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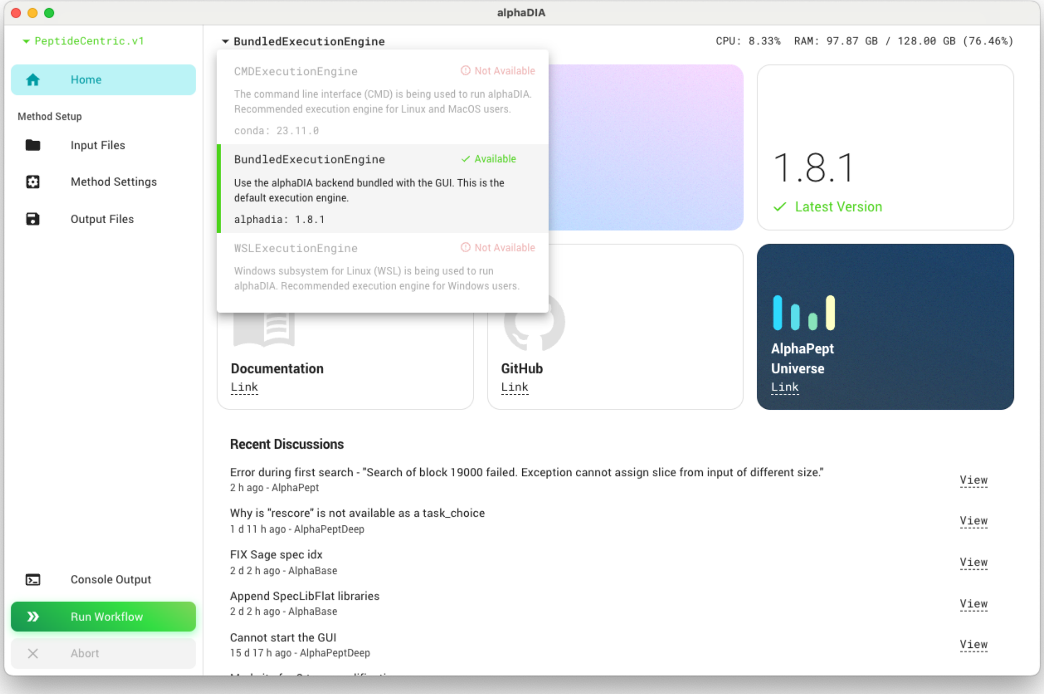
DEVELOPMENT
Modules

Library-free DIA search using the GUI

This tutorial was created using alphaDIA 1.8.1 - please be aware that there might be changes in your version

1. Prerequisites

Make sure that you have a machine with at least 64 gigabytes of memory. Please download the test data for this tutorial here. will be using replicates of label-free bulk DIA data of HeLa digests acquired on the Orbitrap Astral. Also make sure you have a valid alphaDIA installation including the GUI. The easiest option is the one-click installer, and a summary of all installation optic can be found here. Also ensure the right execution engine has been selected and your version is up to date.



CMDExecutionEngine ⓘ Not Available
The command line interface (CMD) is being used to run alphaDIA. Recommended execution engine for Linux and MacOS users.
conda: 23.11.0

BundledExecutionEngine ✓ Available
Use the alphaDIA backend bundled with the GUI. This is the default execution engine.
alphadia: 1.8.1

WSLExecutionEngine ⓘ Not Available
Windows subsystem for Linux (WSL) is being used to run alphaDIA. Recommended execution engine for Windows users.

Recent Discussions

Error during first search - "Search of block 19000 failed. Exception cannot assign slice from input of different size." View
2 h ago - AlphaPept

Why is "rescore" is not available as a task_choice View
1 d 11 h ago - AlphaPeptDeep

