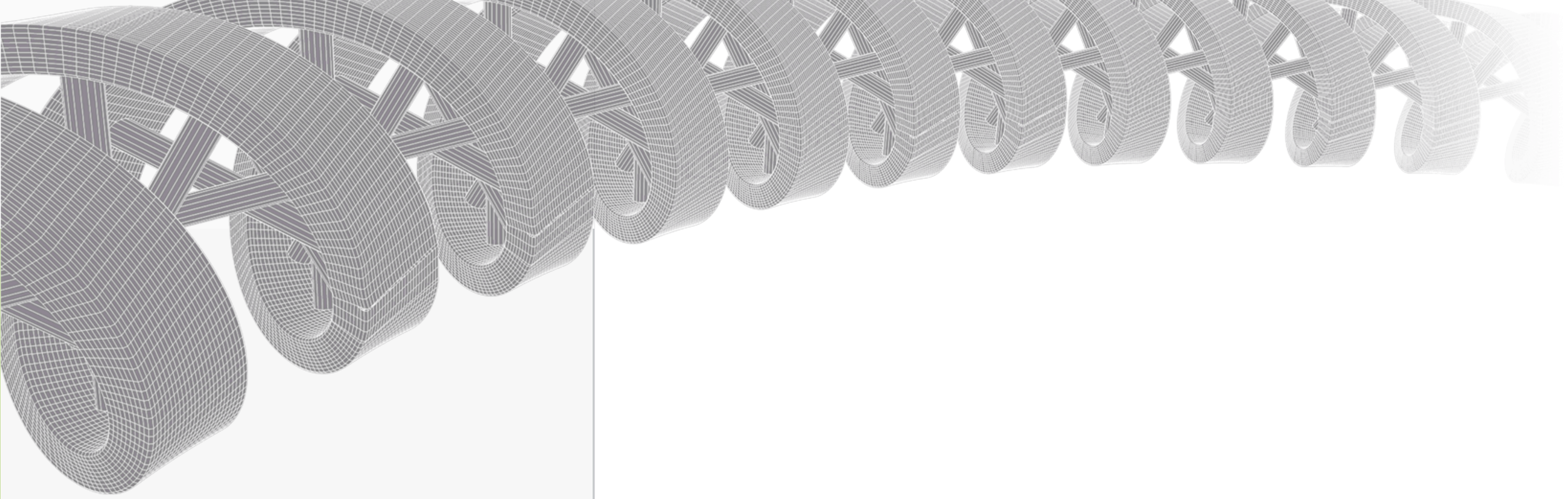

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GenViz Module 4:
Expression profiling, visualization, and interpretation

