Linnarsson Data Analysis

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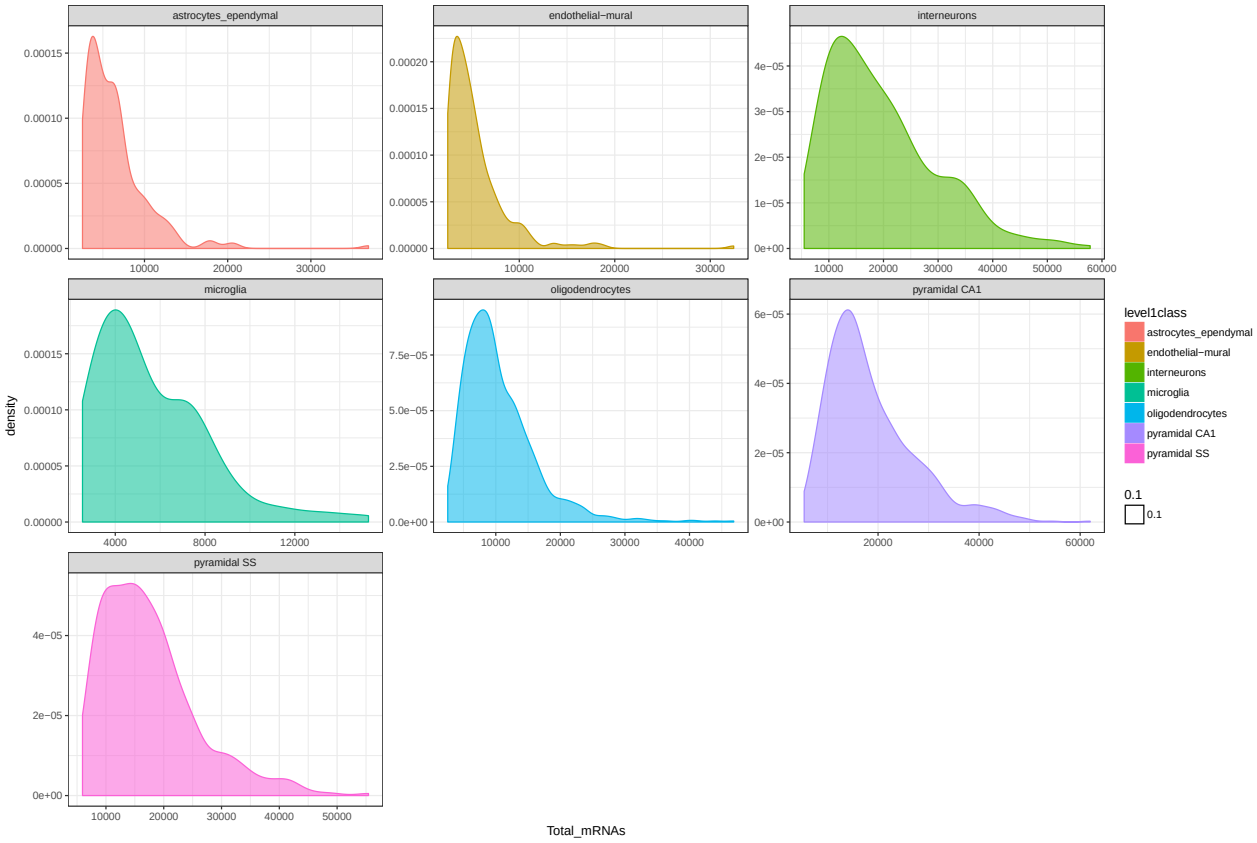
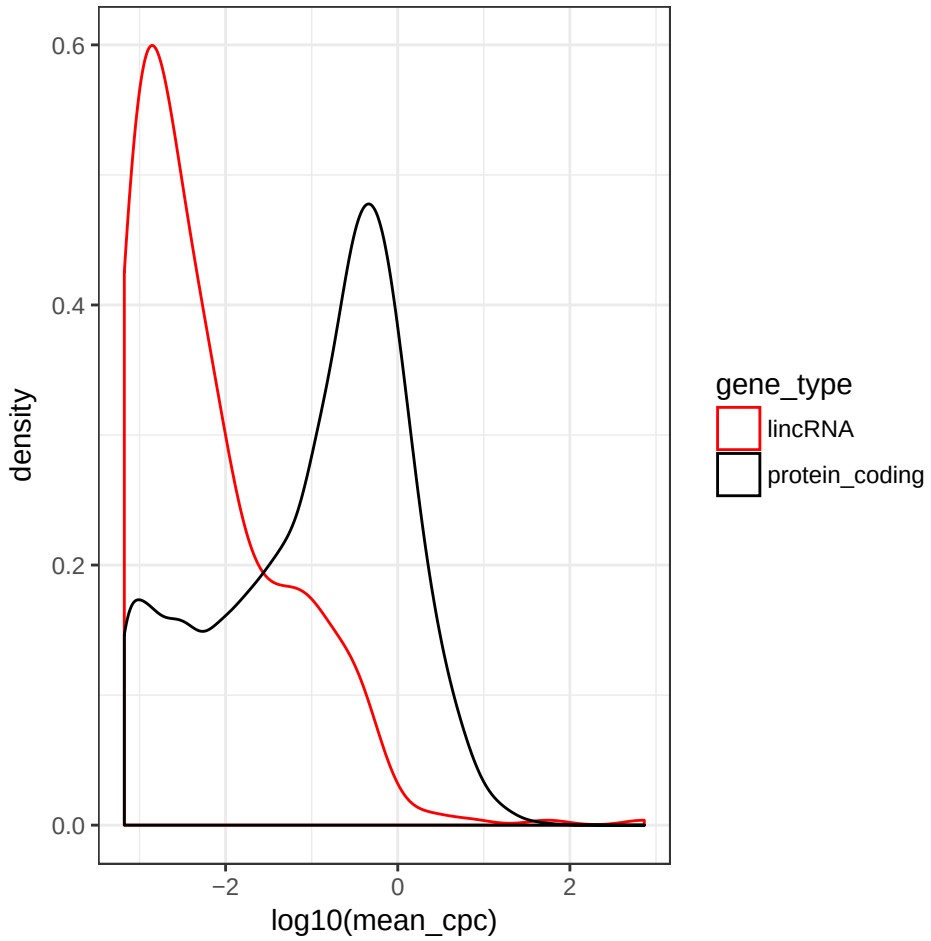
Generating the CellDataSet object

```
# QC of data after
pData(dat)$Total_mRNAs <- Matrix::colSums(exprs(dat))

qplot(Total_mRNAs, data = pData(dat), color = level1class, fill = level1class,
    geom = "density", alpha = 0.1) + facet_wrap("level1class", scales = "free") +
    theme_bw()
```




```
# Calculate the mean copies per cell among all classes (Bulk) and draw the
# density plot for lincRNAs vs protein coding
dat.means <- detectGenes(dat, min_expr = 0.001)
dat.means <- dat.means[fDataa(dat.means)$num_cells_expressed >= 1, pData(dat.means)$num_genes_expressed >
    250]
fData(dat.means)$mean_cpc <- apply(exprs(dat.means), 1, mean)

tmp <- data.frame(gene_short_name = fData(dat.means)$gene_short_name, gene_type = fData(dat.means)$trans
    mean_cpc = fData(dat.means)$mean_cpc)

dat_means <- subset(tmp, gene_type %in% c("protein_coding", "lincRNA"))
```

```