FIX Sage spec idx View
2 d 2 h ago - AlphaBase

Append SpecLibFlat libraries View
2 d 2 h ago - AlphaBase

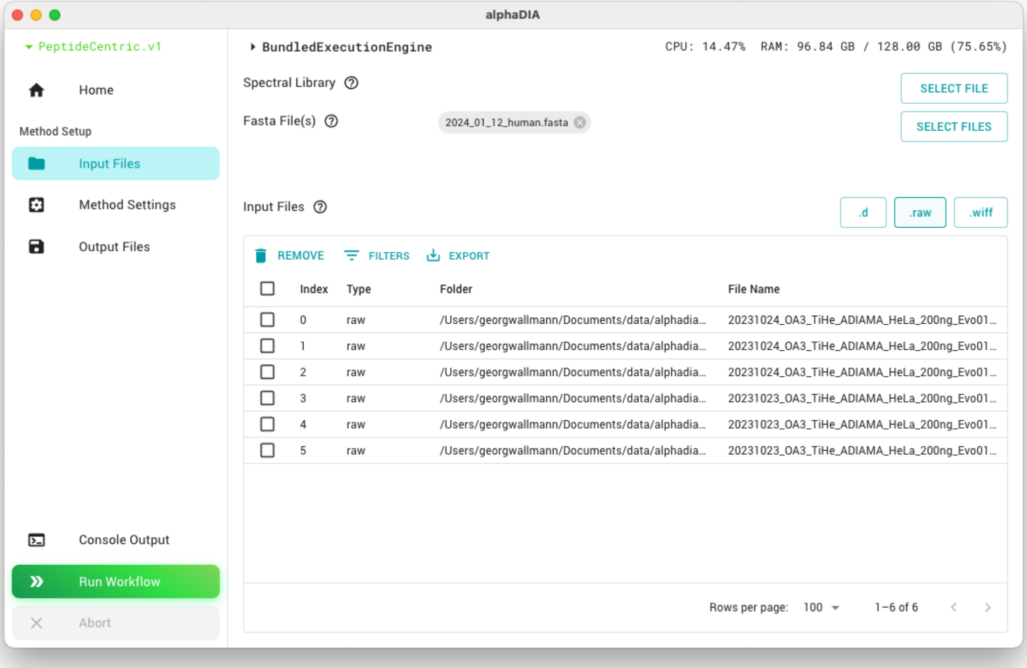
Cannot start the GUI View
15 d 17 h ago - AlphaPeptDeep

2. Project Structure

We will be performing two DIA searches: a first search for library generation and a second search for joined quantification. To accommodate this, we prepare the project directory to have a `first_pass` and a `second_pass` folder.

3. First search

To set up the first search, select all raw files and add them to the file list. Also add the FASTA file which will be used for library prediction.



▸ BundledExecutionEngine CPU: 14.47% RAM: 96.84 GB / 128.00 GB (75.65%)

