BCC pre-processing

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Data

Read in the data and check the format:

```
data_loc <- "../Raw_data/"
filenames <- c(
  "BRCA.exp.348.med.csv",
  "BRCA.348.precursor.txt",
  "rppaData-403Samp-171Ab-Trimmed.txt",
  "BRCA.Methylation.574probes.802.txt"
)

GE <- read.csv(paste0(data_loc, "BRCA.exp.348.med.csv"), header = TRUE)
miRNA <- read.csv(paste0(data_loc, "BRCA.348.precursor.txt"), header = TRUE)
Protein <- read.table(paste0(data_loc, "rppaData-403Samp-171Ab-Trimmed.txt"), header = TRUE)
Meth <- read.table(paste0(data_loc, "BRCA.Methylation.574probes.802.txt"), header = TRUE)

orig_datafiles <- list(GE, miRNA, Protein, Meth) %>%
  magrittr::set_names(c("GE", "miRNA", "Protein", "Meth"))

dim(GE)
```

```
## [1] 17814   349
```

```
dim(miRNA)
```

```
## [1] 1046  349
```

```
dim(Protein)
```

```
## [1] 171 404
```

```
dim(Meth)
```

```
## [1] 574 802
```

```
# View the head of the data
head(GE[, 1:5])
```

```
##       NAME TCGA.A1.A0SH.01A.11R.A084.07 TCGA.A1.A0SJ.01A.11R.A084.07
## 1    ELMO2                     0.162208                     0.577708
## 2  CREB3L1                     1.338000                    -0.483500
## 3    RPS11                     0.044063                    -0.258062
## 4    PNMA1                     0.249500                     0.682250
## 5     MMP2                     1.843833                    -0.531000
## 6 C10orf90                     0.184500                     0.127500
```

```
##   TCGA.A1.A0SK.01A.12R.A084.07 TCGA.A1.A0SO.01A.22R.A084.07
## 1                     1.113042                    -0.375208
## 2                    -1.558500                    -1.628750
## 3                     0.119813                    -0.542063
## 4                     0.867750                    -0.139750
## 5                    -1.674833                    -1.838167
## 6                    -0.843750                    -0.363750
```

```
head(miRNA[, 1:5])
```

```
##           Gene TCGA.A8.A07E.01A.11R TCGA.A8.A09C.01A.11R TCGA.A8.A084.01A.21R
## 1 hsa-let-7a-1            4629.4498            5437.9616             7340.861
## 2 hsa-let-7a-2            9384.9411           10872.5343            14676.347
## 3 hsa-let-7a-3            4742.0152            5596.5660             7457.106
## 4   hsa-let-7b           31208.3577           25291.9778            71135.465
## 5   hsa-let-7c            2414.6069            1250.5346             1003.195
## 6   hsa-let-7d             537.4601             597.8165              606.083
##   TCGA.A8.A091.01A.11R
## 1            9232.1420
## 2           18276.1584
## 3            9207.9908
## 4           85803.7544
## 5            1438.2719
## 6             419.4695
```

```
# head(Protein[, 1:5])
# head(Meth[, 1:5])
```

For integrative clutstering we need common samples across datasets. Find these.

```
# Match columns (samples) between sources
namesExp <- names(GE)[2:349]
namesmiRNA <- names(miRNA)[2:349]
namesProtein <- names(Protein)[2:404]
namesMeth <- names(Meth)

head(namesExp)
```

```
## [1] "TCGA.A1.A0SH.01A.11R.A084.07" "TCGA.A1.A0SJ.01A.11R.A084.07"
## [3] "TCGA.A1.A0SK.01A.12R.A084.07" "TCGA.A1.A0SO.01A.22R.A084.07"
## [5] "TCGA.A2.A04N.01A.11R.A115.07" "TCGA.A2.A04P.01A.31R.A034.07"
```

```
head(namesProtein)
```

```
## [1] "TCGA.C8.A138.01A.21.A13D.20" "TCGA.AO.A03L.01A.31.A13A.20"
## [3] "TCGA.A2.A0SV.01A.21.A13A.20" "TCGA.A2.A0SW.01A.21.A13A.20"
## [5] "TCGA.BH.A0C0.01A.11.A13B.20" "TCGA.AN.A0AK.01A.11.A13B.20"
```

```
# Matching samples present
namesExp <- substr(namesExp, 1, 16)
namesmiRNA <- substr(namesmiRNA, 1, 16)
namesProtein <- substr(namesProtein, 1, 16)

MatchProt <- match(namesExp, namesProtein, nomatch = 0)
MatchMeth <- match(namesExp, namesMeth, nomatch = 0)
```

Convert to matrix format and set row names

```r
miRNA_names <- miRNA[, 1]
miRNA <- miRNA[, 2:349]
miRNA.mat <- as.matrix(miRNA[, order(namesmiRNA)]) %>%
  set_rownames(miRNA_names)

Protein.mat <- Protein[, 2:404]
Protein.mat <- as.matrix(Protein.mat[, MatchProt]) %>%
  set_rownames(Protein[, 1])

Meth.mat <- as.matrix(Meth[, MatchMeth]) %>%
  set_rownames(row.names(Meth))

Exp.mat <- as.matrix(GE[, 2:349])

reduced_matrix_data <- list(Exp.mat, miRNA.mat, Protein.mat, Meth.mat) %>%
  magrittr::set_names(c("GE", "miRNA", "Protein", "Meth"))
```

How much missingness is present?

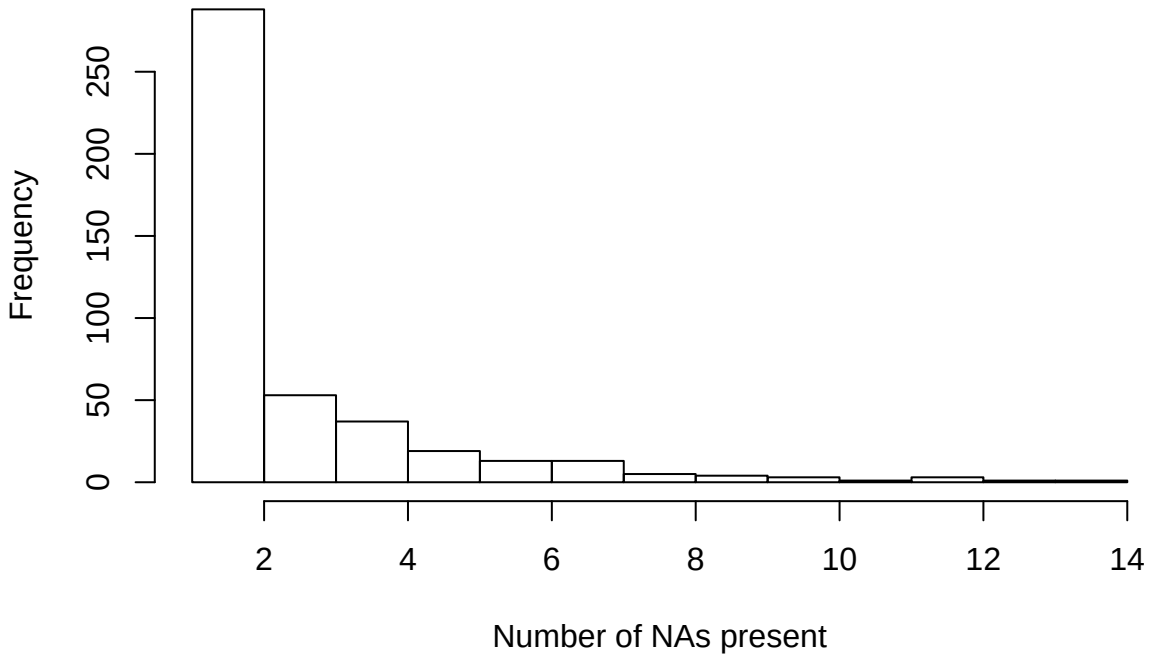
```r
# How many missing entries in our data
print(lapply(reduced_matrix_data, function(x) {
  sum(is.na(x))
}))
```

```
## $GE
## [1] 1119
##
## $miRNA
## [1] 0
##
## $Protein
## [1] 0
##
## $Meth
## [1] 0
```

```r
# The only dataset with NAs is the Gene expression data
dim(reduced_matrix_data$GE)
```

```
## [1] 17814   348
```

```r
ge_missingness <- rowSums(is.na(reduced_matrix_data$GE))
ge_missingness[ge_missingness > 0] %>%
  hist(
    main = "Count of NAs in each gene in GE data (0's excluded)",
    xlab = "Number of NAs present"
  )
```



As the Gene Expression contains actual NA's we will impute missing values using `knn` with $k = 10$ (the default setting).

```
# Impute missing values via KNN (K=10) for the Gene expression data
Exp.mat <- impute.knn(Exp.mat)
Exp.mat <- Exp.mat$data %>%
  set_rownames(GE[, 1])

reduced_matrix_data$GE <- Exp.mat
```

We might also be interested in the number of 0's present in the datasets as this could represent missing data.

```
# Check how many 0's there are in the datasets (possibly these are missing points too!)
print(lapply(reduced_matrix_data, function(x) {
  sum(x == 0)
}))
```

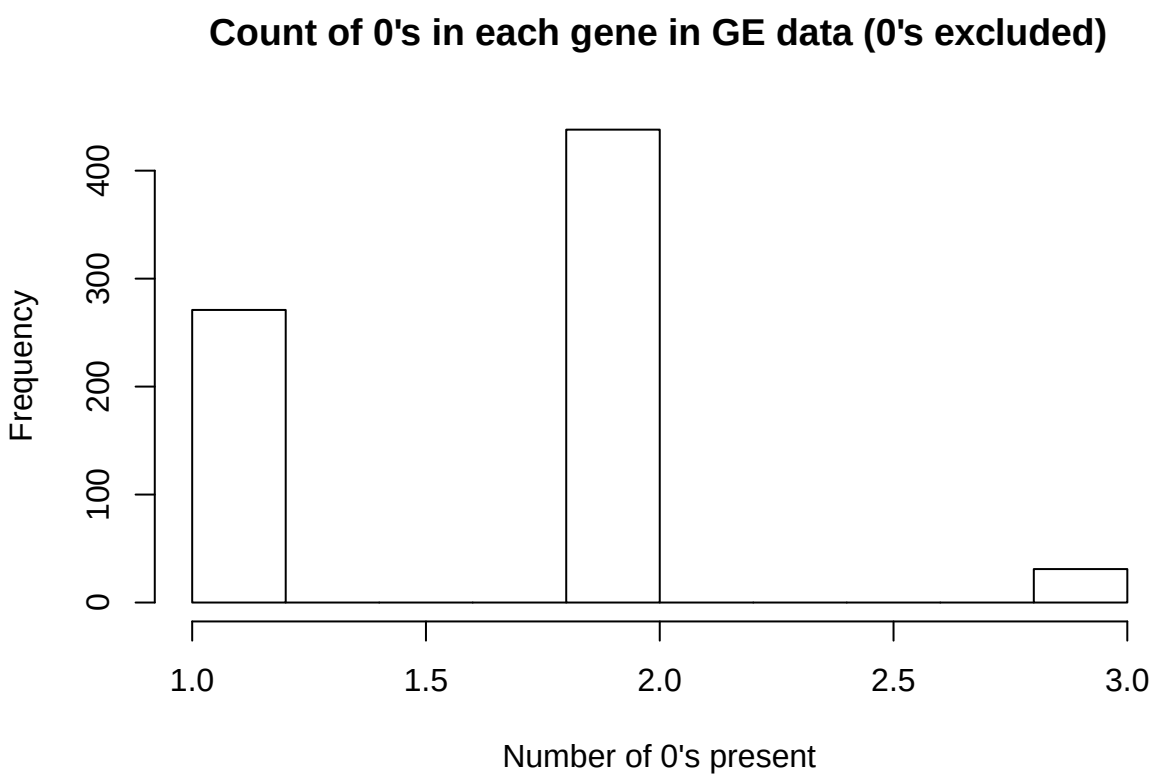
```
## $GE
## [1] 1240
##
## $miRNA
## [1] 211498
##
## $Protein
## [1] 0
##
## $Meth
## [1] 0
```

```
# The GE and miRNA datasets both have 0 entries, check how many genes have 0's
print(lapply(reduced_matrix_data, function(x) {
```

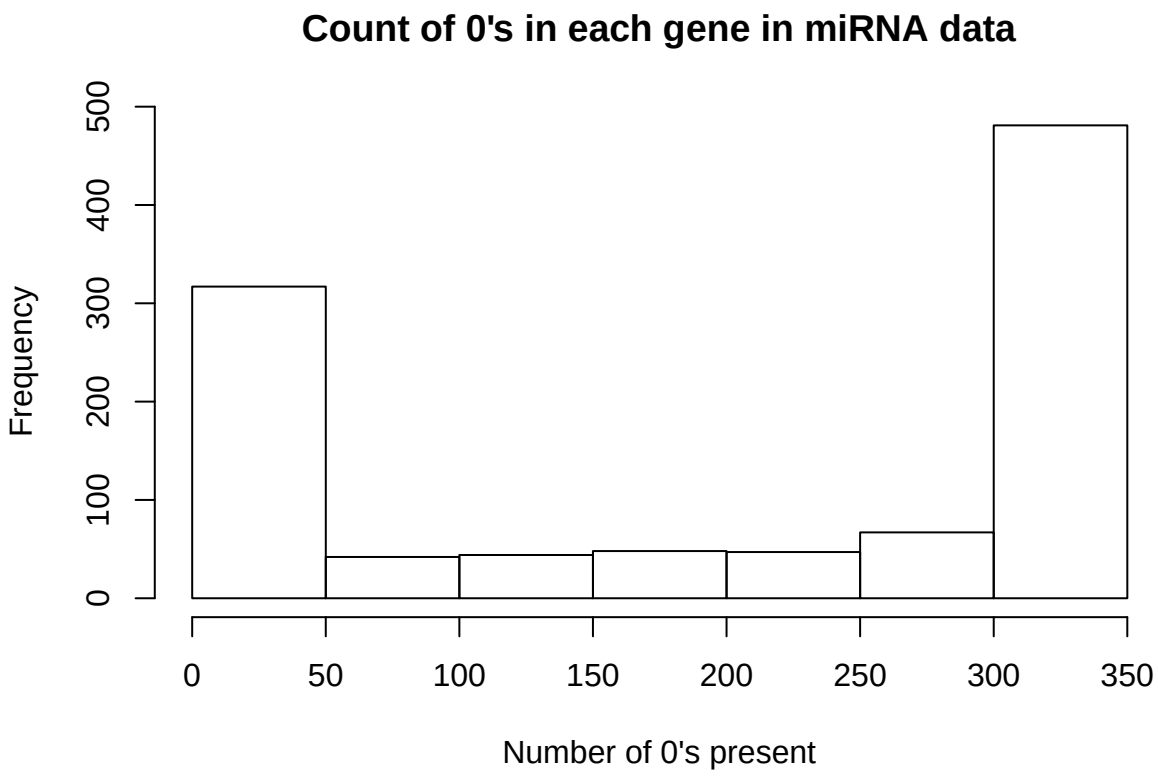
```
  sum(rowSums(x == 0) > 0)
}))
```

```
## $GE
## [1] 740
##
## $miRNA
## [1] 840
##
## $Protein
## [1] 0
##
## $Meth
## [1] 0
```

