# Assembly

Shortest common superstring

# Assembly

Reads

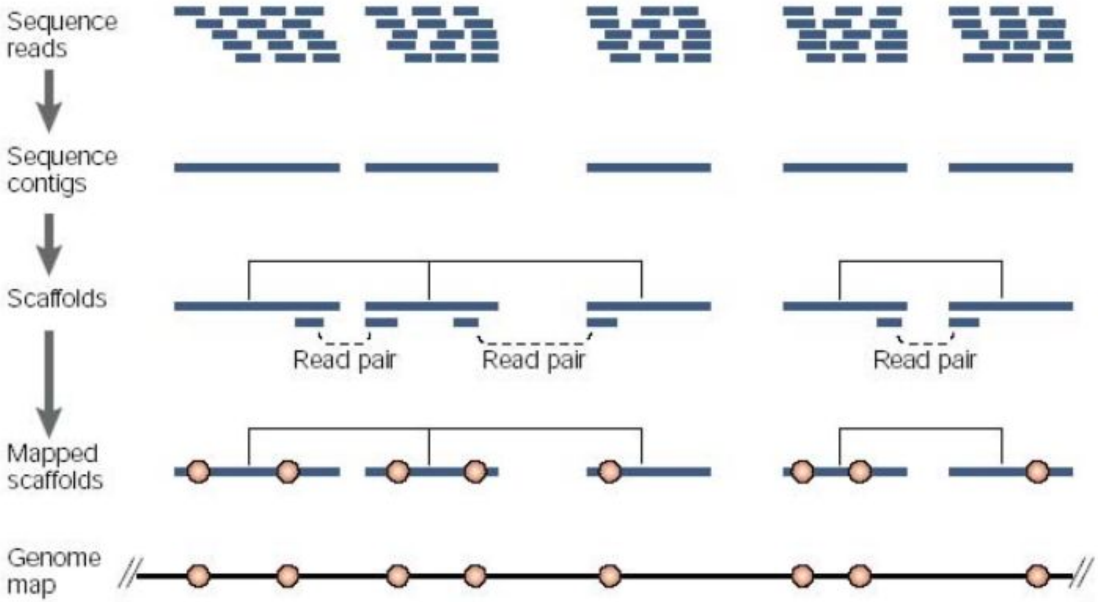
+

Reference genome

Input DNA

How to assemble puzzle without the benefit of knowing what the finished product looks like?

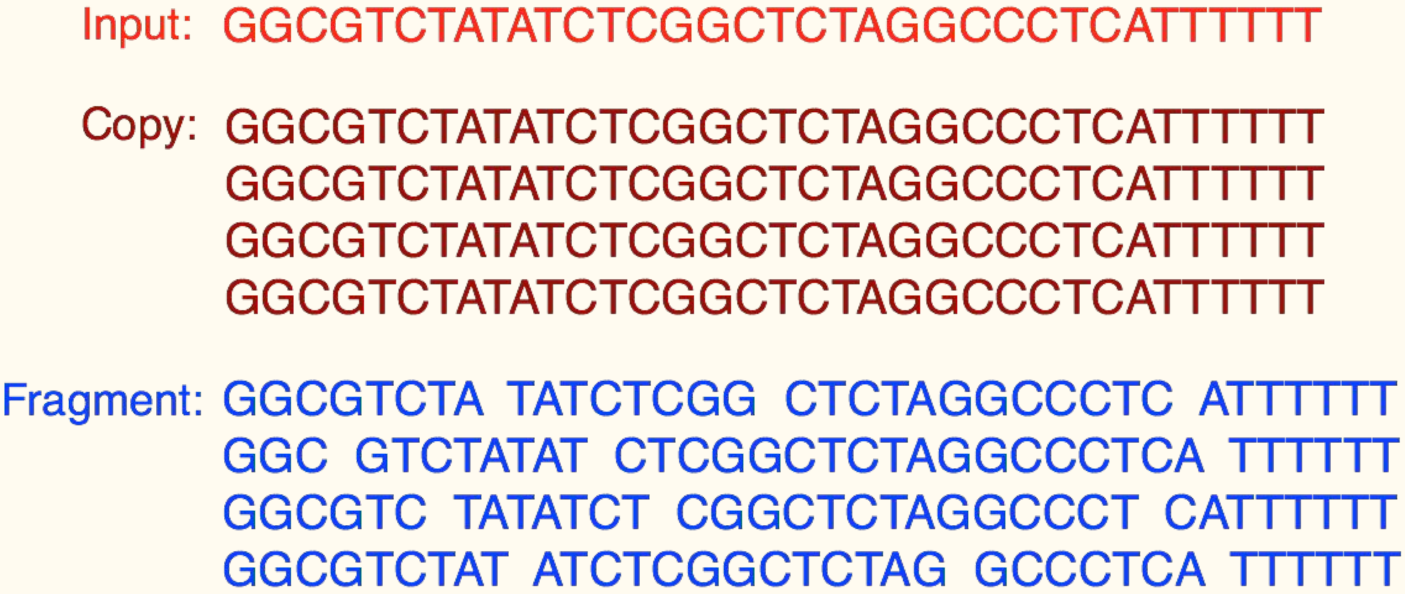
# *de novo* whole-genome shotgun assembly



# Assembly

Whole-genome “shotgun” sequencing starts by copying and fragmenting the DNA

(“Shotgun” refers to the random fragmentation of the whole genome; like it was fired from a shotgun)



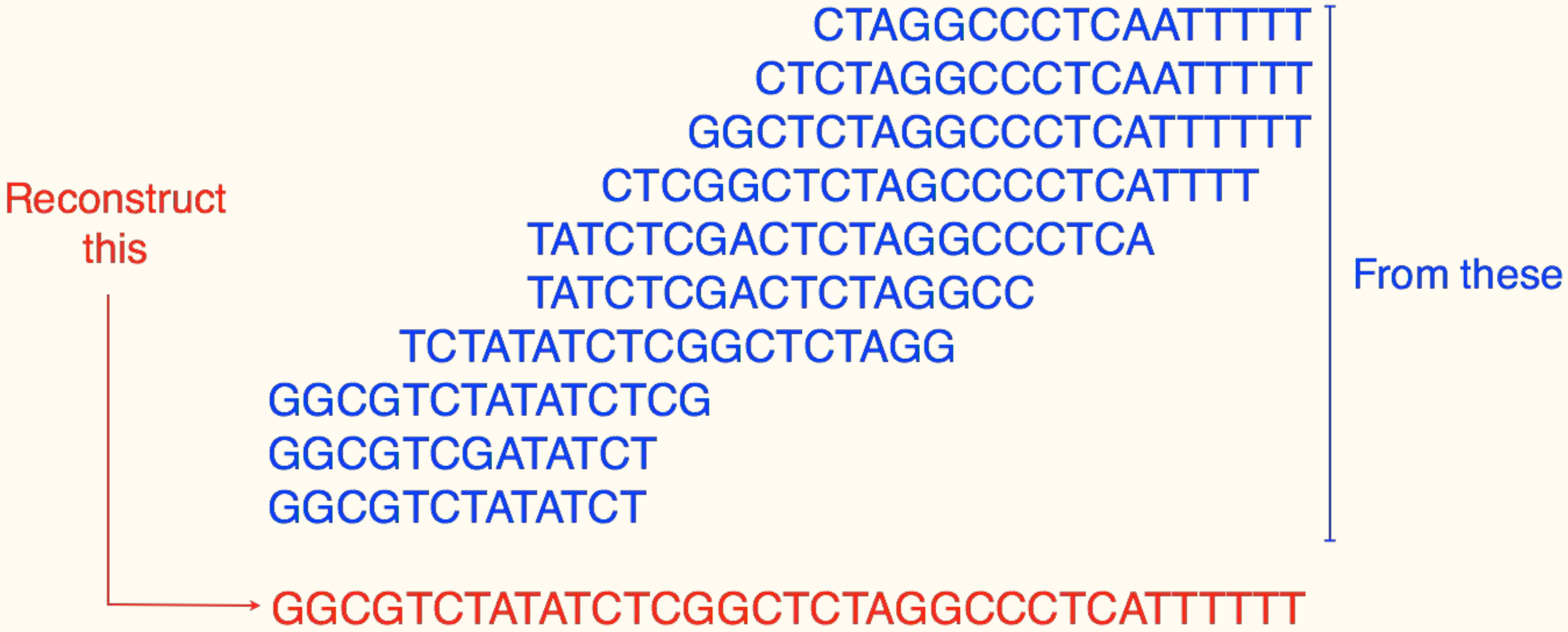
```
Input:    GGCGTCTATATCTCGGCTCTAGGCCCTCATTTTTT

Copy:     GGCGTCTATATCTCGGCTCTAGGCCCTCATTTTTT
          GGCGTCTATATCTCGGCTCTAGGCCCTCATTTTTT
          GGCGTCTATATCTCGGCTCTAGGCCCTCATTTTTT
          GGCGTCTATATCTCGGCTCTAGGCCCTCATTTTTT

Fragment: GGCGTCTA TATCTCGG CTCTAGGCCCTC ATTTTTT
          GGC GTCTATAT CTCGGCTCTAGGCCCTCA TTTTTT
          GGCGTC TATATCT CGGCTCTAGGCCCT CATTTTTT
          GGCGTCTAT ATCTCGGCTCTAG GCCCTCA TTTTTT
```

# Assembly

Assume sequencing produces such a large number fragments that almost all genome positions are *covered* by many fragments...



