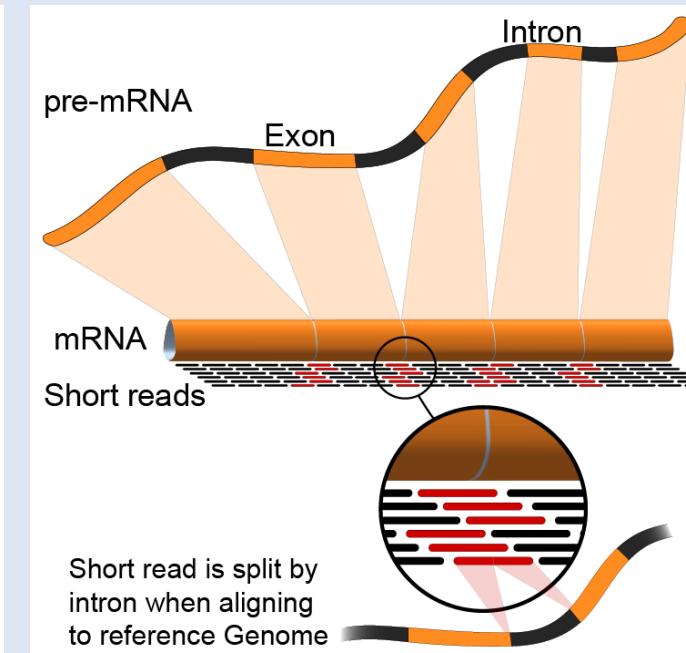
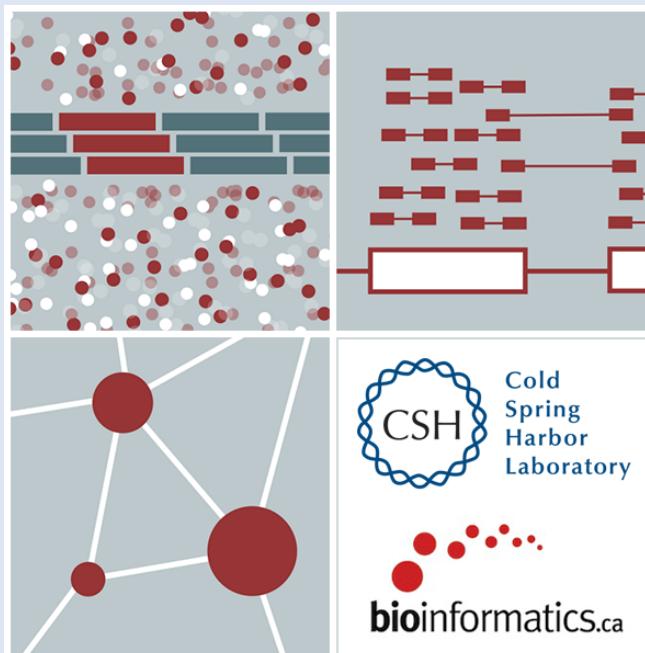




Cold
Spring
Harbor
Laboratory



RNA-Seq Module 2: Alignment vs Assembly vs Pseudoalignment

Felicia Gomez, Charlz Jerold, Obi Griffith, Malachi Griffith,

My Hoang, Mariam Khanfar, Chris Miller, Kartik Singhal, Jennie Yao

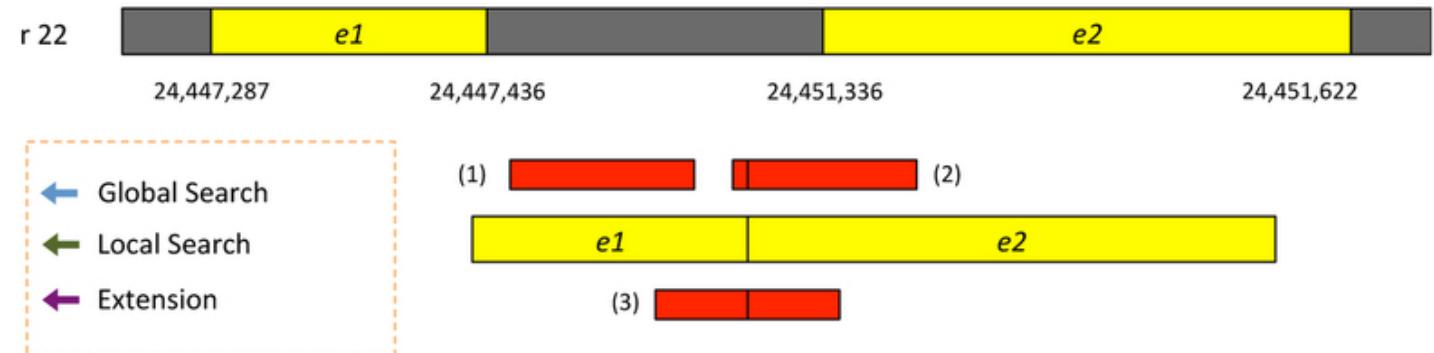
Advanced Sequencing Technologies & Bioinformatics Analysis November 10-21, 2025



