De Bruijn Graph Assembly

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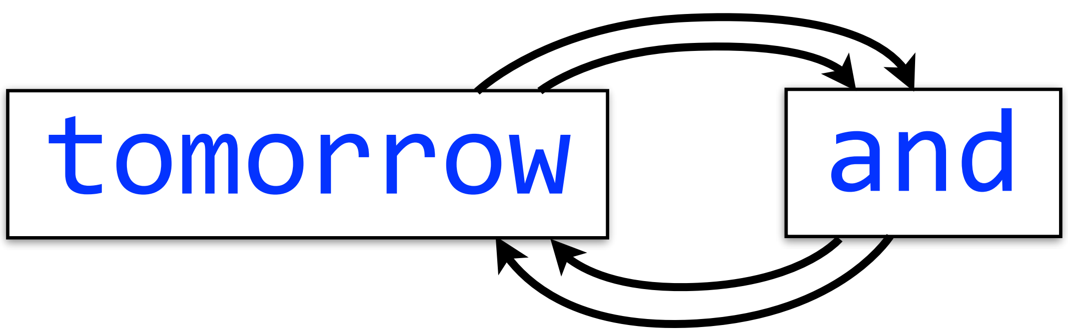
Different kind of graph

"tomorrow and tomorrow and tomorrow"



An edge represents an ordered pair of adjacent words in the input

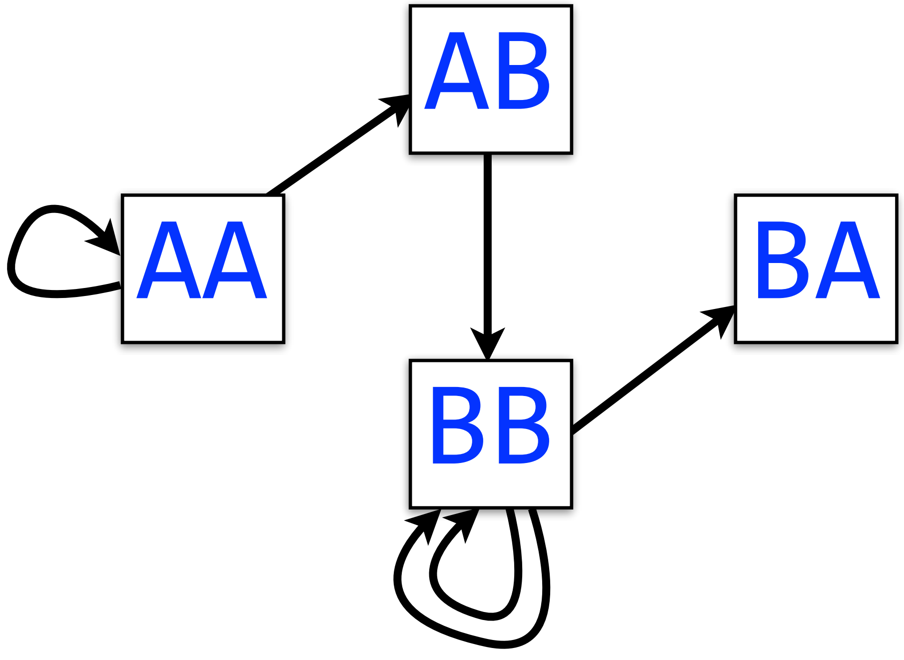
Multigraph: there can be more than one edge from node A to node B

De Bruijn graph

genome: AAABBBBA

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

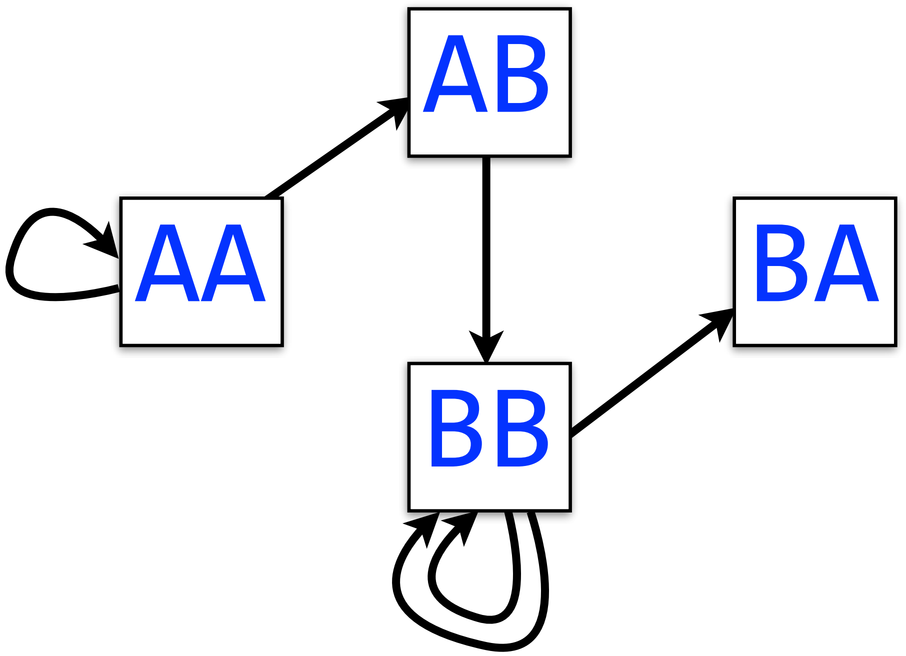
L/R 2-mers: AA, AA AA, AB AB, BB BB, BB BB, BB BB, BA



One edge per *k*-mer

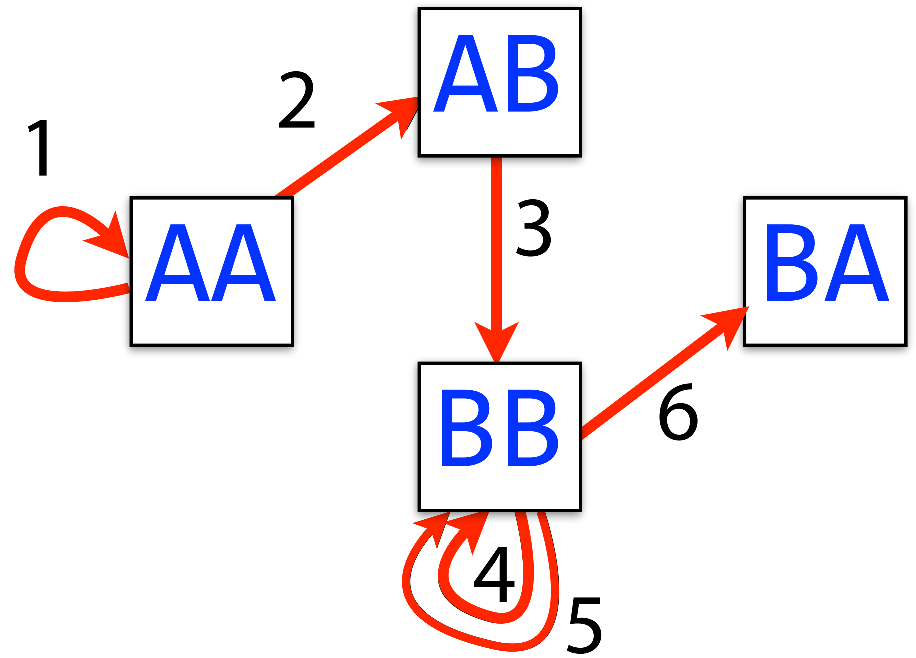
One node per distinct *k*-1-mer

De Bruijn graph



Walk crossing each edge exactly once gives a reconstruction of the genome

De Bruijn graph



AAABBBBA

Walk crossing each edge exactly once gives a reconstruction of the genome. This is an *Eulerian walk*.

De Bruijn graph

Aside: how do you pronounce "De Bruijn"?

