# De-Novo Genome Assembly and its Current State

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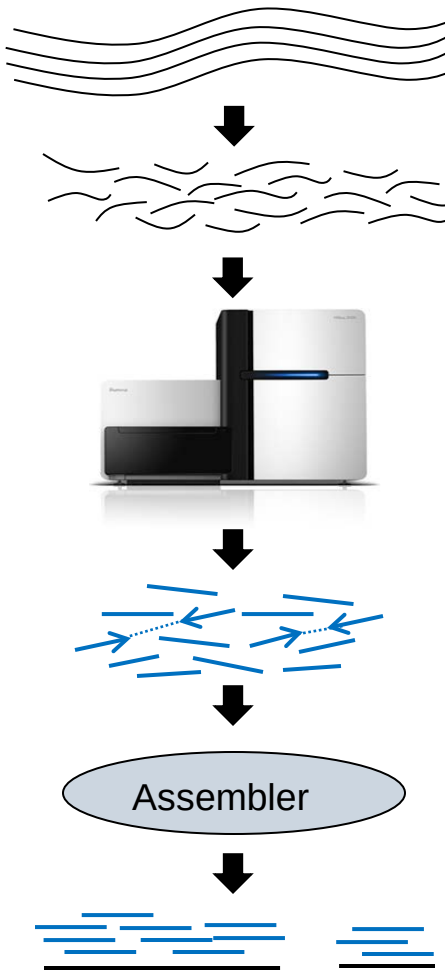
# What is genome assembly and why is it hard?

Original genome sequence (in multiple copies)

Sequence short fragments

Reconstruct original sequence from *reads*




Difficulties:

- Genomes are very long and repeat-rich

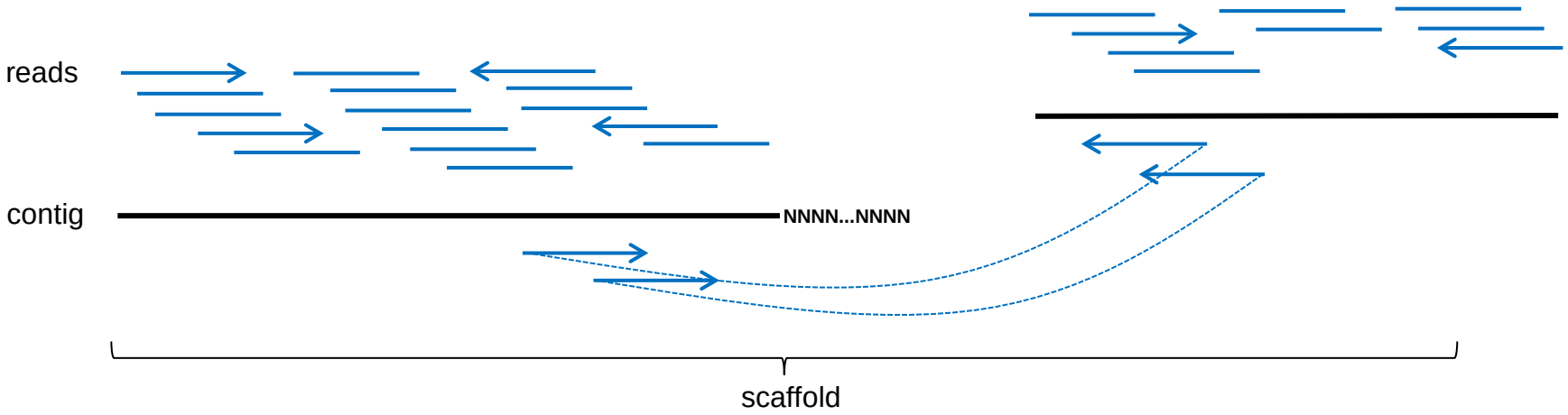
- Reads are very short and may contain errors and biases

# Assembly algorithms - introduction

Assemblers use **overlaps** between reads,

to first produce **contigs**,

that are then used to build **scaffolds**.



