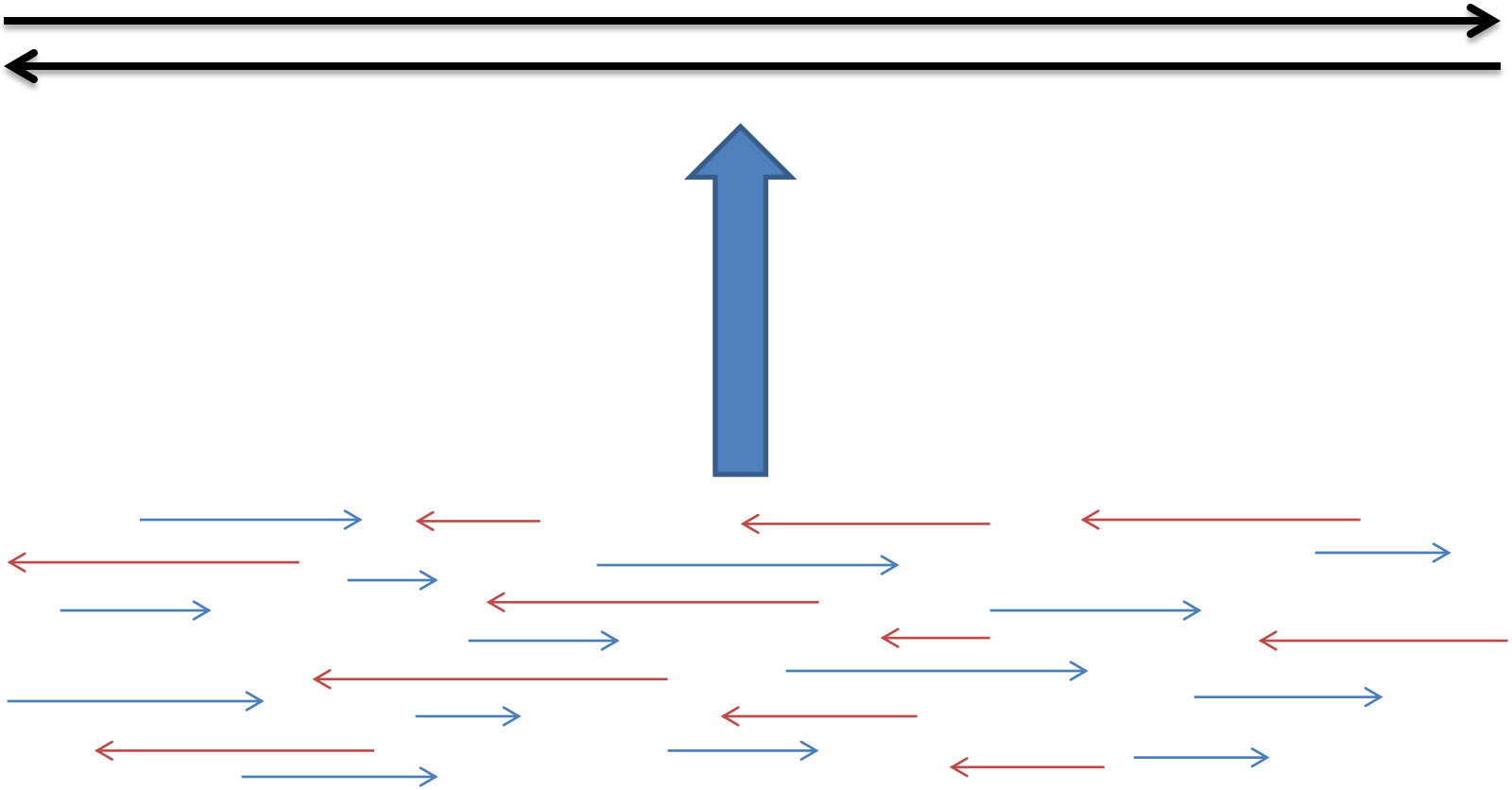
Velvet: Algorithms for de novo short read assembly using de Bruijn graphs

Matthew Tien

Zerbino DR, Birney E. Velvet: Algorithms for de novo short read assembly using de Bruijn graphs. Cold Spring Harbor Press 18 (2008): 821-29.

Genome Assembly Problem



Overlap Graphs

- Construction
  - Nodes= reads
  - Edges= overlap between reads
- Disadvantages
  - Hard overlap problem with large amount of reads
  - Pair-wise computation too much
  - Hamiltonian Path Problem

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De Bruijn Graphs

- K-mer approach
  - Define set length nucleotides of length k
- Construction
  - Node= k-mer
  - Edges= connect nodes by the path created from a read overlapping with the k-mer
- Advantages
  - Set length nodes, so no overlap algorithm needed
  - Eulerian path problem
- Disadvantages
  - Loss of information
  - Shorter contigs

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De Bruijn Graphs useful for Short Reads

- Short reads (~100-200bp long)
  - 25-50 is too short for de Bruijn
- Short Reads
  - Many reads will have only a single or no base difference
  - More ambiguous connections

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Velvet approach to de Bruijn graphs

