CS4330: Combinatorial Methods in Bioinformatics

Primer on de novo genome assembly

Wong Limsoon

Types of genome assembly

Sequencing produces reads which are quite short

These reads need to be assembled into a genome

De novo assembly

Mapping or reference-based assembly

Reference-guided assembly for long reads

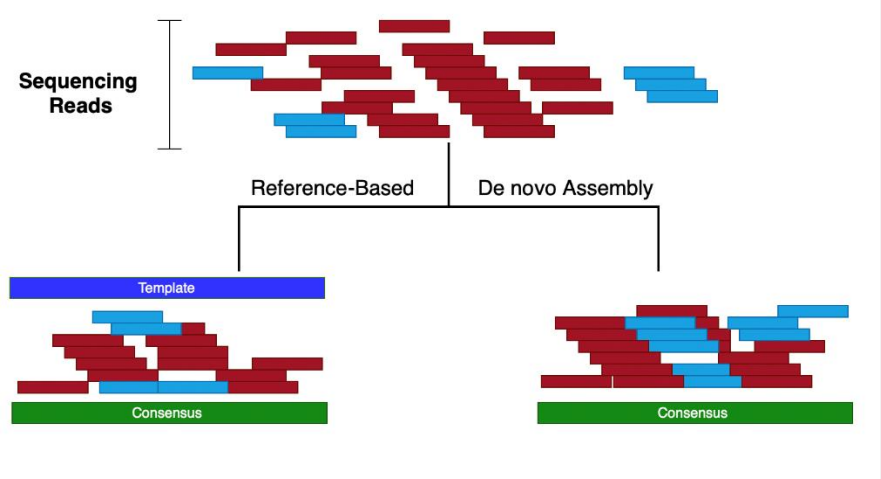


Image credit: Wikipedia

De novo genome assembly

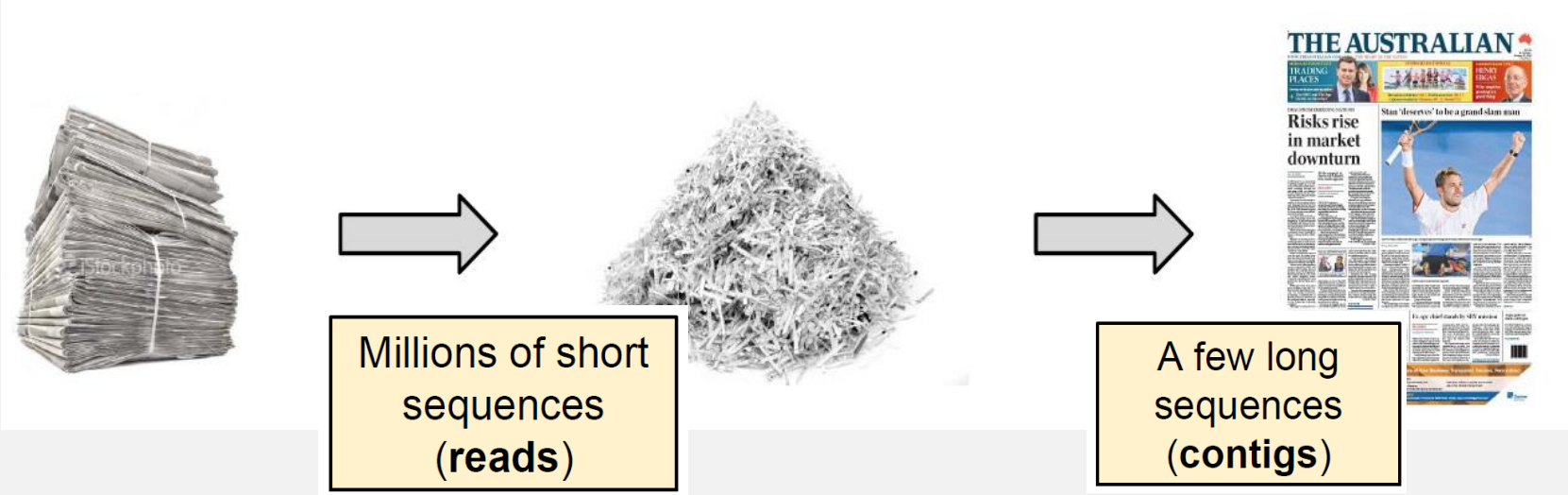


Image credit: Torsten Seemann

Reconstruct original genome from sequence reads only

“Shakespearomics”

