# Genome Assembly

From theory to practice (and back)

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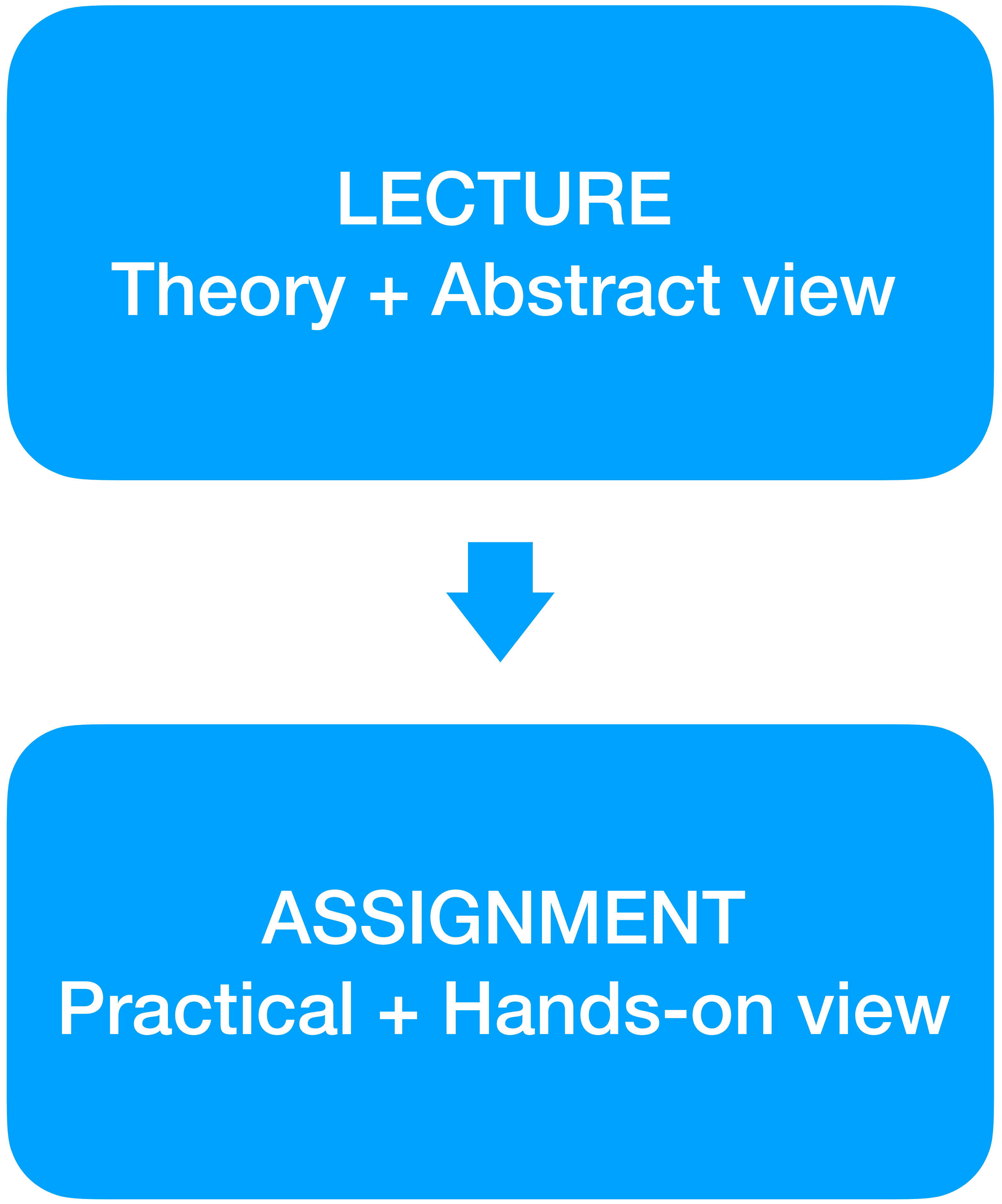
# Lecture outline

1. The problem
2. Practical issues
3. Theoretical problem formulations
4. Practical genome assembly
   - Contig assembly
   - Scaffolding
   - Gap filling
5. And back: a more "practical" theoretical formulation

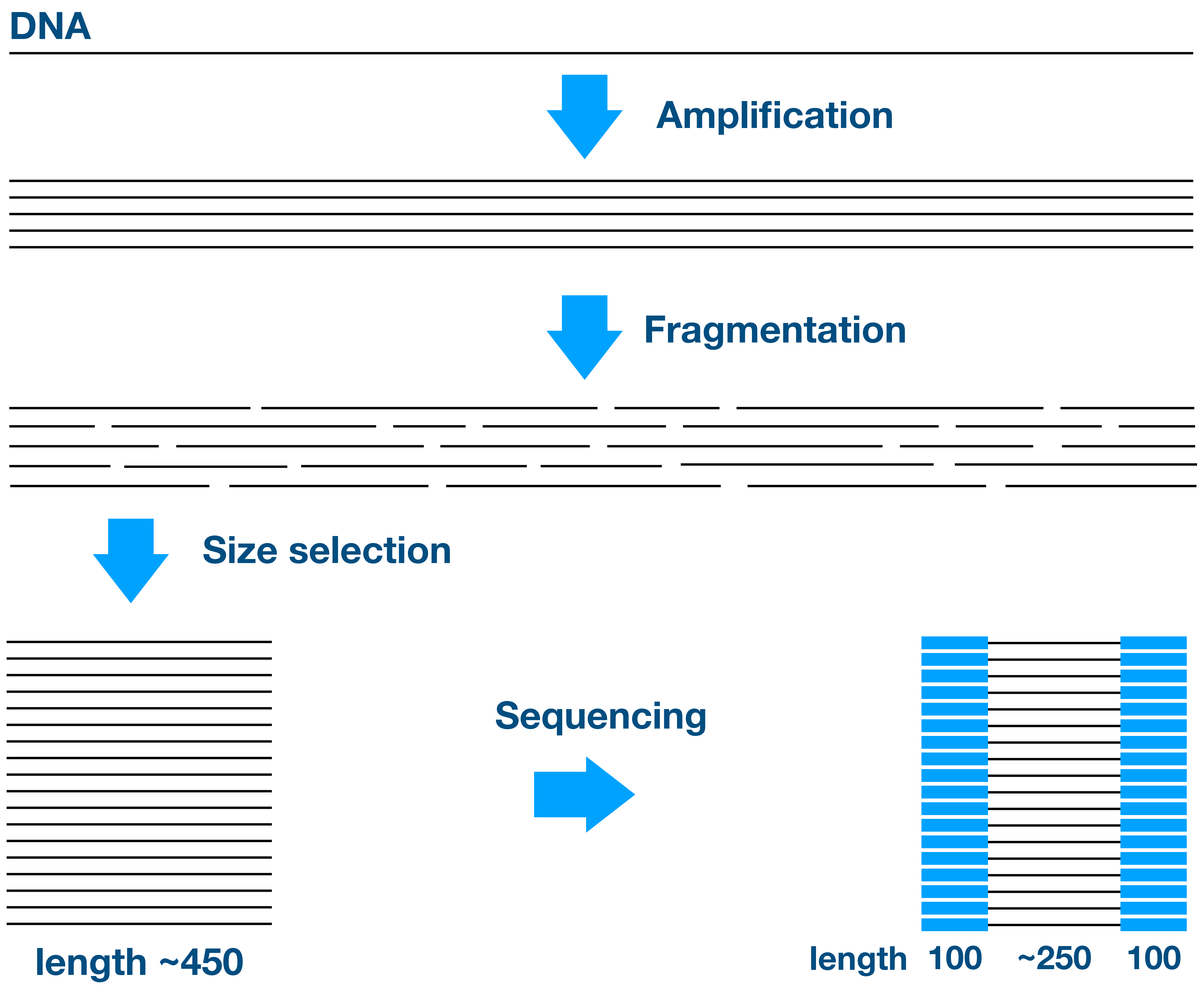


## The problem

(A general description of the input and output)

# Short-read sequencing

(Second-generation sequencing)



## The assembly problem

**INPUT:** A collection of paired-end reads
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(We will see precise computational formulations later)

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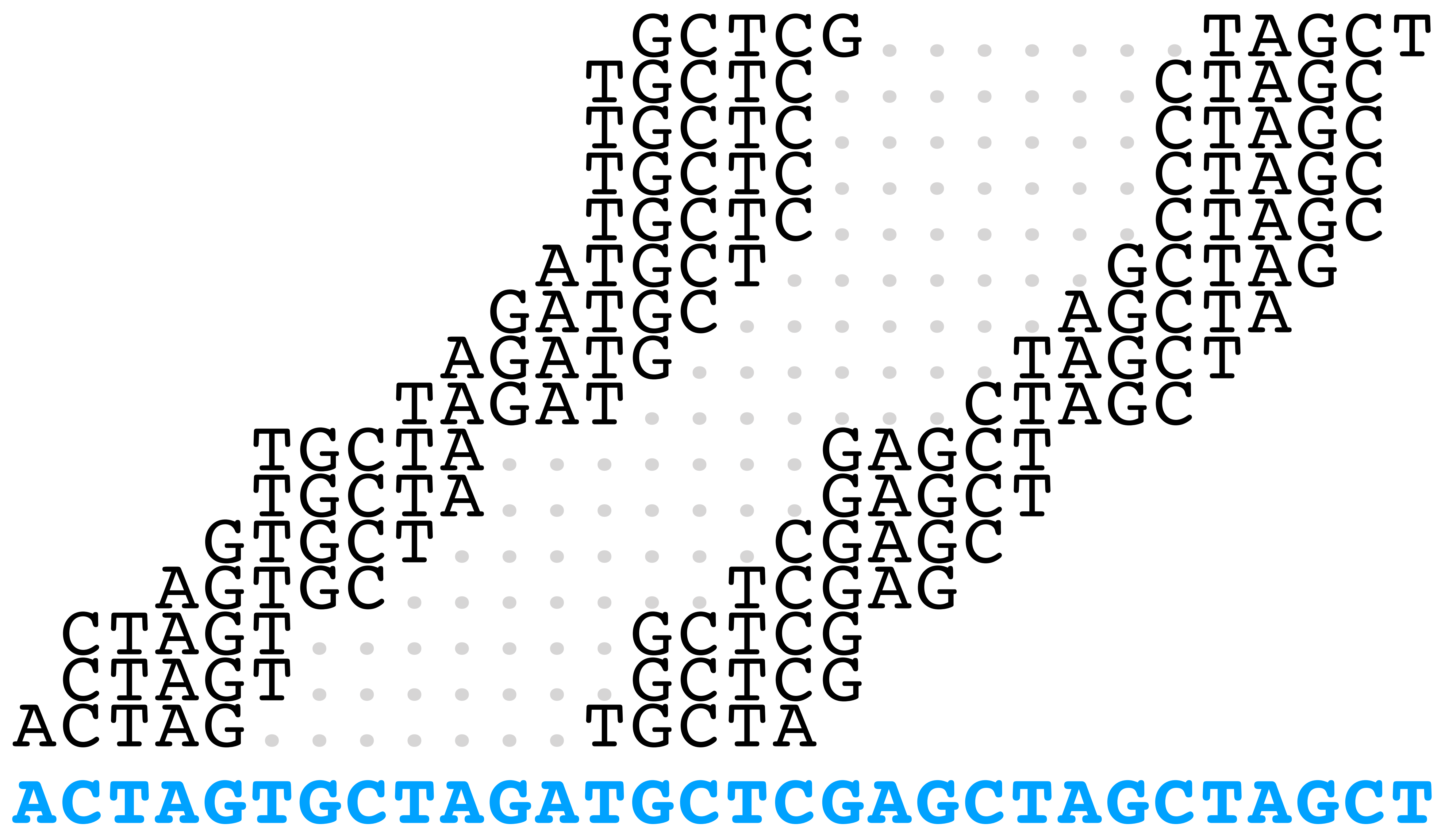

# The assembly problem

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```
TGCTC . . . . . . . CTAGC
TGCTC . . . . . . . CTAGC
TGCTA . . . . . . . GAGCT
CTAGT . . . . . . . GCTCG
ATGCT . . . . . . . GCTAG
GATGC . . . . . . . AGCTA
AGTGC . . . . . . . TCGAG
AGATG . . . . . . . TAGCT
TAGAT . . . . . . . CTAGC
TGCTC . . . . . . . CTAGC
TGCTA . . . . . . . GAGCT
GTGCT . . . . . . . CGAGC
TGCTC . . . . . . . CTAGC
GCTCG . . . . . . . TAGCT
CTAGT . . . . . . . GCTCG
ACTAG . . . . . . . TGCTA
```

???

## Practical issues

(several of which are not covered in this course)

# Repeats longer than read length

MAIN TOPIC

- If every substring of the genome of length = **read length - 1** is unique → trivial

```
AATTGAATTTACACCAC
AATTG
 ATTGA
  TGAAT
   TGAAT
    GAATT
     AATTT
      ATTTA
       TTTAC
        TTACA
         TACAC
          ACACC
           CACCA
            ACCAC
            └──┘
       read length - 1
```

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```
TAGTGCTTAGCGCGTAGATGCTTTAGCGCGTCGAGCTAGCT
TAGTGCTTA      AGATGCTTTA
      TTAGC           TTAGC
       TAGCG           TAGCG
         GCGCGT          GCGCGT
```

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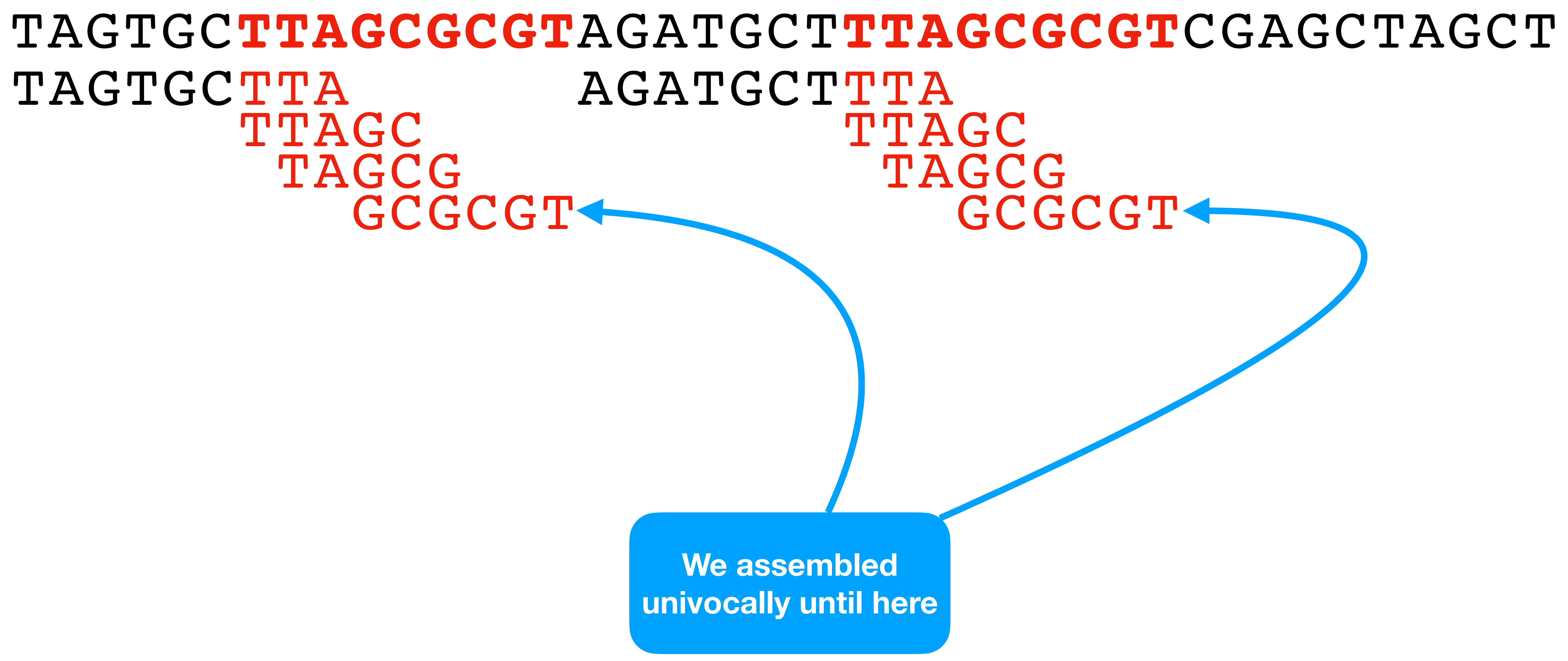
TAGTGCTTAGCGCGTAGATGCTTTAGCGCGTCGAGCTAGCT
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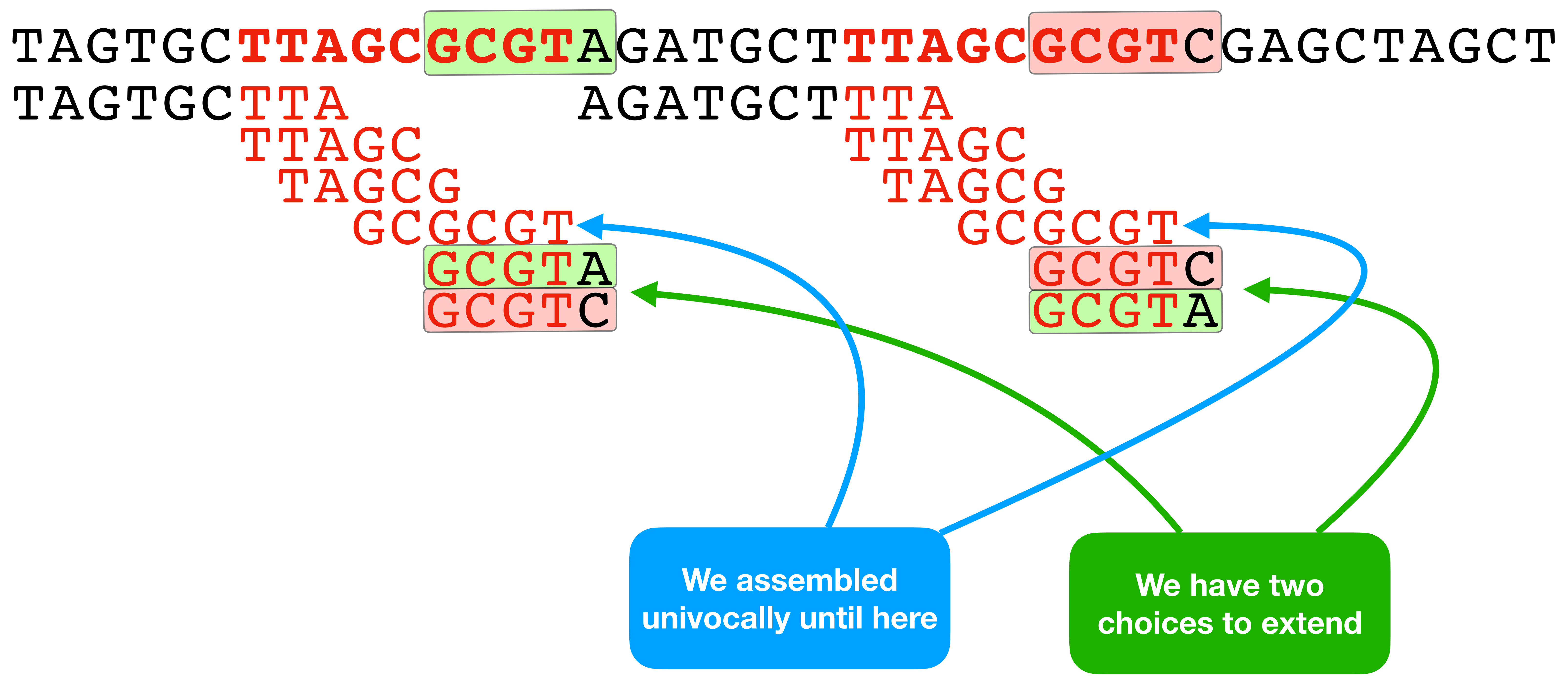
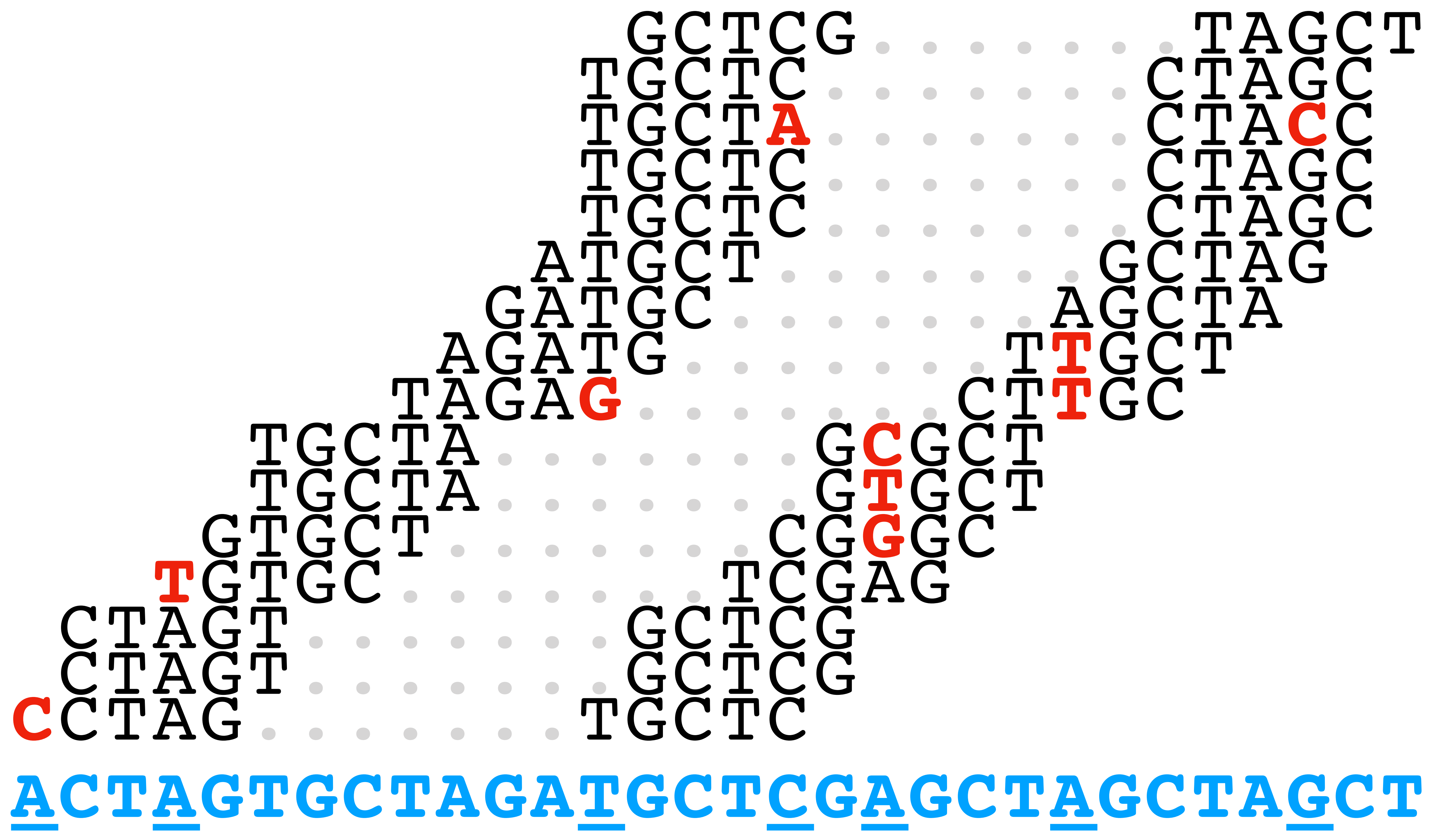

TAGTGCTTAGCGCGTAGATGCTTTAGCGCGTCGAGCTAGCT
TAGTGCTTA
TTAGC
TAGCG
GCGCGT
GCGTA
GCGTC
AGATGCTTTA
TTAGC
TAGCG
GCGCGT
GCGTC
GCGTA
We assembled univocally until here
We have two choices to extend

# Sequencing errors

ASSIGNMENT



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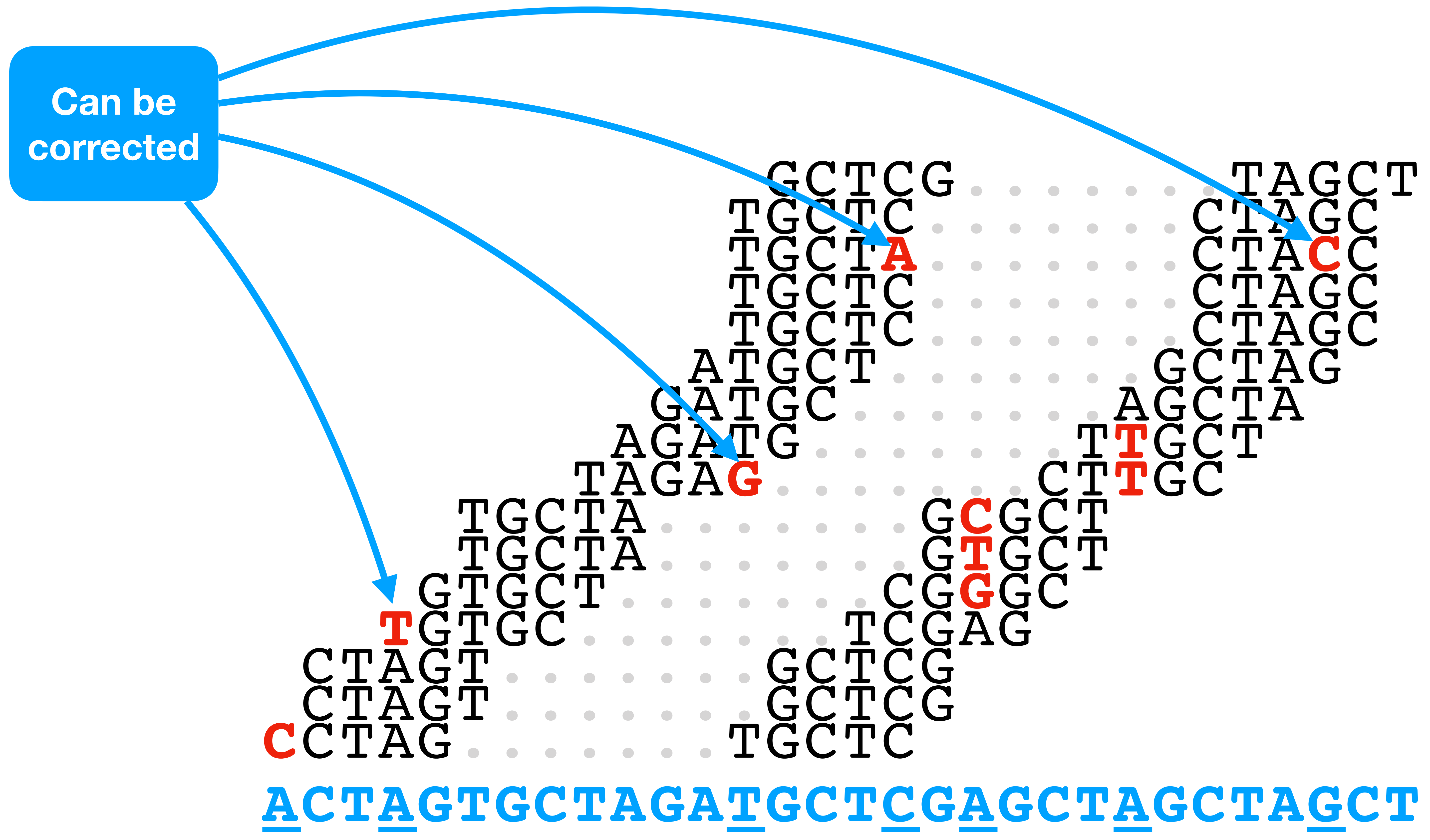

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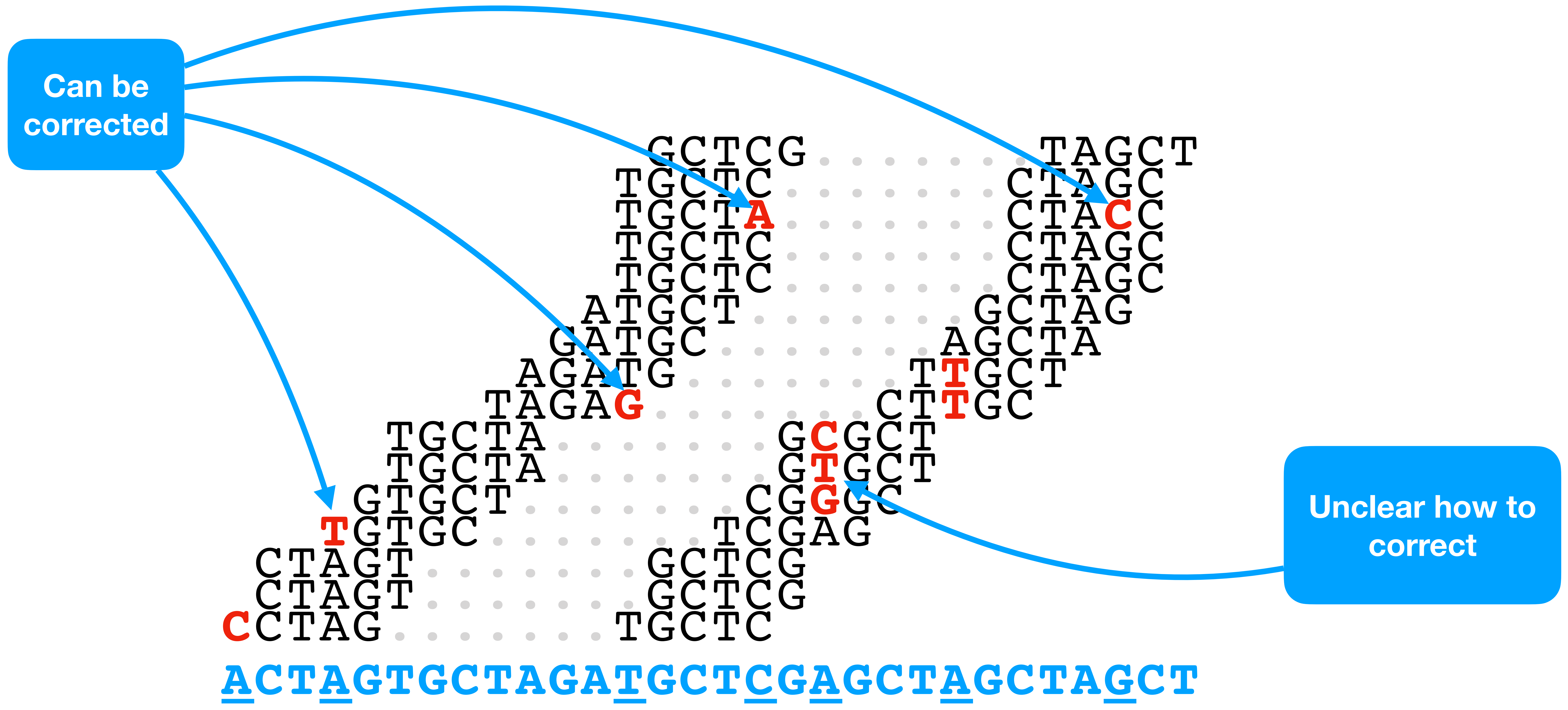

