Metabolomics: Advancing Technology for Disease Diagnosis and Therapeutics

Laboratory for Mass Spectrometry and Metabolomics
质谱与代谢组学实验室 朱正江研究员课题组

zhu lab

Metabolic Reaction Network-based Metabolite Annotation for Untargeted Metabolomics: from Knowns to Unknowns

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Early-Career Members Network, Metabolomics Society (29 March, 2022)

Outline

1. **MetDNA**: metabolic reaction network-based metabolite annotation
2. **MetDNA2**: metabolite annotation from knowns to unknowns
3. The step-by-step instruction of MetDNA2 webserver

Step-by-step instruction

Use of MetDNA2

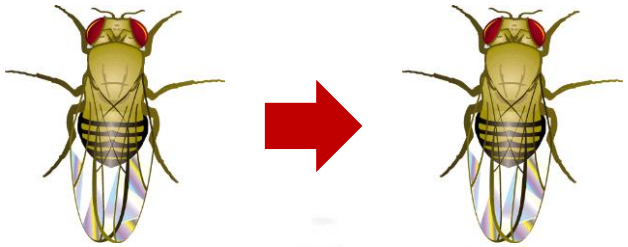
Website:
http://metdna.zhulab.cn/

Tutorial (English/Chinese):
http://metdna.zhulab.cn/metdna/help

The MetDNA2 has been released on Jul. 19, 2021.

Demo data

Study design



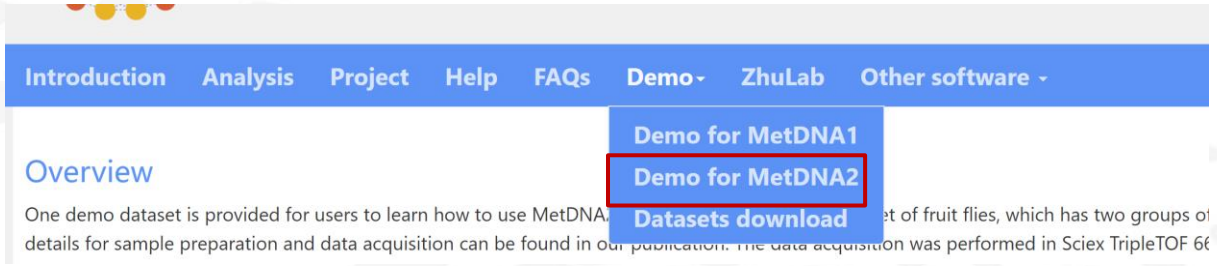
Day03 Day30

- **Group “W03”**: Fruit fly (3-days, n=10)
- **Group “W30”**: Fruit fly (30-days, n=10)

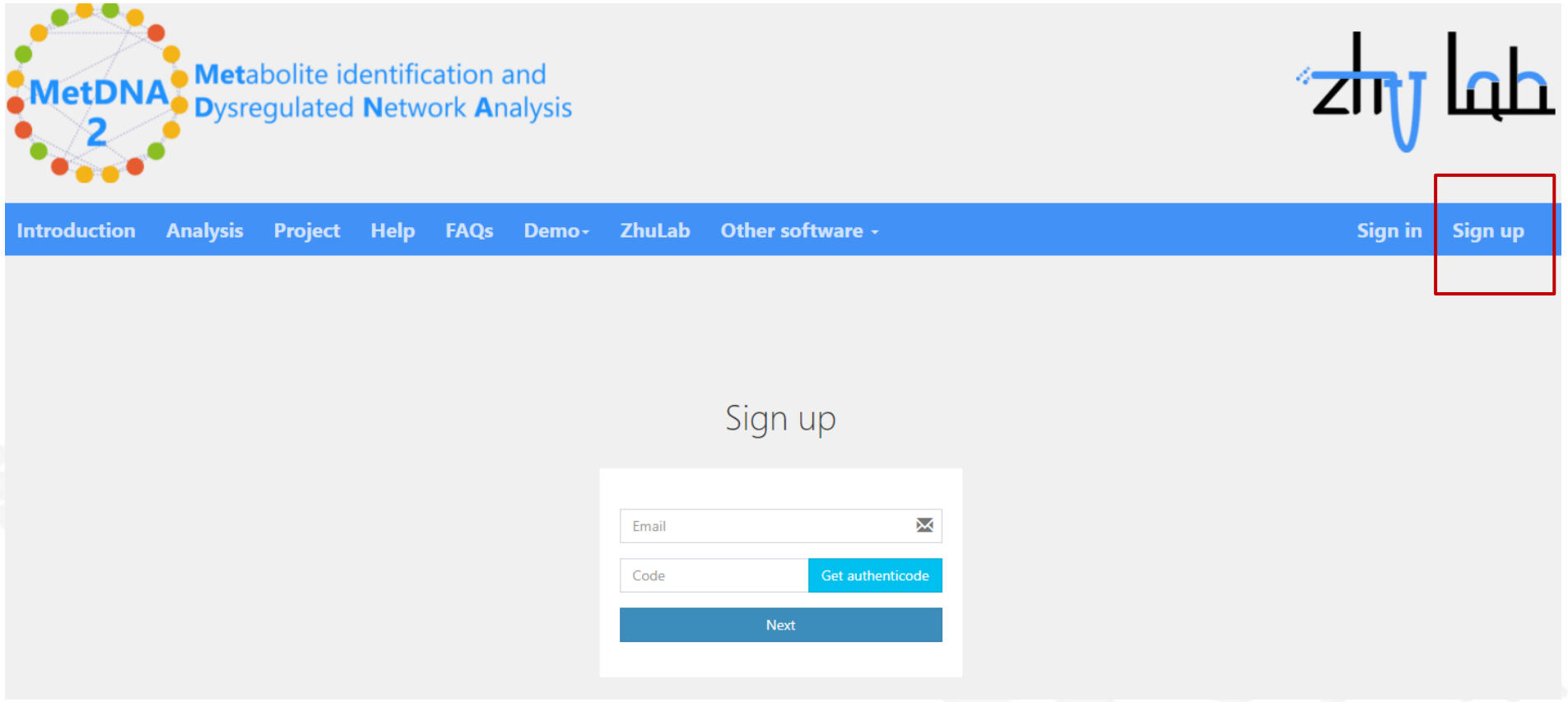
Shen et al., *Nat. Comm.*, **2019**, 10: 1516

