

PEC_1

Juan Gómiz

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https://github.com/Pakillo2345/Juan_Gomiz_PEC1

ABSTRACT

Tras la descarga de los catos human_cachexia.csv, se creó un SummarizedExperiment para explorar los datos de expresión de metabolitos. Comenzamos comprobando que el SummarizedExperiment está correctamente creado. A continuación, se normalizan los datos para poder realizar los siguientes procedimientos: PCA, HEATMAP, BoxPlot, CorrelationMatrix y Lima. Los resultados muestran cómo la expresión diferencial de diferentes metabolitos (glucosa, succinato y creatina) están ligados a la cachexia en humanos.

OBJETIVOS

Comprobar las diferencias en la expresión de metabolitos entre los pacientes con cachexia y los del grupo control (no cachexia)

MATERIALES Y METODOS

Se descargó el fichero human_cachexia.csv desde el siguiente enlace:
<https://github.com/nutrimetabolomics/metaboData/tree/main/Datasets/2024-Cachexia>

Tras la descarga, se cargaron los datos en R utilizando el siguiente comando: cachexia
<- read.csv("C:/Users/Usuario/Downloads/human_cachexia.csv")

Una vez descargados los datos, procedemos a crear el SummarizedExperiment. Los únicos metadatos que contenía el dataset eran el grupo de los pacientes (control o

cachexia). Además, para crear la matriz de assay, tuvimos que transponer filas con columnas del dataset original para adecuarlos al formato.

Una vez hecho eso, comprobamos la estructura de nuestra clase (dimensiones, valores, resumen, etc.). Una vez comprobado esto, se cargó el paquete “POMA” para el resto de análisis.

Ya con poma cargado, se normalizaron los datos del SummarizeExperiment (method = “log_pareto”). Ya con los datos normalizados, realizamos un PCA, un Heatmap, un BoxPlot, una matriz de correlación para comprobar si los metabolitos están correlacionados entre sí y un análisis Lima para comprobar los metabolitos con mayor expresión diferencial.

Paquetes utilizados: SummarizedExperiment, knitr, POMA, ggplot2, ComplexHeatmap, pheatmap, circlize, tidyr

RESULTADOS

```
cachexia <- read.csv("C:/Users/Usuario/Downloads/human_cachexia.csv")

library(SummarizedExperiment)

## Cargando paquete requerido: MatrixGenerics
## Cargando paquete requerido: matrixStats
##
## Adjuntando el paquete: 'MatrixGenerics'
## The following objects are masked from 'package:matrixStats':
##
##   colAlls, colAnyNAs, colAnys, colAvesPerRowSet, colCollapse,
##   colCounts, colCummaxs, colCummins, colCumprods, colCumsums,
##   colDiffs, colIQRDiffs, colIQRs, colLogSumExps, colMadDiffs,
##   colMads, colMaxs, colMeans2, colMedians, colMins, colOrderStats,
##   colProds, colQuantiles, colRanges, colRanks, colSdDiffs, colSds,
##   colSums2, colTabulates, colVarDiffs, colVars, colWeightedMads,
##   colWeightedMeans, colWeightedMedians, colWeightedSds,
##   colWeightedVars, rowAlls, rowAnyNAs, rowAnys, rowAvesPerColSet,
##   rowCollapse, rowCounts, rowCummaxs, rowCummins, rowCumprods,
##   rowCumsums, rowDiffs, rowIQRDiffs, rowIQRs, rowLogSumExps,
##   rowMadDiffs, rowMads, rowMaxs, rowMeans2, rowMedians, rowMins,
##   rowOrderStats, rowProds, rowQuantiles, rowRanges, rowRanks,
##   rowSdDiffs, rowSds, rowSums2, rowTabulates, rowVarDiffs, rowVars,
##   rowWeightedMads, rowWeightedMeans, rowWeightedMedians,
##   rowWeightedSds, rowWeightedVars
## Cargando paquete requerido: GenomicRanges
## Cargando paquete requerido: stats4
```

```
## Cargando paquete requerido: BiocGenerics

##
## Adjuntando el paquete: 'BiocGenerics'

## The following objects are masked from 'package:stats':
##
##     IQR, mad, sd, var, xtabs

## The following objects are masked from 'package:base':
##
##     anyDuplicated, aperm, append, as.data.frame, basename, cbind,
##     colnames, dirname, do.call, duplicated, eval, evalq, Filter, Find,
##     get, grep, grepl, intersect, is.unsorted, lapply, Map, mapply,
##     match, mget, order, paste, pmax, pmax.int, pmin, pmin.int,
##     Position, rank, rbind, Reduce, rownames, sapply, saveRDS, setdiff,
##     table, tapply, union, unique, unsplit, which.max, which.min

## Cargando paquete requerido: S4Vectors

##
## Adjuntando el paquete: 'S4Vectors'

## The following object is masked from 'package:utils':
##
##     findMatches

## The following objects are masked from 'package:base':
##
##     expand.grid, I, unname

## Cargando paquete requerido: IRanges

##
## Adjuntando el paquete: 'IRanges'

## The following object is masked from 'package:grDevices':
##
##     windows

## Cargando paquete requerido: GenomeInfoDb

## Cargando paquete requerido: Biobase

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

##
## Adjuntando el paquete: 'Biobase'
```

```

## The following object is masked from 'package:MatrixGenerics':
##
##      rowMedians

## The following objects are masked from 'package:matrixStats':
##
##      anyMissing, rowMedians

col_data <- data.frame(Muscle.loss = cachexia$Muscle.loss, row.names =
cachexia$Patient.ID)
cachexia$Muscle.loss <- NULL
rownames(cachexia) <- cachexia$Patient.ID
cachexia$Patient.ID <- NULL
assayData <- t(as.matrix(cachexia))

SE <- SummarizedExperiment(assays = list(counts = assayData), colData =
col_data)

save(SE, file = "data.Rda")

# Creamos la clase

# Comprobamos dimensiones
dim(SE)

## [1] 63 77

# Comprobamos los nombres de filas y columnas
rownames(SE)

## [1] "X1.6.Anhydro.beta.D.glucose" "X1.Methylnicotinamide"
## [3] "X2.Aminobutyrate" "X2.Hydroxyisobutyrate"
## [5] "X2.Oxoglutarate" "X3.Aminoisobutyrate"
## [7] "X3.Hydroxybutyrate" "X3.Hydroxyisovalerate"
## [9] "X3.Indoxylsulfate" "X4.Hydroxyphenylacetate"
## [11] "Acetate" "Acetone"
## [13] "Adipate" "Alanine"
## [15] "Asparagine" "Betaine"
## [17] "Carnitine" "Citrate"
## [19] "Creatine" "Creatinine"
## [21] "Dimethylamine" "Ethanolamine"
## [23] "Formate" "Fucose"
## [25] "Fumarate" "Glucose"
## [27] "Glutamine" "Glycine"
## [29] "Glycolate" "Guanidoacetate"
## [31] "Hippurate" "Histidine"
## [33] "Hypoxanthine" "Isoleucine"
## [35] "Lactate" "Leucine"
## [37] "Lysine" "Methylamine"
## [39] "Methylguanidine" "N.N.Dimethylglycine"
## [41] "O.Acetylcarnitine" "Pantothenate"

