DTU

DTU Health Technology
Bioinformatics

***de novo* assembly**

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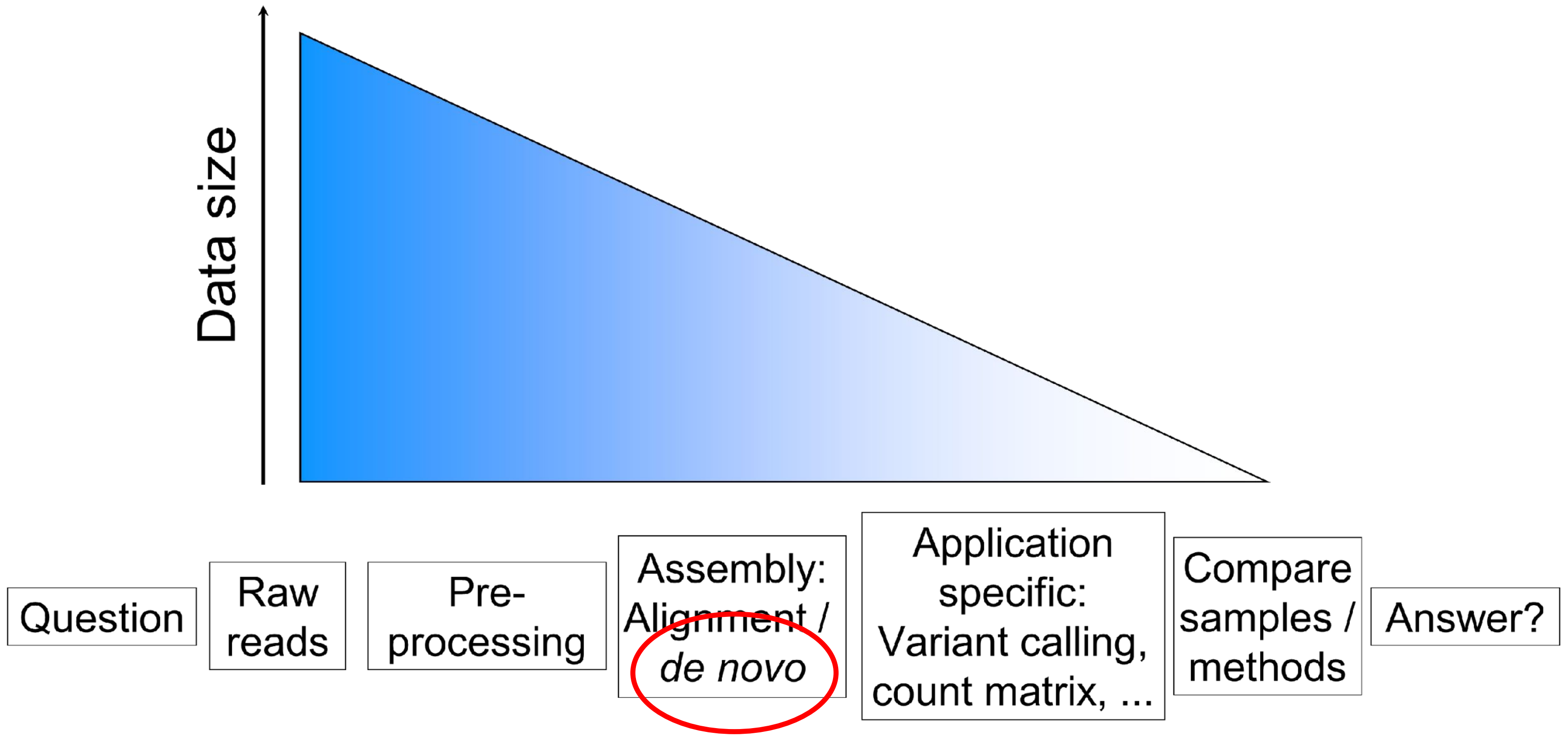
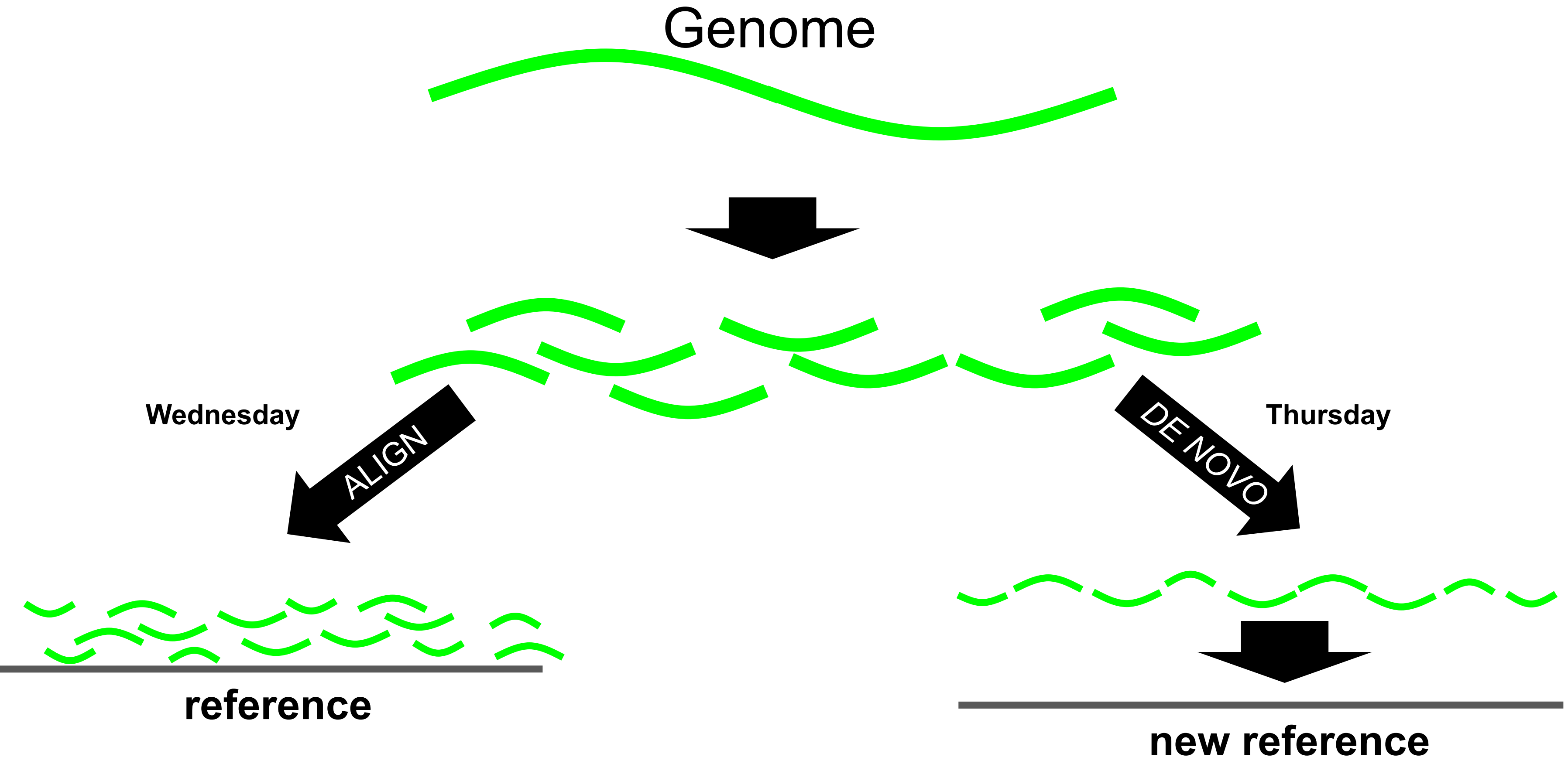
Menu

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

Generalized NGS analysis

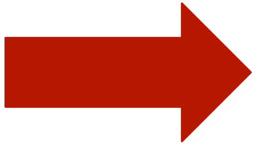

Data size
Question
Raw reads
Pre-processing
Assembly: Alignment / *de novo*
Application specific: Variant calling, count matrix, ...
Compare samples / methods
Answer?

