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Step by Step from Environmental Samples to Preservation Vials

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Protocol status: Working

We use this protocol and it's working

Created: April 05, 2023

Last Modified: September 13, 2023

Protocol Integer ID: 80039

Keywords: microbes isolation from environmental sample, microbes isolation, environmental samples to preservation vial, environmental sample, preservation vial, lab collection, isolation, sample, lab manager

Abstract

This protocol shows how to generate a MICROBES ISOLATION FROM ENVIRONMENTAL SAMPLES into LAB COLLECTION. Please follow the instructions and do not modify the protocols by yourself. If any changes are needed please inform the lab manager or admin.

Troubleshooting



Isolate Primary Culture from Environmental Samples

1 Once you received your environmental samples.

Please catalog them in your Environmental Samples spreadsheet.

Name	Owner	Last modified	File size
Archived	黃尹則	Jun 10, 2022 黃尹則	—
HuangLabNanoporeData storage	黃尹則	Apr 1, 2023 吳昕懋	536 KB
5. HuangLab Item borrowing	黃尹則	Feb 24, 2023 陳緒宇Baldwi...	1 KB
4. HuangLab Things to order	ythuangmycolab User	Apr 14, 2023 范羽萱	4 KB
3. Huang Lab accomplishments	Yin-Tse Huang	Apr 13, 2023 黃尹則	27 KB
2-step PCR barcoding template for nanopor...	洪子純	Apr 4, 2023	14 KB
2 Field Collection Label Citizen Sciecnce Fu...	黃尹則	Mar 27, 2023 黃尹則	29 KB
1 MolecularWork (Direct edit) 分生實驗號碼 ...	黃尹則	3:14 PM Silmi Yusri Rahmada...	1.5 MB
1 InsectCollection Template (Copy it, DON'T...	黃尹則	Oct 27, 2022	101 KB
1 FungiCollection Template (Copy it, DON'T ...	黃尹則	Aug 3, 2022 黃尹則	28 KB
1 Fungi Isolation Plates (Direct edit) 菌株分...	黃尹則	Apr 14, 2023 Silmi Yusri Rah...	1.1 MB
1 EnvirnalmentalSamples Template (Copy it, ...	范羽萱	Apr 13, 2023 me	7 KB

Make a copy of the template for yourself. The template "1 EnvironmentalSamples Template" marked with blue box is available in HuangLab Collection.

2 Fill in the columns of the spreadsheet step by step (As shown in the following steps 2.1-2.15)

*In the template, some template data are there for your reference, please **delete them in your copy** and fill in only your data.

* Remember to scroll to the right and fill in as much as information you can.



	A	B	C	D	E	F	G	H	I	J	K	L
1	Catalog Number	Accession Number	Field Vial	Vial Extracted From	Genus 1	Species 1	Habitat / Substrate	Isolation Method	Verbatim Date	Cataloged Date	Collector First Name	Collector Last Name
3	HMW002						Hospital	Direct	12/08/2021	04/11/2023	Hsin-Mao	Wu
4	HMW003						Park	Direct	12/09/2021	04/11/2023	Hsin-Mao	Wu
5	HMW004						Park	Direct	12/09/2021	04/11/2023	Hsin-Mao	Wu
6	HMW005						Hospital	Direct	03/01/2022	04/11/2023	Hsin-Mao	Wu
7	HMW006						Hospital	Direct	03/01/2022	04/11/2023	Hsin-Mao	Wu
8	HMW009						Park	Direct	03/05/2022	04/11/2023	Hsin-Mao	Wu
9	HMW010						Park	Direct	03/05/2022	04/11/2023	Hsin-Mao	Wu
10	HMW011						Park	Direct	04/24/2022	04/11/2023	Hsin-Mao	Wu
11	HMW012						Park	Direct	04/24/2022	04/11/2023	Hsin-Mao	Wu
12	HMW013						Park	Direct	06/30/2022	04/11/2023	Hsin-Mao	Wu
13	HMW014						Park	Direct	06/30/2022	04/11/2023	Hsin-Mao	Wu
14	HMW015						Park	Direct	07/10/2022	04/11/2023	Hsin-Mao	Wu
15	HMW016						Park	Direct	07/10/2022	04/11/2023	Hsin-Mao	Wu
16	YTH001	129107092					Trail	Direct	07/30/2022	04/11/2023	Yin-Tse	Huang
17	YTH002	129106880					Trail	Direct	07/30/2022	04/11/2023	Yin-Tse	Huang
18	YTH003	129108922					Badland	Direct	07/31/2022	04/11/2023	Yin-Tse	Huang
19	HMW017	129523381					Park	Direct	08/05/2022	04/11/2023	Hsin-Mao	Wu
20	HMW018	129523381					Park	Direct	08/05/2022	04/11/2023	Hsin-Mao	Wu
21	HMW019	129790495					Trail	Direct	08/07/2022	04/11/2023	Hsin-Mao	Wu
22	HMW020	129790495					Trail	Direct	08/07/2022	04/11/2023	Hsin-Mao	Wu
23	HMW021	129791181					ForestUnderston	Direct	08/07/2022	04/11/2023	Hsin-Mao	Wu
24	HMW022	129791181					ForestUnderston	Direct	08/07/2022	04/11/2023	Hsin-Mao	Wu

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EnvironmentalSamples Template (Copy it, DON'T edit) 環境樣品採集Specify樣板 (請複製，不可直接編輯)

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Scrolling to the right and fill them in

- 2.1 Leave the Catalog Number (Column A) blank. The Catalog Number will be generated automatically from Specify. Copy and paste it directly after the upload.



	A
1	Catalog Number
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- 2.2 Accession Number (Column B) is for your own reference, you can name your sample as you want, there is no specific format for this.



B

Accession Number

HMW002

HMW003

HMW004

HMW005

HMW006

HMW009

HMW010

HMW011

HMW012

HMW013

HMW014

HMW015

HMW016

YTH001

YTH002

YTH003

HMW017

HMW018

HMW019

HMW020

HMW021

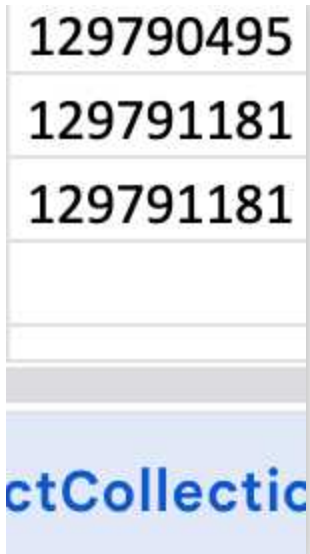
HMW022

6 HuangLabInsec

- 2.3 Field Vial (Column C) is the iNaturalist code. You only need to fill this column when you got the samples from citizen scientists.



C
Field Vial
129107092
129106880
129108922
129523381
129523381
129790495

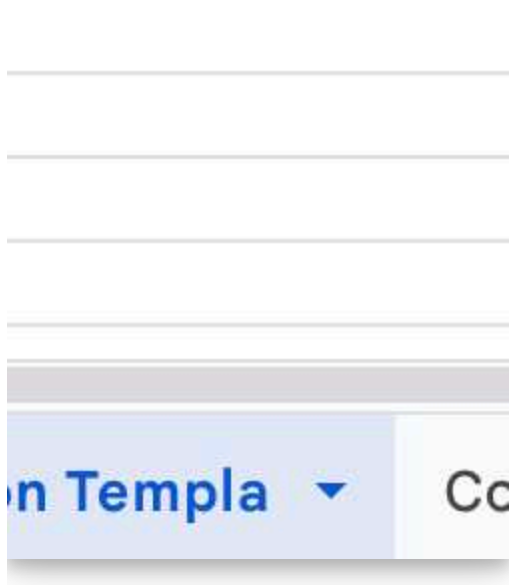


2.4 Leave the Vial Extracted From (Column D) blank.



D

Vial Extracted From



- 2.5 For Column E, F, G, G:
If your samples are **SOIL SAMPLES**, please follow this example.

E	F	G	H
Genus 1	Species 1	Habitat / Substrate	Isolation Method
		Hospital ▼	Direct ▼
		Park ▼	Direct ▼
		Park ▼	Direct ▼
		Hospital ▼	Direct ▼
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		Park ▼	Direct ▼
		Trail ▼	Direct ▼
		Trail ▼	Direct ▼
		Badland ▼	Direct ▼
		Park ▼	Direct ▼
		Park ▼	Direct ▼
		Trail ▼	Direct ▼
		Trail ▼	Direct ▼
		ForestUnderstory ▼	Direct ▼
		ForestUnderstory ▼	Direct ▼
		▼	▼

CollectionMethodPickList ▼

This is the example for Soil Samples.

Genus 1 & Species 1: Leave it blank.

Habitat/Substrate: What is the environment you collected your samples.

Isolation: How you isolate your samples (the default is Direct).

If your samples are **ENVIRONMENTAL SAMPLES**, please follow this example.



E	F	G	H
Genus 1	Species 1	Habitat / Substrate	Isolation Method
Anisandrus	ursulus	Ficus fistulosa	Direct ▼
Euwallacea	interjectus	Ficus fistulosa	Direct ▼
Planiculius	bicolor	Ficus fistulosa	Direct ▼
Euwallacea	fornicatus	Mucuna macrocarpa	Direct ▼
Corticoid		Dried fruiting bodies	Direct ▼
Phellinus		Dried fruiting bodies	Direct ▼
Oligoporus		Dried fruiting bodies	Direct ▼
Phellinus		Dried fruiting bodies	Direct ▼
Agaricales		Dried fruiting bodies	Direct ▼
Phellinus		Dried fruiting bodies	Direct ▼
Polyporus	squamosus	Dried fruiting bodies	Direct ▼
Phellinus		Dried fruiting bodies	Direct ▼
Oligoporus		Dried fruiting bodies	Direct ▼
Agaricales		Dried fruiting bodies	Direct ▼
Polyporus		Dried fruiting bodies	Direct ▼
Oligoporus		Dried fruiting bodies	Direct ▼
Irpex	laceratus	Dried fruiting bodies	Direct ▼
Hyphodontia		Dried fruiting bodies	Direct ▼
Cerrena	zonata	Dried fruiting bodies	Direct ▼
Trametes		Dried fruiting bodies	Direct ▼
Trichaptum		Dried fruiting bodies	Direct ▼
Pleurotus		Dried fruiting bodies	Direct ▼
Polyporoid		Dried fruiting bodies	Direct ▼
Polyporales		Dried fruiting bodies	Direct ▼
Laetiporus		Dried fruiting bodies	Direct ▼
Oligoporus		Dried fruiting bodies	Direct ▼

This is the example for Environmental Samples (e.g., ambrosia & bark beetles, fruiting bodies, etc).

Genus 1 & Species 1: Fill in the scientific name of your samples (unable to identify just leave it blank).

Habitat/Substrate: What is the environment you collected your samples (e.g., the host plant for the ambrosia & bark beetles).

Isolation: How you isolate your samples (the default is Direct).

- 2.6 Fill in the Verbatim Date (Column I) with the date **you COLLECTED the samples**.
Fill in the Cataloged Date (Column J) with the date **you UPLOADED the data to Specify**.



I	J
Verbatim Date	Cataloged Date
12/08/2021	04/11/2023
12/09/2021	04/11/2023
12/09/2021	04/11/2023
03/01/2022	04/11/2023
03/01/2022	04/11/2023
03/05/2022	04/11/2023
03/05/2022	04/11/2023
04/24/2022	04/11/2023
04/24/2022	04/11/2023
06/30/2022	04/11/2023
06/30/2022	04/11/2023
07/10/2022	04/11/2023
07/10/2022	04/11/2023
07/30/2022	04/11/2023
07/30/2022	04/11/2023
07/31/2022	04/11/2023
08/05/2022	04/11/2023
08/05/2022	04/11/2023
08/07/2022	04/11/2023

08/07/2022	04/11/2023
08/07/2022	04/11/2023
08/07/2022	04/11/2023

2.7 Collector First Name (Column K) and Collector Last Name (Column L)
Fill in the name of the collector.



K	L
Collector First Name	Collector Last Name
Hsin-Mao	Wu
Hsin-Mao	Wu
Hsin-Mao	Wu
Hsin-Mao	Wu
Hsin-Mao	Wu
Hsin-Mao	Wu
Hsin-Mao	Wu
Hsin-Mao	Wu
Hsin-Mao	Wu
Hsin-Mao	Wu
Hsin-Mao	Wu
Hsin-Mao	Wu
Hsin-Mao	Wu
Yin-Tse	Huang
Yin-Tse	Huang
Yin-Tse	Huang
Hsin-Mao	Wu
Hsin-Mao	Wu
Hsin-Mao	Wu
Hsin-Mao	Wu
Hsin-Mao	Wu

Hsin-Mao	Wu

Templa ▼
ColletionMethodPickLis

- 2.8 Fill in these columns when the sample is identified with a scientific name, remember to fill Column E and F (Genus and Species names) as well. If not, leave it blank. Fill in the date you did the determination (identification).

