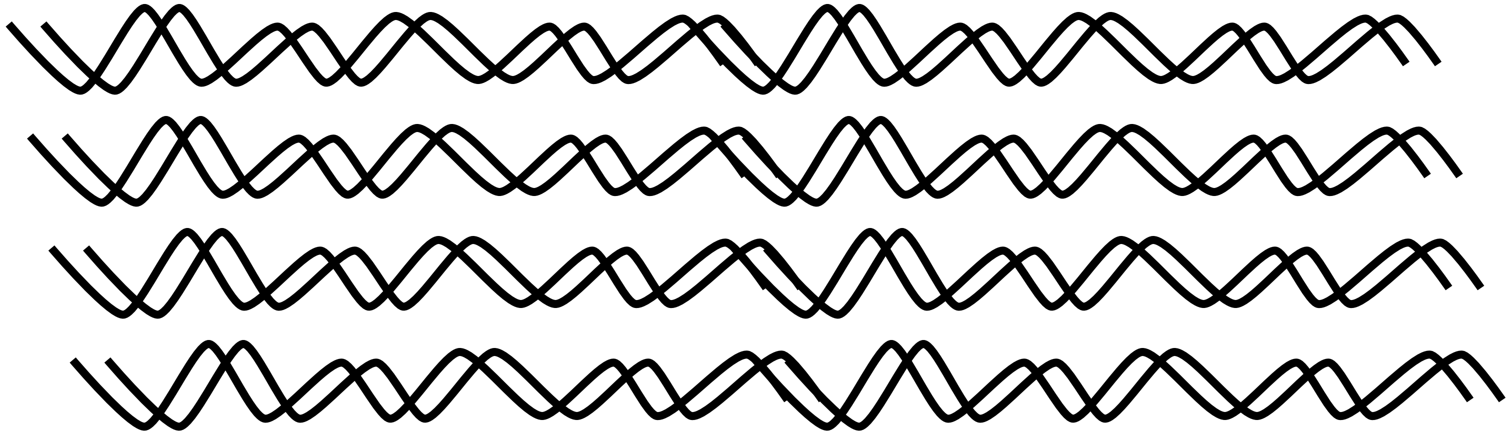
CSE 549: Genome Assembly
Intro & OLC

Stony Brook University

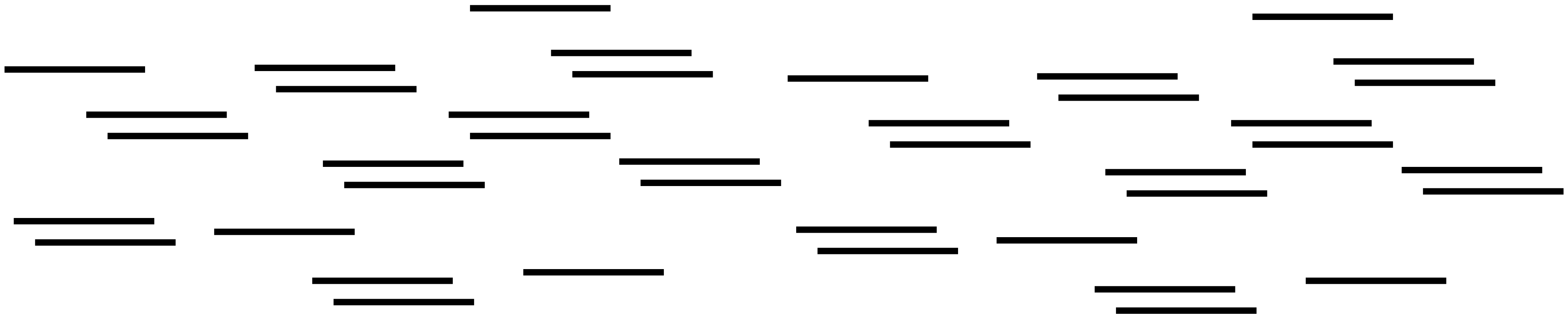
All slides in this lecture **not** marked with "*" courtesy of Ben Langmead.

Shotgun Sequencing

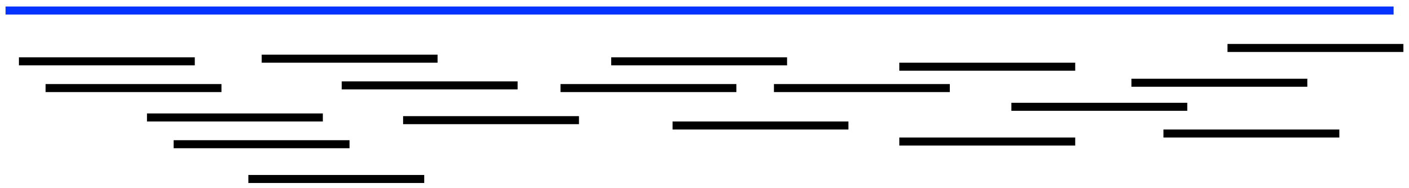
Many copies
of the DNA



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

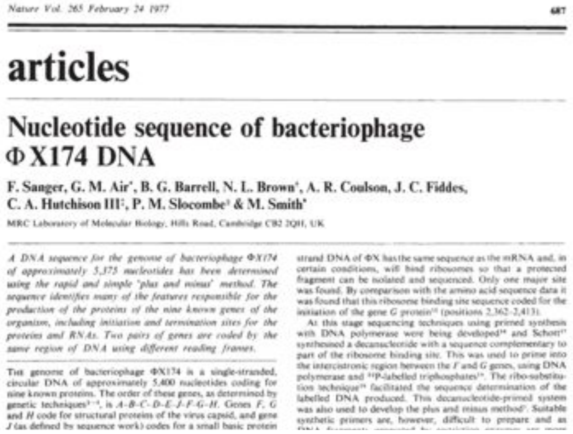


Assemble into original genome:



* Slide from Carl Kingsford

Milestones in Genome Assembly



Nature Vol. 265 February 24 1977 687

articles

Nucleotide sequence of bacteriophage ΦX174 DNA

F. Sanger, G. M. Air[*], B. G. Barrell, N. L. Brown[*], A. R. Coulson, J. C. Fiddes, C. A. Hutchison III[‡], P. M. Slocombe[†] & M. Smith[*]

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

A DNA sequence for the genome of bacteriophage ΦX174 of approximately 5,375 nucleotides has been determined using the rapid and simple 'plus and minus' method. The sequence identifies many of the features responsible for the production of the proteins of the nine known genes of the organism, including initiation and termination sites for the proteins and RNAs. Two pairs of genes are coded by the same region of DNA using different reading frames.

THE genome of bacteriophage ΦX174 is a single-stranded, circular DNA of approximately 5,400 nucleotides coding for nine known proteins. The order of these genes, as determined by genetic techniques[2-4], is *A-B-C-D-E-J-F-G-H*. Genes *F*, *G* and *H* code for structural proteins of the virus capsid, and gene *J* (as defined by sequence work) codes for a small basic protein

strand DNA of ΦX has the same sequence as the mRNA and, in certain conditions, will bind ribosomes so that a protected fragment can be isolated and sequenced. Only one major site was found. By comparison with the amino acid sequence data it was found that this ribosome binding site sequence coded for the initiation of the gene *G* protein[13] (positions 2,362-2,413).

At this stage sequencing techniques using primed synthesis with DNA polymerase were being developed[14] and Schott[15] synthesised a decanucleotide with a sequence complementary to part of the ribosome binding site. This was used to prime into the intercistronic region between the *F* and *G* genes, using DNA polymerase and ^{32}P-labelled triphosphates[16]. The ribo-substitution technique[16] facilitated the sequence determination of the labelled DNA produced. This decanucleotide-primed system was also used to develop the plus and minus method[1]. Suitable synthetic primers are, however, difficult to prepare and as

1977. Sanger *et al.*
1st Complete Organism
5375 bp

1995. Fleischmann *et al.*
1st Free Living Organism
TIGR Assembler. 1.8Mbp

1998. C.elegans SC
1st Multicellular Organism
BAC-by-BAC Phrap. 97Mbp

2000. Myers *et al.*
1st Large WGS Assembly.
Celera Assembler. 116 Mbp

2001.Venter *et al.*, IHGSC
Human Genome
Celera Assembler/GigaAssembler. 2.9 Gbp

2010. Li *et al.*
1st Large SGS Assembly.
SOAPdenovo 2.2 Gbp

Like Dickens, we must computationally reconstruct a genome from short fragments

* Slide from Mike Schatz

Assembly Applications

- Novel genomes

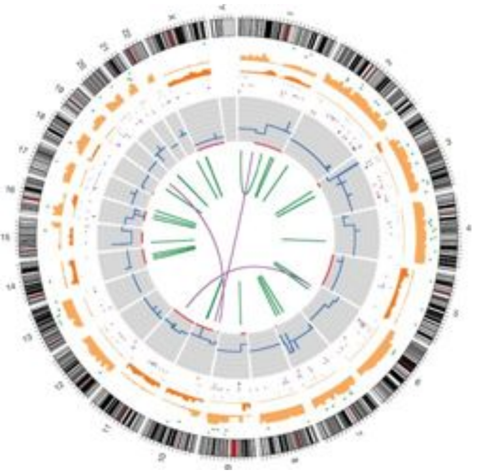
GENOME 10K

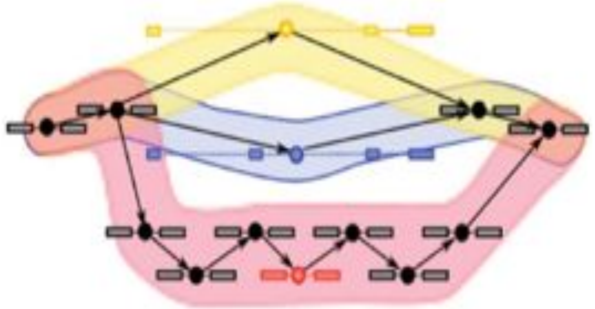
i5K

- Metagenomes

HMP

- Sequencing assays
  - Structural variations
  - Transcript assembly
  - …

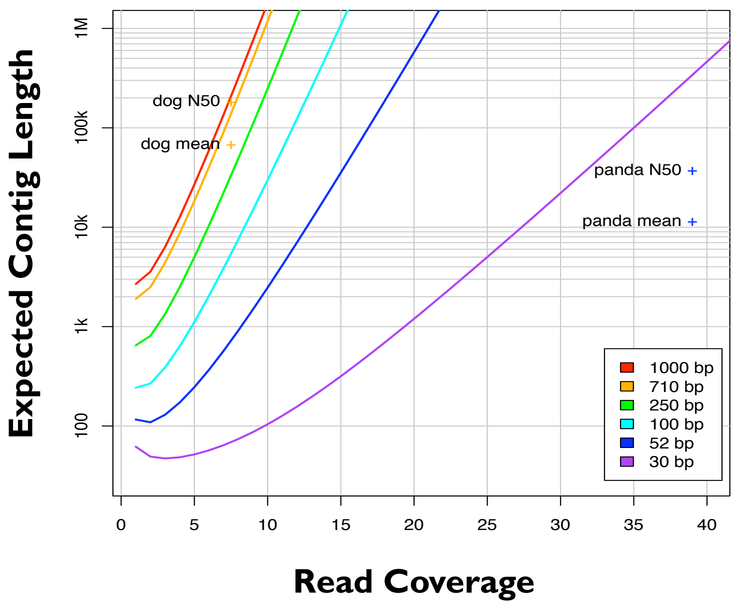




* Slide from Mike Schatz

Ingredients for a good assembly

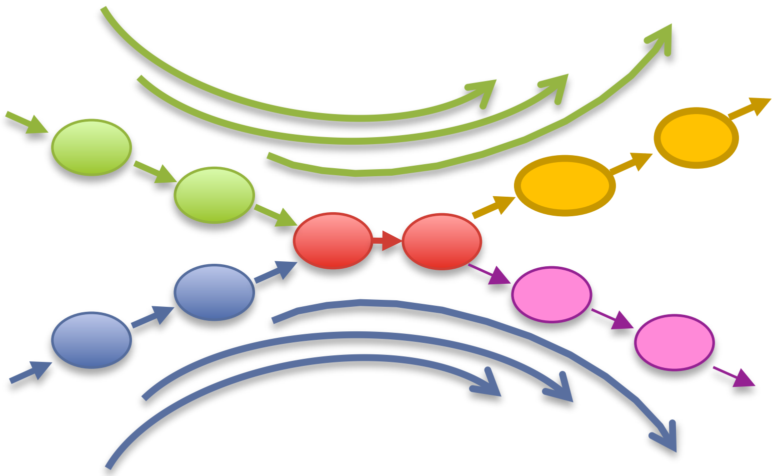
Coverage



High coverage is required

- Oversample the genome to ensure every base is sequenced with long overlaps between reads
- Biased coverage will also fragment assembly

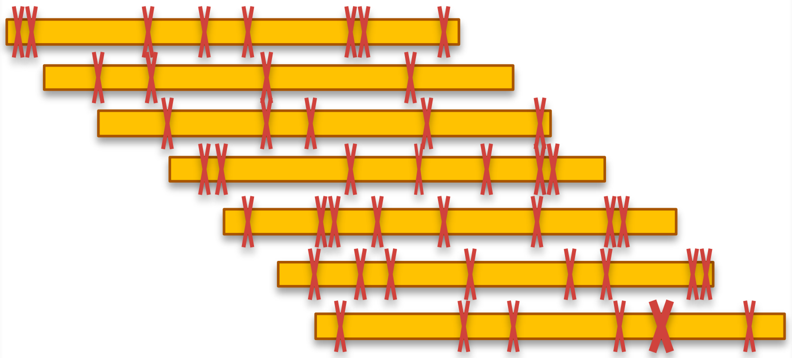
Read Length



Reads & mates must be longer than the repeats

- Short reads will have ***false overlaps*** forming hairball assembly graphs
- With long enough reads, assemble entire chromosomes into contigs

Quality



Errors obscure overlaps

- Reads are assembled by finding kmers shared in pair of reads
- High error rate requires very short seeds, increasing complexity and forming assembly hairballs

Current challenges in *de novo* plant genome sequencing and assembly
Schatz MC, Witkowski, McCombie, WR (2012) *Genome Biology*. 12:243

* Slide from Mike Schatz

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

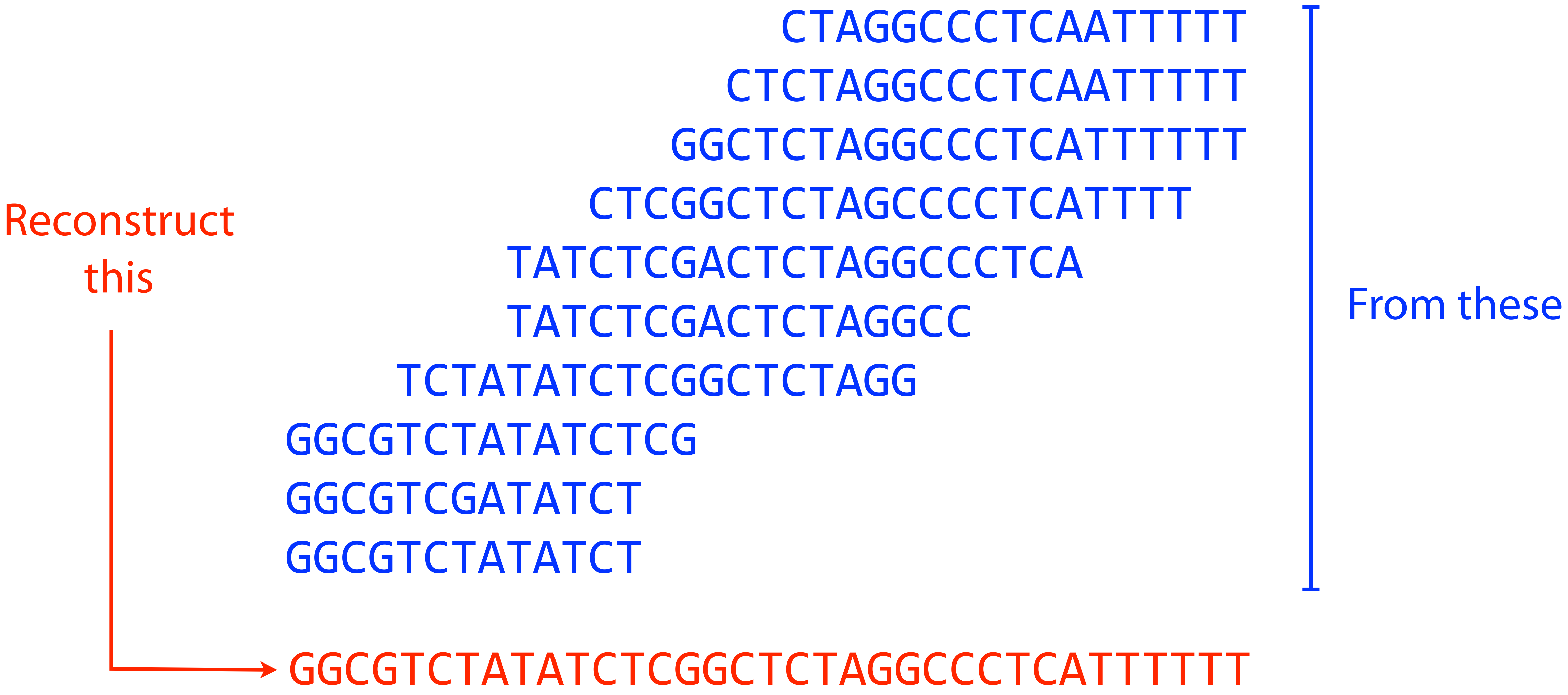
GGC GTCTATAT CTCGGCTCTAGGCCCTCA TTTTTT

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...



