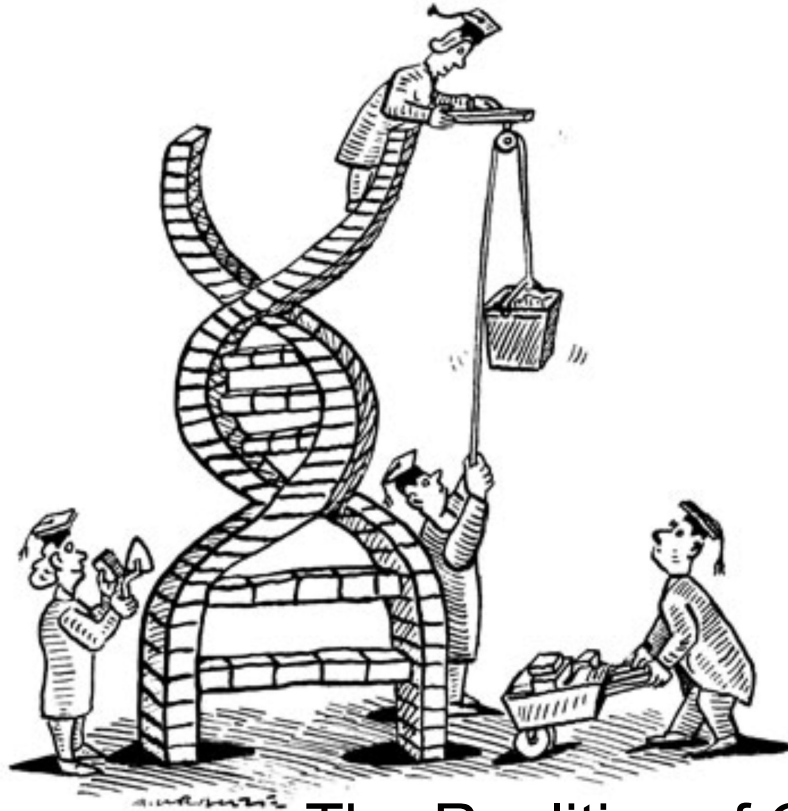
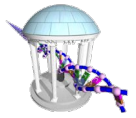


Comp 555 - BioAlgorithms - Spring 2020



- **PROBLEM SET #2
IS ON-LINE**

The Realities of Genome Assembly

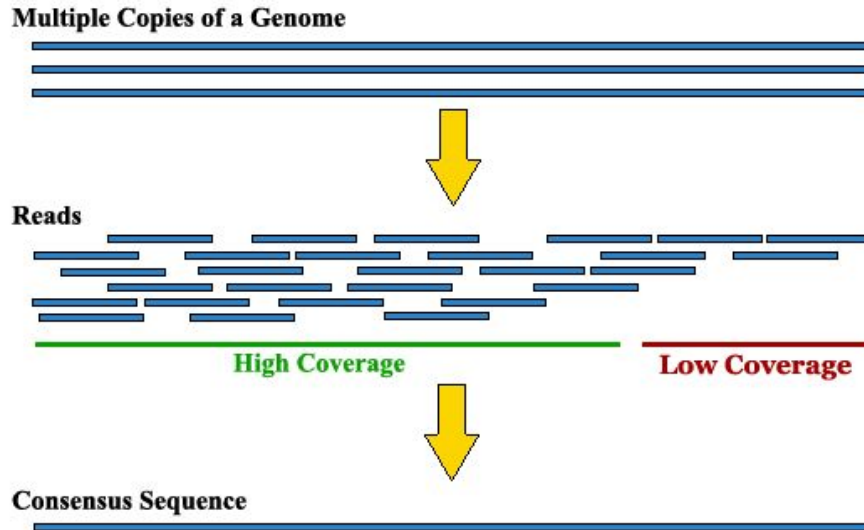
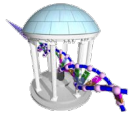
From Last Time



What we learned from a related "Minimal Superstring" problem

- Can be constructed by finding a Hamiltonian path of an k -dimensional De Bruijn graph over σ symbols
 - Brute-force method explores all $V!$ paths through V vertices
 - Branch-and-Bound method considers only paths composed of edges in the graph
 - Finding a Hamiltonian path is an NP-complete problem
 - There is no known method that can solve it efficiently as the number of vertices grows
- Can be solved by finding a Eulerian path of a $(k-1)$ -dimensional De Bruijn graph where k -mers are edges.
 - Euler's method finds a path using all edges in $O(E) \leq O(V^2)$ steps
 - Graph must satisfy constraints to be sure that a solution exists
- All but two vertices must be balanced
- The other two must be semi-balanced

Applications to Assembling Genomes



- Extracted DNA is broken into random small fragments
- 100-200 bases are read from one or both ends of the fragment
- Typically, each base of the genome is covered by 10x - 30x fragments