[illegible]

2.9 Fill in these columns with the geographical information of your samples. Leave it blank, when you don't have the information or the materials is outsourced lacking this info.

CollectionMethodPickList

*Inform admin when other Prep Type is needed.

W	X	Y	Z
Prep Type ▼	Prep Count	Room	Storage
Fridge ▼	1	927	4C
Fridge ▼	1	927	4C
Fridge ▼	1	927	4C
Fridge ▼	1	927	4C
Fridge ▼	1	927	4C
Fridge ▼	1	927	4C
Fridge ▼	1	927	4C
Fridge ▼	1	927	4C
Fridge ▼	1	927	4C
Fridge ▼	1	927	4C
Fridge ▼	1	927	4C
Fridge ▼	1	927	4C
Fridge ▼	1	927	4C
Fridge ▼	1	927	4C
Fridge ▼	1	927	4C
Fridge ▼	1	927	4C
Fridge ▼	1	927	4C
Fridge ▼	1	927	4C
Fridge ▼	1	927	4C
Fridge ▼	1	927	4C
Fridge ▼	1	927	4C

Fridge	▼	1	927	4C
Fridge	▼	1	927	4C
Fridge	▼	1	927	4C
	▼			
	▼			
	▼			

methodPickList ▼

2.11 Fill in the name (Column AA and AB) of who uploaded the data to Specify.



AA	AB
Cataloger First Name	Cataloger Last Name
Hsin-Mao	Wu
Hsin-Mao	Wu
Hsin-Mao	Wu
Hsin-Mao	Wu
Hsin-Mao	Wu
Hsin-Mao	Wu
Hsin-Mao	Wu
Hsin-Mao	Wu
Hsin-Mao	Wu
Hsin-Mao	Wu
Hsin-Mao	Wu
Hsin-Mao	Wu
Hsin-Mao	Wu
Hsin-Mao	Wu
Hsin-Mao	Wu
Hsin-Mao	Wu
Hsin-Mao	Wu
Hsin-Mao	Wu
Hsin-Mao	Wu
Hsin-Mao	Wu
Hsin-Mao	Wu

Hsin-Mao	Wu
Hsin-Mao	Wu

2.12 You can leave any informative notes of your samples. If not, leave it blank.

AC

Note

25

4

25

4

25

4

25

4

25

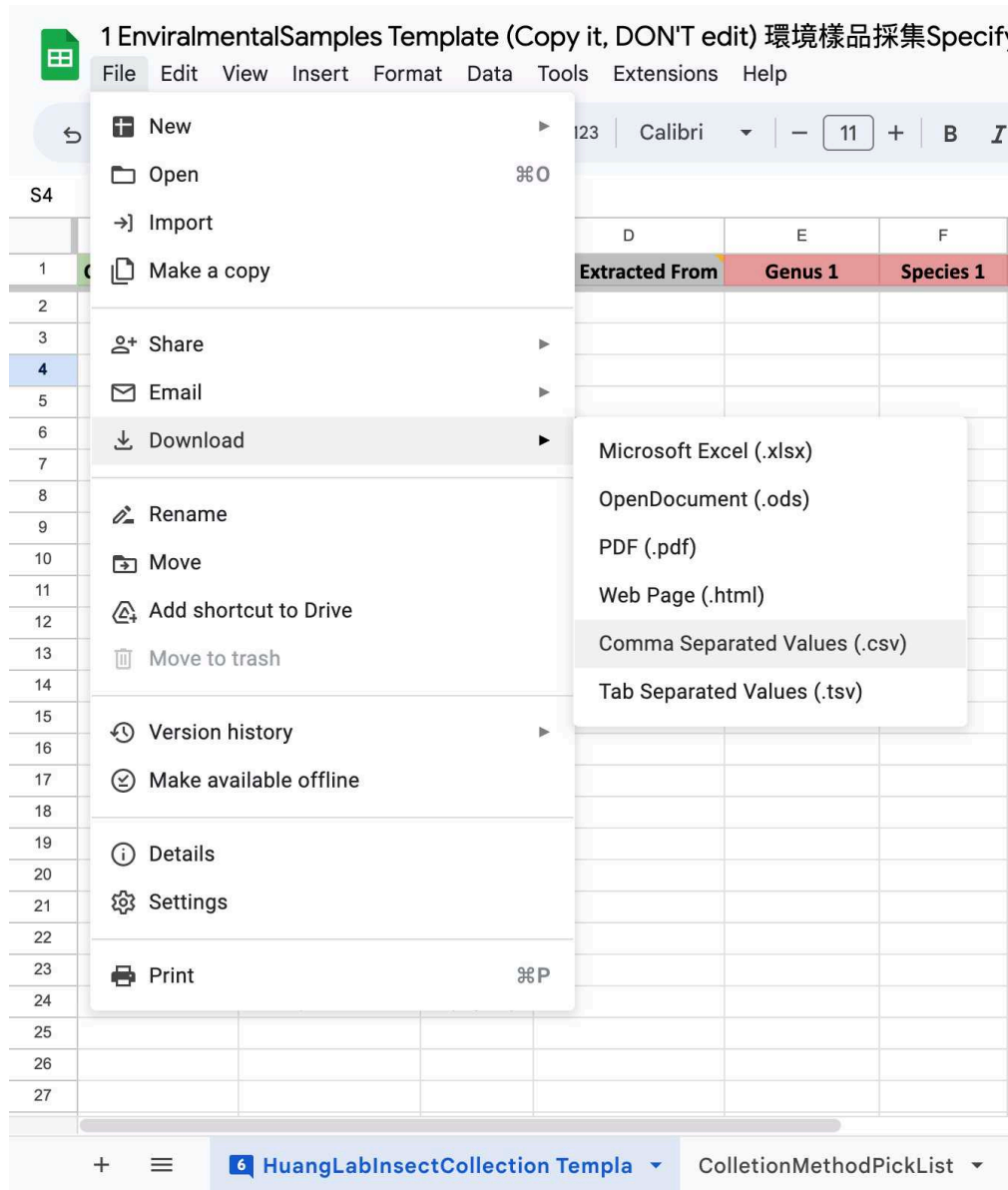
4

25

4

254254

2.13 Done!! Download the spreadsheet as .csv format.

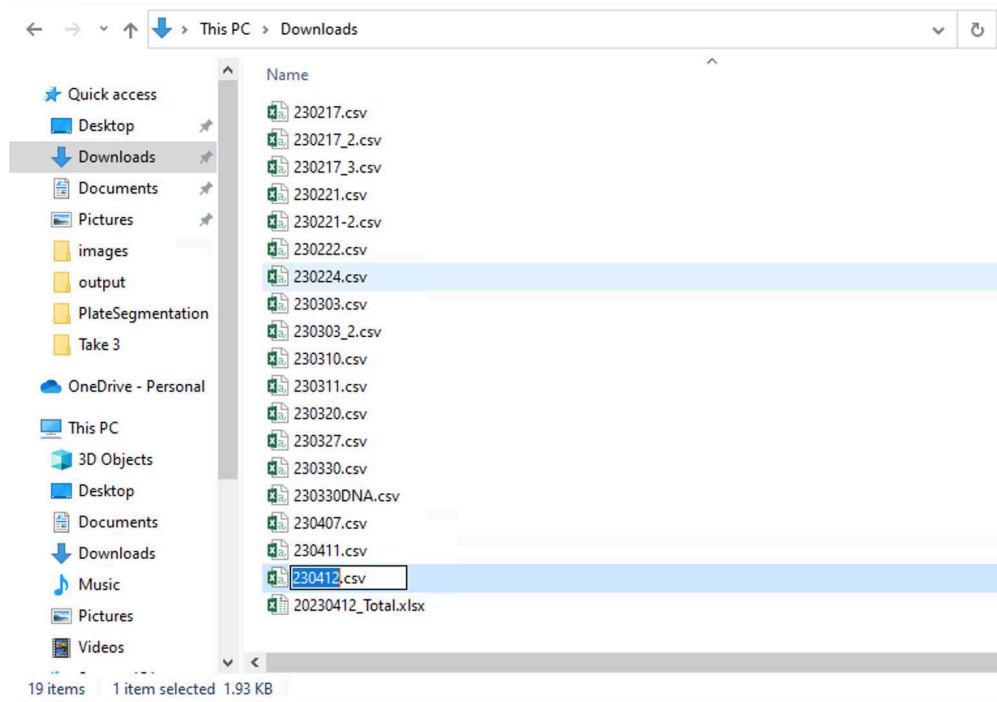


2.14 Delete the row with existed Catalog Number if any.

****Leave the not-uploaded rows only.**

	A	B	C	D	E	F	G	H	I	J	K	L
1	Catalog Number	Accession Nu	Field Vial	Vial Extracte	Genus 1	Species 1	Habitat / Sub	Isolation Met	Verbatim Da	Cataloged Da	Collector	Firs Collector
2	2023-000133	HMW002					Hospital	Direct	12/08/2021	04/12/2023	Hsin-Mao	Wu
3		HMW003					Park	Direct	12/09/2021	04/12/2023	Hsin-Mao	Wu
4		HMW004					Park	Direct	12/09/2021	04/12/2023	Hsin-Mao	Wu
5		HMW005					Hospital	Direct	03/01/2022	04/12/2023	Hsin-Mao	Wu
6		HMW006					Hospital	Direct	03/01/2022	04/12/2023	Hsin-Mao	Wu
7		HMW009					Park	Direct	03/05/2022	04/12/2023	Hsin-Mao	Wu

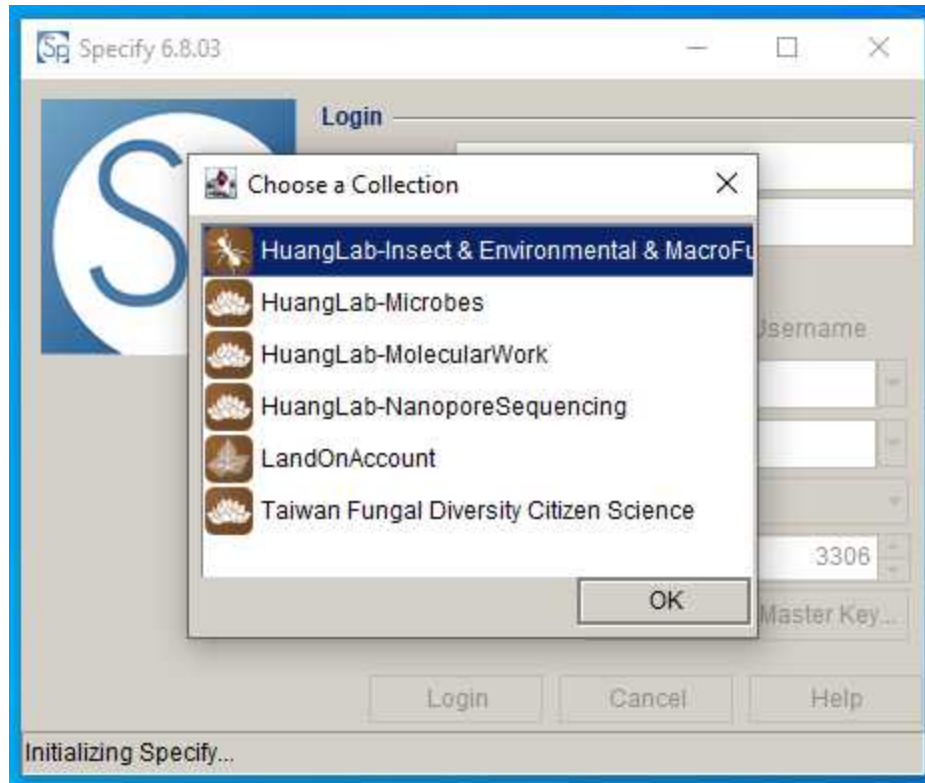
2.15 Rename the file with the date you are going to upload the datasheet to Specify.



