Takara Bio USA, Inc.

# Cogent™ NGS Immune Profiler v1.6 User Manual

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software v1.6
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# I. Introduction

## A. Welcome to the Cogent NGS Immune Profiler Software

**Cogent NGS Immune Profiler Software v1.6** (referred to as Immune Profiler or CogentIP in this guide) is designed to analyze sequence data stored in FASTQ files generated by Illumina® sequencing platforms from libraries prepared using Takara Bio's human and mouse repertoire immune profiling kits (refer to the software compatibility table on the bioinformatics portal page at takarabio.com for more details). The output from CogentIP can then be imported into the Cogent NGS Immune Viewer (Section VII) to visualize the sequence data, such as in chord diagrams.

Written in Python3, Immune Profiler can be launched from a command line interface (CLI); MacOS users also have the option of running it via a graphical user interface (GUI). Immune Profiler incorporates two third-party programs, MIGEC and MiXCR, packaged and included for use only with this software under an end-user license agreement (EULA), acceptance of which requires the Immune Profiler user to be bound by and to comply with the terms before downloading and using Profiler.

**IMPORTANT:** Checking the box next to the EULA acceptance statement before submitting the completed form constitutes accepting and legally binding the Immune Profiler user to the terms of the EULA.

We recommend new users to read through this document prior to starting. There is also a quick start guide available to download, which is a streamlined reference document for installation and usage of the software.

## B. What's New

Unless otherwise noted, the current version of software contains all features included in previous versions.

- **Cogent NGS Immune Profiler v1.6**
  - Support for SMART-Seq® Mouse TCR (with UMIs)
- **Cogent NGS Immune Profiler v1.5**
  - Support for SMART-Seq Human BCR (with UMIs)
  - A full additional set of output files compliant with Adaptive Immune Receptor Repertoire (AIRR) Community standards (Section VI)
  - Single installation package—compatible with both Linux and MacOS

**NOTE:** Find release notes for prior versions of CogentIP in the table on the Cogent NGS Immune Profiler product page.

# II. Before You Begin

## A. Supported Operating Systems

- Mac OS X: El Capitan (Version 10.11 and up)
- Linux: CentOS 6 or higher, Redhat 7.5 or higher

**NOTE:** If the library is sequenced with more than 25 million reads, use the Linux version

## B. Hardware Requirements

- Memory: 16 GB RAM
- Free disk space: at least 100 GB available hard drive space

  **NOTE:** Required free disk space depends on the aggregate size of the input FASTQ files and should be 4X the total size of the FASTQ files to guarantee completion. If the total size of your input FASTQ files is greater than 100 GB, then the larger value from that calculation is the amount of recommended free disk space.

See Section V.D for information on performance benchmark results.

## C. Additional Software Dependencies

- Java 1.8–1.15, i.e., Java SE 8 through Java SE 15

  **NOTE:** This will NOT work with Java 1.16 (Java SE 16) or higher. If running a version of Java 1.16+ on the target install server, please downgrade. Uninstall the later Java version and install a supported version from the range noted above by downloading the earlier version's installation executable from Oracle.

- Python 3.6 or higher

## D. User Account Requirements

The account used to install Immune Profiler needs to have administrative privileges on the server or workstation where it will be installed, including read/write (R/W) permissions for the folder in which it will be located.

Once installed, regular user accounts can be used to run the Immune Profiler executable, but these accounts need to have R/W permissions for the folder where the source metadata CSV is located.

If you are uncertain if the account being used to install or use Immune Profiler meets these criteria, please consult with your local IT for additional assistance.

## E. Required Input Files

Immune Profiler requires paired FASTQ and metadata files as input.

### 1. FASTQ files

The Profiler has been validated to use FASTQ files with up to 25 million total reads on MacOS with 16 GB RAM, equivalent to Illumina MiSeq® platform sequencing capability. For deeper sequencing, we recommend using the Linux version.

The input files, either compressed (`*.fastq.gz`) or decompressed (`*.fastq`) format, can be stored in any directory on the server or workstation as long as the folder is not private or has read-write user restrictions that would prevent the files from being accessed by Immune Profiler.

### 2. Metadata file

The metadata file is a comma-separated value (CSV) file with the following characteristics:

- The output folder of results from running Immune Profiler will be written to the same directory location as the metadata file.

- The metadata CSV file needs to be created by the user in any directory on the server where Immune Profiler is installed and holds user-defined sample names and the path information to the matching FASTQ file names.
- It should have a header consisting of the following three elements: `sampleID`, `read1_file_name`, and `read2_file_name`.
  - `sampleID` is a user-defined, unique identifier for each sample; it should be less than 20 characters in length and only contain alphanumeric characters or hyphens. During the analysis stage, Immune Profiler scans the metadata file to check for the following conditions:
    1) Duplicate sampleIDs
    2) Underscores in the sampleID name
    3) All sampleIDs are 20 characters or less in length
    4) Blank lines

    If any match is found to Conditions 1–3, Immune Profiler will display an error message noting which condition failed and terminate analysis. Edit the metadata file to fix the issue and relaunch Profiler.

    If a blank line is found in the metadata file (Condition 4), Immune Profiler will ignore the empty line, display a warning, and continue processing the rest of the samples.
  - The `read1_file_name` and `read2_file_name` values should match the FASTQ file names corresponding to the sample specified by `sampleID`; directory location information should not be included. Immune Profiler will check and make sure these specified files exist in the FASTQ directory (described above); if no file matching the name is found, an error message is displayed, prompting the user to double-check both the FASTQ directory and FASTQ file names.

An example of the metadata file contents is shown in Table 1.

**Table 1. Example contents of a metadata CSV file (above), as would be viewed in a spreadsheet program.**

| sampleID | read1_file_name | read2_file_name |
|---|---|---|
| S1 | S1_R1.fastq.gz | S1_R2.fastq.gz |
| S2 | S2_R1.fastq.gz | S2_R2.fastq.gz |
| S3 | S3_R1.fastq.gz | S3_R2.fastq.gz |

Additional examples can be viewed in the metadata CSV files of mini dataset samples included with the software. See Appendix A for more information about the mini dataset sample and result files.

# III. Software Overview

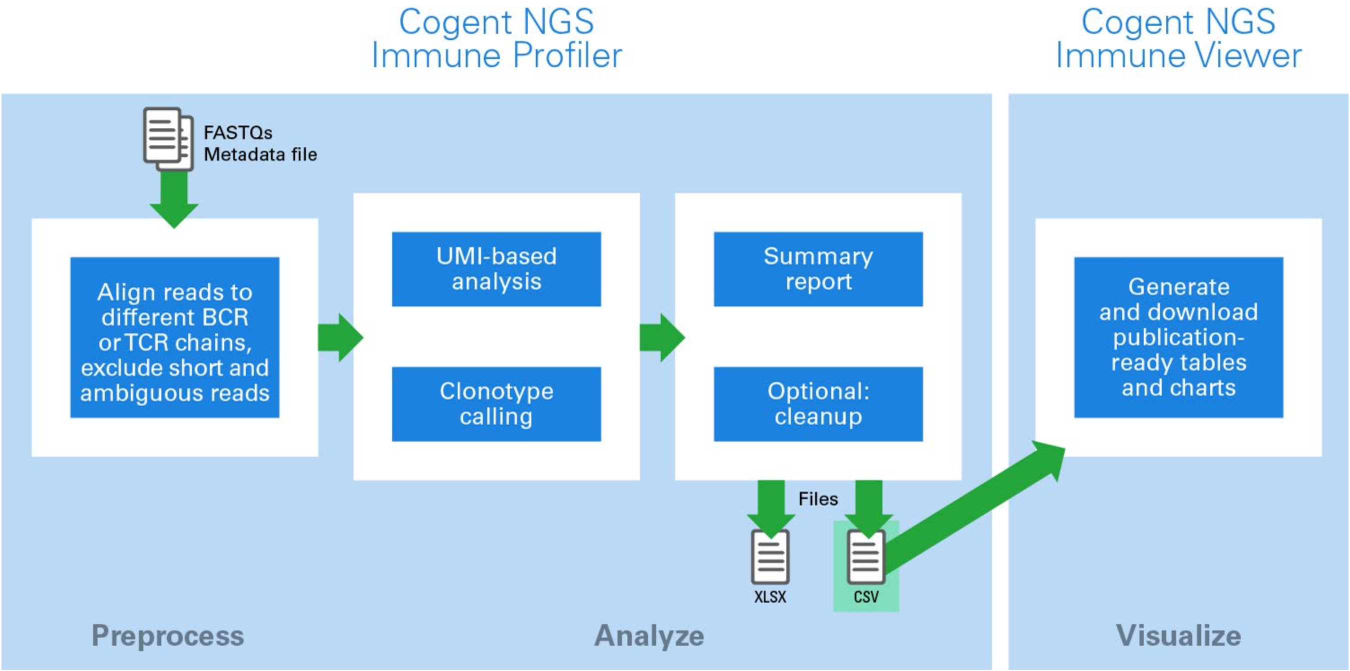

Cogent NGS Immune Profiler
Cogent NGS Immune Viewer
FASTQs
Metadata file
Align reads to different BCR or TCR chains, exclude short and ambiguous reads
UMI-based analysis
Clonotype calling
Summary report
Optional: cleanup
Files
XLSX
CSV
Generate and download publication-ready tables and charts
Preprocess
Analyze
Visualize

**Figure 1. Cogent NGS Immune Profiler analysis workflow.**

The CogentIP workflow consists of five steps and can be invoked using a graphical user interface (GUI) or can be run on the command line. Figure 1 illustrates at a high-level what the workflow consists of, while the list below expands on each step.

1. Preprocess:
   - Splits reads by matching read sequence to different receptor chains (allows one mismatch)
   - Excludes short reads (<30 bp) and reads ambiguously matched to multiple receptor chains
   - If linker-based correction is enabled, excludes read-failed correction
2. UMI-based analysis:
   - Groups reads into molecular identifier groups (MIG) using UMIs (Unique Molecular Identifiers)
   - Conducts sequencing error correction and exclude reads that fail abundance check
   - Generates collapsed-read FASTQs for downstream analysis
3. Clonotype calling:
   - Aligns reads to V(D)J sequences
   - Assembles alignments
   - Defines and reports clonotypes
4. Summary report:
   - Summarizes read QC statistics: chain-specific, short, undetermined, full-length chain (flc)
   - Summarizes mapping statistics: aligned, overlapped, mapped
   - Summarizes clonotype details: numbers, percentage, nucleotide, and amino acid sequence
   - Two output formats: file set identical to CogentIP v1.0, second set with AIRR-compliant header names
5. (Optional) Cleanup:
   - Configuration options available to user on a per run basis
   - Deletes intermediate files generated during processing (see Section V.B (GUI), Section V.C (CLI), and Appendix C for more information)

# IV. Installation and Configuration Requirements

**REMINDER:** Administrative privileges on the server or workstation is required (Section II.D).

## A. Uninstall Previous Instances of Immune Profiler

If an earlier version of Immune Profiler was installed on the server, it will need to be uninstalled prior to installing Cogent NGS Immune Profiler v1.6.

