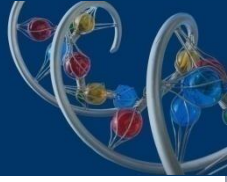


De novo genome assembly

Andrew Tritt /Alicia Clum

CGRL Workshop

October 22, 2014

JGI Programs & Infrastructure

DOE Mission Areas

Bioenergy

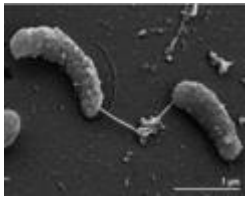
Carbon Cycling

Biogeochemistry

Why Assemble ?

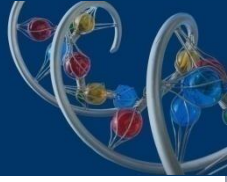
Metagenomes (**600/yr**)
assembly supports understanding community structure and metabolic capabilities

Fungi (**200/yr**)
reference genomes for understanding taxonomic relationships, and identification of genes involved in carbon cycling



Microbes (**>1000/yr**)
reference genomes for clarifying and expanding taxonomic understanding of microbial life

Outline



I. Background

- Graphs

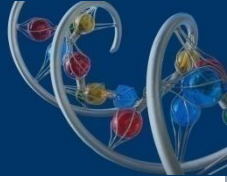
II. Genome assembly

- Old → Assembly in the Sanger days
  - Overlap-layout-consensus
- New → Assembly in the era of big data
  - De Bruijn graphs
- Scaffolding → utilizing paired-end data

III. Hands On

- Assembly of short-read data

Graphs



Not like that of a function or a chart

- a representation of a set of objects where pairs of objects are connected by links
- consists of a set of ***nodes*** (aka vertices) that represent the objects and a set of ***edges*** that represent the links or relationships between objects
- used to represent many real-life problems and systems

Graphs

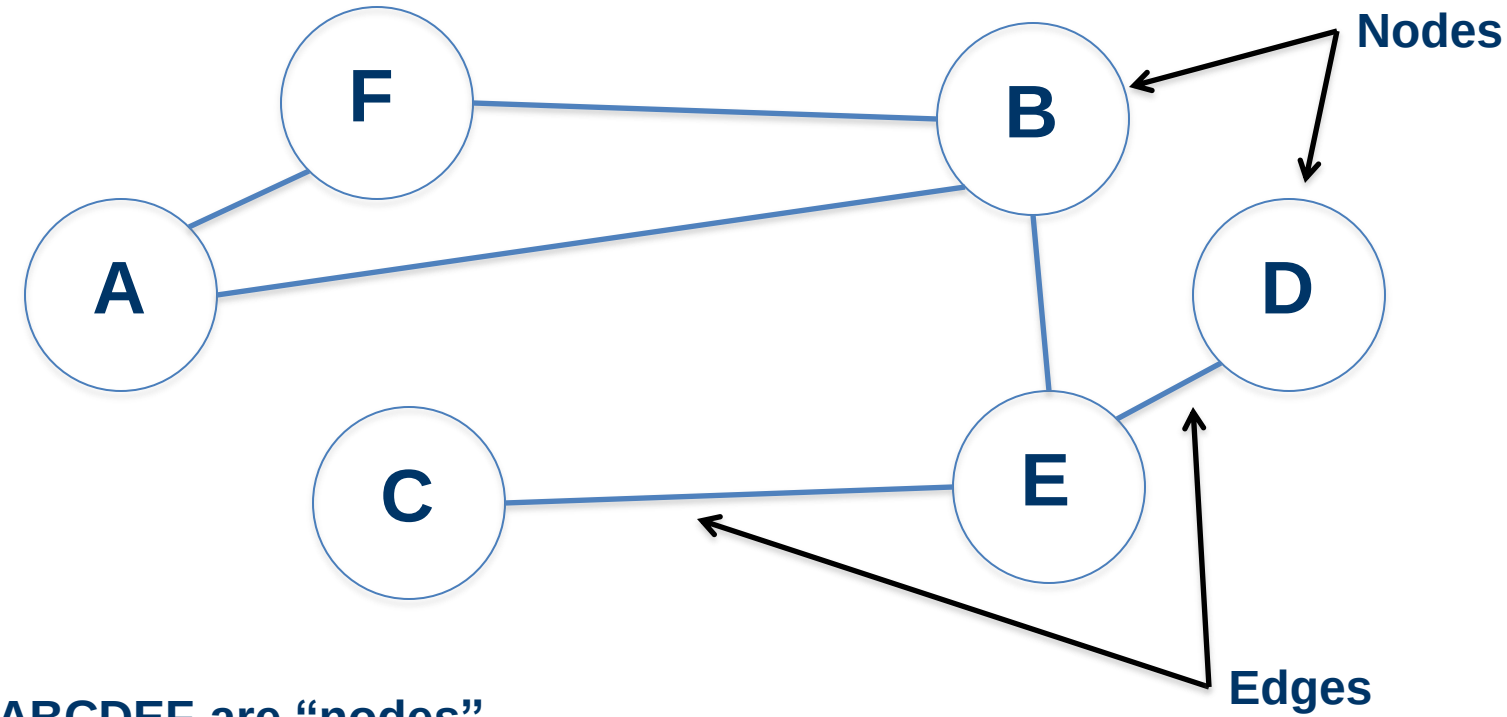
Examples:

- Biology: gene regulatory networks, neuron networks, species migration pattern
- Chemistry/Physics: three dimensional molecules
- Computer science: websites, data structures, electrical circuits and many many more
- Sociology: social networks

Image from ://socialnetworking.lovetoknow.com/What_is_Social_Network_Theory

Graphs

The visual representation:

