PANDAA Software

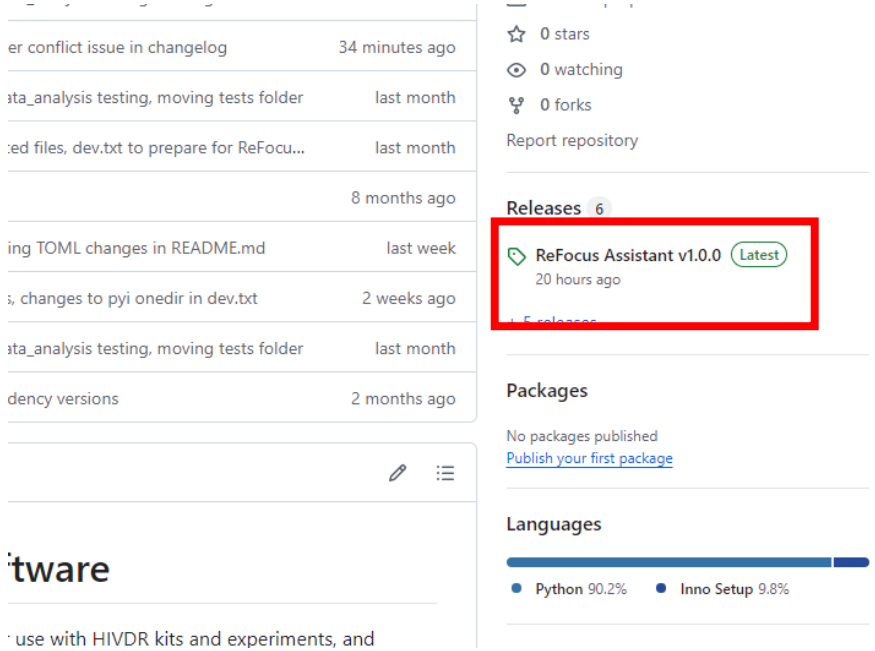
ReFocus Assistant and EpiFocus Assistant:
automated qualitative results analysis tools

Where to get the software

GitHub

[aldatubio/PANDAA-qPCR-Results: Development of a script to auto-analyze qPCR results from tsv / csv files. (github.com)]

