COM SCI 122 Week 4

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Graph Algorithms in Bioinformatics

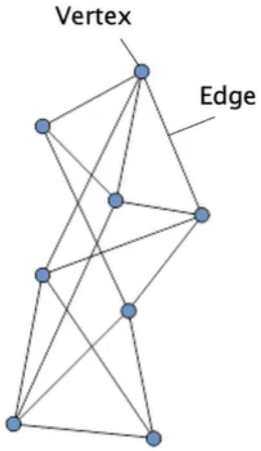
Introduction

A **graph** is a collection (V, E) of two sets:

- V is simply a set of objects, which we call the **vertices** of G.
- E is a set of pairs of vertices which we call the **edges** of G.

Simpler: Think of G as a network:

- Nodes = vertices
- Edges = segments connecting the nodes



Hamiltonian Cycle Problem

- Input: A graph G = (V, E)
- Output: A **Hamiltonian cycle** in G, which is a cycle that visits every vertex exactly once.
- This problem is NP-Complete.
  - This result explains why knight tours were so difficult to find; there is no known quick method to find them!

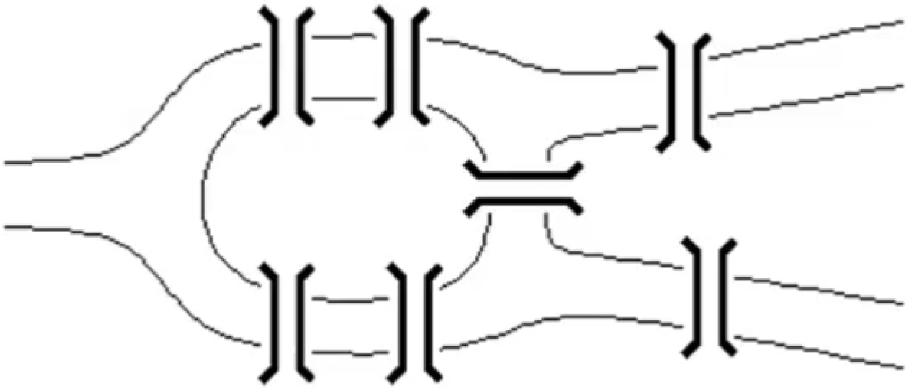
Traveling Salesman Problem (TSP)

- n cities
- Cost of traveling from i to j is given by $\mathrm{c}(i, j)$
- Goal: Find the tour of all the cities of lowest total cost
- Example below: One busy salesman!

So we might like to think of the Hamiltonian Cycle Problem as a TSP with all costs = 1, where we have some edges missing (there doesn't always exist a flight between all pairs of cities).

The Bridges of Konigsberg

- The city of Konigsberg, Prussia (today: Kaliningrad, Russia) was made up of both banks of a river, as well as two islands.
- The riverbanks and the islands were connected with bridges, as follows:



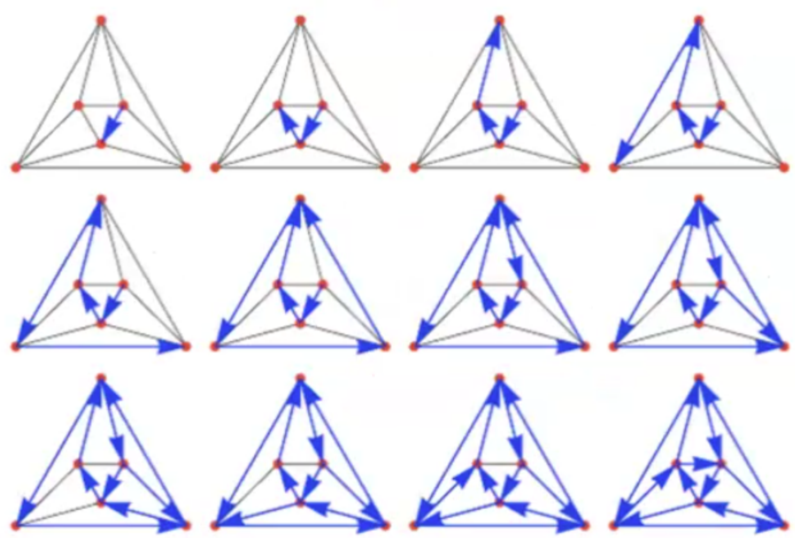
- The residents wanted to know if they could take a walk from anywhere in the city, cross each bridge exactly once, and wind up where they started.

In 1735, enter Euler... his idea: compress each land area down to a single point, and each bridge down to a segment connecting two points. This is just a graph! We are now looking for a cycle in this graph which covers each edge exactly once.

Using this setup, Euler showed that such a cycle cannot exist.

Eulerian Cycle Problem

- Input: A graph G = (V, E)
- Output: A cycle in G that touches every edge in E (called an **Eulerian cycle**), if one exists.
- Example: below is a demonstration of an Eulerian cycle.



