

SUBTYPING PIPELINE

Contents



Quick Guide



Supplementary



Conda



GitHub Repo



Notes

Quick Guide

Important!

Before you start the pipeline, make absolutely sure that all file names, sequence names, structures of input tables are correct and **follow exactly the patterns** as described in examples.

Pipeline Directory

Locate to Pipeline directory:

```
$ cd ~/rki_subtyping/Pipeline
```

Open IDE (Visual Code Studio)

```
$ code ../
```

Input Folders

Make sure you have 5 directories:

```
$ tree -d
```

```
Pipeline/  
├── AllSeqsC020  
├── InputFasta  
├── ManualRega  
├── References  
└── Scripts
```

AllSeqsC020 Folder

Provide this folder with .xlsx files as listed (from NGS pipeline):

```
AllSeqsC020/  
├── MS95_Seqs_ENV_C020_V5.xlsx  
├── MS95_Seqs_INT_C020_V5.xlsx  
└── MS95_Seqs_PRRT_C020_V5.xlsx
```

Refer to Supplementary part of the guide, if you need more information (Page 21).

InputFasta Folder

Provide this folder with files as listed (from NGS pipeline):

```
InputFasta/  
├── MS95_ENV_20.fasta  
├── MS95_INT_20.fasta  
└── MS95_PRRT_20.fasta
```

Refer to Supplementary part of the guide, if you need more information (Page 22).

Conda Environment

Activate `subtyping_pipeline` environment.

```
$ conda activate subtyping_pipeline
```

Be sure you have change in prompt:

```
(subtyping_pipeline) beast2@Beast2:~/rki_sybtyping/Pipeline$
```

Pipeline with `--outdir` parameter

Parameter `--outdir` determines a name of an output folder. The command will generate four enumerated output folders within `Results` folder. Without specifying an output folder you can get a warning message.

```
$ nextflow Scripts/subtyping_pipeline.nf --outdir Results
```

ManualRega Folder (1)

Provide the folder with `.csv` files (separator: comma) generated by [Rega](#) using marked `.fasta` files from the folder procuded by the pipeline:

```
~/rki_subtyping/Pipeline/Results/1_marked_fasta
```

These `.fasta` files have M at the end of the file name:

```
1_marked_fasta/  
├── MS95_ENV_20M.fasta  
├── MS95_INT_20M.fasta  
└── MS95_PRRT_20M.fasta
```

ManualRega Folder (2)

Rega online tool always generates files with the same name **results**, e.g. `results.csv`.

Rename these files accordingly, using the pattern as in the example below:

```
ManualRega/  
├─ Manual_Rega_MS95_ENV_20M.csv  
├─ Manual_Rega_MS95_INT_20M.csv  
└─ Manual_Rega_MS95_PRRT_20M.csv
```

Refer to Supplementary part of the guide, if you need more information (Page 23).

Pipeline with `--fullpipeline` parameter

Repeat the previous command with `--fullpipeline` parameter and `-resume` flag. The latter allows for generating an output up to `12_mafft` folder. The complete processes are cached.

```
$ nextflow Scripts/subtyping_pipeline.nf --outdir Result --fullpipeline -resume
```

Check the output of `12_mafft` folder before you run iqtree analysis (msa files - multiple sequence alignments)!

Pipeline with `--iqtree` parameter

Parameter `--iqtree` allows for running the iqtree process that produces `13_iqtree` folder within `Results`. The folder contains `.iqtree`, `.treefile`, and `.log` files. The parameter can be added at this point, as the last command with report and plot outputs being produced or not added at all (no `13_iqtree` folder then).

```
$ nextflow Scripts/subtyping_pipeline.nf --outdir Results --fullpipeline --iqtree -resume
```

You can monitor the `.log` file while running iqtree within `work` folder using respective process ID (68), e.g [68/72f0eb].

Decision

Manually modify files (see below) which contain `Manual` tag in PRRT_Subtype, INT_Subtype, and ENV_Subtype columns. Save changes and close `.xlsx` files.

```
9_joint_with_tags/  
├── full_MS95_ENV_20M.xlsx  
├── full_MS95_INT_20M.xlsx  
└── full_MS95_PRRT_20M.xlsx
```

Report and Plot

```
$ nextflow Scripts/subtyping_pipeline.nf --outdir Results --fullpipeline --iqtree -resume
```

Repeating the command above generates `14_report` folder with `MS95_subtype_uploads.xlsx` report file.