Genome Assembly vs Minimal Superstring

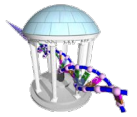


binary3 = {'000', '001', '010', '011', '100', '101', '110', '111'}

101 100
001 111
Solution #1: **0001011100**
000 011
010 110

111 100
001 101
Solution #2: **0001110100**
000 110
011 010

- Minimal substring problem
 - Every k-mer is present and used as a vertex, (all σ^k)
 - Paths, and there may be multiple, are solutions
- Read fragments
 - No guarantee that we will ever see every k-mer
 - Can't disambiguate repeats



Recall our “Toy” example

GACGGCGGGCGCACGGCGCAA - Our toy 20 base sequence from 2 lectures ago
GACGG CGCAC
ACGGC GCACG
CGGCG CACGG - The complete set of 16 5-mers
GGCGG ACGGC
GCGGC CGGCG
CGGCG GGCGC
GGCGC GCGCA
GGCGA CGCAA

Issues:

- All k -mers is equivalent to $k \times$ coverage, ignoring boundaries
- Four repeated k-mers {ACGGC, CGGCG, GCGCA, GGCGC}

Some Code



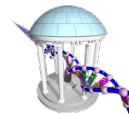
First let's add a function to uniquely label repeated k-mers

```
In [4]: def kmersUnique(seq, k):
    kmers = sorted([seq[i:i+k] for i in range(len(seq)-k+1)])
    for i in range(1,len(kmers)):
        if (kmers[i] == kmers[i-1][0:k]):
            t = kmers[i-1].find('_')
            if (t >= 0):
                n = int(kmers[i-1][t+1:]) + 1
                kmers[i] = kmers[i] + "_" + str(n)
            else:
                kmers[i-1] = kmers[i-1] + "_1"
                kmers[i] = kmers[i] + "_2"
    return kmers

kmers = kmersUnique("GACGGCGGCGCACGGCGCAA", 5)
print(kmers)

['ACGGC_1', 'ACGGC_2', 'CACGG', 'CGCAA', 'CGCAC', 'CGGCG_1', 'CGGCG_2', 'CGGCG_3', 'GACGG', 'GCACG', 'GCGCA_1', 'GCGCA_2',
'GCGGC', 'GGCGC_1', 'GGCGC_2', 'GGCGG']
```

Our Graph class from last lecture



In [25]:

```
import itertools

class Graph:
    def __init__(self, vlist=[
        """ Initialize a Graph
        self.index = {v:i for
        self.vertex = {i:v for
        self.edge = []
        self.edglabel = []
    def addVertex(self, label)
        """ Add a labeled vert
        index = len(self.index
        self.index[label] = in
        self.vertex[index] = v
    def addEdge(self, vsrc, vd
        """ Add a directed edg
        Repeated edges are dis
        e = (self.index[vsrc],
        if (repeats or (e not
            self.edge.append(e
            self.edglabel.app
    def hamiltonianPath(self):
        """ A Brute-force meth
        Basically, all possibl
        for edges. Since edges
        made for *which* versi
        for path in itertools.
            for i in xrange(le
                if ((path[i],p
                    break
            else:
                return [self.v
        return []
    def SearchTree(self, path,
        """ A recursive Branch
        Paths are extended one
        edges from the graph.
        if (len(verticesLeft)
            self.PathV2result
            return True
        for v in verticesLeft:
            if (len(path) == 0
                if self.Search
                    return Tru
        return False
```

```
def hamiltonianPathV2(s
    """ A wrapper funct
    Hamiltonian Path se
    self.PathV2result =
    self.SearchTree([],
    return self.PathV2r

def degrees(self):
    """ Returns two dic
    of each node from t
    inDegree = {}
    outDegree = {}
    for src, dst in sel
        outDegree[src] =
        inDegree[dst] =
    return inDegree, ou

def verifyAndGetStart(s
    inDegree, outDegree
    start = 0
    end = 0
    for vert in self.ve
        ins = inDegree.
        outs = outDegre
        if (ins == outs
            continue
        elif (ins < out
            end = vert
        elif (outs < in
            start = ver
        else:
            start, end
            break
    if (start >= 0) and
        return start
    else:
        return -1

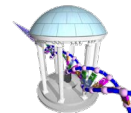
def eulerEdges(self, pa
    edgeId = {}
    for i in xrange(len
        edgeId[self.edg
    edgeList = []
    for i in xrange(len
        edgeList.append
    return edgeList
```

```
def eulerianPath(self):
    graph = [(src,dst) for src,dst in
    currentVertex = self.verifyAndGet
    path = [currentVertex]
    # "next" is where vertices get in
    # it starts at the end (i.e. it i
    # but later "side-trips" will ins
    next = 1
    while len(graph) > 0:
        for edge in graph:
            if (edge[0] == currentVer
                currentVertex = edge[
                graph.remove(edge)
                path.insert(next, cur
                next += 1
                break
            else:
                for edge in graph:
                    try:
                        next = path.index
                        currentVertex = e
                        break
                    except ValueError:
                        continue
                else:
                    print "There is no pa
                    return False
    return path

def render(self, highlightPath=[]):
    """ Outputs a version of the grap
    using graphviz tools (http://www.
    edgeId = {}
    for i in xrange(len(self.edge)):
        edgeId[self.edge[i]] = edgeId
    edgeSet = set()
    for i in xrange(len(highlightPath
        src = self.index[highlightPat
        dst = self.index[highlightPat
        edgeSet.add(edgeId[src,dst].p
    result = ''
    result += 'digraph {\n'
    result += '    graph [nodesep=2, s
    for index, label in self.vertex.i
        result += '        N%d [shape="bc
```

```
for i, e in enumerate(self.edge):
    src, dst = e
    result += '    N%d -> N%d' % (src, dst)
    label = self.edglabel[i]
    if (len(label) > 0):
        if (i in edgeSet):
            result += ' [label="%s", penwidth=3.0]' % (label)
        else:
            result += ' [label="%s"]' % (label)
    elif (i in edgeSet):
        result += ' [penwidth=3.0]'
        result += ';\n'
        result += '    overlap=false;\n'
        result += '};\n'
    return result
```

