TRANSCRIPTOMICS

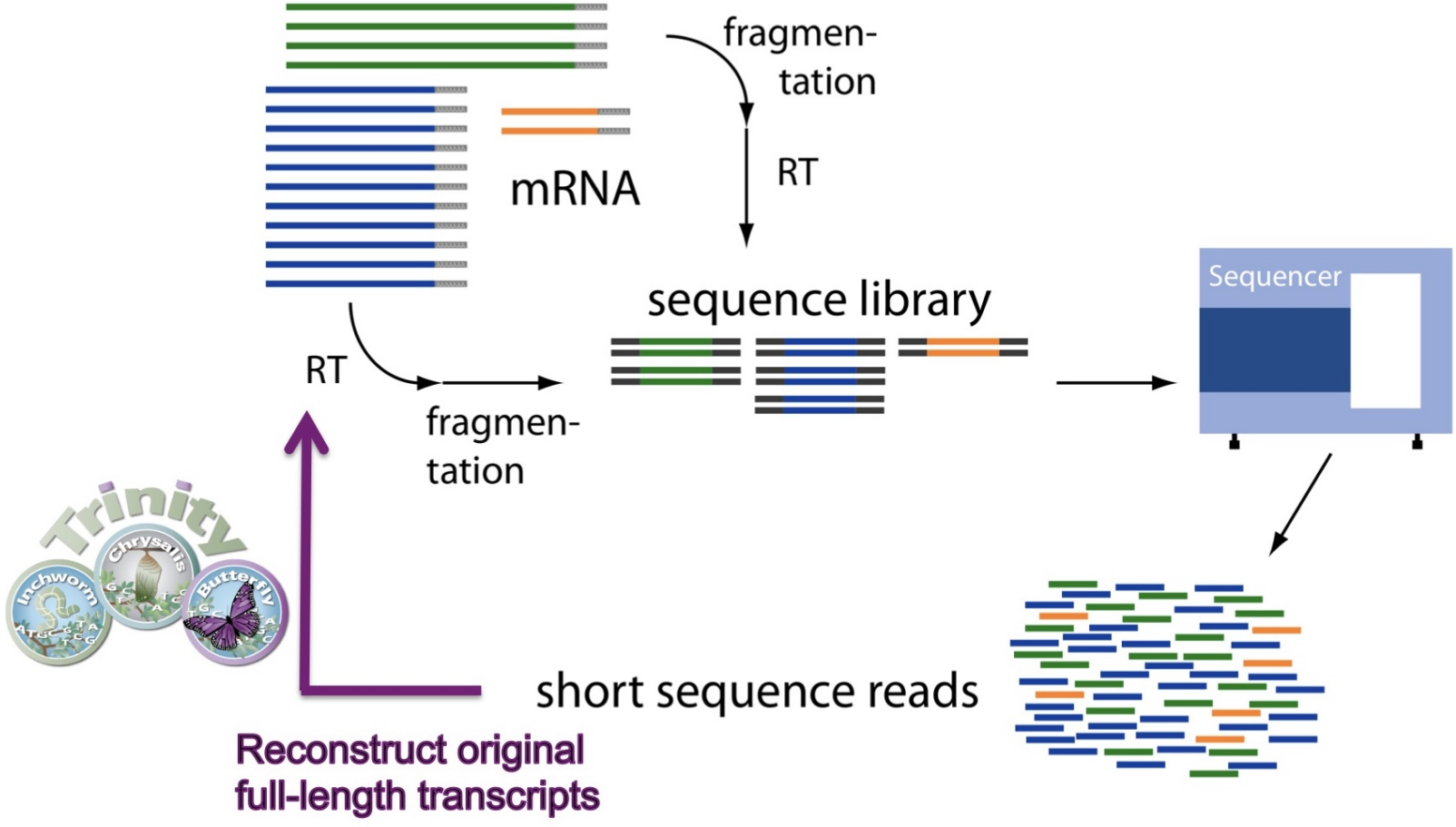
Assembly with Trinity
Part II

Day 03

https://tttorres.github.io/transcriptomics/

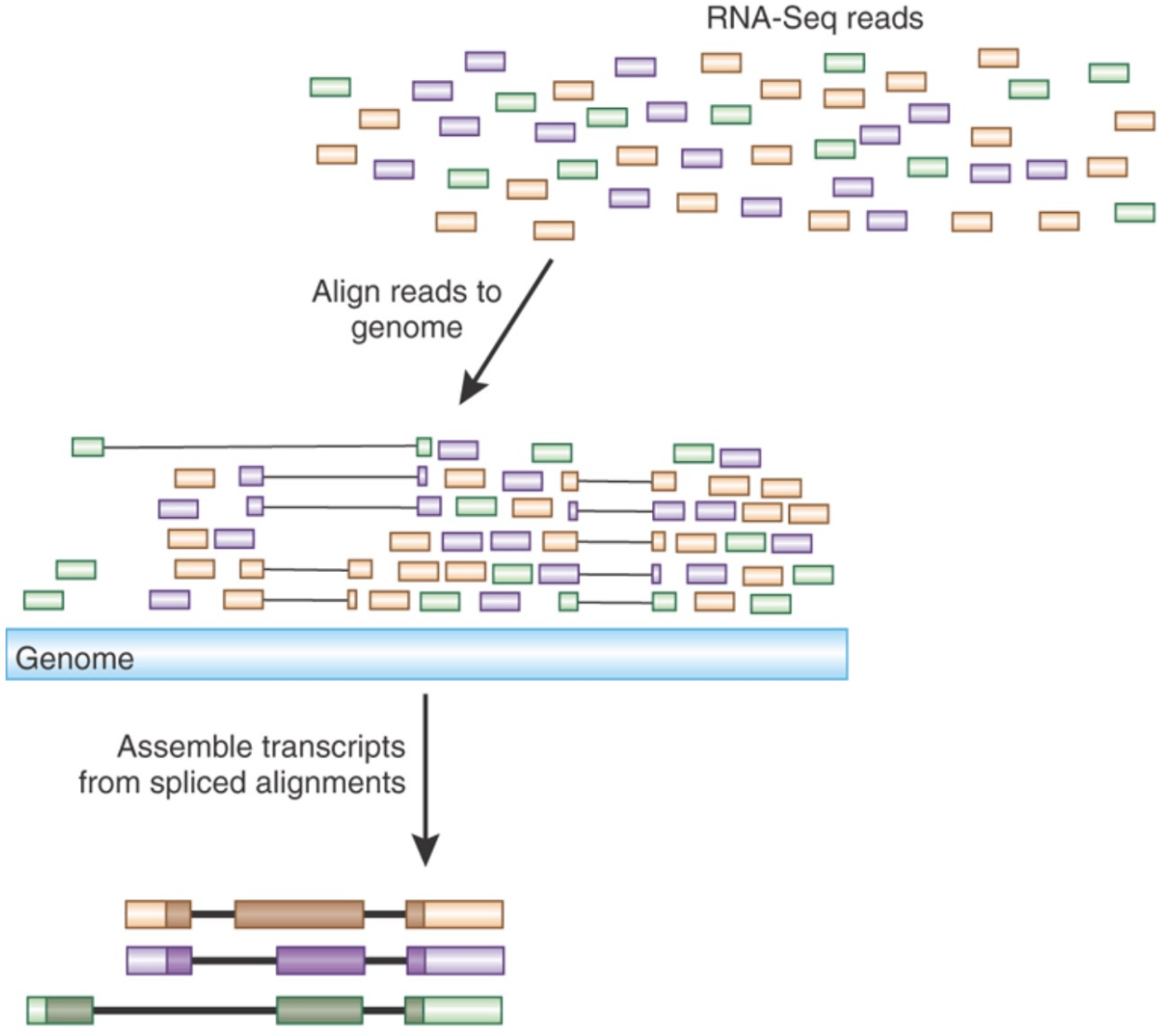
Assembly of RNA-seq reads

Overview



Assembly of RNA-seq reads

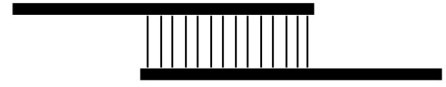
Reference genome



Assembly by overlap

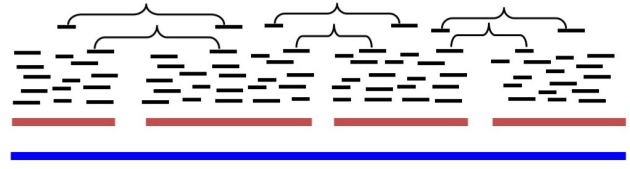
Usual steps

1. Finding overlaps between read pairs



2. Combining overlaping reads into contigs

3. Joining contigs to form scaffolds

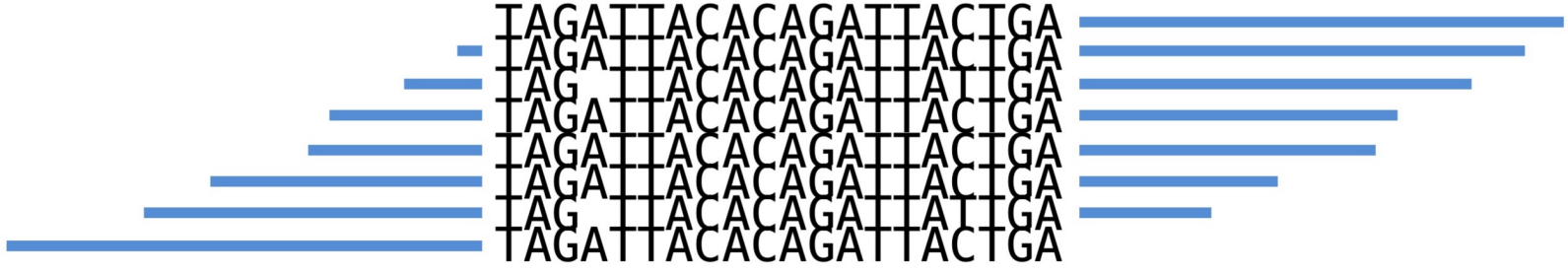


4. Determining the consensus sequence ..ACGATTACAATAGGTT..

Assembly by overlap

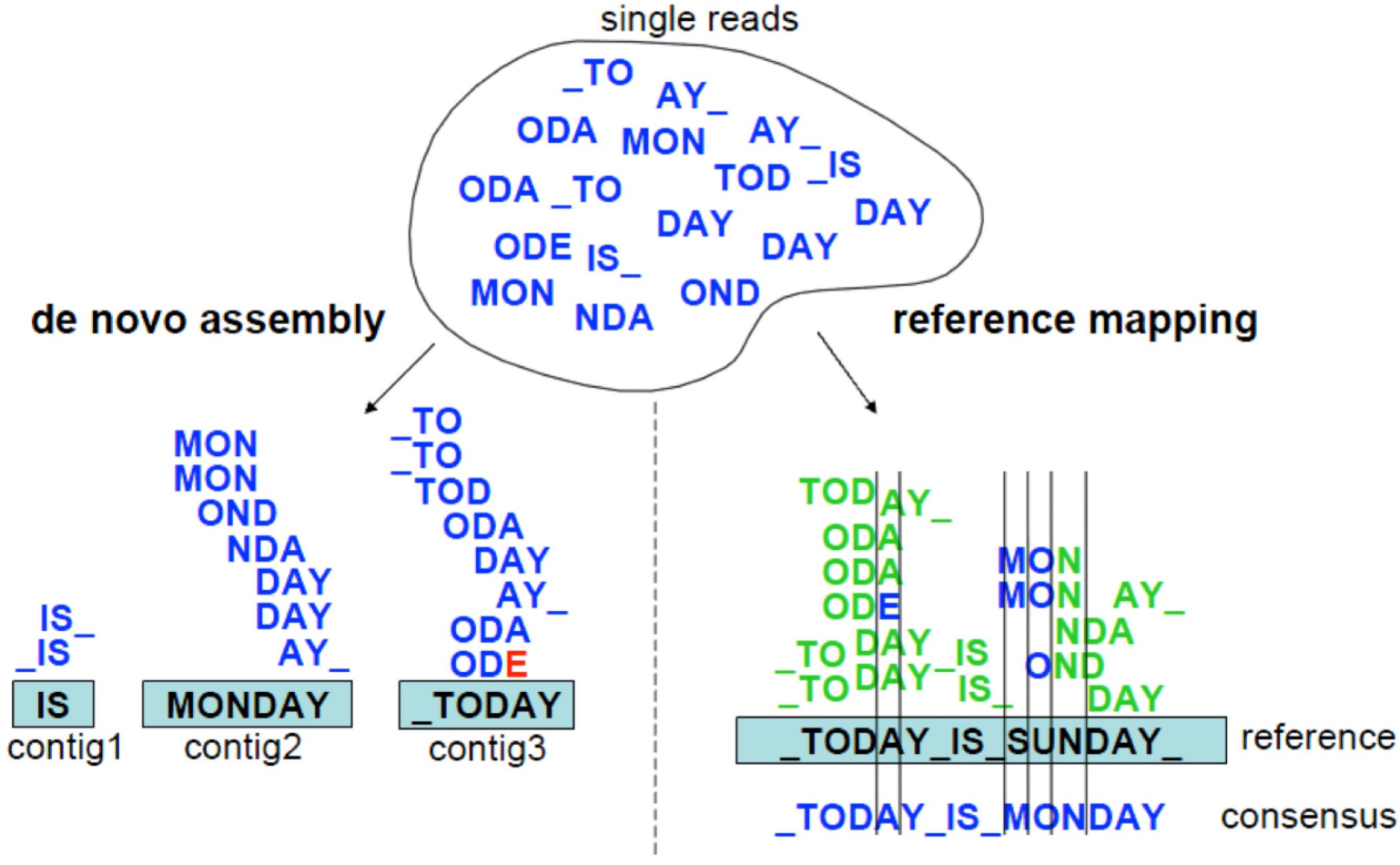
Usual steps

Creating multiple local alignments



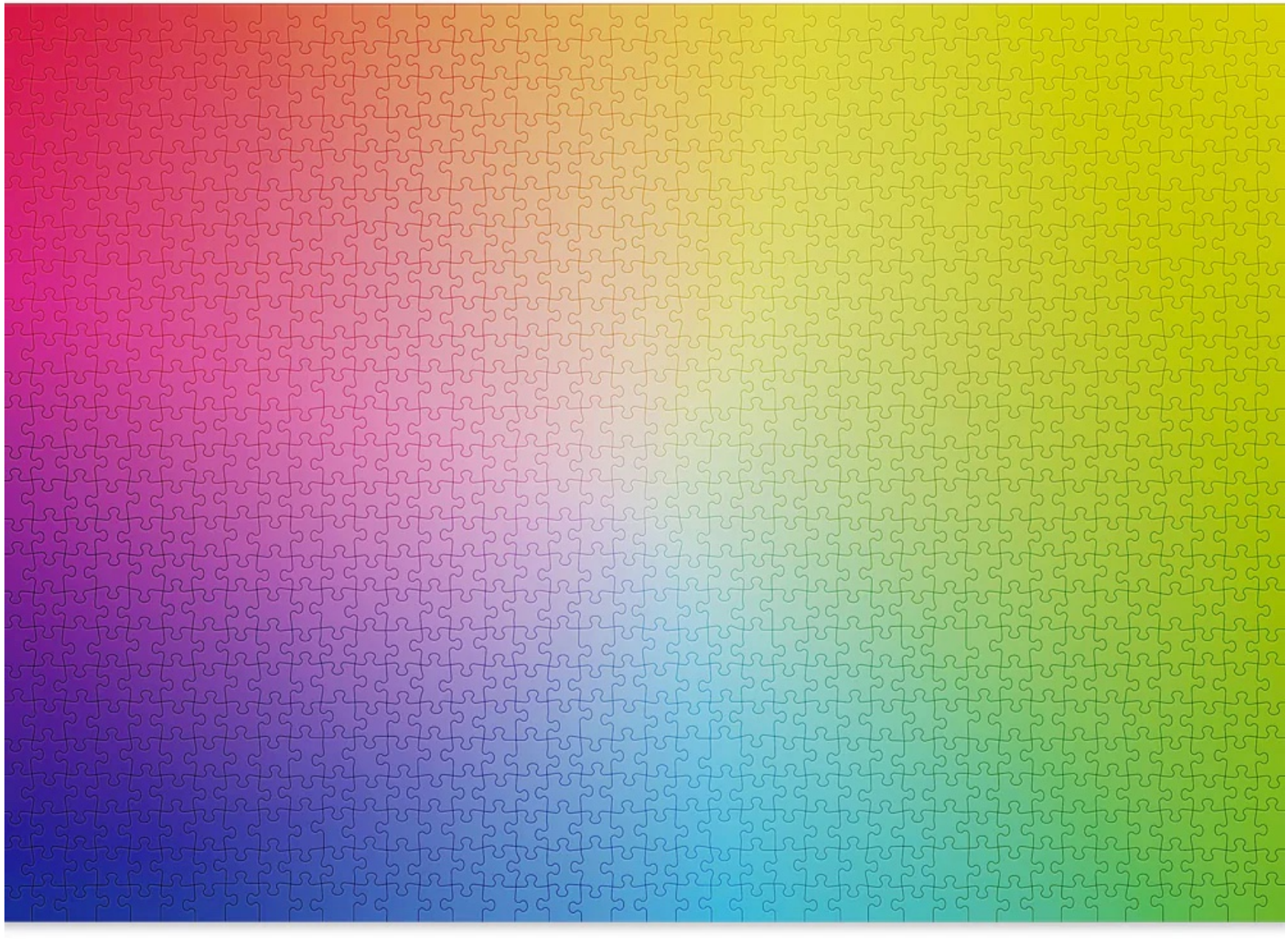
Assembly by overlap

Usual steps



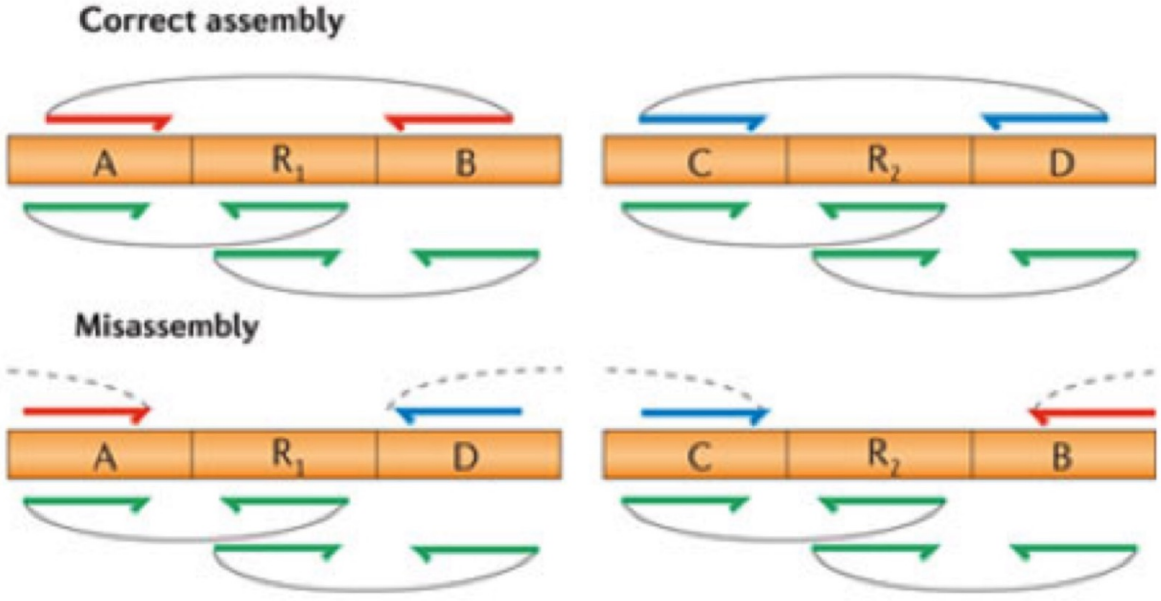
Assembly by overlap

How do you assemble a puzzle with identical pieces?