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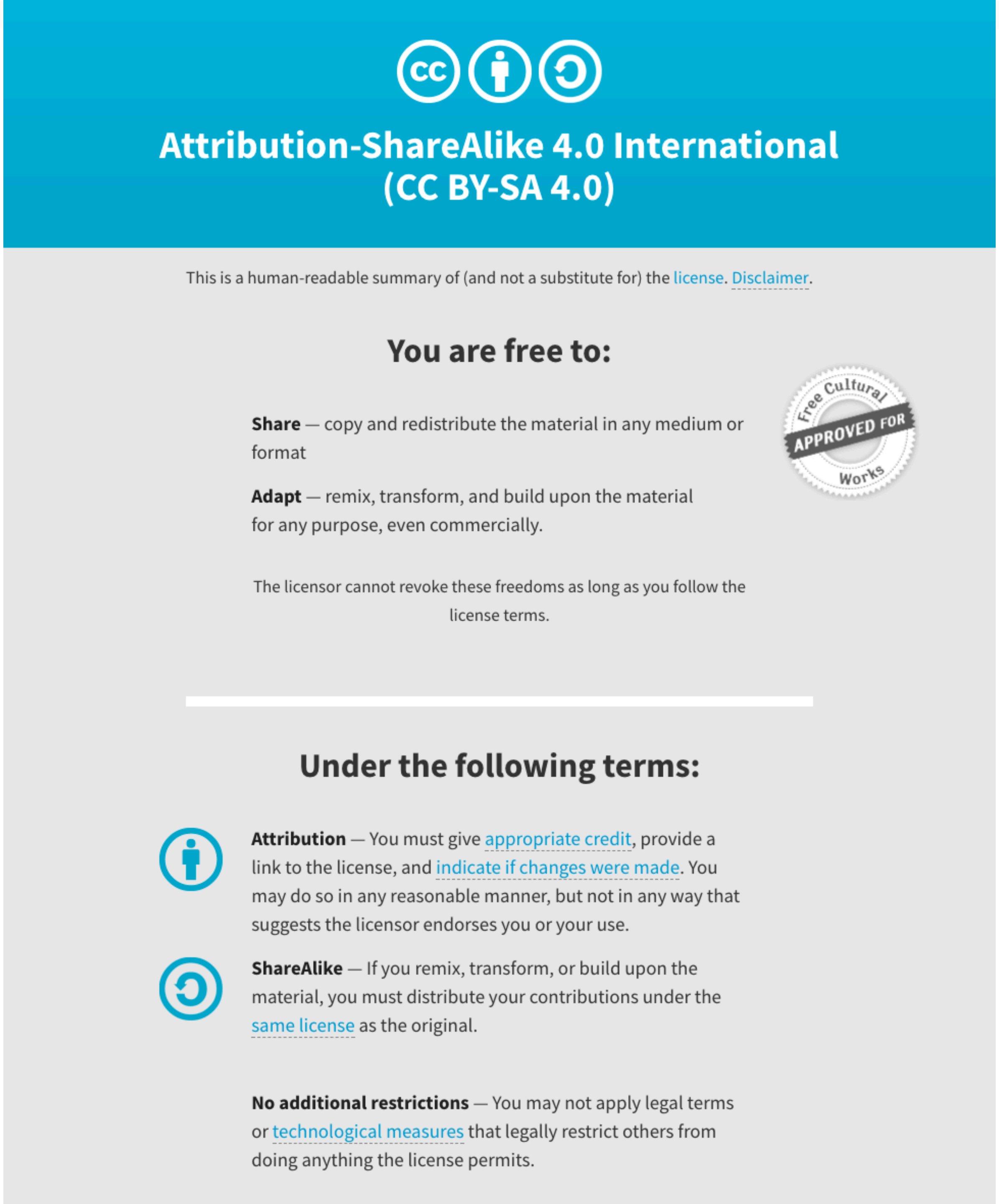
Genomic Data Visualization and Interpretation
September 11-15, 2017
Berlin



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Learning objectives of the course