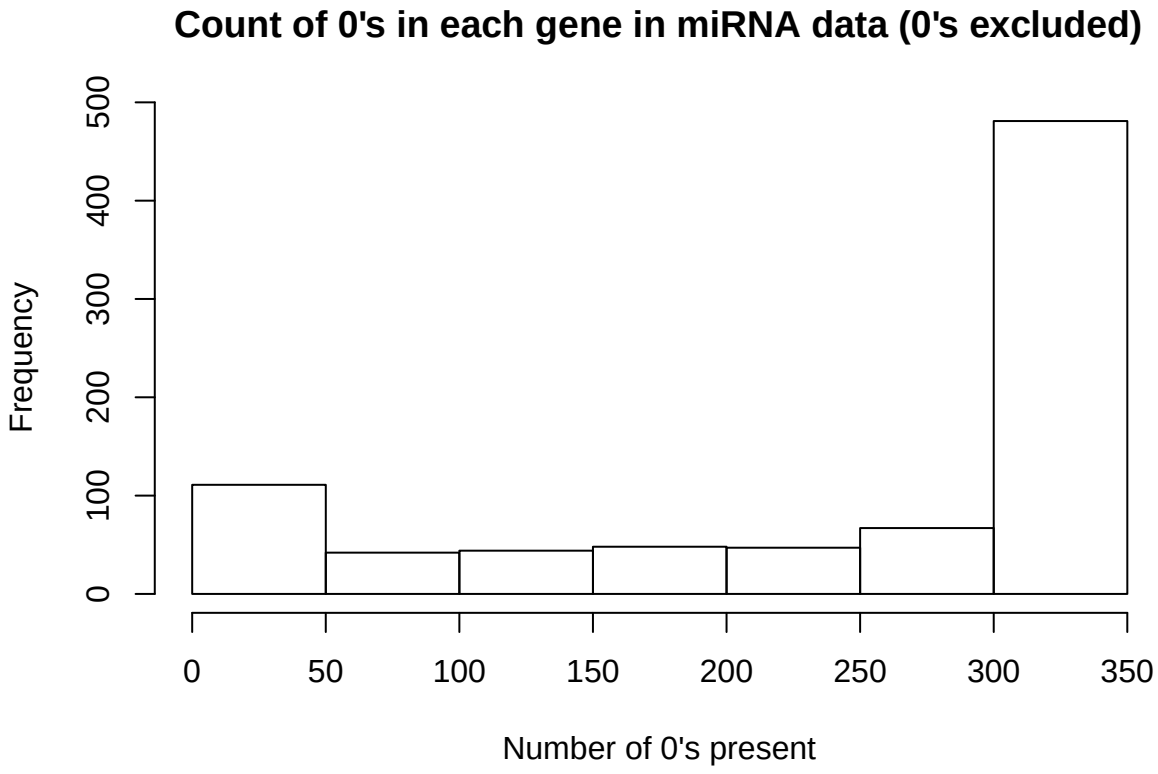
```
ge_zeroness <- rowSums(reduced_matrix_data$GE == 0)
ge_zeroness[ge_zeroness > 0] %>%
  hist(
    main = "Count of 0's in each gene in GE data (0's excluded)",
    xlab = "Number of 0's present"
  )
```




```
miRNA_zeroness <- rowSums(reduced_matrix_data$miRNA == 0)
miRNA_zeroness %>%
  hist(
    main = "Count of 0's in each gene in miRNA data",
    xlab = "Number of 0's present"
  )
```




```
miRNA_zeroness[miRNA_zeroness > 0] %>%
  hist(
    main = "Count of 0's in each gene in miRNA data (0's excluded)",
    xlab = "Number of 0's present"
  )
```



```
# There's a far greater number of 0 entries in the miRNA compared to the GE;
# also, no gene in the GE dataset has more than 3 associated 0 entries
```

Processing

We consider the impact of the processing upon the data in three ways. First we visualise the effect of the preprocessing steps by mapping the points to a 2D UMAP and colouring based upon their UMAP coordinates. We split the points based upon the median of the points in each direction, so points are assigned values based upon:

Table 1: Labels defined by UMAP coordinates.

Condition.in.UMAP.1	Condition.in.UMAP.2	Label
Greater than median	Greater than median	1
Greater than median	Less than median	2
Less than median	Greater than median	3
Less than median	Less than median	4

We then visualise the impact of the processing upon:

1. global structure - through visualisation of the first two principal components (PCs) from a PCA plot;
2. local structure - through visualisation of the first two dimensions of a UMAP reduction; and
3. the relationship between the mean and standard deviation of each gene and their marginal histograms.

Gene expresion

Note: We have used `knn.impute` to fill any NAs already.

Processing step: remove any genes with a standard deviation below 1.5. The argument is that genes with high standard deviation contribute more to the clustering.

First apply the filtering to the dataset and inspect the number of genes dropped.

```
processedExpression <- Exp.mat[apply(Exp.mat, 1, sd) > 1.5, ] ### Filter to select only most variable g
print(nrow(Exp.mat) - nrow(processedExpression))
```

```
## [1] 17169
```

Now generate labels for the data based upon the first two UMAP components (as described in the table above).

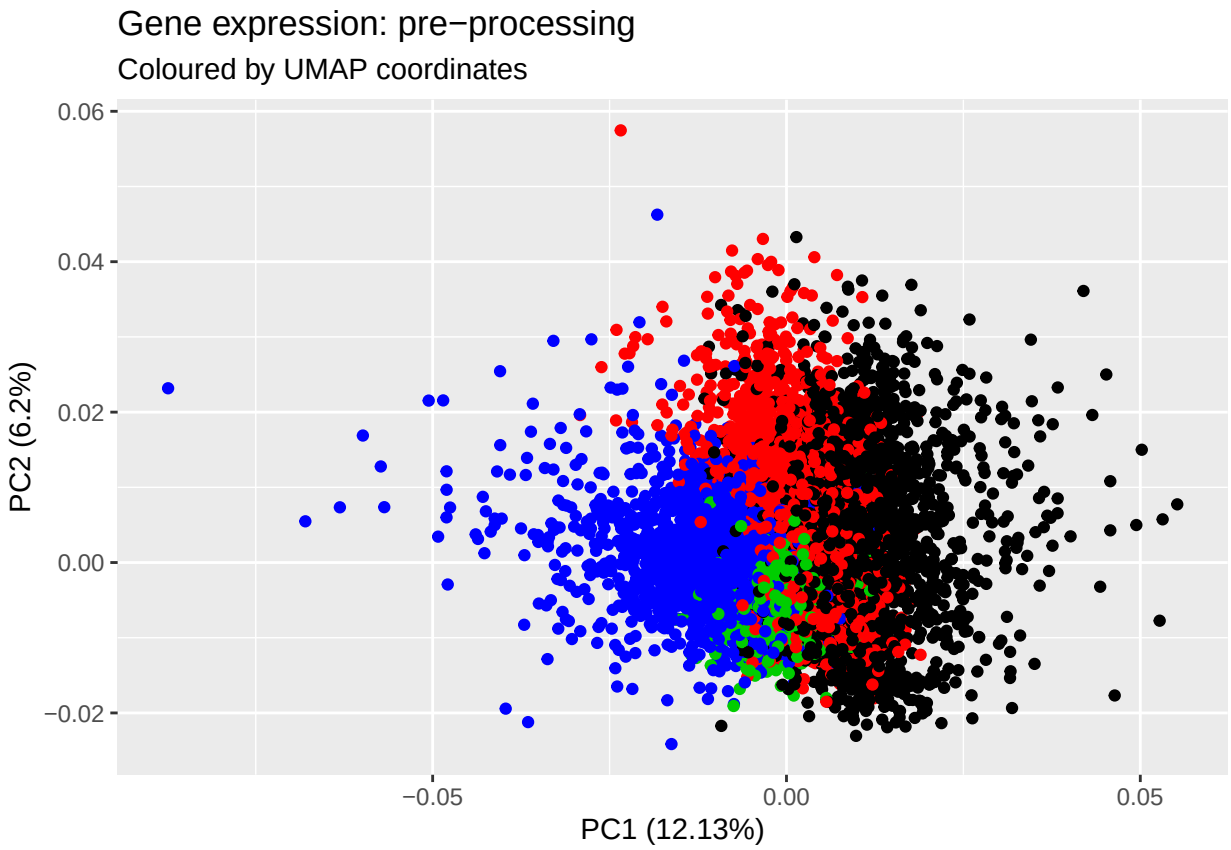
```
# Apply UMAP to the data
ge_umap <- umap(Exp.mat)

# Find labels from the UMAP to track how transform changes layout of data
ge_labels <- makeUMAPLabels(ge_umap$layout)
```

We visualise the global structure pre- and post-processing using the first two components of PCA:

```
# Do PCA
ge_pca <- prcomp(Exp.mat)

# Plot PCA with UMAP defined labels
autoplot(ge_pca, data = Exp.mat, colour = ge_labels) +
```

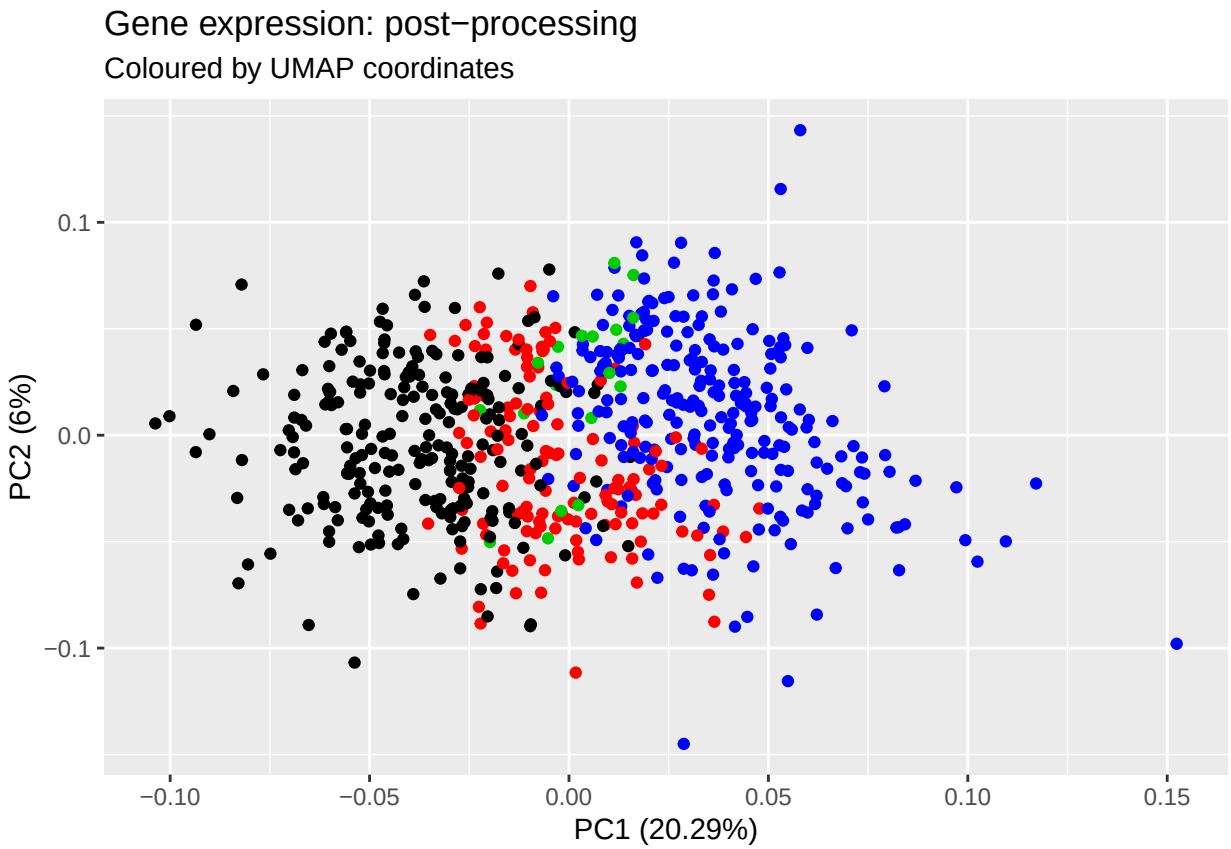
```
  labs(
    title = "Gene expression: pre-processing",
    subtitle = "Coloured by UMAP coordinates"
  )
```

```
## Warning in if (value %in% columns) {: the condition has length > 1 and only the
## first element will be used
```




```
# ggsave(file_names[[1]][1])

# Look at PCs after transform with the same labelling
p_ge_pca <- prcomp(processedExpression)
autoplot(p_ge_pca, data = processedExpression, colour = ge_labels[apply(Exp.mat, 1, sd) > 1.5]) +
  labs(
    title = "Gene expression: post-processing",
    subtitle = "Coloured by UMAP coordinates"
  )
```

