Long Read Genome Assembly

A Case Study in Python

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Background

- Short-read sequencing is known for its cost-effectiveness, accuracy
- However, complexities of genomes, such as repetitive regions, pose a challenge when assembling the genomes from short amplified fragments
- Long reads enhance de novo assembly, overcoming amplification bias even though with higher error rates

Problem Statement

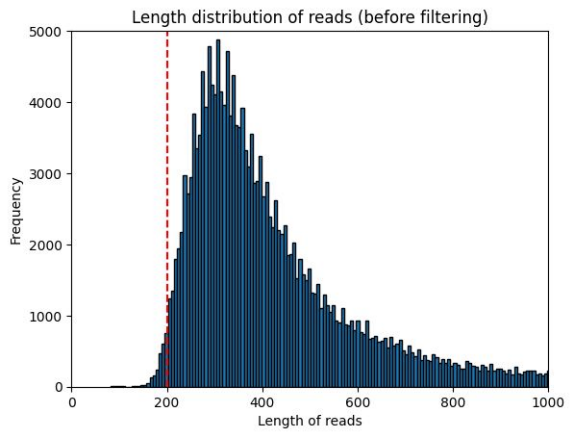
De Novo Genome Reconstruction Problem: Reconstruct a genome from error-prone, single-molecule DNA sequencing reads (long reads) without a reference genome

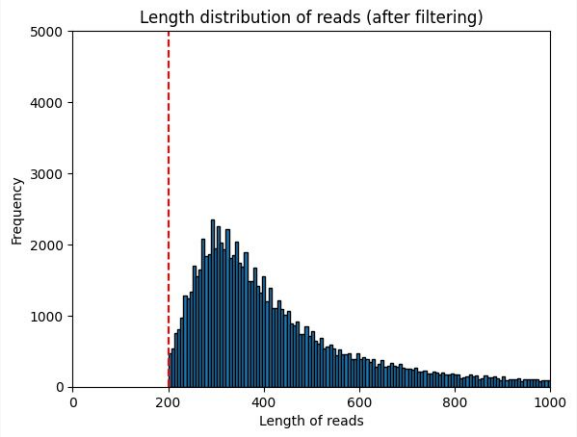
- Input: fastq file consisting of
  - N single-molecule sequencing reads
  - quality scores of base calls for each read
- Output: Long contiguous sequences (contigs) that are highly likely to be in the genome used to produce these reads

Methods

Methods Overview

- Quality Control (NanoFilt -q 10 -l 200 --minGC 0.3)
- Overlap-Layout-Consensus (OLC)
  - Overlap: Detect overlapping reads
  - Error Correction: FalconSense
  - Layout: Group overlapped reads into layouts of reads coming from same genomic region
  - Consensus: Overlay reads in each layout and compute consensus seq (contigs)





Whole-genome, long-read

Oxford Nanopore

106,084 reads

Salmonella enterica serotype Hadar

Ref. 1

Find Overlap Using MHAP

S_1: CATGGACCGA

CAT GAC
ATG ACC
TGG CCG
GGA CGA

GCAGTACCGA :**S_2**

GTA CGA
AGT CCG
CAG ACC
GCA TAC

S_1: CATGGACCGA

| | | | |

S_2 GCAGTACCGA

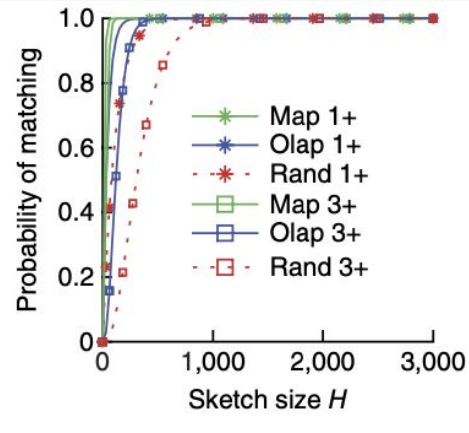
$J(S_1, S_2) \approx 2/4 = 0.5$

min-mers

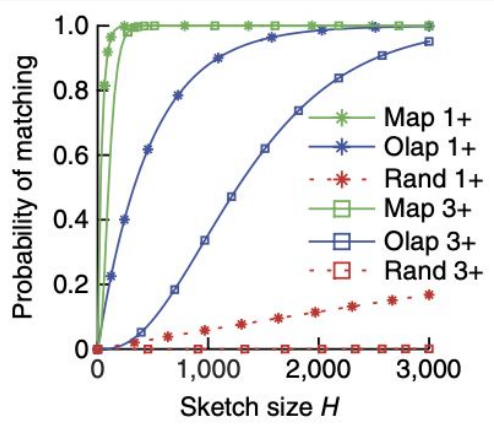
[5, 1, 2, 15]
Sketch (S_1)

