Experimental design

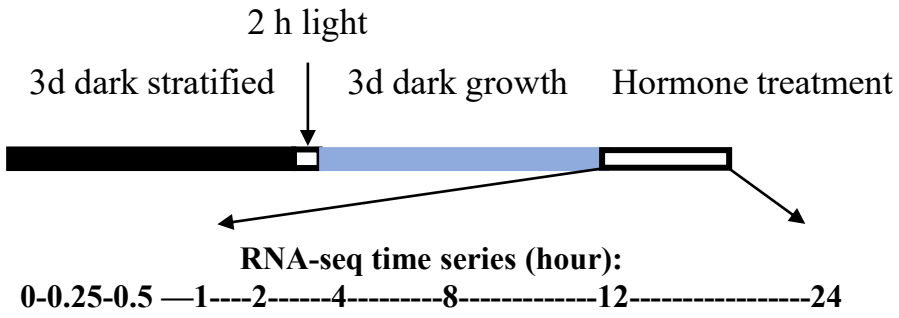
- Research model: whole etiolated(dark-grown) Arabidopsis seedlings

1 RNA-sequencing (RNA-Seq)

➢ Treatment: six hormones

☑ unpublished
- Brassinosteroid (BR)
- Salicylic acid (SA)
- GR24 - Strigolactone/Karrikin (SL/KAR)

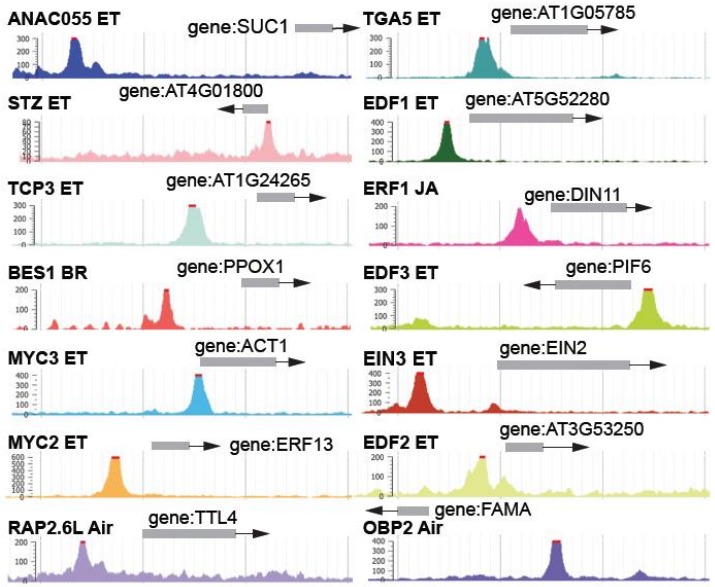
☑ published
- Abscisic acid (ABA)
- Ethylene (ET)
- Jasmonic acid (JA)