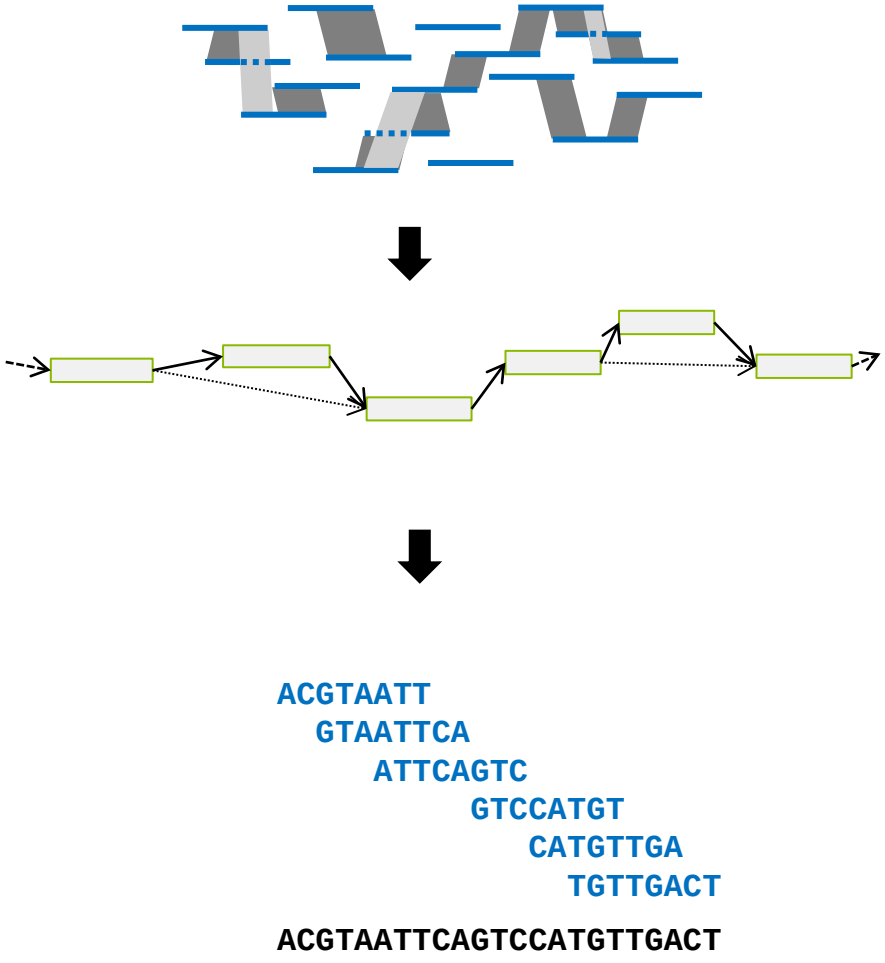

*Read pairs* contribute long-range linking information, especially in the scaffolding phase.

# Algorithms – Overlap Graph

1) **Overlap phase**: pairwise overlap alignments

2) **Layout phase**: overlap graph construction and finding relative placement of reads

3) **Consensus phase**: Produce multiple read alignment and compute contig consensus sequences

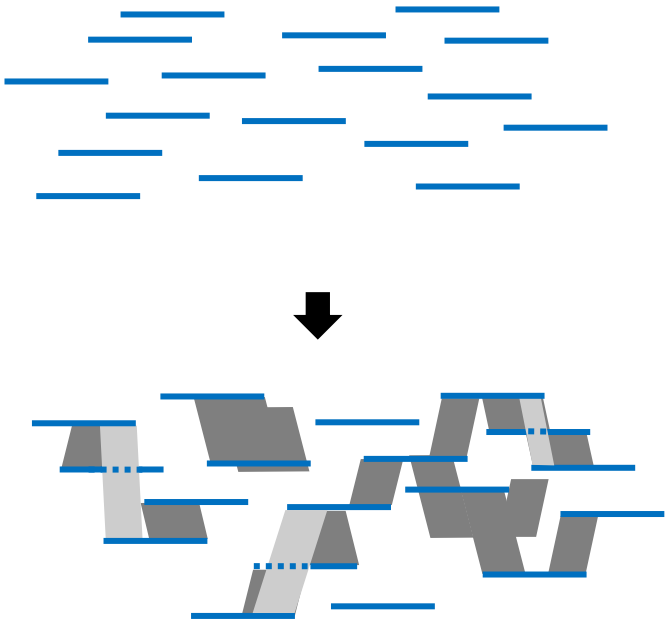



*Kececioglu and Myers, 1995*

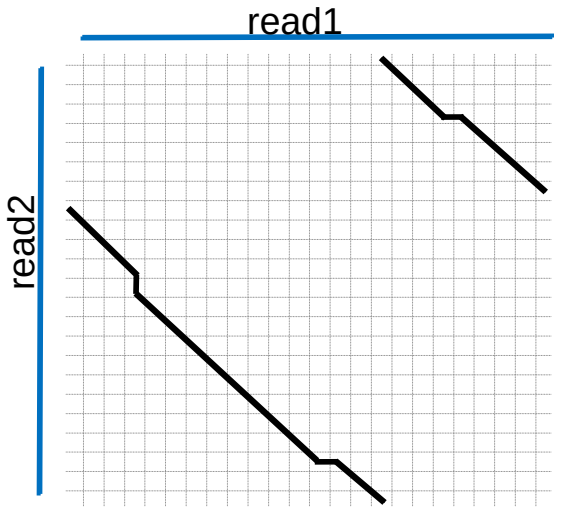
# Algorithms – Overlap Graph

1) **Overlap phase**:

for all pairs of reads



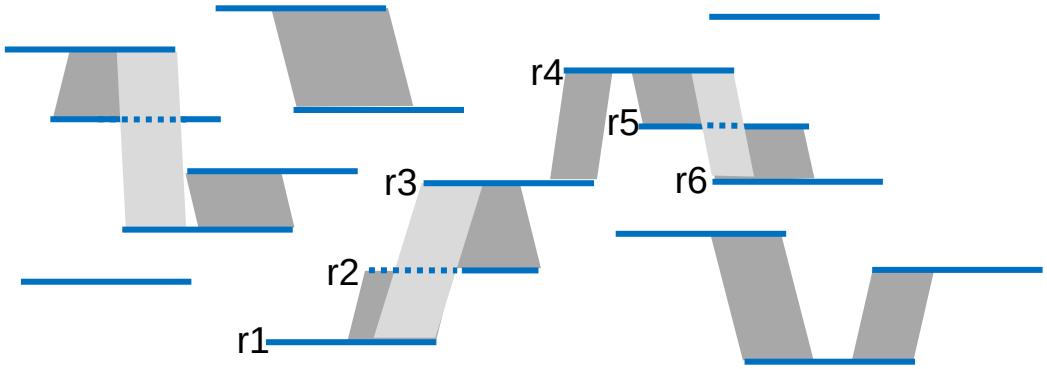
pairwise overlap alignments




Computationally expensive!