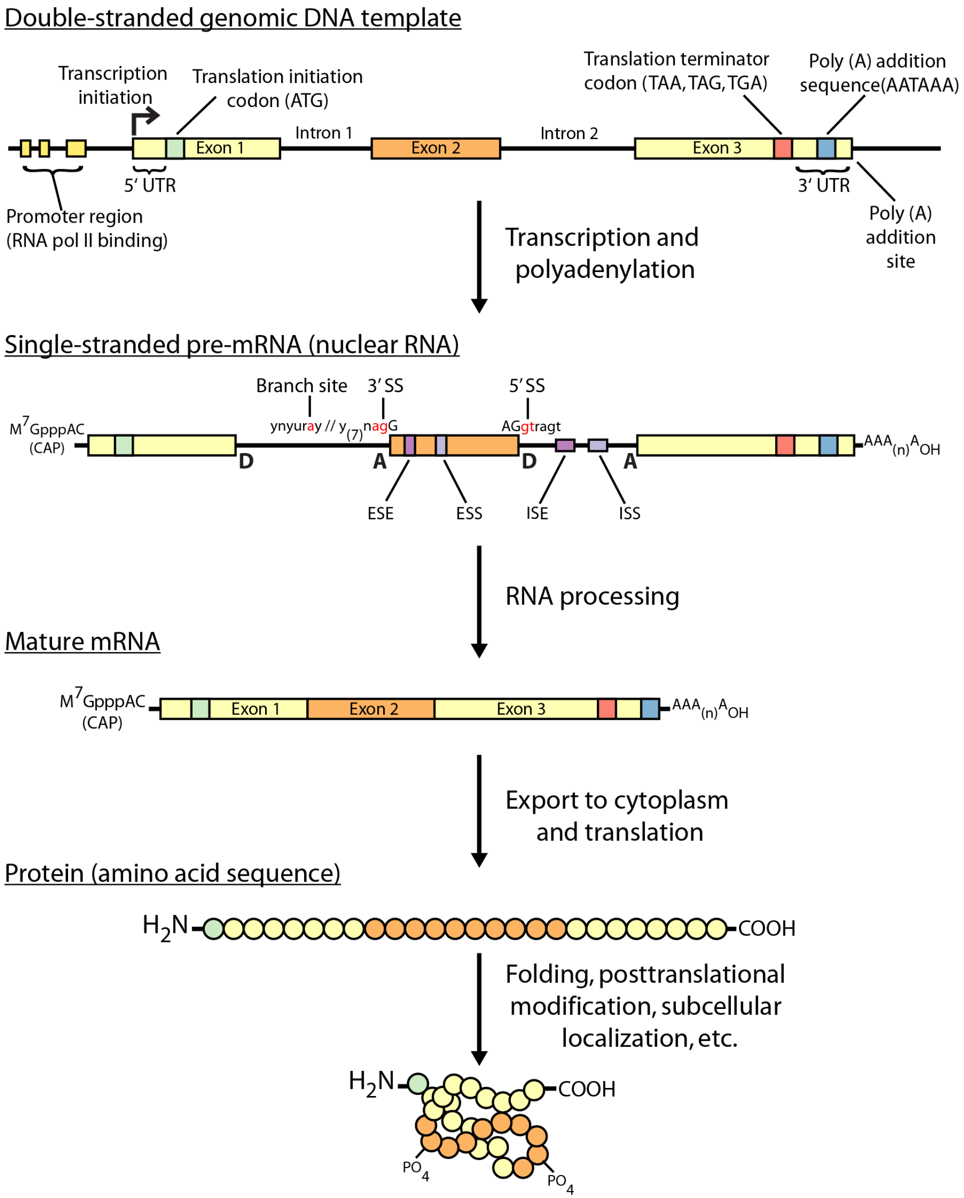
- Module 1: Introduction to genomic data visualization and interpretation
- Module 2: Using R for genomic data visualization and interpretation
- Module 3: Introduction to GenVisR
- **Module 4: Expression profiling, visualization, and interpretation**
- Module 5: Variant annotation and interpretation
- 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



Double-stranded genomic DNA template

Transcription initiation
Translation initiation codon (ATG)
Translation terminator codon (TAA, TAG, TGA)
Poly (A) addition sequence(AATAAA)
Exon 1
Intron 1
Exon 2
Intron 2
Exon 3
5′ UTR
3′ UTR
Promoter region (RNA pol II binding)
Poly (A) addition site

Transcription and polyadenylation

Single-stranded pre-mRNA (nuclear RNA)

