Assembly

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DNA-seq

raw reads

trimmed and cleaned reads

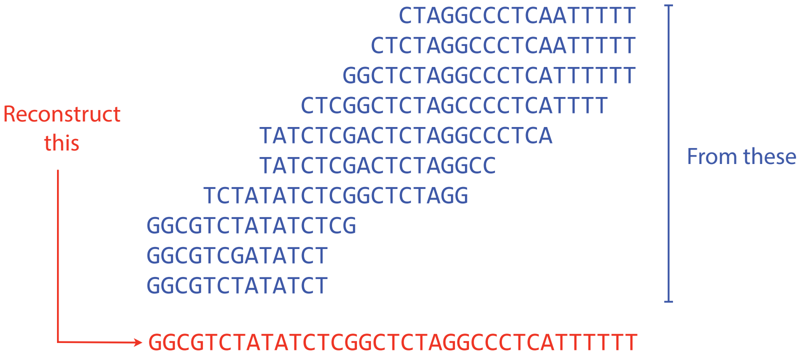
reference genome → mapping

find location of the read and align them with respect to the reference

no reference genome → assembly

reconstruct the initial sequence

The assembly problem



CTAGGCCCTCAATTTTT
CTCTAGGCCCTCAATTTTT
GGCTCTAGGCCCTCATTTTTT
CTCGGCTCTAGCCCCTCATTTT
TATCTCGACTCTAGGCCCTCA
TATCTCGACTCTAGGCC
TCTATATCTCGGCTCTAGG
GGCGTCTATATCTCG
GGCGTCGATATCT
GGCGTCTATATCT

From these

Reconstruct this

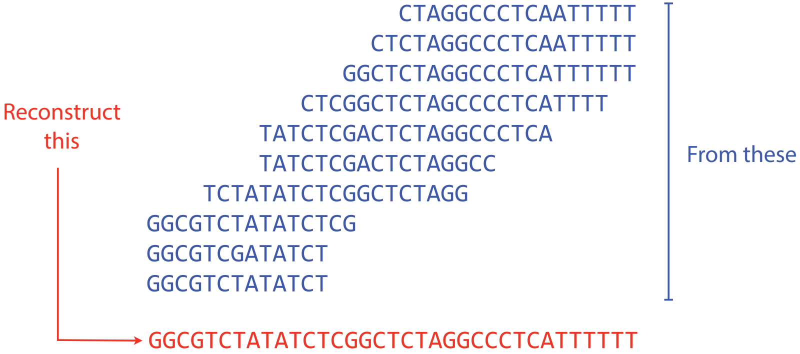
GGCGTCTATATCTCGGCTCTAGGCCCTCATTTTTT

Courtesy of Ben Langmead (Johns Hopkins University)

Why assembling reads ?

- annotation of genomes
    - discovery of new genes
    - gene order, structural variants
    - noncoding regions
    - evolutionnary genomics, phylogenomics
- transcriptome
    - reconstruction of transcripts
    - identification of alternative transcripts
- metagenomics
    - identification of species

The assembly problem



CTAGGCCCTCAATTTTT
CTCTAGGCCCTCAATTTTT
GGCTCTAGGCCCTCATTTTTT
CTCGGCTCTAGCCCCTCATTTT
TATCTCGACTCTAGGCCCTCA
TATCTCGACTCTAGGCC
TCTATATCTCGGCTCTAGG
GGCGTCTATATCTCG
GGCGTCGATATCT
GGCGTCTATATCT

From these

Reconstruct this

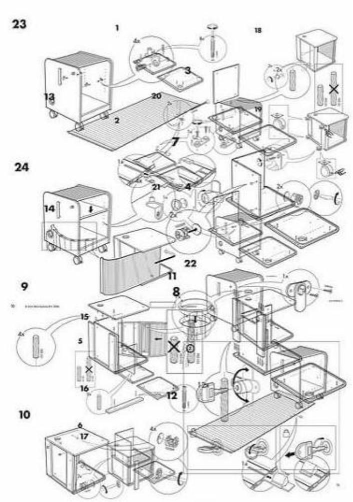
GGCGTCTATATCTCGGCTCTAGGCCCTCATTTTTT

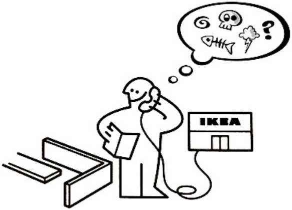
CTAGGCCCTCAATTTTT
GGCGTCTATATCT
CTCTAGGCCCTCAATTTTT
TCTATATCTCGGCTCTAGG
GGCTCTAGGCCCTCATTTTTT
CTCGGCTCTAGCCCCTCATTTT
TATCTCGACTCTAGGCCCTCA
GGCGTCGATATCT
TATCTCGACTCTAGGCC
GGCGTCTATATCTCG

