CS 364 COMPUTATIONAL BIOLOGY

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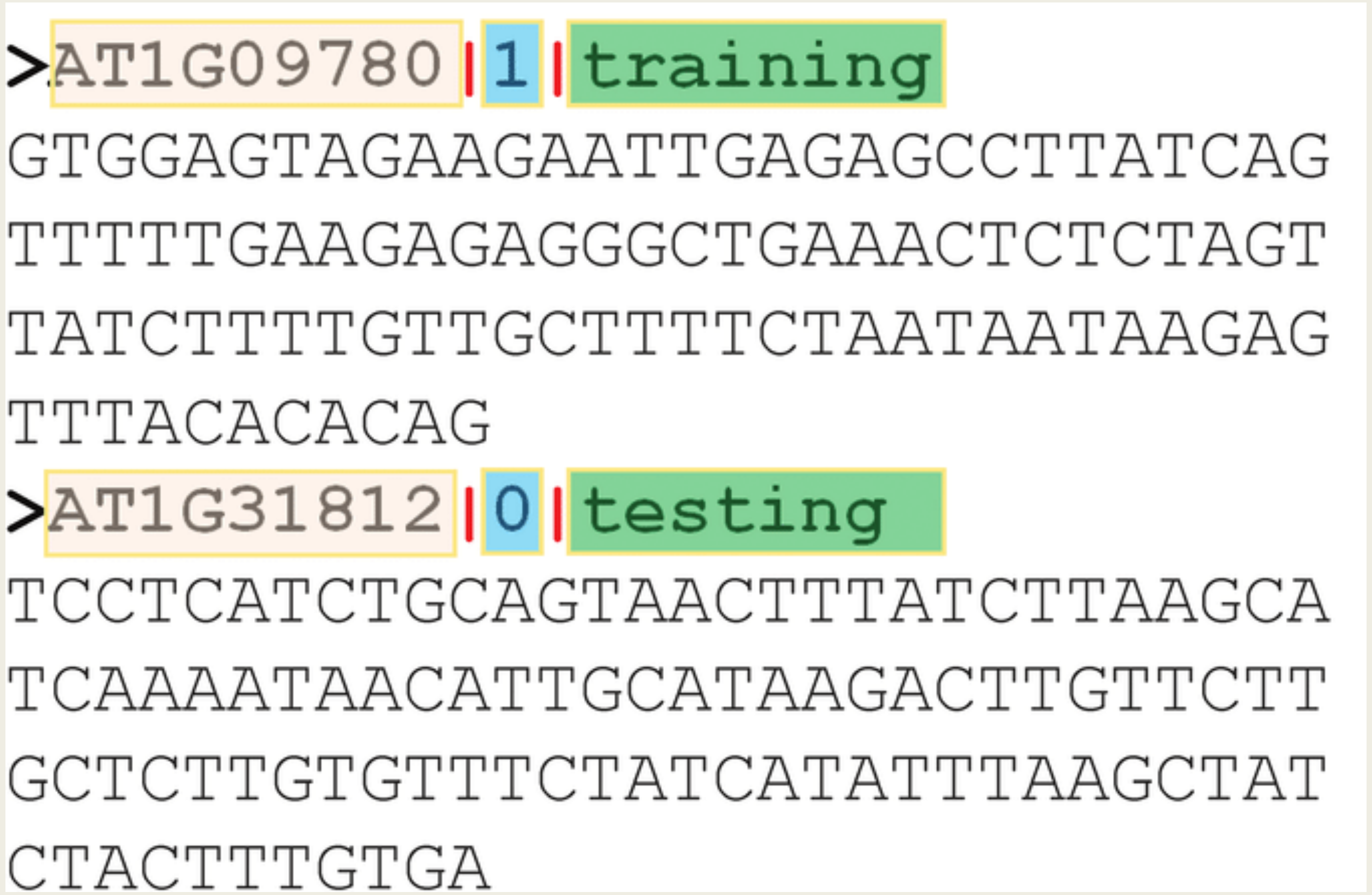
Lab 3 notes

- Fasta file format (can be used for the reads or the reference, or any other sequence)



```
>AT1G09780|1|training
GTGGAGTAGAAGAATTGAGAGCCTTATCAG
TTTTTGAAGAGAGGGCTGAAACTCTCTAGT
TATCTTTTGTTGCTTTTCTAATAATAAGAG
TTTACACACAG
>AT1G31812|0|testing
TCCTCATCTGCAGTAACTTTATCTTAAGCA
TCAAAATAACATTGCATAAGACTTGTTCTT
GCTCTTGTGTTTCTATCATATTTAAGCTAT
CTACTTTGTGA
```

Credit: Jiangning Song

Outline

- de Bruijn graphs (DBGs)
- Traversing DBGs
- Assembling contigs with DBGs

Review Overlap Graph Assembly

Steps of Overlap Graph Assembly (also called "overlap-layout-consensus")

1) Compute **overlaps between all pairs of reads**. With R = number of reads and m = length of reads, this is naively $O(R^2m^2)$. We will learn better ways of "aligning" sequences next week.

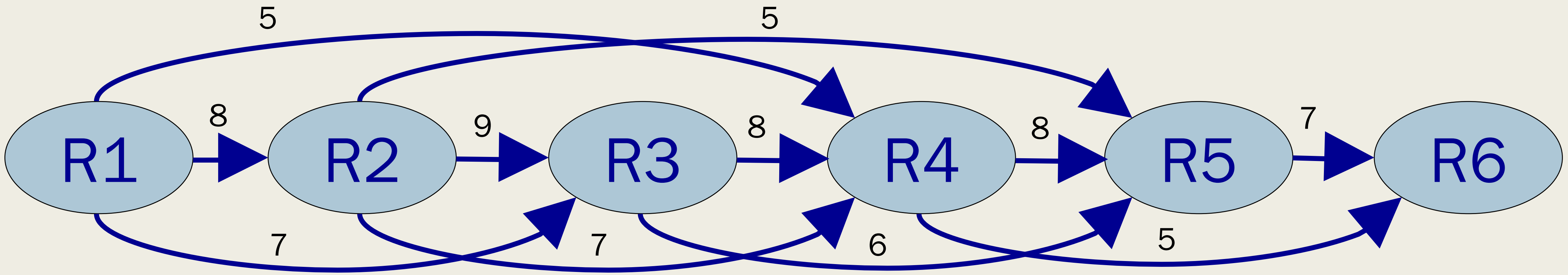
2) Construct a **graph with reads as the nodes** and **directed, weighted edges** between reads with >= T overlap.

