Superbubbles

and their linear-time detection

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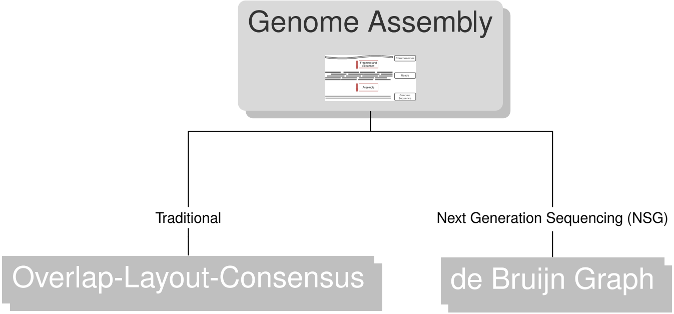
Outline

Outline

Motivation


Genome Assembly
Chromosomes
Fragment and Sequence
Reads
Assemble
Genome Sequence
Traditional
Next Generation Sequencing (NSG)
Overlap-Layout-Consensus
de Bruijn Graph

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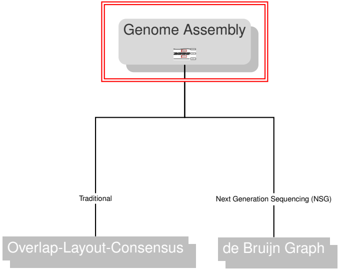

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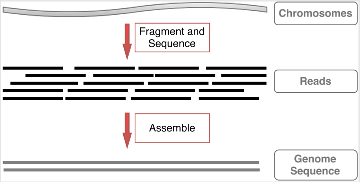

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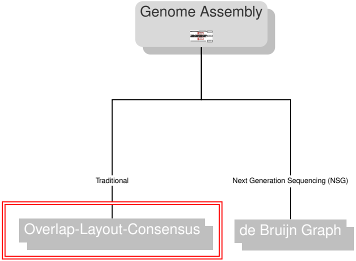

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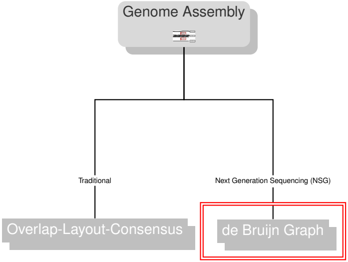

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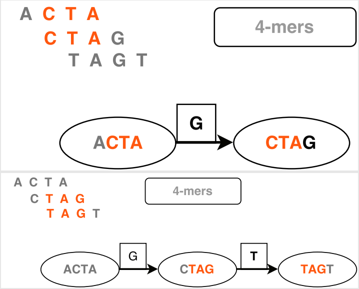

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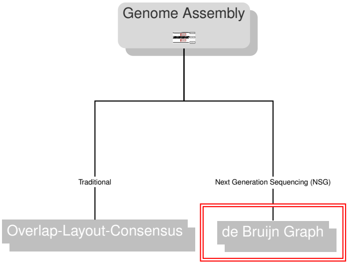
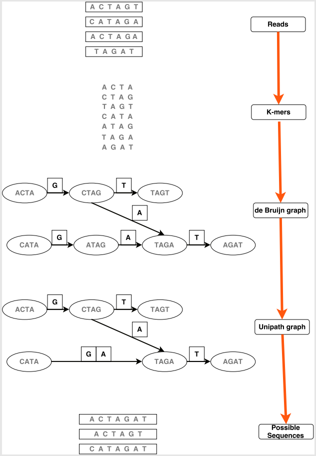


NSG



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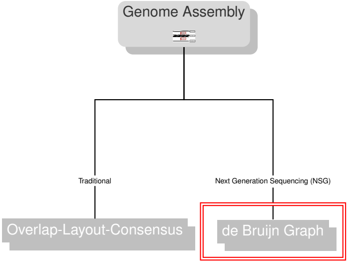

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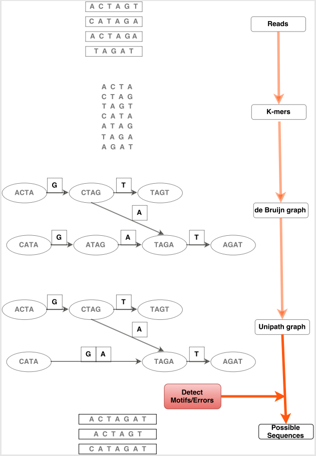

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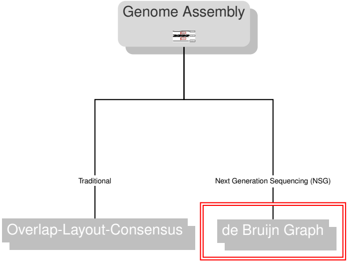
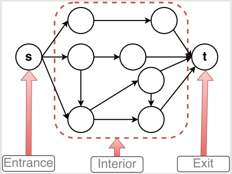