CTAGGCCCTCAATTTTT

CTCTAGGCCCTCAATTTTT

GGCTCTAGGCCCTCATTTTTT

CTCGGCTCTAGCCCCTCATTTT

TATCTCGACTCTAGGCCCTCA

TATCTCGACTCTAGGCC

TCTATATCTCGGCTCTAGG

GGCGTCTATATCTCG

GGCGTCGATATCT

GGCGTCTATATCT

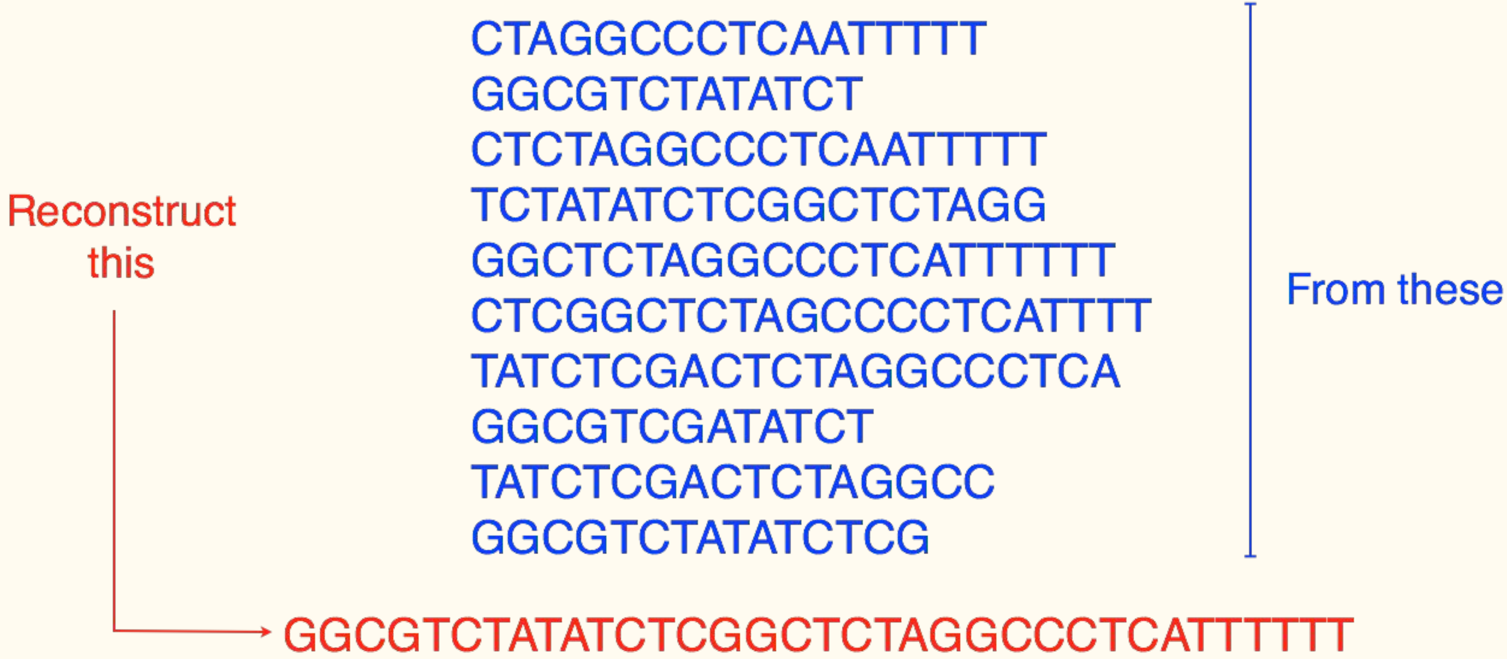
From these

Reconstruct this

GGCGTCTATATCTCGGCTCTAGGCCCTCATTTTTT

# Assembly

...but we don't know what came from where



Reconstruct this

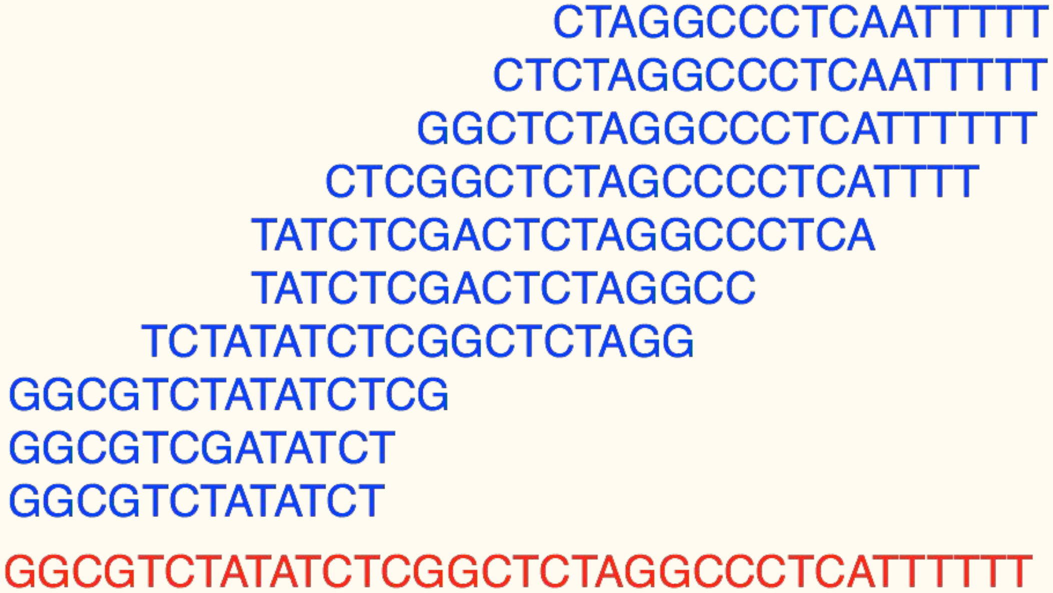
CTAGGCCCTCAATTTTT
GGCGTCTATATCT
CTCTAGGCCCTCAATTTTT
TCTATATCTCGGCTCTAGG
GGCTCTAGGCCCTCATTTTTT
CTCGGCTCTAGCCCCTCATTTT
TATCTCGACTCTAGGCCCTCA
GGCGTCGATATCT
TATCTCGACTCTAGGCC
GGCGTCTATATCTCG

From these

GGCGTCTATATCTCGGCTCTAGGCCCTCATTTTTT

# Assembly

Key term: *coverage*. Usually it's short for *average coverage*: the average number of reads covering a position in the genome



CTAGGCCCTCAATTTTT

CTCTAGGCCCTCAATTTTT

GGCTCTAGGCCCTCATTTTTTT

CTCGGCTCTAGCCCCTCATTTT

TATCTCGACTCTAGGCCCTCA

TATCTCGACTCTAGGCC

TCTATATCTCGGCTCTAGG

GGCGTCTATATCTCG

GGCGTCGATATCT

GGCGTCTATATCT

GGCGTCTATATCTCGGCTCTAGGCCCTCATTTTTT

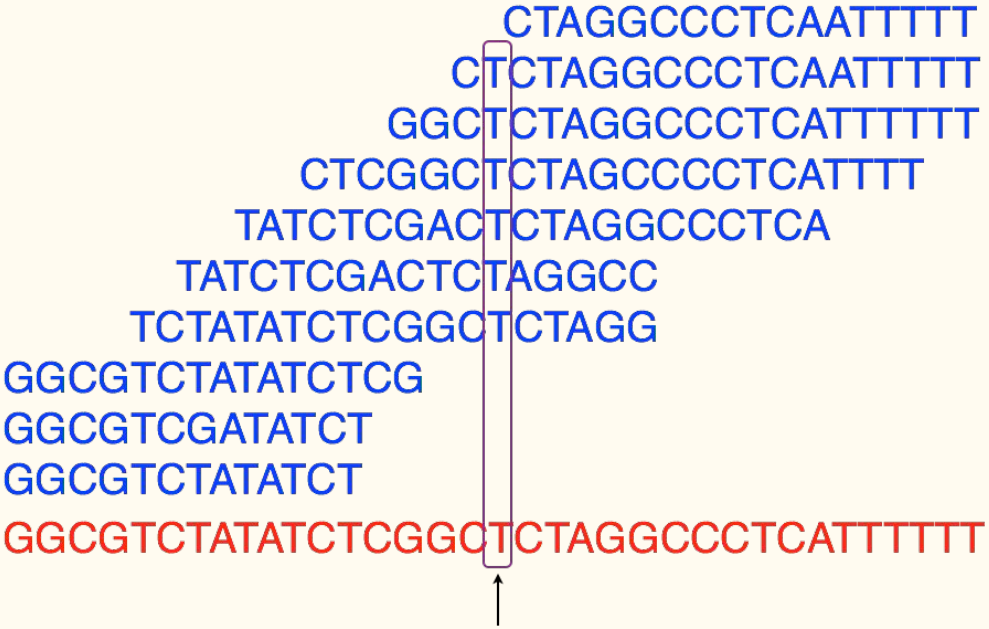
177 nucleotides

35 nucleotides

Average coverage = 177 / 35 ≈ 7x

# Assembly

*Coverage* could also refer to the number of reads covering a particular position in the genome:



CTAGGCCCTCAATTTTT

CTCTAGGCCCTCAATTTTT

GGCTCTAGGCCCTCATTTTTT

CTCGGCTCTAGCCCCTCATTTT

TATCTCGACTCTAGGCCCTCA

TATCTCGACTCTAGGCC

TCTATATCTCGGCTCTAGG

GGCGTCTATATCTCG

GGCGTCGATATCT

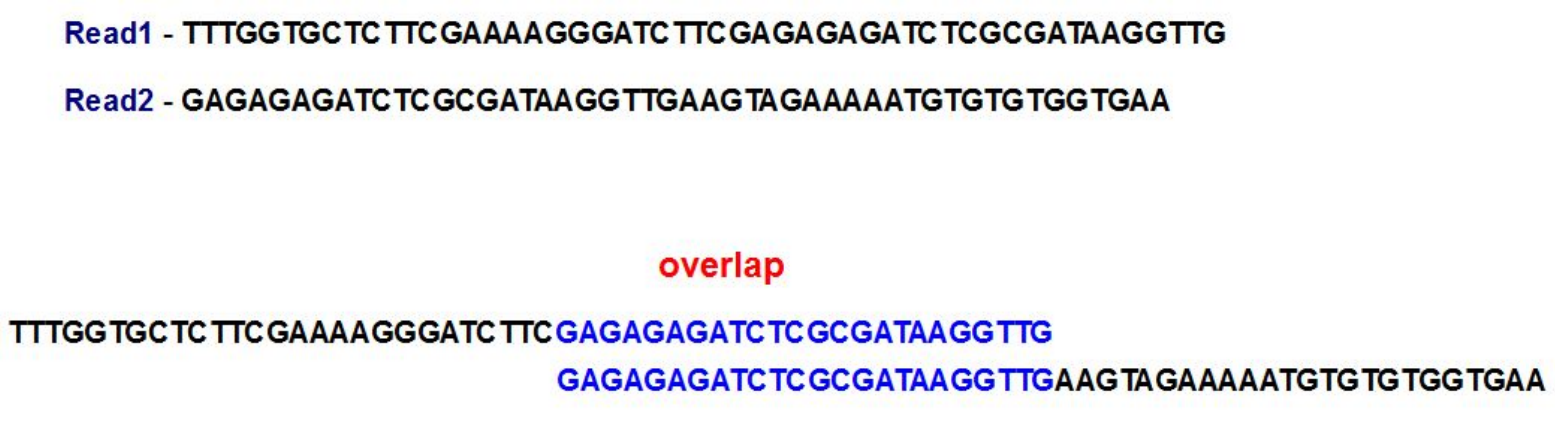
GGCGTCTATATCT

GGCGTCTATATCTCGGCTCTAGGCCCTCATTTTTT

Coverage at this position $= 6$

# Assembly

- Best case scenario: check every pair of (long) reads for overlaps
- Computationally expensive: **n** reads, **~n**2** operations
- Our task: how to get the **best assemblies** for the **smallest expense** – in terms of sequencing and computational expenses



Read1 - TTTGGTGCTCTTCGAAAAGGGATCTTCGAGAGAGATCTCGCGATAAGGTTG

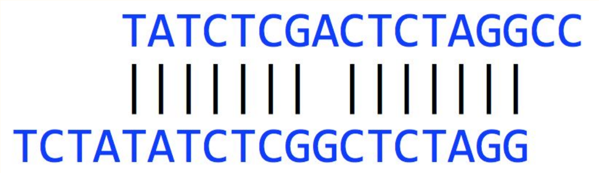
Read2 - GAGAGAGATCTCGCGATAAGGTTGAAGTAGAAAAATGTGTGTGGTGAA

overlap

```
TTTGGTGCTCTTCGAAAAGGGATCTTCGAGAGAGATCTCGCGATAAGGTTG
                           GAGAGAGATCTCGCGATAAGGTTGAAGTAGAAAAATGTGTGTGGTGAA
```

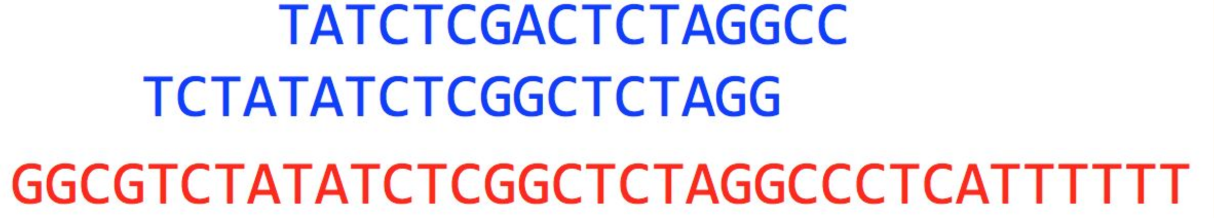
# Assembly

Basic principle: the more similarity there is between the end of one read and the beginning of another...



```
    TATCTCGACTCTAGGCC
    ||||||| |||||||
TCTATATCTCGGCTCTAGG
```

