introduction to (de novo) assembly

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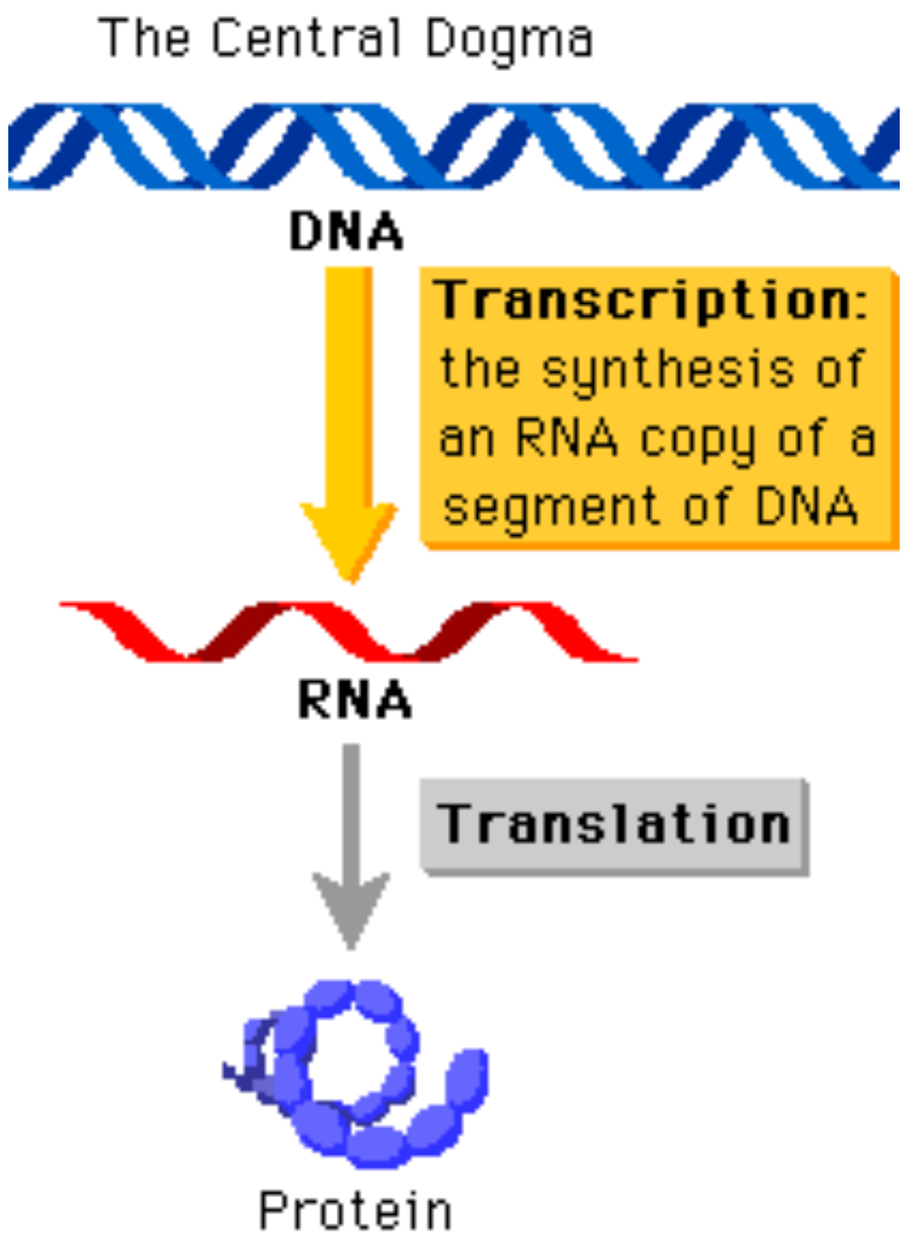
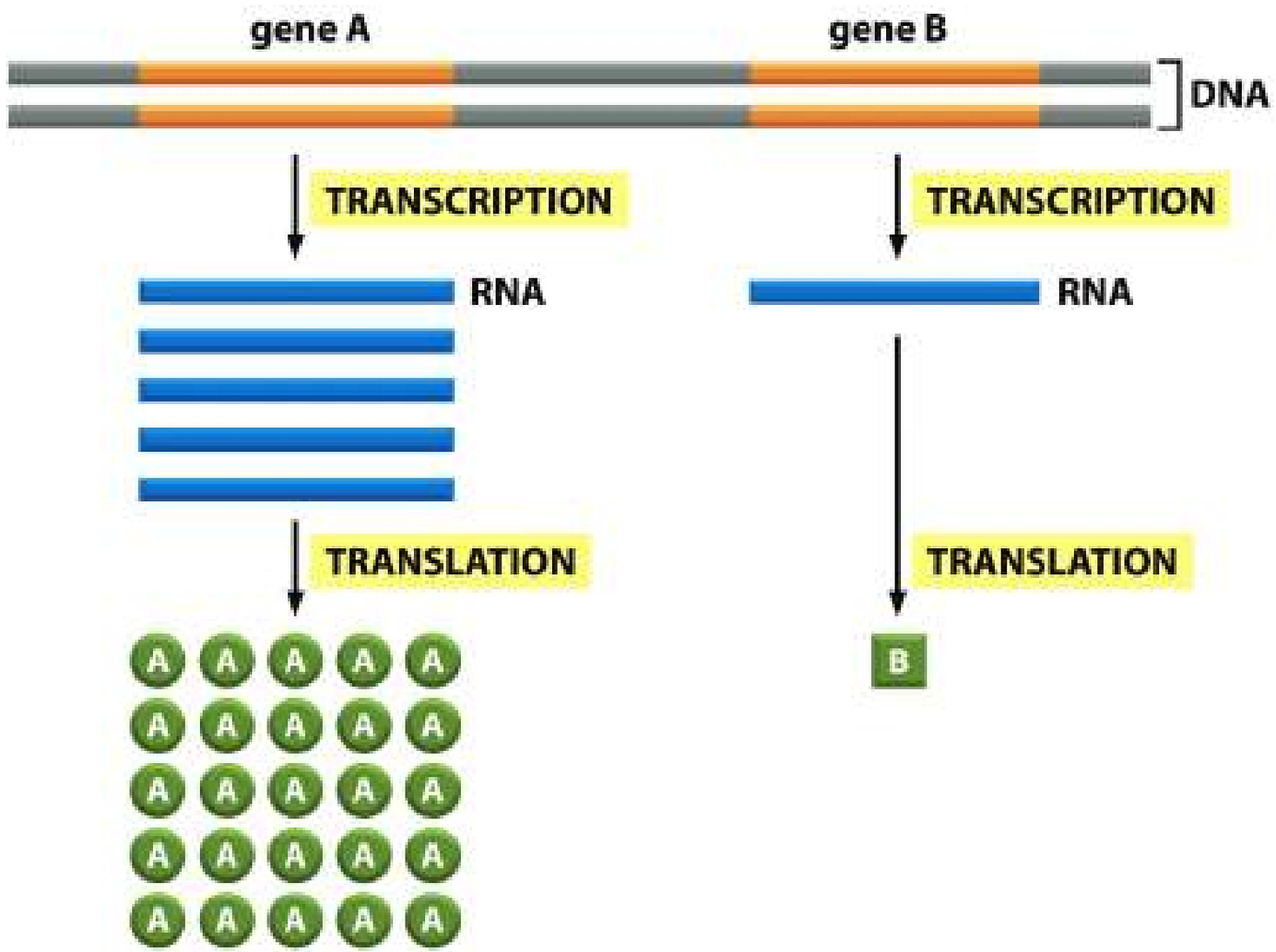
The central dogma of molecular biology

