How to use MISO

RUNNING MISO

1 Get annotation of alternative events to run MISO on (in GFF format)

Use MISO provided annotations of alternative events or your own annotations of transcripts/events to quantify

2 Align RNA-Seq reads using read mapper (e.g. Tophat) to create BAM file

Use `samtools` to create sorted, indexed BAM files if necessary

3 Run MISO

Feed GFF with annotations and BAM file with reads into MISO. If running on paired-end reads, compute insert length distribution mean and standard deviation for each sample

ANALYZING RESULTS

Summarize MISO output

Get exon/isoform expression levels (Ψ values) in each sample along with confidence intervals

Detect differentially expressed exons/isoforms across samples

Compute Bayes factors to determine significance of changes and magnitude of changes ($\Delta\Psi$ values)

Filter events by significance and order of magnitude of expression change

Get set of events that meet certain Bayes factor and $\Delta\Psi$ cutoff values

VISUALIZATION

4 Plot the results

Visualize the results alongside the raw RNA-Seq data with sashimi-plot