

Tiny Earth website: tinyearth.wisc.edu Tiny Earth Database website: data.tinyearth.wisc.edu

Questions? Email tinyearth@wid.wisc.edu

# Tiny Earth Database Tutorial

A step-by-step guide for students entering soil, culture, and isolate data into the global database



## Welcome to the Tiny Earth network!

This is a guide for students using the Tiny Earth Database. The Database is a resource for students, instructors, and the public, therefore accurate and thorough information is expected. The more we know about your isolates, the further we can chase their antibiotic capabilities.

Some information in this database is required, but some is optional based upon what data you are collecting in your classroom, so don't worry if you are missing information for some fields on the site. Please fill in all data your instructor had you collect and/or relevant to your particular experiments.

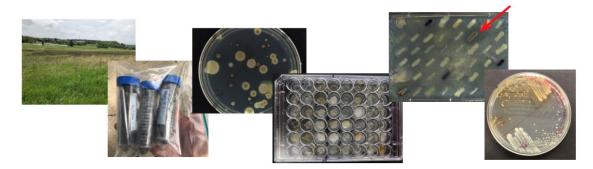
You are encouraged to take photos of your samples--soil, plates, tubes, individual isolates--along the way, as there are several opportunities to upload photos to the Database. Please follow appropriate PPE guidelines for your lab when taking photos.

Once your data is entered into the Database and the Tiny Earth Chemistry Hub (TECH) receives your isolates, data may be updated with further analysis completed at TECH. So stay connected!



## In the Tiny Earth Database, you will submit data about your...

- 1. Soil sample collection site and soil characteristics
  - Most students will work with one soil sample over their Tiny Earth course
- 2. Culturing conditions
  - You may have one culture condition or multiple per soil sample. Be sure to record all culture conditions and keep isolates aligned with their culture conditions.
- 3. Isolate information
  - Once you have found an antibiotic producer against at least one ESKAPE safe-relative, you may have many types of data about the isolate. Isolate ESKAPE screen data is required, but there are options to enter 16S rRNA PCR results, chemical extract screens, and eukaryotic tests.
  - You may have more than one Isolate per culture condition.





## **Data Entry Process**

## SOIL SAMPLE 1





Media: PDA Temperature: 28C Incubation time: 24 hours

## **CULTURE CONDITION 2**

Media: TSA10% Temperature: 28C Incubation time: 24 hours





Create master plate if isolates. Screen against ESKAPE relative. Identify antibiotic producing isolates.

## ISOLATE 1 Enter relevant data, add photos



## ISOLATE 2

Enter relevant data, add photos





Create master plate if isolate: Screen against ESKAPE relative. Identify antibiotic producing isolates.



## **ISOLATE 3**

Enter relevant data, add photos



## **ISOLATE 4**

Enter relevant data, add photos





## **ISOLATE 5** Enter relevant data, add

photos



1. SOIL DATA Soil and environmental characteristics, sample date and location

#### 2. CULTURE DATA Media and other

culture conditions, number of bacteria screened against ESKAPE relatives, optional antibiotic resistance testing.

#### 3. ISOLATE DATA ESKAPE screen results, including ESKAPE strains with positive and negative results.

Option to enter 16S rRNA PCR, chemical extraction, and eukaryotic test data.



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# Make an Account

You should receive an email from tiny-earth-no-reply@discovery.wisc.edu inviting you to create an account on the Tiny Earth database.

Click on the "Accept Invitation" link and you'll be directed to www.data.tinyearth.wisc.edu to set up your account.

Creating an account and entering your Tiny Earth data will ensure your soil isolates stay in the antibiotic pipeline.

### Invitation instructions



tiny-earth-no-reply@discovery.wisc.edu Wed 5/29/2019 11:42 AM

Hello tinyearth@wid.wisc.edu,

You are invited to create an account on the Tiny Earth Database. You can accept the invitation through the link below.

### Accept invitation

If you don't want to accept the invitation, please ignore this email. Your account won't be created until you access the link above and set your password.

# Make an Account

Enter your name and create a password.