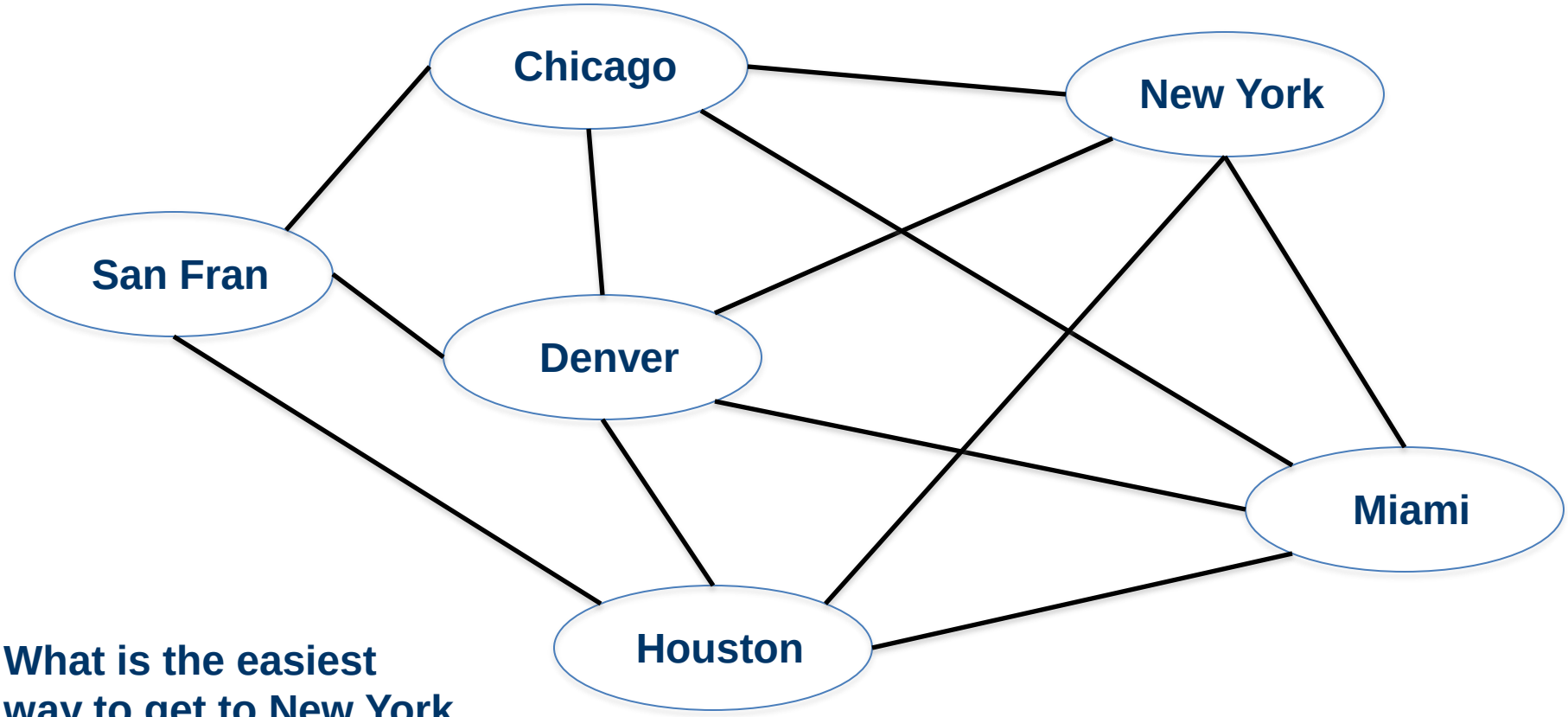


ABCDEF are “nodes”

A and B have some relationship, but A and C do not.

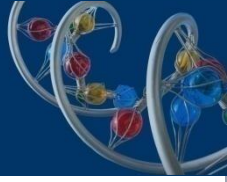
Graphs

Example: Airports and flights



What is the easiest way to get to New York from San Francisco?

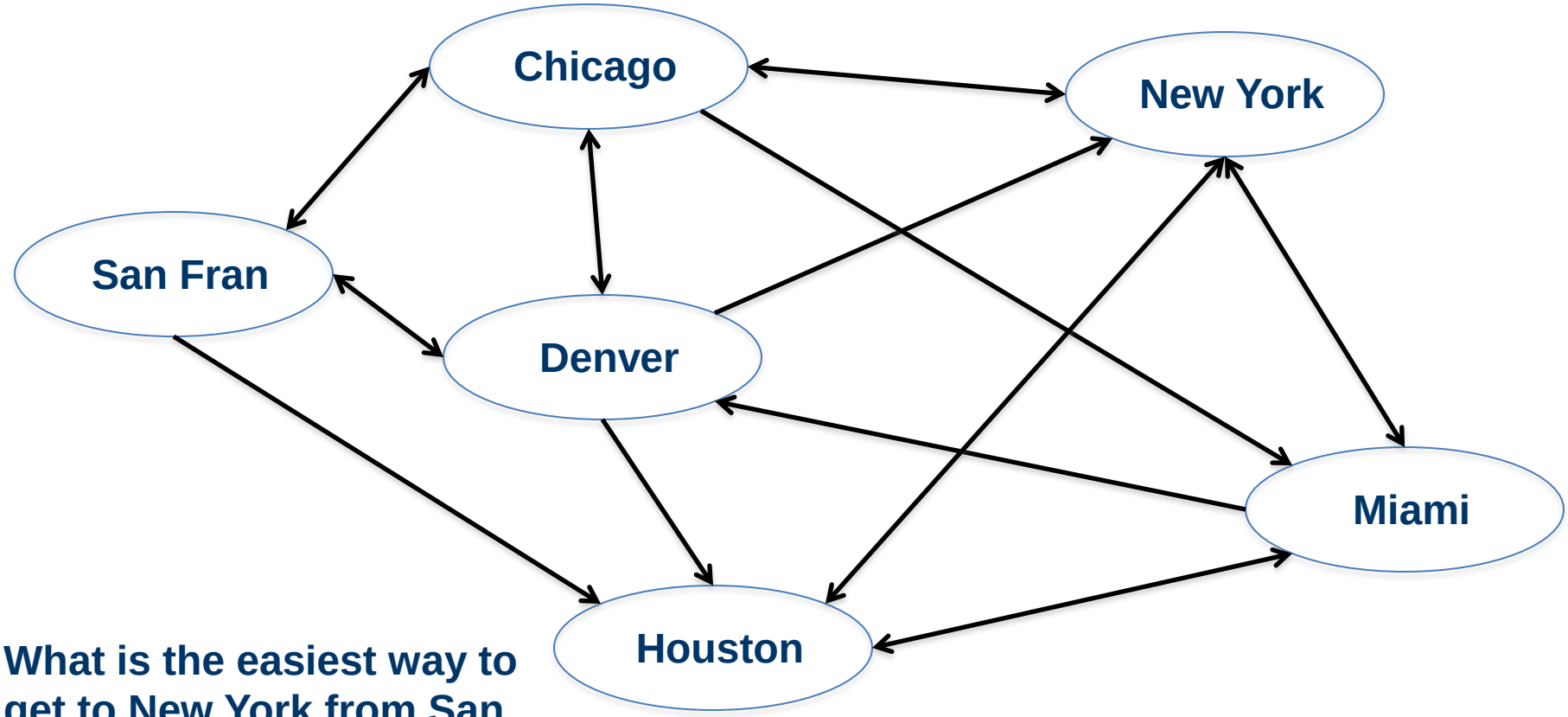
Graphs



In addition to connecting nodes with edges, we can *weight* edges and *direct* them.