There is debate:

https://www.biostars.org/p/7186/

I still don't quite know. I say "De Broin" (rhymes with "groin")

I asked a Dutch person once; his pronunciation sounded more like "De Brown"

Nicolaas Govert
de Bruijn
1918 -- 2012

Directed multigraph

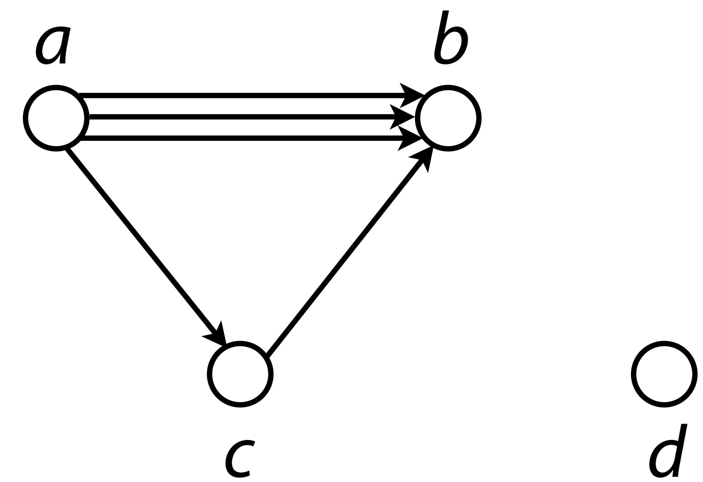
Directed **multigraph** $G(V, E)$ consists of set of *vertices*, V and **multiset** of *directed edges*, E

Otherwise, like a directed graph

Node's *indegree* = # incoming edges

Node's *outdegree* = # outgoing edges

De Bruijn graph is a directed multigraph



$V = \{ a, b, c, d \}$

$E = \{ (a, b), (a, b), (a, b), (a, c), (c, b) \}$

Repeated

Eulerian walk definitions and statements

Node is *balanced* if indegree equals outdegree

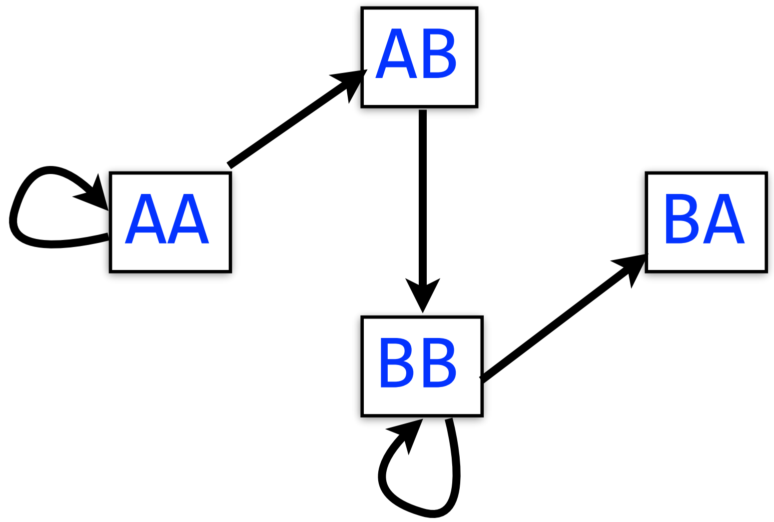
Node is *semi-balanced* if indegree differs from outdegree by 1

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.)

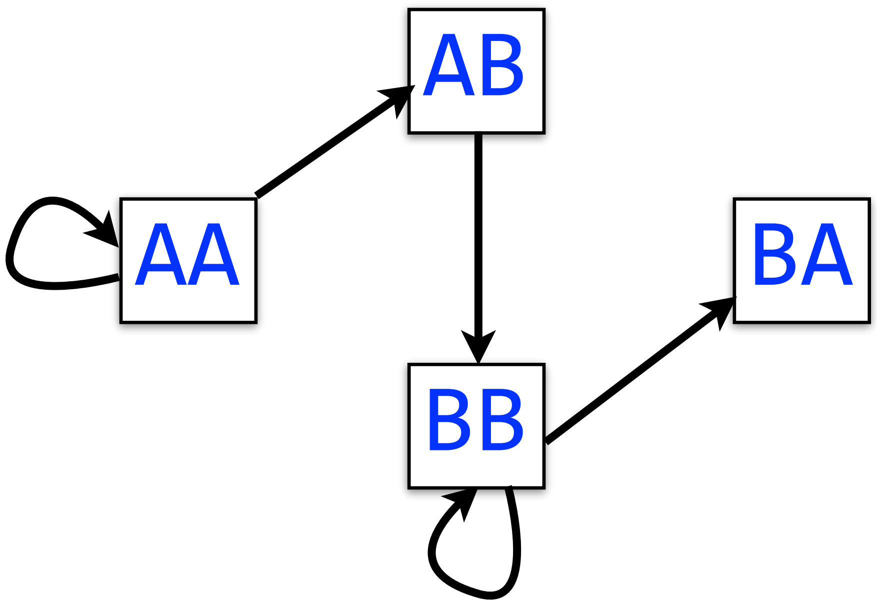
A directed, connected graph is Eulerian if and only if it has at most 2 semi-balanced nodes and all other nodes are balanced

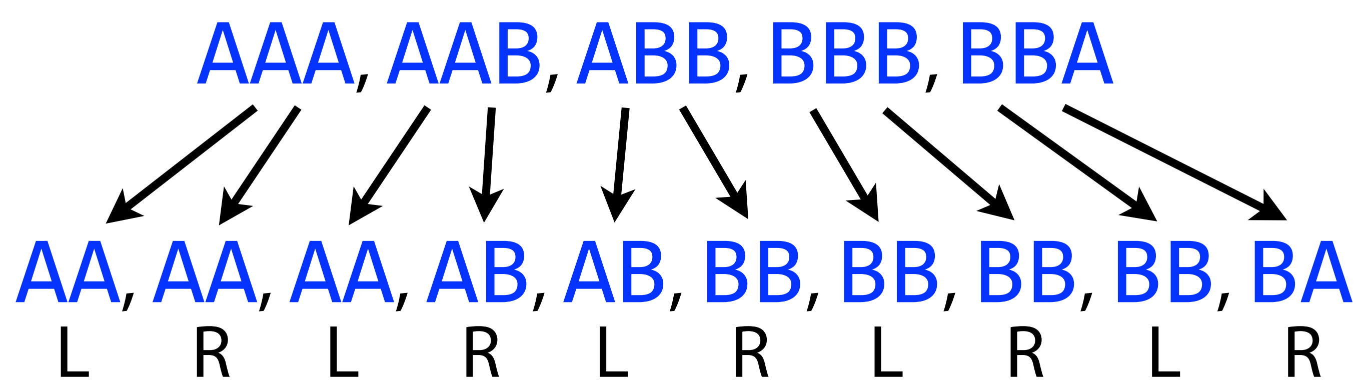


Jones and Pevzner section 8.8

De Bruijn graph

Back to 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": each genome *k*-mer is sequenced exactly once with no errors

