A little tour of assembly methods

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EVOMICS Workshop on Genomics 2020 - Český Krumlov

Content of this course

- How to reconstruct a genome with sequencing data?
- What are the main challenges?
- Which solutions have been proposed?

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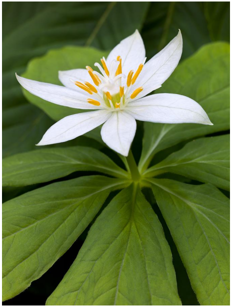
Vol. 554, No. 7690

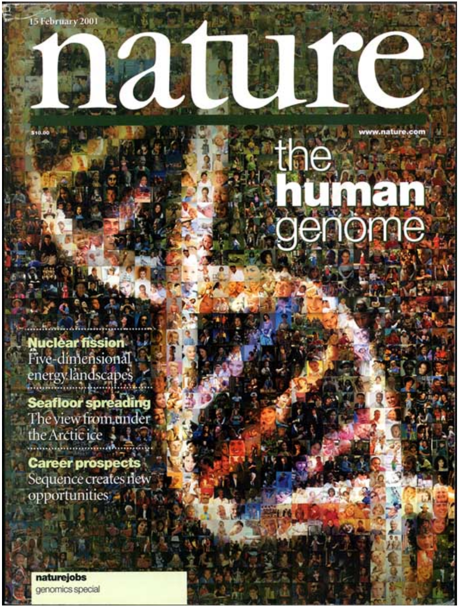
genome size: $\sim$ 32 gigabases

Content of this course

- What is assembly?
- What are the main problematics to bear in mind when conducting an assembly?
- What are the current limits of assembly?



Assembly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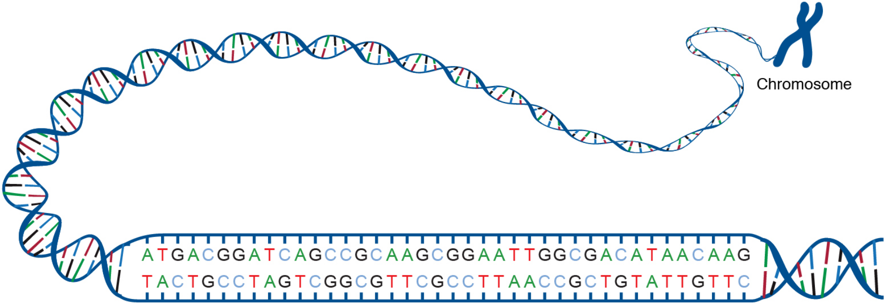
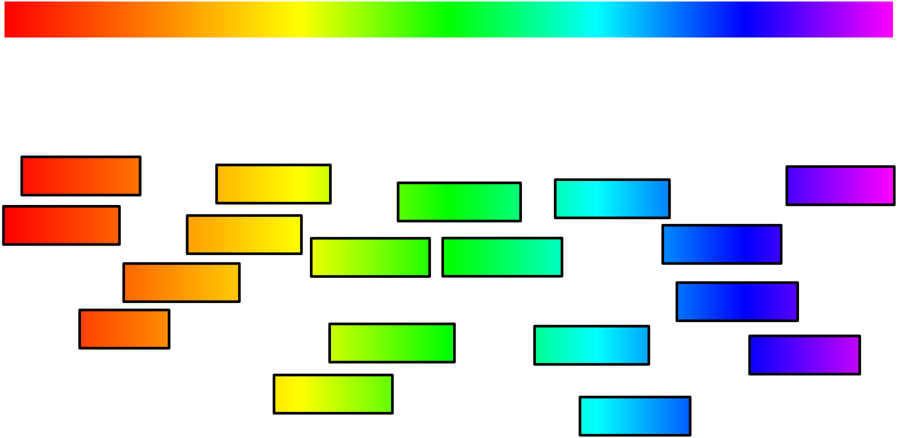
Accessing a genome

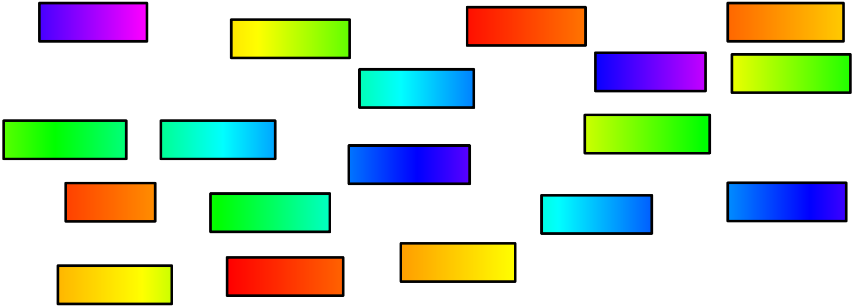

Chromosome
ATGACGGATCAGCCGCAAGCGGAATTGGCGACATAACAAG
TACTGCCTAGTCGGCGTTCGCCTTAACCGCTGTATTGTTC

From `www.genome.gov/genetics-glossary/acgt`

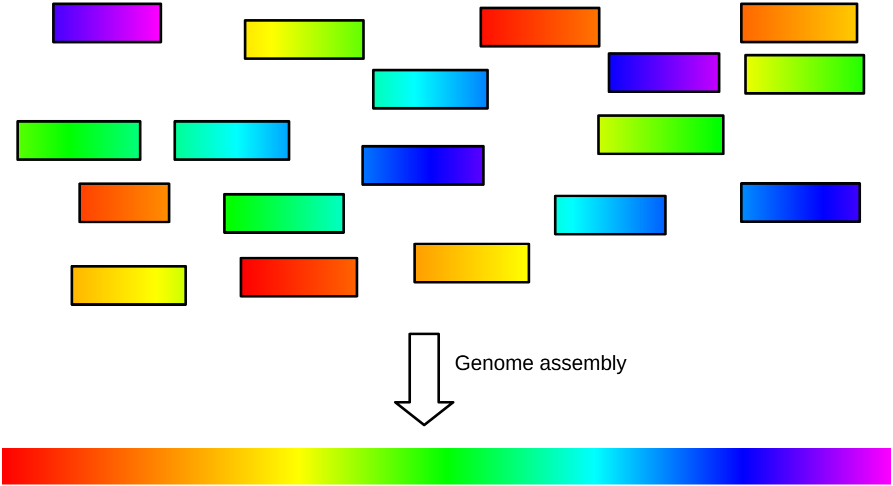
Reads are words from the genome



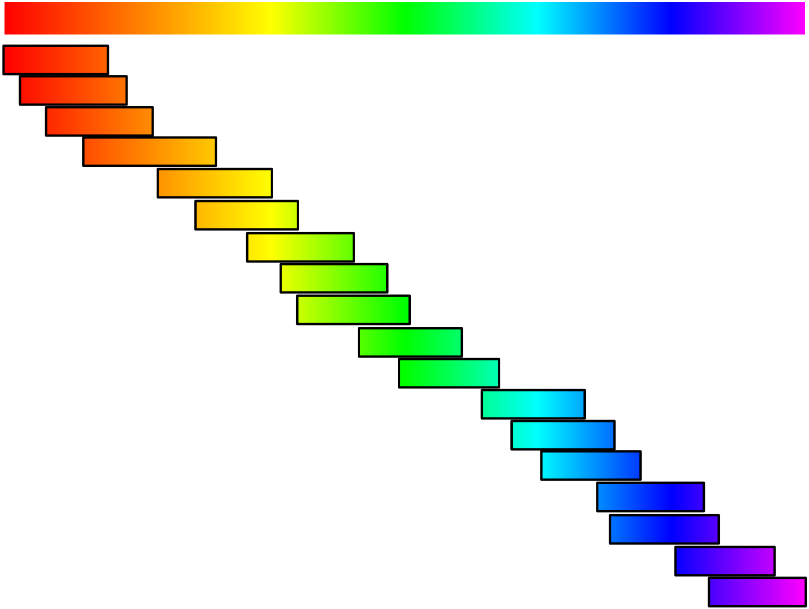
Reads are **shuffled** words from the genome



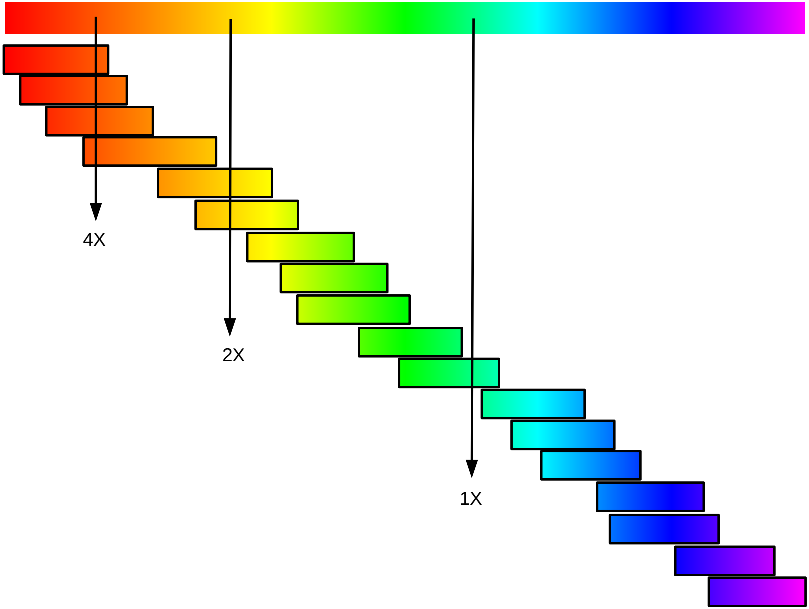
Genome assembly task



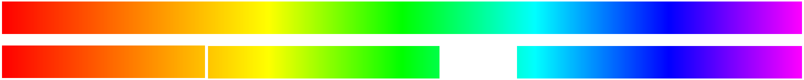
Using read overlaps



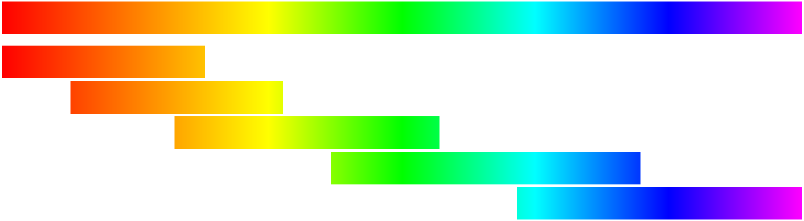
Genome sequencing: coverage



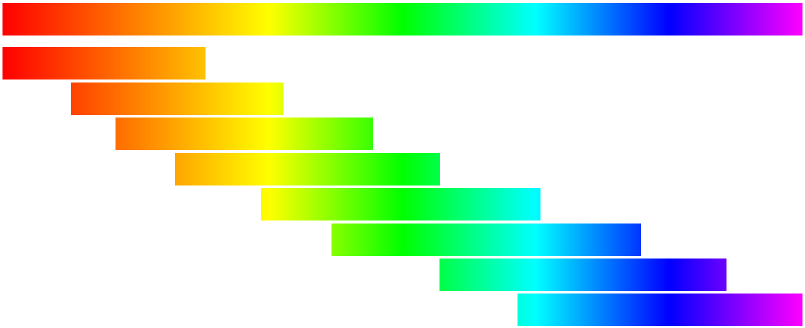
Genome sequencing: coverage



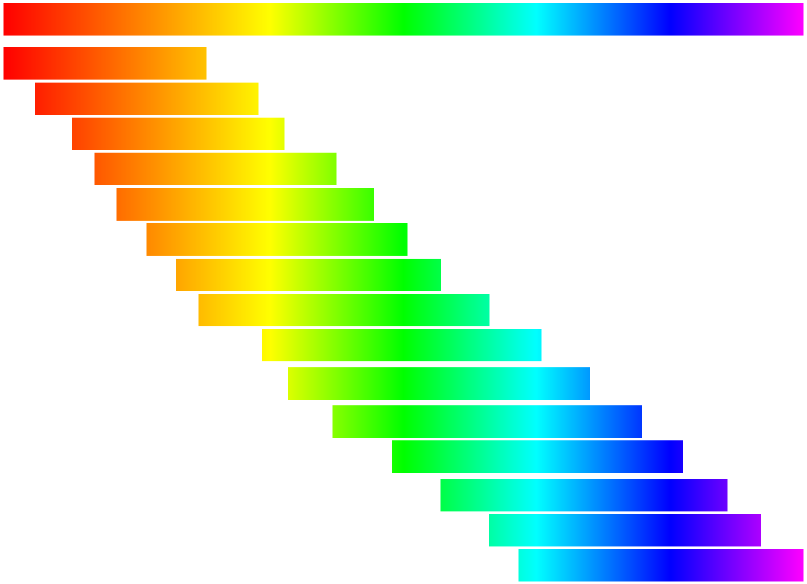
Genome sequencing: coverage



Genome sequencing: coverage



Genome sequencing: coverage



Assembly **idea number 1: Select the longest overlaps**

```
Reads:
1: ATCGGTATCG
2: GGTATCGTTA
3: ATCGTTACGG
4: GTTACGGTAT
5: ACGGTATACC
```

```
1: ATCGGTATCG                Overlap length:7
2:    GGTATCGTTA

1: ATCGGTATCG                Overlap length:4
3:       ATCGTTACGG

1: ATCGGTATCG                Overlap length:1
4:          GTTACGGTAT


1: ATCGGTATCG                No Overlap
5:           ACGGTATACC
```

Assembly idea number 1: assemble the longest overlaps

```
Reads:
1: ATCGGTATCG
2: GGTATCGTTA
3: ATCGTTACGG
4: GTTACGGTAT
5: ACGGTATACC

Best overlaps:
1: ATCGGTATCG
2:   GGTATCGTTA
3:      ATCGTTACGG
4:         GTTACGGTAT
5:            ACGGTATACC

Output “genome”:
ATCGGTATCG + TTA + CGG + TAT + ACC
ATCGGTATCGTTACGGTATACC
```

