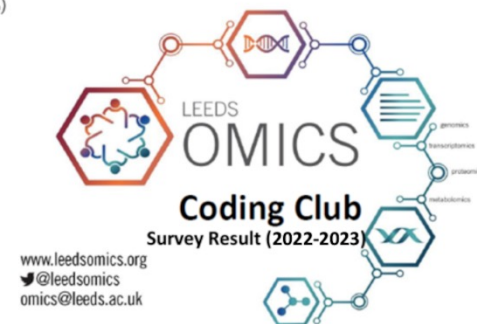
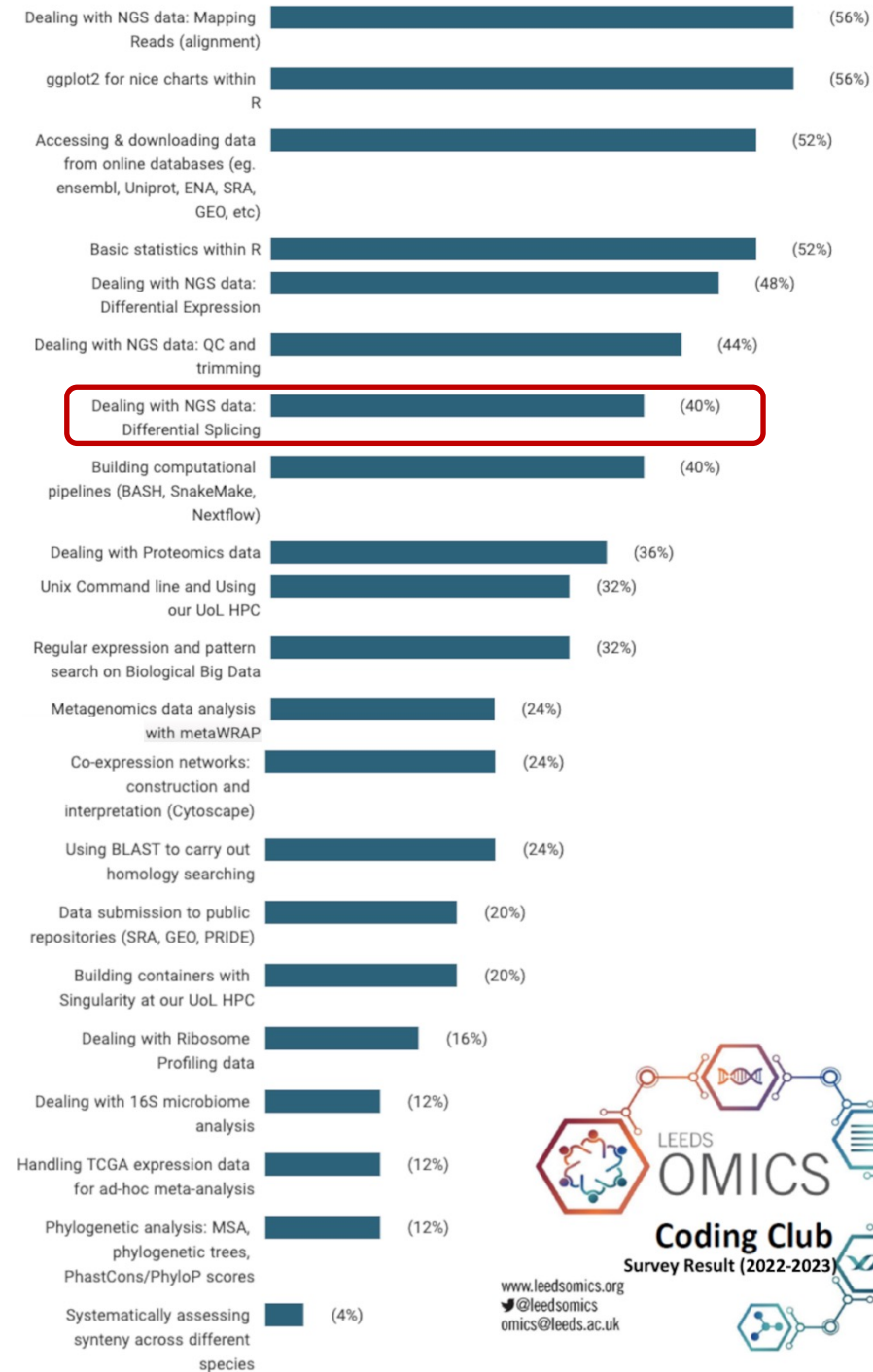


