SRT-Server User Manual

Sheng Yang & Xiang Zhou

Nanjing Medical University

University of Michigan

1 Introduction

1.1 What is SRT-Server

Spatially resolved transcriptomics (SRT) encompasses a suite of innovative technologies that enable gene expression profiling of complex tissues with spatial localization information. As these technologies rapidly advance, numerous computational methods and software tools have been developed to facilitate various SRT analyses. Unfortunately, analyzing SRT datasets using these developed computational methods and tools remains a dauting task for many biologists, as these methods and tools are not easily accessible to researchers with limited statistical and computational backgrounds. SRT-Server allows users to carry out comprehensive SRT analyses for a wide variety of SRT technologies with minimal prior computational knowledge. SRT-Server is user-friendly and features nine analytic modules along with 51 datasets that can perform a range of SRT analyses. The nine analytic modules have hierarchical relationship.

1.2 How to cite SRT-Server

Yang S, Zhou X. SRT-Server: Powering the analysis of spatial transcriptomic data. 2023

2 Login SRT-Server

2.1 Create account

Before you used SRT-Server, we recommend you to login the server. After you inputting

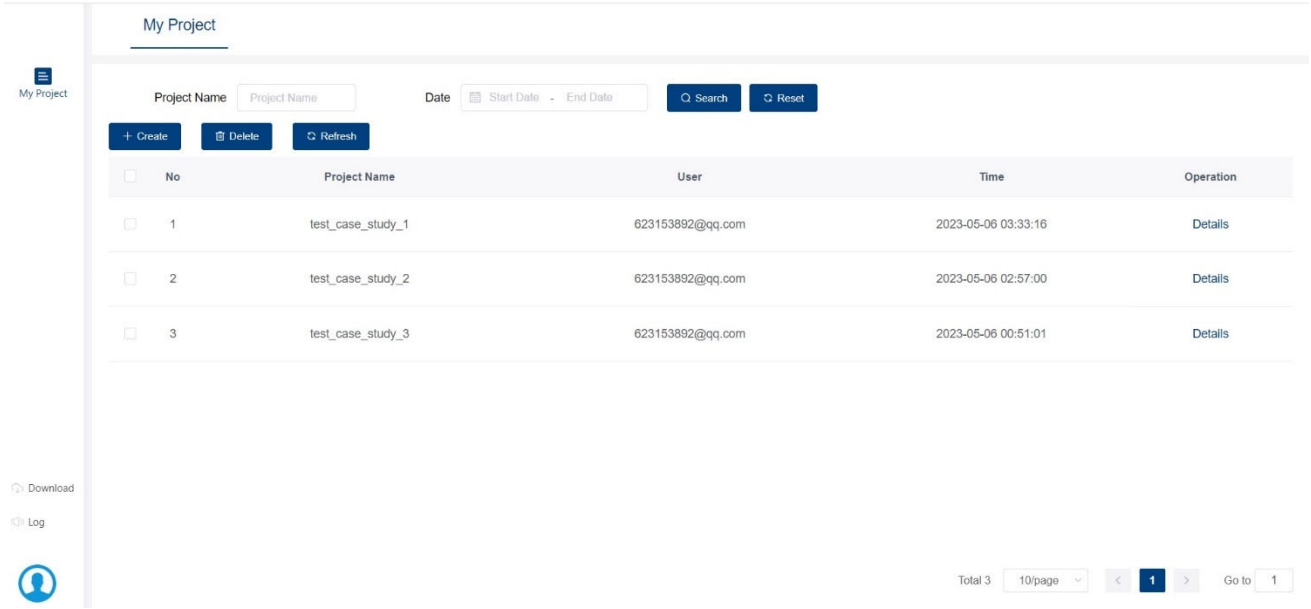
If you create an account, SRT-Server will store your analytic results for 14 days. If you do not create an account, SRT-Server will send you an e-mail for downloading the analytic results. Whatever login or without login, user could create the project.

2.2 Create project

- When user click "**Create**", SRT-Server will pop-up massage box in which the user inputs the project name and description. Clicking "**Details**", SRT-Server provides five key steps: **Project**, **Sample**, **Flow**, **Parameters** and **Result**. For **Sample**, user should select the sample for the project. For **Flow**, user constructs the analytic pipeline by dragging the analytic modules. For **Parameters**, user set parameters for each module. For **Result**, user could check or download the results for each module.
- The "**Sample**" page supports four key functions: **Refresh**, **New Folder**, **Upload files** and **Upload Folder**. After uploading new files or building new folder, user click "**Refresh**" to check whether the folder or file is existing. Click "**New Folder**" to build a new folder. Click "**Upload files**" or "**Upload Folder**" to upload the SRT data from the user's local machine. SRT-Server supports not only four specific SRT platform (i.e. MERFISH, 10x Visium, Slide-seqV2 and SeqFISH) but generic format with expression matrix and spatial location.

