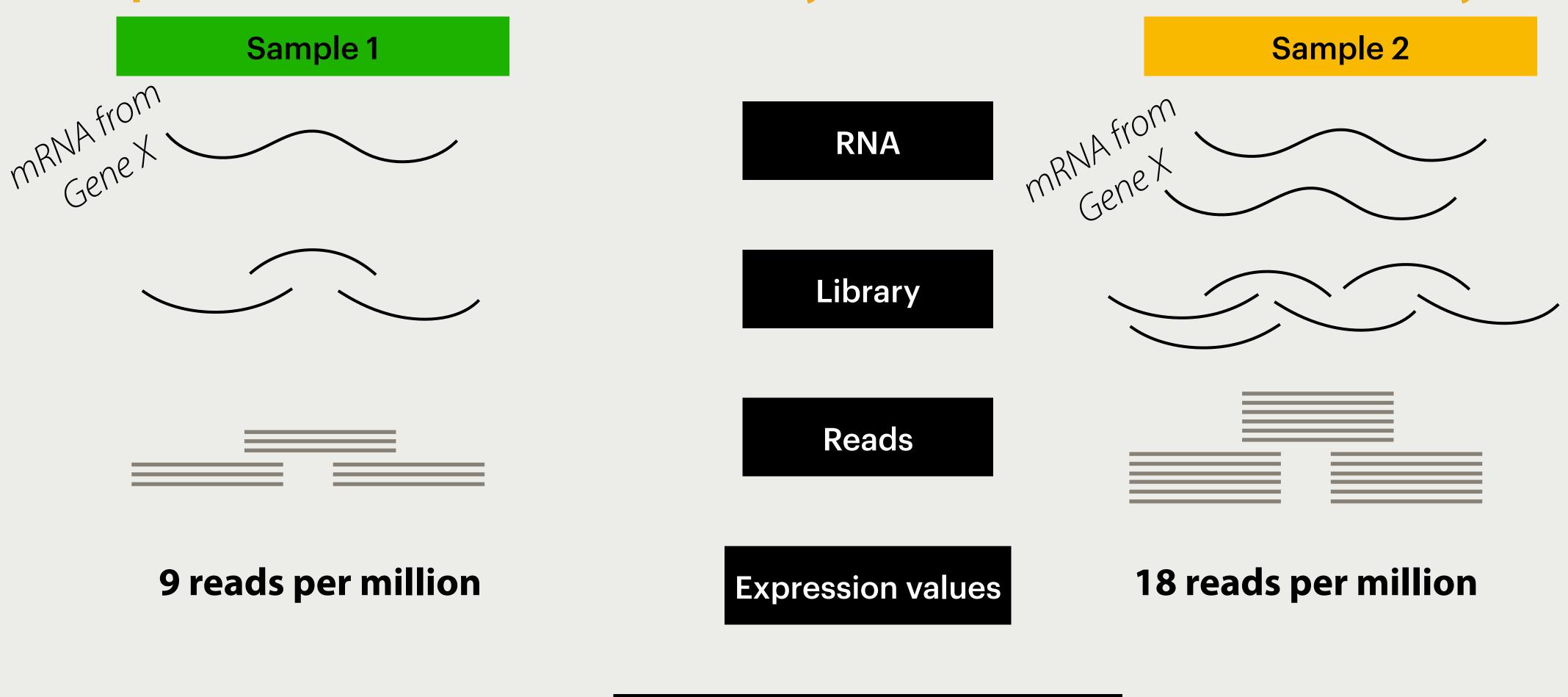
# BIOL 343 Applied Bioinformatics I Counting