3) "Layout" the graph and try to "group" stretches of the graph into "**contigs**" (short for contiguous), these are (hopefully) long portions of the original genome

4) Find a "consensus" *sequence* for each contig

Activity example: m = 10, T = 5

```
ATATATACTGGCGTATCGCAGTAAACGCGCCG
  R1: ACTGGCGTAT
  R2:   TGGCGTATCG
  R3:    GGCGTATCGC
  R4:      CGTATCGCAG
  R5:        TATCGCAGTA
  R6:           CGCAGTAAAC
```



$$\begin{tikzcd}
R1 \arrow[r, "8"] \arrow[rr, bend right, "7"'] \arrow[rrr, bend left, "5"] & R2 \arrow[r, "9"] \arrow[rr, bend right, "7"'] \arrow[rrr, bend left, "5"] & R3 \arrow[r, "8"] \arrow[rr, bend right, "6"'] & R4 \arrow[r, "8"] \arrow[rr, bend right, "5"'] & R5 \arrow[r, "7"] & R6
\end{tikzcd}$$

Li et al, *Briefings in Functional Genomics* (2012)

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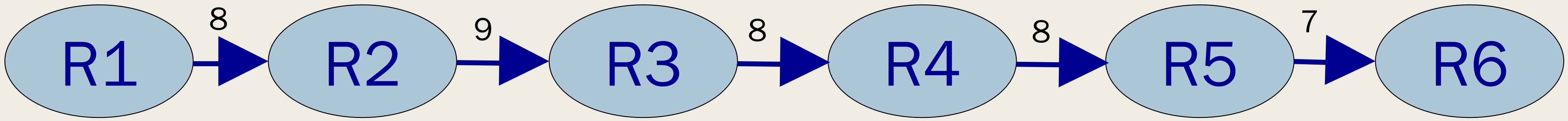
  R6:           CGCAGTAAAC
```

First simplification: remove edges that can be (transitively) inferred from other edges



$$\begin{tikzcd} R1 \arrow[r, "8"] & R2 \arrow[r, "9"] & R3 \arrow[r, "8"] & R4 \arrow[r, "8"] & R5 \arrow[r, "7"] & R6 \end{tikzcd}$$

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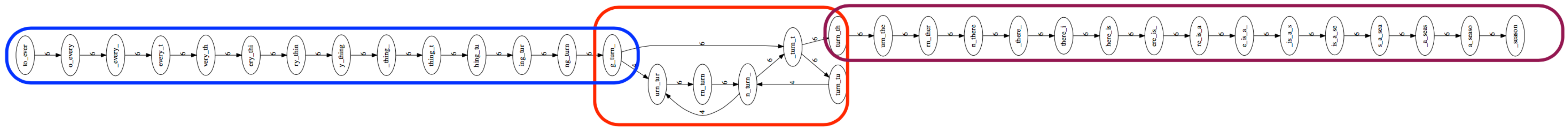
Layout

Emit *contigs* corresponding to the non-branching stretches



Contig 1
to_every_thing_turn_

Contig 2
turn_there_is_a_season

Unresolvable repeat

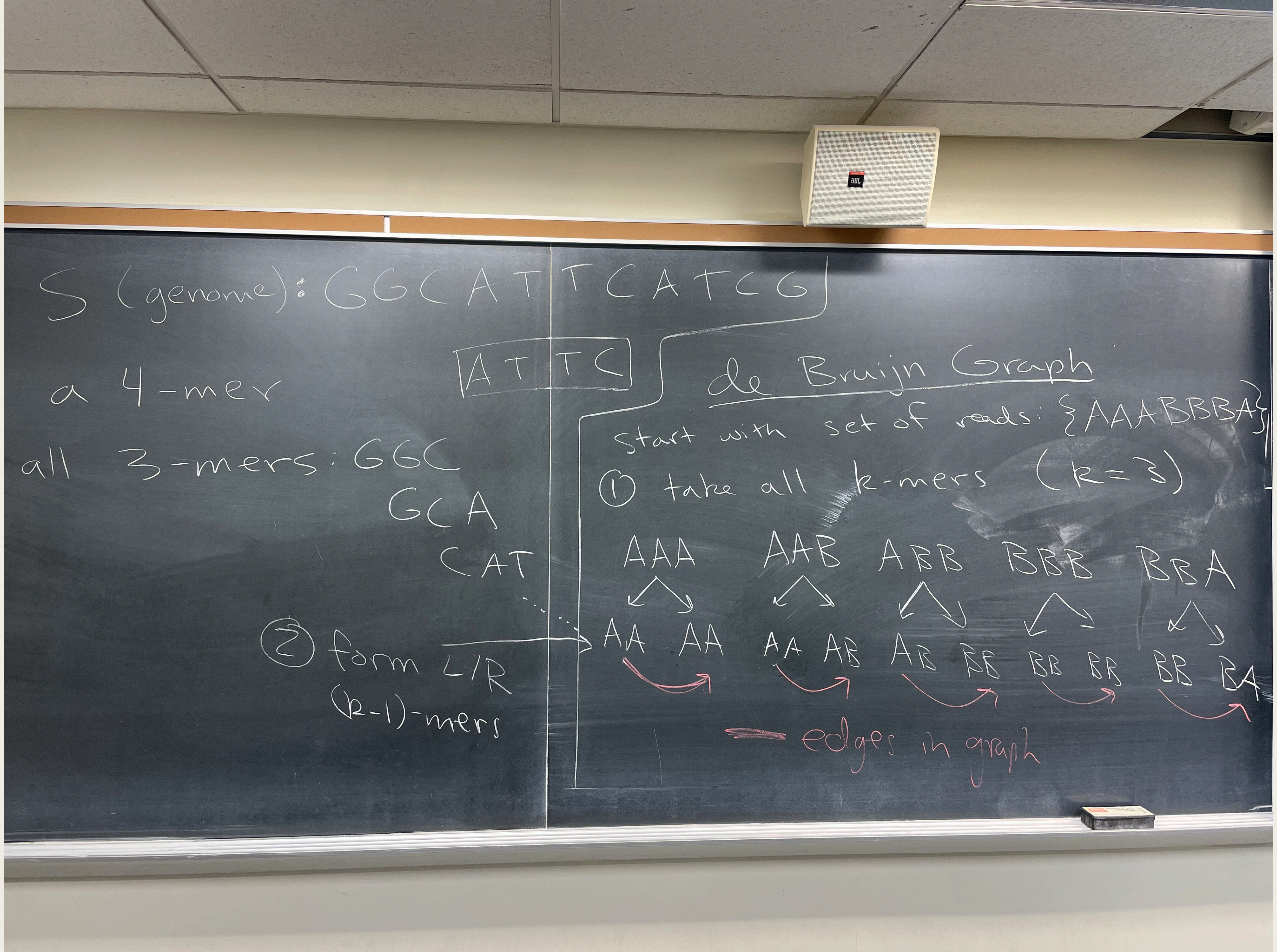
