582670 Algorithms for Bioinformatics

Lecture 5: Graph Algorithms and DNA Sequencing

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Adapted from slides by Alexandru Tomescu, Leena Salmela and Veli Mäkinen, which are partly from `http://bix.ucsd.edu/bioalgorithms/slides.php`

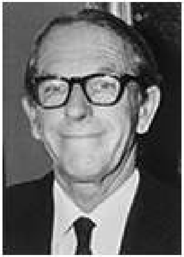
DNA Sequencing: History

Sanger method (1977):

- Labeled ddNTPs terminate DNA copying at random points.

Gilbert method (1977):

- Chemical method to cleave DNA at specific points (G, G+A, T+C, C).

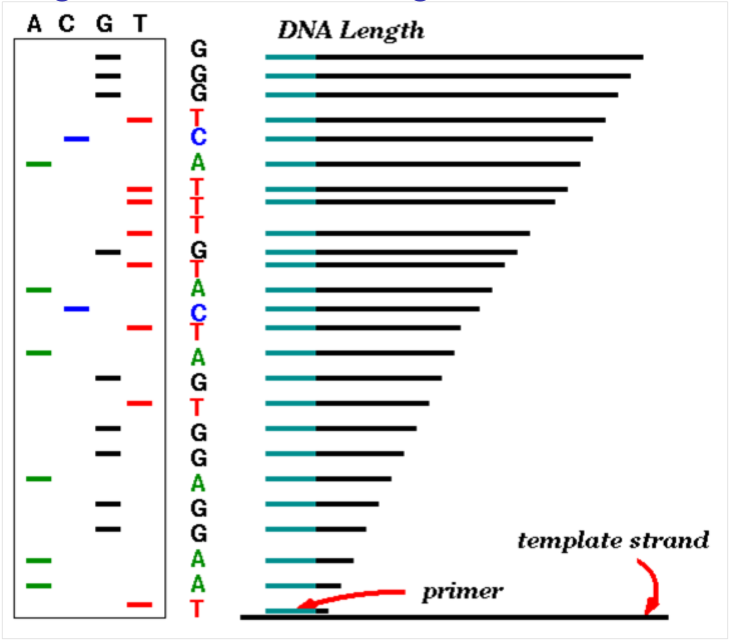


- Both methods generate labeled fragments of varying lengths that are further measured by electrophoresis.

Sanger Method: Generating a Read

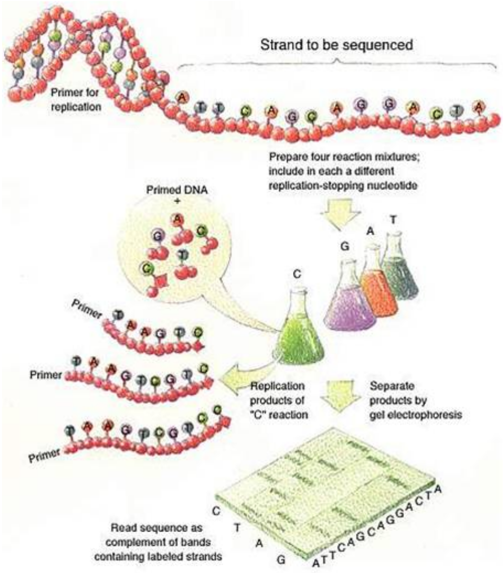
1. Divide DNA sample into four.
2. Each sample will have available all normal nucleotides and modified nucleotides of one type (A, C, G or T) that will terminate DNA strand elongation.
3. Start at primer (restriction site).
4. Grow DNA chain.
5. In each sample the reaction will stop at all points ending with the modified nucleotide.
6. Separate products by length using gel electrophoresis.