Spectral Library

Fasta File(s) 2024_01_12_human.fasta

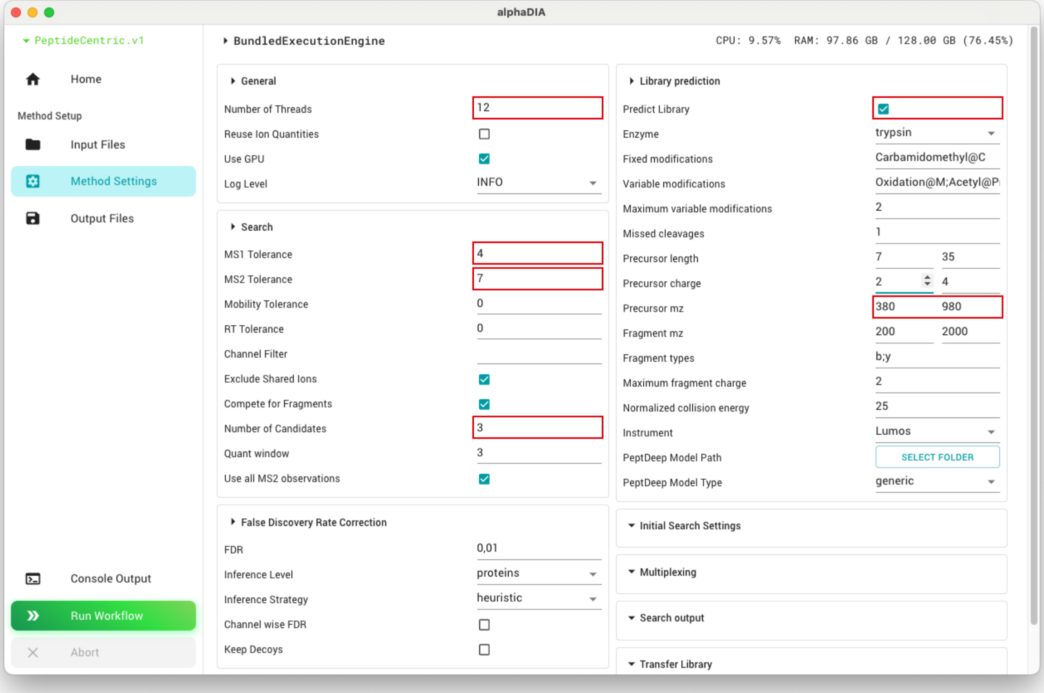
Input Files

☐	Index	Type	Folder	File Name
☐	0	raw	/Users/georgwallmann/Documents/data/alphadia...	20231024_OA3_TiHe_ADIAMA_HeLa_200ng_Evo01...
☐	1	raw	/Users/georgwallmann/Documents/data/alphadia...	20231024_OA3_TiHe_ADIAMA_HeLa_200ng_Evo01...
☐	2	raw	/Users/georgwallmann/Documents/data/alphadia...	20231024_OA3_TiHe_ADIAMA_HeLa_200ng_Evo01...
☐	3	raw	/Users/georgwallmann/Documents/data/alphadia...	20231023_OA3_TiHe_ADIAMA_HeLa_200ng_Evo01...
☐	4	raw	/Users/georgwallmann/Documents/data/alphadia...	20231023_OA3_TiHe_ADIAMA_HeLa_200ng_Evo01...
☐	5	raw	/Users/georgwallmann/Documents/data/alphadia...	20231023_OA3_TiHe_ADIAMA_HeLa_200ng_Evo01...

Rows per page: 100 1–6 of 6

For this search, most parameters can be left at their default values. To speed up processing, set `thread_count` to the number logical cores you have available in your system. Also enable library prediction from FASTA and set the `precursor_mz` range to t range of the dataset `380` - `980` to predict only the relevant subset of precursors. By default, this search will have `Carbamidomethyl@C` as a fixed modification and up to two variable modifications of `Oxidation@M` and `Acetyl@Protein_N-term`.

For the search, we will use known `target_ms1_tolerance` of 4ppm and `target_ms2_tolerance` of 7ppm. These values are optim for Orbitrap Astral data and can be reused. For lower resolution instruments, 10ppm or 15ppm might be optimal. If the optimal mass tolerance is not known, it can be set to `0` to activate automatic optimization. We recommend noting down the optimized value across multiple runs and reusing it for the same instrument/resolution combination. The `target_rt_tolerance` will also b to `0` for automatic optimization. Lastly, we increase the number of peak groups `target_num_candidates` to use for deep-learni based scoring to `3`.



▸ BundledExecutionEngine CPU: 9.57% RAM: 97.86 GB / 128.00 GB (76.45%)

▸ General

Number of Threads	12
Reuse Ion Quantities	☐
Use GPU	☑
Log Level	INFO

▸ Search

MS1 Tolerance	4
MS2 Tolerance	7
Mobility Tolerance	0
RT Tolerance	0
Channel Filter	
Exclude Shared Ions	☑
Compete for Fragments	☑
Number of Candidates	3
Quant window	3
Use all MS2 observations	☑

▸ False Discovery Rate Correction

FDR	0,01
Inference Level	proteins
Inference Strategy	heuristic
Channel wise FDR	☐
Keep Decoys	☐

▸ Library prediction

Predict Library	☑	
Enzyme	trypsin	
Fixed modifications	Carbamidomethyl@C	
Variable modifications	Oxidation@M;Acetyl@P	
Maximum variable modifications	2	
Missed cleavages	1	
Precursor length	7	35
Precursor charge	2	4
Precursor mz	380	980
Fragment mz	200	2000
Fragment types	b;y	
Maximum fragment charge	2	
Normalized collision energy	25	
Instrument	Lumos	
PeptDeep Model Path	SELECT FOLDER	
PeptDeep Model Type	generic	

