

Differential Expression Analysis

Aspergillus fumigatus conidia infection of airway epithelial cells

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Introduction

Aspergillus fumigatus is a ubiquitous fungus and opportunistic pathogen that causes serious infections in people with weakened immune systems. The interaction between its inhaled conidia and respiratory epithelial cells is a critical step in the infectious process. Understanding the molecular changes in these cells after contact provides insight into the pathogenesis of aspergillosis and identifies new therapeutic targets (Dagenais & Keller (2009)).

In this report, I analyzed RNA-seq data from primary human bronchial epithelial (HBEC) cells following interaction with *A. fumigatus* conidia at 2, 6, and 12 hours. The goal is to identify differentially expressed involved in the host response to infection.

Methodology

To fulfill the objectives of this project, I performed an analysis using `limma` and `recount3` R packages. The analysis was conducted on the `recount3` datasets, which provides access to a large collection of RNA-seq data. The `limma` package was used to identify differentially expressed genes between the conditions of interest.

To select a dataset I first explored some of the available projects, I runned the following command:

```
if (!requireNamespace("BiocManager", quietly = TRUE)) {  
    install.packages("BiocManager")  
}  
  
if (!requireNamespace("recount3", quietly = TRUE)) {  
    BiocManager::install("recount3")  
}
```

```
}
```

```
options(recount3_url = "https://data.idies.jhu.edu/recount3/data/")
```

```
invisible(library(recount3))
```

```
## Selecting a project of interest
```

```
human_projects <- available_projects()
```

```
project_name <- "SRP048565"
```

```
project_info <- subset(
```

```
    human_projects,
```

```
    project == project_name & project_type == "data_sources"
```

```
)
```

```
## Cleaning unused variables
```

```
rm(human_projects, project_name)
```

```
invisible(gc())
```

```
project_info
```

project	organism	file_source	project_home	project_type	n_samples
29 SRP048565	human	sra	data_sources/sra	data_sources	18

Analysis

The project SRP048565 was selected for the analysis, which contains 18 samples from primary human bronchial epithelial (HBEC) cells following interaction with *A. fumigatus* conidia at 2, 6, and 12 hours in order to examine the infection of airway epithelial cells at multiple time points of co-incubation (see the [NCBI site](#)).

```
## Generating the object for the selected project
```

```
rse_SRP048565 <- create_rse(project_info)
```

```
## Exploring the object
```

```
rm(project_info)
```

```
rse_SRP048565
```

```

class: RangedSummarizedExperiment
dim: 63856 18
metadata(8): time_created recount3_version ... annotation recount3_url
assays(1): raw_counts
rownames(63856): ENSG00000278704.1 ENSG00000277400.1 ...
ENSG00000182484.15_PAR_Y ENSG00000227159.8_PAR_Y
rowData names(10): source type ... havana_gene tag
colnames(18): SRR1594040 SRR1594041 ... SRR1594056 SRR1594057
colData names(175): rail_id external_id ...
recount_pred.curated.cell_line BigWigURL

```

With the RSE object generated, I proceeded to transform the counts per nucleotide to counts per read.

```

assay(rse_SRP048565, "counts") <- compute_read_counts(rse_SRP048565)

## Exploring the sample attributes
head(rse_SRP048565$sra.sample_attributes)

```

```

[1] "cell type;;NHBE|infection;;Control|source_name;;NHBE cells, negative control, 2 hr|time
[2] "cell type;;NHBE|infection;;Control|source_name;;NHBE cells, negative control, 2 hr|time
[3] "cell type;;NHBE|infection;;Control|source_name;;NHBE cells, negative control, 2 hr|time
[4] "cell type;;NHBE|infection;;Control|source_name;;NHBE cells, negative control, 2 hr|time
[5] "cell type;;NHBE|infection;;Control|source_name;;NHBE cells, negative control, 6 hr|time
[6] "cell type;;NHBE|infection;;Control|source_name;;NHBE cells, negative control, 6 hr|time

```

Because there is consistency in the sample attributes, I expanded them to have a more detailed view of the experimental conditions and prepared the data for the statistical modeling.

```

## As in the class example, here I used the experiment data
rse_SRP048565 <- expand_sra_attributes(rse_SRP048565)
colData(rse_SRP048565)[
  ,
  grepl("^sra_attribute",
  colnames(colData(rse_SRP048565)))
]

```

```

DataFrame with 18 rows and 5 columns
  sra_attribute.cell_type sra_attribute.infection
  <character>           <character>

