ReFocus Assistant and EpiFocus Assistant: PANDAA Automated Analysis Tools

Overview

ReFocus Assistant is an automated qPCR results analysis tool. Given the results of a qPCR run with HIVDR PANDAAs, the application will use the VQ and DRM standard curve included on the plate (identified by the software as samples that have identified quantities) to calculate percent DRMs for each well, based on wells' Cq values for different targets. Positive, negative, or indeterminate qualitative calls are made for each well based on DRM percentages.

EpiFocus Assistant is a companion tool: it similarly performs automated analysis, given instead the results of a qPCR run with one of Aldatu's viral hemorrhagic fever assays. A standard curve is not required, and positive or negative calls for individual wells are based on wells' Cq values as well as the presence/absence of the internal control signal.
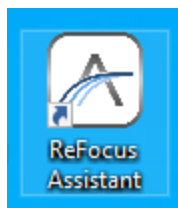
Both tools allow for qualitative and quantitative results to be generated in CSV and/or traceable PDF format.
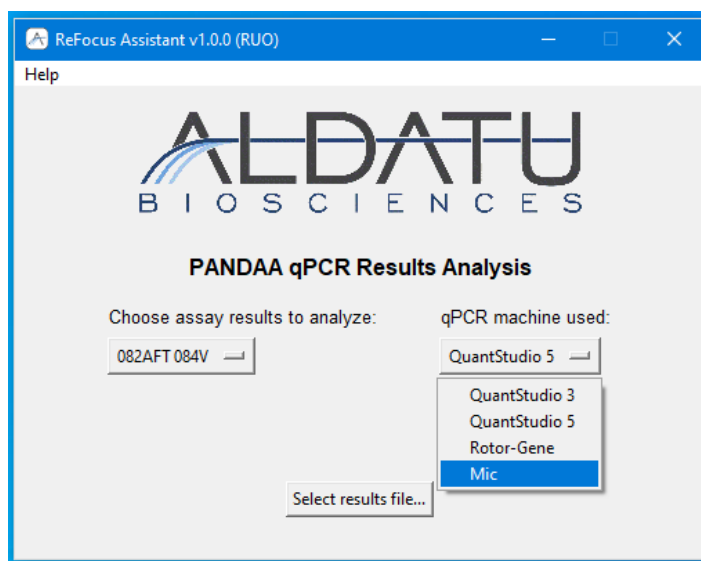
Installation

Each program has its own installer executable. To install, double-click the respective installer, which will walk the user through installation steps. If the installer file isn't readily available in OneDrive, it can also be found on the company's GitHub repository for this project (click the "Releases" heading on the right side of the page).

Workflow: Using PANDAA Assistant Tools

1. Launch the tool by double-clicking the executable.

2. Select the qPCR machine and assay used.

3. Choose **raw, unedited** results file(s) from file selection menu.

   Valid file types include Excel files, CSVs, and text files – click the file type drop-down to see which formats are available, based on the selected machine type.

   QuantStudio and Mic selections allow the user to select one file. The RotorGene selection requires as many files as there are fluorophores/assays in the experiment.

4. Dialog box shows the location of analyzed results files (CSV and optional PDF), then the program automatically closes.

5. Results files are modeled after similar outputs from standard qPCR machine softwares.

Data Analysis Algorithms

ReFocus Assistant (HIVDR)