[5, 1, 6, 6]
Sketch (S_2)

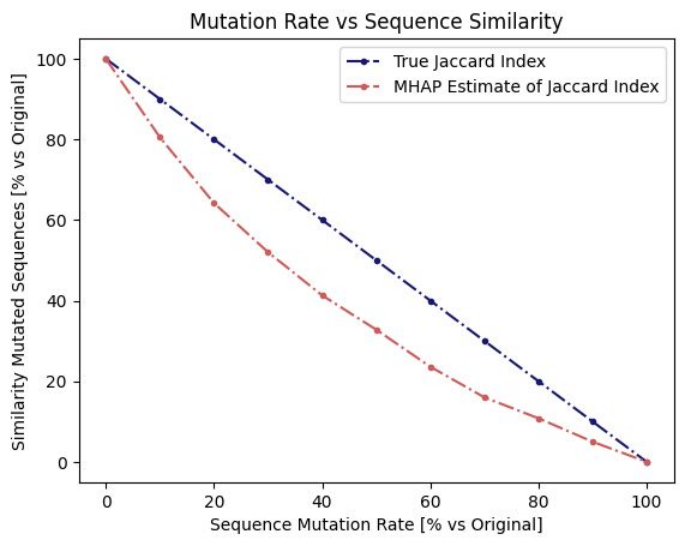
Choose Parameter k, *H*



k = 10



k = 16



H = 1256

Ref. 2

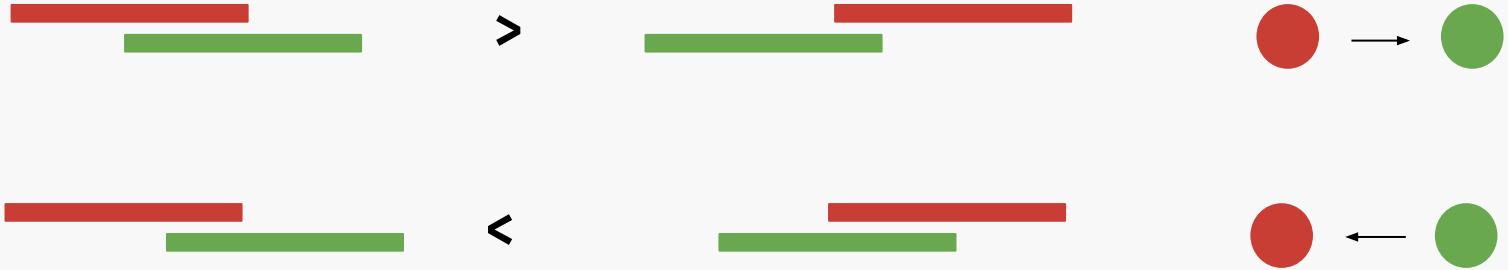
Parallelization

- Assume MHAP with sketch size H tells us (on average) each read has Z candidate reads to overlap with (N = 100,000, H = 1,256, Z = 1,000):
  - O(N*H) = 125,600,000 hashes to computed (for all N sketches)
  - $O(N^2H^2)$ = 1,577,536,000,000,000 calcs for Jaccard mtx (N^2 elements)
  - O(N*Z) = 100,000,000 alignments will be necessary (2% of total)
    - Better than $100{,}000^2/2$ = 5,000,000,000 alignments!
- Multiple embarrassingly parallel steps for MHAP
  - Pairwise Jaccard Index computations are independent & symmetric
  - Computing sketches for each read is independent
- Future parallelization: Pairwise alignments from MHAP independent
  - Efficient memoization should allow us to avoid recomputation