# Algorithms – Overlap Graph

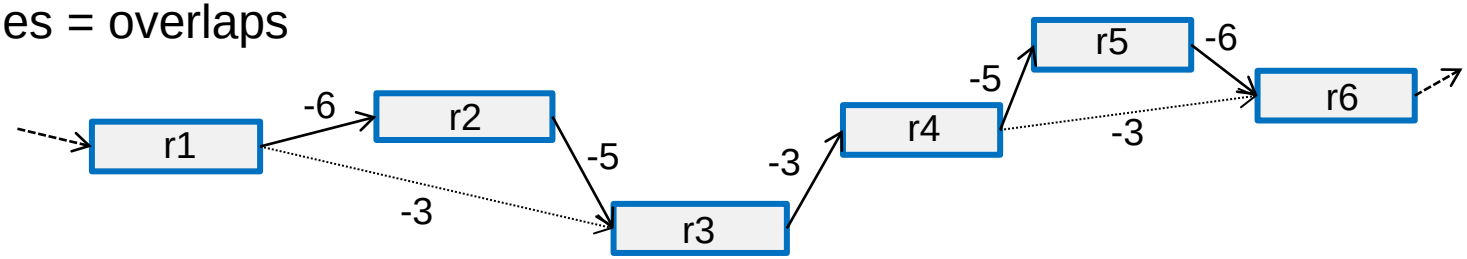
1) **Overlap phase**:




2) **Layout phase:**

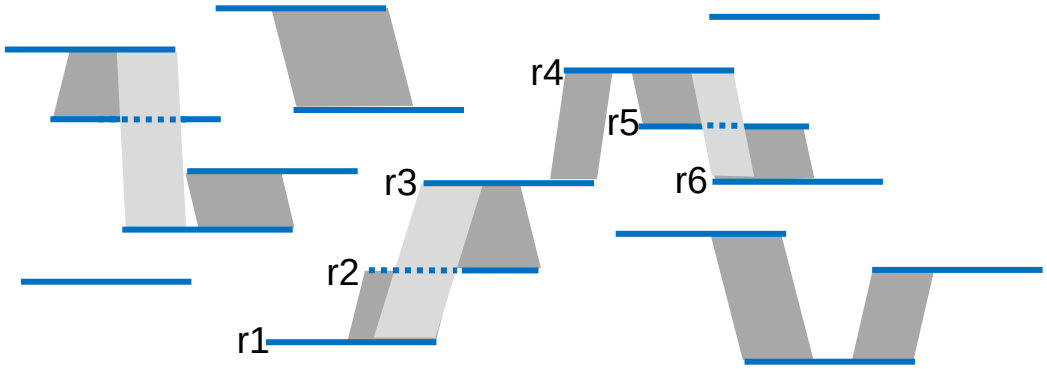
nodes = reads

edges = overlaps



# Algorithms – Overlap Graph

1) **Overlap phase**:




2) **Layout phase:**

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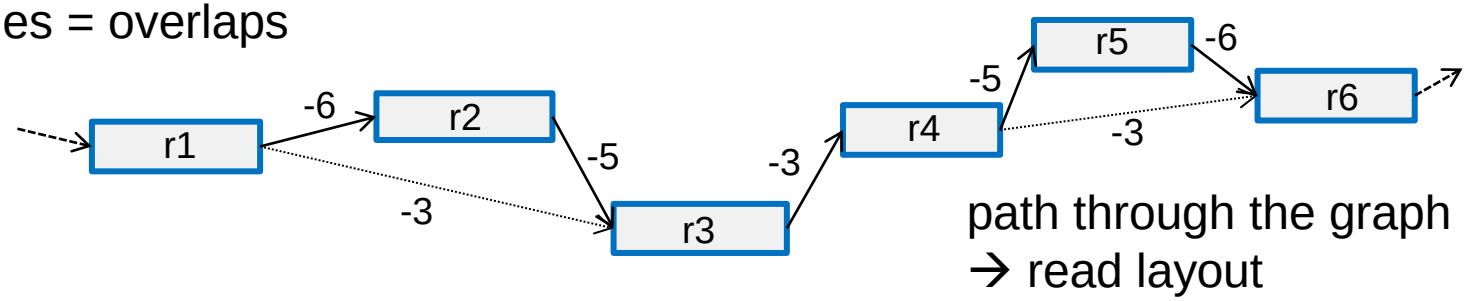
edges = overlaps




path through the graph
→ read layout

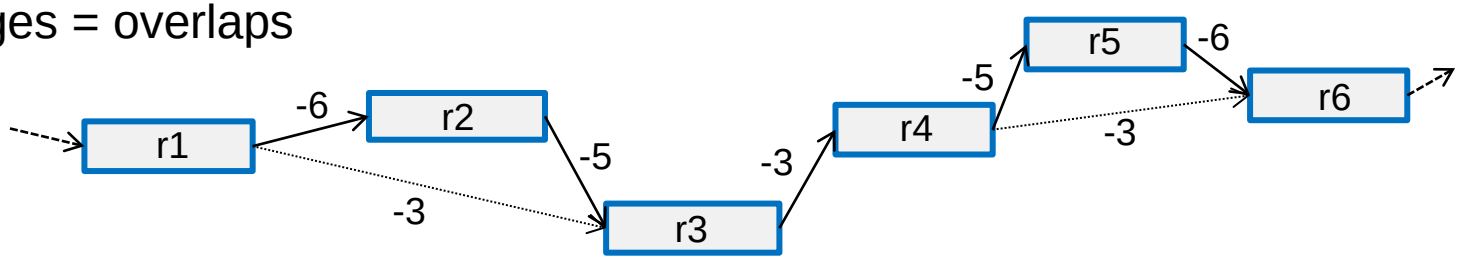
In theory Hamiltonian path (NP-complete), in practice heuristics

