MDA 2013

IX International Course of Massive Data Analysis FOR GENOMICS

Course Presentation

er ciberer
CENTRO DE INVESTIGACIÓN BIOMÉDICA EN RED
DE ENFERMEDADES RARAS

PRINCIPE FELIPE
CENTRO DE INVESTIGACION

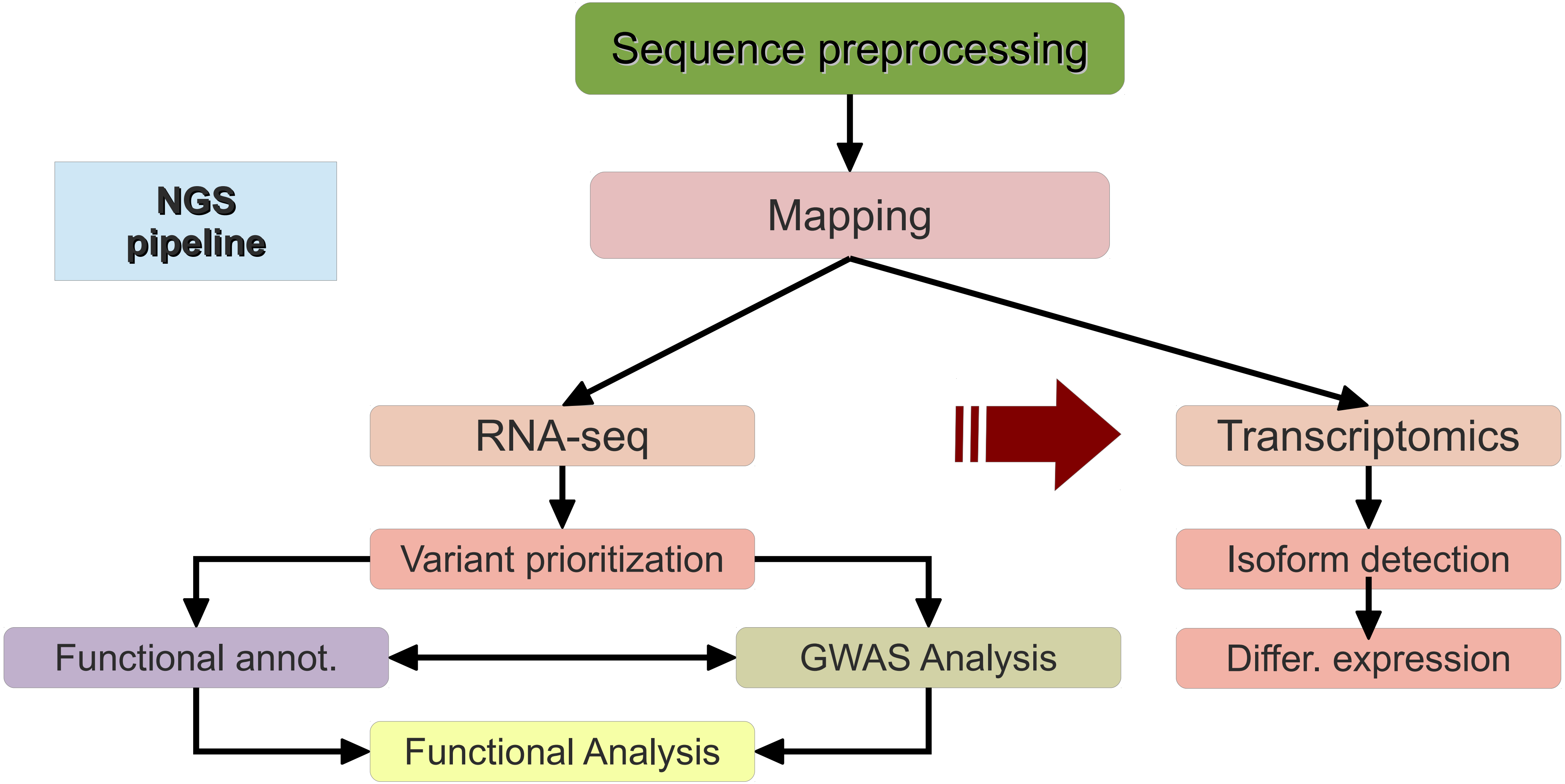
INB

David Montaner
dmontaner@cipf.es

RNA-seq

Where are we?