Reconstruct this

CTAGGCCCTCAATTTTT

CTCTAGGCCCTCAATTTTT

GGCTCTAGGCCCTCATTTTTT

CTCGGCTCTAGCCCCTCATTTT

TATCTCGACTCTAGGCCCTCA

TATCTCGACTCTAGGCC

TCTATATCTCGGCTCTAGG

GGCGTCTATATCTCG

GGCGTCGATATCT

GGCGTCTATATCT

From these

GGCGTCTATATCTCGGCTCTAGGCCCTCATTTTTT

Assembly

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

CTAGGCCCTCAATTTTT
GGCGTCTATATCT
CTCTAGGCCCTCAATTTTT
TCTATATCTCGGCTCTAGG
GGCTCTAGGCCCTCATTTTTT
CTCGGCTCTAGCCCCTCATTTT
TATCTCGACTCTAGGCCCTCA
GGCGTCGATATCT
TATCTCGACTCTAGGCC
GGCGTCTATATCTCG

From these

Reconstruct this

GGCGTCTATATCTCGGCTCTAGGCCCTCATTTTTT

Assembly

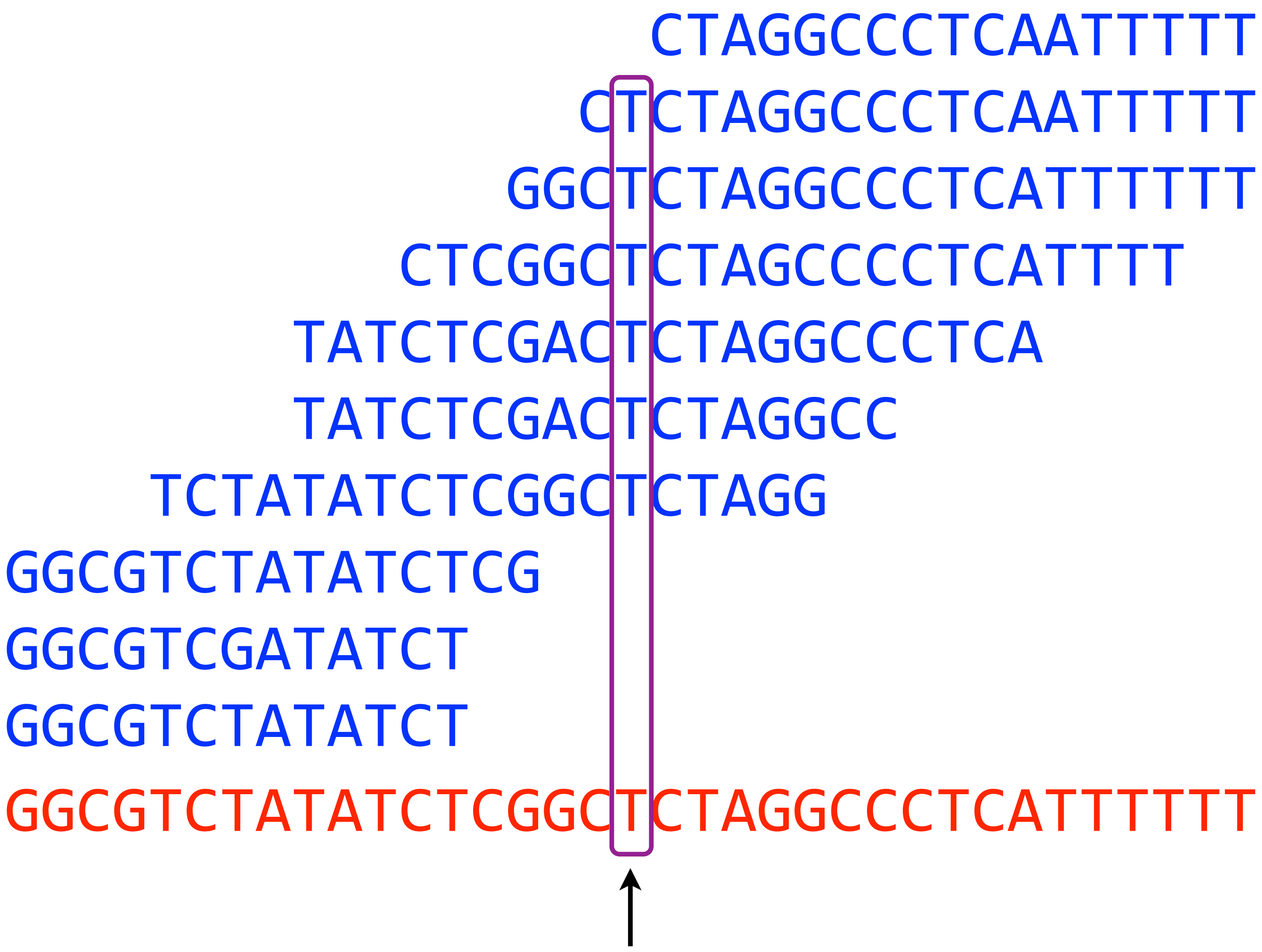
Key term: *coverage*. Usually it’s short for *average coverage*: the average number of reads covering a position in the genome.

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

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:

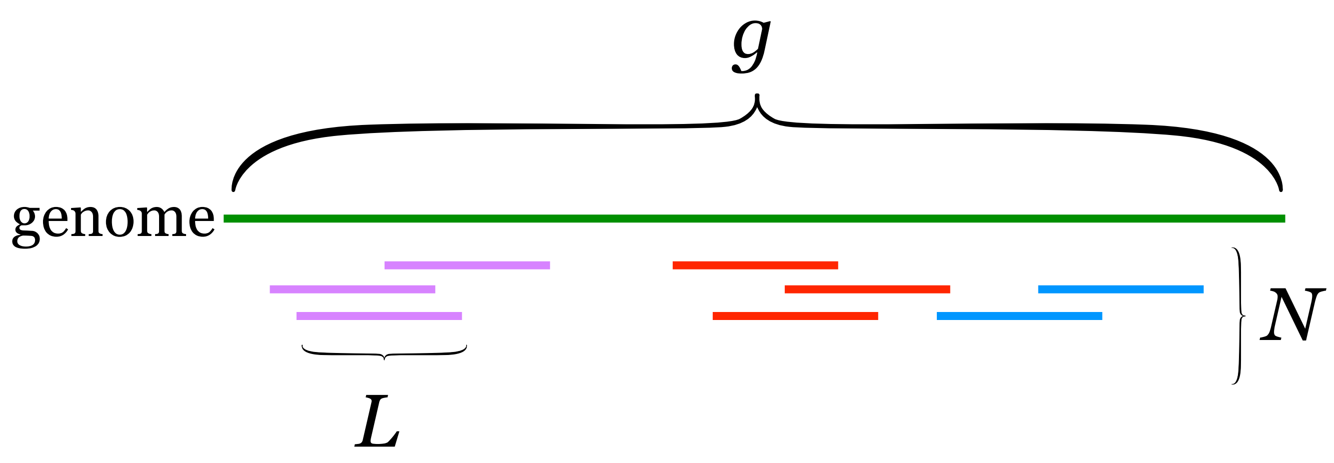
```
Read from Mother:        TATCTCGACTCTAGGCC
                         ||||||| |||||||
Read from Father:    TCTATATCTCGGCTCTAGG

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

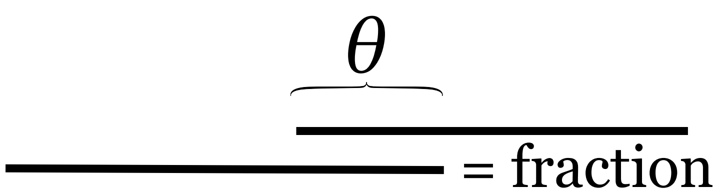
We'll mostly ignore ploidy, but real tools must consider it

How Much Coverage is Enough? Lander-Waterman Statistics

Lander ES, Waterman MS (1988). "Genomic mapping by fingerprinting random clones: a mathematical analysis". Genomics 2 (3): 231–239

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





= fraction of L required to detect an overlap

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.

* Slide from Carl Kingsford

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)

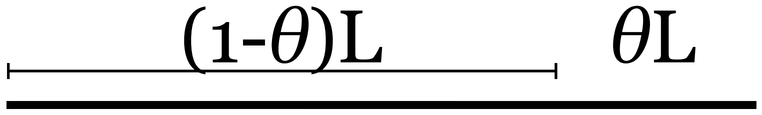
x trials, want k “successes”, small probability λ of success

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 $(1\text{-}\theta)L$)

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

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

$$= Ne^{-(1-\theta)LN/g}$$

← LN/g is called the **coverage** c.

* Slide from Carl Kingsford

Expected # of Islands, 2

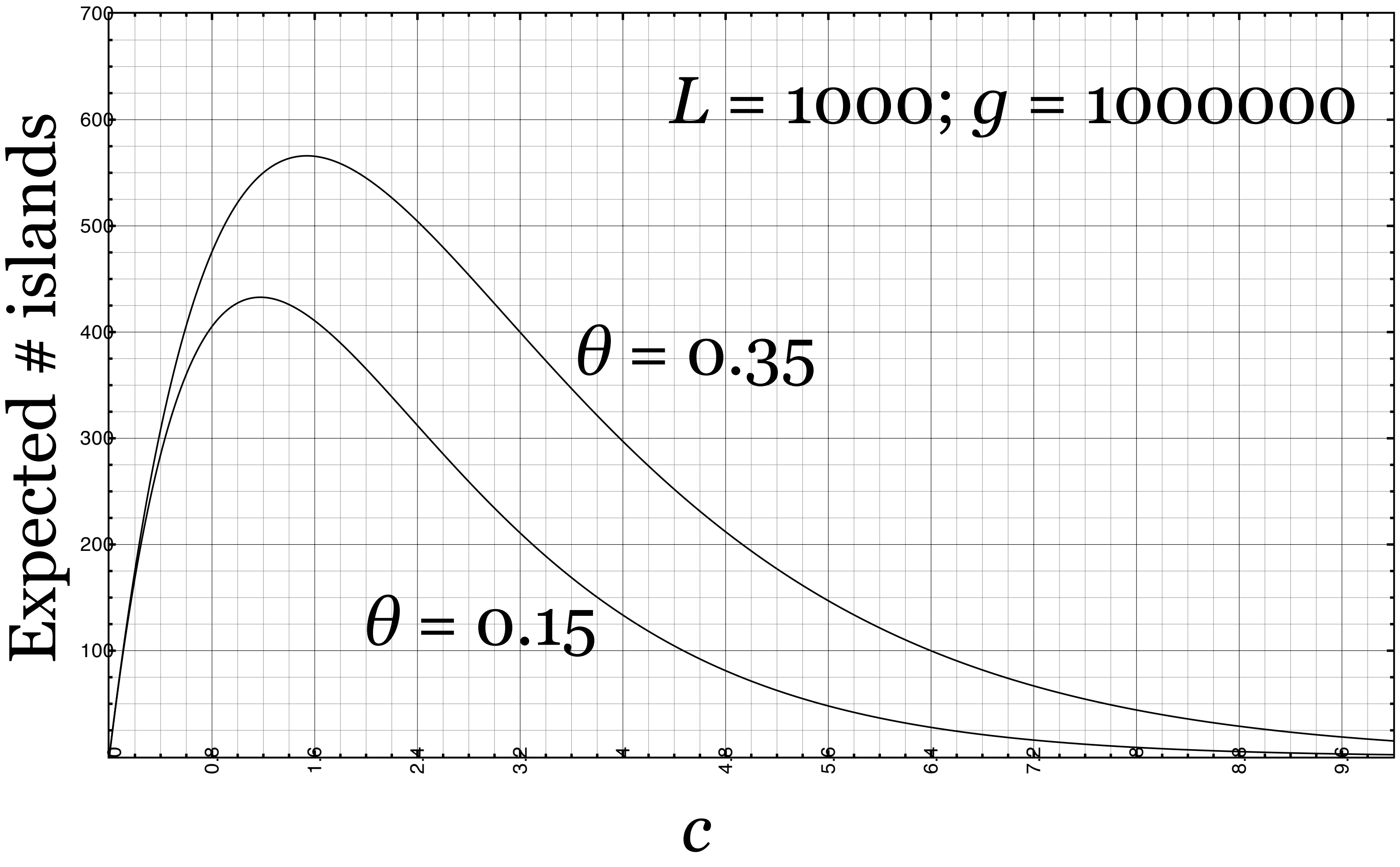
We can rewrite this expression to depend more directly on the things we can control: c and θ

$$\text{Expected \# of islands} = Ne^{-(1-\theta)LN/g}$$

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

$$= \frac{L/g}{L/g} Ne^{-(1-\theta)c}$$