- Visit “Releases” for downloads

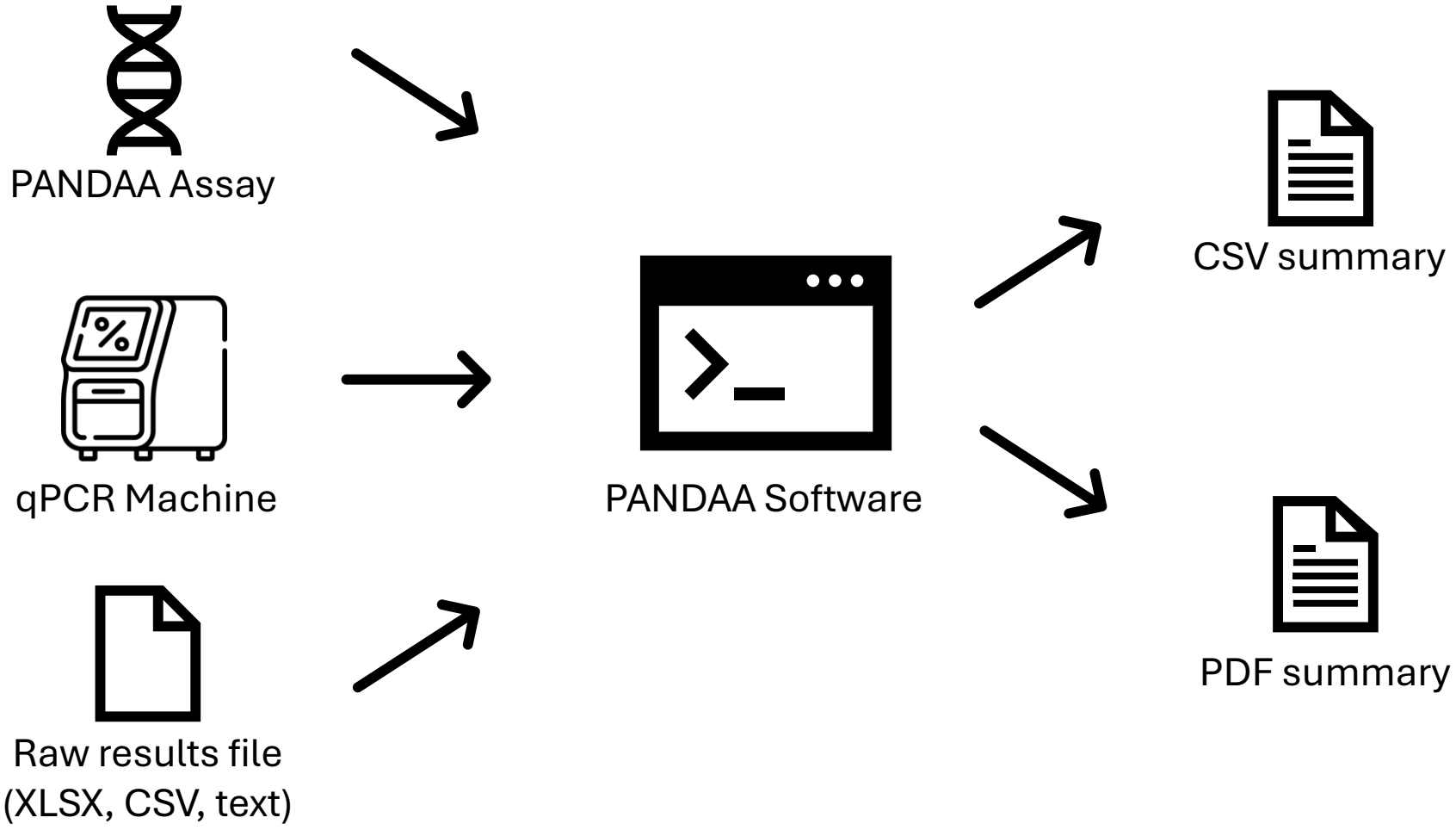


Workflow Overview

Choose tool based on product family:

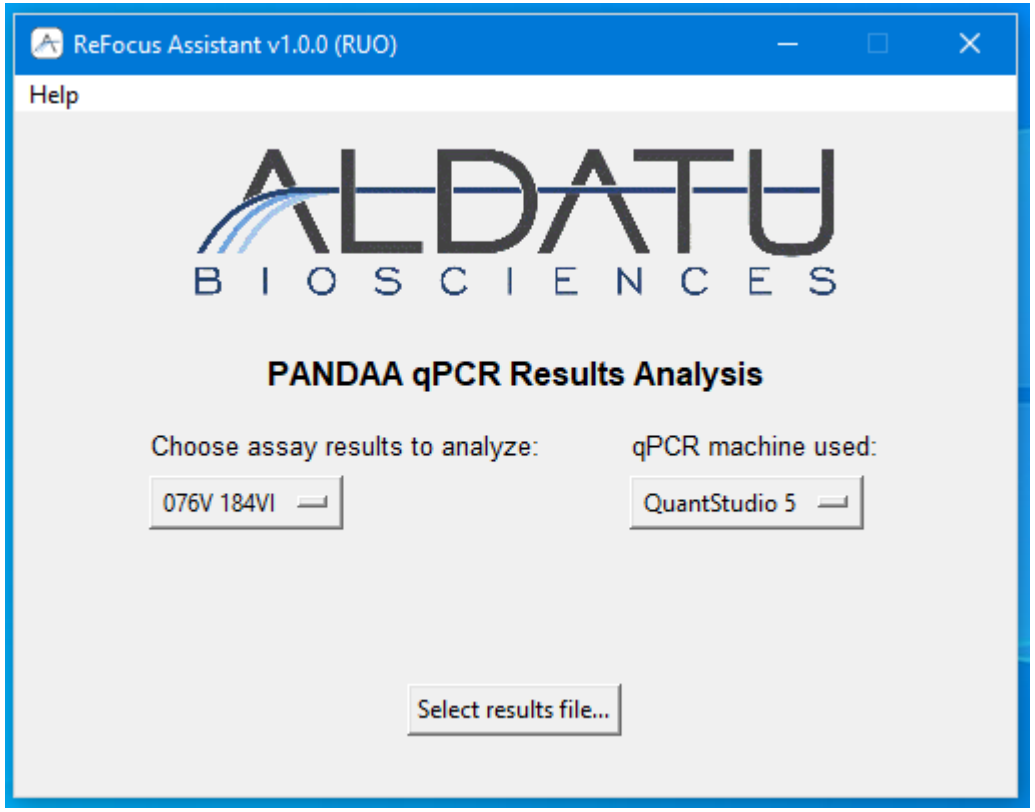
ReFocus Assistant
HIV drug resistance

EpiFocus Assistant
Viral hemorrhagic fevers

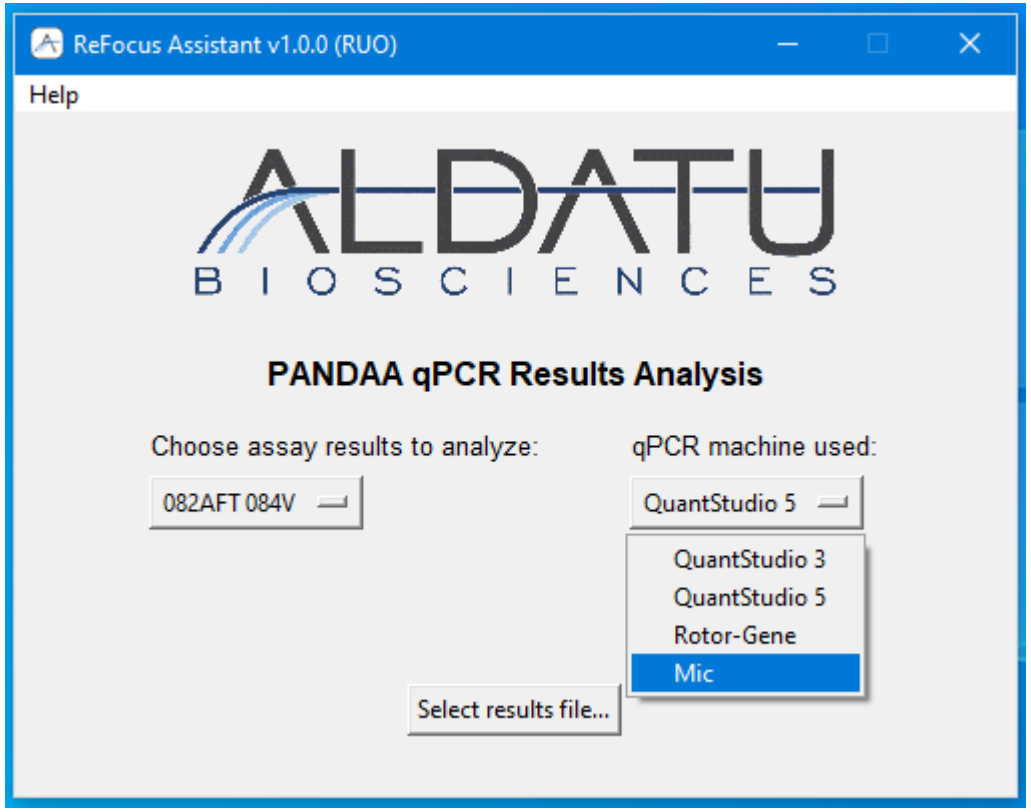


Workflow

Double-click icon to start app and open main window

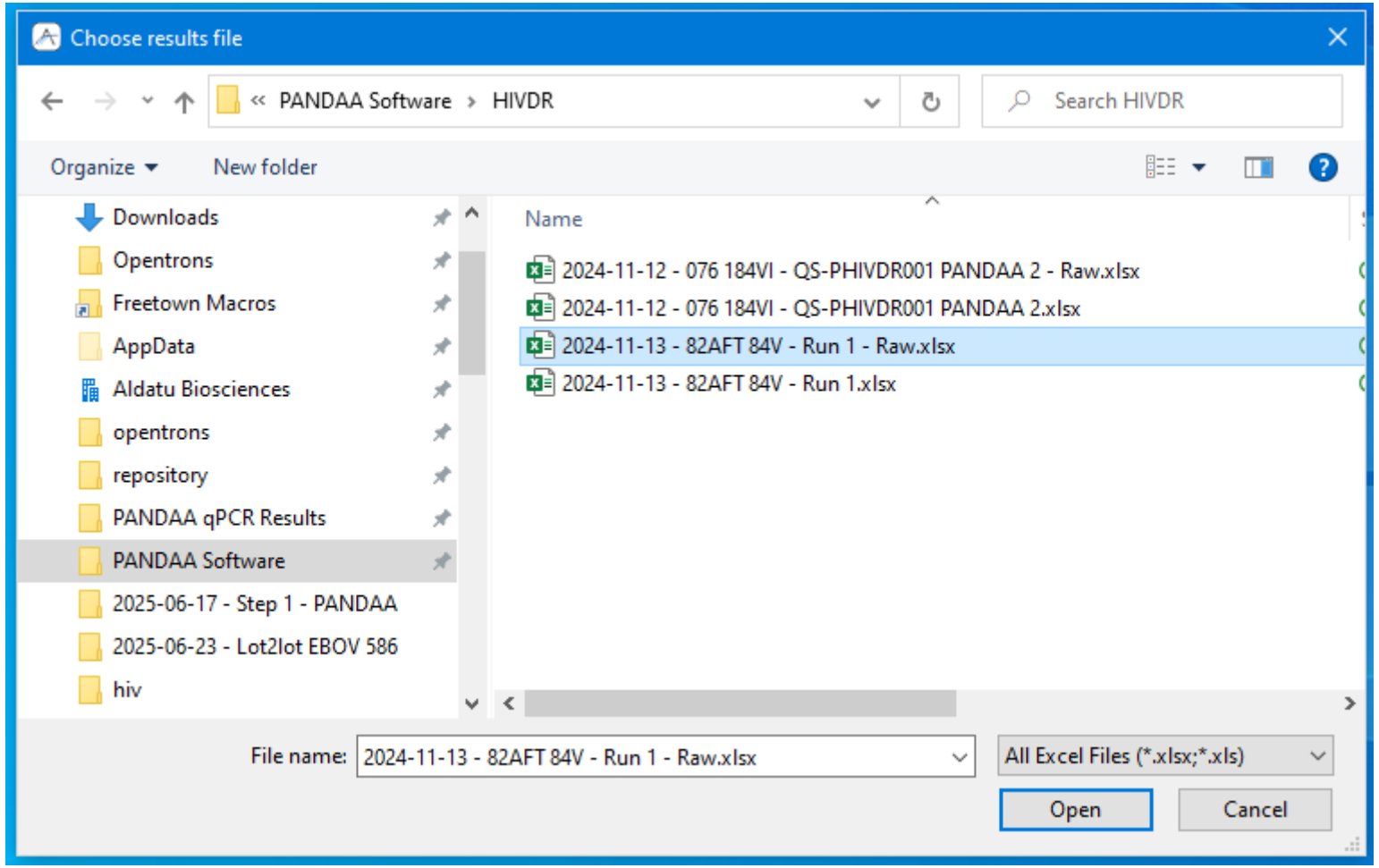