- Error Correction Algorithm
  - Merge sequences that belong together

- Repeat solver
  - Separates paths sharing local overlaps

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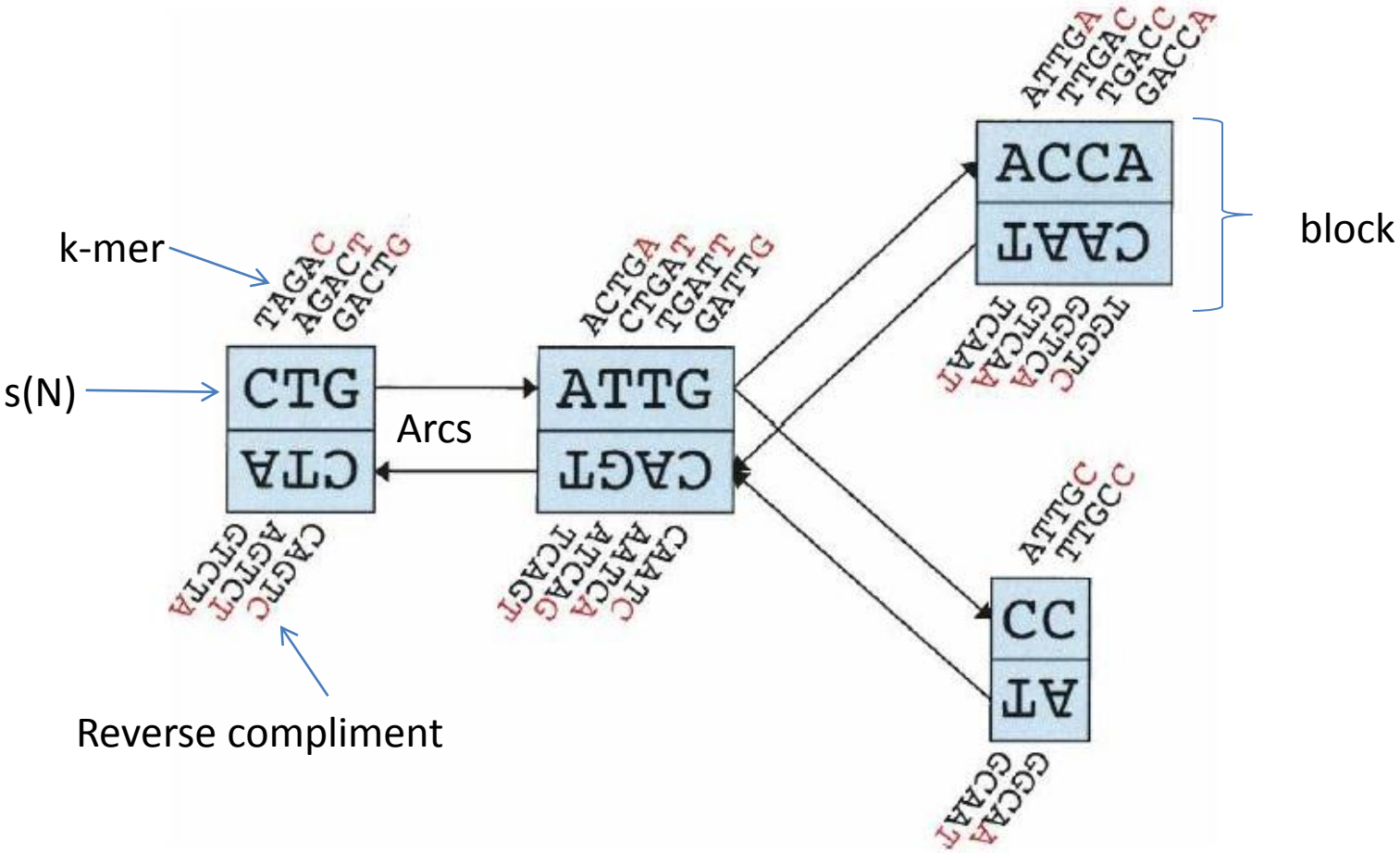
Structure of de Bruijn Graph



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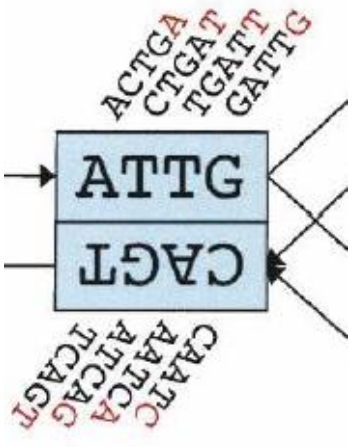
Construction (part 1)

- Two step process:
  1. Reads are hashed to a predefined k-mer length (k=21 or 25 bp)
     a. Each k-mer has an ID that maps the k-mer back to the read and its position in the read.
     b. Simultaneously recorded to its reverse complement

This is just to get a set of k-mers for the graph



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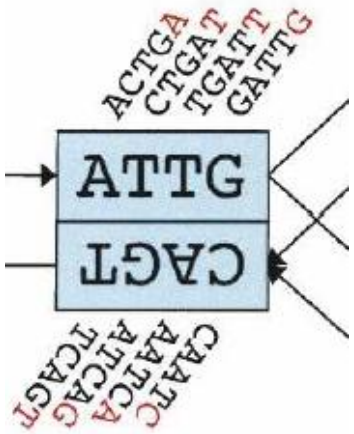
Construction (part 2)

- Two step process:

