Idury-Waterman Algorithm (1995)

CS1820/2820: Algorithmic Foundations of Computational Biology
Spring 2022

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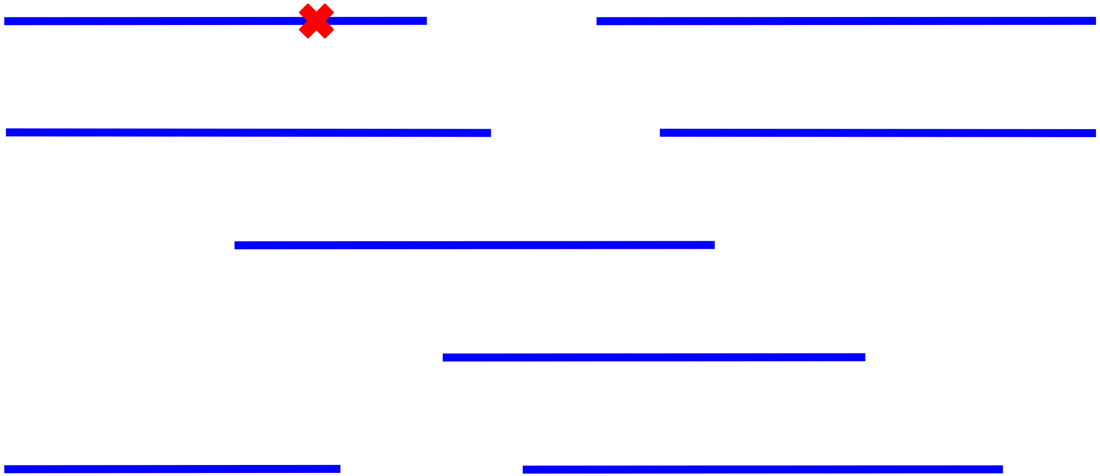
The Algorithm

Inputs: Reads (fragments) f_1, f_2, … f_N and length k

1. Obtain the union of the **spectrum** of all reads
2. Construct the **sequence graph** with (k-1)-tuples as nodes from spectra in (1)
3. Perform a variant of **Eulerian tour** to infer sequence(s)
4. Align the reads to the inferred sequence(s) in (3)

An example:

TTCATGGACATCGAC



An example:

```
TTCATGGACATCGAC
TTCAGG  CATCGAC
TTCATGG   TCGAC
   ATGGACA
      GACATC
TTCAT  ACATCGA
```

An example:

f_1 TTCAGG

f_2 TTCATGG

f_3 ATGGACA

f_4 TTCAT

f_5 CATCGAC

f_6 TCGAC

f_7 GACATC

f_8 ACATCGA

An example:

f_1	TTCAGG
f_2	TTCATGG
f_3	ATGGACA
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f_5	CATCGAC
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1) Obtain the union of spectra for all reads

f_1	TTCAGG
f_2	TTCATGG
f_3	ATGGACA
f_4	TTCAT
f_5	CATCGAC
f_6	TCGAC
f_7	GACATC
f_8	ACATCGA

First, we identify the set of all k-mers (substrings of length k) present in the data.

Let’s choose k = 4.

1) Obtain the union of spectra for all reads

f_1	TTCAGG	→	TTCA, TCAG, CAGG
f_2	TTCATGG	→	TTCA, TCAT, CATG, ATGG
f_3	ATGGACA	→	ATGG, TGGA, GGAC, GACA
f_4	TTCAT	→	TTCA, TCAT
f_5	CATCGAC	→	CATC, ATCG, TCGA, CGAC
f_6	TCGAC	→	TCGA, CGAC
f_7	GACATC	→	GACA, ACAT, CATC
f_8	ACATCGA	→	ACAT, CATC, ATCG, TCGA

1) Obtain the union of spectra for all reads

f_1	TTCAGG	⇨	TTCA, TCAG, CAGG
f_2	TTCATGG	⇨	TTCA, TCAT, CATG, ATGG
f_3	ATGGACA	⇨	ATGG, TGGA, GGAC, GACA
f_4	TTCAT	⇨	TTCA, TCAT
f_5	CATCGAC	⇨	CATC, ATCG, TCGA, CGAC
f_6	TCGAC	⇨	TCGA, CGAC
f_7	GACATC	⇨	GACA, ACAT, CATC
f_8	ACATCGA	⇨	ACAT, CATC, ATCG, TCGA

⇨

TTCA
TCAG
CAGG
TCAT
CATG
ATGG
TGGA
GGAC
GACA
CATC
ATCG
TCGA
CGAC
ACAT

2) Construct the sequence graph on (k-1)-mers

f_1	TTCAGG
f_2	TTCATGG
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f_6	TCGAC
f_7	GACATC
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⇨

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GACA
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TCGA
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For each k-mer ($a_1 \ldots a_k$), we create an edge between nodes labeled $a_1 \ldots a_{k-1}$ and $a_2 \ldots a_k$.

If those nodes do not exist yet, we add them to the graph.

We label the edge by its k-mer, $a_1 \ldots a_k$.

We also store the set of position values (f, i, j) in each edge, which identify all occurrences of that k-mer by (fragment index, start position, end position)*

2) Construct the sequence graph on (k-1)-mers

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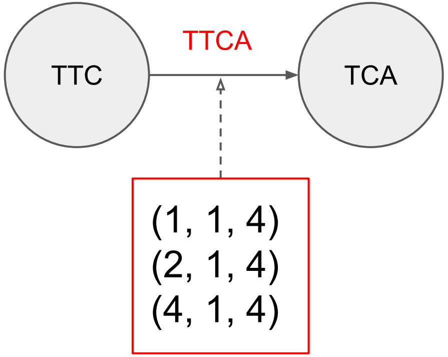

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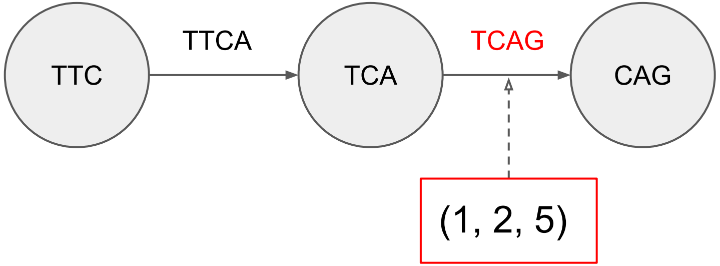

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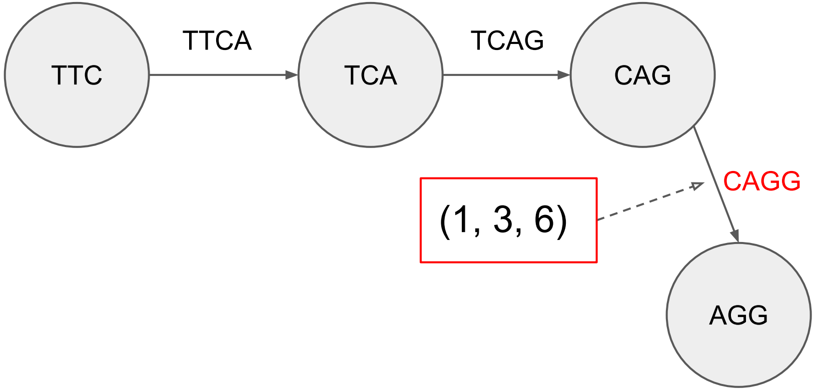

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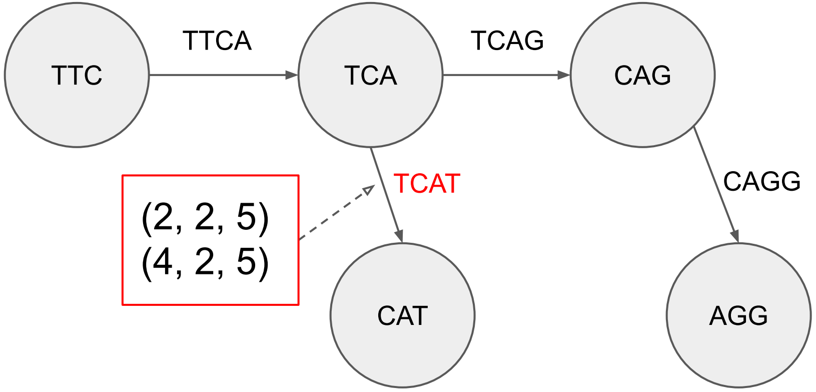

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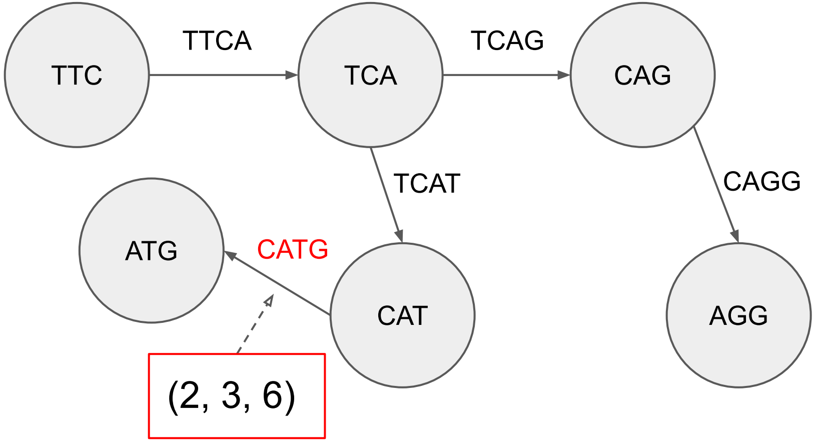

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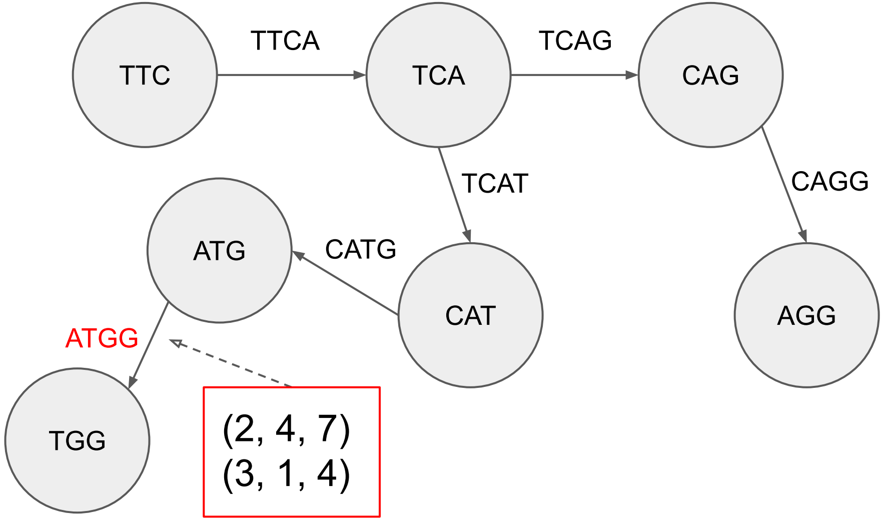

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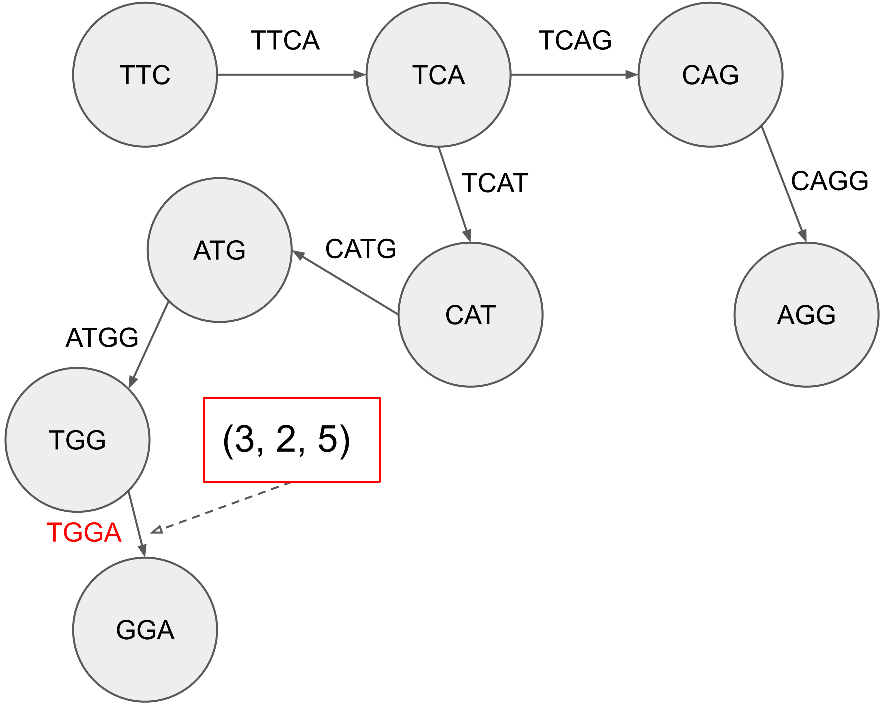


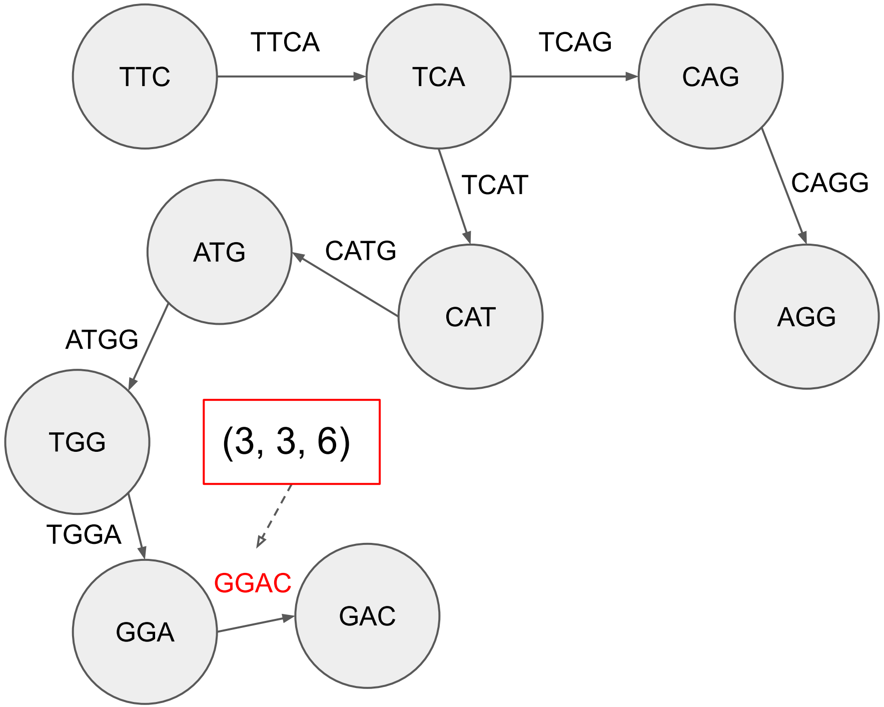
(3, 2, 5)

2) Construct the sequence graph on (k-1)-mers

f_1	TTCAGG
f_2	TTCATGG
f_3	ATGGACA
f_4	TTCAT
f_5	CATCGAC
f_6	TCGAC
f_7	GACATC
f_8	ACATCGA

⇨

TTCA
TCAG
CAGG
TCAT
CATG
ATGG
TGGA
GGAC
GACA
CATC
ATCG
TCGA
CGAC
ACAT



2) Construct the sequence graph on (k-1)-mers

f_1	TTCAGG
f_2	TTCATGG
f_3	ATGGACA
f_4	TTCAT
f_5	CATCGAC
f_6	TCGAC
f_7	GACATC
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GACA
CATC
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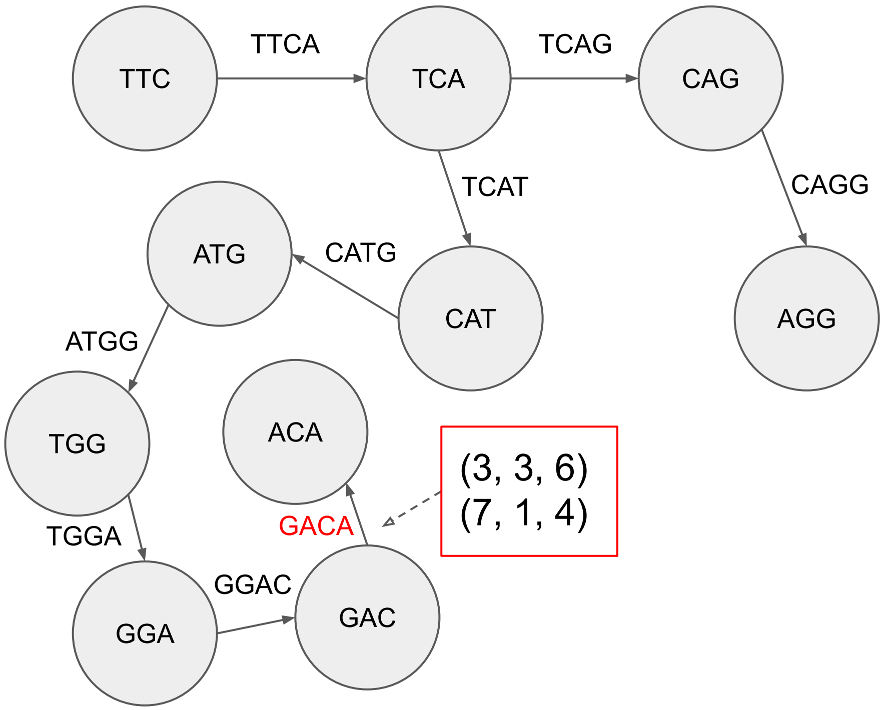
ACAT



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f_2	TTCATGG
f_3	ATGGACA
f_4	TTCAT
f_5	CATCGAC
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f_7	GACATC
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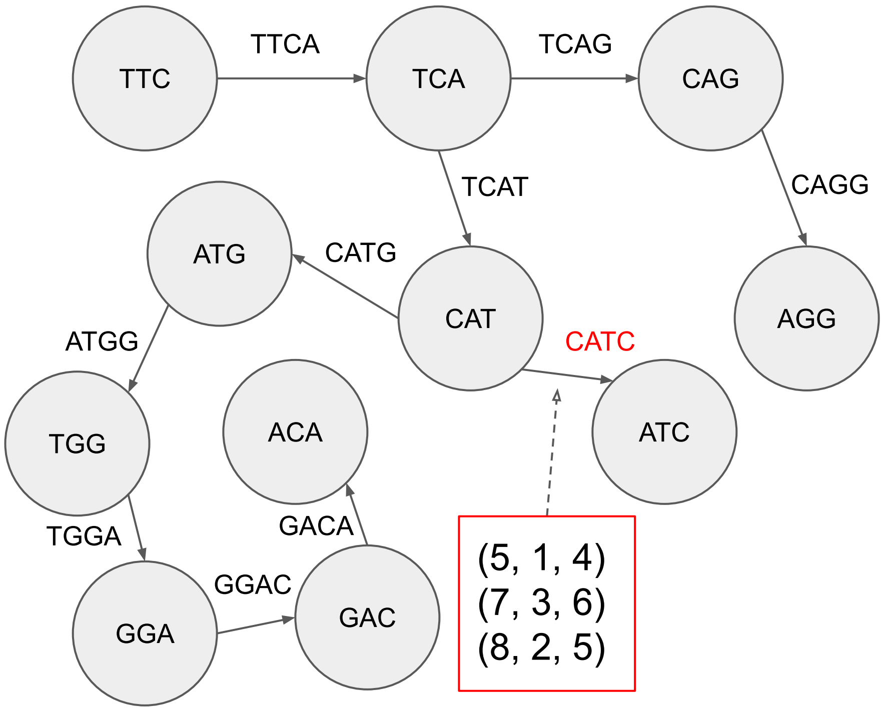

