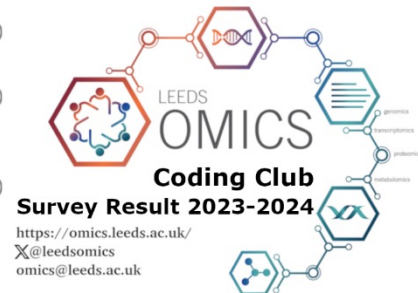
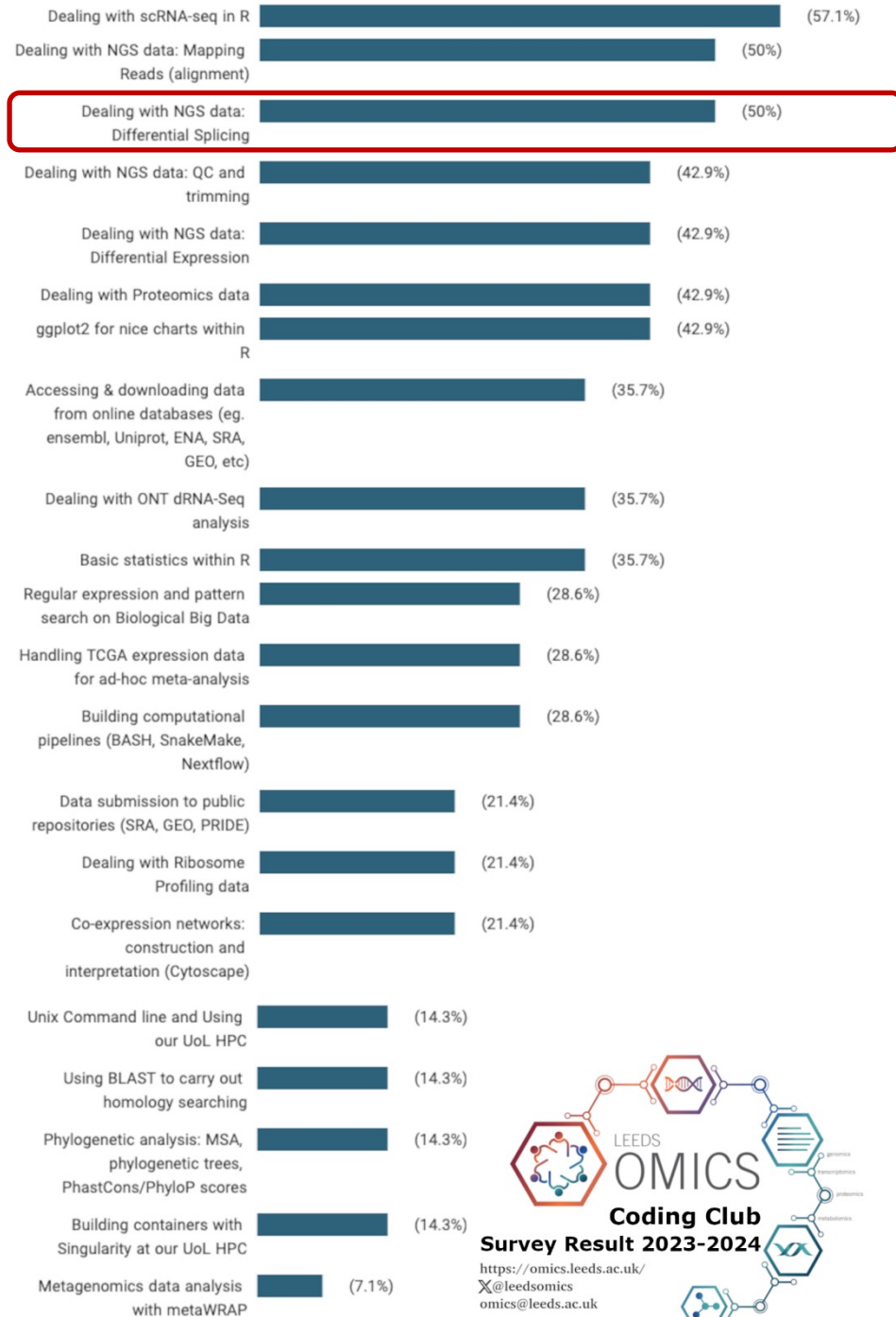


[www.leedsomics.org](http://www.leedsomics.org)  
@leedsomics  
omics@leeds.ac.uk