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Dealing with NGS data: Differential Splicing

Club Moderator: Elton Vasconcelos

Topics to be addressed on the 2022-23 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

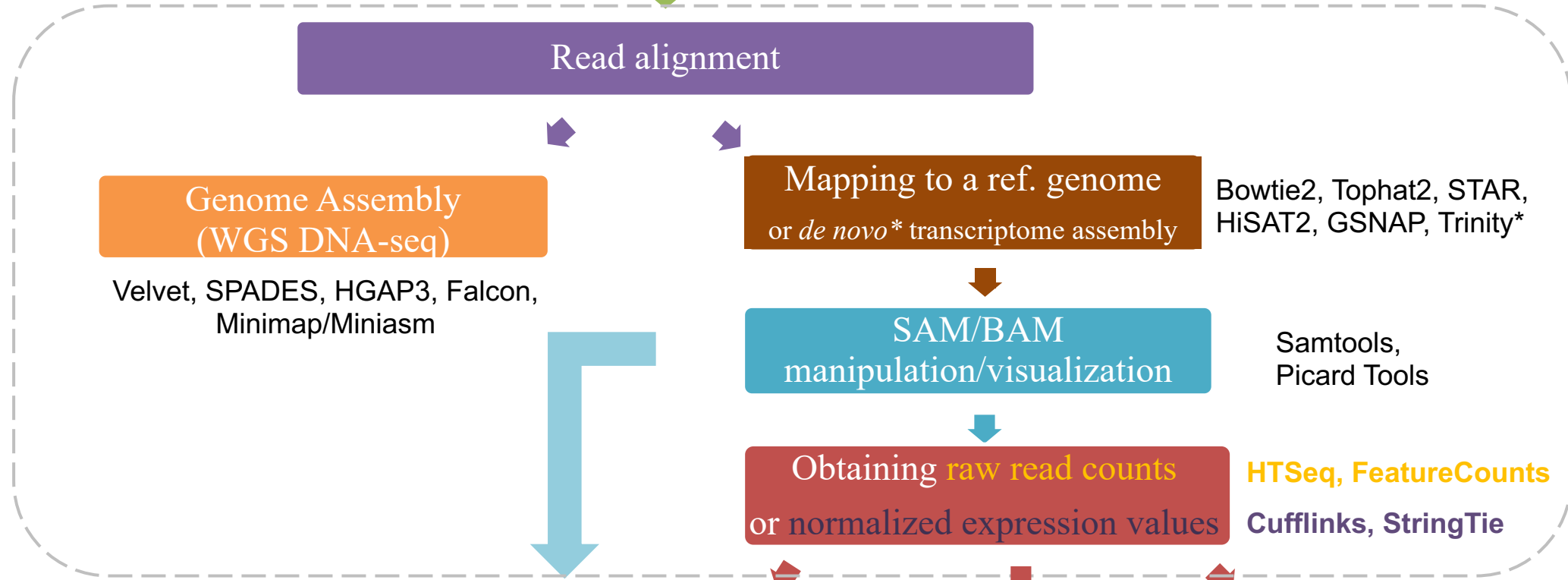
Differential expression
(RNA-seq)