NGS pipeline

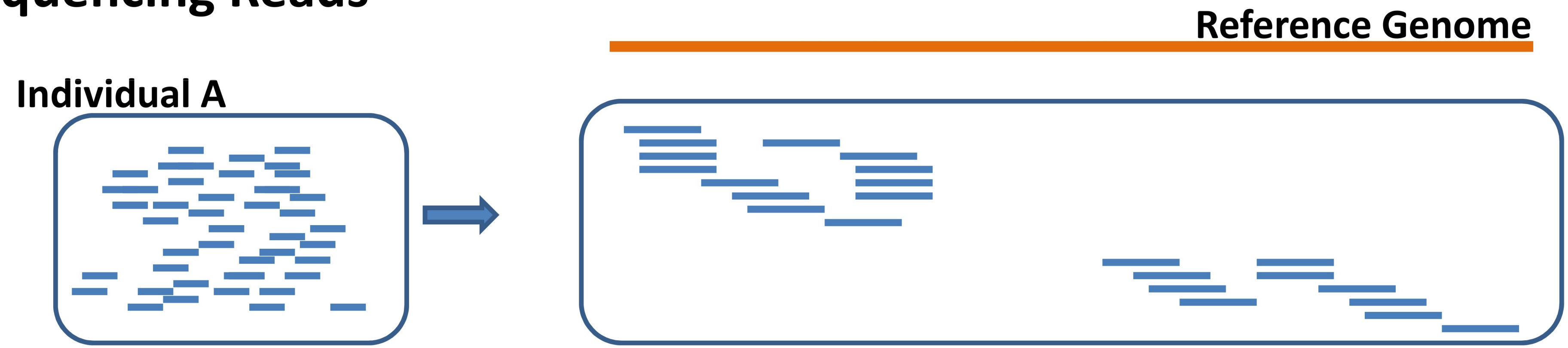


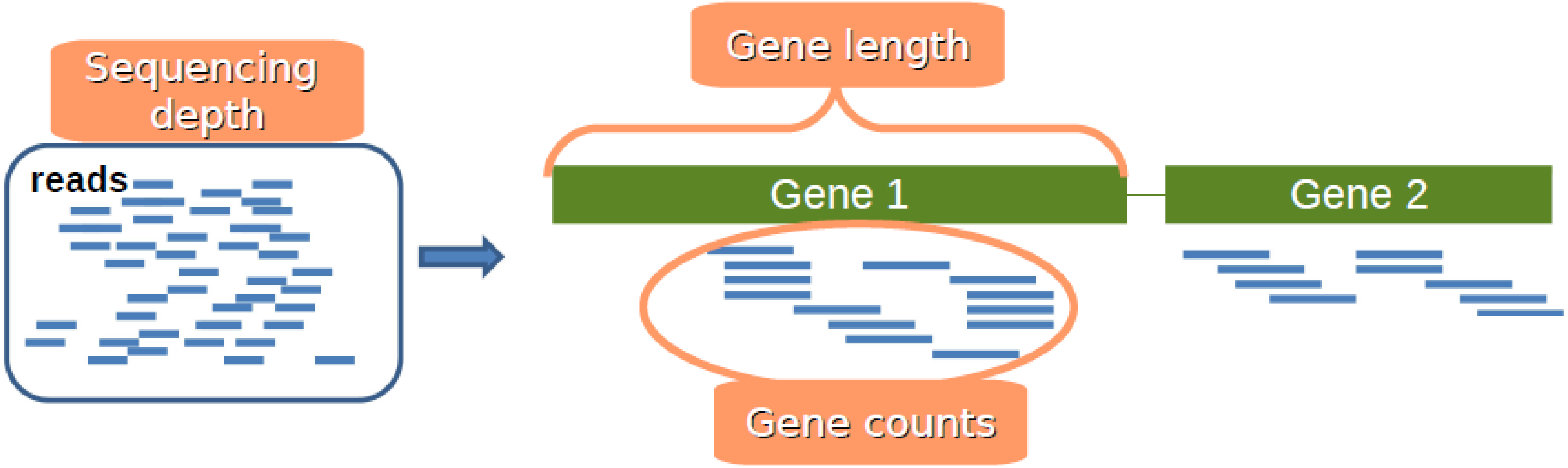
General context

- Reference genome
  - Yes: map against it
  - No: try to assemble transcripts an then map
- Reference transcriptome
  - Yes: estimate known transcripts abundance &
    discover new transcripts
  - No: find expressed regions &
    their transcript combination