  2. For each read, it records which k-mer are overlapped by subsequent reads

     a. original set of k-mers is cut each time an overlap with another read beings or ends

This is to get the s(N) part of each k-mer



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Construction (part 3)

- Use reads to add connections to the nodes

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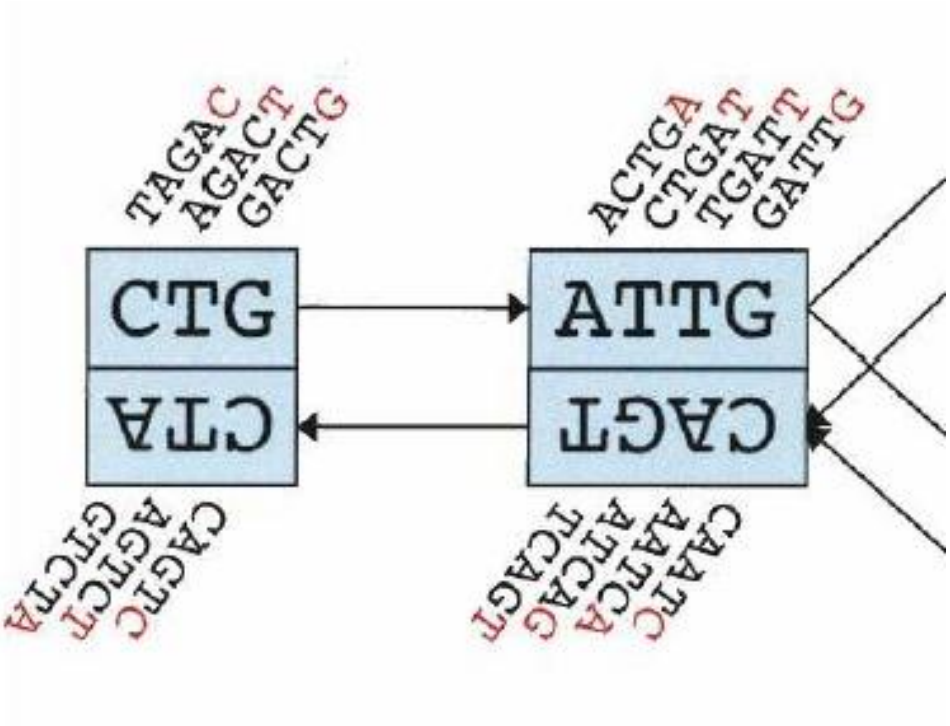
Simplification

- Chains



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Velvet approach to de Bruijn graphs

- **Error Correction Algorithm**
  - **Merge sequences that belong together**

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Error Removal

- Kinds of errors
  - Process: sequencing errors
  - Natural: single nucleotide polymorphisms
- Distinguishing the two is hard
  - Previous methods used to use a probabilistic chance of encounter such errors
  - Velvet: use topological cues to find errors and remove them

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Three kinds of Topological Errors

- Tips
  - Errors at the edge of reads
- Bulges
  - Internal read errors or nearby tips connecting
- Erroneous Connections
  - Cloning errors or to distant merging Tips

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Removing Tips

- Tips is a chain of nodes that is disconnected at one end
- Two criterion Length and “Minority count”:
  - Remove read if it is shorter than 2k. Tips longer that 2k represent genuine sequence or an accumulation of errors
  - The tip is removed if a more common path is present in the node with the out going tip

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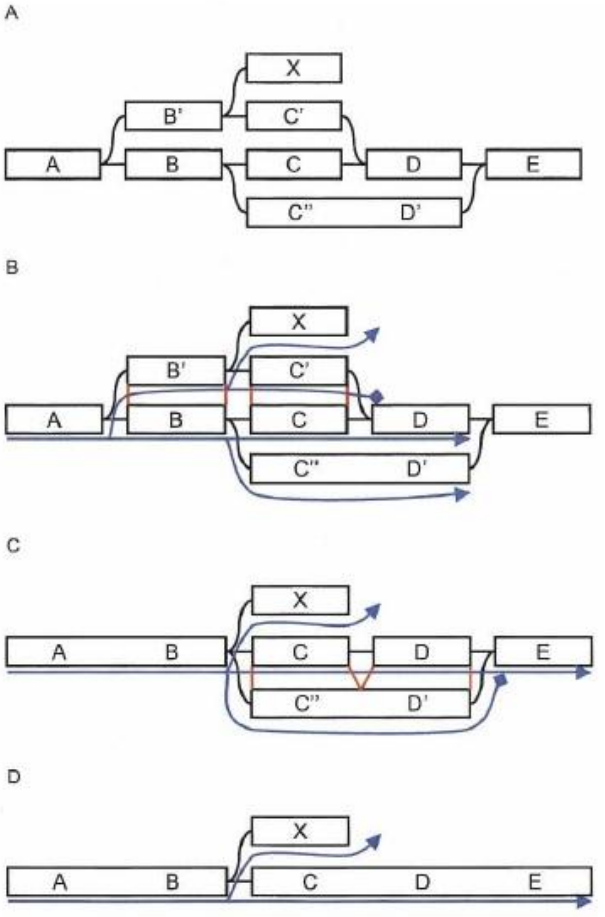
Removing bulges with “Tour Bus” algorithm