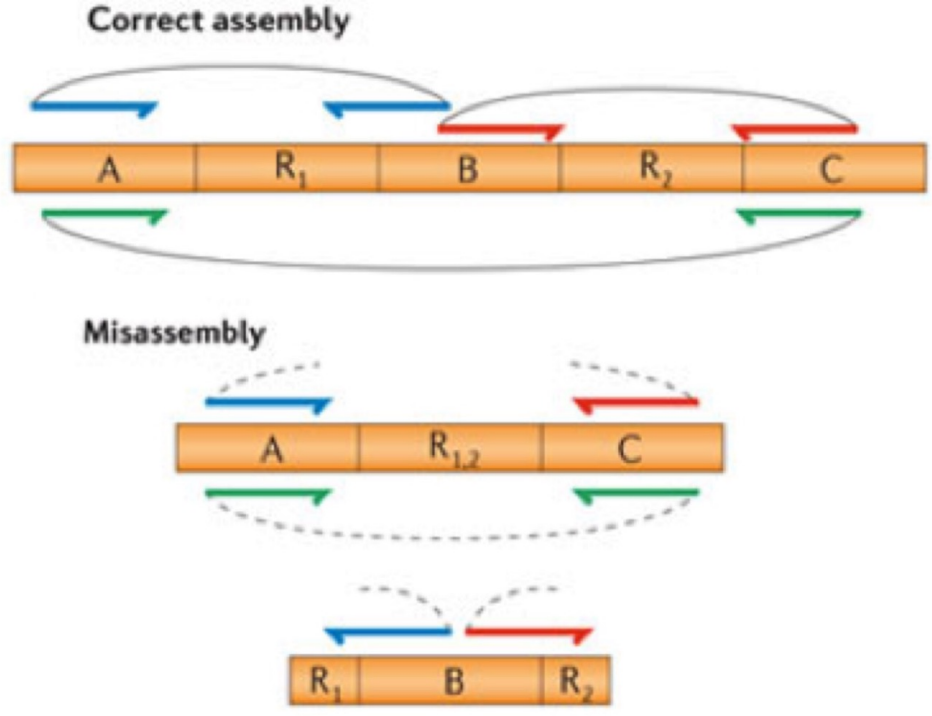
Assembly by overlap

Assembly challenges



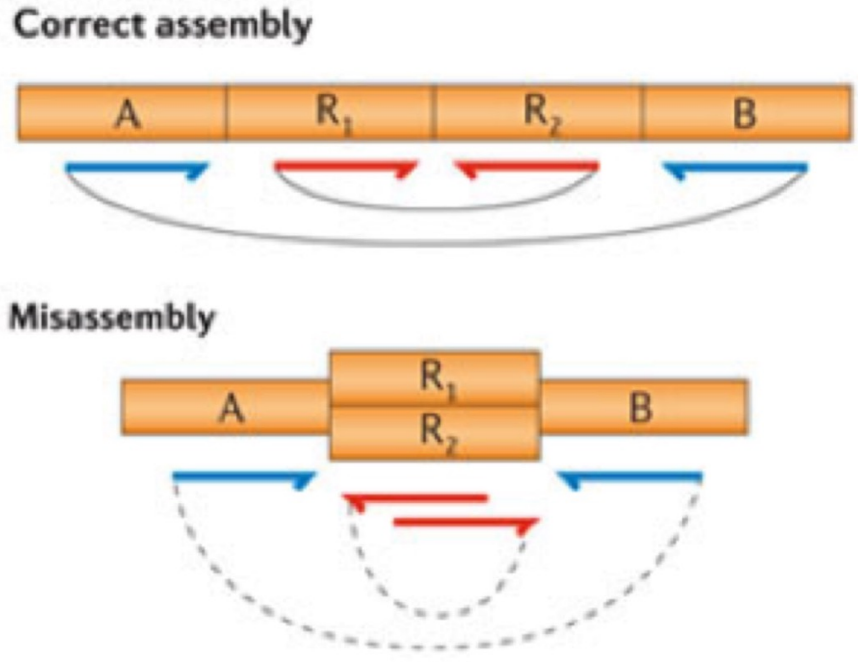
Assembly by overlap

Assembly challenges



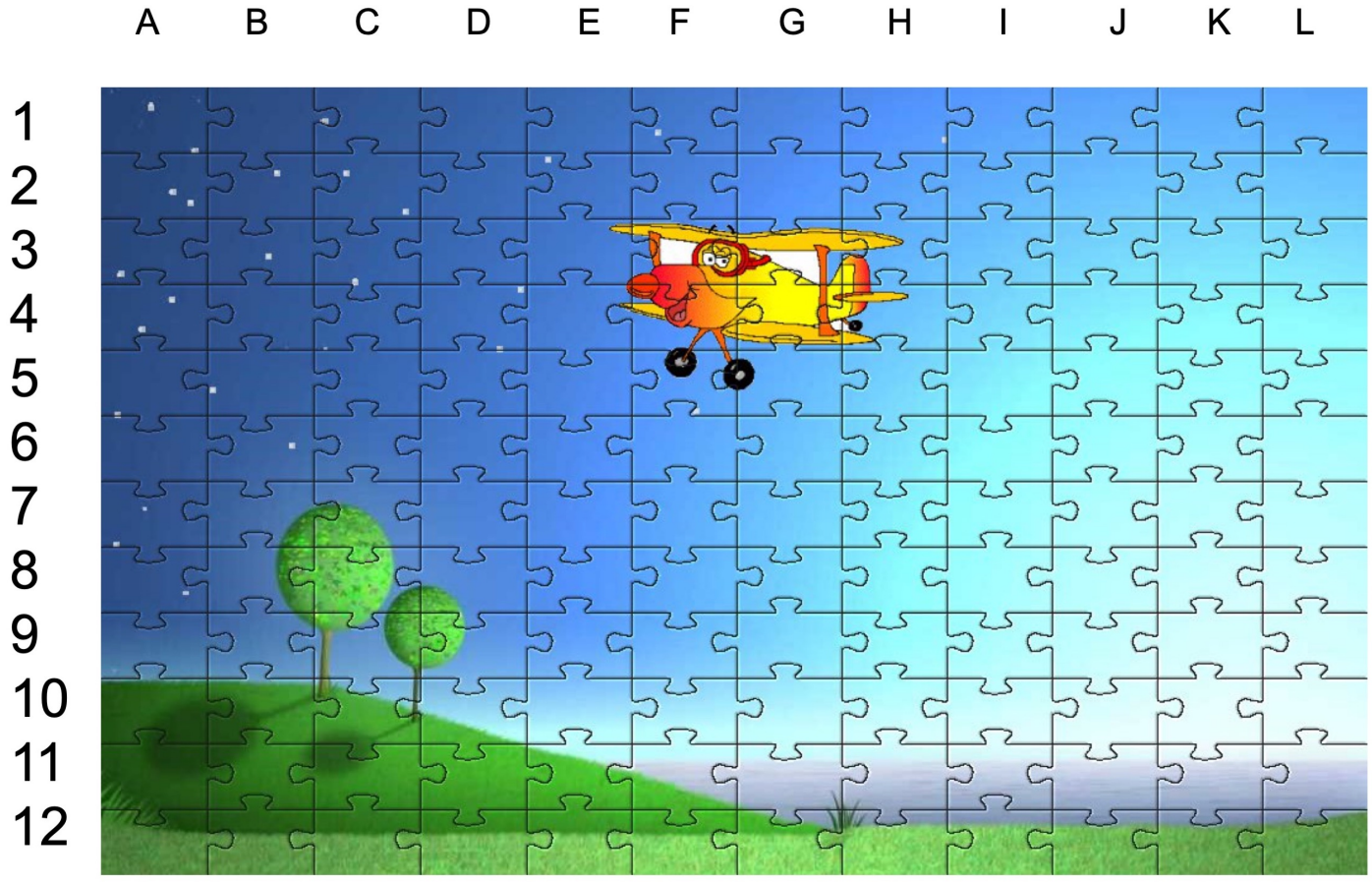
Assembly by overlap

Assembly challenges



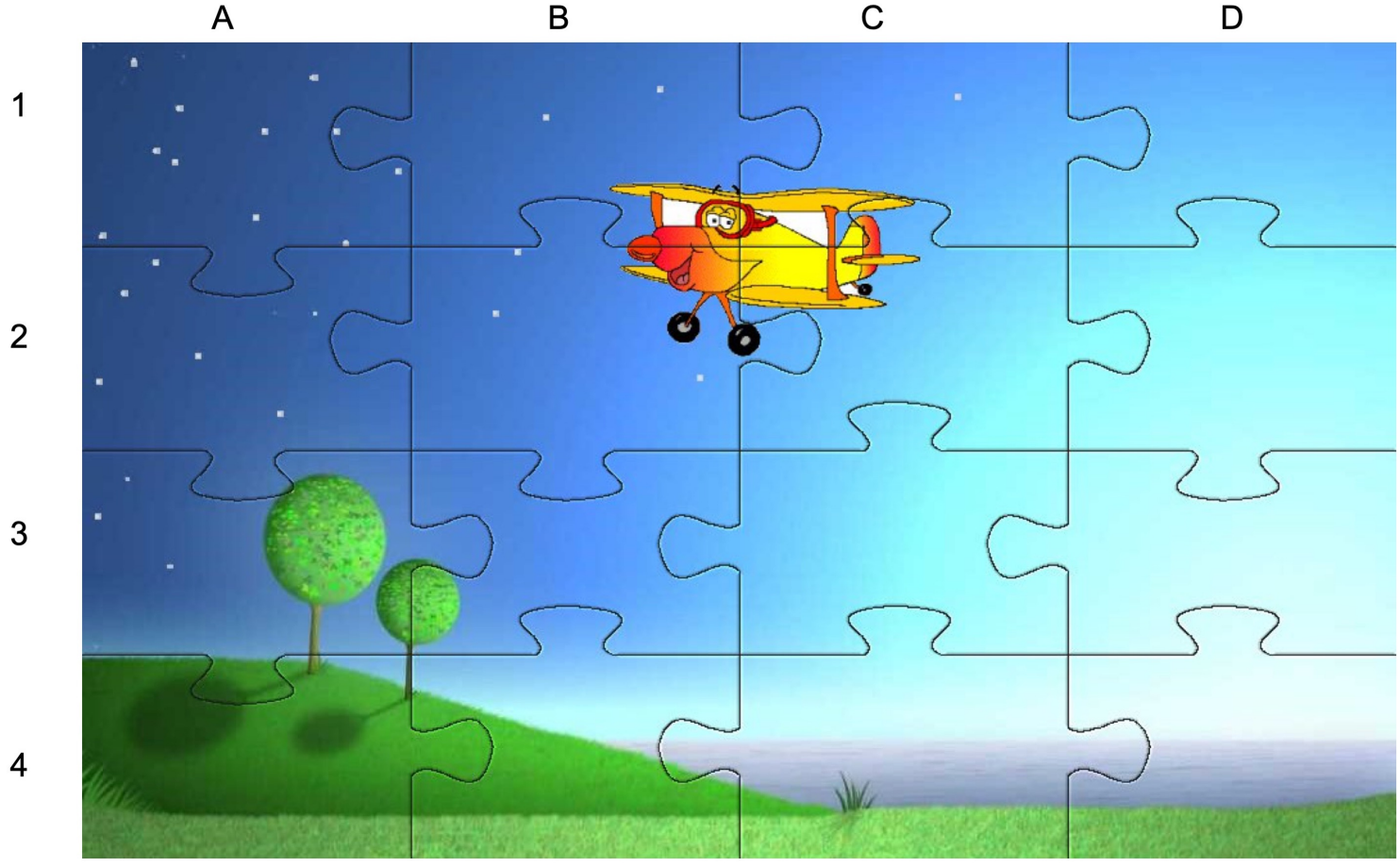
Assembly by overlap

Assembly challenges



Assembly by overlap

Assembly challenges



Assembly by overlap

Assembly challenges

Assembly challenges

Millions of really short sequences

MiniSeq

MiSeq

1-25 million reads
150 - 250bp paired-end
24 hr run time

NextSeq

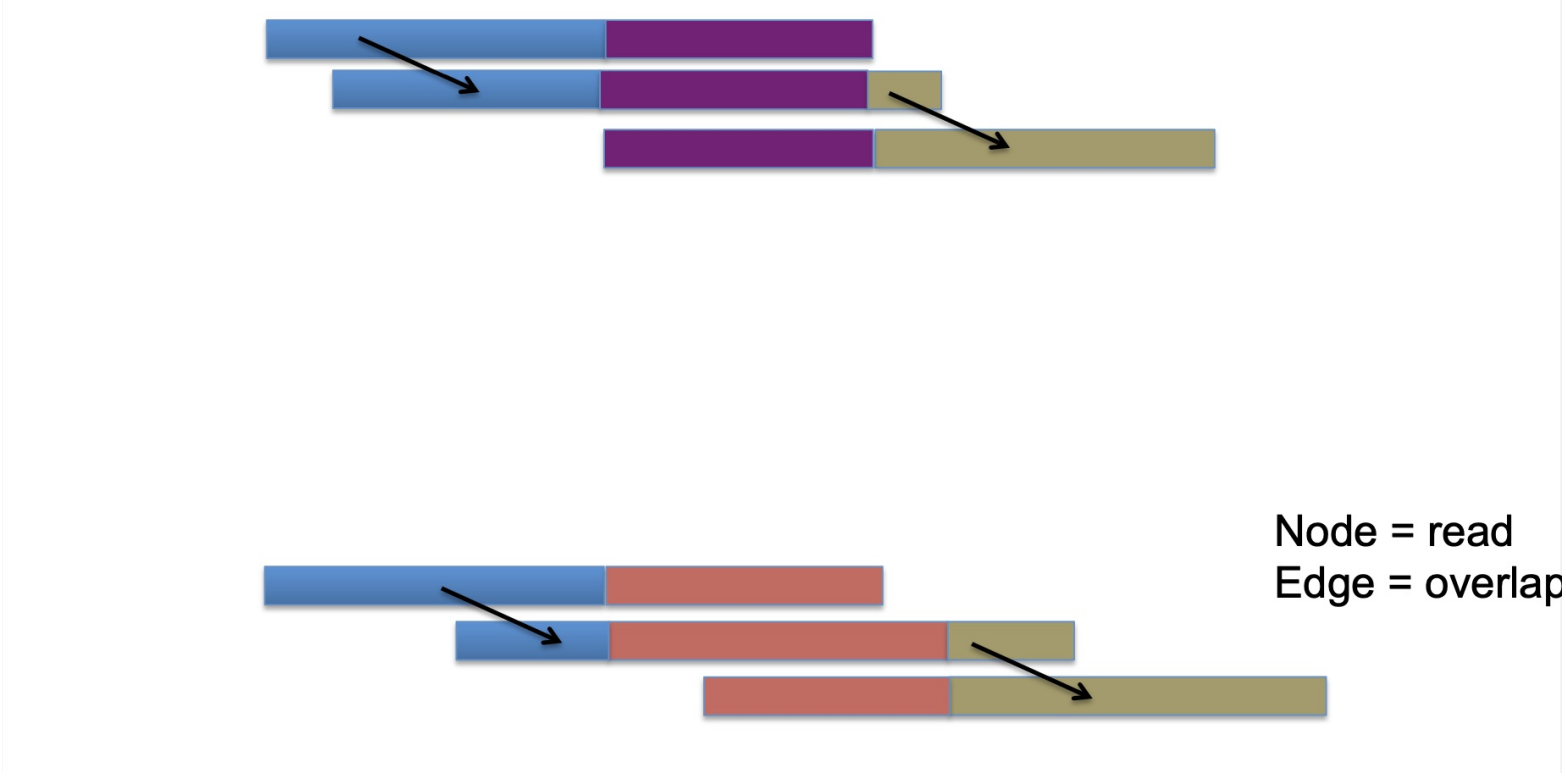
