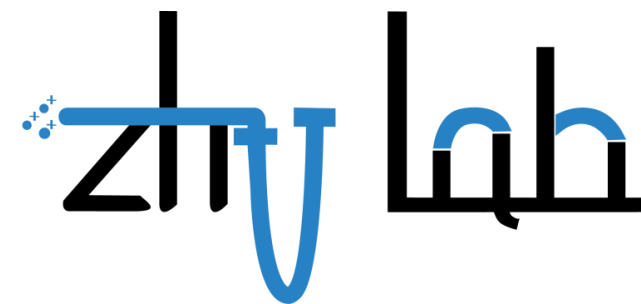


**METABOLOMICS: ADVANCING TECHNOLOGY FOR  
DISEASE DIAGNOSIS AND THERAPEUTICS**

**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**



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

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**Shanghai Institute of Organic Chemistry, CAS, Shanghai**

**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/>

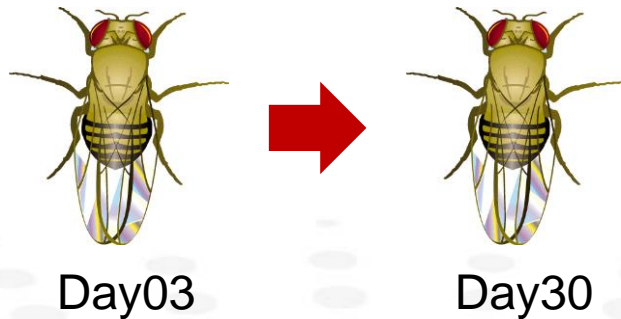
**Tutorial (English/Chinese):**

<http://metdna.zhulab.cn/metdna/help>

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

# Demo data

## Study design



- **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  $\mu\text{m}$ ; 100 mm  $\times$  2.1 mm)
- **Data preprocessing:** xcms (version 1.46.0)
- **Download:**



