De Bruijn Graph assembly

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Real-world assembly methods

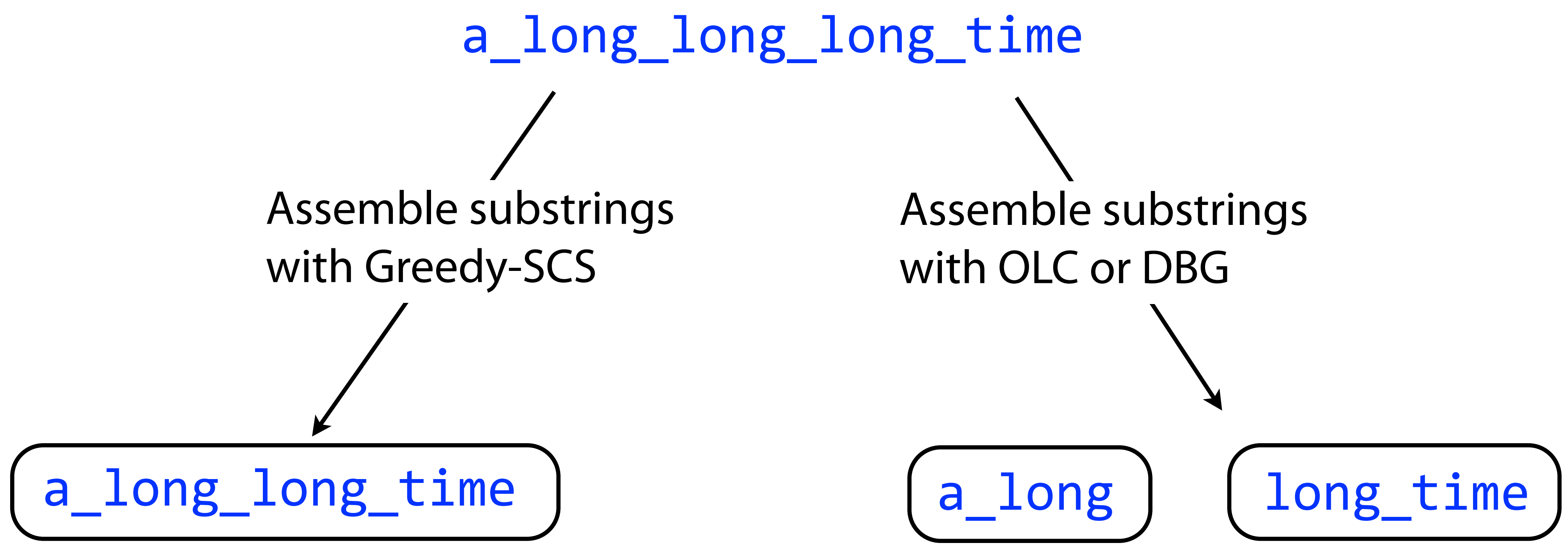
OLC: Overlap-Layout-Consensus assembly

DBG: De Bruijn graph assembly

Both handle unresolvable repeats by essentially *leaving them out*

Unresolvable repeats break the assembly into fragments

Fragments are *contigs* (short for *contiguous*)


a_long_long_long_time
Assemble substrings with Greedy-SCS
Assemble substrings with OLC or DBG
a_long_long_time
a_long
long_time

De Bruijn graph assembly

A formulation conceptually similar to overlapping/SCS, but has some potentially helpful properties not shared by SCS.

k-mer

"*k*-mer" is a substring of length *k*

mer: from Greek meaning "part"

```
             S:  GGCGATTCATCG
    A 4-mer of S:     ATTC
All 3-mers of S:  GGC
                   GCG
                    CGA
                     GAT
                      ATT
                       TTC
                        TCA
                         CAT
                          ATC
                           TCG
```

I'll use "*k*-1-mer" to refer to a substring of length *k* - 1

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.

$$\begin{tikzcd}
& \text{AAB} \arrow[dl] \arrow[dr] & \text{3-mer} \\
\text{AA} & & \text{AB} \\
\text{L} & & \text{R}
\end{tikzcd}$$

AAB's left 2-mer AAB's right 2-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

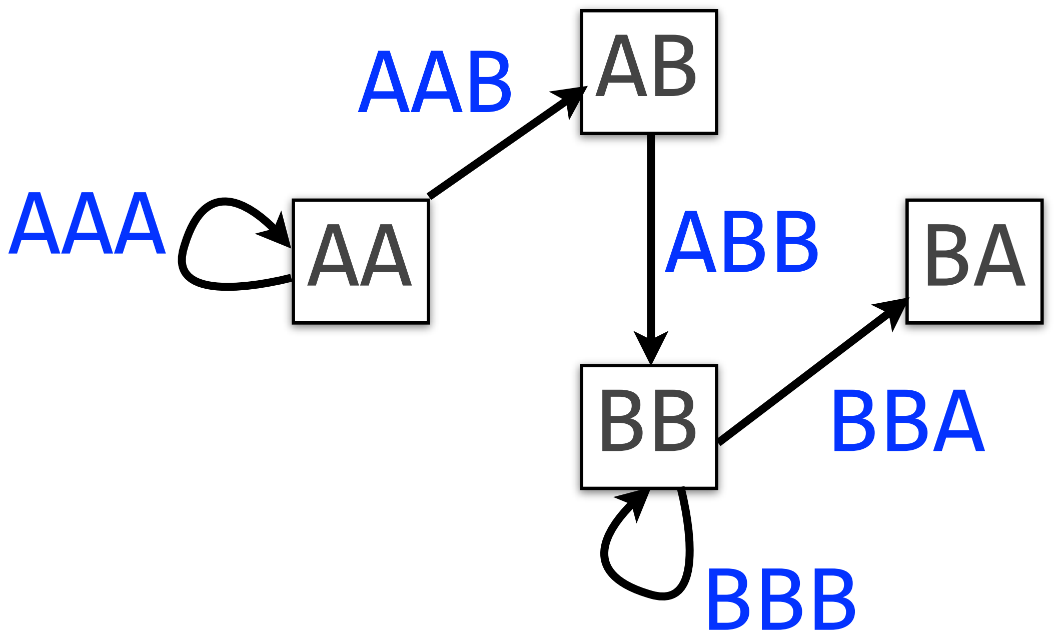
AA	AA	AA	AB	AB	BB	BB	BB	BB	BA
L	R	L	R	L	R	L	R	L	R

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

$$\begin{tikzcd}
 & \boxed{AB} \arrow[dd] & \\
\boxed{AA} \arrow[loop left] \arrow[ur] & & \boxed{BA} \\
 & \boxed{BB} \arrow[loop below] \arrow[ur] &
\end{tikzcd}$$

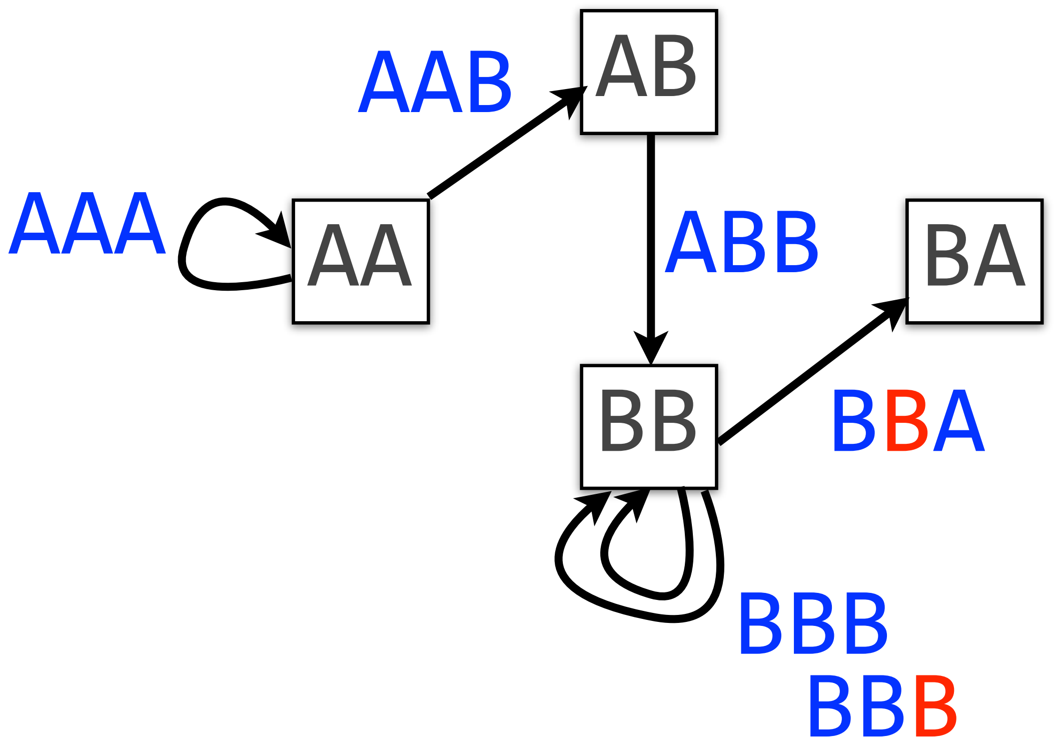
Each *edge* in this graph corresponds to a length-3 input string

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*.

