
DTU

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Bioinformatics

de novo assembly

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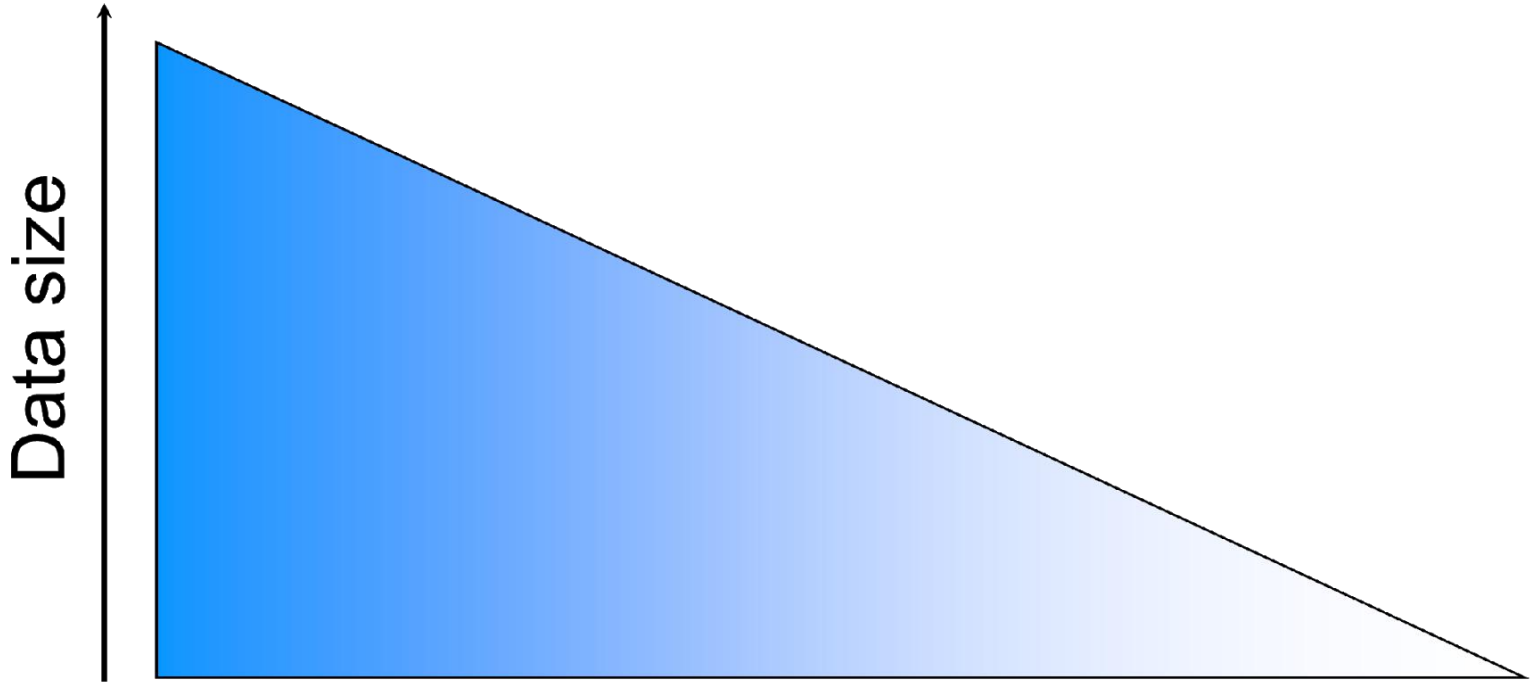
Menu

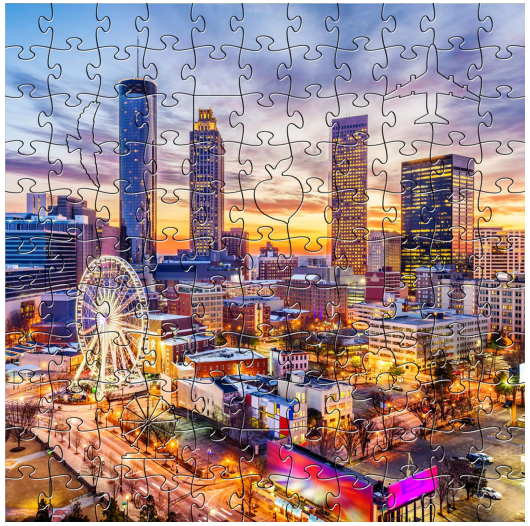
- Assembly approaches
- Assembly graphs
- Graph postprocessing filtering
- The woes of repetition
- Benchmarking your assembly