density.plot <- ggplot(dat_means) + geom_density(aes(x = log10(mean_cpc), color = gene_type)) +
    scale_color_manual(values = c("red", "black")) + theme_bw()

density.plot
```




```
# List the lincRNAs that are expressed with a mean_cpc greater than 1
dat_lincRNA_sort <- subset(dat_means, gene_type %in% "lincRNA")
dat_mRNA_sort <- subset(dat_means, gene_type %in% "protein_coding")

# length(dat_lincRNA_sort$gene_short_name)
print("Number of lncRNAs = 441")
```

```
## [1] "Number of lncRNAs = 441"
```

```
# length(dat_mRNA_sort$gene_short_name)
print("Number of mRNAs = 17091")
```

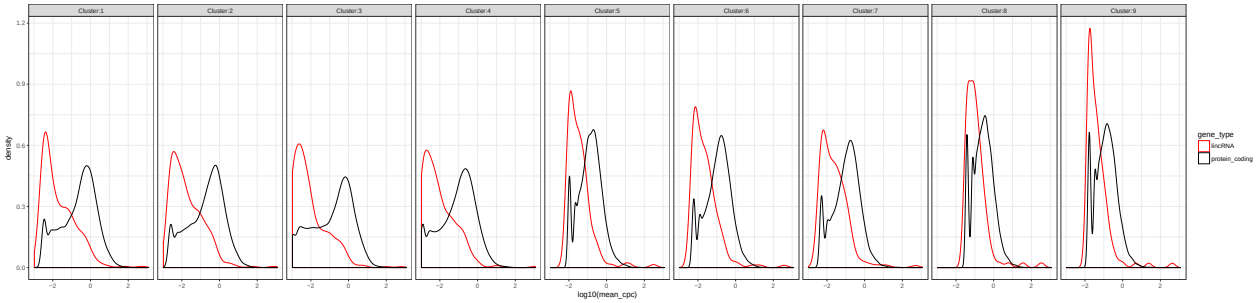
```
## [1] "Number of mRNAs = 17091"
```

Seperate the "dat" CellDataSet by "Cluster" and calculate mean expression of genes

```
# Seperate the 'dat' CellDataSet by 'Cluster' and calculate mean expression
# of genes
level1.split <- lapply(unique(pData(dat)$group_num), function(x) {
    dat[, pData(dat)$group_num == x]
})
```

```
level1.split <- lapply(c(1:length(level1.split)), function(x) {
    detectGenes(level1.split[[x]], min_expr = 0.01)
})

