Towards More Effective Formulations of the Genome Assembly Problem

Alexandru Tomescu
Department of Computer Science
University of Helsinki, Finland

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VIKING LINE

Central dogma of biology

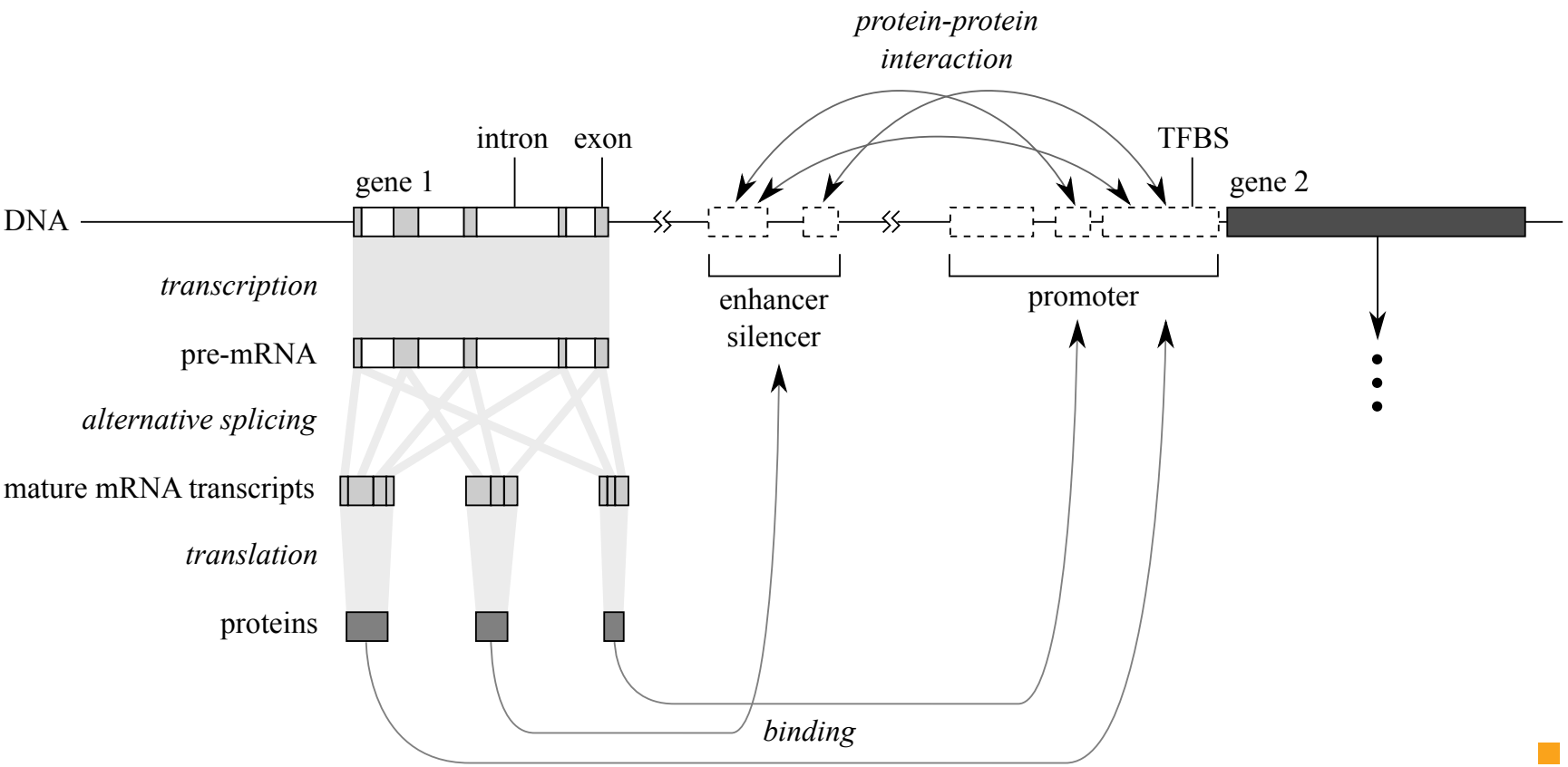
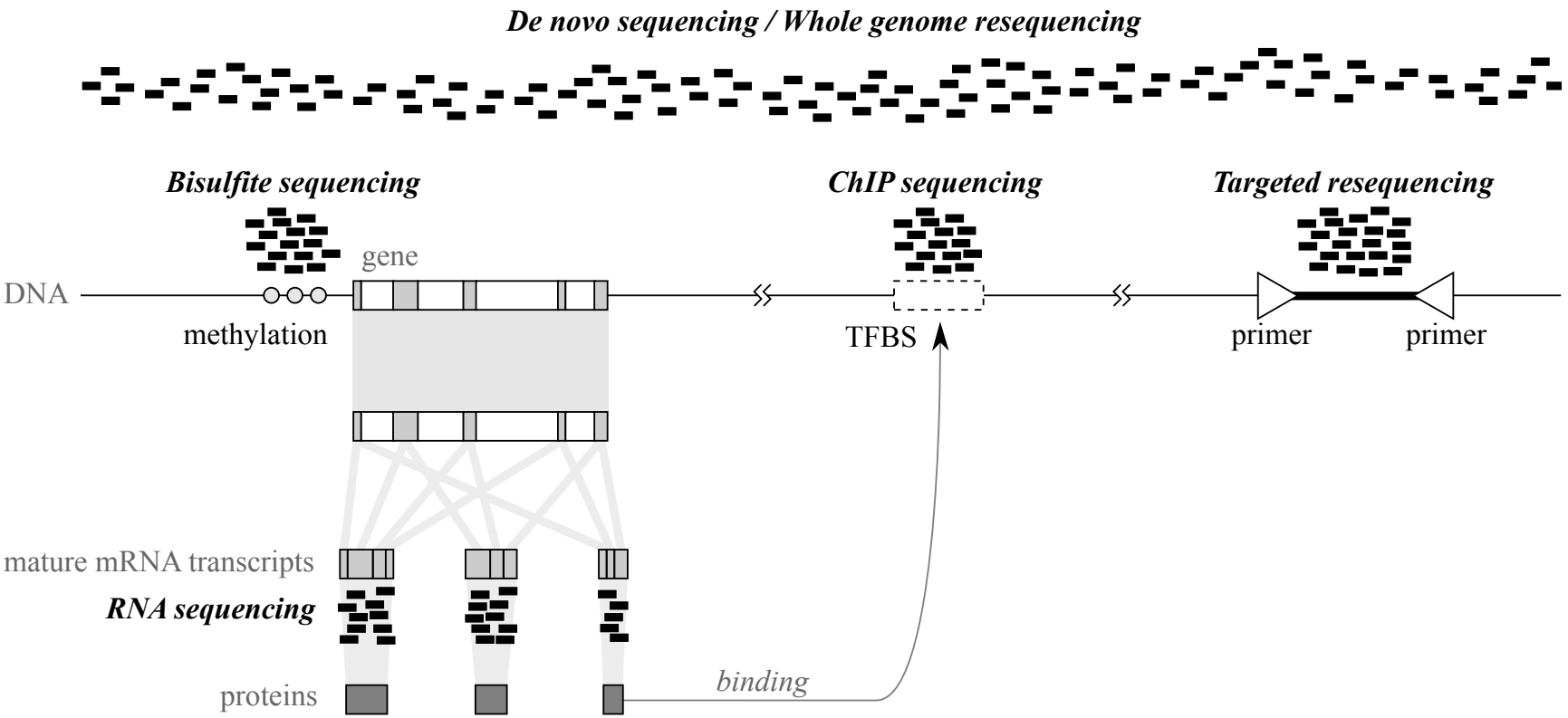