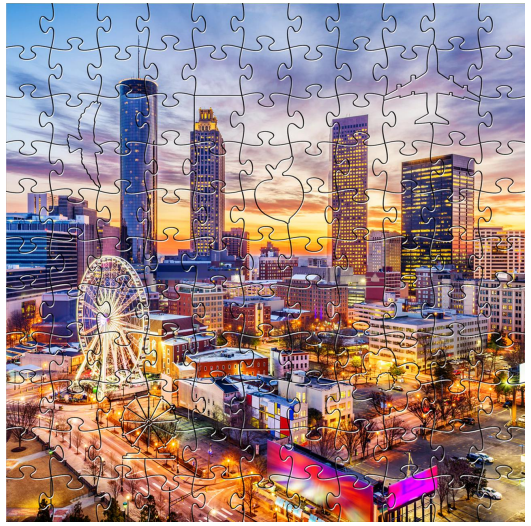
Generalized NGS analysis



Question | Raw reads | Pre-processing | Assembly: Alignment / *de novo* | Application specific: Variant calling, count matrix, ... | Compare samples / methods | Answer?

Alignment vs *de novo* assembly



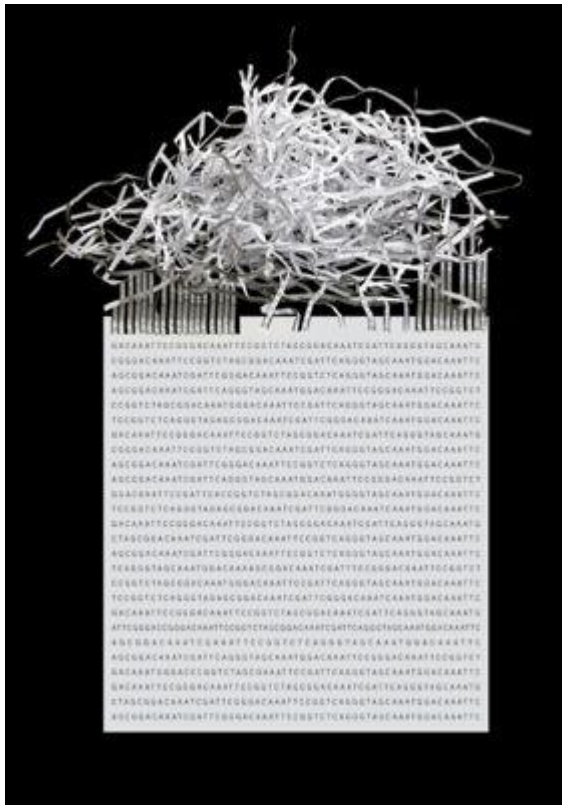


Alignment vs *de novo* assembly

What is *de novo* assembly?

Merge small DNA fragments together so they form a previously unknown sequence

Merge millions reads together so they form previously unknown sequences



de novo assembly

- Assemble reads into longer fragments
- Find overlap between reads
- Many approaches

Rethinking assembly: Shortest superstring problem

Given a set of strings, find the shortest string that contains all the strings as substrings.

This is known as the shortest superstring problem (SSP)

SSP is NP-hard

Which approaches?

- Greedy (“Simple” approach)
- Overlap-Layout-Consensus (OLC)
- de Bruijn graphs

Simple approach - Greedy

- Principle:
    1. Pairwise alignment of all reads
    2. Identify fragments that have largest overlap
    3. Merge these
    4. Repeat until all overlaps are used
- Can only resolve repeats smaller than read length
- High computational cost with increasing no. reads