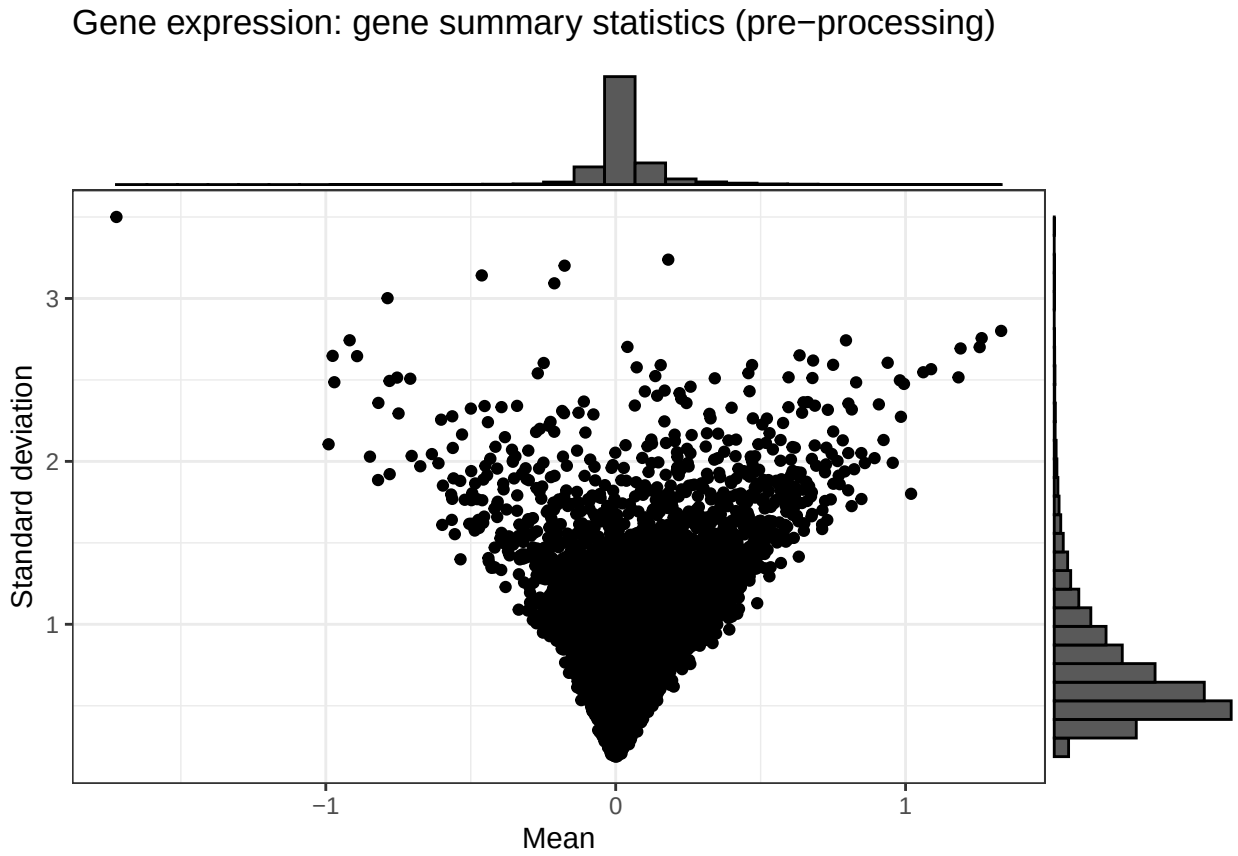
```
## Warning in if (value %in% columns) {: the condition has length > 1 and only the
## first element will be used
```



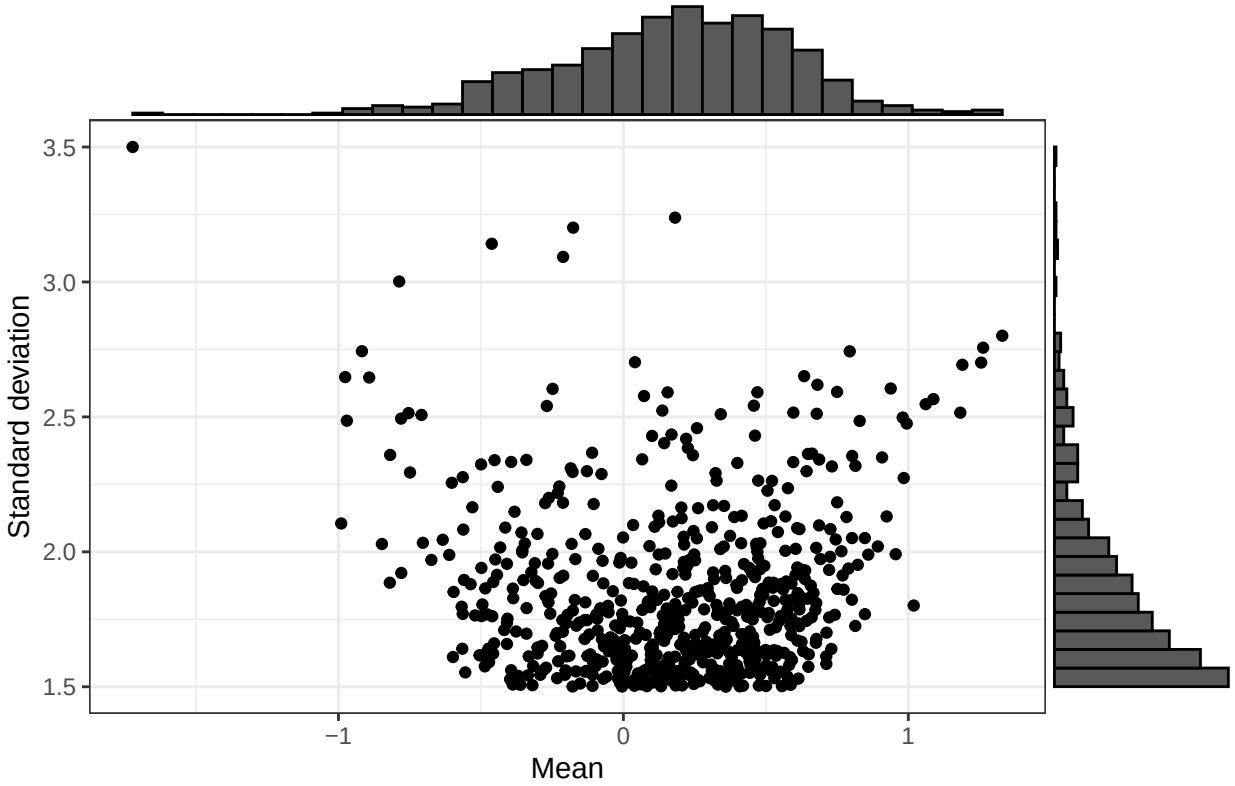

```
# ggsave(file_names[[2]][1])
```

We visualise the summary statistics relationship to each other. We see that $\sigma^2 \propto \mu$.

```
# Plot summary statistics
plotMarginals(Exp.mat,
  main = "Gene expression: gene summary statistics (pre-processing)"
)
```

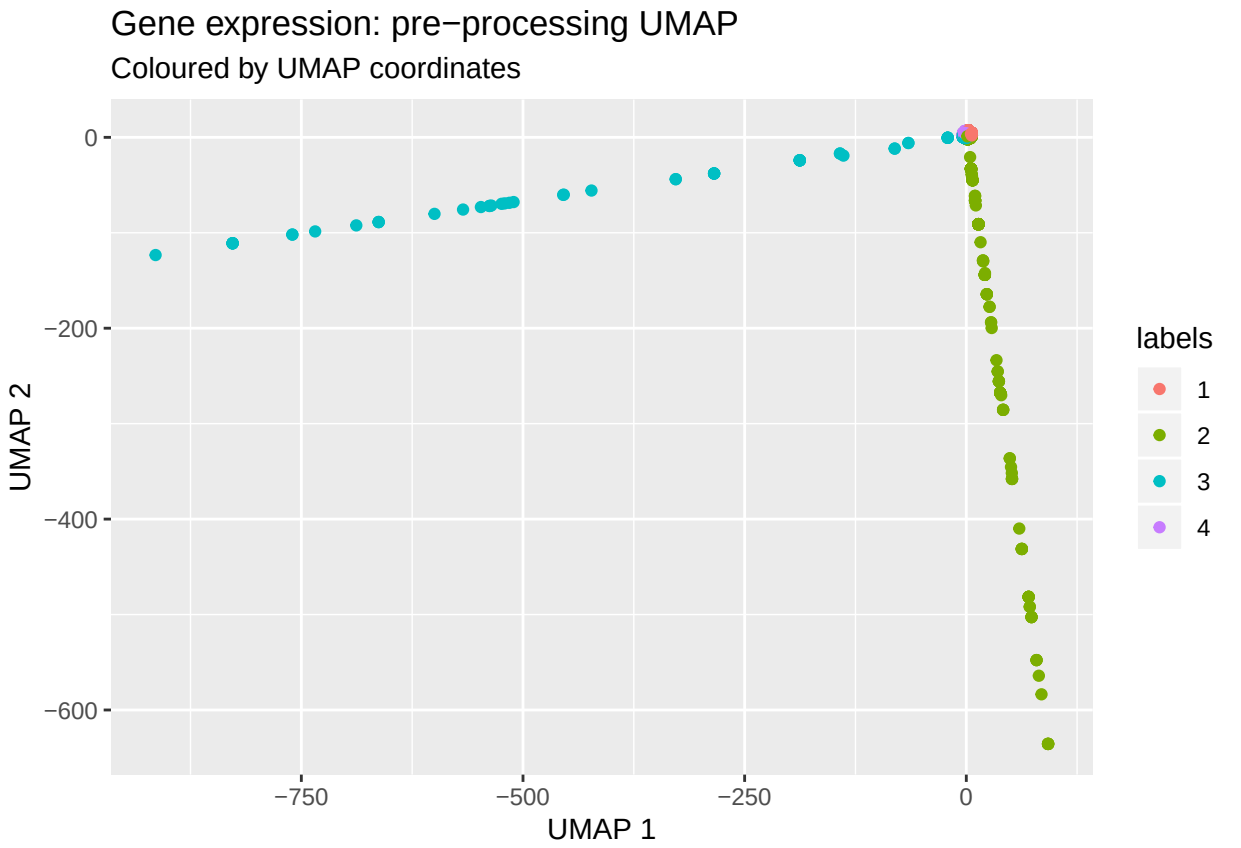



```
plotMarginals(processedExpression,
  main = "Gene expression: gene summary statistics (post-processing)"
)
```

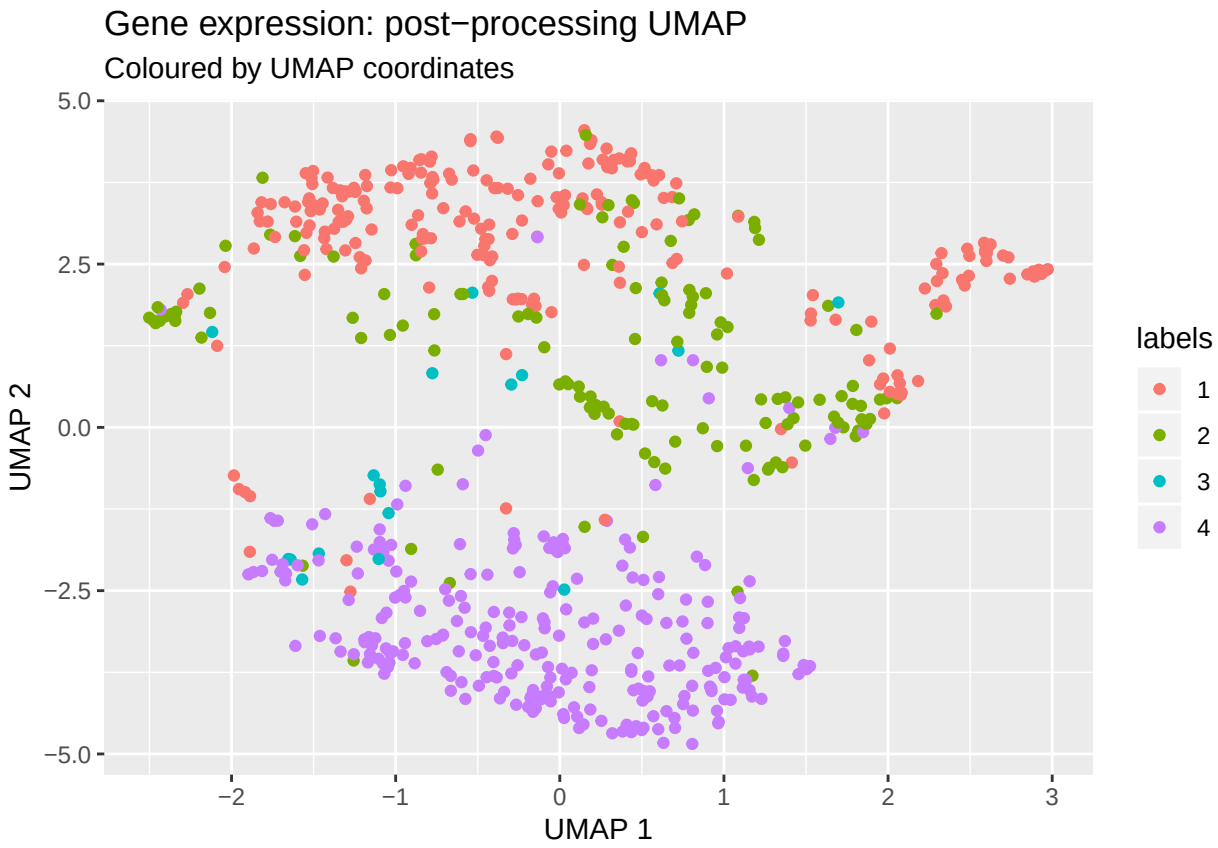


To inspect the local structure within the data we see how the data appears within a UMAP 2D representation:

```
# UMAP visualisation
ge_plt_data <- makeUMAPPlotData(ge_umap$layout, ge_labels)
plotUMAP(ge_plt_data) +
  labs(
    title = "Gene expression: pre-processing UMAP",
    subtitle = "Coloured by UMAP coordinates",
    x = "UMAP 1",
    y = "UMAP 2"
  )
```



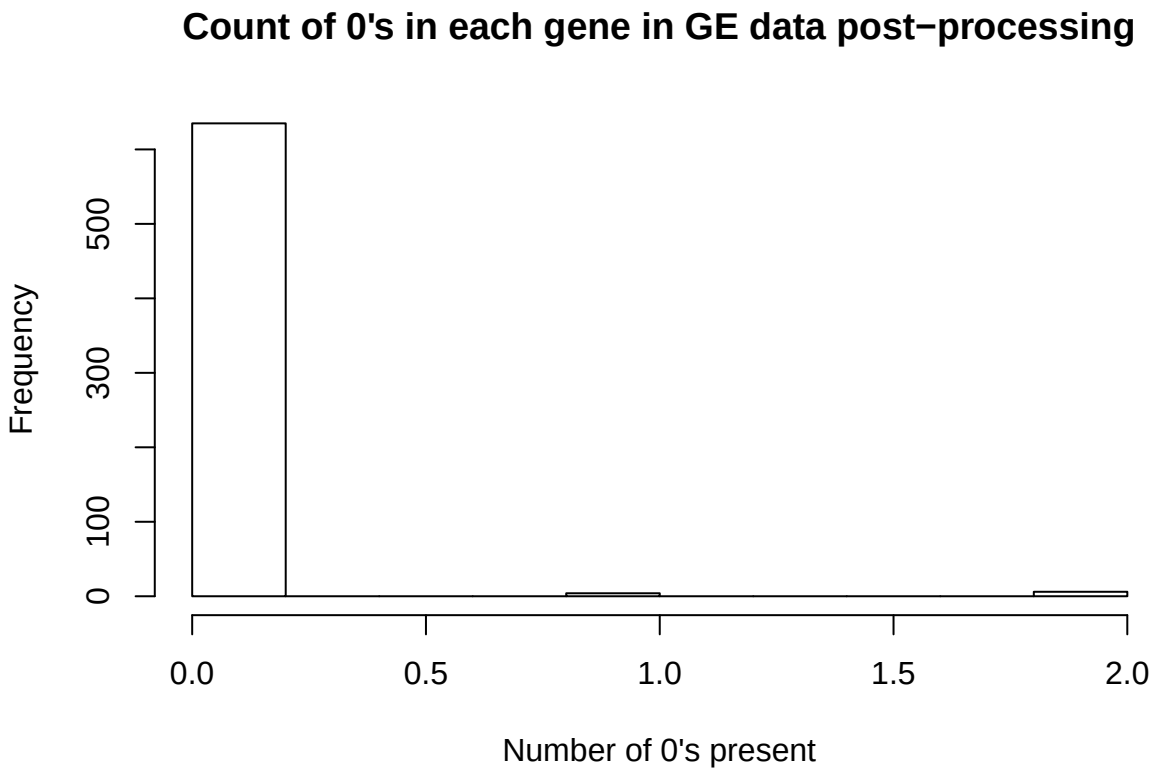

```
# Save UMAP plot
# ggsave(umap_file_names[1])

p_ge_umap <- umap(processedExpression)
p_ge_plt_data <- makeUMAPPlotData(p_ge_umap$layout, ge_labels[apply(Exp.mat, 1, sd) > 1.5])
plotUMAP(p_ge_plt_data) +
  labs(
    title = "Gene expression: post-processing UMAP",
    subtitle = "Coloured by UMAP coordinates",
    x = "UMAP 1",
    y = "UMAP 2"
  )
```



