Searching plexDIA data with DIA-NN

Please see https://github.com/vdemichev/DiaNN
for more information about DIA-NN

Last updated December 8th, 2021

1. Download and install DIA-NN v1.8.1 beta7

DIA-NN v1.8.1 beta7 is available for download on the plexDIA website. A link to the download can be found in the “Download data” section with the hyperlinked “DIA-NN”.
https://plexdia.slavovlab.net

Or, a direct link is here:
https://drive.google.com/drive/u/1/folders/1p538GxhbZ7CllodVKVc0zFjRm_OmoQo1

2. Download plexDIA data

Data can be found on MassIVE (MSV000088302)

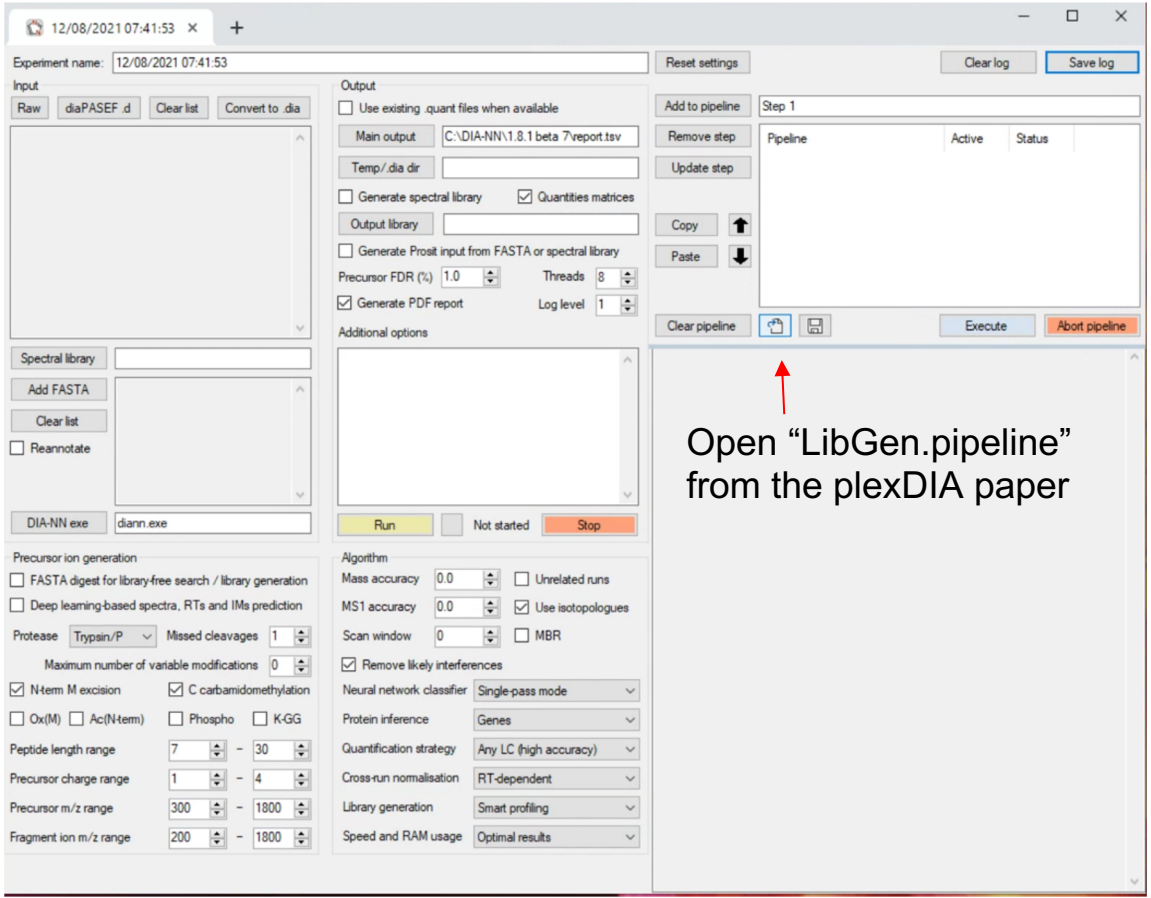
1. FASTA
2. Raw files
3. Spectral libraries (optional; will show how to generate these from FASTA next)
4. .pipeline files (this makes it easier to set up searches)

3. In-silico spectral library generation

In DIA-NN, it is possible to save search settings as a pipeline to make reproducibility easier.

Before, we search the raw data, we can generate the spectral libraries from the human, yeast, ecoli FASTA we provide at MSV000088302.