0.4 - 1.1 billion reads
150bp paired-end
24 hr run time

NovaSeq

0.6 - 10 billion reads
50-250bp paired-end
5 day run time

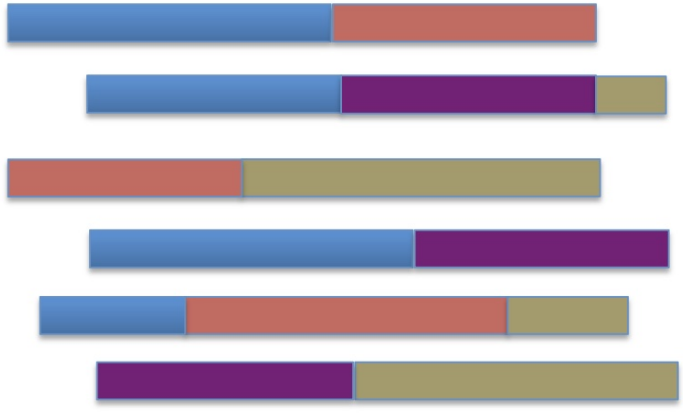
Assembly challenges

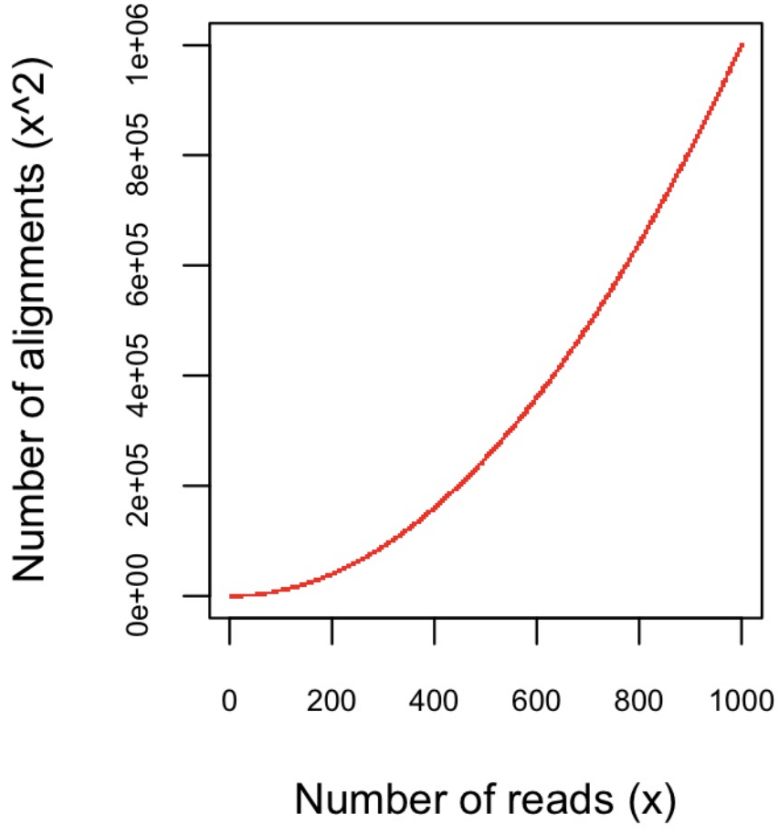
Millions of really short sequences



Assembly challenges

Millions of really short sequences

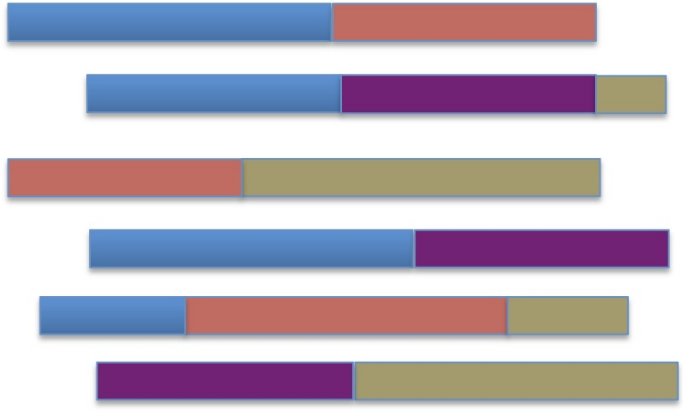


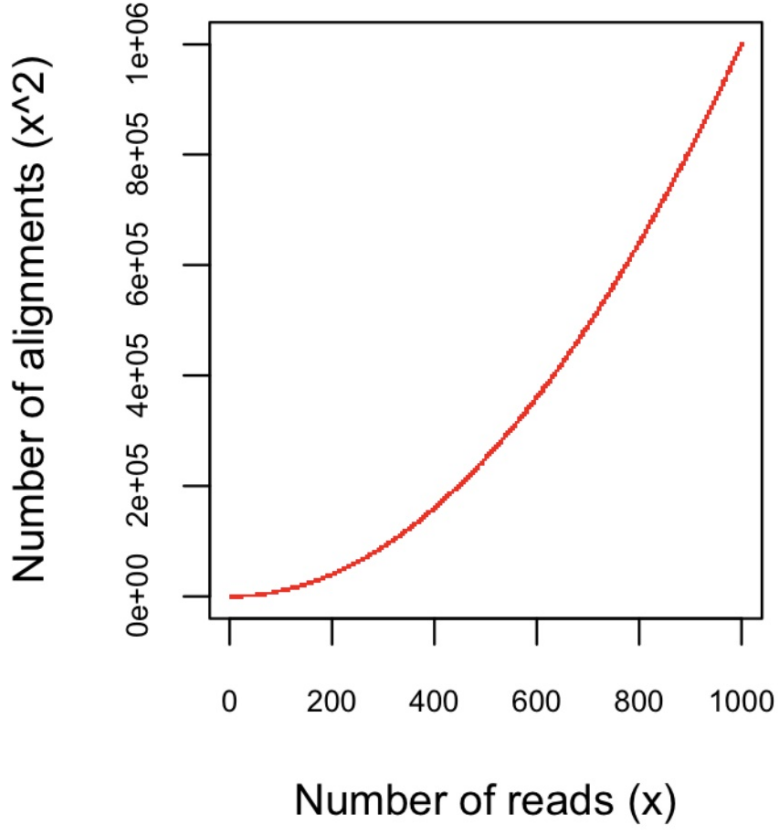


Finding pairwise overlaps between n reads involves ~ n^2 comparisons

Assembly challenges

Millions of really short sequences

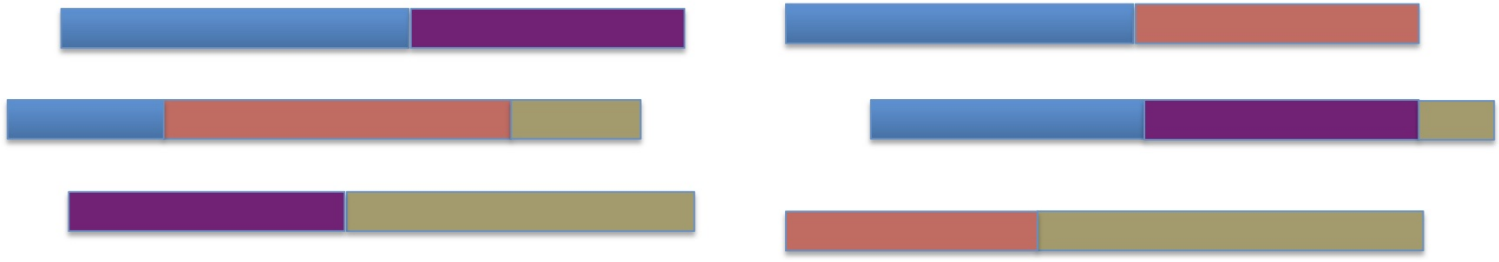




Impractical for typical RNA-Seq data (50M reads)

