CS123A Bioinformatics Module 5 – Week 15 – Presentation 2

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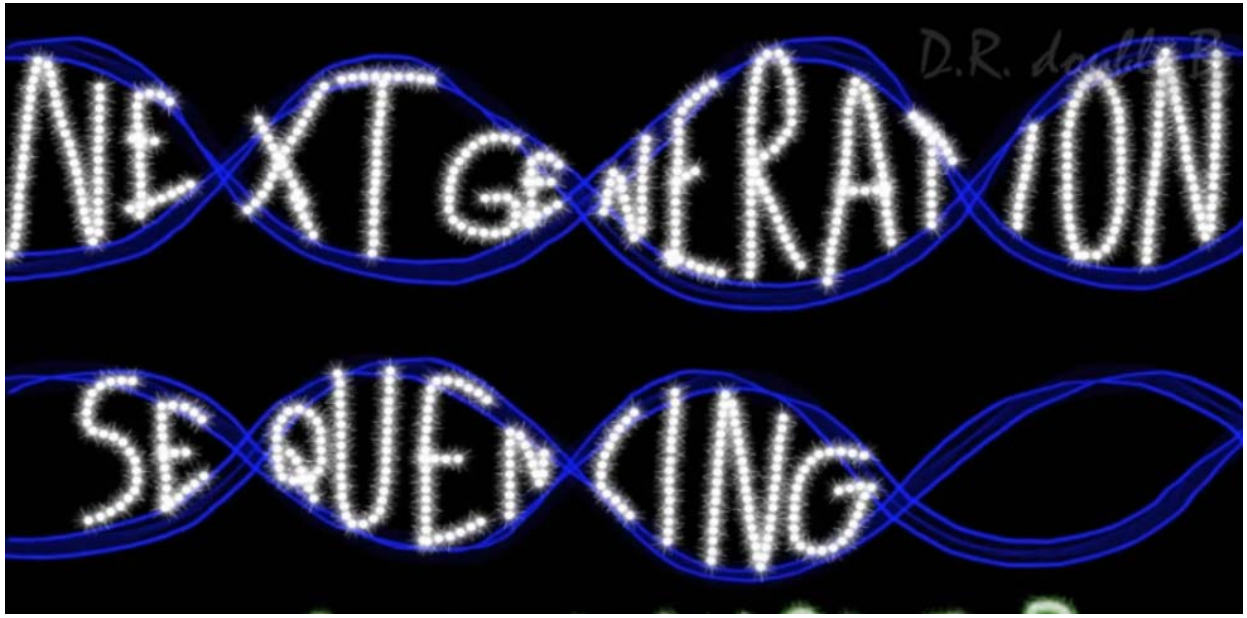


Agenda

- Next Generation Sequencing (cont.)

NGS Technologies

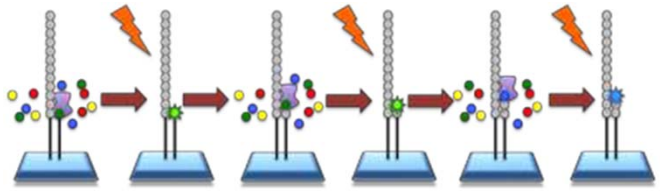
Illumina Sequencing

https://www.youtube.com/watch?v=fCd6B5HRaZ8

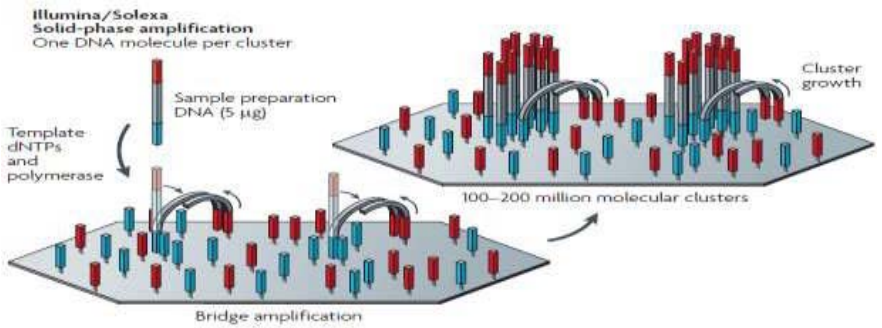
Illumina Sequencing Technology

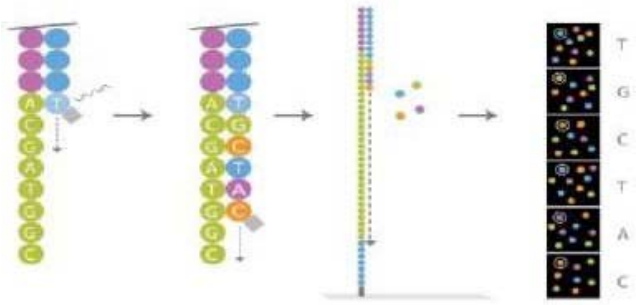
ILLUMINA/SOLEXA SEQUENCING

Solid-phase amplification can produce 100-200 million spatially separated clusters, providing free ends to which a universal sequencing primer can be hybridized to initiate the NGS reaction

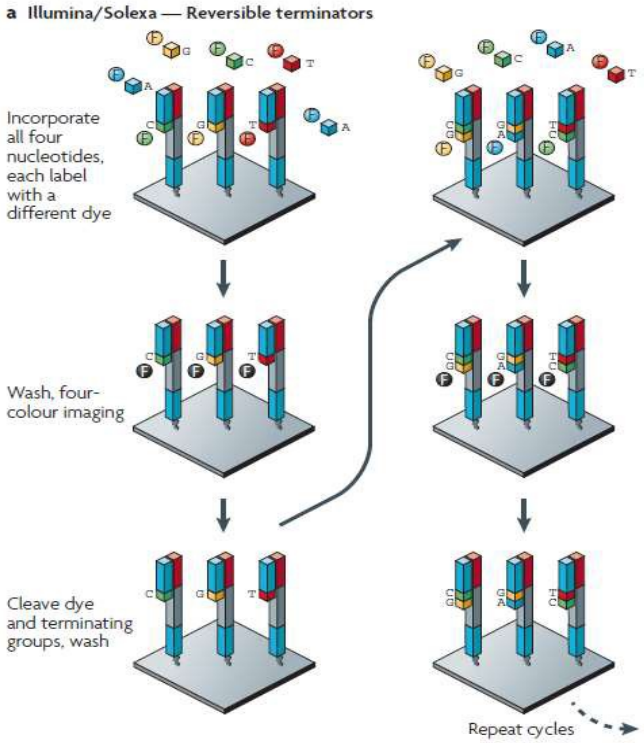


Bridge PCR Clustal Amplification



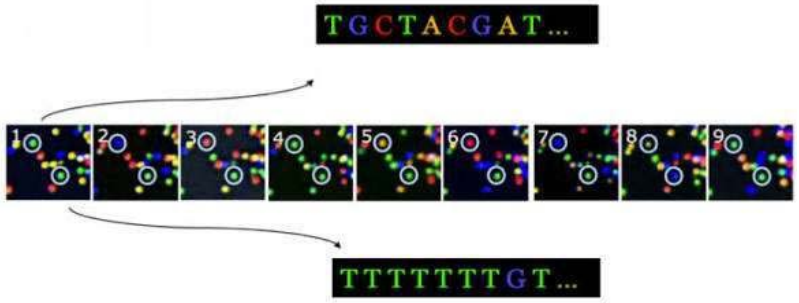


- Run time: 1–10 days
- Produces: 2–1000 Gb of sequence
- Read length: 2 x 50 bp – 2 x 250 bp (paired-end)
- Cost: $0.05–$0.40/Mb



Over 1800 publications.

- **Applications**
  - DNA sequencing
  - Gene Regulation Analysis
  - Sequencing-based Transcriptome Analysis
  - SNPs and SVs discovery
  - Cytogenetic Analysis
  - ChIP-sequencing
  - Small RNA discovery analysis



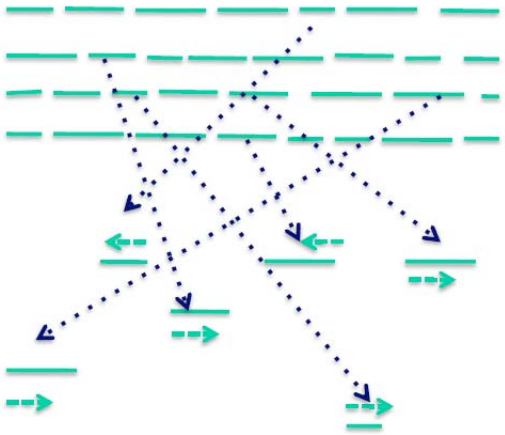
- A whole human genome sequence was determined in **8 weeks** to an average depth of ~ 40X, discovering ~ 4 new million SNPs and ~400000 SVs (with an accuracy <1% for both over-calls and under-calls)

What Is Next?

Assembly Of Sequenced Data

Multiple copies of sample DNA

Randomly fragment DNA



Sequence sample of fragments

Assemble reads

The Fragment Assembly Problem

- Given: A set of reads (strings) {*s1*, *s2*, … , *sn* }
- Do: Determine a large string *s* that "best explains" the reads

- What do we mean by "*best explains*"?
- What *assumptions* might we require?

