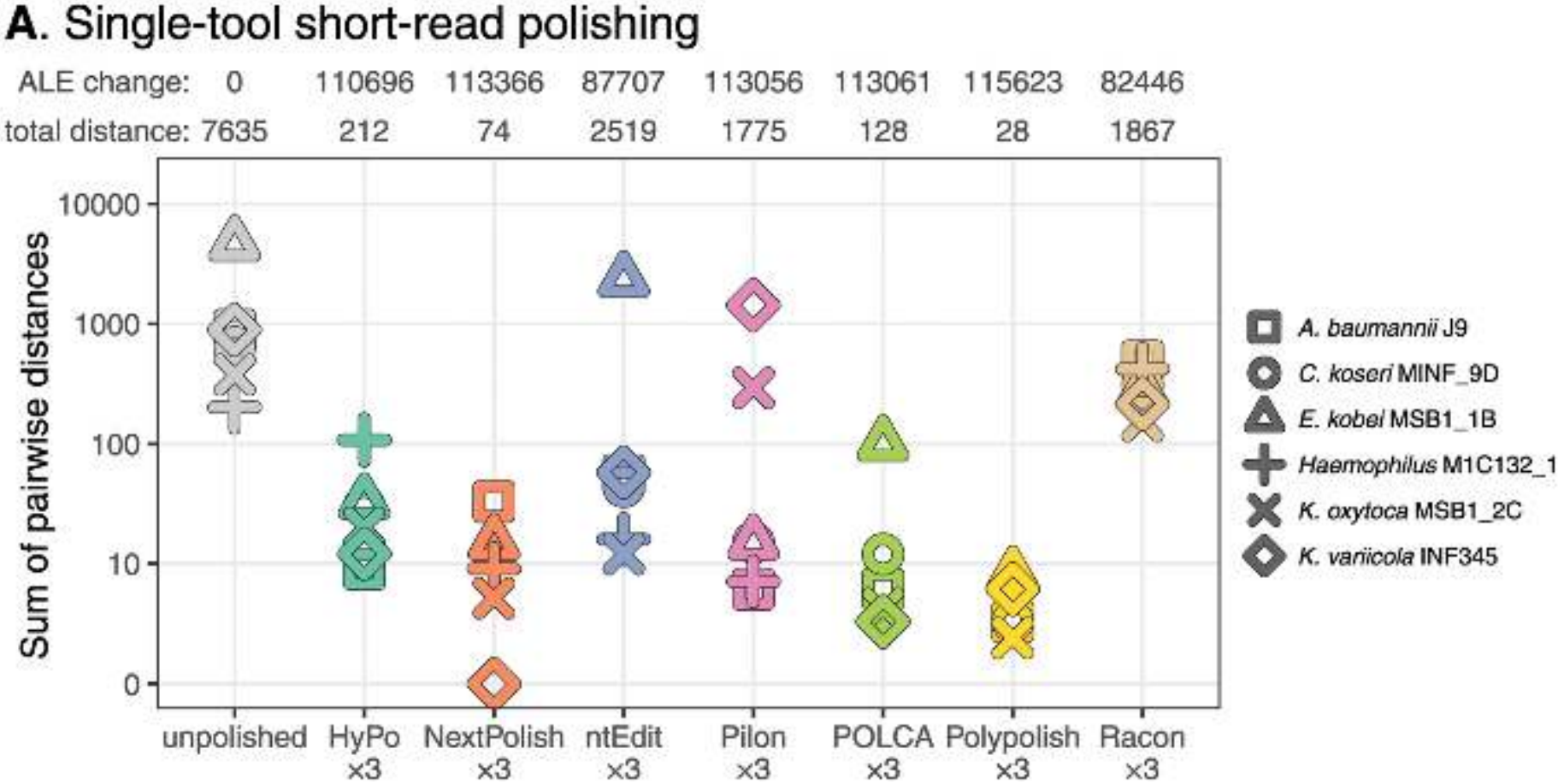
- Homopolymers are hard to read



- Polishing using accurate reads

Systematics errors

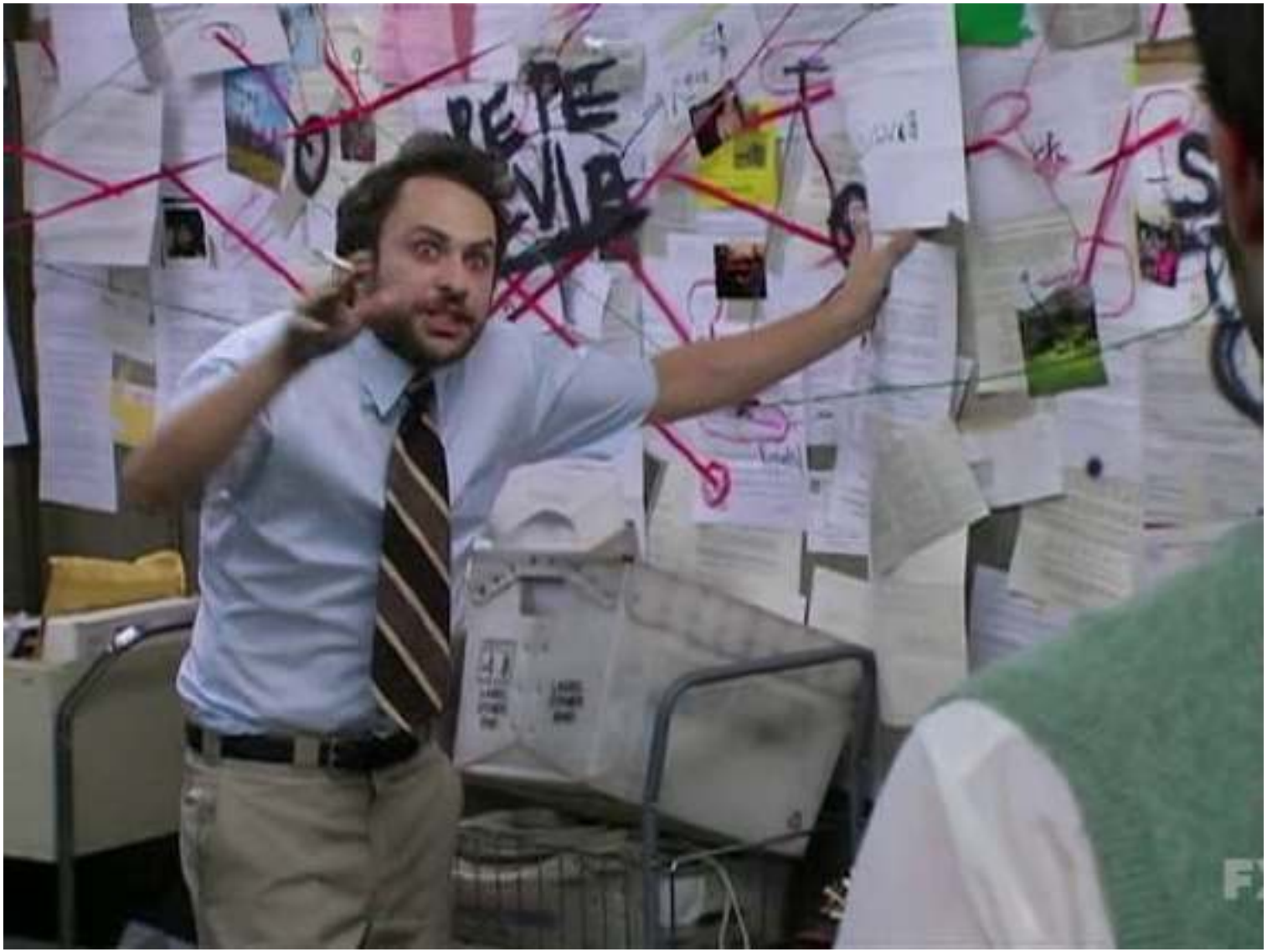
Polishing with Illumina data can improve the final error rate



