2020 生物醫學大數據淘金工作坊 Part 3 Web portal for Single cell RNA-seq

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Outline

01 Introduction

single-cell RNA sequencing

02 Galaxy-based pre-processing system

03 Seashell: web portal for single cell RNA-seq analysis and visualization



Introduction

Single-cell RNA sequencing (scRNA-seq)

scRNA-seq technologies have combined effective single-cell isolation strategies with highly sensitive molecule detection approaches, showing promise in unravelling the heterogeneity of complex tissues or organs.



Single-cell RNA sequencing

A variety of scRNA-seq protocols



Kumar, Pavithra, Yuqi Tan, and Patrick Cahan. "Understanding development and stem cells using single cell-based analyses of gene expression." *Development* 144.1 (2017): 17-32.

Single-cell RNA sequencing

> A growing number of application

Heterogenity of cancer cells



https://www.rna-seqblog.com/new-single-cell-rna-sequencing-methods-could-lead-to-better-regenerative-therapies/

Diversity of T cell receptor

Vallejo, Abbe N. "Immune remodeling: lessons from repertoire alterations during chronological aging and in immune-mediated disease." *Trends in molecular medicine* 13.3 (2007): 94-102.

Differentiation of stem cells



https://www.bio-connect.nl/stem-cell-and-the-regenerative-medicine-ready-for-the-patients/cnt/page/5050

Single-cell RNA sequencing

> Increasing difficulties of processing sequencing data

- Serious batch effect
- Demanding computing power
- etc





Workflow of analysis

The portal system can be divided into two parts:



Galaxy-based docker image for data preprocessing

Users can run this docker image by a Galaxy-based GUI, and then easily obtain expression matrices for further analysis.



RShiny-based web portal system

The web-portal system was well-developed by R language, JavaScript D3 library, and other visualization tools, allowing researchers to automatically perform a user-friendly and up-to-date scRNA-seq analysis pipeline on scRNA-seq experiments. The analysis pipeline contains: cell quality control, normalization, cluster analysis, differentially expressed genes analysis, marker identification, gene ontology analysis, and other popular tools.

Galaxy-based preprocessing system

1. Start the galaxy web server



Download docker image and Create a directory first for data acquisition

\$ docker pull lsbnb/galaxy_sc
\$ mkdir YOUR_DIR



Run the docker

port can be changed # "YOUR_DIR" will be the directory shared with docker container \$ docker run -d -t -i -p 8081:80 -v \ ('pwd')/YOUR_DIR/:/root/galaxy/database/ftp/rnaanalysis@galaxy.org/ \ Isbnb/galaxy_sc /bin/bash



Access to the galaxy web server

http://[Your IP]:8081 will be the access to galaxy web server

1. Start the galaxy web server



The Galaxy Project is supported in part by NHGRI, NSF, The Huck Institutes of the Life Sciences, The Institute for CyberScience at Penn State, and Johns Hopkins University.

1. Start the galaxy web server

• If successfully login

	= Galaxy	Analyze Data Workflow Shared Data - Visualization - Help - User -
(Tools	Markyze Data Workflow Shared Data Visualization Help User Hello, Galaxy for single-cell RNA-seq preprocessing is running! To use scRNA-seq preprocessing workflow, please login with: • Username: "rnaanalysis@galaxy.org" • Password: "rnaanalysis" If you don't have matrix, barcodes, and features data produced from 10x Cellranger, please start from 'Workflows/Cellranger to Seashell'. But if you do, start from 'scRNA-seq/CellrangerToSeashell' directly. This customized Galaxy version is provided by Institute of Information Science, Academia Sinica.
		 Contact information: Chung-Yen Lin (cylin@iis.sinica.edu.tw); LAB website: http://eln.iis.sinica.edu.tw
		<u>Galaxy</u> is an open platform for supporting data intensive research. Galaxy is developed by <u>The Galaxy Team</u> with the support of <u>many contributors</u> . The <u>Galaxy Project</u> is supported in part by <u>NHGRI</u> , <u>NSF</u> , <u>The Huck Institutes of the Life Sciences</u> , <u>The Institute for CyberScience at Penn State</u> , and <u>Johns Hopkins University</u> .