```

```
## [43] "Pyroglutamate"      "Pyruvate"
## [45] "Quinolate"          "Serine"
## [47] "Succinate"          "Sucrose"
## [49] "Tartrate"           "Taurine"
## [51] "Threonine"           "Trigonelline"
## [53] "Trimethylamine.N.oxide" "Tryptophan"
## [55] "Tyrosine"            "Uracil"
## [57] "Valine"              "Xylose"
## [59] "cis.Aconitate"        "myo.Inositol"
## [61] "trans.Aconitate"      "pi.Methylhistidine"
## [63] "tau.Methylhistidine"
```

colnames(SE)

```
## [1] "PIF_178"      "PIF_087"      "PIF_090"      "NETL_005_V1"
"PIF_115"
## [6] "PIF_110"      "NETL_019_V1"  "NETCR_014_V1" "NETCR_014_V2"
"PIF_154"
## [11] "NETL_022_V1"  "NETL_022_V2"  "NETL_008_V1"  "PIF_146"
"PIF_119"
## [16] "PIF_099"      "PIF_162"      "PIF_160"      "PIF_113"
"PIF_143"
## [21] "NETCR_007_V1" "NETCR_007_V2" "PIF_137"      "PIF_100"
"NETL_004_V1"
## [26] "PIF_094"      "PIF_132"      "PIF_163"      "NETCR_003_V1"
"NETL_028_V1"
## [31] "NETL_028_V2"  "NETCR_013_V1" "NETL_020_V1"  "NETL_020_V2"
"PIF_192"
## [36] "NETCR_012_V1" "NETCR_012_V2" "PIF_089"      "NETCR_002_V1"
"PIF_179"
## [41] "PIF_114"      "NETCR_006_V1" "PIF_141"      "NETCR_025_V1"
"NETCR_025_V2"
## [46] "NETCR_016_V1" "PIF_116"      "PIF_191"      "PIF_164"
"NETL_013_V1"
## [51] "PIF_188"      "PIF_195"      "NETCR_015_V1" "PIF_102"
"NETL_010_V1"
## [56] "NETL_010_V2"  "NETL_001_V1"  "NETCR_015_V2" "NETCR_005_V1"
"PIF_111"
## [61] "PIF_171"      "NETCR_008_V1" "NETCR_008_V2" "NETL_017_V1"
"NETL_017_V2"
## [66] "NETL_002_V1"  "NETL_002_V2"  "PIF_190"      "NETCR_009_V1"
"NETCR_009_V2"
## [71] "NETL_007_V1"  "PIF_112"      "NETCR_019_V2" "NETL_012_V1"
"NETL_012_V2"
## [76] "NETL_003_V1"  "NETL_003_V2"
```

colData(SE)

```
## DataFrame with 77 rows and 1 column
##           Muscle.loss
##           <character>
```

```
## PIF_178      cachexic
## PIF_087      cachexic
## PIF_090      cachexic
## NETL_005_V1  cachexic
## PIF_115      cachexic
## ...          ...
## NETCR_019_V2 control
## NETL_012_V1  control
## NETL_012_V2  control
## NETL_003_V1  control
## NETL_003_V2  control
```

