

# Expressed RNAs from RNAseq (TPM calculation)

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## 1 Libraries and settings

```
# -----  
# libraries  
# -----  
library(tidyverse)  
library(GenomicFeatures)  
library(GenomicRanges)  
# -----  
# settings  
# -----  
out <- "/Users/melinaklostermann/Documents/projects/AgoCLIP_miR181/R_github/miR181_paper/Figure5/TPMs-RN
```

## 2 What was done?

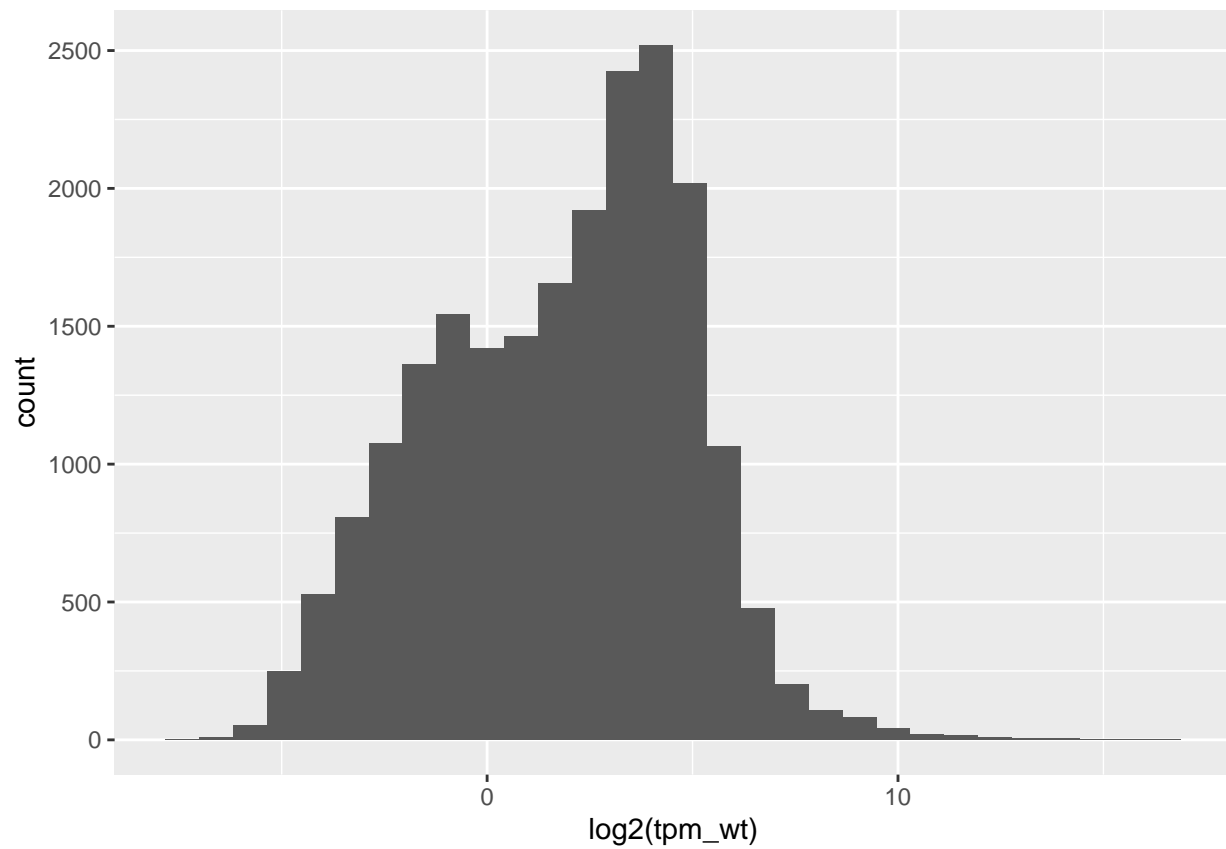
- Calculate TPMs
- Use TPM filter to get a list of expressed RNAs

## 3 Files

```
RNAseq_read_counts <- read_csv("/Users/melinaklostermann/Documents/projects/AgoCLIP_miR181/R_github/miR  
  
#annotation  
anno <- readRDS("/Users/melinaklostermann/Documents/projects/AgoCLIP_miR181/R_github/miR181_paper/Method
```

## 4 Calculate tpm

```
#####  
# get gene transcript length  
#####  
anno <- anno[anno$type == "exon"]  
anno <- split(anno, anno$geneID)  
anno <- reduce(anno)  
  
gene_length <- lapply(anno, function(x) sum(width(x))) %>% unlist()  
  
# get count matrix  
RNAseq_read_counts$...1 <- sub("\\\\.\\.*", "", RNAseq_read_counts$...1)  
RNAseq_read_counts <- as.data.frame(RNAseq_read_counts)  
rownames(RNAseq_read_counts) <- RNAseq_read_counts$...1  
RNAseq_read_counts$...1 <- NULL  
RNAseq_read_counts <- as.matrix(RNAseq_read_counts)  
  
# order gene length  
gene_length <- gene_length[rownames(RNAseq_read_counts)]  
  
# calculate tpm  
x <- RNAseq_read_counts / gene_length  
tpm <- t( t(x) * 1e6 / colSums(x, na.rm = T) )  
  
# tpm of all wt  
tpm <- as.data.frame(tpm) %>%  
  rownames_to_column(var = "gene_id") %>%  
  rowwise() %>%  
  mutate(tpm_wt = median(c(WT_1411, WT_1601, WT_1710), na.rm = T))  
  
# plot tpm of wt  
ggplot(tpm, aes(x = log2(tpm_wt)))+  
  geom_histogram()
```



```
# expressed genes  
expressed_genes <- tpm %>% subset(tpm_wt >= 1) %>% pull(gene_id)  
  
saveRDS(expresses_genes, paste0(out, "expressed_genes.rds"))
```