Follow the uninstall directions in Section IV.F ("Uninstalling Immune Profiler").

**NOTE:** If no version of Immune Profiler has ever been installed on the server, skip to the next section (Section IV.B).

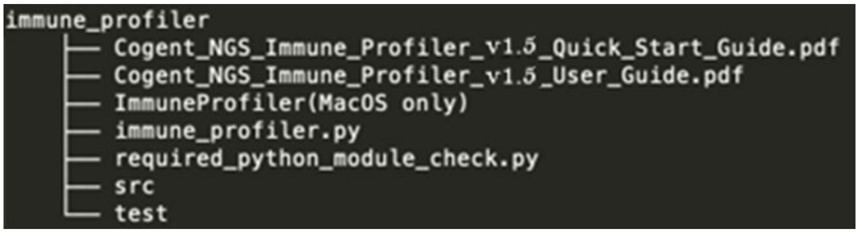
## B. Immune Profiler Download and Installation

Immune Profiler is available for download as a compressed file from takarabio.com/ngs-immune-profiler**.**

1. Download the installation ZIP file (`Cogent_NGS_Immune_Profiler_Software_v1.6.zip`), following the directions (a) on the page seen after submitting the sign-up form on the CogentIP product page or (b) in the confirmation email sent to the email address submitted in the form.
2. If this software is to be used by multiple users, put the package in a location anyone who will be using Immune Profiler can access and has computer permissions to use.
3. Decompress the software package in the directory it is to be installed in.

   The following files should be included in the resulting `immune_profiler/` directory:

   - `Cogent NGS Immune Profiler v1.6 Quick Start Guide.pdf.`
   - `Cogent NGS Immune Profiler v1.6 User Manual.pdf.`
   - `ImmuneProfiler` : the GUI launcher (for use on MacOS only).
   - `immune_profiler.py` : main analysis script (Mac and Linux) .
   - `required_python_module_check.py`: a script for to check for required python modules.
   - `src/`: folder storing dependencies required by Immune Profiler.
   - `test/` : folder contains a directory of test dataset files (`test_input/human/`) and a directory of example outputs generated by the test dataset files (`test_output/human/`). More information about this folder can be found in Appendix A.



```
immune_profiler
     ├── Cogent_NGS_Immune_Profiler_v1.5_Quick_Start_Guide.pdf
     ├── Cogent_NGS_Immune_Profiler_v1.5_User_Guide.pdf
     ├── ImmuneProfiler(MacOS only)
     ├── immune_profiler.py
     ├── required_python_module_check.py
     ├── src
     └── test
```

**Figure 2. Visual diagram of the `immune_profiler` directory, including files and folders.**

## C. Verify Java Installation

1. Open a terminal window on the computer on which Immune Profiler will be installed:
   a. **Mac:** The Terminal application is typically found under **Applications > Utilities > Terminal**. Alternatively, search for `terminal` in Spotlight search.

b. **Linux:** If using Linux with a GUI shell, use the keyboard shortcut **[Ctrl][Alt][T]**.

Alternatively, you can find the Terminal by opening the Dash (upper left on most desktops), typing `terminal`, and selecting the Terminal application.

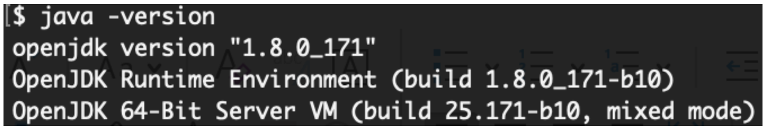
If using Linux on the command line interface (CLI), the CLI is the terminal window.

**NOTE:** You will need to use this terminal window for the next two sections; it is recommended not to close it until directed.

2. Verify the version of Java installed by typing:

```
java -version
```

into the terminal window. Text similar to Figure 3 should display.



```
$ java -version
openjdk version "1.8.0_171"
OpenJDK Runtime Environment (build 1.8.0_171-b10)
OpenJDK 64-Bit Server VM (build 25.171-b10, mixed mode)
```

**Figure 3. How to verify the Java version of your OS.** After typing `java -version` into your terminal, you should see 'java version' and a number displayed in double-quotes. Verify that the first two number values are 1.8 or higher.

For assistance installing Java, visit the website:

https://java.com/en/download/help/download_options.xml

## D. Python Installation and Verification

If Python is already installed on the system, skip to Verifying Python Installation and Version.

### 1. Download and Install Python

1. Download Python from the website:

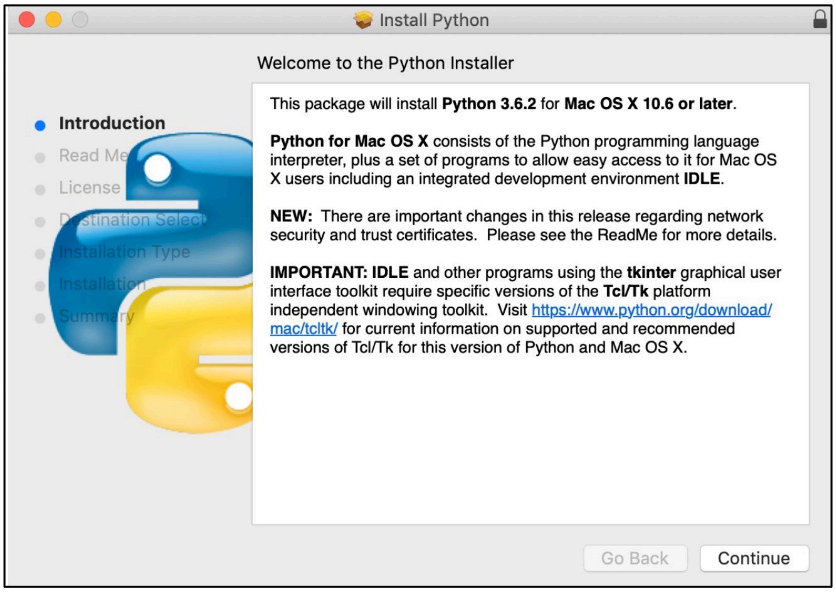
   https://www.python.org/downloads/

   Select the correct installation package for the operating system on which it will be installed. It should be version 3.6.0 or higher.

2. Install Python.

   **Mac users:** accept the default settings.

*(continued on next page)*



Welcome to the Python Installer

This package will install **Python 3.6.2** for **Mac OS X 10.6 or later**.

**Python for Mac OS X** consists of the Python programming language interpreter, plus a set of programs to allow easy access to it for Mac OS X users including an integrated development environment **IDLE**.

**NEW:** There are important changes in this release regarding network security and trust certificates. Please see the ReadMe for more details.

**IMPORTANT: IDLE** and other programs using the **tkinter** graphical user interface toolkit require specific versions of the **Tcl/Tk** platform independent windowing toolkit. Visit https://www.python.org/download/mac/tcltk/ for current information on supported and recommended versions of Tcl/Tk for this version of Python and Mac OS X.

**Figure 4. The Python 3.6 installation pop-up on MacOS.**

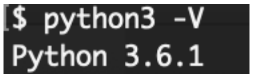
For additional assistance installing Python, visit the website:

https://www.python.org/about/gettingstarted/

## 2. Verifying Python installation and Version

In the same terminal window used to verify the version of Java, verify the version of Python installed by typing:

`python3 -V` **or** `python3 --version`



```
$ python3 -V
Python 3.6.1
```

**Figure 5. How to verify the Python version of your OS.** After typing `python3 -V` into your terminal, you should see a version number displayed. Verify that it is 3.6.0 or higher.

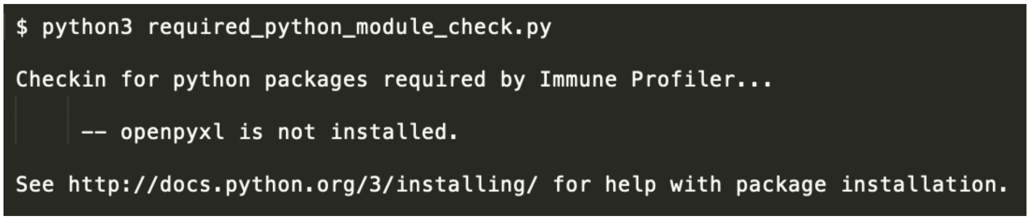
## 3. Verifying Installed Python Modules

In addition to the default modules installed by Python3, Immune Profiler also requires the module 'openpyxl' to be installed.

1. In the same terminal window used to verify the version of Java and Python, change to the directory where Immune Profiler was installed (`immune_profiler/`).
2. Type the command:

   `python3` **`required_python_module_check.py`**

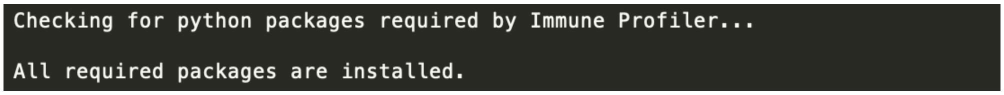
   If any required modules are missing, the script will list them on the terminal, as in Figure 6.



```
$ python3 required_python_module_check.py

Checkin for python packages required by Immune Profiler...

        -- openpyxl is not installed.

See http://docs.python.org/3/installing/ for help with package installation.
```

**Figure 6. Running the required Python module check script and finding a module not installed.**

Otherwise, it will return, "All required packages are installed." (Figure 7).



```
Checking for python packages required by Immune Profiler...

All required packages are installed.
```

Figure 7. Output to a successful check of required python modules.

3. If any packages are reported as missing by the check, install each of them (individually) with the following command typed into the terminal window:

    `python3 -m pip install <package_name>`

   or

    `pip3 install <package_name>`

   where `<package_name>` is replaced by the name of the package. E.g.,

    `python3 -m pip install openpyxl`

   or

    `pip3 install openpyxl`

   For additional assistance installing these modules, please refer to the website:

   https://docs.python.org/3/installing/

4. If you will be using the MacOS GUI version of Immune Profiler, the terminal window can be closed at this point. If using the CLI, keep it open and proceed to the next section.

## E. Conduct Test Run with the Mini Datasets

After installation, an analysis should be done to verify the install using one of the the mini dataset sample files provided in `test/test_input/human/`. See Appendix A for more information about the sample mini dataset files. Table 2 can be used as an aid to determine which mini dataset may be of most interest to you.

**NOTE:** The five-character prefix value (abbreviation) in Table 2 will be used throughout the manual as shorthand for the output of the listed reagent kits, in terms of options within the software.

