

ebrennan_haosmc Analysis

BM

12/28/2021

Analysis

Overview

Salmon merged gene counts were created using Nextflow nf-core_rnaseq ([commit: 8094c42add](#)) using hg38 Homo_sapiens.GRCh38.dna_sm.primary_assembly.fa.gz and Homo_sapiens.GRCh38.96.gtf.gz. These data are used here in RDS format to run DESeq2 @Love2014.

Data comprise of mRNA from Human aortic smooth muscle cells (haosmc) in **group** ‘scrambled miRNA mimic’ (**Scramble**) or ‘let-7d miRNA mimic’ (**Let7d**), and treated (**drug**) with no drug (**None**), TNF alpha alone (**TNFa**; 10ng/ml, 24hr), or in combination with one of the statins Atorvastatin or Lovastatin (**Ator**, **Lova**; 1uM, 24hr). This leads to 10 contrasts (i.e. comparisons) of interest: between **group** with **None** and with **TNFa** (2), and within **group** comparing **TNFa** with **None** (2), **Ator** (2), and **Lova** (2), and **Ator** with **Lova** (2).

Data is saved as XLSX and RDS for ongoing analysis in the **output** directory. Plots are displayed herein and saved to **output** as PDF.

Differential Expression Analysis

The DESeq2 package (@love2014) was used to determine ‘differentially expressed’ genes (DEG) between each group and treatment. The full analysis code is available from www.github.com/brucemoran/ebrennan_haosmc. Table 1a outlines the number of DEG between each ‘contrast’, i.e. group/treatment being compared, at three levels of false discovery rate (FDR) adjusted p-values. Thousands of DEG are evident between each contrast. Table 1b shows overlap of DEG found between treatments between groups (e.g. **Ator** vs. **Lova** in **Scramble** and **Let7d**) are shown to indicate <level of similarity?>

Table 1b: Total DE Genes Found per Contrast

	p < 0.001	p < 0.01	p < 0.05
Scramble_None_vs_Scramble_TNFa	3070	3902	4947
Let7d_None_vs_Let7d_TNFa	2281	3028	4114
Let7d_None_vs_Scramble_None	5580	6891	8283
Let7d_TNFa_vs_Scramble_TNFa	5694	6901	8270
Scramble_Ator_vs_Scramble_TNFa	6397	7586	8895
Scramble_Lova_vs_Scramble_TNFa	6154	7374	8740
Let7d_Ator_vs_Let7d_TNFa	5133	6355	7763
Let7d_Lova_vs_Let7d_TNFa	5181	6386	7718
Let7d_Ator_vs_Scramble_Ator	5175	6397	7840

