Lecture 10: Transcriptome analysis

BIOTECH-7005-BIOINF-3000

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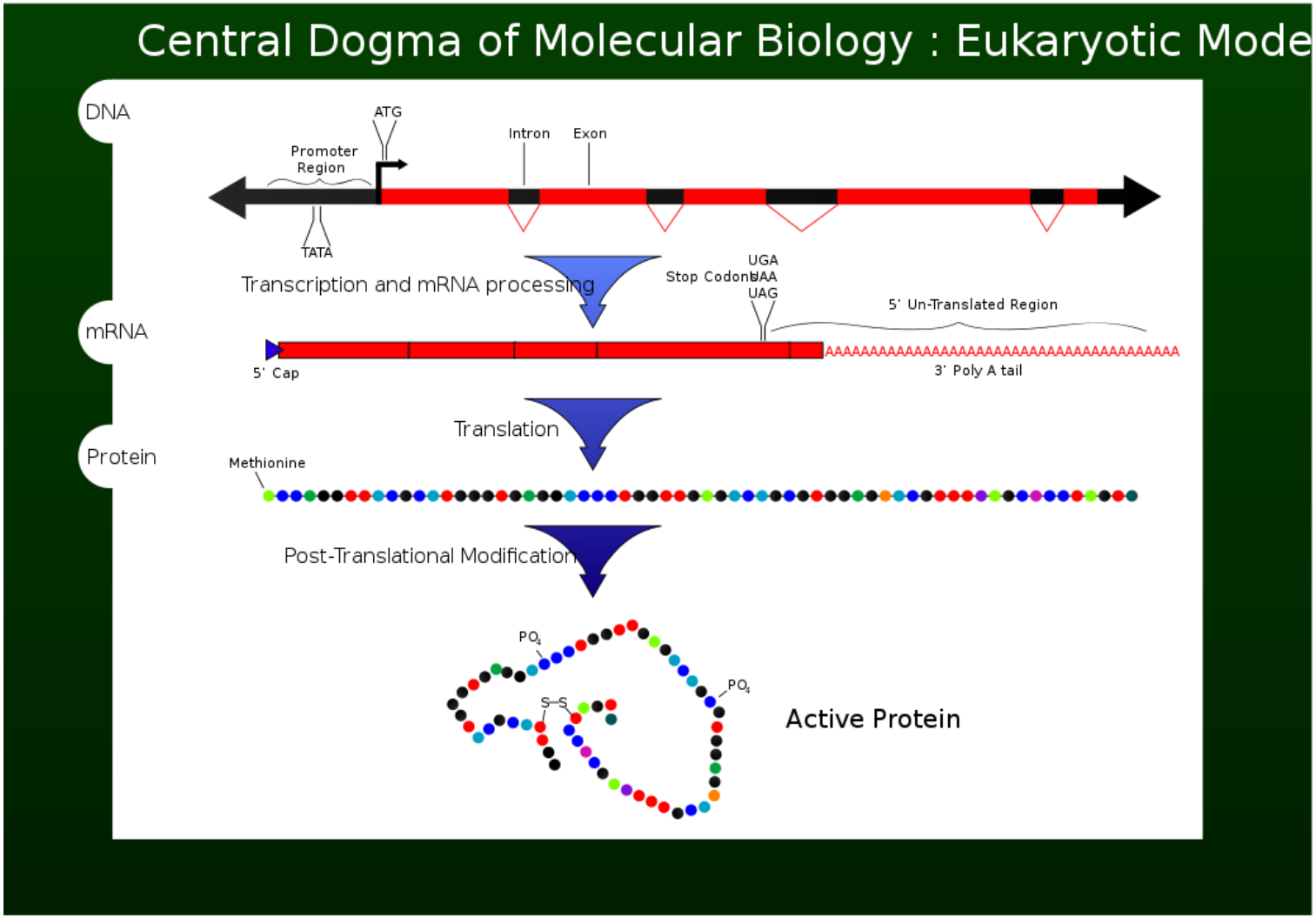
Outline

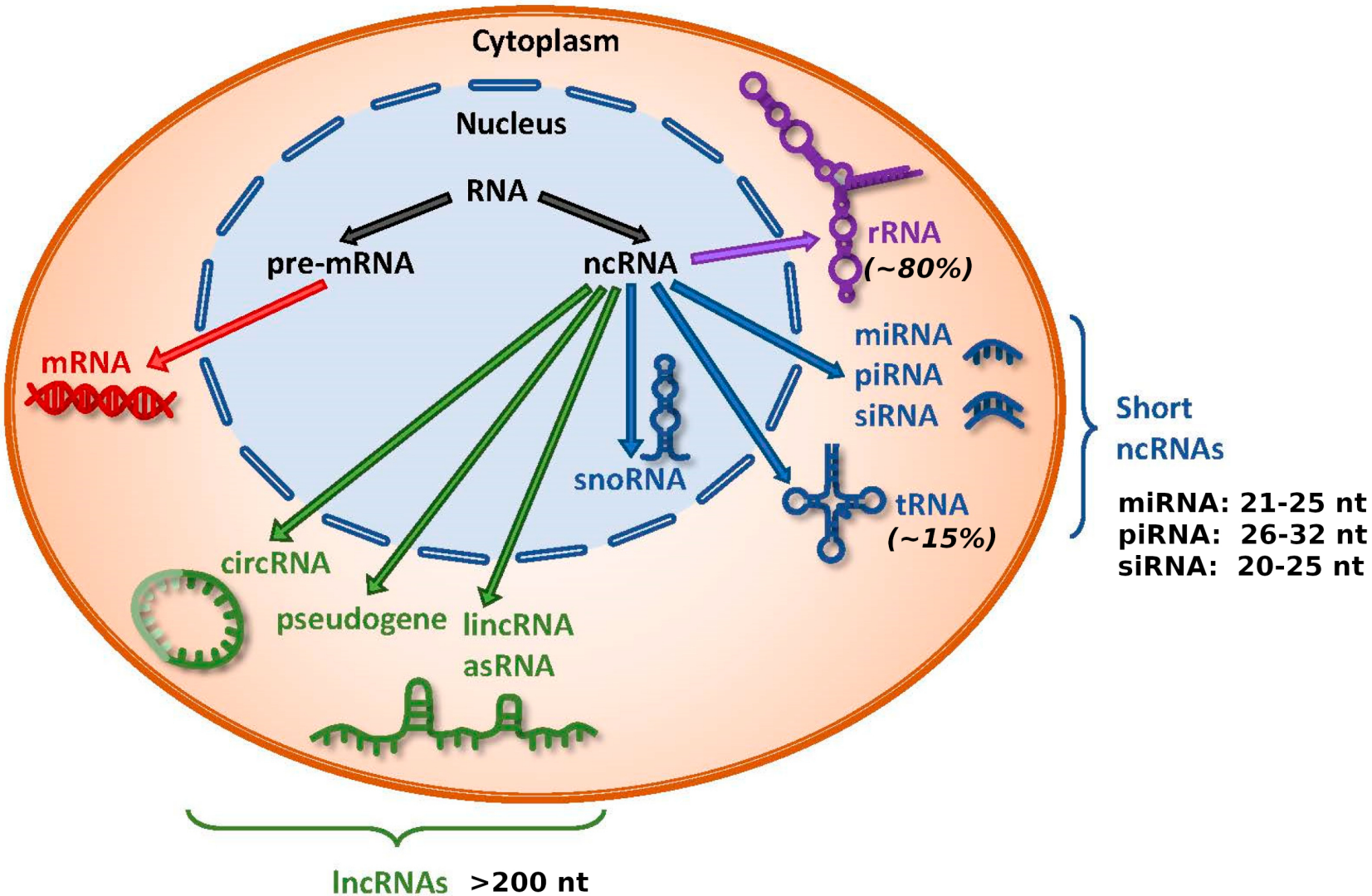
- Introduction to transcriptome and transcriptome analysis
- Transcriptome assembly
  - Genome assembly vs transcriptome assembly
  - De novo transcriptome assembly
  - Genome guided transcriptome assembly
  - Assess assembly quality using BUSCO
- Other transcriptome analysis

Central dogma of biology



By Adenosine at English Wikipedia, CC BY-SA 2.5, https://commons.wikimedia.org/w/index.php?curid=32026515