2 Chromatin immunoprecipitation sequencing (ChIP-Seq)

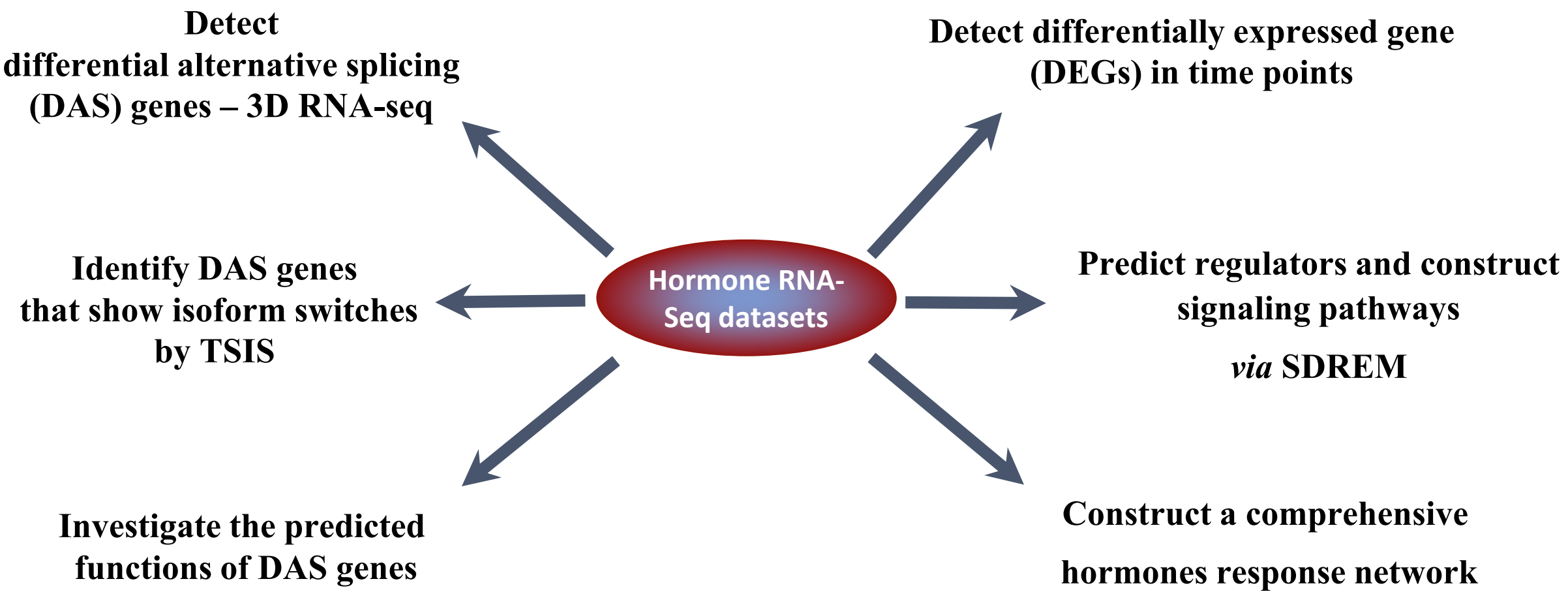
➢ Treatment: air/hormone treatment for 2 h (BR, ET, JA, SA)

☑ 14 key TFs across multiple hormones:



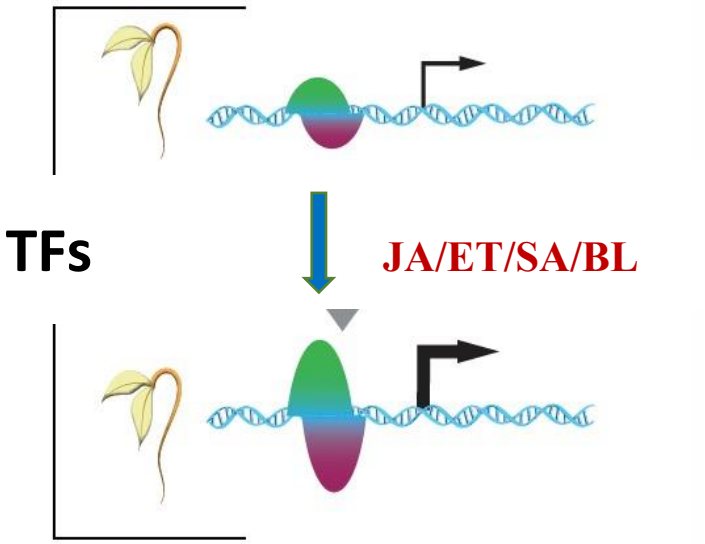
Public RNA-Seq data: Zander et al., 2020; Song et al., 2016; Chang et al., 2013

