Use Case of a RNA-Seq assembly

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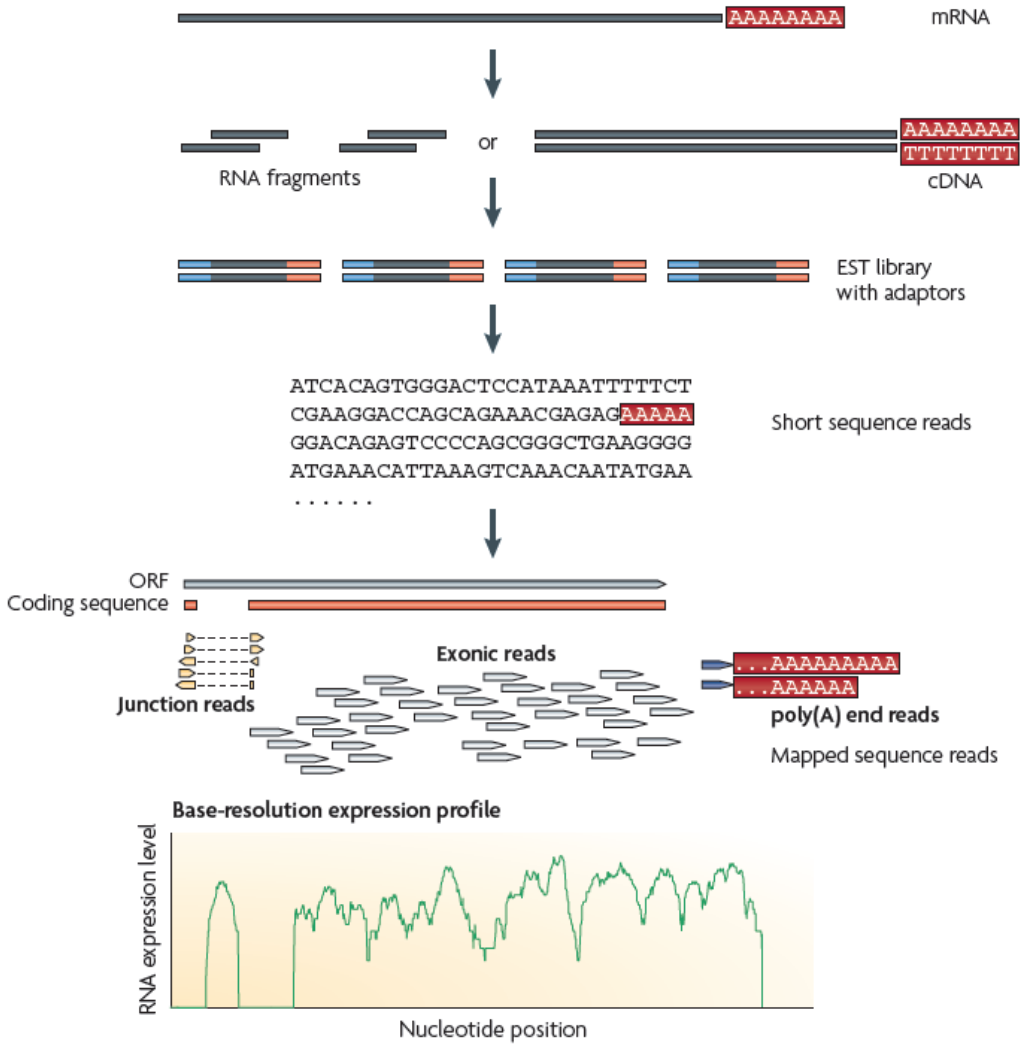
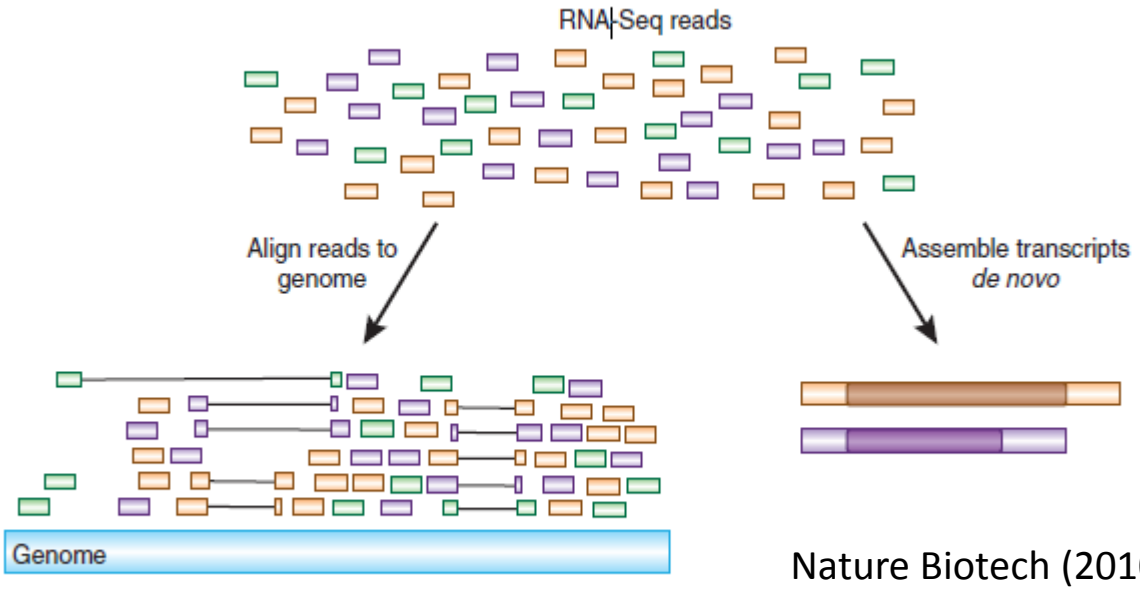
RNASeq technology