Whole genome sequencing



Input

Output



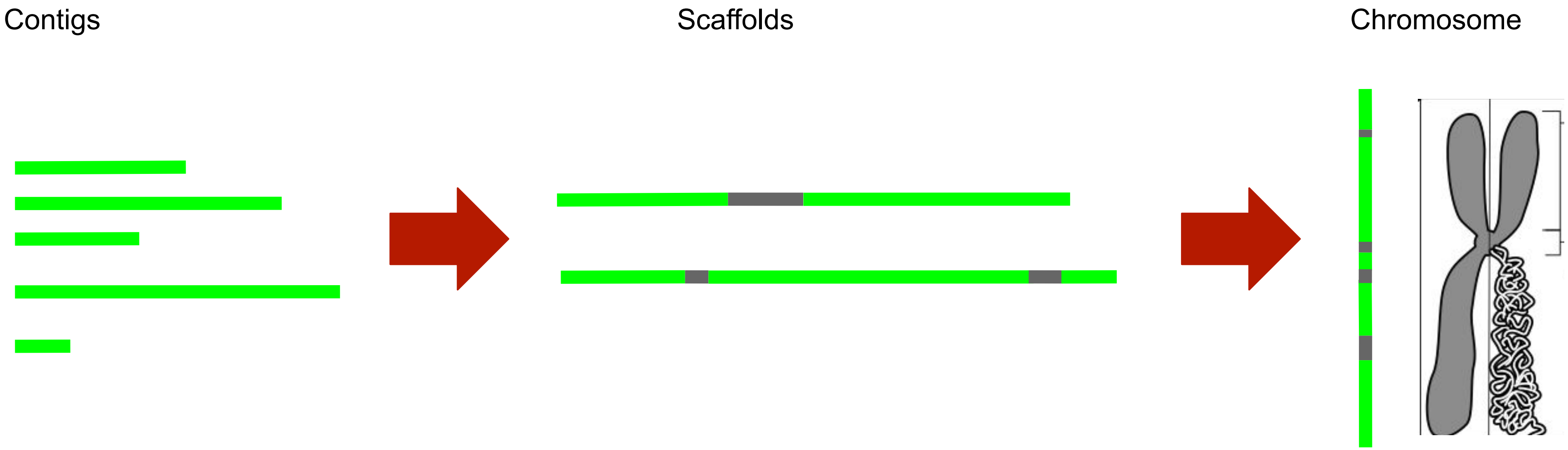
Input

```
@MISEQ423_0:+:7218:7278:60-2
GTTACTCGGACTACCCCGATGCATACACCACATGAAACA
T
+
]V]P]]\]]]]]]]\]]]]]]]]][]]]\]][]]]]]]]]\
]
@MISEQ423_0:-:15245:15305:60-2
AGGGCAAGATGAAGTGAAAGGTAAAGAATCGTGTGAGGG
T
+
]]]][Z]]]]]]]]]]]]][]]]]]]]]]]]]]]]]]]]]
]
@MISEQ423_0:-:242:302:60-2
TTTGGTGGAAATTTTTTGTTATGATGTCTGTGTGGAAAG
T
+
]]]]]]]Z]]]]]]]]]]]]]Z]]]\]]]]Z]]]]]]]]
]
@MISEQ423_0:-:1729:1789:60-2
TGCGGTACTATATCTATTGCGCCAGGTTTCAATTTCTAT
C
+
]]]]]]]]]X]]]]]]]]]]]]]]]]]]]]]]]]]]]]]]
```

Output

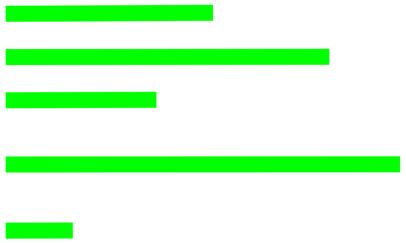
```
>contig#25_0
GATCACAGGTCTATCACCCTATTAACCACTCACGGGAGCTCTCCA
GTATGCACGCGATAGCATTGCGAGACGCTGGAGCCGGAGCACCCT
CTGCCTCATCCTATTATTTATCGCACCTACGTTCAATATTACAGG
ATTAATTAATGCTTGTAGGACATAATAATAACAATTGAATGTCTG
ATAACAAAAAATTTCCACCAAACCCCCCCTCCCCCGCTTCTGGCC
AACCCCAAAAACAAAGAACCCTAACACCAGCCTAACCAGATTTCA
TTTTAACAGTCACCCCCCAACTAACACATTATTTTCCCCTCCCAC
CAACCCCCGCCCATCCTACCCAGCACACACACACCGCTGCTAACC
AAAGACACCCCCCACAGTTTATGTAGCTTACCTCCTCAAAGCAAT
ACATCACCCCATAAACAAATAGGTTTGGTCCTAGCCTTTCTATTA
GCATCCCCGTTCCAGTGAGTTCACCCTCTAAATCACCACGATCAA
AATGCAGCTCAAAACGCTTAGCCTAGCCACACCCCCACGGGAAAC
ACGAAAGTTTAACTAAGCTATACTAACCCCAGGGT
```

Important definitions



Important definitions

Contigs

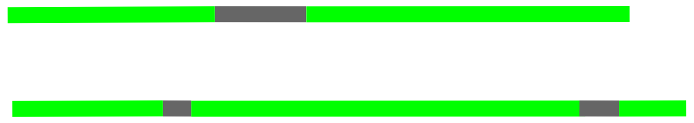


```
>contig#1
GATCACAGGTCTATCACCCTATTAACCACTCACGGGAGCTCTCCA
GTATGCACGCGATAGCATTGCGAGACGCTGGAGCCGGAGCACCCT
CTGCCTCATCCTATTATTTATCGCACCTACGTTCAATATTACAGG
>contig#2
ATTAATTAATGCTTGTAGGACATAATAATAACAATTGAATGTCTG
ATAACAAAAAATTTCCACCAAACCCCCCCTCCCCCGCTTCTGGCC
>contig#3
AACCCCAAAAACAAAGAACCCTAACACCAGCCTAACCAGATTTCA
TTTTAACAGTCACCCCCCAACTAACACATTATTTTCCCCTCCCAC
CAACCCCCGCCCATCCTACCCAGCACACACACACCGCTGCTAACC
AAAGACACCCCCCACAGTTTATGTAGCTTACCTCCTCAAAGCAAT
>contig#4
ACATCACCCCATAAACAAATAGGTTTGGTCCTAGCCTTTCTATTA
GCATCCCCGTTCCAGTGAGTTCACCCTCTAAATCACCACGATCAA
AATGCAGCTCAAAACGCTTAGCCTAGCCACACCCCCACGGGAAAC
ACGAAAGTTTAACTAAGCTATACTAACCCCAGGGT
```

Important definitions

```
>scaffold#1
AACCCCAAAAACAAAGAACCCTAACACCAGCCTAACCAGATTTCA
TTTTAACAGTCACCCCCCAACTAACACATTATTTTCCCCTCCCAC
CAACCCCCGCCCATCCTACCCAGCACACACACACCGCTGCTAACC
AAAGACACCCCCCACAGTTTATGTAGCTTACCTCCTCAAAGCAAT
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNGATCACAGGTCTATC
ACCCTATTAACCACTCACGGGAGCTCTCCA
>scaffold#2
GTATGCACGCGATAGCATTGCGAGACGCTGGAGCCGGAGCACCCT
CTGCCTCATCCTATTATTTATCGCACCTACGTTCAATATTACAGG
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNATTAATTAATGCT
GTAGGACATAATAATAACAATTGAATGTCTGATAACAAAAAATTC
CACCAAACCCCCCCTCCCCCGCTTCTGGCCNNNNNNNACATCACC
CATAAACAAATAGGTTTGGTCCTAGCCTTTCTATTAGCATCCCCT
TCCAGTGAGTTCACCCTCTAAATCACCACGATCAAAATGCAGCTA
AAACGCTTAGCCTAGCCACACCCCCACGGGAAACACGAAAGTTTA
ACTAAGCTATACTAACCCCAGGGT
```

