cellhub

Release 0.1

Sansom lab

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CONTENTS

CHAPTER

ONE

INSTALLATION

1.1 Installation

1.1.1 Dependencies

Core dependencies include:

- Cellranger (from 10X Genomics) >= 6.0
- Python3
- Various Python packages (see python/requirements.txt)
- R >= 4.0
- Various R libraries (see R/install.packages.R)
- Latex
- The provided cellhub R library

1.1.2 Installation

1. Install the cgat-core pipeline system following the instructions here https://github.com/cgat-developers/cgat-core/.
2. Clone and install the cellhub-devel repository e.g.:

```
git clone https://github.com/COMBATOxford/cellhub-devel.git
cd cellhub-devel
python setup.py develop
```

Note: Running "python setup.py develop" is necessary to allow pipelines to be launched via the "cellhub" command.

3. In the same virtual or conda environment as cgat-core install the required python packages:

```
pip install -r cellhub-devel/python/requirements.txt
```

4. To install the required R packages (the "BiocManager" and "devtools" libraries must be pre-installed):

```
Rscript cellhub-devel/R/install.packages.R
```

5. Install the cellhub R library:

```
R CMD INSTALL R/cellhub
```

CHAPTER

TWO

OVERVIEW

2.1 Workflow Overview

2.1.1 Philosophy

Cellhub is designed to efficiently parallelise the processing of large datasets. Once processed different data slices can be easily extracted directly from the original matrices, aligned and exported for downstream analysis. At the heart of operations is an sqlite database which warehouses the experiment metadata and per-cell statistics.

The workflow can be divided into eight main steps.

2.1.2 1. Quantitation of per-channel libraries

The workflow begins with *pipeline_cellranger_multi.py*. Input 10X capture channel library identifiers "library_id" and their associated fastq files are specified in the pipeline_cellranger_multi.yml configuration file. The reads from the different libraries will be mapped in parallel.

Note: The channel library is considered to be the fundamental "batch" unit of a 10X experiment. Cells captured from the same channel are exposed to the same ambient RNA. Separate genomic libraries are prepared for each 10x channel.

- It is recommend to inspect patterns of ambient RNA using *pipeline_ambient_rna.py*.
- Per-channel velocity matrices can be prepared using *pipeline_velocity.py*.
- Cell identification can also be performed with *pipeline_emptydrops.py*.

2.1.3 2. Computation of per-cell statistics

Per-cell QC statistics are computed in parallel for each channel library using *pipeline_cell_qc.py*. The pipeline computes various statistics including standard metrics such as percentage of mitochondrial reads, numbers of UMIs and numbers of genes per cell. In addition it can compute scores for custom genesets.

Per-cell celltype predictions are computed in parallel for each channel library using *pipeline_singleR.py*

Note: all per-channel matrices containing computed cell statistics are required to contain "library_id" and "barcode_id" columns.

Note: file names of the per-channel matrices are specified as “library_id.tsv.gz” (matrices for different analyses such as e.g. qcmetrics and scrublet scores are written to separate folders).

2.1.4 3. Cell demultiplexing [optional]

If samples have been multiplexed within channels either genetically or using hash tags a table of barcode_id -> sample_id assignments are prepared using pipeline_demux.py [not yet written].

2.1.5 4. Preparation of the cell database

The library and sample metadata, per cell statistics (and demultiplex assignments) are loaded into an sqlite database using *pipeline_celldb.py*. The pipeline creates a view called “final” which contains the qc and metadata needed for cell selection and downstream analysis.

Note: The user is required to supply a tab-separated sample metadata file (e.g. “samples.tsv”) via a path in the pipeline_celldb.yml configuration file. It should have columns for library_id, sample_id as well as any other relevant experimental metadata such as condition, genotype, age, replicate, sex etc.

2.1.6 5. Initial assessment of cell quality

This is performed manally by the data analyst as it is highly dataset-specific. Per cell QC statistics can be easily retrieved from the celldb for plotting along with singleR scores from the cellhub API.

2.1.7 6. Fetching of cells for downstream analysis

Cells are fetched using *pipeline_fetch_cells.py*. The user specifies the cells that they wish to retrieve from the “final” table (see step 4) via an sql statement in the pipeline_fetch_cells.yml configuration file. The pipeline will extract the cells and metadata from the original matrices and combine them into market matrices and anndata objects for downstream analyses.

It is recommended to fetch cells into a new directory. By design fetching of a single dataset per-directory is supported.

The pipeline supports fetching of velocity information.

Note: The retrieved metadata will include a “sample_id” column. From this point onwards it is natural to think of the “sample_id” as the unit of interest. The “library_ids” remain in the metadata along with all the qc statistics to facilitate downstream investigation of batch effects and cell quality.

2.1.8 7. ADT normalization [optional]

If samples included the ADT modality, *pipeline_adt_norm.py* normalizes the antibody counts for the high-quality fetched cells in the previous step. Normalized ADT can be then used for downstream integration. The pipeline implements 3 normalization methodologies: DSB, median-based, and CLR. The user can specify the feature space.

2.1.9 8. Integration

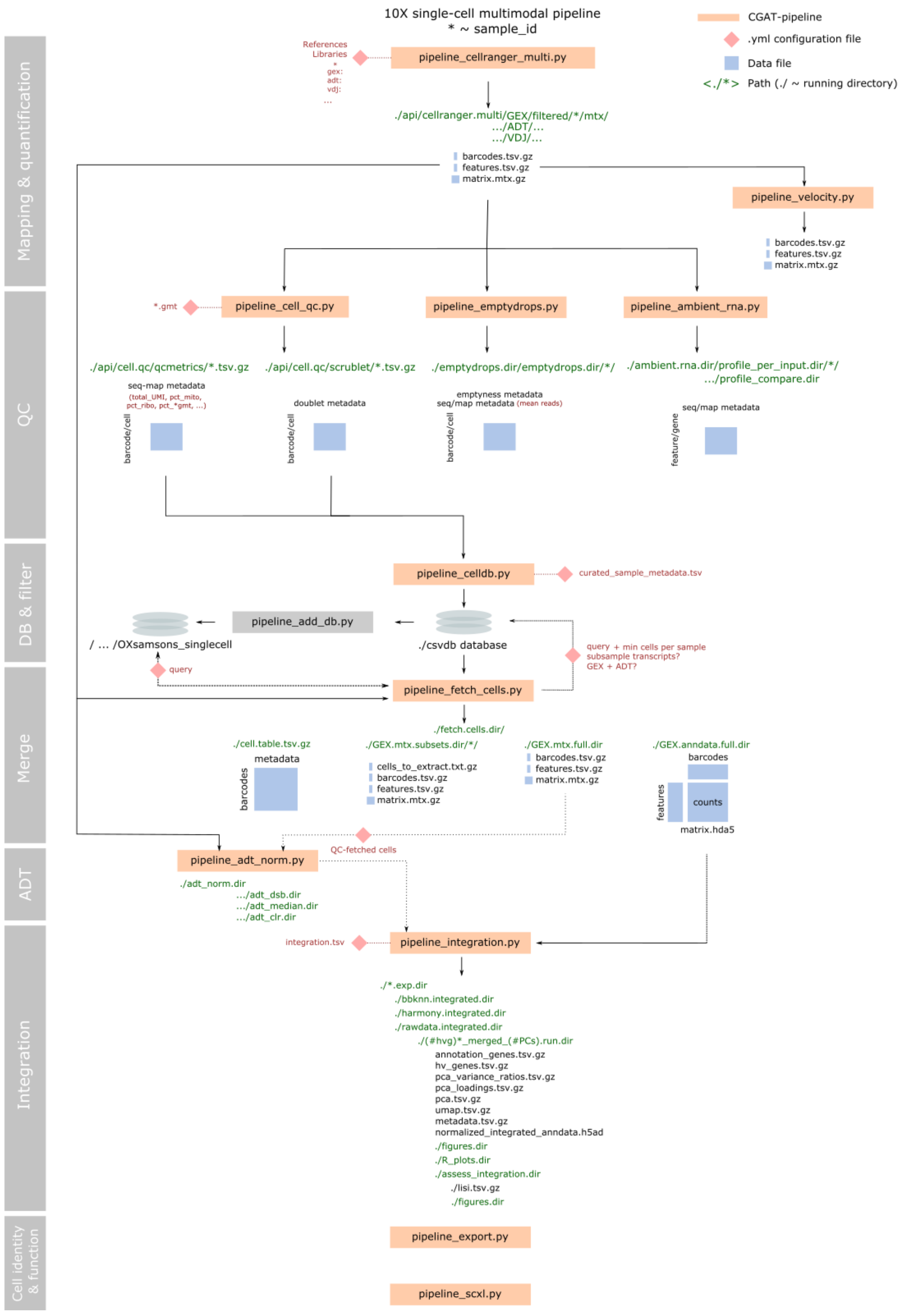
Integration of samples is performed manually by the user because it is highly dataset specific. Different integration algorithms are needed for different contexts. Strategies for HVG selection and modelling of covariates need to be considered by the data analyst on a case by case basis.