RNASeq technology

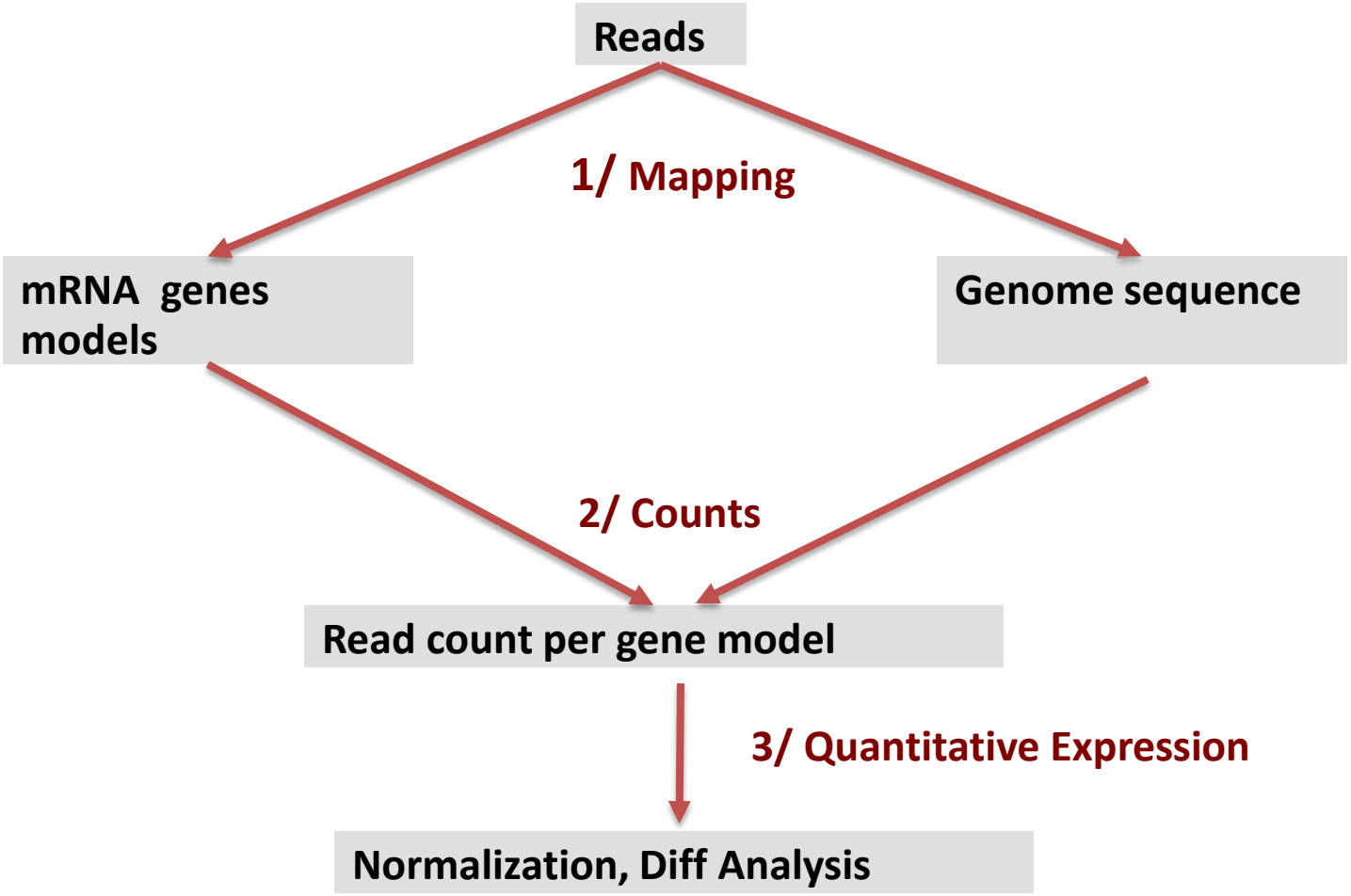


Nature Biotech (2010)

Many Applications :

- quantification,
- detection de novo “new gene, new transcript”
- Meta-transcriptomic (TARA Oceans project Genoscope)