Objects in RNA-seq analysis



Guo, et al., *RNA Biol*, 2020

Objects in ChIP-seq analysis

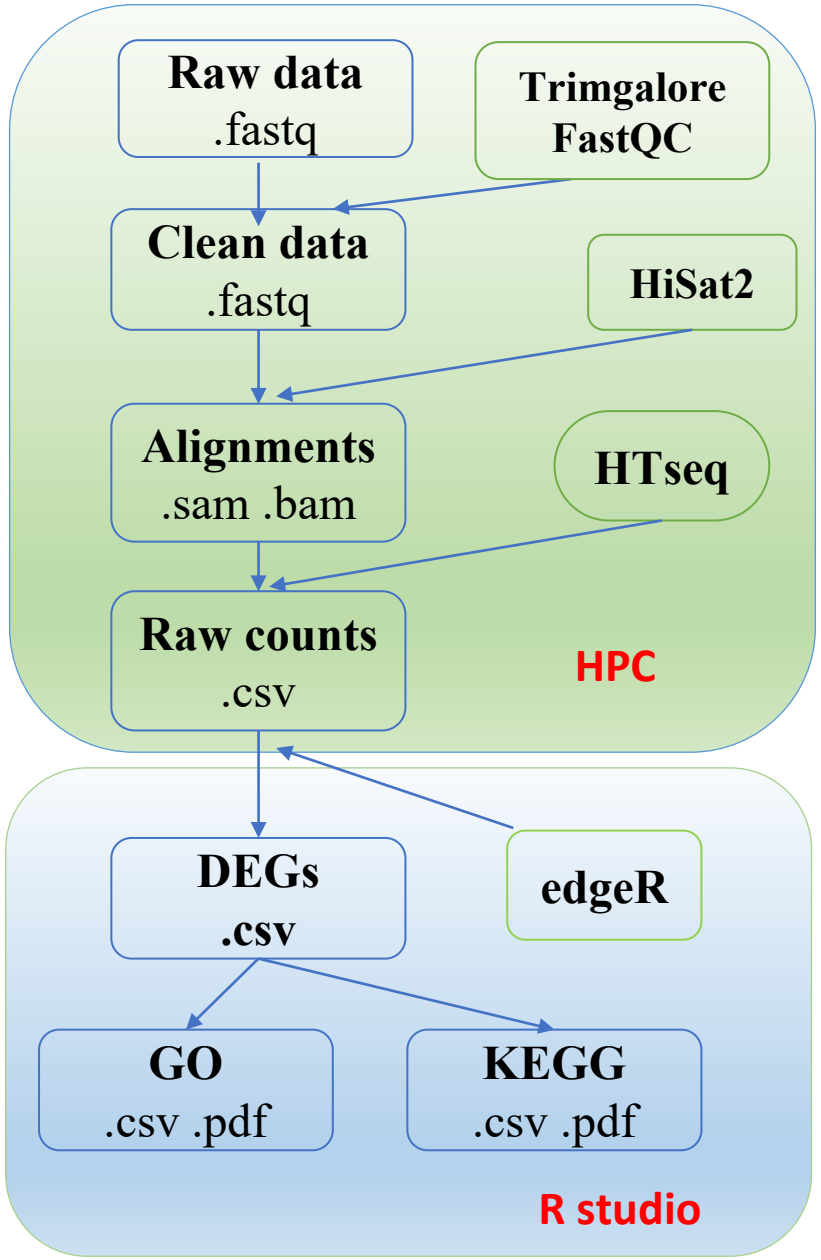