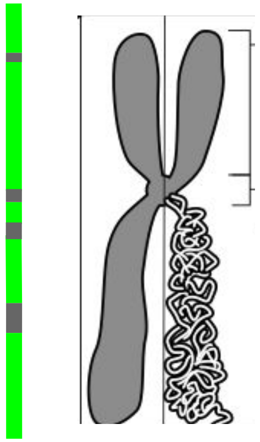
Scaffolds



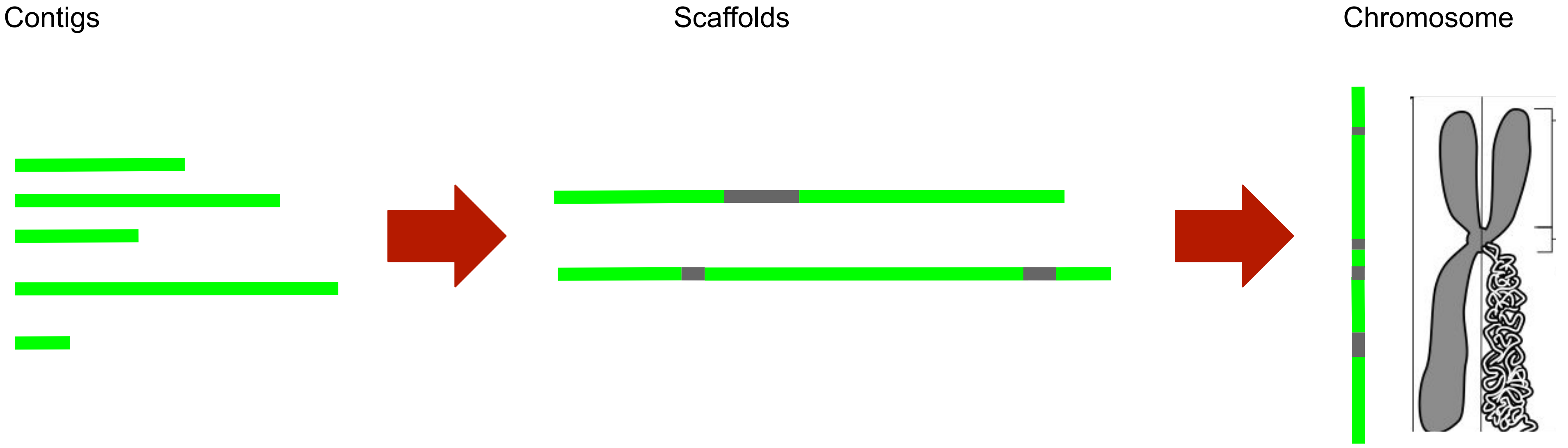
Important definitions

```
>chr22
GTATGCACGCGATAGCATTGCGAGACGCTGGAGCCGGAGCACCCT
T
CTGCCTCATCCTATTATTTATCGCACCTACGTTCAATATTACAGG
G
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNATTAATTAATGCT
TGTAGGACATAATAATAACAATTGAATGTCTGATAACAAAAAATT
TCCACCAAACCCCCCCTCCCCCGCTTCTGGCCNNNNNNNACATCA
CCCCATAAACAAATAGGTTTGGTCCTAGCCTTTCTATTAGCATCC
CCGTTCCAGTGAGTTCACCCTCTAAATCACCACGATCAAAATGCA
GCTCAAAACGCTTAGCCTAGCCACACCCCCACGGGAAACACGAAA
GTTTAACTAAGCTATACTAACCCCAGGGTNNNNNNNAACCCCAAA
AACAAAGAACCCTAACACCAGCCTAACCAGATTTCATTTTAACAG
TCACCCCCCAACTAACACATTATTTTCCCCTCCCACCAACCCCCG
CCCATCCTACCCAGCACACACACACCGCTGCTAACCAAAGACACC
CCCCACAGTTTATGTAGCTTACCTCCTCAAAGCAATNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNGATCACAGGTCTATCACCCTATTA
ACCACTCACGGGAGCTCTCCA
```

Chromosom
e



Important definitions



Which approaches?

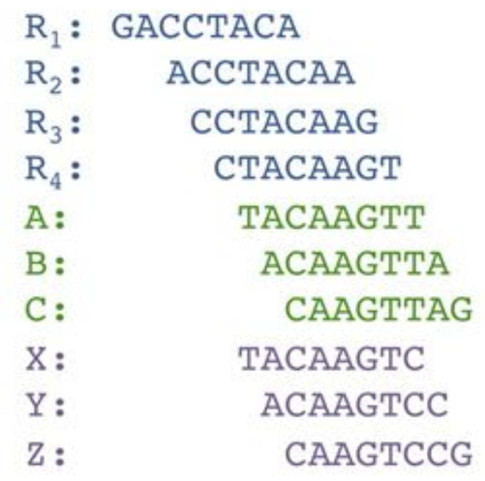
- Greedy (“Simple” approach)
- Overlap-Layout-Consensus (OLC)
- de Bruijn graphs

Simple approach - Greedy

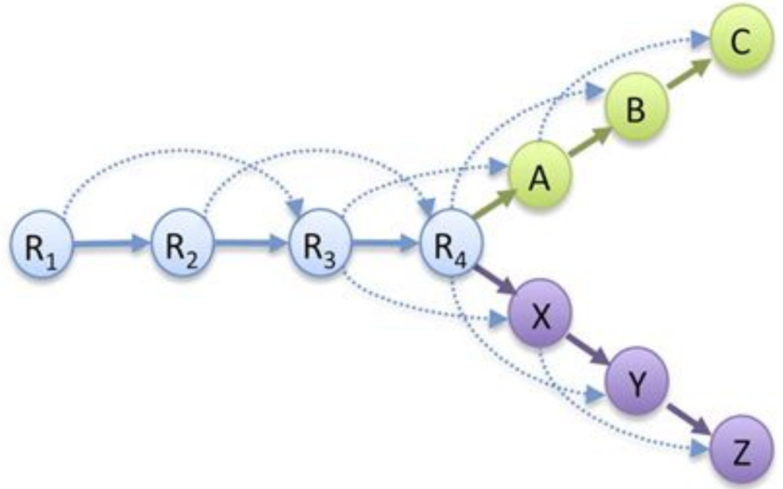
- Principle:
  1. Pairwise alignment of all reads
  2. Identify fragments that have largest overlap
  3. Merge these
  4. Repeat until all overlaps are used
- Can only resolve repeats smaller than read length
- High computational cost with increasing no. reads