From these

Reconstruct this

GGCGTCTATATCTCGGCTCTAGGCCCTCATTTTTT


23
1
18
3
20
13
2
19
7
24
21
4
14
22
11
9
8
15
5
16
12
10
6
17



Some assembly is required

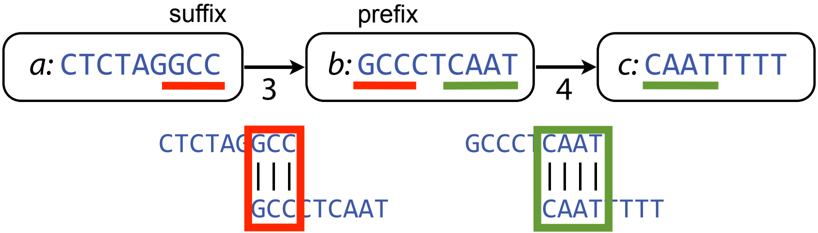
How to assemble reads ?

Historical perspective

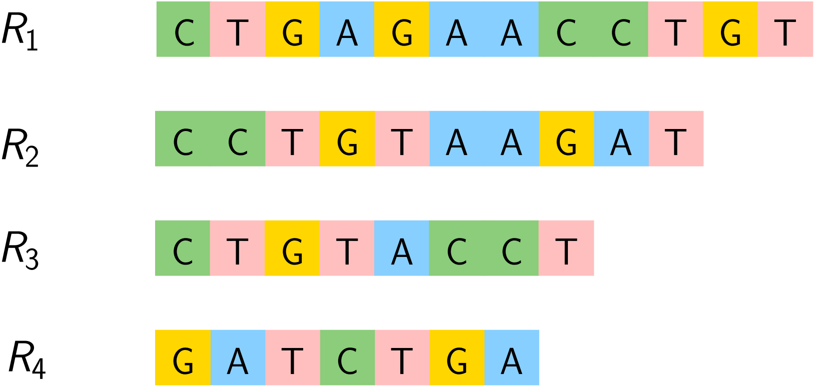
Key features : overlaps between reads

How to assemble reads ?

Historical perspective


suffix
prefix
a: CTCTAGGCC
b: GCCCTCAAT
c: CAATTTTT
3
4
CTCTAGGCC
GCCCTCAAT
GCCCTCAAT
CAATTTTT

Key features : overlaps between reads

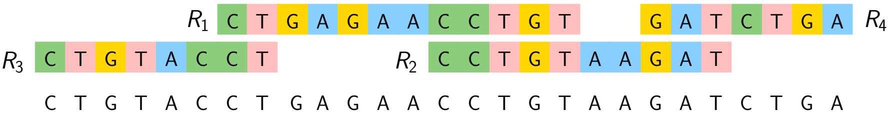


R_1 C T G A G A A C C T G T

R_2 C C T G T A A G A T

R_3 C T G T A C C T

R_4 G A T C T G A

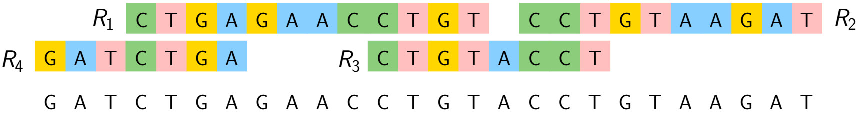


R_1 C T G A G A A C C T G T G A T C T G A R_4

R_3 C T G T A C C T R_2 C C T G T A A G A T

C T G T A C C T G A G A A C C T G T A A G A T C T G A

Length of the assembly : 27

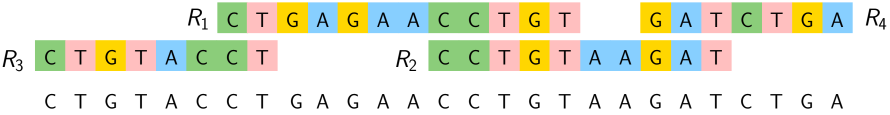


R_1 C T G A G A A C C T G T C C T G T A A G A T R_2

R_4 G A T C T G A R_3 C T G T A C C T

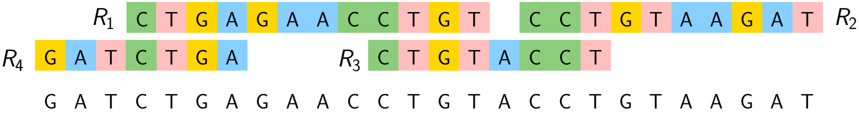
G A T C T G A G A A C C T G T A C C T G T A A G A T