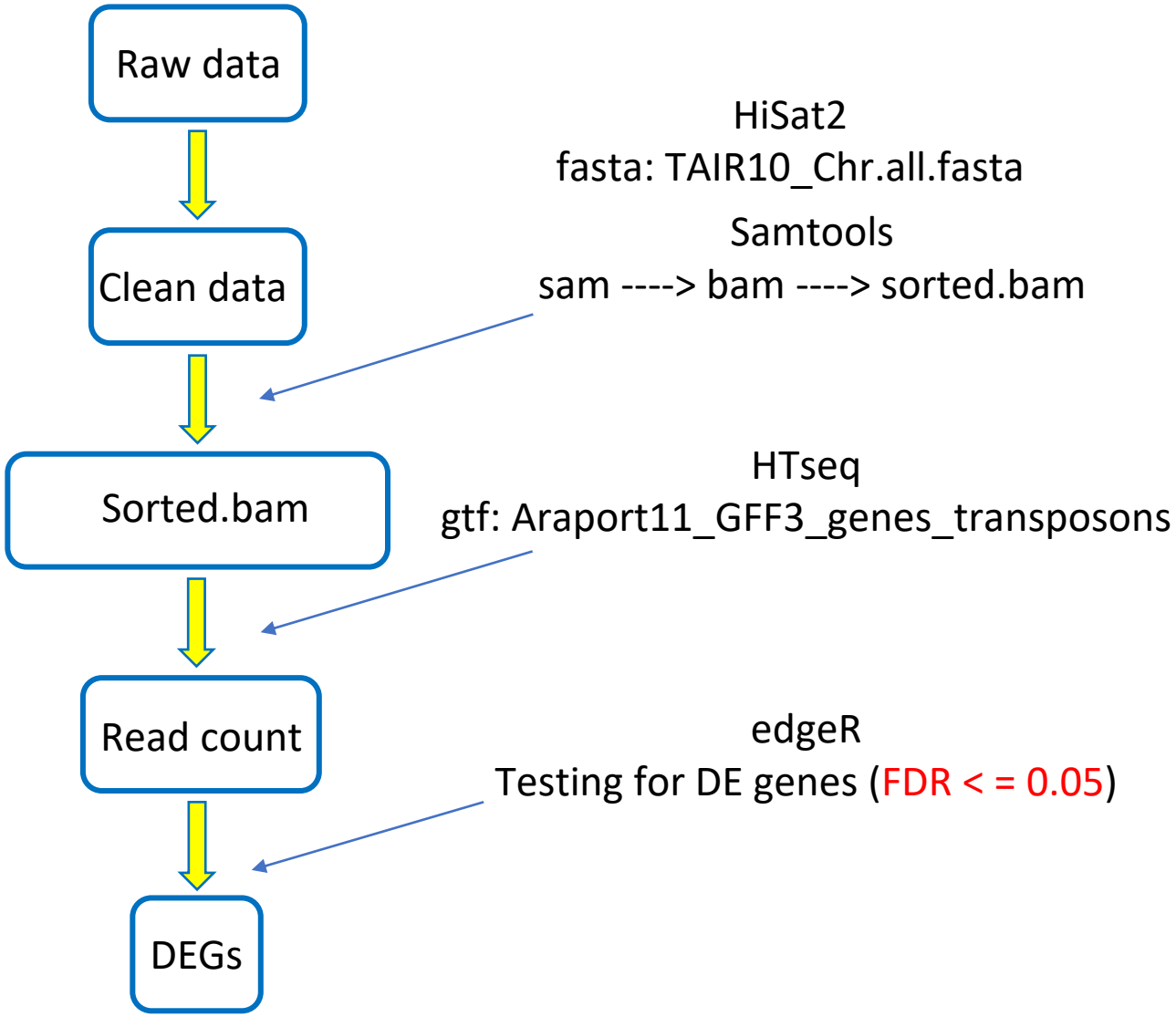
ChIP-seq data analysis

Target genes

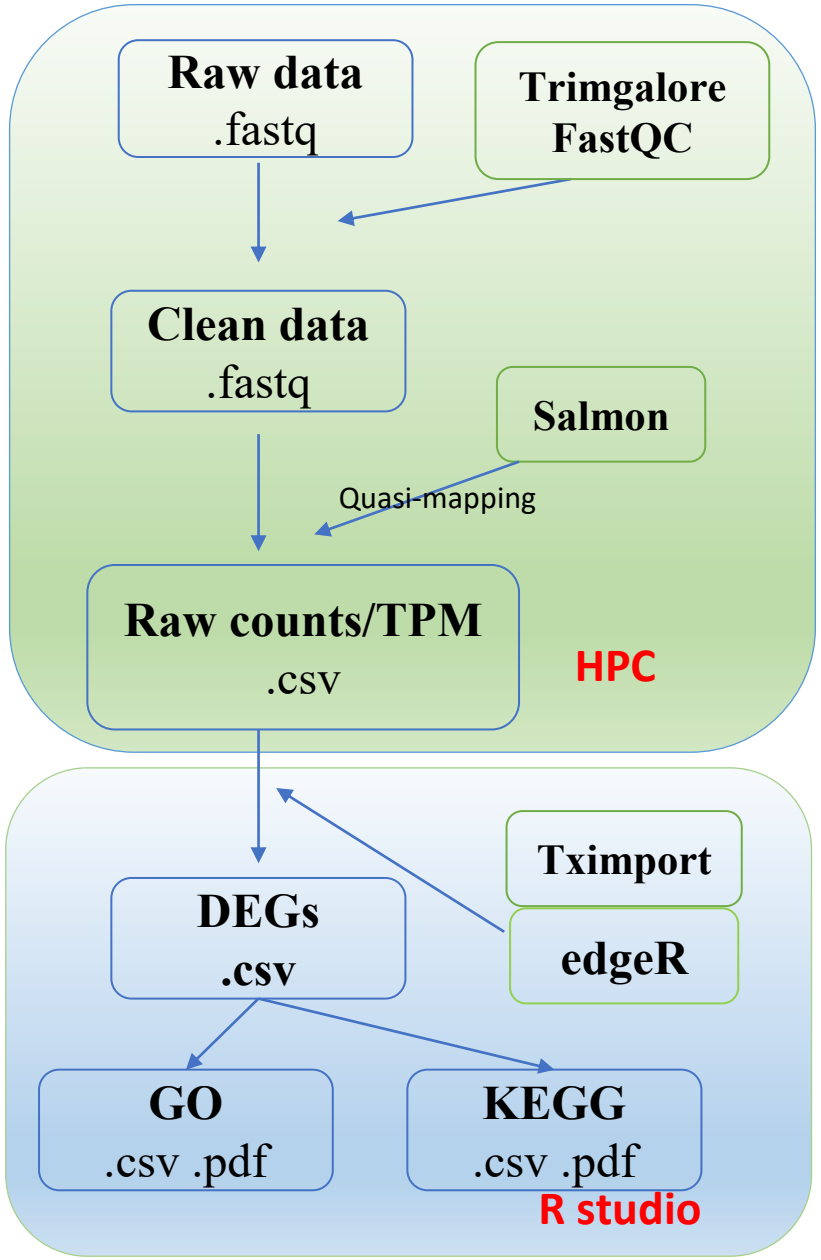