Reads > Contigs > Scaffolds

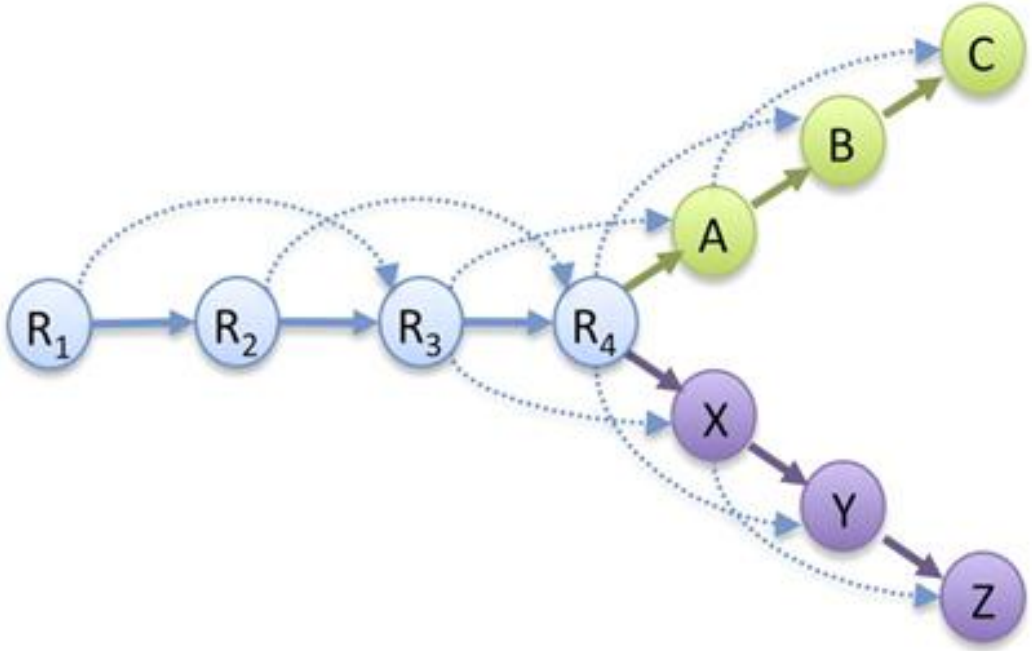
- **Overlap Layout Consensus** and **de Bruijn** use a similar general approach.

1. Try to correct sequence errors in reads with high coverage
2. Assemble reads to contiguous sequence fragments “contigs”
3. Identify repeat contigs
4. Combine and order contigs to “scaffolds”, with gaps representing regions of uncertainty

Overlap-Layout-Consensus

- Create overlap graph by all-vs-all alignment (Overlap)
- Build graph where each node is a read, edges are overlaps between reads (Layout)

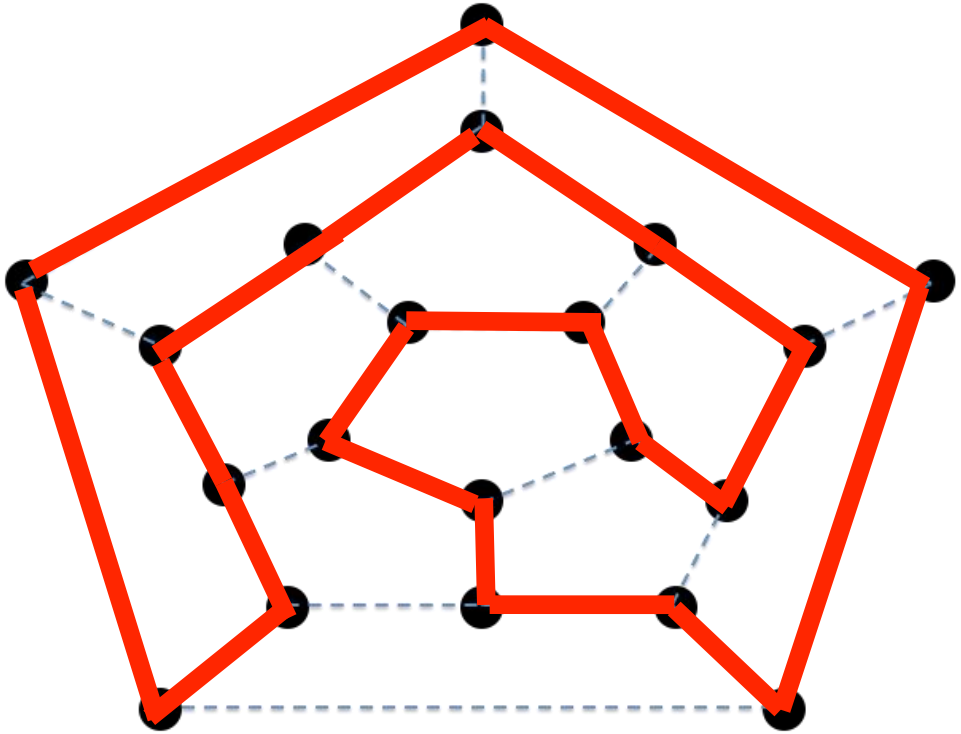
```
R1: GACCTACA
R2:   ACCTACAA
R3:    CCTACAAG
R4:     CTACAAGT
A:       TACAAGTT
B:        ACAAGTTA
C:         CAAGTTAG
X:       TACAAGTC
Y:        ACAAGTCC
Z:         CAAGTCCG
```



