**Supplementary File 5.**  Commands for STAR mapping.

**First round**:

Generating genome index:

STAR --runThreadN 3 --runMode genomeGenerate --genomeDir genome\_index\_STAR\_r1 --genomeFastaFiles dm3.fa --sjdbGTFfile dm3\_genes.gtf --sjdbOverhang 50

Mapping:

STAR --runThreadN 3 --genomeDir genome\_index\_STAR\_r1 --outFileNamePrefix DN1\_A --readFilesIn DN1\_A\_R01.fastq DN1\_A\_R02.fastq --outSJfilterReads Unique

**Second round**:

Re-generating genome index:

STAR --runThreadN 3 --runMode genomeGenerate --genomeDir genome\_index\_STAR\_r2 --genomeFastaFiles dm3.fa --sjdbFileChrStartEnd combined\_SJ\_out\_tab\_unannotated\_for\_2nd\_pass\_genome\_generation.txt --sjdbGTFfile dm3\_genes.gtf --sjdbOverhang 50

* *combined\_SJ\_out\_tab\_unannotated\_for\_2nd\_pass\_genome\_generation.txt is an output file from running JUM. For details, see JUM manual at: https://github.com/qqwang-berkeley/JUM*

Mapping:

STAR --runThreadN 3 --genomeDir genome\_index\_STAR\_r2 --outFileNamePrefix ctrl\_2nd --readFilesIn ctrl\_R01.fastq ctrl\_R02.fastq --outSJfilterReads Unique --outSAMstrandField intronMotif --outFilterMultimapNmax 1