1. Open\load “LibGen.pipeline” which is available at MSV000088302



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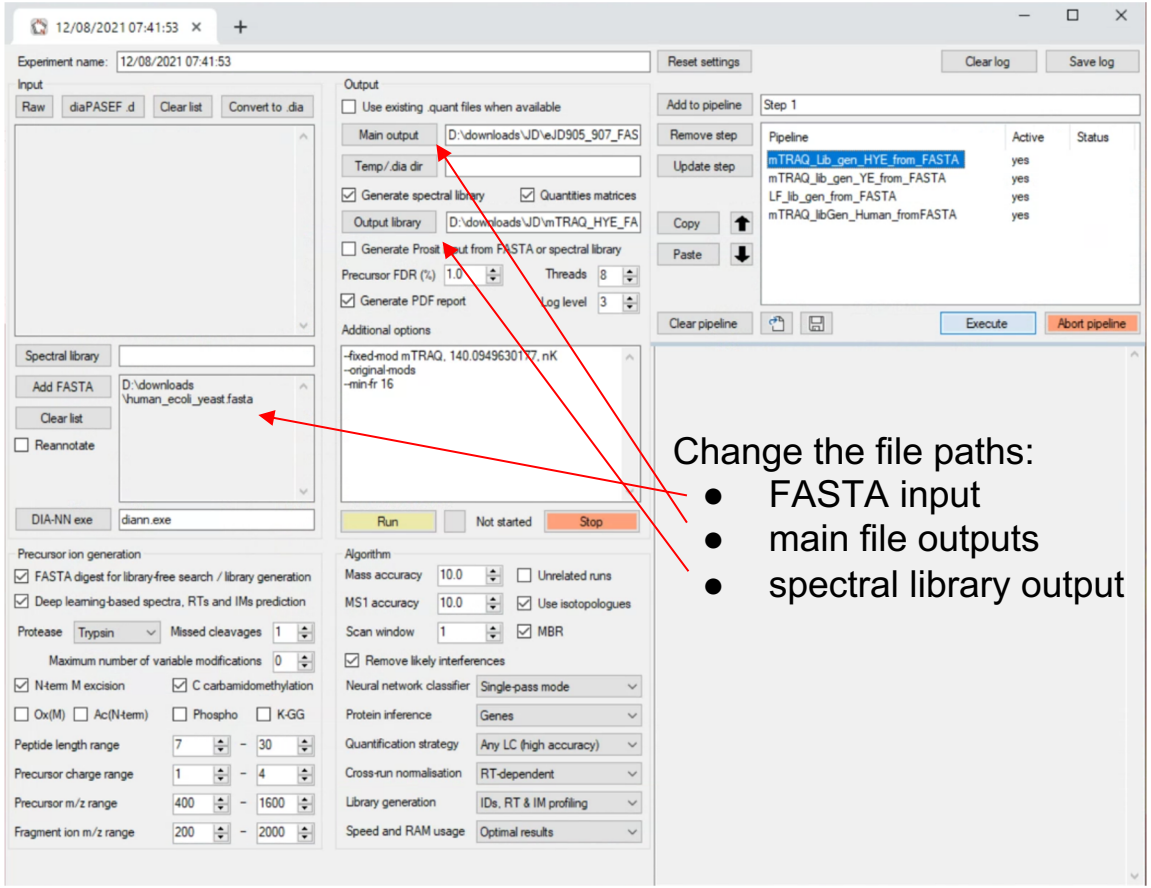
1. Open\load “LibGen.pipeline” which is available at MSV000088302
2. Change the input and output files paths



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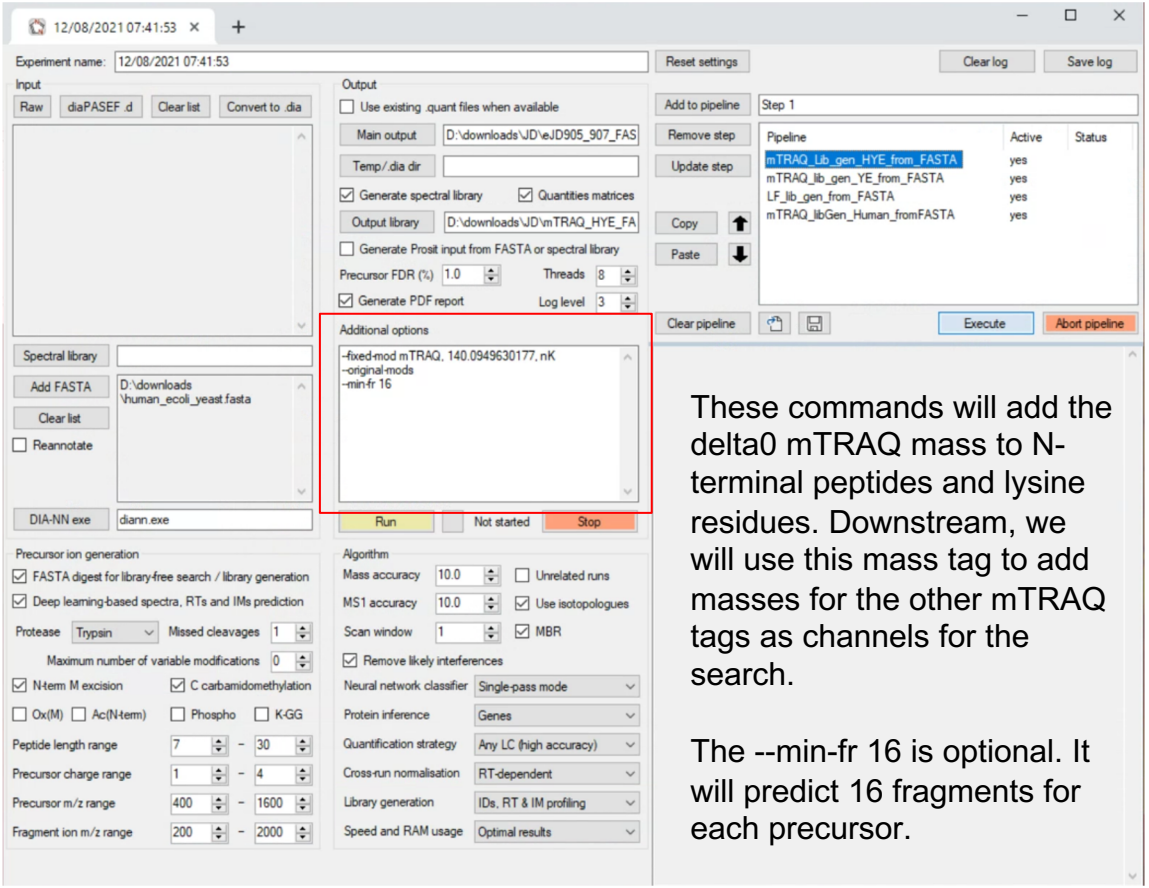
2. Change the input and output files paths
3. Check the commands



These commands will add the delta0 mTRAQ mass to N-terminal peptides and lysine residues. Downstream, we will use this mass tag to add masses for the other mTRAQ tags as channels for the search.

The --min-fr 16 is optional. It will predict 16 fragments for each precursor.