Table 2. Immune profiling kit prefix reference, mini dataset data directory, and mini dataset metadata file names.

| Reagent kit | Prefix | Mini dataset data directory | Mini dataset metadata file |
|---|---|---|---|
| SMART-Seq Human BCR (with UMIs) | BCRv2 | BCRv2_mini/ | bcrv2_mini_meta.csv |
| SMARTer® Human BCR IgG IgM H/K/L Profiling Kit | BCRv1 | BCRv1_mini/ | bcrv1_mini_meta.csv |
| SMART-Seq Human TCR (with UMIs)<br>SMARTer Human TCR a/b Profiling Kit v2 | TCRv2 | TCRv2_mini/ | tcrv2_mini_meta.csv |
| SMART-Seq Human TCR (with UMIs) | mTCRv2 | mTCRv2_mini | mtcrv2_mini_meta.csv |

1. Select one of the mini datasets to test with (e.g., SMART-Seq Human BCR (with UMIs)). You will then use the FASTQ files in the mini dataset directory (e.g., `BCRv2_mini/`) and the mini dataset metadata file (e.g., `bcrv2_mini_meta.csv`) corresponding to your selected reagent kit.
2. Follow the steps in Section V.B (UI) or Section V.C (CLI) to setup and execute an analysis run on your system.
3. Compare the analysis results (1) generated by your run to the results provided in the `report/` subfolder (Section VI.B) to (2) the mini dataset output in `test/test_output/human/`.

Table 3 can be used to determine which mini dataset results directory with which to compare your output. Refer to Appendix A for more information about the mini dataset results data.

**Table 3. Mini dataset data results directory correlations to the immune profiling kits.**

| Reagent kit | Prefix | Mini dataset results directory |
|---|---|---|
| SMART-Seq Human BCR (with UMIs) | `BCRv2` | `BCRv2_mini_results/` |
| SMARTer Human BCR IgG IgM H/K/L Profiling Kit | `BCRv1` | `BCRv1_mini_results/` |
| SMART-Seq Human TCR (with UMIs)<br>SMARTer Human TCR a/b Profiling Kit v2 | `TCRv2` | `TCRv2_mini_results/` |
| SMART-Seq Human TCR (with UMIs) | `mTCRv2` | `mTCRv2_mini_results/` |

The installation is considered to be successful if the output to your test run matches the results stored in `test/test_output/human/`**`<kit-folder>`**`/`, where **`<kit-folder>`** is one of the mini dataset result directories listed in Table 3 relating to the dataset data you ran as a test (e.g., `test/test_output/human/BCRv2_mini_results/`).

## F. Uninstalling Immune Profiler

1. Move any output files that you want to keep that are located in the `immune_profiler/` directory to another location outside that folder.
2. Delete the `immune_profiler/` folder, all its subfolders, and the files contained in it.

**NOTE:** If an older version of Immune Profiler was uninstalled in order to upgrade to a newer version, return to Section IV.B to continue the installation.

# V. Using Immune Profiler

## A. Best Practices

- As a reminder, after the initial installation and before analyzing your own data the first time, conduct a test run using the provided sample datasets and compare your output to the sample results (Section IV.E).
- The computer Immune Profiler is installed on should be plugged in and not running on battery (if a laptop) when a run is initiated. Since the profiling process may take some time to complete, depending on the size of the dataset being analyzed (see Section V.D), this recommendation is to prevent the computer from shutting down before the analysis is finished.
- The metadata and FASTQ files should be stored locally (on the same computer) to the Immune Profiler installation. Immune Profiler is designed to run on a single machine, and the computing speed and stability of the program degrade if analyzing data stored in a remote network location, even if on a mapped drive.
- For MacOS, the recommended upper input data limit is 25 million total reads (MiSeq-size data) due to processing constraints. If you have more data than this—e.g., ten FASTQ pairs, each with 5 million reads (50 million reads in total)—you can create two metadata files (Section II.E), with five samples in each, and launch two analysis runs sequentially to process all the data.

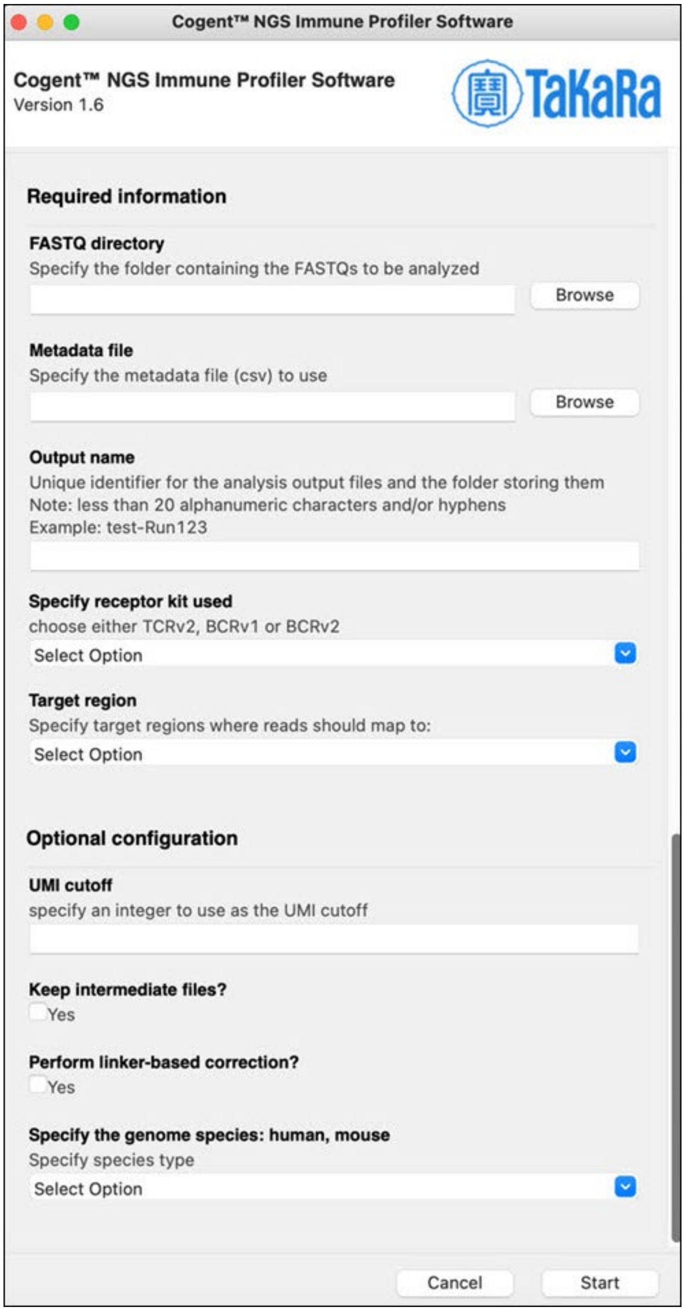
  Once the first run is complete, check the free disk space to make sure there is enough for the second run (Section II.B) before launching it.

## B. Using the GUI (MacOS only)

### 1. Procedure

**NOTE:** The examples used in this section to walk through the analysis procedure include file names corresponding to the mini dataset sample files included with the Immune Profiler package, detailed in Appendix A.

1. Double-click on the executable file `ImmuneProfiler` located in the `immune_profiler/` folder. This will launch the user interface (Figure 8). Depending on the dimension of your screen, there may be a scroll bar on the right side of the interface window.



Cogent™ NGS Immune Profiler Software

Cogent™ NGS Immune Profiler Software
Version 1.6

Required information

FASTQ directory
Specify the folder containing the FASTQs to be analyzed

Metadata file
Specify the metadata file (csv) to use

Output name
Unique identifier for the analysis output files and the folder storing them
Note: less than 20 alphanumeric characters and/or hyphens
Example: test-Run123

Specify receptor kit used
choose either TCRv2, BCRv1 or BCRv2
Select Option

Target region
Specify target regions where reads should map to:
Select Option

Optional configuration

UMI cutoff
specify an integer to use as the UMI cutoff

Keep intermediate files?
□ Yes

Perform linker-based correction?
□ Yes

Specify the genome species: human, mouse
Specify species type
Select Option

Figure 8. Immune Profiler GUI.

2. Populate the required information fields.
   a. FASTQ directory: use the [Browse] button to locate and specify the FASTQ directory for the Immune Profiler.

      Example:

      ```
      /immune_profiler/test/test_input/human/BCRv2_mini/
      ```

   b. Metadata file: use [Browse] to locate and select the metadata `.csv` file associated with the FASTQ files in the directory from 2a. When doing the test run, refer to Table 2 for help determining which metadata file is of most interest to you.

      Example:

      ```
      /immune_profiler/test/test_input/human/bcrv2_mini_meta.csv
      ```

   c. Choose an "Output name" for this analysis run. This is an alphanumeric string, not a file path. The output name string is used to name the output folder created by the analysis and as a prefix for all the results files. Note that:
      - the output folder will be created in the same directory location as the metadata CSV file (Section II.E). User accounts used to run Immune Profiler, therefore, must have read/write permissions to the folder so the output folder can be created (Section II.D).
      - the output name should be less than 20 characters in length and only contain alphanumeric characters and/or hyphens.
      - the output name should be different from the name of the parent folder of the metadata file.
      - Immune Profiler will check if:
        1) A folder matching the output name string already exists in the metadata file location.
        2) The output name is identical to the parent folder name.

        If a match is found to either case, Immune Profiler will terminate the analysis with the corresponding error message:

        1) `Analysis dir already exists: <folder name>`
        2) `The output folder name you defined is identical to its upper folder, please rename.`

   d. "Specify receptor type to analyze" using the dropdown menu, choose '`BCRv2`', '`BCRv1`', or '`TCRv2`'. Refer to Tables 4 & 5, if needed, to correlate these abbreviations with a particular kit type.

   e. Specify "Target region" using the dropdown menu: '`CDR3`', '`Full_length`', or '`Both`'

      This option allows a user to specify analysis on the CDR3 region only, the full-length transcript, or to analyze both CDR3 and full-length sequences in a single run (Both).

      - For BCR, the read will cover the full-length of the V(D)J sequence.
      - For TCR, it will depend on the actual read length; please refer to the SMART-Seq Human TCR (with UMIs) User Manual or SMART-Seq Mouse TCR (with UMIs) User Manual, Appendix B ("Guidelines for Library Sequencing and Data Analysis") for more information about this concept.

      **NOTE:** If you are interested in both CDR3 and full-length results, select `Both`. This will decrease the total processing time compared to launching two independent runs.

3. (Optional) Make selections in the optional configuration fields.

   a. "Keep intermediate files?": default = unchecked (false)

      During analysis, Immune Profiler will create interim files to hold data temporarily until used to generate the final output. These intermediate files include preprocessing FASTQs and binary-form alignment and assembling data files.