Graphs

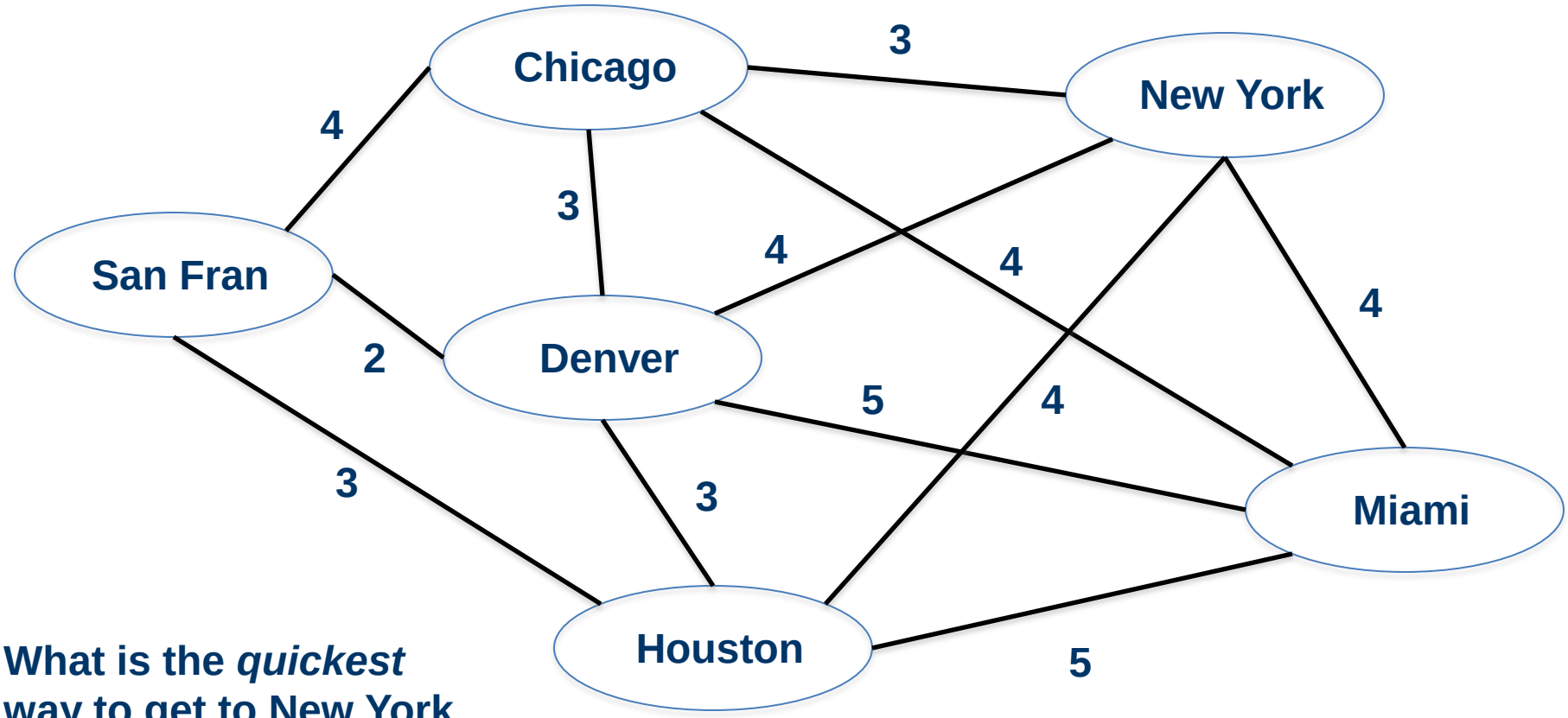
Airports and flights - directed edges



What is the easiest way to get to New York from San Francisco, *round-trip*?

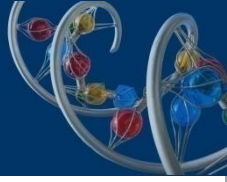
Graphs

Airports and flights - weighted edges

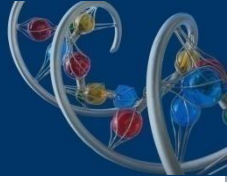


What is the *quickest* way to get to New York from San Francisco?

Graphs



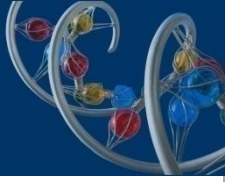
So, graphs are great if you want to plan a trip, but they are also great if you want to assemble a genome!



Graphs and genome assembly

- **Nodes represent some part of a sequence**
  - In Overlap-Layout-Consensus (OLC), represent "reads"
  - In de Bruijn graphs, represent "*k-1* mers"
- **Edges represent some overlap between two sequences**
  - In OLC, represents overlap between "reads"
  - In de Bruijn graphs, represent "*k* mers"

Overlap-Layout-Consensus

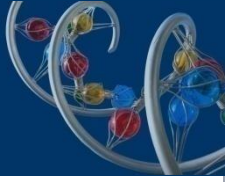


Assembly done by

1. **Overlapping**
   - Align each read to all other reads
2. **Layout**
   - Construct a graph to get an approximate read layout
3. **Consensus**
   - Traverse the graph to compute a consensus sequence

✸ **Sanger data assembled using this algorithm, new long read technology is making these popular again**

Overlap-Layout-Consensus



Example:

Genome: ATGCTAGTGGCAGAGCTTGATCGATCGAT

Reads:

ATGCTAG

TAGTGGCAGA

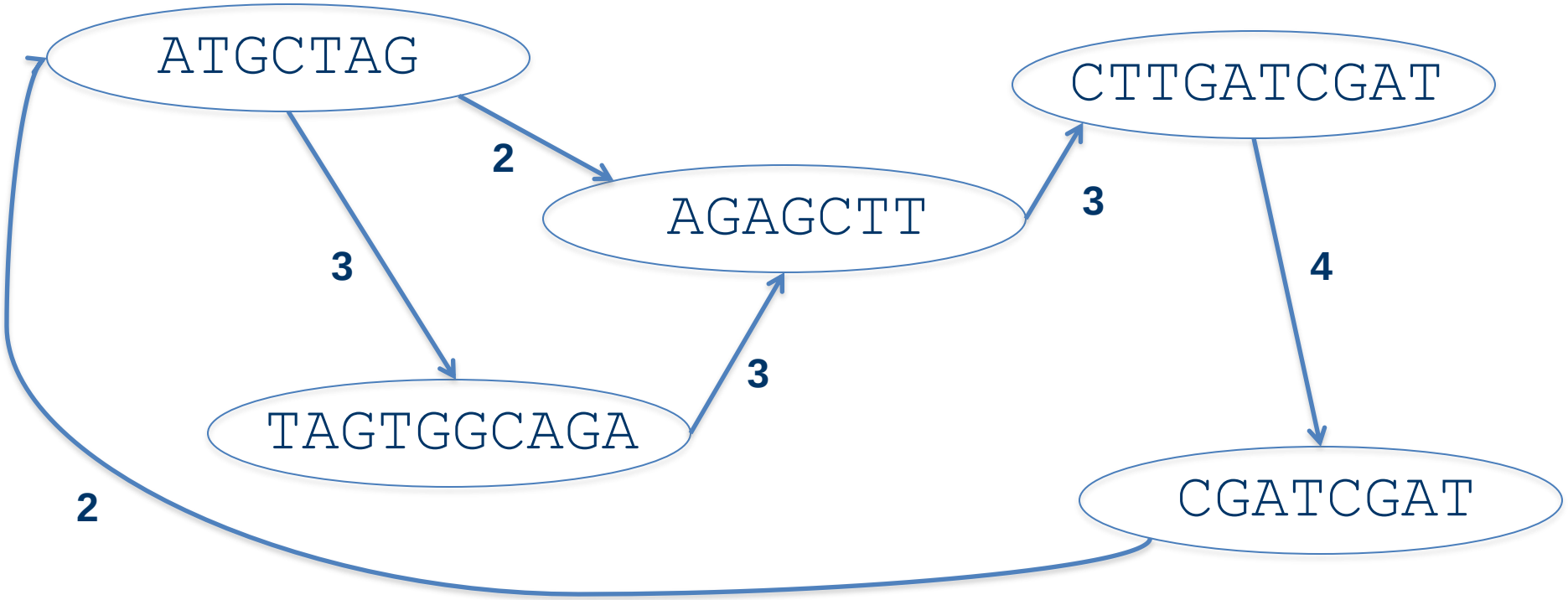
AGAGCTT

CTTGATCGAT

CGATCGAT

Overlap-Layout-Consensus

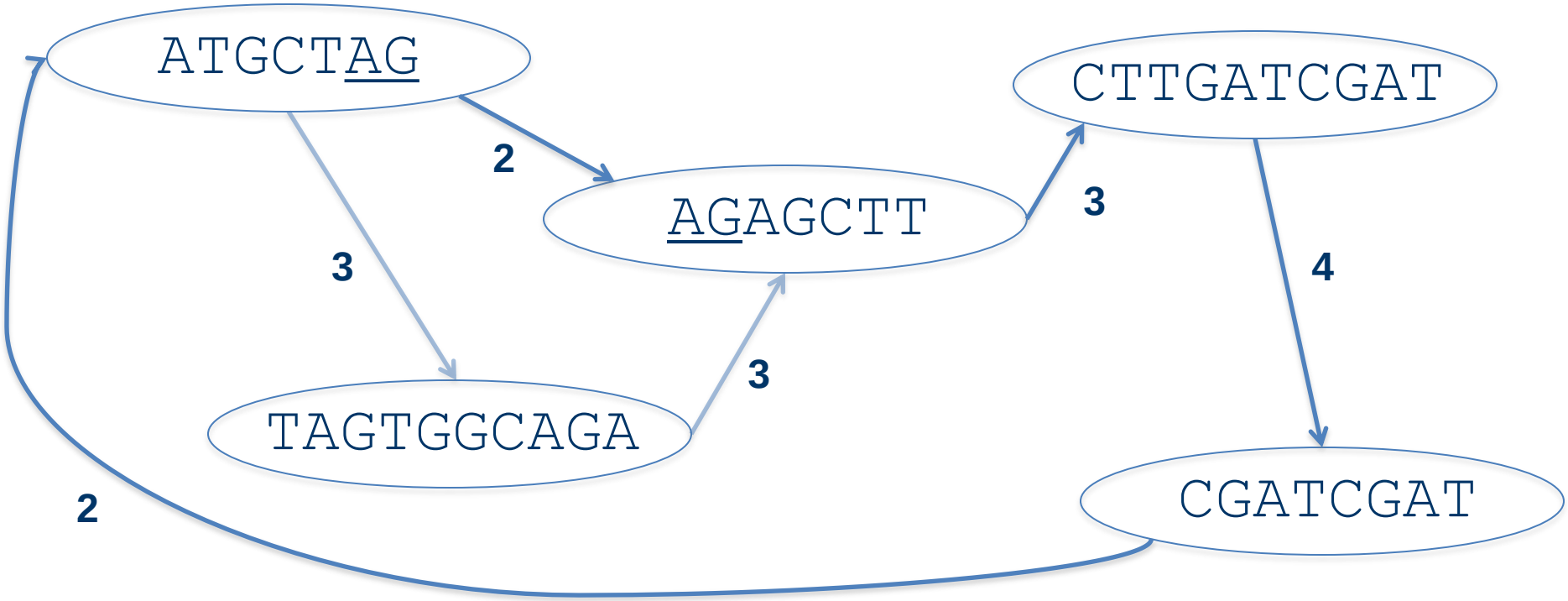
Genome: ATGCTAGTGGCAGAGCTTGATCGATCGAT



Visit each node exactly once, and require the maximum overlap between nodes

Overlap-Layout-Consensus

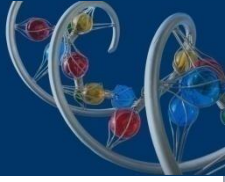
Genome: ATGCTAGTGGCAGAGCTTGATCGATCGAT



What happens if we don't require the maximum overlap between nodes?