Upload your datasheet to Specify database

3 ****The account setup is skipped****

Login to the Specify database. Choose the **first collection (HuangLab-Insect & Environmental & MacroFungi)**.



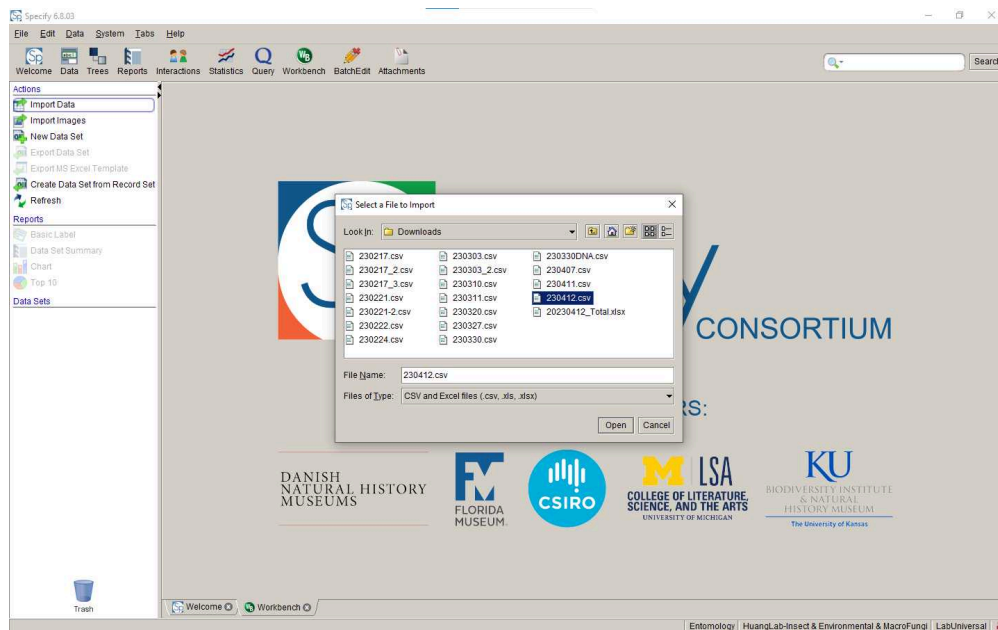
3.1 Go to the **Workbench** tab.



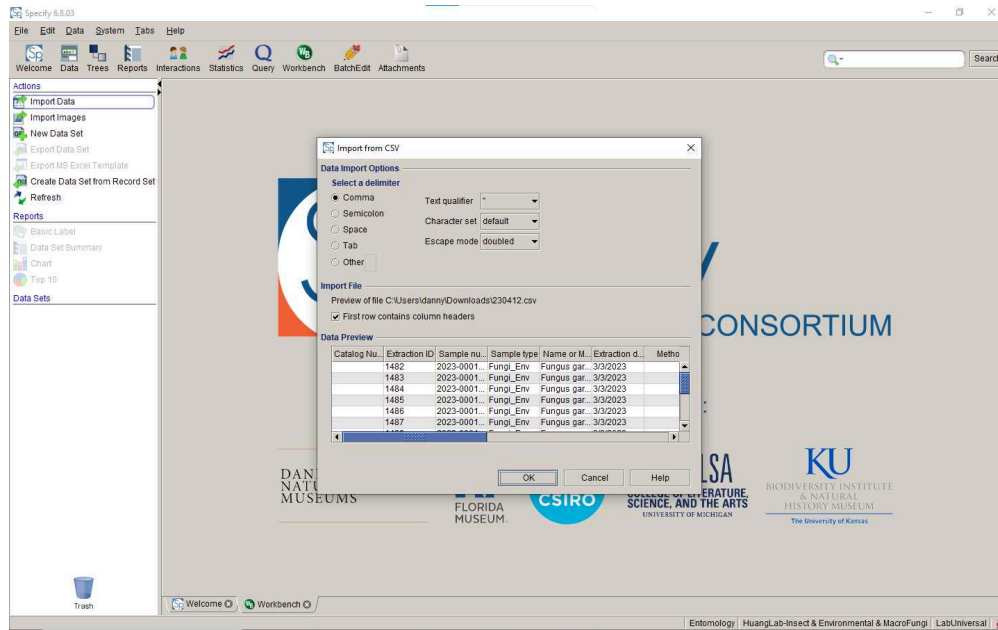
3.2 Click **Import Data**.



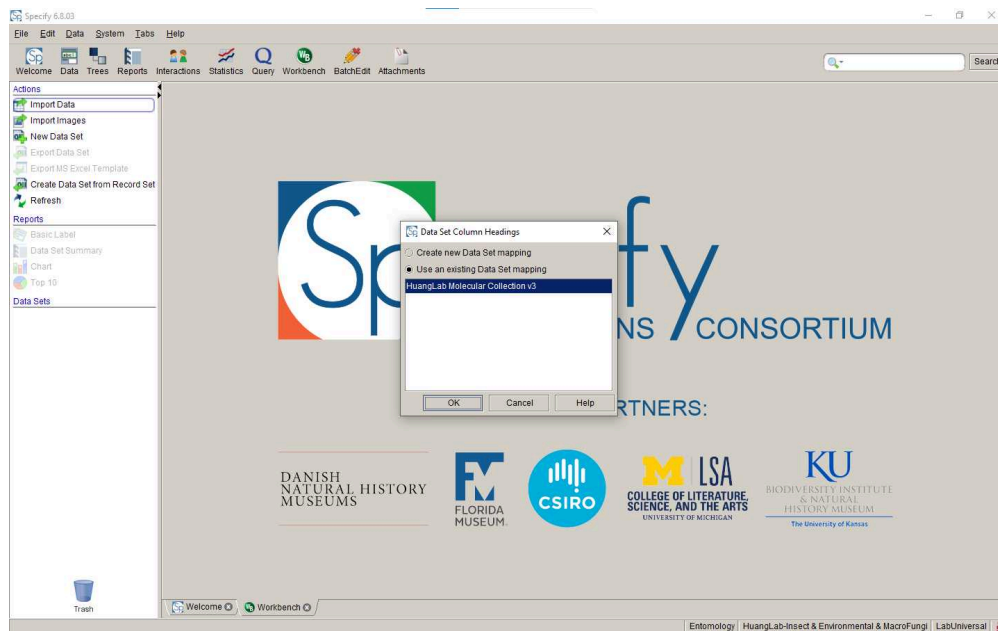
3.3 Choose the file you wanted to import (upload), and click **Open**.



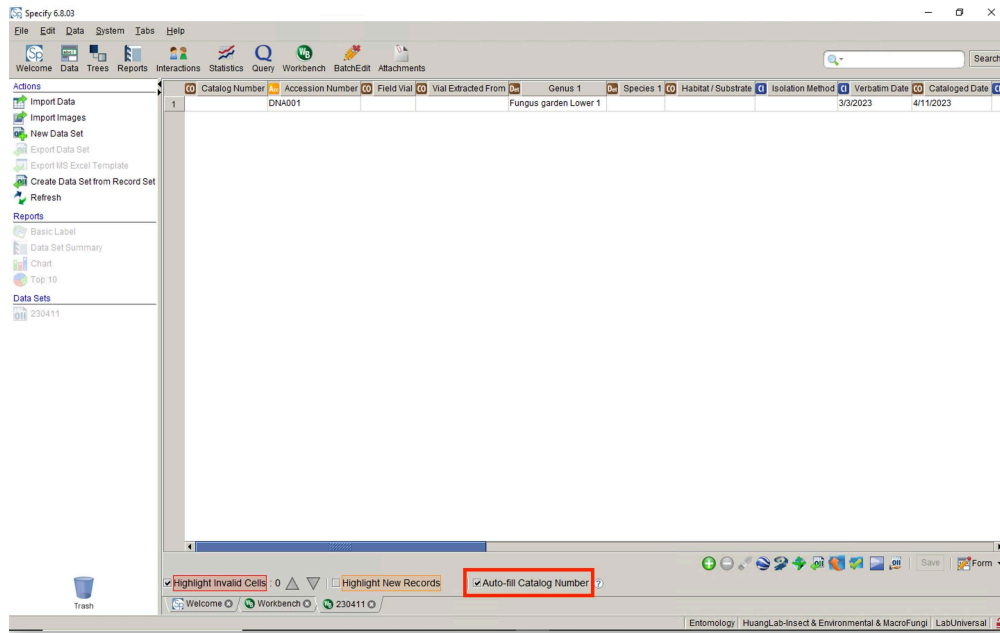
3.4 Let everything as default. Then, click **OK**.



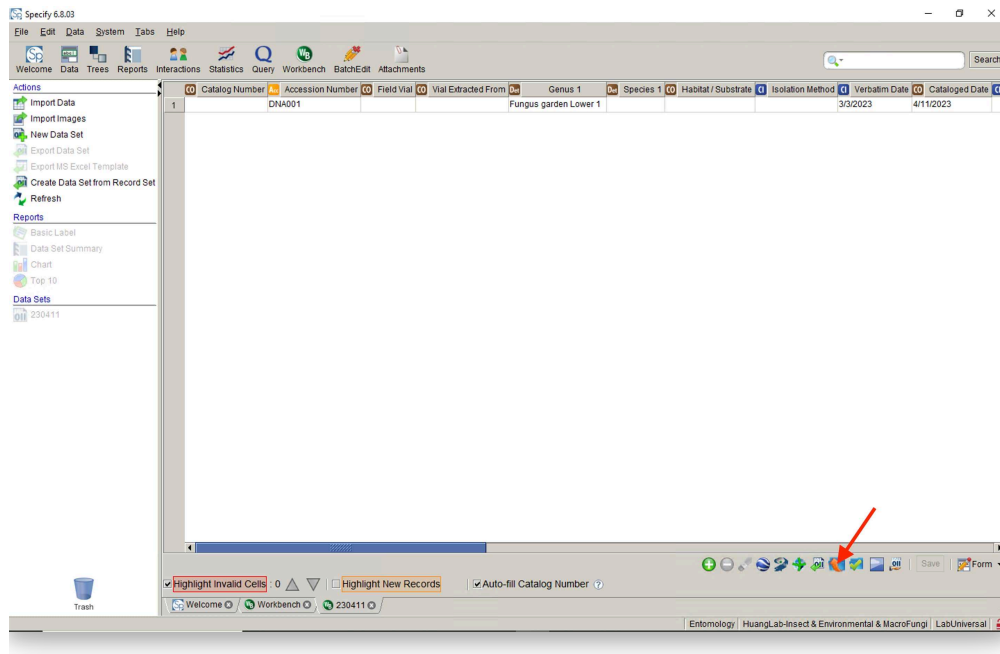
3.5 Select the second option: **Use an existing Data Set mapping.**



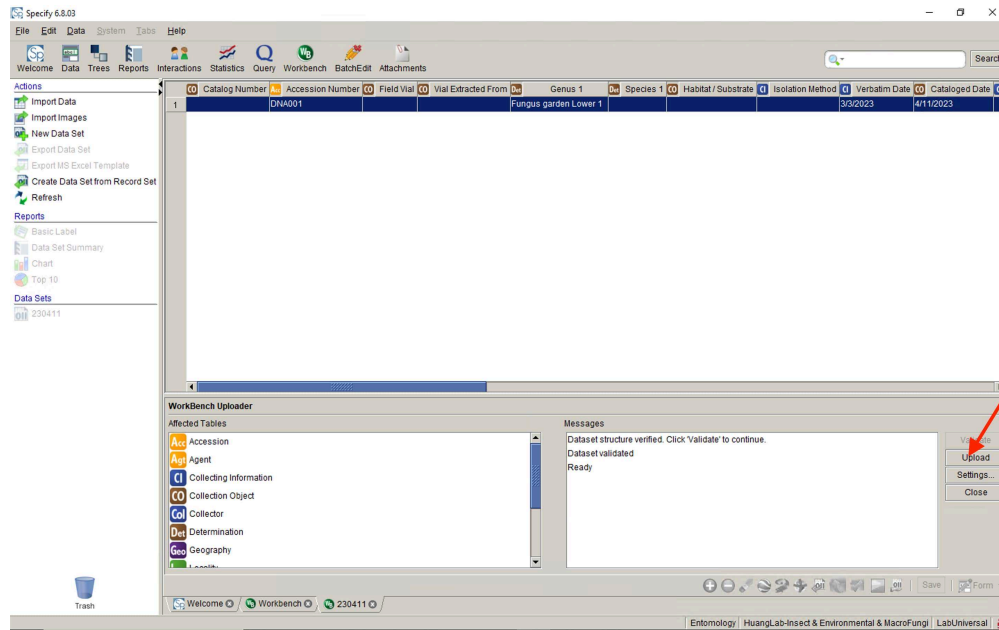
3.6 Check for any invalid cells (red highlighted cells).
Remember to tick Auto-fill Catalog Number (marked with red box).