Image taken from
Genome-Scale Algorithm Design, Cambridge University Press, 2015

Sequencing atlas



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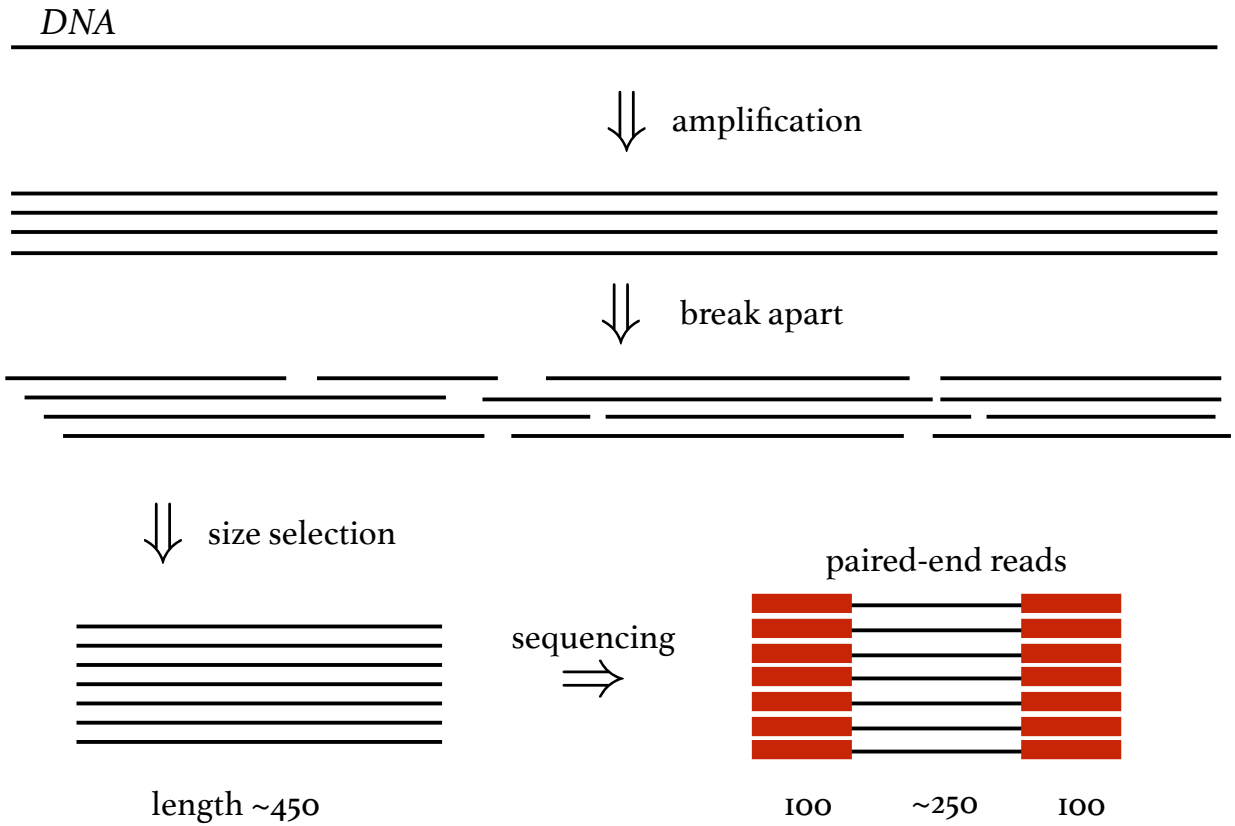
High-throughput sequencing