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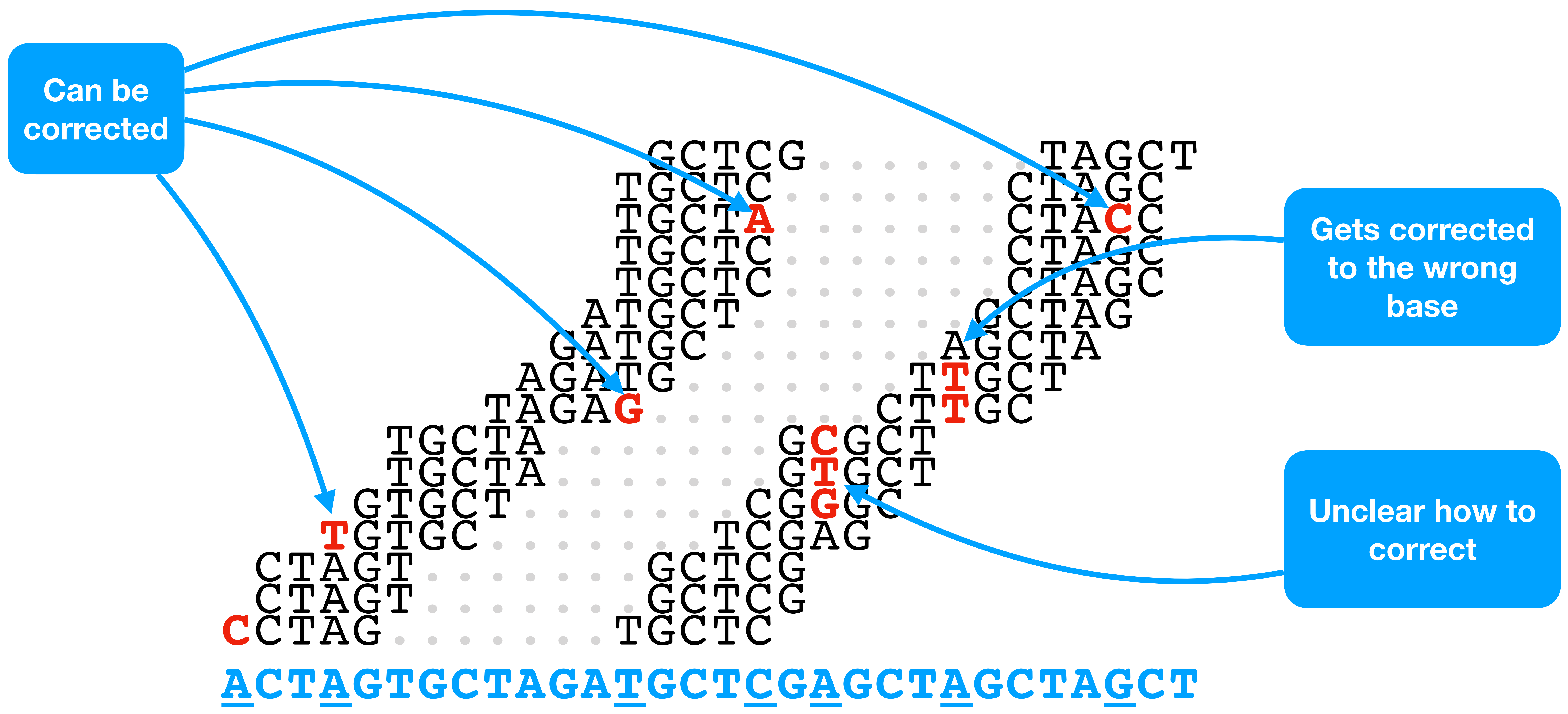

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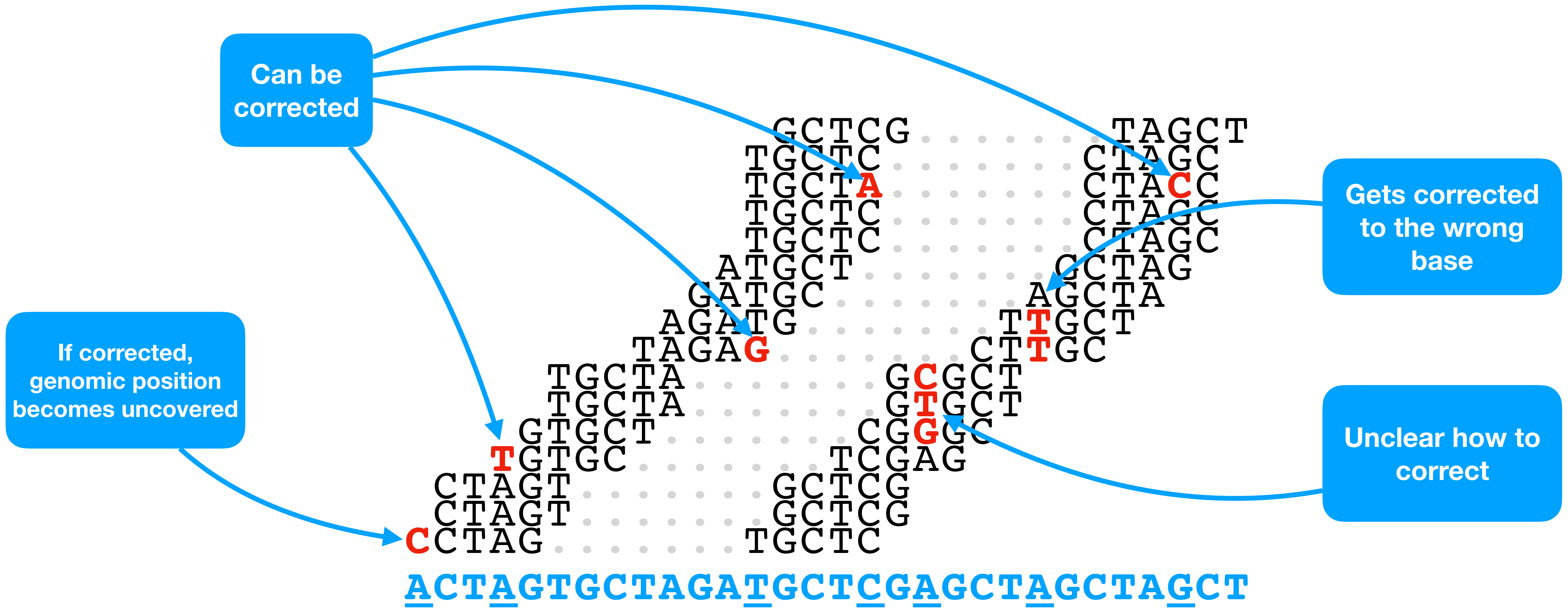

# Polyploidy

LATER IN THE LECTURE

| | |
|---|---|
| Mother | ACTACTGCTAGAAGCTCGAGCTAGCTAGCT |
| Father | ACTAGTGCTAGATGCTCGAGCTAGCTAGCT |

# Polyploidy

LATER IN THE LECTURE

```
             GCTCG.......TAGCT
            AGCTC.......CTAGC
            AGCTC.......CTAGC
            AGCTC.......CTAGC
            AGCTC.......CTAGC
           ATGCT.......GCTAG
          GATGC.......AGCTA
         AGATG.......TAGCT
        TAGAT.......CTAGC
     TGCTA.......GAGCT
     TGCTA.......GAGCT
    CTGCT.......CGAGC
   ACTGC.......TCGAG
 CTAGT.......GCTCG
 CTAGT.......GCTCG
ACTAG.......TGCTA
```

```
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Father  ACTAGTGCTAGATGCTCGAGCTAGCTAGCT
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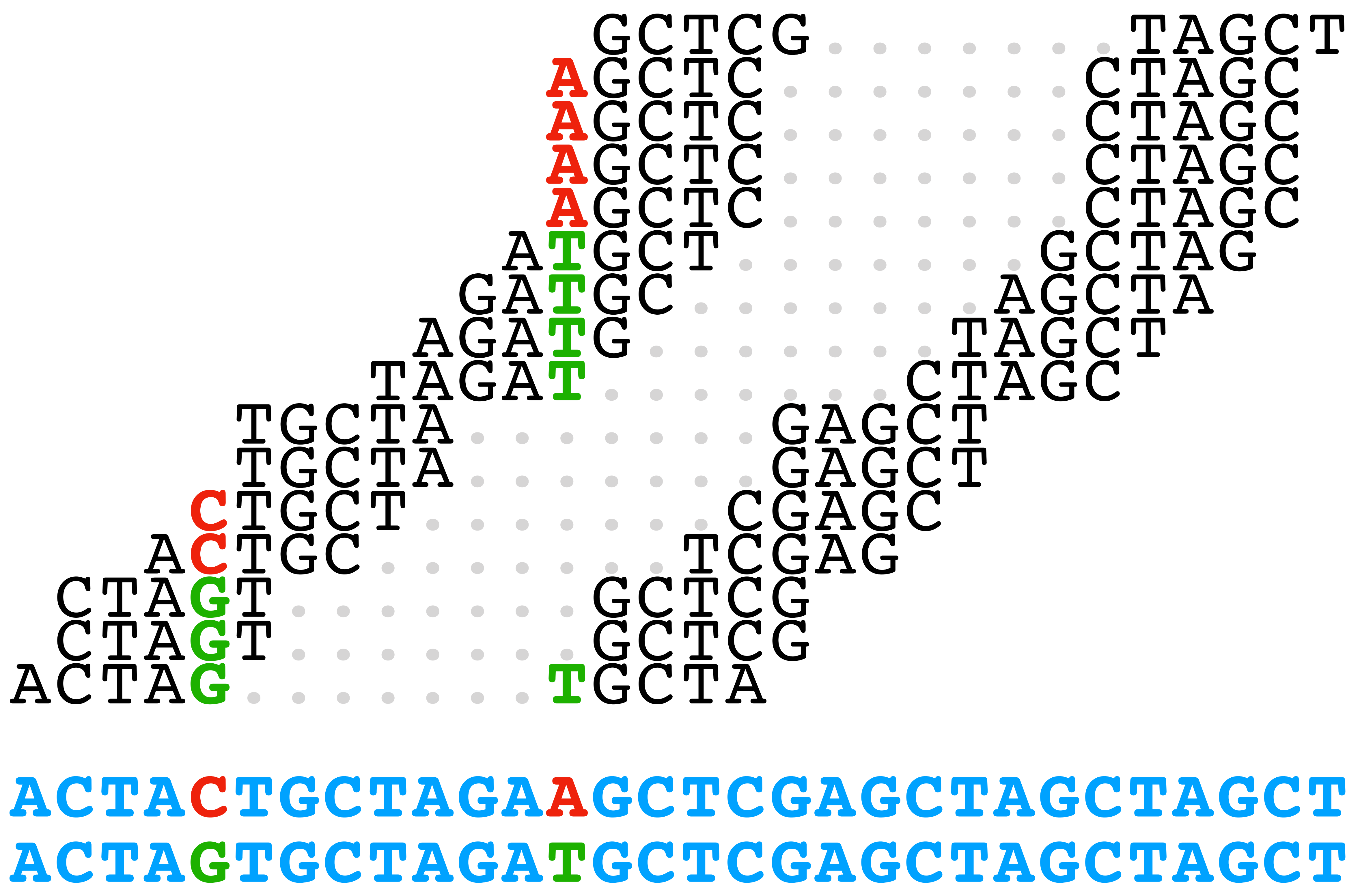


- Sequencing errors + polyploidy at the same time
- Phasing SNPs (C and A from same haplotype, **NOT** e.g. C and T) is a separate problem, called *haplotype assembly* or *haplotype phasing*

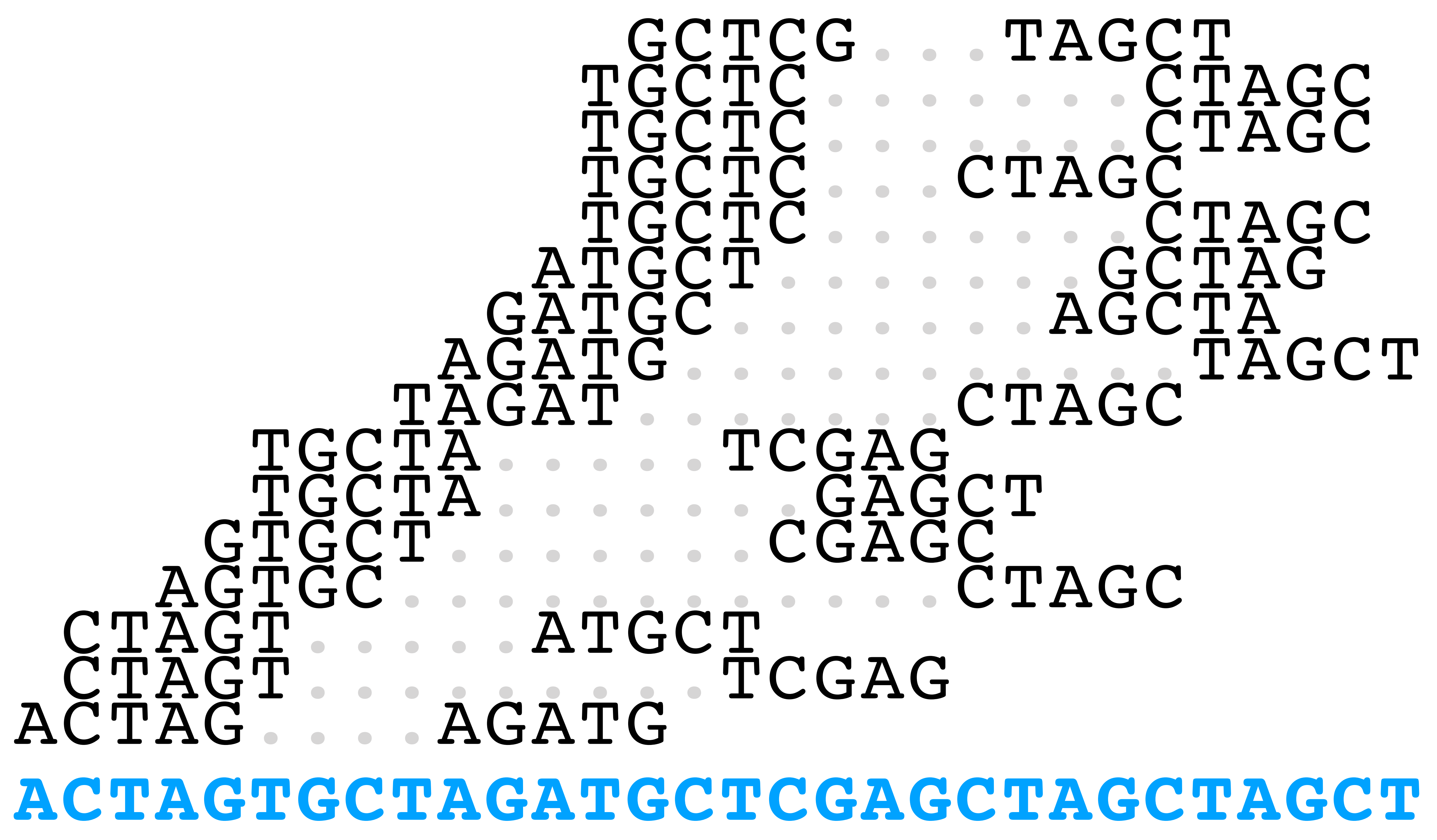
# Unsequenced areas

```
             GCTCG.......TAGCT
            TGCTC.......CTAGC
            TGCTC.......CTAGC
            TGCTC.......CTAGC
            TGCTC.......CTAGC
           ATGCT.......GCTAG
          GATGC.......AGCTA
         AGATG.......TAGCT
        TAGAT.......CTAGC



 CTAGT.......GCTCG
 CTAGT.......GCTCG
ACTAG.......TGCTA
ACTAGT..TAGATGCTCG..CTAGCTAGCT
```

# Non uniform paired-end distance


GCTCG . . . TAGCT
TGCTC . . . . . . . CTAGC
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ATGCT . . . . . . . GCTAG
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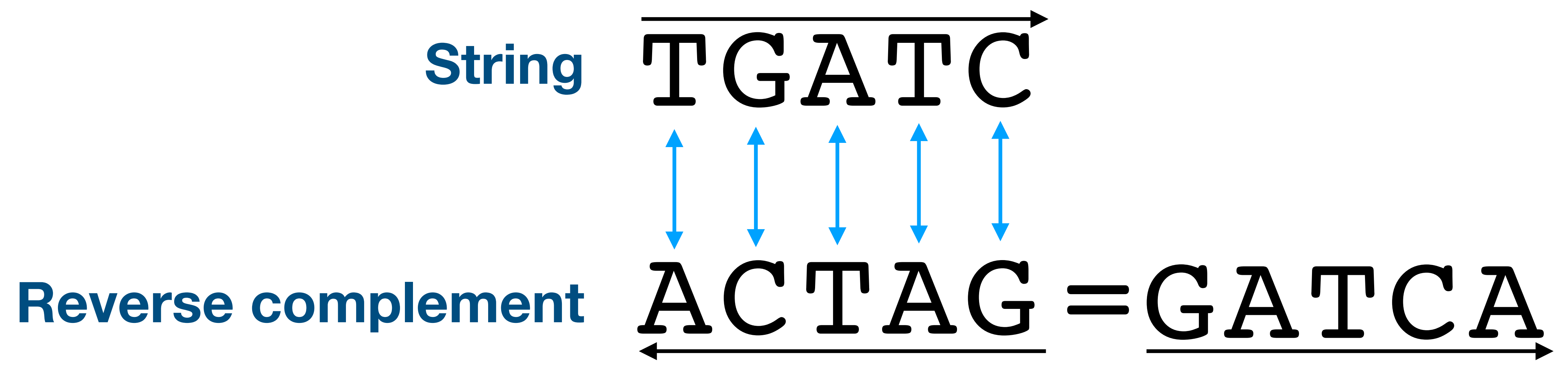
AGATG . . . . . . . . . . . TAGCT
TAGAT . . . . . . . CTAGC
TGCTA . . . . . TCGAG
TGCTA . . . . . . . GAGCT
GTGCT . . . . . . . CGAGC
AGTGC . . . . . . . . . . . . CTAGC
CTAGT . . . . . ATGCT
CTAGT . . . . . . . . . TCGAG
ACTAG . . . AGATG
ACTAGTGCTAGATGCTCGAGCTAGCTAGCT

- Distance between each pair not known precisely from the sequencer

# Double-stranded DNA

NOT IN THE LECTURE

- Reads consist of strings and their reverse complements:



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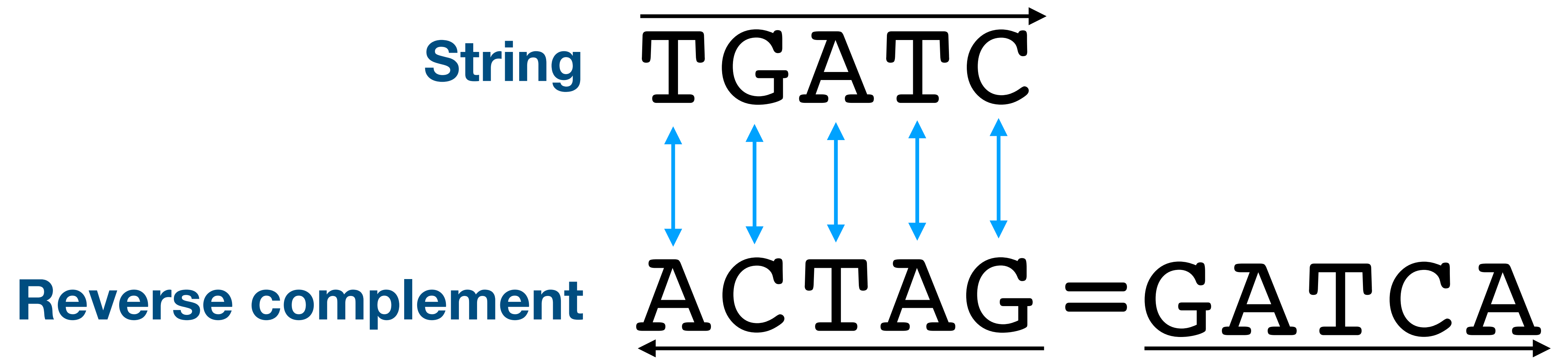

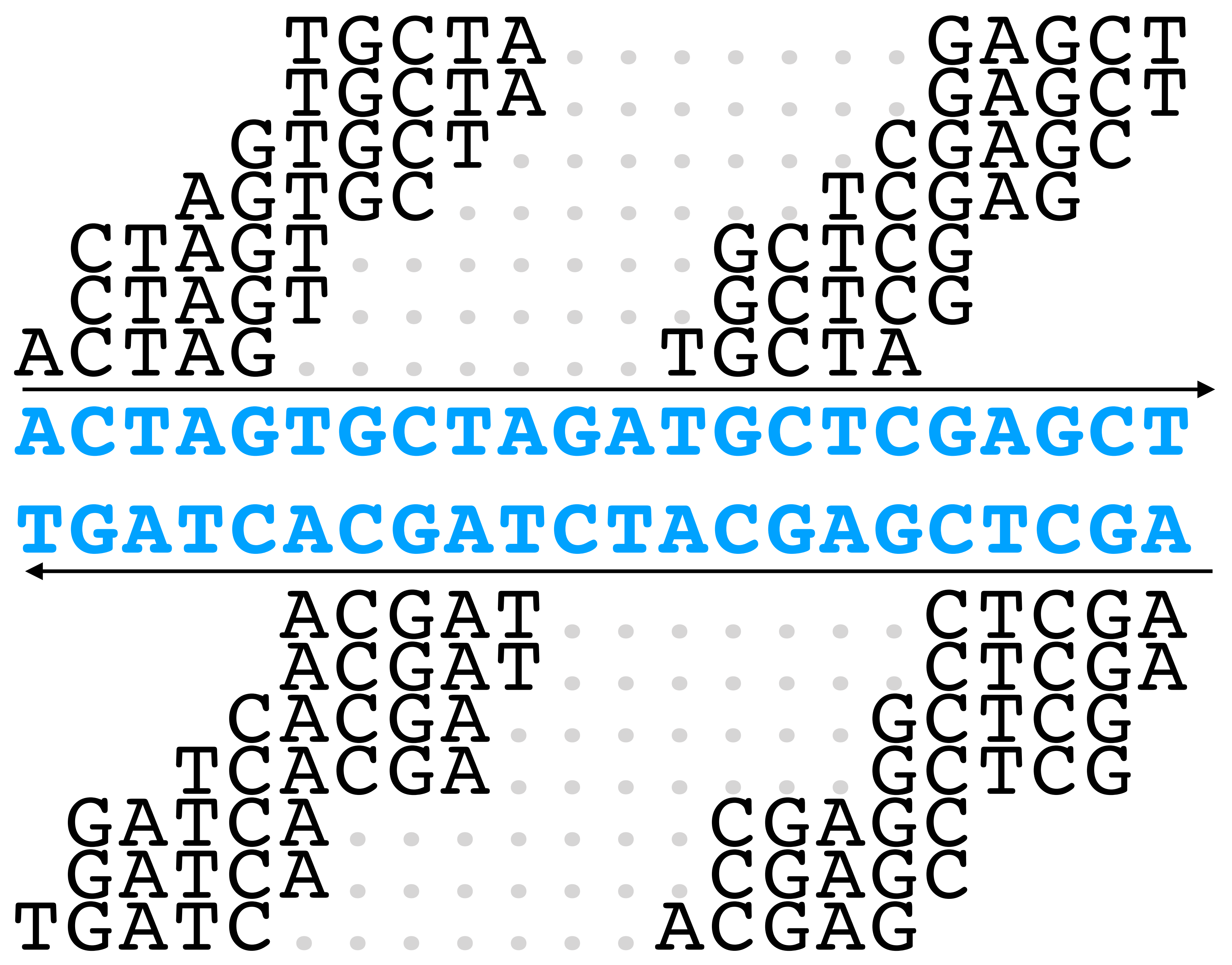

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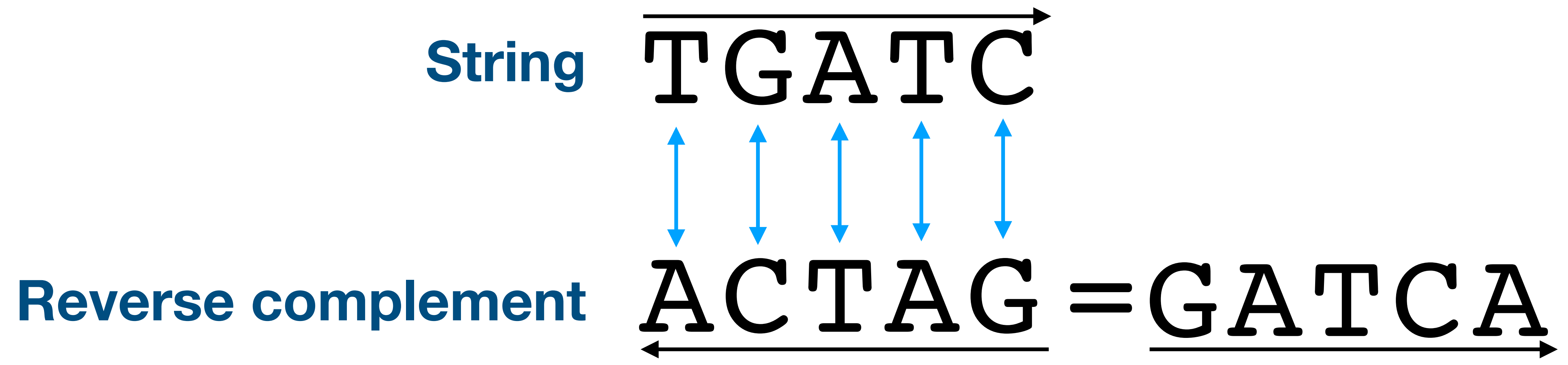

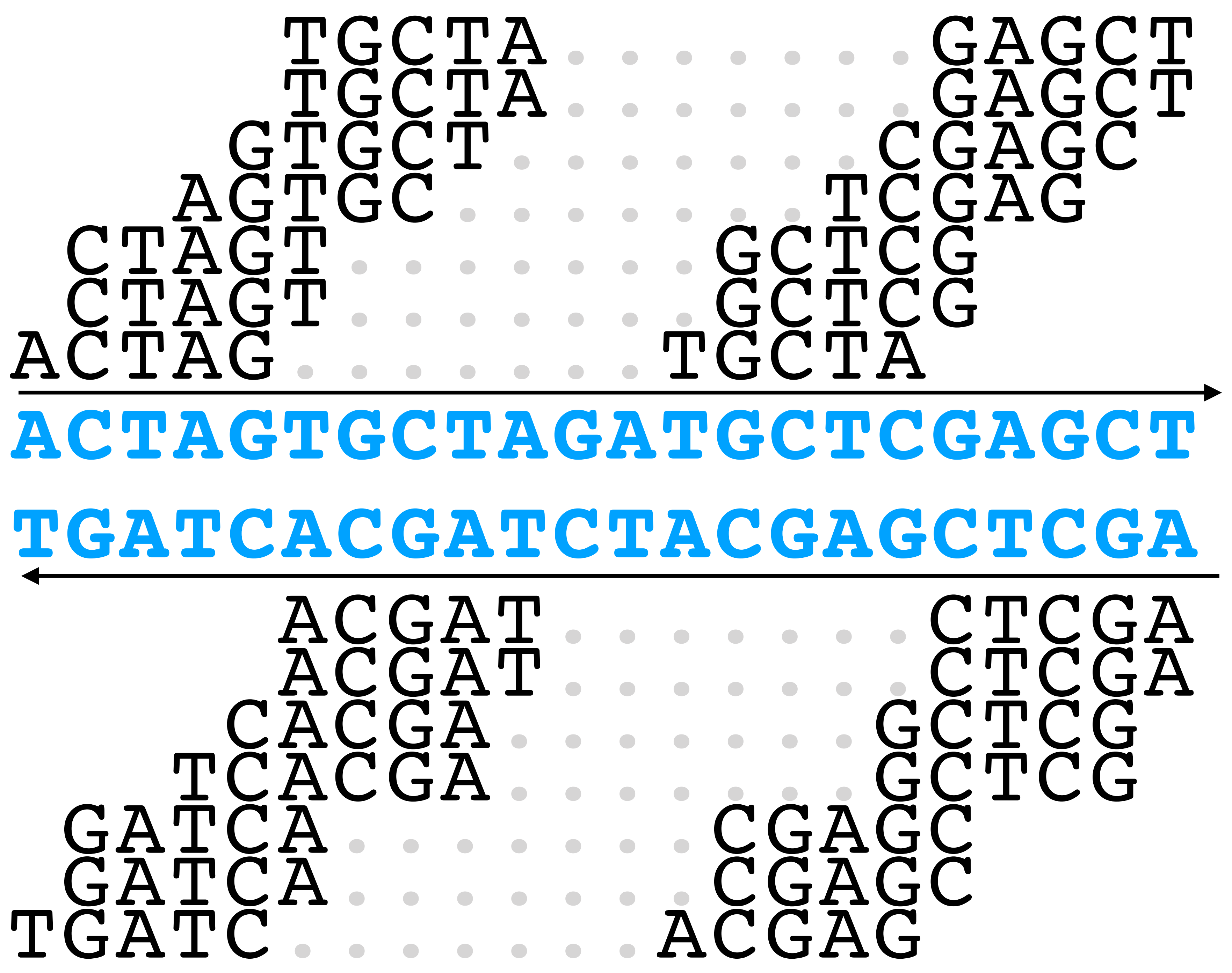

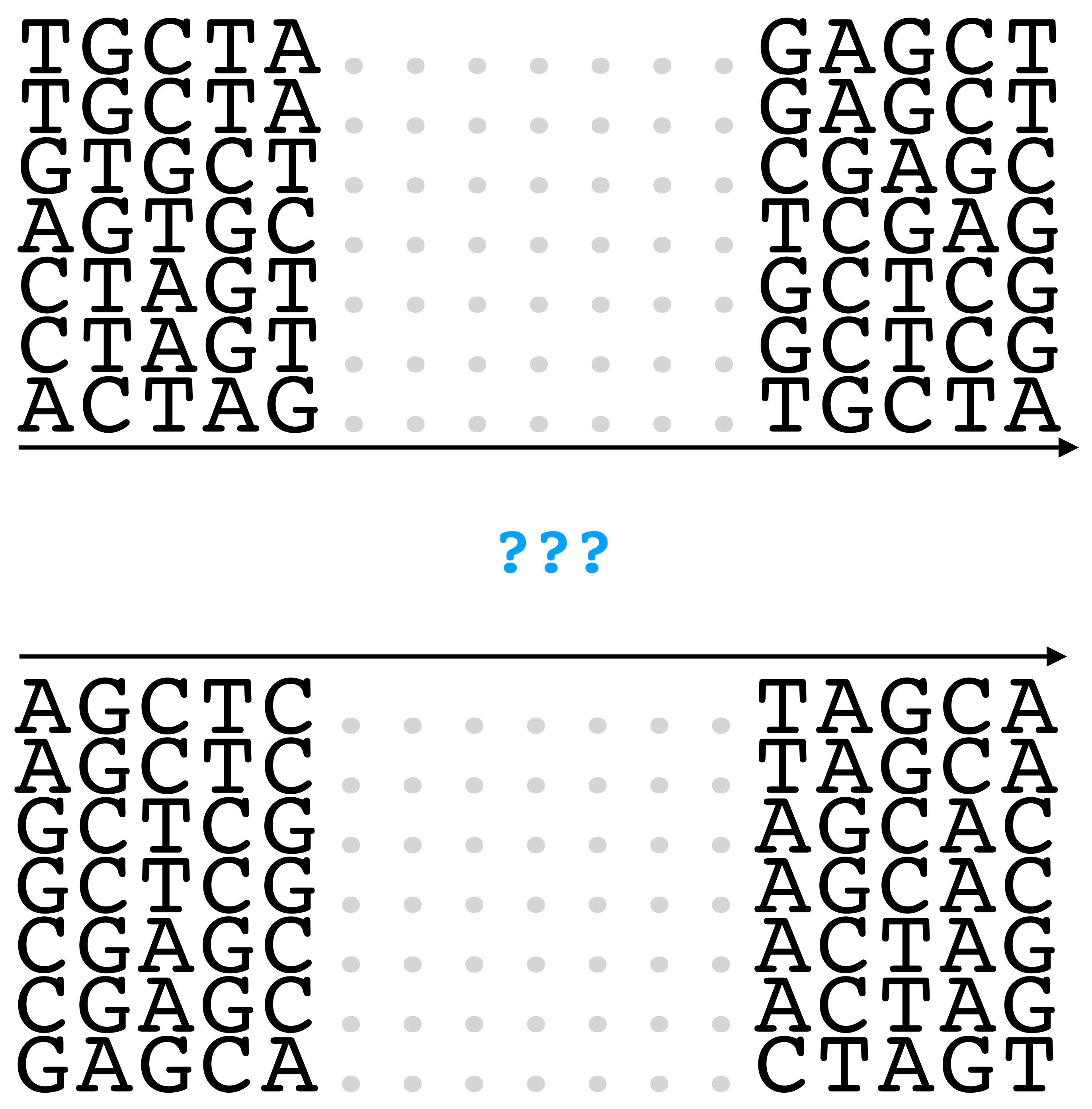

## Large amount of data

NOT IN THE LECTURE

| | Genome length | Total bases at 30x coverage | Size if each base takes 2 bits |
|---|---|---|---|
| **E. coli** | $4.6 \cdot 10^6$ | $138 \cdot 10^6$ | 34 MBytes |
| **Human** | $3.2 \cdot 10^9$ | $96 \cdot 10^9$ | 24 GBytes |
| **Spruce** | $25 \cdot 10^9$ | $750 \cdot 10^9$ | 187.5 GBytes |
| **Axolotl** | $32 \cdot 10^9$ | $960 \cdot 10^9$ | 240 GBytes |

## Theoretical problem formulations

("Classical" computational formulations of how to obtain the output from the input)

## Shortest superstring

**INPUT:** A collection of strings (the reads)

**OUTPUT:** A string $S$ such that every given string is a substring of $S$ ($S$ is a *superstring*), and $S$ is shortest

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```
TAGA
ATAG
CATA
TCAT
```

**TCATAGA**

**Input** **Output $S$**

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ATAG        ATAG
CATA       CATA
TCAT      TCAT
          TCATAGA
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- NP-hard to compute (i.e. it cannot be solved efficiently)
- Not practical: it collapses repeats (main drawback)