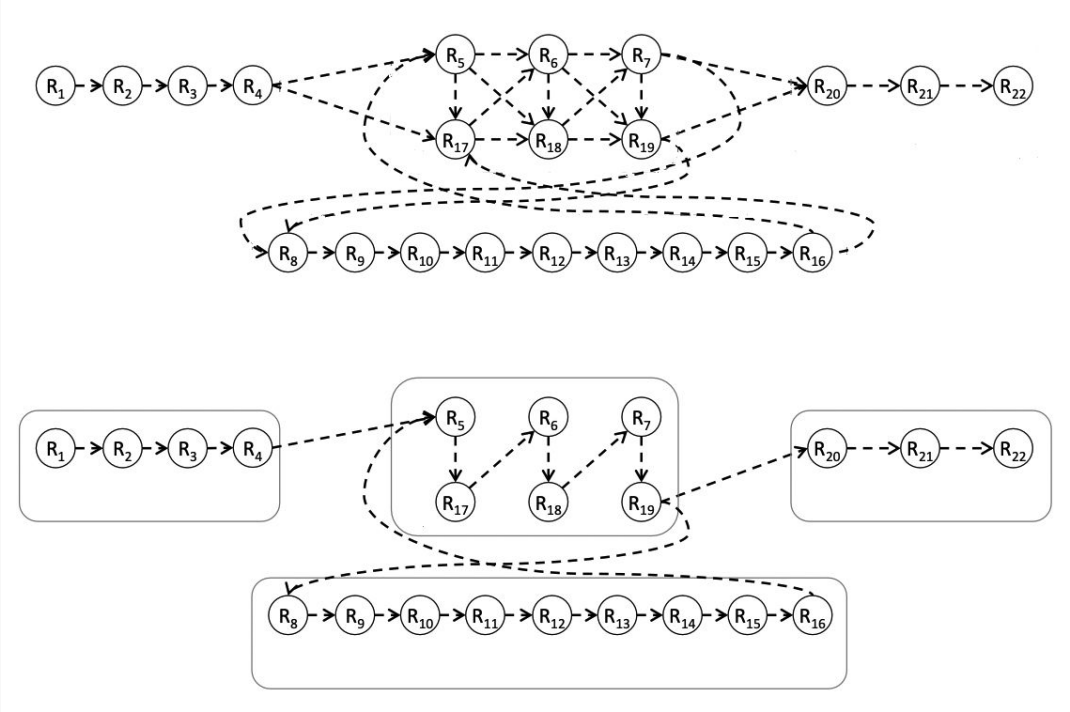
Overlap: Generating directed overlap graph

Error Correction by FalconSense: for each read, generate fitting alignment from all overlapping reads, then obtain consensus for each position in the read

Additionally perform **overlap alignments** in *both* directions to ascertain direction of edge that should be drawn in overlap graph, based on higher score