Theorem:

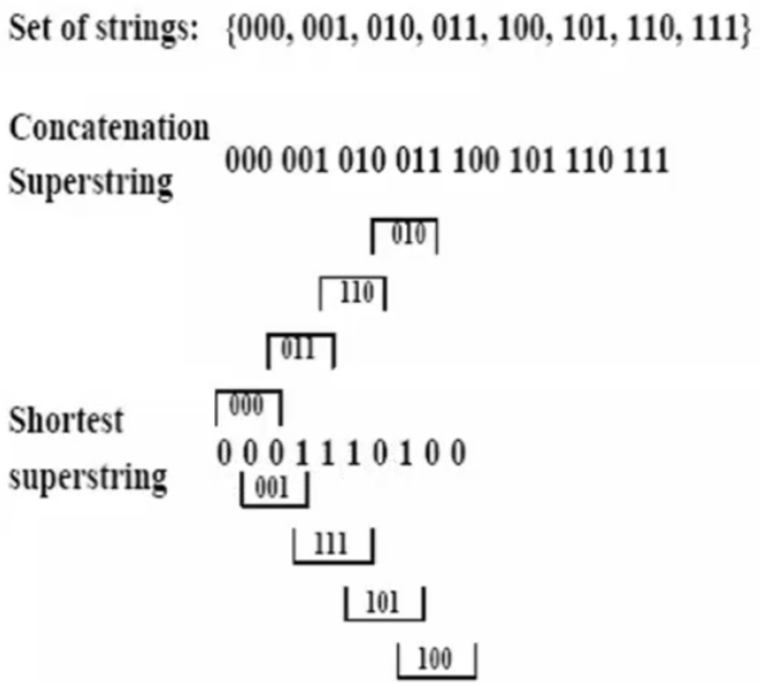
The Eulerian Cycle Problem can be solved in linear time

- So whereas finding a Hamiltonian cycle quickly becomes intractable for an arbitrary graph, finding an Eulerian cycle is relatively much easier

Shortest Superstring Problem (SSP)

- Problem: Given a set of strings, find a shortest string that contains all of them,
- Input: Strings s_1, s_2, ..., s_n
- Output: A "superstring" s that contains all strings s_1, s_2, ..., s_n as substrings, such that the length of s is minimized.

Example:



- So our greedy guess of concatenating all the strings together turns out to be substantially suboptimal (length 24 vs. 10).

To do this, we can define an *overlap function*.

Overlap Function

- Given strings s_i and s_j, define $overlap(s_i, s_j)$ as the length of the longest prefix of s_j that matches a suffix of s_i.
- Example:

```
s_1 = AAAGGCATCAAATCTAAAGGCATCAAA
s_2 =                AAAGGCATCAAATCTAAAGGCATCAAA
```

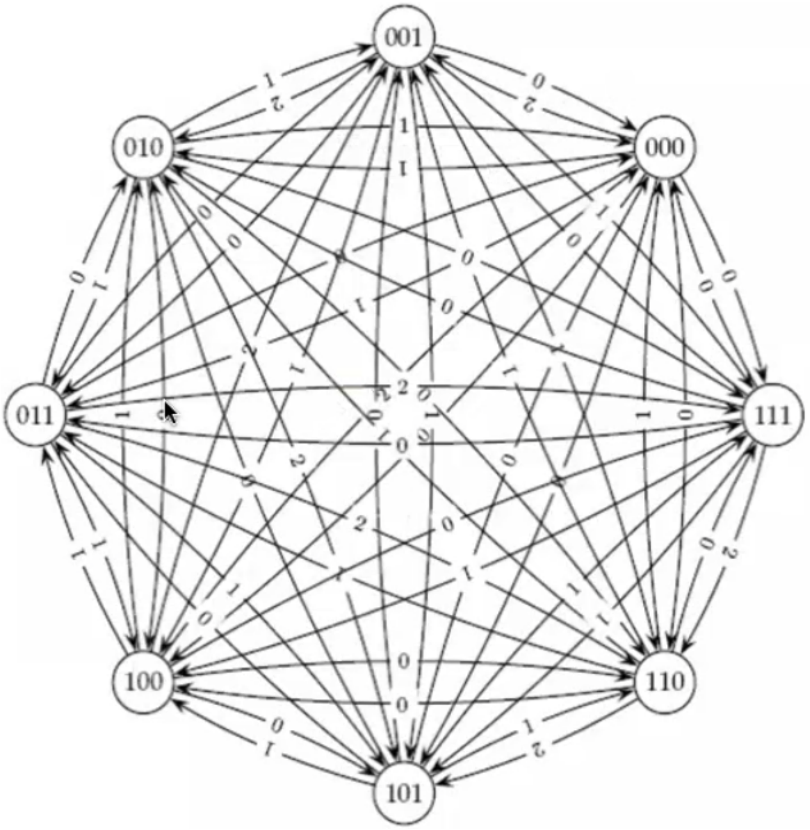
  - Therefore, $overlap(s_1, s_2) = 12$.