Repeating it again generates a `MS95_subtype_counts.png` plot and adds it to the `14_report` folder.

Clean Up

Once the pipeline has generated **Results** folder with all desired output files (**save** all needed outputs first), the input files can be removed from the input folders.

```
$ rm -rf InputFasta/* AllSeqsC020/* ManualRega/* Results/
```

The same is true for dot and nextflow temp files/folders:

```
$ rm -rf .nextflow work .nextflow.log .nextflow.log.*
```

Processes Overview

[1b/f2f10a]	process > mark_fasta (2)	[100%]	3 of 3, cached: 3	✓
[a9/dd644a]	process > get_tags (3)	[100%]	3 of 3, cached: 3	✓
[97/70bbdd]	process > comet (3)	[100%]	3 of 3, cached: 3	✓
[73/a28f41]	process > stanford (3)	[100%]	2 of 2, cached: 2	✓
[e6/e4af1d]	process > json_to_csv (3)	[100%]	2 of 2, cached: 2	✓
[65/e0eb90]	process > clean_rega (3)	[100%]	3 of 3, cached: 3	✓
[87/4d2fcf]	process > join_env (1)	[100%]	1 of 1, cached: 1	✓
[34/36991e]	process > join_int (1)	[100%]	1 of 1, cached: 1	✓
[62/e59285]	process > join_prirt (1)	[100%]	1 of 1, cached: 1	✓
[8d/dad394]	process > make_decision (1)	[100%]	1 of 1, cached: 1	✓
[d8/983216]	process > join_with_tags	[100%]	1 of 1, cached: 1	✓
[e6/ceaa42]	process > fasta_for_mafft (2)	[100%]	3 of 3, cached: 3	✓
[54/89322b]	process > env_concat_panel (1)	[100%]	1 of 1, cached: 1	✓
[a4/b7aaee]	process > int_concat_panel (1)	[100%]	1 of 1, cached: 1	✓
[f7/9e1ccf]	process > prirt_concat_panel (1)	[100%]	1 of 1, cached: 1	✓
[c0/786bcd]	process > mafft (3)	[100%]	3 of 3, cached: 2	✓
[68/72f0eb]	process > iqtree (3)	[100%]	3 of 3, cached: 3	✓
[3c/0fb71f]	process > report	[100%]	1 of 1, cached: 1	✓
[c5/462a18]	process > countplot (1)	[100%]	1 of 1	✓

Supplementary

Example of .xlsx within AllSeqsCO20

Scount	Fragment	Cutoff	Header	Lauf	NGS-ID	Index	GenBank-ID	Sequenz
20-02944	PRRT	20	20-02944_PRRT_20	95		1		CCCCT...
20-02945	PRRT	20	20-02945_PRRT_20	95		2		CCCCT...
20-02947	PRRT	20	20-02947_PRRT_20	95		3		CCCCT...
20-02949	PRRT	20	20-02949_PRRT_20	95		4		CCCCT...
20-02950	PRRT	20	20-02950_PRRT_20	95		5		CCCCT...

Example of .fasta within InputFasta

```
>20-02955_ENV_20  
GGAATTAGGCCAGTGGTGTCAACCCAACTATTGTTAAATGGCAGCCTAGCAGAAGAAGAT  
GTGGTCATTAGATCTGAAAATTTACAAACAATGCTAAAACCATAATAGTACAGCTTAAT  
GAAACAGTAGTGATTAATTGTACAAGACCCGGCAACAATACAAGAAAAAGTATACATATA  
GGACCAGGAAAAGCATGGTATGCAACAGGAGAGATAATAGGAGATATAAGACAAGCACAT  
TGTAAACTTAATAAAACACAATGGGAAAAAACTTTAAAAAGGGTAGCTAGTAAATTAAGG  
AAACAATCCAACCTTACAACAGTAATCTTTAAGAACTCCTCAGGGGGGGACCCAGAAATT  
GTAATGCACAGTTTTAACTGTGGAGGGGAATTTTTCTATTGTAACACAACACAGTTGTTC  
AATAGTATTTGGAATGACACTACTAATAGTACTGACACAAATGAACTATCACACTCCCA  
TGCAGAATAAAACAAATTATAAATAGATGGCAGGAAGCAGGAAGGG
```