Assembly challenges

Millions of really short sequences

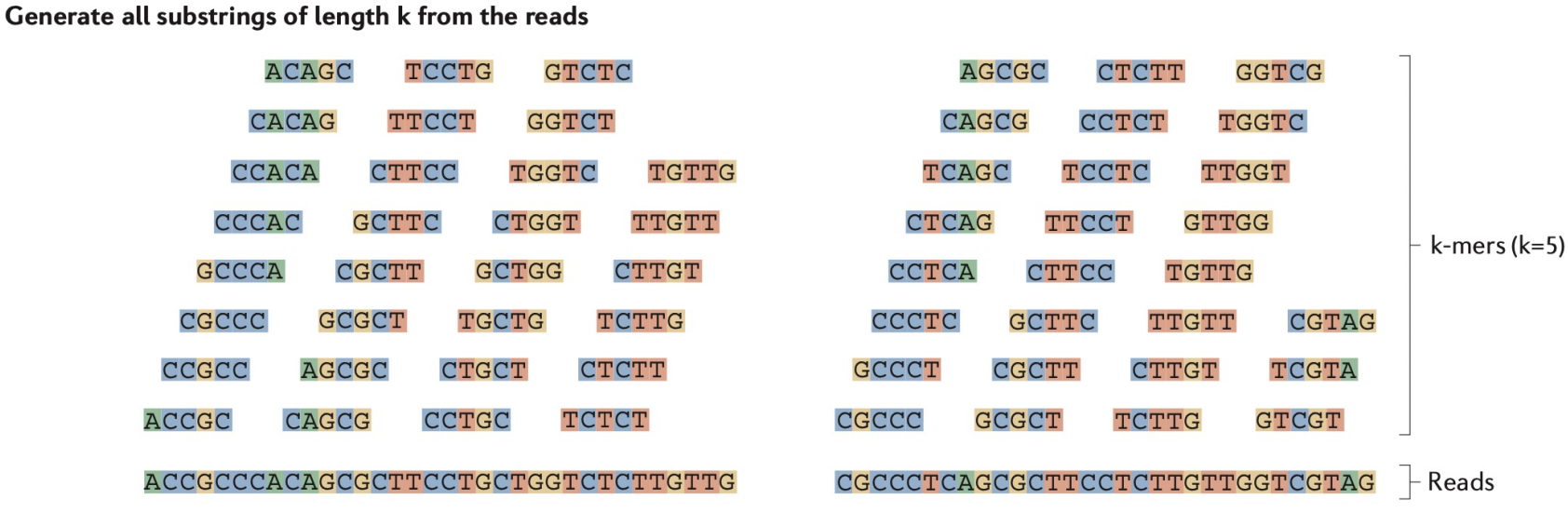


Want to avoid n^2 read alignments to define overlaps

Use a de Bruijn graph

De novo transcriptome assembly

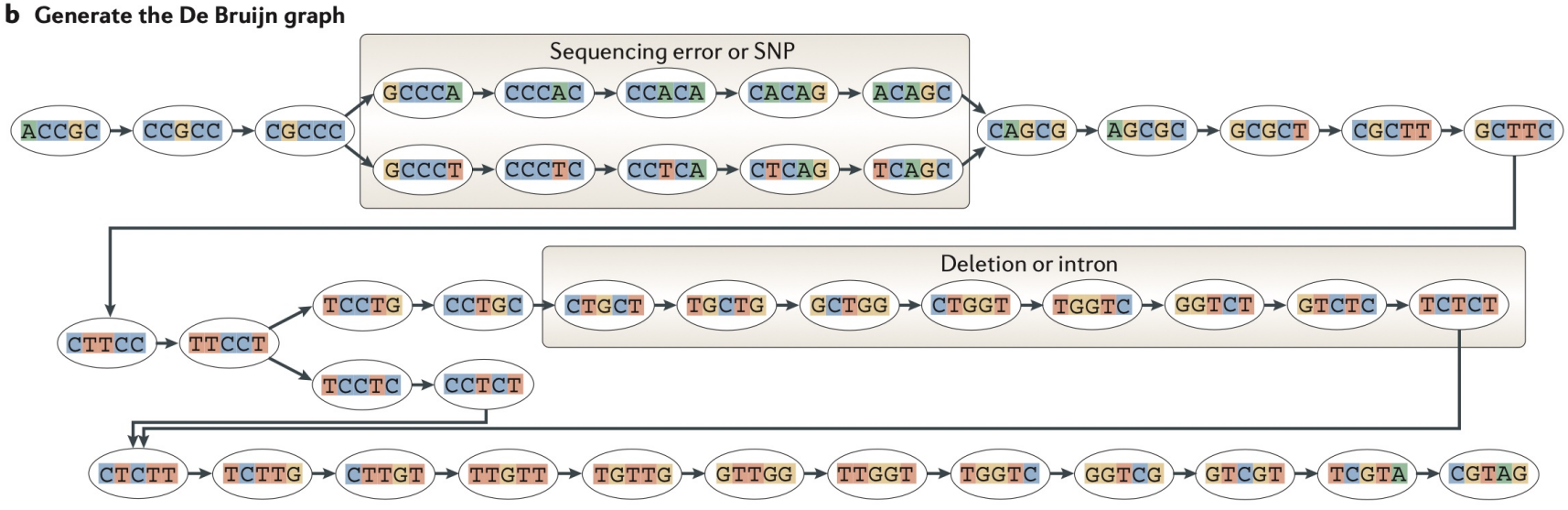
Assembly strategy



Martin, Wang (2011) Nat Rev Genet, 12, 671-682.

De novo transcriptome assembly

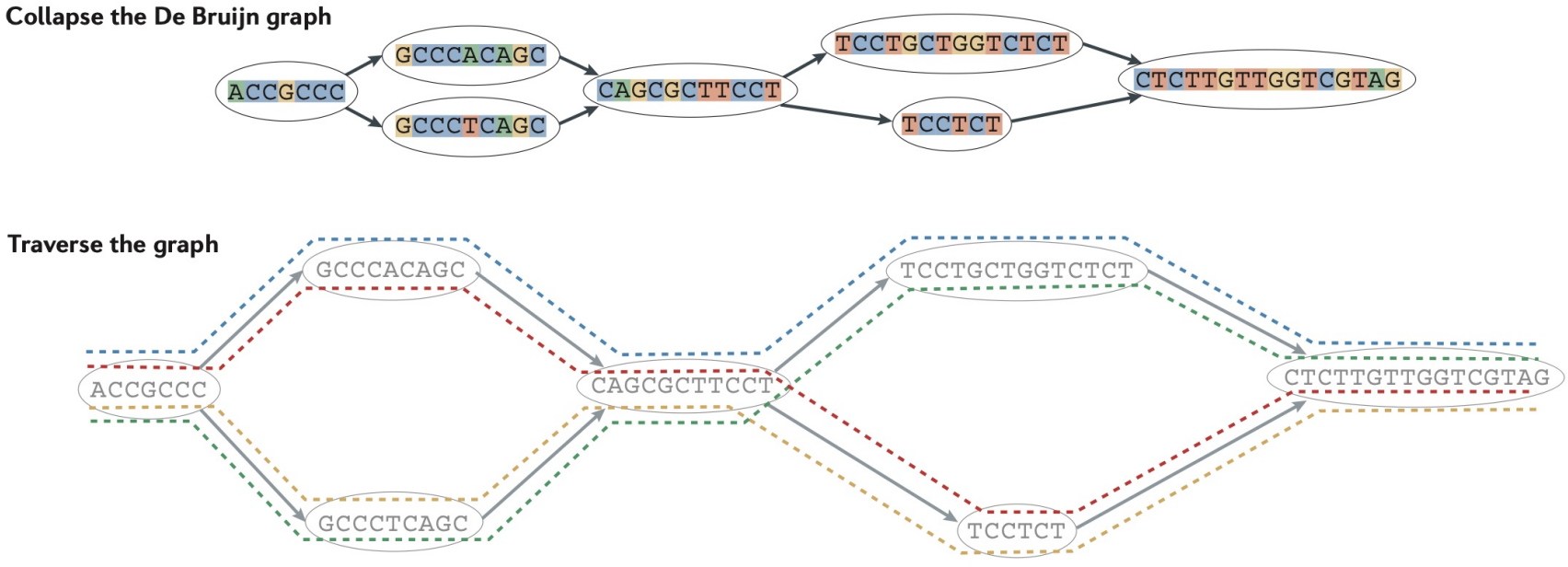
Assembly strategy



Martin, Wang (2011) Nat Rev Genet, 12, 671-682.

De novo transcriptome assembly

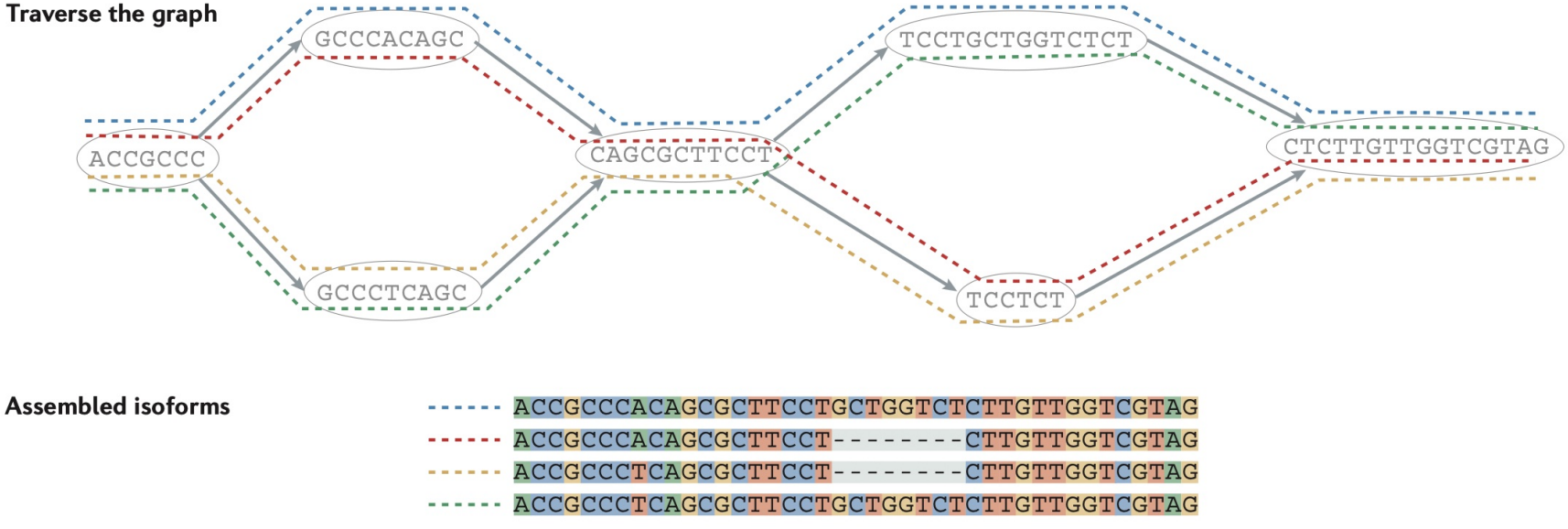
Assembly strategy



Martin, Wang (2011) Nat Rev Genet, 12, 671-682.

De novo transcriptome assembly

Assembly strategy



Martin, Wang (2011) Nat Rev Genet, 12, 671-682.

RNA-Seq: Assembly

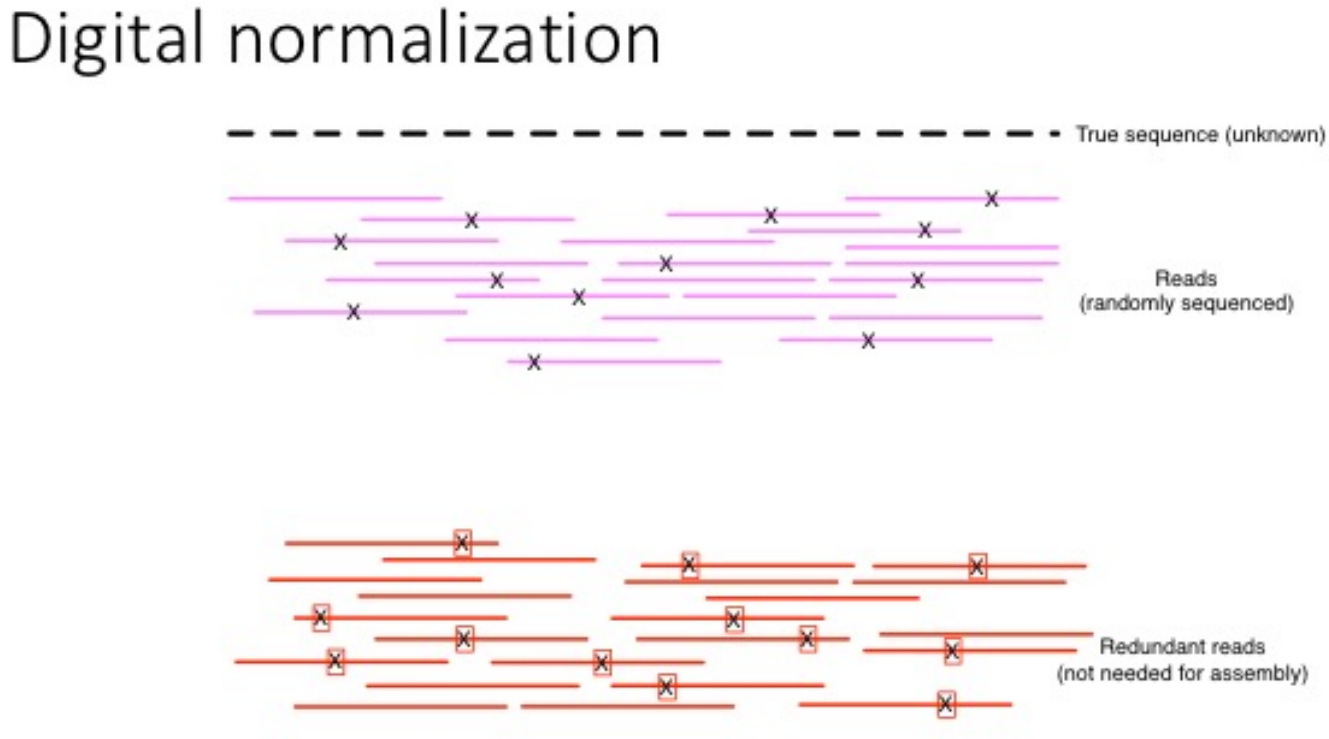
Trinity

Uses of Trinity are:

- Inchworm assembles the RNA-seq data into the unique sequences of transcripts, often generating full-length transcripts for a dominant isoform.
- Chrysalis clusters the Inchworm contigs into clusters and constructs complete de Bruijn graphs for each cluster. Each cluster represents the full transcriptonal complexity for a given gene.
- Butterfly reports full-length transcripts for alternatively spliced isoforms, and teasing apart transcripts that corresponds to paralogous genes.

De novo transcriptome assembly

Assembly strategy

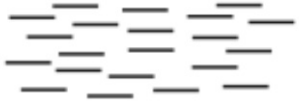


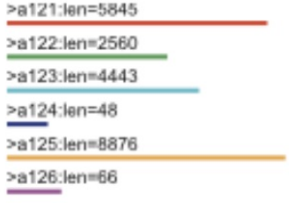
http://ivory.idyll.org/blog/trinity-in-silico-normalize.html

De novo transcriptome assembly

Trinity

RNA-Seq reads → **Linear contigs** → **de-Bruijn graphs** → **Transcripts + Isoforms**





Thousands of disjoint graphs

...CTTCGCAA...TGATCGGAT...
...ATTCGCAA...TCATCGGAT...

Transcriptome assembly with Trinity

Inchworm Algorithm

Read: **AATGTGAAAACTGGATTACATGCTGGTATGTC**...

AATGTGA

ATGTGAA Overlapping kmers of length (k)

TGTGAAA

...

Kmer Catalog (hashtable)

Kmer	Count among all reads
AATGTGA	4
ATGTGAA	2
TGTGAAA	1
GATTACA	9

Transcriptome assembly with Trinity

Inchworm Algorithm

- Decompose all reads into overlapping Kmers => hashtable(kmer, count)

Read: **AATGTGAAAACTGGATTACATGCTGGTATGTC**...

AATGTGA

ATGTGAA Overlapping kmers of length (k)

TGTGAAA

...

Kmer Catalog (hashtable)

Kmer	Count among all reads
AATGTGA	4
ATGTGAA	2
TGTGAAA	1
GATTACA	9

Transcriptome assembly with Trinity

Inchworm Algorithm

- Decompose all reads into overlapping Kmers => hashtable(kmer, count)
- Identify seed kmer as most abundant Kmer, ignoring low-complexity kmers.

GATTACA
9

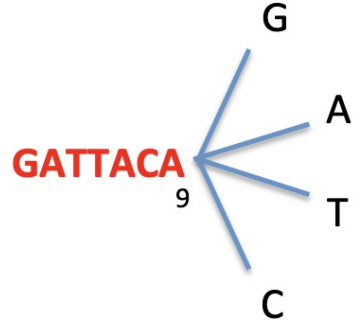
Kmer Catalog (hashtable)

Kmer	Count among all reads
AATGTGA	4
ATGTGAA	2
TGTGAAA	1
GATTACA	9

Transcriptome assembly with Trinity

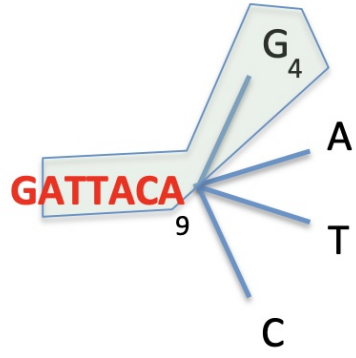
Inchworm Algorithm

- Decompose all reads into overlapping Kmers => hashtable(kmer, count)
- Identify seed kmer as most abundant Kmer, ignoring low-complexity kmers.
- Extend kmer at 3' end, guided by coverage.