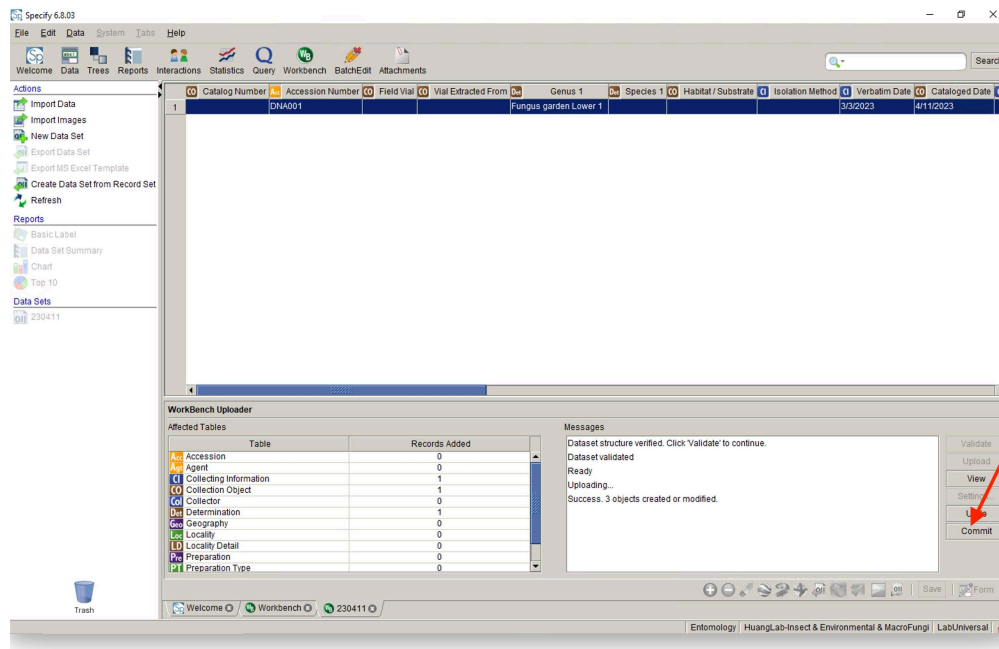
3.7 Click **Upload** (pointed with red arrow).



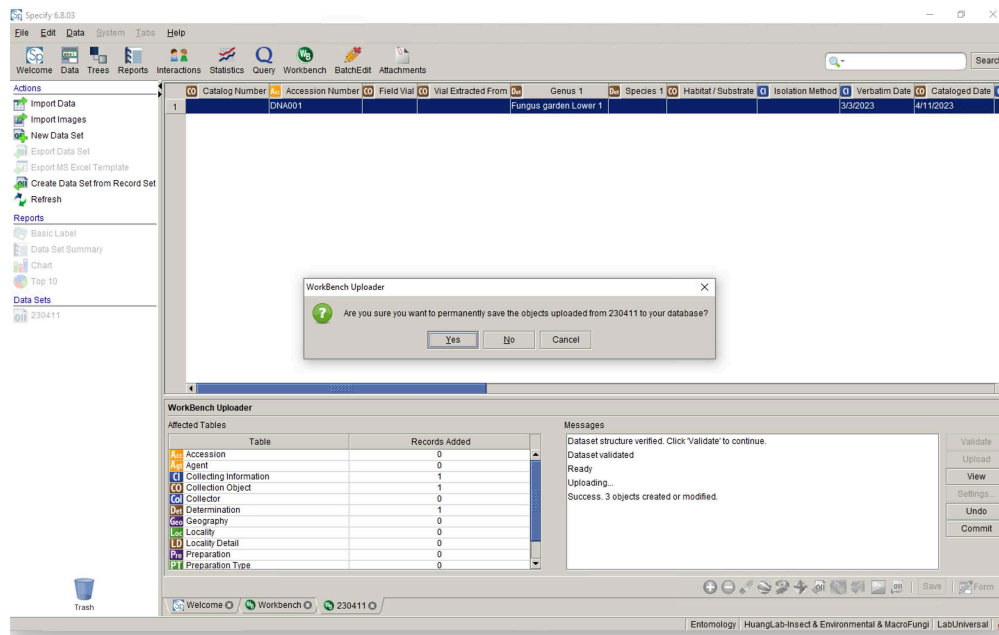
3.8 Click **Upload** again (pointed with red arrow).



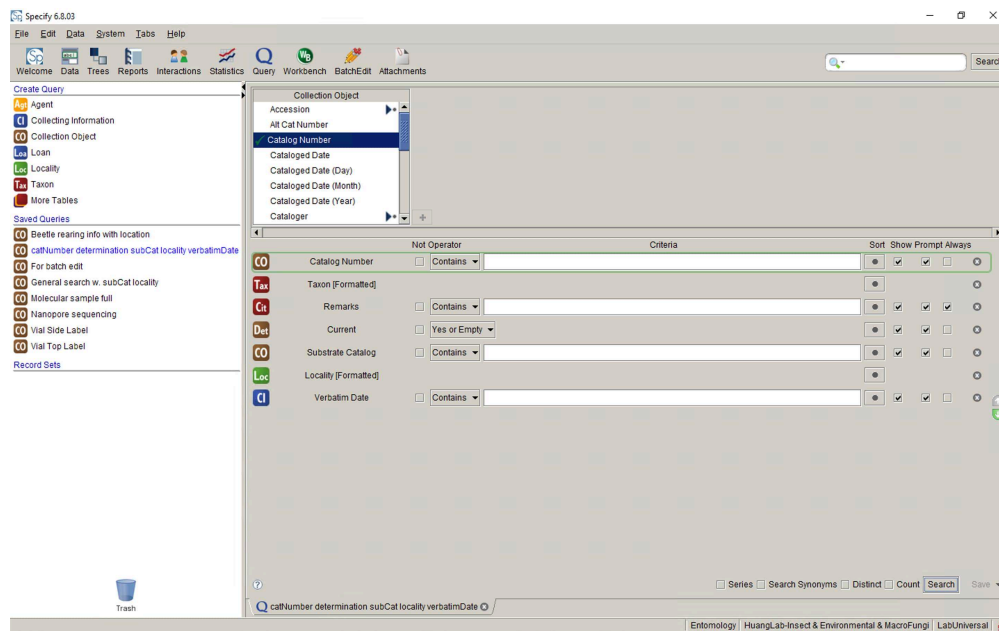
3.9 Then Click **Commit** (marked with red arrow).



3.10 Click **Yes** to finish the upload progress.



- 3.11 Now you can go to **Query** → **catNumber determination...** (under the Saved Queries in left panel) to check if the upload succeeded.
Click **Search** to start querying.



3.12 Ta-Da!! The upload was succesful.

Catalog Number	Taxon (Formatted)	Remarks	Current	Substrate Catalog	Locality (Formatted)	Verbatim Date
2023-000097	Eccectoporus spinosus		true		Dagangshan, Taiwan, Kaohsiung, 22.8701...	3/12/2023
2023-000098	Xyleborinus andrewesi		true		Dagangshan, Taiwan, Kaohsiung, 22.8701...	3/12/2023
2023-000099	Hypothenemus		true		Dagangshan, Taiwan, Kaohsiung, 22.8701...	3/12/2023
2023-000100	Eremothecium gossypii	seed of Koeleria elegans	true		ND, Taiwan, Taoyuan, 25.016720000, 121...	3/13/2023
2023-000101	Eremothecium leptocordis	Leptocoris augur	true		ND, Taiwan, Tainan, 23.3568850000, 120.4...	3/13/2023
2023-000102	Eremothecium leptocordis	seed of Cardiospermum halicababum	true		ND, Taiwan, Taichung, 24.095560000, 122...	3/13/2023
2023-000103	Eremothecium leptocordis	Jadera haematoloma	true		ND, Taiwan, Kaohsiung, 22.7420750000, 1...	3/13/2023
2023-000104		Park			Wei Wu Ying, Taiwan, Kaohsiung, 22.61778...	03/13/2023
2023-000105		Field			Yi He Li, Taiwan, Kaohsiung, 22.640355000...	03/13/2023
2023-000106		Hospital			Chang Gung Memorial Hospital, Taiwan, Ka...	03/13/2023
2023-000107	Mangifera indica		true	Roadside	Namasha National High School, Taiwan, K...	2023-03-11
2023-000108	Mangifera indica		true	Roadside	Namasha National High School, Taiwan, K...	2023-03-11
2023-000109	Mangifera indica		true	Roadside	Namasha National High School, Taiwan, K...	2023-03-11
2023-000110	Bidens alba		true	Roadside	Namasha National High School, Taiwan, K...	2023-03-11
2023-000111	Heterotrigona itama		true	Bee bread	Rumah Amami, Indonesia, West Sumatera...	2/7/2023
2023-000112	Geniotrigona thoracica		true	Bee bread	Rumah Amami, Indonesia, West Sumatera...	2/7/2023
2023-000113	Tetragonula laevis		true	Bee bread	Rumah Amami, Indonesia, West Sumatera...	2/7/2023
2023-000114	Tetragonula fuscobalteata		true	Bee bread	Melipo bee, Indonesia, West Sumatera, -0.0...	2/7/2023
2023-000115	Tetragonula testaceolaris		true	Bee bread	Melipo bee, Indonesia, West Sumatera, -0.0...	2/7/2023
2023-000116	Tetragonula minanglabau		true	Bee bread	Anton Farm, Indonesia, West Sumatera, -0...	2/7/2023
2023-000117	Tritonini		true	Lentinus squamosulus	Xibaoshan, Taiwan, Hualien, 24.212417000...	3/19/2023
2023-000118				Forest land	South Shoushan, Taiwan, Kaohsiung, 22.6...	04/03/2023
2023-000119				Public land	National Sun Yat-sen University, Taiwan, Ka...	04/03/2023
2023-000120	Fungus garden Lower 1		true			3/3/2023
2023-000121	Fungus garden Lower 2		true			3/3/2023
2023-000122	Fungus garden Lower 3		true			3/3/2023
2023-000123	Fungus garden Lower 4		true			3/3/2023
2023-000124	Fungus garden Upper 7		true			3/3/2023
2023-000125	Fungus garden Upper 8		true			3/3/2023
2023-000126	Fungus garden Upper 9		true			3/3/2023
2023-000127	Fungus garden Upper 10		true			3/3/2023
2023-000128	Fungus garden Lower 5		true			3/3/2023
2023-000129	Fungus garden Lower 6		true			3/3/2023
2023-000130	Fungus garden Upper 11		true			3/3/2023
2023-000131	Fungus garden Lower 12		true			3/3/2023
2023-000132	Fungus clay 13		true			3/3/2023
2023-000133	Fungus clay 14		true			3/3/2023
2023-000134	Fungus garden Lower 1		true			3/3/2023
2023-000125	Fungus garden Lower 1		true			3/3/2023

****Remember to delete the Data Sets & Record Sets before closing the Specify.**



3.13 Paste the **Catalog Number** of uploaded data back to your spreadsheet.

Isolate the microbes from your environmental samples

- 4 Prepare the equipment and materials first for sample isolation. Put them on another bench first.