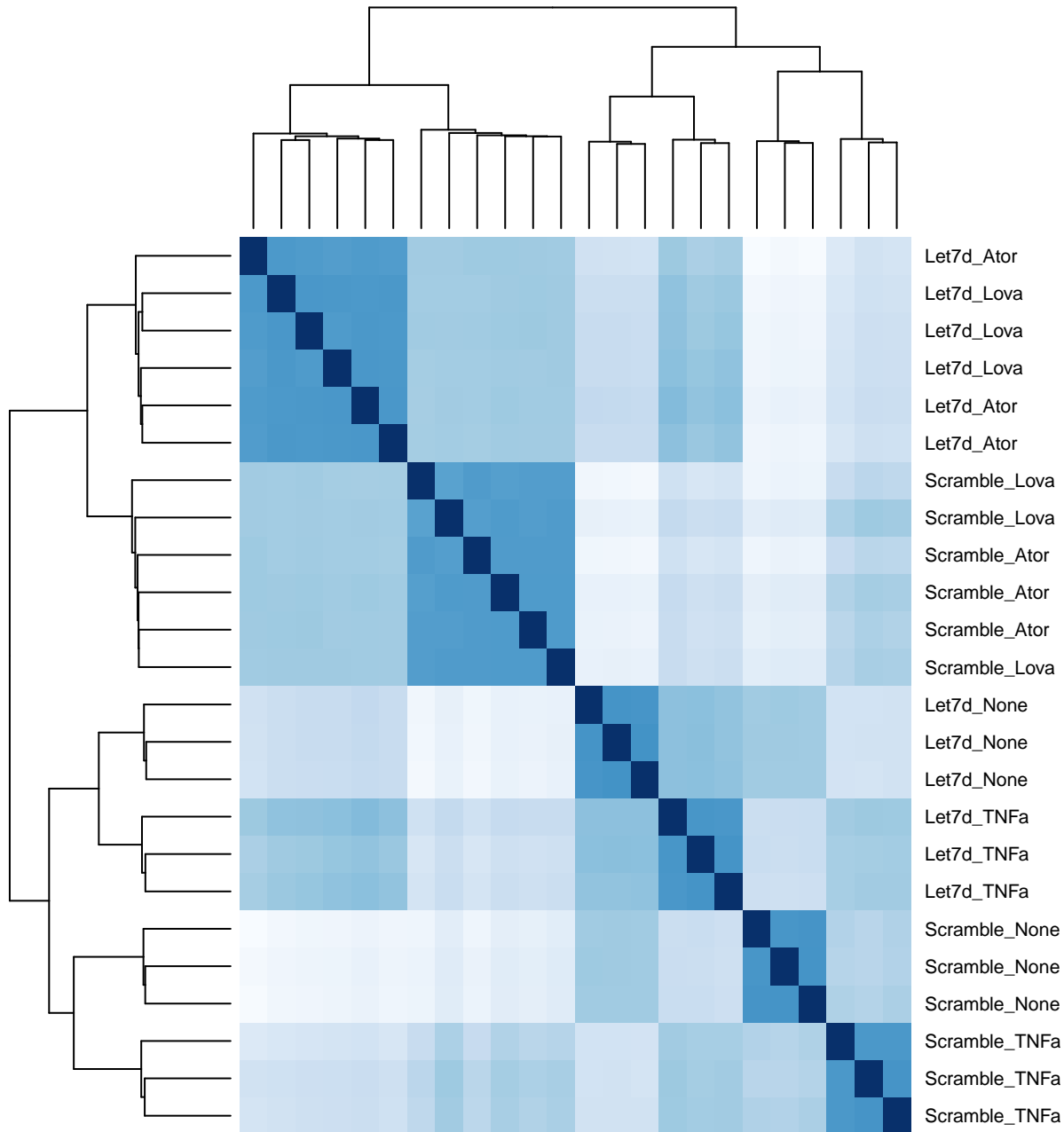
	p < 0.001	p < 0.01	p < 0.05
Let7d_Lova_vs_Scramble_Lova	5193	6373	7782
Let7d_Ator_vs_Let7d_Lova	0	0	1
Scramble_Ator_vs_Scramble_Lova	1	2	2

Table 1b: Overlap of DE Genes Found per Contrast Between Groups (padj < 0.01)

	Unique	Overlap	Overlap %
None_vs_TNFa	1343	1727	47.65
Ator_vs_TNFa	2109	4288	59.21
Lova_vs_TNFa	1876	4278	60.62