Length of the assembly : 26


R_1
C T G A G A A C C T G T
G A T C T G A
R_4
R_3
C T G T A C C T
R_2
C C T G T A A G A T
C T G T A C C T G A G A A C C T G T A A G A T C T G A

Length of the assembly : 27

joining together the reads in decreasing order of the quality of their overlaps


R_1
C T G A G A A C C T G T
C C T G T A A G A T
R_2
R_4
G A T C T G A
R_3
C T G T A C C T
G A T C T G A G A A C C T G T A C C T G T A A G A T

Length of the assembly : 26

trying to maximize the total length of read overlaps

R_1 C T G A G A A C C T G T

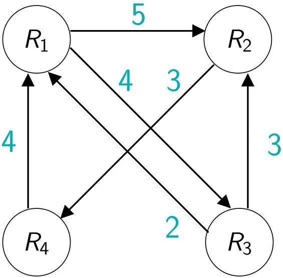
R_2 C C T G T A A G A T

R_3 C T G T A C C T

R_4 G A T C T G A

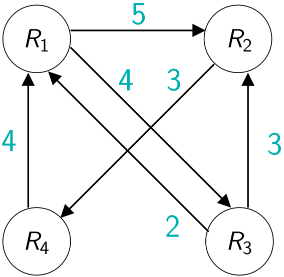
↗	R_1	R_2	R_3	R_4
R_1		5	4	0
R_2	0		0	3
R_3	2	3		0
R_4	4	0	0	

Length of the longest suffix of R_i which is also a prefix of R_j

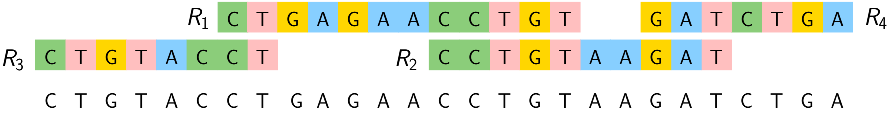


Overlap graph

Paths in the graph

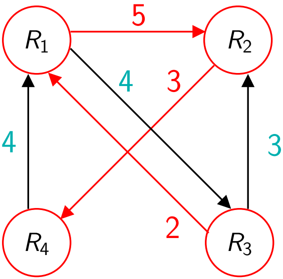


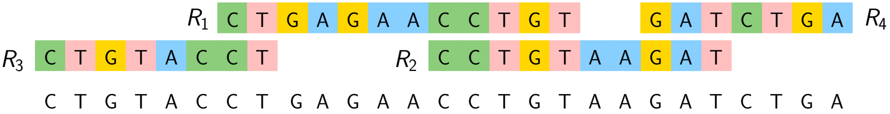
R_1
R_2
R_3
R_4
5
4
3
4
3
2



```
            R1 C T G A G A A C C T G T     G A T C T G A R4
R3 C T G T A C C T        R2 C C T G T A A G A T
   C T G T A C C T G A G A A C C T G T A A G A T C T G A
```

Paths in the graph


5
R_1
R_2
4
3
4
3
R_4
2
R_3

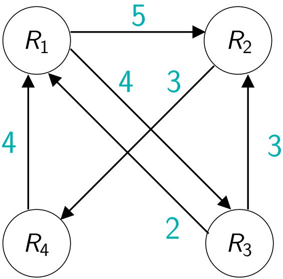


R_1 C T G A G A A C C T G T G A T C T G A R_4

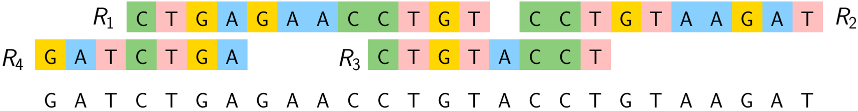
R_3 C T G T A C C T R_2 C C T G T A A G A T