DESeq2, EdgeR, NOISeq, CuffDiff

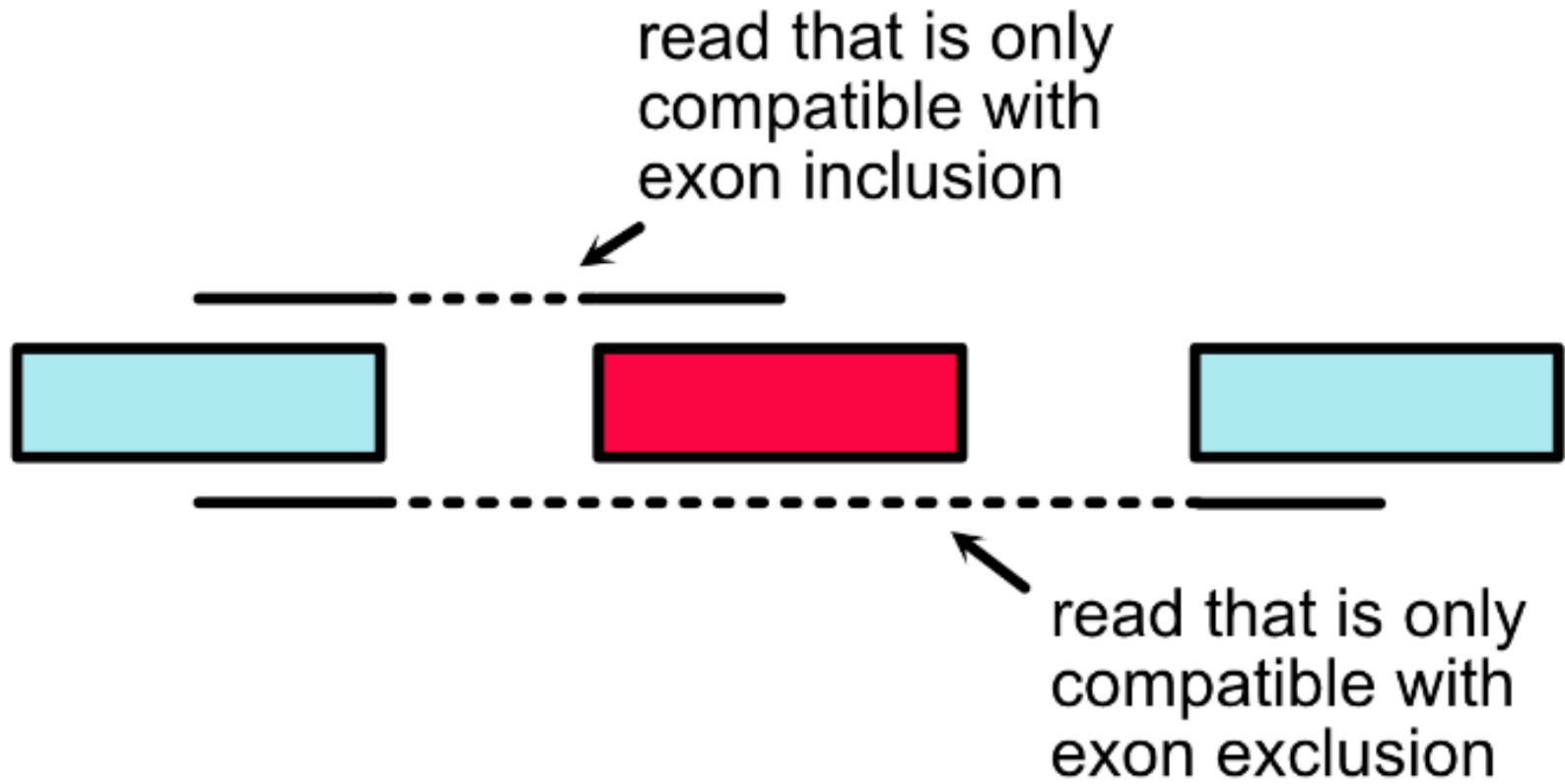
Differential splicing
(RNA-seq)