```

SRR1594040	NHBE	Control	
SRR1594041	NHBE	Control	
SRR1594042	NHBE	Control	
SRR1594043	NHBE	Control	
SRR1594044	NHBE	Control	
...	
SRR1594053	NHBE	Afum	
SRR1594054	NHBE	Afum	
SRR1594055	NHBE	Afum	
SRR1594056	NHBE	Afum	
SRR1594057	NHBE	Afum	
	sra_attribute.source_name	sra_attribute.time	sra_attribute.tissue
	<character>	<character>	<character>
SRR1594040	NHBE cells, negative..	2hr	lung
SRR1594041	NHBE cells, negative..	2hr	lung
SRR1594042	NHBE cells, negative..	2hr	lung
SRR1594043	NHBE cells, negative..	2hr	lung
SRR1594044	NHBE cells, negative..	6hr	lung
...
SRR1594053	NHBE cells, A. fumig..	2hr	lung
SRR1594054	NHBE cells, A. fumig..	6hr	lung
SRR1594055	NHBE cells, A. fumig..	6hr	lung
SRR1594056	NHBE cells, A. fumig..	12hr	lung
SRR1594057	NHBE cells, A. fumig..	12hr	lung

```

## Statistical modeling preparation of my interest variables
rse_SRP048565$sra_attribute.time <- factor(
  rse_SRP048565$sra_attribute.time,
  levels = c("2hr", "6hr", "12hr")
)
rse_SRP048565$sra_attribute.infection <- factor(
  rse_SRP048565$sra_attribute.infection,
  levels = c("Control", "Afum")
)

## Summary of this variables
summary(as.data.frame(colData(rse_SRP048565) [
  ,
  grep("sra_attribute.(time|infection)$",
  colnames(colData(rse_SRP048565)))
]))
```

```

sra_attribute.infection sra_attribute.time
Control:12           2hr :6
Afum    : 6           6hr :6
                      12hr:6

```

As it is observed, there are 6 samples for each time point (2, 6, and 12 hours) and for each condition (infected and non-infected). This allows for a balanced design in the differential expression analysis. Because of the latter, time and infection were used as the main variables of interest in the statistical modeling.

Data Quality Assessment and Filtering

```

## Assessing data quality through QC
rse_SRP048565$assigned_gene_prop <-
  rse_SRP048565$recount_qc.gene_fc_count_all.assigned /
  rse_SRP048565$recount_qc.gene_fc_count_all.total
summary(rse_SRP048565$assigned_gene_prop)

```

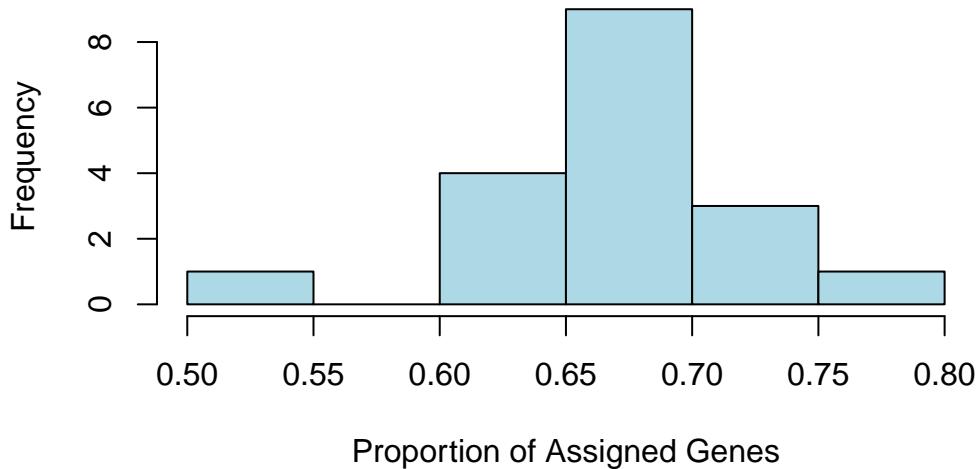
	Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
	0.5471	0.6484	0.6625	0.6692	0.6858	0.7764

```

## Visualizing the distribution of assigned gene proportions
hist(
  rse_SRP048565$assigned_gene_prop,
  main = "Distribution of Assigned Gene Proportions",
  xlab = "Proportion of Assigned Genes",
  col = "lightblue"
)

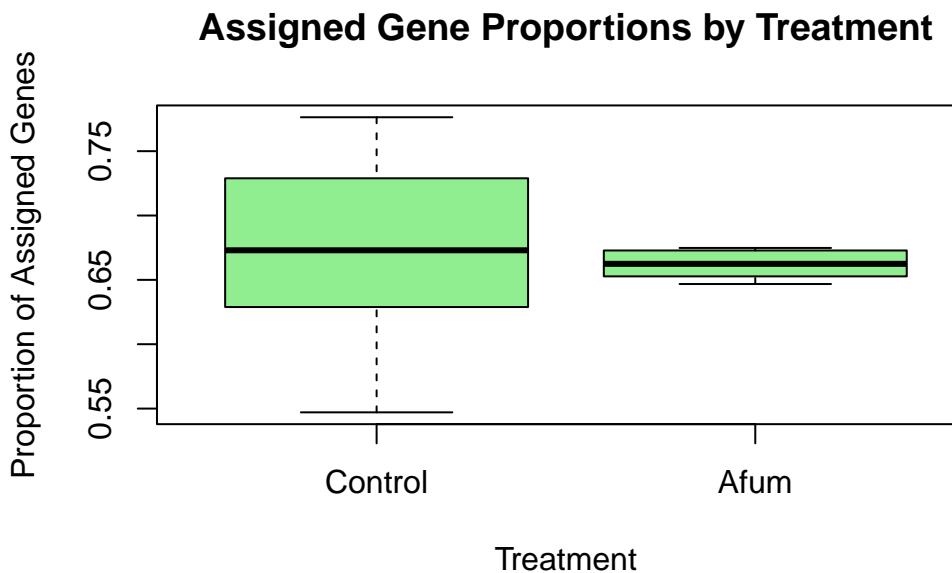
```