(TF-Gene interaction information)

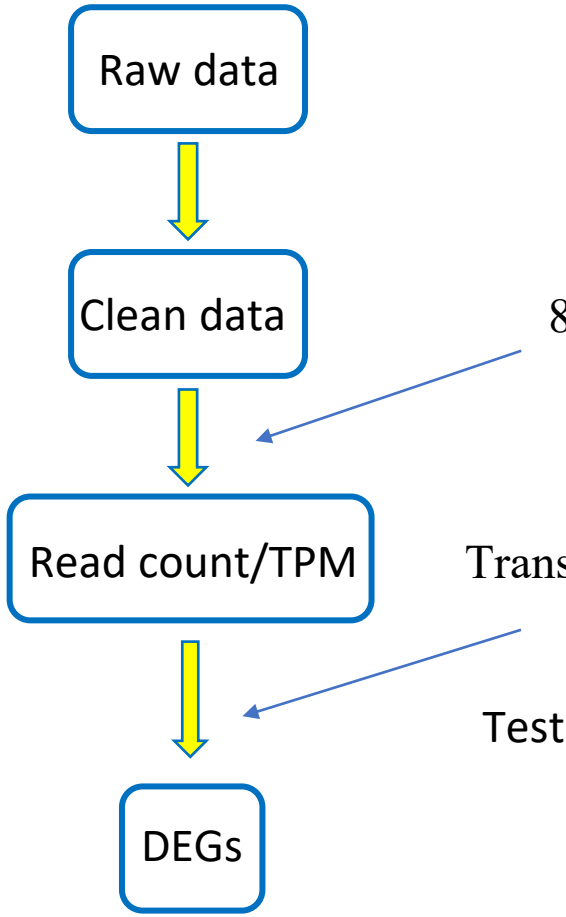
Workflow of RNA-seq analysis (ET RNA-seq data)





