Sequencing and Sequence Assembly

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Lecture based on Mark Craven's class at University of Wisconsin

IDA
Intelligent Data Analysis
RESEARCH GROUP

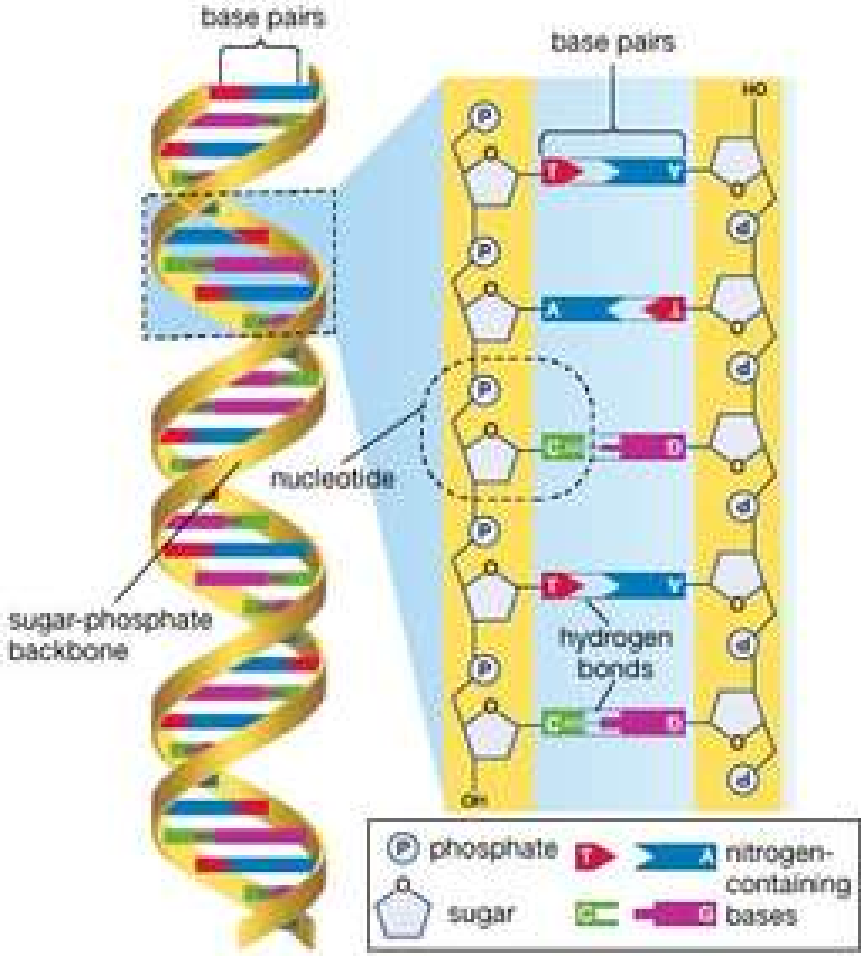
http://cw.felk.cvut.cz/wiki/courses/b4m36bin/start

Overview

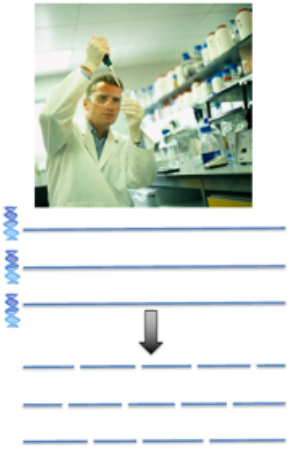
- DNA sequencing
  - before – slow and expensive,
  - next-generation sequencing (NGS) – massively parallel, faster and cheaper,
- sequence assembly
  - we cannot read off the sequence of an entire molecule all at once,
  - the sequence has to be **assembled** from shorter reads,
  - stems from redundancy of the read set = overlaps between reads,
- assembly methods
  - greedy methods,
  - graph-based method
    - the de Bruijn graph method most popular in the age of NGS.

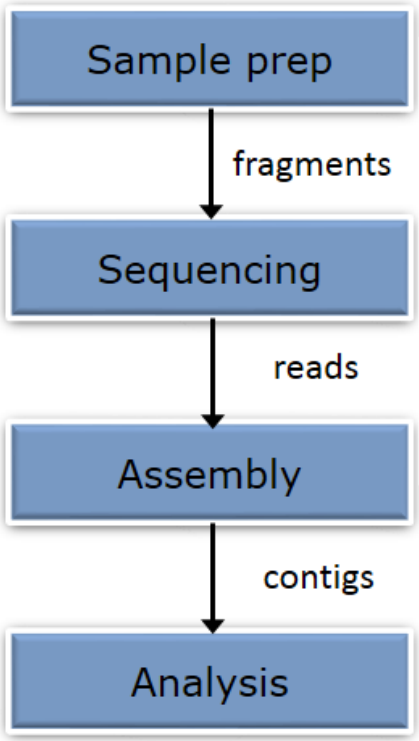
DNA sequencing

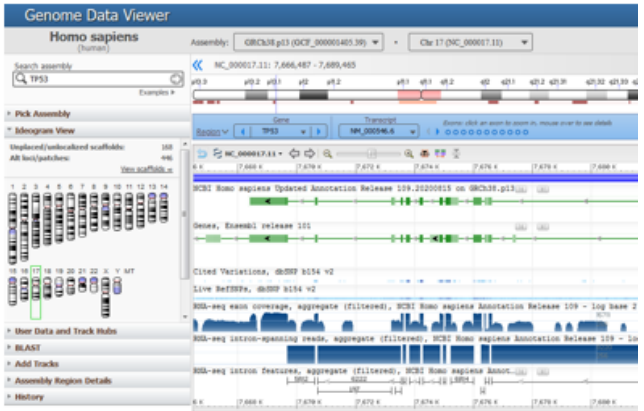
- The basic objective
  - to determine the order of the nucleobases (A, C, G, T) in the DNA molecule,
- the subsequent analytical objectives
  - recognition of DNA structure (genes, introns, exons, regulatory regions, RNA genes), study of differences between species, organisms and individuals, understanding of function,
- sequencing methods
  - de novo vs resequencing.



DNA sequencing – the basic steps related with it



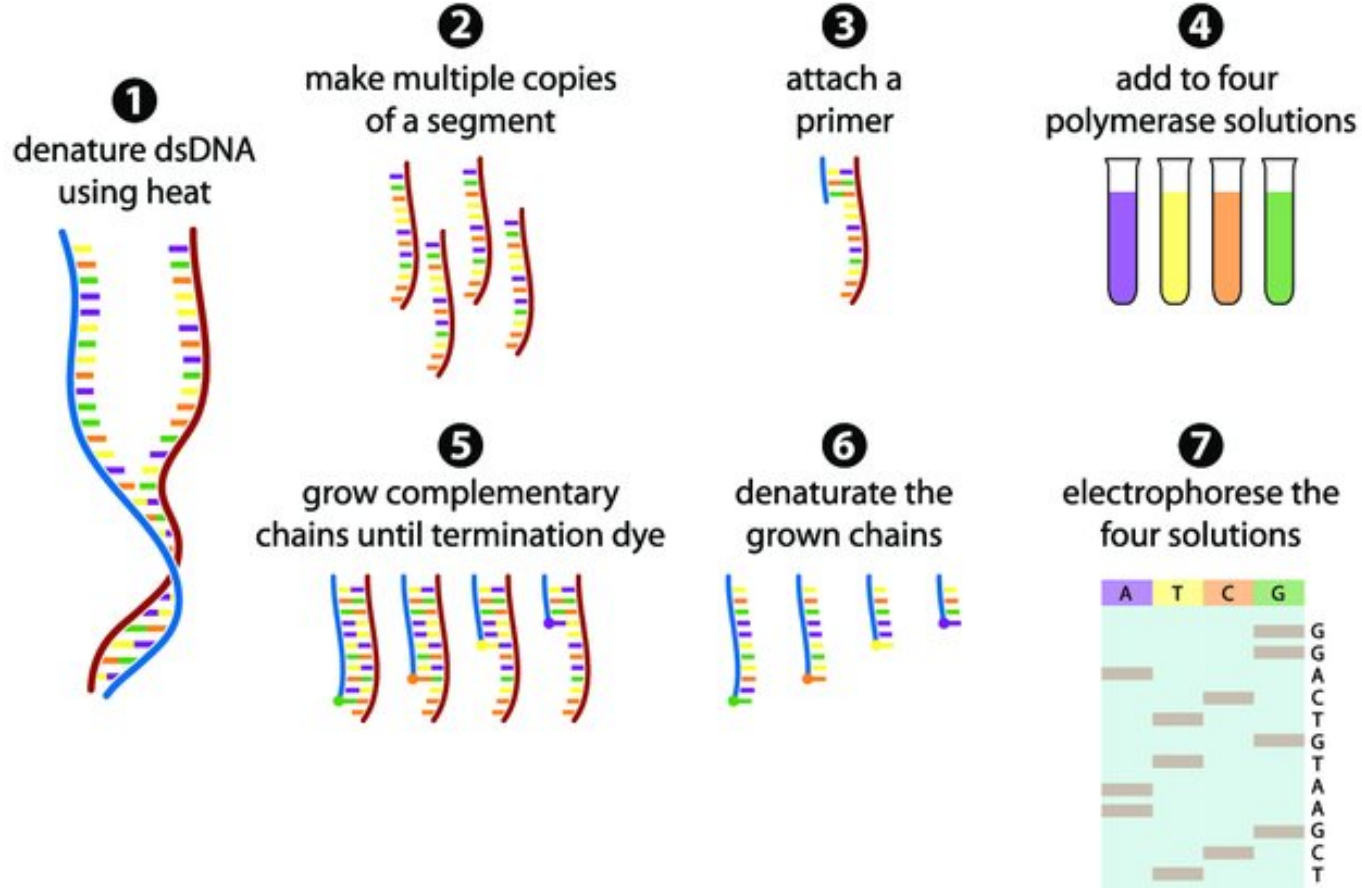




Bioinformatics Algorithms, Computer Science Department, Colorado State University, CS548.

