# Comp 555 - BioAlgorithms - Spring 2022

- Assembling genomes from short sequence fragments
- Using graphs to represent sequences
- Graph algorithms

PROBLEM SET #1 IS DUE THURSDAY BEFORE MIDNIGHT.

PROBLEM SET #2 WILL COME OUT THURSDAY.

## Assembling a Genome

# What we know about Genomes

- **DNA sequences are a biological system's hard drive**
  - They contain an operating system with all the low-level support for growing, dividing, and reproducing
  - They contain application programs for making cells that move our bodies, remember our mother's face, and store energy for use in lean times
  - They are robust. They have programs for repairing and replicating themselves. They even have backups!
- **DNA sequences vary in size**
  - Human nuclear DNA is composed of roughly 6 billion base-pairs distributed over 46 pairs of chromosomes
  - These 6 billion bases are comprised of 2 nearly identical copies
  - One of these copies is called a haplotype and its sequence is called a genome
  - Among humans, any two haplotypes are are 99.9% identical
- **How can we read off the sequence of DNA?**

# DNA Sequencing History

- **DNA sequencing was one of the most significant breakthroughs of the 20th century**
- **This was so inherently obvious it was awarded a Nobel prize only 3 years after its development**

## Sanger method (1977):

Uses labeled dideoxynucleotide-triphosphates (ddNTPs) terminate DNA copying at random points.

Fredrick Sanger

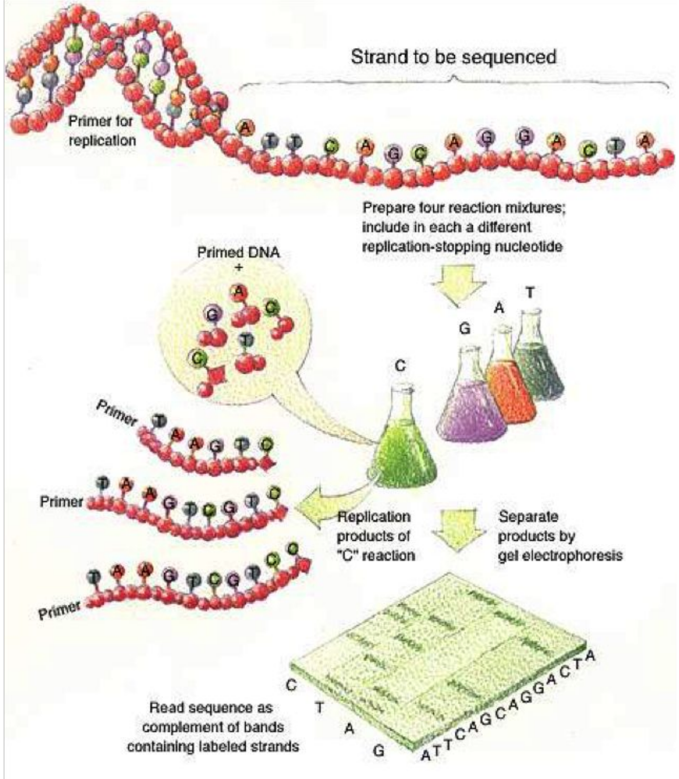
## Gilbert method (1977):

Used various chemicals (Dimethyl Sulfate, Hydrazine) to modify and then cleave DNA at specific points (G, G+A, T+C, C).

Walter Gilbert

# Sanger Method

1. Use the polymerase chain reaction (PCR) to make billions of copies of a DNA sequence
2. Starting at *custom* primer, sort of like our the *origin of replication*, we initiate one last replication
3. Include *chemically altered* and *fluorescently labelled nucleotides*, called dideoxynucleotide-triphosphates (ddNTPs)
4. If a ddNTP gets incorporated into a sequence it stops further replication
5. Separate replication products by length, using gel electrophoresis
6. Good for 500-1000 bases, then the error rates grow and extension rate slows
7. About 10 bases-per-second or 9.5 years to read an entire genome if we could do it from beginning to end


Strand to be sequenced
Primer for replication
Prepare four reaction mixtures; include in each a different replication-stopping nucleotide
Primed DNA
+
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

# Sanger Method

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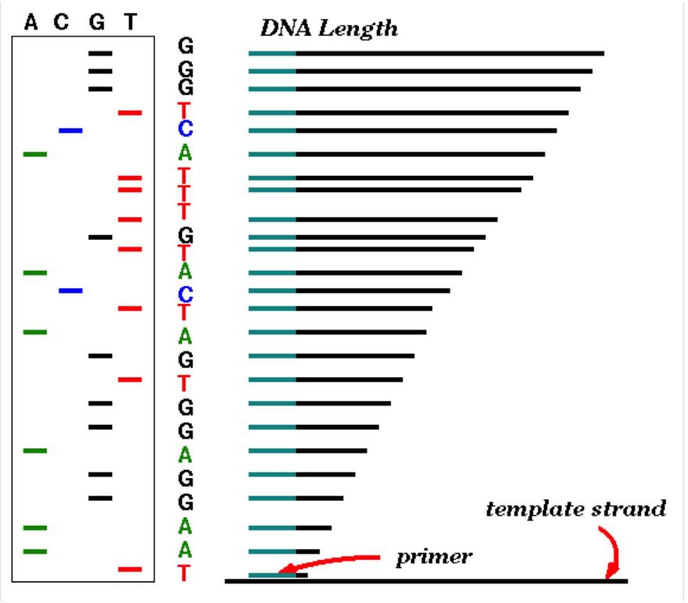


# Assembling the Human Genome

In 1990, a moon-shot-like project was begun to sequence the entire Human Genome.