Why is SSP an NP-Complete Problem?

- Construct a graph G as follows:
  - The n vertices represent the n strings $s_1, s_2, \dots, s_n$.
  - For every pair of vertices s_i and s_j, insert an edge of length $overlap(s_i, s_j)$ connecting the vertices.
- Then finding the shortest superstring will correspond to finding the shortest Hamiltonian path in G.
- But this is the **Traveling Salesman Problem** (TSP), which we know to be NP-complete.
  - Hence SSP must also be NP-complete!

Reducing SSP to TSP: Example 1

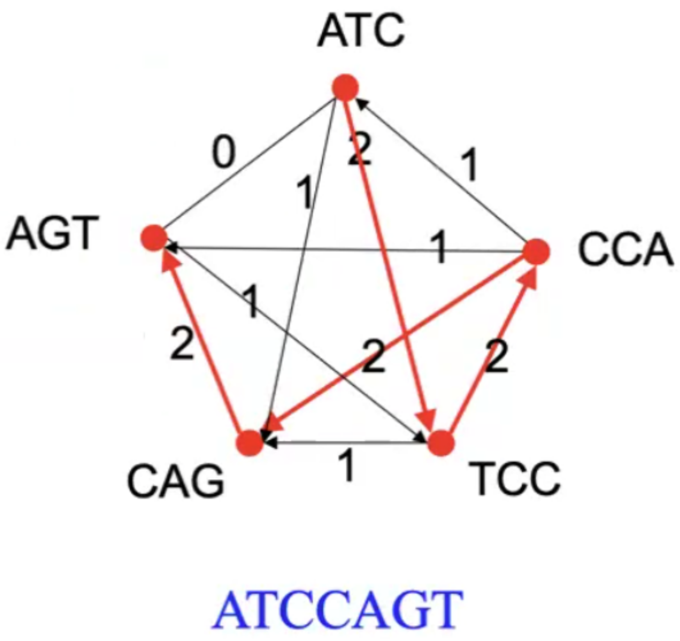
- Take our previous set of strings S = {000, 001, 010, 011, 100, 101, 110, 111}
- Then the graph for S is below:



- One minimal Hamiltonian path gives our previous superstring, 0001110100.

Reducing SSP to TSP: Example 2

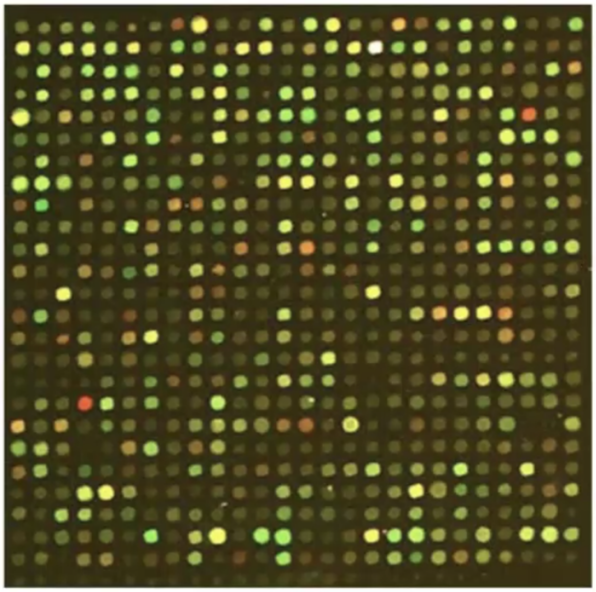
- S = {ATC, CCA, CAG, TCC, AGT}
- This graph is provided below.



- A minimal Hamiltonian path gives as shortest superstring ATCCAGT.

Sequencing By Hybridization

- Using a spectroscopic detector, determine which probes hybridize to the DNA fragment to obtain the l-mer composition of the target DNA fragment.
- Reconstruct the sequence of the target DNA fragment from the l-mer composition.