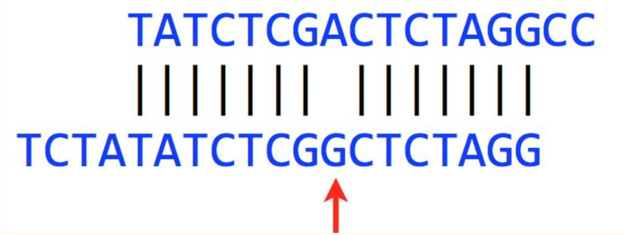
...the more likely they are to have originated from overlapping stretches of the genome:



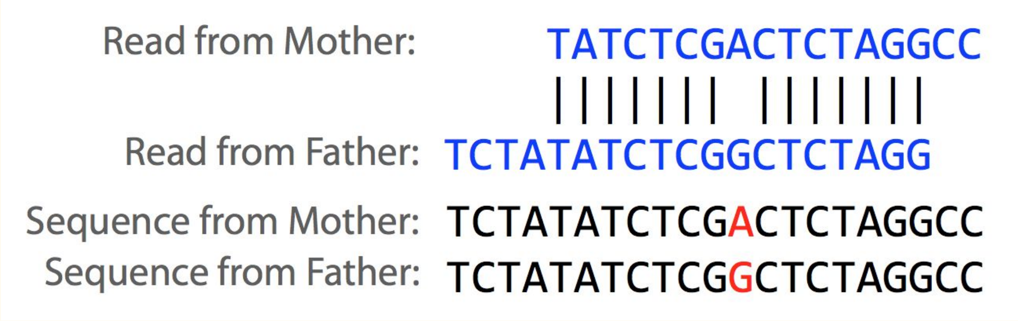
```
        TATCTCGACTCTAGGCC
    TCTATATCTCGGCTCTAGG
GGCGTCTATATCTCGGCTCTAGGCCCTCATTTTTT
```

# Assembly

Say two reads truly originate from overlapping stretches of the genome. Why might there be differences?



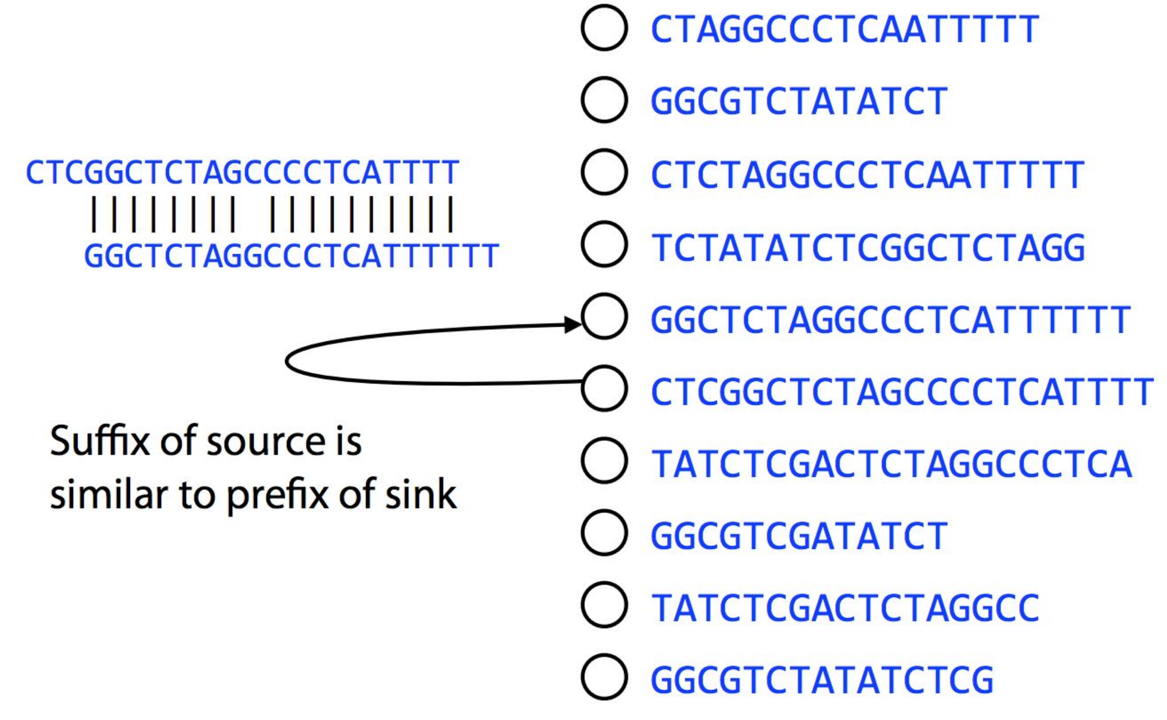
1. Sequencing error
2. Difference between inherited *copies* of a chromosome. E.g. humans are diploid; we have two copies of each chromosome, one from mother, one from father. The copies can differ:


Read from Mother: TATCTCGACTCTAGGCC
Read from Father: TCTATATCTCGGCTCTAGG
Sequence from Mother: TCTATATCTCGACTCTAGGCC
Sequence from Father: TCTATATCTCGGCTCTAGGCC

We'll mostly ignore ploidy, but real tools must consider it

# Assembly

Finding all overlaps is like building a *directed graph* where directed edges connect overlapping nodes (reads)



```
CTCGGCTCTAGCCCCTCATTTT
   |||||||| ||||||||||
   GGCTCTAGGCCCTCATTTTTT
```

Suffix of source is similar to prefix of sink

○ CTAGGCCCTCAATTTTT

○ GGCGTCTATATCT

○ CTCTAGGCCCTCAATTTTT

○ TCTATATCTCGGCTCTAGG

○ GGCTCTAGGCCCTCATTTTTT

○ CTCGGCTCTAGCCCCTCATTTT

○ TATCTCGACTCTAGGCCCTCA

○ GGCGTCGATATCT

○ TATCTCGACTCTAGGCC

○ GGCGTCTATATCTCG

# Graphs

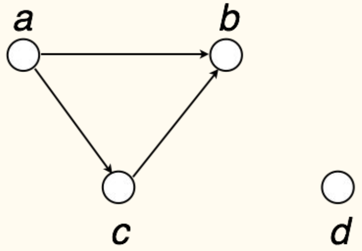
**Directed graph** G=(V, E) is a pair consisting of *node set* (or vertex set) V and *edge set (*or *arc set)* $E \subseteq VxV$.

An **edge** $e = (u, v) \in E$ represents a connection from $u$ to $v$.
**Directed edge** is an ordered pair of vertices.
We call $u$ and $v$ the *source* and the *target (sink)*, respectively, of $e$.

Directed graph also called *digraph*.

An *outdegree* of the node is the number of edges leaving it, and *indegree* of the node is the number of edges ending at it.

*Graph theory* developed by Euler (more info about this later...)



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

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

Source Sink

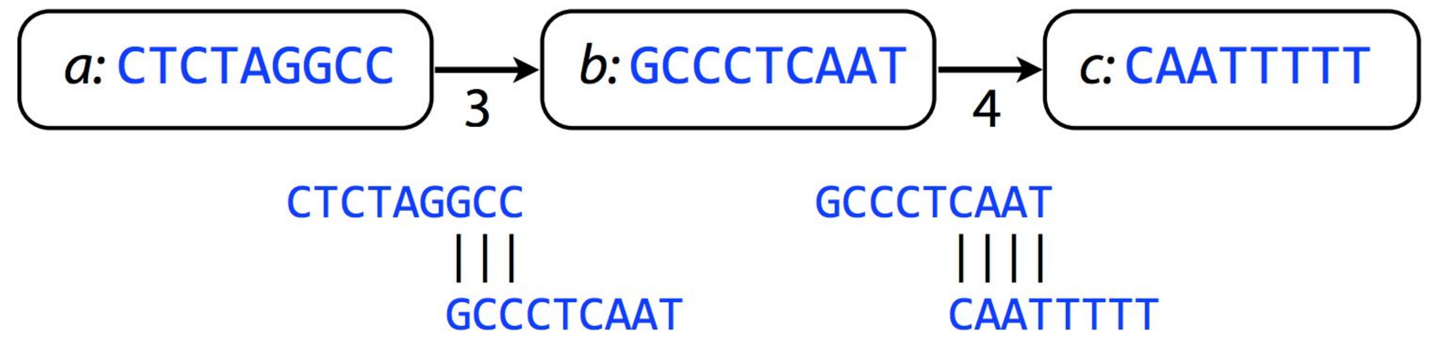
# Overlap graph

Below: overlap graph, where an overlap is a suffix/prefix match of at least 3 characters.

In **overlap graph**:
A **vertex** is a read.
A **directed edge** is an overlap between suffix of source and prefix of sink.

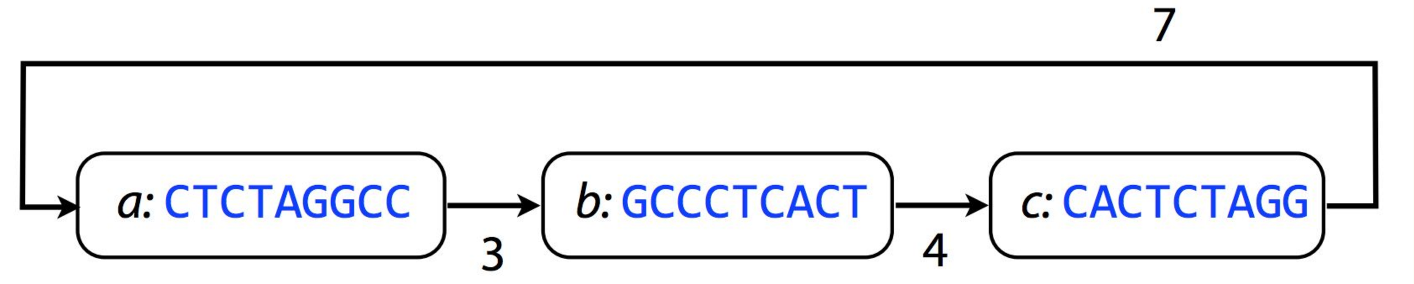
Vertices (reads): { *a:* CTCTAGGCC, *b:* GCCCTCAAT, *c:* CAATTTTT }

Edges (overlaps): {($a$, $b$), ($b$, $c$)}



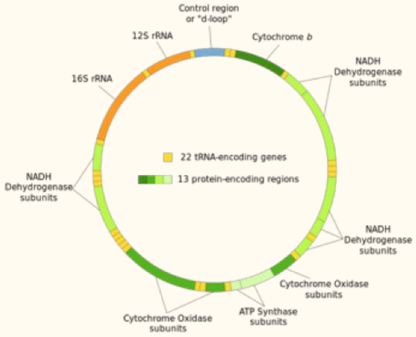
# Overlap graph - cycles in graph

Overlap graph could contain *cycles*.  A cycle is a path beginning and ending at the same vertex.

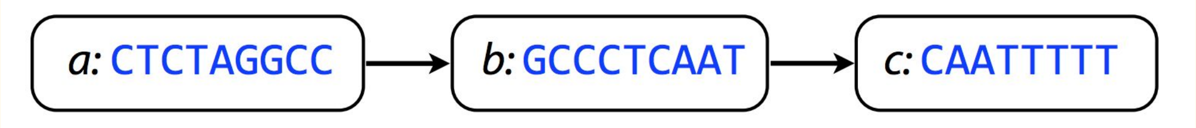


These happen when the DNA string itself is circular.  E.g. bacterial genomes are often circular; mitochondrial DNA is circular.

Cycles could also be due to *repetitive* DNA, as we'll see.



# Finding overlaps



How do we build the overlap graph?

What constitutes an overlap?

