Genome Assembly Overview - cont

BCB 5250 Introduction to Bioinformatics II

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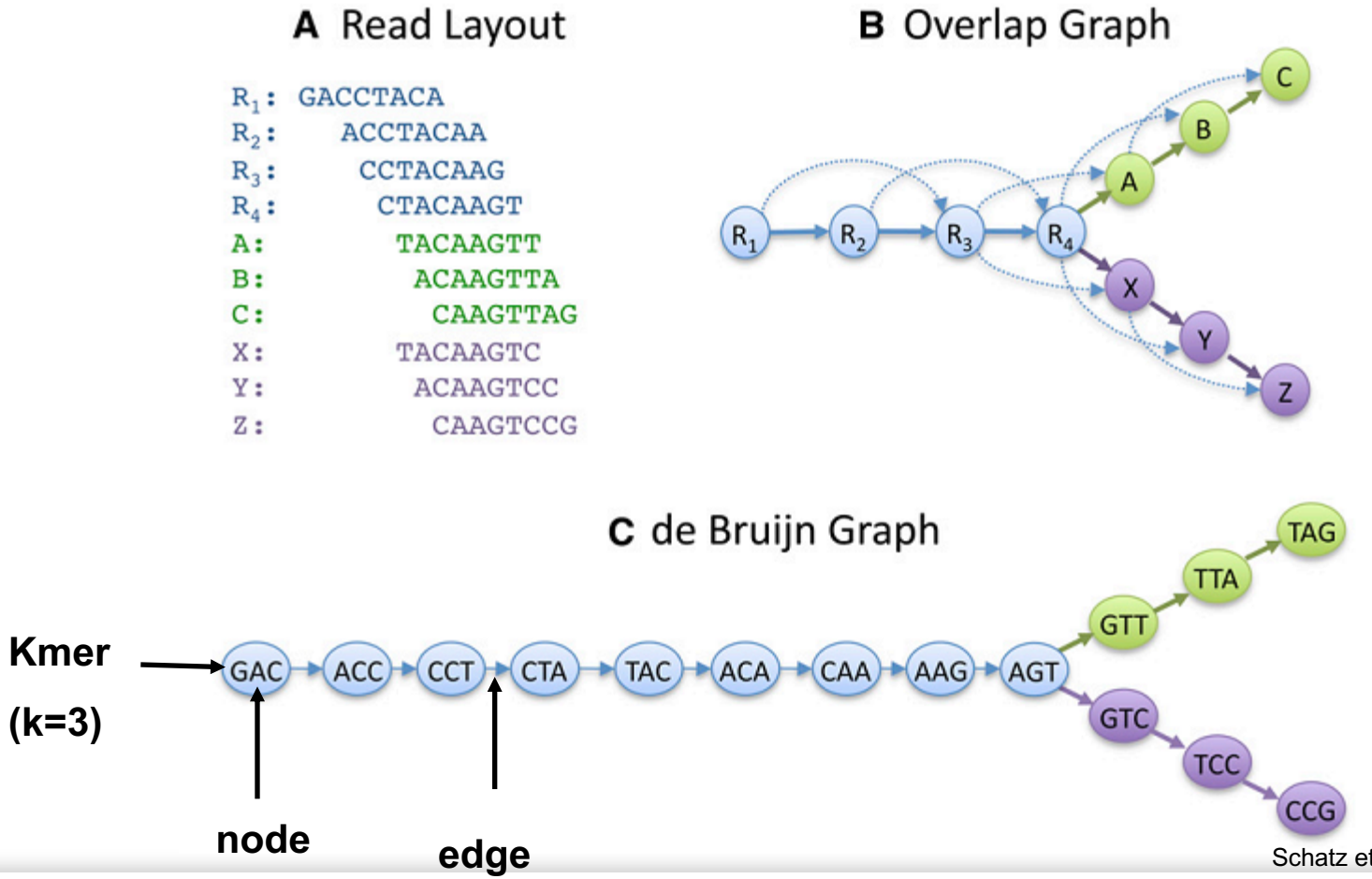
Learning Outcome:

- Learn general knowledge about *de novo* genome assembly.

Assembly outline

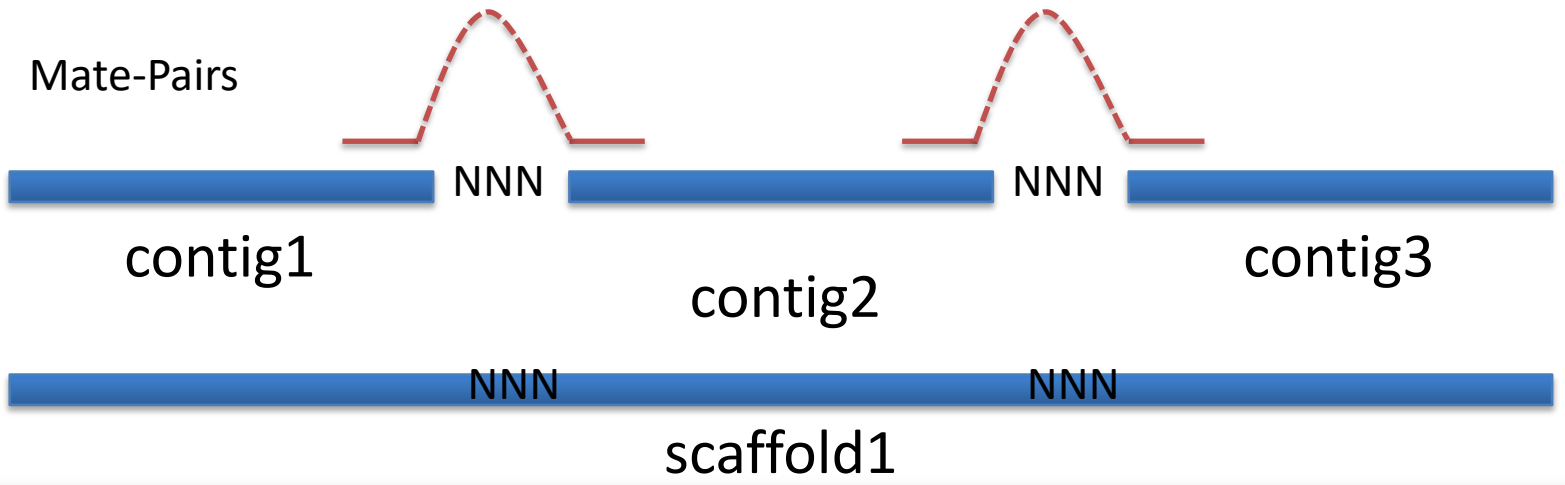
- **K-mer**: a substring of defined length. For the purposes of this talk a substring of the sequence read
- **de Bruijn graph**: a graph representing overlaps between kmers



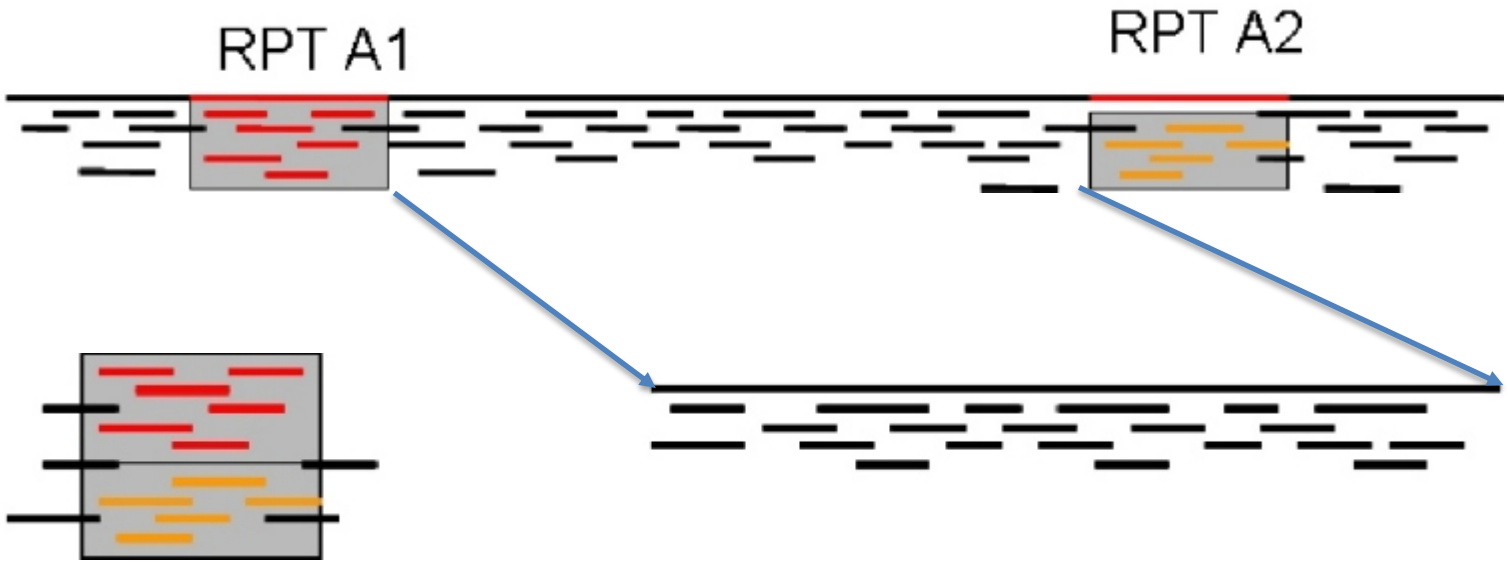
Schatz et al., Genome Res. (2010)

Contig vs Scaffold

- Assembler outputs are contigs.
- A contig (from contiguous) is a set of overlapping DNA segments that together represent a consensus region of DNA.
- A scaffold is composed of contigs and gaps.
  - Gap length can be guessed by incorporating information from paired ends or mate pairs of different insert sizes.
  - Several contigs stitched together with NNNs in between



Repeats often split genome into contigs



Reads from multiple repeats collapse into artifactual contig

Contig derived from unique sequences

How good is my assembly?

- How much total sequence is in the assembly relative to estimated genome size?
- How many pieces, and what is their size distribution?
- Are the contigs assembled correctly?
- Are the scaffolds connected in the right order / orientation?
- How were the repeats handled?
- Are all the genes I expected in the assembly?

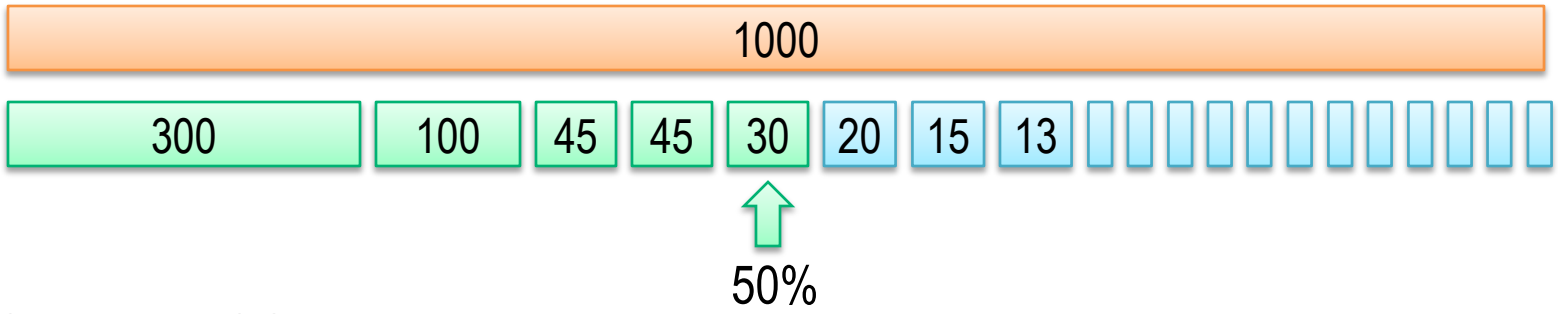
Quality of Assembly? What is N50?

Genome assembly evaluation tool

- GAGE, REARP, QUAST

Def: 50% of the genome is in contigs as large as the N50 value

Example: 1Mbp genome



N50 size = 30 kbp

(300k + 100k + 45k + 45k + 30k = 520k >= 500kbp)

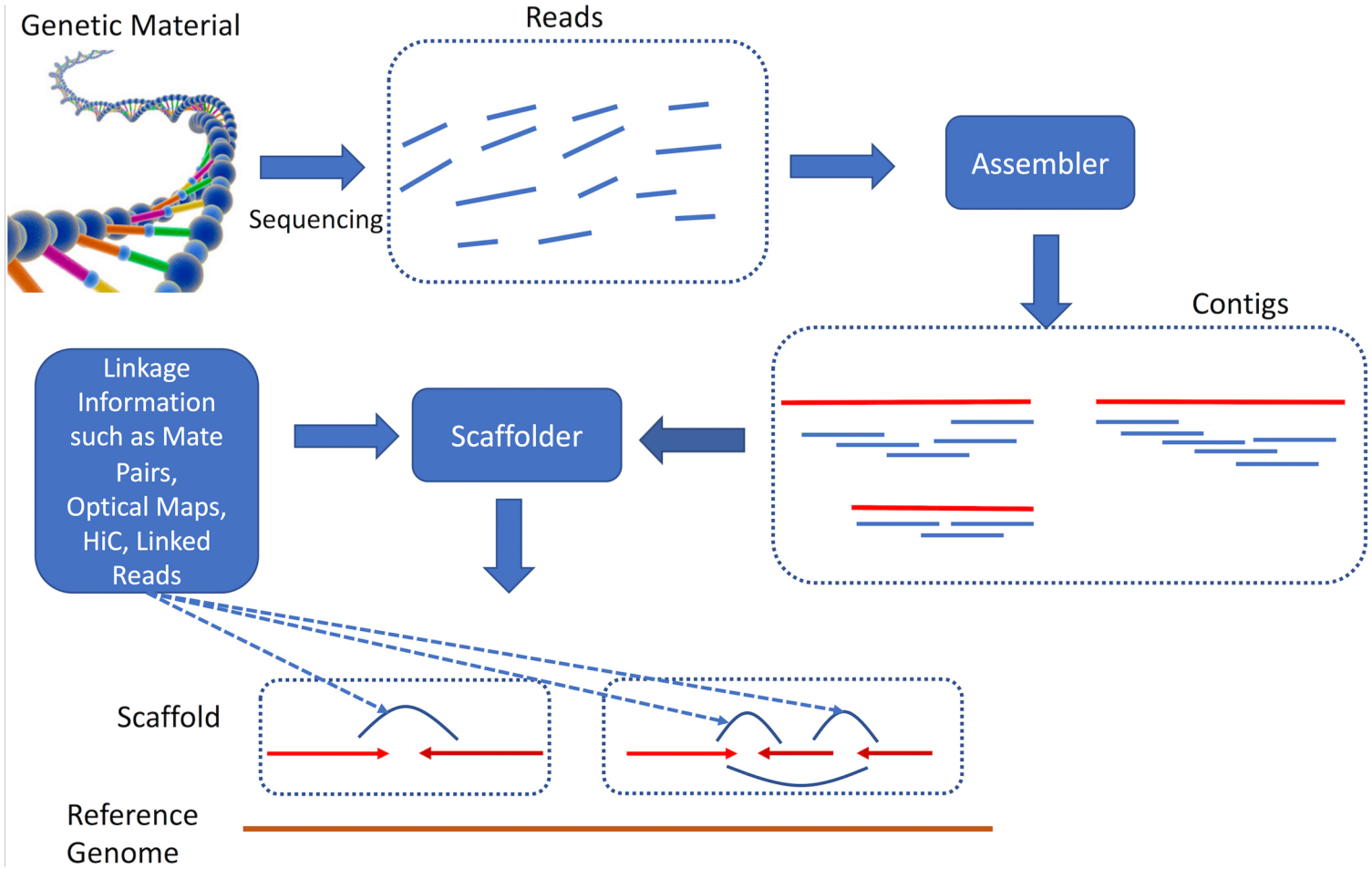
Quality of Assembly

- Example

Our Result

# contigs	71,326	13,507
Largest contig	46,067	1,038,831
N50	5,508	**130,100**
GC (%)	33.26	33.55
# N's per 100 kbp	0	5099.96
Size(MB)		**341**

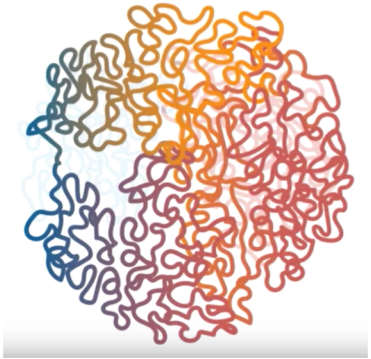
Overview of the genome assembly process.

