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GenViz Module 4: Expression profiling, visualization, and interpretation

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Genomic Data Visualization and Interpretation
September 11-15, 2017
Berlin

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Learning objectives of the course

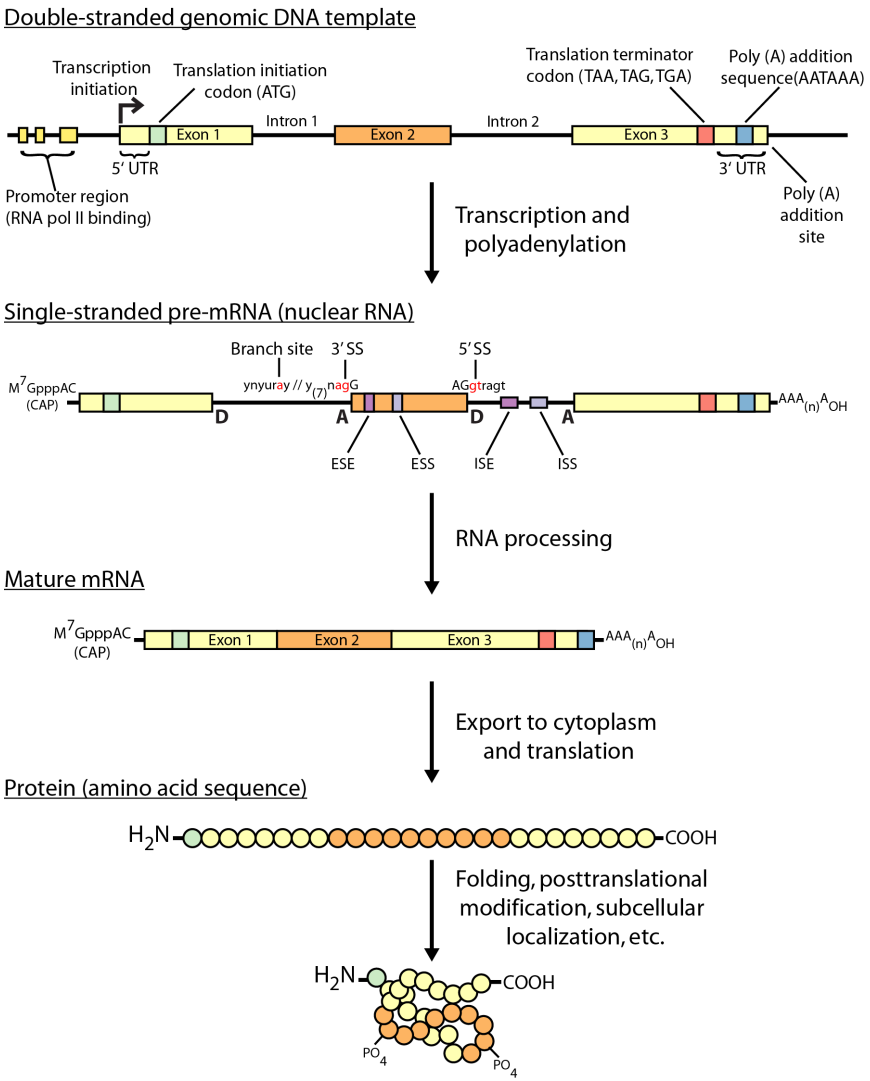
- Module 1: Introduction to genomic data visualization and interpretation
- Module 2: Using R for genomic data visualization and interpretation
- Module 3: Introduction to GenVisR
- **Module 4: Expression profiling, visualization, and interpretation**
- Module 5: Variant annotation and interpretation
- Module 6: Q & A, discussion, integrated assignments, and working with your own data

- Tutorials
  - Provide working examples of data visualization and interpretation
  - Self contained, self explanatory, portable

