

# 3D RNA-seq workshop provides training for better tools and improved analysis for scientists in SEFARI institutions to tackle large scale experimental data

## Introduction

RNA-sequencing (RNA-seq) data analysis is often complex, leaving biologists reliant on in-demand bioinformaticians with highly specialised skills, which can be a source of frustration. Researchers from the James Hutton Institute developed the 3D RNA-seq App, an easy-to-use graphical interface designed for biologists to carry out complex analysis of RNA-seq data.

The 3D RNA-seq workshop funded by the SEFARI Responsive Fund was originally planned to be carried out in-person in May 2020., but due to the Covid-19 pandemic, the workshop was eventually carried out fully online in November, hosted by Wenbin Guo, Linda Milne, Runxuan Zhang and Iain Milne from the Information & Computational Sciences (ICS) department at the Hutton. It brought together 14 researchers from a wide range of SEFARI and other institutions, including the James Hutton Institute, Royal Botanic Garden of Edinburgh, Moredun, the Universities of Dundee, Aberdeen, and Birmingham, as well as IPK in Germany.

The collage consists of four main panels:

- Top Left:** A slide titled "Accurate and fast quantification of transcriptomes using RNA-seq" by Runxuan Zhang, Information and Computational Sciences, The James Hutton Institute, UK. It is dated Nov 12<sup>th</sup>, 2020 and is part of Virtual SEFARI Training.
- Top Right:** A slide titled "What is '3D'?" for the 3D RNA-seq App for expression analysis. It lists: RNA-seq – method of transcriptome profiling; Study transcriptome quantification at levels (3D): Differential gene expression (DE, abundance change); Differential transcript usage (DTU, isoform level % change); Differential alternative splicing (DAS, genes have DTU); Isoform switch (reverse relative abundance). It includes a diagram of alternative splicing and 3D analysis.
- Bottom Left:** A screenshot of the Galaxy web interface showing a "Demo: transcript quantification with Galaxy" workflow. It includes instructions like "https://usegalaxy.eu/" and "Register a Galaxy account to upload big data > 2G".
- Bottom Right:** A screenshot of the "Demo: 3D RNA-seq analysis" interface. It features a bar chart showing transcript abundance and a volcano plot. A specific transcript is highlighted with a 75% isoform switch.

## Body

The workshop's main goal was to train participants in the effective use of 3D RNA-seq and empower them to perform complex differential expression and alternative splicing analyses and produce experimental results in a fast, robust and reproducible way.

We faced significant challenges delivering training that involves a big practical component and ideally would require continuous interaction and exchange; with only one screen being shared, you can only really help one participant at a time. To deliver the training more effectively, we redesigned the workshop and divided it across two days. The main lecture session was held first and then follow-

up one-to-one sessions were arranged later so that participants could discuss their own data with us in more detail.

We encouraged participants to introduce their research background and aim of attending the workshop, allowing us to tailor the presentation to maximize the uptakes for participants. Their research areas cover a wide range of topics and species, from resistance in potato, genetic studies in identifying QTLs in leaf shapes, a gut organoid system in cattle, viral diseases of ruminant livestock, to human intestinal epithelium in response to chemical exposures and gut bacteria studies in human.

In the first part of the session, Runxuan gave an introductory talk to familiarize the participant with transcriptomics analysis. Then Wenbin performed a demo of transcript quantification on the Galaxy platform using two popular programs Salmon and Kallisto. In the second part, Wenbin presented the 3D RNA-seq App, followed by a demo of 3D RNA-seq analysis of an example dataset. He also demonstrated two case-studies of applying 3D RNA-seq App to a time-series study of Arabidopsis in response to cold and an RNA-seq study of dexamethasone treatment on mouse brain tissues. During the Q&A, we discussed frequently asked questions relating to input data preparation and pre-processing, how to get gene and transcript information and how to use the help information on the interface and Github page.

At the one-to-one session, we met with researchers from RBGE and University of Birmingham to help them with issues of 3D RNA-seq analysis specific to the RNA-seq datasets of their own. An oral feedback was obtained. Most of the participants are able to finish the analysis by following the YouTube tutorial video and step-by-step materials we have provided.

A survey was also sent out to collect feedbacks after the workshop. The comments were very positive in terms of the workshop organizing, training contents and skill and responsiveness of the instructors. Participants considered that the 3D RNA-seq App is a useful tool since it is easy to use and follow, particularly with graphical manual and YouTube tutorial. Most participants would like to recommend the 3D RNA-seq App to their colleagues. Some of them have already managed to finish the 3D RNA-seq analysis using the example data or their own data and posted the results and figures on Twitter. We also received suggestions to improve the 3D RNA-seq App and the training. For example, they are looking forward of interactive hands on. It would be good to include theoretical session of terminology and methods in the training for beginners of RNA-seq analysis. They recommended the App to include more colour options of the plot.

We believed that this workshop could increase skill capacity in SEFARI institutions to analyse their RNA-seq data. The software tool allows significant improvement of speed and productivity for biologists using RNA-seq investigating various research questions. The pipeline can be universally applied to all species. 3D RNA-seq has been used for teaching in several universities to improve the skills for undergraduates and Biologists alike. We look forward to carrying out this workshop regularly in the future in person for SEFARI institutions too.

### **Related link**

- 3D RNA-seq App: <https://ics.hutton.ac.uk/3drnaseq>
- 3D RNA-seq App Github: <https://github.com/wyguo/ThreeDRNaseq>
- 3D RAN-seq video tutorial: <https://youtu.be/rqeXECX1-T4>
- SEFARI 3D RNA-seq workshop video: <https://youtu.be/WUwo5N1njR0>

### **Organizers**

- Dr Wenbin Guo, Bioinformatician, <https://www.hutton.ac.uk/staff/wenbin-guo>

- Dr Linda Milne, Bioinformatics Specialist, <https://www.hutton.ac.uk/staff/linda-milne>
- Dr Runxuan Zhang, Computational Biologist, <https://www.hutton.ac.uk/staff/runxuan-zhang>
- Dr Iain Milne, Research Software Engineer, <https://www.hutton.ac.uk/staff/iain-milne>