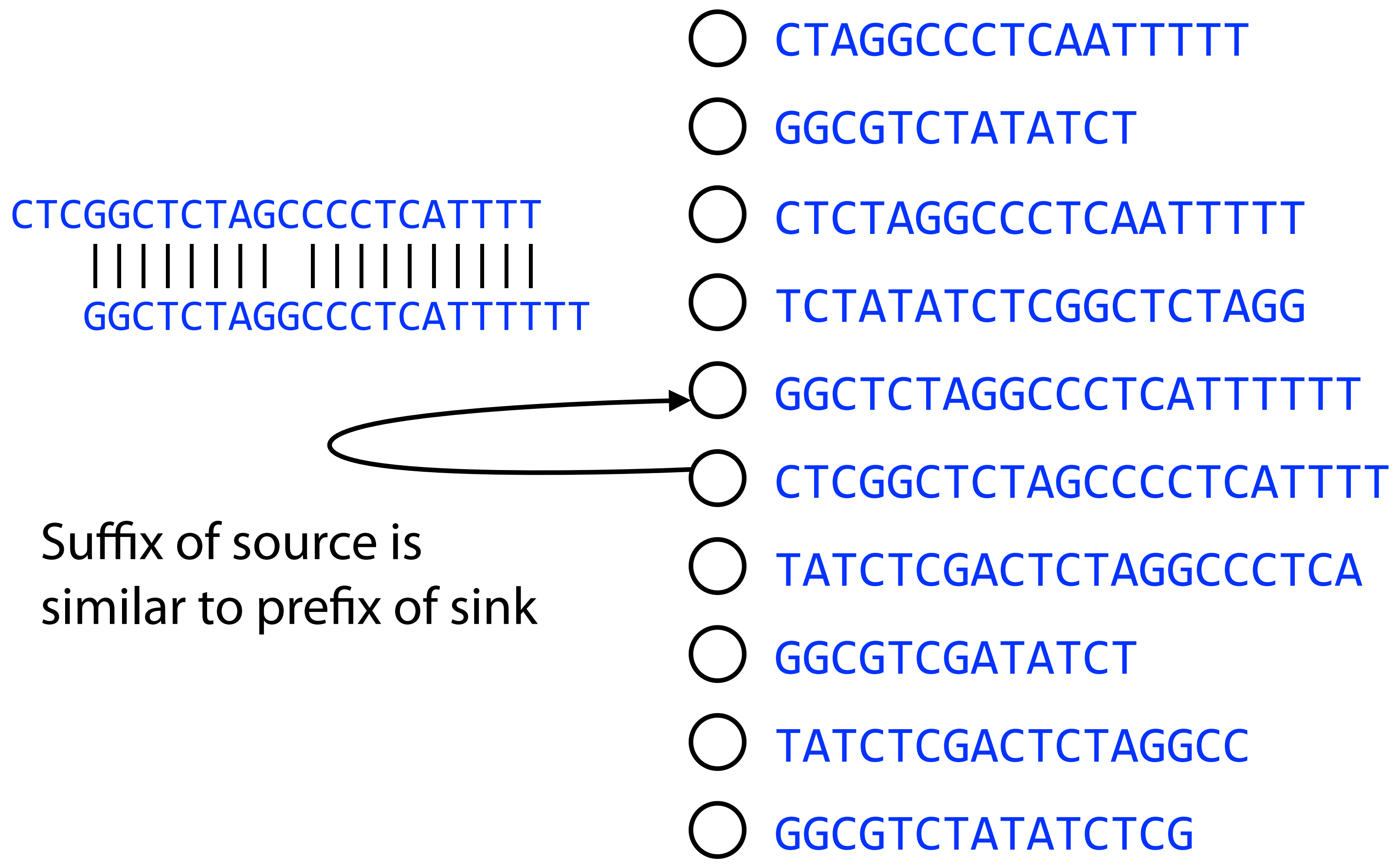
$$= \frac{g}{L} ce^{-(1-\theta)c}$$



* Slide from Carl Kingsford

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

○ CTAGGCCCTCAATTTTT

○ GGCGTCTATATCT

○ CTCTAGGCCCTCAATTTTT

○ TCTATATCTCGGCTCTAGG

○ GGCTCTAGGCCCTCATTTTTT

○ CTCGGCTCTAGCCCCTCATTTT

○ TATCTCGACTCTAGGCCCTCA

○ GGCGTCGATATCT

○ TATCTCGACTCTAGGCC

○ GGCGTCTATATCTCG

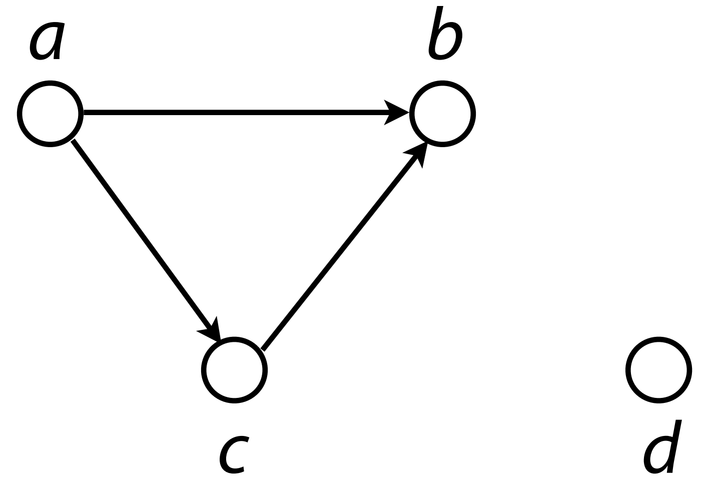
Directed graph review

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



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

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

Source   Sink

Vertex also called *node* or *point*

Edge also called *arc* or *line*

Directed graph also called *digraph*

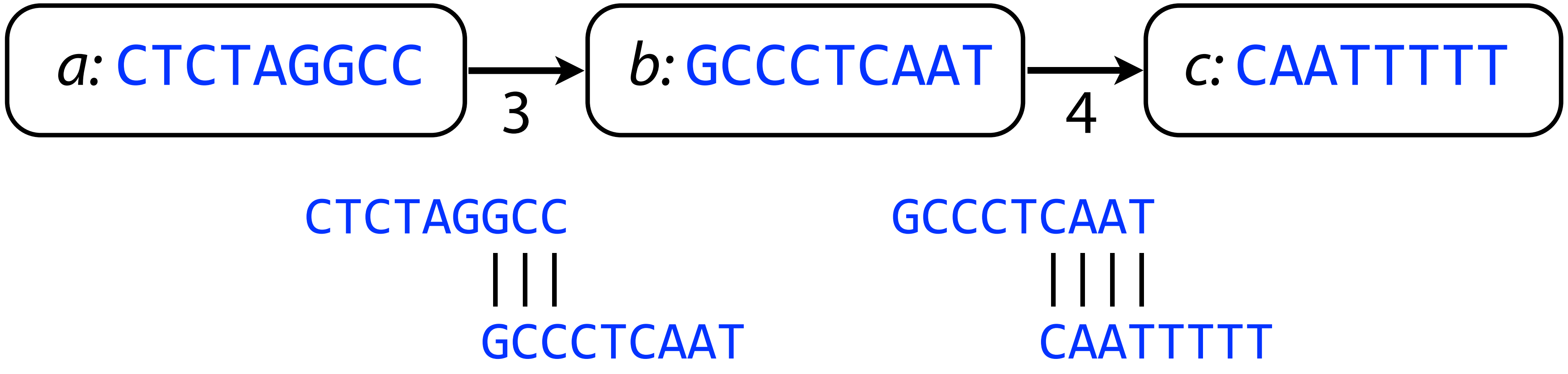
Overlap graph

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*) }

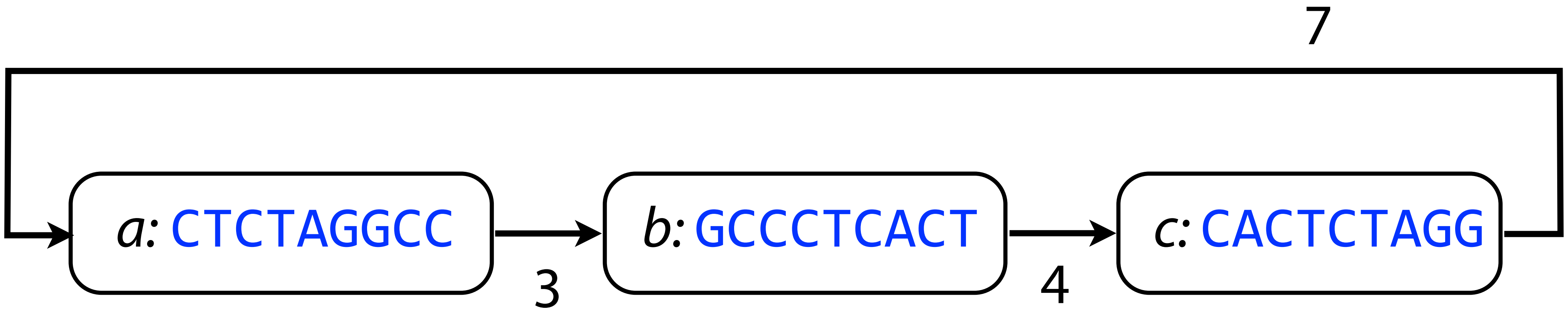


a: CTCTAGGCC → 3 → *b:* GCCCTCAAT → 4 → *c:* CAATTTTT

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

Overlap graph

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



7

a: CTCTAGGCC

3

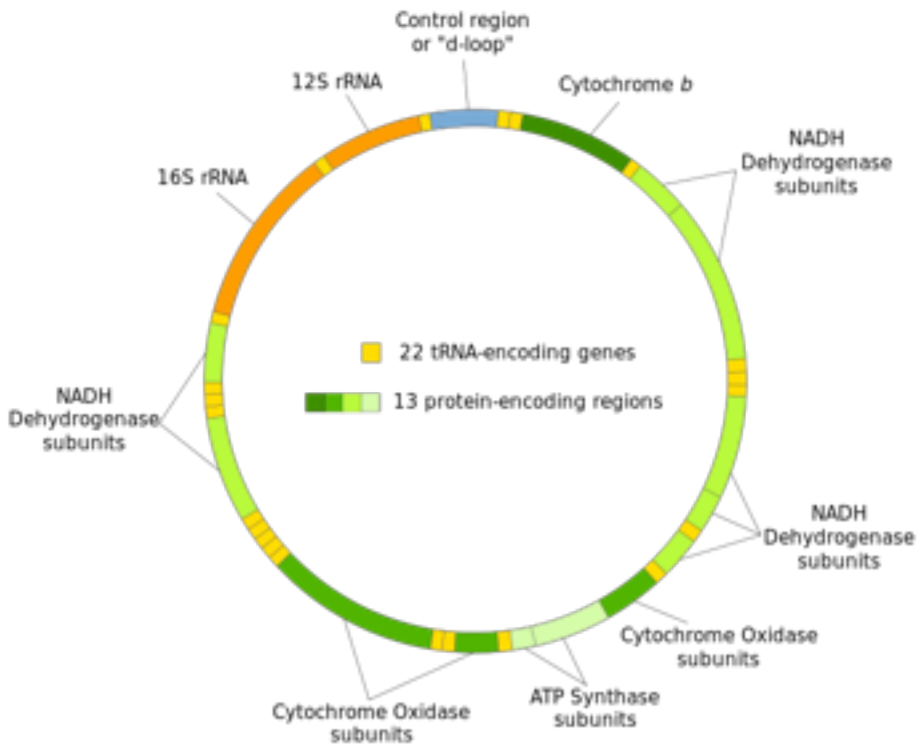
b: GCCCTCACT

4

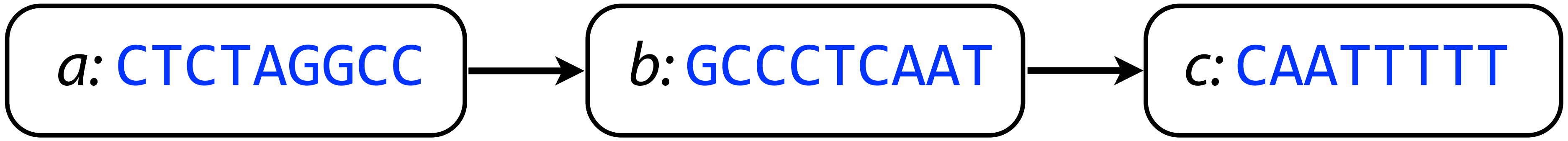
c: CACTCTAGG

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


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

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

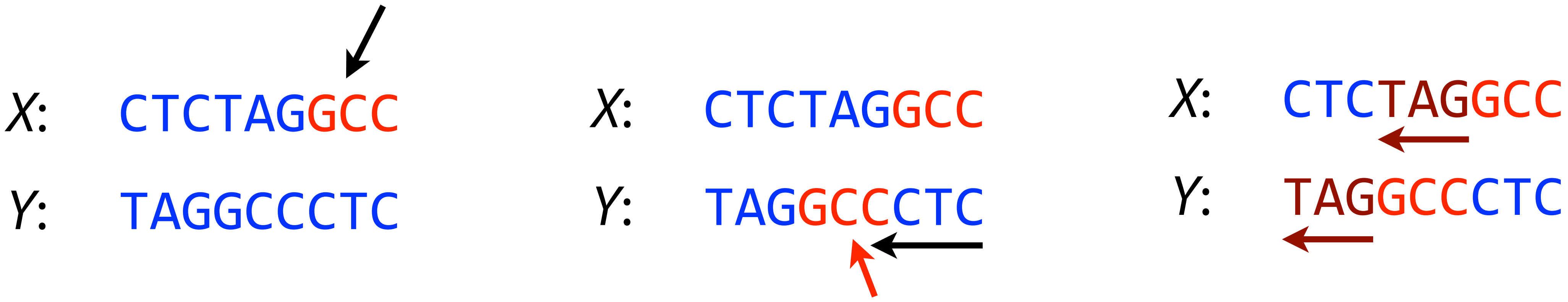
Overlap: length-l suffix of *X* matches length-l prefix of *Y*, where l is given

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*

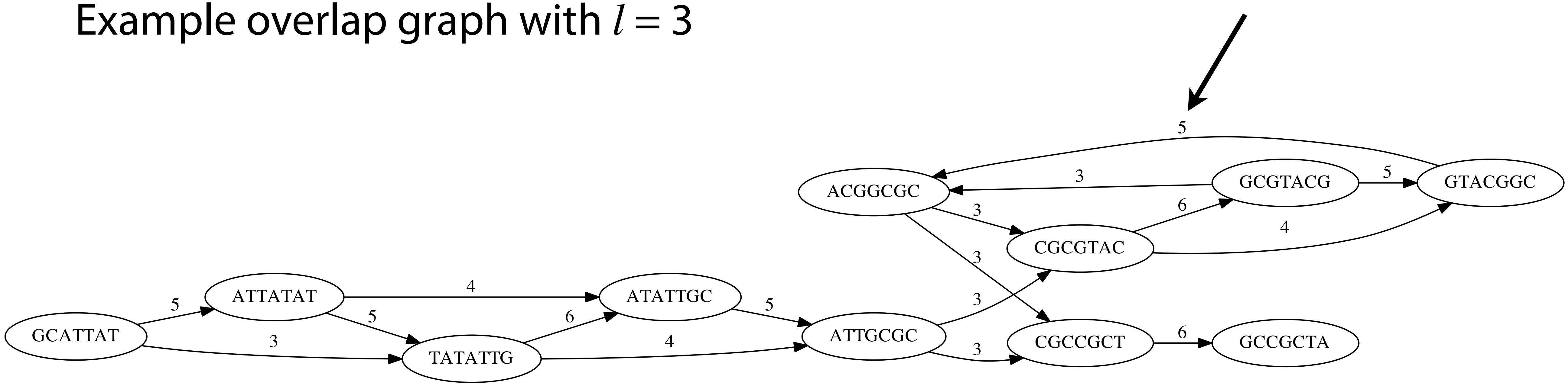


Found it

Finding overlaps

Example overlap graph with $l = 3$

Edge label is overlap length



Original string: GCATTATATATTGCGCGTACGGCGCCGCTACA

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

Can we solve it?

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

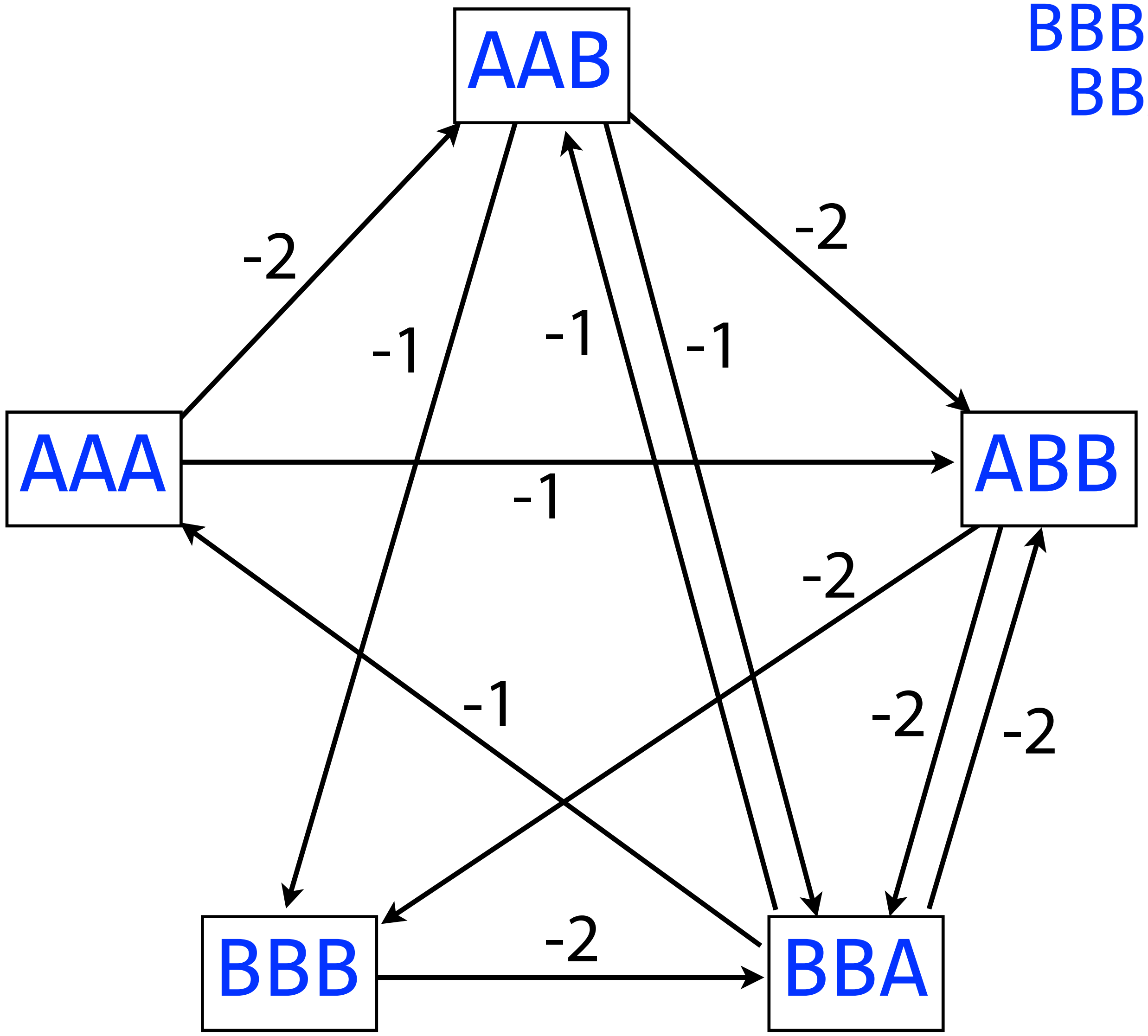
SCS corresponds to a path that visits every node once, minimizing total cost along path

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

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

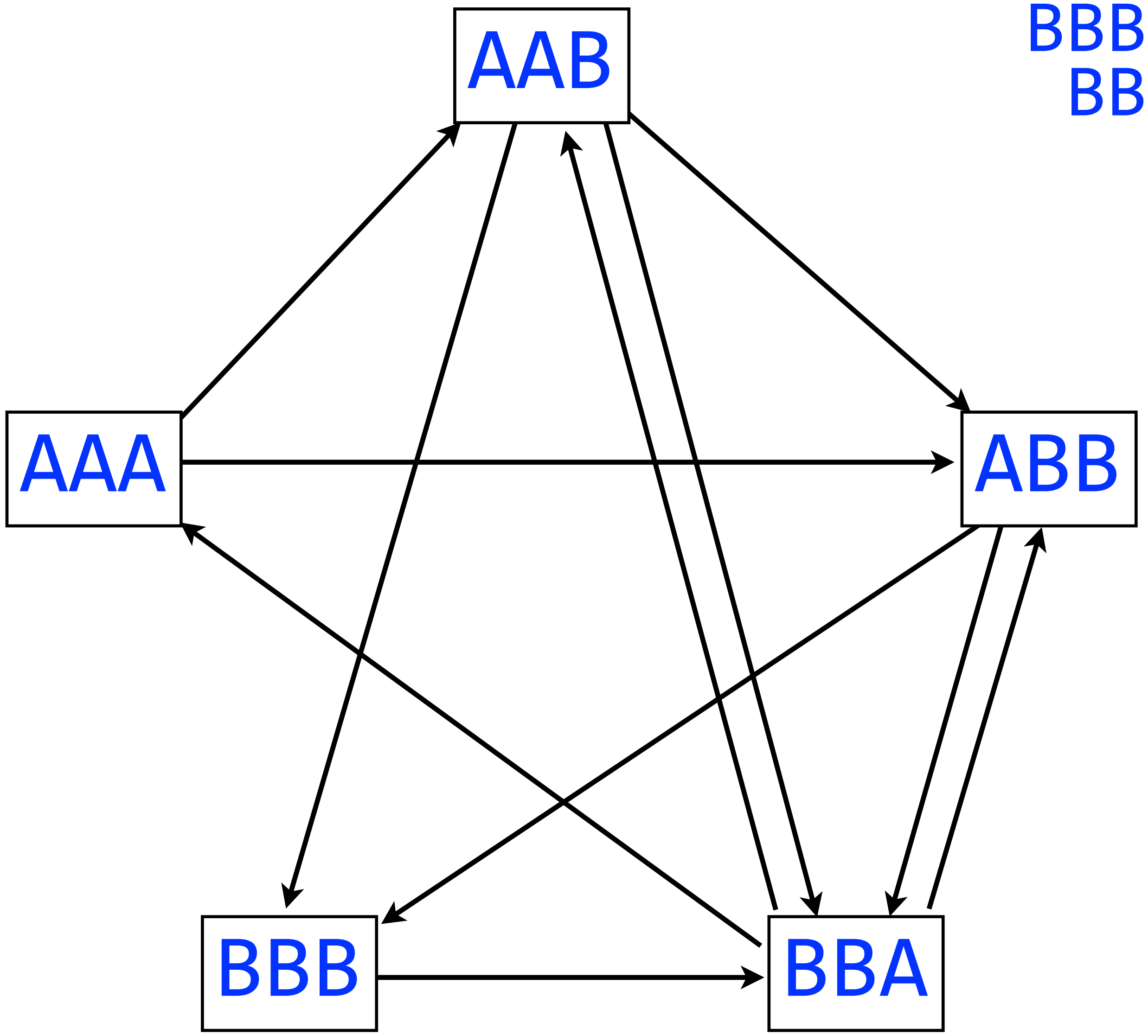
That's the *Hamiltonian Path* problem: NP-complete

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

S: AAA AAB ABB BBB BBA

SCS(S): AAABBBA