Workflow of RNA-seq analysis (ABA, BR, JA, SA, SL_KAR RNA-seq data)





Salmon V0.8.1
Reference:
AtRTDv2_QUASI
82190 transcripts for 34212 genes

Tximport
Transcripts abundance to Gene abundance

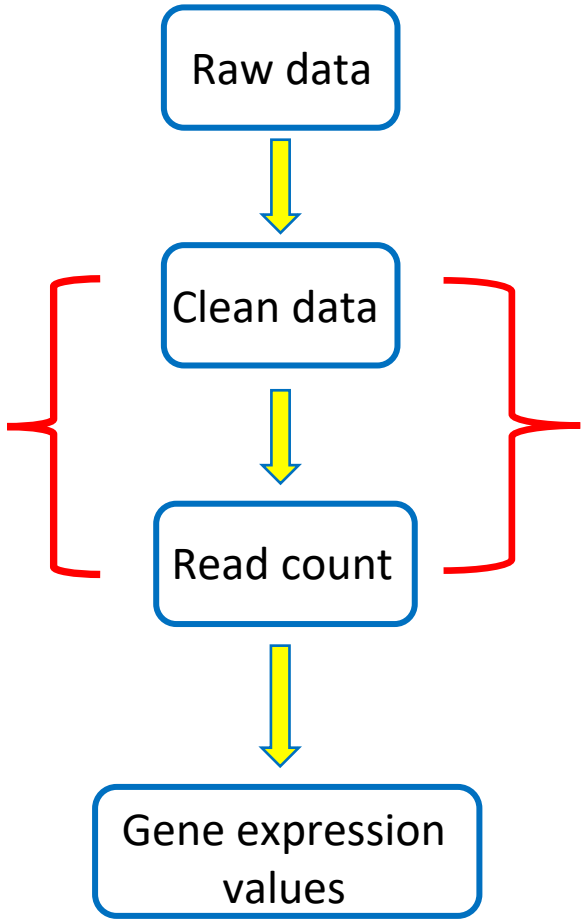
edgeR
Testing for DE genes (FDR < = 0.05/0.01)