(5, 1, 4)
(7, 3, 6)
(8, 2, 5)

2) Construct the sequence graph on (k-1)-mers

f_1	TTCAGG
f_2	TTCATGG
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f_4	TTCAT
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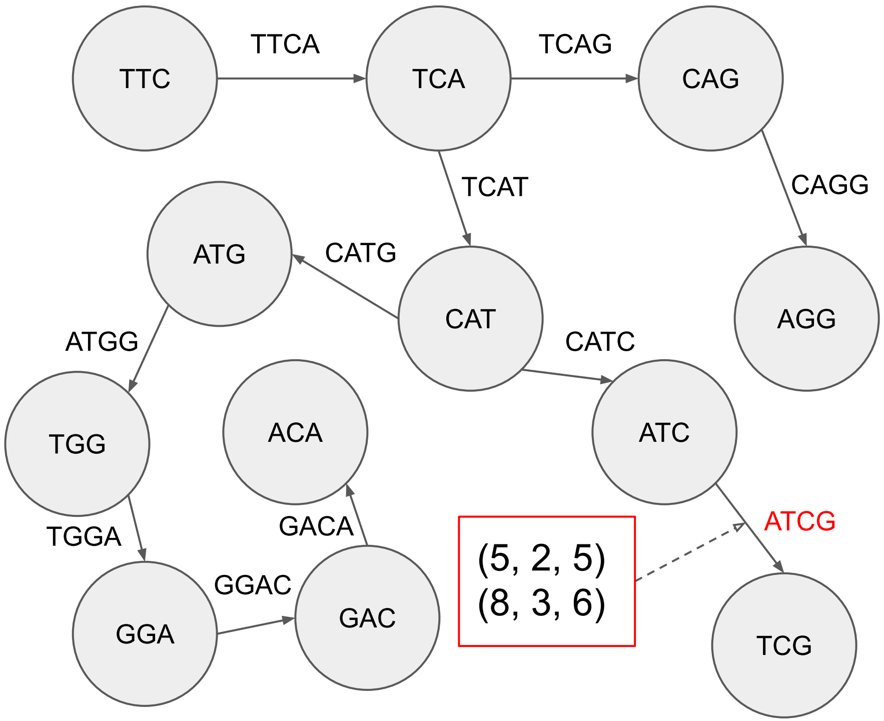

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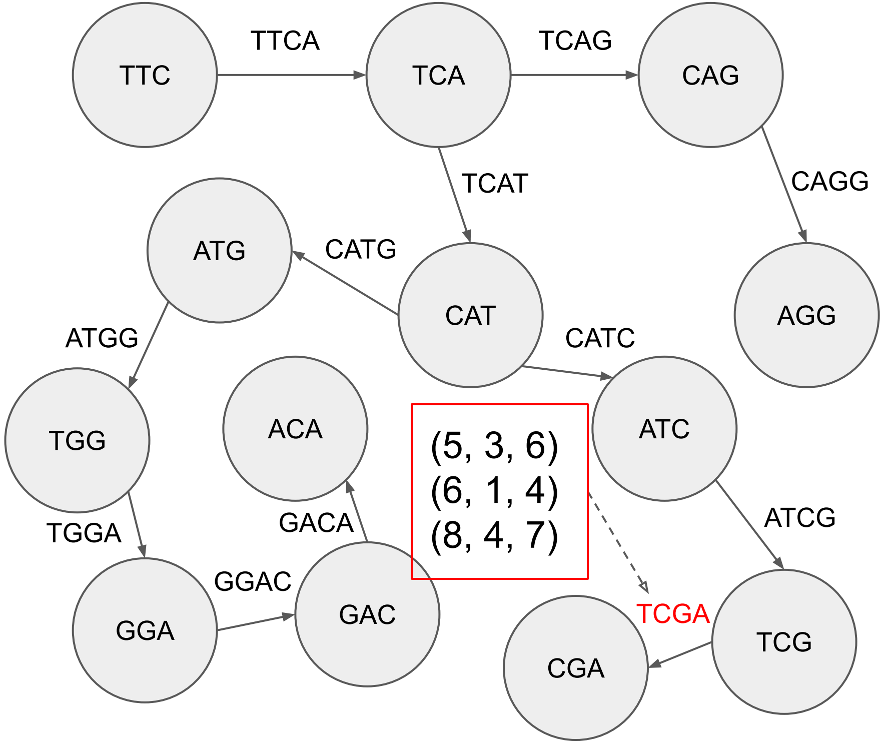

(5, 3, 6)
(6, 1, 4)
(8, 4, 7)

2) Construct the sequence graph on (k-1)-mers

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⇒

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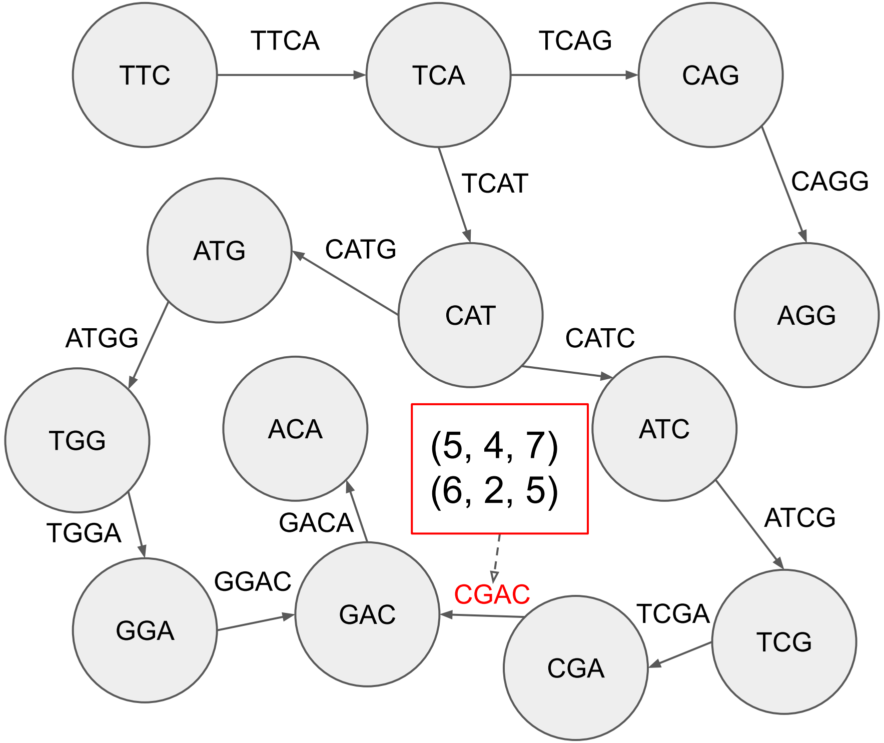

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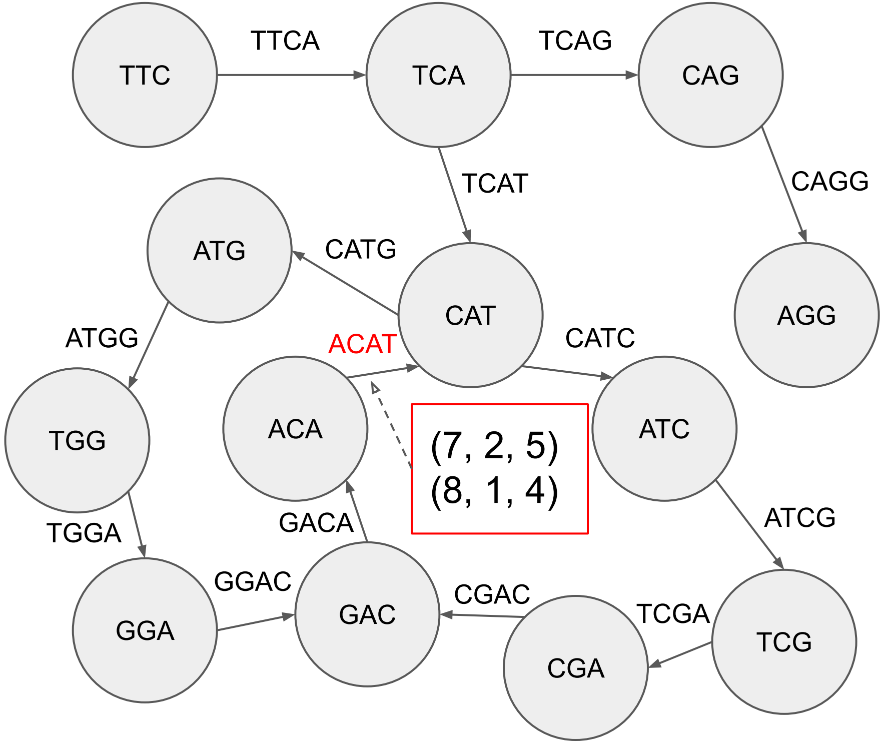

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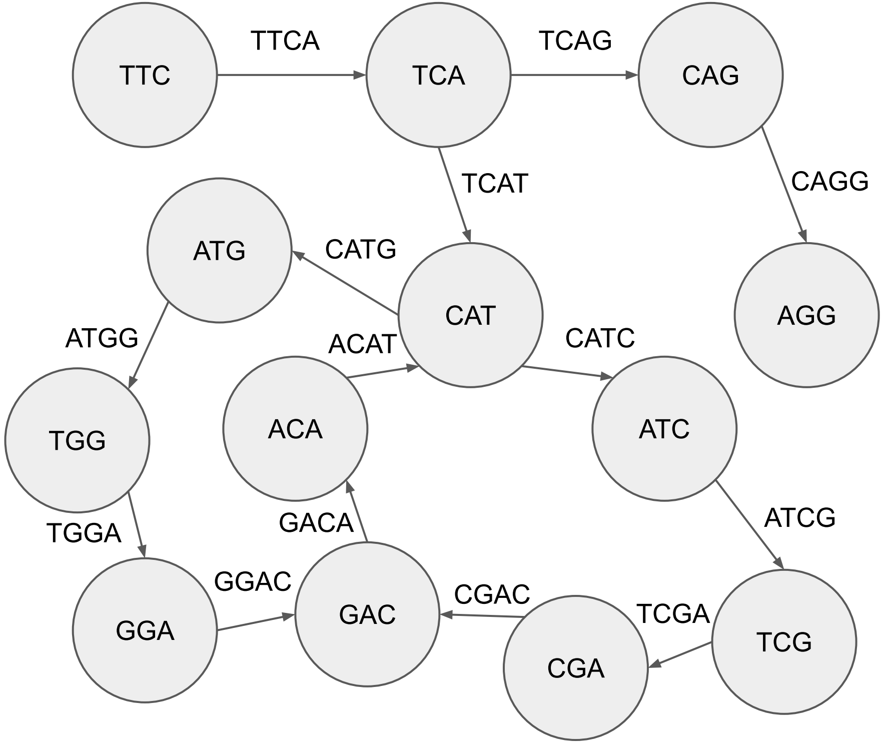

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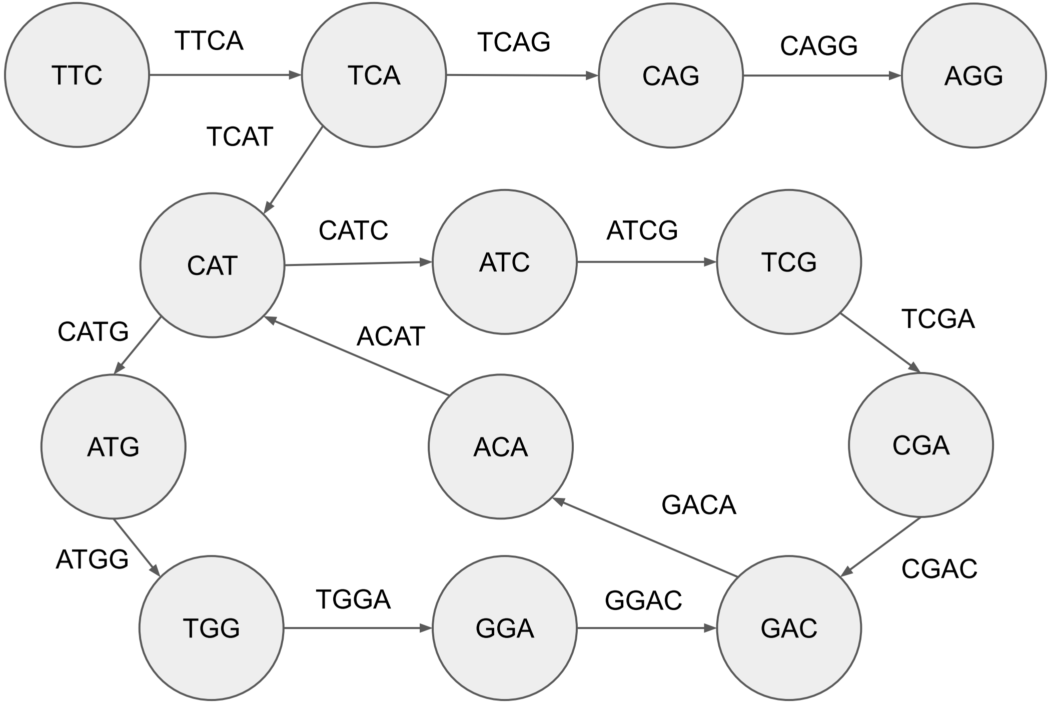
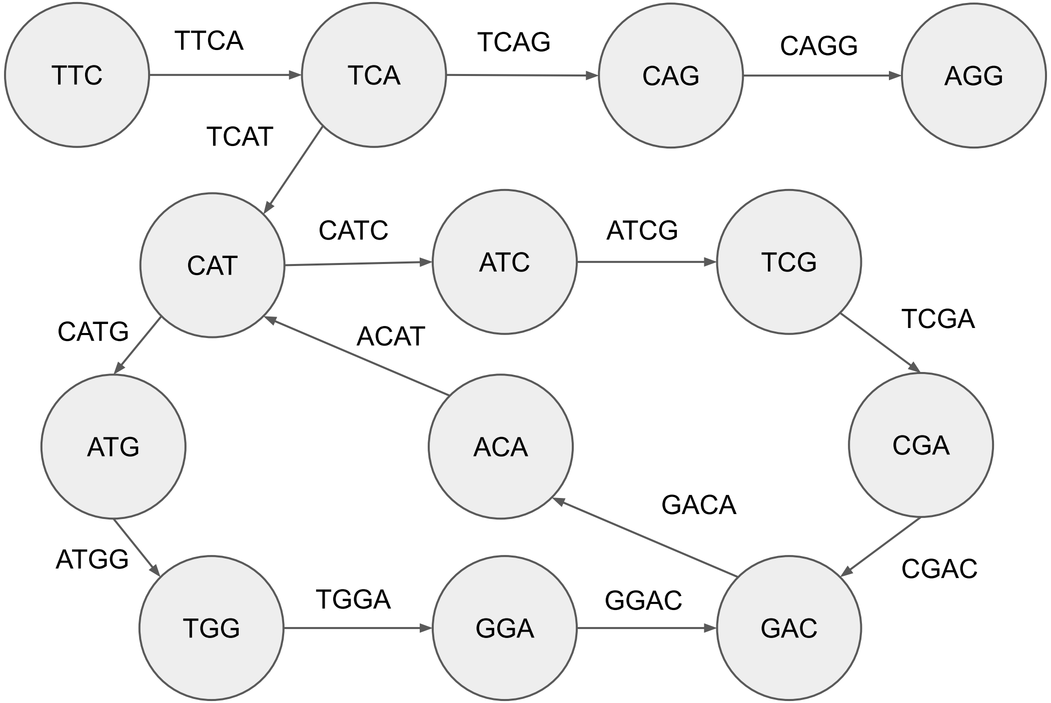


2) Construct the sequence graph on (k-1)-mers

This graph is sufficient to begin searching for an Eulerian path, but we can simplify the graph beforehand to make inference simpler.

There are three types of graph reductions that are possible, which we will illustrate next.



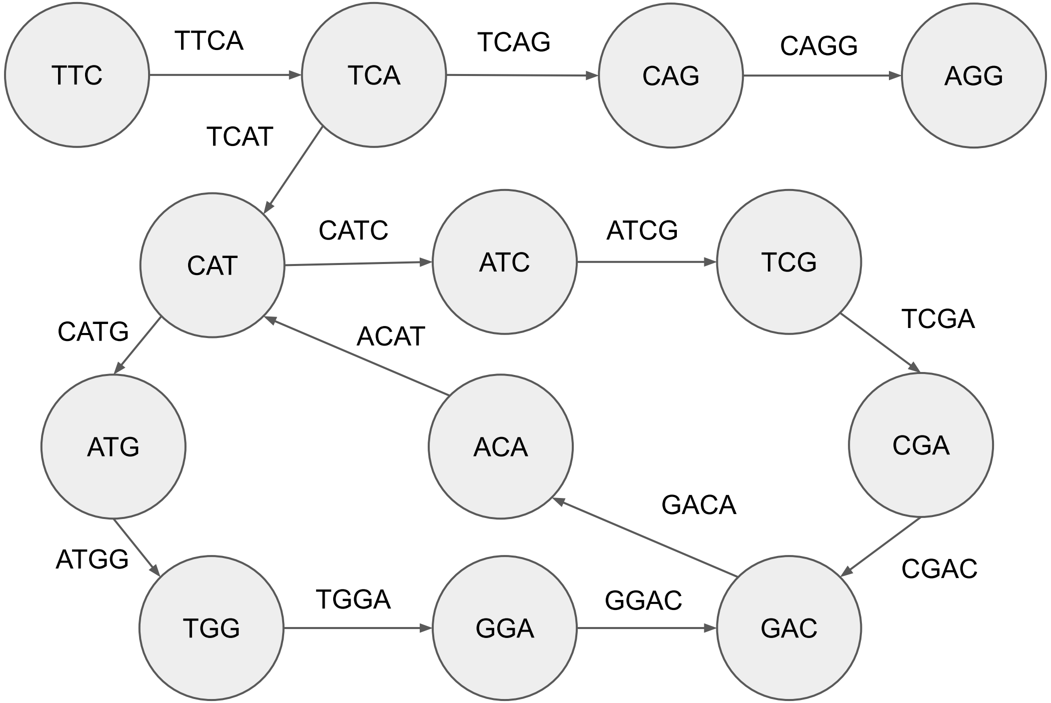
2) Construct the sequence graph on (k-1)-mers

This graph is sufficient to begin searching for an Eulerian path, but we can simplify the graph beforehand to make inference simpler.

