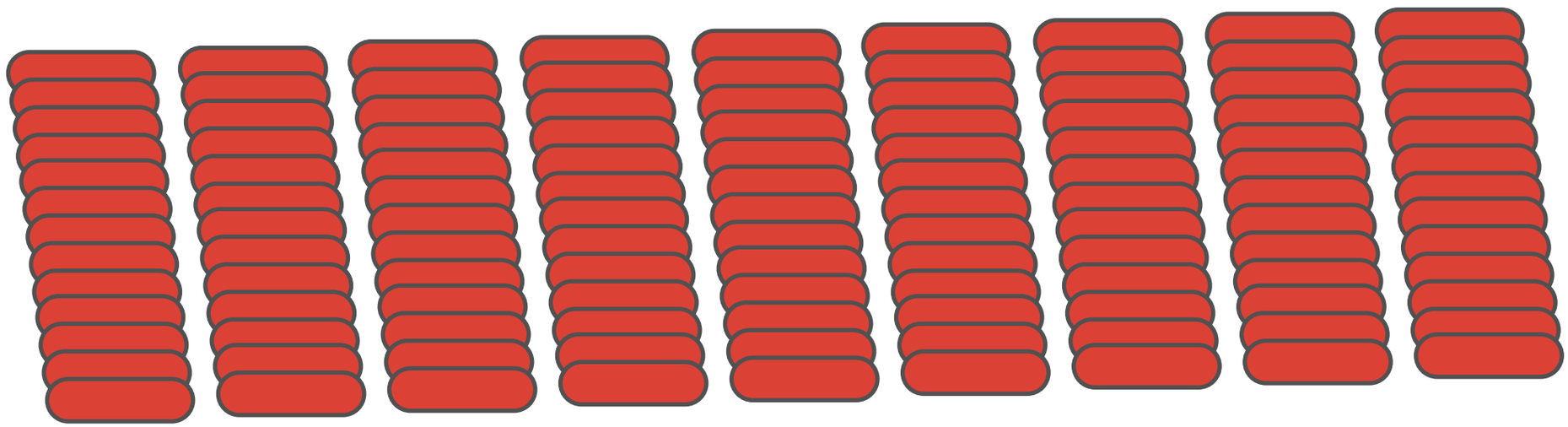
Introduction to genome assembly

CMSC423

Many slides courtesy of Ben Langmead and Carl Kingsford

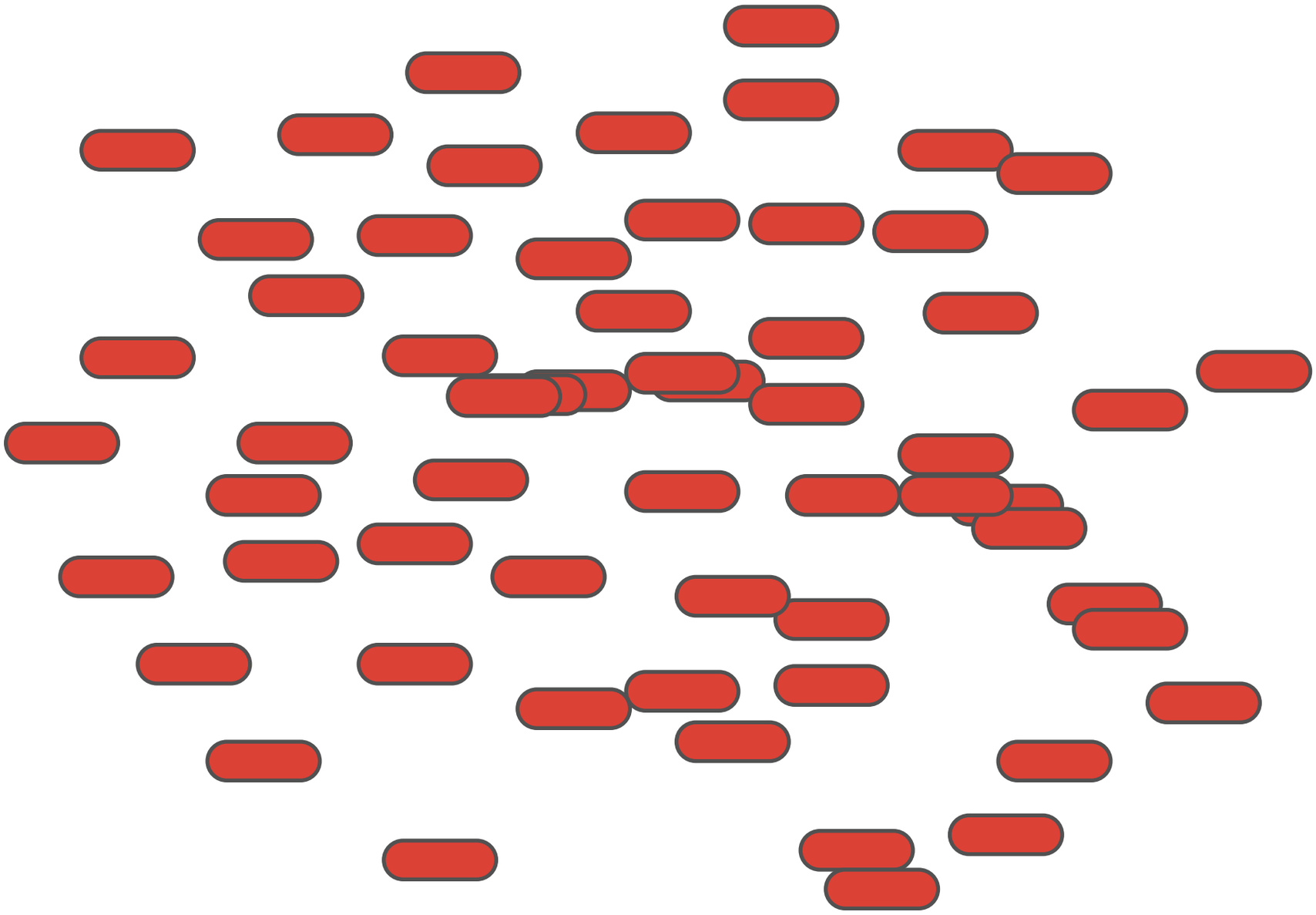
Sec-gen Sequencing

Sec-gen Sequencing



Fragmentation is random,
i.e., not equal-sized (but hard to draw)

Sec-gen Sequencing



Second-Generation Sequencing

- "Ultra high throughput" DNA sequencing
  - 6 gigabases / day vs.
  - 3 gigabases / 13 years (human genome project, more or less)
  - 200 bp long reads

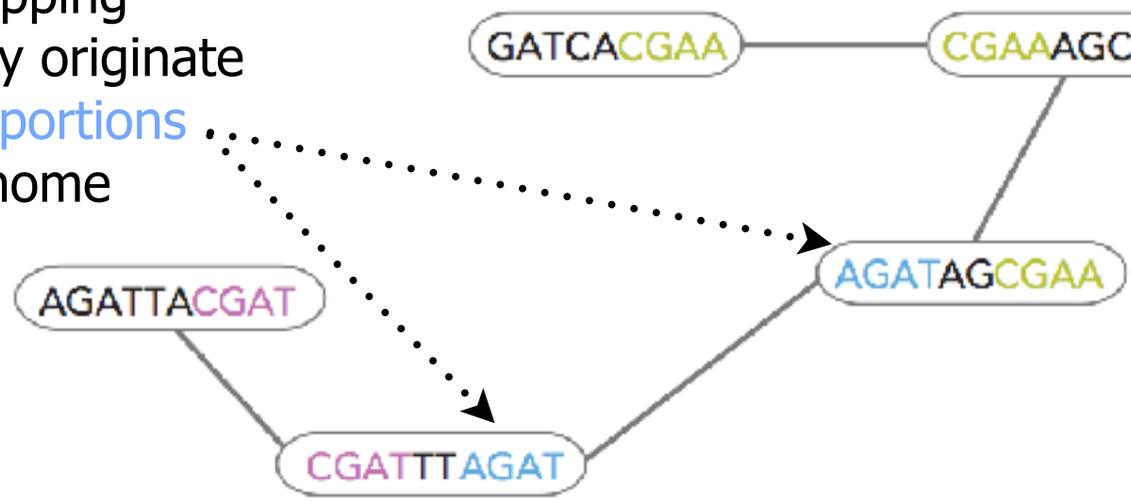
From reads to evidence

From reads to evidence

I. *de novo*

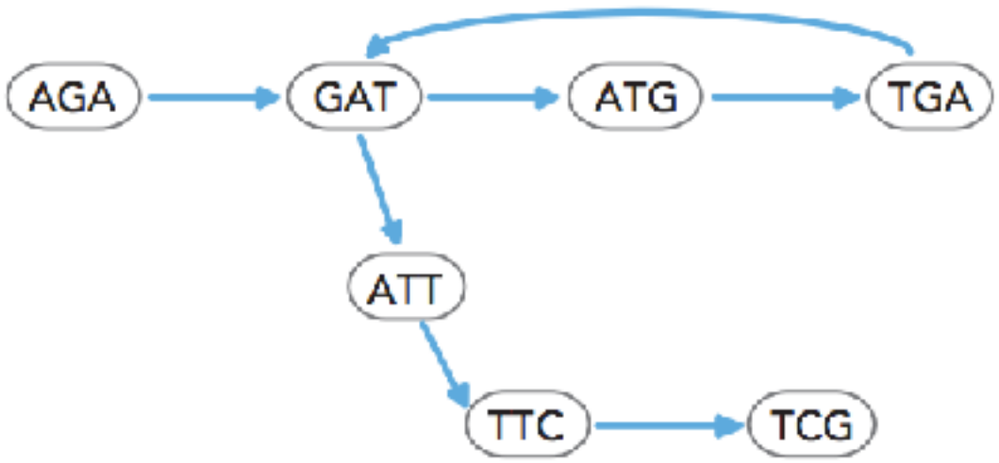
Assume nothing! - let reads tell us everything