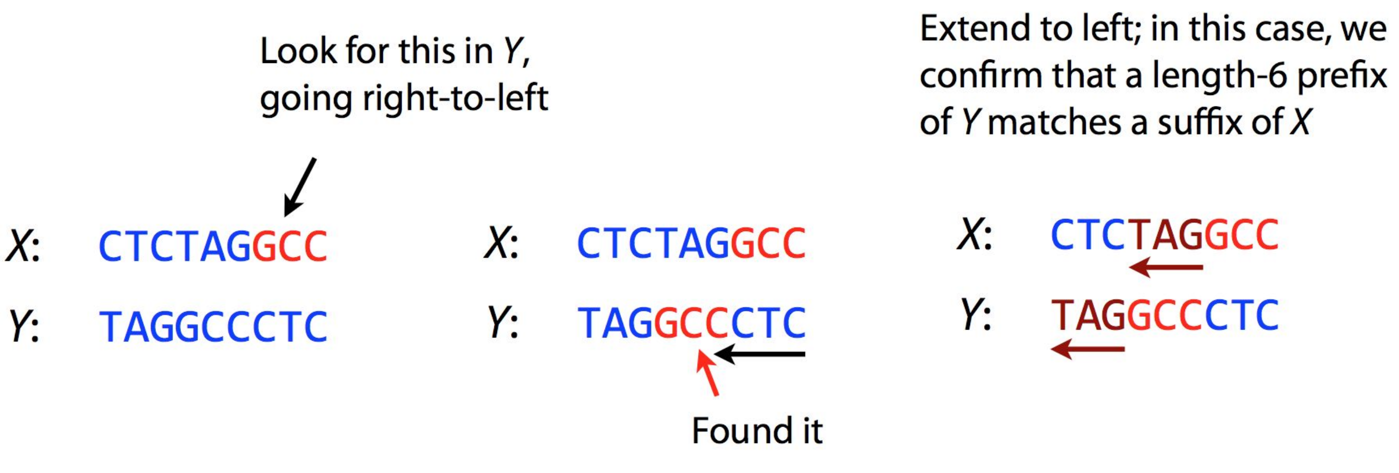
(**Def:**?) Assume for now an “overlap” is when a suffix of $\boldsymbol{X}$ of **length** $\geqslant \boldsymbol{l}$ exactly matches a prefix of $\boldsymbol{Y}$, where $l$ is given.

# Finding overlaps

**Overlap:** length-$l$ suffix of X matches length-$l$ prefix of Y, where $l$ is given.

Simple idea: look in Y for occurrences of length-$l$ suffix of X. Extend matches to the left to confirm whether entire prefix of Y matches.

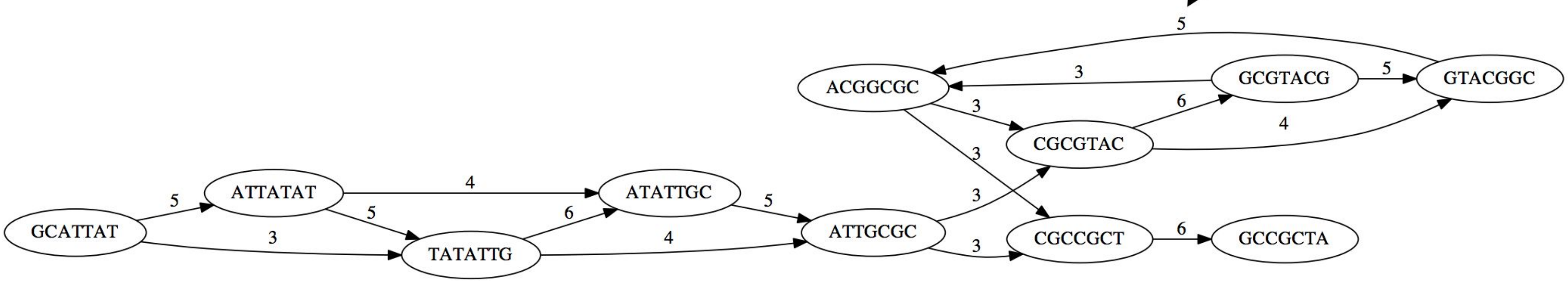
For l=3


Look for this in *Y*, going right-to-left
*X*: CTCTAGGCC
*Y*: TAGGCCCTC
*X*: CTCTAGGCC
*Y*: TAGGCCCTC
Found it
Extend to left; in this case, we confirm that a length-6 prefix of *Y* matches a suffix of *X*
*X*: CTCTAGGCC
*Y*: TAGGCCCTC

# Finding overlaps

Example overlap graph with $l = 3$

Edge label is overlap length



Original string: GCATTATATATTGCGCGTACGGCGCCGCTACA

# Shortest common superstring

Finding overlaps is important, and we'll return to it, but our ultimate goal is to recreate (assemble) the genome.

Every sequence (read) is a part of the genome, and we're trying to recreate a genome from reads. We need a *superstring* of the reads - string (genome) that contains all reads we have. Reasonable approximation is that we want not any, but *shortest superstring*.

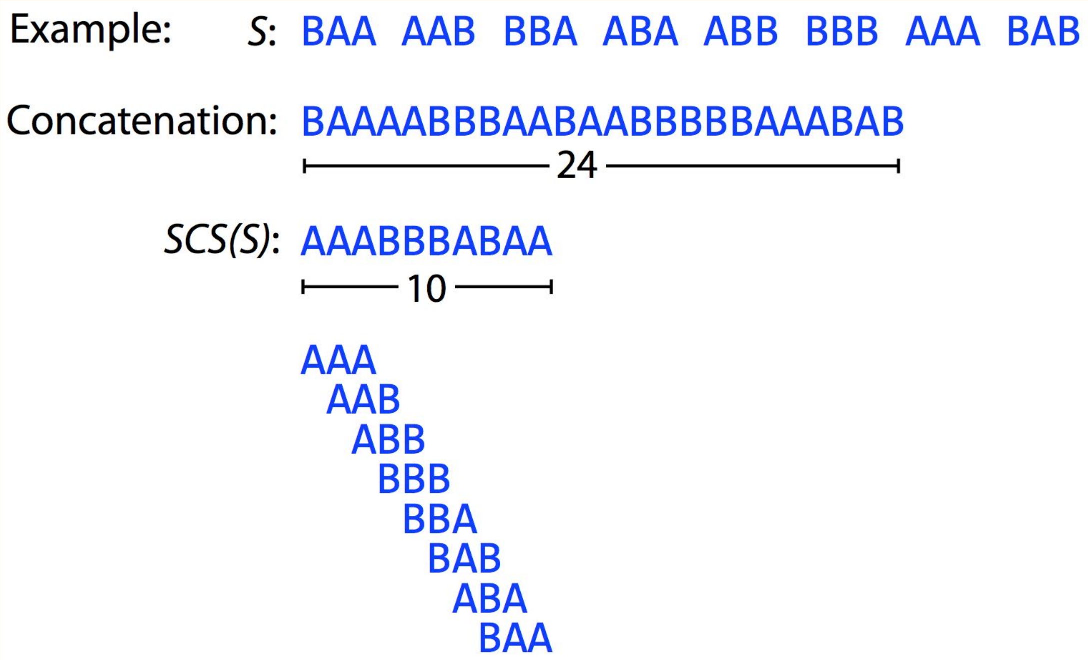
How do we formulate this problem?

**First attempt:** the s**hortest common superstring (SCS)** problem:
Given a set of strings, find a shortest string that contains all of them.

# Shortest common superstring

Given a collection of strings S, find SCS(S): the shortest string that contains all strings in S as substrings.

Without requirement of “shortest,” it’s easy: just concatenate them



Example: *S*: BAA AAB BBA ABA ABB BBB AAA BAB

Concatenation: BAAAABBBAABAABBBBBAAABAB

24

*SCS(S)*: AAABBBABAA

10

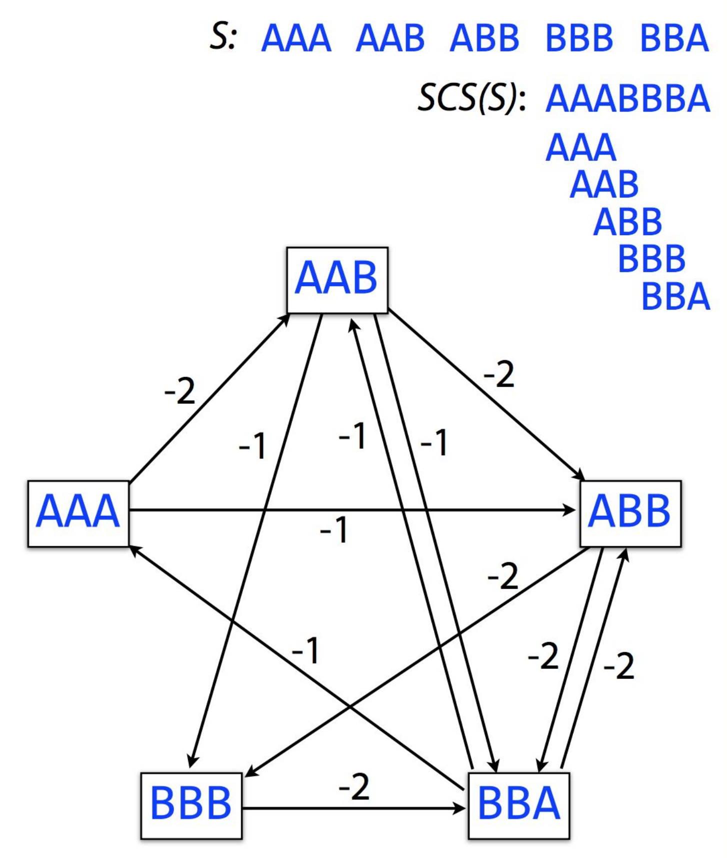
```
AAA
 AAB
  ABB
   BBB
    BBA
     BAB
      ABA
       BAA
```

# Shortest common superstring

How can we solve it?

Imagine a modified overlap graph where each edge has **cost** = - (length of overlap)

SCS corresponds to a path that visits every node once, minimizing total cost along path That's the Traveling Salesman Problem (TSP), which is NP-hard!

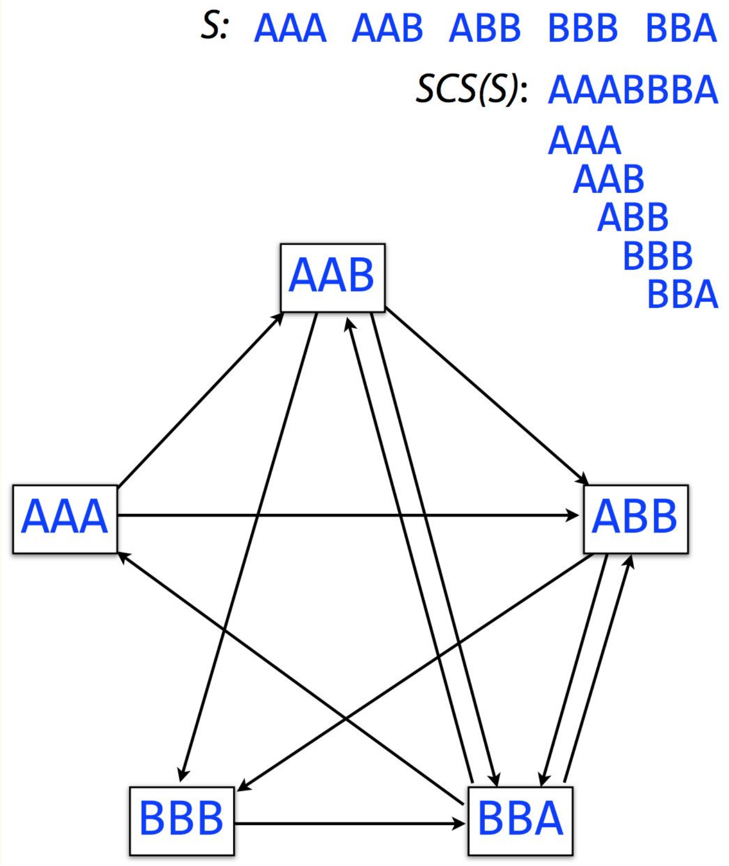


# Shortest common superstring

Say we disregard edge weights and just look for a path that visits all the nodes exactly once

That’s the Hamiltonian Path problem: NP-complete

Indeed, it’s well established that SCS is NP-hard



*S:* AAA AAB ABB BBB BBA

*SCS(S):* AAABBBA

```
AAA
 AAB
  ABB
   BBB
    BBA
```

# NP problems

NP: Decision problems (yes or no answers), if “yes” can be checked in polynomial time

NP complete: Solve one, solved them all!