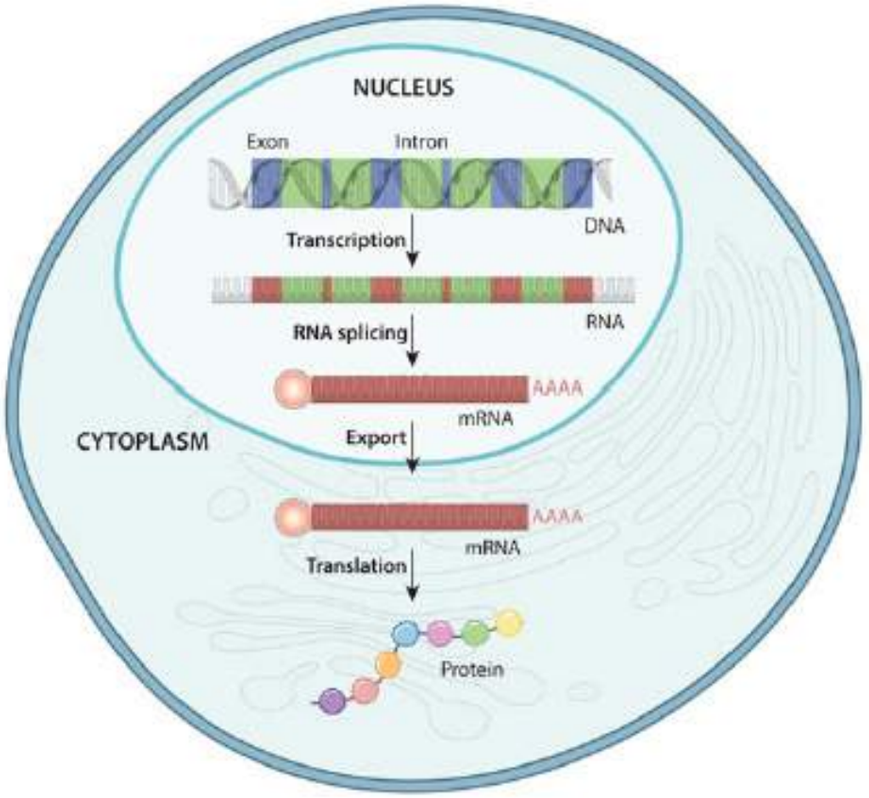


Gene Expression

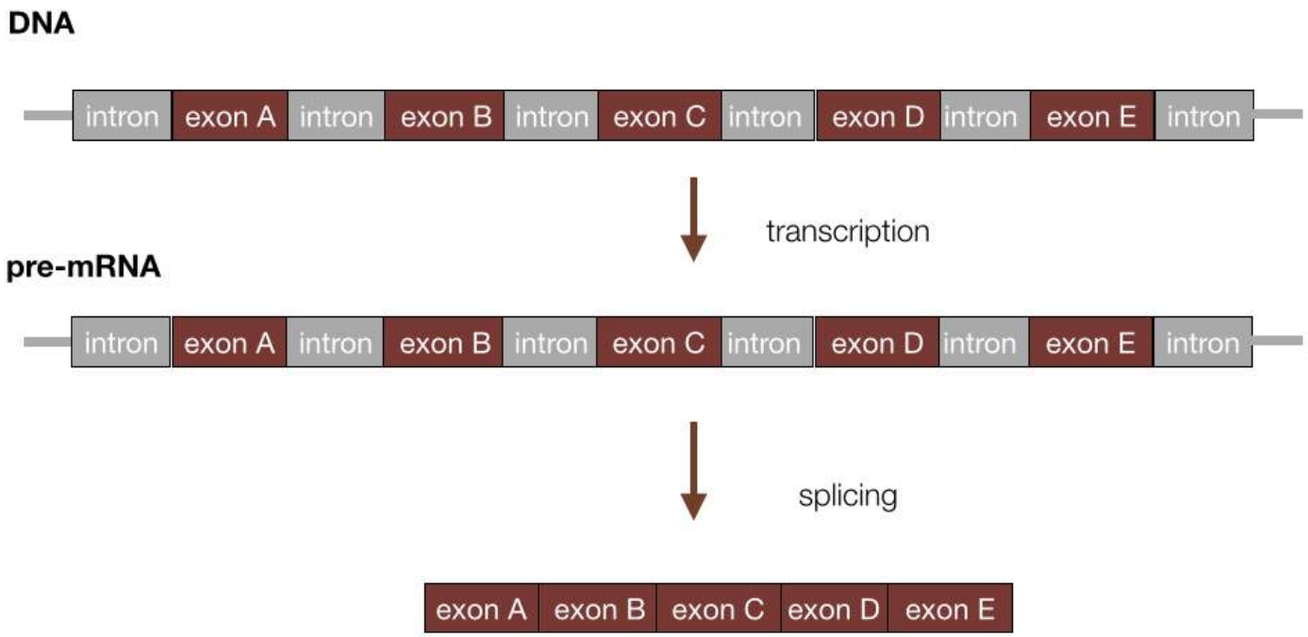


From DNA to RNA to proteins in eucaryotes

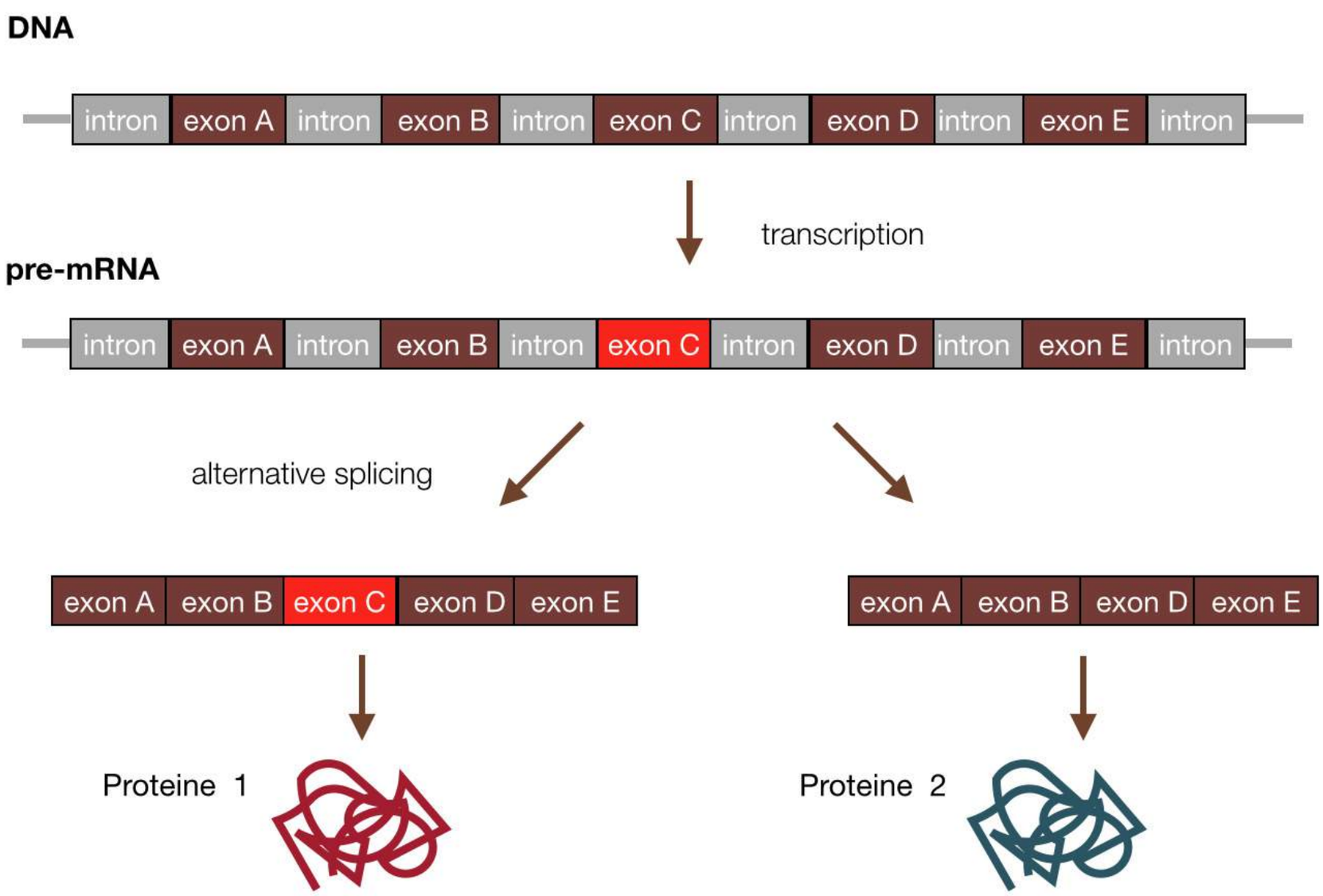
From DNA to proteins in eucaryotes



RNA-splicing in eucaryotes



Alternative splicing (AS) in RNA



Why sequencing?

Perfect Word

+

AACGGTAACTGAAC
GTACGTACTACGTA
CGTACCATGAACGG
TAACTGAACGTACG
TACTACGTACGTAC
CATGAACGGTAACT
GAACGTACGTACTA
CGTACGTACCATG

Reality

- Cannot sequence full length DNA
- But we can sequence short fragments of it