Expression of different RNAs in Eukaryotic cells

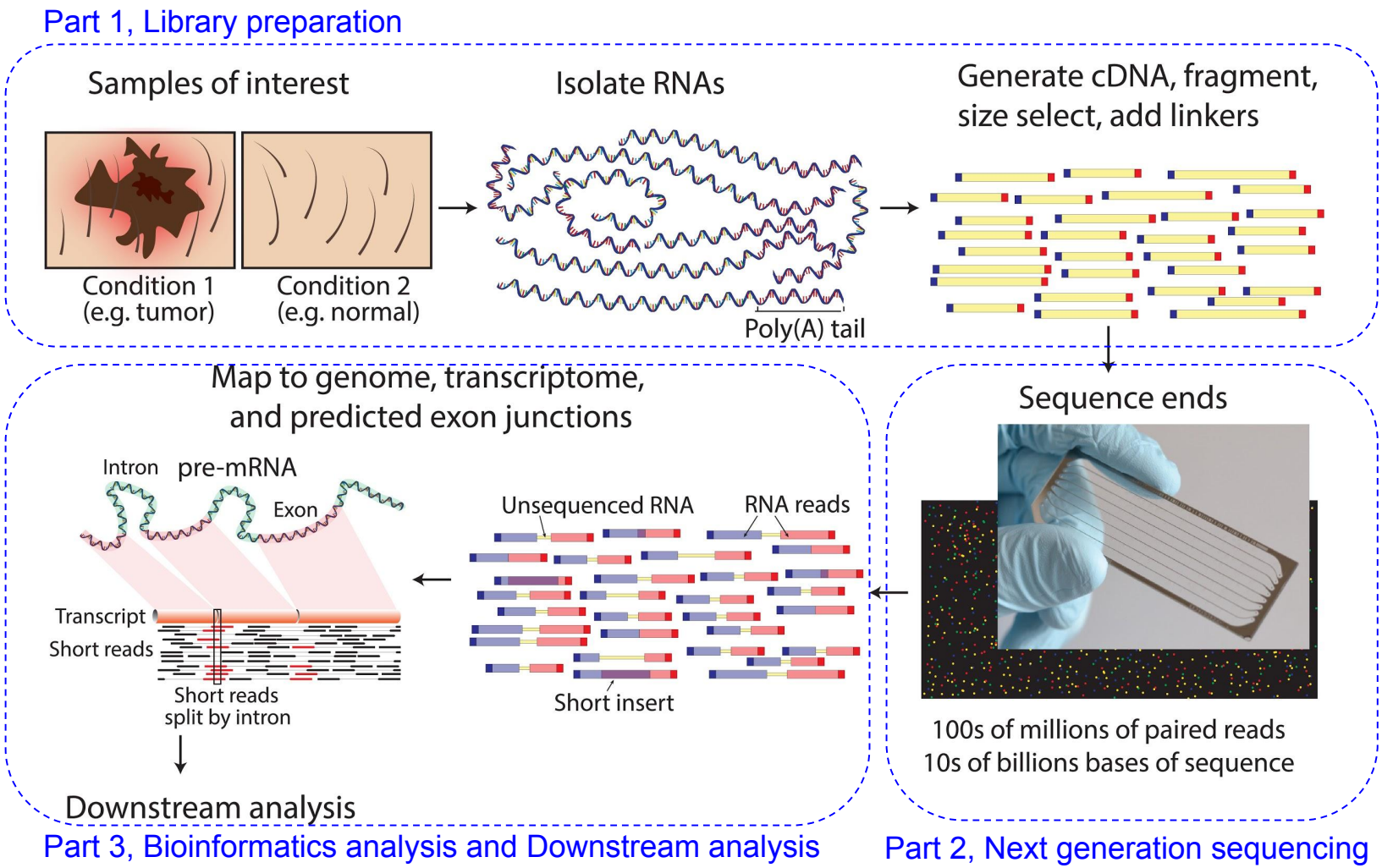


Chan, J.J.; Tay, Y. Noncoding RNA:RNA Regulatory Networks in Cancer. Int. J. Mol. Sci. 2018, 19, 1310.

"Transcriptome Analysis is the study of the transcriptome, of the complete set of RNA transcripts that are produced by the genome, under specific circumstances or in a specific cell, using high-throughput methods. "

https://www.intechopen.com/chapters/66606

Transcriptome analysis using RNA-Seq



Malachi Griffith*, Jason R. Walker, Nicholas C. Spies, Benjamin J. Ainscough, Obi L. Griffith*. 2015. Informatics for RNA-seq: A web resource for analysis on the cloud. PLoS Comp Biol. 11(8).

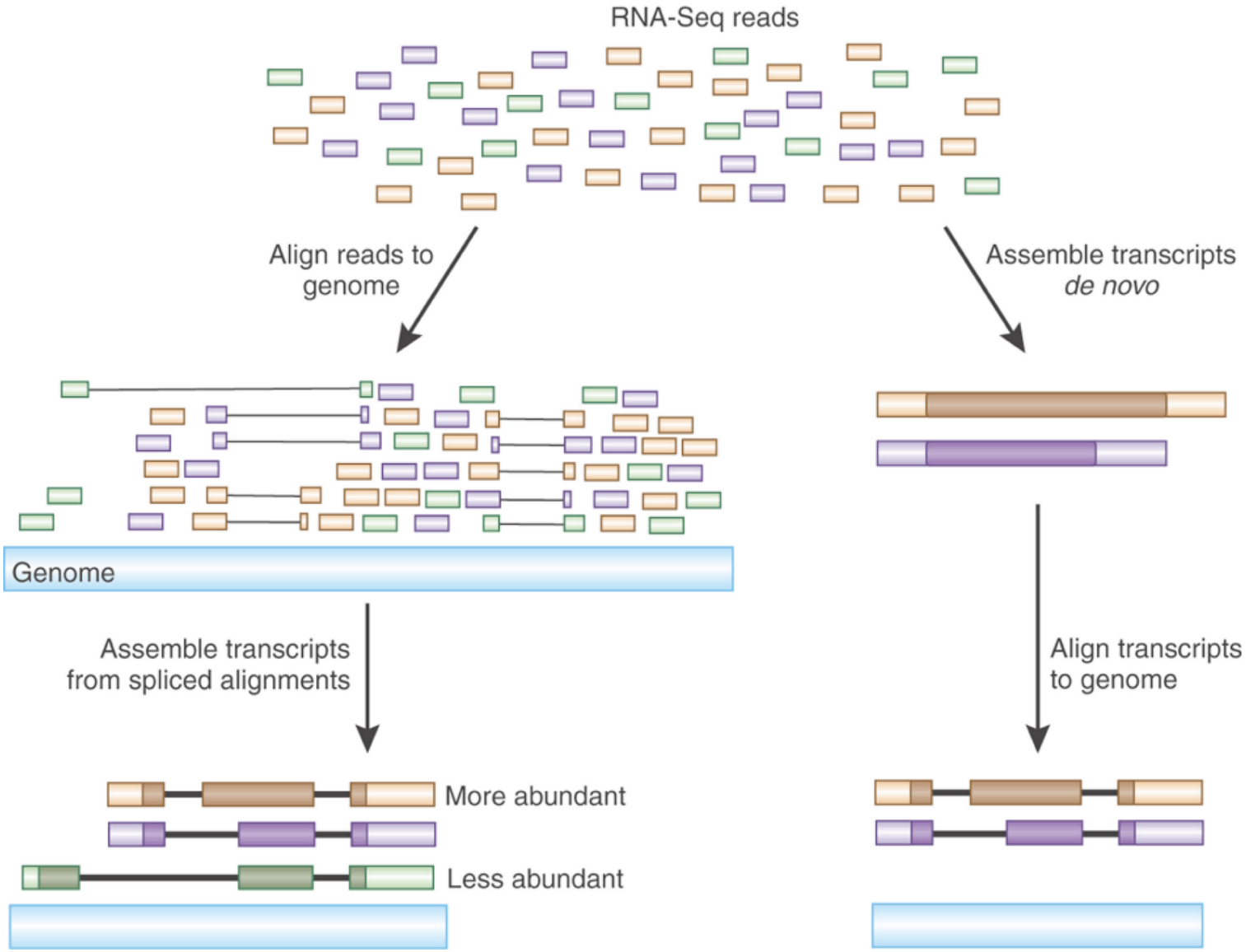
Different types of transcriptome analysis

- transcriptome assembly
- differential gene expression analysis
- alternative splicing
- non-coding RNAs (ncRNAs)
- other analysis

Different types of transcriptome analysis

- **transcriptome assembly**
- differential gene expression analysis
- alternative splicing
- non-coding RNAs (ncRNAs)
- other analysis

Transcriptome assembly using RNA-Seq



Haas, B., Zody, M. Advancing RNA-Seq analysis. Nat Biotechnol 28, 421–423 (2010).