- Traveling salesman problem
- Knapsack problem
- Integer linear programming
- SAT problem …

(Karp’s 21 NP-complete problems)

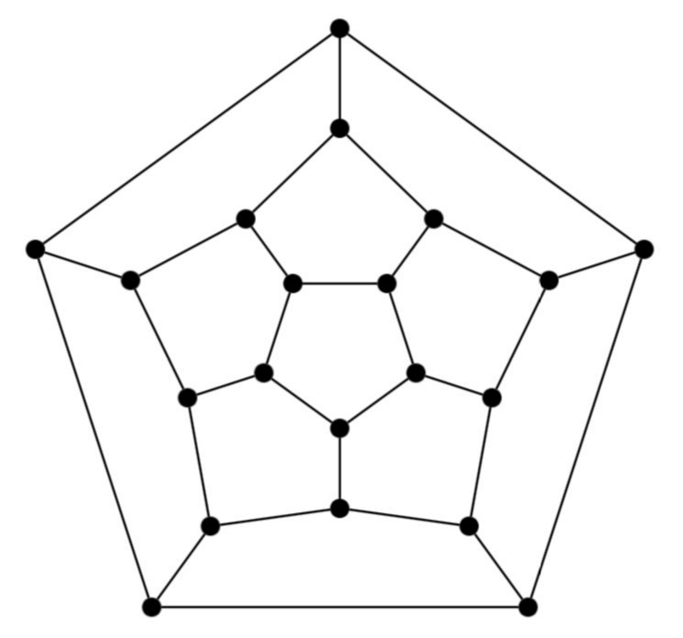
NP hard: at least hard as NP

# Hamiltonian Path

**Hamiltonian Cycle Problem:**
Find a cycle in a graph that visits every vertex exactly once.

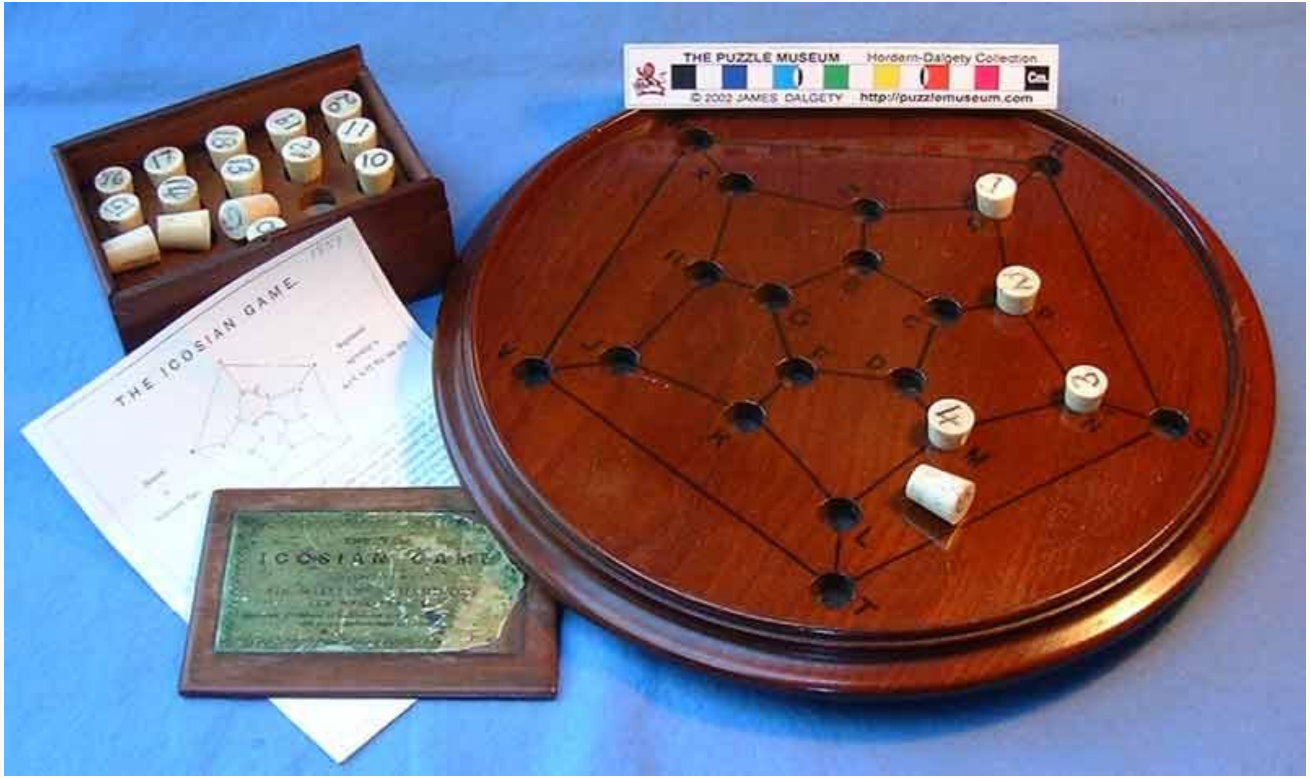
**Input**: A graph G.
**Output**:A cycle in G that visits every vertex exactly once (if exists).

- Sir Richard Hamilton created a game:
  - Graph whose vertices are labeled with the name of 25 cities
  - Visit every city only once
  - Sold via London game dealer for 25 pounds
  - Failed miserably, but defined "Hamiltonian Path" problem which occupied mathematicians and engineers in the times to come
  - NP-complete



Sir Richard Hamilton's Icosian Game

# Hamiltonian Path



Sir Richard Hamilton’s Icosian Game

# NP, NP-hard, NP-complete

Shortest common superstring & friends: For refreshers on Traveling Salesman, Hamiltonian Path, NP-hardness and NP-completeness, see Chapters 34 and 35 of “Introduction to Algorithms” by Cormen, Leiserson, Rivest and Stein;

or Chapters 8 and 9 of “Algorithms” by Dasgupta, Papadimitriou and Vazirani (free online: http://www.cs.berkeley.edu/∼vazirani/algorithms)

Tl;dr - stack overflow - What are the differences between NP, NP-Complete and NP-Hard?

# Greedy approach

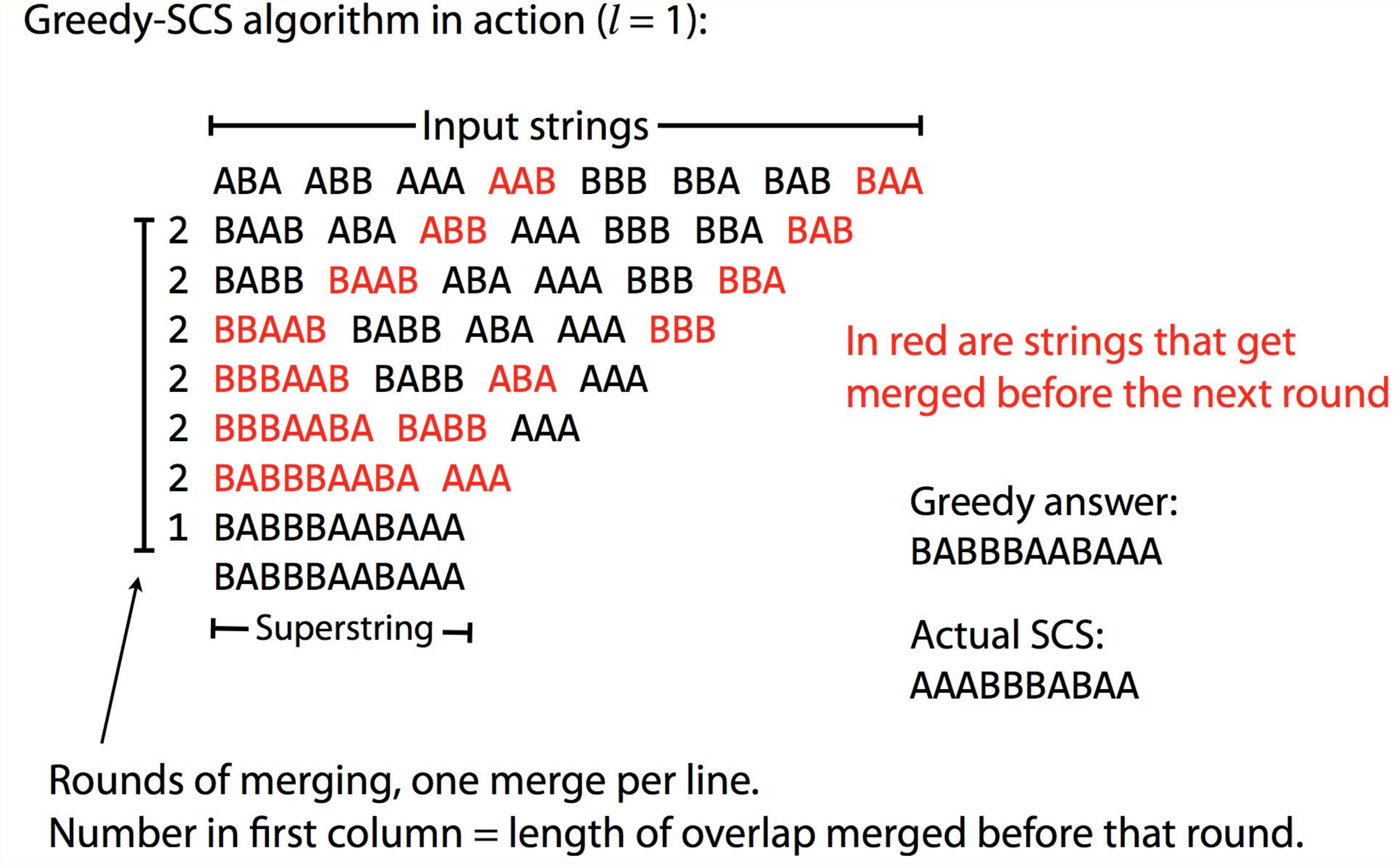
# Shortest common superstring

Let’s take the hint, give up on finding the *shortest* ***possible*** *superstring*.

Non-optimal superstrings can be found with a *greedy* algorithm.

At each step, the greedy algorithm “*greedily*” chooses longest remaining overlap, merges its source and sink.

# Shortest common superstring: greedy



Greedy-SCS algorithm in action ($l = 1$):

Input strings

ABA ABB AAA AAB BBB BBA BAB BAA

2 BAAB ABA ABB AAA BBB BBA BAB

2 BABB BAAB ABA AAA BBB BBA

2 BBAAB BABB ABA AAA BBB

2 BBBAAB BABB ABA AAA

2 BBBAABA BABB AAA

2 BABBBAABA AAA

1 BABBBAABAAA

BABBBAABAAA

Superstring

In red are strings that get merged before the next round

Greedy answer:
BABBBAABAAA

Actual SCS:
AAABBBABAA

Rounds of merging, one merge per line.
Number in first column = length of overlap merged before that round.