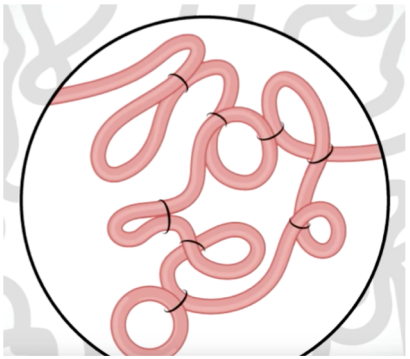


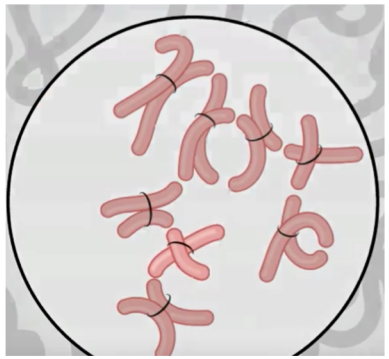
https://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1006994

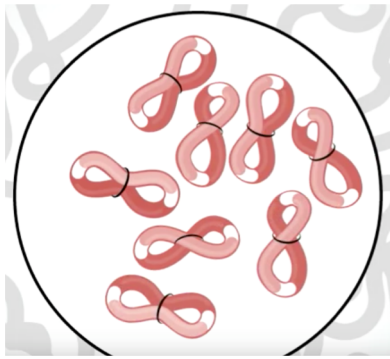
HI-C

https://www.youtube.com/watch?v=-MxEw3IXUWU



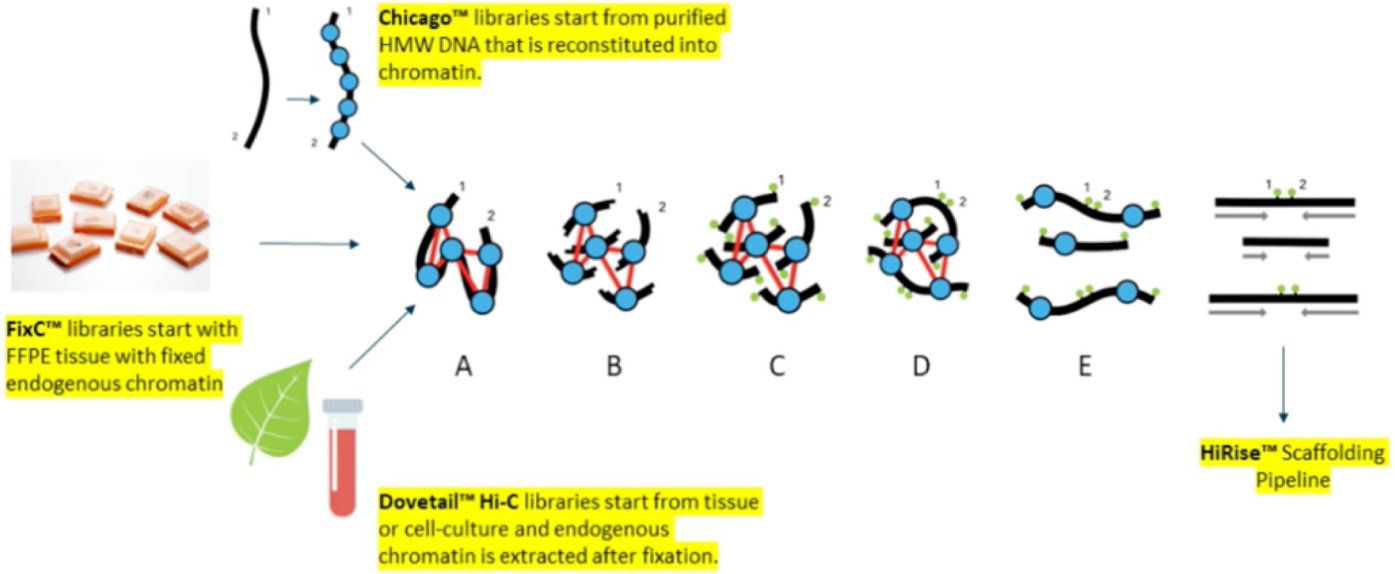






Proximity Ligation Approaches

Dovetail GENOMICS



Merge Paired-End Reads

Bioinformatics. 2014 Mar 1; 30(5): 614–620.
Published online 2013 Oct 18. doi: 10.1093/bioinformatics/btt593
PMCID: PMC3933873
PMID: 24142950

PEAR: a fast and accurate Illumina Paired-End reAd mergeR

Jiajie Zhang,[1,2,3] Kassian Kobert,[1] Tomáš Flouri,[1,*] and Alexandros Stamatakis[1,4]

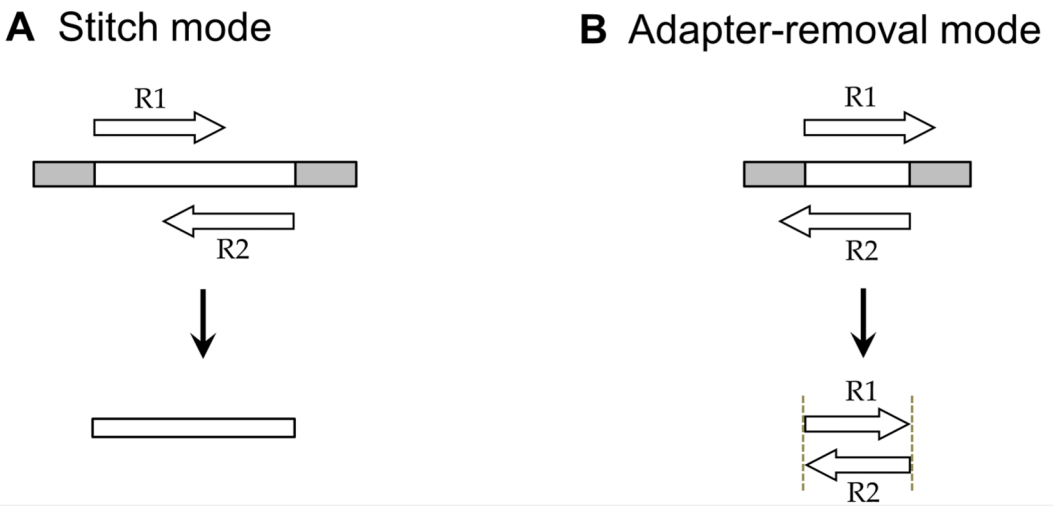
Bioinformatics

Methodology article | Open Access | Published: 20 December 2018

NGmerge: merging paired-end reads via novel empirically-derived models of sequencing errors

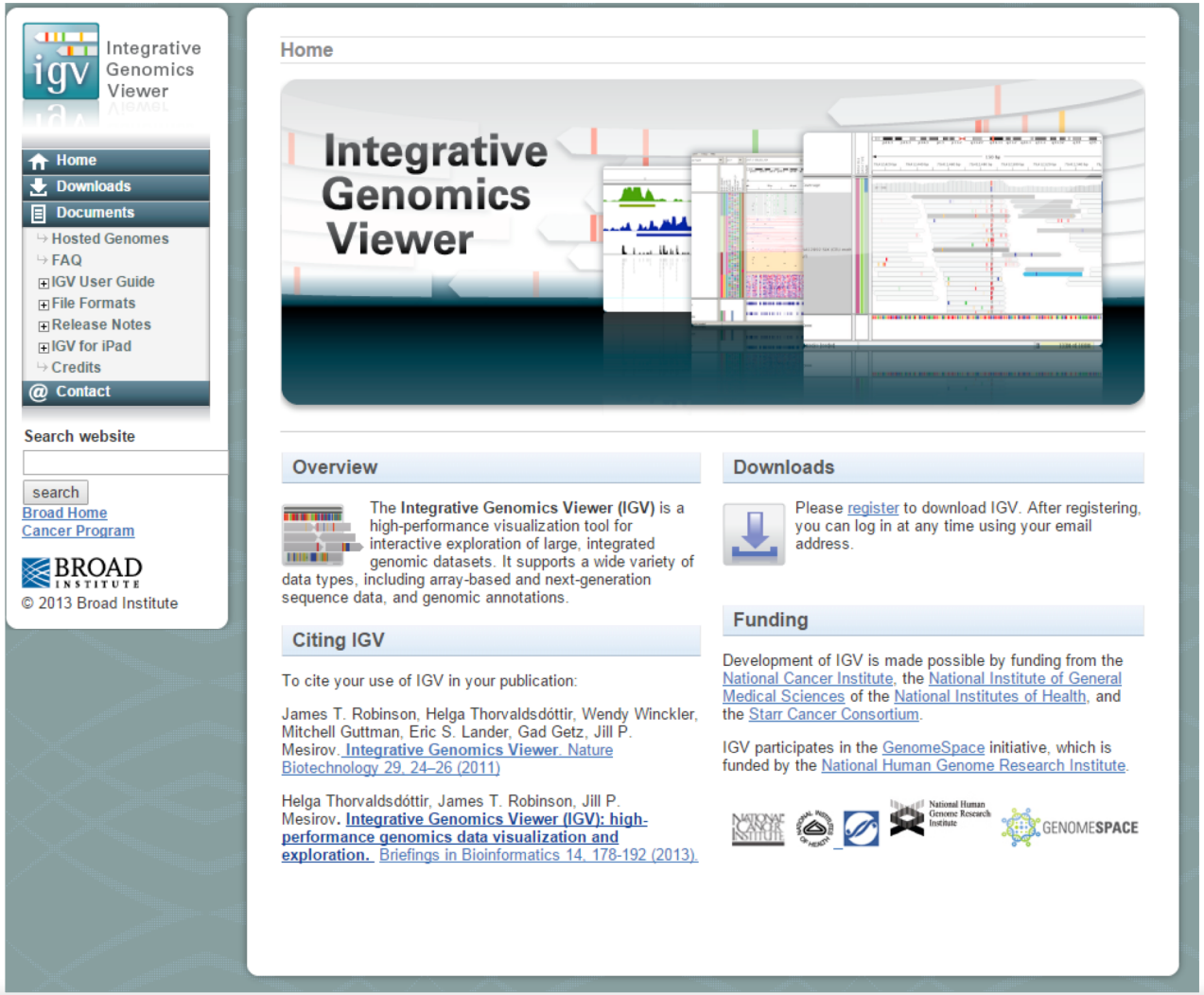
John M. Gaspar

BMC Bioinformatics 19, Article number: 536 (2018) | Cite this article



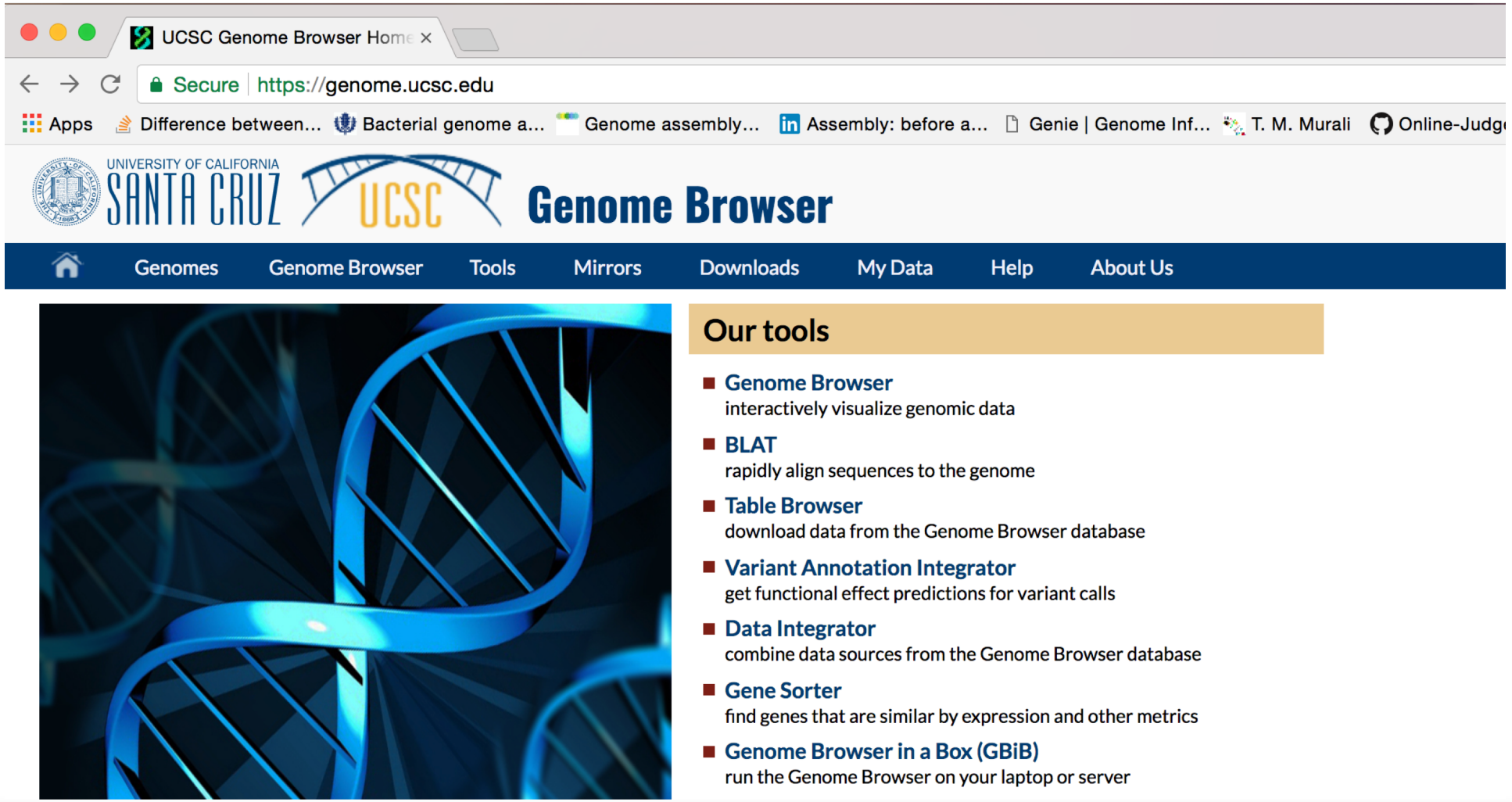
Genome Browser: IGV

- https://www.broadinstitute.org/igv/



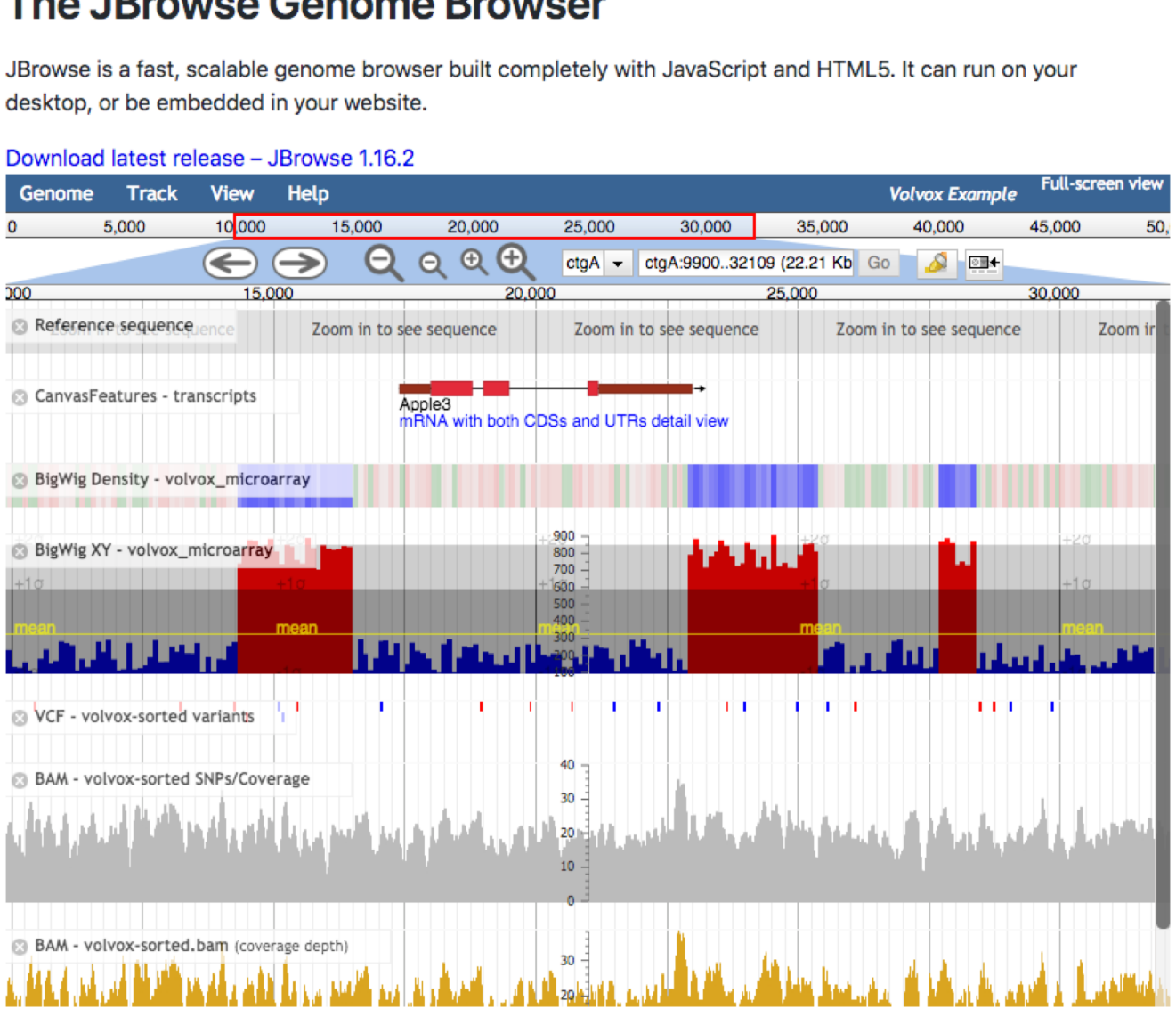
Web: UCSC Genome Browser

- https://genome.ucsc.edu/



Web: JBrose

- https://jbrowse.org/



Example:

- http://www.yeastss.org/

YeasTSS

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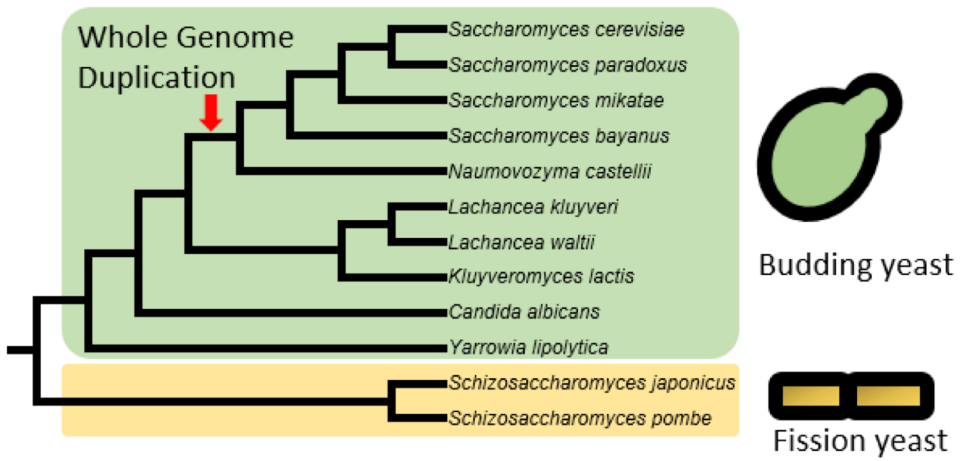
Welcome to the YeasTSS Atlas



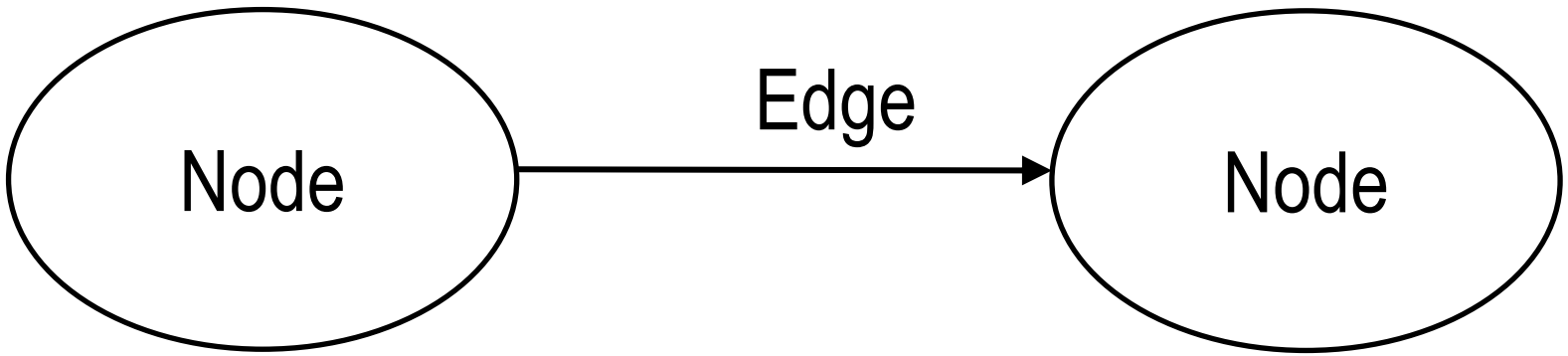
Click species name to visualize its TSS maps in a new window.

The YeasTSS Atlas (Yeast Transcription Start Site Atlas) is a primary depository of yeast transcription initiation sites (TSS) data generated by Dr. Zhenguo Lin's lab at Saint Louis University. These data are valuable for precisely determining the 5'boundary and the 5'untranslated region (5'UTR) of protein coding genes, improving genome annotation quality, and predictions of novel genes, core promoter elements, transcription factor binding sites, and other motifs associated with transcription and inferring gene regulatory network.

More Details

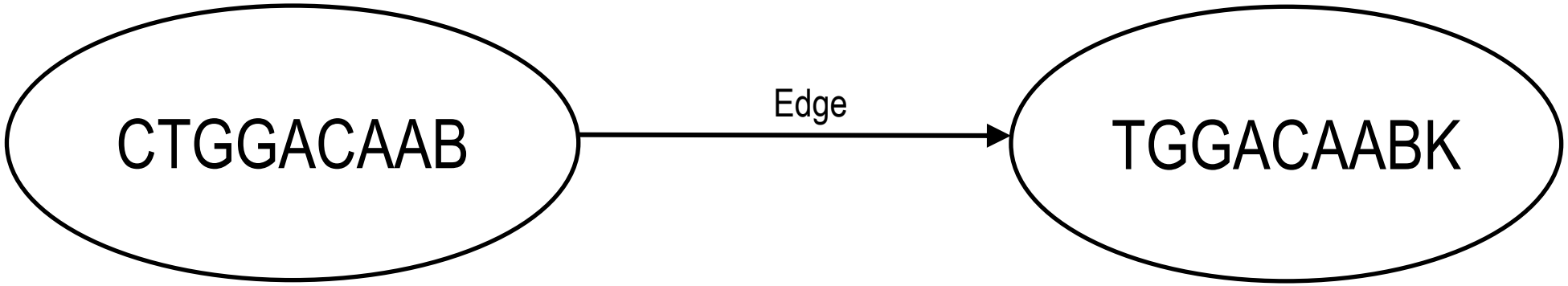
Overlap Graph

- Directed Graph



Overlap Graph

- Each node is a read



- Draw edge A → B when suffix of A overlaps prefix of B

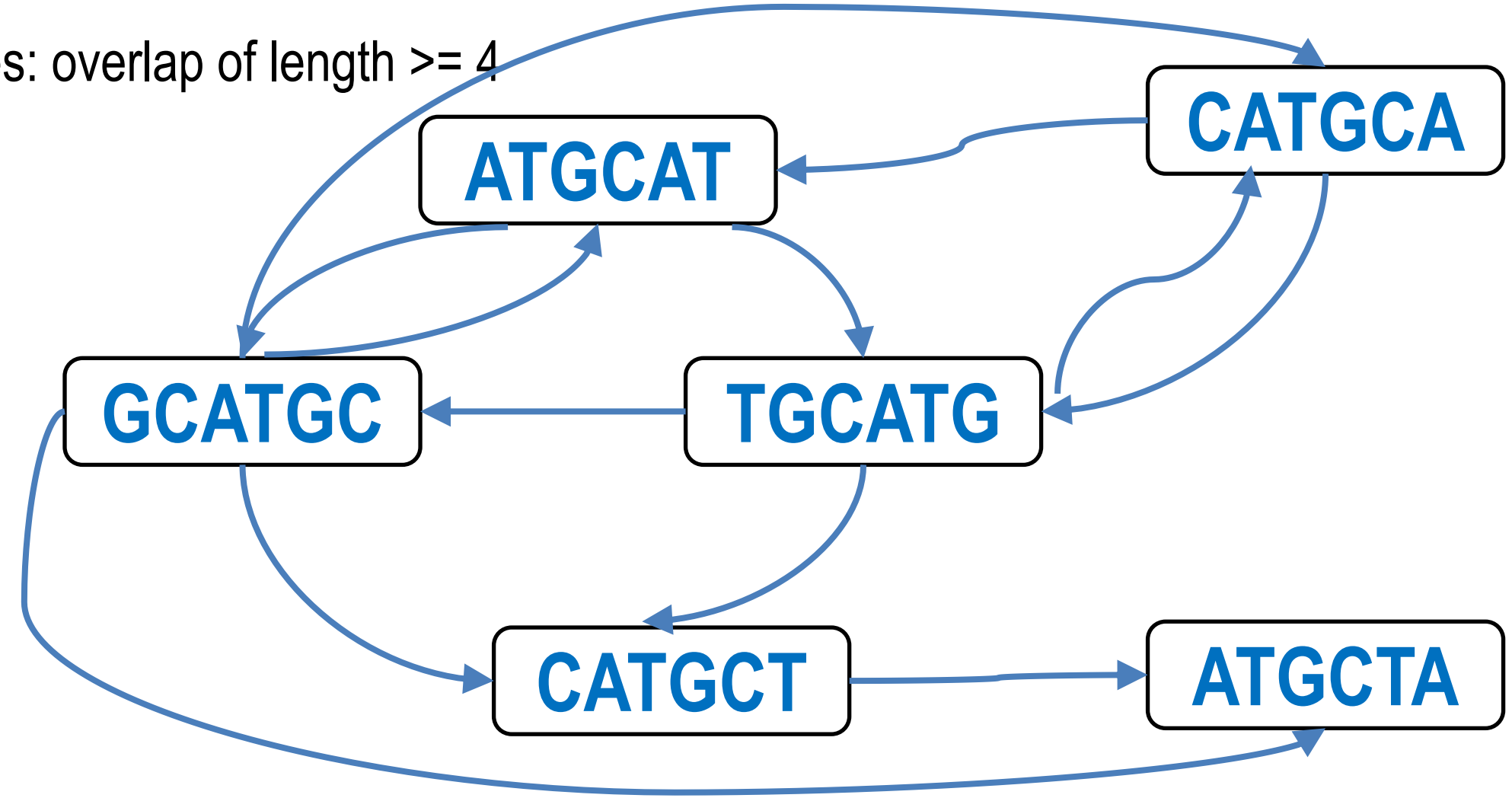
CTGGACAAB
↓
TGGACAABK

Overlap Graph

- Genome: Nodes: **CATGCATGCTA**
- Reads (Nodes): all 6-mers from **CATGCATGCTA**
  - **CATGCA**
  - **ATGCAT**
  - **TGCATG**
  - **GCATGC**
  - **CATGCT**
  - **ATGCTA**
- Edges: overlap of length >= 4

Overlap Graph

- Nodes: all 6-mers from **CATGCATGCTA**
- Edges: overlap of length >= 4



Overlap Graph

- Nodes: all 6-mers from **CATGCATGCTA**
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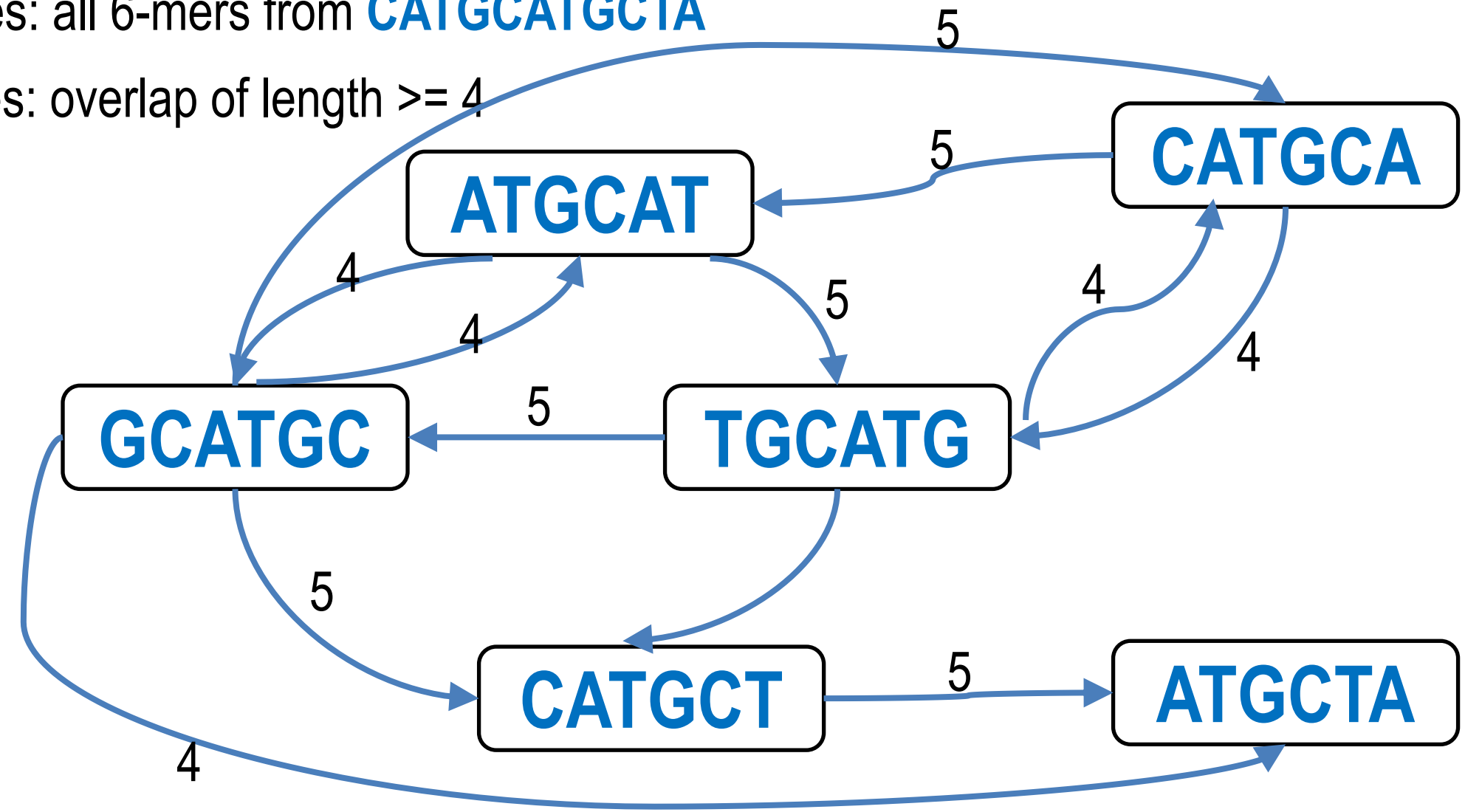

Overlap Graph

- Nodes: all 6-mers from **CATGCATGCTA**
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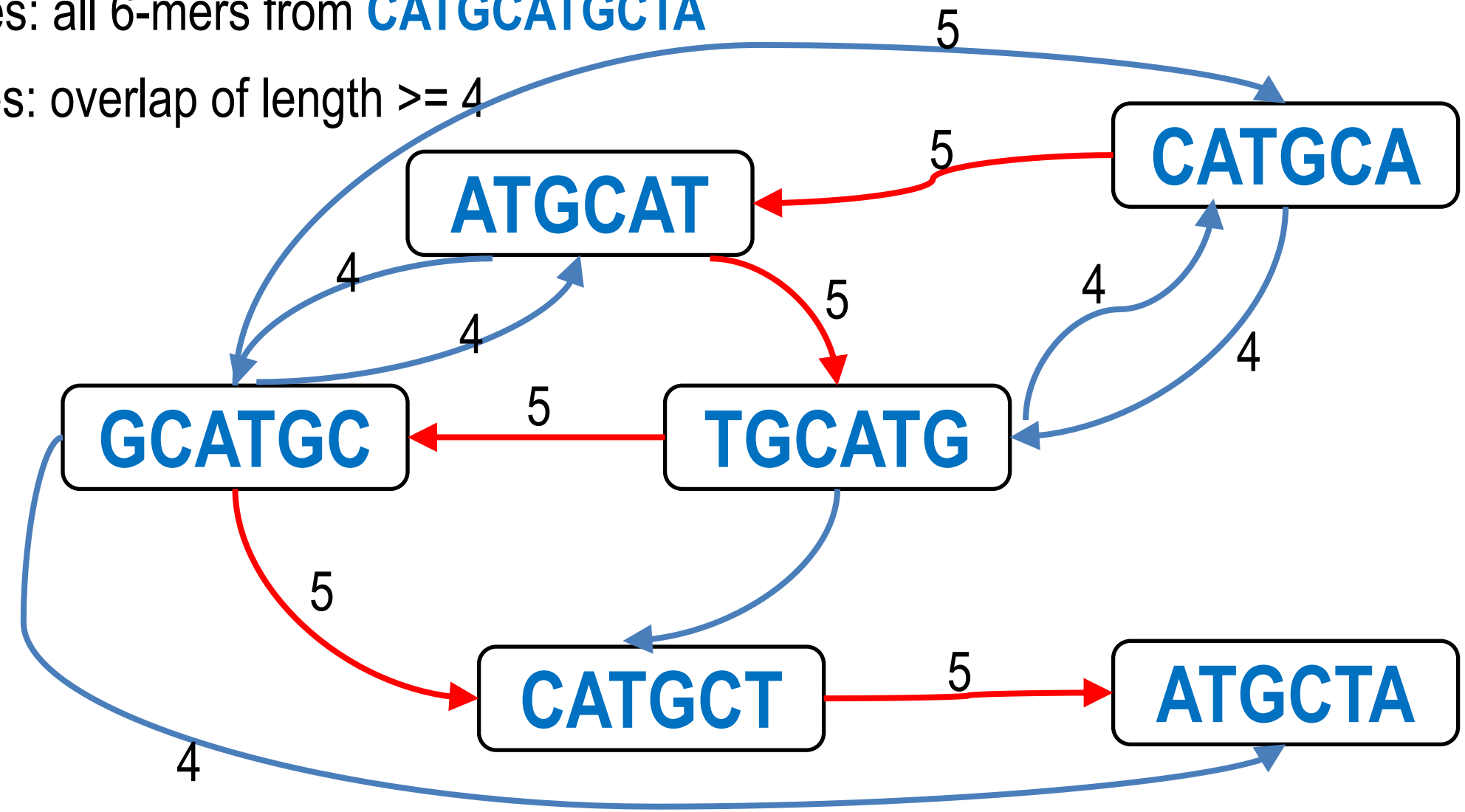
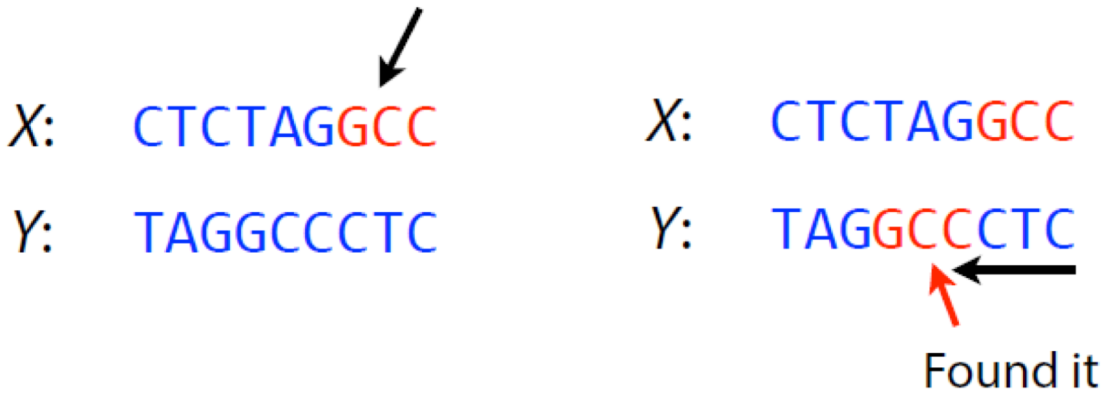


Finding Overlaps

Can we be less naive than this?