Sanger Method: Generating a Read



DNA Sequencing

- Shear DNA into millions of small fragments.
- Read 500-700 nucleotides at a time from the small fragments (Sanger method)



Strand to be sequenced
Primer for replication
A T T C A G C A G G A C T A
Prepare four reaction mixtures; include in each a different replication-stopping nucleotide
Primed DNA +
A G C G T C
G A T C
Primer
Primer
Primer
Replication products of "C" reaction
Separate products by gel electrophoresis
Read sequence as complement of bands containing labeled strands
C T A G
ATTCAGCAGGACTA

Fragment Assembly

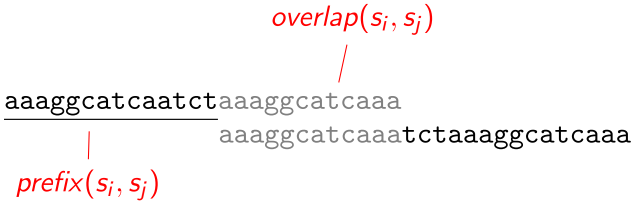
- **Computational Challenge:** assemble individual short fragments (reads) into a single genomic sequence ("superstring")
- Until late 1990s the shotgun fragment assembly of human genome was viewed as intractable problem
  - Now there exists "complete" sequences of human genomes of several individuals
- For small and "easy" genomes, such as bacterial genomes, fragment assembly is tractable with many software tools
- Remains to be difficult problem for more complex genomes

Shortest Superstring Problem

- Problem: Given a set of strings, find a shortest string that contains all of them
- Input: Strings $S = \{s_1, s_2, \ldots, s_n\}$
- Output: A string s that contains all string $s_1, s_2, \ldots s_n$ as substrings, such that the lenght of s is minimized
- **Complexity**: NP-hard
- Recall:
  - Greedy approximation algorithm at the study group
  - Extension to approximate case in the exercises

Overlaps and prefixes

- Define *overlap*(s_i, s_j) as the longest prefix of s_j that matches a suffix of s_i



- Define *prefix*(s_i, s_j) as the part of s_i after its longest overlap with s_j is removed.

SSP as a Graph Problem

- Construct a *prefix graph* with
  - n vertices representing the n strings $s_1, s_2, \ldots, s_n$ and
  - directed edges of length $|prefix(s_i, s_j)|$ from s_i to s_j
- Add a dummy vertex d to prefix graph with edges of length $|s_i|$ from each s_i to d.
- Find the shortest path which visits every vertex exactly once. Such a path is called a *Hamiltonian path*.
- This is the *Asymmetric Travelling Salesman Problem (ATSP)*, which is also NP-complete

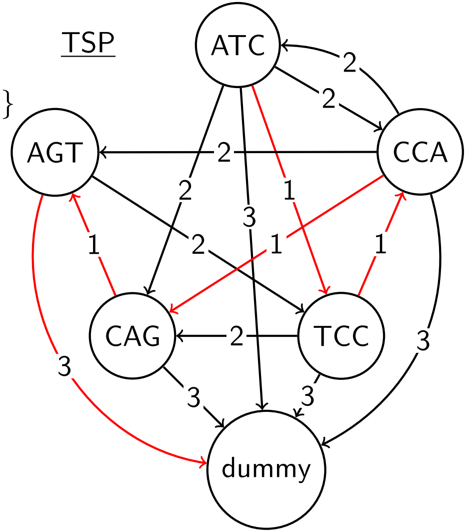
SSP to TSP: An example

$S = \{\mathrm{ATC}, \mathrm{CCA}, \mathrm{CAG}, \mathrm{TCC}, \mathrm{AGT}\}$

SSP

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

TSP



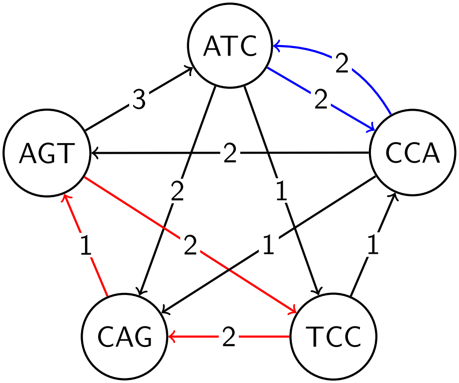
ATCCAGT
(note: only subset of edges shown)