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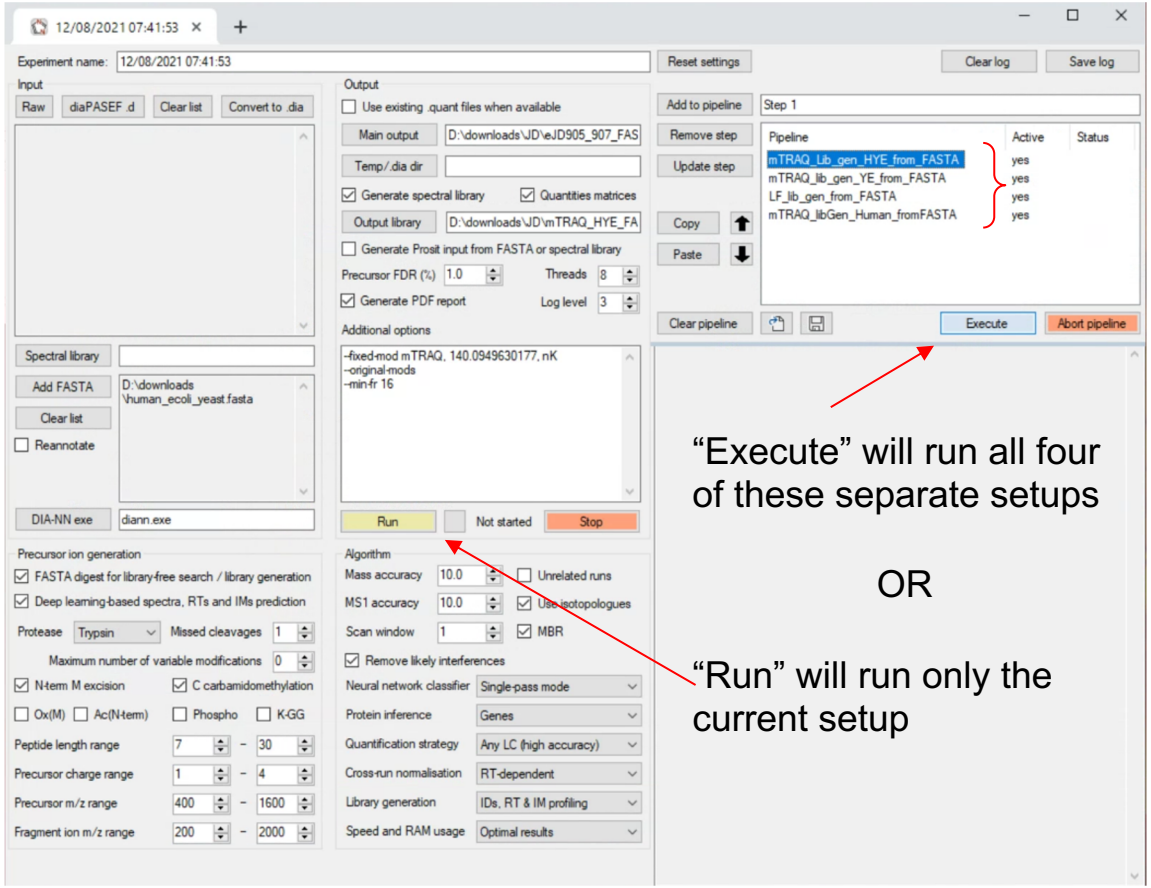
2. Change the input and output files paths
3. Check the commands
4. Click run to run the current setup, or click execute to run the entire pipeline.



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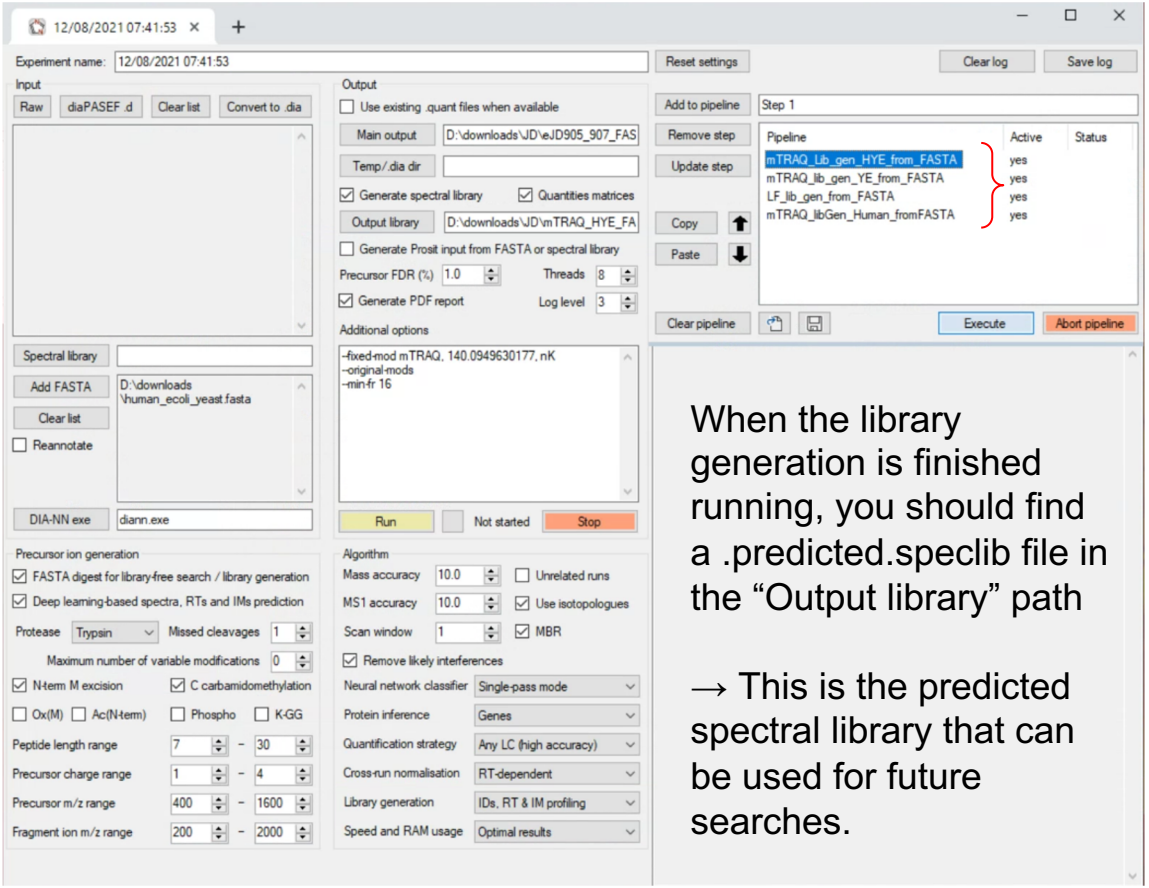

When the library generation is finished running, you should find a .predicted.speclib file in the “Output library” path

→ This is the predicted spectral library that can be used for future searches.