Finding Paths in our K-mer De Bruijn Graphs



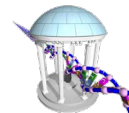
```
In [8]: ▶ k = 5
target = "GACGGCGGCGCACGGCGCAA"
kmers = kmersUnique(target, k)
G1 = Graph(kmers)
for vsrc in kmers:
    for vdst in kmers:
        if (vsrc[1:k] == vdst[0:k-1]):
            G1.addEdge(vsrc,vdst)
path = G1.hamiltonianPathV2()

print(path)
seq = path[0][0:k]
for kmer in path[1:]:
    seq += kmer[k-1]
print(seq)
print(seq == target)
```

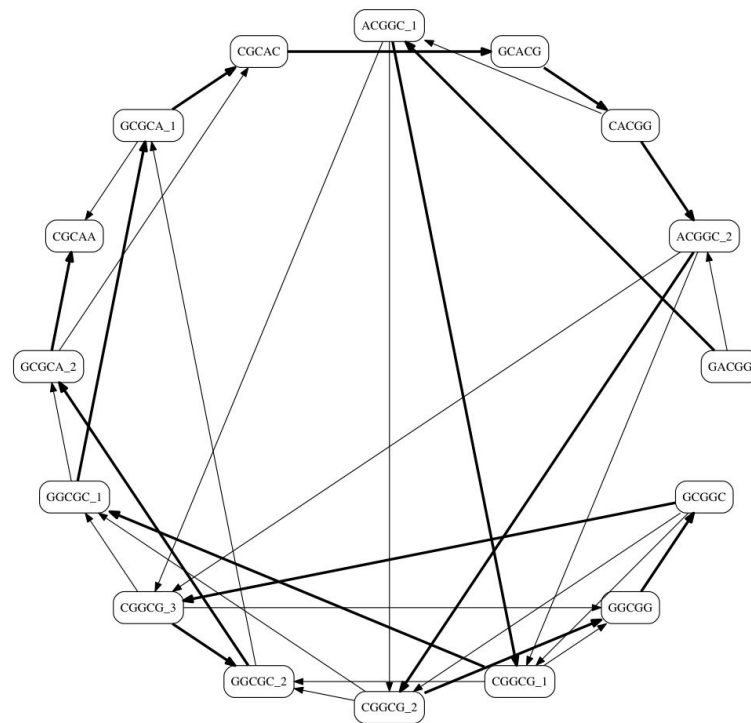
```
['GACGG', 'ACGGC_1', 'CGGCG_1', 'GGCGC_1', 'GCGCA_1', 'CGCAC', 'GCACG', 'CACGG', 'ACGGC_2', 'CGGCG_2', 'GGCGG', 'GCGGC', 'CGGCG_3', 'GGCGC_2', 'GCGCA_2', 'CGCAA']
GACGGCGCACGGCGGCGCAA
False
```

Not the sequence we expected ...