Directed multigraph

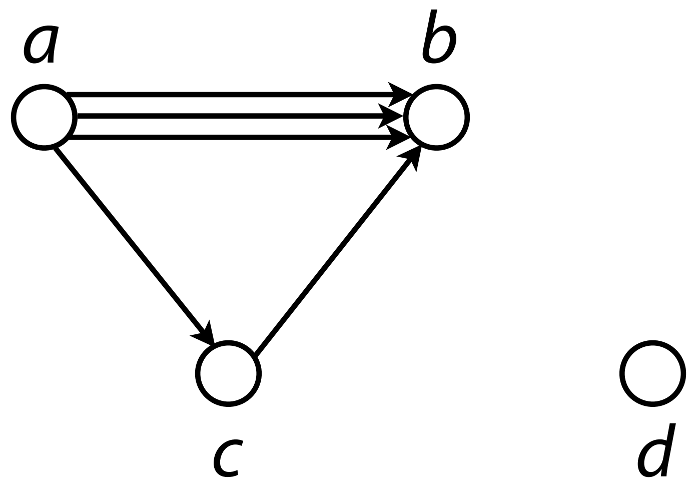
Directed **multigraph** *G*(*V*, *E*) consists of set of *vertices, V* and **multiset** of *directed edges, E*

Otherwise, like a directed graph

Node's *indegree* = # incoming edges

Node's *outdegree* = # outgoing edges

De Bruijn graph is a directed multigraph



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

$$E = \{ \underbrace{(a, b), (a, b), (a, b)}_{\text{Repeated}}, (a, c), (c, b) \}$$

Eulerian walk definitions and statements

Node is *balanced* if indegree equals outdegree

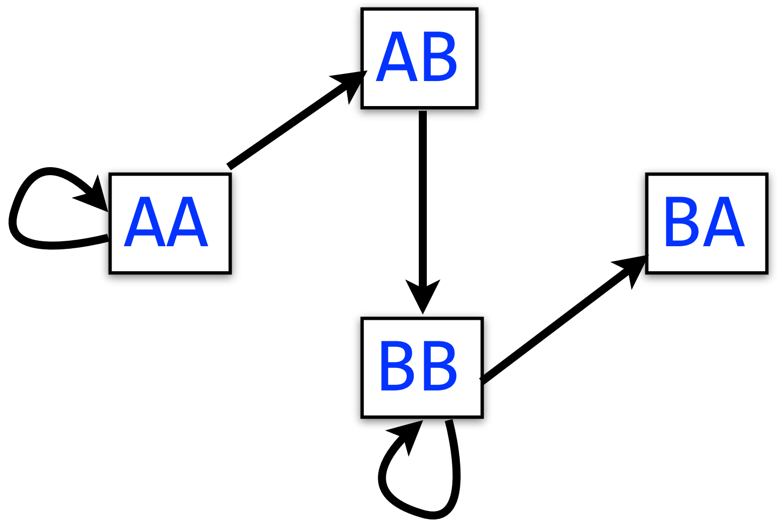
Node is *semi-balanced* if indegree differs from outdegree by 1

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.)

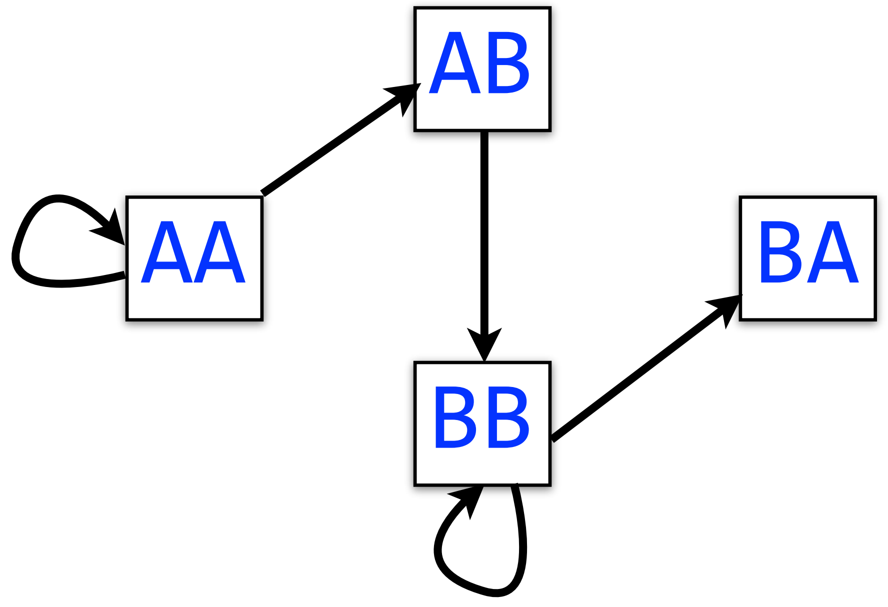
A directed, connected graph is Eulerian if and only if it has at most 2 semi-balanced nodes and all other nodes are balanced

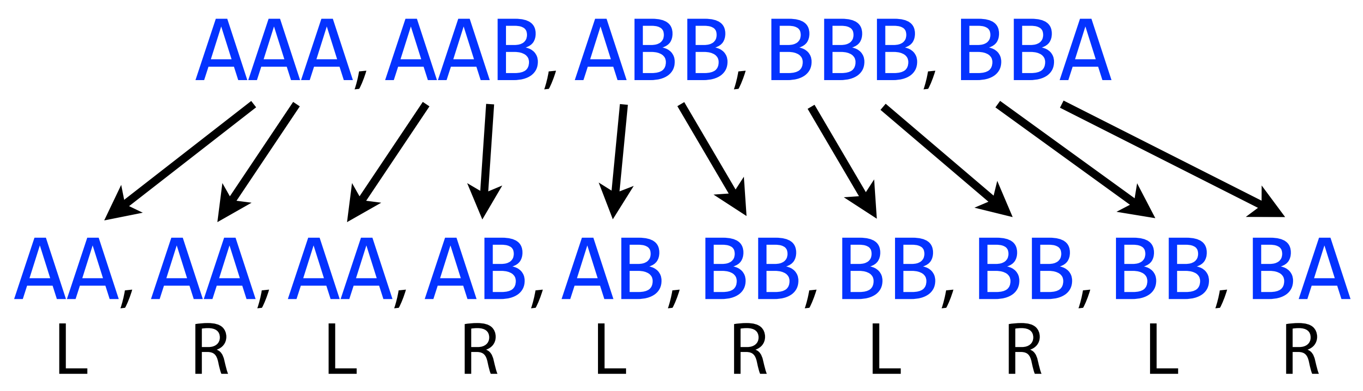


Jones and Pevzner section 8.8

De Bruijn graph

Back to 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

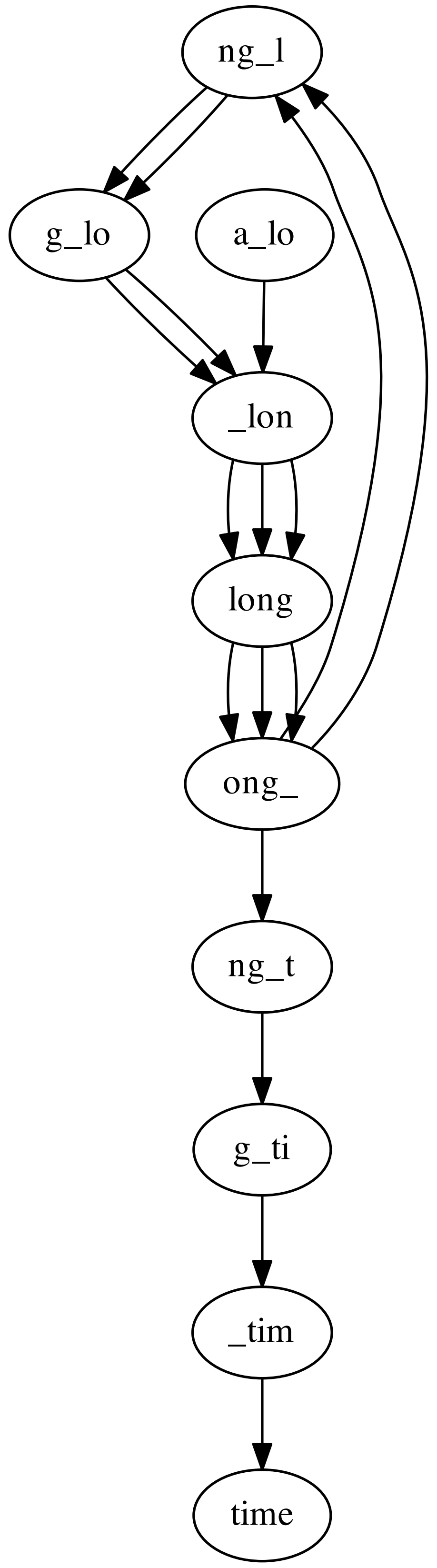
Pick a substring length *k*: 5

Start with an input string: a_long_long_long_time

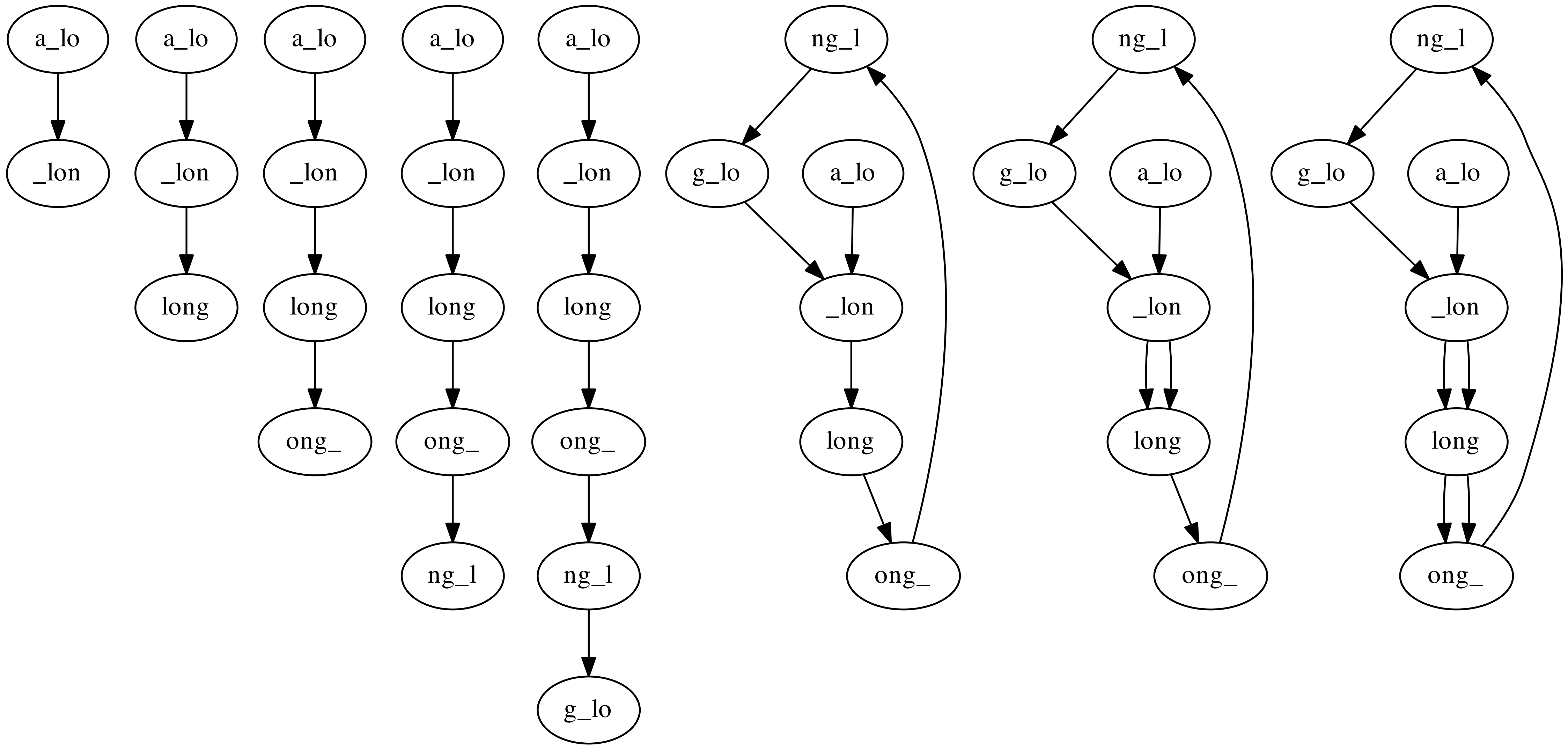
Take each *k* mer and split into left and right *k*-1 mers