From Polypolish: Short-read polishing of long-read bacterial genome assemblies

- I know we said it was the end

Just one or two more graphs



- An overlap graph limitation. Swept under the carpet?

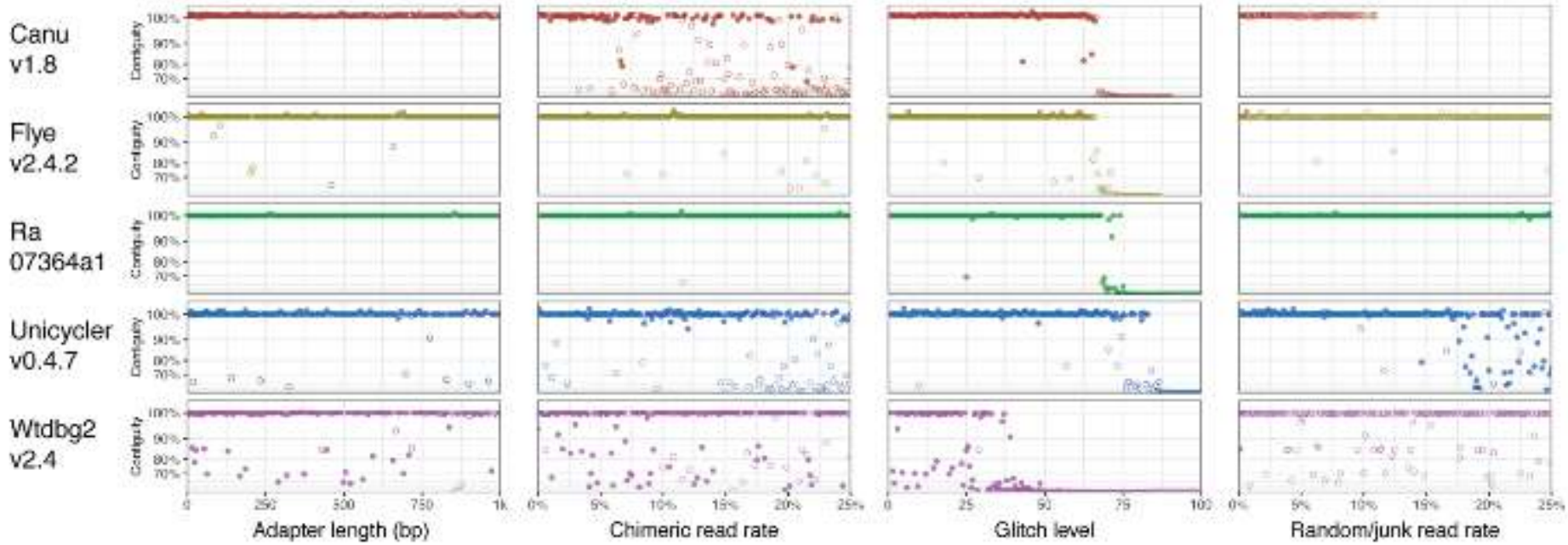
- An olverlap graph limitation. Swept under the carpet?