Your time to shine!

```
Let assemble this genome!

Your read set:

1: ATTTACGGGT
2: TTACGGGTGG
3: ACGGGTCCTT
4: GTCCTTTCTT
5: TTTCTTACGG

 For each read:
    Find the best overlap (length>5)
    Merge the two reads
```

The Greedy solution

```
The best overlaps:
ATTTACGGGT
  TTACGGGTGG

ACGGGTCCTT
    GTCCTTTCTT
        TTTCTTACGG
```

```
Output "genome"
ATTTACGGGTGG
ACGGGTCCTTTCTTACGG
```

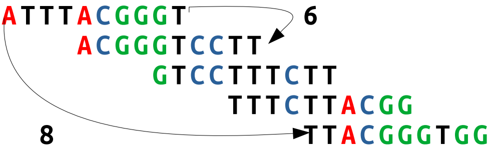
The actual solution

```
The actual genome:
ATTTACGGGTCCTTTCTTACGGGTGG

How the reads should be ordered:
ATTTACGGGT     6
    ACGGGTCCTT
        GTCCTTTCTT
            TTTCTTACGG
                TTACGGGTGG
```

What happened?

```
The actual genome:
ATTTACGGGTCCTTTCTTACGGGTGG
```

How the reads should be ordered:

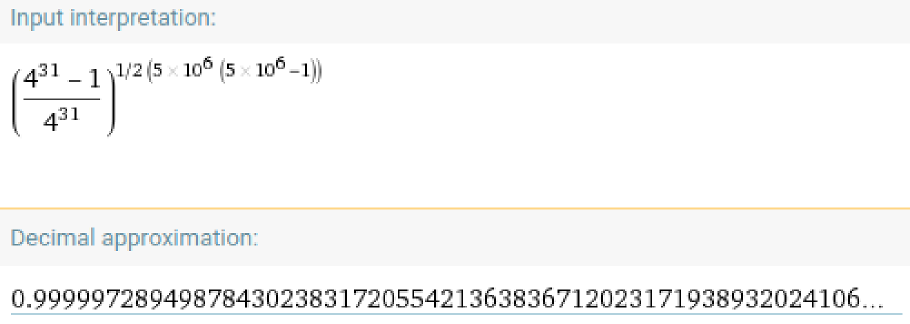


```
ATTTACGGGT      6
    ACGGGTCCTT
        GTCCTTTCTT
            TTTCTTACGG
  8             TTACGGGTGG
```

```
ATTTACGGGT                    ATTTACGGGTGG
  TTACGGGTGG                  Not in the genome


ACGGGTCCTT                    ACGGGTCCTTTCTTACGG
    GTCCTTTCTT                Not in the genome
        TTTCTTACGG
```

Do we expect many repeats?

Probability to have NO repeated word of size 31 in a 5 megabases genome



Input interpretation:

$$\left(\frac{4^{31}-1}{4^{31}}\right)^{1/2\left(5\times 10^{6}\left(5\times 10^{6}-1\right)\right)}$$

Decimal approximation:

0.999997289498784302383172055421363836712023171938932024106…

From en.wikipedia.org/wiki/Birthday_problem

The burden of assembly: genomic repeats

Amount of repeats larger than a given size in *E. coli* genome

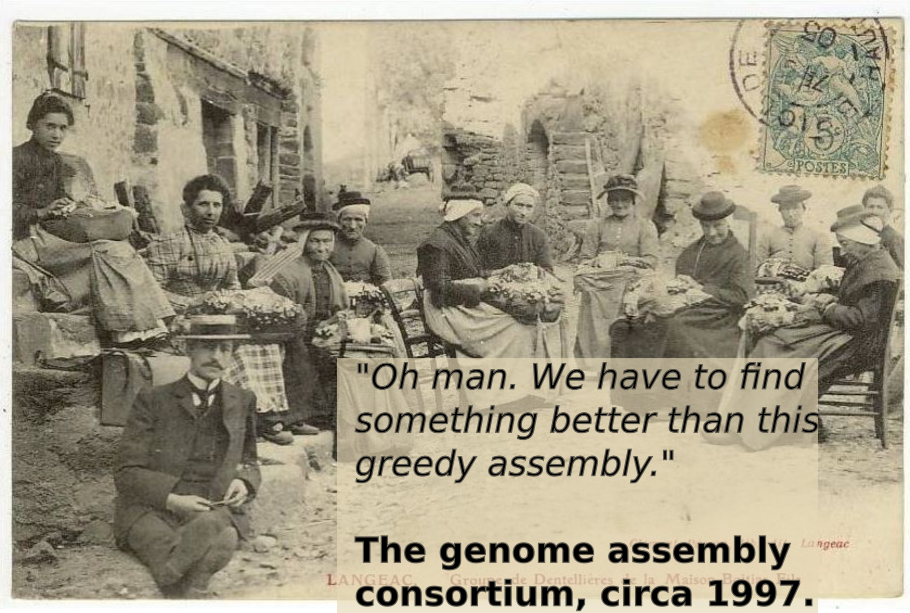
- 15: 44,994
- 21: 1,169
- 31: 559
- 41: 323
- 51: 225
- 61: 192

Genomic repeats are NOT random events

Greedy assemblers

- Simple and efficient scheme
- Rely on **local** best choice (greedy)
- May create errors because of local choices when there are repeats

History from the last century



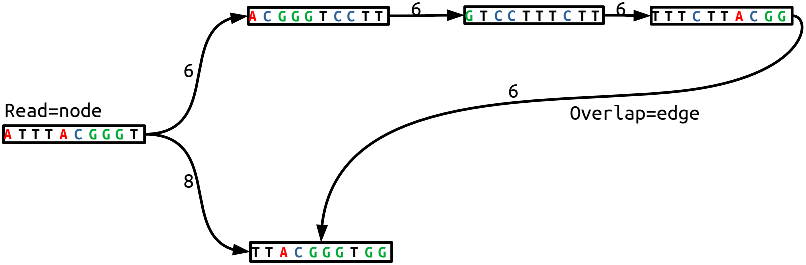
"Oh man. We have to find something better than this greedy assembly."

The genome assembly consortium, circa 1997.

Assembly **idea number 2: consider all overlaps**

Genome:
ATTTACGGGTCCTTTCTTACGGGTGG

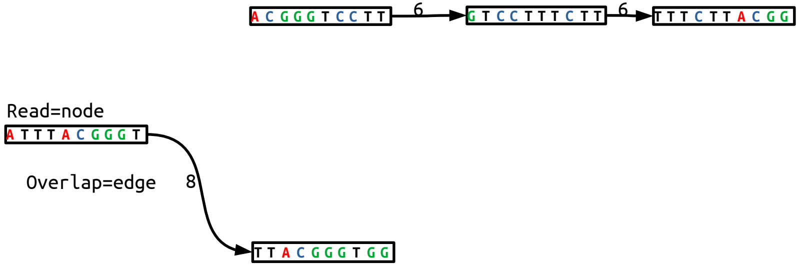
Overlap graph:



Greedy solution

Genome:
ATTTACGGGTCCTTTCTTACGGGTGG

Overlap graph:

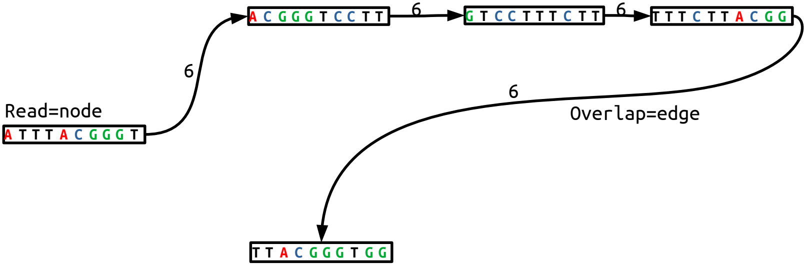


Greedy assembly output:
ATTTACGGGTGG
ACGGGTCCTTTCTTACGG

One piece solution

Genome:
ATTTACGGGTCCTTTCTTACGGGTGG

Overlap graph:



Read=node

ATTTACGGGT

6

ACGGGTCCTT

6

GTCCTTTCTT

6

TTTCTTACGG

6

Overlap=edge

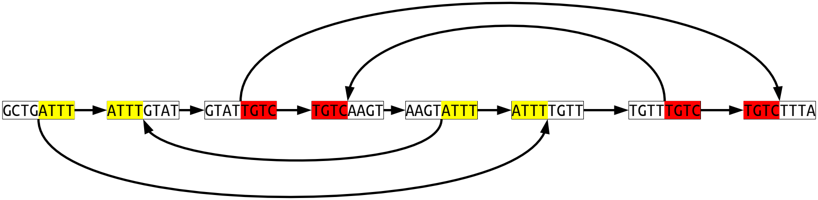
TTACGGGTGG

Overlap graph output:
ATTTACGGGTCCTTTCTTACGGGTGG

Multiple repeats

```
Reads:
GCTGATTT
ATTTGTAT
GTATTGTC
TGTCAAGT
AAGTATTT
ATTTTGTT
TGTTTGTC
TGTCTTTA
```

Overlap graph:



GCTGATTT

ATTTGTAT

GTATTGTC

TGTCAAGT

AAGTATTT

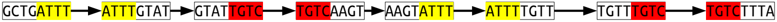
ATTTTGTT

TGTTTGTC

TGTCTTTA

First solution

```
Reads:
GCTGATTT
ATTTGTAT
GTATTGTC
TGTCAAGT
AAGTATTT
ATTTTGTT
TGTTTGTC
TGTCTTTA Overlap graph:
```



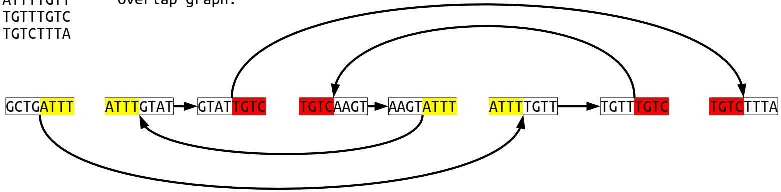
GCTGATTT → ATTTGTAT → GTATTGTC → TGTCAAGT → AAGTATTT → ATTTTGTT → TGTTTGTC → TGTCTTTA

```
Possible assemblies:
GCTGATTTGTATTGTCAAGTATTTTGTTTGTCTTTA
```

Second solution

```
Reads:
GCTGATTT
ATTTGTAT
GTATTGTC
TGTCAAGT
AAGTATTT
ATTTTGTT
TGTTTGTC
TGTCTTTA
```

Overlap graph:

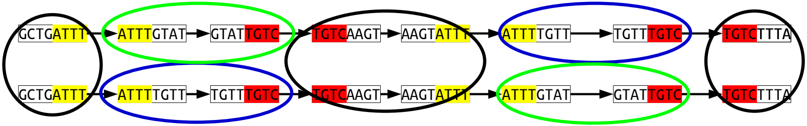


```
Possible assemblies:
GCTGATTTGTATTGTCAAGTATTTTGTTTGTCTTTA
GCTGATTTTGTTTGTCAAGTATTTGTATTGTCTTTA
```

Those two solutions are indistinguishable

Parsimonious solution: do not assemble

Possible assemblies:



GCTGATTT → ATTTGTAT → GTATTGTC → TGTCAAGT → AAGTATTT → ATTTTGTT → TGTTTGTC → TGTCTTTA

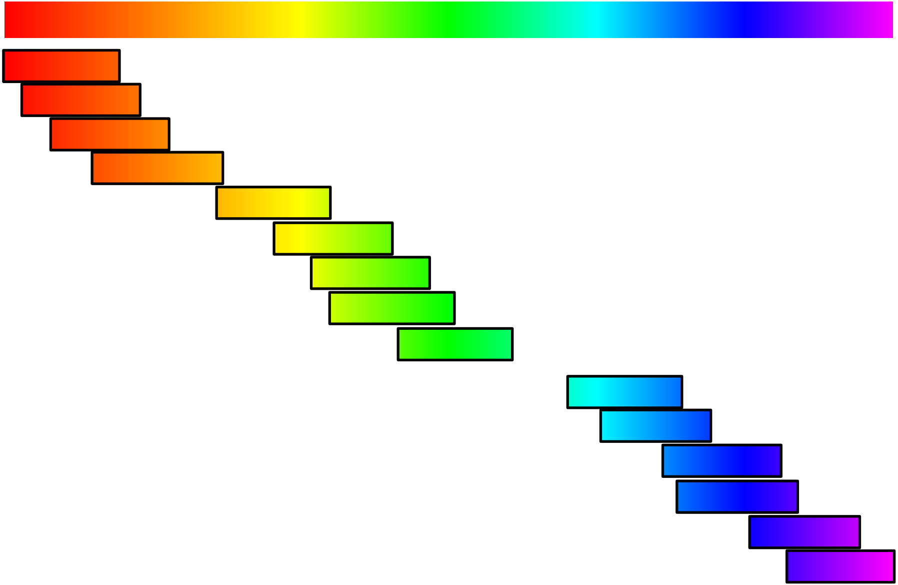
GCTGATTT → ATTTTGTT → TGTTTGTC → TGTCAAGT → AAGTATTT → ATTTGTAT → GTATTGTC → TGTCTTTA

Genome pieces:

GCTGATTT ATTTGTATTGTC TGTCAAGTATTT ATTTTGTTTGTC

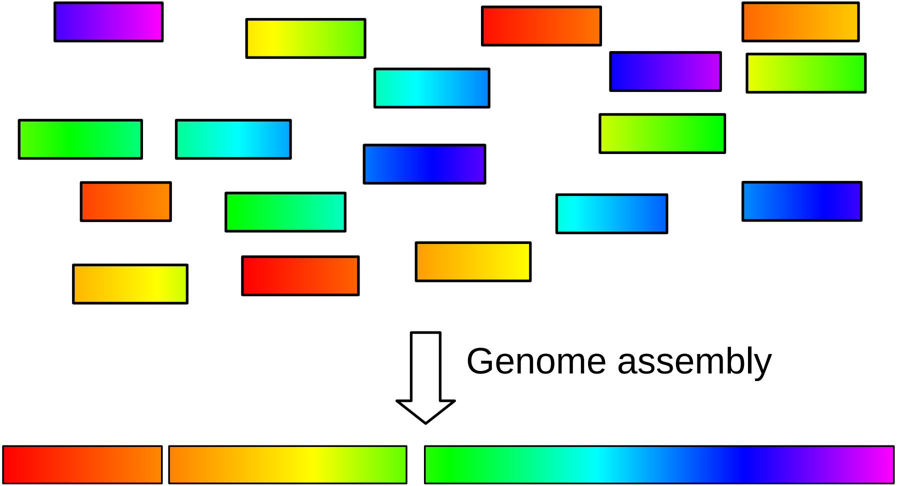
Repeats lead to the fragmentation of the assembly

Missing information also fragments the assembly

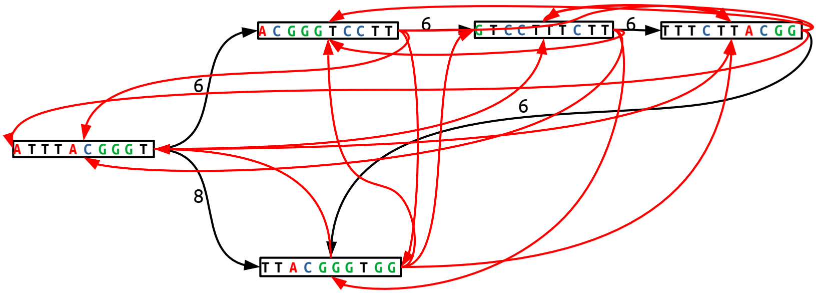


Assembly **concession number 1: output fragments**

In the real world, assemblers often provide pieces of genomes rather than complete ones

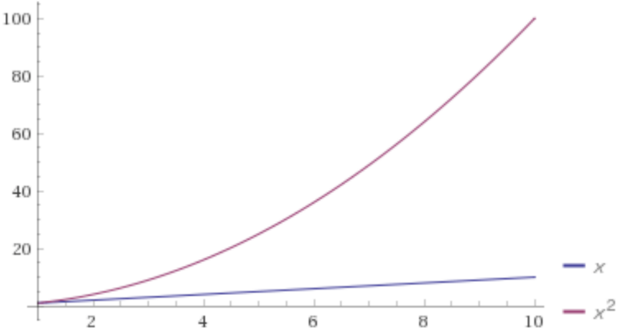


Overlap graph prerequisite : all overlaps



Overlap graph burden: number of reads

$n(n-1)/2 = \mathcal{O}(n^2)$ possible overlaps for n reads



Linear: 2X data 2X time
Quadratic: 2X data 4X time

Overlap graph burden: number of reads

$n(n-1)/2 = \mathcal{O}(n^2)$ possible overlaps for n reads

# Reads	# Overlaps
1000	499,500
10,000	50 million
100,000	5 billion
1 million	500 billion
10 million	50 trillion. . .

Most overlaps are too small to be considered...

The overlap computation is not linear

Overlap graphs in a nutshell

- Graphs of overlaps between the reads
- Can provide a global solution for assembly
- Can be difficult in real cases because it requires a lot of computation (overlaps)

S. cerevisiae, *D. melanogaster*, human could be assembled using overlap graphs approaches (Celera (Myers et al. 2000), SGA (Simpson & Durbin 2011), ...)

Fast forward

Assembly **idea number 3: Focus on genome words**

```
AGATACAGCCATGACCGTAGCATGCTAACTGTGACGGCATTAC
       GCCATGACC read
```

a read is a word from the genome

words of size 7 from the genome:

```
AGATACA
 GATACAG
  ATACAGC
   TACAGCC
         ...
```

k-mer definition

A *k*-mer is a word of size *k*

Exercise

List all **distinct** 4-mers and 5-mers of this set of reads:
CATAATGCA
TGCACATAA
CATAATGCA

Solution

CATAATGCA, TGCA**CATAA**, **CATAATGCA**

5-mers:

CATAA, ATAAT, TAATC, AATGC, ATGCA, TGCAC, GCACA, ACATA

CATAATGCA, **TGCA**CATAA, **CATAATGCA**

4-mers:

CATA, ATAA, TAAT, AATG, ATGC, TGCA, GCAC, CACA, ACAT

Genome words / read words

Let's select a word from the genome:

AGATACAGCCATGACCG**TAGCATG**CTAACTGTGACGGCATTAC

in the genome, after **TAGCAT**

only **AGCATG** appears

Genome words / read words

In real cases we don't have the genome

AGATACAGCCATGACCG**TAGCATG**CTAACTGTGACGGCATTAC

in the genome, after **TAGCAT**
only **AGCATG** appears

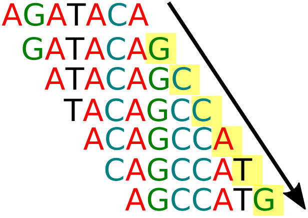
but we have the reads

ATGACCG**TAGCATG**CT
ATGACCG**TAGCATG**CT
GACCG**TAGCATG**CTAA

in the reads, after **TAGCAT**
only **AGCATG** appears

Reconstitute larger genomic words

AGATACAGCCATGACCGTAGCATGCTAACTGTGACGGCATTAC



AGATACA
GATACAG
ATACAGC
TACAGCC
ACAGCCA
CAGCCAT
AGCCATG

AGATACA + G + C + C + A + T + G

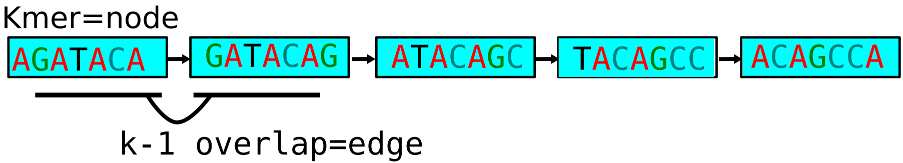
$\boxed{\text{AGATACAGCCATG}}$ a sequence from the genome

The De Bruijn graph

Read

AGATACAGCCA

De Bruijn graph



Kmer=node

AGATACA → GATACAG → ATACAGC → TACAGCC → ACAGCCA

k-1 overlap=edge

AGATACA + G + C + C + A

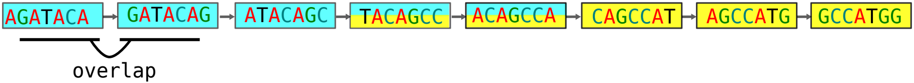
=AGATACAGCCA

De Bruijn graph assembly

Overlapping reads

```
AGATACAGCCA
   TACAGCCATGG
```

De Bruijn graph



Resulting sequence

AGATACAGCCATGG

De Bruijn graph time!

Reads

GCCATGGGTTT
TACAGCCATGG
AGCCATGGGTT
GCCATGGGTTT
AGATACAGCCA
ACAGCCATGGG
GATACAGCCATG
CATGGGTTTAA
ACAGCCATGGG
GATACAGCCATG
CATGGGTTTAA
CAGCCATGGGT

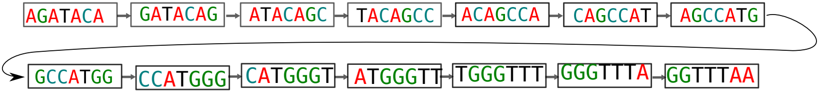
Use 7-mers

Solution

Overlapping reads

```
AGATACAGCCA
 GATACAGCCATG
 GATACAGCCATG
   TACAGCCATGG
    ACAGCCATGGG
    ACAGCCATGGG
     CAGCCATGGGT
      AGCCATGGGTT
       GCCATGGGTTT
       GCCATGGGTTT
         CATGGGTTTAA
         CATGGGTTTAA
```

De Bruijn graph

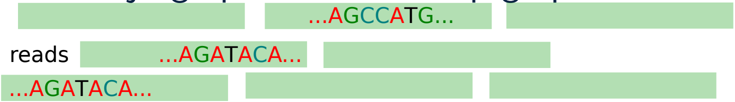


AGATACA → GATACAG → ATACAGC → TACAGCC → ACAGCCA → CAGCCAT → AGCCATG → GCCATGG → CCATGGG → CATGGGT → ATGGGTT → TGGGTTT → GGGTTTA → GGTTTAA

Resulting sequence

AGATACAGCCATGGGTTTAA

De Bruijn graph versus overlap graph


...AGCCATG...

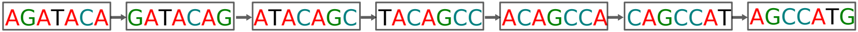
reads ...AGATACA...

...AGATACA...

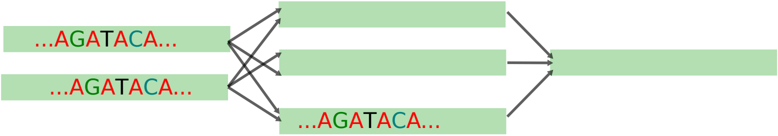
AGATACA
GATACAG   TACAGCC   AGCCATG
ATACAGC   ACAGCCA   CAGCCAT
...

words from the reads

word graph (De Bruijn graph)


AGATACA → GATACAG → ATACAGC → TACAGCC → ACAGCCA → CAGCCAT → AGCCATG

Overlap graph from the reads


...AGATACA...

...AGATACA...

...AGATACA...

De Bruijn graphs abstract redundancy

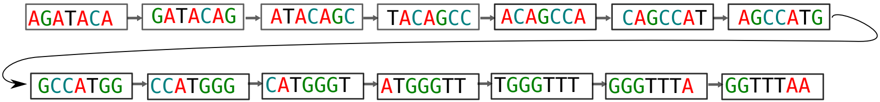
Overlapping reads

```
AGATACAGCCA
 GATACAGCCATG
 GATACAGCCATG
   TACAGCCATGG
    ACAGCCATGGG
    ACAGCCATGGG
     CAGCCATGGGT
      AGCCATGGGTT
       GCCATGGGTTT
       GCCATGGGTTT
         CATGGGTTTAA
         CATGGGTTTAA
```

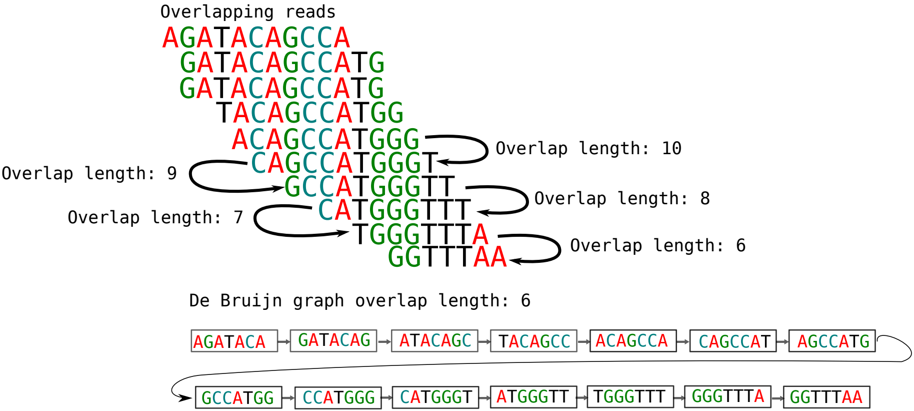
62 **(non distinct)** 7-mers in the reads

De Bruijn graph

14 **distinct** 7-mers in the De Bruijn graph



De Bruijn graphs only rely on $k-1$ overlaps



Overlapping reads

AGATACAGCCA
GATACAGCCATG
GATACAGCCATG
TACAGCCATGG
ACAGCCATGGG
CAGCCATGGGT
GCCATGGGTT
CATGGGTTT
TGGGTTTA
GGTTTAA

Overlap length: 10

Overlap length: 9

Overlap length: 8

Overlap length: 7

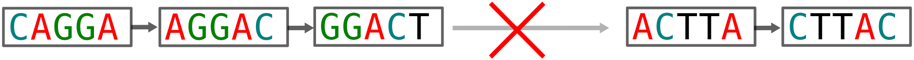
Overlap length: 6

De Bruijn graph overlap length: 6

AGATACA → GATACAG → ATACAGC → TACAGCC → ACAGCCA → CAGCCAT → AGCCATG → GCCATGG → CCATGGG → CATGGGT → ATGGGTT → TGGGTTT → GGGTTTA → GGTTTAA

De Bruijn graphs limitation

Fixed overlaps



CAGGA → AGGAC → GGACT → ACTTA → CTTAC

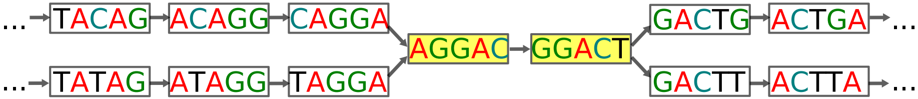
GACTT missing

not k-1 overlap

De Bruijn graphs limitation

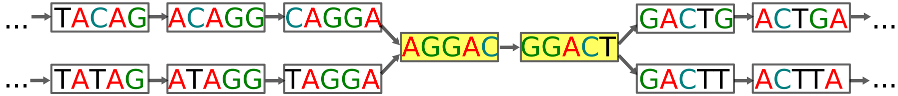
Repeats...

...TACAGGACTTA... ...TATAGGACTGA...



De Bruijn graph limitation

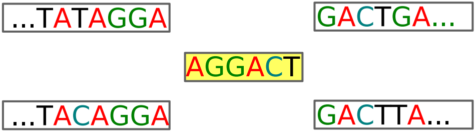
...TACAGGACTTA... ...TATAGGACTGA...



... TACAG ACAGG CAGGA AGGAC GGACT GACTG ACTGA ...