$$\begin{tikzcd}
 & \texttt{a\_long\_long\_long\_time} \arrow[d] & \\
 & \texttt{long\_} \arrow[dl] \arrow[dr] & \\
\texttt{long} & & \texttt{ong\_}
\end{tikzcd}$$

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



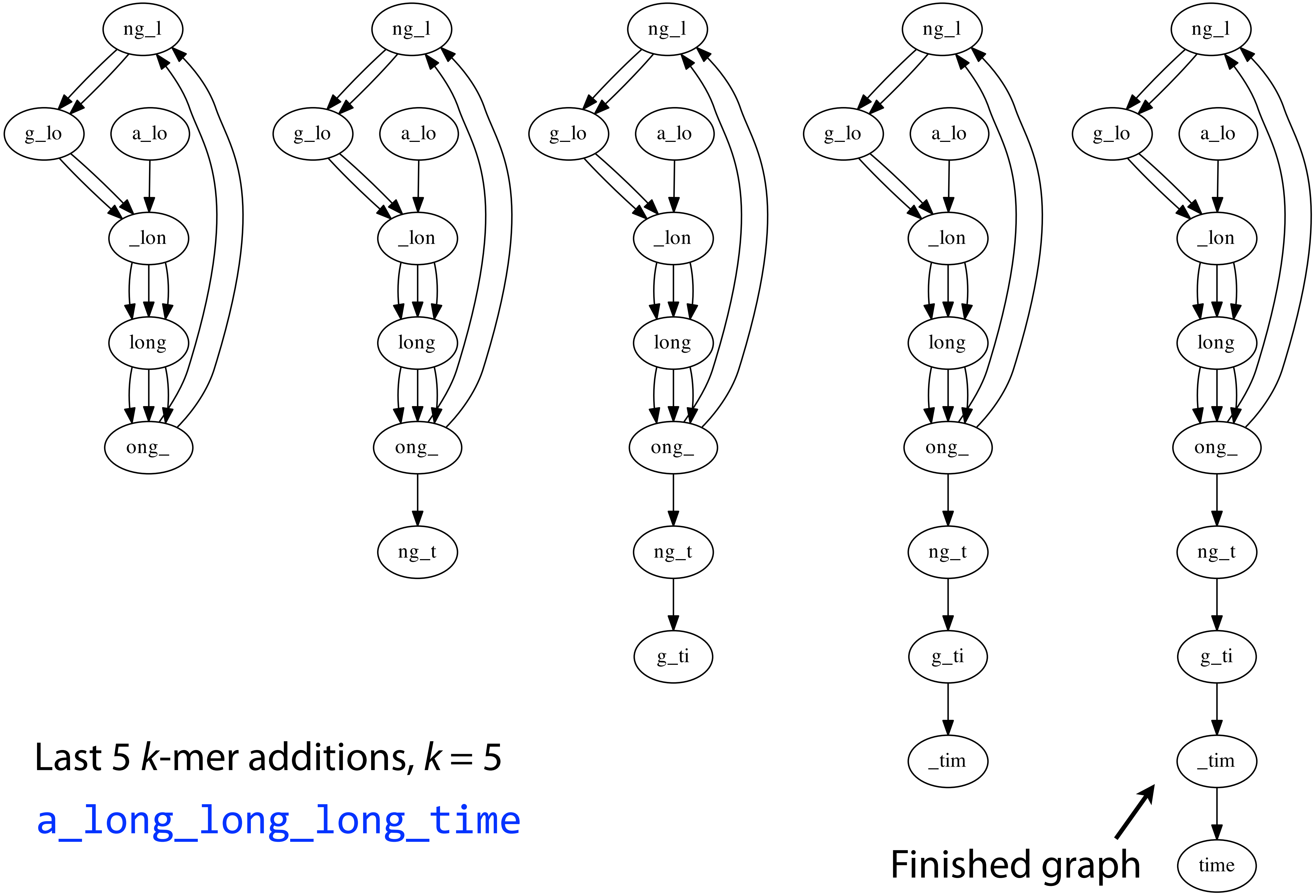
De Bruijn graph



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

De Bruijn graph

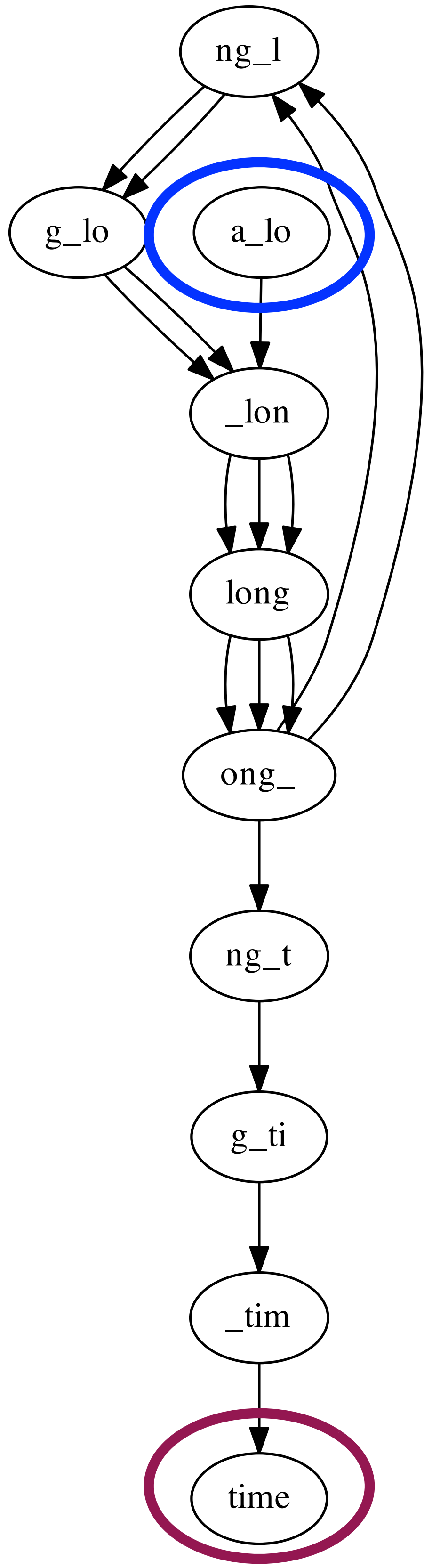
With perfect sequencing, this procedure always yields an Eulerian graph. Why?

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



De Bruijn graph 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

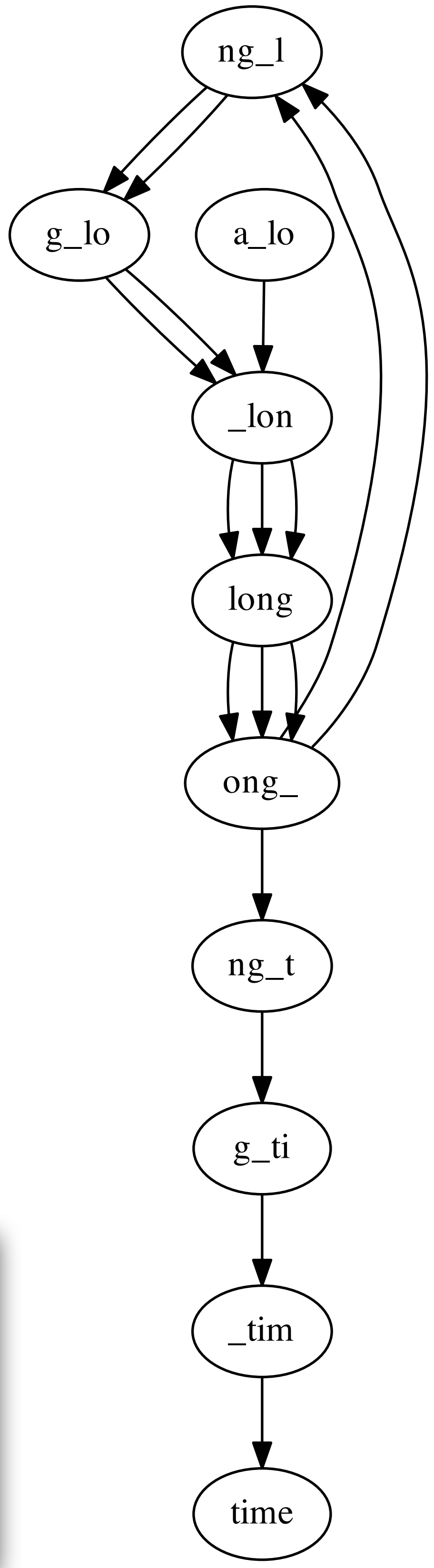
De Bruijn graph

Full illustrative De Bruijn graph and Eulerian walk:

http://nbviewer.ipython.org/7237207

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

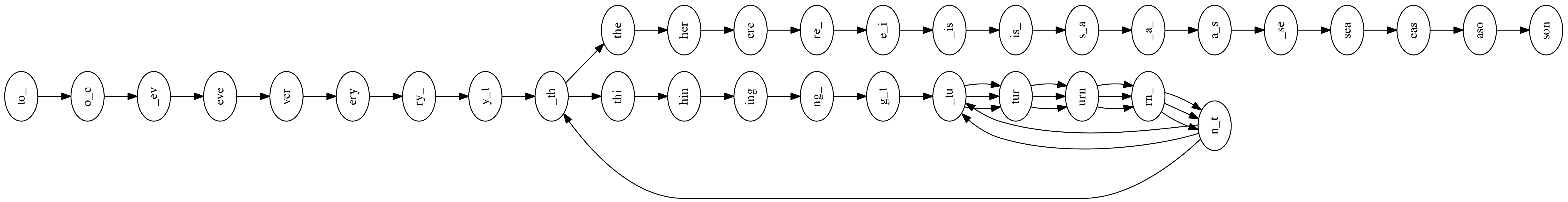
```
>>> G = DeBruijnGraph(["a_long_long_long_time"], 5)
>>> print G.eulerianWalkOrCycle()
['a_lo', '_lon', 'long', 'ong_', 'ng_l', 'g_lo',
'_lon', 'long', 'ong_', 'ng_l', 'g_lo', '_lon',
'long', 'ong_', 'ng_t', 'g_ti', '_tim', 'time']
```



De Bruijn graph

Another example Eulerian walk:

```
>>> 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
```

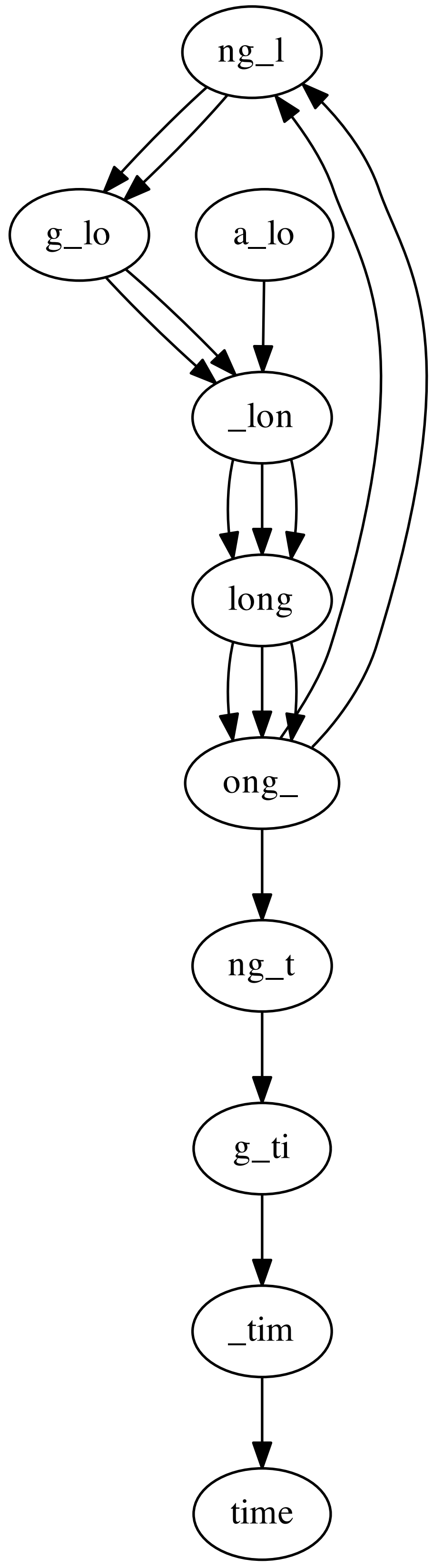


Recall: This is not generally possible or tractable in the overlap/SCS formulation

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

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

Right: graph for ZABCDABEFABY, $k = 3$

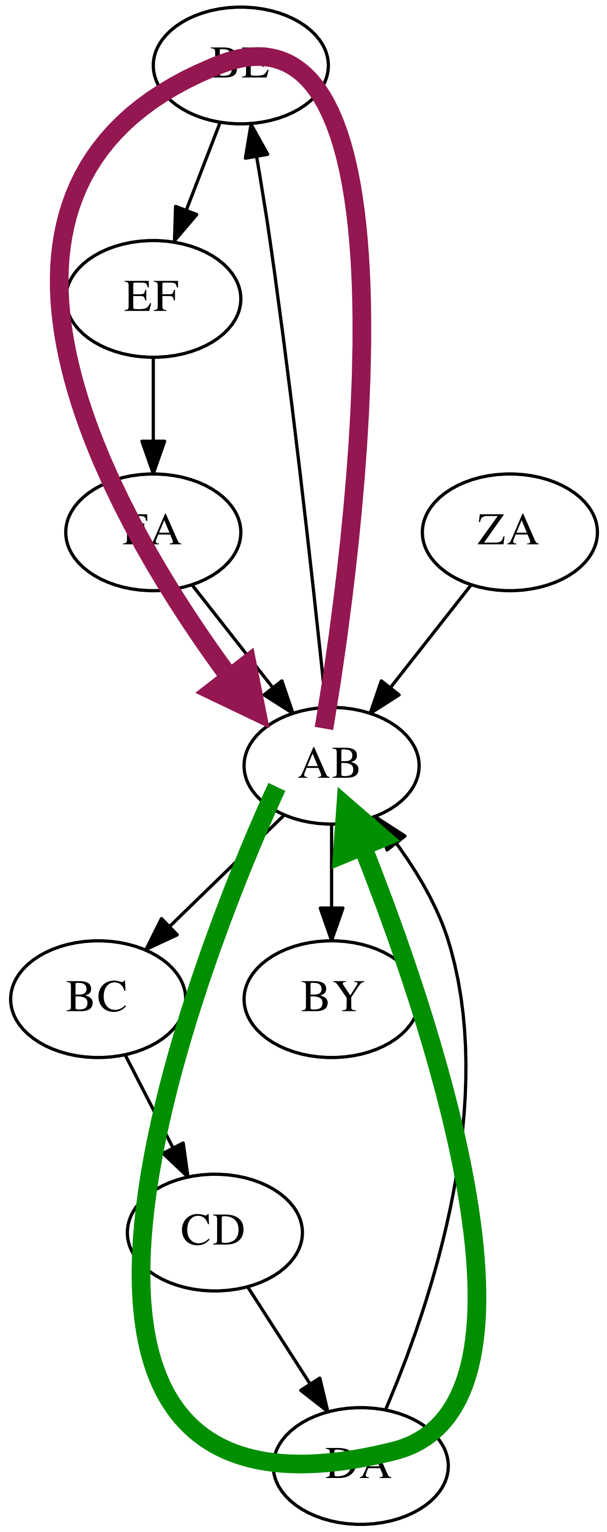
Alternative Eulerian walks:

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



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:

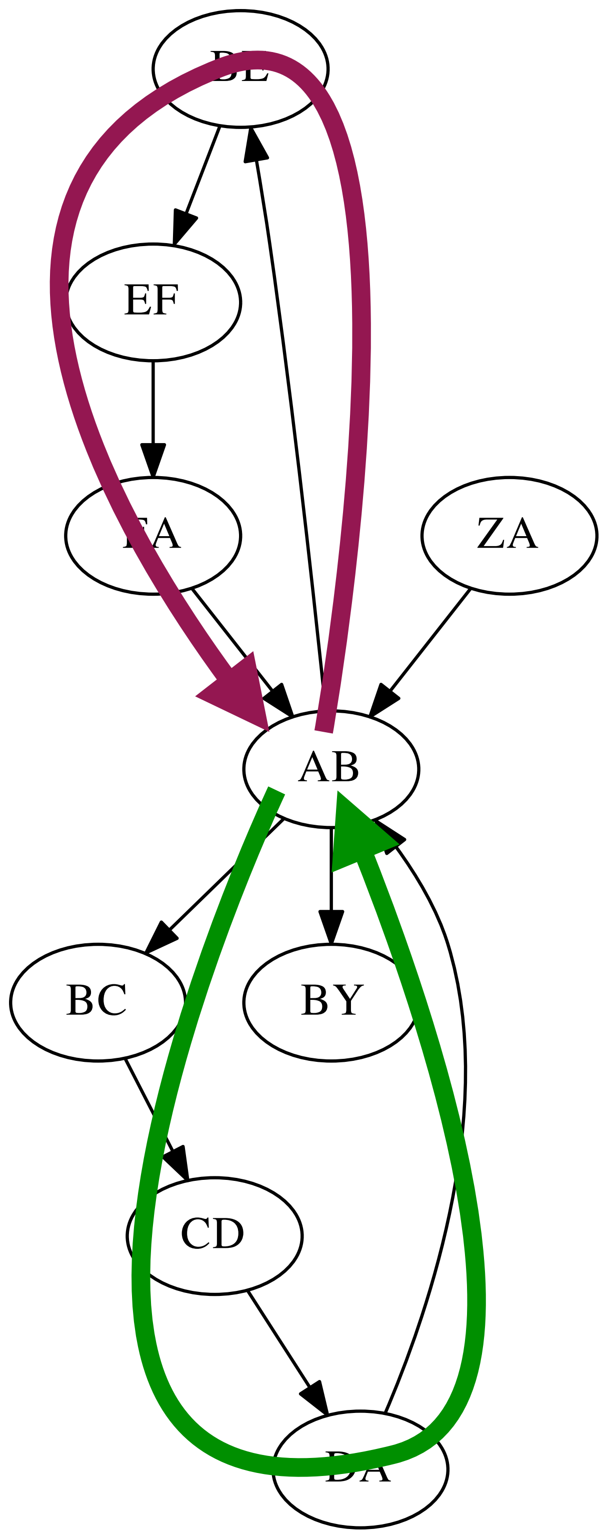
```
>>> st = "to_every_thing_turn_turn_turn_there_is_a_season"
>>> G = DeBruijnGraph([st], 3)
>>> path = G.eulerianWalkOrCycle()
>>> superstring = path[0] + ''.join(map(lambda x: x[-1], path[1:]))
>>> print superstring
to_every_turn_turn_thing_turn_there_is_a_season
```