level1.split <- lapply(c(1:length(level1.split)), function(i) {
    x <- level1.split[[i]]
    x[fData(x)$num_cells_expressed >= 1, pData(x)$num_genes_expressed >= 1000]
})

level1.split <- lapply(level1.split, function(x) {
    mean_cpc <- apply(exprs(x), 1, mean)
    fData(x)$mean_cpc <- mean_cpc
    return(x)
})

tmp <- data.frame()
group_means <- lapply(c(1:length(level1.split)), function(i) {
    x <- level1.split[[i]]
    res <- data.frame(gene_short_name = fData(x)$gene_short_name, gene_type = fData(x)$transcript_type,
        mean_cpc = fData(x)$mean_cpc, group_num = unique(pData(x)$group_num))
    tmp <- rbind(tmp, res)
})

tmp <- plyr::ldply(group_means, data.frame)

group_means <- subset(tmp, gene_type %in% c("protein_coding", "lincRNA"))

density.plot_level1class <- ggplot(group_means) + geom_density(aes(x = log10(mean_cpc),
    color = gene_type)) + facet_grid(. ~ group_num, labeller = labeller(group_num = function(x) {
    paste("Cluster", x, sep = ":")
})) + scale_color_manual(values = c("red", "black")) + theme_bw()

density.plot_level1class
```

