BIOL 343
Applied Bioinformatics I
Post-alignment QC

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Alignment is the most important step in RNA-seq analysis

Counting (also important) and DEG ID relies on high-confidence mapping

- 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
- ***Filtering*** out spurious or redundant alignments is an important QC step after mapping

Browsing RNA-seq alignments

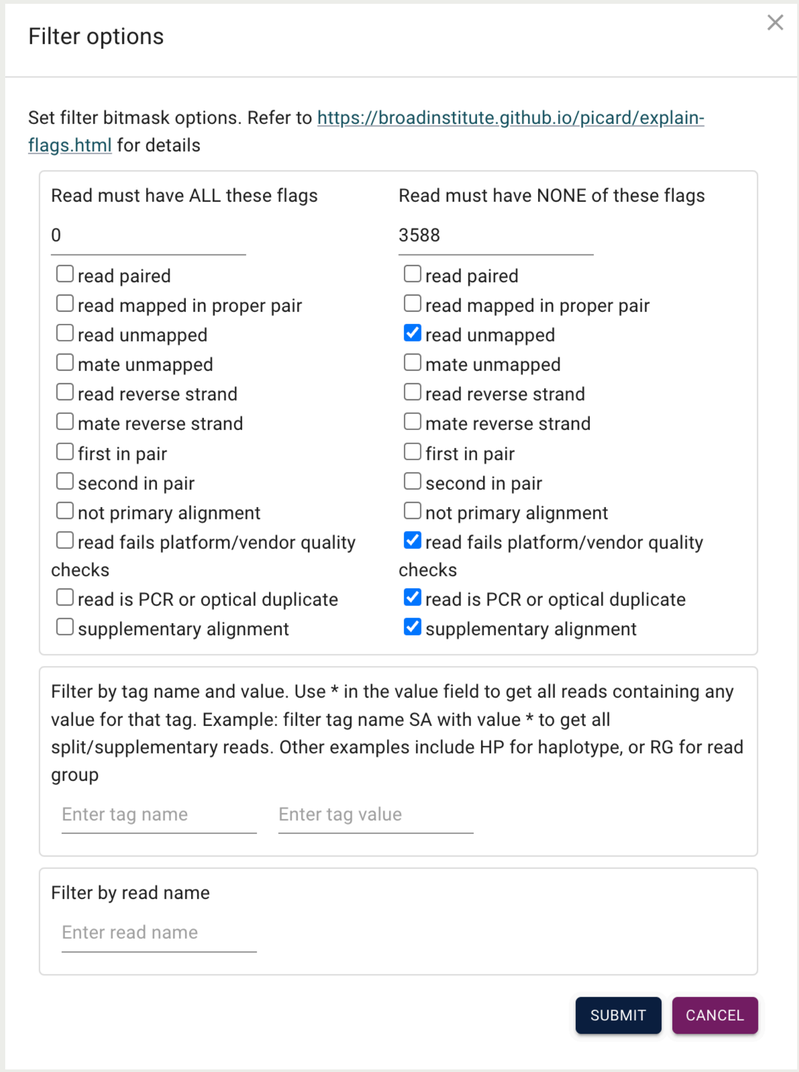
JBrowse2

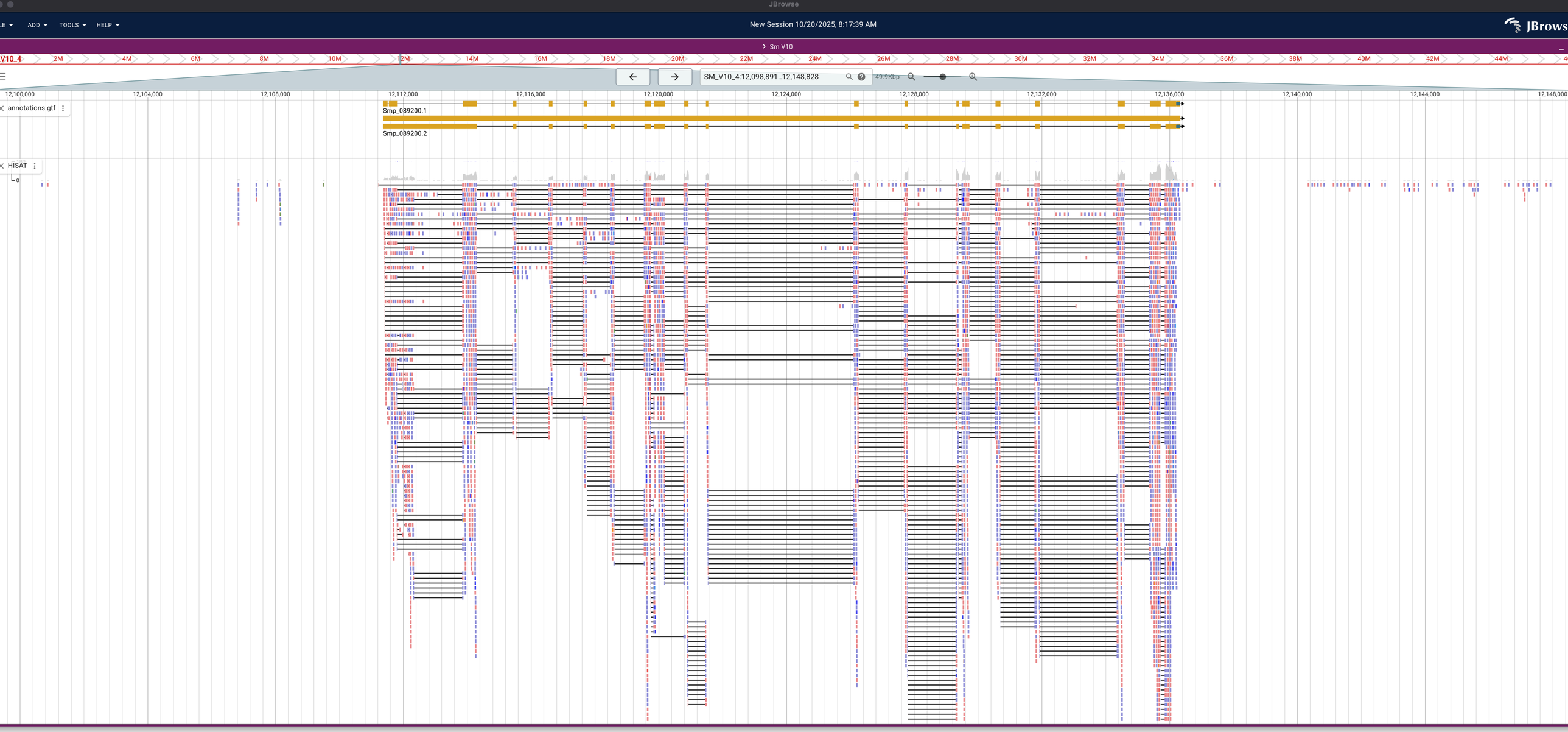
1. Open JBrowse2
2. Download the genome files (.fa & .gtf)
3. Download the STAR alignment (Aligned.sorted.bam) and indexes
4. Load the genome (.fa)
5. Add tracks:
    1. GTF
    2. Alignments

Browsing RNA-seq alignments

JBrowse2

1. Navigate to SM_V10_4:12,110,496..12,137,215
2. Three dots on RNA-seq track…
    1. Pileup settings > Filter by…
    2. Pileup settings > Color by > Strand
    3. SNPCoverage settings > Draw arcs (deselect)




JBrowse
ADD
TOOLS
HELP
New Session 10/20/2025, 8:17:39 AM
JBrows
Sm V10