Let's look at the resulting graphs

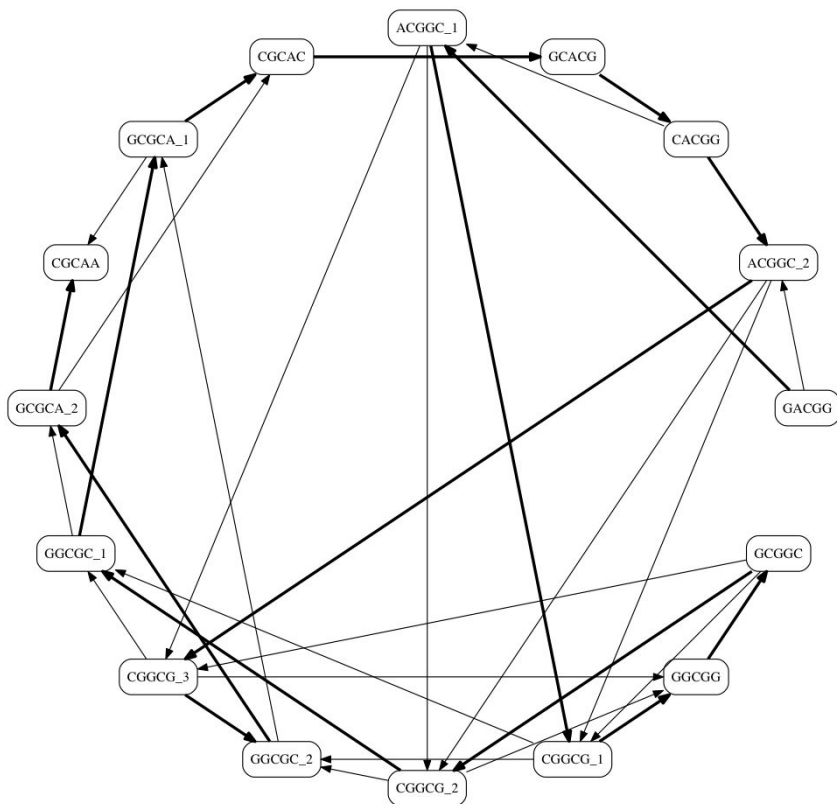


The one we hoped for. Visits $CGGCG_3$ before $CGGCG_2$

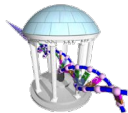


The one we found visits $CGGCG_2$ before $CGGCG_3$

What's the Problem?



- There are many possible Hamiltonian Paths
- How do they differ?
 - There were two possible paths leaving any [CGGCG] node
 - [CGGCG] → [GGCGC]
 - [CGGCG] → [GGCGG]
 - A valid solution can be found down either path
- There might be even more solutions
- Genome assembly appears ambiguous like the Minimal Substring problem, but is it?



How about an Euler Path?

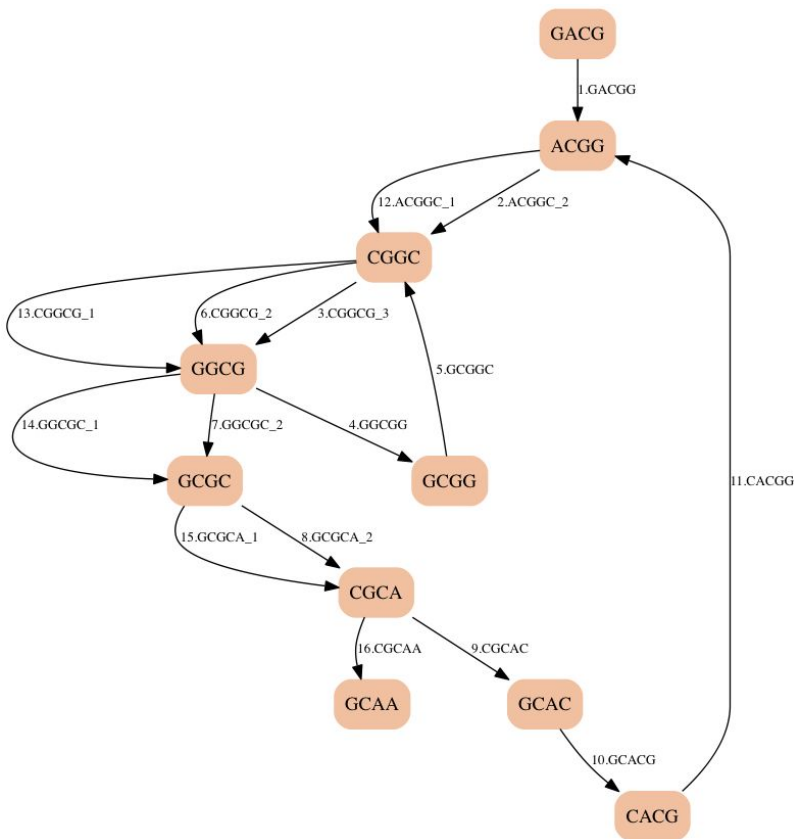
```
In [20]: ▶ k = 5
target = "GACGGCGGCGCACGGCGCAA"
kmers = kmersUnique(target, k)
print(kmers)

nodes = sorted(set([code[:k-1] for code in kmers] + [code[1:k] for code in kmers]))
print(nodes)
G2 = Graph(nodes)
for code in kmers:
    G2.addEdge(code[:k-1],code[1:k],code)
path = G2.eulerianPath()
print(path)
path = G2.eulerEdges(path)
print(path)

seq = path[0][0:k]
for kmer in path[1:]:
    seq += kmer[k-1]
print(seq)
print(seq == target)

['ACGGC_1', 'ACGGC_2', 'CACGG', 'CGCAA', 'CGCAC', 'CGGCG_1', 'CGGCG_2', 'CGGCG_3', 'GACGG', 'GCACG', 'GCGCA_1', 'GCGCA_2',
'GCGGC', 'GGCGC_1', 'GGCGC_2', 'GGCGG']
['ACGG', 'CACG', 'CGCA', 'CGGC', 'GACG', 'GCAA', 'GCAC', 'GCGC', 'GCGG', 'GGCG']
[4, 0, 3, 9, 8, 3, 9, 7, 2, 6, 1, 0, 3, 9, 7, 2, 5]
['GACGG', 'ACGGC_2', 'CGGCG_3', 'GGCGG', 'GCGGC', 'CGGCG_2', 'GGCGC_2', 'GCGCA_2', 'CGCAC', 'GCACG', 'CACGG', 'ACGGC_1', 'CG
GCG_1', 'GGCGC_1', 'GCGCA_1', 'CGCAA']
GACGGCGGCGCACGGCGCAA
True
```

