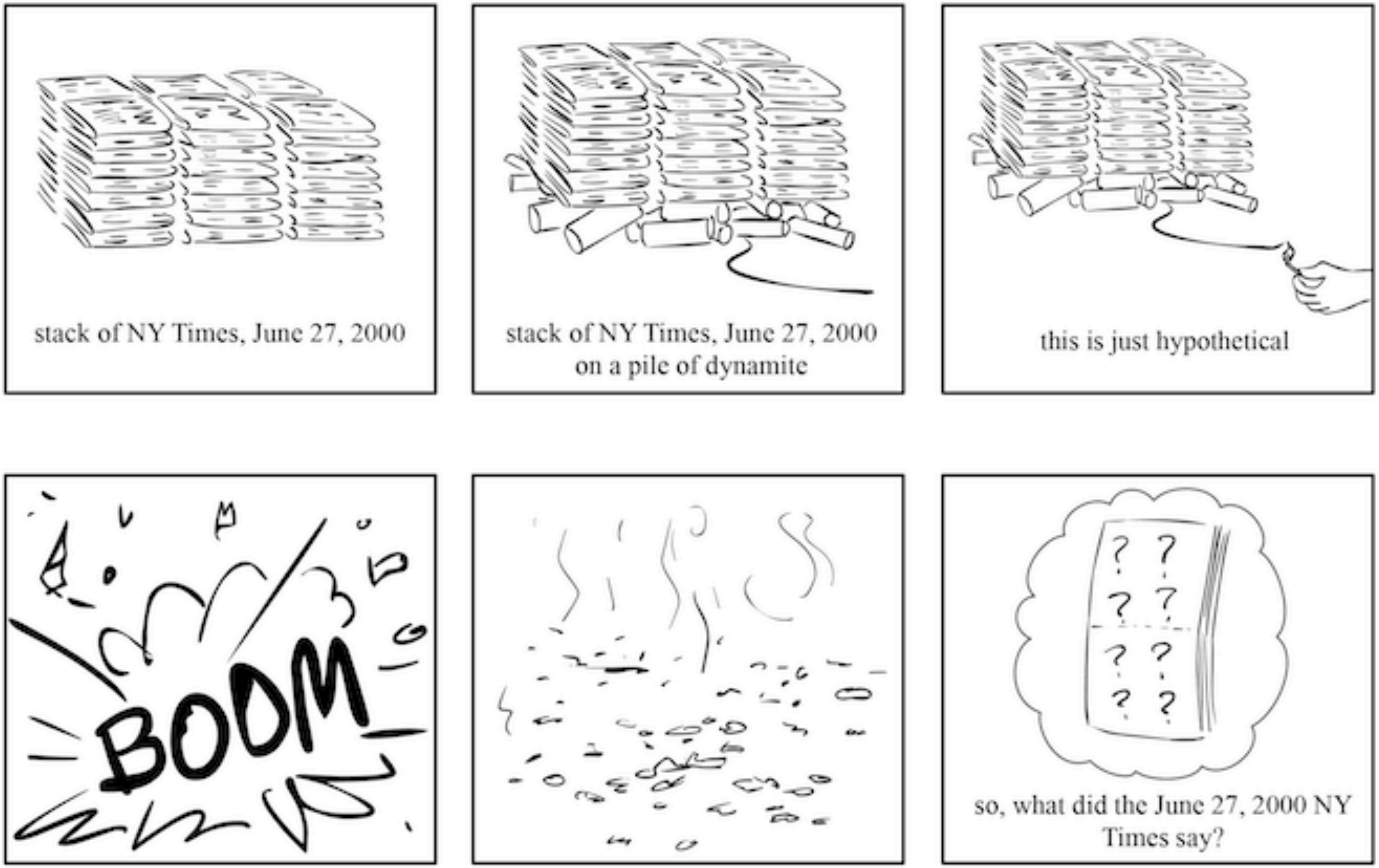
# UBC Bioinformatics Class

## Topic 5: de novo assembly

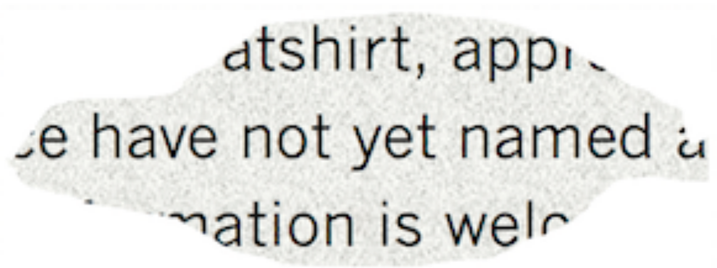
# Outcomes

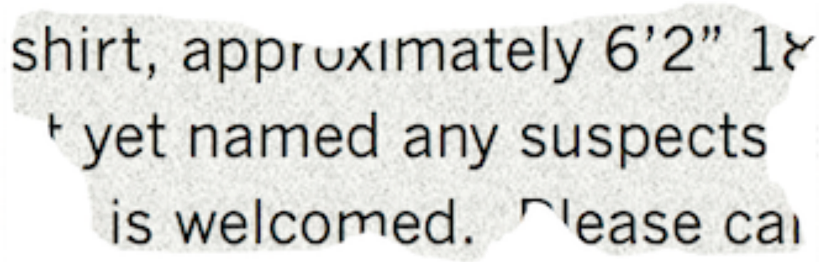
- Identify the difference between de novo assembly and reference guided alignment
- Evaluate two different approaches to de novo genome assembly
- Describe how repetitive elements can hamper proper assembly and compare approaches that can overcome this problem
- Describe approaches for transcriptome/ GBS de novo assembly

