# NextFlow pipeline for SARS-CoV-2 Illumina data

# Table of contents

# Quickstart

## Installation and usage

1. Install NextFlow

```
curl -s https://get.nextflow.io | bash
mv nextflow ~/bin
```

2. Copy `third-party/modeller/config.py.template` to `third-party/modeller/config.py` and replace the line

`license = 'YOUR_MODELLER_KEY'`

with the actual Modeller key you own. If you don't have one, you can get a free academic license here (https://salilab.org/modeller/registration.html).

3. Build three containers:

```
docker build --target production -f Dockerfile-main -t
nf_illumina_sars-3.0-main .
docker build --target prodcution -f Dockerfile-manta -t
nf_illumina_sars-3.0-manta .
docker build --target updater -f Dockerfile-main -t nf_illumina_sars-
3.0-updater:latest .
```

4. Download latest version of external databases:

In project root dir run:

```
./update_external_databases.sh
```

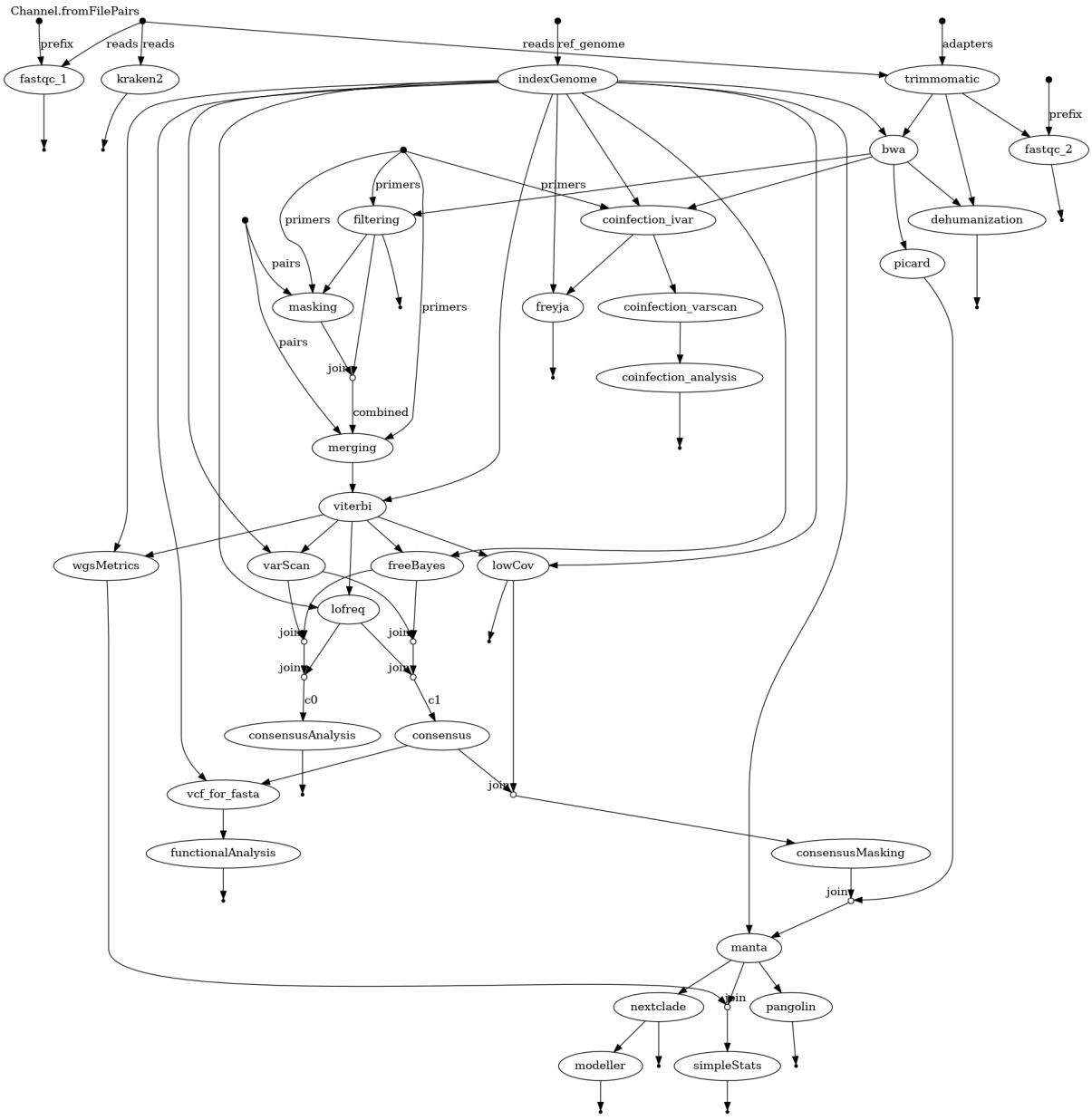
This should fill directories in `data/pangolin` and `data/nextclade`. For more details read the chapter External databases updates.