rMATs, MAJIQ, Suppa2,
isoformSwitchAnalyzeR

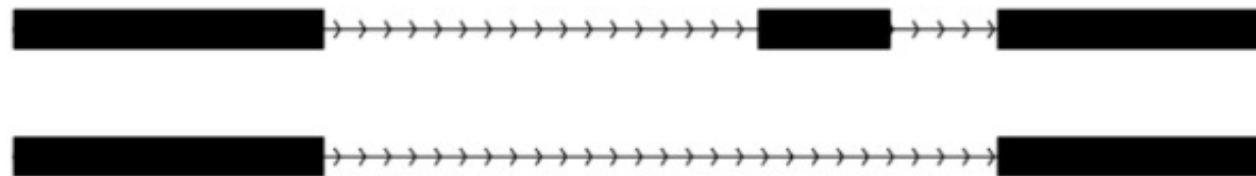
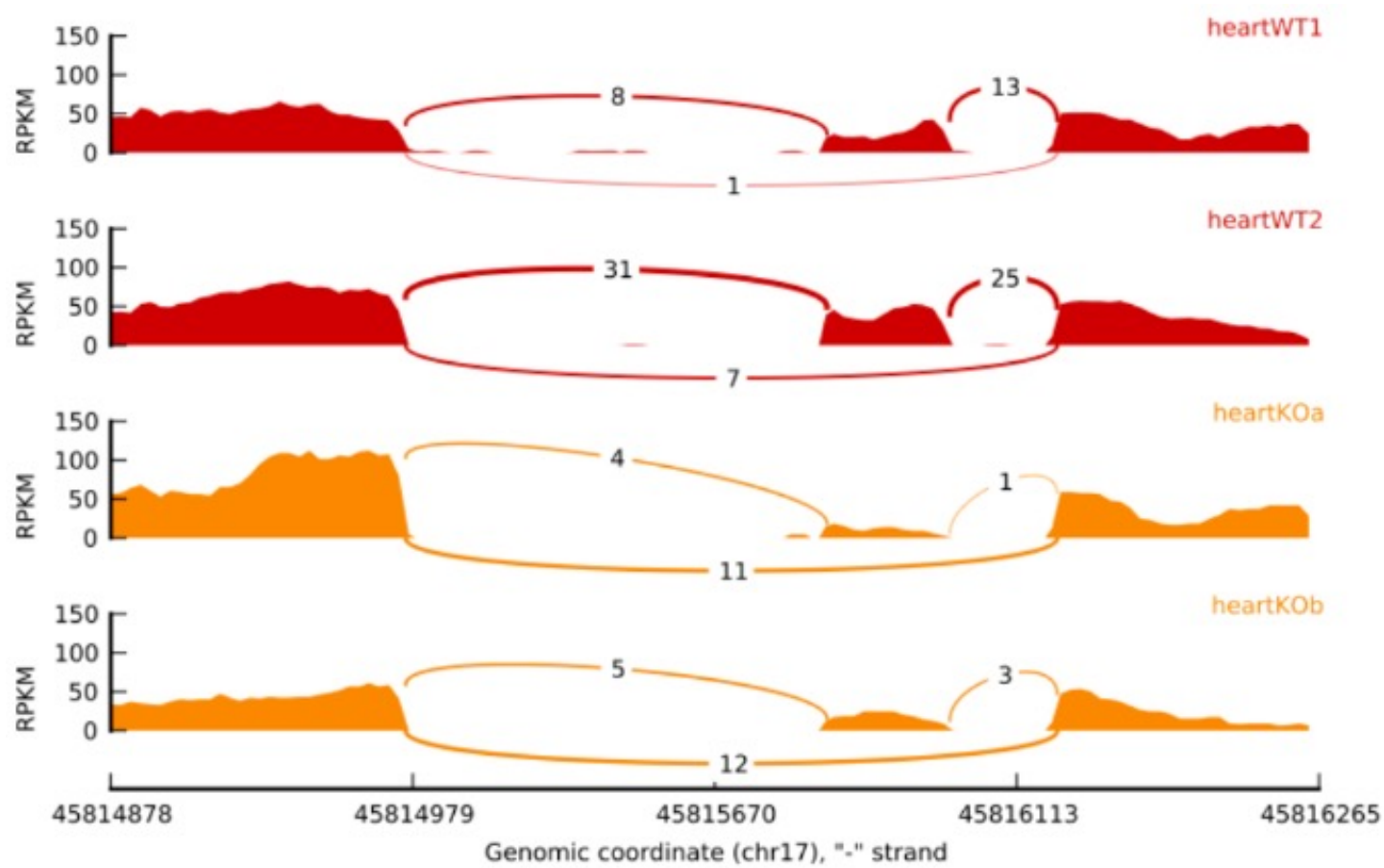
Today's session