Choose assay and qPCR machine from menus



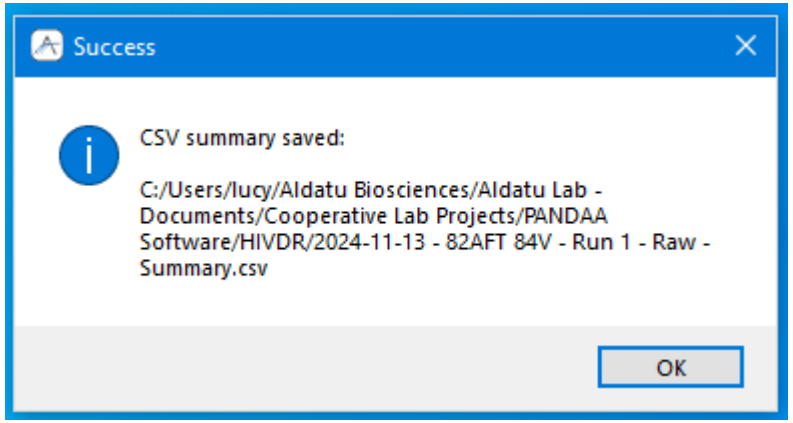
Workflow

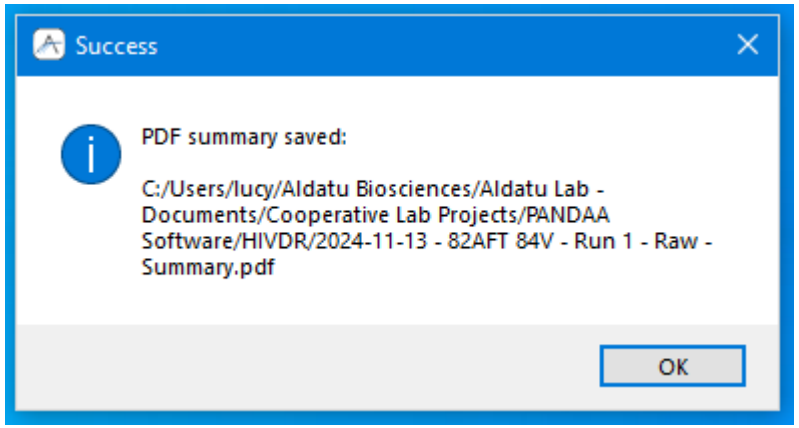
Choose **raw, unedited** results file from file selection menu (can be Excel file, CSV, or text file)