Metabolomics data:

- **Instrument:** Sciex TripleTOF 6600
- **Column:** Waters BEH Amide column (1.7 μm; 100 mm× 2.1 mm)
- **Data preprocessing**: xcms (version 1.46.0)
- **Download:**

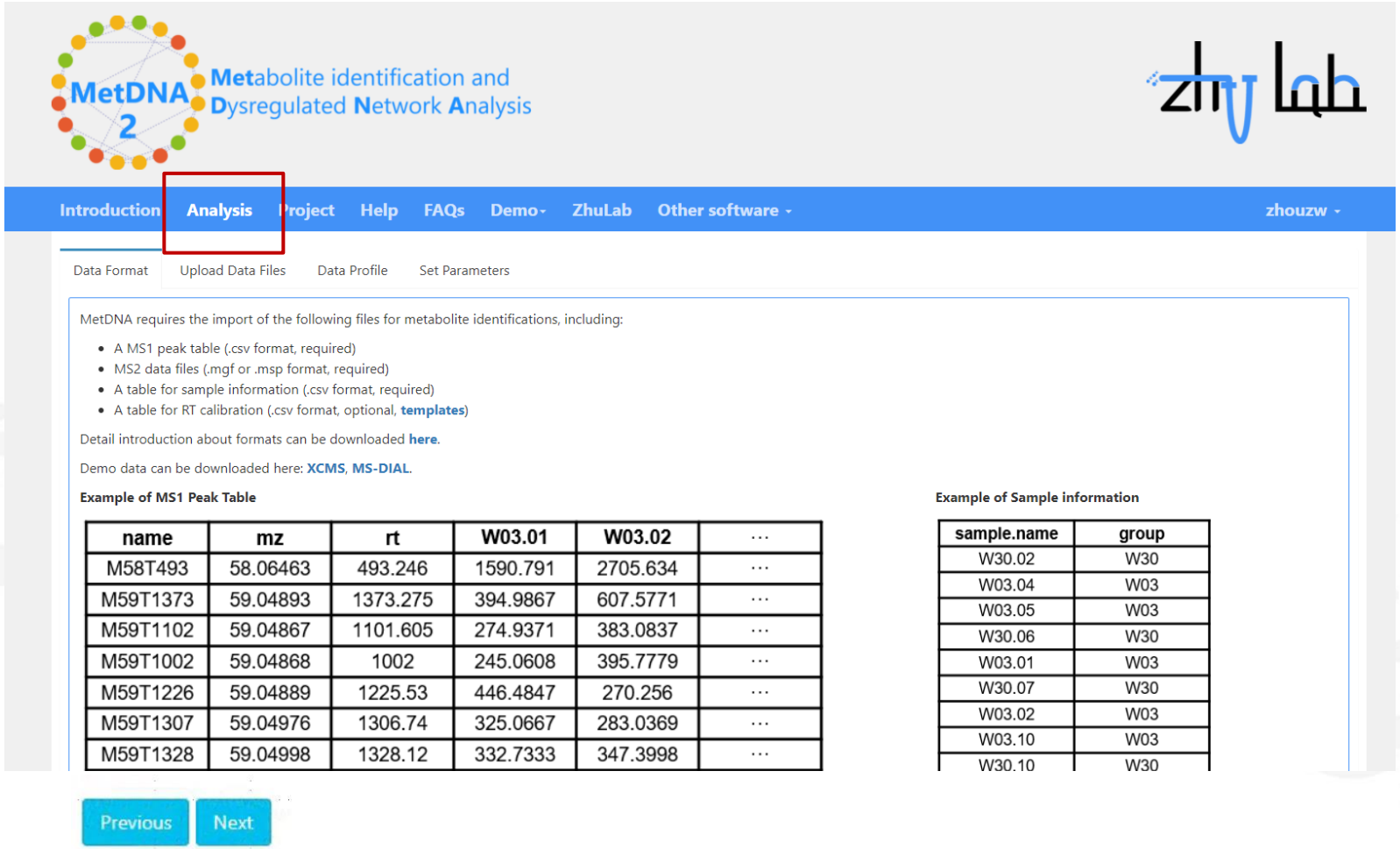


Step 1: register and login



Step 2: data preparing

Data Format → Upload Data File → Data Profile → Set Parameter



Required files:

- A MS1 peak table
- MS2 data files
- A table for sample information
- A table for RT calibration (Optional)

Step 2: data preparing (data format)

Data Format → Upload Data File → Data Profile → Set Parameter

MS1 peak table

- Exported from common peak-picking tools such as XCMS, MZmine, and MS-DIAL

name	mz	rt	W03.01	W03.02	…
M58T493	58.06463	493.246	1590.791	2705.634	…
M59T1373	59.04893	1373.275	394.9867	607.5771	…
M59T1328	59.04998	1328.12	332.7333	347.3998	…
M59T1048	59.04985	1047.56	333.8955	2608.083	…
M59T1188	59.04931	1187.76	149.056	312.12	…
M59T981	59.05934	980.529	1025.758	2449.929	…
M59T1187	59.06076	1187.12	1196.694	477	…
M60T1238	60.04342	1238.085	2475.192	1870.353	…
M60T1189	60.04467	1188.83	1425.861	1540.744	…
…	…	…	…	…	…

- First 3 columns must be "name" , "mz" , and "rt“
- A csv file with comma separator

MS2 data files

- MS2 files in **.mgf** or **.msp** format are acceptable
- **Recommend**: upload MS2 files of QC samples instead of all MS2 files

Sample information

- “sample.name” column must be exactly same

sample.name	group
W30.02	W30
W03.04	W03
W03.05	W03
W30.06	W30
W03.01	W03
W30.07	W30
W03.02	W03
…	…

- First 2 columns must be “sample.name”, and “group”