# Introduction

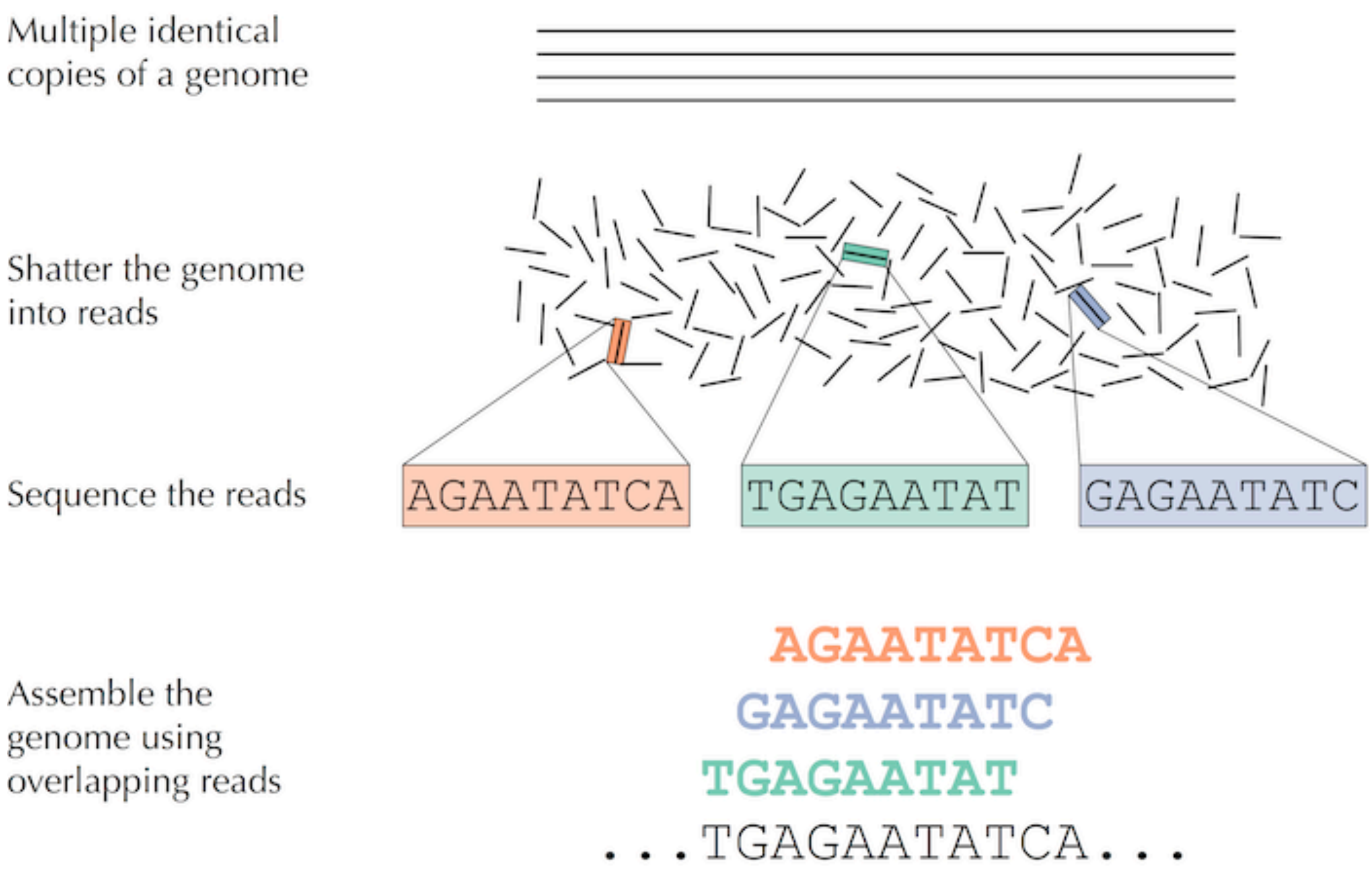


# Introduction



# Introduction



# Alignment vs assembly

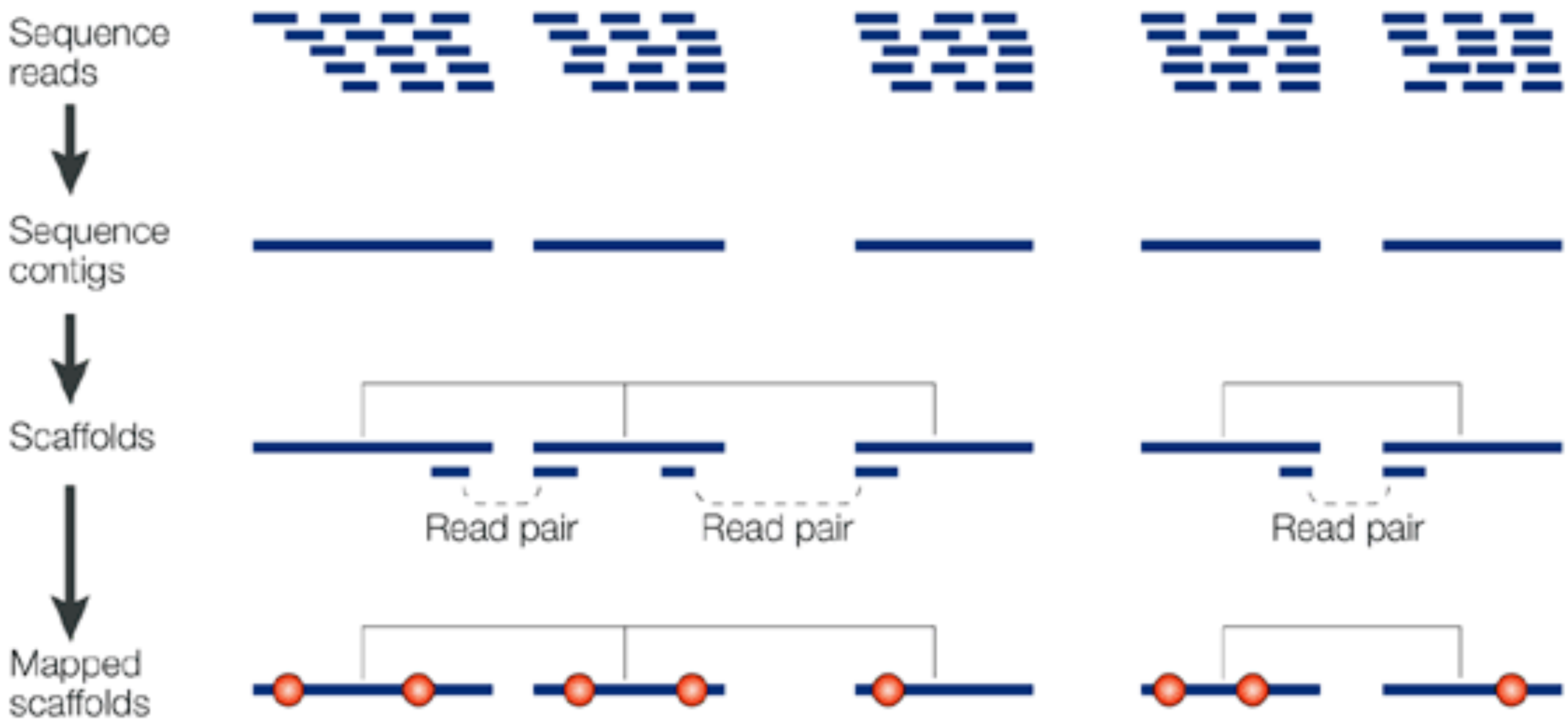
Aligning to a reference:

- Reference guided alignments: align the reads to a reference genome and looks for differences

Building a reference:

- *De novo* assembly: no previous genome assembly is used
- Comparative genome assembly: assemble a newly sequenced genome by mapping it on to a reference
- Hybrid approach: reference-guided and *de novo* for unused reads or *de novo* and then reference guided alignments

# Introduction




Adams 2008

# Introduction

Original sequence

GATAGAAGGGTCCGCTCGCTCAGCTACCGGTTTTTATAGATCTA

GATAGAAGGGTCCGCT
AGAAGGGTCCGCTC
GGGTCCGCTCGCTCA
CCGCTCGCTCAGC
CTCGCTCAGCTACC
TCAGCTACCGGTTT
CTACCGGTTTTT
AGCTACCGGTTTTTAT
TTTTTATAGATCTA

fragmented sequences
from sequencer
(reads)

# Introduction

**assembled**

fragmented sequences
from sequencer
(reads)

TTTTTATAGATCTA
AGCTACCGGTTTTTAT
CAGCTACCGGTTTTT
TCAGCTACCGGTTT
CTCGCTCAGCTACC
CCGCTCGCTCAGC
GGGTCCGCTCGCTCA
AGAAGGGTCCGCTC
GATAGAAGGGTCCGCT

GATAGAAGGGTCCGCTCGCTCAGCTACCGGTTTTTATAGATCTA

We want to reconstruct this from the reads

# Introduction

Simplified scenario

- Single strand
- Error free
- Complete coverage

TTTTTATAGATCTA
AGCTACCGGTTTTTAT
CAGCTACCGGTTTTT
TCAGCTACCGGTTT
CTCGCTCAGCTACC
CCGCTCGCTCAGC
GGGTCCGCTCGCTCA
AGAAGGGTCCGCTC
GATAGAAGGGTCCGCT

GATAGAAGGGTCCGCTCGCTCAGCTACCGGTTTTTATAGATCTA

# Introduction

Coverage: reads "covering" a position in the genome (average or at a single base or region)

TTTTTATAGATCTA
AGCTACCGGTTTTTAT
CAGCTACCGGTTTTT
TCAGCTACCGGTTT
CTCGCTCAGCTACC
CCGCTCGCTCAGC
GGGTCCGATCGCTCA
AGAAGGGTCCGCTC
GATAGAAGGGTCCGCT

131 bases in the reads

44 bases in the "genome"

GATAGAAGGGTCCGCTCGCTCAGCTACCGGTTTTTATAGATCTA

What is our average coverage?

What is the coverage at the arrow?

# Introduction

TTTTTATAGATCTA

AGCTACCGGTTTTTAT

CAGCTACCGGTTTTT

TCAGGTACCGGTTT

CTCGCTCAGCTACC

CCGCTCGCTCAGC

GGGTCCGATTGCTCA

AGAAGGGTCCGCTC

GATAGAAGGGTCCGCT

GATAGAAGGGTCCGCTCGCTCAGCTACCGGTTTTTATAGATCTA

Why might there be differences among reads covering the same position?

# Introduction

CCGCTCGCTCAGC

TCAGCTACCGGTTT

CTCGCTCAGCTACC

CAGCTACCGGTTTTT

AGAAGGGTCCGCTC

GATAGAAGGGTCCGCT

AGCTACCGGTTTTTAT

TTTTTATAGATCTA

GGGTCCGCTCGCTCA

How would you go about "assembling" these reads when you have no reference?

# Code break

Write some code to find all the overlaps exactly 4 bp in length between CTCTAGGCC and a list of other sequences in the file /home/biol525d/Topic_5/data/overlaps.fa

# Overlap-layout-consensus

Overlap: make an overlap graph

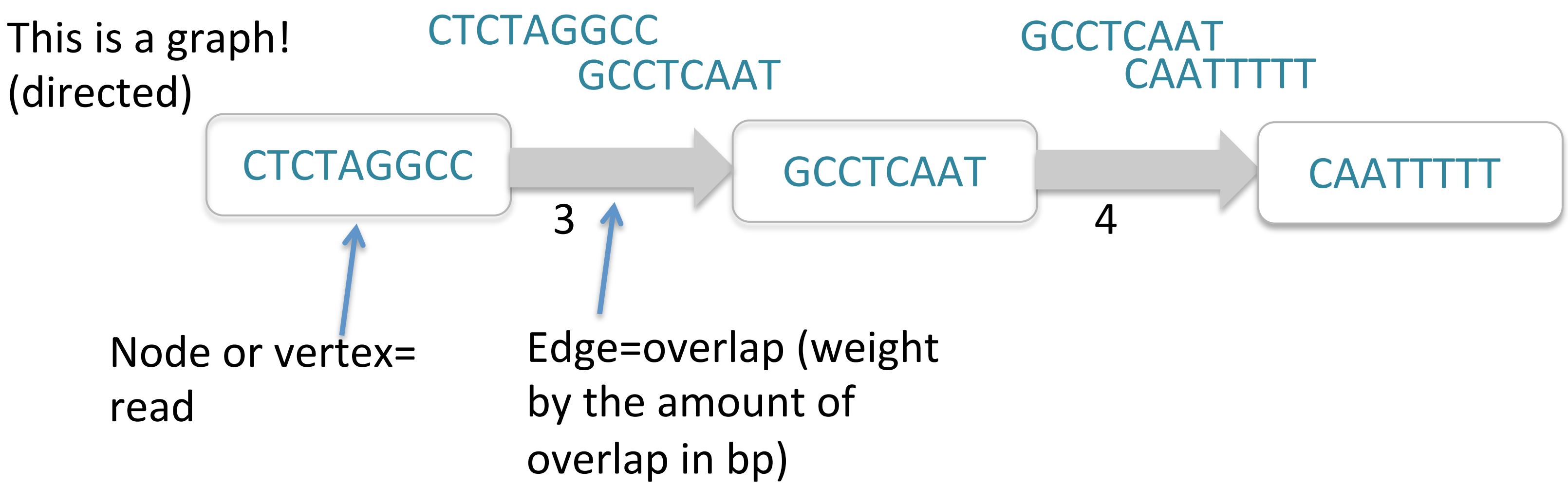
Layout: find the path through the graph

Consensus: find the most likely contig sequence

OLC programs:
ARACHNE, PHRAP, CAP, TIGR, CELERA

# **Overlap**-layout-consensus

Reads: CTCTAGGCC GCCTCAAT CAATTTTT

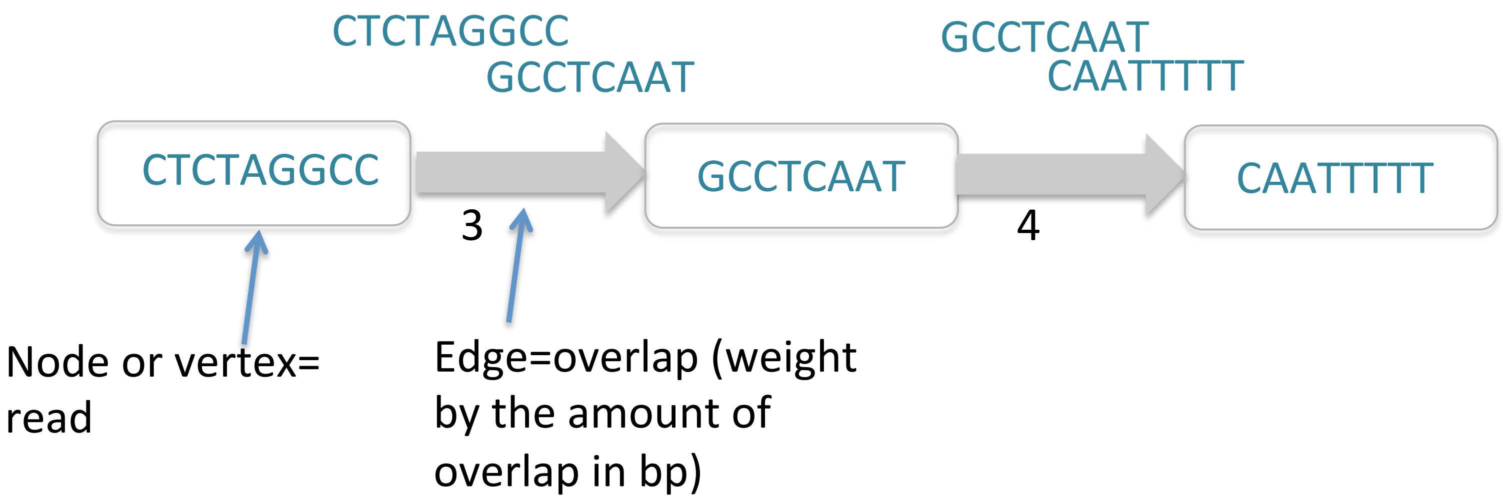



Can pick a minimum overlap length (e.g. 3 bp)

Finding overlaps can be computationally challenging when you have millions of reads!