Original string: “`to_every_thing_turn_turn_turn_there_is_a_season`”

Slide: adapted from Ben Langmead, John Hopkins

Issues with overlap graph assembly

- Next-generation sequencing produces 100’s of millions (or even billions) of reads
- With one node per read this is computationally intractable for large genomes
- What if the nodes in our graph were not reads?

de Bruijn graphs



S (genome): GGCAT|TCATCG

a 4-mer: $\boxed{\text{ATTC}}$

all 3-mers: GGC
GCA
CAT

② form L/R
(k-1)-mers

de Bruijn Graph

Start with set of reads: {AAABBBA}

① take all k-mers (k=3)

$$\begin{tikzcd}[column sep=small] & AAA \arrow[dl] \arrow[dr] & & & AAB \arrow[dl] \arrow[dr] & & & ABB \arrow[dl] \arrow[dr] & & & BBB \arrow[dl] \arrow[dr] & & & BBA \arrow[dl] \arrow[dr] & \\ AA \arrow[rr, bend right] & & AA & AA \arrow[rr, bend right] & & AB & AB \arrow[rr, bend right] & & BB & BB \arrow[rr, bend right] & & BB & BB \arrow[rr, bend right] & & BA \end{tikzcd}$$

— edges in graph

③ form DBG

- nodes: (k-1)-mers
- edges: k-mers (all)

↙ unique

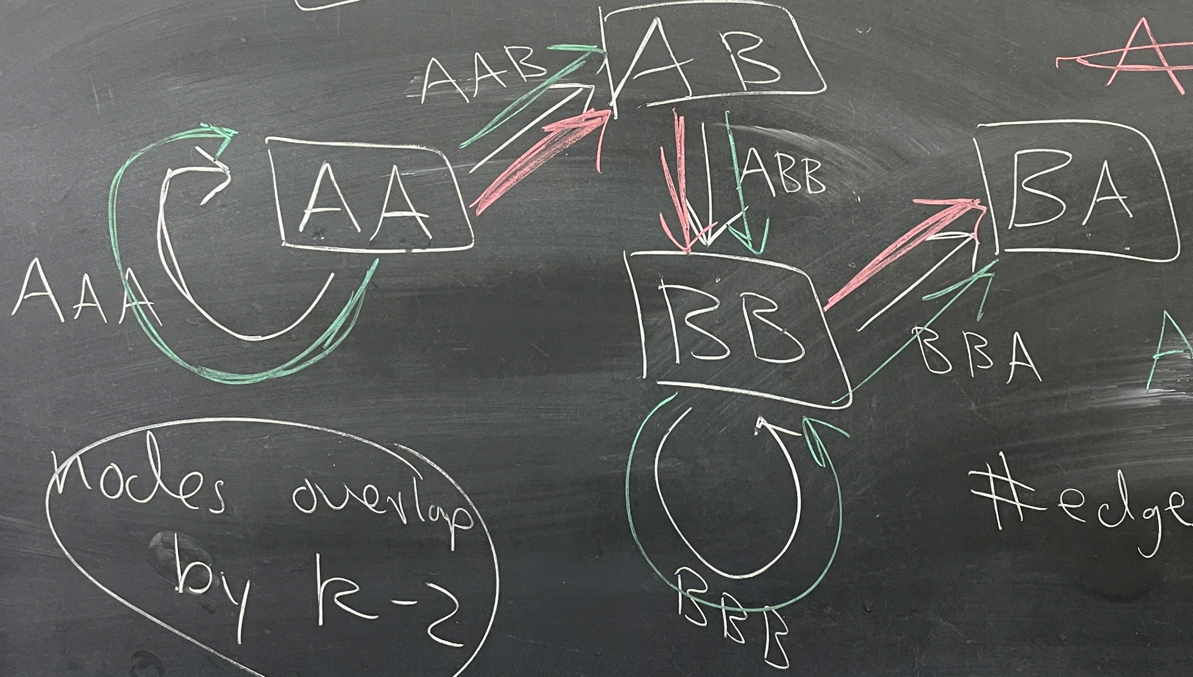
all possible

AA, AB, BA, BB

for us: use ones we see

$\boxed{\text{directed edge from } L \to R}$

~~AABBA~~



AAABBBA☆

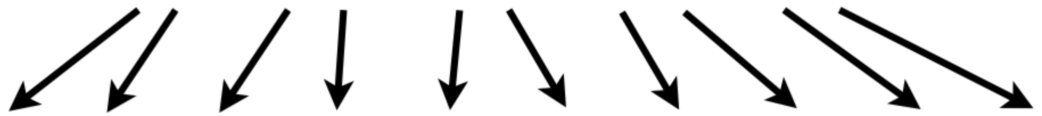
$\boxed{\text{nodes overlap by } k-2}$

$\#\text{edges} = \underbrace{\#\text{ k-mers}}_{\text{total}}$

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