▾ Initial Search Settings

▾ Multiplexing

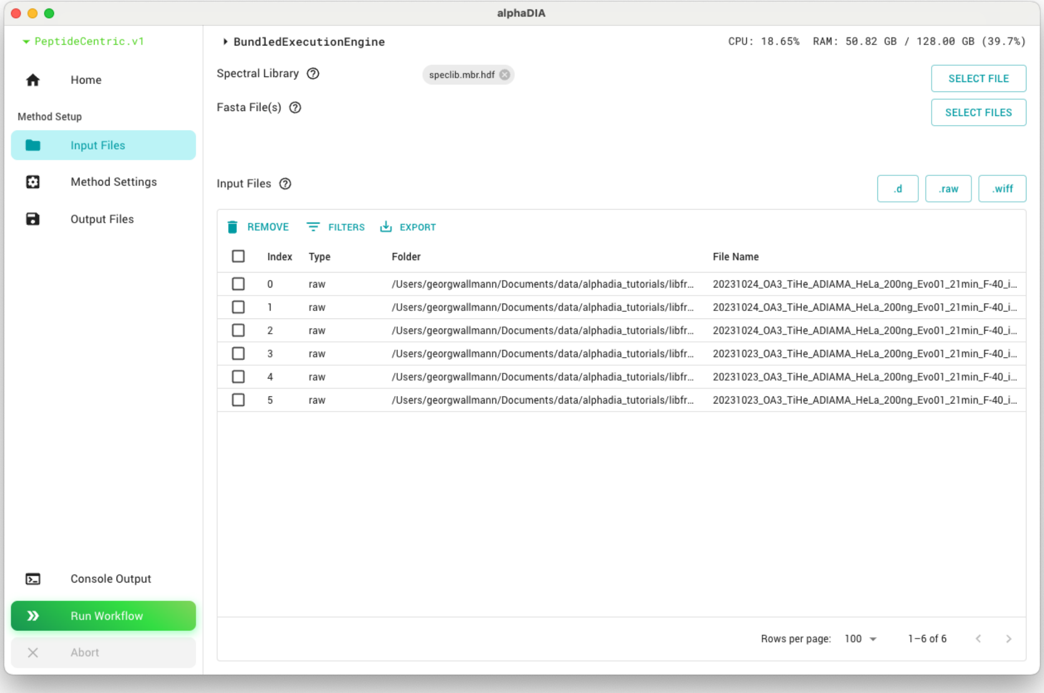
▾ Search output

▾ Transfer Library

Start the first search by clicking the “Run Workflow” button. This will take between one and two hours depending on your syst

4. Second search

For the second search, we will use the library generated in the first search to quantify precursors across samples. Load all raw files as previously but remove the FASTA file. Instead, select the `speclib.mbr.hdf` as the spectral library.



BundledExecutionEngine CPU: 18.65% RAM: 50.82 GB / 128.00 GB (39.7%)

Spectral Library: speclib.mbr.hdf

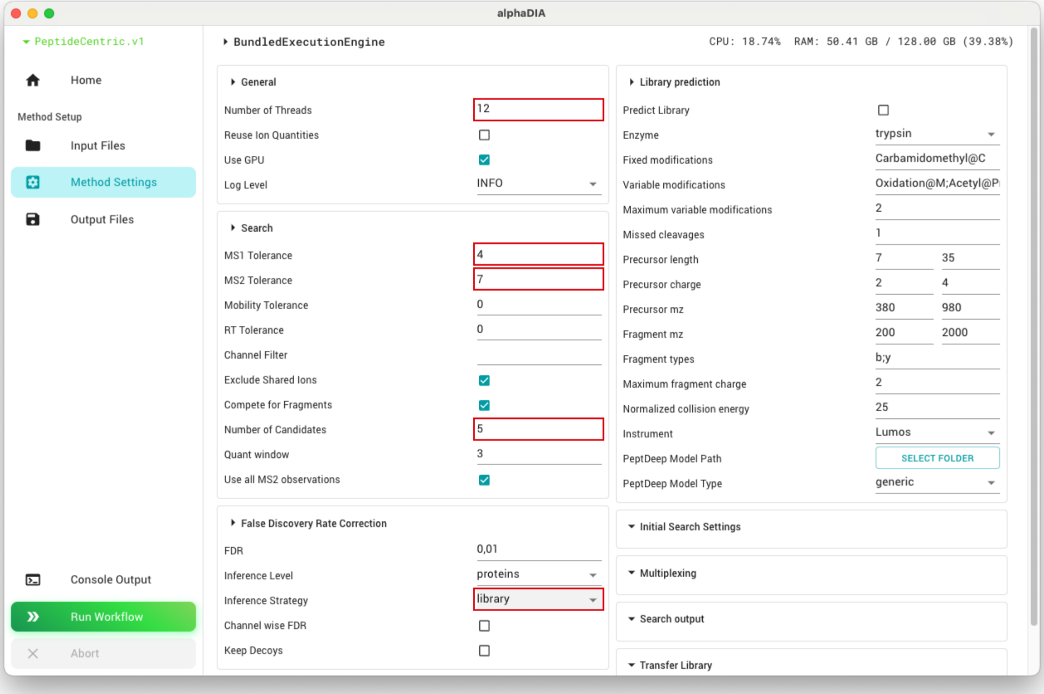
Fasta File(s)