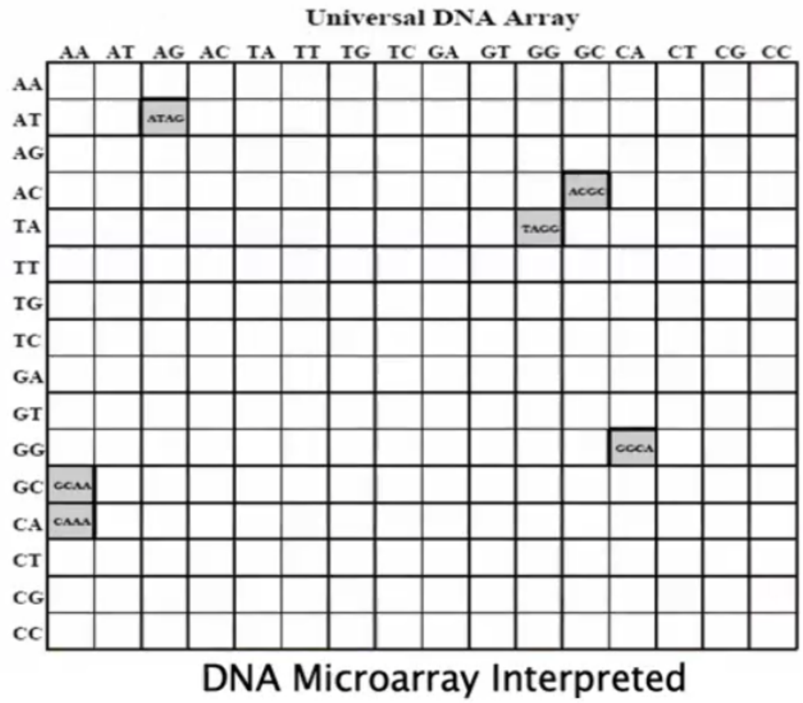


DNA Microarray

How SBH Works: Example

- Say our DNA fragment hybridizes to indicate that it contains the following substrings: GCAA, CAAA, ATAG, TAGG, ACGC, GGCA.

- Then the most logical explanation is that our fragment is the shortest superstring containing these strings!
- Here the superstring is: ATAGGCAAACGC.



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 Microarray Interpreted

l-mer Composition

- *Spectrum*(s, l): The *unordered* of all l-mers in a string s of length n.
- The order of individual elements in *Spectrum*(s, l) does not matter.
- For $s =$ TATGGTGC all of the following are equivalent representations of *Spectrum*$(s, 3)$:
  - {TAT, ATG, TGG, GGT, GTG, TGC}
  - {ATG, GGT, GTG, TAT, TGC, TGG}
  - {TGG, TGC, TAT, GTG, GGT, ATG}
- Which ordering do we choose? Typically the one that is *lexicographic*, meaning in alphabetical order (think of a phonebook).

Different Sequences, Same Spectrum

- Different sequences may share a common spectrum
- Example:

$$\left.\begin{array}{l} \textit{Spectrum}(\text{GTATCT}, 2) = \\ \textit{Spectrum}(\text{GTCTAT}, 2) = \end{array}\right. \{\text{AT, CT, GT, TA, TC}\}$$

The SBH Problem

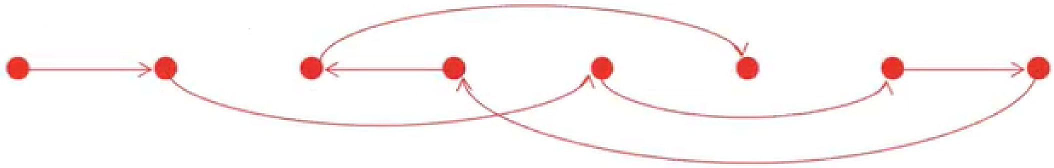
- Problem: Reconstruct a string from its l-mer composition
- Input: A set S, representing all l-mers from an (unknown) string s
- Output: A string s such that $Spectrum(s, l) = S$
- **Note:** As we have seen, there may be more than one correct answer. Determining which DNA sequence is actually correct is another matter.

SBH: Hamiltonian Path Approach

- Create a graph G as follows:
  - Create one vertex for each member of S.
  - Connect vertex v to vertex w with a *directed* edge (arrow) if the last $l - 1$ elements of v match the first $l - 1$ elements of w.
- Then a Hamiltonian path in this graph will correspond to a string s such that $Spectrum(s, l)$!

Example:

$S = \{$ATG TGG TGC GTG GGC GCA GCG CGT$\}$



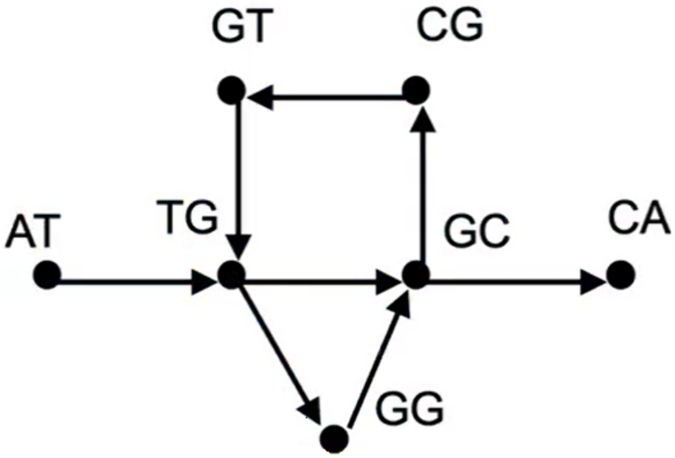
- There are actually two Hamiltonian paths in this graph:
  - Path 1: Gives the string S = ATGCGTGGCA
  - Path 2: Gives the string S = ATGGCGTGCA

SBH: A Lost Cause?