Transcriptome assembly vs genome assembly

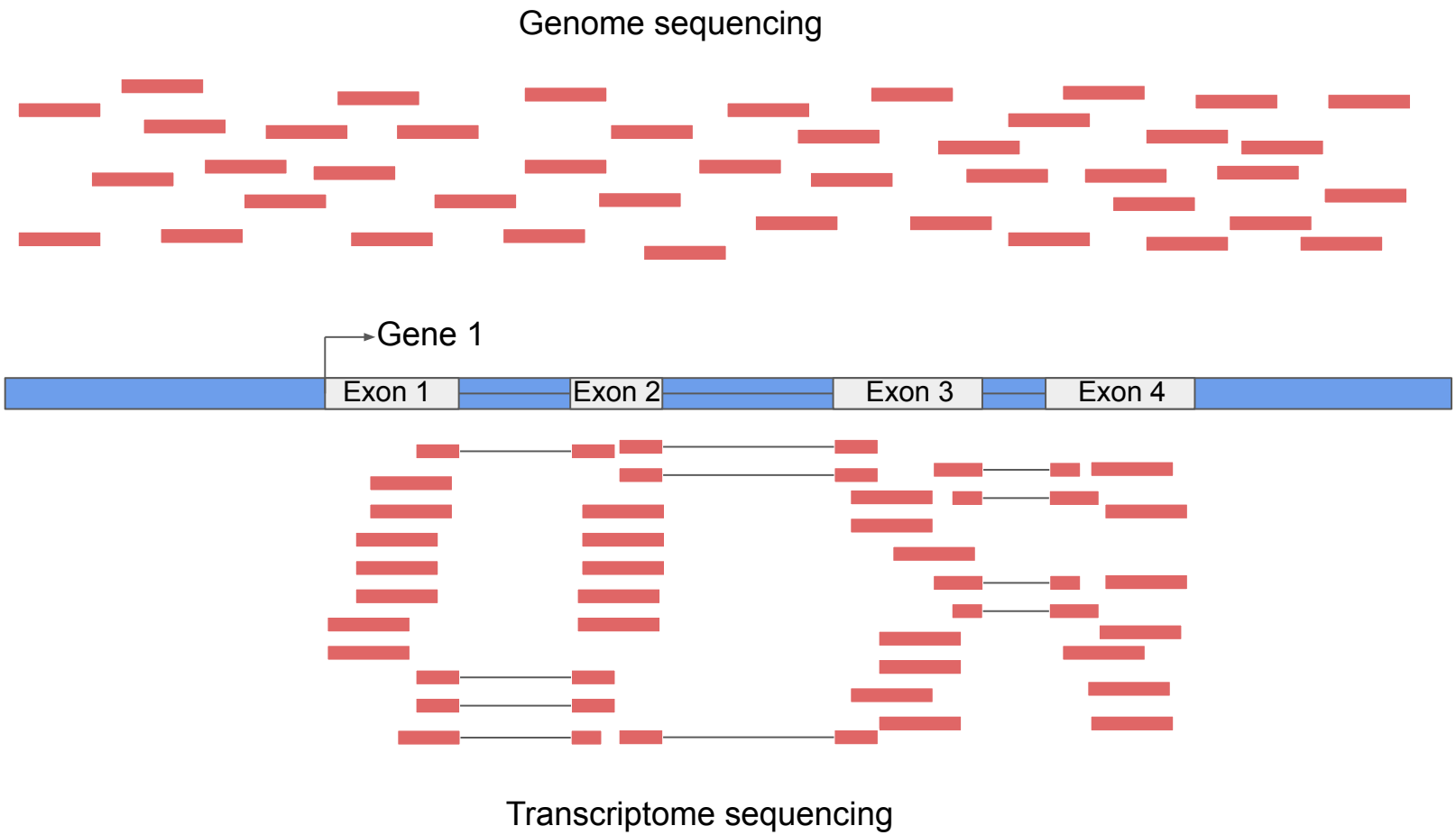
Genome assembly

- Uniform coverage of genome

Transcriptome assembly

- Transcribed regions only

Coverage difference between genome sequencing and transcriptome sequencing



Transcriptome assembly vs genome assembly

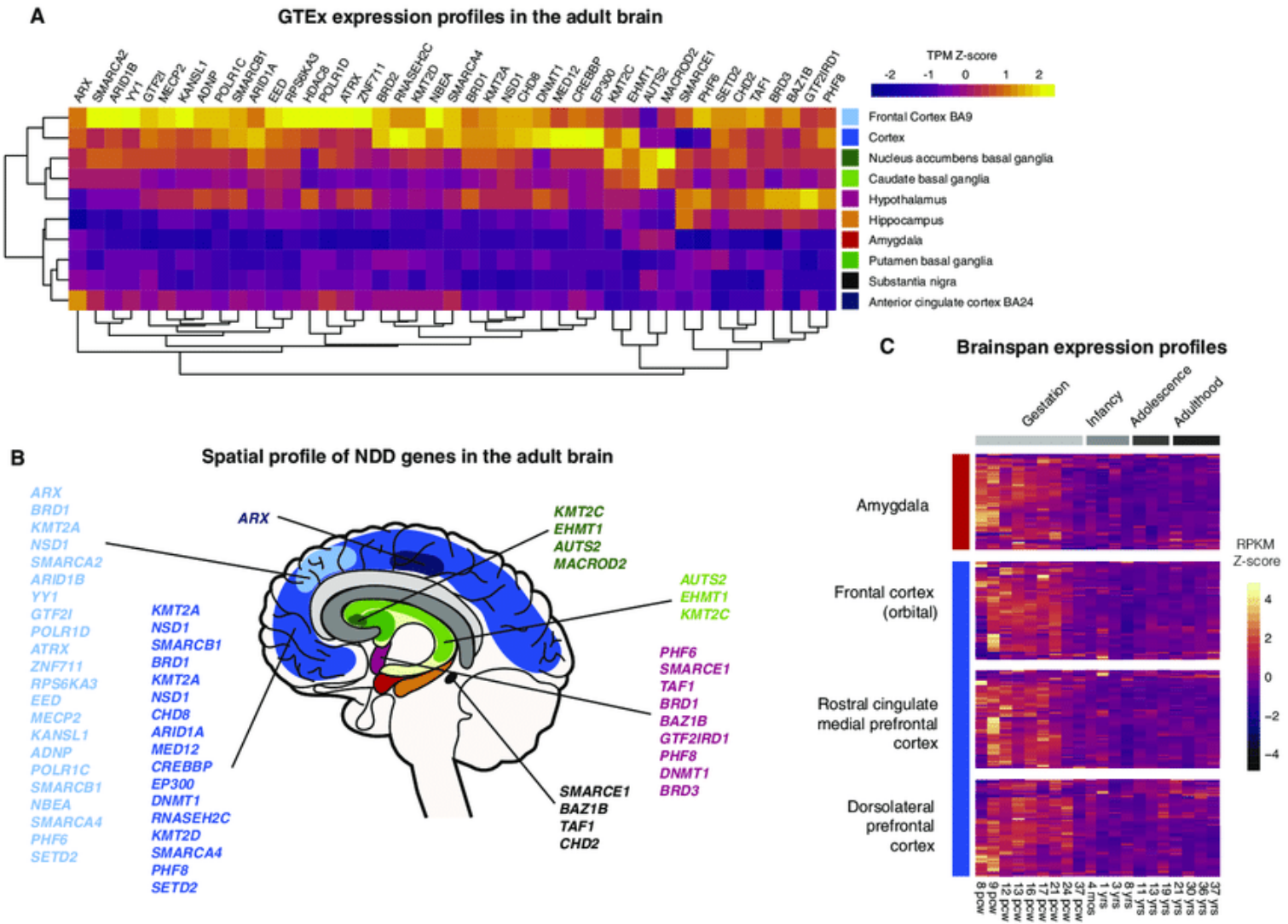
Genome assembly

- Uniform coverage of genome
- Same genome from one individual

Transcriptome assembly

- Transcribed regions only
- Temporal- and spatial-specific

Temporal- and spatial-specific expression of the transcriptome



Gabriele, M., Tobon, L. et al., The chromatin basis of neurodevelopmental disorders: Rethinking dysfunction along the molecular and temporal axes. Progress in Neuro-Psychopharmacology and Biological Psychiatry, (2017).

Transcriptome assembly vs genome assembly

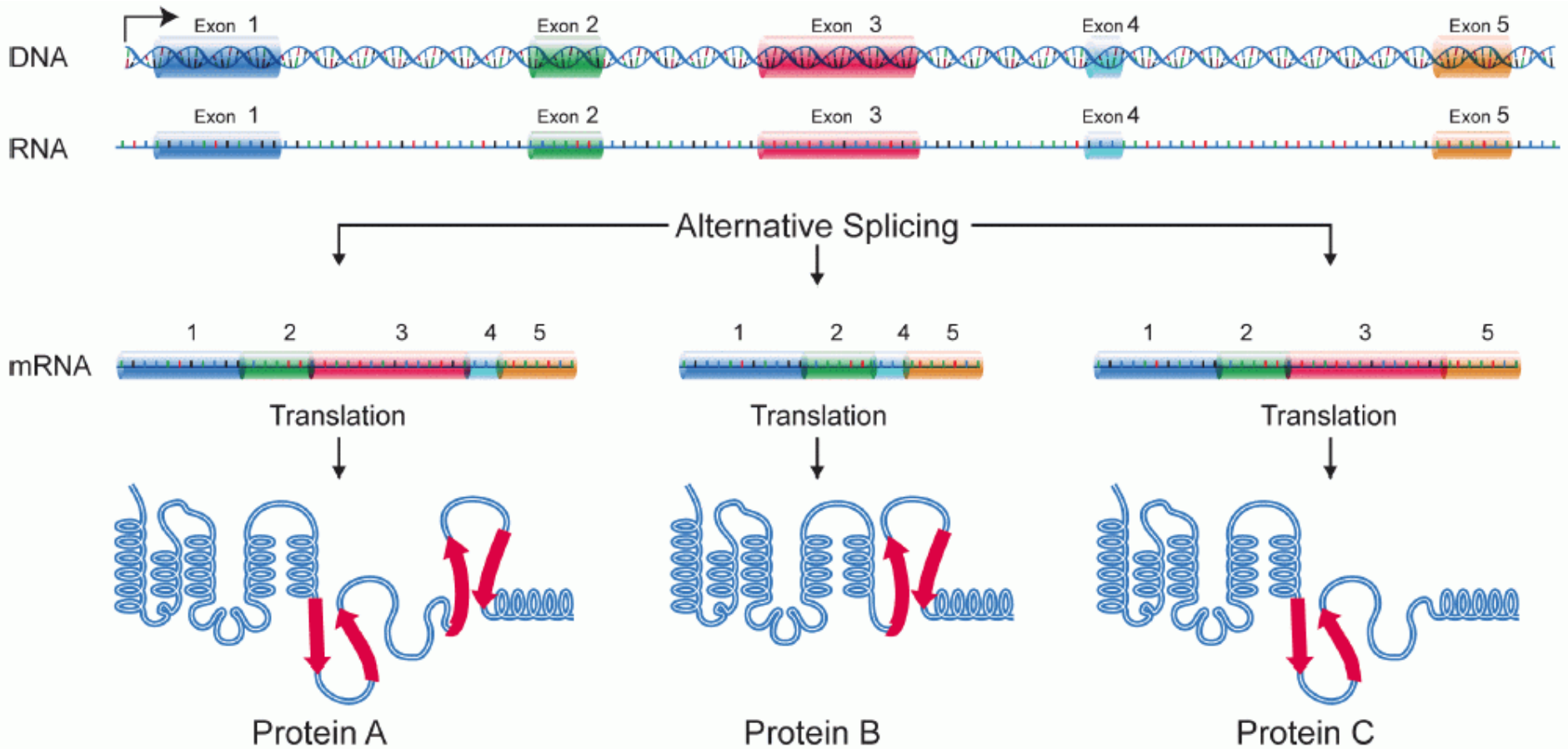
Genome assembly

- Uniform coverage of genome
- Same genome from one individual
- Single copy per locus

Transcriptome assembly

- Transcribed regions only
- Temporal- and spatial-specific
- Single/Multiple copy(ies) per locus (Alternative splicing)