- An overlap graph limitation. Swept under the carpet?

- Long reads for assembly: assembly solved?

Assembly is not solved yet

Sometimes the software fails



From `github.com/rrwick/Long-read-assembler-comparison`

- Long reads for assembly: assembly solved?

Assembly is not solved yet

Sometimes the data cannot solve the problem

- Very large repeated region
- Low local coverage
- Chimeric/noisy reads

- 20 years later

- Telomere-to-Telomere consortium

Has produced in 2021 a complete human genome with one contig per chromosomes !

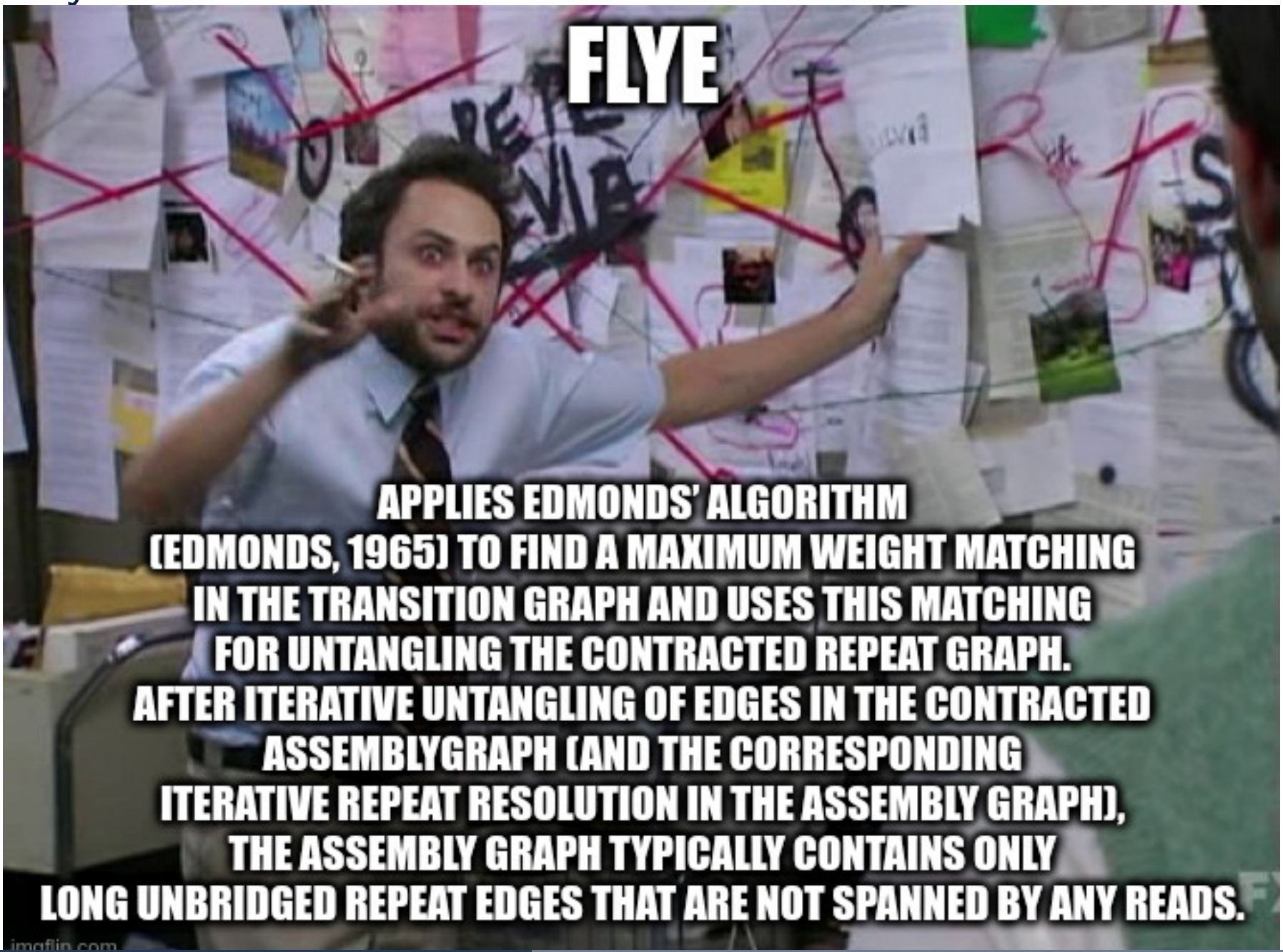
- 30x PacBio HiFi
- 120x coverage of Oxford Nanopore (ultra long reads)
- 70x PacBio CLR
- 10X Genomics, BioNano DLS and Arima Genomics HiC
- 100 authors from 50 labs

- Long reads assemblers

- Flye (Repeat graph) [Kolmogorov et al 2019]
- Raven (OLC) [Vaser et al 2021]
- NECAT/MECAT(OLC) [Xiao et al 2017]

- Canu (Greedy) [Koren et al 2017]
- Shasta (OLC) [Saffin et al 2020]
- Redbean (fuzzy de Bruijn graph) [Ruan 2019]
- ...