# Overlap-**layout**-consensus

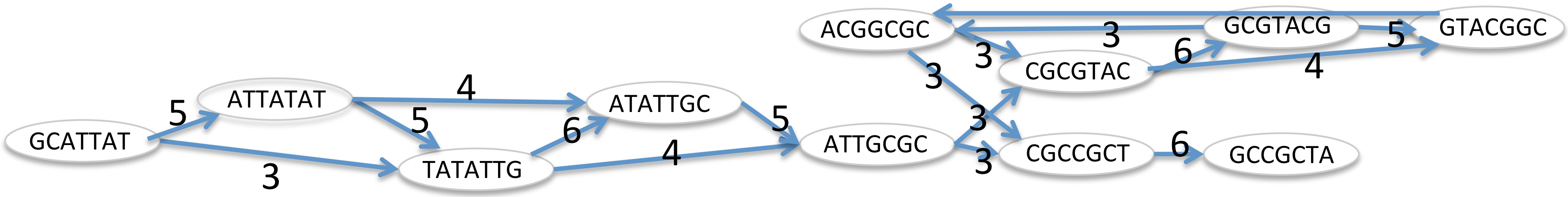
Reads: CTCTAGGCC GCCTCAAT CAATTTTT




Here we have only one path through the graph

# Overlap-**layout**-consensus

These graphs get complicated!




Minimum
overlap = 3
Read length = 7

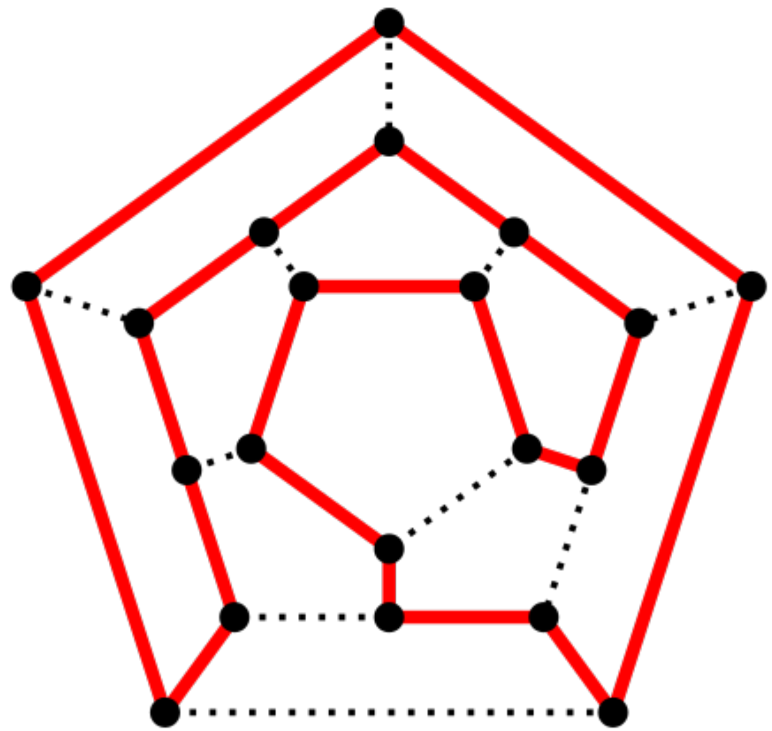
GCATTATATATTGCGCGTACGGCGCCGCTACA

Original sequence

How can we find the best path?

# Overlap-**layout**-consensus

Hamiltonian path: hit each node (read) once
–no quick way to figure it out (NP-complete)
–not practical and not implemented



# Overlap-**layout**-consensus

Shortest superstring: find the shortest final sequence (greatest overlap between reads)
-hit each node (read) once
-NP-hard

# Overlap-**layout**-consensus

Greedy algorithm (example)

1) Pairwise alignments between all fragments
2) Pick the two with the largest overlap
3) Merge chosen fragments
4) Repeat

# Overlap-layout-**consensus**

## Join sequences together into one sequence

Reads: CTCTAGGCC GCCTCAAT CAATTTTT

CTCTAGGCC
GCCTCAAT

GCCTCAAT
CAATTTTT

CTCTAGGCC → 3 → GCCTCAAT → 4 → CAATTTTT

# Limitations of OLC

- require overlaps to be scored between all possible pairs of reads. This is a problem when you have millions of reads

- finding the best path through the graph with a huge number of nodes (reads) is computationally challenging

Is there a faster way to assemble many short reads?

# De Bruijn graphs

What are all the 5-mers (5 bp fragments) in these reads?

2 reads of 9 bp

| read 1 | read 2 |
|---|---|
| ATGGGGAAC | GGGAACCCC |
| ATGGG | GGGAA |
| TGGGG | GGAAC |
| GGGGA | GAACC |
| GGGAA | AACCC |
| GGAAC | ACCCC |

If a read is L bp long, how many kmers of size k can you make?

# Code break

Find all the unique 9mers in a fasta sequence and sort them alphabetically /home/biol525d/Topic_5/data/kmer.fa

1. Find all the kmers in this fasta sequence.

   Hints: test out the following commands

   ```
   cut -c2- kmer.fa
   cut -c1-4 kmer.fa

   for num in {1..10}
   do
   echo $num >> file.txt
   done
   ```

2. Sort them and keep the unique ones

   Hint: try sort

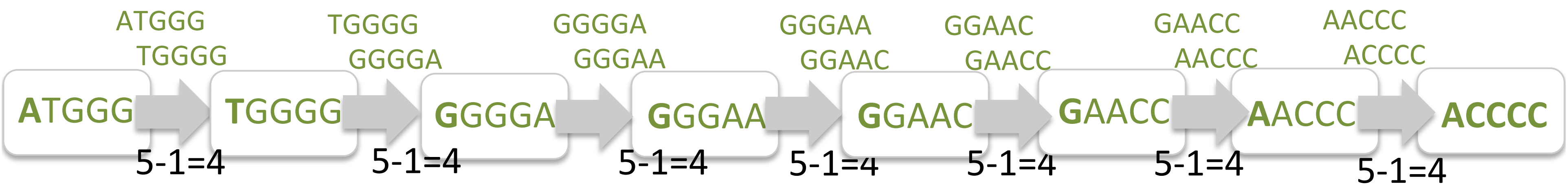
# De Bruijn graphs

- Join up all the k-mers (length = k bp) into a graph with an overlap of k-1 (here k=5)

read 1
ATGGGGAAC

read 2
GGGAACCCC

ATGGG
TGGGG
GGGGA
GGGAA
GGAAC
GAACC
AACCC
ACCCC

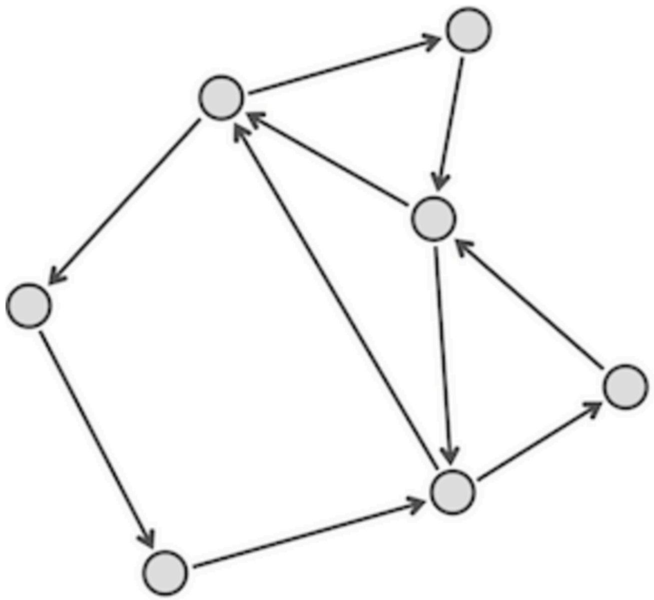


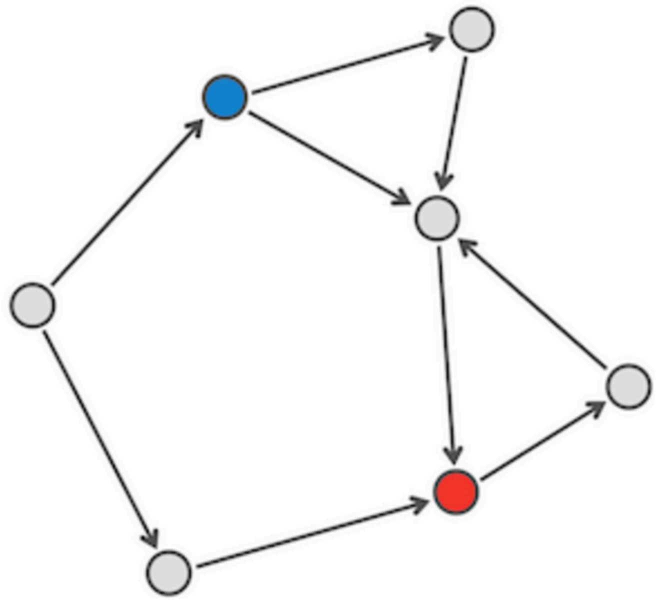

- Traverse through the graph
- The first base of each node spells out the sequence