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
```

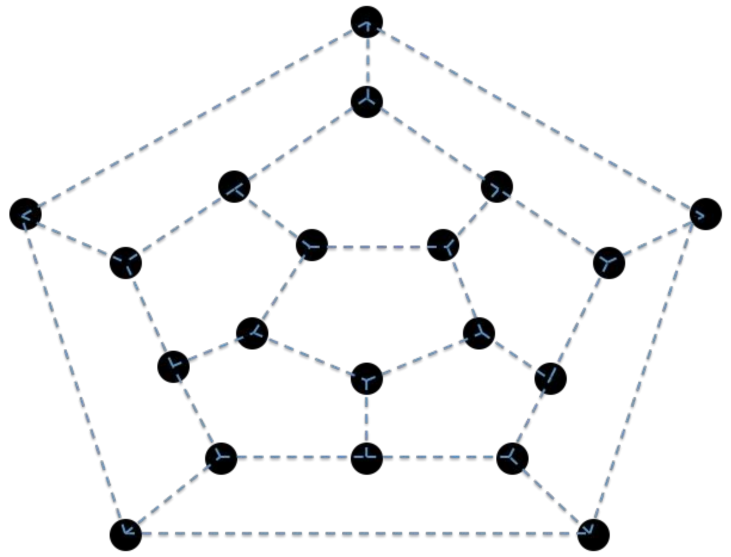


$$\begin{tikzcd}
 & & & & & & C \\
 & & & & & B \arrow[ru] & \\
 & & & & A \arrow[ru] \arrow[rru, dotted, bend left] & & \\
R_1 \arrow[r] \arrow[rr, dotted, bend left] & R_2 \arrow[r] \arrow[rr, dotted, bend left] & R_3 \arrow[r] \arrow[ru, dotted] \arrow[rd, dotted] & R_4 \arrow[ru] \arrow[rd] \arrow[rruu, dotted, bend left] \arrow[rrdd, dotted, bend right] & & & \\
 & & & & X \arrow[rd] \arrow[rrdd, dotted, bend right] & & \\
 & & & & & Y \arrow[rd] & \\
 & & & & & & Z
\end{tikzcd}$$

Schatz et al., Genome Res, 2010

Overlap-Layout-Consensus

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



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

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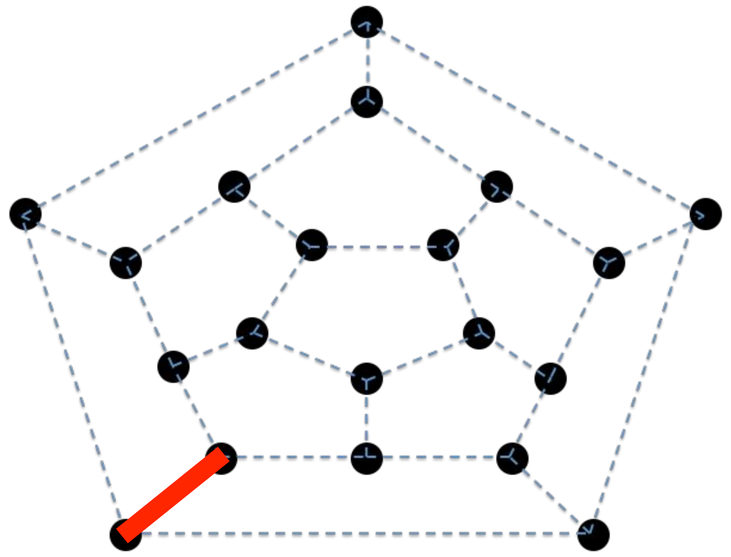

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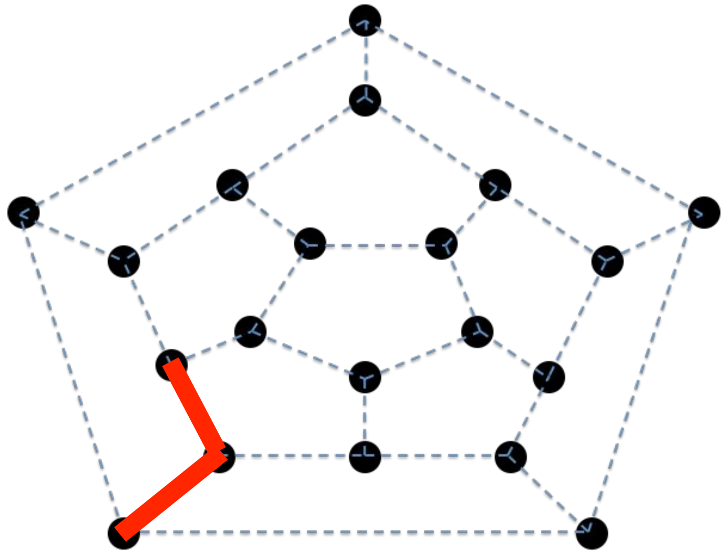

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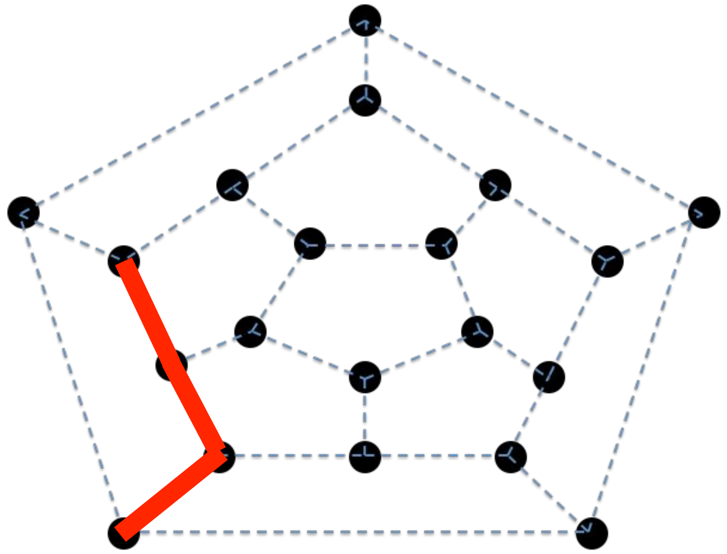

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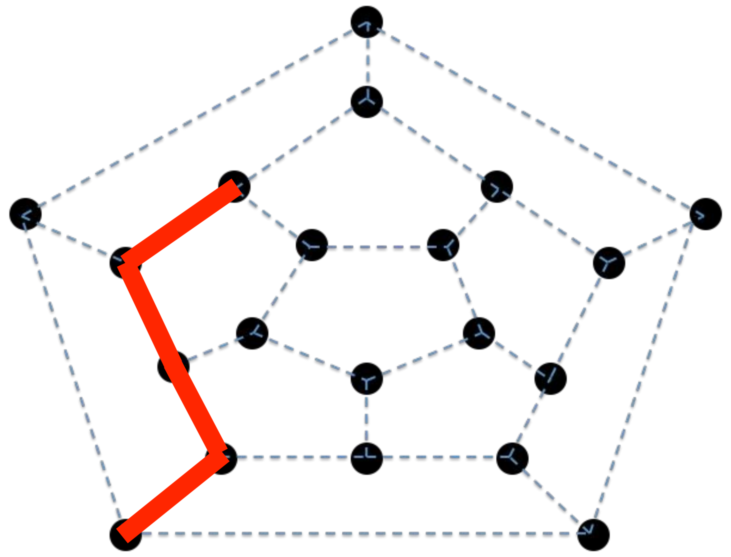

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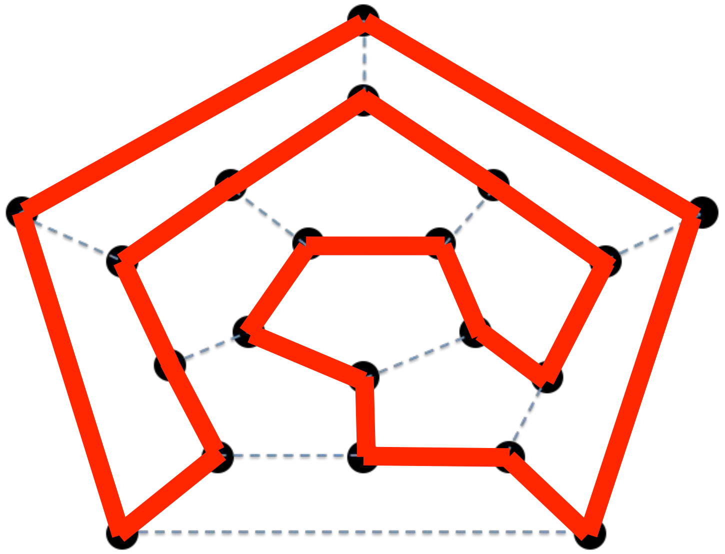


Imagine trying to solve this for a graph of hundred of thousands of nodes (=reads)

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

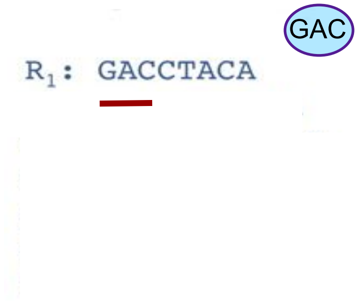
- Directed graph of overlapping items (here DNA sequences)
- Instead of comparing reads, decompose reads into *k*-mers
  - Graph is created by mapping the *k*-mers to the graph
  - Each *k*-mer only exists once in the graph
  - Problem is reduced to walking Eulerian path (visiting each edge once) - this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



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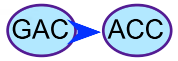
R_1: GACCTACA



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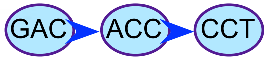

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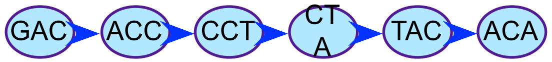

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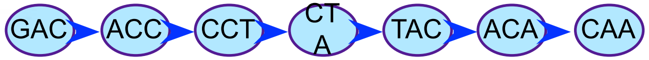

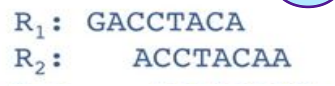
```
R1: GACCTACA
R2:    ACCTACAA
```

How is the graph constructed?

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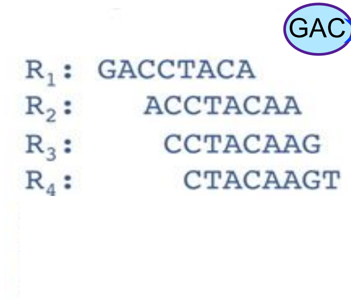
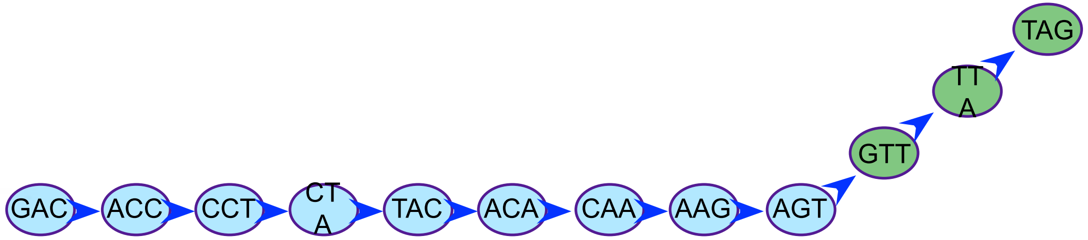
$$\begin{tikzcd}
GAC \arrow[r] & ACC \arrow[r] & CCT \arrow[r] & CTA \arrow[r] & TAC \arrow[r] & ACA \arrow[r] & CAA \arrow[r] & AAG \arrow[r] & AGT
\end{tikzcd}$$

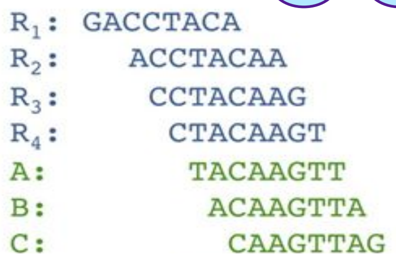