Reads with overlapping sequence probably originate from overlapping portions of the subject genome



Encode overlap relationships as a graph

Source: De Novo Assembly Using Illumina Reads. Illumina. 2010



The full genome sequence is a "tour" of the graph

Source: De Novo Assembly Using Illumina Reads. Illumina. 2010
http://www.illumina.com/Documents/products/technotes/technote_denovo_assembly.pdf

What we'll cover

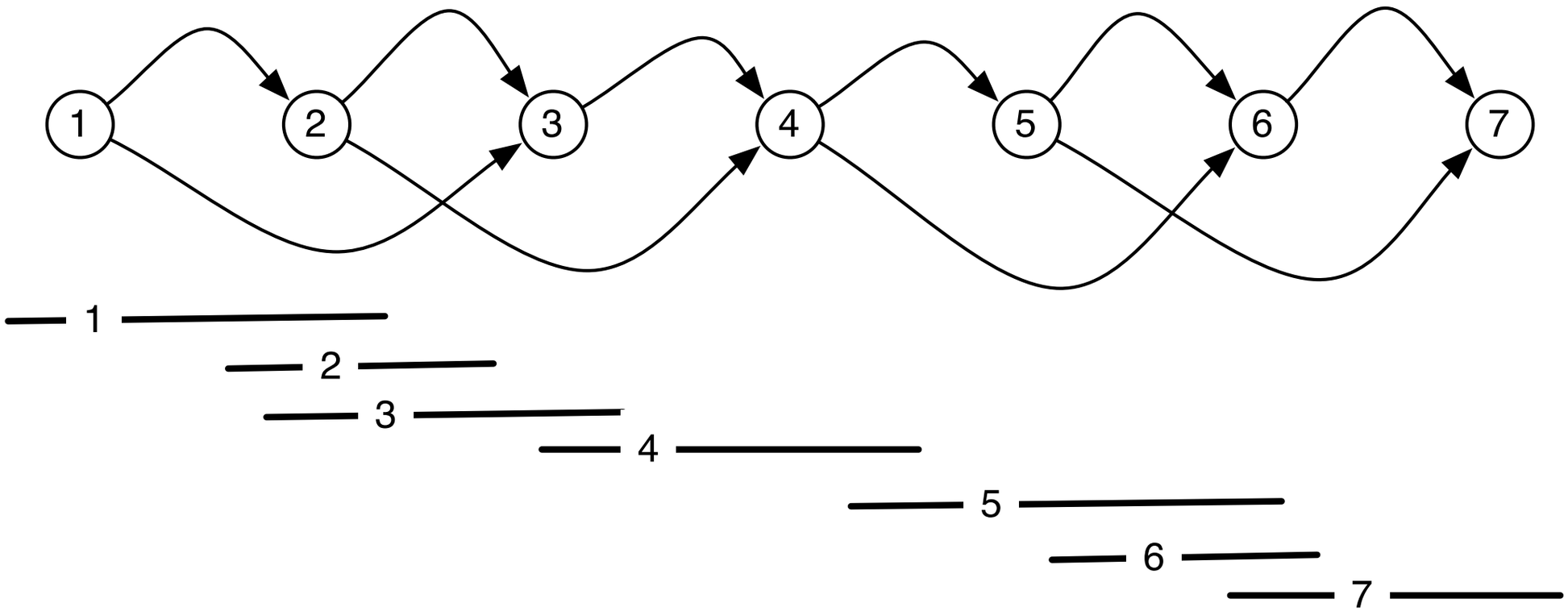
- Genome assembly as graph problems
  - Two representations:
    - Overlap graph
      - How much sequencing required for assembly
    - DeBruijn graph
- How to get assemblies from solutions to graph problems

Overlap Graph

Overlap graph:

Nodes = reads

Edges = overlaps



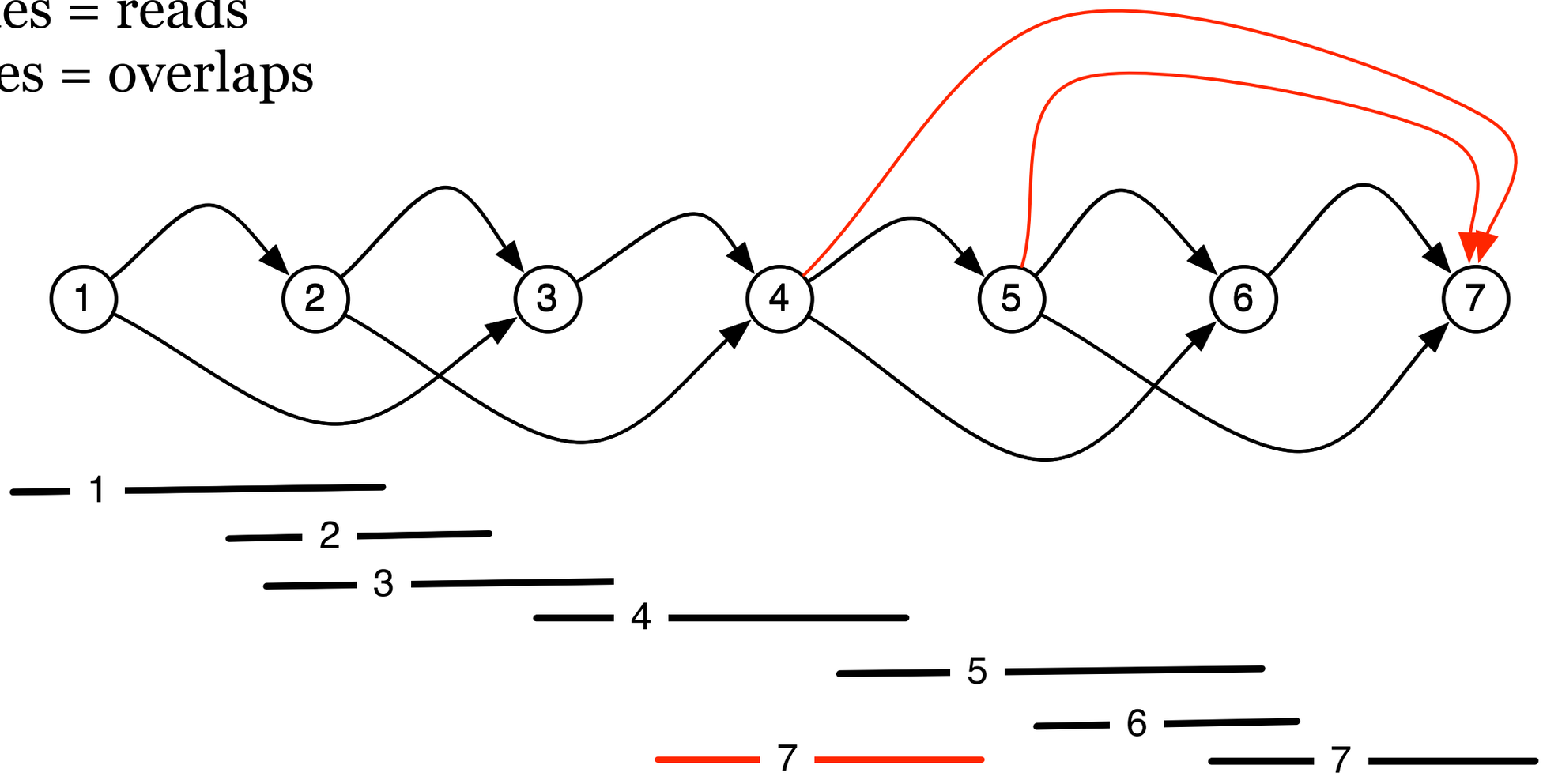
Given overlap graph, how can we find a good candidate assembly?