Step 2: data preparing (data format)

Data Format → Upload Data File → Data Profile → Set Parameter

RT recalibration table

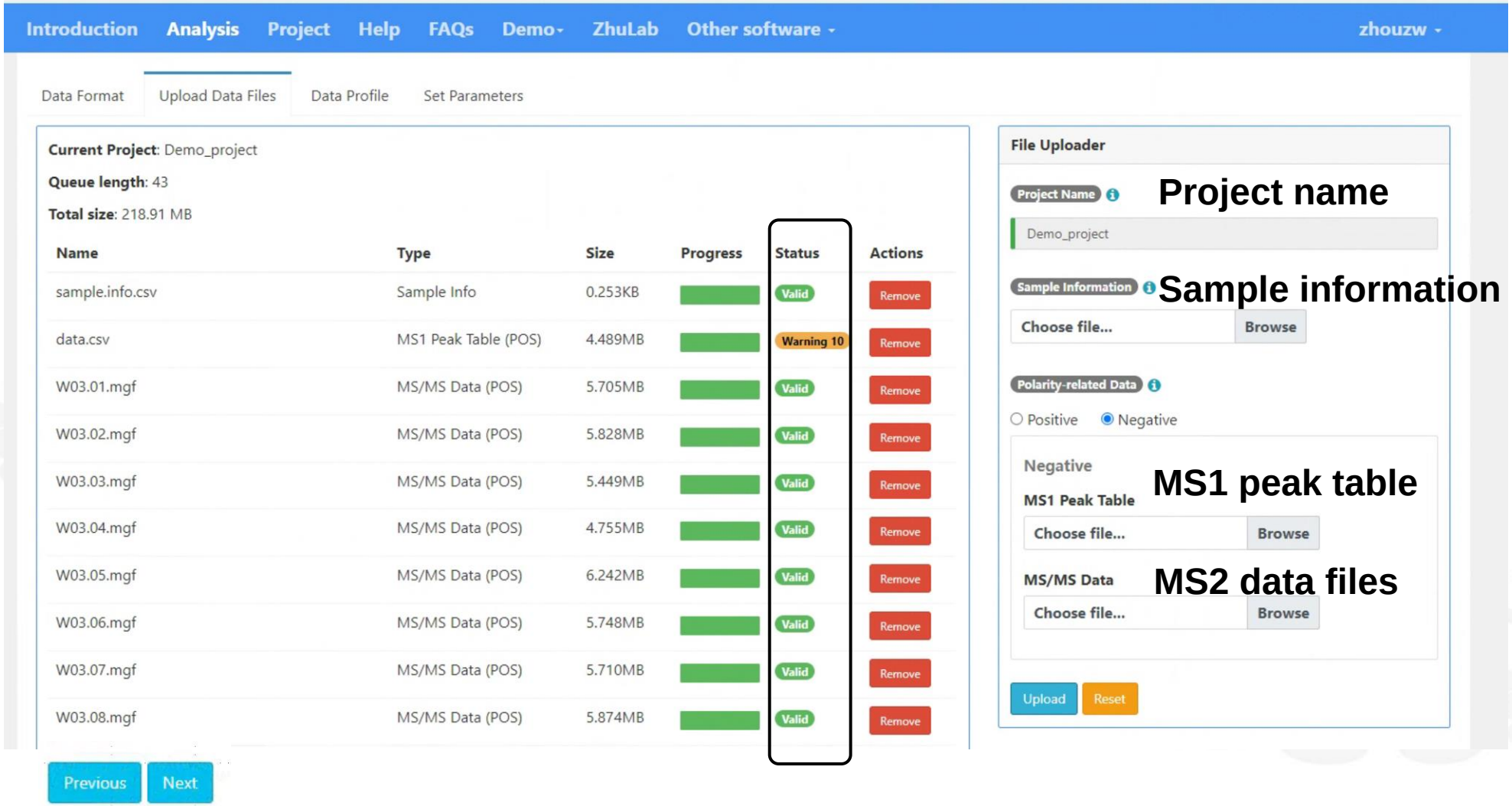
- A csv file: **a set of RT values of reference compounds**
- It is **optional file** to obtain level 1 metabolite annotation (required by RT library match)
- If you want to use ZhuLab RT library, please follow the **EXACTLY SAME** LC condition and gradient.

Example: reference RT table for ZhuLab_Amide23min system

compound.name	id.zhulab	id.pubchem	ref.mz	rt	polarity
4-Pyridoxic acid	L0127	6723	184.0605	0.961	positive
3'-O-methyladenosine	L0251	82530	282.1197	3.026	positive
Creatinine	S0042	588	114.0662	4.768	positive
Nicotinate	S0008	938	124.0393	6.232	positive
Guanosine	S0071	135398635	284.099	7.775	positive
N-Acetylcadaverine	S0249	189087	145.1336	9.54	positive
Beta-Alanine	L0320	239	90.055	10.484	positive
dAMP	L0140	12599	332.0755	13.179	positive
gamma-L-Glutamyl-L-glutamic acid	L0352	92865	277.1031	14.074	positive
N6,N6,N6-Trimethyl-L-lysine	L0190	440120	189.1598	17	positive

