notebook

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[ ]:

```
library(GEOquery)
library(limma)
library(umap)
library(pheatmap)
library(gplots)
library(ggplot2)
library(reshape2)
library(plyr)
library(repr)
library(gridExtra)
library(ggpubr)
library(Rtsne)
library(MASS)
```

```
Loading required package: Biobase

Loading required package: BiocGenerics


Attaching package: ‘BiocGenerics’


The following objects are masked from ‘package:stats’:

    IQR, mad, sd, var, xtabs


The following objects are masked from ‘package:base’:

    Filter, Find, Map, Position, Reduce, anyDuplicated, append,
    as.data.frame, basename, cbind, colnames, dirname, do.call,
    duplicated, eval, evalq, get, grep, grepl, intersect, is.unsorted,
    lapply, mapply, match, mget, order, paste, pmax, pmax.int, pmin,
    pmin.int, rank, rbind, rownames, sapply, setdiff, sort, table,
    tapply, union, unique, unsplit, which.max, which.min


Welcome to Bioconductor

    Vignettes contain introductory material; view with
    'browseVignettes()'. To cite Bioconductor, see
    'citation("Biobase")', and for packages 'citation("pkgname")'.


Setting options('download.file.method.GEOquery'='auto')

Setting options('GEOquery.inmemory.gpl'=FALSE)


Attaching package: ‘limma’


The following object is masked from ‘package:BiocGenerics’:

    plotMA



Attaching package: ‘gplots’


The following object is masked from ‘package:stats’:

    lowess



Attaching package: ‘gridExtra’


The following object is masked from ‘package:Biobase’:

    combine


The following object is masked from ‘package:BiocGenerics’:

    combine
```

```
Attaching package: 'ggpubr'


The following object is masked from 'package:plyr':

    mutate
```

```
[ ]: gset <- getGEO("GSE48558", GSEMatrix =TRUE, getGPL=T, destdir='../Data/')
     gset <- gset[[1]]
```

```
Found 1 file(s)

GSE48558_series_matrix.txt.gz

Using locally cached version: ../Data//GSE48558_series_matrix.txt.gz

Using locally cached version of GPL6244 found here:
../Data//GPL6244.soft.gz
```

```
[ ]: gsms <- paste0("0000000000000XXXXXXXXXXXXXXXXXXXXXXXXXXX1XXX1XXXXX",
                    "XXXXXXXXXXXXXXXXXX2X3XXX1X1442X3XX33XX33X2X3X2X3X5",
                    "XXX5XXX5XXXXXXXXXXXXXXXXXXXXXXXXXXXXX1111111003000",
                    "22222223444413333333")
     sml <- strsplit(gsms, split="")[[1]]
     sel <- which(sml != "X")
     sml <- sml[sel]
     gset <- gset[ ,sel]
```

```
[ ]: gs <- factor(sml)
     groups <- make.names(c("AML","Granulocytes","B Cells","T␣
       ↪Cells","Monocytes","CD34"))
     levels(gs) <- groups
     gset$group <- gs
```

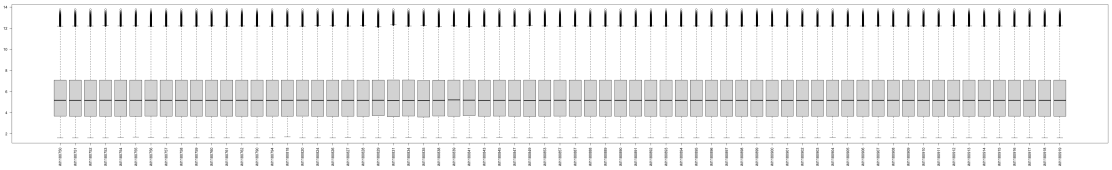
```
[ ]: ex <- exprs(gset)
     print(min(ex))
     print(max(ex))
```

```
[1] 1.611473
[1] 13.76154
```

Question 2)

According to the plot min value is 1.6 and max value is 13.76, so they are logarithmic already and data is normalized so normalization is not necessary.

