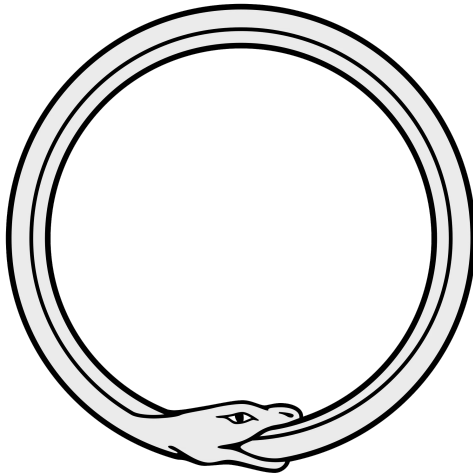


Genome assembly

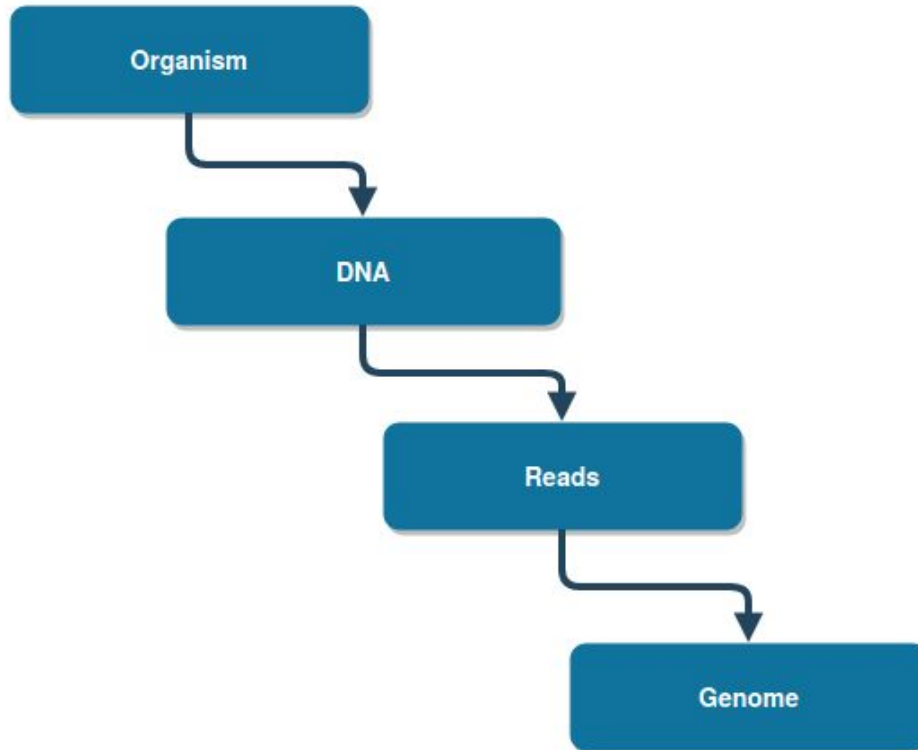


Why do we need it?

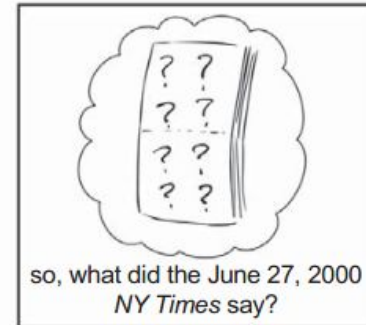
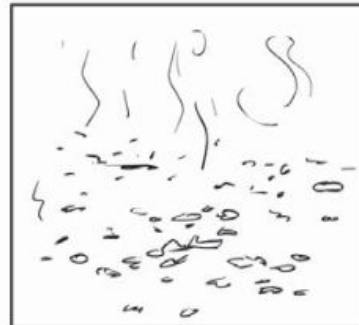
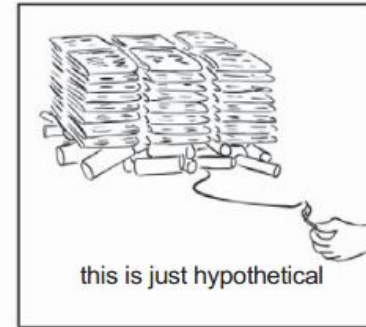
No need to ask this question - genome sequence knowledge is crucial or beneficial for many applications including