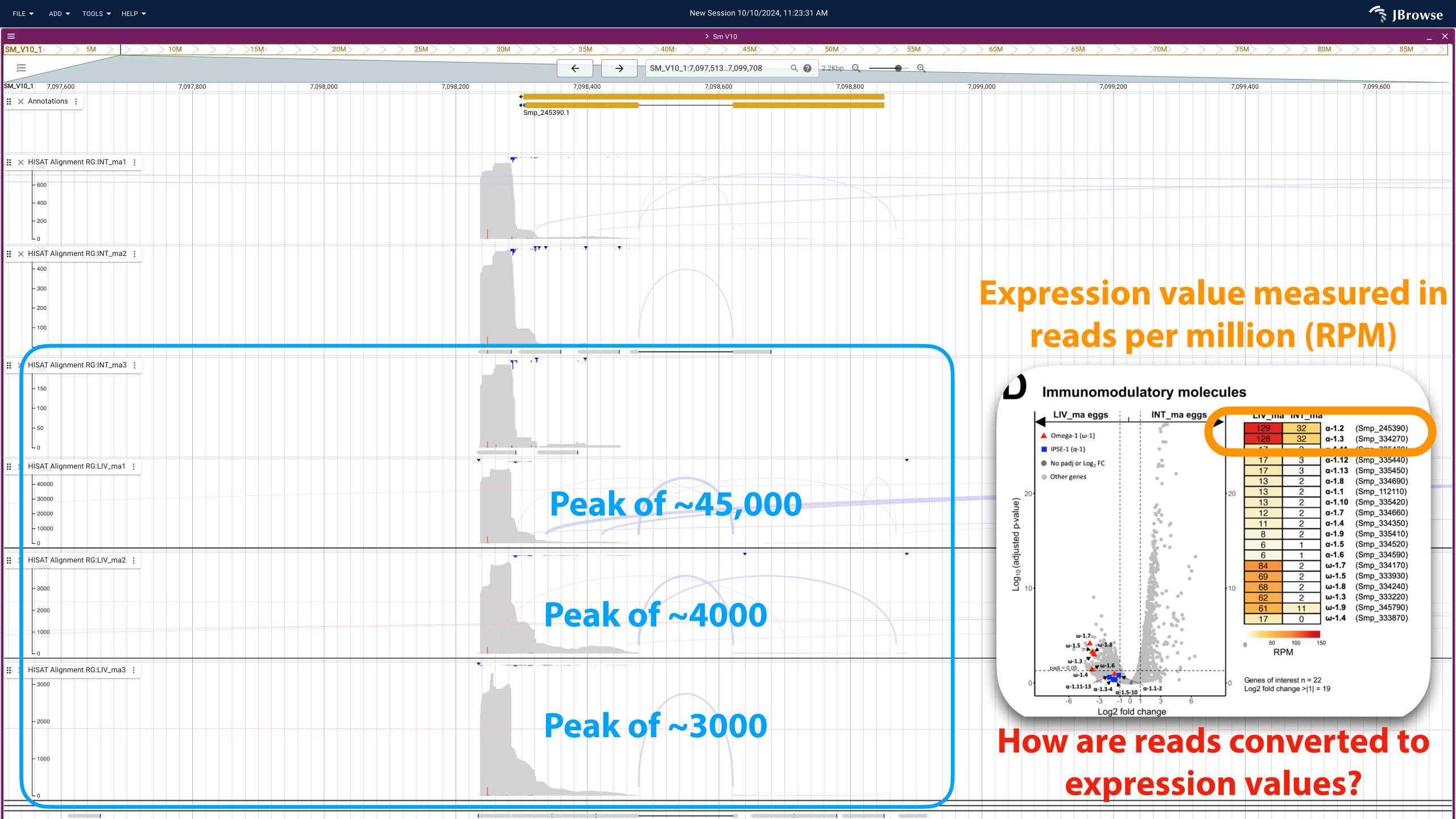
#### Counting converts mapped reads to expression values Expression values will be used by statistical models to identify DEGs

- Recall the goal of our RNA-seq experiments...
  - Treatment vs Control
  - Mutant vs Wild type
  - Identify differentially expressed genes (DEGs)
- DEGs will be identified using statistical tests comparing *expression values* of transcripts/genes
- Expression values will be calculated based on the number of reads that *align/map* to a given genomic locus
- There are different ways to count reads

#### Counting converts mapped reads to expression values Expression values will be used by statistical models to identify DEGs