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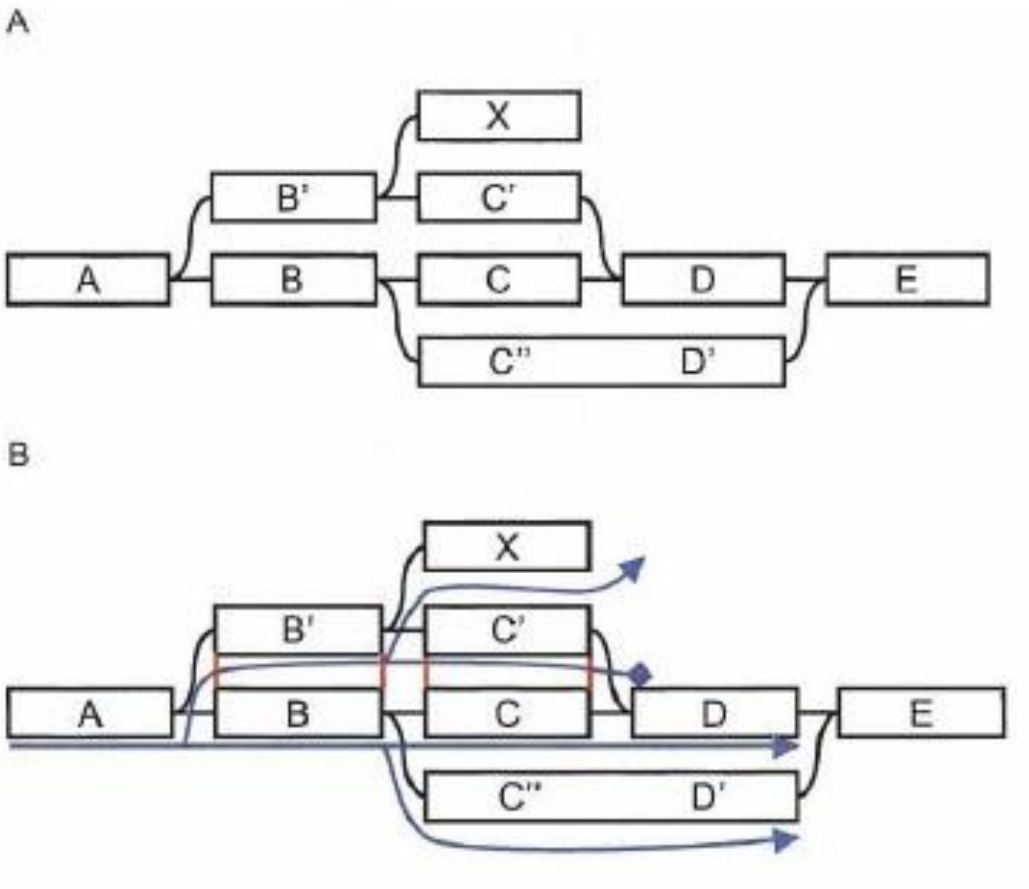
Dijkstra-like Depth First Search

Depth First Search



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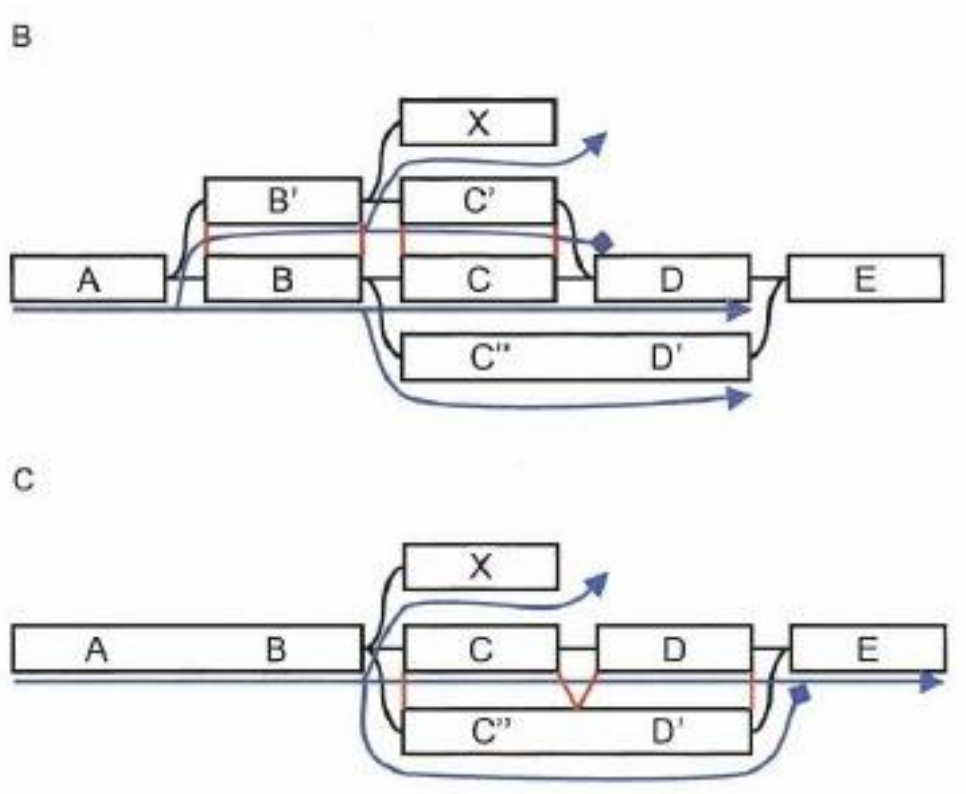
Converge Sequences when you hit a previously visited node