# Algorithms – Overlap Graph

2) **Layout phase:**

nodes = reads

edges = overlaps




3) **Consensus phase**:

multiple read alignment

```
r1  ACGTAATT
   r2  GTAATTCA
      r3  ATTCAGTC
          r4 GTCCATGT
             r5  CATGTTGA
                r6  TGTTGACT
```

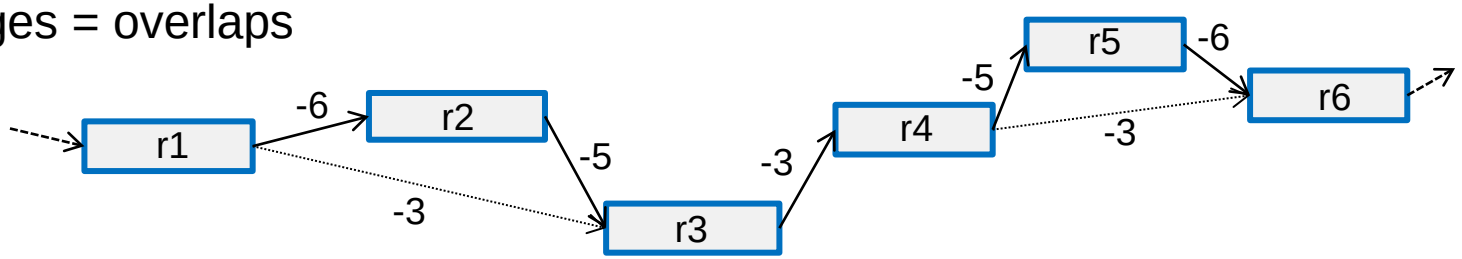
contig **ACGTAATTCAGTCCATGTTGACT**

# Algorithms – Overlap Graph

2) **Layout phase:**

nodes = reads

edges = overlaps




3) **Consensus phase**:

multiple read alignment

```
r1  ACGTAATT
  r2  GTAATTCA
    r3  AT-CAGTC
        r4 GTCCATGT
          r5  CATATTGA
            r6  TGTTGACT
```

robust with alignment errors

contig **ACGTAATTCAGTCCATGTTGACT**

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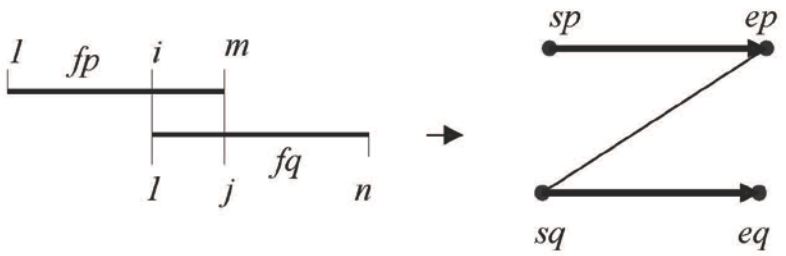
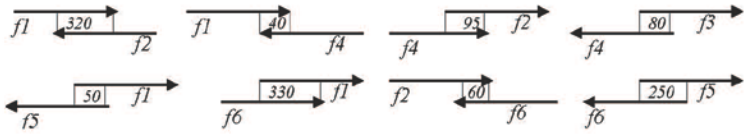



The label (or *"length"*) of the overlap edge $e$ is defined to be $-1$ times the overlap length, e.g. $-(\frac{m-i+j-1}{2}+1)$ in the figure.

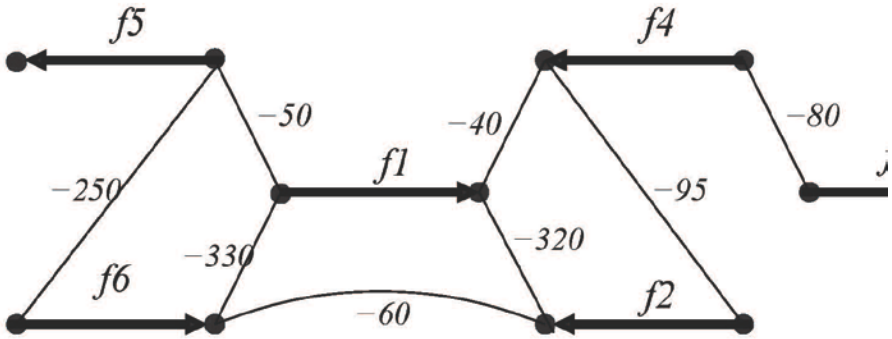
## 10.14 Example

Assume we are given 6 reads $\mathcal{F} = \{f_1, f_2, \ldots, f_6\}$, each of length 500, together with the following overlaps:




Here, for example, the last 320 bases of read $f_1$ align to the first 320 bases of the reverse complement $\overline{f_2}$ of $f_2$, whereas $f_1$ and $\overline{f_5}$ overlap in the first 50 bases of each.