Shortest superstring problem

- Objective: Find a string *s* such that
  - all reads *s1*, *s2*, … , *sn* are substrings of *s*
  - *s* is as short as possible

- Assumptions:
  - Reads are 100% accurate
  - Identical reads must come from the same location on the genome
  - “best” = “simplest”

Shortest superstring example

- Given the reads:
  - {ACG, CGA, CGC, CGT, GAC, GCG, GTA, TCG}

- What is the shortest superstring you can come up with? (HINT: Length=10)

ANSWER: Shortest superstring example

- Given the reads:
  - {ACG, CGA, CGC, CGT, GAC, GCG, GTA, TCG}

- What is the shortest superstring you can come up with? (HINT: Length=10)
  - **TCGACGCGTA**

Algorithms for shortest superstring problem

- This problem turns out to be *NP*-complete
  - Simple *greedy* strategy:

    while # strings > 1 do

        merge two strings with maximum overlap

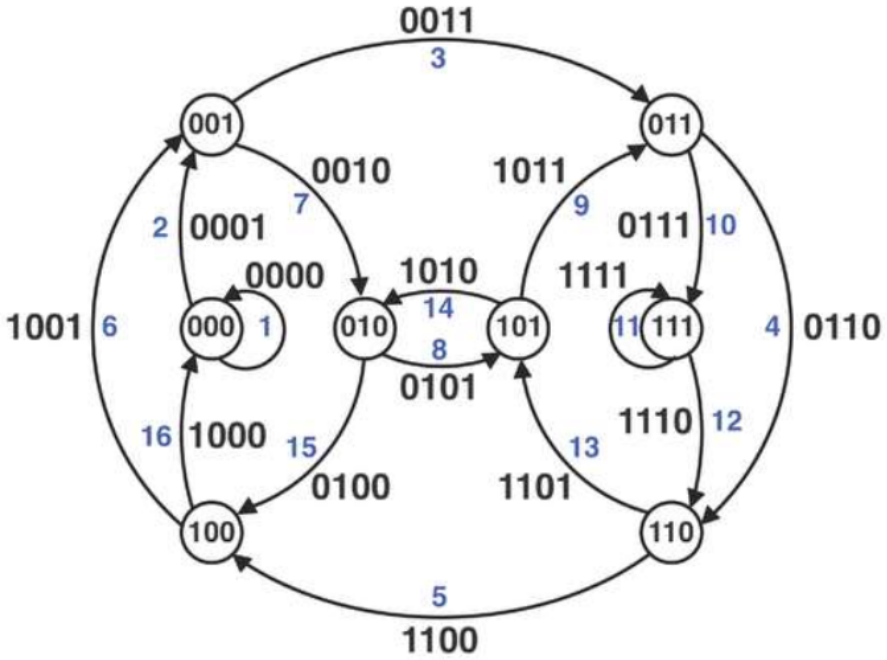
    loop
- Conjectured to give strings with

    length $\leq$ 2 x minimum length

- Other approaches are based on *graph theory… e.g., de Bruijn graphs*

What are de Bruijn graphs ?

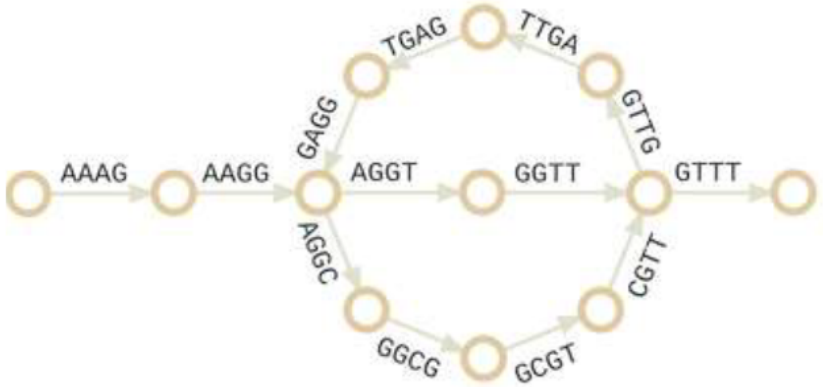
De Bruijn graph is a **directed graph** representing overlaps between sequences of symbols



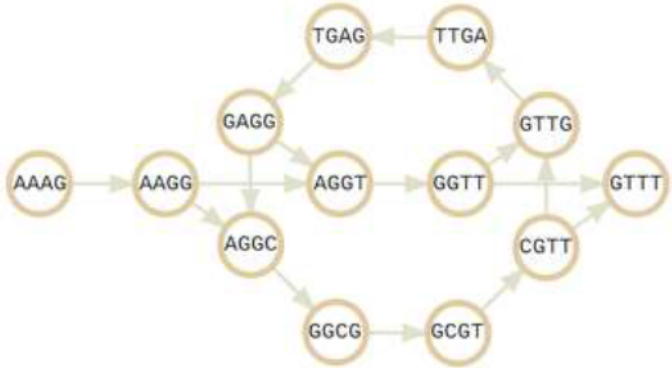
Types of De Bruijn Graphs

- Eulerian Path
- Hamiltonian Trail

B. Eulerian de Bruijn graph



C. Hamiltonian de Bruijn graph



INPUT/OUTPUT

Input :

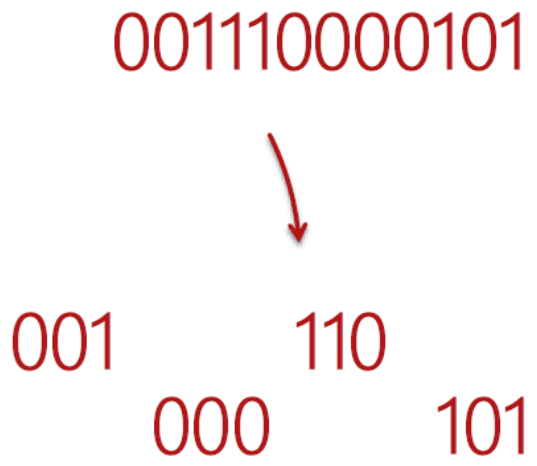
A set of k-mers patterns.

Output :

De Bruijn graphs

K-mers

- All the possible subsequences (of length k) from a read obtained through **DNA Sequencing**
- Small chunks



INPUT

- **Whole Sequence :**

  If whole sequence is given we have to divide it into k-mers

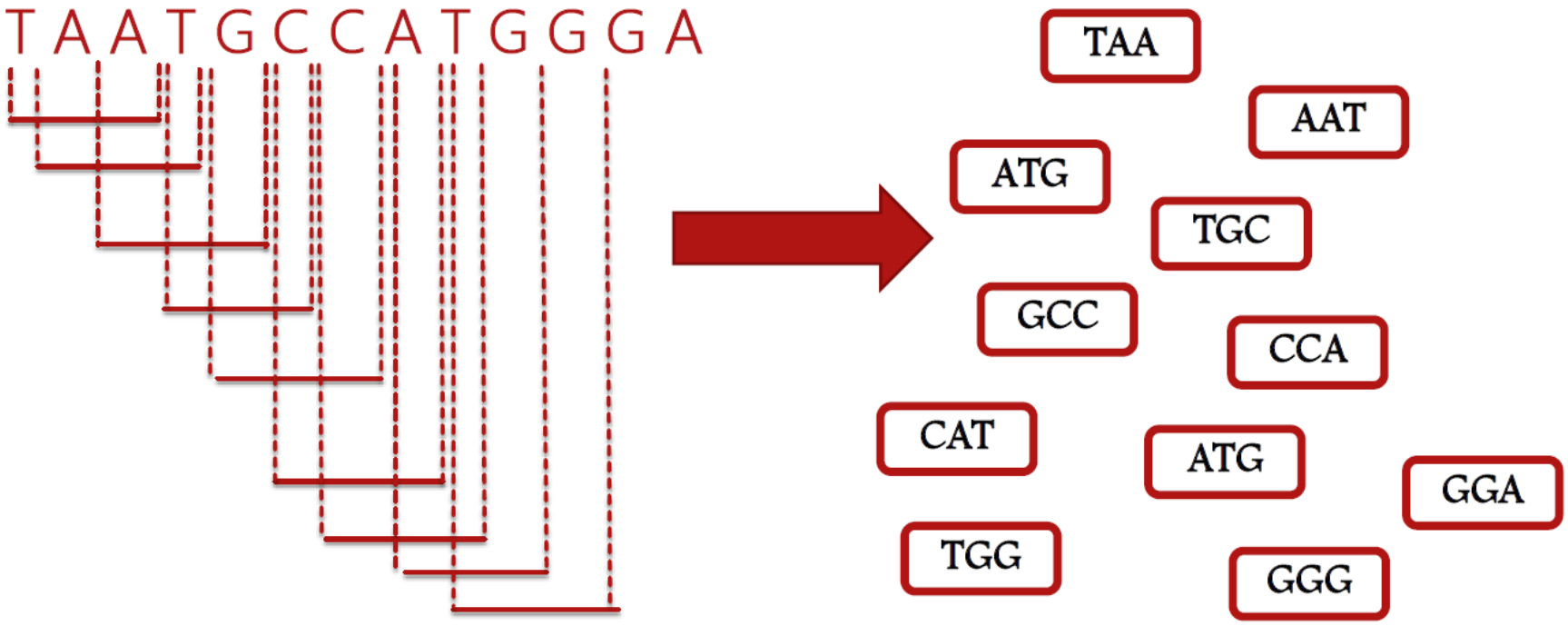
- **K-mers**

1st Step

The first step is to choose a k-mer size, and split the original sequence into its k-mer components(if sequence is given)