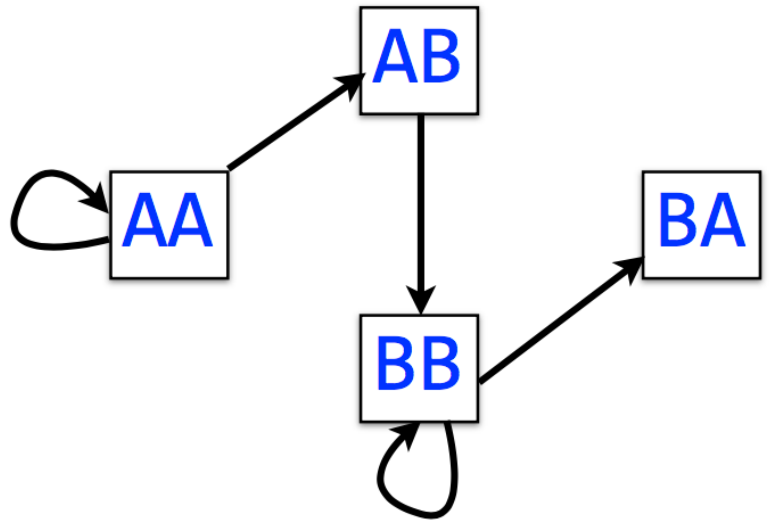
take all 3-mers: AAA, AAB, ABB, BBB, BBA



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:



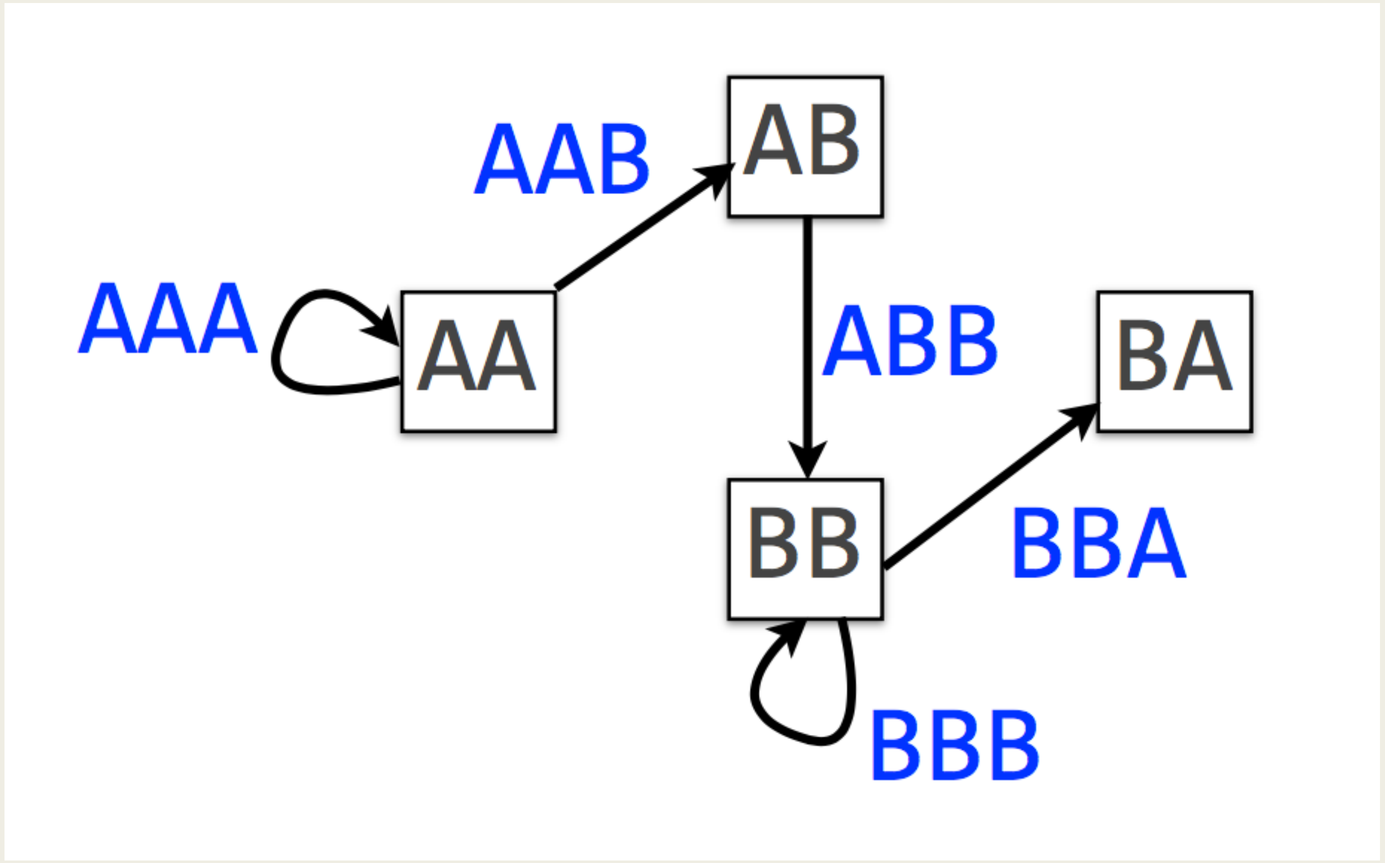
Each *edge* in this graph corresponds to a length-3 input string

Slide: adapted from Ben Langmead, John Hopkins

DBG:

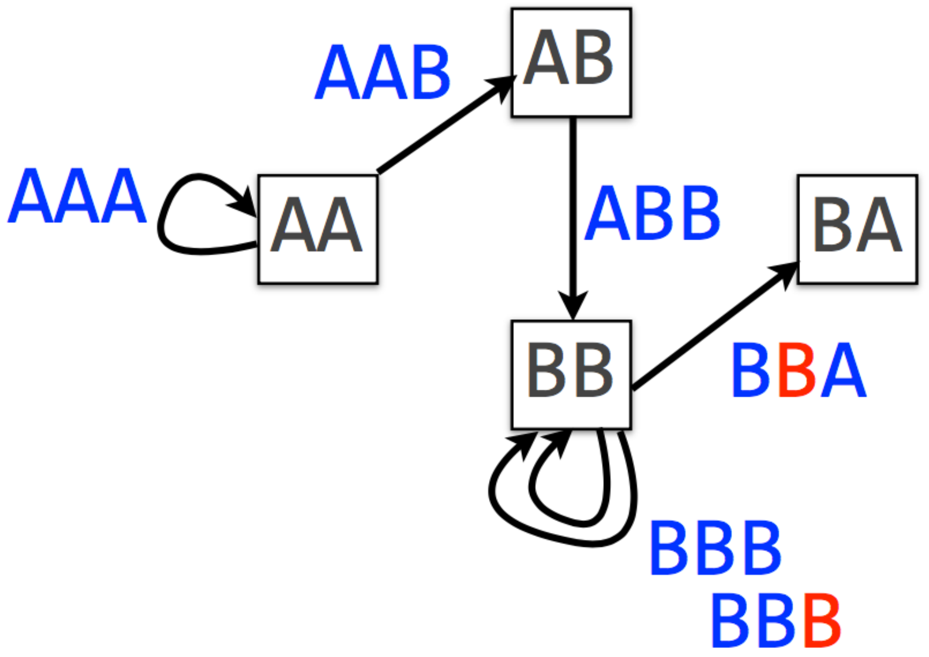
-Nodes: (k-1)-mers

-Edges: k-mers of the genome or reads



Slide: adapted from Ben Langmead, John Hopkins

DBGs can have multi-edges, making them multi-graphs



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

Slide: adapted from Ben Langmead, John Hopkins

Graph terminology

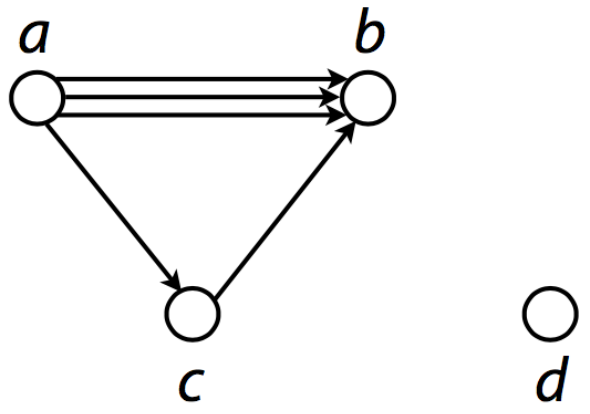
Directed **multigraph** *G*(*V*, *E*) consists of set of *vertices, V* and **multiset** of *directed edges, E*

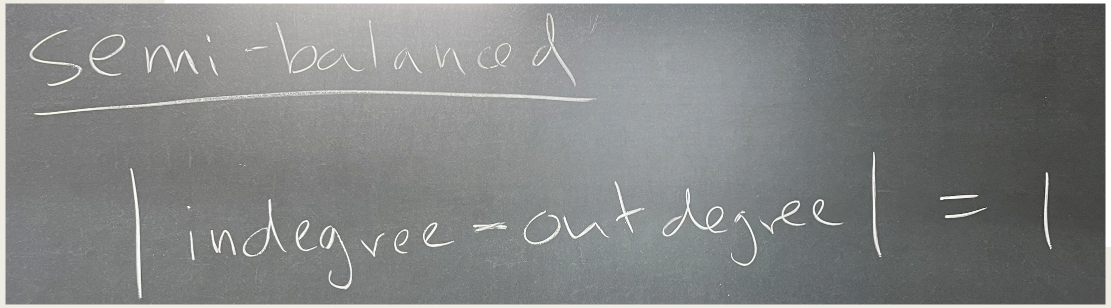
Otherwise, like a directed graph

Node's *indegree* = # incoming edges

Node's *outdegree* = # outgoing edges

De Bruijn graph is a directed multigraph




semi-balanced

$|\text{indegree} - \text{outdegree}| = 1$

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

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

Slide: adapted from Ben Langmead, John Hopkins

Graph terminology (cont.)

Node is *balanced* if indegree equals outdegree

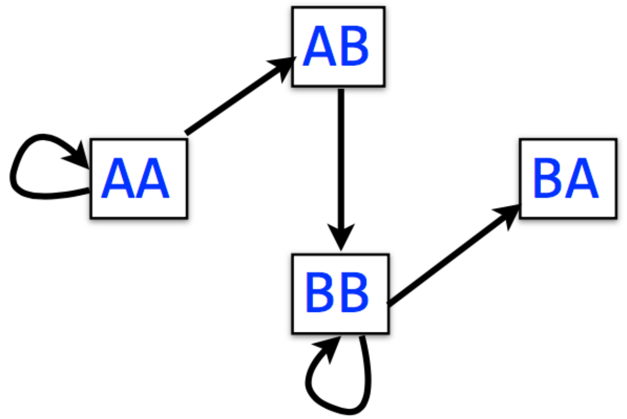
Node is *semi-balanced* if indegree differs from outdegree by 1

