

Universitat  
Oberta  
de Catalunya

## PEC2 ANÁLISIS DE DATOS ÓMICOS

Raúl Gil Caraballo

“2024-12-22”

- 1 Introducción y Objetivos
  - 1.1 Diseño del estudio:
- 2 Métodos
  - 2.1 Lectura de datos
  - 2.2 Exploración y control de calidad
    - 2.2.1 Funciones ad-hoc
    - 2.2.2 QC con arrayQualityMetrics
  - 2.3 Normalización
  - 2.4 Filtrado
  - 2.5 Selección de genes
- 3 Resultados
  - 3.1 Anotación de resultados
  - 3.2 Visualización de resultados
    - 3.2.1 Volcano-plot
  - 3.3 Análisis de significación biológica
- 4 Discusión
- 5 Referencias
- 6 Apendices

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# 1 Introducción y Objetivos

El estudio investiga cómo los antibióticos linezolid y vancomicina afectan la expresión génica del huésped y la inmunomodulación durante una infección por *Staphylococcus aureus* resistente a meticilina (MRSA). Utilizando el modelo murino A/J y el aislado USA300 de MRSA, se analizaron diferencias en la producción de toxinas bacterianas, citoquinas del huésped y vías de señalización relacionadas con la infección.

## 1.1 Diseño del estudio:

35 ratones divididos en siete grupos (sin infección, sin infección con antibióticos, infección con MRSA a las 2 y 24 horas, e infección tratada con linezolid o vancomicina). Los ratones se infectaron con MRSA y se trataron intravenosamente con linezolid o vancomicina a las 2 horas post-infección (hpi). Se analizaron CFUs en sangre y riñón, citoquinas en suero (IL-1 $\beta$ , IL-6, TNF- $\alpha$ ) y toxinas bacterianas (leucocidina Pantón-Valentine y alfa hemolisina) mediante ELISA.

## 2 Métodos

Se usaron 5 muestras de RNA obtenidas de ratones infectados con MRSA y tratados con linezolid o vancomicina. Estas muestras permitieron identificar diferencias en la expresión génica del huésped entre los tratamientos y en comparación con los controles.

Propósito: El objetivo fue evaluar cómo los tratamientos con linezolid y vancomicina afectan la respuesta génica del huésped en un contexto de sepsis por MRSA, proporcionando datos clave para entender la modulación inmunológica asociada a cada antibiótico.

Etapas a cubrir: Preparación de los datos

Descargar datos crudos desde GEO. Eliminar muestras a las 2 horas. Seleccionar 24 muestras usando la función `selectSamples`.

Análisis exploratorio y control de calidad

Decidir si los datos requieren transformación. Visualizar separación de los grupos. Aplicar control de calidad (usando `arrayqualitymetrics`).

Filtrado de los datos

Eliminar sondas menos variables (top 10% en variabilidad). Construcción de matrices de diseño y contrastes

Crear matrices necesarias para las comparaciones especificadas. Identificación de genes diferencialmente expresados

Usar `limma` para identificar genes diferencialmente expresados en las tres comparaciones. Anotación de los genes

Asociar identificadores como Symbol, EntrezID, o EnsemblID. Análisis de significación biológica

Realizar enriquecimiento funcional y análisis GSEA con `clusterProfiler`. Informe del análisis

Generar informe en Rmarkdown (HTML).

## 2.1 Lectura de datos

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GSM944831 blood at T0, biological rep1      GSM944831 Public on Jun 05 2014
                                submission_date last_update_date type channel_count
GSM944831      Jun 06 2012      Jun 05 2014  RNA                                1
                                source_name_ch1 organism_ch1 characteristics_ch1
GSM944831 mouse blood of uninfected control Mus musculus  inbred strain: A/J
                                characteristics_ch1.1
GSM944831 drug treatment (linezolid/vancomycin/no): no
                                characteristics_ch1.2 characteristics_ch1.3
GSM944831 time (0h/2h/24h post infection): 0      replicate: 1
                                characteristics_ch1.4 characteristics_ch1.5 characteristics_ch1.6
GSM944831      host gender: male      age: 8-10 weeks

```

#### treatment\_protocol\_ch1

GSM944831 To mimic the natural course of *S. aureus* infection in humans, which typically arise from a primary focus of infection and disseminates to other sites, we employed an intraperitoneal (i.p.) route of infection in our animal model. Briefly, blood from 5 individual mice from A/J will be collected by intracardiac puncture after infection with USA300, and 24h post-treatment of linezolid vs vancomycin (i.v.) and stored in RNAlater at -20°C

#### growth\_protocol\_ch1

GSM944831 One methicillin-resistant *S. aureus* strain (USA300) was used. Overnight *S. aureus* cultures were inoculated into fresh tryptic soy broth and incubated aerobically at 30°C to log-phase growth (optical density 600nm of ~1.0) (Rice et al., 2003). Cells were harvested by centrifugation, rinsed, and resuspended in phosphate-buffered saline (PBS).

#### molecule\_ch1

GSM944831 total RNA

#### extract\_protocol\_ch1

GSM944831 Total RNA was extracted from mouse blood using the Mouse RiboPure Blood RNA kit (Ambion, Austin, TX) according to the manufacturer's instructions. Globin mRNA was removed from whole blood RNA using the Globinclear kit (Ambion, Austin, TX). All samples passed the quality criteria of the Agilent Bioanalyzer and were used for microarray analysis. Since the total RNA yield of many samples was low, one round of linear amplification was performed for all samples using the MessageAmp Premier kit (Ambion, Austin, TX).

#### label\_ch1

GSM944831 biotin

#### label\_protocol\_ch1

GSM944831 Biotinylated cRNA were prepared according to the standard Affymetrix protocol from 0.5 ug total RNA (Affymetrix).

#### taxid\_ch1

GSM944831 10090

#### hyb\_protocol

GSM944831 Biotin-labeled cDNA was hybridized to the arrays for 16 hours at 45°C according to the manufacturer's instruction. Arrays were then washed and labeled with streptavidinphycoerythrin (strep-PE), and the signal was amplified using biotinylated antistreptavidin followed by another round of staining with strep-PE. These steps were performed on the Affymetrix fluidics station according to the recommended protocol.

#### scan\_protocol

GSM944831 Amplification and microarray hybridization were performed at the Duke University Microarray Core. Labeled gene chips were scanned using an Affymetrix Genechip Scanner 7G (Santa Clara, CA).

data\_processing

GSM944831 Data processing was conducted using the Robust Multichip Average (RMA) generated by Affymetrix Expression Console software. Gene expression data were imported into Partek Genomics Suite 6.5 (Partek, St Louis, Mo) as CEL files using default parameters. Raw data were preprocessed, including background correction, normalization, and summarization using robust multiarray average analysis, and expression data were log2 transformed. Differential expression analysis for the whole blood cells was performed using 1-way ANOVA (either infection status or drug treatment alone). Gene lists were created using a cutoff of  $P < .05$ , 2-fold change, although second-level analysis using a false-discovery rate of  $<0.05$ , 2-fold change was also performed.

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	contact_department	contact_institute	contact_address	
GSM944831	Medicine	Duke University	Stead Bldg, RM 1546A	
	contact_city	contact_state	contact_zip/postal_code	contact_country
GSM944831	Durham	NC	27710	USA

supplementary\_file

GSM944831 ftp://ftp.ncbi.nlm.nih.gov/geo/samples/GSM944nnn/GSM944831/suppl/GSM944831\_2564\_6914\_32297\_Ctl-1\_Mouse430+2.CEL.gz

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	drug treatment	(linezolid/vancomycin/no):ch1	host gender:ch1
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	inbred strain:ch1	pathogen strain:ch1	replicate:ch1
GSM944831	A/J	<NA>	1
	time (0h/2h/24h post infection):ch1		
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## 2.2 Exploración y control de calidad

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## 2.2.1 Funciones ad-hoc

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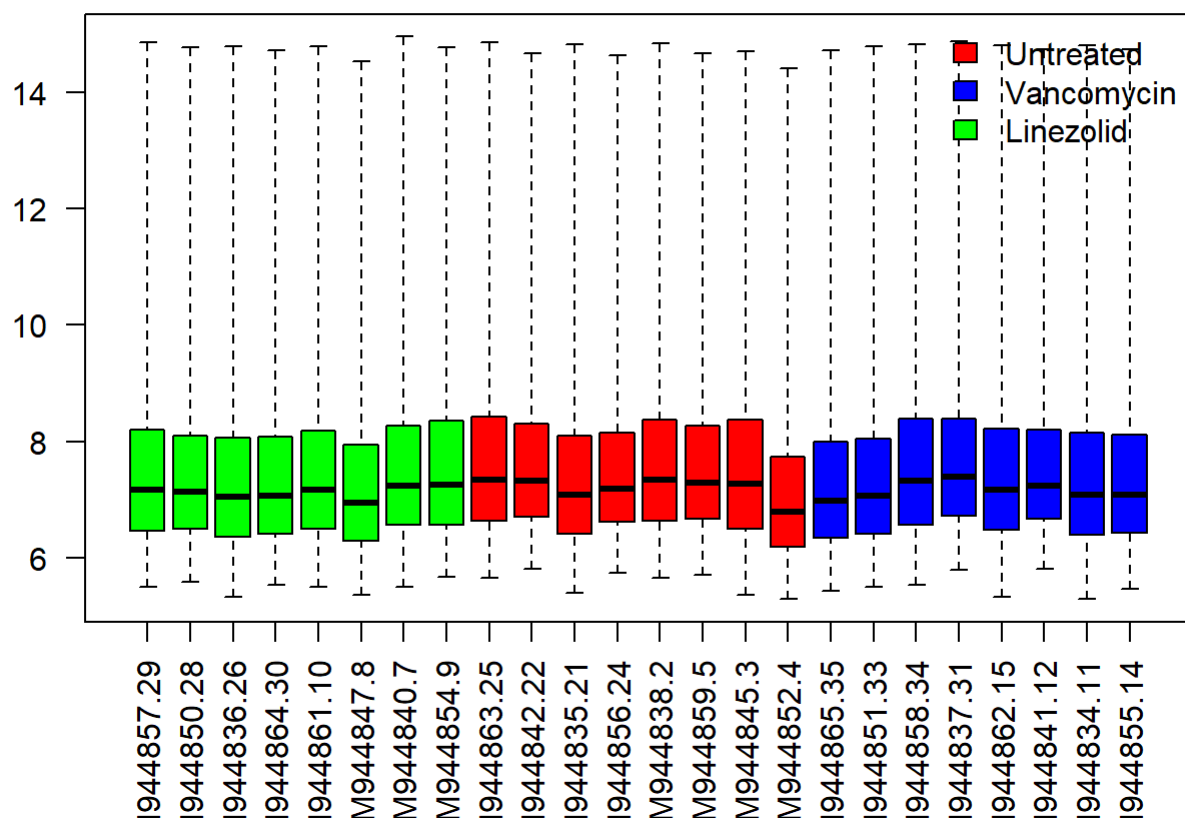
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[19] "blue"  "blue"  "blue"  "blue"  "blue"  "blue"