- Gene searching
- Transcriptome analysis
- Primer construction
- Mobile elements investigation
- Genome stability exploration
- Phylogeny

Typical information for genome assembly



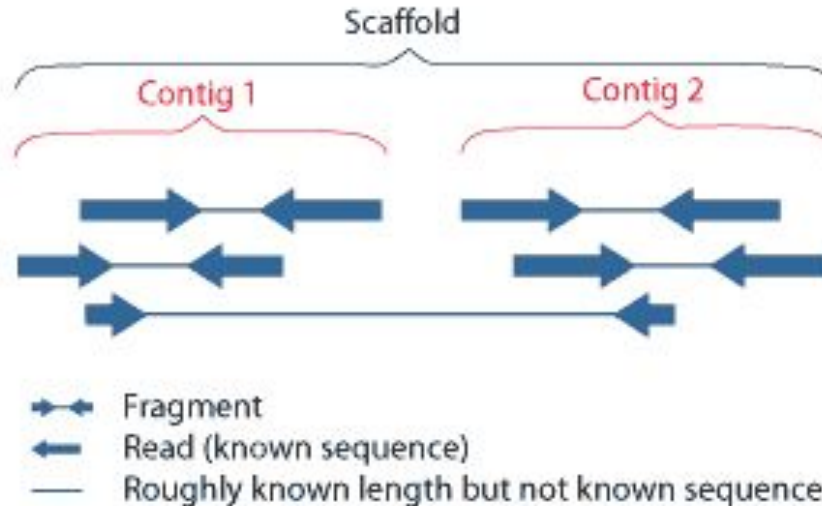
So, something like this...



Levels of genome reconstruction

Just some definitions to be honest

1. read - stretch of DNA from the sequenator
2. contig - overlapped reads which are merged together
3. scaffold - contigs and gaps between them



Assembly strategies

Depend on read type (for example, their length)

1. Naive
2. Greedy
3. Overlap Layout Consensus (OLC)
4. de Bruijn graph (DBG)
- 5.

Naive approach

Well, looks pretty simple

1. Align locally each read vs another
2. Take alignments with scores higher than some threshold and unite pair of reads
3. Repeat previous steps

Sequencing example

Let's allocate reads near their origin. Read length is equal to 5 and genome is БИОИНФОРМАТИКА

| | |
|----|----------------|
| | БИОИНФОРМАТИКА |
| 1 | БИОИН |
| 2 | ИОИНФ |
| 3 | ОИНФО |
| 4 | ИНФОР |
| 5 | НФОРМ |
| 6 | ФОРМА |
| 7 | ОРМАТ |
| 8 | РМАТИ |
| 9 | МАТИК |
| 10 | АТИКА |

So we just align each read vs all others, take best alignments and merge them into contigs. After this we obtain scaffold, in our simple case we have good result

| | | | | |
|-------|--------|-------|--------|-----|
| БИОИН | | ОИНФ | | |
| + | БИОИНФ | + | ОИНФОР | ... |
| ИОИНФ | | ИНФОР | | |

| | | |
|--------|-------------|----------------|
| БИОИНФ | | |
| + | БИОИНФОР... | БИОИНФОРМАТИКА |
| ОИНФОР | | |

OLC

Improved version of previous algo

1. Align reads with each other
2. Create a graph (map) of reads connections
3. Fix inconsistencies of overlaps via multiple alignment

So in our simple case it looks like

read1 -> read2 -> read3 -> read4 -> read5 -> read6 -> read7 -> read8 -> read9
|
v
read10

Ambiguous overlaps are resolved with alignment of reads and taking consensus
We don't have such problems here (we have precise bioinformatics here =))