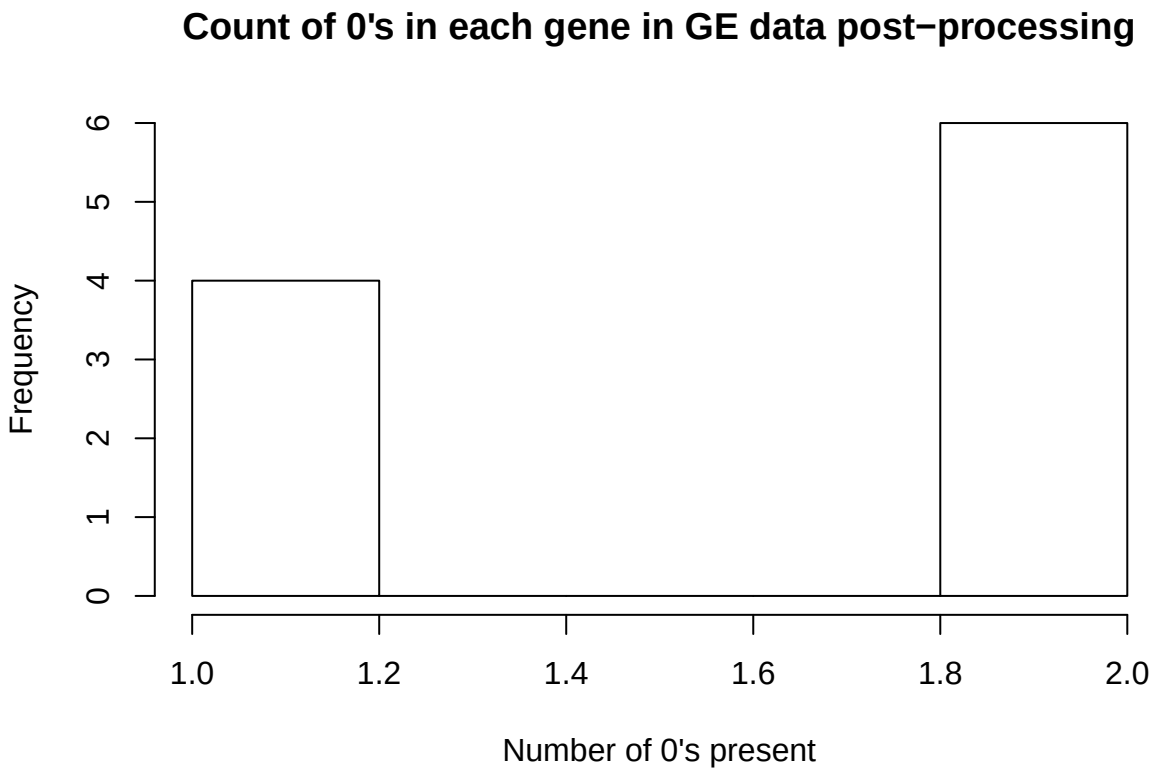

```
# ggsave("~/Documents/PhD/Year_1/Consensus_clustering/Analysis/BCC_TCGA_data/Data/gene_expression_umap_
```

We are also interested in the number of 0's present within the dataset:

```
ge_zeroness_post <- rowSums(processedExpression == 0)

ge_zeroness_post %>%
  hist(
    main = "Count of 0's in each gene in GE data post-processing",
    xlab = "Number of 0's present"
  )
```




```
ge_zeroness_post[ge_zeroness_post > 0] %>%
  hist(
    main = "Count of 0's in each gene in GE data post-processing",
    xlab = "Number of 0's present"
  )
```



Methylation

Processing step: Take the square root of all values.

```
# take square root of methylation data
processedMethylation <- sqrt(Meth.mat)
```

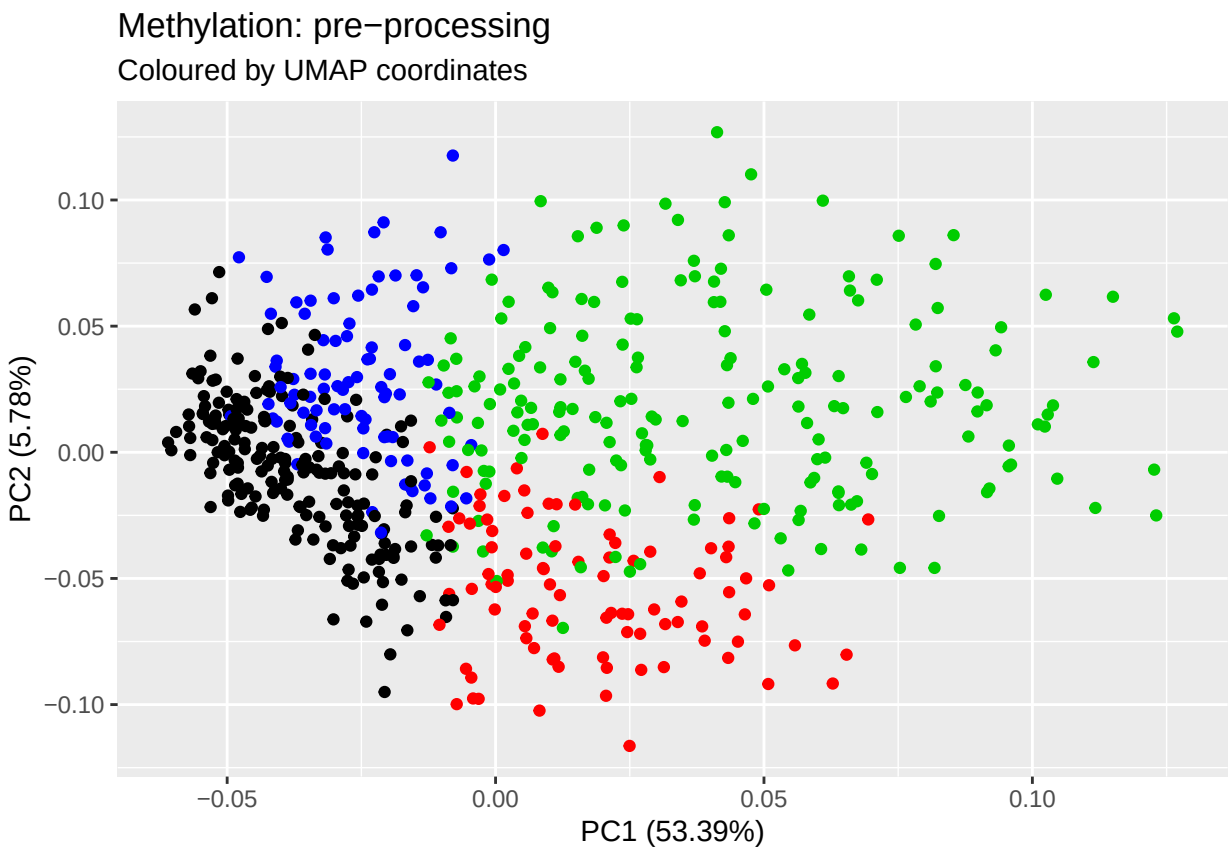
As previosuly we create a set of labels based upon the UMAP representation of the data to see how points move after the transform.

```
meth_umap <- umap(Meth.mat)
meth_labels <- makeUMAPLabels(meth_umap$layout)
```

We visualise the first two PCs of the data pre- and post-processing:

```
meth_pca <- prcomp(Meth.mat)
autoplot(meth_pca, data = Meth.mat, colour = meth_labels) +
  labs(
    title = "Methylation: pre-processing",
    subtitle = "Coloured by UMAP coordinates"
  )
```

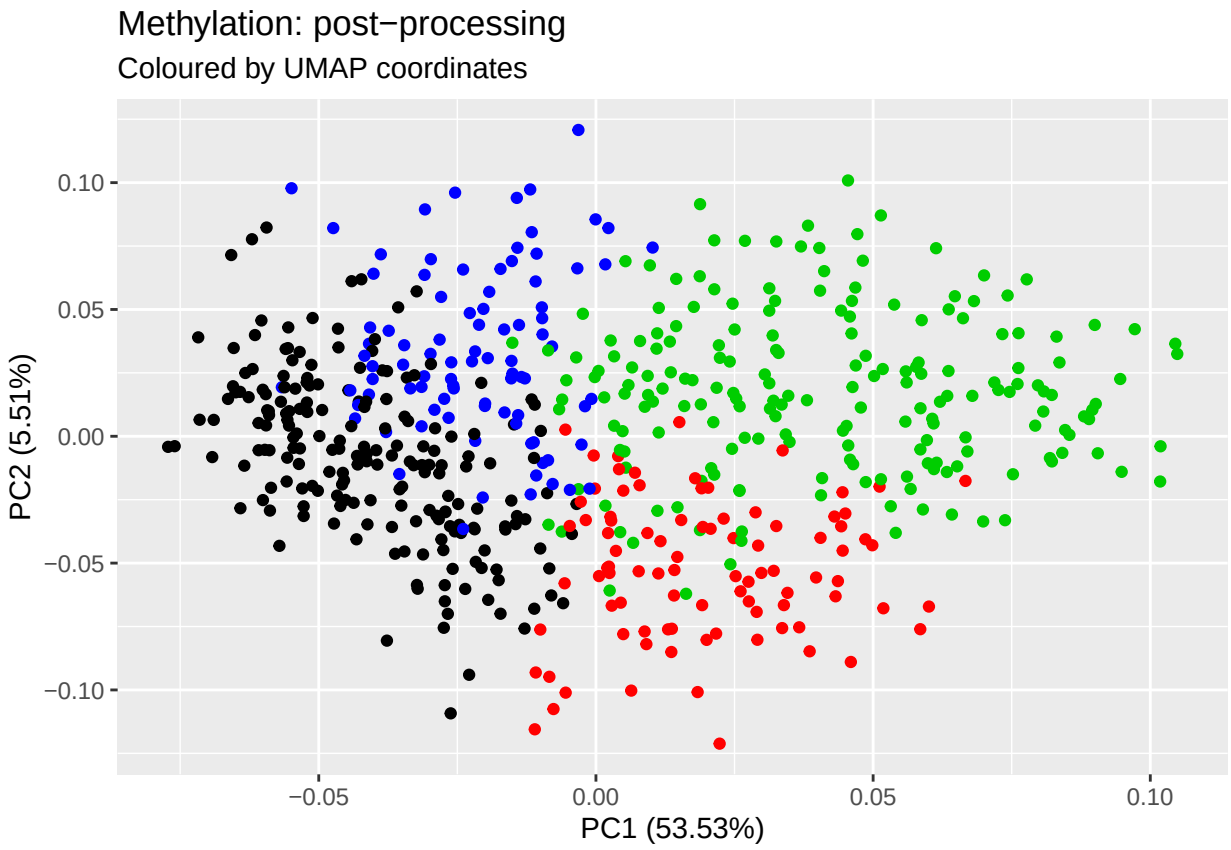
```
## Warning in if (value %in% columns) {: the condition has length > 1 and only the
## first element will be used
```




```
# ggsave(file_names[[1]][2])

p_meth_pca <- prcomp(processedMethylation)
autoplot(p_meth_pca, data = processedMethylation, colour = meth_labels) +
  labs(
```

```
    title = "Methylation: post-processing",
    subtitle = "Coloured by UMAP coordinates"
  )
```

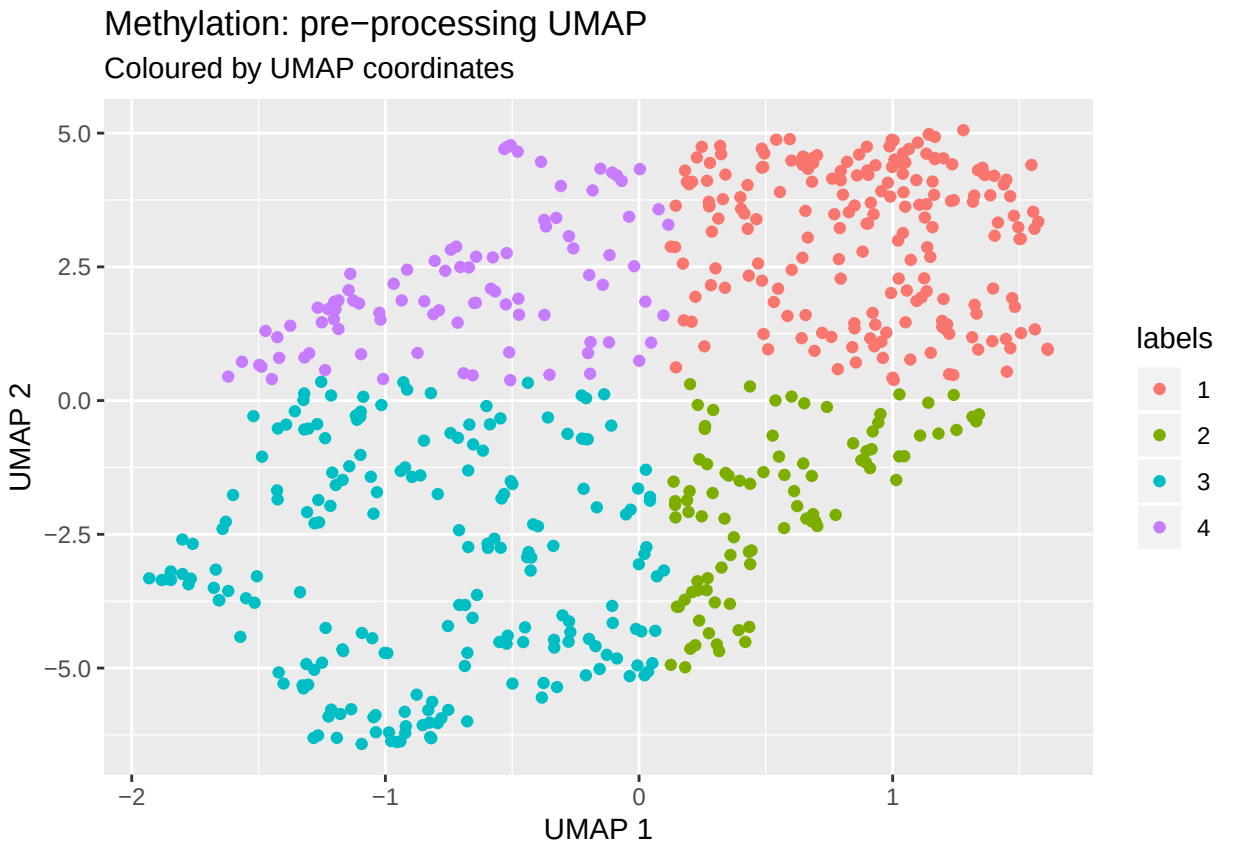
```
## Warning in if (value %in% columns) {: the condition has length > 1 and only the
## first element will be used
```