```

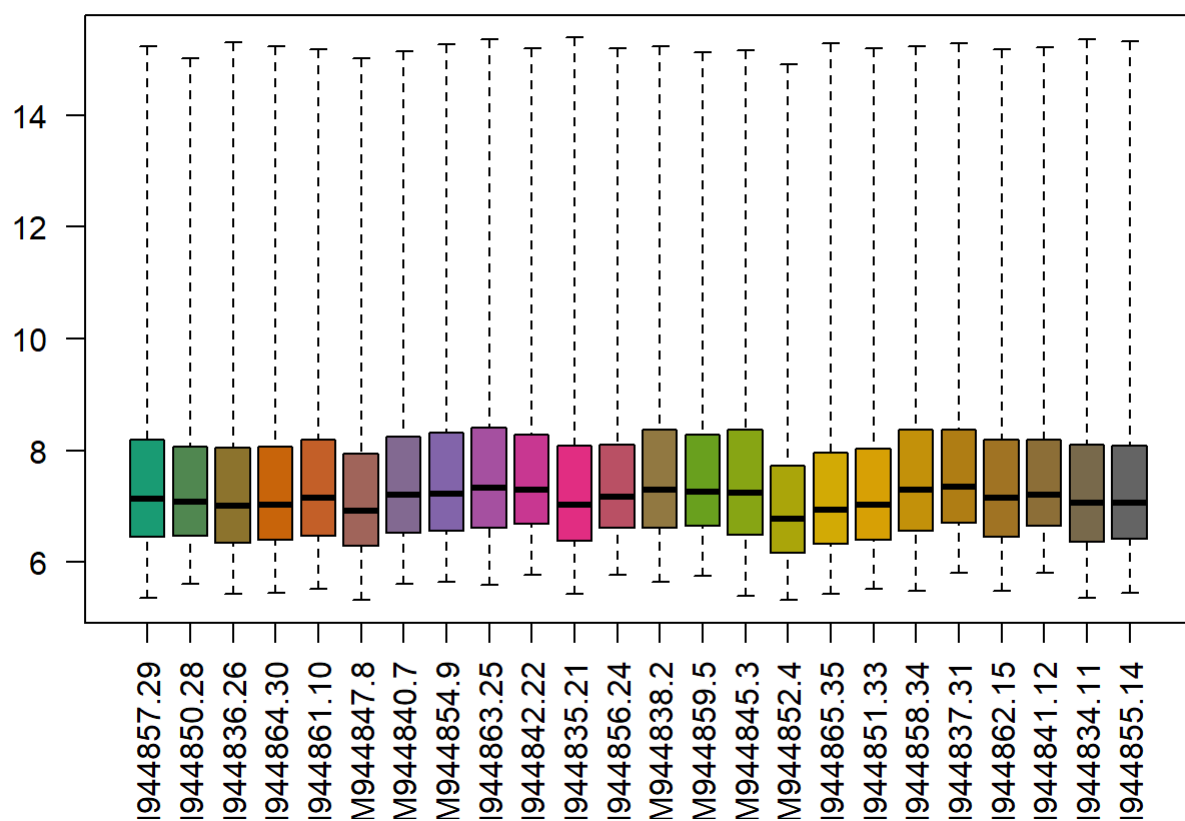
Show



## Distribución de intensidades crudas

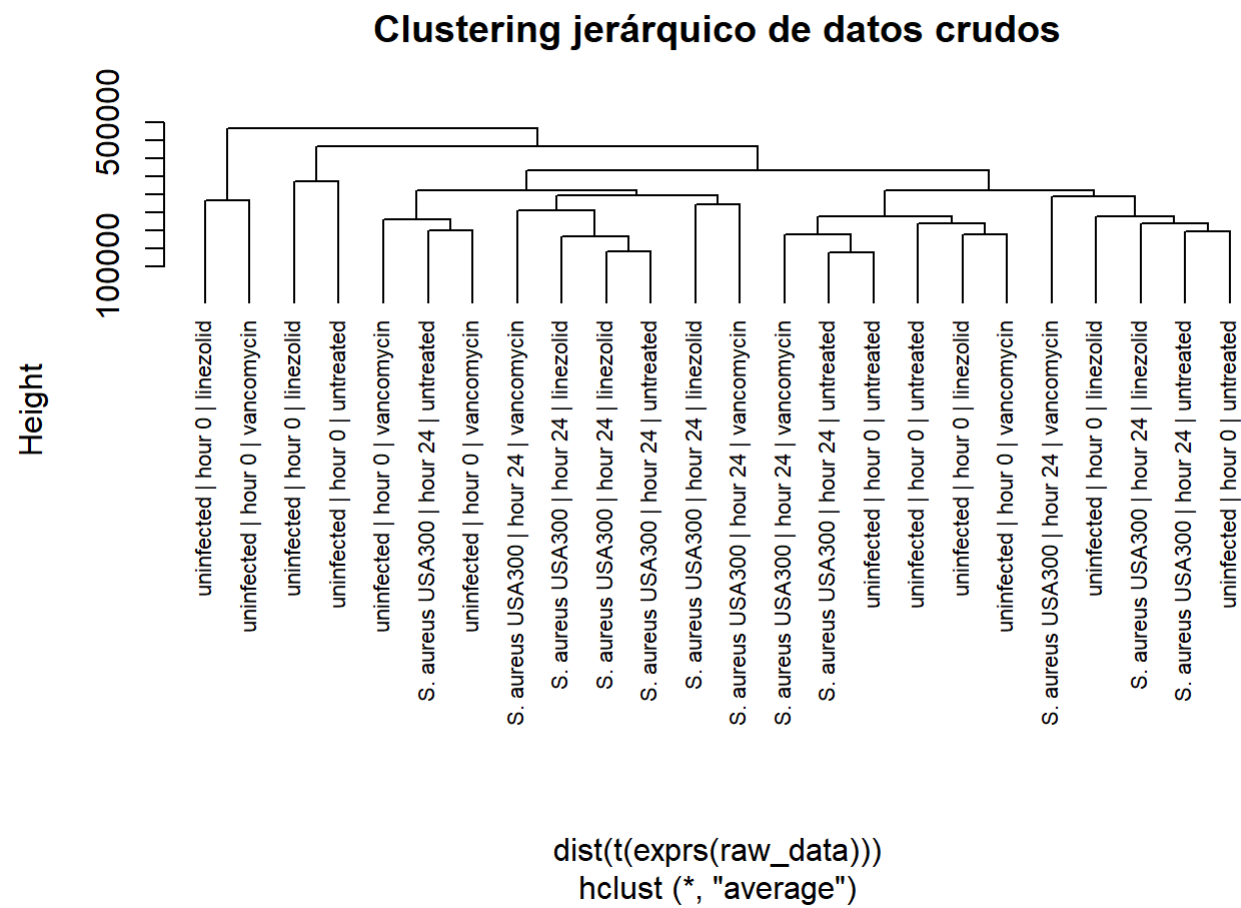

[Show](#)