[ ]:
```
options(repr.plot.width=50, repr.plot.height=8)
boxplot(ex, las = 2)
```



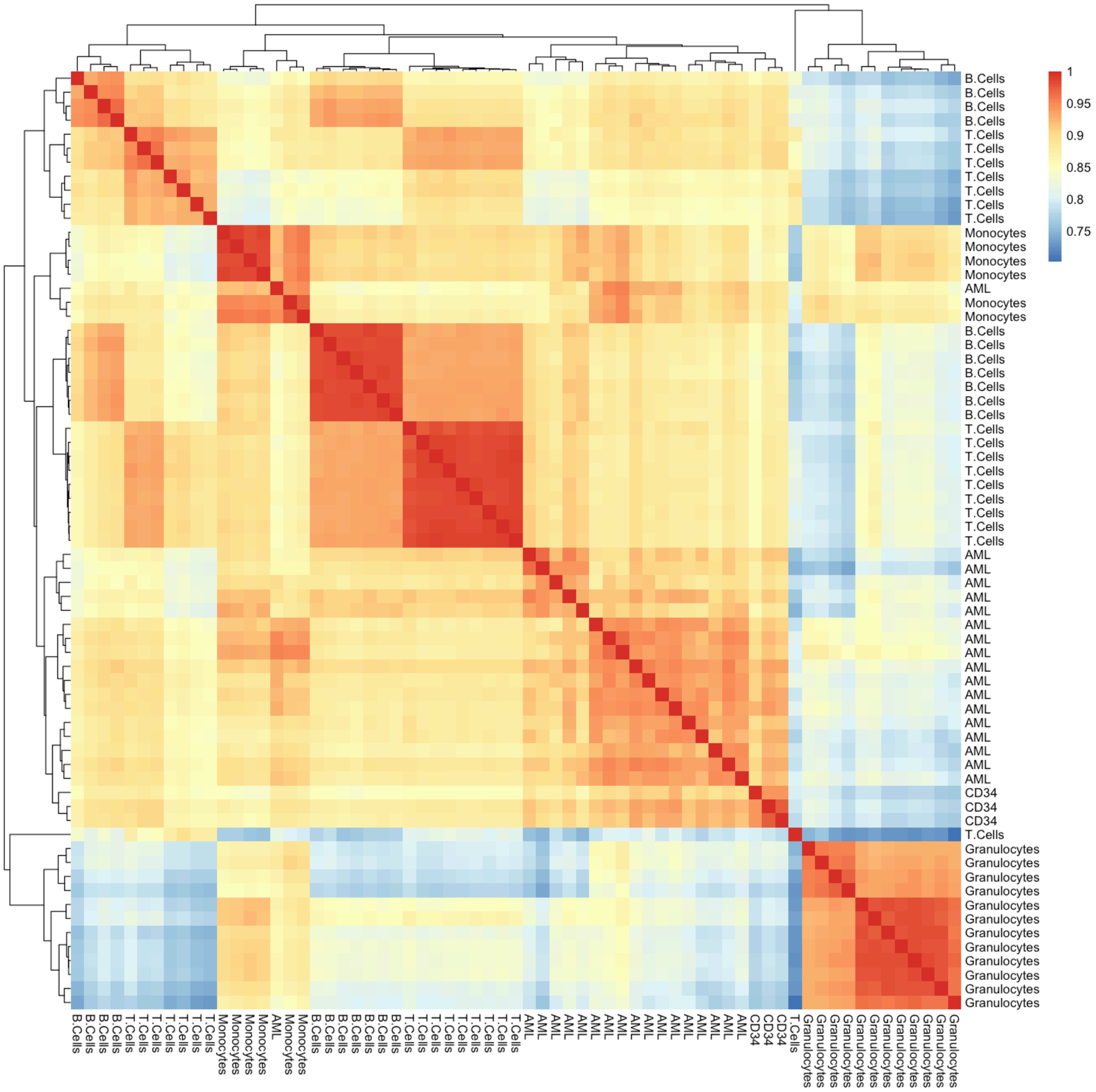
Question 4)