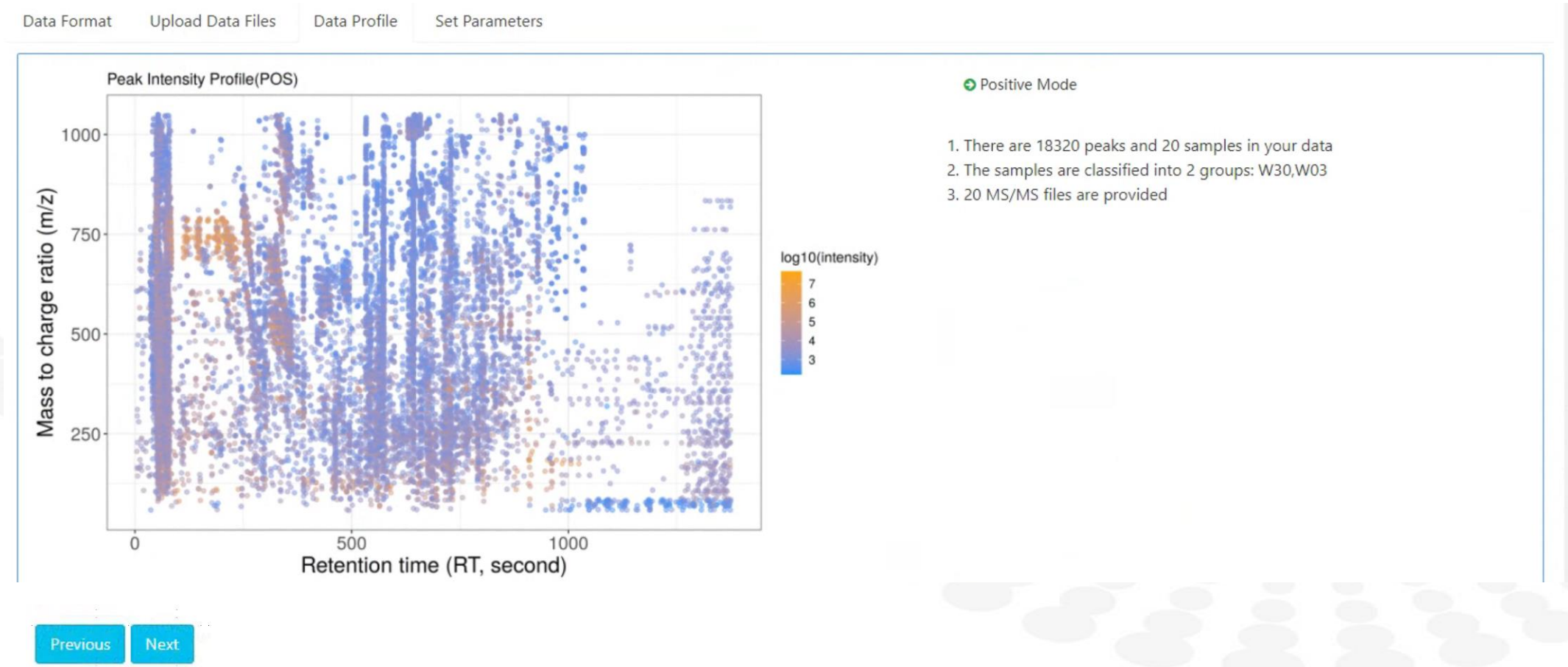
Step 3: data uploading

Data Format → **Upload Data File** → Data Profile → Set Parameter



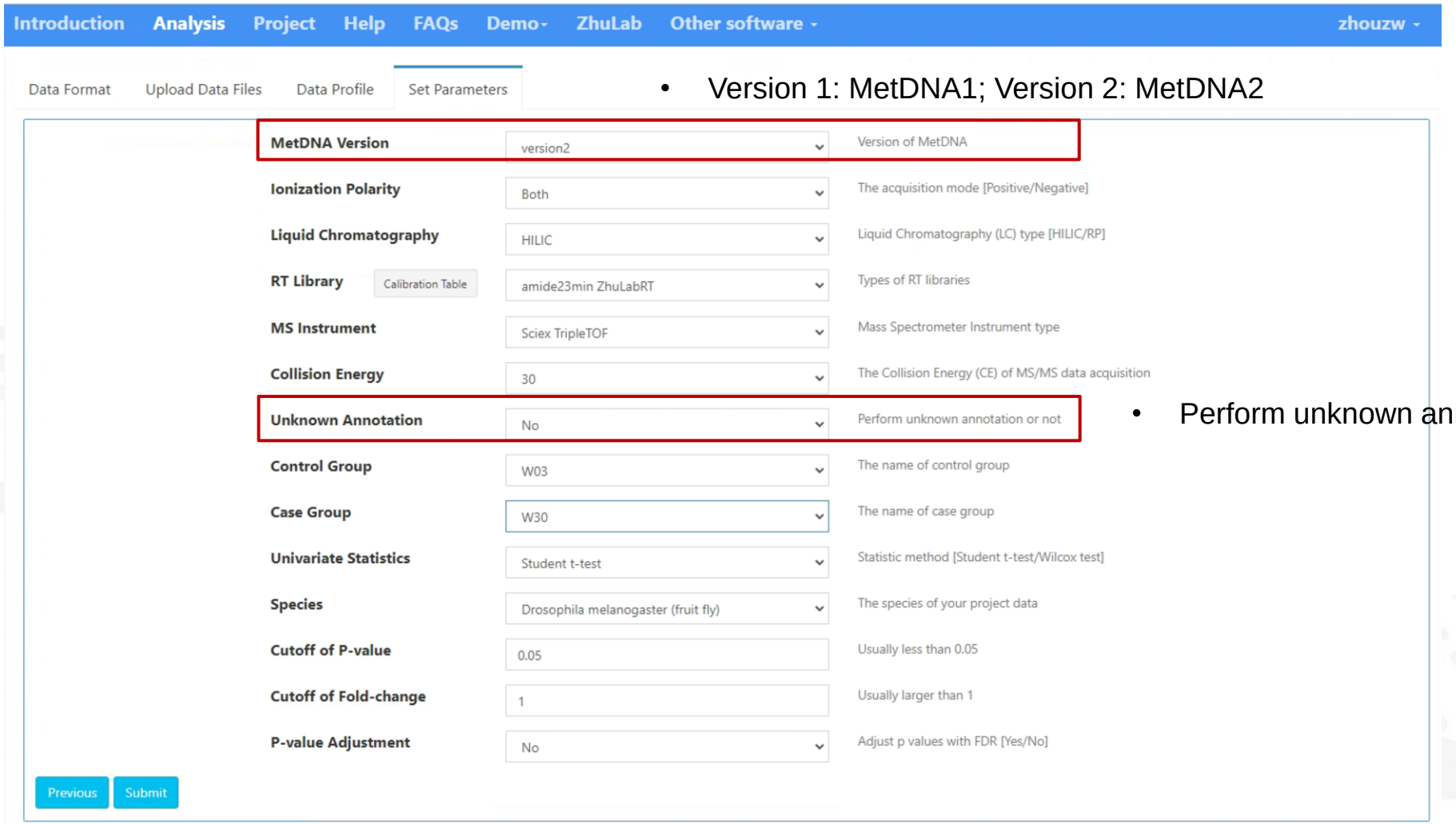
Step 3: data uploading