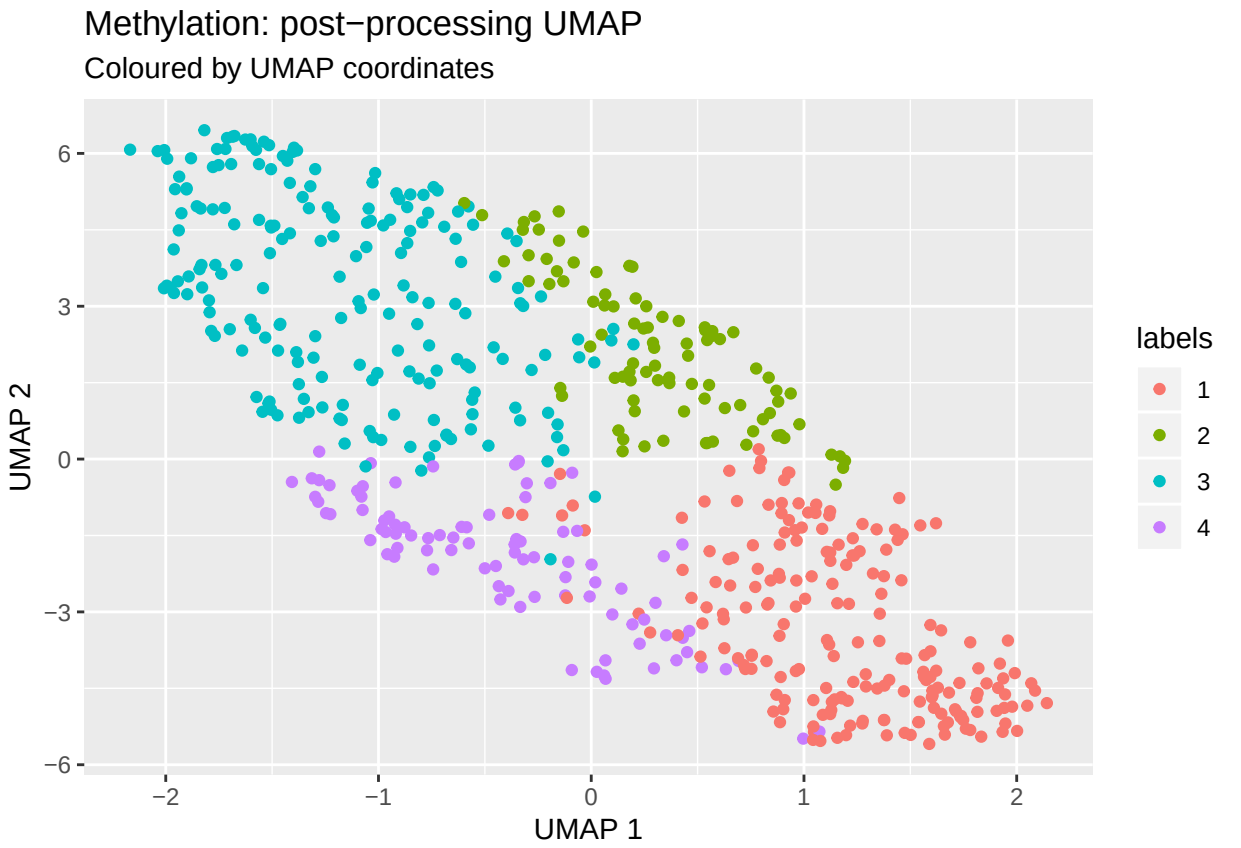

```
# ggsave(file_names[[2]][2])
```

We consider the impact upon local structure through the UMAP:

```
meth_plt_data <- makeUMAPPlotData(meth_umap$layout, meth_labels)
plotUMAP(meth_plt_data) +
  labs(
    title = "Methylation: pre-processing UMAP",
    subtitle = "Coloured by UMAP coordinates",
    x = "UMAP 1",
    y = "UMAP 2"
  )
```



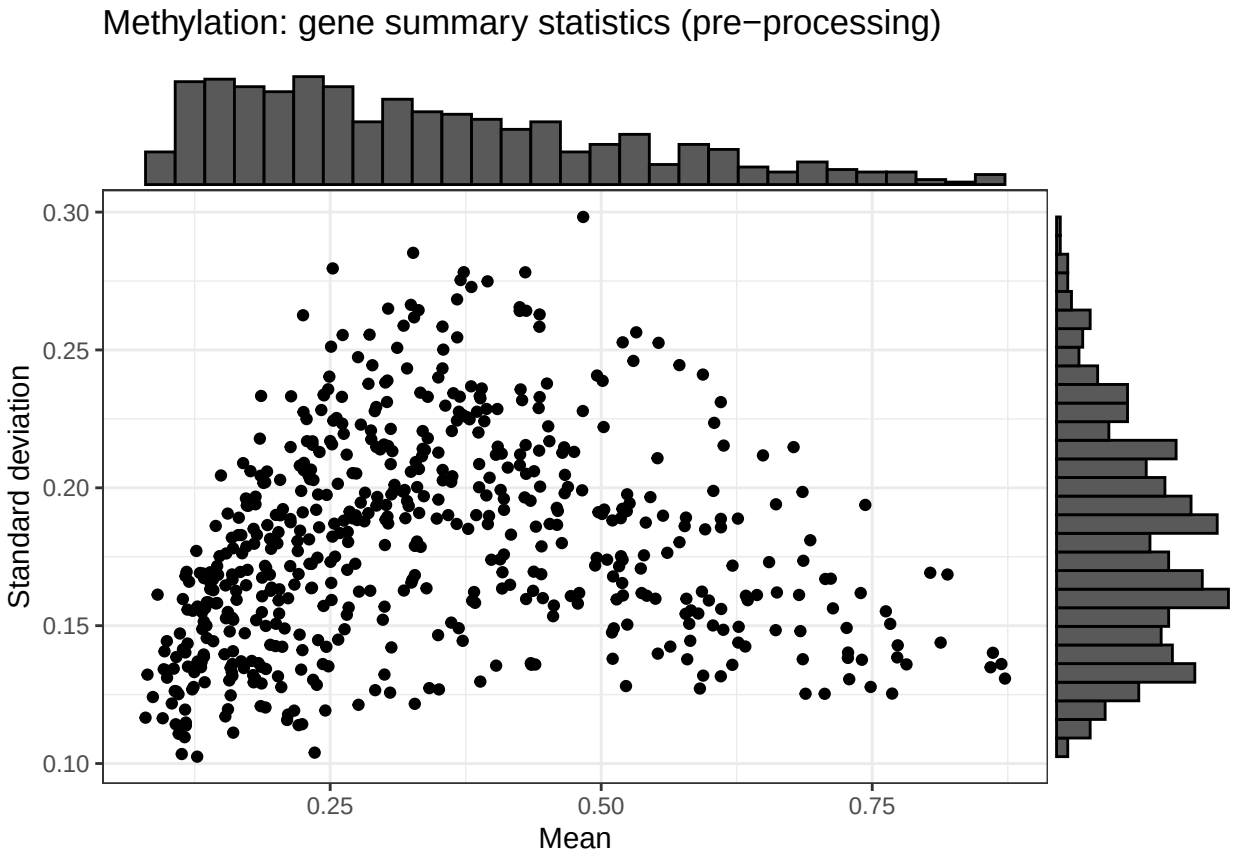

```
p_meth_umap <- umap(processedMethylation)
p_meth_plt_data <- makeUMAPPlotData(p_meth_umap$layout, meth_labels)
plotUMAP(p_meth_plt_data) +
  labs(
    title = "Methylation: post-processing UMAP",
    subtitle = "Coloured by UMAP coordinates",
    x = "UMAP 1",
    y = "UMAP 2"
  )
```



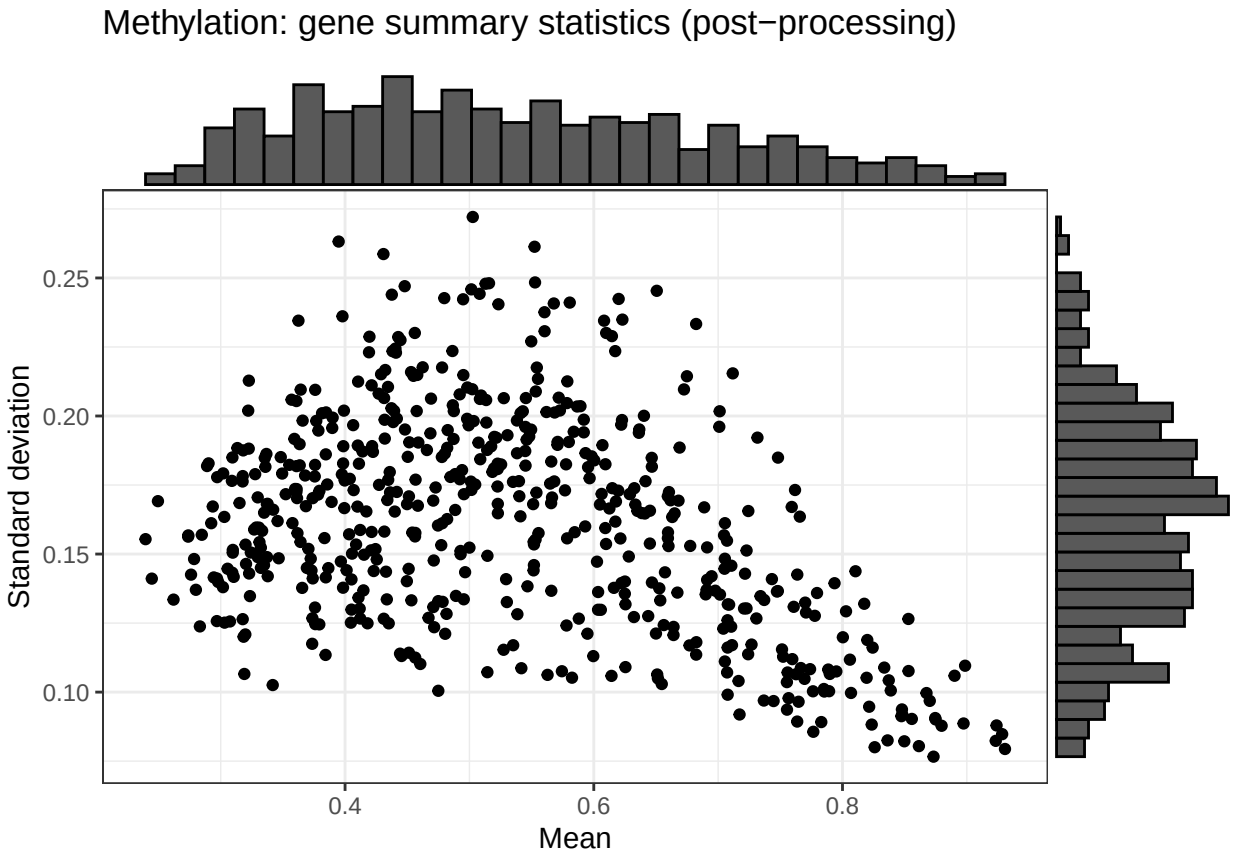

```
# ggsave(umap_file_names[2])
```

Again we are interested in the relationship between the standard deviation of each gene and the mean value:

```
# Plot summary statistics
plotMarginals(Meth.mat,
  main = "Methylation: gene summary statistics (pre-processing)"
)
```




```
plotMarginals(processedMethylation,
  main = "Methylation: gene summary statistics (post-processing)"
)
```



miRNA

Processing step: Filter by the number of 0 entries (any row with more than half of entries being 0 are removed); transform by $\log(x+1)$.

```
# Transform the data

miRNA_to_drop <- rowSums(miRNA.mat == 0) < 348 * 0.5
processedmiRNA <- log(1 + miRNA.mat[which(miRNA_to_drop), ]) ## take log of miRNA data

print(dim(miRNA.mat))
```

```
## [1] 1046  348
```

```
print(dim(processedmiRNA))
```

```
## [1] 423 348
```