- It would require 30x coverage to provide enough sequences
- Recall there are sequence differences-- Approximately 1:1000 bases
- Redundancy was needed to find the majority base from 16 different individuals (32 genomes)
- Also needed the extra coverage to assure that there is enough overlap to assemble the 500 base-pair reads

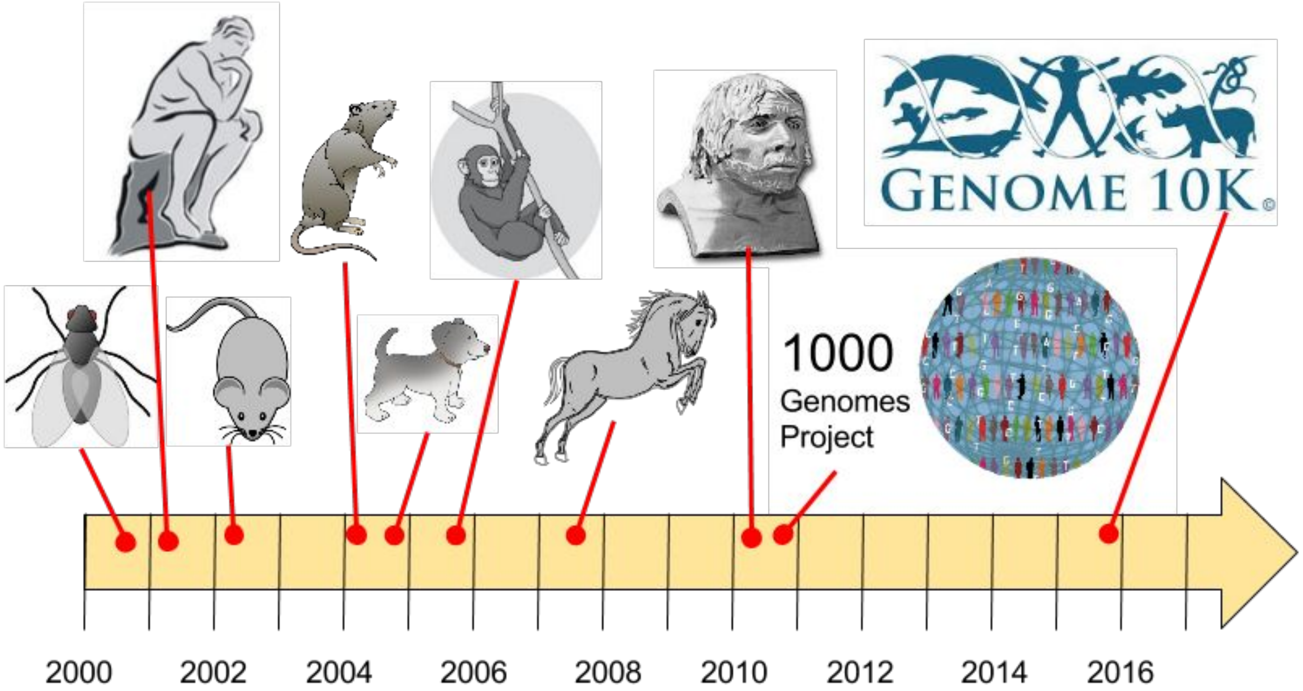
A $3 billion dollar NIH funded public effort led by Francis Collins with a 15-year plan. It would distribute the work across several labs in a community effort by assigning primers to groups on a first-come basis. New sequencing results yielded new primers, so the project required a central coordination.

In 1997 a private company, Celera, lead by Craig Venter, suggested they could beat the public effort by dispensing with primers. They'd just randomly fragment DNA and sequence each with no idea of the how sequenced fragments would fit together. In other words, they were going to rely on computer science to assemble their reads algorithmically.

The result was that, despite tensions, the groups ended up sharing data and technologies.
And the competition led to a completed draft 5 years ahead of schedule.

# The Sequencing Race

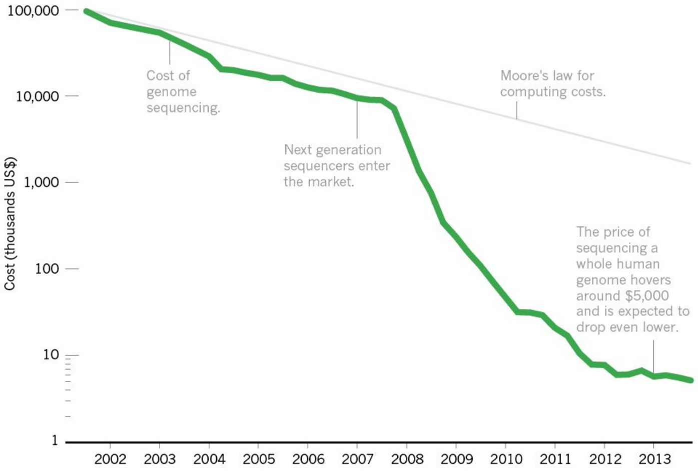
Since the Human Genome project there have been an explosion of genomes sequenced. Initially, the focus was on model organisms, then favorites, then all of human diversity, and finally a catalog of life's diversity.



# The secret behind this explosion of genomes

Next generation sequencing machines have revolutionized the DNA sequencing process. They work in various ways including massively-parallel single-base extension methods, to captured Dnases whose motions suggest a the base being replicated, to microholes that only a single DNA molecule can pass through, and the bases are determined by detectable charge differences.