The k-1 De Bruijn Graph with k-mer edges

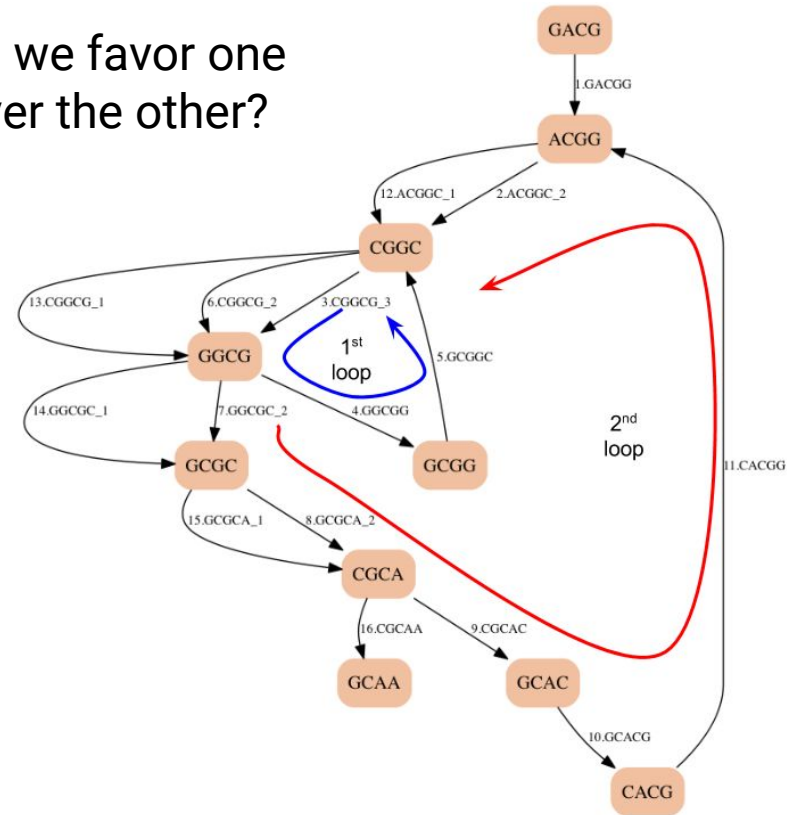
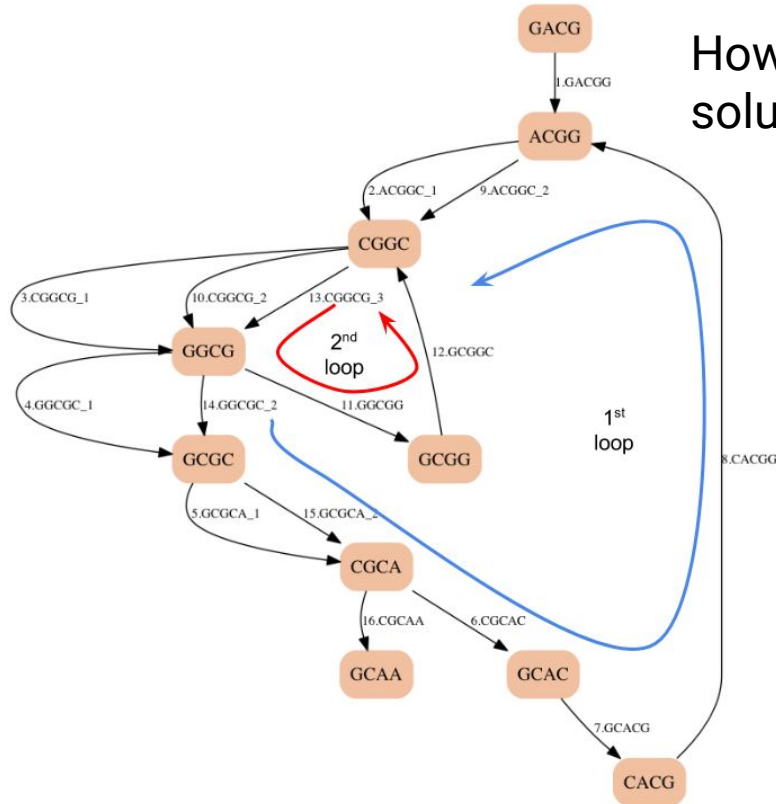


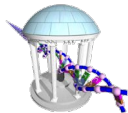
- We got the right answer, but we were lucky.
- There is a path in this graph that matches the Hamiltonian path that we found before



What are the Differences?