DNA sequencing before

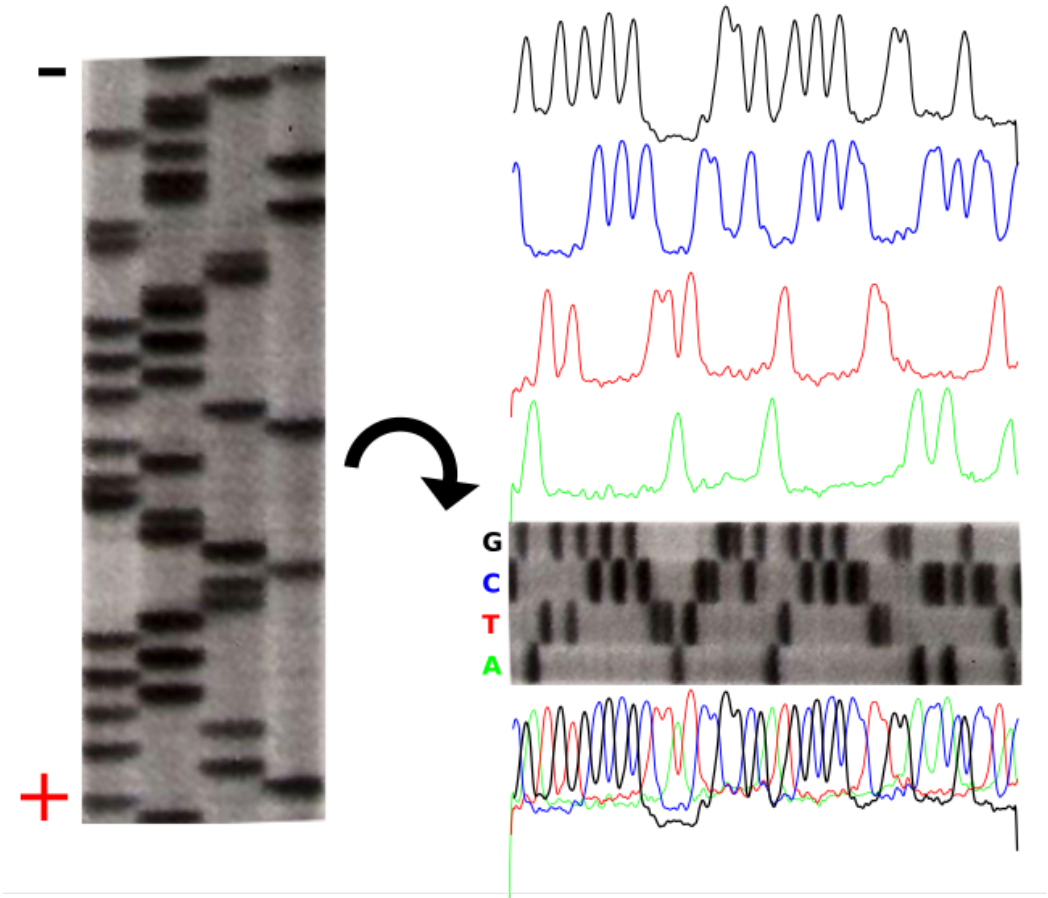
- Sanger sequencing
  - 1977, still used for the Human Genome project until 2004.



M. Gauthier: Simulation of polymer translocation through small channels, 2008.

DNA sequencing before

- Sanger sequencing
  - the last step, electrophoresis.



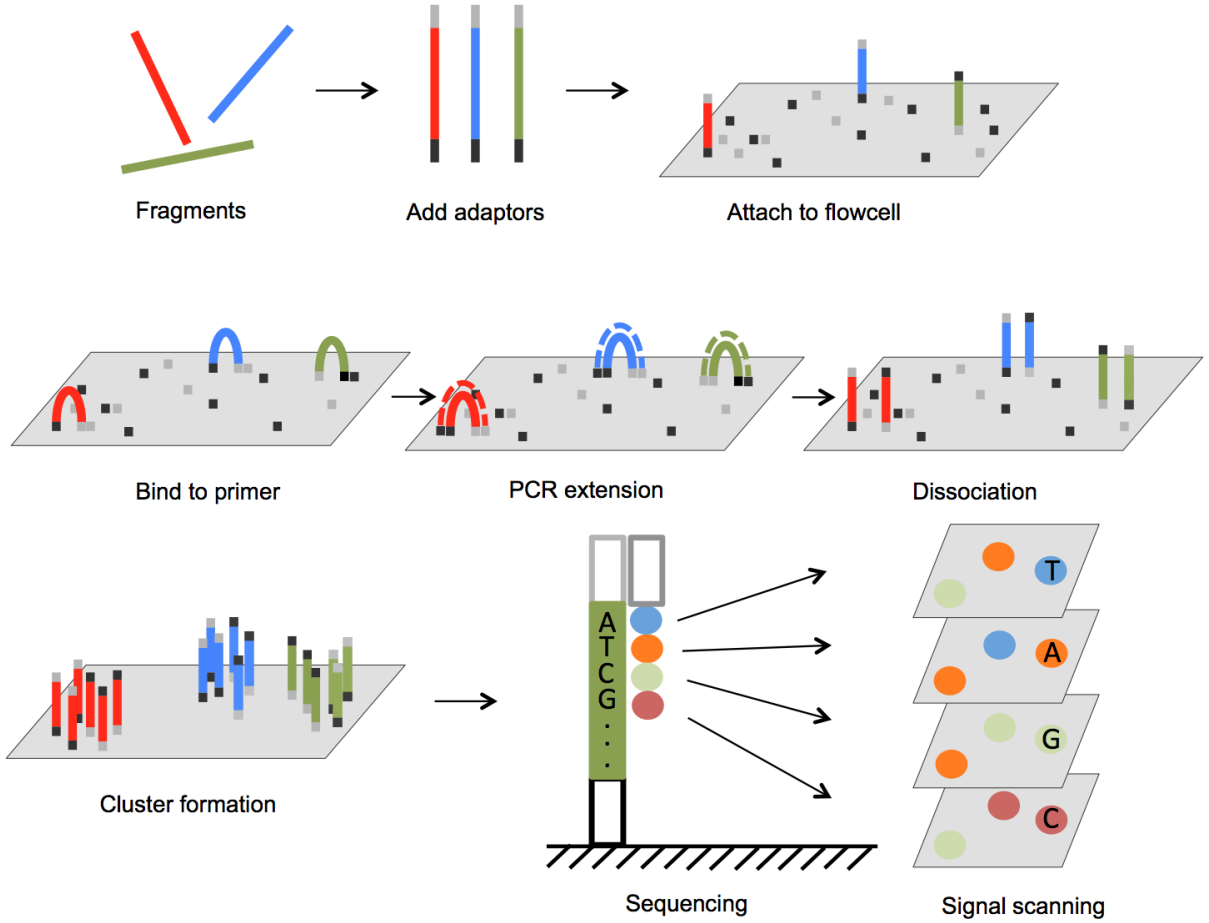
https://bio.libretexts.org/

Statistics for shotgun sequencing

- Shotgun sequencing
  - a general strategy to subdivide a long sequence into **random** fragments,
- Given: G – genome length (3×10^9 nts), L – read length (500 nts), N – number of reads (tbd)
- Calculate: coverage – a=NL/G
- Questions tbd by stats (Lander-Waterman):
  - How many contigs are there?
  - How big are the contigs?
  - How many reads are in each contig?
  - How big are the gaps?
- Requirement: 99% in contigs, 1% in gaps
  - a=4.6, N=3×10^7, mean contig length 10^4,
  - 100 reads/contig on average.

DNA sequencing today

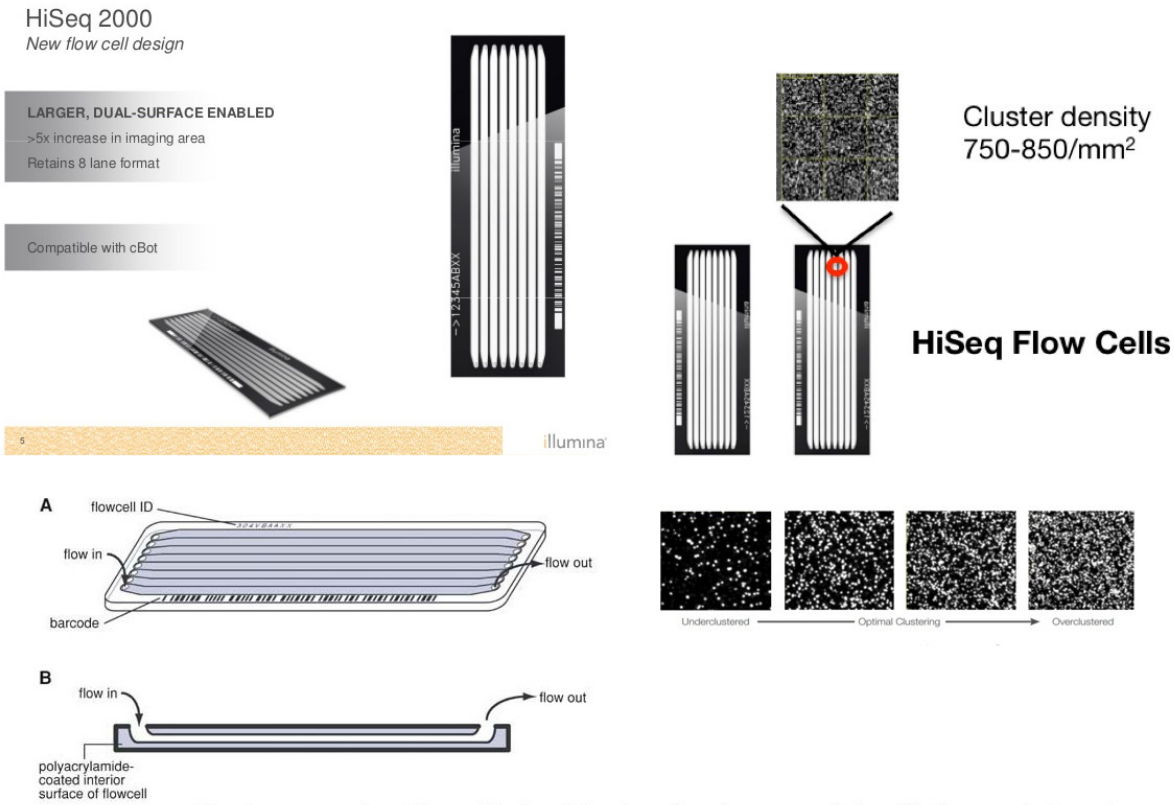
- Next/Second Generation sequencing
  - the main progress in massive parallelization (**high-throughput**).