Overlap Graph

Overlap graph:

Nodes = reads

Edges = overlaps



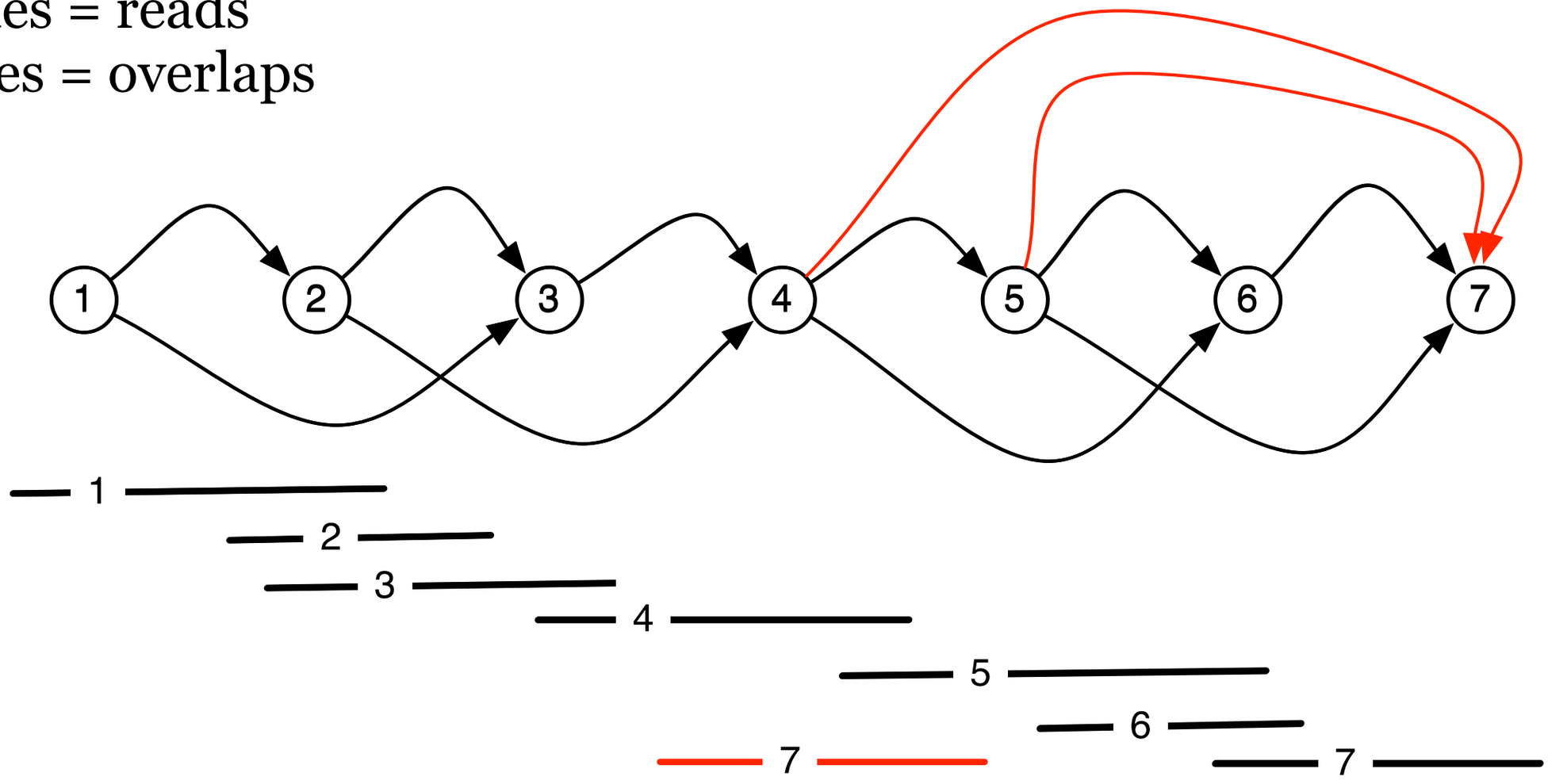
Given overlap graph, how can we find a good candidate assembly?

Overlap Graph

Overlap graph:

Nodes = reads

Edges = overlaps



Given overlap graph, how can we find a good candidate assembly?

Hamiltonian Path (aka Traveling Salesman Path): visit every node in the graph exactly once.

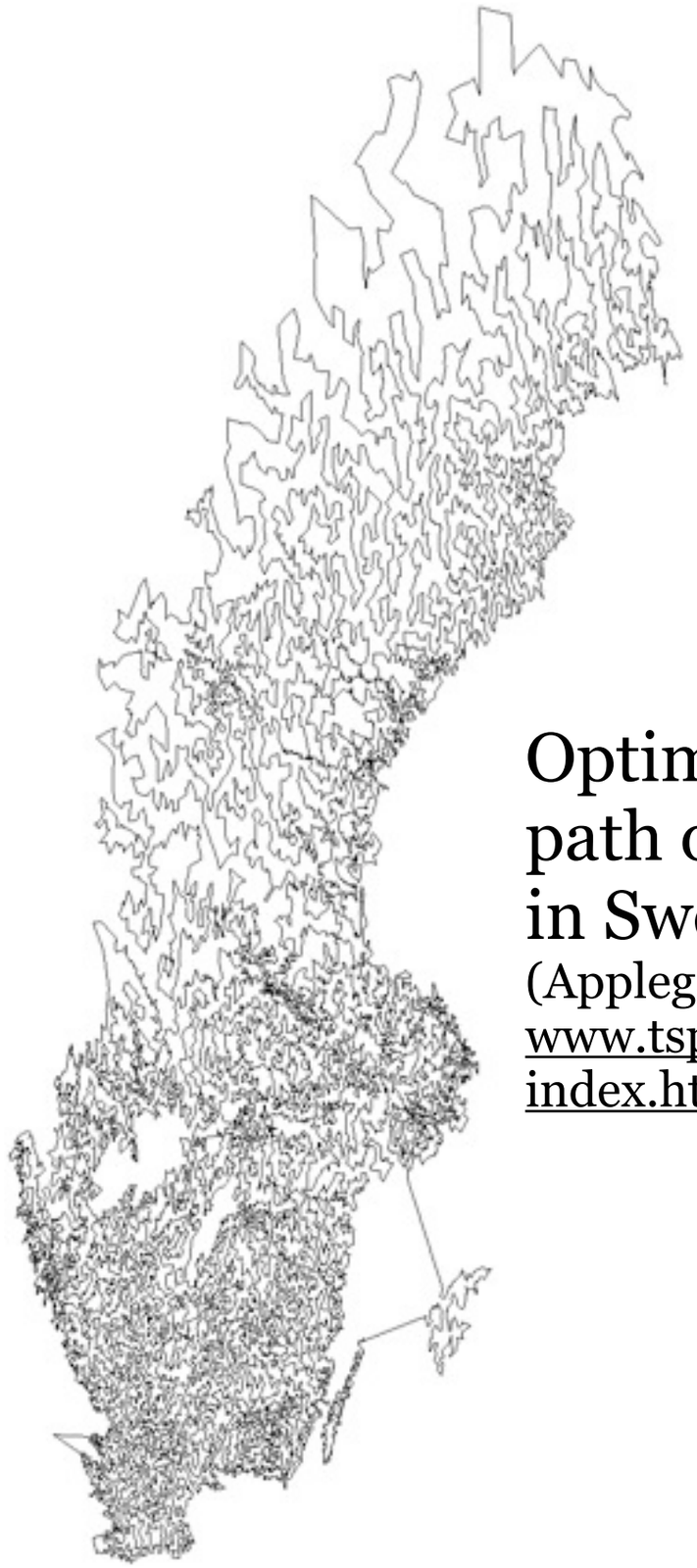
Hamiltonian Path

- Motivation: Every read must be used in exactly one place in the genome.

- Hamiltonian Path is NP-hard.

- Though good solvers exist, they can't operate on the millions of reads from a sequencing project.

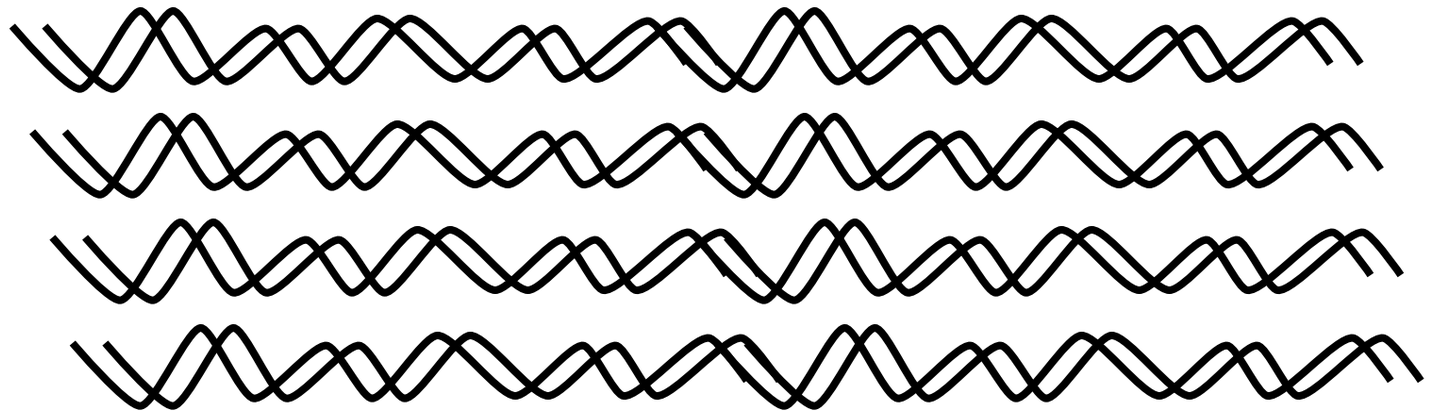
- Solution: greedy walk along the graph.