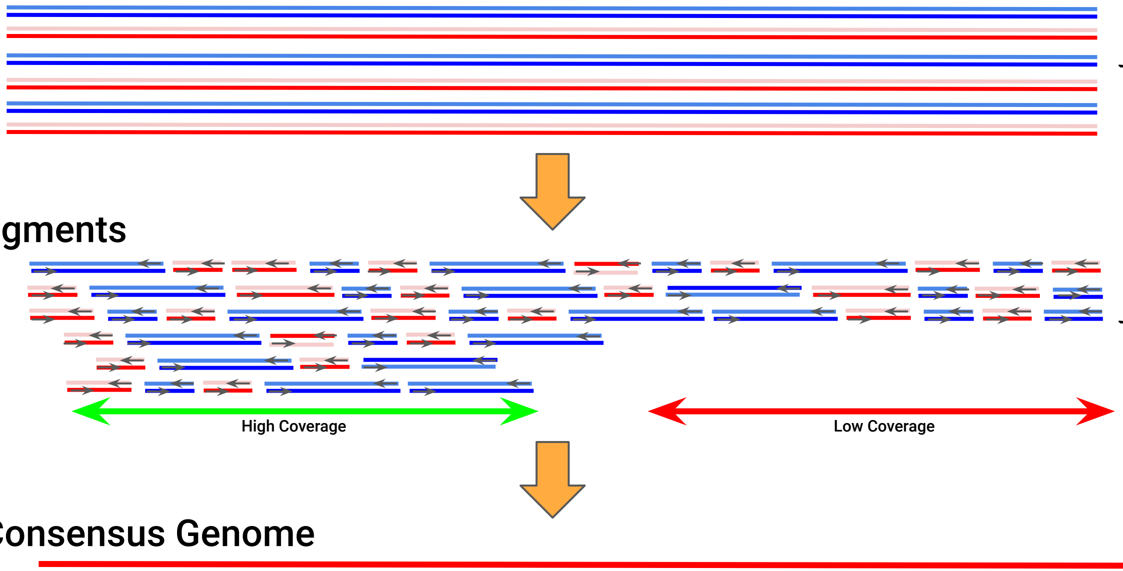
In a way, the *genome moonshot* was far more successful than the real moonshot. The rate at which genomes can be sequenced, and the cost per base has seen unprecedented improvements. Faster than even Moore's Law.



Falling fast

In the first few years after the end of the Human Genome Project, the cost of genome sequencing roughly followed Moore's law, which predicts exponential declines in computing costs. After 2007, sequencing costs dropped precipitously.

# How does it all work?



Many DNA molecules from an organism

Fragments

Consensus Genome

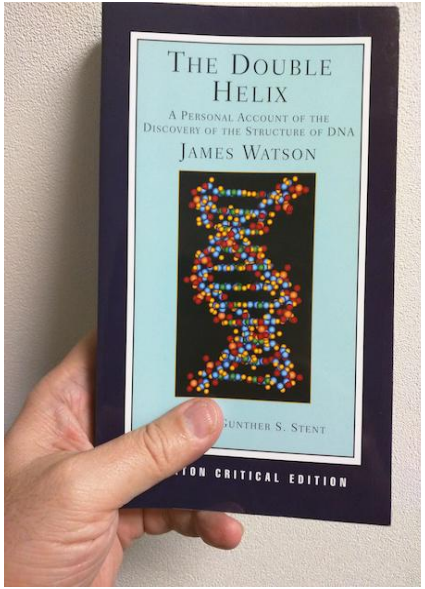
There are actually four sequences that contribute. Two diploid chromosomes, each with a forward and reverse strand

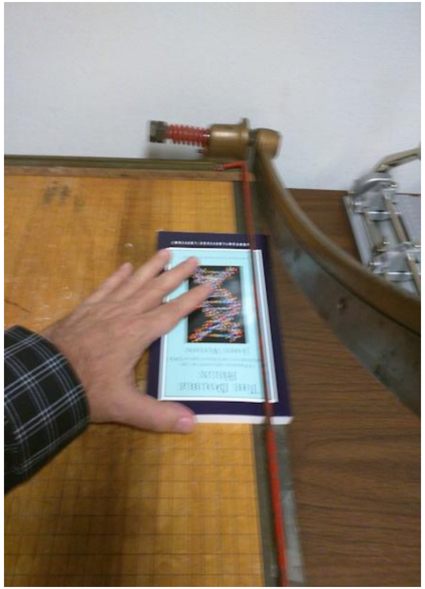
DNA is broken into randomly sized fragments from which uniform sized reads are sequenced.

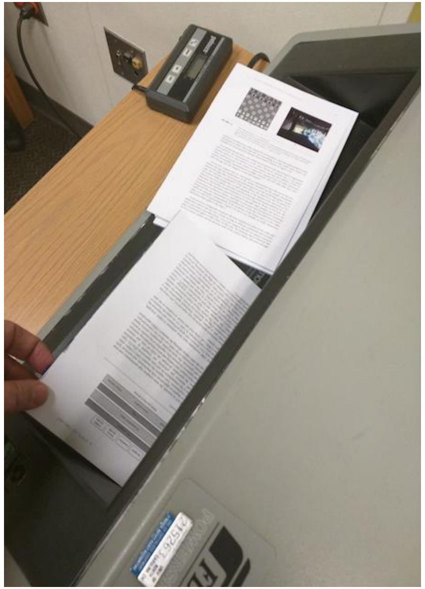
Reads from unknown chromosomes and unknown strands are assembled into a genome sequence

It is as if we must first smash a grecian urn in order to completely see it.