Lu et al.: Next Generation Sequencing in Aquatic Models, 2016.

DNA sequencing today

- Next/Second Generation sequencing
  - technical equipment.



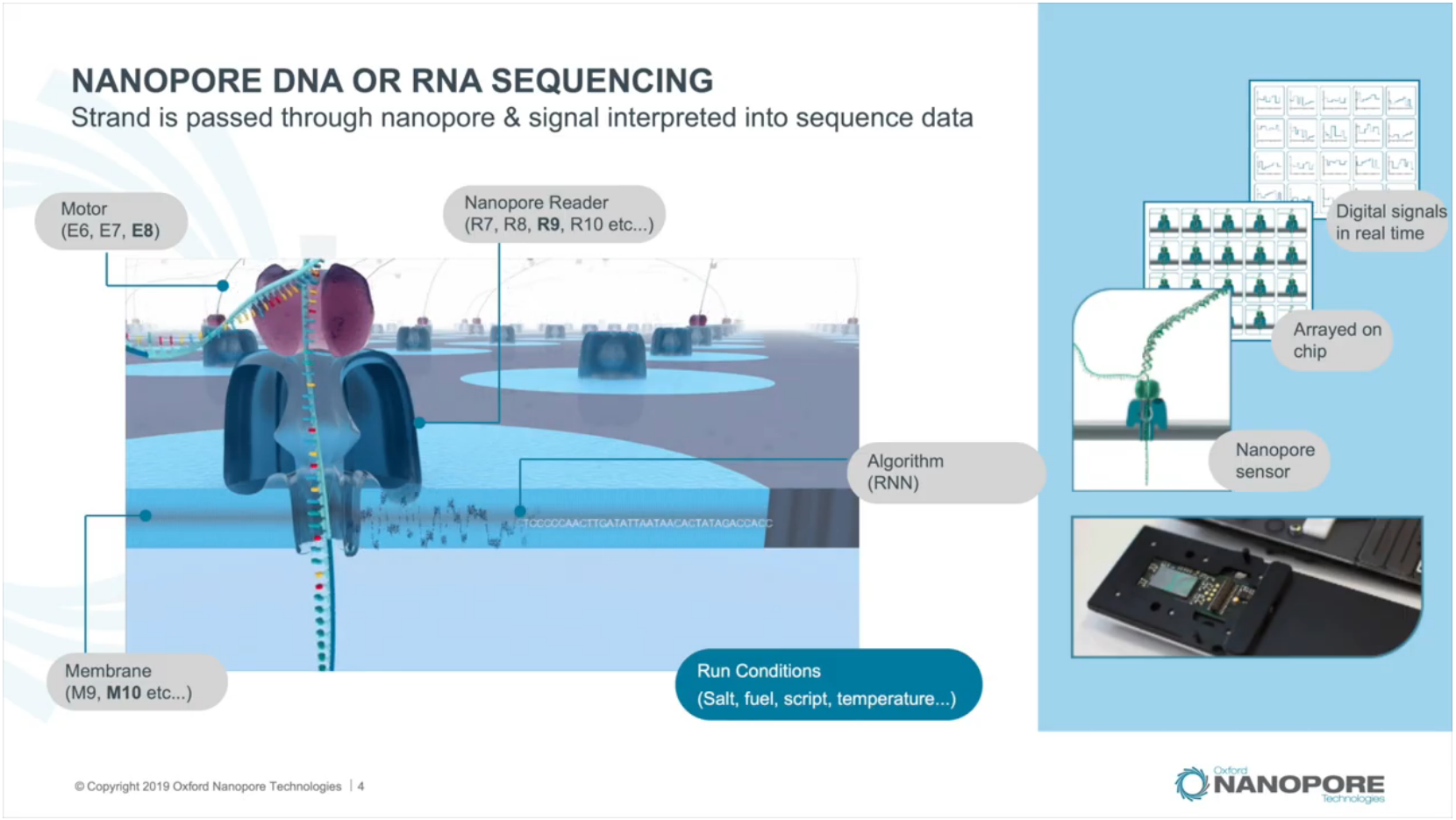
Illumina uses a glass 'flowcell', about the size of a microscope slide, with 8 separate 'lanes'.

The HiSeq instrument scans both upper and lower surfaces of each flowcell lane.

HiSeq2000 - Next Level Hacking.

Modern methods under development

- Nanopore sequencing (the main idea from 2012, still evolving).



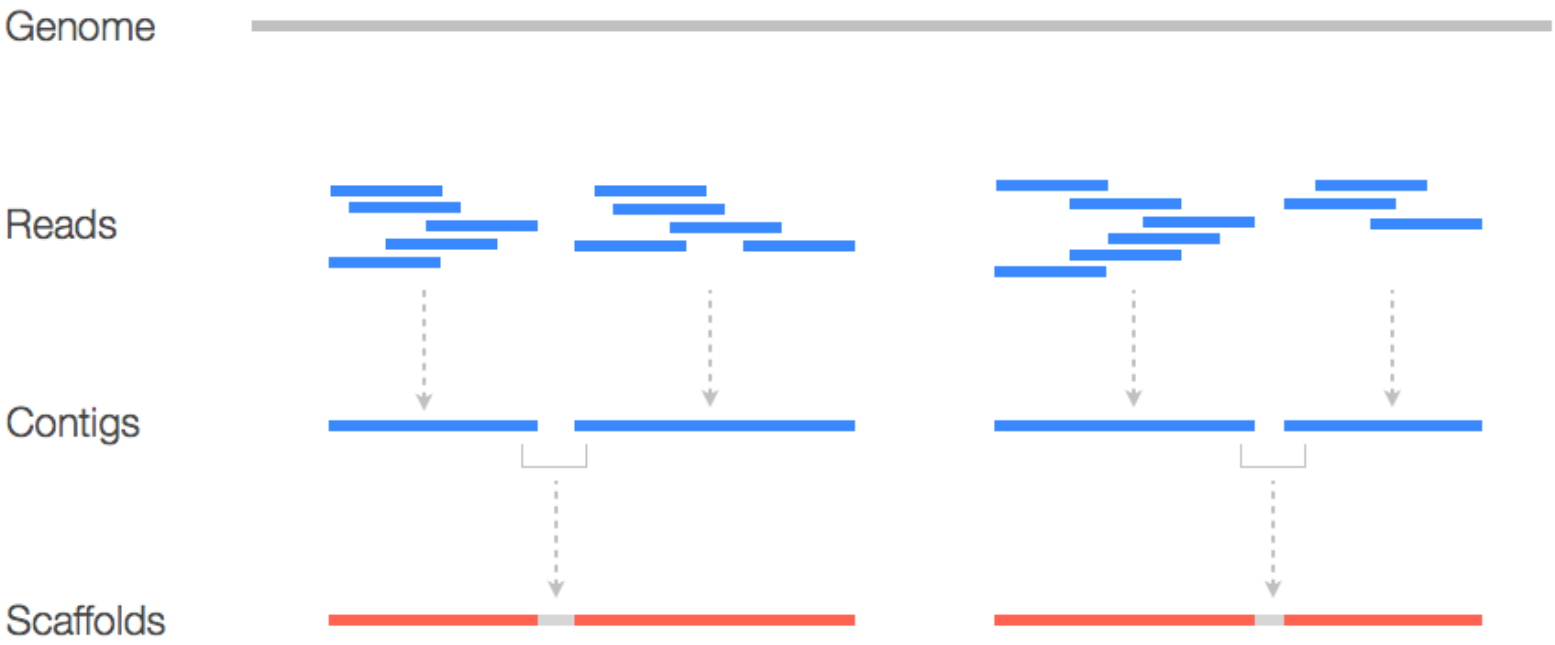
Oxford Nanopore video.

Comparison of sequencing approaches

- Jsme schopni přímo číst jen krátké úseky DNA (tzv. čtení = reads)
  - klíčové parametry: délka čtení v bp (bazické páry), chybovost v %, cena v $ za milion bp, rychlost v bp za den,
- Sanger sequencing
  - 500-800 bp, 1%, $2400, ∼1 Mbp/day,
  - very slow and very expensive,
- next generation technology
  - 454 Genome Sequencer: 250-600 bp, 1%, $10, ∼1 Gbp/day,
  - Illumina Genome Analyzer: 35-150 bp, 1%, $0.15, ∼100 Gbp/day,
  - breakthrough in mass usability, places demands on the assembly of DNA sequences,
- third generation sequencing
  - Oxford Nanopore: x10 kbp, up to 20%,
  - a small portable sequencer with low acquisition costs.

Sequence assembly

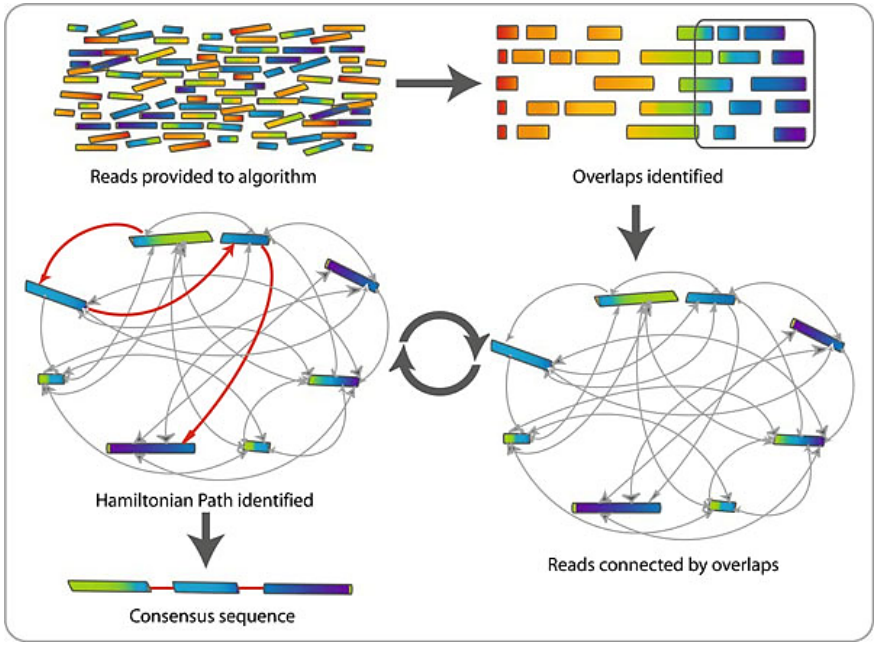
- assembles sequences whose length is close to the original sequence
  - contig = a set of concordant overlapping reads, a contiguous DNA stretch,
  - scaffold = links contiguous sections of DNA separated by gaps, the direction and length of the gaps are clear.