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```
The genome  TCATATATAGA

The reads   TCAT
             CATA
              ATAT
               TATA
                ATAT
                 TATA
                  ATAG
                   TAGA
```

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```
The genome  TCATATATAGA          The shortest   TCATATAGA
                                 superstring S
            TCAT                                TCAT
             CATA                                CATA
              ATAT                                ATAT
               TATA                                TATA
The reads       ATAT             The input        ATAT
                 TATA                              TATA
                  ATAG                              ATAG
                   TAGA                              TAGA
```

# Overlap graphs + Hamiltonian path

```
ACTAGACTAGACC
ACTA
 CTAG
  TAGA
   AGAC
    GACT
     ACTA
      CTAG
       TAGA
        AGAC
         GACC
```

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**INPUT:** Overlap graph of order $t$:

- Every read is a node
- Every suffix-prefix overlap of length $\geq t$ is an edge

**OUTPUT:** A path going through every node (i.e. read) exactly one (*Hamiltonian*)

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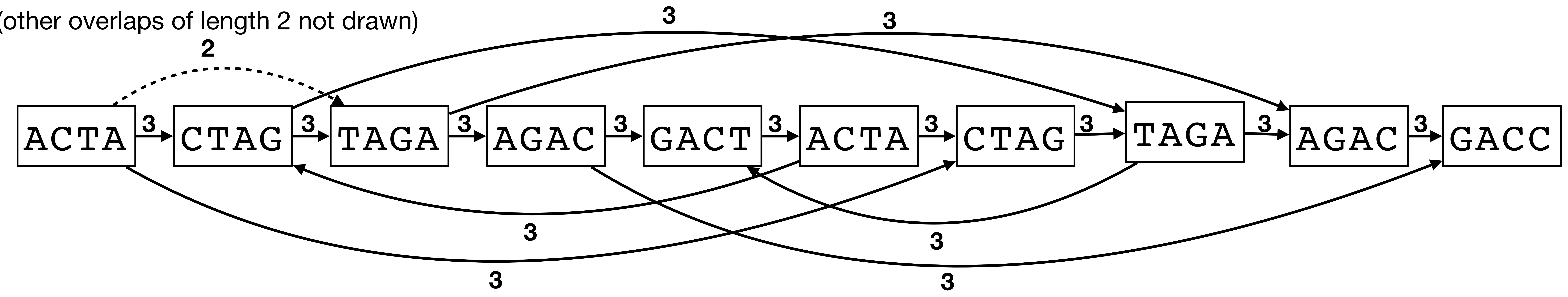
Overlap graph of order 2:

(other overlaps of length 2 not drawn)



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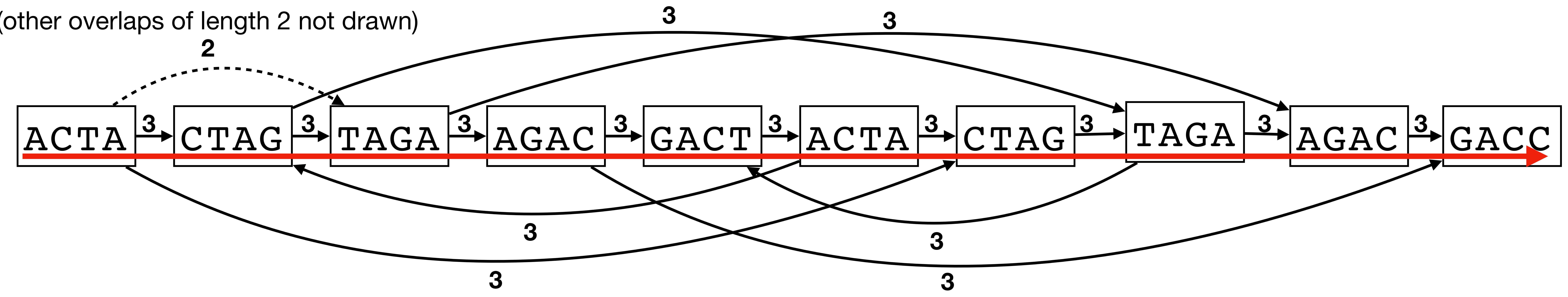

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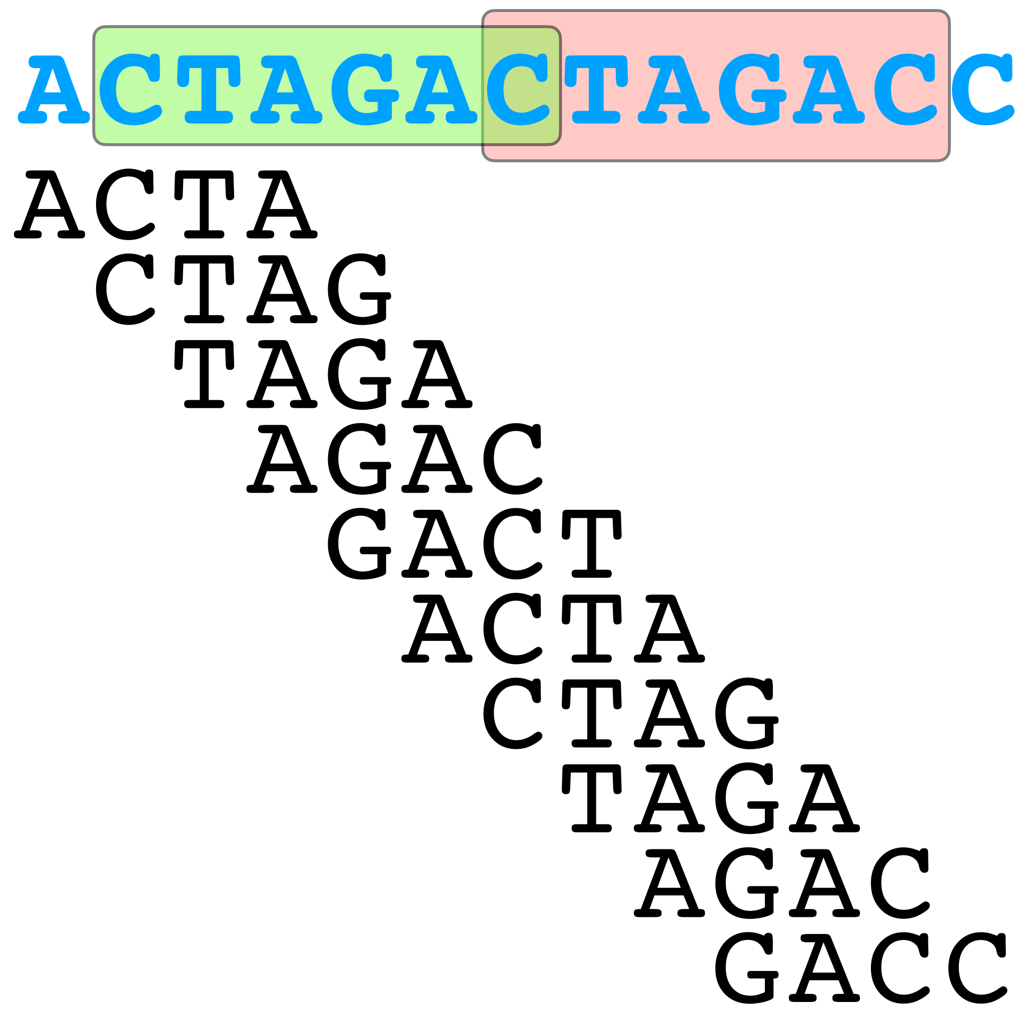

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- Not practical: usually graph has no Hamiltonian path (missing coverage, errors)

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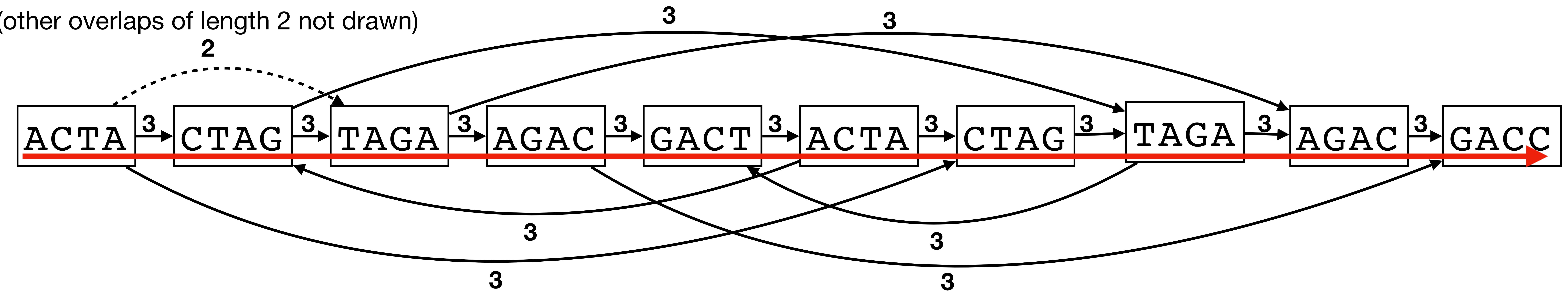

# De Bruijn graphs + Eulerian path

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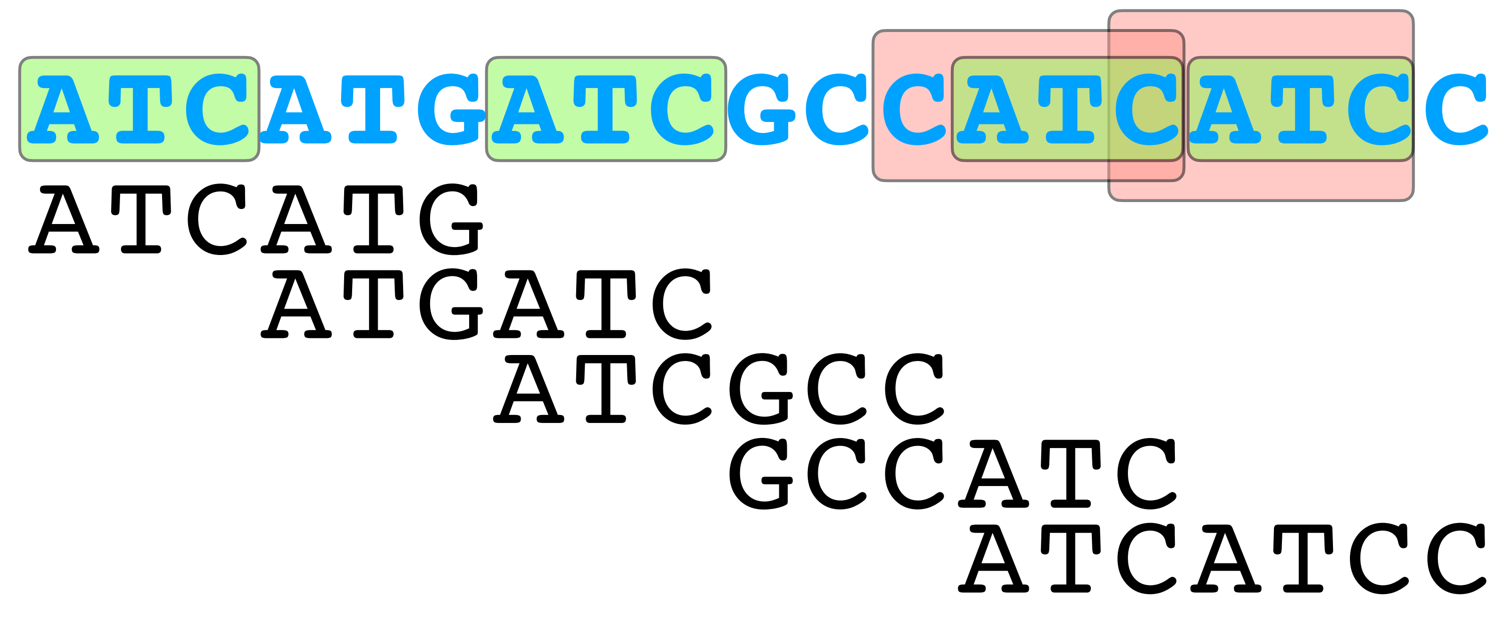

**INPUT:** De Bruijn graph of order $k$:

- Every $k$-mer (substring of length $k$) in the reads is a ***single*** node
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**ASSUMPTION:** Every length-$(k+1)$ interval of the genome appears the same number of times in the reads (*uniform coverage*)

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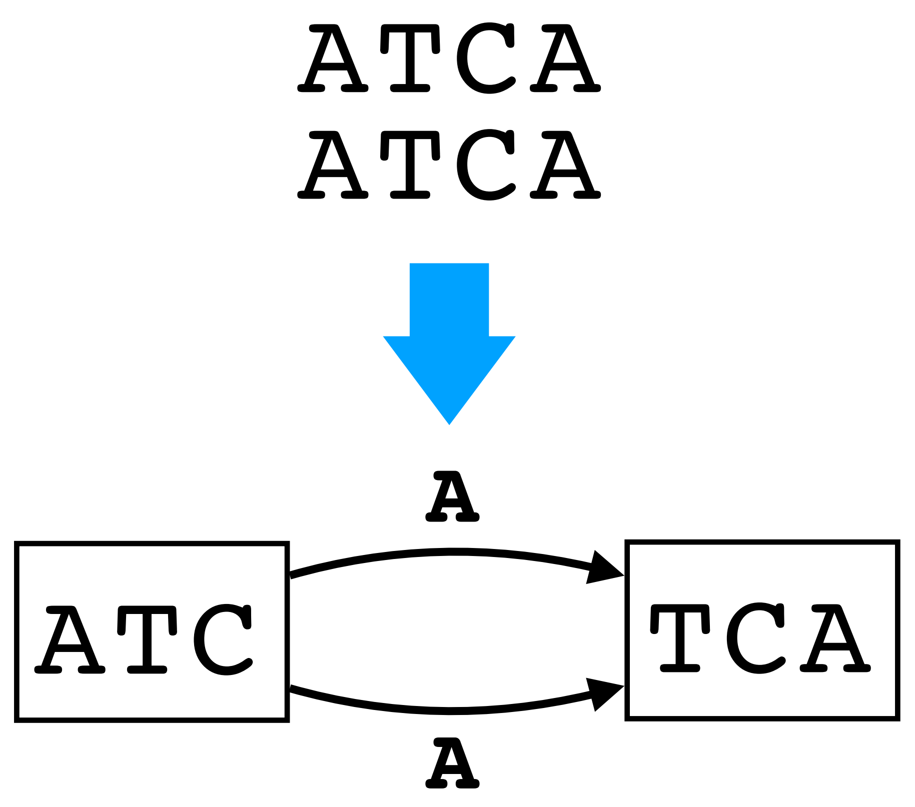

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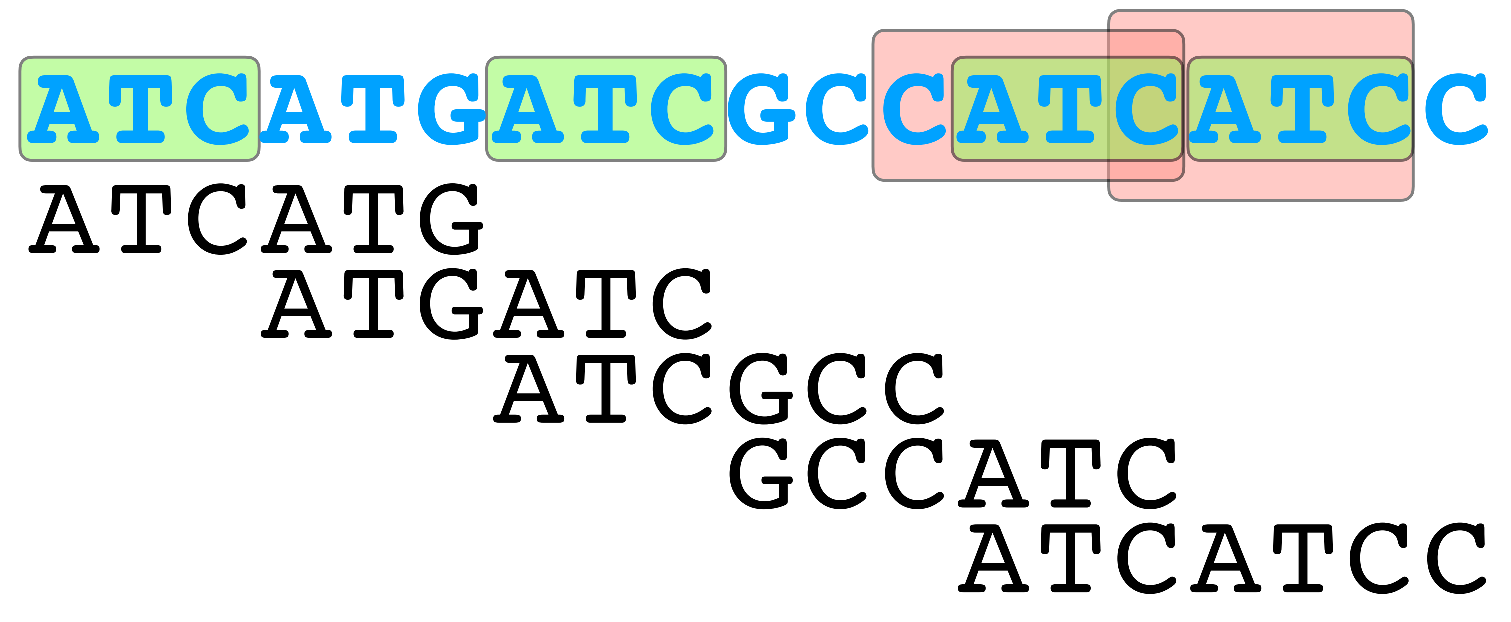

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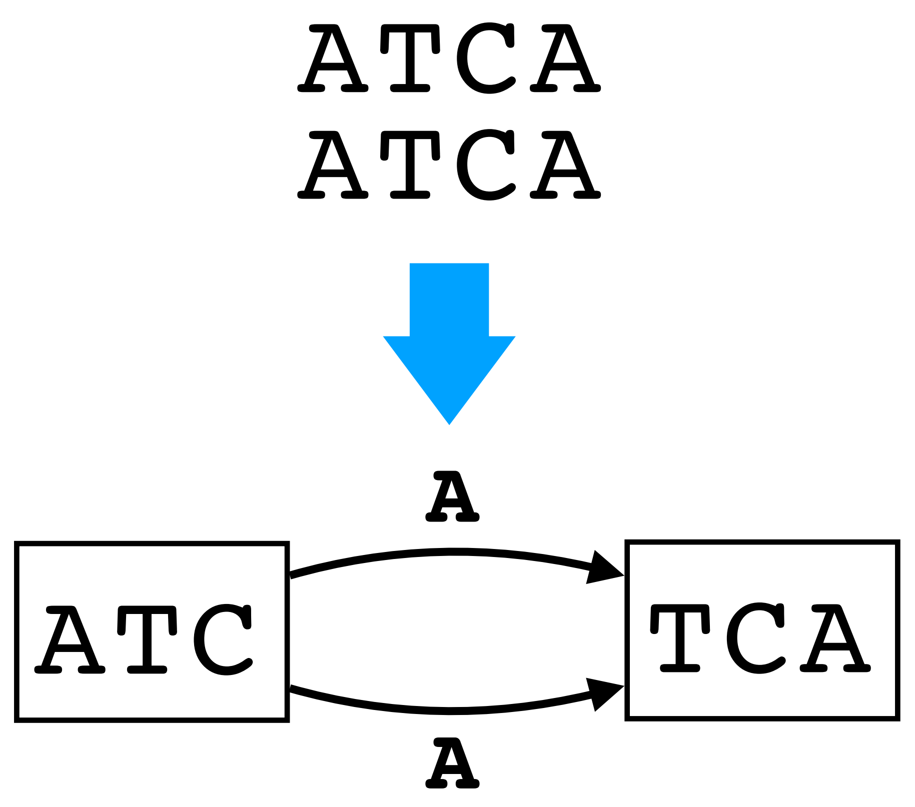
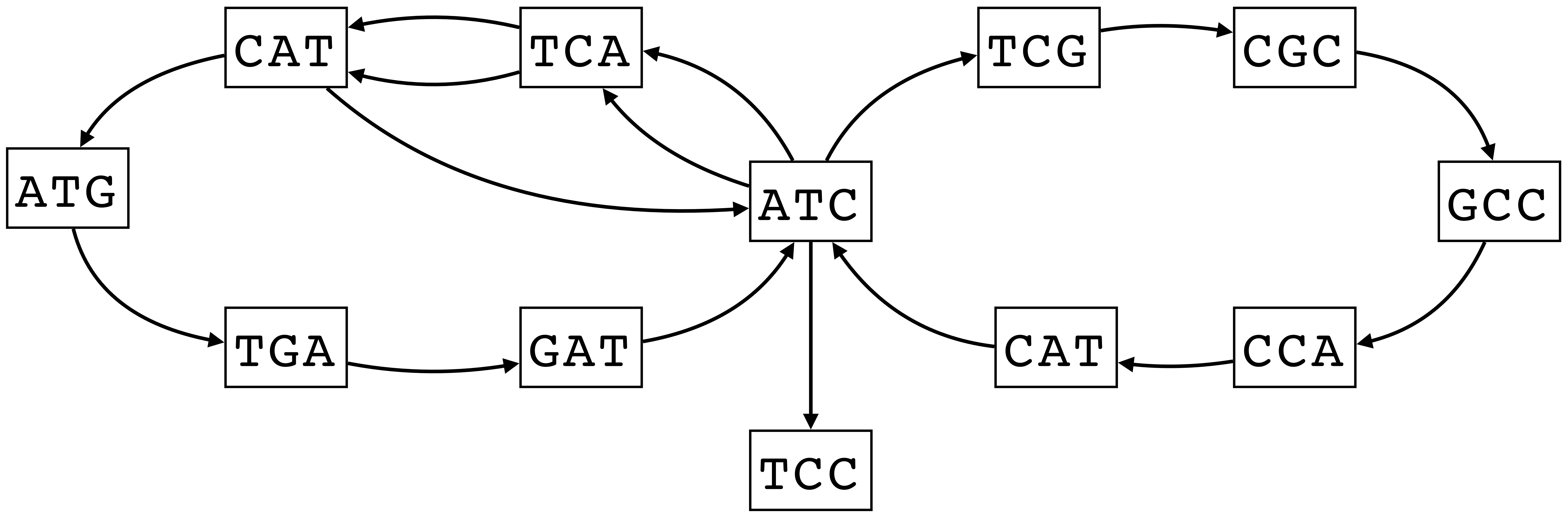


De Bruijn graph of order 3:



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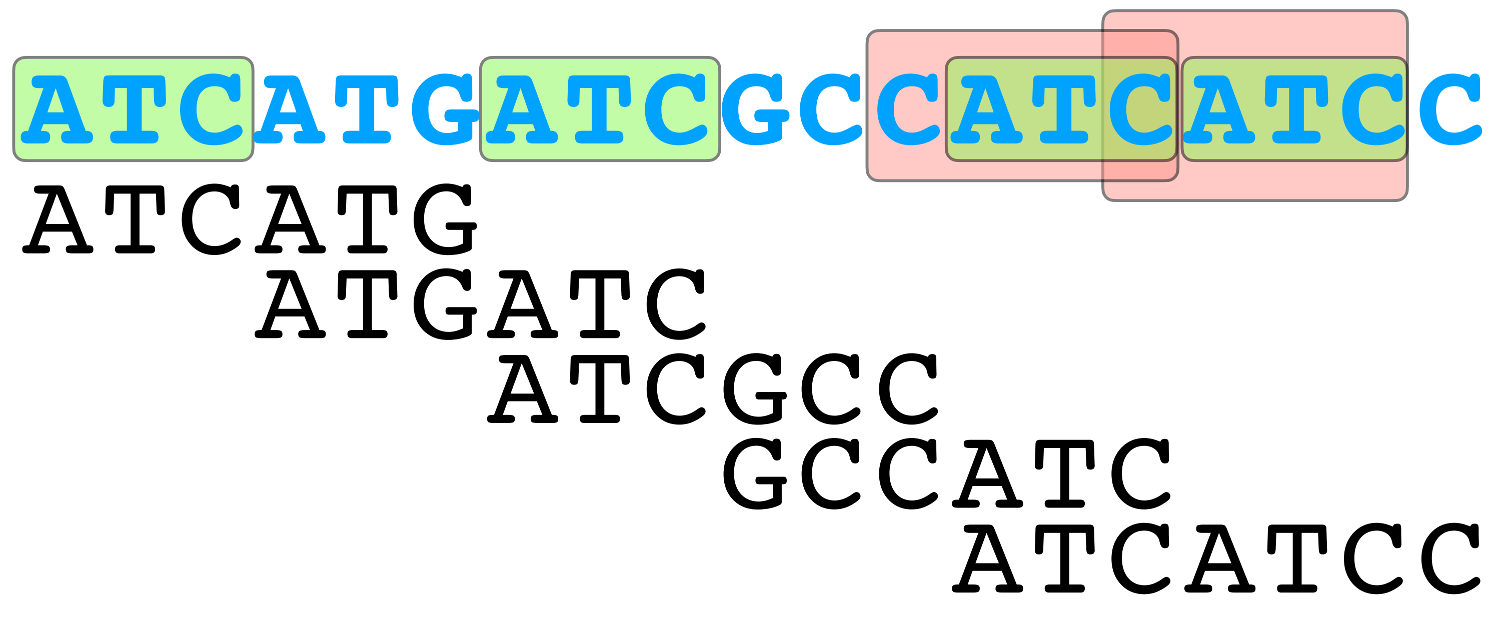

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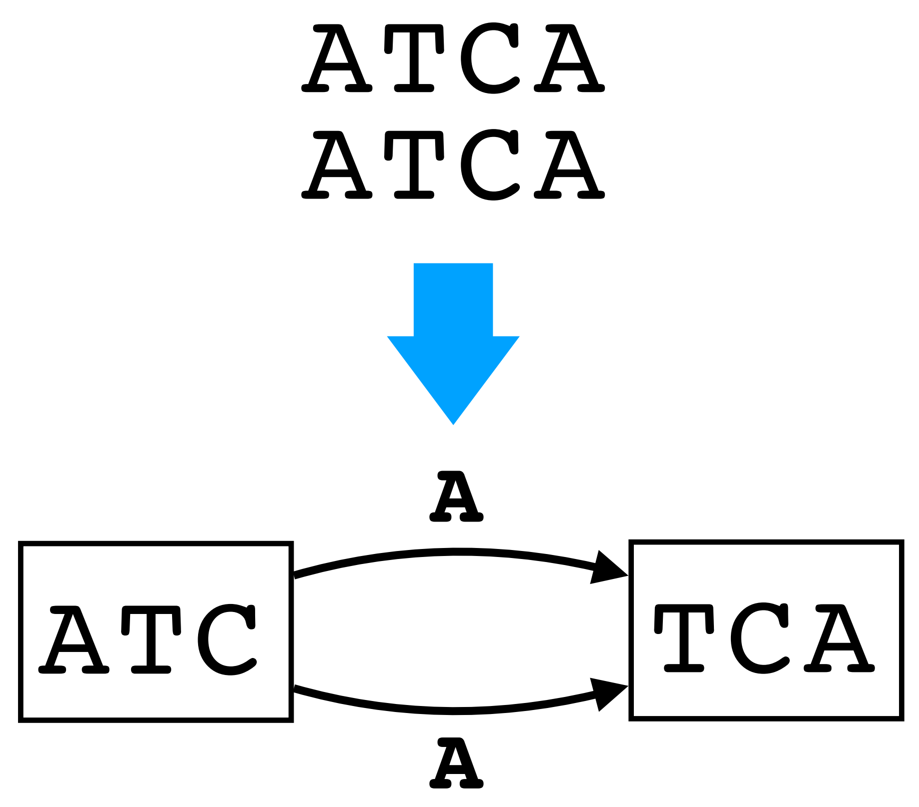

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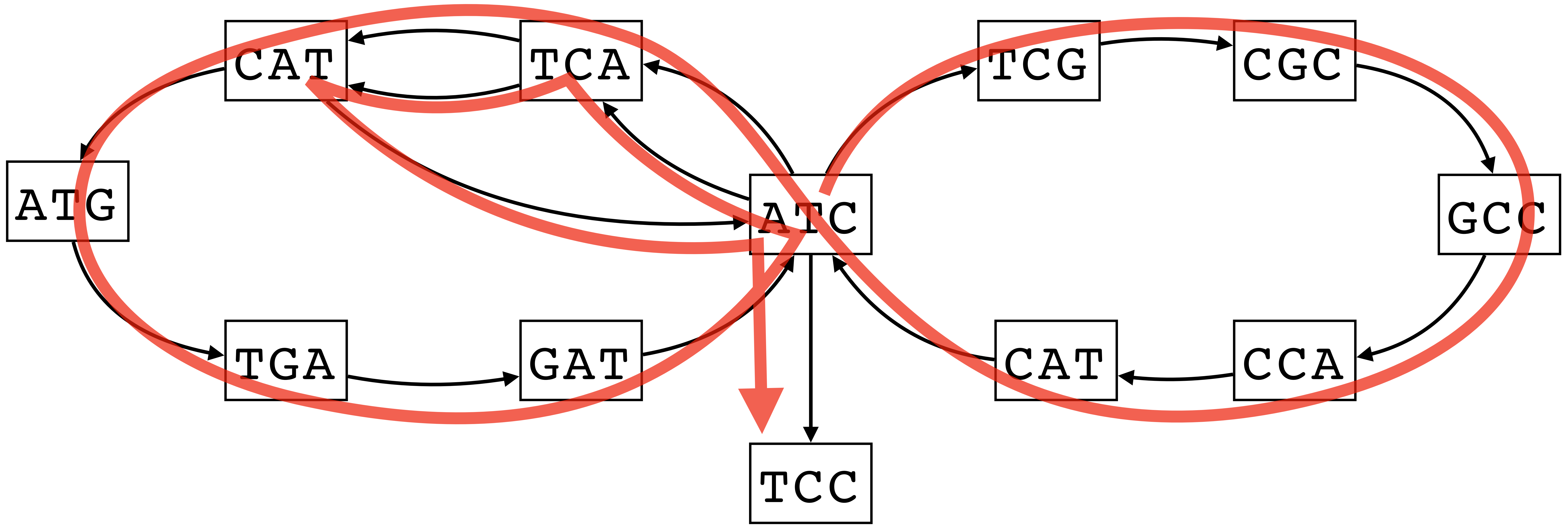

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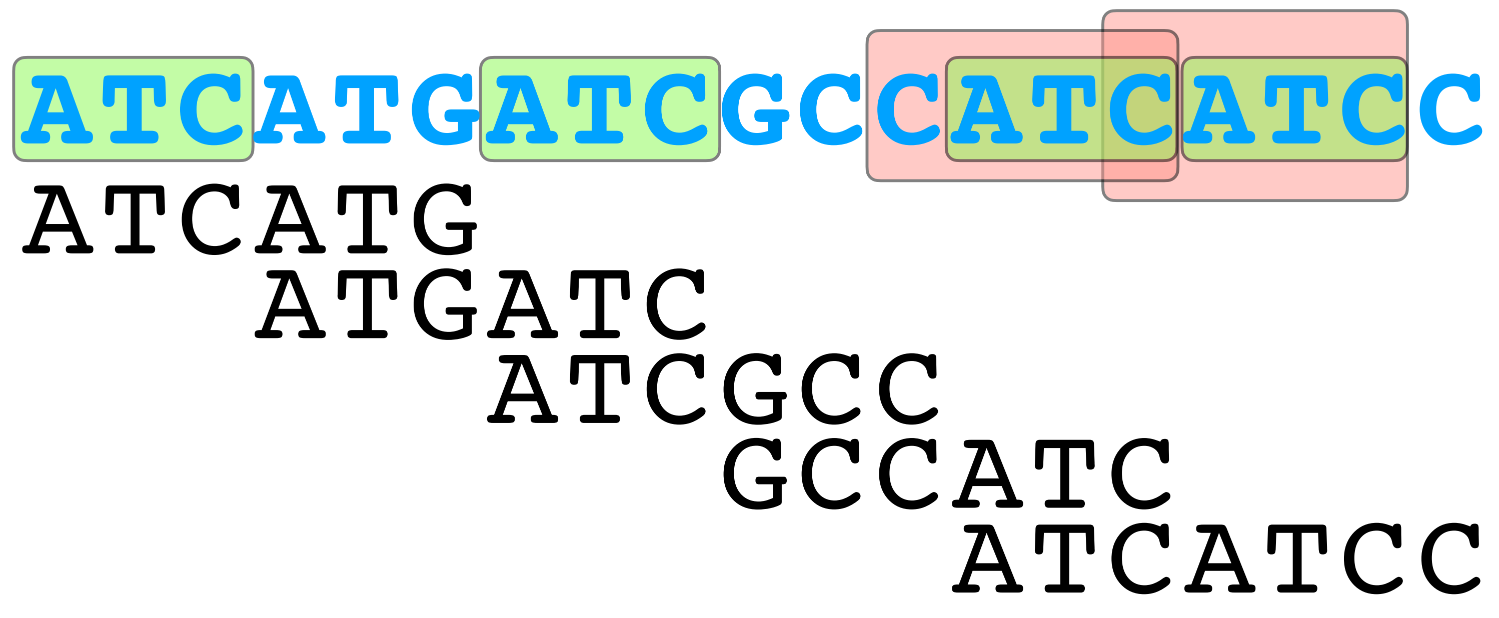