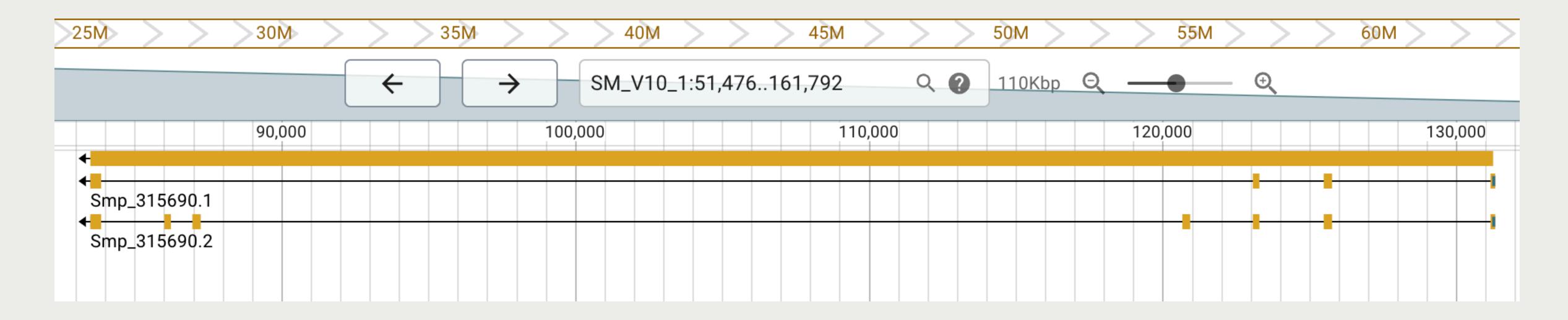
Differentially expressed gene?



## How are reads converted to expression values? Many different situations must be considered

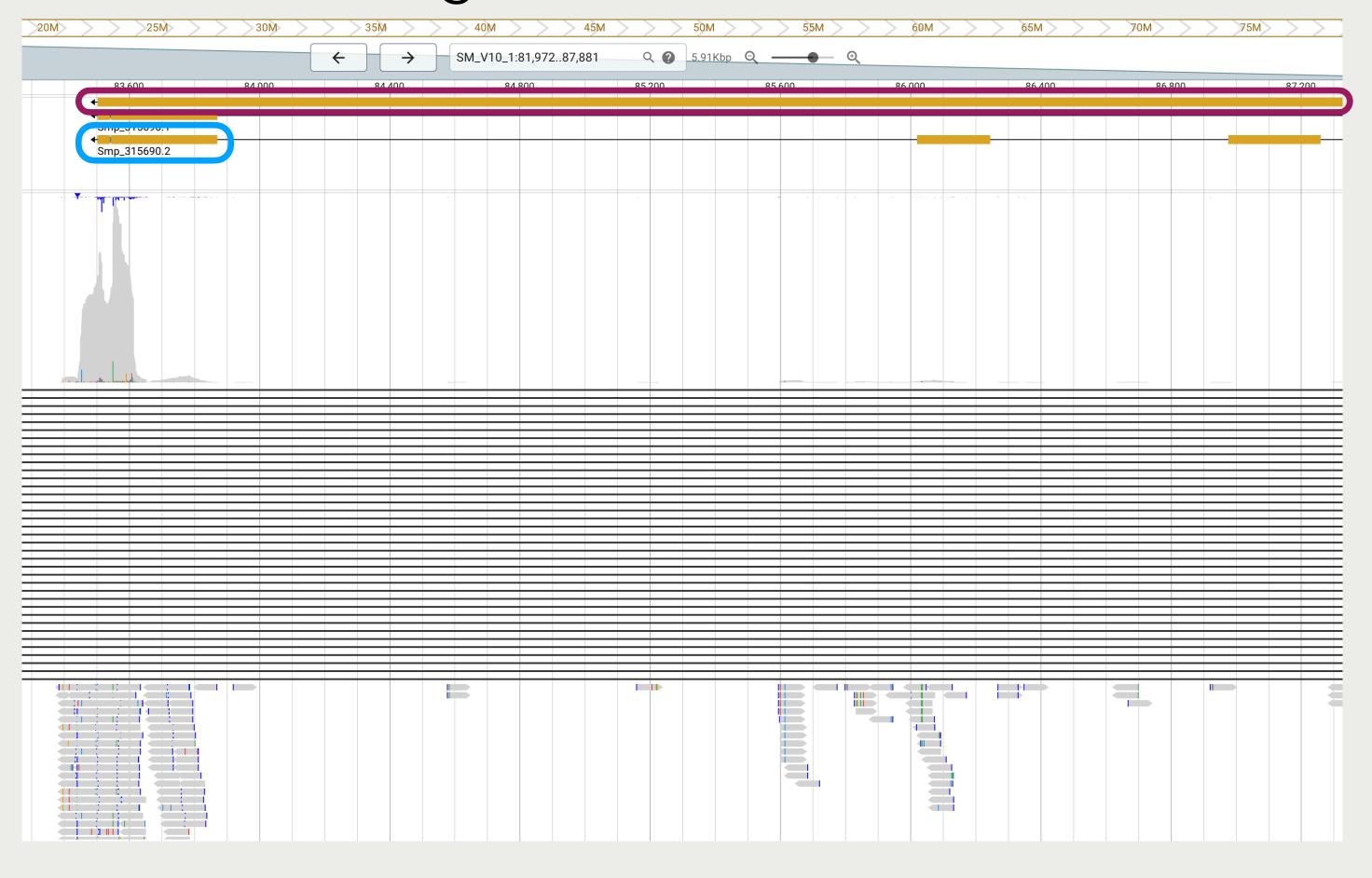
- What if a read only overlaps 1 nt of the gene?
- What if a read overlaps two genes?
- What if a gene has a lot of reads at one location and no reads at a different location?
- What if reads overlap a gene but the originated from the opposite strand?
- What if a read overlaps mostly intron?
- How do you link reads to different isoforms of the same gene?
- How do you normalize for genes that have variable lengths?