Neumann: Genome Assembly Tutorial.

Sequence assembly

- you can simply create the shortest superstring for an existing read set
  - ideally assumes error-free reading and that identical reads come from the same position in the genome,
  - assumptions not met (read error rate, repeats), yet NP-hard problem,
  - can be solved hungrily, or using graph theory (see **overlap graphs** below).



Commins et al., Biological Procedures Online, 2009.

Shortest superstring problem

- Objective: find a string s such that
  - all reads s_1, s_2, ..., s_n are substrings of s,
  - s is as short as possible,
- assumptions:
  - "best" = "simplest",
  - reads are 100% accurate,
  - identical reads must come from the same location on the genome,
- example:
  - given the reads: {**ACG, CGA, CGC, CGT, GAC, GCG, GTA, TCG**},
  - the shortest superstring is **TCGACGCGTA** (length 10).

Algorithms for shortest superstring problem

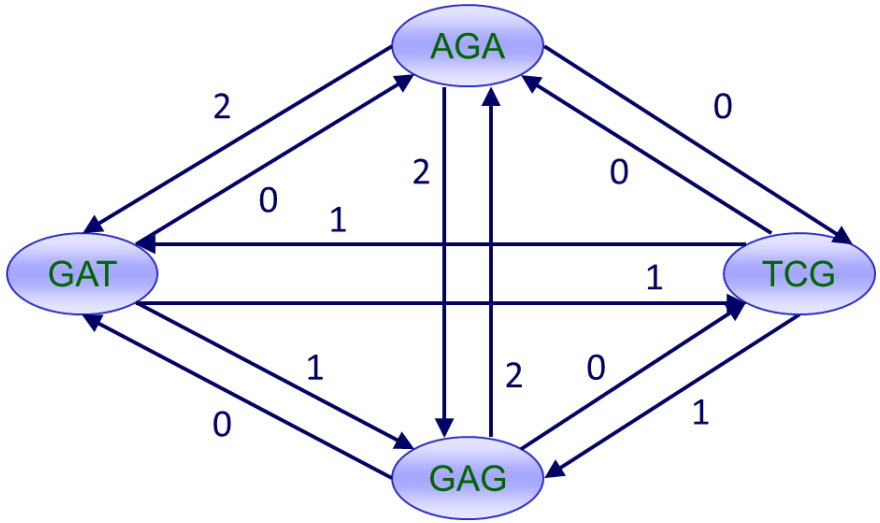
- This problem turns out to be NP-hard
- simple greedy strategy
  - uses a locally optimal problem-solving heuristic,
  - two strings are overlapping if prefix of one string is same suffix of other string or vice versa,

```
while # strings > 1 do
    merge two strings with maximum overlap
loop
```

- conjectured to give string with length $\leq 2\times$ minimum length,
- other approaches are based on graph theory . . . globally optimal solutions.

Overlap graph

- For a set of reads S, construct a directed weighted graph $G = (V, E, w)$
  - with one vertex per read ($v_i \in V$ corresponds to $s_i \in S$),
  - edges between all vertices (a complete graph),
  - $w(v_i, v_j) = \text{overlap}(s_i, s_j)$,
  - $\text{overlap}(s_i, s_j) =$ length of longest suffix of s_i that is a prefix of s_j,
- overlap graph example: let $S = \{$**AGA, GAT, TCG, GAG**$\}$.

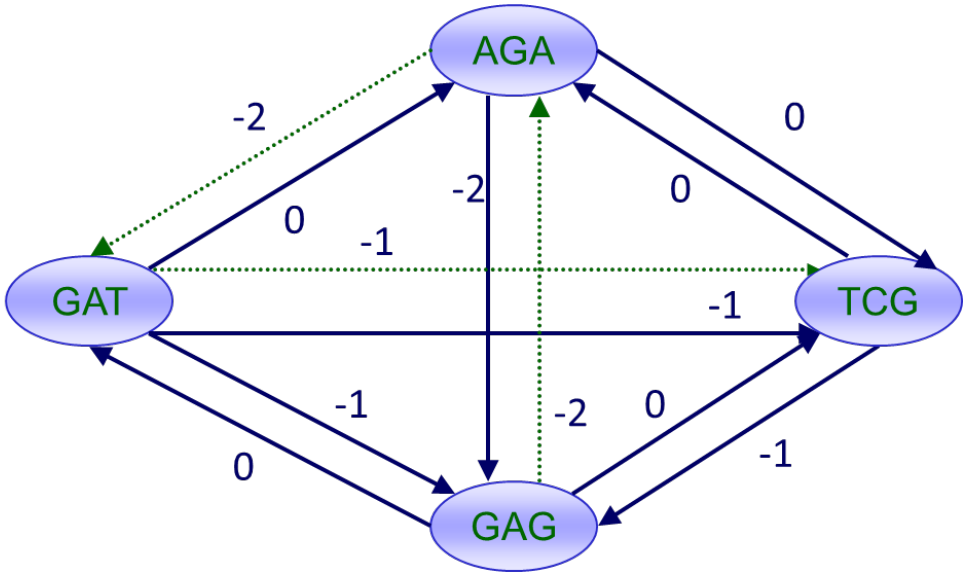


Marc Craven, BMI/CS 576, www.biostat.wisc.edu/bmi576.

Assembly as finding a Hamiltonian path

- Hamiltonian path: path through graph that visits each vertex exactly once,
- minimize superstring length
  - minimize weight of Hamiltonian path in overlap graph with edge weights negated,
  - this is essentially the Traveling Salesman Problem (also NP-complete),

path: GAGATCG
path weight: -5
string length: 7



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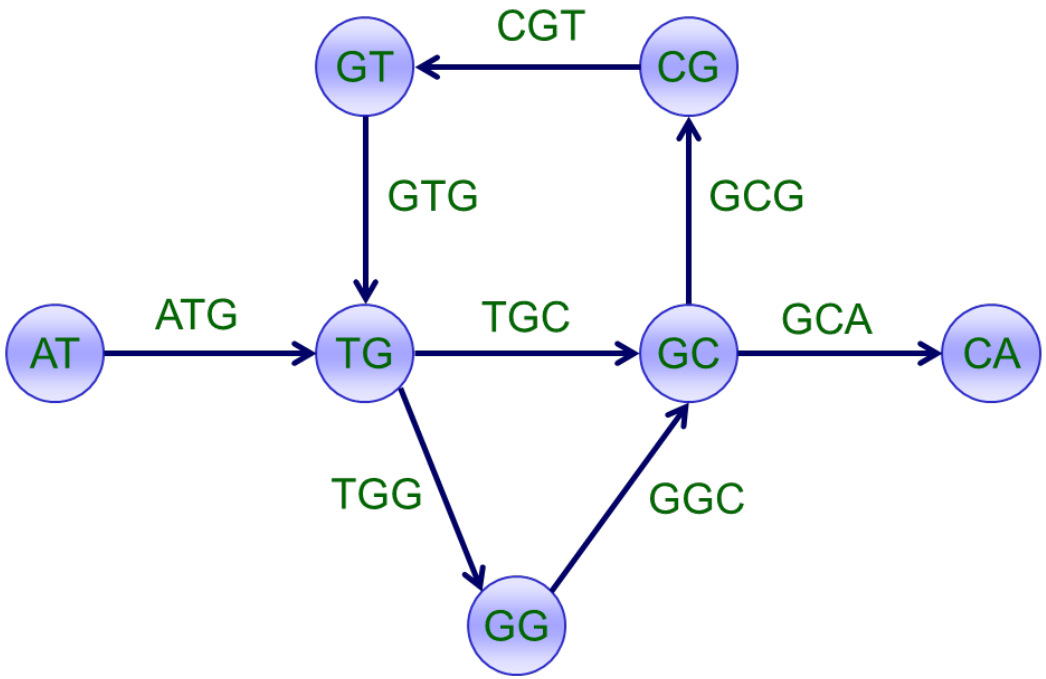
Assembly as finding a Hamiltonian path

- Finding Hamiltonian path is an NP-complete problem,
- nevertheless overlap graphs are often used for sequence assembly
  - can detect repeats,
  - heuristical hierarchical decomposition
    - unitigs (no forks, no conflicts) solved first,
  - mate-pairs to scaffold.

de Bruijn graph

- spectrum(s,k) = set of all k-mers (substrings of length k) from a string s,
- in a de Bruijn graph
  - edges = k-mers that occur in spectrum(s, k), vertices = (k-1)-mers,
- example: spectrum={**ATG, TGG, TGC, GTG, GGC, GCA, GCG, CGT**}.