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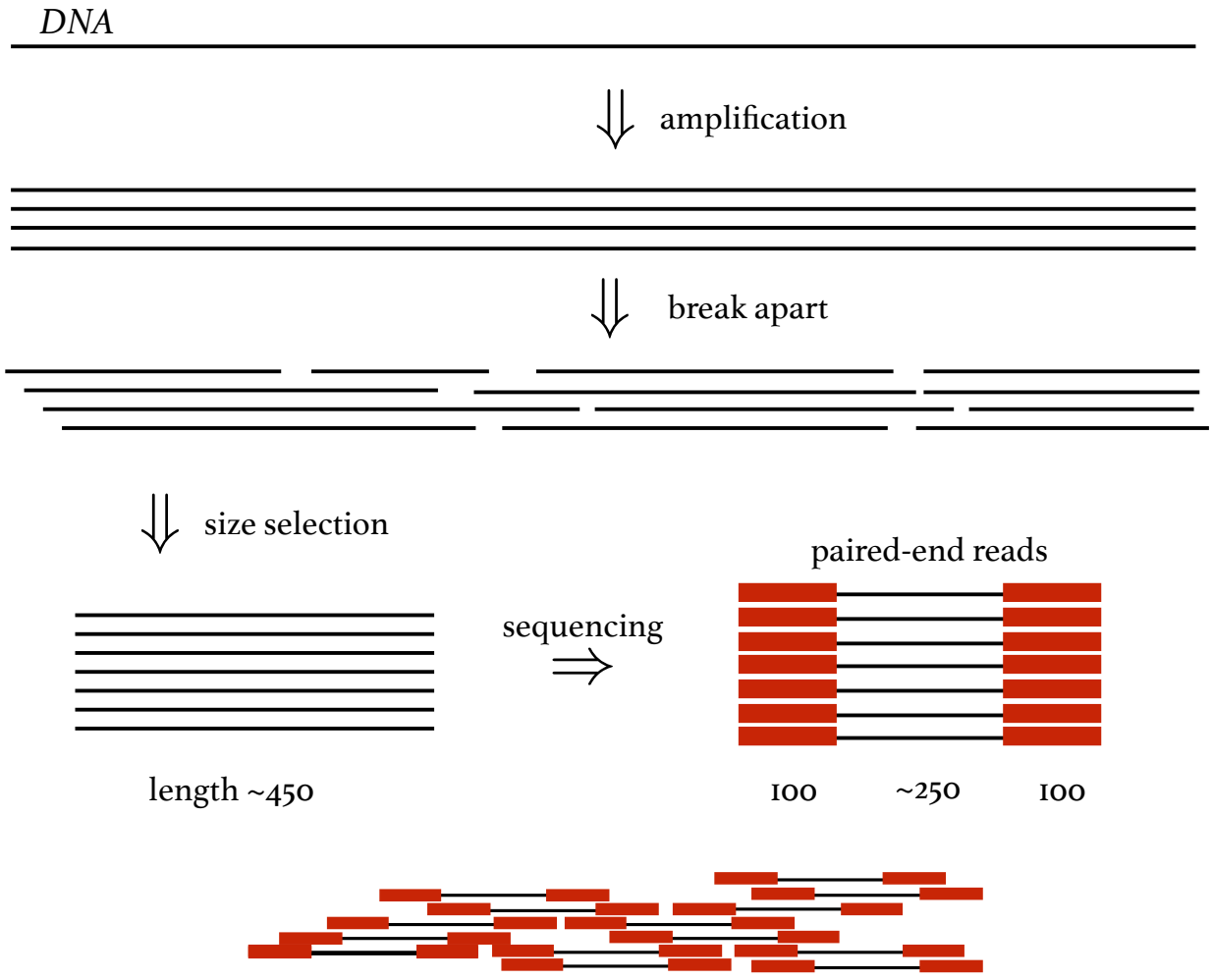

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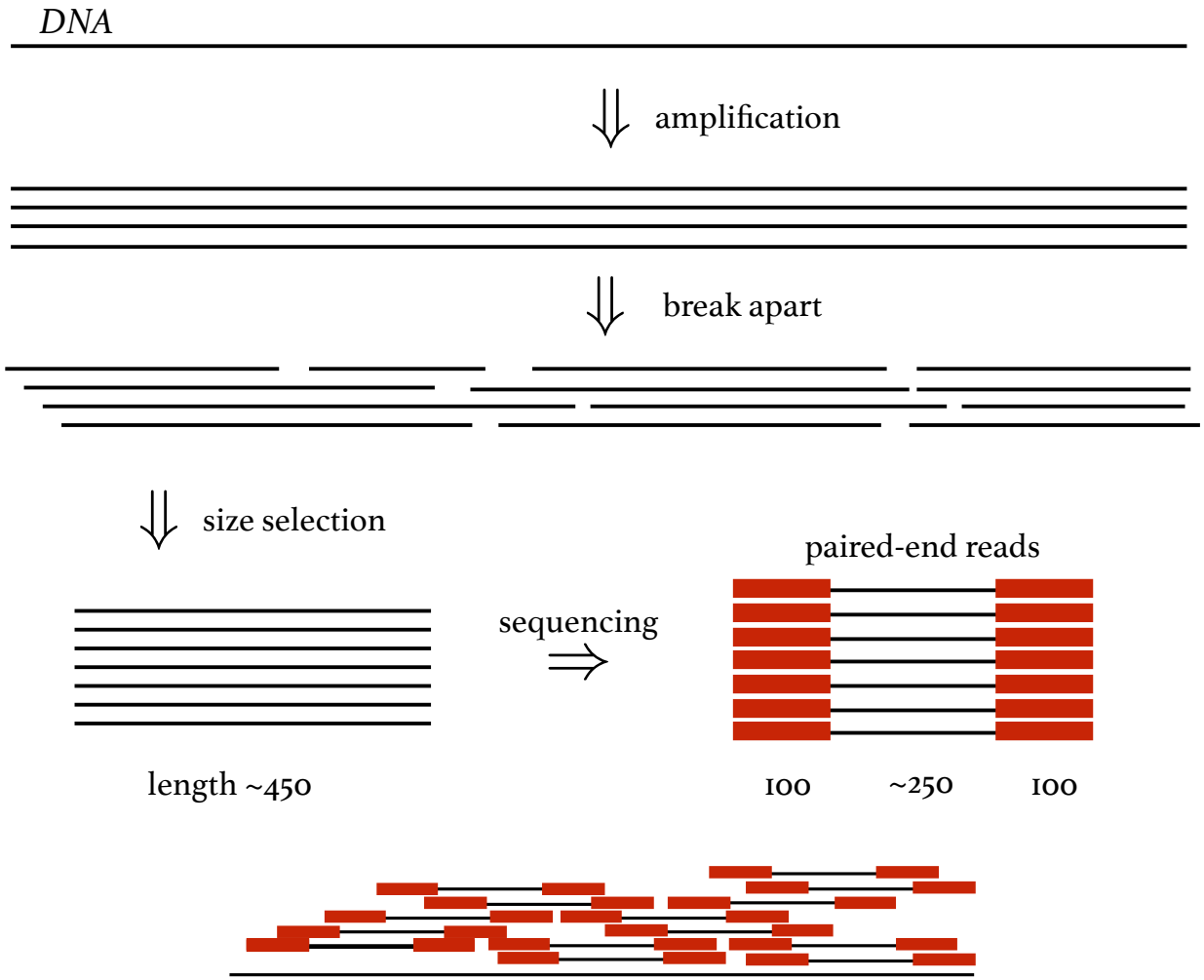