Distribution of Assigned Gene Proportions



The histogram of the assigned gene proportions shows that most samples have a high proportion of assigned genes, which is a **good indicator of data quality**. However, there is some variability among the samples, which could be due to differences in sequencing depth or sample quality. To further investigate this, I created a boxplot to compare the assigned gene proportions between the infected and non-infected samples across the different time points.

```
boxplot(  
  rse_SRP048565$assigned_gene_prop ~ rse_SRP048565$sra_attribute.infection,  
  main = "Assigned Gene Proportions by Treatment",  
  xlab = "Treatment",  
  ylab = "Proportion of Assigned Genes",  
  col = "lightgreen")
```



Because no evidence supported a difference in the assigned gene proportions between the infected and non-infected samples, I decided to filter the data using `edgeR::filterByExpr()` for gene expression, as the lowly expressed genes may not be biologically relevant and could introduce noise into the analysis.

```

if (!requireNamespace("edgeR", quietly = TRUE)) {
  BiocManager::install("edgeR", update = FALSE)
}

invisible(library("edgeR"))

## Extracting the count matrix
counts_matrix <- assay(rse_SRP048565)

## Information about the groups
group <- as.factor(rse_SRP048565$sra_attribute.infection)

## Expression filter
keep_genes <- filterByExpr(counts_matrix, group=group)

## Fetching the filtered data
rse_SRP048565_filtered <- rse_SRP048565[keep_genes,]

```

```

## Exploring dimensions of the filtered data
dim(rse_SRP048565_filtered)

[1] 32710     18

rm(group, keep_genes)
invisible(gc())

## Percentage of genes retained after filtering
round(nrow(rse_SRP048565_filtered) * 100 / nrow(rse_SRP048565), 2)

```

[1] 51.22

After applying the filtration function to the data, I retained approximately 51.2% of the genes, which indicates that a significant portion of the lowly expressed genes were removed from the analysis. This step is crucial for improving the statistical power of the differential expression analysis and reducing the likelihood of false positives.

Normalization

```

## Scaling factors for normalization
dge <- DGEList(
  counts = assay(rse_SRP048565_filtered, "counts"),
  genes = rowData(rse_SRP048565_filtered)
)
dge <- calcNormFactors(dge)

```

Normalization of RNA-seq expression data is necessary because compositional bias and differences in library sizes can distort the results of differential expression analysis (Robinson & Oshlack (2010)). The `calcNormFactors` function from the `edgeR` package estimates scaling factors (by default using the TMM method) to adjust for these biases between samples. These normalization factors are incorporated into the statistical model, ensuring that expression levels are comparable across samples and enabling accurate identification of differentially expressed genes.

Differential Expression Analysis

Differential expression was assessed by comparing infected vs non-infected samples within each time point (2, 6, and 12 hours). To enable time-specific contrasts, we modeled each time-infection combination as a separate group in the design matrix. This approach provides straightforward contrasts for infected vs. control at each time point while accounting for baseline differences among time points.

```
if (!requireNamespace("limma", quietly = TRUE)) {
  BiocManager::install("limma", update = FALSE)
}

library("limma")

## Fetching metadata for the model matrix
time <- droplevels(rse_SRP048565_filtered$sra_attribute.time)
infection <- droplevels(rse_SRP048565_filtered$sra_attribute.infection)

## Combined group factor of time x infection
group <- interaction(time, infection, sep = "_", drop = TRUE)
```

The design matrix was structured to include separate groups for each time-infection combination, so there was no intercept as each computed coefficient corresponds to a specific time-infection group average expression.

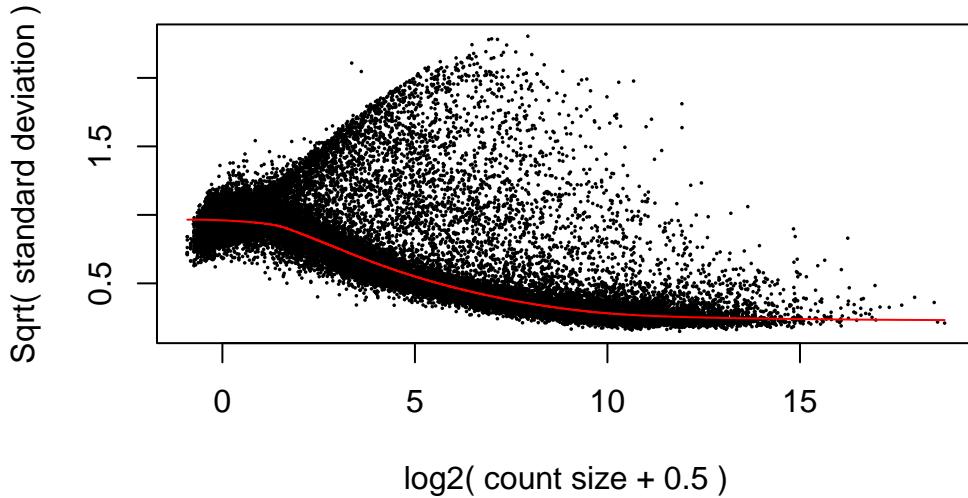
```
## Design matrix without intercept
mod <- model.matrix(~ 0 + group)

## Viewing the model matrix levels
colnames(mod) <- levels(group)
colnames(mod) <- paste0("tr", colnames(mod))
colnames(mod)

[1] "tr2hr_Control"  "tr6hr_Control"  "tr12hr_Control" "tr2hr_Afum"
[5] "tr6hr_Afum"      "tr12hr_Afum"

vGene <- voom(dge, mod, plot = TRUE)
```

