Cold Spring Harbor Laboratory

Advanced Sequencing Technologies & Applications

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Cold Spring Harbor Laboratory

RNA-Seq Module 4
Isoform Discovery and Alternative Expression (tutorial)

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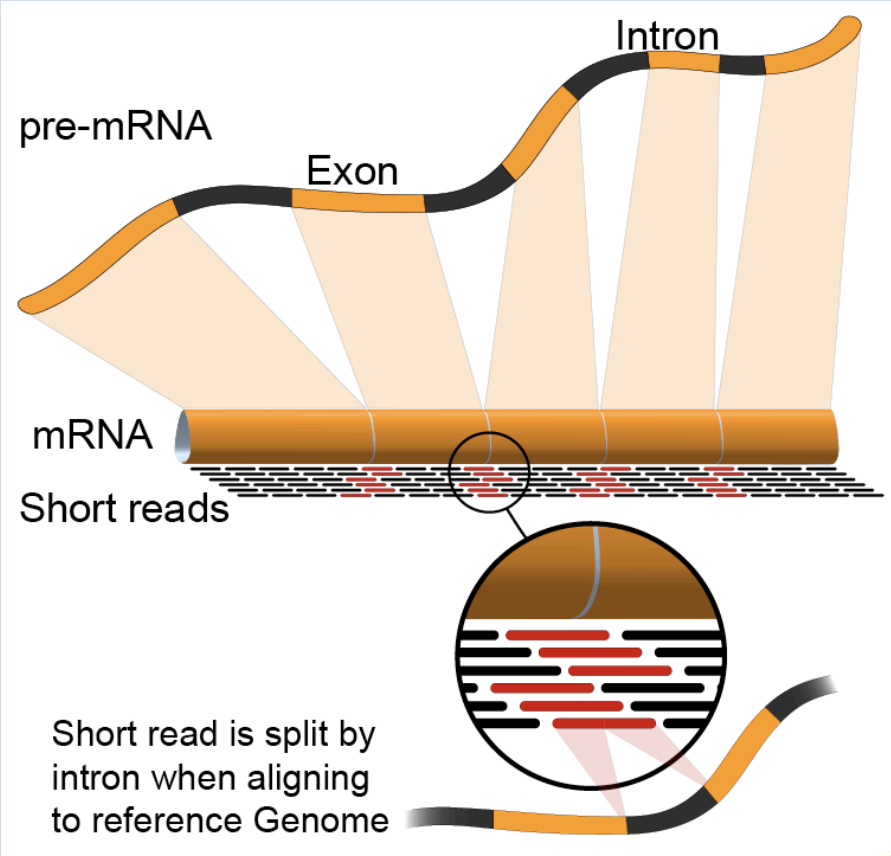


THE GENOME INSTITUTE
at Washington University

Learning Objectives of Tutorial

- Learn how to run StringTie in 'reference only', 'reference guided', and 'de novo' modes
- Learn how to use Cuffmerge to combine transcriptomes from multiple StringTie runs and compare assembled transcripts to known transcripts
- Learn how to perform differential splicing analysis with Ballgown
- Examine junctions counts with RegTools and StringTie alternative transcript files at the command line
- Visualize junction counts and StringTie assembled transcripts in IGV

5-i,ii. Running stringtie in 'ref-guided' and 'de-novo' mode

- In Module 3 we ran StringTie in 'ref-only' mode. This mode gives us an expression estimate for each known gene/transcript
- Now we want to be able to potentially identify novel genes, and novel isoforms of known genes
- To accomplish this we will re-run cufflinks in 'ref-guided' and 'de-novo' modes
  - In 'ref-guided' mode a known transcriptome will be used as a guide
  - In 'de-novo' mode no knowledge of the transcriptome will be used at all

Options that govern use of existing transcript information

- During indexing of the genome with hisat2, transcript information is provided
  - A transcriptome GTF file is used to extract splice sites and exons
  - These are supplied during the index step to build a better index
  - These will be used to **assist the alignment** step by allowing alignment to both transcriptome and genome sequences
  - Coordinates from alignments to transcriptomes will be converted back to genome coordinates
  - Even though we supply transcriptome info, hisat2 will not be limited in to known transcripts or splice sites
- Stringtie '-G' option
  - Used to supply a transcriptome GTF file
  - If specified, uses the reference annotation file (in GTF or GFF3 format) to guide the assembly process. We call this the '**ref-guided**' analysis mode
- Stringtie '-e' option
  - Limits the processing of read alignments to only estimate and output the assembled transcripts matching the reference transcripts given with the -G option
  - We call this '**reference-only**' analysis mode
- Running StringTie with neither '-G' or '-e'
  - We call this '**de-novo**' analysis mode