# Step 1: register and login




Metabolite identification and  
Dysregulated Network Analysis



Introduction Analysis Project Help FAQs Demo - ZhuLab Other software -

Sign in Sign up

## Sign up

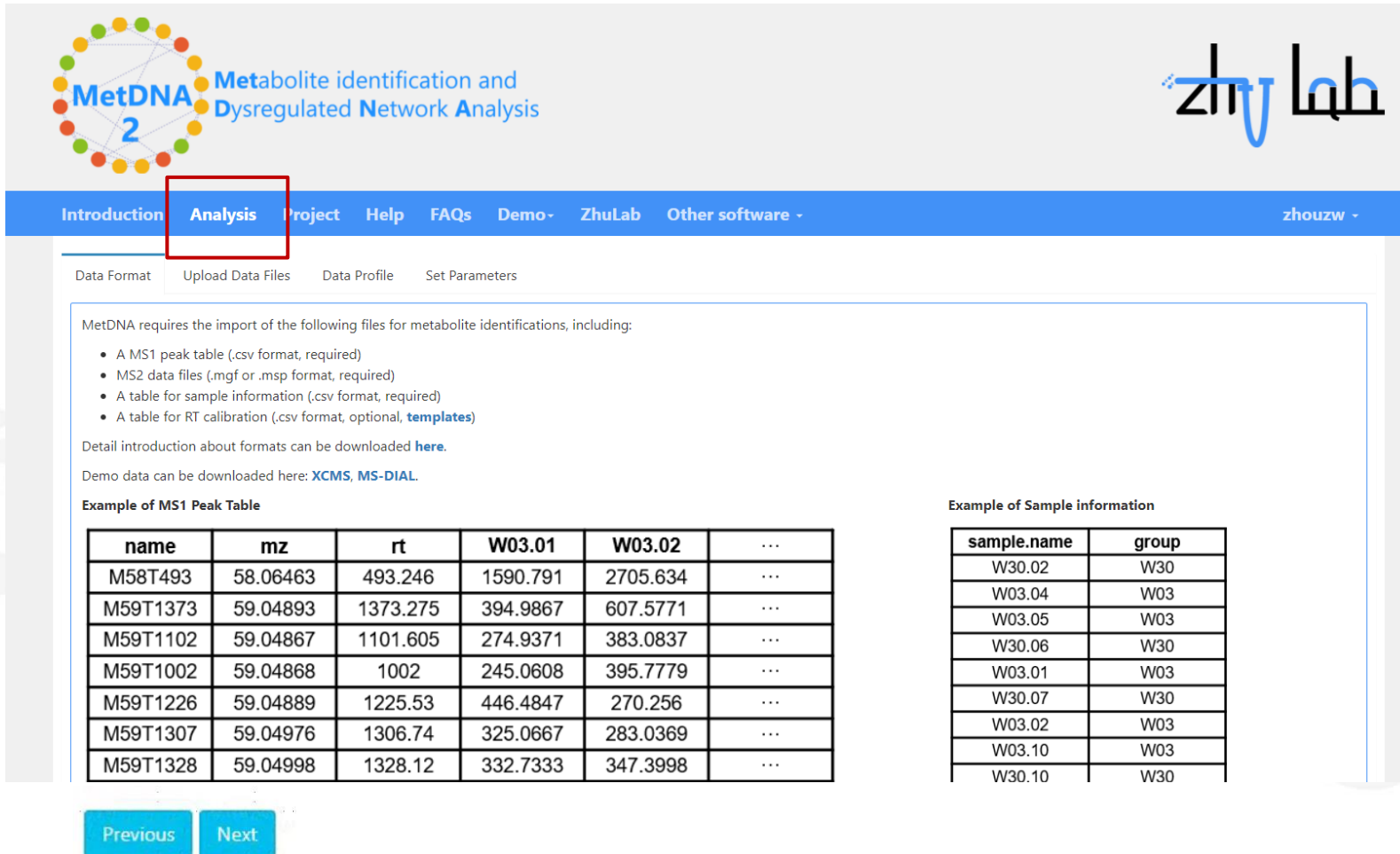
Email 

Code [Get authenticode](#)

Next

# Step 2: data preparing

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



The screenshot shows the MetDNA 2 web interface. The 'Analysis' tab is selected in the top navigation bar. Below it, the 'Data Format' sub-tab is active. The main content area lists the required files for metabolite identification: an MS1 peak table (CSV), MS2 data files (MGF or MSP), a sample information table (CSV), and an optional RT calibration table (CSV). It also provides links for format details and demo data. Two example tables are shown: 'Example of MS1 Peak Table' and 'Example of Sample information'.

MetDNA 2 Metabolite identification and Dysregulated Network Analysis

Introduction Analysis Project Help FAQs Demo- ZhuLab Other software - zhouzw -

Data Format Upload Data Files Data Profile Set Parameters

MetDNA requires the import of the following files for metabolite identifications, including:

- A MS1 peak table (.csv format, required)
- MS2 data files (.mgf or .msp format, required)
- A table for sample information (.csv format, required)
- A table for RT calibration (.csv format, optional, [templates](#))

Detail introduction about formats can be downloaded [here](#).

Demo data can be downloaded here: [XCMS](#), [MS-DIAL](#).

**Example of MS1 Peak Table**

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	...
M59T1102	59.04867	1101.605	274.9371	383.0837	...
M59T1002	59.04868	1002	245.0608	395.7779	...
M59T1226	59.04889	1225.53	446.4847	270.256	...
M59T1307	59.04976	1306.74	325.0667	283.0369	...
M59T1328	59.04998	1328.12	332.7333	347.3998	...

**Example of Sample information**

sample.name	group
W30.02	W30
W03.04	W03
W03.05	W03
W30.06	W30
W03.01	W03
W30.07	W30
W03.02	W03
W03.10	W03
W30.10	W30

Previous Next

## 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

[Introduction](#) [Analysis](#) [Project](#) [Help](#) [FAQs](#) [Demo](#) [ZhuLab](#) [Other software](#) zhouzw

[Data Format](#) [Upload Data Files](#) [Data Profile](#) [Set Parameters](#)

**Current Project:** Demo\_project

**Queue length:** 43

**Total size:** 218.91 MB

Name	Type	Size	Progress	Status	Actions
sample.info.csv	Sample Info	0.253KB	<div></div>	Valid	<a href="#">Remove</a>
data.csv	MS1 Peak Table (POS)	4.489MB	<div></div>	Warning 10	<a href="#">Remove</a>
W03.01.mgf	MS/MS Data (POS)	5.705MB	<div></div>	Valid	<a href="#">Remove</a>
W03.02.mgf	MS/MS Data (POS)	5.828MB	<div></div>	Valid	<a href="#">Remove</a>
W03.03.mgf	MS/MS Data (POS)	5.449MB	<div></div>	Valid	<a href="#">Remove</a>
W03.04.mgf	MS/MS Data (POS)	4.755MB	<div></div>	Valid	<a href="#">Remove</a>
W03.05.mgf	MS/MS Data (POS)	6.242MB	<div></div>	Valid	<a href="#">Remove</a>
W03.06.mgf	MS/MS Data (POS)	5.748MB	<div></div>	Valid	<a href="#">Remove</a>
W03.07.mgf	MS/MS Data (POS)	5.710MB	<div></div>	Valid	<a href="#">Remove</a>
W03.08.mgf	MS/MS Data (POS)	5.874MB	<div></div>	Valid	<a href="#">Remove</a>

[Previous](#) [Next](#)

**File Uploader**

**Project Name**

Demo\_project

**Sample Information**

Choose file...