Motifs

- Tips
- Cross-links
- Bubbles
- More Complex Structures?
  Superbubbles

Superbubble

Superbubble: $\langle s,\ t \rangle$

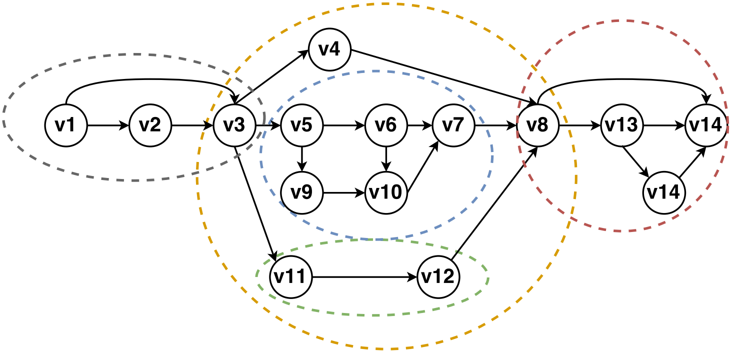


Definition [Onodera et al., 2013]

Let $G = (V, E)$ be a directed graph. For any ordered pair of distinct nodes s and t, $\langle s, t \rangle$ is called a *superbubble* if it satisfies the following:

- **reachability:** t is reachable from s;
- **matching:** the set of nodes reachable from s without passing through t is equal to the set of nodes from which t is reachable without passing through s;
- **acyclicity:** the subgraph induced by U is acyclic, where U is the set of nodes satisfying the matching criterion;
- **minimality:** no node in U other than t forms a pair with s that satisfies the conditions above;

Example



Background

$\mathcal{O}(nm)$-time algorithm [Onodera et al., 2013]

Topological sorting, starting from each vertex, to test if it is an entrance.

$\mathcal{O}(m \log m)$-time algorithm [Sung et al., 2015]

- Partition graphs into a set of sub-graphs -
  - subgraphs corresponding to each non-singleton strongly connected component
  - a subgraph corresponding to the set of all the nodes involved in singleton strongly connected components.
- Convert each subgraph into acyclic if it is cyclic.
- Find superbubbles in each of the subgraph.

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Topological sorting, starting from each vertex, to test if it is an entrance.

$\mathcal{O}(m \log m)$-time algorithm [Sung et al., 2015]

- Partition graphs into a set of sub-graphs - → linear
  - subgraphs corresponding to each non-singleton strongly connected component
  - a subgraph corresponding to the set of all the nodes involved in singleton strongly connected components.
- Convert each subgraph into acyclic if it is cyclic. → linear
- Find superbubbles in each of the subgraph. → $\mathcal{O}(m \log m)$

Outline

2 Linear-Time Algorithm
- Properties
- Description

Properties of superbubbles

Lemma ([Onodera et al., 2013])

Any node can be the entrance (respectively exit) of at most one superbubble.

Lemma ([Sung et al., 2015])

Let G be a directed acyclic graph. We have the following two observations.
1) Suppose (p, c) is an edge in G, where p has one child and c has one parent, then $\langle p, c\rangle$ is a superbubble in G.
2) For any superbubble $\langle s, t\rangle$ in G, there must exist some parent p of t such that p has exactly one child t.

Lemma ([Brankovic et al., 2015])

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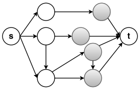

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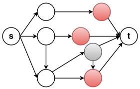

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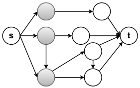

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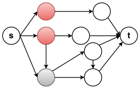

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Abstract Description

Conceptual Idea

- **Input:** $G = (V, E)$, a Directed Acyclic Graph (DAG) where V and E are sets of vertices and edges resp.($|V| = n,\ |E| = m$)
- **Output:** Superbubbles in G
- **Assumption:** Single-source and single-sink *(if not, add dummy vertices)*
- **Work-Flow:**
  - Topologically order the vertices
  - Identify possible entrance and exit candidates: *Candidate-list*
  - Traverse candidate-list (in reverse topological order) to find superbubbles using subroutines:
    - *Report*
    - *Validate*

Detailed Description

Conceptual Idea

- Topological ordering
- Identify candidates
- Find superbubbles using subroutines:
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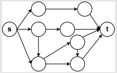
Topological-order

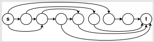
For every edge (a, b), $ord[a] < ord[b]$

TopologicalSort

Recursive DFS (Depth First Search)

Example





Lemma (4)

Given a directed graph $G = (V, E)$ containing a superbubble $\langle s, t \rangle$, a topological ordering obtained by TopologicalSort has the following properties.
- For all x such that $x \in U \setminus \{s, t\}$, ordD$[s]$ < ordD$[x]$ < ordD$[t]$
- For all y such that $y \notin U$, ordD$[y]$ < ordD$[s]$ or ordD$[y]$ > ordD$[t]$.

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Candidate

A node v is an
- **exit candidate:** if it has at least one parent with exactly one child (out-degree 1)
- **entrance candidate:** if it has at least one child with exactly one parent (in-degree 1).