Pick a substring length *k*: 5

Start with an input string: `a_long_long_long_time`

↓

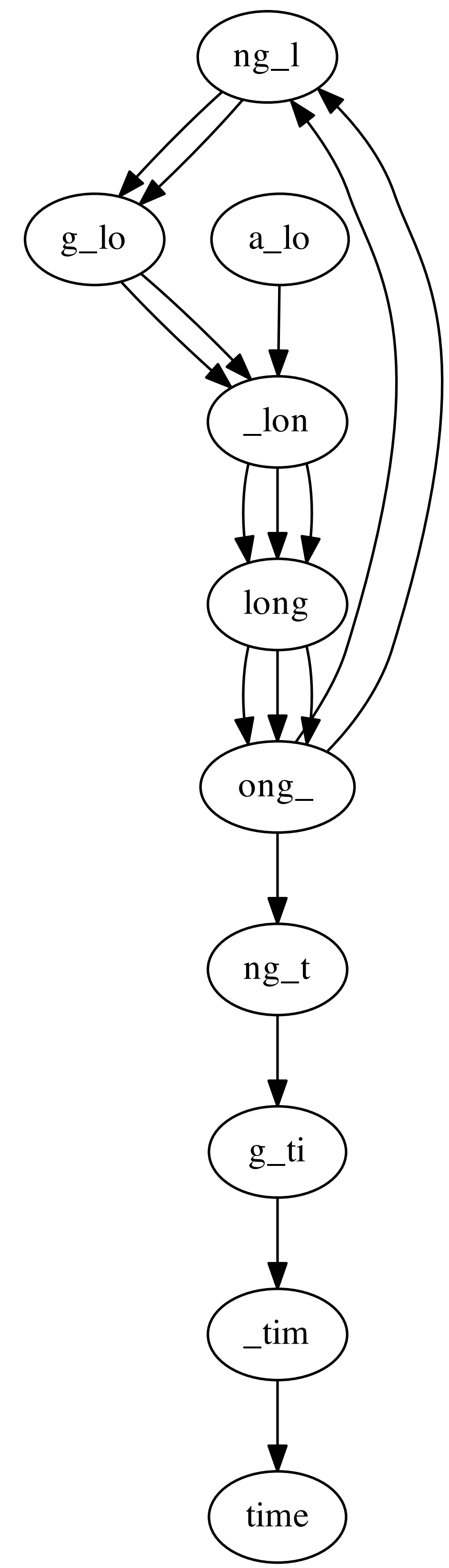
`long_`

↙ ↘

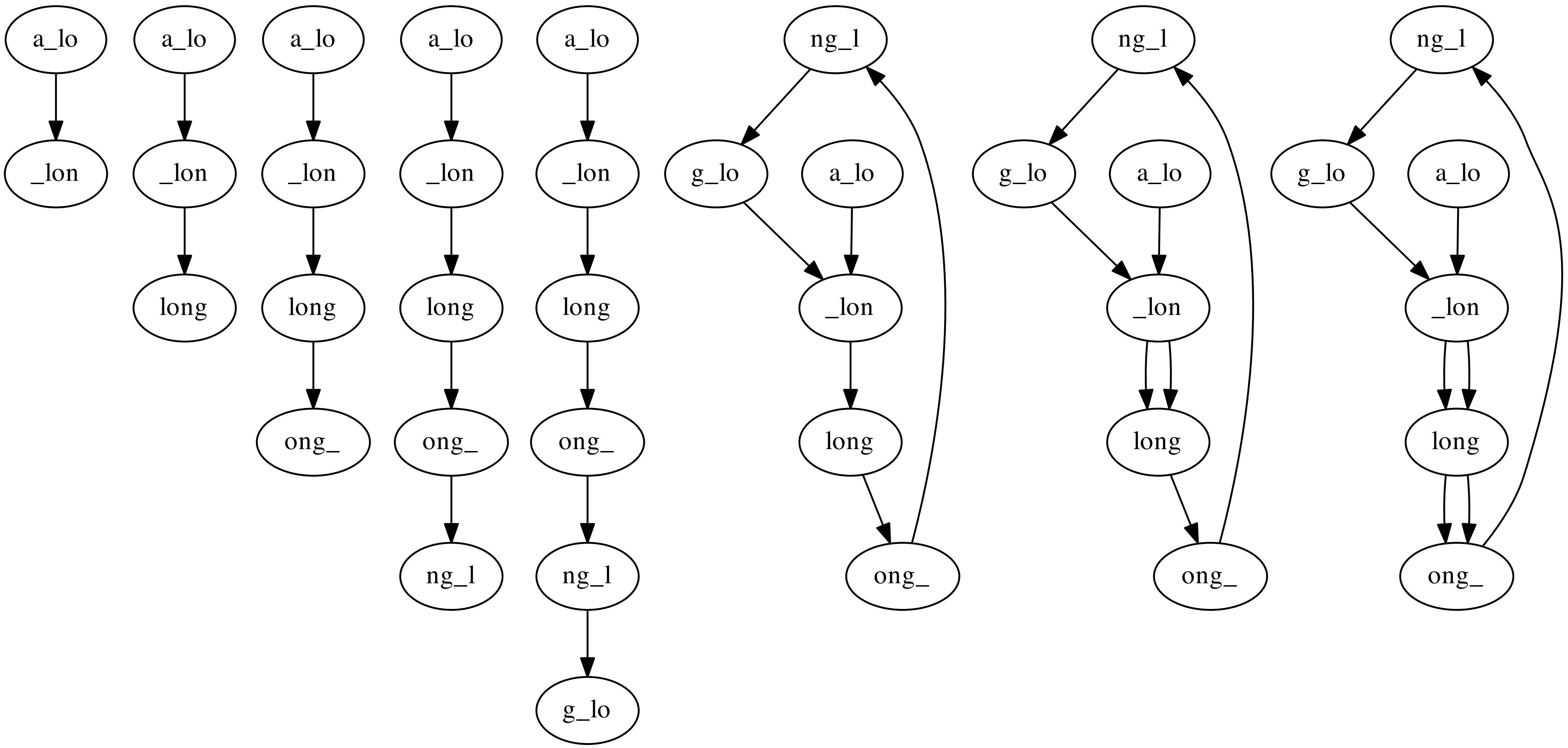
`long` `ong_`

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

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



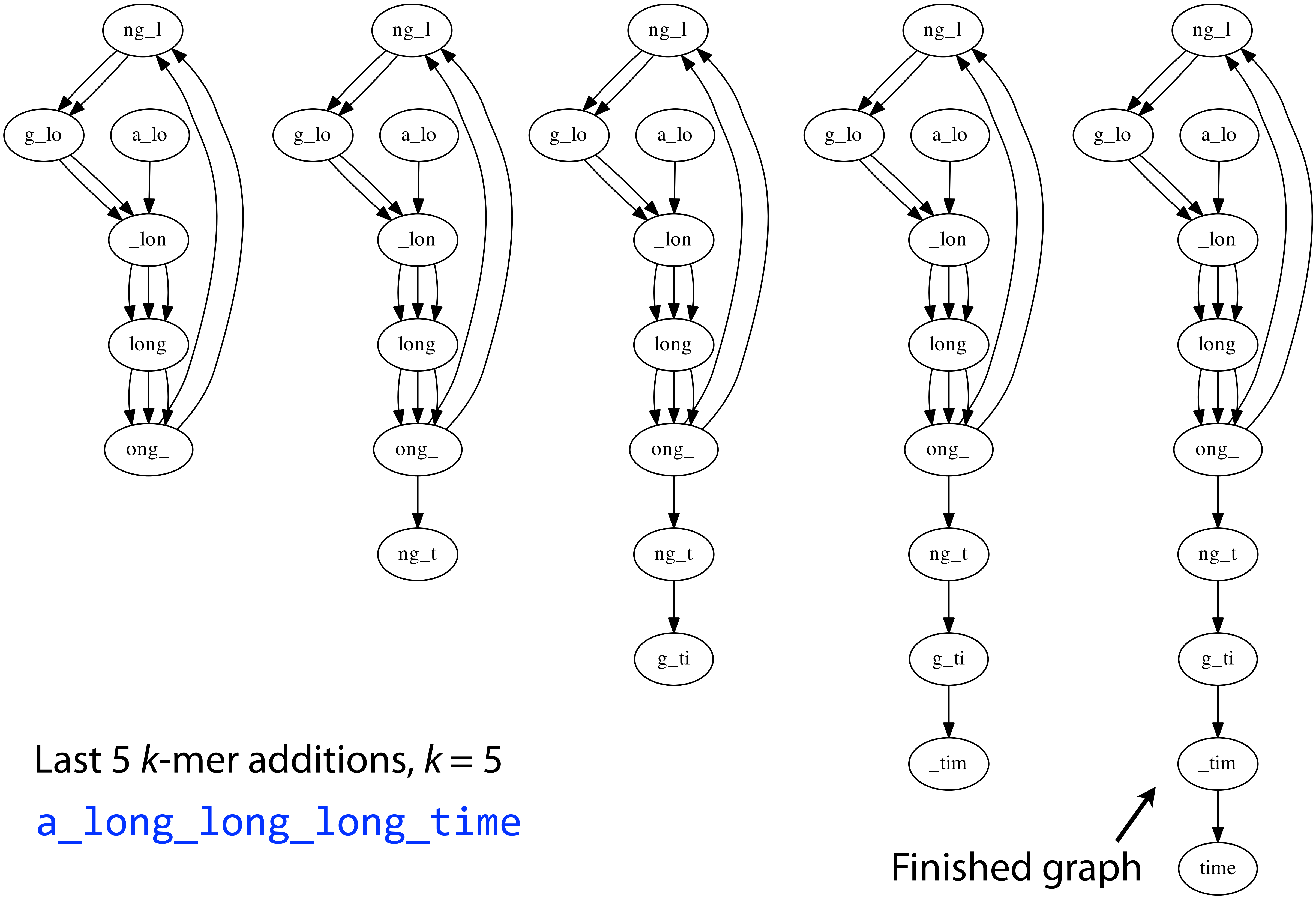
De Bruijn graph



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

Finished graph

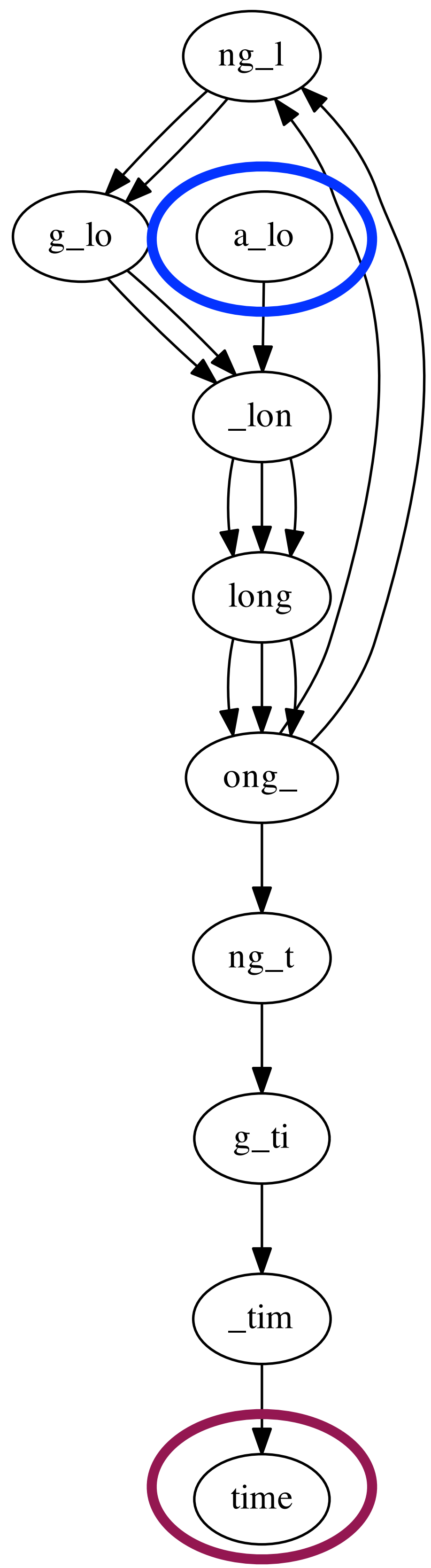
De Bruijn graph

Procedure yields 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



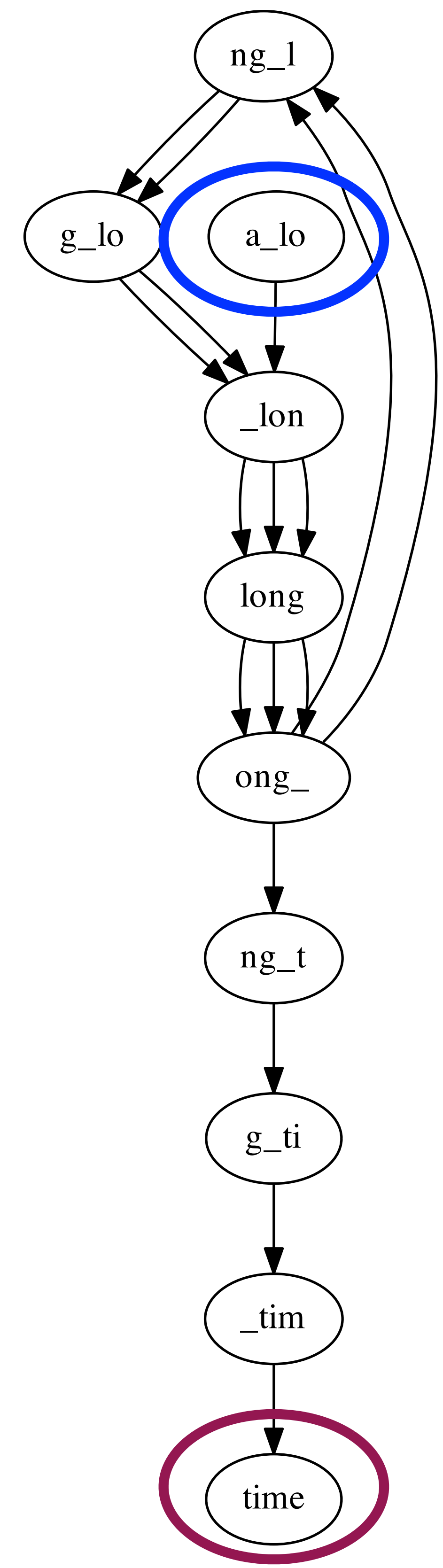
* Unless left- and right-most *k*-1-mers are equal

De Bruijn graph

What string does the Eulerian path spell out?

a_long_long_long_time

The original string! No collapsing!



De Bruijn graph builder implementation

```
class DeBruijnGraph:
    """ A de Bruijn multigraph built from a collection of strings.
        User supplies strings and k-mer length k.  Nodes of the de
        Bruijn graph are k-1-mers and edges join a left k-1-mer to a
        right k-1-mer. """

    @staticmethod
    def chop(st, k):
        """ Chop a string up into k mers of given length """
        for i in xrange(0, len(st)-(k-1)): yield st[i:i+k]

    class Node:
        """ Node in a de Bruijn graph, representing a k-1 mer """
        def __init__(self, km1mer):
            self.km1mer = km1mer

        def __hash__(self):
            return hash(self.km1mer)

    def __init__(self, strIter, k):
        """ Build de Bruijn multigraph given strings and k-mer length k """
        self.G = {}     # multimap from nodes to neighbors
        self.nodes = {} # maps k-1-mers to Node objects
        self.k = k
        for st in strIter:
            for kmer in self.chop(st, k):
                km1L, km1R = kmer[:-1], kmer[1:]
                nodeL, nodeR = None, None
                if km1L in self.nodes:
                    nodeL = self.nodes[km1L]
                else:
                    nodeL = self.nodes[km1L] = self.Node(km1L)
                if km1R in self.nodes:
                    nodeR = self.nodes[km1R]
                else:
                    nodeR = self.nodes[km1R] = self.Node(km1R)
                self.G.setdefault(nodeL, []).append(nodeR)
```

Chop string into *k*-mers

For each *k*-mer, find left and right *k*-1-mers

Create corresponding nodes (if necessary) and add edge

De Bruijn graph

For Eulerian graph, Eulerian walk can be found in O(| *E* |) time. | *E* | is # edges.