General context

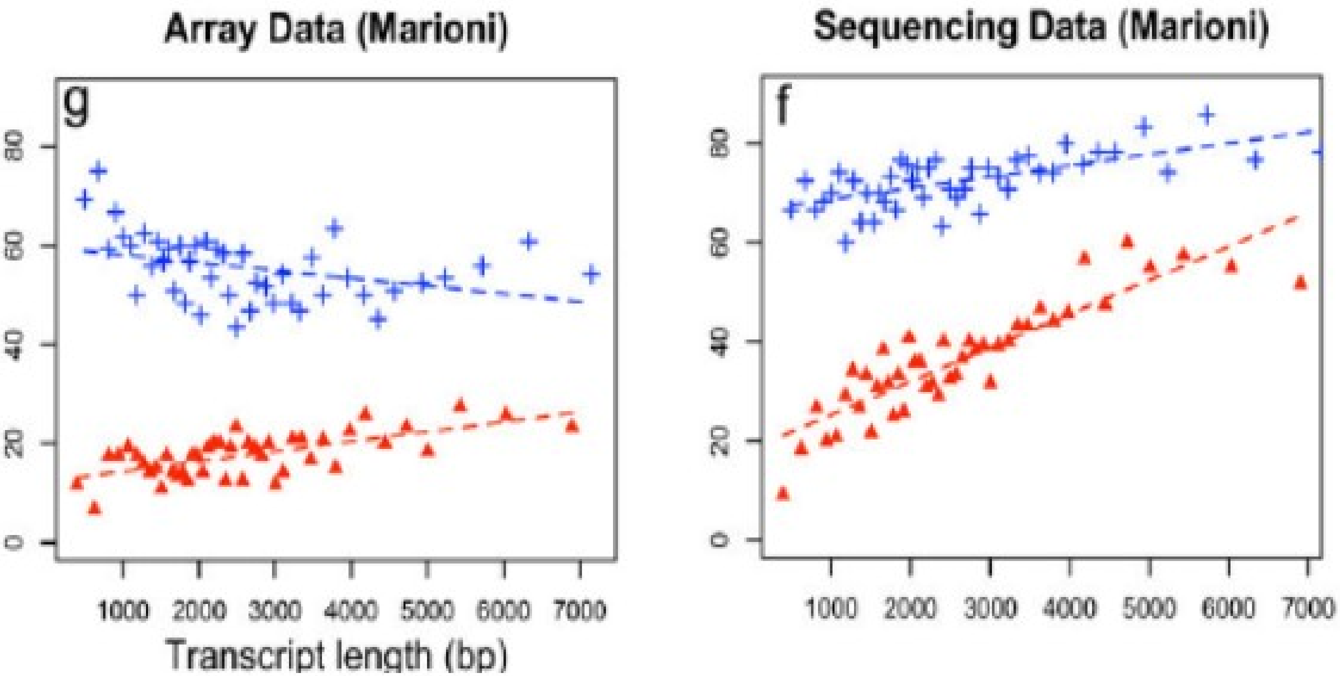
Sequencing Reads

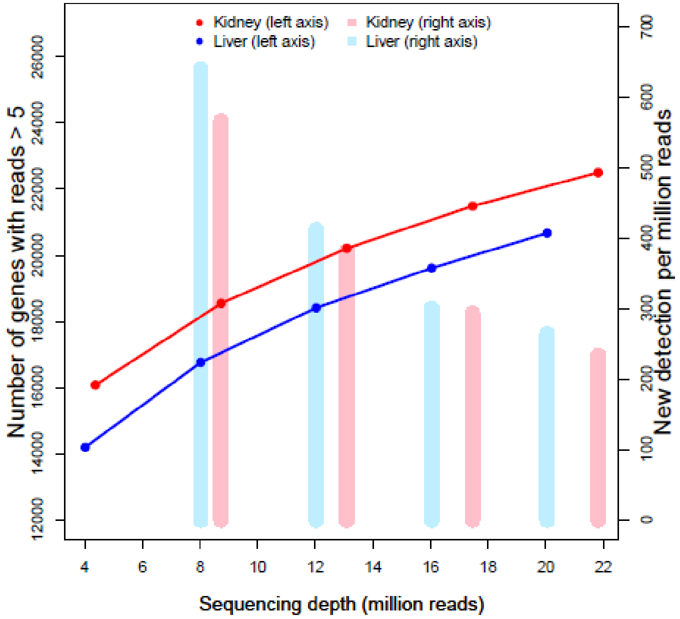




Gene/transcript length dependence

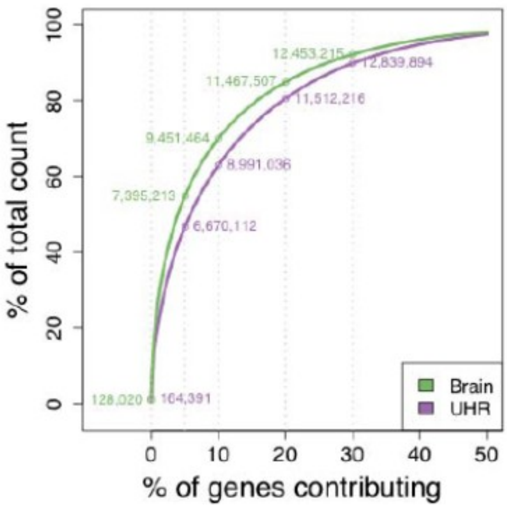
- Counts are proportional to...
  - the transcript length
  - the mRNA expression level.