      By default, Immune Profiler deletes these files once analysis steps associated with them have been completed as they are large and may quickly consume available disk space. By checking this option, the intermediate files will be retained.

   b. "Perform linker-based correction?": default = unchecked (false)

      PCR errors, sequencing errors, or deletion or insertion of one or more nucleotides could cause a frameshift of final read sequences. To benchmark and conduct quality control on these kinds of errors, Immune Profiler offers linker-based correction, which compares the read sequence in certain regions on the read with the designed linker sequence (Arguel 2017; Turchaninova 2016; Vander Heiden 2014).

      By default, this linker-based correction is not performed. When the option is selected, this check is performed and if a frameshift is identified in a read, it is removed from downstream analysis.

4. Once all desired parameters are populated, click [Start] to begin the analysis.

## 2. Example

To generate results identical to the ones in `test/test_output/human/`, choose the parameters for the desired receptor type from the appropriate column in Tables 4 or 5 (next page):

Table 4. UI parameters for the BCR mini dataset samples to match mini dataset sample output.

| Parameters | BCRv2 | BCRv1 |
|---|---|---|
| Root dataset directory | `$PROFILER_HOME/`<br>`test/test_input/` | `$PROFILER_HOME/`<br>`test/test_input/` |
| FASTQ directory | `human/BCRv2_mini` | `human/BCRv1_mini` |
| Metadata file | `human/bcrv2_mini_meta.csv` | `human/bcrv1_mini_meta.csv` |
| Output name | test-run | test-run |
| Specify receptor type to analyze | BCRv2 | BCRv1 |
| Target region | CDR3 | CDR3 |
| Keep intermediate files? | Unchecked | Unchecked |
| Perform linker-based corrections? | Unchecked | Unchecked |
| Species | human | human |

Table 5. UI parameters for the TCR mini dataset samples to match mini dataset sample output.

| Parameters | Human TCR (with UMIs) | Mouse TCR (with UMIs) |
|---|---|---|
| Root dataset directory | `$PROFILER_HOME/ test/test_input/` | `$PROFILER_HOME/ test/test_input/` |
| FASTQ directory | `human/TCRv2_mini` | `mouse/mTCRv2_mini` |
| Metadata file | `human/tcrv2_mini_meta.csv` | `mouse/mtcrv2_mini_meta.csv` |
| Output name | test-run | test-run |
| Specify receptor type to analyze | TCRv2 | TCRv2 |
| Target region | CDR3 | CDR3 |
| Keep intermediate files? | Unchecked | Unchecked |
| Perform linker-based corrections? | Unchecked | Unchecked |
| Species | human | mouse |

`$PROFILER_HOME` is an abbreviation for the full directory path where the Immune Profiler software is installed.

E.g., if Immune Profiler is installed in `/home/user/bin`, then:

`$PROFILER_HOME` = `/home/user/bin/immune_profiler`

1. The output string (folder name and file prefix) will be:

   `test-run`

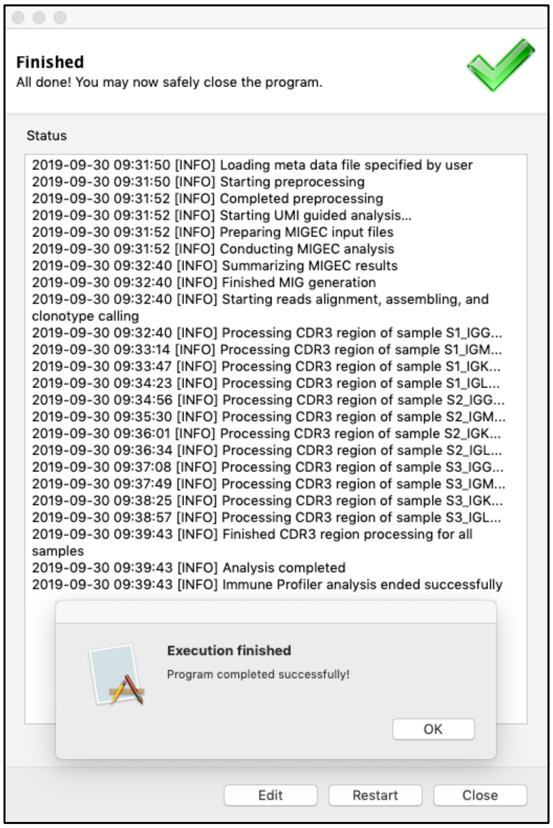
   and the output folder will be created in:

   `/immune_profiler/test/test_input/human/`

   as:

   `/immune_profiler/test/test_input/human/test-run/`
2. "Specify the receptor type" to analyze using the dropdown menu.
3. Specify the "Target region" using the dropdown menu: '`CDR3`'.
4. Keep the defaults for the optional configuration fields (unchecked)
5. **Once all parameters are populated, click [Start] to begin the analysis.**
6. An analysis progress window will replace the Immune Profiler GUI (Figure 9, background).
7. Once the analysis is finished, a pop-up window will display over the progress window with the message "Execution finished / Program completed successfully!" (Figure 9, foreground)

*(continued on next page)*



**Finished**

All done! You may now safely close the program.

Status

```
2019-09-30 09:31:50 [INFO] Loading meta data file specified by user
2019-09-30 09:31:50 [INFO] Starting preprocessing
2019-09-30 09:31:52 [INFO] Completed preprocessing
2019-09-30 09:31:52 [INFO] Starting UMI guided analysis...
2019-09-30 09:31:52 [INFO] Preparing MIGEC input files
2019-09-30 09:31:52 [INFO] Conducting MIGEC analysis
2019-09-30 09:32:40 [INFO] Summarizing MIGEC results
2019-09-30 09:32:40 [INFO] Finished MIG generation
2019-09-30 09:32:40 [INFO] Starting reads alignment, assembling, and
clonotype calling
2019-09-30 09:32:40 [INFO] Processing CDR3 region of sample S1_IGG...
2019-09-30 09:33:14 [INFO] Processing CDR3 region of sample S1_IGM...
2019-09-30 09:33:47 [INFO] Processing CDR3 region of sample S1_IGK...
2019-09-30 09:34:23 [INFO] Processing CDR3 region of sample S1_IGL...
2019-09-30 09:34:56 [INFO] Processing CDR3 region of sample S2_IGG...
2019-09-30 09:35:30 [INFO] Processing CDR3 region of sample S2_IGM...
2019-09-30 09:36:01 [INFO] Processing CDR3 region of sample S2_IGK...
2019-09-30 09:36:34 [INFO] Processing CDR3 region of sample S2_IGL...
2019-09-30 09:37:08 [INFO] Processing CDR3 region of sample S3_IGG...
2019-09-30 09:37:49 [INFO] Processing CDR3 region of sample S3_IGM...
2019-09-30 09:38:25 [INFO] Processing CDR3 region of sample S3_IGK...
2019-09-30 09:38:57 [INFO] Processing CDR3 region of sample S3_IGL...
2019-09-30 09:39:43 [INFO] Finished CDR3 region processing for all
samples
2019-09-30 09:39:43 [INFO] Analysis completed
2019-09-30 09:39:43 [INFO] Immune Profiler analysis ended successfully
```

**Execution finished**

Program completed successfully!

**Figure 9. Immune Profiler GUI progress window at the completion of a successful analysis.**

## C. From the Command Line (Linux or Mac)

### 1. CLI Overview

With Linux or as an alternate method on Mac, Immune Profiler is launched via command line interface (CLI) utilizing the `immune_profiler.py` script. This script can be launched either from within the `immune_profiler/` directory or from any location (working directory) on the Linux server where Immune Profiler software is installed if the full path to the script is specified.

E.g., if `$PROFILER_HOME` = `/home/user/bin/immune_profiler`, the script can be called with:

```
python3 $PROFILER_HOME/immune_profiler.py
```

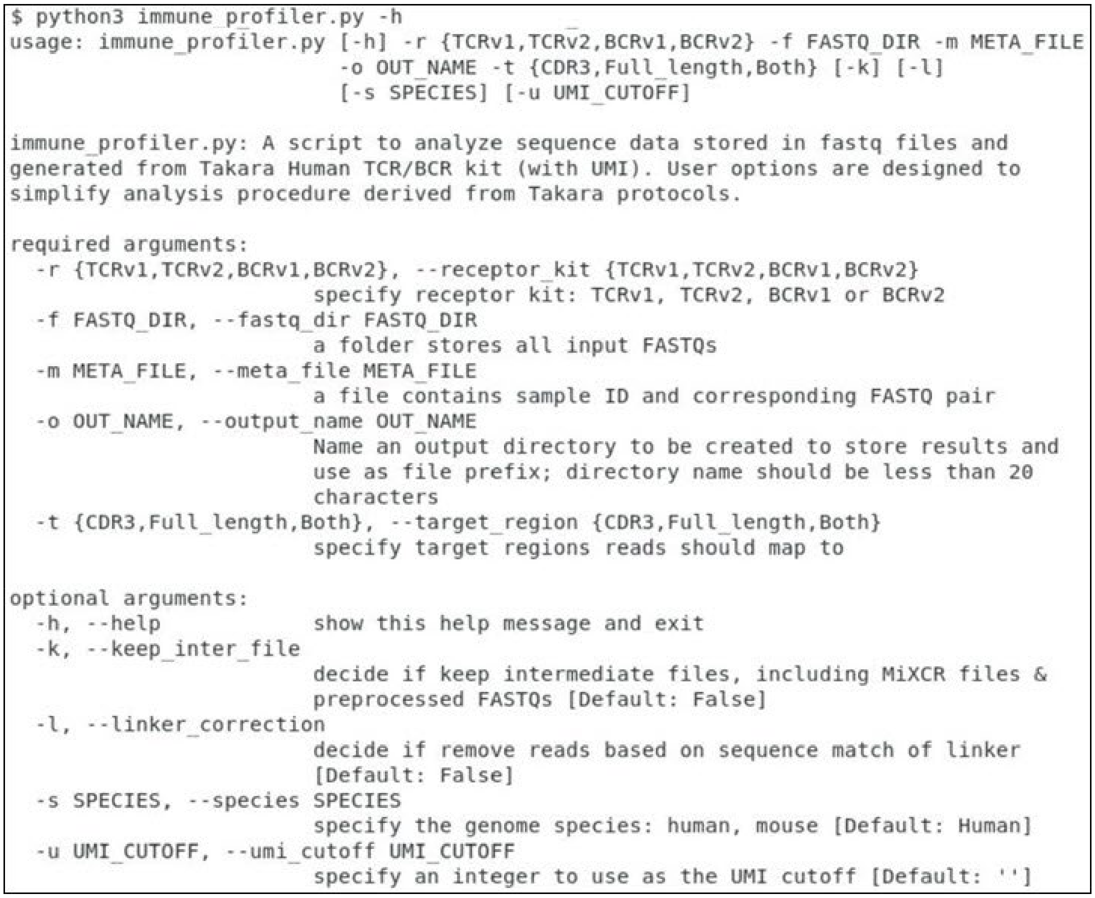
The full list of arguments can be accessed with the `-h` option (Figure 10):

