BOX

Genome assembly (*or on modeling biological problems in CS*)

Léo Ackermann

N.B. Credits of figures are missing

Before starting

If you want to contact me. By email (leo.ackermann@inria.fr) or by Discord (tag me)

A few review you may find interesting. (Theoretical questions related to bioinformatics)

- *Parameterized Algorithms in Bioinformatics: An Overview.* Bulteau & Weller, 2019.
- *Theoretical analysis of edit distance algorithms. Medvedev, 2023.*
- *Theoretical analysis of sequencing bioinformatics algorithms and beyond. Medvedev, 2023.*
- *Modeling Biological Problems in Computer Science: A Case Study in Genome Assembly. Medvedev, 2018.*
- *Information theory in computational biology: where we stand today. Chanda et al. 2020.*
- *Sketching and sublinear data structures in genomics. Marçais et al. 2019.*
- *When less is more: sketching with minimizers in genomics. Ndiaye et al. 2024.*
- *Advancements in practical k-mer sets: essentials for the curious. Marchet. 2024.*
- *Indexing highly repetitive string collections, part I: repetitiveness measures. Navarro. 2021.*
- *Indexing highly repetitive string collections, part II: compressed indexes. Navarro. 2021.*

*blueprint of this lecture

Today's program

A. Discovering genome assembly

B. Trying to formulate it as a mathematical/CS problem

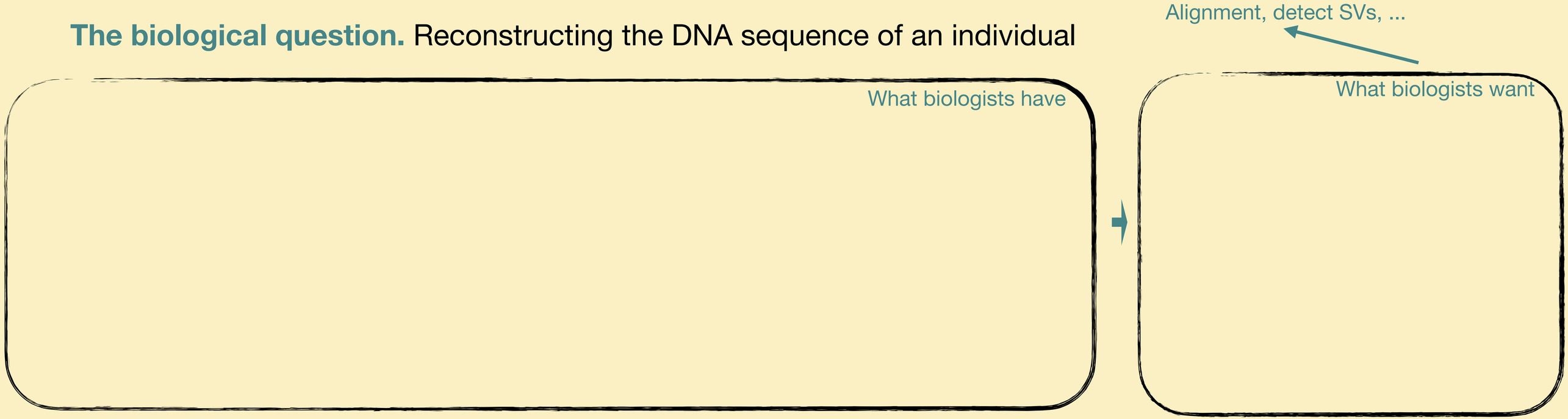
C. Discovering the two main approaches used in practice to solve this problem

Part I

Modeling the biological question

The *De Novo* Sequence Assembly problem

The biological question. Reconstructing the DNA sequence of an individual



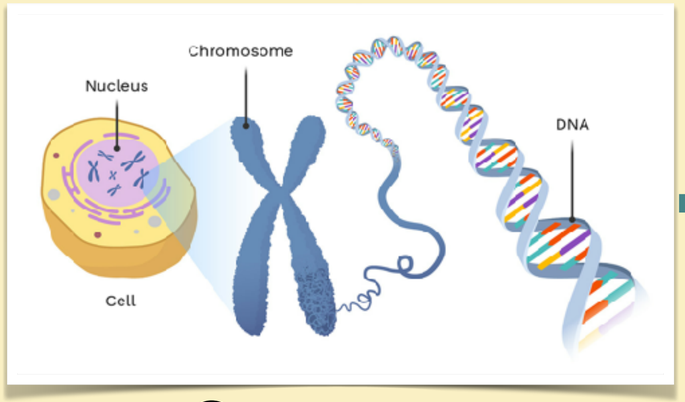
The *De Novo* Sequence Assembly problem

The biological question. Reconstructing the DNA sequence of an individual

Alignment, detect SVs, ...

What biologists have

What biologists want



Genome

Unknown DNA "strings"

DNA Sequencing

Reads

DNA "substrings"

(1M of ±200nt reads for a 5M-nt genome)

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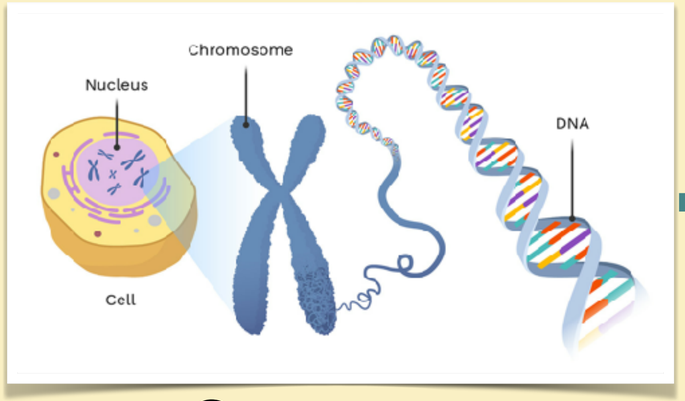

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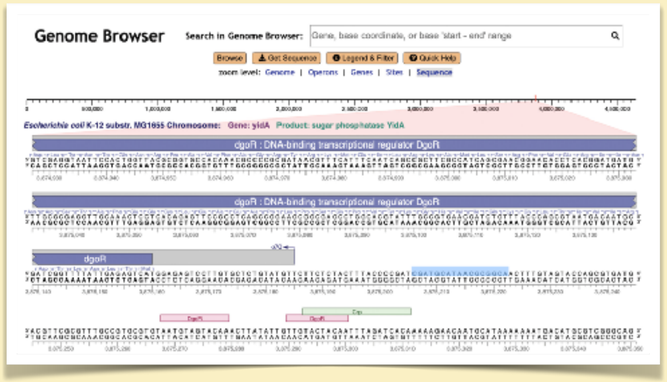

Assembly

Computational representation of genome

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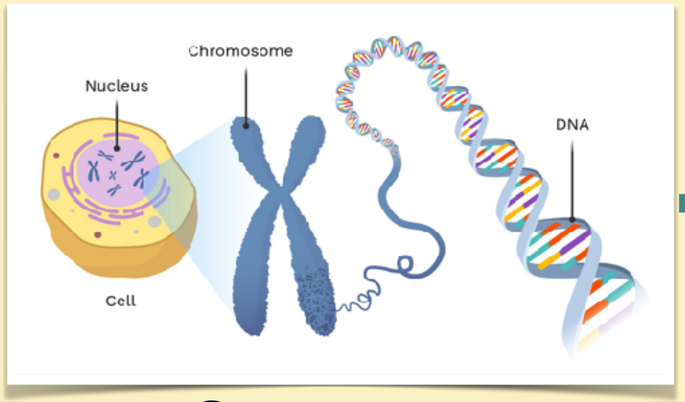

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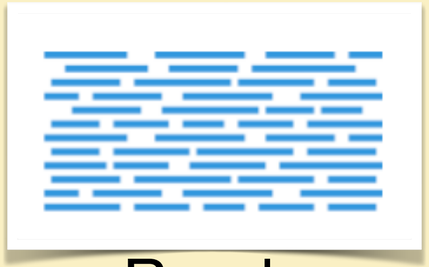

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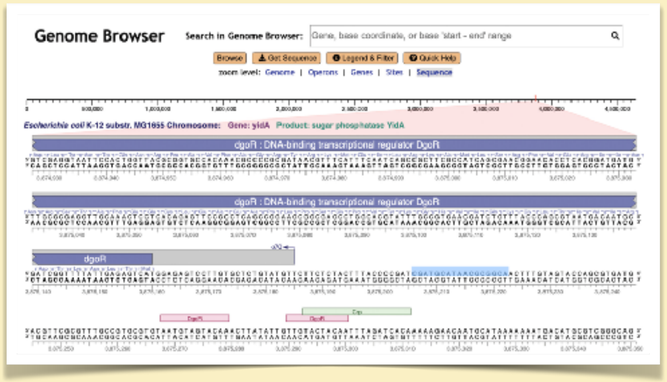

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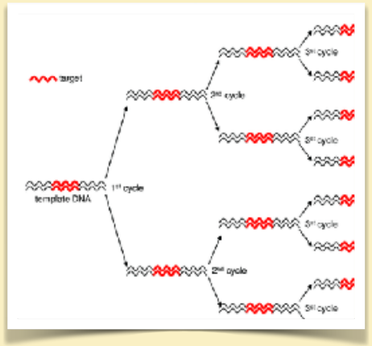
A (quick) zoom on DNA Sequencing.



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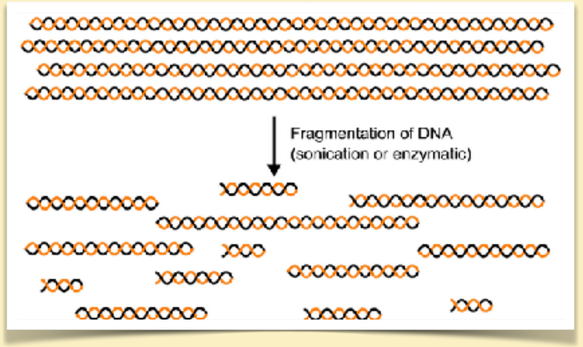

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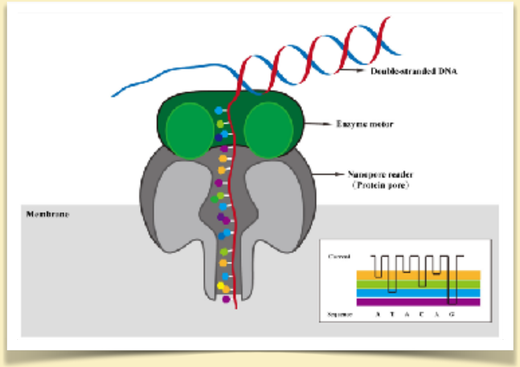

Signal acquisition

(+ base calling)

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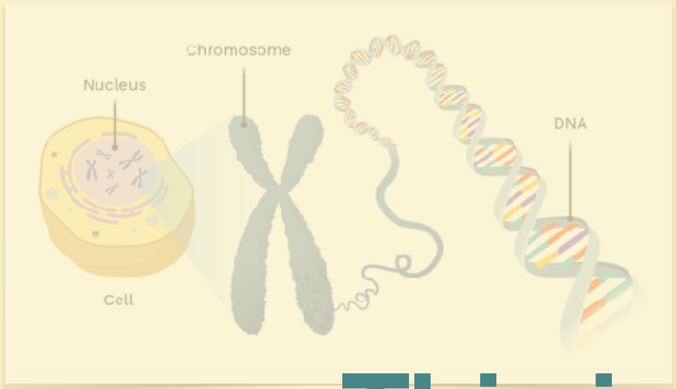

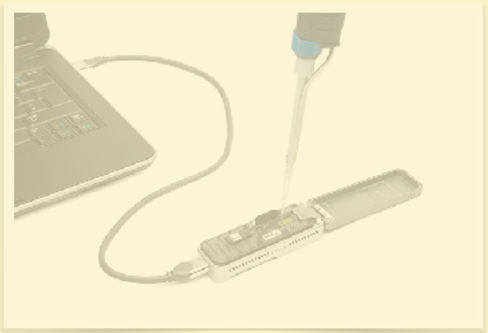

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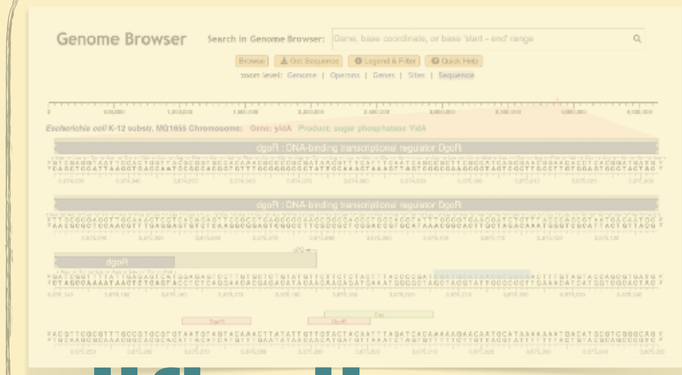

Genome

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DNA Sequencing

Reads

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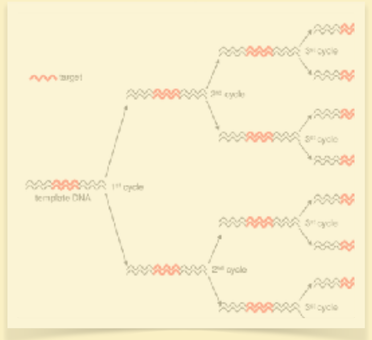
Millions of ~200nt reads for a ~5-nt genome

Assembly

Computational representation of genome

This is already extremely simplified!

A (quick) zoom on DNA Sequencing.



