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.

Define the promoter search region

Characterize the promoter region

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