```
R₁: GACCTACA
R₂:    ACCTACAA
R₃:     CCTACAAG
R₄:      CTACAAGT
```

How is the graph constructed?

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




R_1: GACCTACA
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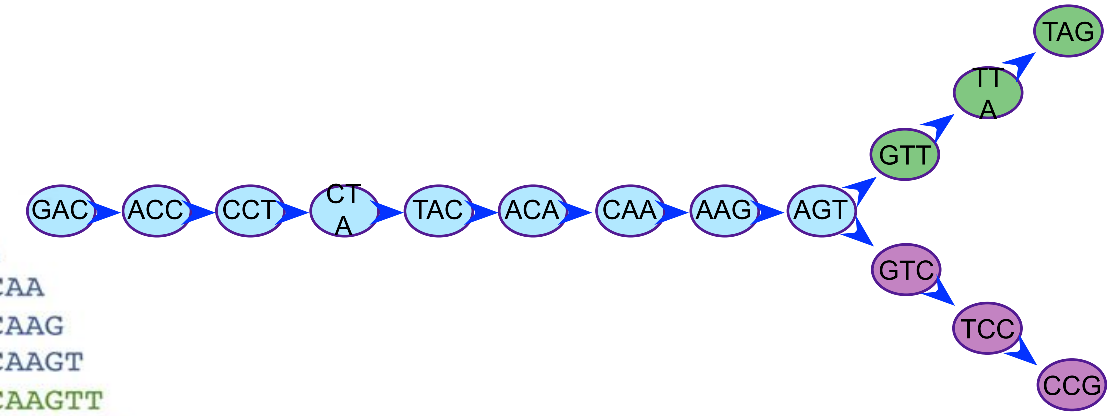

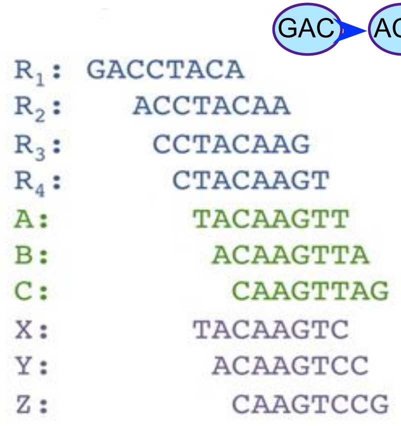

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A:	TACAAGTT
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X:	TACAAGTC
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Z:	CAAGTCCG

Complicated graphs

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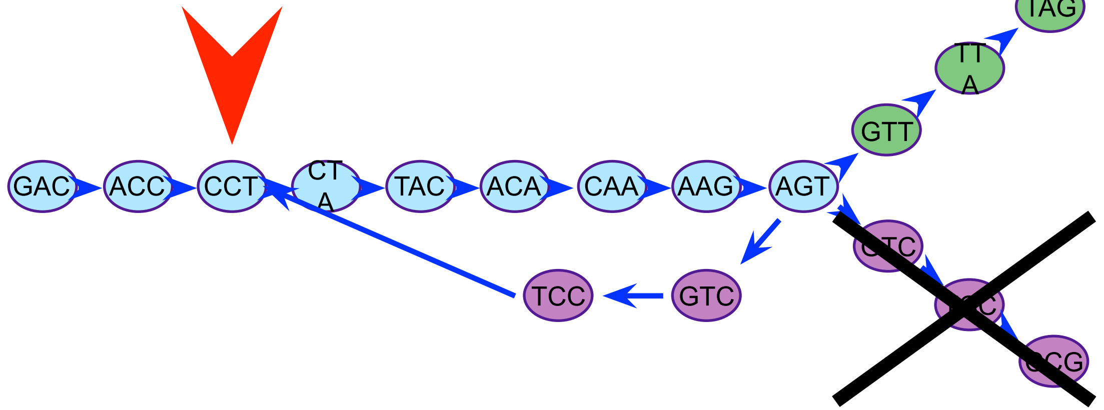

G to T

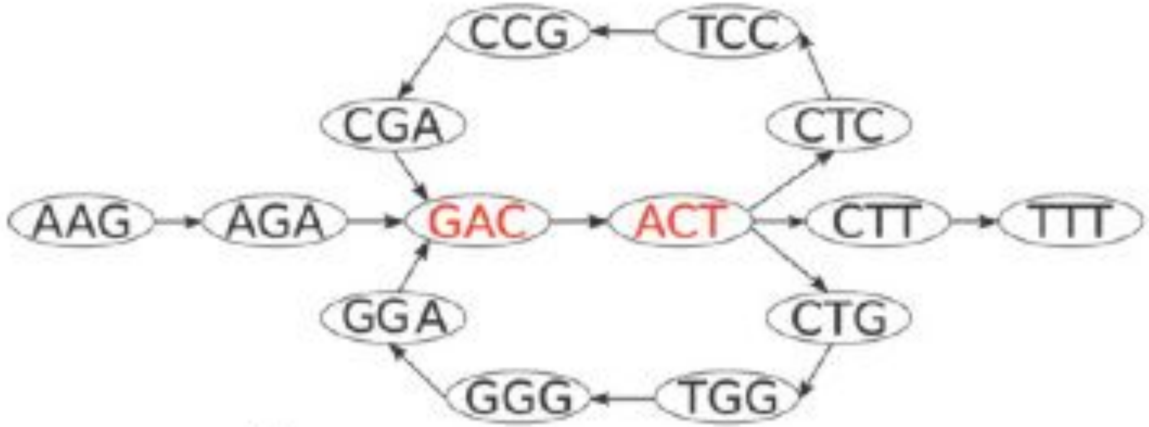


Large genomes with many repeats/errors create very large graphs

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

AAGACTCCGACTGGGACTTT

AAGACTCCGACTGGGACTTT

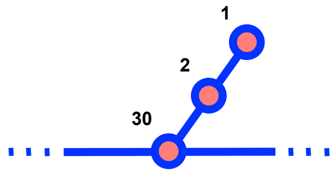


A de Bruijn graph of a sequence

After building: Simplify

Clip tips