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

De Bruijn graph

This is the first sign that Eulerian walks can't solve all our problems

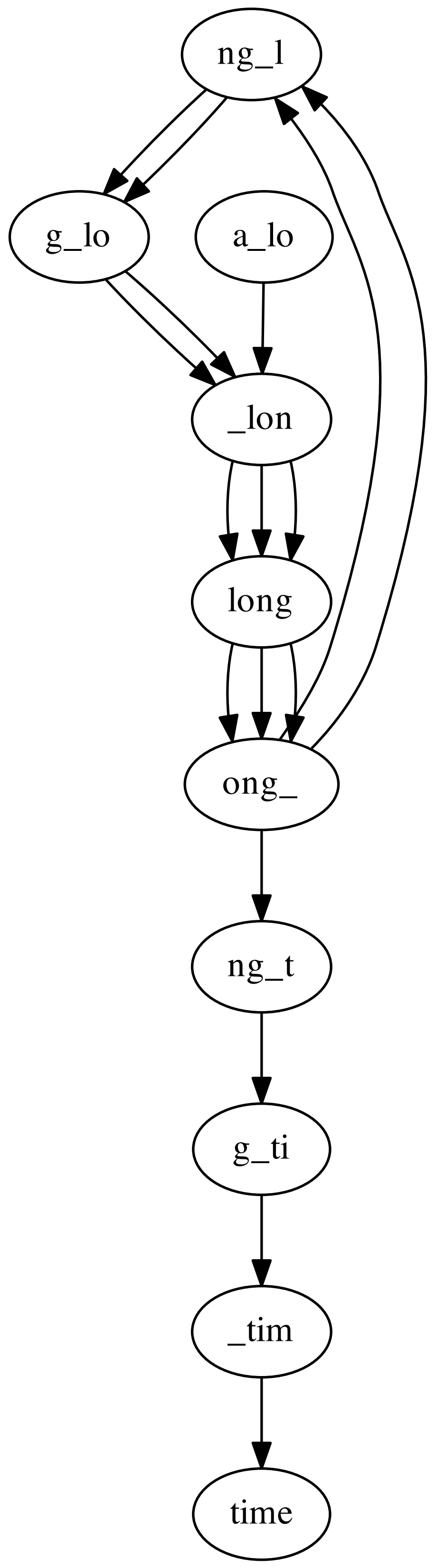
Other signs emerge when we think about how actual sequencing differs from our idealized construction



De Bruijn graph

Gaps in coverage can lead to *disconnected* graph

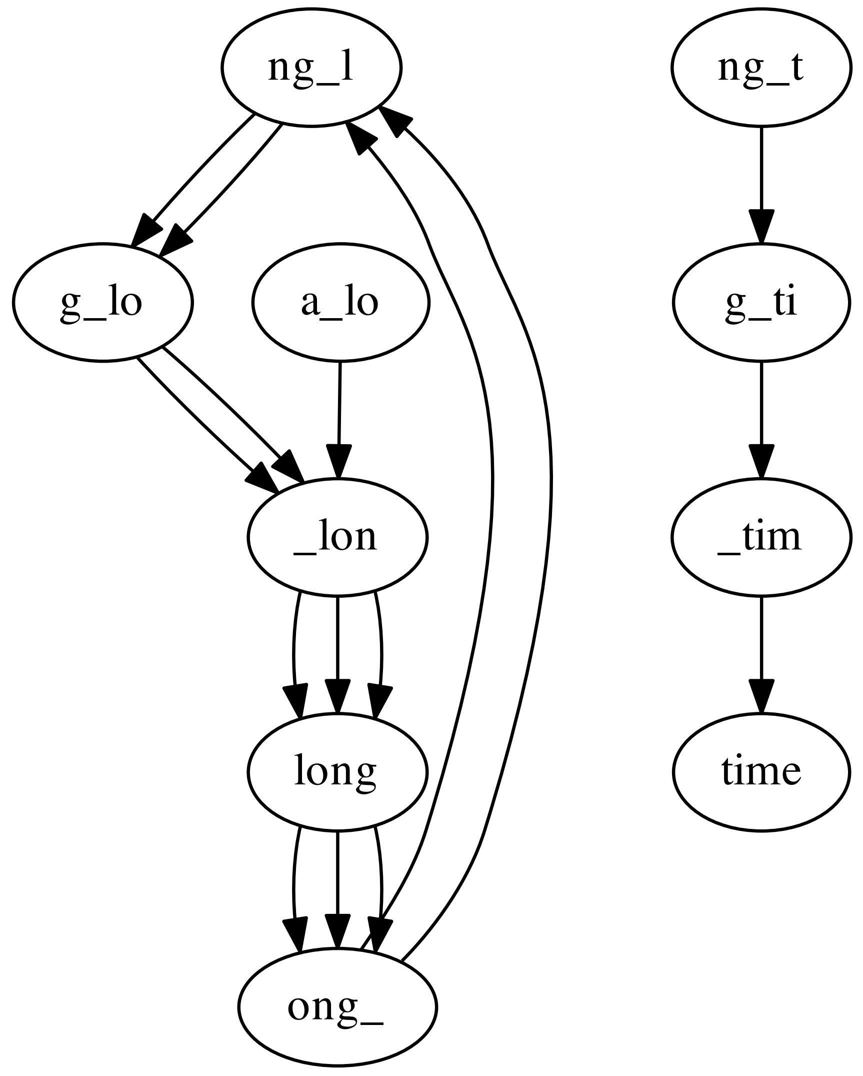
Graph for `a_long_long_long_time`, $k = 5$:



De Bruijn graph

Gaps in coverage can lead to *disconnected* graph

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



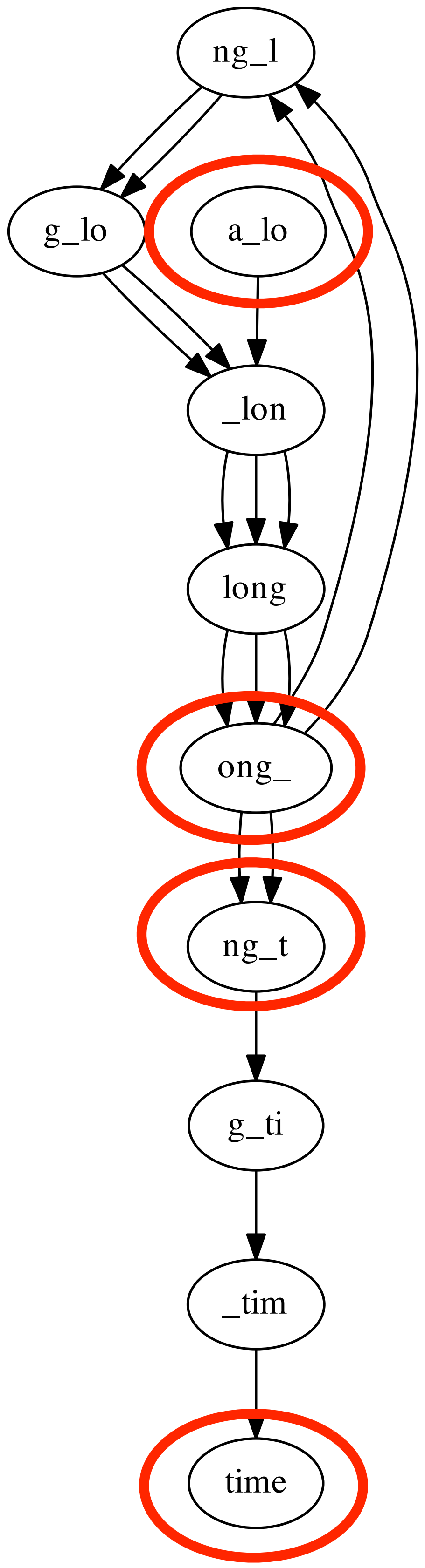
Connected components are individually Eulerian, overall graph is not