Schatz et al., Genome Res, 2010

Overlap-Layout-Consensus

- Create consensus sequence
- We need to use **graph theory** to solve the graph
- *Walk the Hamiltonian path*
- Eg. visit each node *exactly once*



Imagine trying to solve this for a graph of hundred of thousands of nodes (=reads)

Overlap-Layout-Consensus

- Not good with many short reads -> lots of alignment!
- With short read lengths, hard to resolve repeats

- Good for large read lengths:
  – PacBio, Oxford Nanopore, 10X Genomics, 454, Ion Torrent, Sanger
- Example assemblers: Canu, Celera, Newbler

de Bruijn graph

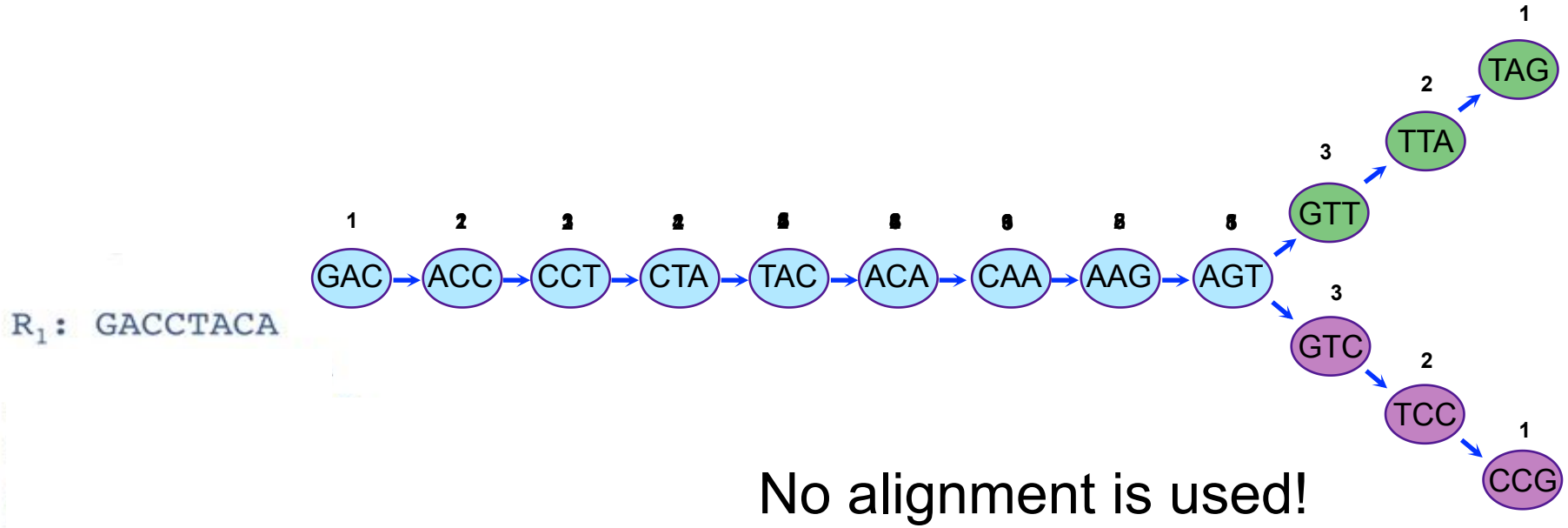
- Directed graph of overlapping items (here DNA sequences)
- Instead of comparing reads, decompose reads into *k*-mers
  - Graph is created by mapping the *k*-mers to the graph
  - Each *k*-mer only exists once in the graph
  - Problem is reduced to walking Eulerian path (visiting each edge once) - this is a solvable problem

Drawbacks ...

– Lots of RAM required (**1-1000 GB !**)
– Optimal *k* can not be identified *a priori*, must be experimentally tested for each dataset
– small *k*: very complex graph, large *k*: limited overlap in low coverage areas
– Iterative approach to find best assembly

How is the graph constructed?