Convert graph into one with Eulerian *cycle* (add an edge to make all nodes balanced), then use this recursive procedure

Insight: If C is a cycle in an Eulerian graph, then after removing edges of C, remaining connected components are also Eulerian

```
# Make all nodes balanced, if not already

tour = []
# Pick arbitrary node
src = g.iterkeys().next()

def __visit(n):
  while len(g[n]) > 0:
    dst = g[n].pop()
    __visit(dst)
  tour.append(n)

__visit(src)
# Reverse order, omit repeated node
tour = tour[::-1][:-1]

# Turn tour into walk, if necessary
```

http://www.algorithmist.com/index.php/Eulerian_tour

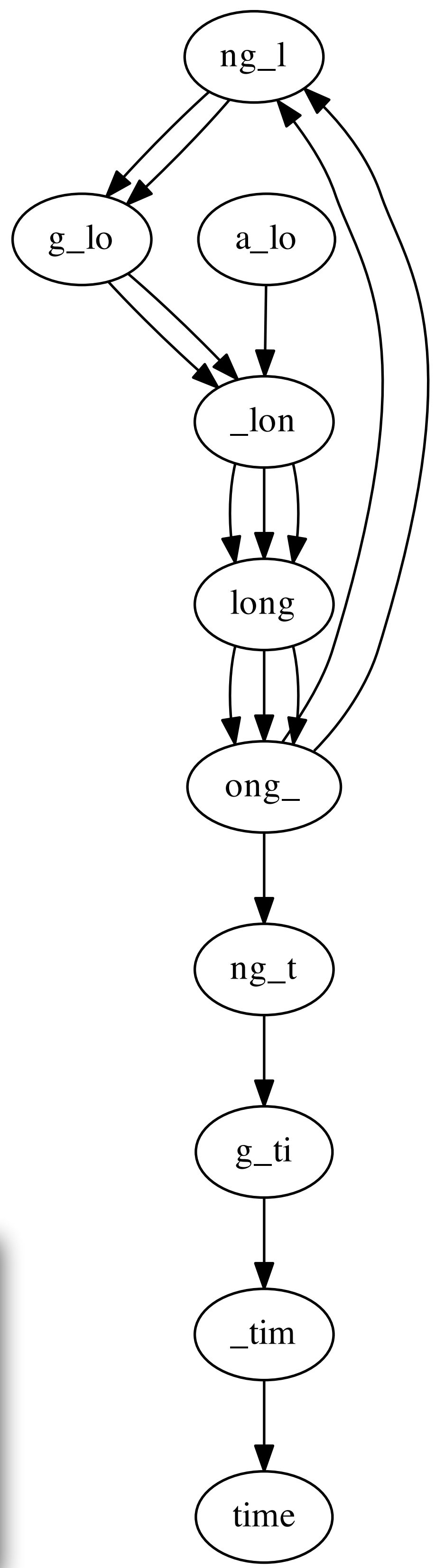
De Bruijn graph

Full illustrative de Bruijn graph and Eulerian walk implementation:

http://bit.ly/CG_DeBruijn

Example where Eulerian walk gives correct answer for small *k* whereas Greedy-SCS could spuriously collapse repeat:

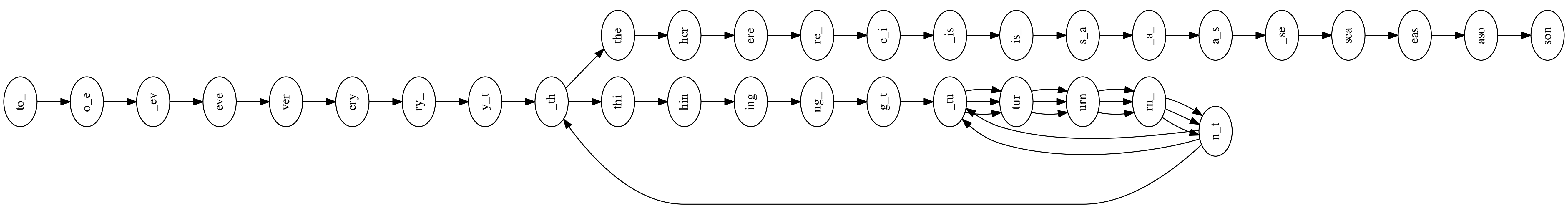
```
>>> G = DeBruijnGraph(["a_long_long_long_time"], 5)
>>> print G.eulerianWalkOrCycle()
['a_lo', '_lon', 'long', 'ong_', 'ng_l', 'g_lo',
'_lon', 'long', 'ong_', 'ng_l', 'g_lo', '_lon',
'long', 'ong_', 'ng_t', 'g_ti', '_tim', 'time']
```



De Bruijn graph

```
>>> st = "to_every_thing_turn_turn_turn_there_is_a_season"
>>> G = DeBruijnGraph([st], 4)
>>> path = G.eulerianWalkOrCycle()  # Fast! Linear in # edges
>>> superstring = path[0] + ''.join(map(lambda x: x[-1], path[1:]))
>>> print superstring
to_every_thing_turn_turn_turn_there_is_a_season
```

http://bit.ly/CG_DeBruijn

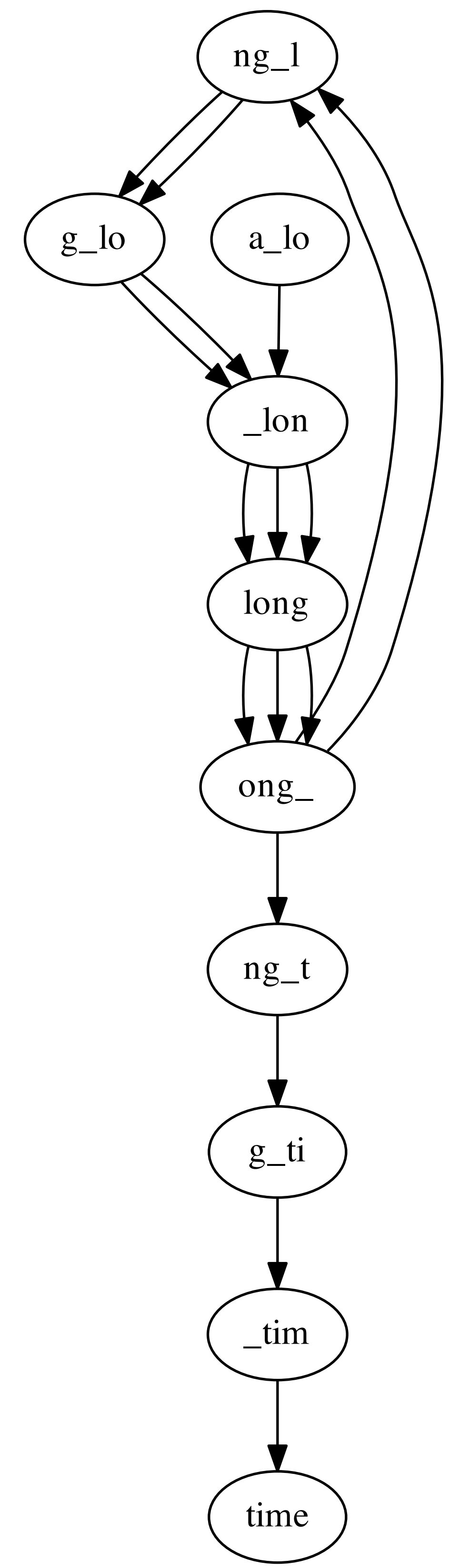


Recall: This is not generally possible or tractable in the overlap/SCS formulation

De Bruijn graph

Assuming perfect sequencing, procedure yields graph with Eulerian walk that can be found efficiently.

We saw cases where Eulerian walk corresponds to the original superstring. Is this always the case?