Input Files

☐	Index	Type	Folder	File Name
☐	0	raw	/Users/georgwallmann/Documents/data/alphadia_tutorials/libfr...	20231024_OA3_TiHe_ADIAMA_HeLa_200ng_Evo01_21min_F-40_i...
☐	1	raw	/Users/georgwallmann/Documents/data/alphadia_tutorials/libfr...	20231024_OA3_TiHe_ADIAMA_HeLa_200ng_Evo01_21min_F-40_i...
☐	2	raw	/Users/georgwallmann/Documents/data/alphadia_tutorials/libfr...	20231024_OA3_TiHe_ADIAMA_HeLa_200ng_Evo01_21min_F-40_i...
☐	3	raw	/Users/georgwallmann/Documents/data/alphadia_tutorials/libfr...	20231023_OA3_TiHe_ADIAMA_HeLa_200ng_Evo01_21min_F-40_i...
☐	4	raw	/Users/georgwallmann/Documents/data/alphadia_tutorials/libfr...	20231023_OA3_TiHe_ADIAMA_HeLa_200ng_Evo01_21min_F-40_i...
☐	5	raw	/Users/georgwallmann/Documents/data/alphadia_tutorials/libfr...	20231023_OA3_TiHe_ADIAMA_HeLa_200ng_Evo01_21min_F-40_i...

Rows per page: 100 1–6 of 6

For the second search, configure the `thread_count`, `target_ms1_tolerance`, and `target_ms2_tolerance` as before. Do not activ library prediction and instead set the `inference_strategy` to `library` to reuse the protein grouping from the first search. In th second search, it can be beneficial to increase the number of peak groups `target_num_candidates` to `5`. Values larger than `5` most likely not have an effect, and we expect that future versions of alphaDIA will have an improved peak group selection mak this step unnecessary.



BundledExecutionEngine CPU: 18.74% RAM: 50.41 GB / 128.00 GB (39.38%)

General

Number of Threads	12
Reuse Ion Quantities	☐
Use GPU	☑
Log Level	INFO

Search

MS1 Tolerance	4
MS2 Tolerance	7
Mobility Tolerance	0
RT Tolerance	0
Channel Filter	
Exclude Shared Ions	☑
Compete for Fragments	☑
Number of Candidates	5
Quant window	3
Use all MS2 observations	☑

False Discovery Rate Correction

FDR	0,01
Inference Level	proteins
Inference Strategy	library
Channel wise FDR	☐
Keep Decoys	☐

Library prediction

Predict Library	☐	
Enzyme	trypsin	
Fixed modifications	Carbamidomethyl@C	
Variable modifications	Oxidation@M;Acetyl@P	
Maximum variable modifications	2	
Missed cleavages	1	
Precursor length	7	35
Precursor charge	2	4
Precursor mz	380	980
Fragment mz	200	2000
Fragment types	b;y	
Maximum fragment charge	2	
Normalized collision energy	25	
Instrument	Lumos	
PeptDeep Model Path		
PeptDeep Model Type	generic	

Initial Search Settings

Multiplexing

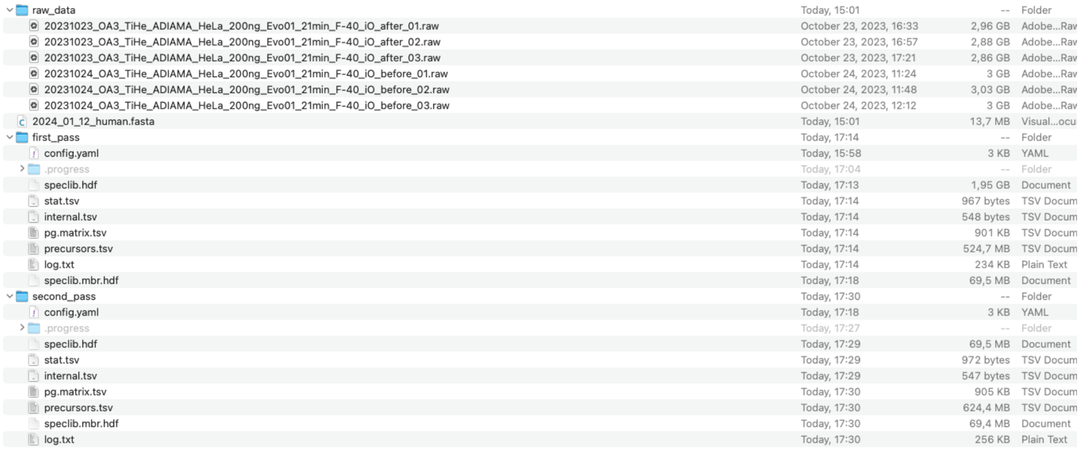
Search output

Transfer Library

Finally, start the search as before. This search will take only around 2 minutes per file.