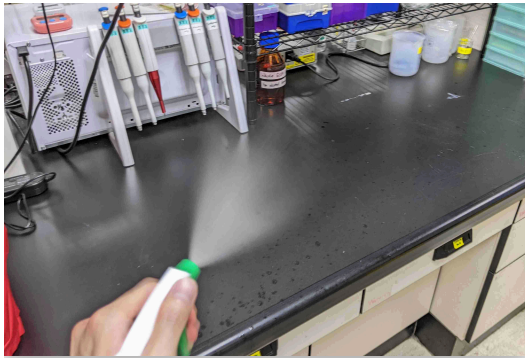


1. Alcohol lamps
2. 95% ethanol cooling bottle
3. Beakers (small & large)
4. Tweezers
5. Scalpel with blade
6. Separator
7. Grafting tube
8. Lighter
9. Marker pen
10. Media

- 4.1 Sterilize the microbes isolation area (air and bench) using a 75% ethanol sprayer.
****Make sure the mist is emitted.****



Spray it a few times up to the air.



Spray it a few times onto the bench.



Wipe the bench unidirectionally using paper towels.

- 4.2 Now, move the equipment and materials for microbes isolation back to the sterilized area.



- 4.3 Before isolation, key the information about your isolation (how many plates you are going to isolate, what kind of media you will use, etc) into the **1 Fungi Isolation Plates (Direct edit) 菌株分離培養基編號 (直接編輯)** spreadsheet first

A	B	C	D	E	F	G	H	I	
Plate numl	Project	Media	Isolation part	Insect_Env_Cataloger	Dilutio	Cataloge	Cataloger	Cataloged Di	Notes
P701	Trichosporon project	DG18	Bird feces	2023-000007	0.25	Yu-Hsuan	Fan	1/5/2023	
P702	Trichosporon project	DG18	Bird feces	2023-000007	0.25	Yu-Hsuan	Fan	1/5/2023	
P703	Trichosporon project	DG18	Bird feces	2023-000007	0.25	Yu-Hsuan	Fan	1/5/2023	
P704	Trichosporon project	DG18	Bird feces	2023-000008	0.25	Yu-Hsuan	Fan	1/5/2023	
P705	Trichosporon project	DG18	Bird feces	2023-000008	0.25	Yu-Hsuan	Fan	1/5/2023	
P706	Trichosporon project	DG18	Bird feces	2023-000008	0.25	Yu-Hsuan	Fan	1/5/2023	
P707	General Isolation	PDA	Fungus	2023-000009	Direct	Yu-Hsuan	Fan	1/6/2023	
P708	General Isolation	PDA	Fungus	2023-000009	Direct	Yu-Hsuan	Fan	1/6/2023	
P709	General Isolation	PDA	Fungus	2023-000010	Direct	Yu-Hsuan	Fan	1/6/2023	
P710	General Isolation	PDA	Fungus	2023-000010	Direct	Yu-Hsuan	Fan	1/6/2023	
P711	General Isolation	PDA	Fungus	2023-000011	Direct	Yu-Hsuan	Fan	1/6/2023	
P712	General Isolation	PDA	Fungus	2023-000011	Direct	Yu-Hsuan	Fan	1/6/2023	
P713	General Isolation	PDA	Fungus	2023-000012	Direct	Yu-Hsuan	Fan	1/6/2023	
P714	General Isolation	PDA	Fungus	2023-000012	Direct	Yu-Hsuan	Fan	1/6/2023	
P715	General Isolation	PDA	Fungus	2023-000013	Direct	Yu-Hsuan	Fan	1/6/2023	
P716	General Isolation	PDA	Fungus	2023-000013	Direct	Yu-Hsuan	Fan	1/6/2023	
P717	General Isolation	PDA	Fungus	2023-000014	Direct	Yu-Hsuan	Fan	1/6/2023	
P718	General Isolation	PDA	Fungus	2023-000014	Direct	Yu-Hsuan	Fan	1/6/2023	
P719	General Isolation	PDA	Fungus	2023-000015	Direct	Yu-Hsuan	Fan	1/6/2023	
P720	General Isolation	PDA	Fungus	2023-000015	Direct	Yu-Hsuan	Fan	1/6/2023	
P721	General Isolation	PDA	Fungus	2023-000016	Direct	Yu-Hsuan	Fan	1/6/2023	

Plate number: The number is **auto-generated**.

Project: Choose an existing one or create one for yourself for latter identified.

Media: The media to isolate your microbes.

Isolation part: The part of the environment samples you isolated your microbes.

Insect_Env_Cataloged #: The catalog number of the environmental samples.

Dilution: The dilution factor of this particular plate for isolation (e.g., 0.001, 0.01, or Direct).

Cataloger First Name & Cataloger Last Name: The name of who uploads the data.

Cataloged Date: The date on which the data is uploaded.

Notes: Any information you wanted to remark on the certain plate. Leave it when you didn't have any notes.

- 4.4 Write the plate information on the downside of the plate following the example photo shown below.

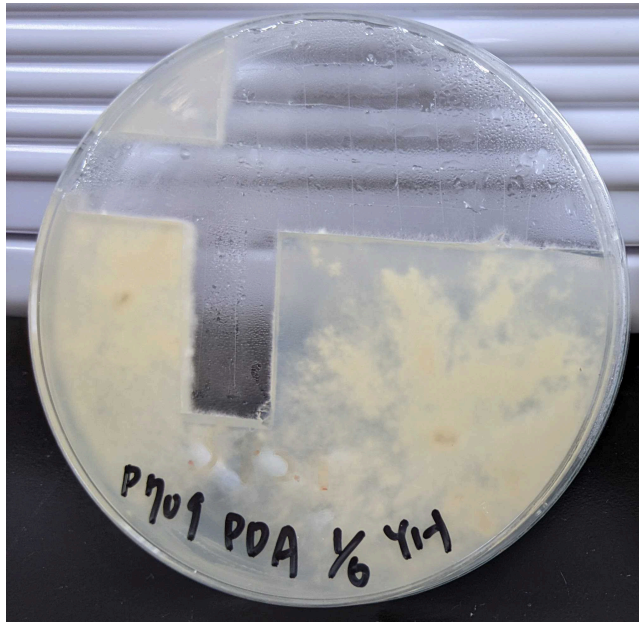


Plate number, Media, Date, Your name

- 4.5 **Make sure that you sterilize every tool before using them for each isolation:**
1. Dip the scalpel, tweezer, or spreader into 95% ethanol cooling bottle, and flame it immediately.
 2. Repeat the step(dip and flame) **THREE times** for sterilization of equipment.
- 4.6 Now, you can start to isolate microbes from your environmental samples. We called them primary cultures.
- **Isolation method varied depend on the type of sample, check published papers or consult with seniors if needed****
- 4.7 After finishing all the isolation, seal the plates with grafting tape for 2 rounds.



4.8 Pack all the plates using a PP bag (no. 8, 10, or 12 depending on how many plates you have). Incubate the plates in your slot in the incubator for 7~21 days (depending on the growth rate of the microbes).

no. 8: 8 plates

no. 10: 24 or 32 plates

no. 12: 48 or 56 plates



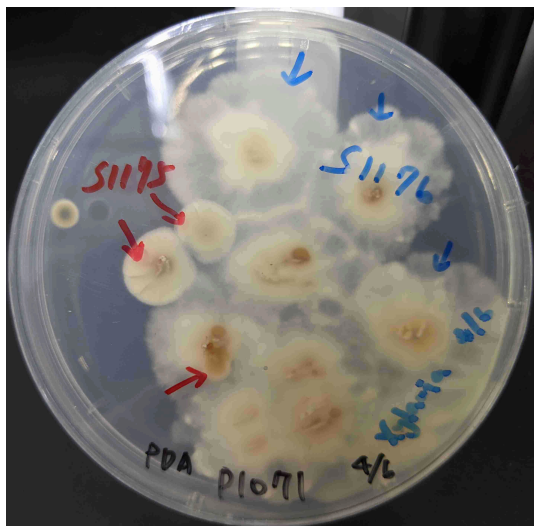
Flip the PP bag over when keeping it in the incubator. The cover of the plates needed to be facing downwards to prevent the microbes from drowning by the water vapors.

4.9 Check the growth condition of the primary cultures every day. **Make sure they are not overgrown.**

Once they are matured, you can start to subculture them.

Record and upload the data of primary cultures

- 5
 1. Examine the microbes colonies under a dissecting microscope.
 2. Choose your desired colonies, which are your targeted colonies.
 3. Mark the targeted colonies with their later corresponding secondary plate number.
 4. If there is more than one morphotype targeted colony in a certain plate, you need to record them into a distinct plate number, e.g., **TWO morphotypes will have TWO distinct secondary plate numbers** from the same primary plate number.



- 5.1 Record all the data of primary cultures to the second worksheet: **Secondary Isolate PDA** (in 1 Fungi Isolation Plates (Direct edit) 菌株分離培養基編號 (直接編輯)).
Fill in all the columns following the photos and instructions shown below.

A	B	C	D	E	F	G	H	I
Plate number	Source primary plate	Project	Media	Isolation Method	Substrate Catalog	Dilution	Cataloger Fir	Cataloger La
S1016	P894	General Isolation	MEA	Head	2023-000073	1	Guan Jie	Phang
S1017	P895	General Isolation	PDA	Head	2023-000073	0.1	Guan Jie	Phang
S1018	P896	General Isolation	MEA	Head	2023-000073	Direct	Guan Jie	Phang
S1019	P897	General Isolation	PDA	Head	2023-000073	Direct	Guan Jie	Phang
S1020	P898	General Isolation	MEA	Head	2023-000070	1	Guan Jie	Phang
S1021	P899	General Isolation	PDA	Head	2023-000070	0.1	Guan Jie	Phang
S1022	P900	General Isolation	MEA	Pronotum	2023-000070	1	Guan Jie	Phang
S1023	P901	General Isolation	PDA	Pronotum	2023-000070	0.1	Guan Jie	Phang
S1024	P902	General Isolation	MEA	Abdomen	2023-000070	1	Guan Jie	Phang
S1025	P903	General Isolation	PDA	Abdomen	2023-000070	0.1	Guan Jie	Phang

Plate number: It is **auto-generated**.

Column B to Column I: You can copy and paste directly from the Column A to Column H of the first worksheet **Primary Isolate MEA**; (Hint: **GREEN columns to GREEN columns**)

A	B	J	K	L	M	N	O
Plate number	Source primary plate	Morph description	CF	CFU_tr	CFU_pro	Cataloged Date	Notes
S1018	P896	P896_contaminated				02/27/2023	discard
S1019	P897	P897_contaminated				02/27/2023	discard
S1020	P898	P898_fusarium_like	64	640	31.68	02/27/2023	
S1021	P899	P899_fusarium_like	16	1600	40.00	02/27/2023	
S1022	P900	P900_fusarium_like	50	500	25.00	02/27/2023	
S1023	P901	P901_fusarium_like	50	5000	50.00	02/27/2023	
S1024	P902	P902_fusarium_like	60	600	30.00	02/27/2023	
S1025	P903	P903_Fusarium	40	4000	25.00	02/27/2023	
S1026	P884	P884_hairy_yeast	1	10	5.56	02/27/2023	
S1027	P890	P890_thick_aerial_hyphae	16	160	18.82	02/27/2023	
S1028	P892	P892_spiky_yeast	10	100	1.26	02/27/2023	
S1029	P893	P893_circular_yeast	2	200	0.25	02/27/2023	
S1030	P894	P894_circular_yeast	12	120	0.75	02/27/2023	
S1031	P894	P894_white_aerial_hyphae	1	10	0.06	02/27/2023	
S1032	P898	P898_circular_yeast	138	1380	68.32	02/27/2023	
S1033	P899	P899_circular_yeast	24	2400	60.00	02/27/2023	
S1034	P900	P900_circular_yeast	150	1500	75.00	02/27/2023	
S1035	P901	P901_circular_yeast	50	5000	50.00	02/27/2023	
S1036	P902	P902_hemisphere_yeast	60	600	30.00	02/27/2023	
S1037	P902	P902_cerebriform_yeast	10	100	5.56	02/27/2023	

Morph description: This is generated automatically by the formula. Replace **YOUR DESCRIPTION** with what you do observe on your targeted colony under a dissecting microscope. Type **contaminated** if you got others than your target or all the colonies were overgrown by common molds.