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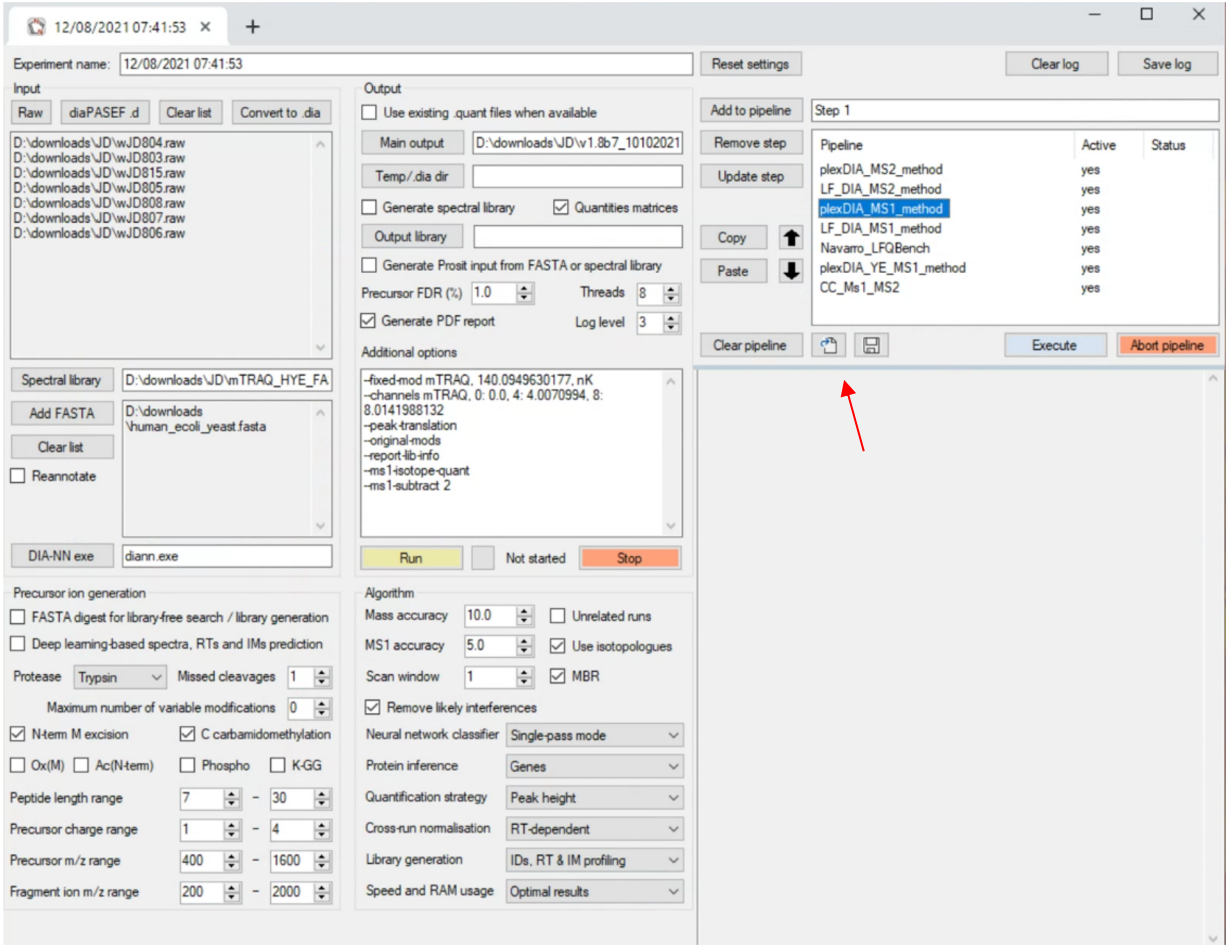
Before, we search the raw data, we can generate the spectral libraries from the human, yeast, ecoli FASTA we provide at MSV000088302.

1. Open\load “LibGen.pipeline” which is available at
2. Change the input and output files paths
3. Check the commands
4. Click run to run the current setup, or click execute to run the entire pipeline.

Note:
The predicted.speclib file can be converted to .tsv by loading the predicted spectral library, deleting any existing commands, and unchecking the two boxes in the “Precursor Generation” tab, then clicking “Run”.

4. Search raw plexDIA data

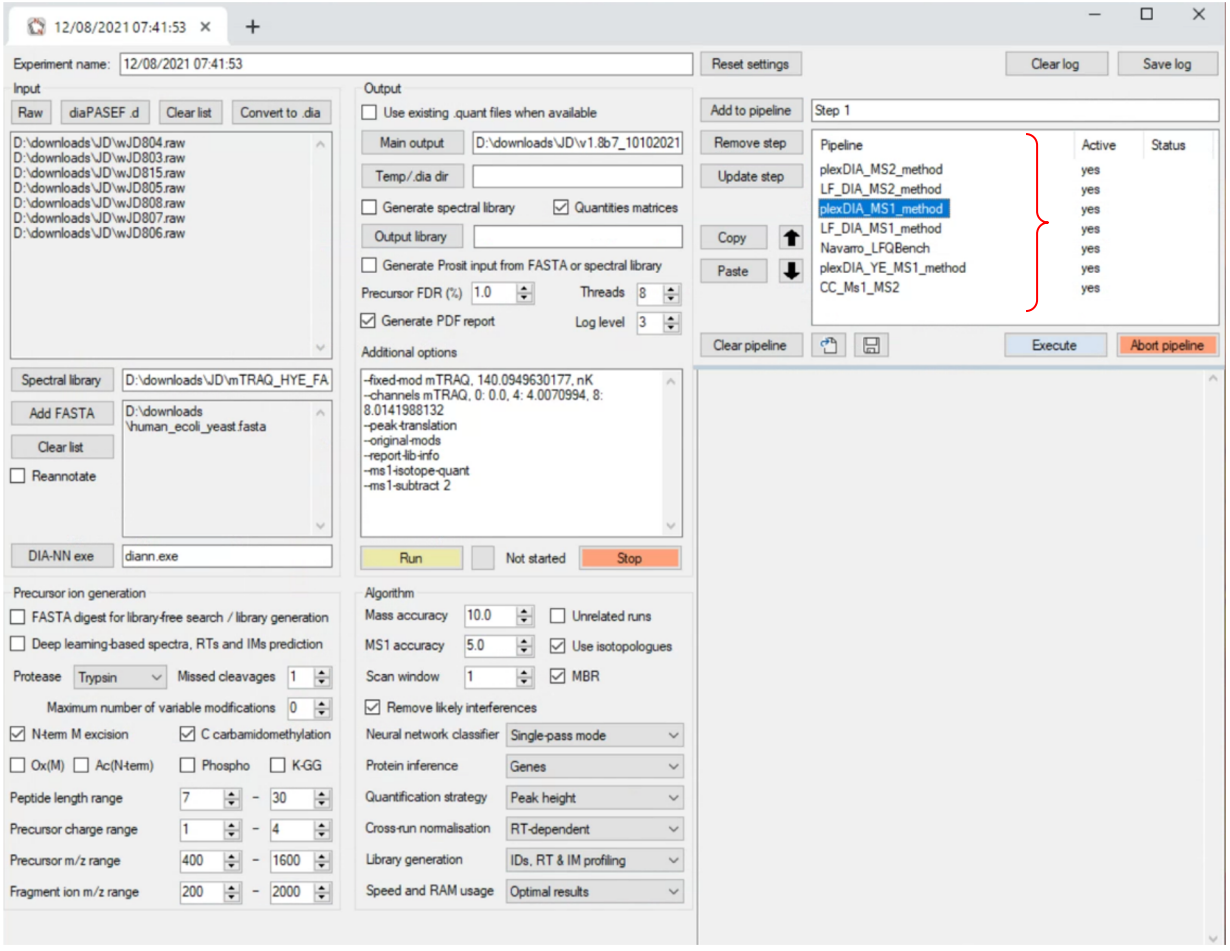
1. Open\load “Searches.pipeline”