... TATAG ATAGG TAGGA GACTT ACTTA ...

genome pieces

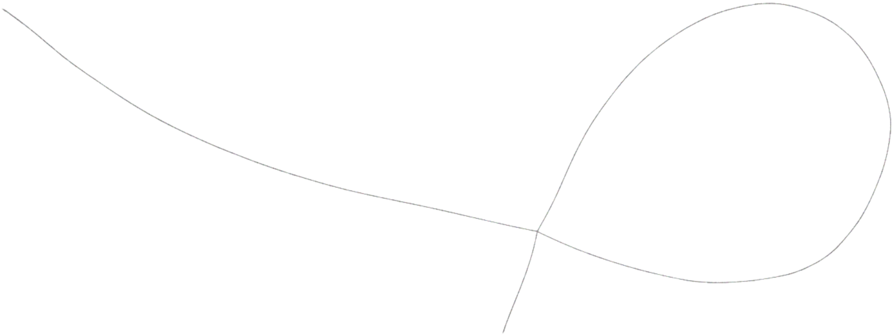


...TATAGGA GACTGA...

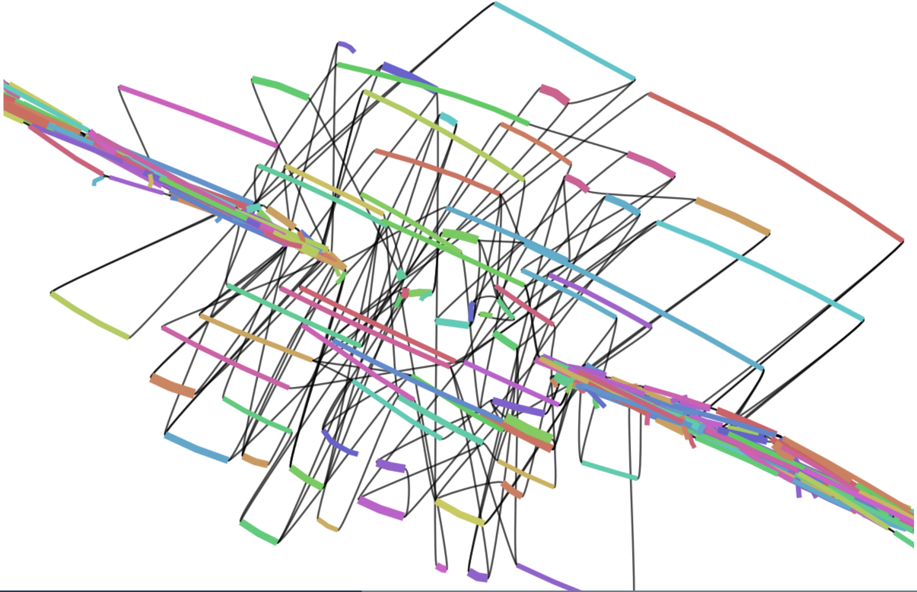
AGGACT

...TACAGGA GACTTA...

De Bruijn graph on a real dataset

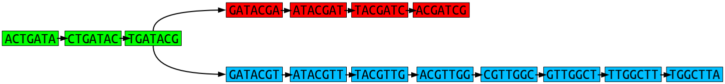


De Bruijn graph on real dataset ZOOMED IN



On the representation of De Bruijn graphs

De Bruijn graph:

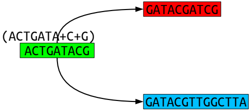


ACTGATA → CTGATAC → TGATACG

GATACGA → ATACGAT → TACGATC → ACGATCG

GATACGT → ATACGTT → TACGTTG → ACGTTGG → CGTTGGC → GTTGGCT → TTGGCTT → TGGCTTA

Compacted De Bruijn graph:



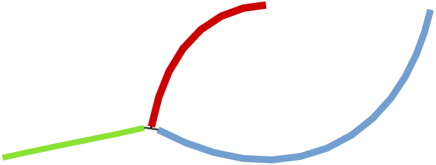
(ACTGATA+C+G)

ACTGATACG

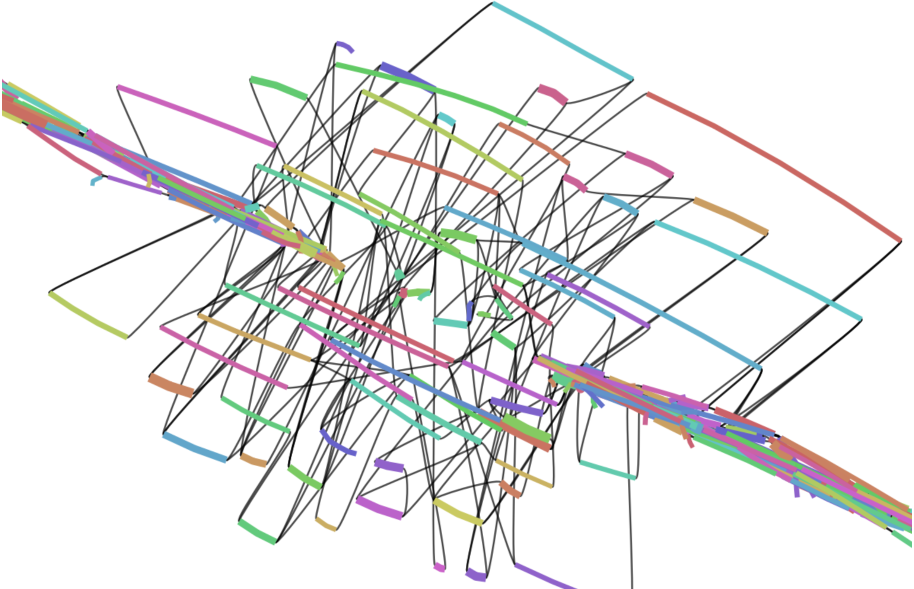
GATACGATCG

GATACGTTGGCTTA

Graphical representation
(.gfa plot using Bandage):



De Bruijn graph on a real dataset ZOOMED IN



Dealing with sequencing errors

```
Genome:
 ATCGGTATCGTTACGGTATACC

Reads:
 ATCGCTATCG
    GGTTTCGTTA
       ATCGATACGG

TCGCTA
GGTTTC
ATCGAT
...
Are not genomic kmers...
```

Erroneous *k*-mers vs genomic *k*-mers

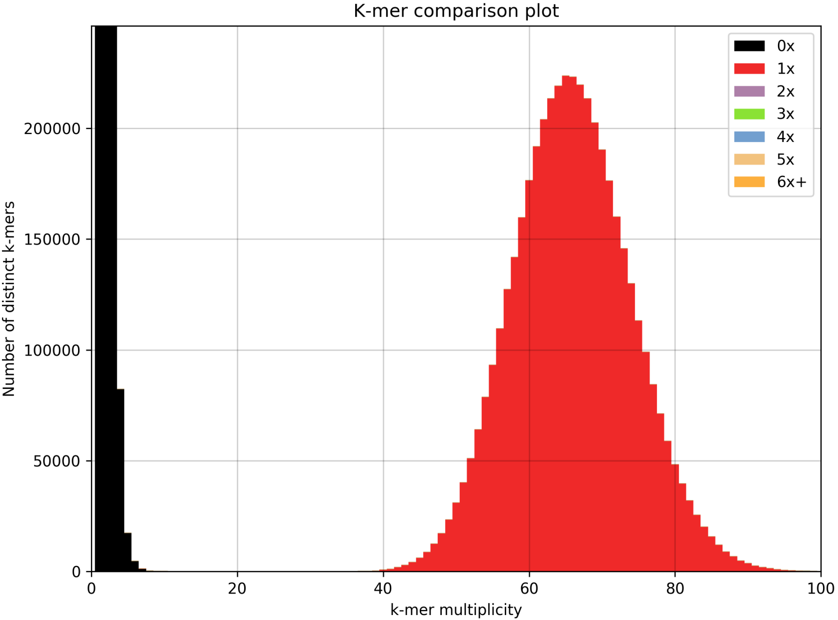
Genome:
TAAGAAAGCTCTGAATCAACGGACTGCGACA

```
Reads:
TAAGAAAGCTCTGAATCA
 AAGAAAGCTCTAAATCAAC
  AGAAAGCTCTGAATCAACG
   GAAAGCTCTGAATCAACGGA
    AAAGCTCTGAATCAACGGAC
     AAGCTCTGAATCAACGGACT
      AGCTCTGAATCAACGGACTG
       GCTCTGAATCAACGGTCTGC
        CTCTGAATCAACGGACTGCG
         TCTGAATCAACGGACTGCGA
```

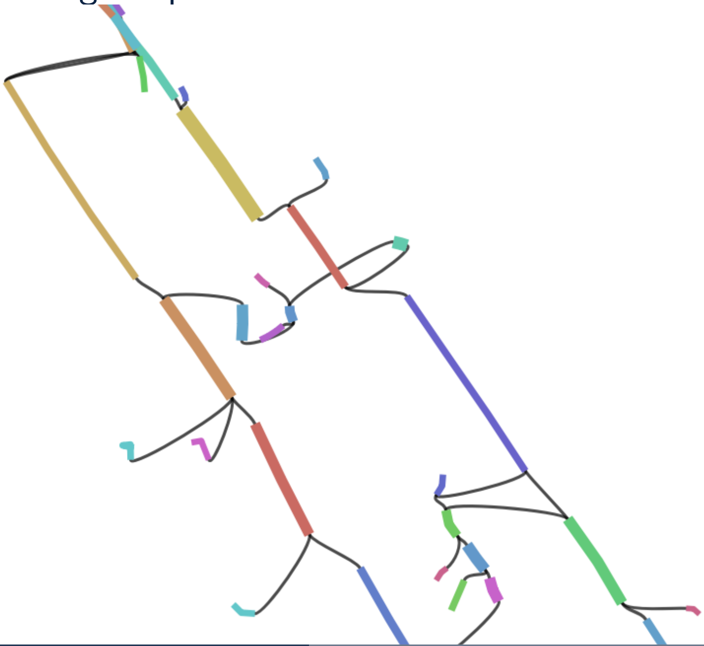
```
9 times  TCTGAAT
1 time   TCTAAAT

6 times         CAACGGA
1 time          CAACGGT
```

Erroneous *k*-mers are seen less than genomic ones

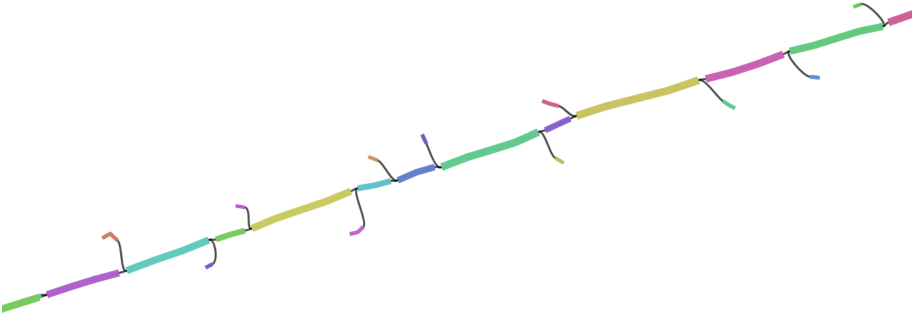
K-mer histogram



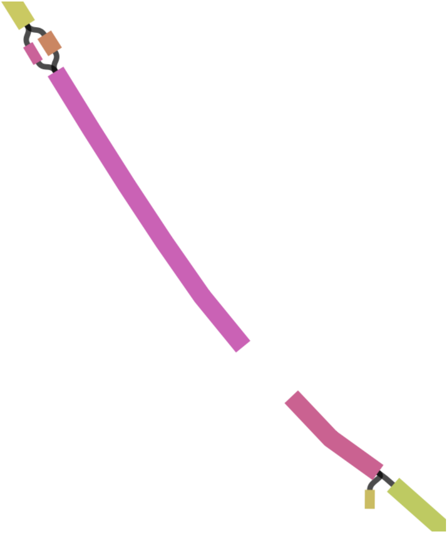
Removing unique *k*-mers



Removing *k*-mers seen less than 3 times

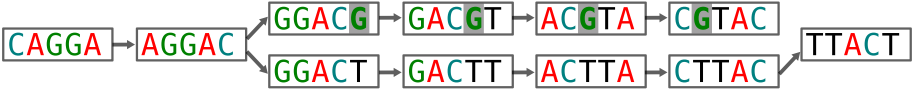


Removing *k*-mers seen less than 4 times



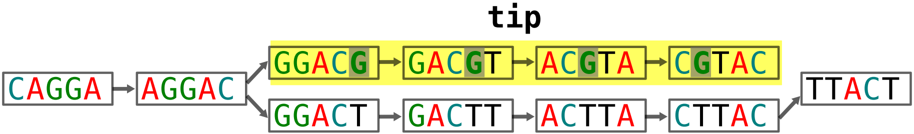
Errors in De Bruijn graphs

```
...TACAGGACTTACTGA...  genome

        CAGGACTTA
reads    AGGACGTAC ←—— sequencing error
         AGGACTTAC
          GGACTTACT
```



Errors in De Bruijn graphs

```
      ...TACAGGACTTACTGA...  genome

          CAGGACTTA
reads      AGGACGTAC ←—— sequencing error
           AGGACTTAC
            GGACTTACT
```



tip

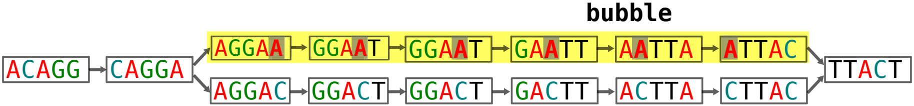
CAGGA → AGGAC

GGACG → GACGT → ACGTA → CGTAC

GGACT → GACTT → ACTTA → CTTAC → TTACT

Errors in De Bruijn graphs

```
        ...TACAGGACTTACTGA...  genome

              ACAGGACTTA
       reads   CAGGAATTAC ←—— sequencing error
               CAGGACTTAC
                AGGACTTACT
```