Data Format → Upload Data File → **Data Profile** → Set Parameter



Step 4: parameter setting

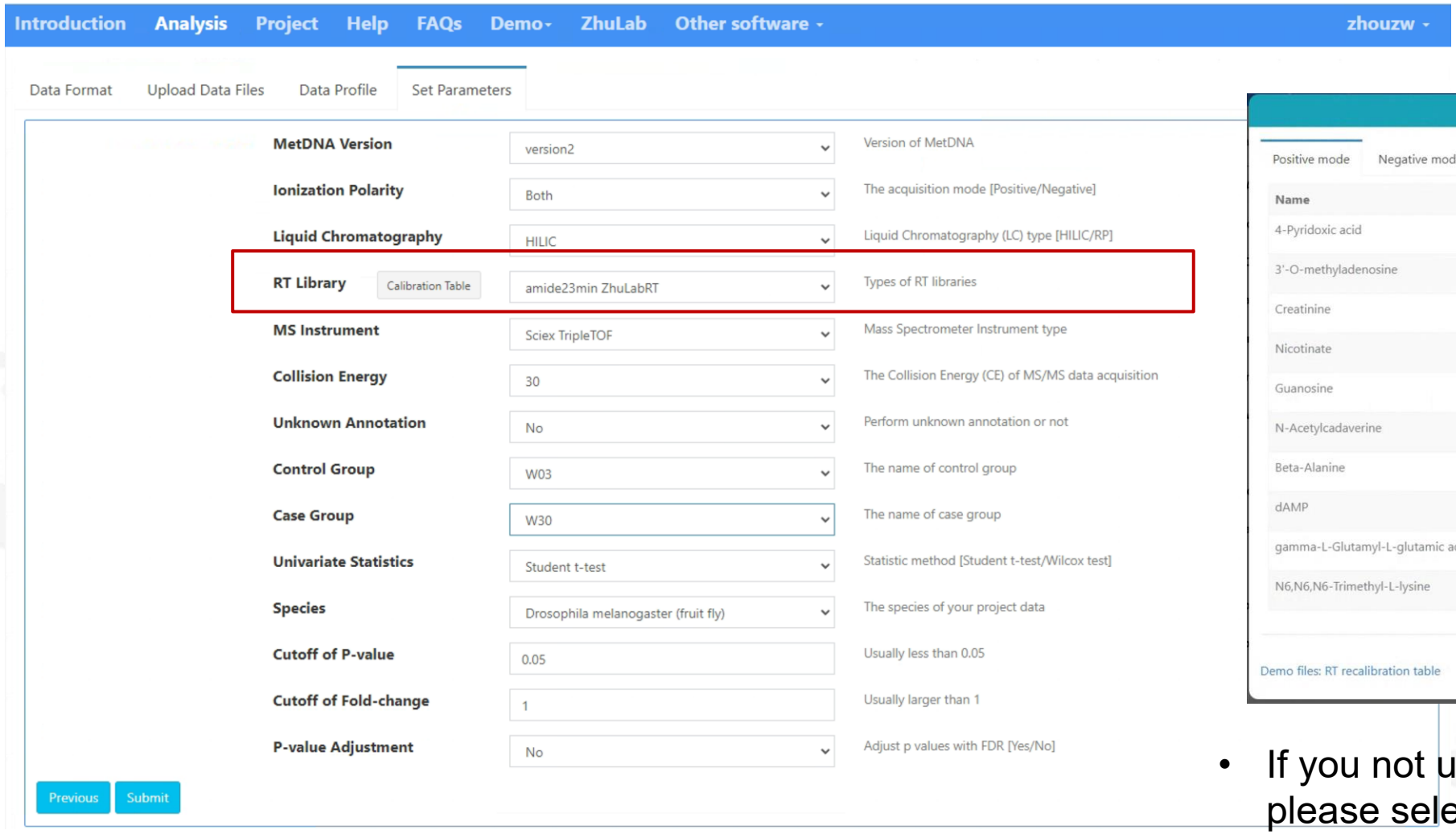
Data Format → Upload Data File → Data Profile → **Set Parameter**