We obtain the following overlap graph $OG$:

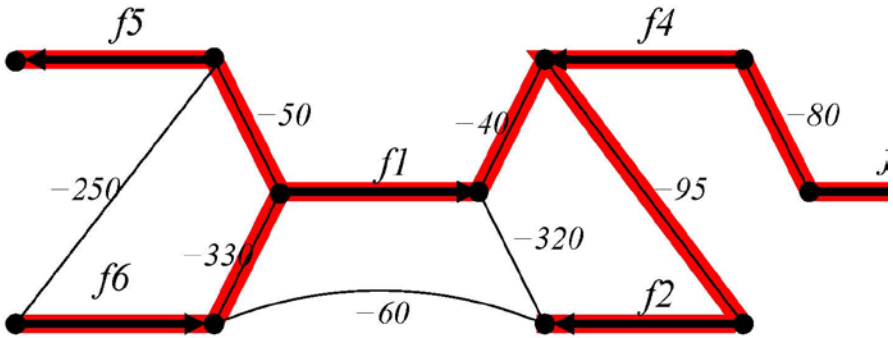



Each read $f_p$ is represented by a read edge $(s_p, e_p)$ of length $|f_p|$. Overlaps off the start $s_p$, or end $e_p$, of $f_p$ are represented by overlap edges starting at the node $s_p$, or $e_p$, respectively. Each overlap edge is labeled by $-1$ times the overlap length.

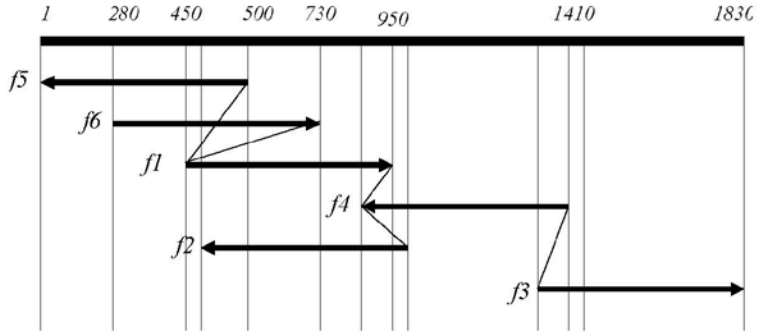
## 10.15 The layout phase

The goal of the layout phase is to arrange all reads into an approximate multi-alignment. This involves assigning coordinates to all nodes of the overlap graph $OG$, and thus, determining the value of $s_i$ and $e_i$ for each read $f_i$.

A simple heuristic is to select a *spanning forest* of the overlap graph $OG$ that contains all read edges. [1]



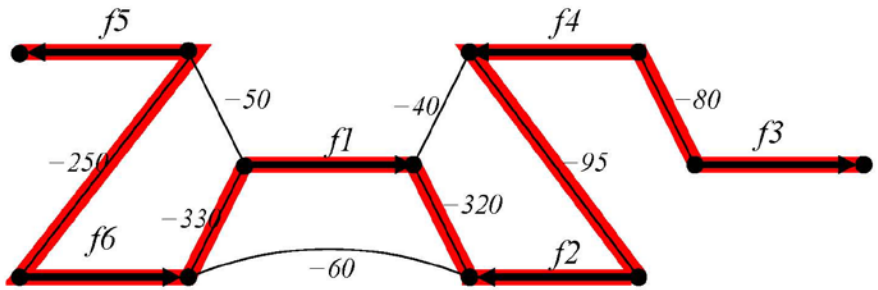

Such a subset of edges positions every read with respect to every other, within a given connected component of the graph:




Such a putative alignment of reads is called a *contig*.

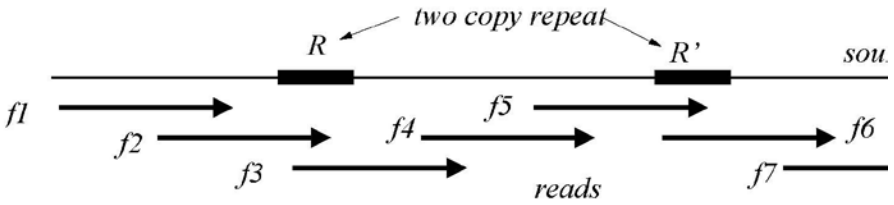
The spanning tree is usually constructed using a *greedy heuristic* in which the overlap edges are chosen in order of decreasing overlap length (i.e., increasing edge "length").

[1] (A spanning forest is a set $F$ of edges such that any two nodes in the same connected component of $OG$ are connected by a unique simple, unoriented path of edges in $F$.)

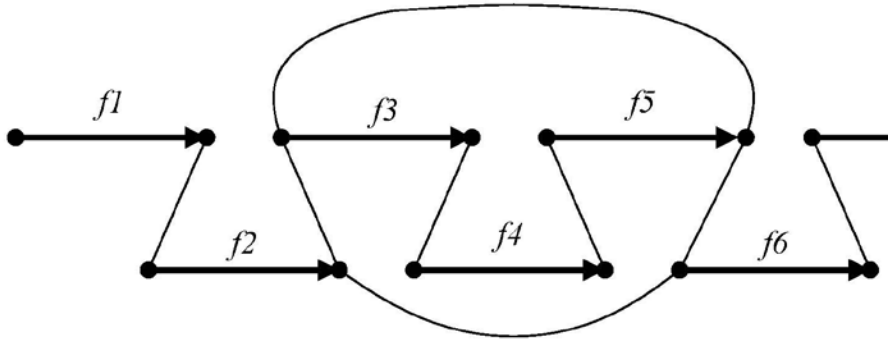



## 10.16 Repeats and the layout phase

Consider the following situation:



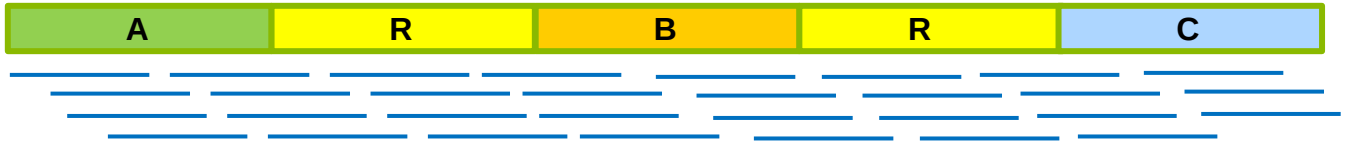

This gives rise to the following overlap graph:



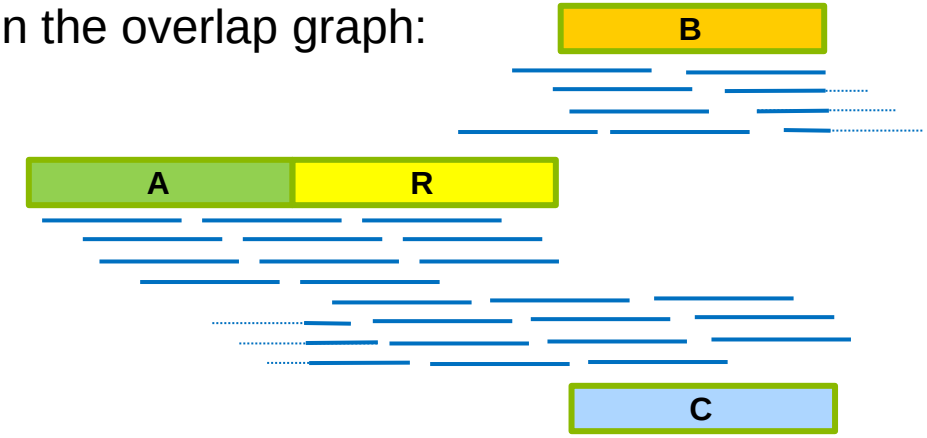

Consider this spanning tree:

# Overlap Graph

There are not only simple paths…




Approximate ordering
in the overlap graph:

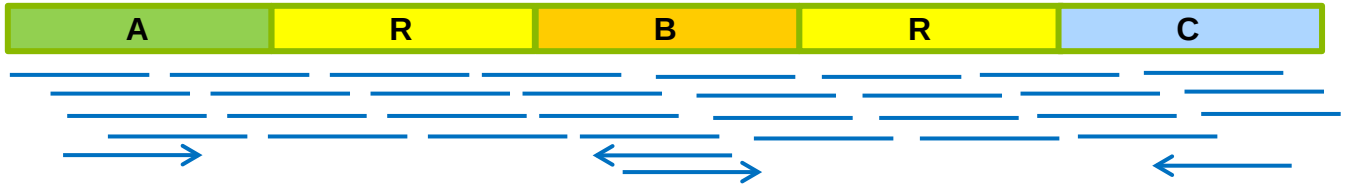



- Coverage is high
- Path branches

R is a repeat!

# Overlap Graph

There are not only simple paths…

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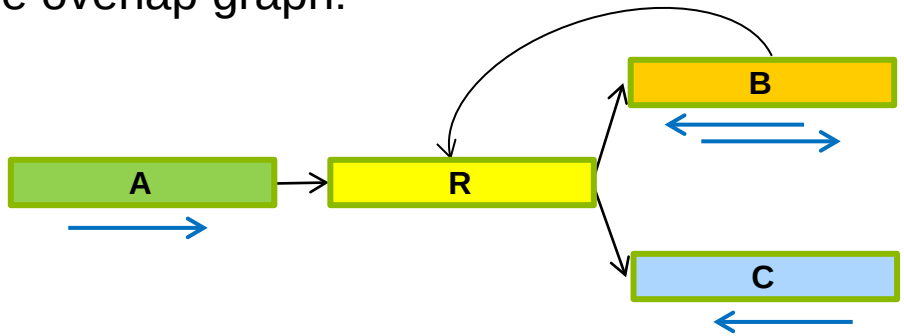
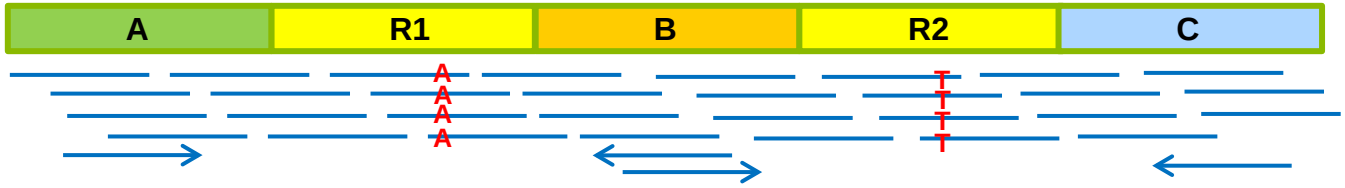
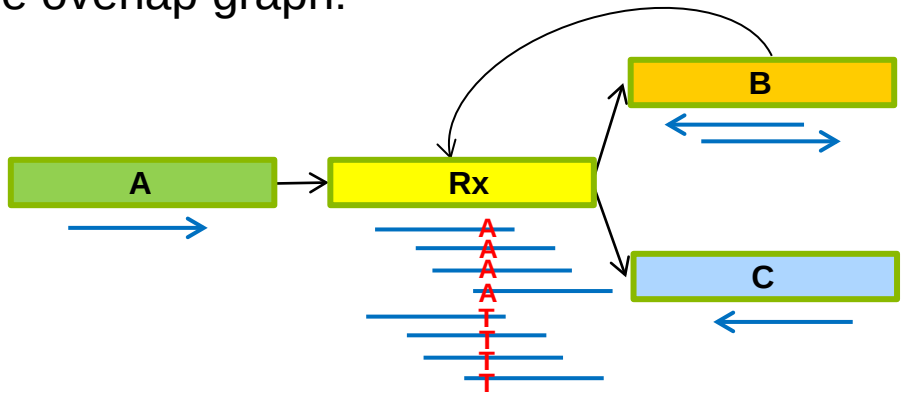