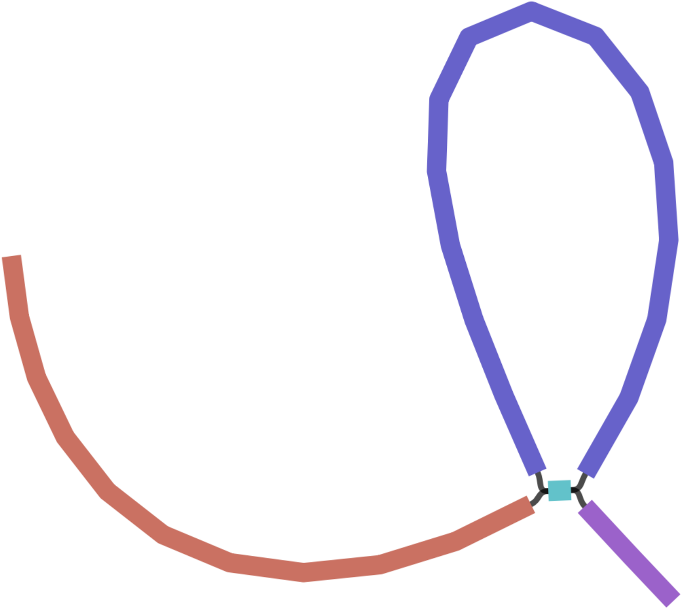
bubble

ACAGG → CAGGA

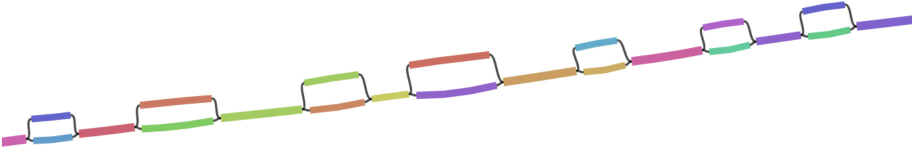
CAGGA → AGGAA → GGAAT → GGAAT → GAATT → AATTA → ATTAC → TTACT

CAGGA → AGGAC → GGACT → GGACT → GACTT → ACTTA → CTTAC → TTACT

(Almost assembled phage !)



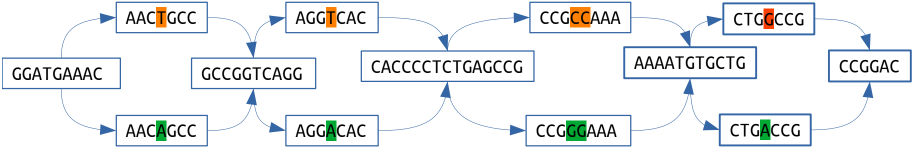
De Bruijn graph on an eukaryota



Two or more genomes per individual

♀ GGATGAAACTGCCGGTCAGGTCACCCCTCTGAGCCGCCAAAATGTGCTGGCCGGAC

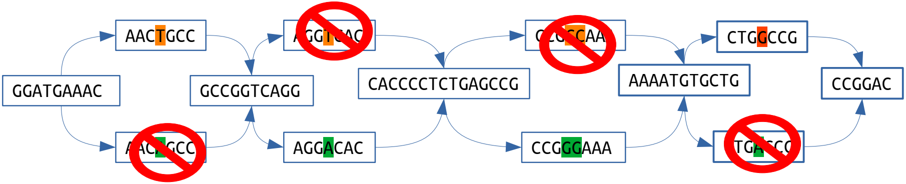
♂ GGATGAAACAGCCGGTCAGGACACCCCTCTGAGCCGGGAAAATGTGCTGACCGGAC



Two or more genomes per individual

♀ GGATGAAACTGCCGGTCAGGTCACCCCTCTGAGCCGCCAAAATGTGCTGGCCGGAC

♂ GGATGAAACAGCCGGTCAGGACACCCCTCTGAGCCGGGAAAATGTGCTGACCGGAC



GGATGAAAC

AACTGCC

AACAGCC

GCCGGTCAGG

AGGTCAC

AGGACAC

CACCCCTCTGAGCCG

CCGCCAA

CCGGGAAA

AAAATGTGCTG

CTGGCCG

TGACCG

CCGGAC

Assembly:

GGATGAAACTGCCGGTCAGGACACCCCTCTGAGCCGGGAAAATGTGCTGGCCGGAC

Assembly **concession number 2**: collapse variability

♀ GGATGAAACTGCCGGTCAGGTCACCCCTCTGAGCCGCCAAAATGTGCTGGCCGGAC

♂ GGATGAAACAGCCGGTCAGGACACCCCTCTGAGCCGGGAAAATGTGCTGACCGGAC

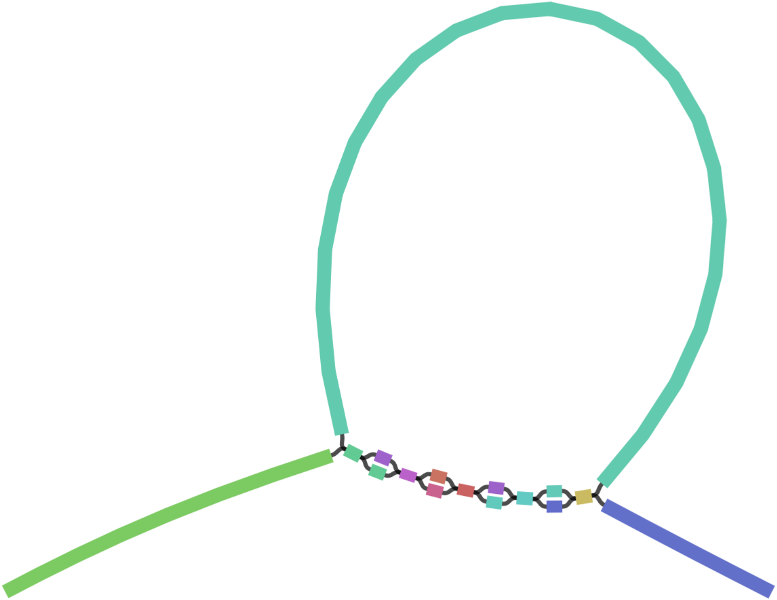
Assembly:

```
GGATGAAACTGCCGGTCAGGACACCCCTCTGAGCCGGGAAAATGTGCTGGCCGGAC
```

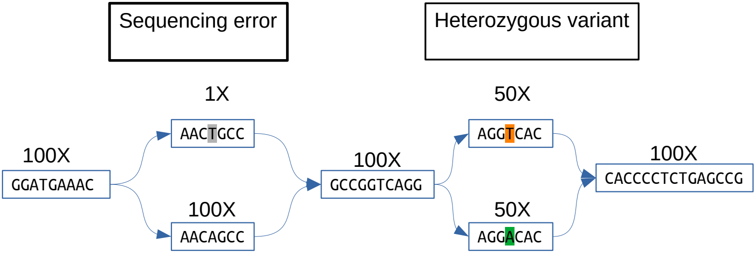
Reads:

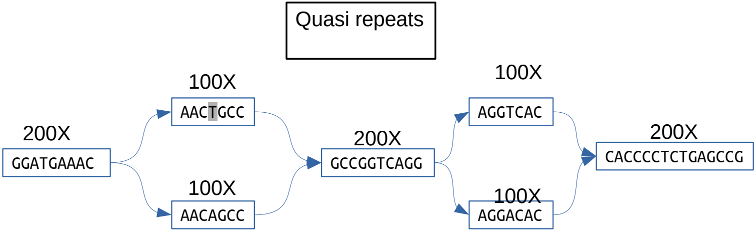
```
 GATGAAACTG
  ATGAAACAGC
   TGAAACAGCCG
    GAAACTGCCGG
     AAACTGCCGGT
      AACAGCCGGTC
       ACAGCCGGTCA
        CTGCCGGTCAG
```

Paralog genes/repeats



Paralog genes/repeats in graph





An assembler is a set of heuristics

Graph cleaning heuristics

- Nodes coverage
- Graph local/global topology
- Reads that can be mapped on nodes
- Estimated coverage/genome size
- . . .

An assembly is a model

Different tools can produce very similar assemblies
A single tool can produce very different assemblies with small changes of parameters(!)

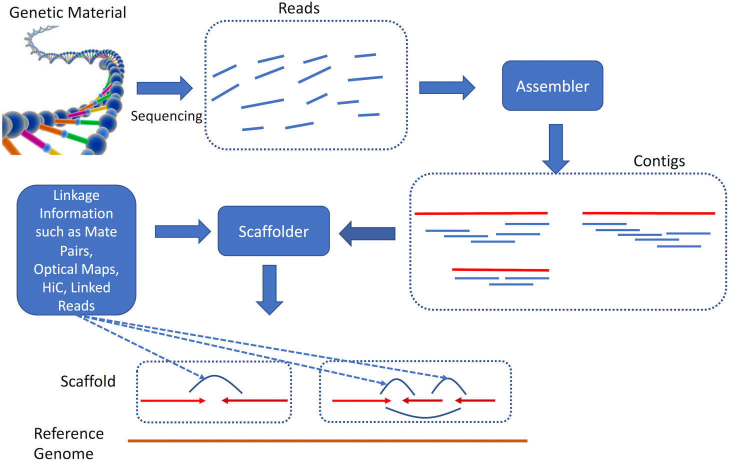
De Bruijn graphs in a nutshell

- Graph of words of size k, k-1 overlaps
- Collapses identical k-mers
- Very successful, have replaced the overlap graphs with high throughput sequencing data
- Still outputs fragments of the genome

white spruce, 20 gigabases

Scaffolding

Softwares can improve the assembly continuity by using other kinds of information



From "Modern technologies and algorithms for scaffolding assembled genomes" Plos Computational Biology

Second generation sequencing

NextSeq Series ⊕

HiSeq 4000 System

HiSeq X Series[‡]

NovaSeq 6000 System

- Short reads ≈ 150*bp*
- Low error rate ≈ 1%
- High throughput (up to billion of reads per run)
- GC bias

Mainly assembled using De Bruijn graphs

State-of-the-art

Your toolkit for the practical session

- SPAdes
- Megahit
- Minia

Other notable assemblers

- SGA
- Discovar denovo
- Abyss

Third generation sequencing

- Long reads $\approx 10 - 100kbp$
- High error rate $\approx 10 - 12\%$
- High throughput (up to millions of reads per run)

Nanopore VS Pacbio

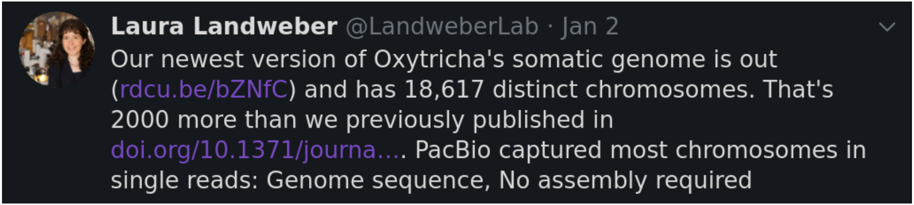
Nanopore

- Portable
- Ultra long reads (100kbases, some reads reach the megabase level)
- Mostly deletions

Pacbio

- More mature
- HiFi reads (99% identity)
- Mostly insertions

Long reads killed the assembly star



Laura Landweber @LandweberLab · Jan 2

Our newest version of Oxytricha's somatic genome is out (rdcu.be/bZNfC) and has 18,617 distinct chromosomes. That's 2000 more than we previously published in doi.org/10.1371/journa.... PacBio captured most chromosomes in single reads: Genome sequence, No assembly required

Repeats spanning

Genome:

GGTAATGGTTTTTTGGTGCTAATGCGTTTTTTCATGGATGTCGTAATTTTTTATCTG

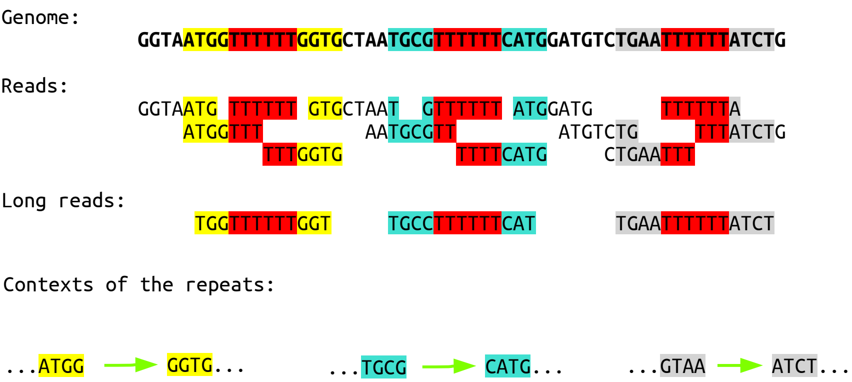
Reads:

```
GGTAATG TTTTTT GTGCTAAT  GTTTTTT ATGGATG      TTTTTTA
    ATGGTTT         AATGCGTT         ATGTCGT     TTTATCTG
           TTTGGTG          TTTTCATG     CGTAATTT
```

Contexts of the repeat:

```
                                          ATCT...
      ...ATGG
                    ???TTTTTT???          GGTG...
       ...TGCG
                                          CATG...
       ...GTAA
```

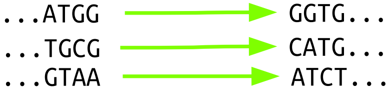
Repeats spanning



```
Genome:
            GGTAATGGTTTTTTGGTGCTAATGCGTTTTTTCATGGATGTCTGAATTTTTTATCTG

Reads:
            GGTAATG TTTTTT GTGCTAAT  GTTTTTT ATGGATG      TTTTTTA
                ATGGTTT         AATGCGTT         ATGTCTG     TTTATCTG
                       TTTGGTG          TTTTCATG     CTGAATTT

Long reads:
                 TGGTTTTTTGGT     TGCCTTTTTTCAT       TGAATTTTTTATCT

Contexts of the repeats:

...ATGG → GGTG...        ...TGCG → CATG...        ...GTAA → ATCT...
```