Workflow

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





Workflow

Results files

	A	B	C	D	E	F	G	H	I
1	Experiment Barcode								
2	Experimen	Experiment 588FAM Threshold - 50,000VIC Threshold - 20,000Cy5 Threshold - 20,000R(							
3	Experimen	C:\Users\lucy\Aldatu Biosciences\Project Freetown - Documents\2. Research and Devel							
4	Experimen	2024-06-17 - Inclusivity SUDV Boniface							
5	Experimen	2024-06-17 16:15:50 PM EDT							
6	Experimen	Standard Curve							
7	Instrument	Aldatu-QS5							
8	Instrument	2.73E+08							
9	Instrument	QuantStudio™ 5 System							
10	Passive Reference								
11	Post-read Stage/Step								
12	Pre-read Stage/Step								
13	Quantificat	Ct							
14	Signal Sm	TRUE							
15	Stage whe	Stage2							
16	Stage/ Cyc	Stage3, Step2							
17									
18									
19	Well	Sample Na	Internal Co	EBOV Cq	MARV Cq	Result			
20	A1	SUDV.Bor	11.8	4.6	35	EBOV Positive			
21	A2	SUDV.Bor	11.7	4.7	35	EBOV Positive			
22	A3	SUDV.Bor	11.8	4.7	35	EBOV Positive			
23	A4	SUDV.Bor	11.7	4.7	35	EBOV Positive			
24	A5	SUDV.Bor	11.8	6.6	35	EBOV Positive			
25	A6	SUDV.Bor	11.9	6.6	34.5	EBOV Positive			
26	A7	SUDV.Bor	11.9	6.8	33.4	EBOV Positive			
27	A8	SUDV.Bor	11.9	6.6	35	EBOV Positive			
28	A9	SUDV.Bor	12.1	8.8	35	EBOV Positive			
29	A10	SUDV.Bor	12.3	8.9	19.2	EBOV Positive			
30	A11	SUDV.Bor	12.2	8.9	17	EBOV Positive			
31	A12	SUDV.Bor	12.4	9.1	35	EBOV Positive			
32	B1	SUDV.Bor	11.6	10.9	18.6	EBOV Positive			
33	B2	SUDV.Bor	12.3	11.2	21.2	EBOV Positive			
34	B3	SUDV.Bor	12.1	11.3	35	EBOV Positive			
35	B4	SUDV.Bor	12.1	11.4	35	EBOV Positive			

Report

Run Information

Experiment Comment	Experiment 588FAM Threshold - 50,000VIC Threshold - 20,000Cy5 Threshold - 20,000ROX OffThermocycler program: RT: 50C for 15 min Activation: 95C for 2 min Adaptation (10x): 90C for 3 sec; 55C for 30 sec; 60C for 30 sec Amplification (35x): 90C for 3 sec; 60C for 1 min Ramp Rate: 1.6C/sec
Experiment File Name	C:\Users\lucy\Aldatu Biosciences\Project Freetown - Documents\2. Research and Development\3. Performance Evals\Analytical Inclusivity\SUDV - Boniface\2024-06-17 - Inclusivity SUDV Boniface r1.eds
Experiment Name	2024-06-17 - Inclusivity SUDV Boniface
Experiment Run End Time	2024-06-17 16:15:50 PM EDT
Experiment Type	Standard Curve
Instrument Name	Aldatu-QS5
Instrument Serial Number	272510135
Instrument Type	QuantStudio 5 System
Quantification Cycle Method	Ct
Signal Smoothing On	true
Stage where Melt Analysis is performed	Stage2
Stage/ Cycle where Ct Analysis is performed	Stage3, Step2