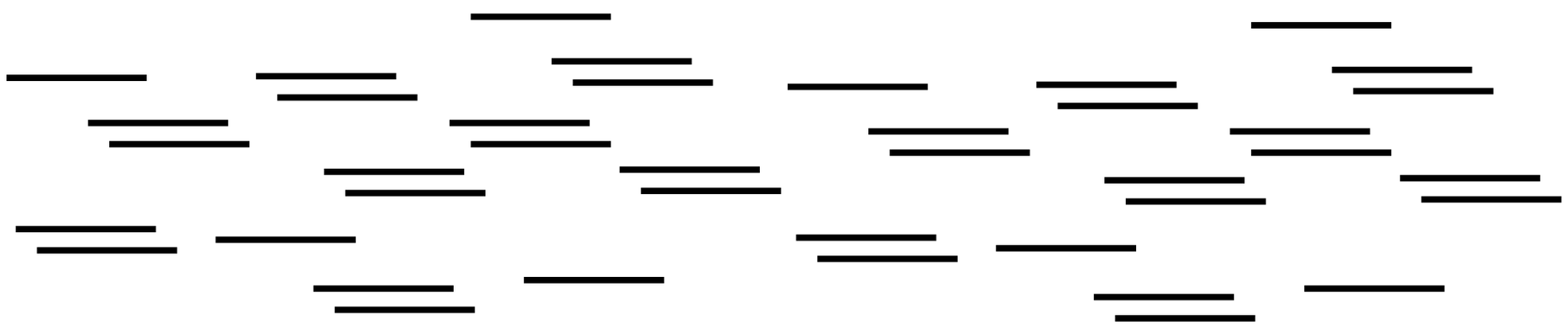
Optimal Hamiltonian path of 24,978 cities in Sweden
(Applegate et al, 2004, www.tsp.gatech.edu/sweden/index.html).

Shotgun Sequencing

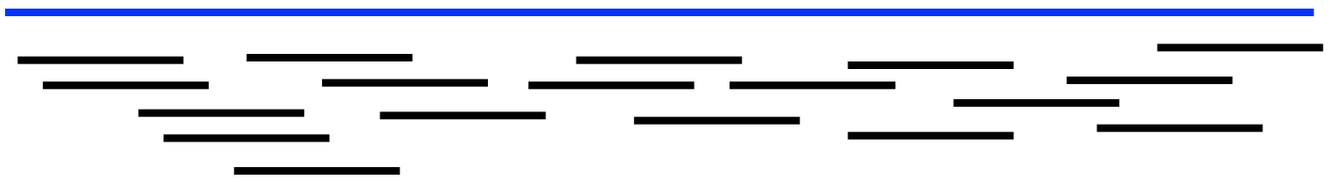
Many copies of the DNA



Shear it, randomly breaking them into many small pieces, read ends of each:

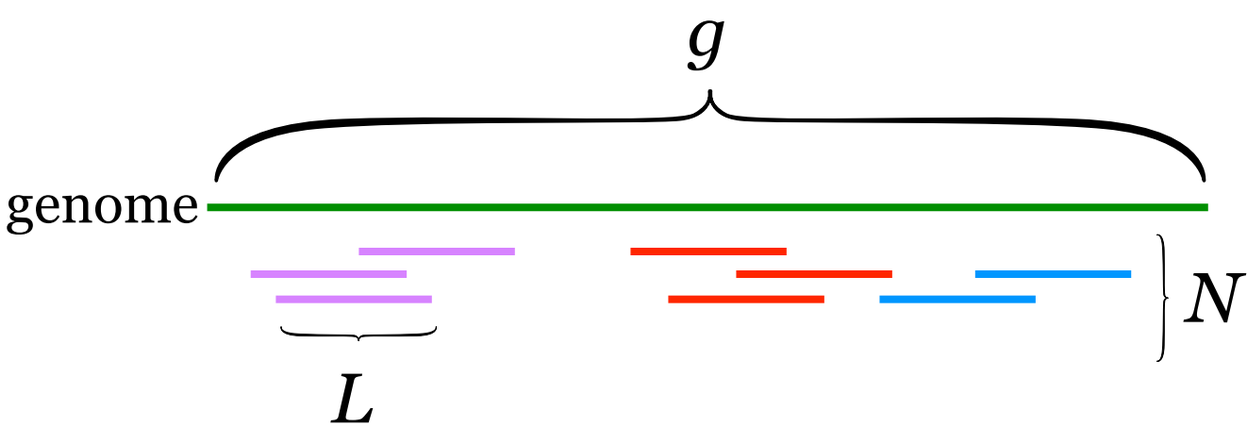


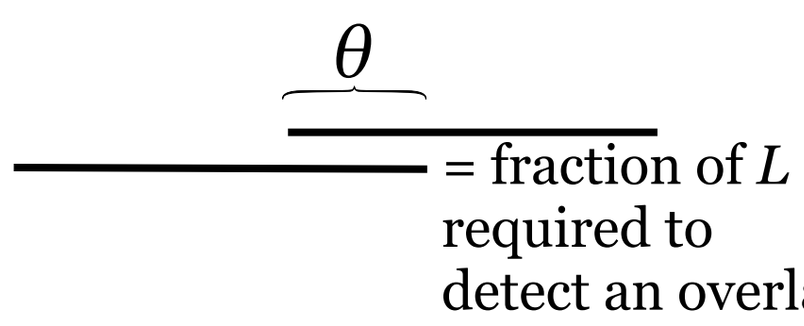
Assemble into original genome:



Lander-Waterman Statistics

How many reads to we need to be sure we cover the whole genome?





An ***island*** is a contiguous group of reads that are connected by overlaps of length ≥ θL.
(Various colors above)

Want: Expression for expected # of islands given N, g, L, θ.

Expected # of Islands

$\lambda := N/g$ = probability a read starts at a given position (assuming random sampling)

Pr(*k* reads start in an interval of length *x*)

x trials, want *k* "successes," small probability λ of success

Expected # of successes = λx

Poisson approximation to binomial distribution:

$$\Pr(k \text{ reads in length } x) = e^{-\lambda x}\frac{(\lambda x)^k}{k!}$$

Expected # of islands = $N \times$ Pr(read is at rightmost end of island)

$= N \times$ Pr(0 reads start in $(1-\theta)L$)

$$= Ne^{-\lambda(1-\theta)L}\frac{(\lambda(1-\theta)L)^0}{0!}$$

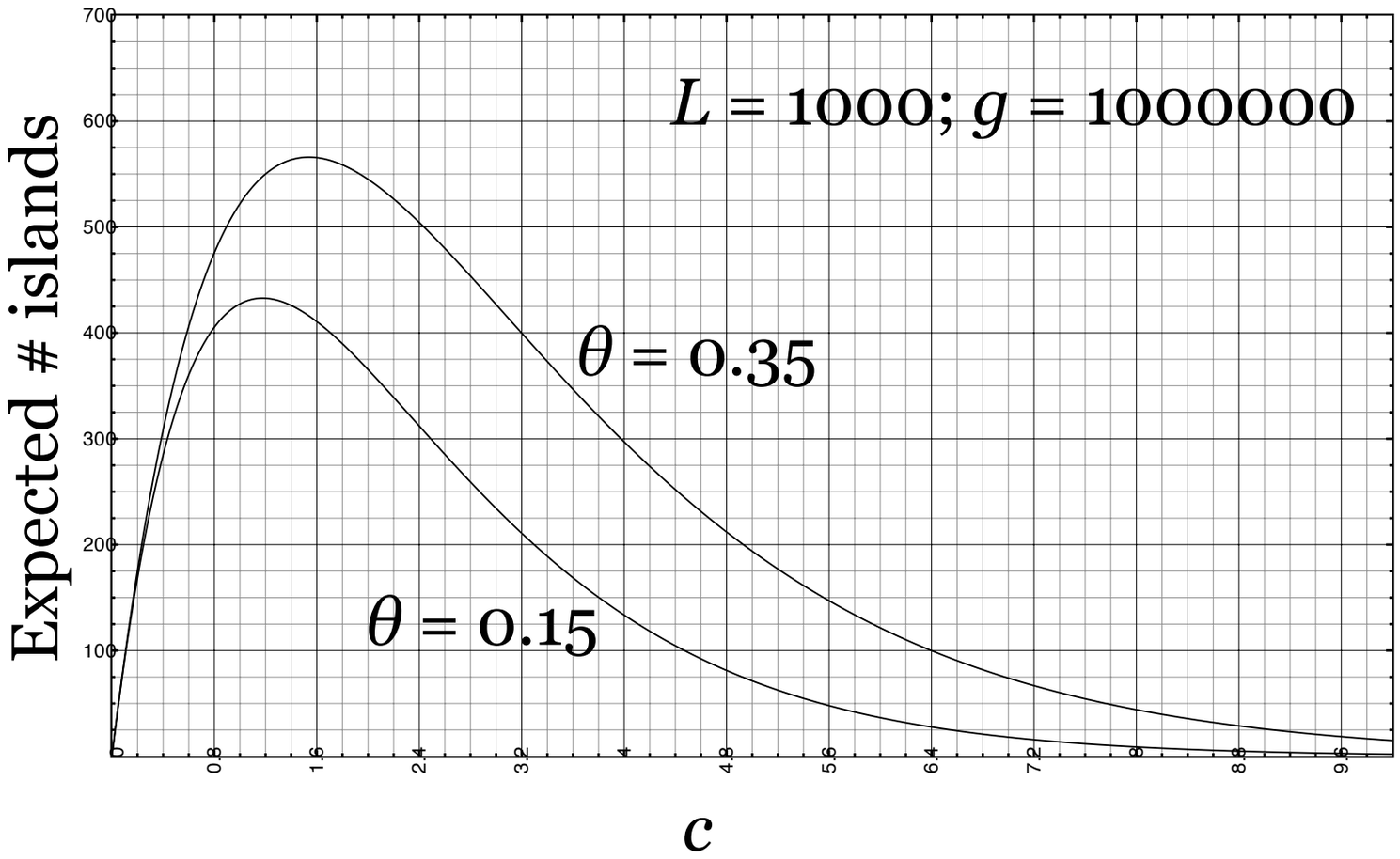
$$= Ne^{-\lambda(1-\theta)L}$$

$$= Ne^{-(1-\theta)LN/g}$$ ← LN/g is called the **coverage** *c*.