Size = 3

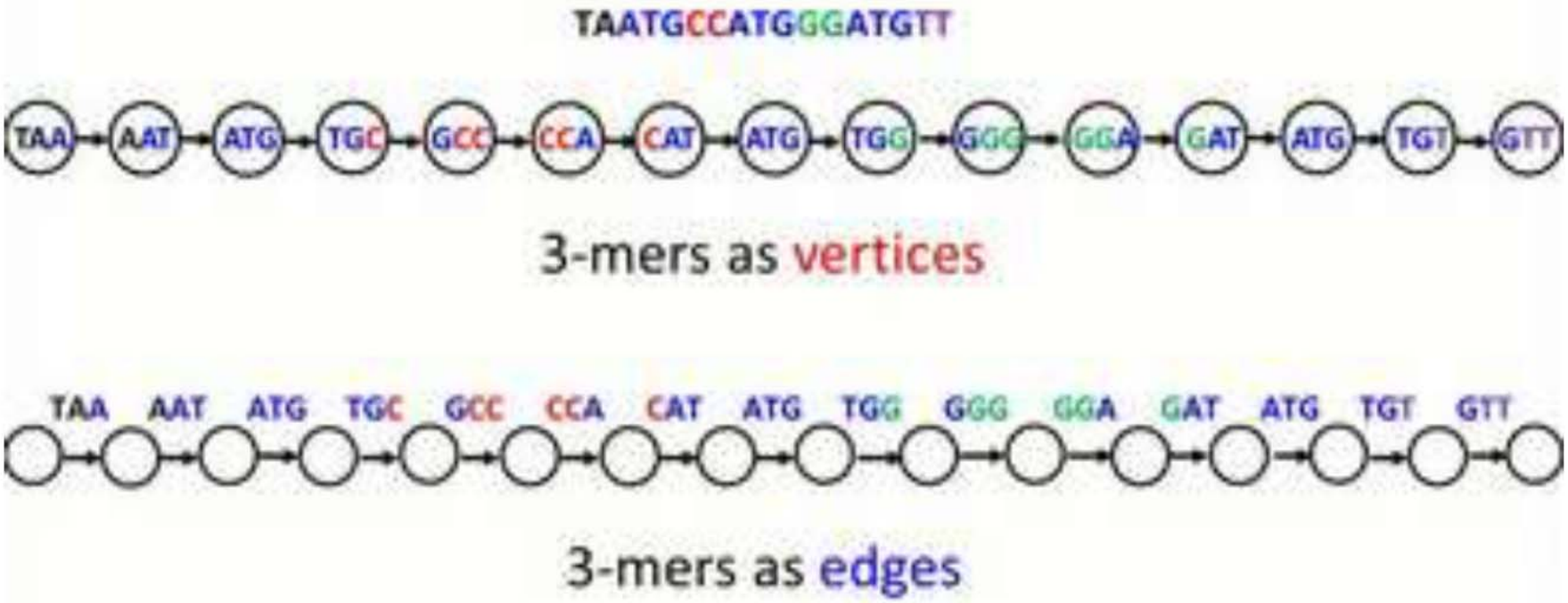
Whole sequence



Construction of graph

- Create Nodes.
- Build edges.
- Create directions.