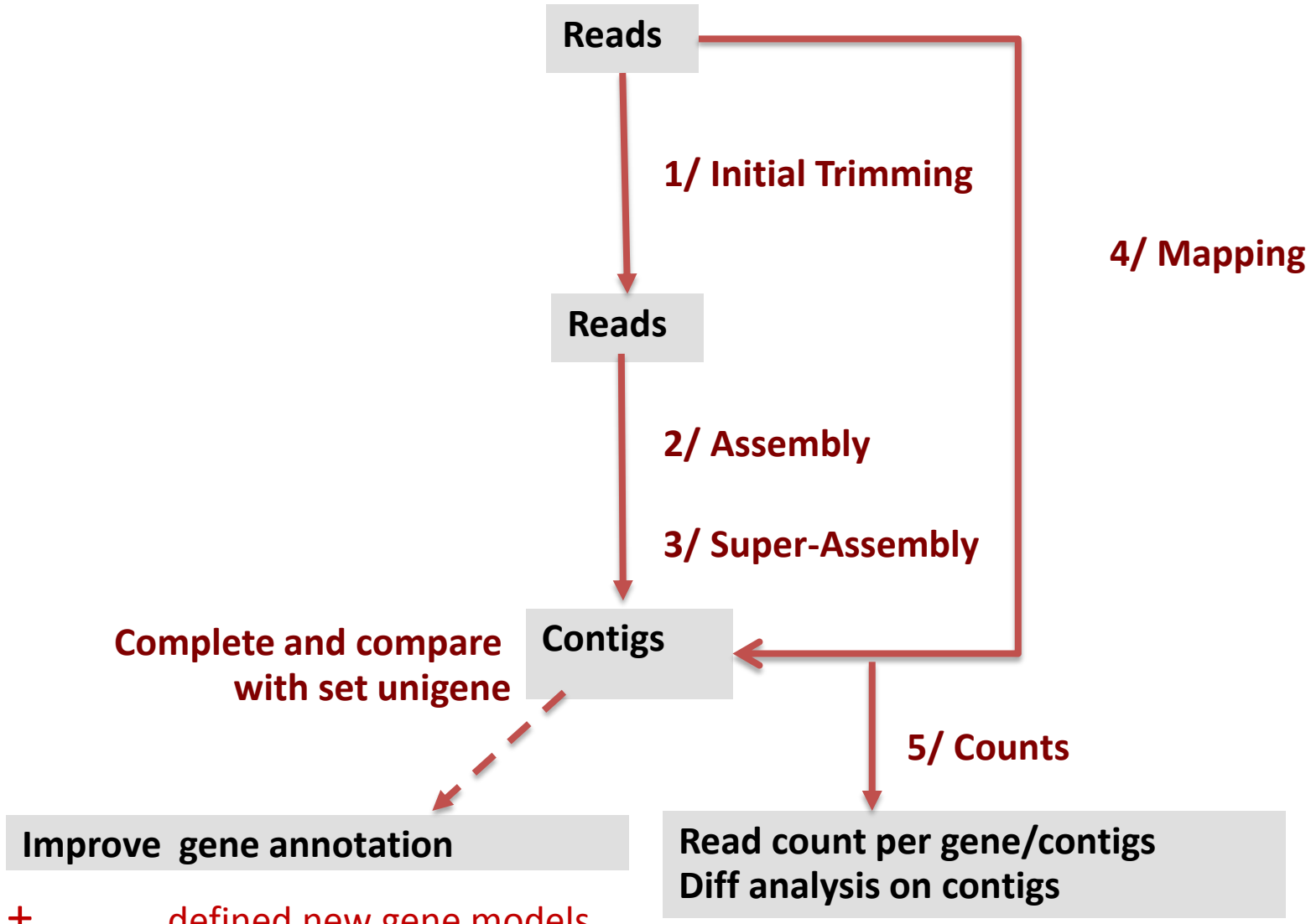
1st strategy : mapping RNA-Seq against a genome (transcripts or genome)