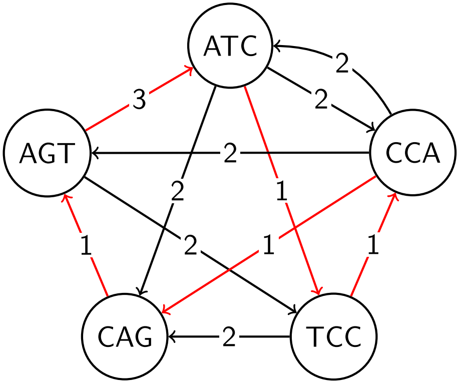
Shortest superstring: 4-approximation

- There are logarithm-factor approximation algorithms for ATSP, but the prefix graph instances admit constant factor approximation algorithms:
  - Resulting superstring is at most c times longer than the optimal OPT, for some constant c.
- 4-approximation algorithm:
  - Construct the prefix graph corresponding to strings in S
  - Find a *minimum weight cycle cover* on the prefix graph
  - Read the superstring defined by the cycle cover
  - Proof of approximation ratio in a study group.

Cycle cover

- A cycle cover is a set of disjoint cycles covering all vertices.
- ATSP tour is a special case: cycle cover with exactly one cycle. It is also known as a *Hamiltonian cycle*.

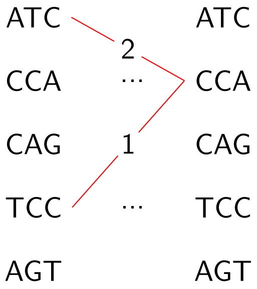




Minimum weight cycle cover

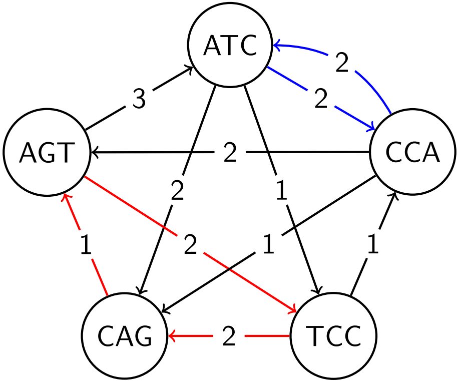
- Minimum weight cycle cover is polynomial time solvable!
- Reduction to *minimum weight perfect mathing on a bipartite graph*:
  - Bipartite graph: vertices can be divided into two sets so that all edges have one endpoint in one set and the other endpoint in the other set
  - Perfect matching: a set of disjoint edges that covers all vertices

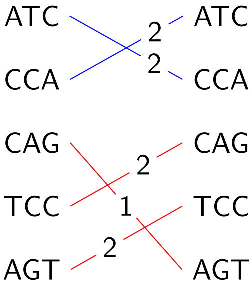
- Create two vertices u_i and v_i for each string s_i to a graph H
- Add edge (u_i, v_j) with weight $|prefix(s_i, s_j)|$ for $i \neq j$
- Each cycle cover in prefix graph corresponds to a minimum weight perfect matching on H and vice versa.



Minimum weight perfect matching

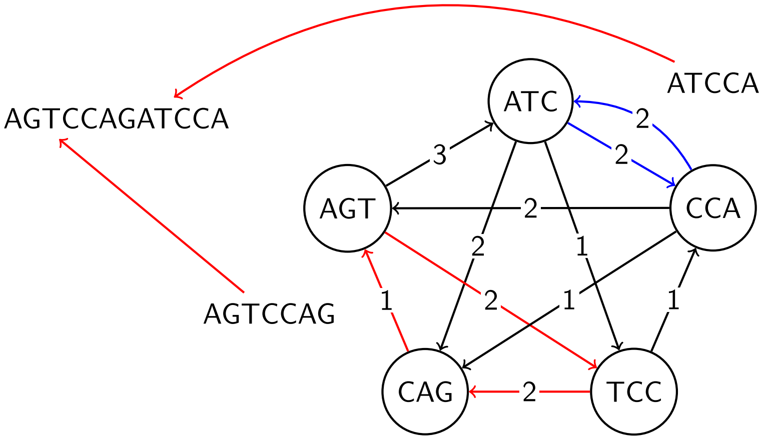
- Classical non-trivial graph problem with polynomial time solutions.





Reading superstring from cycle cover

- Construct a superstring for each cycle
  - concatenate prefixes corresponding to weight starting from any vertex
  - append the overlap of last and first vertex
- Concatenate the cycle superstrings

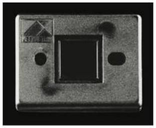


Sequencing by Hybridization (SBH): History

- 1988: SBH suggested as an alternative sequencing method. Nobody believed it will ever work.
- 1991: Light directed polymer synthesis developed by Steve Fodor and colleagues.
- 1994: Affymetrix develops first 64-kb DNA microarray.