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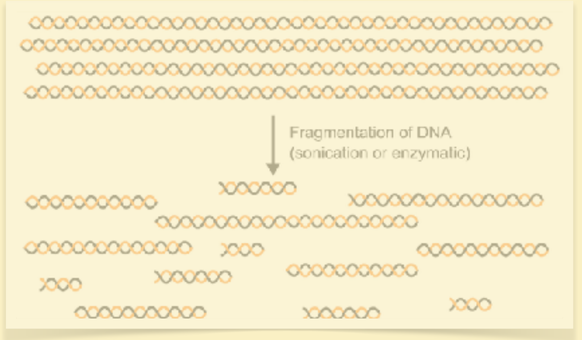

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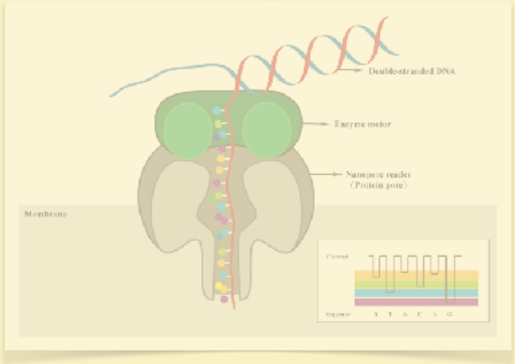

Signal acquisition

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[1] Double-strands vs single-strand

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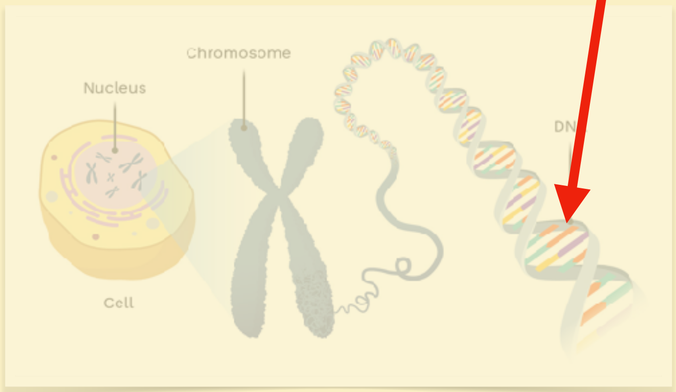

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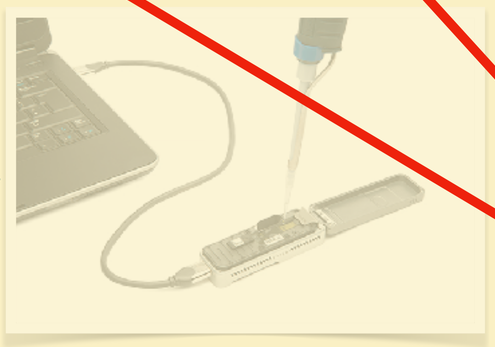

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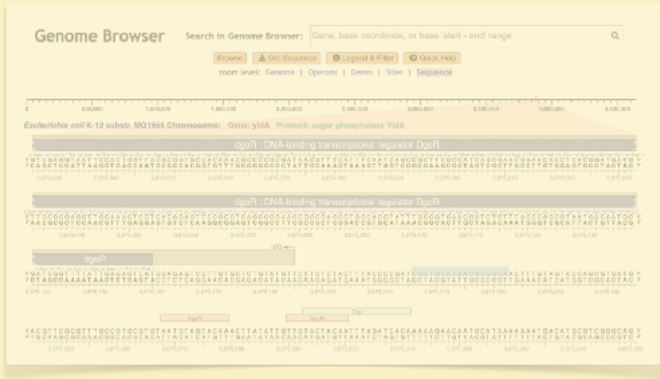

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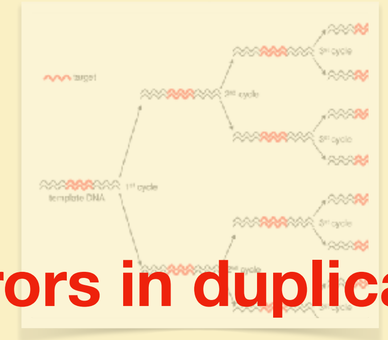

DNA Amplification

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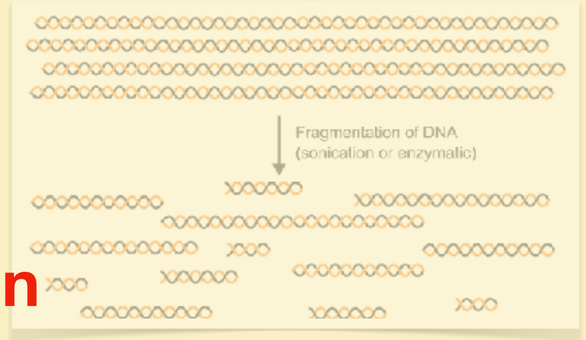

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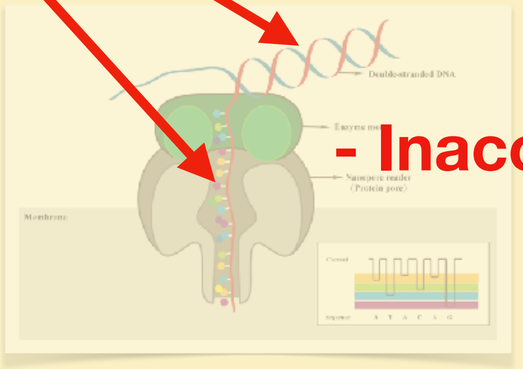

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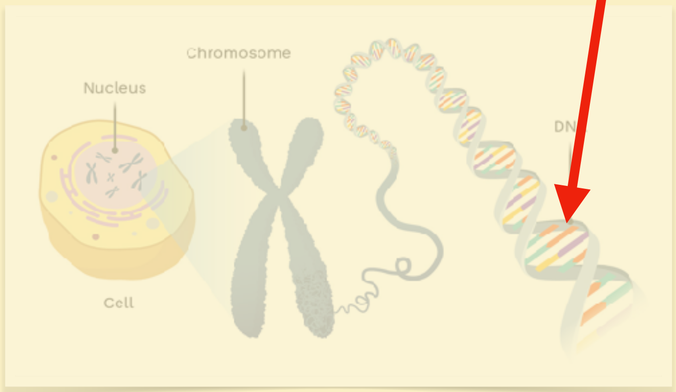

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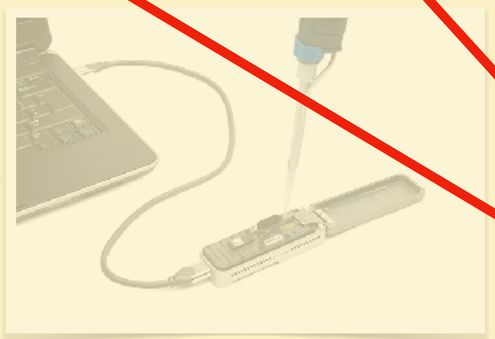

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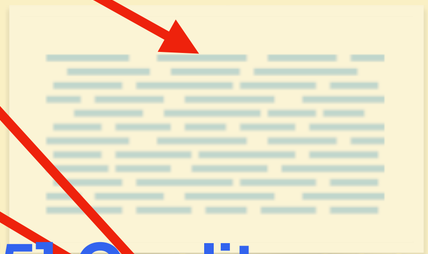

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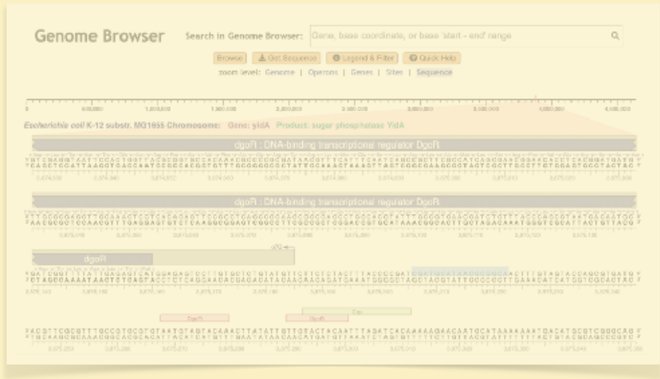

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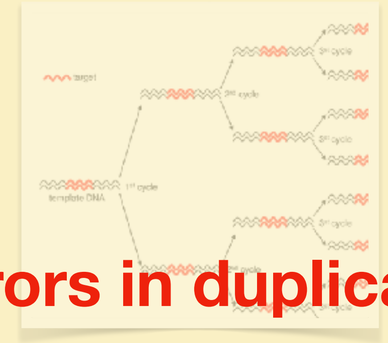

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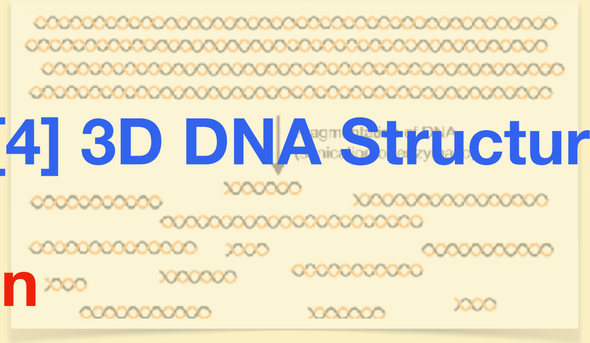

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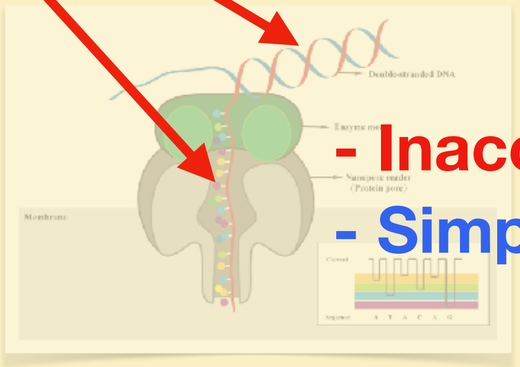
[4] 3D DNA Structure



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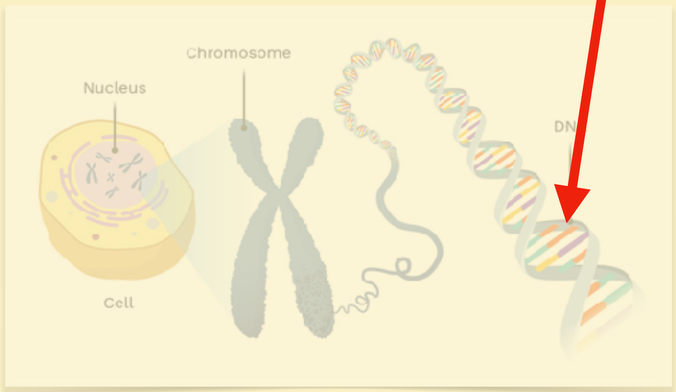

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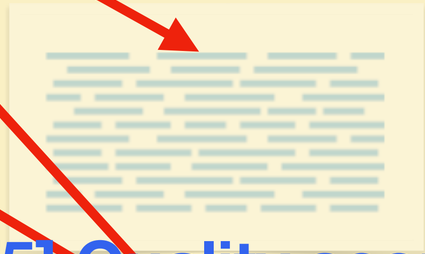

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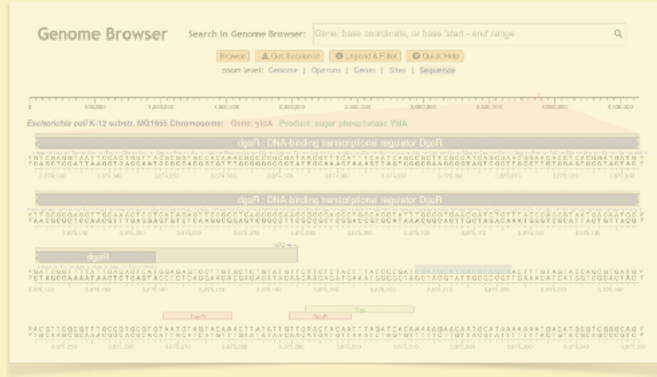

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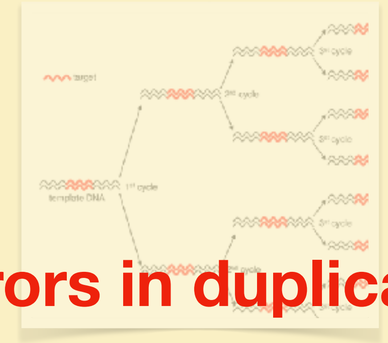

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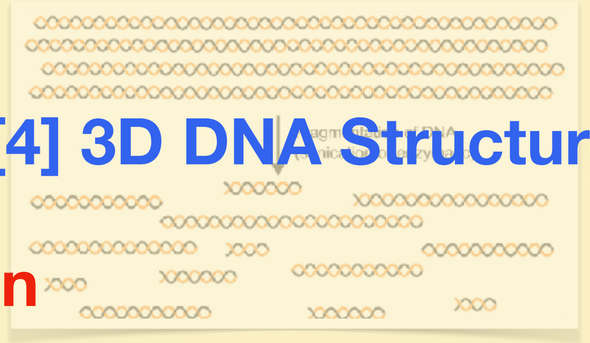

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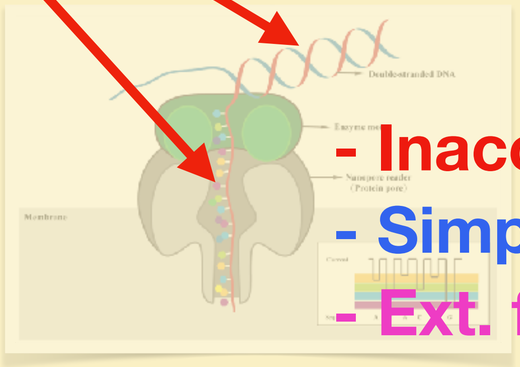

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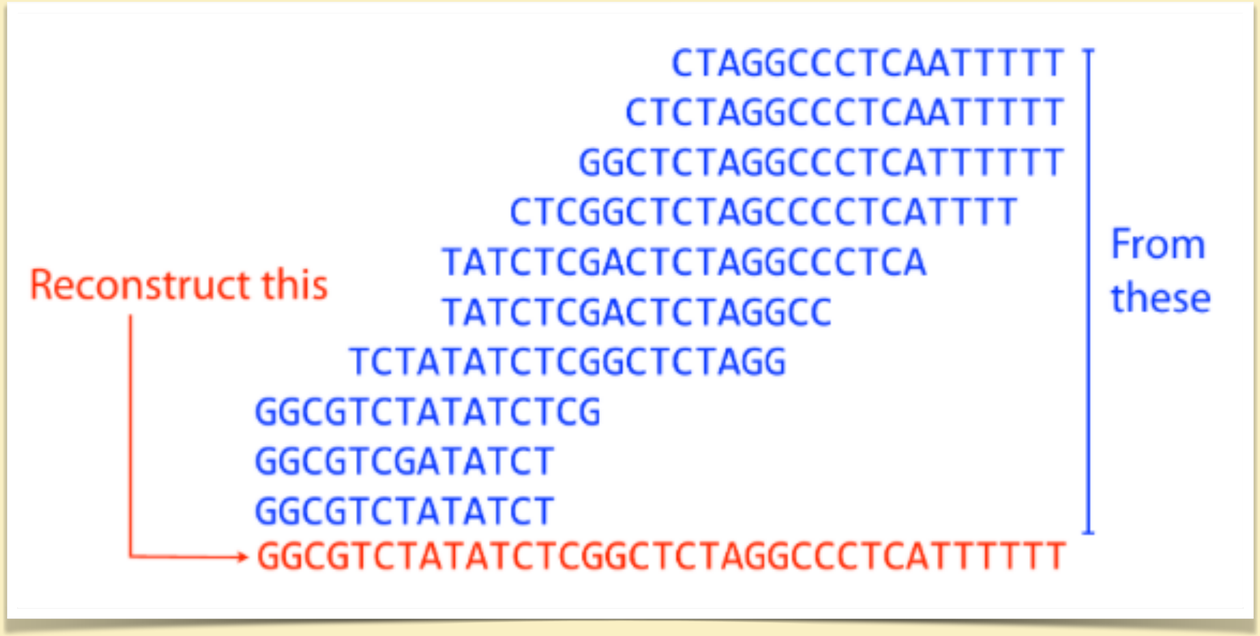
- Inaccuracies

- Simplifications

- Ext. factors

The *De Novo* Sequence Assembly problem

A computer science perspective (draft).