Comprobamos Los metadatos

#visualizamos los primeros datos

```
assay(SE, "counts")[1:5, 1:5]
```

```
##                PIF_178 PIF_087 PIF_090 NETL_005_V1
PIF_115
## X1.6.Anhydro.beta.D.glucose  40.85   62.18  270.43      154.47
22.20
## X1.Methylnicotinamide        65.37  340.36   64.72      52.98
73.70
## X2.Aminobutyrate             18.73   24.29   12.18      172.43
15.64
## X2.Hydroxyisobutyrate        26.05   41.68   65.37      74.44
83.93
## X2.Oxoglutarate              71.52   67.36   23.81     1199.91
33.12
```

```
summary(assay(SE, "counts"))
```

```
##      PIF_178      PIF_087      PIF_090      NETL_005_V1
## Min.   :  5.58  Min.   :  7.69  Min.   :  4.44  Min.   :
25.03
## 1st Qu.: 52.72  1st Qu.: 78.66  1st Qu.: 31.50  1st Qu.:
102.51
## Median : 154.47  Median : 208.51  Median : 141.17  Median :
247.15
## Mean   : 699.86  Mean   : 708.30  Mean   : 771.79  Mean   :
1021.28
## 3rd Qu.: 416.24  3rd Qu.: 412.10  3rd Qu.: 308.03  3rd Qu.:
673.71
## Max.   :16481.60  Max.   :15835.35  Max.   :24587.66  Max.
:20952.22
##      PIF_115      PIF_110      NETL_019_V1      NETCR_014_V1
## Min.   :  4.53  Min.   :  5.05  Min.   :  2.10  Min.   :
1.73
## 1st Qu.: 44.26  1st Qu.: 35.34  1st Qu.: 26.73  1st Qu.:
7.14
## Median : 84.77  Median : 113.30  Median : 91.84  Median :
```

```

18.17
## Mean : 441.22 Mean : 537.48 Mean : 400.85 Mean :
82.77
## 3rd Qu.: 196.62 3rd Qu.: 325.58 3rd Qu.: 223.63 3rd Qu.:
52.52
## Max. :6836.29 Max. :15677.78 Max. :8022.46 Max.
:2208.35
## NETCR_014_V2 PIF_154 NETL_022_V1 NETL_022_V2
## Min. : 2.41 Min. : 9.12 Min. : 4.26 Min. :
7.17
## 1st Qu.: 14.63 1st Qu.: 43.82 1st Qu.: 31.98 1st Qu.:
38.77
## Median : 39.65 Median : 117.92 Median : 83.93 Median :
127.74
## Mean : 207.80 Mean : 478.07 Mean : 367.52 Mean :
650.75
## 3rd Qu.: 102.00 3rd Qu.: 405.50 3rd Qu.: 182.20 3rd Qu.:
283.05
## Max. :6634.24 Max. :8690.62 Max. :8433.78 Max.
:19341.34
## NETL_008_V1 PIF_146 PIF_119 PIF_099
## Min. : 6.05 Min. : 3.49 Min. : 1.48 Min. :
2.230
## 1st Qu.: 36.30 1st Qu.: 28.09 1st Qu.: 5.17 1st Qu.:
5.965
## Median : 83.93 Median : 71.52 Median : 17.46 Median :
18.360
## Mean : 484.70 Mean : 355.17 Mean : 53.48 Mean :
56.669
## 3rd Qu.: 218.11 3rd Qu.: 152.94 3rd Qu.: 40.65 3rd Qu.:
37.155
## Max. :15677.78 Max. :12209.87 Max. :1480.30 Max.
:1635.980
## PIF_162 PIF_160 PIF_113 PIF_143
## Min. : 3.29 Min. : 3.39 Min. : 2.92 Min. :
3.10
## 1st Qu.: 17.91 1st Qu.: 26.06 1st Qu.: 18.82 1st Qu.:
39.26
## Median : 64.07 Median : 78.26 Median : 74.44 Median :
82.27
## Mean : 318.71 Mean : 424.13 Mean : 356.55 Mean :
461.16
## 3rd Qu.: 164.90 3rd Qu.: 193.47 3rd Qu.: 176.13 3rd Qu.:
296.43
## Max. :9701.15 Max. :10198.54 Max. :6974.39 Max.
:11158.98
## NETCR_007_V1 NETCR_007_V2 PIF_137 PIF_100
## Min. : 4.85 Min. : 5.26 Min. : 4.35 Min. :
1.55
## 1st Qu.: 28.80 1st Qu.: 44.97 1st Qu.: 39.41 1st Qu.:

```

8.85				
## Median : 64.72	Median : 98.49	Median : 75.94	Median :	
17.81				
## Mean : 460.75	Mean : 645.12	Mean : 546.23	Mean :	
153.92				
## 3rd Qu.: 210.62	3rd Qu.: 397.55	3rd Qu.: 267.15	3rd Qu.:	
53.80				
## Max. :9798.65	Max. :14328.42	Max. :13359.73	Max. :	
:5943.18				
## NETL_004_V1	PIF_094	PIF_132	PIF_163	
## Min. : 4.71	Min. : 4.57	Min. : 6.42	Min. :	
2.41				
## 1st Qu.: 16.20	1st Qu.: 25.03	1st Qu.: 69.42	1st Qu.:	
31.00				
## Median : 31.19	Median : 72.97	Median : 196.37	Median :	
97.51				
## Mean : 183.79	Mean : 350.55	Mean : 1237.54	Mean :	
516.61				
## 3rd Qu.: 108.31	3rd Qu.: 186.53	3rd Qu.: 641.11	3rd Qu.:	
330.37				
## Max. :4865.87	Max. :8349.86	Max. :33860.35	Max. :	
:11271.13				
## NETCR_003_V1	NETL_028_V1	NETL_028_V2	NETCR_013_V1	
## Min. : 0.790	Min. : 10.07	Min. : 1.82	Min. :	
2.69				
## 1st Qu.: 6.425	1st Qu.: 46.06	1st Qu.: 13.33	1st Qu.:	
31.82				
## Median : 15.180	Median : 115.58	Median : 45.15	Median :	
70.81				
## Mean : 62.813	Mean : 738.89	Mean : 199.61	Mean :	
376.69				
## 3rd Qu.: 29.370	3rd Qu.: 336.99	3rd Qu.: 119.20	3rd Qu.:	
267.74				
## Max. :1737.150	Max. :21590.31	Max. :4188.09	Max. :	
:11731.12				
## NETL_020_V1	NETL_020_V2	PIF_192	NETCR_012_V1	
## Min. : 2.32	Min. : 3.19	Min. : 2.08	Min. : 2.01	
## 1st Qu.: 14.82	1st Qu.: 28.64	1st Qu.: 20.19	1st Qu.: 12.94	
## Median : 37.34	Median : 61.56	Median : 45.60	Median : 24.05	
## Mean : 227.97	Mean : 327.88	Mean : 191.82	Mean : 148.51	
## 3rd Qu.: 104.17	3rd Qu.: 153.72	3rd Qu.: 110.89	3rd Qu.: 64.39	
## Max. :5431.66	Max. :8349.86	Max. :5014.05	Max. :4315.64	
## NETCR_012_V2	PIF_089	NETCR_002_V1	PIF_179	
## Min. : 5.53	Min. : 4.01	Min. : 3.67	Min. :	
2.18				
## 1st Qu.: 42.32	1st Qu.: 49.70	1st Qu.: 21.02	1st Qu.:	
14.88				
## Median : 101.49	Median : 116.75	Median : 62.80	Median :	
50.91				
## Mean : 496.29	Mean : 581.79	Mean : 270.28	Mean :	