Transcriptome assembly with Trinity

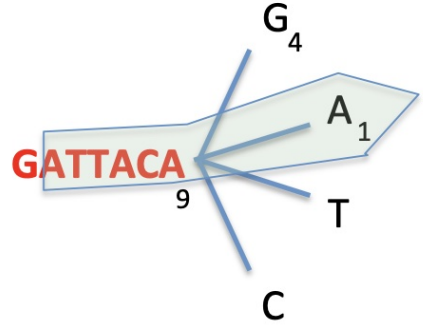
Inchworm Algorithm



Brian Haas

Transcriptome assembly with Trinity

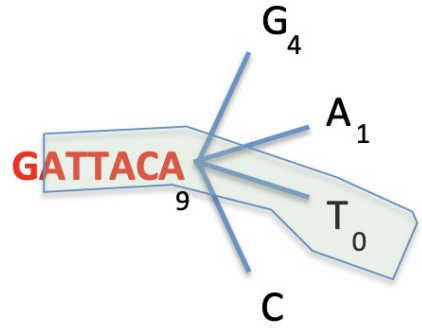
Inchworm Algorithm



Brian Haas

Transcriptome assembly with Trinity

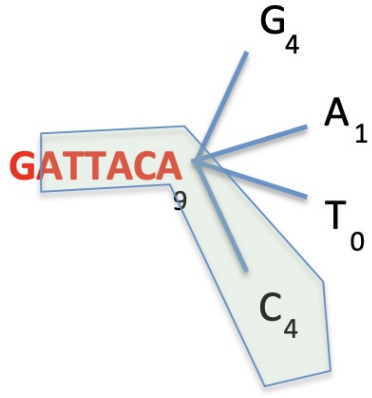
Inchworm Algorithm



Brian Haas

Transcriptome assembly with Trinity

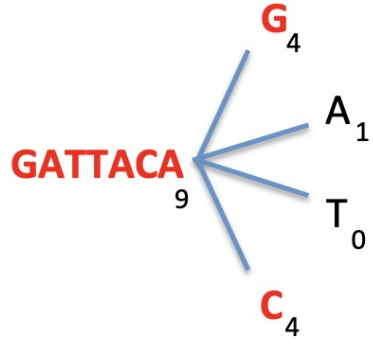
Inchworm Algorithm



Brian Haas

Transcriptome assembly with Trinity

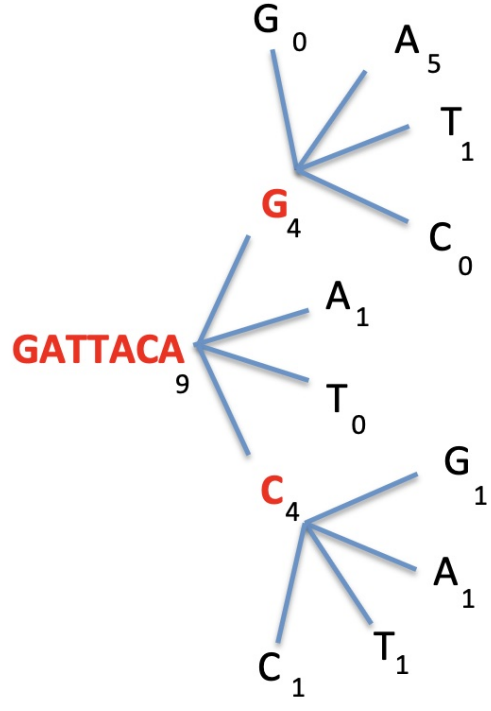
Inchworm Algorithm



Brian Haas

Transcriptome assembly with Trinity

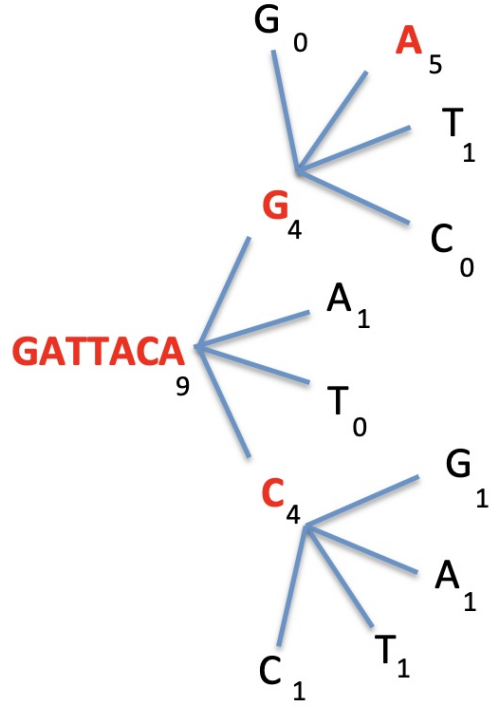
Inchworm Algorithm



Brian Haas

Transcriptome assembly with Trinity

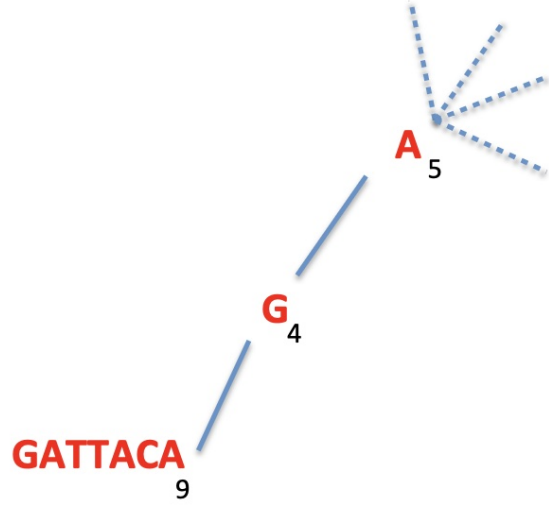
Inchworm Algorithm



Brian Haas

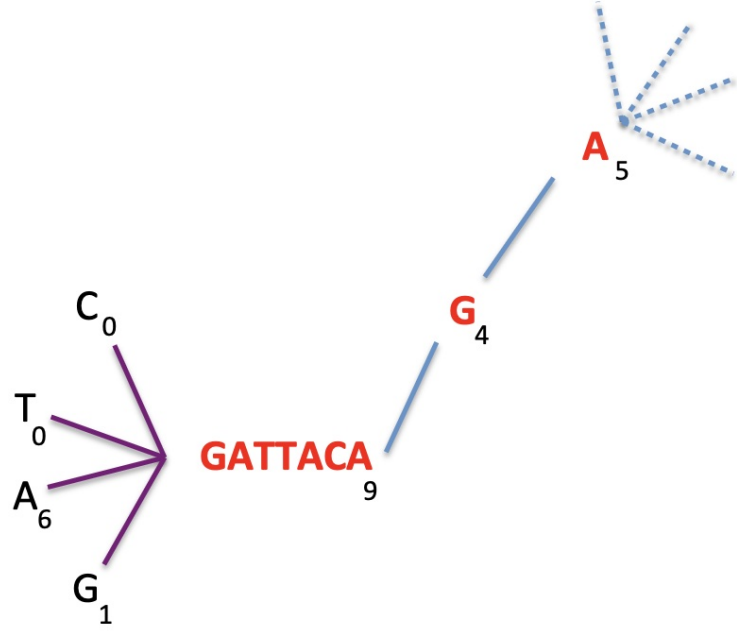
Transcriptome assembly with Trinity

Inchworm Algorithm



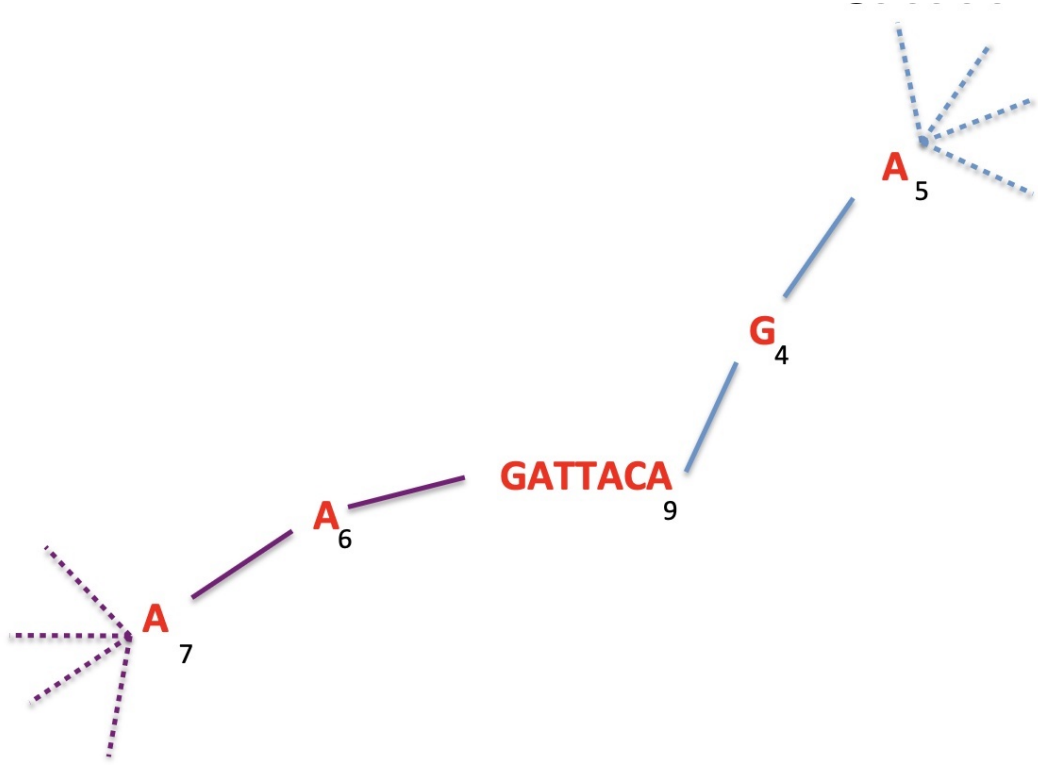
Transcriptome assembly with Trinity

Inchworm Algorithm



Transcriptome assembly with Trinity

Inchworm Algorithm



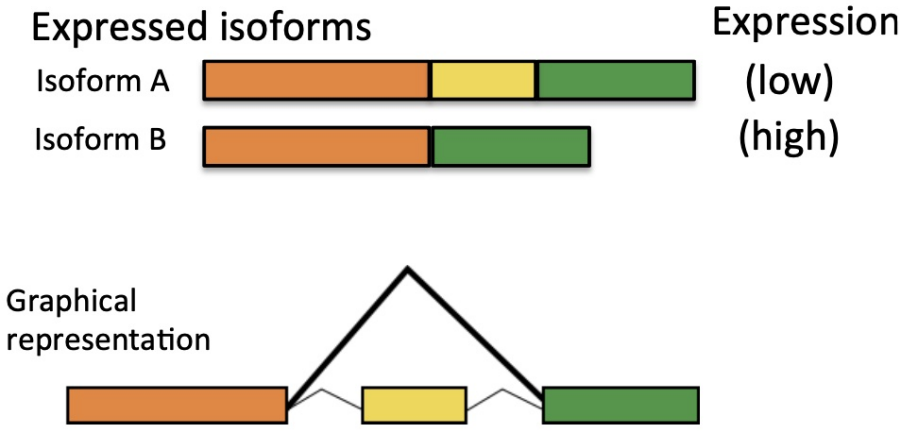
Report contig: ....AAGATTACAGA....

Remove assembled kmers from catalog, then repeat the entire process.

