Unlock the power of single-cell RNA-seq data analysis

Day 1

9:00 – 10:00 Introduction to scRNAseq

- **Overview of scRNAseq Technology**: Learn about the evolution of RNA sequencing from bulk to single-cell approaches.
- **Importance in Research**: Discuss how scRNAseq contributes to understanding cellular heterogeneity in complex tissues.
- **Methodologies and Platforms**: Review different scRNAseq platforms like 10 Genomics, Drop-seq, and Smart-seq.
- **Experimental Design Considerations**: Understand sample preparation, library construction, and sequencing depth requirements.

10:00 – 10:30 Installation of Kallisto/BUStools

- **Setting Up the Environment**: Instructions on installing necessary dependencies and tools on Windows, macOS, and Linux.
- **Installation Walkthrough**: Step-by-step guide to installing Kallisto and BUStools.
- **Verification**: How to test installations to ensure everything is working correctly.
- **Troubleshooting**: Common issues and their solutions during installation.

10:30 – 11:00 Break

11:00 – 12:00 Quantification of scRNAseq Libraries

- **Data Preparation**: Introduction to raw scRNAseq data formats and preprocessing steps.
- **Using Kallisto**: Hands-on session running Kallisto for pseudoalignment.
- **BUStools Workflow**: Learn how to use BUStools for generating count matrices.
- **Interpreting Results**: Understanding the output files and basic result interpretation.

12:00 – 13:00 Lunch Break

13:00 – 14:00 Exploration of Data and Quality Control

- **Quality Metrics**: Identify key metrics like gene counts per cell, mitochondrial gene expression, and doublet detection.
- **Data Visualization**: Use tools like Seurat or Scanpy to visualize data distributions.
- **Filtering Strategies**: Learn criteria for filtering out low-quality cells and genes.
- **Batch Effect Analysis**: Introduction to detecting and correcting batch effects.

14:00 – 15:00 Normalization and Clustering

- **Normalization Techniques**: Discuss methods like log-normalization and scaling.
- **Dimensionality Reduction**: Apply PCA, t-SNE, and UMAP for data simplification.
- **Clustering Algorithms**: Explore clustering methods such as K-means, hierarchical clustering, and graph-based approaches.
- **Cluster Evaluation**: How to determine the optimal number of clusters and assess their biological relevance.

Day 2

9:00 – 9:30 Recap of Day 1

- **Summary Review**: Revisit key concepts and workflows covered on Day 1.
- **Q&A Session**: Opportunity to ask questions and clarify doubts.

9:30 – 10:30 Cell Type Classification Part I

- **Marker Gene Identification**: Learn how to identify genes that define specific cell types.
- **Reference Databases**: Introduction to using databases like CellMarker and PanglaoDB.
- **Manual Annotation**: Techniques for annotating cell clusters based on known markers.

10:30 – 11:00 Break

11:00 – 12:00 Cell Type Classification Part II

- **Automated Annotation Tools**: Use tools like Azimuth, SingleR and Garnett for cell type prediction.
- **Integrating Multiple Datasets**: Strategies for comparing and combining datasets from different sources.
- **Validation**: Methods to validate cell type assignments experimentally or computationally.

12:00 – 13:00 Lunch Break

13:00 – 14:00 Differential Expression Analysis of scRNAseq Data

- **Statistical Methods**: Discuss models suitable for single-cell data, like the negative binomial distribution.
- **Comparative Analysis**: Steps to identify differentially expressed genes between clusters or conditions.
- **Multiple Testing Correction**: Understanding false discovery rates and adjusting p-values.
- **Pathway Analysis**: Introduction to gene set enrichment analysis to interpret DE genes.

14:00 – 14:30 Trajectory Analysis

- **Pseudotime Concept**: Learn how to model temporal processes in cells without time-series data.
- **Tools and Algorithms**: Hands-on with Monocle or Slingshot for trajectory inference.
- **Interpreting Trajectories**: Understand how to read and analyze trajectory plots.

14:30 – 15:00 Velocity Analysis

- **RNA Velocity Theory**: Introduction to the concept of RNA velocity and its significance.
- **Computational Implementation**: Use tools like scVelo to calculate and visualize RNA velocities.
- **Case Studies**: Review examples where velocity analysis provided insights into dynamic biological processes.

Additional Notes:

- **Preparation**: Participants should ensure that their laptops have the required software installed prior to the workshop. Installation guides will be provided ahead of time.
- **Datasets**: Sample datasets will be available for hands-on sessions.
- **Resources**: Supplementary materials, including slides and code scripts, will be shared after the sessions.

We hope these additional details enhance your understanding of the program and help you prepare for an informative and productive experience!