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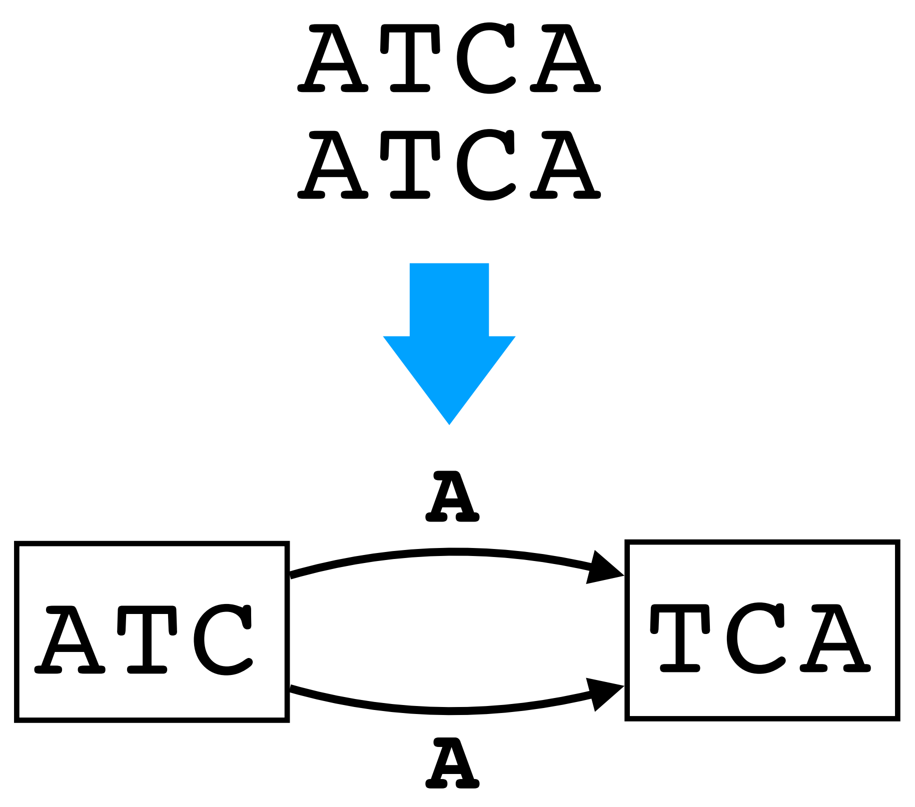

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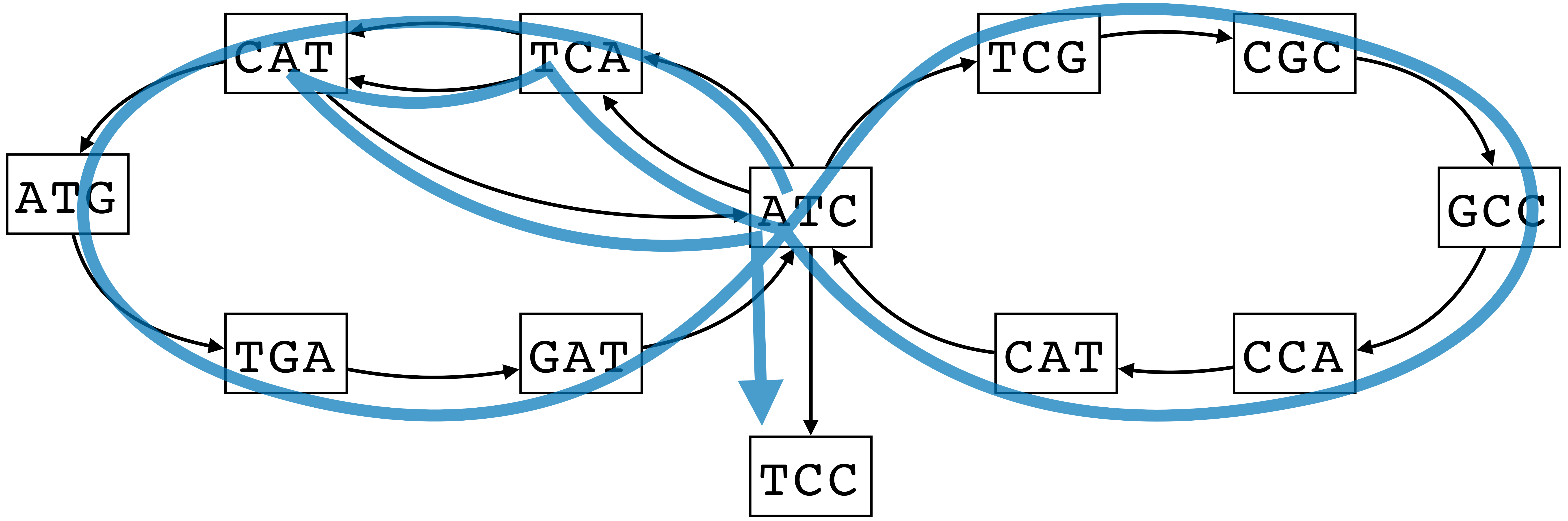

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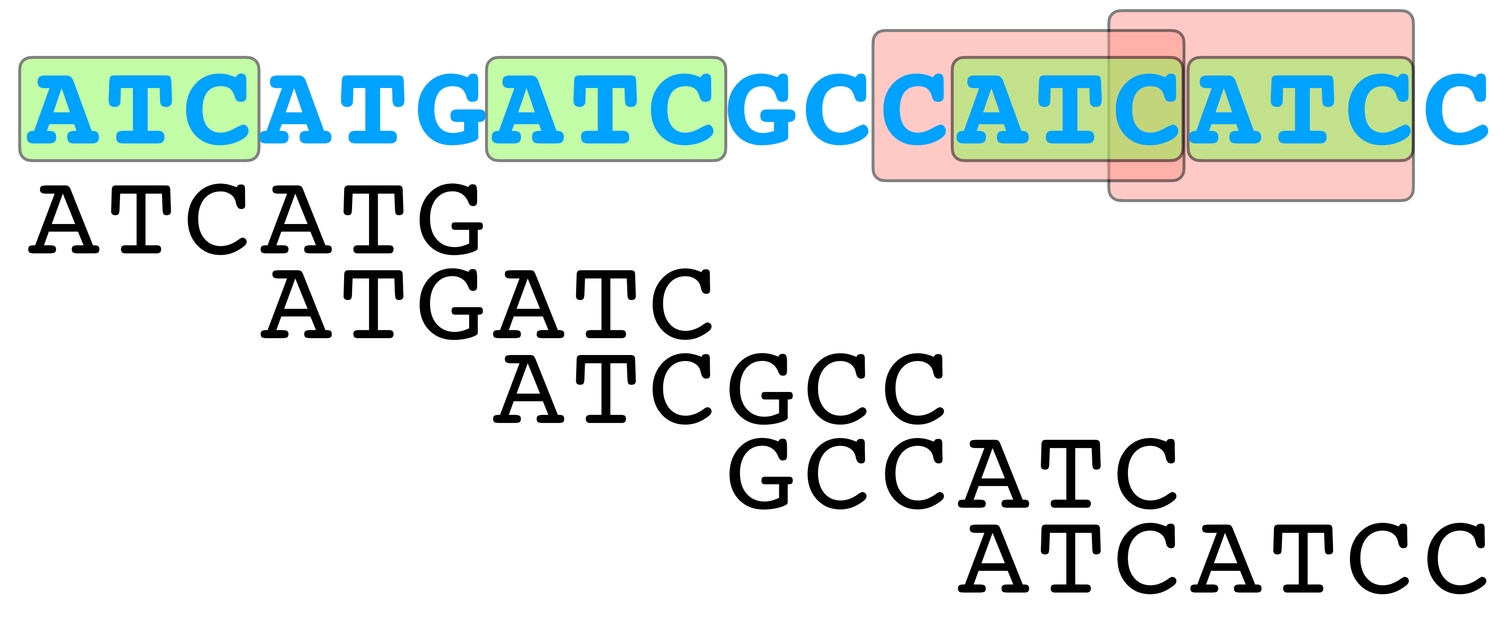

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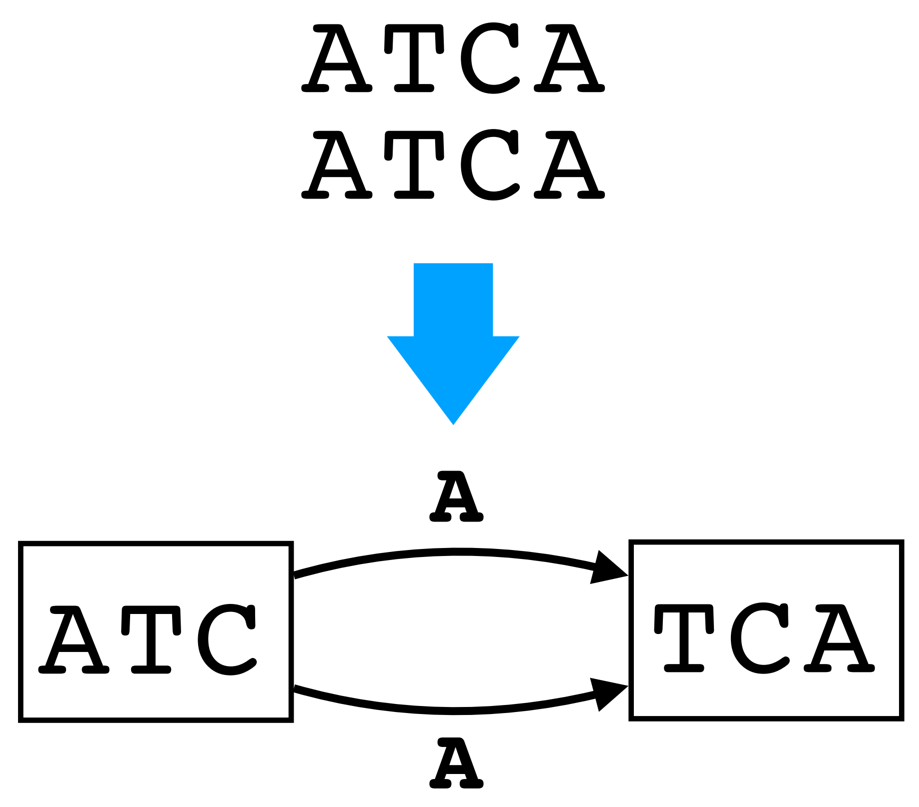
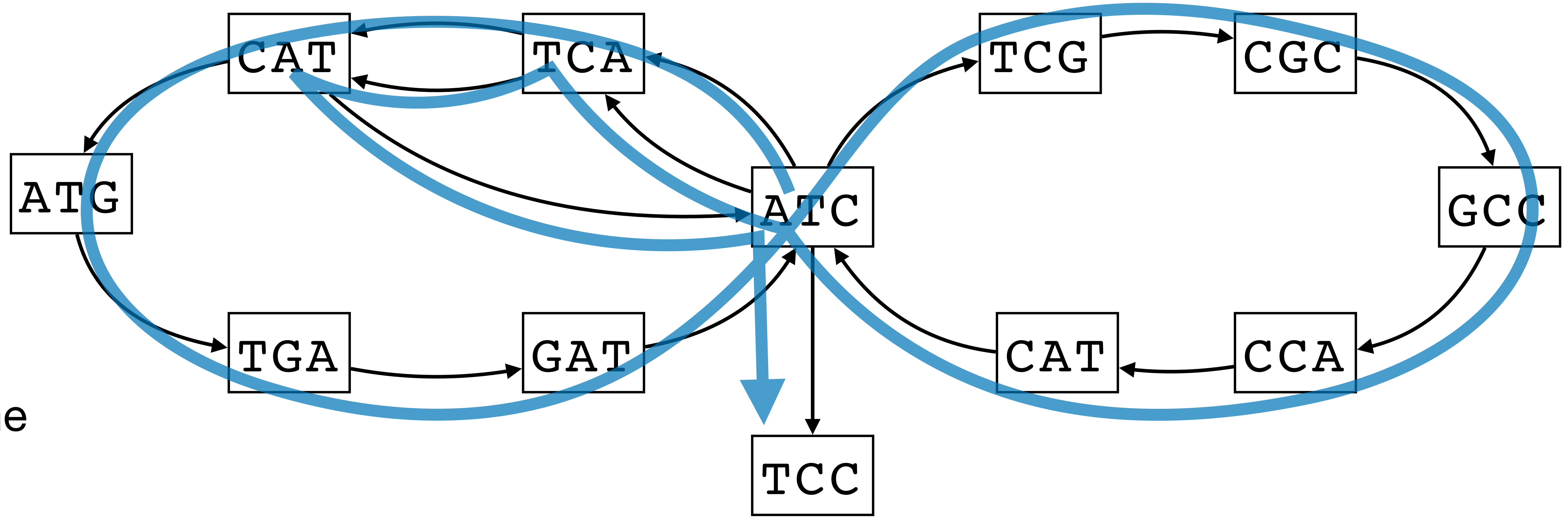
**OUTPUT:** A path going through every ***edge*** (i.e. $(k+1)$-mer) exactly one (*Eulerian*)



De Bruijn graph of order 3:



- Can be solved in *O(|edges|)* time
- Too restrictive assumption

# Section summary

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- Computational complexity ranges from NP-hard to linear
- Not robust to practical issues, and hard to integrate them into the formulations
- Most importantly (even if the above are solved):
    - Many solutions, which one is the true genome?
    - We will see a different "theoretical" approach, closer to practice **AT END OF LECTURE**

# Practical genome assembly

(The sequence of algorithmic steps behind "real" genome assemblers)

# Contig assembly

Focus on single-end reads

Assembling a full genomic sequence in one shot is hopeless

→

Forget about the assembly model (i.e. the problem formulation)

Assemble only parts about which we are sure (contigs → **contig**uous sequences)

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- Assume an assembly graph (here de Bruijn with parallel edges collapsed into one)
- Focus on ***unitigs*** $=_{\text{def}}$ "non-branching" path
- (Usually) Contig $=_{\text{def}}$ unitig in a graph "corrected" for polyploidy

## Unitigs

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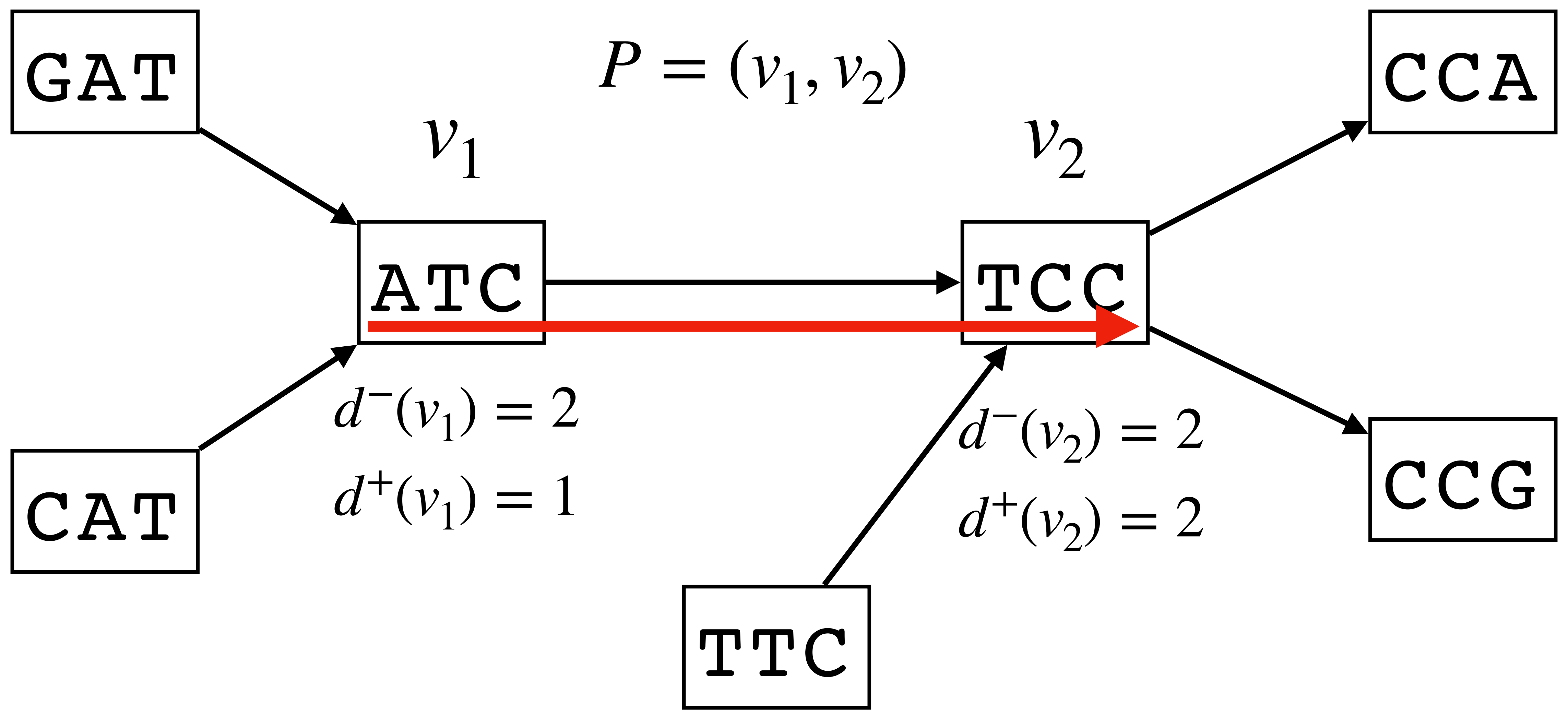

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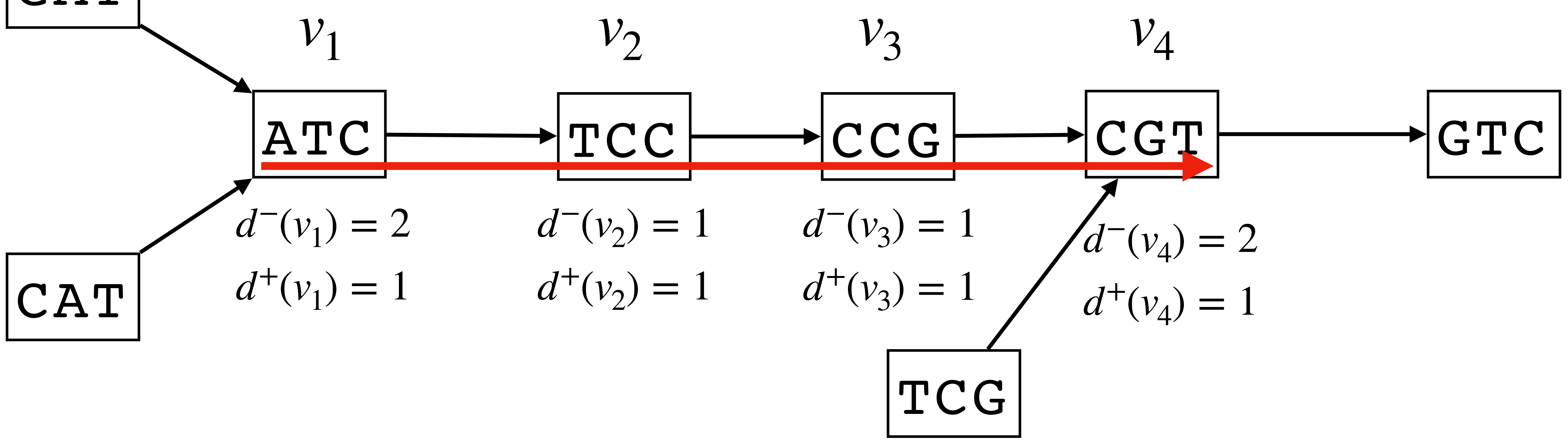
$$P = (v_1, v_2, v_3, v_4)$$



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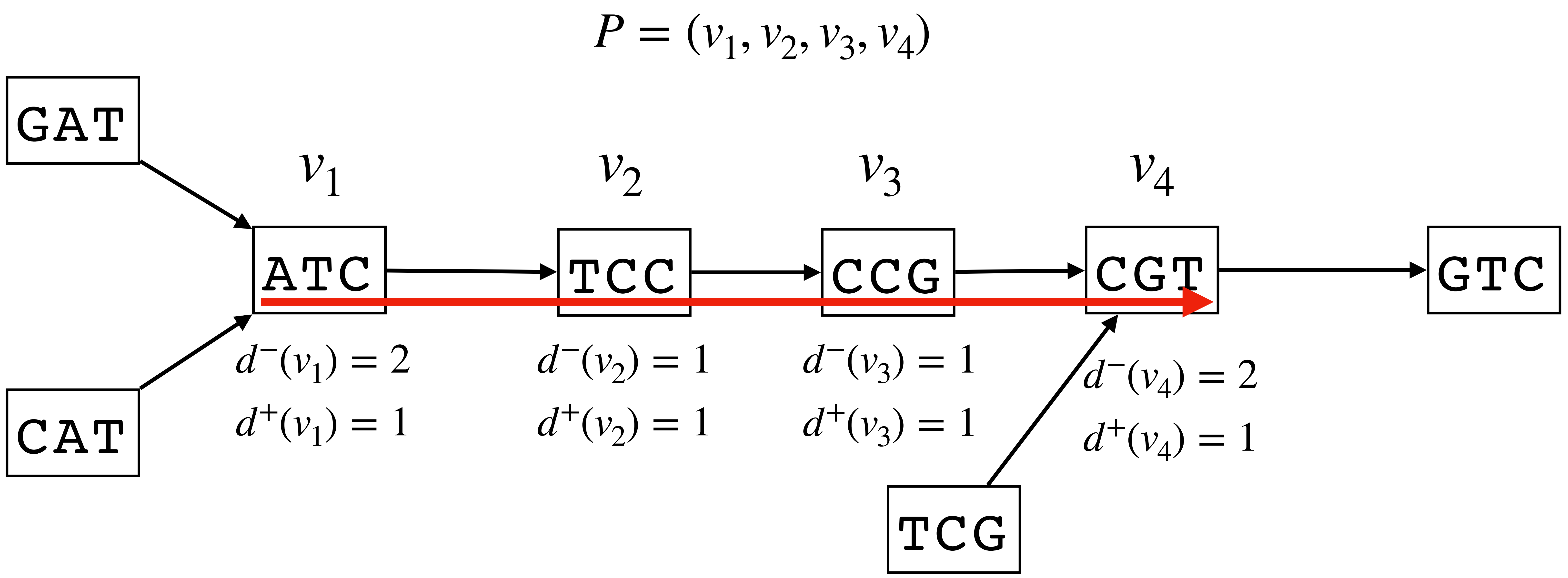

- We want maximal (longest) unitigs

# Example

$$\begin{tikzcd}
 & \mathtt{CAT} \arrow[dl] \arrow[drr, bend right] & \mathtt{TCA} \arrow[l, bend right] & & \mathtt{TCG} \arrow[r, bend left] & \mathtt{CGC} \arrow[dr, bend left] & \\
\mathtt{ATG} \arrow[dr, bend right] & & & \mathtt{ATC} \arrow[ul, bend right] \arrow[ur, bend left] \arrow[dd] & & & \mathtt{GCC} \arrow[dl, bend left] \\
 & \mathtt{TGA} \arrow[r, bend right] & \mathtt{GAT} \arrow[ur, bend right] & & \mathtt{CAT} \arrow[ul, bend left] & \mathtt{CCA} \arrow[l, bend left] & \\
 & & & \mathtt{TCC} & & &
\end{tikzcd}$$

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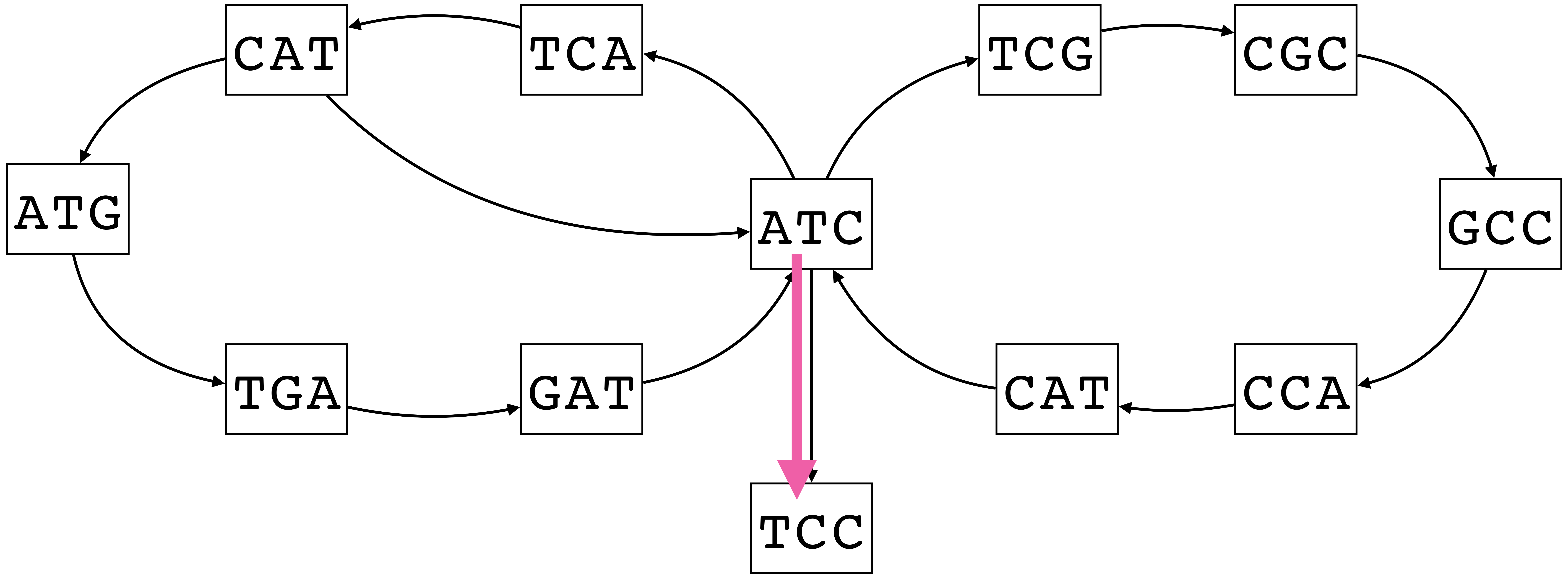

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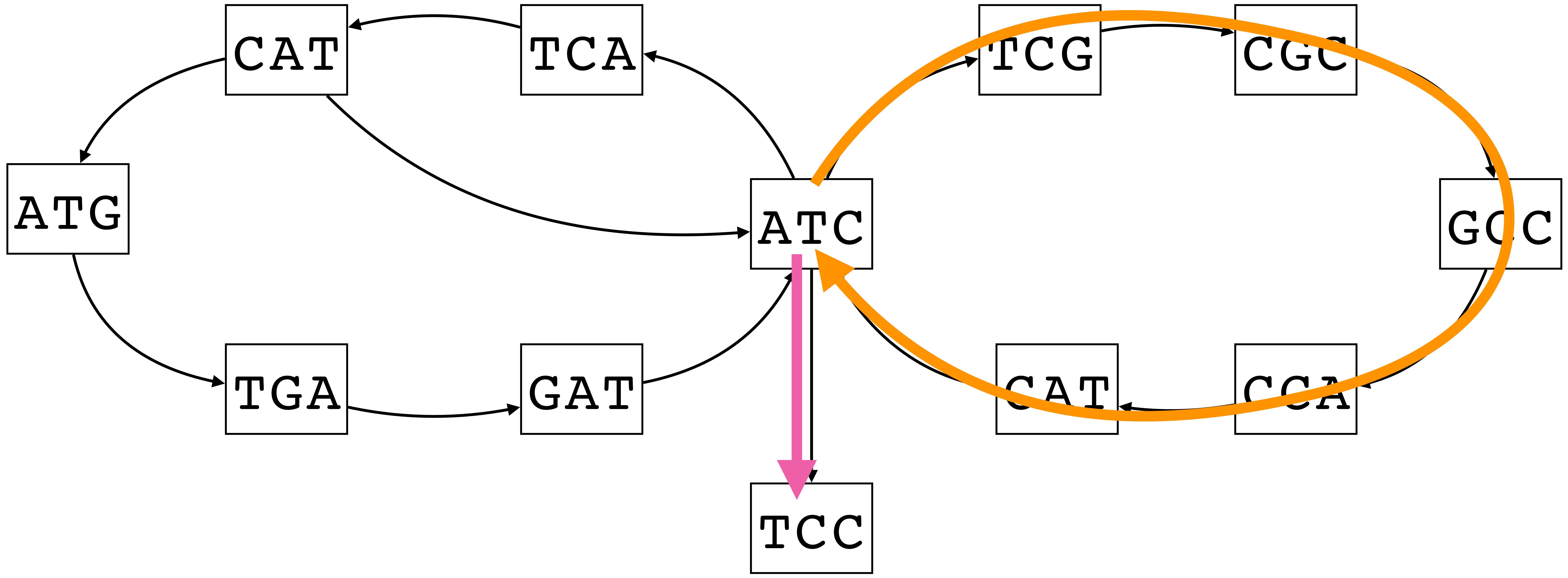

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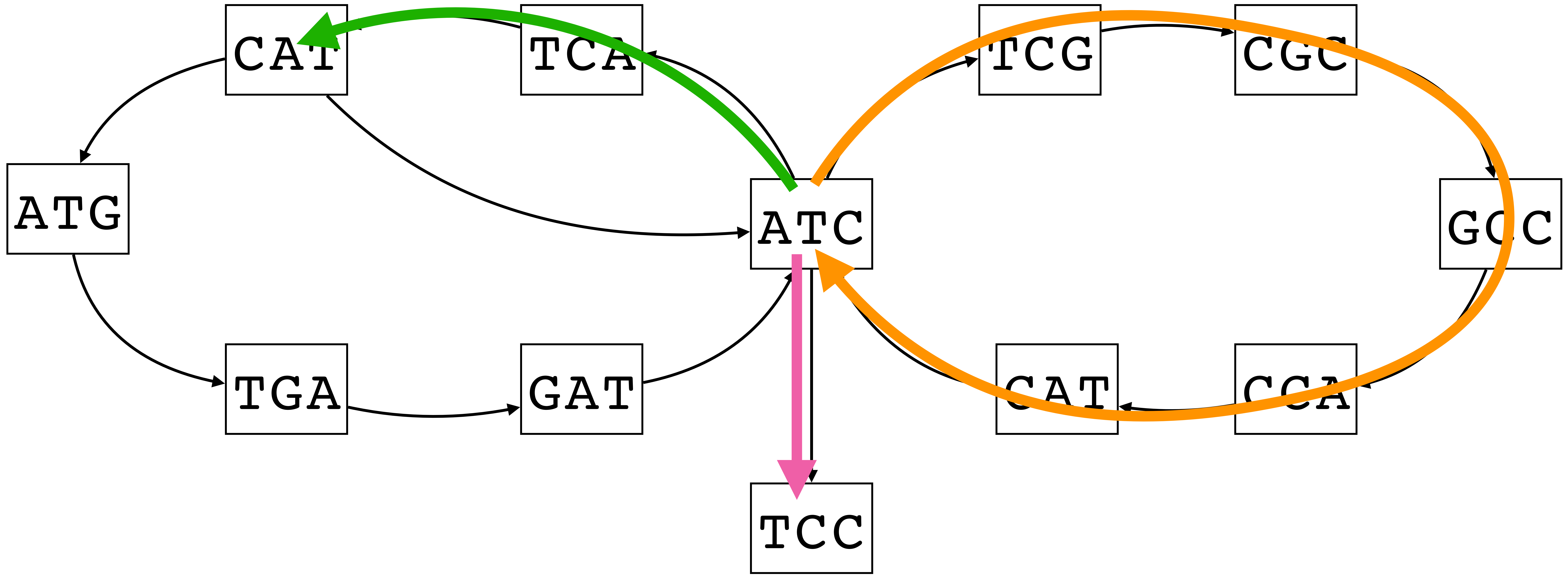