There are three types of graph reductions that are possible, which we will illustrate next.

Why did we bother storing positional information, (f, i, j)?

These are useful for establishing continuity when performing reductions and the Eulerian tour.



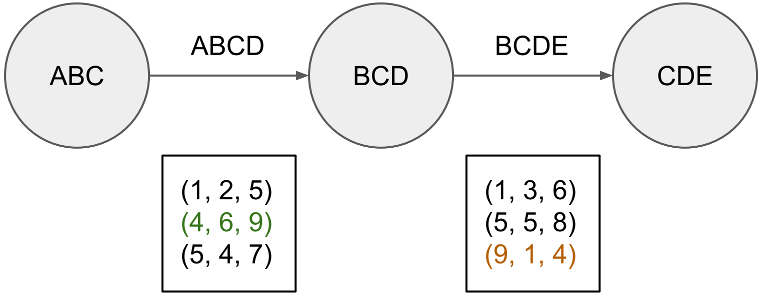
2a) Graph reductions: singletons

Singletons are nodes with indegree = 1 and outdegree = 1 (i.e., a node v with exactly one incoming edge [u, v] and exactly one outgoing edge [v, w]).

We simplify this feature by removing the node v and its incident edges, replacing it with a new edge [u, w].

This new edge has a label which merges the two labels of the previous edges.

Likewise, it stores the position tuples of the previous edges, merged where possible.*



Formally, this is:
{(f, i, m) | (f, i, j+k-2) ∊ [u, v] and (f, j+k-2, m) ∊ [v, w]}
∪ {fragment ends of [u, v]} ∪ {fragment starts of [v, w]}

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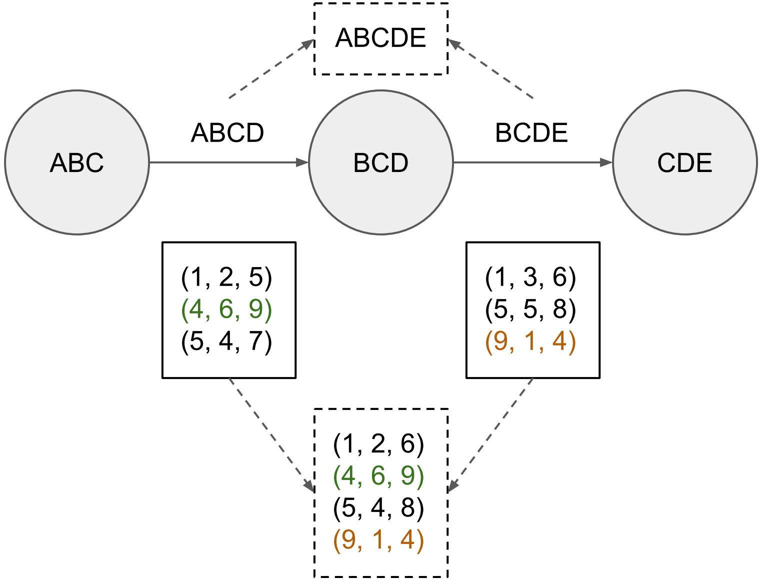

Formally, this is:
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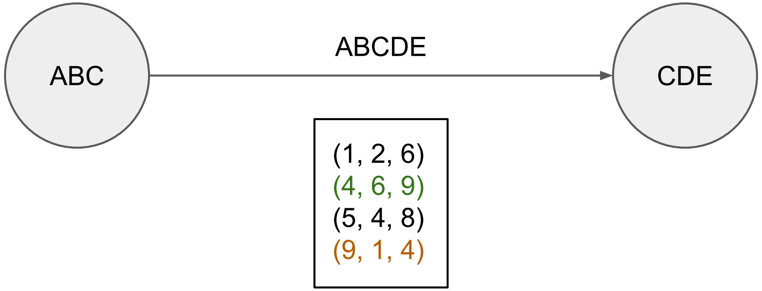

Formally, this is:

$$\{(f, i, m) \mid (f, i, j+k-2) \in [u, v] \text{ and } (f, j+k-2, m) \in [v, w]\} \cup \{\text{fragment ends of } [u, v]\} \cup \{\text{fragment starts of } [v, w]\}$$

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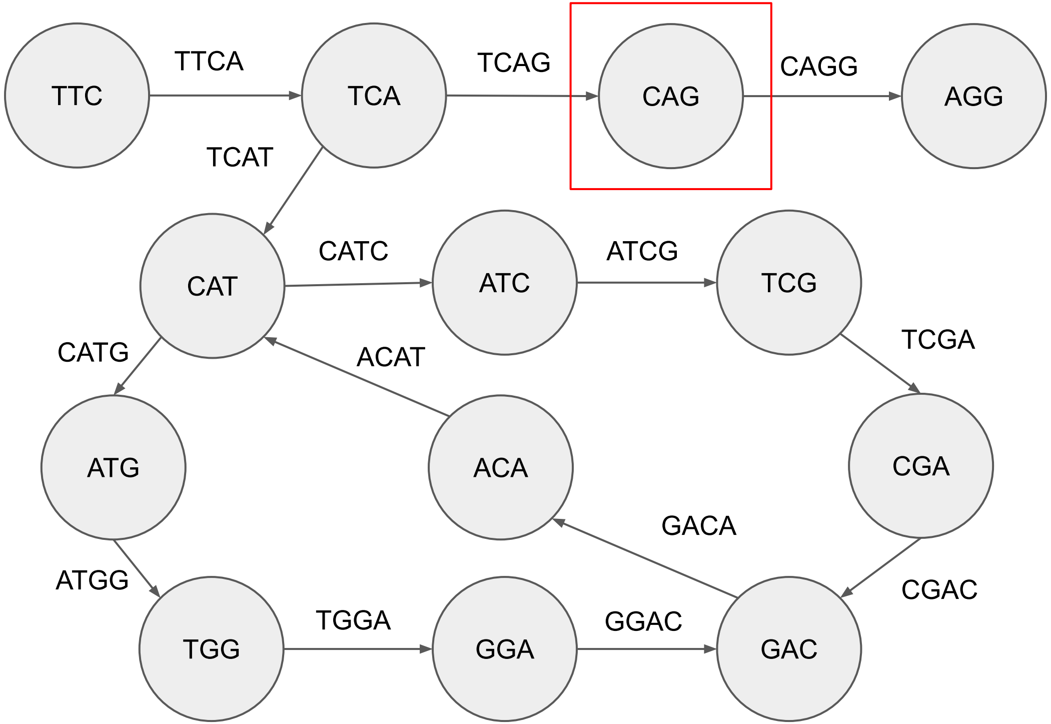

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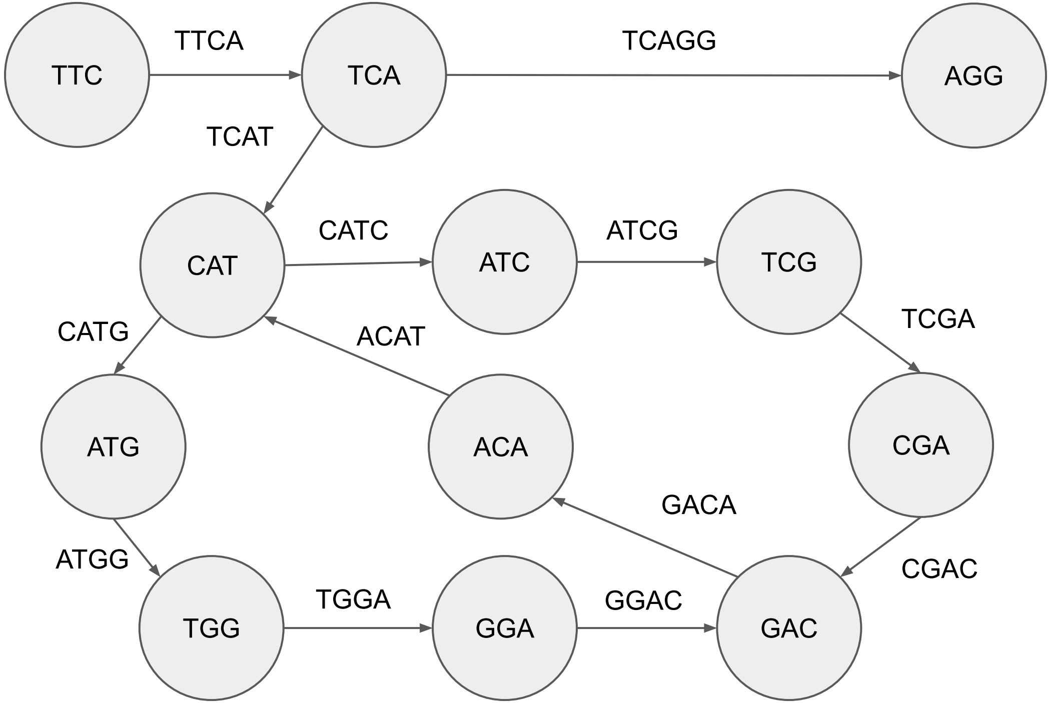

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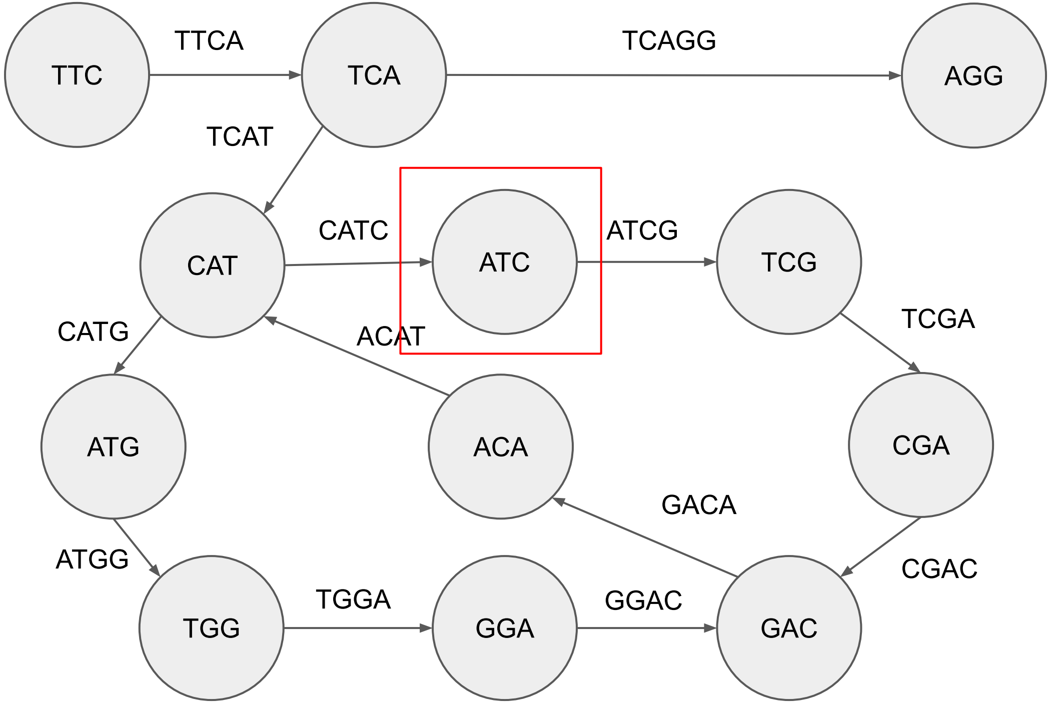

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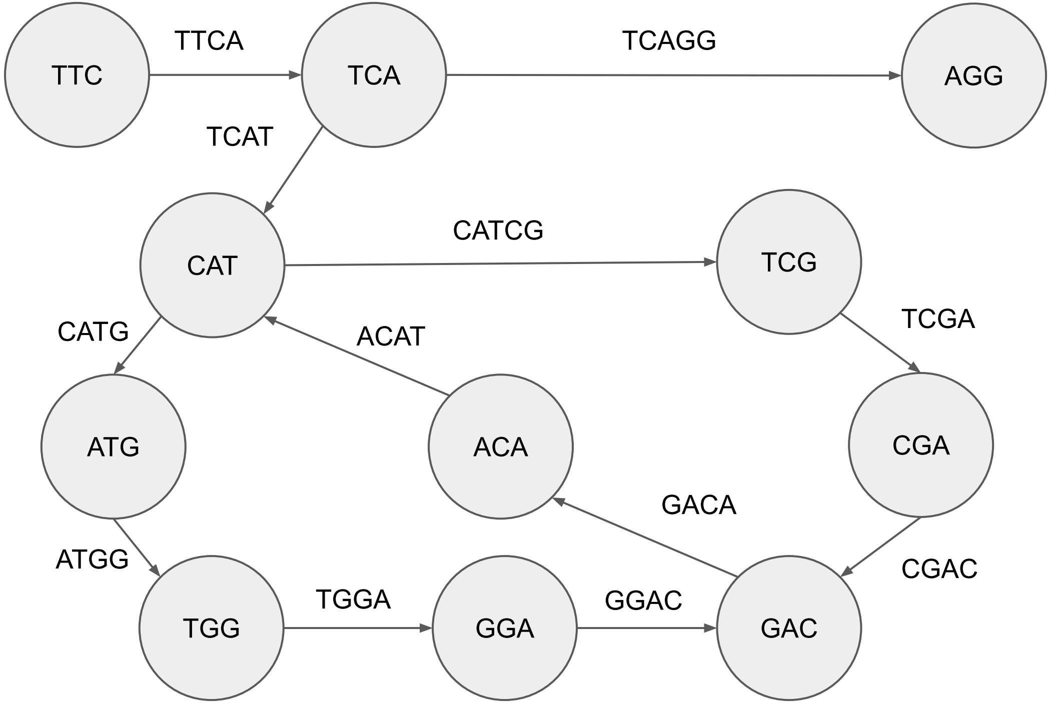

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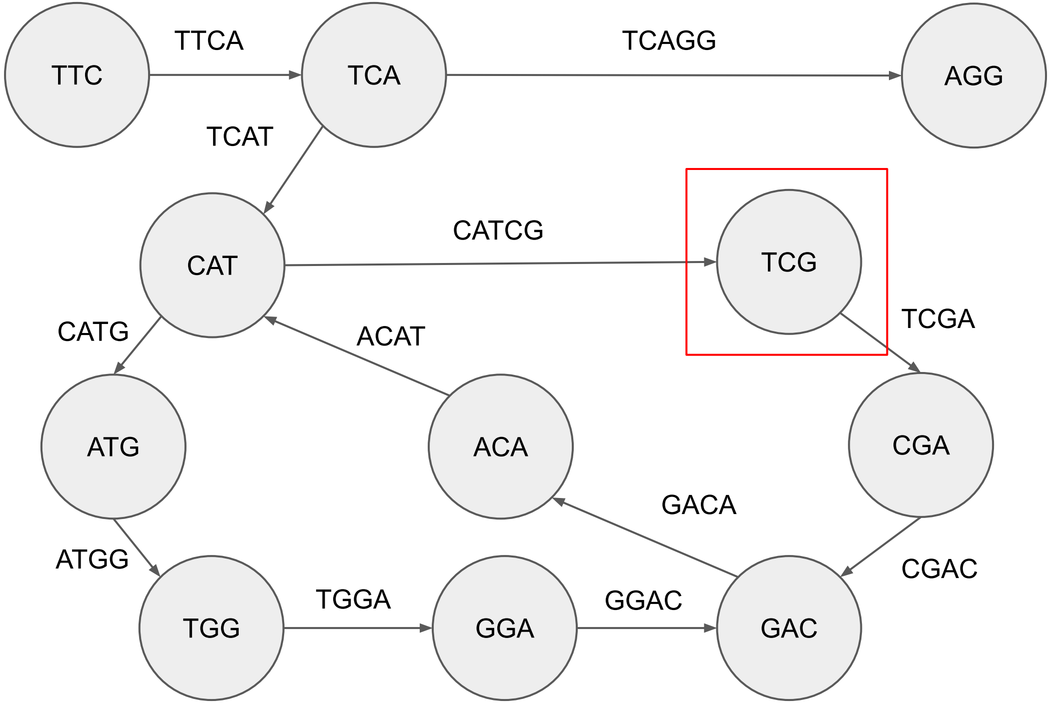

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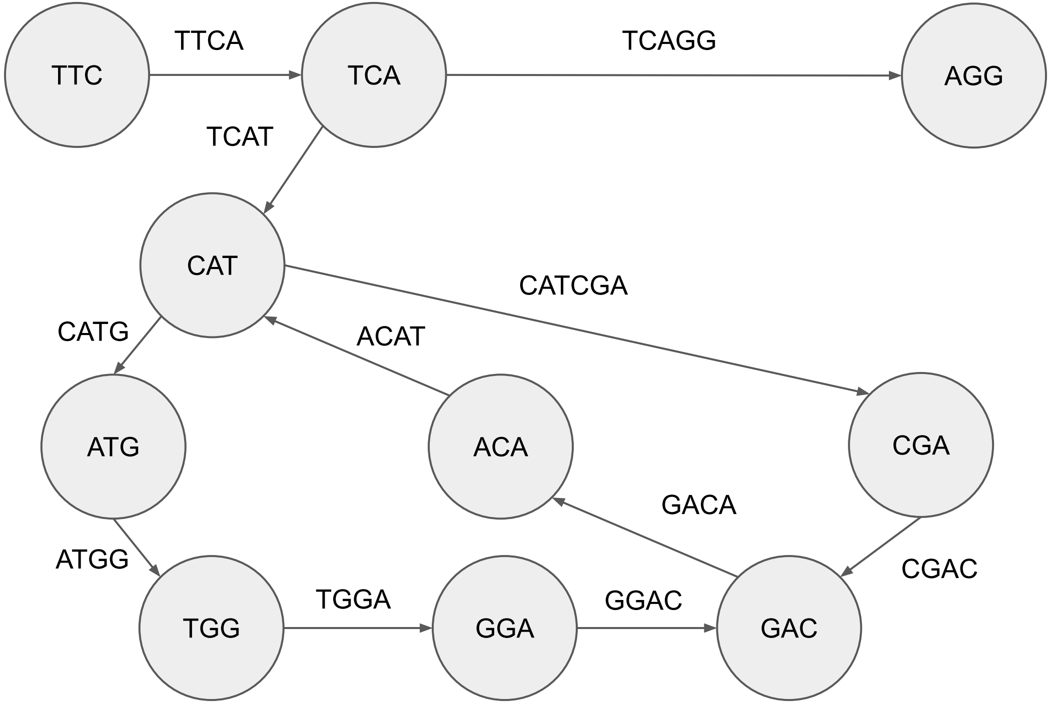