# Dealing with NGS data: Differential Splicing

**Club Moderators:** Elton Vasconcelos and Eilidh Ward

# Topics to be addressed on the 2023-24 season - Survey Result



# Important steps on NGS data analysis workflow

Other club sessions

Data QC and preprocessing

FastQC, Trim\_Galore, Trimmomatic

Other club sessions

Read alignment

Genome Assembly  
(WGS DNA-seq)

Velvet, SPADes, HGAP3, Falcon,  
Minimap/Miniasm

Mapping to a ref. genome  
or *de novo*\* transcriptome assembly

Bowtie2, Tophat2, STAR,  
HiSAT2, GSNAP, Trinity\*

SAM/BAM  
manipulation/visualization

Samtools,  
Picard Tools

Obtaining raw read counts  
or normalized expression values

HTSeq, FeatureCounts  
Cufflinks, StringTie

Other club sessions

Differential peak  
calling (ChIP-seq)

MACS2, Clipper, HOMER

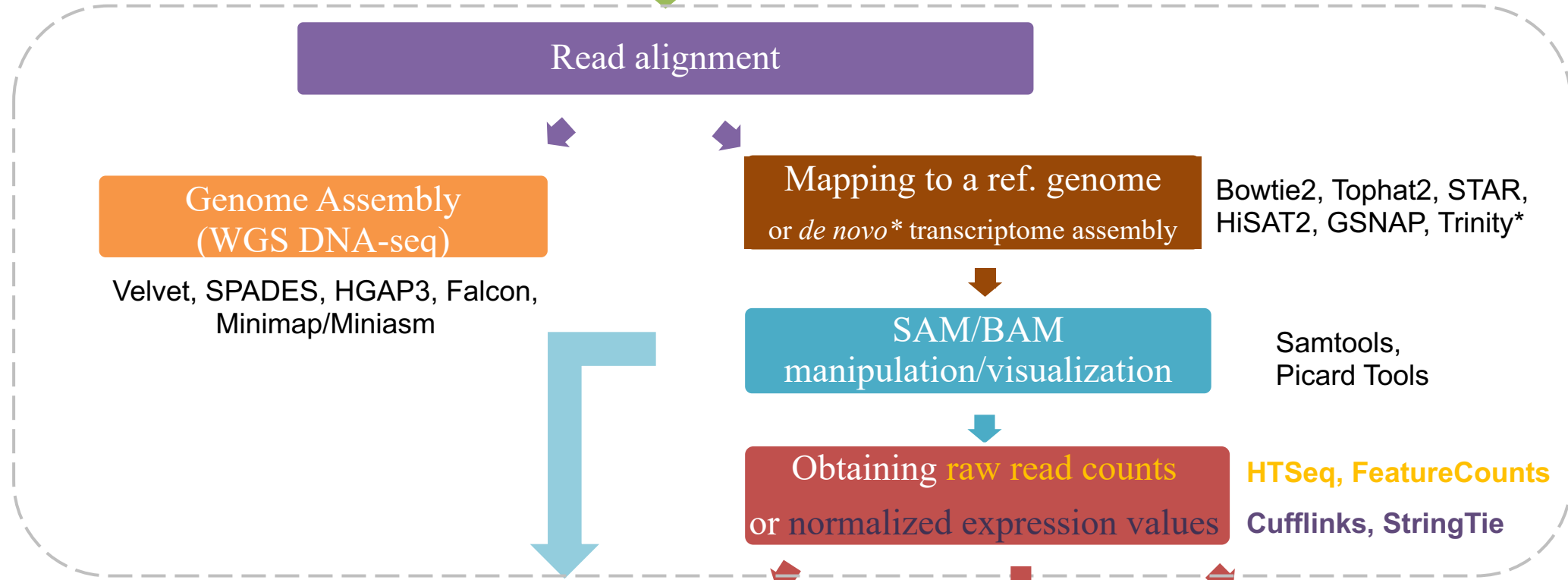
Differential expression  
(RNA-seq)