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.*

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

Slide: adapted from Ben Langmead, John Hopkins

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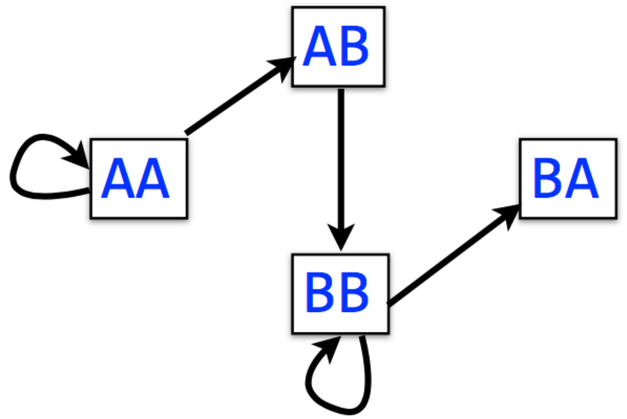
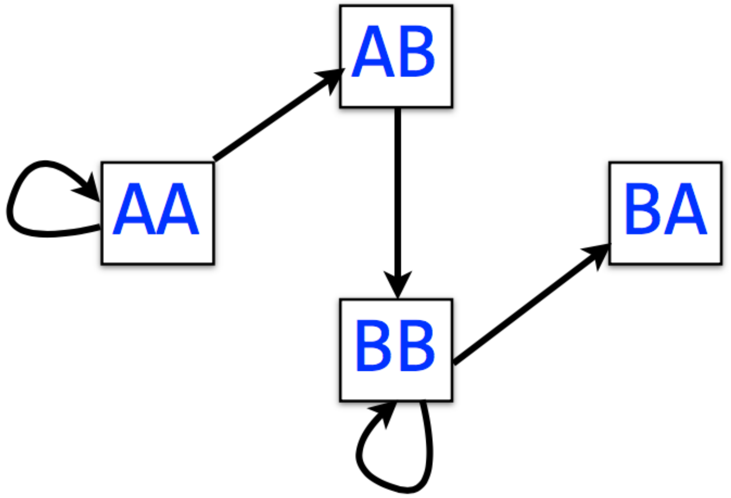
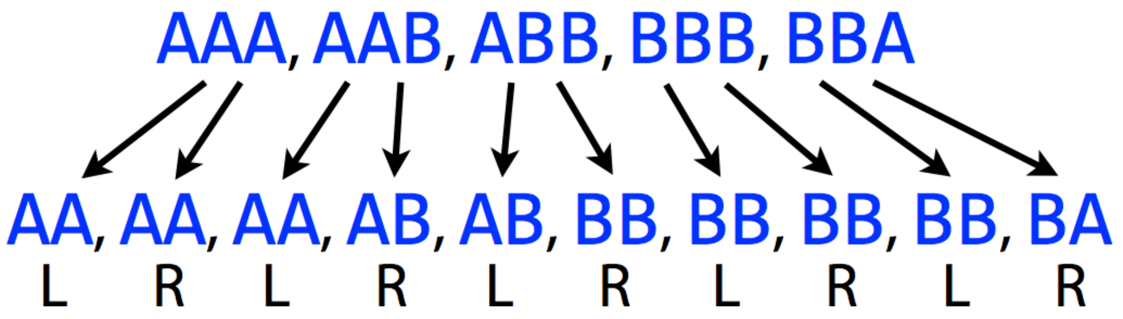


Slide: adapted from Ben Langmead, John Hopkins

Back to our De Bruijn graph





Is it Eulerian? Yes

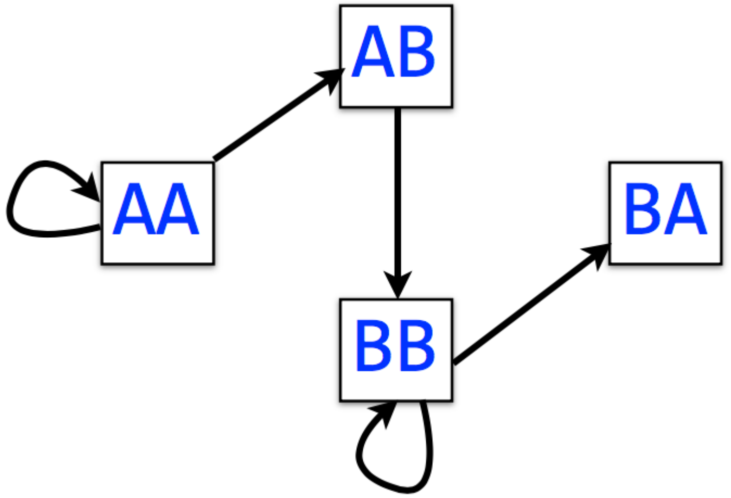
Argument 1: AA → AA → AB → BB → BB → BA

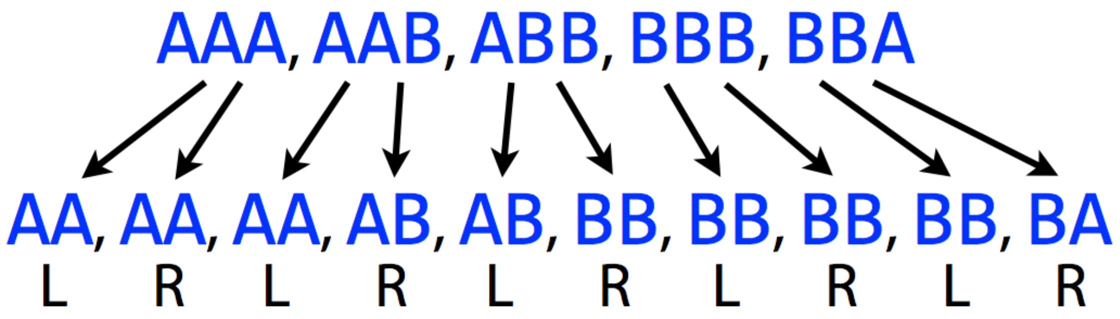
Argument 2: AA and BA are semi-balanced, AB and BB are balanced

Slide: adapted from Ben Langmead, John Hopkins

How to get the sequence from an Eulerian path?

Back to our De Bruijn graph





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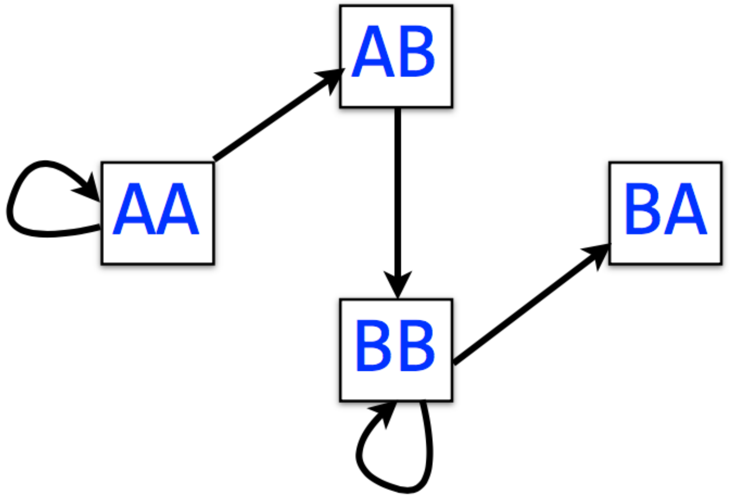
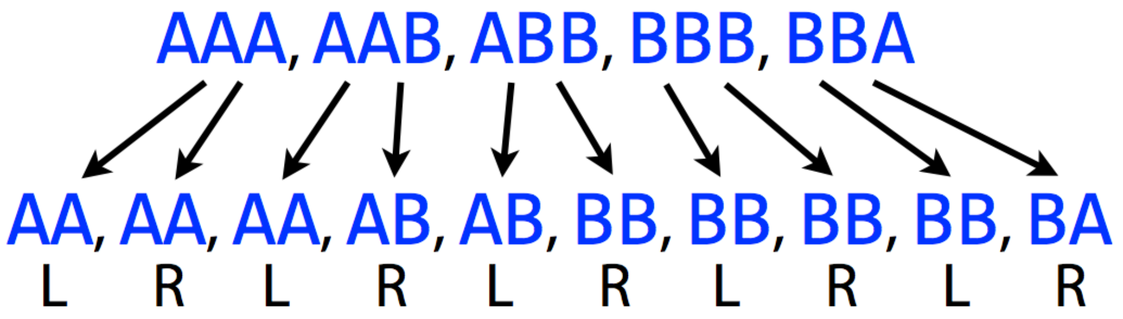




Start with the sequence in the first node. Follow the path, adding on one base each time.

AAABBBA

Is it Eulerian? Yes

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

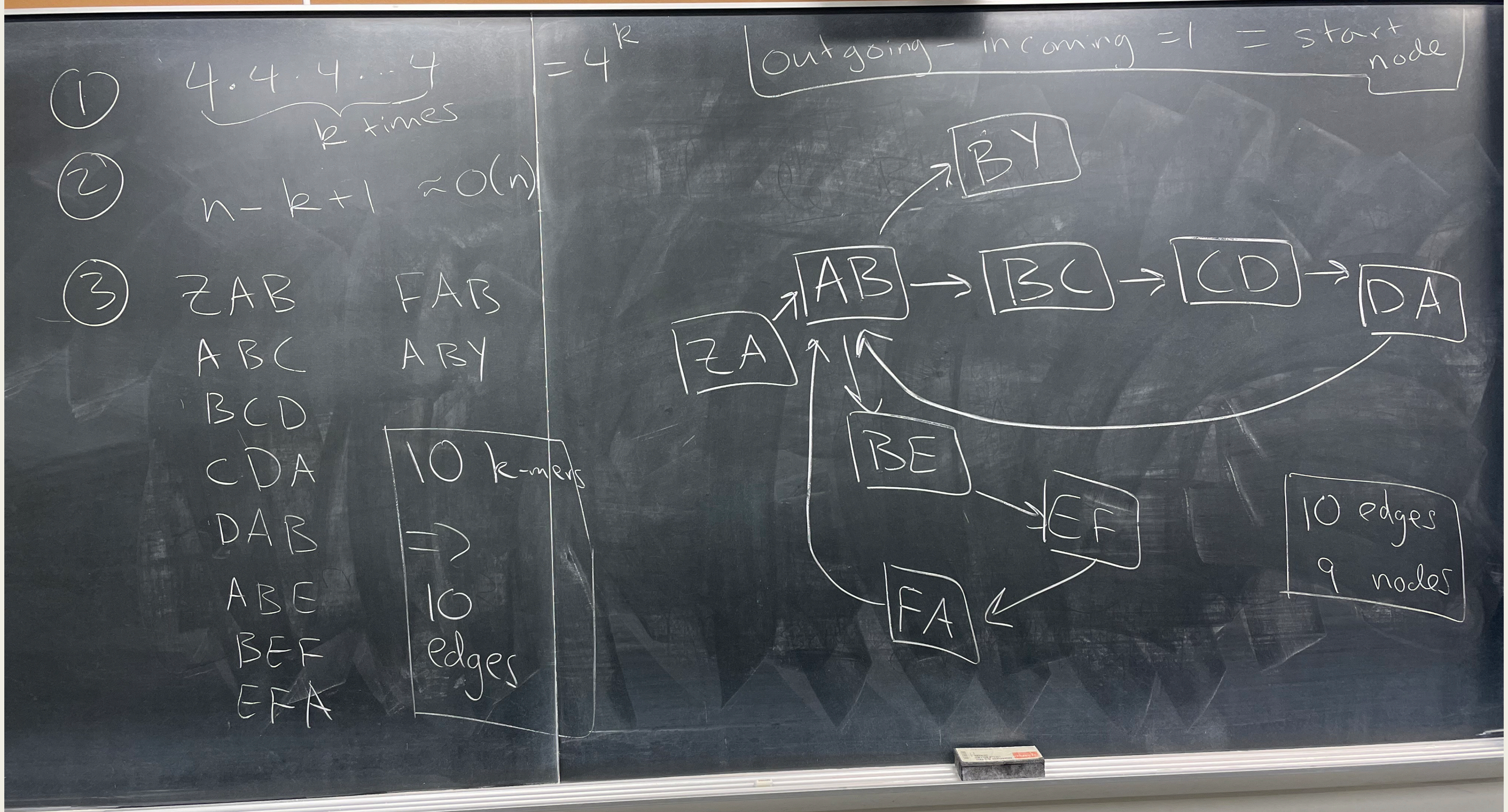
Argument 2: AA and BA are semi-balanced, AB and BB are balanced

Slide: adapted from Ben Langmead, John Hopkins

Handout 5: page 1

<u>Goals:</u>

1) Practice the mechanics of constructing a de Bruijn graph
2) See issues that that affect both OLC and DBG assembly
3) Think about how we would ask a computer to find an Eulerian path