Password should be at least 8 characters, and contain at least 1 character from 3 of the following categories: Uppercase (A-Z), Lowercase (a-z), Digit (0-9), Special Characters (#?!@\$%^&\*-)

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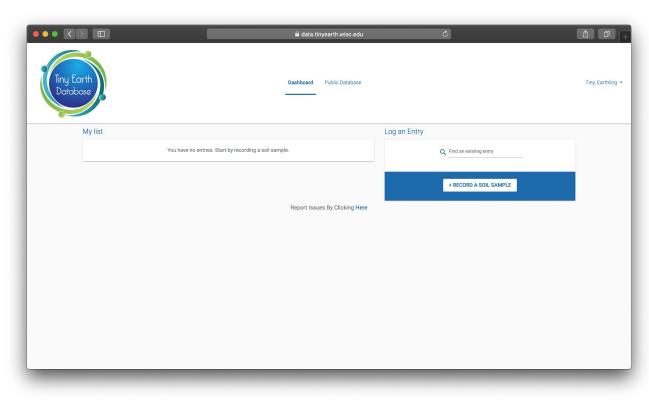


# Enter Soil Data

Yay! You have successfully registered for a Tiny Earth Database account. Now it's time to enter your data.

First, you must enter a soil sample.

Click "RECORD A SOIL SAMPLE"



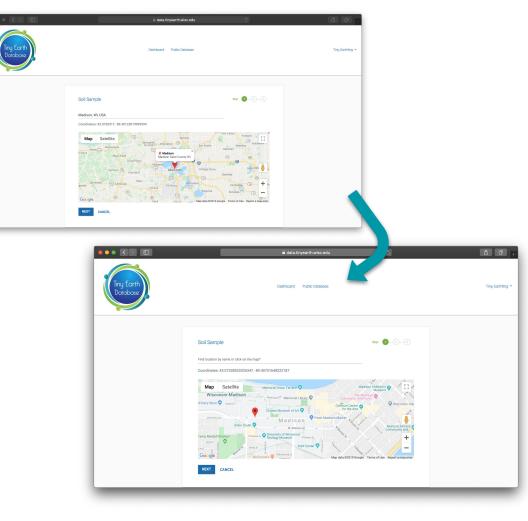


# Enter Soil Data Step 1: Location

You can enter an address or city in the search bar, or click on the map and drag the pin to the specific soil collection site.

If you move the pin, assure that the coordinates are changing to the appropriate location.

Click "NEXT"





# Enter Soil Data Step 2: Environmental Characteristics

- Fri. Nov 08 6 0 lovember 2019 Tirry Earthling no 0-0 Soil Sample CANCEL with a lot of foot traffic and near a Fri. Nov 08 NEXT BACK 15
- 1. Select the date and time of your collection
  - Click on the calendar icon to select from the calendar and clock.
- 2. Describe the area surrounding the soil collection site
  - Provide information regarding vegetation, traffic, weather conditions, etc.
- 3. Enter air temperature in celsius

Note: clicking on the "i" (on any page) will clue you as to what should be included in that field.

If soil was collected from private land, click "UPLOAD PRIVATE SOIL DONOR FORM" and upload signed soil donor form.

Click cone to find a link to download the private soil donor form template.



# Enter Soil Data: Step 2: Private Soil Forms

If you collected soil from private land, you MUST submit a form with approval from the land owner to use the soil.

If your soil sample is from private land, this form will be provided by your instructor.



#### TinyEarthSoilRecruitmentTemplateGeneric\_12.10.2018.pdf (page 2 of 2)

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## You can help!

Instead of taking small samples from soil on campus, **Tiny Earth** students are maximizing microbial diversity and collecting soil samples from across our region. (Photo by Bryce Richter / UW-Madison)



### We'd like a piece of your dirt!

If you agree to donate a soil sample to our research, we will update you throughout the semester on our findings, including description of the antibiotic-producing bacteria discovered. At the end of the semester, we will invite you to our poster session on campus where **Tiny Earth** students will explain details of our findings and you can explain the soil's unique characteristics and uses.

Thank you for supporting Tiny Earth!

Soil Donor's Information:
Name:
Email Address:
Signature:

In return for your donation, we promise that any money we may earn on discoveries made using your dirt will be used exclusively for non-profit education and research.