Now: labeling edges by *k*-mers



Nodes

- We consider each k-mer as edge.
- 2 nodes for each edge

Nodes

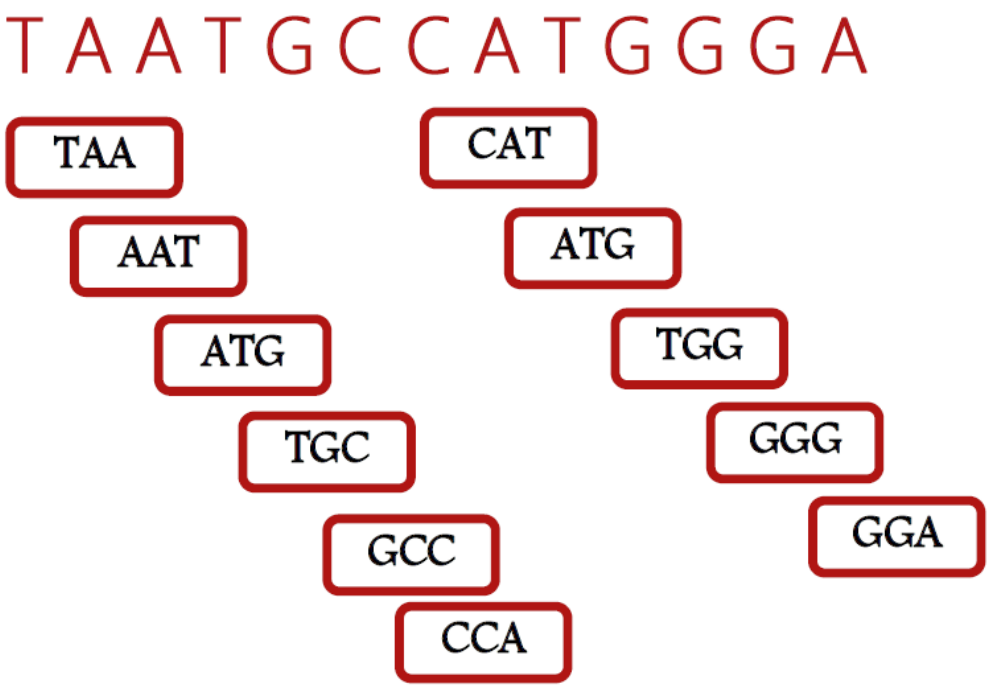
- **First :**

  Prefix of edge

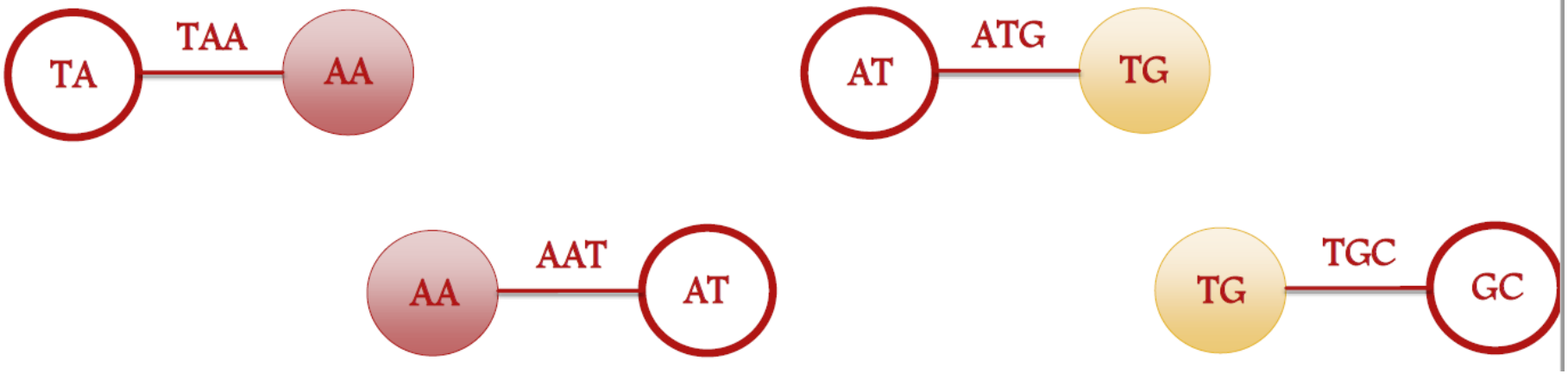
- **Second :**

  Suffix of edge

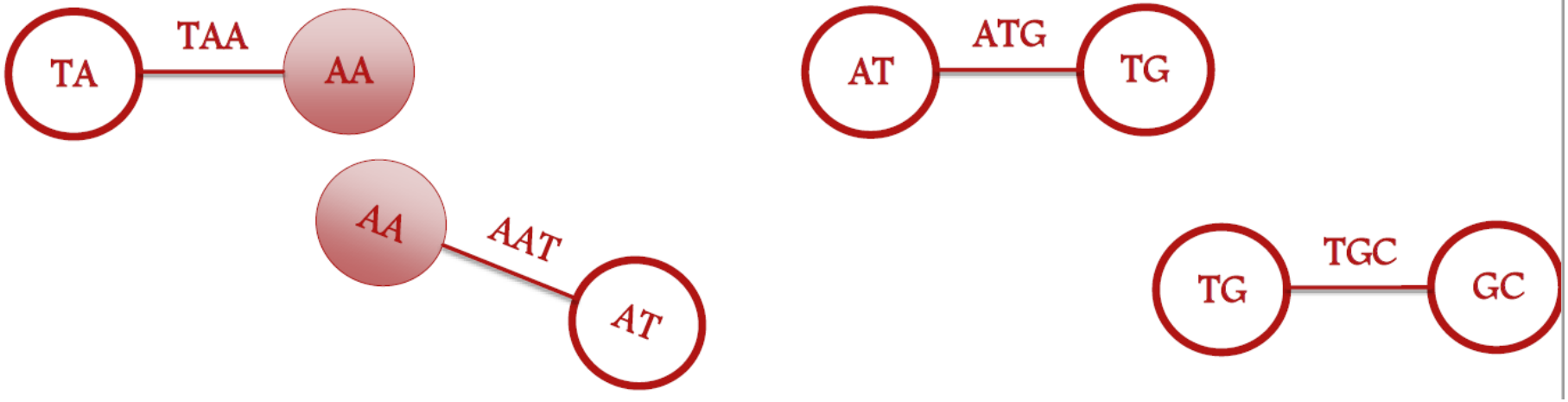
K-mers



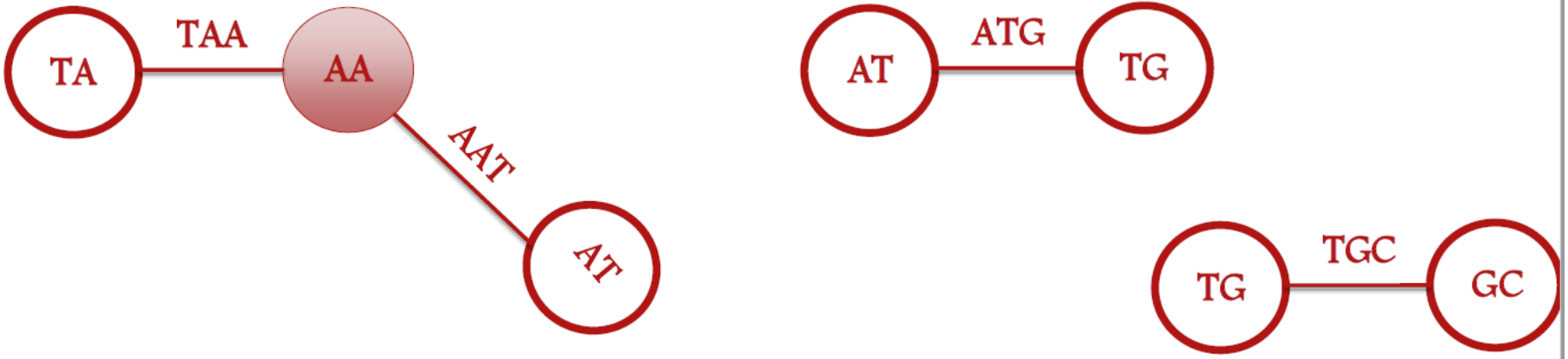
Highlight the similar nodes closer



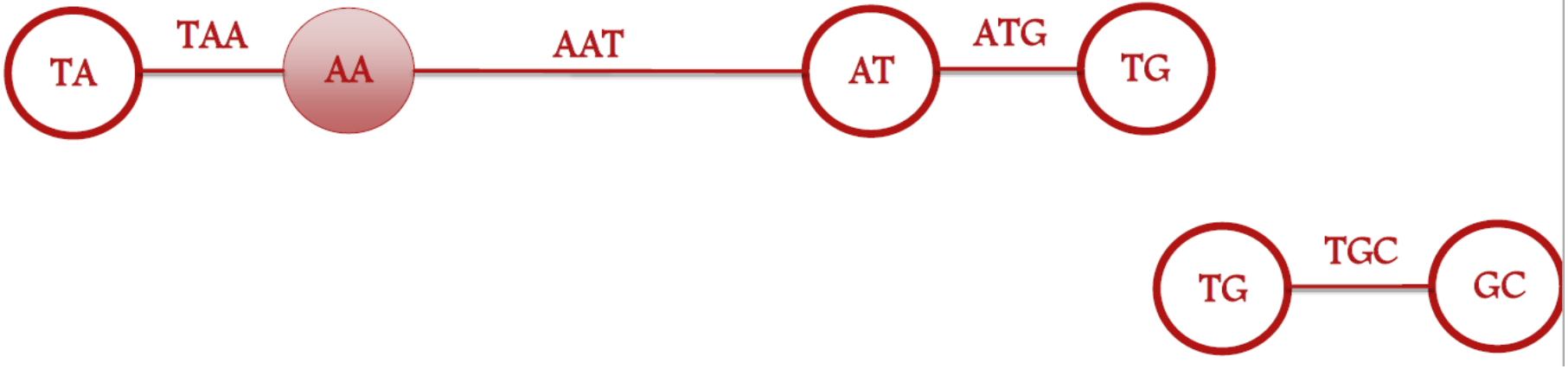
Make the similar nodes closer



Glue the similar nodes



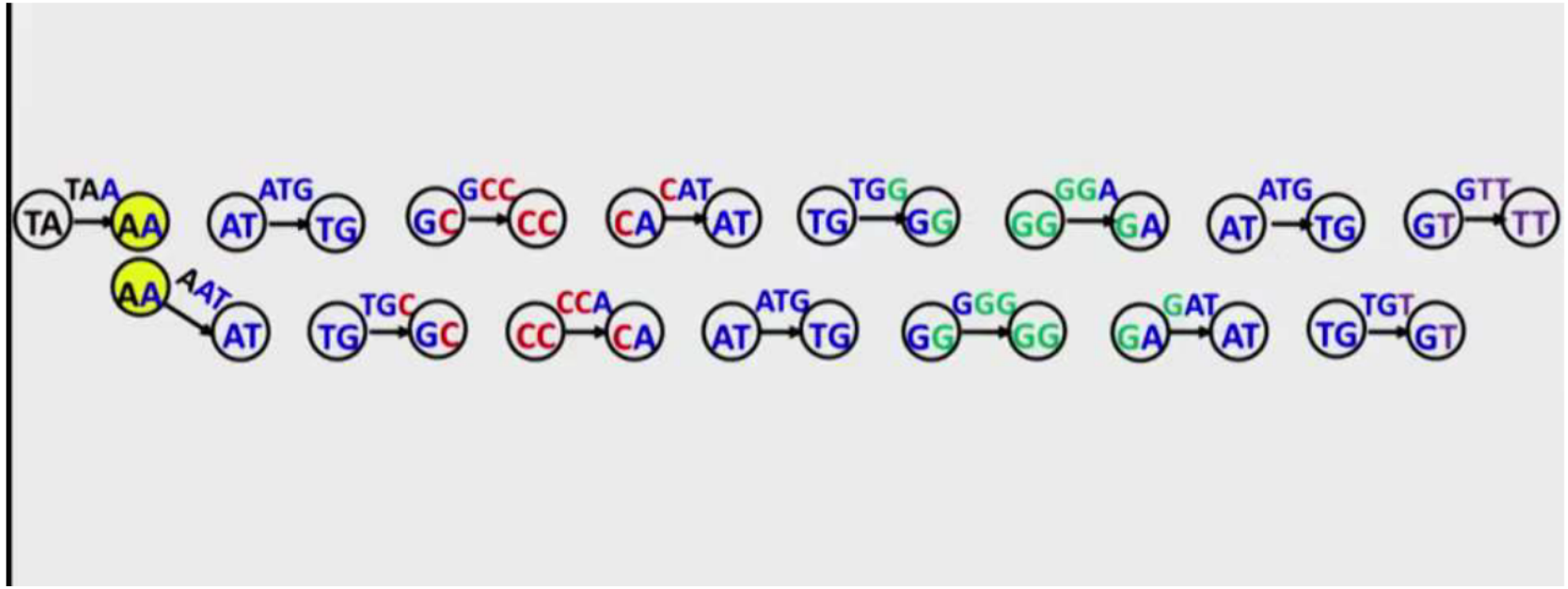
Glue the similar nodes



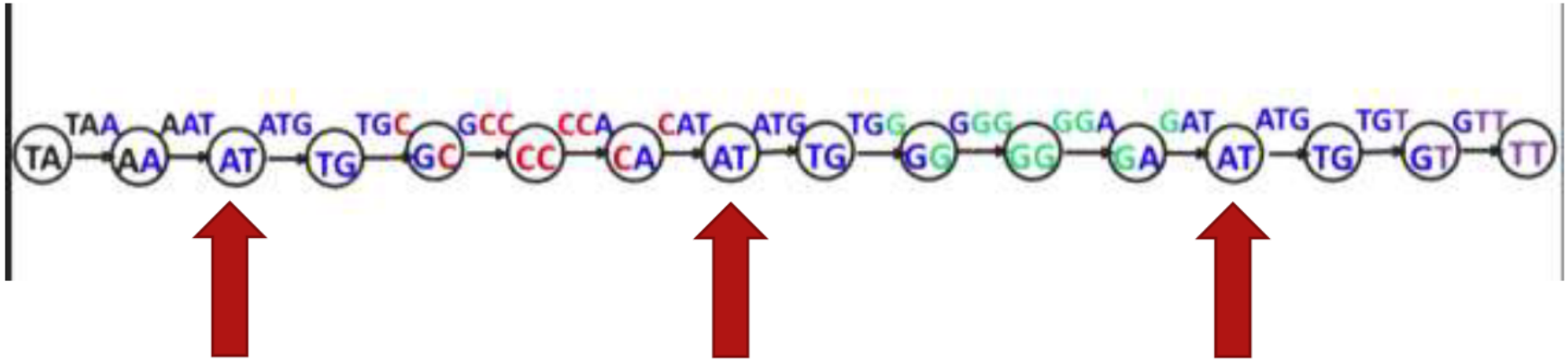
2nd Step

Then a directed graph is constructed by connecting pairs of k-mers with overlaps between the first k-1 nucleotides and the last k-1 nucleotides