198.65				
## 3rd Qu.: 290.56	3rd Qu.: 330.69	3rd Qu.: 177.69	3rd Qu.:	
121.56				
## Max. :13359.73	Max. :16481.60	Max. :7631.20	Max.	
:3533.34				
## PIF_114	NETCR_006_V1	PIF_141	NETCR_025_V1	
## Min. : 5.47	Min. : 7.32	Min. : 1.95	Min. :	
4.01				
## 1st Qu.: 32.62	1st Qu.: 50.91	1st Qu.: 21.66	1st Qu.:	
36.88				
## Median : 98.49	Median : 119.10	Median : 48.42	Median :	
94.63				
## Mean : 502.98	Mean : 697.47	Mean : 279.24	Mean :	
579.72				
## 3rd Qu.: 234.00	3rd Qu.: 404.56	3rd Qu.: 144.90	3rd Qu.:	
242.27				
## Max. :12332.58	Max. :19930.37	Max. :7115.28	Max.	
:14764.78				
## NETCR_025_V2	NETCR_016_V1	PIF_116	PIF_191	
## Min. : 2.53	Min. : 6.62	Min. : 1.120	Min. :	
0.90				
## 1st Qu.: 61.26	1st Qu.: 45.40	1st Qu.: 7.885	1st Qu.:	
9.68				
## Median : 120.30	Median : 127.74	Median : 27.390	Median :	
21.98				
## Mean : 745.91	Mean : 525.02	Mean : 143.280	Mean :	
72.36				
## 3rd Qu.: 337.24	3rd Qu.: 497.73	3rd Qu.: 68.400	3rd Qu.:	
44.70				
## Max. :22247.84	Max. :14328.42	Max. :2864.070	Max.	
:1702.75				
## PIF_164	NETL_013_V1	PIF_188	PIF_195	
## Min. : 6.89	Min. : 1.21	Min. : 1.28	Min. :	
1.51				
## 1st Qu.: 47.94	1st Qu.: 7.15	1st Qu.: 7.03	1st Qu.:	
7.30				
## Median : 121.51	Median : 15.18	Median : 18.92	Median :	
18.54				
## Mean : 639.13	Mean : 76.81	Mean : 71.90	Mean :	
170.47				
## 3rd Qu.: 306.53	3rd Qu.: 42.85	3rd Qu.: 44.26	3rd Qu.:	
62.80				
## Max. :15063.05	Max. :2392.27	Max. :2489.91	Max.	
:4817.45				
## NETCR_015_V1	PIF_102	NETL_010_V1	NETL_010_V2	
## Min. : 6.17	Min. : 7.10	Min. : 1.36	Min. : 0.79	
## 1st Qu.: 27.12	1st Qu.: 35.34	1st Qu.: 7.58	1st Qu.: 5.56	
## Median : 106.70	Median : 101.49	Median : 14.30	Median : 11.25	
## Mean : 396.24	Mean : 343.37	Mean : 64.50	Mean : 54.02	
## 3rd Qu.: 250.44	3rd Qu.: 231.62	3rd Qu.: 35.17	3rd Qu.: 24.31	

## Max. :9996.60	Max. :7480.09	Max. :1480.30	Max. :1064.22
## NETL_001_V1	NETCR_015_V2	NETCR_005_V1	PIF_111
## Min. : 1.97	Min. : 4.39	Min. : 4.10	Min. :
1.77			
## 1st Qu.: 20.19	1st Qu.: 29.52	1st Qu.: 26.57	1st Qu.:
15.34			
## Median : 54.05	Median : 87.36	Median : 49.40	Median :
35.87			
## Mean : 289.17	Mean : 347.33	Mean : 361.04	Mean :
137.42			
## 3rd Qu.: 115.00	3rd Qu.: 234.28	3rd Qu.: 202.39	3rd Qu.:
79.56			
## Max. :6974.39	Max. :8266.78	Max. :11849.01	Max. :
:3827.63			
## PIF_171	NETCR_008_V1	NETCR_008_V2	NETL_017_V1
## Min. : 4.31	Min. : 1.23	Min. : 1.14	Min. :
2.05			
## 1st Qu.: 21.45	1st Qu.: 4.00	1st Qu.: 15.93	1st Qu.:
10.48			
## Median : 62.18	Median : 13.46	Median : 46.06	Median :
23.57			
## Mean : 357.12	Mean : 42.80	Mean : 316.91	Mean :
159.57			
## 3rd Qu.: 177.72	3rd Qu.: 28.08	3rd Qu.: 107.86	3rd Qu.:
56.26			
## Max. :10614.75	Max. :1339.43	Max. :7785.36	Max. :
:5115.34			
## NETL_017_V2	NETL_002_V1	NETL_002_V2	PIF_190
## Min. : 1.55	Min. : 3.29	Min. : 6.23	Min. :
3.03			
## 1st Qu.: 5.78	1st Qu.: 22.43	1st Qu.: 50.41	1st Qu.:
9.30			
## Median : 15.96	Median : 49.90	Median : 100.48	Median :
24.05			
## Mean : 63.52	Mean : 240.74	Mean : 467.35	Mean :
97.24			
## 3rd Qu.: 34.65	3rd Qu.: 125.86	3rd Qu.: 284.31	3rd Qu.:
63.47			
## Max. :1571.84	Max. :6768.26	Max. :13359.73	Max. :
:2121.76			
## NETCR_009_V1	NETCR_009_V2	NETL_007_V1	PIF_112
## Min. : 3.10	Min. : 0.920	Min. : 1.21	Min. :
1.230			
## 1st Qu.: 45.45	1st Qu.: 6.795	1st Qu.: 10.54	1st Qu.:
6.145			
## Median : 152.93	Median : 17.990	Median : 26.05	Median :
17.460			
## Mean : 511.55	Mean : 110.017	Mean : 118.81	Mean :
56.986			
## 3rd Qu.: 323.05	3rd Qu.: 60.700	3rd Qu.: 60.37	3rd Qu.:

```

30.725
## Max. :13493.99 Max. :2298.470 Max. :3165.29 Max.
:1002.250
## NETCR_019_V2 NETL_012_V1 NETL_012_V2 NETL_003_V1
## Min. : 3.67 Min. : 1.84 Min. : 2.69 Min. :
2.51
## 1st Qu.: 22.12 1st Qu.: 10.70 1st Qu.: 9.30 1st Qu.:
14.88
## Median : 58.56 Median : 21.33 Median : 24.05 Median :
34.12
## Mean : 342.26 Mean : 142.84 Mean : 147.55 Mean :
159.46
## 3rd Qu.: 180.37 3rd Qu.: 63.44 3rd Qu.: 59.20 3rd Qu.:
90.47
## Max. :10097.06 Max. :3789.54 Max. :3498.19 Max.
:3498.19
## NETL_003_V2
## Min. : 1.62
## 1st Qu.: 12.55
## Median : 24.29
## Mean : 121.70
## 3rd Qu.: 80.70
## Max. :2864.07