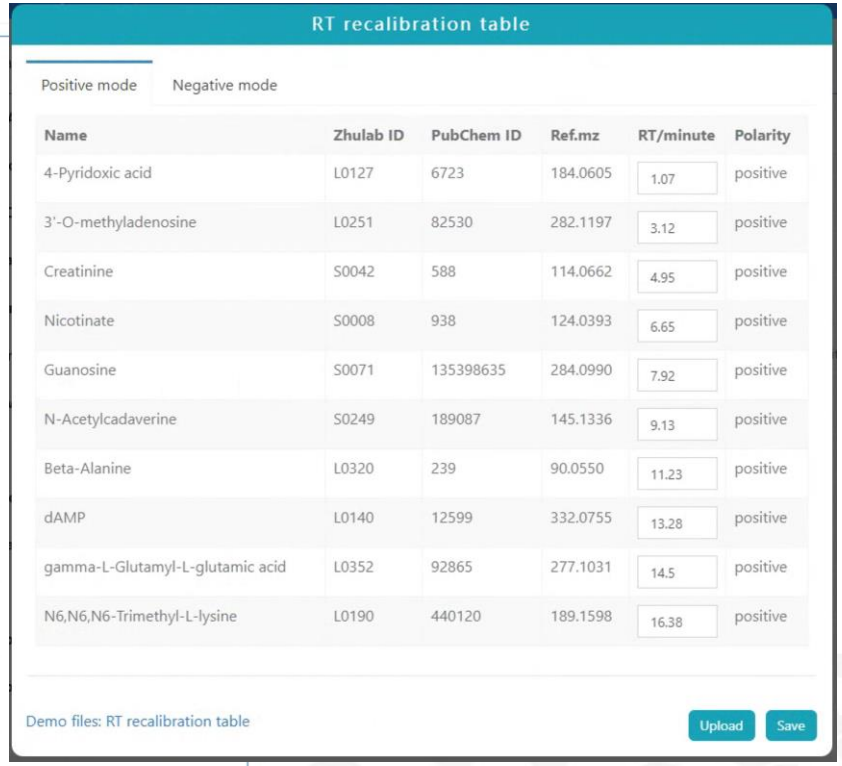


- Version 1: MetDNA1; Version 2: MetDNA2
- Perform unknown annotation or not

Step 4: parameter setting

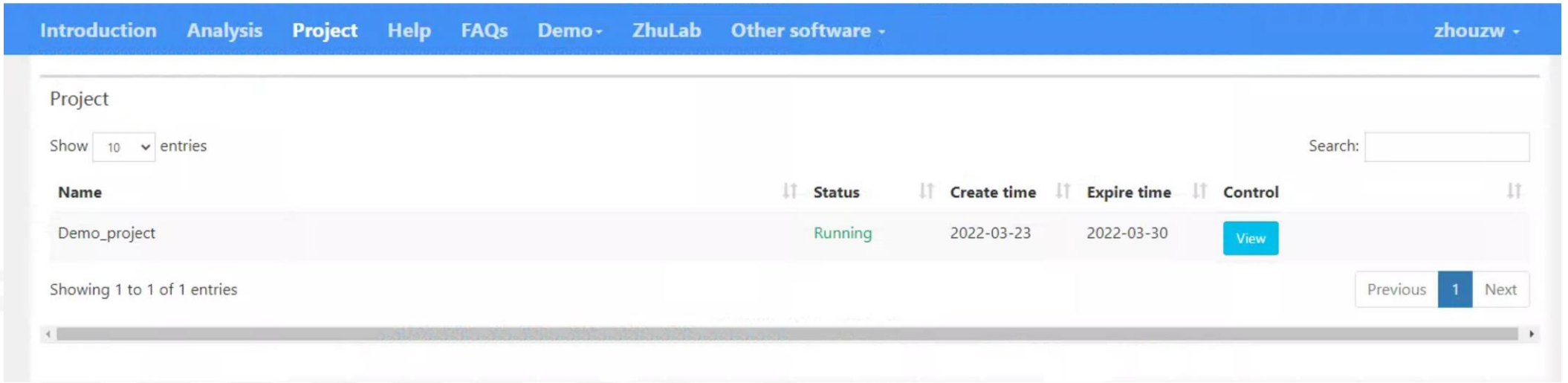
Data Format → Upload Data File → Data Profile → **Set Parameter**





- If you not use the “Zhulab RT system”, please select “No”

Step 5: data analysis



- It usually takes ~1-5 h/project depending on the number of features

Step 6: Result download and interpretation

Demo_project Success

MetDNA <metdna@sioc.ac.cn>
收件人 zhouzw@sioc.ac.cn
8:32

Dear MetDNA user,
Thank you for using MetDNA!
Your project Demo_project has been finished and the analysis results can be downloaded within 7 days by loging in your account.
The detailed description of the analysis results can be found here.
If you have any questions, please see the FAQs of MetDNA, or send email to us (metdna@sioc.ac.cn).

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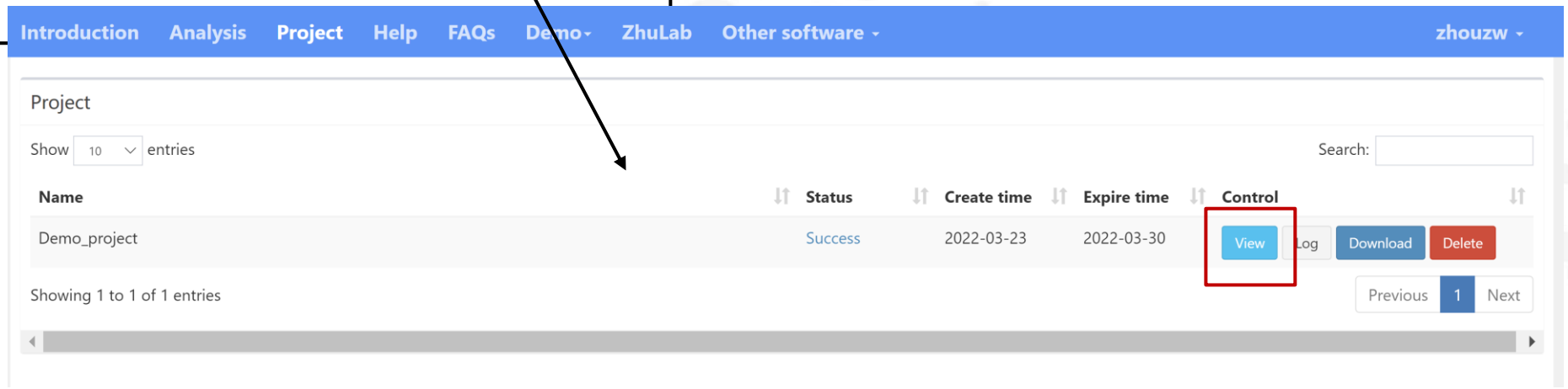
Chinese Academy of Sciences (CAS)