Original text

“Friends, Romans, countrymen, lend me your ears”

-

- ***Reads***

```
ds, Romans, count
ns, countrymen, le
Friends, Rom
send me your ears;
crymen, lend me
```

- ***Overlaps***

```
Friends, Rom
     ds, Romans, court
             ns, courtrymen, le
                     crymen, lend me
                             send me your ears;
```

- ***Majority consensus***

```
Friends, Romans, courtrymen, lend me your ears;
```

(1 contig)

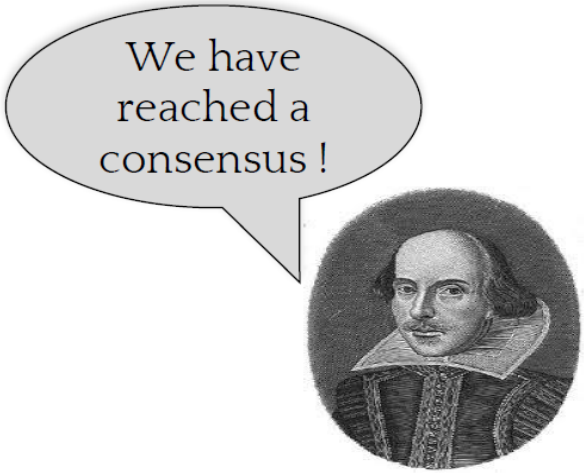
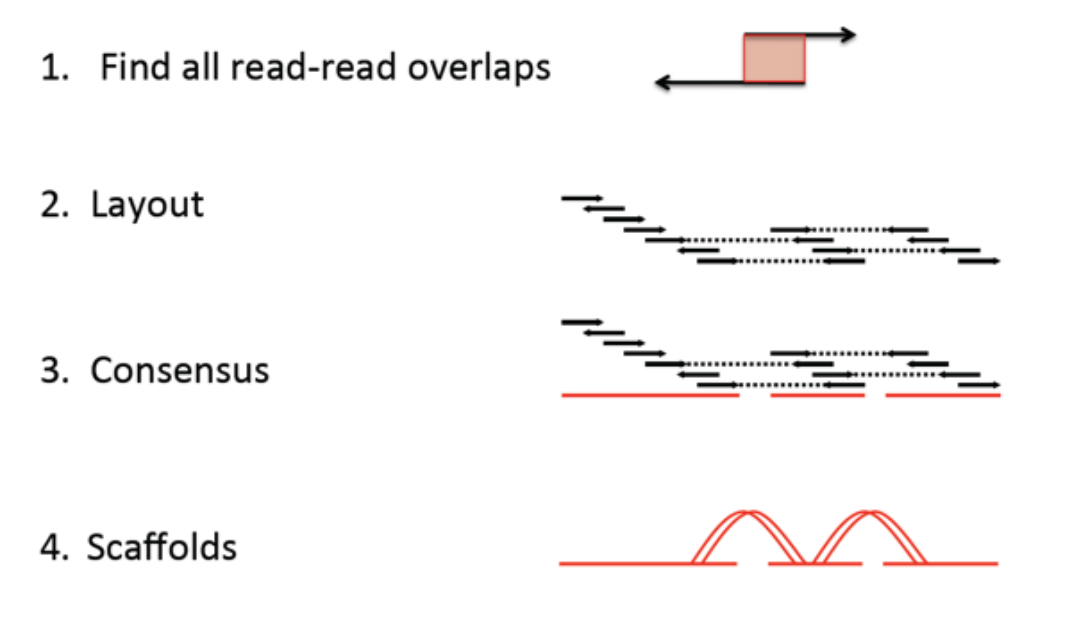


Image credit: Torsten Seemann

Overlap layout consensus

Overlap layout consensus is an assembly method that takes all reads and finds overlaps between them, then builds a consensus sequence from the aligned overlapping reads



https://bioinformaticsworkbook.org/dataAnalysis/GenomeAssembly/Intro_GenomeAssembly.html#gsc.tab=0