① $\underbrace{4 \cdot 4 \cdot 4 \cdots 4}_{k \text{ times}} = 4^k$

② $n-k+1 \approx O(n)$

③

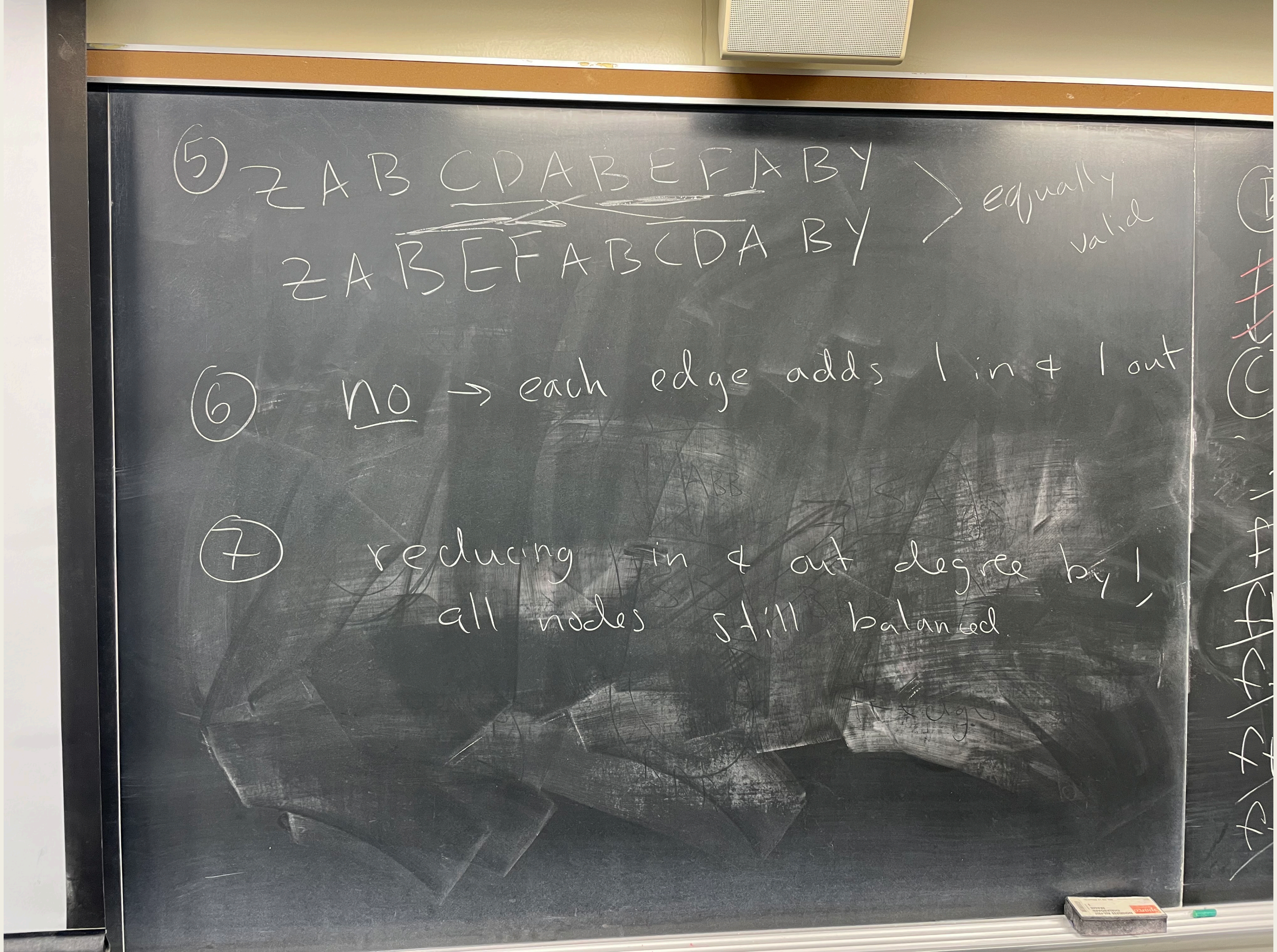
ZAB	FAB
ABC	ABY
BCD	
CDA	
DAB	
ABE	
BEF	
EFA	

$\boxed{\begin{array}{l}10 \text{ k-mers} \\ \Rightarrow \\ 10 \\ \text{edges}\end{array}}$

$\boxed{\text{outgoing} - \text{incoming} = 1 = \text{start node}}$

$$\begin{tikzcd}
 & & \boxed{BY} & & \\
\boxed{ZA} \arrow[r] & \boxed{AB} \arrow[ur] \arrow[r] \arrow[d] & \boxed{BC} \arrow[r] & \boxed{CD} \arrow[r] & \boxed{DA} \arrow[lll, bend left] \\
 & \boxed{BE} \arrow[r] & \boxed{EF} \arrow[dl] & & \\
 & \boxed{FA} \arrow[uu, bend left] & & &
\end{tikzcd}$$

$\boxed{\begin{array}{l}10 \text{ edges} \\ 9 \text{ nodes}\end{array}}$



5. ZABCDABEFABY
   ZABEFABCDABY } equally valid

6. no → each edge adds 1 in & 1 out

7. reducing in & out degree by 1, all nodes still balanced.

Fleury’s Algorithm

- Start with vertex ***u*** where (outdegree – indegree) = 1
- Until there are no more edges:

    Choose ***e = (u,v)*** where removing ***e*** will not disconnect the graph (if possible)
    Traverse ***e***, delete ***e***, and proceed with ***u = v***

Fleury's Algorithm

- Start with vertex ***u*** where (outdegree – indegree) = 1
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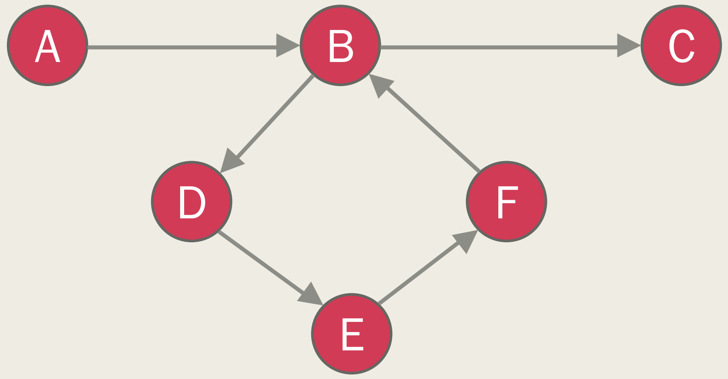


Avoids going from:
A -> B -> C
And then getting stuck

Recursive algorithm