2. Download your reference genome

= Galaxy	Analyze Data Workflow Shared Data - Visualization -	Help -	User
Tools	DownloadRef : Download reference files from server (Galaxy Version 1.0.1)		5
search tools	Referece		
Tools of RNA-Seq	CellRanger Human(hg38)	•	
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DownloadRef : Download reference files from server	Download reference files from server.		_
<u>CellRanger</u> : CellRanger <u>zUMIs</u> : zUMIs	here		
<u>CellrangerToSeashell</u> : Use cellranger output files to Seashell file			
<u>zUMIsToSeashell</u> : Use zUMIs output files to Seashell file			
Workflows			
<u>All workflows</u>			
<u>CellRanger to SeaShell</u>			
 <u>zUMIs to SeaShell</u> 			

2. Download your reference genome

А	Analyze Data Workflo	w Shared Data v	Visualization -	Help∓ User¬	 Usi	ng 698.8 MB
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CellRanger Human(hg38)				-	My history	
✓ Execute					1 shown, 33 <u>deleted</u>	
					023.05 MB	
Download reference files from server.					34: Download CellR anger Human Reference	
					ē	

Downloading

3. Prepare your sequencing data



Create "cellranger_input" and "YOUR_SAMPLE" folder in the directory shared with docker

./YOUR_DIR/cellranger_input/YOUR_SAMPLE/



Put your fastq files in "cellranger_input"

./YOUR_DIR/cellranger_input/YOUR_SAMPLE /[Sample Name]_S1_L00[Lane Number]_R1_001.fastq.gz # ./YOUR_DIR/cellranger_input/YOUR_SAMPLE /[Sample Name]_S1_L00[Lane Number]_R2_001.fastq.gz # ./YOUR_DIR/cellranger_input/YOUR_SAMPLE /[Sample Name]_S1_L00[Lane Number]_I1_001.fastq.gz # If you don't know how to name your fastq files, please refer to 10x Genomics: # https://support.10xgenomics.com/single-cell-gene-expression/software/pipelines/latest/using/fastq-input



Enter name of "YOUR_SAMPLE" and other parameters to galaxy workflow

3. Prepare your sequencing data

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4. Run the galaxy pipeline

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small_test_rds_out		small_test_rds_out

4. Run the galaxy pipeline



5. Download the .rds file

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<u>48: Download CellRan</u> g <u>er Human Reference</u>	• / ×

Download

From pre-processed 10x data

01 Put (1) matrix.mtx.gz (2) barcodes.tsv.gz (3) features.tsv.gz in "YOUR_DIR"

Upload these files to galaxy server by ftp

02

03)

Run galaxy workflow "scRNA-seq/CellrangerToSeashell"

Upload files to galaxy server by ftp

Galaxy Analyze Data Workflow Shared Data - Visualization - Help - User -1 Tools 0 search tools Tools of RNA-Seq Hello, Galaxy for single-cell RNA-seq preprocessing is running! scRNA-seq Upload File from your computer DownloadRef : Download To use scRNA-seg preprocessing workflow, please login with: reference files from server CellRanger : CellRanger • Username: "rnaanalysis@galaxy.org" zUMIs : zUMIs • Password: "rnaanalysis" CellrangerToSeashell : Use cellranger output files to Seashell file zUMIsToSeashell : Use zUMIs If you don't have matrix, barcodes, and features data produced from 10x Cellranger, please start from 'Workflows/Cellranger output files to Seashell file to Seashell'. But if you do, start from 'scRNA-seg/CellrangerToSeashell' directly. Workflows All workflows CellRanger to SeaShell This customized Galaxy version is provided by Institute of Information Science, Academia Sinica. zUMIs to SeaShell Contact information: • Chung-Yen Lin (cylin@iis.sinica.edu.tw); LAB website: http://eln.iis.sinica.edu.tw

Upload files to galaxy server by ftp

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search tools			
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Upload files to galaxy server by ftp

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Tools		Download from web	or upload	from disk					
search tools	Hello, Gala	Regular Composite	Collection						
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	• Chung-Ye		□ Choose	local file 🕞 Choose	FTP file 🛛 🕜 Paste/Fetch	data Pause	Rese	Close	

Run galaxy workflow

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Tools	CellrangerToSea	shell : Use cellranger output files to Seashell	file (Galaxy Ve	rsion 1.0.1)			- Optio	ns	
search tools	matrix.mtx.gz	Choose correct file							
Tools of RNA-Seq	0 4 0	55: matrix.mtx						-	
<u>scRNA-seq</u> <u>Upload File</u> from your computer	barcodes.tsv.gz								
DownloadRef : Download reference files from server	features.tsv.gz	54: features.tsv						•	
CellRanger : CellRanger		53: barcodes.tsv						•	
<u>zUMIs</u> : zUMIs	Expect cell num	bers							
CellrangerToSeashell : Use	20000								
cellranger output files to Seashell file	Output Name								
zUMIsToSeashell : Use zUMIs	test								
output files to Seashell file	✓ Execute								
Workflows									

Use cellranger output files to Seashell file.