Overview of RNA-seq analysis

For ET

HiSat2
fasta: TAIR10

HTseq
gtf: Araport11



For ABA, BR, JA, SA, SL_KAR

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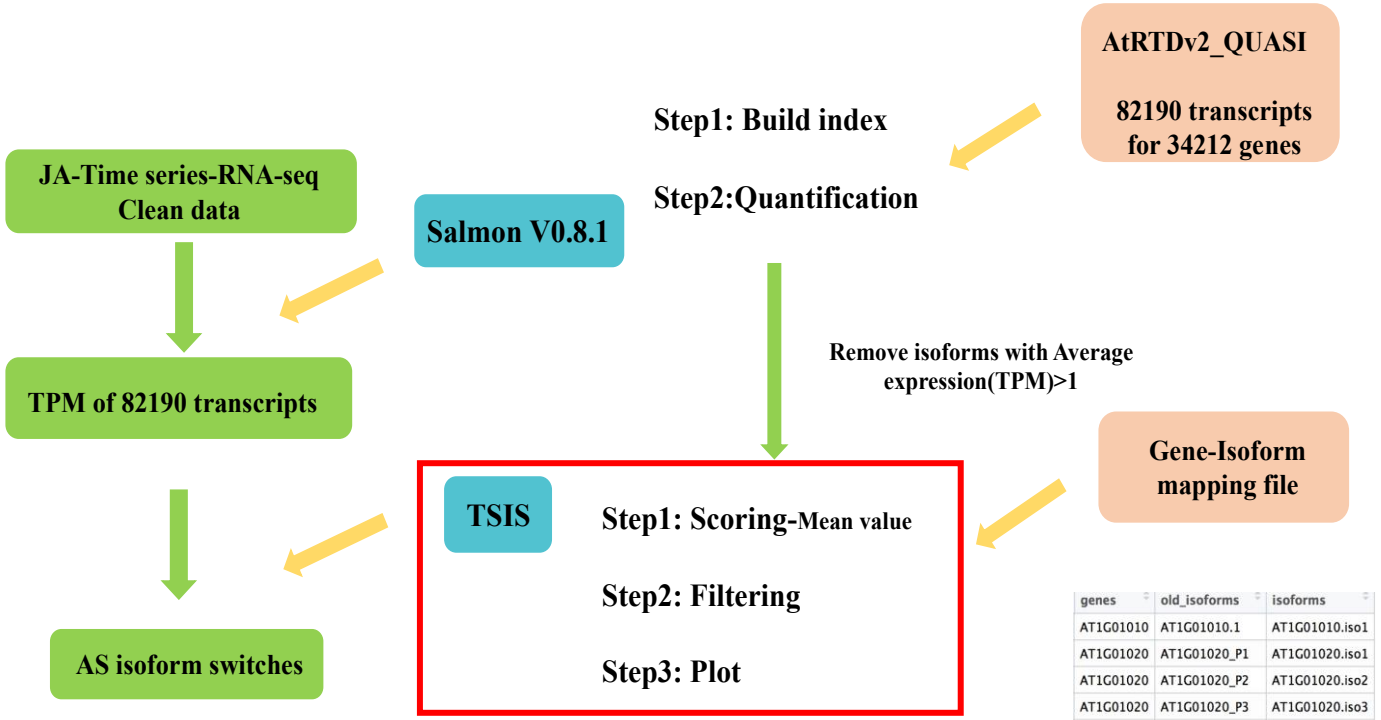
Workflow of detecting alternative splicing isoform switch events



genes	old_isoforms	isoforms
AT1G01010	AT1G01010.1	AT1G01010.iso1
AT1G01020	AT1G01020_P1	AT1G01020.iso1
AT1G01020	AT1G01020_P2	AT1G01020.iso2
AT1G01020	AT1G01020_P3	AT1G01020.iso3
AT1G01020	AT1G01020_P4	AT1G01020.iso4
AT1G01020	AT1G01020_P5	AT1G01020.iso5
AT1G01020	AT1G01020_P6	AT1G01020.iso6
AT1G01030	AT1G01030.1	AT1G01030.iso1
AT1G01030	AT1G01030_ID1	AT1G01030.iso2

Step 1: Scoring

Calculate the average expression values of the replicates for each time-point for each isoform.
Determine the switch points

