**#################### Heatmap of genes with ROC** ####################### header should be like this #####

##### id,s1\_Stage1,s2\_Stage1,s3\_Stage1,…####

############################# **Cancer\_Normal samples** #####################

library(ComplexHeatmap)

library(circlize)

data <- read.table("C:/Users/lenovo/Desktop/final\_",sep=",",header=TRUE,row.names=1)

mat = as.matrix(data[, grep("s", colnames(data))])

mat\_scaled = t(apply(mat, 1, scale))

type = gsub("s\\d+\_", "", colnames(mat))

ha = HeatmapAnnotation(df = data.frame(type = type), col = list(type = c("Cancer" = "pink", "Normal" = "cyan")))

Heatmap(mat\_scaled, cluster\_columns = FALSE, show\_row\_dend = FALSE, name = "Expression", km = 5, col = colorRamp2(c(-3, 0, 3), c("dark green", "white", "red")),

top\_annotation = ha, top\_annotation\_height = unit(4, "mm"),

show\_row\_names = TRUE, row\_names\_side = "left", row\_names\_gp = gpar(fontsize = 8), show\_column\_names = FALSE , column\_title = 'Expression pattern of top 20 RNA transcripts \n in Cancer v/s Normal tissue samples', ) +

Heatmap(data$ROC, name = "ROC", width = unit(5, "mm"), col = colorRamp2(c(1.0, 0.8), c("dark blue", "light blue")))

#############################################################

library(ComplexHeatmap)

library(circlize)

data <- read.table("C:/Users/lenovo/Desktop/final\_",sep=",",header=TRUE,row.names=1)

mat = as.matrix(data[, grep("s", colnames(data))])

mat\_scaled = t(apply(mat, 1, scale))

type = gsub("s\\d+\_", "", colnames(mat))

ha = HeatmapAnnotation(df = data.frame(type = type), col = list(type = c("Cancer" = "pink", "Normal" = "cyan")))

Heatmap(mat\_scaled, cluster\_columns = FALSE, show\_row\_dend = FALSE, name = "Expression", km = 5, col = colorRamp2(c(-3, 0, 3), c("dark green", "white", "red")),

top\_annotation = ha, top\_annotation\_height = unit(4, "mm"),

show\_row\_names = TRUE, row\_names\_side = "left", row\_names\_gp = gpar(fontsize = 1.2), show\_column\_names = FALSE, ) +

Heatmap(data$ROC, name = "ROC", width = unit(5, "mm"), col = colorRamp2(c(1.0, 0.8), c("dark blue", "light blue")))

#######################################################

library(ComplexHeatmap)

library(circlize)

data <- read.table("C:/Users/lenovo/Desktop/final\_",sep=",",header=TRUE,row.names=1)

mat = as.matrix(data[, grep("s", colnames(data))])

mat\_scaled = t(apply(mat, 1, scale))

type = gsub("s\\d+\_", "", colnames(mat))

ha = HeatmapAnnotation(df = data.frame(type = type), col = list(type = c("Stage1" = "pink", "Late\_Stage" = "cyan")))

Heatmap(mat\_scaled, cluster\_columns = FALSE, show\_row\_dend = FALSE, name = "Expression", km = 5, col = colorRamp2(c(-3, 0, 3), c("dark green", "white", "red")),

top\_annotation = ha, top\_annotation\_height = unit(4, "mm"),

show\_row\_names = TRUE, row\_names\_side = "left", row\_names\_gp = gpar(fontsize = 4.5), show\_column\_names = FALSE , column\_title = 'Expression pattern of top99 RNA transcripts \n in Early stage v/s Late stage tissue samples', ) +

Heatmap(data$ROC, name = "ROC", width = unit(5, "mm"), col = colorRamp2(c(0.7, 0.5), c("dark blue", "light blue")))

library(ComplexHeatmap)

library(circlize)

data <- read.table("C:/Users/lenovo/Desktop/final\_",sep=",",header=TRUE,row.names=1)

mat = as.matrix(data[, grep("s", colnames(data))])

mat\_scaled = t(apply(mat, 1, scale))

type = gsub("s\\d+\_", "", colnames(mat))

ha = HeatmapAnnotation(df = data.frame(type = type), col = list(type = c("Stage1" = "pink", "Late\_Stage" = "cyan")))