- From within the `immune_profiler/` directory:

```
python3 immune_profiler.py -h
```

- From any location on the server:

```
python3 $PROFILER_HOME/immune_profiler.py -h
```



```
$ python3 immune_profiler.py -h
usage: immune_profiler.py [-h] -r {TCRv1,TCRv2,BCRv1,BCRv2} -f FASTQ_DIR -m META_FILE
                          -o OUT_NAME -t {CDR3,Full_length,Both} [-k] [-l]
                          [-s SPECIES] [-u UMI_CUTOFF]

immune_profiler.py: A script to analyze sequence data stored in fastq files and
generated from Takara Human TCR/BCR kit (with UMI). User options are designed to
simplify analysis procedure derived from Takara protocols.

required arguments:
  -r {TCRv1,TCRv2,BCRv1,BCRv2}, --receptor_kit {TCRv1,TCRv2,BCRv1,BCRv2}
                        specify receptor kit: TCRv1, TCRv2, BCRv1 or BCRv2
  -f FASTQ_DIR, --fastq_dir FASTQ_DIR
                        a folder stores all input FASTQs
  -m META_FILE, --meta_file META_FILE
                        a file contains sample ID and corresponding FASTQ pair
  -o OUT_NAME, --output_name OUT_NAME
                        Name an output directory to be created to store results and
                        use as file prefix; directory name should be less than 20
                        characters
  -t {CDR3,Full_length,Both}, --target_region {CDR3,Full_length,Both}
                        specify target regions reads should map to

optional arguments:
  -h, --help            show this help message and exit
  -k, --keep_inter_file
                        decide if keep intermediate files, including MiXCR files &
                        preprocessed FASTQs [Default: False]
  -l, --linker_correction
                        decide if remove reads based on sequence match of linker
                        [Default: False]
  -s SPECIES, --species SPECIES
                        specify the genome species: human, mouse [Default: Human]
  -u UMI_CUTOFF, --umi_cutoff UMI_CUTOFF
                        specify an integer to use as the UMI cutoff [Default: '']
```

**Figure 10. Output for the `python3 immune_profiler.py -h` command.**

There are two types of arguments: required information and optional configurations. Users must specify all required arguments to launch Immune Profiler. The optional arguments have default values and can be omitted or included based on the analysis needs.

## 2. Required Information Arguments

See the example commands below ("Command Line Examples") for how each of these arguments might be configured.

- `-r` : the receptor type of the data files to be analyzed: `BCRv1`, `BCRv2`, or `TCRv2`

  Example:

  If the input files represent data for samples from SMART-Seq Human TCR (with UMIs), SMART-Seq Mouse TCR (with UMIs), or SMARTer Human TCR a/b Profiling Kit v2, then this argument and parameter would be typed:

  `-r TCRv2`

- `-f` : path to the FASTQ folder

  If there is an error message regarding files, check either the FASTQ folder path or metadata file for typos.

  **NOTE:** Only FASTQs listed in the metadata file are processed for analysis, even if additional FASTQ files are stored in the specified FASTQ folder.

- `-m` : the path to and name of the metadata file (described in Section II.E)

  **REMINDER:** FASTQ files configured in the metadata file should exactly match the name of the FASTQ file in the directory above. Immune Profiler is case-sensitive.

- `-o` : output name

  This is an alphanumeric string, not a file path. The output name string is used to name the output folder created by the analysis and as a prefix for all the results files. Note that:

  - The output folder will be created in the same directory location as the metadata CSV file (Section II.E). User accounts used to run Immune Profiler, therefore, must have read/write permissions to the folder so the output folder can be created (Section II.D).
  - The output name should be less than 20 characters in length and only contain alphanumeric characters and/or hyphens
  - The output name should be different from the name of the parent folder of the metadata file
  - Immune Profiler will check if:
    1) A folder matching the output name string already exists in the metadata file location
    2) The output name is identical to the parent folder name.

    If a match is found to either case, Immune Profiler will terminate the analysis with the corresponding error message:

    1) `Analysis dir already exists: <folder name>`
    2) `The output folder name you defined is identical to its upper folder, please rename.`

- `-t` : specify target regions reads should map to: `CDR3`, `Full_length`, or `Both`

  This option allows a user to specify analysis on only the CDR3 region, the full-length transcript, or to analyze both CDR3 and full-length sequences in a single run (Both). For BCR, the read will cover the full-length of the V(D)J sequence. For TCR, it will depend on the actual read length; please refer to the SMART-Seq Human TCR (with UMIs) User Manual, or SMART-Seq Mouse TCR (with UMIs) User Manual, Appendix B ("Guidelines for Library Sequencing and Data Analysis") for more information about this concept.

  **NOTE:** If you are interested in both CDR3 and full-length results, select `Both`. This will decrease the total processing time compared to launching two independent runs.

  Example:
  If you want to do a full-length analysis, then this argument and parameter would be typed:

  ```
  -t Full_length
  ```

### 3. Optional configuration arguments

- `-k` : condition to keep intermediate files

  (default: false)

  During the analysis, Immune Profiler will create interim files to hold data temporarily until used to generate the final output. These intermediate files include preprocessing FASTQs and binary-form alignment and assembling data files.

  By default, Immune Profiler deletes these files once analysis steps associated with them have been completed as they are large and may quickly consume available disk space. By checking this option, the intermediate files will be retained.

- `-l` : condition to perform linker-based correction

  (default: false)

  PCR errors, sequencing errors, or deletion or insertion of one or more nucleotides could cause a frameshift of final read sequences. To benchmark and conduct quality control on these kinds of errors, Immune Profiler offers linker-based correction, which compares the read sequence in certain regions on the read with the designed linker sequence (Arguel 2017; Turchaninova 2016; Vander Heiden 2014).

  By default, this linker-based correction is not performed. When the option is selected, this check is performed and if a frameshift is identified in a read, it is removed from downstream analysis.

- `-s` : specify the genome species

  (default: human)

  The current options are 'human' or 'mouse'.

- `-u` : specify an integer to use as umi_cutoff.

  **NOTE:** If this variable is not included in the command, a value is auto-generated by MIGEC from a statistical analysis of your data to remove reads with aberrant UMIs caused by sequencing errors.

  The optimal umi_cutoff value is dependent on sequencing depth. To increase the confidence of the analysis result, the umi_cutoff can be increased; however, umi_cutoff values that are too high may result in insufficient reads per UMI to obtain meaningful data.

## 4. Command line examples

To generate results identical to the ones in `test/test_output/`, choose the parameters for the desired receptor type from the appropriate column in Tables 6 or 7:

**Table 6. CLI parameters for the BCR mini dataset samples to match mini dataset sample output.**

| Parameters | BCRv2 | BCRv1 |
|---|---|---|
| Root dataset directory | `$PROFILER_HOME/ test/test_input/` | `$PROFILER_HOME/ test/test_input/` |
| FASTQ directory | `human/BCRv2_mini` | `human/BCRv1_mini` |
| Metadata file | `human/bcrv2_mini_meta.csv` | `human/bcrv1_mini_meta.csv` |
| Output name | `BCRv2` | `BCRv1` |
| Specify receptor type to analyze | `BCRv2` | `BCRv1` |
| Target region | `CDR3` | `CDR3` |
| Keep intermediate files? | `unchecked` | `unchecked` |
| Perform linker-based corrections? | `unchecked` | `unchecked` |
| Species | `human` | `human` |

**Table 7. CLI parameters for the TCR mini dataset samples to match mini dataset sample output.**

| Parameters | Human TCR (with UMIs) | Mouse TCR (with UMIs) |
|---|---|---|
| Root dataset directory | `$PROFILER_HOME/ test/test_input/` | `$PROFILER_HOME/ test/test_input/` |
| FASTQ directory | `human/TCRv2_mini` | `mouse/mTCRv2_mini` |
| Metadata file | `human/tcrv2_mini_meta.csv` | `mouse/mtcrv2_mini_meta.csv` |
| Output name | `TCRv2` | `mTCRv2` |
| Specify receptor type to analyze | `TCRv2` | `TCRv2` |
| Target region | `CDR3` | `CDR3` |
| Keep intermediate files? | `unchecked` | `unchecked` |
| Perform linker-based corrections? | `unchecked` | `unchecked` |
| Species | `human` | `mouse` |

`$PROFILER_HOME` is a variable corresponding to the full directory path where the Immune Profiler software is installed.

E.g., if Immune Profiler is installed in `/home/user/bin`, then:

```
$PROFILER_HOME = /home/user/bin/immune_profiler
```

**NOTE:** The following commands should be typed all at one CLI prompt.

- The first example command will analyze the BCRv2 mini dataset to generate identical report files to `$PROFILER_HOME/test/test_output/human/BCRv2_mini_results.`

```
$ python3 immune_profiler.py -r BCRv2
  -f $PROFILER_HOME/test/test_input/human/BCRv2_mini
  -m $PROFILER_HOME/test/test_input/human/bcrv2_mini_meta.csv
  -o BCRv2
  -t CDR3
  -s human
```

- This second example will analyze the human TCRv2 sample dataset to generate identical report files to `$PROFILER_HOME/test/test_output/TCRv2_mini_results`

```
$ python3 immune_profiler.py -r TCRv2
  -f $PROFILER_HOME/test/test_input/human/TCRv2_mini
  -m $PROFILER_HOME/test/test_input/human/tcrv2_mini_meta.csv
  -o TCRv2
  -t CDR3
  -s human
```

- This third example will analyze the mouse TCRv2 sample dataset to generate identical report files to `$PROFILER_HOME/test/test_output/mTCRv2_mini_results`

```
$ python3 immune_profiler.py -r TCRv2
  -f $PROFILER_HOME/test/test_input/mouse/mTCRv2_mini
  -m $PROFILER_HOME/test/test_input/mouse/mtcrv2_mini_meta.csv
  -o mTCRv2
  -t CDR3
  -s mouse
```

## D. Processing Time

The run time of the pipeline will vary widely based on the specifications of the computer or server on which it is run. The information in this section is provided for comparison purposes to extrapolate for your own system.

### 1. Test parameters

The following files specifications were used to test a small and large dataset:

Table 8. Dataset parameters used for benchmark testing.

| Dataset parameters | Dataset 1 (small) | Dataset 2 (large) |
|---|---|---|
| Number of FASTQ files | 4 | 2 |
| Reads per file | ~2.6 million | ~13.4 million |
| Aggregate FASTQ file size (GB) | 4.1 | 11.4 |

The following two machines were used to test both datasets:

Table 9. Machine specifications used for benchmark testing. Both machines were 64-bit (x86_64).

| Hardware specification | Linux 1 | MacOS X |
|---|---|---|
| Operating System | CentOS 6.10 | MacOS Mojave 10.14.6 |
| CPU | 32-Core Intel Xeon CPU E7-8837 @ 2.67 GHz | Intel Core i5 @ 2.7 GHz |
| Memory (RAM) | 128 GB | 16 GB |

### 2. Test Results

The following benchmark data was generated on the two machines:

Table 10. Benchmark results, per machine for each dataset

| | Total runtime | |
|---|---|---|
| **Dataset** | **Linux 1** | **MacOS X** |
| Dataset 1 (small) | 27 min | 47 min |
| Dataset 2 (large) | 2 hr 50 min | 4 hr 5min |

# VI. Immune Profiler Output

**NOTE:** In this section, the following shortcut references are used:

– `$PROFILER_HOME` is a variable corresponding to the full directory path where the Immune Profiler software is installed.