Alternative splicing in the transcriptome



https://en.wikipedia.org/wiki/Alternative_splicing

Transcriptome assembly vs genome assembly

Genome assembly

- Uniform coverage of genome
- Same genome from one individual
- Single copy per locus
- Assemble small number of large contigs (Mb)

Transcriptome assembly

- Transcribed regions only
- Temporal- and spatial-specific
- Single/Multiple copy(ies) per locus (Alternative splicing)
- Assemble thousands of shorter transcripts (Kb)

Transcriptome length distribution

Transcript distribution

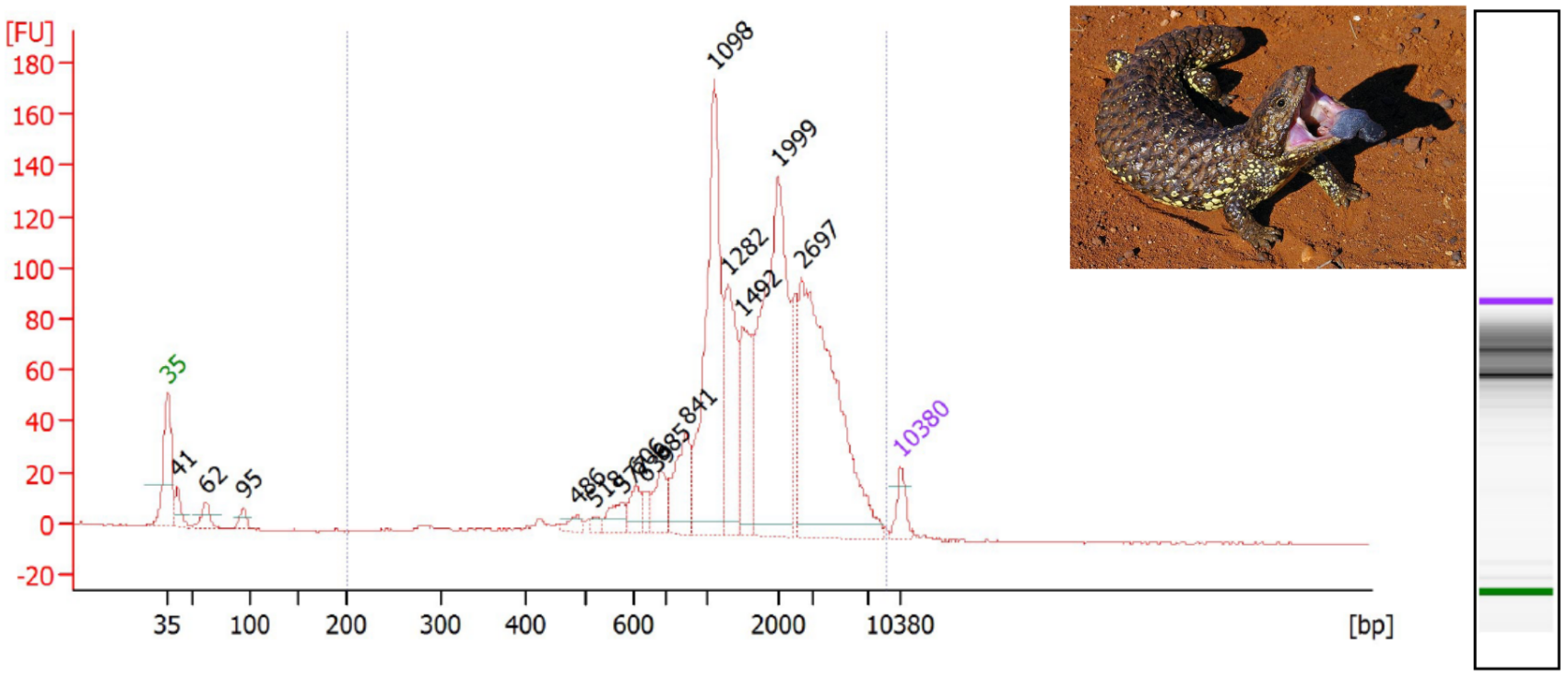


Figure courtesy of Dr Terry Bertozzi

Transcriptome assembly vs genome assembly

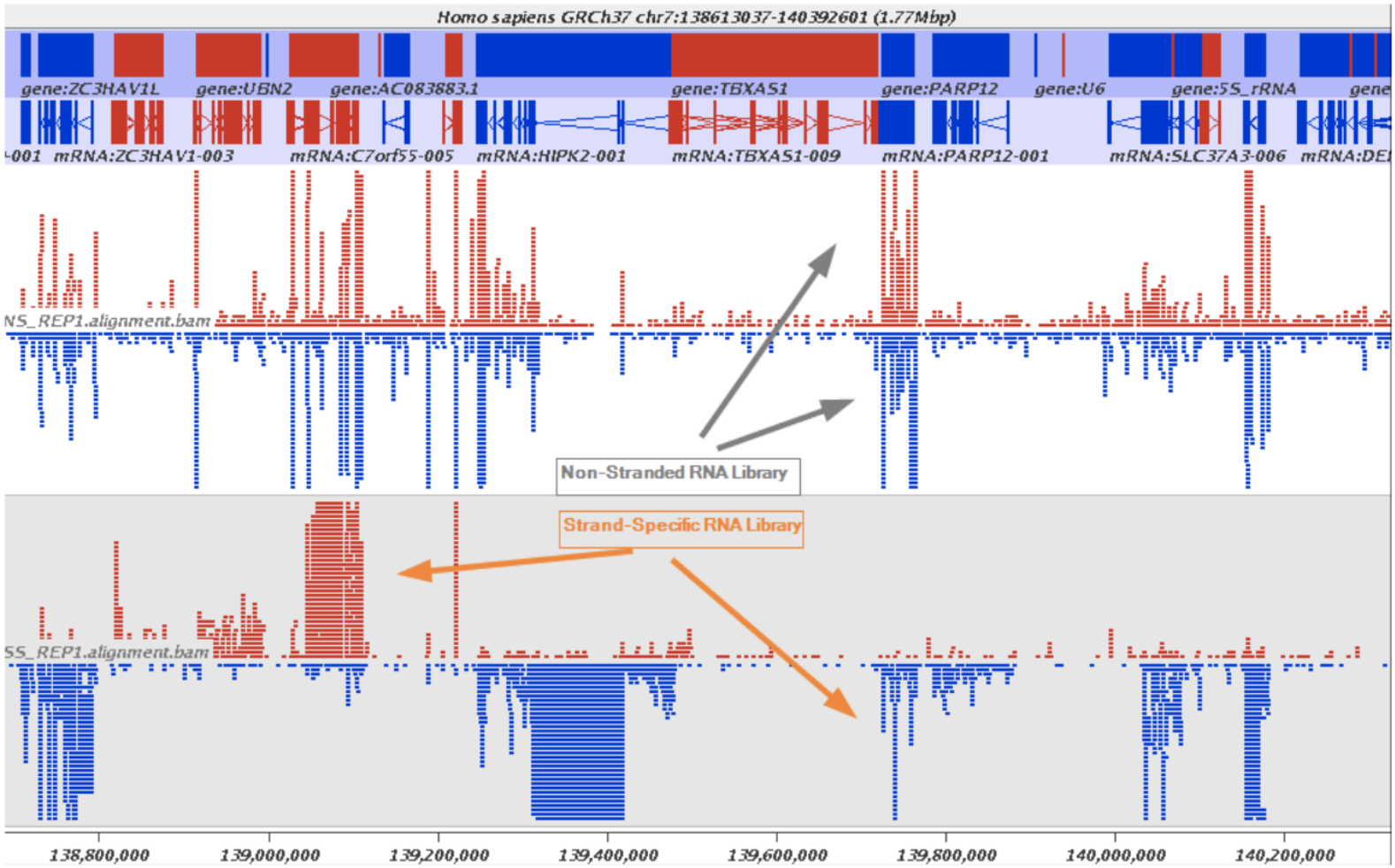
Genome assembly

- Uniform coverage of genome
- Same genome from one individual
- Single copy per locus
- Assemble small number of large contigs (Mb)
- Double stranded

Transcriptome assembly

- Transcribed regions only
- Temporal- and spatial-specific
- Single/Multiple copy(ies) per locus (Alternative splicing)
- Assemble thousands of shorter transcripts (Kb)
- Strand specific

Strand specific expression in transcriptome



https://training.galaxyproject.org/training-material/topics/transcriptomics/tutorials/rb-rnaseq/tutorial.html

De novo transcriptome assembly

Why do we need *de novo* transcriptome assembly?