Heatmap(mat\_scaled, cluster\_columns = FALSE, show\_row\_dend = FALSE, name = "Expression", km = 5, col = colorRamp2(c(-3, 0, 3), c("dark green", "white", "red")),

top\_annotation = ha, top\_annotation\_height = unit(4, "mm"),

show\_row\_names = TRUE, row\_names\_side = "left", row\_names\_gp = gpar(fontsize = 7.5), show\_column\_names = FALSE , ) +

Heatmap(data$ROC, name = "ROC", width = unit(5, "mm"), colorRamp2(c(0.7, 0.65, 0.6), c("magenta4", " magenta3", " linen")))

mat = as.matrix(data[, grep("s", colnames(data))])

mat\_scaled = t(apply(mat, 1, scale))

type = gsub("s\\d+\_", "", colnames(mat))

ha = HeatmapAnnotation(df = data.frame(type = type), col = list(type = c("Cancer" = "pink", "Normal" = "cyan")))

**or**

ha = HeatmapAnnotation(df = data.frame(type = type), col = list(type = c("Stage1" = "pink", "Late\_Stage" = "cyan")))

Heatmap(mat\_scaled, cluster\_columns = FALSE, show\_row\_dend = FALSE, name = "Expression", km = 5, col = colorRamp2(c(-2, 0, 2), c("dark green", "white", "red")),

top\_annotation = ha, top\_annotation\_height = unit(4, "mm"),

show\_row\_names = TRUE, row\_names\_side = "left", row\_names\_gp = gpar(fontsize = 7), show\_column\_names = FALSE ,) +

Heatmap(data$ROC, name = "ROC", width = unit(5, "mm"), col = colorRamp2(c(0.7, 0.5), c("dark blue", "light blue")))

library(ComplexHeatmap)

library(circlize)

data <-read.table("C:/Users/lenovo/Desktop/Heatmaps\_matrices/top10\_cn\_mat",sep=",",header=TRUE,row.names=1)

mat = as.matrix(data[, grep("s", colnames(data))])

mat\_scaled = t(apply(mat, 1, scale))

type = gsub("s\\d+\_", "", colnames(mat))

ha = HeatmapAnnotation(df = data.frame(type = type), col = list(type = c("Cancer" = "pink", "Normal" = "cyan")))

Heatmap(mat\_scaled, cluster\_columns = FALSE, cluster\_rows = FALSE,

show\_row\_dend = FALSE, name = "Methylation \n (beta value)", km = 5, col = colorRamp2(c(-2, 0, 2), c("dark green", "white", "red")),

top\_annotation = ha, top\_annotation\_height = unit(4, "mm"),

show\_row\_names = TRUE, row\_names\_side = "left", row\_names\_gp = gpar(fontsize = 7), show\_column\_names = FALSE , column\_title = 'Methylation pattern of top 10 CpG sites \n in Cancer v/s Normal tissue samples', ) +

Heatmap(data$ROC, name = "ROC", width = unit(5, "mm"), col = colorRamp2(c(1.0, 0.9), c("dark blue", "light blue")))

#######**CpG sites** #####

data <-read.table("C:/Users/lenovo/Desktop/Heatmaps\_matrices/top10\_cn\_mat",sep=",",header=TRUE,row.names=1)

mat = as.matrix(data[, grep("s", colnames(data))])

mat\_scaled = t(apply(mat, 1, scale))

type = gsub("s\\d+\_", "", colnames(mat))

ha = HeatmapAnnotation(df = data.frame(type = type), col = list(type = c("Cancer" = "pink", "Normal" = "cyan")))

**or**

ha = HeatmapAnnotation(df = data.frame(type = type), col = list(type = c("Stage1" = "pink", "Late\_Stage" = "cyan")))

Heatmap(mat\_scaled, cluster\_columns = FALSE, cluster\_rows = FALSE,

show\_row\_dend = FALSE, name = "Methylation \n (beta value)", km = 5, col = colorRamp2(c(-4, 0, 2), c("dark green", "white", "red")),

top\_annotation = ha, top\_annotation\_height = unit(4, "mm"),

show\_row\_names = TRUE, row\_names\_side = "left", row\_names\_gp = gpar(fontsize = 0.8), show\_column\_names = FALSE ,) +

Heatmap(data$ROC, name = "ROC", width = unit(5, "mm"), col = colorRamp2(c(1.0, 0.9), c("dark blue", "light blue")))