Brian Haas

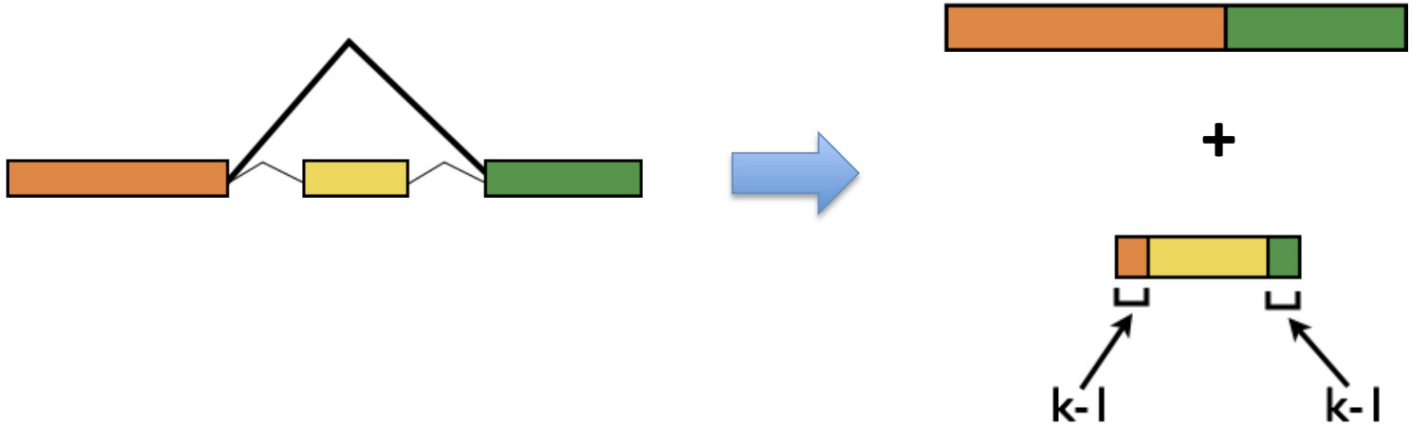
Transcriptome assembly with Trinity

Inchworm Algorithm



Transcriptome assembly with Trinity

Inchworm Algorithm

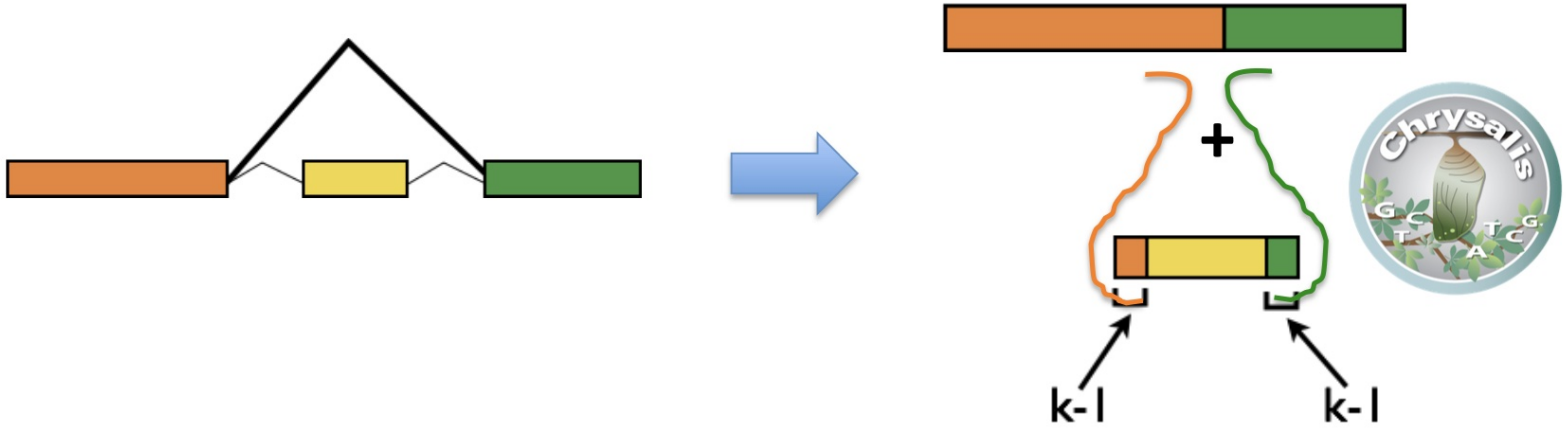


Brian Haas

Transcriptome assembly with Trinity

Chrysalys Algorithm

Chrysalys Re-groups Related Inchworm Contigs

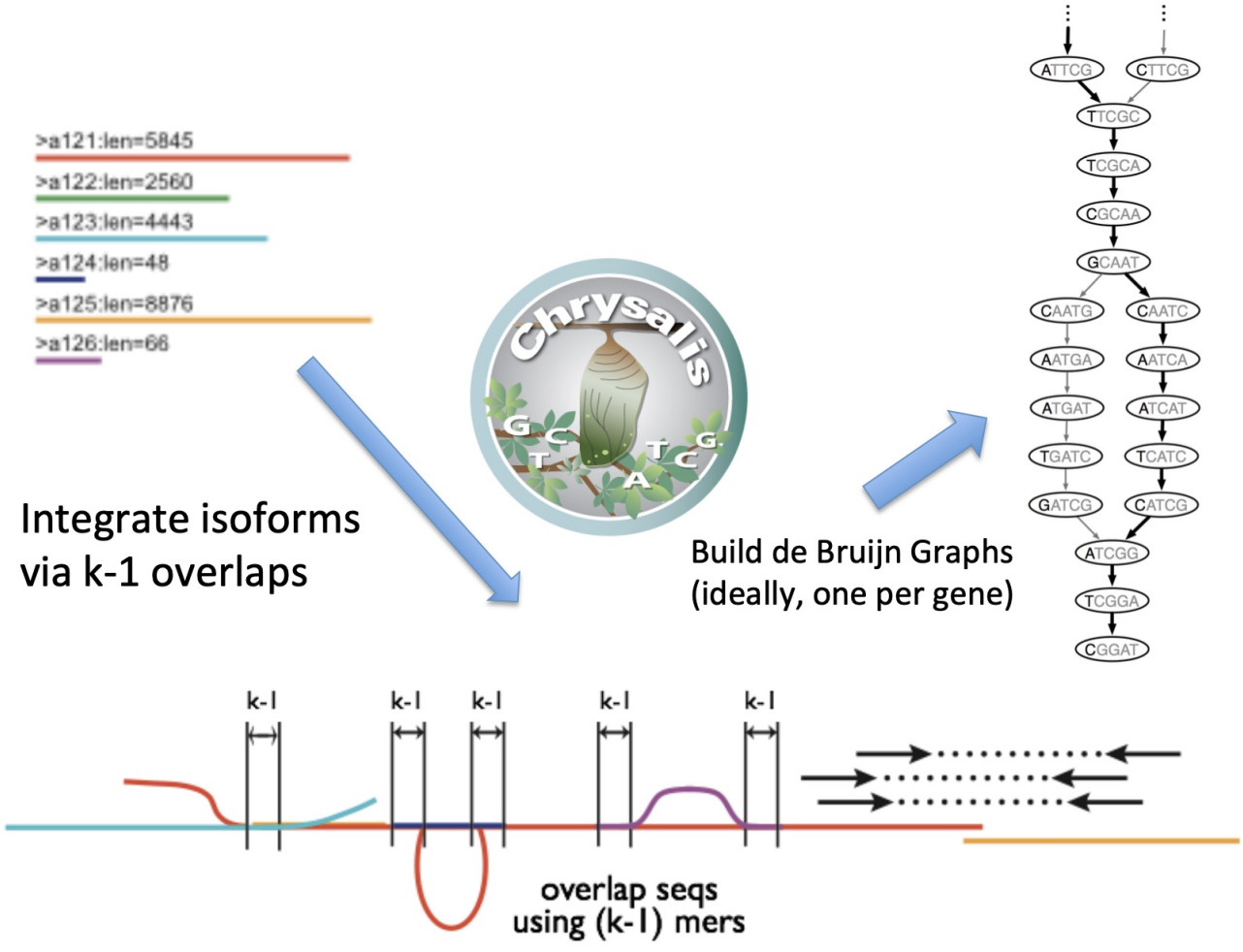


Chrysalis uses (k-1) overlaps and read support to link related Inchworm contigs

Brian Haas

Transcriptome assembly with Trinity

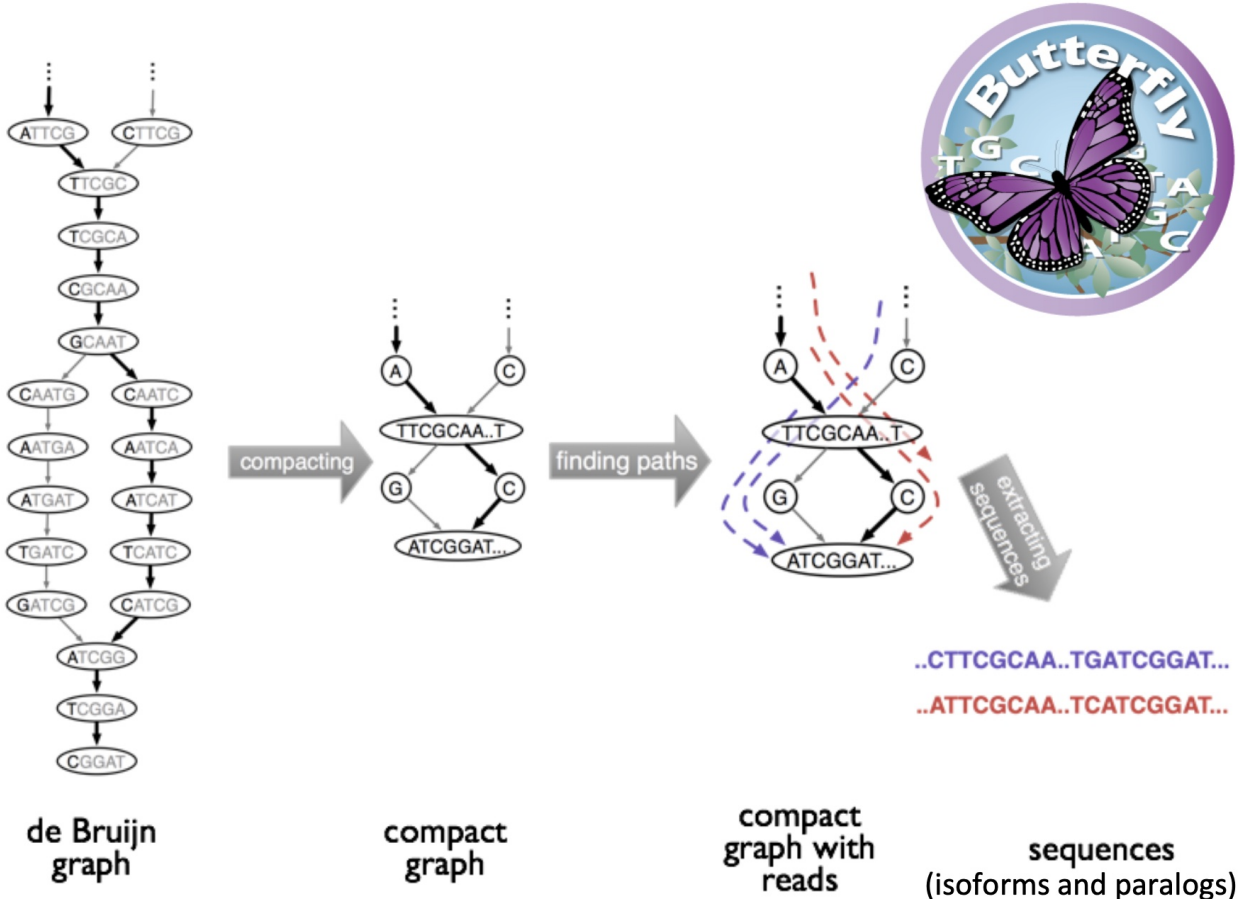
Chrysalys Algorithm



Brian Haas

Transcriptome assembly with Trinity

Butterfly Algorithm



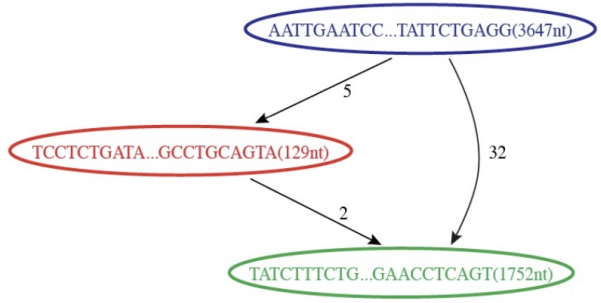
Brian Haas

Transcriptome assembly with Trinity

Butterfly Algorithm

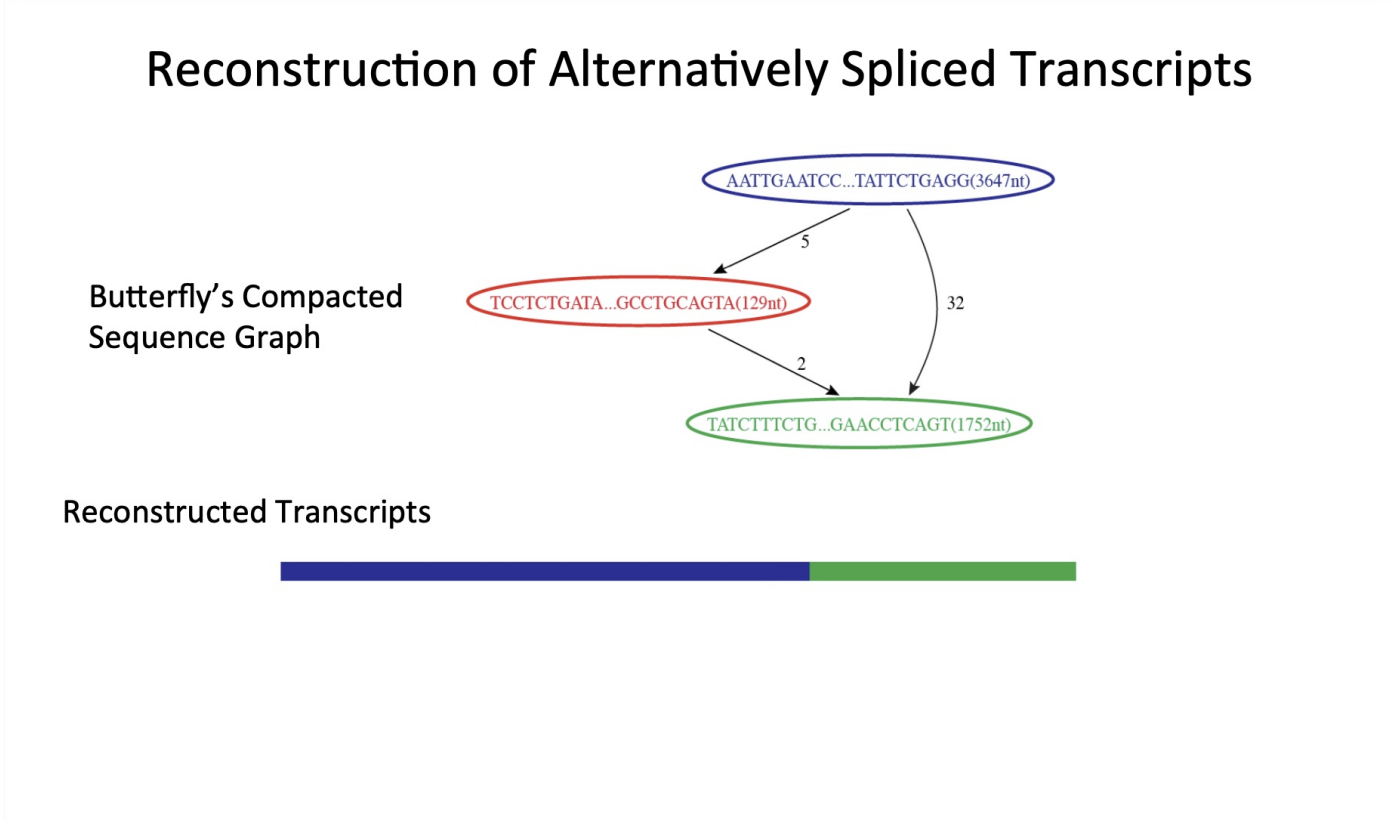
Butterfly Example 1:
Reconstruction of Alternatively Spliced Transcripts