CFU: The number of colonies of your target in a plate.

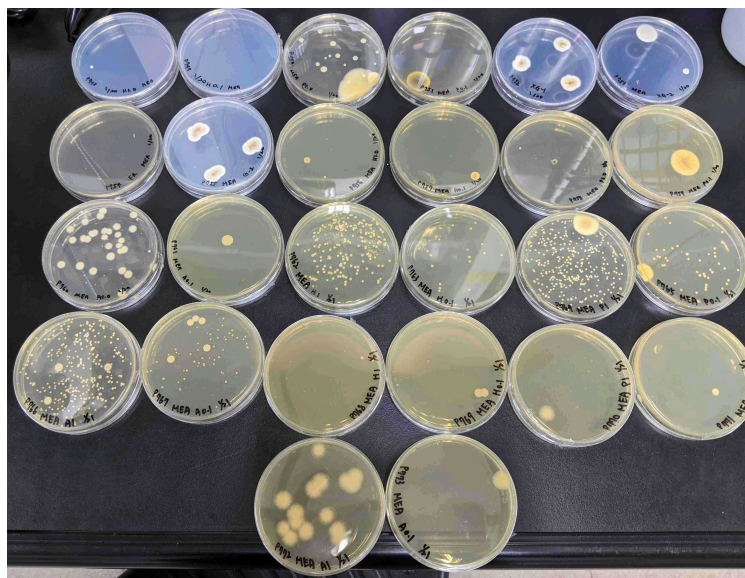
CFU_true: It is **auto-filled** based on the dilution factor of each plate.

CFU_proportion: The proportion of your targeted colony in a certain plate (i.e. what percentage of your target among all the observed colonies on the plate).

Cataloged Date: The date you do the subculture and upload the data.

Notes: Any information you think is important to record. If you have **contaminated** in Morph description, please type discard here.

- 5.2 Record or take a photo of the order of your plates that are ready for scanning. **6** plates as a group, so you can refer back during the photo editing.

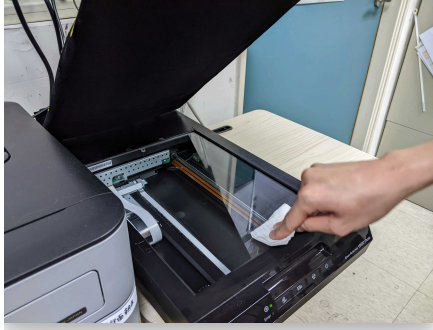


- 5.3 Before scanning plate, you need to sterilize the environment and scanner glass using paper towels with 75% ethanol.



- 5.4 Wipe the scanner glass using paper towels with dishwashing detergent (this prevents water condensation on the glass).



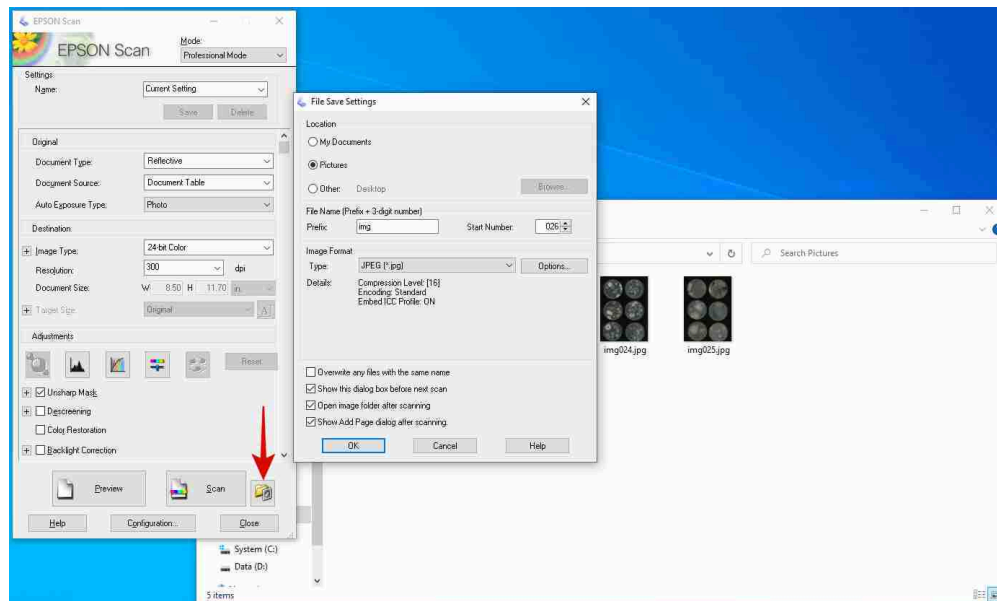


- 5.5 Keep the order of the plates, remove the plates cover, and put the plates upside down onto the scanner glass.

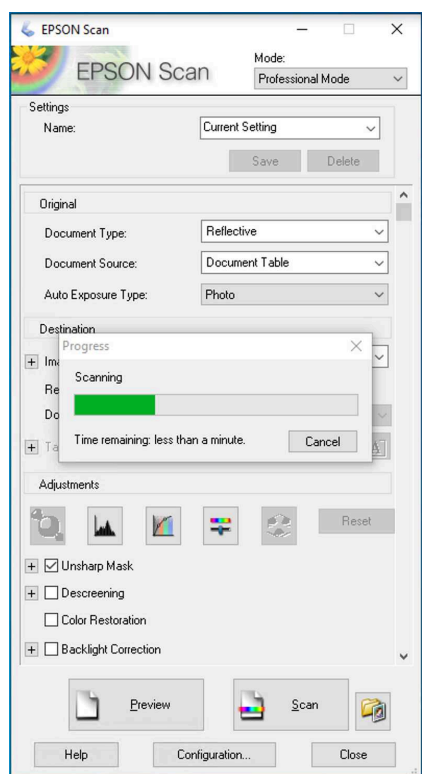
****Do not change the order before and after scanning****



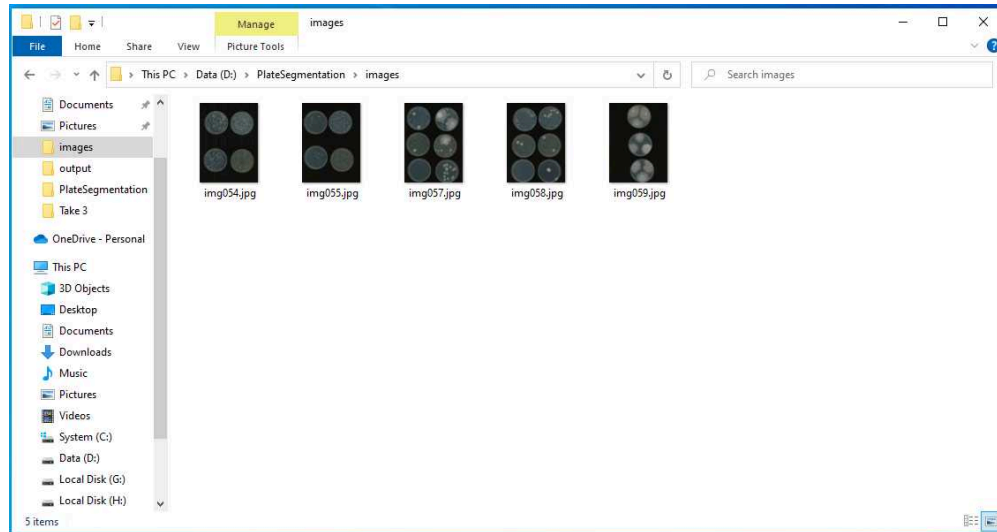
- 5.6 Search and open **EPSON Scan** software. Choose **Professional mode**. Go to the **File Save Settings** (red arrow), and make sure the image format is **.jpg** (follow all the settings as the photo below shown).



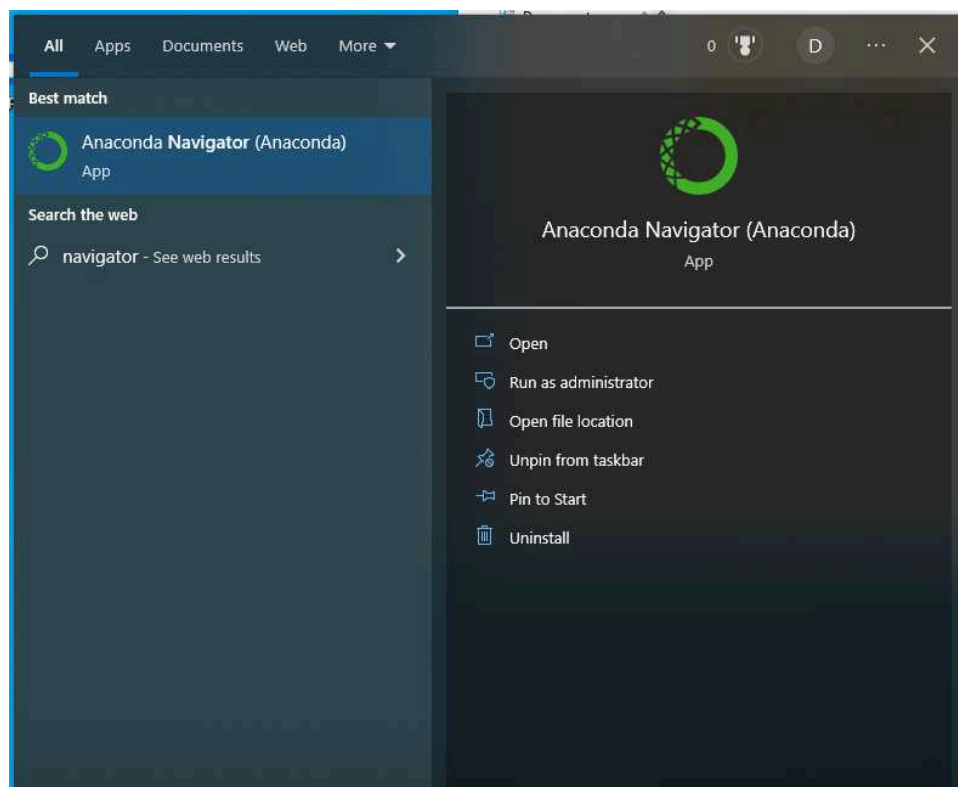
5.7 Then, click **OK**. (It will start scanning like the photo shown below).