Repeats spanning

Contexts of the repeats:



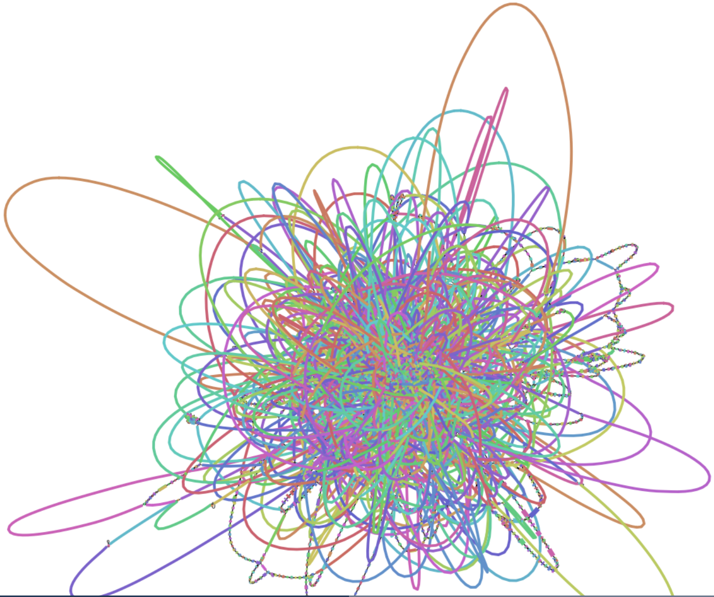
Overlapping Reads:

```
GGTAATG       GTGCTAAT
    ATGGTTT          AATGCGTT         ATGTCTG
     TGGTTTTTTGGT    TGCCTTTTTTCAT        CTGAATTT
           TTTGGTG         TTTTCATG        TGAATTTTTTATCT
                                ATGGATG         TTTATCTG
```

Output assembly:
GGTAATGGTTTTTTGGTGCTAATGCGTTTTTTCATGGATGTCTGAATTTTTTATCTG

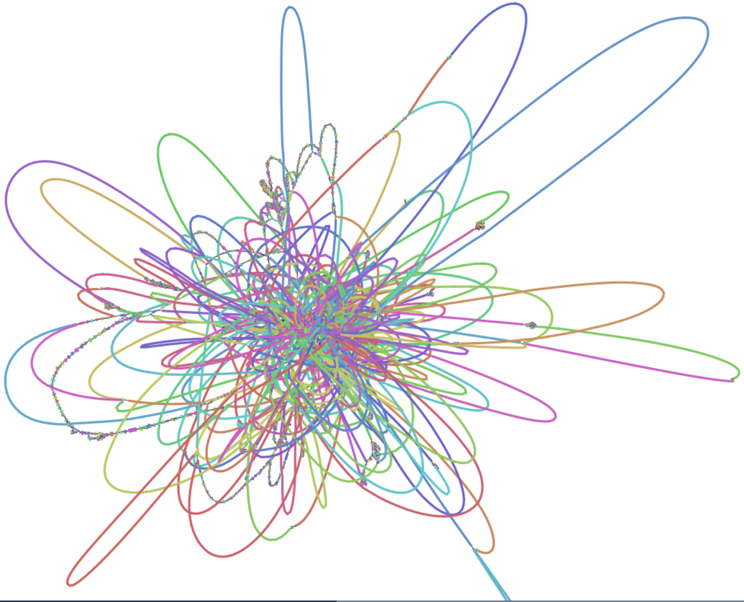
Read length matter

Read size=21



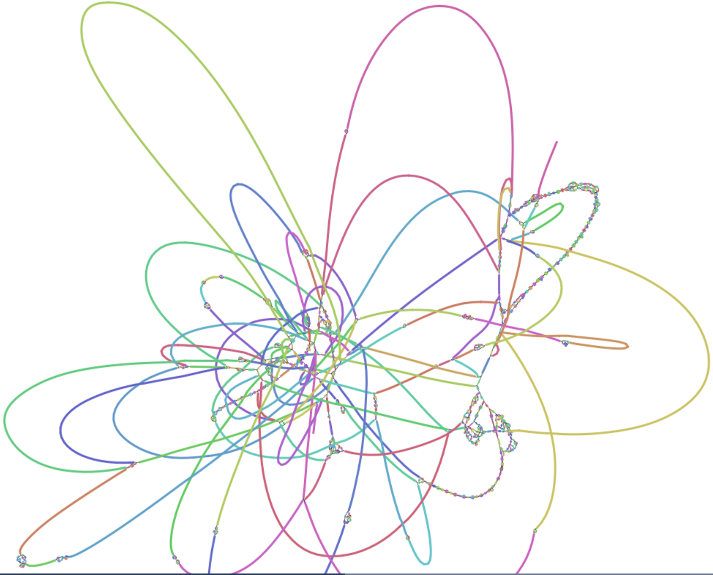
Read length matter

Read size=31



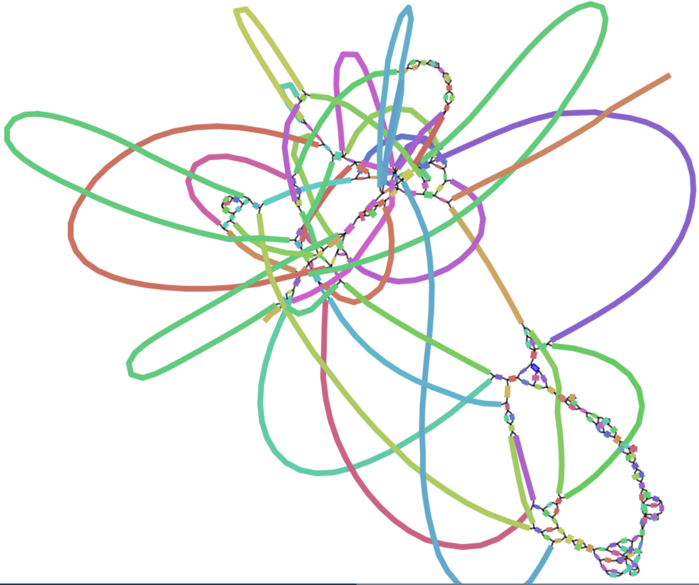
Read length matter

Read size=63



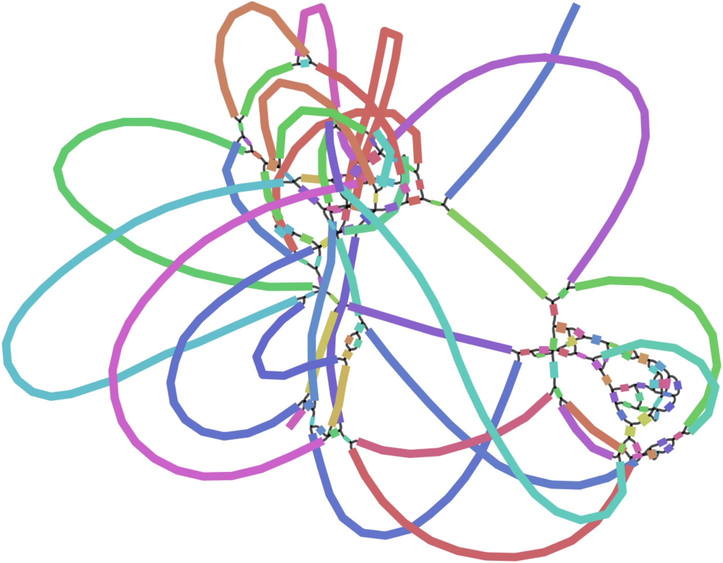
Read length matter

Read size=255



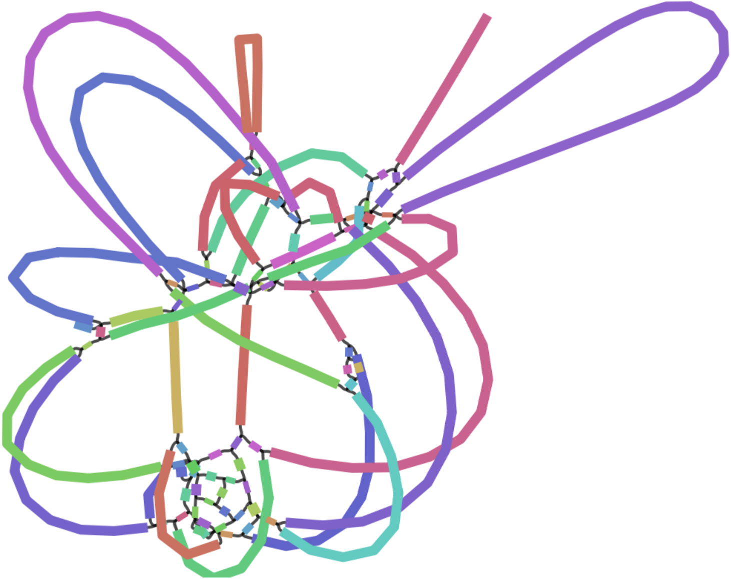
Read length matter

Read size=500



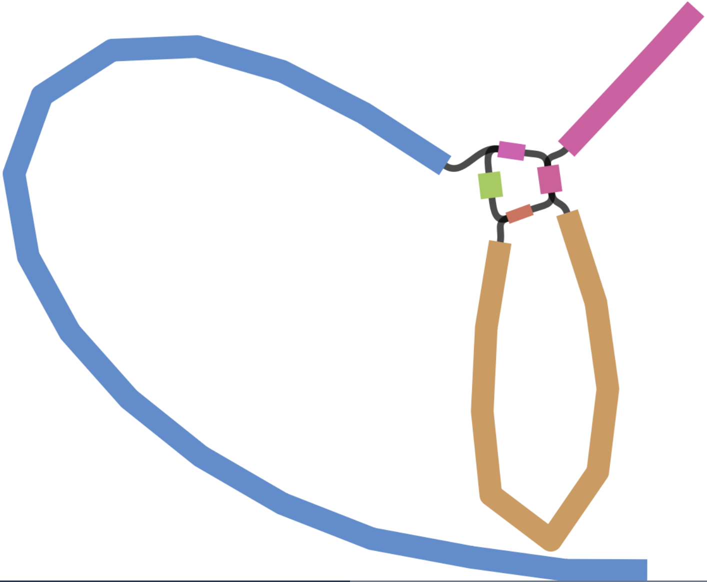
Read length matter

Read size=1000

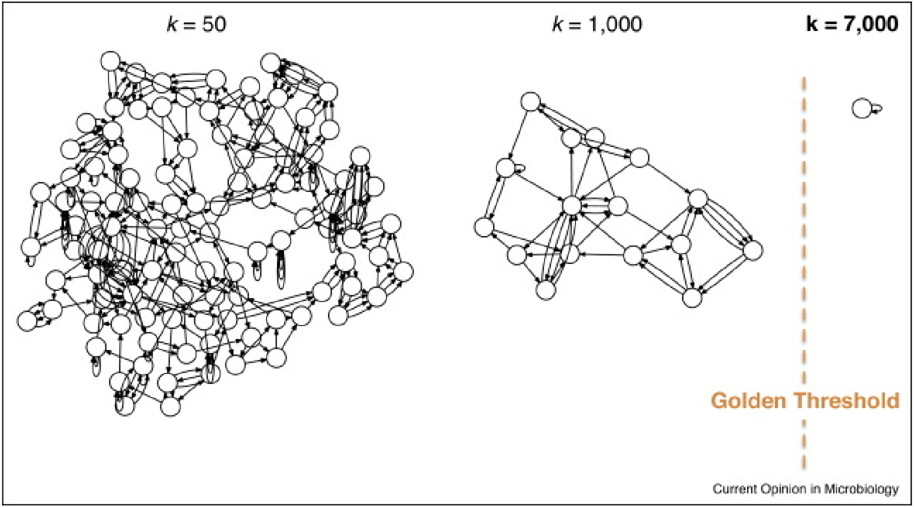


Read length matter

Read size=2000

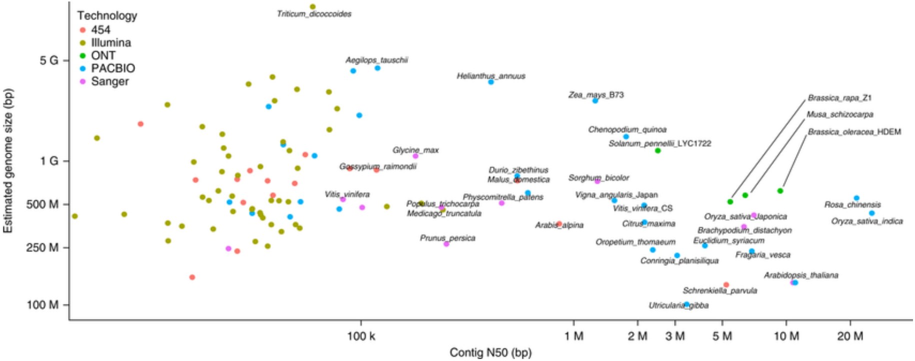


Great hope for assembly



From "One chromosome, one contig: complete microbial genomes from long-read sequencing and assembly"Current Opinion in Microbiology 2015

Great hope for assembly



From "Chromosome-scale assemblies of plant genomes using nanopore long reads and optical maps" Nature Plants 2018

Which assembly strategy is best suited?