Browse

**Polarity-related Data**

☐ Positive ☒ Negative

**Negative**

**MS1 Peak Table**

Choose file...

Browse

**MS/MS Data**

Choose file...

Browse

Upload

Reset

**Project name**

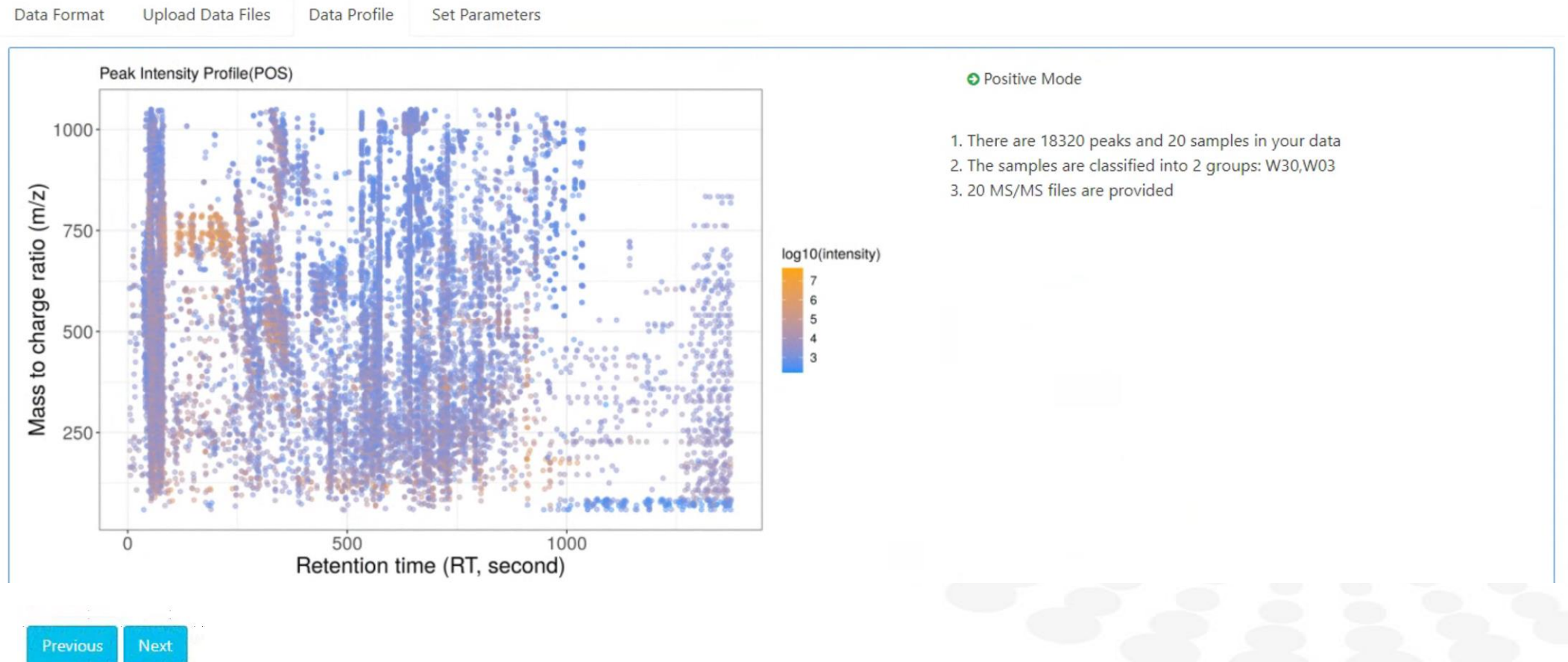
**Sample information**

**MS1 peak table**

**MS2 data files**

# 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**

Introduction Analysis Project Help FAQs Demo- ZhuLab Other software - zhouzw -

Data Format Upload Data Files Data Profile Set Parameters

- Version 1: MetDNA1; Version 2: MetDNA2

MetDNA Version	version2	Version of MetDNA
Ionization Polarity	Both	The acquisition mode [Positive/Negative]
Liquid Chromatography	HILIC	Liquid Chromatography (LC) type [HILIC/RP]
RT Library	Calibration Table amide23min ZhuLabRT	Types of RT libraries
MS Instrument	Sciex TripleTOF	Mass Spectrometer Instrument type
Collision Energy	30	The Collision Energy (CE) of MS/MS data acquisition
Unknown Annotation	No	Perform unknown annotation or not
Control Group	W03	The name of control group
Case Group	W30	The name of case group
Univariate Statistics	Student t-test	Statistic method [Student t-test/Wilcox test]
Species	Drosophila melanogaster (fruit fly)	The species of your project data
Cutoff of P-value	0.05	Usually less than 0.05
Cutoff of Fold-change	1	Usually larger than 1
P-value Adjustment	No	Adjust p values with FDR [Yes/No]