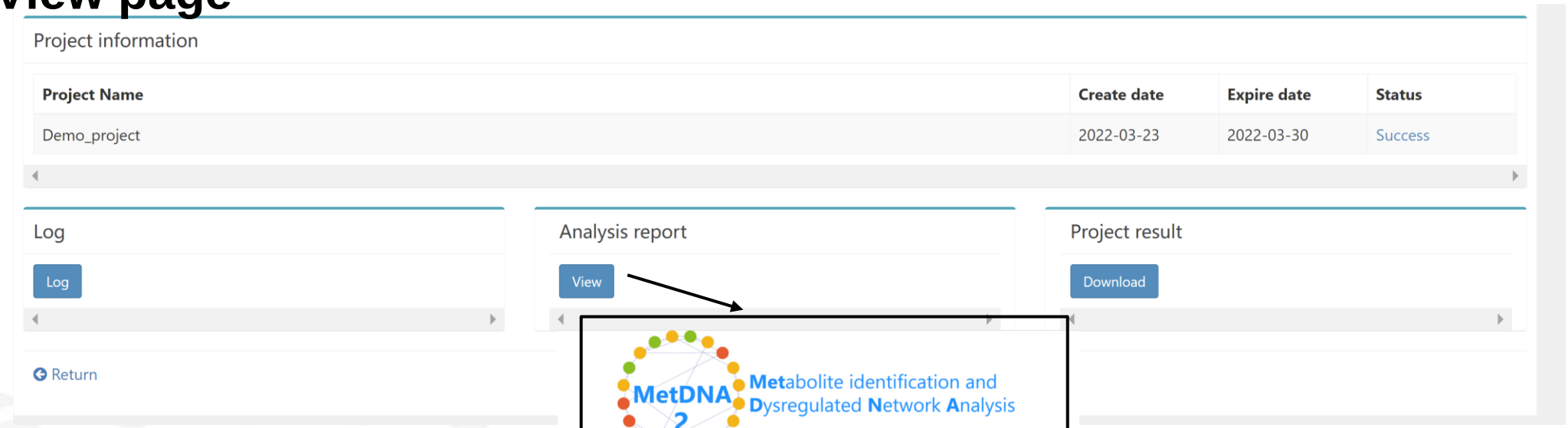
100 Haike Road, Pudong, Shanghai, China 201210
Website: www.zhulab.cn

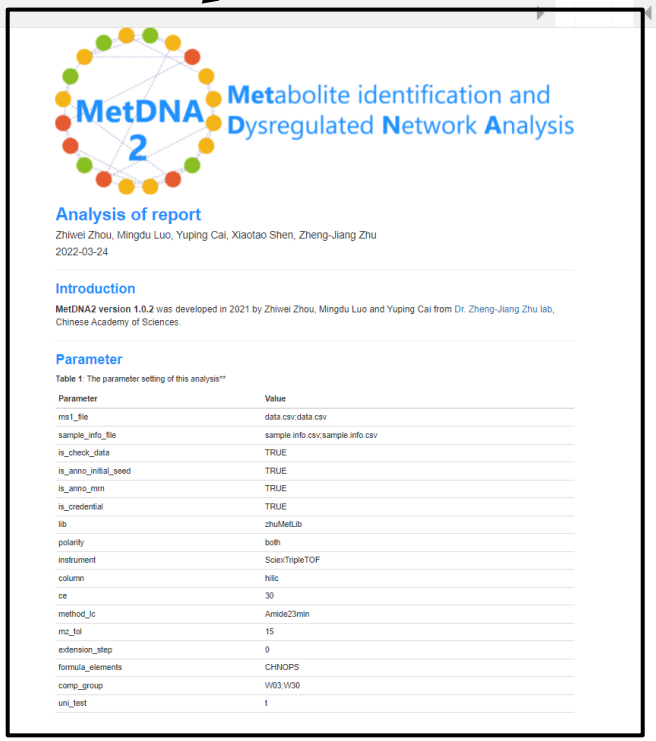
- You will receive a notification email after the project completed.
- You can scan and download results through clicking the link.
- The project will be stored for 7 days to download



