

ENCODE miRNA-seq read alignment using STAR aligner

The ENCODE miRNA-seq data were processed using STAR aligner v. 2.4.2a. This document provides the parameters used to index the genome and align the adapter trimmed reads.

Genome index generation using STAR aligner:

The genome was indexed using the comprehensive GENCODE annotations (M4 for mouse and v.24 for human). The following parameters were used:

```
STAR \  
  --runThreadN 16 \  
  --runMode genomeGenerate \  
  --genomeDir /path/to/genomeDir \  
  --sjdbGTFfile gencode.comprehensive.annotation.gtf \  
  --sjdbOverhang 1 \  
  --genomeFastaFiles genome.fasta
```

Alignment step using STAR aligner:

Prior to alignment, reads were trimmed for 3' and 5' adapters using Cutadapt. You can find the details here (<https://github.com/sorenar/miRNA-seq-adapters>) The miRNA subsets of GENCODE annotations were used for the alignment step:

```
params=' --runThreadN 16  
  --sjdbGTFfile /path/to/GENCODE_miRNA_subset.gtf  
  --alignEndsType EndToEnd  
  --outFilterMismatchNmax 1  
  --outFilterMultimapScoreRange 0  
  --quantMode TranscriptomeSAM GeneCounts  
  --outReadsUnmapped Fastx  
  --outSAMtype BAM SortedByCoordinate  
  --outFilterMultimapNmax 10  
  --outSAMunmapped Within  
  --outFilterScoreMinOverLread 0  
  --outFilterMatchNminOverLread 0  
  --outFilterMatchNmin 16  
  --alignSJDBoverhangMin 1000  
  --alignIntronMax 1  
  --outWigType wiggle  
  --outWigStrand Stranded  
  --outWigNorm RPM  
,
```

```
STAR --genomeDir /path/to/genomeDir --readFilesIn trimmed.reads.fq $params
```