4. Search raw plexDIA data

1. Open\load “Searches.pipeline”

1. Select an appropriate search-setup from the pipeline

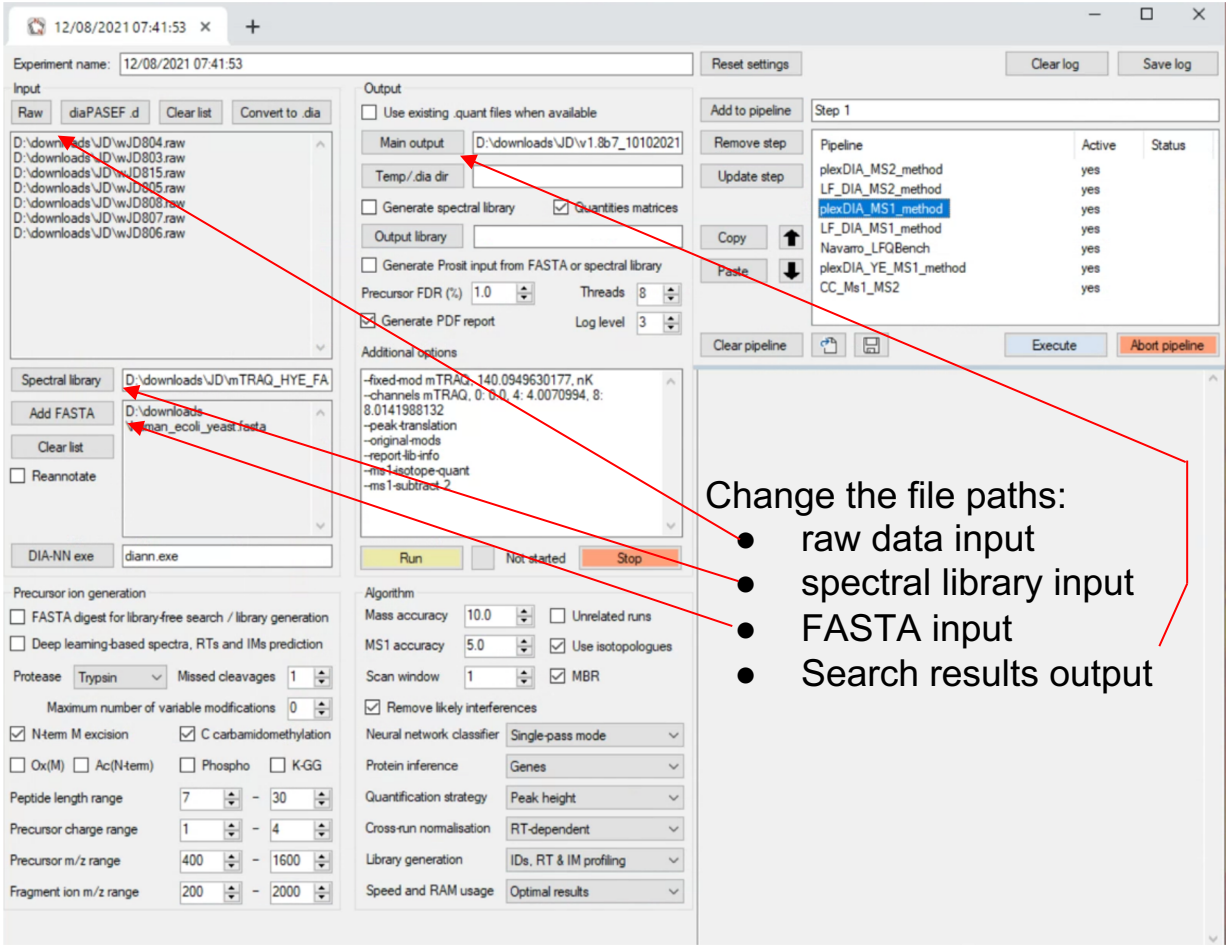


4. Search raw plexDIA data

1. Open\load “Searches.pipeline”

1. Select an appropriate search-setup from the pipeline

1. Change the file paths



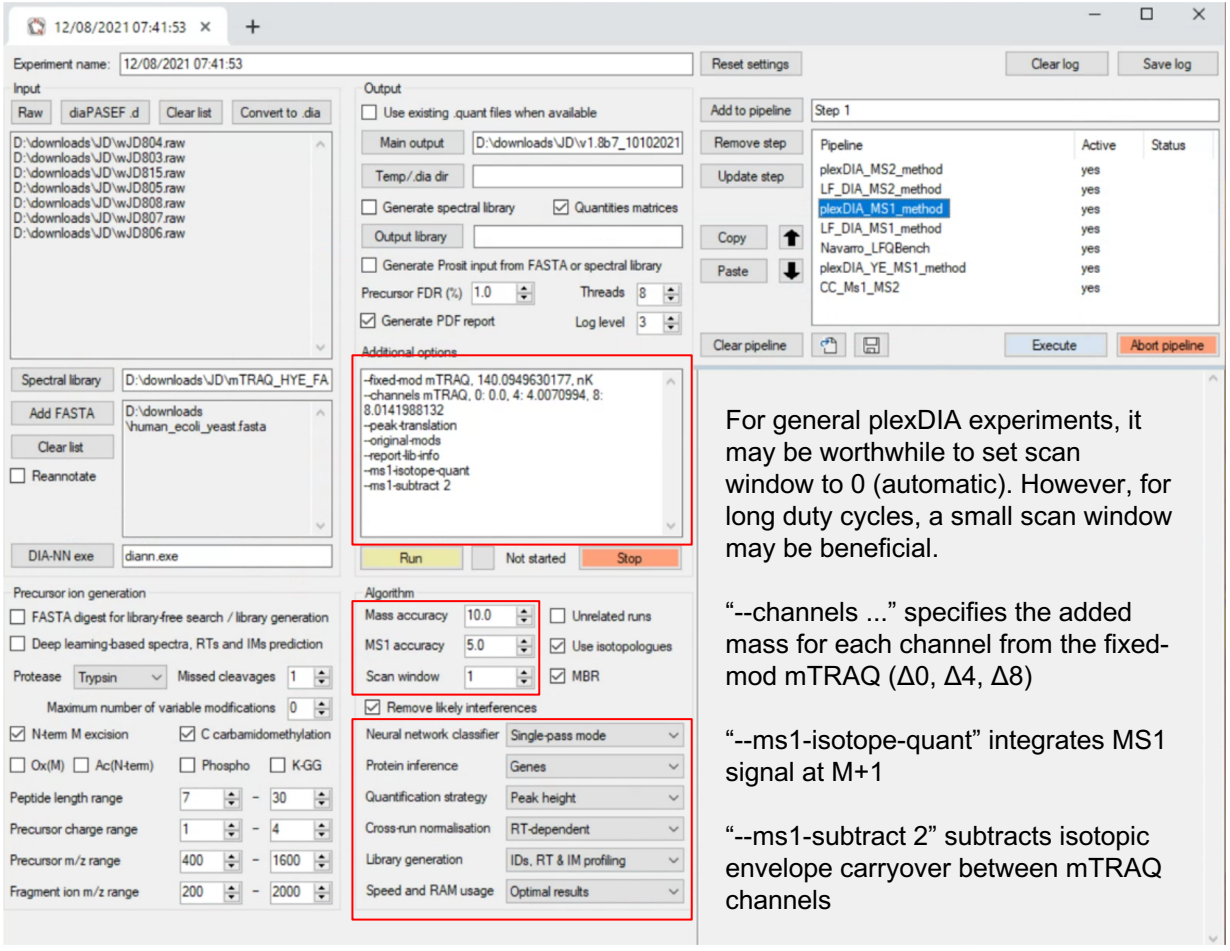
4. Search raw plexDIA data

1. Open\load “Searches.pipeline”

1. Select an appropriate search-setup from the pipeline

1. Change the file paths

1. Modify the commands and settings (optional)



For general plexDIA experiments, it may be worthwhile to set scan window to 0 (automatic). However, for long duty cycles, a small scan window may be beneficial.

“--channels ...” specifies the added mass for each channel from the fixed-mod mTRAQ (Δ0, Δ4, Δ8)