# De Bruijn graphs

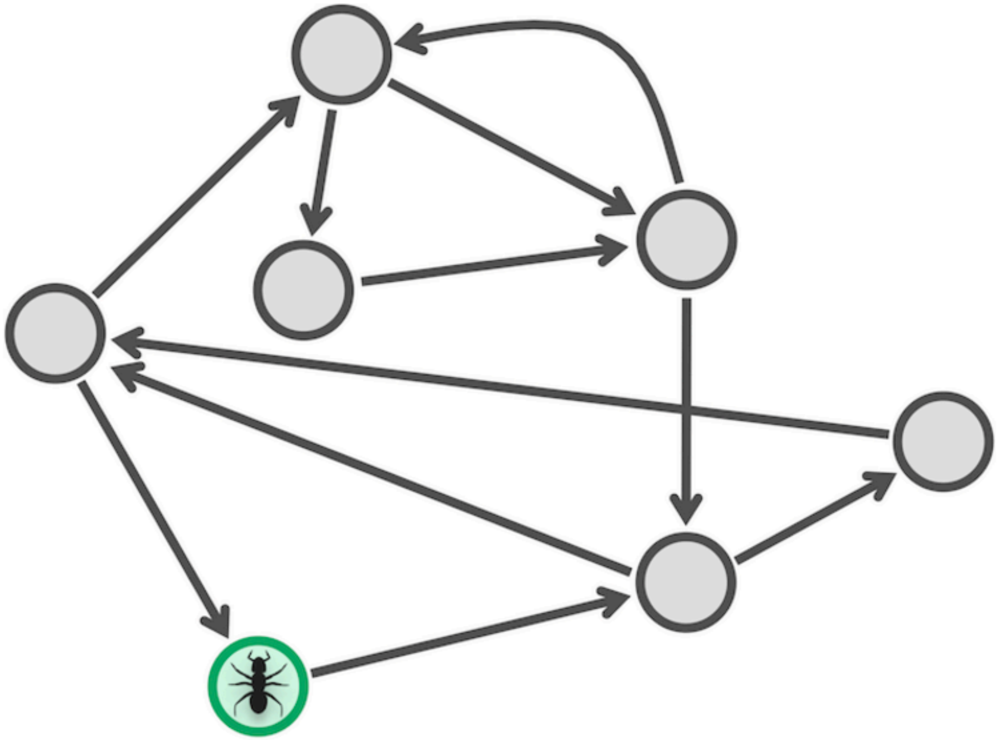
# De Bruijn graphs

Eulerian graph must be both balanced and strongly connected



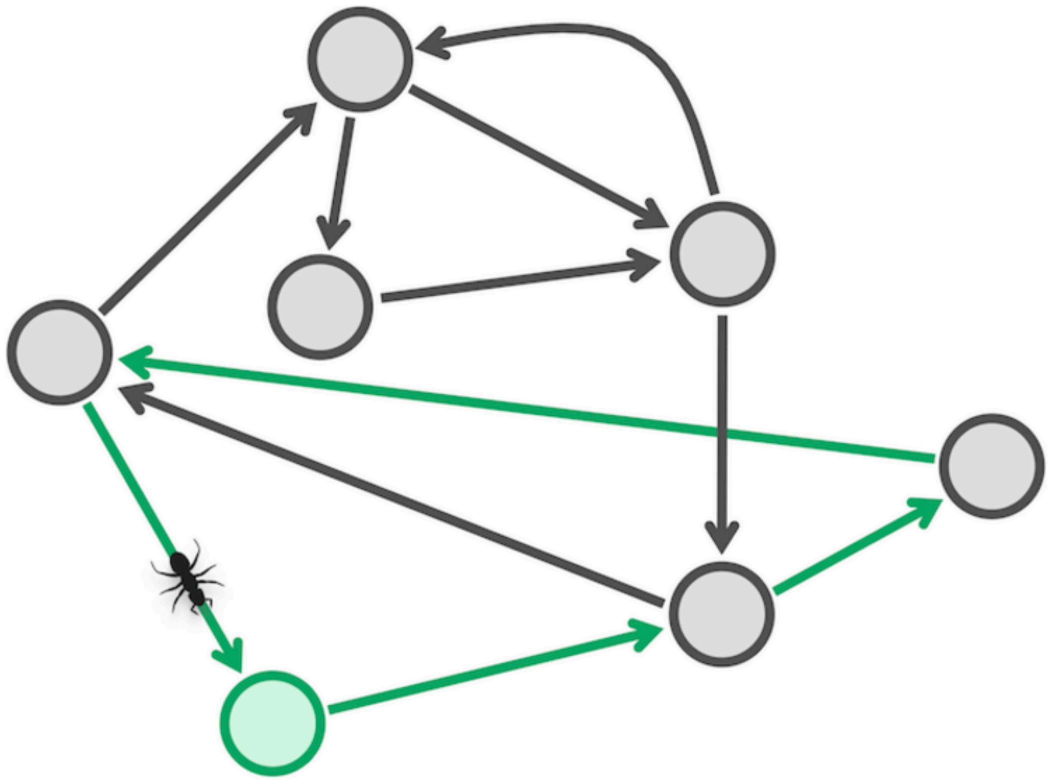


# De Bruijn graphs



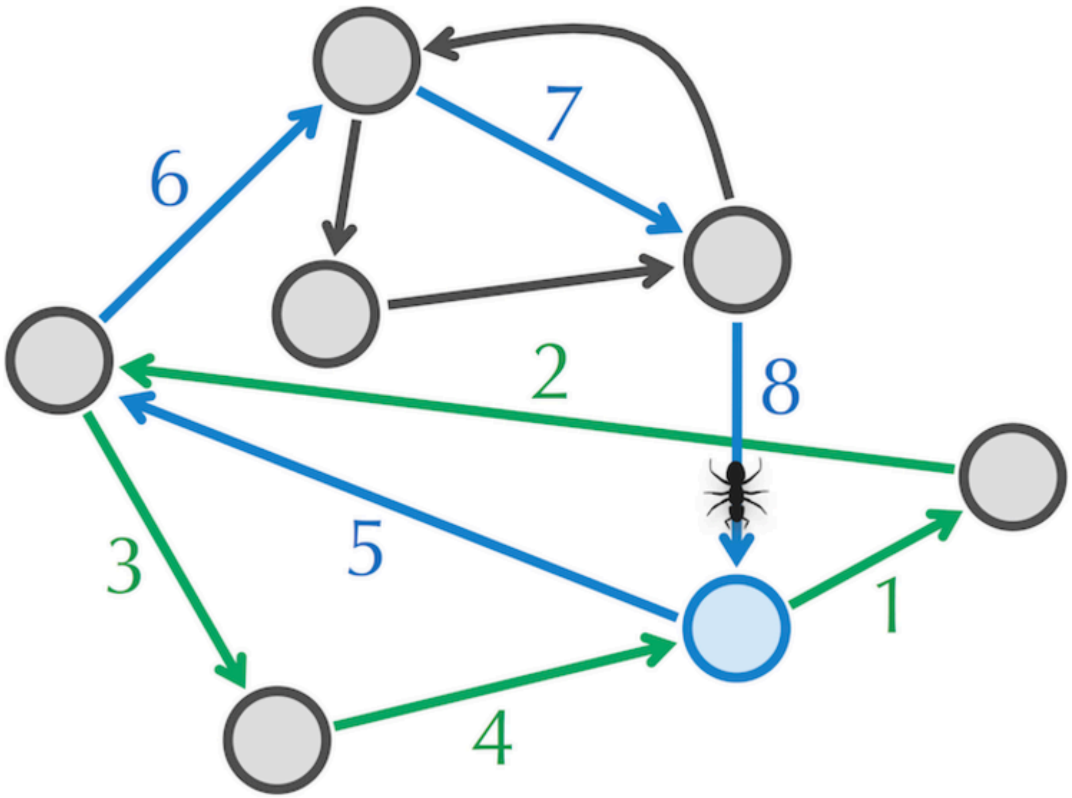
Algorithm to find a path through an Eulerian graph

# De Bruijn graphs



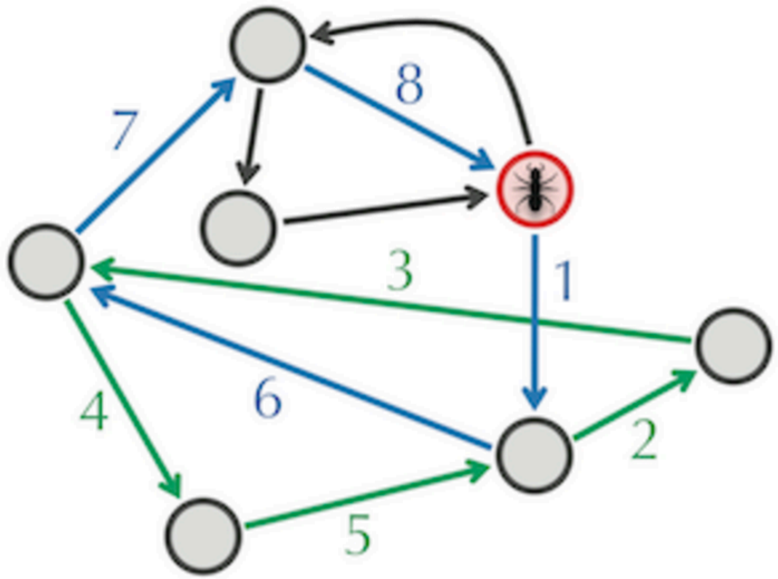
Algorithm to find a path through an Eulerian graph