- Consider SM\_V10\_1:80,002..135,160
  - Smp\_315690 (gene)
  - Smp\_315690.1 and Smp\_315690.2 (transcripts)
    - Differ by the 3 exons (found in .2 but not .1)



SM_V10_1	WormBase	gene	83503	131262	gene_id "Smp_315690"; gene_version "1"; gene_source "WormBase"; gene_biotype "protein_coding";
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SM_V10_1	WormBase	stop_codon	83541	83543	O gene_id "Smp_315690"; gene_version "1"; transcript_id "Smp_315690.2"; exon_number "7"; gene_source "WormBase"; gene_biotype "protein_coding"; transcript_id "Smp_315690.2"; exon_number "7"; gene_source "WormBase"; gene_source "YormBase"; gene_source "
SM V10 1	WormBase	five prime utr	131233	131262	gene id "Smn 315690", gene version "1", transcript id "Smn 315690.2", gene source "WormBase", gene biotype "protein coding", transcript source "Wo

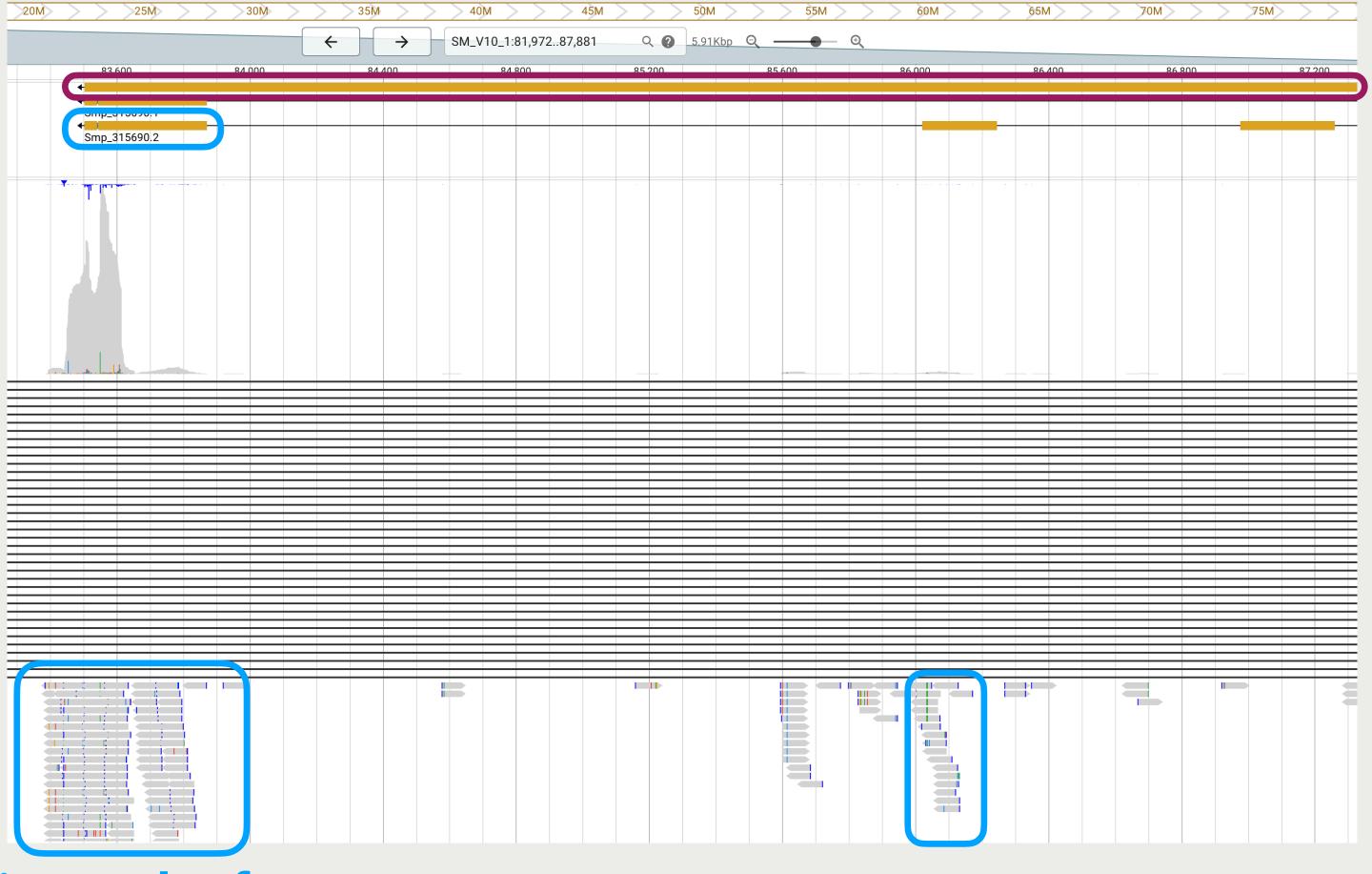
Most reads align to the 3' end:



Feature = exon (by default) Meta-feature = gene\_id (by default)

- featureCounts first counts "hits" to features (i.e., exons)
- "Hit" is any read that overlaps by 1 bp (by default)
- It then counts "hits" to metafeatures (i.e., genes)
  - Tallies all hits to an exon that is apart of that gene

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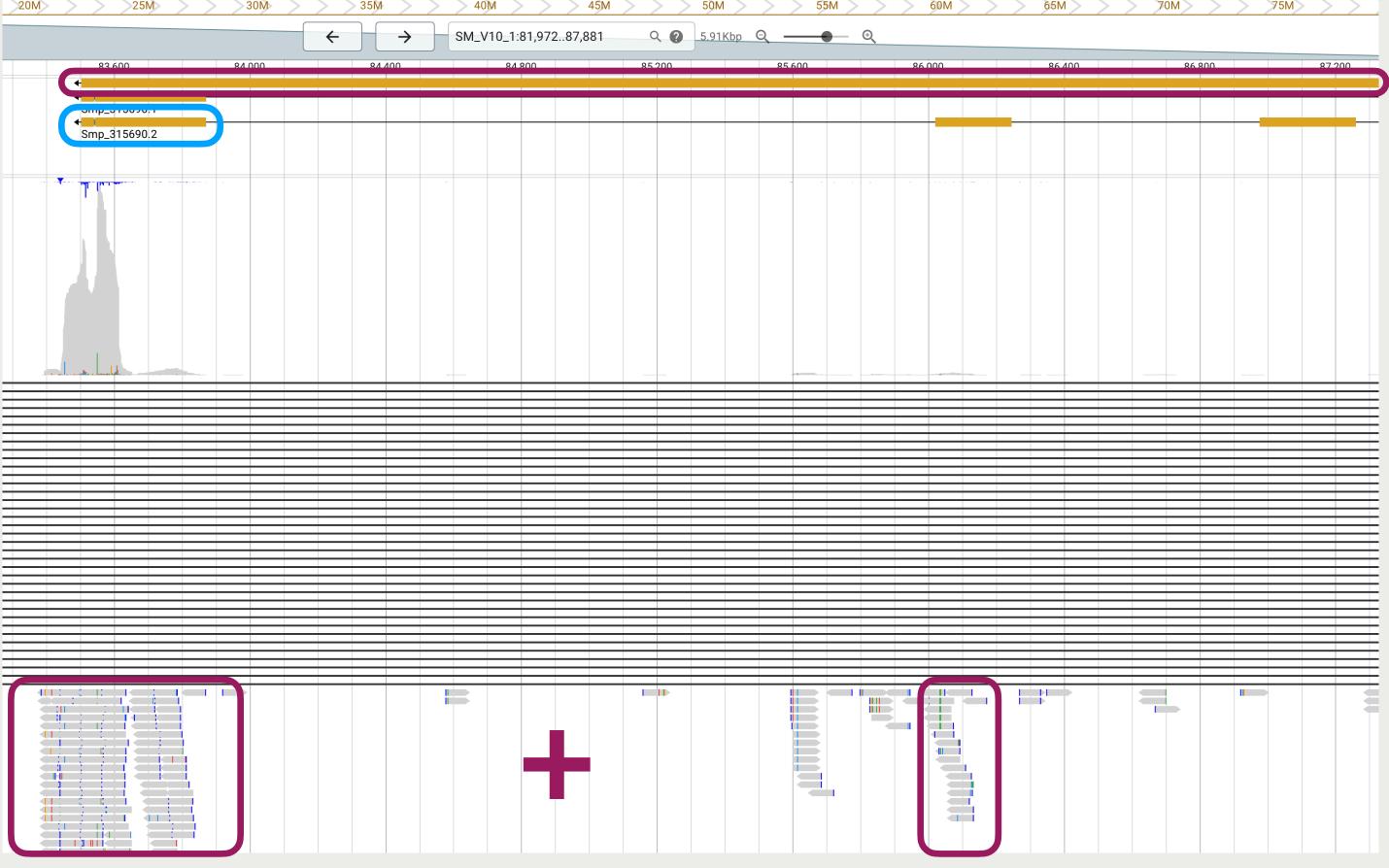


