Genome Assembly Algorithms

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Introduction

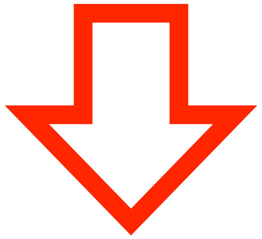
- Last month: algorithms to map and align reads to a reference genome
- What do we do if we don’t have a reference genome?
  - we need to reconstruct the sequence of the genome from the reads
  - this is called *de novo* genome assembly and we’ll discuss algorithms to solve this problem for the next two weeks

Assembly

Reads

+

Reference genome



Input DNA

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

Assembly

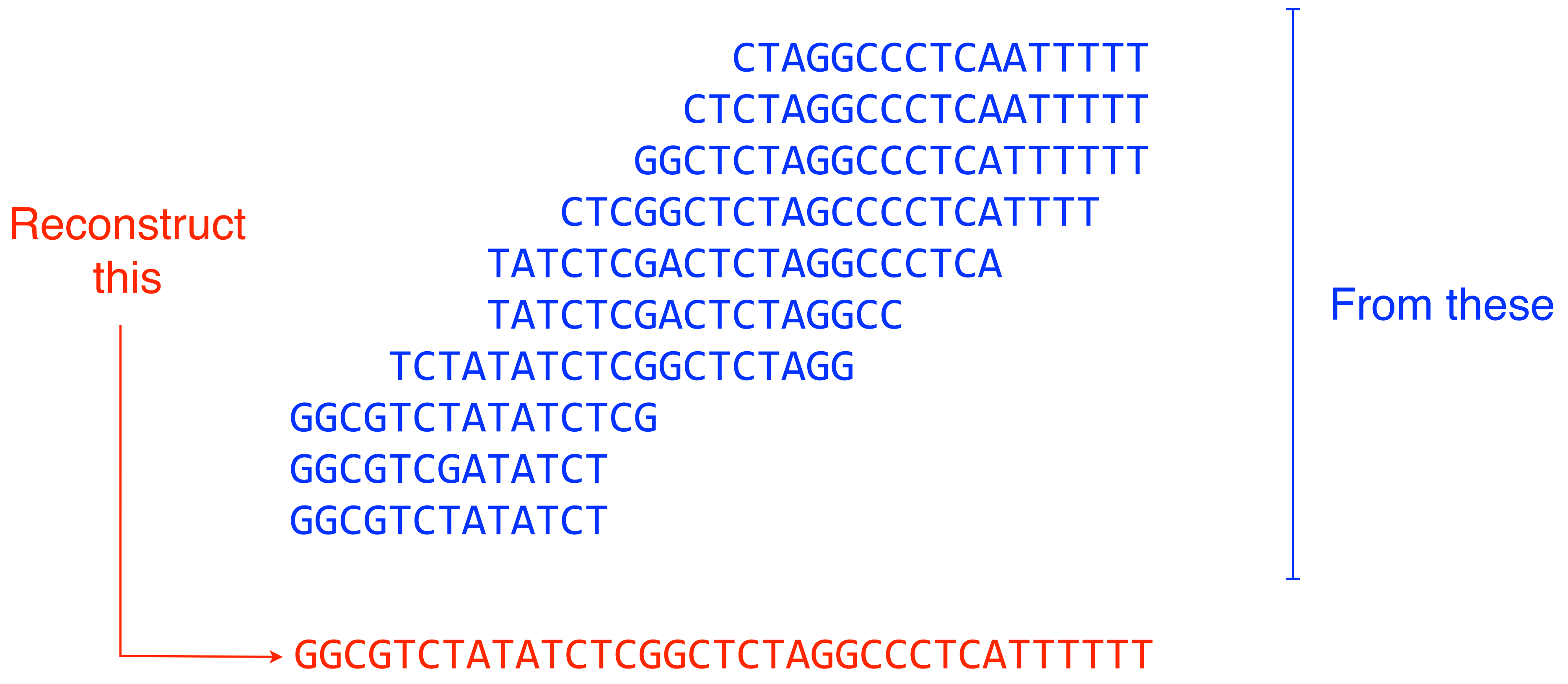
Whole-genome “shotgun” sequencing starts by copying and fragmenting the DNA

(“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 # fragments that almost all genome positions are *covered* by many fragments...



```
                  CTAGGCCCTCAATTTTT
                CTCTAGGCCCTCAATTTTT
              GGCTCTAGGCCCTCATTTTTTT
           CTCGGCTCTAGCCCCTCATTTT
        TATCTCGACTCTAGGCCCTCA
        TATCTCGACTCTAGGCC
    TCTATATCTCGGCTCTAGG
GGCGTCTATATCTCG
GGCGTCGATATCT
GGCGTCTATATCT

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
              GGCTCTAGGCCCTCATTTTTT
           CTCGGCTCTAGCCCCTCATTTT
        TATCTCGACTCTAGGCCCTCA
        TATCTCGACTCTAGGCC
    TCTATATCTCGGCTCTAGG
GGCGTCTATATCTCG
GGCGTCGATATCT
GGCGTCTATATCT

GGCGTCTATATCTCGGCTCTAGGCCCTCATTTTTT
```

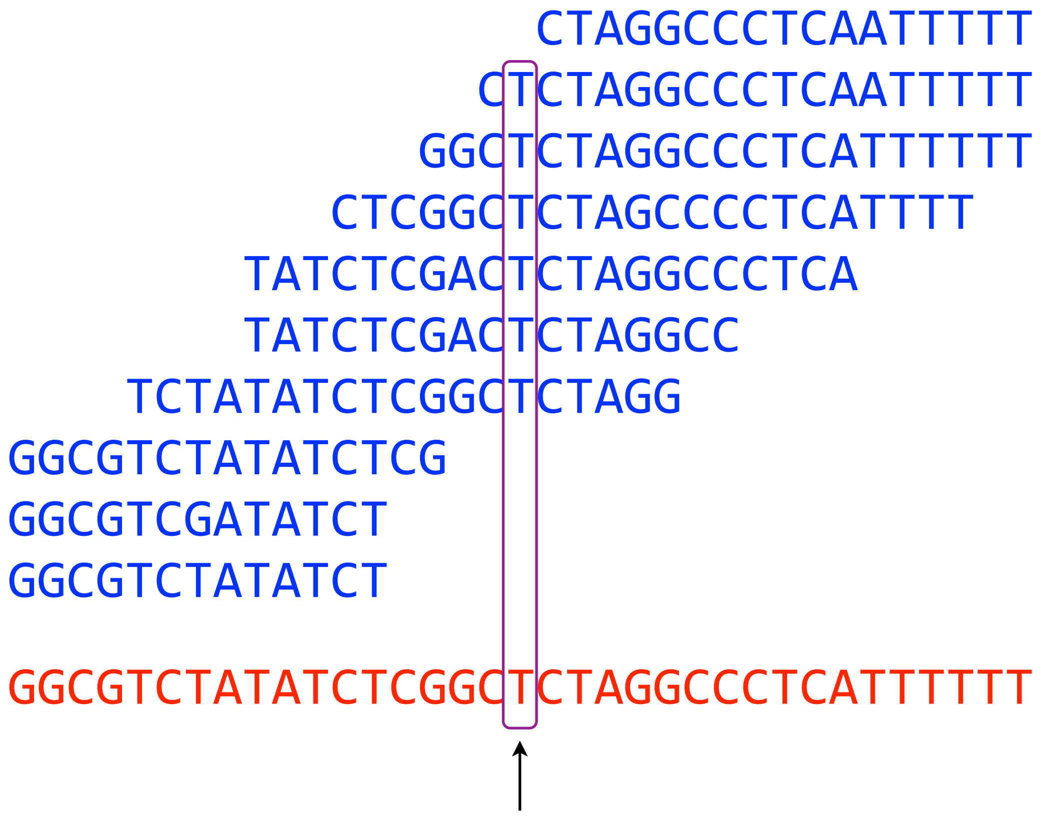
177 nucleotides

35 nucleotides

Average coverage = 177 / 35 ≈ 7x

Assembly

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



Coverage at this position = 6

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?

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

1. Sequencing error

2. Difference between inhereted *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:

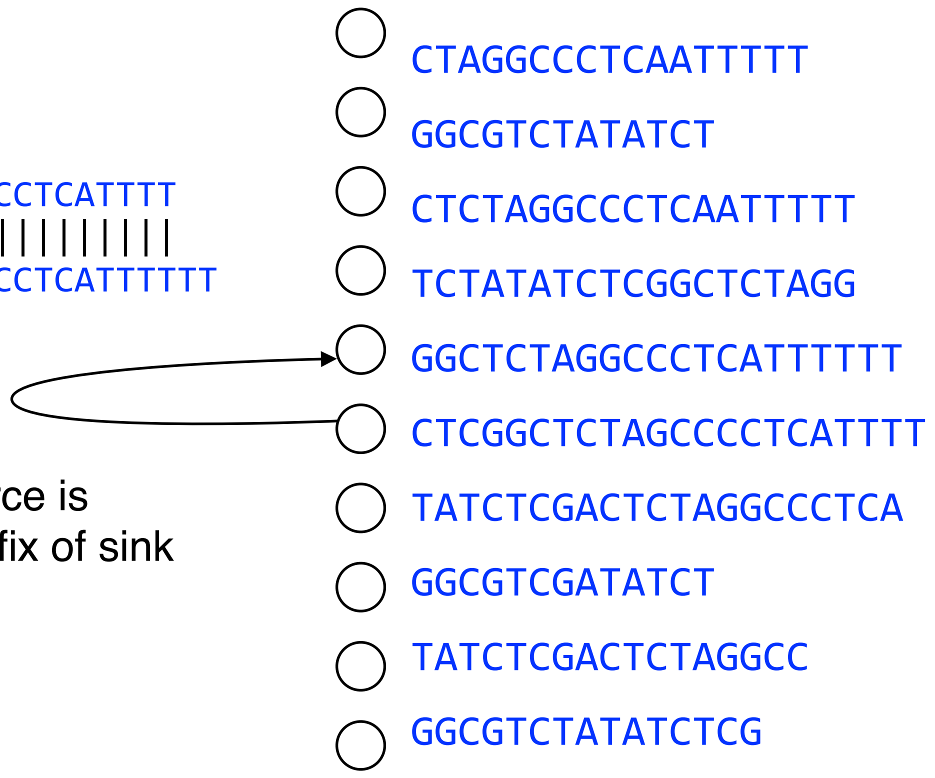
```
Read from Mother:          TATCTCGACTCTAGGCC
                           ||||||| |||||||
Read from Father:      TCTATATCTCGGCTCTAGG

Sequence from Mother:  TCTATATCTCGACTCTAGGCC
Sequence from Father:  TCTATATCTCGGCTCTAGGCC
```

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

Overlaps

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

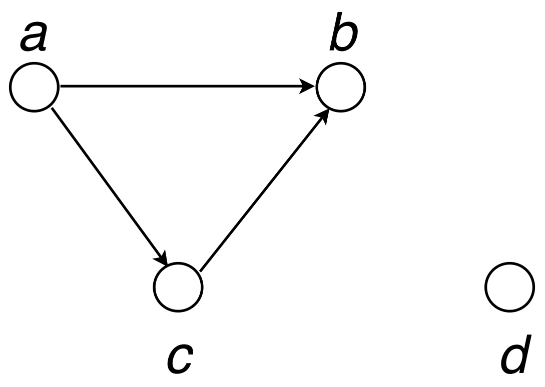
Directed graph review

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

Directed edge is an *ordered pair* of vertices.
First is the *source*, second is the *sink*.