Step 2: Filtering

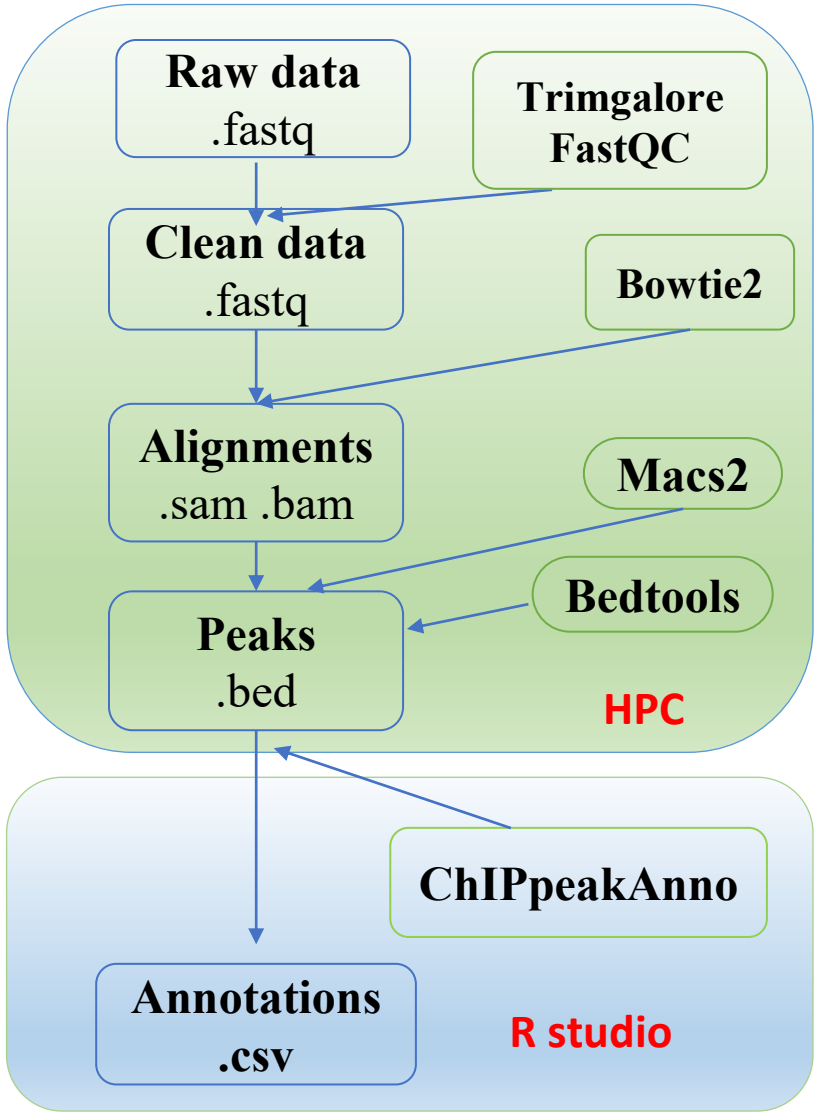
The switch points divide the time series frame into intervals.
The probability: the sum of the frequencies that one isoform is more or less abundant than the other > 0.5
The differences: the sum of average abundance differences of the two isoforms > 1
The p-values: the significance of the differences between the isoform abundances >0.05
The min time point in interval: a measure of whether the effect of the switch is transient or long lived > 1

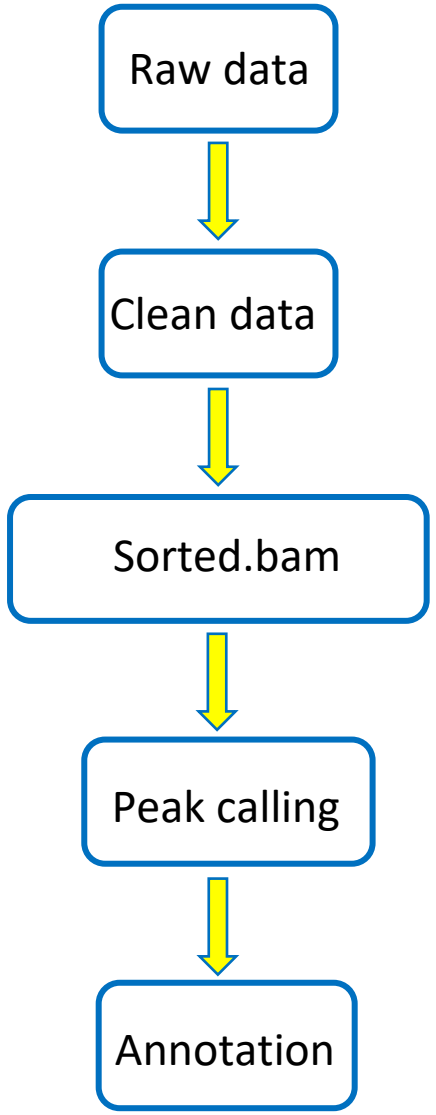
Step 3: Plot

Interactive visualizations of the isoform switch profiles.

Guo, et al., *Bioinformatics*, 2017

Workflow of ChIP-seq data analysis





Bowtie2
fasta: TAIR10_Chr.all.fasta

Samtools
sam --> bam -->
sorted.bam --> confident.bam
(mapq > 10)

Macs2
call peaks with mock air IP
(-log 10 (q value) >= 15 or
$q < 10^{-15}$)

bedtools
overlap between replicates
(f = 0.5)

ChIPpeakAnno
TAIR10 annotated genes