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| **PROTOCOL TEMPLATE** |

LOGO

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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** |
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| **Revision / History** | | **Review** | |
| **Version** | **Change description** | **Date** | **Initials** |
| 01 | First issue | 18-02-2025 |  |
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Contents

[1. INTRODUCTION 2](#_Toc119389311)

[2. EQUIPEMENT / MATERIAL / SOFTWARE / DATA / SAMPLES (select what is applicable) 2](#_Toc119389312)

[3. HEALTH AND SAFETY (if applicable) 2](#_Toc119389313)

[4. SPECIFIC RECOMMENDATIONS / WARNING (if applicable) 2](#_Toc119389314)

[5. PROCEDURE TO FOLLOW 2](#_Toc119389315)

[6. DATA ANALYSIS AND STATISTICS (if applicable) 3](#_Toc119389316)

[7. LITERATURE 4](#_Toc119389317)

[8. APPENDIX (if required) 4](#_Toc119389318)

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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. EQUIPEMENT / MATERIAL / SOFTWARE / DATA / SAMPLES (select what is applicable) |

Detail here specific instrumentation / material / software / data or biomedical samples you obtained or used to perform the experiments.

*Here are some examples…*

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| **Name** | **Description** | **Supplier / Reference** |
| Leica 3001 Fluorescence microscope | Microscope equipped for fluorescence microscopy | Leica Ltd., Tours, France |
| Lung tissue samples | Lung tissue samples from patients with severe COVID19 infection and healthy controls. Samples were obtained within a clinical trial, permission of METC granted (permission number 2022-8299) | MUMC, Department of pulmonology |
| Cytoscape (version 9.2) | Software for creation and analysis of networks. | <https://cytoscape.org/>  Shannon et al. 2013 |
| GEO-E-32998 | Transcriptomics dataset comparing SARS-CoV2 infected lung epithelial cells with healthy control, originally published by Doe et al. 2021 and available on GEO database. | Doe et al. 2021  GEO-E-32998 |
| MES buffer solution | PH buffered MES (2-(N-morpholino)ethanesulfonic acid) solution. | BASF, Ludwigsburg, Germany |

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| 3. HEALTH AND SAFETY (if applicable) |

For lab work - indicate here any health and safety related issues you need to pay attention while executing the tasks in the protocol.

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

Indicate here specific cautions that have to be taken such as to prevent any damage of the instrument (like a maximum recommended temperature, a typical flow rate, etc…)

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| 5. PROCEDURE TO FOLLOW |

Please indicate here all the necessary steps needed to perform the experiments. Someone should be able to reproduce the experiments following your protocol.

Tips: Use bullet points; make it straightforward, and add screenshots, schematic drawings or photos to guide the user. Use headings to separate different tasks in a logical order.

E.g.

1. Tissue sample preparation

* Open the container and take out the tissue sample using sterile tweezers
* Rinse the sample 3 times with buffered NaCl.
* ……….

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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.