## Distribución de intensidades crudas



Clusterin Jerarquico

Show

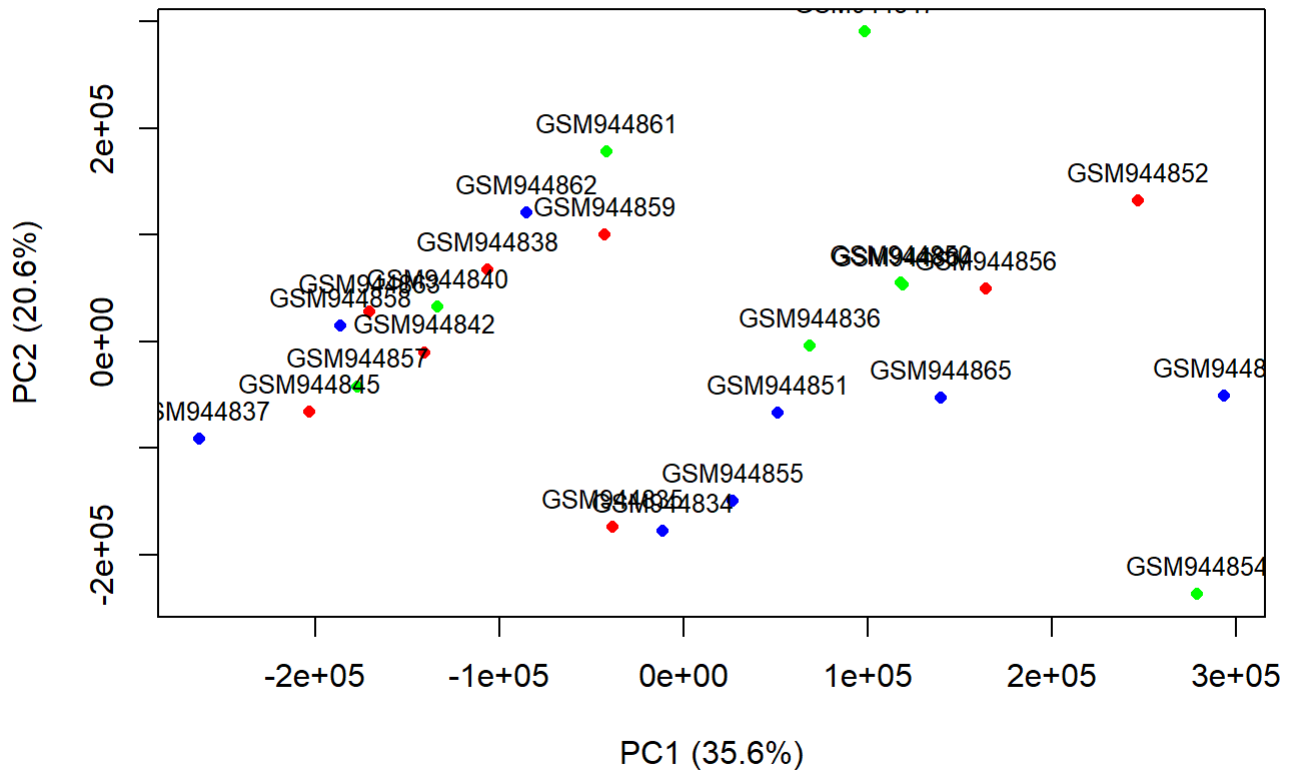


PCA

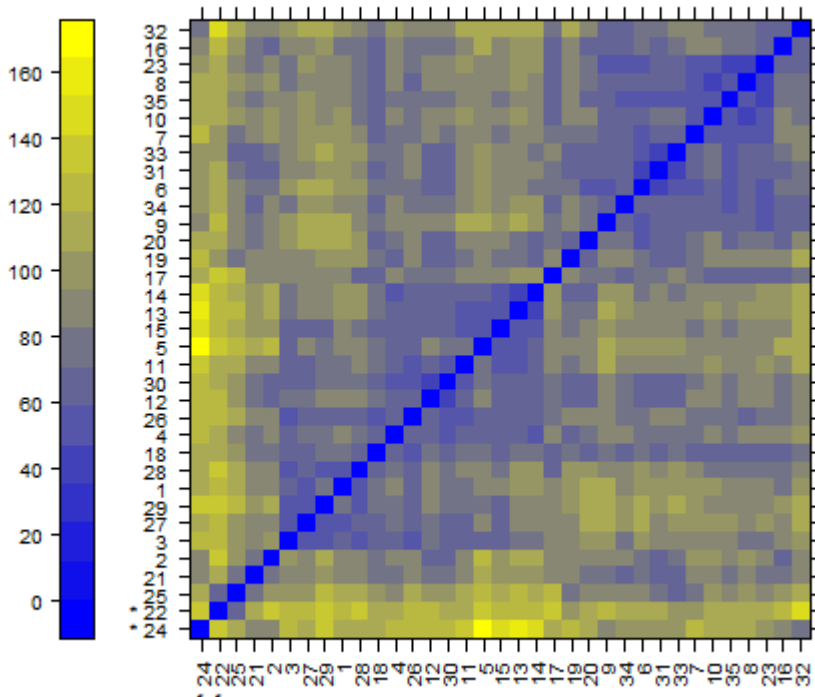
Show

Show

## PCA - Raw Data

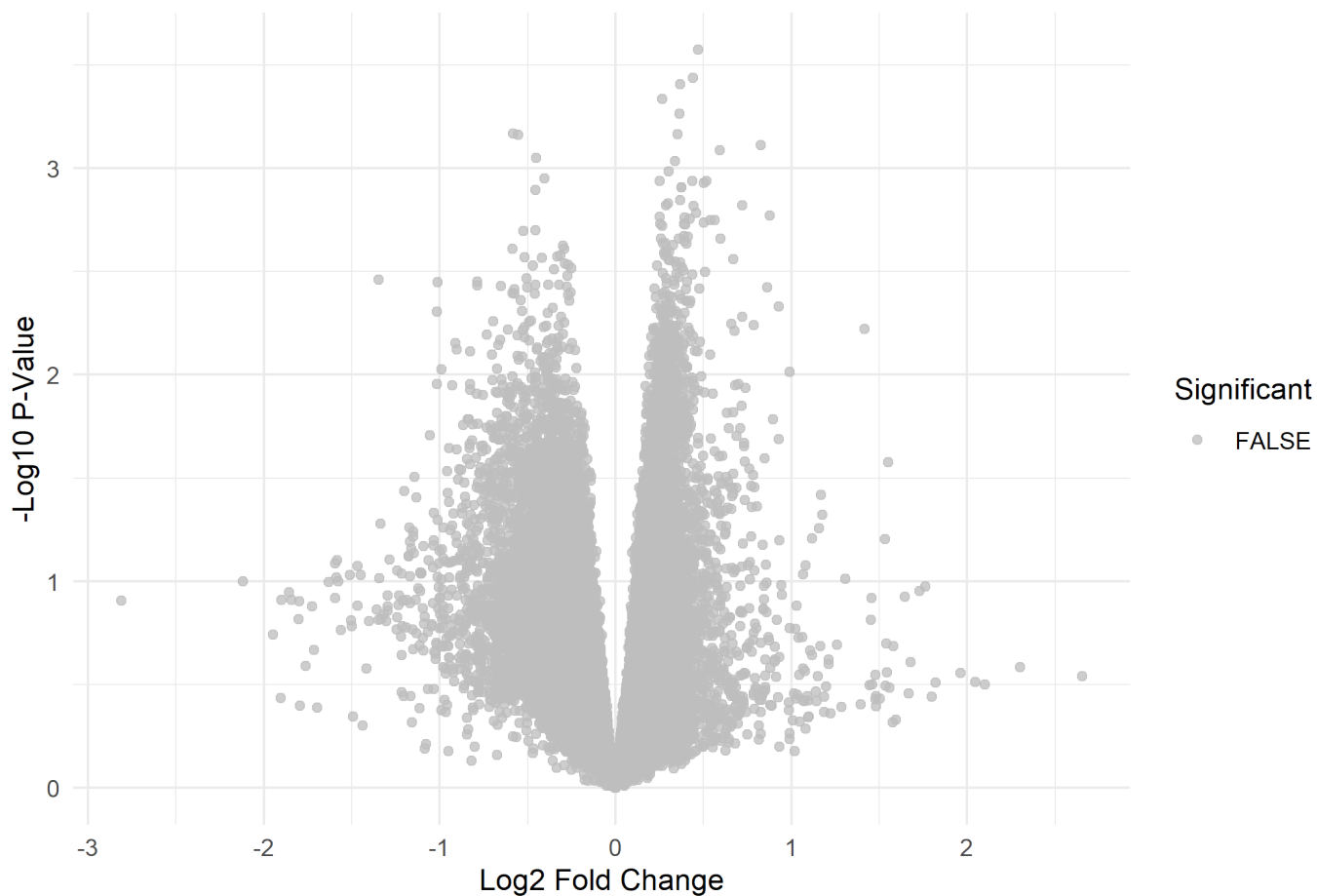


## 2.2.2 QC con arrayQualityMetrics

[Show](#)