E.g., if Immune Profiler is installed in `/home/user/bin`, then:

```
$PROFILER_HOME = /home/user/bin/immune_profiler
```

– `<sampleID>` is a generic phrase that represents the value of the corresponding sample ID as defined in the metadata file (see Section II.E).

## A. Overview

The output files and folder structure depend on the optional configuration arguments selected when running the tool. The potential folders found in the output folder include:

- `report/` : This folder summarizes major statistics collected by the previous workflow steps and merges information into files that can be viewed in a spreadsheet program (CSV and MS-Excel formats). This folder is covered in greater detail in Section VI.B (below).
- `airr_report/`: The contents of the folder are same as the folder `report/`. Files inside this folder use AIRR Community standard column names.
- `preprocess/` : contains intermediate FASTQ files created during preprocessing. If the "Keep intermediate files?" option is set to the default (not checked) in the configuration, this folder will be deleted prior to analysis completion.
- `run_migec/` : stores files generated during UMI-based read correction and collapsed-read FASTQs.
- `run_mixcr/` : stores files for read alignment, assembling, and clonotype calling during processing.

For more information about the `preprocess/`, `run_migec/`, and `run_mixcr/` folders, please refer to Appendix C.

## B. report and airr_report Folders

Immune Profiler summarizes major statistics collected by the workflow steps (Section V) and merges the results into comma-separated value (CSV) files and MS-Excel XLSX files that can be viewed in a spreadsheet program. These files are written to and can be found in the `report/` and `airr_report/` folders.

There are slight differences between the files in the two folders, but many have the same or similar names and they contain identical information. The two primary differences between the two sets of output files in the folders are the following:

1. All files in the `airr_report/` folder use column names compliant with AIRR community standards. For more information about this, please refer to:

   https://docs.airr-community.org/en/stable/swtools/airr_swtools_standard.html

2. In keeping with AIRR standards, all files in the `airr_report/` folder are in CSV format. Sample-level report files in the `report/` folder are in XLSX format and have multiple sheets (tabs).

Example output files for each receptor type are included in the mini dataset sample output in `$PROFILER_HOME/test/test_output/human/` (see Appendix A). These sample files follow the output format of the `report/` folder.

**1. `<output name>_sample_QC_stats.csv`**

The QC (quality control) statistics table. For each sample (row), Immune Profiler summarizes the number of reads and read % that are assigned to different chains*, chains shorter than 30 bp in length (short), from undetermined chain (undetermined), failed linker-based correction (flc), and total chains.

*The different chains, by kit type, are listed below:

- **BCRv1:** IgG, IgM, IgK, and IgL
- **BCRv2:** IgG, IgM, IgK, IgL, IgA, IgD, IgE
- **TCRv2:** TRA and TRB

**2. `<output name>_mig_[cdr3|fl]_mapping_stats.csv`**

The mapping statistics table for all samples that are analyzed in the same Immune Profiler run for the target region, i.e., CDR3 or Full_length, specified in the configuration. The string `[cdr3|fl]` means either CDR3 or Full_length. Table 11 lists which files will be seen depending on the target region option selected.

**Table 11. Mapping statistics table files created based on the optional target region configuration**

| Target region | File name |
|---|---|
| Both (default) | `<output name>_mig_cdr3_mapping_stats.csv`<br>`<output name>_mig_fl_mapping_stats.csv` |
| CDR3 | `<output name>_mig_cdr3_mapping_stats.csv` |
| Full_length | `<output name>_mig_fl_mapping_stats.csv` |

The output file contains mapping information and a clonotype summary for all combinations of samples and chains. The columns names for each folder (`report/` and `airr_report/`) and what information the columns represent are listed in Table 12.

Table 12. <output name>_mig_[cdr3|fl]_mapping_stats.csv column names and their descriptions.

| Column name (report/) | Column name (airr_report/) | Description |
|---|---|---|
| — | organism_id | (airr_report only) This value will be 'human' or 'mouse' |
| — | sample_processing_id | (airr_report only) The value <sampleID> |
| sample type | sample_type | User-defined sampleID plus chain type, i.e., <sampleID>_<chain type>. |
| total reads | total_read | Total reads in original FASTQs of corresponding sample + chain type. |
| total MIG | total_mig | Total molecular identifier groups (MIG) classified by MIGEC after read check. |
| UMI threshold | umi_threshold | The UMI threshold used to discard rare reads with too few UMIs. Efficient error correction preferably requires more than five reads per UMI, and the minimum requirement is three (Shugay 2014; Turchaninova 2016). |
| number of reads after MIG collapse | number_of_reads_after_mig_collapse | After MIG collapse, reads belonging to the same MIG are collapsed into a single read. These reads are stored in FASTQs regenerated under run_migec/assemble; this column reports total reads in these files. |
| aligned | aligned | Number of reads aligned to target region by MiXCR. |
| pair-read overlap | pair-read_overlap | Number of pair-reads that overlap with each other. |
| overlapped and aligned | overlapped_and_aligned | Number of reads that have overlap and also were aligned to the target region by MiXCR. |
| clonotype count | clonotype_count | The total number of the clonotype identified after MiXCR procedure. |
| <chain counts> | <chain counts> | Column names vary here: if BCR, count of IgG, IgM, IgK, IgL,IgA, IgD, IgE, and IgH (lack of a constant region) will be listed, or in all caps (IGG, IGM, etc.) for the AIRR format; If TCR, columns of TRA and TRB will be listed. |

3. **report/<output name>_<sampleID>_mig_[cdr3|fl]_report.xlsx**

A sample-level report file summarizing mapping statistics of the particular sample specified by <sampleID> and clonotype details for each chain.

- The first tab, stats, contains sample-specific mapping statistics. It is a subset of the previous file (<output name>_mig_[cdr3|fl]_mapping_stats.csv) and identical to it in table structure.

- The rest of the tabs are clonotype details for each chain type, with the columns in Table 13.

**Table 13. `report/<output name>_<sampleID>_mig_[cdr3|fl]_report.xlsx` column names and descriptions.**

| Column name | Description |
|---|---|
| Read Count* | Number of reads with which a particular clonotype is identified. |
| Fraction | Fraction of read count over total reads. |
| Clonal Sequence | User-defined sampleID plus chain type, i.e., `<sampleID>_<chain type>`.<br>• If target region is CDR3 only, this sequence is the sequence in CDR3 region only.<br>• If target region is Full_length, the corresponding clonotype sequence is reported here. |
| Clonal Sequence Quality | Sequence quality score†. |
| CDR3 Min Quality | Minimal quality score used as a threshold. Only sequence reads with their quality score above this threshold would be aligned and assembled. |
| CDR3 Sequence | CDR3 region sequence. |
| CDR3 Amino Acid Sequence | Amino Acid Sequence of CDR3 region. |
| Clonal type | Chain type categories. For BCR, the value could be:<br>• **BCRv2:** IgG, IgH‡, IgM, IgK, IgL, IgA, IgD, or IgE<br>• **BCRv1:** IgG, IgH‡, IgM, IgK, or IgL |
| Frame Shift | A mark indicates if any frameshift is found in a read. |
| Stop Codon | A mark indicates if any stop codon is found in a read. |
| Amino Acid Length | Total amino acid length for the corresponding clonotype. |
| [V\|D\|J\|C] segment | the most likely segment type for each of the four categories. There is a column for each type (i.e., `V segment`, `D segment`, etc.) |
| all [V\|D\|J\|C] hits | all possible V segment types for each of the four categories. There is a column for each type (i.e., `all J hits`). |

*The read here is a collapsed read, mapped to the FASTQs in the `run_migec/assemble/` folder.
Immune Profiler assumes UMIs are on Read 1, while the reagent kit chemistries have UMIs on Read 2. The FASTQ file names, therefore, correspond to their opposite read.

†Since the collapsed read is used, the quality score is an artificial assignment by the software.

‡A clonal_type value of IgH means the sequence is a heavy chain region that doesn't contain a constant region that would identify the chain as either an IgG or IgM.

### 4. `airr_report/<output name>_<sampleID>_mig_[cdr3|fl]_report.csv`

A sample-level report file summarizing mapping statistics of the particular sample specified by `<sampleID>` and clonotype details for each chain.

Unlike the XLSX file in the report/ folder, this similarly named CSV file contains only one tab representing an aggregate of the clonotype details for each chain type, with the columns in Table 14.

Table 14. airr_report/<output name>_<sampleID>_mig_[cdr3|fl]_report.csv column names and descriptions.

| Column name | Description |
|---|---|
| organism_id | Value will be 'human' or 'mouse' |
| sample_processing_id | Value of <sampleID> |
| read_count[*] | Number of reads with which a particular clonotype is identified. |
| fraction | Fraction of read count over total reads. |
| clonal_sequence | User-defined sampleID plus chain type, i.e., <sampleID>_<chain type>.<br>• If target region is CDR3 only, this sequence is the sequence in CDR3 region only.<br>• If target region is Full_length, the corresponding clonotype sequence is reported here. |
| clonal_sequence_quality | Sequence quality score[†]. |
| cdr3_min_quality | Minimal quality score used as a threshold. Only sequence reads with their quality score above this threshold would be aligned and assembled. |
| cdr3_sequence | CDR3 region sequence. |
| cdr3_amino_acid_sequence | Amino Acid Sequence of CDR3 region. |
| clonal_type | Chain type categories. For BCR, the value could be:<br>• **BCRv2:** IgG, IgH[‡], IgM, IgK, IgL, IgA, IgD, or IgE<br>• **BCRv1:** IgG, IgH[‡], IgM, IgK, or IgL |
| frame_shift | A mark indicates if any frameshift is found in a read. |
| stop_codon | A mark indicates if any stop codon is found in a read. |
| junction_length_aa | Total amino acid length for the corresponding clonotype. |
| [v\|d\|j\|c]_segment | The most likely segment type for each of the four categories. There is a column for each type (i.e., v_segment, d_segment, etc.) |
| all_[v\|d\|j\|c]_hits | All possible V segment types for each of the four categories. There is a column for each type (i.e., all_j_hits). |

[*] The read here is a collapsed read, mapped to the FASTQs in the run_migec/assemble/ folder.
Immune Profiler assumes UMIs are on Read 1, while the reagent kit chemistries have UMIs on Read 2. The FASTQ file names, therefore, correspond to their opposite read.