- Flye


FLYE
APPLIES EDMONDS' ALGORITHM
(EDMONDS, 1965) TO FIND A MAXIMUM WEIGHT MATCHING
IN THE TRANSITION GRAPH AND USES THIS MATCHING
FOR UNTANGLING THE CONTRACTED REPEAT GRAPH.
AFTER ITERATIVE UNTANGLING OF EDGES IN THE CONTRACTED
ASSEMBLYGRAPH (AND THE CORRESPONDING
ITERATIVE REPEAT RESOLUTION IN THE ASSEMBLY GRAPH),
THE ASSEMBLY GRAPH TYPICALLY CONTAINS ONLY
LONG UNBRIDGED REPEAT EDGES THAT ARE NOT SPANNED BY ANY READS.

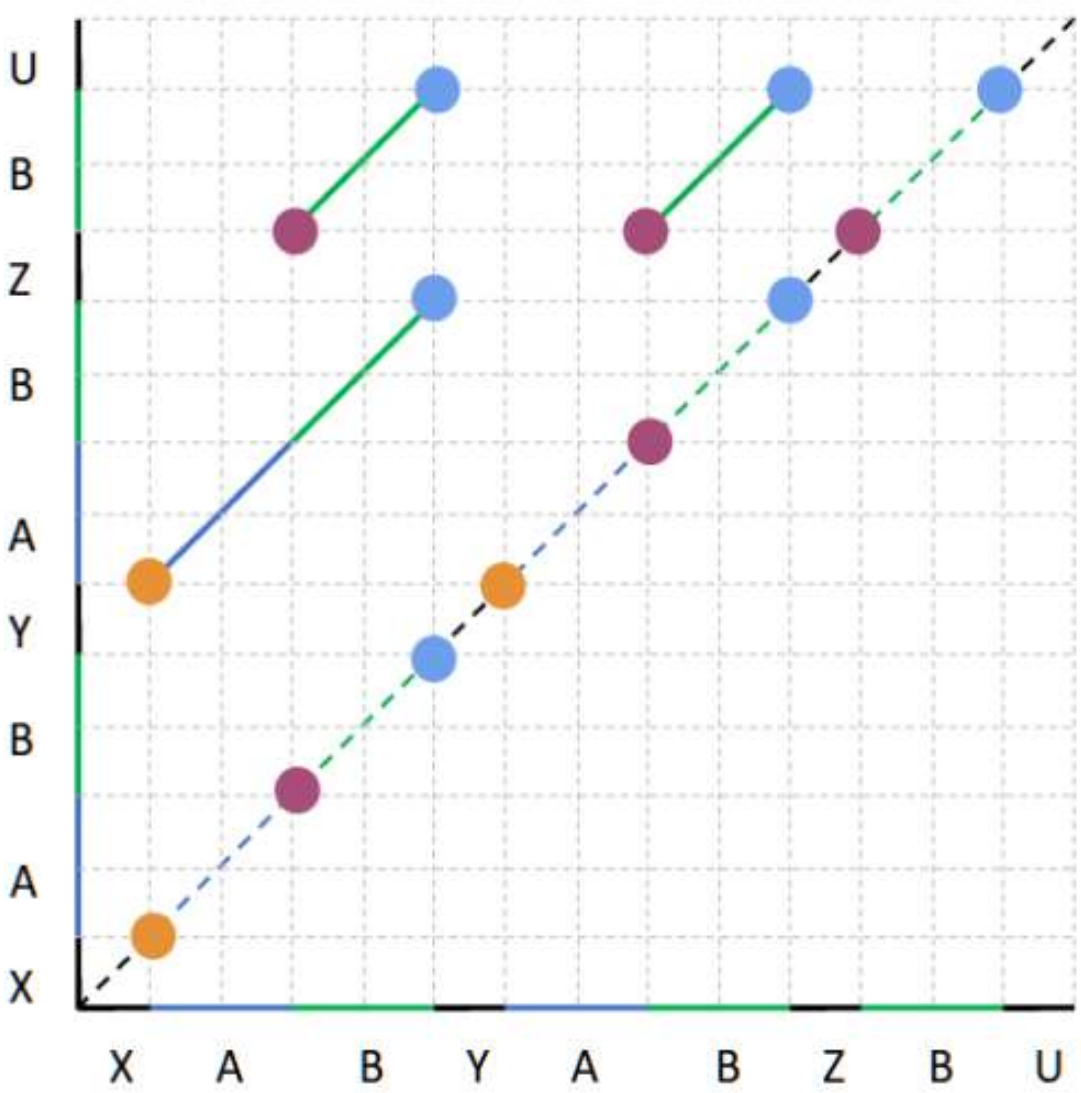
- Repeat graph