# Enter Soil Data: Step 3: Soil **Characteristics**

Enter

- Depth of sample collection
- Type of soil
  - Choose from the  $\bigcirc$ drop-down menu

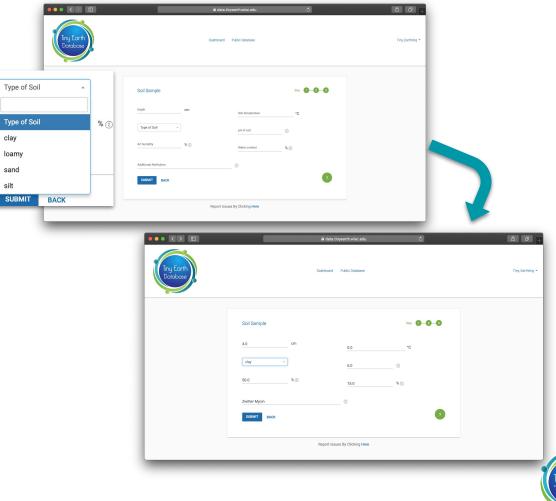
clay

sand

silt

- Air humidity
- Soil temperature
- pH of soil
- Soil water content

Include your partner's name in "additional attribution" if another person was involved with making or using these isolates



# Enter Soil Data: **Step 3: Soil Characteristics**

For extra assistance with entering soil characteristic data, click on 
and more information for each field will appear.

### Tips

#### Type of Soil

Soil type usually refers to the different sizes of mineral particles such as sand (particle size > 63  $\mu$ m), silt (particle size > 2  $\mu$ m) and clay (particle size < 2  $\mu$ m). Sandy soil is composed mostly of sand. Loamy soil is composed mostly of a mix of sand and silt. Clay soil is mostly composed of clay. Silt soil is mostly composed of silt.

#### **Air Humidity**

Air humidity is the amount of water vapor present in air, and it is presented as the percentage of the current absolute humidity to the highest possible absolute humidity. Air humidity can be easily found on a weather

app.

#### Soil Temperature

Measuring soil temperture requires inserting a thermometer into the soil at the time and point of collection.

#### pH of Soil

Soil pH can be measured in the lab using a pH meter or test strip.

#### Water Content

Soil water content can be measured by comparing wet mass of soil to dry mass of soil (soil can be dried in an oven or by leaving at room temperature for a period of time).



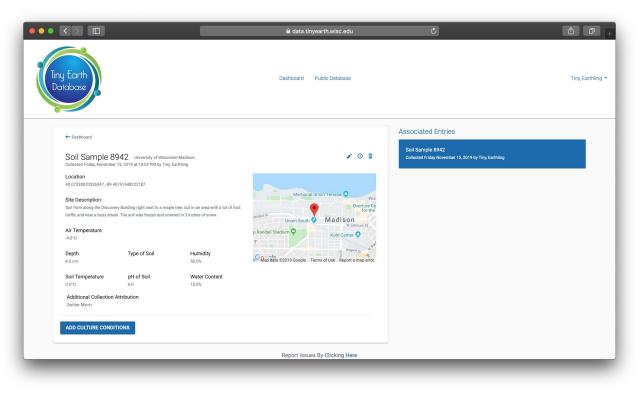
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# Enter Soil Data Finished Page

Once you have completed all 3 steps for soil sample data entry, you will be directed to a page that looks like this.

You can always go back and edit information for this sample by clicking on the  $\checkmark$  or deleting the entry by clicking on the  $\boxed{10}$ .

The soil sample number, in this case 7636, is specific to your soil sample and can be searched in the database by others in your class.



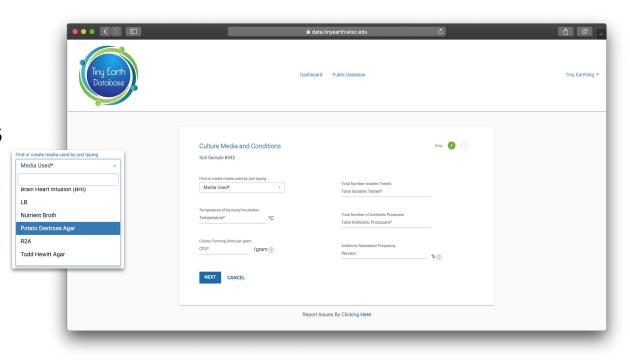


# Enter Culture Conditions **Step 1: Media and quantity of isolates tested**

On the Soil Sample page, click "ADD CULTURE CONDITIONS"

First, select the media you grew your soil dilutions on. You may type a different media and click on it if your media is not on this list.