```

resumen estadístico del assay

```

library(knitr)
group_counts <- table(colData(SE)$Muscle.loss)

kable(group_counts, caption = "Tabla de frecuencias:")

```

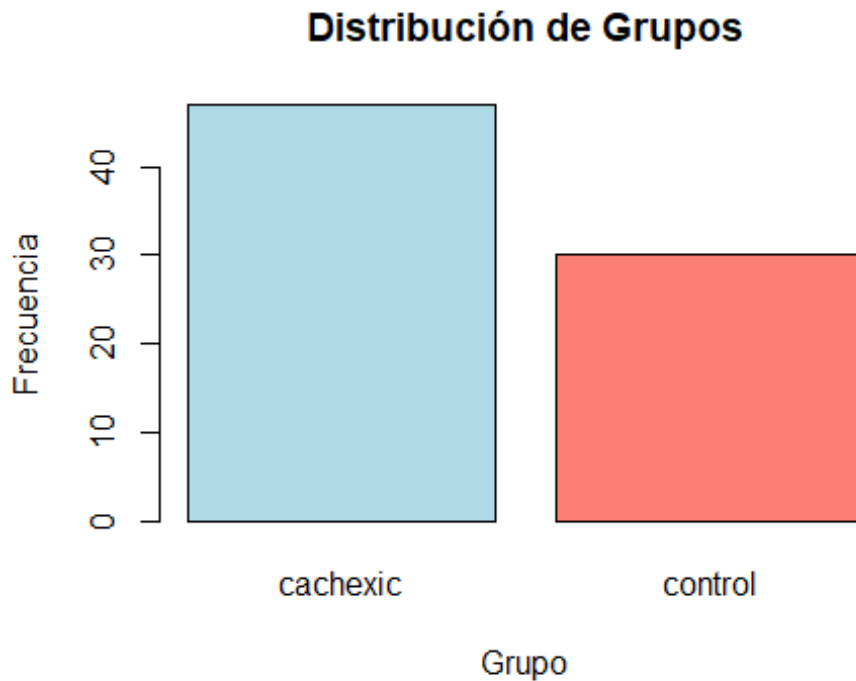
Tabla de frecuencias:

Var1	Freq
cachexic	47
control	30

```

barplot(group_counts, main = "Distribución de Grupos", xlab = "Grupo",
ylab = "Frecuencia", col = c("lightblue", "salmon"))

```



```
# Se crea una tabla de frecuencias absolutas para ver la distribución de los pacientes en ambos grupos
```

```
# Creamos un barplot para ver esa distribución gráficamente
```

```
library(POMA)
```

```
## Warning: package 'POMA' was built under R version 4.4.1
```

```
## Welcome to POMA!
```

```
## Version 1.16.0
```

```
## POMAShiny app: https://github.com/pcastellanoescuder/POMAShiny
```

```
# Normalizamos el SummarizedExperiment con la función PomaNorm y el método elegido ha sido log_pareto
```

```
normaliced <- PomaNorm(SE, method = "log_pareto")
```

```
# Creamos la variable expression_data, la cual contiene los datos transpuestos del assay normalizado para poder hacer el PCA
```

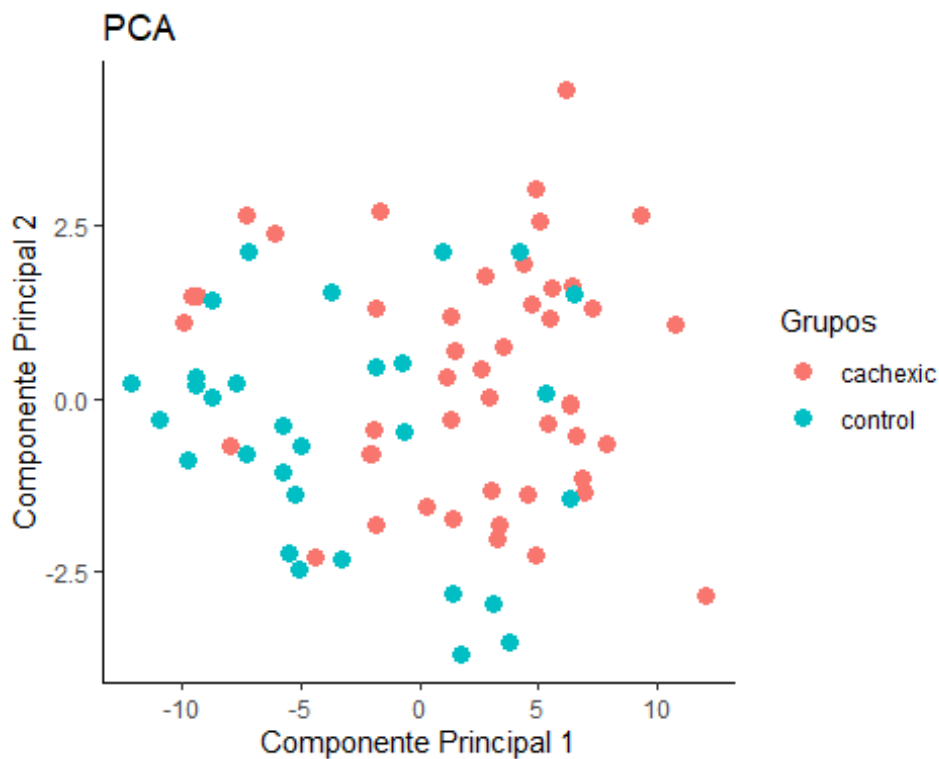
```
expression_data <- t(assay(normaliced))
```

```
pca_result <- prcomp(expression_data, center = TRUE, scale. = FALSE)
```

```
pca_data <- data.frame(PC1 = pca_result$x[,1], PC2 = pca_result$x[,2],  
  Grupos = colData(SE)$Muscle.loss)
```

```
# Pasamos el pca a data.frame para poder graficarlo con ggplot2

library(ggplot2)
ggplot(pca_data, aes(x = PC1, y = PC2, color = Grupos)) +
  geom_point(size = 3) +
  labs(title = "PCA", x = "Componente Principal 1", y = "Componente Principal 2") +
  theme_classic()
```



```
#Visualizamos los resultados de los dos componentes principales del PCA
```