2.1.10 9. Clustering analysis

Clustering analysis is performed with *pipeline_cluster*.

2.2 Workflow Diagram

The diagram is now a little out of date with respect to configuration of the pipeline inputs but provides a useful depiction of the overall workflow.


10X single-cell multimodal pipeline
* ~ sample_id
CGAT-pipeline
.yml configuration file
Data file
<./*> Path (./ ~ running directory)
Mapping & quantification
QC
DB & filter
Merge
ADT
Integration
Cell identity & function
References
Libraries
*
gex:
adt:
vdj:
...
pipeline_cellranger_multi.py
./api/cellranger.multi/GEX/filtered/*/mtx/
.../ADT/...
.../VDJ/...
barcodes.tsv.gz
features.tsv.gz
matrix.mtx.gz
pipeline_velocity.py
barcodes.tsv.gz
features.tsv.gz
matrix.mtx.gz
*.gmt
pipeline_cell_qc.py
pipeline_emptydrops.py
pipeline_ambient_rna.py
./api/cell.qc/qcmetrics/*.tsv.gz
./api/cell.qc/scrublet/*.tsv.gz
./emptydrops.dir/emptydrops.dir/*/
./ambient.rna.dir/profile_per_input.dir/*/
.../profile_compare.dir
seq-map metadata
(total_UMI, pct_mito, pct_ribo, pct_*gmt, ...)
doublet metadata
emptyness metadata
seq/map metadata (mean reads)
seq/map metadata
barcode/cell
barcode/cell
barcode/cell
feature/gene
pipeline_celldb.py
curated_sample_metadata.tsv
pipeline_add_db.py
/ ... /OXsamsons_singlecell
./csvdb database
query + min cells per sample
subsample transcripts?
GEX + ADT?
query
pipeline_fetch_cells.py
./fetch.cells.dir/
./cell.table.tsv.gz
metadata
barcodes
./GEX.mtx.subsets.dir/*/
cells_to_extract.txt.gz
barcodes.tsv.gz
features.tsv.gz
matrix.mtx.gz
./GEX.mtx.full.dir
barcodes.tsv.gz
features.tsv.gz
matrix.mtx.gz
./GEX.anndata.full.dir
barcodes
features
counts
matrix.hda5
pipeline_adt_norm.py
QC-fetched cells
./adt_norm.dir
.../adt_dsb.dir
.../adt_median.dir
.../adt_clr.dir
integration.tsv
pipeline_integration.py
./*.exp.dir
./bbknn.integrated.dir
./harmony.integrated.dir
./rawdata.integrated.dir
./(#hvg)*_merged_(#PCs).run.dir
annotation_genes.tsv.gz
hv_genes.tsv.gz
pca_variance_ratios.tsv.gz
pca_loadings.tsv.gz
pca.tsv.gz
umap.tsv.gz
metadata.tsv.gz
normalized_integrated_anndata.h5ad
./figures.dir
./R_plots.dir
./assess_integration.dir
./lisi.tsv.gz
./figures.dir
pipeline_export.py
pipeline_scxl.py

2.3 Usage

2.3.1 Configuring and running pipelines

Run the cellhub –help command to view the help documentation and find available pipelines to run cellhub.

The cellhub pipelines are written using cgat-core pipelining system. From more information please see the CGAT-core paper. Here we illustrate how the pipelines can be run using the cellranger_multi pipeline as an example.

Following installation, to find the available pipelines run:

```
cellhub -h
```

Next generate a configuration yml file:

```
cellhub cellranger_multi config -v5
```

To fully run the example cellhub pipeline run:

```
cellhub cellranger_multi make full -v5
```

However, it may be best to run the individual tasks of the pipeline to get a feel of what each task is doing. To list the pipline tasks and their current status, use the 'show' command:

```
cellhub cellranger_multi show
```

Individual tasks can then be executed by name, e.g.

```
cellhub cellranger_multi make cellrangerMulti -v5
```

Note: If any upstream tasks are out of date they will automatically be run before the named task is executed.

2.3.2 Getting Started

To get started please see the *IFNb example*.

CHAPTER

THREE

EXAMPLES

3.1 IFNb PBMC example

3.1.1 Setting up

1. Clone the example template to a local folder

Clone the folders and files for the example into a local folder.

> cp -r /path/to/cellhub/examples/ifnb_pbmc/* .

This will create 3 folders:

- "cellhub" where the preprocessing pipelines will be run and where the cellhub database will be created
- "integration" where integration is to be performed.
- "cluster" where we can perform downstream analysis with "cellhub cluster".

3.1.2 Running the pre-processing pipelines and creating the database

2. Running Cellranger

The first step is to configure and run pipeline_cellranger_multi. We already have a pre-configured yml file so we can skip this step but the syntax is included for reference here and also for the other steps:

```
# enter the cellhub directory

cd cellhub

# cellhub cellranger_multi config
```

Edit the pipeline_cellranger_multi.yml file as appropriate to point to folders containing fastq files extracted from the original BAM files submitted by Kang et. al. to GEO (GSE96583). The GEO identifiers are: unstimulated (GSM2560248) and stimulated (GSM2560249). The fastqs can be extracted with the 10X bamtofastq tool.

We run the pipeline as follows:

```
cellhub cellranger_multi make full -v5 -p20
```

If you have not run "python setup.py devel" pipelines can instead be launched directly. In this case the equivalent command would be:

```
python path/to/cellhub-devel/cellhub/pipeline_cellranger_multi.py make full -v5 -p20 --
↪pipeline-log=pipeline_cellranger_multi.py
```

Note: when launching pipelines directly if the "–pipeline-log" parameter is not specified the log file will be written to "pipeline.log".

3. Running the cell qc pipeline

Next we run the cell qc pipeline:

```
# cellhub cell_qc config

cellhub cell_qc -v5 -p20 make full
```

4. Running emptydrops and investigating ambient RNA (optional)

If desired we can run emptydrops:

```
# cellhub emptydrops config

cellhub emptydrops -v5 -p20 make full
```

And investigate the ambient rna:

```
# cellhub ambient_rna config

cellhub ambient_rna -v5 -p20 make full
```

5. Loading the cell statistics into the celldb

The cell QC statistics and metadata ("samples.tsv") are next loaded into a local sqlite database:

```
# cellhub celldb config

cellhub celldb -v5 -p20 make full
```

6. Run pipeline_singleR

Single R is run on all the cells so that the results are avaliable to help with QC as well as downstream analysis:

```
# cellhub singleR config

cellhub singleR -v5 -p20 make full.
```

As noted: *in the pipeline_singleR inputs section* the celldex references neede to be stashed before the pipeline is run.