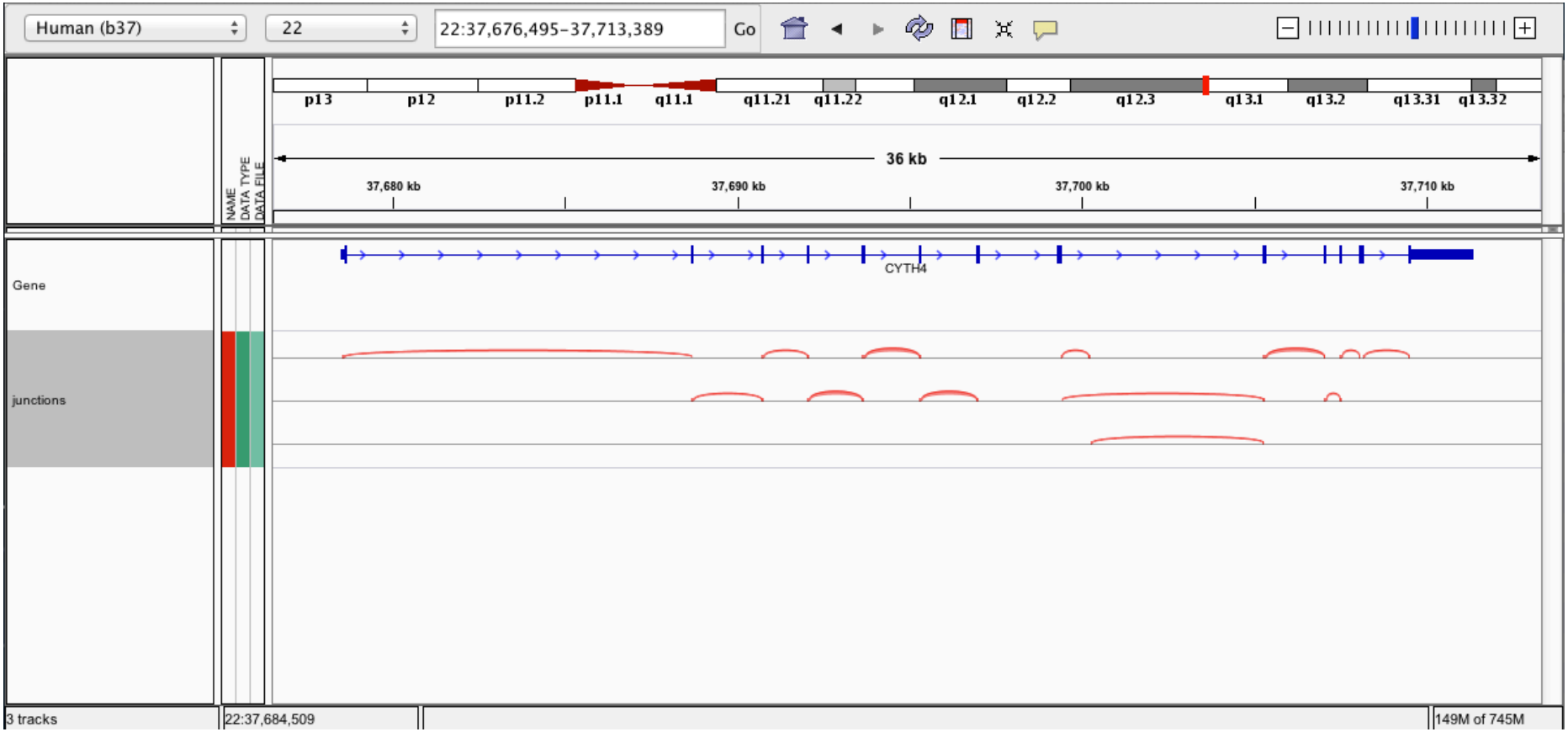
A ‘junctions.bed’ file

- After alignment, we can create a summary of all reads that support exon-exon junctions
  - e.g. exon1-exon2 has 5 reads
  - e.g. exon1-exon3 has 9 reads
- This file reports all of the unique exon-exon junctions observed and the read counts for each
  - In BED format

```
track name=junctions description="TopHat junctions"
22      17062079        17063415        JUNC00000001    3       -       17062079        17063415        255,0,0 2       98,19   0,1317
22      17092740        17095057        JUNC00000002    5       +       17092740        17095057        255,0,0 2       43,91   0,2226
22      17117940        17119543        JUNC00000003    6       +       17117940        17119543        255,0,0 2       40,75   0,1528
22      17152466        17156100        JUNC00000004    3       -       17152466        17156100        255,0,0 2       12,88   0,3546
22      17525819        17528242        JUNC00000005    1       +       17525819        17528242        255,0,0 2       71,29   0,2394
22      17528261        17538007        JUNC00000006    1       +       17528261        17538007        255,0,0 2       55,45   0,9701
22      17566071        17577976        JUNC00000007    10      +       17566071        17577976        255,0,0 2       48,25   0,11880
22      17577951        17578785        JUNC00000008    24      +       17577951        17578785        255,0,0 2       25,99   0,735
22      17578093        17578710        JUNC00000009    1       +       17578093        17578710        255,0,0 2       76,24   0,593
```