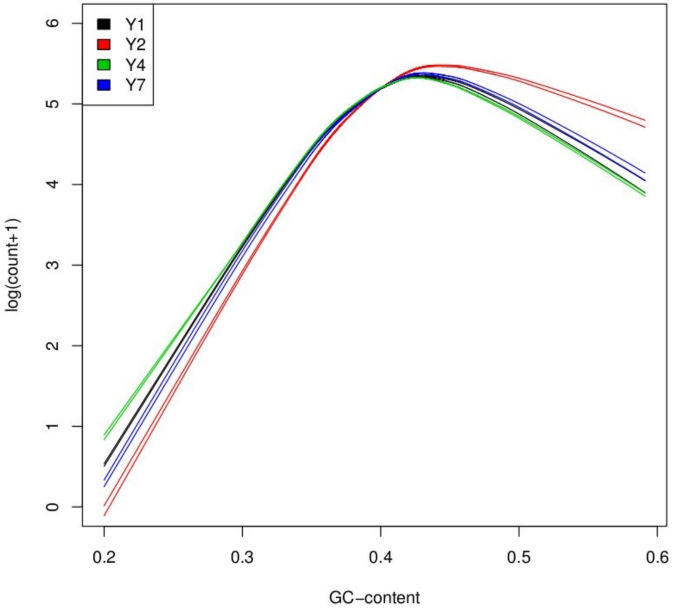




Count Normalization

- Transcript length: *within* library
- Library size: *between* libraries
- Many other biases ...
  - Differences on the read count distribution among samples.
  - GC content of the gene affects the detection of that gene (Illumina)
  - sequence-specific bias is introduced during the library preparation





Count Normalization

- **RPKM**: Reads Per Kilobase of the transcript per Million mapped reads

$$RPKM = 10^9 \times \frac{C}{N*L}$$

- **C** is the number of mappable reads mapped onto the gene's exons.
- **N** is the total number of mappable reads in the experiment.
- **L** is the total length of the exons in base pairs.
- Fragments Per Kilobase of exon per Million fragments mapped (FPKM),