```
find_tour(u, tour):
        for each edge e = (u,v):
                remove e
                find_tour(v, tour)
        push u onto tour
```

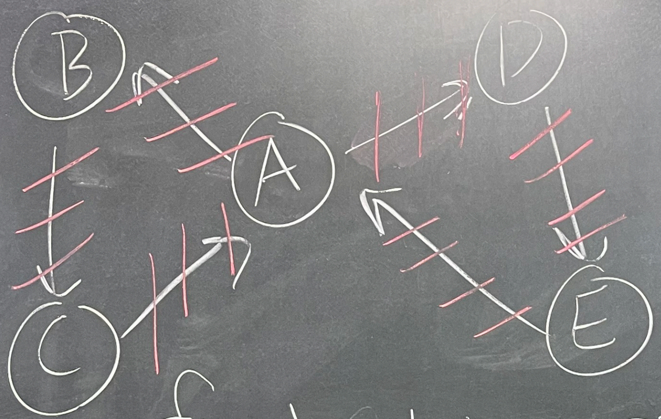
<u>To use in practice</u>:

- Start with tour as an empty stack
- Start at any node u (or start at a node with (outdegree – indegree) = 1 for a path)
- After completion, pop elements off tour to find the correct order

y
lid

l out

y !



function stack

~~ft(A)~~
~~ft(C)~~
~~ft(B)~~
~~ft(D)~~
~~ft(A)~~
~~ft(E)~~
~~ft(D)~~

tour stack

D
E
A
B
C
A
D