######################## **early\_late stage**##############

library(ComplexHeatmap)

library(circlize)

data <- read.table("s2\_tpose",sep=",",header=TRUE,row.names=1)

mat = as.matrix(data[, grep("s", colnames(data))])

mat\_scaled = t(apply(mat, 1, scale))

type = gsub("s\\d+\_", "", colnames(mat))

ha = HeatmapAnnotation(df = data.frame(type = type), col = list(type = c("Early\_stage" = "cyan", "Late\_stage" = "Pink")))

Heatmap(mat\_scaled, cluster\_columns = FALSE, cluster\_rows = FALSE, show\_row\_dend = FALSE, name = "expression", km = 5, col = colorRamp2(c(-2, 0, 2), c("dark green", "white", "red")),

top\_annotation = ha, top\_annotation\_height = unit(4, "mm"),

show\_row\_names = TRUE, row\_names\_side = "left", row\_names\_gp = gpar(fontsize = 10), show\_column\_names = FALSE , column\_title = 'Gene Expression pattern of 30 RNA transcripts\n in Early stage (stage-I) and Late stage tissue samples', ) +

Heatmap(data$ROC, name = "ROC", width = unit(5, "mm"), col = colorRamp2(c(0.75, 0.50), c("dark blue", "light blue")))

library(ComplexHeatmap)

library(circlize)

data <- read.table("s2\_tpose",sep=",",header=TRUE,row.names=1)

mat = as.matrix(data[, grep("s", colnames(data))])

mat\_scaled = t(apply(mat, 1, scale))

type = gsub("s\\d+\_", "", colnames(mat))

ha = HeatmapAnnotation(df = data.frame(type = type), col = list(type = c("Stage1" = "cyan", "Late\_stage" = "Pink")))

Heatmap(mat\_scaled, cluster\_columns = FALSE, show\_row\_dend = FALSE, name = "Expression", km = 5, col = colorRamp2(c(-2, 0, 2), c("dark green", "white", "red")),

top\_annotation = ha, top\_annotation\_height = unit(4, "mm"),

show\_row\_names = TRUE, row\_names\_side = "left", row\_names\_gp = gpar(fontsize = 6), show\_column\_names = FALSE, ) +

Heatmap(data$ROC, name = "ROC", width = unit(5, "mm"), col = colorRamp2(c(0.75, 0.6), c("dark blue", "light blue")))

library(ComplexHeatmap)

library(circlize)

data <- read.table("s2\_tpose",sep=",",header=TRUE,row.names=1)

mat = as.matrix(data[, grep("s", colnames(data))])

mat\_scaled = t(apply(mat, 1, scale))

type = gsub("s\\d+\_", "", colnames(mat))

ha = HeatmapAnnotation(df = data.frame(type = type), col = list(type = c("Early\_stage" = "cyan", "Late\_stage" = "Pink")))

Heatmap(mat\_scaled, cluster\_columns = FALSE, cluster\_rows = FALSE, show\_row\_dend = FALSE, name = "expression", km = 5, col = colorRamp2(c(-2, 0, 2), c("dark green", "white", "red")),

top\_annotation = ha, top\_annotation\_height = unit(4, "mm"),

show\_row\_names = TRUE, row\_names\_side = "left", show\_column\_names = FALSE , ) +

Heatmap(data$ROC, name = "ROC", width = unit(5, "mm"), col = colorRamp2(c(0.75, 0.50), c("dark blue", "light blue")))

##################

library(ComplexHeatmap)

library(circlize)

data <- read.table("s2\_tpose",sep=",",header=TRUE,row.names=1)

mat = as.matrix(data[, grep("s", colnames(data))])

mat\_scaled = t(apply(mat, 1, scale))

type = gsub("s\\d+\_", "", colnames(mat))

ha = HeatmapAnnotation(df = data.frame(type = type), col = list(Sample type = c("Early\_stage" = "cyan", "Late\_stage" = "Pink")))

Heatmap(mat\_scaled, cluster\_columns = FALSE, cluster\_rows = FALSE, show\_row\_dend = FALSE, name = "Expression", km = 5, col = colorRamp2(c(-2, 0, 2), c("dark green", "white", "red")),

top\_annotation = ha, top\_annotation\_height = unit(4, "mm"),

show\_row\_names = TRUE, row\_title = "Protein coding genes", row\_names\_side = "left", row\_names\_gp = gpar(fontsize = 10), show\_column\_names = FALSE , column\_title = 'Gene Expression pattern of top 10 protein coding genes\n in Early stage and Late stage tissue samples', ) +