“--ms1-isotope-quant” integrates MS1 signal at M+1

“--ms1-subtract 2” subtracts isotopic envelope carryover between mTRAQ channels

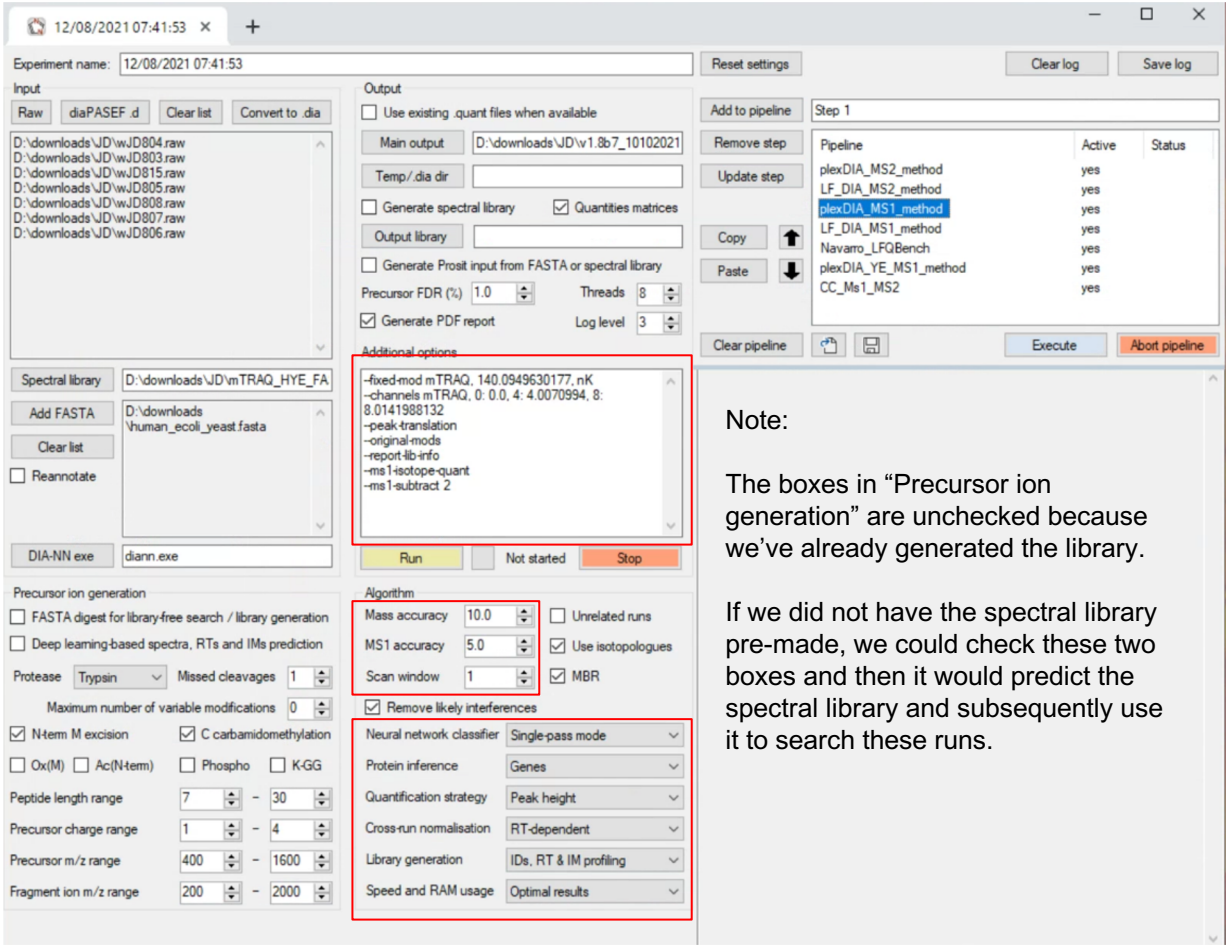
4. Search raw plexDIA data

1. Open\load “Searches.pipeline”

1. Select an appropriate search-setup from the pipeline

1. Change the file paths

1. Modify the commands and settings (optional)



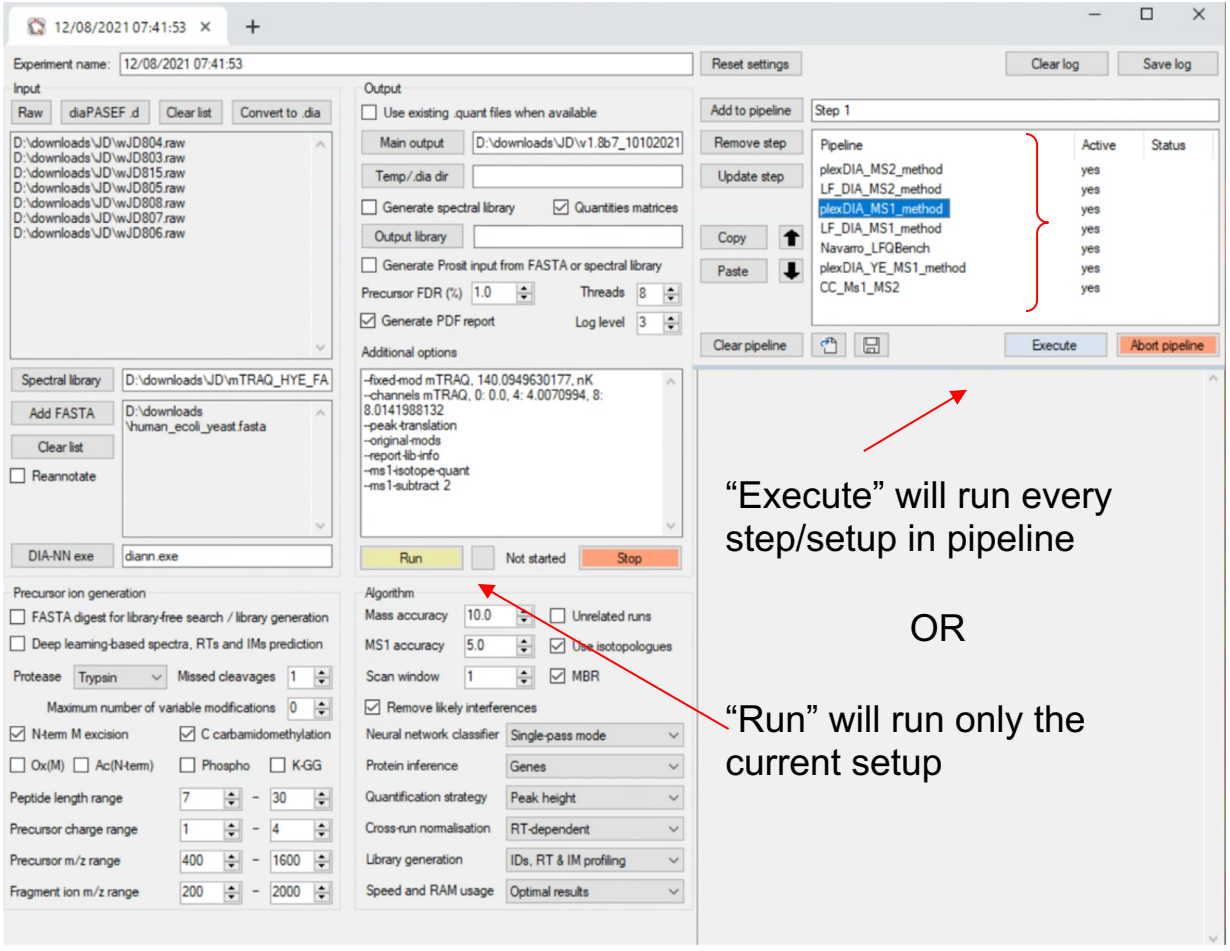
Note:

The boxes in “Precursor ion generation” are unchecked because we’ve already generated the library.

If we did not have the spectral library pre-made, we could check these two boxes and then it would predict the spectral library and subsequently use it to search these runs.

