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| **PROTOCOL CODING IN “R”** |

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| **PROJECT ID** | **18 Bwanya** |
| **Title:** | **Gene Expression Analysis and Visualization of Lung Cancer Pathways Using R and Bioinformatics Pipelines** |
| **Version:** | **01** |
| **Author(s)**  **(+ initials)** | **Silke Römkens i6325117**  **Bart Bruijnen i6330097**  **Jarne Paar i6318995**  **Cecilia Mantovani i6323828**  **Sabya Sherpa i6326426**  **Anne Fleur van Groningen i6315029** |
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| **Revision / History** | | **Review** | |
| **Version** | **Change description** | **Date** | **Initials** |
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| 1. INTRODUCTION |

This protocol describes the steps involved in analyzing differentially expressed genes (DEGs) in lung cancer using RNA-seq data. In the project, we focus on analyzing the publicly available dataset GSE1089 to identify significant DEGs between tumor and healthy tissues. We aim to gain more insight into biological pathways and genes relevant to lung cancer. We try to look at risk factors and characteristics of non-small lung cancer to identify differential gene expression and consequently guide future interventions.

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| 2. SOFTWARE / DATA |

Various programs and databanks were employed to work in and aid the project’s progress:

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| **Name** | **Description** | **Version / Developer** |
| Computer | 16 gb RAM |  |
| Github | Github is an online and desktop application. The program is employed to create a global location to share and work on files collectively. It is primarily used to work on the code, but can also be used to work on other files of interest which are non-code related. |  |
| R | R is a programming language primarily employed for … statistical analysis. To use this programming language, the “R” package first must be fetched online. | 4.2.2. |
| Rstudio | Rstudio is a program which uses the R programming language to develop a working code. |  |
| Rstudio packages: |  |  |
| NCBI |  |  |

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| 3. HEALTH AND SAFETY (if applicable) |

We will be working entirely with computers thus this section is not applicable

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| 4. SPECIFIC RECOMMENDATIONS / WARNING (if applicable) |

We are using GitHub, so we have to take caution with working on our code together. If we work individually, we have push our code not together otherwise the GitHub will not accept multiple edits at once and some of the code will be lost.

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| 5. PROCEDURE TO FOLLOW |

These are the procedure to follow to get results

1. Download the lung cancer data from GEO database​ to perform the analysis of the data
2. Install and require libraries in R, the libraries we will be using are DESeq2, ggplot2, dplyr, pheatmap, clusterProfiler, org.HS.eg.db and GEOquery.
3. Load the count matrix, it has the data from step 1
4. Load metadata, this can be required from the R library GEOquery
5. Create a DESeq2 dataset in R to create a statistical model
6. Perform Quality control
7. Get results and sort by pValue
8. Filter significant genes with adjusted p-value < 0.05
9. Visualize results such as volcano plot, heatmap, MA plot
10. Functional enrichment analysis using GO, KEGG

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| 6. DATA ANALYSIS AND STATISTICS (if applicable) |

Give here a brief overview of how to analyse and present the obtained data. Name the software (e.g. Excel, R, or SPSS – also in section 2.) and statistical methods (e.g. students t-test, Fisher’s test) you used for data analysis.

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| 7. LITERATURE |

Indicate any literature if needed, or reference to other documents needed to understand / complement this protocol. That includes scientific papers, websites, databases or books.

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| 8. APPENDIX (if required) |

Add here any materials or information that are needed to understand/reproduce the protocol, but are too extensive to add to the main text.