Vertex is drawn as a circle

Edge is drawn as a line with an arrow connecting two circles



Vertex also called *node* or *point*

Edge also called *arc* or *line*

Directed graph also called *digraph*

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

$E = \{ (\underset{\text{Source}}{a}, \underset{\text{Sink}}{b}), (a, c), (c, b) \}$

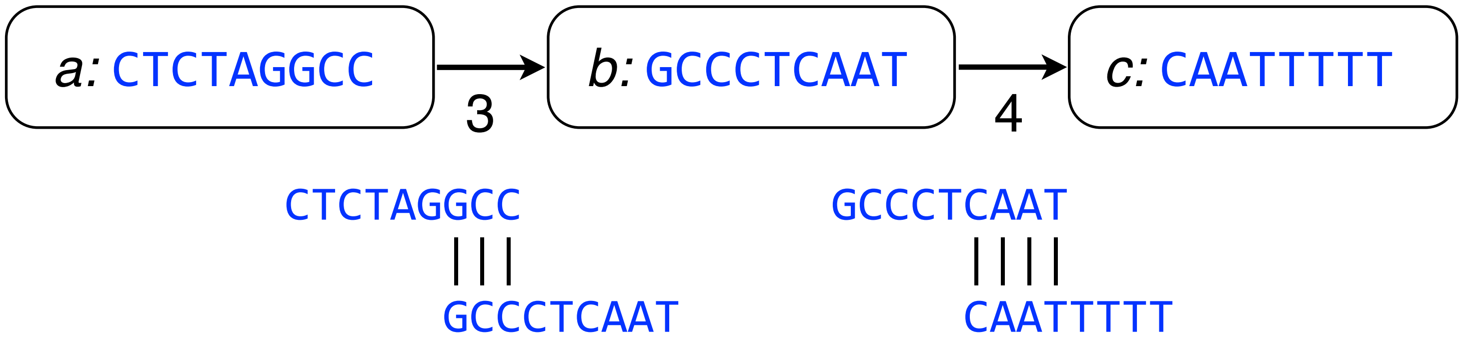
Overlap graph

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

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*) }



```
CTCTAGGCC
      |||
      GCCCTCAAT
```

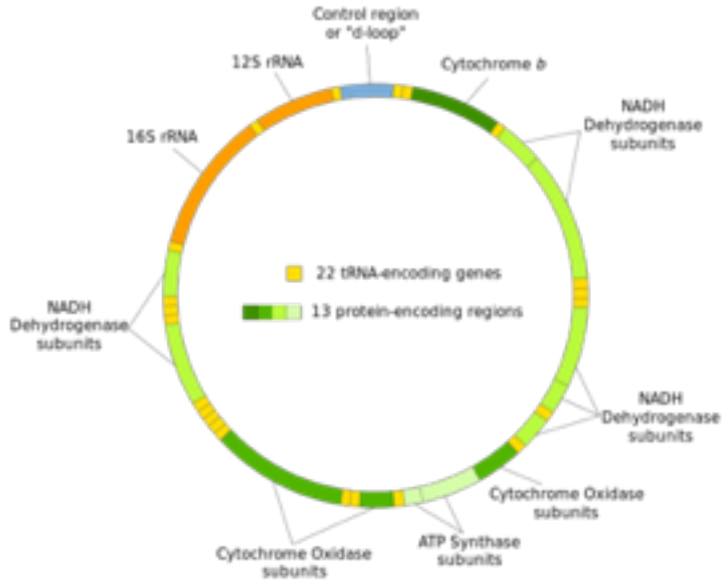
```
GCCCTCAAT
     ||||
     CAATTTTT
```

Overlap graph

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

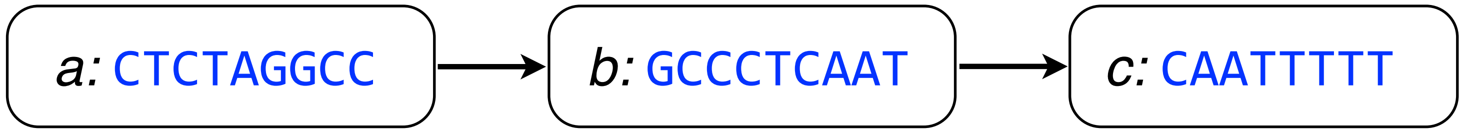
$$\begin{tikzcd} a\text{: CTCTAGGCC} \arrow[r, "3"'] & b\text{: GCCCTCACT} \arrow[r, "4"'] & c\text{: CACTCTAGG} \arrow[ll, bend right, "7"'] \end{tikzcd}$$

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


Control region or "d-loop"
12S rRNA
Cytochrome b
16S rRNA
NADH Dehydrogenase subunits
22 tRNA-encoding genes
13 protein-encoding regions
NADH Dehydrogenase subunits
NADH Dehydrogenase subunits
Cytochrome Oxidase subunits
ATP Synthase subunits
Cytochrome Oxidase subunits

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?

Assume for now an "overlap" is when a suffix of *X* of length ≥ *l* exactly matches a prefix of *Y*, where *l* is given

Finding overlaps

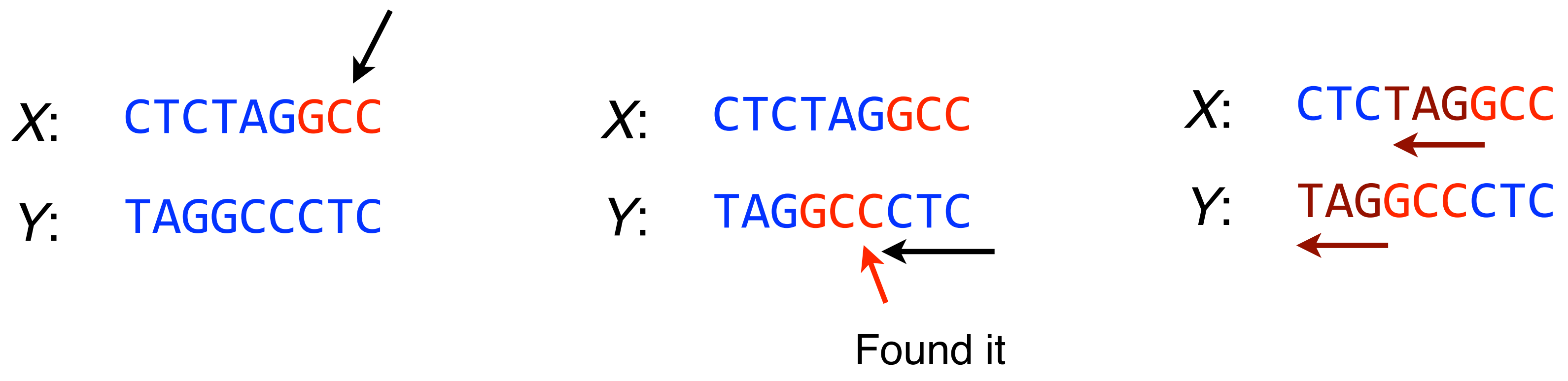
Overlap: length-l suffix of X matches length-l prefix of Y, where l is given

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.

Say $l = 3$

Look for this in Y, going right-to-left

Extend to left; in this case, we confirm that a length-6 prefix of Y matches a suffix of X



Finding overlaps: implementation

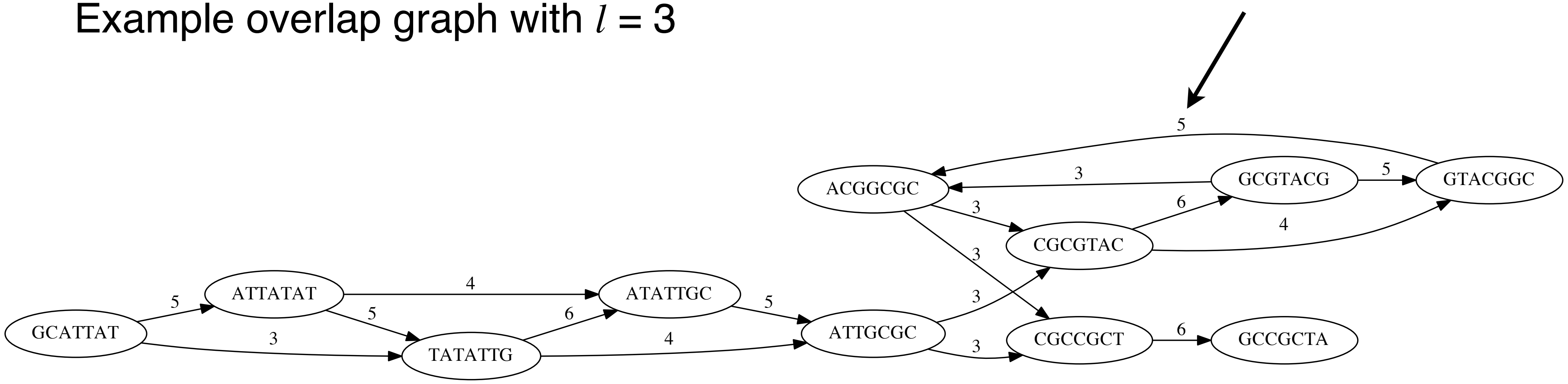
```
def suffixPrefixMatch(x, y, k):
    ''' Return length of longest suffix of x of length at least k that
        matches a prefix of y.  Return 0 if there no suffix/prefix
        match has length at least k. '''
    if len(x) < k or len(y) < k:
        return 0
    idx = len(y) # start at the right end of y
    # Search right-to-left in y for length-k suffix of x
    while True:
        hit = string.rfind(y, x[-k:], 0, idx)
        if hit == -1: # not found
            return 0
        ln = hit + k
        # See if match can be extended to include entire prefix of y
        if x[-ln:] == y[:ln]:
            return ln # return length of prefix
        idx = hit + k - 1 # keep searching to left in Y
    return -1
```

Python example: http://nbviewer.ipython.org/7089885

Finding overlaps

Example overlap graph with $l = 3$

Edge label is overlap length



Original string: GCATTATATATTGCGCGTACGGCGCCGCTACA

Formulating the assembly problem

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

How do we formulate this problem?

First attempt: the *shortest common superstring* (*SCS*) problem

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

$\longleftarrow 24 \longrightarrow$

SCS(S): AAABBBABAA

$\longleftarrow 10 \longrightarrow$

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

Shortest common superstring

Can we solve it?

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

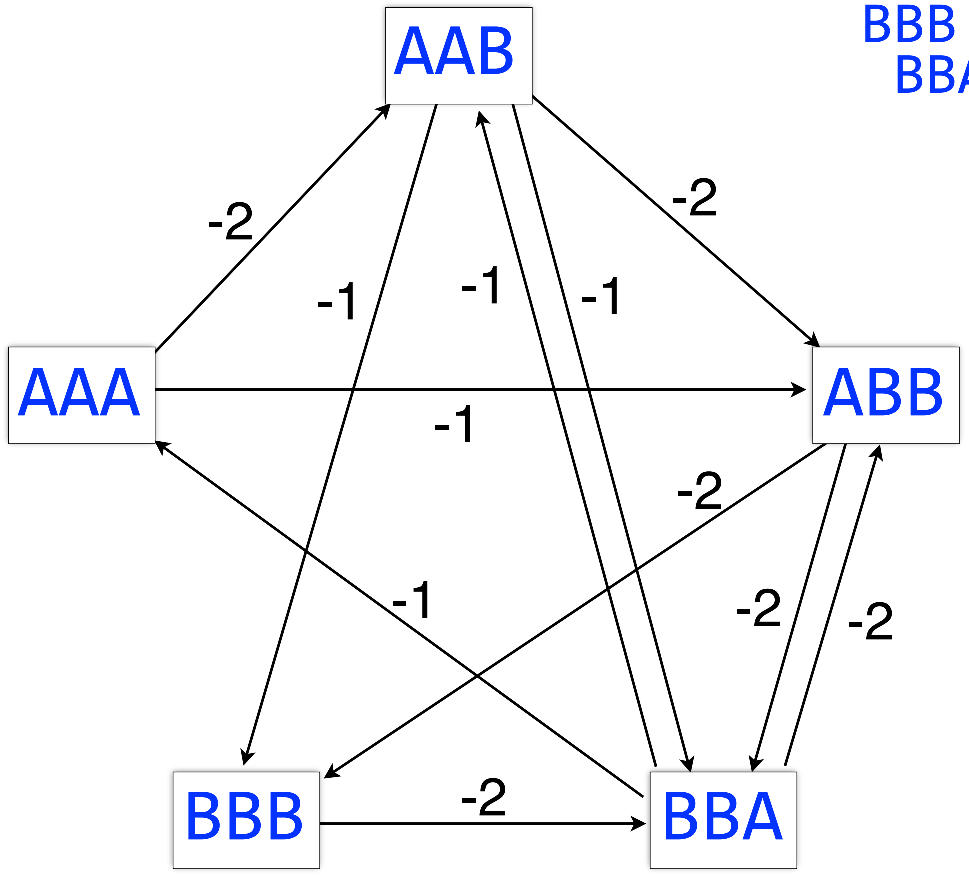
SCS corresponds to a path that visits every node once, minimizing total cost along path

That's the *Traveling Salesman Problem* (*TSP*), which is NP-hard!