heat map

## Volcano Plot: Infected vs Uninfected Linezolid



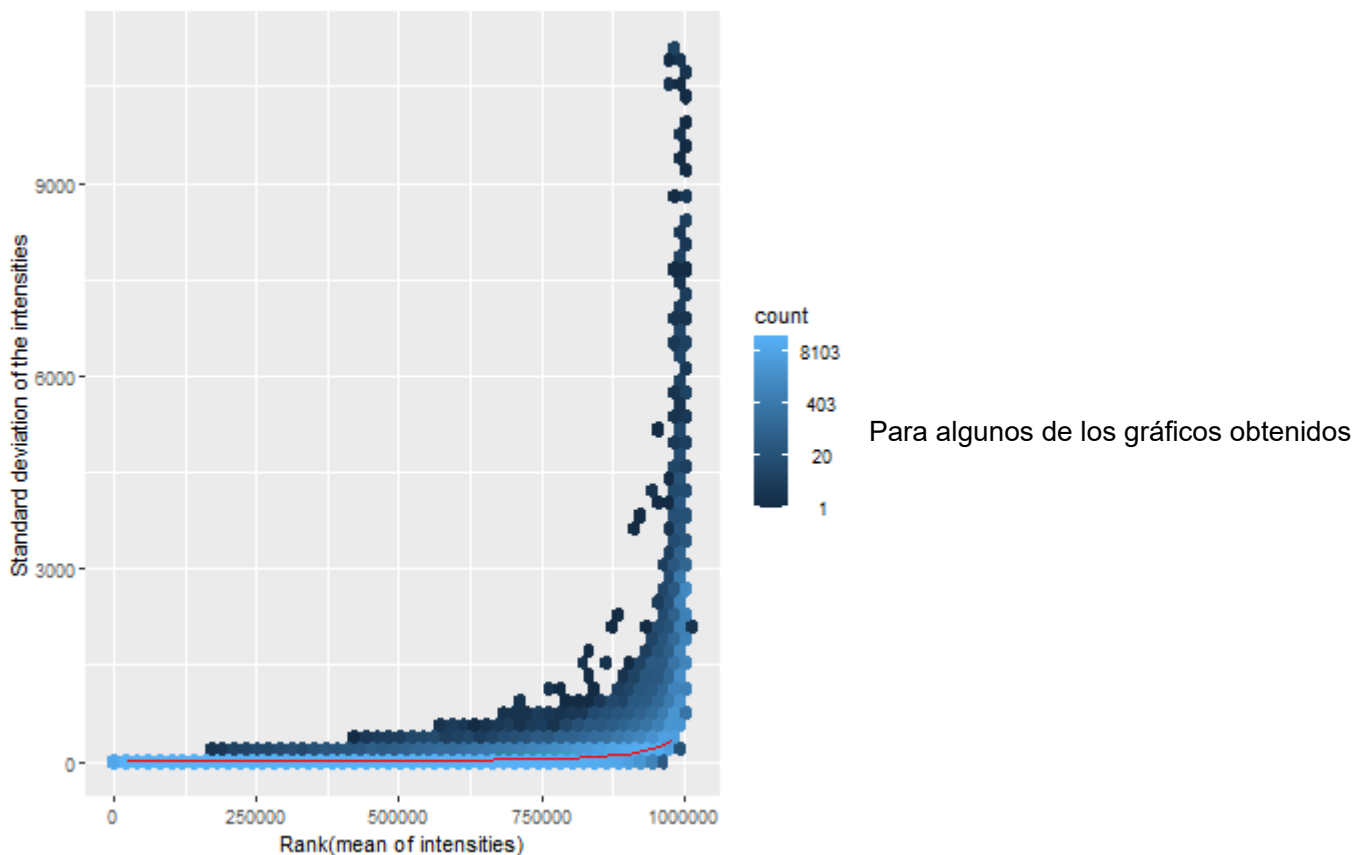
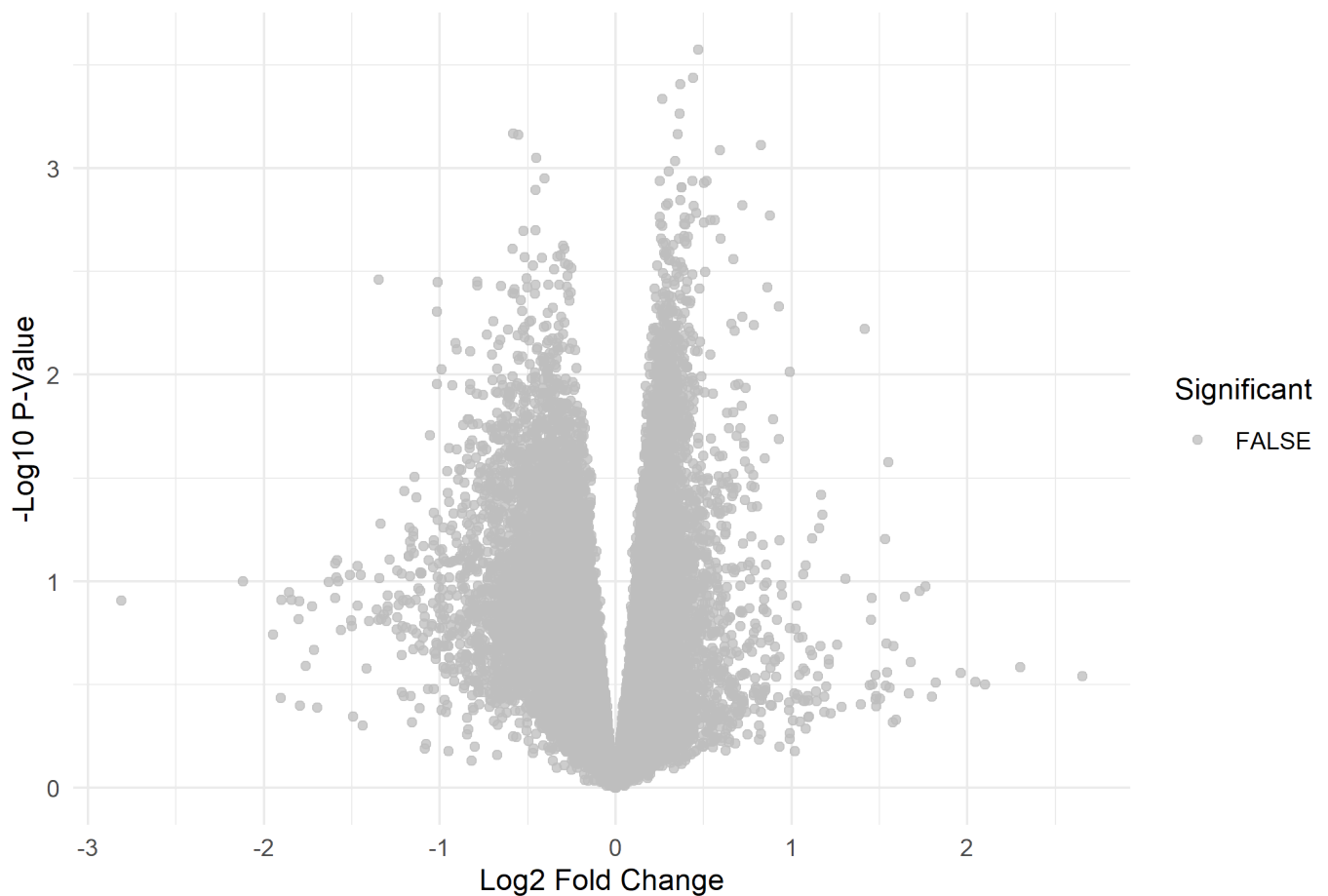
Volcano Vancomizina

## Volcano Plot: Infected vs Uninfected Linezolid



Volcano Linezolid

## Volcano Plot: Infected vs Uninfected Linezolid



con Array Quality Metrix, se observa sobre todo en sin tratamiento que están subexpresadas, además en la parte baja. Esto indica que los cambios observados (la subexpresión) no son considerados estadísticamente relevantes según el umbral. Así pues, los puntos en esa región representan características que están subexpresadas, pero no tienen suficiente evidencia estadística para ser consideradas diferencialmente expresadas con confianza. Estas observaciones podrían ser ruido experimental o requerir más muestras para confirmar su significancia.

## 2.3 Normalización

### Datos Normalizados

[Show](#)

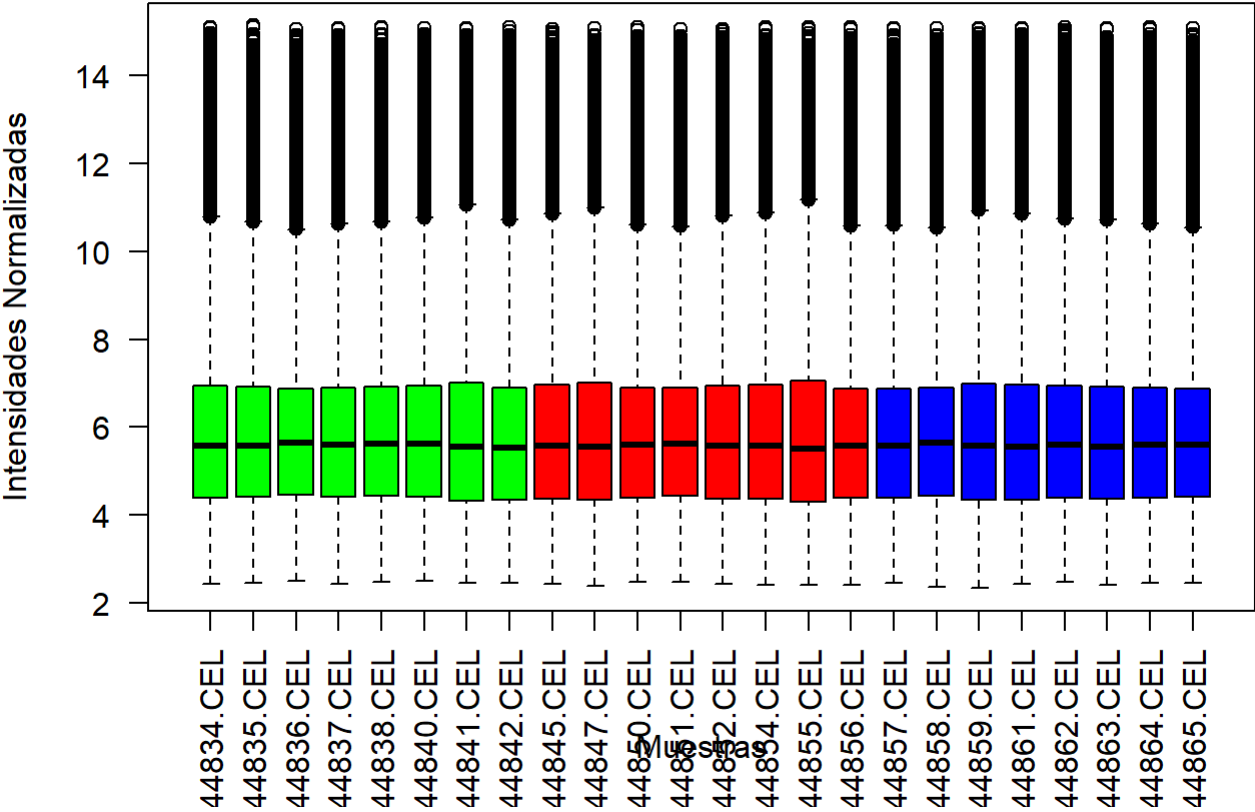
Background correcting  
Normalizing  
Calculating Expression

[Show](#)

```
      muestra      infeccion      hora tratamiento
GSM944857.29 GSM944857 S. aureus USA300 hour 24 linezolid
GSM944850.28 GSM944850 S. aureus USA300 hour 24 linezolid
GSM944836.26 GSM944836 S. aureus USA300 hour 24 linezolid
GSM944864.30 GSM944864 S. aureus USA300 hour 24 linezolid
GSM944861.10 GSM944861      uninfected hour 0 linezolid
GSM944847.8  GSM944847      uninfected hour 0 linezolid
GSM944840.7  GSM944840      uninfected hour 0 linezolid
GSM944854.9  GSM944854      uninfected hour 0 linezolid
GSM944863.25 GSM944863 S. aureus USA300 hour 24 untreated
GSM944842.22 GSM944842 S. aureus USA300 hour 24 untreated
GSM944835.21 GSM944835 S. aureus USA300 hour 24 untreated
GSM944856.24 GSM944856 S. aureus USA300 hour 24 untreated
GSM944838.2  GSM944838      uninfected hour 0 untreated
GSM944859.5  GSM944859      uninfected hour 0 untreated
GSM944845.3  GSM944845      uninfected hour 0 untreated
GSM944852.4  GSM944852      uninfected hour 0 untreated
GSM944865.35 GSM944865 S. aureus USA300 hour 24 vancomycin
GSM944851.33 GSM944851 S. aureus USA300 hour 24 vancomycin
[ reached 'max' / getOption("max.print") -- omitted 6 rows ]
```