voom: Mean–variance trend



The plot shows that the samples with lower count sizes have higher variability, meaning that the genes with low expression levels tend to have more distance with the mean in their expression measurements, as they are more affected by technical noise and sampling variability.

```

## The model matrix is already contained in the voom object, so we can
## directly fit the linear model
fit <- lmFit(vGene)

## Contrasts for infected vs control at each time point
cont <- makeContrasts(
  Inf_vs_Ctrl_2hr  = tr2hr_Afum - tr2hr_Control,
  Inf_vs_Ctrl_6hr  = tr6hr_Afum - tr6hr_Control,
  Inf_vs_Ctrl_12hr = tr12hr_Afum - tr12hr_Control,
  levels = mod
)

## Re-expressing the model fit with the specified contrasts
## Adjust the variance estimates for the contrasts
eb_results <- eBayes(contrasts.fit(fit, cont))

## Tables
tt_2hr  <- topTable(eb_results, coef="Inf_vs_Ctrl_2hr", number=Inf)
tt_6hr  <- topTable(eb_results, coef="Inf_vs_Ctrl_6hr", number=Inf)

```

```
tt_12hr <- topTable(eb_results, coef="Inf_vs_Ctrl_12hr", number=Inf)

dim(tt_12hr)
```

```
[1] 32710    16
```

All genes are considered in each table (all have the same dimensions), sorted by their significance with an FDR < 0.05, this election allows me to filter according to an specified threshold.

Time-specific DEGs

2 hours

```
## DEGs with FDR < 0.05
table(tt_2hr$adj.P.Val < 0.05)
```

```
FALSE  TRUE
32455  255
```

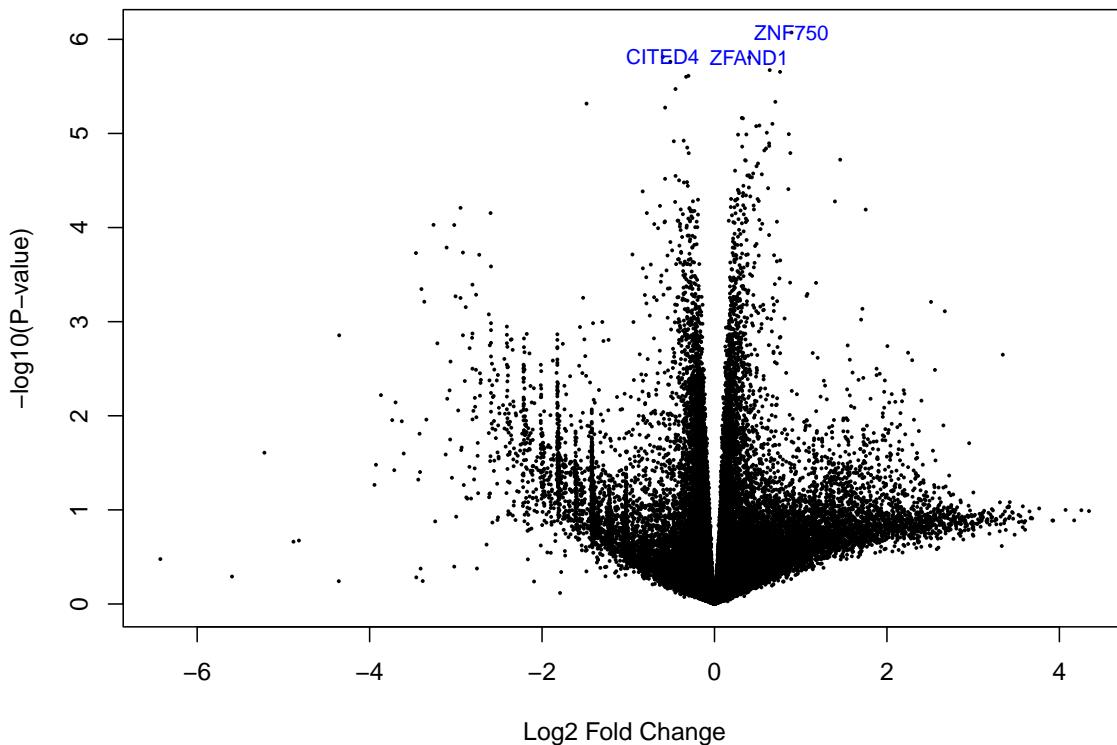
```
## DEGs with FDR < 0.05
head(
  tt_2hr[(tt_2hr$adj.P.Val < 0.05) & (abs(tt_2hr$logFC) > 1), ],
  n = 5
)
```