QC Plots

Heatmap

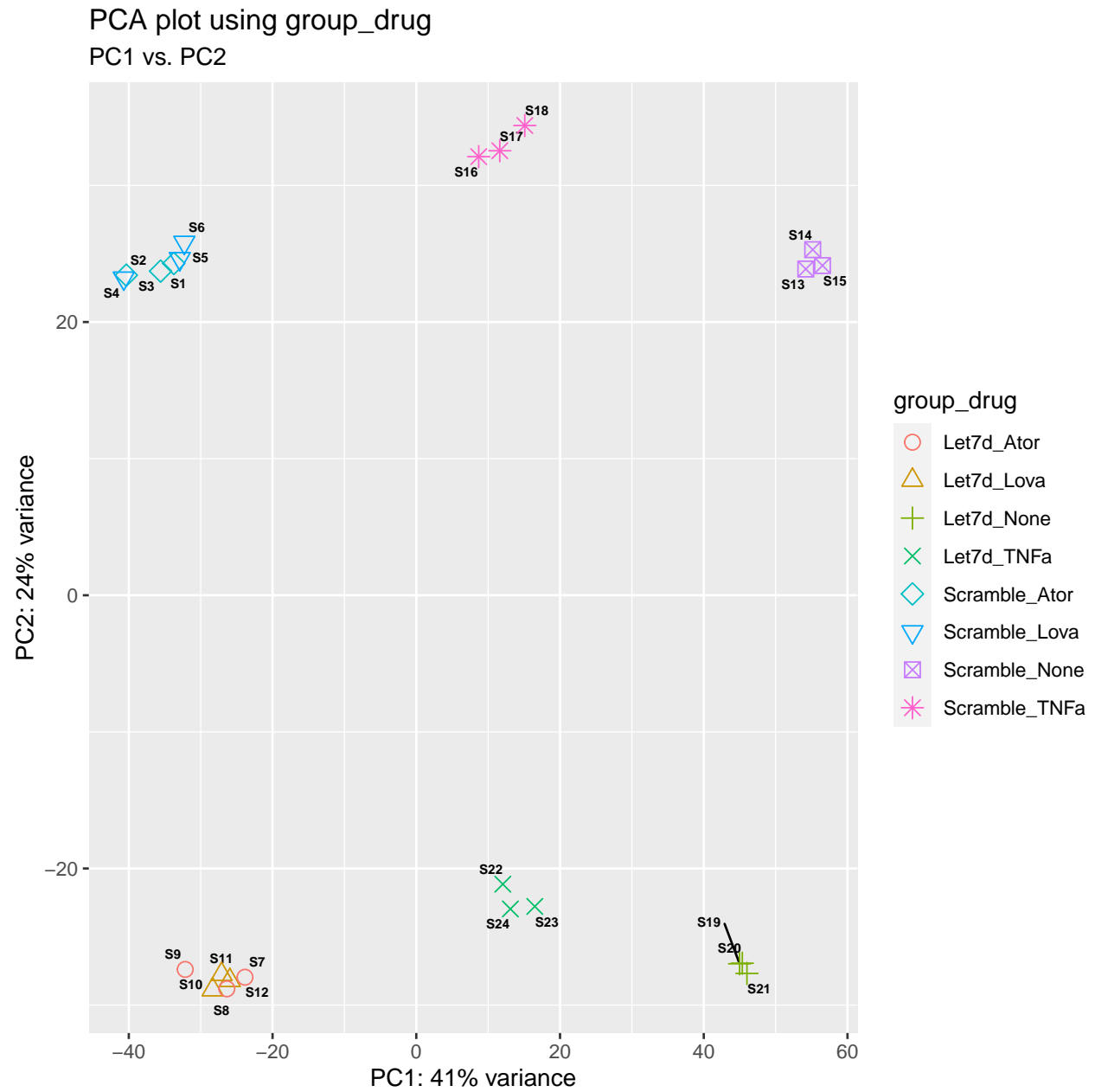


PCA Plots

PCA 1 vs 2

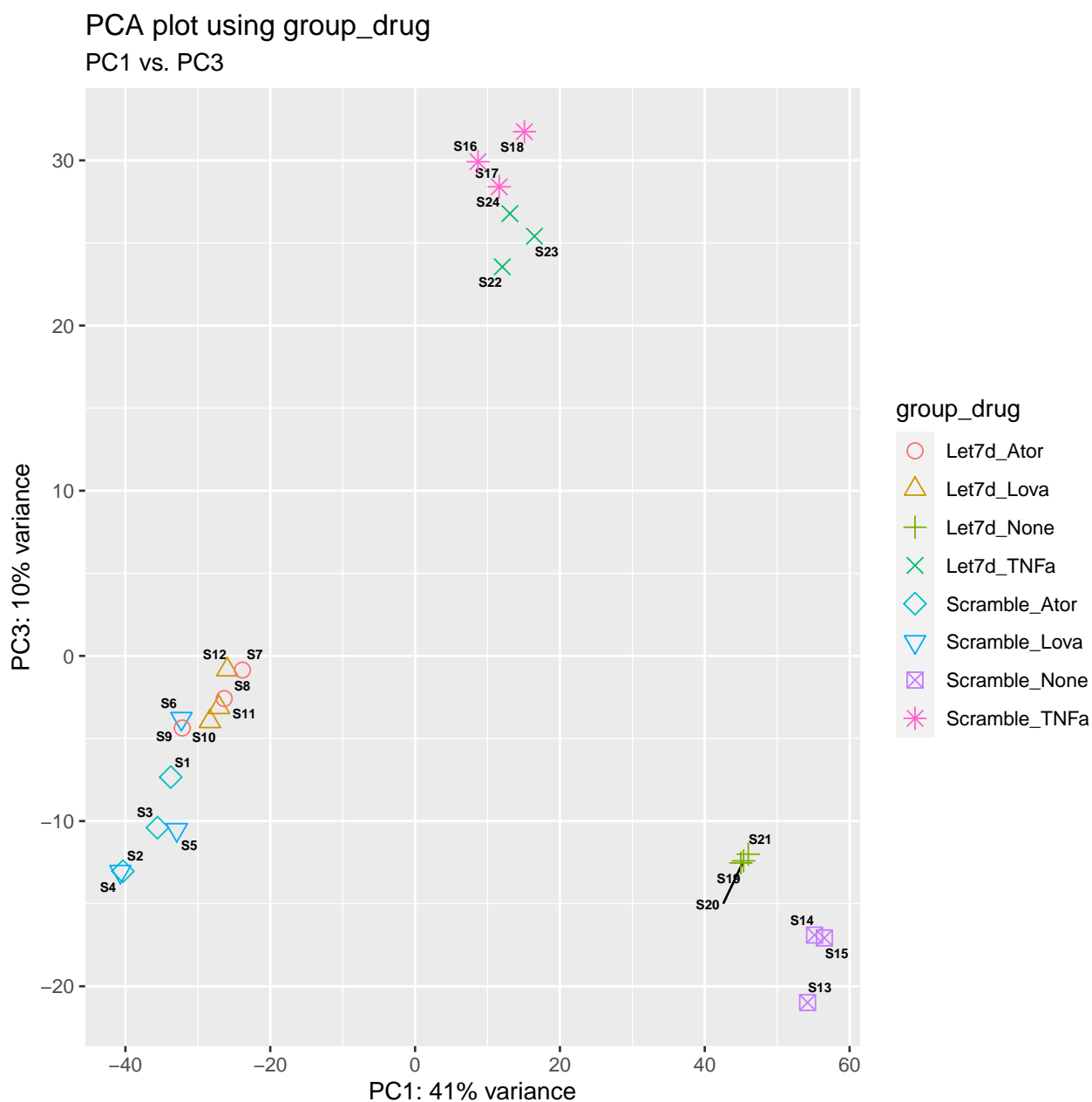
PC 1 accounts for 41% of variance (quite a lot), and very clearly separates based on drug treatment. Evident on the x-axis between -40, -20 are **Ator**, **Lova** treated cells, between 0 - 20 are **TNFa** treated cells, and after 40 are cells treated with no drug (**None**).