De Bruijn graph (DBG) & k-mers

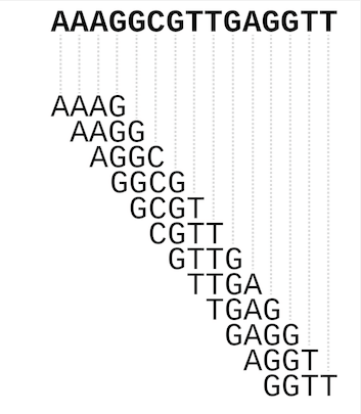
Chop the reads into k-mers

Construct DBG from k-mers

Representing a sequence in terms of its k-mer components

Find Eulerian/Hamiltonian path in graph

Derive the genome sequence from path



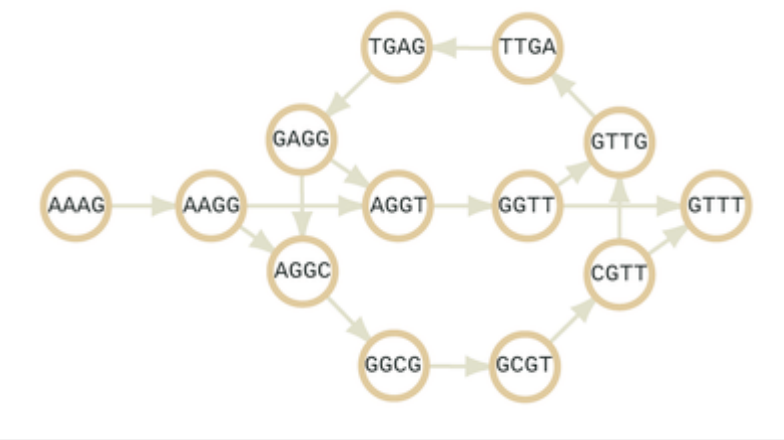


Image credit: Jang Il Sohn

Genome assembly is hard

A puzzle with millions to billions of pieces (N)

Read length is short relative to genome (10^2 vs 10^9 bp)

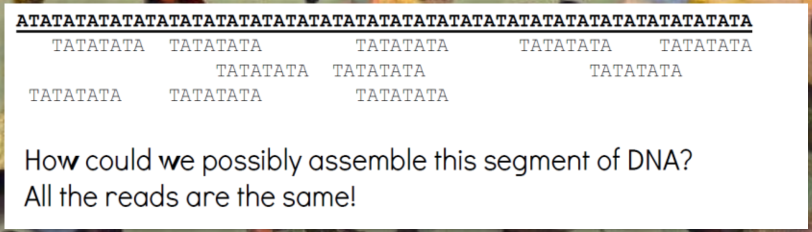
Lots of overlap to check

Compare each pair of pieces to find overlap (N^2)

Lots of sky; i.e. short repeats

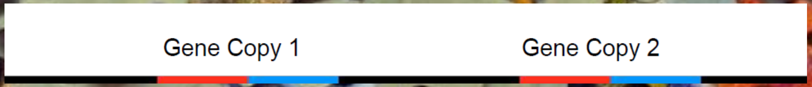
Lots of dirty pieces; i.e. sequencing errors