(seq err,end)



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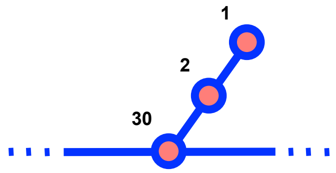
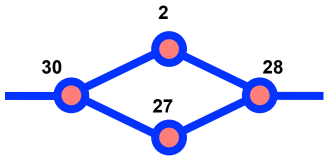


Pinch bubbles

(seq err, middle, SNP)



...

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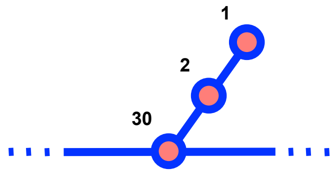

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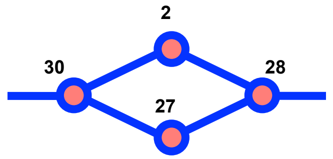
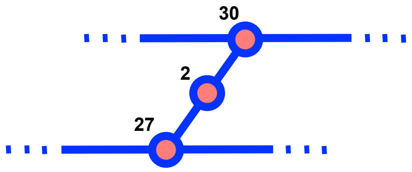
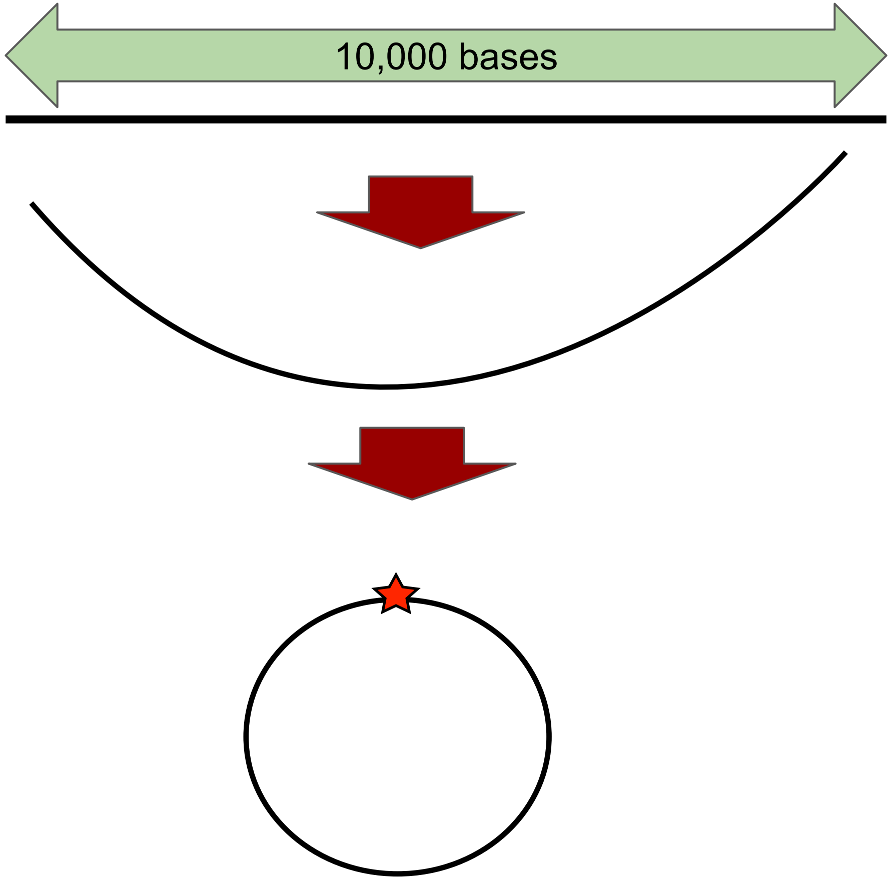


Remove low cov. links



Mate pair reads



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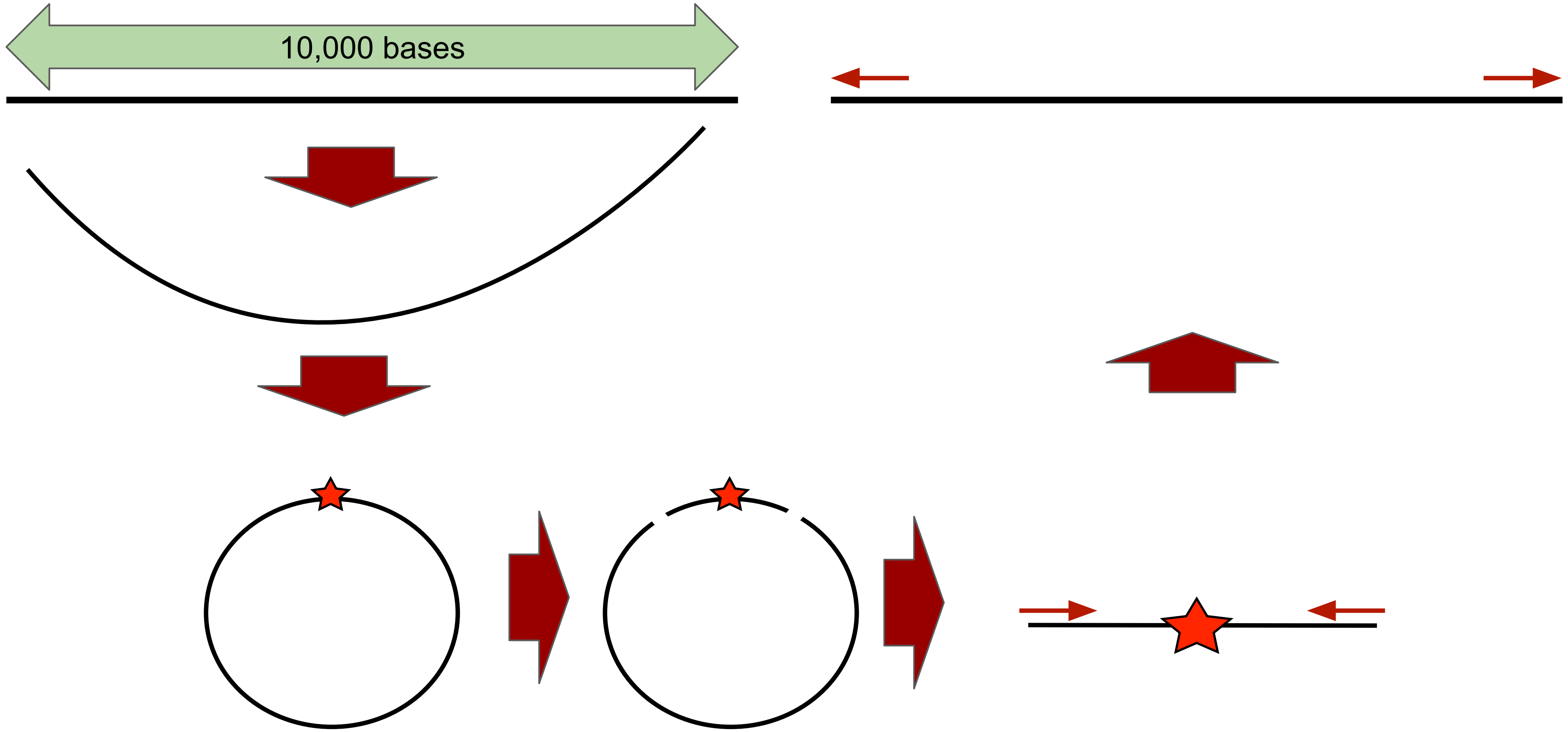

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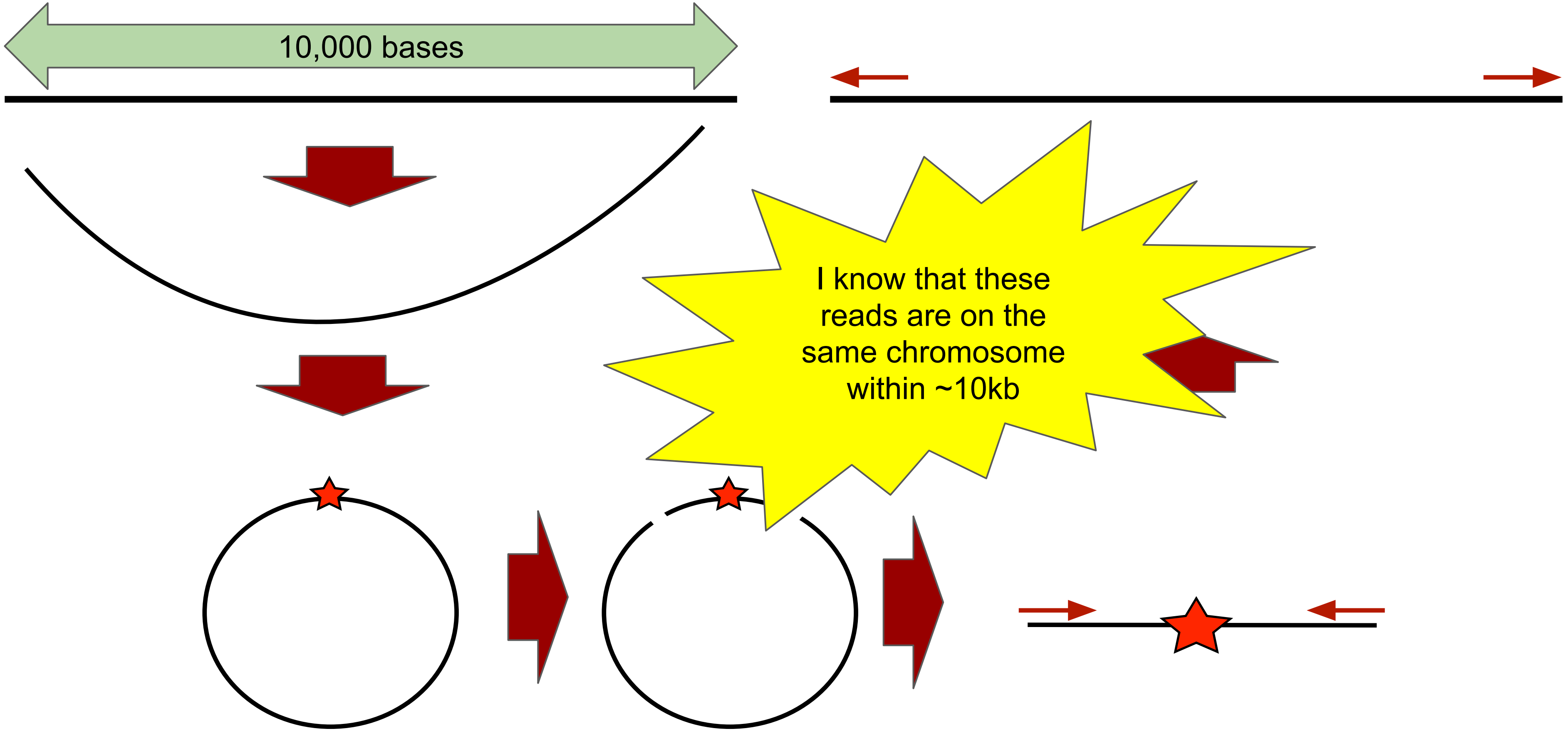
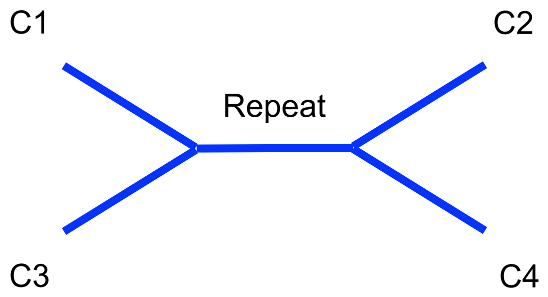

10,000 bases
I know that these reads are on the same chromosome within ~10kb

Create contigs and scaffolds

Which goes with which?



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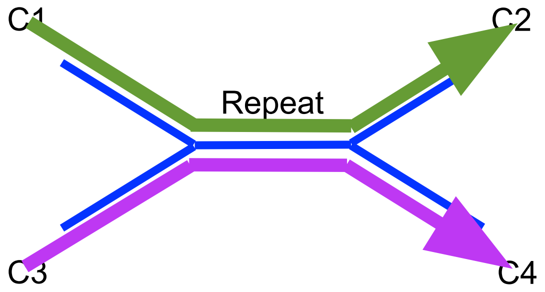