Expected # of Islands, 2

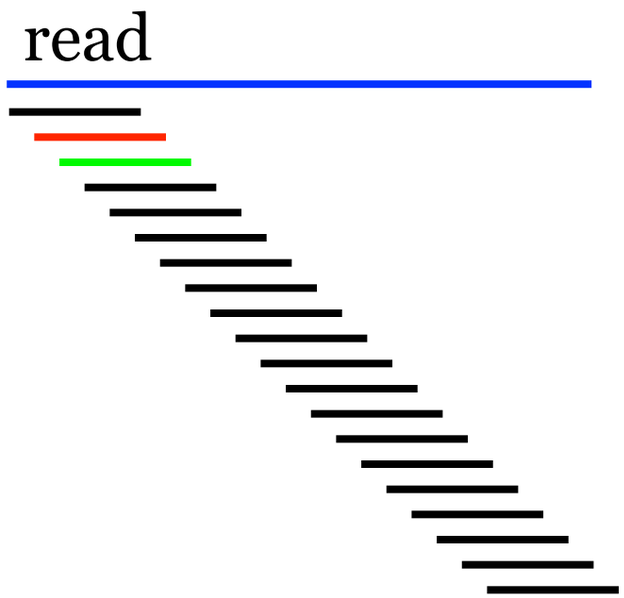
Rewrite to depend more directly on the things we can control: c and θ

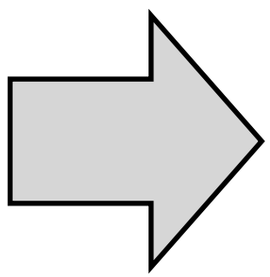
$$\begin{aligned}
\text{Expected \# of islands} &= Ne^{-(1-\theta)LN/g} \\
&= Ne^{-(1-\theta)c} \\
&= \frac{L/g}{L/g} Ne^{-(1-\theta)c} \\
&= \frac{g}{L} ce^{-(1-\theta)c}
\end{aligned}$$

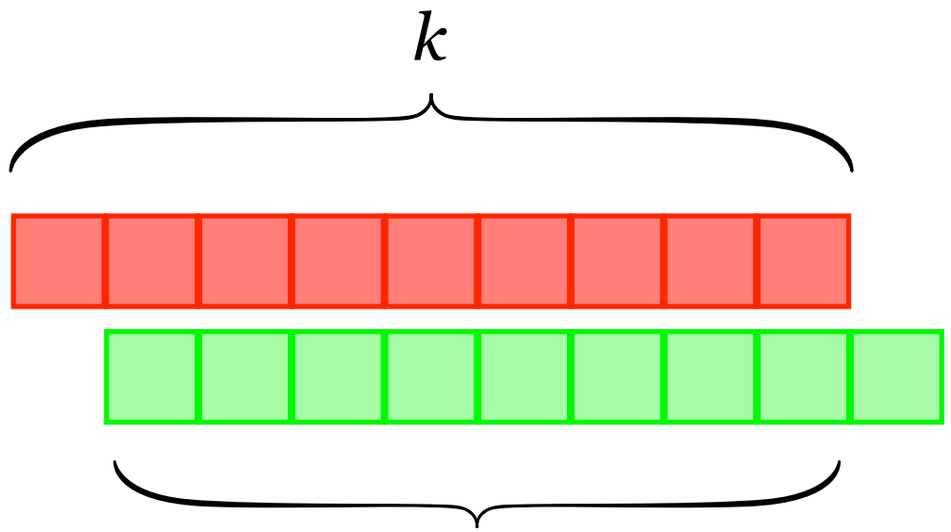


Assembly via Eulerian Path

de Bruijn graph

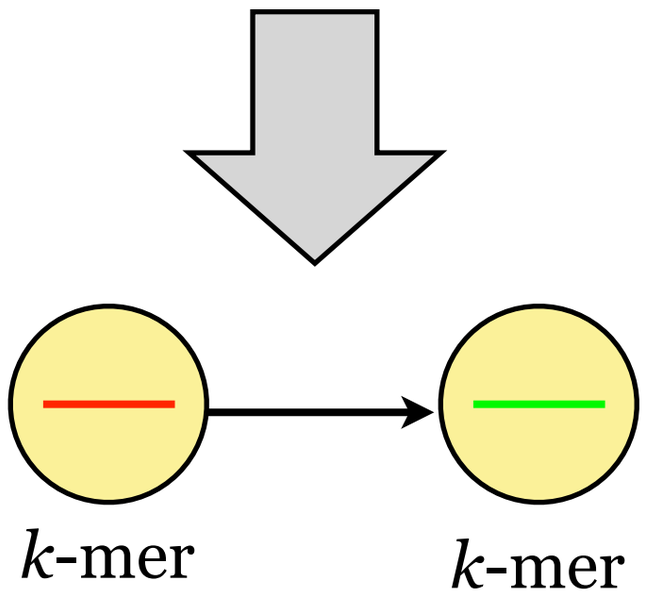




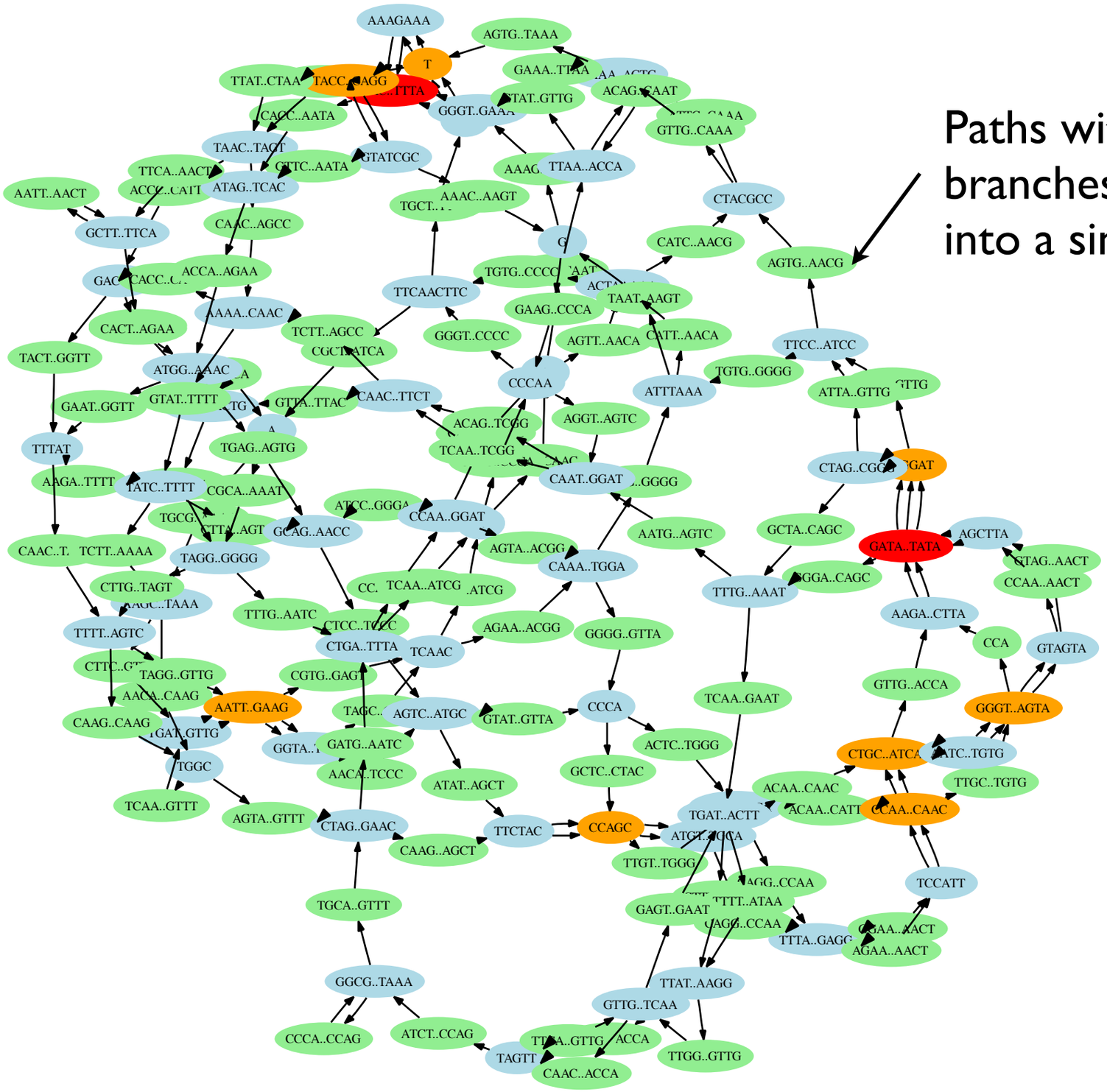


de Bruijn graph: nodes represent kmers, edges connect k-mers that are known to follow each other based on an observed read.