Mis-assembly! ATGCTAGA instead of ATGCTAGT

Overlap-Layout-Consensus



Problems with OLC algorithm

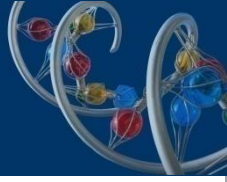
1.Computationally expensive

- **Aligning every read to each other read is expensive**
  - *O(n^2):* for *n* reads, (n^2 – *n*)/2 alignments

2.No straightforward solution

- **Visiting each node exactly once is called a *Hamiltonian path***
  - NP-complete: A solution may or may not exist, but there is no way of knowing, and finding the solution is not deterministic

De Bruijn Graphs



For a given sequence of characters, a *de Bruijn* graph represents all *k*-mers in the sequence

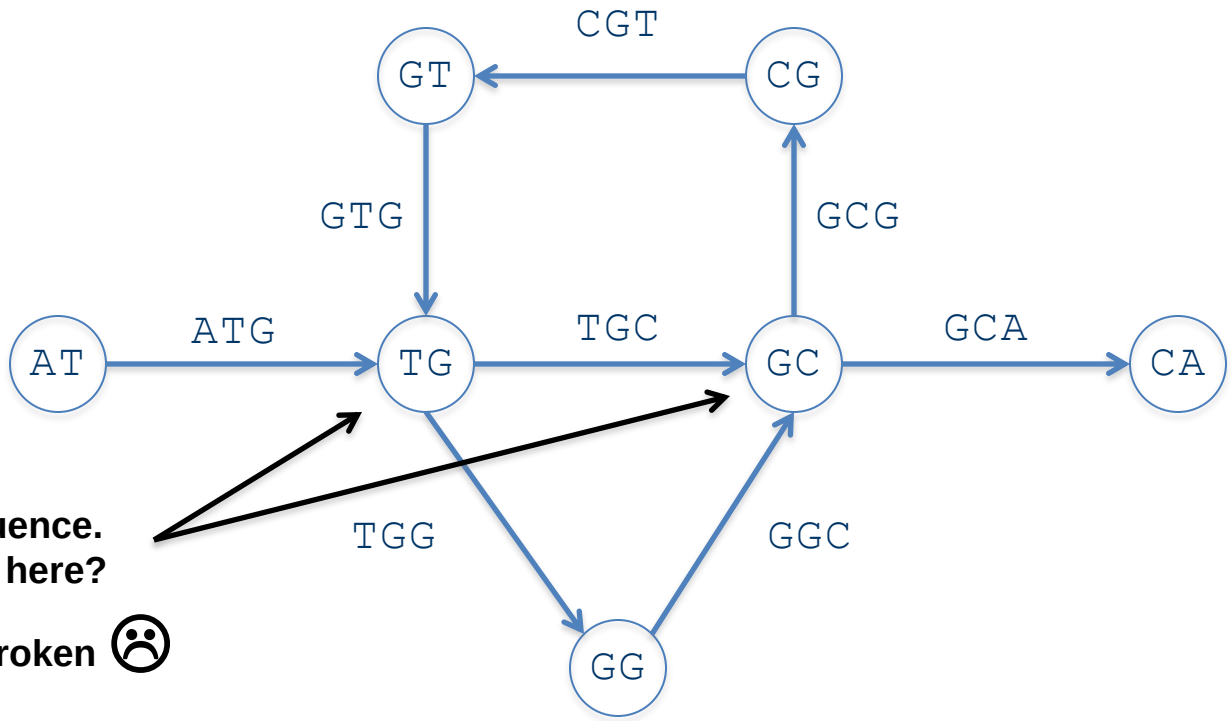
- **Nodes represent (*k*-1)-mers**
- **Edges represent overlap between two *k*-mers**
  - An edge exists between two nodes if the *k*-mer representing the edge is found in the data set
    - (*k*-2)-length suffix of one node is the (*k*-2)-length prefix of the other node
  - Weighted according to *k*-mer frequency
  - Directed from suffix node to prefix node

De Bruijn Graphs

Building a De Bruijn graph with *k*=3

Genome: ATGGCGTGCA

***k*-mers:** {ATG,TGG,GGC,GCG,CGT,GTG,TGC,GCA}

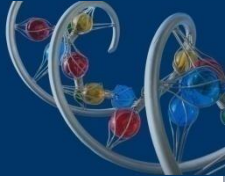


Repetitive sequence.
What do we do here?

Contig gets broken ☹

Once graph is built, traverse it to extract contigs

De Bruijn Graphs



Advantages

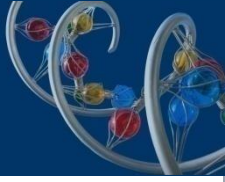
1.Computationally tractable

- **want every *k*-mer in graph to be in assembly → traverse every edge exactly once → *Eulerian path* (solvable problem)**

2.Less expensive computation

- ***k*-mers are *hashed*, no aligning reads.**
  - Hash = efficient storage and comparison of strings
  - *O(n)*
    - for every read of length *L*, $L - k + 1$ *k*-mers to hash.
    - memory requirements scale linearly with genome size

Scaffolding



Thus far, we have talked about how to make *contigs*.

→ **utilizes individual reads**

Generally, we sequence a DNA molecule from both ends

→ **two *paired* reads**

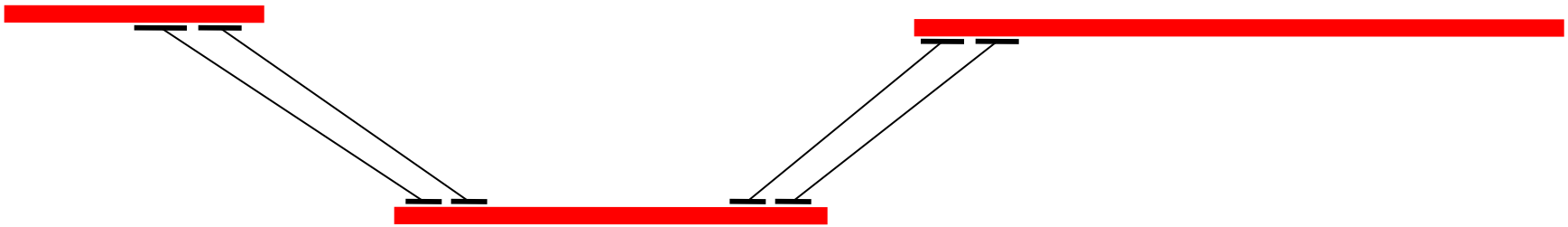
- **use this pairing information to connect contigs → *scaffolding***