PC2 accounts for 24% variance, still quite high, and is based on **Scramble**/**Let7d**.



PCA 1 vs 3

PC3 (y-axis) accounts for 10% variance, so we have attributed 3/4 total variance. This is also a biologically based PC, given that **TNFa** treated cells are clearly separated. There is also some separation of **Ator/Lova** treated cells from untreated **None** cells.



Pathway Analysis

Pathways are taken from [MsigDB](#)(@subramanian2011) 'Hallmark' gene sets (@liberzon2015) and analysis conducted with the `fgSEA` package ()

```
## [1] "Running: fgsea_plot()"
## [1] "Found rank_col: stat"

## [1] "Running: fgsea_plot()"
## [1] "Found rank_col: stat"

## [1] "Running: fgsea_plot()"
## [1] "Found rank_col: stat"
```

```
## [1] "Running: fgsea_plot()"
## [1] "Found rank_col: stat"

## [1] "Running: fgsea_plot()"
## [1] "Found rank_col: stat"

## [1] "Running: fgsea_plot()"
## [1] "Found rank_col: stat"

## [1] "Running: fgsea_plot()"
## [1] "Found rank_col: stat"

## [1] "Running: fgsea_plot()"
## [1] "Found rank_col: stat"

## [1] "Running: fgsea_plot()"
## [1] "Found rank_col: stat"

## [1] "Running: fgsea_plot()"
## [1] "Found rank_col: stat"

## [1] "Running: fgsea_plot()"
## [1] "Found rank_col: stat"

## [1] "Working on: Scramble_None_vs_Scramble_TNFa"
## Estimating ssGSEA scores for 20 gene sets.
## |
## |
## |

## [1] "Working on: Let7d_None_vs_Let7d_TNFa"
## Estimating ssGSEA scores for 19 gene sets.
## |
## |
## |

## [1] "Working on: Let7d_None_vs_Scramble_None"
## Estimating ssGSEA scores for 18 gene sets.
## |
## |
## |

## [1] "Working on: Let7d_TNFa_vs_Scramble_TNFa"
## Estimating ssGSEA scores for 21 gene sets.
## |
## |
## |

## [1] "Working on: Scramble_Ator_vs_Scramble_TNFa"
## Estimating ssGSEA scores for 33 gene sets.
## |
## |
## |
```

```

## [1] "Working on: Scramble_Lova_vs_Scramble_TNFa"
## Estimating ssGSEA scores for 35 gene sets.
## |
## |===

## [1] "Working on: Let7d_Ator_vs_Let7d_TNFa"
## Estimating ssGSEA scores for 36 gene sets.
## |
## |===

## [1] "Working on: Let7d_Lova_vs_Let7d_TNFa"
## Estimating ssGSEA scores for 34 gene sets.
## |
## |===

## [1] "Working on: Let7d_Ator_vs_Scramble_Ator"
## Estimating ssGSEA scores for 17 gene sets.
## |
## |===

## [1] "Working on: Let7d_Lova_vs_Scramble_Lova"
## Estimating ssGSEA scores for 13 gene sets.
## |
## |===

## [1] "Working on: Let7d_Ator_vs_Let7d_Lova"
## [1] "No genesets in pathways, skipping..."
## [1] "Working on: Scramble_Ator_vs_Scramble_Lova"
## [1] "No genesets in pathways, skipping..."

```