Heat map shows correlations between different samples, for example each sample has high corealation with itself that is determined with red color or granulocytes have low corelation between B-cells and T-celss that is determined with blue

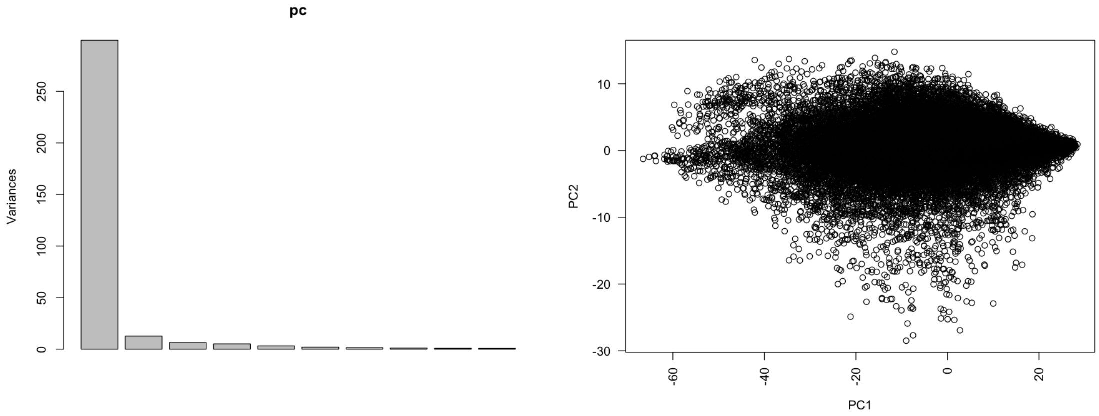
According to the heatmap AML has high corolation with CD34,Monocytes

what is neccesecity?

[ ]:
```
options(repr.plot.width=12, repr.plot.height=12)
pheatmap(cor(ex),
         labels_row = gs,
         labels_col = gs,
         border_color = NA,)
```

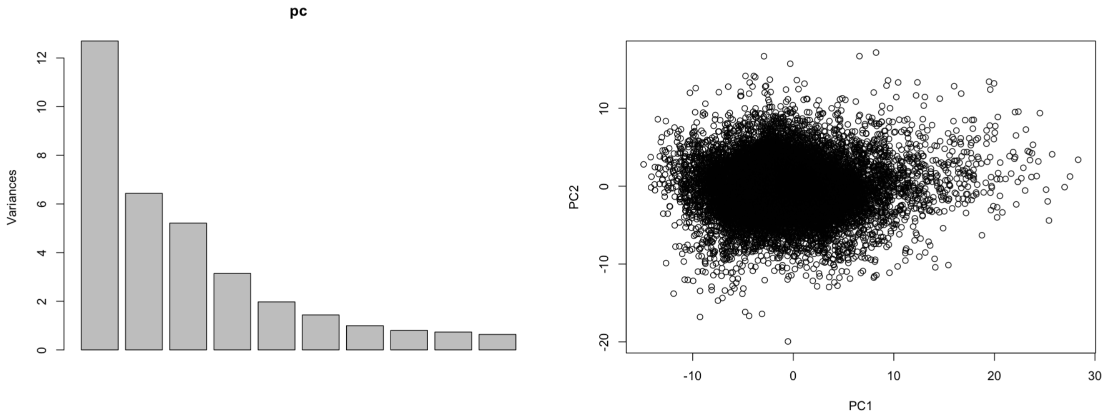


```
[ ]: pc <- prcomp(ex)
     options(repr.plot.width=15, repr.plot.height=6)
     par(mfrow = c(1, 2))
     p1 <- plot(pc)
     p2 <- plot(pc$x[, 1:2], las = 2)
```



```
[ ]: ex.scale <- t(scale(t(ex), scale = F))
     pc <- prcomp(ex.scale)

     options(repr.plot.width=15, repr.plot.height=6)
     par(mfrow = c(1, 2))
     plot(pc)
     plot(pc$x[, 1:2]) # x column are genes (so each point in this plot represents a gene)
```