7. Run pipeline_annotation

This pipeline retrieves Ensembl and KEGG annotations needed for downstream analysis.:

```
# cellhub annotation config

cellhub annotation -v5 -p10 make full
```

Please note that the specified Ensembl version should match that used for the cellranger reference trancriptome.

3.1.3 Performing cell QC

8. Assessment of cell quality

This step is left to the reader to perform manually because it needs to be carefully tailored to individual datasets.

3.1.4 Performing downstream analysis

9. Fetch cells for integration

We use pipeline_fetch_cells to retrieve the cells we want for downstream analysis. (QC thresholds and e.g. desired samples are specified in the pipeline_fetch_cells.yml) file:

```
# It is recommended to fetch the cells in to a seperate directory for integration.
cd ../integration

# cellhub fetch_cells config
cellhub fetch_cells  -v5 -p20 make full
```

10. Integration

Run the provided jupyter notebook to perform a basic Harmony integration of the data and to save it in the appropriate anndata format (see *in the pipeline_cluster inputs section*) is provided.

11. Clustering analysis

Cluster analysis is performed with pipeline cluster (a seperate directory is recommended for this so that multiple clustering runs can be performed as required).:

```
# change into the clustering directory
cd ../cluster.dir

# checkout the yml file
cellhub cluster config

# a suitable yml file has been provided so we can now launch the pipeline
cellhub cluster -v5 -p200 make full
```

The pdf reports and excel files generated by the pipeline can be found in the "reports.dir" subfolder.

For interactive visulation, the results are provided in cellxgene format. To view the cellxgene.h5ad files, you will first need toinstall cellxgene with "pip install cellxgene". The cellxgene viewer can then be launched with:

```
# substitute "{x}" with the number integrated components used for the clustering run.
cellxgene --no-upgrade-check launch out.{x}.comps.dir/cellxgene.h5ad
```

CHAPTER

FOUR

CODING GUIDELINES

4.1 Coding Guidelines

4.1.1 Repository layout

Table 1: Repository layout

Folder	Contents
cellhub	The cellhub Python module which contains the set of CGAT-core pipelines
cellhub/tasks	The cellhub tasks submodule which contains helper functions and pipeline task definitions
cellhub/reports	The latex source files used for building the reports
Python	Python worker scripts that are executed by pipeline tasks
R/cellhub	The R cellhub library
R/scripts	R worker scripts that are executed by pipeline tasks
docsrc	The documentation source files in restructured text format for compilation with sphinx
docs	The compiled documentation
examples	Configuration files for example datasets
conda	Conda environment, TBD
tests	TBD

4.1.2 Coding style

Currently we are working to improve and standardise the coding style:

- Python code should be pep8 compliant. Compliance checks will be enforced soon.
- R code should follow the tidyverse style guide. Please do not use right-hand assignment.
- Arguments to Python scripts should be parsed with argparse.
- Arguments to R scripts should be parsed with optparse or supplied via yaml files.
- Logging in Python scripts should be performed with the standard library "logging" module.
- Logging in R scripts should be performed with the "futile.logger" library.
- If you need to write to stdout from R scripts use message() or warning(). Do not use print() or cat().

4.1.3 Writing pipelines

The pipelines live in the “cellhub” python module.

Auxiliary task functions live in the “cellhub/task” python sub-module.

Note: Tasks of more than a few lines should be abstracted into appropriately named sub-modules.

In the notes below “xxx” denotes the name of a pipeline such as e.g. “cell_qc”.

1. Paths should never be hardcoded in the pipelines - rather they must be read from the yaml files.
2. Yaml configuration files should be named pipeline_xxx.yml
3. The output of individual pipelines should be written to a subfolder name “xxx.dir” to keep the root directory clean (it should only contain these directories and the yml configuration files!).
4. Pipelines that generate cell-level information for down-stream analysis must read their inputs from the api and register their outputs to the API, see *API*. If you need information from an upstream pipeline that is not present on the API please raise an issue.
5. We are documenting the pipelines using the sphinx “autodocs” module so please maintain informative rst docstrings.

4.1.4 Cell barcodes

- We use cell barcodes in the format “UMI-library_id”.
- Barcodes are set upstream e.g. in the market matrix files that are exposed on the API by pipeline_cellranger_multi.py.
- Downstream pipelines shold not need to manipulate barcodes. If you find barcodes being served on the API in an incorrect format please raise an issue.
- We use the field/column name “barcode_id” for the fields/columns that contains the barcodes in tables and databases.

4.1.5 Yaml configuration file naming

The cgat-core system only supports configuration files name “pipeline.yml”.

We work around this by overriding the cgat-core functionality using a helper function in cellhub.tasks.control as follows:

```
import Pipeline as P
import cellhub.tasks.control as C

# Override function to collect config files
P.control.write_config_files = C.write_config_files
```

Default yml files must be located at the path cellhub/yaml/pipeline_xxx.yml

4.1.6 Writing and compiling the documentation

The source files for the documentation are found in:

```
docsrc
```

The documentation source files for the pipelines can be found in:

```
docsrc/pipelines
```

To build the documentation cd to the docsrc folder and run:

```
make github
```

This will build the documentation and copy the latex output to the "docs" folder. You then need to cd to the "docs" folder and run:

```
make
```

To compile the pdf.

When the repo is made public we will switch to using html documentation on readthedocs. Unfortunately there is no straightforward solution for private html hosting.

4.2 API

4.2.1 Overview

The pre-processing pipelines must define and register their outputs via a common api. The *cellhub.tasks.api module* provides the code for doing this. The api comprises of an "api" folder into which pipeline outputs are symlinked (by the "register_dataset" "api" class method).

The api provides a stable and sanitised interface from which the downstream pipelines can access the information.

4.2.2 Example

The "api" folder for the IFNB example dataset looks like this.

```
api
├── cell.qc
│   ├── qcmetrics
│   │   └── filtered
│   │       ├── GSM2560248.tsv.gz -> ../../../../cell.qc.dir/qcmetric.dir/GSM2560248.tsv.gz
│   │       ├── GSM2560249.tsv.gz -> ../../../../cell.qc.dir/qcmetric.dir/GSM2560249.tsv.gz
│   │       └── manifest.yml
│   └── scrublet
│       └── filtered
│           ├── GSM2560248.tsv.gz -> ../../../../cell.qc.dir/scrublet.dir/GSM2560248.tsv.gz
│           ├── GSM2560249.tsv.gz -> ../../../../cell.qc.dir/scrublet.dir/GSM2560249.tsv.gz
│           └── manifest.yml
└── cellranger.multi
    └── GEX
        ├── filtered
        │   ├── GSM2560248
        │   │   └── mtx
        │   │       ├── barcodes.tsv.gz -> ../../../../../../cellranger.multi.dir/out.dir/GSM2560248/GSM2560248/GEX/barcodes.tsv.gz
        │   │       ├── features.tsv.gz -> ../../../../../../cellranger.multi.dir/out.dir/GSM2560248/GSM2560248/GEX/features.tsv.gz
        │   │       ├── manifest.yml
        │   │       └── matrix.mtx.gz -> ../../../../../../cellranger.multi.dir/out.dir/GSM2560248/GSM2560248/GEX/matrix.mtx.gz
        │   └── GSM2560249
        │       └── mtx
        │           ├── barcodes.tsv.gz -> ../../../../../../cellranger.multi.dir/out.dir/GSM2560249/GSM2560249/GEX/barcodes.tsv.gz
        │           ├── features.tsv.gz -> ../../../../../../cellranger.multi.dir/out.dir/GSM2560249/GSM2560249/GEX/features.tsv.gz
        │           ├── manifest.yml
        │           └── matrix.mtx.gz -> ../../../../../../cellranger.multi.dir/out.dir/GSM2560249/GSM2560249/GEX/matrix.mtx.gz
        └── unfiltered
            ├── GSM2560248
            │   └── mtx
            │       ├── barcodes.tsv.gz -> ../../../../../../cellranger.multi.dir/out.dir/GSM2560248/unfiltered/GEX/barcodes.tsv.gz
            │       ├── features.tsv.gz -> ../../../../../../cellranger.multi.dir/out.dir/GSM2560248/unfiltered/GEX/features.tsv.gz
            │       ├── manifest.yml
            │       └── matrix.mtx.gz -> ../../../../../../cellranger.multi.dir/out.dir/GSM2560248/unfiltered/GEX/matrix.mtx.gz
            └── GSM2560249
                └── mtx
                    ├── barcodes.tsv.gz -> ../../../../../../cellranger.multi.dir/out.dir/GSM2560249/unfiltered/GEX/barcodes.tsv.gz
                    ├── features.tsv.gz -> ../../../../../../cellranger.multi.dir/out.dir/GSM2560249/unfiltered/GEX/features.tsv.gz
                    ├── manifest.yml
                    └── matrix.mtx.gz -> ../../../../../../cellranger.multi.dir/out.dir/GSM2560249/unfiltered/GEX/matrix.mtx.gz
```