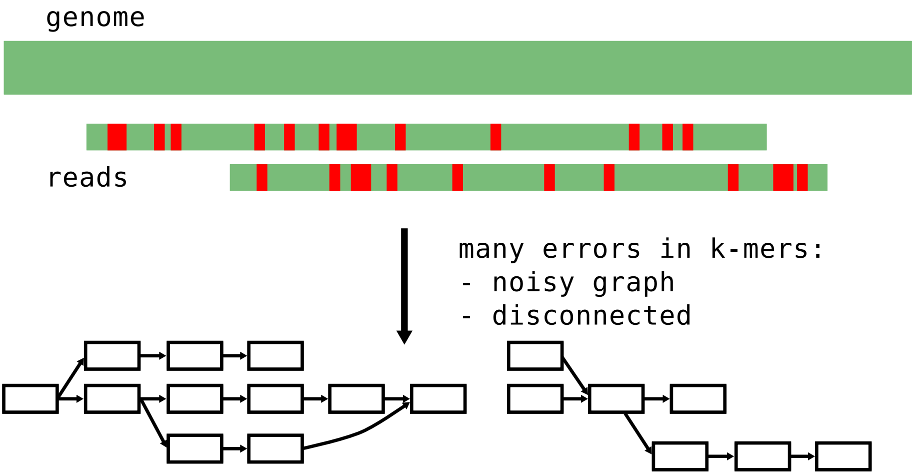
- Long reads $\approx 10kbp$
- High error rate $\approx 12\%$
- High throughput (up to millions of reads per run)

Based on long reads properties, which assembly solution would you choose and why?

Vote!

- ~~Fish~~
- ~~Birbs~~
- Greedy
- Overlap graph
- De Bruijn graph

Long reads for assembly: De Bruijn graph?



genome

reads

many errors in k-mers:
- noisy graph
- disconnected

Long reads for assembly: overlap graph?

Supposed to be super expensive!

```
TAAGAAAGCTCTGAATCAACGGACTGCGACAATAAGTGGTGGTATCCAGAATTTGTCACTTCAAGTAAAAACACCTCACGAGTTAAAACACCTAAGTTC
TAAGAAAGCT
 AAGAAAGCTC
  AGAAAGCTCT
   GAAAGCTCTG
    AAAGCTCTGA
     AAGCTCTGAA
      AGCTCTGAAT
       GCTCTGAATC
        CTCTGAATCA
         TCTGAATCAA
          CTGAATCAAC
           TGAATCAACG
            GAATCAACGG
             AATCAACGGA
              ATCAACGGAC
               TCAACGGACT
                CAACGGACTG
                 AACGGACTGC
                  ACGGACTGCG
                   CGGACTGCGA
                    GGACTGCGAC
                     GACTGCGACA
                      ACTGCGACAA
                       CTGCGACAAT
                        TGCGACAATA
                        …
```

Average coverage: 10
Read length: 10
Average overlap: 9
Read number:100

```
TAAGAAAGCTCTGAATCAACGGACTGCGACAATAAGTGGTGGTATCCAGAATTTGTCACTTCAAGTAAAAACACCTCACGAGTTAAAACACCTAAGTTC
TAAGAAAGCTCTGAATCAACGGACTGCGAC
   GAAAGCTCTGAATCAACGGACTGCGACAAT
      AGCTCTGAATCAACGGACTGCGACAATAAG
         TCTGAATCAACGGACTGCGACAATAAGTGG
            GAATCAACGGACTGCGACAATAAGTGGTGG
               TCAACGGACTGCGACAATAAGTGGTGGTAT
                  ACGGACTGCGACAATAAGTGGTGGTATCCA
                     GACTGCGACAATAAGTGGTGGTATCCAG
                        TGCGACAATAAGTGGTGGTATCCAGAAT
                           GACAATAAGTGGTGGTATCCAGAATTTG
                              AATAAGTGGTGGTATCCAGAATTTGTCA
```

Average coverage: 10
Read length: 30
Average overlap: 27
Read number: 33

Longer reads, better overlaps

- Less reads for the same coverage
- Larger overlaps

5Mb bacteria example with 100X coverage

Short reads

- 5 million 100bp reads
- 99 bp average overlap

Long reads

- 50,000 10kbp reads
- 9,900 bp average overlap

Very long reads

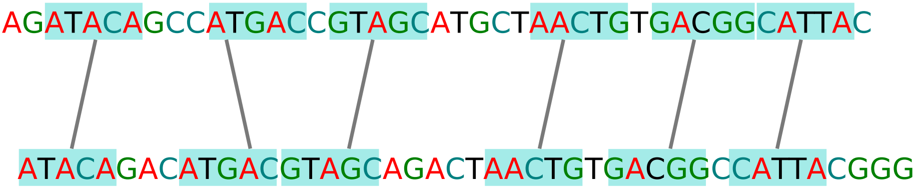
- 5,000 100kbp reads
- 99,000 bp average overlap

Anchors chaining in overlap graph

GCCATGACC

CATGACCGA

short reads



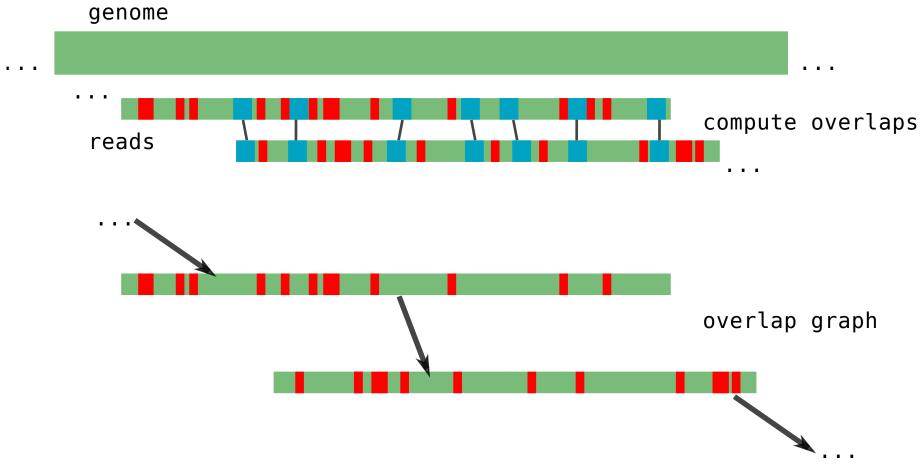
AGATACAGCCATGACCGTAGCATGCTAACTGTGACGGCATTAC

ATACAGACATGACGTAGCAGACTAACTGTGACGGCCATTACGGG

long reads

Minimap's job.

Long reads for assembly: overlap graphs

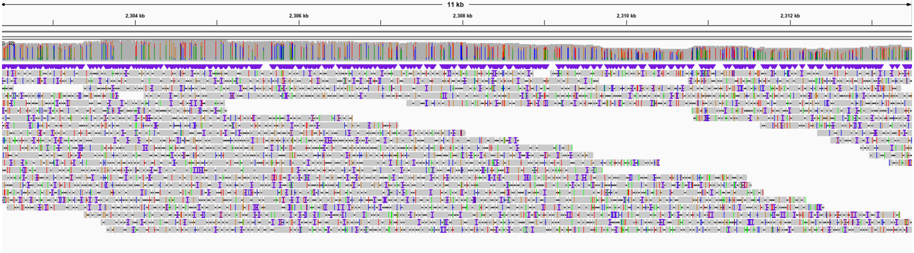


Sequencing errors

```
Genome:
 ATCGGTATCGTTACGGTATACC

Reads:
 ATCGCTATCG              (Substitution)
   GGTATCGTCTA           (Insertion)
      AT GTTACGG         (Deletion)
```

Insertion and deletion made calling almost impossible

Sequencing errors

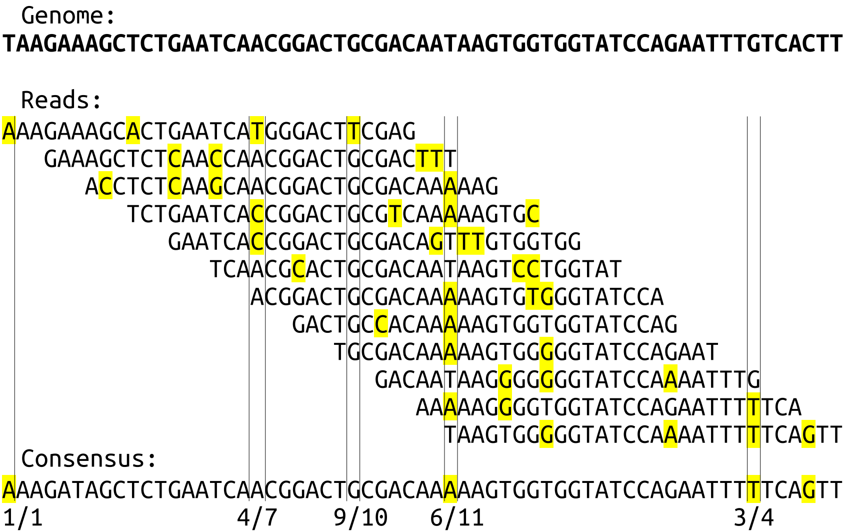


Miniasm

Long reads can be assembled without taking care of the sequencing errors (”Minimap and miniasm: fast mapping and de novo assembly for noisy long sequences”, Bioinformatics ’16)

Genome characteristics and structure can be quickly estimated

Using coverage to remove noise: Consensus



```
 Genome:
TAAGAAAGCTCTGAATCAACGGACTGCGACAATAAGTGGTGGTATCCAGAATTTGTCACTT

 Reads:
AAAGAAAGCACTGAATCATGGGACTTCGAG
   GAAAGCTCTCAACCAACGGACTGCGACTTTT
      ACCTCTCAAGCAACGGACTGCGACAAAAAG
         TCTGAATCACCGGACTGCGTCAAAAAGTGC
            GAATCACCGGACTGCGACAGTTTGTGGTGG
               TCAACGCACTGCGACAATAAGTCCTGGTAT
                  ACGGACTGCGACAAAAAGTGTGGGTATCCA
                     GACTGCCACAAAAAGTGGTGGTATCCAG
                        TGCGACAAAAAGTGGGGGTATCCAGAAT
                           GACAATAAGGGGGGGTATCCAAAATTTG
                              AAAAAGGGGTGGTATCCAGAATTTTTCA
                                TAAGTGGGGGTATCCAAAATTTTTCAGTT
 Consensus:
AAAGATAGCTCTGAATCAACGGACTGCGACAAAAAGTGGTGGTATCCAGAATTTTTCAGTT
1/1              4/7    9/10   6/11                  3/4
```

Exercise: Perform a consensus

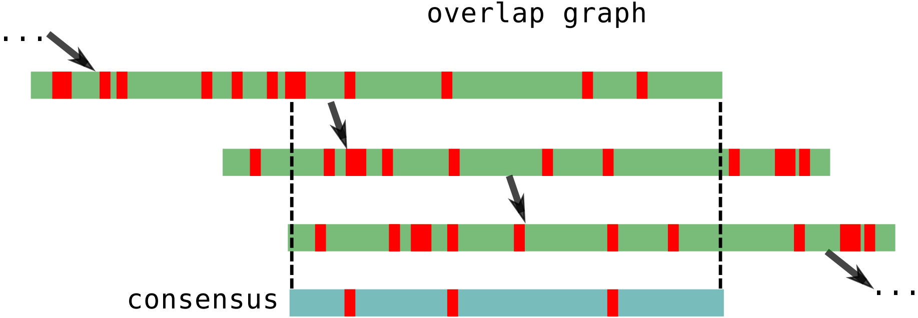
```
Erroneous reads:
TAAGAAAGCTCTGAATCAAACGGTACTGCGA
GAAAGCTTGAATCAAACGGACTGCGACAA
AGCTCTGAATCAACGGACTGCGACAATAA


Contig to polish:
TAAGAAAGCTTGAATCAACGGAATGGCGACAATAA
```

Exercise: Perform a consensus - solution

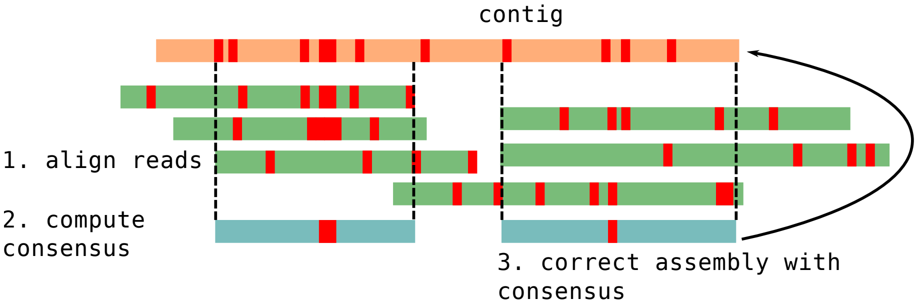
```
Correct contig:
TAAGAAAGCTCTGAATCAA-CGGACTG-CGACAATAA
```

```
Aligned reads:
TAAGAAAGCTCTGAATCAAACGGTACTGCGA
   GAAAGCT-TGAATCAAACGGACTG-CGACAA
      AGCTCTGAATCAA-CGGACTG-CGACAATAA
```