Multiple copies; i.e. long repeats

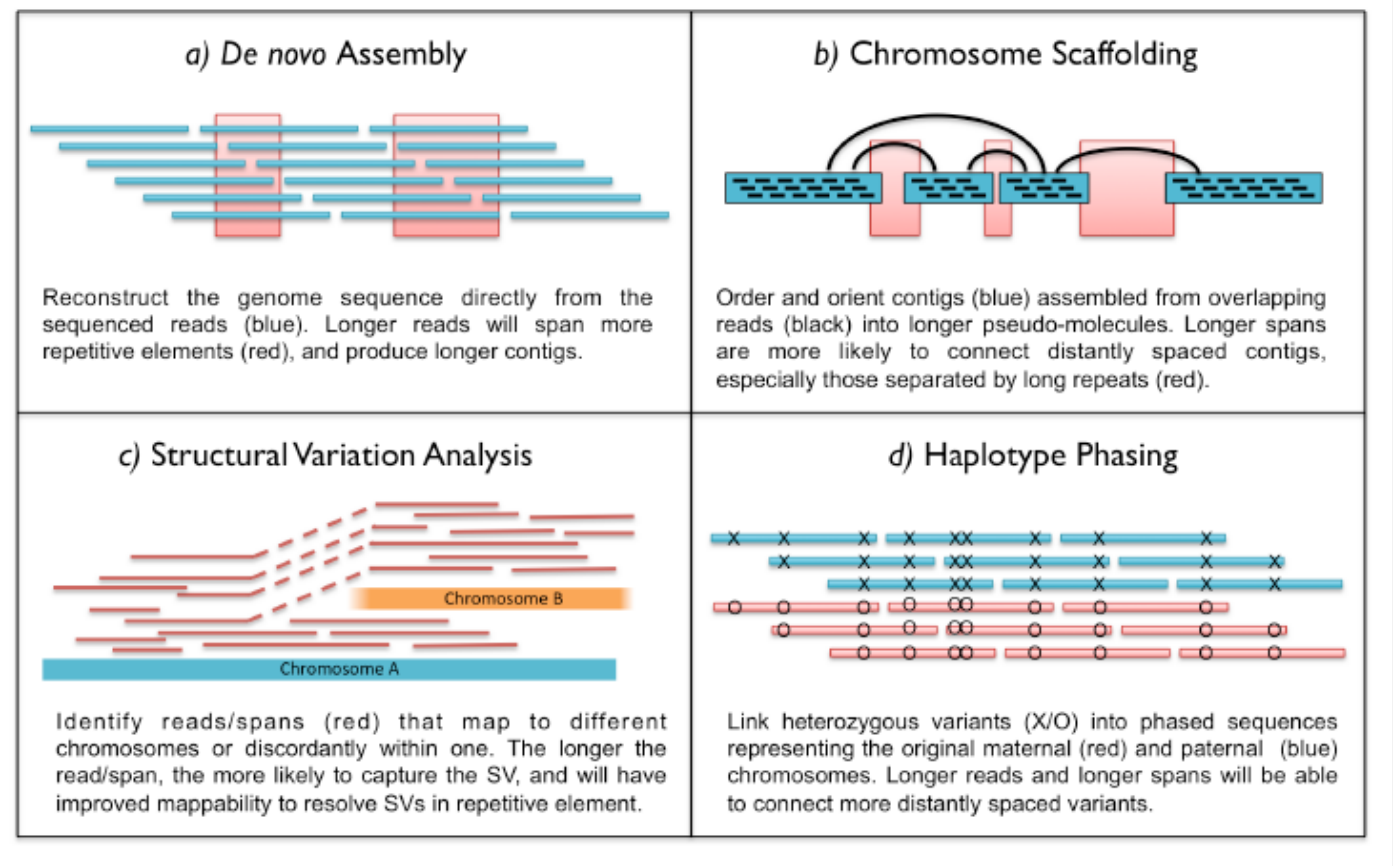


```
ATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATA
   TATATATA  TATATATA        TATATATA      TATATATA    TATATATA
                 TATATATA  TATATATA              TATATATA
 TATATATA    TATATATA        TATATATA
```

How could we possibly assemble this segment of DNA?
All the reads are the same!



Some of these difficulties are mitigated by 3rd-gen sequencing's long read length