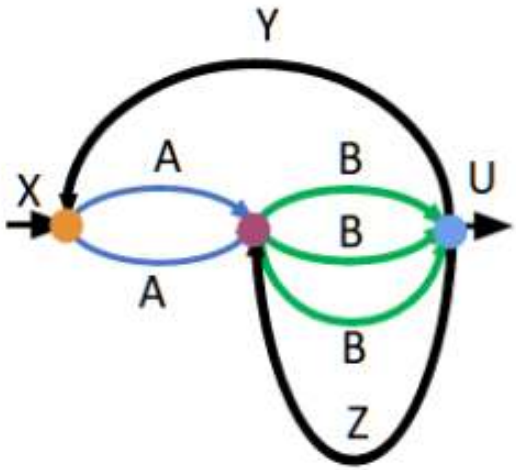
- Repeat graph

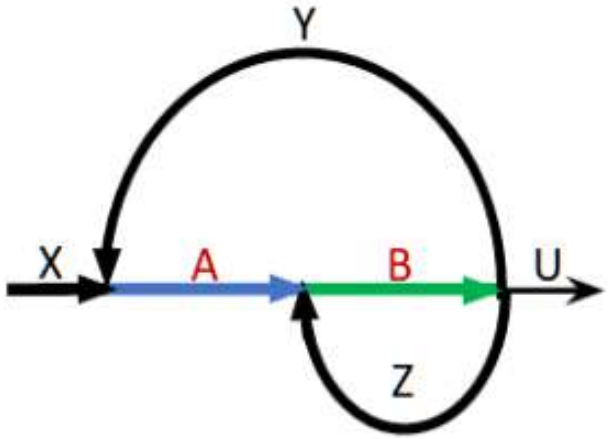
- Repeat graph

- Repeat graph

Repeat graph







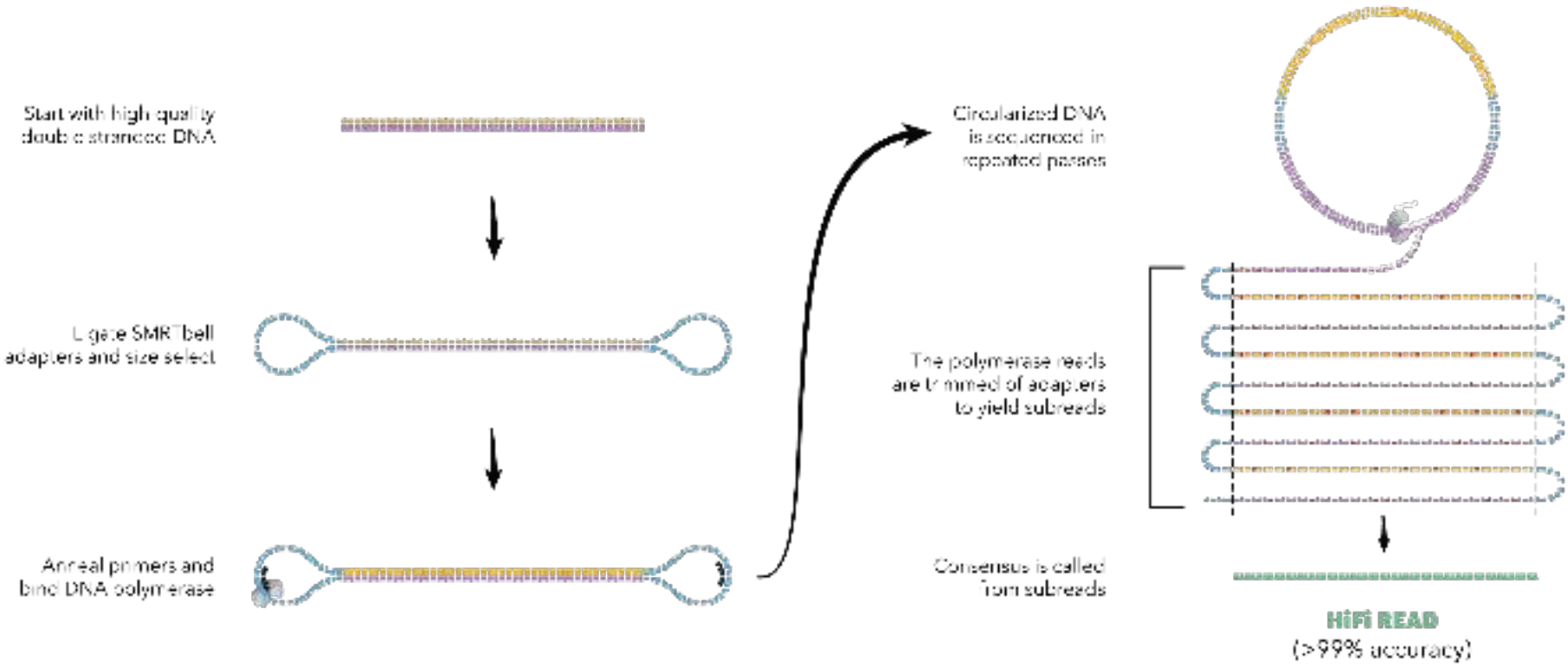
Long read assembly summary

- Overlap graphs with quick overlap computation
- Long reads can span repeats and improve assemblies
- Methods to polish contigs

Future of assembly

The future of genome sequencing according to MidJourney.
Left: yes, but kinda boring. Right: hmmm.

- Consensus during sequencing



Stands for "High Fidelity"