2.3 Manage projects

We provide a simple project management system which supports user to browser projects, to search projects by date or project name and to delete projects.


My Project
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1
test_case_study_1
623153892@qq.com
2023-05-06 03:33:16
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test_case_study_2
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2023-05-06 02:57:00
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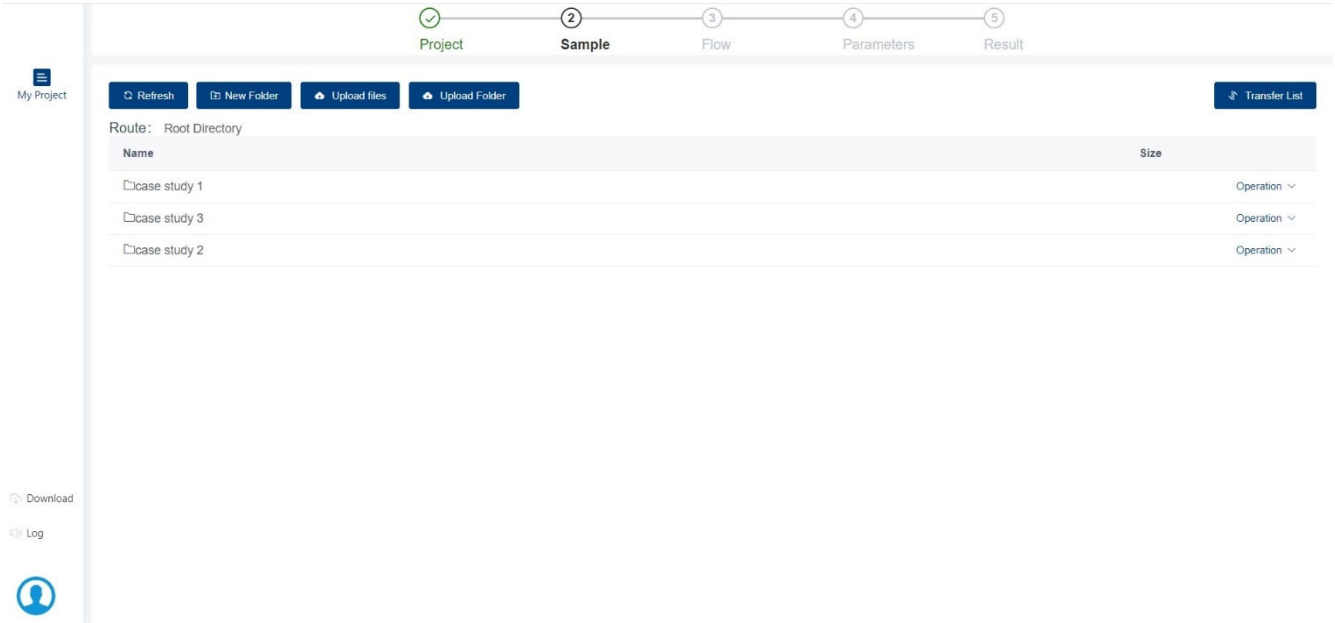
Project
2
Sample
3
Flow
4
Parameters
5
Result
My Project
Refresh
New Folder
Upload files
Upload Folder
Transfer List
Route: Root Directory
Name
Size
case study 1
Operation
case study 3
Operation
case study 2
Operation
Download
Log

3 Nine Modules for SRT-Server

The analytic modules in SRT-Server provide the foundation for building user-defined analytic pipelines. These modules are represented as icons on the sidebar in the graphical interface of the SRT-Server. Users can drag these modules onto a canvas on the interface and use arrows to connect them to form their desired analytic pipeline.

After user build your analytic pipeline, user should select “De”

3.1 QC: quality control

Three required parameters:

- **Platform**: six selections: Visium, Merfish, Seqfish, Slideseq, Generic_Spot, Generic_Cell.
- **Minium Cells**: filtering out spots with a low number of expressed genes
- **Minium Features**: excluding features with a low number of expressed spots.

3.2 SVG: Detecting spatially variable genes

Five required parameters:

- **Methods:** SPARK or SPARK-X. SPARK will take long time (longer than 24 hours for 10x Visium data). SRT-Server recommend user to use SPARK-X to detect SVGs.
- **P Threshold:** The cutoff to define the significant SVGs.
- **Permutation for qq Plot:** SVG module could generate qq plot to show the type I error of the SRT data for SPARK or SPARK-X.
- **Pattern Plot** SVG module could generate the pattern plot for the SRT data. If user select *true*, user should set “**Pattern number**”. If user select *false*, this module will not generate pattern plot.
- **Pattern Number:** When user need the pattern plot, user should set the pattern number.