# An Analogy





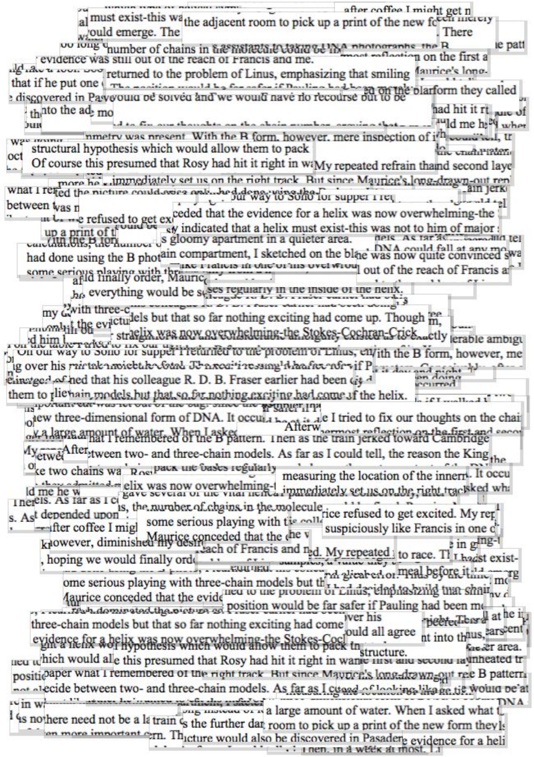


Some important differences

- A better analogy would have been to shred 100's of books
- Shuffle the pages before shredding
- Oh yeah, my book has approximately 850,000 characters.
- The entirety of Encyclopedia Britannica is approximately 250,000,000 characters.
- Your genome is approximately 12 times larger

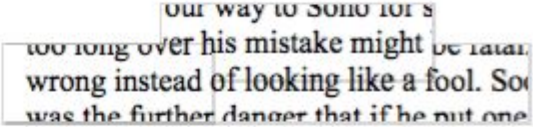
# How would you Reassemble our Book?

**Each paper shred is like a DNA fragment, or read.**



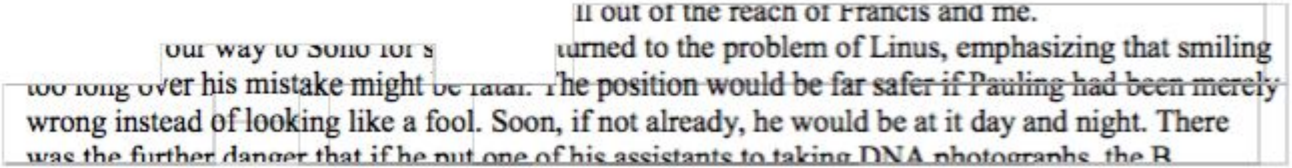
# Searching for overlaps

You'd look for fragments that fit together based on some overlapping context that they share.



our way to Soho for s
too long over his mistake might be fatal.
wrong instead of looking like a fool. Soo
was the further danger that if he put one

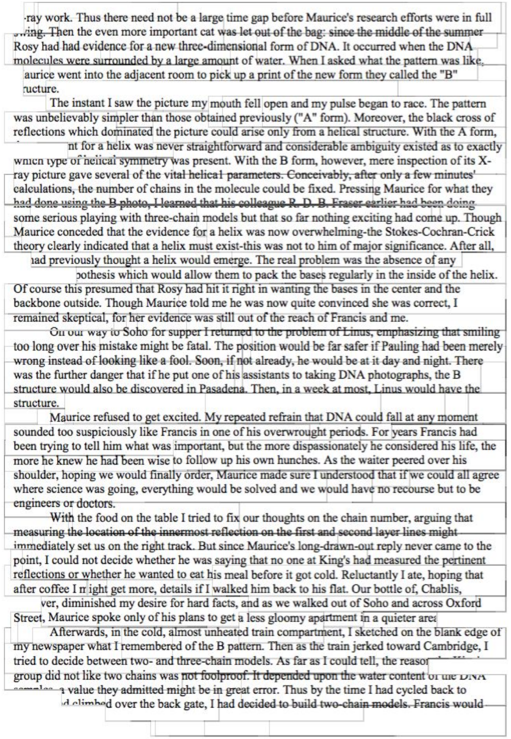
And then, build upon those to assemble a more complete picture.



ll out of the reach of Francis and me.
our way to Soho for s turned to the problem of Linus, emphasizing that smiling
too long over his mistake might be fatal. The position would be far safer if Pauling had been merely
wrong instead of looking like a fool. Soon, if not already, he would be at it day and night. There
was the further danger that if he put one of his assistants to taking DNA photographs, the B

# Finally you assemble a *nearly* complete version

- How can we code such an approach?
- What is *overlapping context* in our DNA fragments?
- How would we represent and manage these overlaps?



-ray work. Thus there need not be a large time gap before Maurice's research efforts were in full swing. Then the even more important cat was let out of the bag: since the middle of the summer Rosy had had evidence for a new three-dimensional form of DNA. It occurred when the DNA molecules were surrounded by a large amount of water. When I asked what the pattern was like, aurice went into the adjacent room to pick up a print of the new form they called the "B" ructure.

The instant I saw the picture my mouth fell open and my pulse began to race. The pattern was unbelievably simpler than those obtained previously ("A" form). Moreover, the black cross of reflections which dominated the picture could arise only from a helical structure. With the A form, nt for a helix was never straightforward and considerable ambiguity existed as to exactly which type of helical symmetry was present. With the B form, however, mere inspection of its X-ray picture gave several of the vital helica1 parameters. Conceivably, after only a few minutes' calculations, the number of chains in the molecule could be fixed. Pressing Maurice for what they had done using the B photo, I learned that his colleague R. D. B. Fraser earlier had been doing some serious playing with three-chain models but that so far nothing exciting had come up. Though Maurice conceded that the evidence for a helix was now overwhelming-the Stokes-Cochran-Crick theory clearly indicated that a helix must exist-this was not to him of major significance. After all, ıad previously thought a helix would emerge. The real problem was the absence of any pothesis which would allow them to pack the bases regularly in the inside of the helix. Of course this presumed that Rosy had hit it right in wanting the bases in the center and the backbone outside. Though Maurice told me he was now quite convinced she was correct, I remained skeptical, for her evidence was still out of the reach of Francis and me.