S: AAA AAB ABB BBB BBA

SCS(S): AAABBBA

```
AAA
 AAB
  ABB
   BBB
    BBA
```



Shortest common superstring

S: AAA AAB ABB BBB BBA

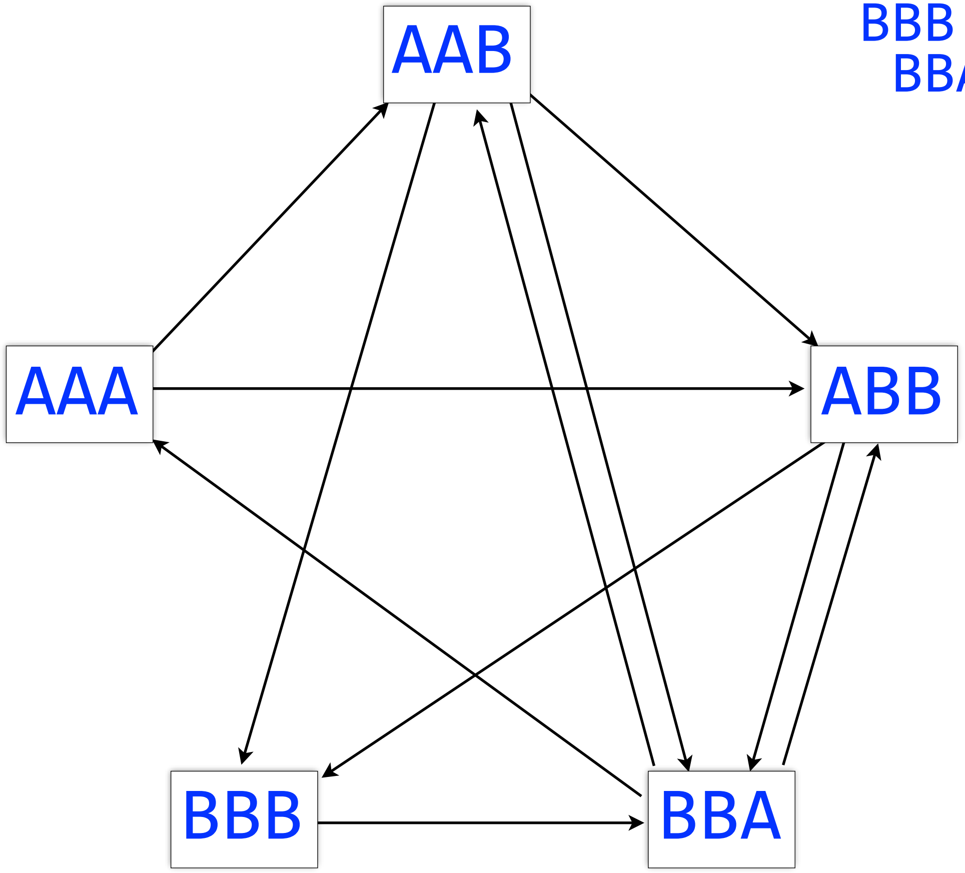
SCS(S): AAABBBA

```
AAA
 AAB
  ABB
   BBB
    BBA
```

Say we disregard edge weights and just look for a path that visits each node exactly once

That's the *Hamiltonian Path* problem: NP-complete

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



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

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

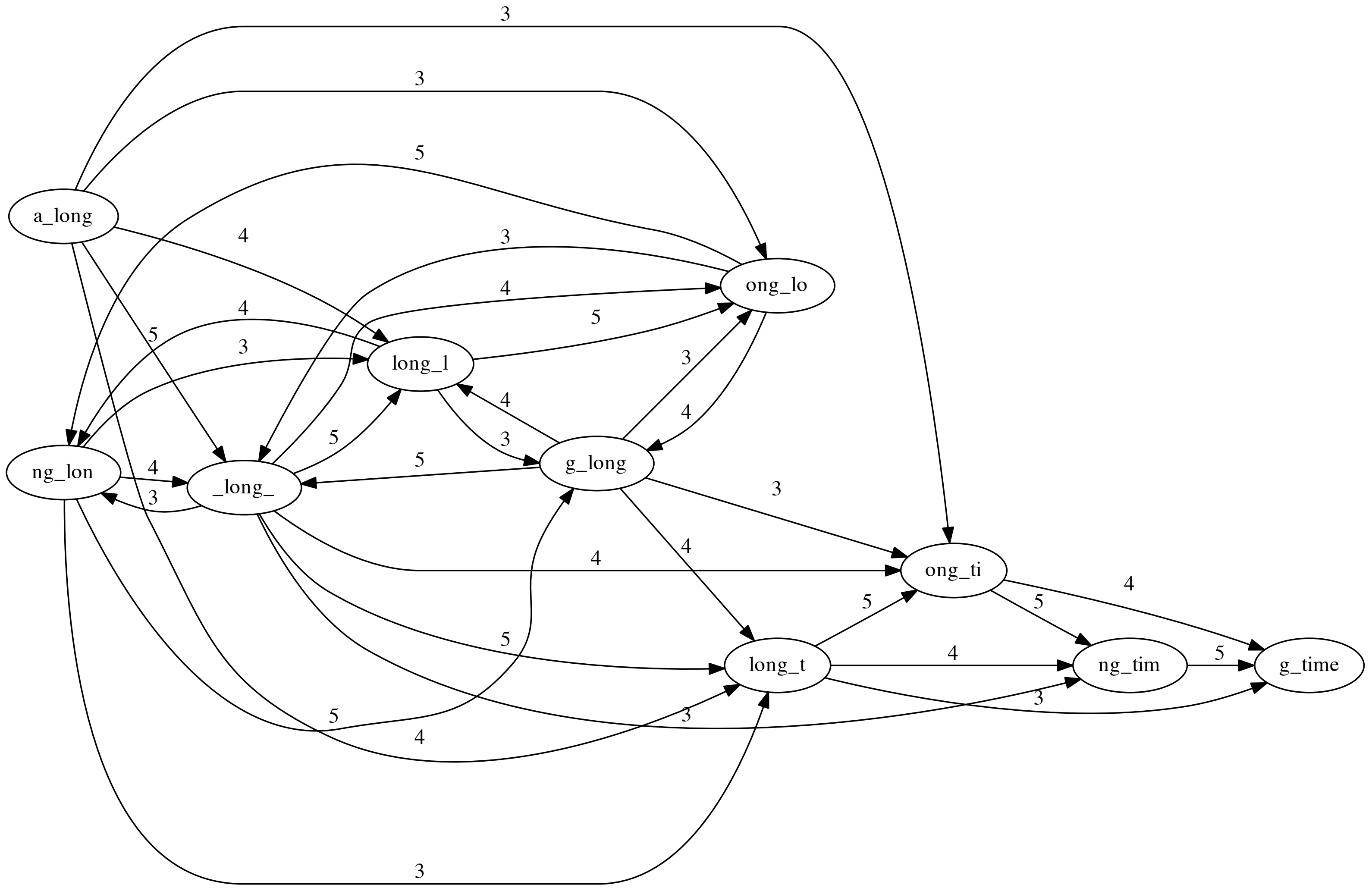
```
  ng_lon _long_ a_long long_l ong_ti ong_lo long_t g_long g_time ng_tim
5 ng_time ng_lon _long_ a_long long_l ong_ti ong_lo long_t g_long
5 ng_time g_long_ ng_lon a_long long_l ong_ti ong_lo long_t
5 ng_time long_ti g_long_ ng_lon a_long long_l ong_lo
5 ng_time ong_lon long_ti g_long_ a_long long_l
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?

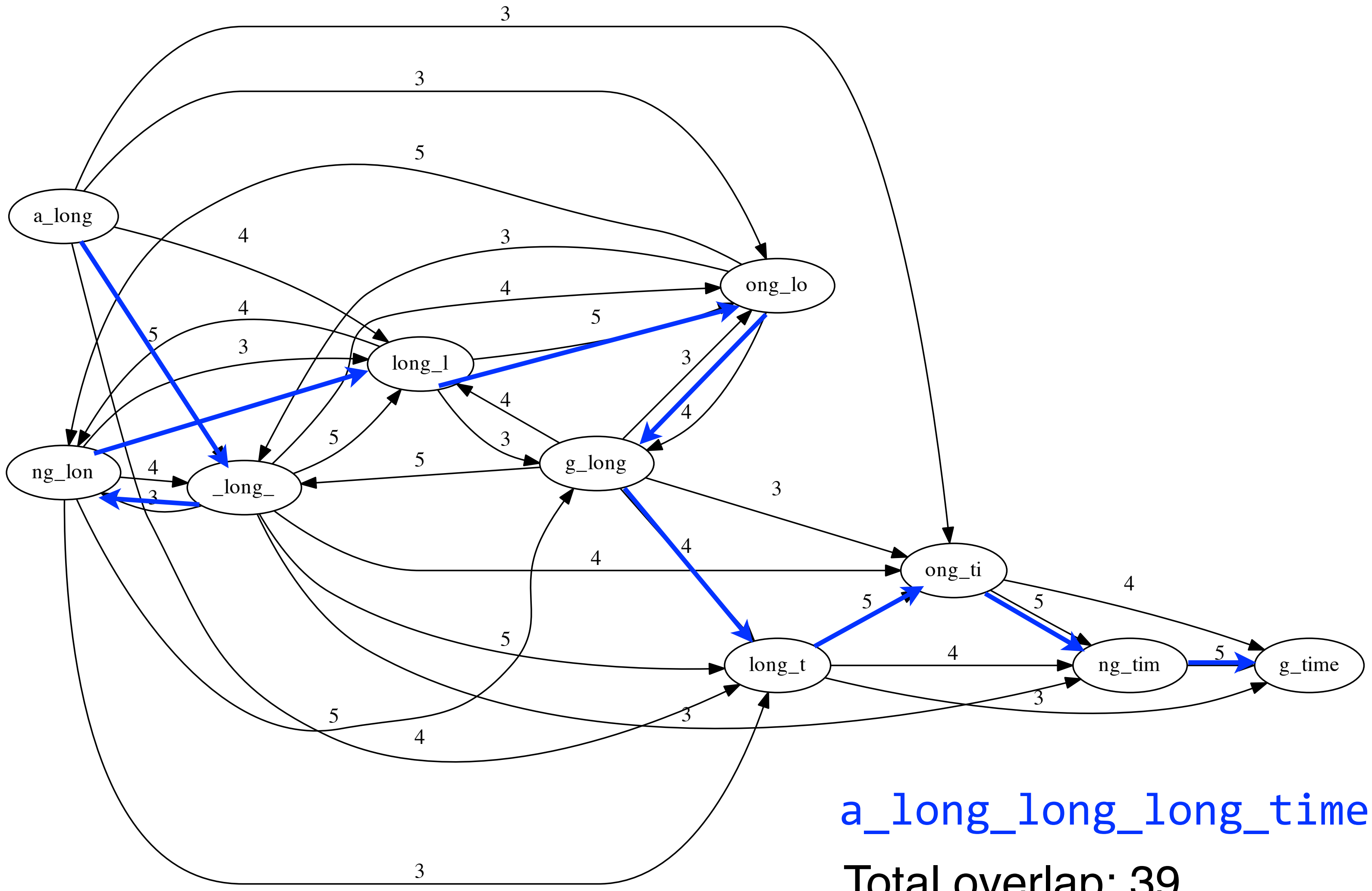
Shortest common superstring: greedy

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



Shortest common superstring: greedy

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

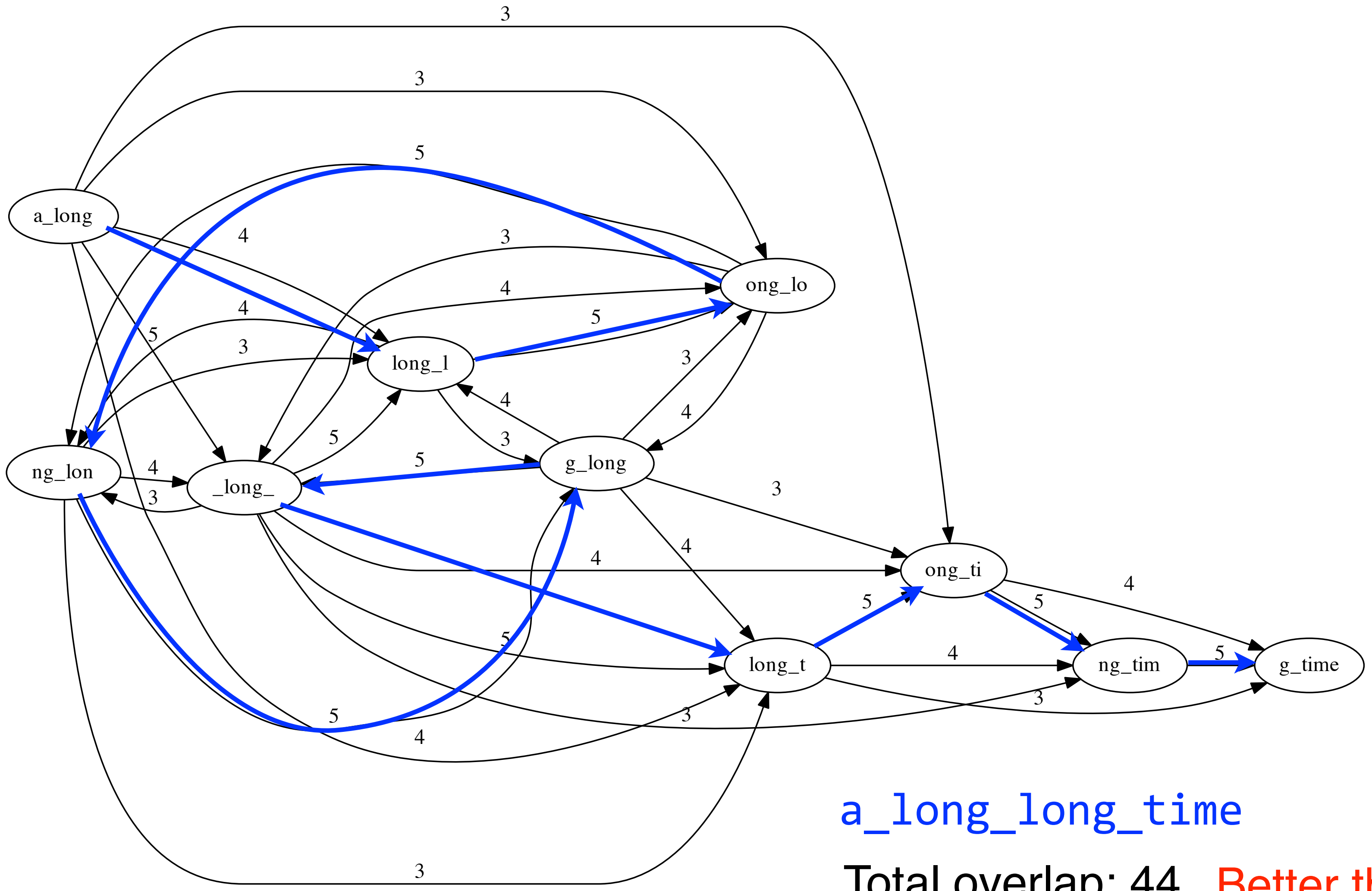


a_long_long_long_time

Total overlap: 39

Shortest common superstring: greedy

The overlap graph for that 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`

Shortest common superstring: greedy

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 `long`s

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

$$\begin{tikzcd} \texttt{a\_long\_long\_long\_time} \arrow[d, "\textit{collapse}"] \\ \texttt{a\_long\_long\_time} \end{tikzcd}$$

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`

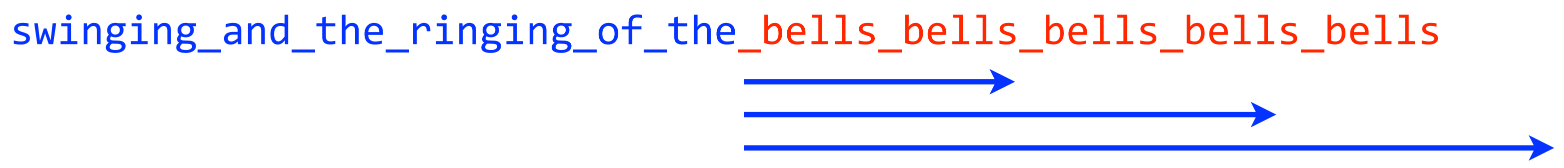
Extract every substring of length k, then run Greedy-SCS.
Do this for various l (min overlap length) and k.

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

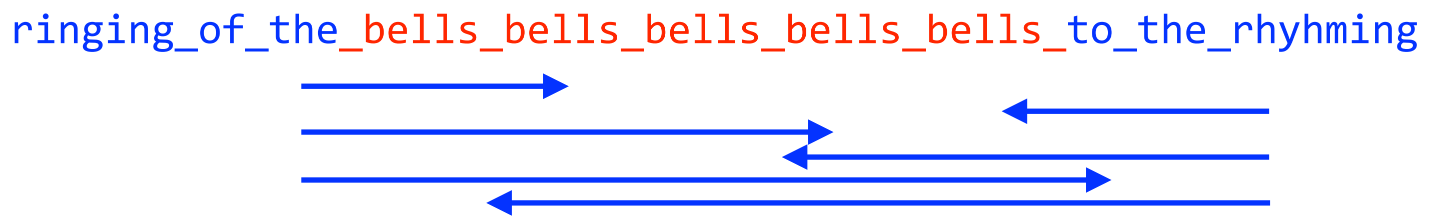
Basic principle: *repeats foil assembly*

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
```