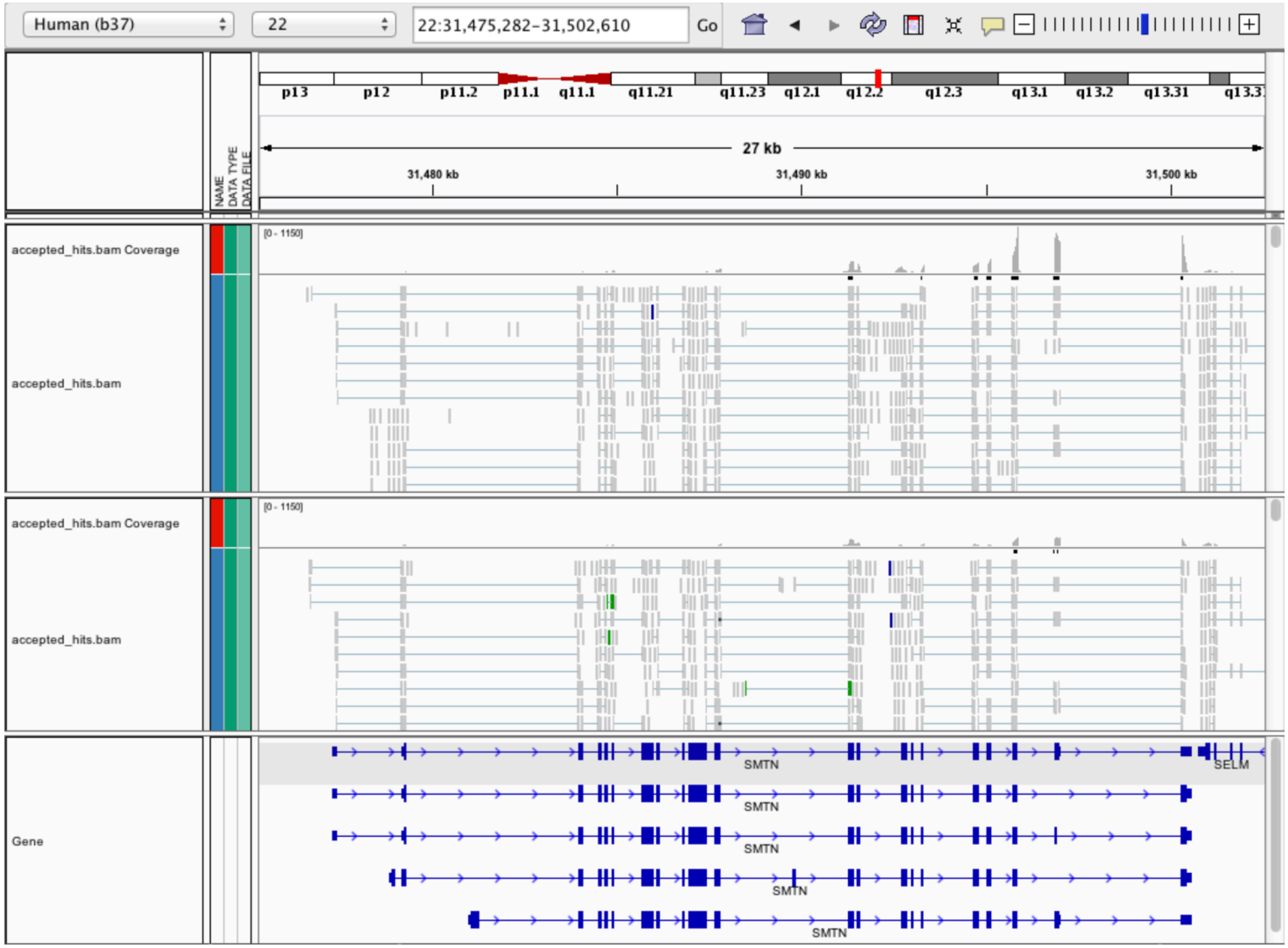
Learning objectives of module 4

- Expression profiling, visualization, and interpretation
  - Expression estimation for known genes (concepts)
  - FPKM' expression estimates vs. 'raw' counts
  - Differential expression methods (DESeq2)
  - Downstream interpretation of expression and differential estimates

Gene expression



Expression estimation for known genes and transcripts



What is FPKM (RPKM)

- RPKM: Reads Per Kilobase of transcript per Million mapped reads.
- FPKM: Fragments Per Kilobase of transcript per Million mapped reads.
- In RNA-Seq, the relative expression of a transcript is proportional to the number of cDNA fragments that originate from it. However:
  - The number of fragments is also biased towards larger genes
  - The total number of fragments is related to total library depth
- FPKM (RPKM) attempt to normalize for gene size and library depth

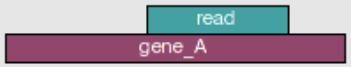
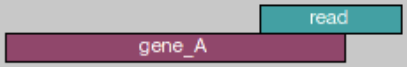
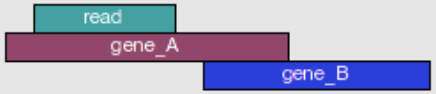
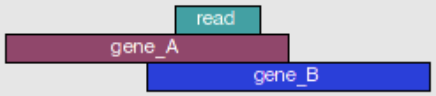
- FPKM (RPKM) = (10^9 * C) / (N * L)
  - C = number of mappable reads/fragments for a gene/transcript/exon/etc
  - N = total number of mappable reads/fragments in the library
  - L = number of base pairs in the gene/transcript/exon/etc

- http://www.biostars.org/p/11378/
- http://www.biostars.org/p/68126/

What are raw counts

- Raw read counts as an alternate for differential expression analysis
  - Instead of calculating FPKM, simply assign reads/fragments to a defined set of genes/transcripts and determine “raw counts”
    - Transcript structures could still be defined by something like cufflinks
- HTSeq (htseq-count)
  - http://www-huber.embl.de/users/anders/HTSeq/doc/count.html
  - htseq-count --mode intersection-strict --stranded no --minaqual 1 --type exon --idattr transcript_id accepted_hits.sam chr22.gff > transcript_read_counts_table.tsv
  - Important caveat of ‘transcript’ analysis by htseq-count:
    - 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

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

Alternative differential expression methods

- Raw count approaches
  - DESeq2 - http://www-huber.embl.de/users/anders/DESeq/
  - edgeR - http://www.bioconductor.org/packages/release/bioc/html/edgeR.html
  - Others…

‘FPKM’ expression estimates vs. ‘raw’ counts

- Which should I use?
  - Long running debate with countless blogs and analyses arguing the advantages of each. The general consensus:
- FPKM
  - Isoform deconvolution
  - Good for straight visualization (e.g., heatmaps)
  - Calculating fold changes, etc.
- Counts
  - More robust statistical methods for differential expression
  - Accommodates more sophisticated experimental designs with appropriate statistical tests

Multiple approaches advisable