

Canadian Bioinformatics Workshops

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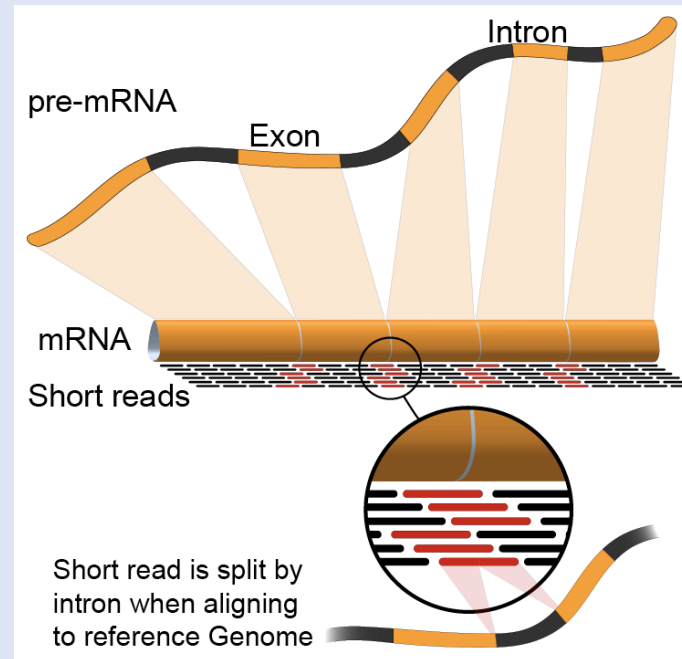
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RNA-Seq Module 3: HTSeq

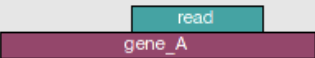
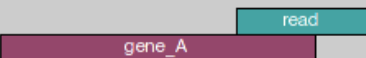


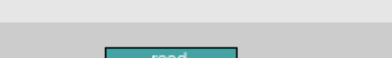
Malachi Griffith, Obi Griffith, Isabel Risch,
Nicolas Ho, Melisa Acun, Varinder Verma, Mobin Khoramjoo
RNA-seq Analysis 2025. July 7-9, 2025



Alternatives to FPKM

- Raw read counts for differential expression analysis
 - Assign reads/fragments to defined genes/transcripts, get “raw counts”
 - Transcript structures could still be defined by something like Stringtie
 - HTSeq (htseq-count)
 - <https://htseq.readthedocs.io/>
- ```
htseq-count --mode intersection-strict --stranded no --minqual 1 --type
exon --idattr transcript_id accepted_hits.sam chr22.gff >
transcript_read_counts_table.tsv
```
- Caveats of ‘transcript’ analysis by htseq-count:
    - Designed for genes - ambiguous reads from overlapping transcripts may not be handled!
    - <http://seqanswers.com/forums/showthread.php?t=18068>

# HTSeq-count basically counts reads supporting a feature (exon, gene) by assessing overlapping coordinates

|                                                                                      | union     | intersection_strict | intersection_nonempty |
|--------------------------------------------------------------------------------------|-----------|---------------------|-----------------------|
|    | gene_A    | gene_A              | gene_A                |
|    | gene_A    | no_feature          | gene_A                |
|    | gene_A    | no_feature          | gene_A                |
|    | gene_A    | gene_A              | gene_A                |
|    | gene_A    | gene_A              | gene_A                |
|    | ambiguous | gene_A              | gene_A                |
|  | ambiguous | ambiguous           | ambiguous             |

Note, if gene\_A and gene\_B on opposite strands, sequence data is stranded, and correct HTSeq parameter set then this read may not be ambiguous

Whether a read is counted depends on the nature of overlap and “mode” selected

# We are on a Coffee Break & Networking Session

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