C T G T A C C T G A G A A C C T G T A A G A T C T G A

Paths in the graph

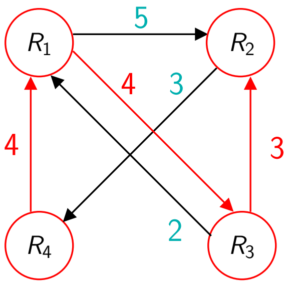


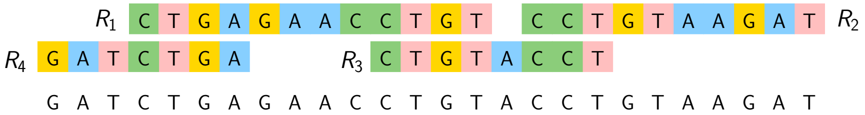
R_1
R_2
R_3
R_4
5
4
3
4
3
2



```
  R1      C T G A G A A C C T G T   C C T G T A A G A T  R2
R4  G A T C T G A      R3 C T G T A C C T
    G A T C T G A G A A C C T G T A C C T G T A A G A T
```

Paths in the graph


R_1
R_2
R_3
R_4
5
4
3
4
3
2



```
R1        C T G A G A A C C T G T   C C T G T A A G A T R2
R4  G A T C T G A      R3 C T G T A C C T
    G A T C T G A G A A C C T G T A C C T G T A A G A T
```

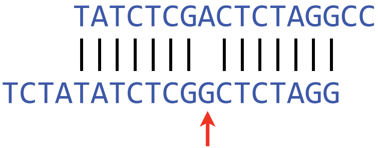
several paths = several assemblies

Overlap assemblies in real life

- risk of contamination
- existence of sequencing errors
- existence of repeats
- diploid and polyploid genomes
- low coverage or unevent coverage

+ unable to handle the large number of NGS sequencing reads

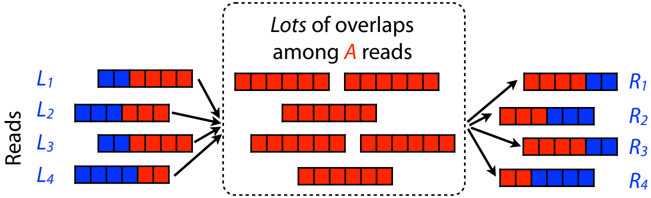
Sequencing errors



```
    TATCTCGACTCTAGGCC
    ||||||| |||||||
TCTATATCTCGGCTCTAGG
```

- Approximate overlaps : Construction of alignments between reads
- Assembly : consensus sequence

Repeats


Lots of overlaps
among *A* reads
Reads
L_1
L_2
L_3
L_4
R_1
R_2
R_3
R_4

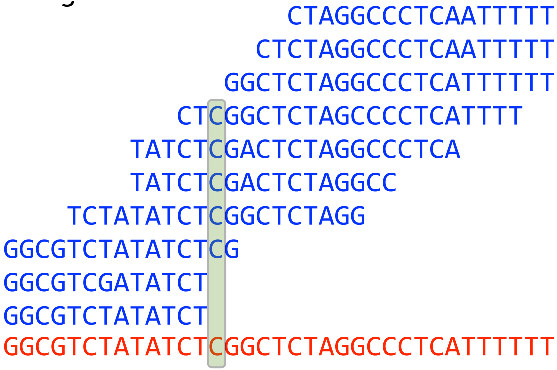
The region A is longer than reads

Diploidy and polyploidy

```
Read from Mother:           TATCTCGACTCTAGGCC
                            ||||||| |||||||
Read from Father:       TCTATATCTCGGCTCTAGG

Sequence from Mother:   TCTATATCTCGACTCTAGGCC
Sequence from Father:   TCTATATCTCGGCTCTAGGCC
```

Coverage



```
                  CTAGGCCCTCAATTTTT
                CTCTAGGCCCTCAATTTTT
              GGCTCTAGGCCCTCATTTTTT
           CTCGGCTCTAGCCCCTCATTTT
        TATCTCGACTCTAGGCCCTCA
        TATCTCGACTCTAGGCC
    TCTATATCTCGGCTCTAGG
GGCGTCTATATCTCG
GGCGTCGATATCT
GGCGTCTATATCT
GGCGTCTATATCTCGGCTCTAGGCCCTCATTTTTT
```

Coverage = 5

```
                  CTAGGCCCTCAATTTTT
                CTCTAGGCCCTCAATTTTT
              GGCTCTAGGCCCTCATTTTTT
           CTCGGCTCTAGCCCTCATTTT
        TATCTCGACTCTAGGCCCTCA
        TATCTCGACTCTAGGCC           177 bases
    TCTATATCTCGGCTCTAGG
GGCGTCTATATCTCG
GGCGTCGATATCT
GGCGTCTATATCT                        35 bases
GGCGTCTATATCTCGGCTCTAGGCCCTCATTTTTT
```