4. Search raw plexDIA data

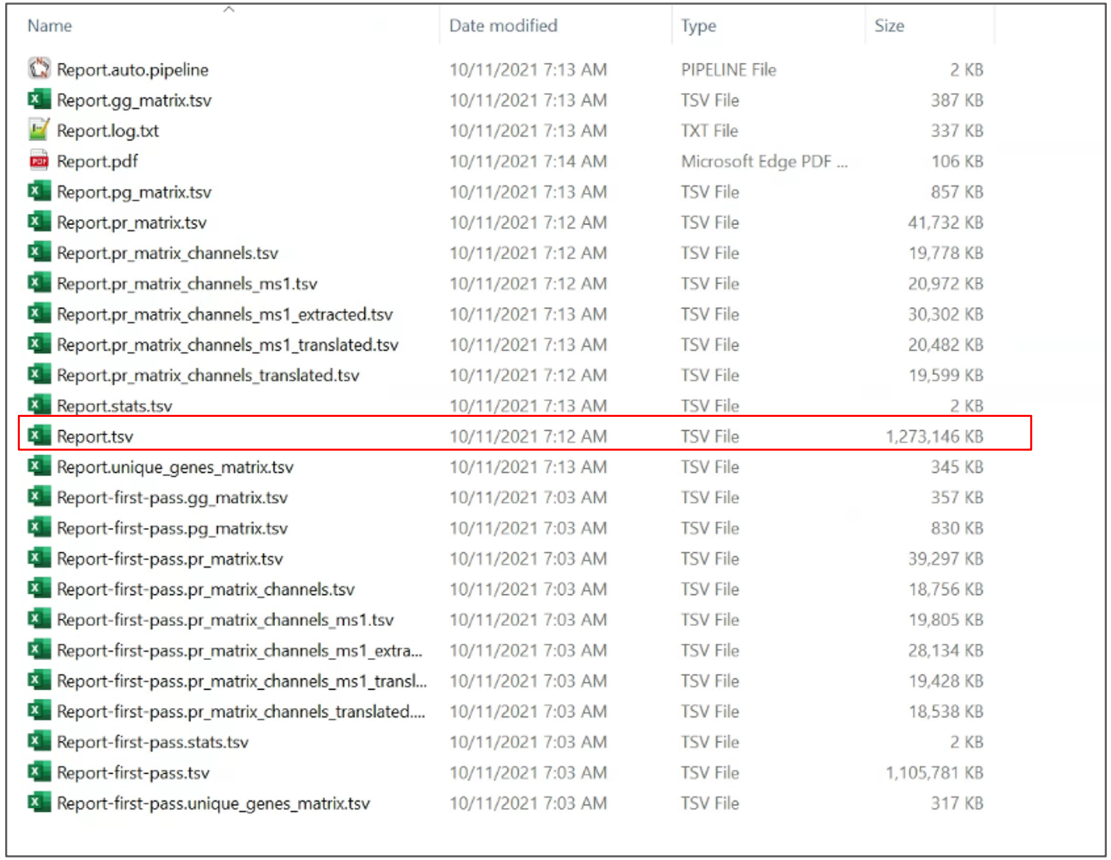
1. Open\load “Searches.pipeline”
1. Select an appropriate search-setup from the pipeline
1. Change the file paths
1. Modify the commands and settings (optional)
1. Click run to run the current setup, or click execute to run the entire pipeline.



5. DIA-NN search outputs

- When the runs finish searching, the output folder should hold most of the following data
- The main .tsv file will have all the required information for analysis.
- For more information about the column outputs, please refer to: https://github.com/vdemichev/DiaNN#main-output-reference

Note: “First-pass” refers to run-specific results (before MBR).



Name	Date modified	Type	Size
Report.auto.pipeline	10/11/2021 7:13 AM	PIPELINE File	2 KB
Report.gg_matrix.tsv	10/11/2021 7:13 AM	TSV File	387 KB
Report.log.txt	10/11/2021 7:13 AM	TXT File	337 KB
Report.pdf	10/11/2021 7:14 AM	Microsoft Edge PDF ...	106 KB
Report.pg_matrix.tsv	10/11/2021 7:13 AM	TSV File	857 KB
Report.pr_matrix.tsv	10/11/2021 7:12 AM	TSV File	41,732 KB
Report.pr_matrix_channels.tsv	10/11/2021 7:12 AM	TSV File	19,778 KB
Report.pr_matrix_channels_ms1.tsv	10/11/2021 7:12 AM	TSV File	20,972 KB
Report.pr_matrix_channels_ms1_extracted.tsv	10/11/2021 7:13 AM	TSV File	30,302 KB
Report.pr_matrix_channels_ms1_translated.tsv	10/11/2021 7:13 AM	TSV File	20,482 KB
Report.pr_matrix_channels_translated.tsv	10/11/2021 7:12 AM	TSV File	19,599 KB
Report.stats.tsv	10/11/2021 7:13 AM	TSV File	2 KB
Report.tsv	10/11/2021 7:12 AM	TSV File	1,273,146 KB
Report.unique_genes_matrix.tsv	10/11/2021 7:13 AM	TSV File	345 KB
Report-first-pass.gg_matrix.tsv	10/11/2021 7:03 AM	TSV File	357 KB
Report-first-pass.pg_matrix.tsv	10/11/2021 7:03 AM	TSV File	830 KB
Report-first-pass.pr_matrix.tsv	10/11/2021 7:03 AM	TSV File	39,297 KB
Report-first-pass.pr_matrix_channels.tsv	10/11/2021 7:03 AM	TSV File	18,756 KB
Report-first-pass.pr_matrix_channels_ms1.tsv	10/11/2021 7:03 AM	TSV File	19,805 KB
Report-first-pass.pr_matrix_channels_ms1_extra...	10/11/2021 7:03 AM	TSV File	28,134 KB
Report-first-pass.pr_matrix_channels_ms1_transl...	10/11/2021 7:03 AM	TSV File	19,428 KB
Report-first-pass.pr_matrix_channels_translated....	10/11/2021 7:03 AM	TSV File	18,538 KB
Report-first-pass.stats.tsv	10/11/2021 7:03 AM	TSV File	2 KB
Report-first-pass.tsv	10/11/2021 7:03 AM	TSV File	1,105,781 KB
Report-first-pass.unique_genes_matrix.tsv	10/11/2021 7:03 AM	TSV File	317 KB

Questions? Please reach out to:

Derks J, Leduc A, Huffman RG, Specht H, Ralser M, Demichev V, Slavov N. (2021) **Increasing the throughput of sensitive proteomics by plexDIA** bioRxiv 2021.11.03.467007; doi: https://doi.org/10.1101/2021.11.03.467007
https://plexdia.slavovlab.net

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