DNA sequencing

1

2

3

4

5

DNA sequencing


The New York Times

MEN WALK ON MOON

ASTRONAUTS LAND ON PLAIN; COLLECT ROCKS, PLANT FLAG

DNA sequencing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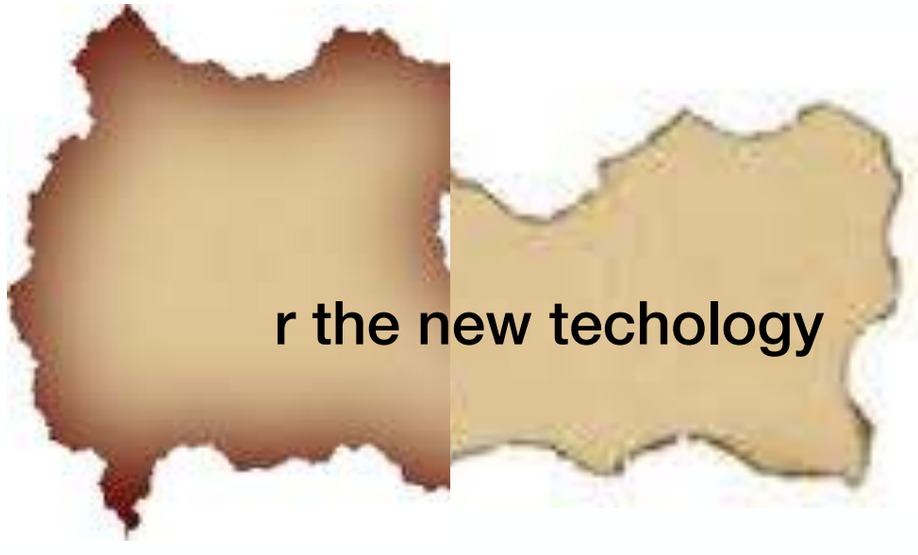


Billions of short reads

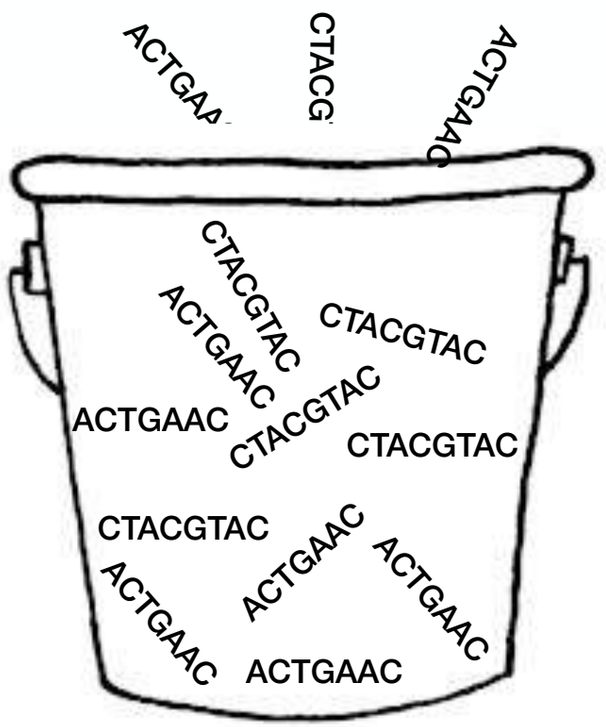
AACGGTAACTGAACGTACGTACTACGTACGTACCATG

AACGGTAACTGAACGTACGTACTACGTACGTACCATG

Billions of short reads

AACGGTAACTGAACGTACGTACTACGTACGTACCATG…..

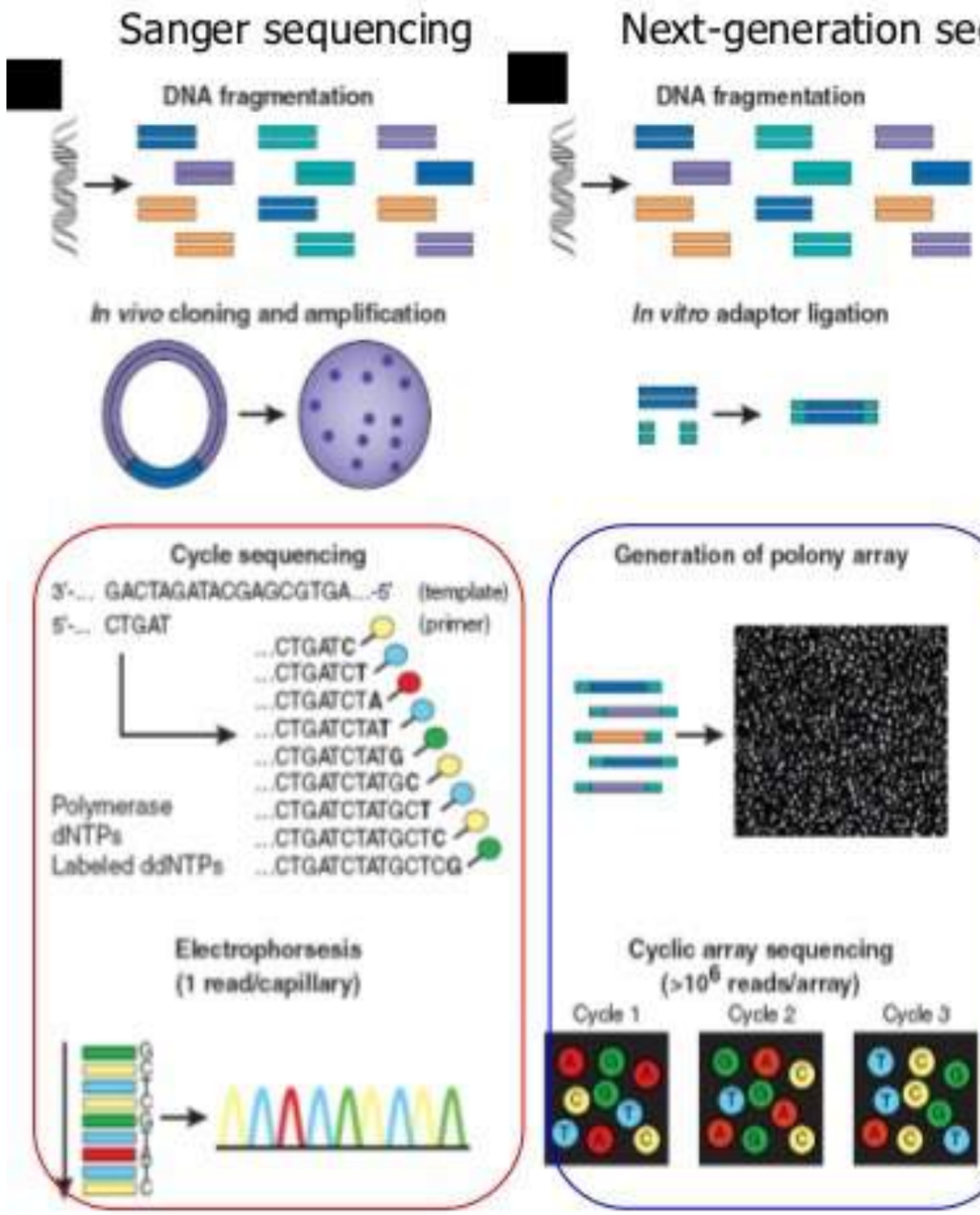
AACGGTAACTGAACGTACGTACTACGTACGTACCATG…..