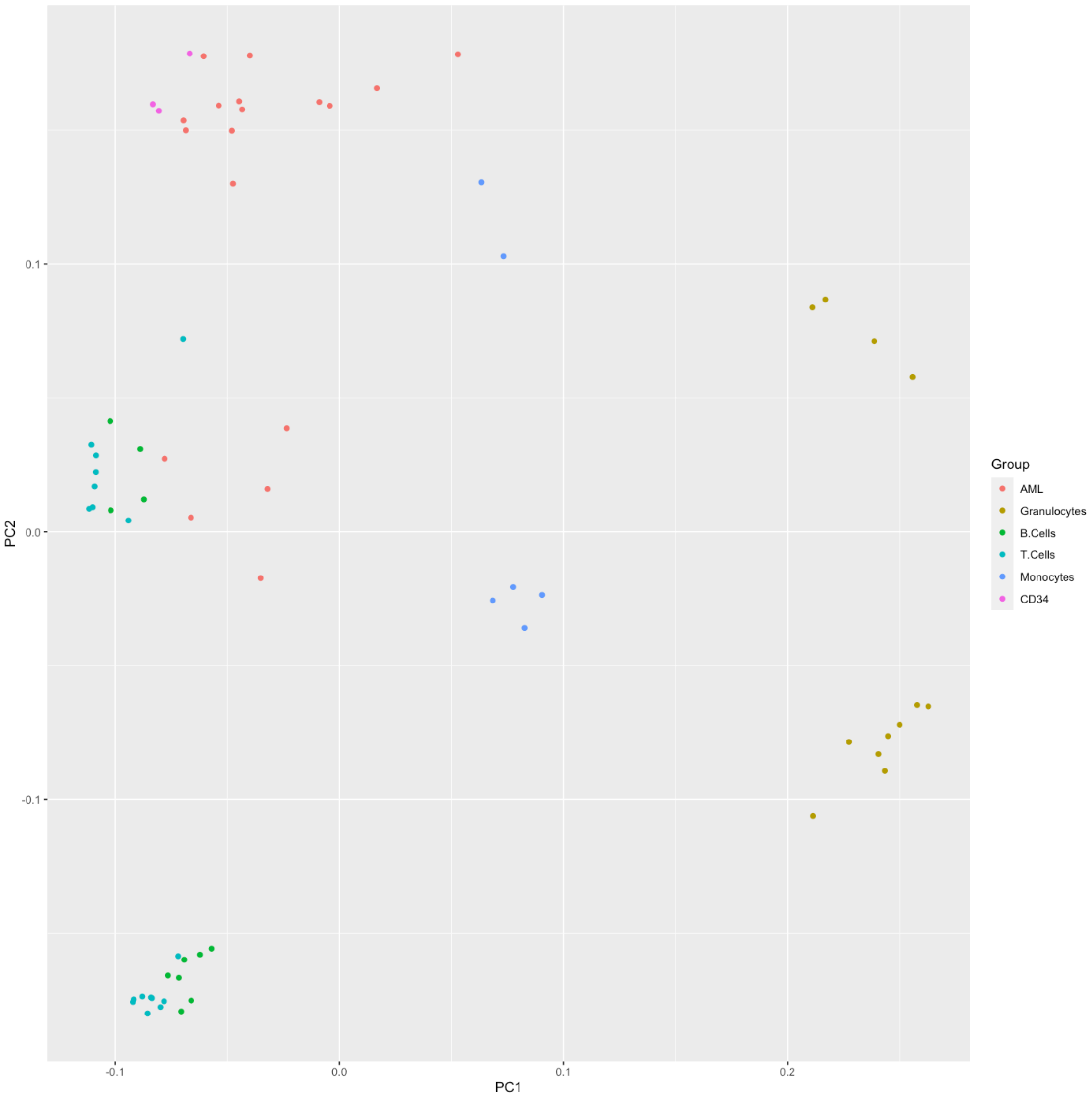
Question 3)

Dimension reduction can visualize data so we can determine if we performed well on experiment or not.