We assume here that DNA is a single stranded, single chromosome

Illumina HiSeqX

Genome assembly problem

E.coli	$4.6 \cdot 10^6$
Human	$3.2 \cdot 10^9$
Spurce	$25 \cdot 10^9$

INPUT: A collection of paired-end reads
OUTPUT: The genome

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Initial formulations:

- Shortest superstring problem (NP-hard)
- Build a graph with reads as nodes, and significant overlaps between reads as directed edges:
  - Find a walk that passes through every node exactly once (NP-complete)
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Unrealistic:

- Longer repeated regions are collapsed
- Genome coverage is not uniform
- We cannot choose between multiple solutions

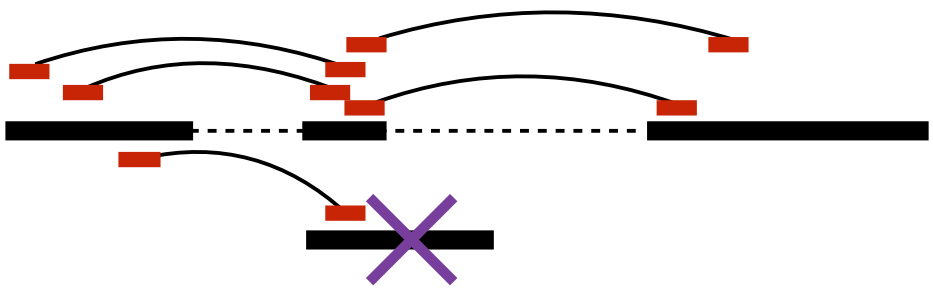
Practical formulations / pipeline

1. **Contig assembly**: assemble the reads into strings (*contigs*) that are *guaranteed* to occur in the genome

Practical formulations / pipeline

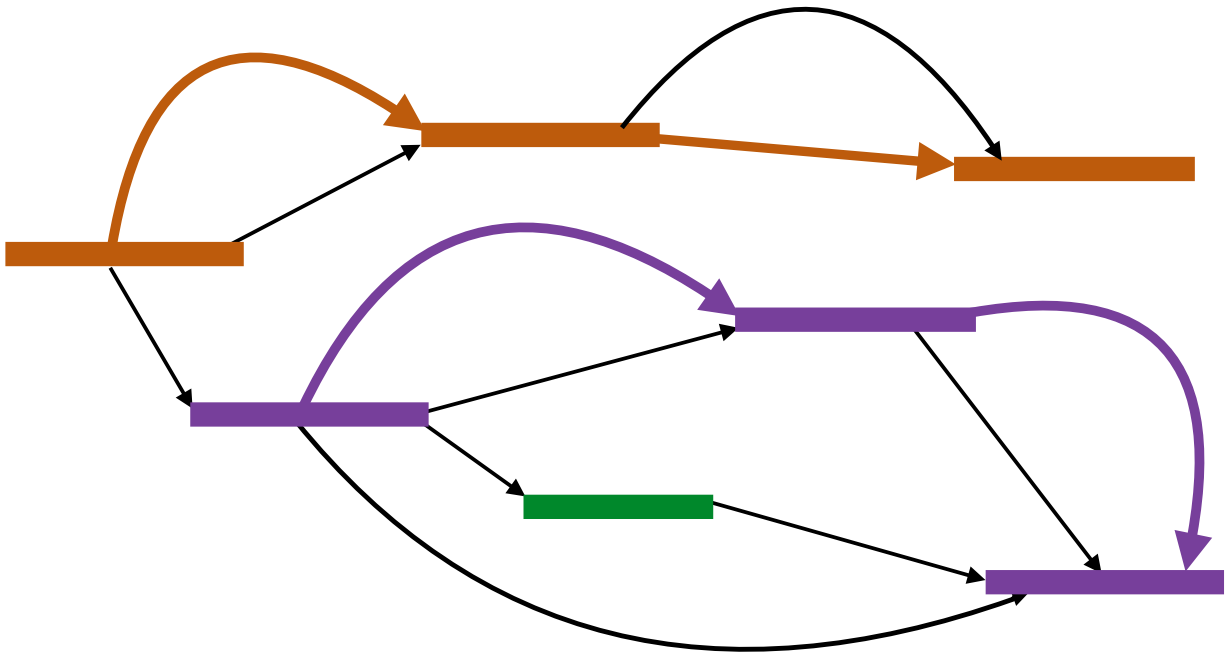
1. **Contig assembly**: assemble the reads into strings (*contigs*) that are *guaranteed* to occur in the genome

2. **Scaffolding**: using paired-end reads, chain the contigs into scaffolds that are *guaranteed* to occur in the genome



Practical formulations / pipeline (2)

3. **Gap filling**: fill the gaps in the scaffolds



Tens of genome assembly programs available: ABySS, Velvet, Allpaths-LG, Bambus2, MSR-CA, SGA, Cortex, SOAPdenovo, Opera-LG, SPADES, ...