```
AAA
 AAB
  ABB
   BBB
    BBA
```



Shortest common superstring

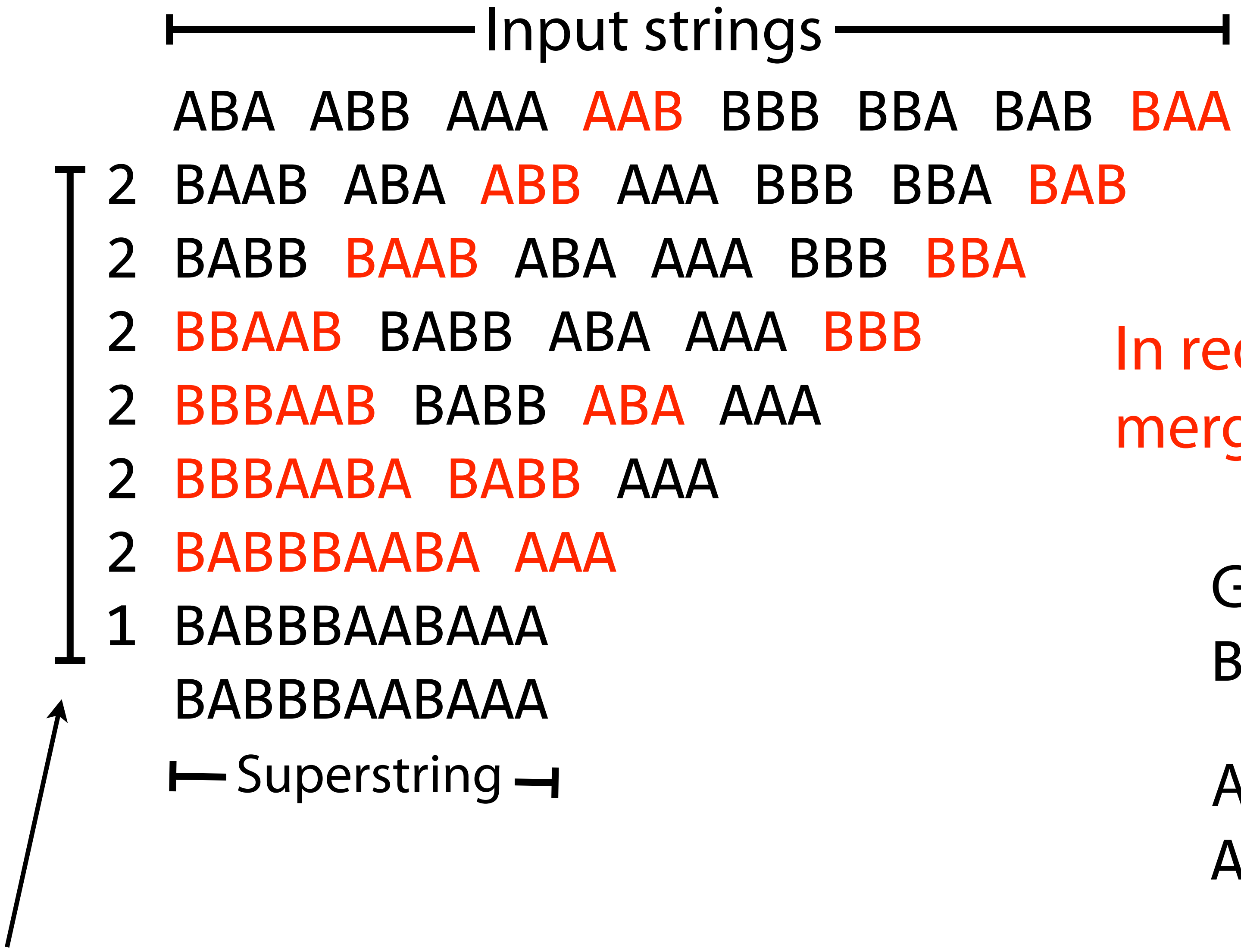
Let’s take the hint give up on finding the *shortest possible* superstring

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. $l = 3$.

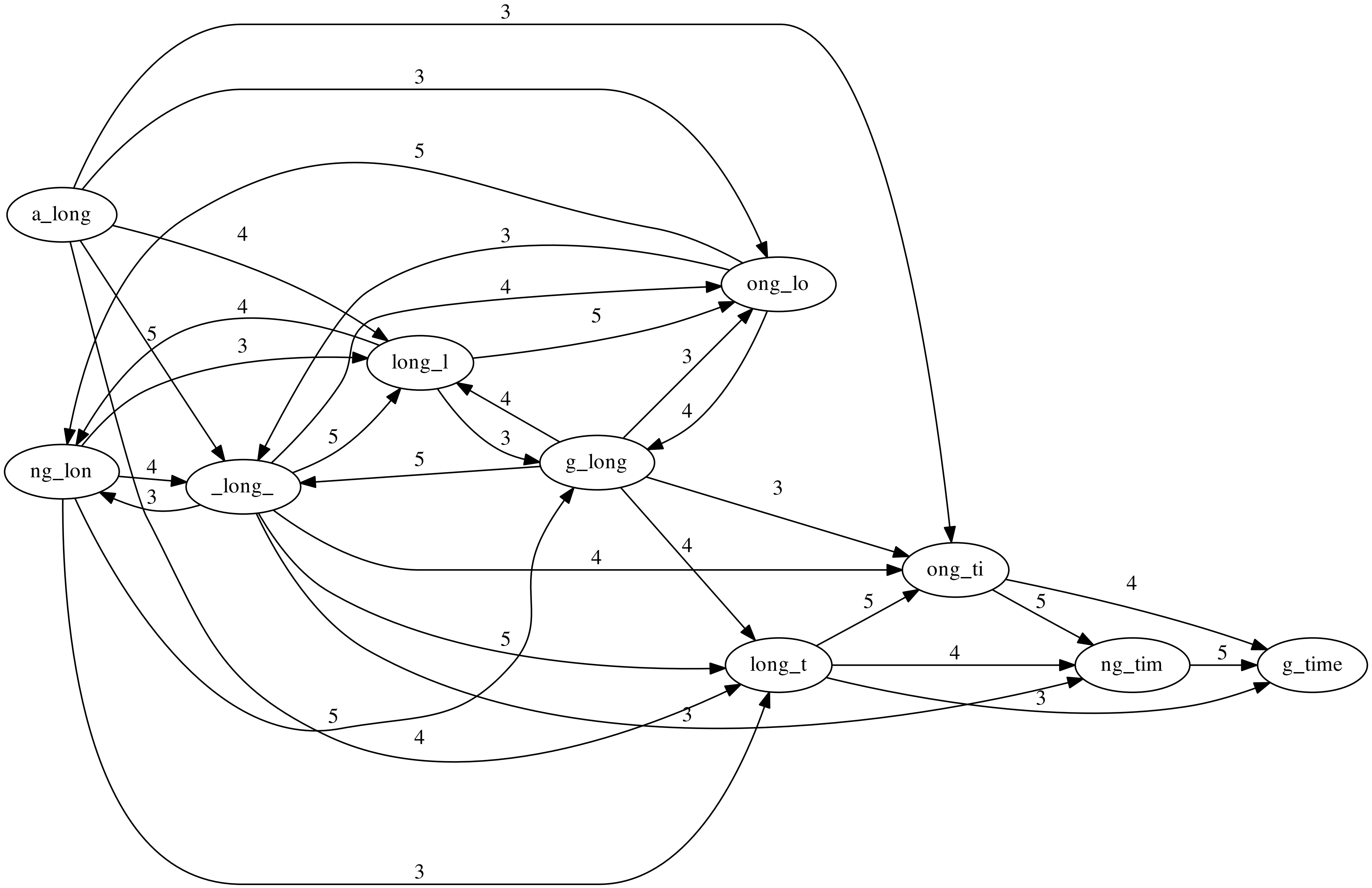
```
  ng_lon _long_ a_long long_l ong_ti ong_lo long_t g_long g_time ng_tim
5 ng_time ng_lon _long_ a_long long_l ong_ti ong_lo long_t g_long
5 ng_time g_long_ ng_lon a_long long_l ong_ti ong_lo long_t
5 ng_time long_ti g_long_ ng_lon a_long long_l ong_lo
5 ng_time ong_lon long_ti g_long_ a_long long_l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