Say $l = 3$

Look for this in *Y*, going right-to-left



Extend to left; in this case, we confirm that a length-6 prefix of *Y* matches a suffix of *X*

We're doing this for *every pair* of input strings

Allow Mismatches?

What if we want to allow mismatches and gaps in the overlap?

I.e. How do we find the best *alignment* of a suffix of *X* to a prefix of *Y*?

```
X: CTCGGCCCTAGG
       ||| |||||
Y:     GGCTCTAGGCCC
```

Dynamic programming

But we must frame the problem such that only backtraces involving a suffix of *X* and a prefix of *Y* are allowed

Finding overlaps

- What if we want to allow mismatches and gaps in the overlap?
- I.e. How do we "nd the best alignment Y: of a suffix of X to a pre"x of Y?:

Dynamic programming

$$D[i,j] = \min \begin{cases} D[i-1,j] + s(x[i-1], -) \\ D[i,j-1] + s(-, y[j-1]) \\ D[i-1,j-1] + s(x[i-1], y[j-1]) \end{cases}$$

$s(a,b)$	A	C	G	T	-
A	0	4	2	4	8
C	4	0	4	2	8
G	2	4	0	4	8
T	4	2	4	0	8
-	8	8	8	8	

```
X: CTCGGCCCTAGG
   ||| |||||
Y:    GGCTCTAGGCCC
```

How to initialize first row & column so suffix of *X* aligns to prefix of *Y*?

First column gets 0s
(any suffix of *X* is possible)

First row gets ∞s
(must be a prefix of *Y*)

Backtrace from last row

		-	G	G	C	T	C	T	A	G	G	C	C	C
	-	0	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞
	C	0	4	12	20									
	T	0	4	8	14									
	C	0	4	8	8									
	G	0		4	12									
	G	0	0		8	16	16	24	26	30	36	44	52	60
X	C	0	4	4		8	16	18	26	30	34	36	44	52
	C	0	4	8	4		8	16	22	30	34	34	36	44
	C	0	4	8	8	6		10	18	26	34	34	34	36
	T	0	4	8	10	8	8		10	18	26	34	36	36
	A	0	2	6	12	14	12	10		10	18	26	34	40
	G	0	0	2	10	16	18	16	10		10	18	26	34
	G	0	0	0	6	14	20	22	18	10		10	18	26


```
X: CTCGGCCCTAGG
   ||| |||||
Y:    GGCTCTAGGCCC
```

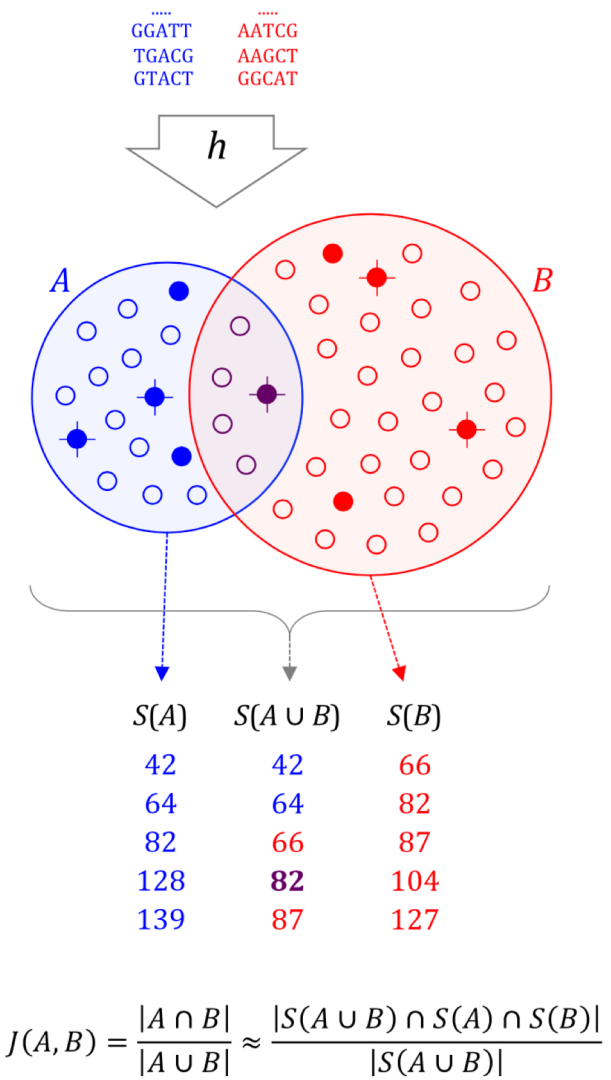
New Techniques

MinHash and mash:

Computing Jaccard distance (=> Average Nucleotide Identity) between any
two genomic samples, very very quickly.

A ∪ B = Union = combined set of A and B

A ∩ B = intersection = set of shared objects

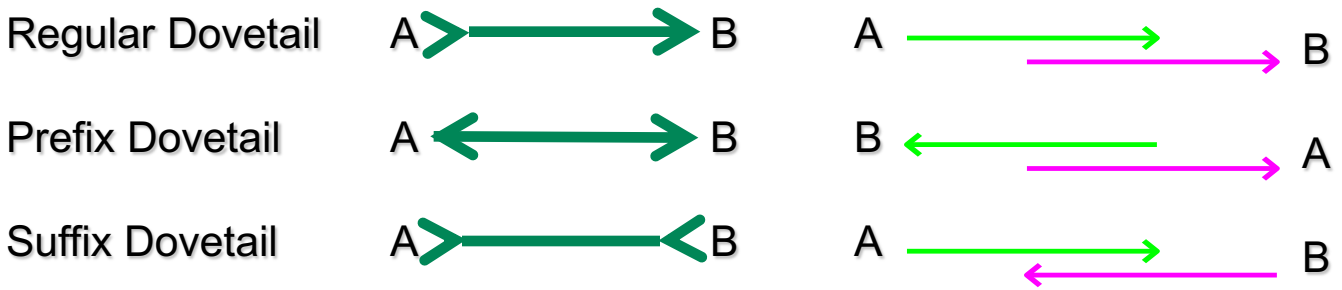


$$J(A,B) = \frac{|A \cap B|}{|A \cup B|} \approx \frac{|S(A \cup B) \cap S(A) \cap S(B)|}{|S(A \cup B)|}$$

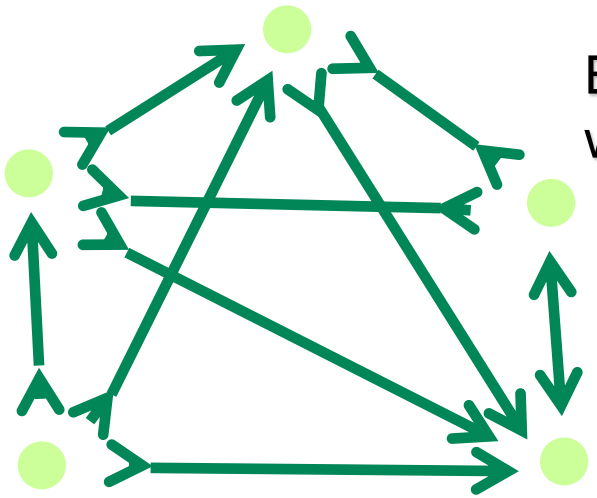
Overlap Graph

Bi-directed Graph Format

Edge Types:



E.G.:



Edges are annotated with deltas of overlaps

Layout

- Overlap graph is big and messy. Contigs don't "pop out" at us.
- Anything redundant about this part of the overlap graph?
- Bundle stretches of the overlap graph into unitigs

Layout

Read r_1 : AGCTAAGCATTTACGATAGCCGATAGCTAAATTAC
Read r_2 : CGTAATTTAGCTATCGGCTATCGTAAATGCTTAGC
Read r_3 : AACGTAATTTAGCTATCGGCTATCGTAAATGCTTA
Read r_4 : GCATTTACGATAGCCGATAGCTAAATTACGTTATA
Read r_5 : GTATAACGTAATTTAGCTATCGGCTATCGTAAATG
Read r_6 : ATTTACGATAGCCGATAGCTAAATTACGTTATACT
Read r_7 : TTTACGATAGCCGATAGCTAAATTACGTTATACTC
Read r_8 : ATATAACGTAATTTAGCTATCGGCTATCGTAAATG
Read r_9 : ATTTACGATAGCCGATAGCTAAATTACGTTATATA
Read r_{10} : CTATATAACGTAATTTAGCTATCGGCTATCGTAAA

Set of given reads

Read r_1 : AGCTAAGCATTTACGATAGCCGATAGCTAAATTAC
Read $\bar{r}_2$: GCTAAGCATTTACGATAGCCGATAGCTAAATTACG
Read $\bar{r}_3$: TAAGCATTTACGATAGCCGATAGCTAAATTACGTT
Read r_4 : GCATTTACGATAGCCGATAGCTAAATTACGTTATA
Read $\bar{r}_5$: CATTTACGATAGCCGATAGCTAAATTACGTTATAC
Read r_6 : ATTTACGATAGCCGATAGCTAAATTACGTTATACT
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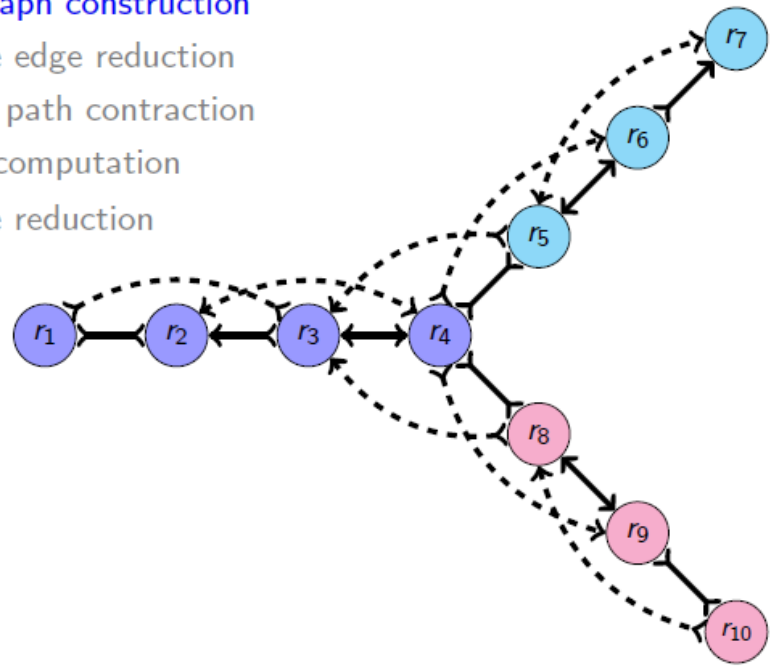
Overlapping reads

Overlap graph construction
Transitive edge reduction
Composite path contraction
Flow computation
Tree reduction



```
constant FUZZ ← 10
1.  for v ∈ V do
2.   { mark[v] ← vacant
3.     for v → w ∈ E do
4.         reduce[v → w] ← false
     }

5.  for v ∈ V do
6.   { for v → w ∈ E do
7.         mark[w] ← inplay
8.     longest ← max_w len(v → w) + FUZZ
9.     for v → w ∈ E in order of length do
10.        if mark[w] = inplay then
11.            for w → x ∈ E in order of length and
12.                len(w → x) + len(v → w) ≤ longest do
13.                if mark[x] = inplay then
14.                    mark[x] ← eliminated

15.    for v → w ∈ E in order of length do
16.        for w → x ∈ E in order of length and
17.            (len(w → x) < FUZZ or
                   w → x is the smallest edge out of w) do
18.            if mark[x] = inplay then
19.                mark[x] ← eliminated

20.    for v → w ∈ E do
21.     { if mark[w] = eliminated then
22.           reduce[v → w] ← true
23.       mark[w] ← vacant
        }
     }
```

Fig. 4. Transitive reduction algorithm.

```
ATAGCTAAATTAC
CGTAAATGCTTAGC
TCGTAAATGCTTA
AAATTACGTTATA
GCTATCGTAAATG
ATTACGTTATACT
TTACGTTATACTC
GCTATCGTAAATG
ATTACGTTATATA
CGGCTATCGTAAA
```

```
ATAGCTAAATTAC
ATAGCTAAATTACG
ATAGCTAAATTACGTT
ATAGCTAAATTACGTTATA
ATAGCTAAATTACGTTATAC
```

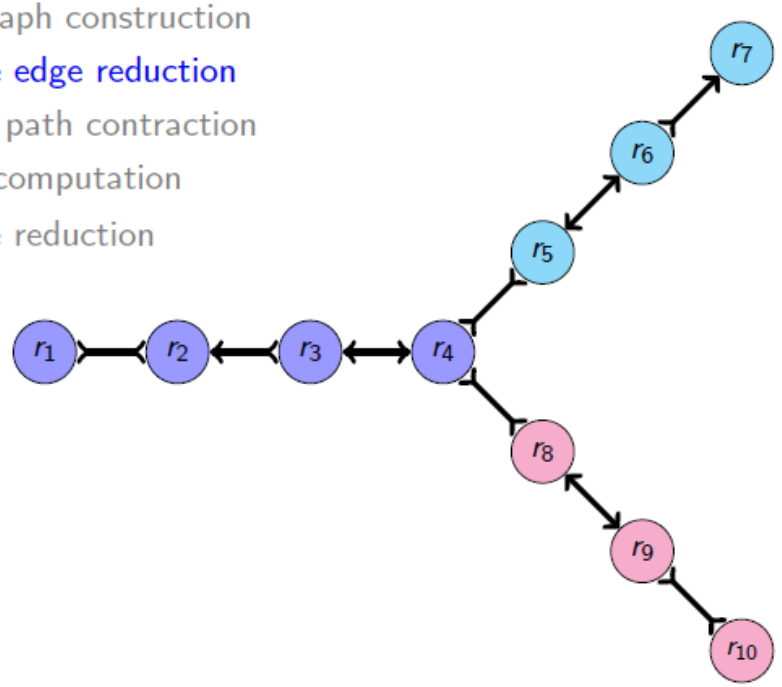
Overlap graph construction

Transitive edge reduction

Composite path contraction

Flow computation

Tree reduction



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doi:10.1093/bioinformatics/bti1114

Genes and Genomes

The fragment assembly string graph

Eugene W. Myers

Department of Computer Science, University of California, Berkeley, CA, USA

Layout

Read r_1 : AGCTAAGCATTTACGATAGCCGATAGCTAAATTAC
Read r_2 : CGTAATTTAGCTATCGGCTATCGTAAATGCTTAGC
Read r_3 : AACGTAATTTAGCTATCGGCTATCGTAAATGCTTA
Read r_4 : GCATTTACGATAGCCGATAGCTAAATTACGTTATA
Read r_5 : GTATAACGTAATTTAGCTATCGGCTATCGTAAATG
Read r_6 : ATTTACGATAGCCGATAGCTAAATTACGTTATACT
Read r_7 : TTTACGATAGCCGATAGCTAAATTACGTTATACTC
Read r_8 : ATATAACGTAATTTAGCTATCGGCTATCGTAAATG
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Set of given reads

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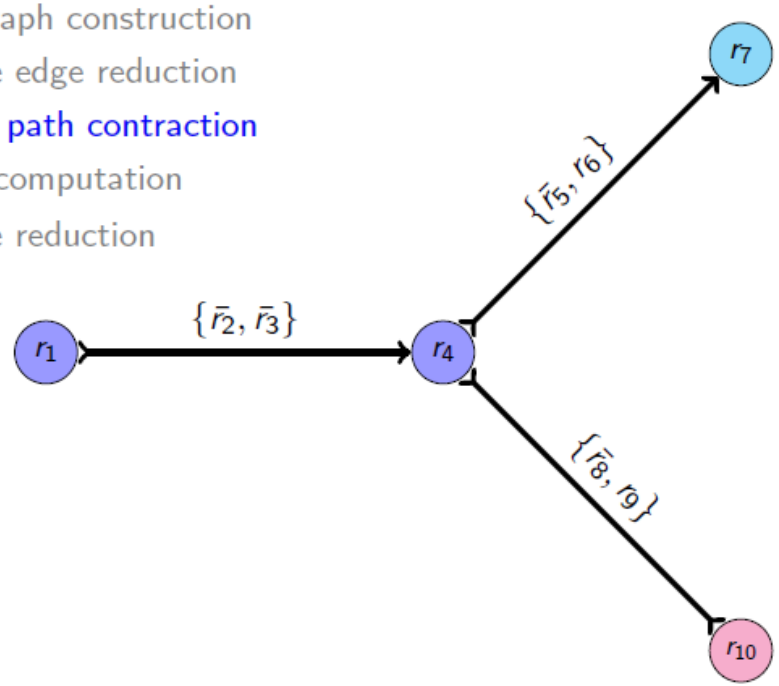
Overlapping reads