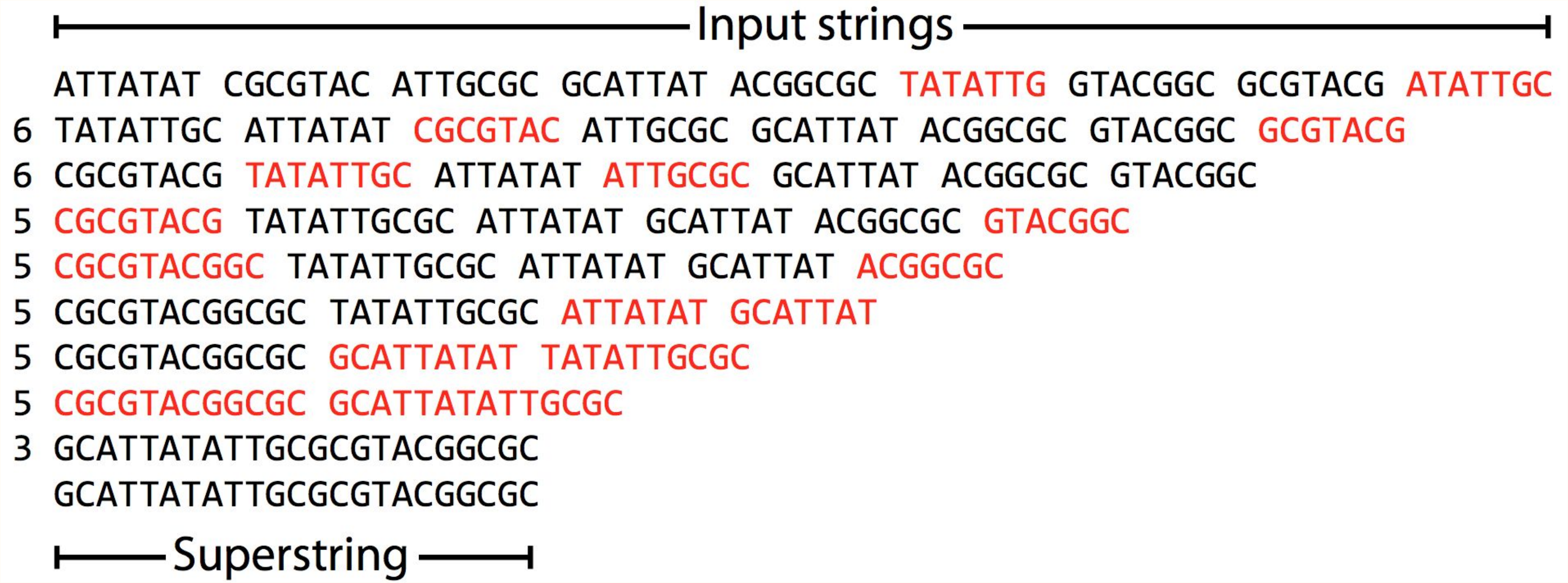
# Shortest common superstring: greedy

Greedy algorithm is not guaranteed to choose overlaps yielding SCS.

But greedy algorithm is a good approximation; i.e. the superstring yielded by the greedy algorithm won’t be more than ~2.5 times longer than true SCS (see *Gusfield 16.17.1*)

# Shortest common superstring: greedy

Greedy-SCS algorithm in action again (l = 3):



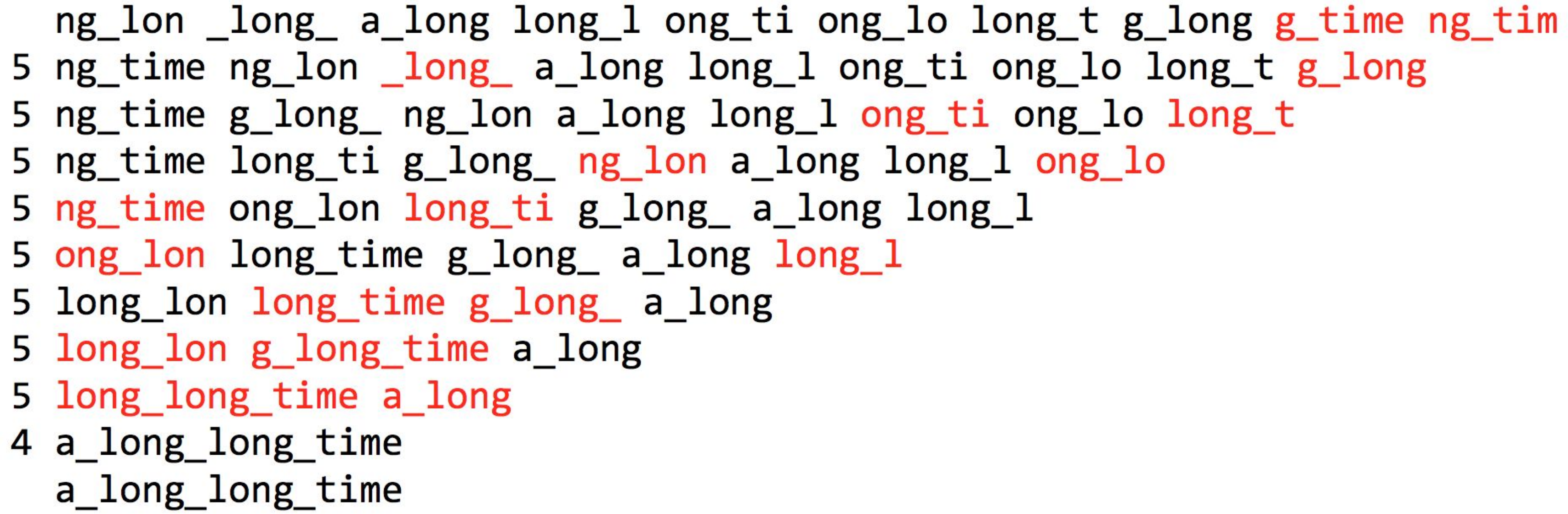
Input strings

```
  ATTATAT CGCGTAC ATTGCGC GCATTAT ACGGCGC TATATTG GTACGGC GCGTACG ATATTGC
6 TATATTGC ATTATAT CGCGTAC ATTGCGC GCATTAT ACGGCGC GTACGGC GCGTACG
6 CGCGTACG TATATTGC ATTATAT ATTGCGC GCATTAT ACGGCGC GTACGGC
5 CGCGTACG TATATTGCGC ATTATAT GCATTAT ACGGCGC GTACGGC
5 CGCGTACGGC TATATTGCGC ATTATAT GCATTAT ACGGCGC
5 CGCGTACGGCGC TATATTGCGC ATTATAT GCATTAT
5 CGCGTACGGCGC GCATTATAT TATATTGCGC
5 CGCGTACGGCGC GCATTATATTGCGC
3 GCATTATATTGCGCGTACGGCGC
  GCATTATATTGCGCGTACGGCGC
```

Superstring

# Shortest common superstring: greedy

Another setup for Greedy-SCS: assemble all substrings of length 6 from string a_long_long_long_time. $l = 3$.



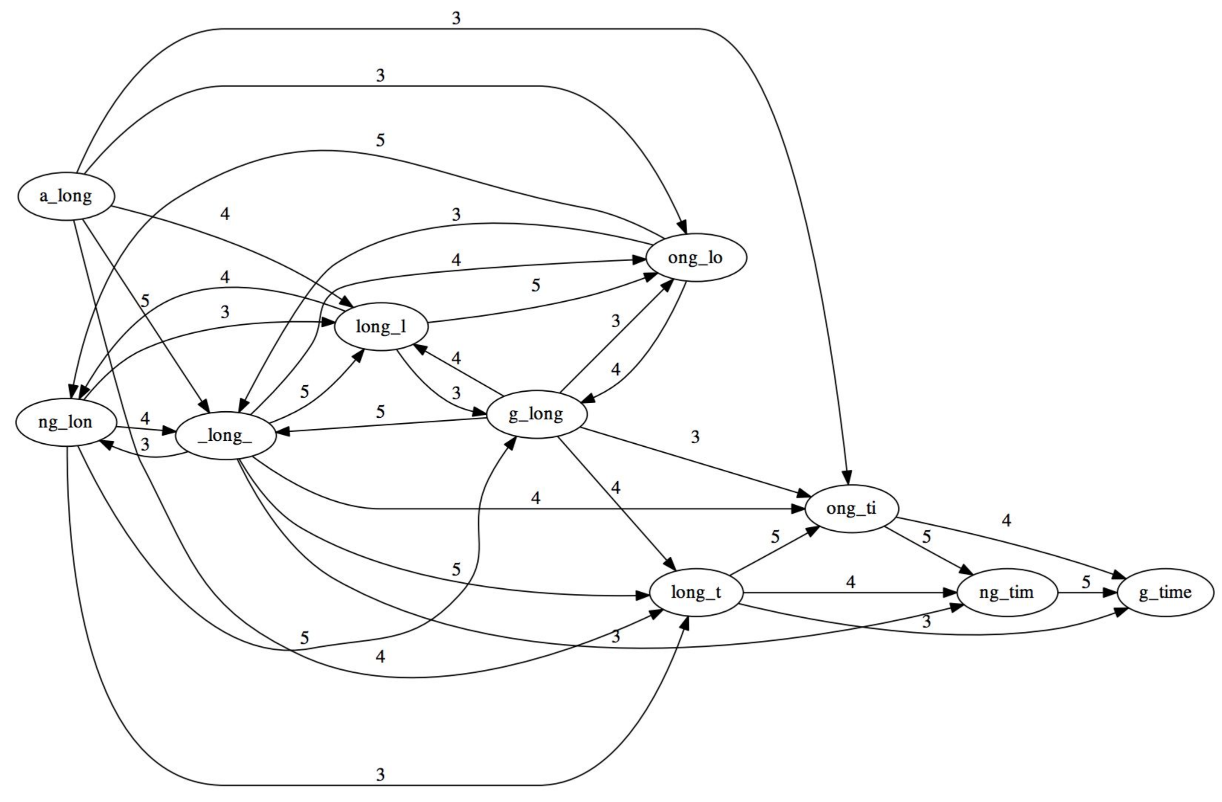
```
  ng_lon _long_ a_long long_l ong_ti ong_lo long_t g_long g_time ng_tim
5 ng_time ng_lon _long_ a_long long_l ong_ti ong_lo long_t g_long
5 ng_time g_long_ ng_lon a_long long_l ong_ti ong_lo long_t
5 ng_time long_ti g_long_ ng_lon a_long long_l ong_lo
5 ng_time ong_lon long_ti g_long_ a_long long_l
5 ong_lon long_time g_long_ a_long long_l
5 long_lon long_time g_long_ a_long
5 long_lon g_long_time a_long
5 long_long_time a_long
4 a_long_long_time
  a_long_long_time
```

I only got back: a_long_long_time (missing a _long )

What happened?