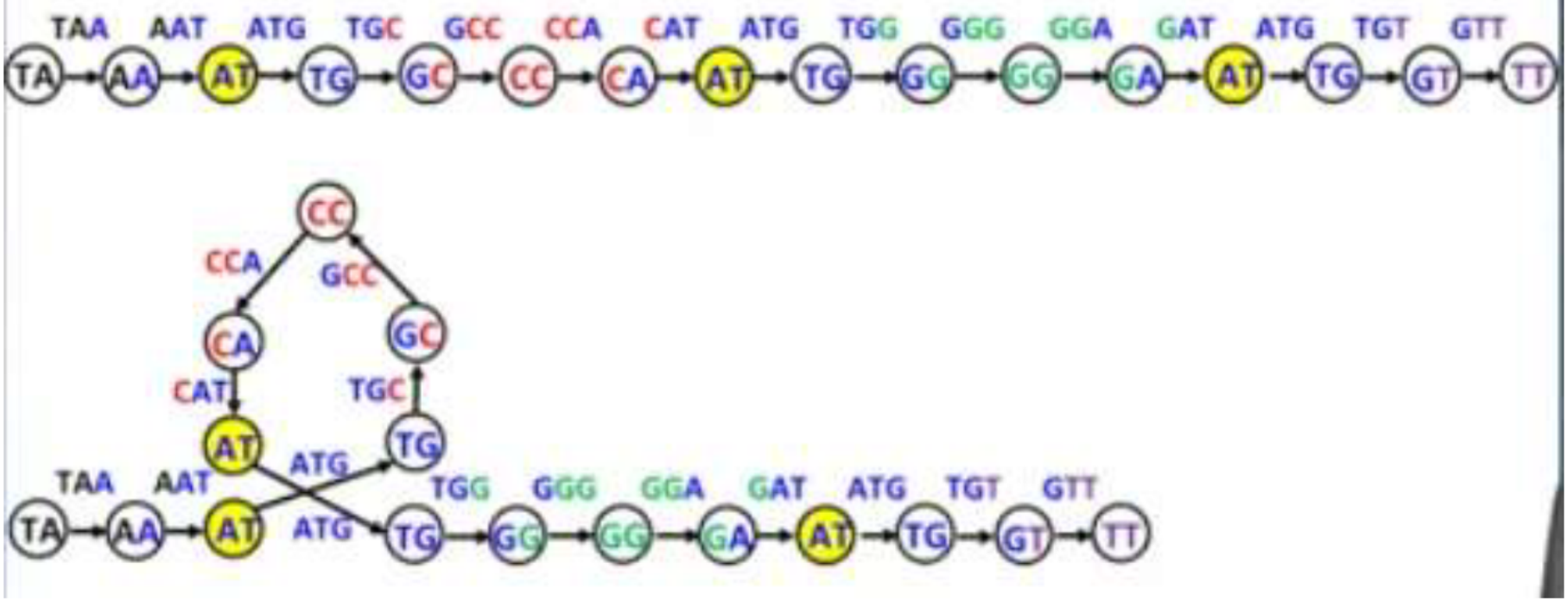
For All K-mers

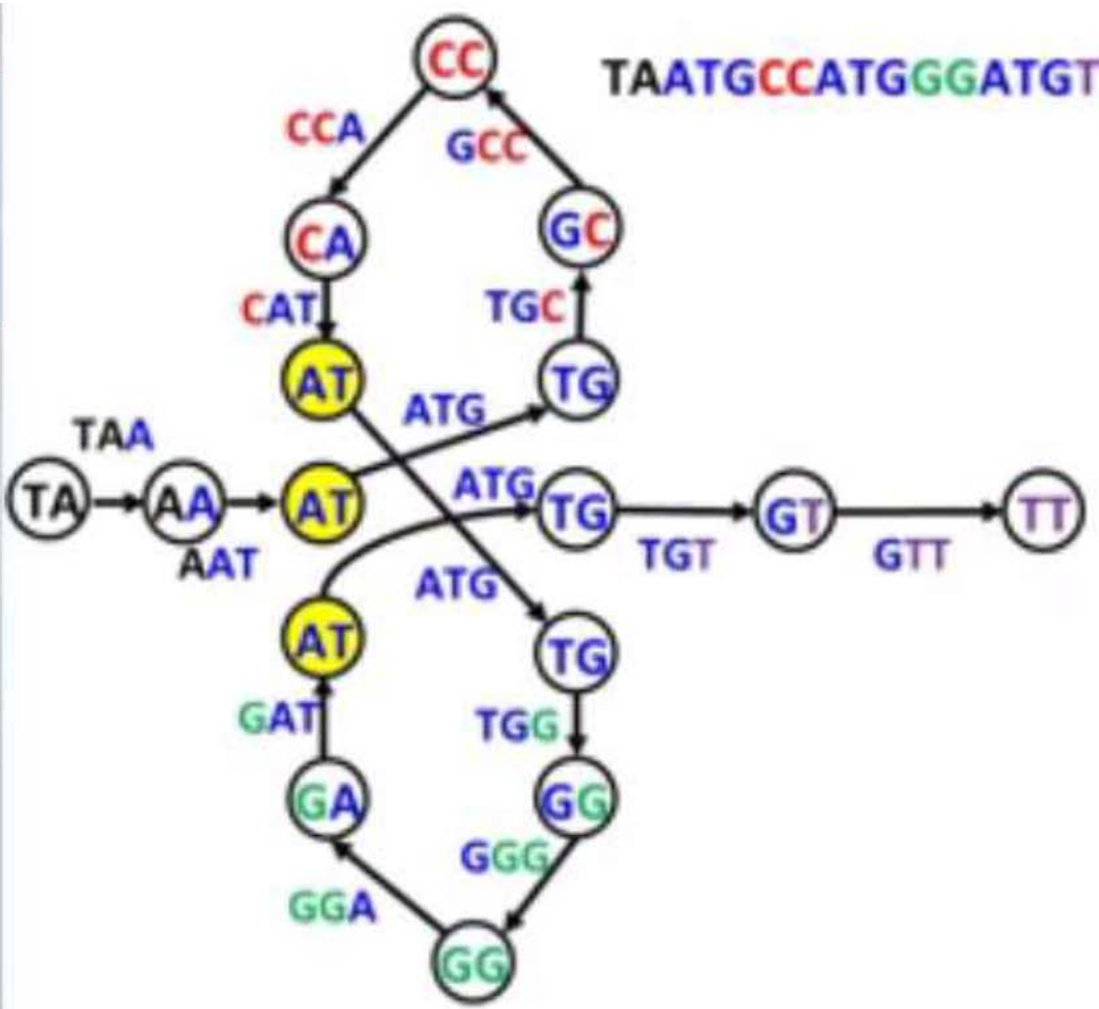


Glue the similar nodes again.