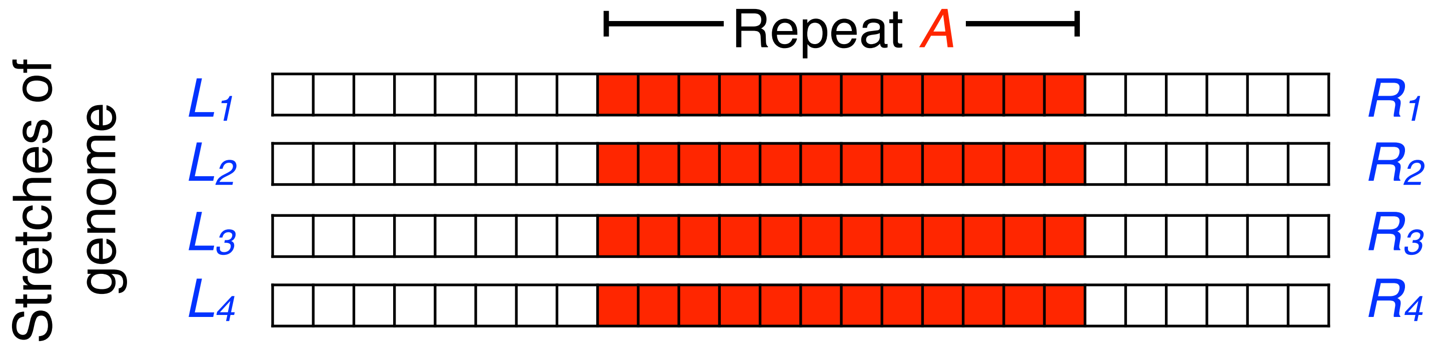
Often we can “walk in” from both sides. When we meet in the middle, the repeat is resolved:



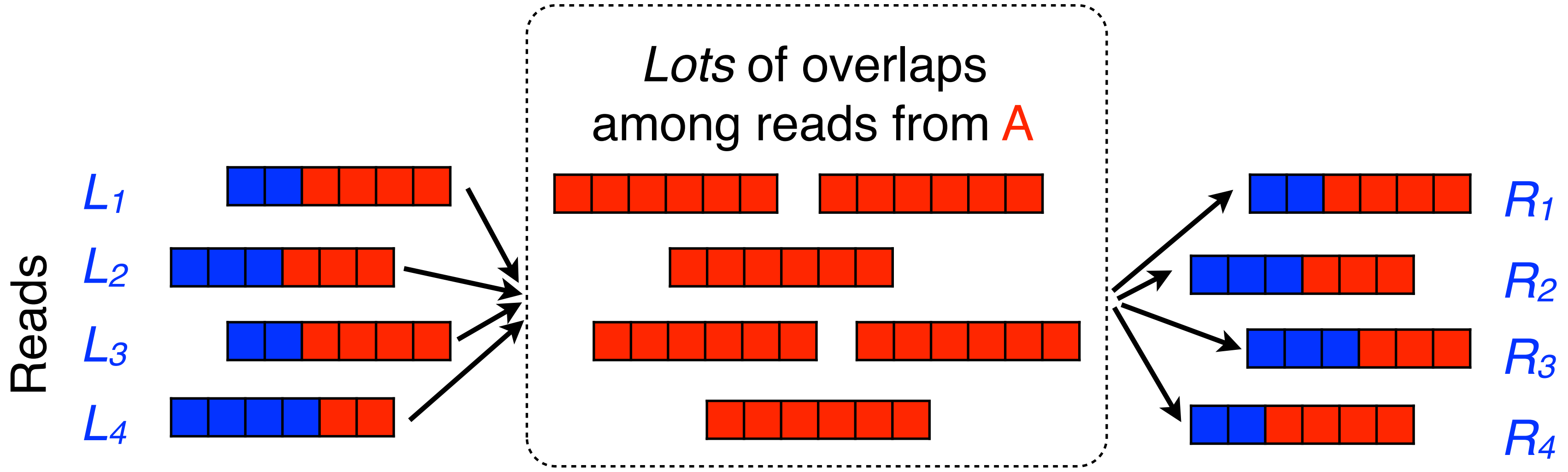
```
ringing_of_the_bells_bells_bells_bells_bells_to_the_rhyhming
```

Repeats

Picture the portion of the overlap graph involving repeat *A*



Assume *A* is longer than read length



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

Shortest common superstring: post mortem

SCS is flawed as a way of formulating the assembly problem

- No tractable way to find optimal SCS
  - Had to use Greedy-SCS. Answers might be too long.
- SCS spuriously collapses repetitive sequences
  - Answers might be too short, by a lot!

Need formulations that are (a) tractable, and (b) handle repeats as gracefully as possible

Remember: repeats foil assembly no matter the algorithm. This is a property of read length and repetitiveness of the genome.

Real-world assembly methods

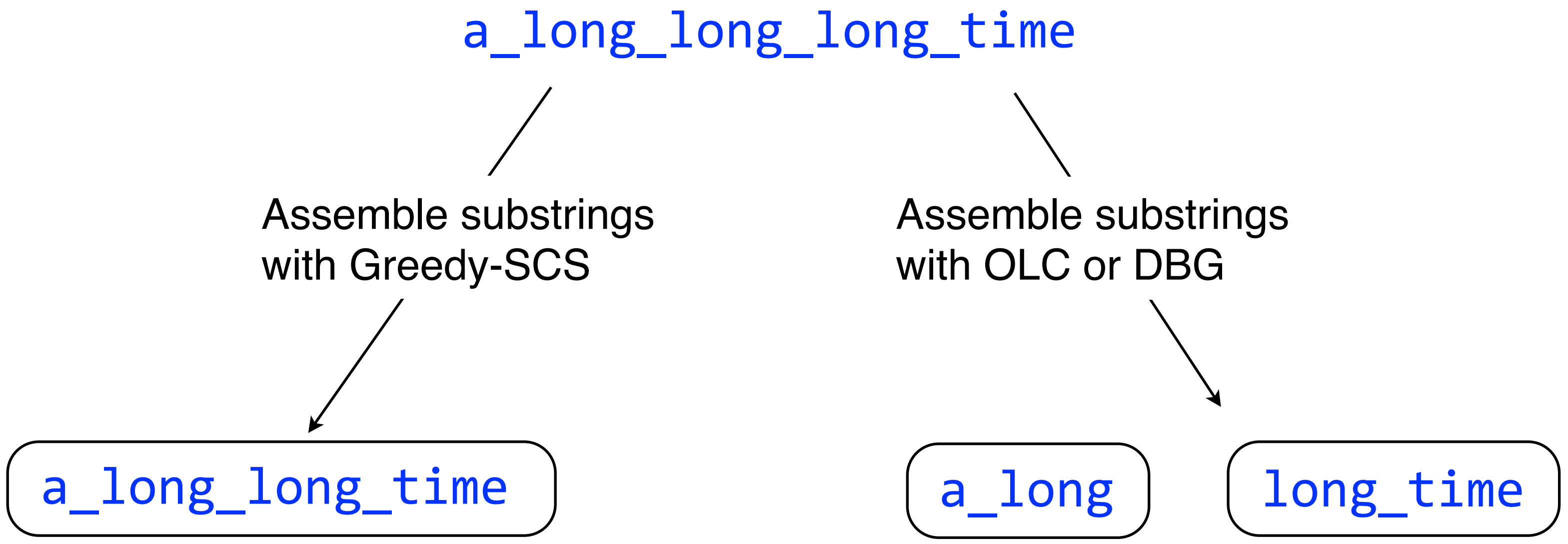
OLC: Overlap-Layout-Consensus assembly

DBG: De Bruijn graph assembly

Both handle unresolvable repeats by essentially *leaving them out*

Unresolvable repeats break the assembly into fragments

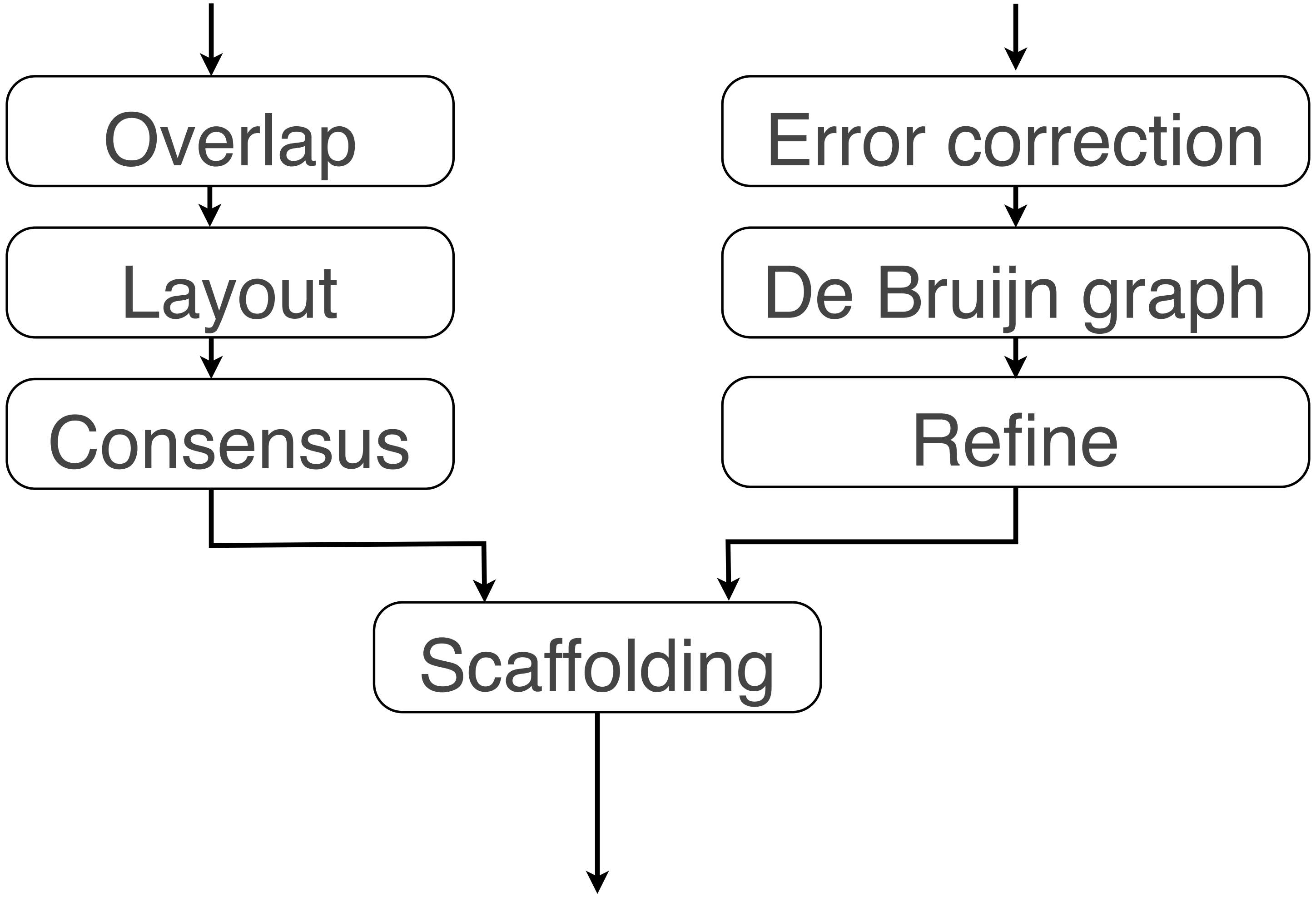
Fragments are *contigs* (short for *contiguous*)