Como se puede observar, de manera genral, los datos se ven distribuidos uniformemente, aunque existen cierta tendencia de los controles a encontrarse en la parte menor-izquierda de la tabla. Con esto se puede observar que existen ligeras diferencias entre ambos grupos.

```
library(ComplexHeatmap)
library(pheatmap)
library(circlize)

# Asignamos el assay normalizado a la variable expresion_norm
expresion_norm <- assay(normaliced)

#Definimos los colores de los diferentes grupos
```


Este Heatmap nos muestra cómo hay cierta tendencia de los metabolitos a expresarse en mayor cantidad en los pacientes del grupo cachexia. Existen algunos pacientes control que tienen sobreexpresados estos metabolitos. Habría que realizar otro tipo de estudio y estudiar otras variables para comprobar qué ocurre.

```
library(ggplot2)
library(tidyr)

# Creamos un dataframe con el assay normalizado para realizar un boxplot
score <- as.data.frame(t(assay(normaliced)))

# Añadimos Los metadatos
score$Grupo <- colData(SE)$Muscle.loss

# Realizamos score_long para que aparezcan cajas por metabolitos
individuales
score_long <- pivot_longer(score,
                           cols = -Grupo,
                           names_to = "Metabolito",
                           values_to = "Expresion")

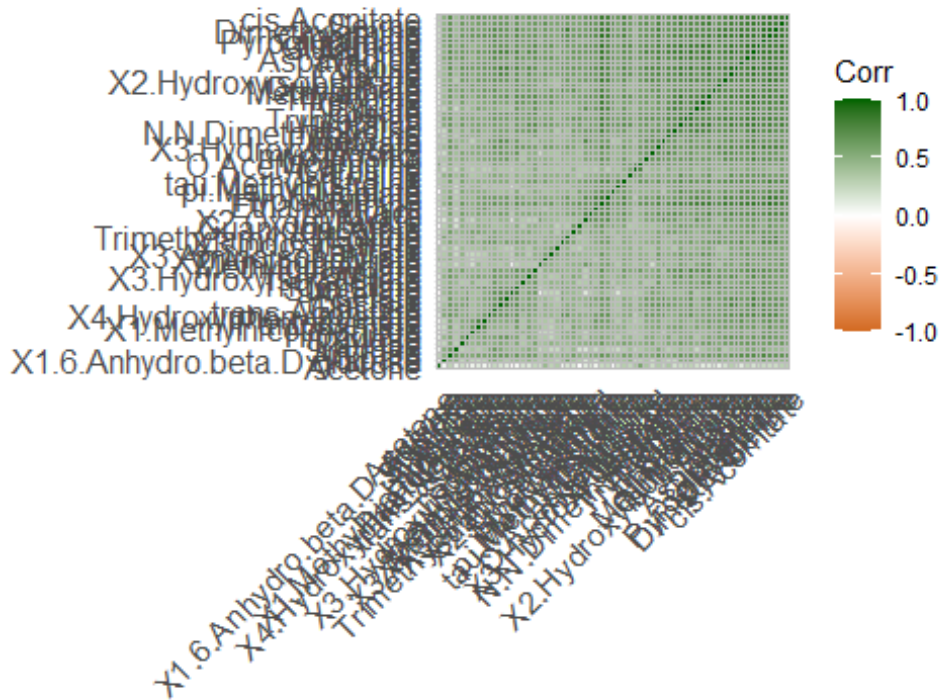
score_long$Metabolito <- factor(score_long$Metabolito)

ggplot(score_long, aes(x = Metabolito, y = Expresion, fill = Grupo)) +
  geom_boxplot(alpha = 0.7) +
  theme_classic() +
  labs(x = "Metabolito", y = "Expresion", title = "Expresion por
metabolito") +
  theme(axis.text.x = element_text(angle = 90, hjust = 1))
```



```
## 8 Creatinine      cis.Aconitate 0.897 2.68e-28 6.55e-26
## 9 Creatinine      Fucose         0.881 4.75e-26 1.03e-23
## 10 Asparagine     Glutamine      0.877 1.23e-25 2.40e-23
## # i 1,943 more rows
```

```
poma_cor$corrplot
```



Visualizamos la matriz de correlación.

En líneas generales, podemos observar que las expresiones de varios metabolitos están correlacionadas entre sí, llegando a ser algunas cercanas a 1. Sin embargo, también existen metabolitos que están poco correlacionados entre sí.