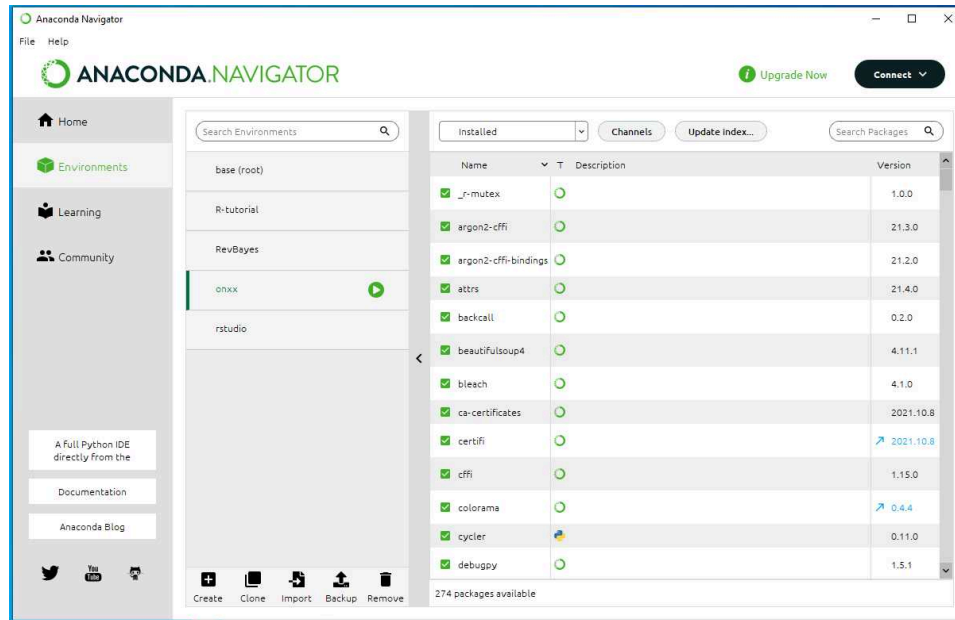
5.8 Move the files from your **Pictures** folder to the **D:\PlateSegmentation\images**



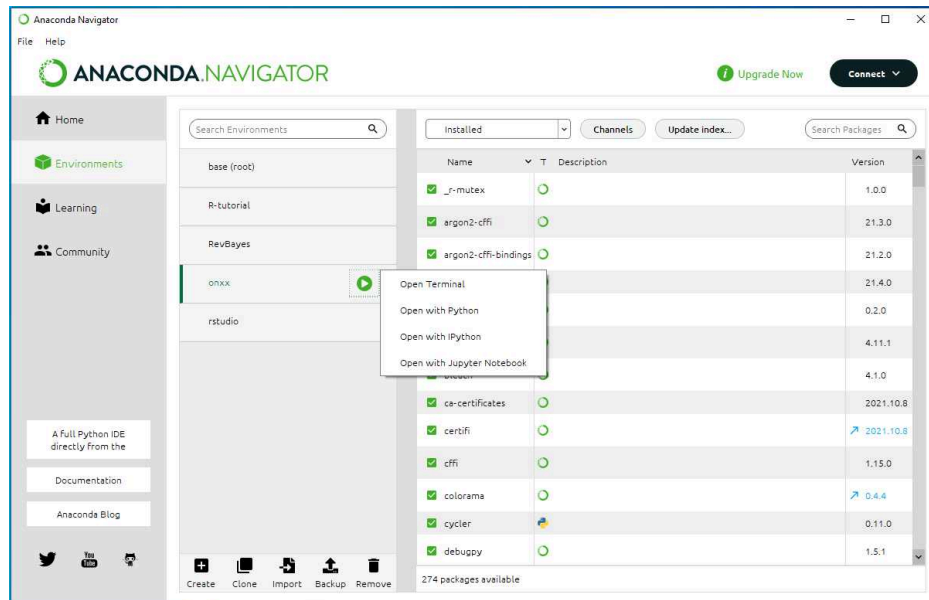
5.9 Search and open **Anaconda Navigator** software.



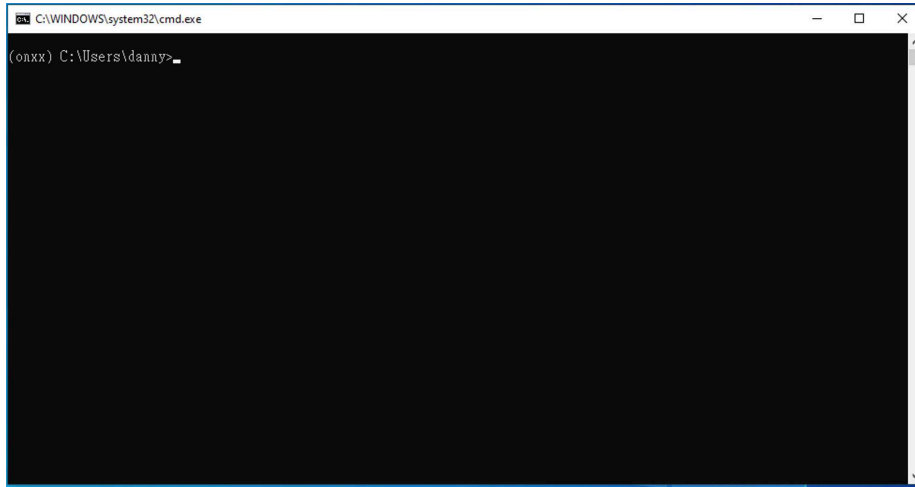
You will see the interface as the photo shown below.



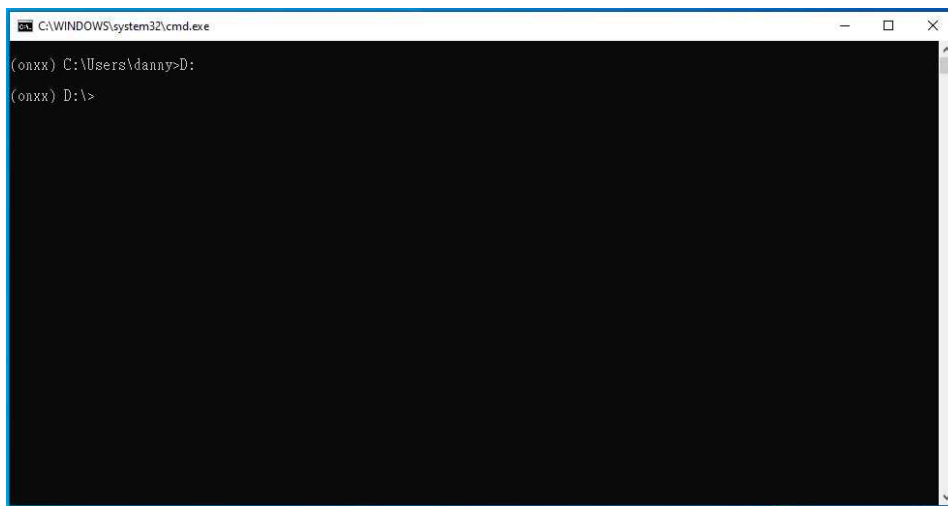
5.10 Select **onnx** under **Environments** tab (as the photo shown below).



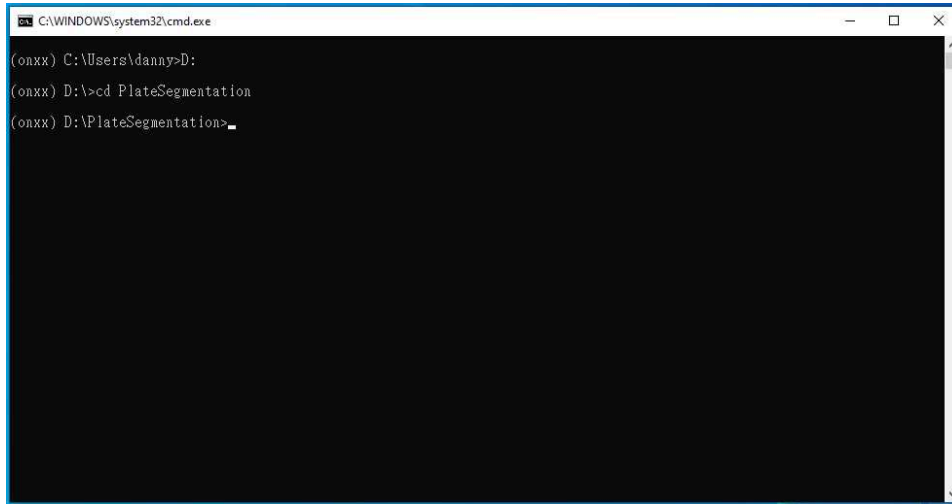
Left click the **play button**. Select **Open Terminal**.



- 5.11 Follow the commands, and you will have the similar output as the photos shown below.
Type **D:** , then press **enter**



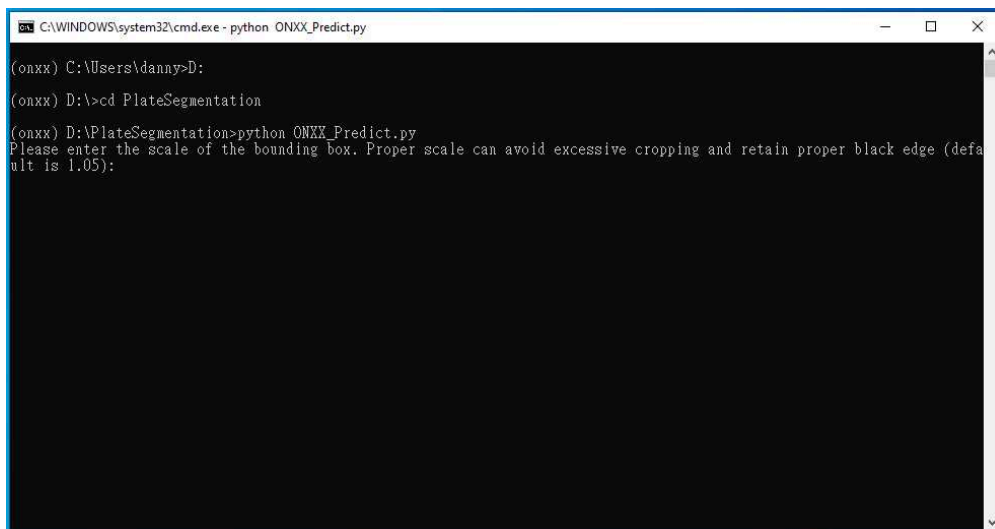
Type **cd PlateSegmentation** , then press **enter**



```
C:\WINDOWS\system32\cmd.exe

(onxx) C:\Users\danny>D:
(onxx) D:\>cd PlateSegmentation
(onxx) D:\PlateSegmentation>_
```

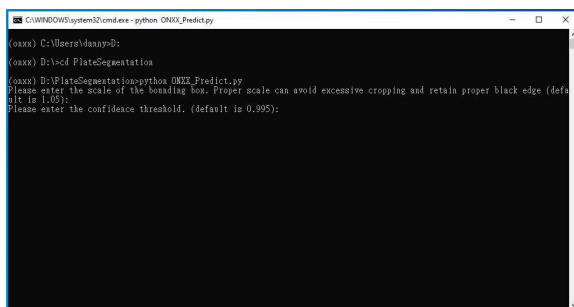
Type **python ONXX_predict.py** , then press **enter**



```
C:\WINDOWS\system32\cmd.exe - python ONXX_Predict.py

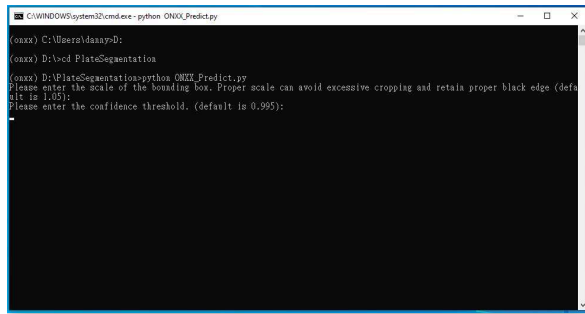
(onxx) C:\Users\danny>D:
(onxx) D:\>cd PlateSegmentation
(onxx) D:\PlateSegmentation>python ONXX_Predict.py
Please enter the scale of the bounding box. Proper scale can avoid excessive cropping and retain proper black edge (default is 1.05):
```

Press **enter** again and again. You will see a **_** flashing.