Very low error rates $\approx$ 0.1% 0.01%

Almost only homopolymer errors remain

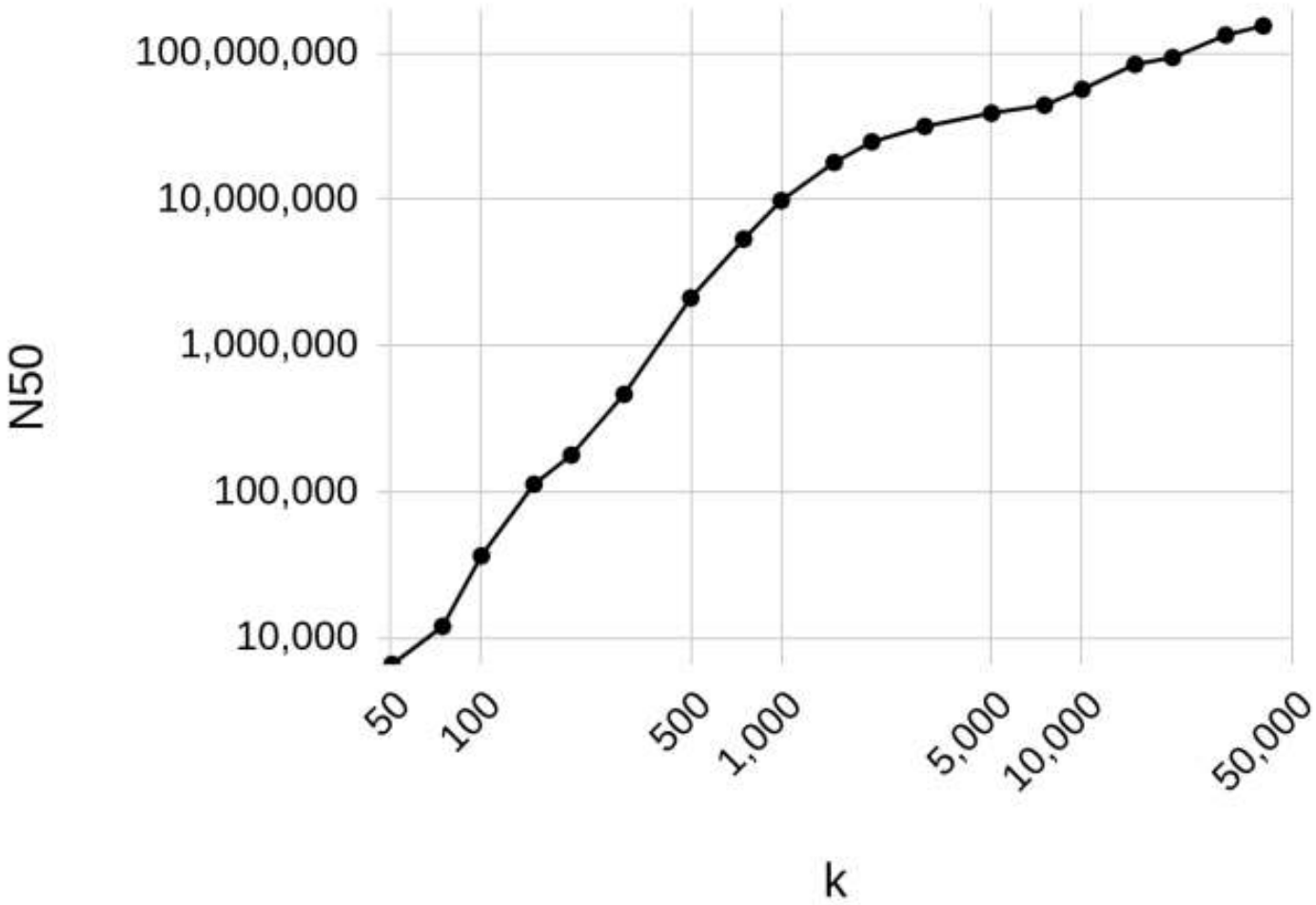
HiFi Assembly

With almost error-less long reads we have several promising improvements ahead:

- Use de Bruijn graph (more efficient data structures)
- Assemble large genomes very fast
- Perform diploid assembly