RNA processing

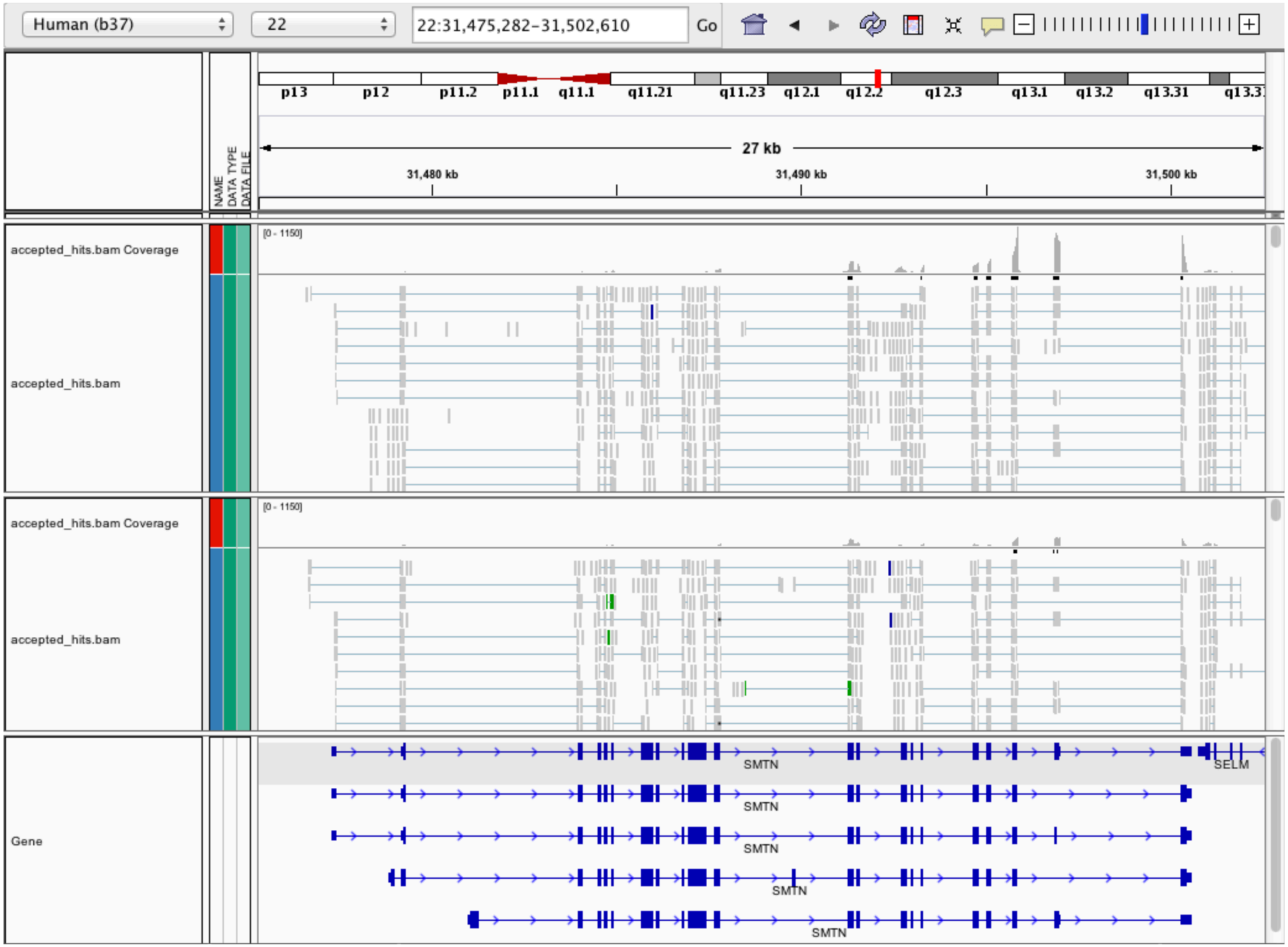
Mature mRNA

Export to cytoplasm and translation

Protein (amino acid sequence)

Folding, posttranslational modification, subcellular localization, etc.