Gluing identically labeled vertices

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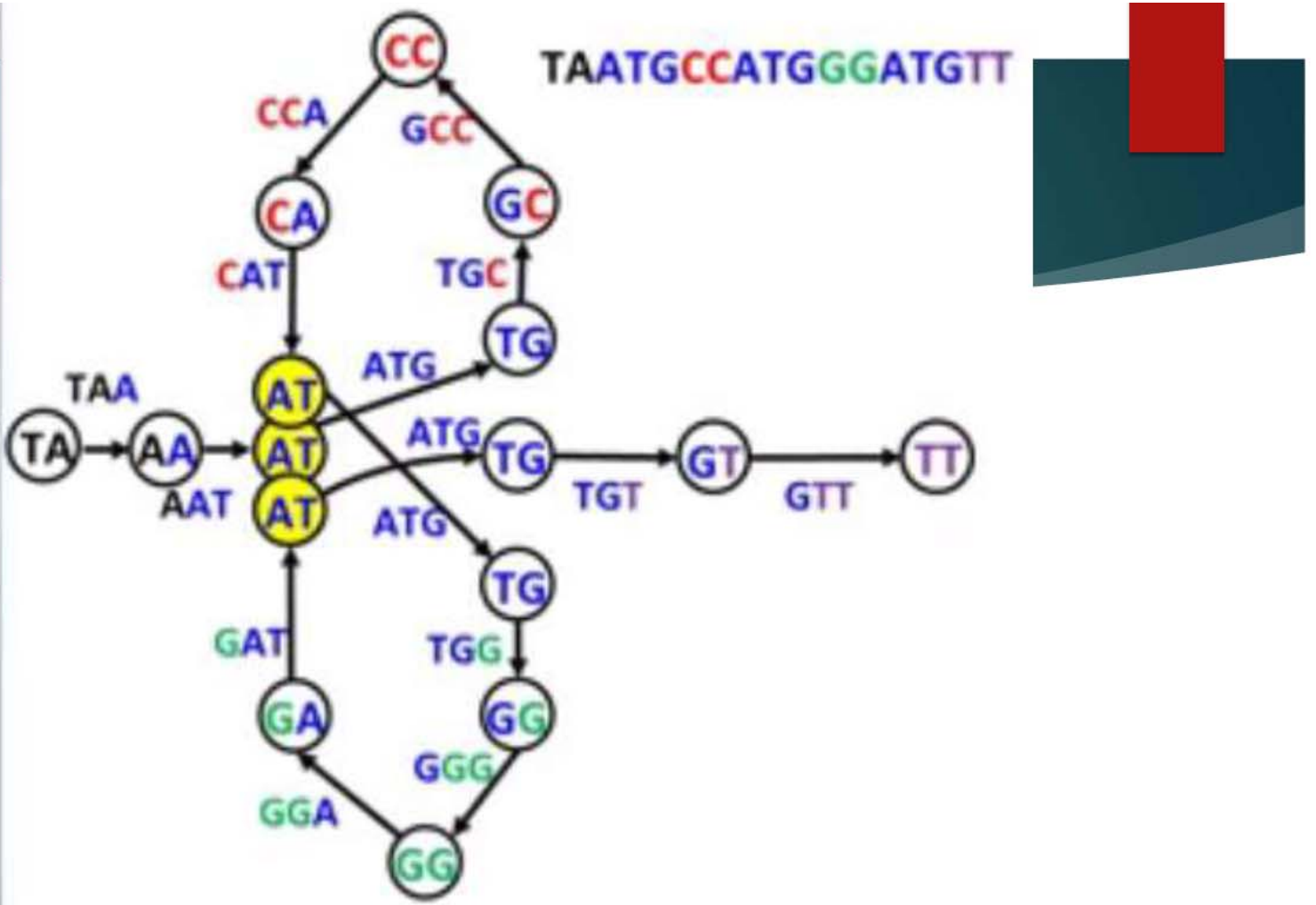
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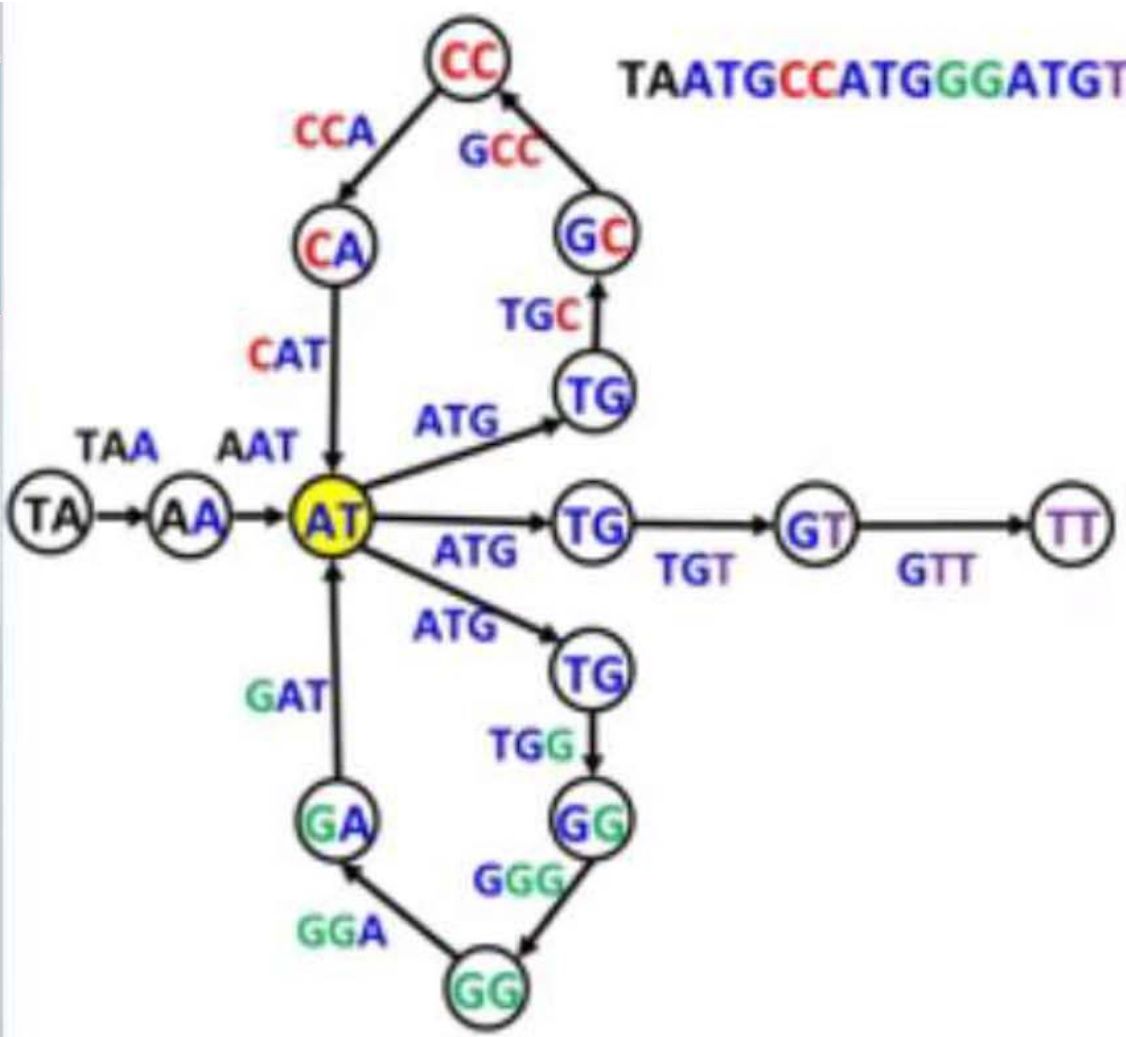
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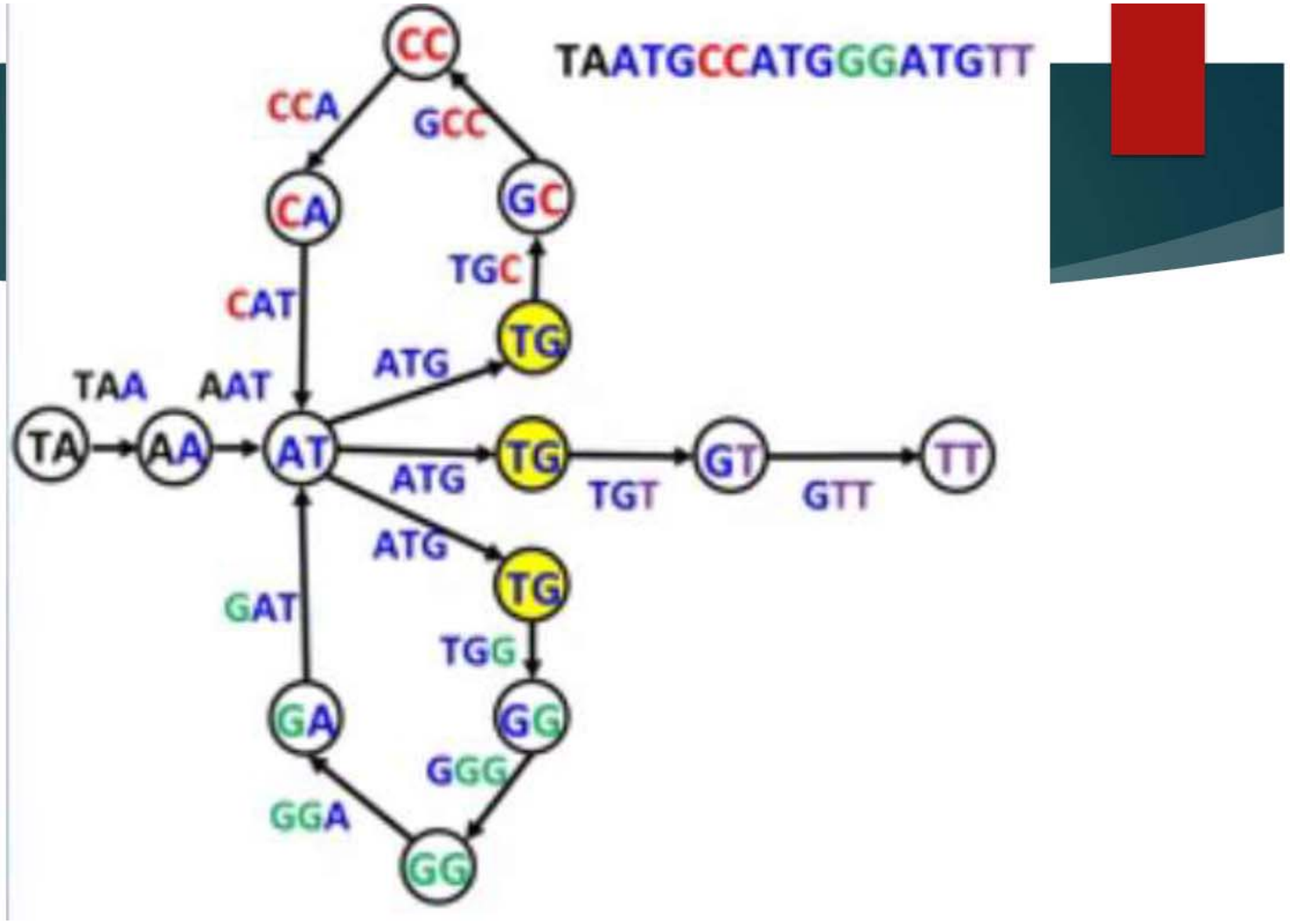