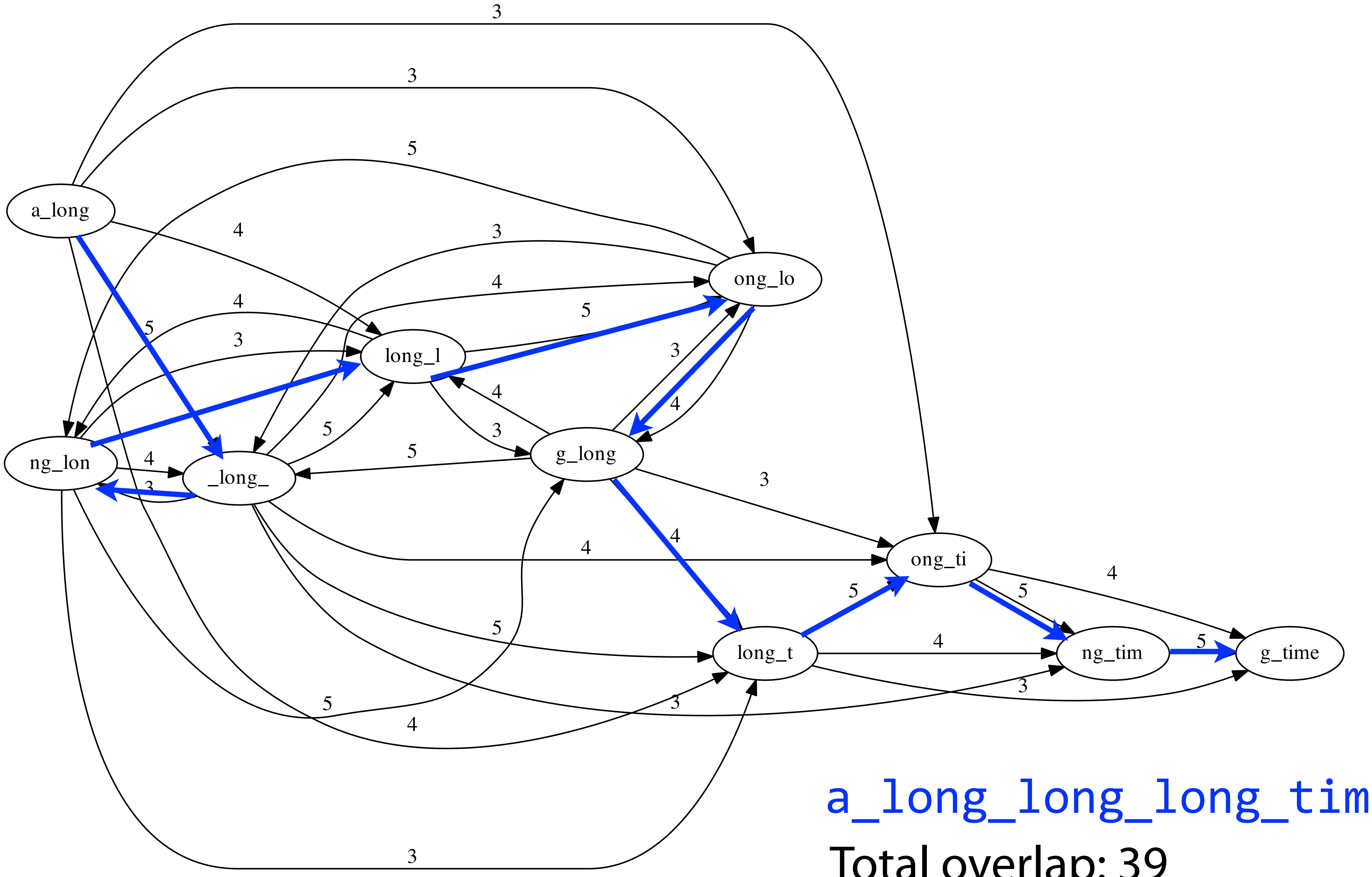
Shortest common superstring: greedy

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



Shortest common superstring: greedy

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

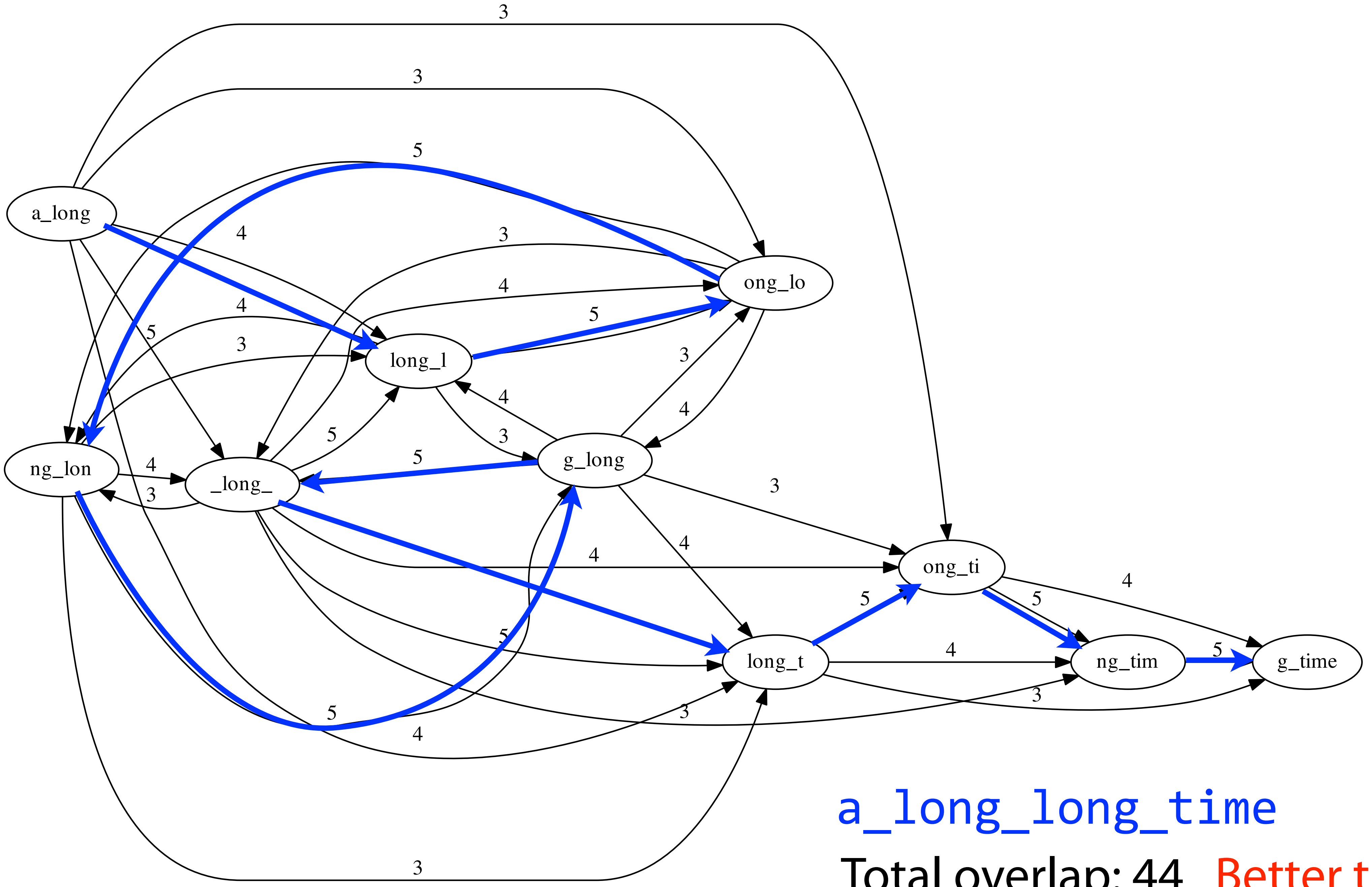


a_long_long_long_time

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
```

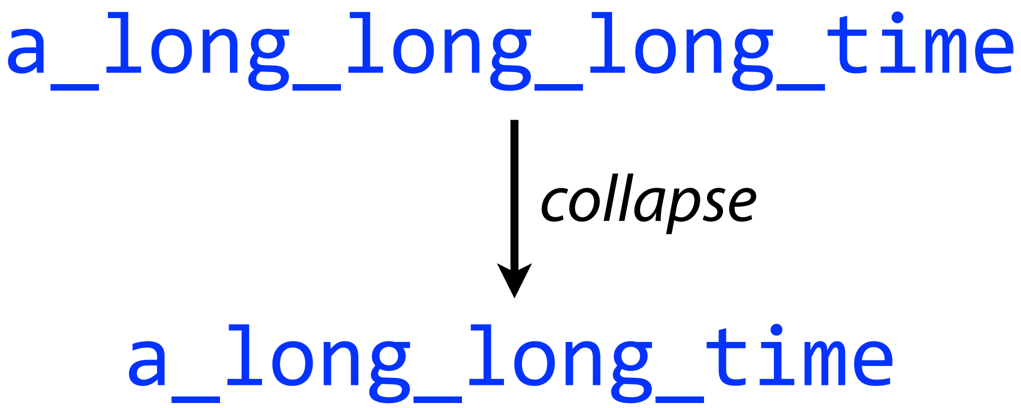
One length-8 substring spans all three `long`s

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



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

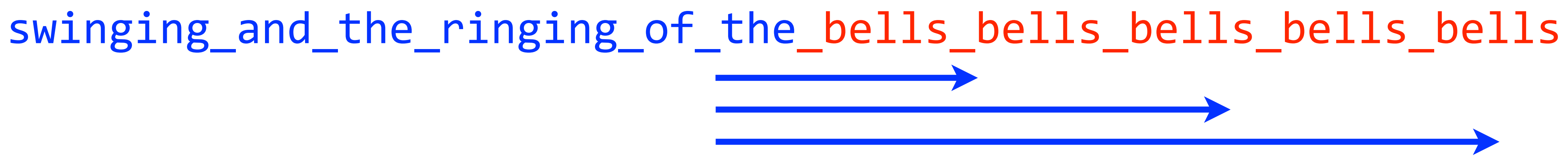
Extract every substring of length k, then run Greedy-SCS.
Do this for various l (min overlap length) and k.

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

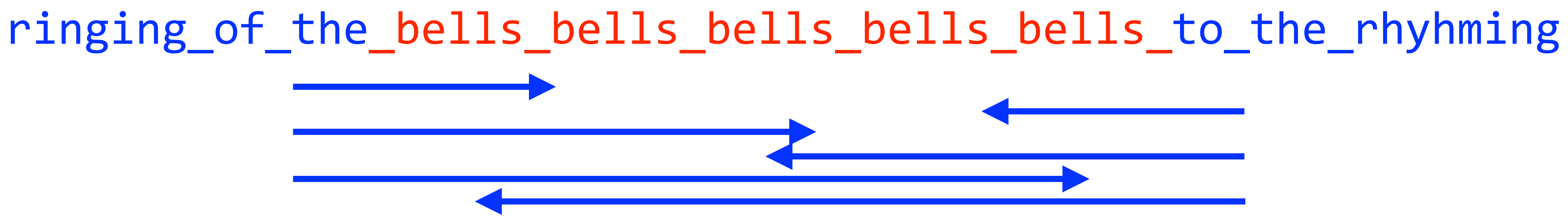
Basic principle: *repeats foil assembly*

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`

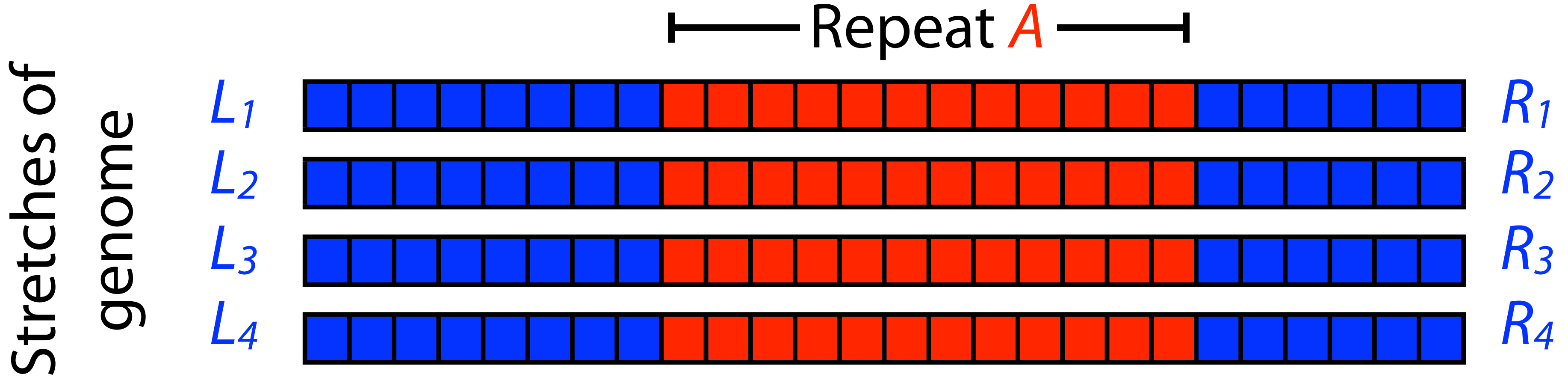
read length

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`

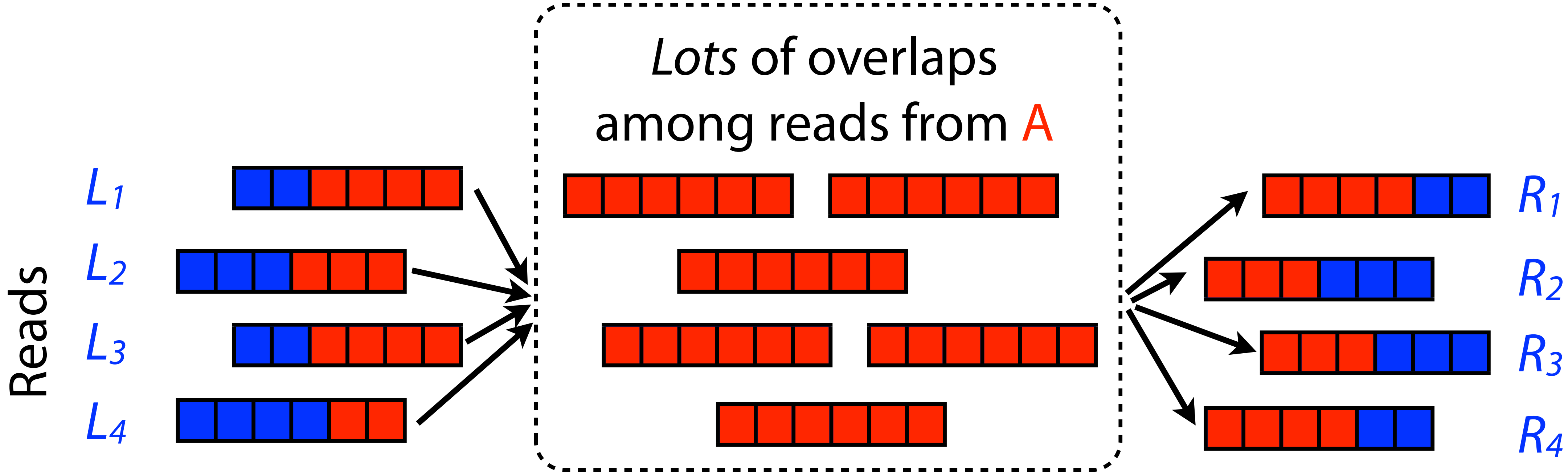
longer and longer substrings allow us to “reach” further into the repeat

Repeats

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



Assume *A* is longer than read length


Lots of overlaps among reads from A

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.

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

> Boža, Vladimír, Broňa Brejová, and Tomáš Vinař. "GAML: Genome Assembly by Maximum Likelihood." Algorithms in Bioinformatics. Springer Berlin Heidelberg, 2014. 122-134.
>
> Medvedev, Paul, and Michael Brudno. "Maximum likelihood genome assembly." Journal of computational Biology 16.8 (2009): 1101-1116.

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

Real-world assembly methods

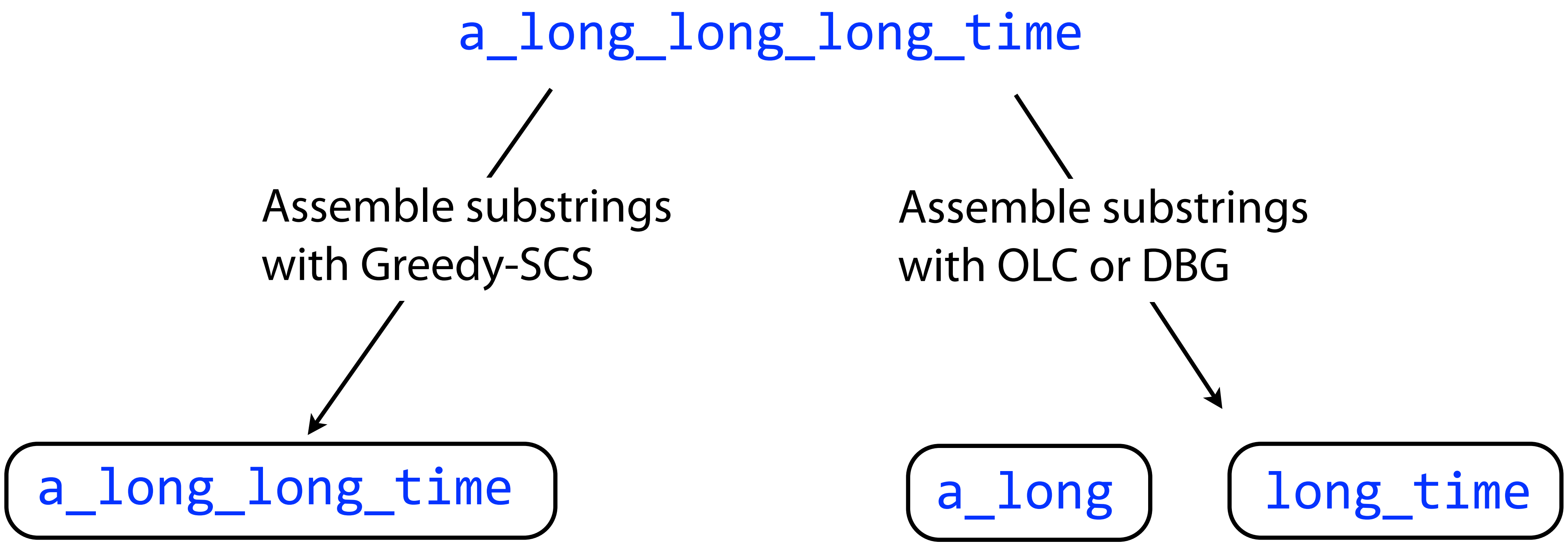
OLC: Overlap-Layout-Consensus assembly

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

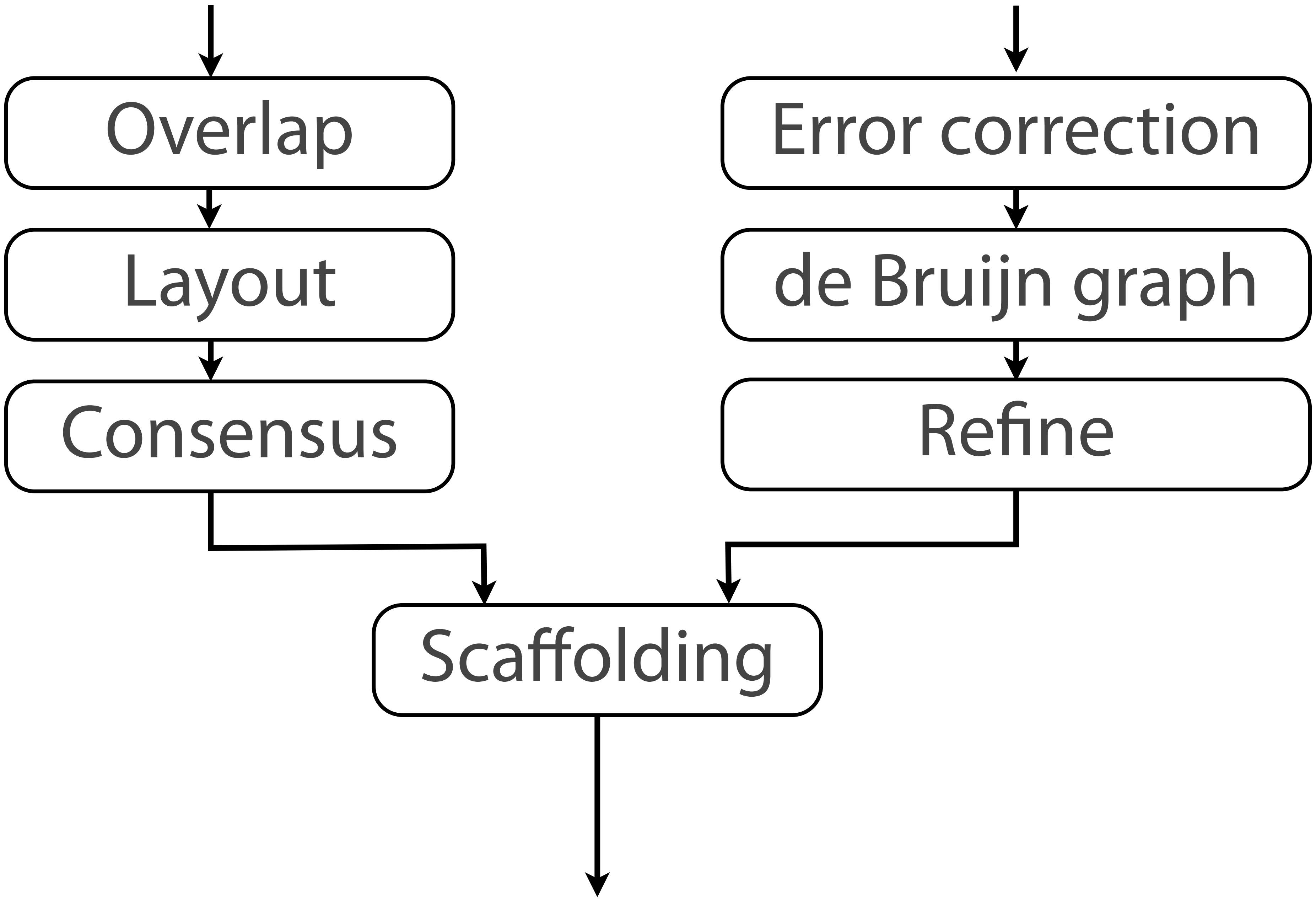
a_long

long_time

Assembly alternatives

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

Alternative 2: de Bruijn graph (DBG) assembly



Overlap

Layout

Consensus

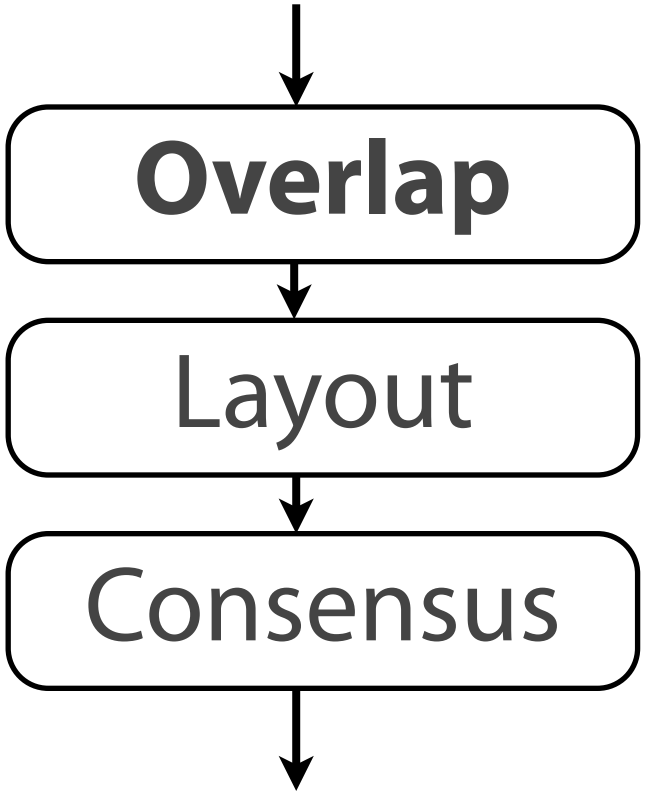
Error correction

de Bruijn graph

Refine

Scaffolding

Overlap Layout Consensus



Overlap

Layout

Consensus

Build overlap graph

Bundle stretches of the overlap graph into *contigs*

Pick most likely nucleotide sequence for each contig

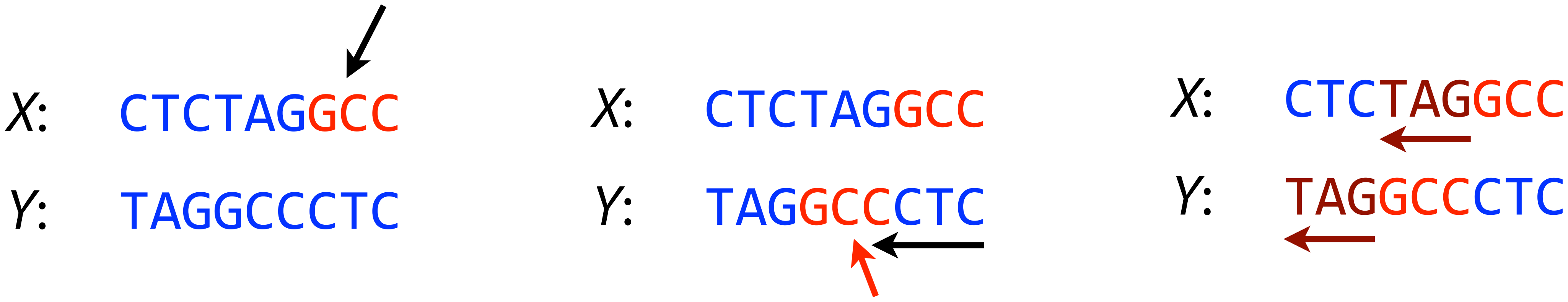
Finding overlaps

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?

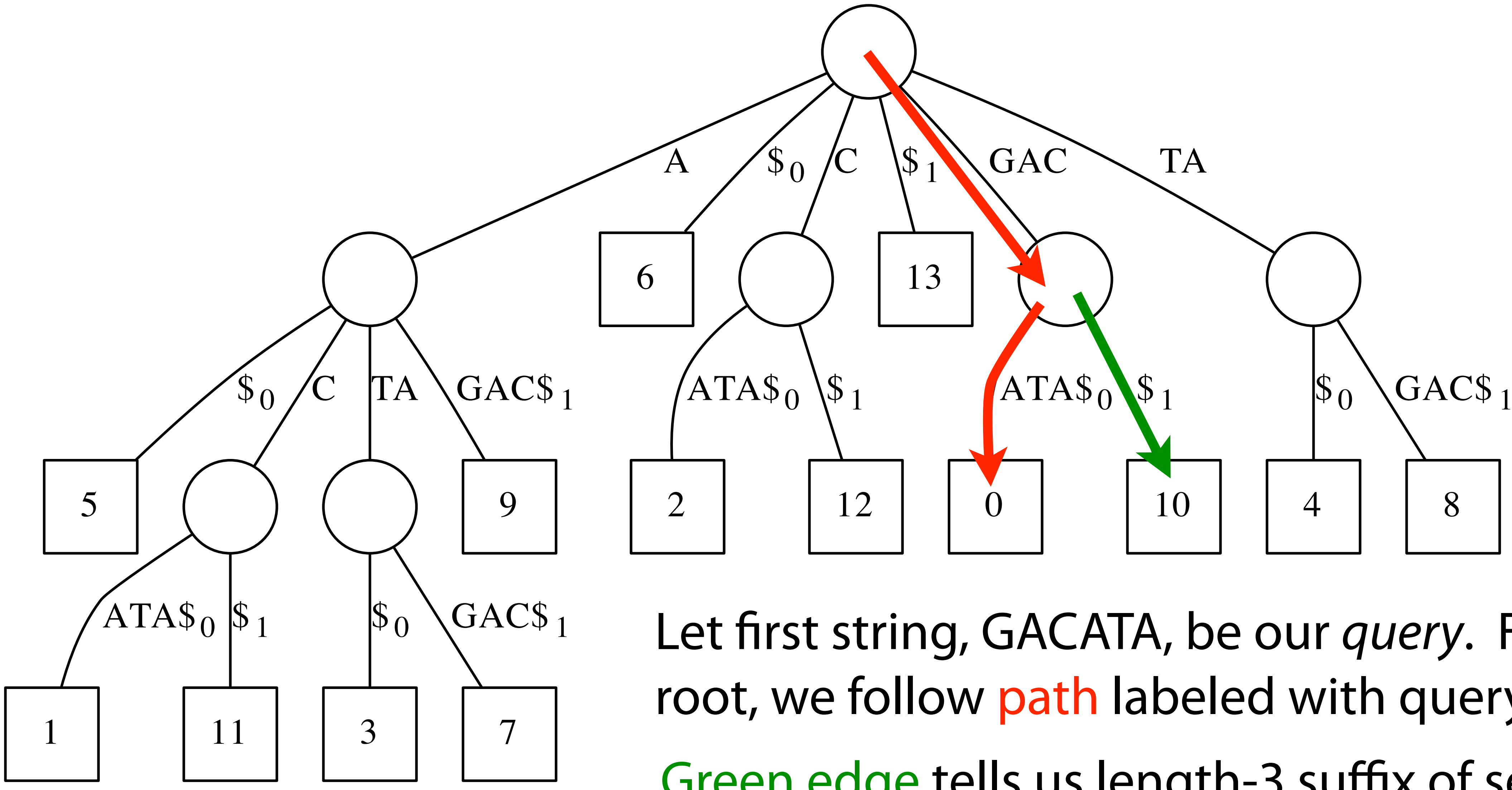
Problem: Given a collection of strings *S*, for each string *x* in *S* find all overlaps involving a prefix of *x* and a suffix of another string *y*

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$_1$



Let first string, GACATA, be our *query*. From root, we follow path labeled with query.

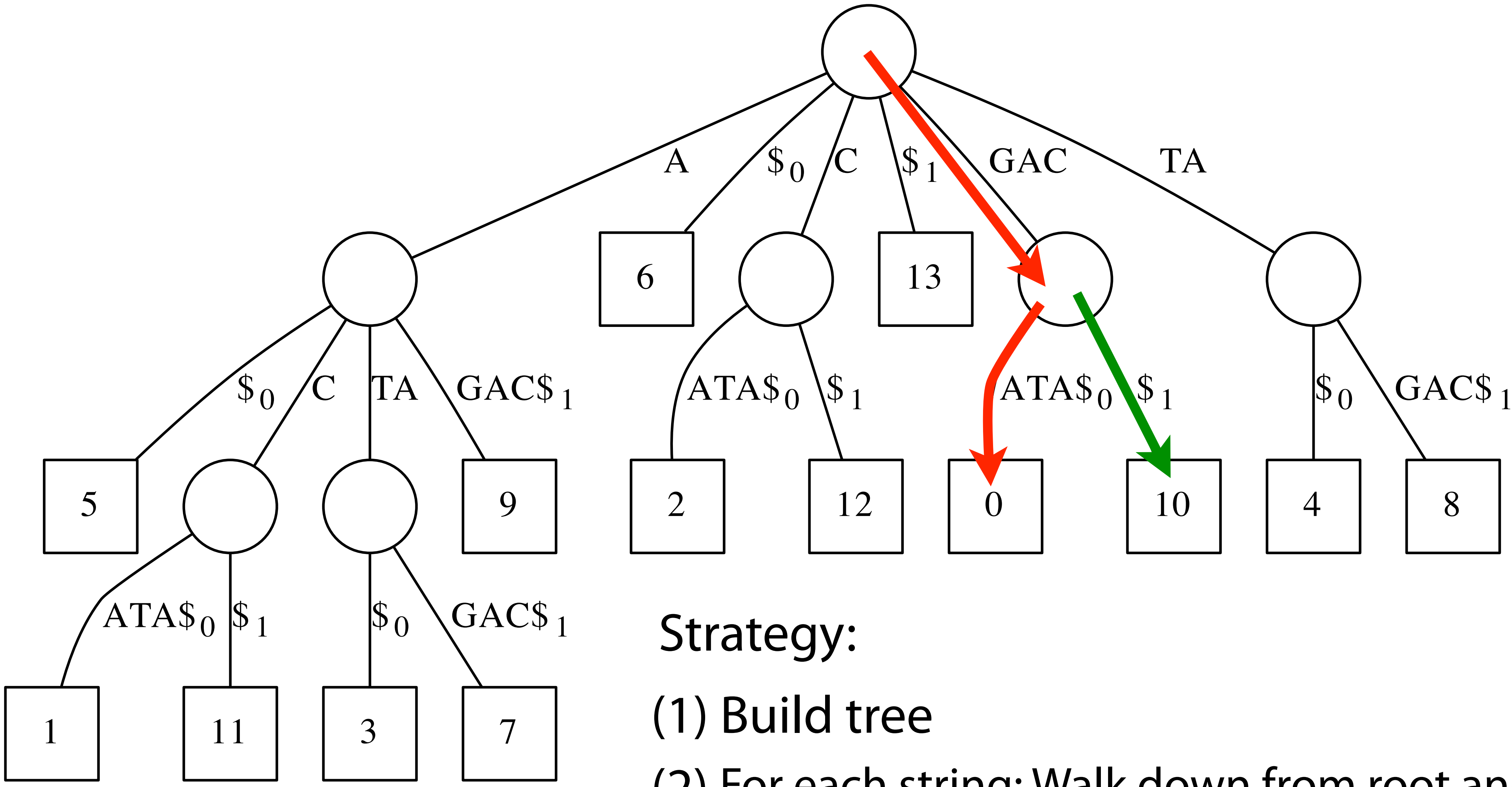
Green edge tells us length-3 suffix of second string equals length-3 prefix of query