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

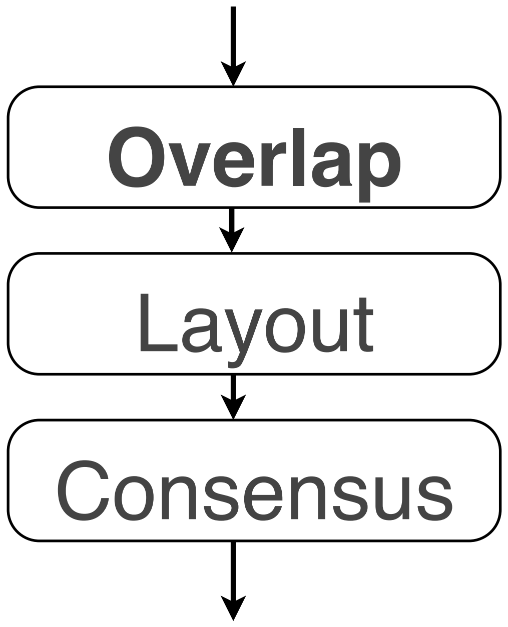
Assembly alternatives

Alternative 1: Overlap-Layout-Consensus (OLC) assembly

Alternative 2: De Bruijn graph (DBG) assembly



Overlap Layout Consensus



Build overlap graph

Bundle stretches of the overlap graph into *contigs*

Pick most likely nucleotide sequence for each contig

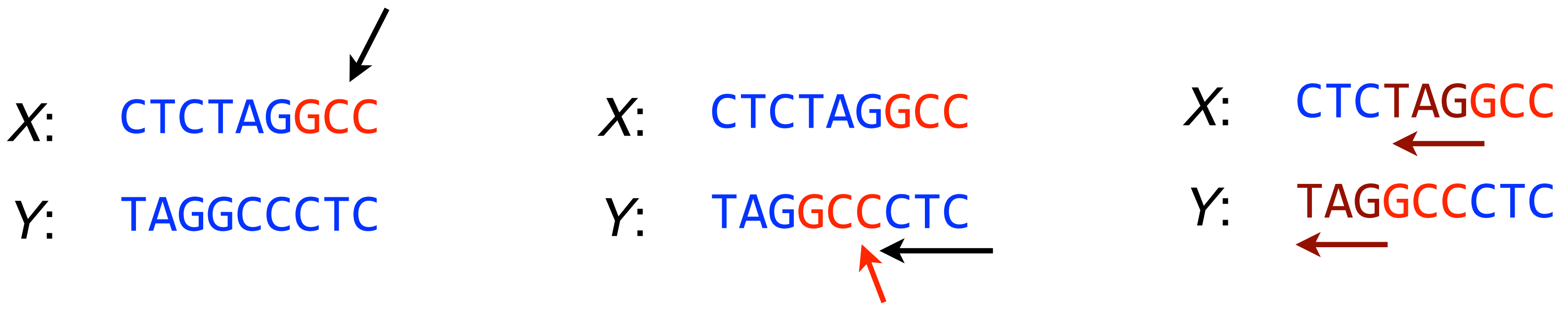
Finding overlaps

Can we be less naive than this?

Say $l = 3$

Look for this in *Y*, going right-to-left

Extend to left; in this case, we confirm that a length-6 prefix of *Y* matches a suffix of *X*



Found it

We're doing this for *every pair* of input strings

Finding overlaps

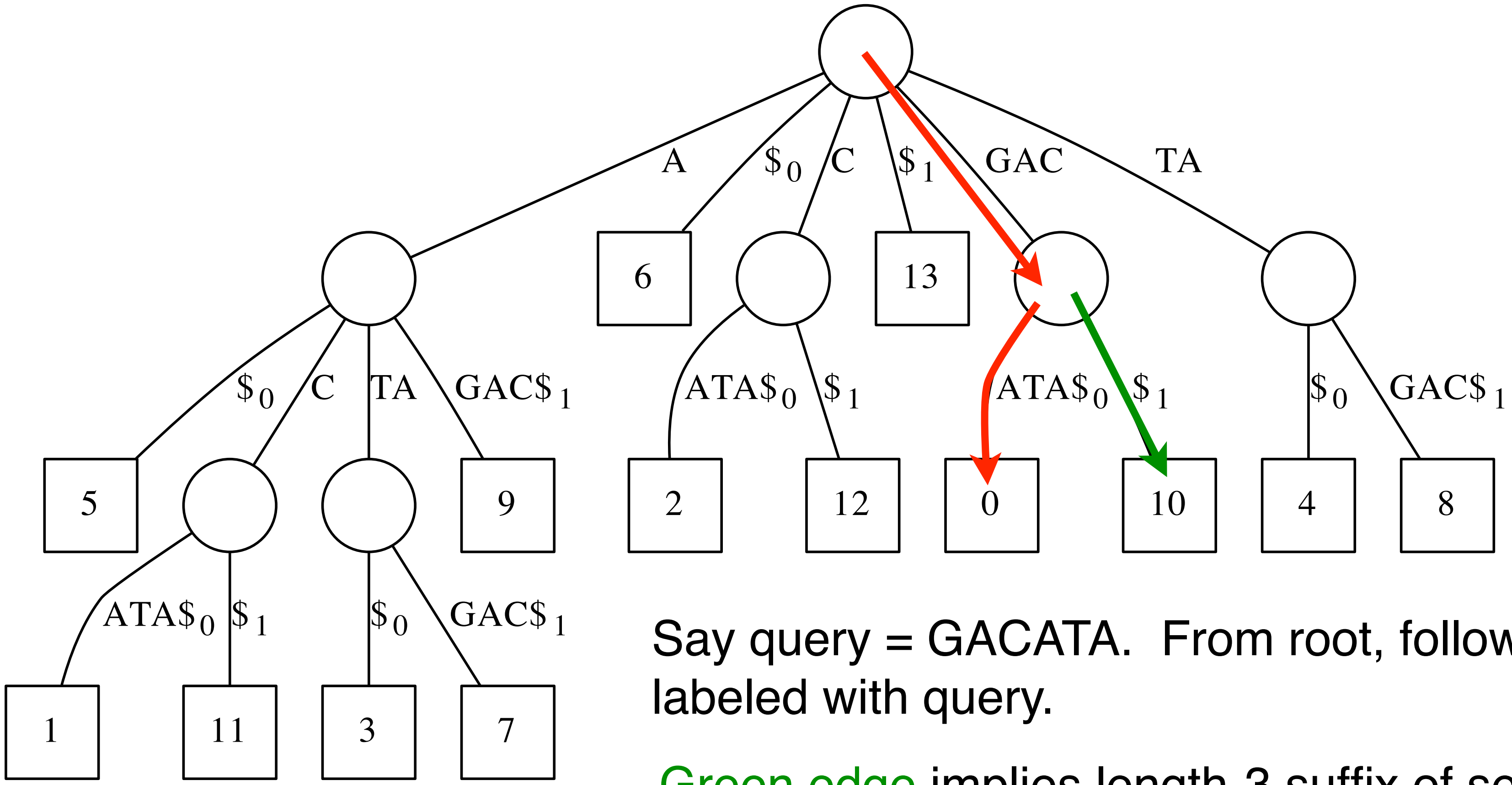
Can we use suffix trees for overlapping?

Problem: Given a collection of strings *S*, for each string *x* in *S* find all overlaps involving a prefix of *x* and a suffix of another string *y*

Hint: Build a generalized suffix tree of the strings in *S*

Finding overlaps with suffix tree

Generalized suffix tree for { "GACATA", "ATAGAC" }　　GACATA$\$_0$ATAGAC$\$_1$



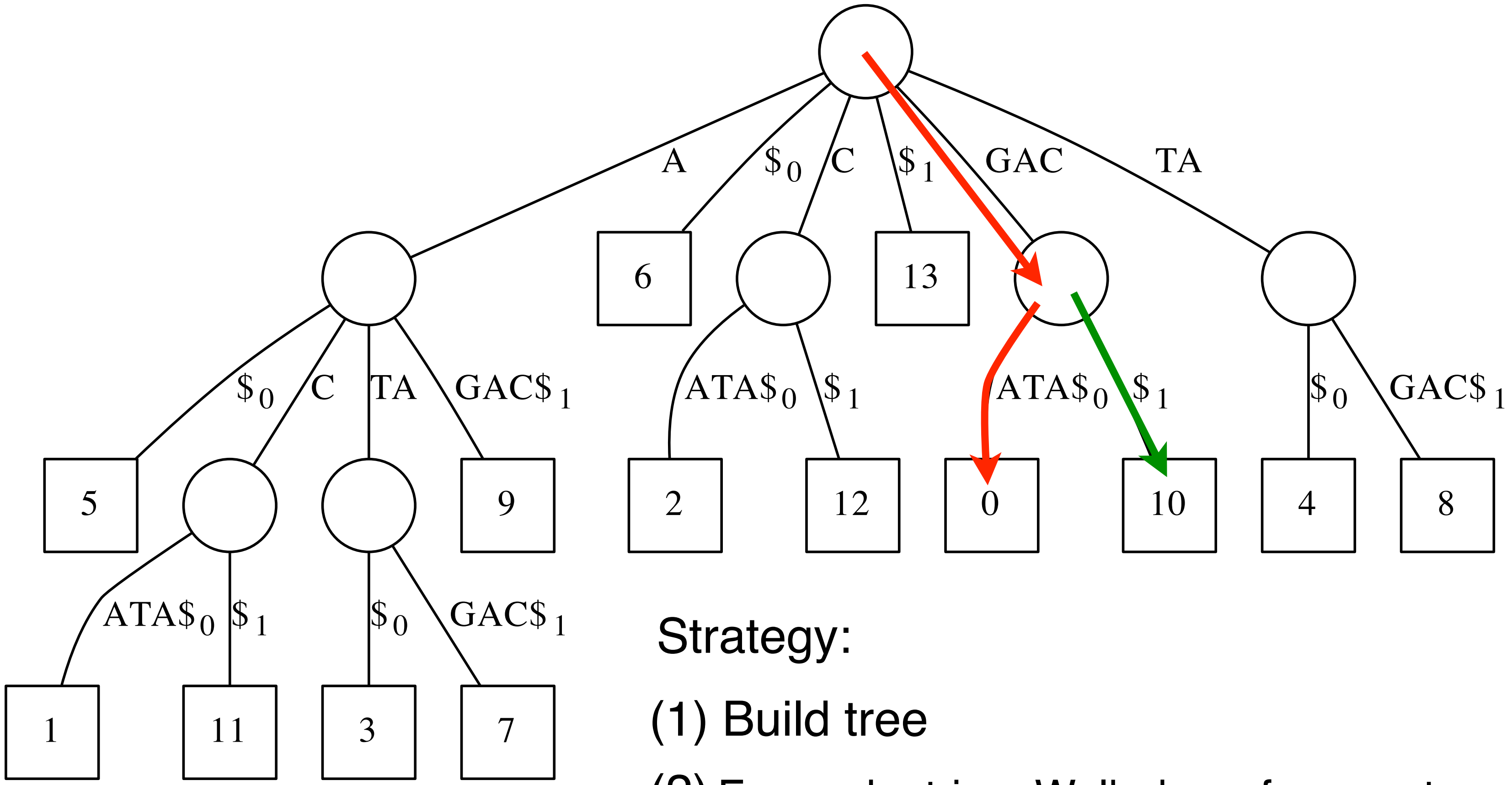
Say query = GACATA. From root, follow path labeled with query.