	source	type	bp_length	phase	gene_id	gene_type	gene_name	level	havana_gene	tag
ENSG00000283480.1	HAVANA	gene	1780	NA	ENSG00000283480.1					
ENSG00000136688.10	HAVANA	gene	1380	NA	ENSG00000136688.10					
ENSG00000125740.13	HAVANA	gene	5553	NA	ENSG00000125740.13					
ENSG00000169429.10	HAVANA	gene	2274	NA	ENSG00000169429.10					
ENSG00000023445.13	HAVANA	gene	7518	NA	ENSG00000023445.13					
ENSG00000283480.1		antisense	RP11-204E9.3	2	OTTHUMG00000191690.1	<NA>				
ENSG00000136688.10		protein_coding		IL36G	2	OTTHUMG00000131336.4	<NA>			
ENSG00000125740.13		protein_coding		FOSB	1	OTTHUMG00000182122.2	<NA>			
ENSG00000169429.10		protein_coding		CXCL8	2	OTTHUMG00000151316.1	<NA>			

	protein_coding	BIRC3	1	OTTHUMG00000167324.4	<NA>
	logFC	AveExpr	t	P.Value	adj.P.Val
ENSG00000283480.1	-1.484047	0.6380456	-7.043327	4.817394e-06	0.01432518
ENSG00000136688.10	1.457682	0.9990654	6.224062	1.900956e-05	0.01869481
ENSG00000125740.13	1.396777	1.0245256	5.647477	5.279353e-05	0.02461517
ENSG00000169429.10	1.753640	2.6075758	5.537929	6.443203e-05	0.02474002
ENSG00000023445.13	1.177662	1.3533451	4.587902	3.870321e-04	0.04998812
	B				
ENSG00000283480.1	3.8265465				
ENSG00000136688.10	3.1315447				
ENSG00000125740.13	2.0916660				
ENSG00000169429.10	1.9010806				
ENSG00000023445.13	0.3127872				

```
## Reorganizing the gene names with the LM results order
gene_names <- rowData(rse_SRP048565_filtered)$gene_name
names(gene_names) <- rownames(rse_SRP048565_filtered)

volcanoplot(
  eb_results,
  coef = "Inf_vs_Ctrl_2hr",
  highlight = 3,
  names = gene_names[rownames(eb_results)]
)
```



At the 2 hour time point, there are 255 DEGs with an FDR < 0.05, indicating an early host response to *A. fumigatus* infection. The volcano plot shows that most of the DEGs have a log fold change (logFC) close to zero, suggesting that the changes in gene expression are relatively modest at this early stage of infection. However, from the three most significant DEGs two are upregulated (*ZNF750* and *ZFAND1*) and one is downregulated for the infected samples compared to controls.

ZNF750 and *ZFAND1* both are transcription factors with zinc finger domains critical for regulating epithelial homeostasis and stress adaptation. The first contributes to epithelial maturation and barrier integrity. While the second regulates cytoplasmic stress granule turnover and facilitating proteasome recruitment during acute cellular stress, which supports protein quality control and recovery from exogenous insults.

In the other hand, the downregulated gene *CITED4* functions as a transcriptional coactivator interacting with *CBP/p300* and *TFAP2*, modulating RNA polymerase II-dependent gene expression and influencing transcriptional programs linked to cellular differentiation and stress responses.

There is also a gene with a $\log FC > 1$ and an $adj.P.Val < 0.05$, which is **IL36G**, a member of the IL-1 cytokine family, functions as a pro-inflammatory epithelial-derived cytokine that activates *NF-B* and *MAPK* signaling.

In the context of *Aspergillus fumigatus* conidia exposure in HBECs (an interaction known to **trigger inflammatory signaling, oxidative stress, and epithelial remodeling**), the upregulation of stress-response regulators such as *ZFAND1* and differentiation-associated factors like *ZNF750* may reflect **activation of epithelial defense and repair mechanisms**. Conversely, the downregulation of *CITED4* in infected cells could indicate **suppression or reprogramming of specific transcriptional coactivation pathways during the host response**, potentially shifting the epithelial transcriptional landscape toward stress and innate immune programs.