Take out BC and B'C' sequences, align them, if there is a good alignment then merge them with consensus sequence. The longer sequence is always merged into the smaller and connectivity is conserved

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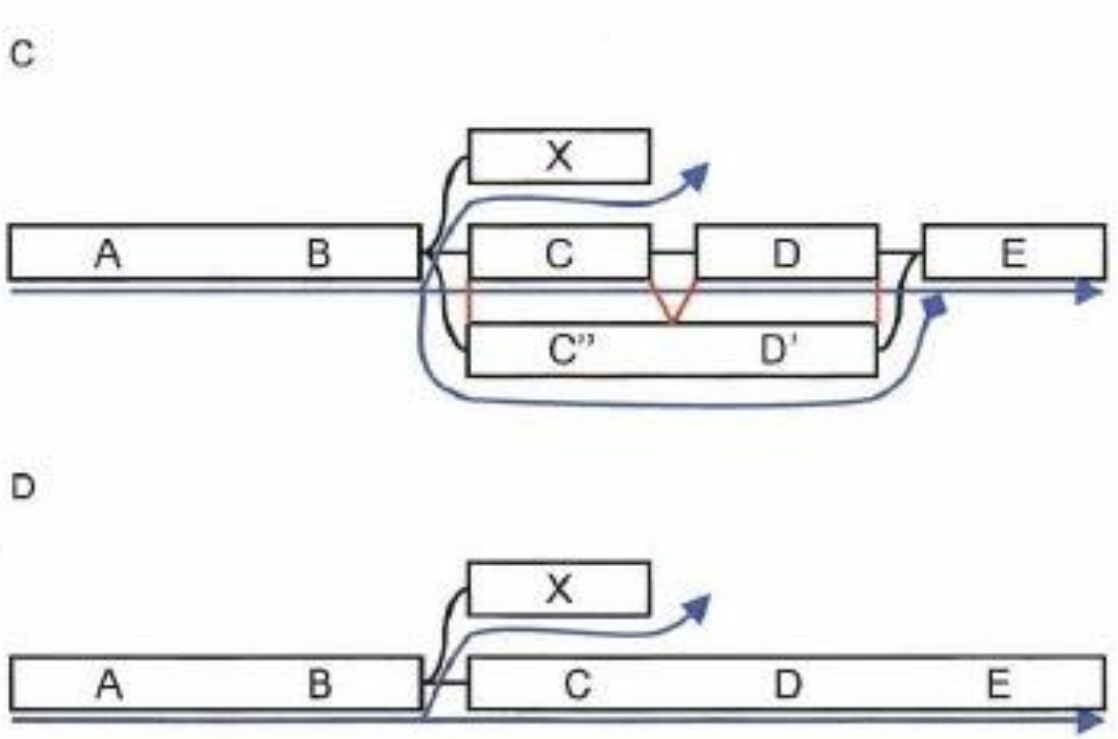
Iterate through to get final graph



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Removing Erroneous Connections

- Removal by a basic coverage cutoff set by the user based on plots of node coverage after the removal of tips and bubbles.

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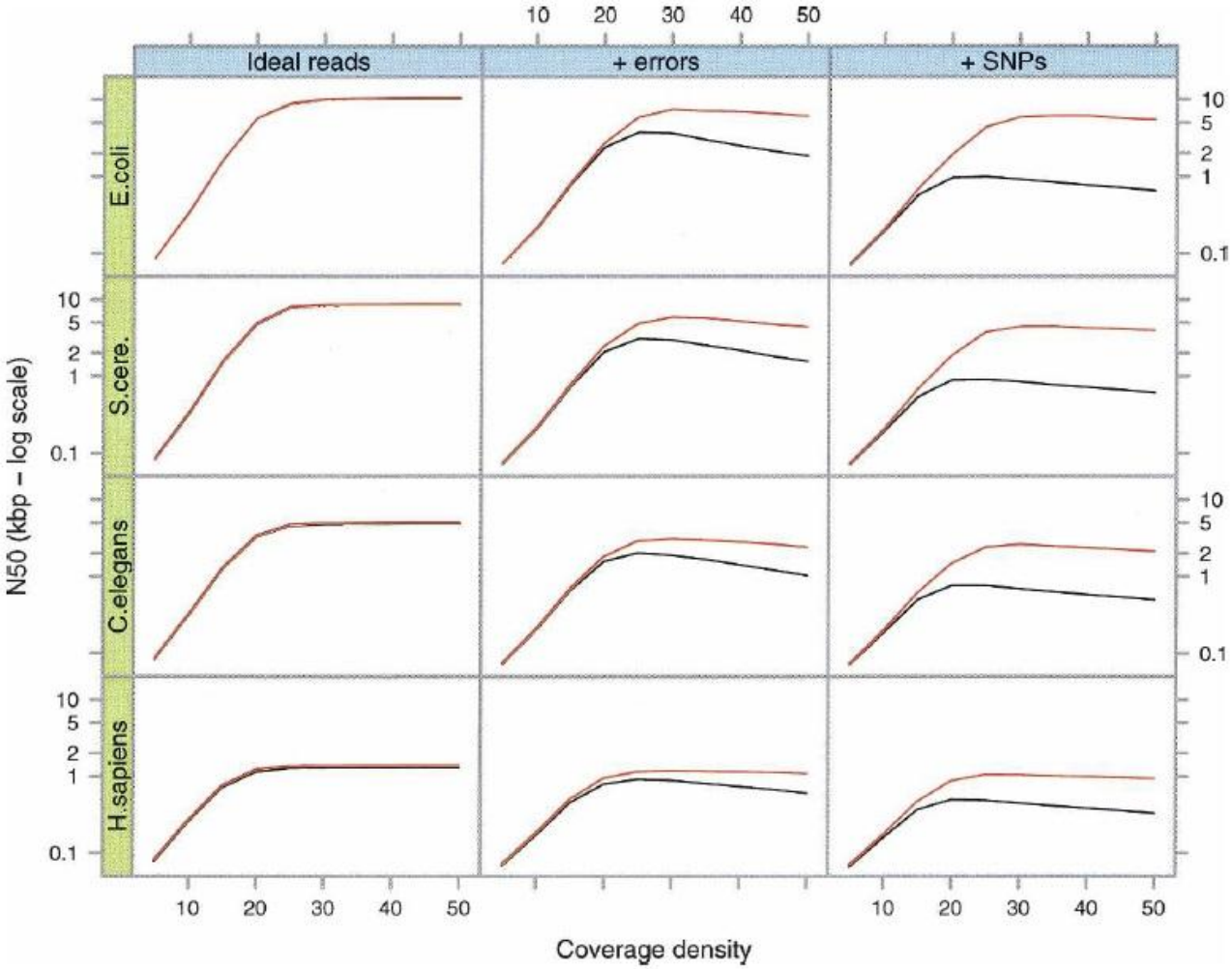
Testing on Simulated Data Results