Green edge implies length-3 suffix of second string equals length-3 prefix of query

```
ATAGAC
   |||
   GACATA
```

Finding overlaps with suffix tree

Generalized suffix tree for { “GACATA”, “ATAGAC” }   GACATA$_0$ATAGAC$_1$



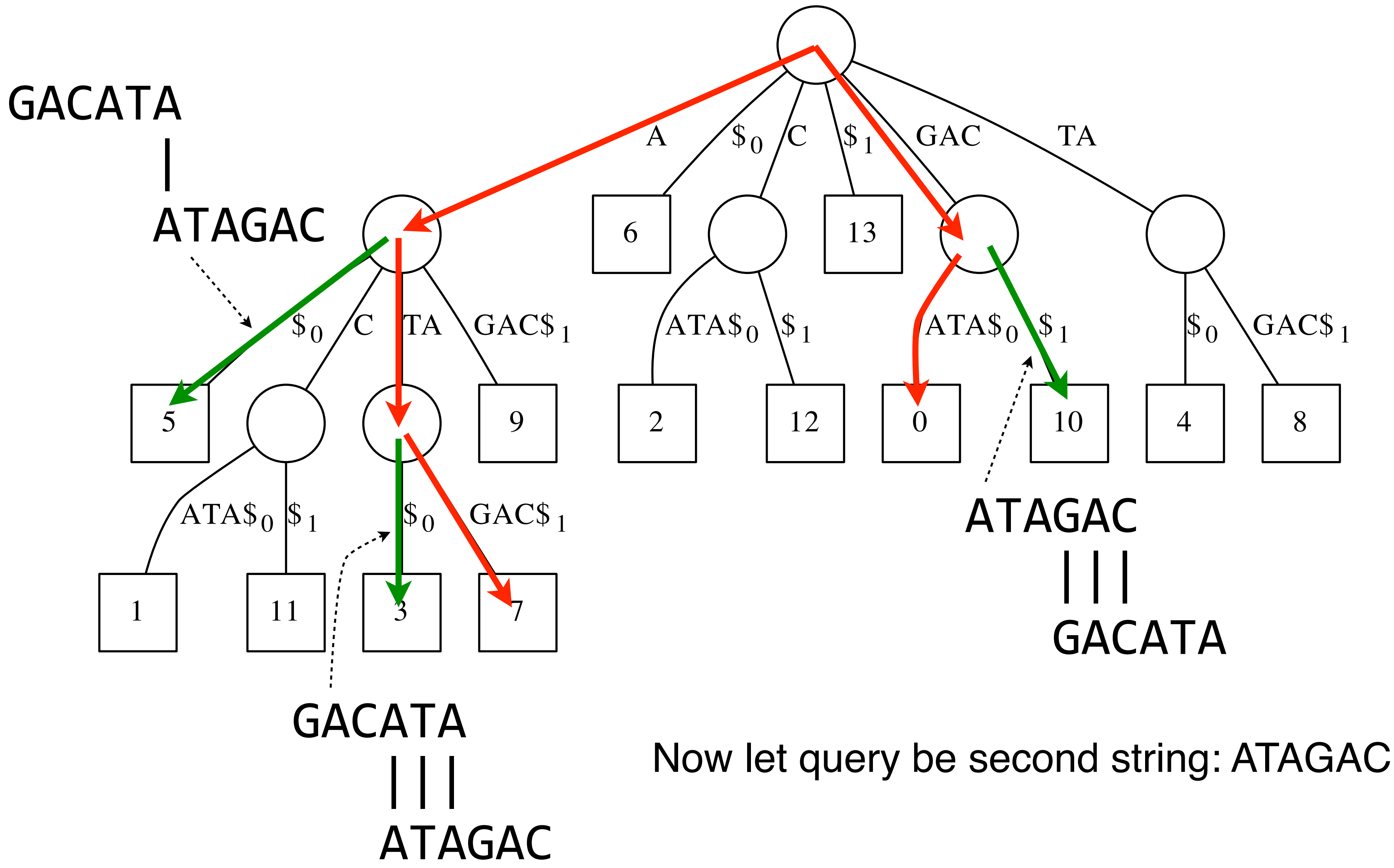
Strategy:

(1) Build tree

(2) For each string: Walk down from root and report any outgoing edge labeled with a separator. Each corresponds to a prefix/suffix match involving prefix of query string and suffix of string ending in the separator.

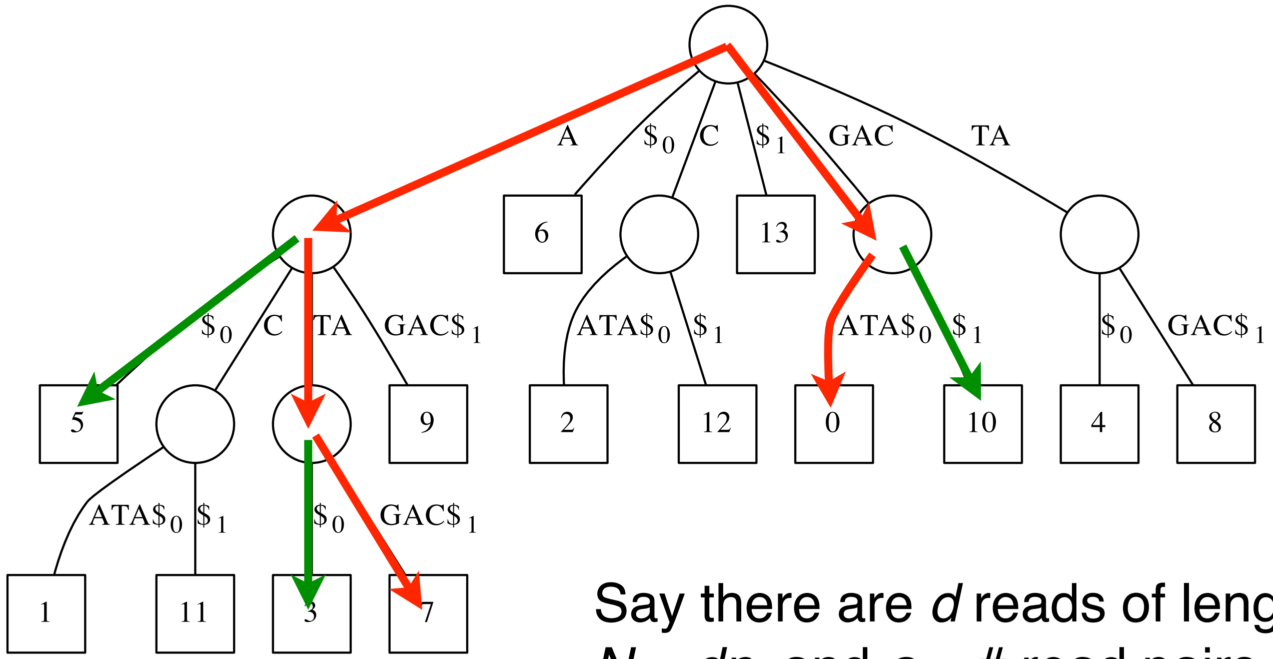
Finding overlaps with suffix tree

Generalized suffix tree for { “GACATA”, “ATAGAC” }   GACATA$\$_0$ATAGAC$\$_1$



Now let query be second string: ATAGAC

Finding overlaps with suffix tree



Say there are *d* reads of length *n*, total length *N* = *dn*, and *a* = # read pairs that overlap

Assume for given string pair we report only the longest suffix/prefix match

Time to build generalized suffix tree:	O(*N*)
... to walk down red paths:	O(*N*)
... to find & report overlaps (green):	O(*a*)
Overall:	O(*N* + *a*)

d^2 doesn't appear explicitly, but *a* is O(d^2) in worst case

Finding overlaps

What if we want to allow mismatches and gaps in the overlap?

I.e. How do we find the best *alignment* of a suffix of *X* to a prefix of *Y*?

```
X: CTCGGCCCTAGG
      ||| |||||
Y:    GGCTCTAGGCCC
```

Dynamic programming

But we must frame the problem such that only backtraces involving a suffix of *X* and a prefix of *Y* are allowed

Finding overlaps with dynamic programming

Find the best alignment of a suffix of *X* to a prefix of *Y*

```
X: CTCGGCCCTAGG
      ||| |||||
Y:    GGCTCTAGGCCC
```

We'll use *global alignment* recurrence and score function

$$D[i,j] = \min \begin{cases} D[i-1,j] + s(x[i-1], -) \\ D[i,j-1] + s(-, y[j-1]) \\ D[i-1,j-1] + s(x[i-1], y[j-1]) \end{cases}$$

$s(a,b)$

	A	C	G	T	-
A	0	4	2	4	8
C	4	0	4	2	8
G	2	4	0	4	8
T	4	2	4	0	8
-	8	8	8	8	

But how do we force it to find prefix / suffix matches?

Finding overlaps with dynamic programming

Find the best alignment of a suffix of *X* to a prefix of *Y*

$$D[i,j] = \min \begin{cases} D[i-1,j] + s(x[i-1], -) \\ D[i,j-1] + s(-, y[j-1]) \\ D[i-1,j-1] + s(x[i-1], y[j-1]) \end{cases}$$

$s(a,b)$	A	C	G	T	-
A	0	4	2	4	8
C	4	0	4	2	8
G	2	4	0	4	8
T	4	2	4	0	8
-	8	8	8	8	

How to initialize first row & column so suffix of *X* aligns to prefix of *Y*?

First column gets 0s
(any suffix of *X* is possible)

First row gets ∞s
(must be a prefix of *Y*)

Backtrace from last row

Y

X

	-	G	G	C	T	C	T	A	G	G	C	C	C
-	0	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞
C	0	4	12	20	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
T	0	4	8	14	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
C	0	4	8	8	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
G	0	0	4	12	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
G	0	0	0	8	16	16	24	26	30	36	44	52	60
C	0	4	4	0	8	16	18	26	30	34	36	44	52
C	0	4	8	4	2	8	16	22	30	34	34	36	44
C	0	4	8	8	6	2	10	18	26	34	34	34	36
T	0	4	8	10	8	8	2	10	18	26	34	36	36
A	0	2	6	12	14	12	10	2	10	18	26	34	40
G	0	0	2	10	16	18	16	10	0	10	18	26	34
G	0	0	0	6	14	20	22	18	10	[illegible]	10	18	26

```
X: CTCGGCCCTAGG
      ||| |||||
Y:    GGCTCTAGGCCC
```

Finding overlaps with dynamic programming

Find the best alignment of a suffix of *X* to a prefix of *Y*

$$D[i,j] = \min \begin{cases} D[i-1,j] + s(x[i-1],-) \\ D[i,j-1] + s(-,y[j-1]) \\ D[i-1,j-1] + s(x[i-1],y[j-1]) \end{cases}$$

$s(a,b)$	A	C	G	T	-
A	0	4	2	4	8
C	4	0	4	2	8
G	2	4	0	4	8
T	4	2	4	0	8
-	8	8	8	8	

Problem: very short matches got high scores by chance...

...which might obscure the more relevant match

Say we want to enforce minimum overlap length *l* = 5

Y

X

	-	G	G	C	T	C	T	A	G	G	C	C	C
-	0	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞
C	0	4	12	20	28	36	44	52	60	68	76	84	92
T	0	4	8	14	20	28	36	44	52	60	68	76	84
C	0	4	8	8	16	20	28	36	44	52	60	68	76
G	0	0	4	12	12	20	24	30	36	44	52	60	68
G	0	0	0	8	16	16	24	26	30	36	44	52	60
C	0	4	4	0	8	16	18	26	30	34	36	44	52
C	0	4	8	4	2	8	16	22	30	34	34	36	44
C	0	4	8	8	6	2	10	18	26	34	34	34	36
T	0	4	8	10	8	8	2	10	18	26	34	36	36
A	0	2	6	12	14	12	10	2	10	18	26	34	40
G	0	0	2	10	16	18	16	10	0	10	18	26	34
G	0	0	0	6	14	20	22	18	10	2	10	18	26

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Finding overlaps with dynamic programming

Find the best alignment of a suffix of *X* to a prefix of *Y*

$$D[i,j] = \min \begin{cases} D[i-1,j] + s(x[i-1], -) \\ D[i,j-1] + s(-, y[j-1]) \\ D[i-1,j-1] + s(x[i-1], y[j-1]) \end{cases}$$

$s(a,b)$	A	C	G	T	-
A	0	4	2	4	8
C	4	0	4	2	8
G	2	4	0	4	8
T	4	2	4	0	8
-	8	8	8	8	

Solve by initializing certain additional cells to ∞

Cells whose values changed highlighted in red

Now the relevant match is the best candidate

Y

X	-	G	G	C	T	C	T	A	G	G	C	C	C
-	0	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞
C	0	4	12	20	28	36	44	52	60	68	76	84	92
T	0	4	8	14	20	28	36	44	52	60	68	76	84
C	0	4	8	8	16	20	28	36	44	52	60	68	76
G	0	0	4	12	12	20	24	30	36	44	52	60	68
G	0	0	0	8	16	16	24	26	30	36	44	52	60
C	0	4	4	0	8	16	18	26	30	34	36	44	52
C	0	4	8	4	2	8	16	22	30	34	34	36	44
C	0	4	8	8	6	2	10	18	26	34	34	34	36
T	∞	4	8	10	8	8	2	10	18	26	34	36	36
A	∞	12	6	12	14	12	10	2	10	18	26	34	40
G	∞	20	12	10	16	18	16	10	0	10	18	26	34
G	∞	∞	∞	∞	∞	20	22	18	10	$\boxed{2}$	10	18	26

Finding overlaps with dynamic programming

Say there are d reads of length n, total length $N = dn$, and a is total number of pairs with an overlap

Number of overlaps to try:	$O(d^2)$
Size of each dynamic programming matrix:	$O(n^2)$
Overall:	$O(d^2 n^2) = O(N^2)$

Contrast $O(N^2)$ with suffix tree: $O(N + a)$, but where a is worst-case $O(d^2)$

But dynamic programming is more flexible, allowing mismatches and gaps

Real-world overlappers mix the two, using indexes to filter out vast majority of non-overlapping pairs, then using dynamic programming for remaining pairs

Finding overlaps

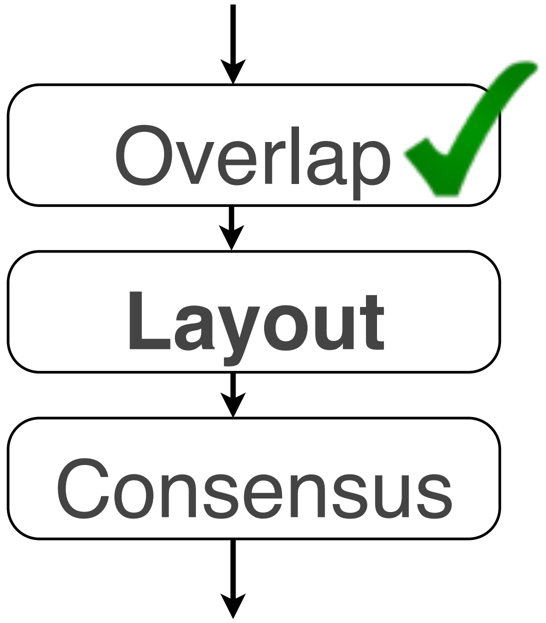
- Overlapping is typically the slowest part of assembly
    - Consider a second-generation sequencing dataset with hundreds of millions or billions of reads!