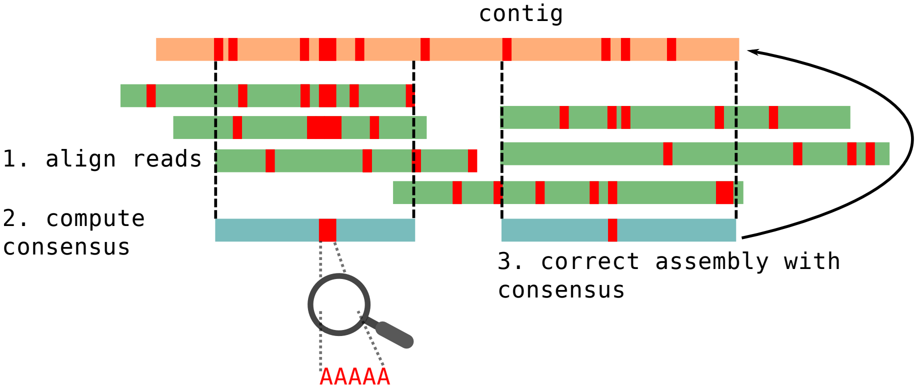
Consensus during assembly



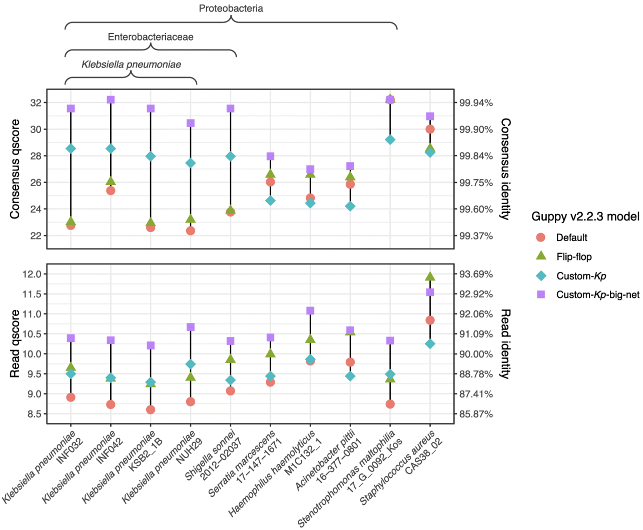
Consensus after assembly: polishing



Consensus after assembly: polishing

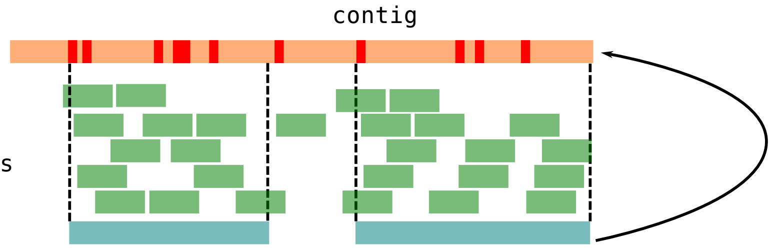


Systematic errors



From "Performance of neural network basecalling tools for Oxford Nanopore sequencing" Genome biology 2019

Polishing using accurate reads



contig

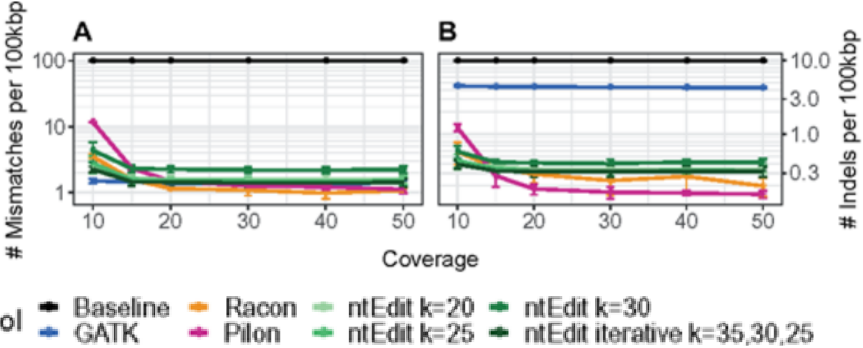
1. align **short** reads

2. compute consensus

3. correct assembly with consensus

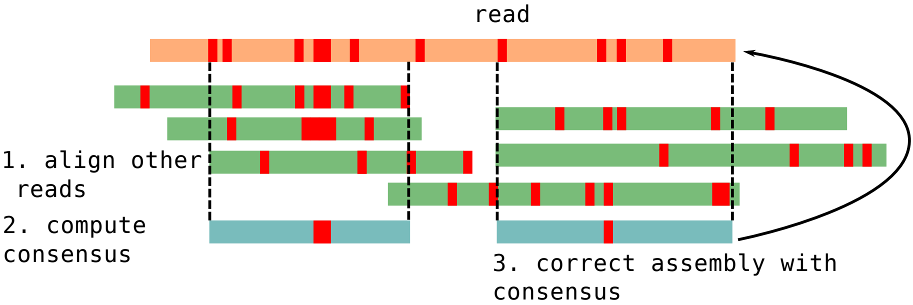
Systematics errors

Polishing with Illumina data can improve the final error rate



From ntEdit: scalable genome sequence polishing Bioinformatics 2019

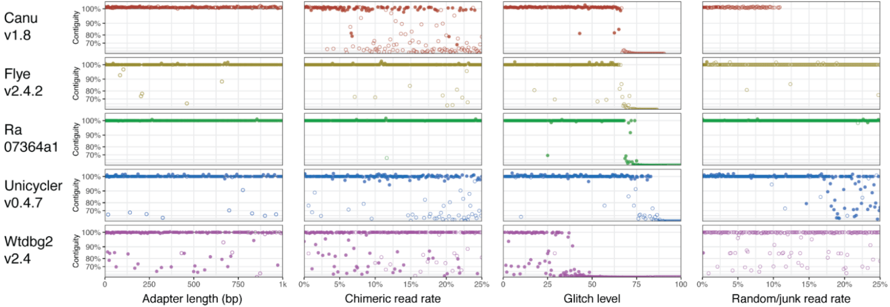
Correction before assembly



Long reads for assembly: assembly solved?

Assembly is not solved yet

Sometime the software fail



From `github.com/rrwick/Long-read-assembler-comparison`

Long reads for assembly: assembly solved?

Assembly is not solved yet

Sometimes the data cannot solve the problem

- Very large repeated region
- Low local coverage
- Chimeric/noisy reads

Long reads assemblers

Your toolkit for the practical

- Flye
- Miniasm
- Raven

Other notable assemblers

- Canu
- Mecat
- Redbean
- . . .

Long read assembly summary

- Not resolved: correction before or after assembly (polishing)
- Overlap graphs with quick overlap computation
- Long reads can span repeats and improve assemblies
- Methods to polish contigs

Conclusions

Take home messages

- Short reads: De Bruijn graphs / Long reads: Overlap graph
- Repeats are the core issue
- Output fragments of genomes (**contigs**)
- Several steps and heuristics in practice

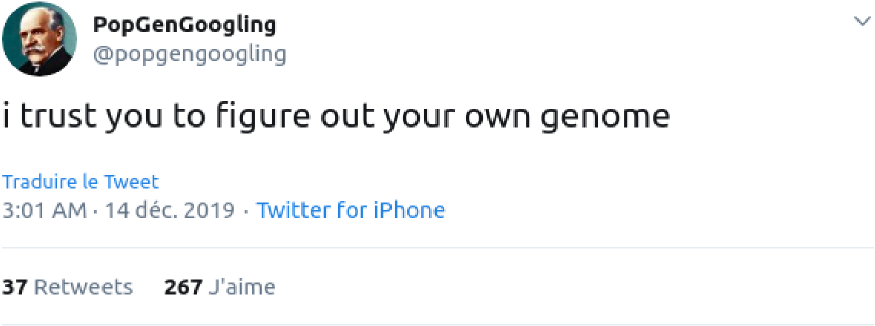
Challenges

- Difficult to reconstruct haplotypes
- Scaling on large genomes
- Robustness to noisy data

Topics we did not review today

- Read correction
- Multi *k* assembly
- Use of paired-end, mate-pair, 10X, Hi-C . . .)

The end



PopGenGoogling
@popgengoogling

i trust you to figure out your own genome

Traduire le Tweet

3:01 AM · 14 déc. 2019 · Twitter for iPhone

37 Retweets **267** J'aime

Practical session

Datasets

Short Illumina reads 150bp
Long PacBio reads

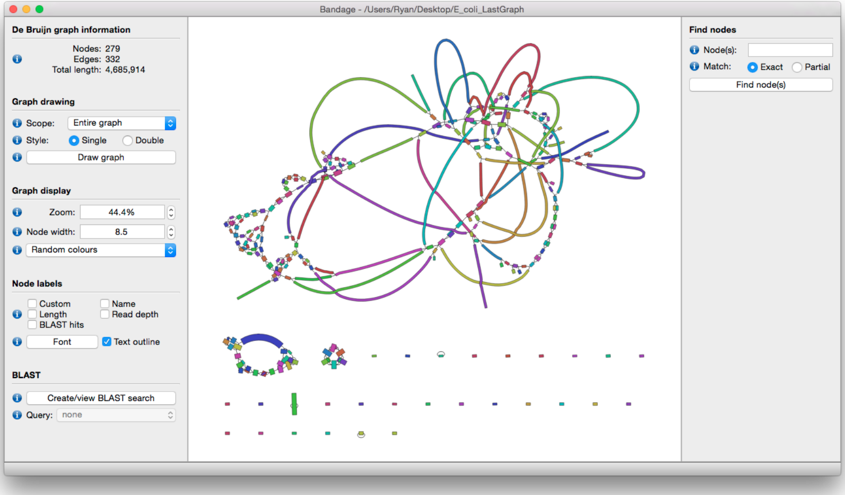
Steps

- Perform a draft assembly
- Evaluate it
- (Try to) Perform a better assembly

You will read the assembler website and papers to decide which one you want to use

Visualize assembly

Bandage tool can visualize assembly graphs (GFA)



Bandage - /Users/Ryan/Desktop/E_coli_LastGraph

De Bruijn graph information

Nodes: 279
Edges: 332
Total length: 4,685,914

Graph drawing

Scope: Entire graph
Style: ● Single ○ Double

Graph display

Zoom: 44.4%
Node width: 8.5
Random colours

Node labels

□ Custom □ Name
□ Length □ Read depth
□ BLAST hits
☑ Text outline

BLAST

Query: none

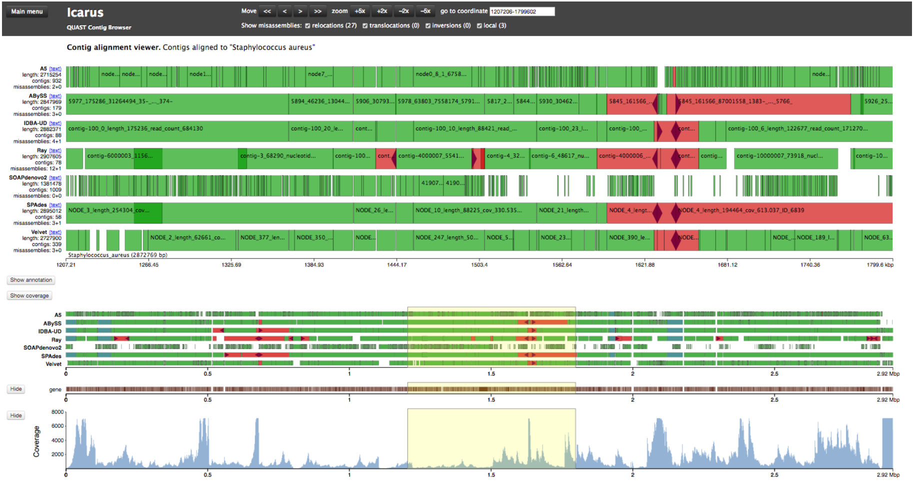
Find nodes

Node(s):
Match: ● Exact ○ Partial

From `rwick.github.io/Bandage`

Evaluate assembly

Contigs can be mapped and compared to a reference/closely related genome



Contig alignment viewer. Contigs aligned to "Staphylococcus aureus"

Go to coordinate: 1207206-1799602

Show misassemblies: ☑ relocations (27) ☑ translocations (0) ☑ inversions (0) ☑ local (3)

A5 (text)
length: 2715254
contigs: 932
misassemblies: 2+0

ABySS (text)
length: 2847969
contigs: 179
misassemblies: 3+0

IDBA-UD (text)
length: 2882371
contigs: 88
misassemblies: 4+1

Ray (text)
length: 2907605
contigs: 78
misassemblies: 12+1

SOAPdenovo2 (text)
length: 1381478
contigs: 1009
misassemblies: 0+0

SPAdes (text)
length: 2895012
contigs: 58
misassemblies: 3+1

Velvet (text)
length: 2727900
contigs: 339
misassemblies: 3+0

Staphylococcus_aureus (2872769 bp)

From quast.bioinf.spbau.ru/manual.html

Assembly continuity

N50

N50 can be described as a weighted median statistic such that 50% of the entire assembly is contained in contigs or scaffolds equal to or larger than this value.

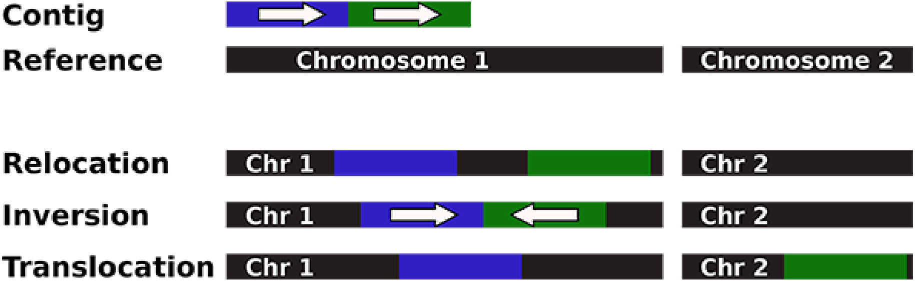
N75

N75 is the same statistic for 75% of the assembly

NGA50

Similar to the N50 but only takes into account contigs/scaffolds that can be aligned on the reference genome and consider 50% of the **genome size** instead of the assembly size

Misasemblies



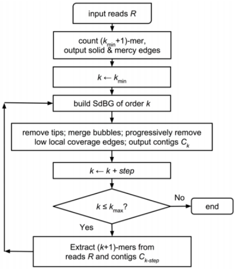
Multiple *k* assembly

Most De Bruijn graph assemblers can now perform several assemblies with different *k*-mer sizes to produce an improved "super" assembly
(will be discussed in metagenomic session)

Advice

Using a single size of *k*-mer will allow the assembly to go way faster during the practical

Fig. 1. The workflow of MEGAHIT


input reads *R*
count (k_{min}+1)-mer, output solid & mercy edges
$k \leftarrow k_{min}$
build SdBG of order *k*
remove tips; merge bubbles; progressively remove low local coverage edges; output contigs C_k
$k \leftarrow k + step$
$k \leq k_{max}$?
No
end
Yes
Extract (*k*+1)-mers from reads *R* and contigs $C_{k\text{-}step}$