5. Results

In the end, both folders should contain a full search output. Use the precursor-level file `precursors.tsv` or the protein matrix i `pg.matrix.tsv` for any downstream analysis.



raw_data	Today, 15:01	--	Folder
20231023_OA3_TiHe_ADIAMA_HeLa_200ng_Evo01_21min_F-40_iO_after_01.raw	October 23, 2023, 16:33	2,96 GB	Adobe...Ra
20231023_OA3_TiHe_ADIAMA_HeLa_200ng_Evo01_21min_F-40_iO_after_02.raw	October 23, 2023, 16:57	2,88 GB	Adobe...Ra
20231023_OA3_TiHe_ADIAMA_HeLa_200ng_Evo01_21min_F-40_iO_after_03.raw	October 23, 2023, 17:21	2,86 GB	Adobe...Ra
20231024_OA3_TiHe_ADIAMA_HeLa_200ng_Evo01_21min_F-40_iO_before_01.raw	October 24, 2023, 11:24	3 GB	Adobe...Ra
20231024_OA3_TiHe_ADIAMA_HeLa_200ng_Evo01_21min_F-40_iO_before_02.raw	October 24, 2023, 11:48	3,03 GB	Adobe...Ra
20231024_OA3_TiHe_ADIAMA_HeLa_200ng_Evo01_21min_F-40_iO_before_03.raw	October 24, 2023, 12:12	3 GB	Adobe...Ra
2024_01_12_human.fasta	Today, 15:01	13,7 MB	Visual...ocu
first_pass	Today, 17:14	--	Folder
config.yaml	Today, 15:58	3 KB	YAML
.progress	Today, 17:04	--	Folder
speclib.hdf	Today, 17:13	1,95 GB	Document
stat.tsv	Today, 17:14	967 bytes	TSV Docum
internal.tsv	Today, 17:14	548 bytes	TSV Docum
pg.matrix.tsv	Today, 17:14	901 KB	TSV Docum
precursors.tsv	Today, 17:14	524,7 MB	TSV Docum
log.txt	Today, 17:14	234 KB	Plain Text
speclib.mbr.hdf	Today, 17:18	69,5 MB	Document
second_pass	Today, 17:30	--	Folder
config.yaml	Today, 17:18	3 KB	YAML
.progress	Today, 17:27	--	Folder
speclib.hdf	Today, 17:29	69,5 MB	Document
stat.tsv	Today, 17:29	972 bytes	TSV Docum
internal.tsv	Today, 17:29	547 bytes	TSV Docum
pg.matrix.tsv	Today, 17:30	905 KB	TSV Docum
precursors.tsv	Today, 17:30	624,4 MB	TSV Docum
speclib.mbr.hdf	Today, 17:30	69,4 MB	Document
log.txt	Today, 17:30	256 KB	Plain Text

You can get a quick overview from the contents of the `stat.tsv` file. This two-step search strategy resulted in more than 115, precursors and 9,300 protein groups across the six files.

run	channel	precursors	proteins	ms1_accuracy	fw
20231024_OA3_TiHe_ADIAMA_HeLa_200ng_Evo01_21min_F-40_iO_before_03	0	118286	9356	0.597696	2.
20231024_OA3_TiHe_ADIAMA_HeLa_200ng_Evo01_21min_F-40_iO_before_02	0	120276	9362	0.594870	2.
20231024_OA3_TiHe_ADIAMA_HeLa_200ng_Evo01_21min_F-40_iO_before_01	0	119902	9355	0.596190	2.
20231023_OA3_TiHe_ADIAMA_HeLa_200ng_Evo01_21min_F-40_iO_after_03	0	118977	9352	0.589555	2.
20231023_OA3_TiHe_ADIAMA_HeLa_200ng_Evo01_21min_F-40_iO_after_02	0	116552	9359	0.590638	2.
20231023_OA3_TiHe_ADIAMA_HeLa_200ng_Evo01_21min_F-40_iO_after_01	0	120054	9355	0.583652	2.

Made with Sphinx and @pradyunsg's Furo