Previous Submit

- Perform unknown annotation or not

# Step 4: parameter setting

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

Introduction Analysis Project Help FAQs Demo ZhuLab Other software zhouzw

Data Format Upload Data Files Data Profile **Set Parameters**

**MetDNA Version** version2 Version of MetDNA

**Ionization Polarity** Both The acquisition mode [Positive/Negative]

**Liquid Chromatography** HILIC Liquid Chromatography (LC) type [HILIC/RP]

**RT Library** Calibration Table amide23min ZhuLabRT Types of RT libraries

**MS Instrument** Sciex TripleTOF Mass Spectrometer Instrument type

**Collision Energy** 30 The Collision Energy (CE) of MS/MS data acquisition

**Unknown Annotation** No Perform unknown annotation or not

**Control Group** W03 The name of control group

**Case Group** W30 The name of case group

**Univariate Statistics** Student t-test Statistic method [Student t-test/Wilcox test]

**Species** Drosophila melanogaster (fruit fly) The species of your project data

**Cutoff of P-value** 0.05 Usually less than 0.05

**Cutoff of Fold-change** 1 Usually larger than 1

**P-value Adjustment** No Adjust p values with FDR [Yes/No]

Previous Submit

**RT recalibration table**

Positive mode Negative mode

Name	Zhulab ID	PubChem ID	Ref.mz	RT/minute	Polarity
4-Pyridoxic acid	L0127	6723	184.0605	1.07	positive
3'-O-methyladenosine	L0251	82530	282.1197	3.12	positive
Creatinine	S0042	588	114.0662	4.95	positive
Nicotinate	S0008	938	124.0393	6.65	positive
Guanosine	S0071	135398635	284.0990	7.92	positive
N-Acetylcadaverine	S0249	189087	145.1336	9.13	positive
Beta-Alanine	L0320	239	90.0550	11.23	positive
dAMP	L0140	12599	332.0755	13.28	positive
gamma-L-Glutamyl-L-glutamic acid	L0352	92865	277.1031	14.5	positive
N6,N6,N6-Trimethyl-L-lysine	L0190	440120	189.1598	16.38	positive

Demo files: RT recalibration table Upload Save

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

# Step 5: data analysis

Project

Show 10 entries

Search:

Name	Status	Create time	Expire time	Control
Demo_project	Running	2022-03-23	2022-03-30	<a href="#">View</a>

Showing 1 to 1 of 1 entries

Previous 1 Next

- 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



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 logging 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](mailto:metdna@sioc.ac.cn)).

Laboratory for Mass Spectrometry and Metabolomics (ZhuLab)  
Interdisciplinary Research Center on Biology and Chemistry (IRCBC)  
Shanghai Institute of Organic Chemistry (SIOC)  
Chinese Academy of Sciences (CAS)

100 Haik Road, Pudong, Shanghai, China 201210  
Website: [www.zhulab.cn](http://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

Navigation bar: Introduction Analysis Project Help FAQs Demo- ZhuLab Other software - zhouzw -

Project

Show 10 entries

Search:

Name	Status	Create time	Expire time	Control
Demo_project	Success	2022-03-23	2022-03-30	<a href="#">View</a> <a href="#">Log</a> <a href="#">Download</a> <a href="#">Delete</a>

Showing 1 to 1 of 1 entries

Previous 1 Next



# Step 6: Result download and interpretation

## View page

Project information

Project Name	Create date	Expire date	Status
Demo_project	2022-03-23	2022-03-30	Success

Log

Log

Analysis report


View

Project result

Download

Return

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



**MetDNA 2** Metabolite identification and  
Dysregulated Network Analysis

**Analysis of report**  
Zhiwei Zhou, Mingdu Luo, Yiping Cai, Xiaotao Shen, Zheng-Jiang Zhu  
2022-03-24

**Introduction**  
MetDNA2 version 1.0.2 was developed in 2021 by Zhiwei Zhou, Mingdu Luo and Yiping Cai from Dr. Zheng-Jiang Zhu lab, Chinese Academy of Sciences.

