

NGS Bioinformatics Course

AFRICA 2021

Module 8: RNA-Seq Expression Analysis (Pathogens)

Practical Assignment

Module topic: RNA-Seq Analysis - Pathogens

Contact session title:

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Participant: <write your name here>

Date: <write today's date here>

Introduction

This practical illustrates the principles of RNA-seq expression analysis using data derived from bacterial isolates. The practical assignment for this module can be found in the file:

['Module8_RNASeq_Pathogen_Practical_Manual.pdf'](#) or
['Module8_RNASeq_Pathogen_Practical_Manual.html'](#)

Follow the instructions outlined in this manual and perform each analysis step as indicated. Answer the questions at the end of each section and submit your answers by the deadline indicated on Vula.

All information is found in the practical material. We will use this document to answer the questions.

Tools used in this session

List any tools and appropriate URLs or software download information. Please note: use web-based online applications as far as possible.

[See Section 1.5 Prerequisites in Module8_RNASeq_Pathogen_Practical_Manual.pdf](#)

Please note

- **Hand-in information** please upload your completed assignment to the Vula 'Assignments' tab. Take note of the final hand-in date for each assignment, which will be indicated on Vula.

Assigned Tasks

Task 1: Introducing The Tutorial Dataset

1. Why is there more than one FASTQ file per sample?
<start typing your answer here>

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2. How many reads were generated for the N2 sample?

<start typing your answer here>

3. The three Resistant samples N2, N6 and N10 represent technical replicates. True or False? Comment on your answer?

<start typing your answer here>

Task 2: Estimate Transcript Abundance With Salmon

Take a look at the ***quant.sf*** file in the **N2** folder and answer these questions:

4. What does **TPM** stand for?

<start typing your answer here>

5. How many reads in total are mapped to the tRNA genes?

(Hint: they start with "rna-")

<start typing your answer here>

6. Do you think this level of tRNA is acceptable?

<start typing your answer here>

Task 3: Differential Expression Analysis with DESeq2

7. How many genes are up- and down-regulated between the resistant and sensitive isolates of the *Mycobacterium tuberculosis*?

<start typing your answer here>

8. How many genes are significantly differentially expressed (i.e., meet the LFC threshold and adjusted p-value cutoffs) between the resistant and sensitive isolates of the *Mycobacterium tuberculosis*? Name these genes.

<start typing your answer here>

9. What are the p-values for the significantly differentially expressed genes?

<start typing your answer here>