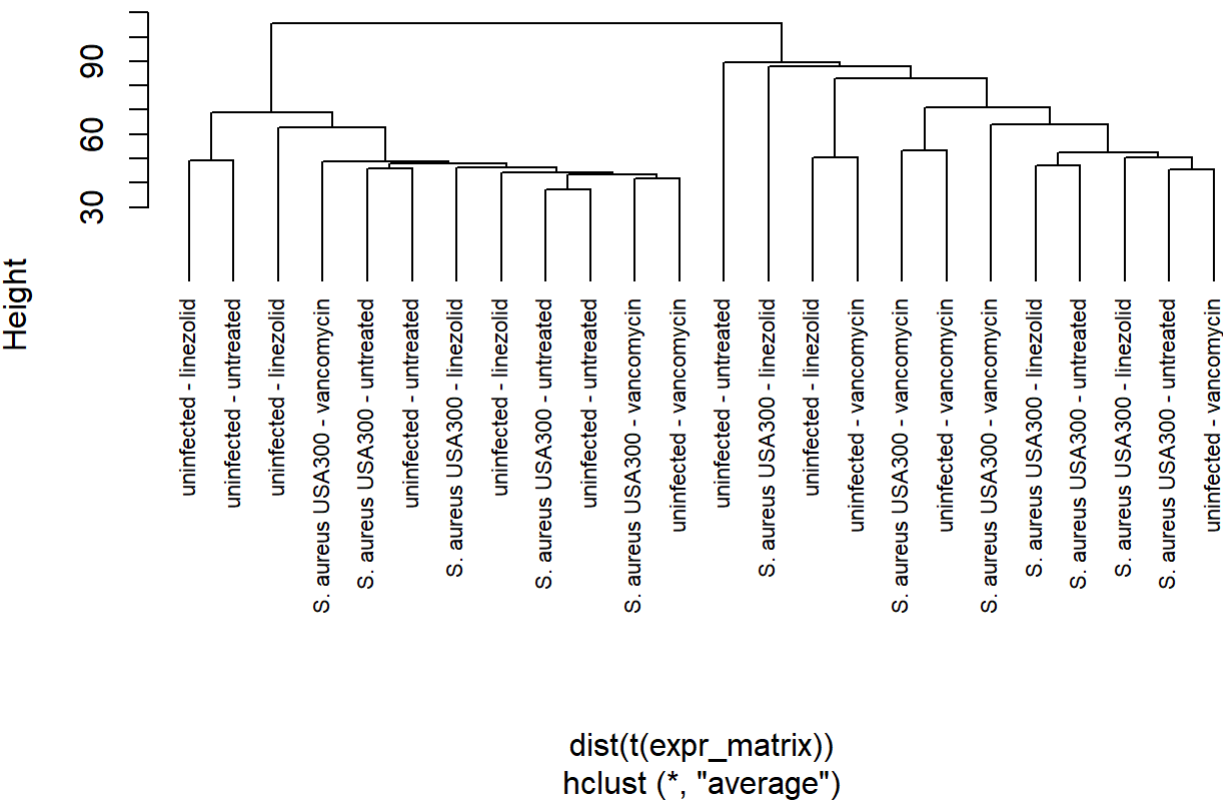
[Show](#)[Show](#)

Distribución de intensidades normalizadas



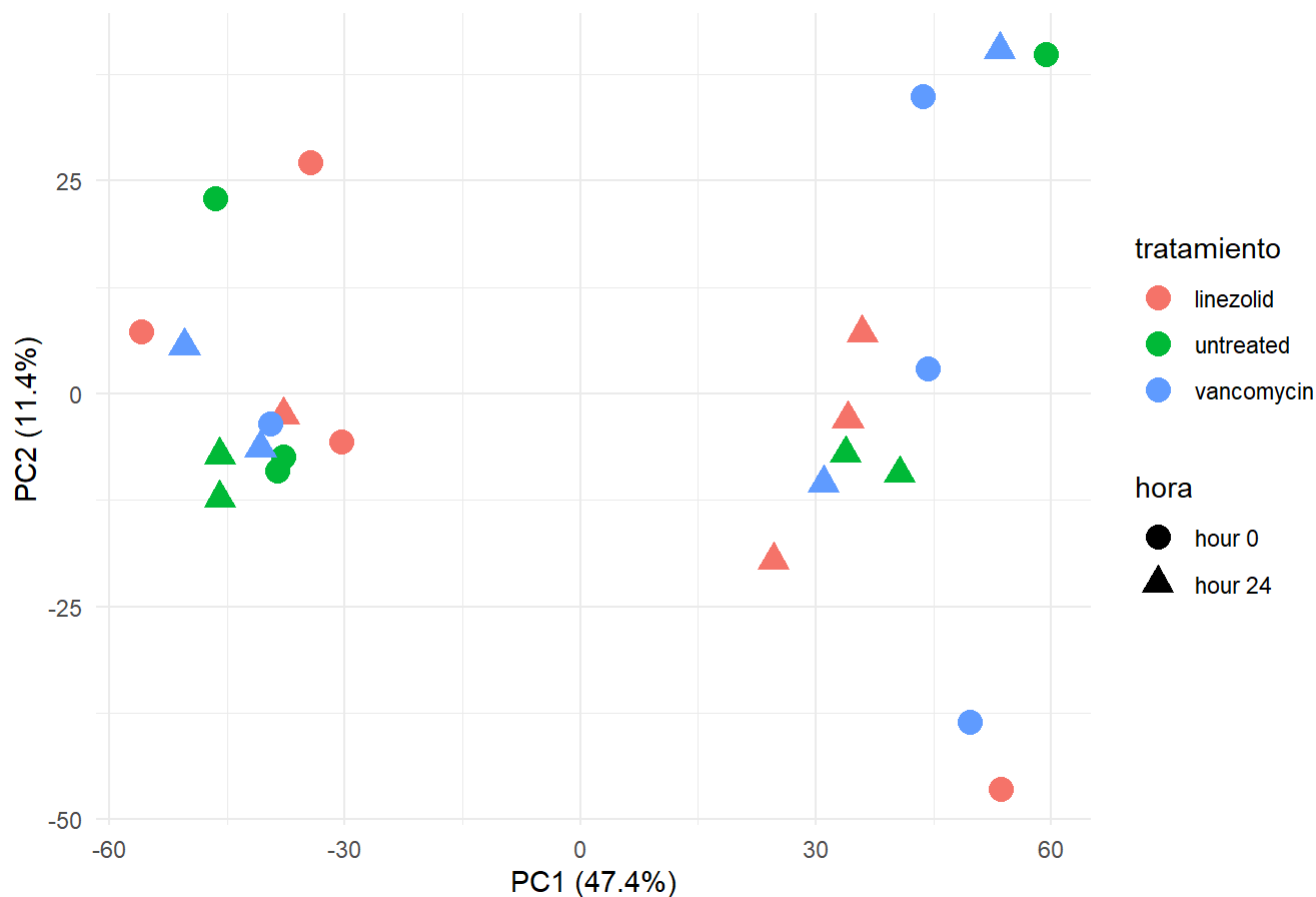
Show

Clustering jerárquico de datos normalizados



Show

### PCA de intensidades normalizadas



Las dos primeras componentes explican casi un 60% de la variabilidad en los grupos, pero no se puede afirmar diferencias en los grupos ni por infectado=24h, no infectado=0h, ni por tratamiento.

## 2.4 Filtrado

[Show](#)[Show](#)[Show](#)



```

$eset
ExpressionSet (storageMode: lockedEnvironment)
assayData: 6144 features, 24 samples
  element names: exprs
protocolData
  sampleNames: GSM944834.CEL GSM944835.CEL ... GSM944865.CEL (24 total)
  varLabels: ScanDate
  varMetadata: labelDescription
phenoData
  sampleNames: GSM944857.29 GSM944850.28 ... GSM944855.14 (24 total)
  varLabels: muestra infeccion hora tratamiento
  varMetadata: labelDescription
featureData: none
experimentData: use 'experimentData(object)'
Annotation: mouse4302.db

```

```

$filter.log
$filter.log$numDupsRemoved
[1] 16958

```

```

$filter.log$numLowVar
[1] 14336

```

```

$filter.log$numRemoved.ENTREZID
[1] 7650

```

```

$filter.log$feature.exclude
[1] 13

```

numDupsRemoved: 16,958 sondas duplicadas eliminadas. numLowVar: 14,336 sondas de baja varianza eliminadas. numRemoved.ENTREZID: 7,650 sondas eliminadas por no tener un ENTREZID. feature.exclude: 13 sondas eliminadas por otros criterios.