\+ trimming not necessary, time saving

\- confidence of gene models or assembly genome, no new genes detected

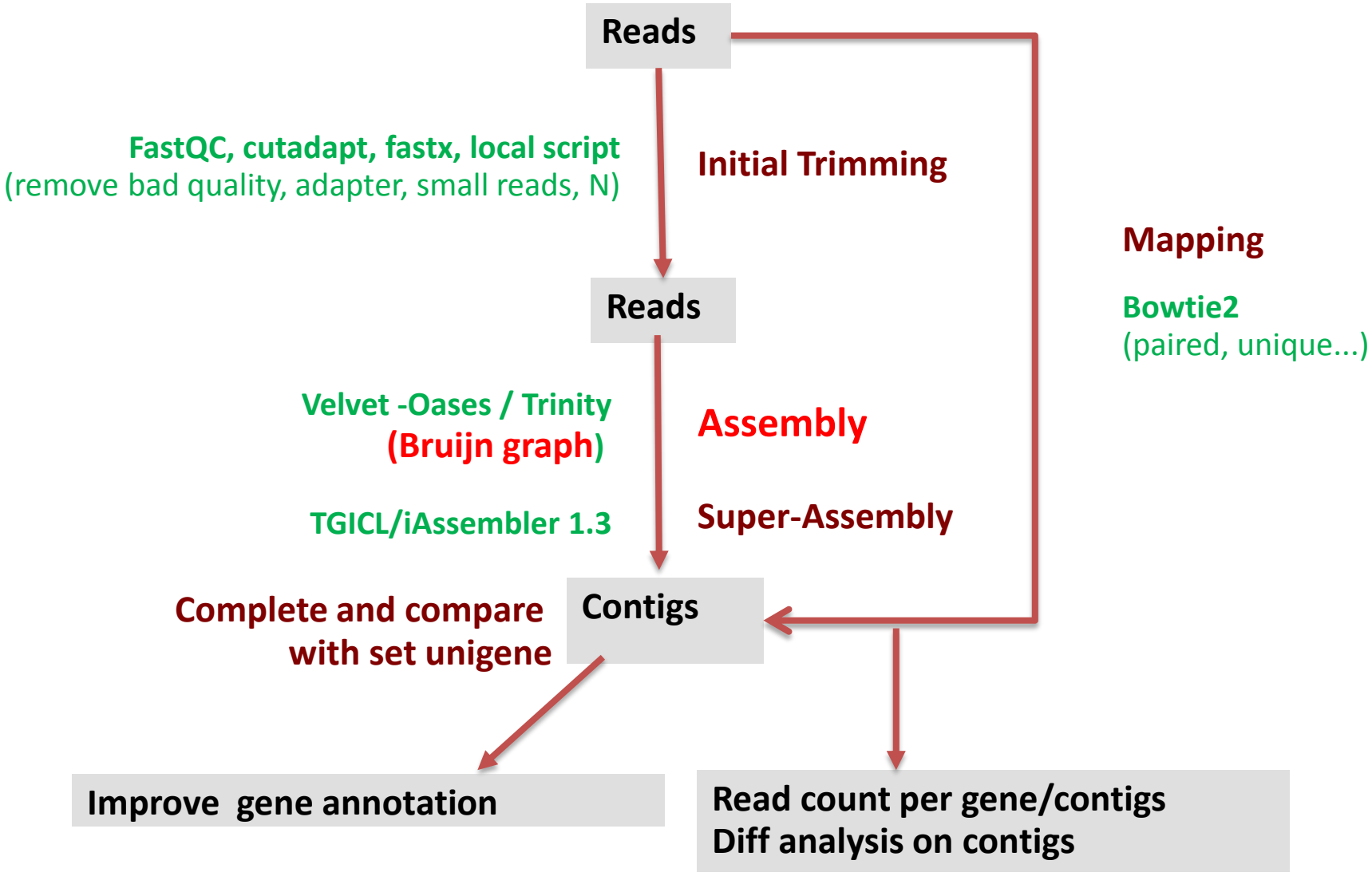
2nd strategy : de novo Assembly of RNAseq (without reference genome)



\+ defined new gene models

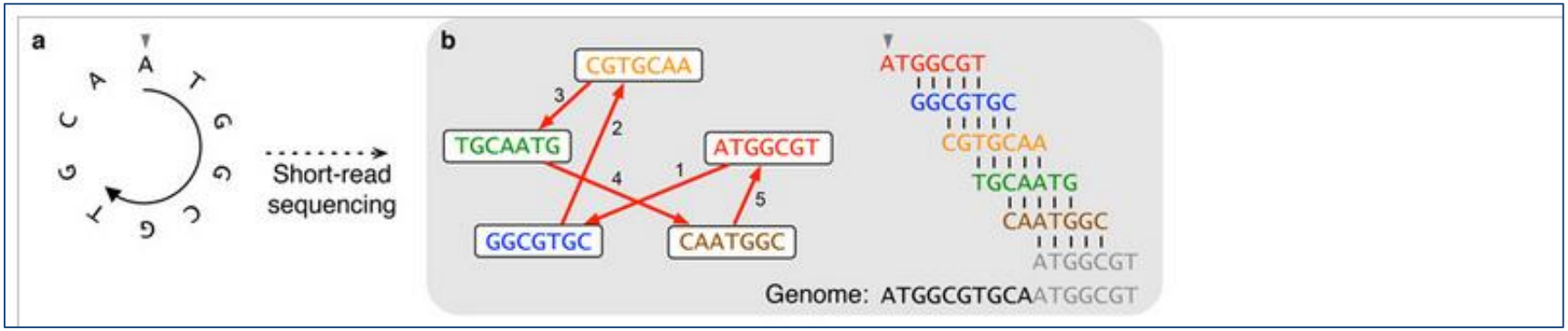
\- Assembly: not perfect, defined kmer, time and memory consuming

de novo Assembly of RNAseq



From reads assembly to contigs → Graph construction methods

Why are de Bruijn graphs useful for genome assembly? Nat Biotechnol. Compeau et al. 2017



