NEXT-GEN SEQUENCING

Cogent™ NGS Immune Profiler and Cogent NGS Immune Viewer Quick Start Guide

The following information is provided as a high-level introduction to the software. For more detailed information, please see the Cogent NGS Immune Profiler User Manual and the Cogent NGS Immune Viewer User Manual.

NOTE: Refer to the "Immune profiling kit compatibility" on the bioinformatics portal to view supported chemistries.

Before You Begin (Immune Profiler)

A. Supported operating systems
  - Mac OS X: El Capitan (Version 10.11 and up)
  - Linux: CentOS 6 or higher, RedHat 7.5 or higher

B. Hardware requirements
  - Memory: 16 GB RAM
  - Free disk space: at least 100 GB available hard drive space

C. Additional software dependencies
  - Java 11 or higher, up to 23
  - Conda 23.7.4 or higher

D. Required input files
  - FASTQ files generated by an Illumina® sequencing platform
  - CSV metadata file

Confirm Java and conda versions

NOTE: To bring up a terminal window on a Mac, go to the search box, type in `Terminal` and press [Enter].

- Verify Java is installed and meets or exceeds the required version by typing the following into a terminal window:

```
java -version
```

- Verify conda is installed and meets or exceeds the required version by typing the following into a terminal window:

```
conda --version
```

Installation of Immune Profiler

1. Sign up to download the installation package from our website.
    - If a previous version of Immune Profiler is installed on the same computer as the Cogent NGS Immune Profiler will be installed, proceed to step 2
    - If this is a new install, skip to step 3
2. (Uninstall the previous version)
    a. If you want to save any previous output files stored in the folder `immune_profiler/`, make sure to move them to another location.
    b. Delete the `immune_profiler/` directory and all subfolders.
3. (Install the new version): On the computer on which Immune Profiler is to be installed, unzip the Immune Profiler software package downloaded in Step 1 into the folder location of your choice.
4. Run the Immune Profiler help command to install the Immune Profiler dependencies.

```
$PROFILER_HOME/bin/cogentip analyze -h
```

To run the Immune Profiler

```
$PROFILER_HOME/bin/cogentip analyze -r
<RECEPTOR> -s <SPECIES> -m <METADATA> -o
<OUTPUT> --threads <THREADS>
```

- `<RECEPTOR>` is the immune cell receptor type of the data being processed (options: `TCRv1, TCRv2, BCRv1, or BCRv2`)
- `<SPECIES>` is the species of the sample (options: `human` or `mouse`)
- `<METADATA>` is the filename (including the full path) of the metadata file
- `<OUTPUT>` is a string; it will be the name of the output folder created by the analysis
- `<THREADS>` is the number of threads to use for TRUST4 steps that can run on multiple threads

To Generate Reports with Custom UMI Cutoffs

1. Evaluate the `umi_group_sizes_frequency.<SAMPLE>.png` file per the recommendations in the user manual.
2. Add determined UMI cutoff values in `umi_cutoffs.template.csv`.

3. Run (all on one command line):

```
$PROFILER_HOME/bin/cogentip report -a
<PREVIOUS_ANALYSIS_OUTPUT_DIRECTORY> -u
<FULL_PATH_UMI_CUTOFFS_CSV> -s <SPECIES>
```

- `<PREVIOUS_ANALYSIS_OUTPUT_DIRECTORY>` is the output folder (`-o <OUTPUT>`) from a `cogent analyze` run.
- `<FULL_PATH_UMI_CUTOFFS_CSV>` is the full path and file name for the `umi_cutoffs.template.csv` file modified in Step 2.
- `<SPECIES>` is the species of the sample (options: `human` or `mouse`)

Before You Begin (Immune Viewer)

A. Supported operating systems
  - Mac OS X: El Capitan (Version 10.11 and up)
  - Window 8 and up

B. Hardware requirements
  - Memory: 2 GB RAM
  - Free disk space: >2 GB

C. Additional software dependencies
  - A program that can zip and unzip files
  - A PDF file reader

D. Required input file:
  `*.immune_viewer_report.csv` file generated by CogentIP, from one of the `reports/<sampleID>/<RECEPTOR>/` folders

To use the Immune Viewer

1. Through a web browser, visit the URL: takarabio.com/ngs-immune-viewer
2. Click **Launch** » to start the tool.
3. Click on [Browse] under “Upload clonotype input file”.
4. In the pop-up window, navigate and select a `*.immune_viewer_report.csv` file.
5. Click [OK] to upload the CSV.
6. Navigate through the tabs to view charts.
7. Click [Current view] or [All views] to download data.

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