Overlap graph construction
Transitive edge reduction
Composite path contraction
Flow computation
Tree reduction



Layout

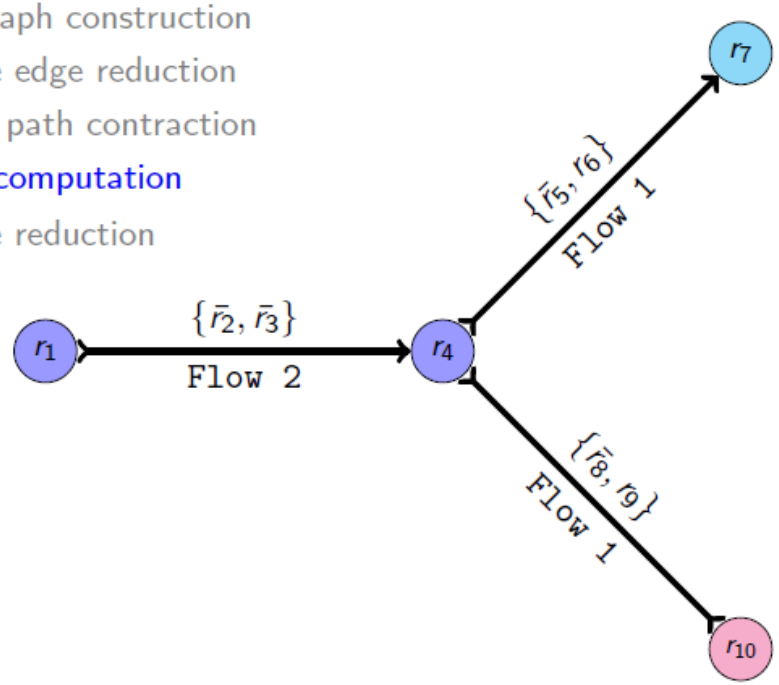
Read r_1 : AGCTAAGCATTTACGATAGCCGATAGCTAAATTAC
Read r_2 : CGTAATTTAGCTATCGGCTATCGTAAATGCTTAGC
Read r_3 : AACGTAATTTAGCTATCGGCTATCGTAAATGCTTA
Read r_4 : GCATTTACGATAGCCGATAGCTAAATTACGTTATA
Read r_5 : GTATAACGTAATTTAGCTATCGGCTATCGTAAATG
Read r_6 : ATTTACGATAGCCGATAGCTAAATTACGTTATACT
Read r_7 : TTTACGATAGCCGATAGCTAAATTACGTTATACTC
Read r_8 : ATATAACGTAATTTAGCTATCGGCTATCGTAAATG
Read r_9 : ATTTACGATAGCCGATAGCTAAATTACGTTATATA
Read r_{10} : CTATATAACGTAATTTAGCTATCGGCTATCGTAAA

Set of given reads

Read r_1 : AGCTAAGCATTTACGATAGCCGATAGCTAAATTAC
Read $\bar{r}_2$: GCTAAGCATTTACGATAGCCGATAGCTAAATTACG
Read $\bar{r}_3$: TAAGCATTTACGATAGCCGATAGCTAAATTACGTT
Read r_4 : GCATTTACGATAGCCGATAGCTAAATTACGTTATA
Read $\bar{r}_5$: CATTTACGATAGCCGATAGCTAAATTACGTTATAC
Read r_6 : ATTTACGATAGCCGATAGCTAAATTACGTTATACT
Read r_7 : TTTACGATAGCCGATAGCTAAATTACGTTATACTC
Read $\bar{r}_8$: CATTTACGATAGCCGATAGCTAAATTACGTTATAT
Read r_9 : ATTTACGATAGCCGATAGCTAAATTACGTTATATA
Read $\bar{r}_{10}$: TTTACGATAGCCGATAGCTAAATTACGTTATATAG

Overlapping reads

Overlap graph construction
Transitive edge reduction
Composite path contraction
Flow computation
Tree reduction



Layout

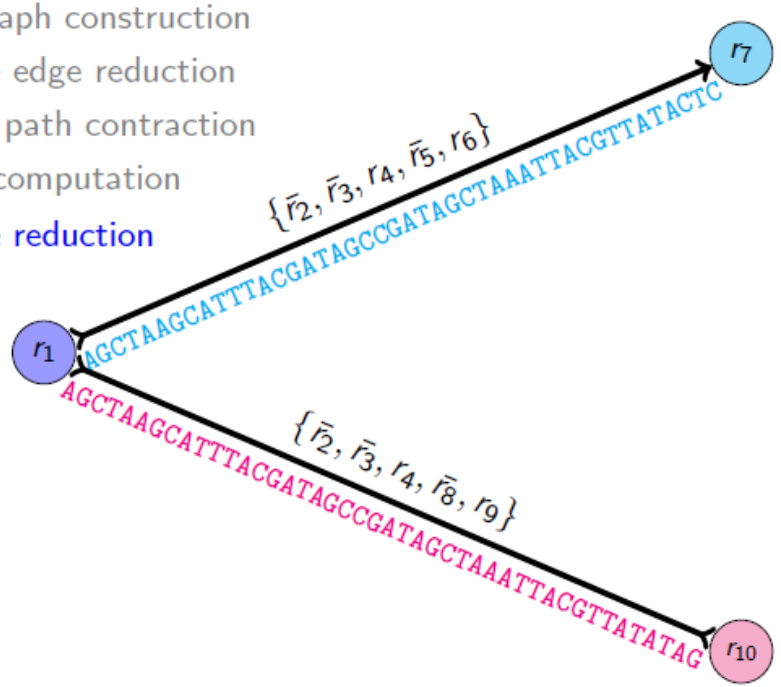
```
Read r1    :  AGCTAAGCATTTACGATAGCCGATAGCTAAATTAC
Read r2    :  CGTAATTTAGCTATCGGCTATCGTAAATGCTTAGC
Read r3    :  AACGTAATTTAGCTATCGGCTATCGTAAATGCTTA
Read r4    :  GCATTTACGATAGCCGATAGCTAAATTACGTTATA
Read r5    :  GTATAACGTAATTTAGCTATCGGCTATCGTAAATG
Read r6    :  ATTTACGATAGCCGATAGCTAAATTACGTTATACT
Read r7    :  TTTACGATAGCCGATAGCTAAATTACGTTATACTC
Read r8    :  ATATAACGTAATTTAGCTATCGGCTATCGTAAATG
Read r9    :  ATTTACGATAGCCGATAGCTAAATTACGTTATATA
Read r10   :  CTATATAACGTAATTTAGCTATCGGCTATCGTAAA
```

Set of given reads

```
Read r1    :  AGCTAAGCATTTACGATAGCCGATAGCTAAATTAC
Read r̄2    :   GCTAAGCATTTACGATAGCCGATAGCTAAATTACG
Read r̄3    :     TAAGCATTTACGATAGCCGATAGCTAAATTACGTT
Read r4    :       GCATTTACGATAGCCGATAGCTAAATTACGTTATA
Read r̄5    :        CATTTACGATAGCCGATAGCTAAATTACGTTATAC
Read r6    :         ATTTACGATAGCCGATAGCTAAATTACGTTATACT
Read r7    :          TTTACGATAGCCGATAGCTAAATTACGTTATACTC
Read r̄8    :        CATTTACGATAGCCGATAGCTAAATTACGTTATAT
Read r9    :         ATTTACGATAGCCGATAGCTAAATTACGTTATATA
Read r̄10   :          TTTACGATAGCCGATAGCTAAATTACGTTATATAG
```

Overlapping reads

Overlap graph construction
Transitive edge reduction
Composite path contraction
Flow computation
Tree reduction




```
Contig 1:
AGCTAAGCATTTACGATAGCCGATAGCTAAATTACGTTATACTC
Contig 2:
AGCTAAGCATTTACGATAGCCGATAGCTAAATTACGTTATATAG
```

Contig is a set of overlapping DNA segments.

Consensus

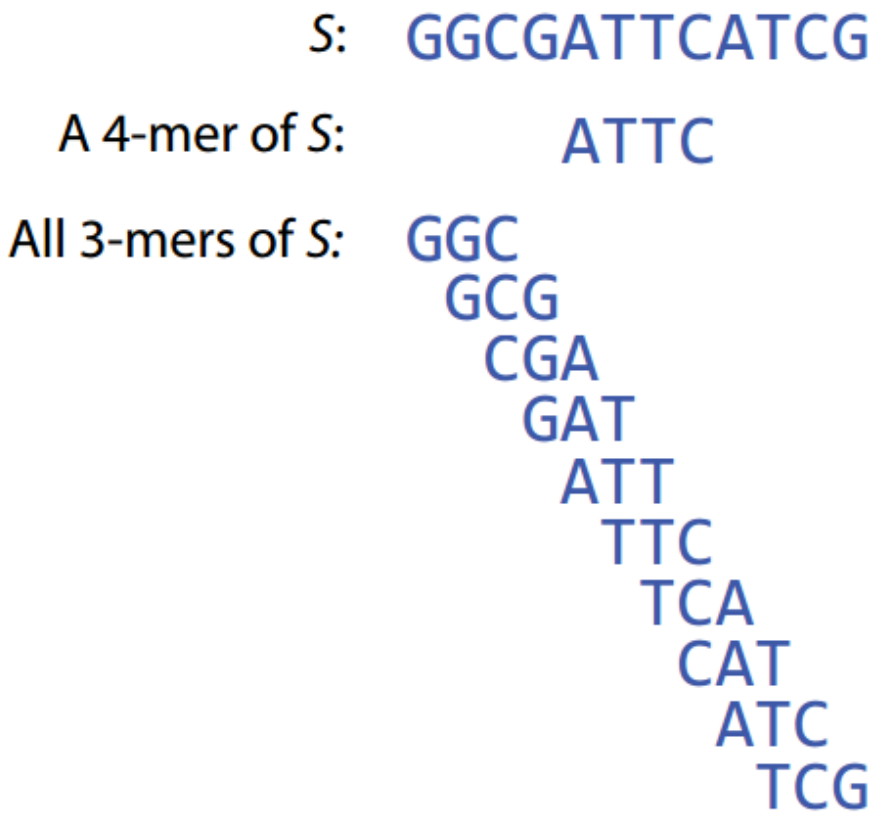
At each position, ask: what nucleotide (and/or gap) is here?

Complications: (a) sequencing error, (b) ploidy

Say the true genotype is AG, but we have a high sequencing error rate and only about 6 reads covering the position.

k-mer

"k-mer" is a substring of length k

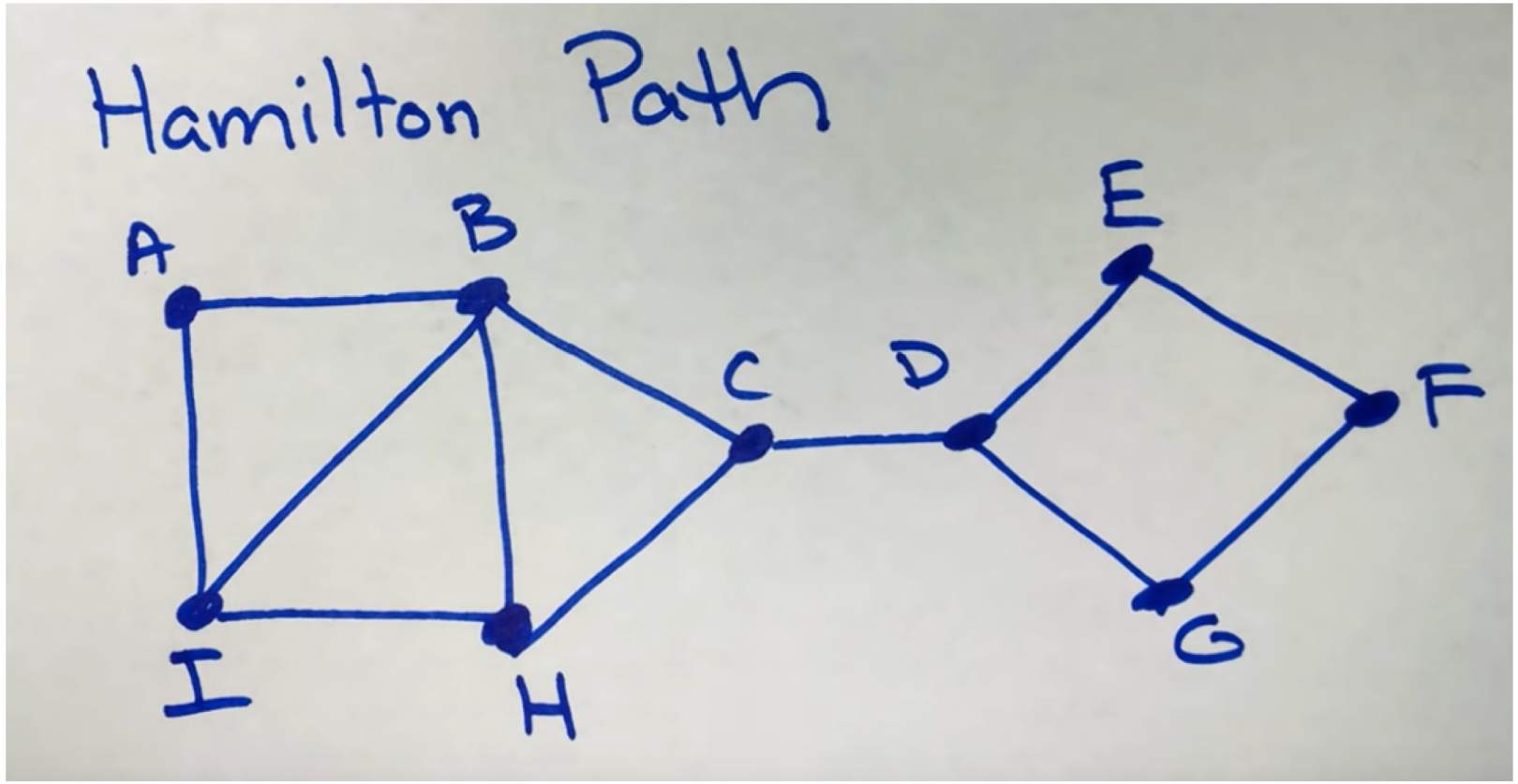


mer: from Greek meaning "part"

Courtesy of Ben Langmead. Used with permission.

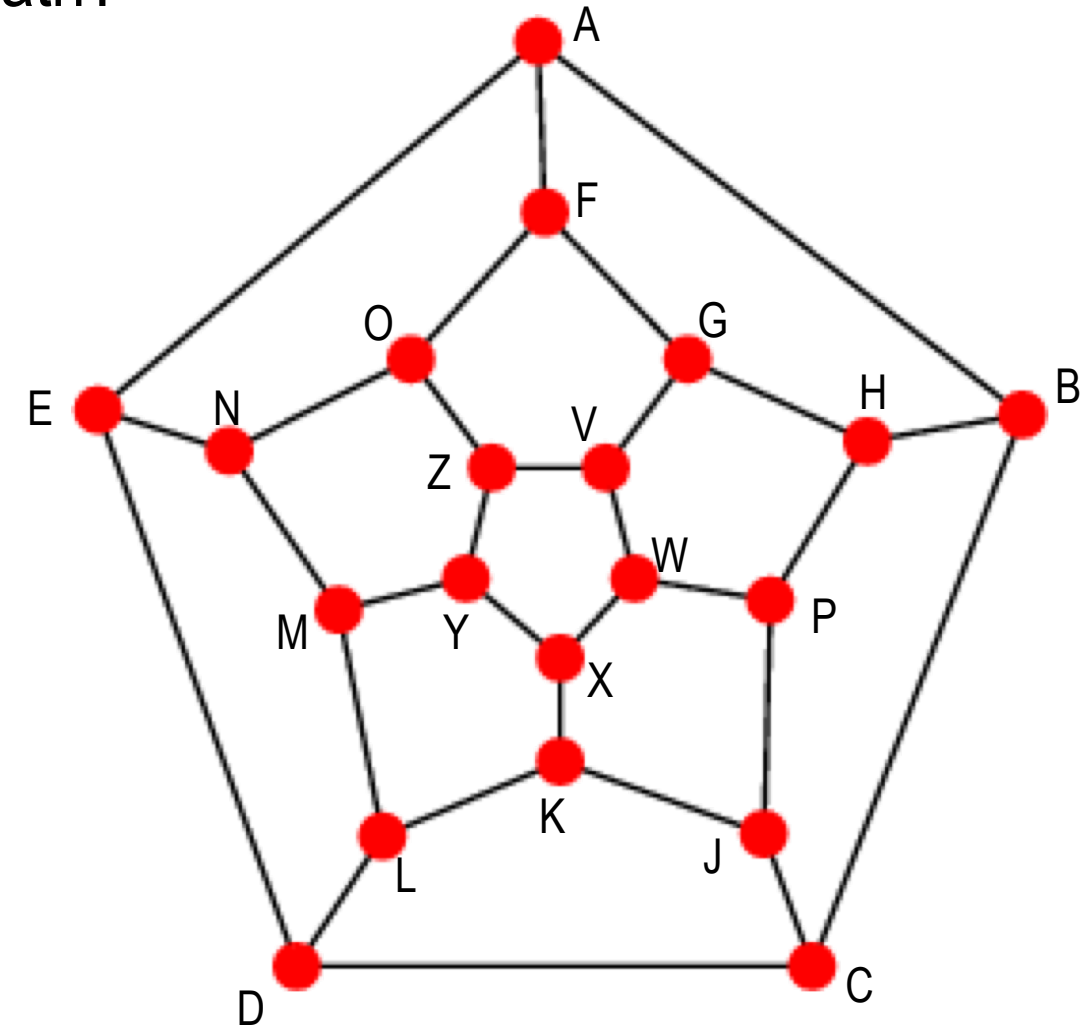
Hamiltonian Path

- A path in a graph visiting every node once is called a Hamiltonian path, in honor of the Irish mathematician William Hamilton. So, it is also called a Hamilton path.