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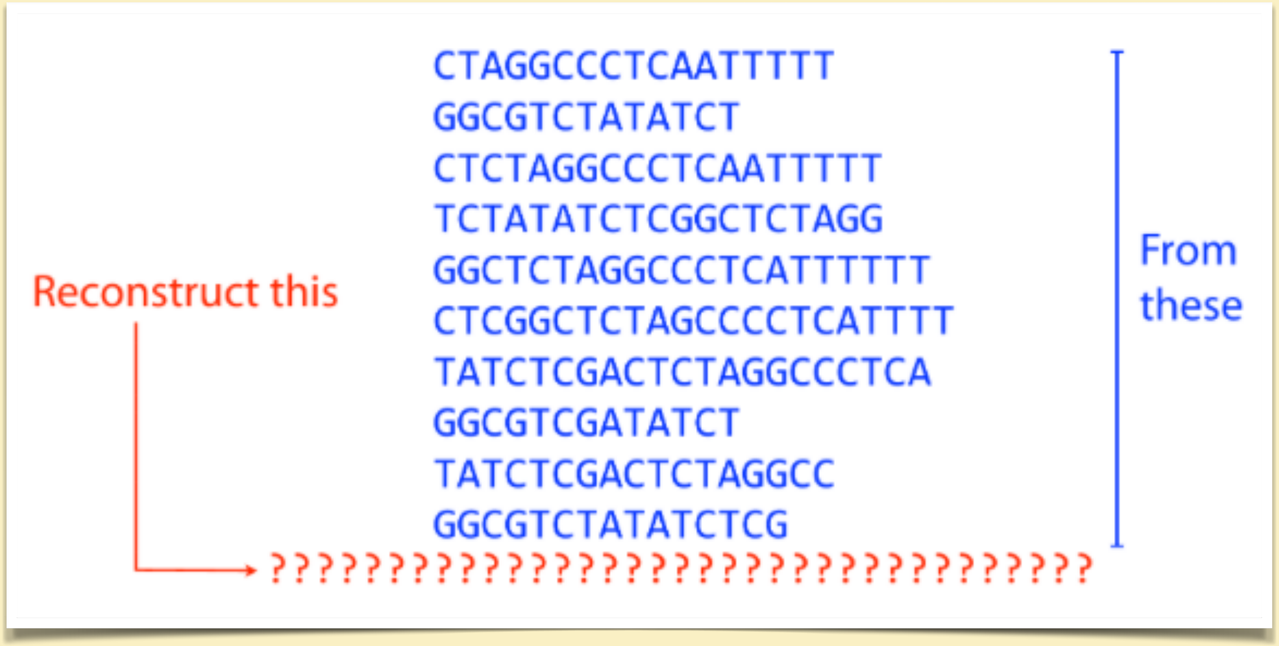

A well-formulated problem

Toward a well-defined problem

Input: A collection of strings $\mathcal{S} = \{s_1, \cdots, s_n\}$ generated by a sequencing experiment on some genome

Output: the genome that generated $\mathcal{S}$

What do you think of it?

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Definition (Genome assembly, v1).

Input: a collection of strings $\mathcal{S} = \{s_1, \cdots, s_n\}$
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Motto. **The ultimate test of a (practical) algorithm is how is performs on real data!**

A useful (?) problem

Optimization criterion

Definition (Genome assembly, v1).

Input: a collection of strings $\mathcal{S} = \{s_1, \cdots, s_n\}$

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Example. Consider $\mathcal{S} = \{ACG, CGT\}$. What is G?

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Many possible answers: ACGT, ACGCGT, ACGTTTTTTTTCGT, CGTACACACG, ...

Which one should we pick?

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Definition (Genome assembly, v2).

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Limitation. This doesn't guarantee the unicity of the solution (but maybe not a problem on real data).

Computational complexity

Lemma. The "Genome Assembly (v2)" is NP-complete (by reduction to Hamiltonian path)

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Still, ideally we would like a tractable formulation of the problem.

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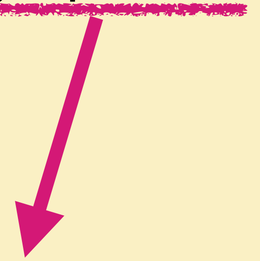

Hidden constants

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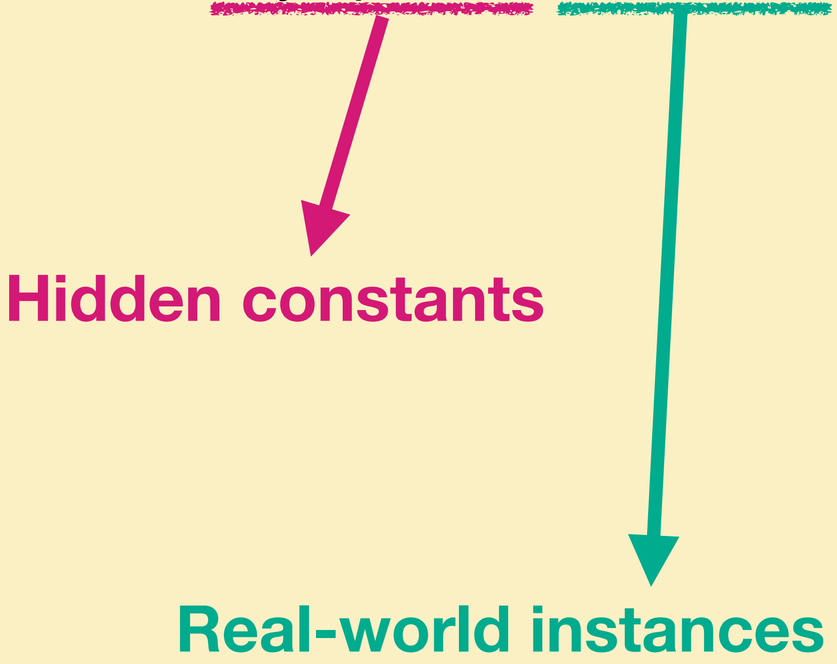

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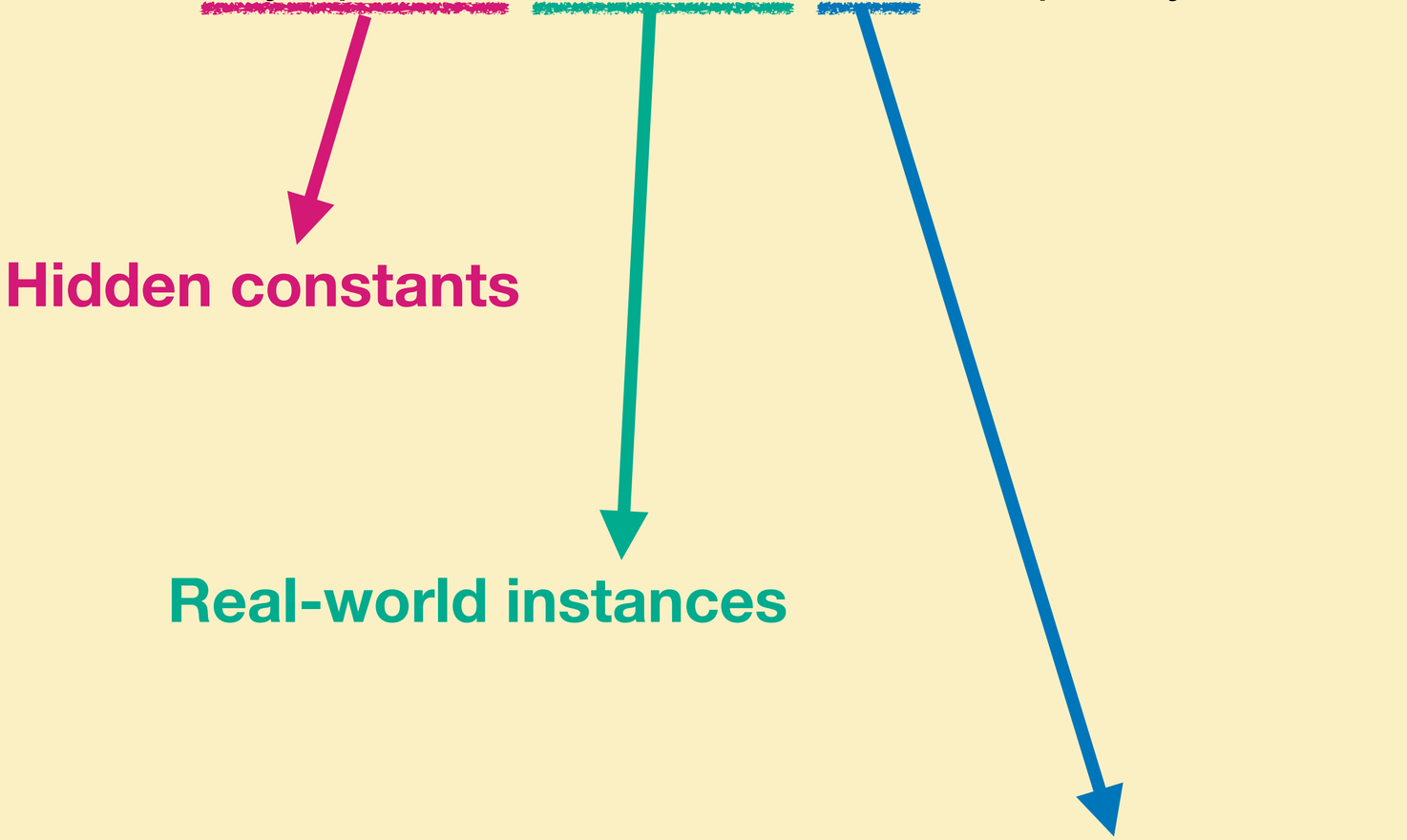

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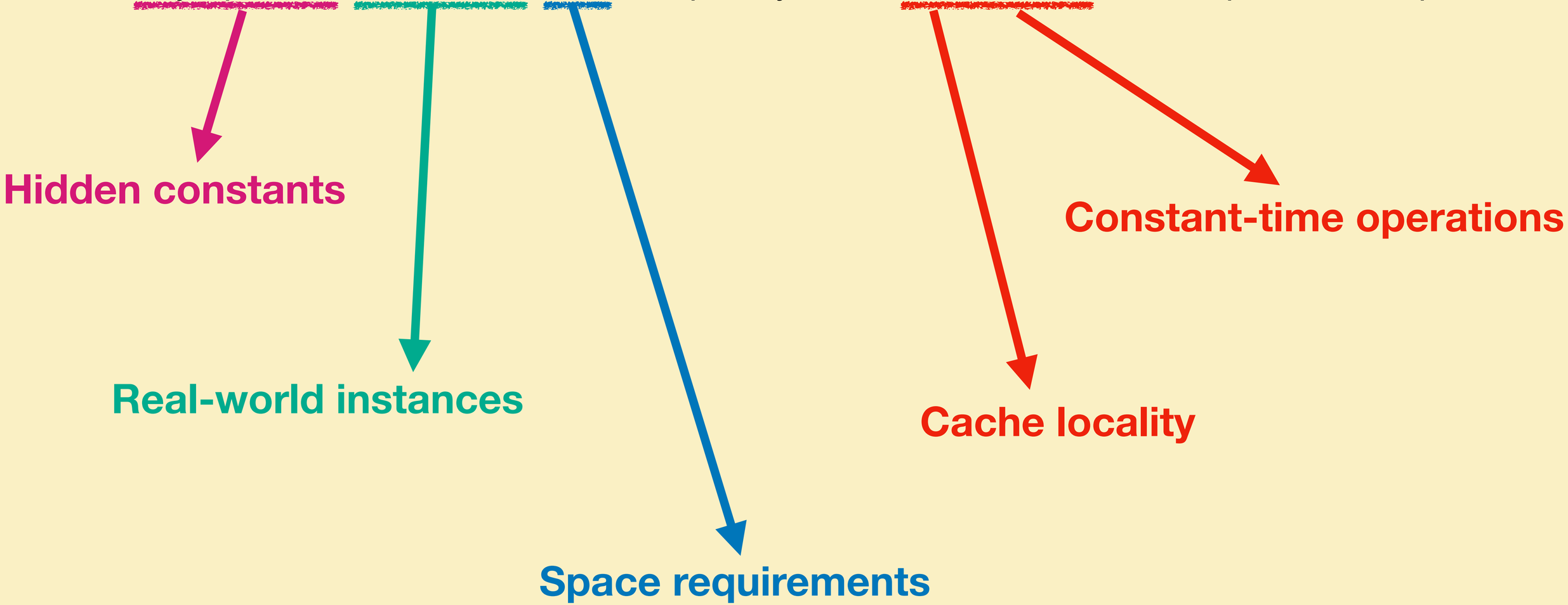