DESeq2, EdgeR, NOISeq, CuffDiff

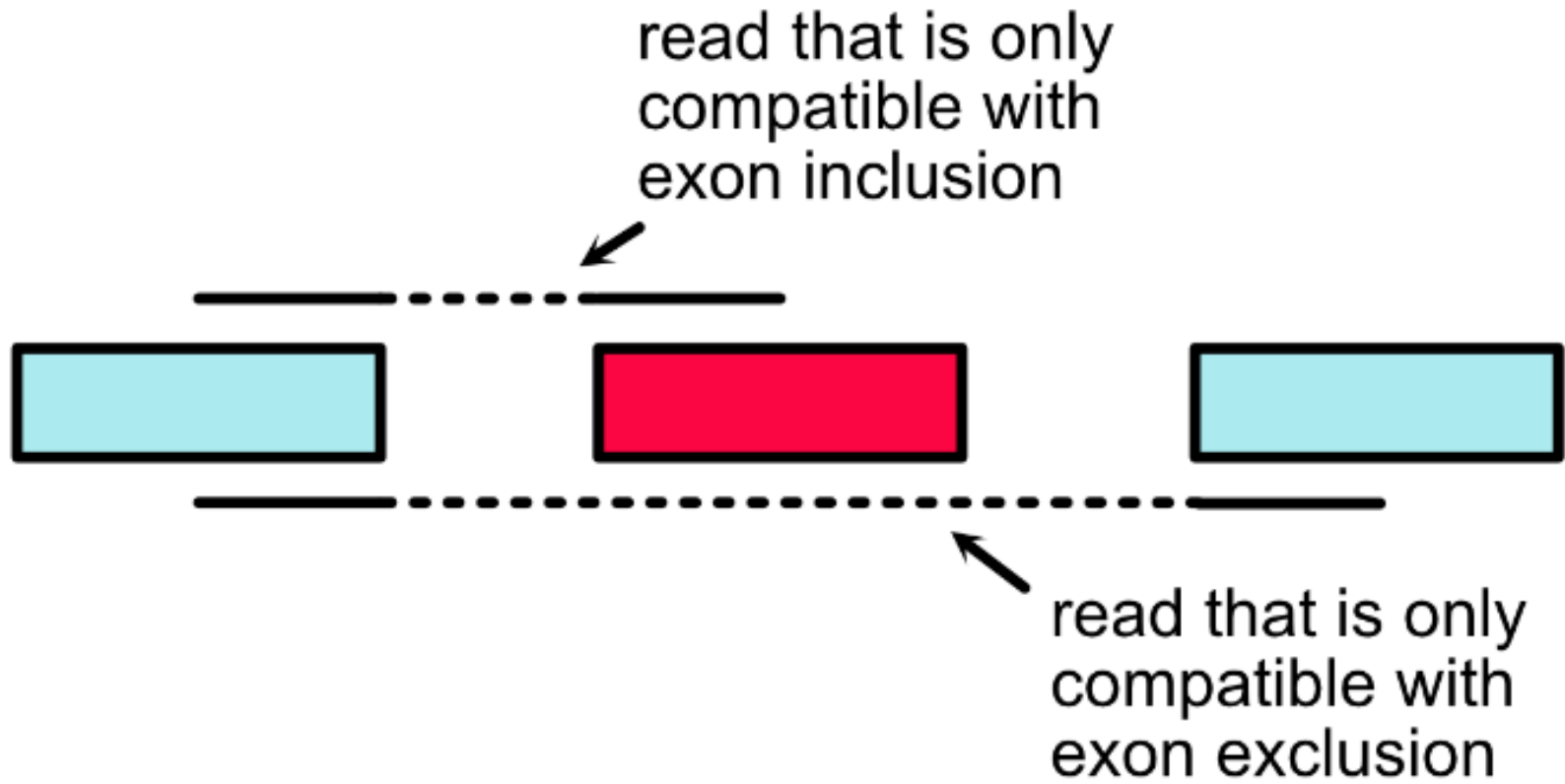
Differential splicing  
(RNA-seq)