# Overlap Graph

Now: R1 and R2 are nearly identical




Approximate ordering
in the overlap graph:




**Overlap strictness:**
Tradeoff between error tolerance and “natural” repeat separation

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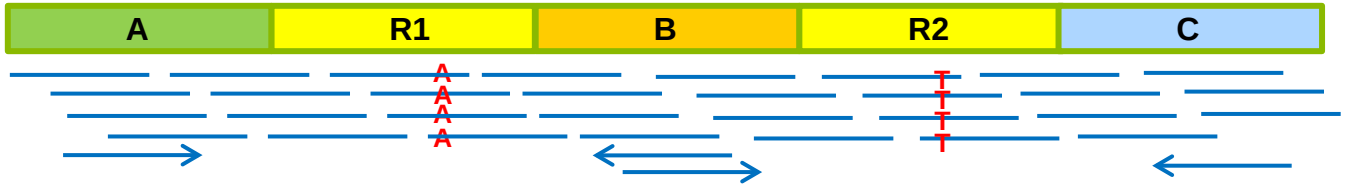


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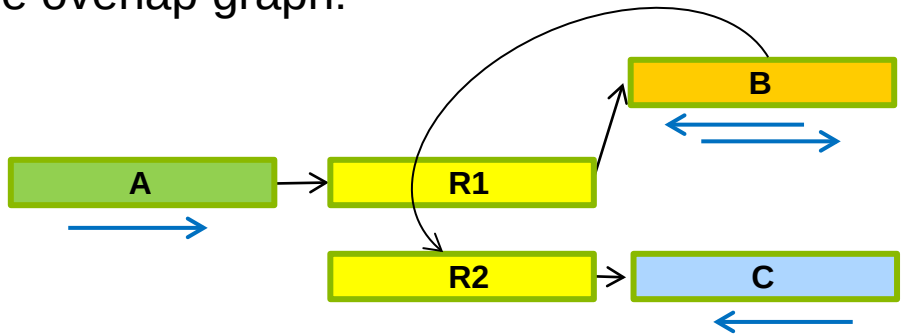



**Overlap strictness:**
Tradeoff between error tolerance and “natural” repeat separation

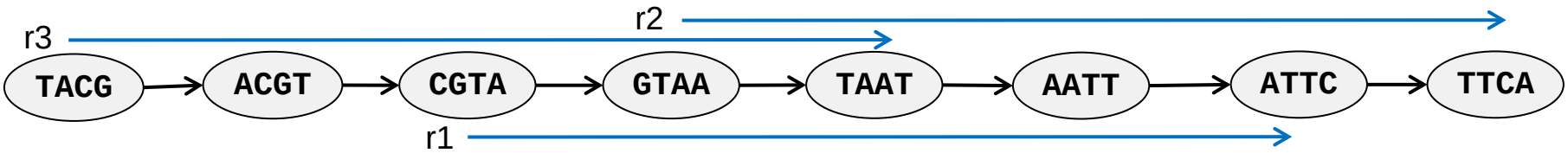
# Algorithms - de Bruijn graph

- No overlap phase, no consensus phase, basically just a layout phase

Nodes = *k*-mers
Edges = (*k+1)*-mers

Given *k* = 4 and three read sequences:

r1 **CGTAATTC**
r2 **GTAATTCA**
r3 **TACGTAAT**




contig: **TACGTAATTCA**
r3 **TACGTAAT**
r1 **CGTAATTC**
r2 **GTAATTCA**

In theory „de Bruijn Superwalk Problem“ (NP-hard), in practice heuristics

*de Bruijn, 1946; Pevzner, 2001; Medvedev 2007*

# de Bruijn graph

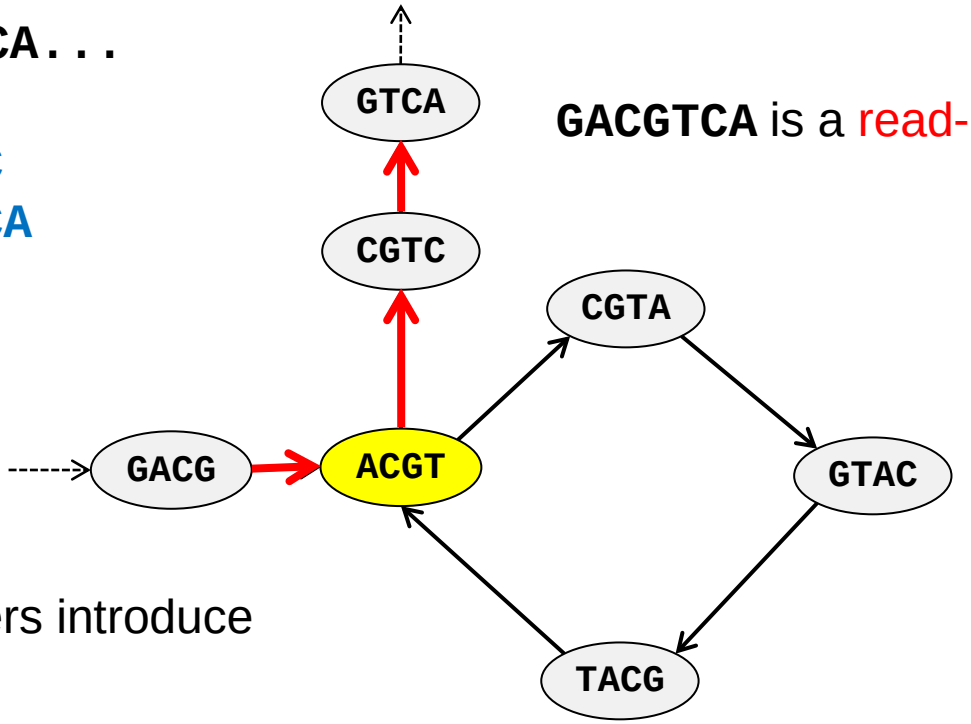
Again, there are not only simple paths…

...G**ACGT** **ACGT**CA...

GACGTACG

CGTACGTC

GTACGTCA




**GACGTCA** is a read-incoherent path

Repetitive k-mers introduce cycles

# de Bruijn graph

What if we increase *k* to 5?

```
...GACGTACGTCA...
   GACGTACG
     CGTACGTC
      GTACGTCA
```

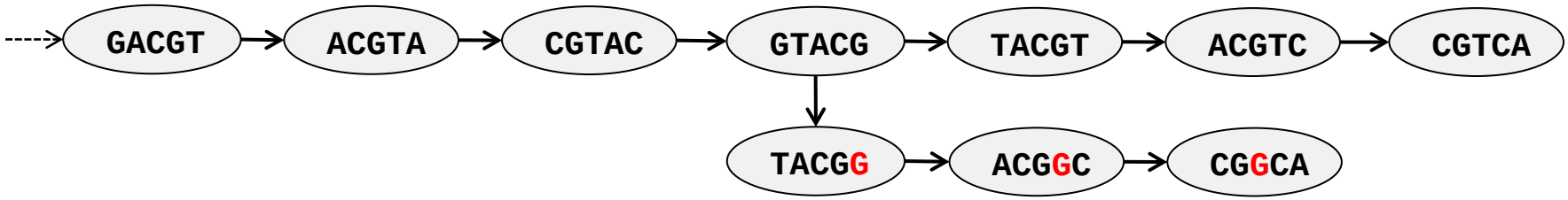

→ Back to a linear graph structure

Increasing *k* leads to better repeat resolution

# de Bruijn graph

What if we have sequencing errors?

```
...GACGTACGTCA...
   GACGTACG
     CGTACGTC
      GTACGGCA
```

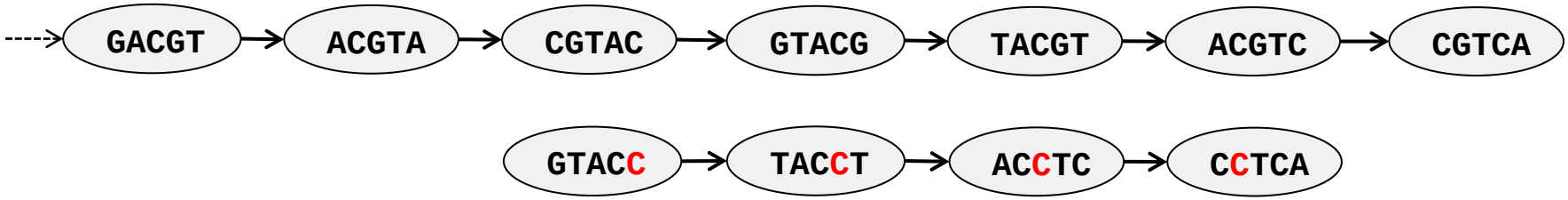



→ Additional nodes

# de Bruijn graph

What if we have sequencing errors?

```
...GACGTACGTCA...
   GACGTACG
     CGTACGTC
      GTACCTCA
```



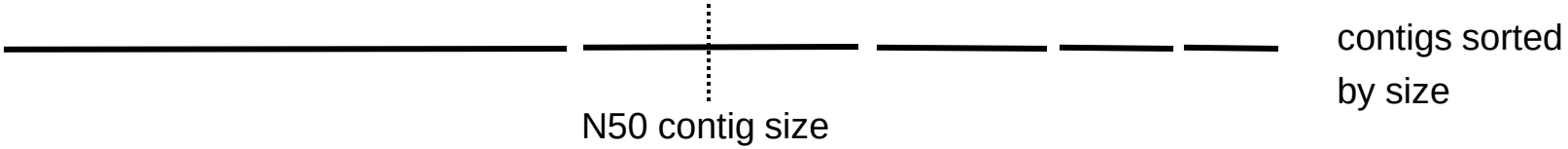

→ Additional nodes
& graph disconnected

**Choice of *k*:**
Tradeoff between error tolerance and repeat resolution

# What is a good assembly?

Some assembly evaluation metrics:

- High N50 contig size (most commonly used metric)




- Low number of assembly errors (sequence errors, structural misjoins)


assembled as 

Only if a reference sequence is known!

# Conclusions

- Most assemblers use either the OLC or de Bruijn graph paradigm, both lead to NP-hard assembly models.

- However, assembler performance is independent of the underlying paradigm and mainly depends on heuristics for repeat resolution and handling noise.

- How to measure assembly accuracy is another important aspect, in general tradeoff between assembly contiguity and correctness.

- Evaluations show that assembly is far from solved, assembler performance still quite inconsistent.

*“For large genomes, the choice of assemblers is often limited to those that will run without crashing” (GAGE paper, 2012)*

# References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Figures:


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