De Bruijn graphs

Definition

Given a set R of strings, the de Bruijn graph of order k of R is the directed graph $DB^k(R)$ with

- node set: the set of k-mers of R
- edge set: the set of $k+1$-mers of the strings of R

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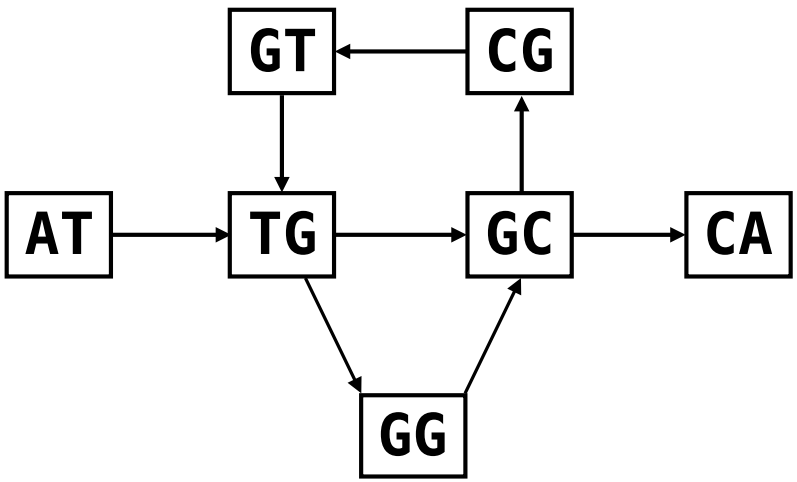
ATGCGTGGCA
ATGCG
CGTG
TGGCA

$k = 2$

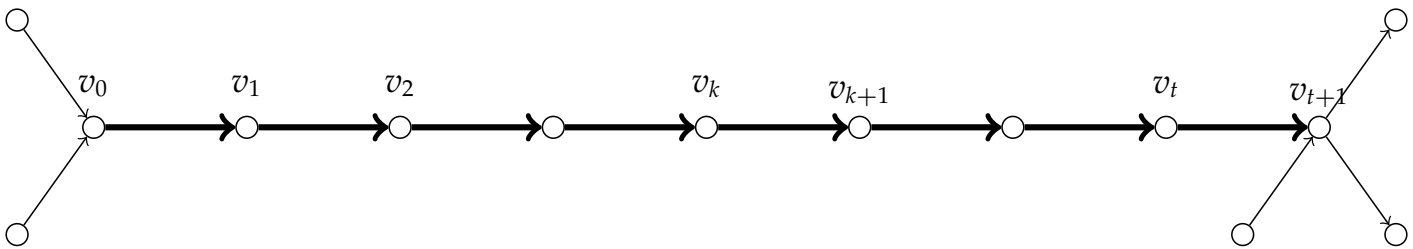


Contig assembly

joint work with Paul Medvedev

Contig assembly

- No previous formal definition of *contig*
- Usually, contigs are maximal, unary paths (i.e., whose internal nodes have in-degree and out-degree 1, aka unitigs)



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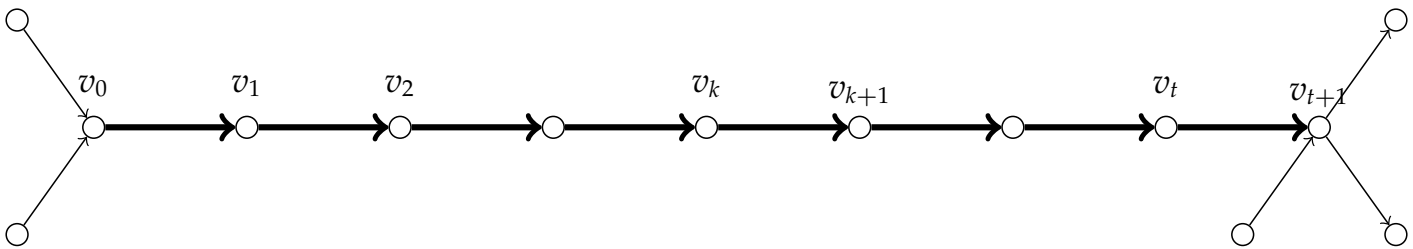

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- a walk is safe if it is a sub-walk of all genomic walks of G

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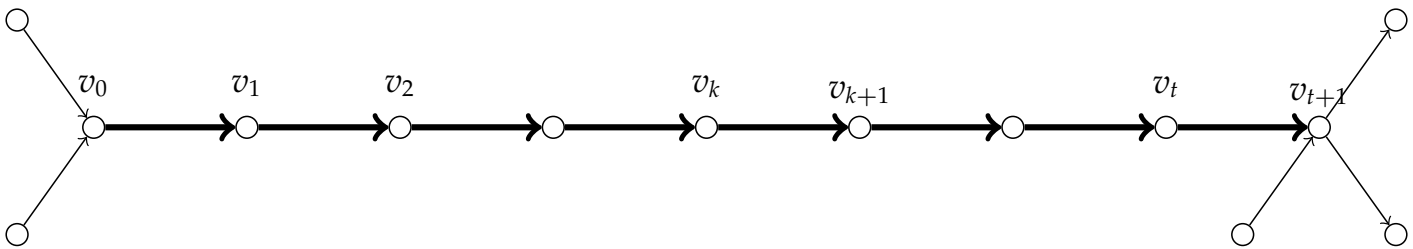

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We now assume that the dBG admits a genomic walk (i.e., is strongly connected) and is not a single cycle.

Contig assembly (2)

We say that a contig assembly algorithm is

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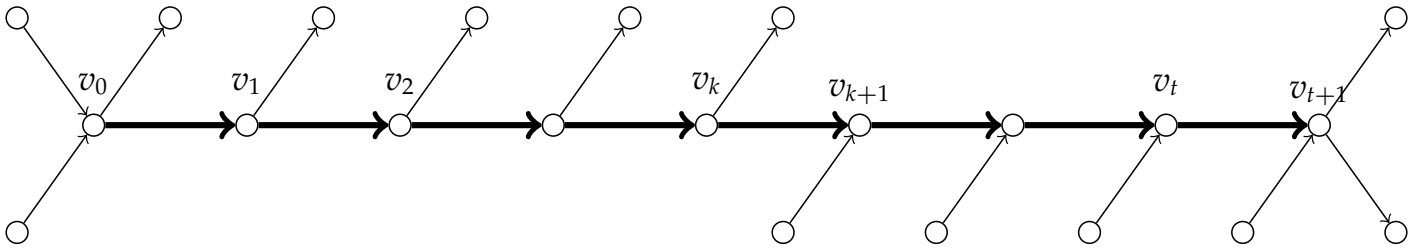
Is there a sound and complete contig assembly algorithm?