(From Lemmas 2 and 3)

Identifying Candidates

Check each node in V, in topological order, to identify whether it is an exit or an entrance candidate (or both).
-If both, add twice (First as entrance and then as exit).

- Maximum size: $2n$

- Candidates are added in topological order in Candidate-list.

Detailed Description

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What?

- Reports all the possible superbubbles (including the nested ones) between given *start* and *exit*
- Called for each exit candidate in decreasing order either by *main* routine or through a recursive call to identify a nested superbubble.

How?

- Checks the possible entrance candidates between given *start* and *exit* candidates starting with the nearest previous entrance candidate (to *exit*), using *Validate*.
  - If *valid*, report it and recursively find nested superbubbles.
  - Otherwise, mark the returned *alternative entrance candidate*

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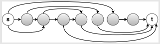
What?

- Returns *start* itself, given *start* and *exit* is a valid superbubble.
- Otherwise returns an alternative possible entrance for *exit*.

How?

- Valid: For a valid superbubble $\langle s, t \rangle$, every $x \in U \backslash \{s, t\}$ has
  - t as its *topologically furthest* child. - s as its *topologically furthest* parent.



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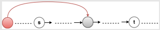

Red Vertex is an *alternate entrance candidate* for the pair (s, t).

- Maintain arrays of topological furthest child and parent resp., for each vertex and using RMQ to verify the conditions for validity.

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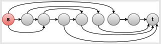

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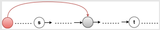

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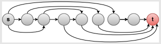

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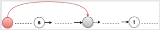

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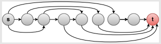

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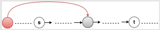

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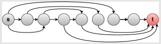

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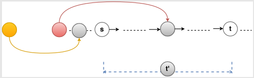
Marking



- *Red Vertex* is an invalid entrance not only for the superbubble ending at *t* but also for all those ending at any other exit node (*t'*) between *s* and *t* for which *s* is not a valid entrance and which also has *Red Vertex* as an alternative entrance.
- Further, any candidate in the sequence of alternative entrance candidates following *Red Vertex* (*Orange Vertex* and so on) can not be a valid entrance for the superbubble ending at *t'*.
- *Marking*: to skip this sequence later.

Outline

3 Analysis
- Correctness
- Running Time

Correctness of the algorithm

Lemma (5)

Given s and t, the candidates for an entrance and an exit of a superbubble in G, respectively, subroutine Validate reports $\langle s, t\rangle$ if and only if $\langle s, t\rangle$ is a superbubble.

Lemma (6)

For a given exit candidate e, let x be the alternative entrance candidate returned by the subroutine VALIDATE(s, e). *Then any entrance candidate between x and e can not be a valid entrance for the superbubble ending at e.*

Theorem

Given a directed acyclic graph $G = (V, E)$, where $n = |V|$ and $m = |E|$, algorithm SUPERBUBBLE *correctly finds all superbubbles in G in decreasing order of the topological ordering of their exit nodes.*

Running Time of the algorithm

Lemma (7)

For the given exit and entrance candidates s and e_1 (respectively), let $mark[s]$ has been set to t_1 which later gets reset to t_2 while considering s with another exit candidate e_2. Then any exit candidate between s and e_2 can not reset $mark[s]$ to t_1 again.

- **Topological sorting:** $\mathcal{O}(n+m)$
- **Computing the candidates list:** $\mathcal{O}(n+m)$
- **All the list's operations:** constant time each, sums up to a linear cost $\mathcal{O}(n)$, as there are at most $2n$ candidates in the list.
- **Each call for *Validate*:** $\mathcal{O}(1)$ [RMQ]
- **Total calls to *Validate*:** $\mathcal{O}(n+m)$.
  - Either validates the entrance candidate ($\mathcal{O}(n)$)
  - Or marks the corresponding position ($\mathcal{O}(m)$)
    - Once 'marked', it will not be considered again in subsequent calls [from lemma (7)].
    - Marking is done every time an edge is found for the first time between a vertex (in between an entrance candidate and an exit candidate) and its topologically furthest parent.
- **Total time:** $\mathcal{O}(n+m)$

Outline

4 Summary

Summary

- A critical step of assembly algorithms utilizing de Bruijn graphs is to detect typical motif structures in the graph caused by sequencing errors and genome repeats, and filter them out; one such complex subgraph class is a so-called *superbubble*.
- A supperbubble $\langle s, t\rangle$ is equivalent to a single source, single sink, acyclic directed subgraph of G with source s and sink t, which does not have any cut nodes and preserves all in-degrees and out-degrees of nodes in $U \backslash \{s, t\}$, as well as the out-degree of s and in-degree of t.
- Given a directed acyclic graph $G = (V, E)$, where $n = |V|$ and $m = |E|$, all superbubbles in G can be identified in $\mathcal{O}(n + m)$-time.
- What's Next: More complex motifs

Paper appears in TCS (Open Access):
[L. Brankovica, C. S. Iliopoulos, R. Kundu, M. Mohamed, S. P. Pissis, F. Vayani: Linear-time superbubble identification algorithm for genome assembly].

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Thank You!