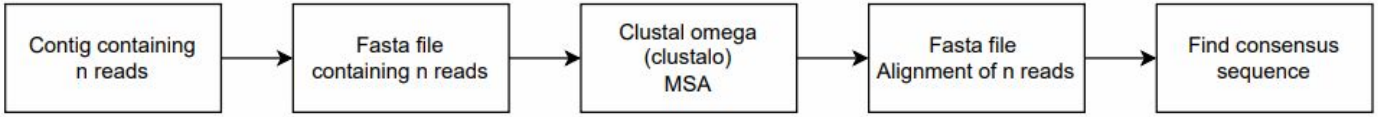
Layout



maximal nonbranching paths = contigs

Ref. 4

Consensus

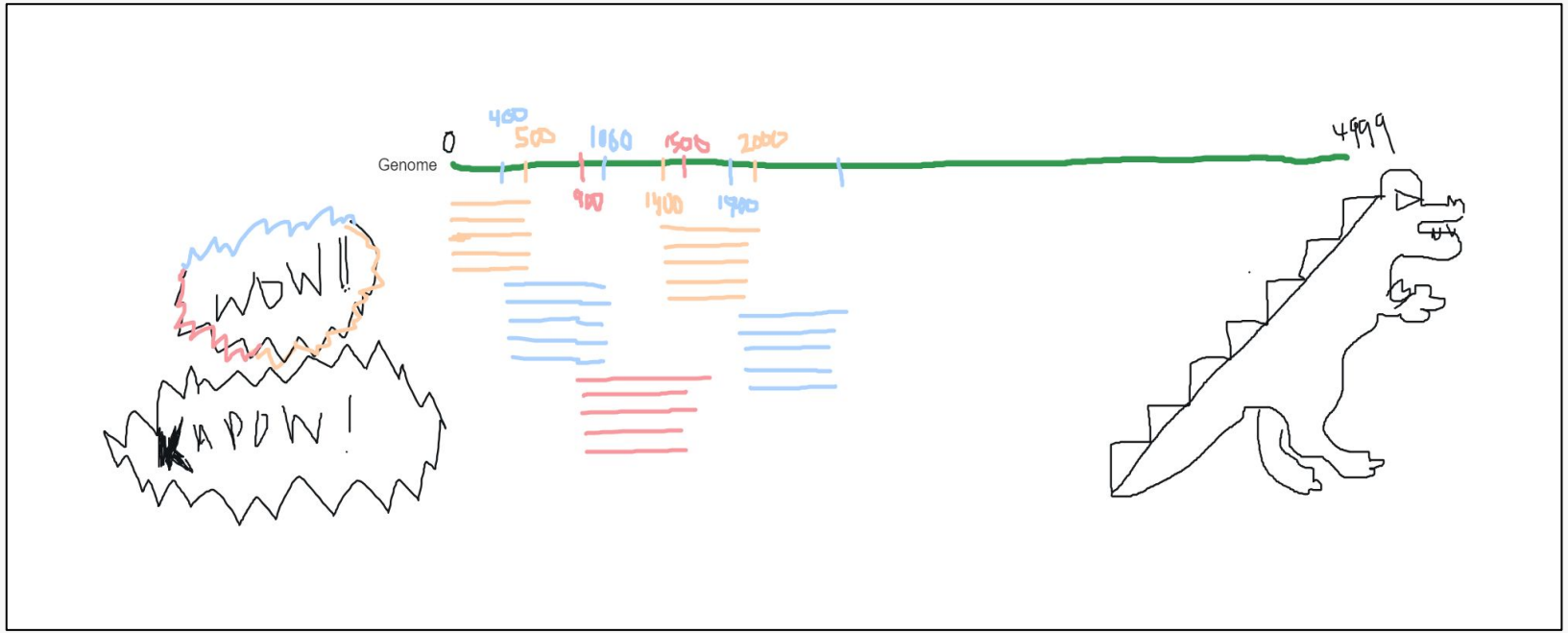


Finding the consensus from the MSA

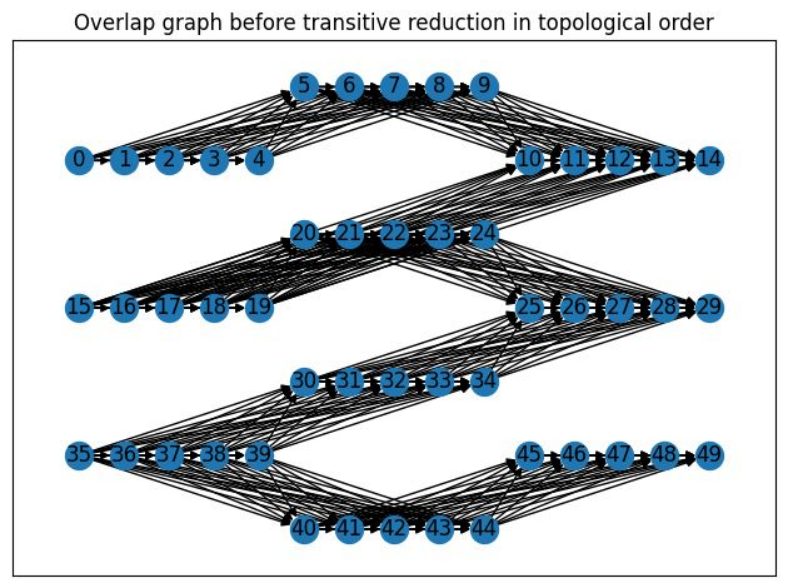
```
TTAACCTTGGTTTTGAACTTGAACACTTAGGGGATTG
        TGGTTTTGAACTTGAACACTTAGGGGATTGAAGATTCAACAA
                AACTTGAACACTTAGGGGATTGAAGATTCAACAACCCTAAAGCT
                        CACTTAGGGGATTGAAGATTCAACAACCCTAAAGCTTGGG
                                ATTGAAGATTCAACAACCCTAAAGCTTGGGGT
                                        ACCCTAAAGCTTGGGGTA
                                                AGCTTGGGGTAAAAC
TTAACCTTGGTTTTGAACTTGAACACTTAGGGGATTGAAGATTCAACAACCCTAAAGCTTGGGGTAAAAC
```