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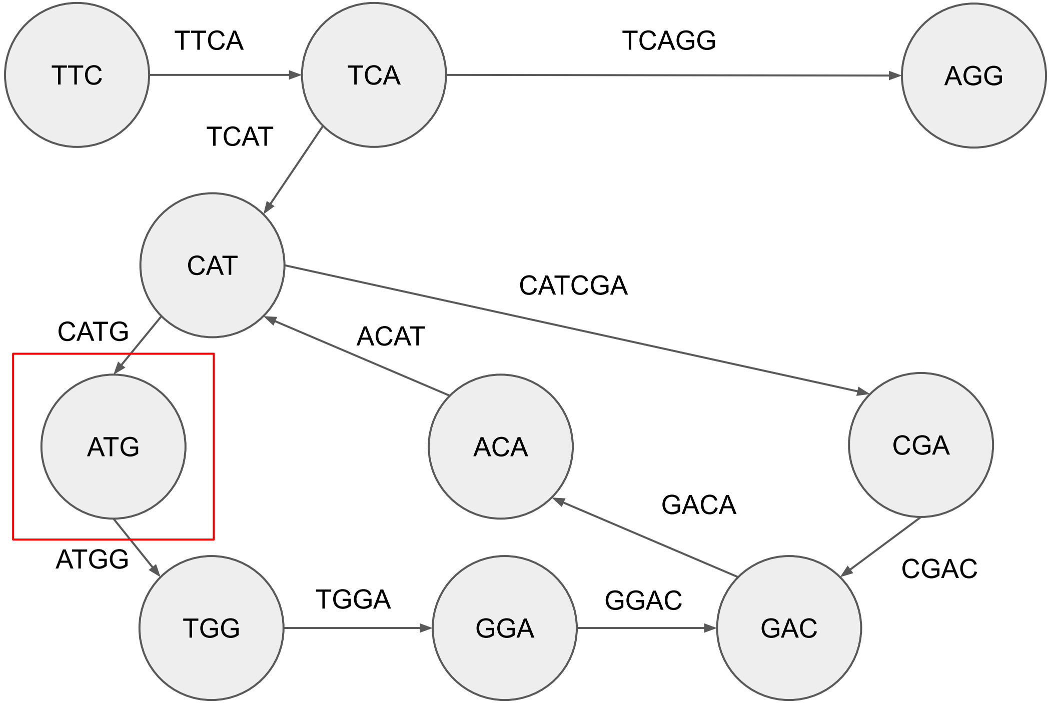

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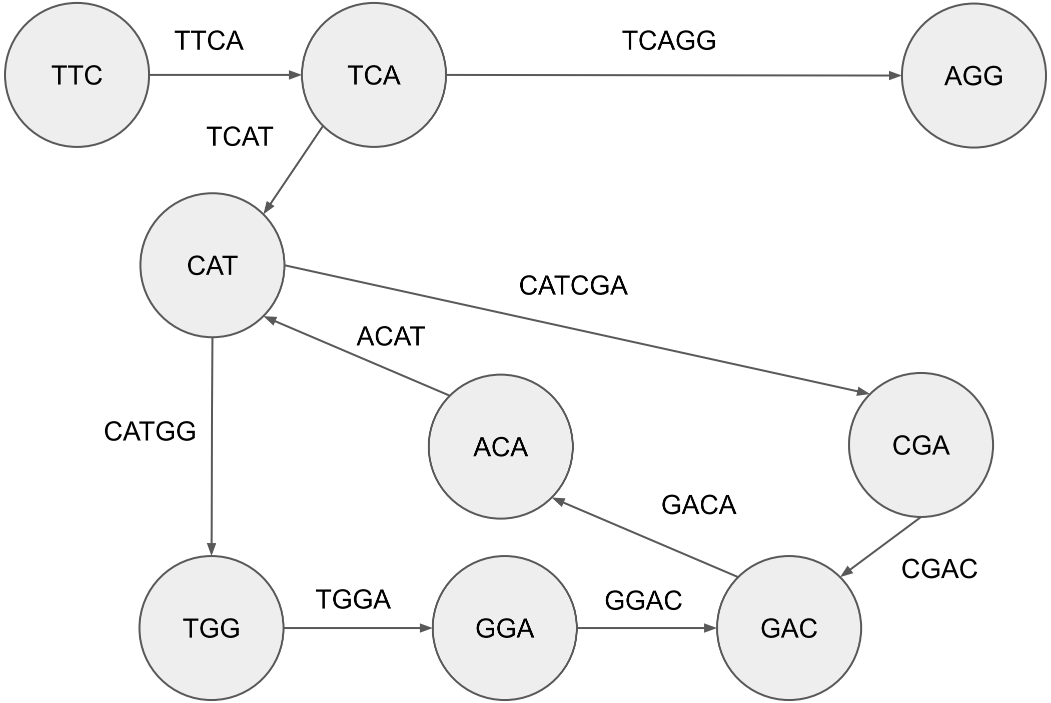

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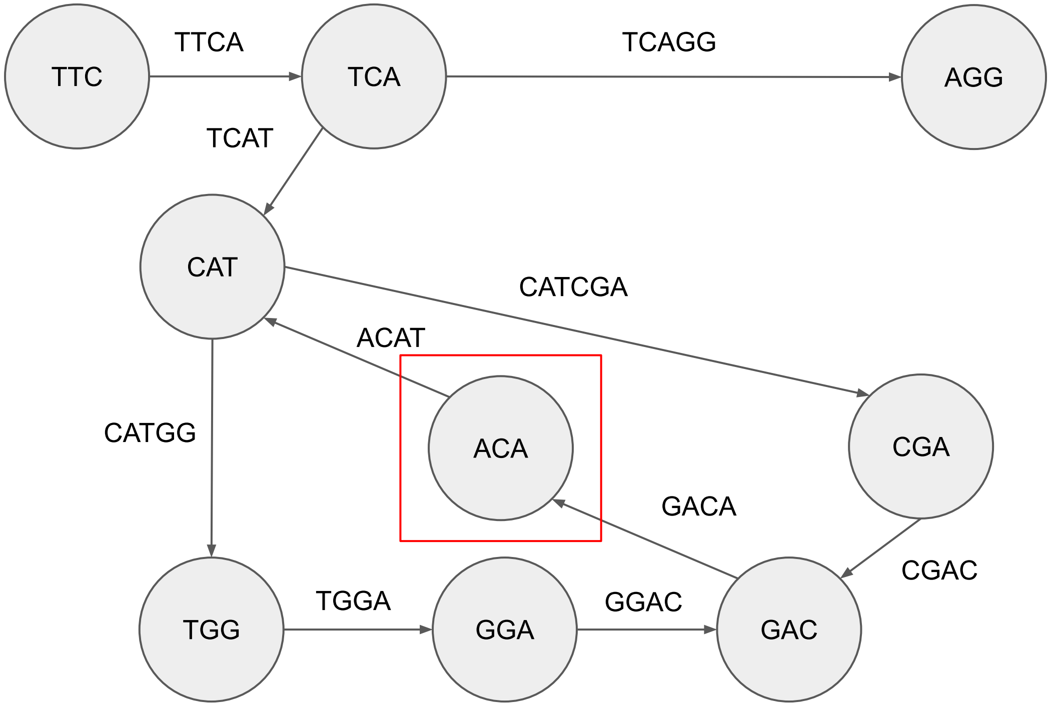

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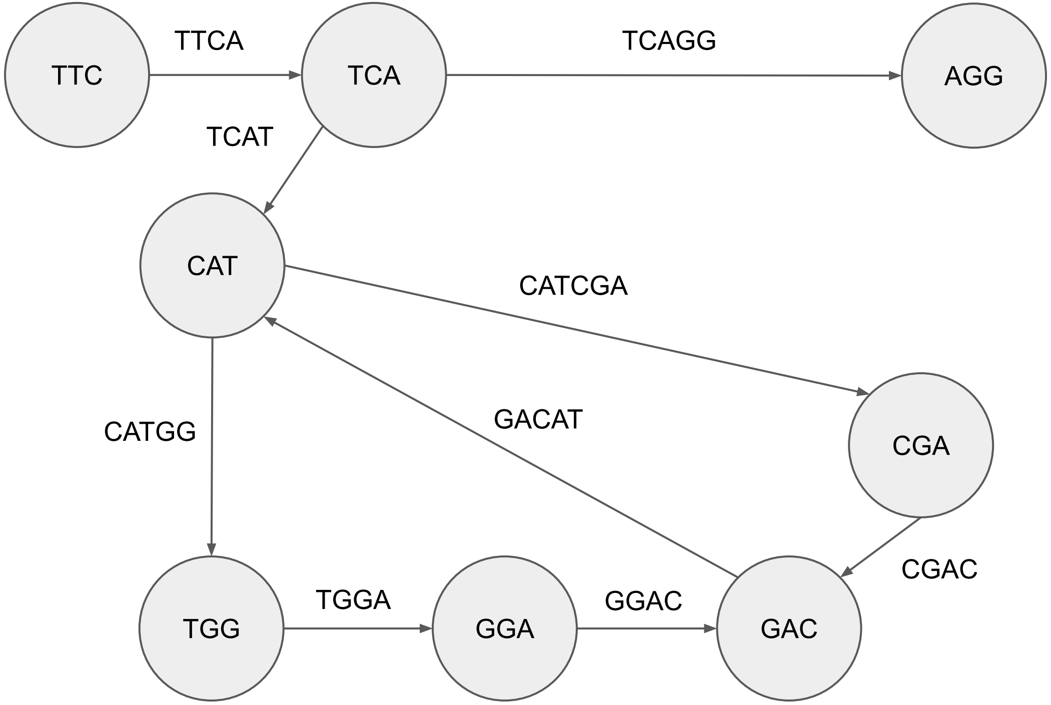

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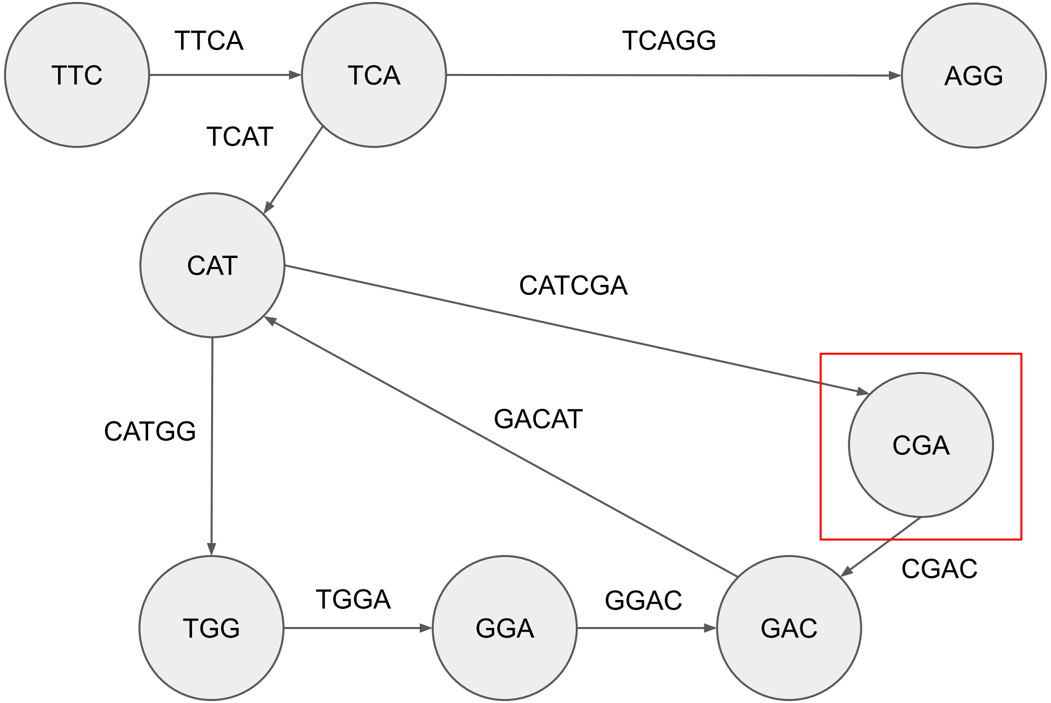

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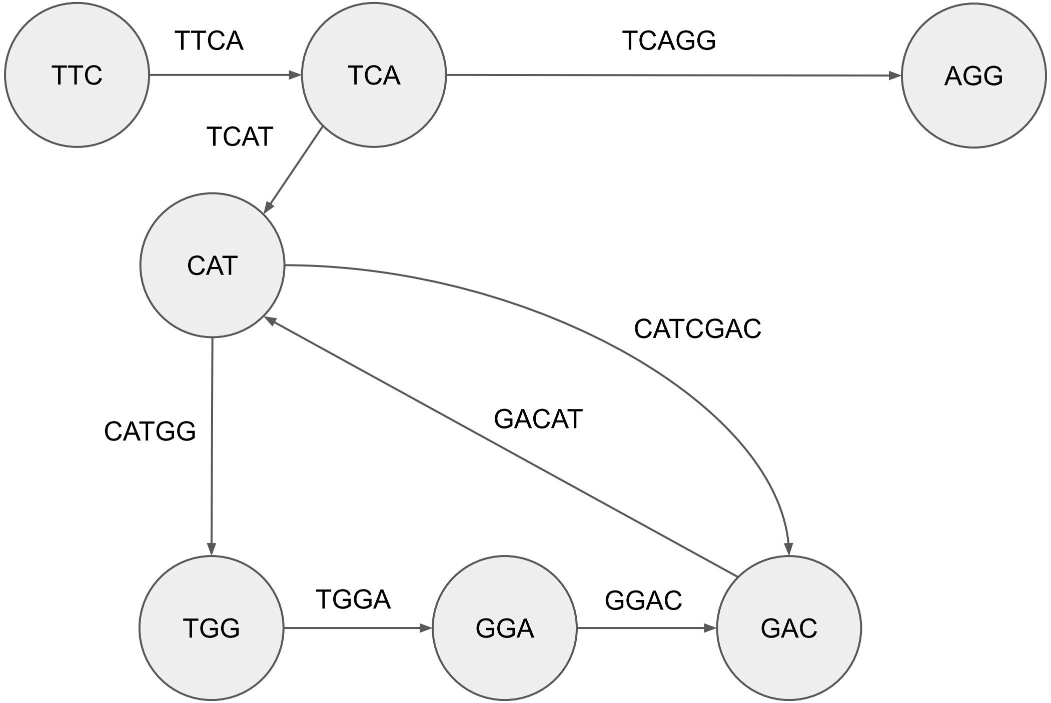

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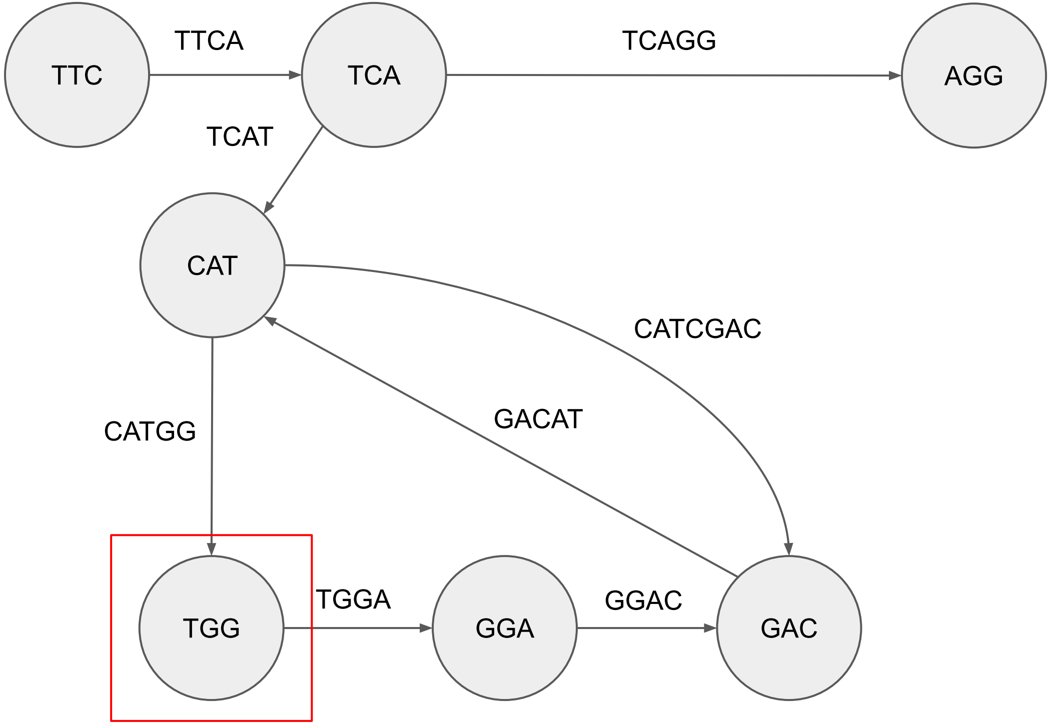

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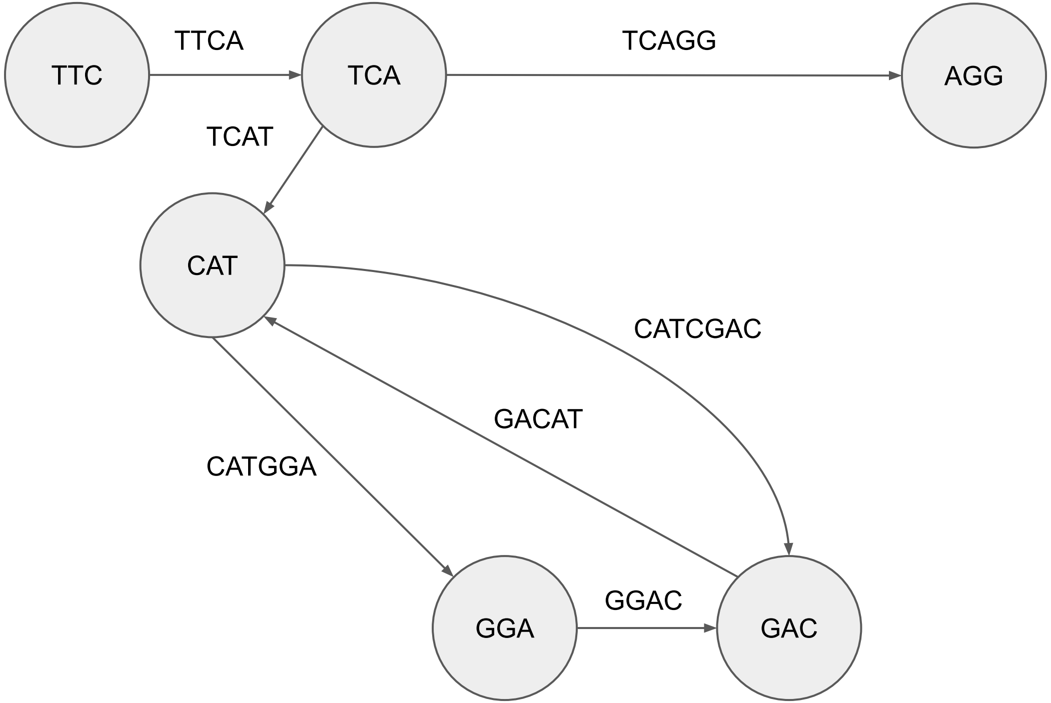