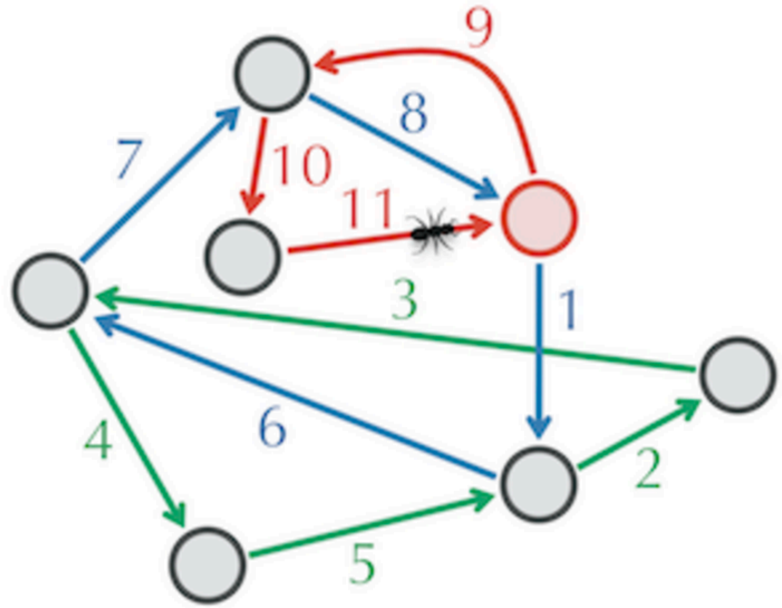
# De Bruijn graphs




Algorithm to find a path through an Eulerian graph

# De Bruijn graphs




Algorithm to find a path through an Eulerian graph

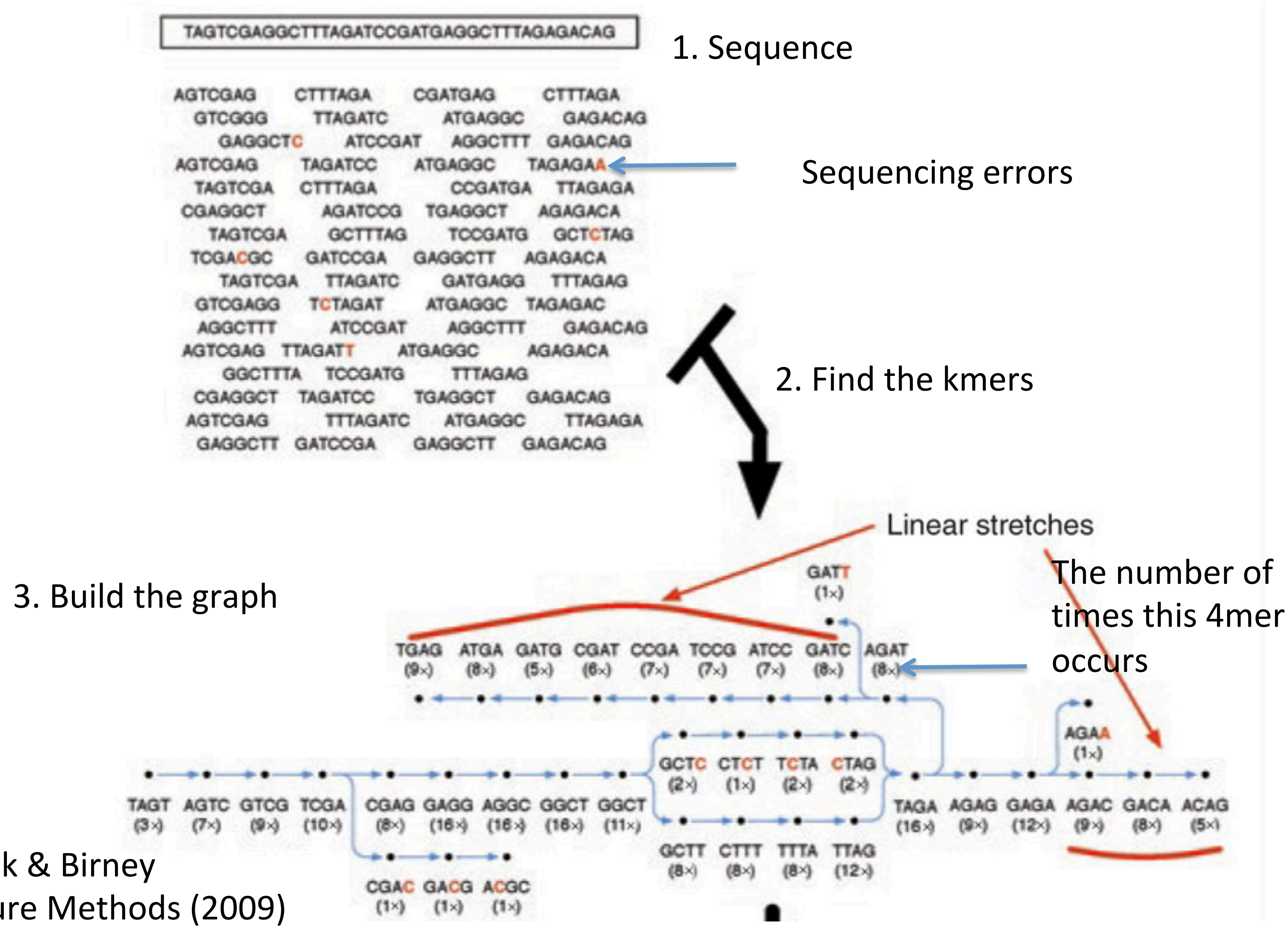
# De Bruijn graphs

Limitations of the Eulerian path:

- With “perfect” genomic data there are usually many Eulerian tours

- Data is not perfect (areas of low coverage, errors, repeats, etc.)

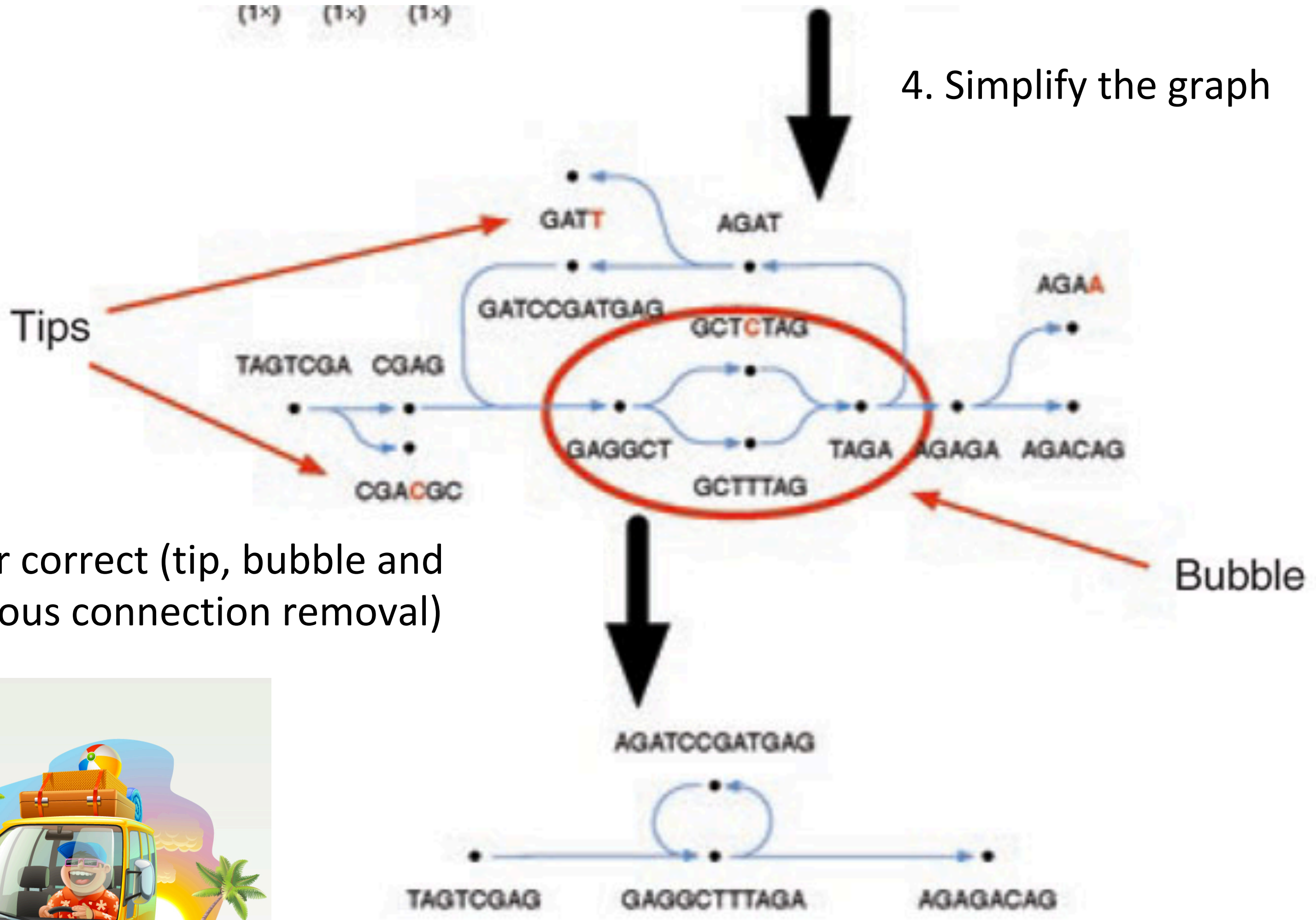
# De Bruijn graphs




Flicek & Birney
Nature Methods (2009)

# De Bruijn graphs

4. Simplify the graph




5. Error correct (tip, bubble and erroneous connection removal)

Flicek & Birney Nature Methods (2009)

# De Bruijn graphs

Advantages:
1) Set node length (no overlap algorithm)
2) Easy approaches for traversing through the graph
3) Simpler representation of repeats in the graph

Disadvantages:
1) Lose information
2) Shorter contigs

For PacBio and other long read sequences, what type of assembly strategy would you use?