Step 6: Result download and interpretation

View page

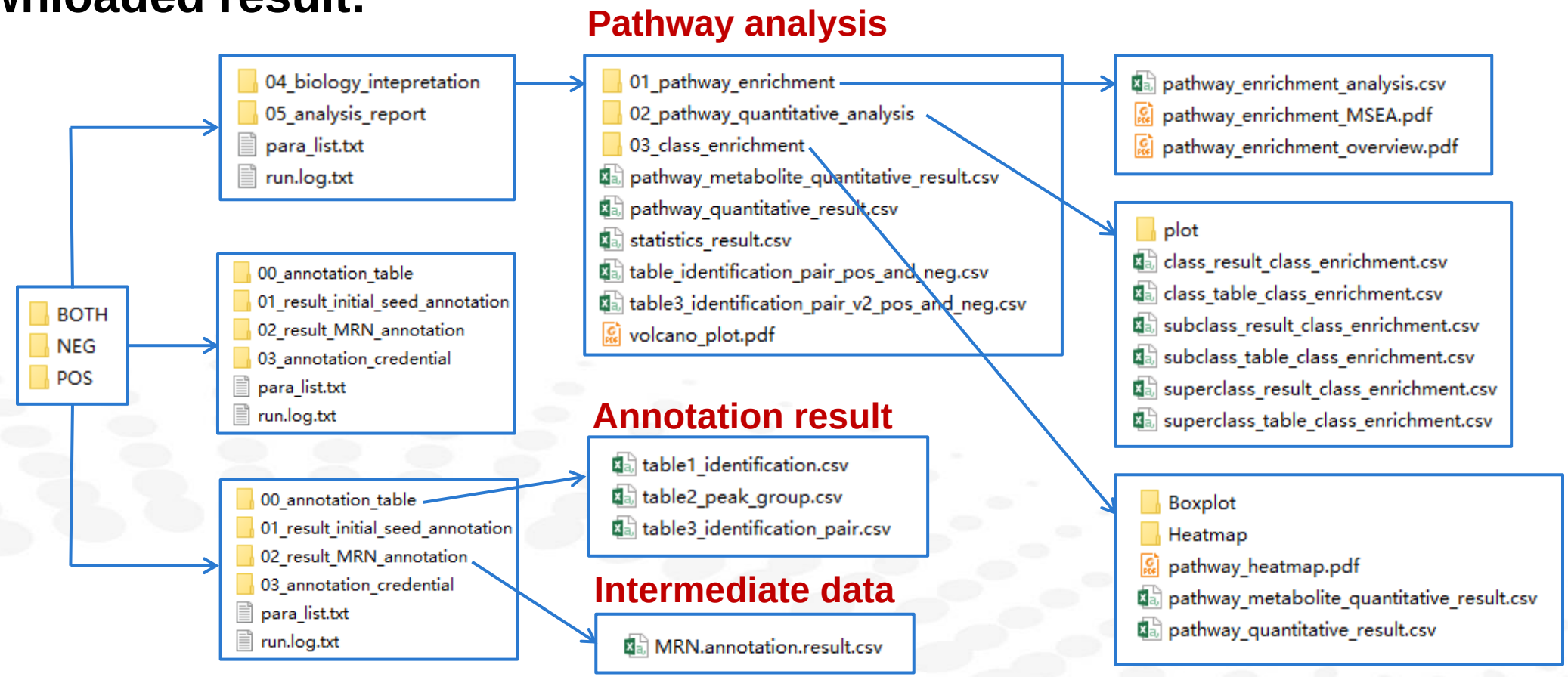




- **Log**: data processing records (Used for debugging)
- **Analysis report**: parameters and brief summary
- **Project result**: annotation results

Step 6: Result download and interpretation

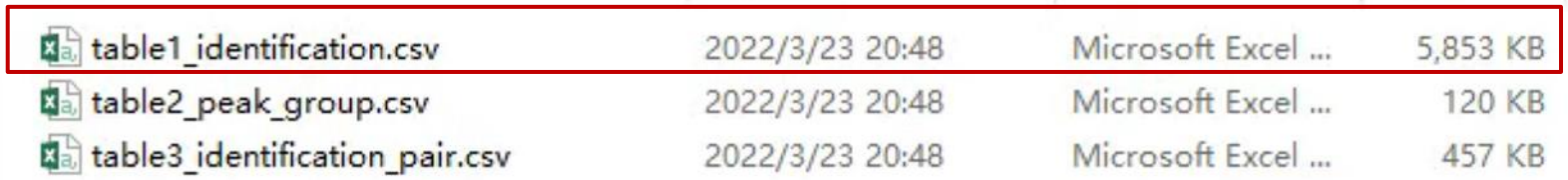
Downloaded result:



- **POS/NEG folder**: the results of metabolite annotation
- **BOTH folder**: the results of pathway analysis and HTML reports

Step 6: Result download and interpretation

Folder of annotation results: /POS or NEG/00_annotation_table/



Annotation files:

- **table1_identification.csv**: annotated metabolites for each feature
- **table2_peak_group.csv**: recognized abiotic peaks in each peak group
- **table3_identification_pair.csv**: separated feature – metabolite pairs for analysis

Bug reporting

Website: http://metdna.zhulab.cn/

Tutorial (English/Chinese):
http://metdna.zhulab.cn/metdna/help

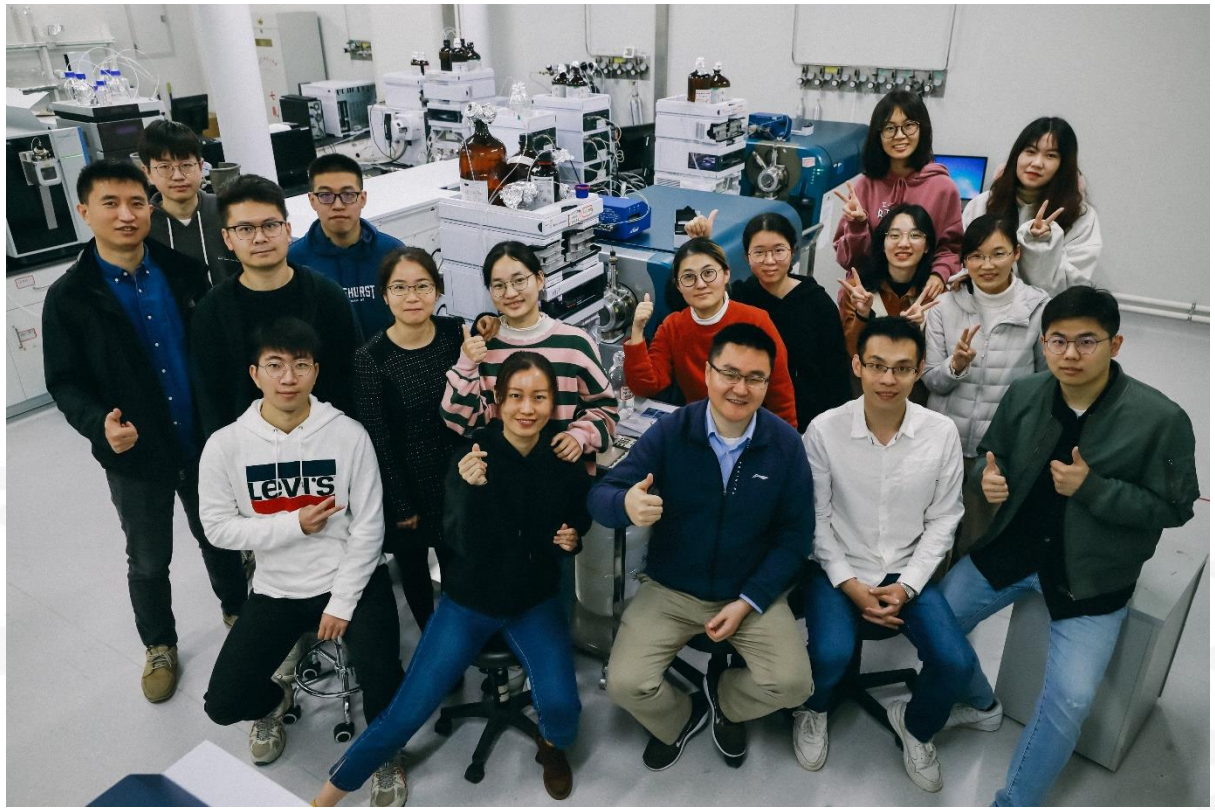
Bug reporting:

Google group: MetDNA

Chinese users: 786156544 (QQ group)

For any technical questions, please email to MetDNA@sioc.ac.cn

Acknowledgements



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