How might we favor one solution over the other?





Choose a bigger k-mer

```
In [22]: ▶ k = 8
target = "GACGGCGGCGCACGGCGCAA"
kmers = kmersUnique(target, k)
print(kmers)
nodes = sorted(set([code[:k-1] for code in kmers] + [code[1:k] for code in kmers]))
print(nodes)
G3 = Graph(nodes)
for code in kmers:
    G3.addEdge(code[:k-1],code[1:k],code)
path = G3.eulerianPath()
print(path)
path = G3.eulerEdges(path)
print(path)

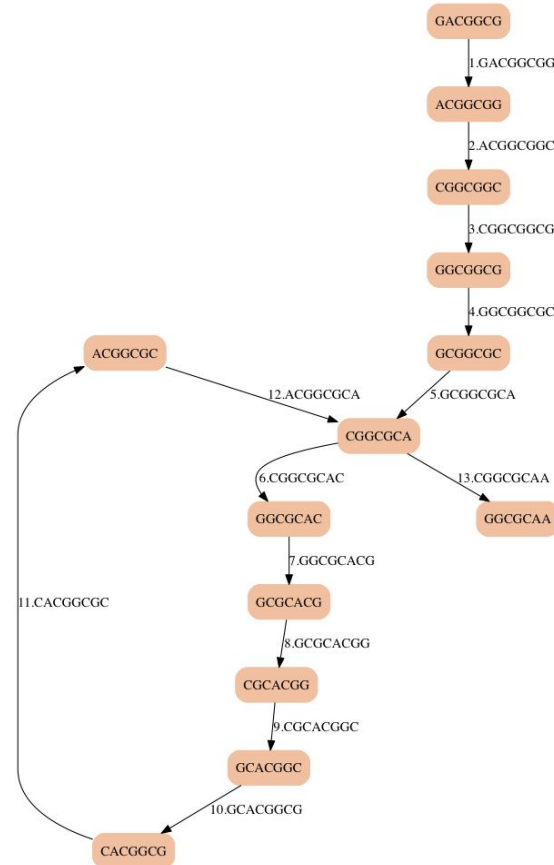
seq = path[0][0:k]
for kmer in path[1:]:
    seq += kmer[k-1]
print(seq)
print(seq == target)

['ACGGCGCA', 'ACGGCGGC', 'CACGGCGC', 'CGCACGGC', 'CGGCGCAA', 'CGGCGCAC', 'CGGCGGCG', 'GACGGCGG', 'GCACGGCG', 'GCGCACGG', 'GC
GGCGCA', 'GGCGCACG', 'GGCGGCGC']
['ACGGCGC', 'ACGGCGG', 'CACGGCG', 'CGCACGG', 'CGGCGCA', 'CGGCGGC', 'GACGGCG', 'GCACGGC', 'GCGCACG', 'GCGGGCG', 'GGCGCAA', 'G
GCGCAC', 'GGCGGCG']
[6, 1, 5, 12, 9, 4, 11, 8, 3, 7, 2, 0, 4, 10]
['GACGGCGG', 'ACGGCGGC', 'CGGCGGCG', 'GGCGGCGC', 'GCGGCGCA', 'CGGCGCAC', 'GGCGCACG', 'GCGCACGG', 'CGCACGGC', 'GCACGGCG', 'CA
CGGCGC', 'ACGGCGCA', 'CGGCGCAA']
GACGGCGGCGCACGGCGCAA
True
```



Advantage of larger k-mers

- Making k larger (8) eliminates the second choice of loops
- There are edges to choose from, but they all lead to the same path of vertices