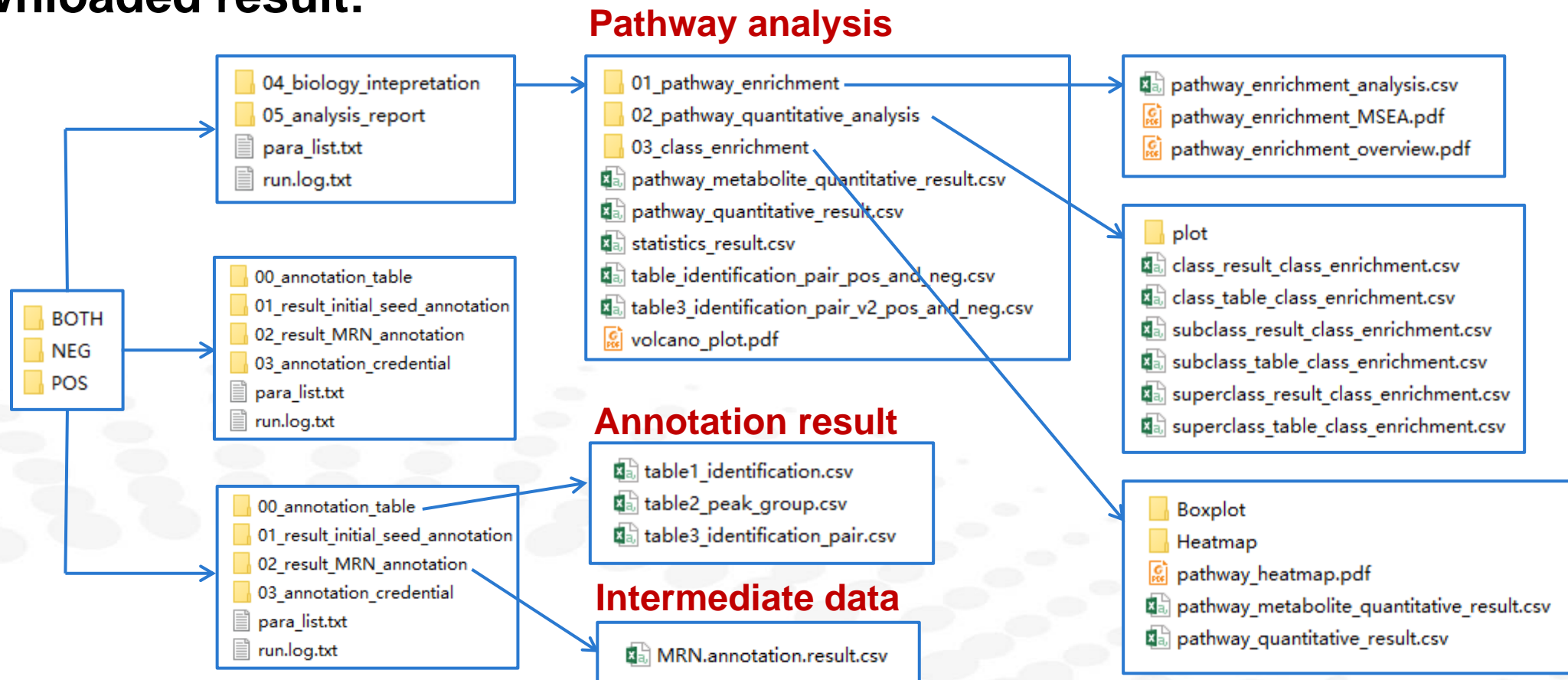
**Parameter**  
Table 1: The parameter setting of this analysis\*\*

Parameter	Value
ms1_file	data.csv\data.csv
sample_info_file	sample_info.csv/sample_info.csv
is_check_data	TRUE
is_anno_initat_seed	TRUE
is_anno_min	TRUE
is_credential	TRUE
lib	zhuMetLib
polarity	both
instrument	SciexTripleTOF
column	hplc
ce	30
method_lc	Amide23min
mz_tol	15
extension_step	0
formula_elements	CHNOPS
comp_group	W03.W30
uni_test	1

- **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/

 table1_identification.csv	2022/3/23 20:48	Microsoft Excel ...	5,853 KB
 table2_peak_group.csv	2022/3/23 20:48	Microsoft Excel ...	120 KB
 table3_identification_pair.csv	2022/3/23 20:48	Microsoft Excel ...	457 KB

## 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](mailto:MetDNA@sioc.ac.cn)**

# Acknowledgements



## Advisor:

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Wang

Shengwei Wan

Haosong Zhang

Ruohong Wang

Tianzhang Kou

Zhangdan Xie

Beizi Xing



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