- At this point, we should be concerned about using a Hamiltonian path to solve SBH.
- After all, recall that SSP was an NP-Complete problem, and we have seen that an instance of SBH is an instance of SSP.
- However, note that SBH is actually a specific case of SSP, so there is still hope for an efficient algorithm for SBH:
  - We are considering a spectrum of only l-mers, and not strings of any other length.
  - Also, we only are connecting two l-mers with an edge if and only if the overlap between them is l - 1, whereas before we connected l-mers if there was any overlap at all.
- **Note:** SBH is not NP-Complete since SBH reduces to SSP, but not vice versa.

SPH: Eulerian Path Approach

- So instead, let us consider a completely *different* graph G:
  - Vertices = the set of $(l-1)$-mers which are substrings of some l-mer from our set S.
  - v is connected to w with a *directed* edge if the final $l-2$ elements of v agree with the first $l-2$ elements of w, and the *union* of v and w is in S.
- **Example:** S = {ATG, TGG, TGC, GTG, GGC, GCA, GCG, CGT}
  - V = {AT, TG, GG, GC, GT, CA, CG}
  - E = shown below.



- **Key Point:** A sequence reconstruction will actually correspond to an *Eulerian* path in this graph
- Recall that an Eulerian path is "easy" to find (one can always be found in linear time)... so we have found a simple solution to SBH!
- In our example, two solutions:
  - ATGGCGTGCA
  - ATGCGTGGCA

But, How do we know an Eulerian Path exists?

- A graph is **balanced** if for every vertex the number of incoming edes equals to the number of outgoign edges. We write this for vertex v as:
$$in(v) = out(v)$$
- **Theorem:** A connected graph is *Eulerian* (i.e., contains an Eulerian cycle) if and only if each of its vertices is balanced.
- We will prove this be demonstrating the following:
  1. Every Eulerian graph is balanced.
  2. Every balanced graph is Eulerian.

Proof: Every Eulerian Graph is Balanced

- Suppose we have an Eulerian graph G. Call C the Eulerain cycle of G, and let v be any vertex of G.
- For every edge e entering v, we can pair e with an edge leaving v, which is simply the edge in our cycle C that follows e.
- Therefore, it directly follows that $in(v) = out(v)$ as needed, and since our choice of v was arbitrary, this relation must hold for all vertices in G, so we are finished with the first part.

Proof: Every Balanced Graph is Eulerian

- Next, suppose that we have a balanced graph G.
- We will actually *construct* an Eulerian cycle in G.
- Start with an arbitrary vertex v and form a path in G without repeated edges until we reach a "dead end," meaning a vertex with no unused edges leaving it.
- G is balanced, so every time we enter a vertex w that isn't v during the course of our path, we can find an edge leaving w. So our dead end is v and we have a *cycle*.
- We have two simple cases for our cycle, which we call C:
  1. C is an Eulerian cycle → G is Eulerian → DONE.
  2. C is not an Eulerian cycle.
- So we can assume that C is not an Eulerian cycle, which means that C contains vertices which have untraversed edges.
- Let w be such a vertex, and start a new path from w. Once again, we must obtain a cycle, say C'.
- Combine our cycles C and C' into a bigger cycle C^* by swapping edges at w.
- Once again, we test C^*:
  1. C^* is an Eulerian cycle → G is Eulerian → DONE
  2. C^* is not an Eulerian cycle.
- If C^* is not Eulerian, we iterate our procedure. Because G has a finite number of edges, we must eventually reach a point where our current cycle is Eulerian (Case 1 above). DONE.