- <u>CellRanger to SeaShell</u>
- <u>zUMIs to SeaShell</u>

All workflows



Authentication

Please authenticate									
Username:									
Password:									
	Login								

Homepage



Welcome to SeaShell !

Our web service provides an user-friendly interface to process and manage your single-cell data.





Workflow

Upload rds file to SeaShell Cell QC, Normalization, and Clustering 02) 03

Visualization and Gene analysis

Create new project

SeaShell 🖀 Home 🗄 Databank 🎓 Visualization 🚯 Help

Databank

The management sysytem of single-cell experiments can be used to pre-process raw sequencing files and link to other data anaylsis module.



										Edit mode			
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	Projects	Experiments	Species	Cells	Status	CellQC	Record	Brief	Normalization	Clustering			
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Create new project

Upload All single-cell experiments (rds files) to one project. (For example: Control and Drug experiments)



Create new project

Upload All single-cell experiments (rds files) to one project. (For example: Control and Drug experiments)

Databank

The management sysytem of single-cell experiments can be used to pre-process raw sequencing files and link to other data analysis module.

					Add or Ren	nove a row
Project		Experiment 1	Pre-proce	ssed data	<u> </u>	
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				Upload complete		



Create new project

Upload All single-cell experiments (rds files) to one project. (For example: Control and Drug experiments)

Databank

The management sysytem of single-cell experiments can be used to pre-process raw sequencing files and link to other data anaylsis module.

+ New Project

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Hint Click to perform cell QC respectively

Real time qualified cell numbers

Plot

+







Check by 2D scatter plot

Step3. Check selected cell population

Main population are defined by UMI counts AND gene numbers

Number of observations





> Secondary cell QC

Step4. Define outliers by statistical distribution

Outliers are defined as cells certain median absolute deviation (MAD) away from the median in the following distribution.





Databank

The management sysytem of single-cell experiments can be used to pre-process raw sequencing files and link to other data analysis module.

+ New Project

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		Projects		Experiments	Species	Cells	Status	CellQC	Record	Brief	Normalization	Clustering
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			•	Drug	Hs	27	QC 🛇				Perform	
				5						n	ormalizat	ion

Success and new tips

Normalization



Normalization

Normalization Normalization will be performed for all cells in the project to remove unwanted techical bias. Method scran Default Default Default Default Default

SAVE CANCEL

Min.

1st Qu.

Median

Mean

3rd Qu.

Max.



RIE plot

Clustering

Cluster analys	sis	PBMC					
Clustering will be performed to unc	over hidden subpopulations of cells.	Project					
Dimensionality Reduction	Number of highly variable genes used						
UMAP	- 500						
UMAP	or tSNE	MAST or wilcox					
Clustering method	Number of clusters	DEG test method					
SC3	• 3	MAST -					
Cell-type classification							
None	•						
Start							

Clustering



DEG preview



$\bullet \bullet \bullet$

Loading your project: PBMC...

It takes about 0.5~1 mins depending on cell numbers



PBMC

Vis



•

Rename your cluster by DEGs

Rank	Gene	Adjusted P-value	Fold change(log)	Туре	DEG filter
					01-Cluster
1	IL7R	0.00e+00	1.83e+00	Positive	T cell
2	TRAC	0.00e+00	1.40e+00	Positive	Rename cluster
3	LDHB	0.00e+00	1.34e+00	Positive	02-FoldChange
4	TCF7	0.00e+00	1.19e+00	Positive	1.5
5	CD3E	0.00e+00	1.11e+00	Positive	03-adjPvalue
6	NOSIP	0.00e+00	9.63e-01	Positive	0.1
7	LEF1	0.00e+00	9.36e-01	Positive	04-Type
8	TRABD2A	0.00e+00	9.23e-01	Positive	Positive only
9	CD3G	0.00e+00	9.09e-01	Positive	Send to GO
10	CD3D	0.00e+00	Use₀these [DEGsefo	r GO ana

1-10 of 462 rows

Previous 1 2 3 4 5 ... 47 Next









Visualization

A plenty of visualization tools can be perform on well-processed project.

-

ed project. PBMC Project Project **3** Clusters **3,456** Qualified cell numbers

PBMC







Contact



中央研究院 資訊科學研究所 系統生物學暨網路生物學實驗室 主持人 林仲彦 研究員