Can have > 1 edge between nodes.



Example bacterial de Bruijn graph

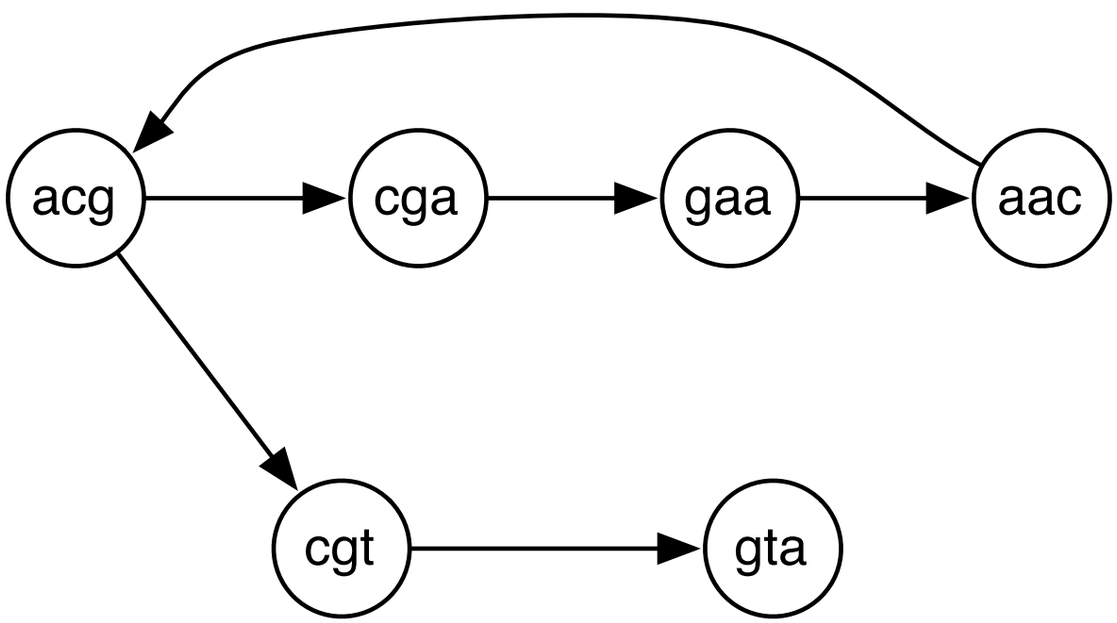


Paths with no branches compressed into a single node

Eulerian path = use every edge exactly once.

With perfect data, the genome can be reconstructed by some Eulerian path through this graph

Assembly via Eulerian Path



acgaacgta

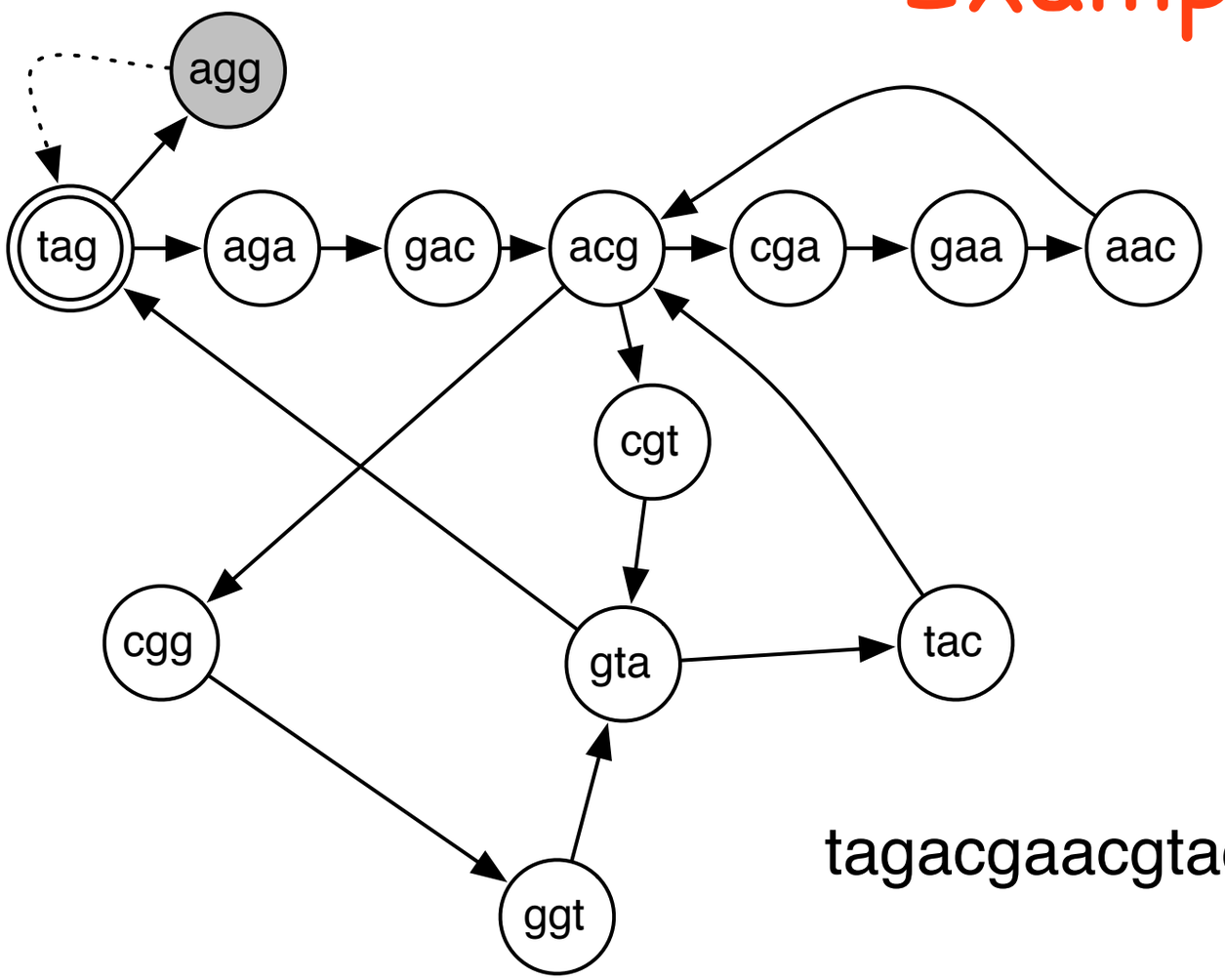
Let dG(s) be the de Bruijn graph of string s. Then s corresponds to some Eulerian path in dG(s).

A directed graph has an Eulerian path if and only if:

- One node has one more edge leaving it than entering
- One node has one more edge entering than leaving
- All other nodes have the same number of edges entering and leaving

How can we find such a path?

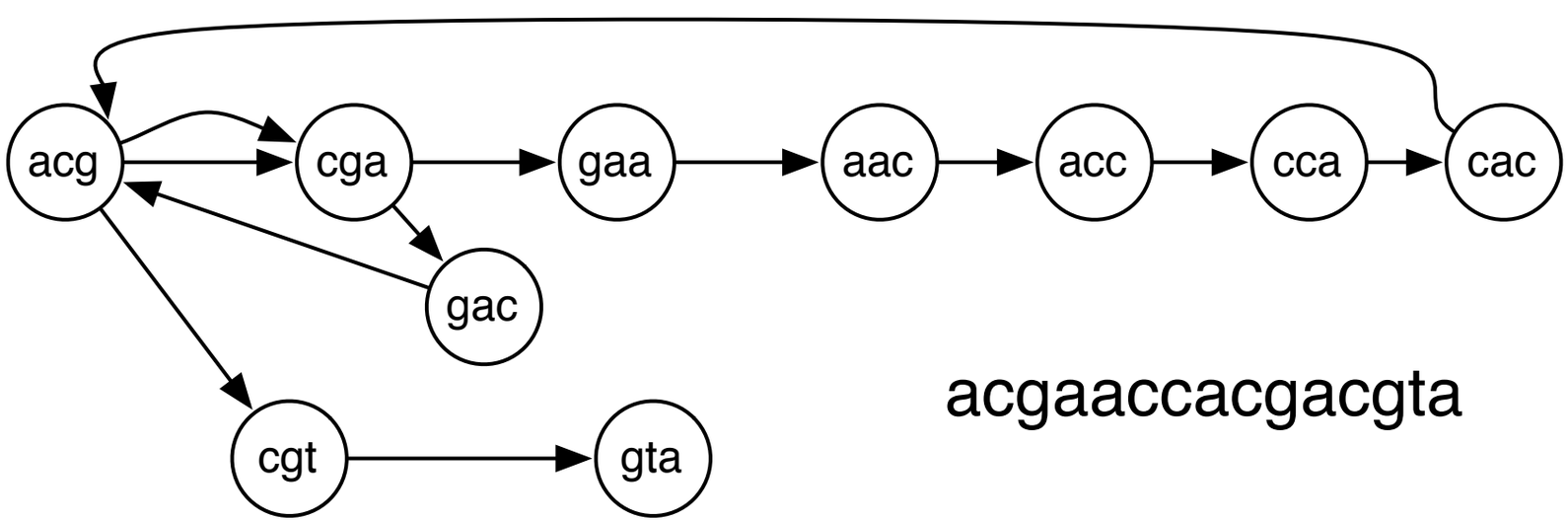
Examples



A directed graph has an Eulerian **cycle** if and only if:

- All nodes have the same number of edges entering and leaving

tagacgaacgtacggtagg



acgaaccacgacgta

Eulerian Path Algorithm

Connect node with out-degree < in-degree to node with out-degree < in-degree. So that we will have an Eulerian cycle.