[Show](#)

Features	Samples
45101	24

[Show](#)

Features	Samples
6144	24

[Show](#)

Número de genes eliminados: 38957

[Show](#)

## 2.5 Selección de genes

[Show](#)

```

      S. aureus USA300.linezolid uninfected.linezolid
GSM944857.29                1                0
GSM944850.28                1                0
GSM944836.26                1                0
GSM944864.30                1                0
GSM944861.10                0                1
GSM944847.8                 0                1
GSM944840.7                 0                1
GSM944854.9                 0                1
GSM944863.25                0                0
GSM944842.22                0                0
GSM944835.21                0                0
GSM944856.24                0                0

      S. aureus USA300.untreated uninfected.untreated
GSM944857.29                0                0
GSM944850.28                0                0
GSM944836.26                0                0
GSM944864.30                0                0
GSM944861.10                0                0
GSM944847.8                 0                0
GSM944840.7                 0                0
GSM944854.9                 0                0
GSM944863.25                1                0
GSM944842.22                1                0
GSM944835.21                1                0
GSM944856.24                1                0

      S. aureus USA300.vancomycin uninfected.vancomycin
GSM944857.29                0                0
GSM944850.28                0                0
GSM944836.26                0                0
GSM944864.30                0                0
GSM944861.10                0                0
GSM944847.8                 0                0
GSM944840.7                 0                0
GSM944854.9                 0                0
GSM944863.25                0                0
GSM944842.22                0                0
GSM944835.21                0                0
GSM944856.24                0                0

[ reached getOption("max.print") -- omitted 12 rows ]
attr("assign")
[1] 1 1 1 1 1 1
attr("contrasts")
attr("contrasts")$lev
[1] "contr.treatment"

```

[Show](#)

Contrasts	
Levels	Infected_vs_Uninfected_Untreated
S..aureus.USA300.linezolid	0
uninfected.linezolid	0
S..aureus.USA300.untreated	1
uninfected.untreated	-1
S..aureus.USA300.vancomycin	0
uninfected.vancomycin	0

Contrasts	
Levels	Infected_vs_Uninfected_Vancomycin
S..aureus.USA300.linezolid	0
uninfected.linezolid	0
S..aureus.USA300.untreated	0
uninfected.untreated	0
S..aureus.USA300.vancomycin	1
uninfected.vancomycin	-1

Contrasts	
Levels	Infected_vs_Uninfected_Linezolid
S..aureus.USA300.linezolid	1
uninfected.linezolid	-1
S..aureus.USA300.untreated	0
uninfected.untreated	0
S..aureus.USA300.vancomycin	0
uninfected.vancomycin	0

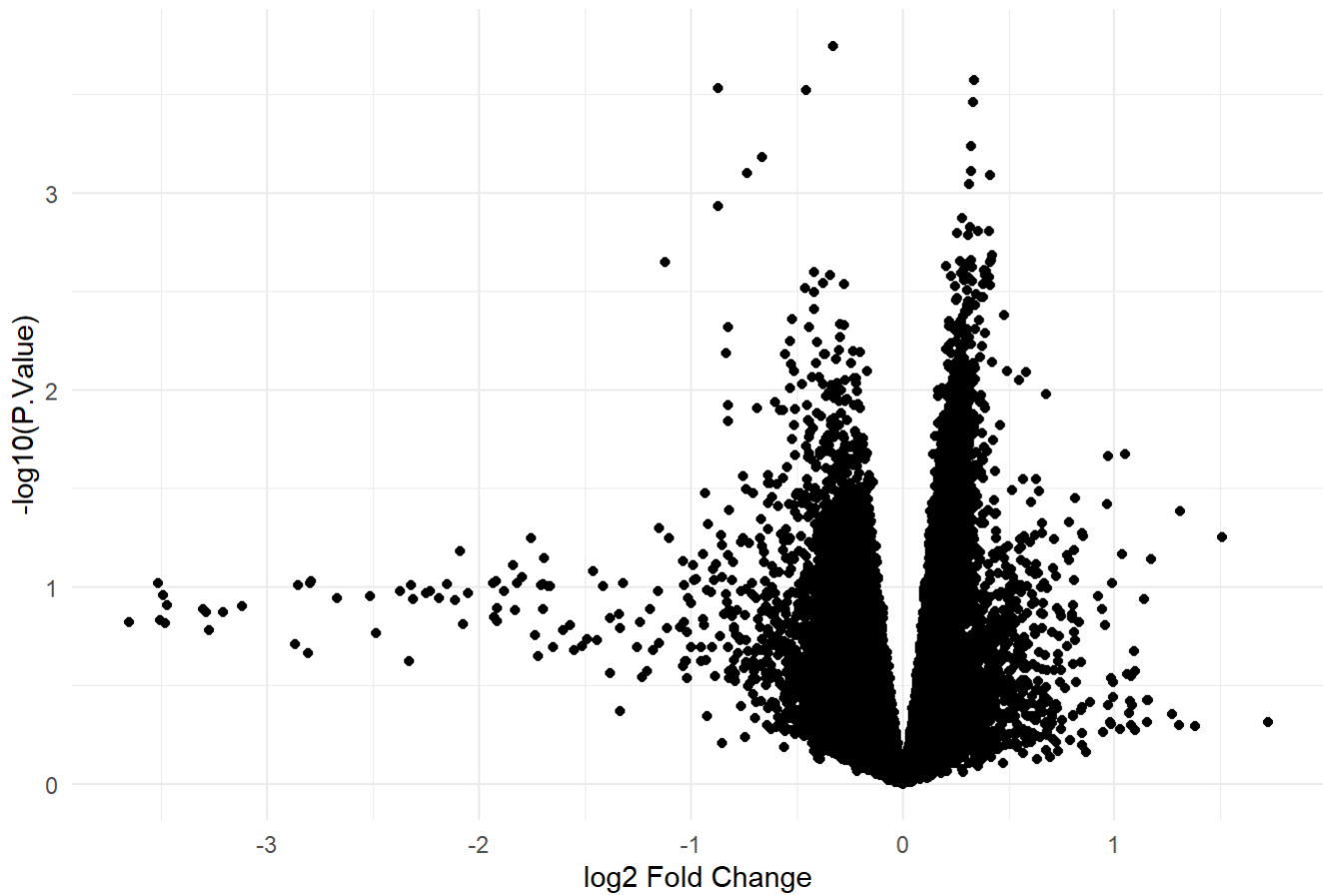
[Show](#)

	logFC	AveExpr	t	P.Value	adj.P.Val	B
1429988_at	-0.3303309	4.183678	-4.549792	0.0001789897	0.999589	-2.326058
1453759_at	0.3354145	6.217915	4.379694	0.0002680904	0.999589	-2.434572
1452565_x_at	-0.8709565	6.693986	-4.342393	0.0002929548	0.999589	-2.458750
1459733_at	-0.4566668	4.839545	-4.331049	0.0003009657	0.999589	-2.466129
1441695_at	0.3320004	4.833566	4.272693	0.0003457820	0.999589	-2.504286
1456211_at	0.3229163	3.916426	4.054857	0.0005806244	0.999589	-2.649500
1448804_at	-0.6633339	5.661398	-4.001101	0.0006598030	0.999589	-2.685979
1421488_at	0.3213472	5.155091	3.932645	0.0007763781	0.999589	-2.732781
1425469_a_at	-0.7361754	8.273785	-3.922565	0.0007951926	0.999589	-2.739704
1444624_at	0.4127577	6.327284	3.913672	0.0008121688	0.999589	-2.745820

logFC: Los genes con valores extremos (positivos o negativos) son los más interesantes para interpretación biológica. P.Value vs adj.P.Val: Aunque los valores p son bajos, los valores ajustados son altos (0.999589), lo que indica que no hay genes significativos tras el ajuste. B: Todos los valores de B son negativos, lo que sugiere una baja confianza en que estos genes sean diferencialmente expresados.

[Show](#)[Show](#)

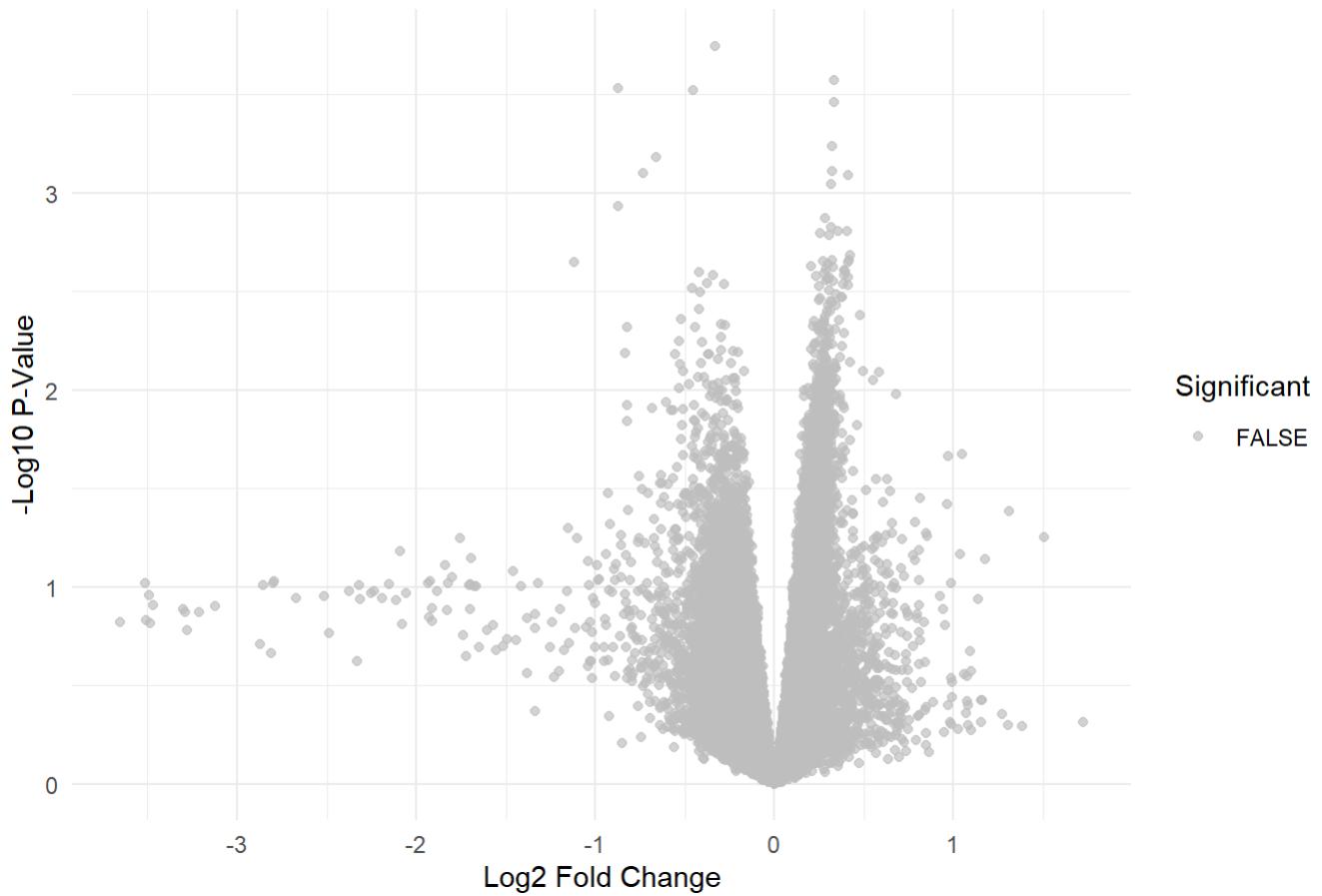
## Volcano Plot

[Show](#)