- Same 10 reads, extract *k*-mers from reads and map onto graph, *k* = 3:



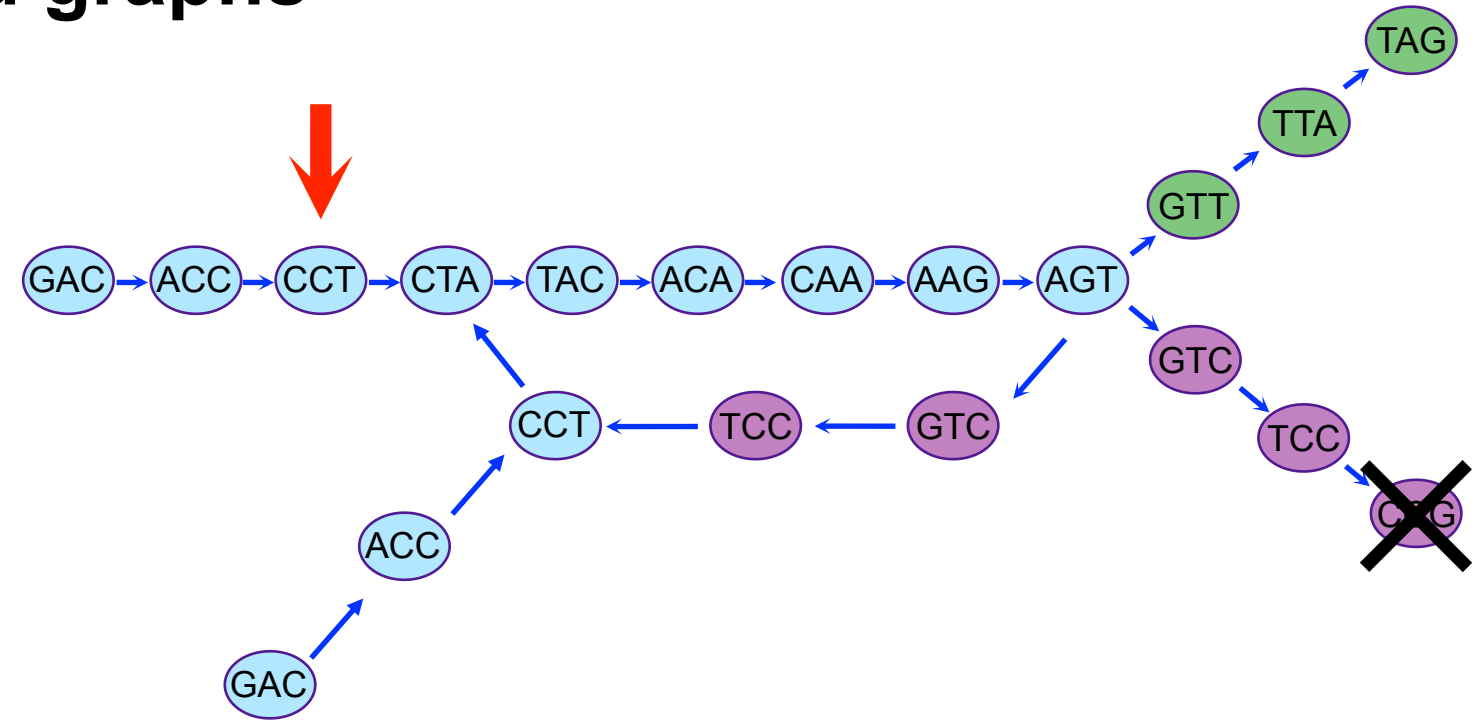
No alignment is used!

Different assemblers uses different modifications of the de Bruijn graphs

Complicated graphs

```
R1: GACCTACA
R2:   ACCTACAA
R3:    CCTACAAG
R4:     CTACAAGT
A:       TACAAGTT
B:        ACAAGTTA
C:         CAAGTTAG
X:       TACAAGTC
Y:        ACAAGTCC
Z:         CAAGTCCT
```