SM_V10_4:12,098,891..12,148,828
49.9Kbp
annotations.gtf
Smp_089200.1
Smp_089200.2
HISAT

Browsing RNA-seq alignments

JBrowse2

1. Close STAR Alignment
2. HISAT Alignment three dots…
    1. Pileup settings > Group by…
        1. Tag name = RG (read group)
        2. Deselect checkbox
3. Close the STAR alignment and all D/E alignments
4. Navigate to SM_V10_4:12,110,496..12,137,215
5. Three dots…
    1. SNPcoverage settings > remove arcs

JBrowse

FILE ADD TOOLS HELP

New Session 10/20/2025, 10:37:29 AM

JBrowse

SM V10

SM_V10_4 2M 4M 6M 8M 10M 12M 14M 16M 18M 20M 22M 24M 26M 28M 30M 32M 34M 36M 38M 40M 42M 44M 46M

SM_V10_4:12,110,496..12,137,215 26.7Kbp

12,112,000 12,114,000 12,116,000 12,118,000 12,120,000 12,122,000 12,124,000 12,126,000 12,128,000 12,130,000 12,132,000 12,134,000 12,136,000

Annotations

Smp_089200.1

Smp_089200.2

STAR RO ADULT_PZQ_A

STAR RO ADULT_PZQ_B

STAR RO ADULT_PZQ_C

STAR RO ADULT_CTRL_A

STAR RO ADULT_CTRL_B

STAR RO ADULT_CTRL_C

Higher expression in CTRL than PZQ…do we see that?