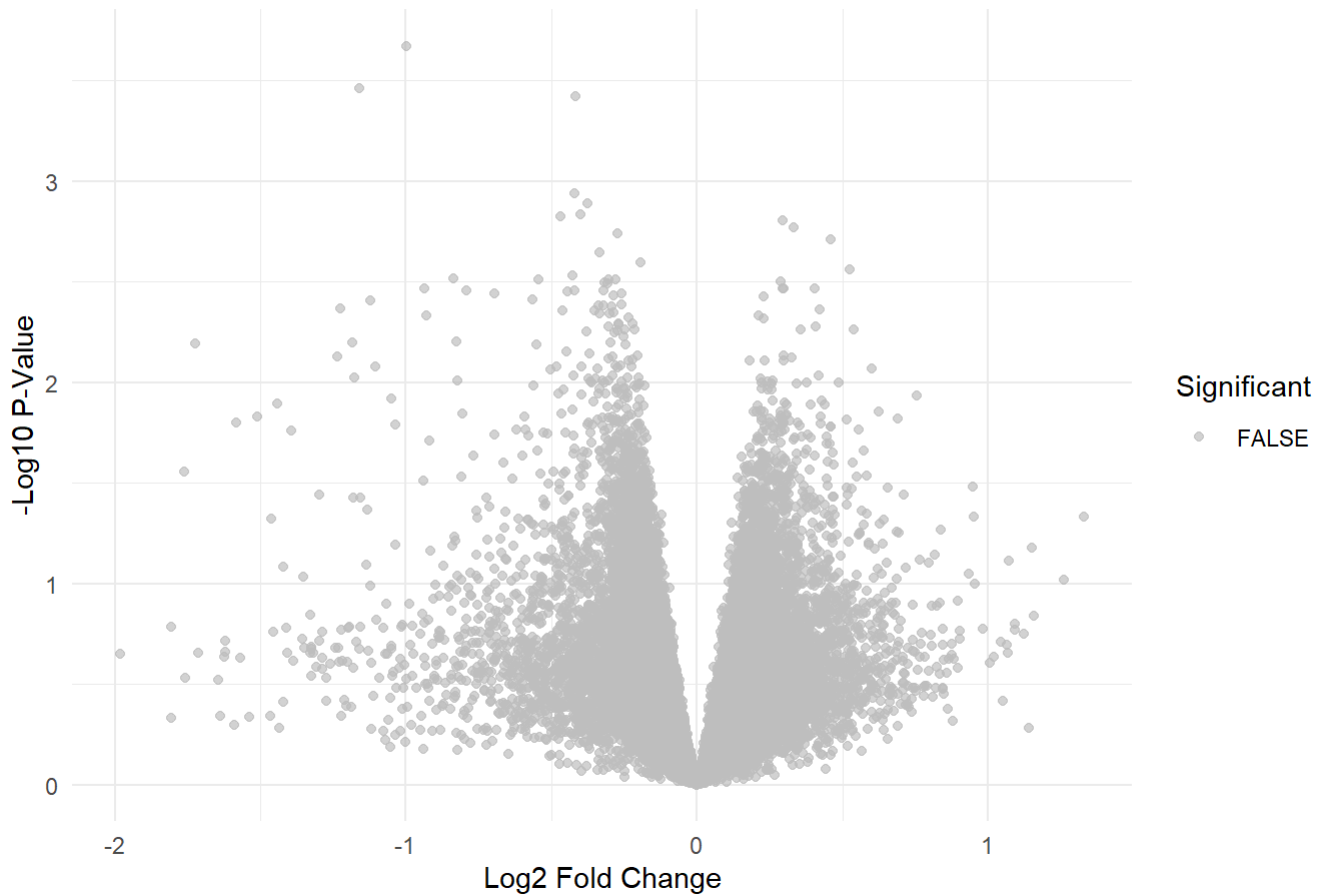
	PROBEID	ENTREZID	SYMBOL
1	1415670_at	54161	Copg1
2	1415671_at	11972	Atp6v0d1
3	1415672_at	57437	Golga7
4	1415673_at	100678	Psph
5	1415674_a_at	60409	Trappc4
6	1415675_at	13481	Dpm2

[Show](#)[Show](#)[Show](#)[Show](#)

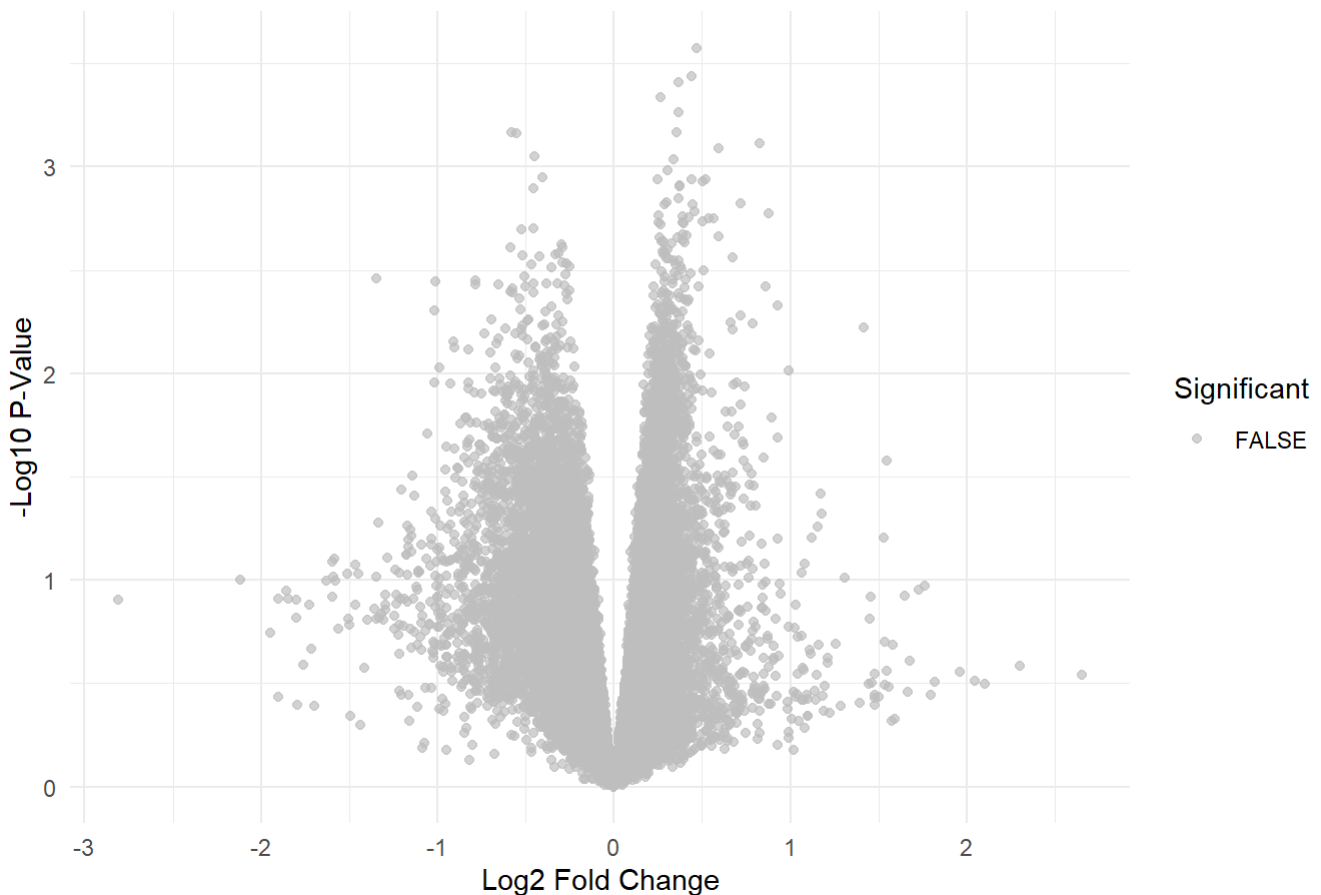
## Volcano Plot: Infected vs Uninfected Untreated

[Show](#)

## Volcano Plot: Infected vs Uninfected Vancomycin

[Show](#)

## Volcano Plot: Infected vs Uninfected Linezolid

[Show](#)

## 3 Resultados

### 3.1 Anotación de resultados

[Show](#)

```
[1] "ACCNUM"      "ALIAS"      "ENSEMBL"    "ENSEMBLPROT" "ENSEMBLTRANS"
[6] "ENTREZID"    "ENZYME"     "EVIDENCE"   "EVIDENCEALL" "GENENAME"
[11] "GENETYPE"    "GO"         "GOALL"      "IPI"         "MGI"
[16] "ONTOLOGY"    "ONTOLOGYALL" "PATH"       "PFAM"        "PMID"
[21] "PROBEID"     "PROSITE"    "REFSEQ"     "SYMBOL"      "UNIPROT"
```

[Show](#)