# Repetitive regions

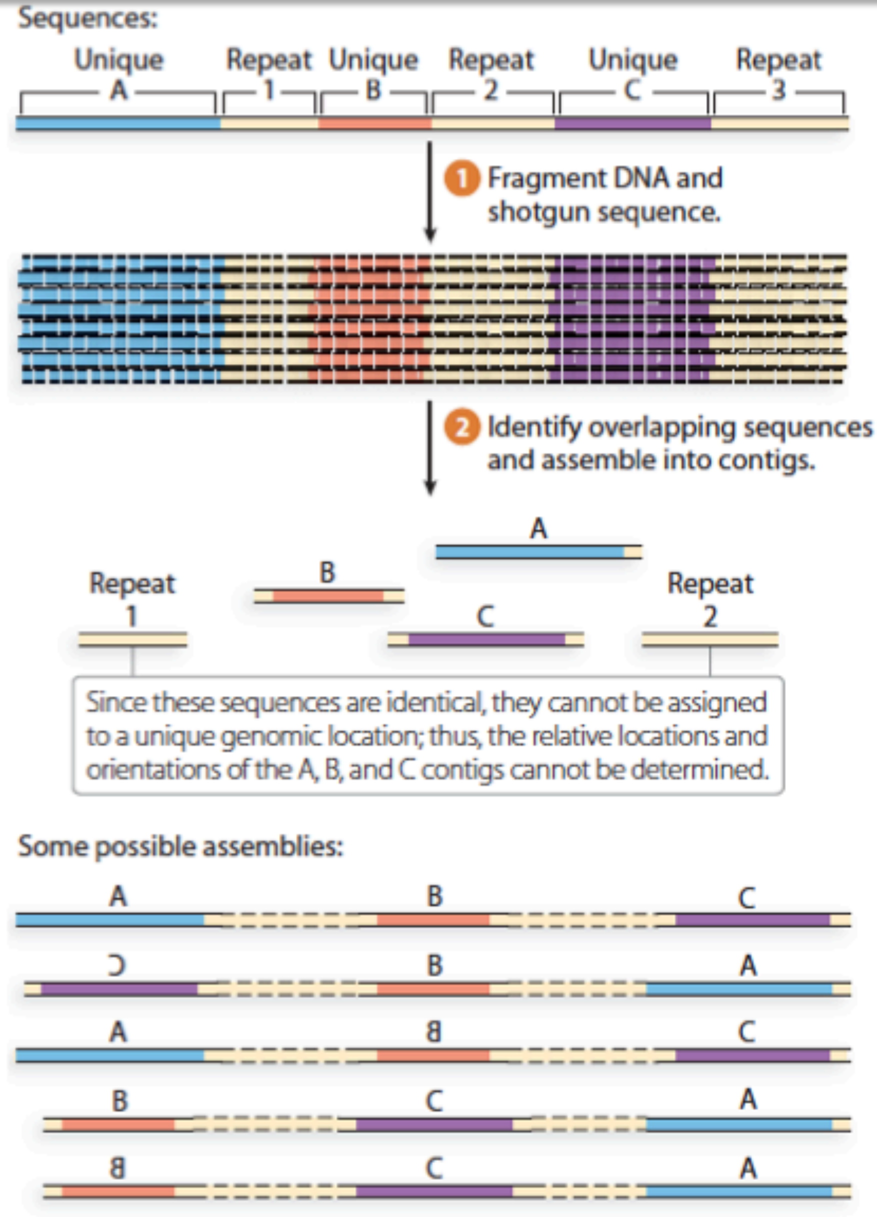



**Figure 18.2** **The problem of repetitive DNA.**

Sanders and Bowman

# Repetitive regions

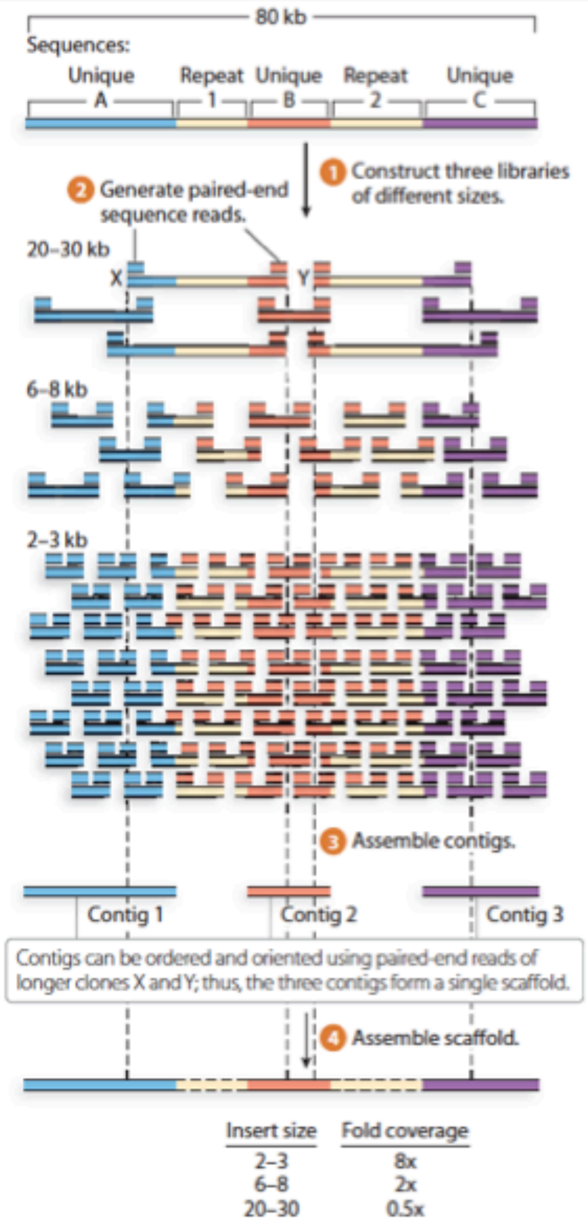


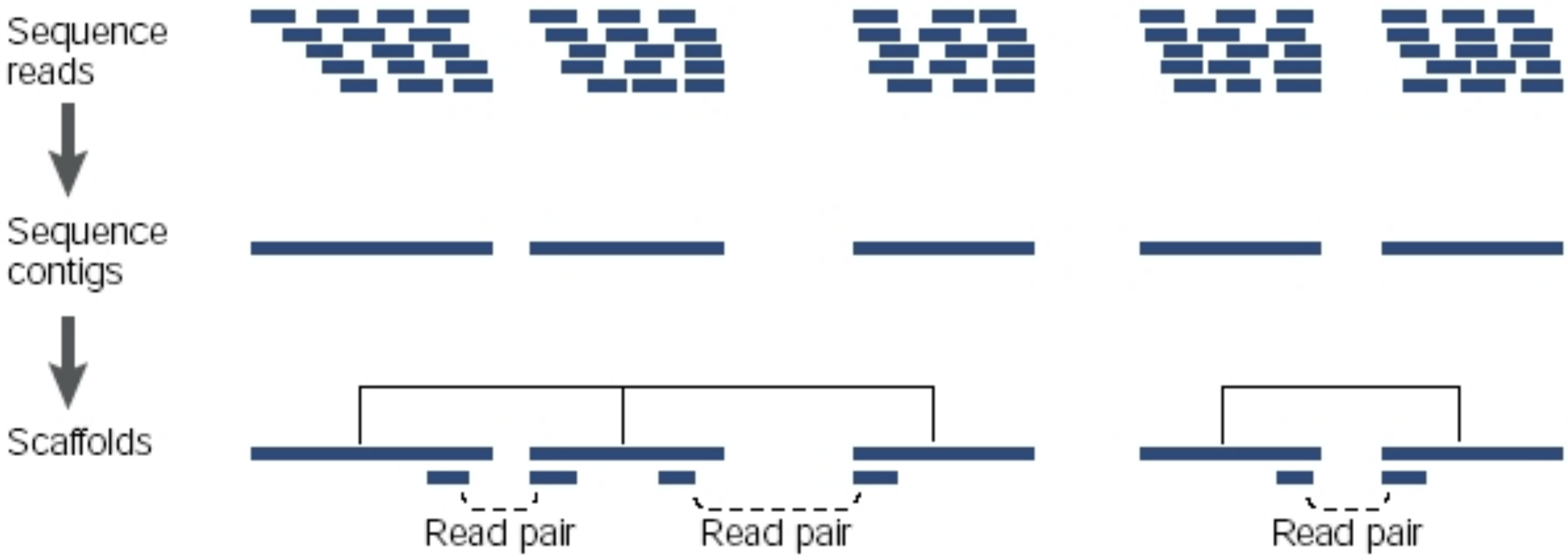

**Figure 18.3** **Paired-end shotgun sequencing strategy.**

Sanders and Bowman

# Repetitive regions

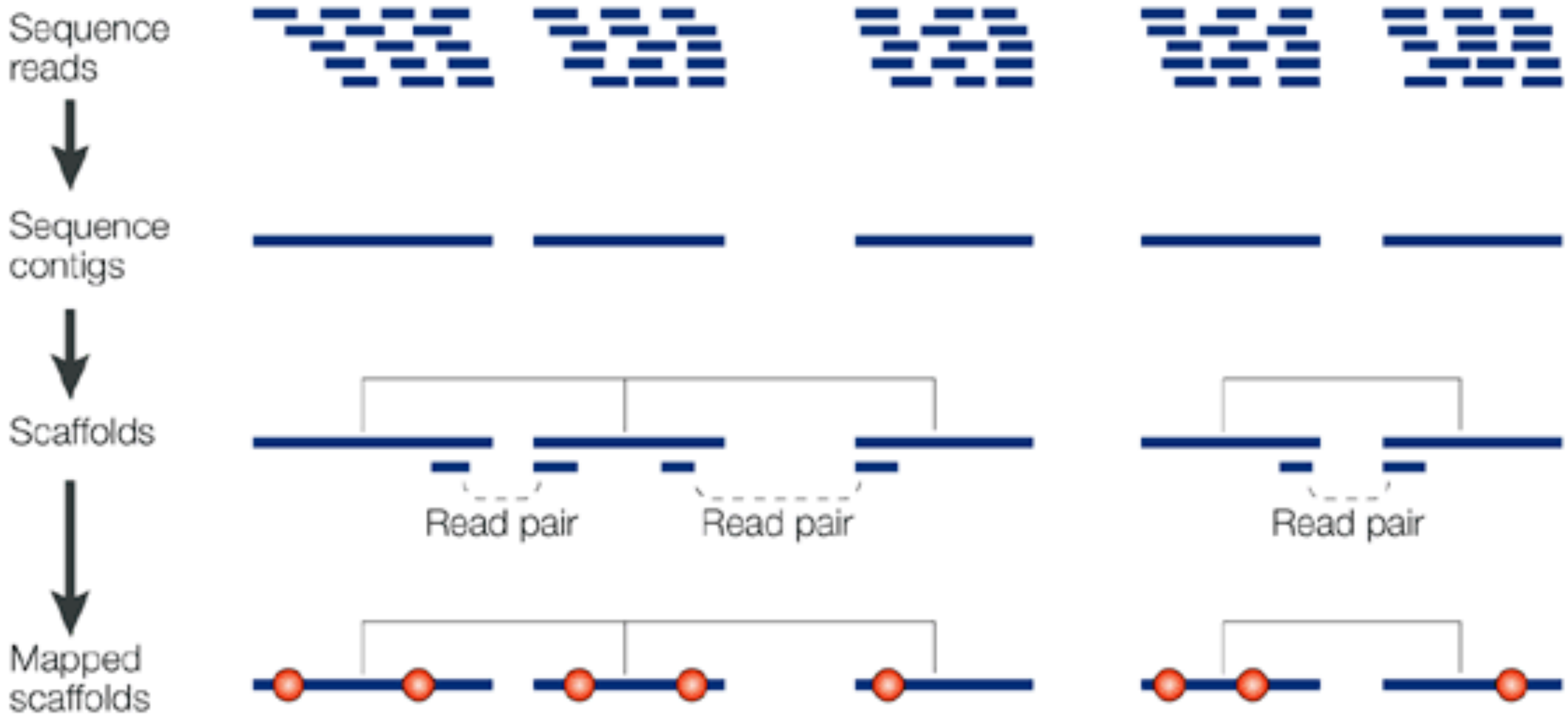
- Finishing eukaryotic genome assemblies can be challenging because much of the genome is repetitive
- This repetitive DNA breaks up the assembly and obscures the order and orientation of the assembled contigs
- Even well studied model organisms can have poorly assembled regions of their genome