rMATs, MAJIQ, Suppa2,  
isoformSwitchAnalyzeR

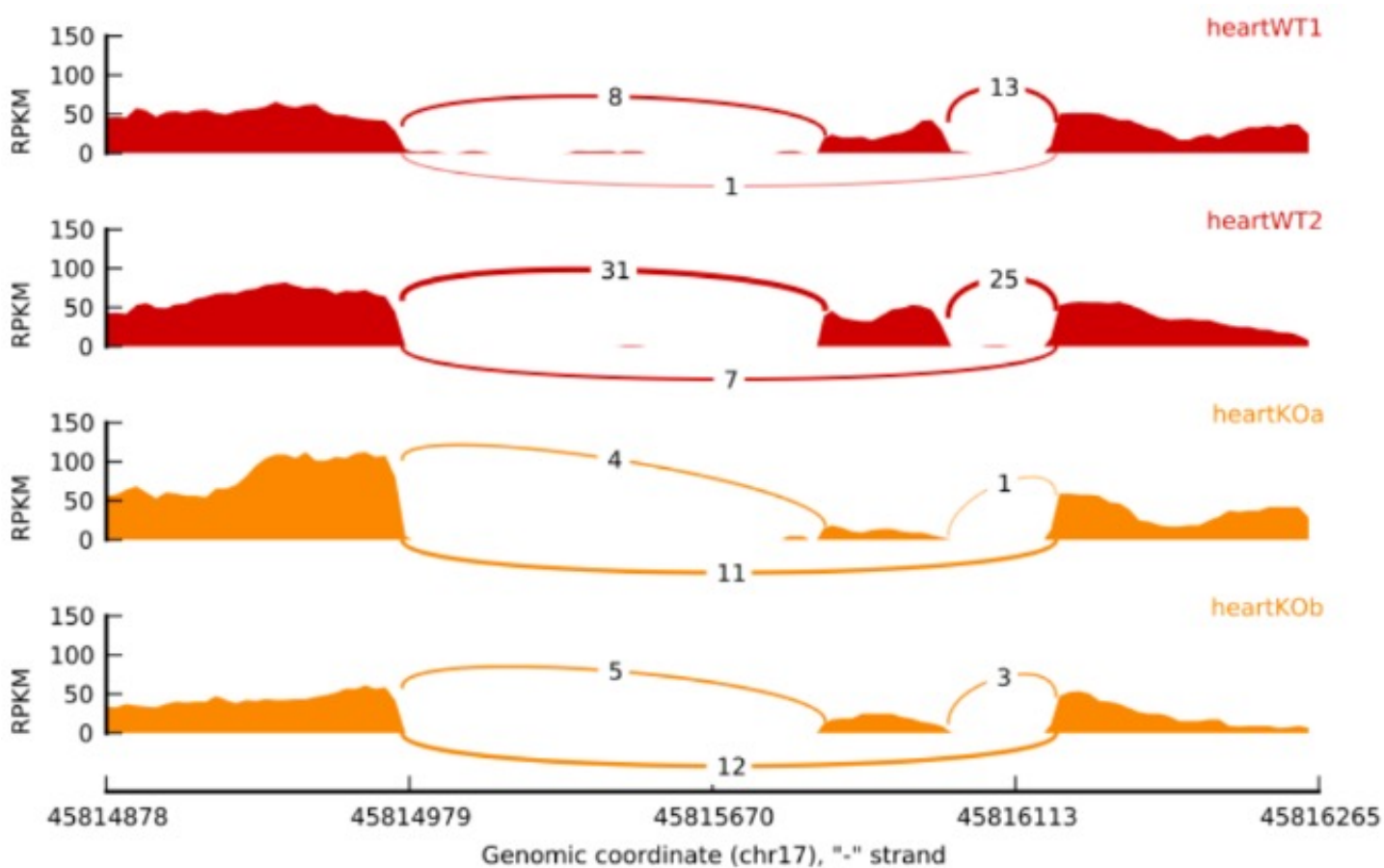
**Today's session**



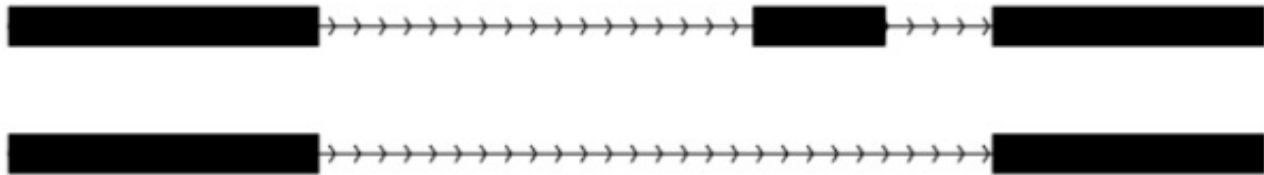
# Alternative Splicing



# Differential Splicing



Sashimi plots



# Differential Splicing

## Software packages for DS analysis

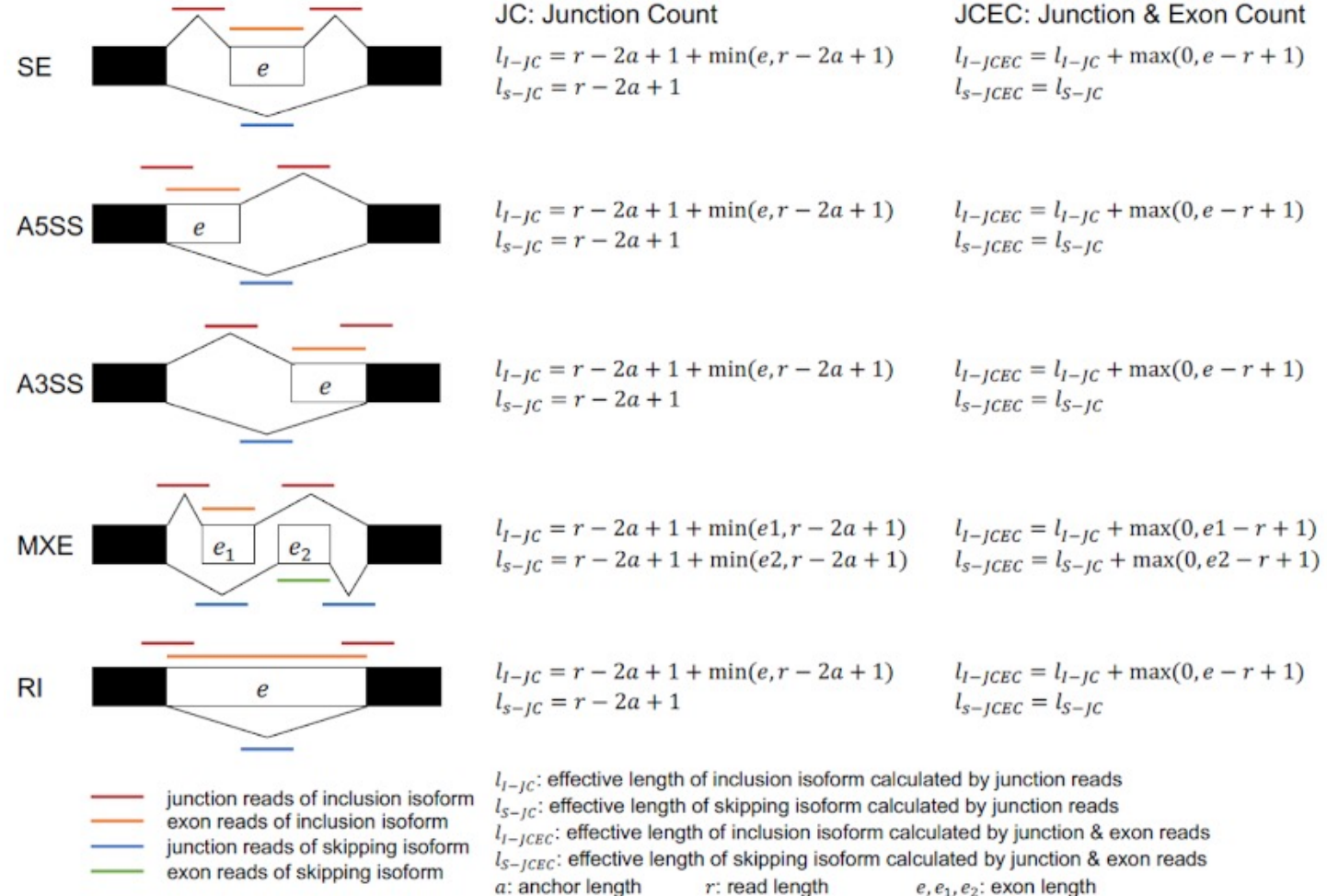
- **rMATs** .....  
<https://github.com/Xinglab/rmats-turbo>

- MAJIQ  
<https://majiq.biociphers.org/>

- Suppa2  
<https://github.com/comprna/SUPPA>

- DEXSeq \*  
<https://bioconductor.org/packages/release/bioc/html/DEXSeq.html>

- isoformSwitchAnalyzeR \*  
<https://github.com/kvittingseerup/IsoformSwitchAnalyzeR>



→ Important Output Metrics: Percent Spliced-in (PSI), IncLevDiff, DEU\*, dIF\*, and adj. p-values

# Running rMATs-turbo

## Command line

```
$ run_rmats --gtf Homo_sapiens.GRCh38.105.gtf --b1 wt.txt --b2 mut.txt --libType fr-  
firststrand --readLength 100 --variable-read-length --novelSS --nthread 16 --od rMATsOUT --  
tmp rMATsTMP
```

- **Content of wt.txt file**

wt\_rep1-vsGRCh38\_Aligned.sortedByCoord.out.bam,wt\_rep2-  
vsGRCh38\_Aligned.sortedByCoord.out.bam,wt\_rep3-vsGRCh38\_Aligned.sortedByCoord.out.bam

- **Content of mut.txt file**

mut\_rep1-vsGRCh38\_Aligned.sortedByCoord.out.bam,mut\_rep2-  
vsGRCh38\_Aligned.sortedByCoord.out.bam,mut\_rep3-vsGRCh38\_Aligned.sortedByCoord.out.bam

Bring your issues on!