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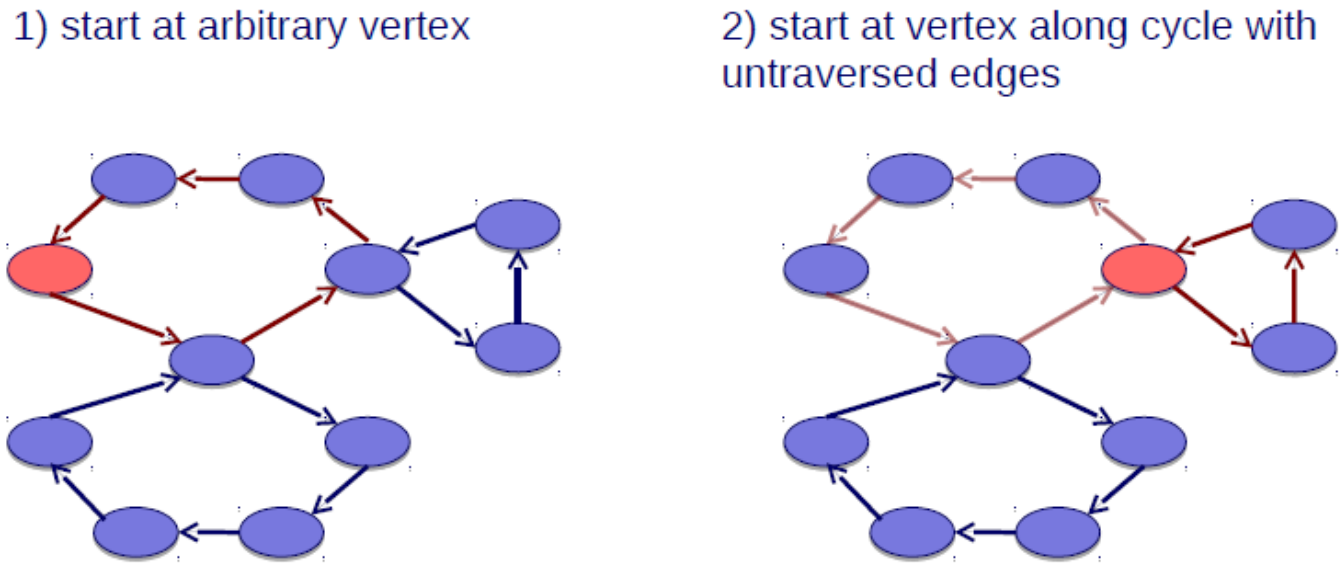
de Bruijn graph

- Can we find a DNA sequence containing all k-mers?
  - in a de Bruijn graph, can we find a path that visits every edge of the graph exactly once?
- the theory of Eulerian graphs
  - cycle: a path in a graph that starts/ends on the same vertex,
  - Eulerian cycle: a path that visits every edge of the graph exactly once,
  - theorem: a connected graph has an Eulerian cycle if and only if each of its vertices are balanced,
  - a vertex v is balanced if indegree(v) $=$ outdegree(v),
  - there is **a linear-time** algorithm for finding Eulerian cycles!
- We have reads, not k-mers ...
  - reads are immediately split into shorter k-mers,
  - certain information loss (read coherence, overlaps).

Eulerian cycle algorithm

- Start at any vertex v, traverse unused edges until returning to v,
- while the cycle c is not Eulerian
  - pick a vertex w along c for which there are untraversed outgoing edges,
  - traverse unused edges until ending up back at w,
  - join two cycles into one cycle c.



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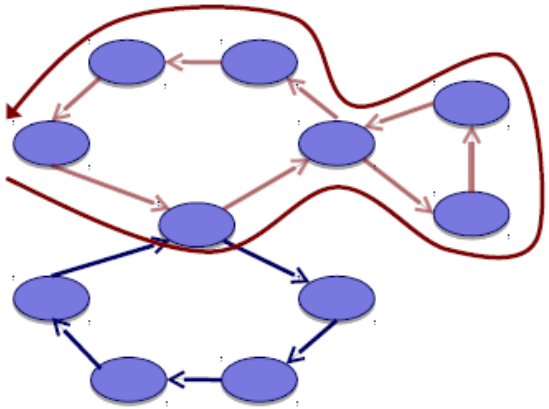
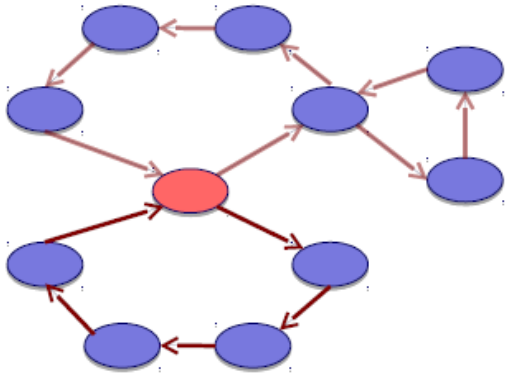
Eulerian cycle algorithm

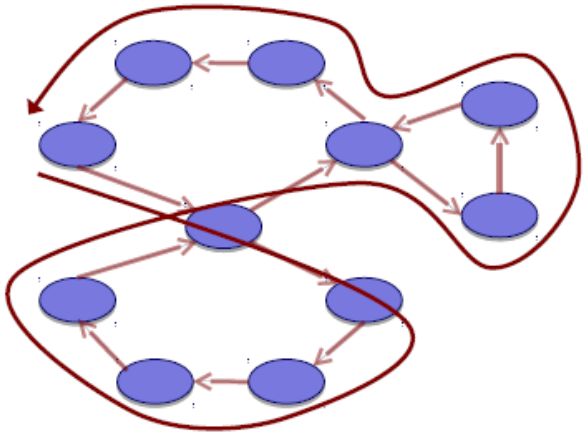
3) join cycles



4) start at vertex along cycle with untraversed edges



5) join cycles



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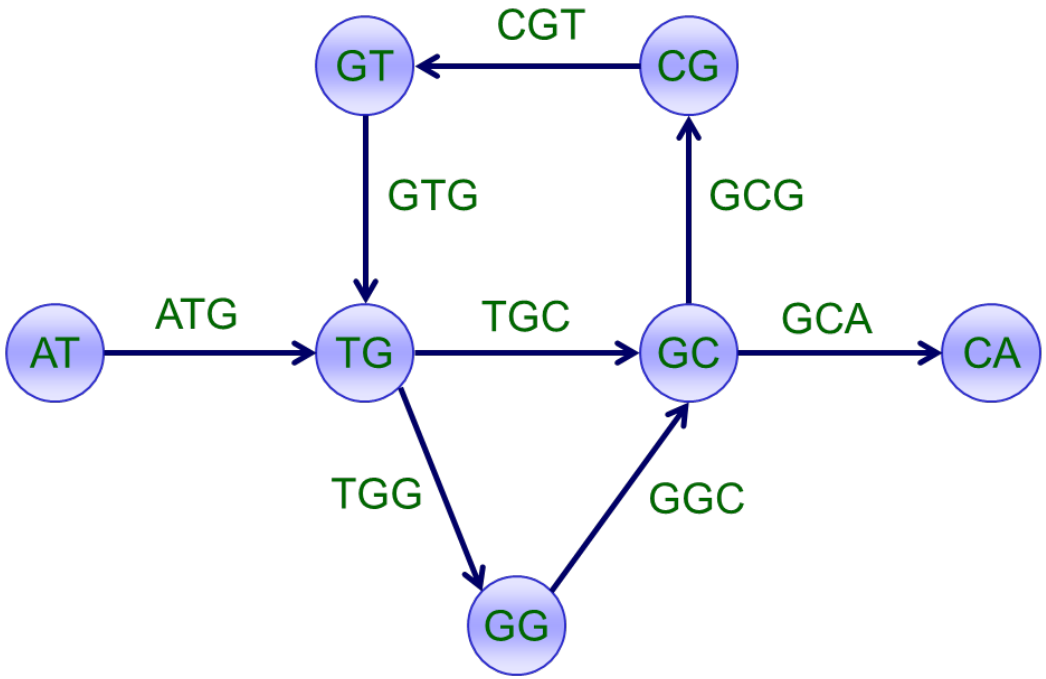
Assembly as finding Eulerian paths

- Eulerian path: path that visits every edge exactly once (actually, a trail),
- the assembly problem = finding Eulerian paths in a de Bruijn graph,
- resulting sequences contain all k-mers.
- example assembly: **ATGGCGTGCA** or **ATGCGTGGCA**.



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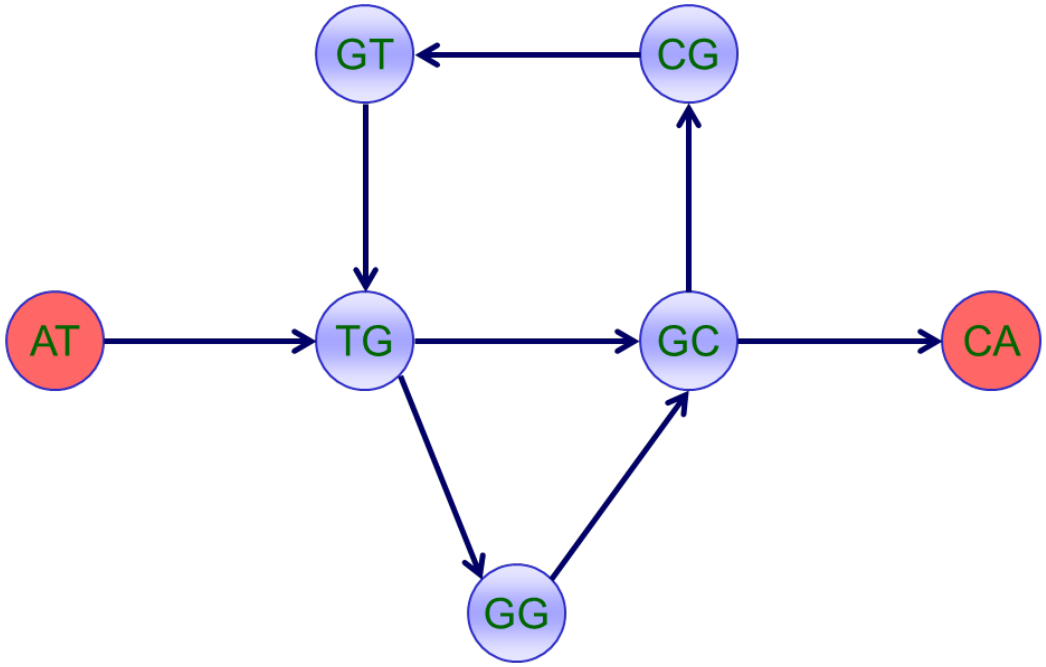
Eulerian paths

- a vertex v is semibalanced if |indegree(v) – outdegree(v)| $= 1$,
- a connected graph has an Eulerian path if and only if it contains at most two semibalanced vertices.