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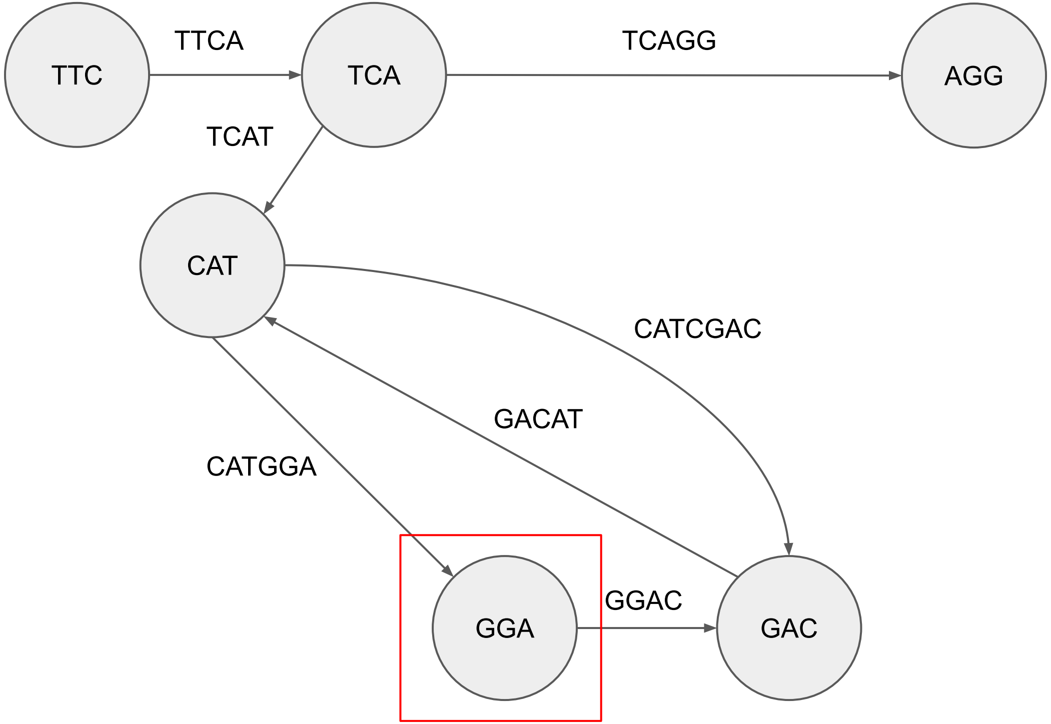

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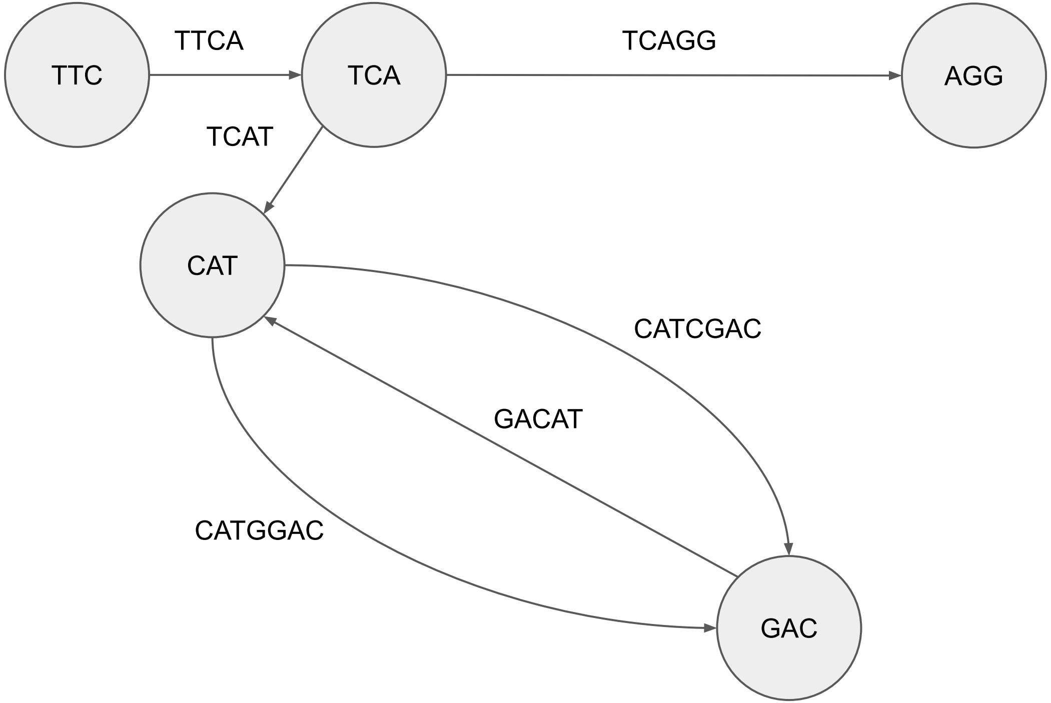
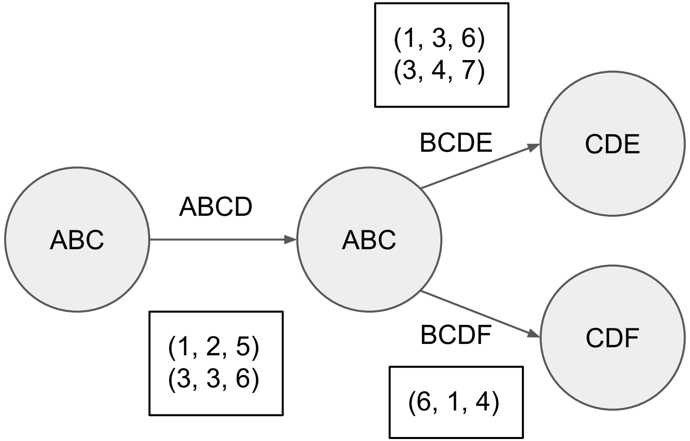


2b) Graph reductions: forks

Forks are nodes with indegree = 1 and outdegree > 1 (i.e., a node v with exactly one incoming edge [u, v] and outgoing edges [v, w_1], [v, w_2], …).*

A node with outdegree > 1 indicates either a sequencing error or a repetitive region. We apply a heuristic approach to resolve this feature.

Of the outgoing edges, keep only the one which has the most occurrences continuing from the incoming edge. If the number of such occurrences is roughly equal for all edges, leave the fork in the graph.*



“Reverse forks” (i.e., nodes with indegree > 1 and outdegree = 1) can also be resolved analogously.

Idury and Waterman formalize this heuristic as the **overlap test**, but the explanation here is the rough intuition behind its behavior.

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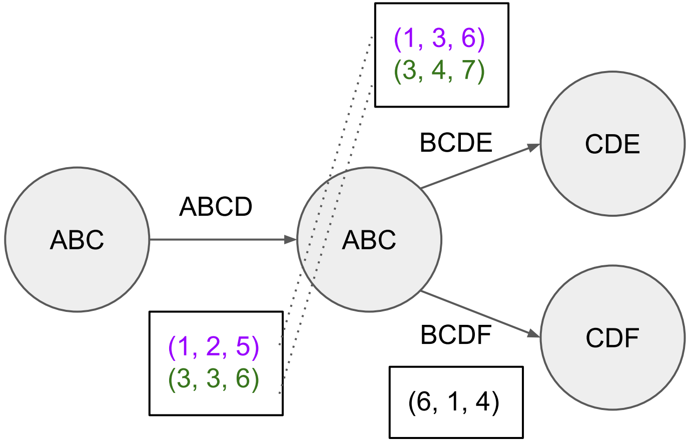

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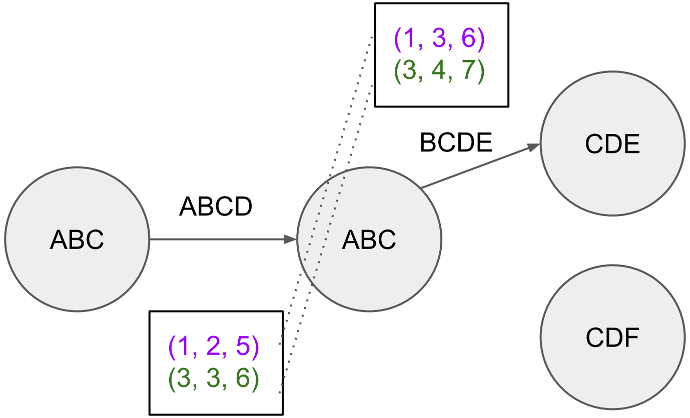

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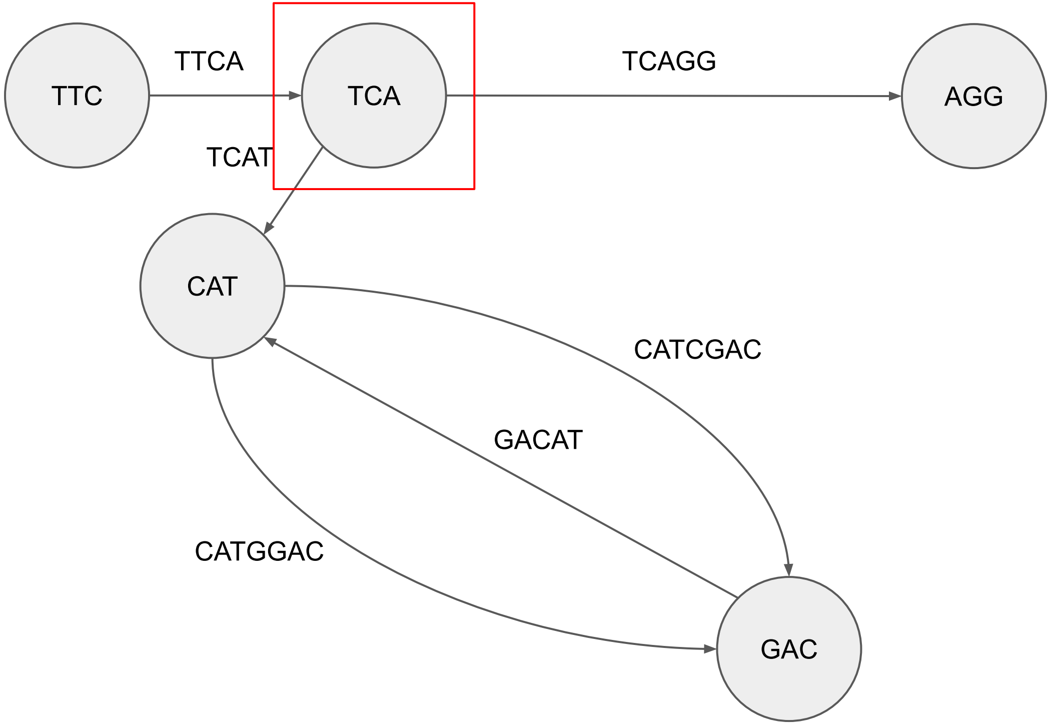

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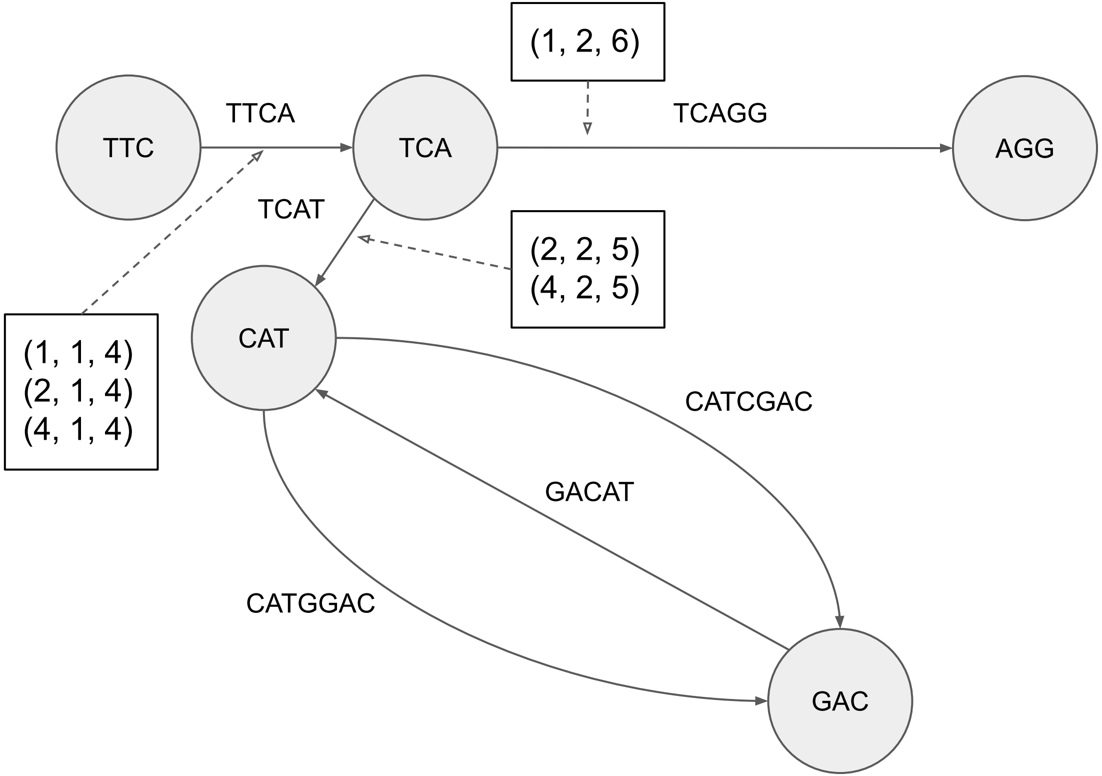

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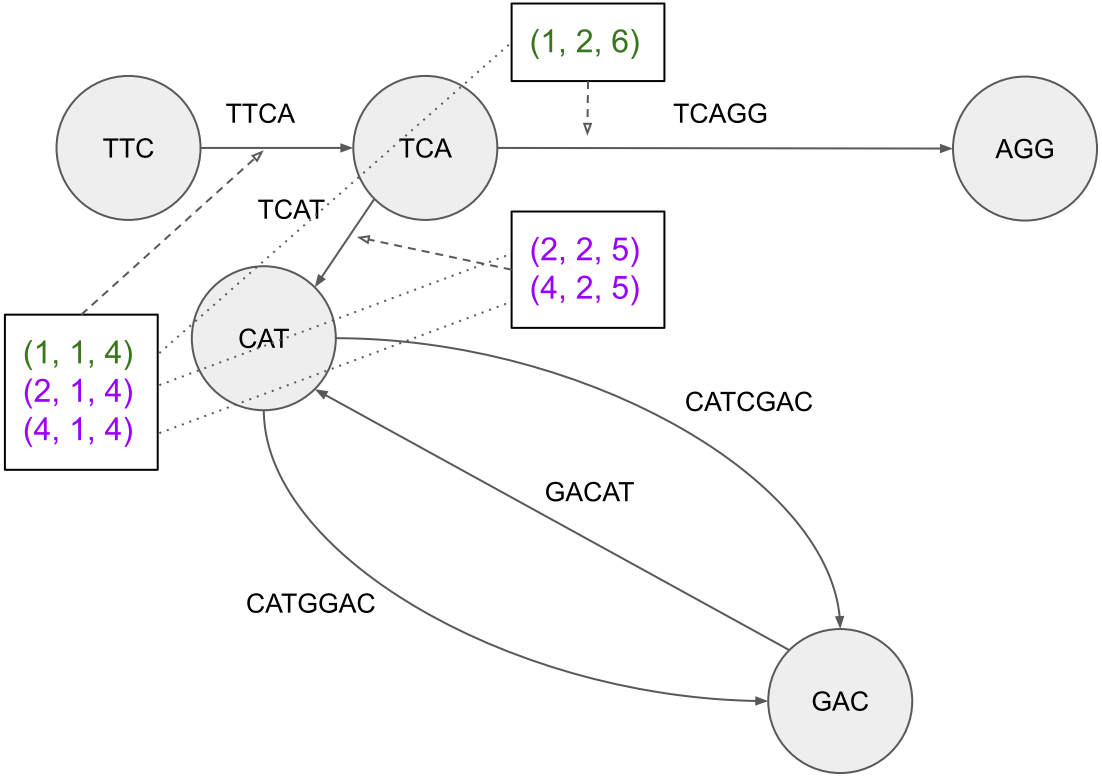

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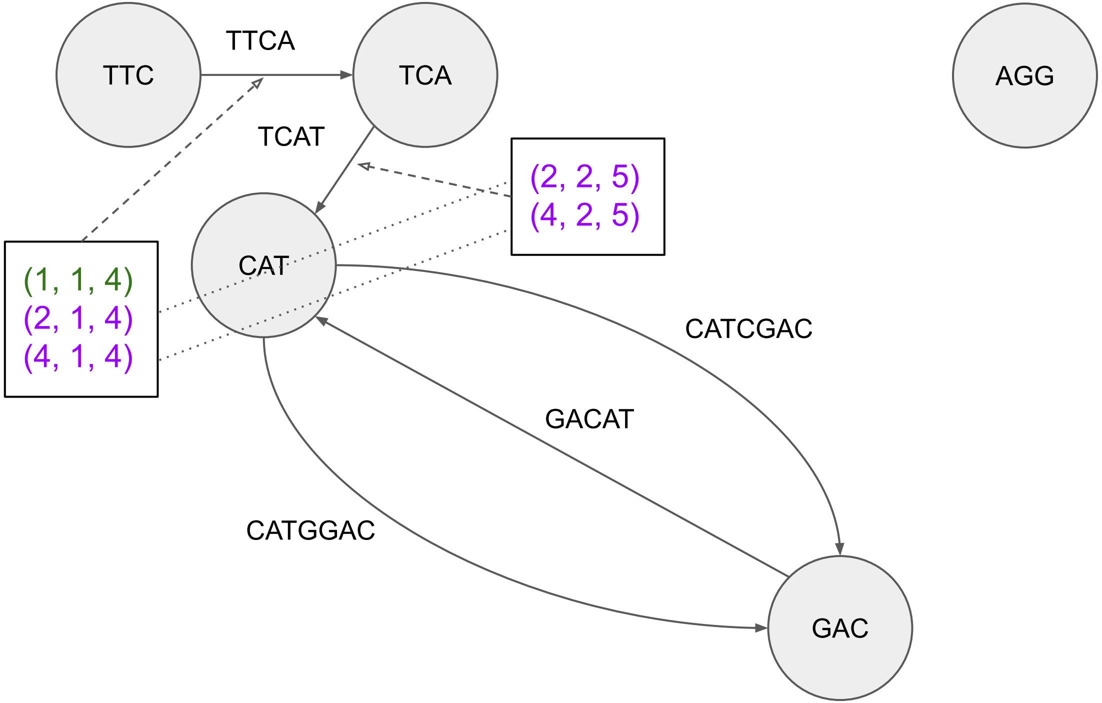