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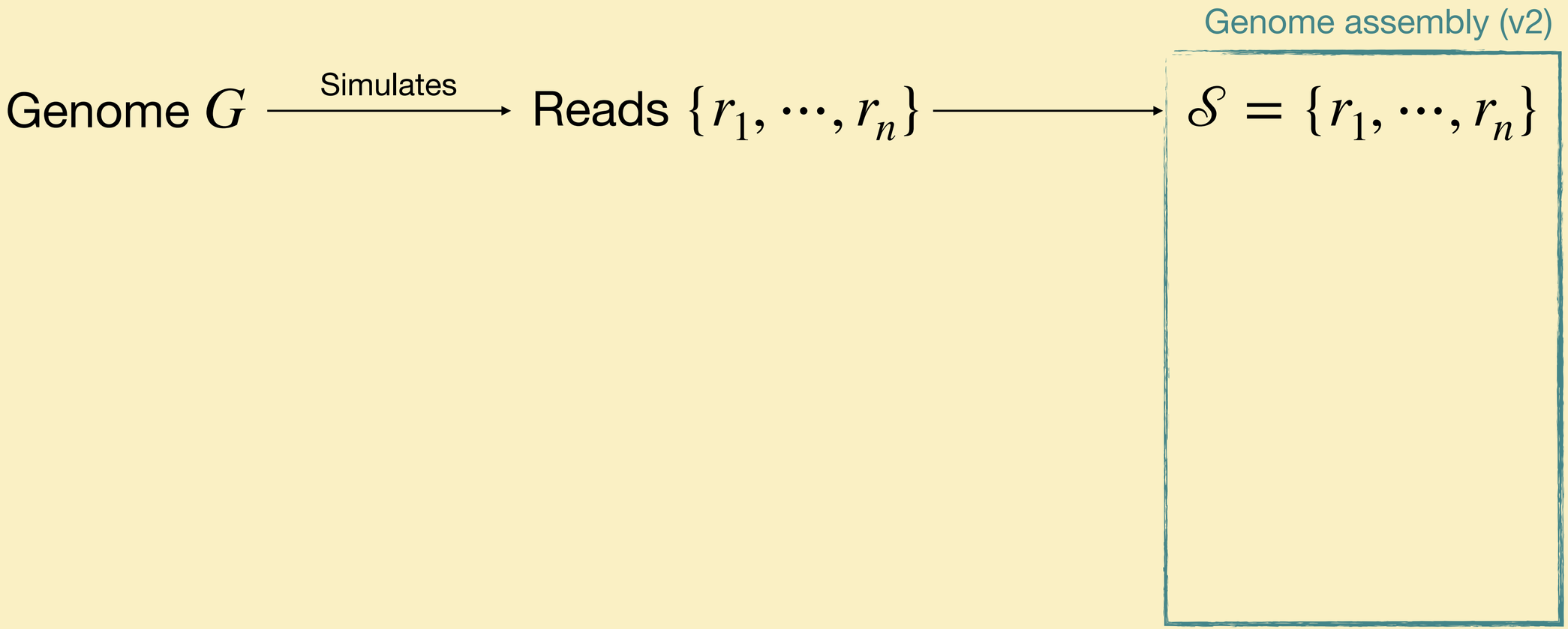

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Genome assembly (v2)

$\mathcal{S} = \{r_1, \cdots, r_n\}$

Very big brain*

SCS

*or brute-force on small instance

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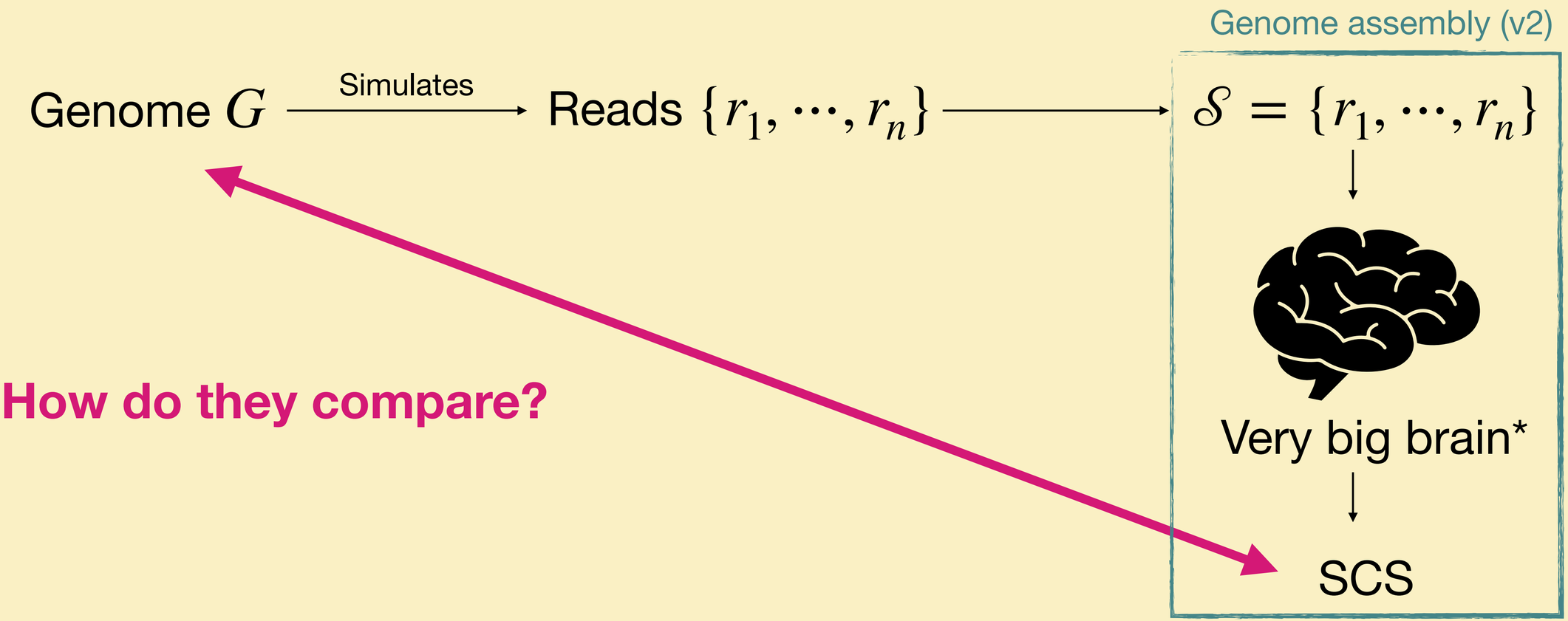

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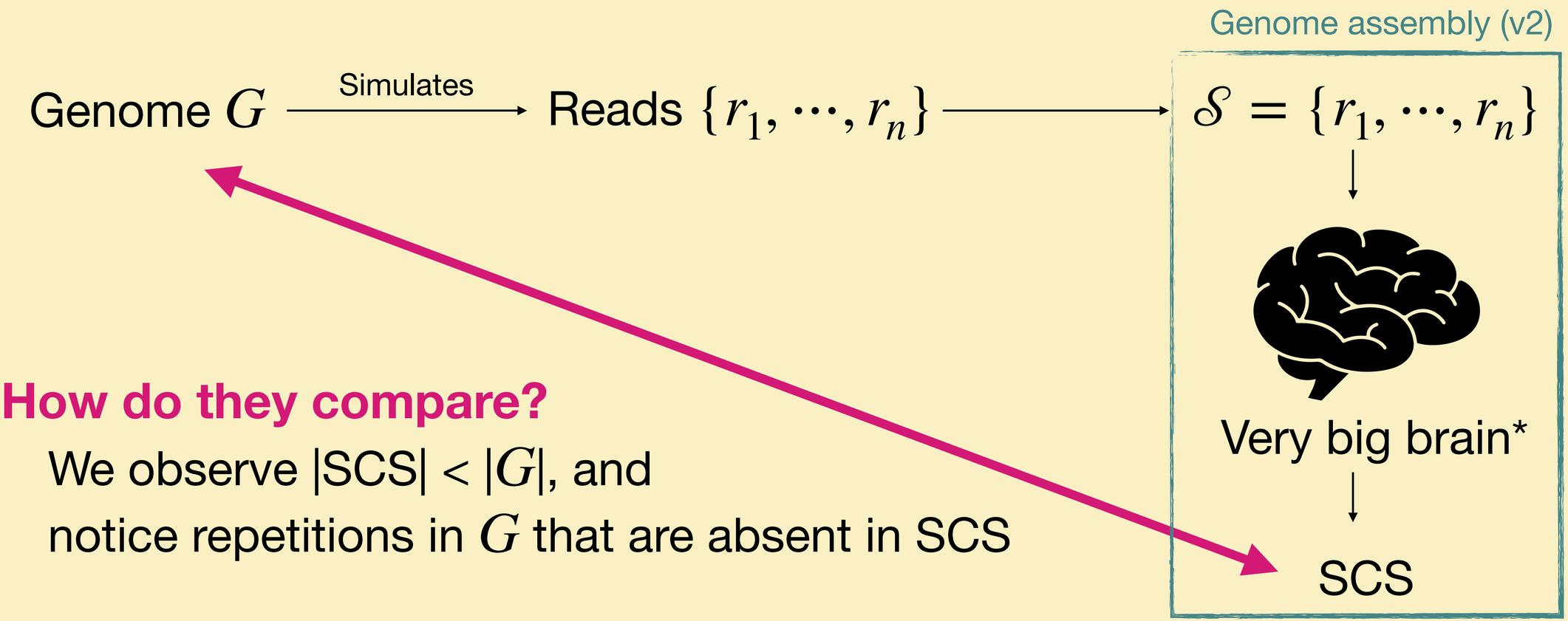

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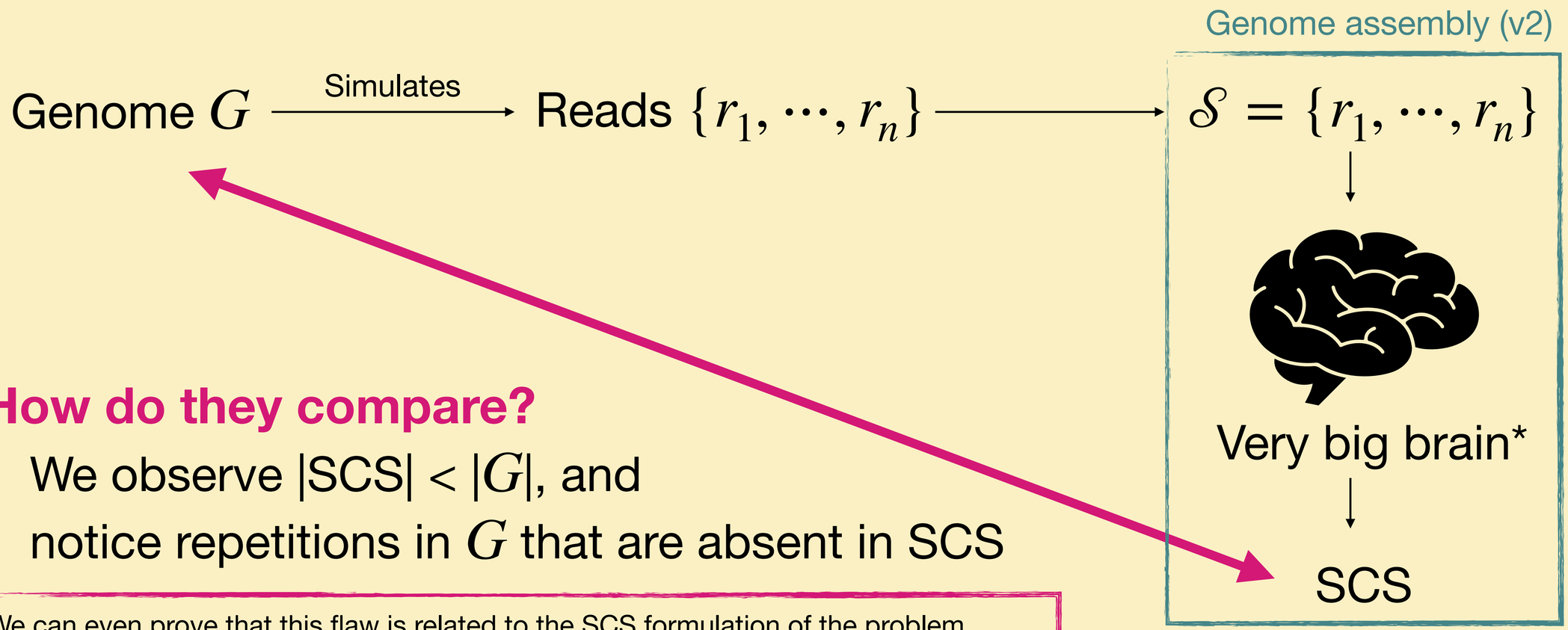

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We can even prove that this flaw is related to the SCS formulation of the problem
==> Even if we were able to solve GAv2, we won't solve the original biological question.