Junction read count

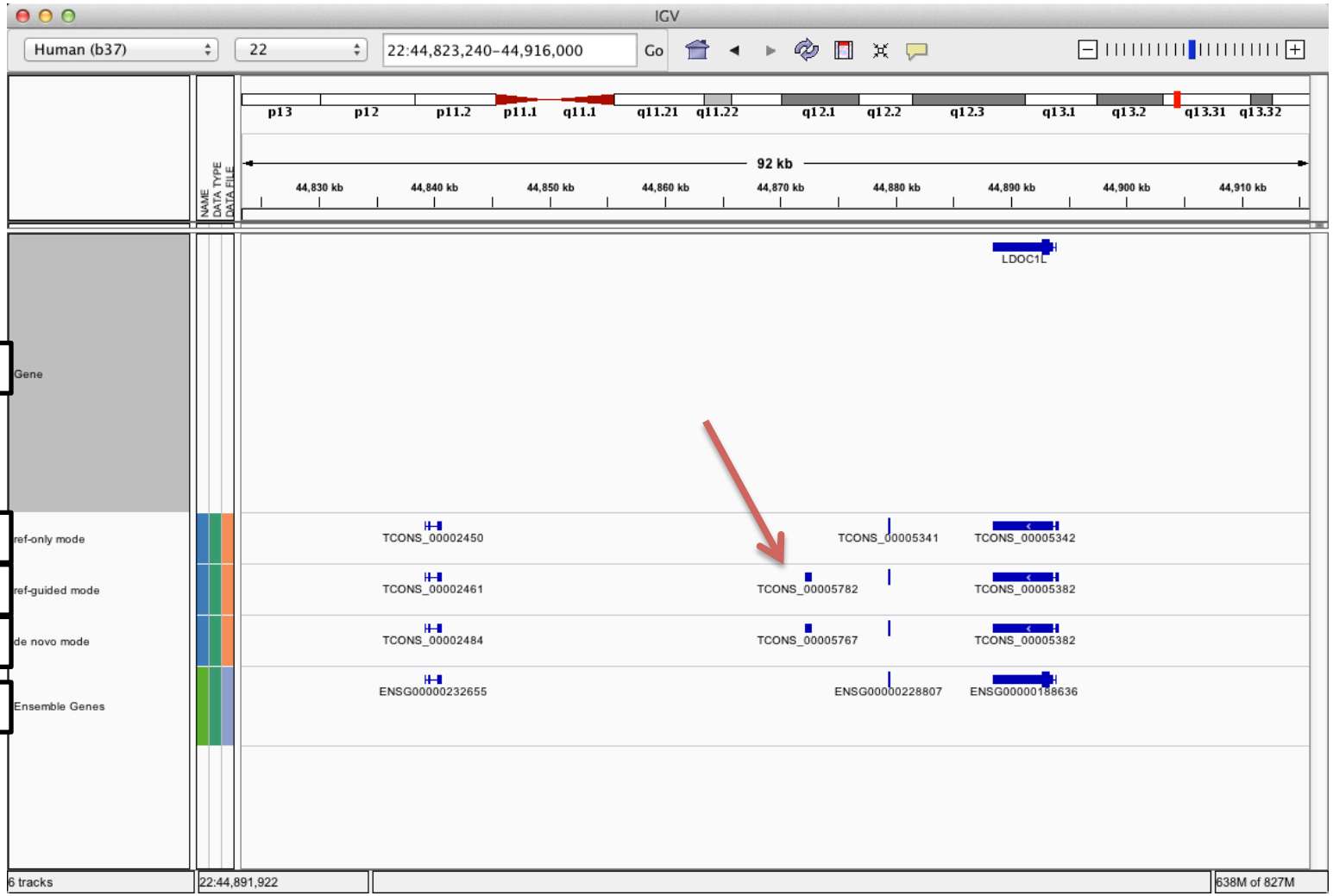
Viewing the junctions.bed in IGV



5-iii,iv. StringTie merge

- https://ccb.jhu.edu/software/stringtie/index.shtml
- StringTie merge combines transcripts predicted from multiple RNA-seq data sets into one view of the transcriptome
  - Do this before running StringTie to compare between multiple conditions
- StringTie merge can also simultaneously compare transcripts to the known transcripts GTF file from Ensembl, etc.
  - http://cufflinks.cbcb.umd.edu/manual.html#class_codes