De Bruijn graph

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

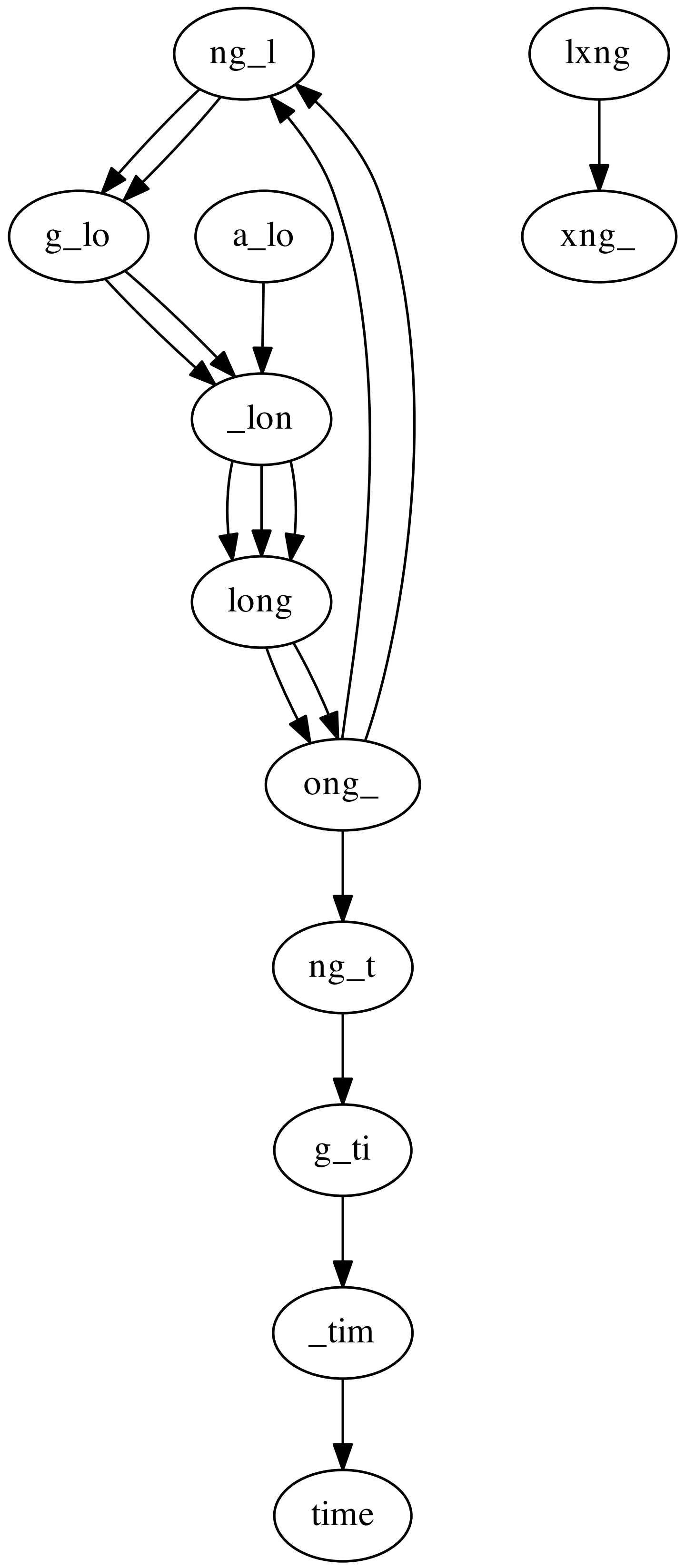


De Bruijn graph

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 a copy of `long_` into `lxng_`

Graph is not connected; largest component is not Eulerian



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) is an improved formulation where we seek 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.

De Bruijn graph

In practice, De Bruijn graph-based tools give up on unresolvable repeats and yield fragmented assemblies, just like OLC tools.

But first we note that using the De Bruijn graph representation has **other advantages**...

De Bruijn graph

Say a sequencer produces **d** reads of length **n** from a genome of length **m**

$$\left.\begin{array}{l}\mathbf{d} = 6 \times 10^9 \text{ reads} \\ \mathbf{n} = 100 \text{ nt}\end{array}\right\} \approx 1 \text{ sequencing run}$$

$\mathbf{m} = 3 \times 10^9$ nt $\approx$ human

To build a De Bruijn graph in practice:

Pick *k*. Assume $k \leq$ shortest read length (k = 30 to 50 is common).

```
For each read:
    For each k-mer:
        Add k-mer's left and right k-1-mers to graph if not there
        already.  Draw an edge from left to right k-1-mer.
```

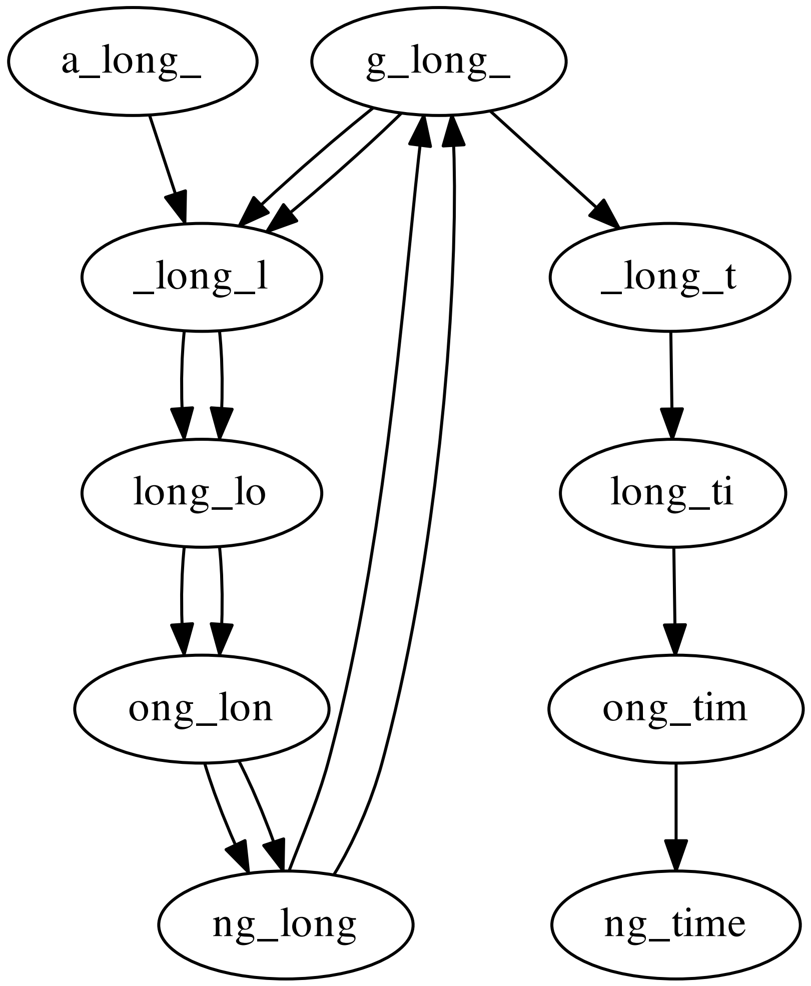
De Bruijn graph

Pick $k = 8$ Genome: `a_long_long_long_time`

Reads: `a_long_long_long`, `ng_long_l`, `g_long_time`

k-mers:

```
a_long_l              ng_long_   g_long_t
 _long_lo              g_long_l   _long_ti
  long_lon                         long_tim
   ong_long                         ong_time
    ng_long_
     g_long_l
      _long_lo
       long_lon
        ong_long
```



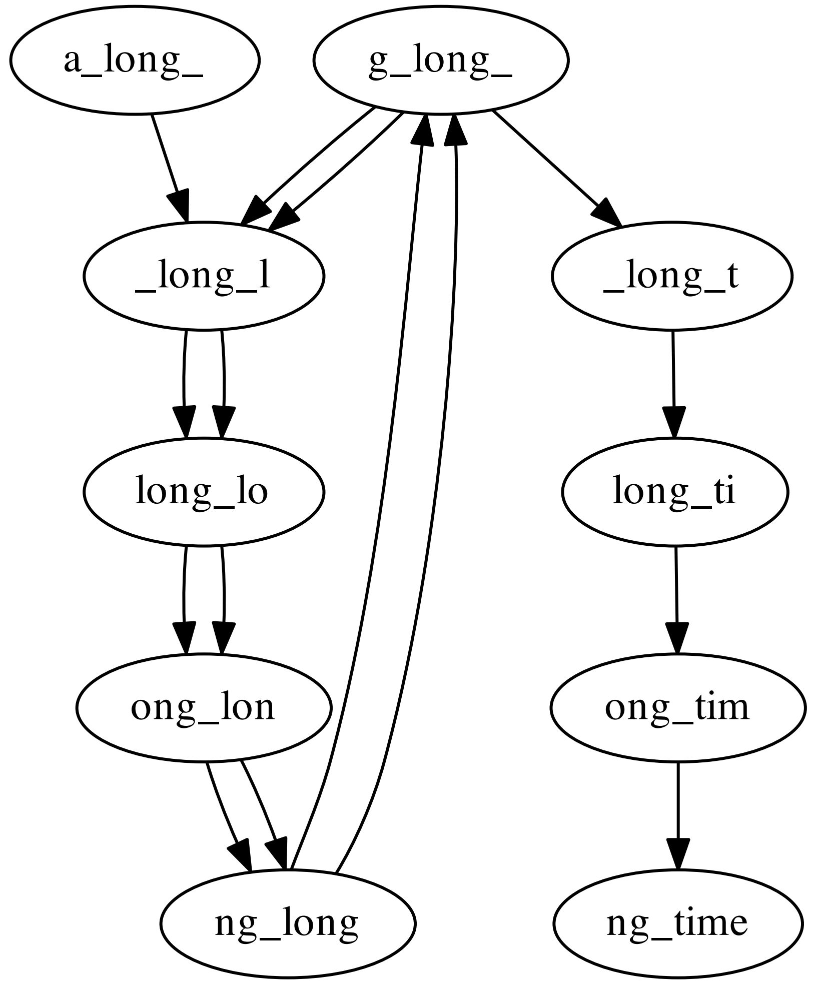
Given n (# reads), N (total length of all reads) and k, and assuming k < length of shortest read:

Exact number of k-mers: $N - n(k-1)$ $O(N)$

This is also the number of edges, $|E|$

Number of nodes $|V|$ is at most $2 \cdot |E|$, but typically much smaller due to repeated k-1-mers

De Bruijn graph



How much work to build graph?

For each *k*-mer, add 1 edge and up to 2 nodes

Reasonable to say this is O(1) expected work

Assume hash map encodes nodes & edges

Assume *k*-1-mers fit in O(1) machine words, and hashing O(1) machine words is O(1) work