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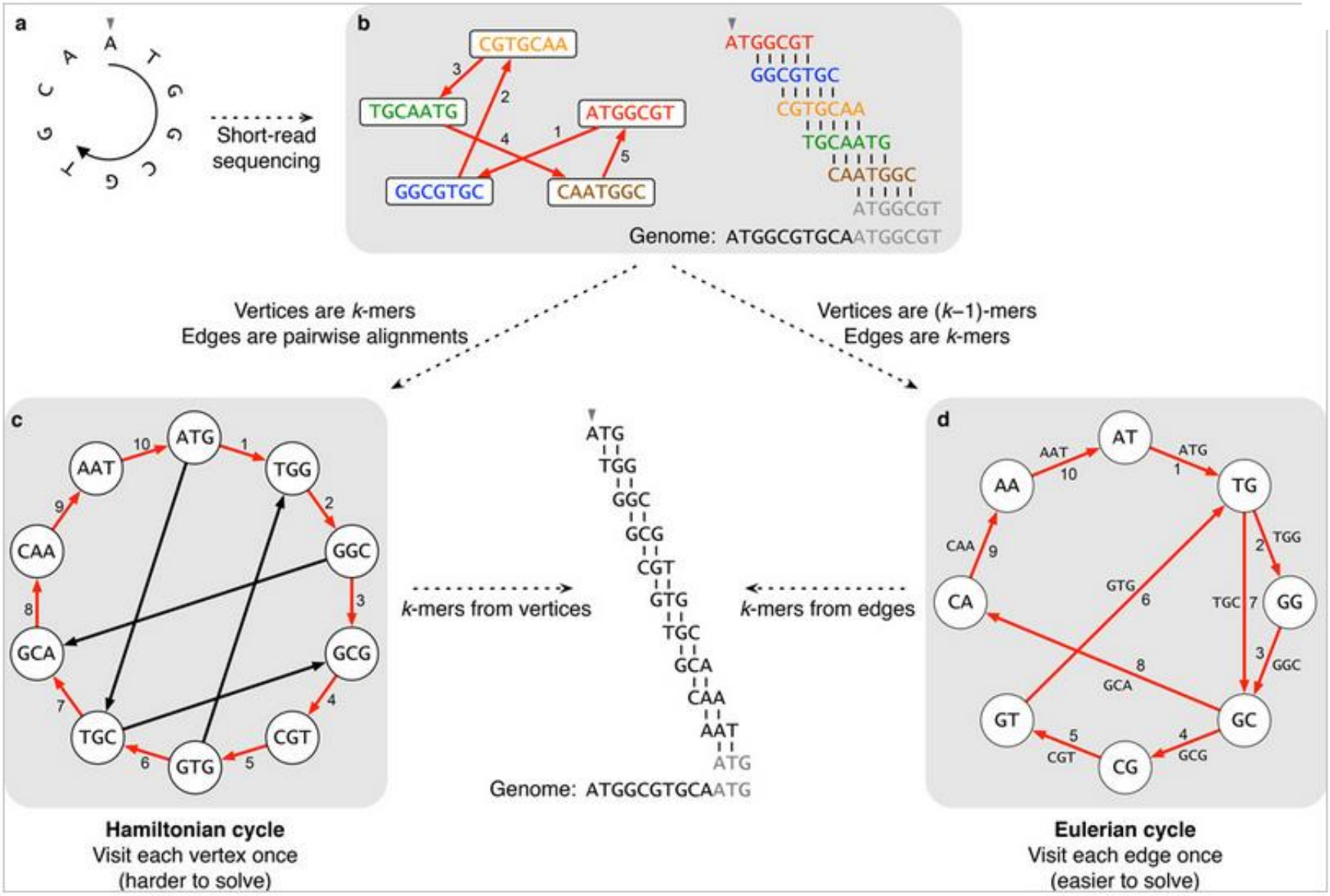


Two strategies for genome assembly: from Hamiltonian cycles to Eulerian cycles

De novo Assembly : 2 methods for Graph path

Hamiltonian: Node= kmer or read; Edge= pairwise between node

- Aligning pairwise = this step is very time/memory consuming
- Path = Cross by all nodes once a time

→ **Overlap Layout Consensus (OLC method)**

→ **Tools : newbler, cap3 adapted for 454 sequencing…not adapted to 2nd generation of sequencer**

Eulerian: Node = (k-1) mer; Edge = kmer

- Easy to contsruct
- Path = Cross by all edges once a time

→ **Graph de Bruijn method**

→ **Adapted to last NGS with 10 to 40 million of reads (50 to 150 bases)**

→ **using by all popular tools : velvet, Trinity, SOAPdenovo**

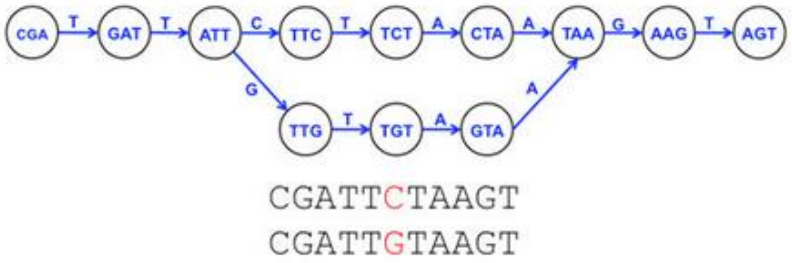
Bruijn Graph :

"Instead of assigning each k-mer to a node, we will now assign each k-mer located within a read to an edge."