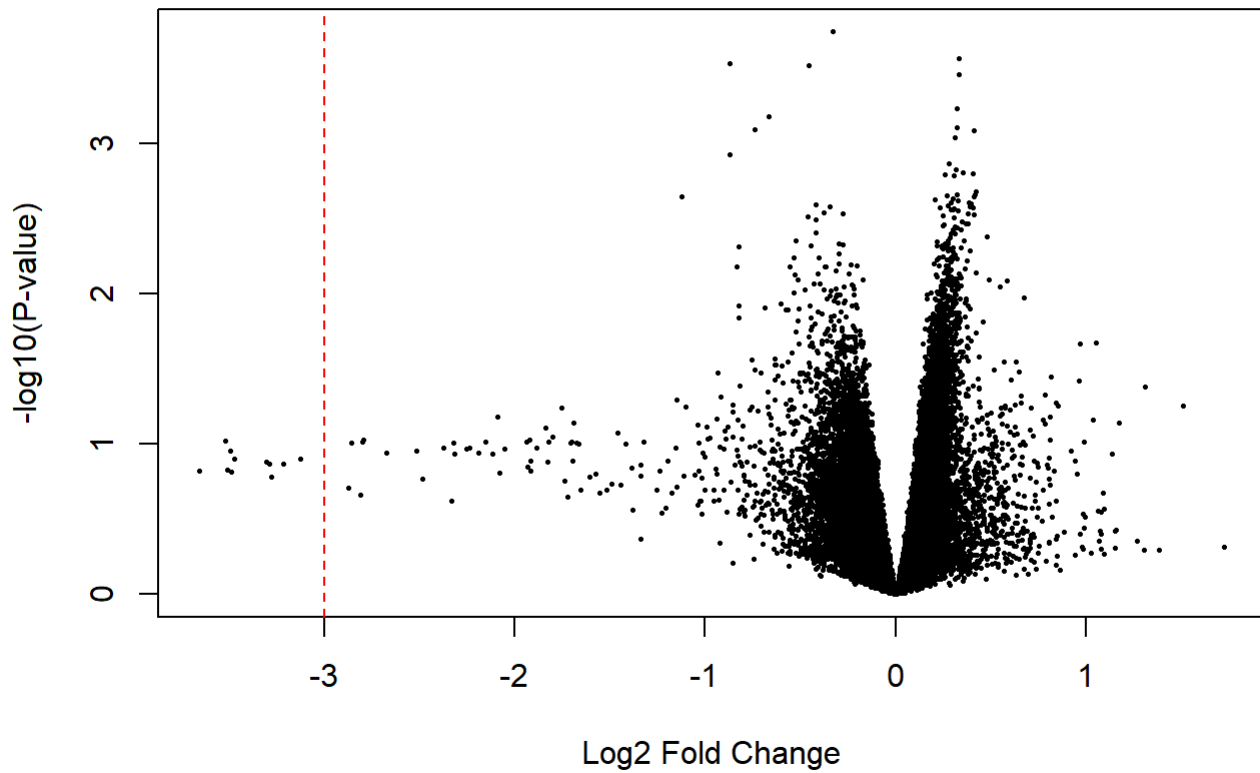
```
[1] PROBEID ENTREZID SYMBOL
<0 rows> (o 0- extensión row.names)
```

### 3.2 Visualización de resultados

#### 3.2.1 Volcano-plot

[Show](#)[Show](#)

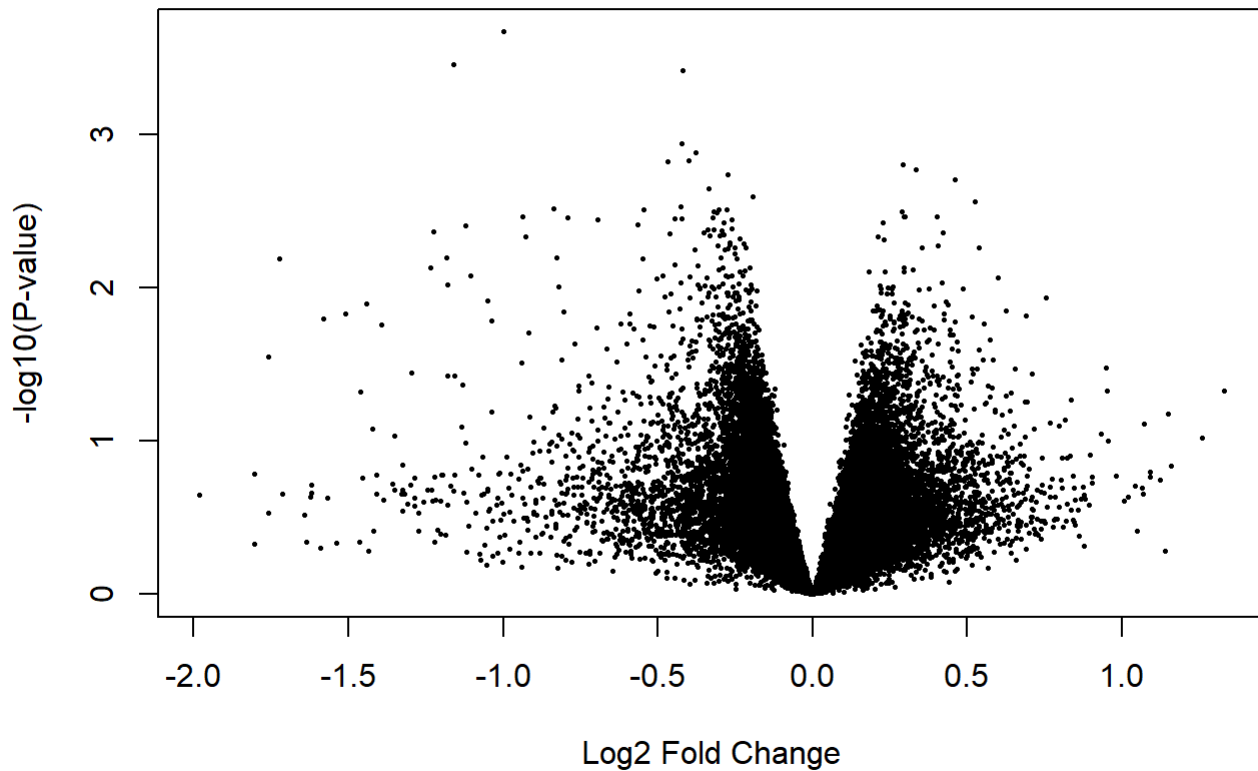
## Volcano Plot: Infected vs Uninfected Untreated

[Show](#)

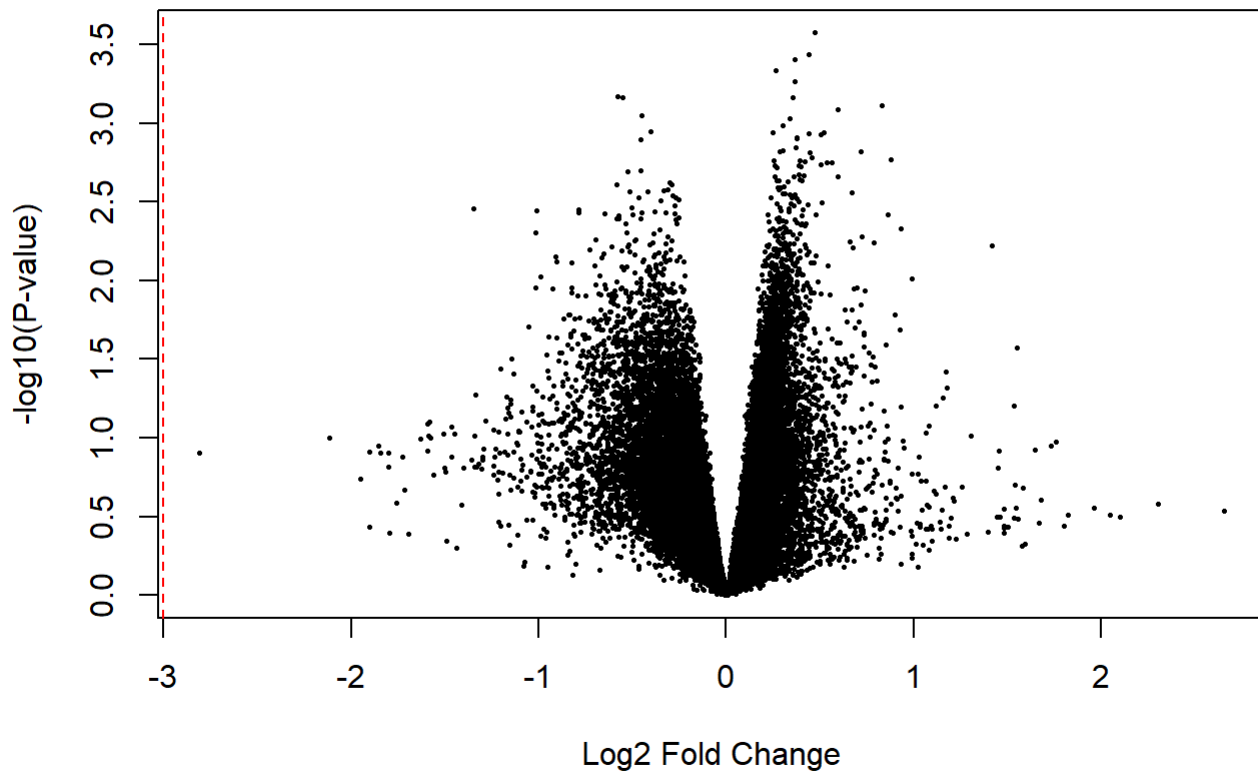
png  
2

[Show](#)

### Volcano Plot: Infected vs Uninfected Vancomycin

[Show](#)

### Volcano Plot: Infected vs Uninfected Linezolid

[Show](#)



```
png
2
```

[Show](#)

```
png
2
```

## 3.3 Analisis de significación biológica

[Show](#)

```
[1] GOBPID      Pvalue      OddsRatio ExpCount  Count      Size      Term
<0 rows> (o 0- extensión row.names)
```

[Show](#)

```
[1] 0 7
```

[Show](#)

## 4 Discusión

## 5 Referencias

<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE38531>

(<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE38531>)

<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GPL1261>

(<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GPL1261>)

<https://pmc.ncbi.nlm.nih.gov/articles/PMC3614971/> (<https://pmc.ncbi.nlm.nih.gov/articles/PMC3614971/>)

[https://github.com/GilCaraballo/PEC2\\_Datos\\_Omicos](https://github.com/GilCaraballo/PEC2_Datos_Omicos) ([https://github.com/GilCaraballo/PEC2\\_Datos\\_Omicos](https://github.com/GilCaraballo/PEC2_Datos_Omicos))

## 6 Apendices