Finding overlaps

Celera Assembler’s overlapper is probably the best documented:

- Inverted substring indexes built on batches of reads
- Only look for overlaps between reads that share one or more substrings of some length
  - http://wgs-assembler.sourceforge.net/wiki/index.php/RunCA#Overlapper

Overlap Layout Consensus



Build overlap graph

Bundle stretches of the overlap graph into *contigs*

Pick most likely nucleotide sequence for each contig

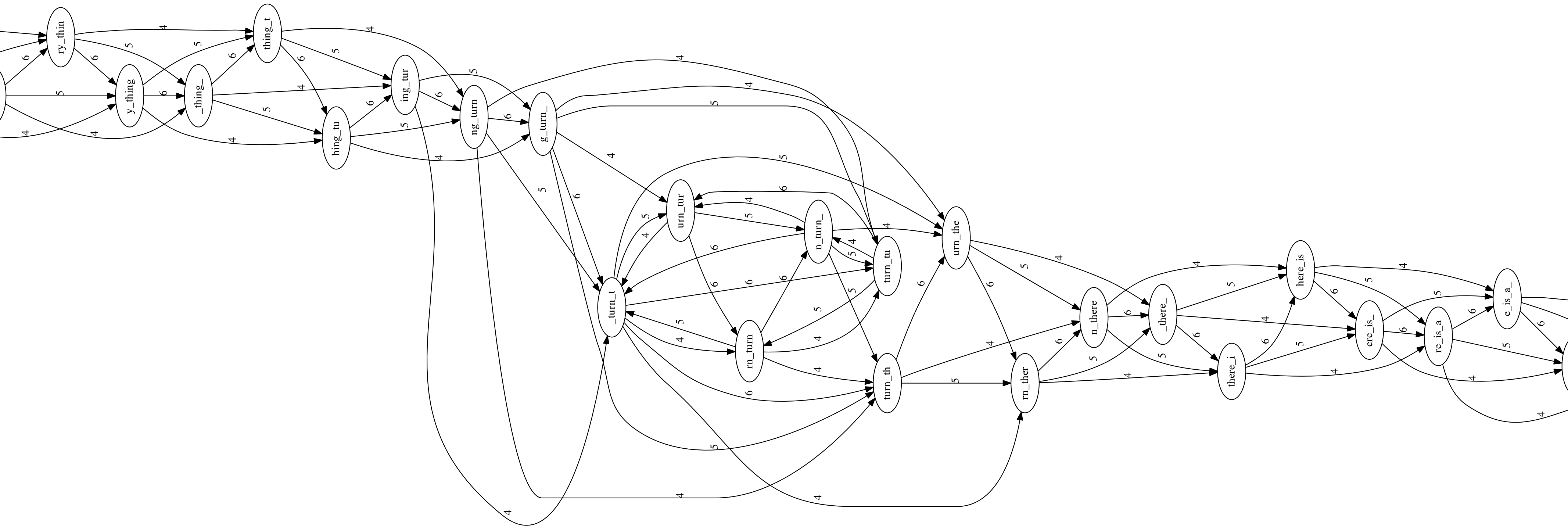
Layout

Overlap graph is big and messy. Contigs don't "pop out" at us.

Below: part of the overlap graph for

`to_every_thing_turn_turn_turn_there_is_a_season`

$l = 4$, $k = 7$

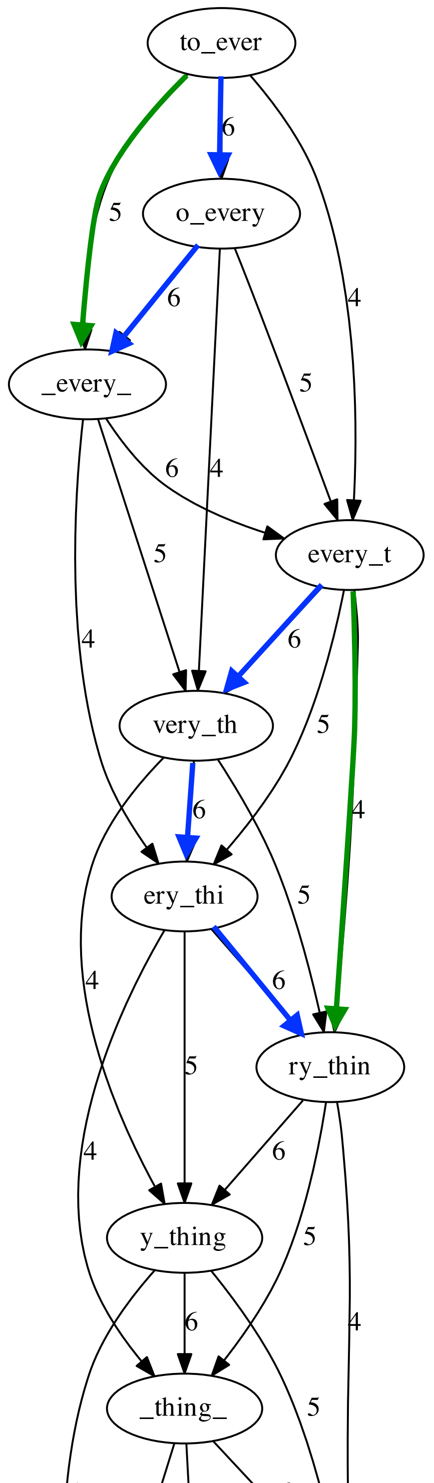


Layout

Anything redundant about this part of the overlap graph?

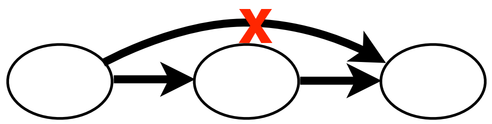
Some edges can be *inferred* (*transitively*) from other edges

E.g. green edge can be inferred from blue

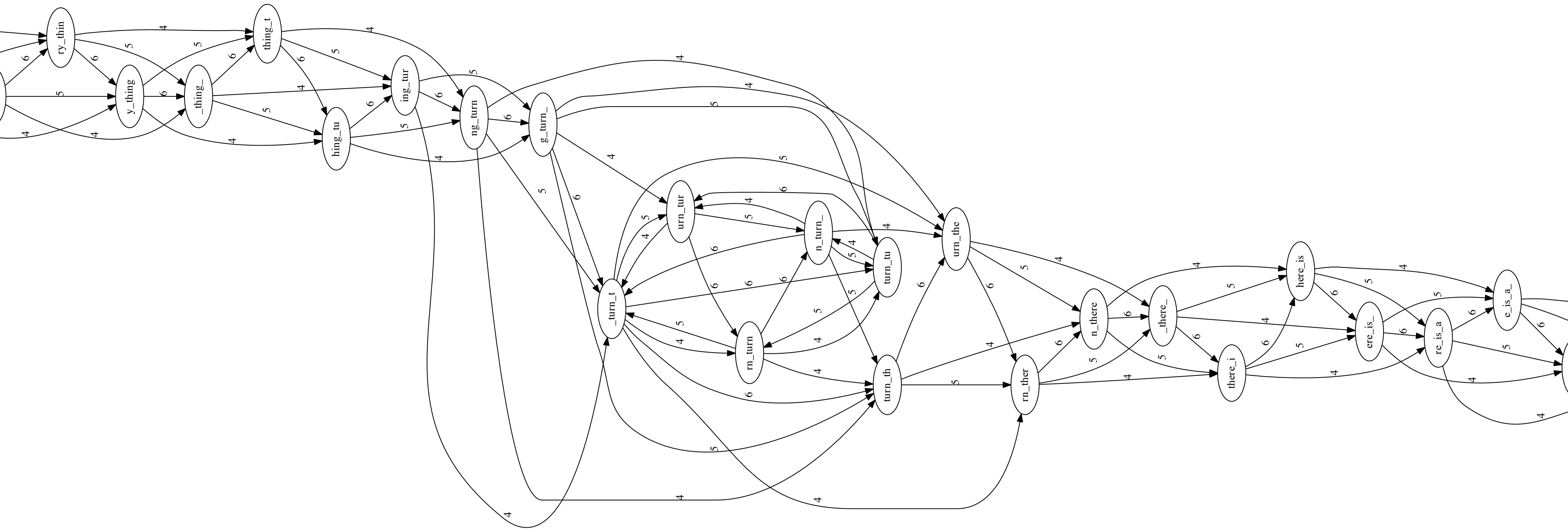


Layout

Remove transitively-inferrible edges, starting with edges that skip one node:

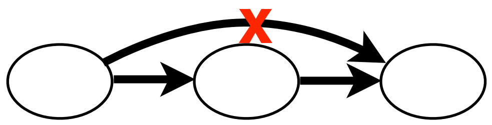


Before:

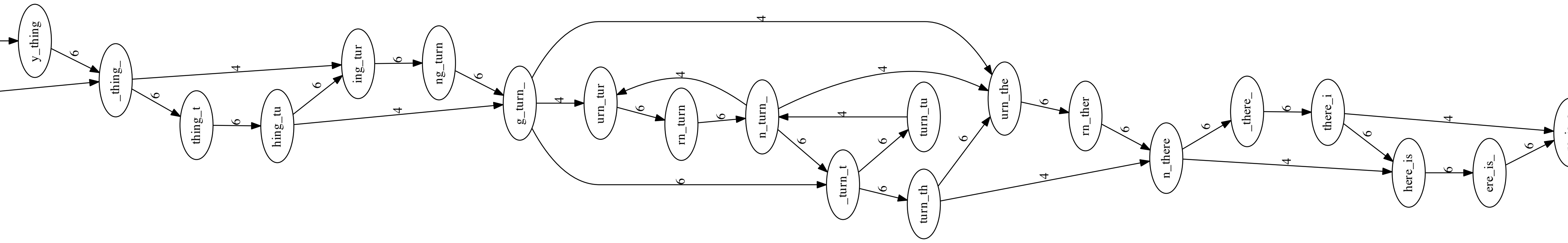


Layout

Remove transitively-inferrible edges, starting with edges that skip one node:

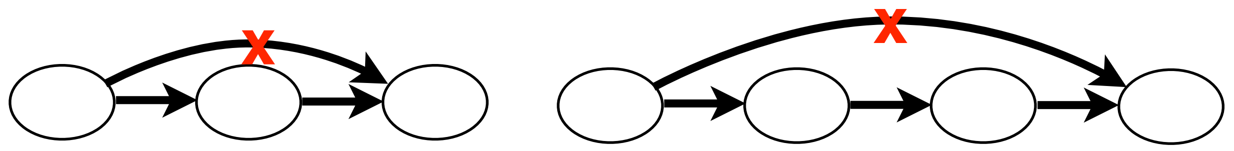


After:

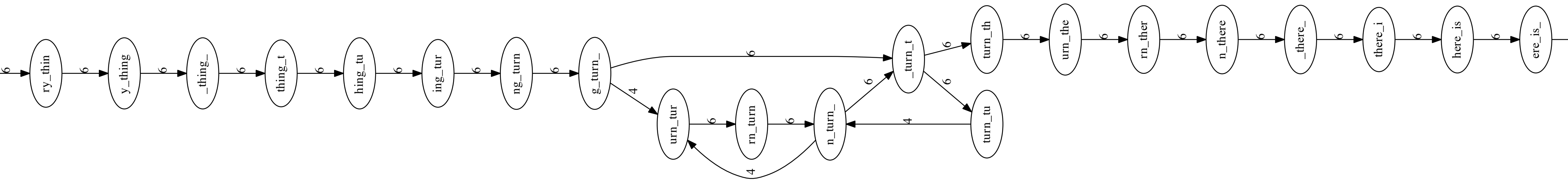


Layout

Remove transitively-inferrible edges, starting with edges that skip one *or two* nodes:



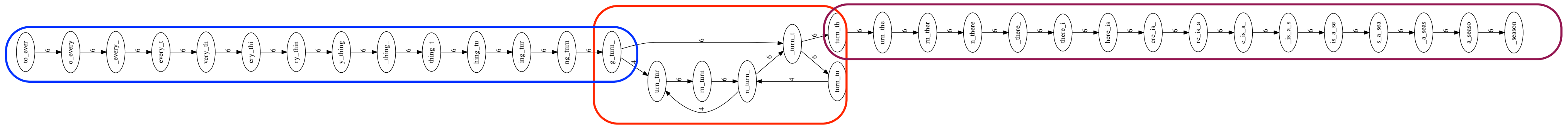
After:



Even simpler

Layout

Emit *contigs* corresponding to the non-branching stretches



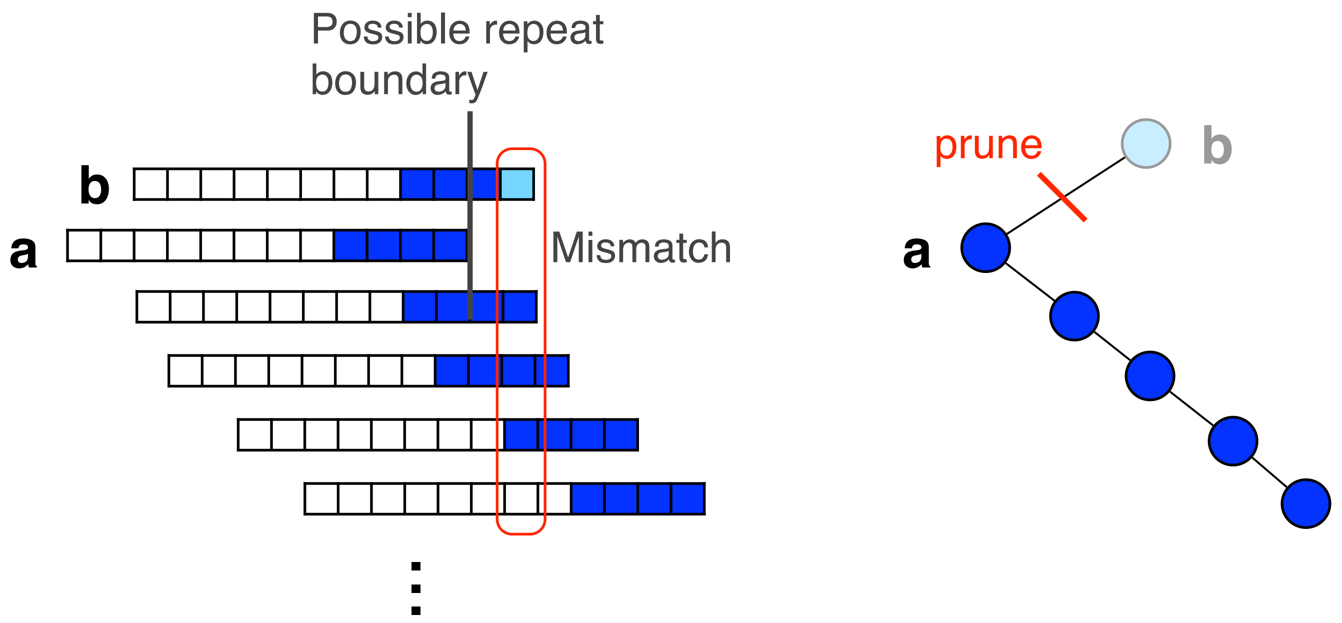
Contig 1
`to_every_thing_turn_`

Contig 2
`turn_there_is_a_season`

Unresolvable repeat

Layout

In practice, layout step also has to deal with spurious subgraphs, e.g. because of sequencing error



Mismatch could be due to sequencing error or repeat. Since the path through **b** ends abruptly we might conclude it's an error and prune **b**.

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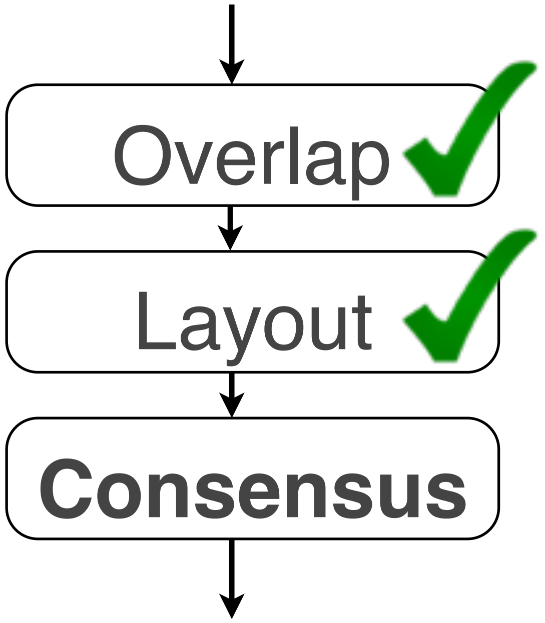
Overlap Layout Consensus

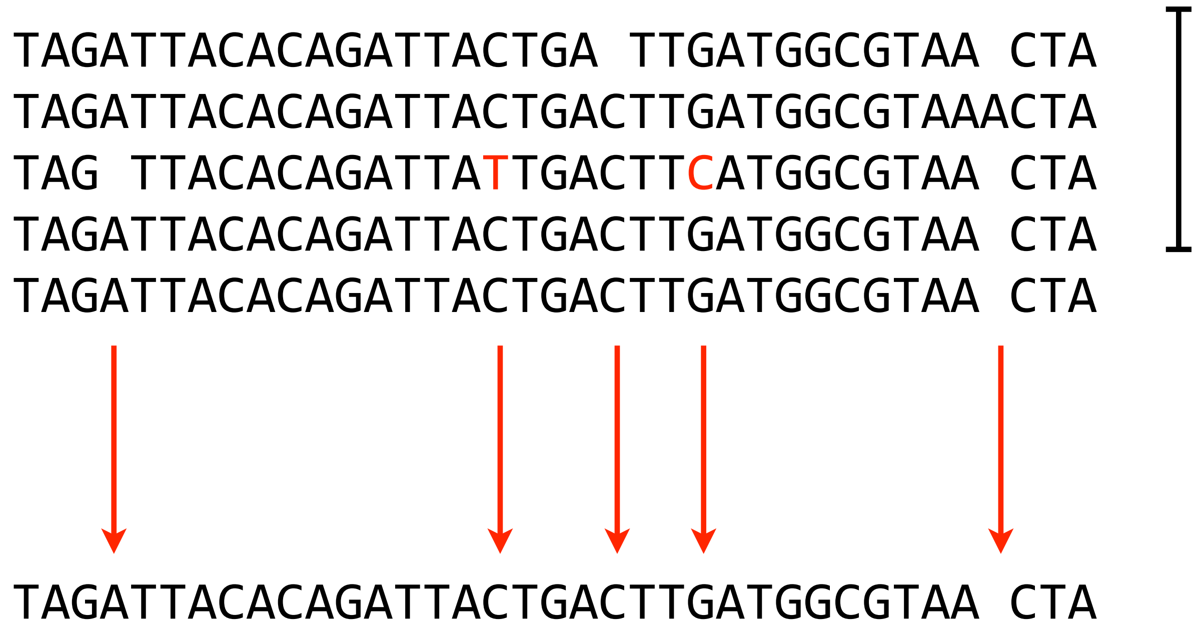


Build overlap graph

Bundle stretches of the overlap graph into *contigs*

Pick most likely nucleotide sequence for each contig

Consensus



```
TAGATTACACAGATTACTGA TTGATGGCGTAA CTA
TAGATTACACAGATTACTGACTTGATGGCGTAAACTA
TAG TTACACAGATTATTGACTTCATGGCGTAA CTA
TAGATTACACAGATTACTGACTTGATGGCGTAA CTA
TAGATTACACAGATTACTGACTTGATGGCGTAA CTA

TAGATTACACAGATTACTGACTTGATGGCGTAA CTA
```

Take reads that make up a contig and line them up

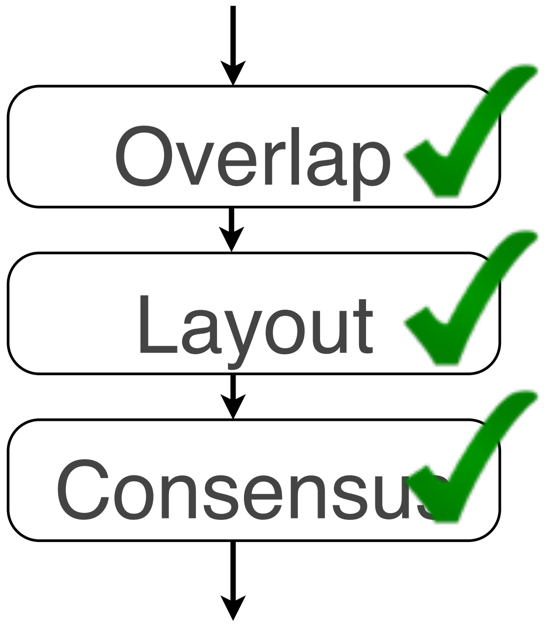
Take *consensus*, i.e. majority vote

At each position, ask: what nucleotide (and/or gap) is here?

Complications: (a) sequencing error, (b) ploidy

Say the true genotype is AG, but we have a high sequencing error rate and only about 6 reads covering the position.

Overlap Layout Consensus



Build overlap graph

Bundle stretches of the overlap graph into *contigs*

Pick most likely nucleotide sequence for each contig

OLC drawbacks

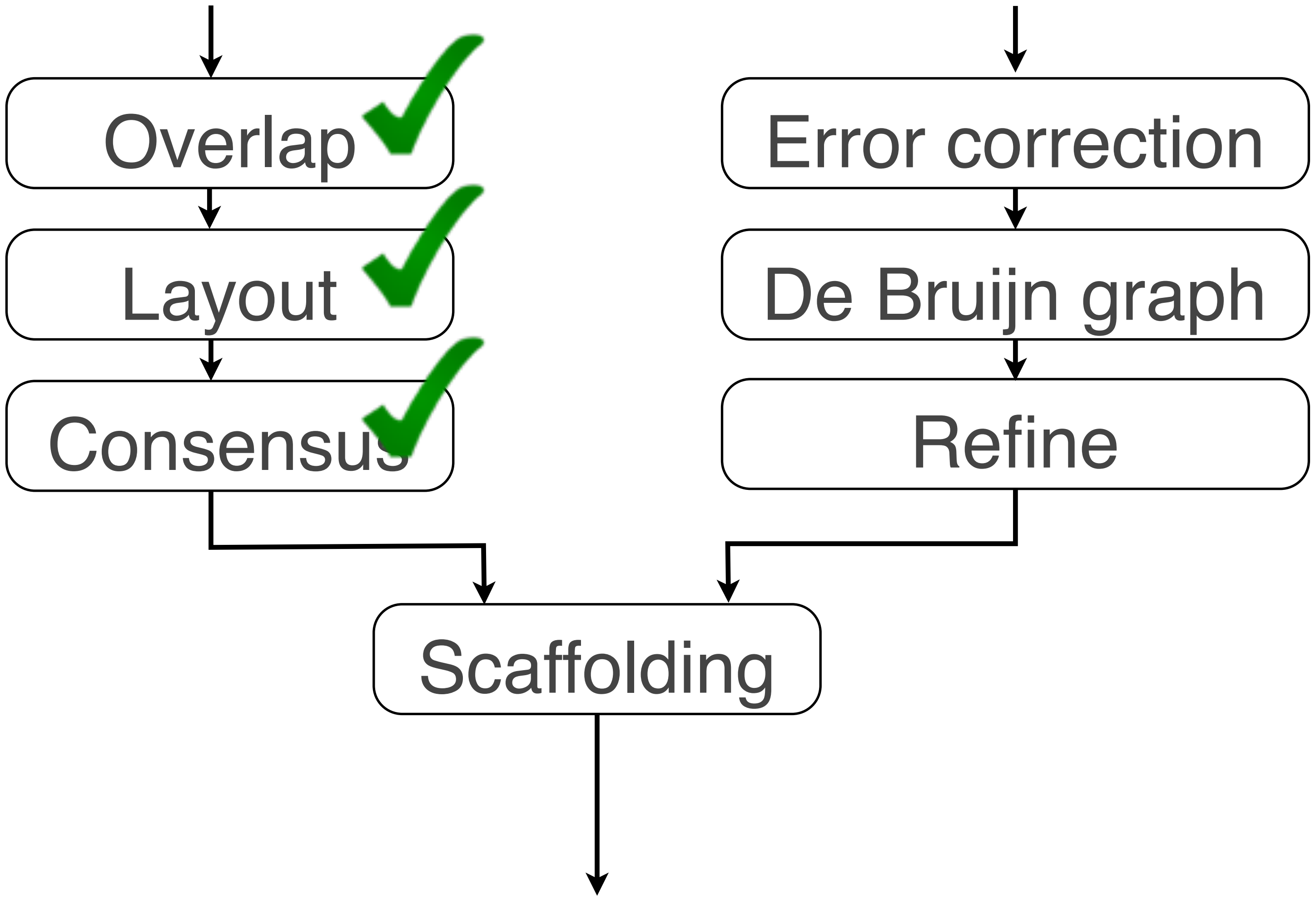
Building overlap graph is slow. We saw $O(N + a)$ and $O(N^2)$ approaches.

Overlap graph is big; one node per read, and in practice # edges grows superlinearly with # reads

2nd-generation sequencing datasets are ~ 100s of millions or billions of reads, hundreds of billions of nucleotides total

Summary

- Discussed assembly as SCS problem
- Discussed OLC assembly
- Next week: de Bruijn assembly and memory efficiency



Overlap
Layout
Consensus
Error correction
De Bruijn graph
Refine
Scaffolding