De Bruijn graph

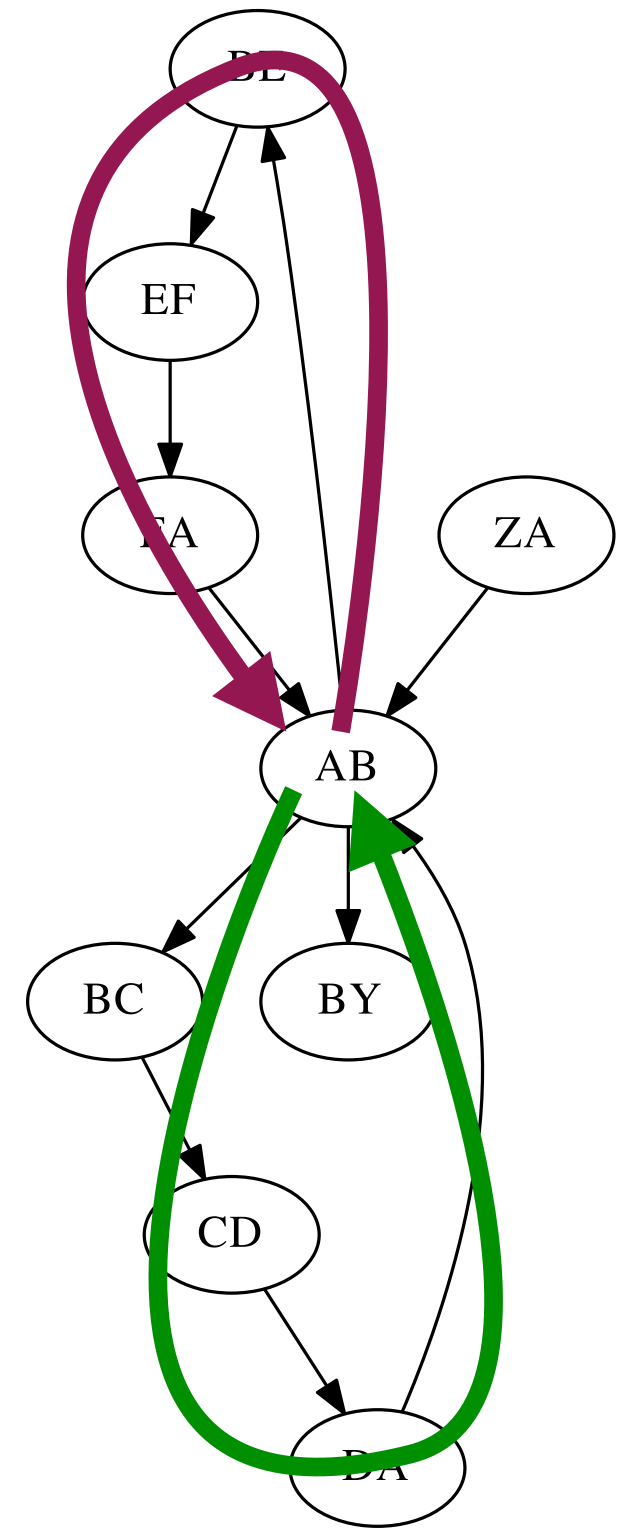
Problem 1: Repeats still cause misassembles

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

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

Problem 2:

We've been building DBGs assuming "perfect" sequencing: each *k*-mer reported exactly once, no mistakes. Real datasets aren't like that.



Third law of assembly

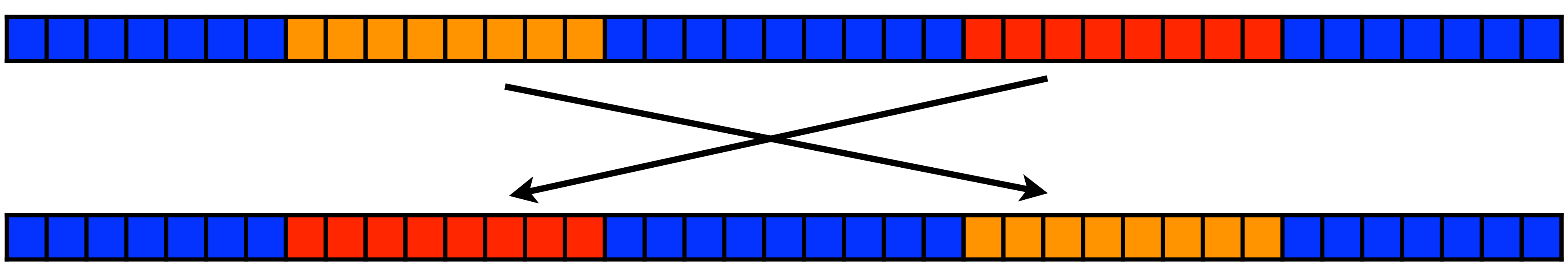
Repeats make assembly difficult; whether we can assemble without mistakes depends on length of reads and repetitive patterns in genome

Collapsing: `a_long_long_long_time`

↓

`a_long_long_time`

Shuffling:

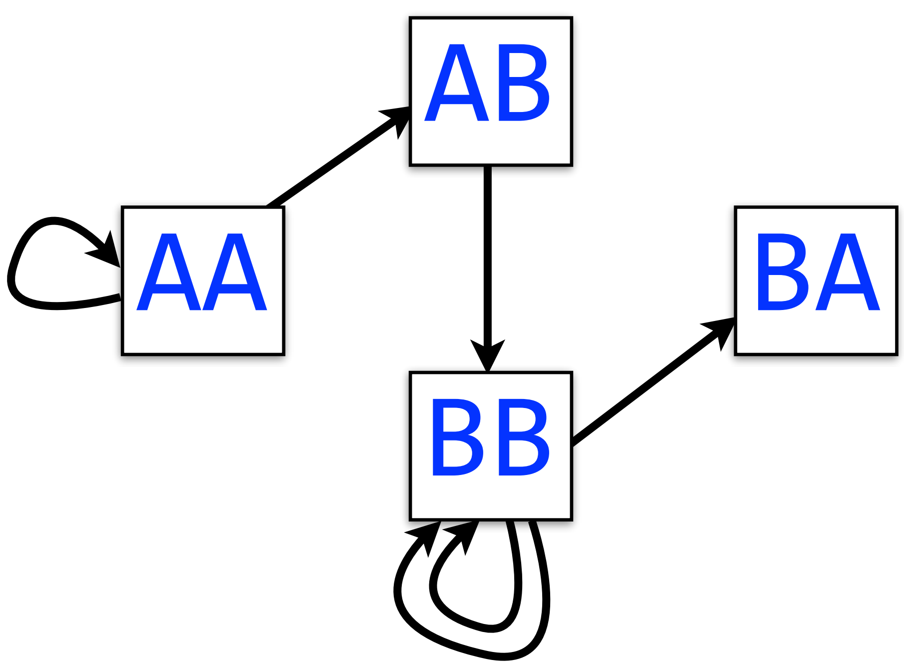


De Bruijn graph

genome: AAABBBBA

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

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



One edge per *k*-mer

One node per distinct *k*-1-mer

De Bruijn graph

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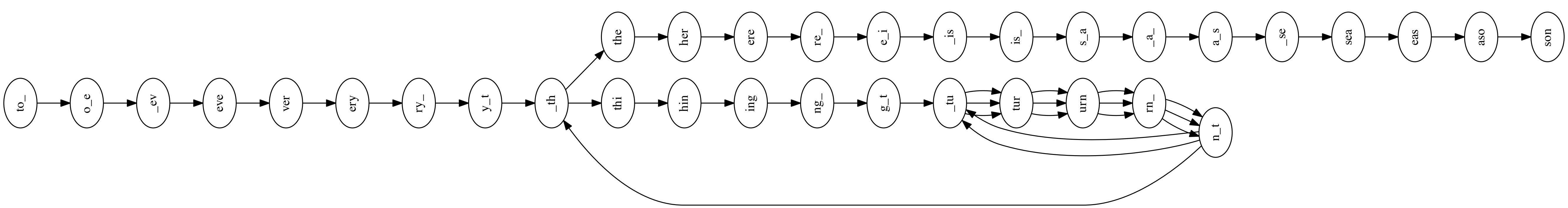

De Bruijn graph

Case where *k* = 4 works:

```
>>> st = "to_every_thing_turn_turn_turn_there_is_a_season"
>>> G = DeBruijnGraph([st], 4)
>>> path = G.eulerianWalkOrCycle()
>>> superstring = path[0] + ''.join(map(lambda x: x[-1], path[1:]))
>>> print superstring
to_every_thing_turn_turn_turn_there_is_a_season
```

But *k* = 3 does not:

```
>>> st = "to_every_thing_turn_turn_turn_there_is_a_season"
>>> G = DeBruijnGraph([st], 3)
>>> path = G.eulerianWalkOrCycle()
>>> superstring = path[0] + ''.join(map(lambda x: x[-1], path[1:]))
>>> print superstring
```