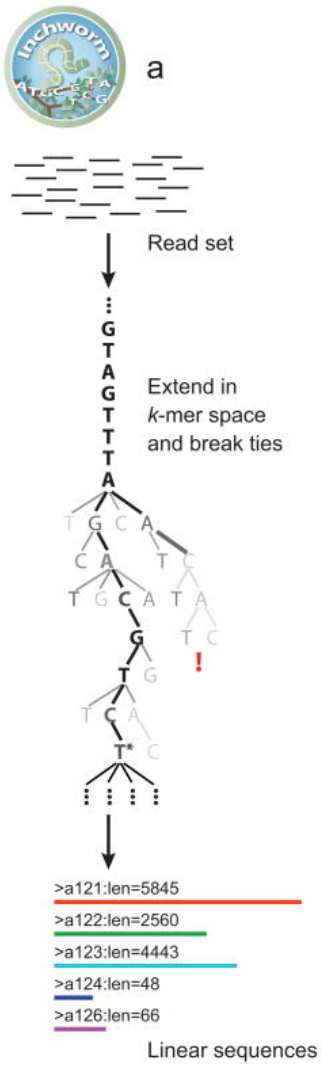
- No available reference genome (non-model organisms)
- Reference genome is not in high quality
- Cheaper than whole genome assembly

De novo transcriptome assembly - Trinity

- Developed at the Broad Institute and the Hebrew University of Jerusalem
- De Bruijn graphs based short read assembler
- Three independent software modules: Inchworm, Chrysalis, and Butterfly
- Outputs a FASTA format sequence file

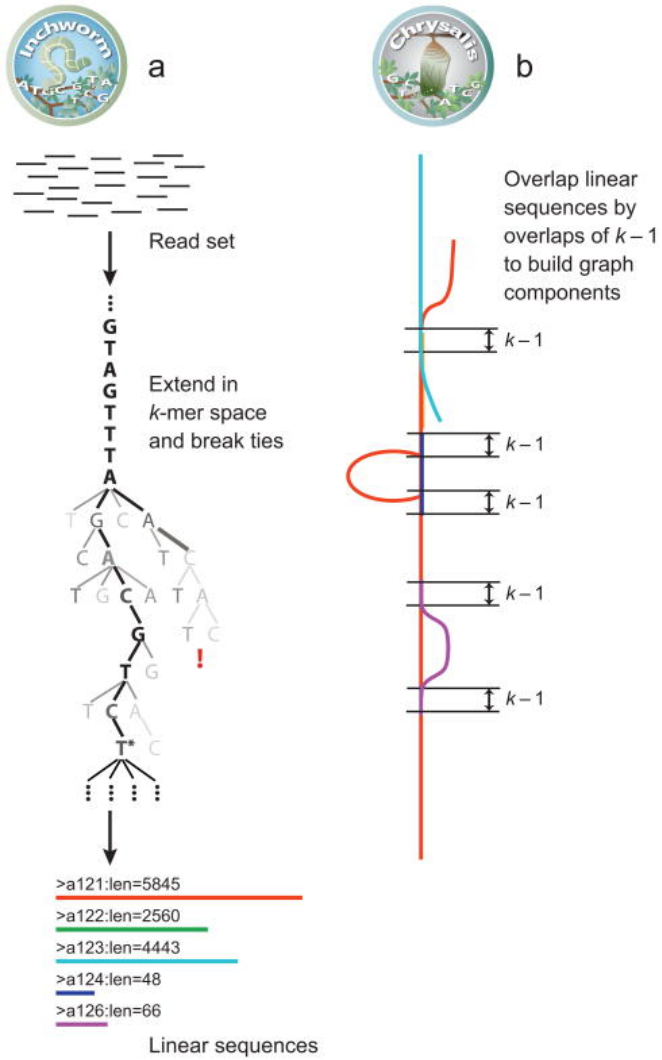
`https://github.com/trinityrnaseq/trinityrnaseq/wiki`

Trinity - step 1



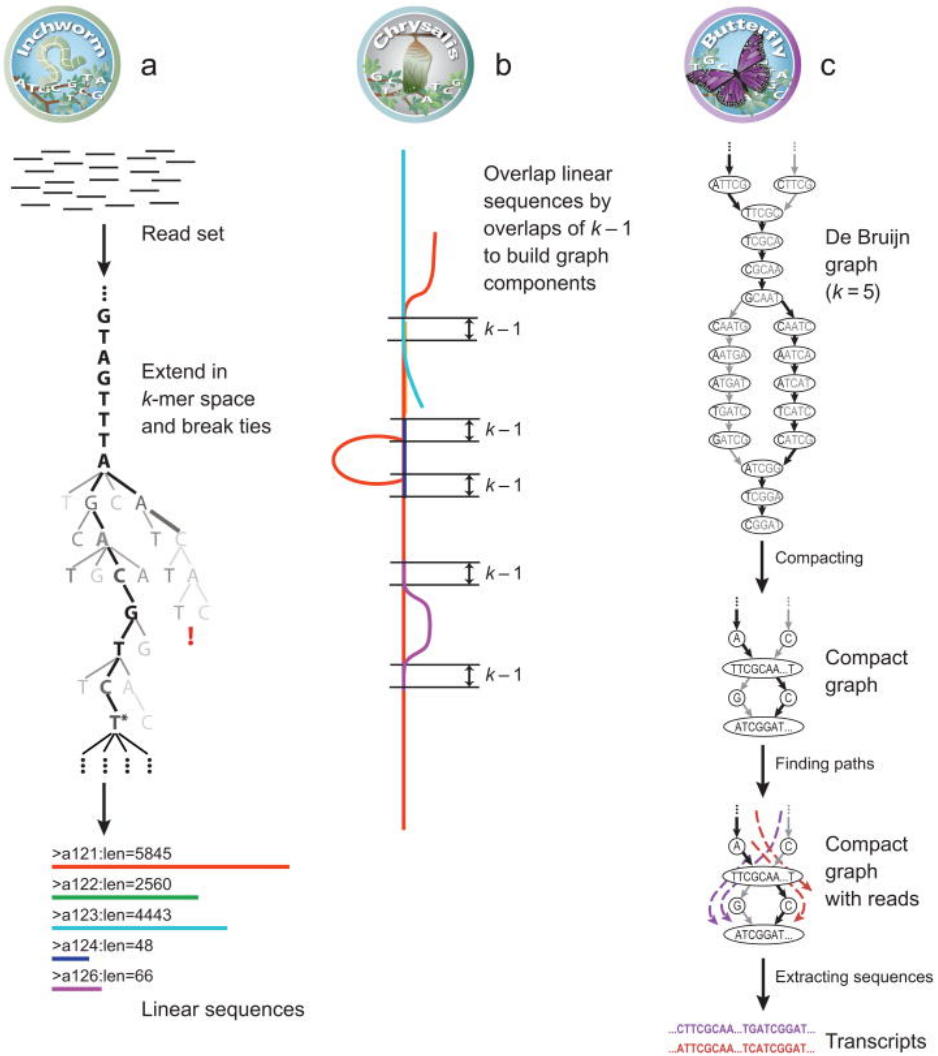
Grabherr M., et al., Trinity: reconstructing a full-length transcriptome without a genome from RNA-Seq data. Nat Biotechnol. 2021 Jul; 29(7): 644-652

Trinity - step 2



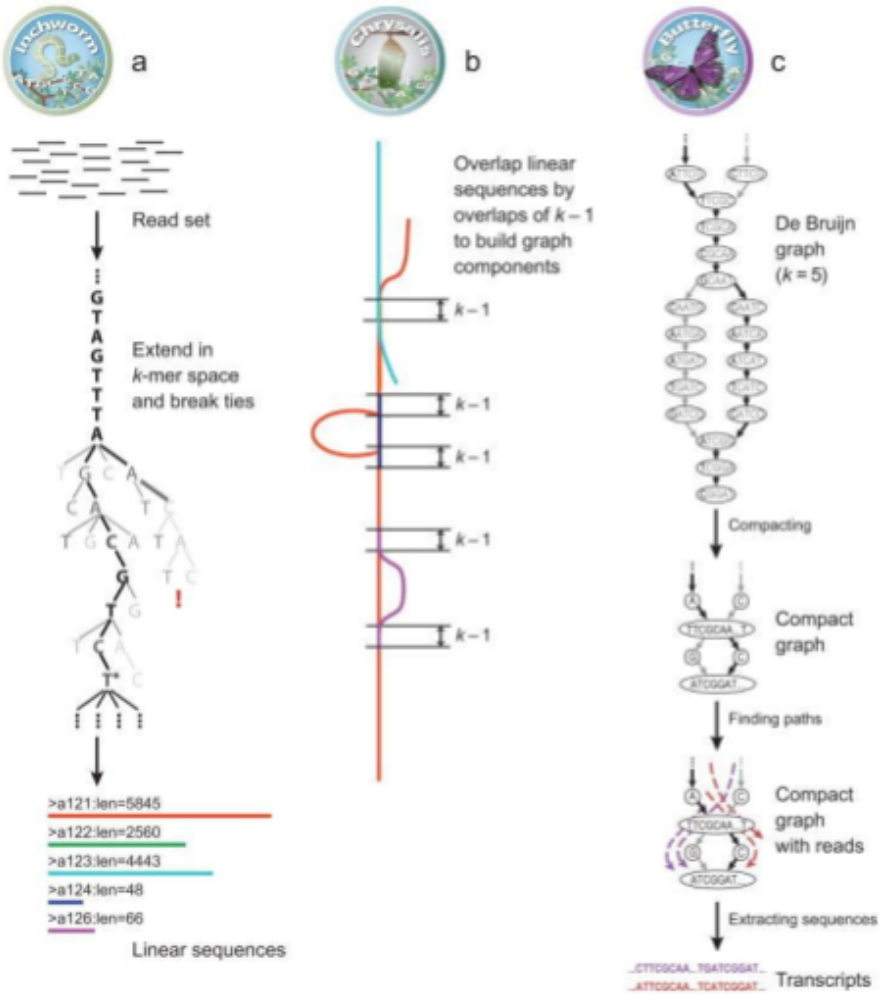
Grabherr M., et al., Trinity: reconstructing a full-length transcriptome without a genome from RNA-Seq data. Nat Biotechnol. 2021 Jul; 29(7): 644-652

Trinity - step 3



Grabherr M., et al., Trinity: reconstructing a full-length transcriptome without a genome from RNA-Seq data. Nat Biotechnol. 2021 Jul; 29(7): 644-652