Scaffolding

1. Build contigs

2. Map (align) reads to contigs

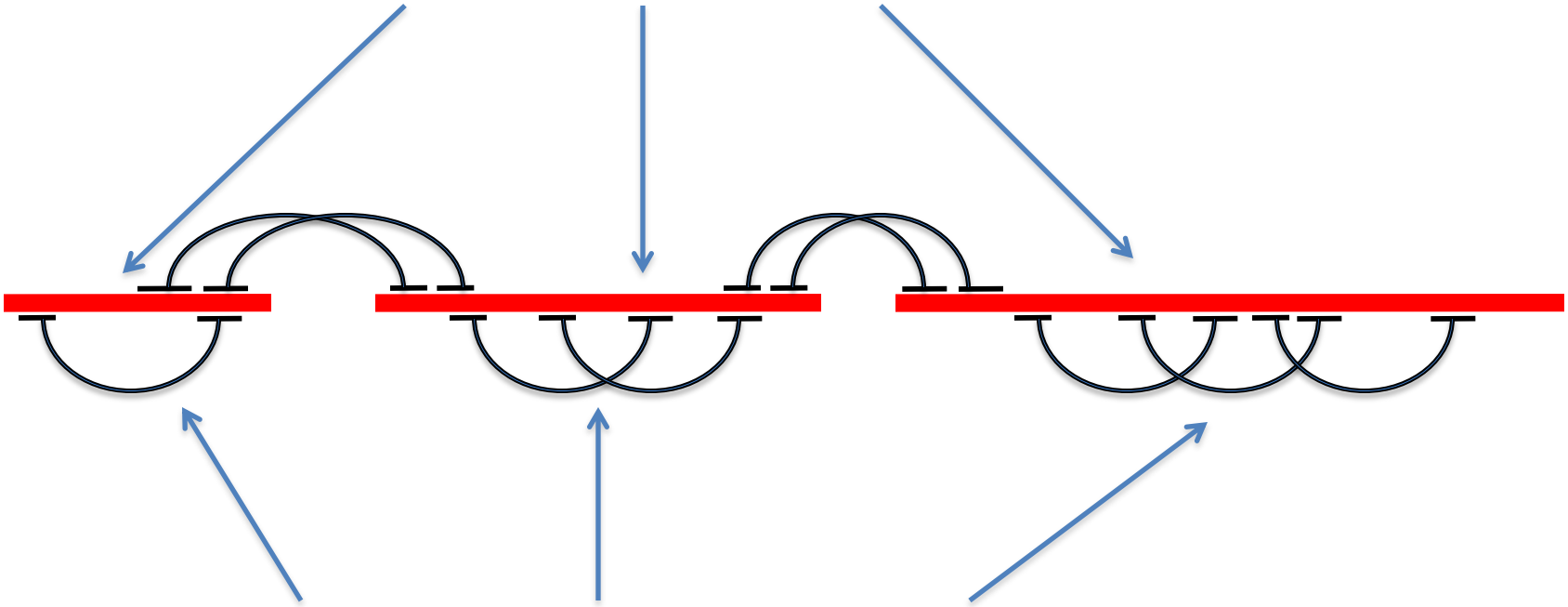
3. Connect with pairing information



Scaffolding

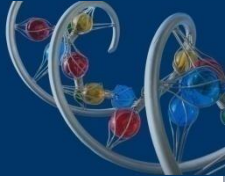
Okay, so now we know which contigs are linked… what next?

Connect these contigs with a sequence of N's



Estimate the number of N's using pairs that map to the same contigs

Scaffolding



An example assembly:

>Scaffold1
:
:
:
GGGATAAAATATGTTGCAGTTTTTCTAGGATACGTAGTAATTTCTTCATT
TACAAAAAAGTATAGTAAAAGTATAGCAATAGTAGAGGAAGAAATAGAAG
CAAAAGAGAATCAAACCACTCCCTATAATATCTTTTGATTTTCTTTGAAT
ATCTCGCTTTTTTCATAGAATTCCTTACCAAACAAACTAGCTC**NNNNNNNN**
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCCTTTCAA
ATTGTAACGTGAGGTCTTCTTTTTGACAAAGATCATTTTTGACTACTACA
GGAATGCTCCTTAAAGCTTTTAAGCCTTATGACATTTTATTAGACAATAA
GTATTGGTAGTATTATGTCCAACATTAGGATTATAGCGGAACAGCACGGA
:
:
:

Our biggest problem

REPEATS!

Repeats present ambiguity in genome sequence

→ E.g. a read is generated from a duplicated region

→ Which specific region did it come from?

"Without repeats, it would just be simple math"