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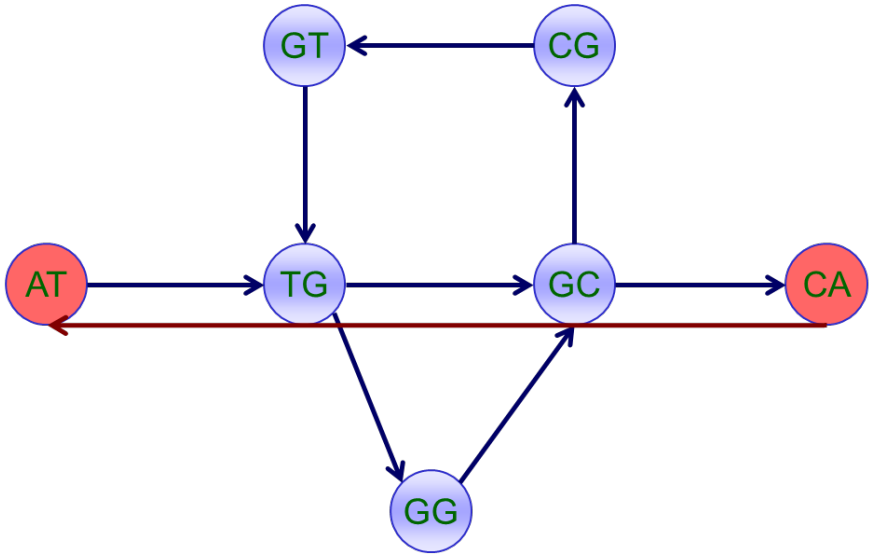
Eulerian path $\rightarrow$ Eulerian cycle

- If a graph has an Eulerian Path starting at w and ending at x then
  - all vertices must be balanced, except for w and x which may have |indegree(v) – outdegree(v)| $= 1$,
  - if w and x are not balanced, add an edge between them to balance,
  - graph now has an Eulerian cycle which can be converted to an Eulerian path by removal of the added edge.



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Violating assumptions in de Bruijn graphs

- Assume a sequence: **a_long_long_long_time**
  - length m=21, the sequence contains repeats,
  - choose k=5, number of 5-mers n=m-k+1=17,
  - taken from [Langmead: de Bruijn graph assembly, 2014].
- Assume different sets of k-mers (see the next slide):

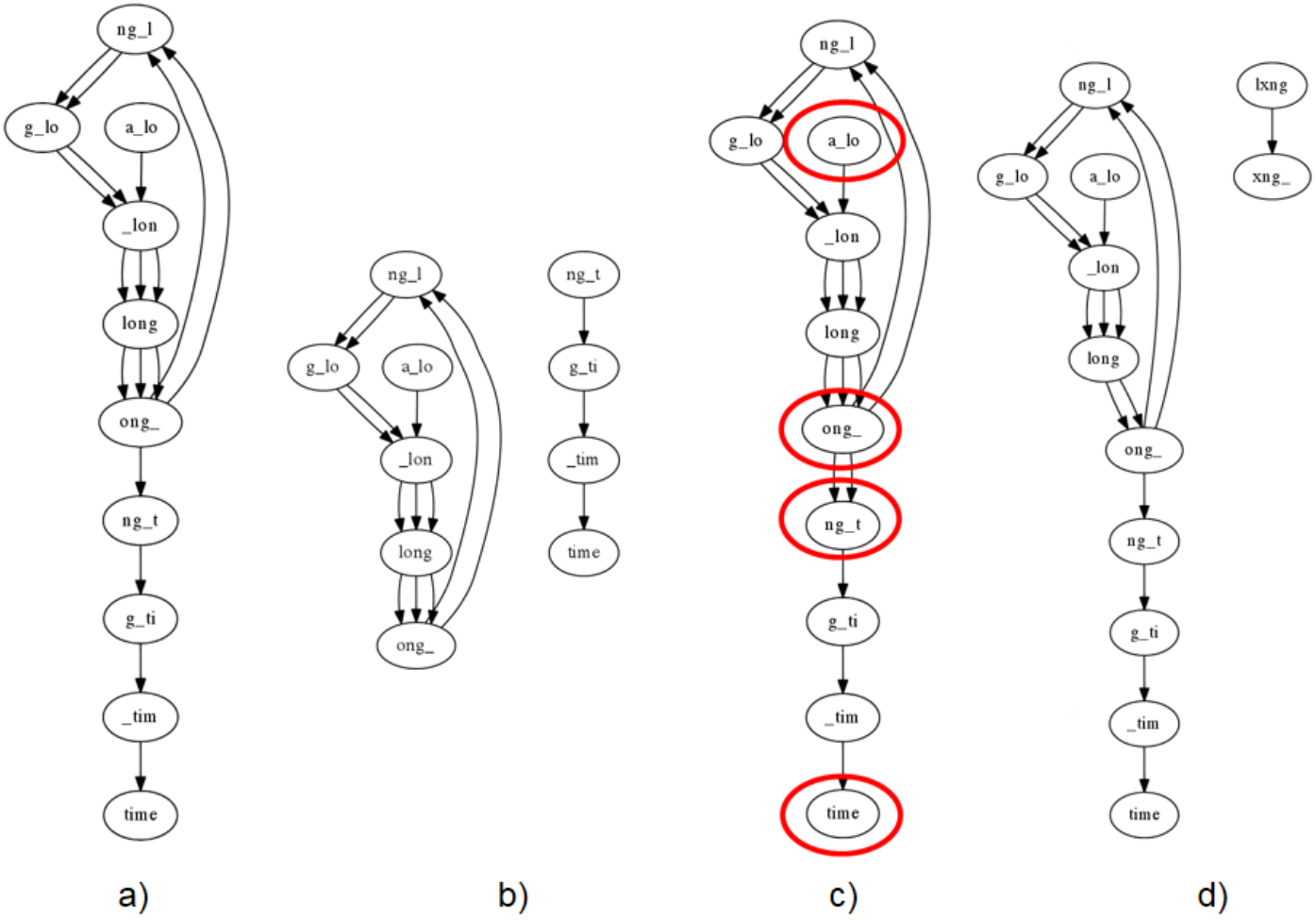
(a) all 5-mers → the correct assembly,

(b) omitting **ong_t** → two graph components, the overall graph not Eulerian,

(c) extra copy of **ong_t** → 4 semi-balanced nodes, graph not Eulerian,

(d) errors and differences between chromosomes, turn a copy of **long_** into **lxng_** → graph not connected, largest component not Eulerian.

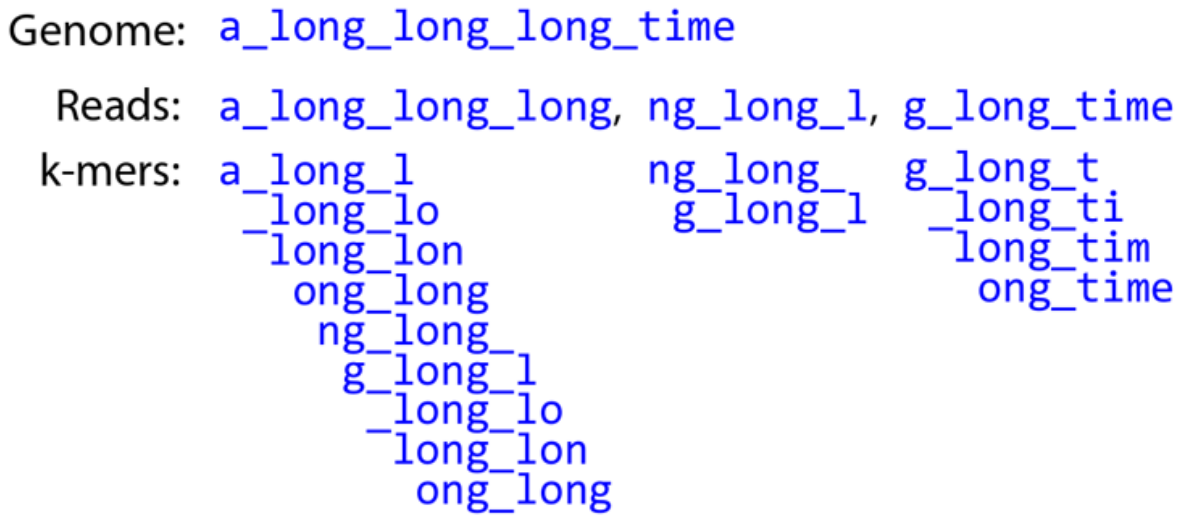
Violating assumptions in de Bruijn graphs



Langmead: de Bruijn graph assembly, 2014.

de Bruijn graphs – short k-mers

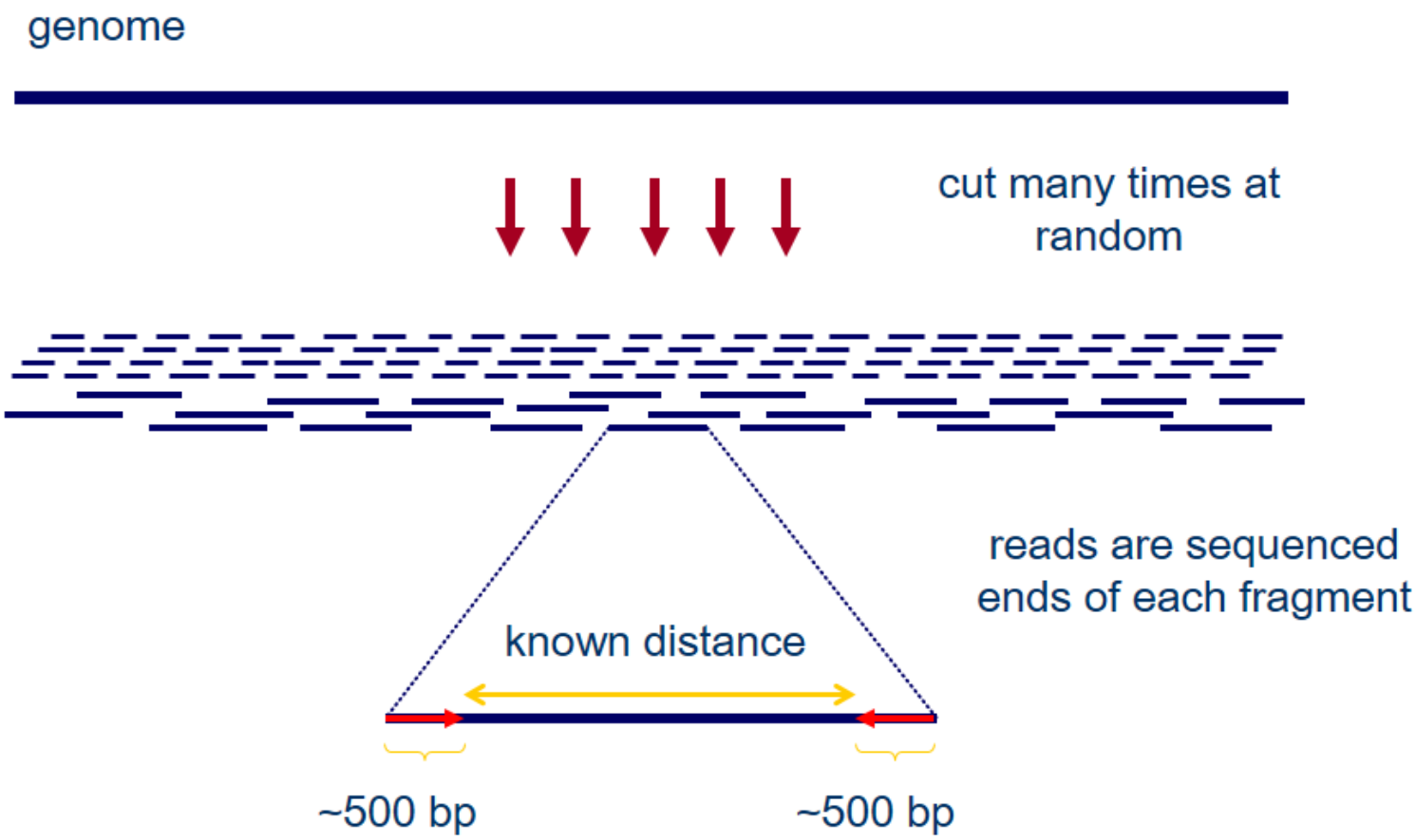
- Only short k-mers guarantee that none is missed,
- still, the number of k-mers remains O(N)
  - N is the total length of reads,
- de Bruijn graph with O(N) edges and O(N) nodes too
  - can be constructed in O(N),
  - Euler cycle found in O(N).