- 35 bp long
- different coverage values (5x – 50x)
- k=21
- Ideal reads- no errors
- +errors, 1% error rate
- +SNPs, 1% error rate on one strand, 1/500 bp on second strand. Fragments taken from both

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Testing on Experimental Data Results

173,428 bp, 970x coverage, Human. 35 bp long reads, k=31. Built a de Bruijn graph from a known finished sequence for comparison.

Table 1. Efficiency of the Velvet error-correction pipeline on the BAC data set

Step	No. of nodes	N50 (bp)	Maximum length (bp)	Coverage (percent >50 bp)	Coverage (percent >100 bp)
Initial	1,353,791	5	7	0	0
Simplified	945,377	5	80	4.3	0.2
Tips clipped	4898	714	5037	93.5	78.7
Tour Bus	1147	1784	7038	93.4	90.1
Coverage cutoff	685	1958	7038	92.0	90.0
Ideal	620	2130	9045	93.7	91.9

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Testing on Experimental Data Results

2,700,036 bp, 48x coverage*, Strep. suis.* 35 bp long reads, k=31. Built a de Bruijn graph from a known finished sequence for comparison.

Table 2. Efficiency of the Velvet error-correction pipeline on the *Streptococcus* data set

Step	No. of nodes	N50 (bp)	Maximum length (bp)	Coverage (percent >50 bp)	Coverage (percent >100 bp)
Initial	3,621,167	16	16	0	0
Simplified	2,222,845	16	44	0.1	0
Tips clipped	15,267	2195	7949	96.2	95.4
Tour Bus	3303	4334	17,811	96.8	96.4
Coverage cutoff	1496	8564	29,856	96.9	96.5
Ideal	1305	9609	29,856	97.0	96.8

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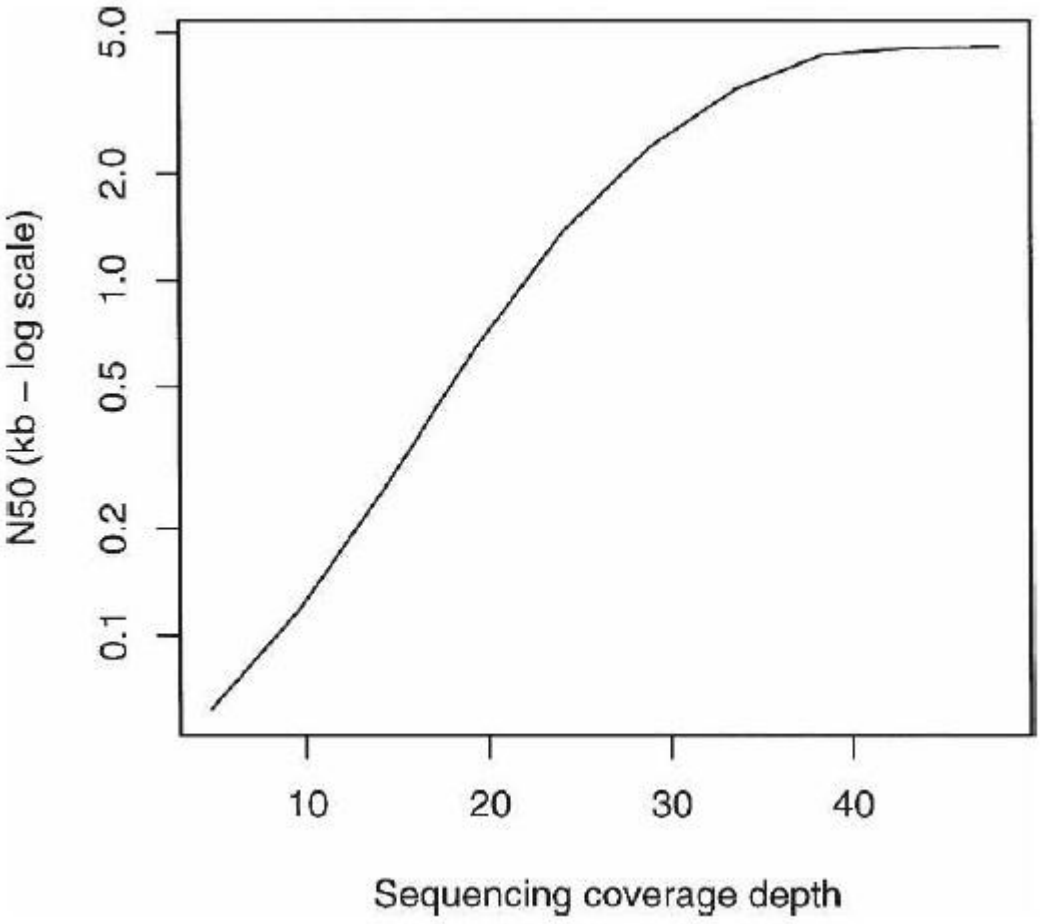
N50 (average sequence length) from Velvet on Strep Data