Imagine we have several reads from 1 genome position

reads

БИОИН

ИОИНΦ

И~~A~~ИНΦ

ИОИНΦ

consensus

БИОИНΦ

De Bruijn Graph

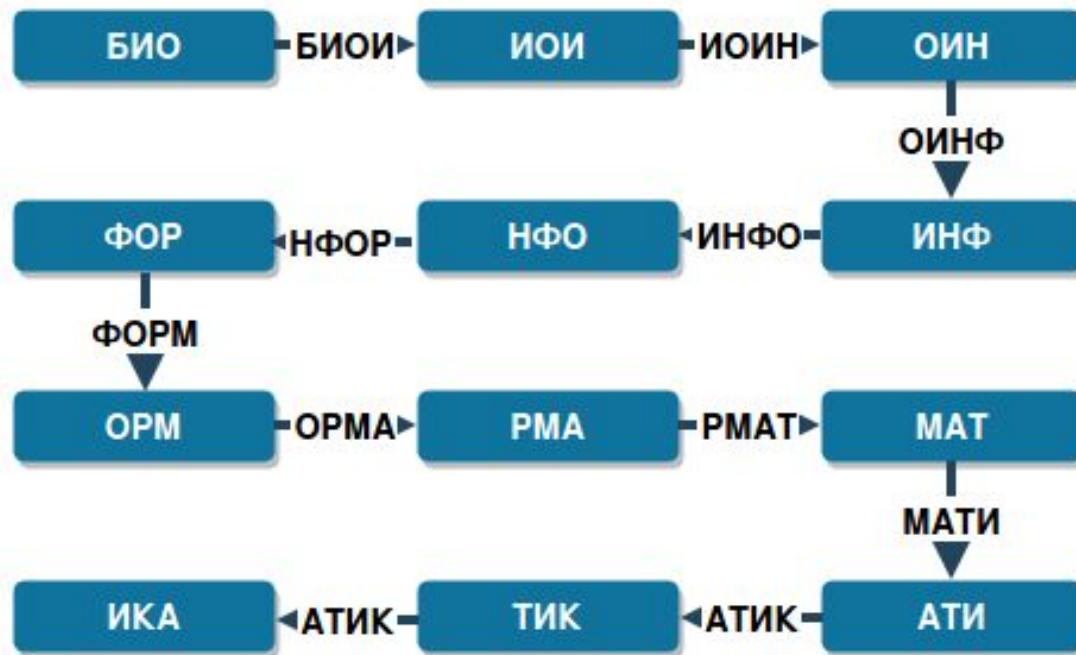
Very popular nowadays, was introduced to genome assembly by Pevzner

1. Split reads into k-mers with fixed k, usually k is odd
2. Build de Bruijn graph from them - $(k - 1)$ -mers are the vertices and edges are k-mers (overlap sequence between edges)
3. Process this graph

Let's look at this strategy on our example with БИОИНФОРМАТИКА genome
read_length = 5, k = 4

| Reads | k-mers | (k - 1)-mers |
|-----------------|--------|--------------------|
| БИОИНБИОН, ИОИН | | БИО, ИОН, ИОИ, ОИН |
| ИОИНФИОИН, ОИНФ | | ИОИ, ОИН, ОИН, ИНФ |
| ОИНФООИНФ, ИНФО | | ОИН, ИНФ, ИНФ, НФО |
| ИНФОРИНФО, НФОР | | ИНФ, НФО, НФО, ФОР |
| НФОРМНФОР, ФОРМ | | НФО, ФОР, ФОР, ОРМ |
| ФОРМАФОРМ, ОРМА | | ФОР, ОРМ, ОРМ, РМА |
| ОРМАТОРМА, РМАТ | | ОРМ, РМА, РМА, МАТ |
| РМАТИРМАТ, МАТИ | | РМА, МАТ, МАТ, АТИ |
| МАТИКМАТИ, АТИК | | МАТ, АТИ, АТИ, ТИК |
| АТИКААТИК, ТИКА | | АТИ, ТИК, ТИК, ИКА |

Graph



About coverage

Let's compute the coverage of our genome and think how it is affected by k-mering

| | |
|----|----------------|
| | БИОИНФОРМАТИКА |
| 1 | БИОИН |
| 2 | ИОИНФ |
| 3 | ОИНФО |
| 4 | ИНФОР |
| 5 | НФОРМ |
| 6 | ФОРМА |
| 7 | ОРМАТ |
| 8 | РМАТИ |
| 9 | МАТИК |
| 10 | АТИКА |

coverage

1 3 5 5 5 4 2

2 4 5 5 5 3 1

Coverage after creating k-mers

Let's compute the coverage of our genome and think how it is affected by k-mering