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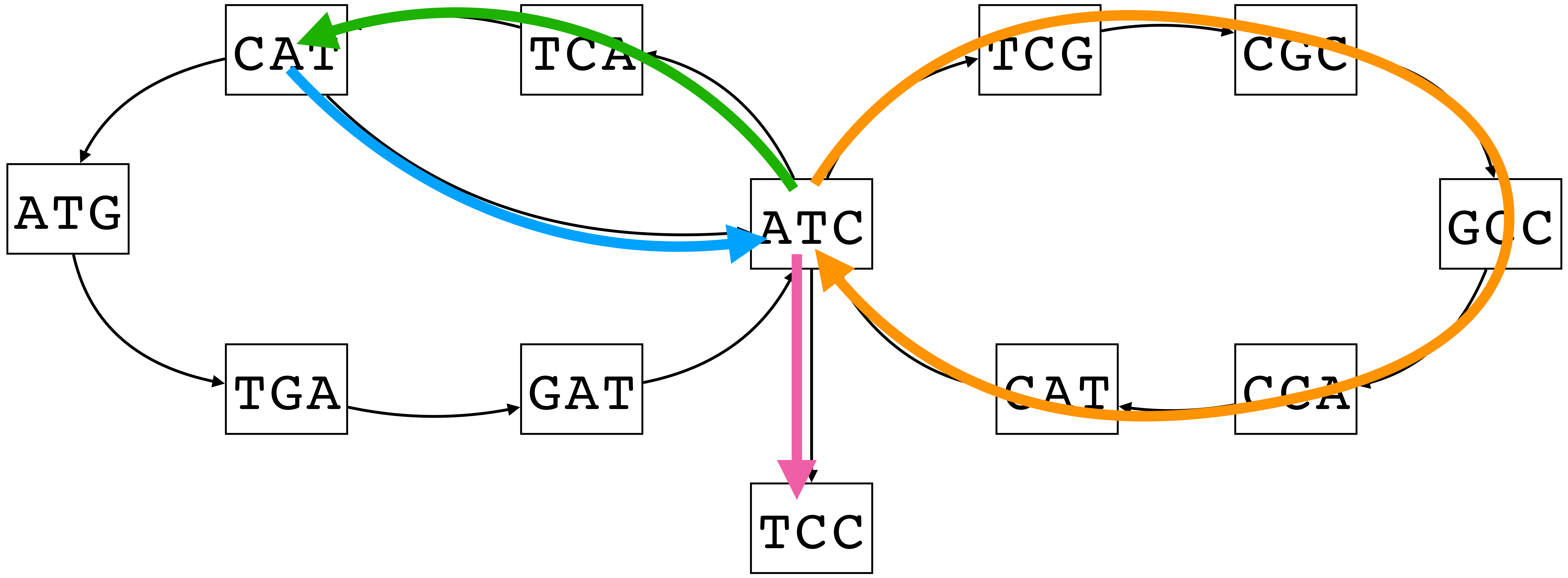

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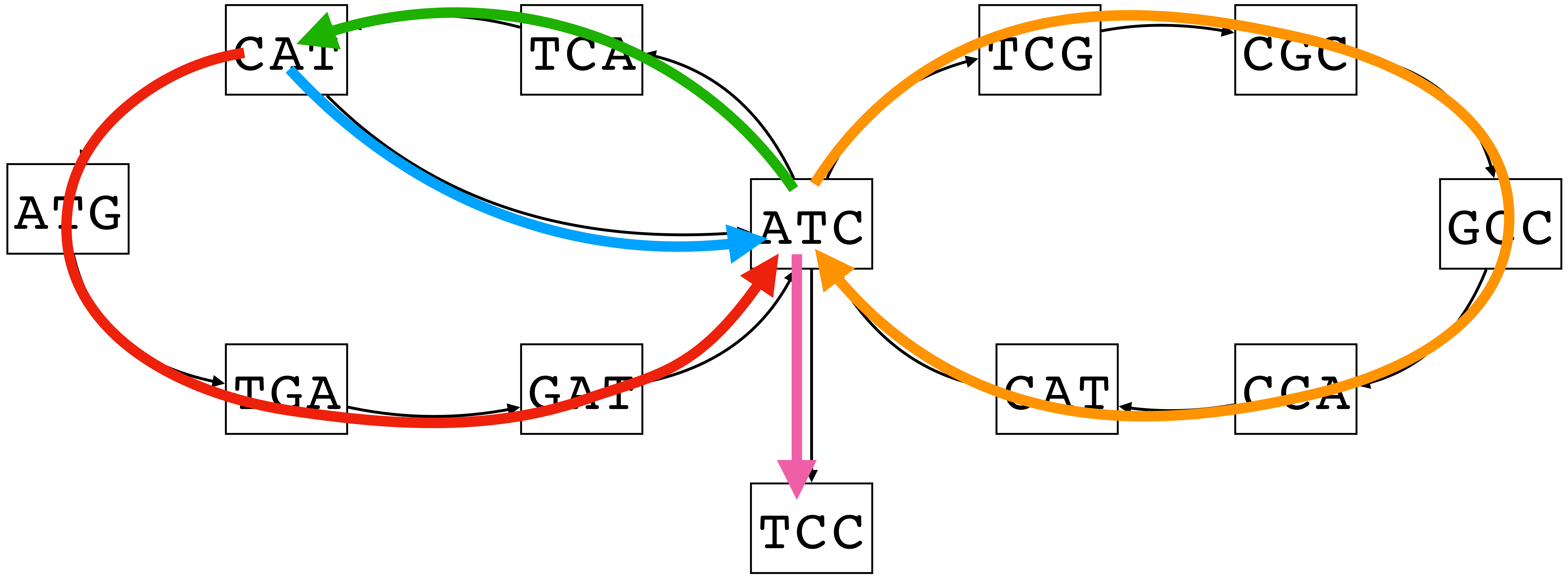

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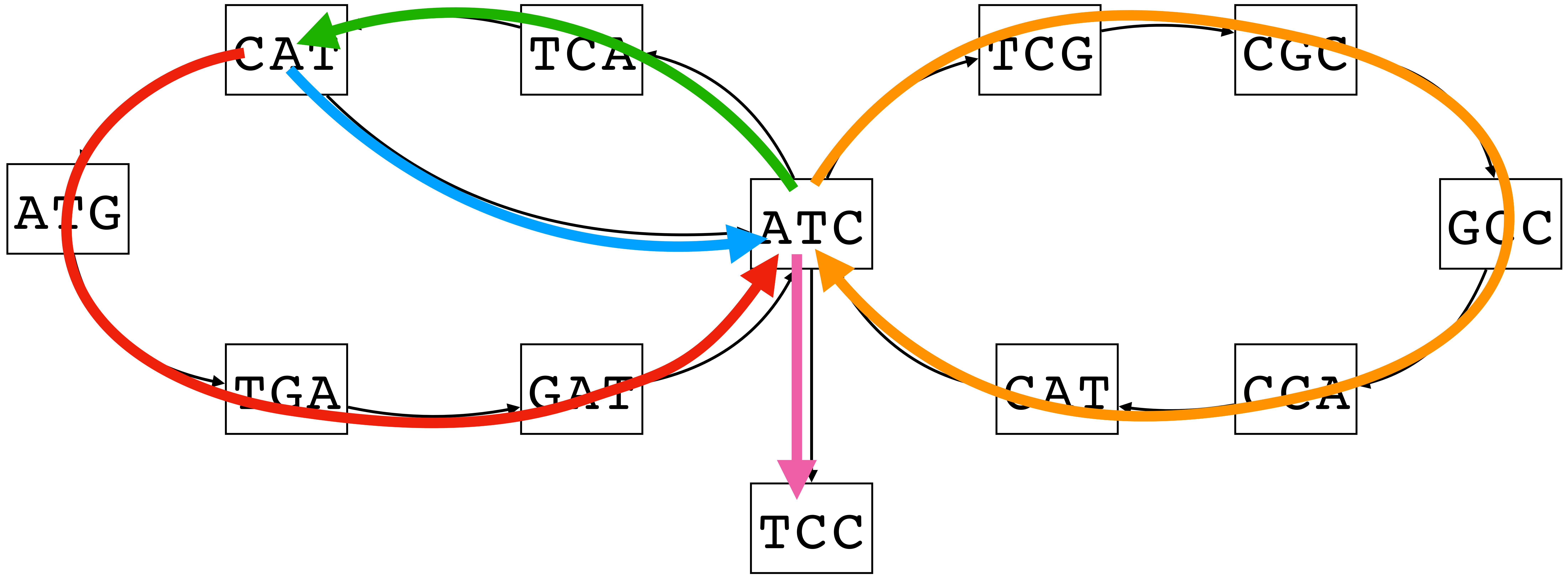

- Unitigs can be found in $O(|\text{edges}|)$ time

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```
             ATGTA
            CATGT
           GCATG
          TGCAT
         TTGCA
        ATTGC
       AATTG
Mother AATTGCATGTA
Father AATTGAATGTA
       AATTG
        ATTGA
         TTGAA
          TGAAT
           GAATG
            AATGT
             ATGTA
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              ATGTA
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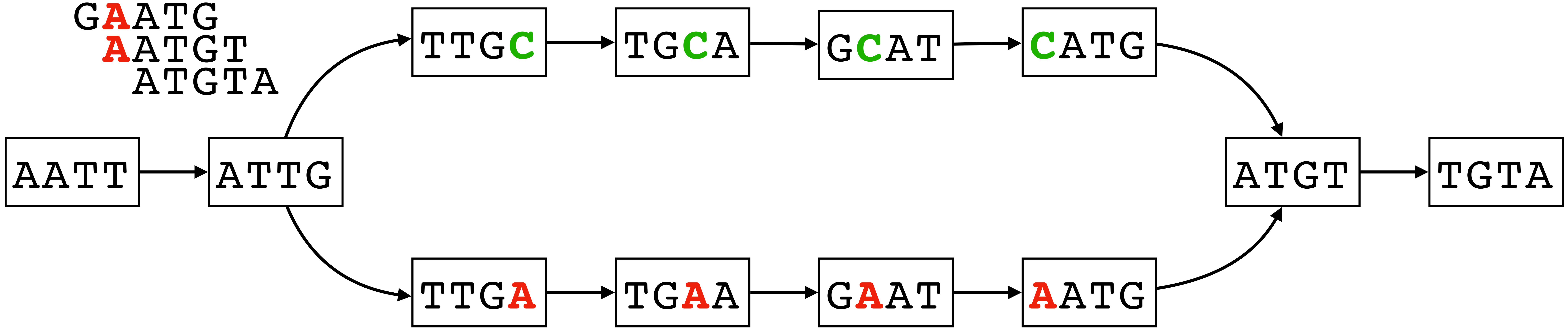

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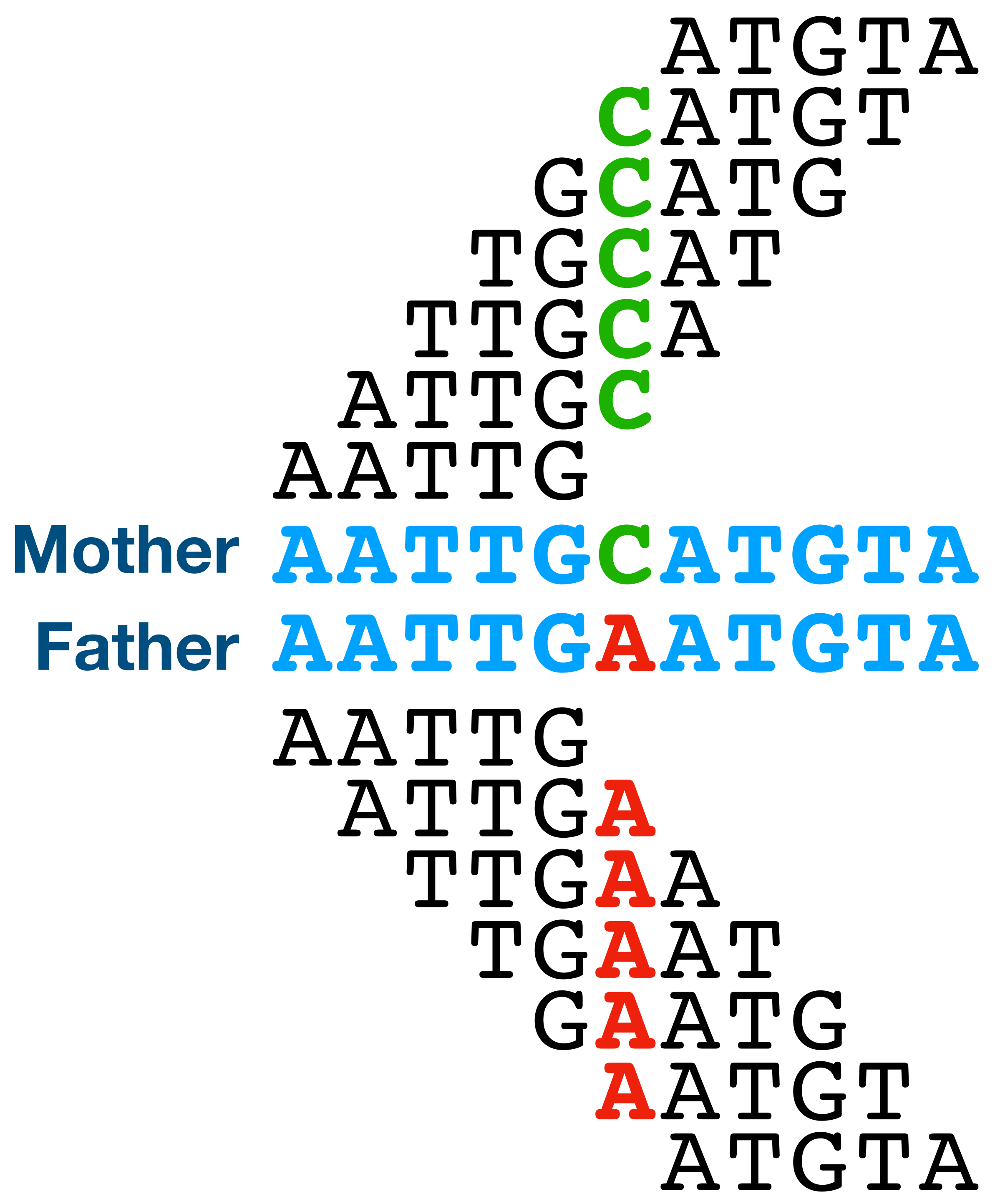

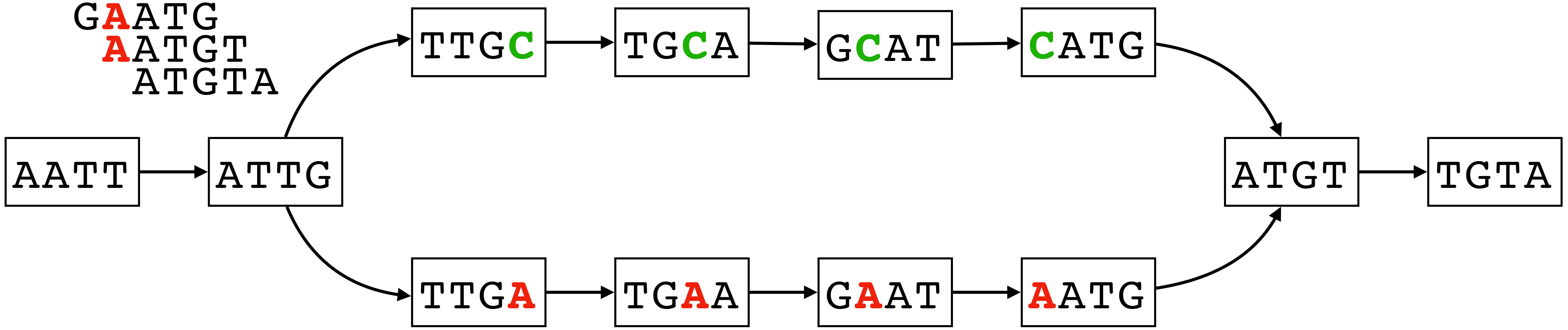

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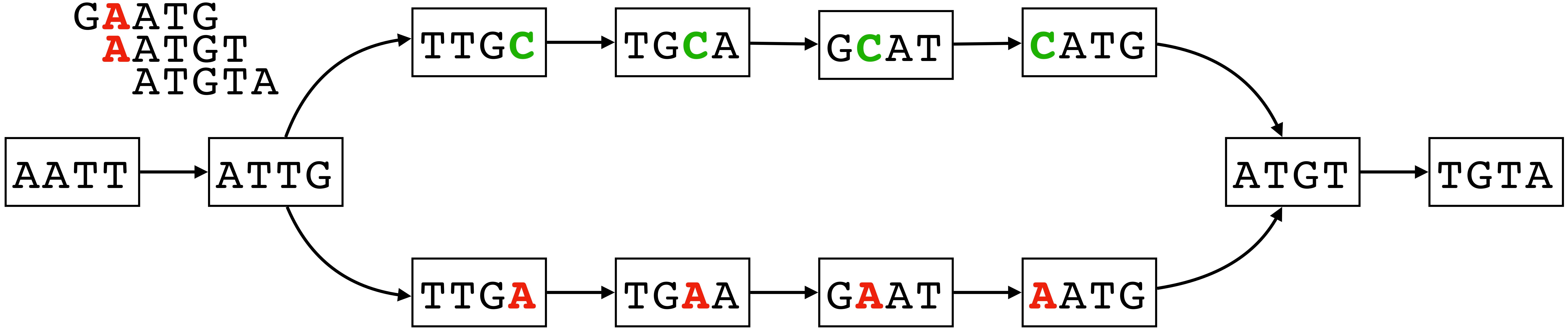

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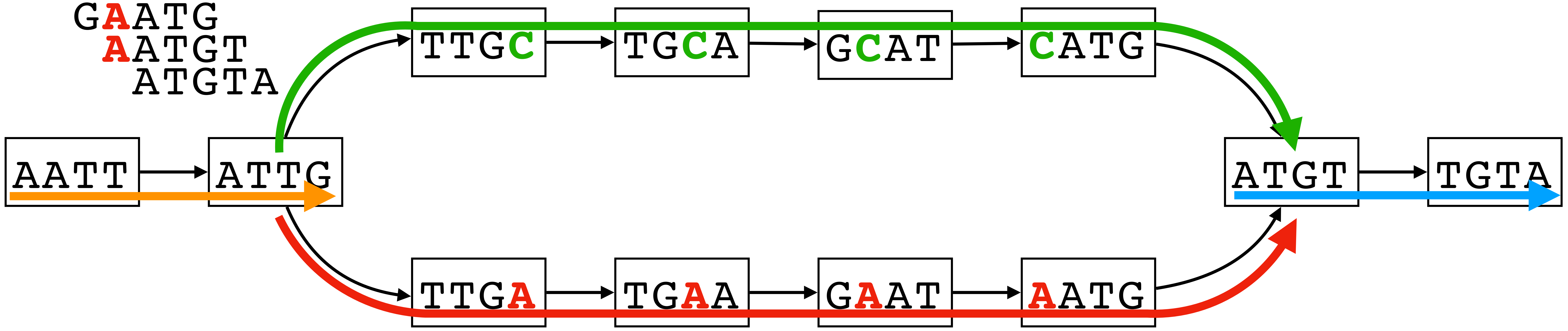

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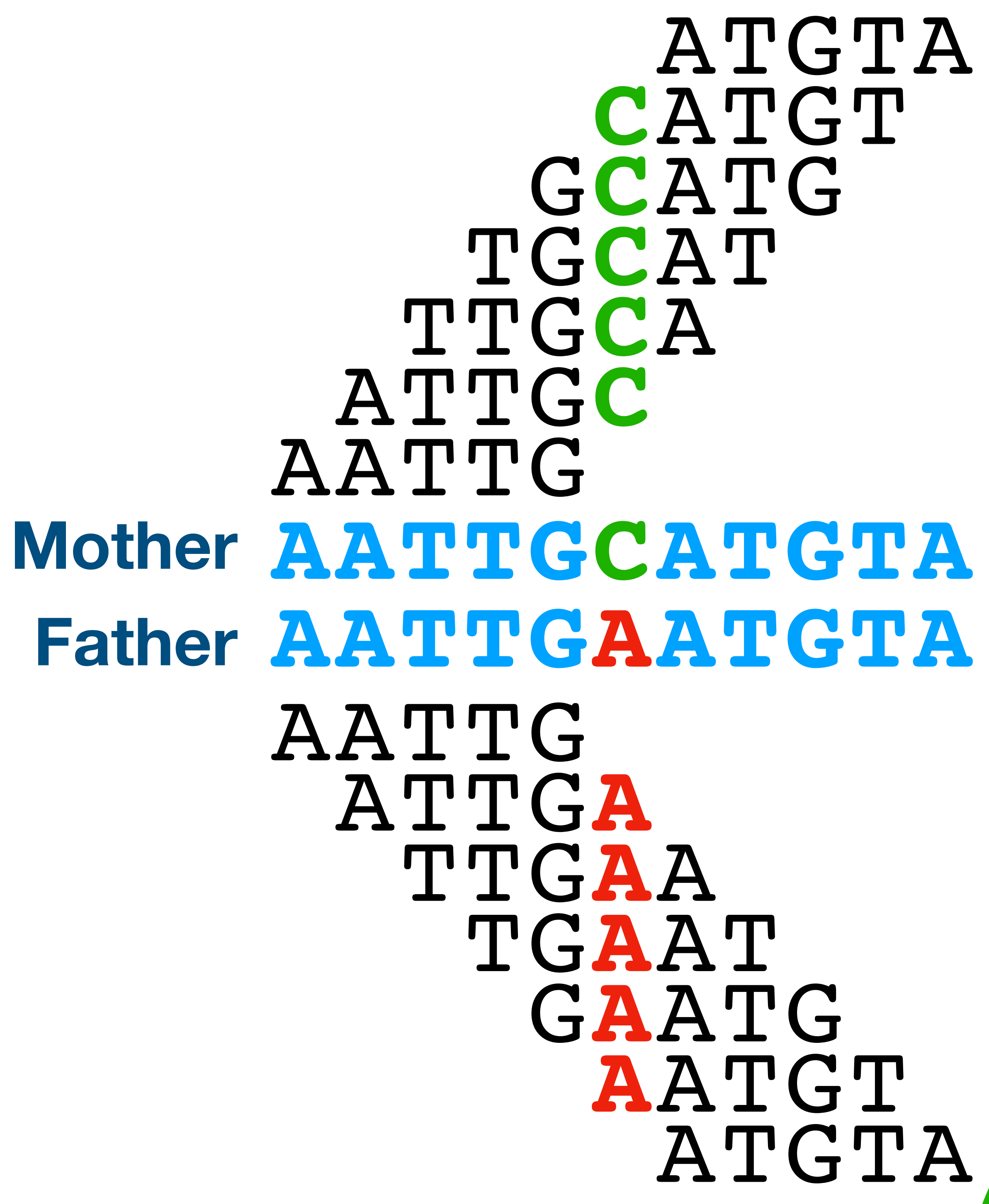

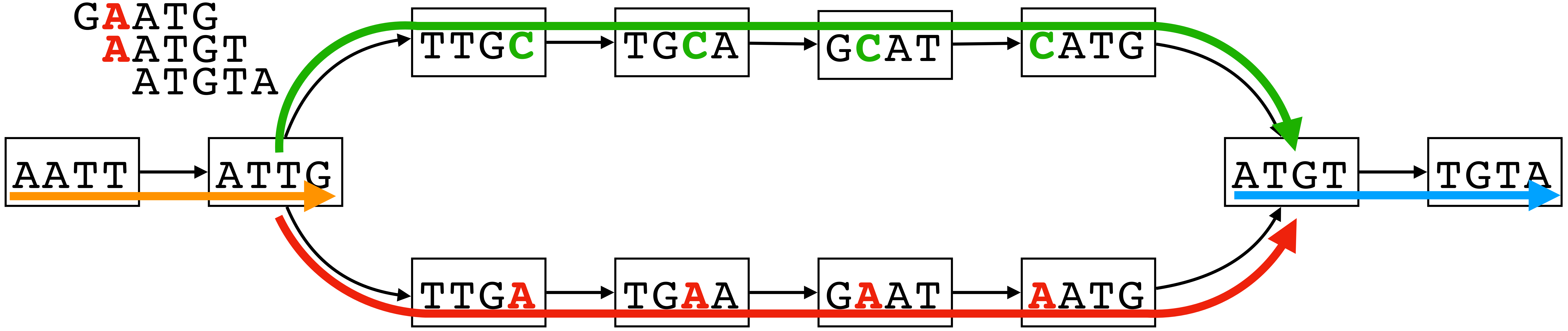

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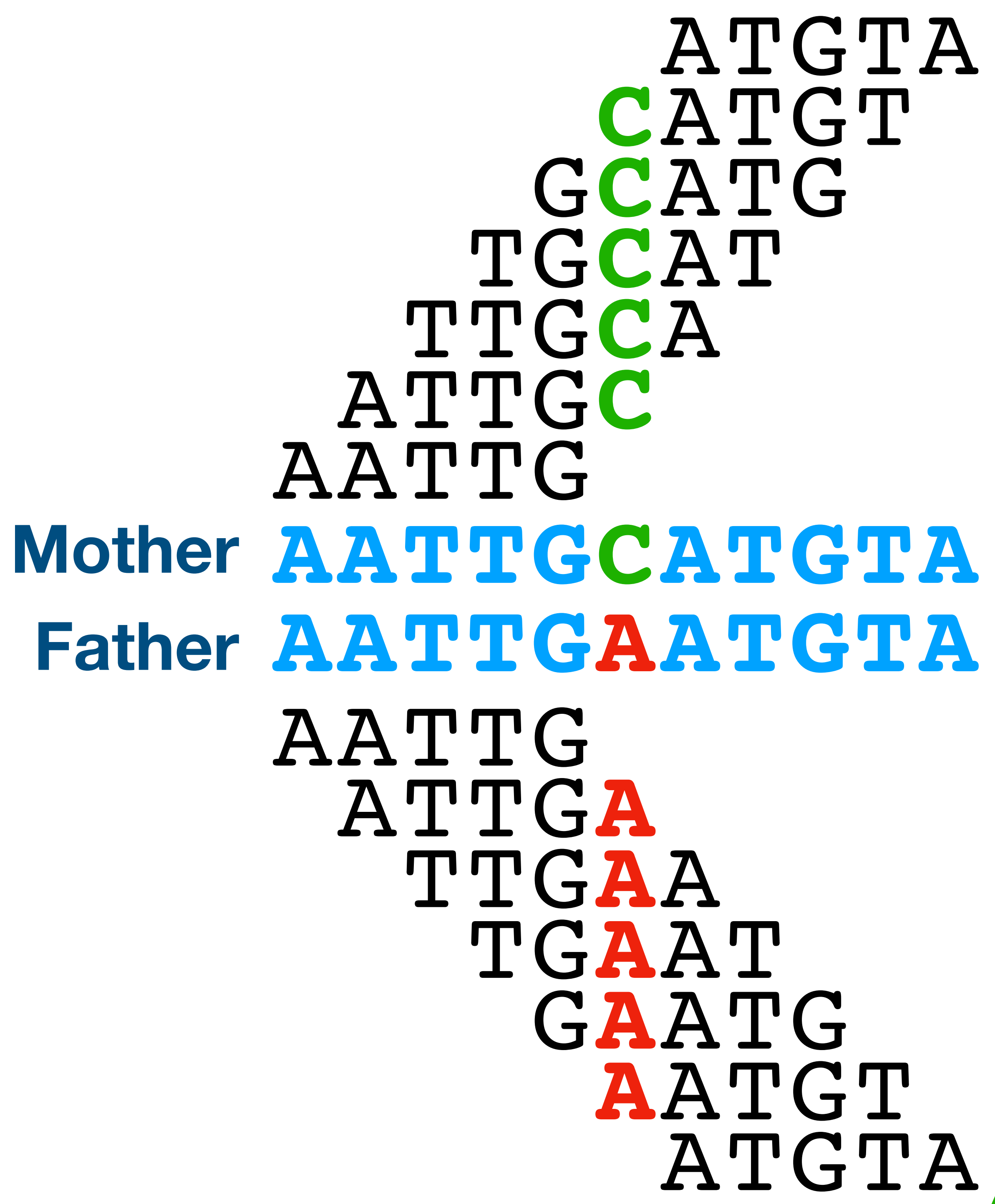

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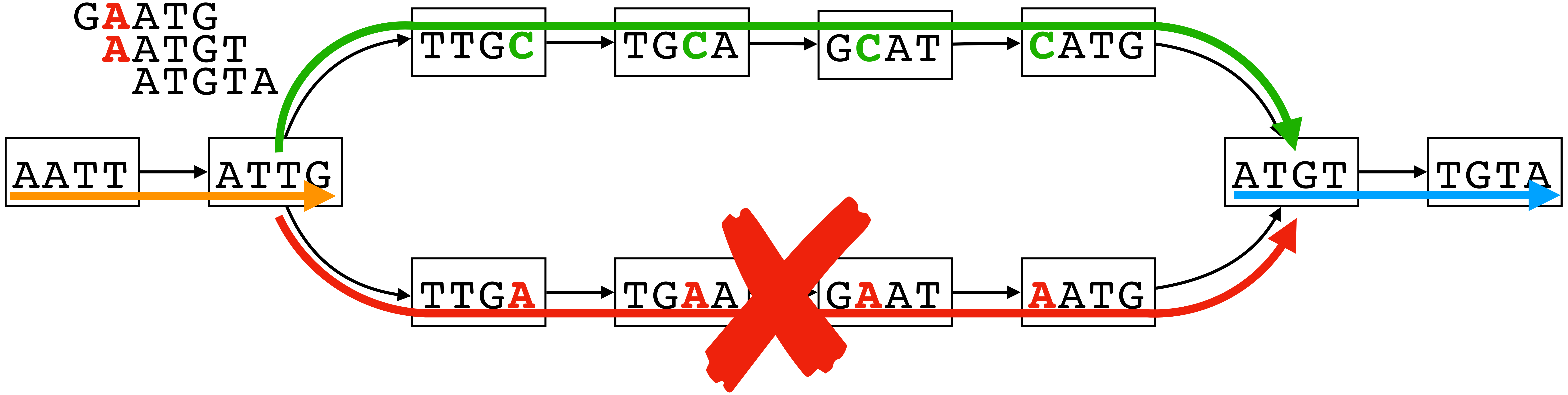

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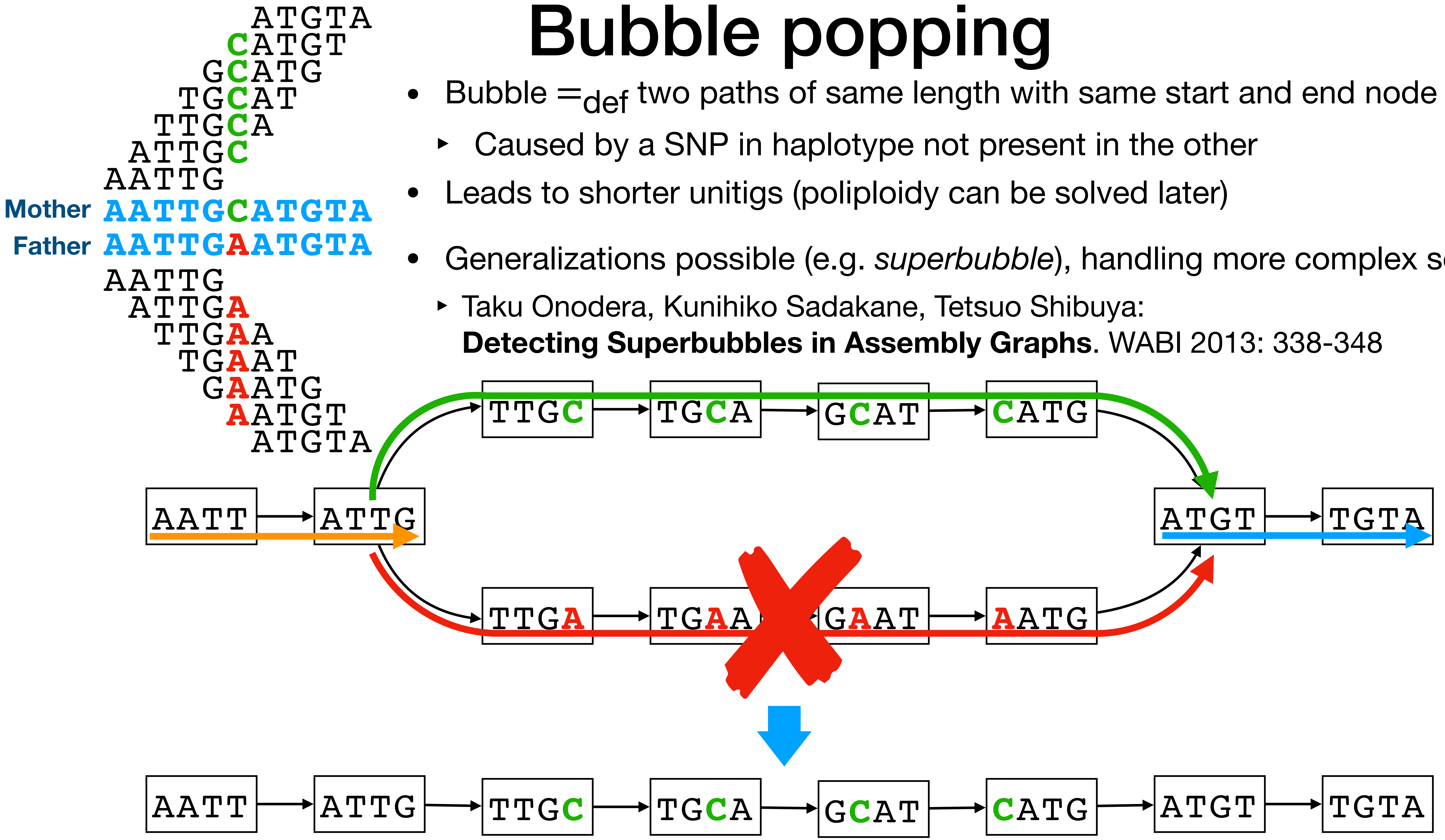