G to T

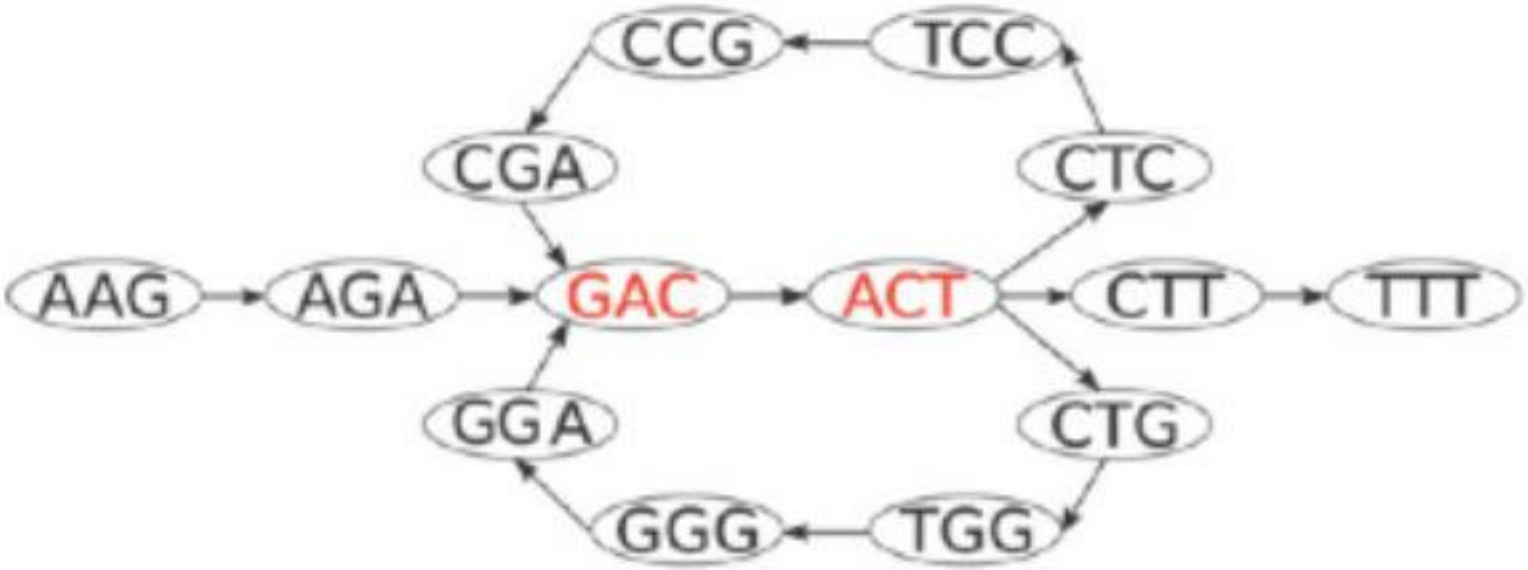


Large genomes with many repeats/errors creates very large graphs

Create the *de* Bruijn graph of this genome using *k*=3

AAGACTCCGACTGGGACTTT

AAGACTCCGACTGGGACTTT

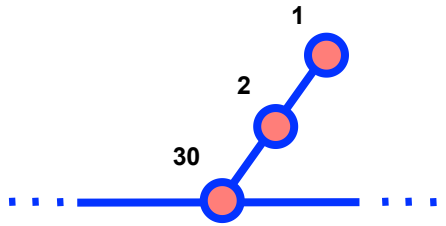


A de Bruijn graph of a sequence

After building: Simplify

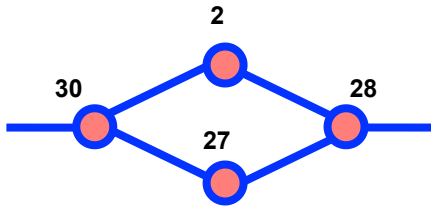
Clip tips

(seq err, end)

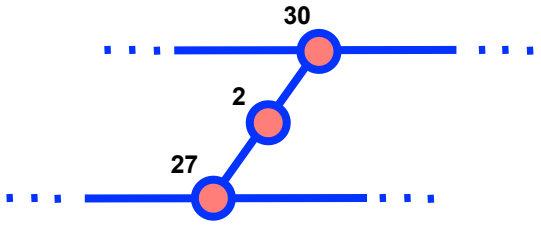


Pinch bubbles

(seq err, middle, SNP)