The upregulated expression of *IL36G* may indicate an early pro-inflammatory response to *A. fumigatus* exposure. This suggests that the infected HBECs are **initiating an inflammatory signaling cascade** in response to the fungal *conidia*, which could contribute to the recruitment of immune cells and the activation of downstream defense mechanisms.

```
if (!requireNamespace("pheatmap", quietly = TRUE)) {
  BiocManager::install("pheatmap", update = FALSE)
}
library("pheatmap")

## Extracting the genes of interest
expression_ht <- vGene$E[rank(tt_2hr$adj.P.Val) <= 50, ]

df <- as.data.frame(
  colData(rse_SRP048565_filtered)[
    ,
    "sra_attribute.infection",
    drop = FALSE
  ]
)
colnames(df) <- "Infection"

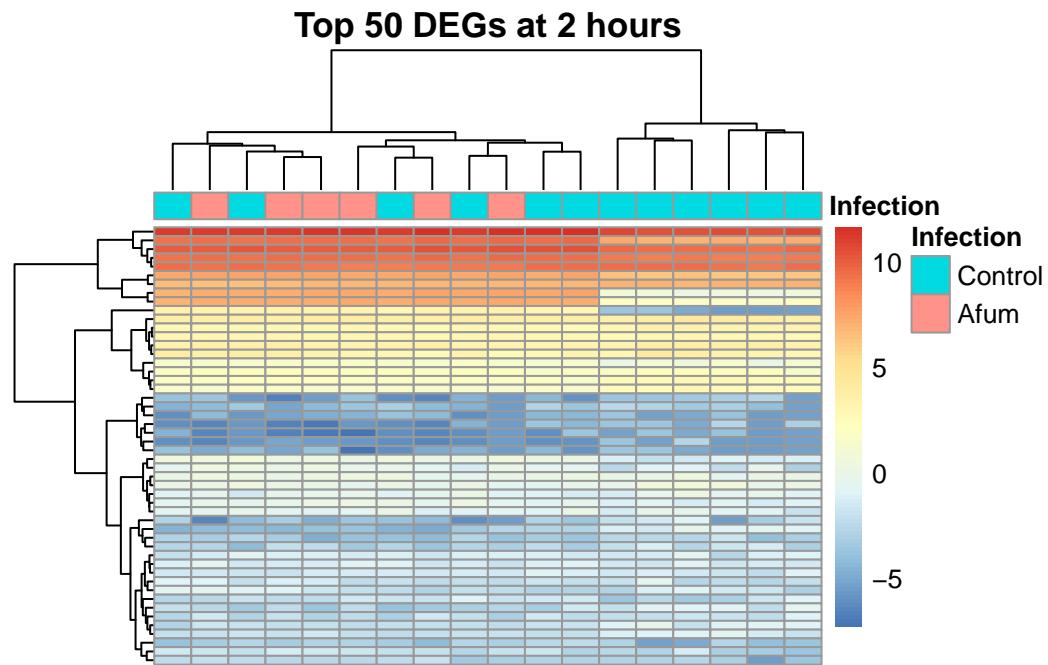
# (opcional pero recomendable) asegurar factor
df$Infection <- factor(df$Infection)

pheatmap(
  expression_ht,
  cluster_rows = TRUE,
  cluster_cols = TRUE,
  annotation_col = df,
  show_rownames = FALSE,
```

```

    show_colnames = FALSE,
    main = "Top 50 DEGs at 2 hours"
)

```



At the 2 hours-time point, two main clusters appear, one full of control samples and the other mixing both treatments. This might suggests that the infection samples have gene expression levels that are similar in a way to those shown in some control samples. If a Venn diagram would be made, several significant genes may be present in control and infection samples.

Still, most control samples cluster together showing low similarity with the infected ones.

6 hours

```
table(tt_6hr$adj.P.Val < 0.05)
```

```

FALSE
32710

```

```

## DEGs with FDR < 0.05
head(
  tt_6hr[(tt_6hr$adj.P.Val < 0.05) & (abs(tt_6hr$logFC) > 1), ],
  n = 5
)

```

```

[1] source      type       bp_length   phase      gene_id     gene_type
[7] gene_name   level      havana_gene tag        logFC       AveExpr
[13] t          P.Value    adj.P.Val  B
<0 rows> (or 0-length row.names)

```

At the 6-hour time point, there are no DEGs with an FDR < 0.05, suggesting an absent response to the pathogen in a more advanced stage of the illness. Because of the latter, it is possible that the host response is transient and peaks at an earlier time point, or that the response is more complex and involves changes in gene expression that are not captured by the statistical analysis at this time point.

12 hours

```
table(tt_12hr$adj.P.Val < 0.05)
```

```

FALSE  TRUE
28095 4615

```

```

head(
  tt_12hr[(tt_12hr$adj.P.Val < 0.05) & (abs(tt_12hr$logFC) > 1), n = 5
)