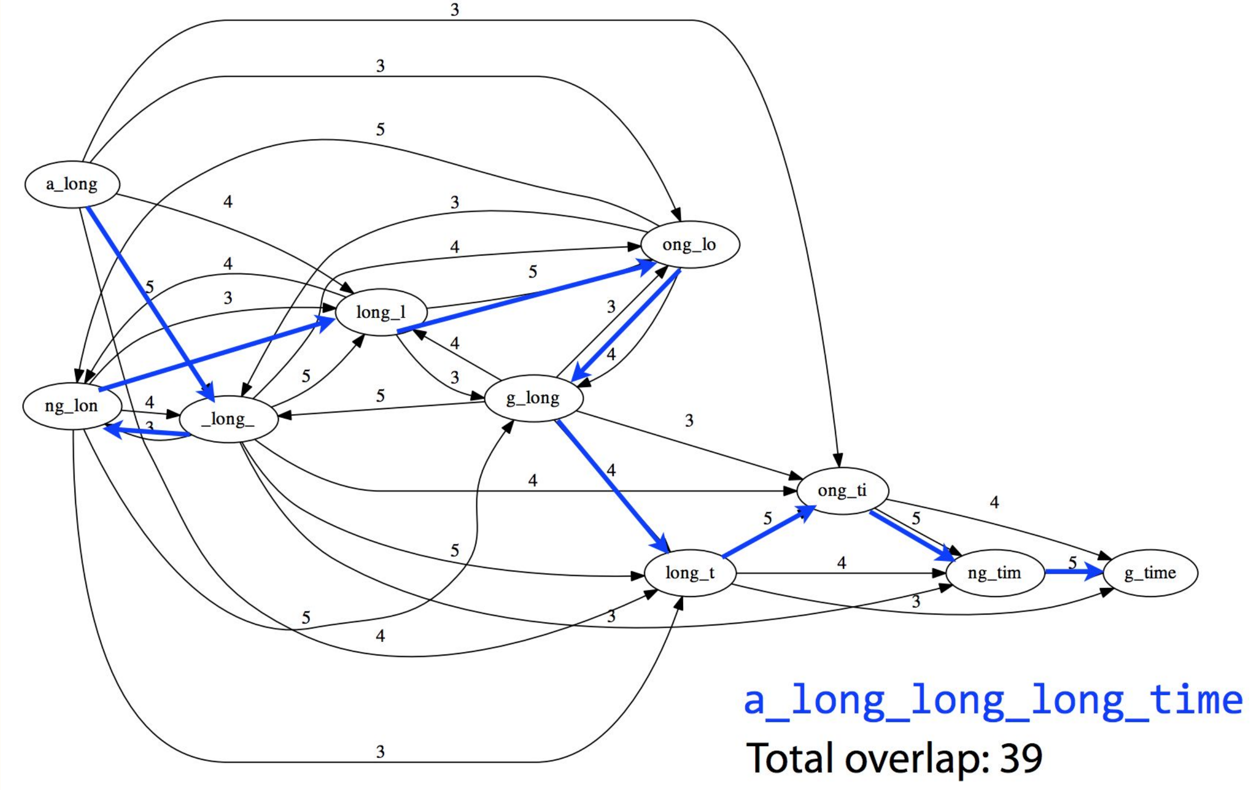
# Shortest common superstring: greedy

The overlap graph for that scenario ($l = 3$):



# Shortest common superstring

The overlap graph for that scenario ($l = 3$):

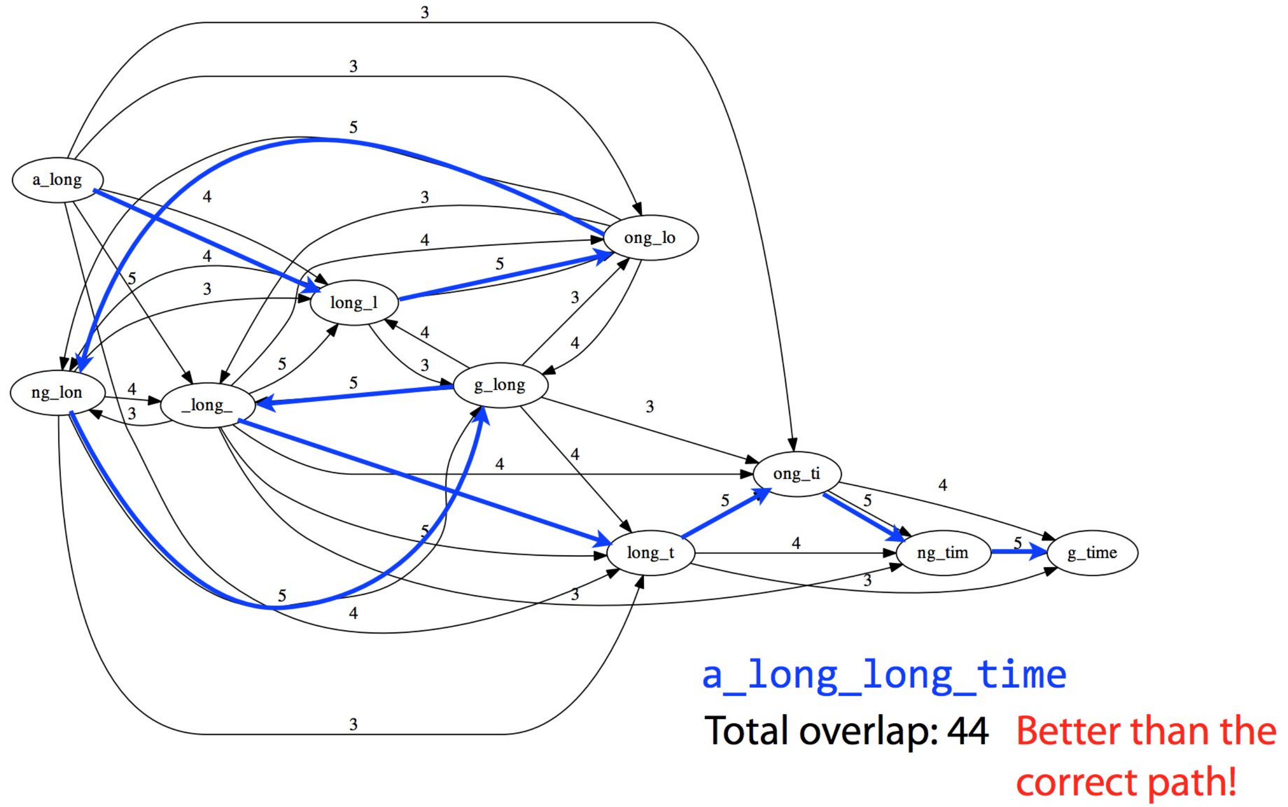


a_long_long_long_time

Total overlap: 39

# Shortest common superstring: greedy

The overlap graph for greedy solution scenario ($l = 3$):

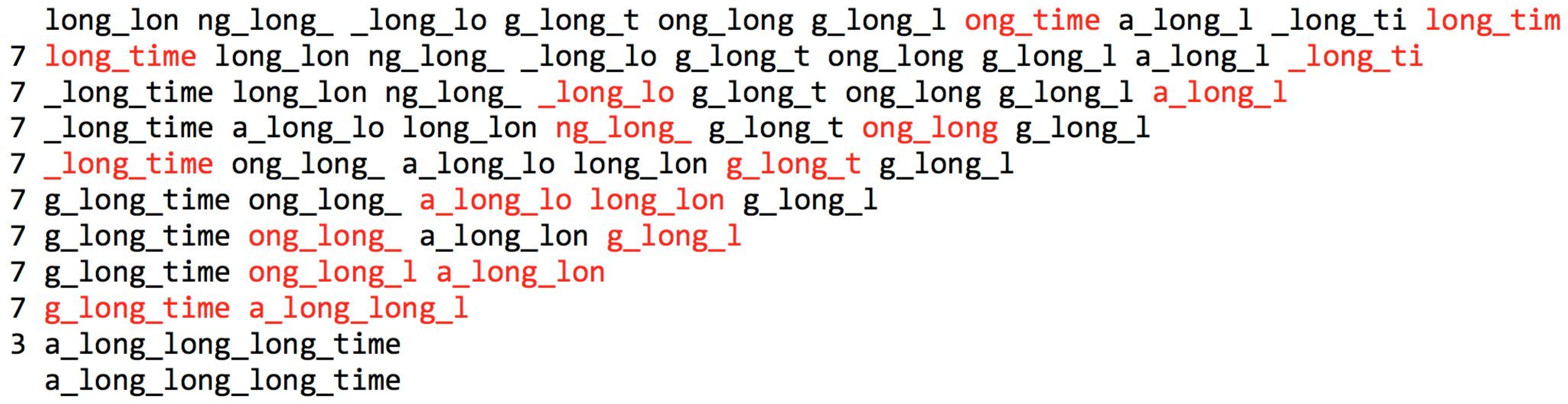


a_long_long_time

Total overlap: 44 Better than the correct path!

# Shortest common superstring: greedy

Same example, but increased the substring length from 6 to 8

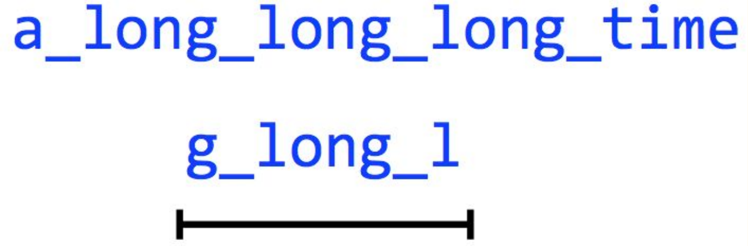


```
  long_lon ng_long_ _long_lo g_long_t ong_long g_long_l ong_time a_long_l _long_ti long_tim
7 long_time long_lon ng_long_ _long_lo g_long_t ong_long g_long_l a_long_l _long_ti
7 _long_time long_lon ng_long_ _long_lo g_long_t ong_long g_long_l a_long_l
7 _long_time a_long_lo long_lon ng_long_ g_long_t ong_long g_long_l
7 _long_time ong_long_ a_long_lo long_lon g_long_t g_long_l
7 g_long_time ong_long_ a_long_lo long_lon g_long_l
7 g_long_time ong_long_ a_long_lon g_long_l
7 g_long_time ong_long_l a_long_lon
7 g_long_time a_long_long_l
3 a_long_long_long_time
  a_long_long_long_time
```

Got the whole thing: a_long_long_long_time

# Repeats

Why are substrings of length 8 long enough for Greedy-SCS to figure out there are 3 copies of long?



a_long_long_long_time

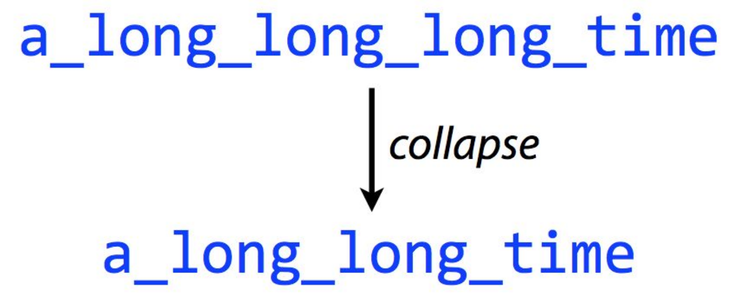
g_long_l

One length-8 substring spans all three longs

# Repeats

Repeats often foil assembly. They certainly foil SCS, with its “shortest” criterion! Reads might be too short to “resolve” repetitive sequences.

This is why sequencing vendors try to increase read length. Algorithms that don’t pay attention to repeats (like our greedy SCS algorithm) might collapse them



a_long_long_long_time

*collapse*

a_long_long_time

The human genome is ~ 50% repetitive!

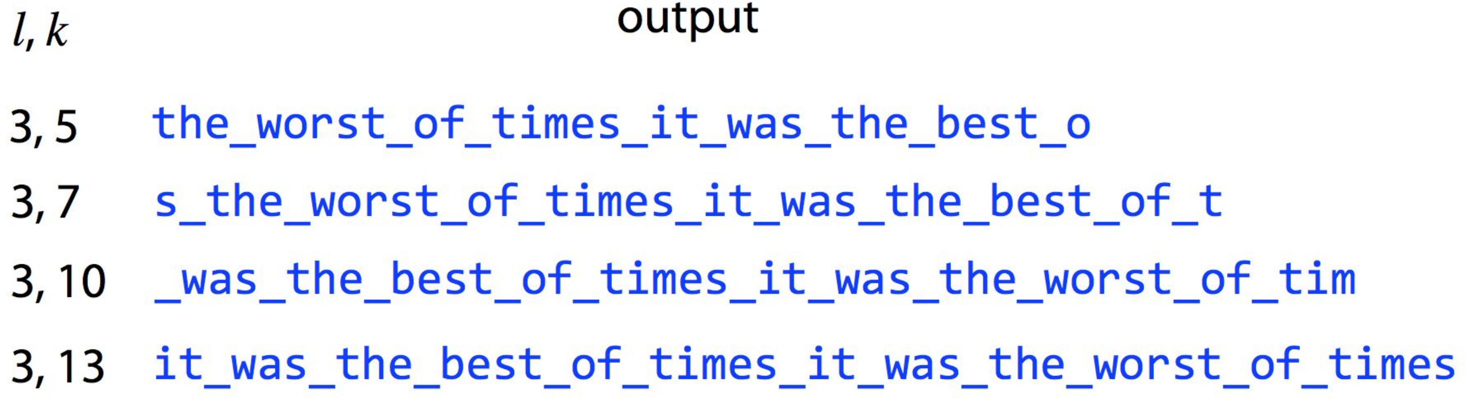
# Repeats

Basic principle: **repeats foil assembly!**

Another example using Greedy-SCS:

Input: it_was_the_best_of_times_it_was_the_worst_of_times

Extract every substring of length k, then run Greedy-SCS. Do this for various *l* (min overlap length) and k.