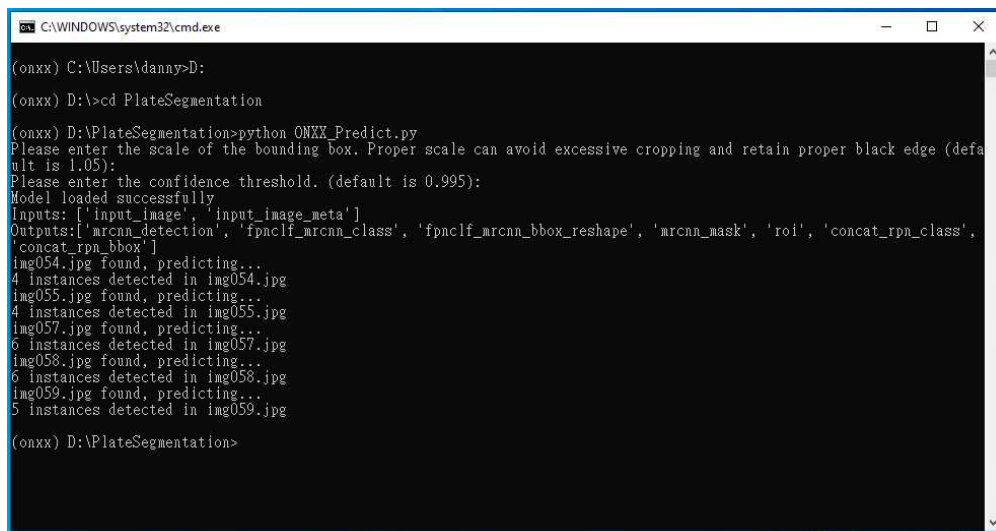
```
C:\WINDOWS\system32\cmd.exe - python ONXX_Predict.py

(onxx) C:\Users\danny>D:
(onxx) D:\>cd PlateSegmentation
(onxx) D:\PlateSegmentation>python ONXX_Predict.py
Please enter the scale of the bounding box. Proper scale can avoid excessive cropping and retain proper black edge (default is 1.05):
Please enter the confidence threshold. (default is 0.995):
```



```
C:\WINDOWS\system32\cmd.exe - python ONXX_Predict.py
(onxxx) C:\Users\danny>D:
(onxxx) D:\>cd PlateSegmentation
(onxxx) D:\PlateSegmentation>python ONXX_Predict.py
Please enter the scale of the bounding box. Proper scale can avoid excessive cropping and retain proper black edge (default is 1.05):
Please enter the confidence threshold. (default is 0.995):
```



Then, a new command line comes out, which represent the program execution was complete.



```
C:\WINDOWS\system32\cmd.exe
(onxxx) C:\Users\danny>D:
(onxxx) D:\>cd PlateSegmentation
(onxxx) D:\PlateSegmentation>python ONXX_Predict.py
Please enter the scale of the bounding box. Proper scale can avoid excessive cropping and retain proper black edge (default is 1.05):
Please enter the confidence threshold. (default is 0.995):
Model loaded successfully
Inputs: ['input_image', 'input_image_meta']
Outputs: ['mrcnn_detection', 'fpnclf_mrcnn_class', 'fpnclf_mrcnn_bbox_reshape', 'mrcnn_mask', 'roi', 'concat_rpn_class', 'concat_rpn_bbox']
img054.jpg found, predicting...
4 instances detected in img054.jpg
img055.jpg found, predicting...
4 instances detected in img055.jpg
img057.jpg found, predicting...
6 instances detected in img057.jpg
img058.jpg found, predicting...
6 instances detected in img058.jpg
img059.jpg found, predicting...
5 instances detected in img059.jpg
(onxxx) D:\PlateSegmentation>
```

5.12 The output files are in the **D:\PlateSegmentation\output**.



- 6 Follow  [go to step #4.4](#) to  [go to step #4.9](#) for the subculture of primary cultures. Now, we called them **secondary cultures**. You may need to keep the primary cultures for a while before the secondary plate are all secured.

Ready for preservation

- 7 After 7-21 days (depending on the growth rate of your targeted microbes), the secondary cultures are ready to be preserved. If the secondary cultures were contaminated, please redo the subculture from the primary cultures.
- 7.1 Before prepare preservation vials, the **For Specify cataloging COPY to MODIFY!!!** worksheet need to be filled.

A	B	C	D	E	F	G	H
Catalog Number	Accession Number	Genus 1	Species 1	Isolation Method	Substrate Cataloged #	Verbatim Date	Cataloged Date
	S1169			Fungus	2023-000117	04/08/2023	
	S1170			Gut	2023-000117	04/08/2023	
	S1171			Gut	2023-000117	04/08/2023	
	S1172			Gut	2023-000117	04/08/2023	
	S1173			Fungus	2023-000117	04/08/2023	
2023-000XXX	S1174			Fungus	2023-000117	04/08/2023	

Catalog Number: It is auto-generated from Specify, fill in after you upload the data.

Column B, Column E to Column G: It is synchronized from the **Secondary Isolate PDA** worksheet.

Genus 1 & Species 1: Only fill in when you can identify them.

Cataloged Date: Same as previous spreadsheet.

I	J	K	L	M
Collector First Name	Collector Last Name	Determination First Name	Determination Last Name	Determination Date
Guan Jie	Phang			
Guan Jie	Phang			
Guan Jie	Phang			
Guan Jie	Phang			
Guan Jie	Phang			
Guan Jie	Phang			

Collector First Name & Collector Last Name: Same as previous spreadsheet.

Column K to Column M: Same as previous spreadsheet.

There is 2 part for all the locality information, you need to replace the each information: spreadsheet code (red arrow pointed), worksheet name (purple arrow pointed) with your own spreadsheet information.

Part 1

The screenshot shows a Google Sheet titled "Environmental Samples". The data table has columns labeled N through T. Column N is "Locality", O is "District", P is "County", Q is "State/Province", R is "Country", S is "Latitude", and T is "Longitude". The formula in row 175, column N is highlighted in blue. Red arrows point to this formula. Purple arrows point to the worksheet name "Environmental Samples" in the bottom left corner.

Part 2

Part 1: Replace the red arrow pointed region of the written formula with your own spreadsheet code (highlighted region).

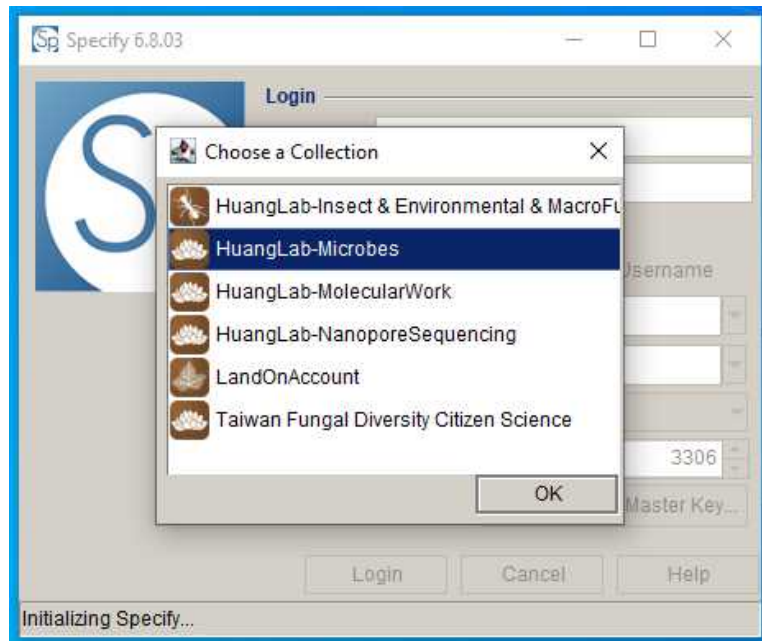
Part 2: Replace the purple arrow pointed region of the written formula with your own worksheet name (blue shaded region).

If the Cataloged Date is filled, the Column U to Column Z is auto-filled with the written formula. Do not change it unless it needed. The Column AA is synchronized with the Column O of the **Secondary Isolate PDA** worksheet.

U1047 `=IFS(H1047="", "", H1047<>"", "Water Viol")`

	U	V	W	X	Y	Z	AA
1	Prep Type	Prep Count	Room	Storage	Cataloger First Name	Cataloger Last Name	Note
1035	Water Vial	2	927	25C	Guan Jie	Phang	
1036	Water Vial	2	927	25C	Guan Jie	Phang	
1037	Water Vial	2	927	25C	Guan Jie	Phang	
1038	Water Vial	2	927	25C	Guan Jie	Phang	
1039	Water Vial	2	927	25C	Guan Jie	Phang	
1040	Water Vial	2	927	25C	Guan Jie	Phang	
1041	Water Vial	2	927	25C	Guan Jie	Phang	
1042	Water Vial	2	927	25C	Guan Jie	Phang	
1043	Water Vial	2	927	25C	Shan-Yu	Chen	
1044	Water Vial	2	927	25C	Yu-Hsuan	Fan	
1045	Water Vial	2	927	25C	Guan Jie	Phang	
1046	Water Vial	2	927	25C	Guan Jie	Phang	
1047							discard
1048							discard

- 7.2 Once the spreadsheet is filled, download the data with .csv format. Open **Specify**, go to **HuangLab-Microbes** collection.

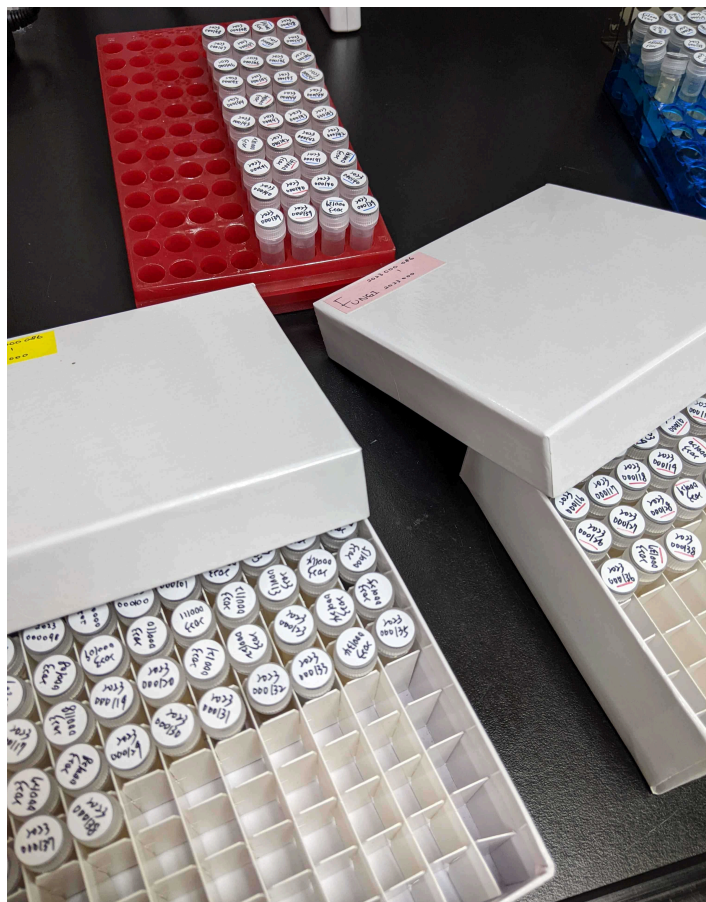


7.3 Follow [⇒ go to step #3.1](#) to [⇒ go to step #3.13](#) for preservation vials data upload.

7.4 For each **Catalog Number**, you must prepare **2** water vials and **2** glycerol vials. You also need a 2 ml microtube with lysis mixture and beads (for details, check **DNA extraction protocols**).

Each tube needed a sticker with its corresponding **Catalog Number** on top of the cap. The label should be written with a black color marker pen or a technical pen alternative.

Please follow the example in the photo shown below.



**For water vials: either tube must have a RED underline.
For glycerol vials: both tubes must have a BLUE underline.**

The lysis mixture microtubes that are ready for use will look like this.



- 7.5 Operate UV sterilization of the laminar flow for 15 minutes. After UV sterilization, you need to ethanol-sterilize the materials that are going into the laminar flow.

Light up the alcohol lamp and flame the material.

****The materials you need are the same as [go to step #4](#) , but you only need a scalpel instead of a tweezer and a spreader.****

Culture preservation

- 8 Preserve the plates with one water and one glycerol vial for one colony. The rest is for DNA extraction. Make sure you flame-sterilize the scalpel every time before slicing the agar.

****The details of culture preservation are not provided here.****

- 8.1 Store the preservation vials in their corresponding freezing box.

For water vials, you can keep them directly.

For glycerol vials, you need to keep them in a freezing container and then freeze them inside a -20°C fridge for 1 day before keeping them in the -80°C fridge collection.

****The freezing boxes of water vials are kept in the steel cabinet in front of Prof. Huang's office; the freezing boxes of glycerol vials are kept in the -80°C fridge outside N927****



The tubes are needed to be kept in the order shown above, but not the other way around.