AACGGTAACTGAACGTACGTACTACGTACGTACCATG

?

Next Generation Sequencing



Advantages:

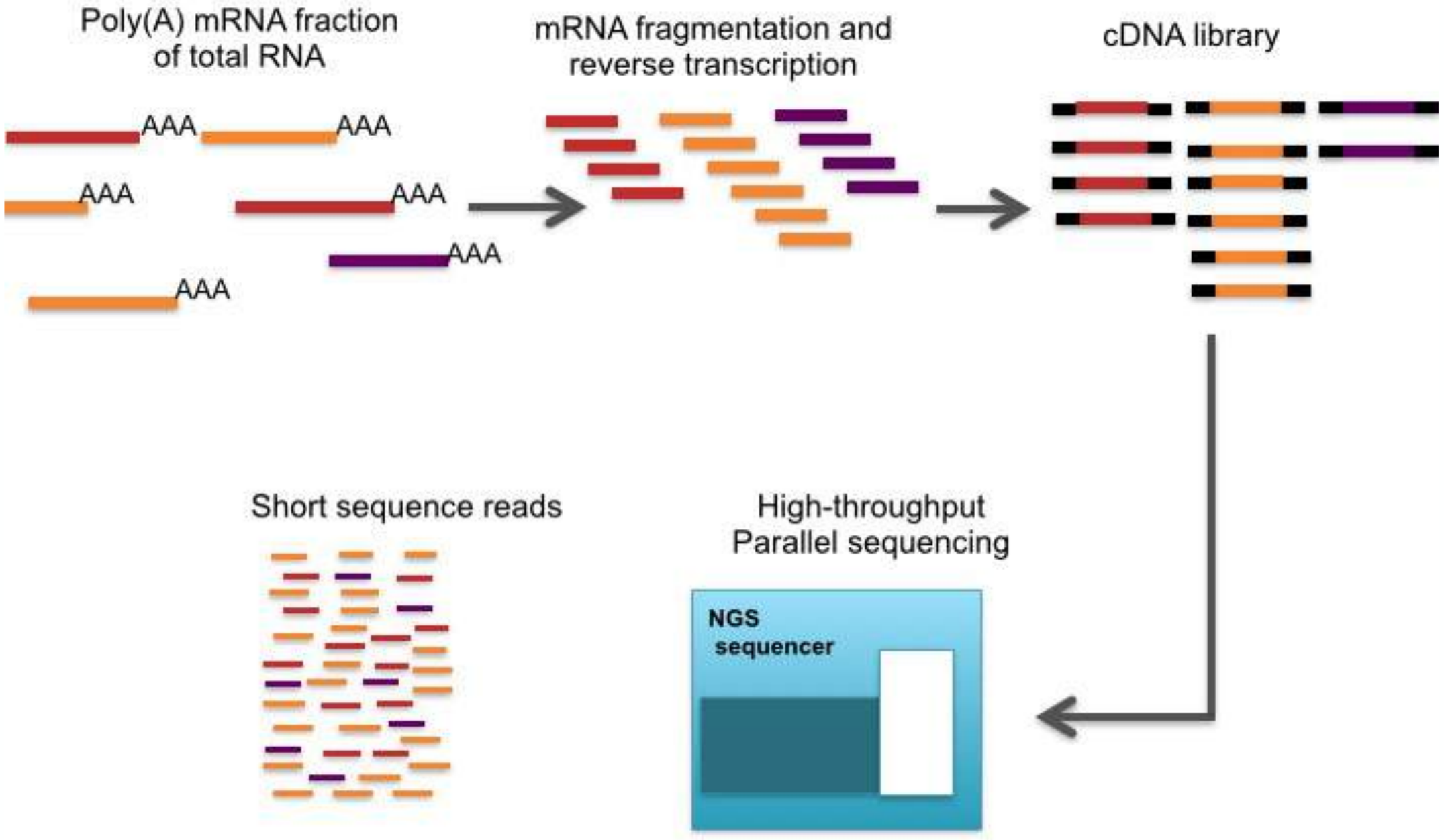
- Construction of a sequencing library → clonal amplification to generate sequencing features

✓ No in vivo cloning, transformation, colony picking...