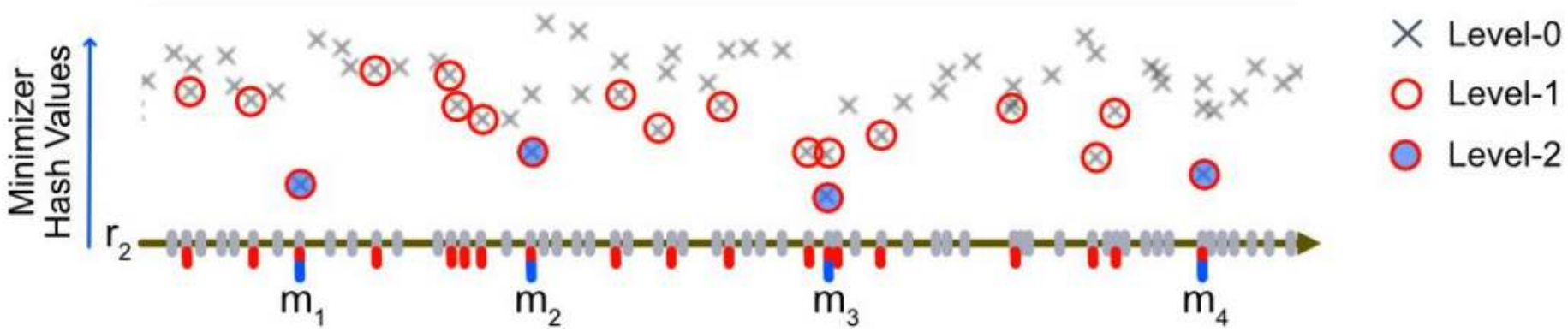
de Bruijn graph Assembly

Using K=500 and K=5000 de Bruijn graphs to assemble

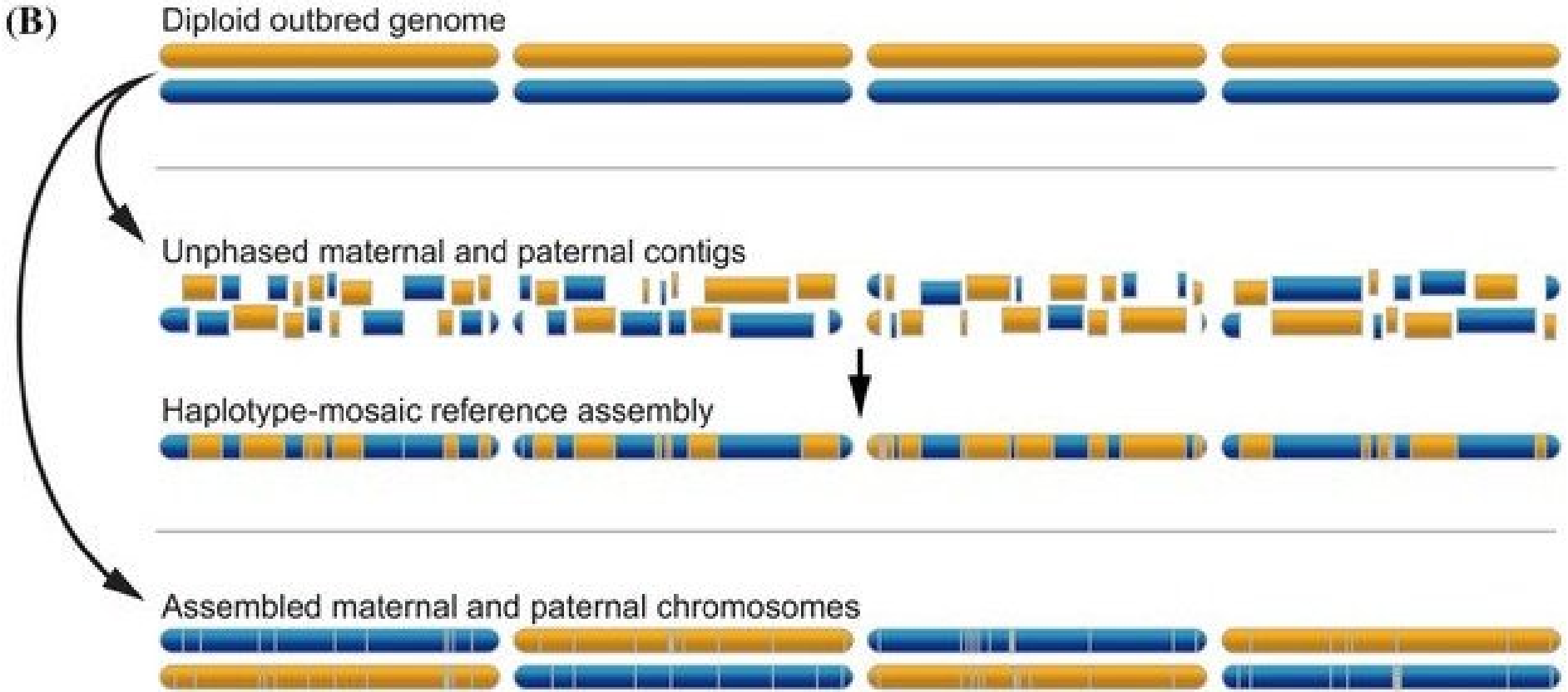


- Very fast genome assembly

Human genome assembled within 2 hours (Peregrine assembler) and 10 minutes (RMBG assembler)