Pasamos los metadatos a factor para que PomaLimma() pueda hacer el análisis

Lima realiza un modelo lineal y para ello definimos el contraste y ajustamos el modelo por el método "fdr"

el cual nos proporciona un p-valor ajustado

```
colData(SE)$Muscle.loss <- factor(colData(SE)$Muscle.loss, levels =
c("control", "cachexic"))
```

```
normaliced <- PomaNorm(SE, method = "log_pareto")
```

```
lima <- as.data.frame(PomaLimma(normaliced, contrast = "control-
```

```
cachexic", adjust = "fdr"))
```

```
lima
```

##		feature	log2FC	AveExpr	t
pvalue					
## 1		Glucose	-1.0322993	-1.513265e-16	-4.6972111
3.205098e-06					
## 2		Succinate	-1.0253957	-1.948297e-16	-4.6413156
4.170937e-06					
## 3		Creatine	-1.0267729	1.694171e-17	-4.6148569
4.720317e-06					
## 4		myo.Inositol	-0.9595430	-4.066012e-16	-4.3761576
1.402117e-05					
## 5		N.N.Dimethylglycine	-0.9445657	2.383555e-17	-4.3334097
1.695020e-05					
## 6		Quinolate	-0.9207049	-1.012673e-16	-4.2726705
2.213322e-05					
## 7		X3.Hydroxyisovalerate	-0.9295781	-1.648213e-16	-4.2643746
2.294887e-05					
## 8		Acetate	-0.9153537	-1.771220e-16	-4.1766707
3.352052e-05					
## 9		Glutamine	-0.9132066	4.075023e-16	-4.1701428
3.446998e-05					
## 10		Betaine	-0.9044539	1.087919e-16	-4.1241231
4.192691e-05					
## 11		cis.Aconitate	-0.9071847	3.898397e-16	-4.1156001
4.346663e-05					
## 12		Adipate	-0.8952628	-1.878007e-16	-4.1076832
4.494489e-05					
## 13		Alanine	-0.8837596	2.393918e-16	-4.0393543
5.984654e-05					
## 14		Valine	-0.8498376	-1.199212e-16	-3.9318847
9.311403e-05					
## 15		Leucine	-0.8392899	-1.511688e-16	-3.8949996
1.081143e-04					
## 16		X3.Hydroxybutyrate	-0.8374432	1.820333e-17	-3.8429463
1.332101e-04					
## 17		Pyroglutamate	-0.8144690	-6.479305e-17	-3.7409742
1.991178e-04					
## 18		Formate	-0.8033174	4.038301e-16	-3.6917013
2.410073e-04					
## 19		Sucrose	-0.8127144	-2.020390e-16	-3.6836201
2.486227e-04					
## 20		Methylamine	-0.7810981	-1.899184e-16	-3.6085448
3.310185e-04					
## 21		Tryptophan	-0.7819707	1.751395e-16	-3.6029018
3.381492e-04					
## 22		Histidine	-0.7836027	1.566658e-16	-3.5478070
4.157517e-04					

## 23	Tyrosine	-0.7732364	-6.860043e-17	-3.5363545
4.338489e-04				
## 24	Dimethylamine	-0.7644788	2.818362e-16	-3.5204135
4.602680e-04				
## 25	Lactate	-0.7707779	-2.879190e-16	-3.5059318
4.855658e-04				
## 26	Creatinine	-0.7542276	1.619376e-16	-3.4735987
5.468118e-04				
## 27	Threonine	-0.7329839	-3.954944e-16	-3.3502781
8.529068e-04				
## 28	Pyruvate	-0.7239614	-1.442749e-16	-3.3006570
1.016084e-03				
## 29	X3.Indoxylsulfate	-0.7044695	-4.690512e-16	-3.2378533
1.264134e-03				
## 30	Trigonelline	-0.7067871	-5.749369e-17	-3.1742261
1.571619e-03				
## 31	Citrate	-0.7006617	-1.486906e-18	-3.1670869
1.610123e-03				
## 32	Lysine	-0.6892809	-2.823995e-17	-3.1496042
1.708118e-03				
## 33	trans.Aconitate	-0.6831981	1.734498e-16	-3.1382837
1.774473e-03				
## 34	Asparagine	-0.6794636	-1.222867e-16	-3.1266120
1.845367e-03				
## 35	Serine	-0.6797276	-1.223881e-16	-3.1222156
1.872741e-03				
## 36	Xylose	-0.6787594	3.921489e-16	-3.1164184
1.909408e-03				
## 37	Hippurate	-0.6824034	-1.330330e-16	-3.0781668
2.168390e-03				
## 38	Fucose	-0.6409561	-4.709436e-16	-2.9352326
3.447869e-03				
## 39	X2.Aminobutyrate	-0.6379206	2.114335e-16	-2.9291253
3.515452e-03				
## 40	tau.Methylhistidine	-0.6363123	-4.369926e-16	-2.9066929
3.774186e-03				
## 41	Fumarate	-0.6179110	1.029457e-16	-2.8454336
4.571511e-03				
## 42	Trimethylamine.N.oxide	-0.6236777	-4.406648e-16	-2.8410734
4.633714e-03				
## 43	X2.Hydroxyisobutyrate	-0.6090136	1.809745e-16	-2.8179748
4.976230e-03				
## 44	O.Acetylcarnitine	-0.6188370	-2.089553e-17	-2.8137694
5.041002e-03				
## 45	Ethanolamine	-0.6055601	-2.368461e-16	-2.7558813
6.013654e-03				
## 46	Glycine	-0.5977372	-3.386090e-16	-2.6994171
7.122508e-03				
## 47	Taurine	-0.6017751	2.300424e-16	-2.6975402
7.162340e-03				