# Repetitive regions




Adams 2008

# Repetitive regions




Adams 2008

# Current assembly approaches

- Long read sequencing
- Synthetic long reads
- Long-range scaffolding technologies

# Long read assemblies

Long read only *de novo* assembly. PacBio/Nanopore reads are assembled using an OLC algorithm (e.g., HGAP).( >50x PacBio)

Hybrid *de novo* assembly. Error correct long reads with more accurate short reads (e.g., PacBioToCA module of Celera) before performing long read assembly. (~20x PacBio)

Gap filling. Starting with an *existing* mate-pair based assembly, the internal gaps (consisting of Ns) inside the scaffolds are filled using PacBio sequences. (~5x PacBio)

Scaffolding. Using an *existing* assembly (such as an assembly based on short read data), PacBio reads are used to join contigs. (~5x PacBio)

# Synthetic long read assemblies

Synthetic long reads (SLRs) technologies [Illumina, 10X Genomics, Loop Genomics, and Universal Sequencing Technology (UST)]

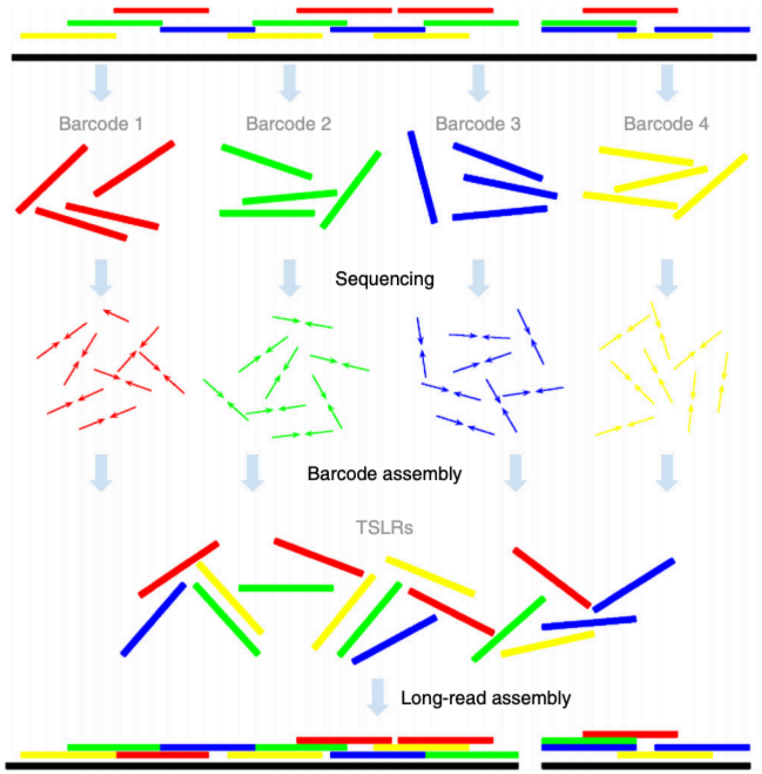


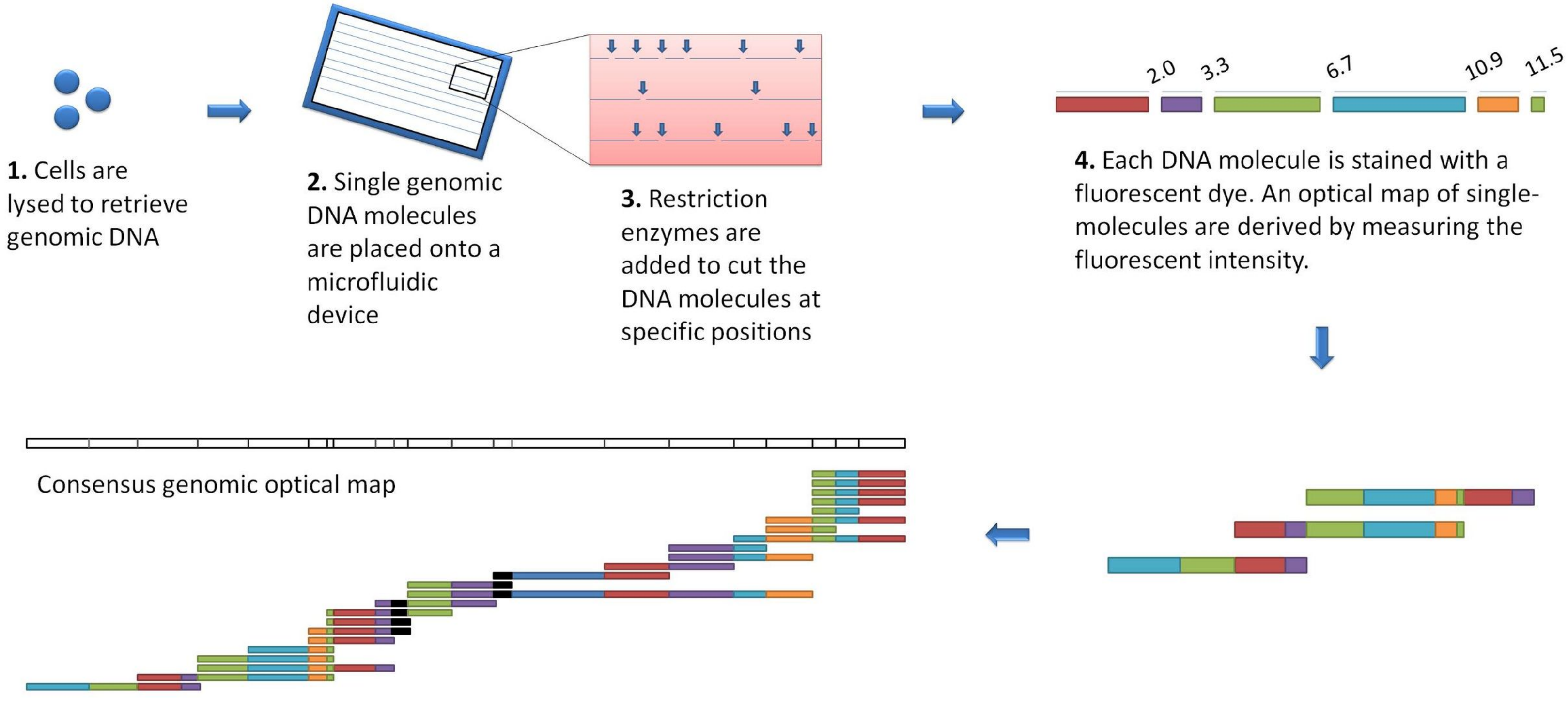

**Figure 1** | The TSLR technology. The barcode assembly step generates virtual long reads. In an idealized scenario, the barcode assembly would result in ~300 TSLRs with lengths of ~10 kb. In reality, it results in 350–450 TSLRs varying in length from 1 to 10 kb.

Anton Bankevich, & Pavel A Pevzner. (2016). TruSPAdes: Barcode assembly of TruSeq synthetic long reads. Nature Methods, 13(3), 248-250.

# Long-range scaffolding technologies

Optical mapping

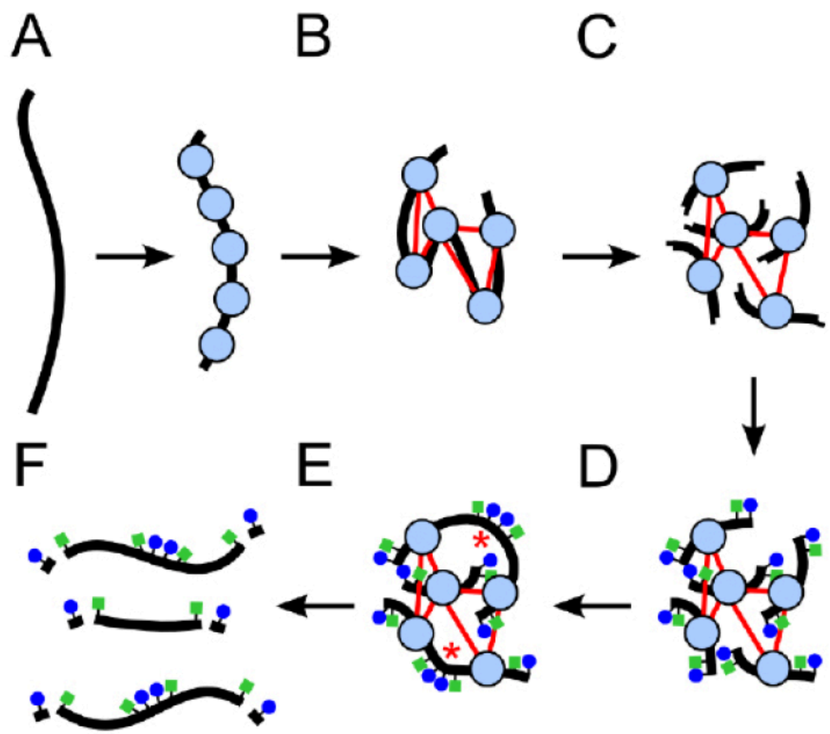
- Bionano Genomics https://vimeo.com/116090215