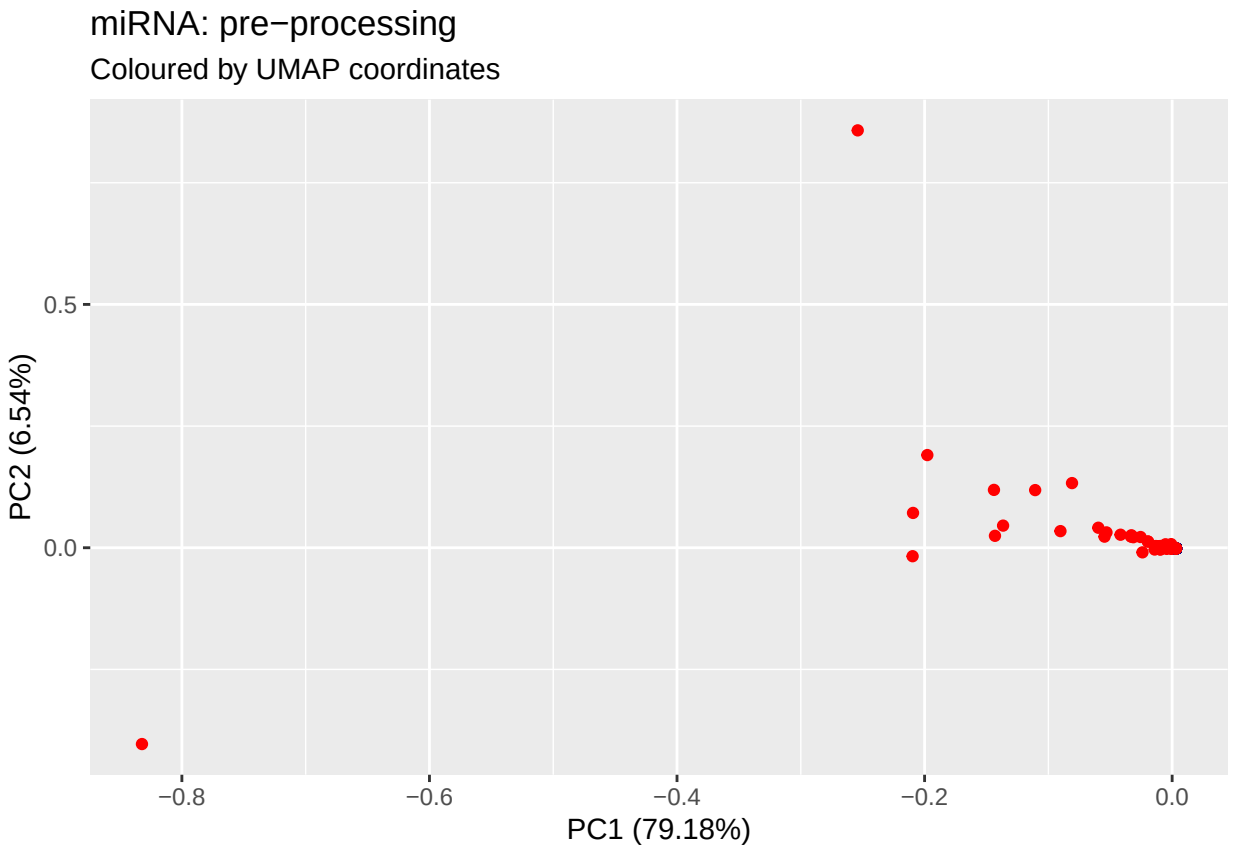
First define the UMAP labels to track the movement of points within the PCA and UMAP plots.

```
# Apply UMAP to see local structure (should not be strongly affected by the transforms)
miRNA_umap <- umap(miRNA.mat)

# Create labels to record approximate clustering from UMAP
miRNA_labels <- makeUMAPLabels(miRNA_umap$layout)
```

Then visualise the PCA:

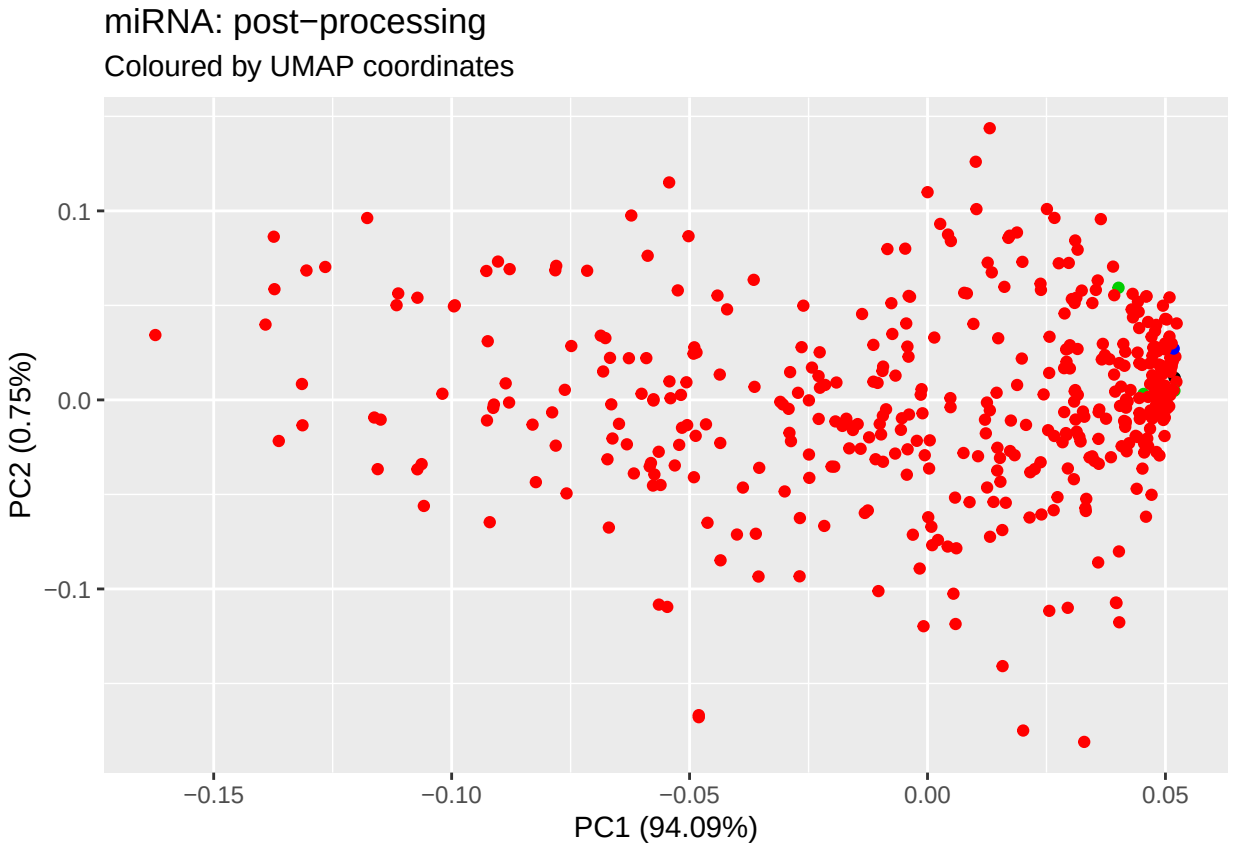
```
# Apply PCA for global structure
miRNA_pca <- prcomp(miRNA.mat)

autoplot(miRNA_pca, data = miRNA.mat, colour = miRNA_labels) + # , scale = 0) +
  labs(
    title = "miRNA: pre-processing",
    subtitle = "Coloured by UMAP coordinates"
  )
```

```
## Warning in if (value %in% columns) {: the condition has length > 1 and only the
## first element will be used
```



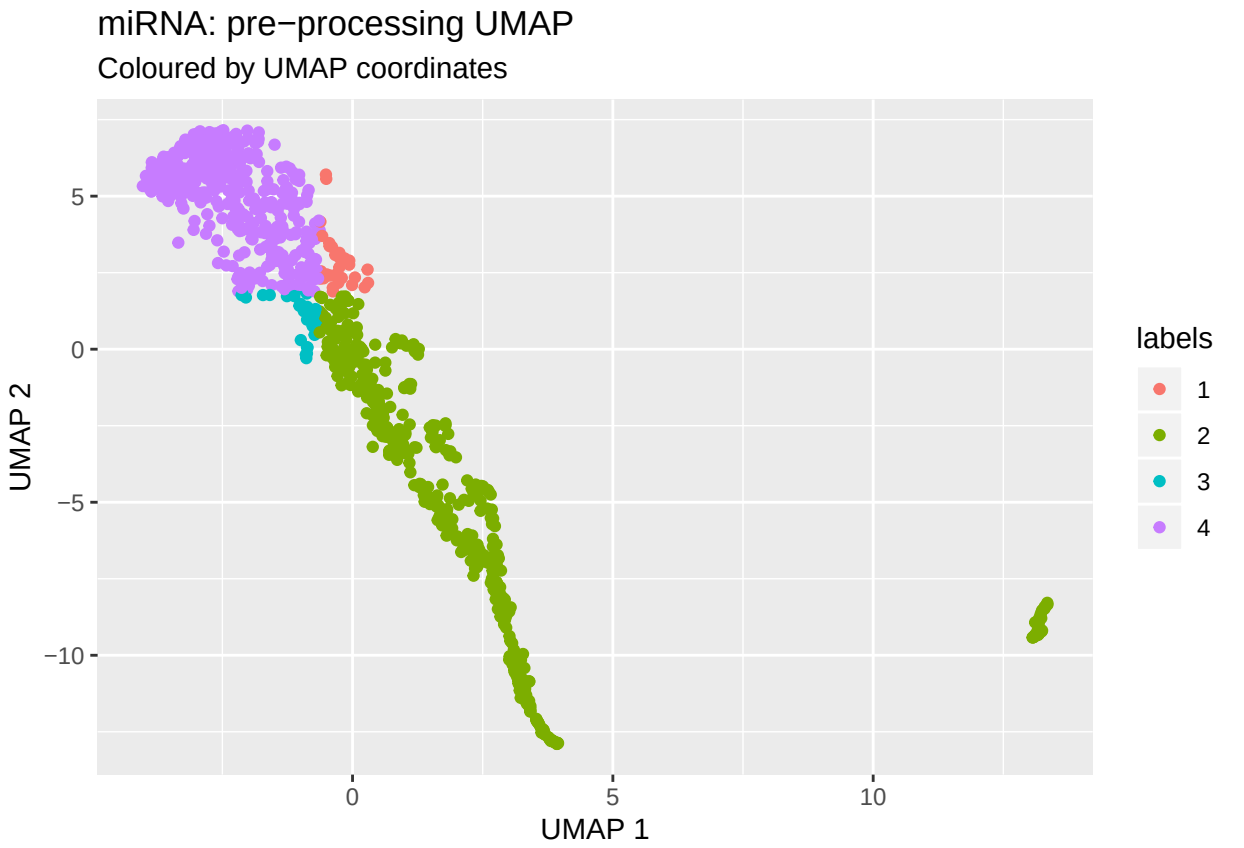

```
# Save the PCA plot with UMAP colouring
# ggsave(file_names[[1]][3])

# Take the PCA of the transformed data
p_miRNA_pca <- prcomp(processedmiRNA)

# Plot with the same labelling as previosuly applied
autoplot(p_miRNA_pca, data = processedmiRNA, colour = miRNA_labels[which(miRNA_to_drop)]) +
  labs(
    title = "miRNA: post-processing",
    subtitle = "Coloured by UMAP coordinates"
  )
```

```
## Warning in if (value %in% columns) {: the condition has length > 1 and only the
## first element will be used
```

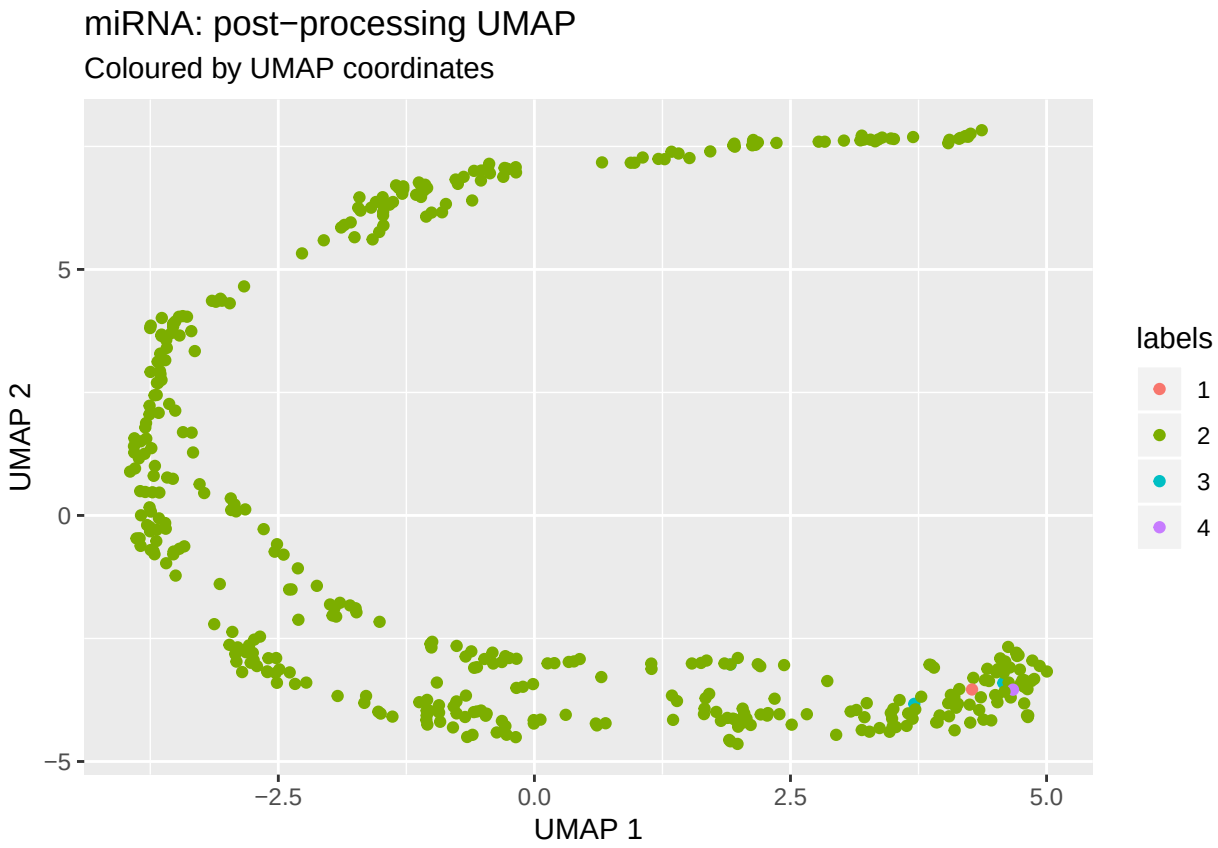



```
# ggsave(file_names[[2]][3])
```

```
# Create a data.frame of UMAP coordinates and labelling
miRNA_plt_data <- makeUMAPPlotData(miRNA_umap$layout, miRNA_labels)

# Plot
plotUMAP(miRNA_plt_data) +
  labs(
    title = "miRNA: pre-processing UMAP",
    subtitle = "Coloured by UMAP coordinates",
    x = "UMAP 1",
    y = "UMAP 2"
  )
```