On our way to Soho for supper I returned to the problem of Linus, emphasizing that smiling too long over his mistake might be fatal. The position would be far safer if Pauling had been merely wrong instead of looking like a fool. Soon, if not already, he would be at it day and night. There was the further danger that if he put one of his assistants to taking DNA photographs, the B structure would also be discovered in Pasadena. Then, in a week at most, Linus would have the structure.

Maurice refused to get excited. My repeated refrain that DNA could fall at any moment sounded too suspiciously like Francis in one of his overwrought periods. For years Francis had been trying to tell him what was important, but the more dispassionately he considered his life, the more he knew he had been wise to follow up his own hunches. As the waiter peered over his shoulder, hoping we would finally order, Maurice made sure I understood that if we could all agree where science was going, everything would be solved and we would have no recourse but to be engineers or doctors.

With the food on the table I tried to fix our thoughts on the chain number, arguing that measuring the location of the innermost reflection on the first and second layer lines might immediately set us on the right track. But since Maurice's long-drawn-out reply never came to the point, I could not decide whether he was saying that no one at King's had measured the pertinent reflections or whether he wanted to eat his meal before it got cold. Reluctantly I ate, hoping that after coffee I m ight get more, details if I walked him back to his flat. Our bottle of, Chablis, ver, diminished my desire for hard facts, and as we walked out of Soho and across Oxford Street, Maurice spoke only of his plans to get a less gloomy apartment in a quieter area

Afterwards, in the cold, almost unheated train compartment, I sketched on the blank edge of my newspaper what I remembered of the B pattern. Then as the train jerked toward Cambridge, I tried to decide between two- and three-chain models. As far as I could tell, the reason the King's group did not like two chains was not foolproof. It depended upon the water content of the DNA samples, a value they admitted might be in great error. Thus by the time I had cycled back to d climbed over the back gate, I had decided to build two-chain models. Francis would

# Key idea: Finding links between read pairs

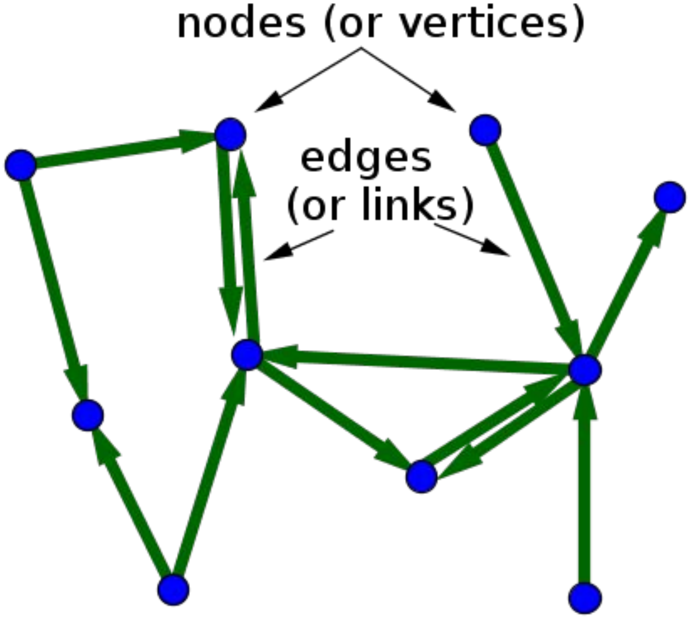
This leads us to a data representation called a graph

A graph is composed of nodes, which can represent entities, in our case read fragments. Nodes are connected by edges that represent some relationship between a pair of nodes, in our case how much of an ***overlap*** is shared between the nodes.

The edges of a graph can be directed.

Objectives: 1) include all nodes
2) prefer edges with large overlaps

Leads to a related graph problem…
2') Uniform large overlaps



# De Bruijn's Problem and his Graphs

**Nicolaas de Bruijn**
**(1918-2012)**

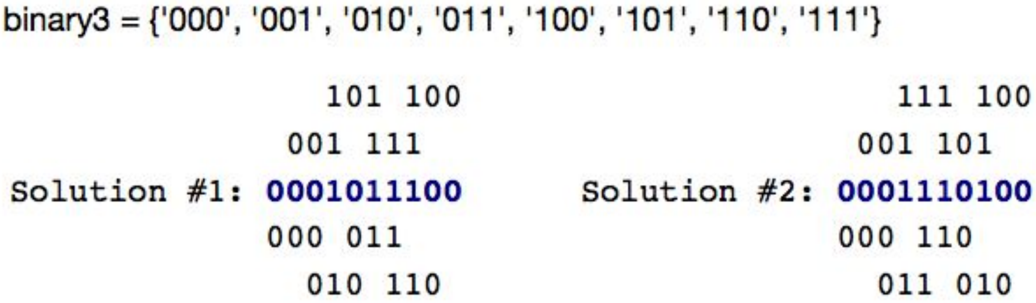
A dutch mathematician noted for his many contributions in the fields of graph theory, number theory, combinatorics and logic.

## Minimal Superstring Problem:

Large fragment overlaps imply a shorter sequence.

Find the *shortest sequence* that contains all $|\Sigma|^k$ strings of length k from the alphabet Σ as a substring.

Example: All strings of length 3 from the alphabet {'0','1'}.



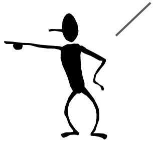
```
binary3 = {'000', '001', '010', '011', '100', '101', '110', '111'}

                  101 100                              111 100
                001 111                              001 101
Solution #1:  0001011100          Solution #2:  0001110100
              000 011                           000 110
                010 110                           011 010
```

He solved this problem by mapping it to a graph. Note, this particular problem leads to cyclic sequence.