Trinity - Output

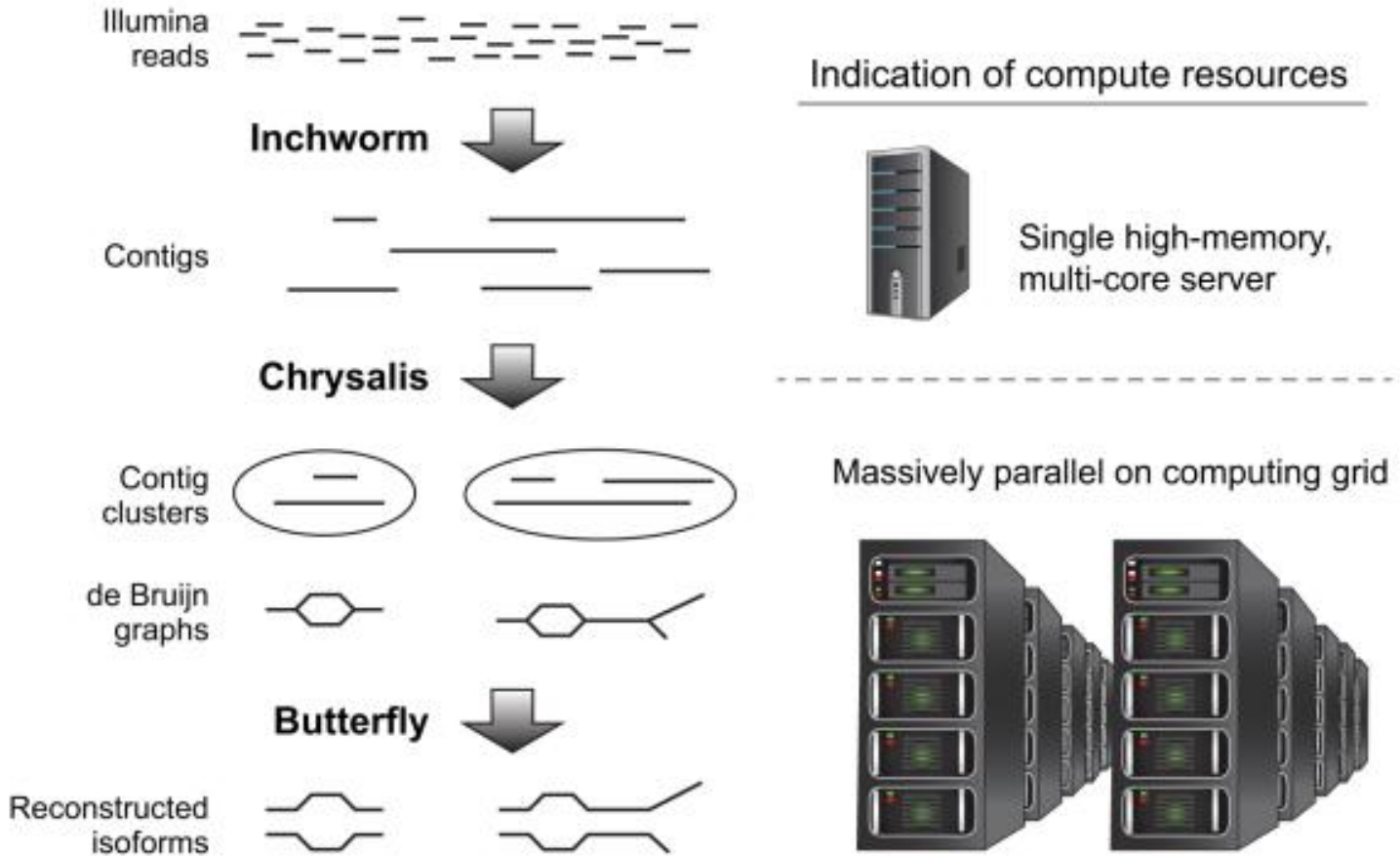


Output: Trinity.fasta

```
>comp0_c0_seq1 len=5528 path=[3647:0-3646 129:3647-3775 1752:3776-5527]
AATTGAATCCCTTTTTGTATTGAAAAAGTTGAAATGAAAGACATATACAGAT
TGAATGTG…TCCTCTGATACACAGCCTCGCAGGGTTCATTTCAAGCCGTGGG
GCTGCGCCACGGGTGCTAAGTCAACTGCATTCGATGCGGCTTTTAAACCCCC
AGGGGACACCTCGGCCAGCTGTTTGCCTGCAGTA…TTGTGTTTCTTCAACAG
TTTATCAGCTTGCTGAATTGCCATTTTATTATTTCCATTATCAAGATAATCG
TAAATGGGCCGGAGGCGCCGGTCGTTAGGGTCCTGCACATGGCCCCGCGTCG
CCATGATGACAAGCGCAGAACCTCAGT
>comp0_c0_seq2 len=5399 path=[3647:0-3646 1752:3647-5398]
AATTGAATCCCTTTTTGTATTGAAAAAGTTGAAATGAAAGACATATACAGAT
TGAATG…TGGTGATTGCAAAATATAATGCAATTTCGAACAATTAAAATTATG
AAAATATAC…TTGTGTTTCTTCAACAGTTTATCAGCTTGCTGAATTGCCATT
TTATTATTTCCATTATCAAGATAATCGTAAATGGGCCGGAGGCGCCGGTCGT
TAGGGTCCTGCACATGGCCCCGCGTCGCCATGATGACAAGCGCAGAACCTCA
GT
```

Grabherr M., et al., Trinity: reconstructing a full-length transcriptome without a genome from RNA-Seq data. Nat Biotechnol. 2021 Jul; 29(7): 644-652

Trinity - required compute resources

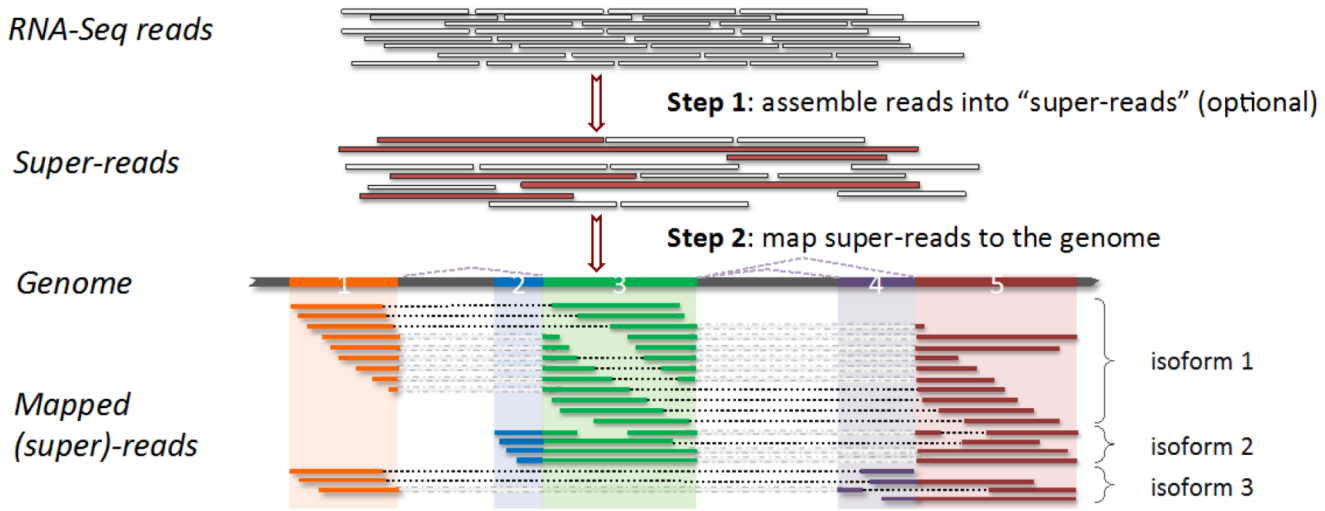


Haas B., et al., De novo transcript sequence reconstruction from RNA-Seq: reference generation and analysis with Trinity. Nat Protoc. 2013 Aug; 8(8)

When do we use genome guided transcriptome assembly?

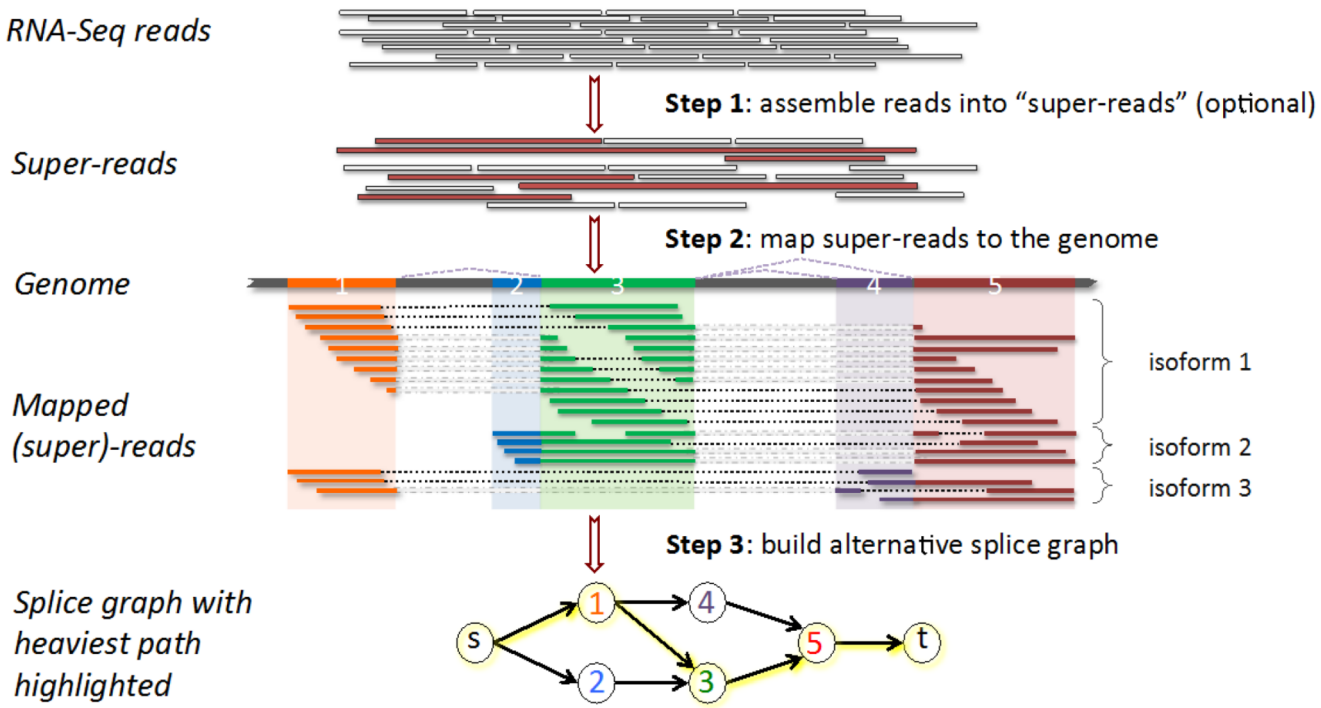
- With an available reference genome
- To improve current gene annotation
- To identify alternative splicing isoforms
- To identify non-coding RNAs (ncRNAs)