- Array-based sequencing

✓ Higher degree of parallelism than capillary-based sequencing

RNA-seq

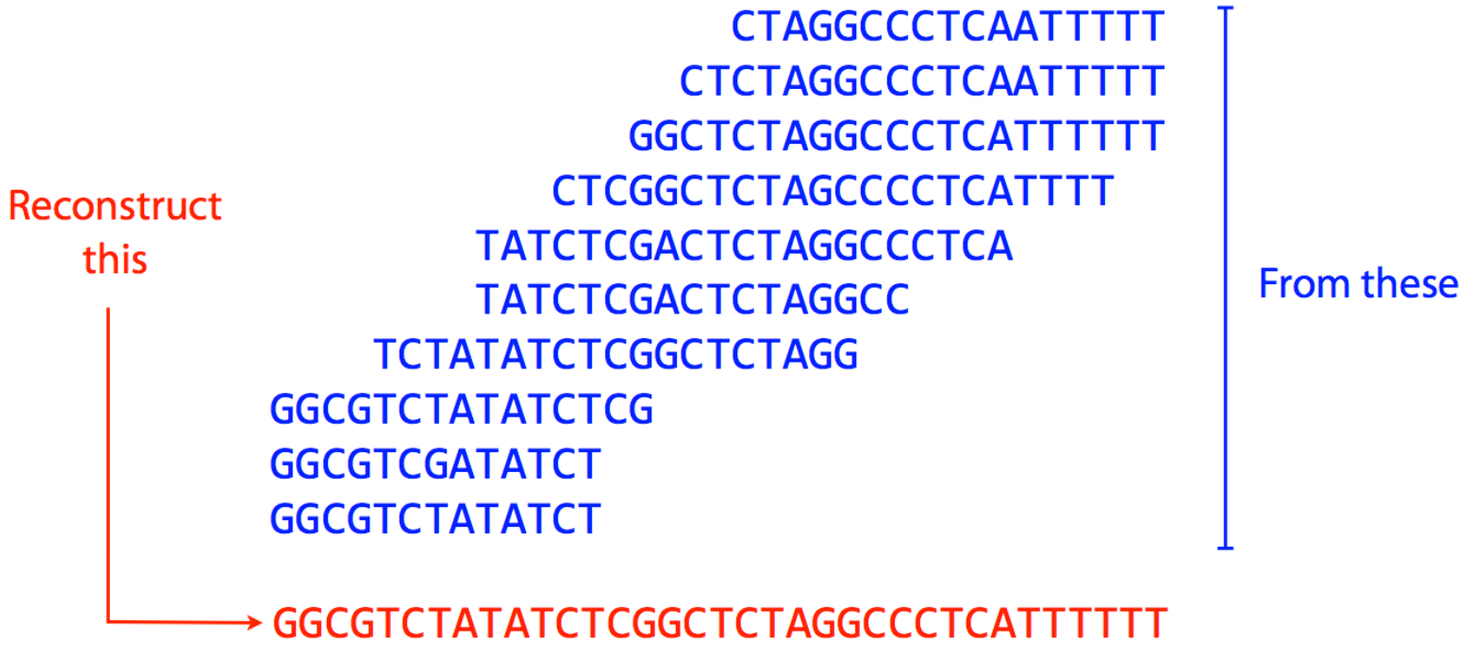

Poly(A) mRNA fraction
of total RNA
AAA
AAA
AAA
AAA
AAA
mRNA fragmentation and
reverse transcription
cDNA library
Short sequence reads
High-throughput
Parallel sequencing
NGS
sequencer

Why RNA-sequencing?

- Which region of the genome is transcribed
- Variability (AS events) of the mRNAs of the same gene
- Expression level of the mRNAs

coverage

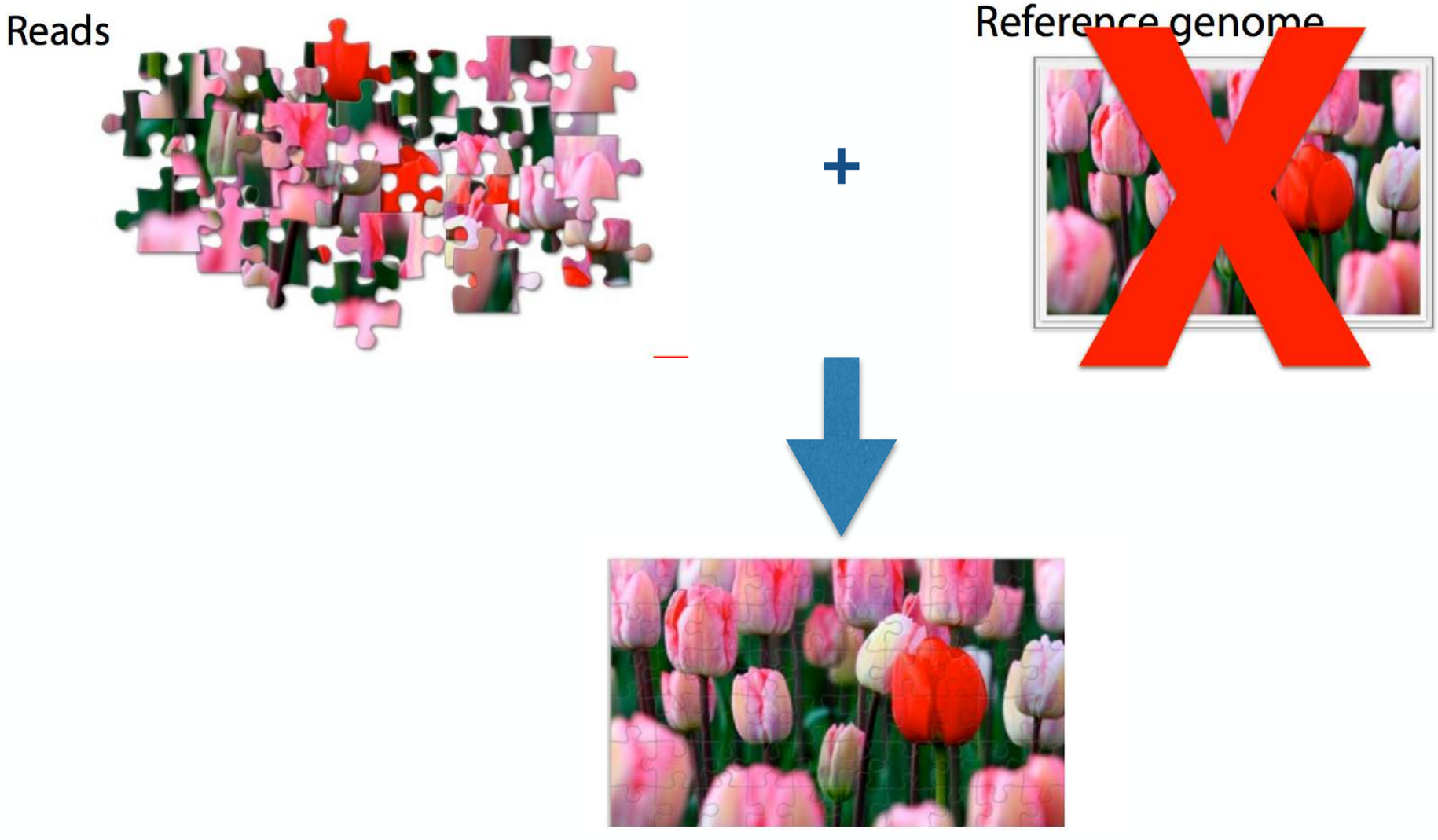
- All genome positions are **covered** many times.
- The **coverage** is the number of reads covering a fixed position.



Some terminology

- **read** - a 100-250 long word that comes out of a NGS machine
- **coverage** - the average number of reads (or inserts) that cover a position in the target DNA piece
- **shotgun sequencing** - the process of obtaining many reads from random locations in DNA, to detect overlaps and assemble
- **mate pair** - a pair of reads from two ends of the same insert fragment (we know approx. distance)
- **contig** - a contiguous sequence formed by several overlapping reads with no gaps
- **consensus sequence** - sequence derived from the multiple alignment of reads in a contig

de novo assembly vs referenced-based mapping



de novo assembly

Methods

Different approaches

- greedy assembly
- Overlap-layout-consensus
- de bruijn graphs
- strings graphs

Basic idea

- Find all overlaps between reads
- Build a graph
- Simplify the graph (sequencing errors)
- Traverse a graph to produce a consensus.

Modelling and assembling NGS data (I)

- Given a set of strings $\mathcal{R} \subset \Sigma^* = \{A, C, T, G\}^*$ each $r \in \mathcal{R}$

  is part of an unknown string $S \in \Sigma^*$ reconstruct the original

  string $S \in \Sigma^*$

Modelling and assembling NGS data (I)

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- **Shortest superstring problem (SSP)**
  - Given a set of strings $r \in \mathcal{R}$ find a **minimum** length string $S \in \Sigma^*$ such that every $r \in \mathcal{R}$ is a substring of S

Shortest Superstring Problem

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Exercise 1

- SSP is NP-hard. (Garey and Johnson 1979)

SSP: Greedy Algorithm (I)

Algorithm

- **Overlap** *ov(s; t)* of two strings *s* , *t* is the longest string *y* such, that *s = xy* and *t = yz* for some non-empty *x* , *z* .