```
ATAGAC
   |||
   GACATA
```

Finding overlaps with suffix tree

Generalized suffix tree for { "GACATA", "ATAGAC" }

GACATA$\$_0$ATAGAC$\$_1$



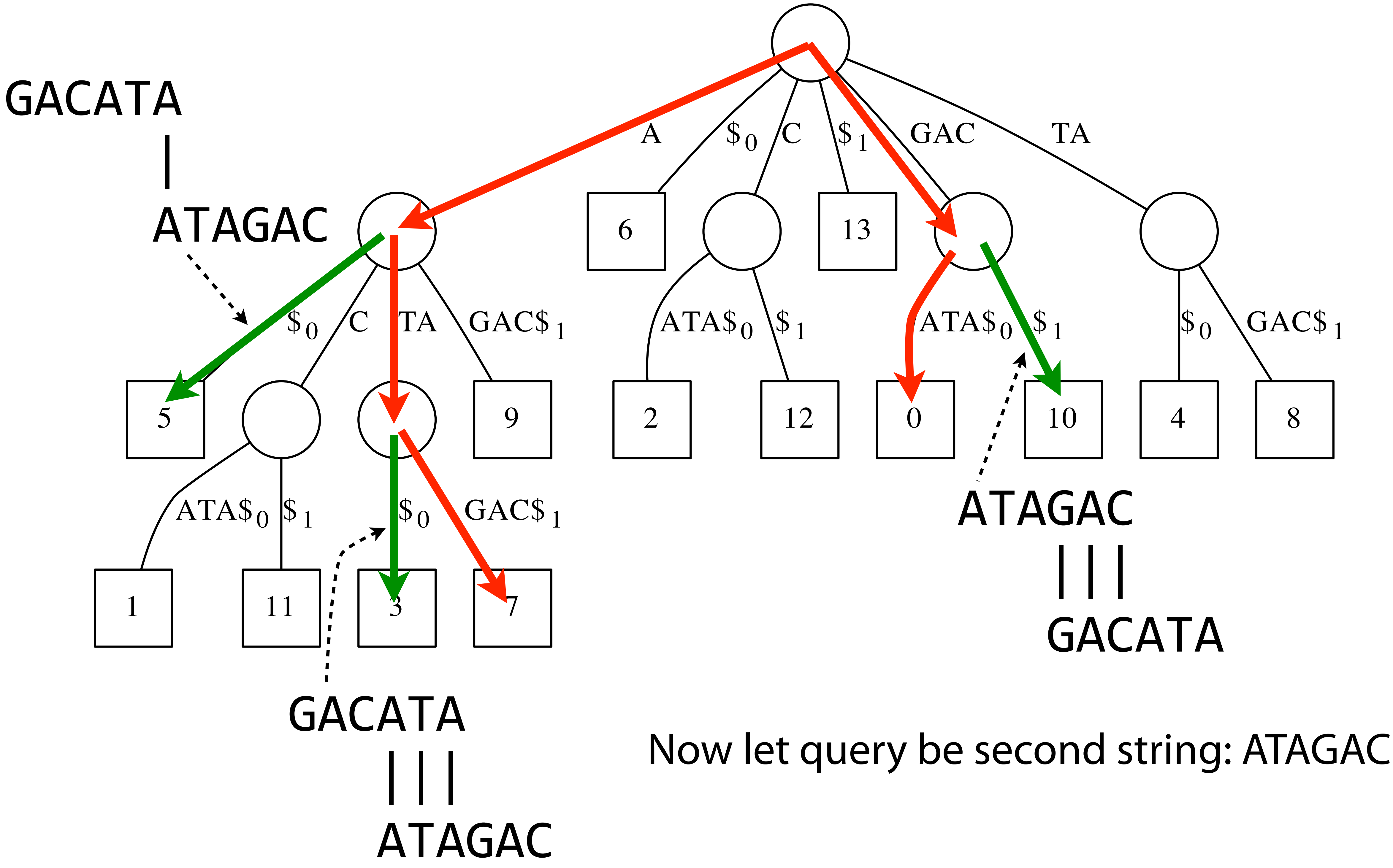
Strategy:

(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$_1$

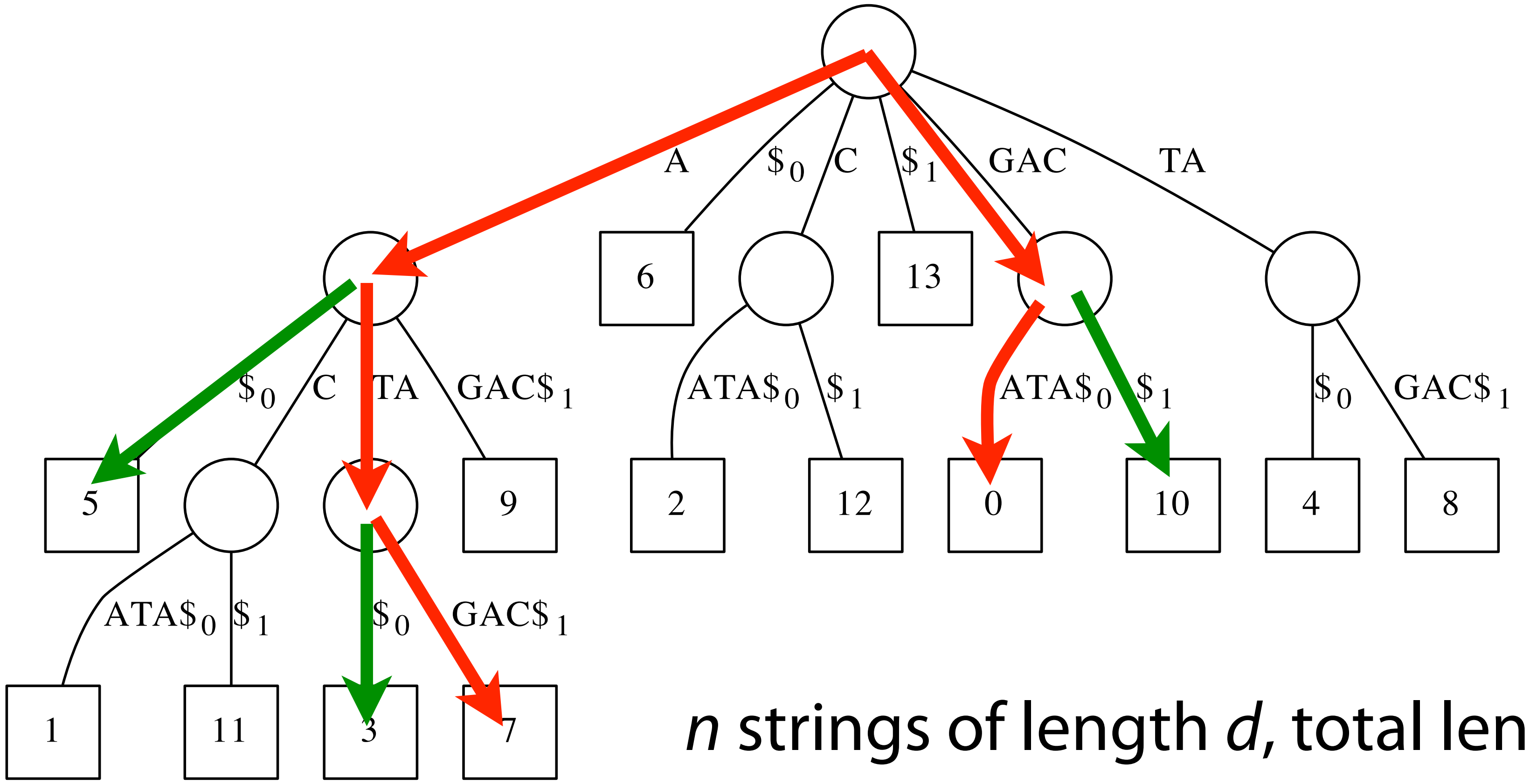


Now let query be second string: ATAGAC

Finding overlaps with suffix tree

Generalized suffix tree for { "GACATA", "ATAGAC" }

GACATA$\$_0$ATAGAC$\$_1$



n strings of length *d*, total length $N = nd$, and
a = # of string pairs that overlap

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

Bounds don't include n^2,
but *a* is O(n^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
```

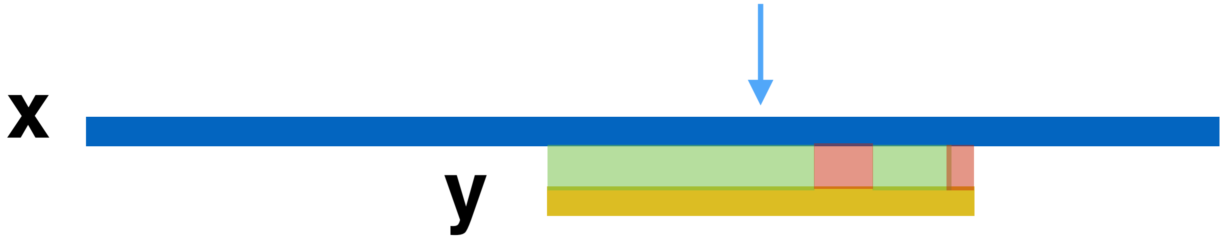
Dynamic programming

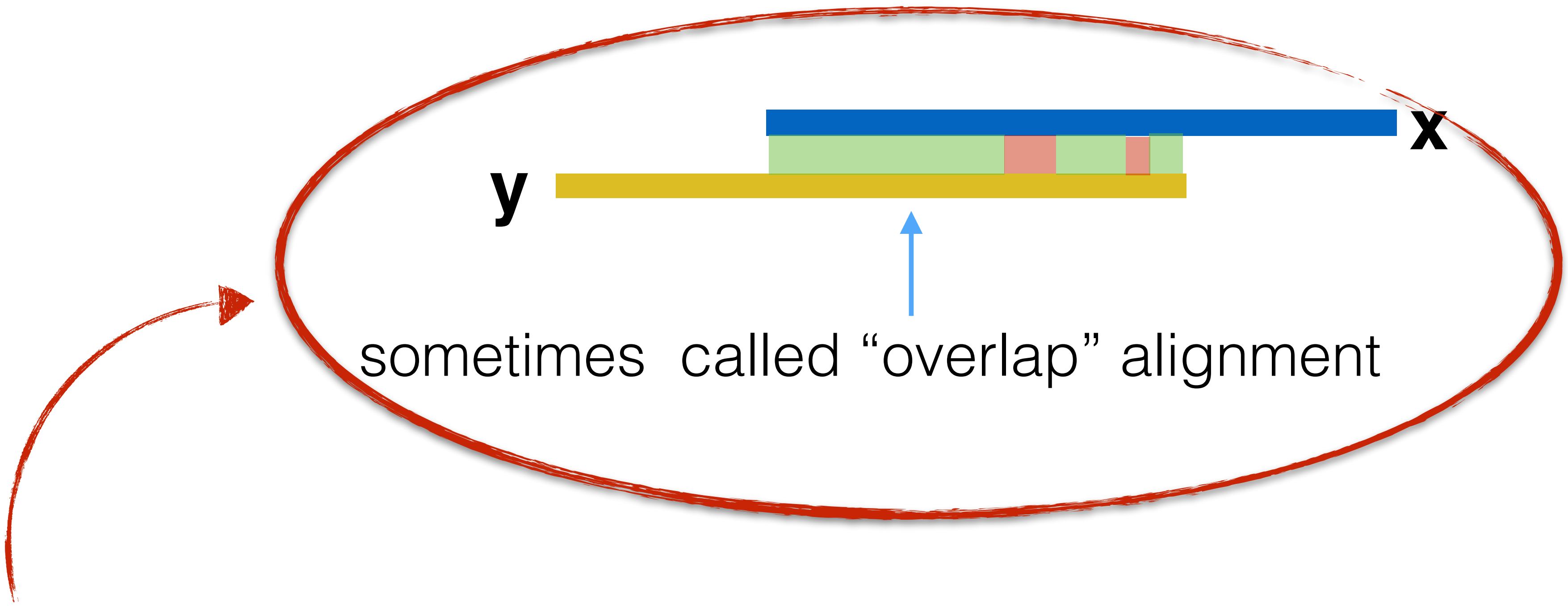
But we must frame the problem such that only backtraces involving a suffix of *X* and a prefix of *Y* are allowed

Recall: Semi-global Alignment

Semi-global (glocal): Gaps at the beginning or end of **x** or **y** are free. Useful when one one string is significantly shorter than the other or we want to find an overlap between the suffix of one string and a prefix of the other

sometimes called "cost-free-ends" or "fitting" alignment





sometimes called "overlap" alignment

This variant is useful for our purposes here

Finding overlaps with dynamic programming

Say there are *n* strings of length *d*, total length *N* = *nd,* and *a* is total number of pairs with an overlap

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

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

But dynamic programming is more flexible, allowing mismatches and gaps

In practice, overlappers are between the two, using indexes to filter away non-overlapping pairs, then dynamic programming for the remainder

Finding overlaps

Overlapping is typically the slowest part of assembly

Consider a second-generation sequencing dataset with hundreds of millions or billions of reads!

Approaches from alignment unit can be adapted to finding overlaps

We saw adaptations of naive exact matching, suffix-tree-assisted exact matching, and dynamic programming

Could also have adapted efficient exact matching, approximate string matching, co-traversal, ...

Finding overlaps

Celera Assembler's overlapper is probably the best documented:

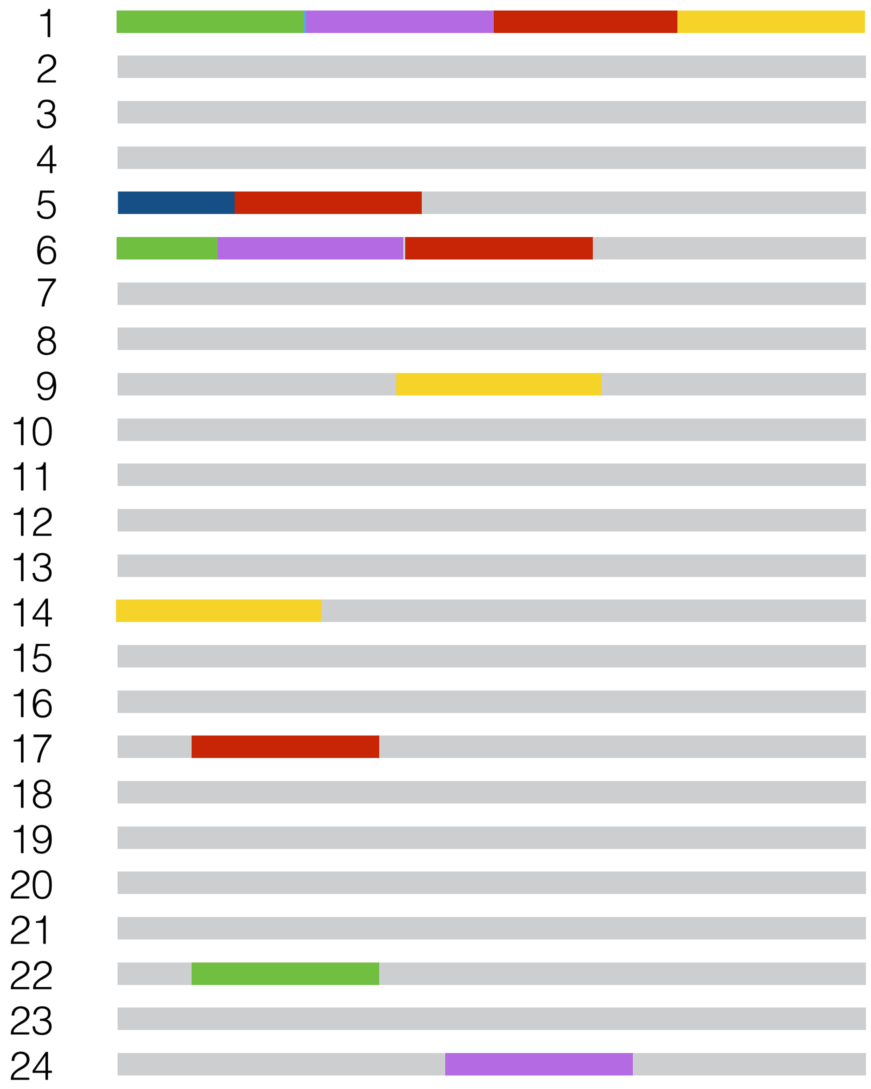
- Inverted substring indexes built on batches of reads
- Only look for overlaps between reads that share one or more substrings of some length

http://sourceforge.net/apps/mediawiki/wgs-assembler/index.php?title=RunCA#Overlapper

Inverted substring index is a "k-mer" lookup table.
It maps every short fixed-length substring to the set of reads where it occurs.

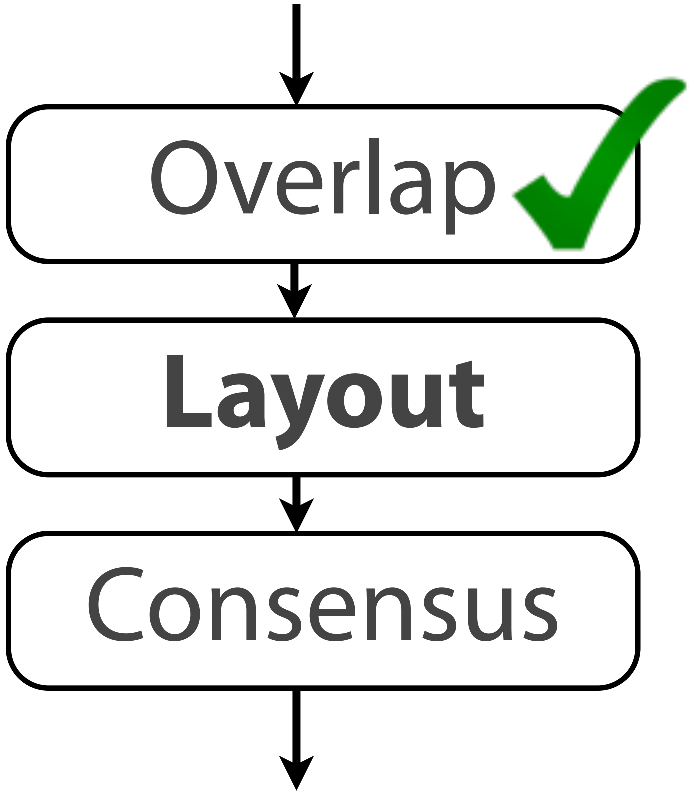
Utility of an inverted index

🟥	1, 5, 6, 17
🟪	1, 6, 24
🟩	1, 6, 22
⋮	



Only reads sharing at least 1 indexed substring can possibly have an exact overlap. Checking only these pairs *greatly* reduces the burden of detecting overlaps. However, overlapping can still be one of the slowest steps in an assembly.