https://doi.org/10.1101/048603

To do the next exercise, you need to know a little about cell-free DNA

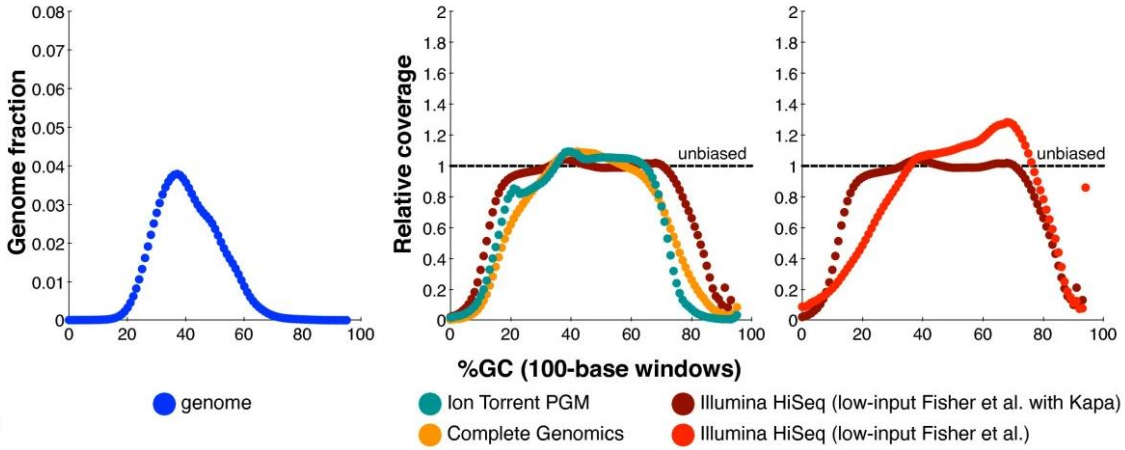
Let's watch this video on cell-free DNA together

https://www.youtube.com/watch?v=F8eNSMWI01g

Watch the first 2-8 minutes

https://www.youtube.com/watch?v=RtabLZcjXDo

Exercise



Does the GC content bias in DNA sequencing coverage have a big impact on genome assembly?

Does it have a big impact on tumour purity determination from cell-free DNA?

Does it have a big impact on non-invasive pre-natal testing using cell-free DNA?

Popular tools

Check these out yourself

1. **SPAdes (St. Petersburg genome assembler):**
   - Website: http://cab.spbu.ru/software/spades/
   - SPAdes is designed to work with various sequencing technologies, including Illumina, Ion Torrent, and PacBio.
2. **Velvet:**
   - Website: https://www.ebi.ac.uk/~zerbino/velvet/
   - Velvet is known for its efficiency in handling large datasets and is often used for short-read de novo assembly.
3. **SOAPdenovo:**
   - Website: https://soap.genomics.org.cn/soapdenovo.html
   - SOAPdenovo is a widely used assembler for de novo genome assembly, and it supports large genomes.
4. **ALLPATHS-LG:**
   - Website: https://software.broadinstitute.org/allpaths-lg/blog/
   - ALLPATHS-LG is particularly useful for high-coverage data and is known for its accuracy in assembling large genomes.
5. **Canu:**
   - Website: https://canu.readthedocs.io/en/latest/
   - Canu is specifically designed for single-molecule sequencing technologies like PacBio and Oxford Nanopore.

Popular tools

Check these out yourself

6. **MaSuRCA (Maryland Super-Read Celera Assembler):**
   - Website: http://www.genome.umd.edu/masurca.html
   - MaSuRCA is designed to assemble genomes from a combination of Illumina and long reads.
7. **ABySS (Assembly By Short Sequences):**
   - Website: https://www.bcgsc.ca/platform/bioinfo/software/abyss
   - ABySS is suitable for assembling genomes using short-read sequencing data.
8. **IDBA (Iterative De Bruijn Graph De Novo Assembler):**
   - Website: http://i.cs.hku.hk/~alse/hkubrg/projects/idba/
   - IDBA is designed to handle metagenomic data and short-read sequences.
9. **Megahit:**
   - Website: https://github.com/voutcn/megahit
   - Megahit is a fast and memory-efficient assembler, often used for assembling large and complex metagenomic datasets.
10. **Unicycler:**
    - Website: https://github.com/rrwick/Unicycler
    - Unicycler is designed for bacterial genome assembly and can handle both short and long reads.

Self-guided practice

Bilal Wajid & Erchin Serpedin, “Do it yourself guide to genome assembly”, *Briefings in Functional Genomics* 15(1):1-9, 2016. Supplementary section

Good to read

Sequence assembly. https://en.wikipedia.org/wiki/Sequence_assembly

B. Wajid & E. Serpedin, “Do it yourself guide to genome assembly”, Briefings in Functional Genomics, 15(1):1-9, 2016. https://doi.org/10.1093/bfgp/elu042