- **Prefix** *pr(s; t)* of *s* w.r.t. *t* be the string *x* in the previous definition.

- *s = pr(s; t)ov(s; t)*. Notice that *pr(s; t)t* is the shortest string containing *s* and *t* in that order. This string is usually called a merge of *s* and *t*.

- **Greedy approach:** We pick two strings s_i s_j with largest overlap from *R* (breaking ties arbitrarily) and replace them with their merge. Stop when there is only one string left.

SSP: Greedy Algorithm (II)

Algorithm

- **Greedy approach:** We pick two strings s_i s_j with largest overlap from R (breaking ties arbitrarily) and replace them with their merge. Stop when there is only one string left.

- How good is this algorithm? Can it have an approximation factor better than two?

Approximation algorithm

- A minimization problem is a problem where we want to find a solution with minimum value.
    - An algorithm for a minimization problem is called a ρ-approximation algorithm, for some ρ > 1, if the algorithm produces for any input I a solution whose value is at most ρ·opt(I).
- A maximization problem is a problem where we want to find a solution with maximum value.
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$$\{ab^k, b^k c, b^{k+1}\}$$

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The Greedy Conjecture [Blum et al. 1991]: The Greedy Algorithm has approximation factor 2.

SSP: Greedy Algorithm (II)

Algorithm

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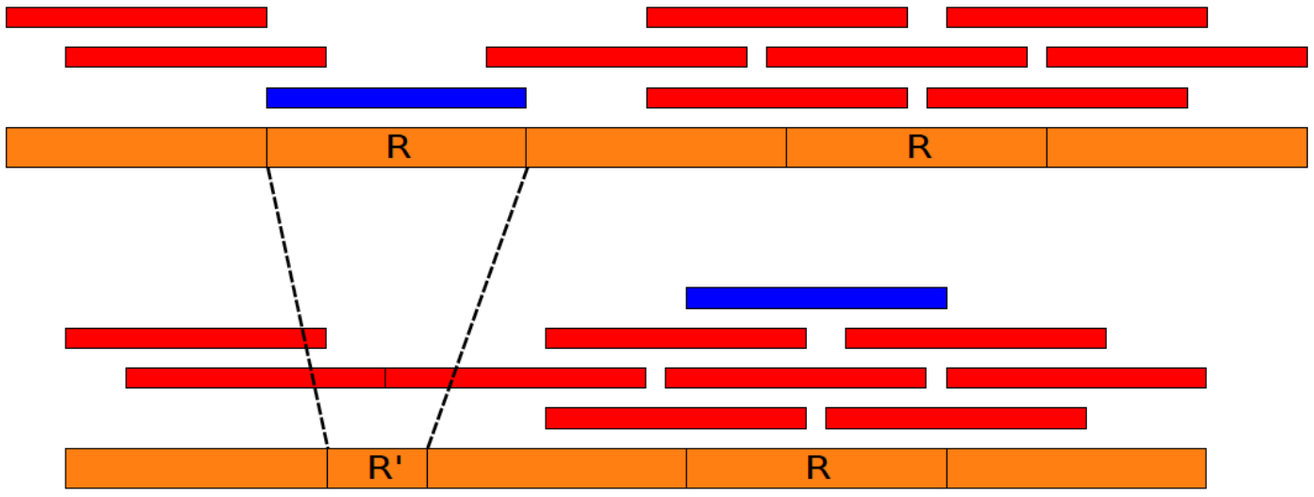
  - How good is this algorithm? Can it have an approximation factor better than two?

Exercise 2

- **What is the best approximation value that you can prove for the greedy algorithm?**

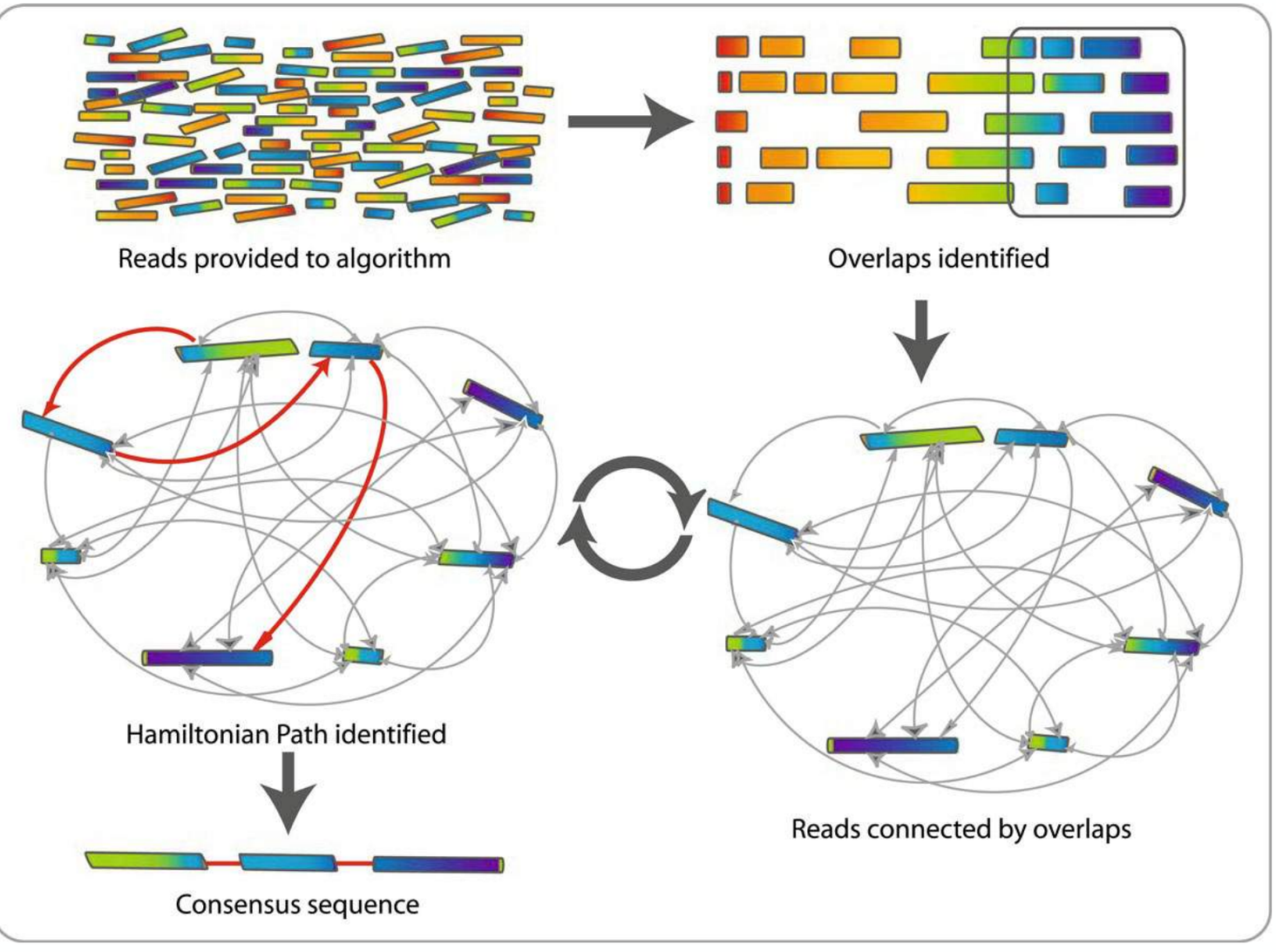
SSP: Problems (III)

- **Minimality :** requiring the superstring to be of minimal length, although motivated by parsimony, is in the best case questionable due to *repeats*.