Limits of using Assembly

Time consuming:
for 40 millions of reads with the most popular tool Trinity
=index kmer 27bases + graph +contigs.
→ 23h to obtain contigs , Graph construction 6h

Bubbles=complexities in graph

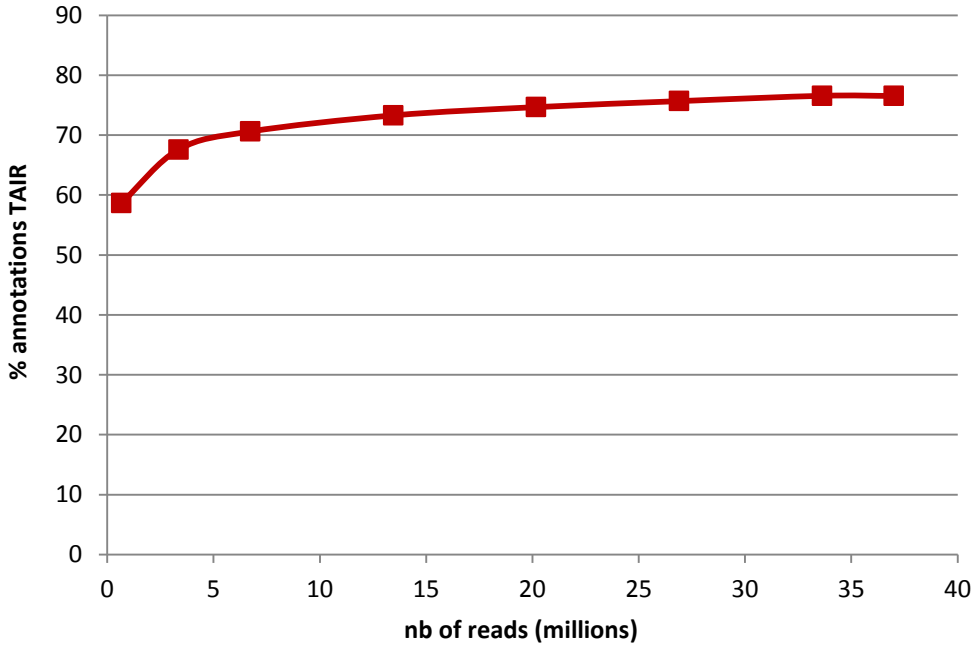


→ **Biological reasons**

- Errors in read sequences : tools exist to remove some errors
- Natural genotype difference : allelic, polyploidy
- Repetitions : many repeat element in eukaryote
- RNA-Seq :
  - No same coverage by gene
  - Alternative transcripts (splicing alternative)
  - Many graph by gene