```
$\overbrace{\text{find\_tour}}^{ft}$(u):
    for edge e=(u,v):
        remove e
        find_tour(v)
    push u onto stack
```

Final seq.

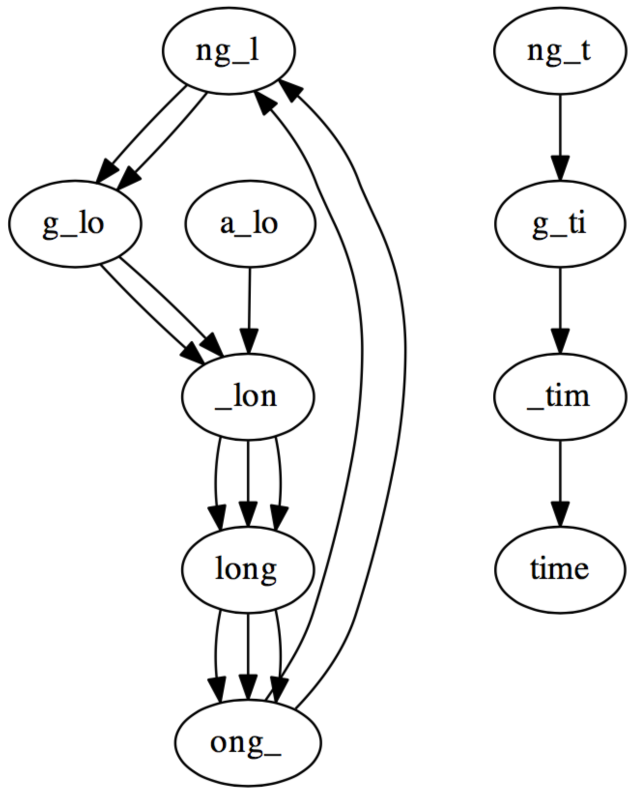
DEABCAD

Handout 5: page 2

Issues with DBGs

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

Slide: adapted from Ben Langmead, John Hopkins

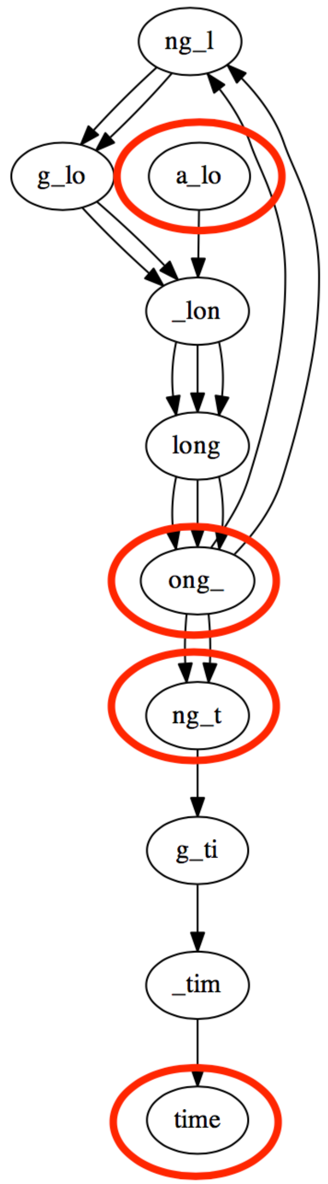
Issues with DBGs

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



Slide: adapted from Ben Langmead, John Hopkins

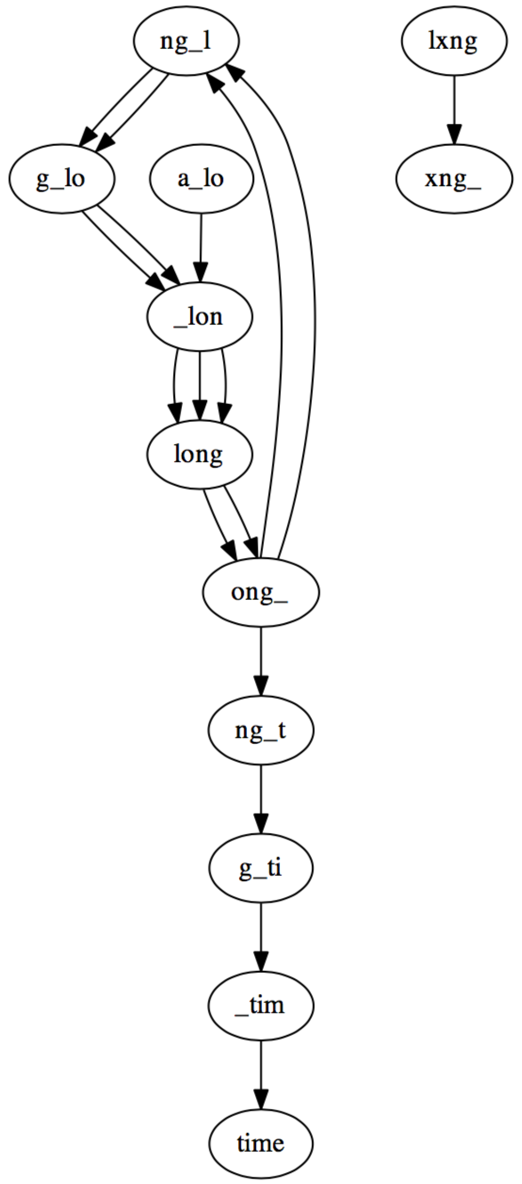
Issues with DBGs

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



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