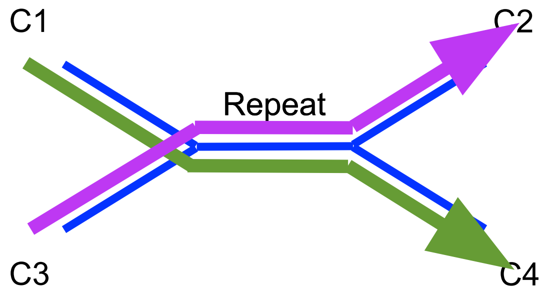


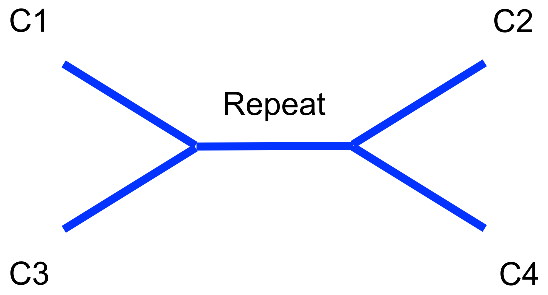
Create contigs and scaffolds

Which goes with which?



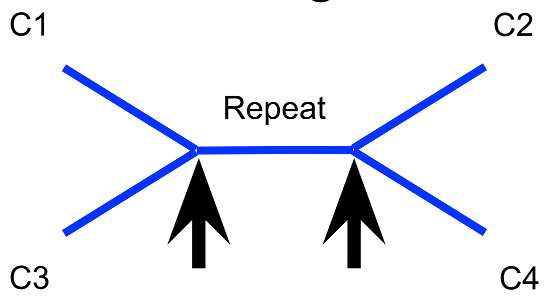
Create contigs and scaffolds

Cut graph at repeat boundaries to create contigs



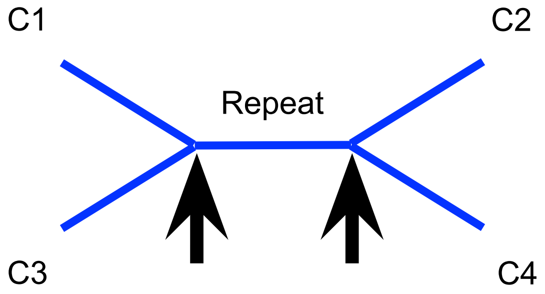
Create contigs and scaffolds

Cut graph at repeat boundaries to create contigs



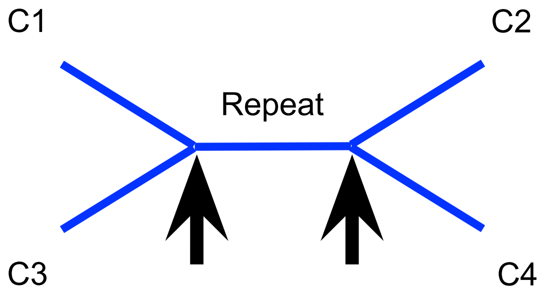
Create contigs and scaffolds

Cut graph at repeat boundaries to create contigs

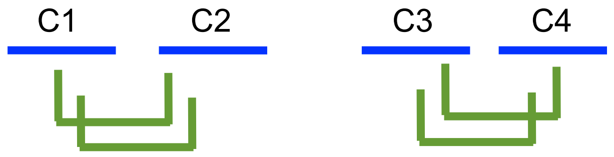


Create contigs and scaffolds

Cut graph at repeat boundaries to create contigs

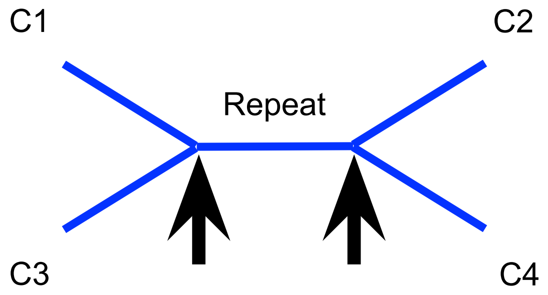


Use paired-end or mate-pair information to resolve repeats and combine to scaffolds

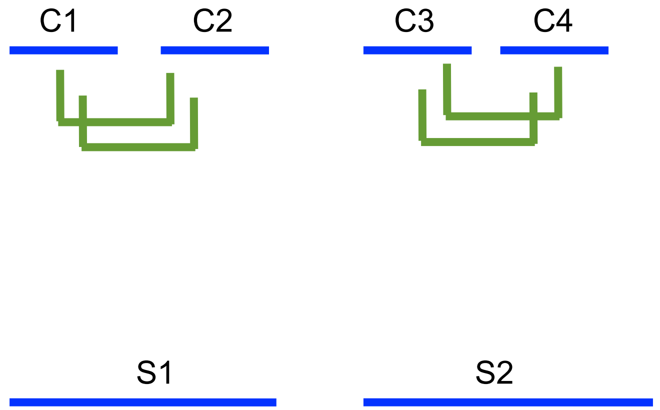


Create contigs and scaffolds

Cut graph at repeat boundaries to create contigs



Use paired-end or mate-pair information to resolve repeats and combine to scaffolds