Non-switching contigs



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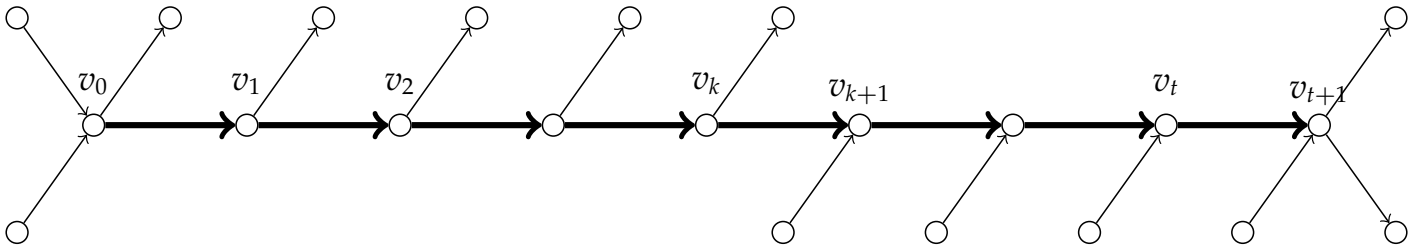
Non-switching contigs



- A path with all out-branching nodes before all in-branching nodes
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  - Kingsford, Schatz, Pop 2010
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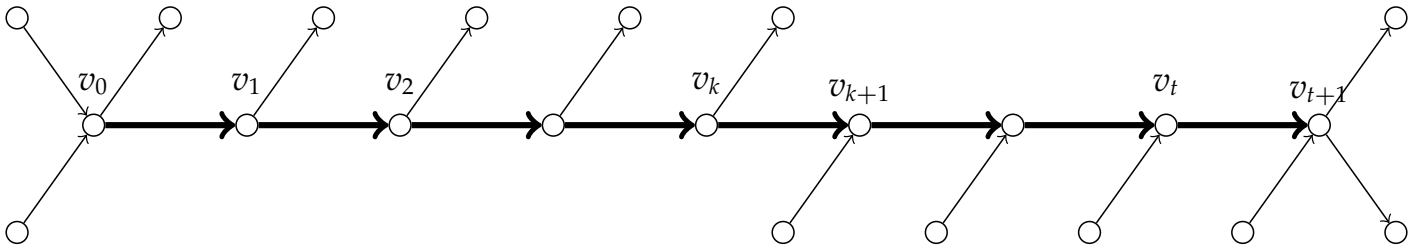

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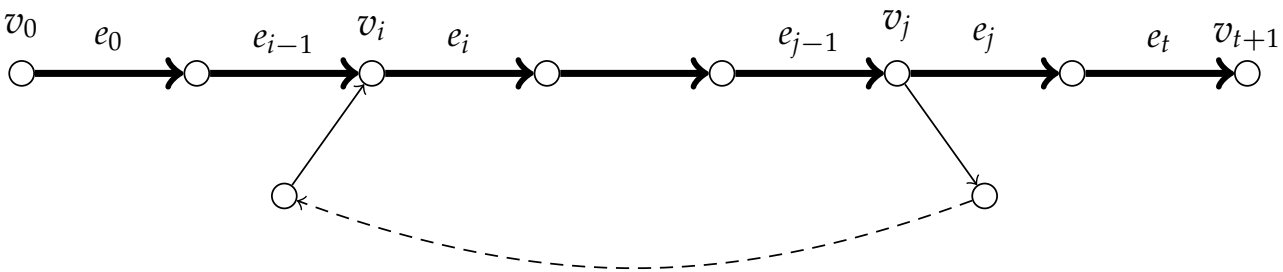
Theorem

There is an $O(|G| + |output|)$-time algorithm to output all maximal non-switching contigs of G.

Theorem

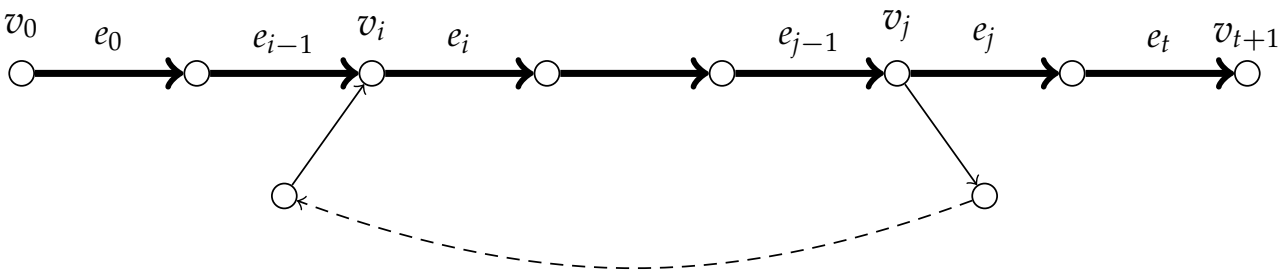
The non-switching contig assembly algorithm is sound but not complete.

*Omni*TIGS



We say that a walk $w = (v_0, e_0, v_1, e_1, \ldots, v_t, e_t, v_{t+1})$ is an omnitig if for all $1 \leq i \leq j \leq t$, there is no proper v_j-v_i path with first edge different from e_j, and last edge different from e_{i-1}.

*Omni*TIGS



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THEOREM

A walk w is safe $\Leftrightarrow$ w is an omnitig.

THEOREM

There is a polynomial time algorithm for outputting all maximal omnitigs.