- Diploid assembly

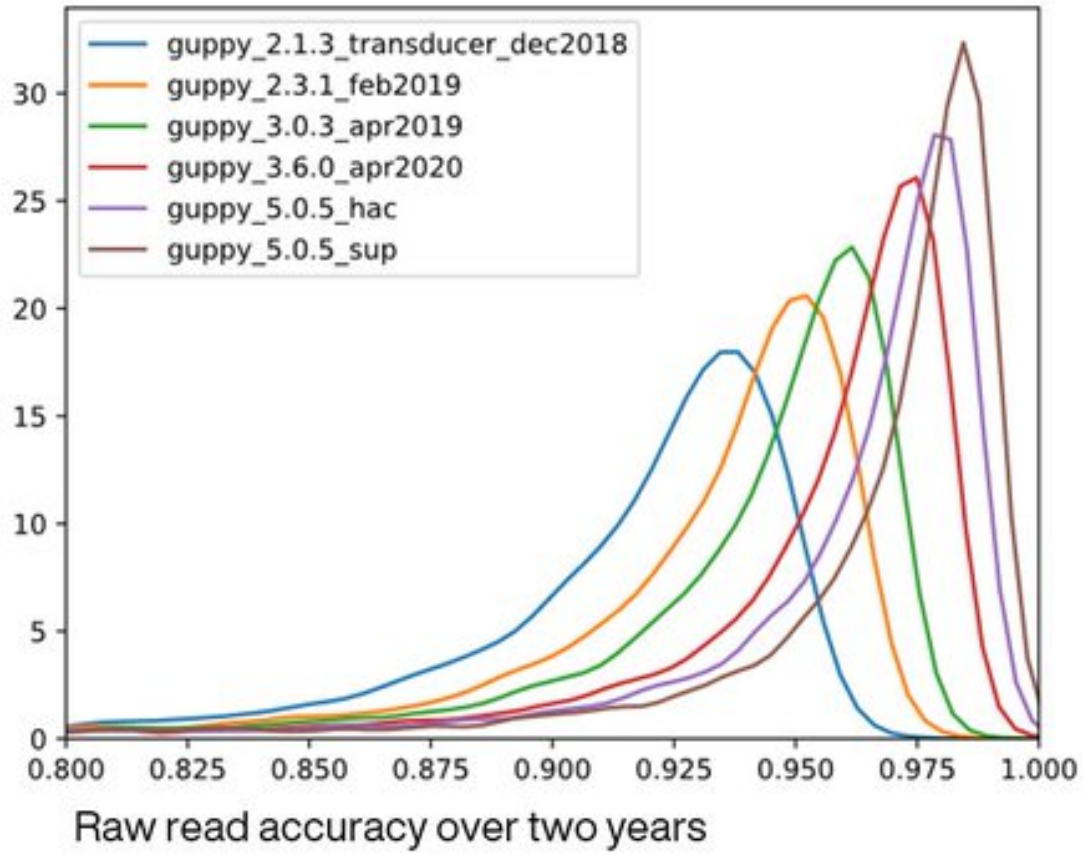


- Human diploid assembly (20/46 chr)

Asm	Contig NG50 (Mb)	Scaffold NG50 (Mb)	Hamming error	QV	#Errors Chr X & Y
Downsampled (35× HiFi / 60× ONT-UL)					
Verkko	12.90		0.13%	52.18	10
Verkko + trio	**80.77**	**102.55**	0.13%	52.40	10
Verkko + Hi-C	58.24	82.42	0.16%	52.48	10
LJA	0.39		0.14%	55.75	7
Hifiasm (unitigs)	0.35		0.23%	**61.05**	**2**
Hifiasm + trio	64.50		**0.06%**	60.86	26
Hifiasm + Hi-C	66.34		0.79%	60.57	37
Full-coverage (>100× HiFi / 85× ONT-UL)					
Verkko	17.35		**0.05%**	54.91	**5**
Verkko + trio	**134.00**	135.80	**0.05%**	55.77	**5**
Verkko + Hi-C	68.32	85.97	**0.05%**	55.57	**5**
HPRC curated	72.70	**146.75**	0.13%	**61.35**	26

- Ongoing progress

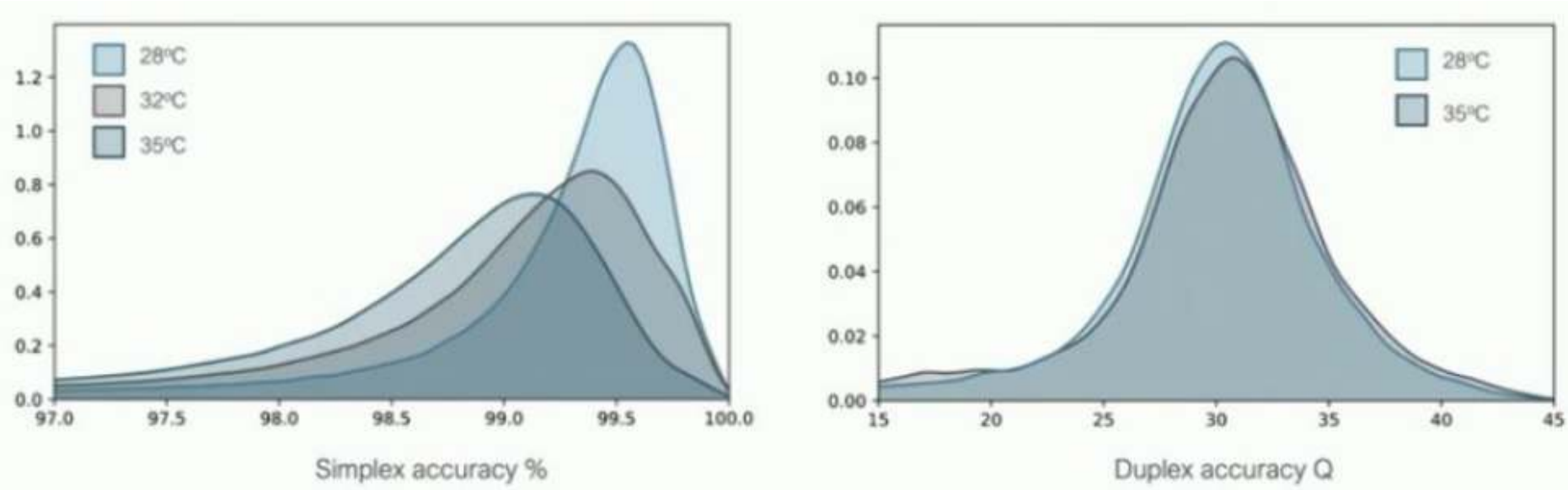
Errors in Nanopore sequencing data are rapidly diminishing



Q20 chemistry achieved modal accuracy $\approx 99\%$

- High fidelity Nanopore incoming?

Nanopore duplex reads could deliver long and precise reads in the future



- Take home messages

- Short reads: de Bruijn graphs / Long reads: Overlap graph
- Repeats are the core issue
- Output fragments of genomes (**contigs**)
- Several parameters and heuristics used in practice

- Ongoing work

- Reconstruct haplotypes
- Scaling on large genomes
- Robustness to noisy data
- Repetitive regions

- The end

PopGenGoogling
@popgengoogling
i trust you to figure out your own genome
Traduire le Tweet
3:01 AM · 14 déc. 2019 · Twitter for iPhone
37 Retweets
267 J'aime