Expression estimation for known genes and transcripts



Abundance/expression estimation methods

- Raw Counts
  - HTSeq-Count
  - FeatureCounts
  - StringTie

- RPKM/FPKM values
  - StringTie

- TPM values
  - Kallisto
  - Salmon
  - StringTie

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

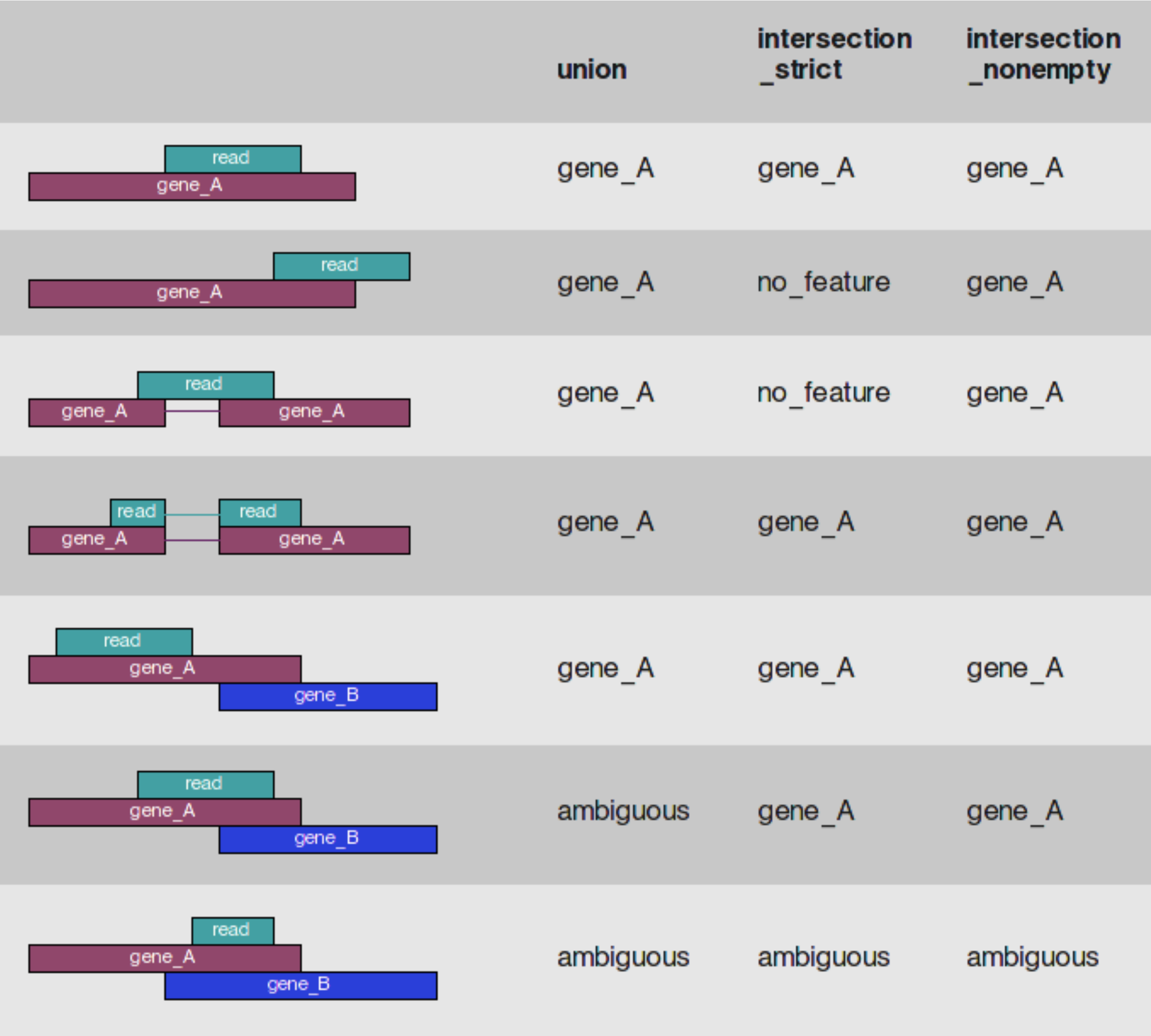
- FPKM (RPKM) = (10^9 * C) / (N * L)
    - C = number of mappable reads/fragments for a gene/transcript/exon/etc
    - N = total number of mappable reads/fragments in the library
    - L = number of base pairs in the gene/transcript/exon/etc

- 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
read gene_A	gene_A	gene_A	gene_A
read gene_A	gene_A	no_feature	gene_A
read gene_A gene_A	gene_A	no_feature	gene_A
read read gene_A gene_A	gene_A	gene_A	gene_A
read gene_A gene_B	gene_A	gene_A	gene_A
read gene_A gene_B	ambiguous	gene_A	gene_A
read gene_A gene_B	ambiguous	ambiguous	ambiguous