```
## Warning: Removed 110 rows containing non-finite values (stat_density).
```

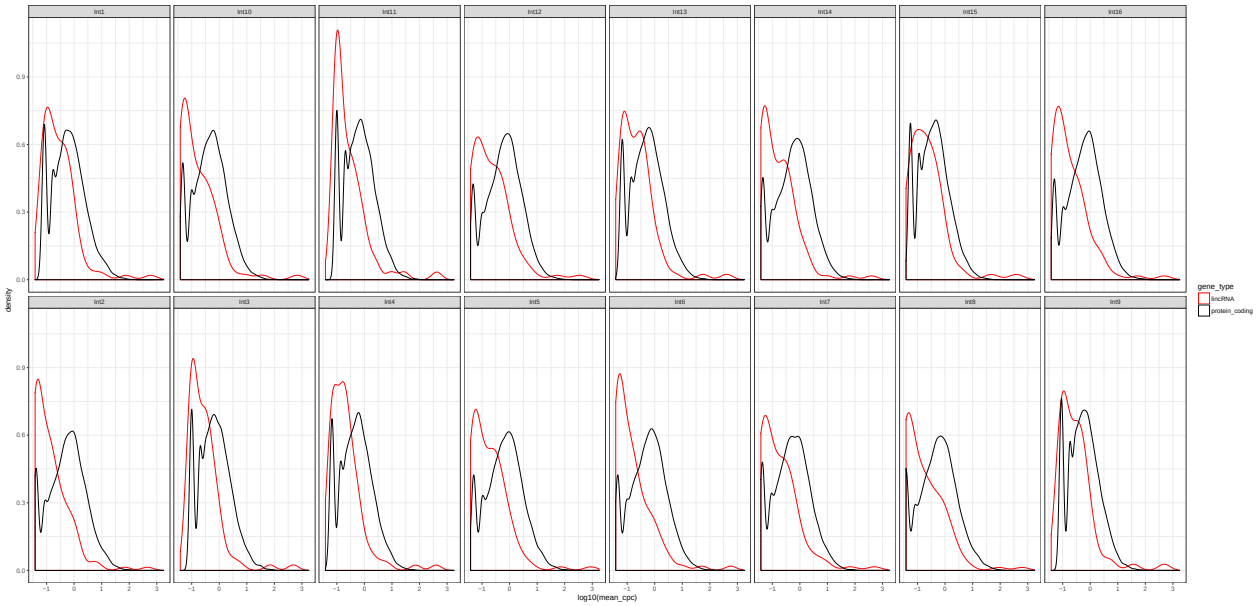


Seperate the "dat" CellDataSet by level2class and calculate mean expression of genes

```
# Seperate the 'dat' CellDataSet by level2class and calculate mean
# expression of genes
level2.split <- lapply(unique(pData(dat)[pData(dat)$level1class == "interneurons",
    ]$level2class), function(x) {
    dat[, pData(dat)$level2class == x]
})
```

```
level2.split <- lapply(c(1:length(level2.split)), function(x) {
    detectGenes(level2.split[[x]], min_expr = 0.01)
})

level1.split <- lapply(c(1:length(level2.split)), function(i) {
    x <- level2.split[[i]]
    x[fData(x)$num_cells_expressed >= 1, pData(x)$num_genes_expressed > 100]
})

level2.split <- lapply(level2.split, function(x) {
    mean_cpc <- apply(exprs(x), 1, mean)
    fData(x)$mean_cpc <- mean_cpc
    return(x)
})

tmp <- data.frame()

group_means_level2class <- lapply(c(1:length(level2.split)), function(i) {
    x <- level2.split[[i]]
    res <- data.frame(gene_short_name = fData(x)$gene_short_name, gene_type = fData(x)$transcript_type,
        mean_cpc = fData(x)$mean_cpc, level2class = unique(pData(x)$level2class))
    tmp <- rbind(tmp, res)
})

tmp <- plyr::ldply(group_means_level2class, data.frame)

group_means_level2class <- subset(tmp, gene_type %in% c("protein_coding", "lincRNA"))

density.plot_level2class <- ggplot(group_means_level2class) + geom_density(aes(x = log10(mean_cpc),
    color = gene_type)) + facet_wrap(~level2class, nrow = 2) + scale_color_manual(values = c("red",
    "black")) + theme_bw()

density.plot_level2class
```

```
## Warning: Removed 107012 rows containing non-finite values (stat_density).
```


lnt1
lnt10
lnt11
lnt12
lnt13
lnt14
lnt15
lnt16
lnt2
lnt3
lnt4
lnt5
lnt6
lnt7
lnt8
lnt9
density
log10(mean_cpc)
gene_type
lincRNA
protein_coding