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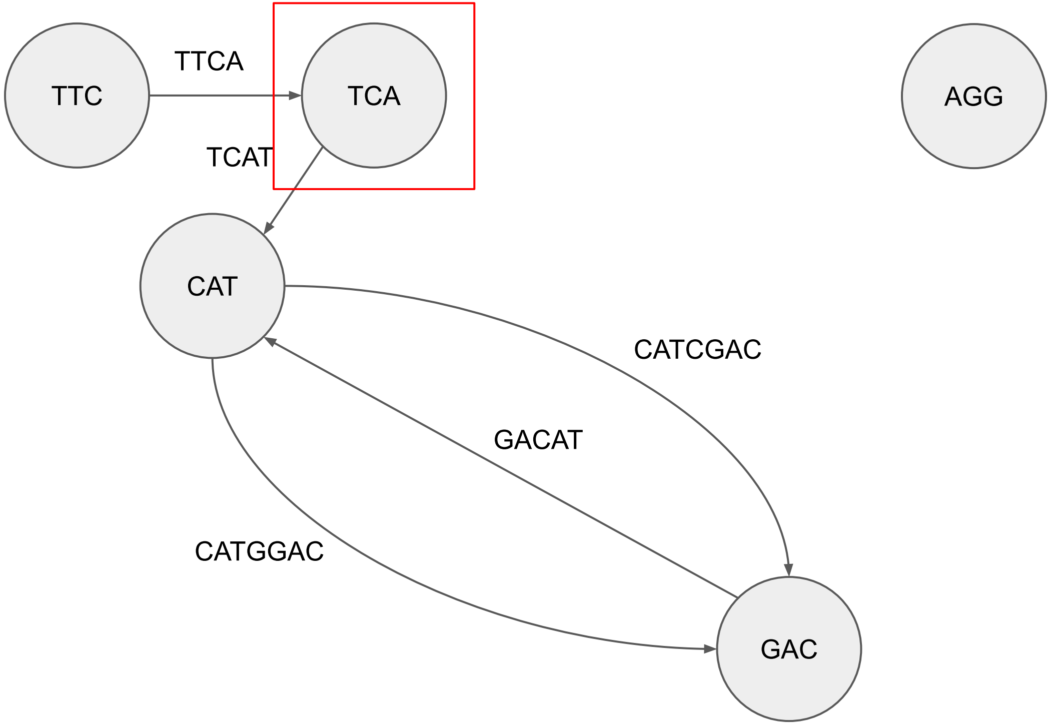

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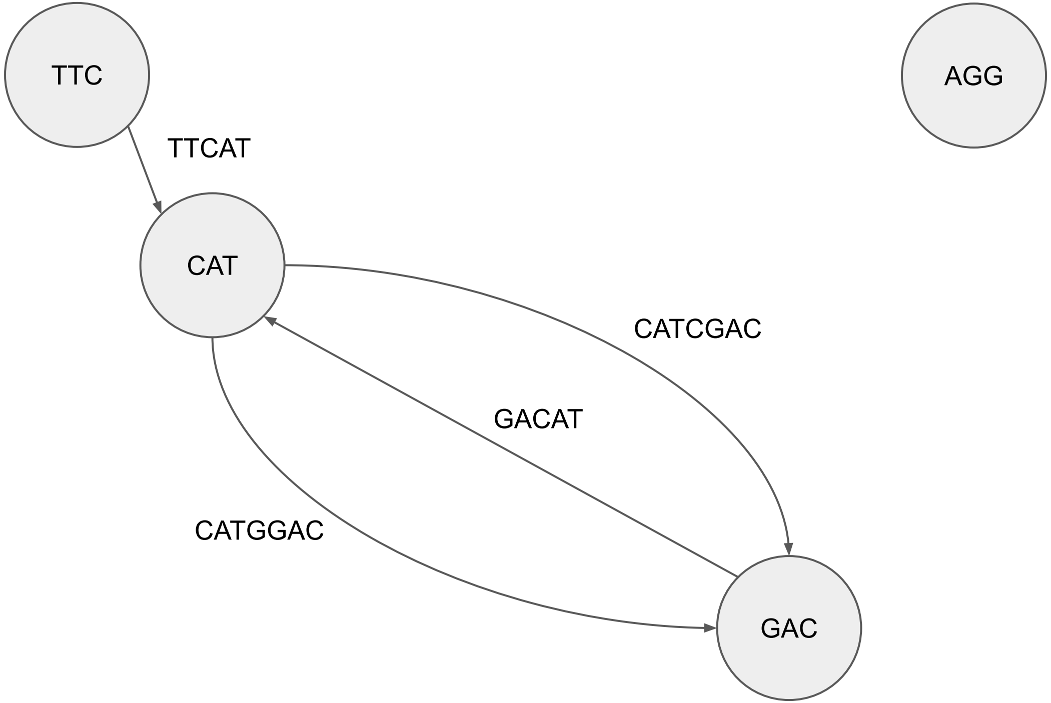
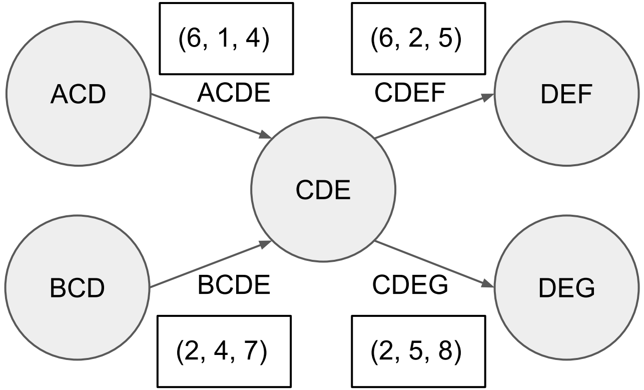


2c) Graph reductions: crosses

Crosses are nodes with indegree > 1 and outdegree > 1 (i.e., a node v with incoming edges $[u_1, v]$, $[u_2, v]$, … and outgoing edges $[v, w_1]$, $[v, w_2]$, …).

We again apply a heuristic approach to resolve this feature, which may be the result of sequencing errors or repetitive regions.

If there are pairs of edges which have continuing occurrences with each other, merge these pairs of edges.*



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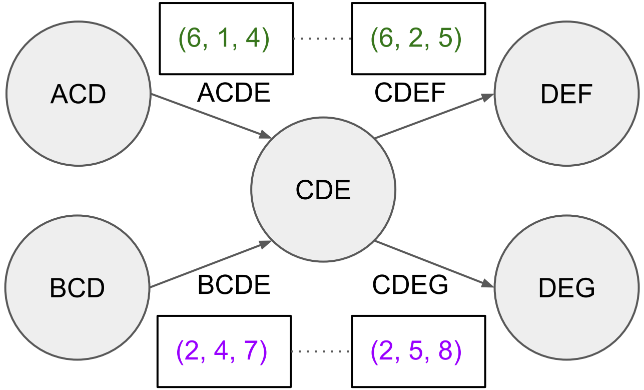

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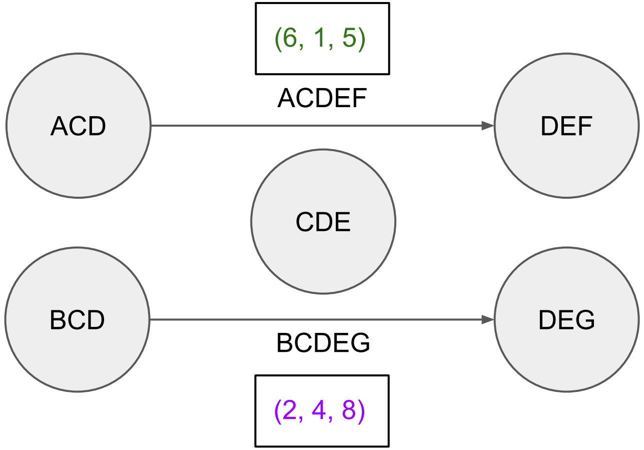

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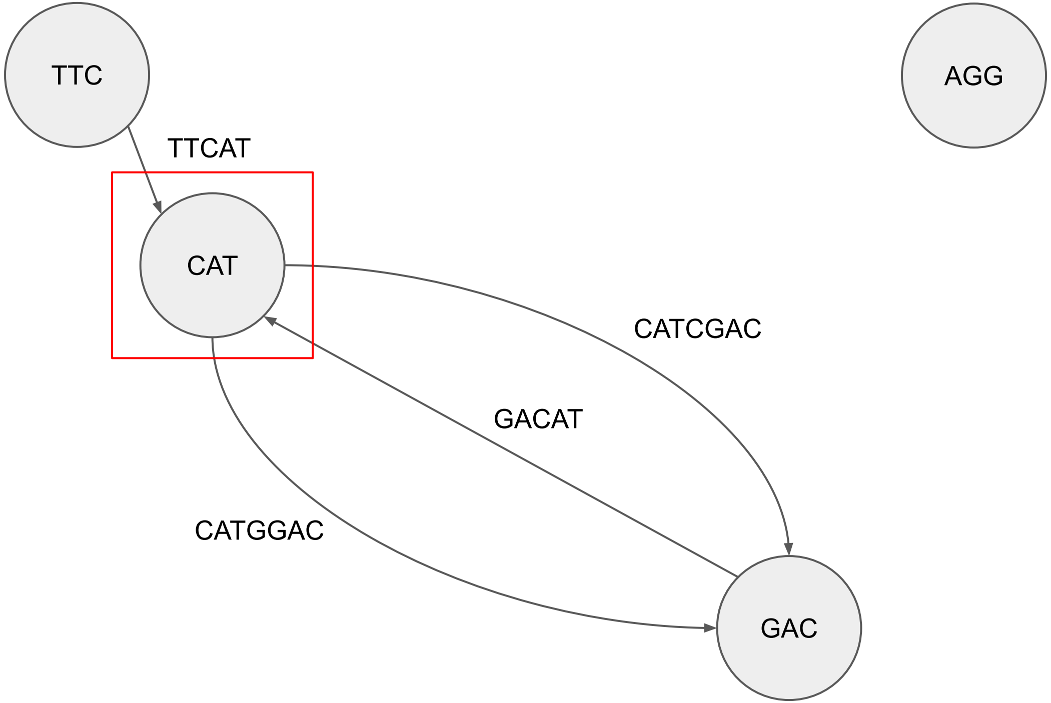

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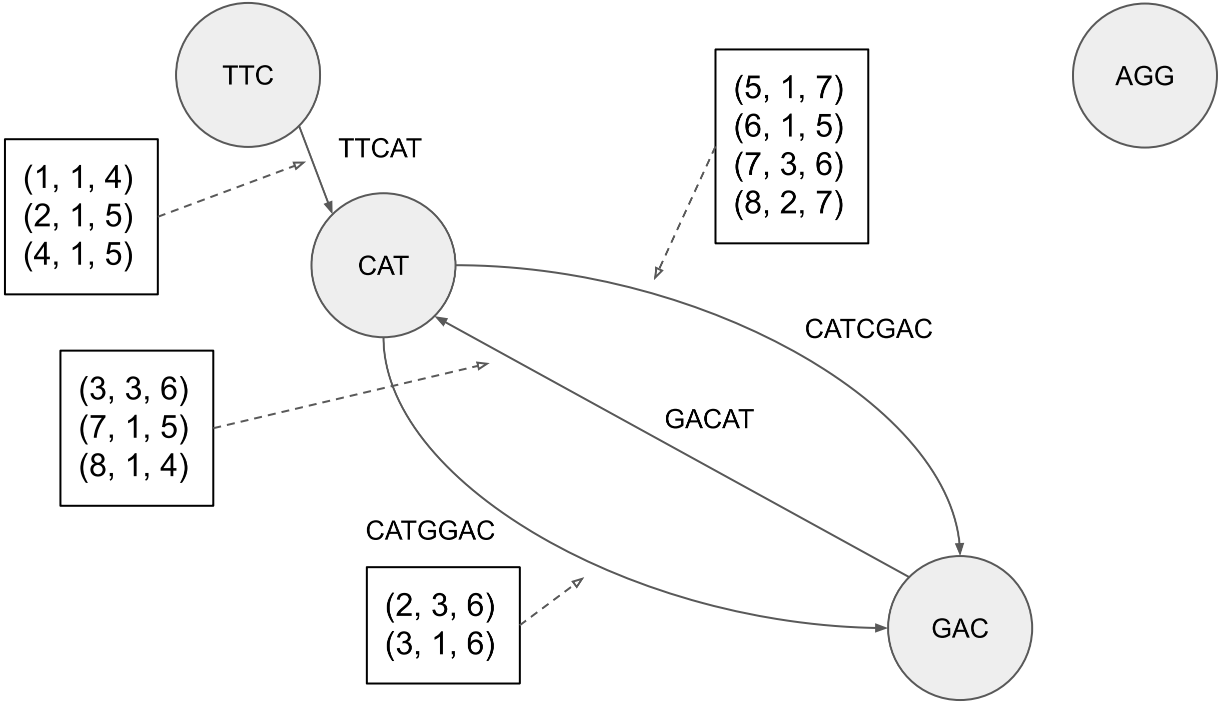

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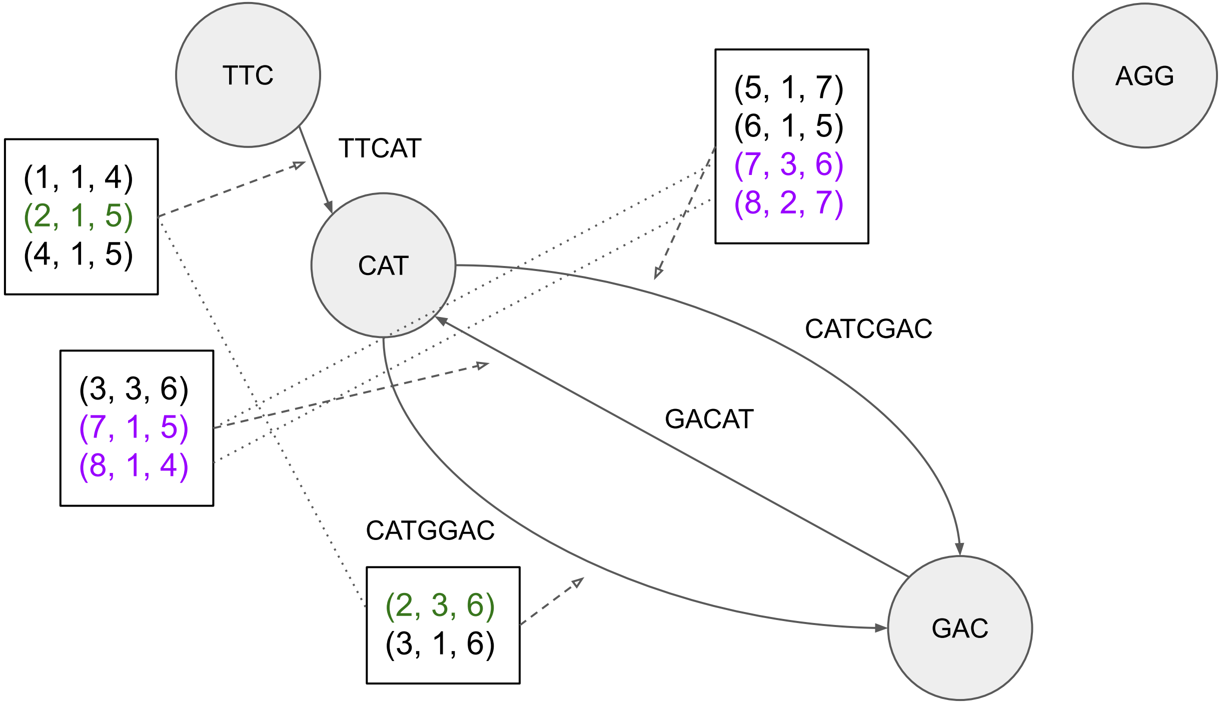

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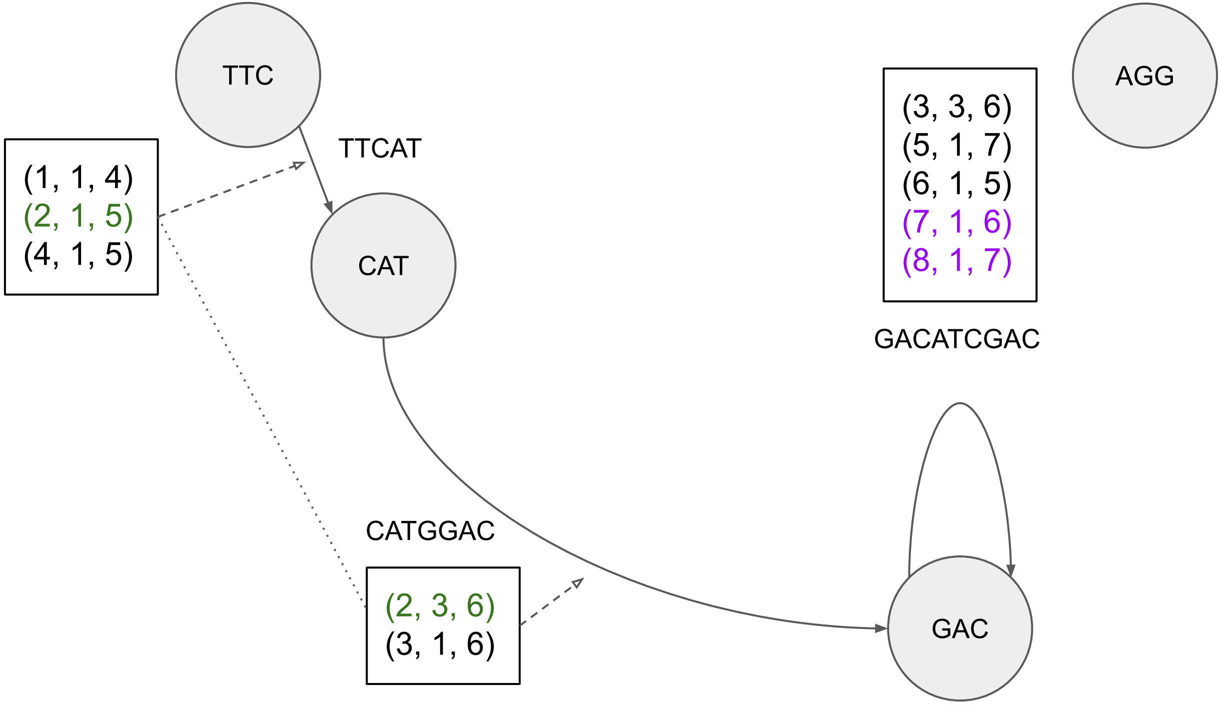