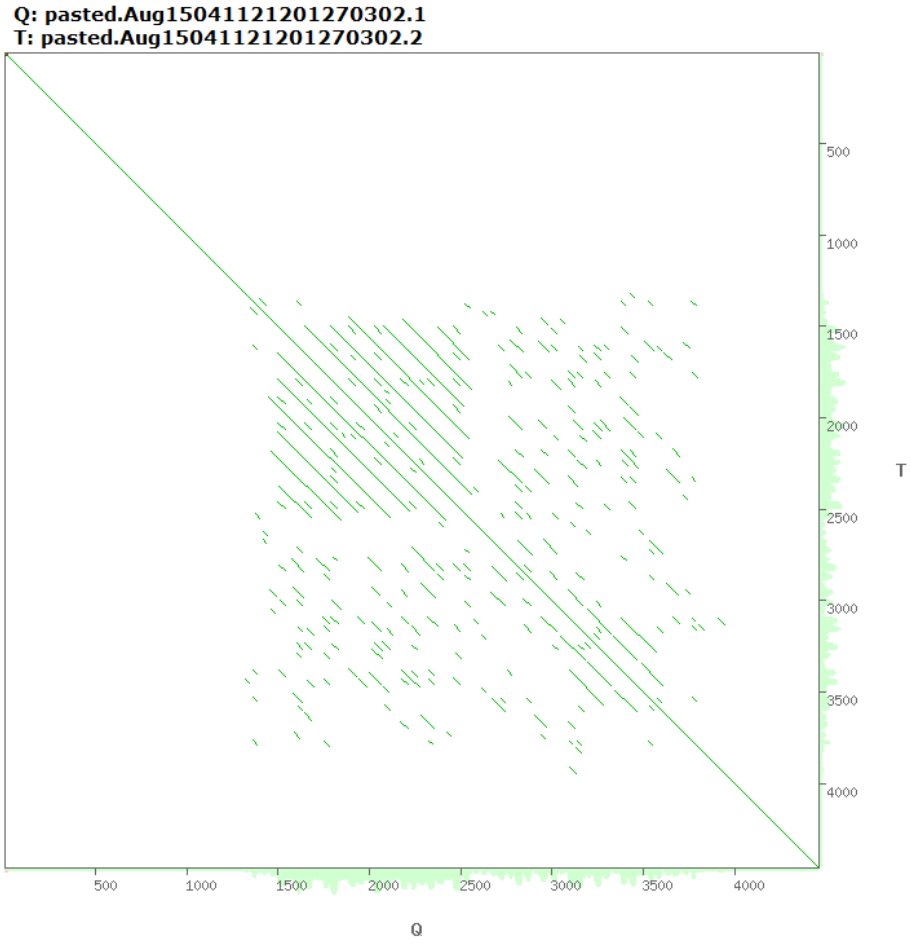


Figure is a *dot-plot* of inaZ gene. Provided by Dr. Wolber

dots represent matching sequence

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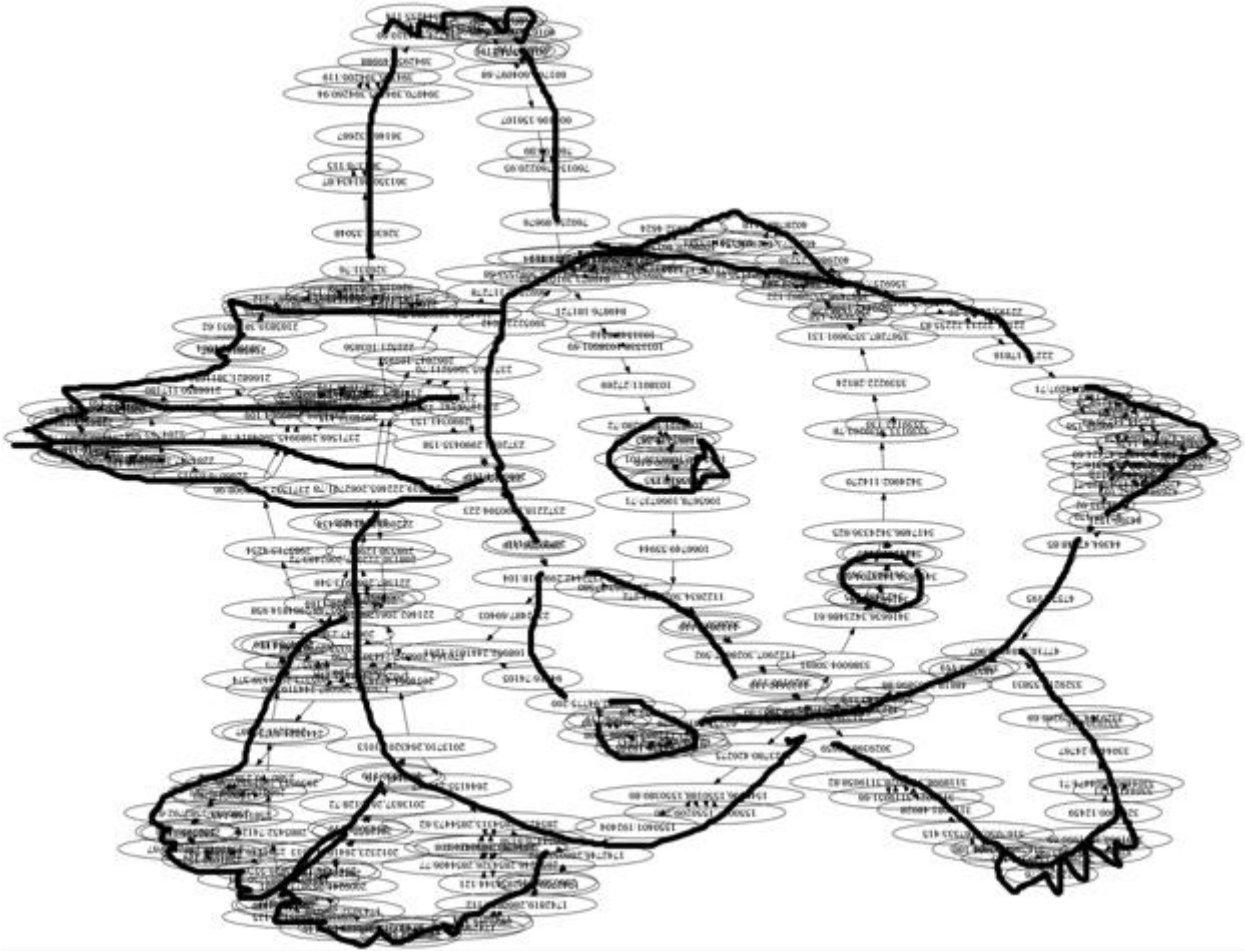
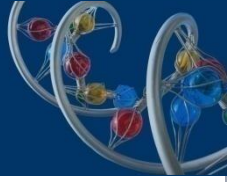
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