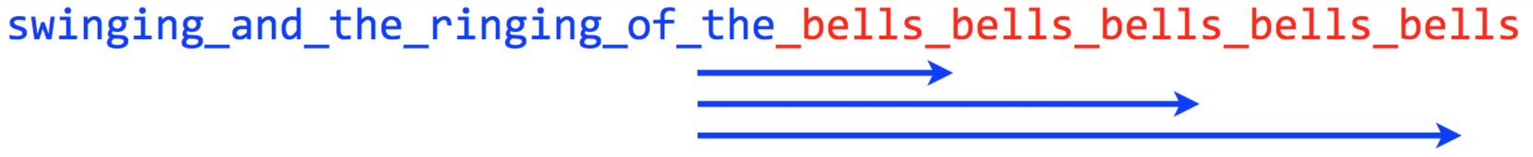


| $l, k$ | output |
|---|---|
| 3, 5 | the_worst_of_times_it_was_the_best_o |
| 3, 7 | s_the_worst_of_times_it_was_the_best_of_t |
| 3, 10 | _was_the_best_of_times_it_was_the_worst_of_tim |
| 3, 13 | it_was_the_best_of_times_it_was_the_worst_of_times |

# Repeats

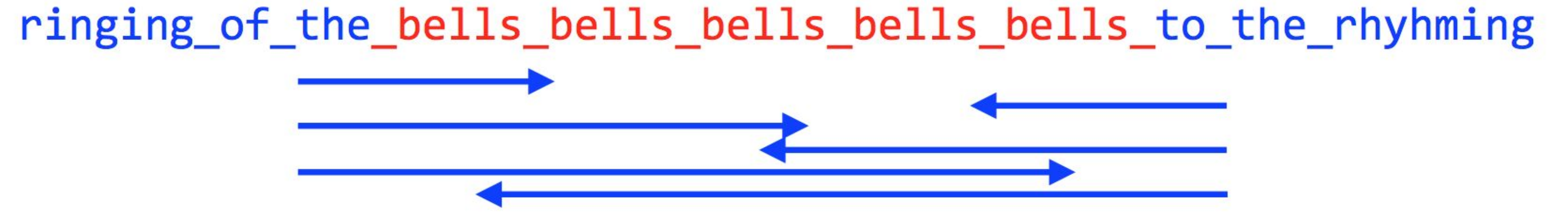
Basic principle: repeats foil assembly

Longer and longer substrings allow us to “anchor” more of the repeat to its non-repetitive context:



swinging_and_the_ringing_of_the_bells_bells_bells_bells_bells

Often we can “walk in” from both sides. When we meet in the middle, the repeat is resolved:

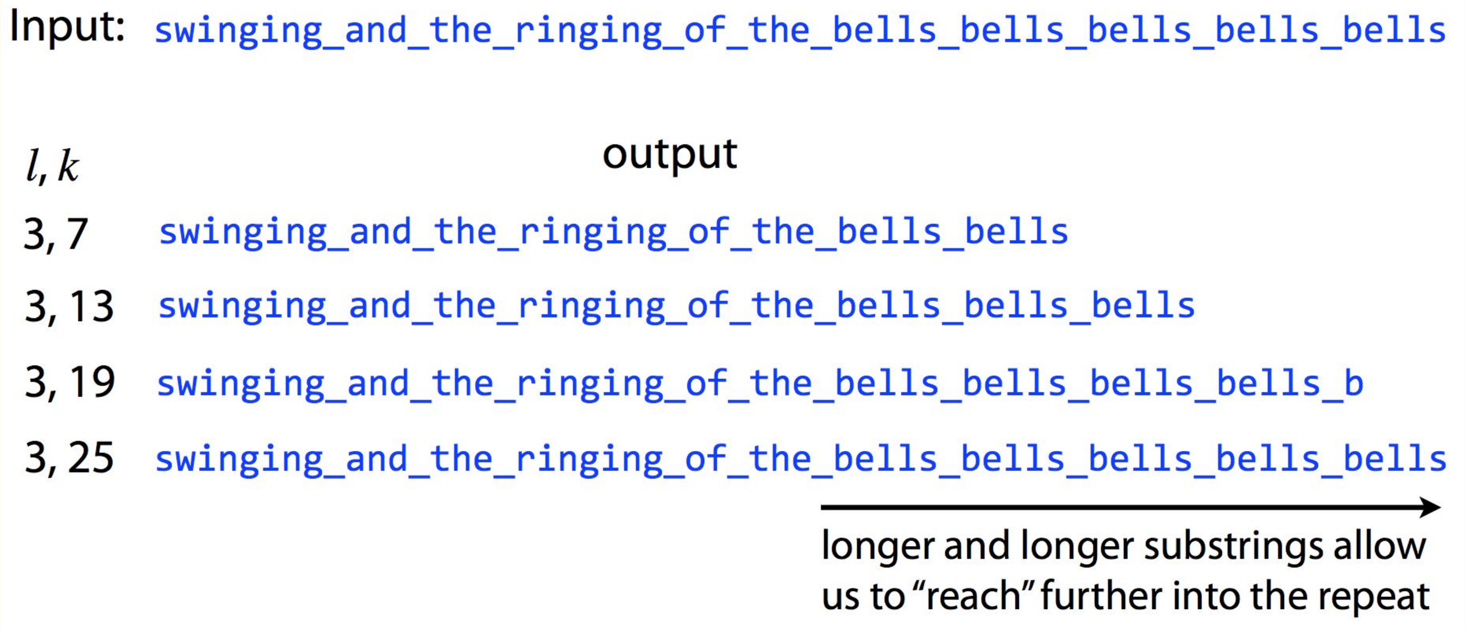


ringing_of_the_bells_bells_bells_bells_bells_to_the_rhyhming

# Repeats

**Basic principle: repeats foil assembly**

Yet another example using Greedy-SCS:



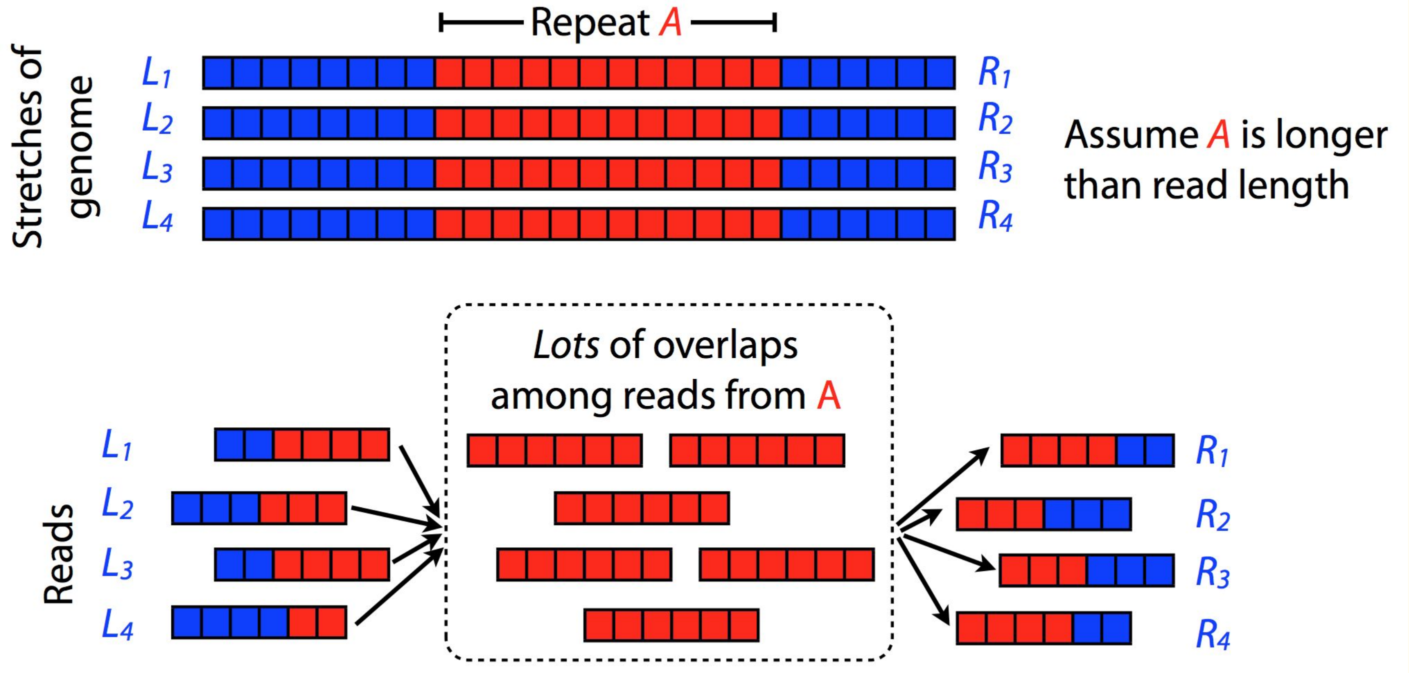
Input: swinging_and_the_ringing_of_the_bells_bells_bells_bells_bells

| $l, k$ | output |
|---|---|
| 3, 7 | swinging_and_the_ringing_of_the_bells_bells |
| 3, 13 | swinging_and_the_ringing_of_the_bells_bells_bells |
| 3, 19 | swinging_and_the_ringing_of_the_bells_bells_bells_bells_b |
| 3, 25 | swinging_and_the_ringing_of_the_bells_bells_bells_bells_bells |

longer and longer substrings allow us to “reach” further into the repeat

# Repeats

Picture the portion of the overlap graph involving repeat A


Repeat A
Stretches of genome
L1
L2
L3
L4
R1
R2
R3
R4
Assume A is longer than read length
Lots of overlaps among reads from A
Reads
L1
L2
L3
L4
R1
R2
R3
R4

Even if we avoid collapsing copies of A, we can't know which paths in correspond to which paths out.

# Shortest common superstring

SCS is too computationally complex as a way of formulating the assembly problem

- There is no practical way to find SCS
- Greedy approach: we might get too long answers (at least ~2.5 times)
- Repat handling: SCS might collapse repeat sequences
    - In this case answers might get too short

Need solutions that are: a) tractable b) handle repeats as best as possible

Note: Repeats are problem in any assembly algorithm. This is a property of read length and repetitiveness of the genome.

# Taxonomy of assembly approaches

Search for most parsimonious explanation of the reads (shortest superstring):

- Exact solutions are intractable (e.g. TSP), but a greedy approximation is possible
- Any solution will collapse repeats spuriously

Search for "maximum likelihood" explanation of the reads; i.e. force solution to be consistent with uniform coverage:

- No solutions (that I know of) are tractable

Give up on unresolvable repeats and use a tractable algorithm to assemble the resolvable portions. This is what real tools do.