Example of .csv within ManualRega

An example of a `.csv` file produced by Rega online tool (names of columns and only one sample for demonstration)

```
"name","length","assignment","rule","support","begin","end","type","pure",  
"pure_support","pure_inner","pure_outer","scan_best_support","scan_assigned_support",  
"scan_assigned_nosupport","scan_best_profile","scan_assigned_profile","crf",  
"crf_support","crf_inner","crf_outer","crfscan_best_support",  
"crfscan_assigned_support","crfscan_assigned_nosupport","crfscan_best_profile",  
"crfscan_assigned_profile","major_id","minor_id"  
"20-02944_PRRT_20","1026.0","HIV-1 CRF 06_CPX","4","98.0","1823.0","2848.0","Human  
immunodeficiency virus 1","HIV-1 Subtype G","93.0","0.0","93.0","0.5","0.357","0.643",  
"G K A1 A1 A1 A1 G A1 A1 G G G G G","G - - - - - - - G G G G -","HIV-1 CRF 06_CPX",  
"98.0","0.0","98.0","1.0","1.0","0.0","06_CPX 06_CPX 06_CPX 06_CPX 06_CPX 06_CPX  
06_CPX 06_CPX 06_CPX 06_CPX 06_CPX 06_CPX 06_CPX 06_CPX","06_CPX 06_CPX 06_CPX 06_CPX  
06_CPX 06_CPX 06_CPX 06_CPX 06_CPX 06_CPX 06_CPX 06_CPX 06_CPX 06_CPX",""
```

References Folder

This folder contains reference panels and does not need any change unless reference panels should be replaced.

References/

- Reference_ENV_Panel_Stanford.fas
- Reference_INT_Panel_Stanford.fas
- Reference_PRRT_Panel_Stanford.fas

Scripts Folder

This folder contains the scripts and does not need any change.

```
Scripts/  
├── comet_rest.py  
├── decision.py  
├── fasta_for_mafft.py  
├── full_join.py  
├── json_parser.py  
├── nexflow.config  
├── plot.py  
├── rega_cleanup.py  
├── repeat_marking.py  
├── report.py  
├── subtyping_pipeline.nf  
└── tag_parser.py
```


Conda

Conda Info

List available conda environments.

```
$ conda info --envs
# conda environments:
#
base                *  /home/beast2/anaconda3
subtyping_pipeline  /home/beast2/anaconda3/envs/subtyping_pipeline
```

Conda Version

Pipeline's version of conda 4.14.0

```
$ conda --version
```

Deactivation of Environment

This command is used to deactivate the current environment.

```
$ conda deactivate
```

Be sure you have change in prompt:

```
(base) beast2@Beast2:~/rki_sybtotyping/Pipeline$
```

GitHub Repo

Repo Link

The project is hosted [here](#). Use this link to clone the repo in case of data loss.

How to Clone

Locate to home directory

```
$ cd
```

Clone the repo

```
$ git clone https://github.com/vera-rykalina/rki_subtyping
```

Modify path of `ProjectDir` within `subtyping_pipeline.nf`

```
projectDir = "/home/beast2/rki_subtyping/Pipeline"
```

Notes

Keep in Mind (1)

- The pipeline does not take into account subsubtypes. If there are subsubtypes they are converted to subtypes. For instance, A1 is converted to A, F2 is converted to F etc.
- The pipeline does not perform a full quality check of `.fasta` sequences. Illegal characters should be excluded (the pipeline takes care only of underscores so far). Sequences with illegal characters are not accepted by Rega online tool.

Keep in Mind (2)

- Make sure that sample names do not exceed 30 characters in length. Long sample names get shorten by Rega online tool that can cause issues. E.g, this sequence name **PK105_F482_23_MiS84_S86_20consensus_PRRT_20** is too long and gets shortern by Rega to **PK105_F482_23_MiS84_S86_20cons**. In such cases a manual change is necessary.

Keep in Mind (3)

- Be sure you are connected to the Internet
- You can always delete the whole `Results` folder or individual subfolder/subfolders within `Results` and repeat the command with `-resume`.