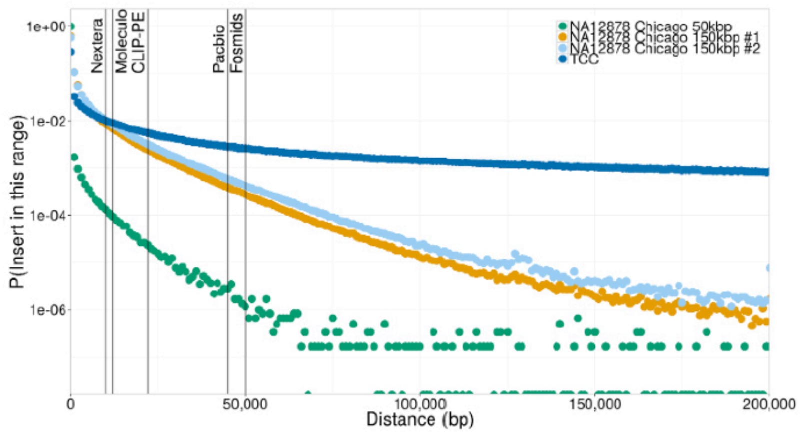



**5.** Overlapping of the multiple single-molecule maps gives us the consensus genomic optical map

# Long-range scaffolding technologies

## Chicago or Hi-C libraries (Dovetail Genomics)



# Other types of de novo assembly

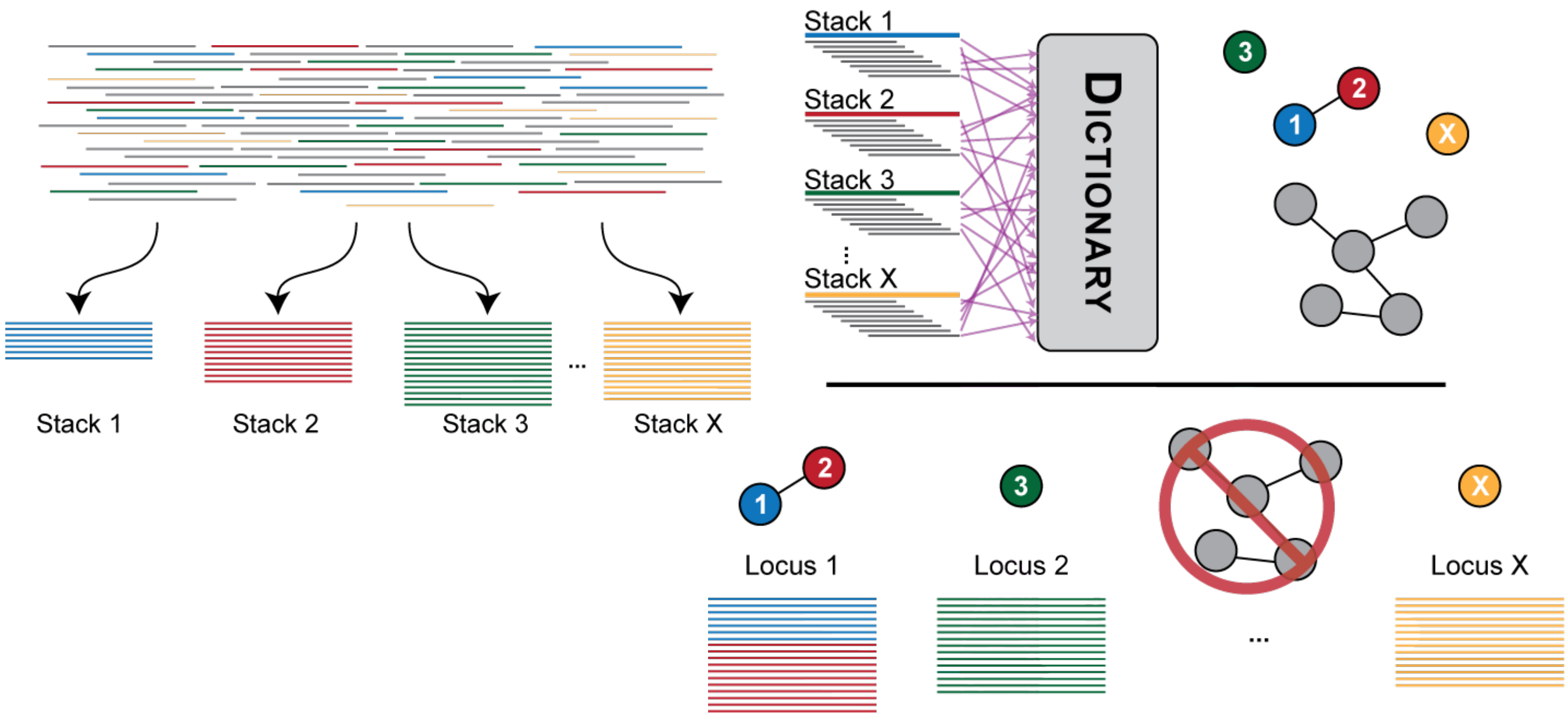
Transcriptome

- Variable coverage among genes/isoforms
- Alternative splicing
  - promotors, exons, and poly(A)

Illumina short reads – Trinity (recommended)
PacBio long reads – full length isoforms (error correct with short reads)

# Other types of de novo assembly

## De novo assembly of GBS reads: Stacks




http://catchenlab.life.illinois.edu/stacks/param_tut.php

# Further Reading

Jiao WB, Schneeberger K. 2017. The impact of third generation genomic technologies on plant genome assembly. Current Opinion in Plant Biology 36: 64–70.

Flicek, P., & Birney, E. (2009). Sense from sequence reads: methods for alignment and assembly. Nature methods, 6, S6-S12.

Zerbino DR, Birney E. Velvet: Algorithms for de novo short read assembly using de Bruijn graphs. Genome Research. 2008;18(5):821-829. doi:10.1101/gr.074492.107.

http://computing.bio.cam.ac.uk/local/doc/velvet.pdf

Li, Z., Chen, Y., Mu, D., Yuan, J., Shi, Y., Zhang, H., ... & Yang, B. (2012). Comparison of the two major classes of assembly algorithms: overlap–layout–consensus and de-bruijn-graph. Briefings in functional genomics, 11(1), 25-37.

Grabherr MG, Haas BJ, Yassour M, et al. Trinity: reconstructing a full-length transcriptome without a genome from RNA-Seq data. Nature biotechnology. 2011;29(7):644-652. doi:10.1038/nbt.1883.

https://github.com/trinityrnaseq/trinityrnaseq/wiki

J. Catchen, A. Amores, P. Hohenlohe, W. Cresko, and J. Postlethwait. Stacks: building and genotyping loci de novo from short-read sequences. G3: Genes, Genomes, Genetics, 1:171-182, 2011.

http://catchenlab.life.illinois.edu/stacks/

# Tutorial

Today you will use the genome assembly program Velvet to assemble a bacterial genome.

Velvet overview:

1. Hash k-mers
2. Construct the graph
3. Correct for errors
4. Resolve the repeats

Refer to Github page or open /home/biol525d/Topic_5/README.txt and follow the instructions

# Tutorial

1) Given the above information, what is the expected coverage?

2) For a k-mer of 21 what is the k-mer coverage for this genome assembly?

3) Can you think of other ways to assess assembly quality? What might be the trouble with only focusing on maximizing N50? Discuss this with your group.

4) Quantify the assembly metrics for your first assembly that you ran without any options. In your group of four, each person should pick different sets of parameters to run. Compare the resulting assemblies with one another and discuss which ones seemed to have improved the assembly and why that might be. Be prepared to share your findings with the class.

# For next class

- Make sure R and Rstudio are installed and working on your computer
- Go over Greg's short R tutorial (Topic 2) if you are not familiar with R

1. ABySS (Assembly By Short Sequencing) (Birol et al): A denovo assembler for short read sequence data which uses a distributed representation of a de Bruijn graph, allowing parallel computation of the assembly algorithm across a network of commodity computers. Developed at Canada's Michael Smith Genome Sciences Centre.

2. ALLPATHS-LG (Gnerre et al): a de Bruijn graph-based *de novo* assembler for large (and small) genomes. ALLPATHS-LG is being developed by scientists at the Broad Institute.

3. Bambus2: The second generation Bambus scaffolder relies on a combination of a novel method for detecting genomic repeats and algorithms that analyze assembly graphs to identify biologically meaningful genomic variants. Bambus2 compares favorably to existing scaffolds generated by CABOG, Newbler and SOAPdenovo with respect to contiguity and error rate. While Bambus 2 was specifically designed for polymorphic and metagenomic scaffolding, its modular and efficient algorithm allows it to be used to scaffold mammalian genomes and used a drop-in replacement scaffolder for CABOG, Newbler, and SOAPdenovo. Bambus2 is being primarily developed by Sergey Koren and Mihai Pop, with input from Todd Treangen,

4. Celera Assembler: an Overlap-Layout-Consenus based de novo whole-genome shotgun (WGS) DNA sequence assembler. It reconstructs long sequences of genomic DNA from fragmentary data produced by whole-genome shotgun sequencing. Celera Assembler has enabled many advances in genomics, including the first whole genome shotgun sequence of a multi-cellular organism (Myers 2000) and the first diploid sequence of an individual human (Levy 2007). Celera Assembler was developed at Celera Genomics starting in 1999. It was released to SourceForge in 2004 as the wgs-assembler under the GNU General Public License. The pipeline revised for 454 data was named CABOG (Miller 2008).

5. MSR-CA (pronounced "MizerKa") is a new technique that pre-processes the short read data and then performs the final assembly using a modified version of Celera Assembler. MSR-CA stands for Maryland Super-Reads + Celera Assembler. The pre-processing steps include error correction and subsequent coverage reduction by creating "super-reads," which are produced using a de Bruijn graph. The algorithm then groups together the reads that map to the same sets of nodes and edges, and for each set replaces them by a single super-read that contains these nodes and edges. This can reduce the number of reads by a factor of 50 or more, resulting in the data set that is much easier to manage.

6. SGA (Simpson et al): stands for String Graph Assembler. Experimental de novo assembler based on string graphs. SGA is being developed by scientists at the Wellcome Trust Sanger Institute.

7. SOAPdenovo (Li et al): is the short-read assembler that was used for the panda genome, the first mammalian genome assembled entirely from Illumina reads, and for several human genomes and other genomes subsequently. It is being developed by scientists at BGI.

8. Velvet (Zerbino et al): Velvet is a *de novo* genome assembler specially designed for short read sequencing technologies, particularly Illumina reads, and was one of the first short-read assemblers to be published. It was developed by Daniel Zerbino and Ewan Birney at the European Bioinformatics Institute (EMBL-EBI), near Cambridge, England.

| Assembler | Contigs | | | | Scaffolds | | | |
|---|---|---|---|---|---|---|---|---|
| | Num | N50 (kb) | Errors | N50 corr. (kb) | Num | N50 (kb) | Errors | N50 corr. (kb) |
| ABySS | 302 | 29.2 | 19 | 24.8 | 246 | 34 | 1 | 28 |
| Allpaths-LG | **60** | 96.7 | 20 | **66.2** | **12** | 1,092 | **0** | **1,092** |
| Bambus2 | 109 | 50.2 | 190 | 16.7 | 17 | 1,084 | **0** | 1,084 |
| CABOG | Could not run: incompatible read lengths in one library | | | | | | | |
| MSR-CA | 94 | 59.2 | 34 | 48.2 | 17 | **2,412** | 3 | 1,022 |
| SGA | 1252 | 4.0 | **10** | 4.0 | 456 | 208 | 1 | 208 |
| SOAPdenovo | 107 | **288.2** | 65 | 62.7 | 99 | 332 | **0** | 288 |
| Velvet | 162 | 48.4 | 42 | 41.5 | 45 | 762 | 17 | 126 |