Average coverage = 177 / 35 ≈ 5-fold

Overlaps - Historical perspectives

- Sanger sequencing
  - Celera (Myers, 2000)
    originally developed for the assembly of the human genome
  - SGA (Simpson, Durbin, 2012)
- not suitable for NGS short reads (Illumina)

  computationally expensive : construction of the graph, size of the graph, path discovery
- comeback with long reads (Nanopore, Pacbio)

De Bruijn graphs

- introduced in bioinformatics to deal with NGS data
- used by allmost modern short-reads assembly tools

  seminal : `Velvet` (2008), `Abyss` (2009), `SOAPdenovo2` (2012)

  state-of-the art : `SPAdes` (2012), `MaSuRCA` (2013), `Megahit` (2015)...

Genome assembly reborn : recent computational challenges. M. Pop, Briefings in Bioinformatics 2009 `https://doi.org/10.1093/bib/bbp026`

How to apply de Bruijn graphs to genome assembly. P.E.C. Compeau, P.A. Pevzner, G. Tesler, Nature Biotechnology 2011 `doi:10.1038/nbt.2023`

Rationale

- The genome can be reconstructed from the *k*-mers it contains
- Reads are decomposed into *k*-mers

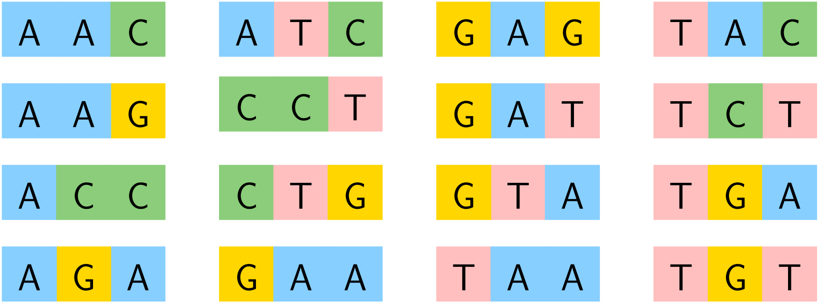
How many distinct 3-mers are they in

R_1 C T G A G A A C C T G T

R_2 C C T G T A A G A T

R_3 C T G T A C C T

R_4 G A T C T G A



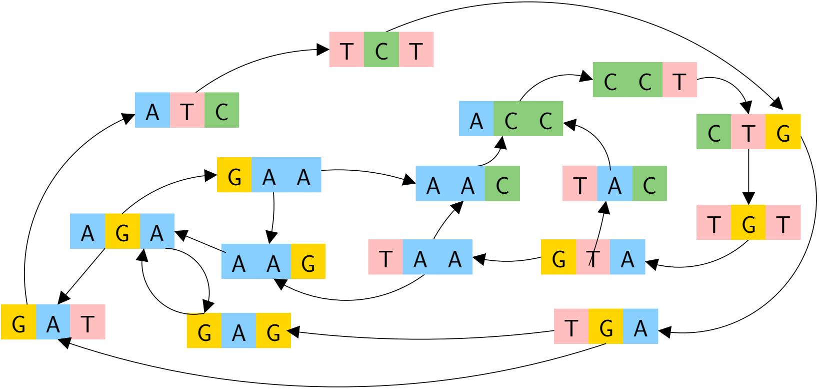
AAC	ATC	GAG	TAC
AAG	CCT	GAT	TCT
ACC	CTG	GTA	TGA
AGA	GAA	TAA	TGT

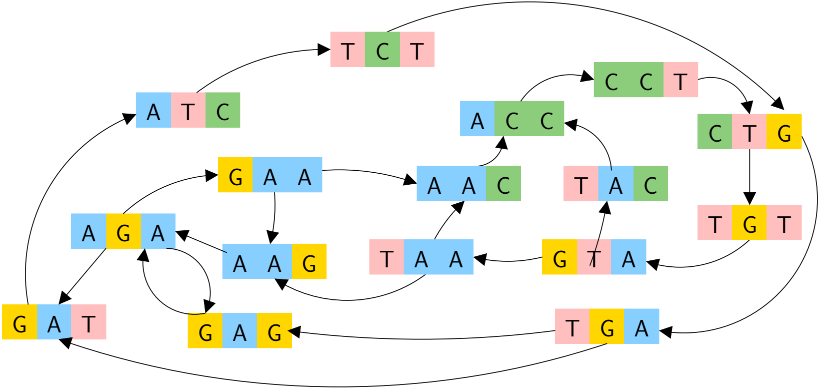
De Bruijn Graph

- Nodes : k-mers present in the reads
- Arcs : overlaps of length $k-1$ between k-mers