Problem of depth sequencing : counts of reads → coverage by gene

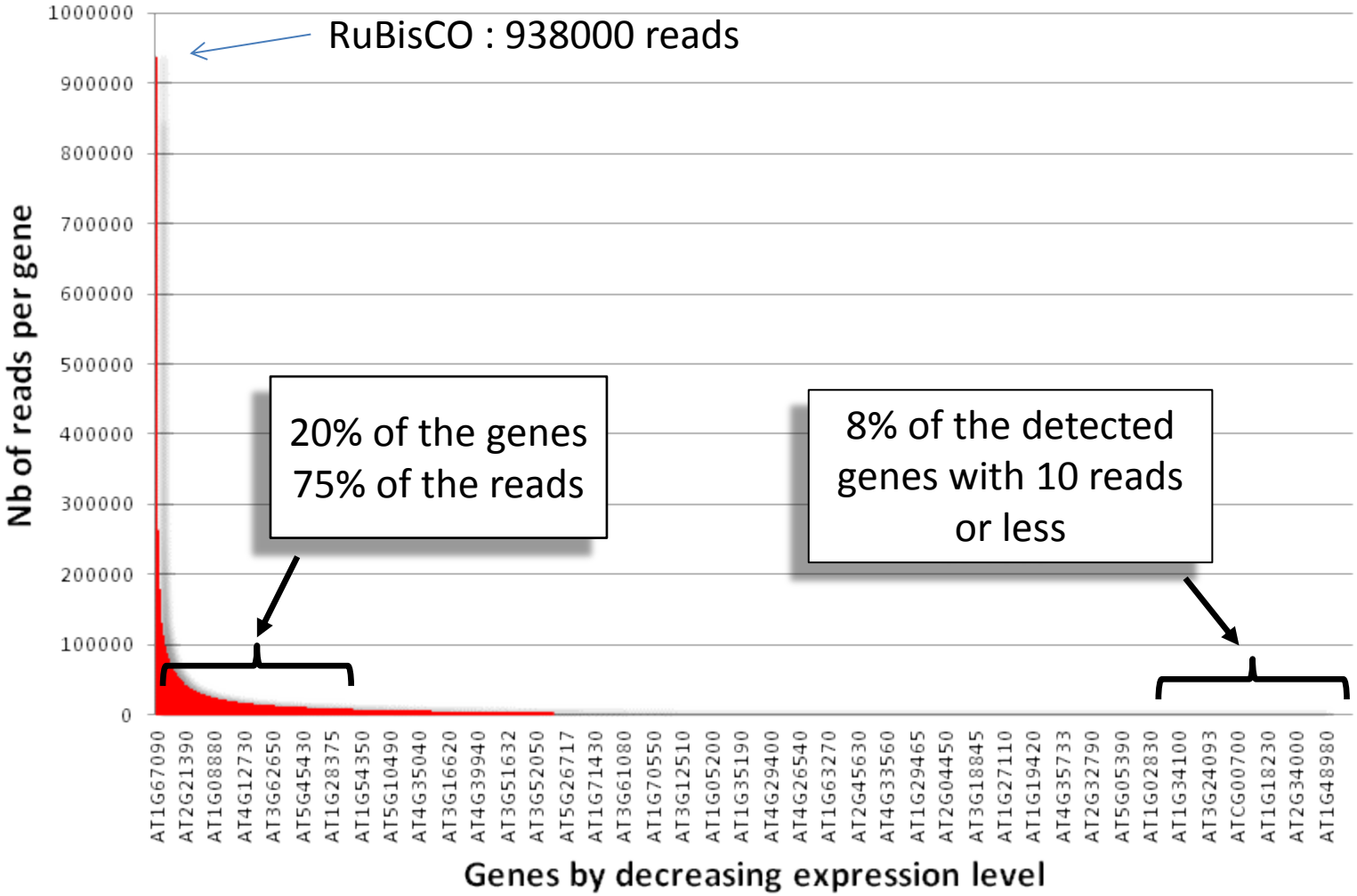
→ Bowtie2 mapping : 98% of reads mapped



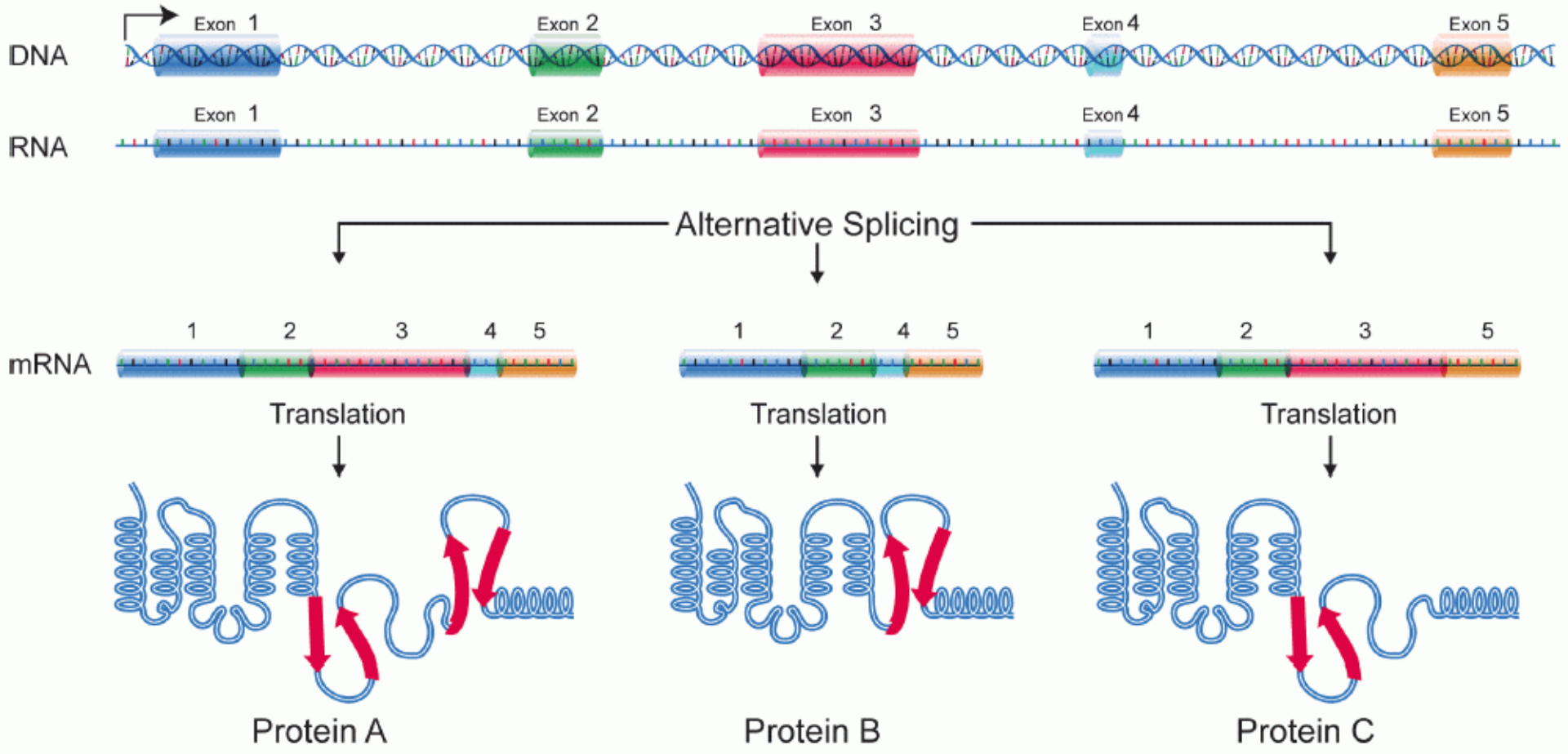
Among 27884 genes on nuclear chromosomes