First microarray prototype (1989)

First commercial DNA microarray prototype with 16,000 features (1994)

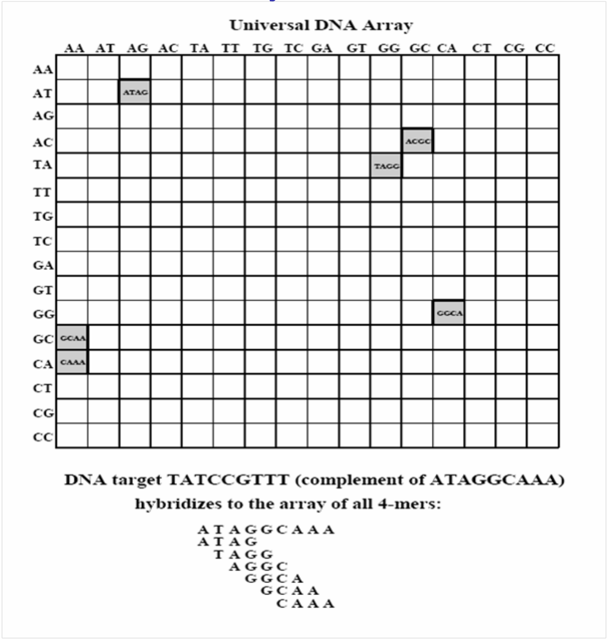


500,000 features per chip (2002)

How SBH works

- Attach all possible DNA probes of length ℓ to a flat surface, each probe at a distinct and known location. This set of probes is called the *DNA microarray*.
- Apply a solution containing fluorescently labeled DNA fragment to the array.
- The DNA fragment hybridizes with those probes that are complementary to substrings of length ℓ of the fragment.
- Using a spectroscopic detector, determine which probes hybridize to the DNA fragment to obtain the ℓ-mer composition of the DNA fragment.
- Reconstruct the sequence of the DNA fragment from the ℓ-mer composition.

Hybridization on DNA Array



Universal DNA Array

	AA	AT	AG	AC	TA	TT	TG	TC	GA	GT	GG	GC	CA	CT	CG	CC
AA																
AT			ATAG													
AG																
AC												ACGC				
TA											TAGG					
TT																
TG																
TC																
GA																
GT																
GG													GGCA			
GC	GCAA															
CA	CAAA															
CT																
CG																
CC																

DNA target TATCCGTTT (complement of ATAGGCAAA)
hybridizes to the array of all 4-mers:

```
ATAGGCAAA
ATAG
 TAGG
  AGGC
   GGCA
    GCAA
     CAAA
```

ℓ-mer composition

- $\mathit{Spectrum}(s, \ell)$ is a multiset of all possible $(n - \ell + 1)$ ℓ-mers in a string s of length n.
- E.g. for $s = \mathrm{TATGGTGC}$, $\mathit{Spectrum}(s, 3)$:

$$S = \{\mathrm{TAT}, \mathrm{ATG}, \mathrm{TGG}, \mathrm{GGT}, \mathrm{GTG}, \mathrm{TGC}\}$$

- Different sequences may have the same spectrum:

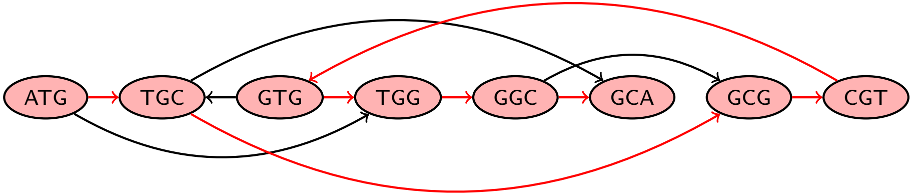
$$\begin{aligned}&\mathit{Spectrum}(\mathrm{GTATCT}, 2) =\\&\mathit{Spectrum}(\mathrm{GTCTAT}, 2) =\\&\{\mathrm{AT}, \mathrm{CT}, \mathrm{GT}, \mathrm{TA}, \mathrm{TC}\}\end{aligned}$$