3.3 DECON: Cell type deconvolution in spot resolution SRT

Five required parameters:

- **CARD without scRNA-seq Panel (Boolean):** DECON moule supports two panels: i) using expression matrix of scRNA-seq; ii) using marker genes information.
- **Species:** Mouse or Human.
- **Status:** Select the expression matrix or marker gene information which is corresponding to the analyzed SRT data.

- **Grid Number** Initial number of grids to construct a refined spatial map. Default is 2000.
- **sc Mapping:** If user select *true*, DECON module will perform single cell resolution mapping. This step will take a long time (about 30 minutes for one slice of 10x Visium data).

Tips:

The platform of datasets determines the CT or SDD module.

We set CT and SDD for cell and spot resolution platform, respectively.

3.4 CT: Cell typing/cell type clustering in single-cell resolution SRT

Five required parameters for Seurat:

- **Methods:** Seurat
- **Batch:** CT_PCA provides four choices: i) None: one sample; ii) Merge: using merge function in Seurat; iii) Normalization_Integration: using integrate method; iv) SCTransform_Integration: using SCT method.
- **Resolution:** different resolutions split by comma without spacing.

Five required parameters for Garnett or scSorter:

- **Methods:** Garnett or scSorter.
- **Species:** Mouse or Human.
- **Status:** Select the expression matrix or marker gene information which is corresponding to the analyzed SRT data.

Five required parameters for BASS

- **Cell Type Number:** the cell type numbers, which are split by comma without spacing.
- **Domain Number:** the domain numbers, which are split by comma without spacing.
- **Beta Methods:** fix or SW
- **Initial Methods:** kmeans or mclust
- **Gene Selection:** CL_jo provides two selections: i) sparkx: using top 2000 SVGS; ii) hvg; using highly variable genes.

3.5 SDD: detecting spatial domains

- **Models:** SDD_sPCA and CL_jo.
- **Methods:** SDD_sPCA includes SpatialPCA. CL_jo includes BASS.

Five required parameters for SpatialPCA

- **Featured Genes:** SDD_sPCA provides three selections: i) spatial: using top 2000 SVGS; ii) hvg; using highly variable genes; iii) custom: using custom genes which can be set by user.
- **Spark Version** or **Custom Genes:** If user set **Featured Genes** as **spatial**, user should select **Spark Version** as SPARK or SPARK-X. If user set **Featured Genes** as **custom**, user should input **Custom Gene**. Specially, all the custom gene names are split by comma.
- **Cluster Methods:** walktrap and louvain.
- **Domain Number:** the domain numbers, which are split by comma without spacing.
- **Kernel:** Select kernel for the relationship between each spot. SDD_sPCA provides three kernels: gaussian, cauchy and delaunday.

Five required parameters for BASS

- **Cell Type Number:** the cell type numbers, which are split by comma without spacing.
- **Domain Number:** the domain numbers, which are split by comma without spacing.
- **beta Methods:** fix or SW
- **Initial Methods:** kmeans or mclust
- **Gene Selection:** CL_jo provides two selections: i) sparkx: using top 2000 SVGS; ii) hvg; using highly variable genes.

Tips:

The next four modules should use the result of CT or SDD.

Different parameters of CT or SDD will generate different results.

The user should set the parameters for the selected CT or SDD results.

3.6 DEG: Identifying genes that are differentially expressed genes in a specific cell type

or spatial domain

- **DE methods:** DEG module provides wilox test.

3.7 ORA: Pathway enrichment analysis with the identified DEGs or SVGs

- **Methods:** svg or de
- **Species:** Mouse or Human
- **Pathway Databases:** All, GO, KEGG, Reactome and WikiPathways. If the SRT data is human, ORA module provides DO pathways.

3.8 CCC: Detecting cell-cell communications in both single-cell resolution and spot resolution SRT

- **Species:** Mouse or Human.
- **Ccc Methods:** spatalk
- **Ligand-receptor Databases:** six datasets: SpaTalk.DB, CellPhoneDB, iTALK, CellChatDB, CellTalkDB and CellCall

3.9 TRAJ: Estimating pseudo-time trajectories across cells or spatial locations

- **Start Domain** or **Start Cell Type:** Input a specific cell type or domain. If user input more than one cell type or domain, the number should be split by comma without spacing.

4 Q&A

Thanks for your consideration for SRT-Server. We hope our server can help you to solve the data analysis problem in for the SRT. If you have any questions, please feel free to tell us in the Github (https://github.com/biostat0903/SRT-Server). We will update SRT-Server or provide new analytic modules.