[†] Since the collapsed read is used, the quality score is an artificial assignment by the software.

[‡] A clonal_type value of IgH means the sequence is a heavy chain region that doesn't contain a constant region that would identify the chain as either an IgG or IgM.

### 5. <sampleID> folders

These subfolders are created for individual samples and contain CSV files that store clonotype details for each chain type. The files have the following naming convention:

```
<sampleID>_<chain-type>_mig_[cdr3|fl]_result.csv
```

The *_result.csv files are made available for use with Cogent NGS Immune Viewer (Section VII) or for advanced users for downstream analysis.

- In the report/ folder—each CSV file is identical to the sample-level clonotype tabs included in <output name>_<sampleID>_mig_[cdr3|fl]_report.xlsx
- In the airr_report/ folder—each CSV file is the clonotype-specific subset of <output name>_<sampleID>_mig_[cdr3|fl]_report.csv

# VII. Cogent NGS Immune Viewer

**Cogent NGS Immune Viewer** (referred to as Immune Viewer) takes as input:

- **(Recommended)** `*_result.csv` files generated by Immune Profiler from the `report/<sampleID>` and `airr_report/<sampleID>` folders (Section VI.B.5, above), -or-
- `airr_report/<output name>_<sampleID>_mig_[cdr3|fl]_report.csv` (Section VI.B.4)

then creates visualizations (charts) or tabulated outputs and publication-ready plots for download.

For more information on the Immune Viewer, please visit the software website page at takarabio.com/ngs-immune-viewer or the Cogent NGS Immune Viewer v1.0 User Manual.

# VIII. References

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Bolotin, D. A. *et al.* MiXCR: software for comprehensive adaptive immunity profiling. *Nat. Methods* **12**, 380–381 (2015).

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Turchaninova, M. A. *et al*. High-quality full-length immunoglobulin profiling with unique molecular barcoding. *Nat. Protoc.* **11**, 1599–1616 (2016).

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# Appendix A. Overview of Mini Dataset Sample Files

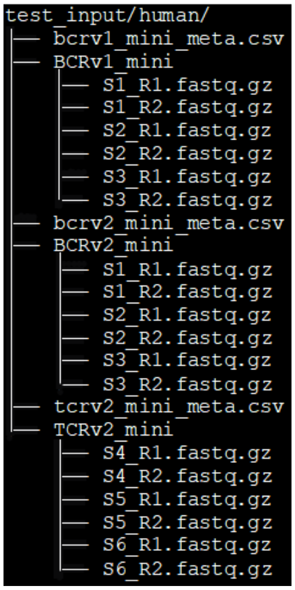
Mini dataset sample files are provided in the Immune Profiler installation within the folder:

```
immune_profiler/test/test_input/human/
```

These should be used both to verify Immune Profiler is installed correctly (Section IV.E) and to familiarize yourself with the operative steps to use the software.

## A. Sample Input Data

Three sets of sample data are provided in the `test/test_input/human/` directory. Refer to Figure 11 for a visual representation of the subfolder contents:



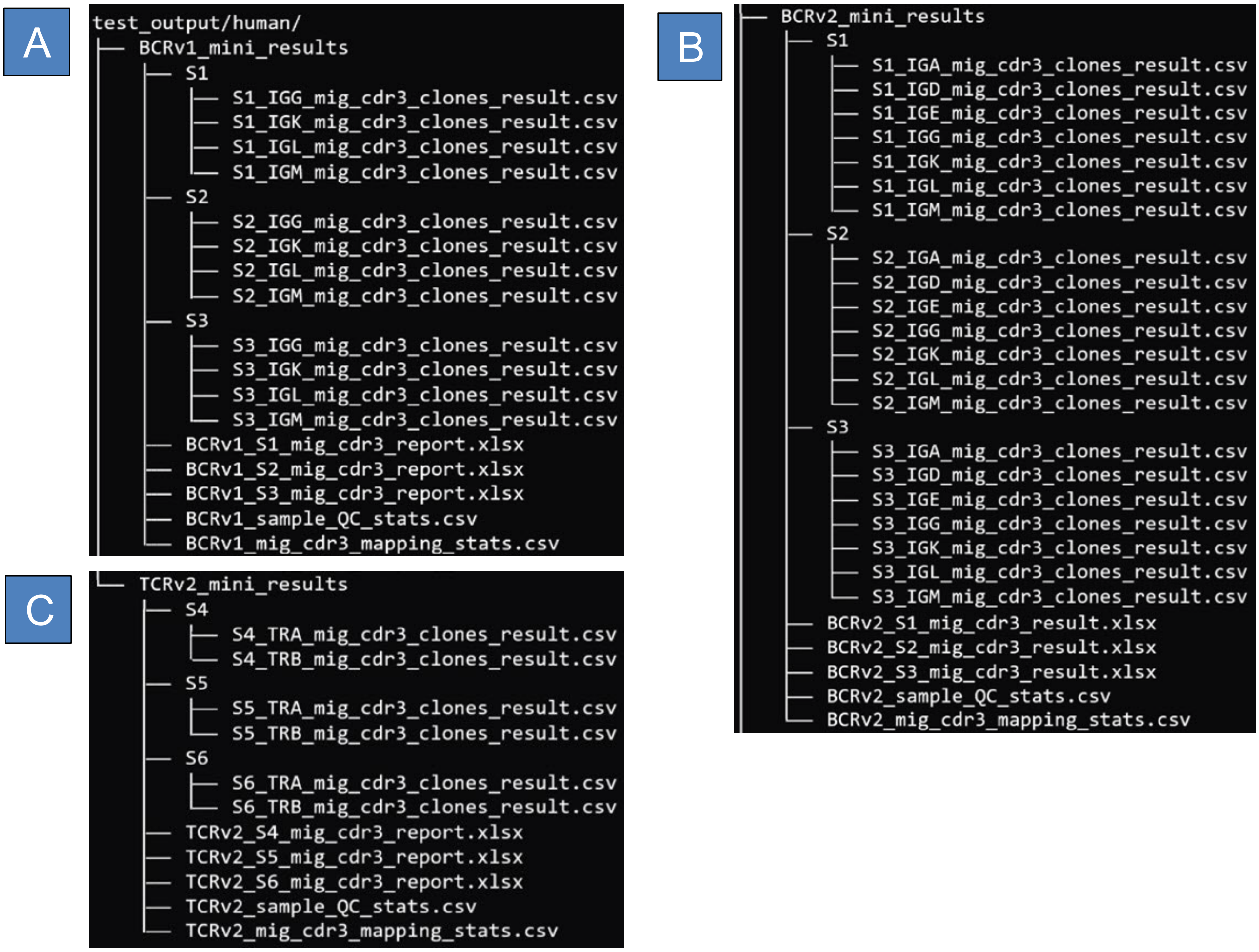
```
test_input/human/
├── bcrv1_mini_meta.csv
├── BCRv1_mini
│   ├── S1_R1.fastq.gz
│   ├── S1_R2.fastq.gz
│   ├── S2_R1.fastq.gz
│   ├── S2_R2.fastq.gz
│   ├── S3_R1.fastq.gz
│   └── S3_R2.fastq.gz
├── bcrv2_mini_meta.csv
├── BCRv2_mini
│   ├── S1_R1.fastq.gz
│   ├── S1_R2.fastq.gz
│   ├── S2_R1.fastq.gz
│   ├── S2_R2.fastq.gz
│   ├── S3_R1.fastq.gz
│   └── S3_R2.fastq.gz
├── tcrv2_mini_meta.csv
└── TCRv2_mini
    ├── S4_R1.fastq.gz
    ├── S4_R2.fastq.gz
    ├── S5_R1.fastq.gz
    ├── S5_R2.fastq.gz
    ├── S6_R1.fastq.gz
    └── S6_R2.fastq.gz
```

**Figure 11. Folder structure and files found in `test_input`/.**

1. Mini dataset BCRv1 input files include:
   - `bcrv1_mini_meta.csv`
   - `BCRv1_mini/` folder : Read1 and Read2 FASTQ files for three samples: S1, S2, and S3, each with 16,000 paired-end reads
2. Mini dataset BCRv2 input files include:
   - `bcrv2_mini_meta.csv`
   - `BCRv2_mini/` folder : Read1 and Read2 FASTQ files for three samples: S1, S2, and S3, each with 20,000 paired-end reads
3. Mini dataset TCRv2 input files include:
   - `tcrv2_mini_meta.csv`
   - `TCRv2_mini/` folder : Read1 and Read2 FASTQ files for three samples: S4, S5, and S6, each with 3,000, 2,500 and 2,000 paired-end reads

## B. Sample Results Data

Three sets of sample result files are provided in the `test/test_output/human/` folder, corresponding to the three sets of input data in Section A. Refer to Figure 12 for a visual representation of the folder contents.



```
test_output/human/
├── BCRv1_mini_results
│   ├── S1
│   │   ├── S1_IGG_mig_cdr3_clones_result.csv
│   │   ├── S1_IGK_mig_cdr3_clones_result.csv
│   │   ├── S1_IGL_mig_cdr3_clones_result.csv
│   │   └── S1_IGM_mig_cdr3_clones_result.csv
│   ├── S2
│   │   ├── S2_IGG_mig_cdr3_clones_result.csv
│   │   ├── S2_IGK_mig_cdr3_clones_result.csv
│   │   ├── S2_IGL_mig_cdr3_clones_result.csv
│   │   └── S2_IGM_mig_cdr3_clones_result.csv
│   ├── S3
│   │   ├── S3_IGG_mig_cdr3_clones_result.csv
│   │   ├── S3_IGK_mig_cdr3_clones_result.csv
│   │   ├── S3_IGL_mig_cdr3_clones_result.csv
│   │   └── S3_IGM_mig_cdr3_clones_result.csv
│   ├── BCRv1_S1_mig_cdr3_report.xlsx
│   ├── BCRv1_S2_mig_cdr3_report.xlsx
│   ├── BCRv1_S3_mig_cdr3_report.xlsx
│   ├── BCRv1_sample_QC_stats.csv
│   └── BCRv1_mig_cdr3_mapping_stats.csv
```

```
├── BCRv2_mini_results
│   ├── S1
│   │   ├── S1_IGA_mig_cdr3_clones_result.csv
│   │   ├── S1_IGD_mig_cdr3_clones_result.csv
│   │   ├── S1_IGE_mig_cdr3_clones_result.csv
│   │   ├── S1_IGG_mig_cdr3_clones_result.csv
│   │   ├── S1_IGK_mig_cdr3_clones_result.csv
│   │   ├── S1_IGL_mig_cdr3_clones_result.csv
│   │   └── S1_IGM_mig_cdr3_clones_result.csv
│   ├── S2
│   │   ├── S2_IGA_mig_cdr3_clones_result.csv
│   │   ├── S2_IGD_mig_cdr3_clones_result.csv
│   │   ├── S2_IGE_mig_cdr3_clones_result.csv
│   │   ├── S2_IGG_mig_cdr3_clones_result.csv
│   │   ├── S2_IGK_mig_cdr3_clones_result.csv
│   │   ├── S2_IGL_mig_cdr3_clones_result.csv
│   │   └── S2_IGM_mig_cdr3_clones_result.csv
│   ├── S3
│   │   ├── S3_IGA_mig_cdr3_clones_result.csv
│   │   ├── S3_IGD_mig_cdr3_clones_result.csv
│   │   ├── S3_IGE_mig_cdr3_clones_result.csv
│   │   ├── S3_IGG_mig_cdr3_clones_result.csv
│   │   ├── S3_IGK_mig_cdr3_clones_result.csv
│   │   ├── S3_IGL_mig_cdr3_clones_result.csv
│   │   └── S3_IGM_mig_cdr3_clones_result.csv
│   ├── BCRv2_S1_mig_cdr3_result.xlsx
│   ├── BCRv2_S2_mig_cdr3_result.xlsx
│   ├── BCRv2_S3_mig_cdr3_result.xlsx
│   ├── BCRv2_sample_QC_stats.csv
│   └── BCRv2_mig_cdr3_mapping_stats.csv
```

```
└── TCRv2_mini_results
    ├── S4
    │   ├── S4_TRA_mig_cdr3_clones_result.csv
    │   └── S4_TRB_mig_cdr3_clones_result.csv
    ├── S5
    │   ├── S5_TRA_mig_cdr3_clones_result.csv
    │   └── S5_TRB_mig_cdr3_clones_result.csv
    ├── S6
    │   ├── S6_TRA_mig_cdr3_clones_result.csv
    │   └── S6_TRB_mig_cdr3_clones_result.csv
    ├── TCRv2_S4_mig_cdr3_report.xlsx
    ├── TCRv2_S5_mig_cdr3_report.xlsx
    ├── TCRv2_S6_mig_cdr3_report.xlsx
    ├── TCRv2_sample_QC_stats.csv
    └── TCRv2_mig_cdr3_mapping_stats.csv
```