De Bruijn graph

Case where $k = 4$ works:

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>>> G = DeBruijnGraph([st], 4)
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```

But $k = 3$ does not:

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>>> G = DeBruijnGraph([st], 3)
>>> path = G.eulerianWalkOrCycle()
>>> superstring = path[0] + ''.join(map(lambda x: x[-1], path[1:]))
>>> print superstring
to_every_turn_turn_thing_turn_there_is_a_season
```

Due to repeats that are unresolvable at $k = 3$

De Bruijn graph

Problem 1: Repeats still cause misassembles

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

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

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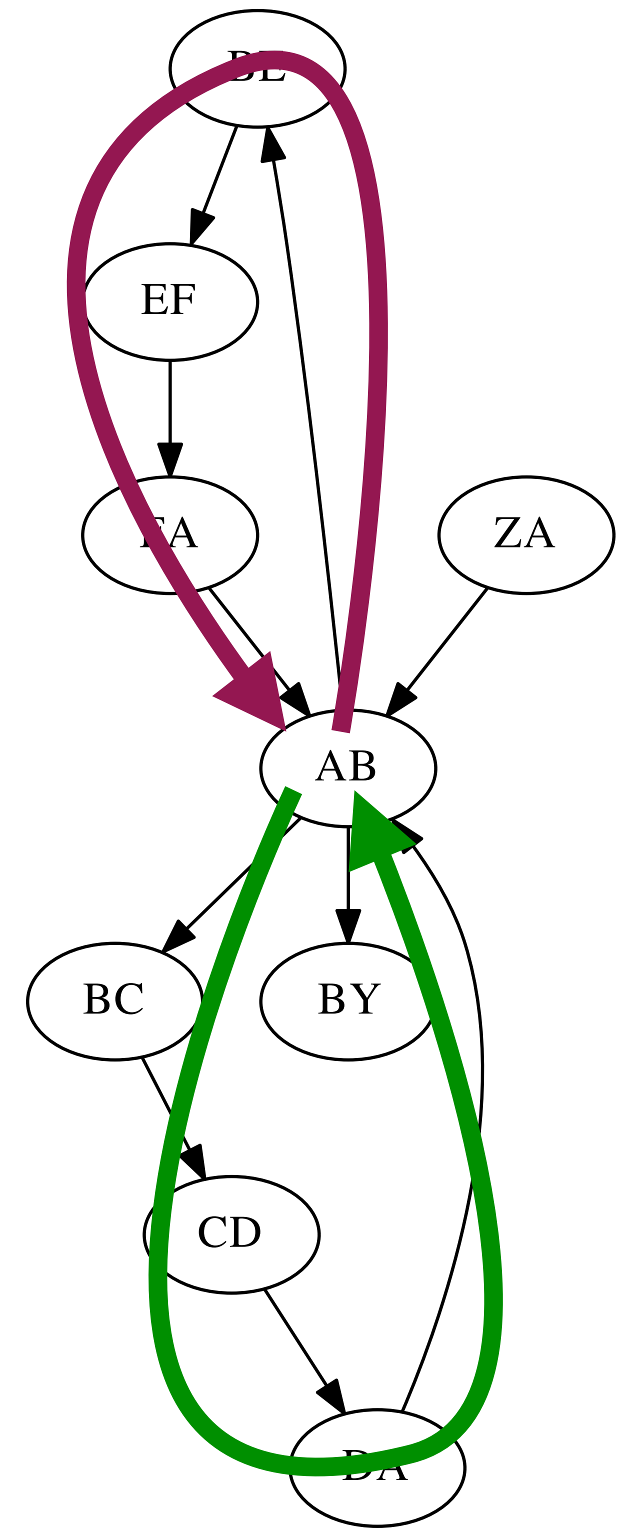
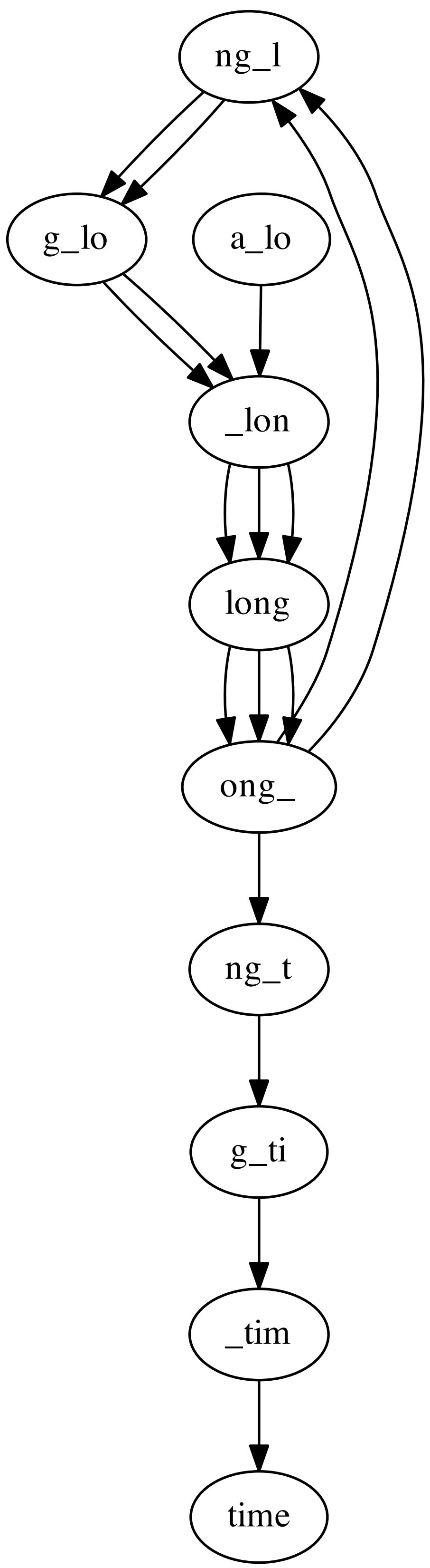


De Bruijn graph

Gaps in coverage (missing *k*-mers) lead to *disconnected* or non-Eulerian graph

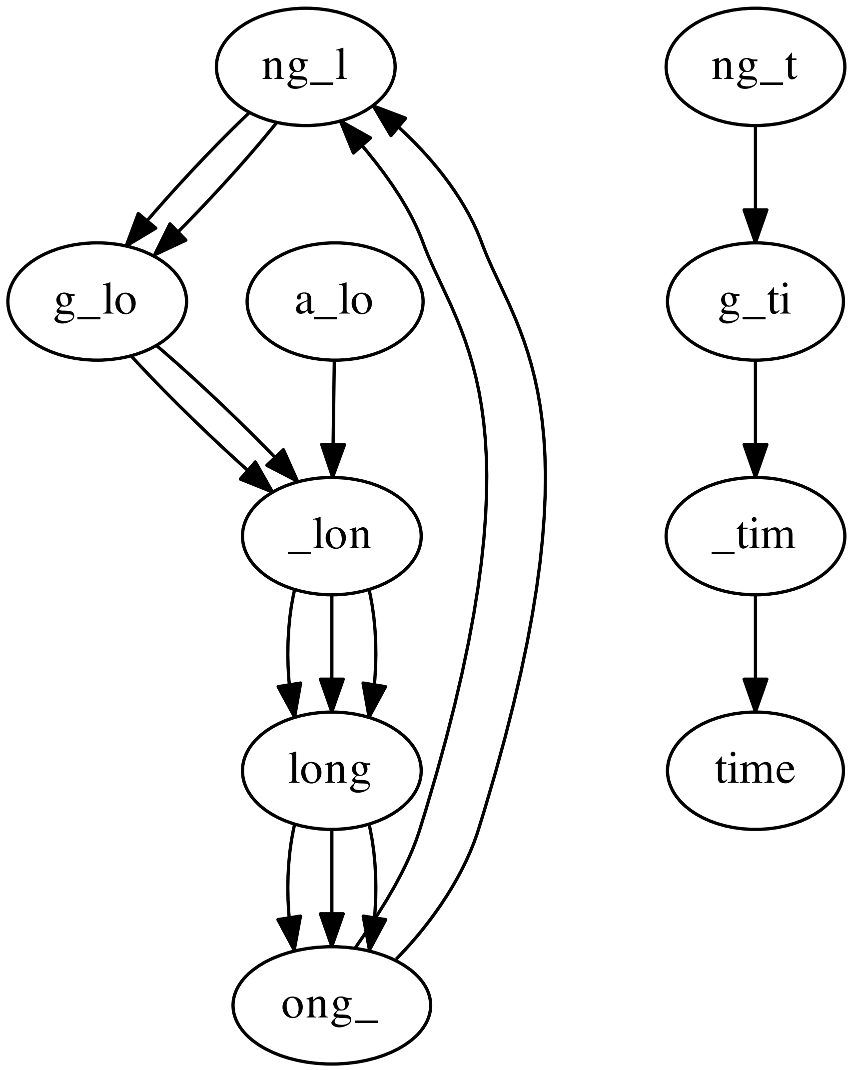
Graph for `a_long_long_long_time`, $k = 5$:



De Bruijn graph

Gaps in coverage (missing *k*-mers) lead to *disconnected* or non-Eulerian graph