StringTie



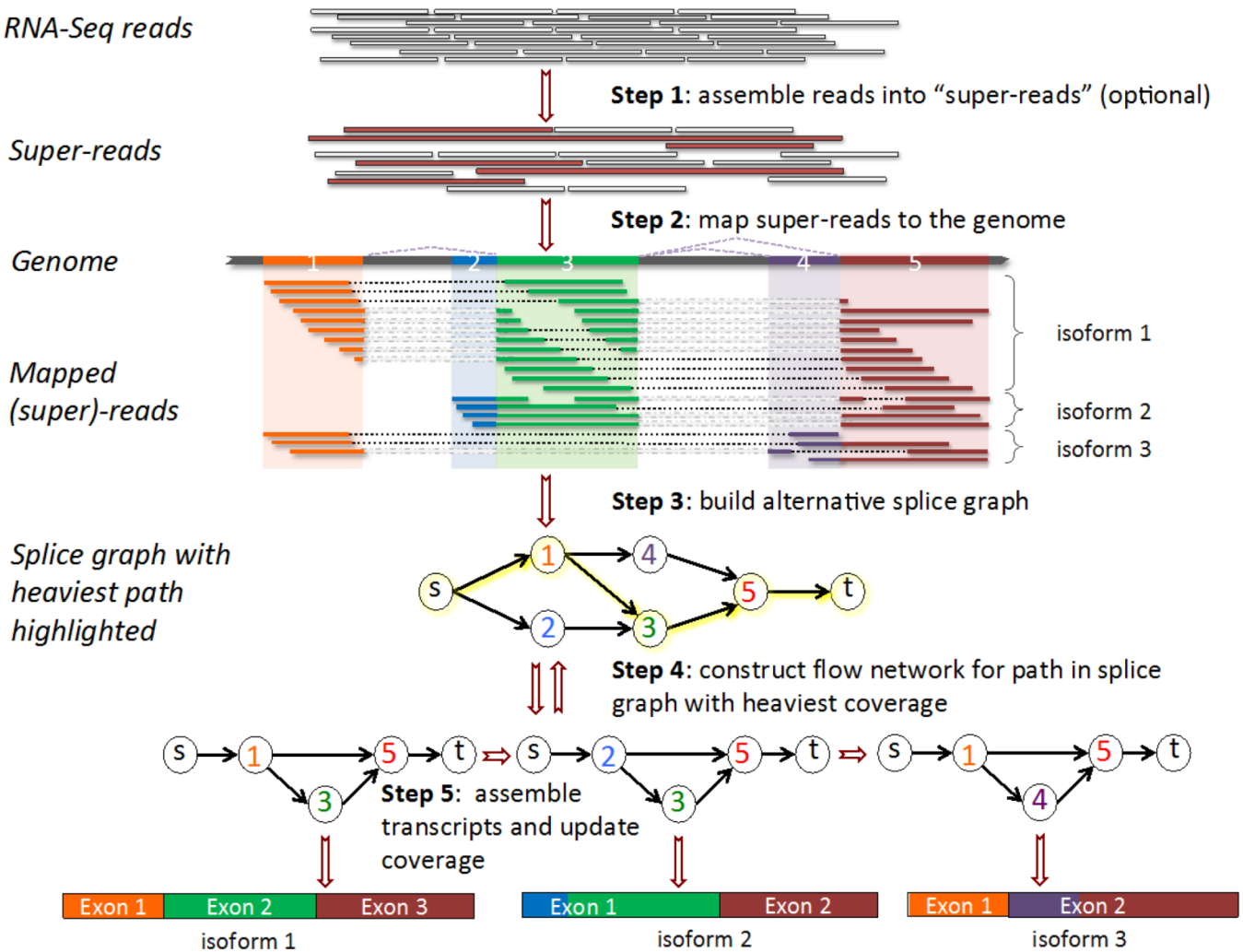
Pertea M., et al., StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. Nat Biotechonol. 2015 Mar; 33(3): 290-295

StringTie



Pertea M., et al., StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. Nat Biotechonol. 2015 Mar; 33(3): 290-295

StringTie



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StringTie

```
seqname source      feature     start   end     score   strand  frame attributes
chrX    StringTie   transcript  281394  303355  1000    +       .     gene_id "ERR188044.1";
chrX    StringTie   exon        281394  281684  1000    +       .     gene_id "ERR188044.1";
...
```

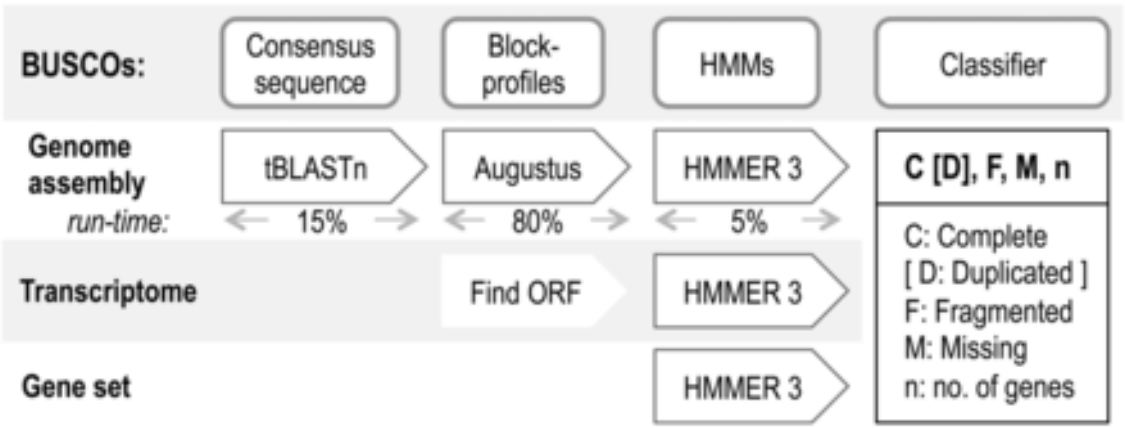
- StringTie outputs a Gene Transfer Format (GTF) file that contains details of the transcripts that StringTie assembles from RNA-Seq data. The definition of each column in this GTF file can be found at ENSEMBL.
- StringTie can also calculate coverage for expression analysis.

Pertea M., et al., StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. Nat Biotechonol. 2015 Mar; 33(3): 290-295

`http://asia.ensembl.org/info/website/upload/gff.html`

Assessing assembly completeness - BUSCO

- Benchmarking Universal Single-Copy Orthologs (BUSCO)
- Can be used on both genome and transcriptome assemblies



Simao F., et al., BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics. 2015 October; 31(19): 3210-3212

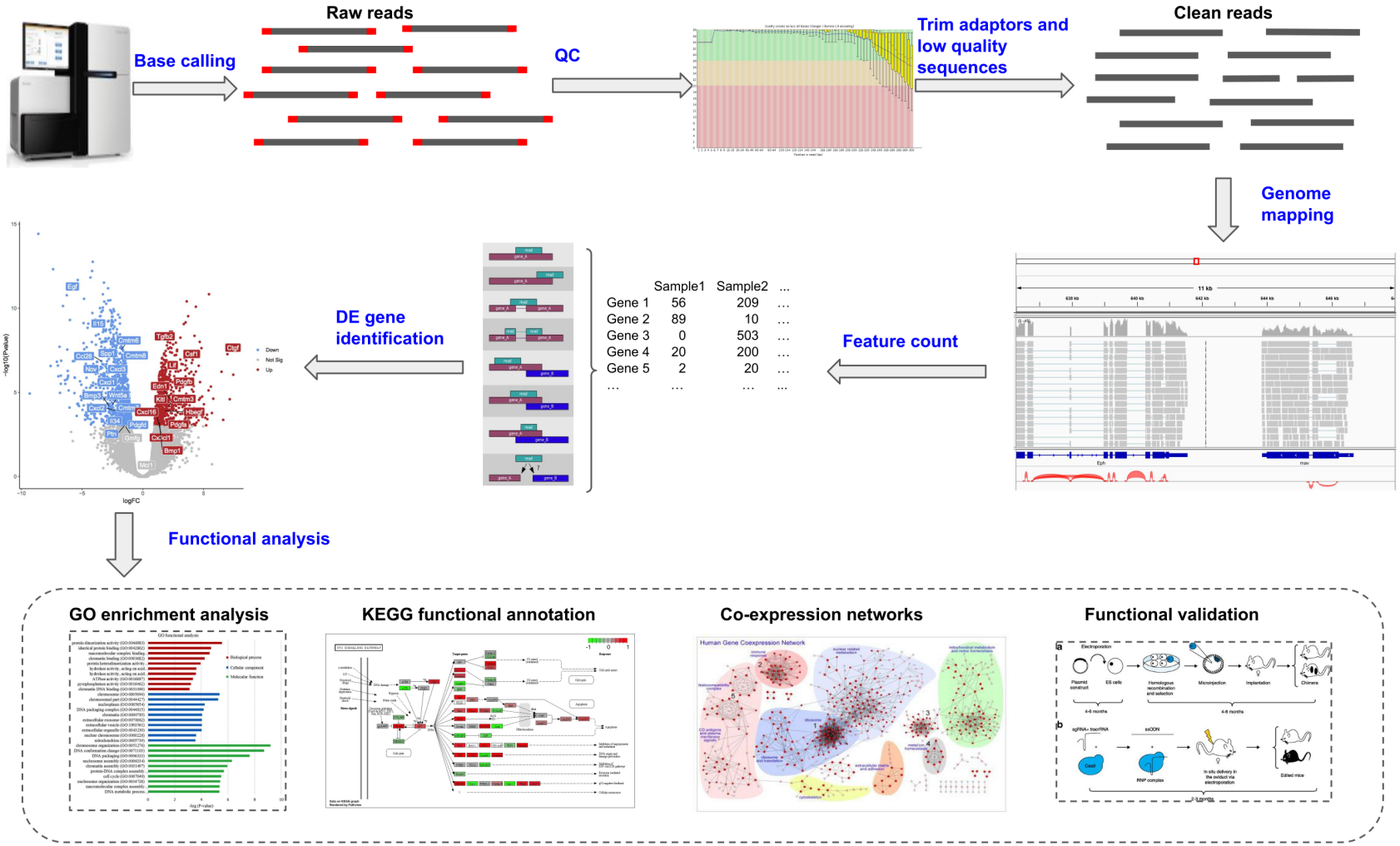
Assessing assembly completeness - BUSCO report