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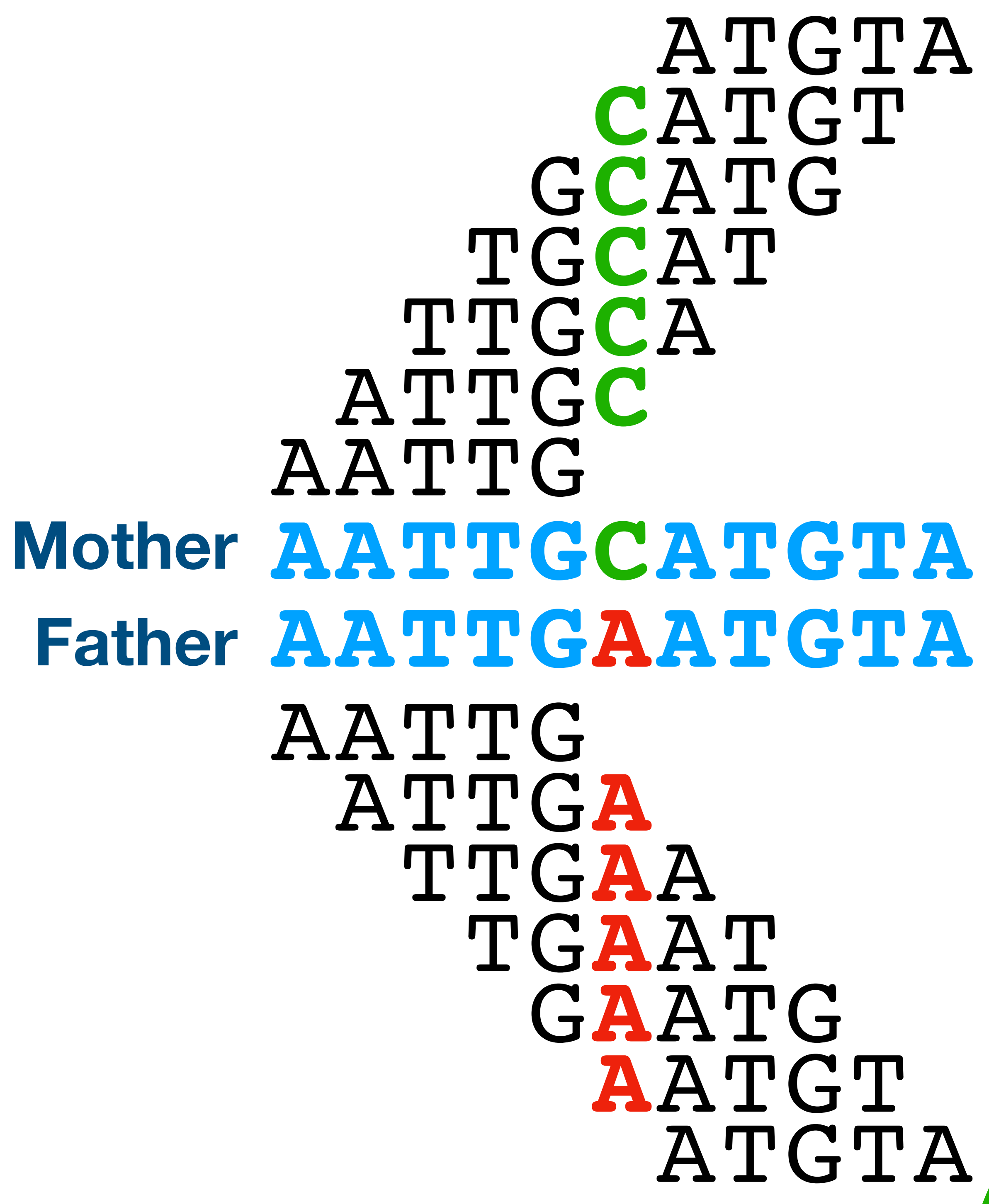

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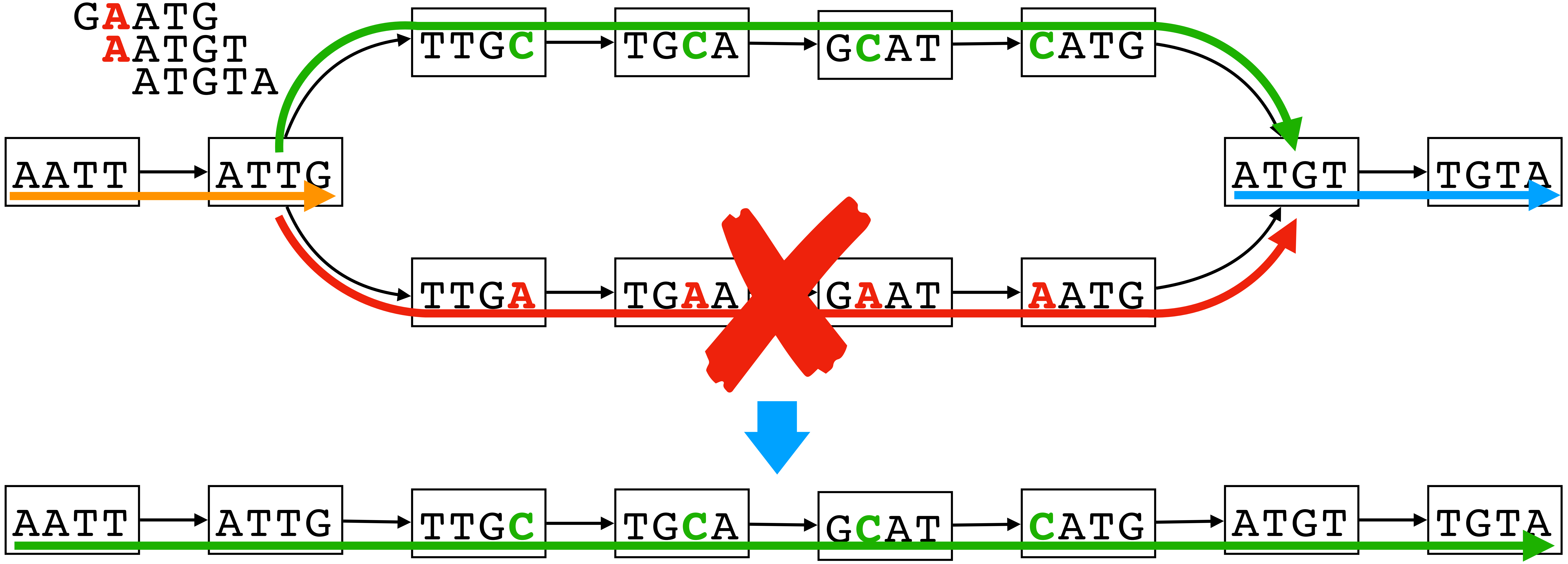

## Tip removal

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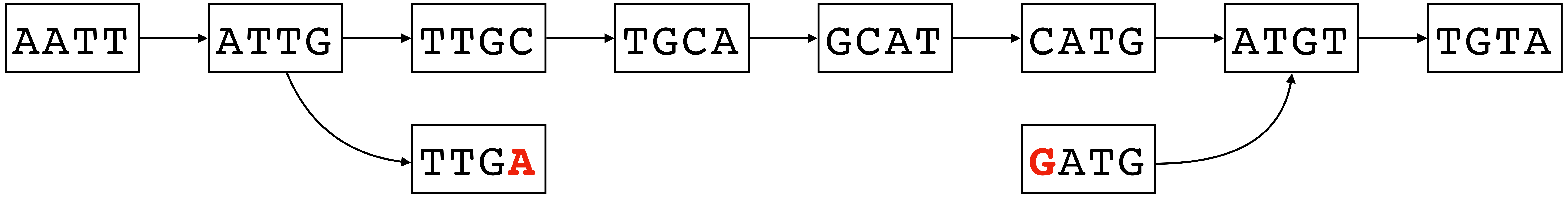

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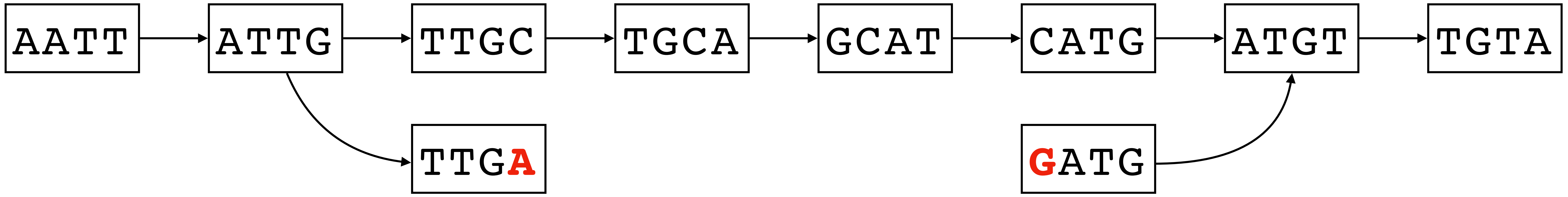

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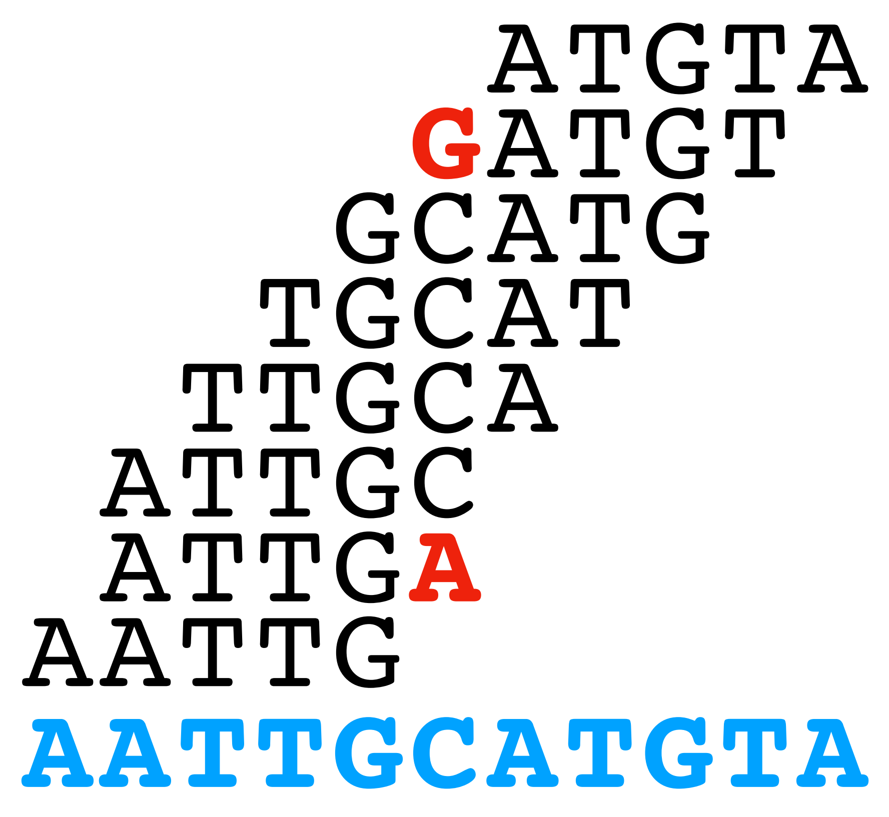

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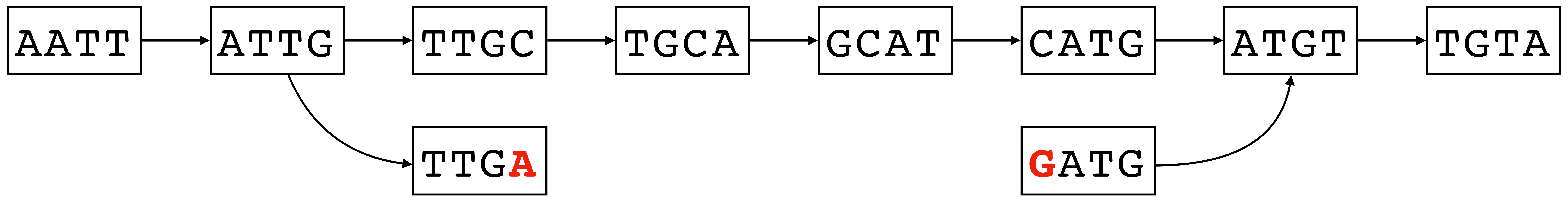

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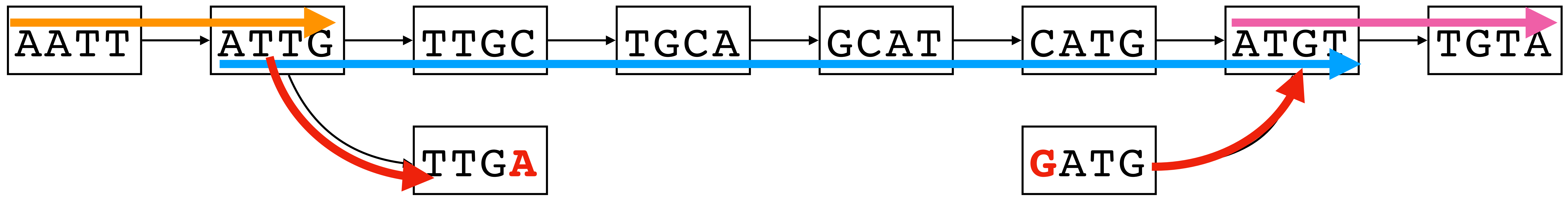

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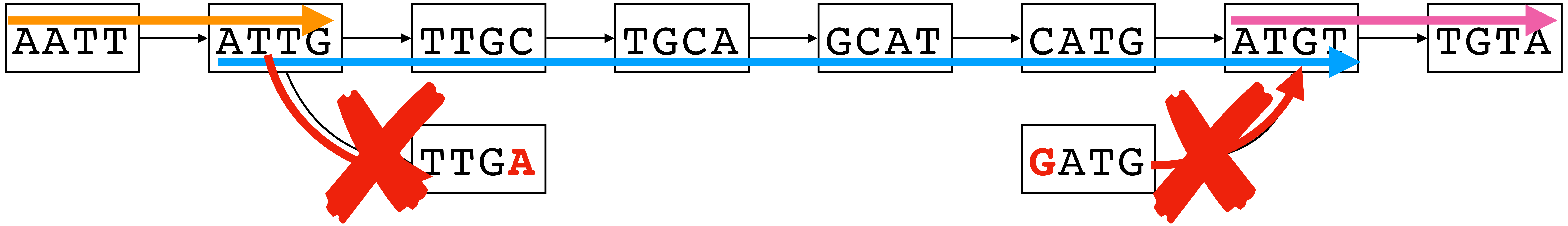

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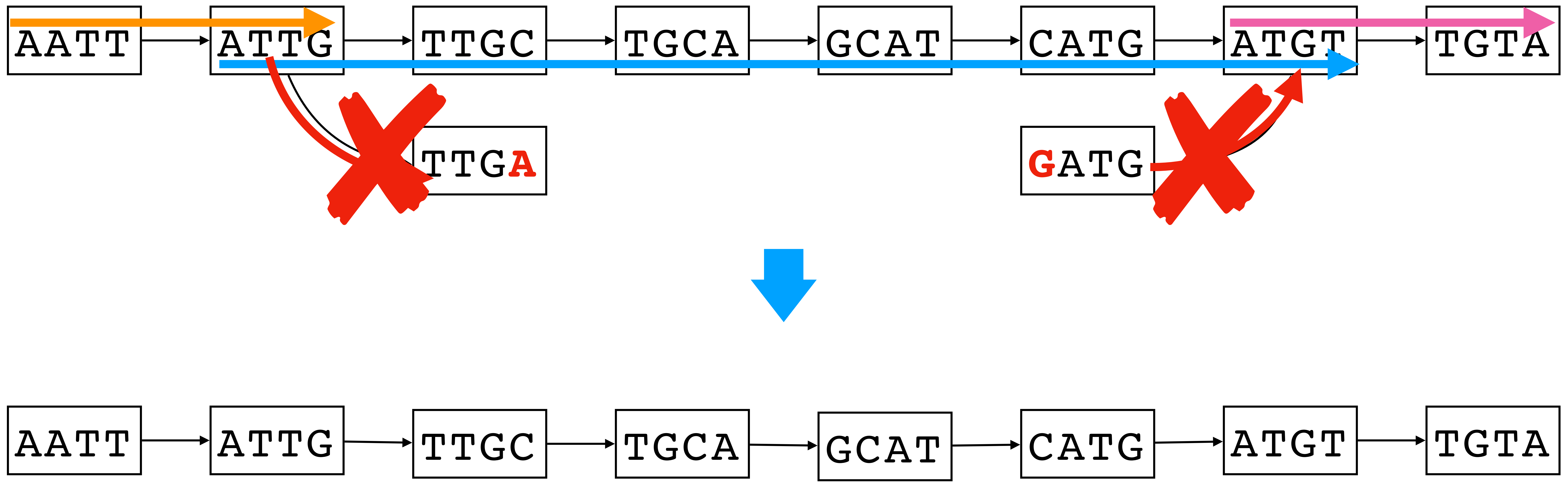

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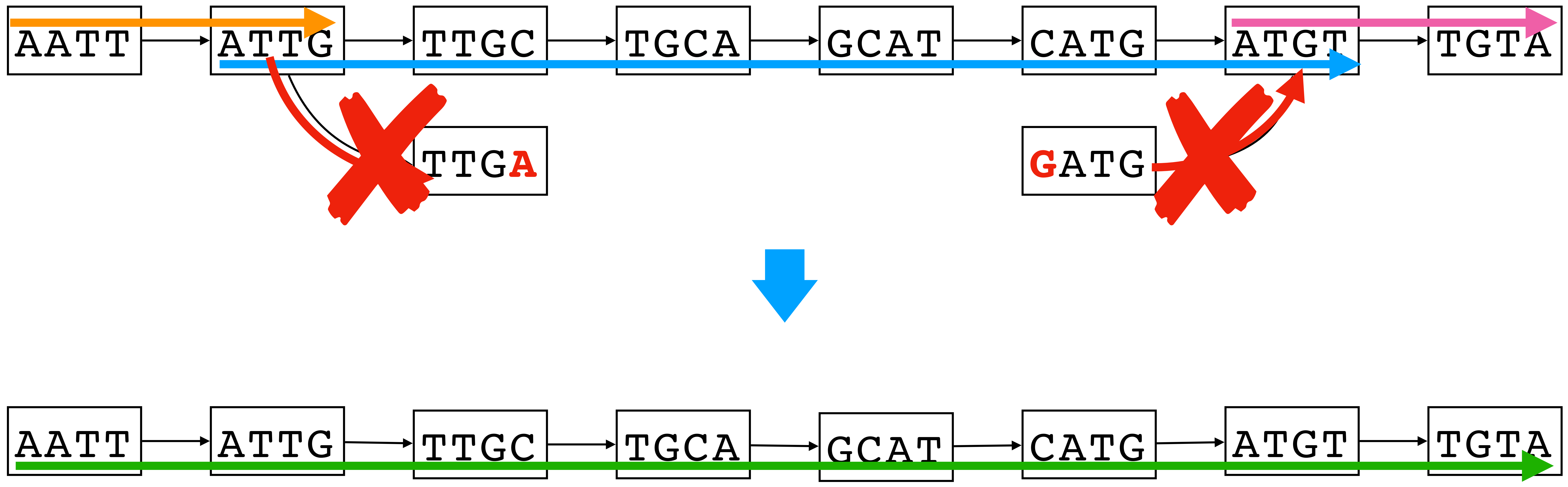
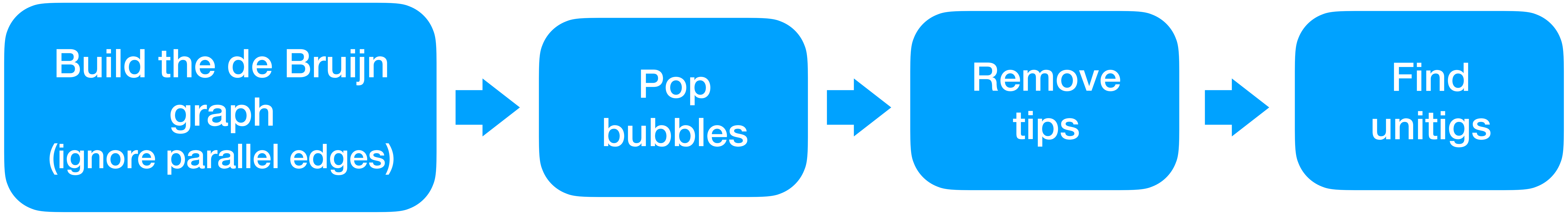


# Contigs assembly

(simplified to ignore some practical issues e.g. errors, reverse complements)



Output the unitigs as "contigs"

# Scaffolding

Bring in paired-end information

Align reads to contigs

→

Chain (order) the contigs

Output chains of contigs with "gaps" (**`NNNN...`**) between them

**Contigs**

```
TCGATAGCTAAAA
AATTGT
ATAGAGATATTT
ATATCGCTAGA
```

→

**Scaffolds**

TCGATAGCTAAAA**NNNNNNNNNNN**AATTGT**NNN**ATAGAGATATTT
ATATCGCTAGA

# Align paired-end reads to contigs

```
                            ATATA......15.......TGCAA
                         AGAAT...........24...........GTAAT
                       ATACA....11.....CTAAT
                      AATAG.......16.......TGCAA
Contig 1 GTGAAGTTAATACAATAGAATATA      CTAATGCAATGGAATGTAAT Contig 2
```

- Align paired-end reads to contigs, focus on read pairs aligning to different contigs

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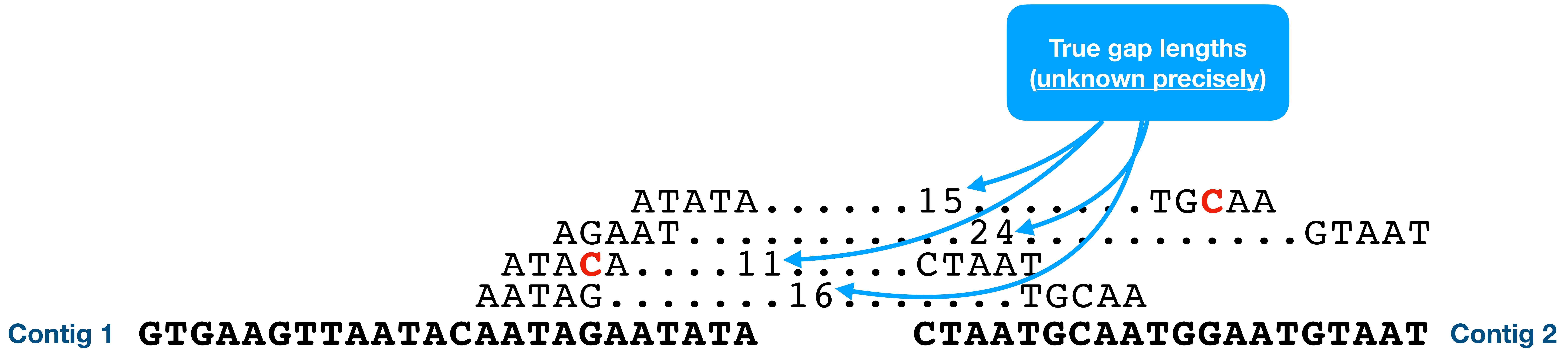

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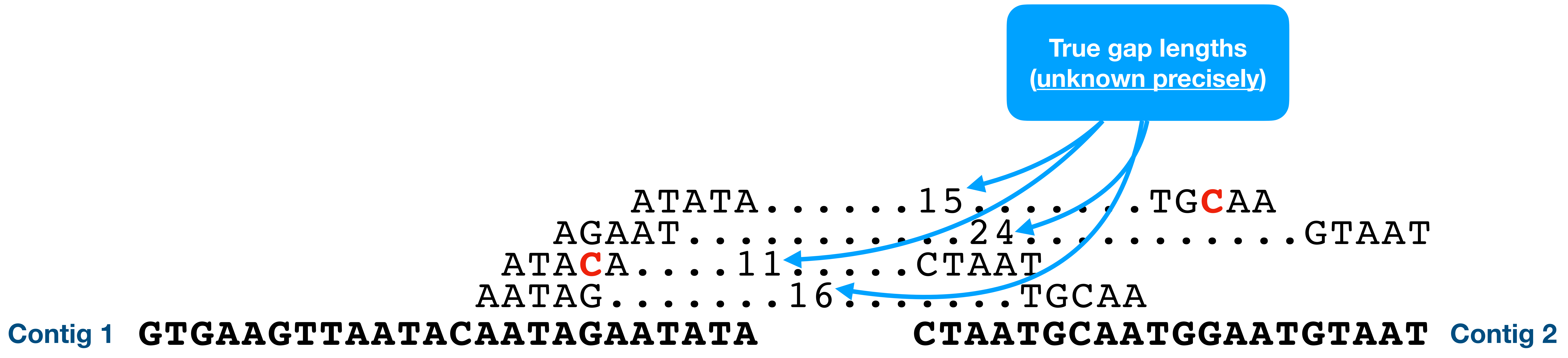

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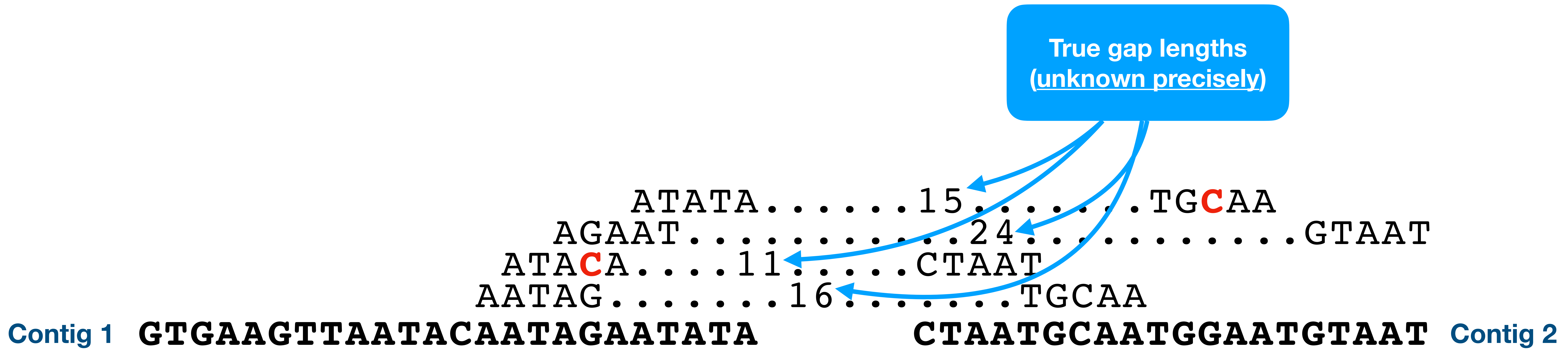

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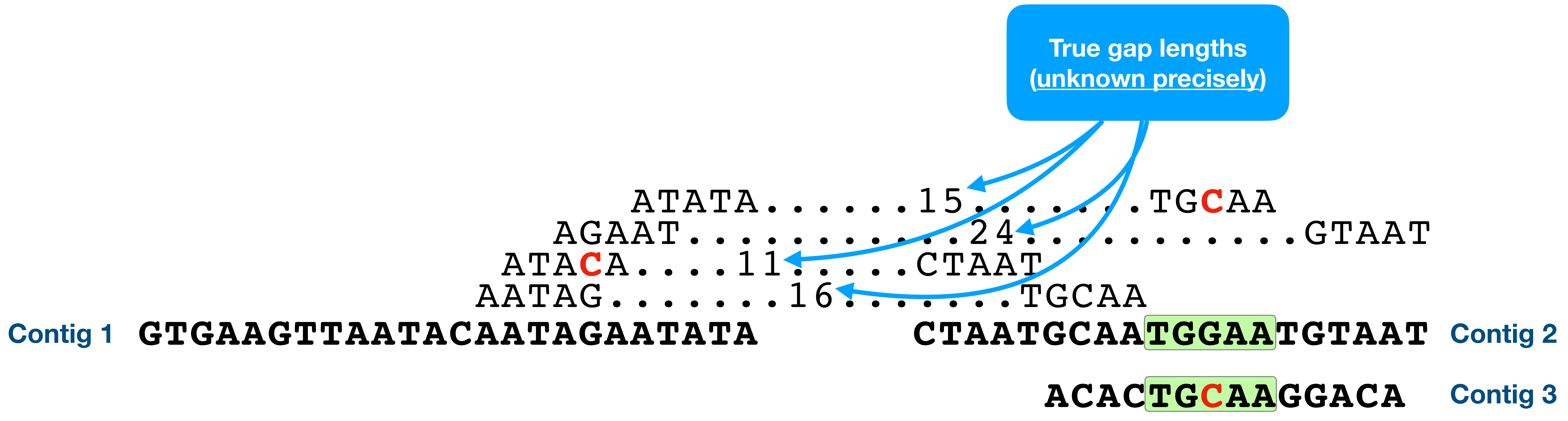

True gap lengths (unknown precisely)
ATATA.......15........TGCAA
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Contig 1 GTGAAGTTAATACAATAGAATATA
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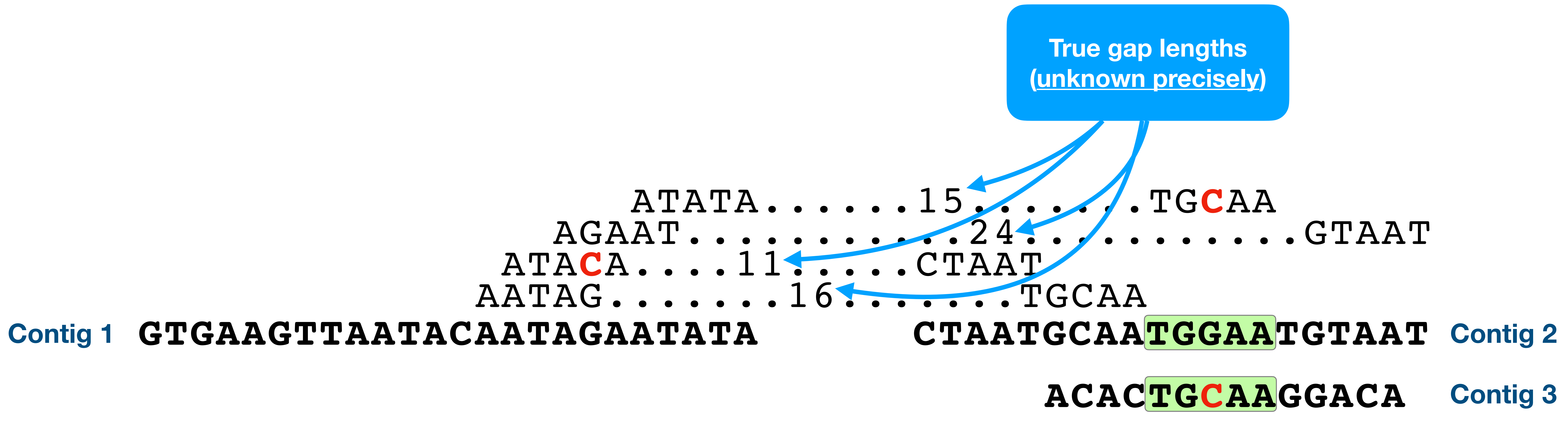
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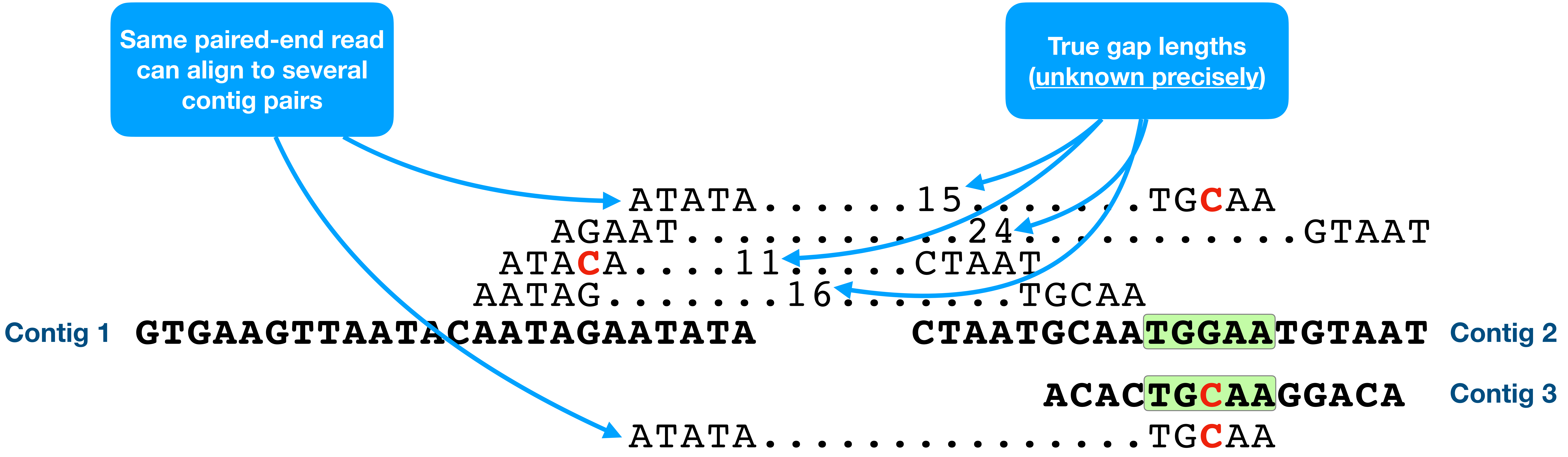