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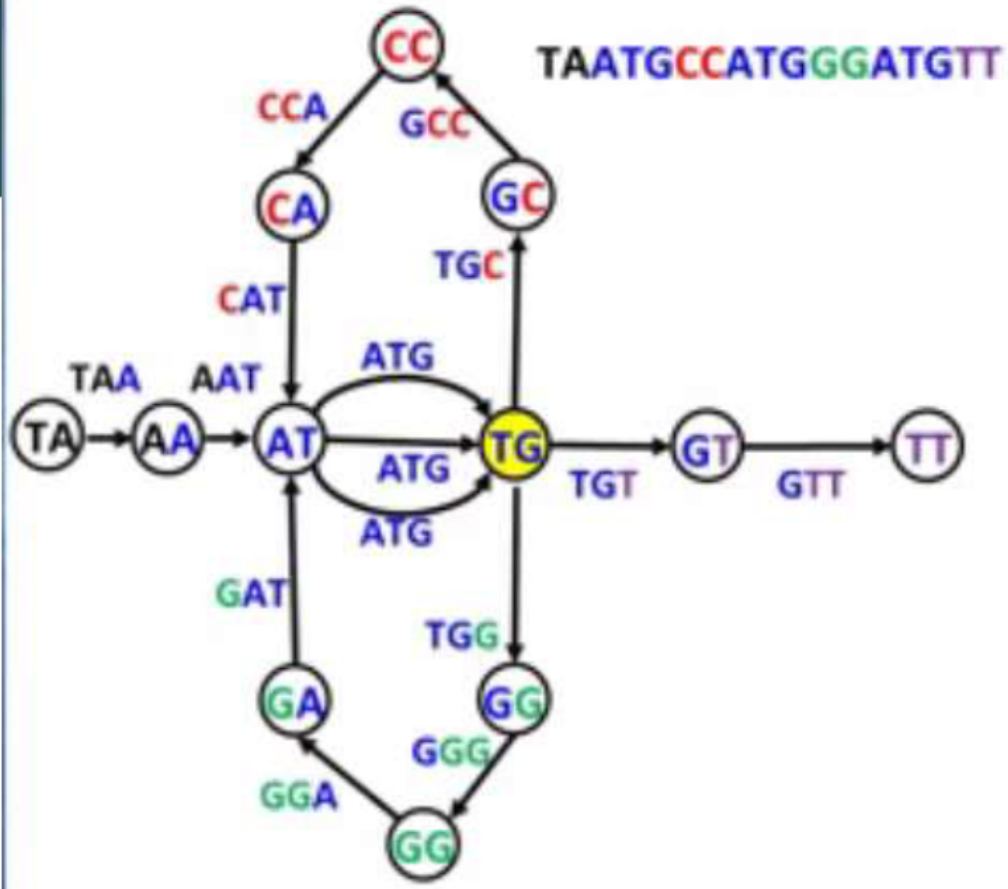
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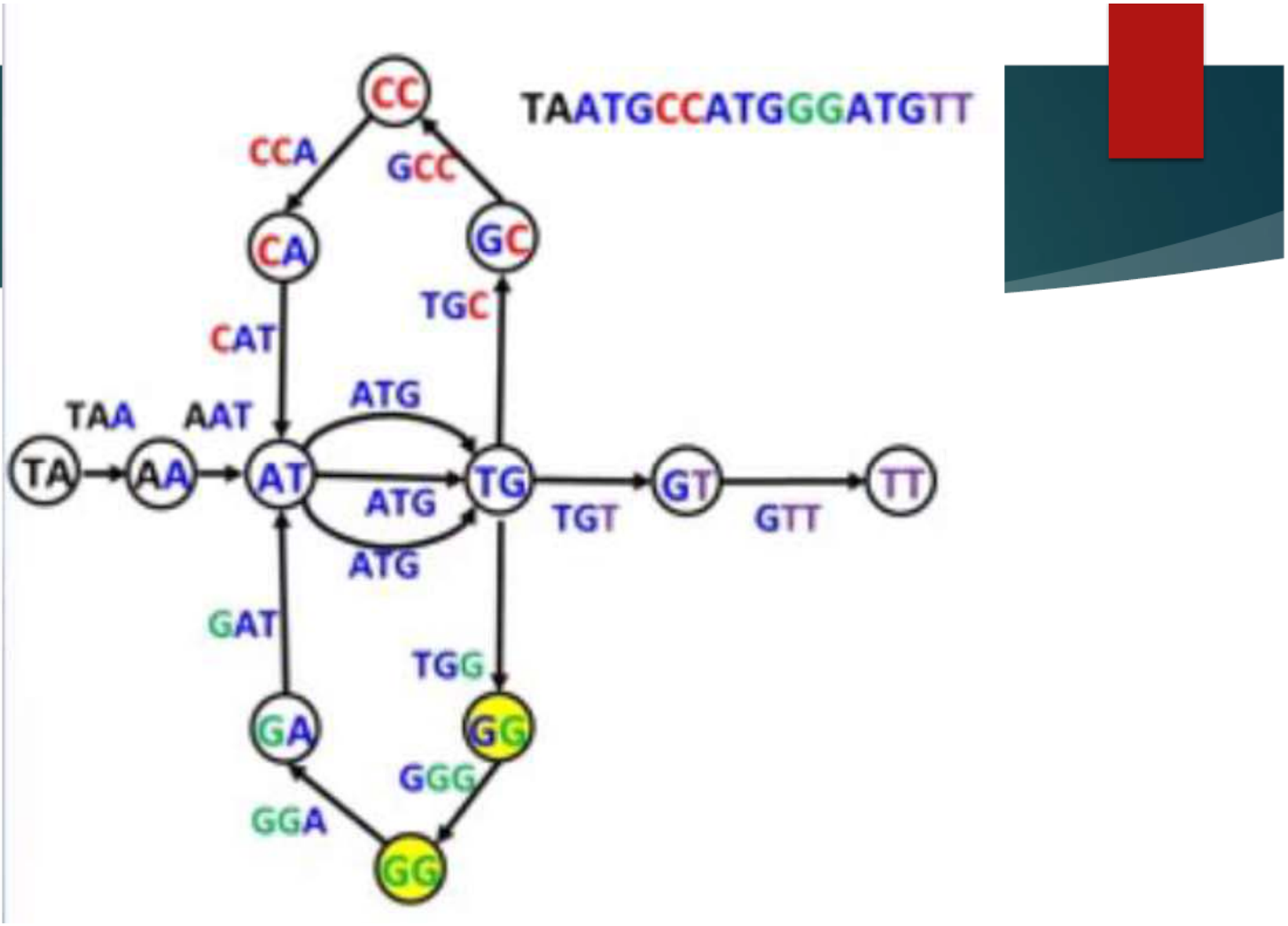
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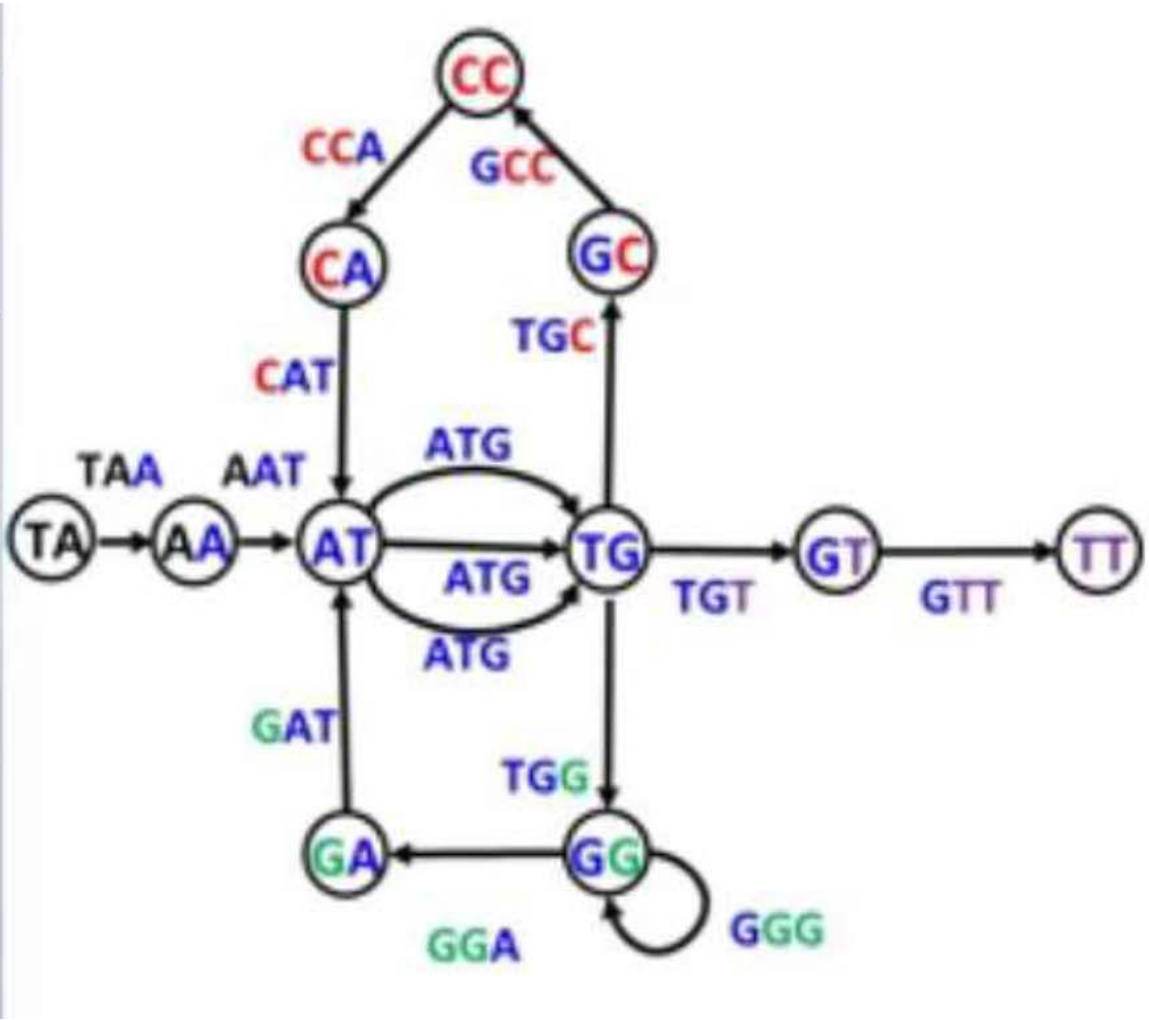
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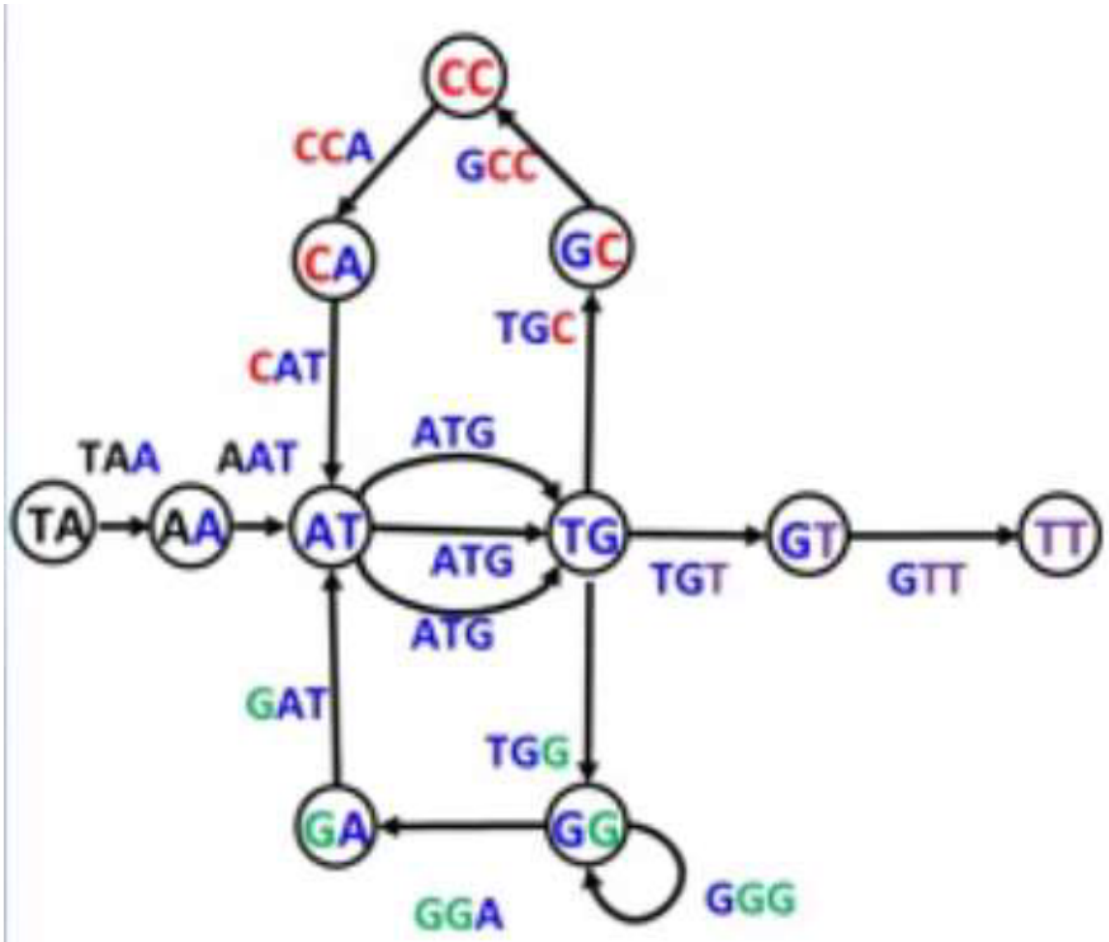
Construction of Genome