Applied to the Hamiltonian Solution



```
In [23]: ▶ k = 8
target = "GACGGCGGCGCACGGCGCAA"
kmers = kmersUnique(target, k)
G4 = Graph(kmers)
for vsrc in kmers:
    for vdst in kmers:
        if (vsrc[1:k] == vdst[0:k-1]):
            G4.addEdge(vsrc,vdst)
path = G4.hamiltonianPathV2()

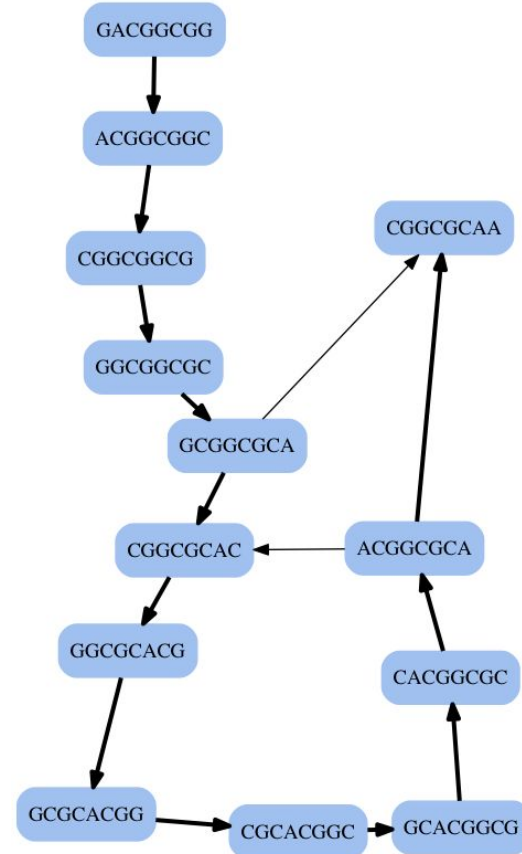
print(path)
seq = path[0][0:k]
for kmer in path[1:]:
    seq += kmer[k-1]
print(seq)
print(seq == target)

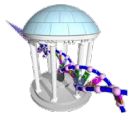
['GACGGCGG', 'ACGGCGGC', 'CGGCGGCG', 'GGCGGCGC', 'GCGGCGCA', 'CGGCGCAC', 'GGCGCACG', 'GCGCACGG', 'CGCACGGC', 'GACAGGCG', 'CA
CGGCGC', 'ACGGCGCA', 'CGGCGCAA']
GACGGCGGCGCACGGCGCAA
True
```




Graph with 8-mers as vertices

- There is only one Hamiltonian path
- There are no repeated k-mers





Assembly in Reality

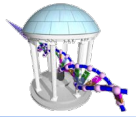
- Problems with repeated k-mers
- We can't distinguish between repeated k-mers
- Recall we knew from our example that were {2:ACGGC, 3:CGGCG, 2:GCGCA, 2:GGCGC}
- Assembling path without repeats:

```
In [26]: ▶ k = 5
target = "GACGGCGGCGCACGGCGCAA"
kmers = set([target[i:i+k] for i in range(len(target)-k+1)])
nodes = sorted(set([code[:k-1] for code in kmers] + [code[1:k] for code in kmers]))
G5 = Graph(nodes)
for code in kmers:
    G5.addEdge(code[:k-1],code[1:k],code)

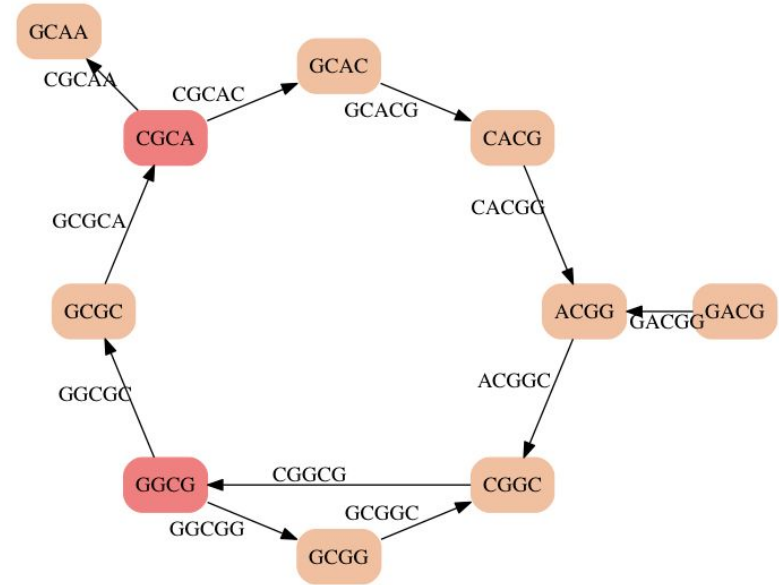
print(sorted(G5.vertex.items()))
print(G5.edge)

[(0, 'ACGG'), (1, 'CACG'), (2, 'CGCA'), (3, 'CGGC'), (4, 'GACG'), (5, 'GCAA'), (6, 'GCAC'), (7, 'GCGC'), (8, 'GCGG'), (9, 'G
GCG')]
[(9, 8), (3, 9), (1, 0), (4, 0), (6, 1), (8, 3), (0, 3), (2, 5), (7, 2), (2, 6), (9, 7)]
```