Butterfly's Compacted Sequence Graph



Transcriptome assembly with Trinity

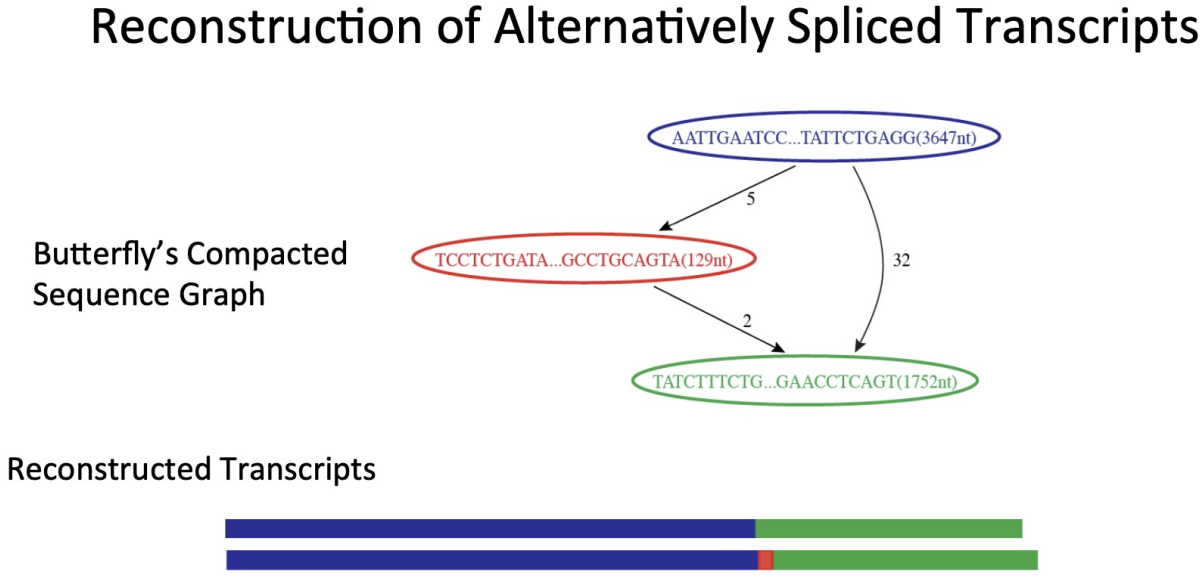
Butterfly Algorithm



Brian Haas

Transcriptome assembly with Trinity

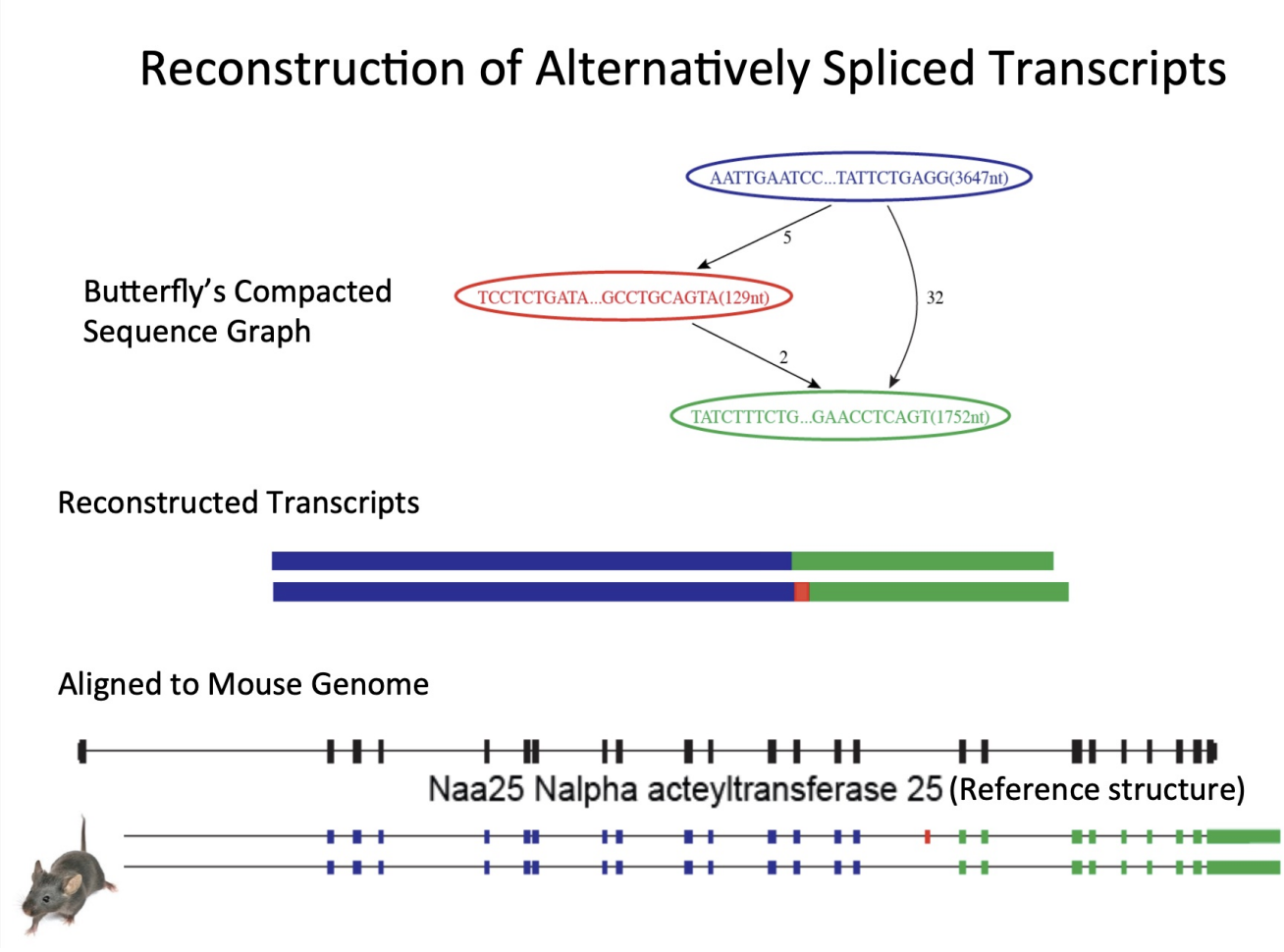
Butterfly Algorithm



Brian Haas

Transcriptome assembly with Trinity

Butterfly Algorithm



Brian Haas

RNA-Seq: Assembly

Trinity

Assembly results

Dois arquivos:

- `${SPECIES}.Trinity.fasta`
- `${SPECIES}.fasta.gene_trans_map`

Exemplo:

- `Chom.Trinity.fasta`
- `Chom.fasta.gene_trans_map`

RNA-Seq: Assembly

Trinity results

1. Check files: `*.Trinity.fasta` :

```
# head ${SPECIES}.Trinity.fasta

head ChomF_1.Trinity.fasta
>TRINITY_DN1870_c0_g1_i1 len=208 path=[0:0-207]
ACTGAATCTATCATGAAACGTACCGCTTTGGTAGCCAACACCTCTAACATGCCTGTC
GCTCGTGAAGCTTCAATTTACACTGGTATTACCTTATCTGAATACTTCCGTGATATG
TACAACGTATCTATGATGGCTGATTCTACCTCTCGTTGGGCTGAAGCTCTTCGTGAA
TCTGGTCGTTTGGCTGAAATGCCTGCCG
>TRINITY_DN1811_c0_g1_i1 len=738 path=[0:0-737]
CAGGATGTCTATAAAATTGGTGGTATTGGTACAGTACCCGTGGGTCGTGTTGAAACT
ATTTTGAAACCTGGTATGGTGGTTAATTTTGCTCCTGTCAATTTGGTAACTGAAGTC
TCGGTGGAAATGCATCATGAAGCCTTGTCGGAAGCAATGCCCGGTGATAATGTTGGT
AATGTTAAAAACGTTTCGGTTAAGGAATTGCGTCGCGGTTATGTTGCCGGTGATACC
```

RNA-Seq: Assembly

Trinity results

The `.Trinity.fasta` file

```
TRINITY_DN1870_c0_g1_i1 len=208 path=[0:0-207]
```

➡️ TRINITY_DN1870_c0_g1_i1:

- DN1870: unique identifier for the de novo assembly cluster to which the contig belongs.
- c0: specific component within the cluster, connected sequences that likely derive from the same gene family.
- g1: gene-like group within the component. Sequences within the same group (g1) are interpreted as splice variants or isoforms of the same gene.
- i1: Represents the specific isoform number (e.g., i1 is isoform 1).

RNA-Seq: Assembly

Trinity results

The `.Trinity.fasta` file

```
TRINITY_DN1870_c0_g1_i1 len=208 path=[0:0-207]
```

➡️ len=208:

- Length of the contig in base pairs (bp). In this example, the contig is 208 bp long.

➡️ path=[0:0-207]:

- Assembly path used to reconstruct the contig.
  0: Represents the node index in the assembly graph.
  0-207: Specifies the position range in the node that was used to assemble this contig.

RNA-Seq: Assembly

Trinity results

2. Check files: `*.Trinity.fasta.gene_trans_map` :

```
# head ${SPECIES}.Trinity.fasta.gene_trans_map

head ChomF_1.Trinity.fasta.gene_trans_map
TRINITY_DN1870_c0_g1    TRINITY_DN1870_c0_g1_i1
TRINITY_DN1811_c0_g1    TRINITY_DN1811_c0_g1_i1
TRINITY_DN1862_c0_g1    TRINITY_DN1862_c0_g1_i1
TRINITY_DN1808_c0_g1    TRINITY_DN1808_c0_g1_i1
TRINITY_DN1869_c0_g1    TRINITY_DN1869_c0_g1_i1
TRINITY_DN1803_c0_g1    TRINITY_DN1803_c0_g1_i1
TRINITY_DN1835_c0_g1    TRINITY_DN1835_c0_g1_i1
TRINITY_DN1892_c0_g1    TRINITY_DN1892_c0_g1_i1
TRINITY_DN1854_c0_g1    TRINITY_DN1854_c0_g1_i1
TRINITY_DN1880_c0_g1    TRINITY_DN1880_c0_g1_i1
```

RNA-Seq: Assembly

Trinity results

The `.gene_trans_map` file

> Mapping between genes and transcripts identified during the assembly. Links individual transcripts to their respective parent genes.

> 📝 Software tools like Trinotate, RSEM, and Salmon use this file to relate the expression of isoforms and genes.

RNA-Seq: Assembly

Trinity results

2. How many contigs were assembled?

RNA-Seq: Assembly

Trinity results

2. How many contigs were assembled?

```
#grep -c "^>" ${SPECIES}.Trinity.fasta

grep -c "^>" Chom.Trinity.fasta

1733
```

RNA-Seq: Assembly

Trinity results

```
TrinityStats.pl Trinity.fasta

################################
## Counts of transcripts, etc.
################################
Total trinity 'genes':  1636
Total trinity transcripts:      1733
Percent GC: 38.72
```

RNA-Seq: Assembly

Trinity results

```
################################################
Stats based on ALL transcript contigs:
################################################

        Contig N10: 1955
        Contig N20: 1400
        Contig N30: 988
        Contig N40: 778
        Contig N50: 620

        Median contig length: 351
        Average contig: 519.83
        Total assembled bases: 900862
```

RNA-Seq: Assembly

Trinity results

```
################################################################
## Stats based on ONLY LONGEST ISOFORM per 'GENE':
################################################################

        Contig N10: 1625
        Contig N20: 1195
        Contig N30: 869
        Contig N40: 684
        Contig N50: 552

        Median contig length: 338
        Average contig: 480.36
        Total assembled bases: 785867
```

RNA-Seq: Assembly

Trinity

Assembly results

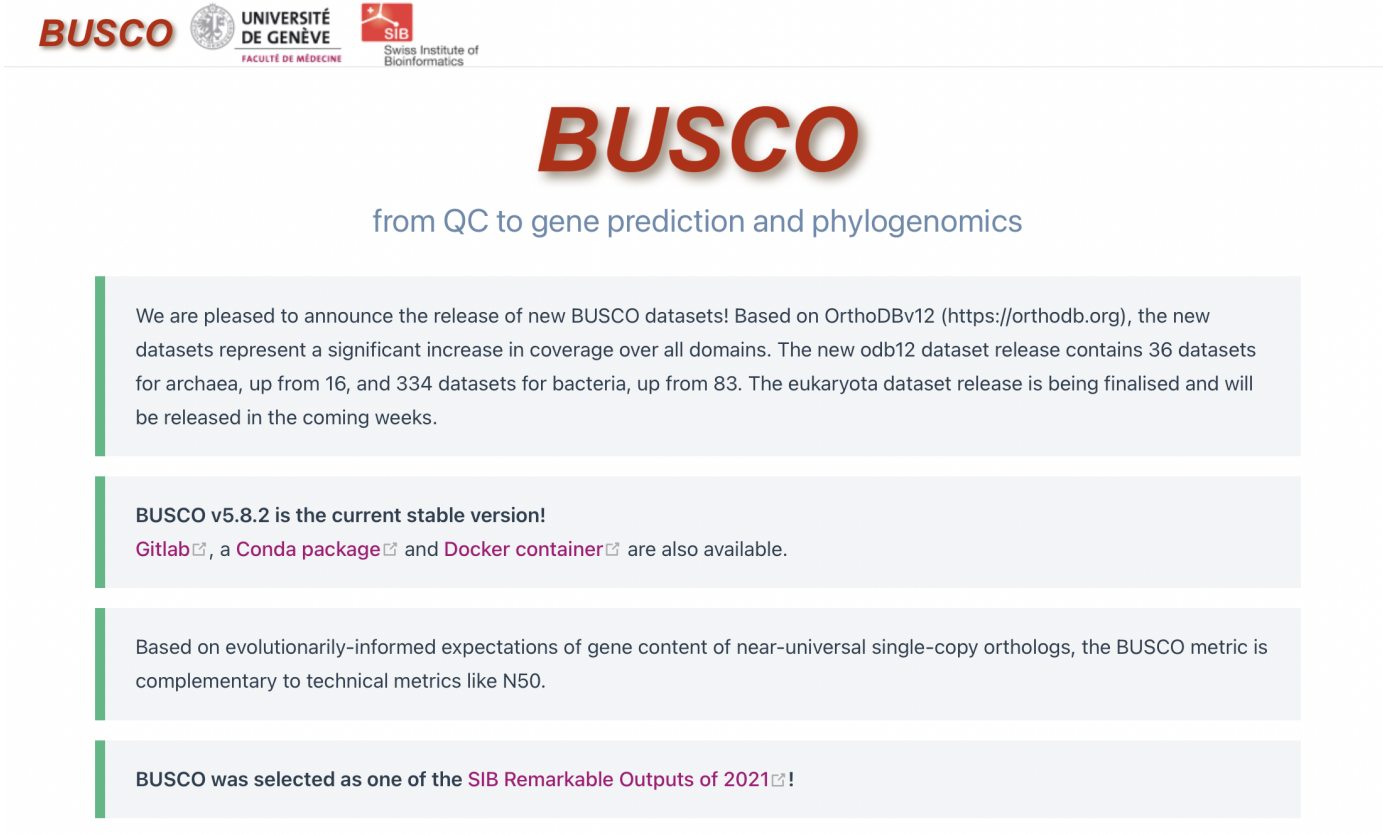
Exemplo:

- `ChomF.Trinity.fasta`
- `ChomF.fasta.gene_trans_map`

Quality assessmens ❓

RNA-Seq: Assembly

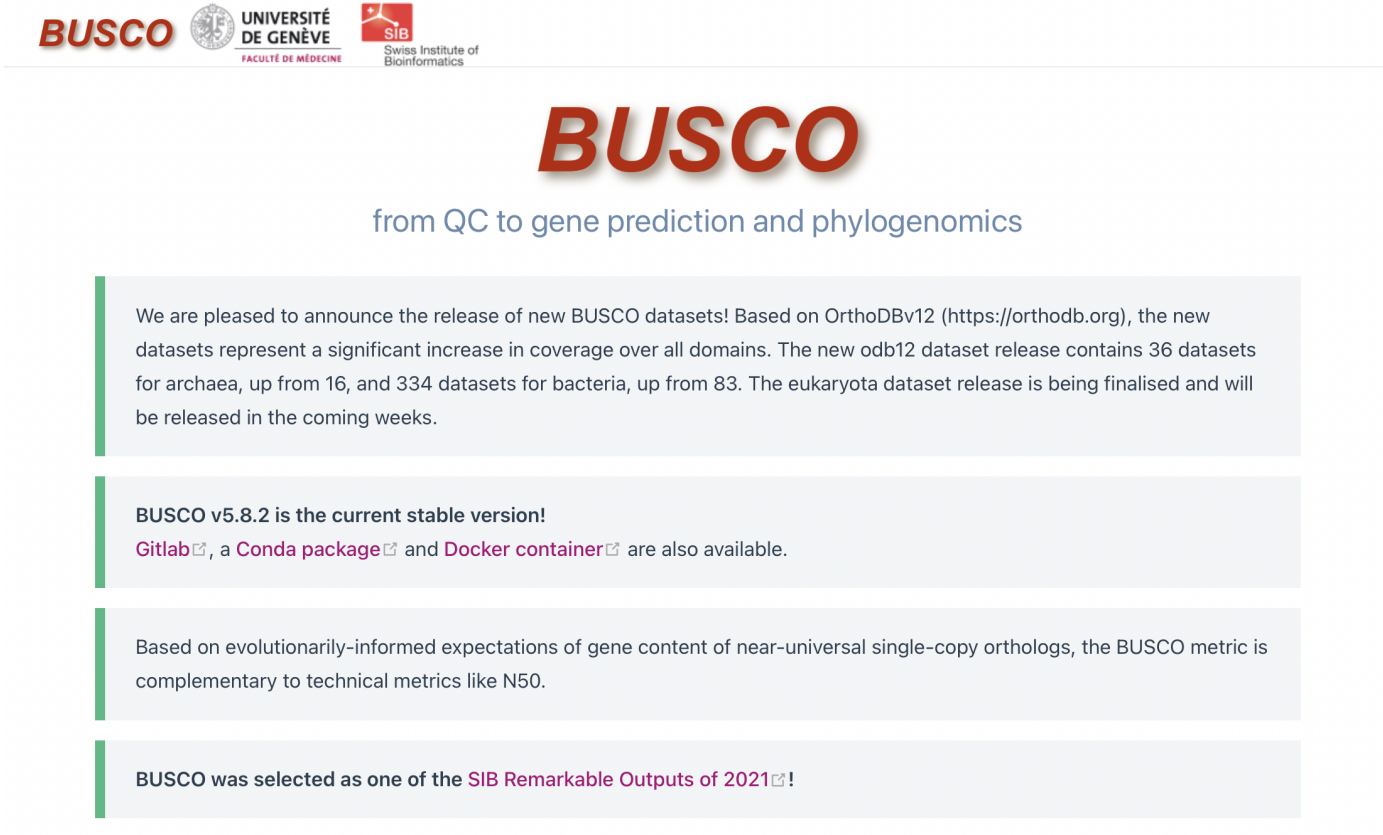
Quality assessment: BUSCO



https://busco.ezlab.org

RNA-Seq: Assembly

Quality assessment: BUSCO



https://busco.ezlab.org

RNA-Seq: Assembly

Quality assessment: BUSCO

Benchmarking Universal Single-Copy Orthologue (BUSCO)

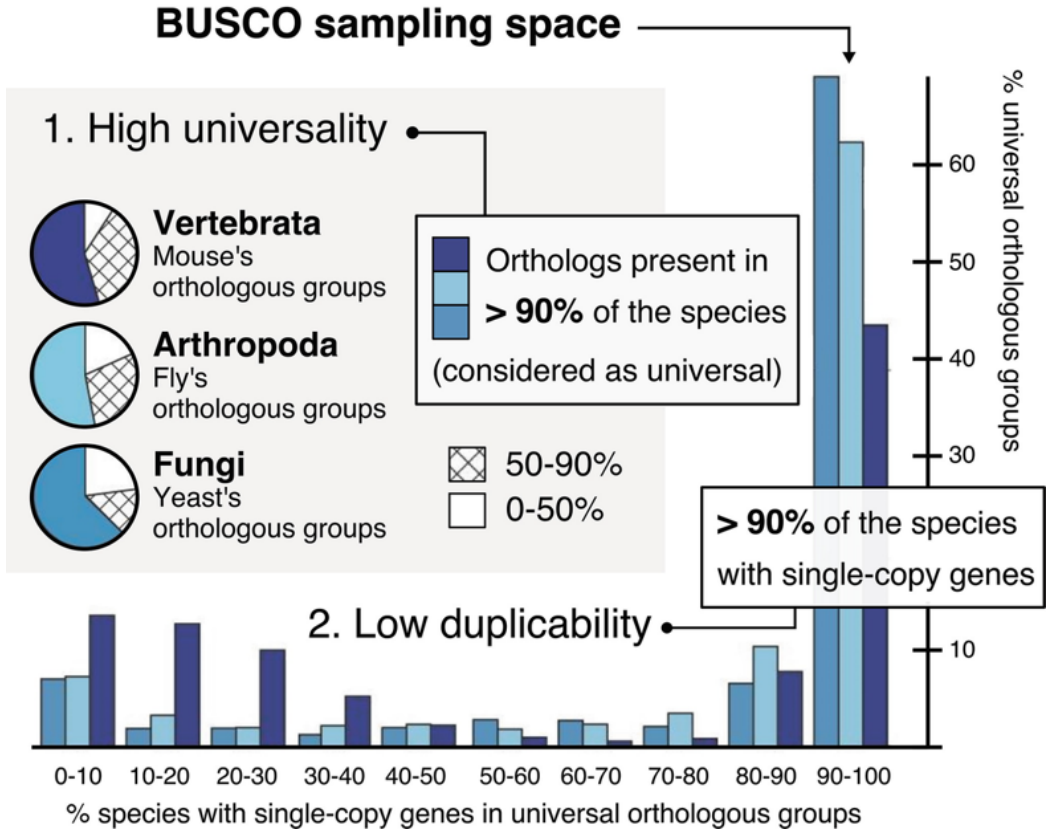
> Provides evolutionarily sound measures of completeness and redundancy in terms of expected gene content

> This is achieved by considering the presence of a predefined and expected set of single-copy marker genes as a proxy for genome-wide completeness.

https://busco.ezlab.org

RNA-Seq: Assembly

Quality assessment: BUSCO



Waterhouse et al (2011) Genome Biology and Evolution, 3:75–86

RNA-Seq: Assembly

Quality assessment: BUSCO

📝 BUSCO uses AUGUSTUS to predict gene models (determine what genes are likely present in your transcriptome).

📝 HMMER is then used to detect orthologs in those predicted genes (checking if the genes are similar to the set of conserved, universal orthologs).

RNA-Seq: Assembly

BUSCO

Running BUSCO

1. Activate environment

```
conda activate annotation
```

2. Call BUSCO to read help

```
busco --help
```

RNA-Seq: Assembly

BUSCO

Running BUSCO

2. Call BUSCO to read help

```
usage: busco -i [SEQUENCE_FILE] -l [LINEAGE] \
             -o [OUTPUT_NAME] -m [MODE] [OTHER OPTIONS]

  -i SEQUENCE_FILE, --in SEQUENCE_FILE
  -o OUTPUT, --out OUTPUT
  -m MODE, --mode MODE  Specify which BUSCO analysis mode
                        There are three valid modes:
                        - geno or genome (DNA)
                        - tran or transcriptome (DNA)
                        - prot or proteins (protein)
  -l LINEAGE, --lineage_dataset LINEAGE
                        Specify the name of the BUSCO
                        lineage to be used
```

RNA-Seq: Assembly

BUSCO

Running BUSCO

3. See available databases

```
busco --list-datasets

- eukaryota_odb10
    - metazoa_odb10
        - arthropoda_odb10
            - arachnida_odb10
            - insecta_odb10
                - endopterygota_odb10
                    - diptera_odb10
                    - hymenoptera_odb10
                    - lepidoptera_odb10
                - hemiptera_odb10
```

RNA-Seq: Assembly

BUSCO

Running BUSCO

3. See available databases

```
busco --list-datasets

- eukaryota_odb10.........................255 BUSCOs
    - metazoa_odb10.......................954 BUSCOs
        - arthropoda_odb10................1013 BUSCOs
            - arachnida_odb10
            - insecta_odb10...............1367 BUSCOs
                - endopterygota_odb10.....2124 BUSCOs
                    - diptera_odb10.......3285 BUSCOs
                    - hymenoptera_odb10
                    - lepidoptera_odb10
                - hemiptera_odb10
```

RNA-Seq: Assembly

BUSCO

Running BUSCO

4. Run BUSCO

```
busco -i Trinity.fasta -o busco_diptera -l diptera_odb10 \
      -m transcriptome -c 8
```

- -i: Transcriptome file
- -o: Output folder
- -l: dataset
- -m: analysis mode
- -c: number of threads

RNA-Seq: Assembly

BUSCO

BUSCO results

```
	--------------------------------------------------
	|Results from dataset diptera_odb10              |
	--------------------------------------------------
	|C:3.4%[S:3.2%,D:0.3%],F:0.8%,M:95.8%,n:3285     |
	|113    Complete BUSCOs (C)                      |
	|104    Complete and single-copy BUSCOs (S)      |
	|9    Complete and duplicated BUSCOs (D)         |
	|25    Fragmented BUSCOs (F)                     |
	|3147    Missing BUSCOs (M)                      |
	|3285    Total BUSCO groups searched             |
	--------------------------------------------------
```

RNA-Seq: Assembly

BUSCO

Plotting BUSCO results

5. Where is `generate_plot.py`?

```
which generate_plot.py
```

6. Test `generate_plot.py`

```
/path/to/script/generate_plot.py -h
```

7. Create a folder for summary files

```
mkdir BUSCO_summaries
```

RNA-Seq: Assembly

BUSCO

Plotting BUSCO results

8. Move summary files to new folder

```
cp short_summary.txt BUSCO_summaries/
```

9. Run `generate_plot.py`

```
generate_plot.py -wd BUSCO_summaries/
```

10. If it doesn't work, run

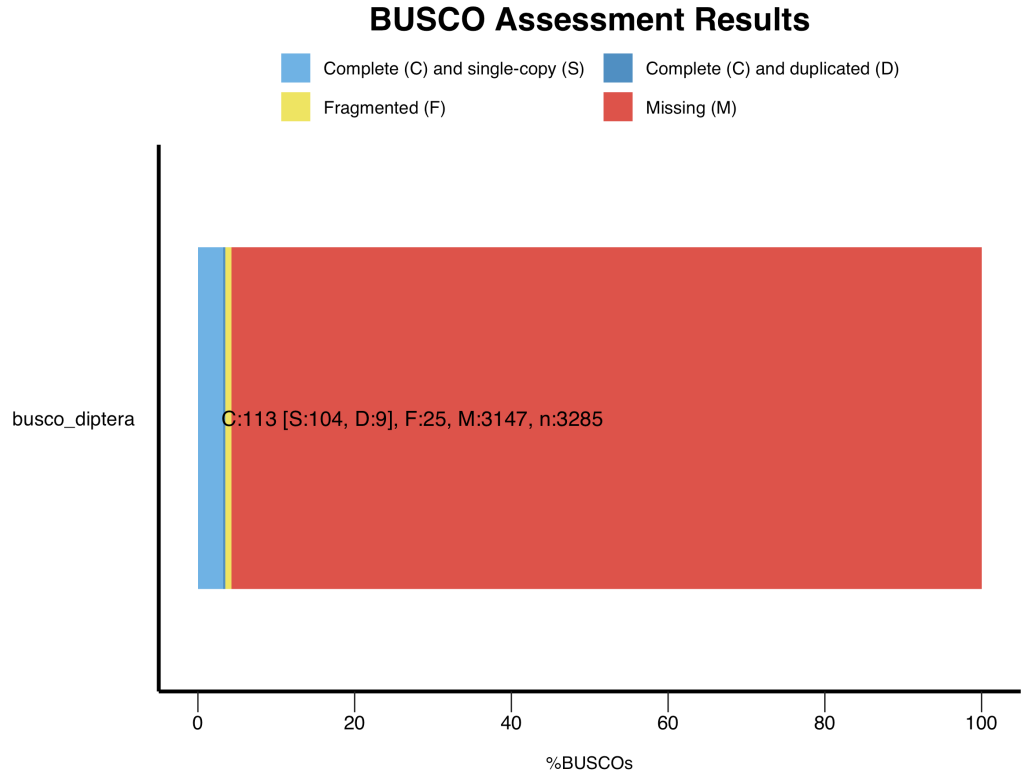
```
generate_plot.py -wd BUSCO_summaries/ --no_r
```

11. Open the R script in Rstudio and run it

RNA-Seq: Assembly

BUSCO

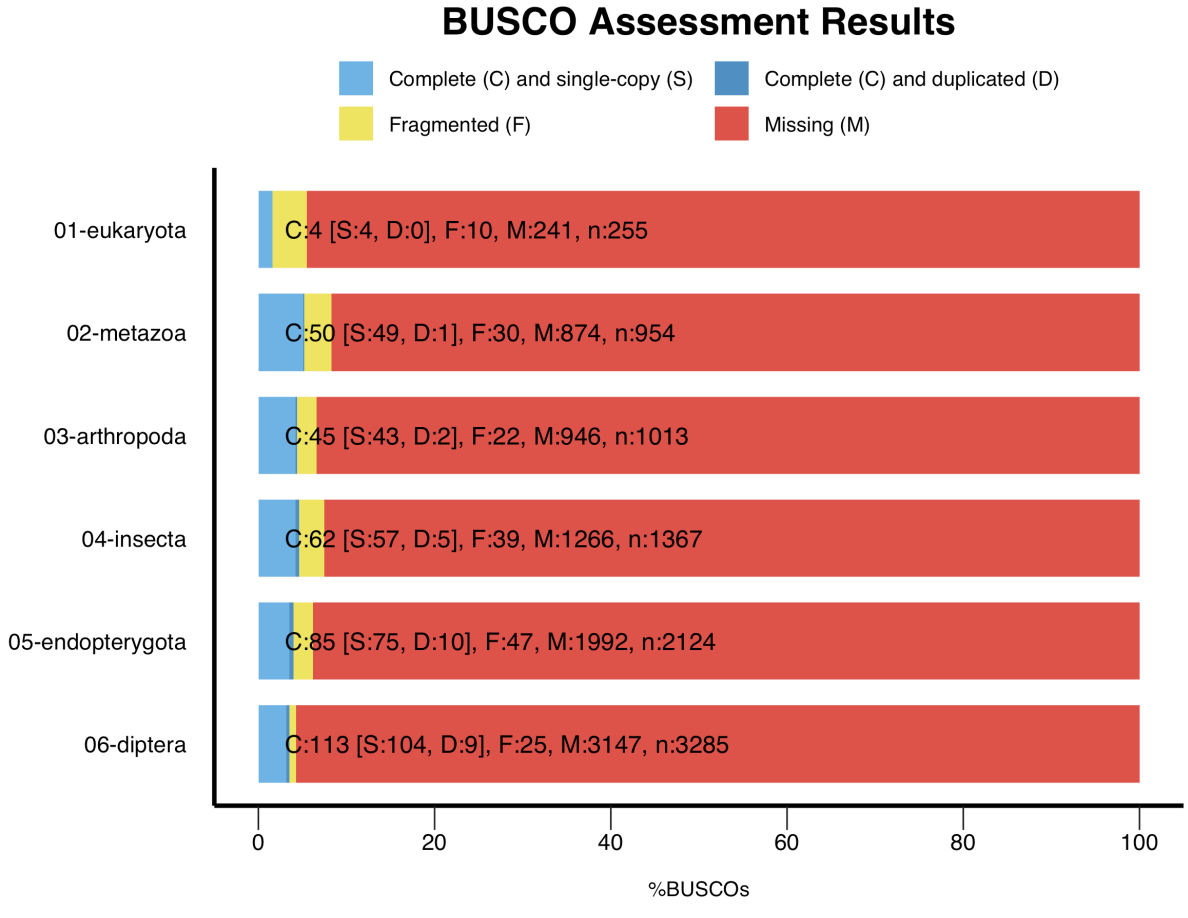
Plotting BUSCO results



RNA-Seq: Assembly

BUSCO

Plotting BUSCO results



RNA-Seq: Assembly

BUSCO

Plotting BUSCO results