5. Copy `run_nf_pipeline.sh.template` to `run_nf_pipeline.sh` and fill in the paths to the reads and output directory.

6. Run the pipeline:

```
./run_nf_pipeline.sh
```

# Pipeline overview




overview of the pipeline

# External databases updates

Some components of the pipeline require access to their two databases, which are updated roughly once every two weeks. Different software pieces need to be updated in different ways. To make this process as smooth and painless as possible, we prepared a dedicated Docker container exactly for this task, along with a bash script for running it with appropriate volume mounts. The script should be placed in either the cron or systemd timer and run on a weekly basis.

## Updates procedure

Build the dedicated container:

```
docker build --target updater -f Dockerfile-main -t nf_illumina_sars-3.0-updater:latest .
```

Run the updater script. The working dir must be in project root directory.

```
update_external_databases.sh nextclade
update_external_databases.sh pangolin
update_external_databases.sh kraken
update_external_databases.sh freyja
```

Total size of downloads is ~55 GiB.

| Database | Size |
|---|---|
| pangolin | ~90 MiB |
| nextclade | ~1.3 MiB |
| kraken | ~55 GiB |
| freyja | ~100 MiB |

If everything work fine in directories `data\pangolin` and `data\nextclade` you should see downloaded content like below:

```
data/nextclade/
└── sars-cov-2.zip

data/pangolin/
├── bin
├── pangolin_data
└── pangolin_data-1.25.1.dist-info

data/kraken
└── k2_standard_20240112.tar.gz

data/freyja/
├── curated_lineages.json
├── lineages.yml
└── usher_barcodes.csv
```

It is recommended to put the following in crontab or equivalently systemd timer.

```
0 3 * * 6 cd /path/to/sars-illumina &&
bin/update_external_databases.sh nextclade
5 3 * * 6 cd /path/to/sars-illumina &&
bin/update_external_databases.sh pangolin
10 3 * * 6 cd /path/to/sars-illumina &&
bin/update_external_databases.sh freyja
15 3 1 */3 * cd /path/to/sars-illumina &&
bin/update_external_databases.sh kraken
```

## Updates internals

The following section contain information what and how is updated. Unless you need to debug or refactor the code, and you followed guides in chapter "Updates procedure" in "External databases updates" you can safely skip it.

### List of components that require updates

- Nextclade
- Pangolin

- Kraken
- Freyja

### Nextclade

Nextclade (https://docs.nextstrain.org/projects/nextclade/) is software for assigning evolutionary lineage to SARS-Cov2. To make it work properly, it requires a database which is updated roughly once every two weeks.

The recommended way of downloading dataset is using `nxtclade` tool.

```
nextclade dataset get --name sars-cov-2 --output-zip sars-cov-2.zip
```

Detailed manual is available https://docs.nextstrain.org/projects/nextclade/en/stable/user/datasets.html. Nextclade is downloading `index.json` from site: https://data.clades.nextstrain.org/v3/index.json, and based on that files it decide what to download and from where. Probably the same data are available directly on GitHub: https://github.com/nextstrain/nextclade_data/tree/master/data/nextstrain/sars-cov-2/wuhan-hu-1/orfs.

The command above will download a `sars-cov-2.zip` file in desired destination (default: `data/nextclade`). That directory have to be mounted inside `main` container. It is done by Nextflow in the `modules/nextclade.nf` module.

```
process nextclade {
    (...)
    containerOptions "--volume
${params.nextclade_db_absolute_path_on_host}:/home/SARS-
CoV2/nextclade_db"
    (...)
```

### Pangolin

Pangolin (https://github.com/cov-lineages/pangolin) (**P**hylogenetic **A**ssignment of **N**amed **G**lobal **O**utbreak **LIN**eages) is alternative to Nextclade software for assigning evolutionary lineage to SARS-Cov2.