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Refinements and reformulations of the problem

SCS introduces non-existing substrings

$$G_{true} = AATTCCAGCTGATTCCAGT$$

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Caution. It's possible that a k-mer of the "underlying genome" is not spanned by any read, just because of the fragmentation

=> for well-chosen k (given $|genome|$ and n), this can be made very unlikely

Changing perspective: graphs

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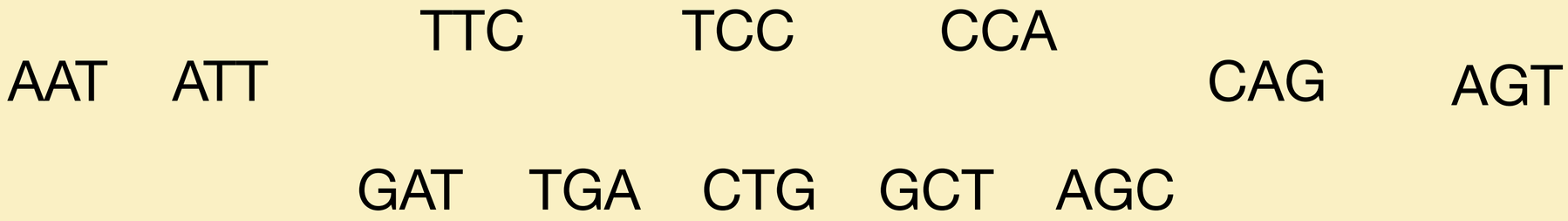

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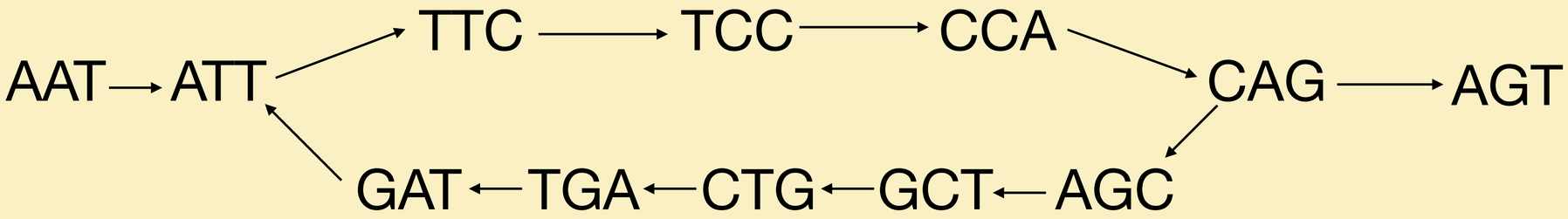

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Looks NP-hard...

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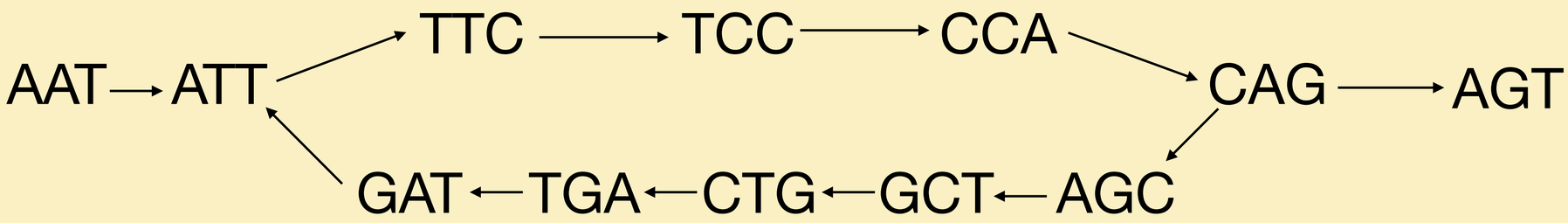

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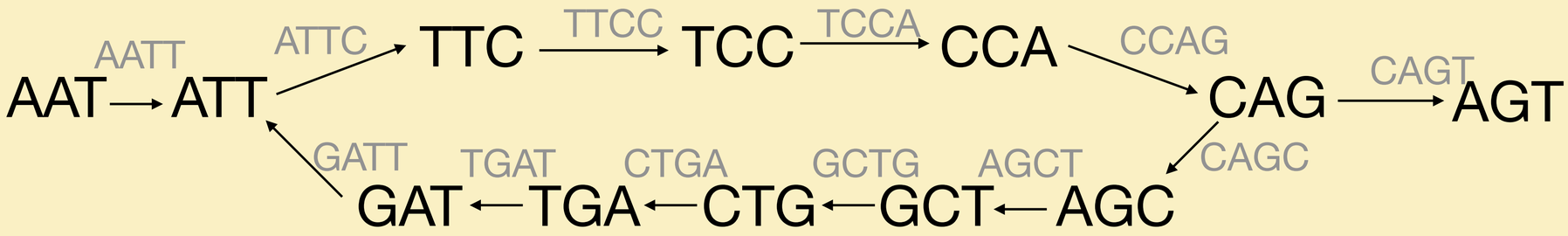

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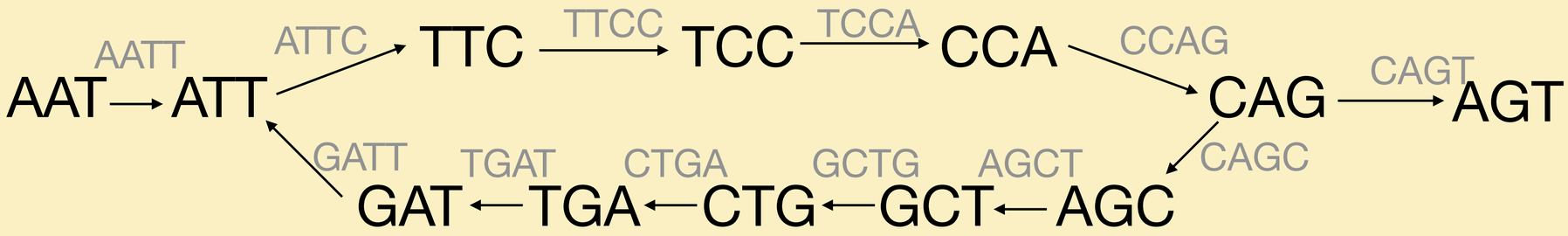

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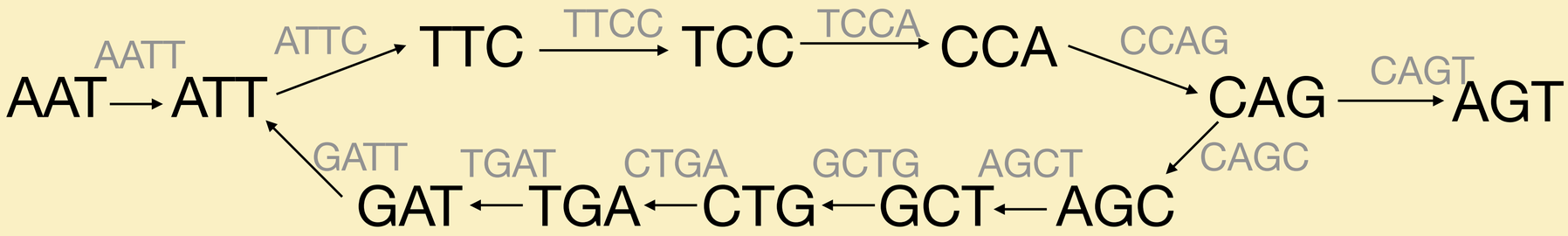

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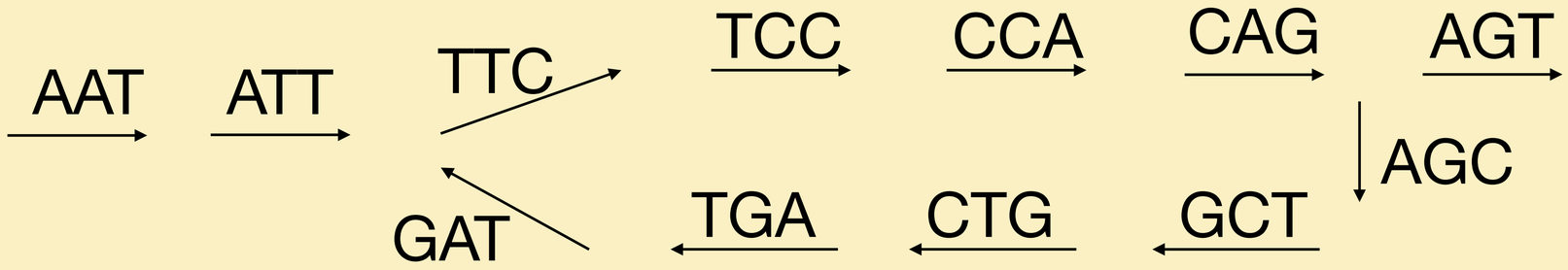

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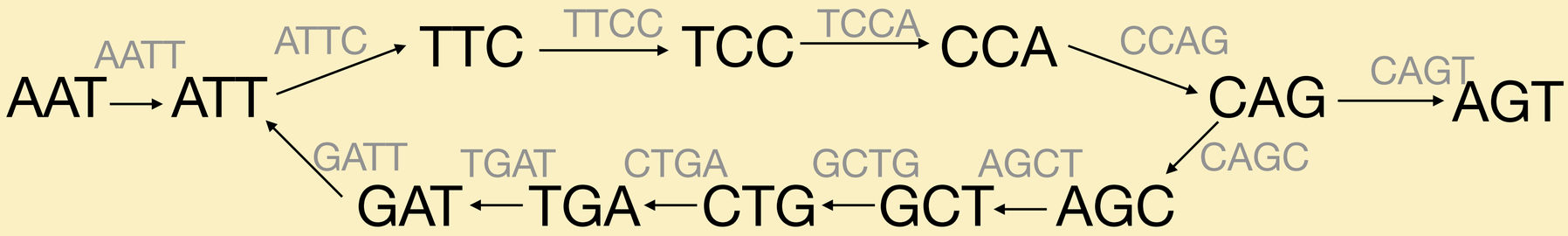

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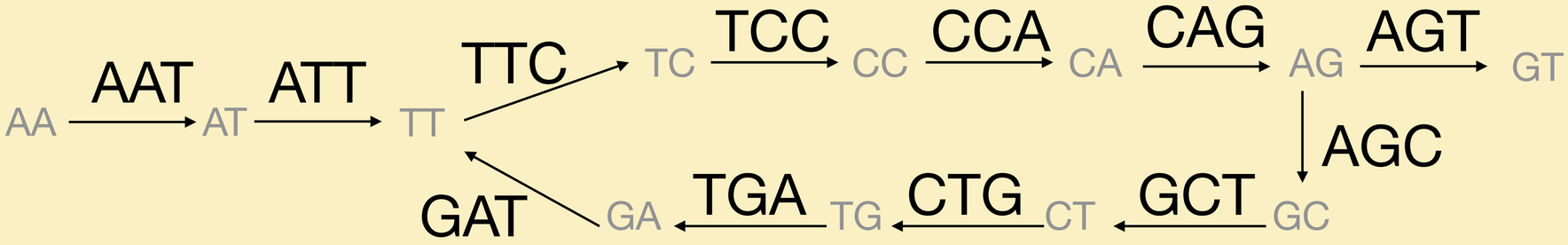

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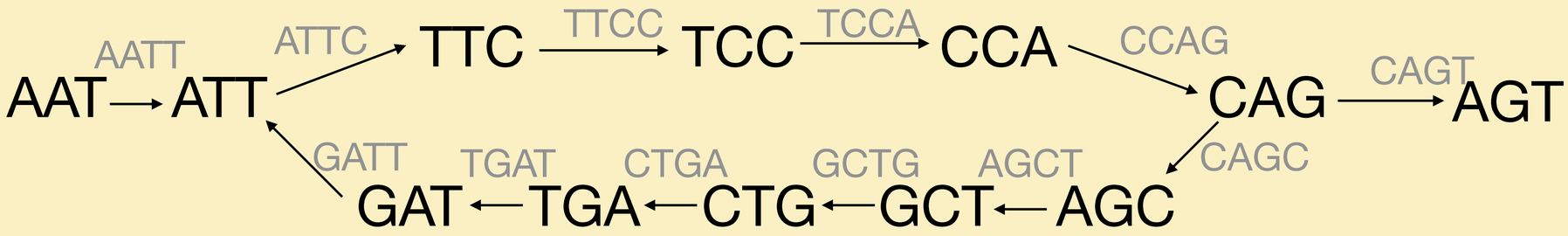

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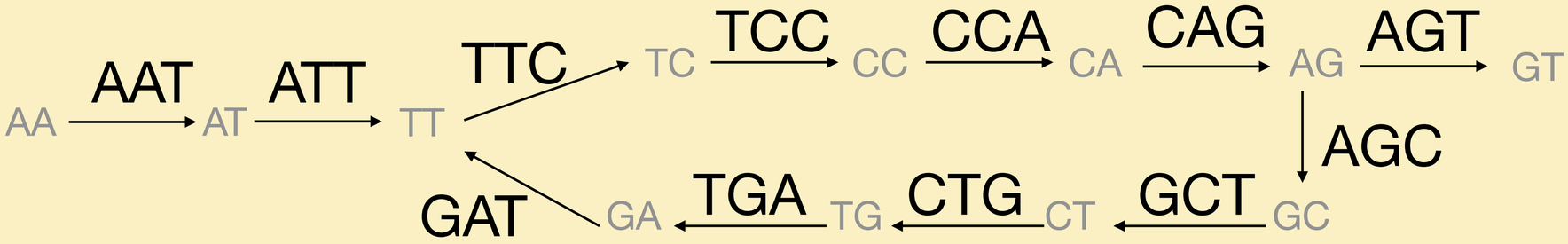

Definition (Genome assembly, v6).

Tractable!