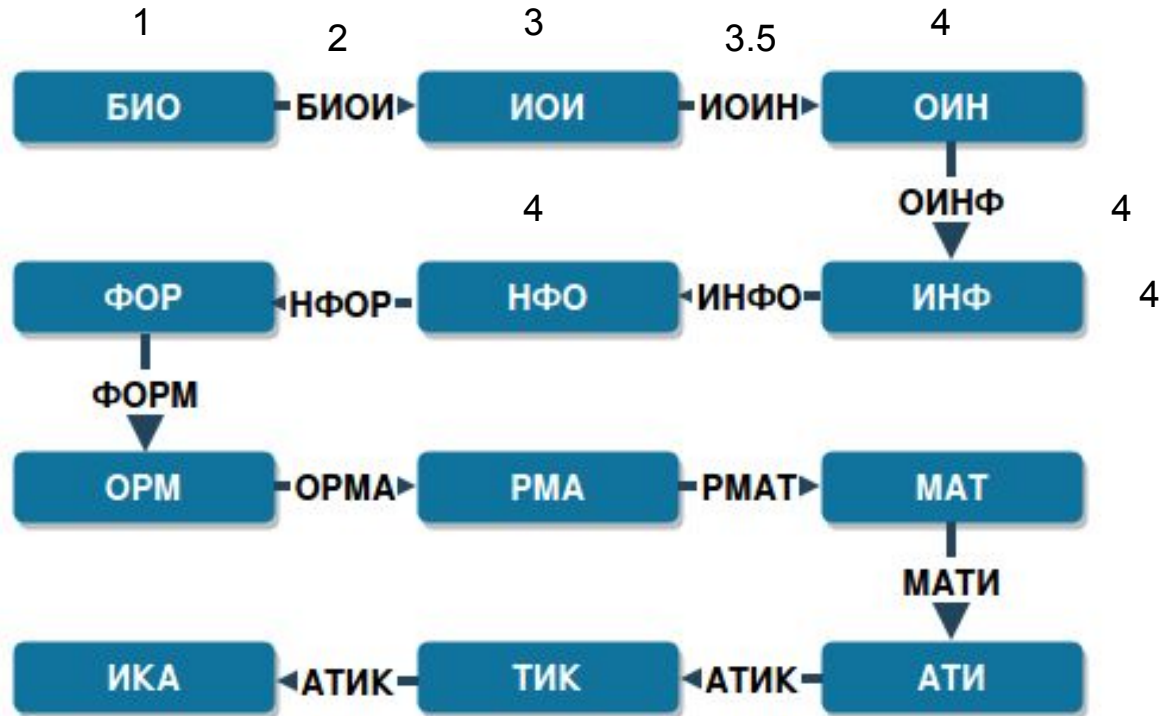
| | |
|---|----------------|
| | БИОИНФОРМАТИКА |
| 1 | БИОИ |
| | ИОИН |
| 2 | ИОИН |
| | ОИНФ |
| 3 | ОИНФ |
| | ИНФО |
| 4 | ИНФО |
| | НФОР |
| 5 | НФОР |
| | ФОРМ |
| | ... |

coverage

1 6 8

3 7 8

Graph with coverage



Coverages

- vertex coverage - number of such sequences (k - 1) in data
- edge coverage - computed from vertex coverage with such formula

$$E_c = \frac{V_{c-in} + V_{c-out}}{parts}$$

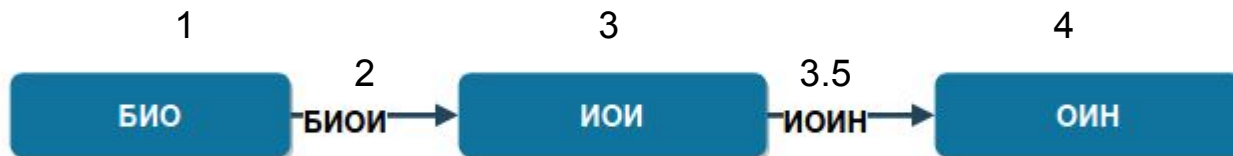
E - coverage of the edge

V - coverage of the vertex

parts - number of vertex which built this edge - 2 by default (each edge's vertex contribute 1)