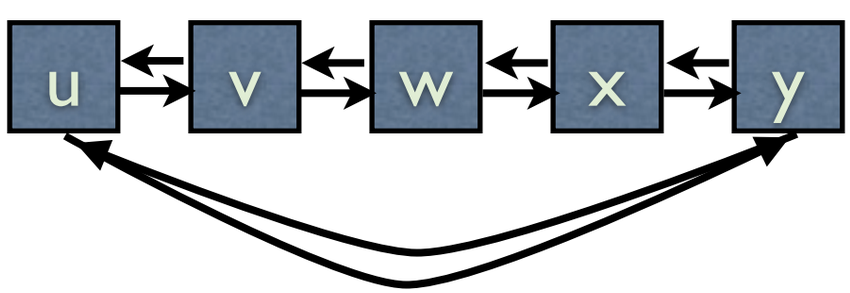
Why will you return to *u*?

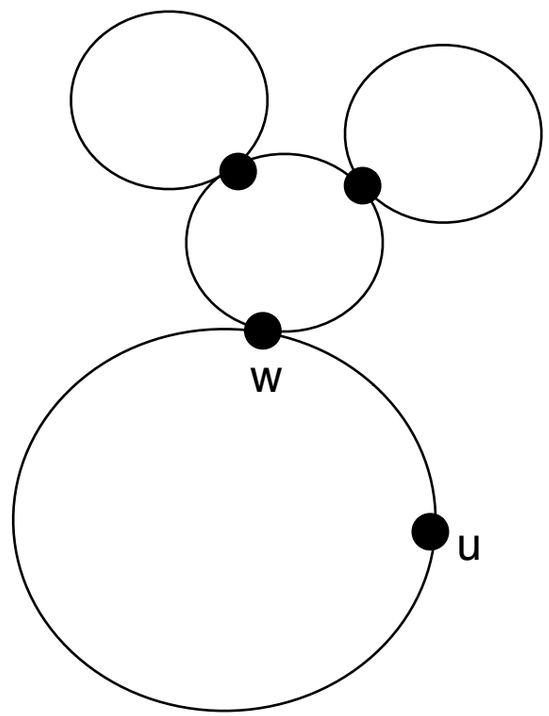
Walk from some arbitrary node *u* until you return to *u*, creating a doubly liked list of the path you visit.

Repeat until all edges used:

- Start from some node *w* on the current tour with unused edges*.
- Walk along unused edges until you return to *w*, inserting the visited nodes after *w* into the current tour list.

*How can find such a node quickly?





Eulerian Path Algorithm

Connect node with out-degree < in-degree to node with out-degree < in-degree. So that we will have an Eulerian cycle.

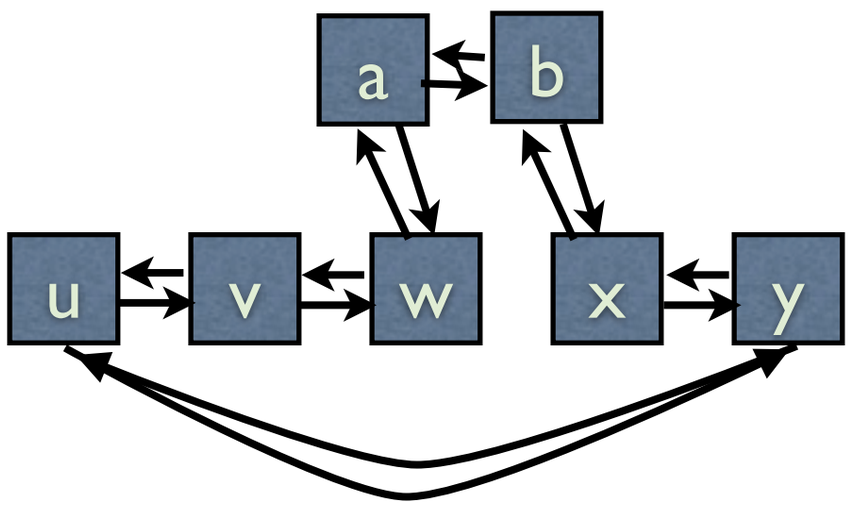
Why will you return to *u*?

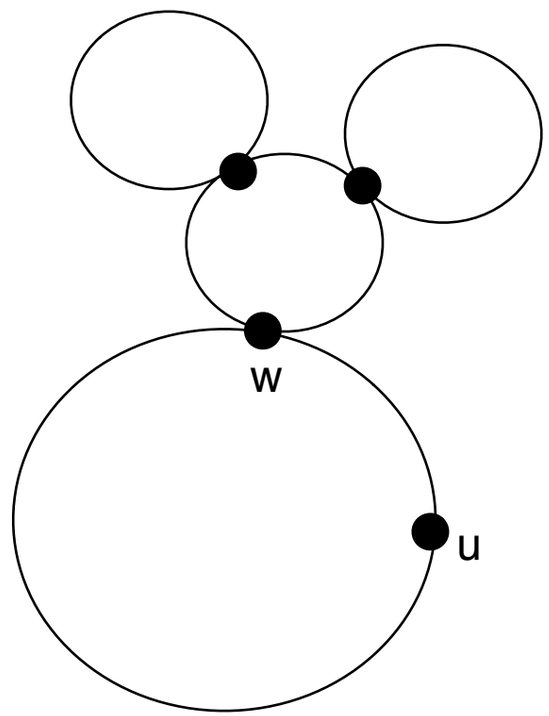
Walk from some arbitrary node *u* until you return to *u*, creating a doubly liked list of the path you visit.

Repeat until all edges used:

*How can find such a node quickly?

- Start from some node *w* on the current tour with unused edges*.
- Walk along unused edges until you return to *w*, inserting the visited nodes after *w* into the current tour list.



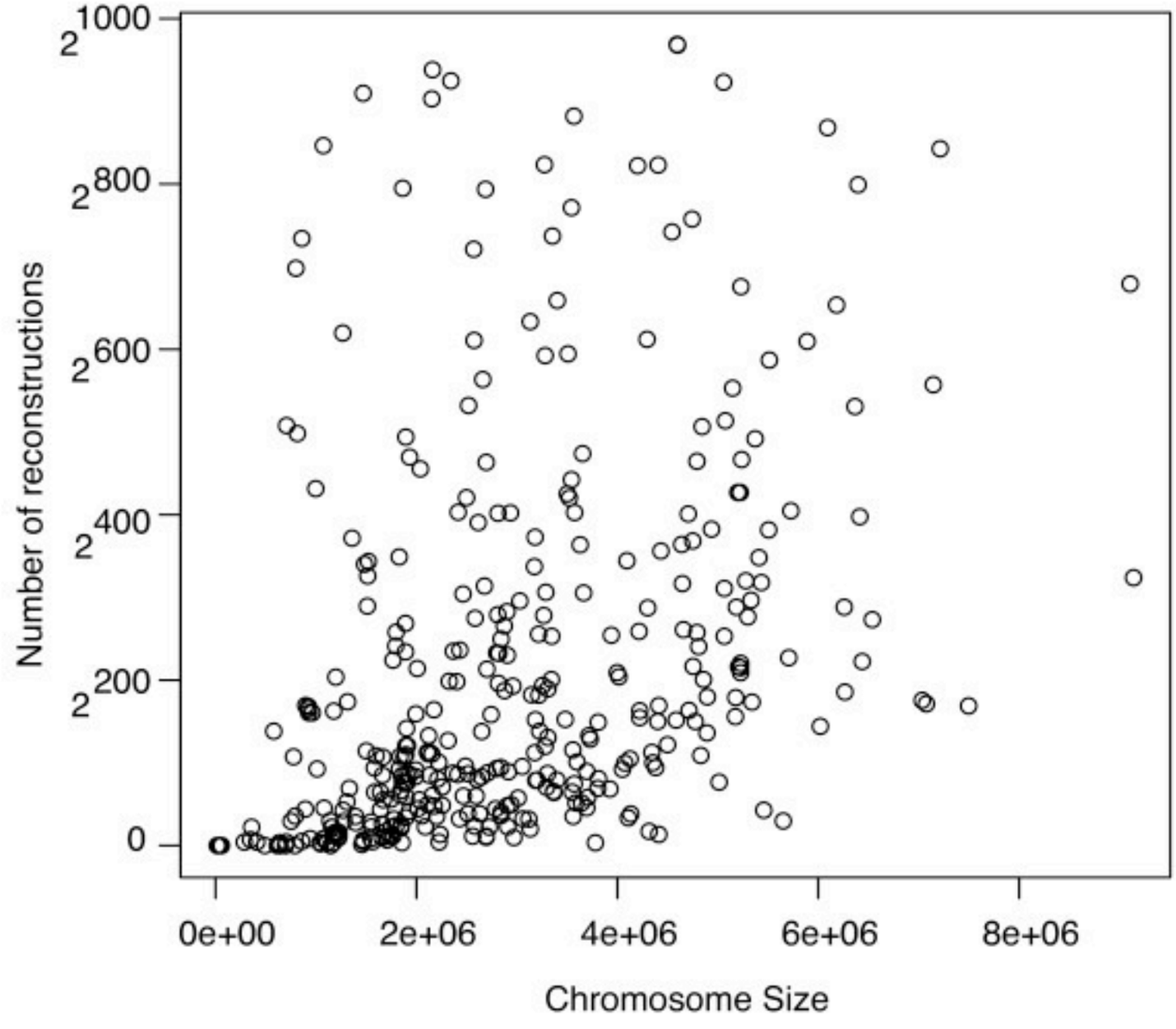


The Problem with Eulerian Paths

There are typically an astronomical number of possible Eulerian tours with perfect data.