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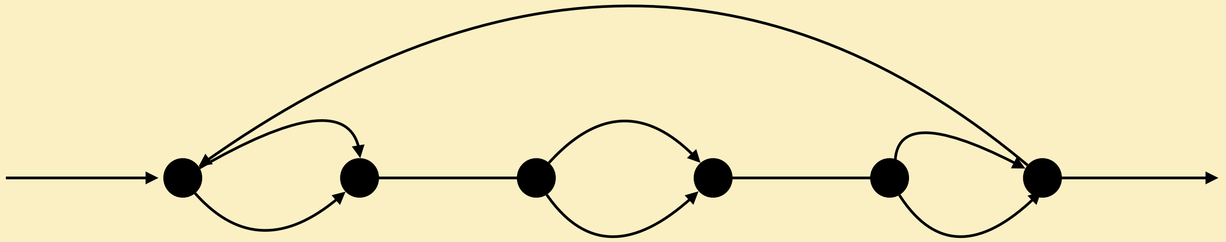

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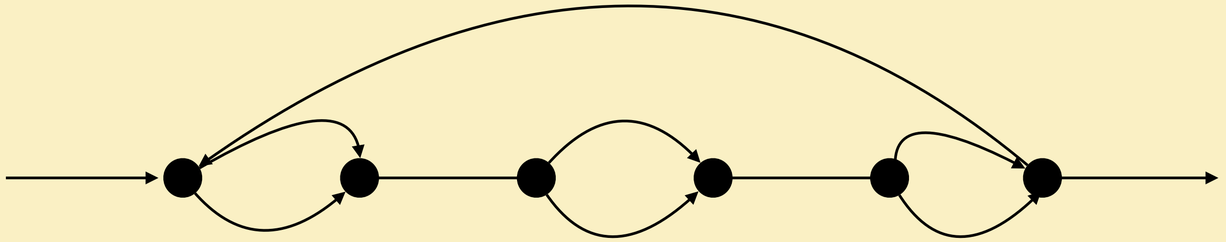

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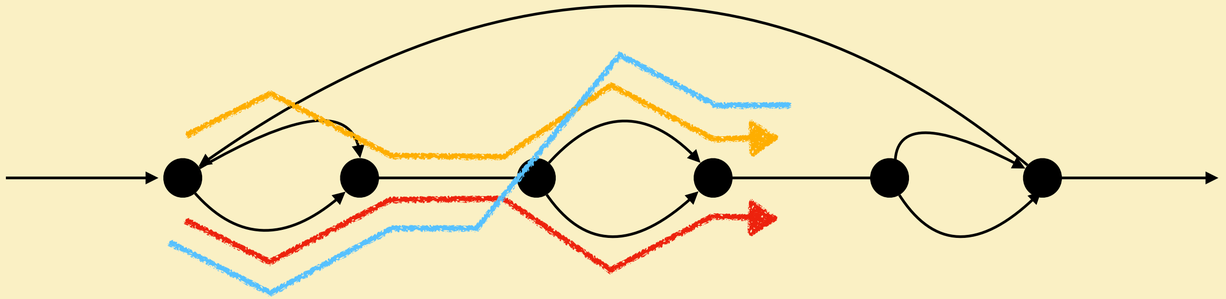

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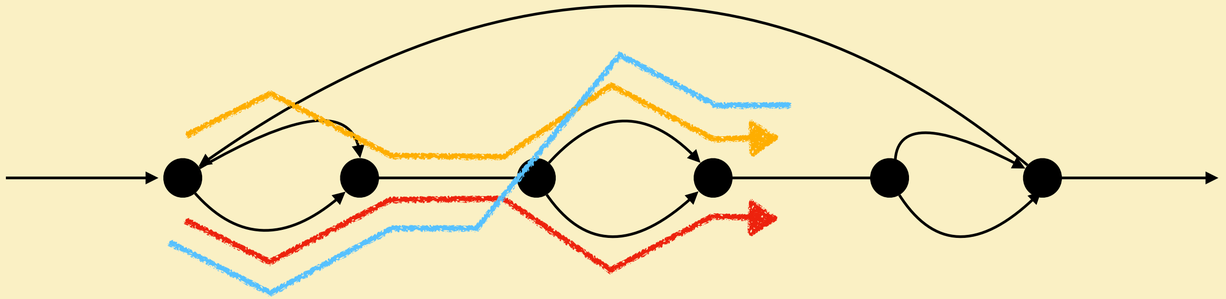

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No matter what we do, if the genome is repetitive, we cannot recover it!

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Morally, these are strings that appear as substring in any genome reconstruction based on the dBG

A last word on "usefulness"

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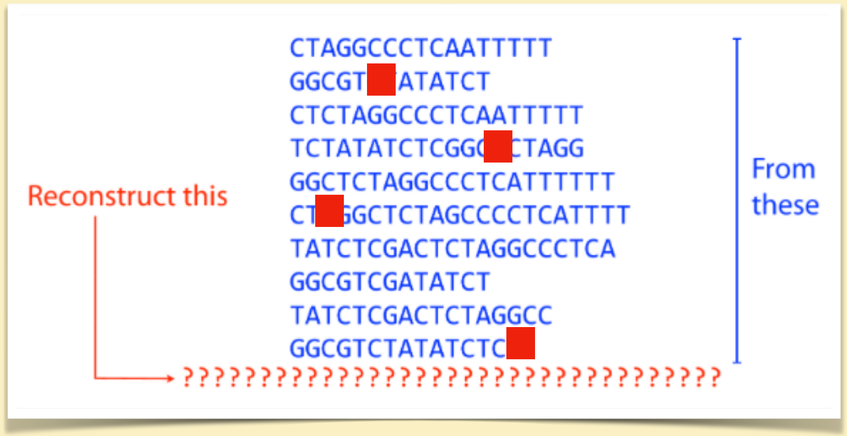

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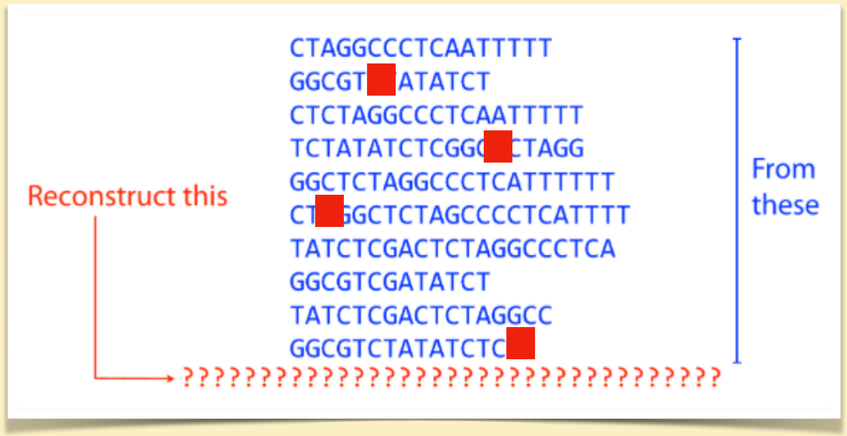
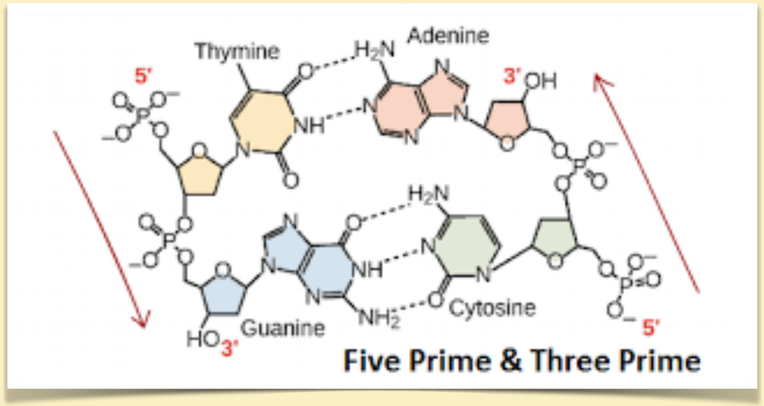


All reads are 5' to 3'



*bidirectional model

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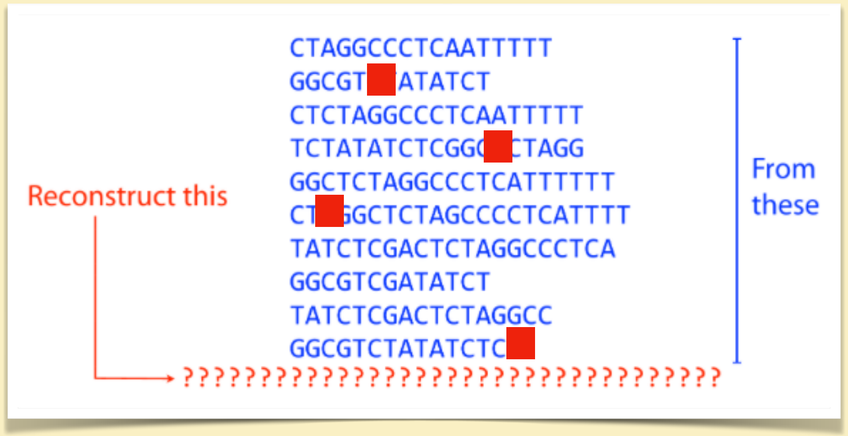

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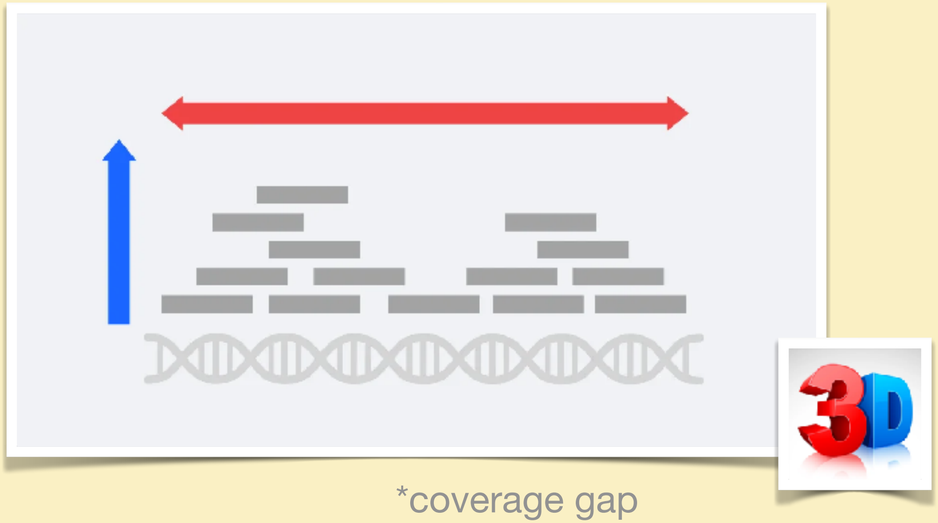

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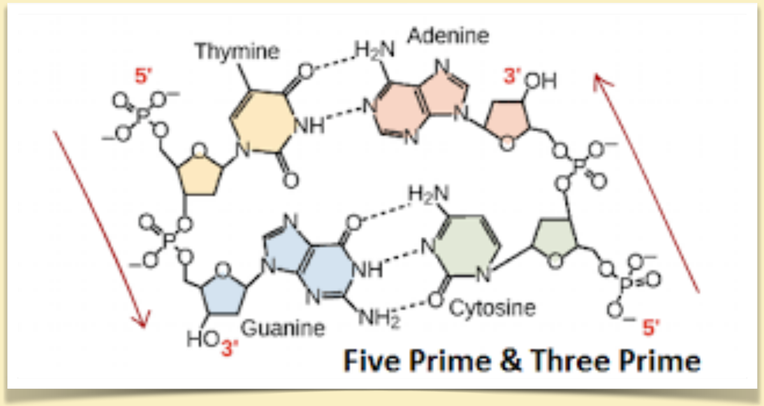

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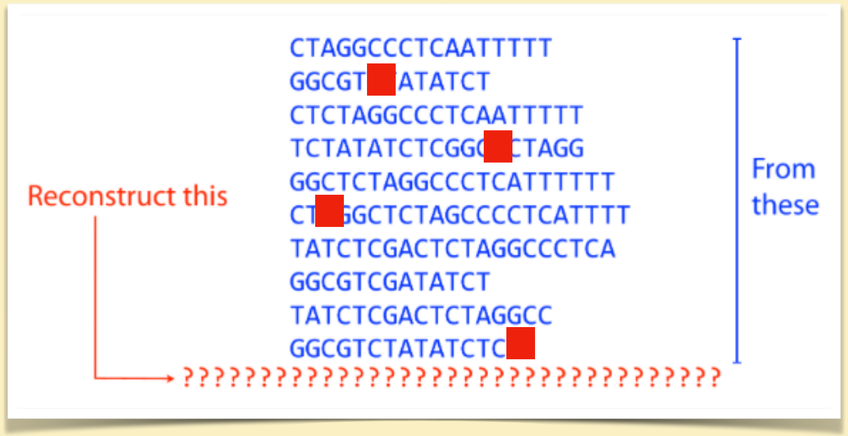

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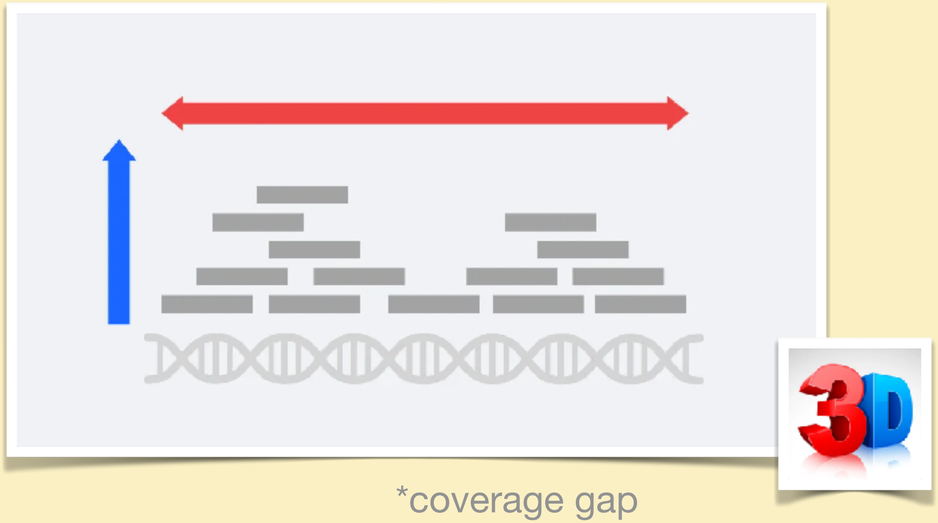

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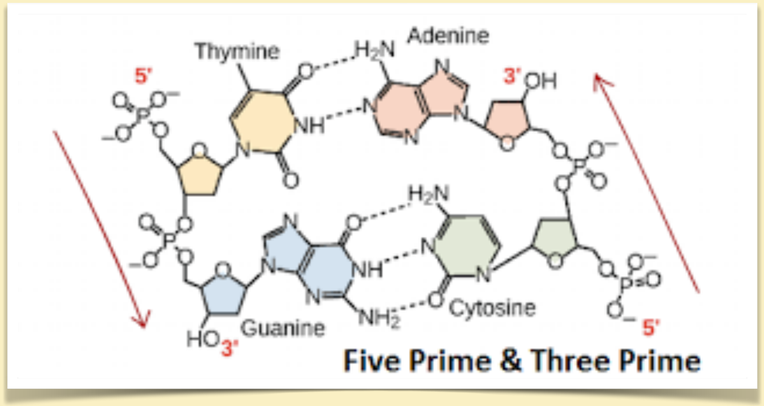

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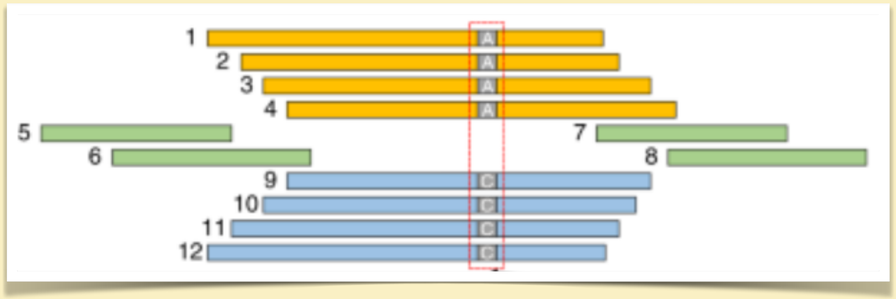
Single haploids



Origin of flaws

Motto. **The ultimate test of a (practical) algorithm is how is performs on real data!**

What if it doesn't work?