C:89.0%[S:85.8%,D:3.2%],F:6.9%,M:4.1%,n:3023

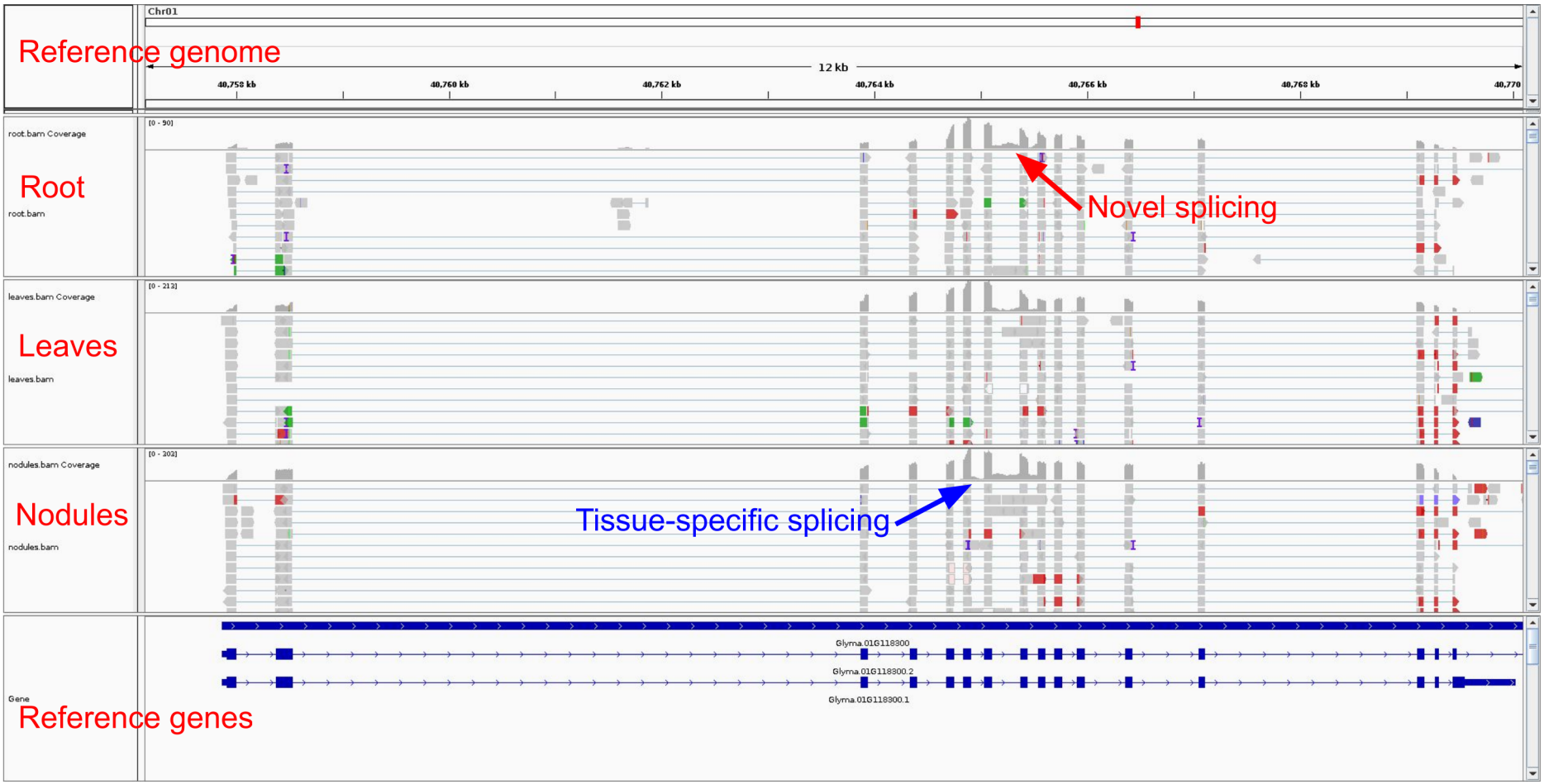
- C: Complete orthologs
  - S: Single copy
  - D: Duplicated copy
- F: Fragmented copy
- M: Missing copy
- n: Total orthologs

Simao F., et al., BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics. 2015 October; 31(19): 3210-3212

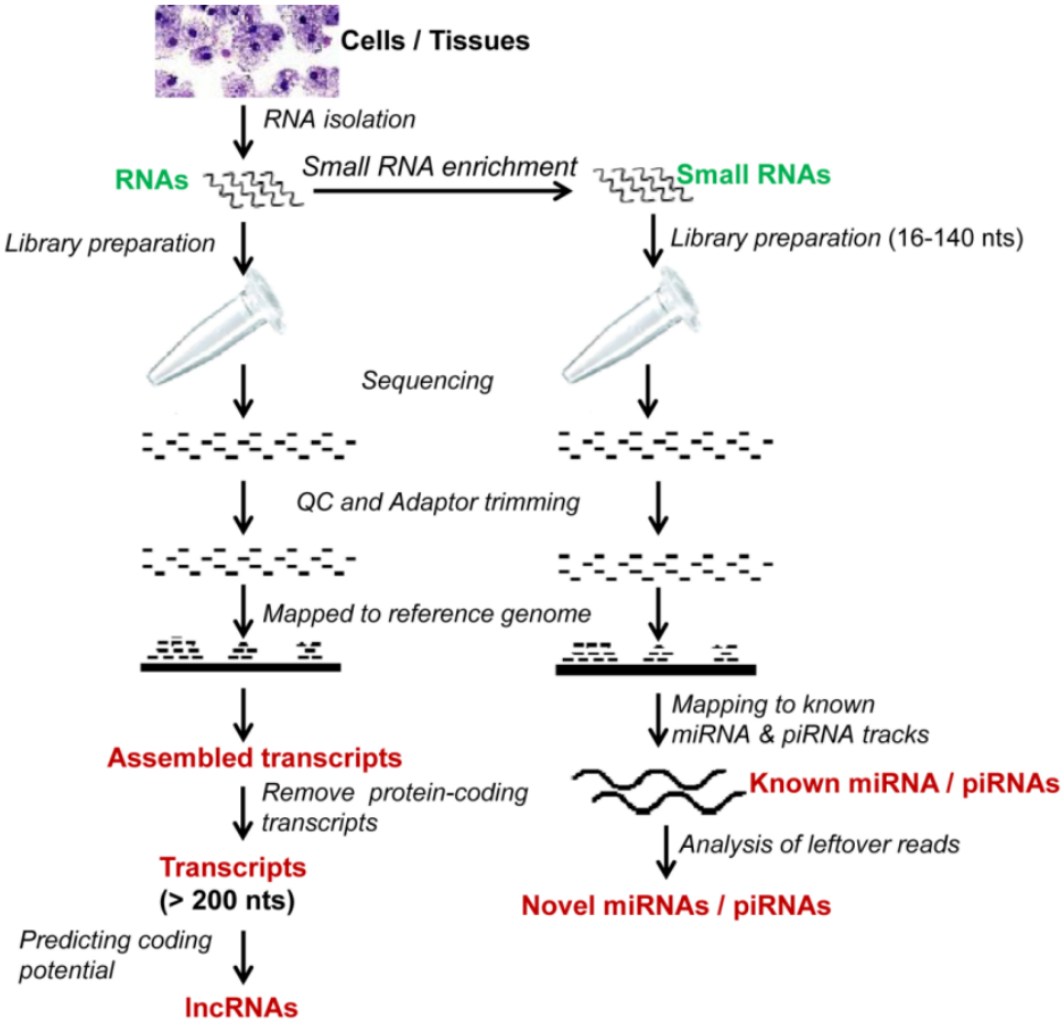
Other transcriptome analysis - Differential gene expression analysis



Other transcriptome analysis - Alternative splicing



Other transcriptome analysis - non-coding RNAs (ncRNAs)



Mallick (2016) Decrypting the Treasures of Regulatory Non-coding RNAs in High-throughput Era. J Data Mining Genomics and Proteomics 7: e124.

Lecture summary

- Transcriptome analysis is the study of the total RNA readout
- Transcriptome assembly has many challenges compared to genome assembly
- De novo transcirptome assembly - Trinity
- Genome guided transcriptome assembly - StringTie
- Assessing assembly using BUSCO