Euler's Theorem: Extension

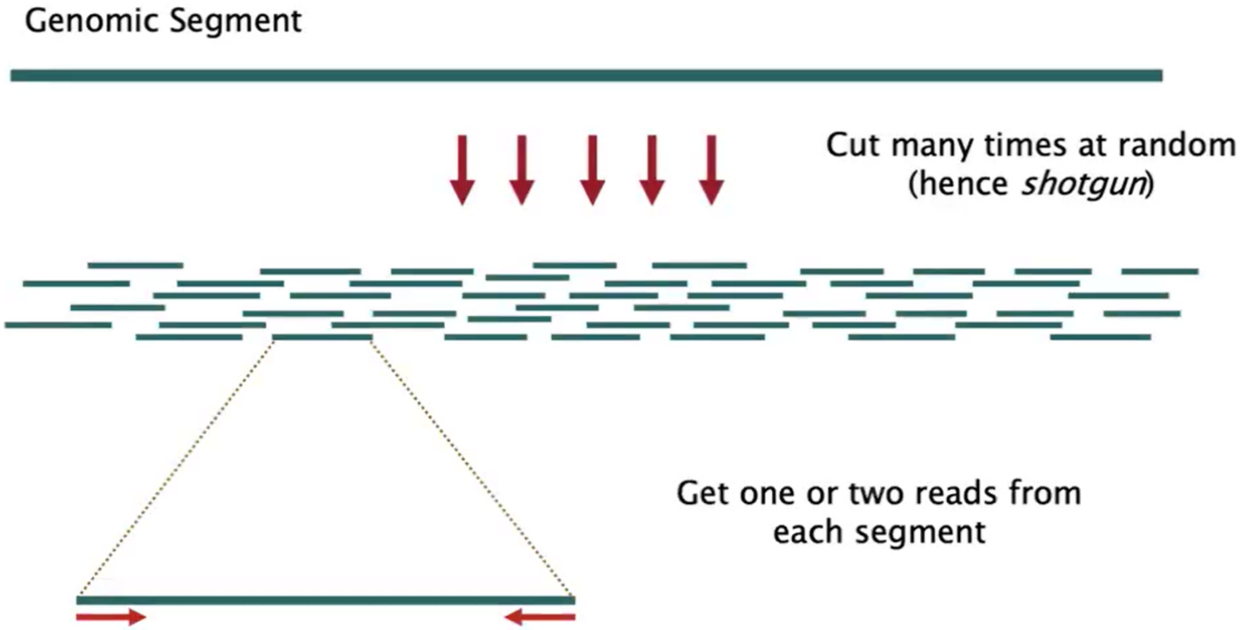
- A vertex v is **semi-balanced** if either $in(v) = out(v) + 1$ or $in(v) = out(v) - 1$.
- **Theorem:** A connected graph has na Eulerian path if and only if it contains at most two semi-balanced vertices and all other vertices are balanced.
  - If G has no semi-balanced vertices, DONE.
  - If G has two semi-balanced vertices, connect them with a new edge, e, so that the graph G + e is balanced and must be Eulerian. Remove e from the Eulerian cycle in G + e to obtain an Eulerian path in G.
- **Think:** Why can G not have just one semi-balanced vertex?

Some Difficulties with SBH

- **Fidelity of Hybridization:** It is difficult to detect differences between probes hybridized with perfect matches and those with one mismatch
- **Array Size:** The effect of low fidelity can be decreased with longer l-mers, but array size increases exponentially in l. Array size is limited with current technology.
- **Practicality:** SBH is still impractical. As DNA microarray technology improves, SBH may become practical in the future.
- **Practicality Again:** Although SBH is still impractical, it spearheaded expression analysis and SNP analysis techniques.
- **Practicality Again and Again:** In 2007 Solexa (now Illumina) developed a new DNA sequencing approach that generates so many short l-mers that they essentially mimic a universal DNA array.

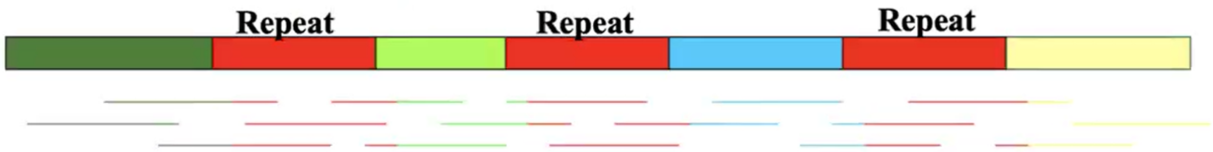
Fragment Assembly and Repeats in DNA

Shotgun Sequencing


Genomic Segment
Cut many times at random (hence *shotgun*)
Get one or two reads from each segment

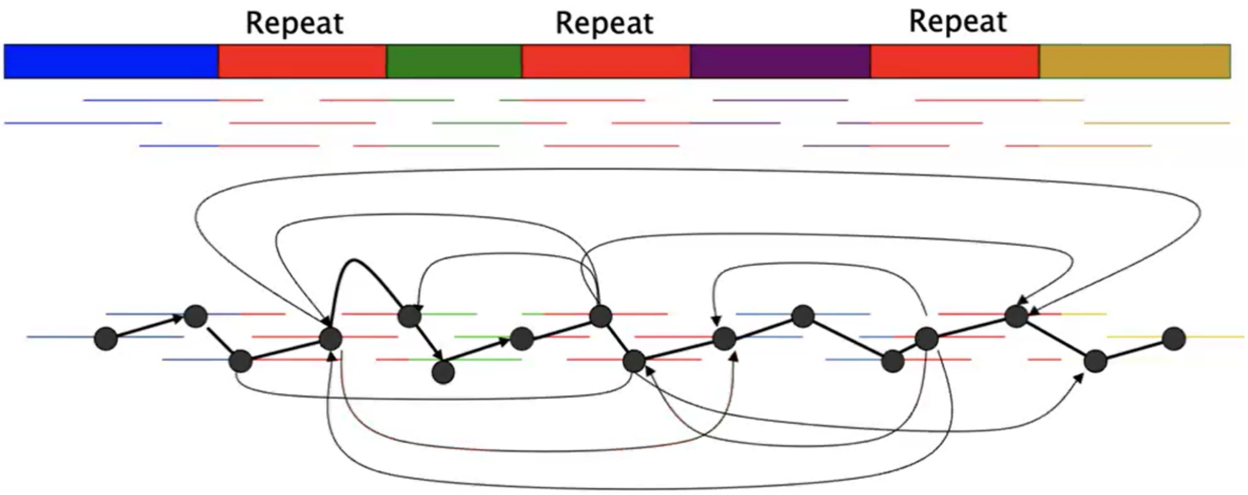
Challenges in Fragment Assembly

- Repeats: A **major** problem for fragment assembly
- More than 50% of human genome are repeats:
  - Over 1 million *Alu* repeats (about 300 bp)
  - About 200,000 LINE repeats (1000 bp and longer)