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

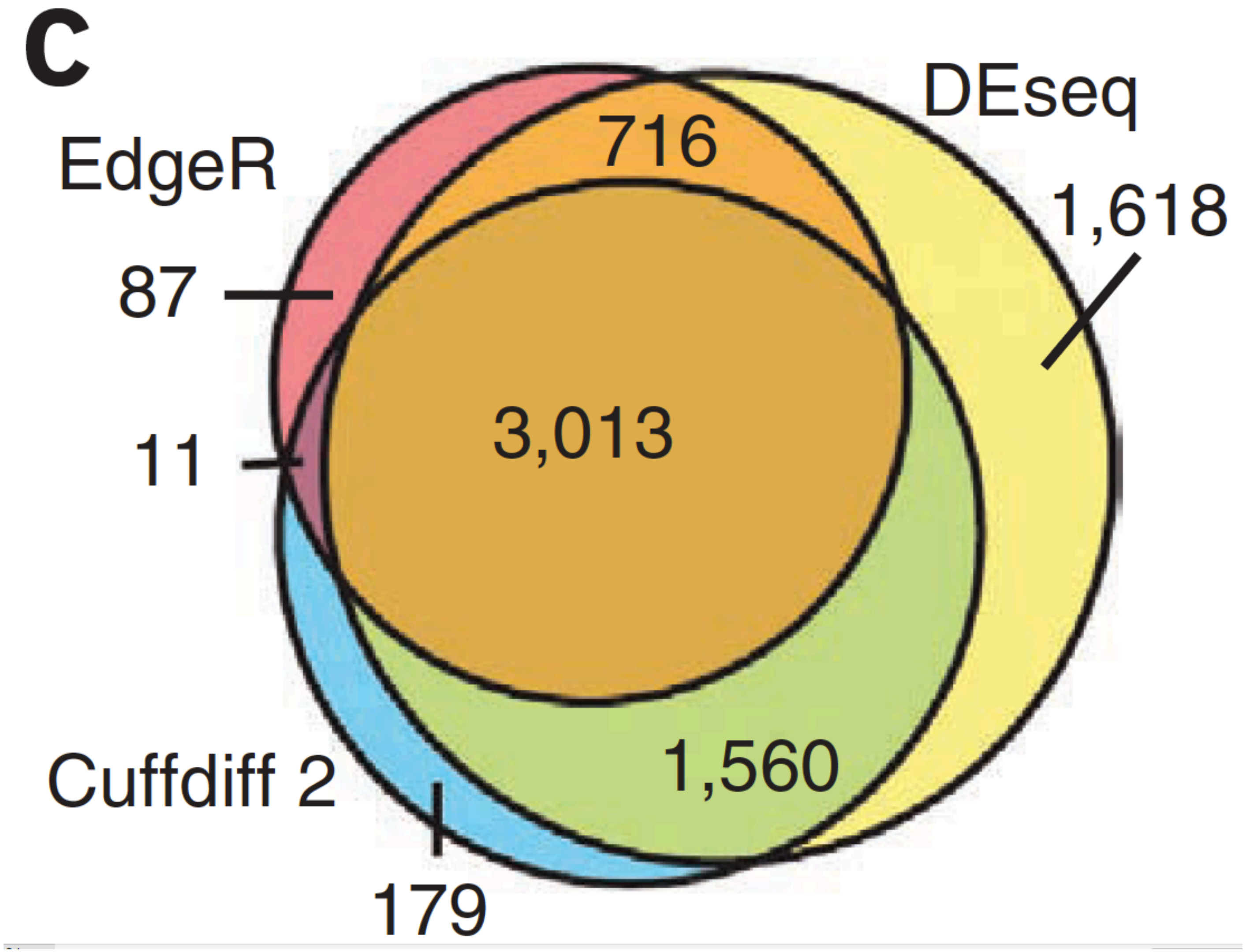
Differential expression methods

- Raw count approaches (gene level)
  - DESeq2
  - edgeR
  - Many others…
- FPKM approaches (for transcript level)
  - Ballgown
    - Helpful explanation (PMID: 25748911)
    - Many others (EBSeq, etc.)
- TPM approaches
  - Kallisto/Sleuth

‘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



Refer to www.rnaseq.wiki for many, many more details, resources and exercises