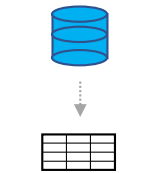


A. Retrieve raw data, quality control, trim, and alignment

Run as needed. Note enumeration follows script names.

1. Retrieve data

Creates time2splice/ folder structure, metadatafile.csv and SraAccList.txt. .fastqs retrieved using SraAccList.txt.

2. Run quality control

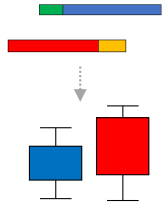
Run ***FastQC*** for all .fastq files in time2splice/ directory.

3. Trim data

Run ***Trim Galore!***, then ***FastQC*** to trim reads below quality threshold. Merge lanes of the same flow cell .fastq files.

4/5. Run & plot alignment

Run ***Bowtie2***, ***BWA***, or ***HISAT2*** on .fastq data in time2splice directory. Plot alignment for one or two aligners.



B. Temporal expression analysis

Run as needed. Note enumeration follows script names.

1. Run transcript quantification

Quantify transcript treatment and control expression with ***Salmon***.

2. Run differential splicing analysis

Run ***SUPPA*** differential splicing analysis across case and controls.

3. Format results

Converts NM_ gene names to flybase name. Merges outputs.

4. Identify differential splicing forms

SUPPA identifies forms of differential splicing (e.g. using PSI and DTU).

5. Calculate total control alternative splicing

Calculate and plot the proportions of alternative splicing in control samples.

6. Calculate total case alternative splicing

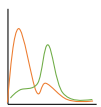
Calculate and plot the proportions of alternative splicing in case samples.

7. Get bias genes

Retrieve male and female biased genes. Create .beds to plot average profiles.

8/9. Plot splicing events

Plots alternative splicing (PSI and DTU), and events in categories (e.g. female sex specific, male new sex specific).



C. Temporal protein-DNA analysis

Run as needed. Note enumeration follows script names.

1. Mark duplicate reads

Run Run ***Picard's MarkDuplicates*** in for all .sorted.bam files in a given directory.

2. Call peaks

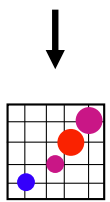
Run ***MACS2*** to call peaks and location (***Chipseeker***) for all .sorted.bam files in a given directory.

3. Find fold enrichment

Generate signal track using ***MACS2*** to profile transcription factor modification enrichment levels genome-wide.

4. Plot profile and location

Plot average profile of TF binding across gene or at TSS/TES with ***deepTools*** and binding location using ***chipseeker***.



D. Temporal multi-omics integration

Run as needed. Miscellaneous tests at README end plot peak intensity and perform chi-squared test

Peak intersections

Run ***Intervene*** to view intersection of each narrowpeak file.

Gene ontology

Perform gene ontology analysis with ***ClusterProfiler*** given a list of genes.

Find motifs *denovo*

Get coordinates of bed file and run through ***MEME***.