## 48	pi.Methylhistidine	-0.5805823	-4.805409e-17	-2.5945048
9.681313e-03				
## 49	Glycolate	-0.5393059	1.278288e-16	-2.4312309
1.531058e-02				
## 50	X1.6.Anhydro.beta.D.glucose	-0.5320398	-1.703408e-16	-2.4103459
1.620757e-02				
## 51	X2.Oxoglutarate	-0.5258000	-1.423825e-16	-2.3474819
1.919280e-02				
## 52	Carnitine	-0.5075868	2.044045e-16	-2.2966210
2.195013e-02				
## 53	Guanidoacetate	-0.4800732	2.854859e-16	-2.1940659
2.857564e-02				
## 54	X4.Hydroxyphenylacetate	-0.4544492	-2.519292e-16	-2.0846137
3.748505e-02				
## 55	X1.Methylnicotinamide	-0.4334957	1.919010e-16	-1.9631778
5.004155e-02				
## 56	Hypoxanthine	-0.3911140	-1.520474e-16	-1.7723967
7.678615e-02				
## 57	Isoleucine	-0.3494710	-2.715180e-16	-1.6241529
1.048167e-01				
## 58	X3.Aminoisobutyrate	-0.3603753	-1.227373e-16	-1.6100669
1.078574e-01				
## 59	Tartrate	-0.3153632	-1.358491e-17	-1.4200982
1.560474e-01				
## 60	Pantothenate	-0.2910809	-4.786260e-17	-1.3142591
1.892118e-01				
## 61	Methylguanidine	-0.2579655	1.203042e-16	-1.1757260
2.401248e-01				
## 62	Uracil	-0.2261089	7.033515e-17	-1.0317271
3.025748e-01				
## 63	Acetone	-0.1526298	-1.080034e-16	-0.7071185
4.797403e-01				
##	adj_pvalue		B	
## 1	9.912666e-05		4.10620934	
## 2	9.912666e-05		3.85851470	
## 3	9.912666e-05		3.74223327	
## 4	2.065398e-04		2.72142998	
## 5	2.065398e-04		2.54400781	
## 6	2.065398e-04		2.29474265	
## 7	2.065398e-04		2.26095549	
## 8	2.359607e-04		1.90756252	
## 9	2.359607e-04		1.88153715	
## 10	2.359607e-04		1.69916273	
## 11	2.359607e-04		1.66559713	
## 12	2.359607e-04		1.63447738	
## 13	2.900256e-04		1.36825850	
## 14	4.190131e-04		0.95814277	
## 15	4.540799e-04		0.81981523	
## 16	5.245148e-04		0.62672220	
## 17	7.379071e-04		0.25565586	

```

## 18 8.243805e-04 0.07978192
## 19 8.243805e-04 0.05115023
## 20 1.014447e-03 -0.21196079
## 21 1.014447e-03 -0.23152713
## 22 1.188369e-03 -0.42101404
## 23 1.188369e-03 -0.46005009
## 24 1.208203e-03 -0.51418254
## 25 1.223626e-03 -0.56315563
## 26 1.324967e-03 -0.67179568
## 27 1.990116e-03 -1.07724013
## 28 2.286190e-03 -1.23638701
## 29 2.746221e-03 -1.43451935
## 30 3.272186e-03 -1.63148990
## 31 3.272186e-03 -1.65335422
## 32 3.341464e-03 -1.70669487
## 33 3.341464e-03 -1.74108140
## 34 3.341464e-03 -1.77640898
## 35 3.341464e-03 -1.78968293
## 36 3.341464e-03 -1.80715850
## 37 3.692123e-03 -1.92167583
## 38 5.678807e-03 -2.33742037
## 39 5.678807e-03 -2.35475583
## 40 5.944343e-03 -2.41812789
## 41 6.950571e-03 -2.58876700
## 42 6.950571e-03 -2.60077757
## 43 7.217799e-03 -2.66410370
## 44 7.217799e-03 -2.67557854
## 45 8.419115e-03 -2.83183421
## 46 9.600583e-03 -2.98118872
## 47 9.600583e-03 -2.98610143
## 48 1.270672e-02 -3.25066134
## 49 1.968503e-02 -3.64920956
## 50 2.042153e-02 -3.69835587
## 51 2.370875e-02 -3.84377163
## 52 2.659342e-02 -3.95865668
## 53 3.396727e-02 -4.18277528
## 54 4.373256e-02 -4.41083543
## 55 5.732032e-02 -4.65038638
## 56 8.638442e-02 -4.99802216
## 57 1.158500e-01 -5.24386020
## 58 1.171555e-01 -5.26611255
## 59 1.666269e-01 -5.54741354
## 60 1.986724e-01 -5.68893138
## 61 2.479978e-01 -5.85769206
## 62 3.074550e-01 -6.01328566
## 63 4.797403e-01 -6.28976043

```

Con esto estamos viendo La expresión diferencial de los diferentes metabolitos y sus p-valores

A raíz de estos resultados, se confirman los análisis anteriores. Los metabolitos, visualmente, están más expresados en el grupo cachexia que en el grupo control. Además, estos resultados son significativos si nos fijamos en el p_ajustado, el cual es menor que 0.05 en todos los casos.

Para saber los metabolitos que verdaderamente se expresan diferencialmente, usamos un método combinado. Siendo los metabolitos relevantes los que su p-valor ajustado sea < 0.05 y su log2FC esté por encima de 1 y por debajo de menos 1.

```
diff <- c()

p_val <- 0.05

umbral <- 1

met_diff <- lima[lima$adj_pvalue <= p_val & (lima$log2FC < -umbral |
lima$log2FC >umbral),]

kable(met_diff, caption = "Metabolitos más expresados")
```

Metabolitos más expresados

feature	log2FC	AveExpr	t	pvalue	adj_pvalue	B
Glucose	-1.032299	0	-4.697211	3.2e-06	9.91e-05	4.106209
Succinate	-1.025396	0	-4.641316	4.2e-06	9.91e-05	3.858515
Creatine	-1.026773	0	-4.614857	4.7e-06	9.91e-05	3.742233

Como resultado obtenemos que los metabolitos expresados diferencialmente entre ambos grupos son: Glucosa, Succinato y Creatina.

CONCLUSIONES

Las conclusiones que se pueden sacar de este trabajo es que los pacientes que están en el grupo cachexia tienen mayor expresión metabólica que los del grupo control. Concretamente, destacan: Glucosa, Succinato y Creatina.

También se subraya la necesidad de realizar más análisis o estudios contemplando otro tipo de variables.