Overlap Layout Consensus



Overlap ✓ — Build overlap graph

Layout — **Bundle stretches of the overlap graph into *contigs***

Consensus — Pick most likely nucleotide sequence for each contig

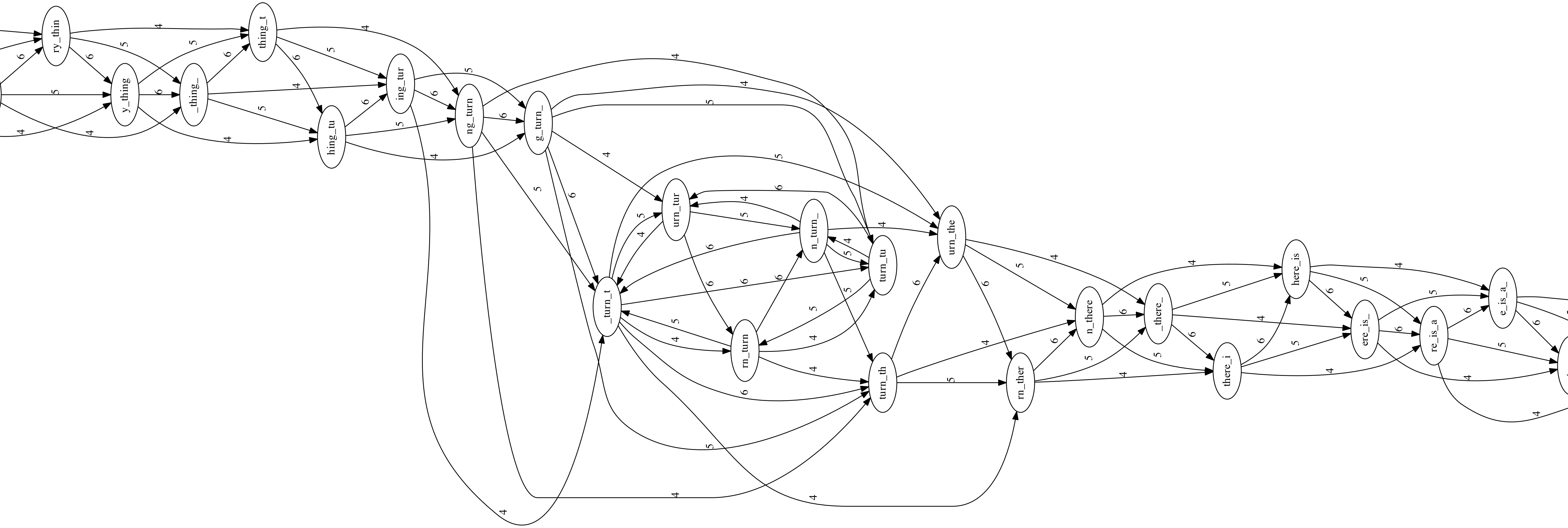
Layout

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

Below: part of the overlap graph for

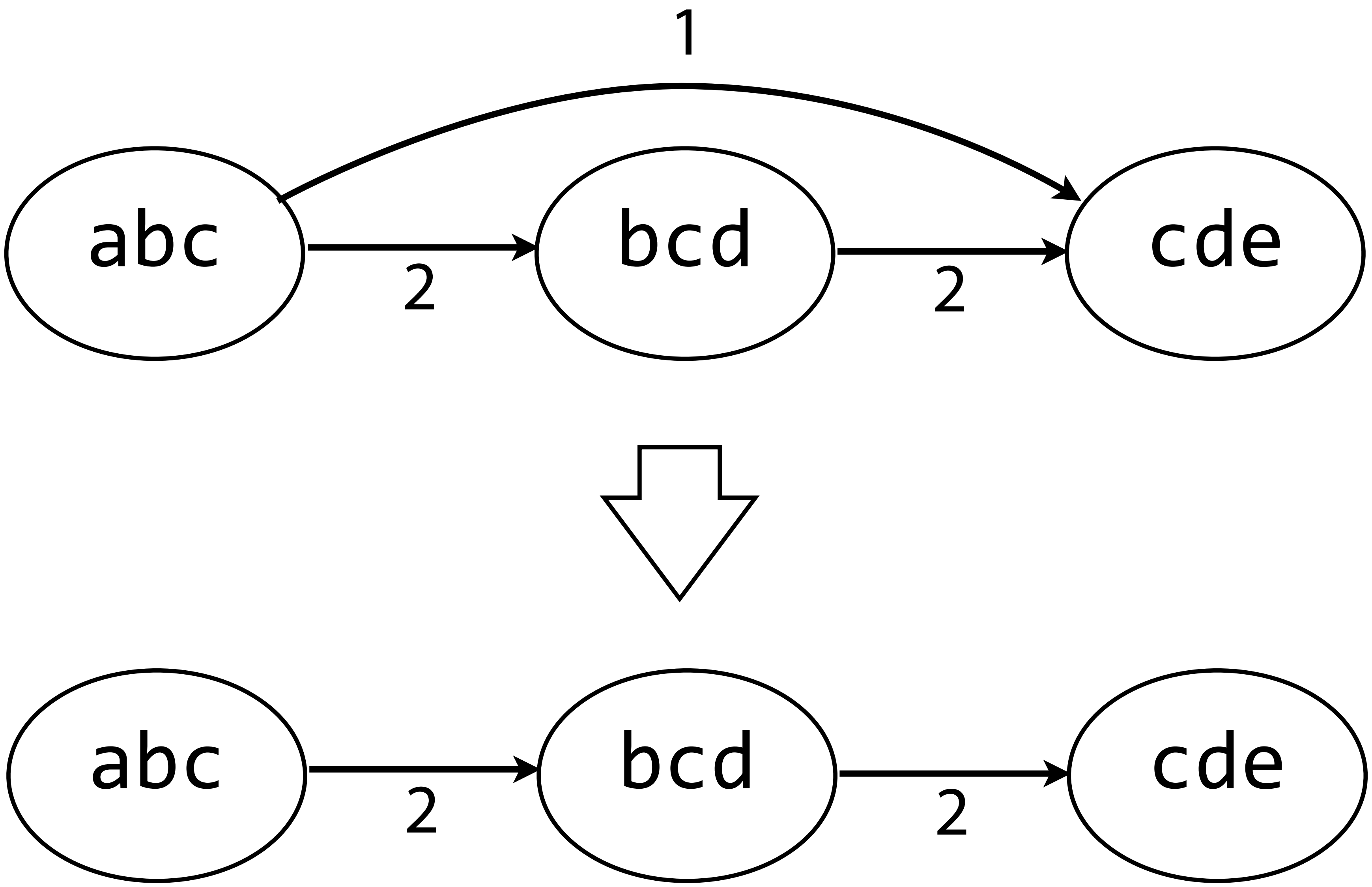
`to_every_thing_turn_turn_turn_there_is_a_season`

$l = 4, k = 7$



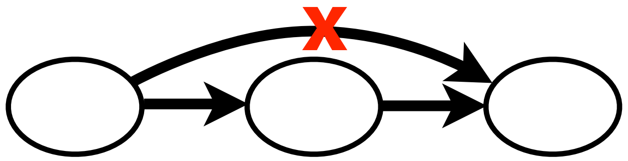
Layout

Picture gets clearer after removing some transitively-inferrible edges

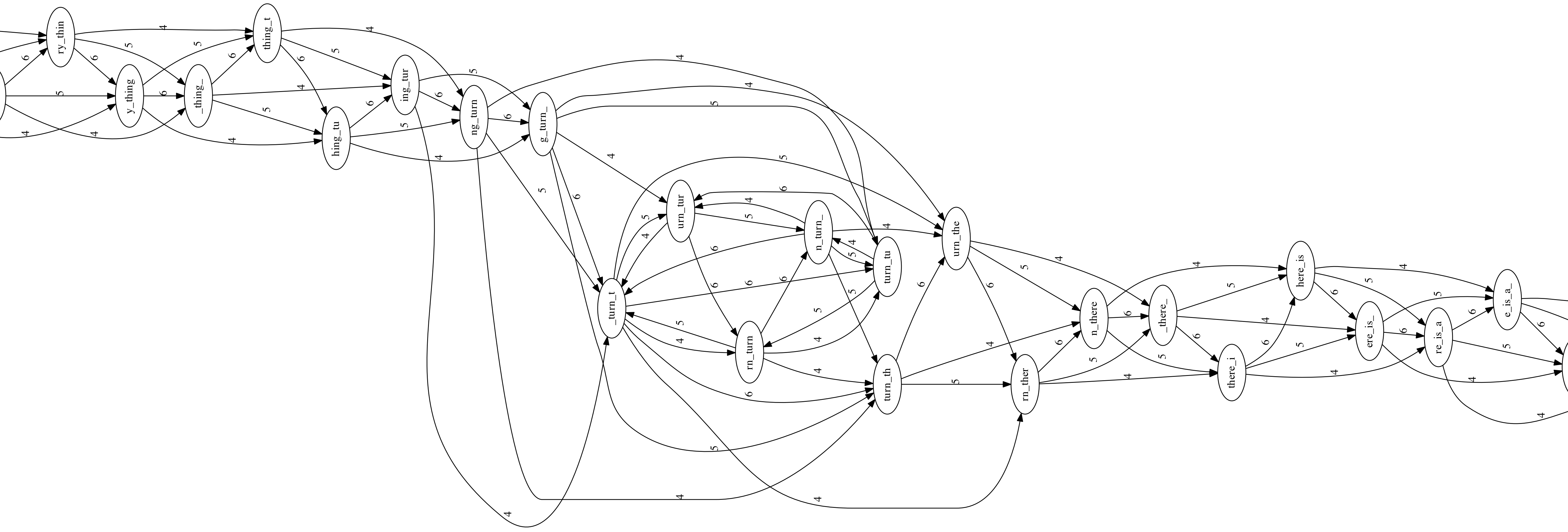


Layout

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

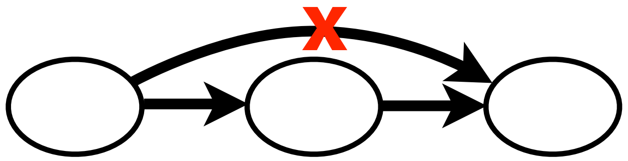


Before:

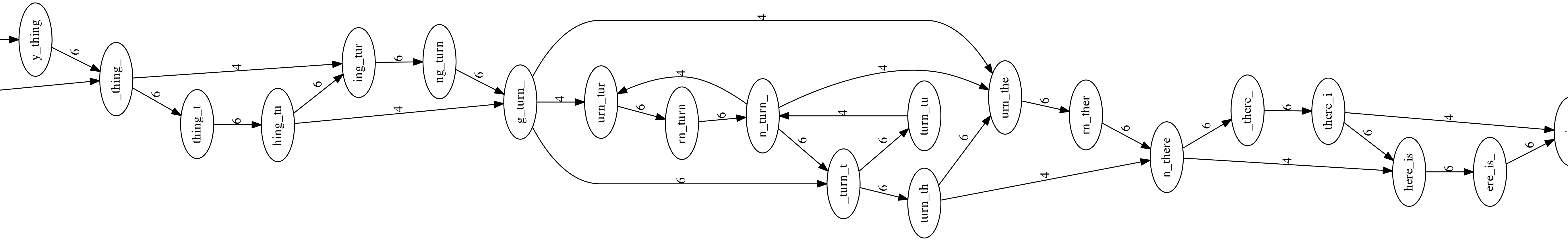


Layout

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



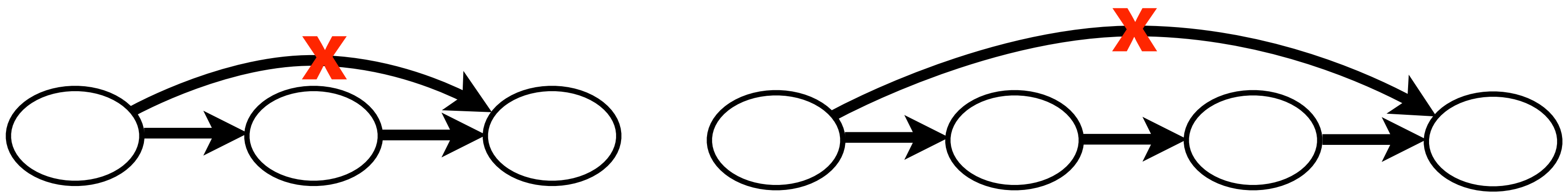
After:



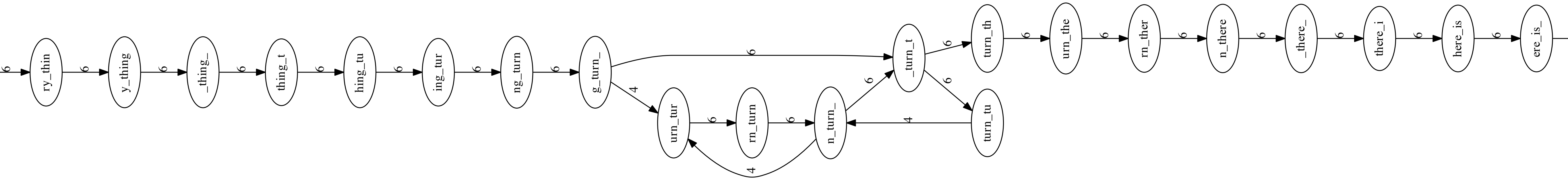
These edges are between reads whose overlaps completely encompass the center node.

Layout

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



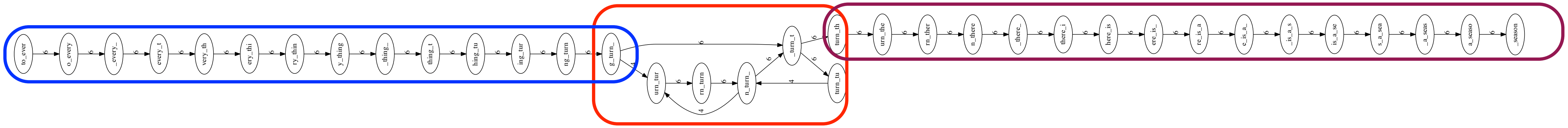
After:



Even simpler

Layout

Emit *contigs* corresponding to the non-branching stretches



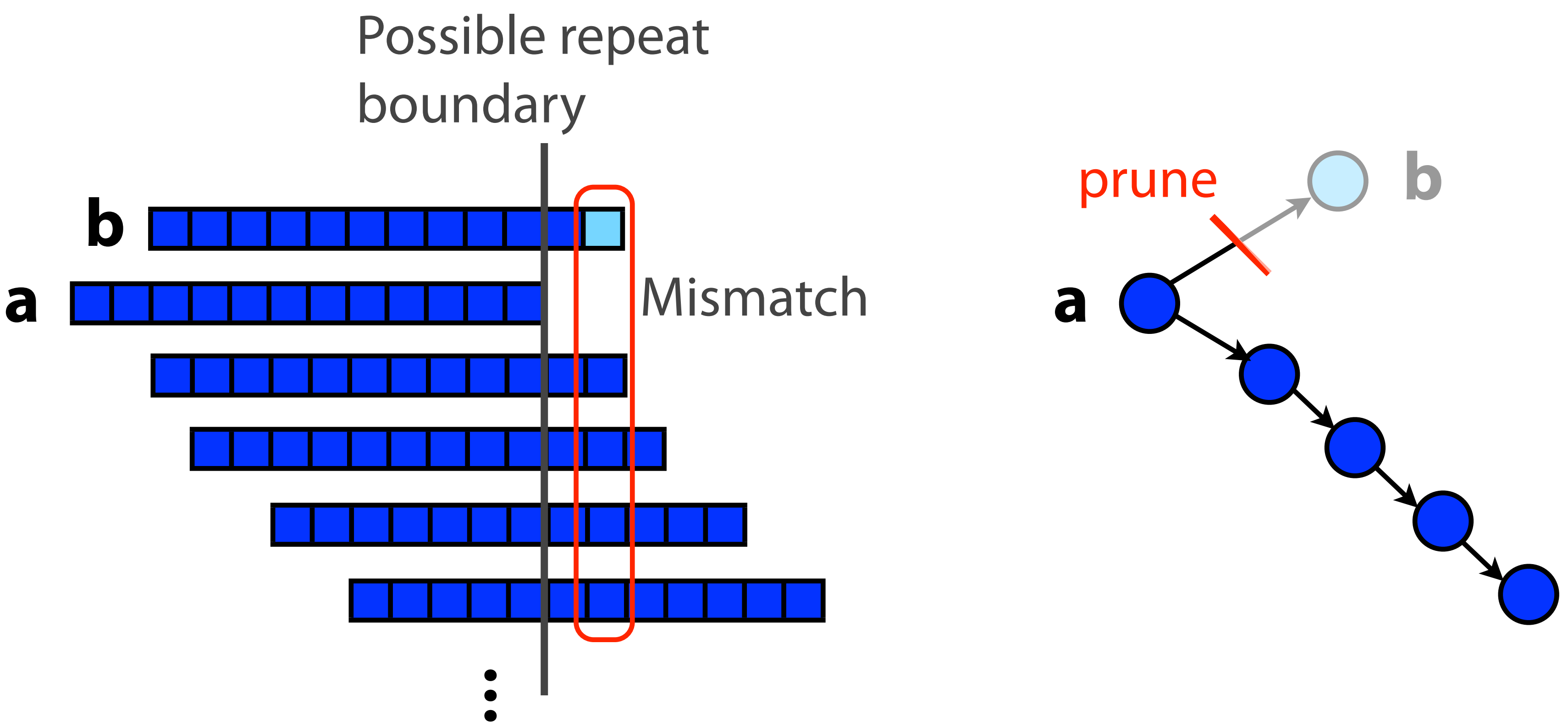
Contig 1
to_every_thing_turn_

Contig 2
turn_there_is_a_season

Unresolvable repeat

Layout

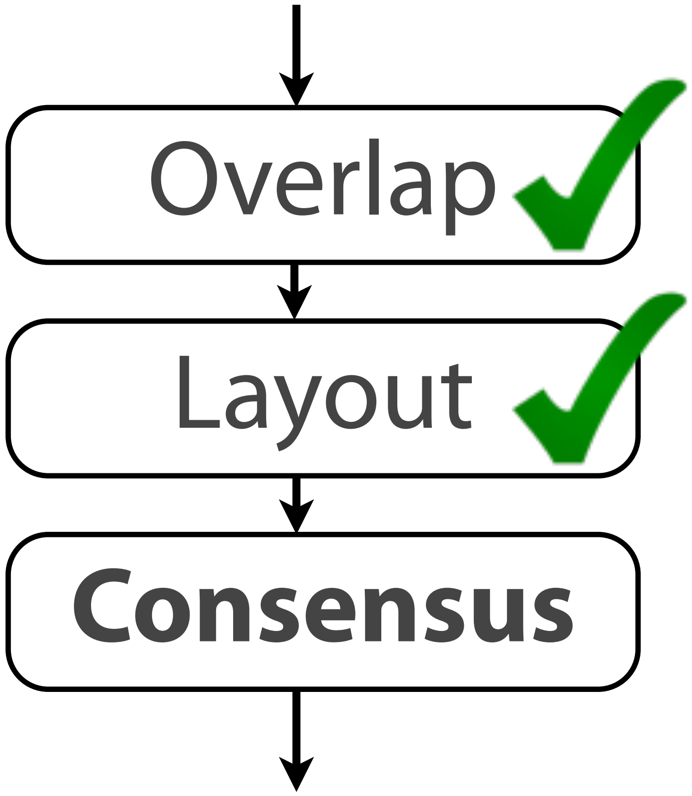
In practice, layout step also has to deal with spurious subgraphs, e.g. because of sequencing error



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**.

Modern assemblers are full of such "heuristics" — wisdom gained from running them on a lot of data.