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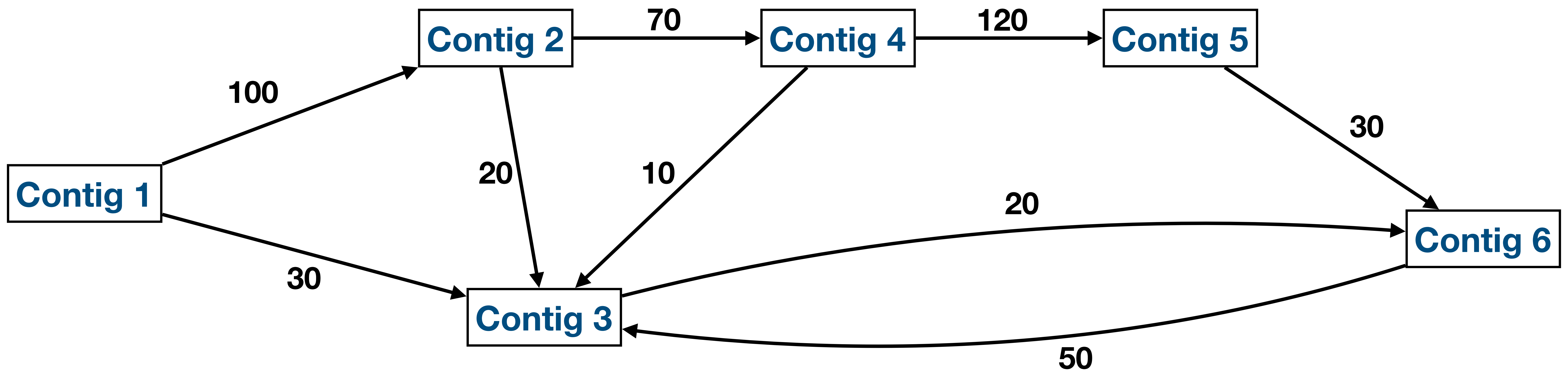

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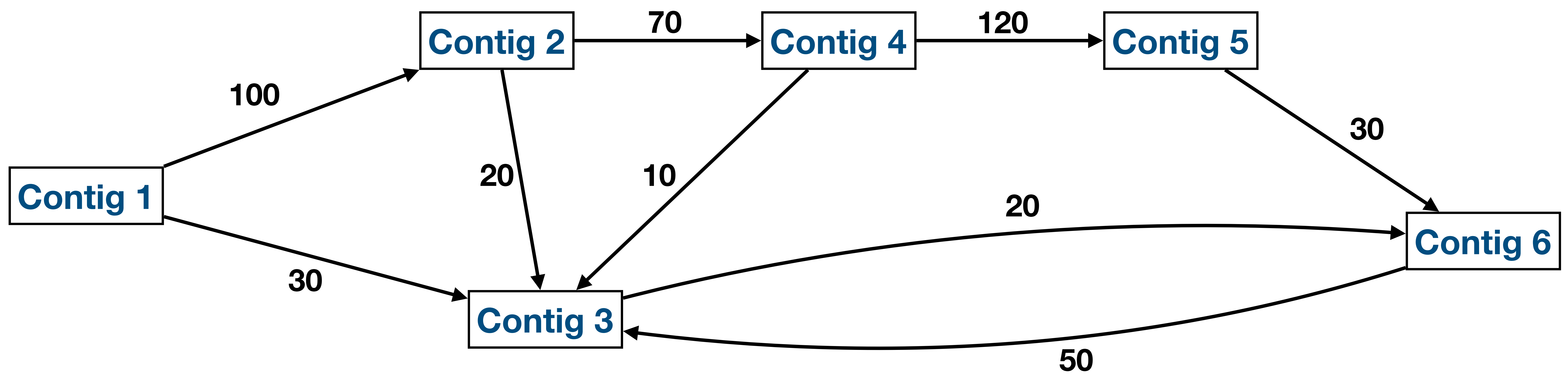

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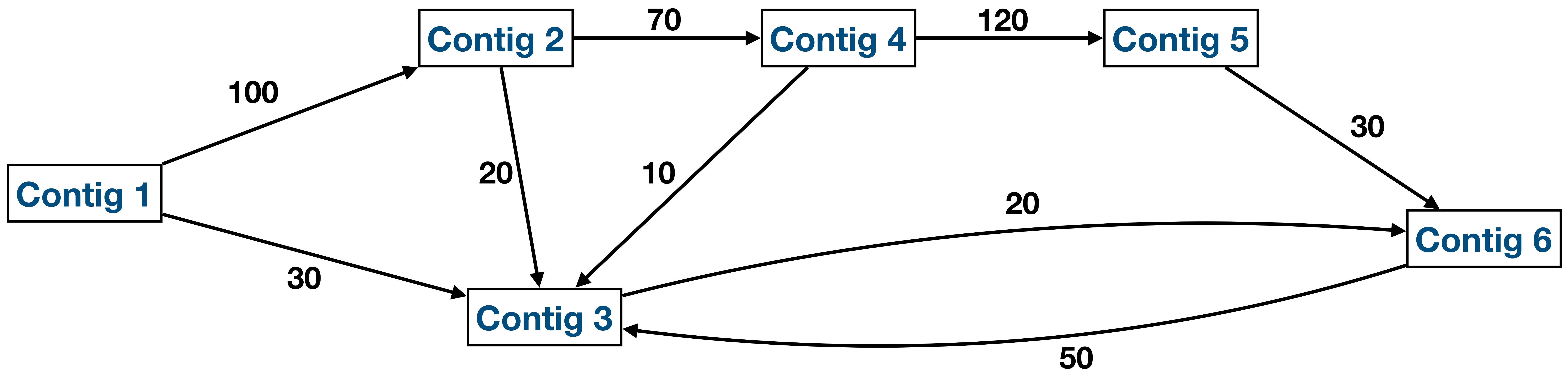

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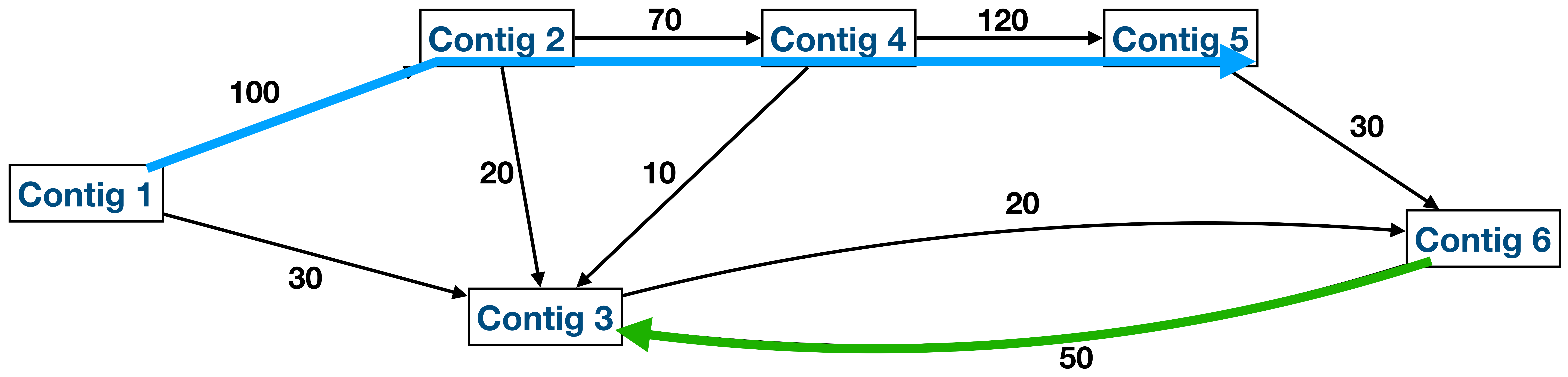

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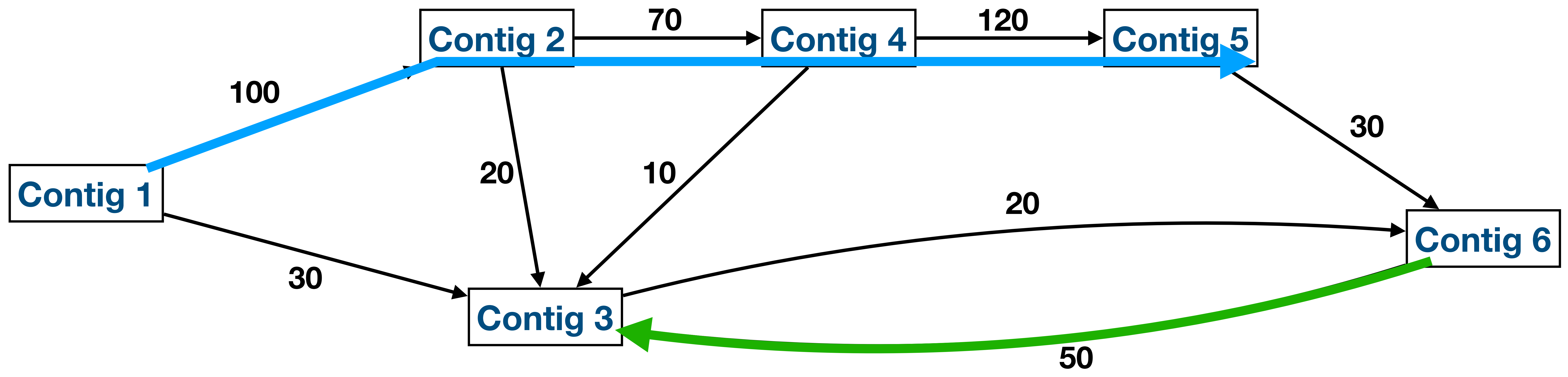

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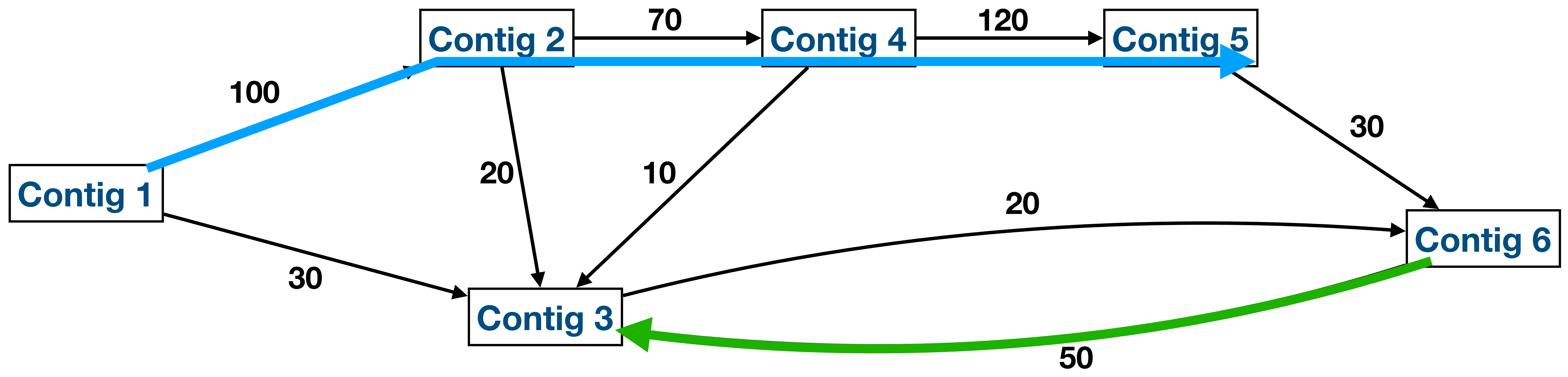

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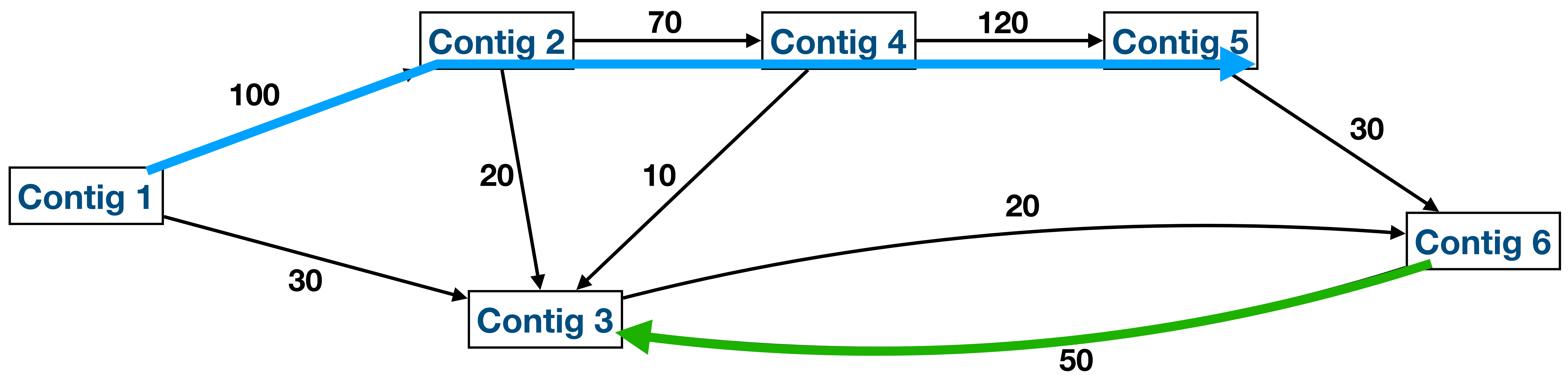

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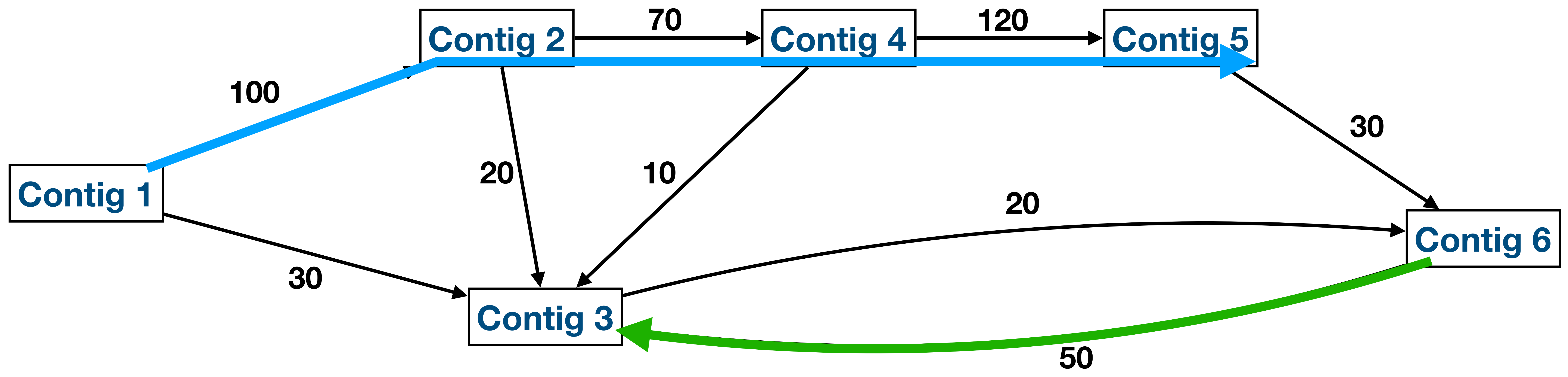

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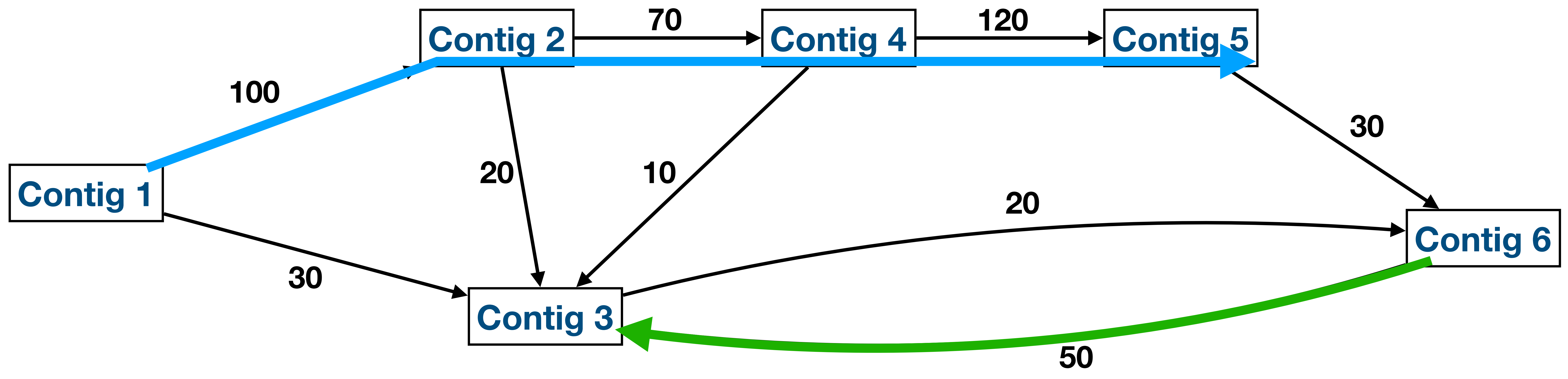

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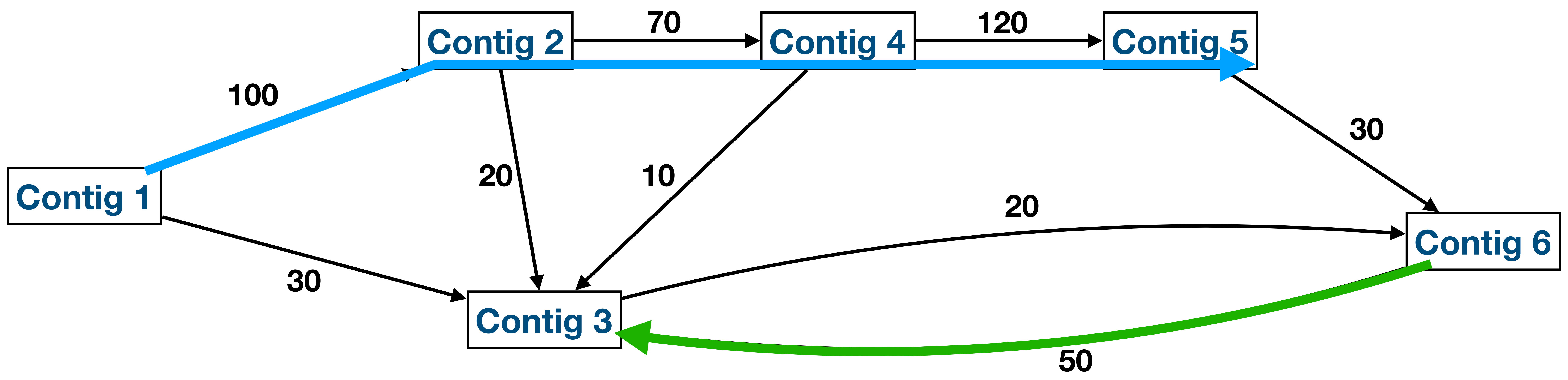

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  - Igor Mandric, Alex Zelikovsky: **ScaffMatch: Scaffolding Algorithm Based on Maximum Weight Matching.** RECOMB 2015: 222-223

# Gap filling

Scaffolds contain gap length estimates (number of **N**s)

Bring back all reads

Find filling paths from the assembly graph

Output the scaffolds in which some gaps are "filled"

TCGATAGCTAAAA**NNNNNNNNNN**AATTGT**NNN**ATAGAGATATTT

TCGATAGCTAAAA**TGCCGTTCGG**AATTGT**NNN**ATAGAGATATTT

## Find path of given length

$$\begin{tikzcd}\text{ACCTAGCCAT} \arrow[r, leftrightarrow, "\text{Gap length 5}"] & \text{ATGCTCGGGT}\end{tikzcd}$$

# Find path of given length

ACCTAGC
CAT
ATC
TCG
CGA
GAT
ATGCTCGGGT
TCT
CTT
TTT
TTA
TAT

- Build the de Bruijn graph of all reads

# Find path of given length

s
t
ACCTAGC
CAT
ATC
TCG
CGA
GAT
TCT
CTT
TTT
TTA
TAT
ATG
CTCGGGT

- Build the de Bruijn graph of all reads
- Take the last $k$-mer of the 1st contig (node $s$) and the first $k$-mer of the 2nd contig (node $t$)

# Find path of given length

ACCTAGC
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$s$
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  - If gap length estimate is $d$, how long should be the path? **ASSIGNMENT**

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- Can be solved by dynamic programming in time $O(d\,|\text{edges}|)$
  - Leena Salmela, Kristoffer Sahlin, Veli Mäkinen, Alexandru I. Tomescu: **Gap Filling as Exact Path Length Problem**. RECOMB 2015: 281-292

# Section summary

**Contig assembly**

Forget about the assembly model (i.e. the problem formulation)

Assemble only parts about which we are sure (contigs → contiguous sequences)

→

**Scaffolding**

Chain (order) the contigs

Output chains of contigs with "gaps" (`NNNN...`) between them

→

**Gap filling**

Find filling paths from the assembly graph

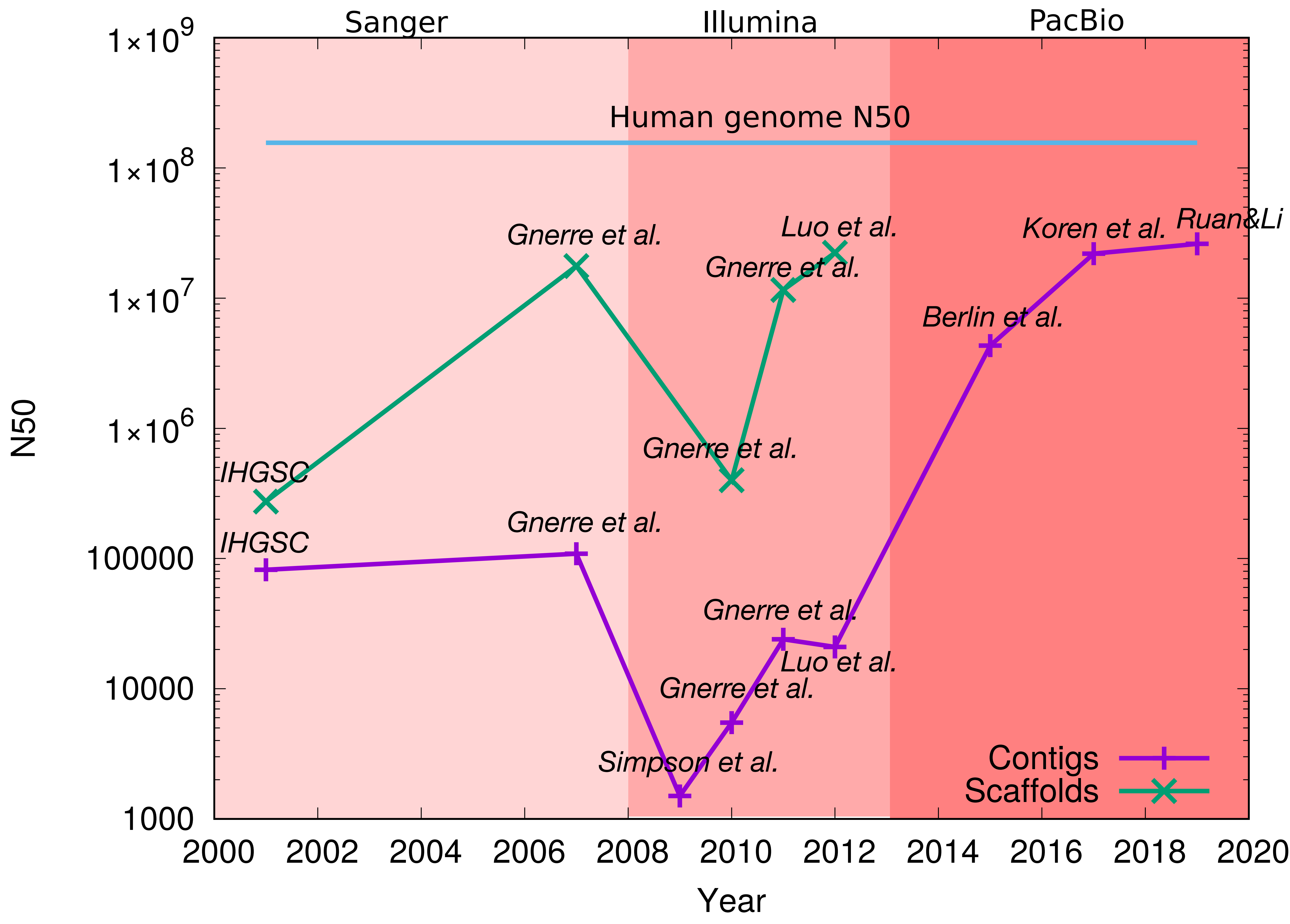
Output the scaffolds in which some gaps are "filled"

- A natural decomposition into subproblems based on the available paired-end information
- One can improve each step individually, thus improving the overall result

# Long-read sequencing
(Third-generation sequencing)

- No paired-end reads (focus is on contig assembly)
- Higher error rate: 15% compared to 0.1% for short reads
  - Still developing: accurate PacBio HiFi reads
- No "clear" best strategy
- Short reads still relevant for some scenarios (e.g. metagenomic sequencing)

**Picture: Leena Salmela**
**https://www.cs.helsinki.fi/u/lmsalmel/**



**N50 measure → ASSIGNMENT**

## A more "practical" theoretical formulation

(A principled approach to contig assembly)

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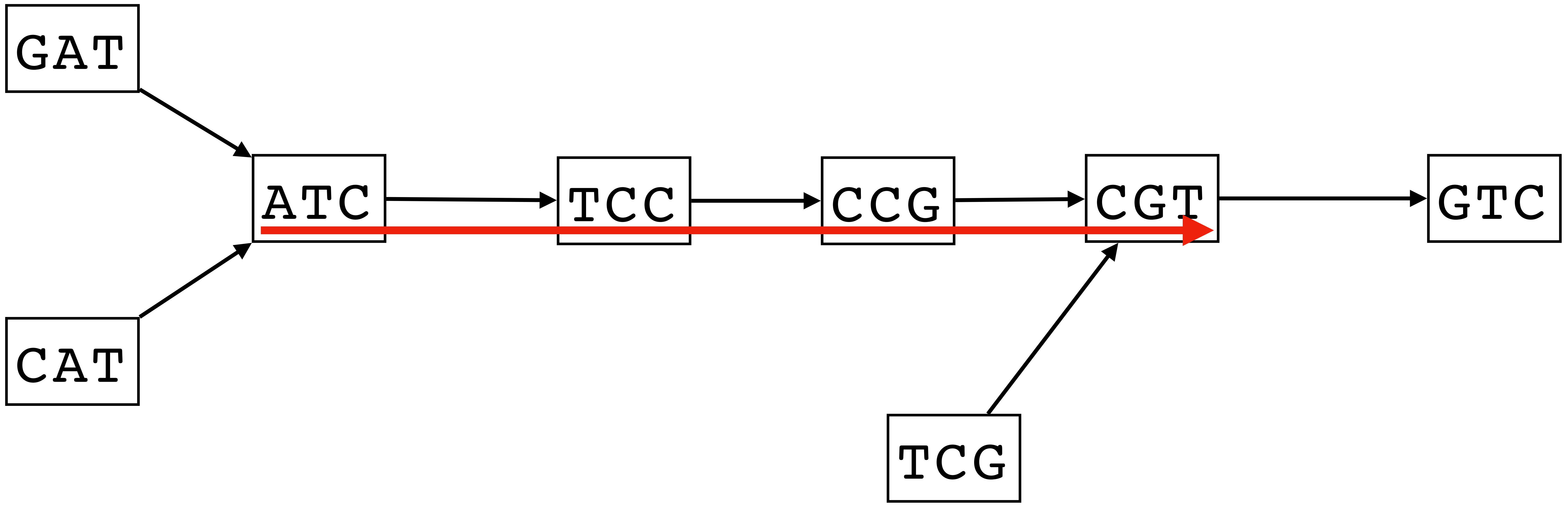

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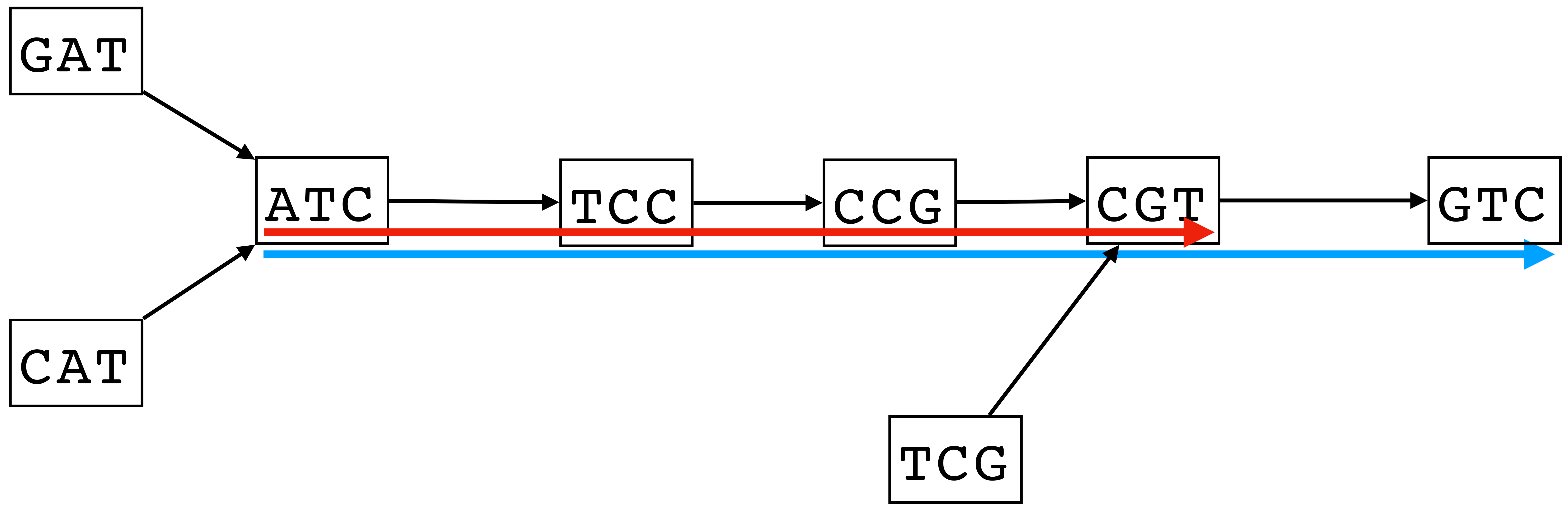

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$$\begin{tikzcd}
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\end{tikzcd}$$

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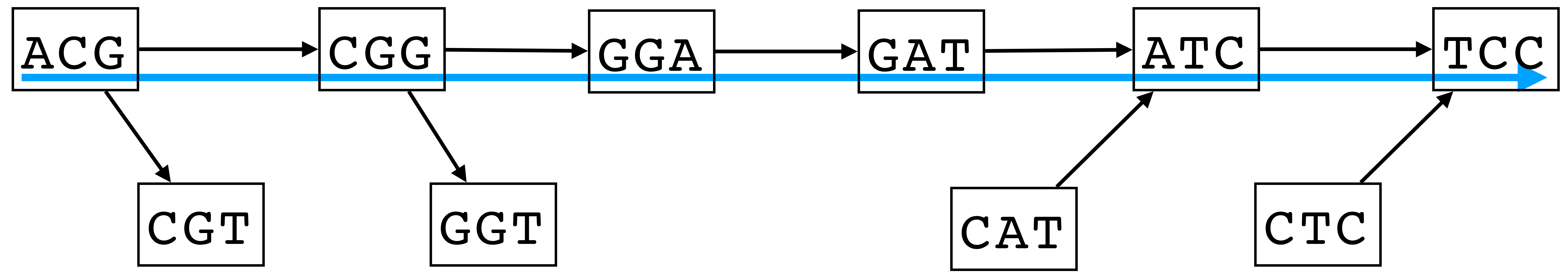