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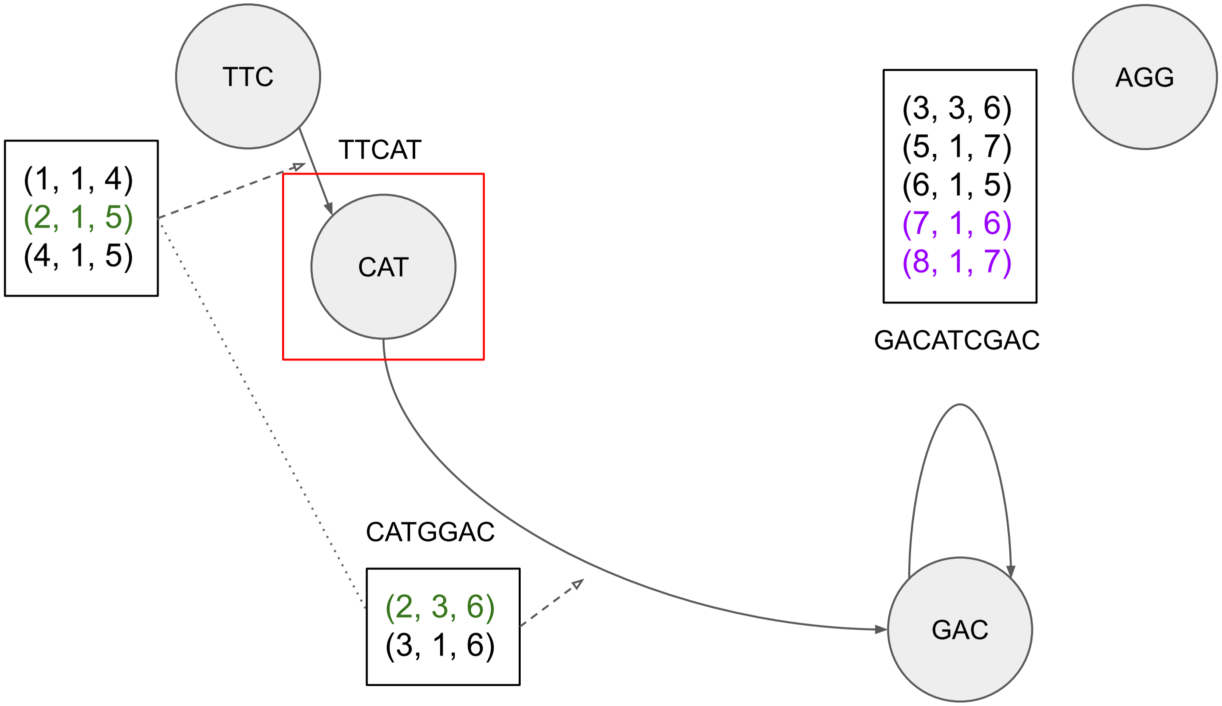

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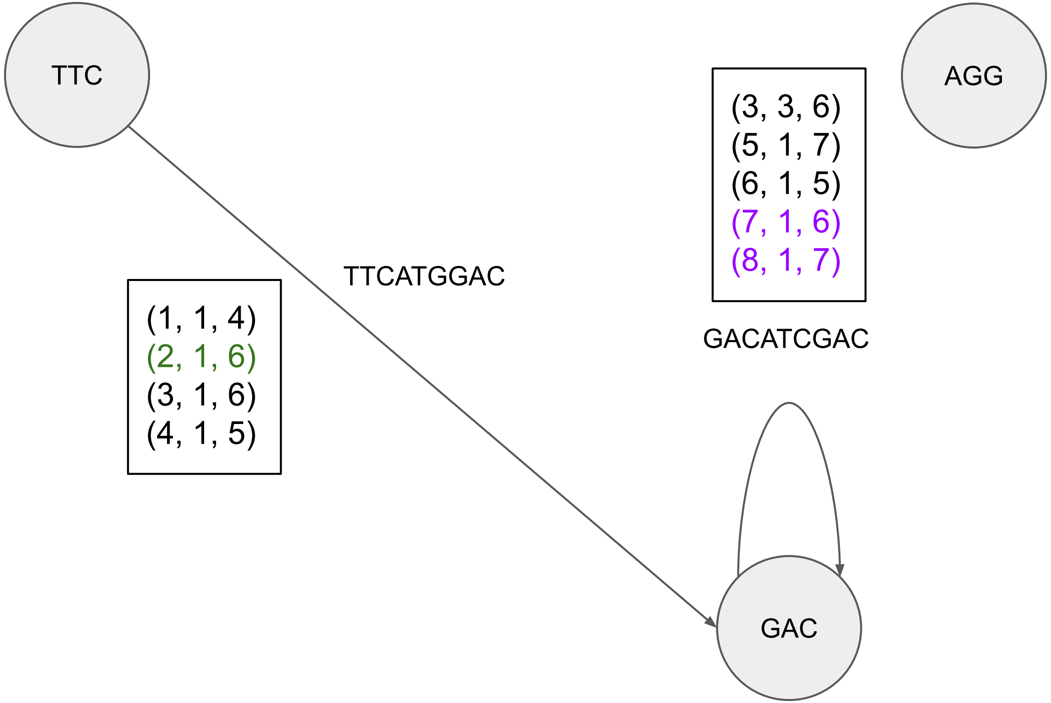


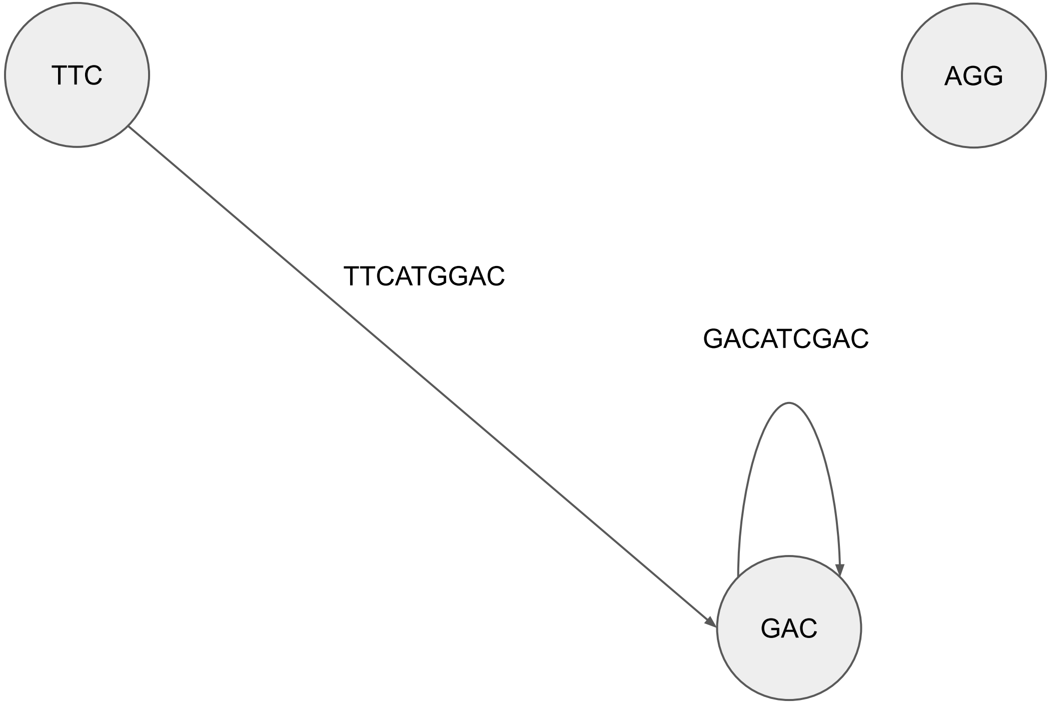
(1, 1, 4)
(2, 1, 6)
(3, 1, 6)
(4, 1, 5)

(3, 3, 6)
(5, 1, 7)
(6, 1, 5)
(7, 1, 6)
(8, 1, 7)

2) Irreducible sequence graph

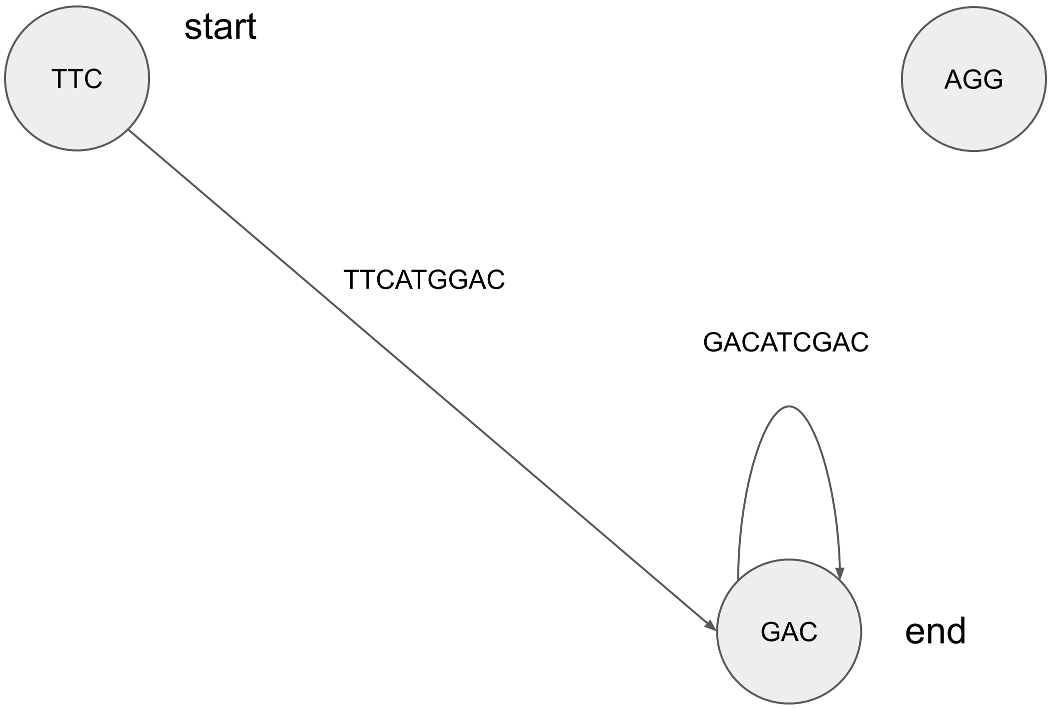
The graph has now been fully reduced, and we can search for an Eulerian path.

You may notice that the reductions accumulate lots of occurrence tuples within the graph edges. These remaining edges are referred to as “super edges,” and in general, we assign more weight (confidence) to edge labels with more occurrences.



3) Perform an Eulerian tour

We identify the start node as having outdegree - indegree = 1 and the terminal node as having indegree - outdegree = 1.



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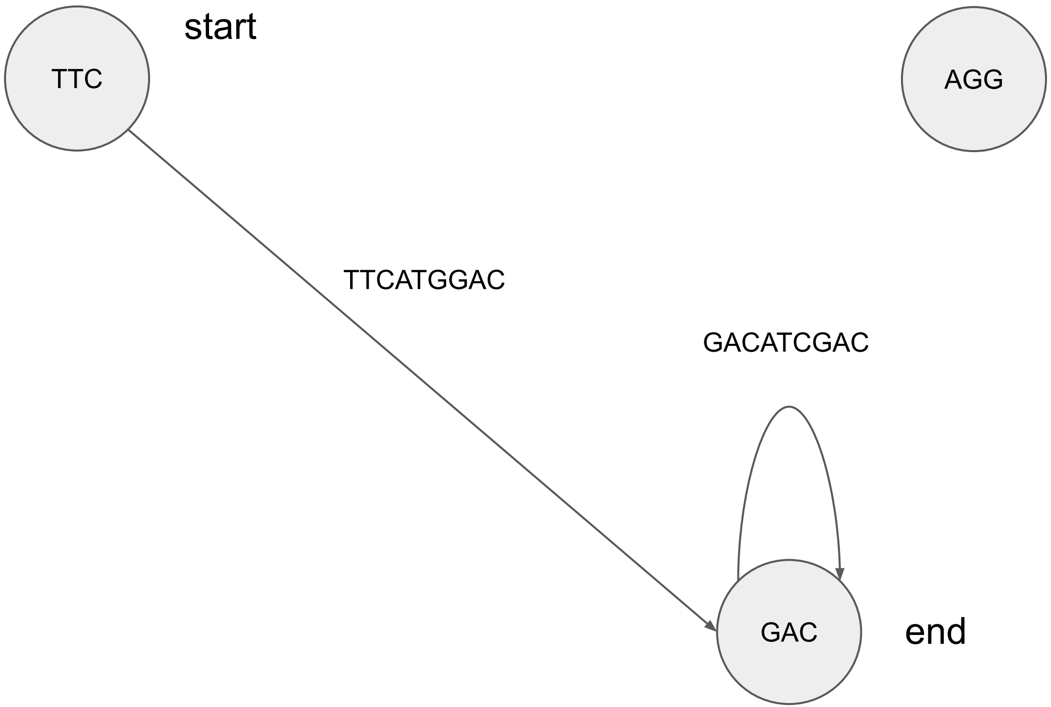
Beginning with the start node, follow outgoing edges until all edges are used once and the terminal node is reached. Infer the sequence by merging edge labels along the path taken.

If there are multiple outgoing edges to follow, choose edges based on the continuity of occurrences and edge weights.



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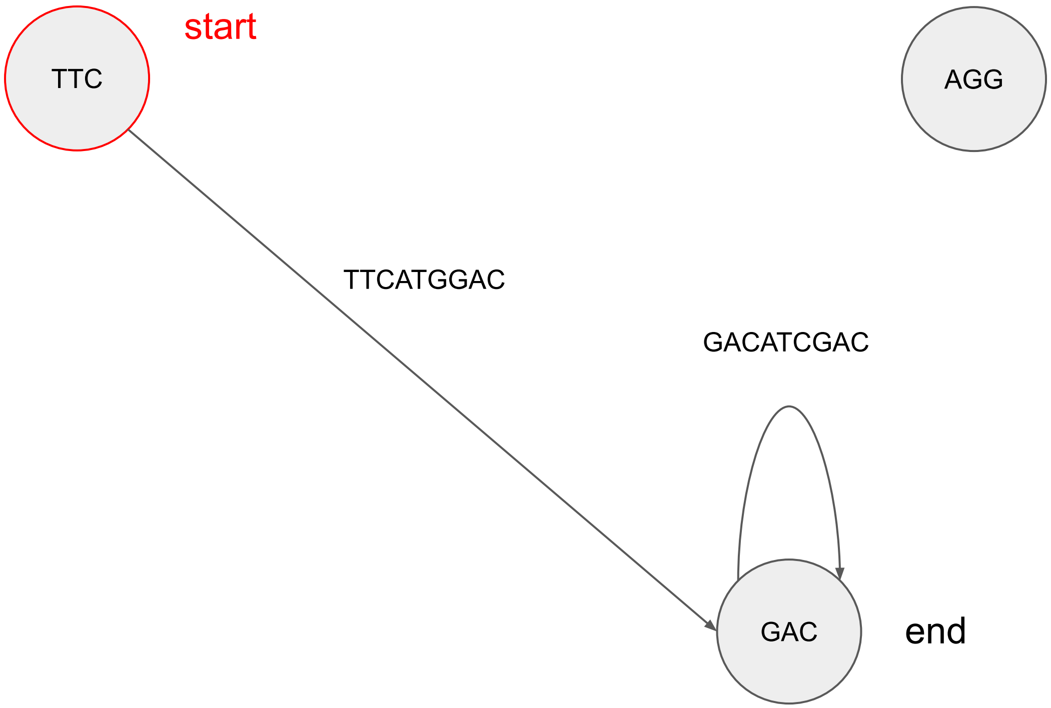

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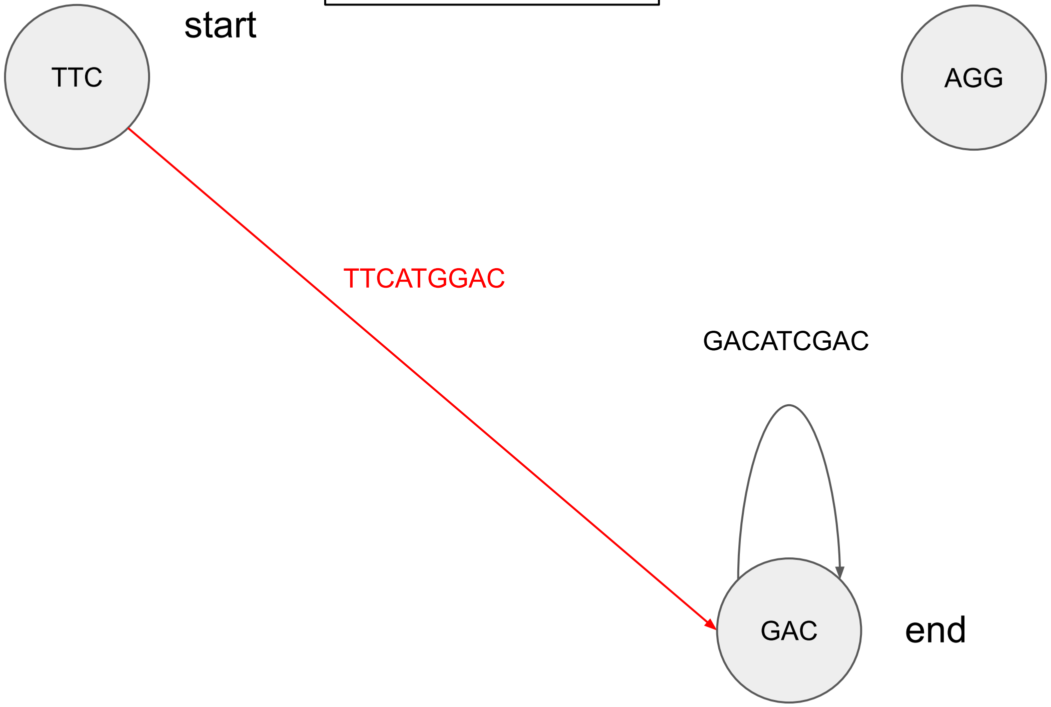