# Construct a "graph" of a sequence

For the moment let's imagine that reads are like k-mers from a sequence, as they do tend to be uniform in length.

```
GACGGCGGCGCACGGCGCAA    - Our toy sequence
GACGG
 ACGGC
  CGGCG
   GGCGG
    GCGGC
     CGGCG
      GGCGC             - The complete set of 16 5-mers, 11 unique
       GCGCA
        CGCAC
         GCACGG
          CACGG
           ACGGC
            CGGCG
             GGCGC
              GCGCA
               CGCAA
```



For the moment we'll pretend that we can tell these three repeated k-mers apart.

Now we can construct a graph where:

1. Each 5-mer is a node
2. There is a directed edge from a k-mer that shares its *(k-1)-base suffix* with the *(k-1)-base prefix* of another

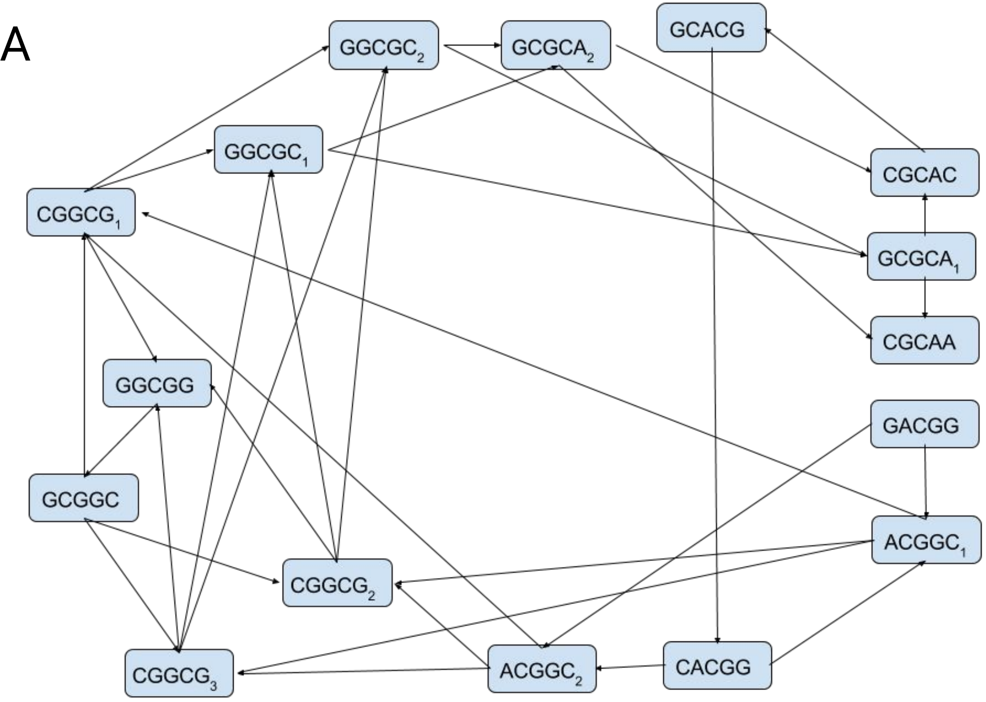
# A read-overlap graph

The read-overlap graph for the 5-mers from:

GACGGCGGCGCACGGCGCAA

The problem is:

***How to infer the original sequence From this graph?***



Our original sequence is just a path in this graph. How would you find it?

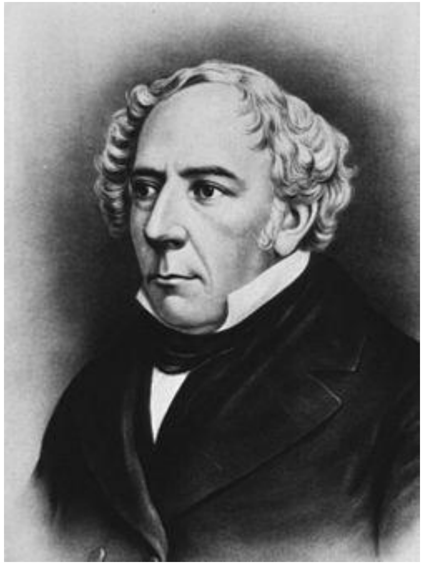
# Parlor games

Once finding paths in graphs was a popular form of entertainment…

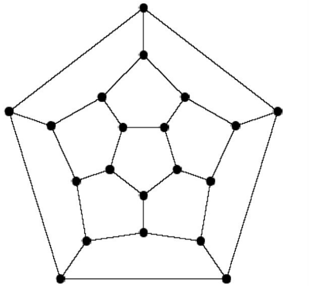
Graphs would be printed in newspapers, and people would try to find paths in them as a game.

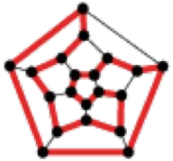
## The rules of our game

- Every node, k-mer, can be used exactly once
- The object is to find a path along edges that visits every node one time
- This game was invented in the mid 1800's by a mathematician called ***Sir William Hamilton***



An example of Hamilton's game:





# Finding a Hamiltonian Path in our graph

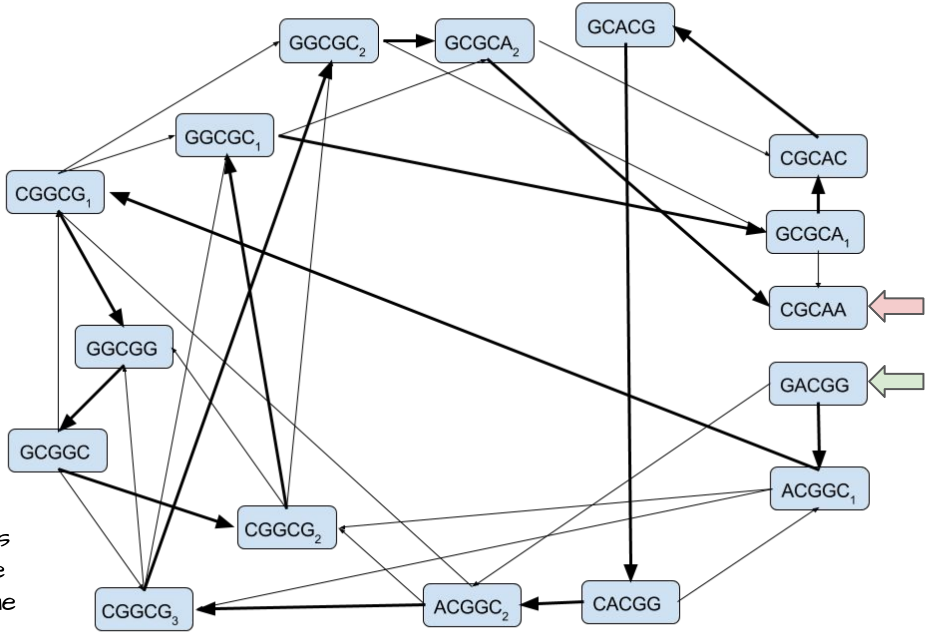
For our desired sequence:

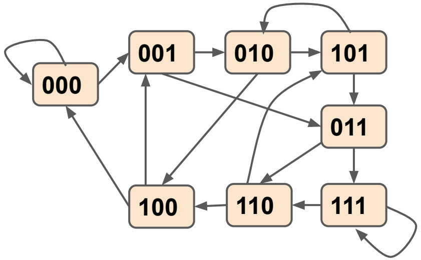
GACGGCGGCGCACGGCGCAA

is indeed a path in this graph.

How would you write a program
To solve Hamilton's puzzles?

Is the solution unique?





de Bruijn knew this was
a hard problem. But, he
also knew another game
he could play.

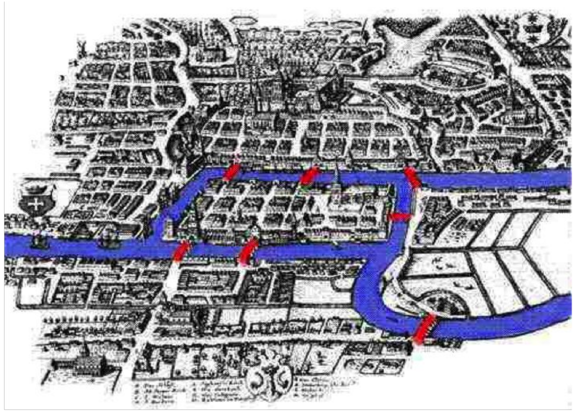
# Euler's Tour

## The rules of a new game: A tour of bridges

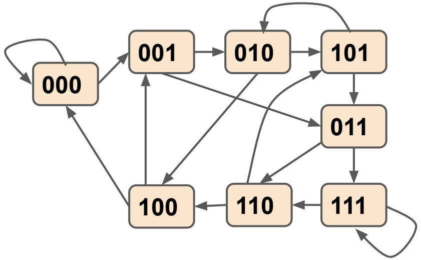
- Every *edge*, k-mer, can be used exactly once
- The object is to find a path in the graph that uses each *edge* only one time
- This game was invented in the late 1700's by a mathematician called Leonhard Euler

Leonhard Euler

A version of Euler's game:



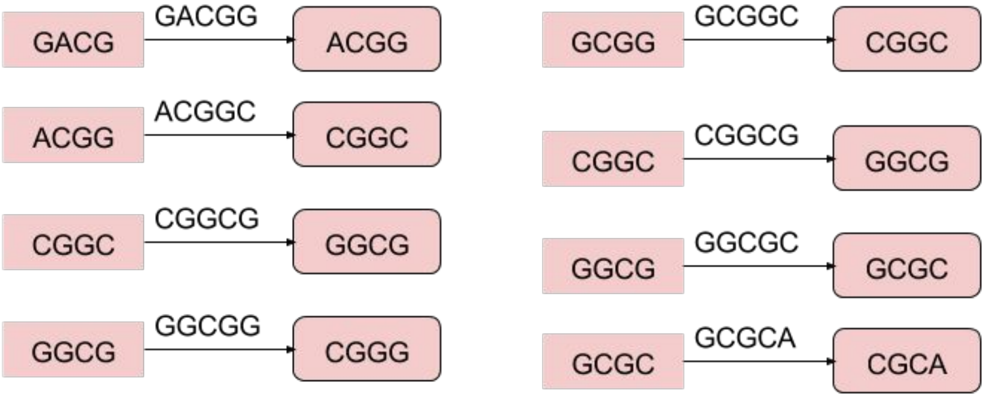
Bridges of Königsberg: Find a city tour that crosses every bridge just once



How can I make my "k-mers" into edges rather than nodes?

# Another representation of k-mers in a graph

- Rather than making each k-mer a node, let's try making them an edge
- That seems odd, but it is related to the overlap idea
  - The 5-mer GACGG has a prefix GACG and a suffix ACGG
  - Think of the k-mer as the edge connecting a prefix node to a suffix node
  - This leads to a series of simple graphs



GACG → ACGG: GACGG

ACGG → CGGC: ACGGC

CGGC → GGCG: CGGCG

GGCG → CGGG: GGCGG

GCGG → CGGC: GCGGC

CGGC → GGCG: CGGCG

GGCG → GCGC: GGCGC

GCGC → CGCA: GCGCA

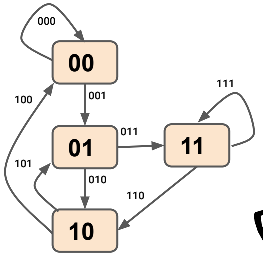
  - Then combine all nodes with the same label