- **Local choices :** in the true solution, the genome from which the reads were generated, several suffix-prefix read overlaps are not locally optimal.

Modelling and assembling NGS data (II)
Overlap-Layout-Consensus



- **Overlap :** Build the overlap graph (find potentially overlapping reads)
- **Layout :** merge reads into contigs and simplify the graph.
- **Consensus :** Derive the DNA sequence and correct read errors

Building the overlap graph

Definition (Overlap Graph)

- Given a set of reads $\mathcal{R} \subset \Sigma^* = \{A, C, T, G\}^*$ the overlap graph is a complete weighted directed graph such that:

- $V = \mathcal{R}$ and $E = \mathcal{R}^2$;

- $w(u, v) =$ length of the maximal suffix of *u* that is equal to a prefix of *v*

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In a more general definition a small number of mismatches is allowed for the suffix-prefix overlap. (Use DP to find the optimal overlap alignment)

Finding the optimal overlap alignment

- Overlap: suffix of *X* matches a prefix of *Y*
- We want to allow mismatches between *X* and *Y*.

X : **CTCGGCCCTGG - -**

Y : **- - CGACCCTAGTT**

```
D[i,j]= min {
                    D[i-1,j]+score(X[i-1],-),
                    D[i, j-1] + score(-,Y[j-1]),
                    D[i-1, j-1] + score(X[i-1],Y[j-1])
}
```

score(a,b)

	-	A	C	T	G
-	8	8	8	8	8
A	8	0	4	4	2
C	8	4	0	2	4
T	8	4	2	0	4
G	8	2	4	4	0

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C	8	4	0	2	4
T	8	4	2	0	4
G	8	2	4	4	0

		-	C	G	A	C	C	C	T	A	G	T	T
-	0	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞
C	0												
T	0												
C	0												
G	0												
G	0												
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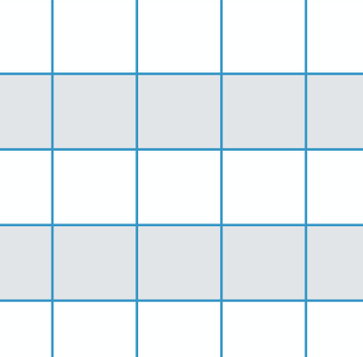

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G	8	2	4	4	0

		-	C	G	A	C	C	C	T	A	G	T	T
-	0	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞
C	0												
T	0												
C	0												
G	0												
G	0												
C	0												
C	0												
C	0												
T	∞												
A	∞												
G	∞												

Prefix of Y

Suffix of X

Asking for a suffix of length at least 4

Layout

- Graph simplification
    - remove all arcs that have weights below a given threshold.
    - remove from the graph all the edges that are transitively inferable. (**String graph**)

Consensus

- Find a *constrained* walk of minimum length in the graph
    - We define a *selection* function *s* that classifies the arcs:
        - *optional* : no constraint
        - *required* : present at leat once
        - *exact* : present exactly once

- Selection function is defined using A-statistics (Meyers et al. 2000)

Overlap-Layout-Consensus
and
Hamiltonian Path

- Given a selection function *s* , a string graph *G* find an *s-walk.*

Finding a minimum s-walk is NP-hard from a reduction from Hamiltonian path (Medvedev et.al. 2007)

- Many assemblers use heuristics however for shorter reads and much deeper coverages, the overlap computation step is a computational bottleneck.

Finding overlaps

- For **N reads of length L** we need:
  - **O(N²)** comparisons
  - each comparison **O(L²)** alignment

Given N reads…
Where N ~ 100 million…

We need to use a linear-time algorithm

Modelling and assembling NGS data (III)
de Bruijn graph

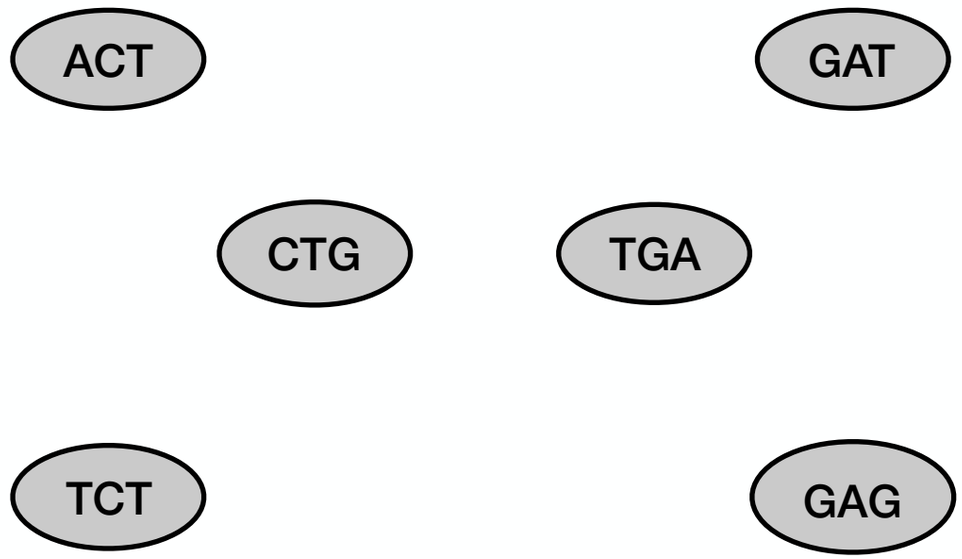
De Bruijn graph

Given a set of reads *R* and an integer *k* we define the de Bruijn graph *B(R,k)*

- Vertices are substrings of length **k** (**k**-mers)
- Arcs are *k-1* suffix-prefix overlaps that appear as a substring in *R*.

Example

R={ACTGAT,TCTGAG}, k=3



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de Bruijn graph *vs* overlap graph

- If all the reads of *R* have length exactly *k + 1*, the line graph of $G_k(R)$ is exactly the overlap graph of *R* with the arcs of weight zero removed.

- In a de Bruijn graph there is a loss of information with regard to the overlap graph: in de Bruijn graphs we do not have the information that two k-mers came from the same read.
    - As a consequence there are walks in the de Bruijn graph that are not read coherent (not entirely covered by reads).

Problem: Find an Eulerian path (visit each arc of the graph once).

- Finding an Eulerian path is polynomial but it may be not “read coherent”.

- A graph may have an exponential number of Eulerian paths.

Computing a de Bruijn graph

- Given a read set *R,* we can build a de Bruijn graph $G_k(R)$ using a hash table to store all (k +1)-mers present in *R*.
- As each insertion and membership query in the hash table takes *O(1)* (expected) time, the de Bruijn graph can be built in time linear in the size of *R*, i.e. $O(\sum_{r \in \mathcal{R}} |r|)$.