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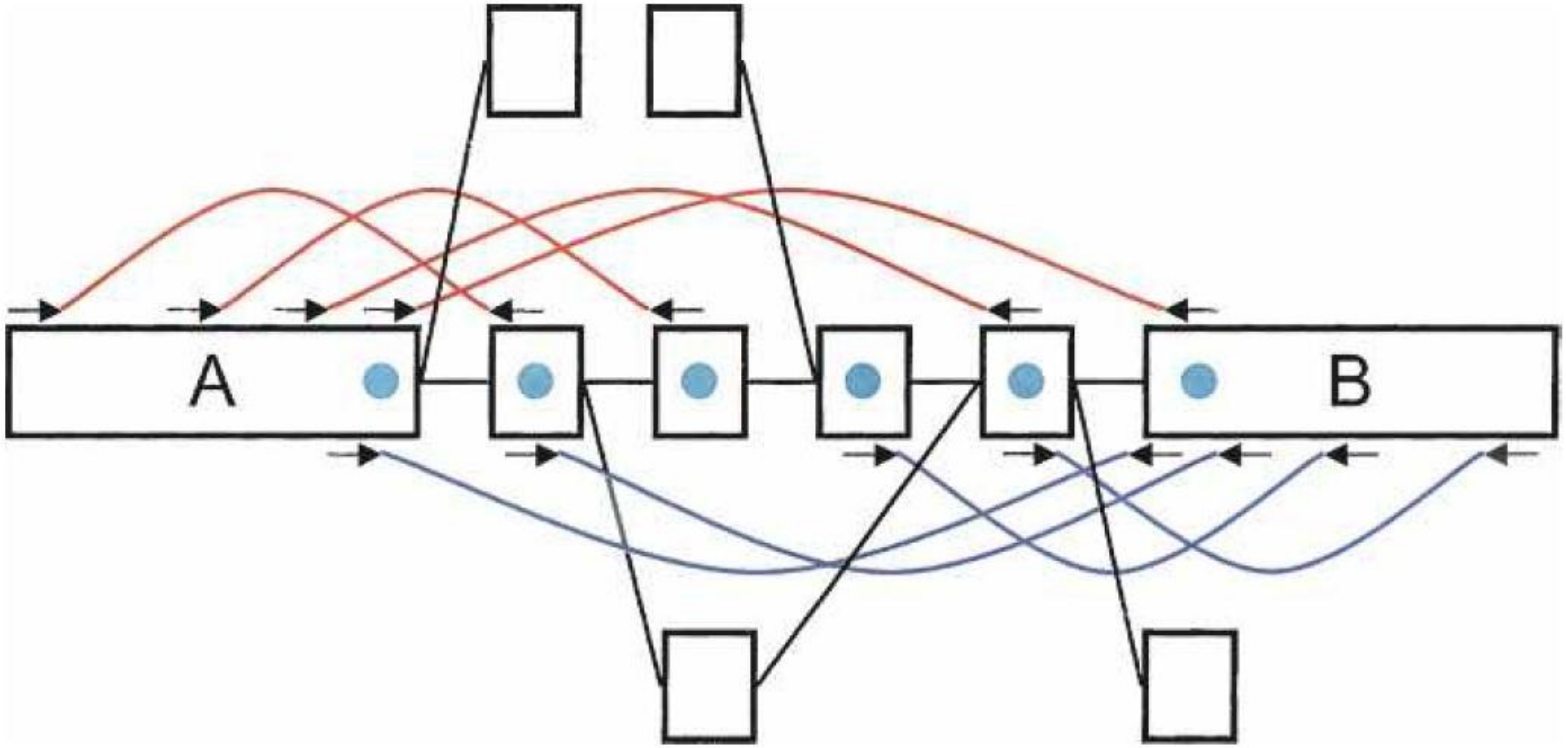
Breadcrumbs: Resolution of repeats