Be sure to fully spell out any media used not on this list.





# Enter Culture Conditions Step 1: Media and quantity of isolates tested

Next, enter:

- Temperature (in celsius) of incubation between the time you plated your soil dilution and counted CFUs
- Colony forming units (CFUs)/gram of soil. CFUs must be entered in scientific notation, for example 1.82e5
- Total number of bacterial isolates tested (aka screened against at least one ESKAPE safe-relative)
- Total number of antibiotic producers against at least one ESKAPE safe-relative

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(Tiny Earth Database)		Dashboard	Public Database		$\neg in_{\mathcal{K}} Earthleg ~ \bullet$
	Culture Media and Conditions Soil Sample 9442 Title or overle media used by just typing Petraio Destrose Ager "amportune of bacterial involution 2 "amportune of bacterial involution 2 Colory formato Units per gram 1.22e5 / gram (5) ENCEL		Total Humber too ates Tested 24 Intel Humber of Ant Socte Hocusers 7 Antibilito Sesticance Pregurecy 21	ang 🌒 — 🔅	
		Report Issu	es By Clicking Here		

Note: If isolates were tested for antibiotic resistance, against streptomycin for example, record the percent resistant out of those tested and indicate which antibiotic was used on Step 2.



# Enter Culture Conditions Step 2: Photos of dilution plates and descriptors

You are encouraged to upload photos of your dilution plates here.

Click "UPLOAD PHOTOS"

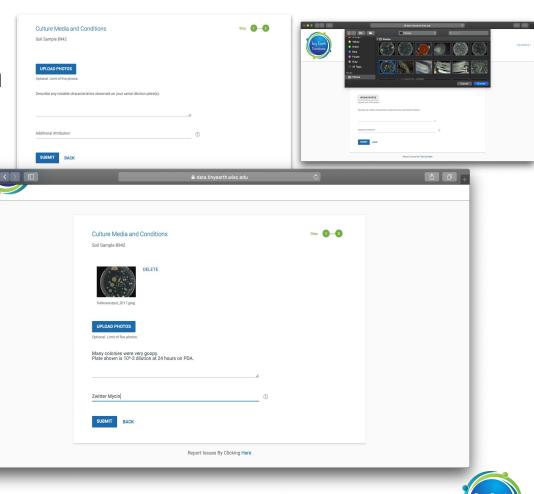
Describe the photo, noting specific characteristics or unique qualities.

Some things to include:

- Hours or days of growth
- Dilution factor

Include your partner's name in "additional attribution" if another person was involved with making or using these isolates

Click "SUBMIT"

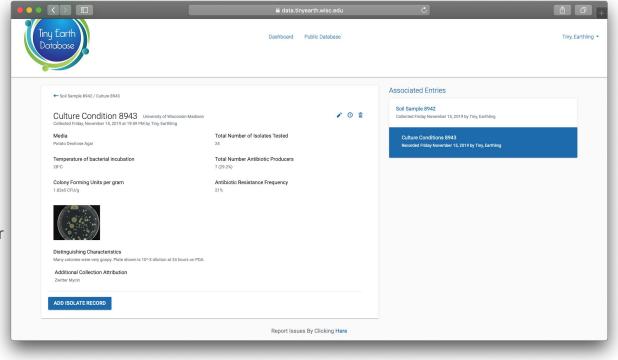


# Enter Culture Conditions Finished Page

Again, you can always edit or delete Culture Condition entries.

You may also go back to the Soil Sample entry and create multiple culture conditions under one soil sample.

Click "ADD ISOLATE RECORD" to enter data for single isolates.