Adding back constraints to limit # of tours leads to a NP-hard problem.

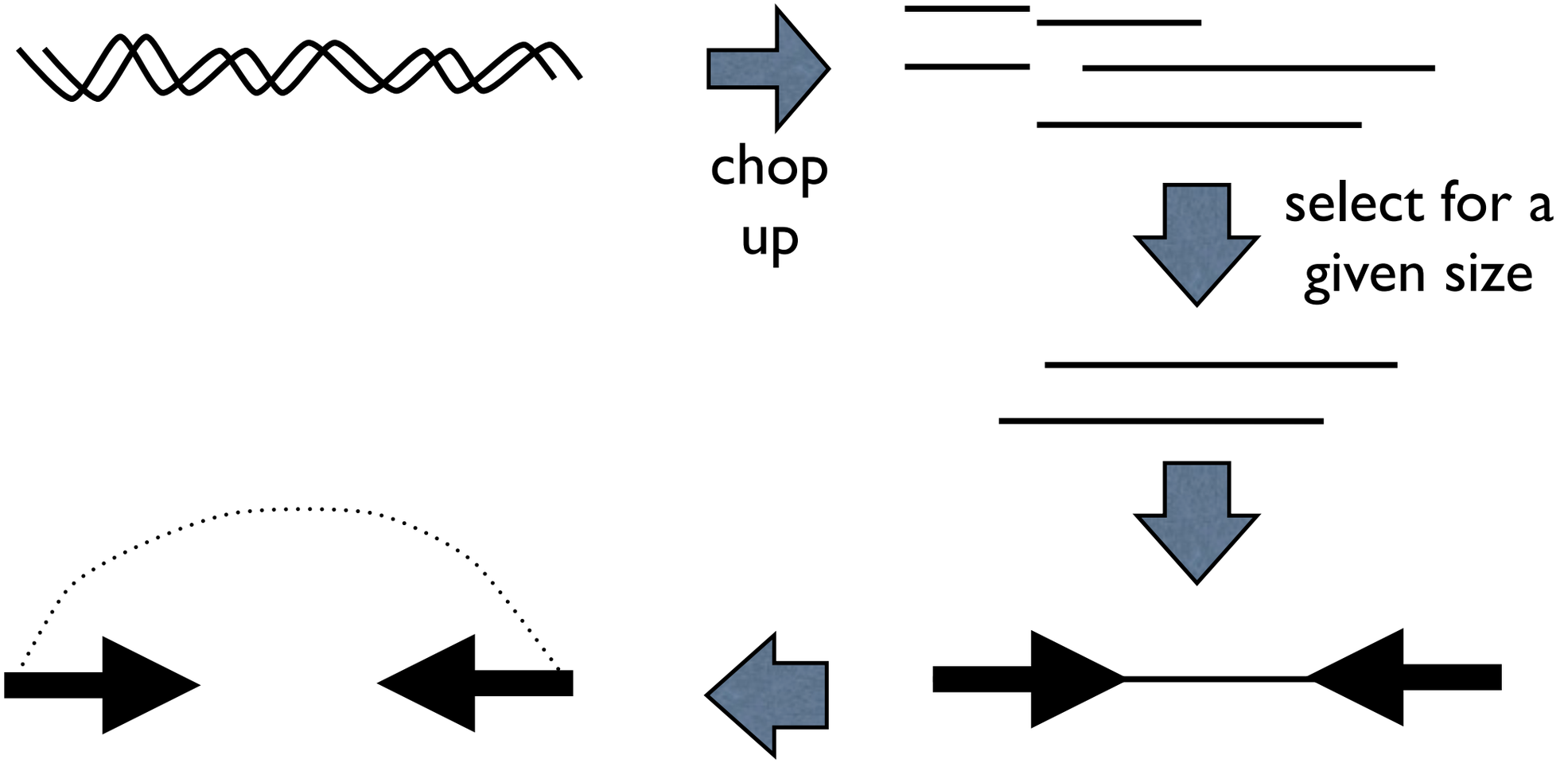
With imperfect data, there are usually NO Eulerian tours.



(Kingsford, Schatz, Pop, 2010)

Aside: counting # of Eulerian tours in a directed graph is easy, but in an undirected graph is #P-complete (hard).

Mate Pairs

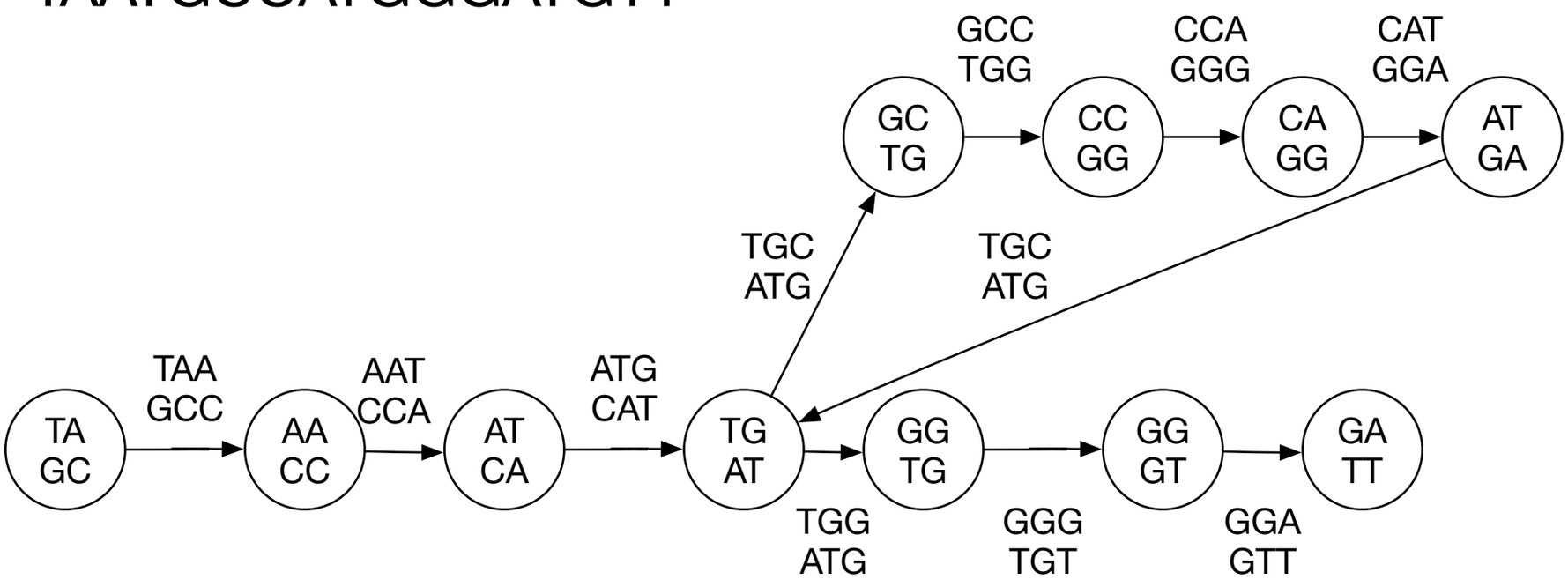


mate pair: 2 reads, of opposite orientation, separated by an approximately known distance

sequence ≈ 1000 bases from each end

⇒ long range information

TAATGCCATGGGATGTT



Gapped Genome Path String Problem

Reconstruct a string from a sequence of (k,d)-mers corresponding to a path in a paired de Bruijn graph.

Given: A sequence of (k, d)-mers $(a_1|b_1)$, ... , $(a_n|b_n)$ such that Suffix$(a_i|b_i)$ = Prefix$(a_{i+1}|b_{i+1})$ for all i from 1 to n-1.

Return: A string Text where the i-th k-mer in Text is equal to Suffix$(a_i|b_i)$ for all i from 1 to n, if such a string exists.

References

- http://www.cbcb.umd.edu/research/assembly_primer
- http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2874646/
- http://www.math.ucsd.edu/~gptesler/186/slides/shotgun_f13-handout.pdf
- http://www.biomedcentral.com/1471-2105/11/21/abstract