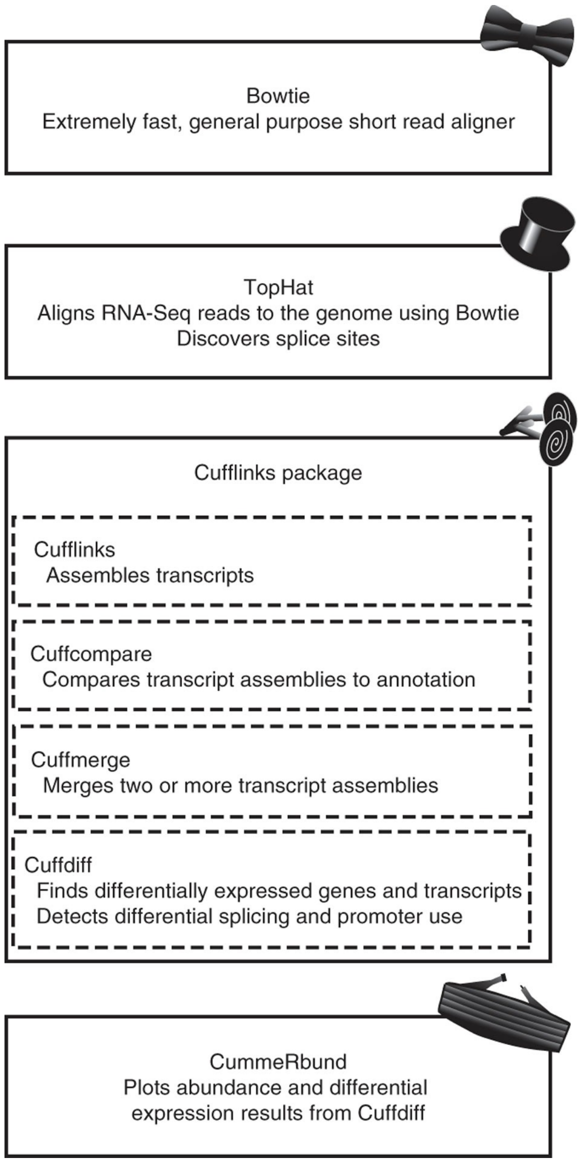
Count Normalization

- **RPKM** (Mortazavi et al., 2008)
- **TC**: Gene counts are divided by the sequencing depth associated to that sample and multiplied by the average of the total counts across all the samples. Gene counts are divided by the gene length (kb) times the total number of millions of mapped reads.
- **Upper-quartile** (Bullard et al., 2010): Gene counts are divided by the upper quartile of counts for genes with at least one read.
- **Median** (Bullard et al., 2010): Gene counts are divided by the median of counts for genes with at least one read.
- **Quantile** (Irizarry et al., 2003): This method matches the gene count distributions across samples.
- **TMM** (Robinson & Oshlack, 2010): Trimmed Mean of M-values. Basd on the hypothesis that most of the genes are not DE. A sample is taken as the reference. For each sample, the scaling factor is the weighted mean of log ratios between this sample and the reference after removing the most expressed genes and those with the largest log ratios. Gene counts are divided by this factor re-scaled by the mean of the normalized library sizes.
- **DESeq** (Anders & Huber, 2010): Based on the hypothesis that most of the genes are not DE. The scaling factor for a given sample is the median of the ratio, for each gene, of its counts over its geometric mean across all the samples. Gene counts are divided by this scaling factor.
- **FPKM** (Trapnell et al., 2010): implemented in Cufflinks, isimilar to RPKM .

Software

- **Cufflinks**:
  - assembly
  - compare with the known transcripts

- **Cuffdiff**: differential expression
  - estimate fragment length distribution
  - calculate transcript abundances FPKM
  - sequence bias correction



Bowtie
Extremely fast, general purpose short read aligner

TopHat
Aligns RNA-Seq reads to the genome using Bowtie
Discovers splice sites

Cufflinks package

Cufflinks
Assembles transcripts

Cuffcompare
Compares transcript assemblies to annotation

Cuffmerge
Merges two or more transcript assemblies

Cuffdiff
Finds differentially expressed genes and transcripts
Detects differential splicing and promoter use

CummeRbund
Plots abundance and differential
expression results from Cuffdiff

Software

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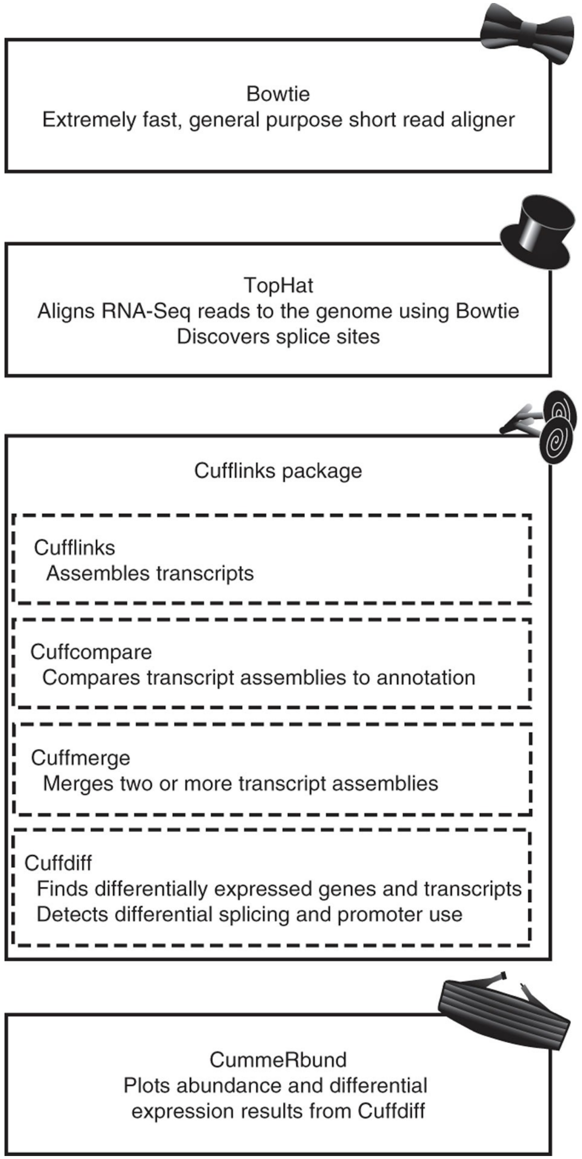
CummeRbund:

an **R** package to handle results



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