  Do not depend on the set of reads
- Easy to construct, low memory footprint

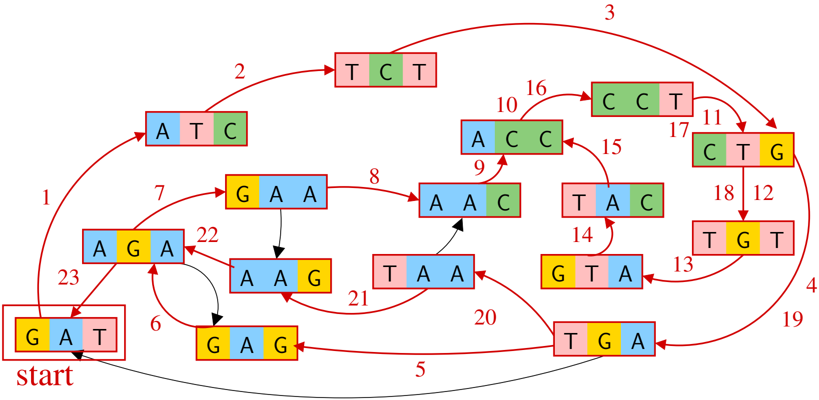
  Great advantage over overlap graphs


T C T
C C T
A T C
A C C
C T G
G A A
A A C
T A C
A G A
A A G
T A A
G T A
T G T
G A T
G A G
T G A


T C T
A T C
C C T
A C C
C T G
G A A
A A C
T A C
A G A
A A G
T A A
G T A
T G T
G A T
G A G
T G A

Assembly = path in the graph

Several paths = several assemblies


3
T C T
2
A T C
16
C C T
10
11
17
A C C
15
C T G
1
7
G A A
8
9
A A C
T A C
18
12
22
A G A
A A G
T A A
14
G T A
T G T
13
23
4
21
20
19
6
G A T
G A G
T G A
start
5

R_1 C T G A G A A C C T G T

C C T G T A A G A T R_2

R_4 G A T C T G A

R_3 C T G T A C C T

G A T C T G A G A A C C T G T A C C T G T A A G A T

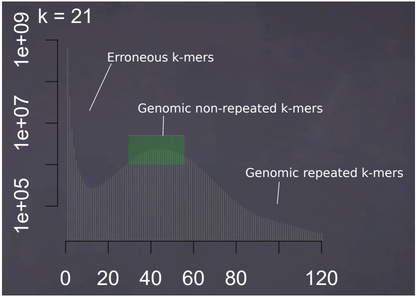
De Bruijn Graphs in practice - choice of k

Length of k-mers

- small k :
  - pro : more non-erroneous k-mers
  - cons : less signal, more random overlaps, repeat collapsing
- large k :
  - pro : higher signal, less random overlaps, less repeat collapsing
  - cons : more erroneous k-mers
- generally $k \geq 20$ (may be longer for large genomes)
- higher sequencing coverage means larger k values can be used
- multi-k assembly ($k = 21 \rightarrow k = 55 \rightarrow k = 72$)
  `IDBA`, `SPAdes`, `Megahit`

In this lecture : `SPAdes`

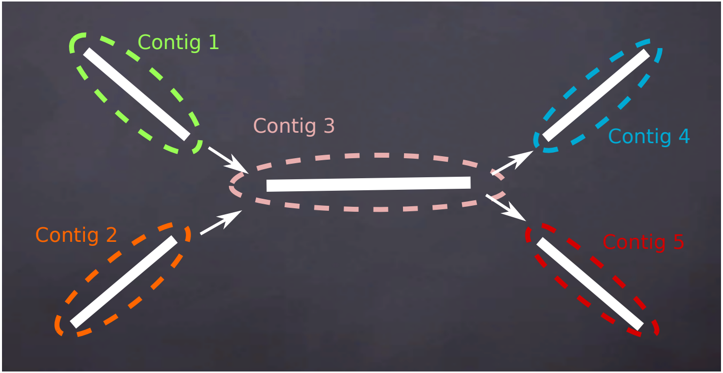
De Bruijn Graphs in practice - cleaning *k*-mers