Best dimension reduction method is tSNE which has most discriminative clustering of samples.

[ ]:

```
pcr <- data.frame(pc$rotation[, 1:3], Group=gs)
options(repr.plot.width=12, repr.plot.height=12)
ggplot(pcr, aes(x = PC1, y = PC2, color = Group)) + geom_point() + theme_gray()
```

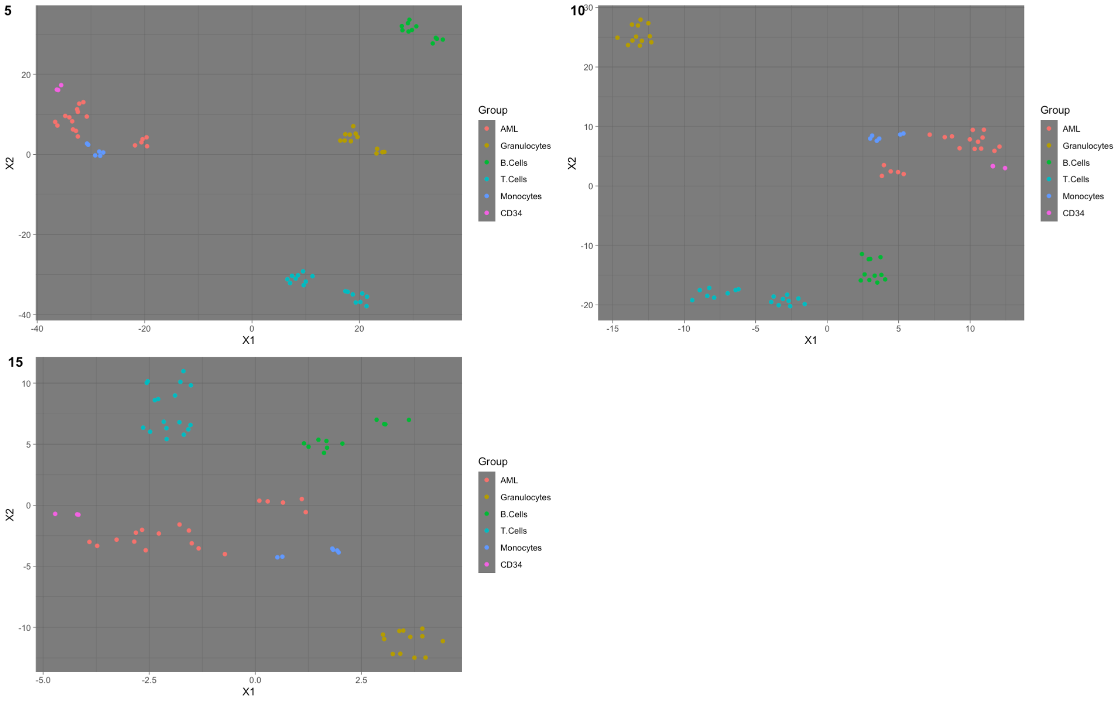


[ ]:

```
tsne_results <- list(Rtsne(t(ex), perplexity=5, check_duplicates = FALSE),
                     Rtsne(t(ex), perplexity=10, check_duplicates = FALSE),
                     Rtsne(t(ex), perplexity=15, check_duplicates = FALSE))

options(repr.plot.width=16, repr.plot.height=10)
plots.list <- list()

for(i in seq_along(tsne_results)) {
  tsne <- data.frame(tsne_results[[i]]$Y[, 1:2], Group=gs)
  plots.list[[i]] <- ggplot(tsne, aes(X1, X2, color = Group)) + geom_point() + theme_dark()
}
ggarrange(plotlist = plots.list,
          ncol = 2,
          nrow = 2,
          labels = c(5, 10, 15))
```


X2
X1
Group
AML
Granulocytes
B.Cells
T.Cells
Monocytes
CD34
X2
X1
Group
AML
Granulocytes
B.Cells
T.Cells
Monocytes
CD34
X2
X1
Group
AML
Granulocytes
B.Cells
T.Cells
Monocytes
CD34