COROLLARY

The omnitig algorithm is sound and complete.

Experimental results

Algorithm	#strings	E-size	AVG length
unitig	41,524	7,806	893
non-switching contigs	32,589	7,822	1,136
omnitigs	24,949	7,850	1,479

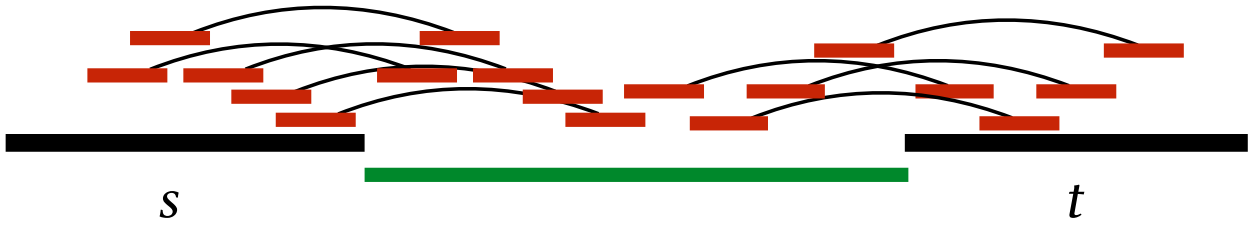
- genome: circularized human chr21 (length $48 \cdot 10^6$)
- graph: dBG^k(chr21) for $k = 55$
- e-size: given a set of substrings of genome, their e-size is the average, over all genomic positions i, of the mean length of the strings spanning position i

GAP FILLING

joint work with Leena Salmela, Kristoffer Sahlin and Veli Mäkinen

Problem formulation

Previous formulations (GapCloser 2012, GapFiller 2012):



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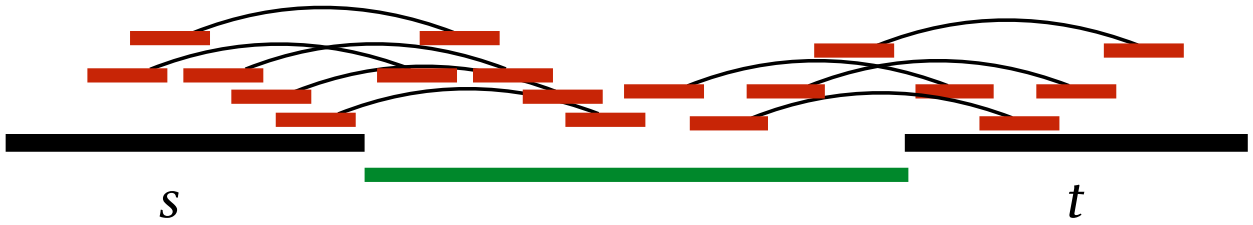
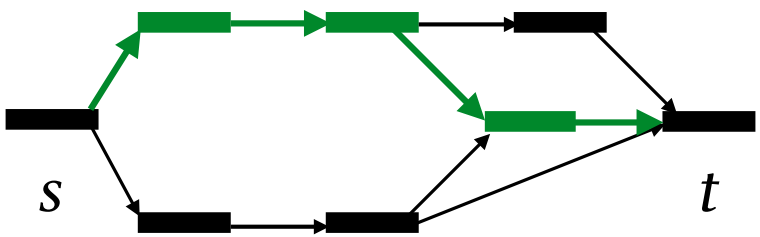


We formulate it as Exact Path Length problem. Given:

- $G = dBG^k(R)$, for some k
- $s, t \in V(G)$, the two k-mers flanking the gap
- $[d'..d]$ an estimate on the gap length

For every $x \in [d'..d]$ find an s-t path spelling a string of length x (i.e. a path of length $x - k$).



Dynamic programming (DP)

Usually, $d < |V(G)|$.

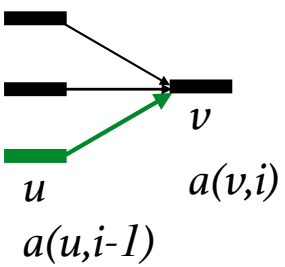
Can be solved by DP in time $O(d \cdot |E(G)|)$:

- for every node v store:

$$a(v,i) := \begin{cases} 1 & \text{if there exists an } s\text{-}v \text{ path of length } i, \\ 0 & \textit{otherwise}. \end{cases}$$