Origin of flaws

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What if it doesn't work?

Finding the origin of flaws.

A. Simulate data under algorithm's assumptions
B. Simulate biological data to the best we understand it
C. Use real data

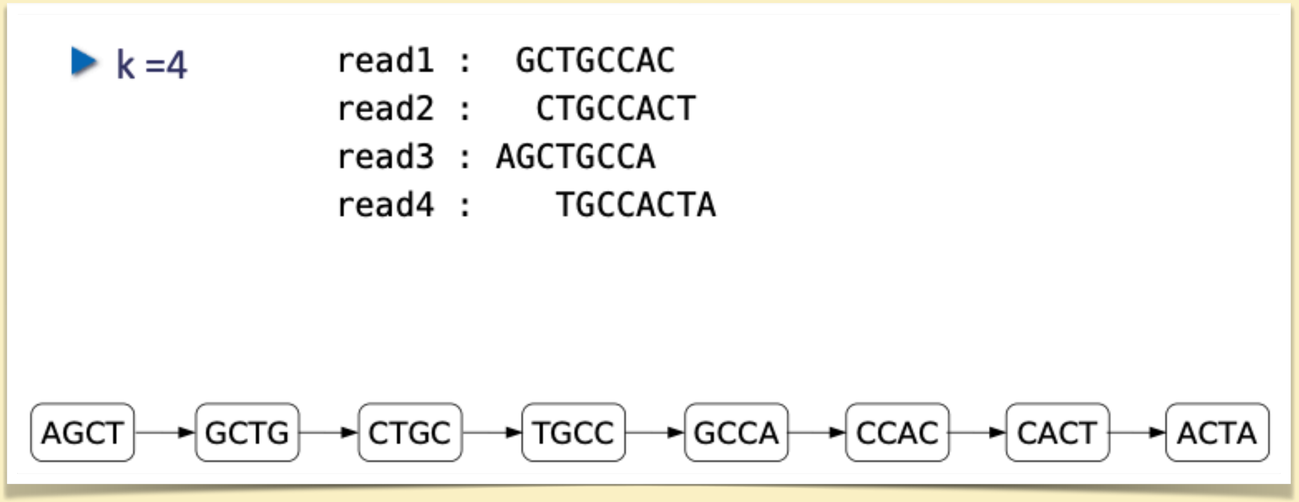
Part II

Solving GAv7 (DNA consensus regions)

De Bruijn graphs

The *de Bruijn* graph

Historical context. Coined in 1946, used in bioinformatics in 1995.



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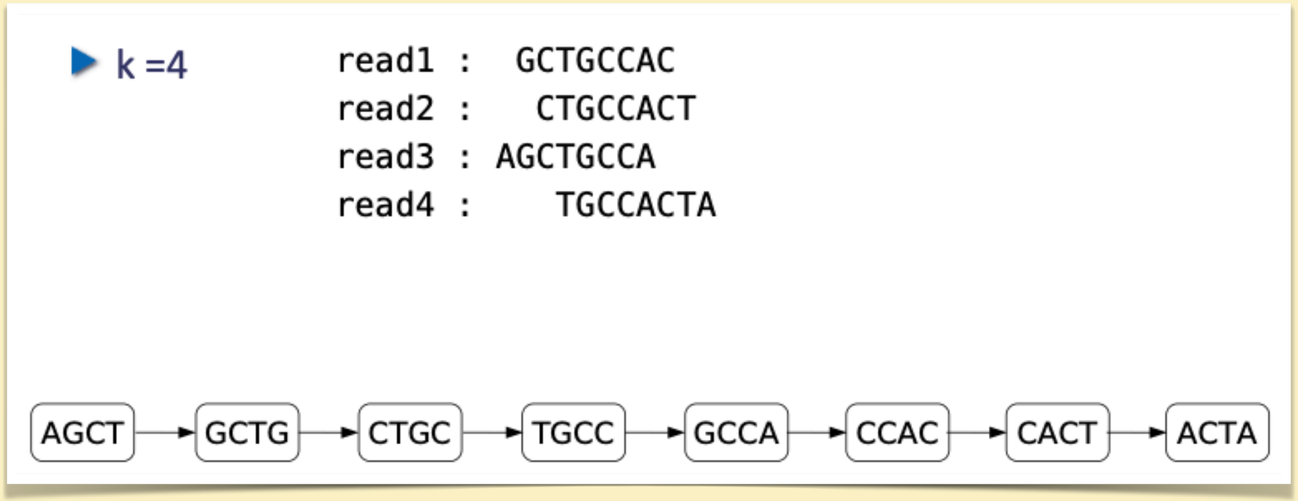


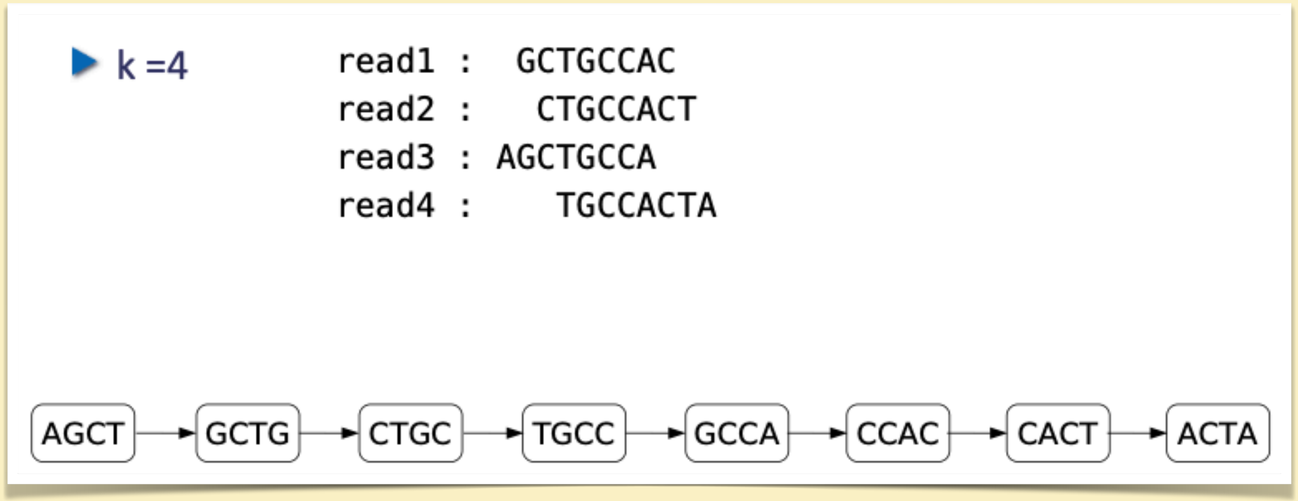
*finding unitigs is easy (compared to HP) -> stick to node-centric

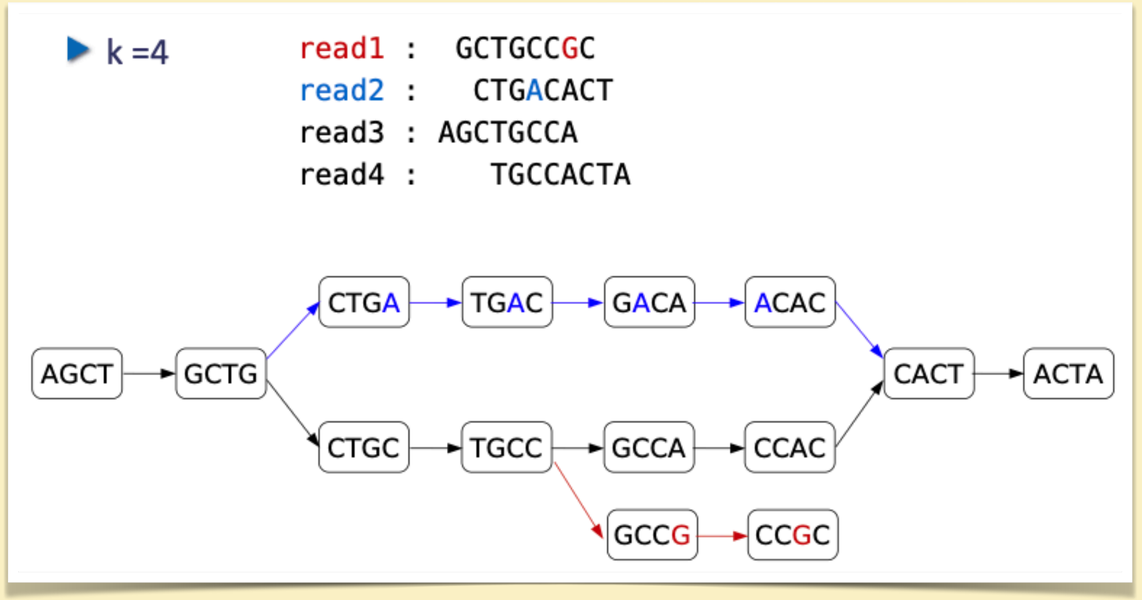
Benefits.

- The graph can be computed efficiently (exact and fixed-length overlaps)
- Roughly $\mathcal{O}(|genome|)$ nodes, doesn't depend on the read coverage

Is there any drawbacks?

Sequencing errors in the *de Bruijn* graph





=> Even single nucleotide errors have great impact on the topology

Up to k new vertices, new branching paths

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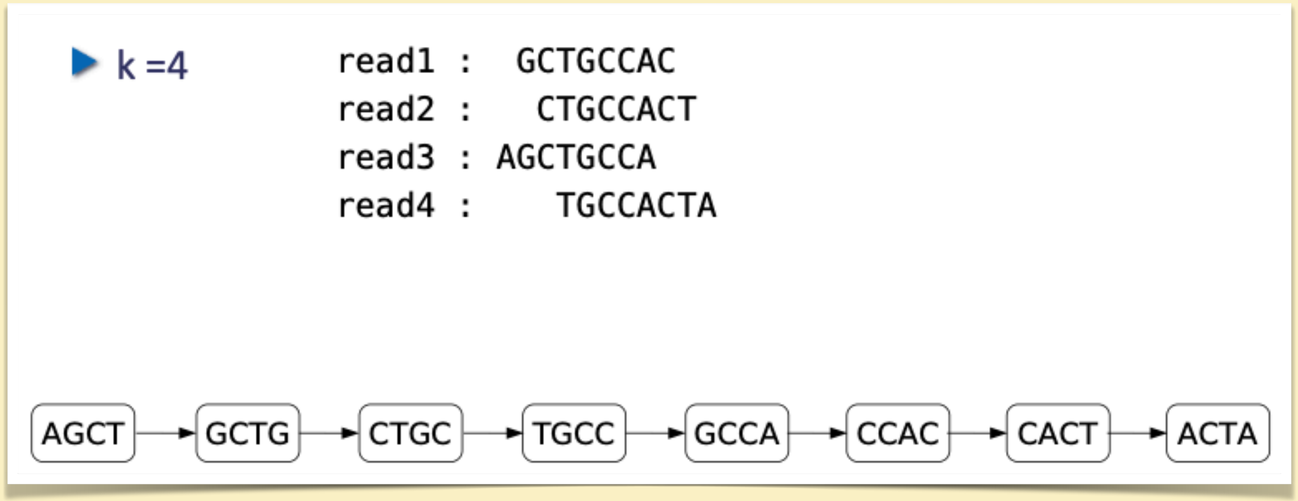

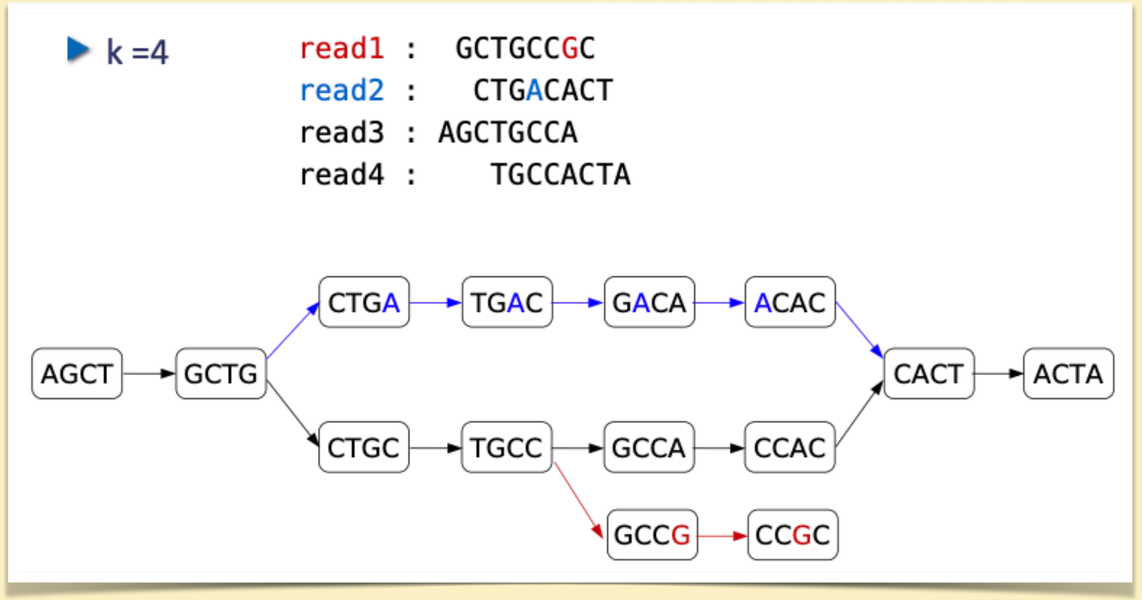

=> Even single nucleotide errors have great impact on the topology

Up to k new vertices, new branching paths

=> Challenging task: clean the graph WITHOUT losing SNPs (biological single nucleotide variants)

Topology (candidate error zones), k-mer abundance (decide if error)

The choice of k in the *de Bruijn* graph

As k grows.