Alternative Splicing



Differential Splicing



Differential Splicing

Software packages for DS analysis

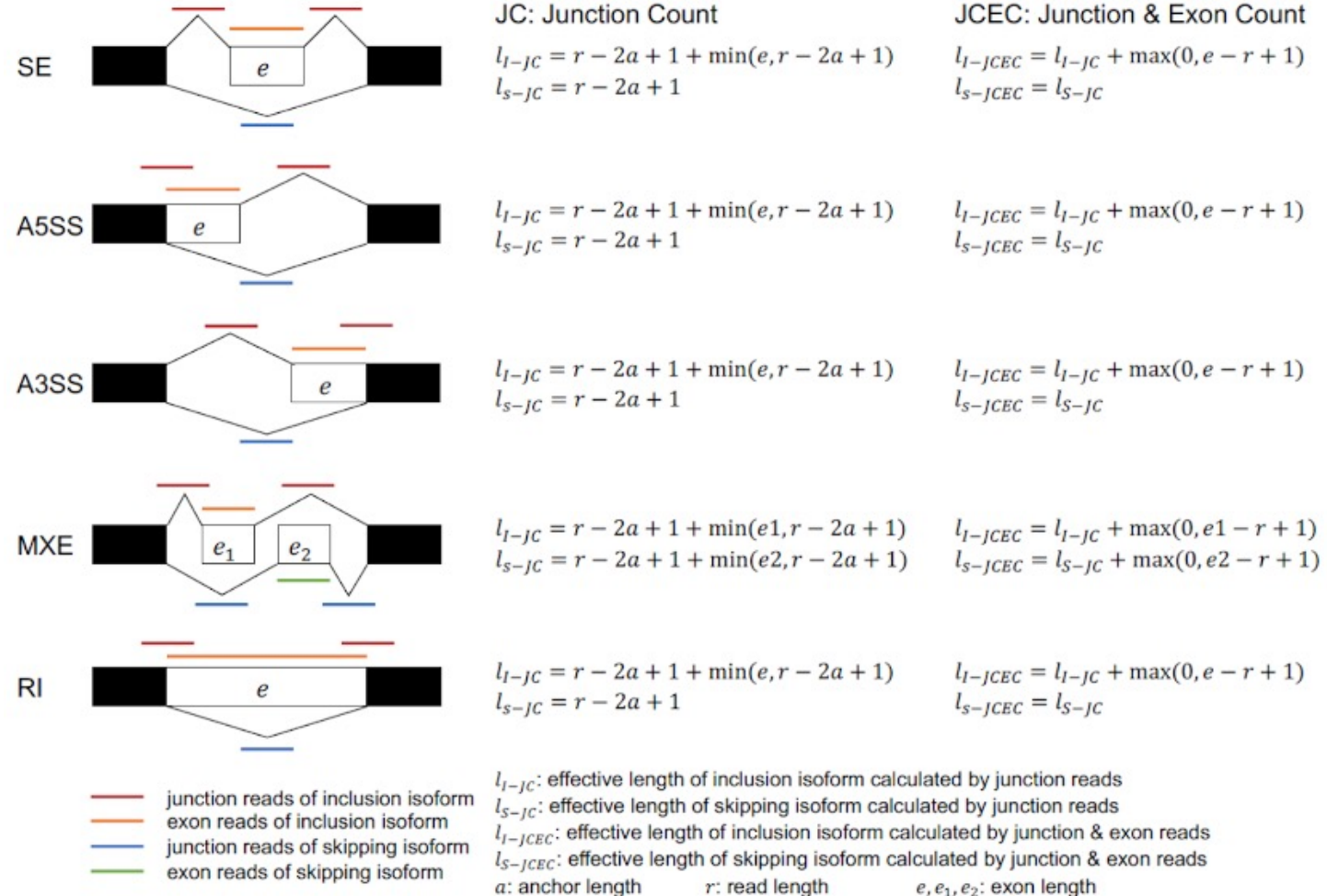
- **rMATs**
<https://github.com/Xinglab/rmats-turbo>

- 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!