Samples

Well	Sample Name	Internal Control Cq	EBOV Cq	MARV Cq	Result
A1	SUDV.Boniface	11.8	4.6	35.0	EBOV Positive
A2	SUDV.Boniface	11.7	4.7	35.0	EBOV Positive
A3	SUDV.Boniface	11.8	4.7	35.0	EBOV Positive
A4	SUDV.Boniface	11.7	4.7	35.0	EBOV Positive
A5	SUDV.Boniface	11.8	6.6	35.0	EBOV Positive
A6	SUDV.Boniface	11.9	6.6	34.5	EBOV Positive

ReFocus Assistant vs EpiFocus Assistant

ReFocus Assistant

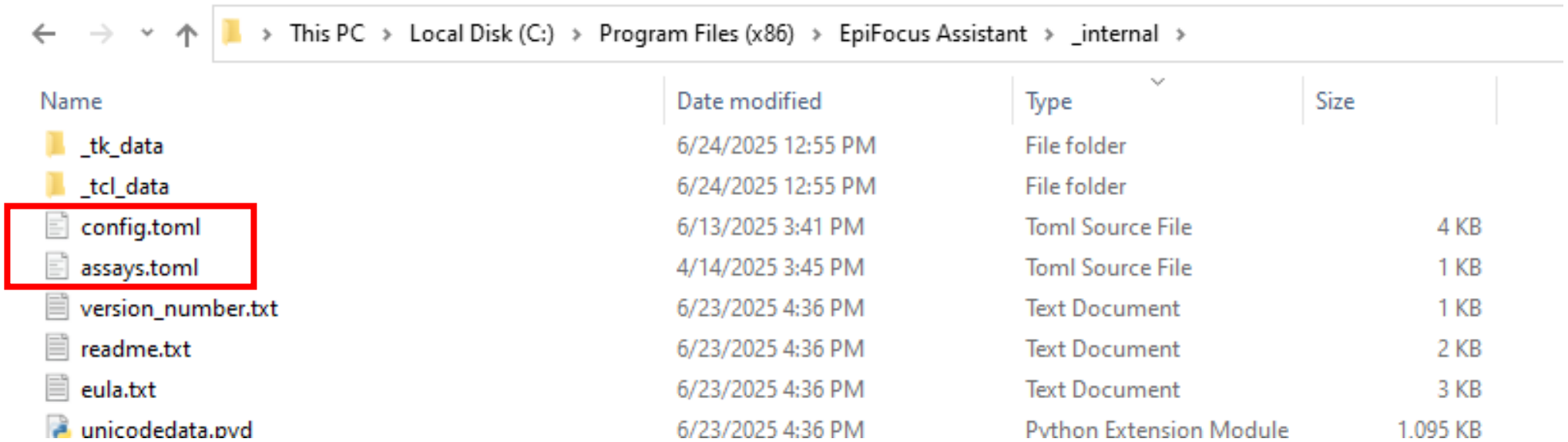
- For HIVDR assays
- Builds standard curve based on wells with specified input quantity
- Cq values in unknown wells are compared to the standard curve to calculate quantity in unknowns
- Qualitative results are given as a percentage of DRM present in a well, compared to the amount of VQ detected

EpiFocus Assistant

- For VHF assays
- No standard curve required; well quantities ignored
- Qualitative results (positive or negative) are given based on Cq value, and whether it is above or below specified cutoffs