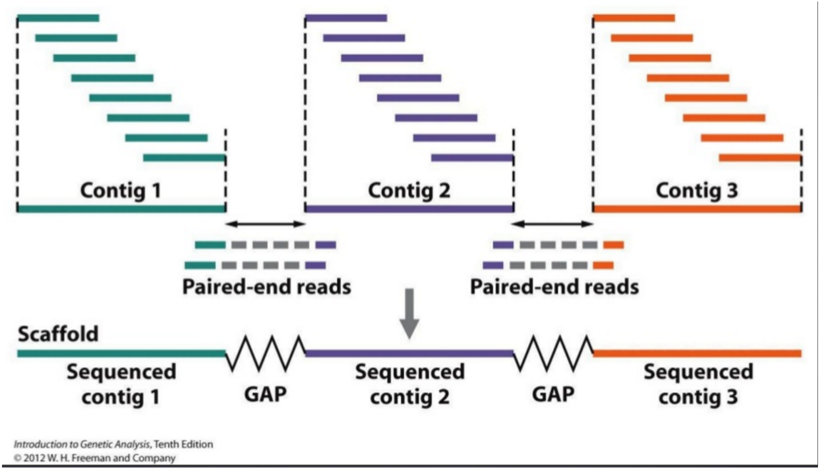
Courtesy of Rayan Chikhi (Institut Pasteur)
horizontal axis : number of occurrences
vertical axis : number of *k*-mers

- *k*-mers with low frequency are likely to contain sequencing errors
- they can be removed before the construction of the graph

De Bruijn Graphs in practice - contigs and scaffolds


Contig 1
Contig 3
Contig 4
Contig 2
Contig 5

Contigs =*simple* paths in the graph


Contig 1
Contig 2
Contig 3
Paired-end reads
Paired-end reads
Scaffold
Sequenced contig 1
GAP
Sequenced contig 2
GAP
Sequenced contig 3

Introduction to Genetic Analysis, Tenth Edition
© 2012 W. H. Freeman and Company

Scaffold = link between contigs using paired-end reads
Error-prone

Short read assembly is still difficult

even with De Bruijn graphs

- risk of contamination
- existence of sequencing errors solved
- existence of repeats
- diploid and polyploid genomes
- low coverage or unevent coverage

+ unable to handle the large number of NGS sequencing reads solved

Short read assembly is still difficult

- library design
  - longest read lengths
  - coverage $\geq$ 50x, x ploidy number
  - for 1 bacterial genome, no point going above 200x
  - BROAD recipe : several mate pairs libraries of increasing size
- assembler
  - SPAdes for small genomes
  - unclear for large genomes
  - try at least two assemblers, try different parameters
  - high computational requirements overall
- an assembly is not the absolute truth, it is a mostly complete, generally fragmented and mostly accurate hypothesis

How to compare/analyse assemblies ?

- no trivial ranking between assemblies
- no simple criteria
- assembly with high coverage and short contigs / assembly with low coverage and long contigs

Quast

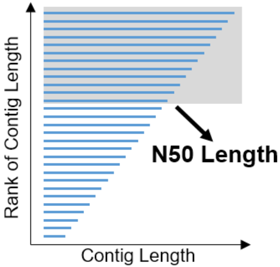
Quality Assessment Tool for Genome Assemblies

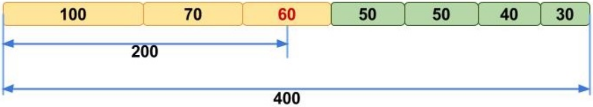
- provides a large number of statistics and metrics : contigs, missamblies, functional elements
- works both with and without a reference genome
- accepts multiple assemblies, thus is suitable for comparison

QUAST : quality assessment tool for genome assemblies. Bioinformatics 2013 `https://doi.org/10.1093/bioinformatics/btt086`

Contigs

- number of contigs
- length of the largest contig
- total number of bases in the assembly (sum of contig lengths)




100
70
60
50
50
40
30
200
400

- $N50$: contig length N for which 50% of all bases in the sequences are in a contig of length $L \geq N$
- $NG50$: contig length such that using equal or longer length contigs produces 50% of the expected length of the reference genome

Miassemblies (requires a reference genome)