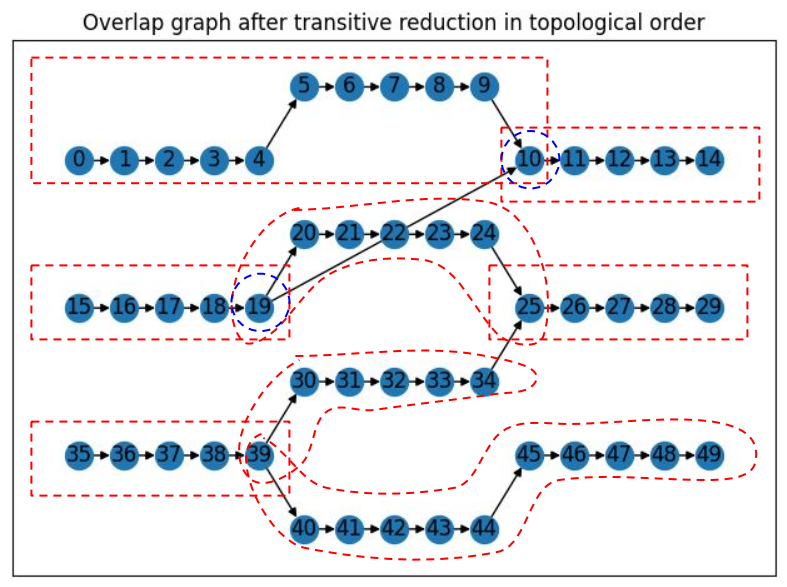
Results

The Perfect OLC Dataset...



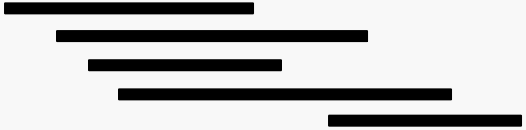
Why Transitive Reduction Matters?





Imperfect OLC Datasets...

- Case 1
  - A synthetic genome is broken down into different sizes of reads (depth x1)
  - No sequencing errors introduced into the reads



- Case 2
  - A synthetic genome is broken down into different sizes of reads (depth x1)
  - Sequencing errors (< 10%) are introduced into the reads

- **Our Results**: (1) More contigs than reads, (2) Most contigs consists of two reads, (3) Many disjoint components (1 large connected component)

Conclusions

- Optimization is an essential step to handle complex biological data.
- Generating the overlap graph is the computational bottleneck of genome assembly.
- The performance of our algorithm is very poor compared to Spades, even on the toy data!
- Python might not be the best language to handle computationally intensive tasks.

Future Steps

- Continue to run the pipeline on the real data (expensive)
- Parallelize Jaccard Index Calculation and alignment step
- Benchmark assembly results to existing tools
- Troubleshoot the layout step for different sets of synthetic data
- Characterize effects of different sequencing error rates and depths on our algorithm's performance for the "Perfect OLC Dataset"

References

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2. Berlin, K., Koren, S., Chin, C., Drake, J., Landolin, J. M., & Phillippy, A. M. (2015). Assembling large genomes with single-molecule sequencing and locality-sensitive hashing. Nature Biotechnology, 33(6), 623–630. https://doi.org/10.1038/nbt.3238
3. Chu, J., Mohamadi, H., Warren, R. L., Yang, C., & Birol, İ. (2016). Innovations and challenges in detecting long read overlaps: an evaluation of the state-of-the-art. Bioinformatics, 33(8), 1261–1270. https://doi.org/10.1093/bioinformatics/btw811
4. Sung, W. (2017). Algorithms for Next-Generation Sequencing. In Chapman and Hall/CRC eBooks. https://doi.org/10.1201/9781315374352
5. Zhang, H., Jain, C., & Aluru, S. (2020). A comprehensive evaluation of long read error correction methods. BMC Genomics, 21(S6). https://doi.org/10.1186/s12864-020-07227-0

k-mer	Γ_1	Γ_2	Γ_3	Γ_4
GCA	36	19	14	57
CAG	18	13	56	39
AGT	11	54	33	28
GTA	44	27	**6**	49
TAC	49	44	27	**6**
ACC	**5**	48	47	26
CCG	22	**1**	60	43
CGA	24	7	50	45