How to configure settings using TOML files

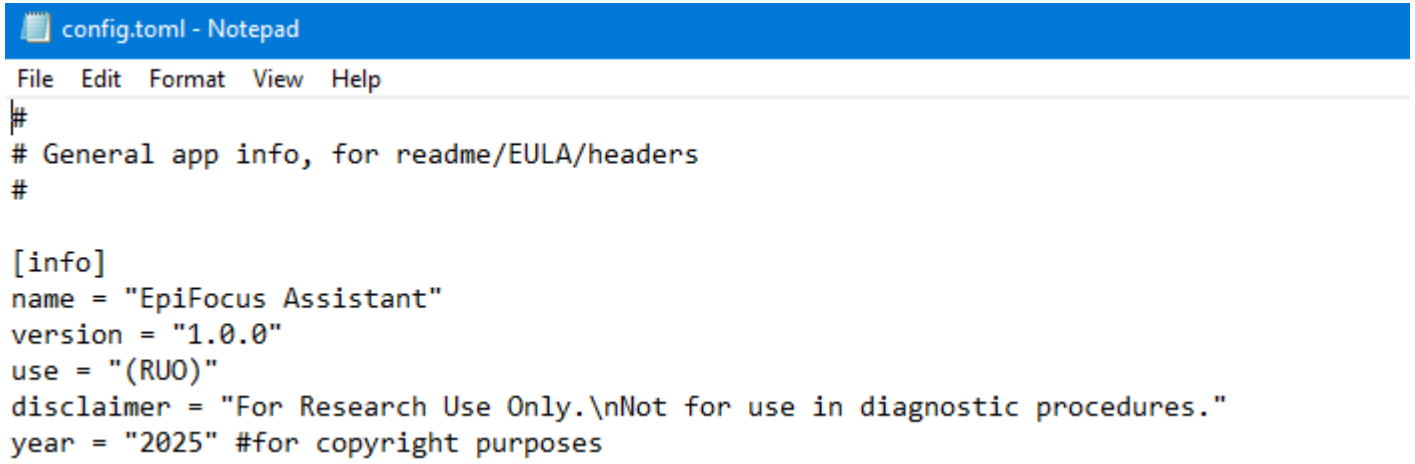
Navigate to the Program Files folder



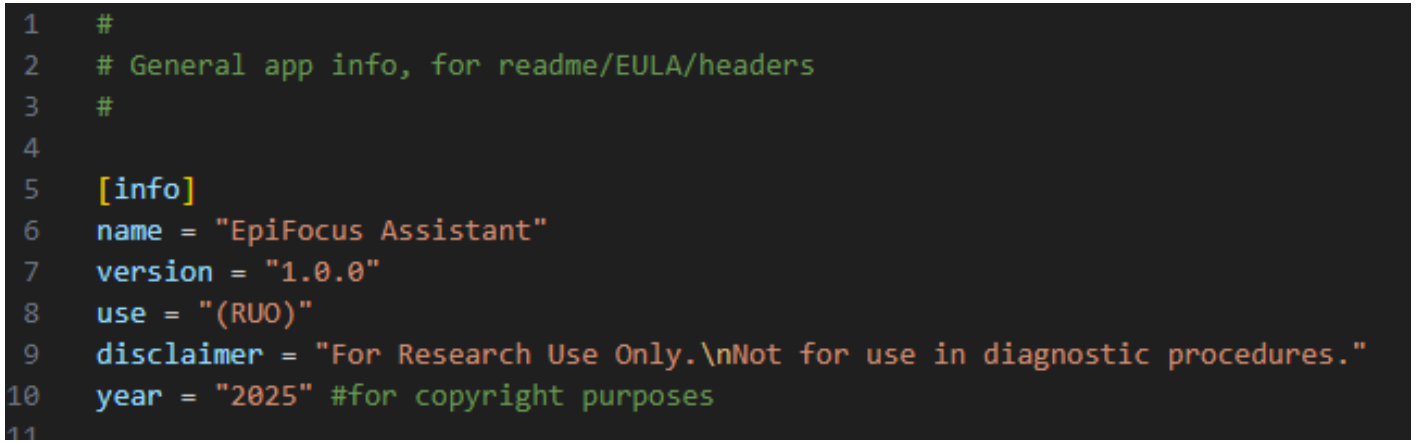
How to configure settings using TOML files

TOMLs can be opened in any text editor, although they can be color-coded in VSCode for easier reading