```

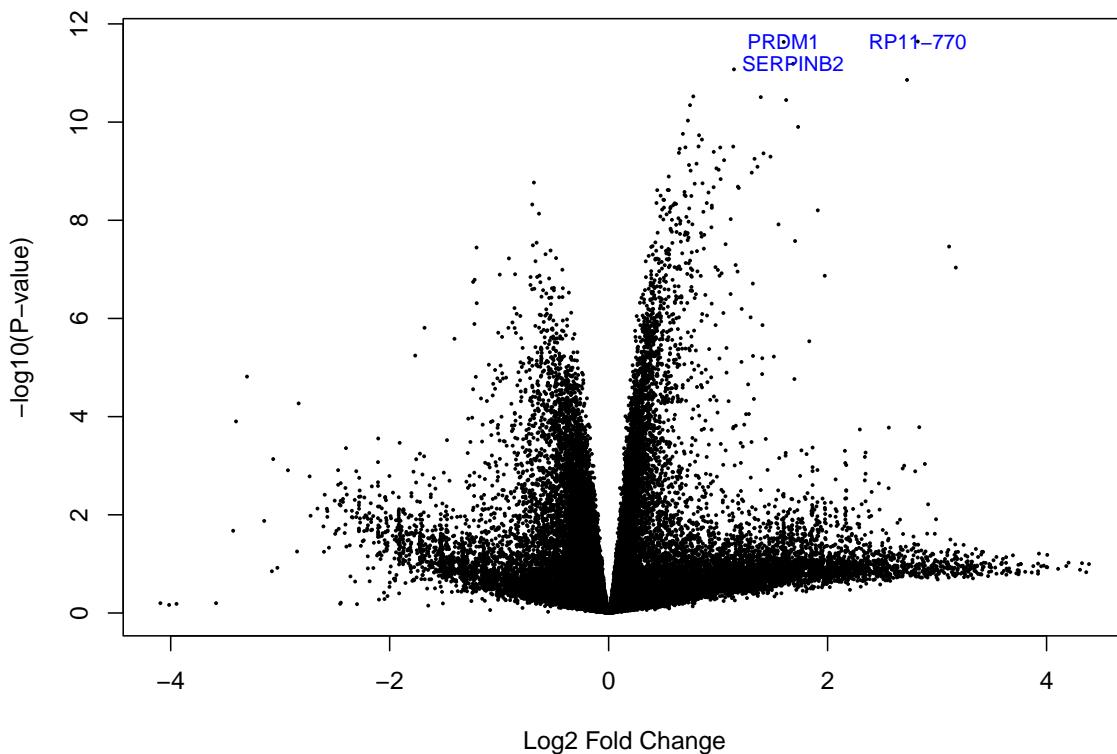
	source	type	bp_length	phase	gene_id
ENSG00000197632.8	HAVANA	gene	2748	NA	ENSG00000197632.8
ENSG00000057657.15	HAVANA	gene	6369	NA	ENSG00000057657.15
ENSG00000167772.11	HAVANA	gene	2482	NA	ENSG00000167772.11
ENSG00000136153.19	HAVANA	gene	14367	NA	ENSG00000136153.19
ENSG00000242550.5	HAVANA	gene	2700	NA	ENSG00000242550.5
	gene_type	gene_name	level	havana_gene	
ENSG00000197632.8	protein_coding	SERPINB2	2	OTTHUMG00000060592.4	
ENSG00000057657.15	protein_coding	PRDM1	1	OTTHUMG00000015299.4	

ENSG00000167772.11	protein_coding	ANGPTL4	2	OTTHUMG00000182273.5		
ENSG00000136153.19	protein_coding	LM07	1	OTTHUMG00000017093.17		
ENSG00000242550.5	protein_coding	SERPINB10	2	OTTHUMG00000060594.6		
	tag	logFC	AveExpr	t	P.Value	
ENSG00000197632.8	overlapping_locus	1.684529	7.656766	19.84717	6.505678e-12	
ENSG00000057657.15	overlapping_locus	1.599230	3.776720	21.35109	2.332487e-12	
ENSG00000167772.11		<NA>	1.144886	7.538975	19.48354	8.428257e-12
ENSG00000136153.19	overlapping_locus	1.389655	6.338621	17.74503	3.103105e-11	
ENSG00000242550.5	overlapping_locus	1.621444	6.177426	17.57186	3.555894e-11	
	adj.P.Val	B				
ENSG00000197632.8		6.892207e-08	17.56929			
ENSG00000057657.15		3.814782e-08	17.50762			
ENSG00000167772.11		6.892207e-08	17.27695			
ENSG00000136153.19		1.450037e-07	16.05946			
ENSG00000242550.5		1.453916e-07	15.90981			

```

volcanoplot(
  eb_results,
  coef = "Inf_vs_Ctrl_12hr",
  highlight = 3,
  names = gene_names[rownames(eb_results)]
)

```



For the 12 hour time point, 4615 DEGs were identified with an FDR < 0.05, indicating a more robust host response to *A. fumigatus* infection at this later stage. This time point three most significant DEGs are all upregulated in the infected samples compared to controls, and they are **SERPINB2**, **PRDM1** and an unidentified gene (*AC091633*). *SERPINB2* acts as a protease inhibitor involved in fibrinolysis and inflammatory signaling pathways, contributing to regulation of extracellular proteolytic activity and tissue remodeling. While *PRDM1* functions as a transcriptional repressor that regulates interferon-responsive genes and immune-related transcriptional programs.

By examining the genes with a $\log_{2}\text{FC} > 1$ and an $\text{adj.P.Val} < 0.05$, the other two most significant DEGs are **ANGPTL4** and **SLC20A2** and also upregulated during infection. The first mediates sodium-dependent phosphate uptake, contributing to cellular metabolic balance and mineral homeostasis, processes that can influence cellular signaling and survival under stress conditions. The second is a secreted regulator of lipid metabolism and endothelial behavior, modulating vascular permeability, extracellular matrix interactions, and cellular adhesion.