Overlap Graph: Hamiltonian Approach

- Each vertex represents a read from the original sequence.
- Vertices are connected by an edge if they overlap.

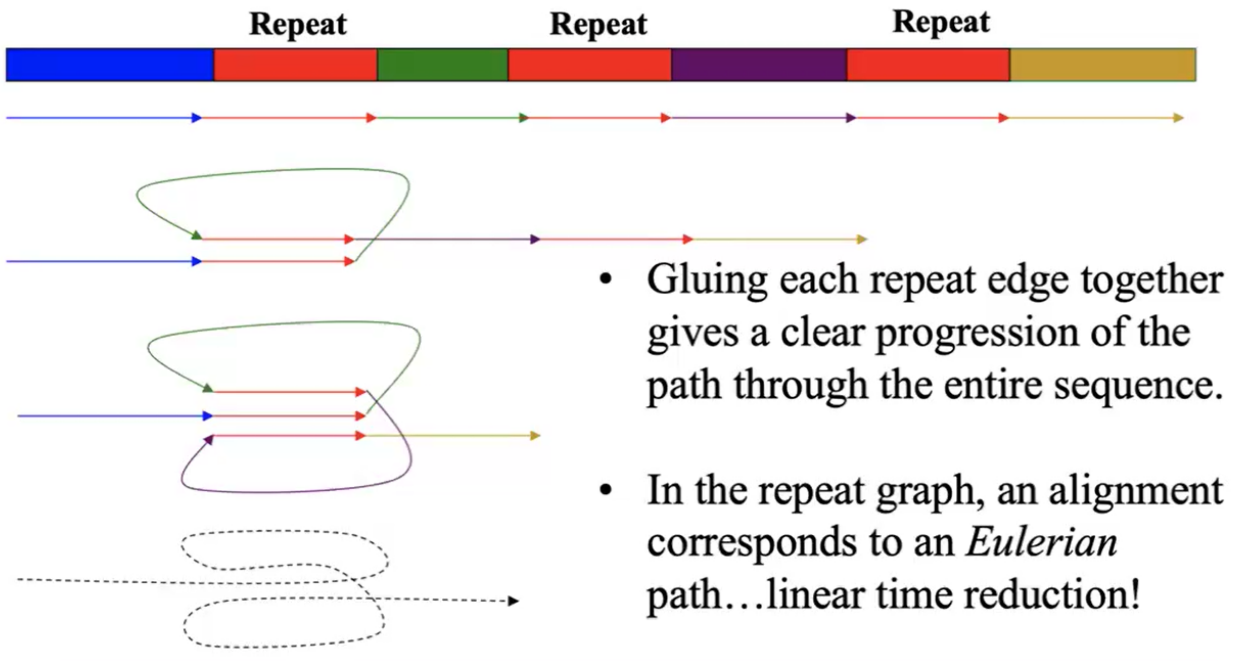


- So finding an alignment corresponds to finding a Hamiltonian path in the overlap graph.
- Recall that the Hamiltonian path/cycle problem is *NP-Complete*: no efficient algorithms are known.

Euler Approach to Fragment Assembly

- The "overlap-layout-consensus" technique implicitly solves the Hamiltonian path problem and has a high rate of mis-assembly.
- Can we adapt the Eulerian Path approach borrowed from the SBH problem?
- Fragment assembly without repeat masking can be done in linear time with greater accuracy.