# A De Bruijn Graph

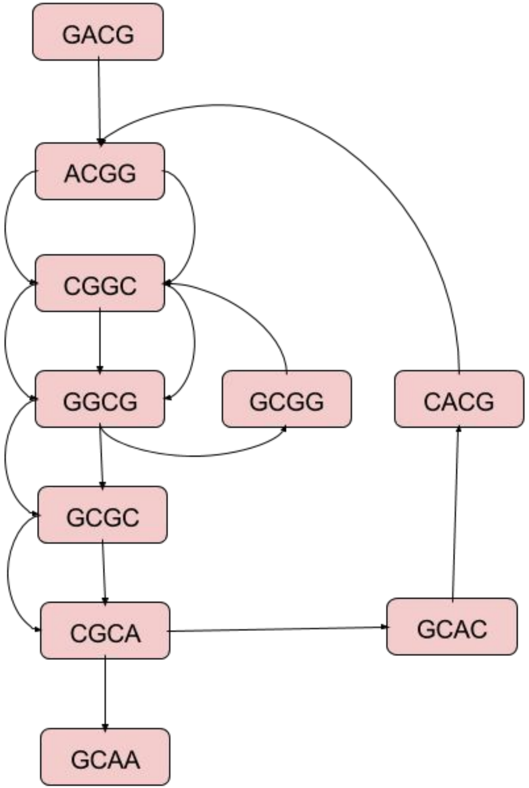
This graph, like the previous one has the property that edges connect nodes where a k-1 suffix matches a k-1 prefix. Graphs of this type are called "De Bruijn" graphs, after our famous mathematician.

Recall that our original 16 5-mers are edges in this graph, whereas they were nodes in the previous one.

Now, how might you infer the original sequence using this graph?



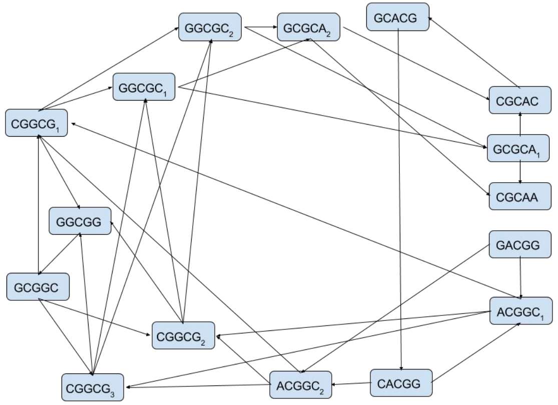
Some of those bridges leave and come back to the same island.



# Two graphs, same problem

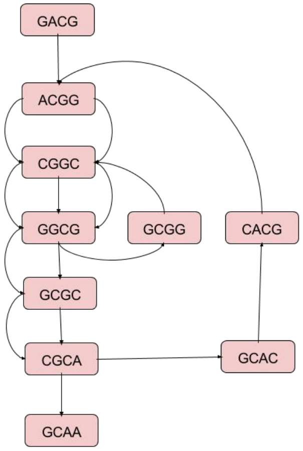
**Two graphs representing 5-mers from the sequence "GACGGCGGCGCACGGCGCAA"**

**Hamiltonian Path:**



Each k-mer is a vertex. Find a path that passes through every *vertex* of this graph exactly once.

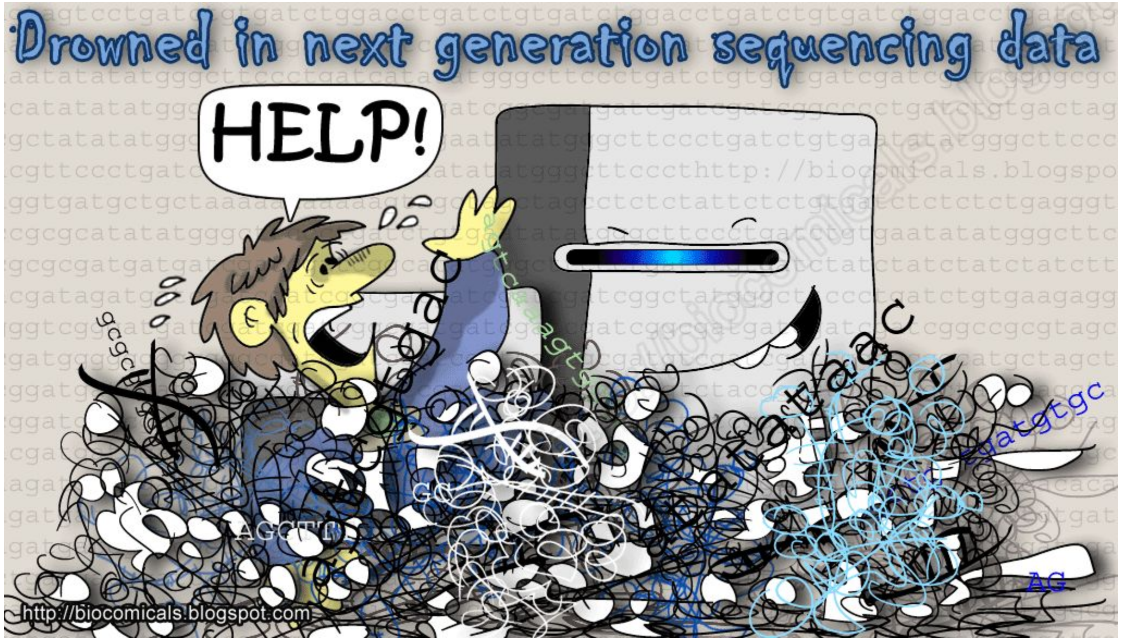
**Eulerian Path:**



Each k-mer is an edge. Find a path that passes through every *edge* of this graph exactly once.

# Next Time

- Code to solve our graph problems
- Code that is simple
- Code that is fast


Drowned in next generation sequencing data
HELP!
http://biocomicals.blogspot.com