Washington University in St. Louis
SCHOOL OF MEDICINE

Alignment

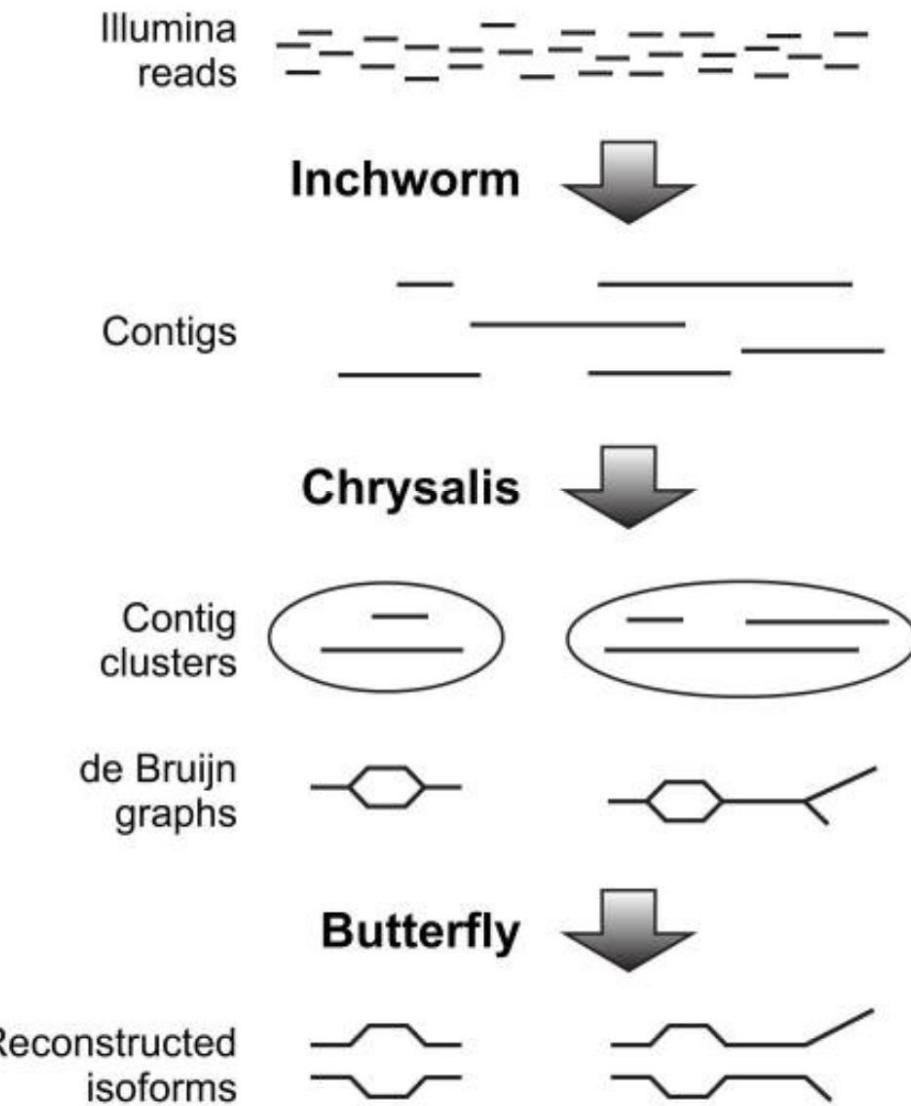
- Uses a reference genome/transcriptome to map reads
- Capable of some novel transcript inference
- Relatively fast runtime
- Tools: HISAT2, STAR, GSNAP



Kim et al. 2015. Nat Methods 12:357–360

Assembly

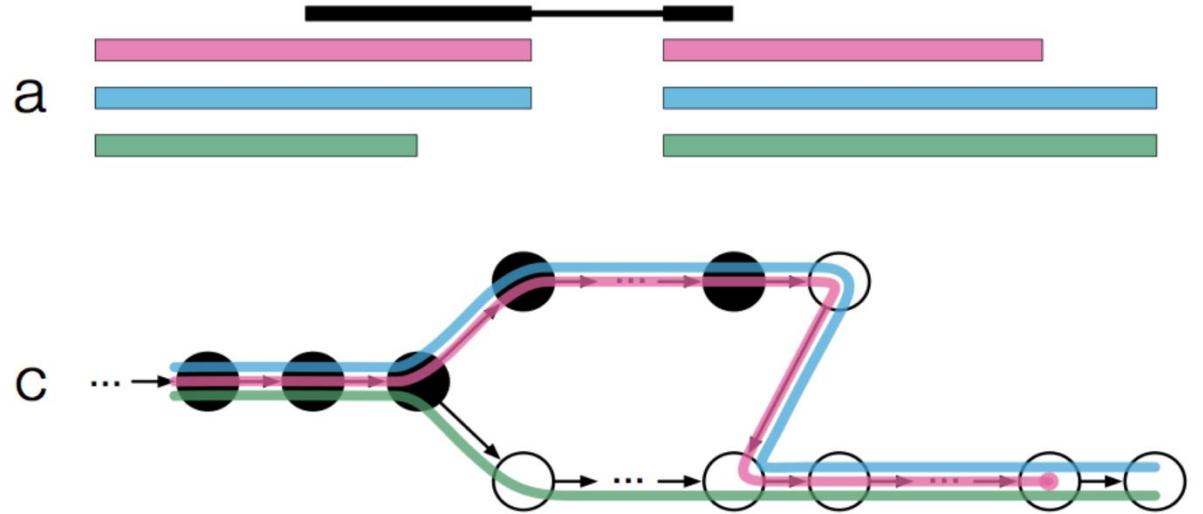
- Infer transcript structure directly from the data
- Useful when you do not have a reference sequence
- Other uses – highly rearranged genomes (some cancers)
- Computationally expensive
- Tools: Trinity, Velvet, SPAdes



Haas, et al (2013) doi: 10.1038/nprot.2013.084

Pseudoalignment

- Does not determine where in the genome a read lies, only which transcripts it is compatible with
- Very fast!
- Does not produce a bam by default (though pseudo-bams can be created), not useful for variant detection.
- Tools: Kallisto, Sailfish



Bray, 2016 doi:10.1038/nbt.3519

<https://tinyheero.github.io/2015/09/02/pseudoalignments-kallisto.html>

We are on a Coffee Break & Networking Session