- initialize $a(s,0) = 1$, and compute

$$a(v,i) := \bigvee_{u \in N^-(v)} a(u, i-1).$$

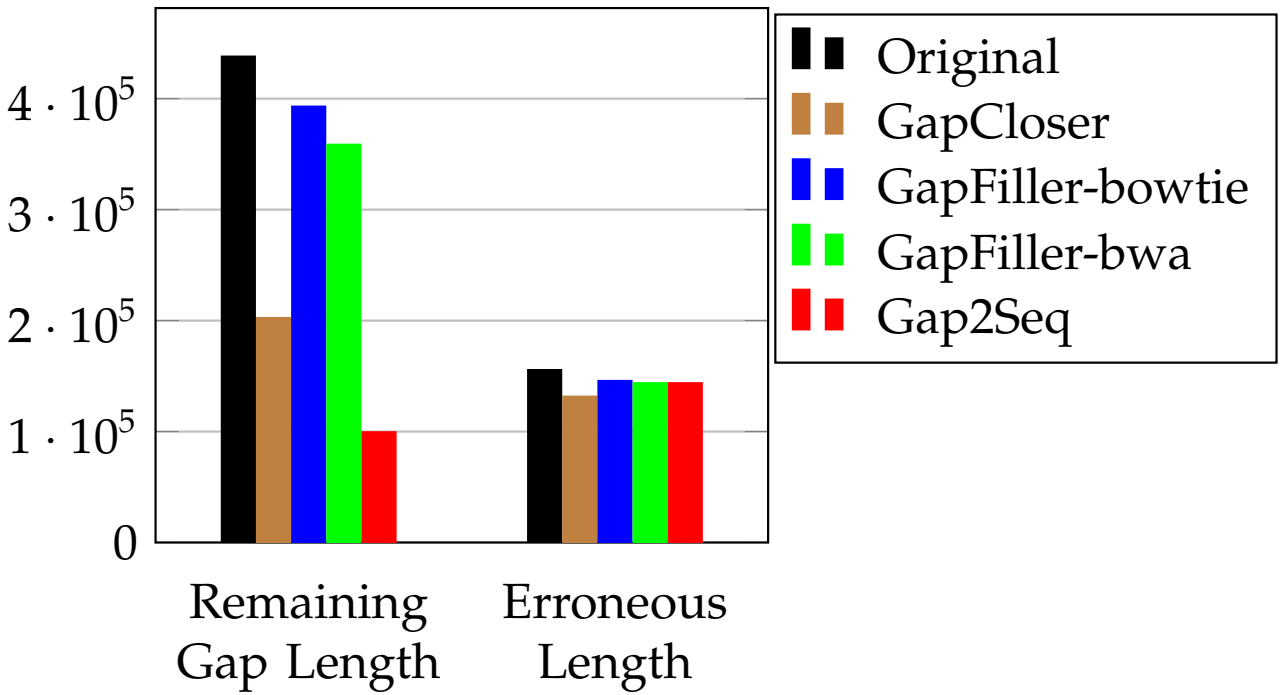


- back-tracking in the DP matrix from $a(t, x-k)$ gives a path (if exists)

Engineered implementation

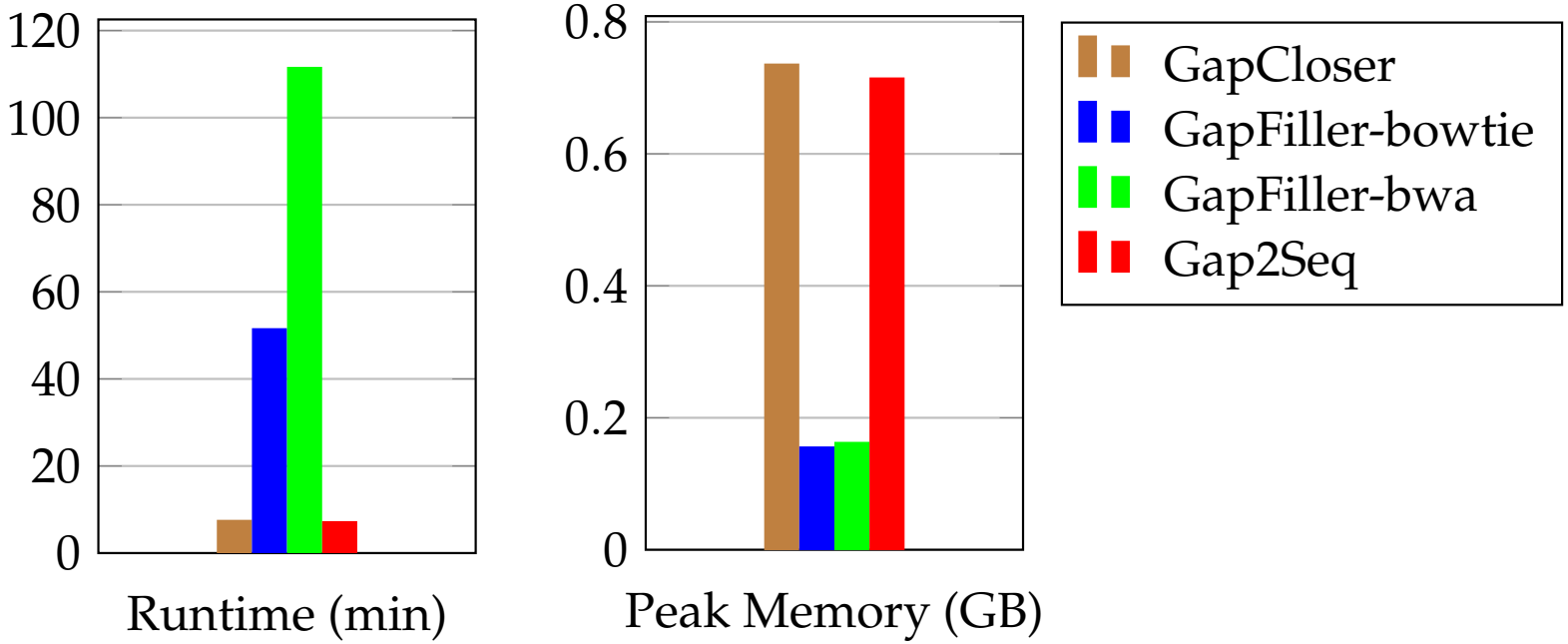
- *k*-mers flanking the gaps can have errors
  - allow paths to start/end at up to *t* flanking *k*-mers
- we should not explore the entire graph!
  - *meet in the middle* optimization
- DP matrix rows are sparse
  - store only the non-zero entries in each row
- parallelization on scaffold level
- limit the memory usage of the DP matrix
  - abandon the search on a gap if limit exceeded

Experimental results (*Gap2Seq*)



- genome: Staphylococcus aureus (length $2.8 \cdot 10^6$)
- graph: dBG with $k = 31$, for $R =$ a collection of real reads
- results are totals over all scaffolds from a benchmark dataset (ABySS, Allpaths-LG, Bambus2, CABOG, MSR-CA, SGA, SOAPdenovo, Velvet)

EXPERIMENTAL RESULTS (*Gap2Seq*)



Conclusions

Potential uses for omnitigs:

- longer contigs = better starting point for scaffolding and gap filling
- more flanking information around loci of interest

Directions for omnitigs:

- robustness to errors, coverage gaps, reverse complements?
- faster omnitig algorithm
- a sound and complete algorithm when the genomic walk is linear?

Gap Filling:

- performance on human data is 'so and so' (but all tools have problems)
- we need to improve the runtime and memory usage
- we need a way to choose between multiple solutions (contig assembly?)

MULŢUMESC