1. The program recognizes wells that have quantities entered (for example, "Standards" in QuantStudio files with specified quantities in that field). The program uses a least-squares regression to construct standard curves for each target (log quantity vs. Cq value). By default, the program uses the raw quantity as the VQ / VIC quantity, and uses 20% of the specified quantity as the quantity for other HIV assays (i.e., 20% DRM for standard curve templates). This percentage can be changed in the TOML configuration file.
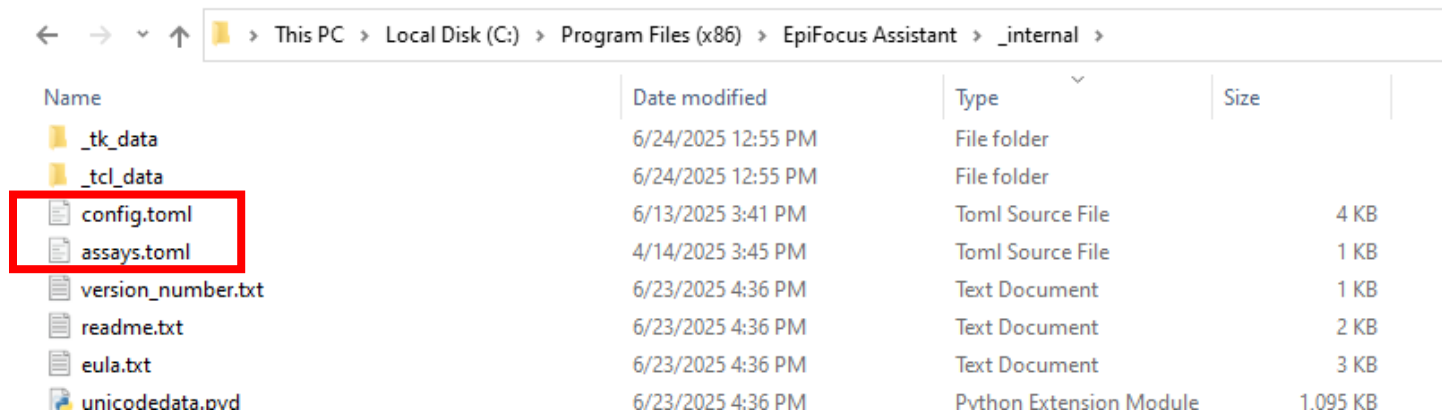
2. Using the standard curves constructed for each target, the quantity for each target is calculated for each unknown well.

3. For each well, percentage DRMs are calculated: for each non-VQ target, the target quantity is divided by the VQ quantity.

4. Percentage DRMs are compared to established minimum and maximum cutoffs (editable in TOML configuration file): if percentage is below minimum, the well is marked negative; if percentage is above maximum, the well is marked positive; if percentage is between minimum and maximum, the well is marked indeterminate.

EpiFocus Assistant (VHFs)

1. For each unknown well, an ordered list is constructed that stores the Cq value of each target in the well.

   a. A dummy value for the internal control Cq value is stored (high number).

   b. For each sample target, if the Cq value is below the positive Cq cutoff (default 30 cycles, editable in TOML configuration file) *and* the dRn for that well is above the minimum cutoff (default 5%, editable in TOML configuration file), the actual Cq value is returned and added to the ordered list. Otherwise, a dummy value is added (high number – exceeds dummy internal control value).

2. The program finds the smallest number (minimum valid Cq) stored in this list and returns its corresponding target name. For wells positive for a non-internal control target, the analysis algorithm stops here.

3. If the minimum valid Cq is the dummy internal control value (well was negative for all other targets), the actual internal control value is checked for amplification. If amplification occurred, the well is marked negative. If internal control amplification did not occur, the well is marked invalid.

Configuration via TOML files

The Assistant tools can be configured via TOML files (TOML: Tom's Obvious Minimal Language). These can be found in the *_internal* subfolder after installation. These TOML files allow for runtime configuration – no recompiling is required (any edits to TOML files will be immediately reflected in the executable).

TOMLs can be configured in any text editor, although editing in Visual Studio Code enables color-coding of file components, which may make more complex edits easier.