Iterate parameters

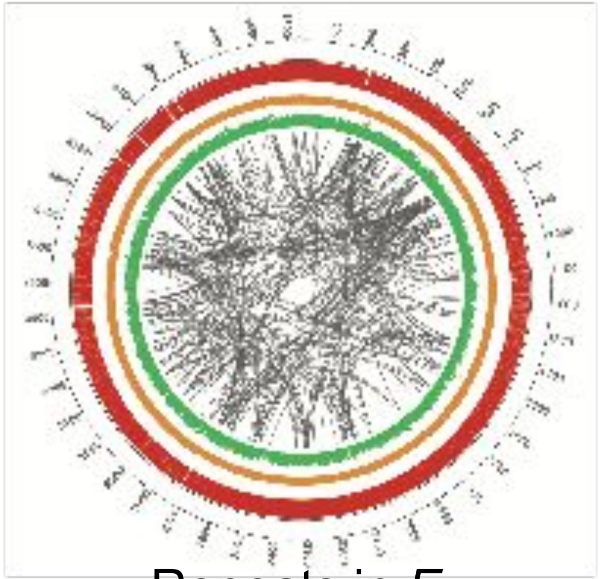
- Re-run with different *k*-sizes, find optimum
- Run with multiple k-mers at the same time! (eg. SPAdes)
- Compare assembly statistics such as, assembly length, N50, no. contigs

- 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

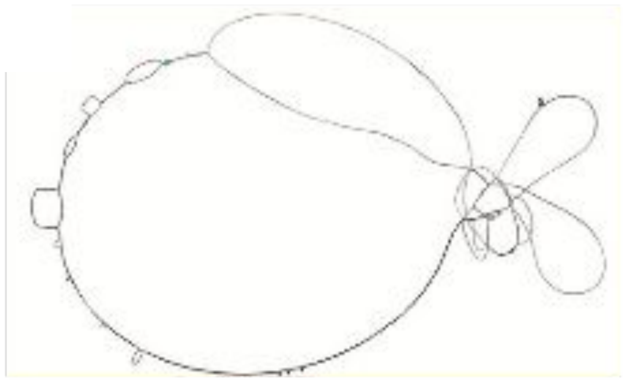
- Paired-end & Mate-pair for long range continuity
- Hybrid approaches (combine Illumina with PacBio/Oxford Nanopore)

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

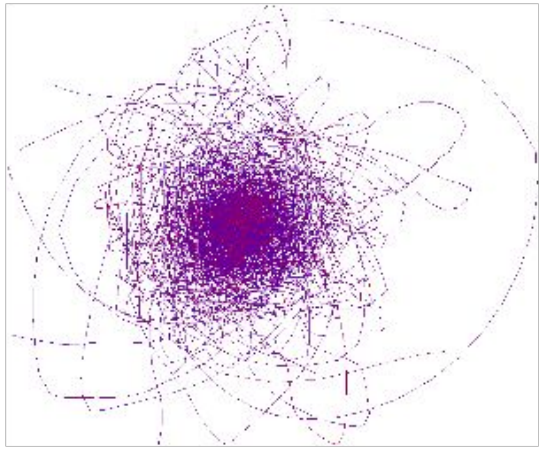
Two bacterial genomes *de Bruijn* graphs

Few
repeats

“more”
repeats



Flicek & Birney, Nat.Methods 2009

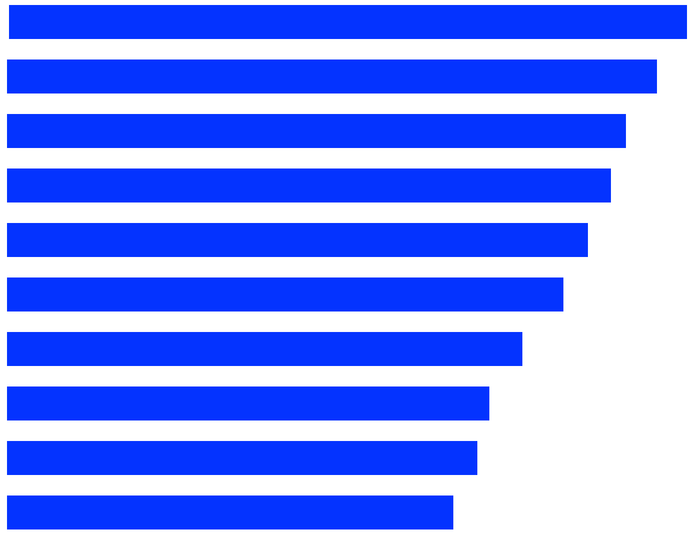


Zerbino, 2009

N50: Assembly quality

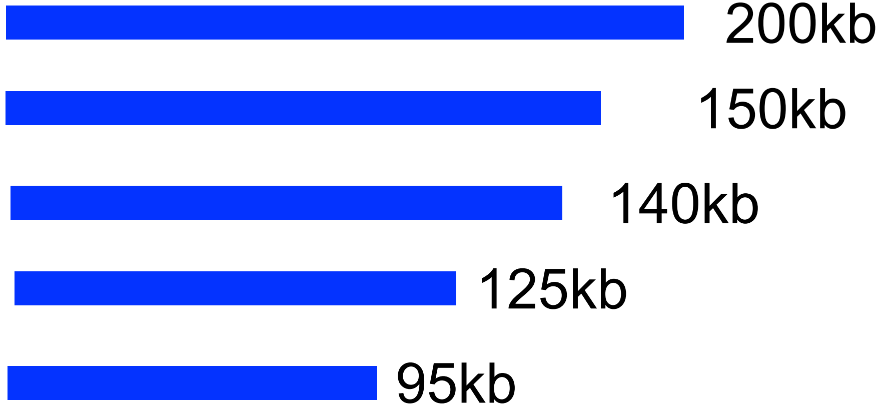
N50: What is the smallest piece in the largest half of the assembly?

- 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!