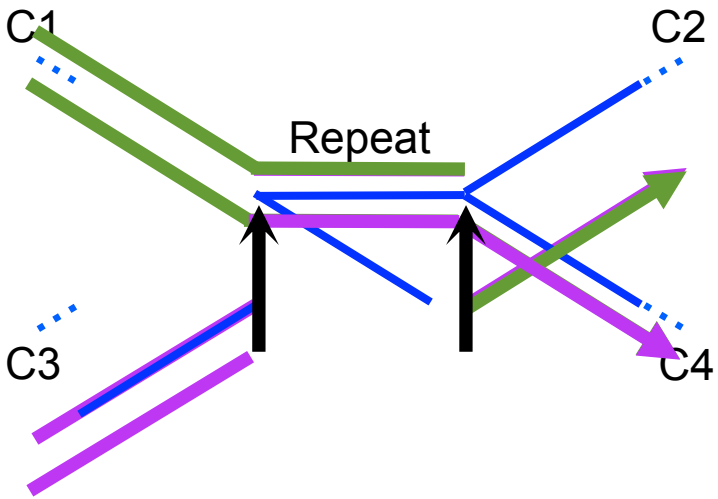


Remove low cov. links

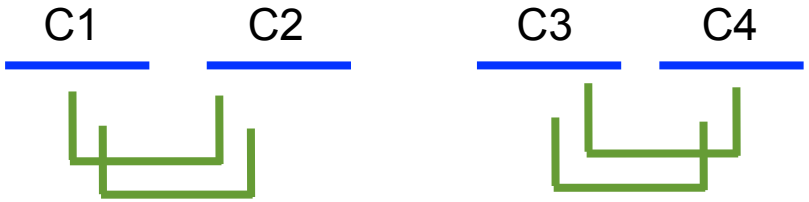


Create contigs and scaffolds

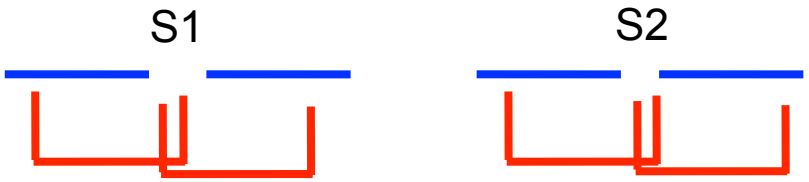
Cut graph at repeat boundaries to create contigs



Use paired end information to resolve repeats and combine to scaffolds



Fill potential gaps using PE reads



The assembly is done

Iterate parameters

- Re-run with different *k*-sizes, find optimum
- Run with multiple k-mers at the same time! (eg. SPAdes)
- Compare assembly statistics such as, assembly length, N50, no. contigs

- Assembly refinement
  - Break contigs not supported by PE/MP reads
  - Analyze assembly using REAPR or QUAST

Successful *de novo* assembly

- Success is a factor of:
  - Genome size, **genomic repeats(!)**, ploidy
  - High coverage, long read lengths, PE/MP libraries

Repeats in *E. coli*

Improving *de novo* assemblies

- Paired end & Mate pair for long range continuity
- Hybrid approaches (combine Illumina with PacBio/Oxford Nanopore)

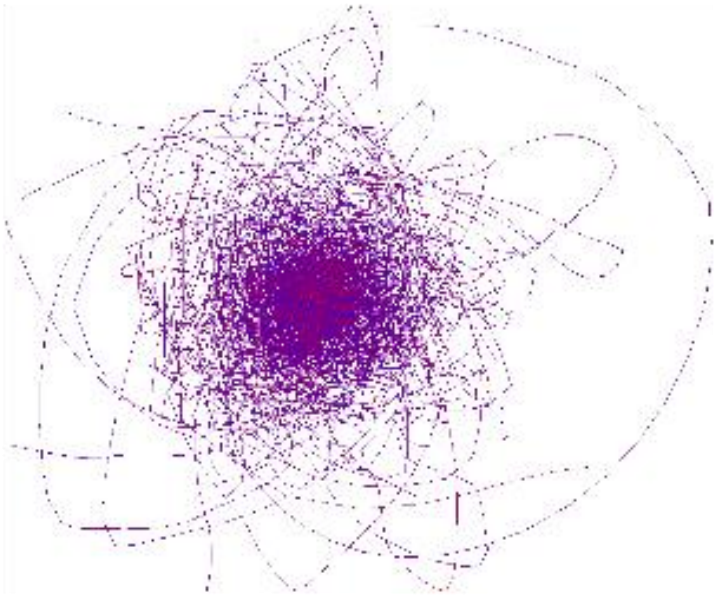
- Synthetic long reads: Illumina Synthetic Reads (Moleculo) or 10X Genomics
- Hi-C contact maps