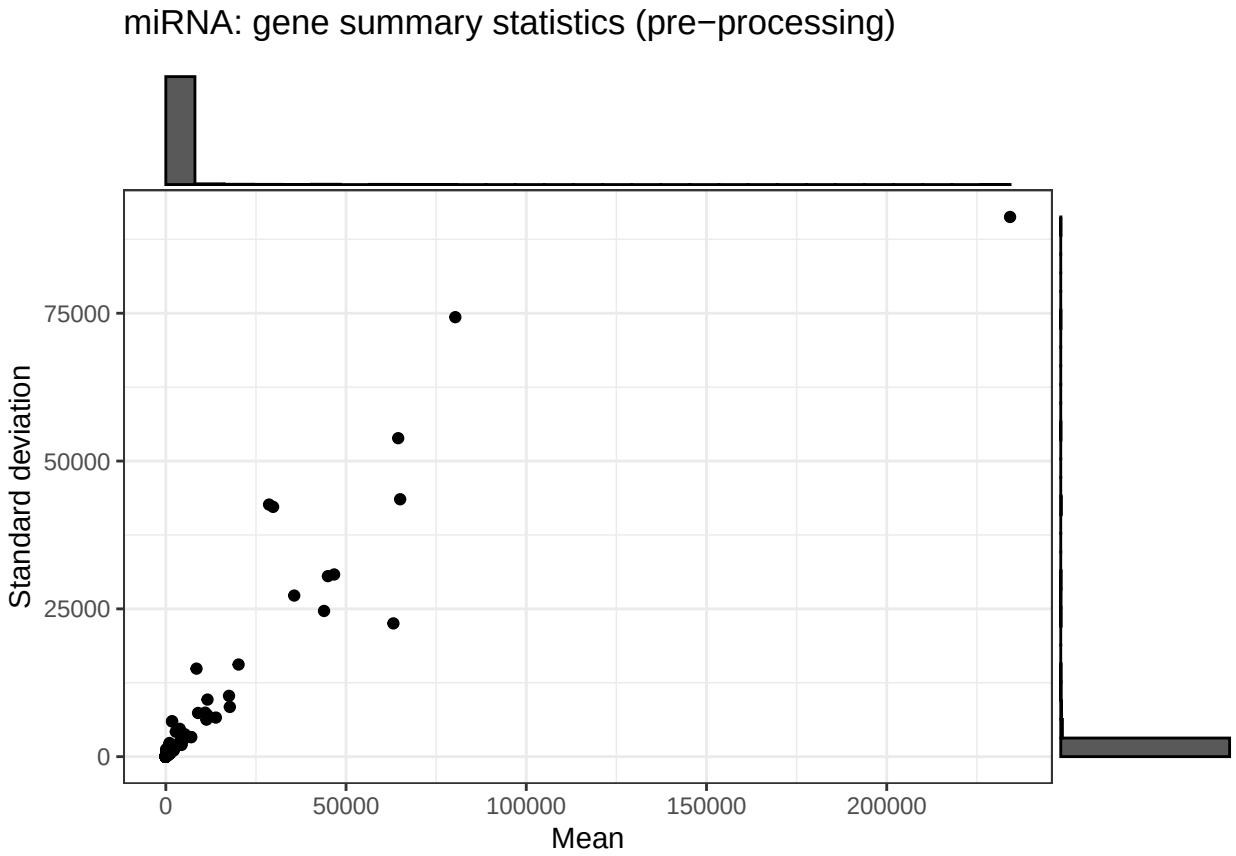

```
# ggsave(umap_file_names[3])

p_miRNA_umap <- umap(processedmiRNA)
p_miRNA_plt_data <- makeUMAPPlotData(p_miRNA_umap$layout, miRNA_labels[which(miRNA_to_drop)])
plotUMAP(p_miRNA_plt_data) +
  labs(
    title = "miRNA: post-processing UMAP",
    subtitle = "Coloured by UMAP coordinates",
    x = "UMAP 1",
    y = "UMAP 2"
  )
```



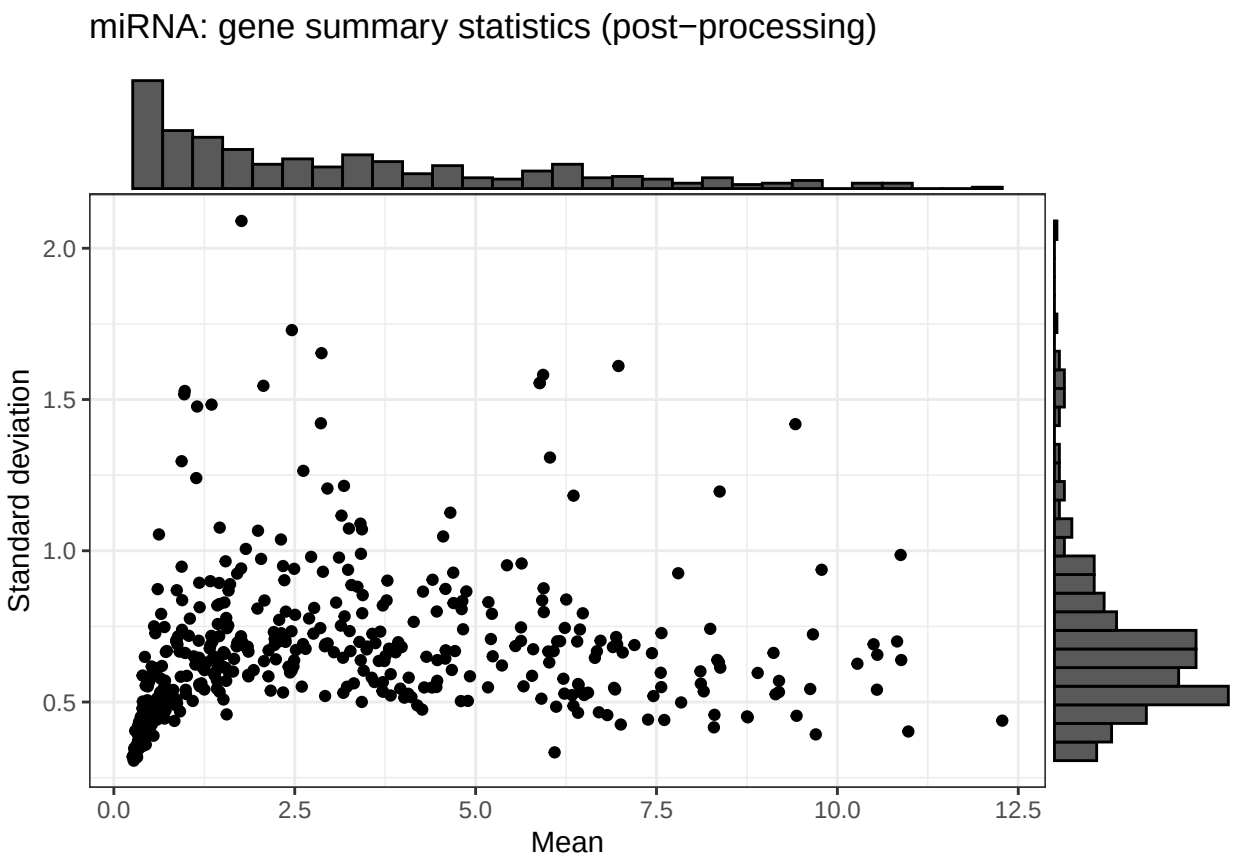

```
# ggsave("~/Documents/PhD/Year_1/Consensus_clustering/Analysis/BCC_TCGA_data/Data/miRNA_umap_post_proce
```

Visualise the relaitonship between the standard deviation and the mean of each gene:

```
# Plot summary statistics
plotMarginals(miRNA.mat,
  main = "miRNA: gene summary statistics (pre-processing)"
)
```




```
plotMarginals(processedmiRNA,
  main = "miRNA: gene summary statistics (post-processing)"
)
```



Protein

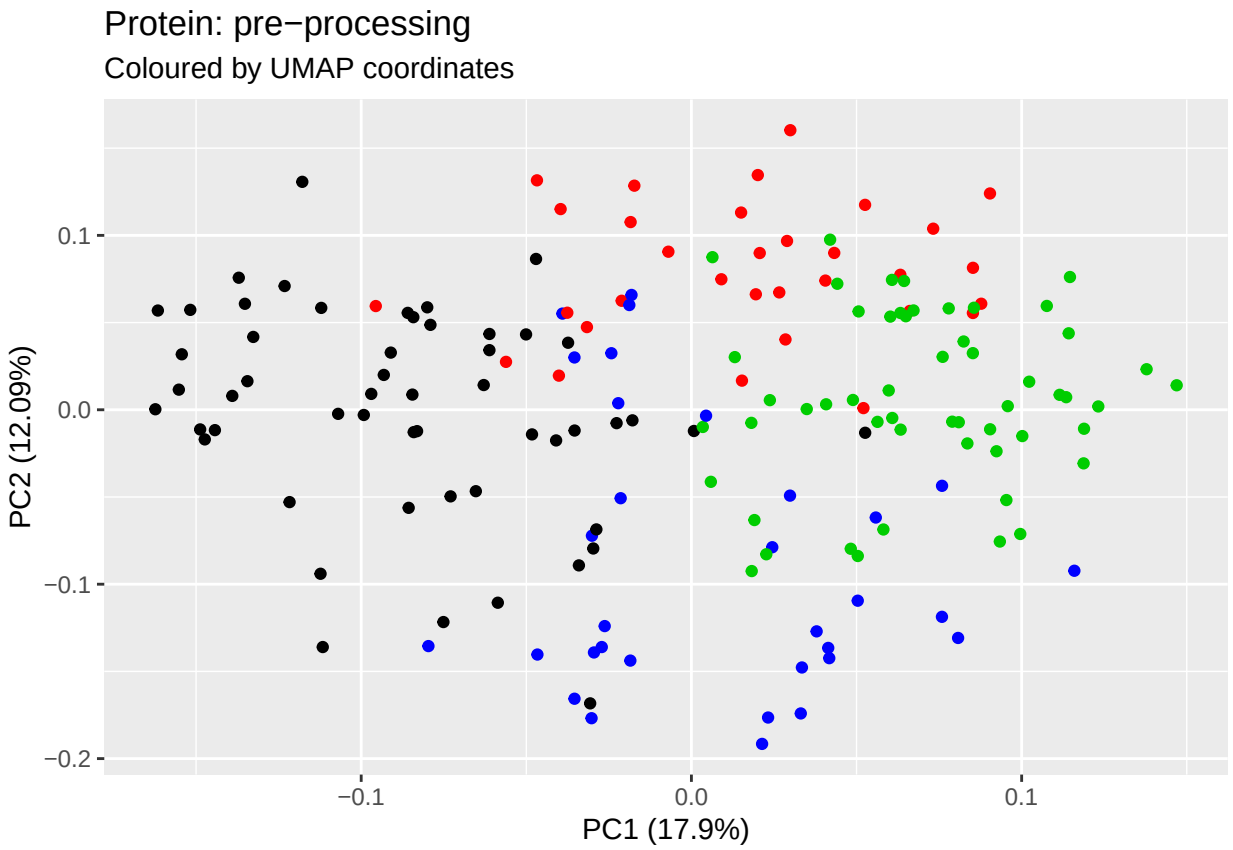
Processing step: scale data by mean centreing and dividing by the standard deviation.

```
processedProtein <- scale(Protein.mat, center = TRUE, scale = TRUE) # Column center/scale protein
```

```
protein_umap <- umap(Protein.mat)
protein_labels <- makeUMAPLabels(protein_umap$layout)
```

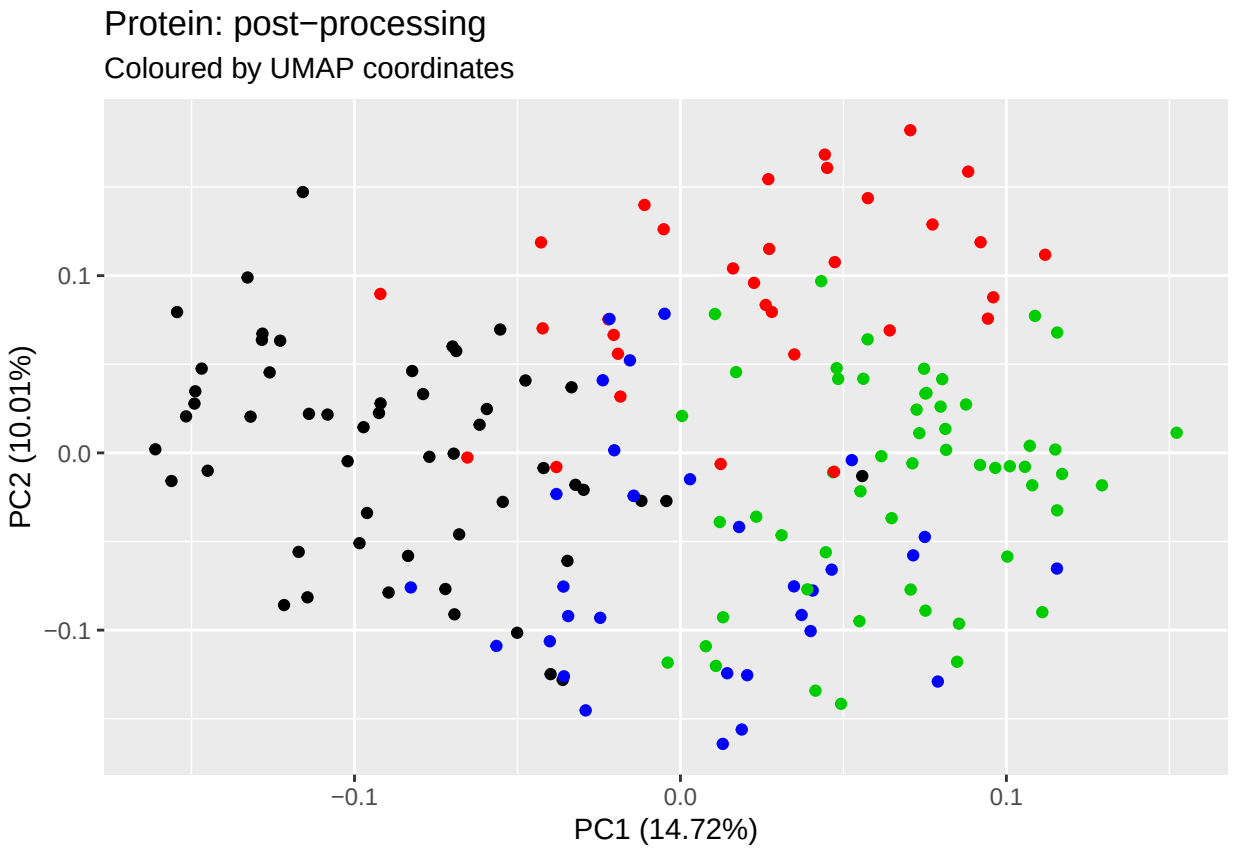
```
protein_pca <- prcomp(Protein.mat)
autoplot(protein_pca, data = Protein.mat, colour = protein_labels) +
  labs(
    title = "Protein: pre-processing",
    subtitle = "Coloured by UMAP coordinates"
  )
```

```
## Warning in if (value %in% columns) {: the condition has length > 1 and only the
## first element will be used
```



