03-CibersortLineGraph

Cibersort based Line Graph - R Markdown

- Read in output from Cibersort online (tumor samples)
- Read in output from Cibersort online (normal samples)
- Create one cibersort object for each group of interest.
- For each group of interest

  - Call function which calculates the means of each immune cell type
  - Cbind all means values

- Plot a line graph of the mean values so you can see how the mean value changes across the groups.
  ## Read in cibersort output from online web - tumor samples

```
# Read in cibersort output - rows are samples, columns are immune cell types
inputBLCAtumor = read.csv("input/CIBERSORTx_Tumor_Results.txt",
                          header=TRUE, row.names=1, sep="\t")
knitr::kable(inputBLCAtumor[1:5, 1:4], caption = "Bladder cancer Tumor")
```

Table 1: Bladder cancer Tumor

	B.cells.naive	B.cells.memory	Plasma.cells	T.cells.CD8
GSM340547	0.0000000	0.1478116	0.0000000	0.0699226
GSM340548	0.0000000	0.1167400	0.1668580	0.0000000
GSM340549	0.0243214	0.0000000	0.0014384	0.1269212
GSM340550	0.1223687	0.0000000	0.0045003	0.1051648
GSM340551	0.0000000	0.0296045	0.0407549	0.0000000

Read in cibersort output from online web - normal samples

```
# Read in cibersort output - rows are samples, columns are immune cell types
inputBLCAnormal = read.csv("input/CIBERSORTx_Normal_Results.txt",
                           header=TRUE, row.names=1, sep="\t")
knitr::kable(inputBLCAnormal[1:5, 1:4], caption = "Bladder cancer Normal")
```

Table 2: Bladder cancer Normal

	B.cells.naive	B.cells.memory	Plasma.cells	T.cells.CD8
GSM340537	0.0595902	0	0.0208277	0.0054900
GSM340538	0.0118495	0	0.0882123	0.2068398
GSM340539	0.0000000	0	0.0652977	0.1781057
GSM340540	0.0000000	0	0.0000000	0.1562189

	B.cells.naive	B.cells.memory	Plasma.cells	T.cells.CD8
GSM340541	0.0440019	0	0.0000000	0.1522319

Create cibersort data objects

```
cibersortDataTumor <- inputBLCAtumor
cibersortDataNormal <- inputBLCAnormal
```

For each group of interest , call function to calculate means

```
# Function Definition
funcMeans <- function(cibersortData) {

    data3 = as.data.frame(cibersortData[,1:22]) #select only 22 cell type data
    data1 = as.data.frame(t(cibersortData[,1:22]))

    colnames(data1) = row.names(cibersortData)
    data1$row <- row.names(data1)

    #melt and reshape data
    melted <- reshape2::melt(data1, id.vars = "row")
    melted$row <-factor(melted$row, levels=c(colnames(cibersortData)))

    #calculate means
    means <- plyr::ddply(melted, c("row"), summarise, mean=mean(value))

    data2a <- reshape2::melt(means, id.vars = "row")

    return(data2a)
}

#calling function to calclulate means - returns a data frame with 22 rows and 3 columns
tumorMeans <- funcMeans(cibersortDataTumor)
normalMeans <- funcMeans(cibersortDataNormal)

#The First column is the cell type, Third column is the mean value
colnames(tumorMeans)
```

```
## [1] "row"      "variable" "value"
```

```
head(tumorMeans)
```

```
##                           row variable      value
## 1              B.cells.naive     mean 0.04250546
## 2             B.cells.memory     mean 0.03937819
## 3               Plasma.cells     mean 0.04435274
## 4                T.cells.CD8     mean 0.07393444
## 5          T.cells.CD4.naive     mean 0.02009520
## 6 T.cells.CD4.memory.resting     mean 0.13176274
```

cbind all means value (third column) from each object

IMPORTANT NOTE - When doing cbind, order them such that the baseline group is on the left most column

```r
#IMPORTANT NOTE - When doing cbind, order them such that the baseline group is on the left most column
meansAll = cbind(normalMeans$value, tumorMeans$value)
row.names(meansAll) <- tumorMeans$row #Set row names - immune cell types
colnames(meansAll) <- c("Adjacent Normal", "Tumor")
```

Call function to Plot Line Graph.