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Breadcrumbs Algorithm

- Using the read pair (from sequencing data), find all long nodes.
  - Ignore the long contigs that pair up with the long nodes
- For each long node, breadcrumb flags all nodes containing the mate reads of the reads in that long node
- Find the simplest path and merge (hopefully)

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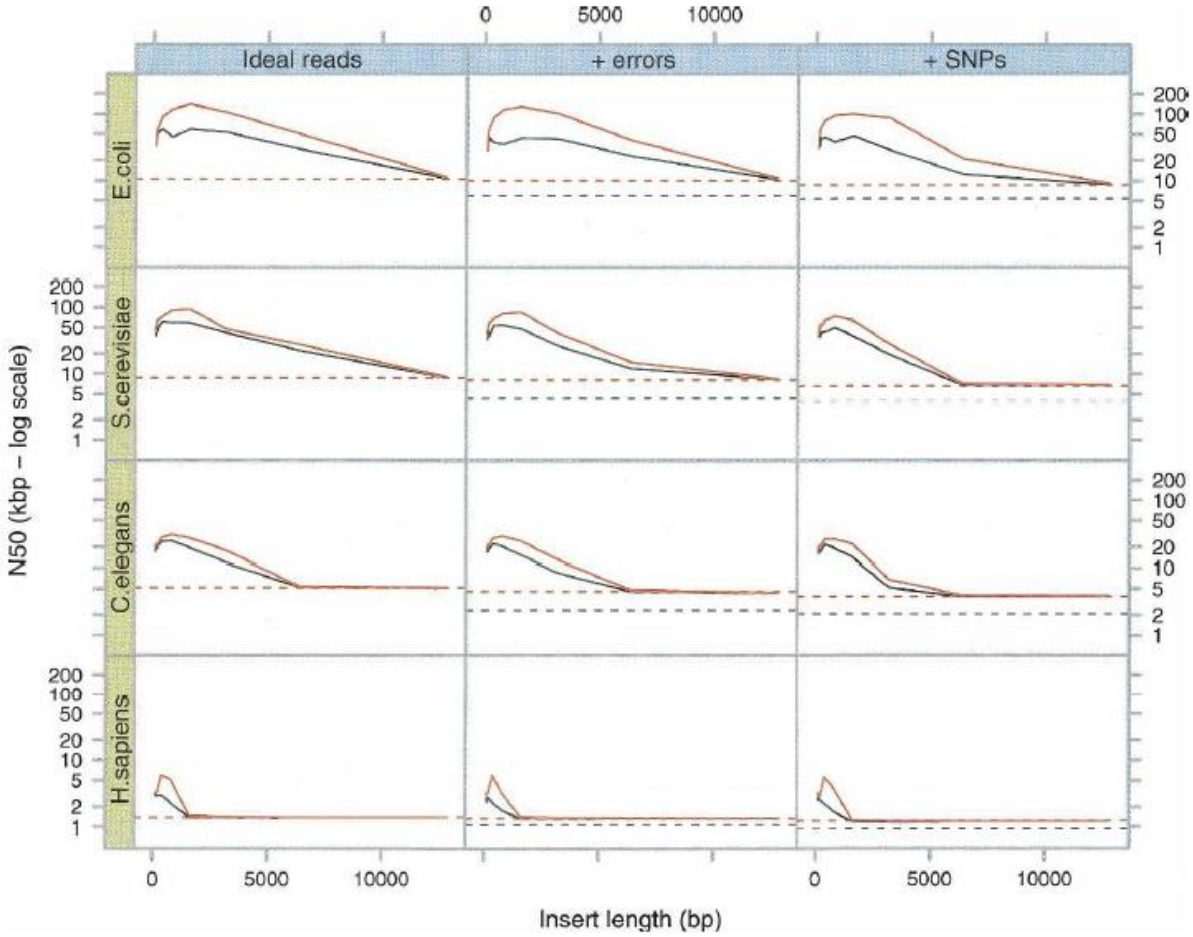
Breadcrumb results



- Same data set as before.
- Horizontal broken lines denote N50 lengths. Black indicates after Tour Bus. Red indicates after 4x coverage cutoff
- Black solid lines indicates N50 after breadcrumbs and Red indicates N50 after super-contigging

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Overview of Velvet

1. Hash k-mers
2. **Construct the graph**
3. Correct for errors
4. Resolve the repeats

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Comparison of short read assemblers on experimental *Strep.*

Table 3. Comparison of short read assemblers on experimental *Streptococcus suis* Solexa reads

Assembler	No. of contigs	N50	Average error rate	Memory	Time	Seq. Cov.
Velvet 0.3	470	8661 bp	0.02%	2.0G	2 min 57 sec	97%
SSAKE 2.0	265	1727 bp	0.20%	1.7G	1 h 47 min	16%
VCAKE 1.0	7675	1137 bp	0.64%	1.8G	4 h 25 min	134%

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Picking k

E(X) = expected number of times a unique k-mer in a genome of length G is observed in a set of n read of length l. C is coverage.

Want to get E(x) of about 10-15. Helps pick k.

$$E(X) = \frac{n(l-k+1)}{G-k+1} \approx \frac{n}{G}(l-k+1) = C\frac{l-k+1}{l}$$

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