We can compute a de Bruijn graph for a set of reads in linear time in the size of the reads.

de Bruijn graph

de Bruijn graph : Theory

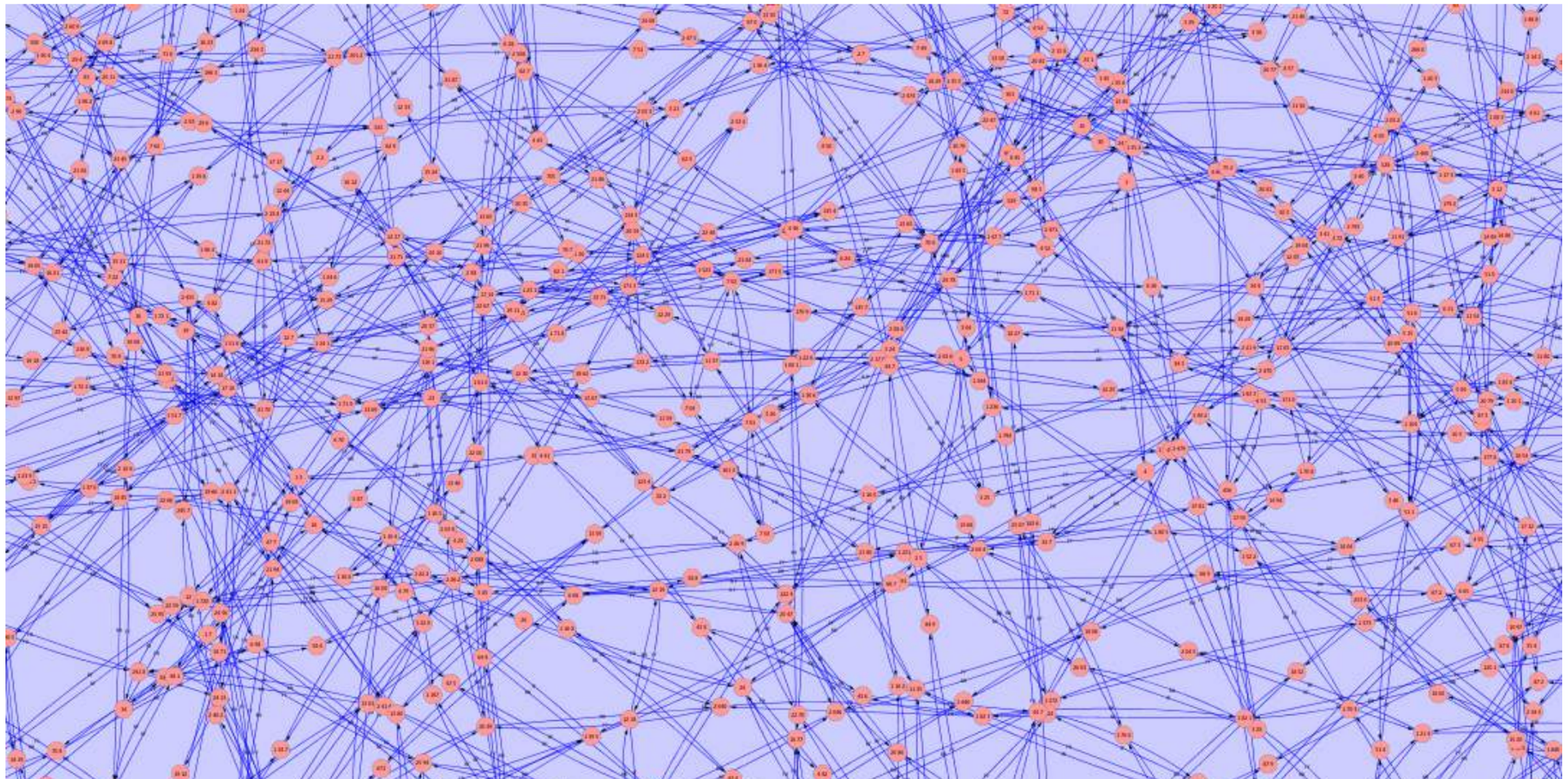
- *Sparse* Graph: out and in degree ≤ 4

de Bruijn graph

de Bruijn graph : Theory

- *Sparse* Graph: out and in degree ≤ 4

de Bruijn graph : Reality



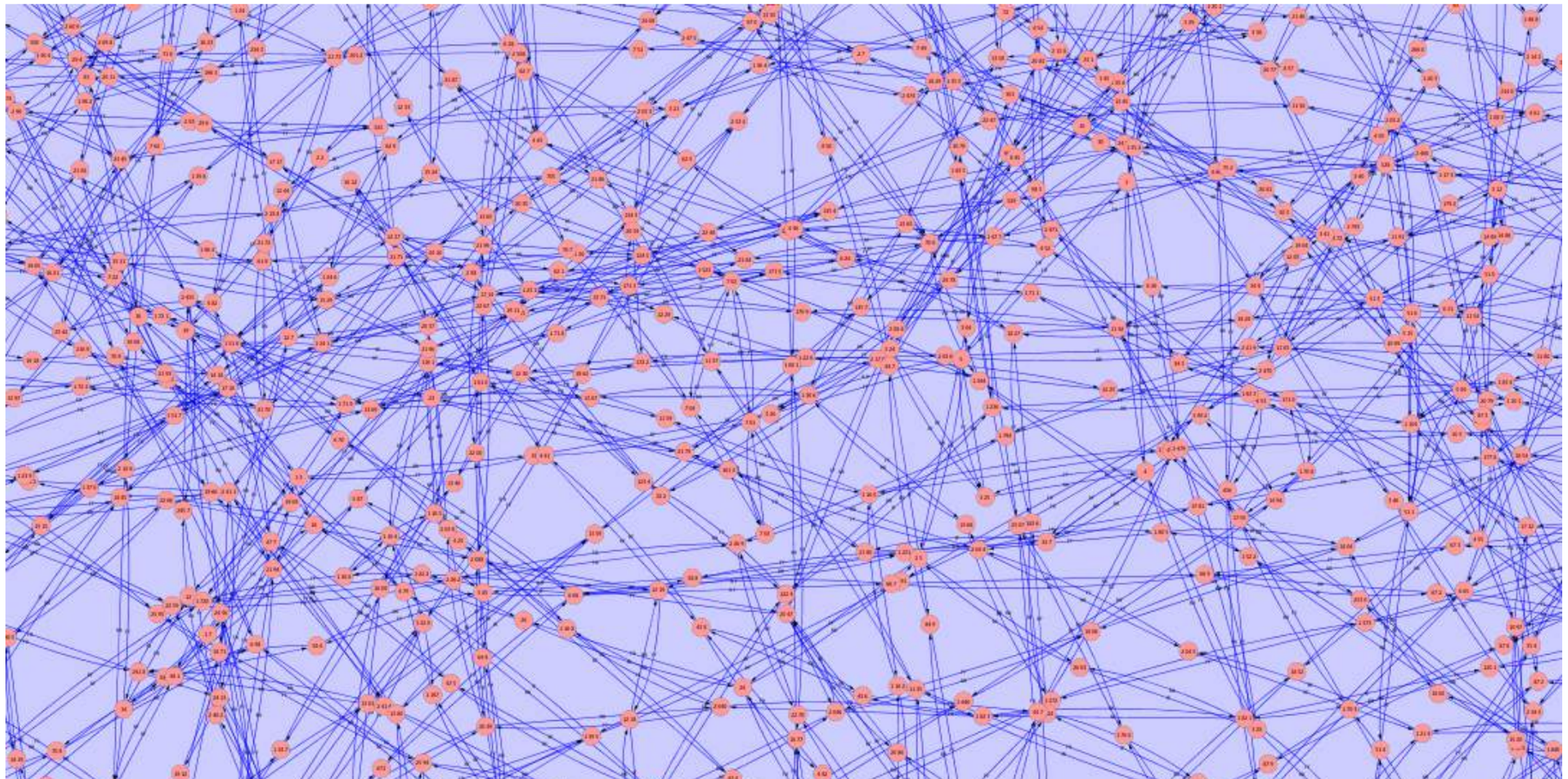
de Bruijn graph

de Bruijn graph : Theory

- *Sparse* Graph: out and in degree ≤ 4

What creates the complexity?

de Bruijn graph : Reality



repeats challenge in assembly