- missassembly breakpoints : position in the contig where the left flanking sequence aligns over 1 kb away from the right flanking sequence on the reference, or they overlap >1 kb, or align on opposite strands or different chromosomes :
- metrics : total number of missambly breakpoints, number of contigs that contain misassembly breakpoints, number of bases contained in all contigs that have one or more misassemblies (`Mummer`)
- number of unaligned contigs : contigs that have no alignment to the reference sequence
- number of ambiguously mapped contigs : contigs that have multiple alignments to the reference genome

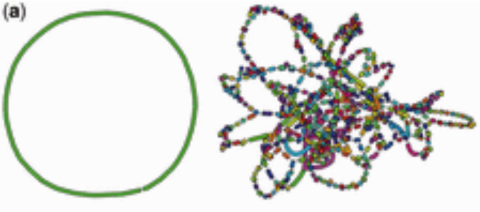
Functional elements

- genome fraction (%) : number of aligned bases in the reference, divided by the genome size.
- duplication ratio : number of aligned bases in the assembly divided by the number of aligned bases in the reference
- number of mismatches and number of indels per 100 kb
- number of genes based on a user-provided annotated list of gene positions in the reference genome
- number of predicted genes in the assembly (`GeneMark.hmm` for prokaryotes and `GlimmerHMM` for eukaryotes)

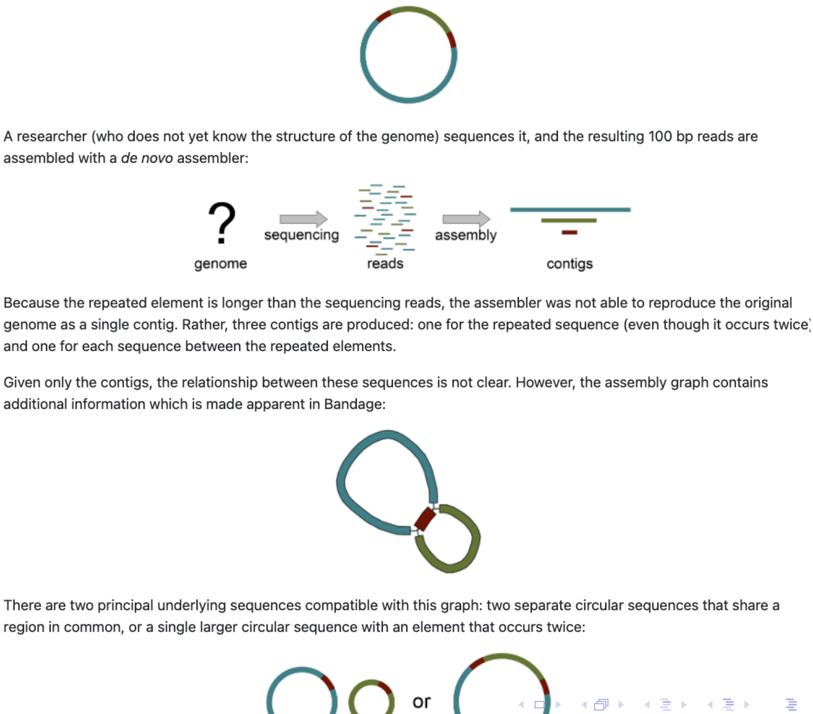
Bandage

Bioinformatics Application for Navigating De novo Assembly Graphs Easily

- interactive visualization of the assembly graph (such as de Bruijn graph)
- `https://github.com/rrwick/Bandage/wiki/`



Left : ideal bacterial assembly with one single contig
Right : poor assembly with many short contigs



A researcher (who does not yet know the structure of the genome) sequences it, and the resulting 100 bp reads are assembled with a *de novo* assembler:

Because the repeated element is longer than the sequencing reads, the assembler was not able to reproduce the original genome as a single contig. Rather, three contigs are produced: one for the repeated sequence (even though it occurs twice) and one for each sequence between the repeated elements.

Given only the contigs, the relationship between these sequences is not clear. However, the assembly graph contains additional information which is made apparent in Bandage:

There are two principal underlying sequences compatible with this graph: two separate circular sequences that share a region in common, or a single larger circular sequence with an element that occurs twice:

Exercise

`https://zenodo.org/record/582600/files/mutant_R1.fastq`
`https://zenodo.org/record/582600/files/mutant_R2.fastq`

- Assembly with `SPAdes`
- Quality metrics with `Quast`
- Visualization with `Bandage image (version 0.8.1 + Galaxy 4)`

Bacterial training dataset for Galaxy training network tutorials on Genome assembly : imaginary *Staphylococcus aureus* bacterium with a miniature genome.