5-v. Comparison of merged GTFs from each StringTie mode



UCSC genes

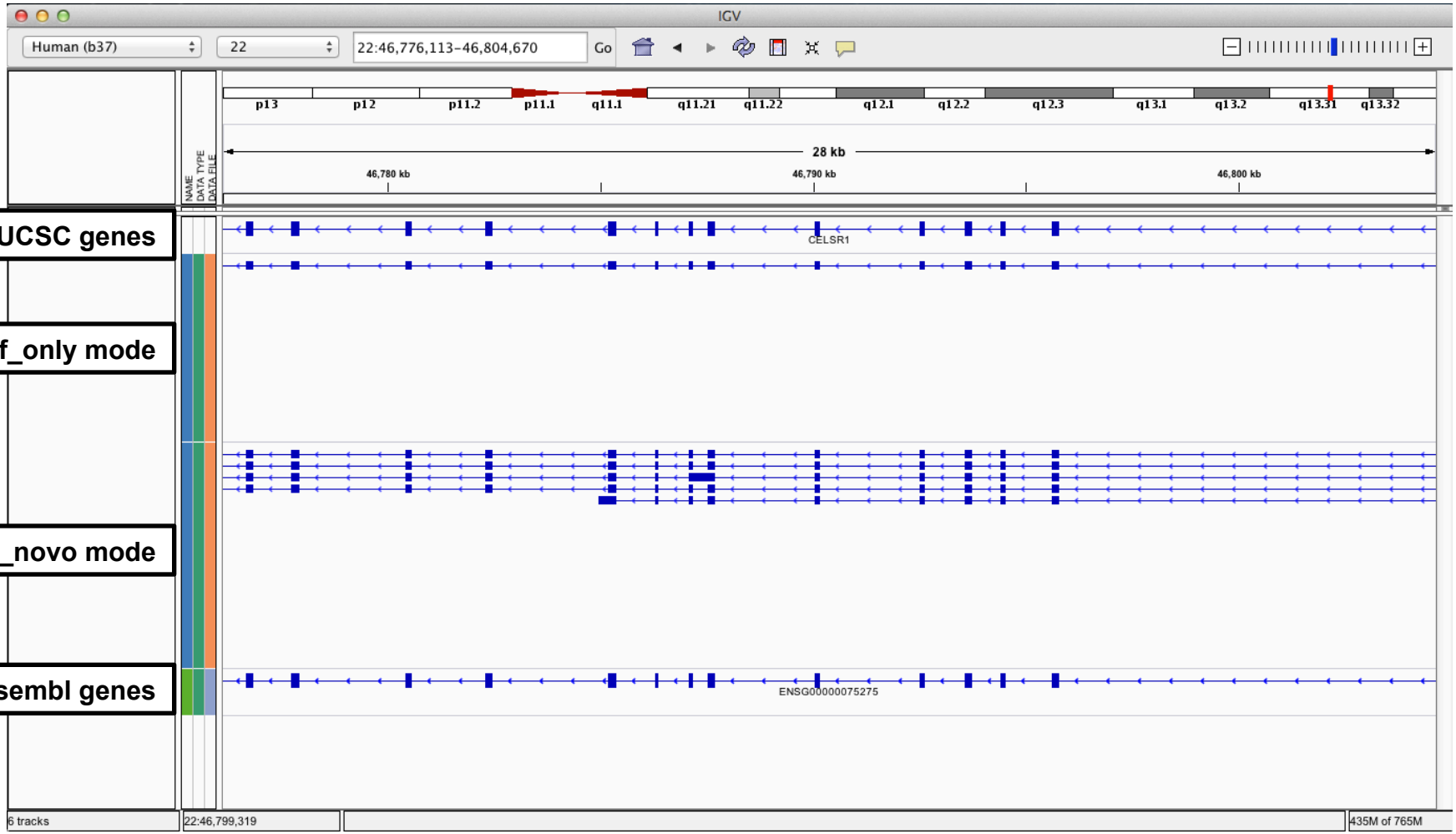
ref_only mode

ref_guided mode

de_novo mode

Ensembl genes

Comparison of merged GTFs from each StringTie mode



We are on a Coffee Break & Networking Session