The SBH Problem

- Goal: Reconstruct a string from its ℓ-mer composition
- Input: A multiset S, representing all ℓ-mers from an (unknown) string s
- Output: A string s such that $Spectrum(s, \ell) = S$

SBH: Hamiltonian Path Approach

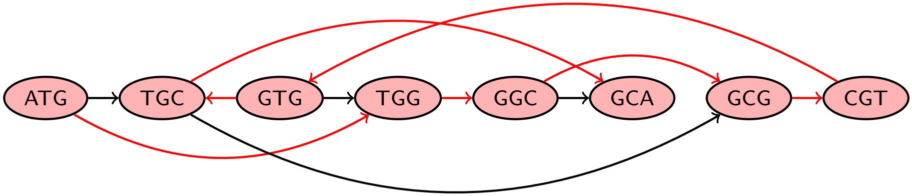
- Construct a graph
  - One vertex for each ℓ-mer in the input spectrum
  - Draw an edge between two vertices if the ℓ-mers overlap by $\ell - 1$ nucleotides
- Find a path that visits each **vertex** once.
- Example: $S = \{\mathrm{ATG}, \mathrm{TGC}, \mathrm{GTG}, \mathrm{TGG}, \mathrm{GGC}, \mathrm{GCA}, \mathrm{GCG}, \mathrm{CGT}\}$



ATGCGTGGCA

SBH: Hamiltonian Path Approach

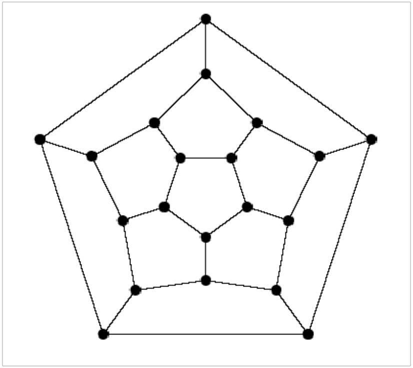
- Another path for:
  $S = \{\mathrm{ATG}, \mathrm{TGC}, \mathrm{GTG}, \mathrm{TGG}, \mathrm{GGC}, \mathrm{GCA}, \mathrm{GCG}, \mathrm{CGT}\}$



ATGGCGTGCA

Hamiltonian Cycle Problem

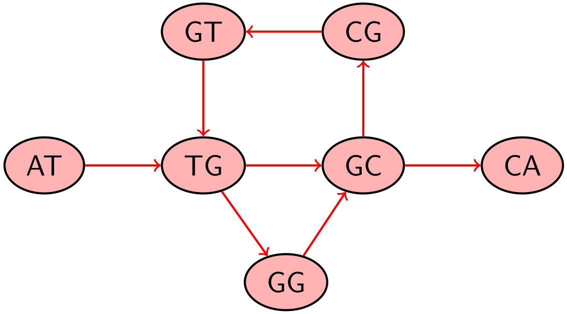
- Find a cycle that visit every **vertex** exactly once.
- NP-complete



Game invented by Sir William Hamilton in 1857

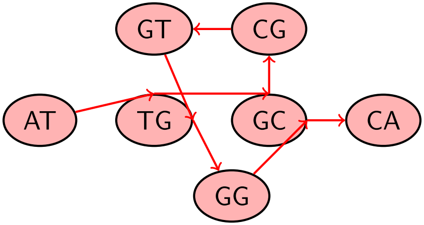
SBH: Eulerian Path Approach

- Construct a graph
    - A vertex for each $(\ell - 1)$-mer
    - An edge between two vertices corresponds to an ℓ-mer from S
    - Find a path that visits each **edge** once.
    - Example: $S = \{\mathrm{ATG}, \mathrm{TGC}, \mathrm{GTG}, \mathrm{TGG}, \mathrm{GGC}, \mathrm{GCA}, \mathrm{GCG}, \mathrm{CGT}\}$

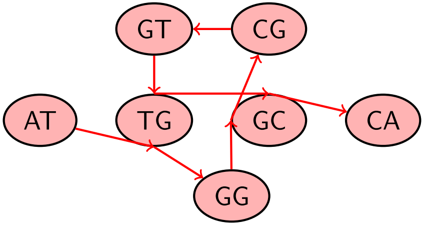


SBH: Eulerian Path Approach

- $S = \{\mathrm{ATG}, \mathrm{TGC}, \mathrm{GTG}, \mathrm{TGG}, \mathrm{GGC}, \mathrm{GCA}, \mathrm{GCG}, \mathrm{CGT}\}$ corresponds to two different paths:



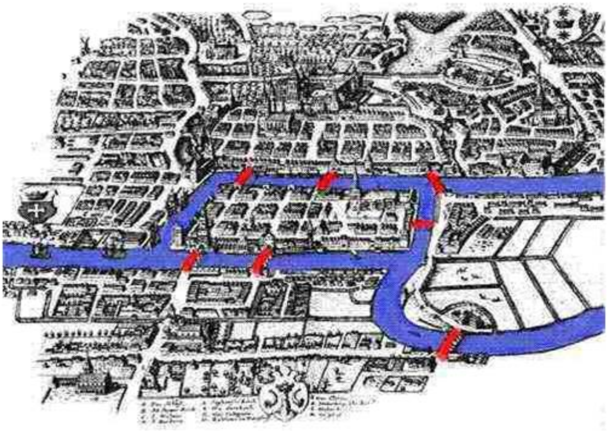
ATGCGTGGCA



ATGGCGTGCA

The Bridge Obsession Problem

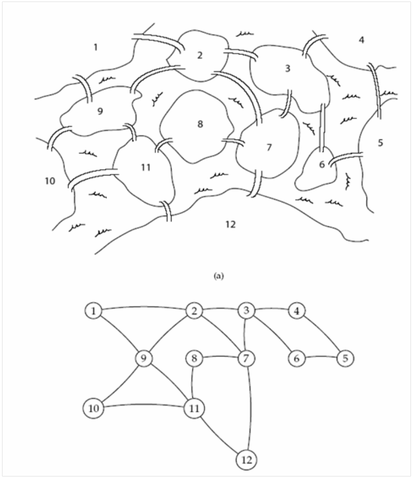
Find a tour crossing every bridge just once
Leonhard Euler, 1735



Bridges of Königsberg

Eulerian Cycle Problem

- Find a cycle that visits every **edge** exactly once
- Linear time



More complicated Königsberg

Euler Theorems

- A vertex is *balanced* if the number of incoming edges equals the number of ougoing edges:

$$in(v) = out(v)$$

- **Theorem**: A connected graph has a *Eulerian cycle* if and only if each of its vertices is balanced.
- Such a graph is called a *Eulerian graph*.

- A vertex is *semi-balanced* if $in(v) = out(v) + 1$ or $in(v) = out(v) - 1$
- **Theorem**: A connected graph has a *Eulerian path* if and only if it contains at most two semi-balanced vertices and all other vertices are balanced.

Some Difficulties with SBH

- In practice, ℓ-mer composition can never be measured with 100% accuracy
  - With inaccurate data, the computational problem is again NP-hard.
    - Find minimum completion (insertion/deletion of edges and vertices) of the graph so that it becomes Eulerian
    - Jacek Błazewicz and Marta Kasprzak: Complexity of DNA sequencing by hybridization. *Theoretical Computer Science*, 290(3):1459–1473, 2003.
- Microarray technology has found other uses:
  - Widely used in expression analysis and SNP analysis
- Virtual ℓ-mer compositions are used in many fragment assembly tools, leading to heuristics exploiting the Eulerian path approach.

Study Group 1: Random assignment at lecture

- Read pages 284–290 from Jones and Pevzner.
  - The peptide sequencing problem
- At study group draw an example spectrum graph.

Study Group 2: Random assignment at lecture

- Read pages 61–64 from Vazirani: Approximation Algorithms, Springer, 2001.
    - Analysis of the 4-approximation algorithm for Shortest Superstring Problem.
- At study group explain visually the proofs of Lemmas 7.2. and 7.3. Explain how Lemma 7.3 leads to the proof of Theorem 7.4.

Study Group 3: Random assignment at lecture

(Ask lecturer for your group if you were not present)

- Read pages 272–275 from Jones and Pevzner.
  - Eulerian cycles and paths.
- At study group explain the algorithm for finding a Eulerian cycle using an example. How can the algorithm be modified for finding a Eulerian path?