Resulting Graph with "unique" 5-mers as edges



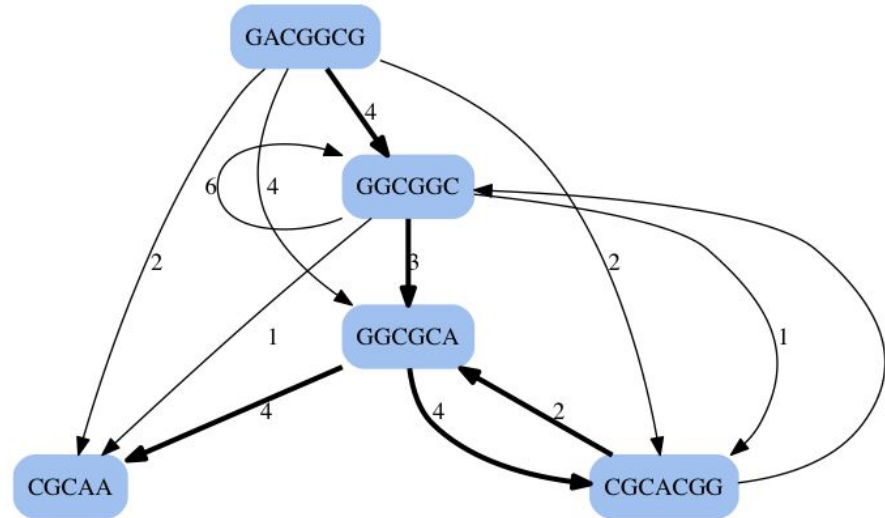
- There is no single Euler Path
- But there is a set of paths that covers all edges ['GACGGCG', 'GGCGGC', 'GGCGCA', 'CGCAA', 'CGCACGG']
 - Extend a sequence from a node until you reach a node with an out-degree \rightarrow in-degree
 - Save these partially assembled subsequences, call them *contigs*
 - Start new contigs following each out-going edge at these branching nodes



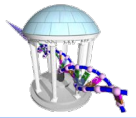


Next assemble contigs

- Use a modified read-overlap graph to assemble these contigs
- Add edge-weights that indicate the amount of overlap



- Usually much smaller than the graph made from k-mers

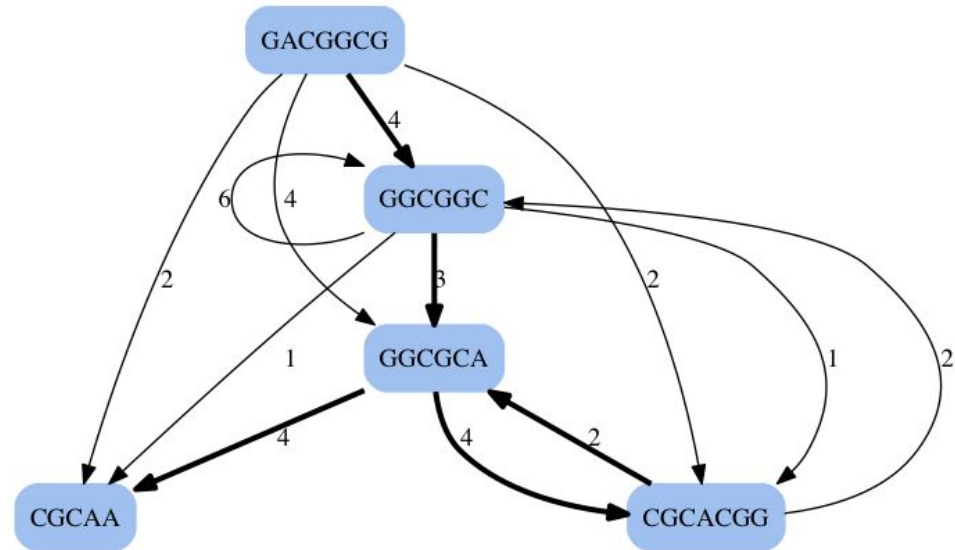


A Heavy Path

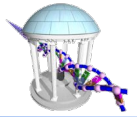
Find the heaviest path touching all vertices in this smaller graph

GACGGCGGCGCACGGCGCAA
GACGGCG
GGCGGC
GGCGCA
CGCACGG
GGCGCA
CGCAA

4
3
4
2
4
17



Discussion



- No simple single algorithm for assembling a real genome sequences
- Generally, an iterative task
 - Choose a k-mer size, ideally such that no or few k-mers are repeated
 - Assemble long paths (contigs) in the resulting graph
 - Use these contigs, if they overlap sufficiently, to assemble longer sequences
- Truly repetitive subsequences are a challenge
 - Leads to repeated k-mers and loops in graphs in the problem areas
 - Often we assemble the "shortest" version of a genome consistent with our k-mer set
- Things we've ignored
 - Our k-mers are extracted from short read sequences that may contain errors
 - Our short read set could be missing entire segments from the actual genome
 - Our data actually supports 2 paths, one through the primary sequence, and a second through it again in reverse complement order.