Notepad

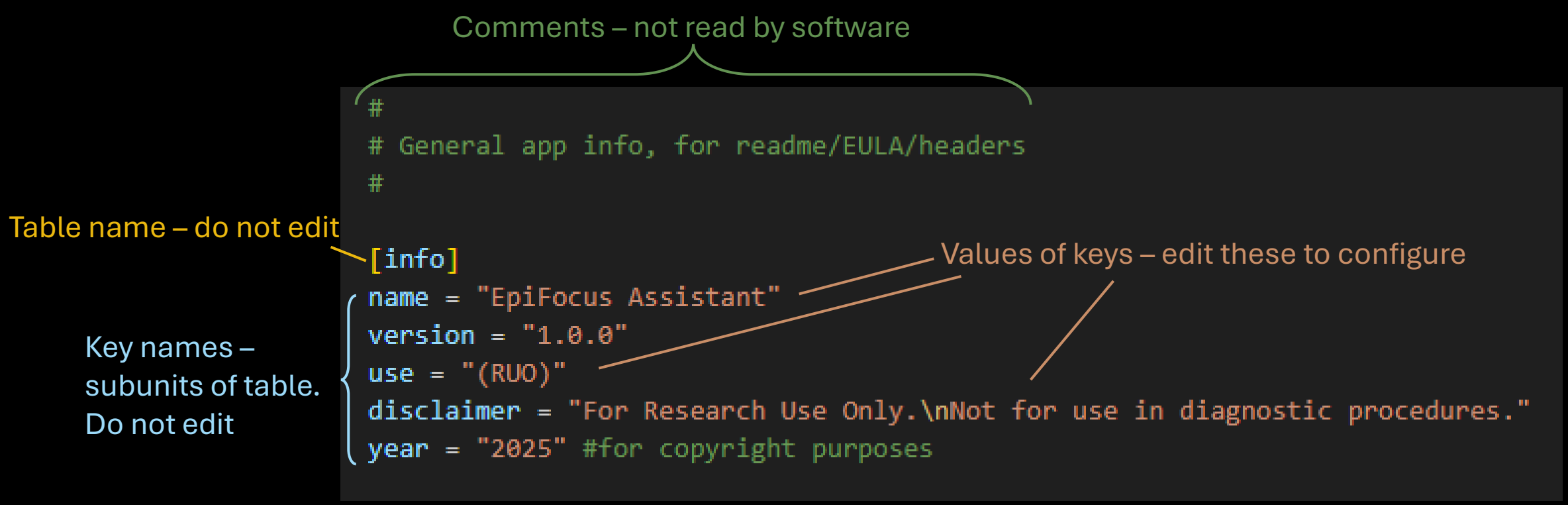


VSCode



How to configure settings using TOML files

Anatomy of a TOML



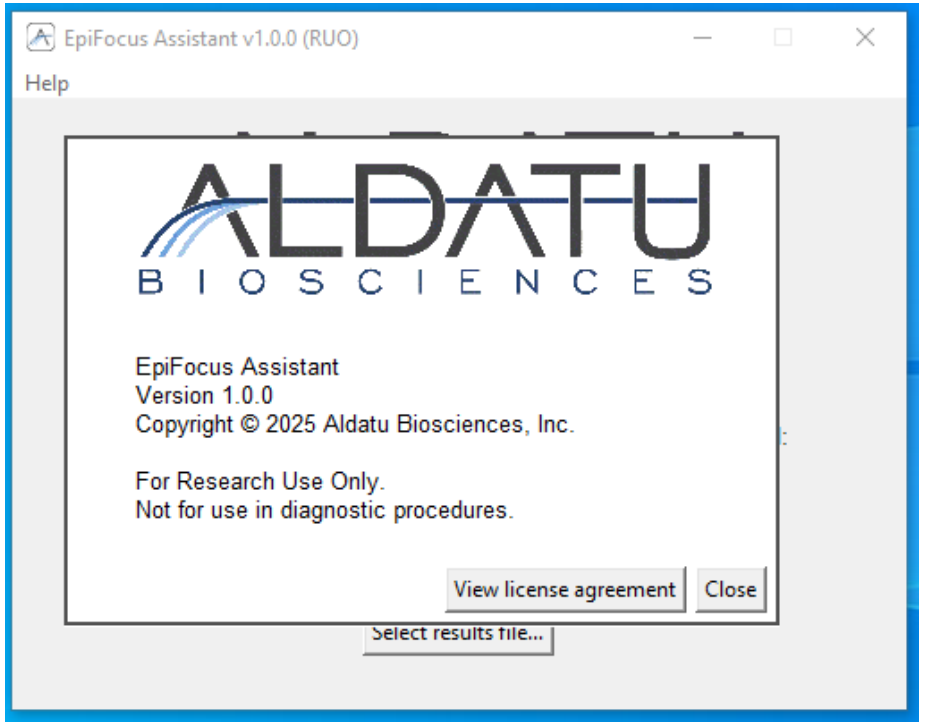
TOML: Tom's Obvious Minimal Language

How to configure settings using TOML files

How TOML settings show up in the software

```
#
# General app info, for readme/EULA/headers
#

[info]
name = "EpiFocus Assistant"
version = "1.0.0"
use = "(RUO)"
disclaimer = "For Research Use Only.\nNot for use in diagnostic procedures."
year = "2025" #for copyright purposes
```



Troubleshooting

In general, errors should be caught and should show up as an error dialog box for the user. Feel free to let me know if I've missed anything!