The TOML files used in these programs are structured as a set of tables. Do not edit the table headers or key names, as these are referenced by program code. Edit key names to change parameters.
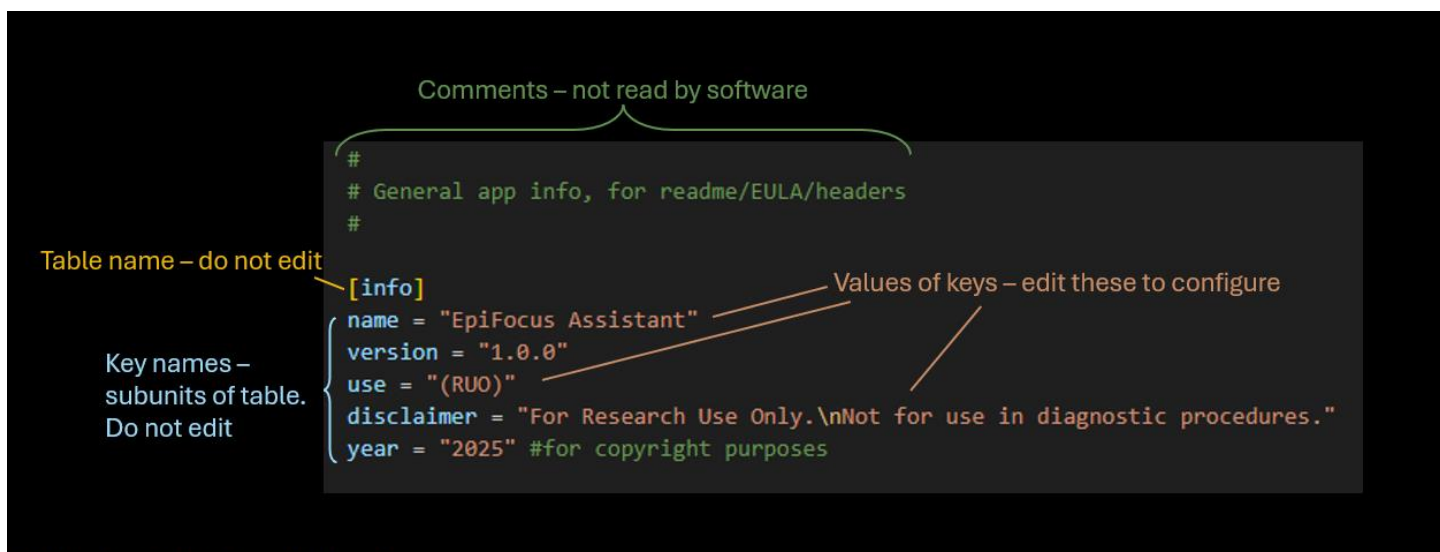
Each TOML file contains detailed comments that describe acceptable key values and their impact.

- *config.toml* contains parameters that can be edited to change displayed text and constant values used in analysis.
- *assays.toml* contains a reference library of assays that can be accessed by the program.

A summary of settings that can be changed in *config.toml* is included below. Additional details about a given parameter are available in the comments of *config.toml.*

Key	Summary
name	App name
version	Version number
use	"RUO," etc.
disclaimer	"For Research Use Only."
year	Used in copyright note
assay_choice_format	Assays listed as radio buttons or drop-down
assay_choices	Assays to choose from
machine_choices	Machines to choose from

Key	Summary
export_columns	Columns for report
create_pdf	True = makes PDF results
wait	Slow program speed slightly
division	Type of analysis: VHF or HIVDR
cq_cutoff	Cq assigned to no amp
pos_cutoff	Cq cutoff for positives
dRn_percent_cutoff	Percentage required for true amplification

Troubleshooting

In general, errors should be caught by the program: error handling generally consists of an error message explaining the error to the user, after which the program automatically closes.

Troubleshooting Tips

- **File format mismatch:** If the program fails to open your input file, ensure you selected the correct qPCR machine and assay. The tool expects raw, unedited exports (XLSX, CSV, or TXT depending on the machine).

- **No output generated:** Check that:
    - You're not running the program from a protected or read-only folder.
    - The input file isn't open in another program (e.g., Excel).
    - You selected a file format compatible with the selected machine type.

- **TOML formatting issues:** Only edit the **values**, not the key names or headers. Use Notepad or Visual Studio Code (recommended for syntax highlighting). Avoid extra quotes, commas, or blank lines between sections.

- **Unexpected qualitative calls:** If results look incorrect, double-check your TOML configuration – especially cutoffs for Cq or percent DRM. Refer to the in-file comments for guidance.

When to contact a developer

The programs are designed to be maintained by editing TOML files. However, changes to the underlying analysis logic (e.g., new curve-fitting methods, output format changes, or adding support for a new qPCR file structure) will require developer assistance.

If I'm no longer available, share the GitHub repository and this guide with your support person. The software is written in Python 3.10; a full list of dependencies can be found in major release notes.