

Classify *Drosophila* Promoters

Define the promoter search region

Perform *blastn* search of the initial exon of the *D. melanogaster* ortholog against the contig from the target species (e.g., *D. kikkawai*) using more sensitive search parameters

Has full-length *blastn* alignment to the exon?

Yes

Define TSS Position based on the start of the *blastn* alignment

No

Has partial *blastn* alignment to the exon?

Yes

Extrapolate TSS Position based on the partial *blastn* alignment

No

Use other evidence (e.g., RNA Polymerase II ChIP-Seq data, ATAC-Seq data, RNA-Seq data, UTR predictions) to define a promoter search region

Characterize the promoter region

The “Combined CSHL RAMPAGE TSS” track has one peak?

Yes

TSS Search Region: extent of RAMPAGE peak
TSS Position: position with the highest read density
Promoter Shape: determined by Shape Index (SI):
Peaked: $SI > -1$; Broad: $SI \leq -1$

No

The “Combined CSHL RAMPAGE TSS” track has multiple peaks?

Yes

TSS Search Region: extent of all RAMPAGE peaks consistent with the orientation of the gene
TSS Position: position with the highest read density among the cluster of consistent RAMPAGE peaks

No

Found signals in the “RAMPAGE Read Density” tracks?

Yes

TSS Search Region: extent of the genomic region after clustering regions with RAMPAGE signals
TSS Position: position with the highest read density within the cluster of RAMPAGE signals

No

Insufficient evidence to locate the TSS

Define promoter search region based on other lines of evidence (e.g., *blastn* alignments, RNA-Seq data, UTR predictions)