In bronchial epithelial cells exposed to *Aspergillus fumigatus conidia*, these upregulated genes may reflect coordinated responses integrating metabolic adaptation (*SLC20A2*), modulation

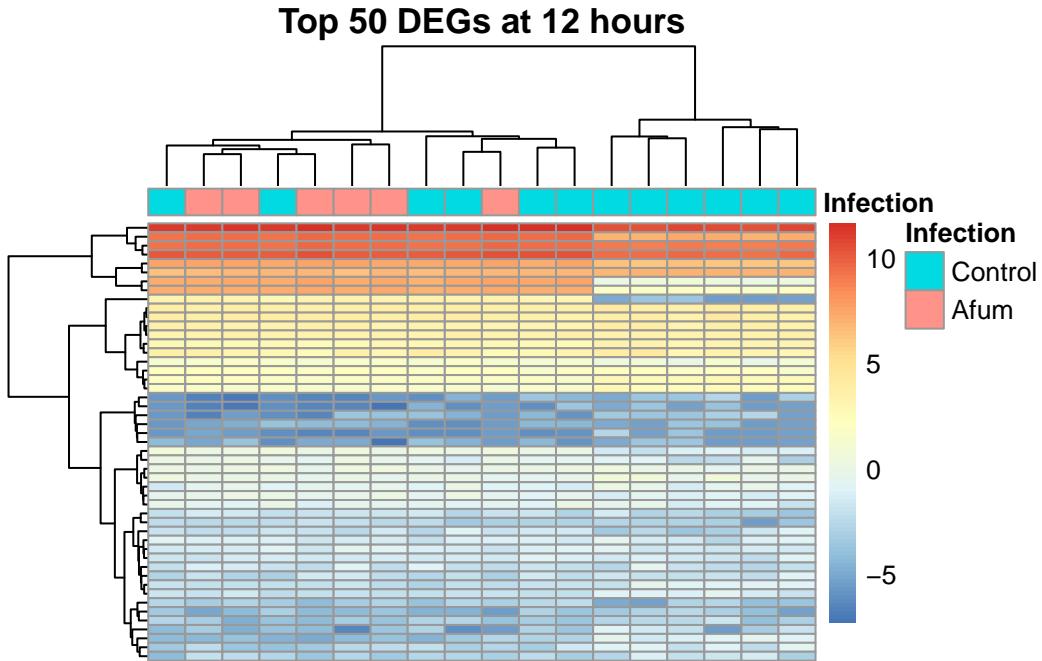
of epithelial-vascular interactions and barrier integrity (*ANGPTL4*), control of extracellular protease activity during inflammation (*SERPINB2*), and transcriptional reprogramming of immune signaling pathways (*PRDM1*). Given that fungal contact induces epithelial activation, cytokine production, oxidative stress, and tissue remodeling, modulation of these genes may contribute to **balancing host defense, barrier maintenance, and inflammatory regulation** during early antifungal responses.

```
## Extracting the genes of interest
expression_ht <- vGene$E[rank(tt_12hr$adj.P.Val) <= 50, ]

df <- as.data.frame(
  colData(rse_SRP048565_filtered)[
    , "sra_attribute.infection",
    drop = FALSE
  ]
)
colnames(df) <- "Infection"

# (opcional pero recomendable) asegurar factor
df$Infection <- factor(df$Infection)

pheatmap(
  expression_ht,
  cluster_rows = TRUE,
  cluster_cols = TRUE,
  annotation_col = df,
  show_rownames = FALSE,
  show_colnames = FALSE,
  main = "Top 50 DEGs at 12 hours"
)
```



This time point shows a very similar behaviour to that in the 2-hours time, which could mean that the dynamics in response to the infection of *A. fumigatus* conidia follows an equivalent expression pattern as the infection evolves.

```
## Cleaning the variables in the NameSpace
rm(list = ls())
invisible(gc())
```

Results

A. fumigatus conidia is an ambient fungus that infects its host through inhalation, and the interaction between its spores and respiratory epithelial cells is a critical step in the infectious process. The scRNA-seq revealed differentially expressed genes in the infected samples compared to controls at 2 and 12 hours, but not at 6 hours. At 2 hours, the DEGs were mostly related to stress response and epithelial homeostasis, while at 12 hours, the DEGs were more associated with metabolic adaptation, vascular interactions, and immune regulation. These findings suggest a dynamic host response to *A. fumigatus* infection that evolves over time, with early activation of defense mechanisms followed by more complex regulatory processes as the infection progresses.

It has been reported that HBECs participates in the attenuation of the inflammatory response to *A. fumigatus* conidia (Richard et al. (2018)), which is consistent with the observed upreg-

ulation of genes involved in immune regulation and barrier maintenance at later time points. The absence of DEGs at 6 hours may indicate a transient phase in the host response, where the initial activation subsides before a more robust response emerges at 12 hours. Overall, these results provide insights into the temporal dynamics of the epithelial response to fungal infection and highlight potential targets for therapeutic intervention in aspergillosis.

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