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Hits to the feature

Most reads align to the 3' end:

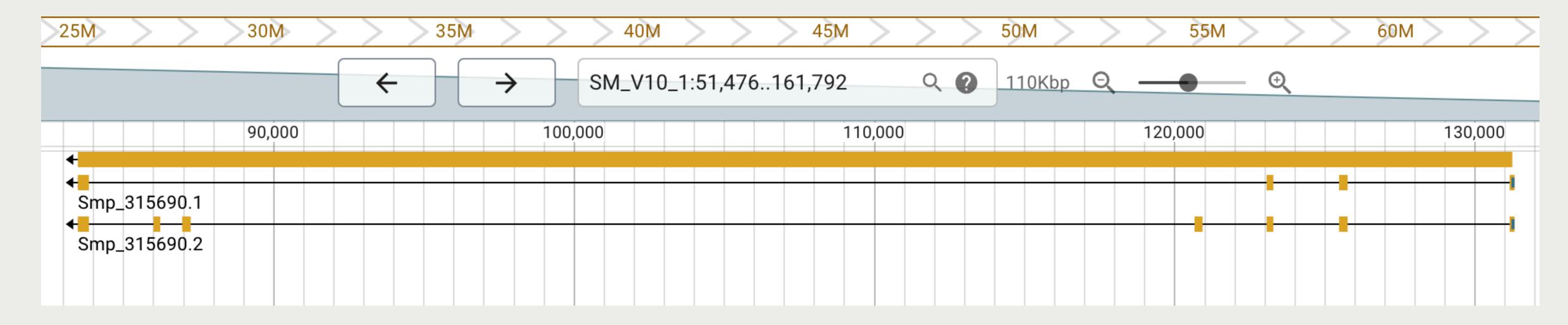


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Hits to the meta-feature

- featureCounts has excellent performance (written in C)
- Works well when transcript-level differential expression is not of interest
  - What about when you want to differentiate isoforms (different transcripts from the same gene) like Smp\_315690.1 and Smp\_315690.2?
  - Only use hits that map unambiguously...



- Output: integer counts at meta-feature level
- Matrix of integer values the value in the *i*-th row and the *j*-th column represents the reads assigned to gene *i* in sample *j*
- Cannot do differential expression with only these counts
  - Unnormalized
    - Longer genes will necessarily have more reads
    - Counts will vary by library size (i.e., number of reads that aligned)
    - Differential expression tools will do the normalization for us