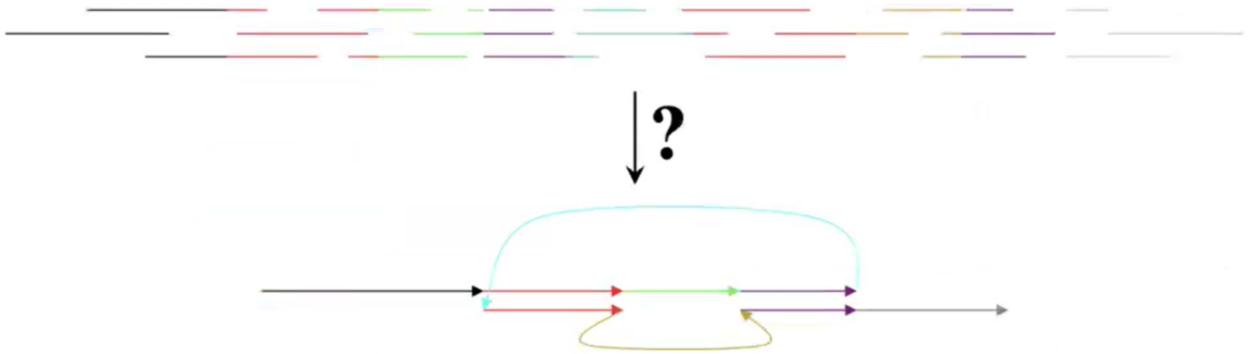
Repeat Graph: Eulerian Approach



- Gluing each repeat edge together gives a clear progression of the path through the entire sequence.
- In the repeat graph, an alignment corresponds to an *Eulerian* path…linear time reduction!

Making Repeat Graph from Reads Only

- **Problem:** In previous slides, we have constructed the repeat graph while *already knowing* the genome structure.
- How do we construct the repeat graph just from fragmets?
- **Solution:** Break the reads into smaller pieces.



Repeat Sequences: Emulating a DNA Chip

- A virtual DNA chip allows one to solve the fragment assembly problem using our SBH algorithm.

Construction of Repeat Graph

- **Construction of Repeat Graph from *k*-mers**: emulates an SBH experiment with a huge (virtual) DNA chip.
- **Breaking reads into *k*-mers**: Transforms sequencing data into virtual DNA chip data.
- Error correction in reads: "Consensus first" approach to fragment assembly.
  - Makes reads (almost) error-free BEFORE the assembly even starts.
- Uses reads and mate-pairs to simplify the repeat graph (Eulerian Superpath Problem.)

Practical Sequence Assembly

- Split reads into kmers
- Error correct kmers based on occurrence threshold
- Construct De Bruijn Graph (Eulerian Graph)
- Find Eulerian Path (or as many long paths as possible)

Project 2 - Sequence Assembly

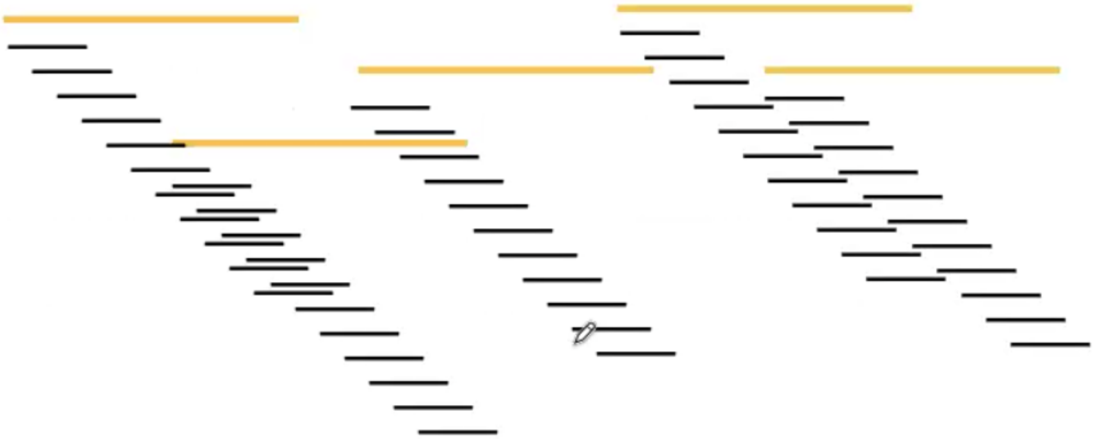
- Given reads - reconstruct the genome using assembly
- Project 2a - Input is "spectrum" of a sequence.
- Project 2b - Input is "reads" of a sequence.
- Output - Sort the "input" in order on the reconstructed sequence.

Practical Genome Assembly

- Convert reads to spectrum
  - Convert reads to kmers
  - Error corretion and copy number count
- Obtain Sequence from Spectrum
  - Generate de Bruijn graph using spectrum
  - Find Eulerian Path and obtain sequence
  - Use original reads to resolve sequence ambiguities

Reads to Kmers

- Break up reads into kmers



Kmer Error Correction and Copy Number

- Assume Coverage is 30x

Kmers	**Count**	**True Count**
ACTAG	29	1
AGGAC	1	0
CGATC	61	2
TCAGA	31	1
TGATC	2	0

- Since coverage is 30x, each kmer that appears in 30 reads would show up in the genome once.
- Kmers that show up once or twice likely result from read errors.

Spectrum to Sequence

- De Bruijn Graph Approach
- Build Graph
- Find Eulerian Paths
- Use reads to "Correct graph/paths" and choose the best path.
  - De Bruijn Graph used only kmers - less information
  - Reads contain how the kmers go together
  - Paired reads contain even more information

Difficulties

- Exponential number of paths through the graph
- Errors in the spectrum/graph
  - No Eulerian Path
  - Many dead ends in graph

  - Unconnected graph

- Read information/paired end sequences