4.2.3 Usage

Please see the *cellhub.tasks.api module documentation* for more details.

..note:: If you are writing a pipeline and information that you need from an upstream pipeline has not been exposed on the api please raise an issue.

CHAPTER

FIVE

TASKS MODULE DOCUMENTATION

5.1 API

5.1.1 Overview

This module contains the code for registering and accessing pipeline outputs from a common location.

There are classes that provide methods for:

(1) registering pipeline outputs to the common service endpoint

(2) discovering the information avaliable from the service endpoint (not yet written)

(3) accessing information from the service endpoint (not yet written)

The service endpoint is the folder "api". We use a rest-like syntax for providing access to the pipline outputs.

5.1.2 Usage

Registering ouputs on the service endpoint

Please see *pipeline_cellranger_multi.py* or *pipeline_cell_qc.py* source code for examples.

As an example the code used for registering the qcmetrics outputs is reproduced with some comments here:

```
import cellhub.tasks.api as api

file_set={}

...

# the set of files to be registered is defined as a dictionary
# the keys are arbitrary and will not appear in the api

file_set[library_id] = {"path": tsv_path,
                        "description":"qcmetric table for library " +
↪                      library_id,
                        "format":"tsv"}

# an api object is created, passing the pipeline name
x = api.api("cell.qc")
```

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```
# the dataset to be deposited is added
x.define_dataset(analysis_name="qcmetrics",
                 data_subset="filtered",
                 file_set=file_set,
                 analysis_description="per library tables of cell GEX qc statistics",
                 file_format="tsv")

# the dataset is linked in to the API
x.register_dataset()
```

Discovering avaliable datasets

TBD.

Accessing datasets

For now datasets can be accessed directly via the "api" endpoint. However in future it will likely be recommended to access them via a subclass of the "read" class (not yet written) which will provide sanity checking.

5.1.3 Class and method documentation

class `cellhub.tasks.api.`**`api`**(*pipeline=None*, *endpoint='api'*)

Bases: `object`

A class for defining and registering datasets on the cellhub api.

When initialising an instance of the class, the pipeline name is passed e.g.:

```
x = cellhub.tasks.api.register("cell_qc")
```

Note: pipeline names are sanitised to replace spaces, underscores and hypens with periods.

`define_dataset`(*analysis_name=None*, *analysis_description=None*, *data_subset=None*, *data_id=None*, *data_format=None*, *file_set=None*)

Define the dataset.

The "data_subset", "data_id" and "data_format" parameters are optional.

The file_set is a dictionary that contains the files to be registered:

```
{ "name": { "path": "path/to/file",
            "format": "file-format",
            "description": "free-text" }
```

the top level "name" keys are arbitrary and not exposed in the API

e.g. for cell ranger output the file_set dictionary looks like this:

```
{"barcodes": {"path":"path/to/barcodes.tsv",
              "format": "tsv",
              "description": "cell barcode file"},
{"features": {"path":"path/to/features.tsv",
              "format": "tsv",
              "description": "features file"},
{"matrix": {"path":"path/to/matrix.mtx",
              "format": "market-matrix",
              "description": "Market matrix file"}
}
```

register_dataset()

Register the dataset on the service endpoint. The method:

1. creates the appropriate folders in the "api" endpoint folder
2. symlinks the source files to the target location
3. constructs and deposits the manifest.yml file

The location at which datasets will be registered is defined as:

```
api/pipeline.name/analysis_name/[data_subset/][data_id/][data_format/]
```

(data_subset, data_id and data_format are [optional])

show()

Print the api object for debugging.

reset_endpoint()

Clean the dataset endpoint

CHAPTER SIX

PIPELINE DOCUMENTATION

6.1 Pipeline ADT normalization

6.1.1 Overview

This pipeline implements three normalization methods:

- DSB (https://www.biorxiv.org/content/10.1101/2020.02.24.963603v3)
- Median-based (https://bioconductor.org/books/release/OSCA/integrating-with-protein-abundance.html)
- CLR (https://satijalab.org/seurat/archive/v3.0/multimodal_vignette.html)

Configuration

The pipeline requires a configured `pipeline_adt_norm.yml` file. Default configuration files can be generated by executing:

```
python <srcdir>/pipeline_adt_norm.py config
```

Input files

This pipeline requires the unfiltered gene-expression and ADT count matrices and a list of high quality barcodes most likely representing single-cells.

This means that ideally this pipeline is run after high quality cells are selected via the pipeline_fetch_cells.py.

This pipeline will look for the unfiltered matrix in the api:

./api/cellranger.multi/ADT/unfiltered/*/mtx/*.gz

./api/cellranger.multi/GEX/unfiltered/*/mtx/*.gz

Dependencies

This pipeline requires: * cgat-core: https://github.com/cgat-developers/cgat-core * R dependencies required in the r scripts

6.1.2 Pipeline output

The pipeline returns a adt_norm.dir folder containing one folder per methodology adt_dsb.dir, adt_median.dir, and adt_clr.dir with per-sample folders conatining market matrices [features, qc-barcodes] with the normalized values.

6.1.3 Code

`cellhub.pipeline_adt_norm.`**`gexdepth`**`(`*infile*, *outfile*`)`

This task will run R/adt_calculate_depth_dist.R, It will describe the GEX UMI distribution of the background and cell-containing barcodes. This will help to assess the quality of the ADT data and will inform about the definition of the background barcodes.

`cellhub.pipeline_adt_norm.`**`gexdepthAPI`**`(`*infiles*, *outfile*`)`

Add the umi depth metrics results to the API

`cellhub.pipeline_adt_norm.`**`adtdepth`**`(`*infile*, *outfile*`)`

This task will run R/adt_calculate_depth_dist.R, It will describe the ADT UMI distribution of the background and cell-containing barcodes. This will help to assess the quality of the ADT data and will inform the definition of the background barcodes.

`cellhub.pipeline_adt_norm.`**`adtdepthAPI`**`(`*infiles*, *outfile*`)`

Add the umi depth metrics results to the API

`cellhub.pipeline_adt_norm.`**`adt_plot_norm`**`(`*infile*, *outfile*`)`

This task will run R/adt_plot_norm.R, It will create a visual report on the cell vs background dataset split and, if the user provided GEX and ADT UMI thresholds, those will be included.

`cellhub.pipeline_adt_norm.`**`dsb_norm`**`(`*infile*, *outfile*`)`

This task runs R/adt_normalize.R. It reads the unfiltered ADT count matrix and calculates DSB normalized ADT expression matrix which is then saved like market matrices per sample.

`cellhub.pipeline_adt_norm.`**`dsbAPI`**`(`*infile*, *outfile*`)`

Register the ADT normalized mtx files on the API endpoint

`cellhub.pipeline_adt_norm.`**`median_norm`**`(`*infile*, *outfile*`)`

This task runs R/adt_get_median_normalization.R, It reads the filtered ADT count matrix and performed median-based normalization. Calculates median-based normalized ADT expression matrix and writes market matrices per sample.

`cellhub.pipeline_adt_norm.`**`medianAPI`**`(`*infile*, *outfile*`)`

Register the ADT normalized mtx files on the API endpoint

`cellhub.pipeline_adt_norm.`**`clr_norm`**`(`*infile*, *outfile*`)`

This task runs R/get_median_clr_normalization.R, It reads the filtered ADT count matrix and performes CLR normalization. Writes market matrices per sample.

`cellhub.pipeline_adt_norm.`**`clrAPI`**`(`*infile*, *outfile*`)`

Register the CLR-normalized ADT mtx files on the API endpoint `cellhub.pipeline_adt_norm.plot`(*infile*, *outfile*)

Draw the pipeline flowchart

`cellhub.pipeline_adt_norm.full`()

Run the full pipeline.

6.2 Pipeline ambient rna

6.2.1 Overview

This pipeline performs the following steps: * Analyse the ambient RNA profile in each input (eg. channel's or library's raw cellrange matrices) * Compare ambient RNA profiles across inputs

Configuration

The pipeline requires a configured `pipeline.yml` file. Default configuration files can be generated by executing:

```
python <srcdir>/pipeline_ambient_rna.py config
```

Input files

An tsv file called 'input_libraries.tsv' is required. This file must have column names as explained below. Must not include row names. Add as many rows as iput channels/libraries for analysis.

This file must have the following columns:

- library_id - name used throughout. This could be the channel_pool id eg. A1
- raw_path - path to the raw_matrix folder from cellranger count
- exp_batch - might or might not be useful. If not used, fill with "1"
- channel_id - might or might not be useful. If not used, fill with "1"
- seq_batch - might or might not be useful. If not used, fill with "1"
- (optional) excludelist - path to a file with cell_ids to excludelist

You can add any other columns as required, for example pool_id

Dependencies

This pipeline requires: * cgat-core: https://github.com/cgat-developers/cgat-core * R dependencies required in the r scripts

6.2.2 Pipeline output

The pipeline returns: * per-input html report and tables saved in a 'profile_per_input' folder * ambient rna comparison across inputs saved in a 'profile_compare' folder

6.2.3 Code

`cellhub.pipeline_ambient_rna.`**`ambient_rna_per_input`**`(`*infile*, *outfile*`)`

Explore count and gene expression profiles of ambient RNA droplets per input - The output is saved in profile_per_input.dir/<input_id> - The output consists on a html report and a ambient_genes.txt.gz file - See more details of the output in the ambient_rna_per_library.R

`cellhub.pipeline_ambient_rna.`**`ambient_rna_compare`**`(`*infiles*, *outfile*`)`

Compare the expression of top ambient RNA genes across inputs - The output is saved in profile_compare.dir - Output includes and html report and a ambient_rna_profile.tsv - See more details of the output in the ambient_rna_compare.R

`cellhub.pipeline_ambient_rna.`**`plot`**`(`*infile*, *outfile*`)`

Draw the pipeline flowchart

`cellhub.pipeline_ambient_rna.`**`full`**`()`

Run the full pipeline.

6.3 Pipeline annotation

6.3.1 Overview

This pipeline retrieves annotation from Ensembl

6.3.2 Usage

The annotation pipeline should be run in the cellhub directory.

Configuration

The pipeline requires a configured `pipeline_cluster.yml` file.

Default configuration files can be generated by executing:

python <srcdir>/pipeline_annotation.py config

The ensembl version specified in the yaml file should match that used to build the reference transcriptome for the mapping algorithm (e.g. Cellranger)

Inputs

This pipeline has no inputs.

Dependencies

This pipeline requires:

6.3.3 Pipeline output

The pipeline produces the following outputs:

1. api/annotation/ensembl/ensembl.to.entrez.tsv.gz

- A mapping of ensembl_id to gene_name and entrez_id. Used by gsfisher for pathway analysis.

2. api/annotation/ensembl/ensembl.gene_name.map.tsv.gz

- A unique mapping of ensembl_id -> gene_name. Missing gene names are replaced with ensembl_ids. The gene names have been made unique.

3. api/annotation/kegg/kegg_pathways.rds

- Kegg pathways in rds format for gsfisher.

`cellhub.pipeline_annotation.`**`fetchEnsembl`**`(`*infile*, *outfile*`)`

Fetch the ensembl annotations from BioMart. This task requires internet access.

`cellhub.pipeline_annotation.`**`ensemblAPI`**`(`*infile*, *outfile*`)`

Add the Ensembl gene annotation results to the cellhub API.

`cellhub.pipeline_annotation.`**`fetchKegg`**`(`*infile*, *outfile*`)`

Fetch the Kegg pathway annotations. This task requires internet access.

`cellhub.pipeline_annotation.`**`keggAPI`**`(`*infile*, *outfile*`)`

Add the kegg pathways to the cellhub API

6.4 Pipeline celldb

6.4.1 Overview

This pipeline uploads the outputs from the upstream single-cell preprocessing steps into a SQLite database.

6.4.2 Usage

See *Installation* and *Usage* for general information on how to use cgat pipelines.

Configuration

The pipeline requires a configured `pipeline.yml` file.

Default configuration files can be generated by executing:
cellhub celldb config

Input files

The pipeline requires the output of the pipelines:
>> pipeline_cellranger.py : sample/10X-chip-channel x design-metadata >> pipeline_qc_metrics.py : barcode/cell x sequencing + mapping metadata >> pipeline_ambient_rna.py : gene/feature x sequencing + mapping metadata

pipeline generates a tsv configured file.

Dependencies

6.4.3 Pipeline output

The pipeline returns an SQLite populated database of metadata and quality features that aid the selection of 'good' cells from 'bad' cells.

Currently the following tables are generated: * metadata

6.4.4 Code

`cellhub.pipeline_celldb.`**`connect`**`()`
connect to database. Use this method to connect to additional databases. Returns a database connection.

`cellhub.pipeline_celldb.`**`load_samples`**`(`*outfile*`)`
load the sample metadata table

`cellhub.pipeline_celldb.`**`load_gex_qcmetrics`**`(`*outfile*`)`
load the gex qcmetrics into the database

`cellhub.pipeline_celldb.`**`load_gex_scrublet`**`(`*outfile*`)`
Load the scrublet scores into database.

`cellhub.pipeline_celldb.`**`load_gmm_demux`**`(`*outfile*`)`
Load the gmm demux dehashing calls into the database.

`cellhub.pipeline_celldb.`**`load_demuxEM`**`(`*outfile*`)`
load the demuxEM dehashing calls into the database

`cellhub.pipeline_celldb.`**`final`**`(`*outfile*`)`
Construct a "final" view on the database from which the cells can be selected and fetched by pipeline_fetch_cells.py

6.5 Pipeline Cellranger Multi

6.5.1 Overview

This pipeline performs the following functions:

- Alignment and quantitation (using cellranger count or cellranger multi)

6.5.2 Usage

See *Installation* and *Usage* on general information how to use CGAT pipelines.

Configuration

The pipeline requires a configured `pipeline_cellranger_multi.yml` file.

Default configuration files can be generated by executing:

> python <srcdir>/pipeline_cellranger_multi.py config

Dependencies

This pipeline requires: * cgat-core: https://github.com/cgat-developers/cgat-core * cellranger: https://support.10xgenomics.com/single-cell-gene-expression/

6.5.3 Pipeline output

The pipeline returns: * the output of cellranger multi

6.5.4 Code

`cellhub.pipeline_cellranger_multi.`**`taskSummary`**`(`*infile*, *outfile*`)`

> Make a summary of optional tasks that will be run

`cellhub.pipeline_cellranger_multi.`**`makeConfig`**`(`*outfile*`)`

> Read parameters from yml file for the whole experiment and save config files as csv.

`cellhub.pipeline_cellranger_multi.`**`cellrangerMulti`**`(`*infile*, *outfile*`)`

> Execute the cellranger multi pipleline for first sample.

`cellhub.pipeline_cellranger_multi.`**`postProcessMtx`**`(`*infile*, *outfile*`)`

> Post-process the cellranger multi matrices to split the counts for the GEX, ADT and HTO modalities into seperate market matrices.
>
> The cellbarcode are reformatted to the “UMI-LIBRARY_ID” syntax.
>
> Inputs:
>
> > The input cellranger.multi.dir folder layout is:
> >
> > **unfiltered “outs”: ::**
> > library_id/outs/multi/count/raw_feature_bc_matrix/

filtered "outs": ::
library_id/outs/per_sample_outs/sample|library_id/count/sample_feature_bc_matrix

Outputs:

This task produces:

unfiltered: ::
out.dir/library_id/unfiltered/mtx/[GEX|ADT|HTO]/

filtered: ::
out.dir/library_id/filtered/sample_id/mtx/[GEX|ADT|HTO]/

`cellhub.pipeline_cellranger_multi.`**`mtxAPI`**`(`*infile*, *outfile*`)`

Register the post-processed mtx files on the API endpoint

`cellhub.pipeline_cellranger_multi.`**`h5API`**`(`*infile*, *outfile*`)`

Put the h5 files on the API

Inputs:

The input cellranger.multi.dir folder layout is:

unfiltered "outs":