Hamiltonian Path Problem:

- Is this a Hamiltonian path?



Eulerian Path

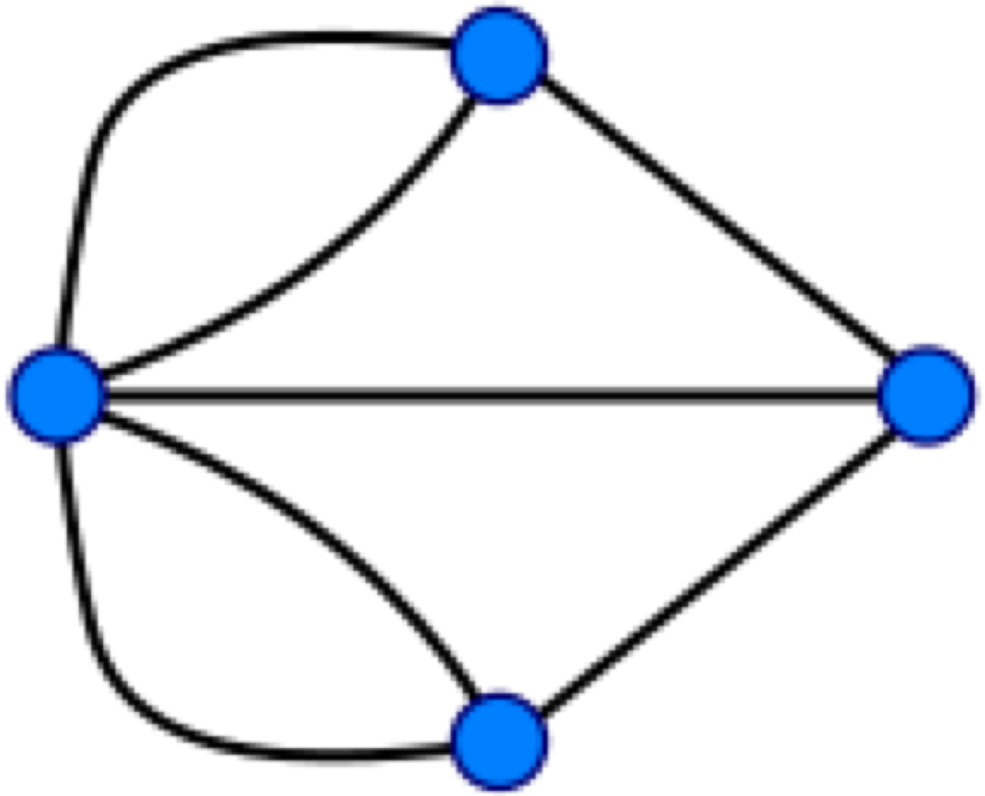
- A path in the graph that visits every edge exactly once.
- Such a path is called an Eulerian Path in honor of the great mathematician Leonhard Euler (pronounced “oiler”).

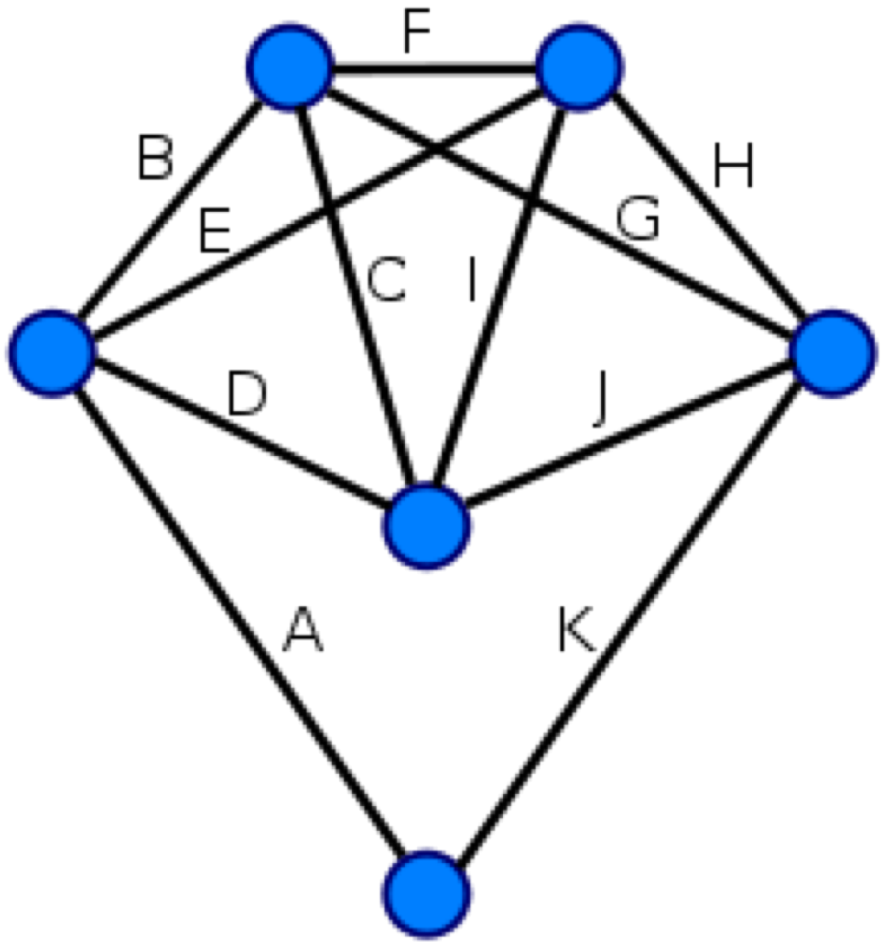
Eulerian Path Problem:
Construct an Eulerian path in a graph.

Input: A directed graph.
Output: A path visiting every edge in the graph exactly once (if such a path exists).

Eulerian Path Problem:

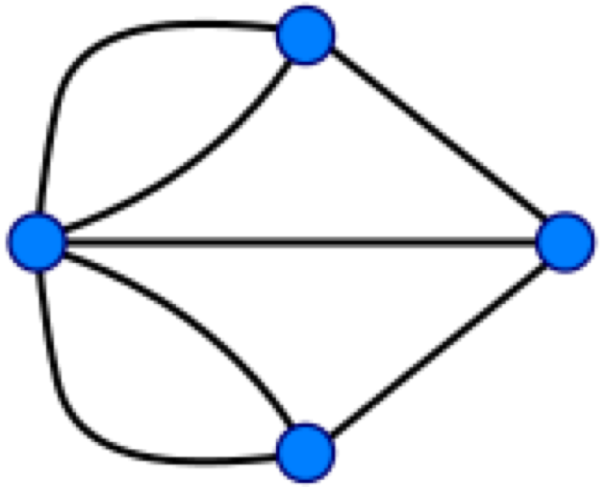
- Are these Eulerian paths?

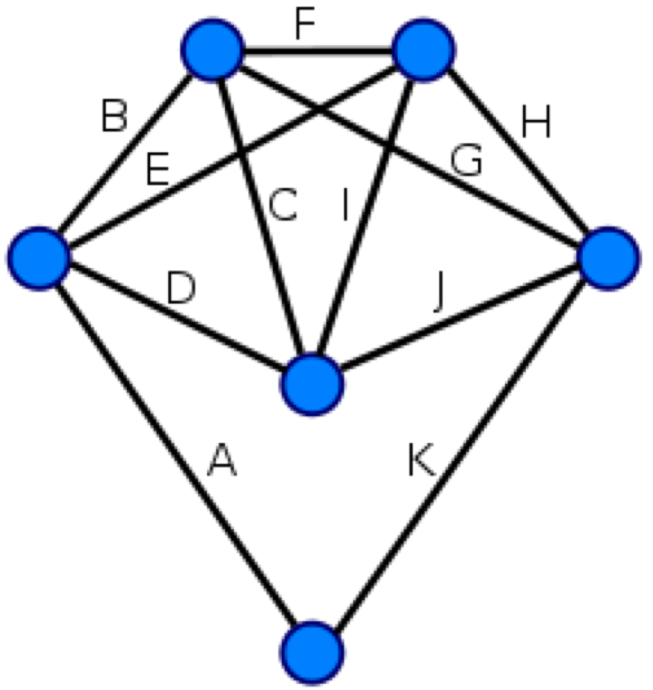




Eulerian Path Problem:

- Are these Eulerian paths?





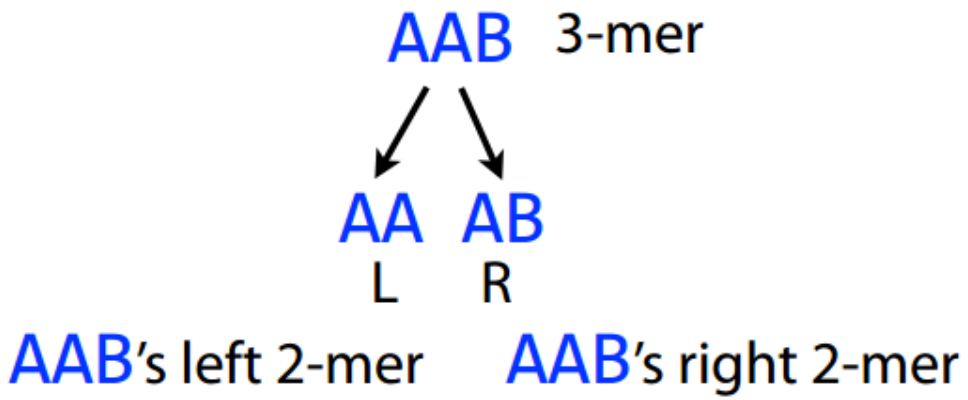
In order for a graph to be Eulerian, the number of incoming edges at any node must be equal to the number of outgoing edges at that node.

De Bruijn graph

As usual, we start with a collection of reads, which are substrings of the reference genome.

AAA, AAB, ABB, BBB, BBA

AAB is a *k*-mer ($k = 3$). AA is its *left* *k*-1-mer, and AB is its right *k*-1-mer.



De Bruijn graph

Take each length-3 input string and split it into two overlapping substrings of length 2. Call these the *left* and *right 2-mers*.

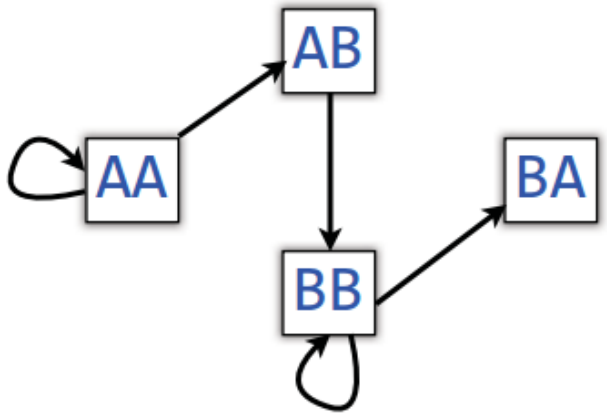
AAABBBA

take all 3-mers: AAA, AAB, ABB, BBB, BBA

form L/R 2-mers: AA, AA, AA, AB, AB, BB, BB, BB, BB, BA

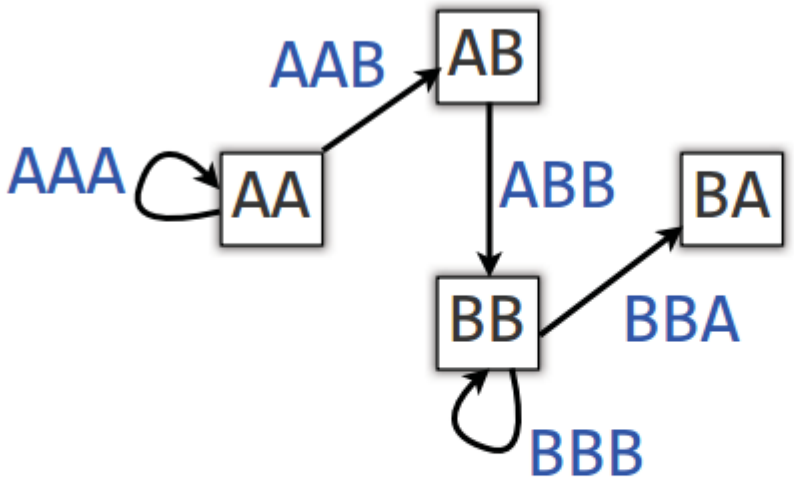
L R L R L R L R L R

Let 2-mers be nodes in a new graph. Draw a directed edge from each left 2-mer to corresponding right 2-mer:



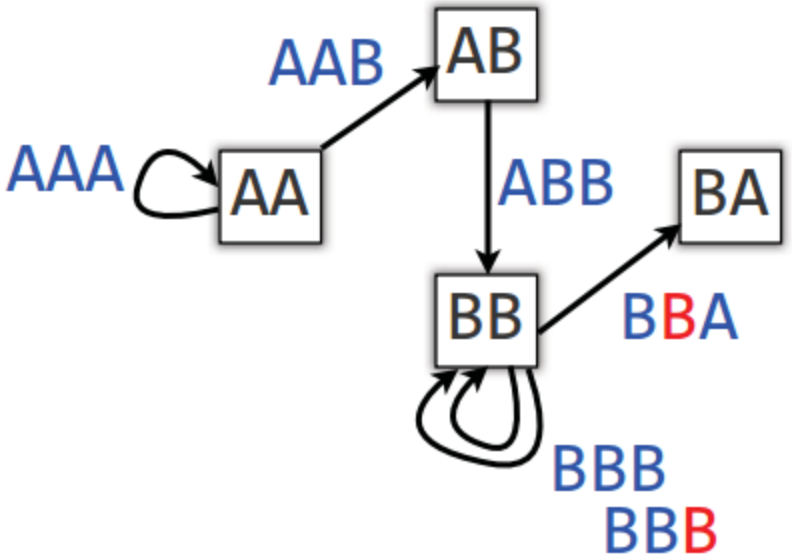
Each *edge* in this graph corresponds to a length-3 input string

De Bruijn graph



An edge corresponds to an overlap (of length *k*-2) between two *k*-1 mers.
More precisely, it corresponds to a *k*-mer from the input.

De Bruijn graph

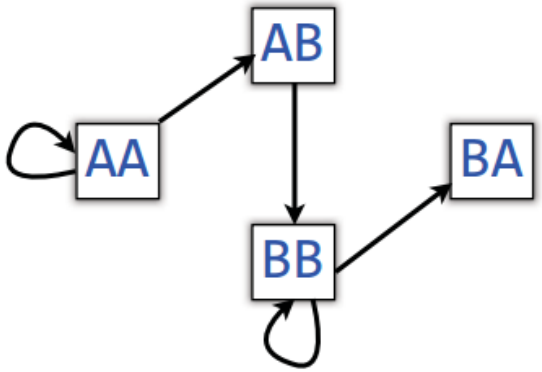


If we add one more B to our input string: AAABBBBA, and rebuild the De Bruijn graph accordingly, we get a *multiedge*.

Eulerian walk definitions and statements

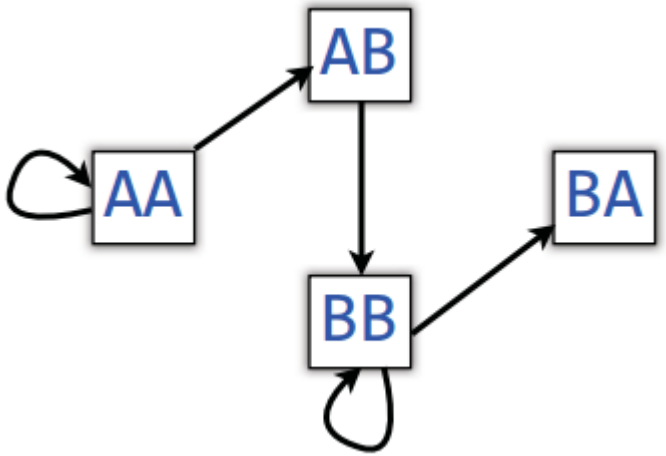
- Node is balanced if indegree equals outdegree
- Node is semi-balanced if indegree differs from outdegree by 1
- Graph is connected if each node can be reached by some other node
- Eulerian walk visits each edge exactly once
- Not all graphs have Eulerian walks. Graphs that do are Eulerian. (For simplicity, we won't distinguish Eulerian from semi-Eulerian.)