Overlap Layout Consensus

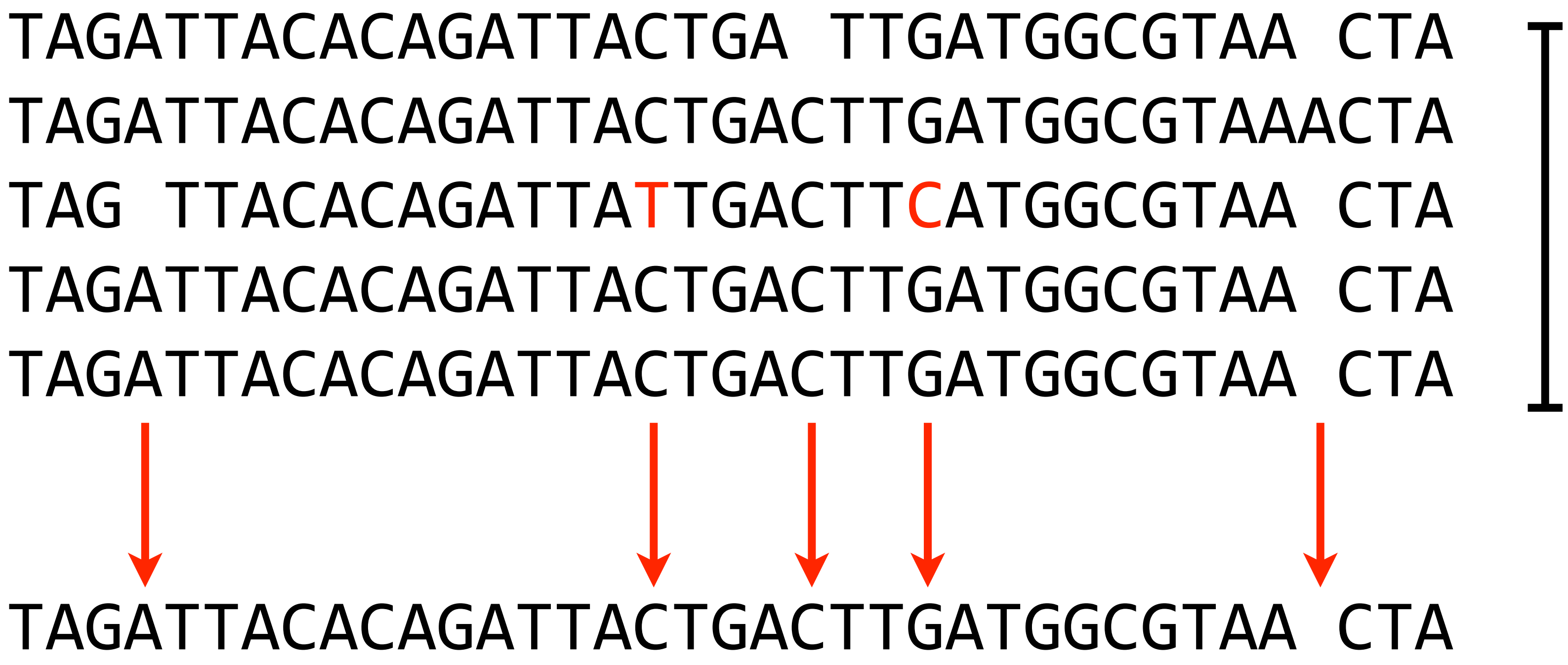


Overlap ✓ Build overlap graph

Layout ✓ Bundle stretches of the overlap graph into *contigs*

Consensus **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

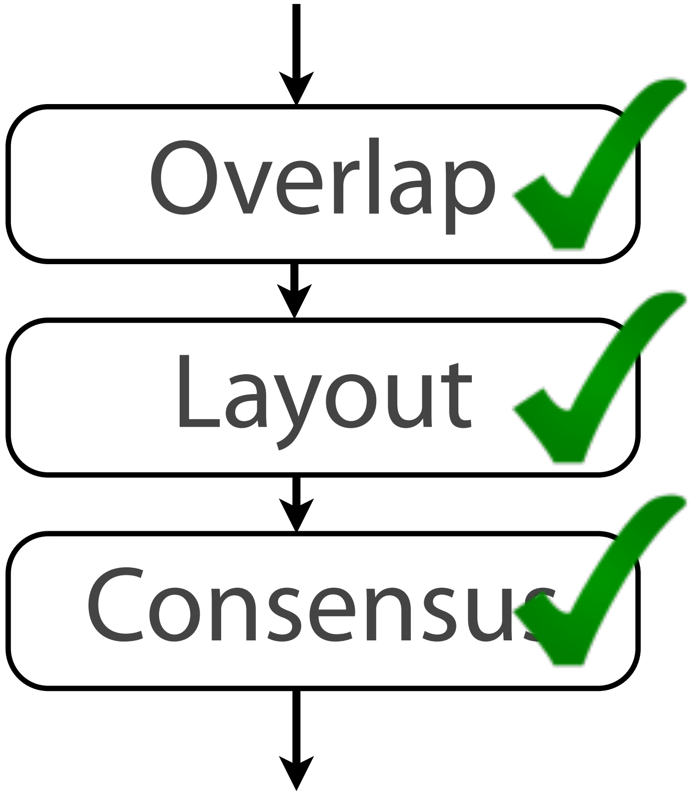
Take *consensus*, i.e. majority vote

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



Overlap ✓

Layout ✓

Consensus ✓

Build overlap graph

Bundle stretches of the overlap graph into *contigs*

Pick most likely nucleotide sequence for each contig

What’s the main drawback of OLC?

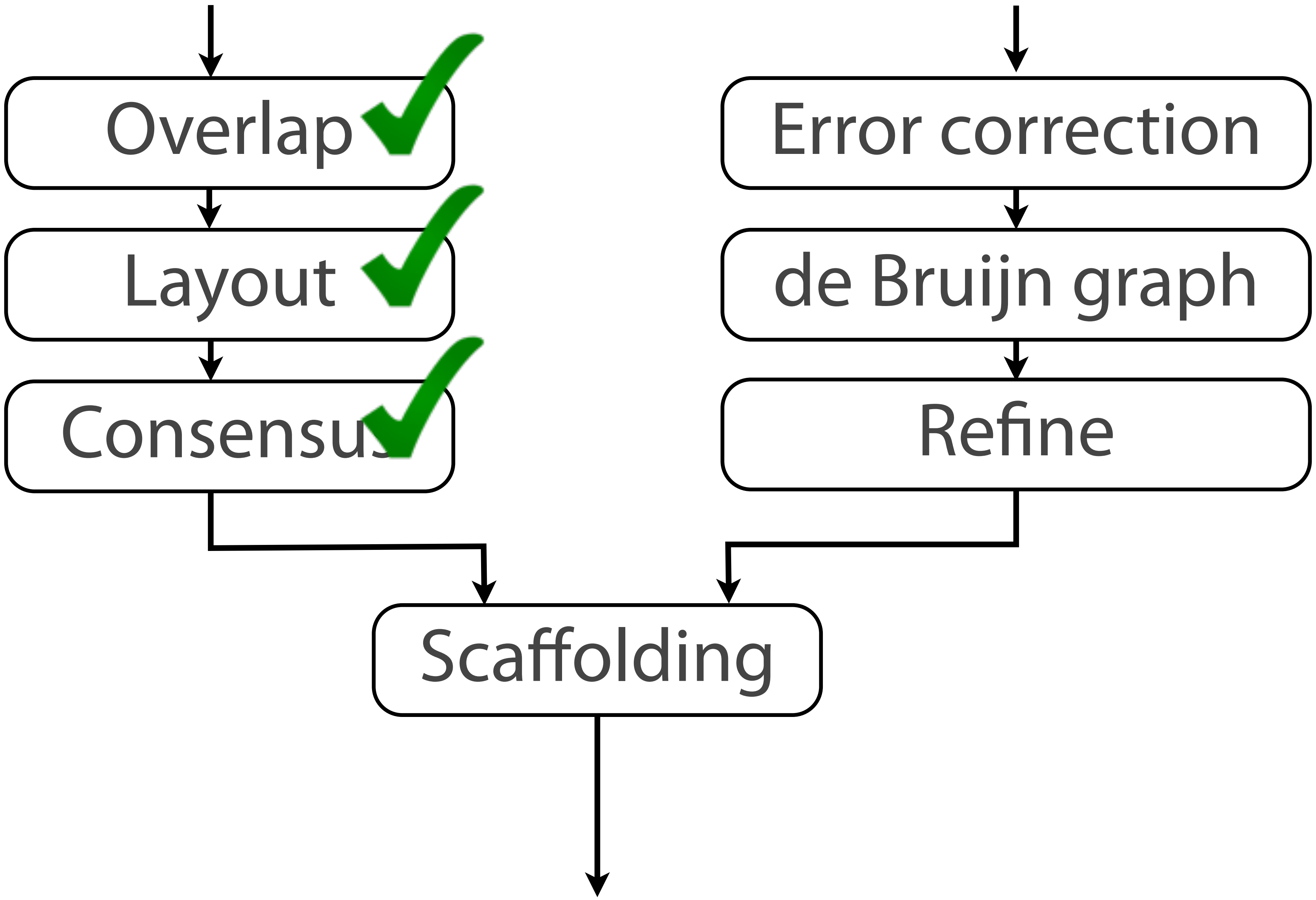
Building overlap graph is *slow.* We saw O($N + a$) and O(N^2) approaches

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

Assembly alternatives

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

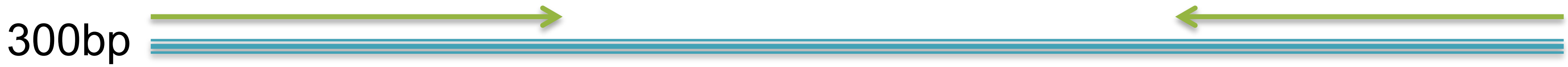
Alternative 2: de Bruijn graph (DBG) assembly



Scaffolding with mate pair information

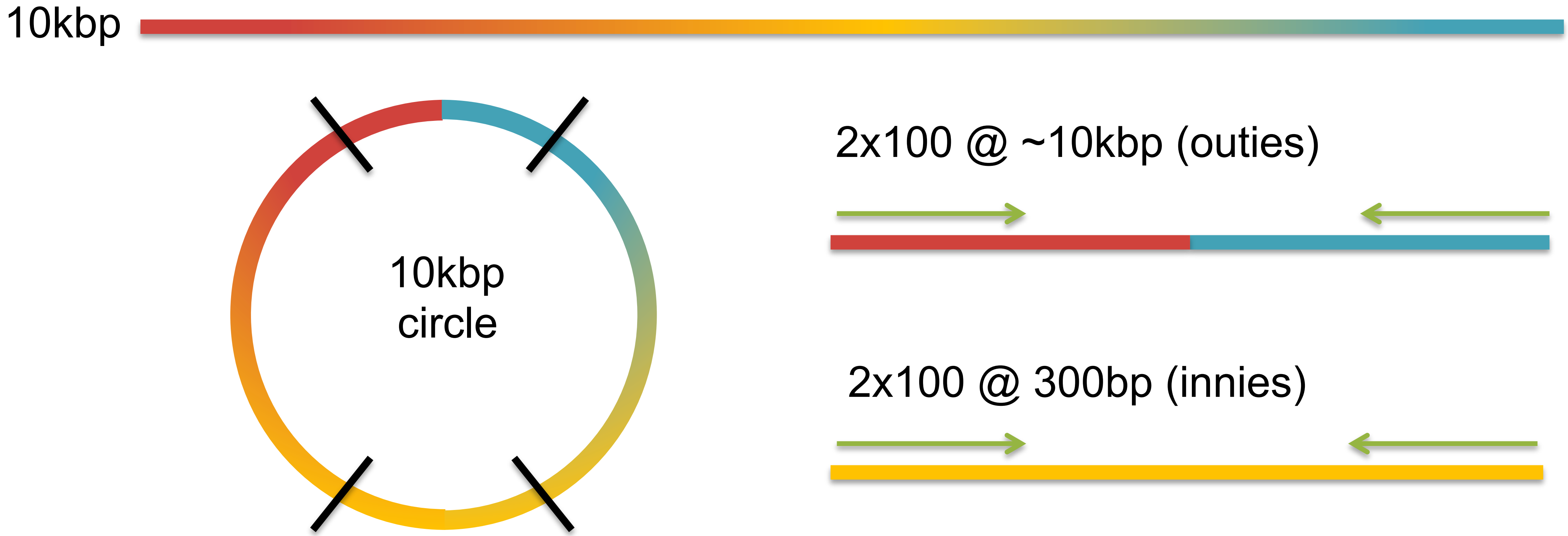
Paired-end sequencing

- Read one end of the molecule, flip, and read the other end
- Generate pair of reads separated by up to 500bp with inward orientation



Mate-pair sequencing

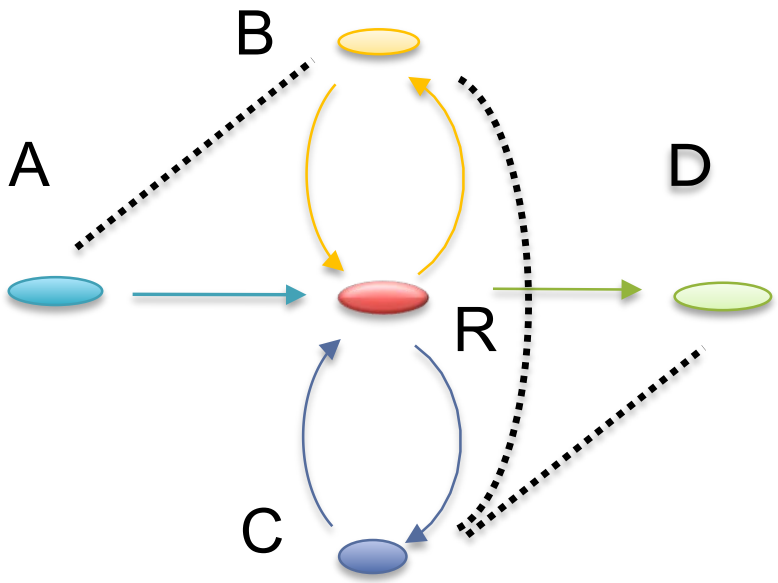
- Circularize long molecules (1-10kbp), shear into fragments, & sequence
- Mate failures create short paired-end reads



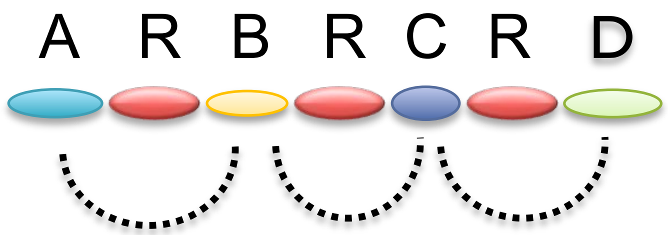
* Slide from Mike Schatz

Scaffolding

- Initial contigs (*aka* unipaths, unitigs) terminate at
  - *Coverage gaps*: especially extreme GC
  - *Conflicts*: errors, repeat boundaries



- Use mate-pairs to resolve correct order through assembly graph
  - Place sequence to satisfy the mate constraints
  - Mates through repeat nodes are tangled



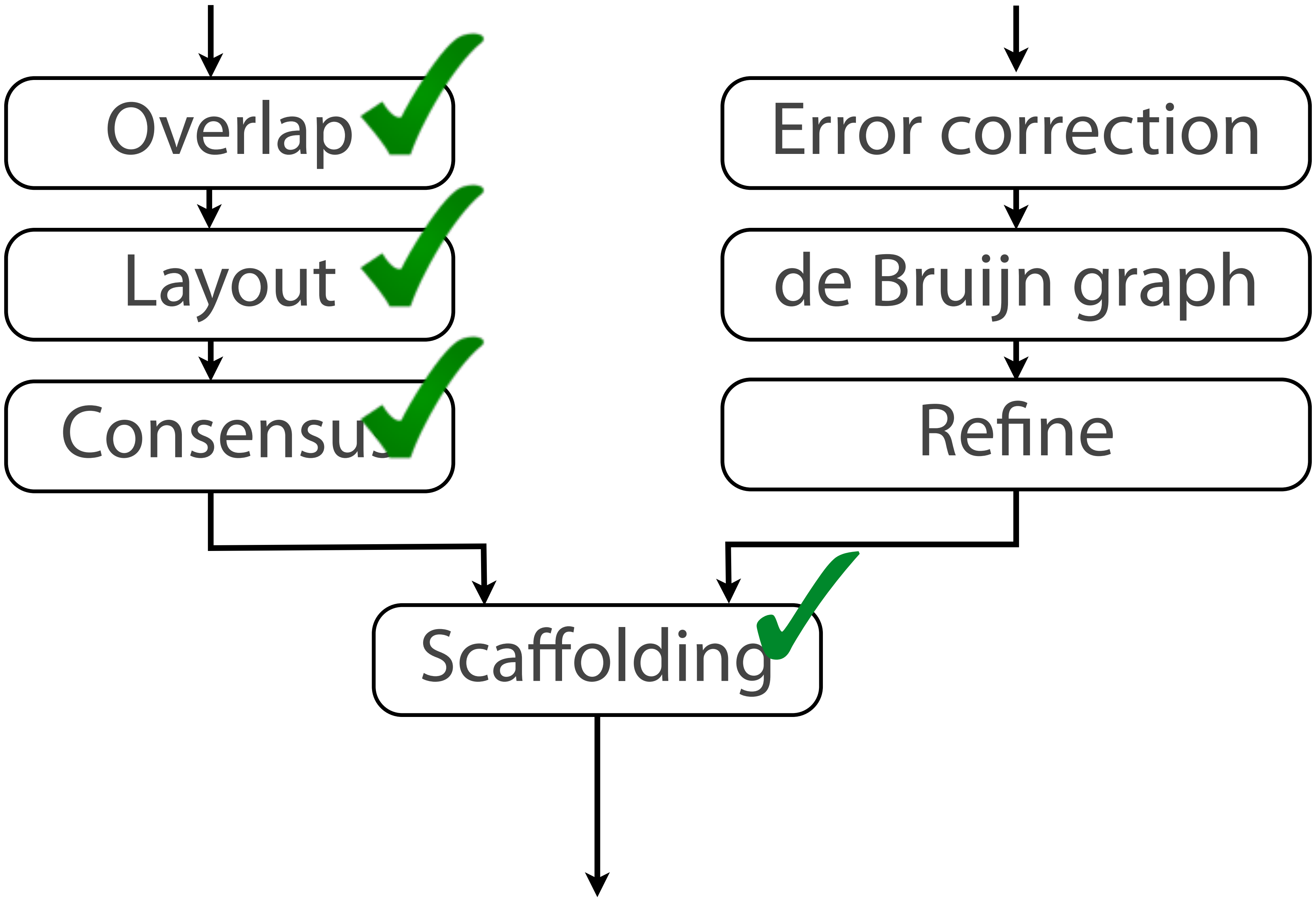
- Final scaffold may have internal gaps called sequencing gaps
  - We know the order, orientation, and spacing, but just not the bases. Fill with Ns instead

* Slide from Mike Schatz

Assembly alternatives

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

Alternative 2: de Bruijn graph (DBG) assembly


Overlap ✓
Layout ✓
Consensus ✓
Error correction
de Bruijn graph
Refine
Scaffolding ✓