- Use whole sequence of first edge

Then

- The Only Use the last alphabet from the intermediate edges.
- Build the genome.



T A A T G C C A T G G G A

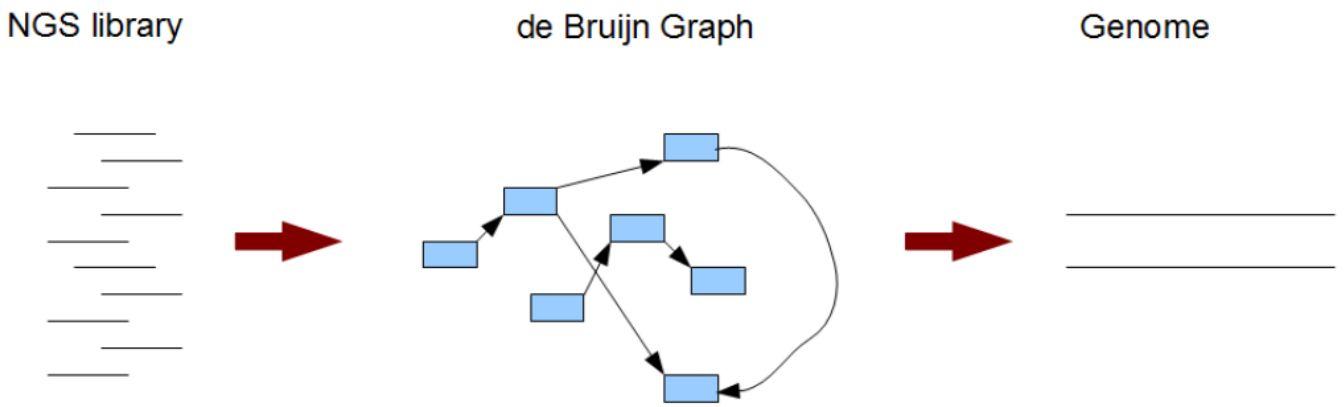
Lets Try An Example

- Consider the read: ATGGCGTGCA
- Build the de Bruijn graph for k=3

ANSWER: Lets Try An Example

- Consider the read: ATGGCGTGCA
- Build the de Bruijn graph for k=3

De bruijn graph based genome assembly algorithm



De Bruijn graph

No: graph can have multiple Eulerian walks, only one of which corresponds to original superstring

Right: graph for ZABCDABEFABY, $k = 3$

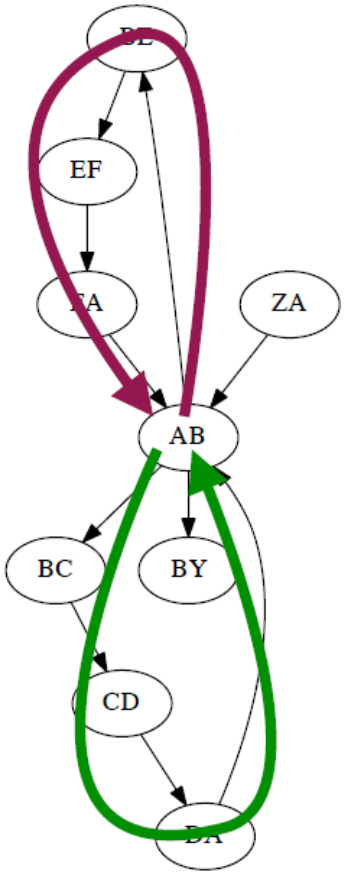
Alternative Eulerian walks:

ZA → AB → BE → EF → FA → AB → BC → CD → DA → AB → BY

ZA → AB → BC → CD → DA → AB → BE → EF → FA → AB → BY

These correspond to two edge-disjoint directed cycles joined by node AB

AB is a repeat: ZABCDABEFABY



De Bruijn graph

Casting assembly as Eulerian walk is appealing, but not practical

- Uneven coverage, sequencing errors, etc make graph non-Eulerian
- Even if graph were Eulerian, repeats yield many possible walks

> Kingsford, Carl, Michael C. Schatz, and Mihai Pop. "Assembly complexity of prokaryotic genomes using short reads." *BMC bioinformatics* 11.1 (2010): 21.

De Bruijn Superwalk Problem (DBSP) is an improved formulation where we seek a walk over the De Bruijn graph, where walk contains each read as a *subwalk*

- Proven NP-hard!

> Medvedev, Paul, et al. "Computability of models for sequence assembly." *Algorithms in Bioinformatics*. Springer Berlin Heidelberg, 2007. 289-301.

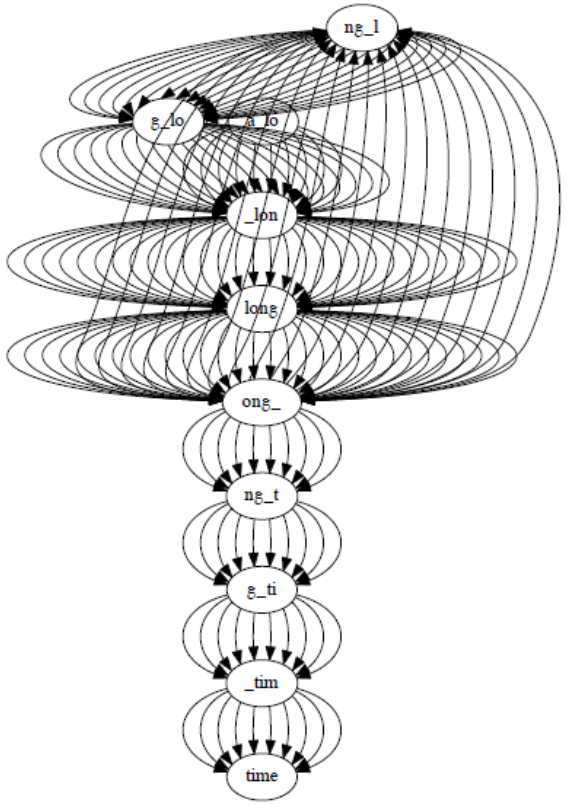
De Bruijn graph

In practice, De Bruijn graph-based tools give up on unresolvable repeats and yield fragmented assemblies, just like OLC tools.

But first we note that using the De Bruijn graph representation has **other advantages**...

De Bruijn graph

In typical assembly projects,
average coverage is ~ 30 - 50



Remaining Genome Assembly Steps

- Mapping: Identify genes and where they are on the genome.
- Annotation: Describe mapped genes, chromosomes, …
- SNV calling: Identify variations

de Bruijn Graph Building Steps

- Break read into k-mers
- Make k-mers edges in graph and prefix node the k-1 prefix of the k-mer, and the suffix node the k-1 suffix. E.g., ABC –ABCD-> BCD
- Merge similar nodes
- Traversed through each edge only once to retrieve the smallest encompassing sequence that yielded the reads.

NEXT: CRISPR