1m	--> **16507 genes (59%)**
5m	--> 19041
10m	--> 19926
20m	--> 20748
30m	--> 21177
40m	--> 21487
50m	--> 21733
55m	--> **21739 (78%)**

Problem of non uniform gene expression
→Biased distribution of the reads by gene



Problem of Isoforms : Alternative splicing



Wikipedia source

Arabidopsis : 30% of genes with 2 to 3 mRNA
Human : 85% of genes encode isoform proteins

Assembly Results on Arabidopsis RNA-Seq

F1_Mplex	
Nb of PE reads	43 030 388 PE
Nb of contigs from assembly	33 736 (length mean 1360)
Nb of mapped contigs Genome TAIR10	33 072 98%

Data from Illumina HiSeq2000

- **Velvet/oases (kmer 61,71)**
- **iAssembler**

Comparison of annotations
TAIR10 annotation versus Contigs from assembly

F1_Mplex	
Nb of PE reads	43 030 388 PE
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Comparaison annotations	
Nb tagged Genes Nb contigs	17 783 32 220 (97%)
Nb of genes with confirmed structure Nb Contigs	15 613 (88%) 20 881 (65%) contigs

Data from Illumina HiSeq2000

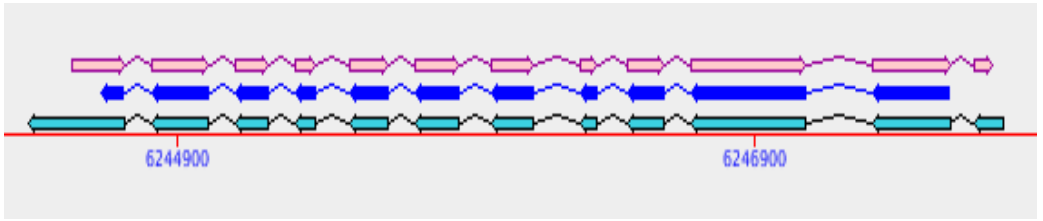
- **Velvet/oases (kmer 61,71)**
- **iAssembler**

→Gene=Locus

→ Model of genes with confirmed exon/intron structure

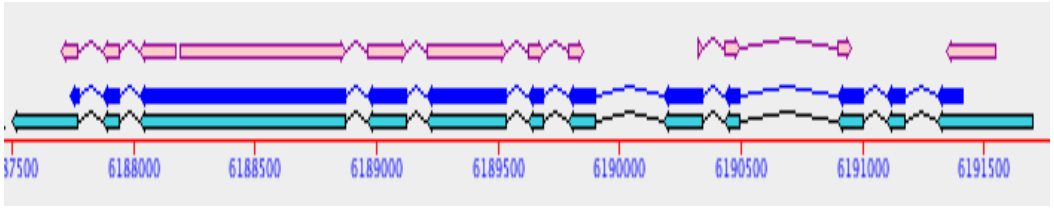
Quality of Assembly : contig versus gene annotation

1 gene – 1 contig
same gene model

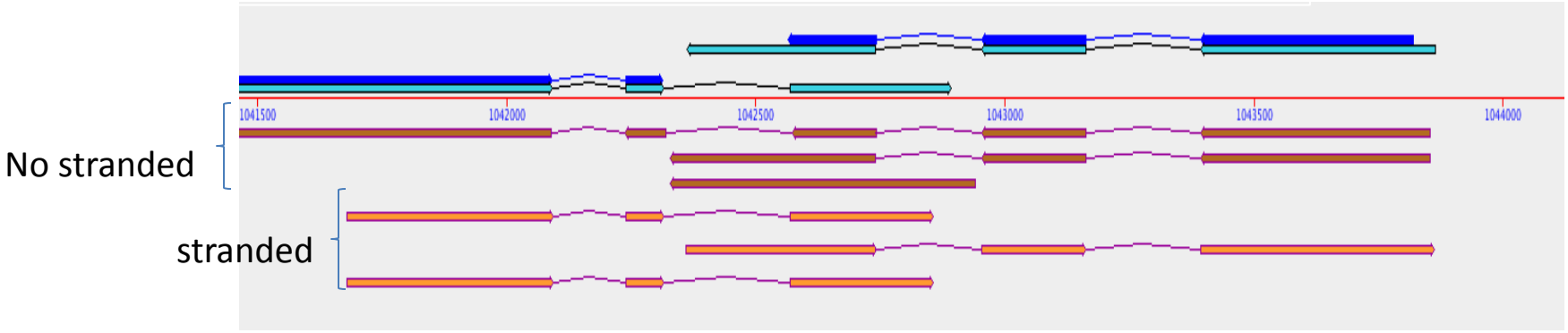


2/3 of contigs correspond to gene structure (partial)

1 gene – 2 or n contigs
same gene model



Library Stranded → remove chimeric genes



Quality of Assembly

35% of contigs with other gene models (isoforms)

1 gene – 1 or n contigs
with other gene models

→3% of contigs without annotation = new genes

Conclusion of assembly on transcriptomic and simple model

→ A good quality of contigs, efficient to detect new gene models

→ Problems: distinct false/good gene models,
chimera that increases with read number

With more complex transcriptome/genome

→ Can not work: too complex graph path

→ Generate too much contigs