Langmead: de Bruijn graph assembly, 2014.

Paired end reads

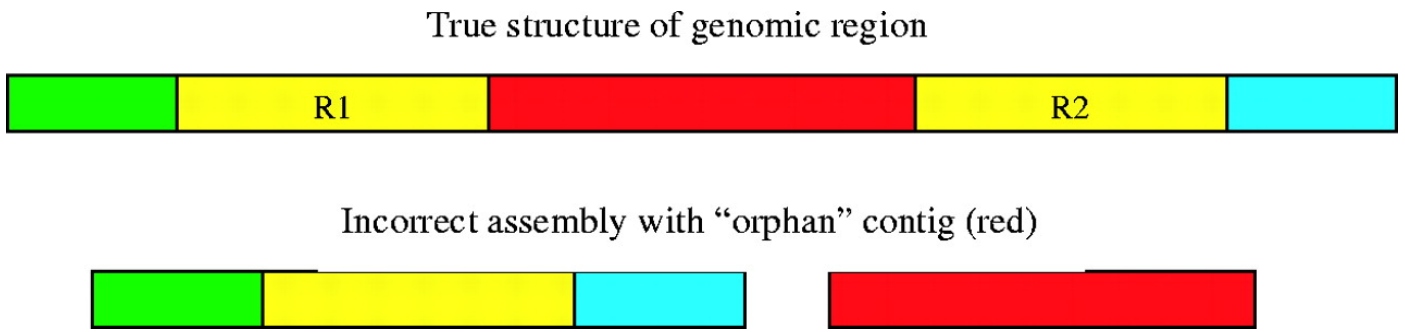
- One approach to reducing ambiguity in assembly is to use paired end reads.



Marc Craven, BMI/CS 576, www.biostat.wisc.edu/bmi576.

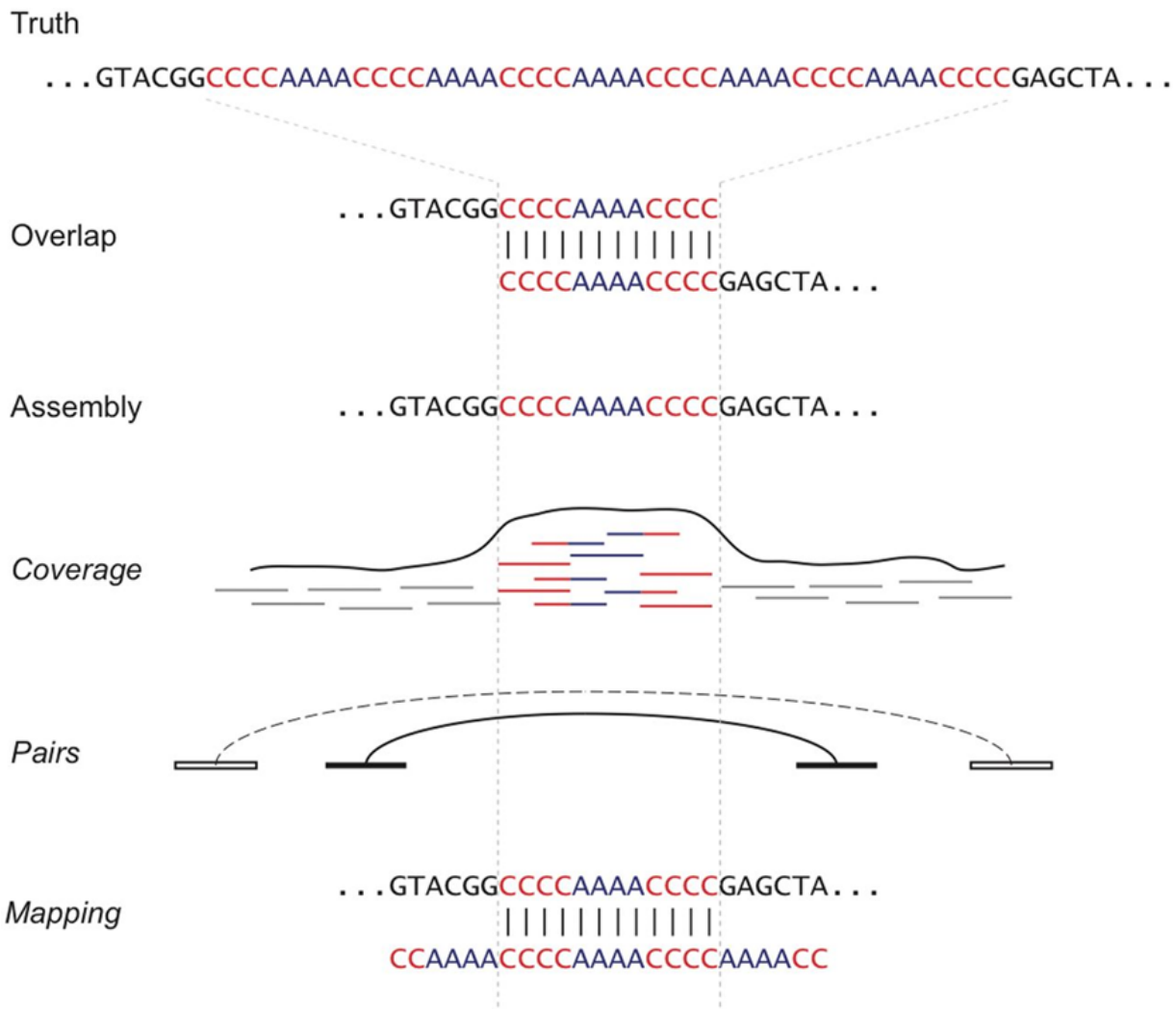
Repetitive sequences (repeats)

- Most common source of assembly errors,
- if sequencing technology produces reads $>$ repeat size, impact is much smaller,
- most straightforward solution:
  - mate pairs with spacing $>$ largest known repeat.



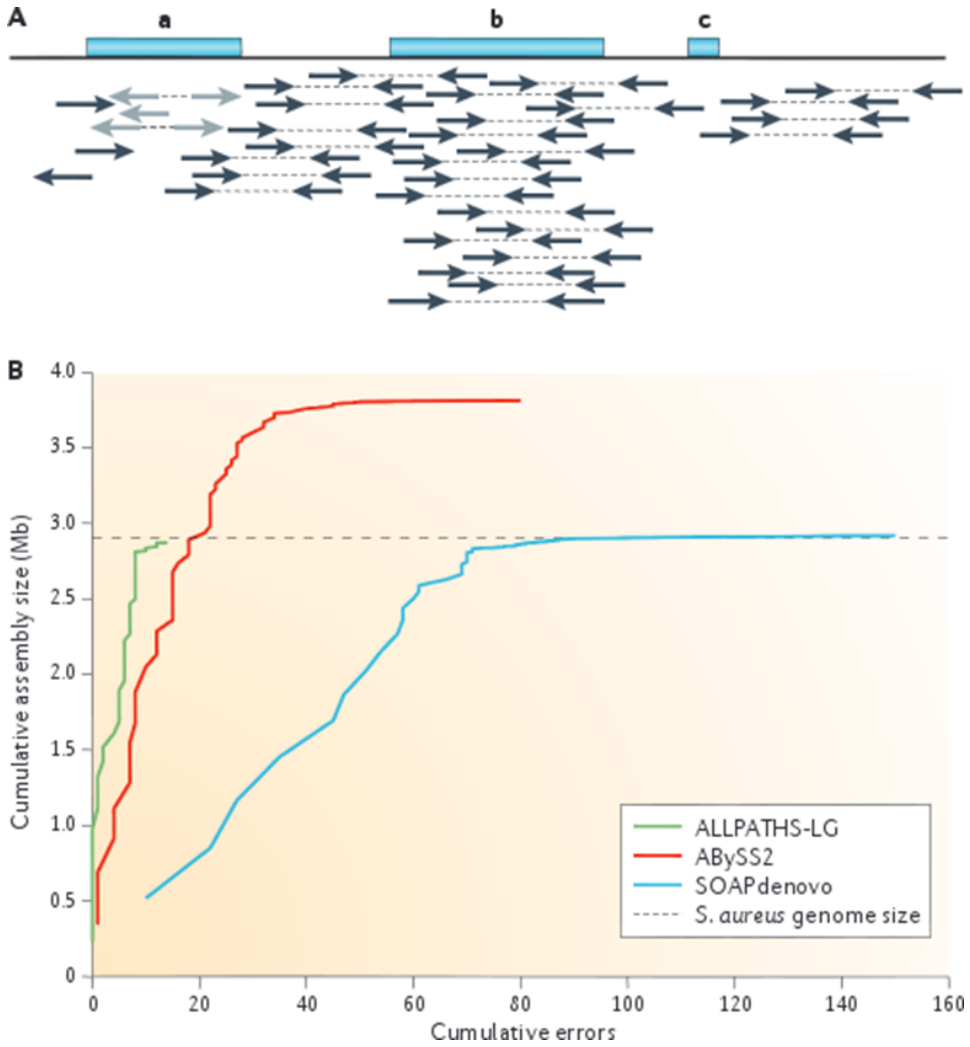
Salzberg and Yorke: Beware of mis-assembled genomes, Bioinformatics, 2005.