Heatmap(data$ROC, name = "ROC", width = unit(5, "mm"), col = colorRamp2(c(0.60, 0.65), c("dark blue", "light blue")))

################# methylation status ##############

ha = HeatmapAnnotation(df = data.frame(type = type), col = list(type = c("Cancer\_samples" = "pink", "Normal\_samples" = "blue")))

############cancer vs normal ###################

library(ComplexHeatmap)

library(circlize)

data <- read.table("s2\_tpose",sep=",",header=TRUE,row.names=1)

mat = as.matrix(data[, grep("s", colnames(data))])

mat\_scaled = t(apply(mat, 1, scale))

type = gsub("s\\d+\_", "", colnames(mat))

ha = HeatmapAnnotation(df = data.frame(type = type), col = list(type = c("Cancer" = "pink", "Normal" = "blue")))

Heatmap(mat\_scaled, cluster\_columns = FALSE, cluster\_rows = TRUE, show\_row\_dend = TRUE, name = "Methylation", km = 5, col = colorRamp2(c (0, 0.5, 1), c("green","white", "red")),

top\_annotation = ha, top\_annotation\_height = unit(5, "mm"),

show\_row\_names = TRUE, row\_names\_side = "left", row\_names\_gp = gpar(fontsize = 10), show\_column\_names = FALSE , column\_title = 'Methylation pattern of top 25 Hypomethylated CpG sites\n in Cancer and Normal tissue samples', )+

Heatmap(data$ROC, name = "ROC", width = unit(5, "mm"), col = colorRamp2(c(0.98, 0.91), c("dark blue", "light blue")))

############ without scaling values ################

library(ComplexHeatmap)

library(circlize)

data <- read.table("s2\_tpose",sep=",",header=TRUE,row.names=1)

mat = as.matrix(data[, grep("s", colnames(data))])

type = gsub("s\\d+\_", "", colnames(mat))

ha = HeatmapAnnotation(df = data.frame(type = type), col = list(type = c("Cancer" = "pink", "Normal" = "cyan")))

Heatmap(mat, cluster\_columns = FALSE, cluster\_rows = FALSE, show\_row\_dend = FALSE, name = "Methylation", km = 5, col = colorRamp2(c (0, 0.5, 1), c("light green","white", "red")),

top\_annotation = ha, top\_annotation\_height = unit(5, "mm"),

show\_row\_names = TRUE, row\_names\_side = "left", row\_names\_gp = gpar(fontsize = 5), show\_column\_names = FALSE , column\_title = 'Methylation pattern of top 100 hypermethylated CpG sites\n in Stage\_I v/s Late stage tissue samples', ) +

Heatmap(data$ROC, name = "ROC", width = unit(5, "mm"), col = colorRamp2(c(0.7, 0.65, 0.6), c("magenta4", " magenta3", " linen")))

Heatmap(data$ROC, name = "ROC", width = unit(5, "mm"), col = colorRamp2(c(0.65, 0.60), c("dark blue", "light blue")))

################# **Final\_heatmap ##################**

################# stage\_I vs late stages ##################

library(ComplexHeatmap)

library(circlize)

data <- read.table("s2\_tpose",sep=",",header=TRUE,row.names=1)

mat = as.matrix(data[, grep("s", colnames(data))])

mat\_scaled = t(apply(mat, 1, scale))

type = gsub("s\\d+\_", "", colnames(mat))

ha = HeatmapAnnotation(df = data.frame(type = type), col = list(type = c("Stage\_I" = "cyan", "Late\_stage" = "Pink")))

Heatmap(mat\_scaled, cluster\_columns = FALSE, cluster\_rows = FALSE, show\_row\_dend = FALSE, name = "expression", km = 5, col = colorRamp2(c(-3, 0, 3), c("dark green", "white", "red")),

top\_annotation = ha, top\_annotation\_height = unit(4, "mm"),

show\_row\_names = TRUE, row\_names\_side = "left", row\_names\_gp = gpar(fontsize = 8), show\_column\_names = FALSE , column\_title = 'Gene Expression pattern of top 50 protein coding genes\n in Stage-I v/s Late stage tissue samples',)+

Heatmap(data$ROC, name = "ROC", width = unit(5, "mm"), col = colorRamp2(c(0.65, 0.60), c("dark blue", "light blue")))