Condense graph

We can condense all straight parts (without branching) of our graphs



$$E_c^* = \frac{E_c \cdot parts_1 + V_{new}}{parts_1 + parts_2 - 1}$$

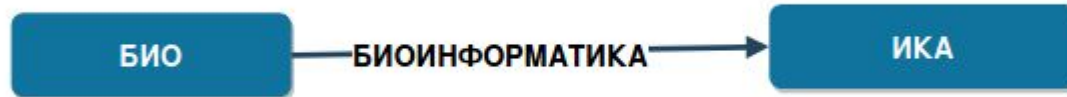
$$\frac{2 \cdot 2 + 4}{2 + 2 - 1} = 2.66$$

2.66



Condensed graph

During this process we reduce number of vertices and edges, elongate our edges and recompute their coverage. After that we have condensed graph possibly with some conflicts



Possible problems

Possible origins - sequencing errors, polymorphism (especially in polyploid organisms), different repeats, contamination

- tips
- bubbles
- chimerism

Bubble

Let's illustrate it with more DNAic example

Genome

AATAGA

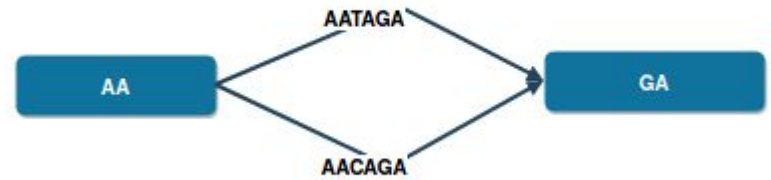
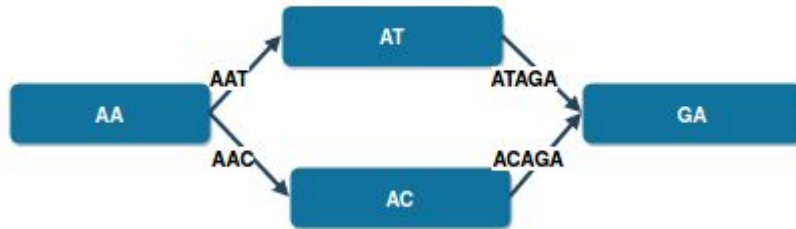
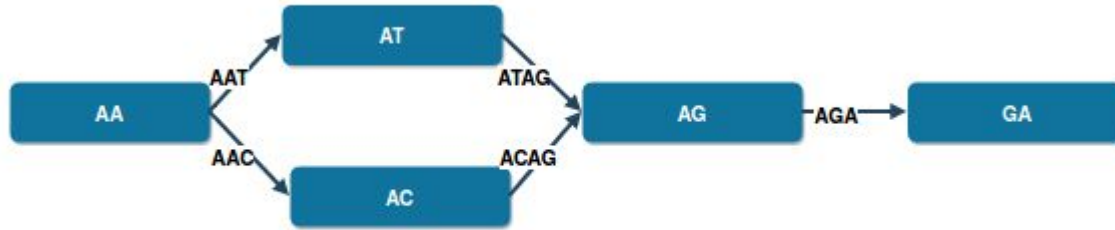
Reads

AATA

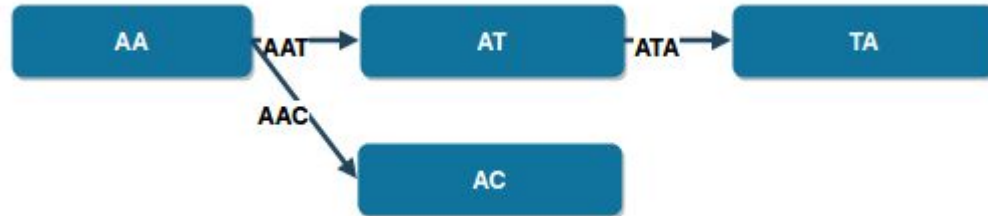
AACA and so on



After condensing



Tip



Possible solutions

Prune tips if their coverage is low (less than some threshold)

Choose way in the bubble with the highest coverage

Possible additional steps

Assemblers would like to get ideal reads. So we can do the following before the assembly

- clean dataset from contamination
- deduplicate reads
- correct reads

Contigs

Just edges of collapsed graph



БИОИНФОРМАТИКА



AATAGA

AACAGA