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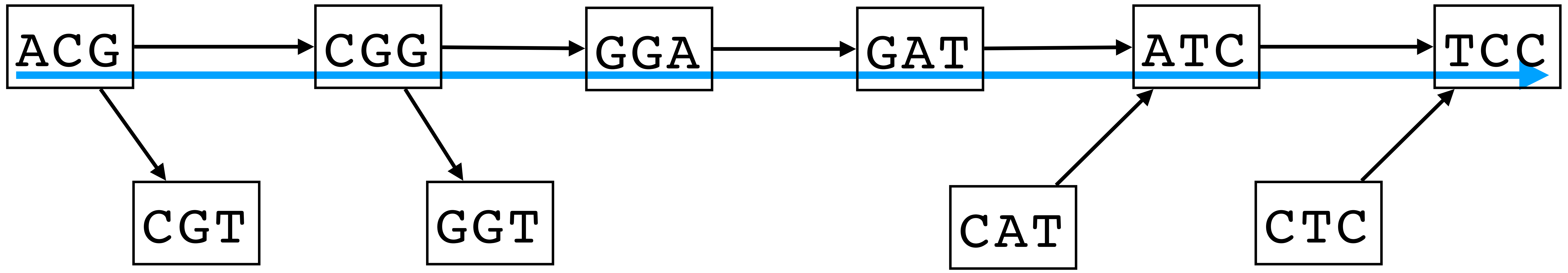

## Omnitigs

- Assume that the "genome assembly solution" is a circular walk covering every edge at least once (walk can repeat nodes)
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  - Massimo Cairo, Paul Medvedev, Nidia Obscura Acosta, Romeo Rizzi, Alexandru I. Tomescu:
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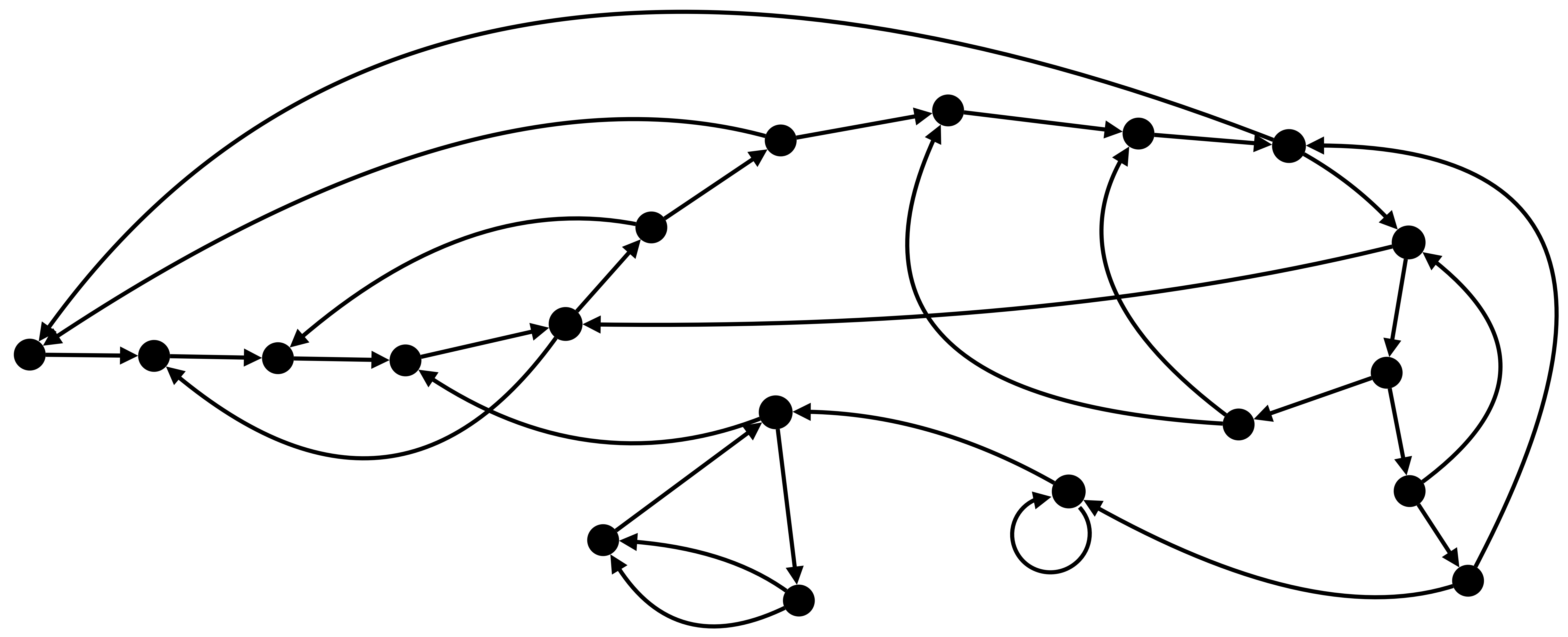
- Can be adapted to deal with practical issues (ongoing work)
  - **<u>Subprojects available as Master thesis topics</u>**

# Example + Results on perfect data



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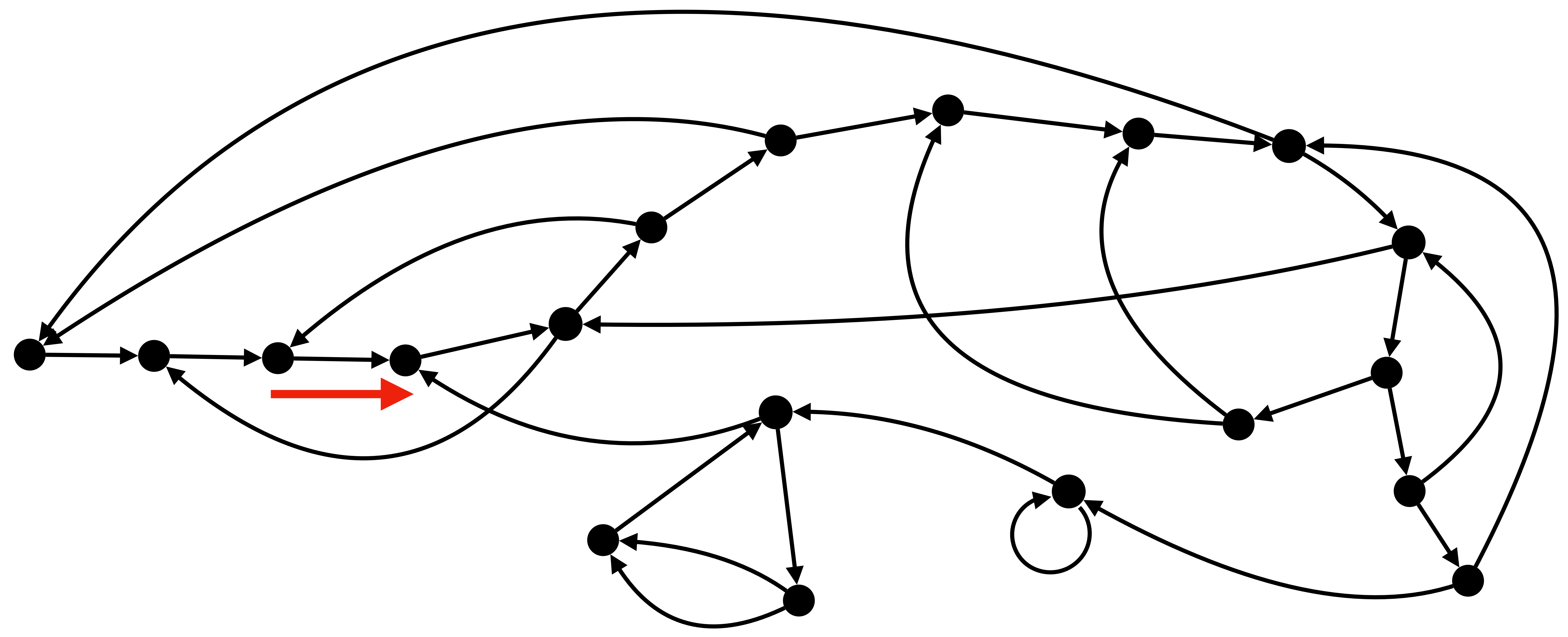

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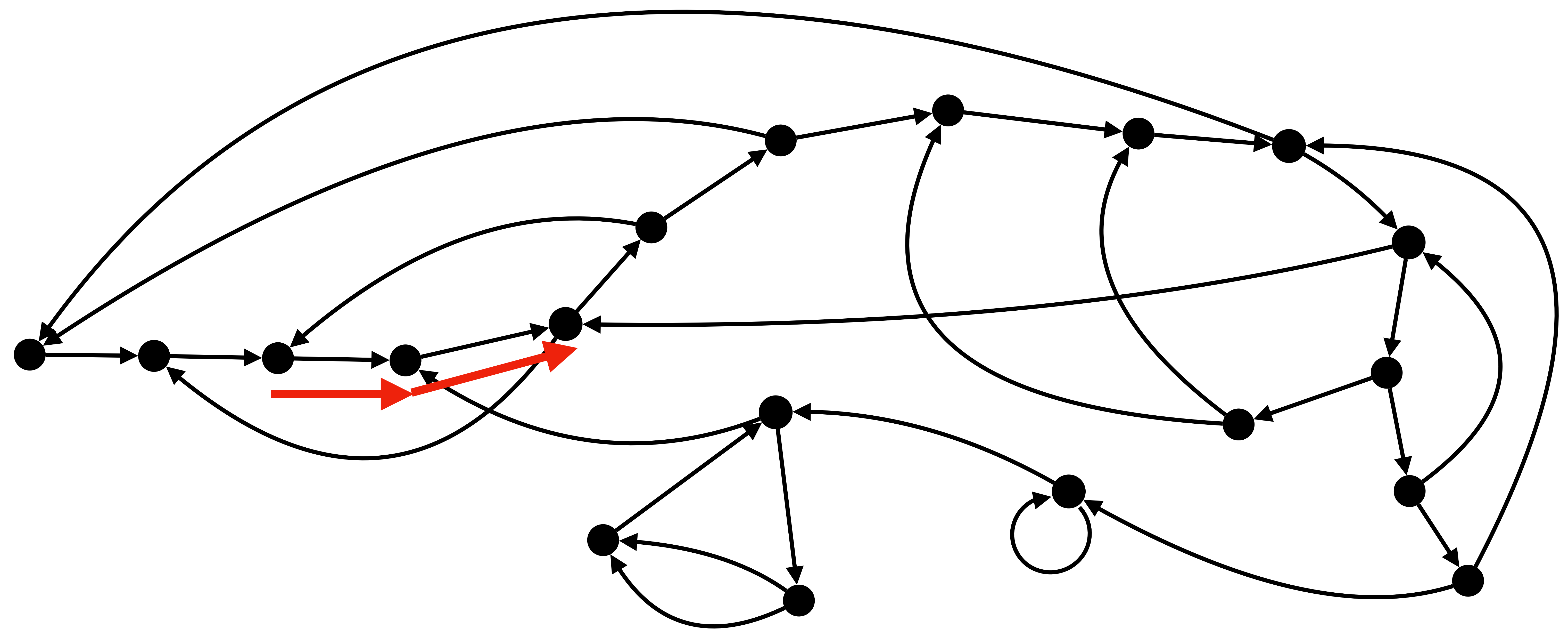

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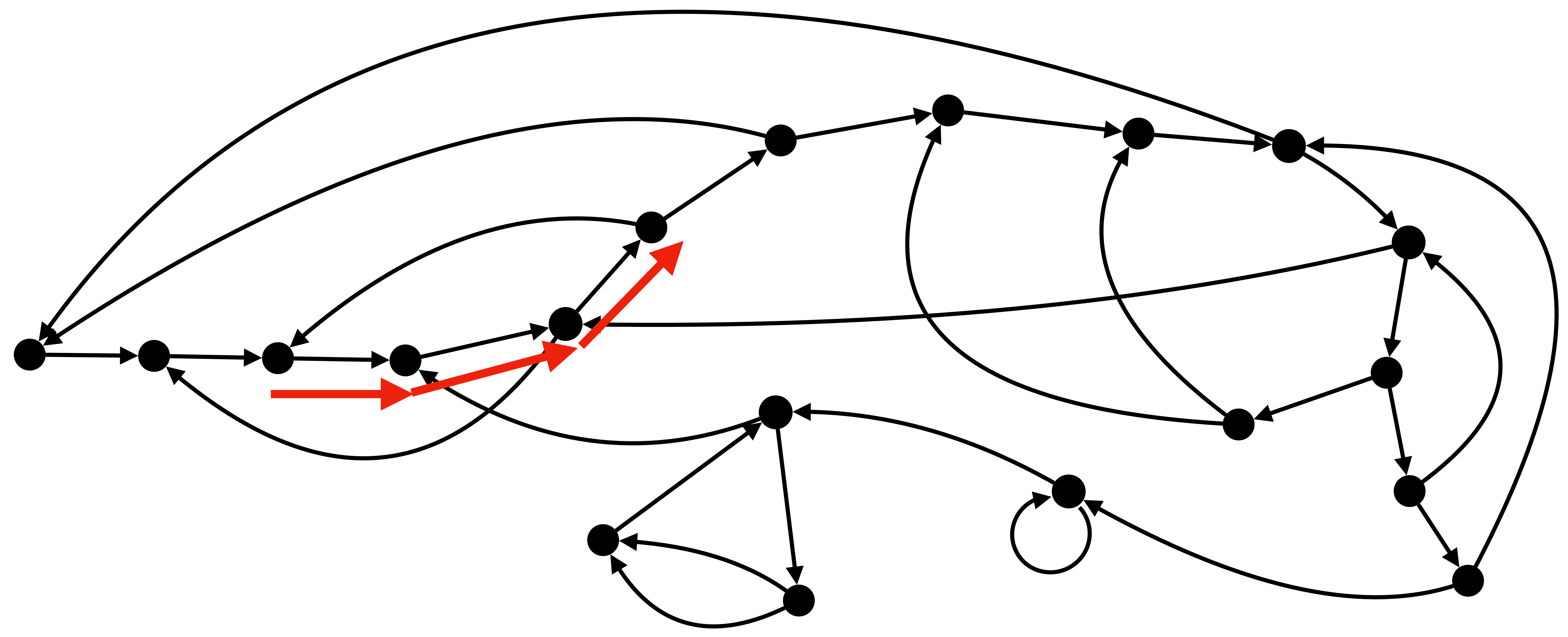

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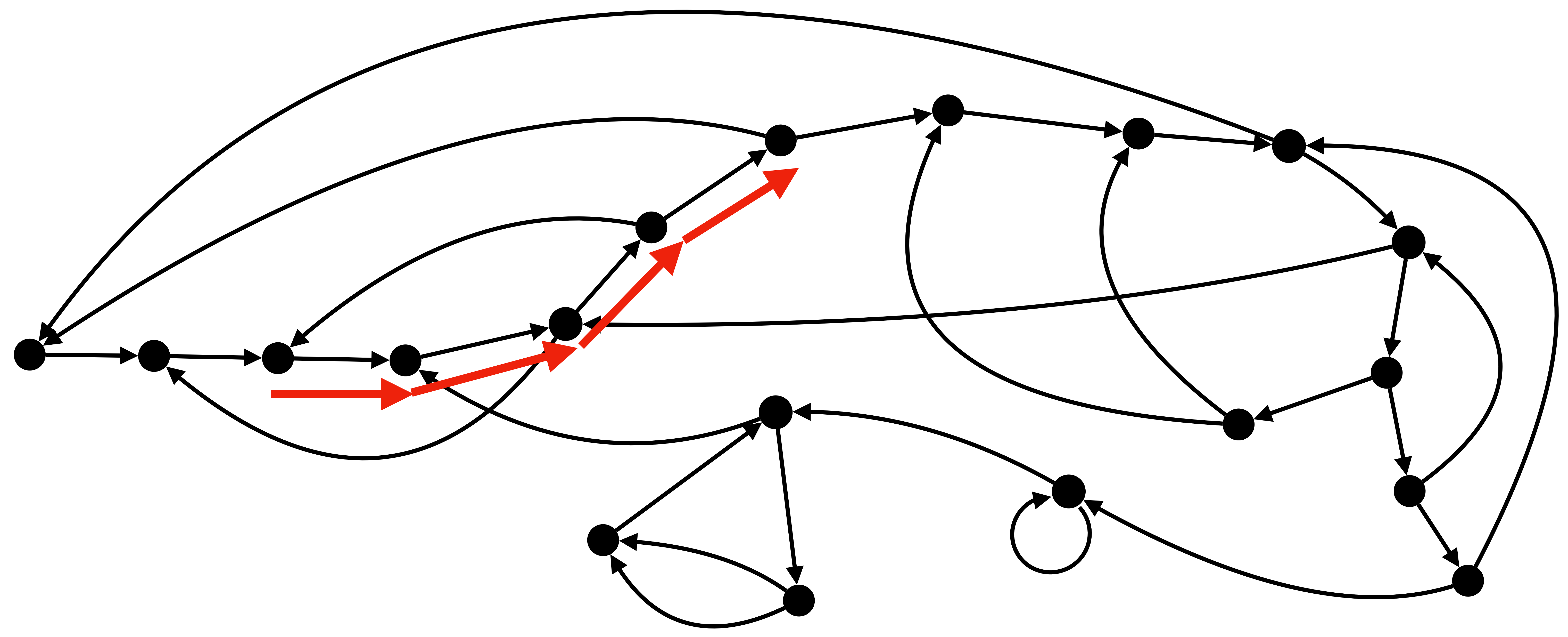

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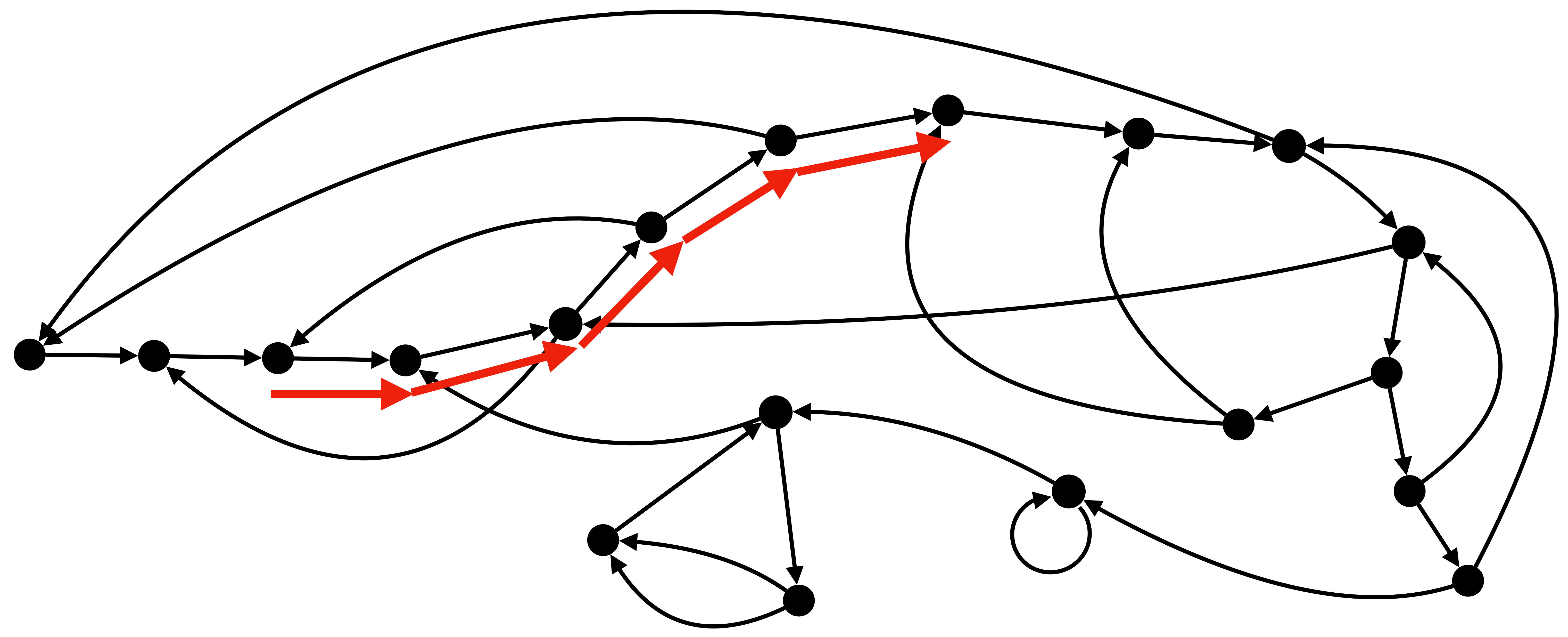

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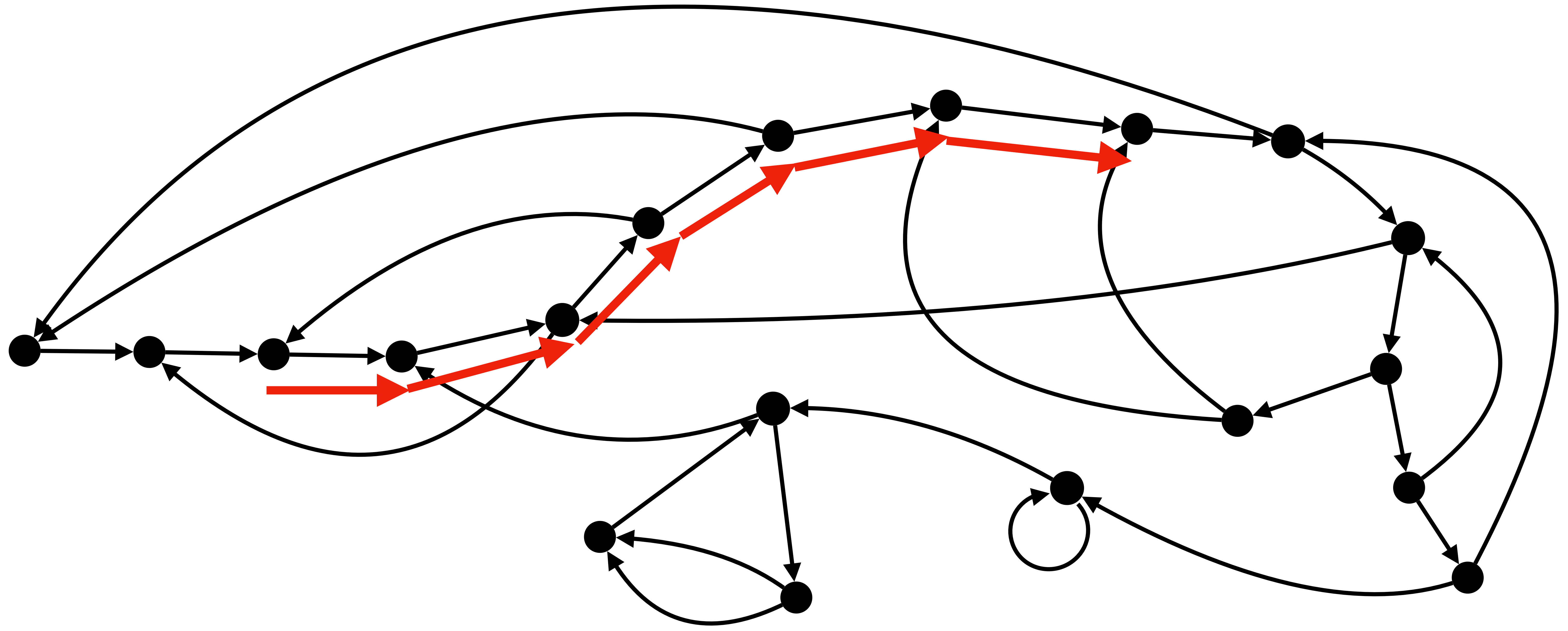

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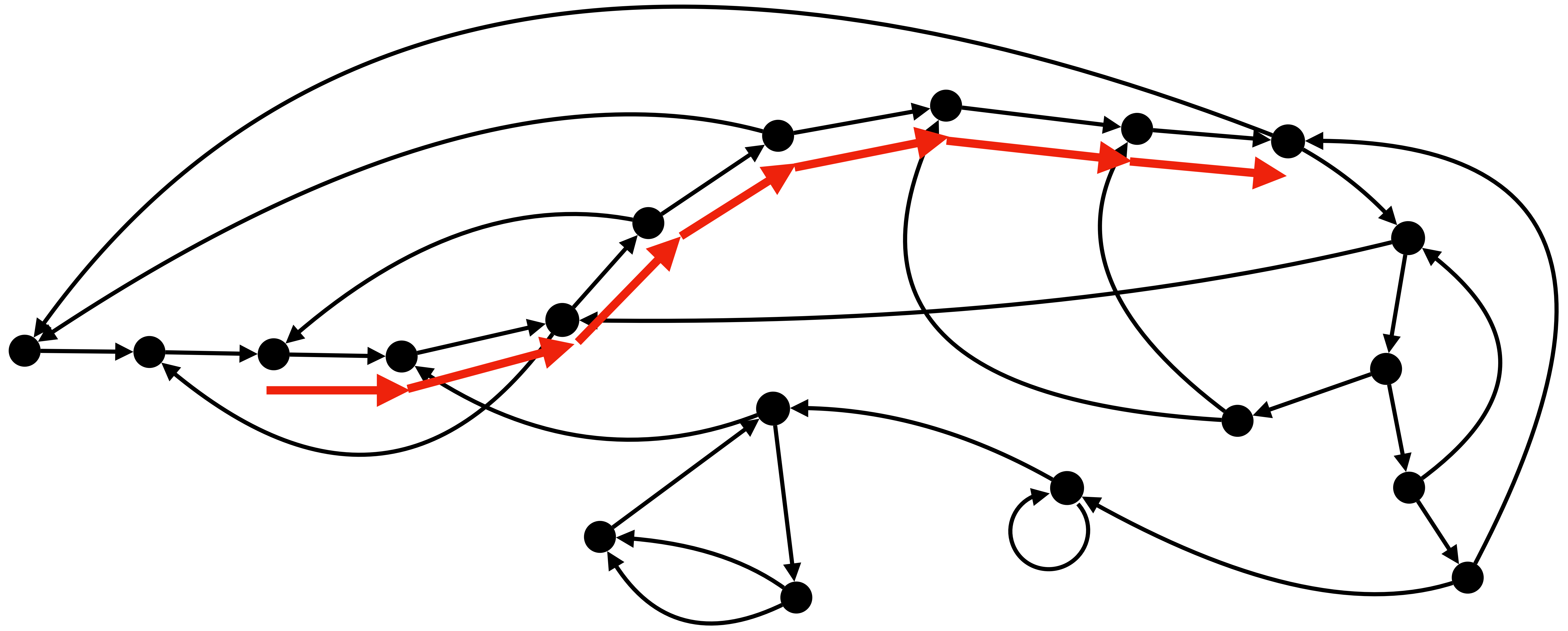

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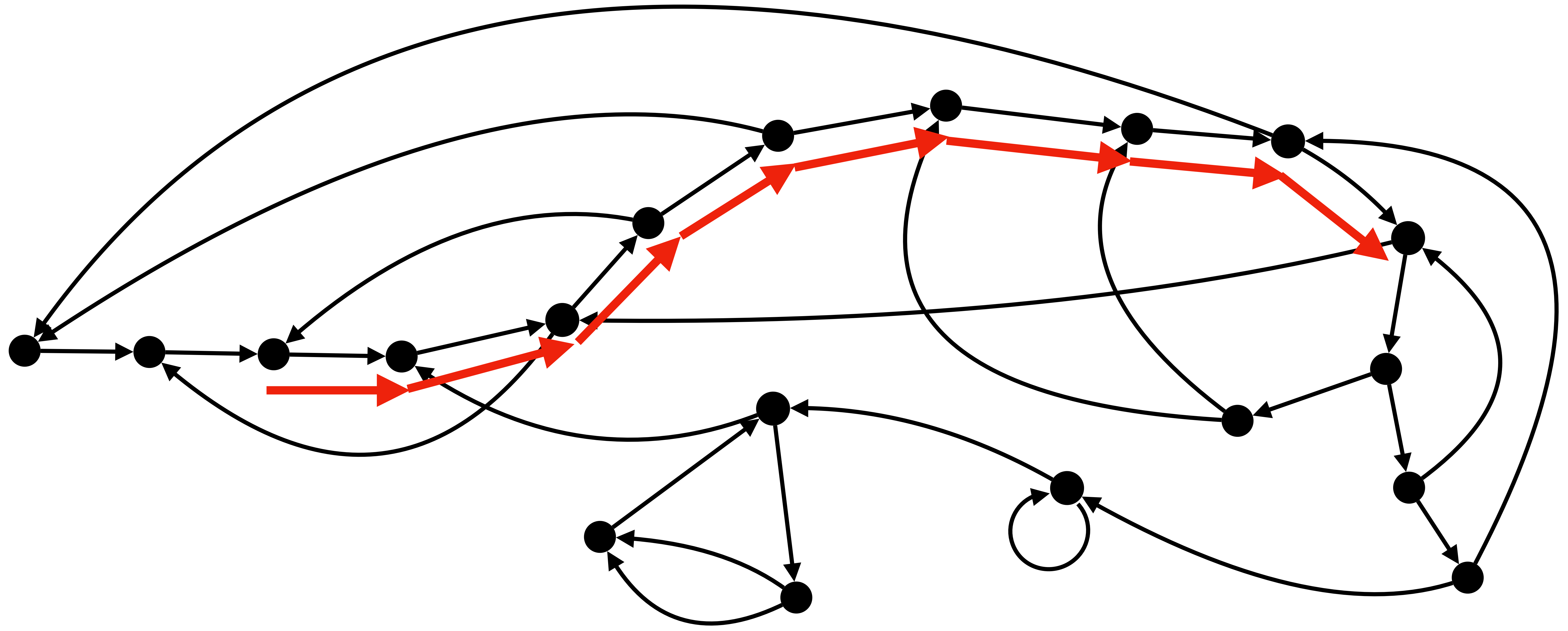

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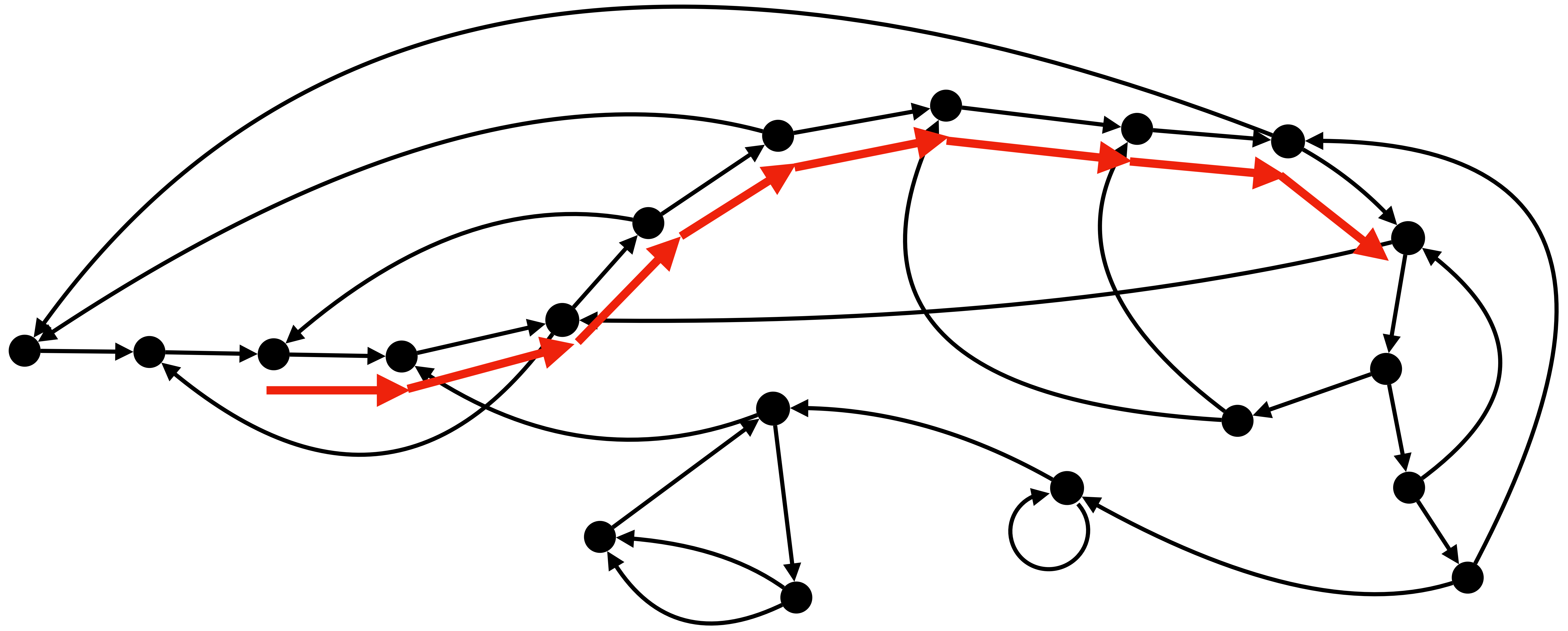

| chr10, length 135M | #Strings | Avg. length | Avg. #SNPs / string |
|---|---|---|---|
| unitigs | 260K | 546 | 26 |
| omnitigs | 158K **(-40%)** | 887 **(+62%)** | 41 **(+58%)** |

## Section summary

Theory is important,
but more so when
it is motivated by practice