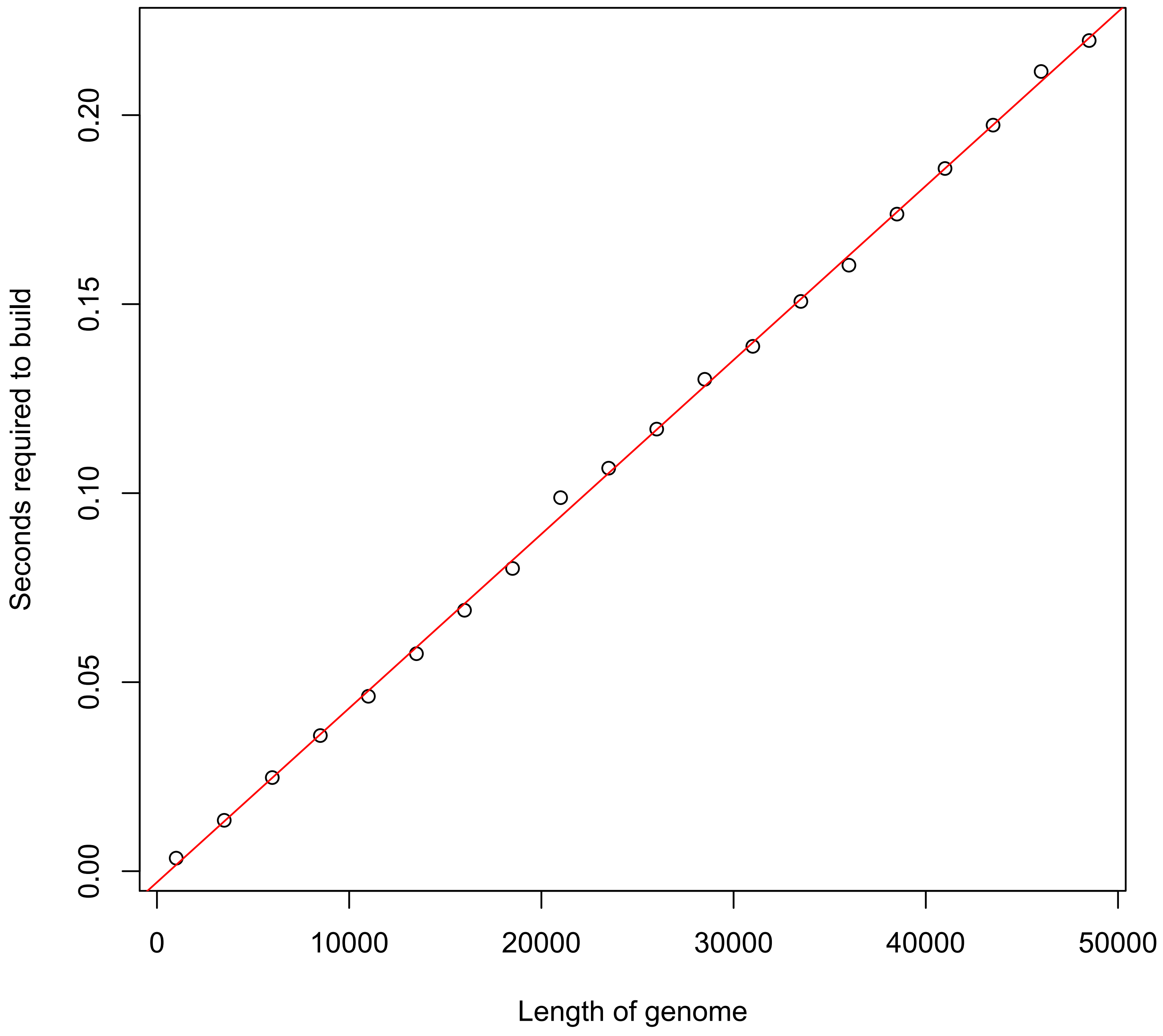
Querying / adding a key is O(1) expected work

O(1) expected work for 1 *k*-mer, O(*N*) overall

De Bruijn graph

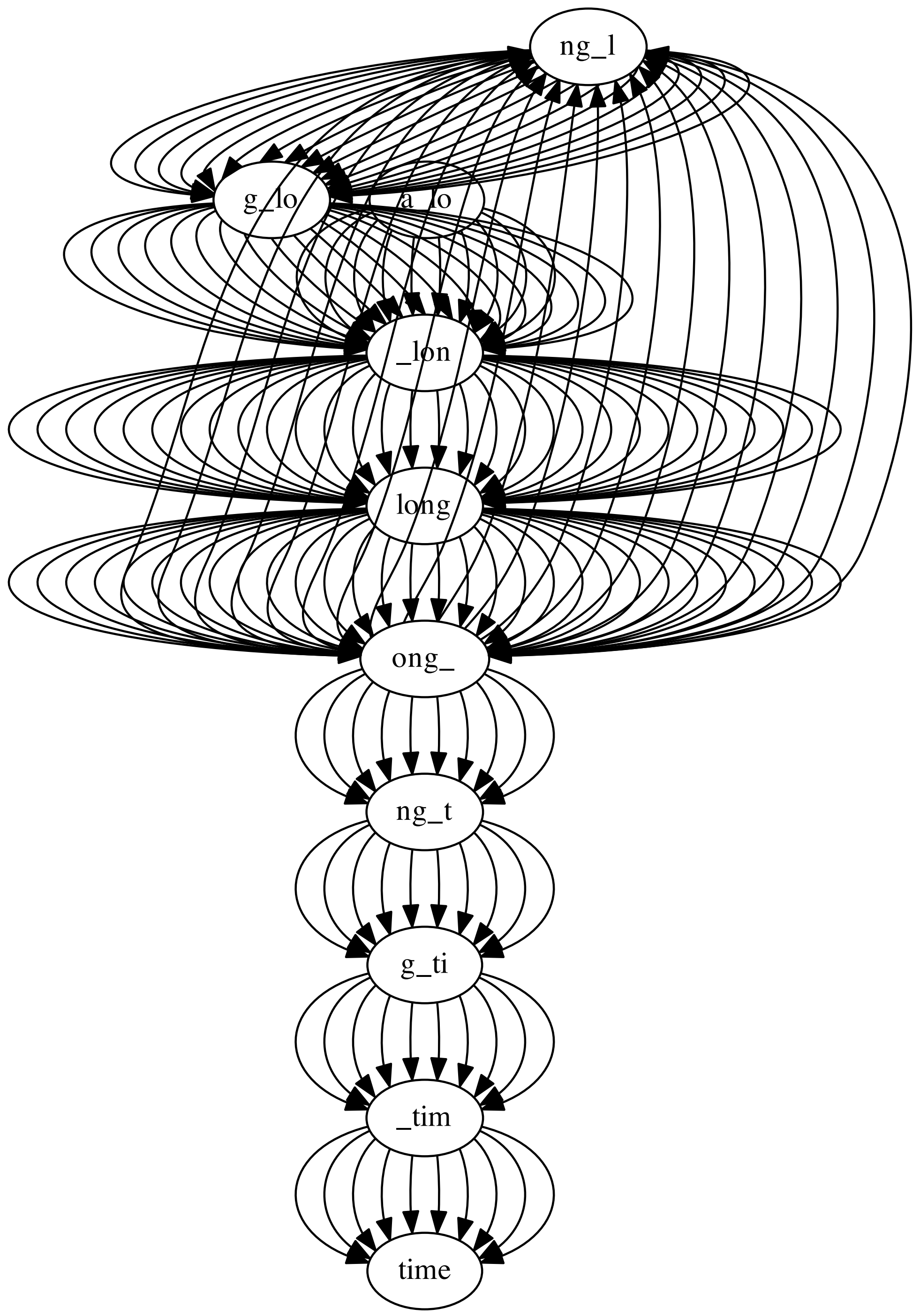
Timed De Bruijn graph construction applied to progressively longer prefixes of lambda phage genome, $k = 14$

O(N) expectation appears to work in practice, at least for this small example



De Bruijn graph

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



De Bruijn graph

Recall *average coverage*: average # reads covering a genome position

```
                  CTAGGCCCTCAATTTTT
                CTCTAGGCCCTCAATTTTT
              GGCTCTAGGCCCTCATTTTTT
           CTCGGCTCTAGCCCTCATTTT
        TATCTCGACTCTAGGCCCTCA                177 nucleotides
        TATCTCGACTCTAGGCC
    TCTATATCTCGGCTCTAGG
GGCGTCTATATCTCG
GGCGTCGATATCT
GGCGTCTATATCT
GGCGTCTATATCTCGGCTCTAGGCCCTCATTTTTT          35 nucleotides
```