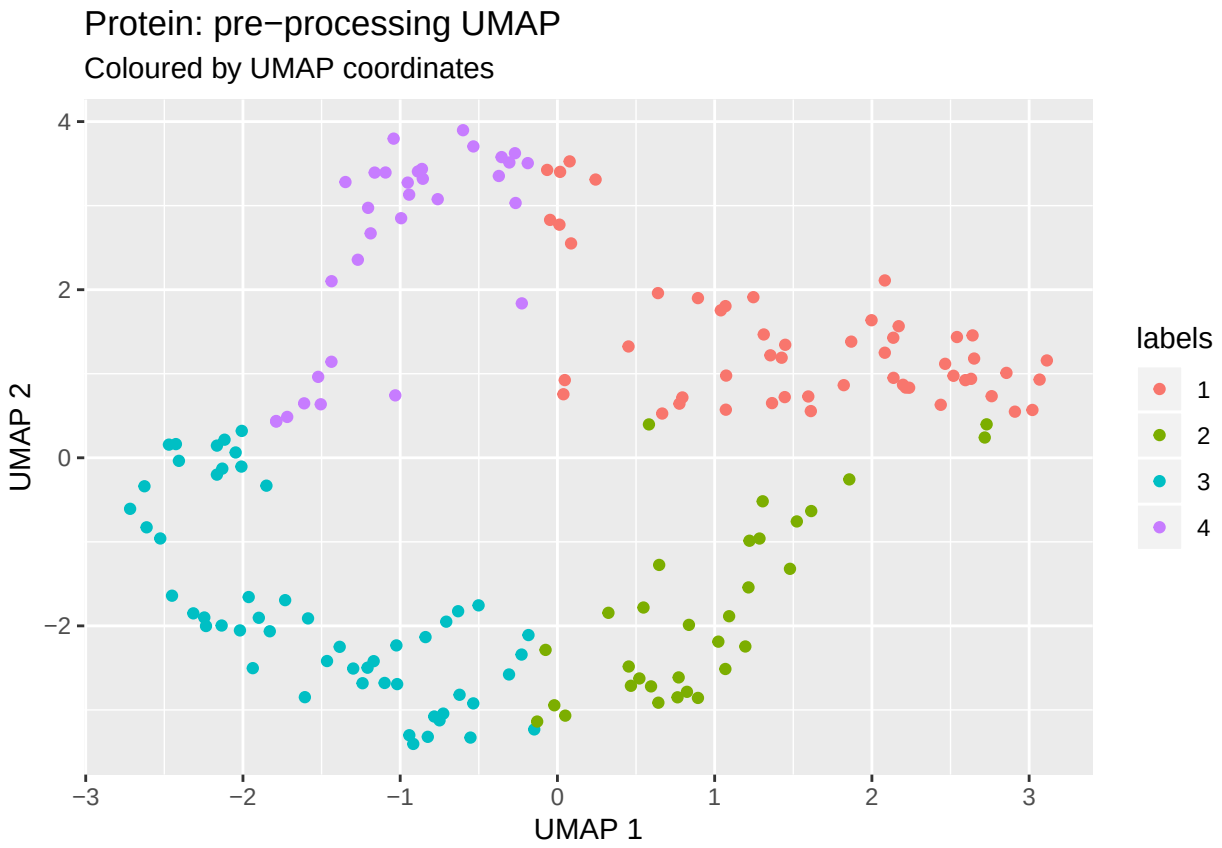

```
# ggsave(file_names[[1]][4])

p_protein_pca <- prcomp(processedProtein)
autoplot(p_protein_pca, data = processedProtein, colour = protein_labels) +
  labs(
    title = "Protein: post-processing",
    subtitle = "Coloured by UMAP coordinates"
  )
```

```
## Warning in if (value %in% columns) {: the condition has length > 1 and only the
## first element will be used
```

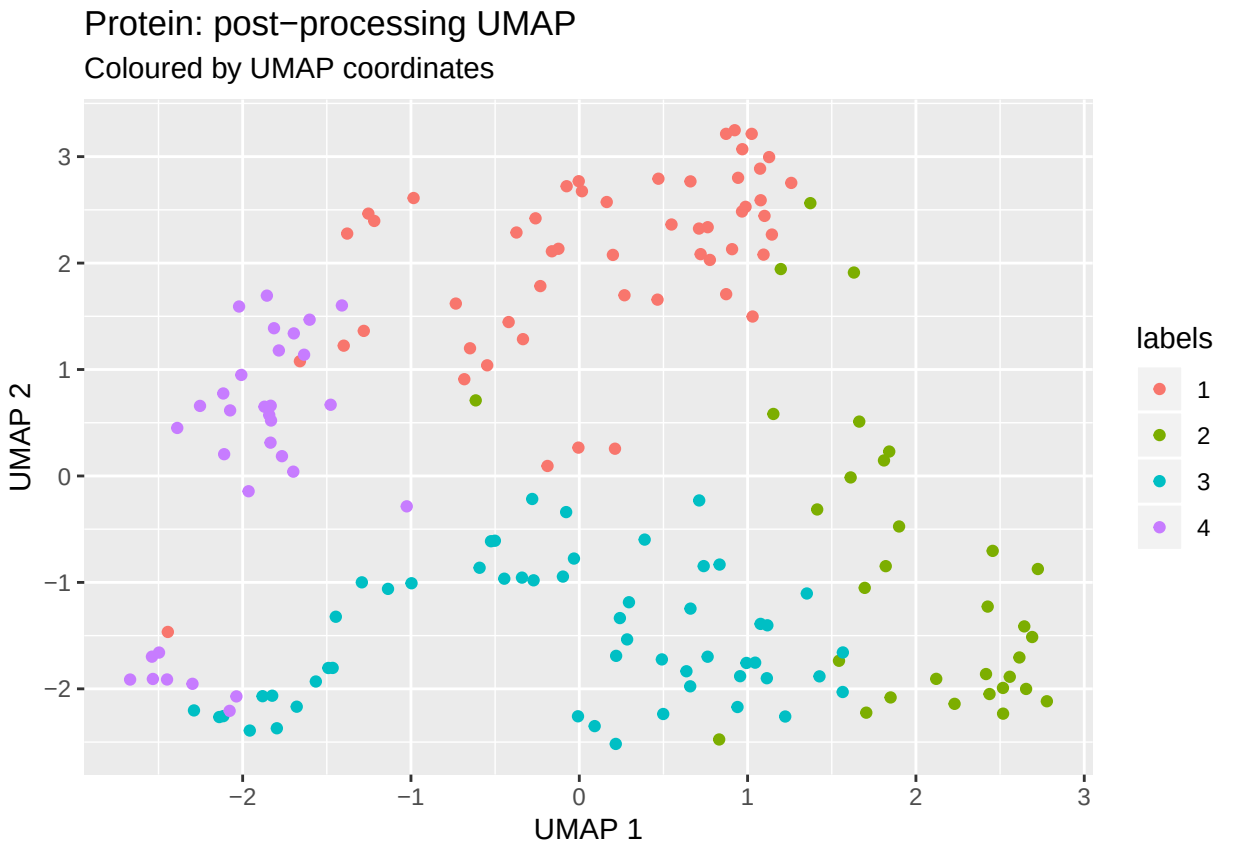



```
# ggsave(file_names[[2]][4])
```

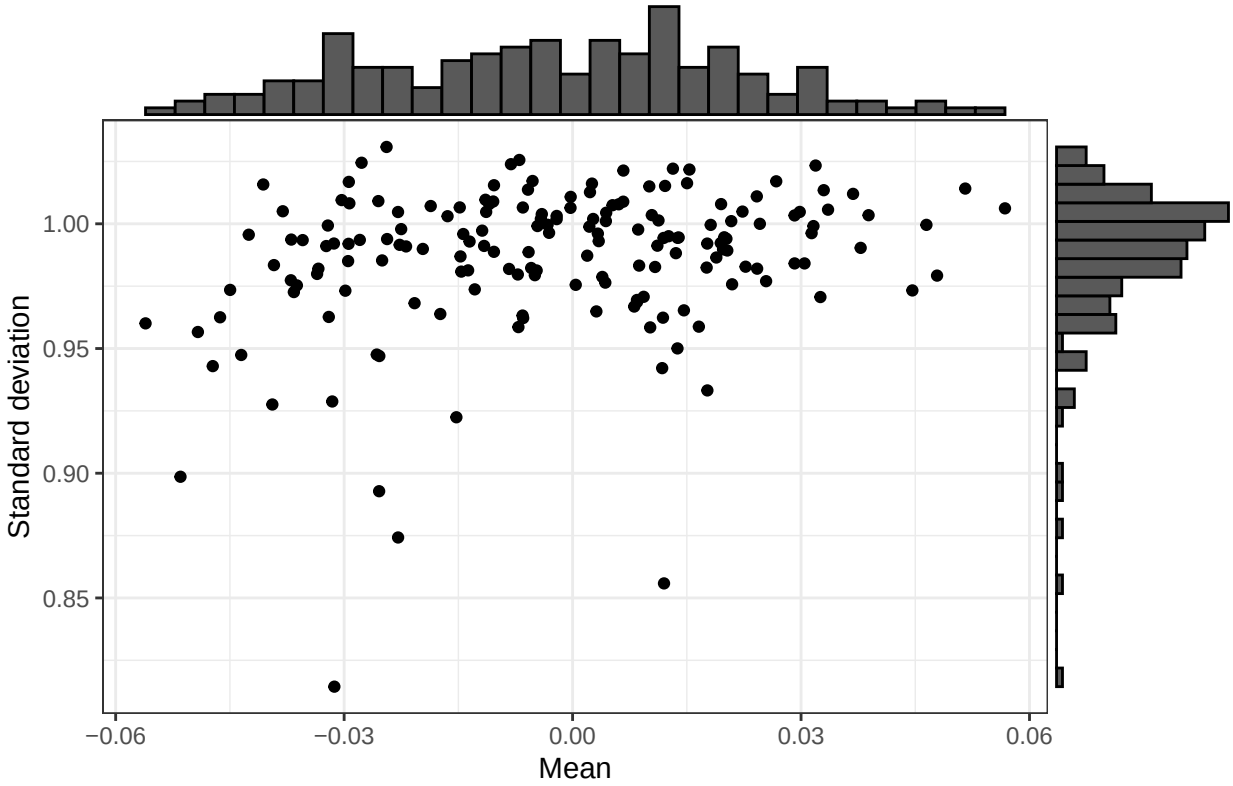
```
protein_plt_data <- makeUMAPPlotData(protein_umap$layout, protein_labels)
plotUMAP(protein_plt_data) +
  labs(
    title = "Protein: pre-processing UMAP",
    subtitle = "Coloured by UMAP coordinates",
    x = "UMAP 1",
    y = "UMAP 2"
  )
```



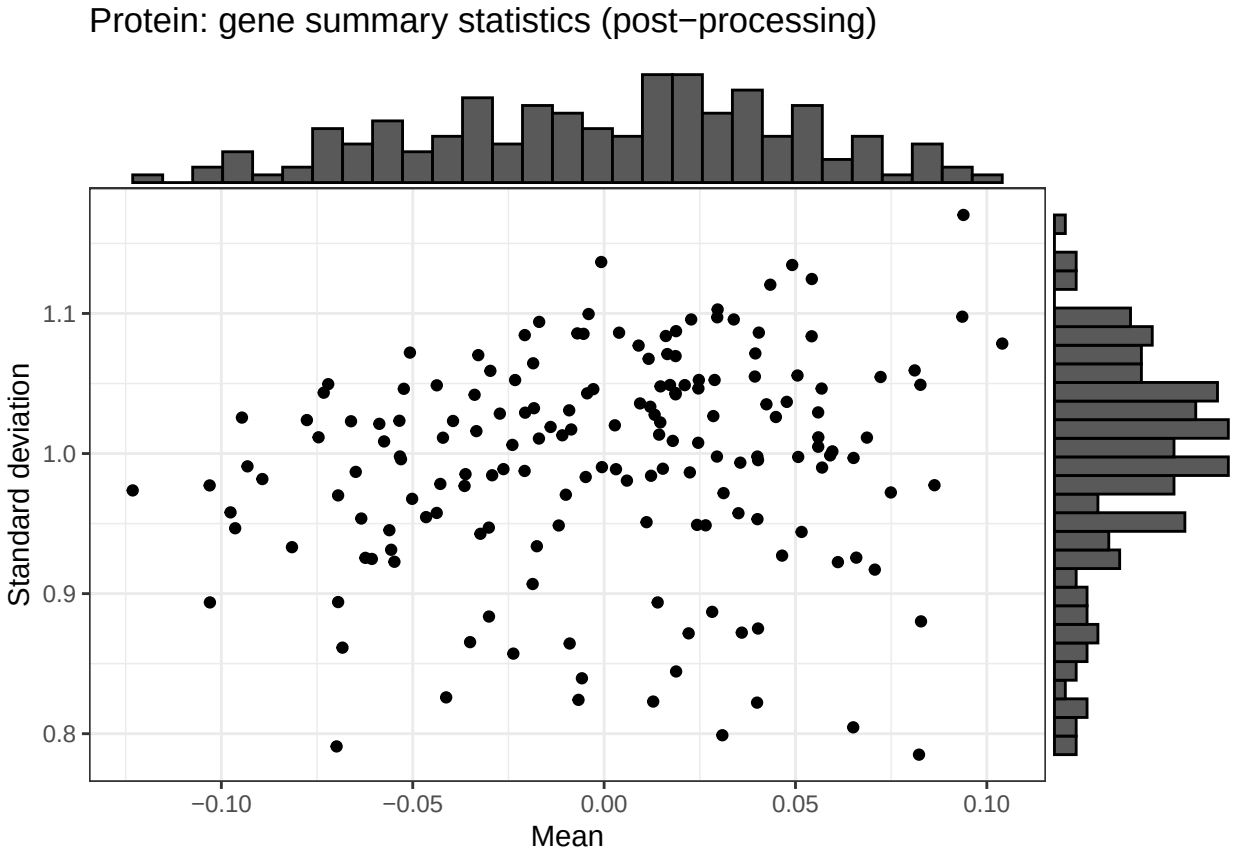

```
# ggsave(umap_file_names[4])

p_protein_umap <- umap(processedProtein)
p_protein_plt_data <- makeUMAPPlotData(p_protein_umap$layout, protein_labels)
plotUMAP(p_protein_plt_data) +
  labs(
    title = "Protein: post-processing UMAP",
    subtitle = "Coloured by UMAP coordinates",
    x = "UMAP 1",
    y = "UMAP 2"
  )
```




```
# Plot summary statistics
plotMarginals(Protein.mat,
  main = "Protein: gene summary statistics (pre-processing)"
)
```




```
plotMarginals(processedProtein,
  main = "Protein: gene summary statistics (post-processing)"
)
```


Protein: gene summary statistics (post−processing)
Standard deviation
1.1
1.0
0.9
0.8
−0.10
−0.05
0.00
0.05
0.10
Mean