CyVerse Documentation

Release 2.0

CyVerse

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In the last two decades, transcriptome-wide profiling has been playing a major role in revolutionizing biology and medicine. Recent single-cell RNA-seq technique has taken the field of transcriptomics to the next level by allowing the quantification of gene expression in individual cells. Albeit, this enormous increase in sequencing of tens of thousands of cells in a single run has brought new challenges in data analysis. In this tutorial, we will use CyVerse Discovery Environment (DE) public apps for analyzing a sample dataset of Peripheral Blood Mononuclear Cells (PBMC). The dataset has 1,222 single cells and is freely available from 10X Genomics website. Access the slides for this tutorial here.

Some experience with R programming and command-line is required to follow this tutorial.

CHAPTER 1

Learning Objectives

- What is single-cell RNA-Seq and why it is useful
- Basic steps for single-cell analysis
- Using single-cell analysis tools in the CyVerse Discovery Environment

CHAPTER 2

Tutorial Maintainer(s)

Who to contact if this guide needs fixing. You can also email learning@CyVerse.org

Maintainer	Institution	Contact
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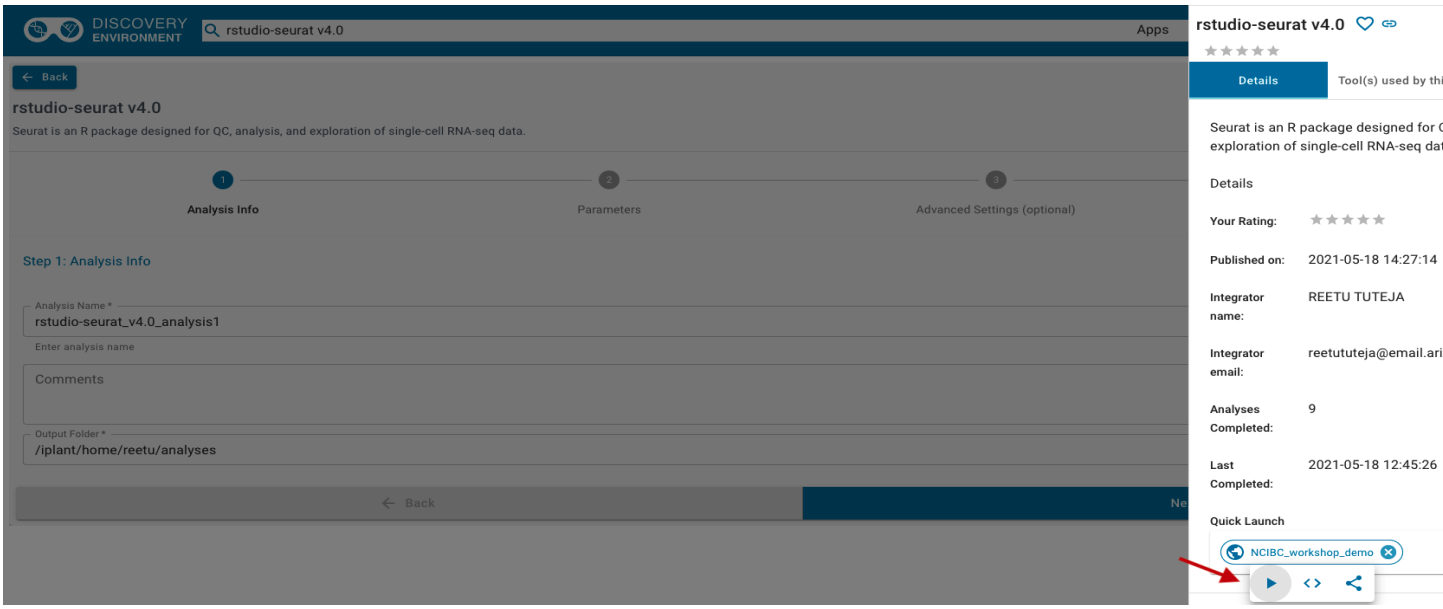
2.1 Launching Seurat app in the Discovery Environment

1. Login to the Discovery Environment.
2. Click on "Apps" tab in the Discovery Environment and search for "rstudio-seurat" or click the app link here.
3. Change the name of the analysis and output folder as needed or leave for defaults. Click Next.
4. Provide the input data as needed or use quick launch to input demo dataset and R markdown file. To use quick launch, click on the Details button on the top right corner. From the App details window, click on Play from the NCIBC_workshop_demo quick launch. This will input the Demo_Folder in the input data section. Click Next.



5. For the next section "Resource Requirements", request resources as needed or leave for defaults and Click Next.
6. Click Launch Analysis. You will receive a notification that the job has been submitted and running. Click on Access your running analysis link, that will take you to the app loading screen.

After the Rstudio is launched, input the username (rstudio) and password (rstudio1). You will find the demo data and R markdown file within the Rstudio IDE.

Input Data:

Input	Description	Location
Healthy.gz	This folder has barcode.tsv, matrix.mtx and features.tsv files from filtered count.	iplantcollaborative > example_data > seurat

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2.2 Further reading

Shalek AK, Benson M. Single-cell analyses to tailor treatments. Sci Transl Med. 2017 Sep 20;9(408):eaan4730. doi: 10.1126/scitranslmed.aan4730. PMID: 28931656; PMCID: PMC5645080.

Svensson V, Vento-Tormo R, Teichmann SA. Exponential scaling of single-cell RNA-seq in the past decade. Nat Protoc. 2018 Apr;13(4):599-604. doi: 10.1038/nprot.2017.149. Epub 2018 Mar 1. PMID: 29494575.

Ilicic T, Kim JK, Kolodziejczyk AA, Bagger FO, McCarthy DJ, Marioni JC, Teichmann SA. Classification of low quality cells from single-cell RNA-seq data. Genome Biol. 2016 Feb 17;17:29. doi: 10.1186/s13059-016-0888-1. PMID: 26887813; PMCID: PMC4758103.

Lun AT, McCarthy DJ, Marioni JC. A step-by-step workflow for low-level analysis of single-cell RNA-seq data with Bioconductor. F1000Res. 2016 Aug 31;5:2122. doi: 10.12688/f1000research.9501.2. PMID: 27909575; PMCID: PMC5112579.

Perraudeau F, Risso D, Street K, Purdom E, Dudoit S. Bioconductor workflow for single-cell RNA sequencing: Normalization, dimensionality reduction, clustering, and lineage inference. F1000Res. 2017 Jul 21;6:1158. doi: 10.12688/f1000research.12122.1. PMID: 28868140; PMCID: PMC5558107.

Bacher R. Normalization for Single-Cell RNA-Seq Data Analysis. Methods Mol Biol. 2019;1935:11-23. doi: 10.1007/978-1-4939-9057-3_2. PMID: 30758817.

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CHAPTER 3

Prerequisites

3.1 Downloads, access, and services

In order to complete this tutorial, you will need access to the following services/software

Prerequisite	Preparation/Notes	Link/Download
CyVerse account	You will need a CyVerse account to complete this exercise	
Cyberduck	Standalone software for upload/download to Data Store	

3.2 Platform(s)

We will use the following CyVerse platform(s):

Platform	Interface	Link	Platform Tour
Data Store	GUI/Command line		
Discovery Environment	Web/Point-and-click		

3.3 Application(s) used

Discovery Environment App(s):

App name	Version	Description	App link	Notes/other links
Rstudio-Seurat	4.0.1	Single-cell Analysis		

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