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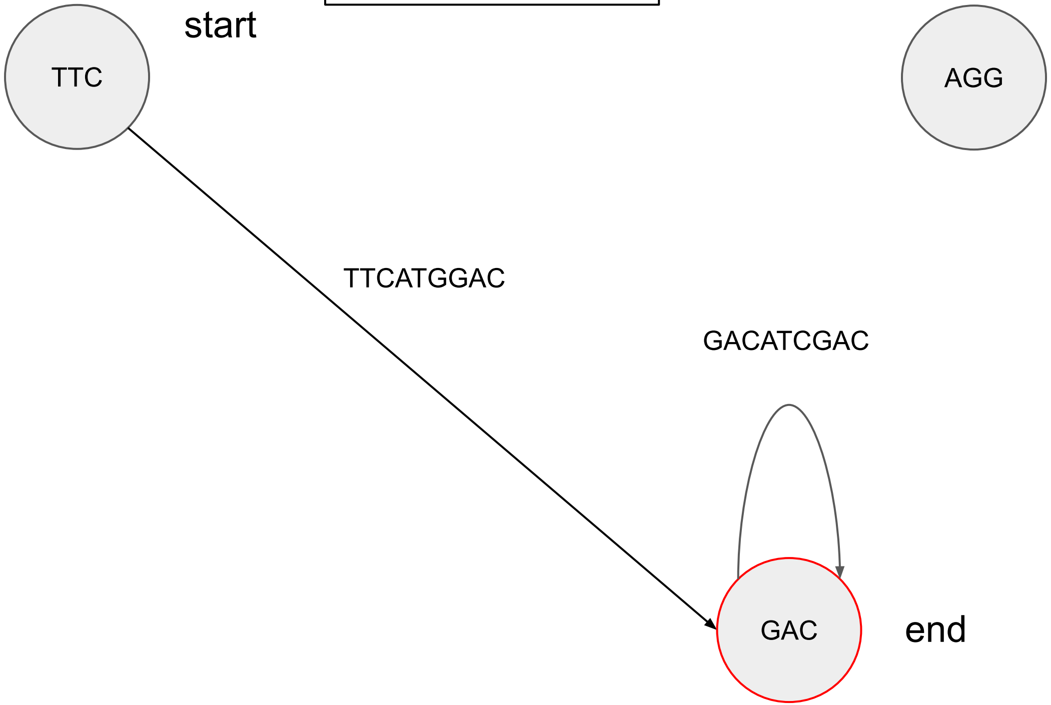

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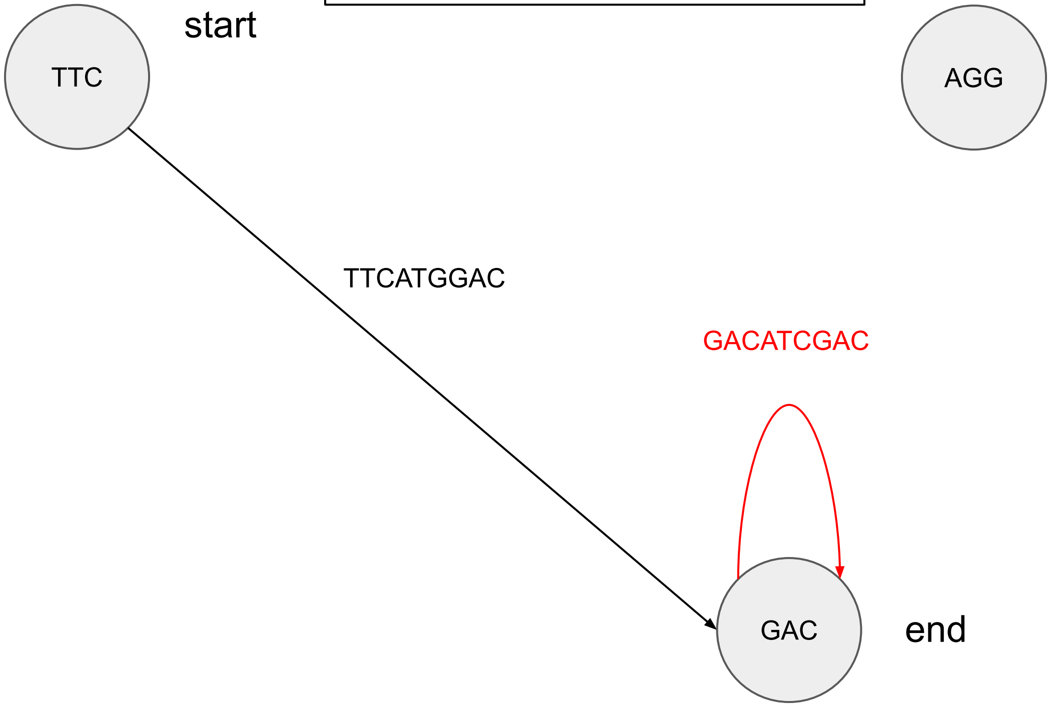

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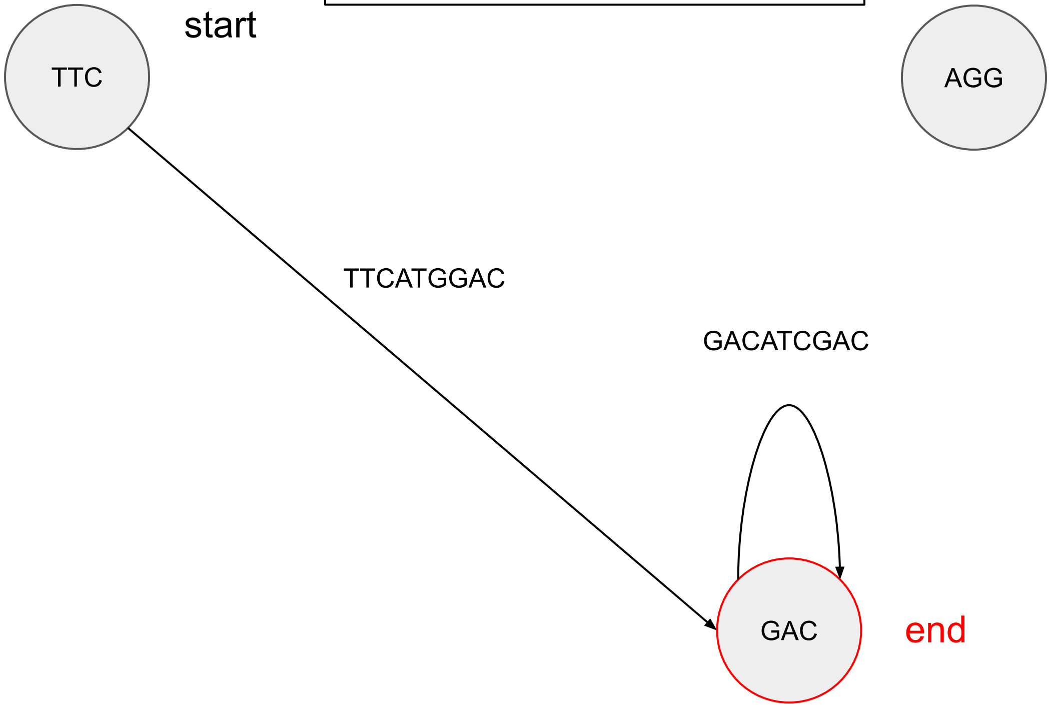

4) Align the reads to the inferred sequence

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f_1	TTCAGG
f_2	TTCATGG
f_3	ATGGACA
f_4	TTCAT
f_5	CATCGAC
f_6	TCGAC
f_7	GACATC
f_8	ACATCGA

First, we apply hashing methods to identify where each fragment might align well to the sequence.

This will produce “candidate diagonals.”

We can then perform alignment along those diagonals, which is more efficient than using the entire edit graph.

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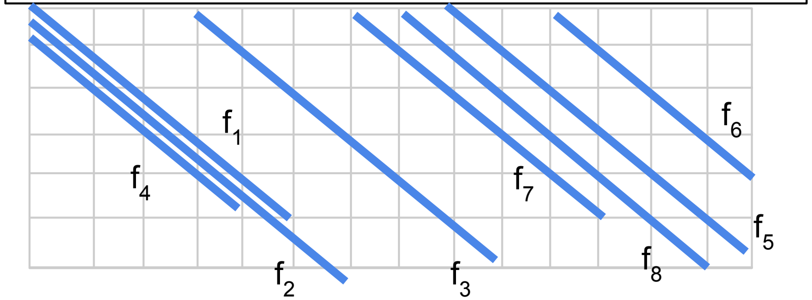

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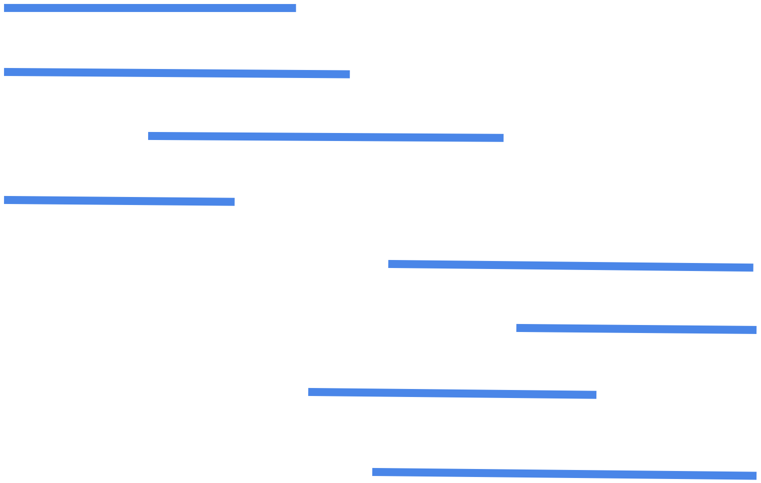

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```

Statistics of Sequence Graphs

We have seen that graph reductions greatly help to limit the size of the sequence graph during assembly. But how many reductions can we expect to make? What do we expect the graph structure to look like?

Define the following:

- k = tuple size, edge label length
- L = length of original sequence
- N = number of fragments (reads)
- ℓ = average fragment length
- c = mean depth of coverage
- T = N(ℓ - k + 2) = number of (k-1) regions
- r = error rate

Assume:

1. Errors are uniformly distributed over fragments and the length of each fragment
2. Error rate r is small
3. For any position i of the original sequence, the number of fragments covering i…(i+k-2) is a Poisson random variable
4. There are no repeats of length at least k
5. The only sequencing errors are substitutions

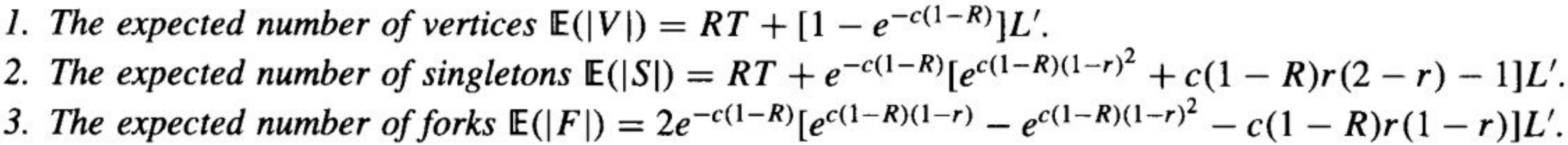
Statistics of Sequence Graphs

Let L’ = L - k + 2 and R = 1 - (1 - r)$^{(k-1)}$

This is the number of (k-1)-mers in the original sequence.

This is the (k-1)-mer error rate.

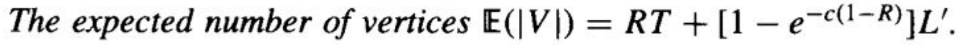
Then we have the following results:



1. *The expected number of vertices* $\mathbb{E}(|V|) = RT + [1 - e^{-c(1-R)}]L'$.
2. *The expected number of singletons* $\mathbb{E}(|S|) = RT + e^{-c(1-R)}[e^{c(1-R)(1-r)^2} + c(1-R)r(2-r) - 1]L'$.
3. *The expected number of forks* $\mathbb{E}(|F|) = 2e^{-c(1-R)}[e^{c(1-R)(1-r)} - e^{c(1-R)(1-r)^2} - c(1-R)r(1-r)]L'$.

Statistics of Sequence Graphs: vertices

Here we will briefly outline the proof of (1):



The expected number of vertices $\mathbb{E}(|V|) = RT + [1 - e^{-c(1-R)}]L'.$

Vertices in the sequence graph can be classified as true (if they are found in the original sequence) or false (if they are generated from a sequencing error).

We can compute the number of expected vertices as the sum of the expected number of true vertices and the expected number of false vertices.

If T is the number of (k-1)-mers in our read set, and R is the false (k-1)-mer rate, then the expected number of false vertices is RT.

Statistics of Sequence Graphs: vertices

To find the expected number of true vertices, we consider that the number of true vertices is equivalent to the number of positions such that at least one fragment has no sequencing errors.

Recall that the depth at a particular position (number of reads covering that position) is a Poisson variable with mean c ⇔ X ~ Poisson(c). So we have:

$$\mathbb{E}(True) = L' \sum_{i=1}^{\infty} (1 - R^i)\mathbb{P}(X = i)$$

Number of (k-1)-mers in the original sequence

Expectation over all possible values for X ~ Poisson

Probability that at least 1 out of i reads is correctly sequenced

Probability that i reads cover the (k-1)-length region

Statistics of Sequence Graphs: vertices

$$\begin{aligned}\mathbb{E}(True) &= L'\sum_{i=1}^{\infty}(1-R^i)\mathbb{P}(X=i)\\ &= L'\sum_{i=1}^{\infty}\left(\frac{e^{-c}c^i}{i!}-\frac{e^{-c}(cR)^i}{i!}\right)\\ &= L'(1-e^{-c(1-R)}).\end{aligned}$$

pmf of Poisson: $\Pr(X{=}k)=\frac{\lambda^k e^{-\lambda}}{k!}$

using Taylor expansion for e: $e^x=\sum_{n=0}^{\infty}\frac{x^n}{n!}$

Summing the number of false vertices and true vertices produces:

The expected number of vertices $\mathbb{E}(|V|) = RT + [1-e^{-c(1-R)}]L'.$

What did we expect from our example sequence graph?

- k = 4
- L = 15
- N = 8
- ℓ = 50/8 = 6.25
- c = 50/15 = 3.33
- T = 32
- r = 1/50 = 0.02

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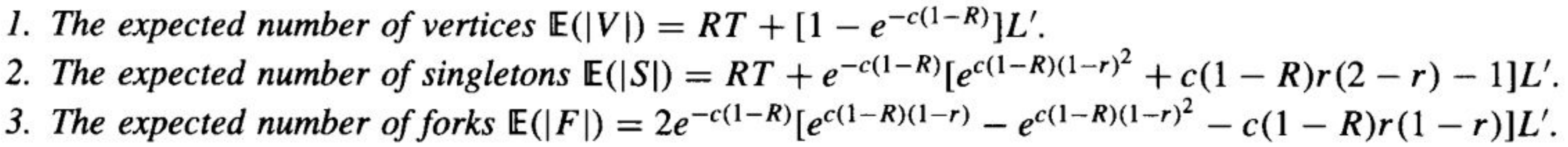

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E(|V|) ≈ 14.318

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$E(|V|) \approx 14.318$

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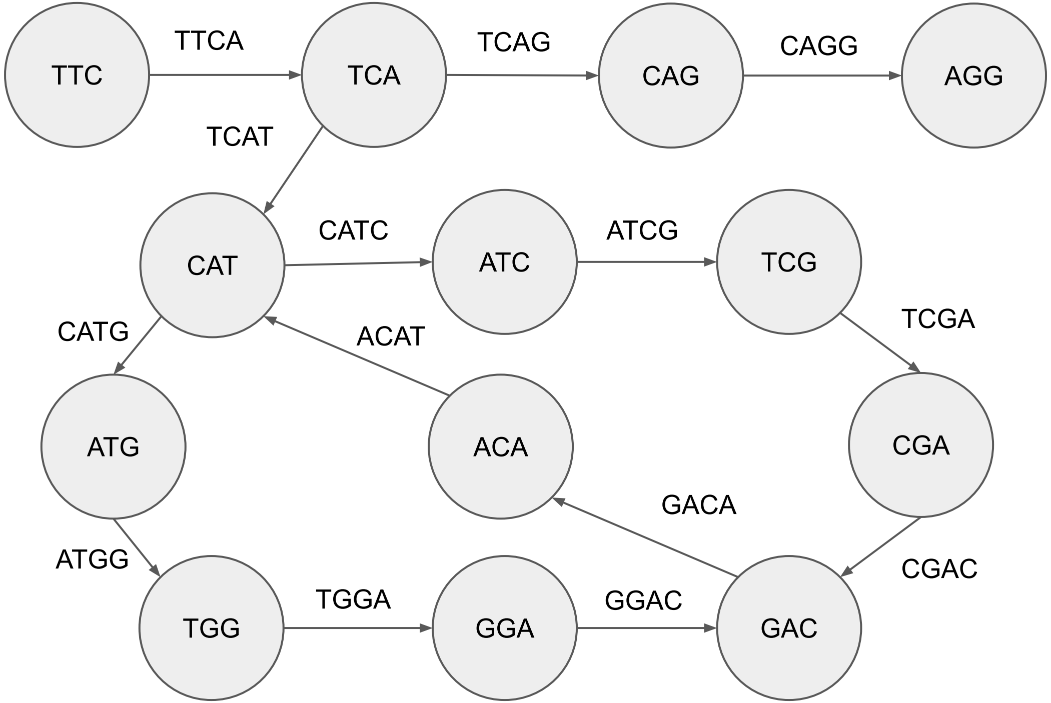
$|V| = 13$

$|S| = 8$

$|F| = 1$



What did we expect from our example sequence graph?

$E(|V|) \approx 14.318$

$E(|S|) \approx 12.869$

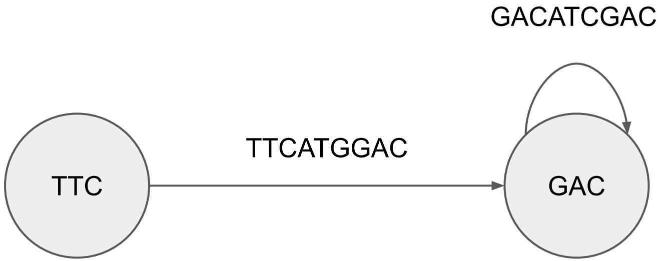
$E(|F|) \approx 1.387$

This also predicts that we could simplify the graph to 1-3 nodes

$|V| = 13$

$|S| = 8$

$|F| = 1$



Practical considerations

- The Idury-Waterman algorithm pioneered the use of De Bruijn graphs in assembly, particularly with the advent of shotgun sequencing over sequencing by hybridization
- The technical challenges associated with repeat regions, cost limitations on sequencing depth, and sequencing errors require heuristics to resolve
    - Idury and Waterman propose utilizing positional information, incorporating both **multiplicity** of k-mers and fragment **continuity** as deciding factors
- The randomness of sequencing errors and sampling reads prevents concrete upper bounds on efficiency but allow for probabilistic estimates of computational performance