A fungal de Bruijn graph

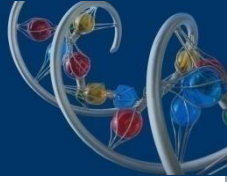


Genome Assembly

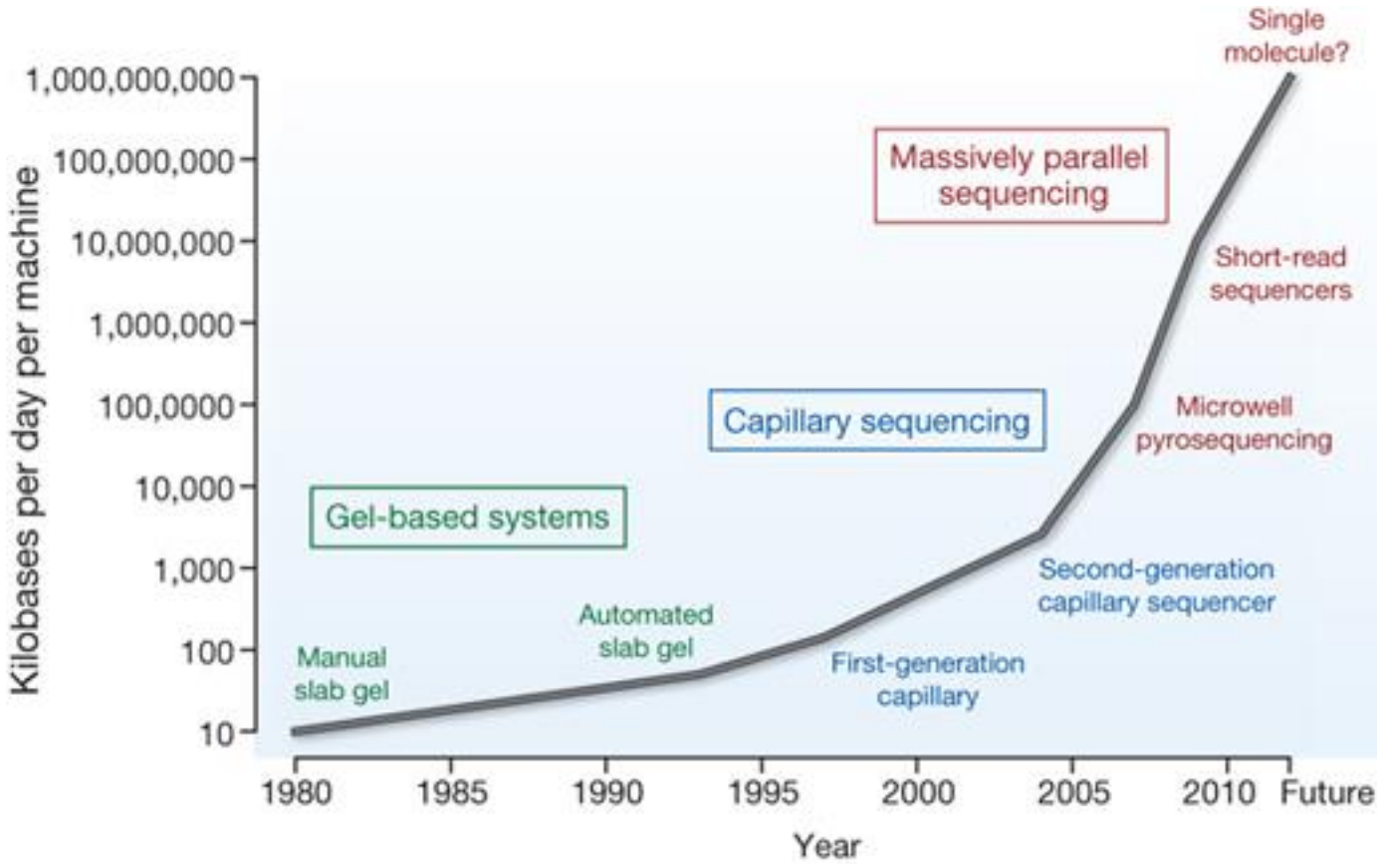


Questions?

Extra

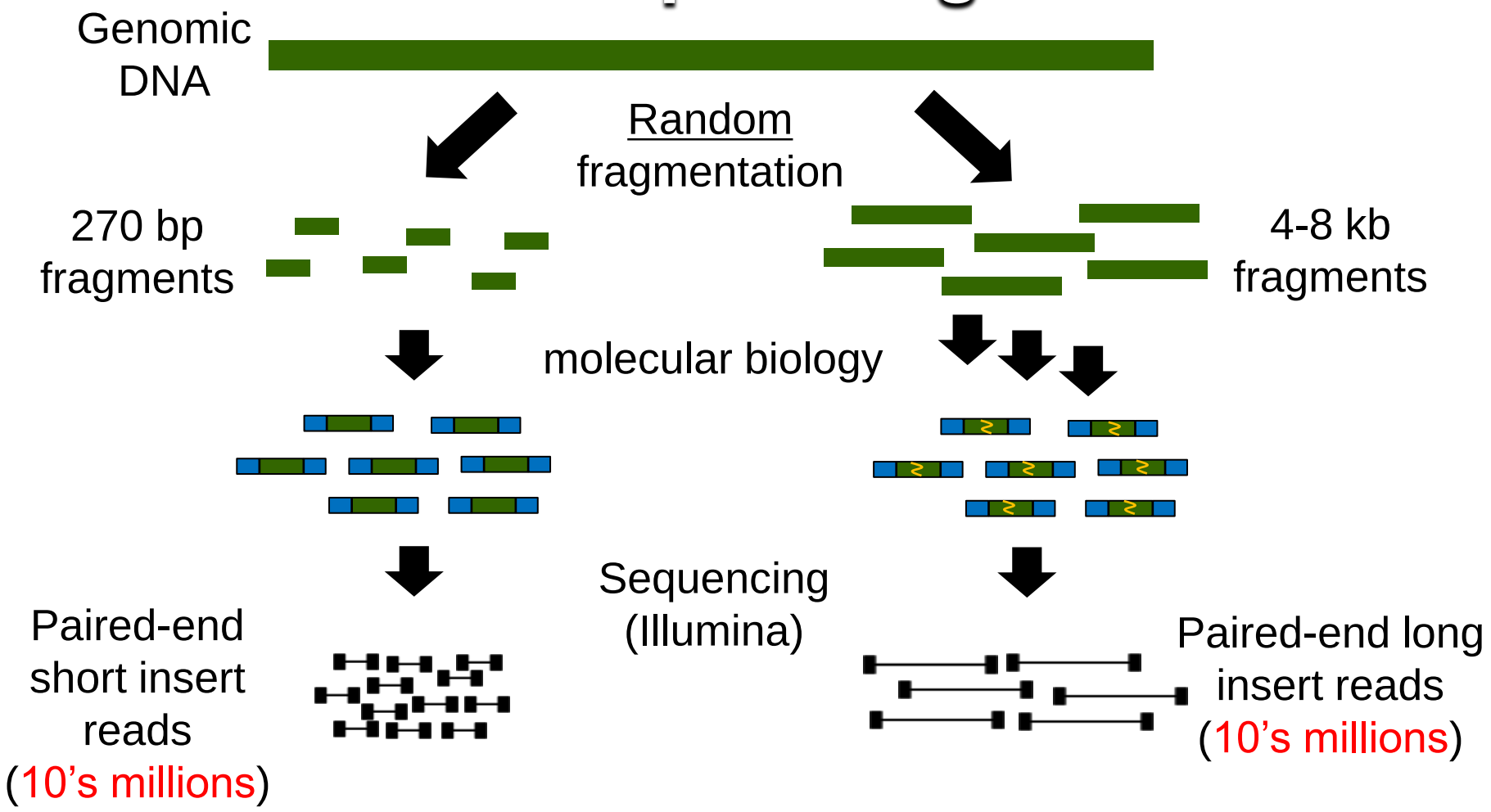


Sequencing capacity



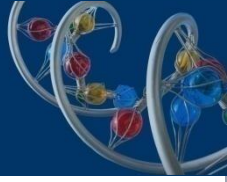
see ftp://ftp.ncbi.nih.gov/genbank/gbrel.txt

Short read genome sequencing



How do we assemble this data back into a genome?

Platform Summary



- Massively parallel short reads
- limited by phasing errors
- read length up to 300bp
- 0.3% error, mostly substitutions and at ends
- Gigabases / run collected in days or weeks

CGRL

- Single molecule long reads
- read length limited by polymerase damage
- reads lengths up to 15 kbp (or longer)
- 15% error, mostly randomly distributed indels
- Megabases / run collected in hours