Output will be a set of graphs in the "outputFolderName" folder

```r
source("functionLineGraph.R")
```

```
## Warning: package 'directlabels' was built under R version 4.3.2
```

```r
objectLineGraph <- functionLineGraph(meansAll,
                                     outputFileName = "2BLCA_Ciber_LinePlot",
                                     outputFolderName = "output") #Set output folder name
```

```
## Warning: Using 'size' aesthetic for lines was deprecated in ggplot2 3.4.0.
## i Please use 'linewidth' instead.
## This warning is displayed once every 8 hours.
## Call 'lifecycle::last_lifecycle_warnings()' to see where this warning was
## generated.
```

```r
#insert object into the Rmarkdown
objectLineGraph
```
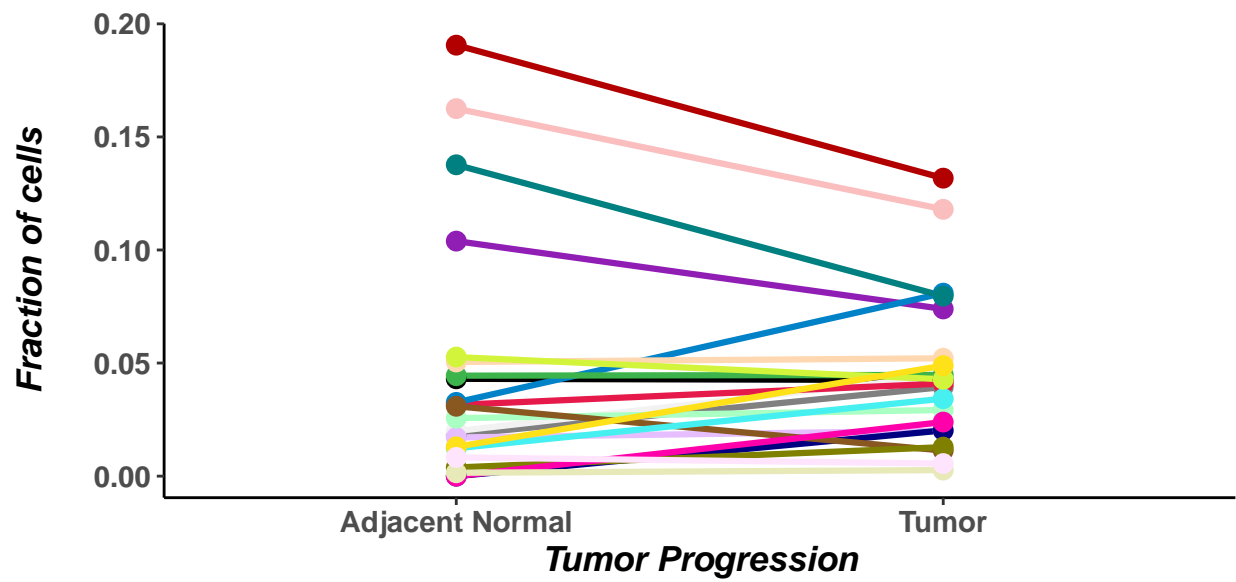
Legend:
- T.cells.CD4.naive
- T.cells.CD4.memory.resting
- T.cells.CD4.memory.activated
- T.cells.follicular.helper
- T.cells.regulatory..Tregs.
- T.cells.gamma.delta
- NK.cells.resting
- NK.cells.activated
- Monocytes
- Macrophages.M0
- Macrophages.M1
- Macrophages.M2
- Den...
- Den...
- Mast...
- Mast...

```
#Build HTML / PDF
```