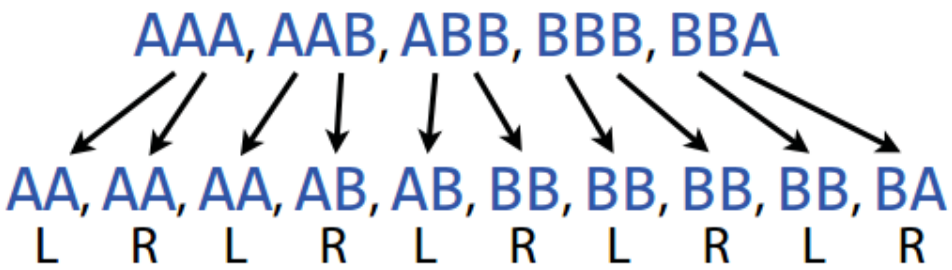
- A directed, connected graph is Eulerian if and only if it has at most 2 semi-balanced nodes and all other nodes are balanced



De Bruijn graph

Back to our De Bruijn graph





Is it Eulerian? Yes

Argument 1: AA → AA → AB → BB → BB → BA

Argument 2: AA and BA are semi-balanced, AB and BB are balanced

De Bruijn graph

A procedure for making a De Bruijn graph for a genome

Assume *perfect sequencing* where each length-*k* substring is sequenced exactly once with no errors

Pick a substring length *k*: 5

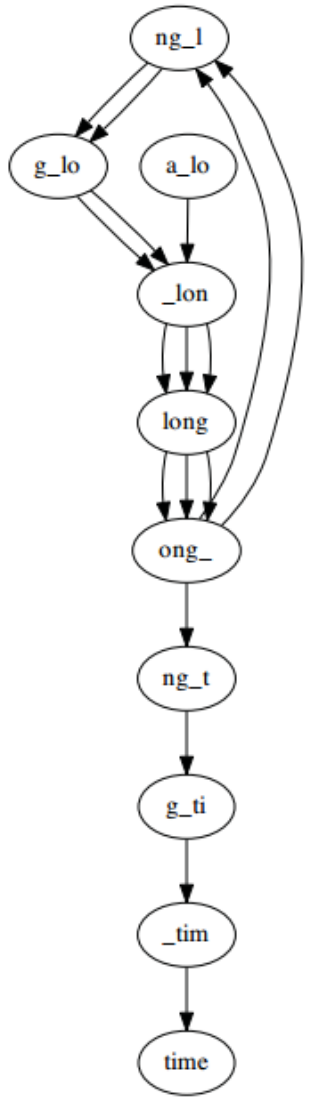
Start with each read: a_long_long_long_time

Take each *k* mer and split into left and right *k*-1 mers

long_

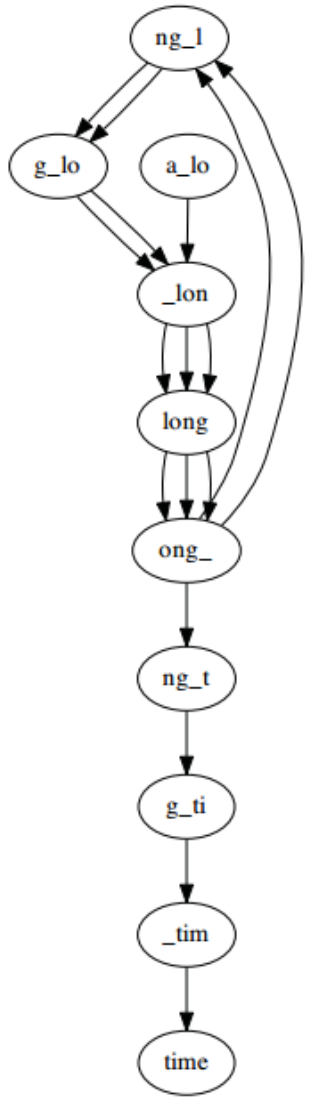
long ong_

Add k-1 mers as nodes to De Bruijn graph (if not already there), add edge from left *k*-1 mer to right *k*-1 mer

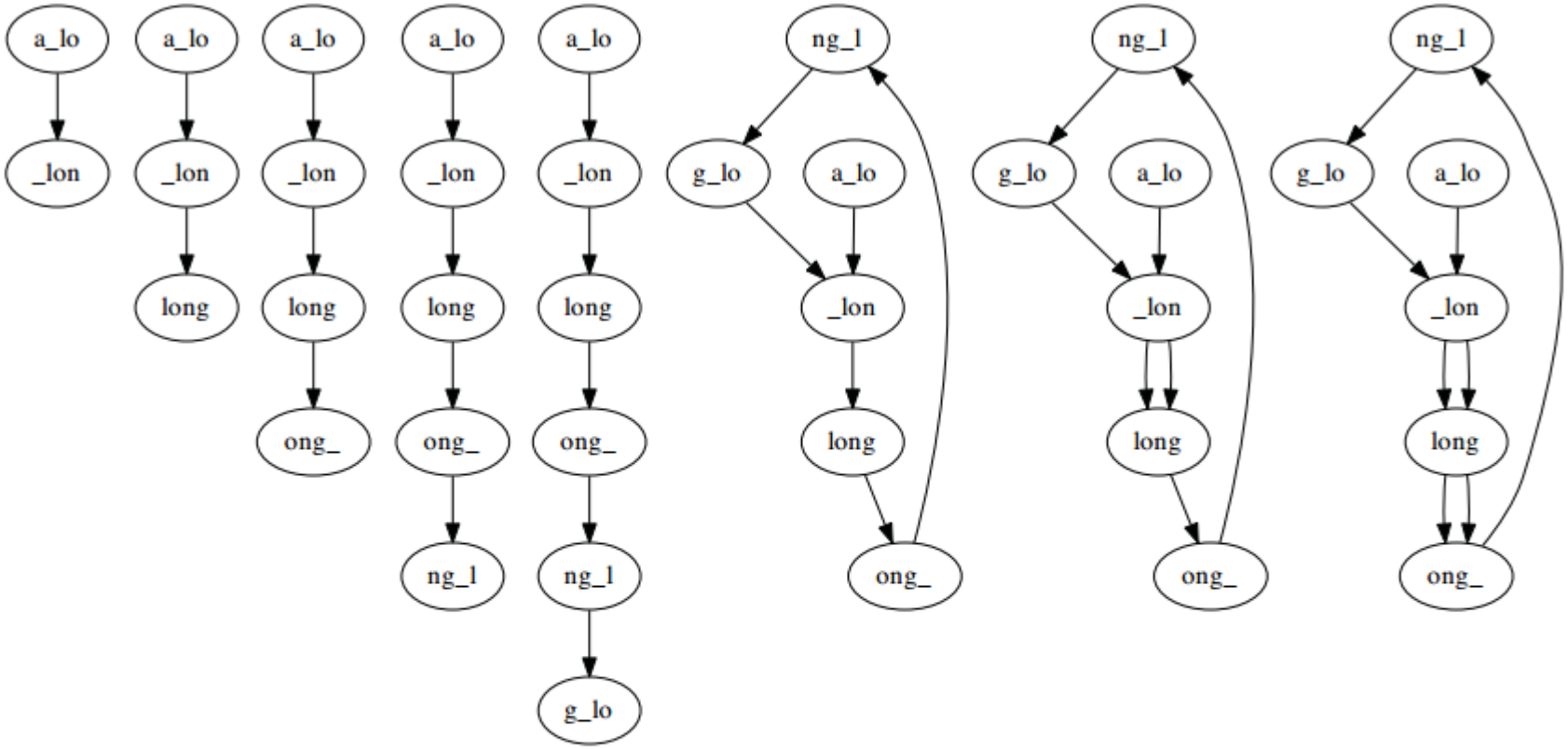


De Bruijn graph

- For genome assembly each k-mer is recorded in “twin” nodes – one node in the forward direction and one node in reverse complement
- k is odd so no node can be its own reverse complement
- We will not show reverse complement twin nodes to cut down on clutter



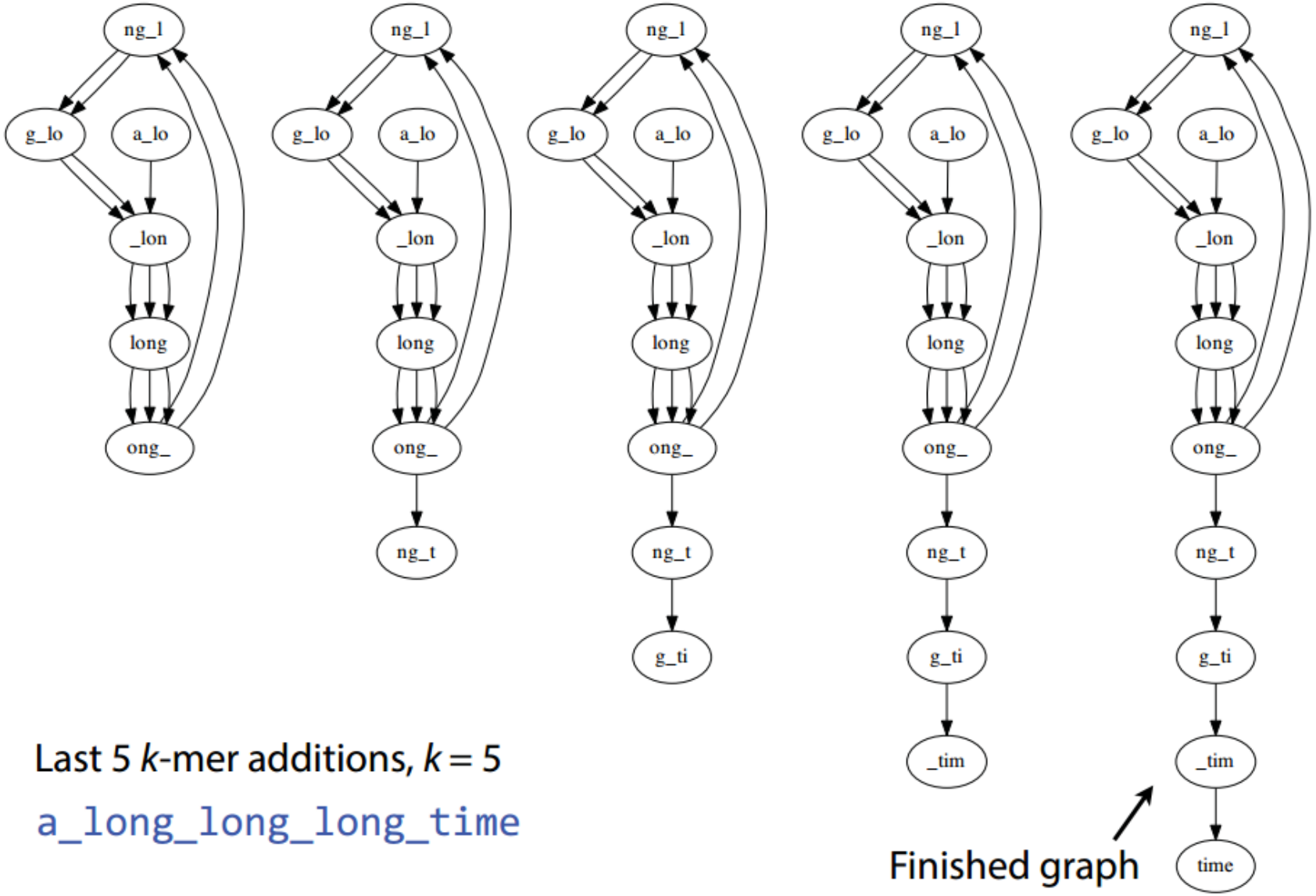
De Bruijn graph



First 8 *k*-mer additions, $k = 5$

```
a_long_long_long_time
```

De Bruijn graph



Last 5 *k*-mer additions, $k = 5$

a_long_long_long_time

De Bruijn graph

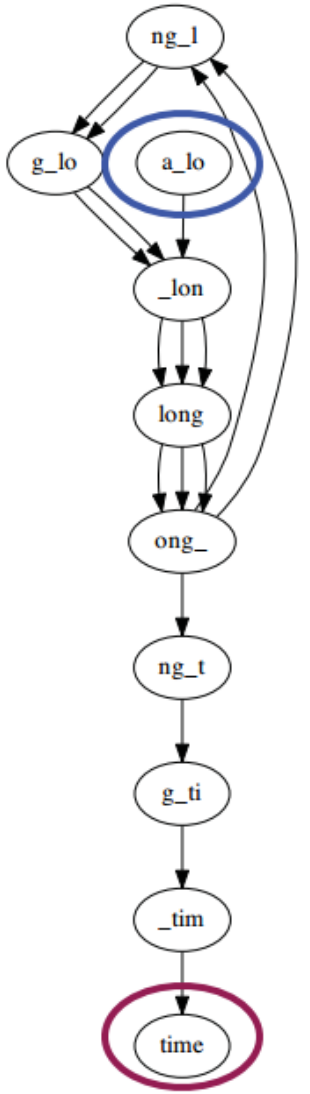
With perfect sequencing, this procedure always yields an Eulerian graph. Why?

Node for *k*-1-mer from left end is semi-balanced with one more outgoing edge than incoming *

Node for *k*-1-mer at right end is semi-balanced with one more incoming than outgoing *

Other nodes are balanced since # times *k*-1-mer occurs as a left *k*-1-mer = # times it occurs as a right *k*-1-mer

* Unless genome is circular



Repeats

No: graph can have multiple Eulerian walks, only one of which corresponds to original superstring

Right: graph for ZABCDABEFABY, $k = 3$

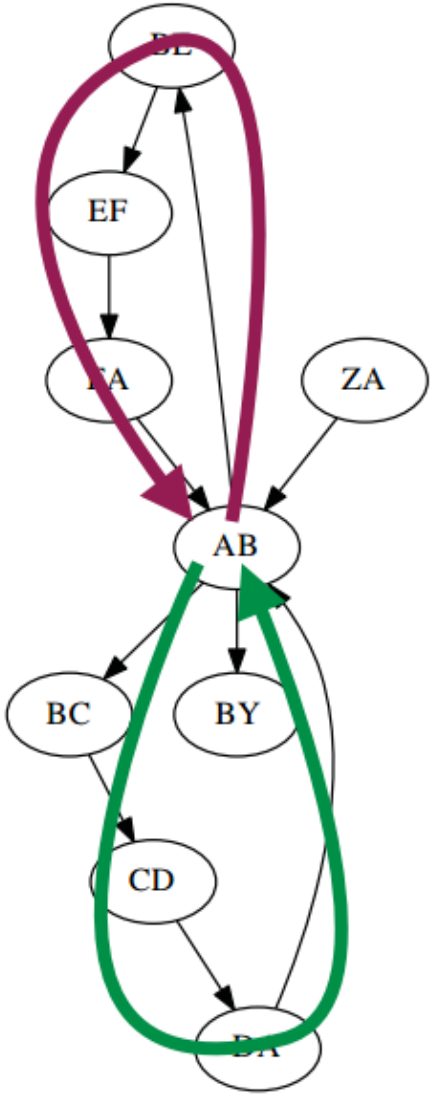
Alternative Eulerian walks:

ZA → AB → BE → EF → FA → AB → BC → CD → DA → AB → BY

ZA → AB → BC → CD → DA → AB → BE → EF → FA → AB → BY

These correspond to two edge-disjoint directed cycles joined by node AB

AB is a repeat: ZABCDABEFABY

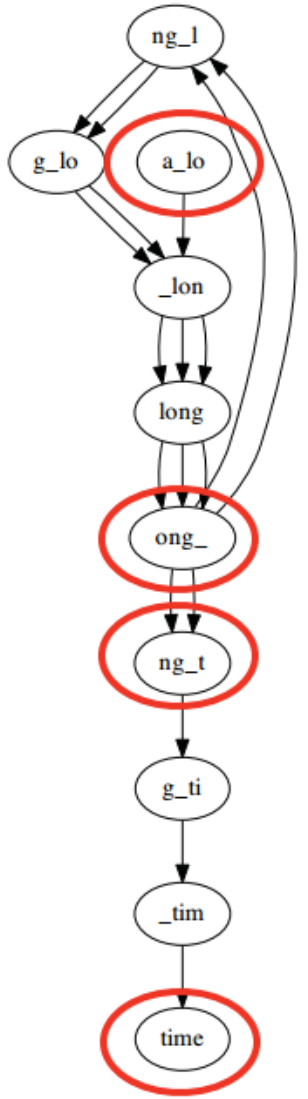


Differences in coverage

Differences in coverage also lead to non-Eulerian graph

Graph for `a_long_long_long_time`, $k = 5$ but with *extra copy* of `ong_t`:

Graph has 4 semi-balanced nodes, isn't Eulerian

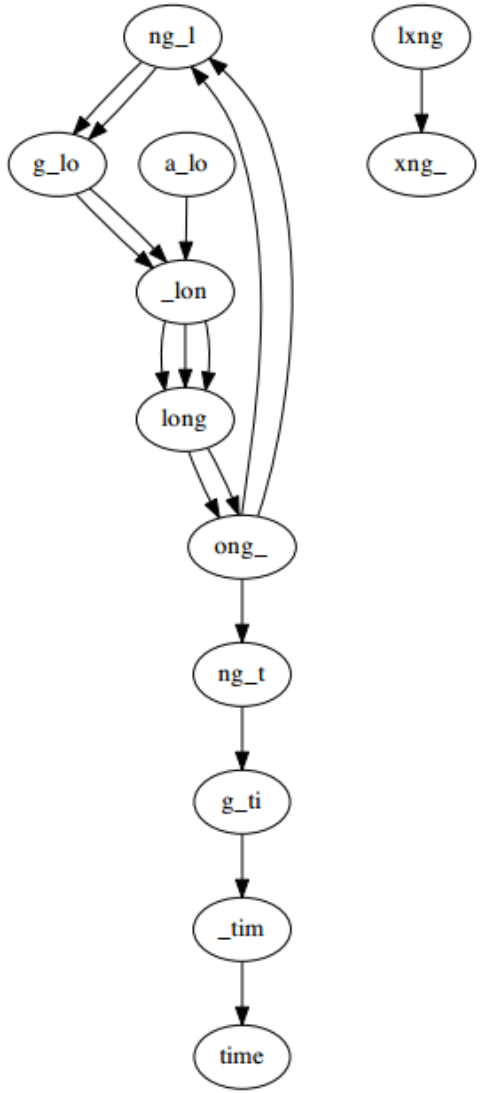


Errors and differences

Errors and differences between chromosomes also lead to non-Eulerian graphs

Graph for `a_long_long_long_time`, $k = 5$ but with error that turns a copy of `long_` into `lxng_`