Mis-assembly of repetitive sequence



Schatz et al.: Hawkeye and AMOS: visualizing and assessing the quality of genome assemblies, Brief Bioinform 2013.

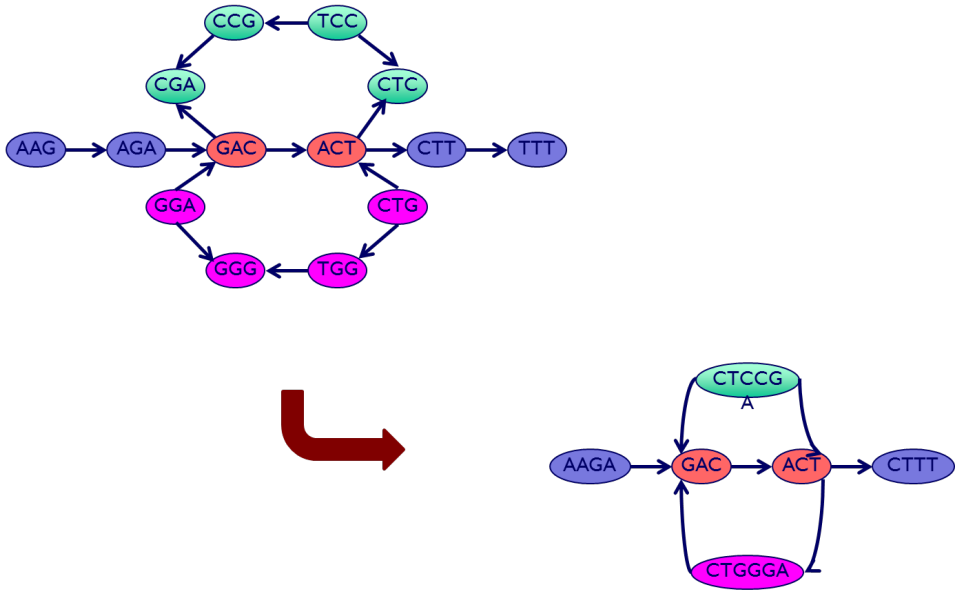
Methods for assembly validation



Nagarajan and Pop: Sequence Assembly Demystified, Nature Reviews, 2013.

The Velvet assembler

- Based on de Bruijn graphs, includes additional tricks for
  - reducing the size of the graph,
  - trying to correct for errors in sequences,
  - taking advantage of paired-end reads,
- compress the graph, collapse linear subgraphs:



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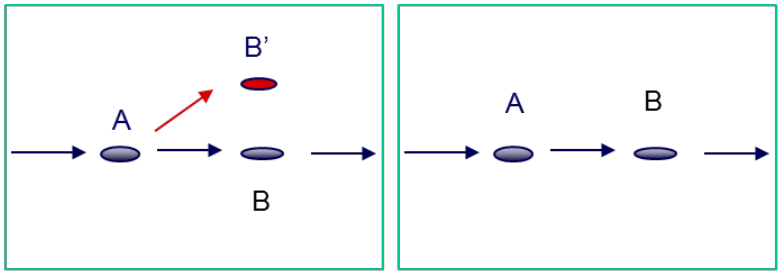
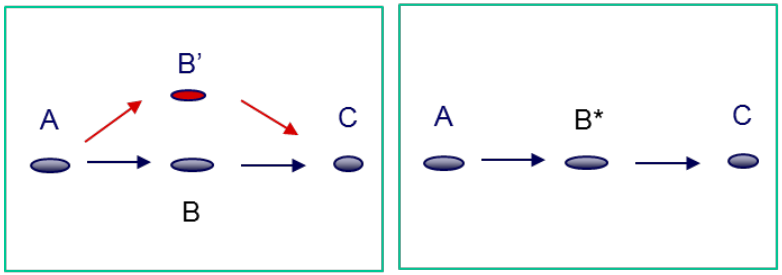
Error correction in Velvet

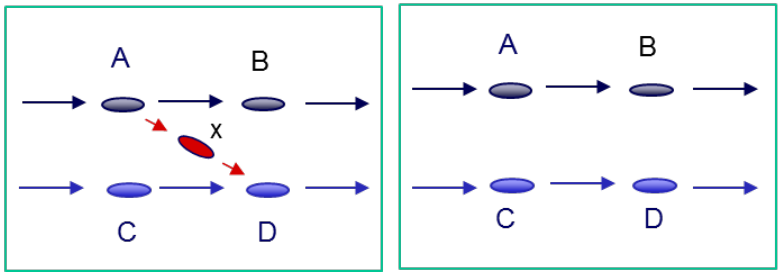
- errors at end of read
  - trim off “dead-end” tips,



- errors in middle of read
  - pop bubbles,



- chimeric edges
  - clip short, low coverage nodes.



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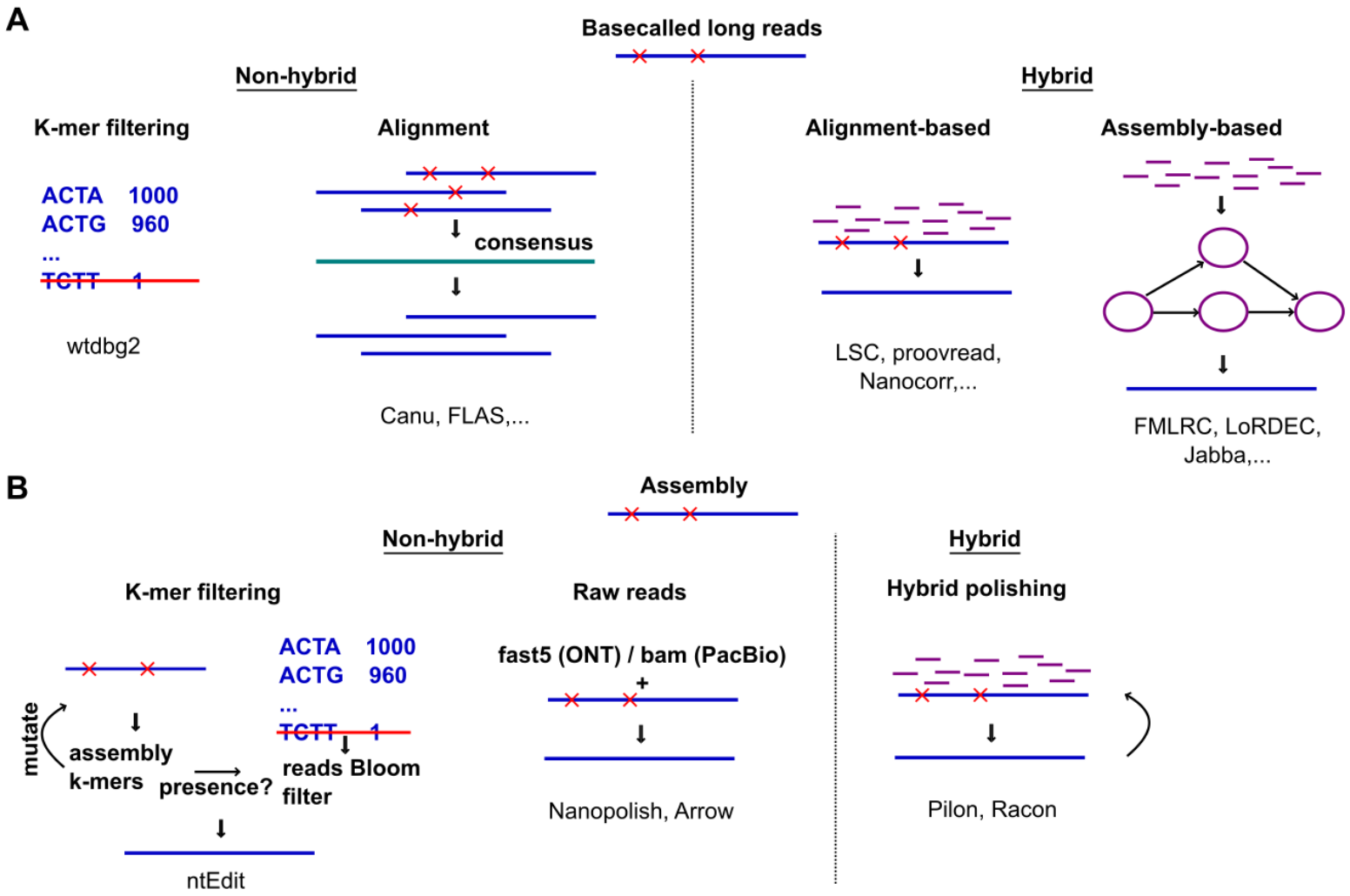
Short vs long read assembly

- Short read assembly
  - assembly most often based on de Bruijn graphs,
  - the main issues
    - large number of reads, efficiency issues,
    - repeat resolution,
- long read assembly
  - assembly based on overlap graphs,
  - the main issue is read correction
    - requires high coverage (50-100x), could be expensive,
    - or hybrid assembly with shorter reads to error-correct.

Long read correction and polishing



Amarasinghe et al.: Opportunities and challenges in long-read sequencing data analysis, Genome Biology, 2020.

Summary

- The sequencing problem
  - sequencing in vitro,
  - sequence assembly in silico,
    - de novo versus resequencing,
    - approaches: greedy, overlap graph, Euler trail,
    - elements: reads, contigs, scaffolding,
  - assembly validation
    - statistical, viewers, comparative methods,
- still open problem
  - costs, efficiency, reliability,
  - changes in sequencing imply changes in assembly.