General workflow

No genome available

RNA-seq

de-novo splicing

needles

Illumina Hiseq 2000   .fastq

Raw files QC
FASTX toolkit

Specificity-Sensitivity trade-off

Different kmer sizes (51-61)

Splicing events identification
KisSplice

ACC → G → CCG → T → GCT → T → CTT

Transcriptome Assembly

Annotation
InterPro, dammit, gotubro-RSQL

Splicing downstream analysis
KissDE, DESeq2, fgsea ...

System-level analysis
Regulatory networks, Integration

Global expression patterns during stress

UpSet, Heatmap

Differential splicing and clustering

KissDE (diff variant), K-Means

Differential expression and functional impact

DESeq2, fgsea, REVIGO

Regulatory networks
Seidr, backbonning, Infomap, neat

Integration
MOFA2

Approach/algorithms to identify most regulatory variables

Crowd-Network (DREAM5)

Network Interpretation (filter edges, unsupervised cluster and functional impact)

Backbonning, Infomap, neat

Is alternative splicing a key component?

Proteome + Metabolome (Escandón *et al*, 2017)

MOFA2 (non-supervised)

Candidates   Candidates   Candidates

Validation

Biological Validation
RT-PCR, Sanger seq

GAT AAAT CT GG TCT TA TTT CC

Validation in-silico
Intron/Event comparative analysis