Graph is not connected; largest component is not Eulerian



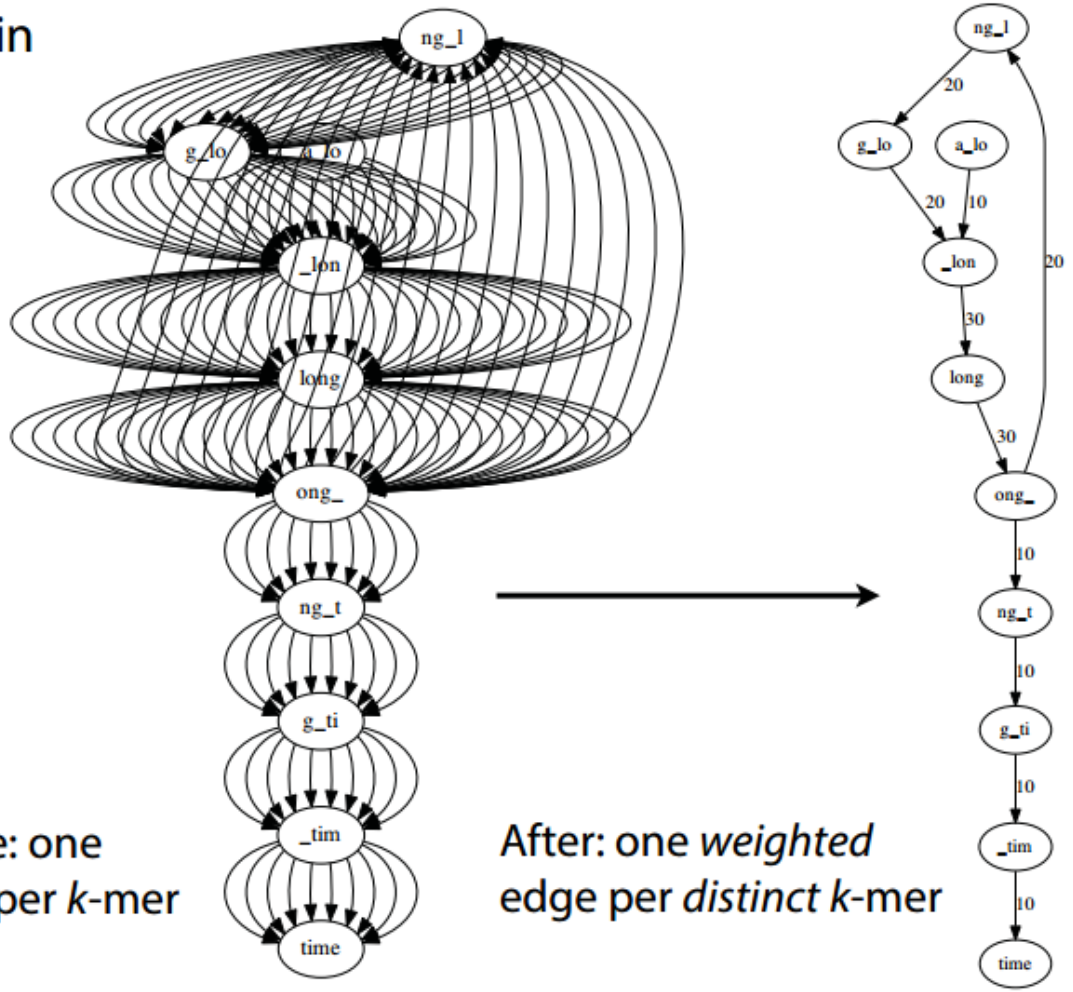
De Bruijn graph

In typical assembly projects, average coverage is ~ 30 - 50

Same edge might appear in dozens of copies; let's use edge *weights* instead

Weight = # times *k*-mer occurs

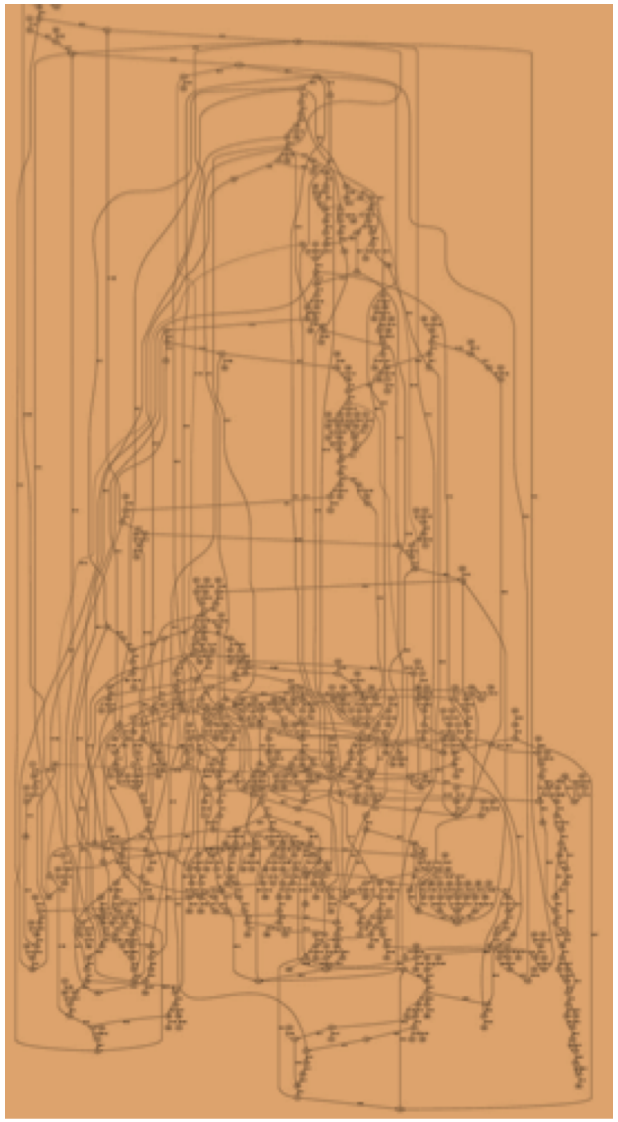
Using weights, there's one *weighted* edge for each *distinct k*-mer



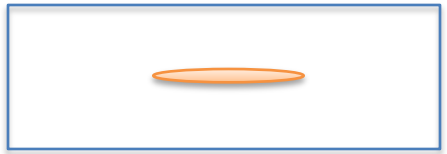
Before: one edge per *k*-mer

After: one *weighted* edge per *distinct k*-mer

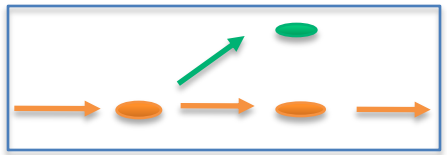
Node Types



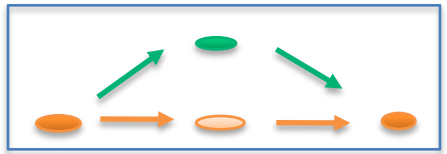
(Chaisson, 2009)



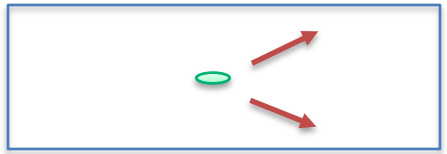
Isolated nodes (10%)



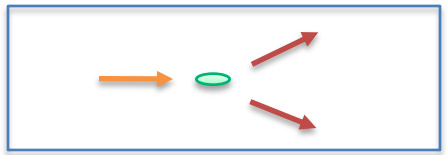
Tips (46%)



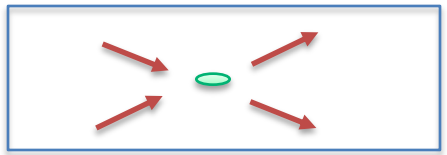
Bubbles/Non-branch (9%)



Dead Ends (.2%)



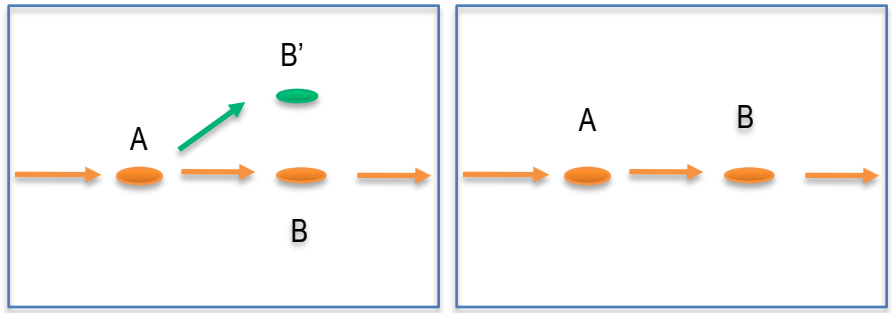
Half Branch (25%)



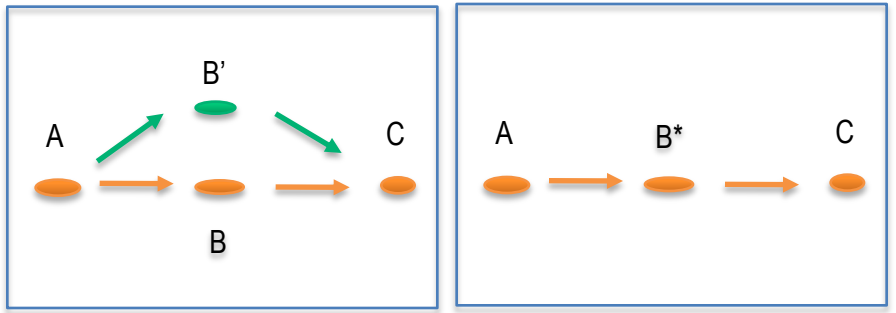
Full Branch (10%)

Error Correction

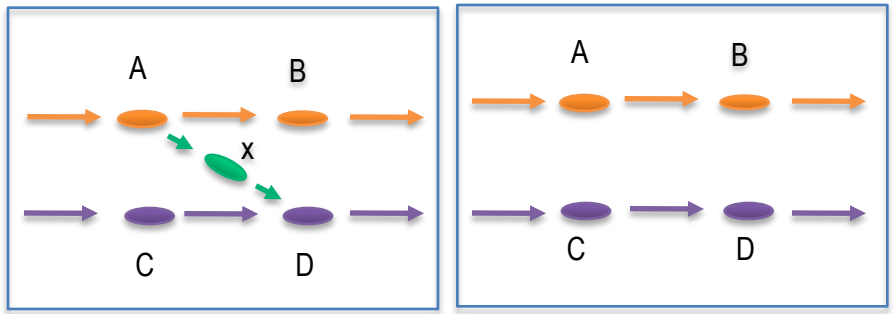
- Errors at end of read
  - Trim off ‘dead-end’ tips



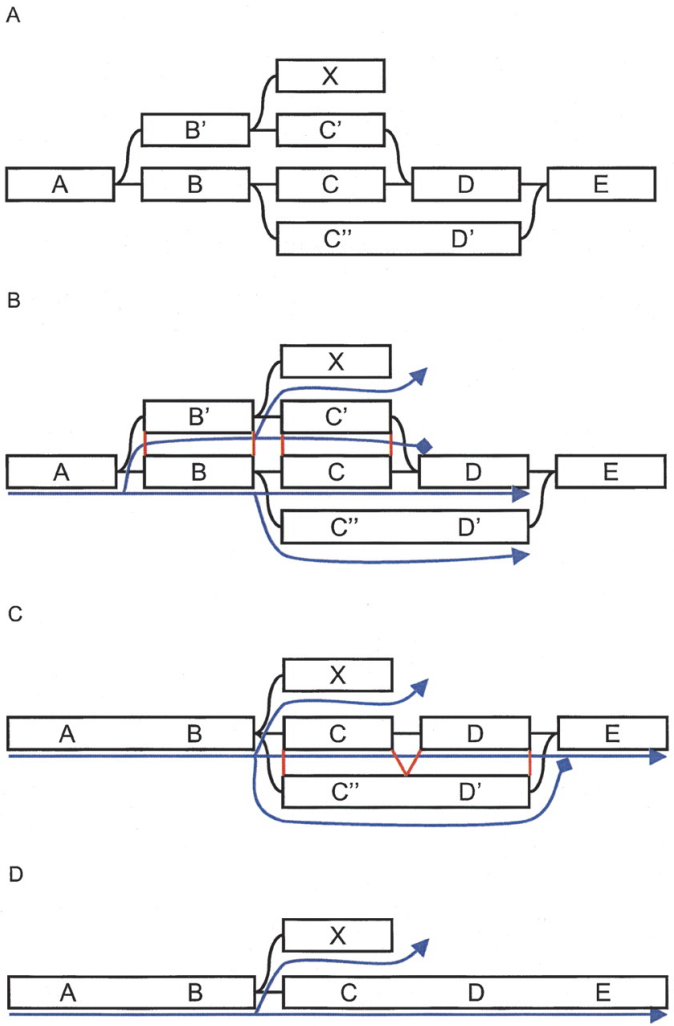
- Errors in middle of read
  - Pop Bubbles



- Chimeric Edges
  - Clip short, low coverage nodes



Example of Tour Bus error correction in Velvet.



Example of Tour Bus error correction. (A) Original graph. (B) The search starts from A and spreads toward the right. The progression of the top path (through B' and C') is stopped because D was previously visited. The nucleotide sequences corresponding to the alternate paths B'C' and BC are extracted from the graph, aligned, and compared. (C) The two paths are judged similar, so the longer one, B'C', is merged into the shorter one, BC. The merging is directed by the alignment of the consensus sequences, indicated in red lines in B. Note that node X, which was connected to node B', is now connected to node B. The search progresses, and the bottom path (through C' and D') arrives second in E. Once again, the corresponding paths, C'D' and CD are compared. (D) CD and C'D' are judged similar enough. The longer path is merged into the shorter one.

Zerbino D R , Birney E Genome Res. 2008;18:821-829

Handling Repeats

1. Repeat detection
   - **pre-assembly:** find fragments that belong to repeats
     - statistically (most existing assemblers)
     - repeat database (*RepeatMasker*)
   - **during assembly:** detect "tangles" indicative of repeats (Pevzner, Tang, Waterman 2001)
   - **post-assembly:** find repetitive regions and potential mis-assemblies.
     - *Reputer, RepeatMasker*
     - "unhappy" mate-pairs (too close, too far, mis-oriented)
2. Repeat resolution
   - find DNA fragments belonging to the repeat
   - determine correct tiling across the repeat
   - Obtain long reads spanning repeats

De Bruijn graph

What are the limitations of De Bruijn graphs?

Reads are immediately split into shorter *k*-mers; can't resolve repeats as well as overlap graph

Only a very specific type of "overlap" is considered, which makes dealing with errors more complicated.

Read coherence is lost. Some paths through De Bruijn graph are inconsistent with respect to input reads. Need to thread reads though De Bruijn graph to recover information lost when reads are fragmented into k-mers.

This is the OLC ↔ DBG tradeoff

Single most important benefit of De Bruijn graph is speed and simplicity.

Example Result

	OLC t >= 75	De Bruijn k = 61	De Bruijn k = 67	De Bruijn k = 59

Table 1. Assembly statistics for *C. elegans* data set

	SGA	Velvet	ABySS	SOAPdenovo
Scaffold N50 size	26.3 kbp	31.3 kbp	23.8 kbp	31.1 kbp
Aligned contig N50 size	16.8 kbp	13.6 kbp	18.4 kbp	16.0 kbp
Mean aligned contig size	4.9 kbp	5.3 kbp	6.0 kbp	5.6 kbp
Sum aligned contig size	96.8 Mbp	95.2 Mbp	98.3 Mbp	95.4 Mbp
Reference bases covered	96.2 Mbp	94.8 Mbp	95.9 Mbp	95.1 Mbp
Reference bases covered by contigs ≥1 kb	93.0 Mbp	92.1 Mbp	93.9 Mbp	92.3 Mbp
Mismatch rate at all assembled bases	1 per 21,545 bp	1 per 8786 bp	1 per 5577 bp	1 per 26,585 bp
Mismatch rate at bases covered by all assemblies	1 per 82,573 bp	1 per 18,012 bp	1 per 8209 bp	1 per 81,025 bp
Contigs with split/bad alignment (sum size)	458 (4.4 Mbp)	787 (7.2 Mbp)	638 (9.1 Mbp)	483 (4.4 Mbp)
Total CPU time	41 h	2 h	5 h	13 h
Max memory usage	4.5 GB	23.0 GB	14.1 GB	38.8 GB

100 MBase genome, 33.8M read pairs, 100bp reads each end, 250bp insert size