Graph for `a_long_long_long_time`, *k* = 5 but *omitting* `ong_t` :

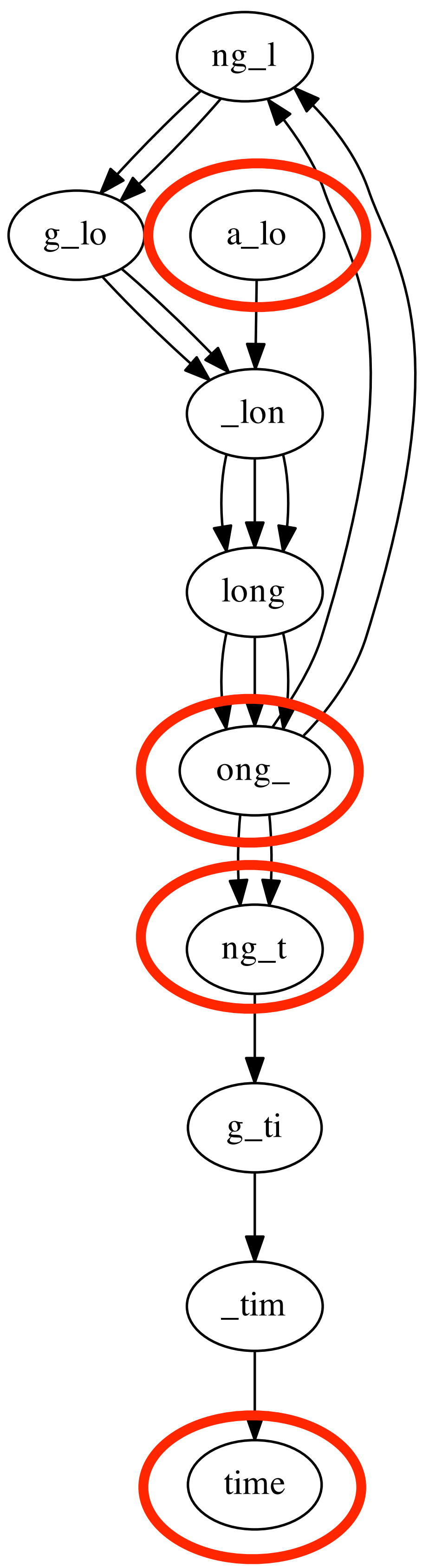


De Bruijn graph

Coverage *differences* make graph non-Eulerian

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

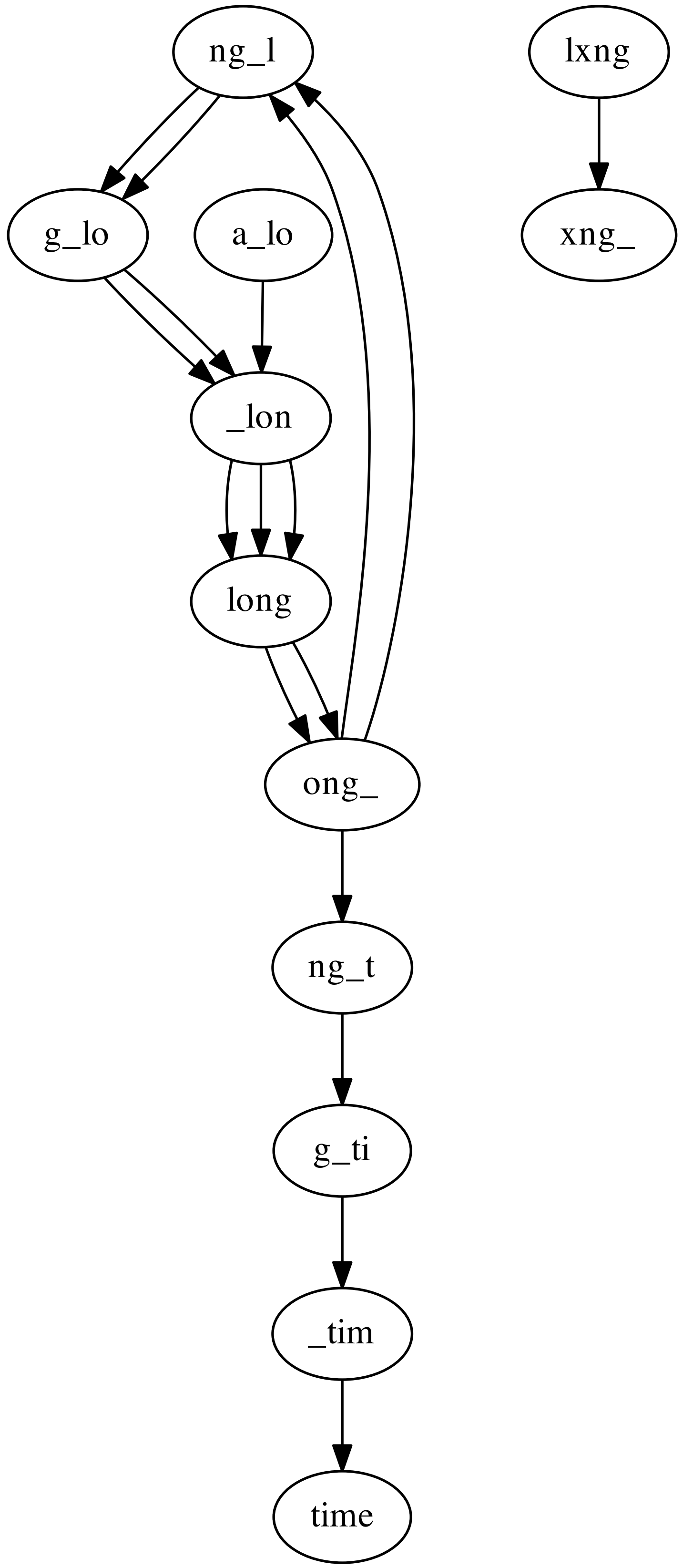
4 semi-balanced nodes



De Bruijn graph

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 one copy of `long_` into `lxng_`



De Bruijn graph

Casting assembly as Eulerian walk is appealing, but not practical

Uneven coverage, sequencing errors, etc make graph non-Eulerian

Even if graph were Eulerian, repeats yield many possible walks

Kingsford, Carl, Michael C. Schatz, and Mihai Pop. "Assembly complexity of prokaryotic genomes using short reads." *BMC bioinformatics* 11.1 (2010): 21.

De Bruijn Superwalk Problem (DBSP) seeks a walk over the De Bruijn graph, where walk contains each read as a *subwalk*

Proven NP-hard!

Medvedev, Paul, et al. "Computability of models for sequence assembly." *Algorithms in Bioinformatics*. Springer Berlin Heidelberg, 2007. 289-301.