To make it work properly, it requires a database that is stored in the Git repository pangolin-data (https://github.com/cov-lineages/pangolin-data).

Pangolin-data is actually a regular python package. Normal update procedure is via command: `pangolin --update-data`. It also can be installed by `pip` command. Keeping it inside main container is slightly tricky. We don't want to rebuild entire container just to update the database. We also don't want to keep the database inside the container, because it would force us to run the update before every pipeline run, which is stupid. The best solution is to mount the database from the host.

To achieve this goal we install the package externally to the container in designated path using host native `pip`.

```
pip install \
    --target data/pangolin \
    --upgrade \
    git+https://github.com/cov-lineages/pangolin-data.git@v1.25.1
```

> Make sure you entered proper version in the end of git url. The version number is also git tag. List of available tags with their release dates is here (https://github.com/cov-lineages/pangolin-data/tags).

Then that dir is mounted as docker volume inside the container (which is done automagically in the Nextflow module file):

```
process variantIdentification {
    containerOptions "--volume
${params.pangolin_db_absolute_path_on_host}:/home/SARS-CoV2/pangolin"
    (...)
```

During container build the `$PYTHONPATH` environment variable is set to indicate proper dir.

```
(...)
ENV PYTHONPATH="/home/SARS-CoV2/pangolin"
(...)
```

So the manual download consist of two steps:

1. Install the desired version of `pangolin-data` package in `data/pangolin` directory.
2. Provide absolute path to that dir during starting pipeline

`--pangolin_db_absolute_path_on_host /absolute/path/to/data/pangolin`

### Kraken

Kraken 2 (https://ccb.jhu.edu/software/kraken2/) is a taxonomic classification system using exact k-mer matches. The pipeline utilizes it to detect contamination in samples. It requires a database of approximately 55 GiB, which could potentially be reduced to 16 GiB or 8 GiB, albeit at the cost of sensitivity and accuracy. It updated roughly quarterly. Skipping updates may result in skipping newer taxa.

Database may be built for user own (not recommended) or be downloaded from aws s3. DB is maintained by Kraken 2 maintainers. Here you can find more here (https://github.com/BenLangmead/aws-indexes), and here (https://benlangmead.github.io/aws-indexes/).

Downloading is via aws cli (`apt install awscli`), python library `boto3` or HTTP protocol. There are several types of databases, that differ with set of organism. We use and recommend using `standard` db, which is quite complete. There is also `nt` db which contain all RefSeq and GenBank sequences, but it's size and processing time is too high for routine surveillance.

Links for http downloads are available here (https://benlangmead.github.io/aws-indexes/k2). They are in form of: `https://genome-idx.s3.amazonaws.com/kraken/k2_DBNAME_YYYMMDD.tar.gz`

where DB name is one of following: `standard`, `standard_08gb`, `standard_16gb`, `viral`, `minusb`, `pluspf`, `pluspf_08gb`, `pluspf_16gb`, `pluspfp`, `pluspfp_08gb`, `pluspfp_16gb`, `nt`, `eupathdb48`.

Refer to official docs for more details.

In case of our pipeline we use python script that is using `boto3` library. It is part of `nf_illumina_sars-3.0-updater` container. For running this script simply pass `kraken` to the container during running.

We recommend using for this dedicated script: `update_externeal_databases.sh`.

Kraken DB will not be updated if local path already contain the file with the same name.

### Freyja

Freyja (https://andersen-lab.github.io/Freyja/index.html) is a tool to recover relative lineage abundances from mixed SARS-CoV-2 samples from a sequencing dataset.

Natively Freyja updates require installing Freyja python package (by default with conda) and running `freyja update` command. This command will download the latest version of curated lineages and usher barcodes. However, in our pipeline we do it simpler way. We download the files directly from the dedicated GitHub repository andersen-lab/Freyja-data

(https://github.com/andersen-lab/Freyja-data) using regular `wget`, which is implemented in the `update_external_databases.sh` script, and `updater` container.