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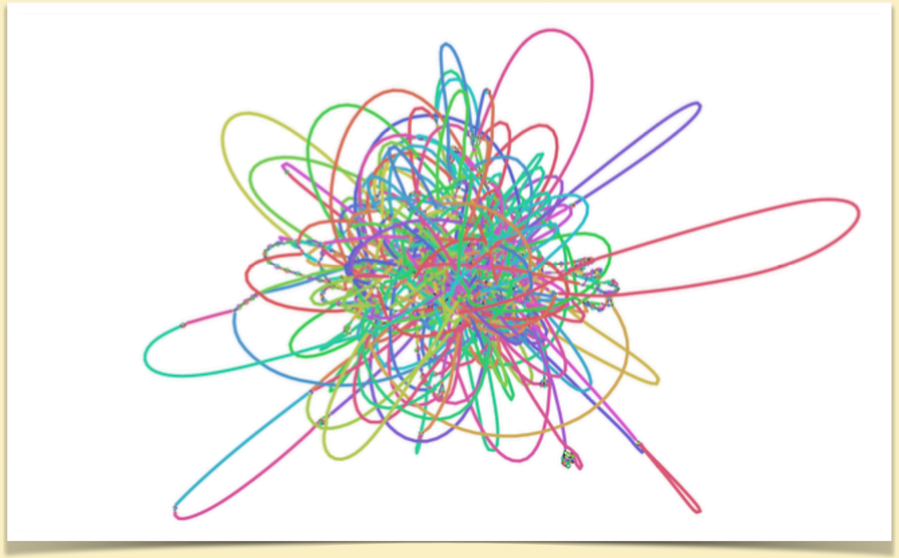

E. Coli, k=31, main component

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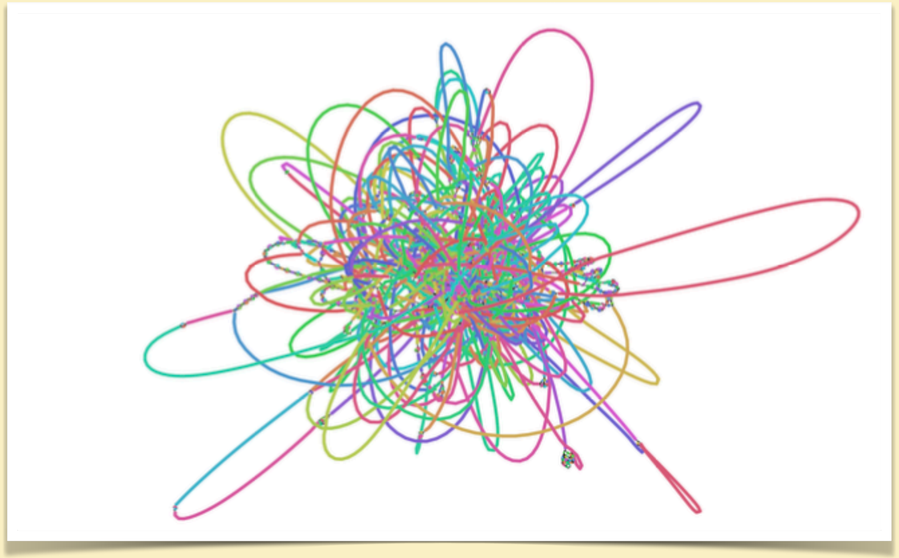

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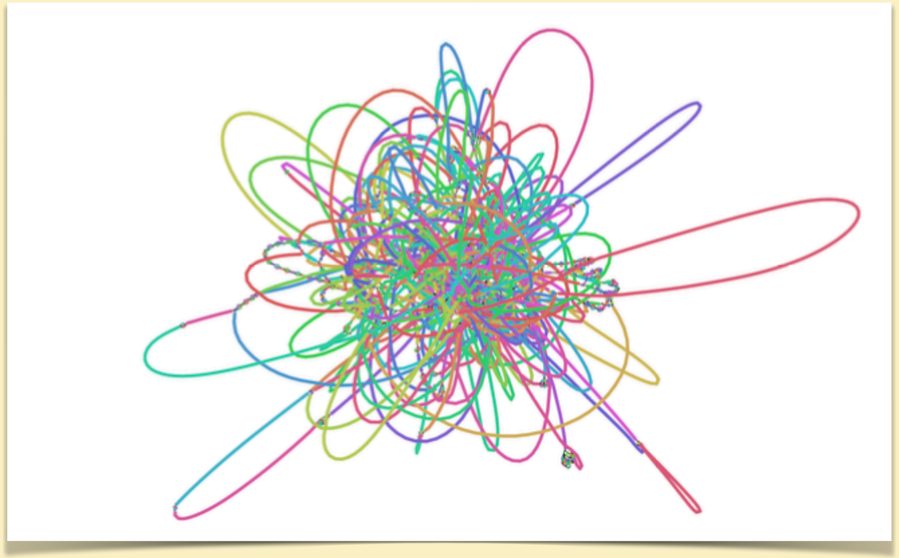

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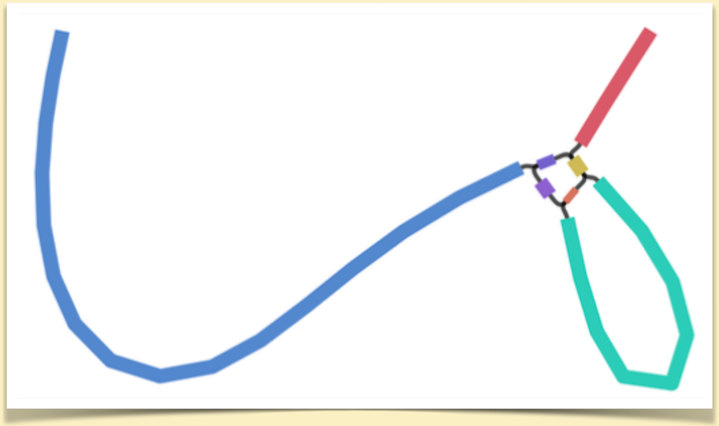


E. Coli, k=2000, main component

Storage requirements

Storing vertices. $\log_2(4) = 2$ bits per k-mer, hence $\approx 2k \cdot |genome|$ bits in total

Storing edges. at least $\log_2(2k \cdot |genome|)$ bits per pointer,
hence $\approx \log_2(2k \cdot |genome|) \cdot 4 \cdot 2k|genome|$ bits in total

Huge!

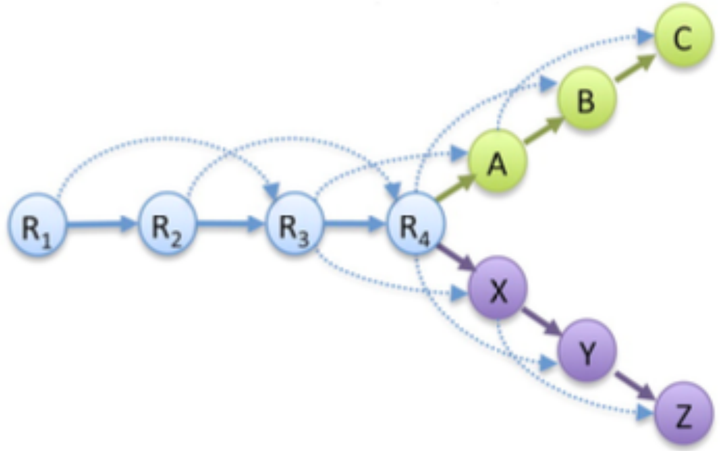
In practice, edges are not stored but computed on the fly (eg. Bloom filter, see later.)

Overlap graphs (overlap-layout-consensus)

A (slightly) different approach

Definition. The **overlap graph** is the graph whose vertices are the reads, and the edges connect any overlapping vertices

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



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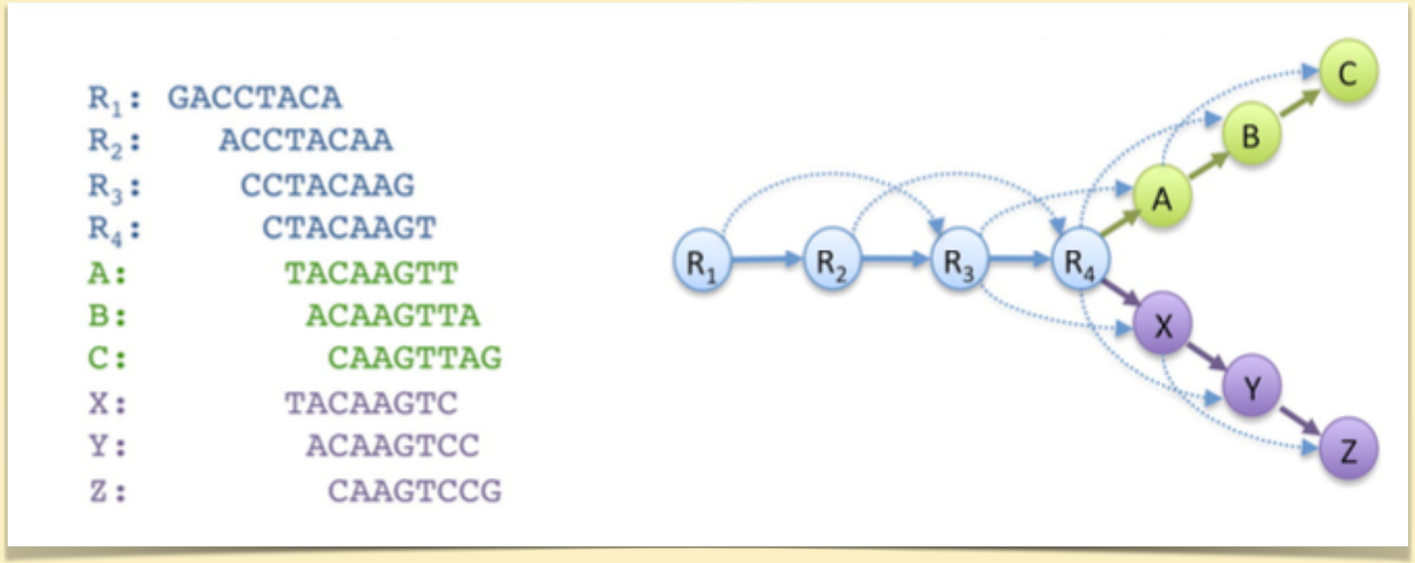
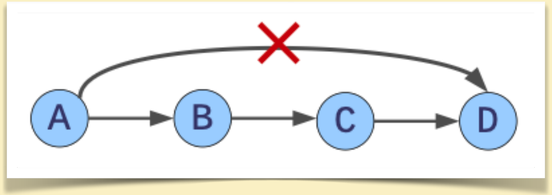


Note. Edges that can be deduced from transitivity are often removed



Vertices (reads) included in others are also removed

Approximate overlap is often used

eg. 2 errors overlap

```
ATTCCTCATCT
||  ||
ACAGCTATTCT
```

More on the overlap graph

Prop. Let N be the number of reads. The graph has N vertices and $\mathcal{O}(N^2)$ edges.

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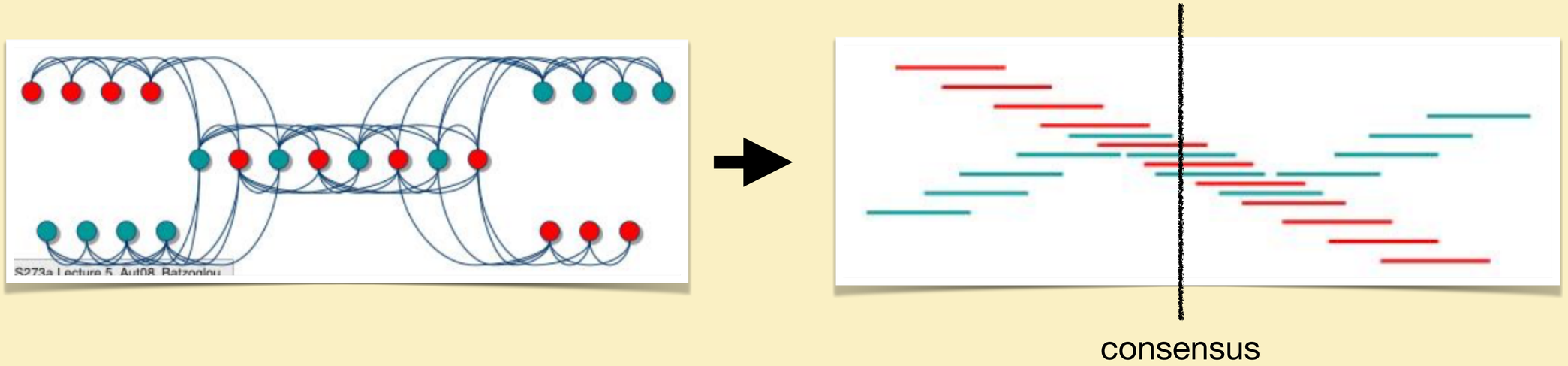
Correcting reads in the approximate overlap setting.



Part III (next time)

Real-world genome assembly

Homework.

A. For each of the $\{\text{short}, \text{long}\} \times \{\text{high quality}, \text{low quality}\}$ types of reads, what method (dBG/OG) is better suited? Why?

B. Propose (on paper) a complete assembly pipeline, that includes graph-cleaning steps. Make design assumptions explicit and expected flaws as clear as possible. Estimate the space/time complexity of each step.