```
library_id/outs/multi/count/raw_feature_bc_matrix/
```

filtered "outs":

```
library_id/outs/per_sample_outs/sample|library_id/count/sample_feature_bc_
↪matrix
```

`cellhub.pipeline_cellranger_multi.`**`postProcessVDJ`**`(`*infile*, *outfile*`)`

Post-process the cellranger contig annotations.

The cellbarcodes are reformatted to the "UMI-LIBRARY_ID" syntax.

Inputs:

The input cellranger.multi.dir folder layout is:

unfiltered "outs": ::
library_id/outs/multi/vdj_[b|t]/

filtered "outs": ::
library_id/outs/per_sample_outs/sample|library_id/vdj_[b|t]/

Outputs:

This task produces:

unfiltered: ::
out.dir/library_id/unfiltered/vdj_[t|b]/

filtered: ::
out.dir/library_id/filtered/sample_id/vdj_[t|b]/

`cellhub.pipeline_cellranger_multi.`**`vdjAPI`**`(`*infile*, *outfile*`)`

Register the post-processed VDJ contigfiles on the API endpoint

`cellhub.pipeline_cellranger_multi.`**`full`**`()`

Run the full pipeline.

6.6 Pipeline Cell QC

6.6.1 Overview

This pipeline performs the following steps:

- Calculates per-cell QC metrics: ngenes, total_UMI, pct_mitochondrial, pct_ribosomal, pct_immunoglobin, pct_hemoglobin, and any specified geneset percentage
- Runs scrublet to calculate per-cell doublet score

Configuration

The pipeline requires a configured `pipeline.yml` file. Default configuration files can be generated by executing:

```
python <srcdir>/pipeline_cell_qc.py config
```

Input files

A tsv file called 'libraries.tsv' is required. This file must have column names as explained below. Must not include row names. Add as many rows as input channels/librarys for analysis. This file must have the following columns: * library_id - name used throughout. This could be the channel_pool id eg. A1 * path - path to the filtered_matrix folder from cellranger count

Dependencies

This pipeline requires: * cgat-core: https://github.com/cgat-developers/cgat-core * R dependencies required in the r scripts

6.6.2 Pipeline output

The pipeline returns: * qcmetrics.dir folder with per-input qcmetrics.tsv.gz table * scrublet.dir folder with per-input scrublet.tsv.gz table

6.6.3 Code

`cellhub.pipeline_cell_qc.`**`qcmetrics`**`(`*infile*, *outfile*`)`

This task will run R/calculate_qc_metrics.R, It uses the input_libraries.tsv to read the path to the cellranger directory for each input Ouput: creates a cell.qc.dir folder and a library_qcmetrics.tsv.gz table per library/channel For additional input files check the calculate_qc_metrics pipeline.yml sections: - Calculate the percentage of UMIs for genesets provided - Label barcodes as True/False based on whether they are part or not of a set of lists of barcodes provided

`cellhub.pipeline_cell_qc.`**`qcmetricsAPI`**`(`*infiles*, *outfile*`)`

Add the QC metrics results to the API

`cellhub.pipeline_cell_qc.`**`scrublet`**`(`*infile*, *outfile*`)`

This task will run python/run_scrublet.py, It uses the input_libraries.tsv to read the path to the cellranger directory for each input Ouput: creates a scrublet.dir folder and a library_scrublet.tsv.gz table per library/channel It also creates a doublet score histogram and a double score umap for each library/channel Check the scrublet section in the pipeline.yml to specify other parameters

`cellhub.pipeline_cell_qc.`**`scrubletAPI`**`(`*infiles*, *outfile*`)`

Add the scrublet results to the API

`cellhub.pipeline_cell_qc.`**`plot`**`(`*infile*, *outfile*`)`

Draw the pipeline flowchart

`cellhub.pipeline_cell_qc.`**`full`**`()`

Run the full pipeline.

6.7 Pipeline cluster

6.7.1 Overview

This pipeline performs clustering of integrated single cell datasets. Starting from an anndata object with integrated coordinates (e.g. from Harmony or scVI) the pipeline:

- Computes the neighbour graph using the HNSW alogrithm
- Performs UMAP compuation and Leiden clustering (with ScanPy)
- Visualises QC statistics on the UMAPs and by cluster
- Visualises singleR results
- Finds cluster marker genes (using the ScanPy 'rank_genes_groups' function)
- Performs pathway analysis of the cluster phenotypes (with gsfisher)
- Prepares marker gene and summary reports
- Export an anndata for viewing with cellxgene

For plotting of data in R, the pipeline saves the input anndata in loom format and reads data in R with the loomR library.

6.7.2 Usage

See *Installation* and *Usage* on general information how to use CGAT pipelines.

Configuration

It is recommended to perform the clustering in a new directory.

The pipeline requires a configured `pipeline_cluster.yml` file.

Default configuration files can be generated by executing:

python <srcdir>/pipeline_cluster.py config

Inputs

The pipeline starts from an anndata object with the following structure.

- annadata.var.index -> ensembl_ids (use of gene names is not supported)
- anndata.X -> scaled data (a dense matrix)
- anndata.layers["counts"] -> raw counts (a sparse matrix)
- anndata.layers["log1p"] -> total count normalised, 1og1p transformed data (a sparse matrix)
- anndata.obs -> metadata (typically passed through from original cellhub object)
- anndata.obsm["X_rdim_name"] -> containing the integrated coordinates (where "rdim_name" matches the "run-spec_rdim_name" parameter). TODO: rename this parameter.

It is strongly recommended to retain the information for all of the genes in all of the matrices (i.e. do not subset to HVGs!). This is important for marker gene discovery and pathway analysis.

6.7.3 Pipeline output

The pipeline produces the following outputs:

1. Summary report

- Containing the overview UMAP plots, visualisation of QC and SingleR information
- The result of the per-cluster pathway analysis

2. Marker report

- Containing heatmaps, violin plots, expression dotplots, MA and volcano plots for each cluster.

3. xlsx spreadsheets for the marker gene and pathway results
4. Anndata objects ready to be viewed with cellxgene

`cellhub.pipeline_cluster.`**`taskSummary`**(*infile*, *outfile*)

Make a summary of optional tasks that will be run

`cellhub.pipeline_cluster.`**`preflight`**(*infile*, *outfile*)

Preflight sanity checks.

`cellhub.pipeline_cluster.`**`metadata`**(*infile*, *outfile*)

Export the metadata (obs) from the source anndata for use in the plotting tasks

`cellhub.pipeline_cluster.`**`loom`**(*infile*, *outfile*)

Export the data to the loom file format. This is used as an exchange format for plotting in R.

`cellhub.pipeline_cluster.`**`neighbourGraph`**(*infile*, *outfile*)

Compute the neighbor graph. A miniminal anndata is saved for UMAP computation and clustering.

`cellhub.pipeline_cluster.`**`scanpyCluster`**(*infile*, *outfile*)

Discover clusters using ScanPy.

`cellhub.pipeline_cluster.`**`cluster`**(*infile*, *outfile*)

Post-process the clustering result.

`cellhub.pipeline_cluster.`**`compareClusters`**(*infile*, *outfile*)

Draw a dendrogram showing the relationship between the clusters.

`cellhub.pipeline_cluster.`**`clustree`**`(`*infile*, *outfile*`)`

Run clustree.

`cellhub.pipeline_cluster.`**`paga`**`(`*infile*, *outfile*`)`

Run partition-based graph abstraction (PAGA) see: https://genomebiology.biomedcentral.com/articles/10.1186/s13059-019-1663-x

`cellhub.pipeline_cluster.`**`UMAP`**`(`*infile*, *outfile*`)`

Compute the UMAP layout.

`cellhub.pipeline_cluster.`**`RDIMS_VIS_TASK`**`(`*infile*, *outfile*`)`

Compute the UMAP layout.

`cellhub.pipeline_cluster.`**`plotRdimsFactors`**`(`*infiles*, *outfile*`)`

Visualise factors of interest on the UMAP.

`cellhub.pipeline_cluster.`**`plotRdimsClusters`**`(`*infile*, *outfile*`)`

Visualise the clusters on the UMAP

`cellhub.pipeline_cluster.`**`plotRdimsSingleR`**`(`*infile*, *outfile*`)`

Plot the SingleR primary identity assignments on a UMAP

`cellhub.pipeline_cluster.`**`plotRdimsGenes`**`(`*infile*, *outfile*`)`

Visualise gene expression levels on the UMAP.

`cellhub.pipeline_cluster.`**`plotSingleR`**`(`*infile*, *outfile*`)`

Make singleR heatmaps for the references present on the cellhub API.

`cellhub.pipeline_cluster.`**`summariseSingleR`**`(`*infile*, *outfile*`)`

Collect the single R plots into a section for the Summary report.

`cellhub.pipeline_cluster.`**`plotGroupNumbers`**`(`*infiles*, *outfile*`)`

Plot statistics on cells by group, e.g. numbers of cells per cluster.

Plots are defined on a case-by-case basis in the yaml.

`cellhub.pipeline_cluster.`**`clusterStats`**`(`*infile*, *outfile*`)`

Compute per-cluster statistics (e.g. mean expression level).

`cellhub.pipeline_cluster.`**`findMarkers`**`(`*infile*, *outfile*`)`

Find per-cluster marker genes. Just execute the rank_genes_groups routine, no filtering here.

`cellhub.pipeline_cluster.`**`summariseMarkers`**`(`*infiles*, *outfile*`)`

Summarise the differentially expressed marker genes. P-values are adjusted per-cluster are filtering out genes with low expression levels and fold changes. Per-cluster gene universes are prepared for the pathway analysis.

`cellhub.pipeline_cluster.`**`topMarkerHeatmap`**`(`*infiles*, *outfile*`)`

Make the top marker heatmap.

`cellhub.pipeline_cluster.`**`dePlots`**`(`*infile*, *outfile*`)`

Make per-cluster diagnoistic differential expression plots (MA and volcano plots).

`cellhub.pipeline_cluster.`**`markerPlots`**`(`*infiles*, *outfile*`)`

Make the per-cluster marker plots TODO: add some version of split dot plots back..

`cellhub.pipeline_cluster.`**`plotMarkerNumbers`**`(`*infile*, *outfile*`)`

Summarise the numbers of marker genes for each cluster.

`cellhub.pipeline_cluster.`**`markers`**`(`*infile*, *outfile*`)`

Target to run marker gene plotting tasks.

`cellhub.pipeline_cluster.`**`parseGMTs`**`(`*param_keys=['gmt_pathway_files_']*`)`

Helper function for parsing the lists of GMT files

`cellhub.pipeline_cluster.`**`genesetAnalysis`**`(`*infile*, *outfile*`)`

Naive geneset over-enrichment analysis of cluster marker genes.

Testing is performed with the gsfisher package.

GO categories and KEGG pathways are tested by default.

Arbitrary sets of genes cat be supplied as GMT files (e.g. such as those from MSigDB).

`cellhub.pipeline_cluster.`**`summariseGenesetAnalysis`**`(`*infile*, *outfile*`)`

Summarise the geneset over-enrichment analyses of cluster marker genes.

Enriched pathways are saved as dotplots and exported in excel format.

`cellhub.pipeline_cluster.`**`genesets`**`(`*infile*, *outfile*`)`

Intermediate target to run geneset tasks

`cellhub.pipeline_cluster.`**`plots`**`(`*infile*, *outfile*`)`

Target to run all the plotting functions.

`cellhub.pipeline_cluster.`**`latexVars`**`(`*infiles*, *outfile*`)`

Prepare a file containing the latex variable definitions for the reports.

`cellhub.pipeline_cluster.`**`summaryReportSource`**`(`*infile*, *outfile*`)`

Write the latex source for the summary report.

`cellhub.pipeline_cluster.`**`summaryReport`**`(`*infile*, *outfile*`)`

Compile the summary report to PDF format.

`cellhub.pipeline_cluster.`**`markerReportSource`**`(`*infile*, *outfile*`)`

Write the latex source file for the marker report.

`cellhub.pipeline_cluster.`**`markerReport`**`(`*infile*, *outfile*`)`

Prepare a PDF report visualising the discovered cluster markers.

`cellhub.pipeline_cluster.`**`export`**`(`*infile*, *outfile*`)`

Link output files to a directory in the “reports.dir” folder.

Prepare folders containing the reports, differentially expressed genes and geneset tables for each analysis.

TODO: link in the cellxgene anndata files.

`cellhub.pipeline_cluster.`**`report`**`()`

Target for building the reports.

`cellhub.pipeline_cluster.`**`cellxgene`**`(`*infile*, *outfile*`)`

Export an anndata object for cellxgene.

6.8 Pipeline Emptydrops

6.8.1 Overview

This pipeline performs the following task:

- run emptydrops on the raw output of cellranger

6.8.2 Usage

See *Installation* and *Usage* on general information how to use CGAT pipelines.

Configuration

The pipeline requires a configured `pipeline.yml` file.

Default configuration files can be generated by executing:

> python <srcdir>/pipeline_emptydrops.py config

Input files

The pipeline is run from the cellranger count output (raw_feature_bc_matrix folder).

The pipeline expects a tsv file containing a column named path and a column named sample_id.

'raw path' should contain the path to each cellranger path to raw_feature_bc_matrix. 'sample_id' is the desired name for each sample (output folder will be named like this).

Dependencies

This pipeline requires: * cgat-core: https://github.com/cgat-developers/cgat-core * R + packages

6.8.3 Pipeline output

The pipeline returns: A list of barcodes passing emptydrops cell identification and a table with barcode ranks including all barcodes (this can be used for knee plots).

6.8.4 Code

`cellhub.pipeline_emptydrops.`**`emptyDrops`**`(`*infile*, *outfile*`)`

> Run Rscript to run EmptyDrops on each library

`cellhub.pipeline_emptydrops.`**`meanReads`**`(`*infile*, *outfile*`)`

> Calculate the mean reads per cell

`cellhub.pipeline_emptydrops.`**`full`**`()`

> Run the full pipeline.

6.9 Pipeline fetch cells

6.9.1 Overview

This pipeline fetches a given set of cells from market matrices or loom files into a single market matrix file.

6.9.2 Usage

See *Installation* and *Usage* on general information how to use CGAT pipelines.

Configuration

It is recommended to fetch the cells into a new directory. Fetching of multiple datasets per-directory is (deliberately) not supported.

The pipeline requires a configured `pipeline_fetch_cells.yml` file.

Default configuration files can be generated by executing:

> python <srcdir>/pipeline_fetch_cells.py config

Inputs

The pipeline will fetch cells from a cellhub instance according to the parameters specified in the local pipeline_fetch_cell.yml file.

The location of the cellhub instances must be specificed in the yml:

```
cellhub:
    location: /path/to/cellhub/instance
```

The specifications of the cells to retrieve must be provided as an SQL statement (query) that will be executed against the “final” table of the cellhub database:

```
cellhub:
    sql_query: >-
        select * from final
        where pct_mitochondrial < 10
        and ngenes > 200;
```

The cells will then be automatically retrieved from the API.

Cell barcodes are set according to the “barcode_id” column which is set by pipeline_cellranger_multi.py and have the format “UMI-1-LIBRARY_ID”

Dependencies

This pipeline requires:

6.9.3 Pipeline output

The pipeline outputs a folder containing a single market matrix that contains the requested cells.

`cellhub.pipeline_fetch_cells.`**`fetchCells`**`(`*infile*, *outfile*`)`

Fetch the table of the user's desired cells from the database effectively, cell-metadata tsv table.

`cellhub.pipeline_fetch_cells.`**`barcodeSubsets`**`(`*infile*, *output_files*, *sentinel*`)`

Generate the sets of barcodes to retrieve from each of the source matrices. (These will be used for extraction of data from all of the specified modalities).

`cellhub.pipeline_fetch_cells.`**`GEXSubsets`**`(`*infile*, *outfile*`)`

Extract the GEX cell subsets from the parent mtx.

`cellhub.pipeline_fetch_cells.`**`mergeGEXSubsets`**`(`*infiles*, *outfile*`)`

Merge the GEX cell subsets into a single mtx.

`cellhub.pipeline_fetch_cells.`**`downsampleGEX`**`(`*infiles*, *outfile*`)`

Down-sample transcripts given a cell-metadata variable

TODO: incorporate down-sampling into the fetching
of the cell subsets.

`cellhub.pipeline_fetch_cells.`**`exportAnnData`**`(`*infiles*, *outfile*`)`

Export h5ad anndata matrices for downstream analysis with scanpy

`cellhub.pipeline_fetch_cells.`**`ADTSubsets`**`(`*infile*, *outfile*`)`

Extract the ADT cell subsets from the parent mtx.

`cellhub.pipeline_fetch_cells.`**`mergeADTSubsets`**`(`*infiles*, *outfile*`)`

Merge the ADT cell subsets into a single mtx.

`cellhub.pipeline_fetch_cells.`**`exportAnnDataADT`**`(`*infiles*, *outfile*`)`

Export h5ad anndata matrices for downstream analysis with scanpy

6.10 Pipeline singleR

6.10.1 Overview

This pipeline runs singleR for cell prediction. Single R:

(1) runs at cell level (cells are score idependently)

(2) Uses a non-paramentric correlation test (i.e. monotonic transformations of the test data have no effect).

Given these facts, in cellhub we run singleR on the raw counts upstream to (a) help with cell QC and (b) save time in the interpretation phase.

This pipeline operates on the ensembl_ids.

6.10.2 Usage

See *Installation* and *Usage* on general information how to use CGAT pipelines.

Configuration

The pipeline should be run in the cellhub directory.

To obtain a configuration file run “cellhub singleR config”.

Inputs

1. Per-sample h5 files (from the cellhub API).
2. References for singleR obtained via the R bioconductor ‘celldex’ library. As downloading of the references is very slow, they need to be manually downloaded and “stashed” as rds files in an appropriate location using the R/scripts/singleR_stash_references.R scripts. This location is then specified in the yaml file.

6.10.3 Pipeline output

The pipeline saves the singleR scores and predictions for each of the specified references on the cellhub API.

`cellhub.pipeline_singleR.`**`genSingleRjobs`**`()`

generate the singleR jobs

`cellhub.pipeline_singleR.`**`singleR`**`(`*infile*, *outfile*`)`

Perform cell identity prediction with singleR.

`cellhub.pipeline_singleR.`**`concatenate`**`(`*infile*, *outfile*`)`

Concatenate the label predictions across all the samples.

`cellhub.pipeline_singleR.`**`singleRapi`**`(`*infiles*, *outfile*`)`

Add the singleR results to the cellhub API.

6.11 Pipeline Velocity

6.11.1 Overview

This pipeline performs the following steps:

- sort bam file by cell barcode
- estimate intronic and exonic reads using velocyto (on selected barcodes)

6.11.2 Usage

See *Installation* and *Usage* on general information how to use CGAT pipelines.

Configuration

The pipeline requires a configured `pipeline_velocity.yml` file.

Default configuration files can be generated by executing:

> python <srcdir>/pipeline_velocity.py config

Input files

The pipeline is run from bam files generated by cellranger count.

The pipeline expects a tsv file containing the path to each cellranger bam file (path) and the respective sample_id for each sample. In addition a list of barcodes is required, this could be the filtered barcodes from cellranger or a custom input (can be gzipped file). Any further metadata can be added to the file. The required columns are sample_id, barcodes and path.

Dependencies

This pipeline requires: * cgat-core: https://github.com/cgat-developers/cgat-core * samtools * veloctyo

6.11.3 Pipeline output

The pipeline returns: * a loom file with intronic and exonic reads for use in scvelo analysis

6.11.4 Code

`cellhub.pipeline_velocity.`**`checkInputs`**`(`*outfile*`)`

> Check that input_samples.tsv exists and the path given in the file is a valid directorys.

`cellhub.pipeline_velocity.`**`genClusterJobs`**`()`

> Generate cluster jobs for each sample

`cellhub.pipeline_velocity.`**`sortBam`**`(`*infile*, *outfile*`)`

> Sort bam file by cell barcodes

`cellhub.pipeline_velocity.`**`runVelocyto`**`(`*infile*, *outfile*`)`

> Run velocyto on barcode-sorted bam file. This task writes a loom file into the pipeline-run directory for each sample.

`cellhub.pipeline_velocity.`**`full`**`()`

> Run the full pipeline.

CHAPTER

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