Two bacterial genomes *de Bruijn* graphs

Few repeats

Flicek & Birney, Nat.Methods 2009

"more" repeats

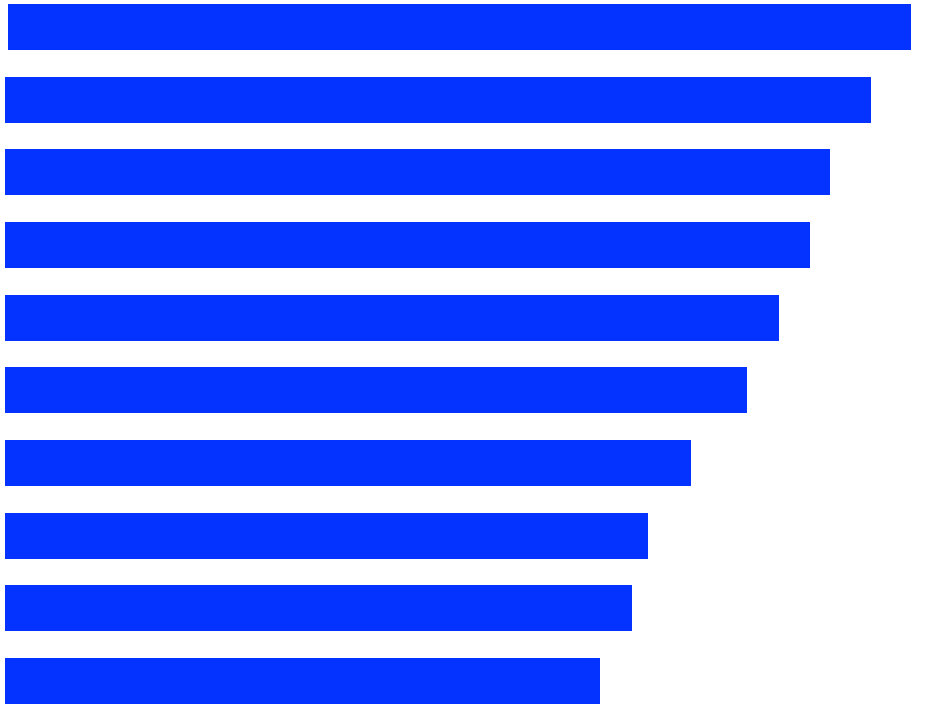


Zerbino, 2009

N50: Assembly quality

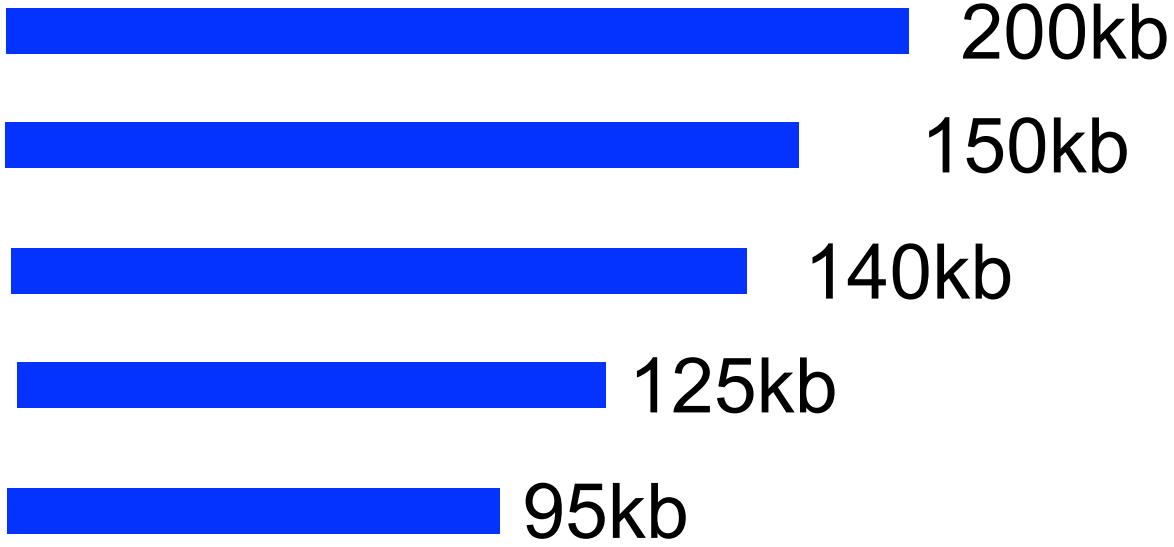
N50: What is the smallest piece in the largest half of the assembly?

- Calculate sum of assembly
- Order contigs by size
- Sum contigs starting by largest
- When half the sum is reached, N50 is the length of the contig



N50 example

5 scaffolds, calculate N50:



Sum: 200+150+140+125+95=710kb

Half: 710 / 2 = 355kb

200kb + 150kb = 350kb

350kb + 140kb = 490kb

490kb > 355kb => **N50: 140kb**

Some assemblers

- OLC: Canu, Newbler
- de Bruijn: Allpaths-LG, SPAdes, Velvet(best), SOAPdenovo, Megahit (very lean), …
- other: MIRA, SGA, Flye (very good for 3g NGS)

Used in exercises today

Exercise time!

http://teaching.healthtech.dtu.dk/22126/index.php/Denovo_exercise