Average coverage = 177 / 35 ≈ 7x

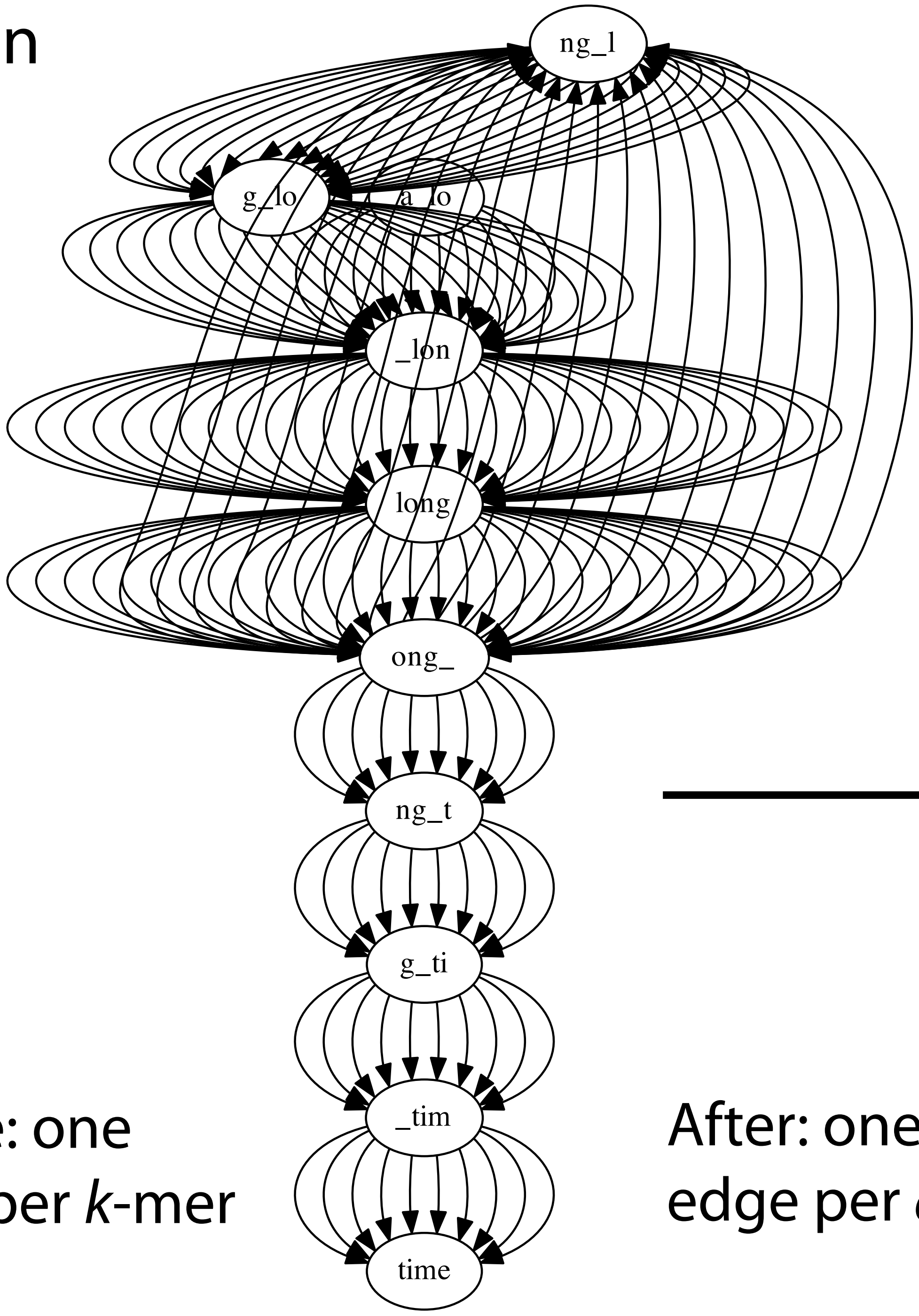
De Bruijn graph

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

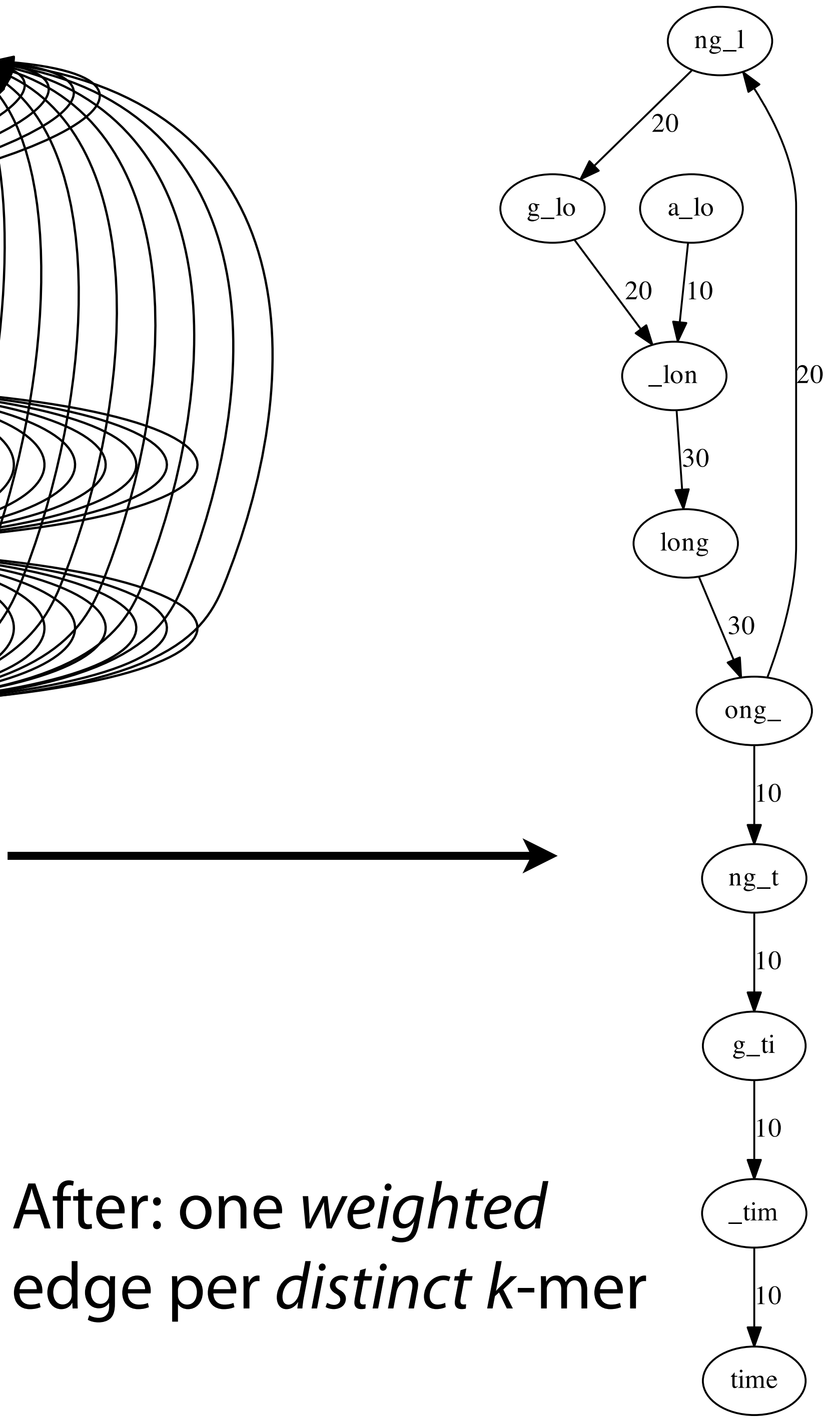
Same edge might appear in dozens of copies; let's use edge *weights* instead

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

De Bruijn graph

# of nodes and edges both O(*N*); *N* is total length of all reads

Say (a) reads are error-free, (b) we have one *weighted* edge for each *distinct* *k*-mer, and (c) length of genome is *G*

- There's one node for each distinct *k*-1-mer, one edge for each distinct *k*-mer

- Can't be more distinct *k*-mers than there are *k*-mers in the genome; likewise for *k*-1-mers

- So # of nodes and edges are also both O(*G*)

- Combine with the O(*N*) bound and the # of nodes and edges are both O(min(*N*, *G*))

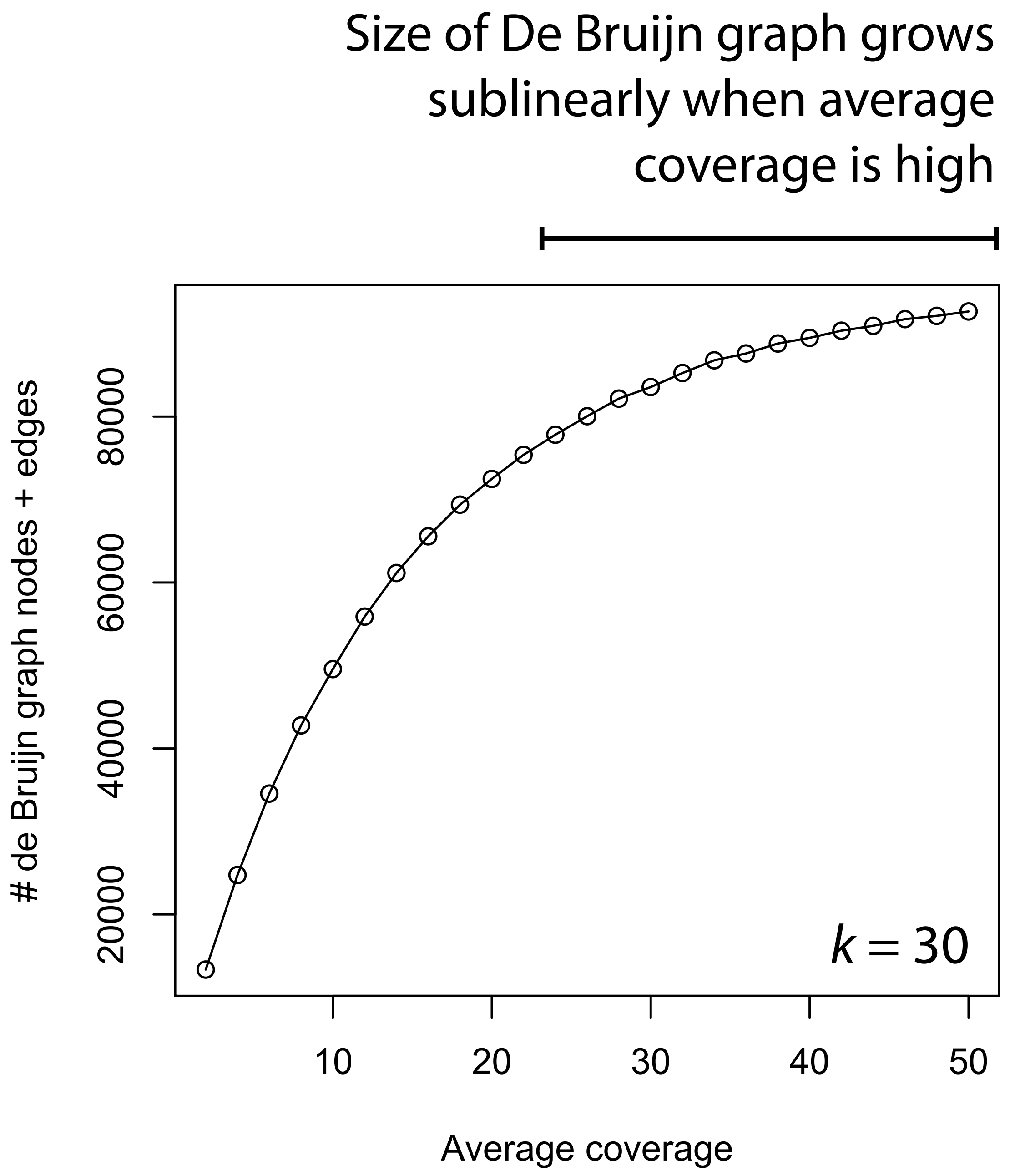
De Bruijn graph

With high average coverage, O(*G*) size bound is advantageous

Genome = lambda phage (~ 48.5 K nt)

Draw random *k*-mers until target average coverage is reached (x axis)

Build De Bruijn graph and total the # of nodes and edges (y axis)



De Bruijn graph

What De Bruijn graph advantages have we discovered?

Can be built in O(N) expected time, N = total length of reads

With perfect data, graph is O(min(N, G)) space; G = genome length

Note: when average coverage is high, $G \ll N$

Compares favorably with overlap graph

Space is O($N + a$).

Fast overlap graph construction (suffix tree) is O($N + a$) time

a is O(n^2)

De Bruijn graph

What did we give up?

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, as we'll see

Read coherence is lost. Some paths through De Bruijn graph are inconsistent with respect to input reads.

This is the OLC $\longleftrightarrow$ DBG tradeoff

Single most important benefit of De Bruijn graph is the O(min(G, N)) space bound, though we'll see this comes with large caveats