**Figure 12. Folder structure and files found in `test_output/human/`. (Panel A)** Mini dataset files for BCRv1. **(Panel B)** Mini dataset files for BCRv2. **(Panel C)** Mini dataset files for TCRv2

In the list below, `<RECEPTOR>` is the immune cell receptor type of the data being processed (options: `BCRv1`, `BCRv2`, -or- `TCRv2`)

- **Files**
  - `<RECEPTOR>_<sampleID>_mig_cdr3_report.xlsx`: summary statistics of individual samples, identified by `sampleID` (as defined in the metadata input file), together with clonotype details derived from all the receptor chains
  - `<RECEPTOR>_sample_QC_stats.csv`: quality control statistics for all samples of the receptor type (i.e, `BCRv2_sample_QC_stats.csv` are QC stats for all BCRv2 samples)
  - `<RECEPTOR>_mig_cdr3_mapping_stats.csv`: mapping statistics for all samples of the receptor type

- **Subfolders**

  Each subfolder is named for and corresponds to the `sampleID` value for the samples defined in the metadata input file. The subfolder of each sample contains multiple CSV files, corresponding to receptor chains.

  - For BCRv1, there are four files: for IGG, IGK, IGL, and IGM
  - For BCRv2, there are seven files: for IGA, IGD, IGE, IGG, IGK, IGL, and IGM
  - For TCRv2, there are two files: for TRA and TRB

  These files are identical to the clonotype worksheets (individual tabs) included in the `<RECEPTOR>_<sampleID>_mig_cdr3_report.xlsx file`. They are provided in CSV format to make it easier for advanced users to import them into other tools for tertiary analysis.

## Appendix B. Log Files

**Table 15. Immune Profiler log files.** The table contains the filename, the location where to find them within the `immune_profiler/` directory, and a brief description of the information stored in each.

| Log filename | Subfolder | Description |
|---|---|---|
| `<output name>_immune_profiler.log` | -- | Immune Profiler analysis progress and important notes |
| `mig_run_migec.log` | `run_migec/` | MIGEC analysis progress |
| `mig_run_migec.error` | `run_migec/` | MIGEC error messages (if any) |
| `assemble.log.txt` | `run_migec/assemble` | MIGEC process status |
| `assemble.cmd.txt` | `run_migec/assemble` | The command call to MIGEC for the assemble function |
| `checkout.cmd.txt` | `run_migec/ checkout_all/` | MIGEC analysis commands used |
| `checkout.log.txt` | `run_migec/ checkout_all/` | MIGEC analysis progress and findings |
| `checkout.filelist.txt` | `run_migec/ checkout_all/` | Documents all intermediate files while MIGEC is processing |
| `run_mixcr_[cdr3|fl].log` | `run_mixcr/` | MIXCR analysis progress and important notes.<br>**NOTE:** Different files are created depending on whether the CDR3 or Full_length target regions are selected during configuration |
| `run_mixcr_[cdr3|fl].error` | `run_mixcr/` | MIXCR error messages (if any)<br>**NOTE:** Different files are created depending on whether the CDR3 or Full_length target regions are selected during configuration |

# Appendix C. More Output Details from Immune Profiler

## A. Preprocess Folder

This folder is generated for results of the first step of the Immune Profiler workflow (Section III), which separates reads in original sample-level FASTQs into different chain-specific FASTQs.

- FASTQs are created for each of the chain types.
    - o BCRv1 `<chain type>` : IgG, IgM, IgK, and IgL
    - o BCRv2 `<chain type>` : IgA, IgD, IgE, IgG, IgM, IgK, and IgL
    - o TCRv2 `<chain type>` : TRA and TRB

  `<sampleID>_<chain type>_R1.fastq` and `<sampleID>_<chain type>_R2.fastq`

- An undetermined FASTQ pair is generated to store reads that cannot be confidently assigned to any chain categories.

  `<sampleID>_undetermined_R1.fastq`

  `<sampleID>_undetermined_R2.fastq`

- Reads that are less than 30 bases in length, too short to be accurately aligned with any V(D)J sequences, are assigned to:

  `<sampleID>_short_R1.fastq`

  `<sampleID>_short_R2.fastq`

- If linker-based correction is turned on, an additional FASTQ pair is created to store reads that failed to correct:

  `<sampleID>_flc_R1.fastq`

  `<sampleID>_flc_R2.fastq`

**REMINDER:** If the "Keep intermediate file?" option is not selected, the `preprocess/` folder is deleted by Immune Profiler to save storage space and computing resources on the workstation or server.

## B. run_migec Folder

This folder is created during the second step of the Immune Profiler workflow. A version of MIGEC embedded in Immune Profiler is deployed to conduct error correction using Unique Molecular Identifiers (UMIs). Algorithm details for this processing can be found at https://migec.readthedocs.io/en/latest/index.html. If you have additional questions after referring to the documentation, please contact technical_support@takarabio.com.

- `barcodes.txt` : an intermediate file generated by Immune Profiler to link sample information with MIGEC processing
- `assemble/` : reads from the same UMI are grouped together and defined as a Molecular Identifier Group (MIG). Reads within the same MIG are cross-referenced, potential sequence errors are corrected, and collapsed reads are deducted. The `assemble/` folder stores the resulting FASTQs.

  `<sampleID>_<chain type>_R1.t*.cf.fastq`

  `<sampleID>_<chain type>_R2.t*.cf.fastq`

For `t*` in the FASTQ file name, the `*` represents a numeric value; the value indicates the UMI threshold used for the corresponding chain-specific samples.

**IMPORTANT:** Immune Profiler assumes UMIs are on Read 1, while SMART-Seq Human BCR (with UMIs), SMART-Seq Human TCR (with UMIs), SMART-Seq Mouse TCR (with UMIs), SMARTer Human BCR IgG IgM H-K-L Profiling Kit, and SMARTer Human TCR a/b Profiling Kit v2 chemistry have UMIs on Read 2. The FASTQ file names, therefore, correspond to their opposite read.
Example:

– The file `<sampleID>_<chain type>_R1.T1.CF.FASTQ` is FASTQ Read 2

– `<sampleID>_<chain type>_R2.t1.cf.fastq` is Read 1

- `checkout_all/` : read screening is performed and trustable reads deducted, creating sample-level clean-up FASTQs.

  `<sampleID>_<chain type>_R1.fastq` and `<sampleID>_<chain type>_R2.fastq`

  Any undetermined reads identified based on UMI algorithms are assigned to these files:

  `undef-R1.fastq` and `undef-R2.fastq`

  **NOTE:** If the "Keep intermediate file" option is not selected, all the FASTQs in this folder are deleted after processing.

- `histogram/` : UMI statistics are collected, and a threshold is determined for read exclusion (Shugay et al. 2014). In total 10 files are created at this step; details about these files can be found at https://migec.readthedocs.io/en/latest/logs.html. If you have additional questions after referring to the documentation, please contact technical_support@takarabio.com.

  - `estimates.txt`
  - `histogram.cmd.txt`
  - `overseq.txt`
  - `overseq-units.txt`
  - `collision1.txt`
  - `collision1-units.txt`
  - `pwm.txt`
  - `pwm-units.txt`
  - `pwm-summary.txt`
  - `pwm-summary-units.txt`

## C. run_mixcr Folder

This folder results from the third step of the analysis workflow. A version of MiXCR embedded in Immune Profiler is called to conduct read alignment, assembling, and clonotype reporting. Algorithm details can be found at https://mixcr.readthedocs.io/_/downloads/en/docs/pdf/ (PDF). If you have additional questions after referring to the documentation, please contact technical_support@takarabio.com.

Depending on the target region specified in the optional configuration arguments, the folders `mig_cdr3/`, `mig_fl/`, or both folders may be created to store corresponding analysis results.

Each folder will contain files with the following:

- Sample- and chain-level alignment statistics report

  `<sampleID>_<chain type>_mig_[cdr3|fl]_align_report.txt`

- Sample- and chain-level clonotype assembling statistics report

  `<sampleID>_<chain type>_mig_[cdr3|fl]_clones_report.txt`

- Sample- and chain-level clonotype detail report

  `<sampleID>_<chain type>_mig_[cdr3|fl]_clones_all.txt`

- Two files in binary format are created for each sample and chain.

  `<sampleID>_<chain type>_mig_[cdr3|fl].vdjca`

  `<sampleID>_<chain type>_mig_[cdr3|fl].clns`

The string `[cdr3|fl]` means either `cdr3` or `fl` will be inserted there, as in the following example:

```
<sampleID>_<chain type>_mig_cdr.vdjca
<sampleID>_<chain type>_mig_fl.clns
```

**NOTE:** If the option to keep intermediate files is selected, the `*.vdjca` and `*.clns` files are retained. By default, they are deleted after processing.

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