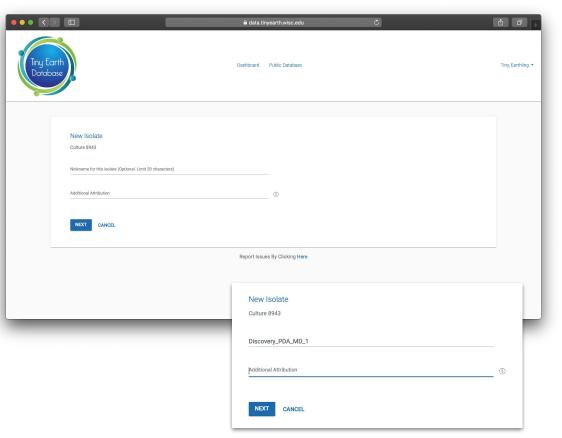


# Enter New Isolate Record

Enter a "nickname" for your antibiotic-producing isolate. You may call it whatever you'd like.

Once your isolates are published, the nickname you give it will be accessible to the public. Choose something that helps you identify your isolate. The Database will give your isolate a number which will be linked to this nickname. Keep both identifiers for your records.

Include an "additional attribution" if another person was involved in finding this isolate.





# Enter New Isolate Record ESKAPE Test

Select appropriate box for ESKAPE screen.

If the isolate showed antibiotic activity against a specific ESKAPE, click "yes" and enter the hours between plating and reading the screen and the media screened on.

If the isolate did not show antibiotic activity, click "no"

If an ESKAPE was not tested, click "not tested."

If you screened your isolate against a bacteria that is not on the list, click "Add another bacteria" and enter the species and screening results.

Once finished, click "NEXT"

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Isolate ESKAPE Screen Culture 8943				5tep <b>()</b> —(2)
Antibiotic Activity* Mycobacterium smegmatis	- Yes - No 👩 Not tested			
Enterbacter ærogenes	Ves 👩 No 🗆 Not tested			
Pseudomonas putida	Yes 👩 No 🗌 Not tested			
Acinetobacter baylyi	Yes 👩 No Not tested			
Escherichia coli	Tes 👩 No Not tested			
Staphylococcus epidermidis	2 Yes No Not tested		hours Potato Dextrose	
Enterococcus raffinosus Bacillus subtilis	Yes No Not tested		hours Potato Dextrose *	
Add another bacteria	Vies No Notiested	27		
NEXT CANCEL				
		Report Issues By Clicking Here		
Erwinia carotovora		□Yes ☑No □Not	tested	
Add another bacteria				

Note: Knowledge of which ESKAPEs your isolate has been screened against will help the Tiny Earth Chemistry Hub greatly. Thank you!



## Enter New Isolate Record ESKAPE Test

On Step 2 of Isolate ESKAPE Screen, enter any defining characteristics of the screen, such as size of zone of inhibition (measured from edge of isolate growth to furthest point of the zone).

Upload photos of the screen plates.

Click "SUBMIT"

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	Isolate ESKAPE Screen Culture 8943		Step <b>()—(2</b>	
	UPLOAD PHOTOS Optional. Limit of five photos. ZOI vs. 8. epidermidis = 3mm ZOI vs. 8. subtilis = 6mm Very distinct ZOIs.	A		
_	SUBMIT BACK	Report Issues By Clicking Here		



# New Isolate Record **Data Entry Options**

Once you have entered Isolate ESKAPE Screen data, you can also enter

- 16S rRNA PCR results,
- Chemical extraction results, and
- Eukaryotic test results.

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y Earth habase	Dashboard Public Database		Tiny, Earthling 👻
← Soil Sample 8942 / Culture 8943 / Isolate 8945		Associated Entries	
Isolate 8945 Discovery_PDA_MD_1 University of Wisconsin-Madison	✓ ① <sup>1</sup> / <sub>1</sub>	Soil Sample 8942 Collected Friday November 15, 2019 by Tiny, Earthling	
ESKAPE Test Recorded Friday, November 15, 2019 by Tiny, Earthling	*	Culture Conditions 8943 Recorded Friday November 15, 2019 by Tiny, Earthling	
Add Test Results ESKAPE 165 RNA TEST ANTIBIOTIC ACTIVITY OF EXTRACT EUKARYOTIC INHIBITION		Isociato 8945 Recorded Friday November 13, 2019 by Tiny. Earthing	
	Report Issues By Clicking Here		
	Soil Sample 8942 / Culture 8943 / Isolate 8945     Isolate 8945 Discovery, PDA-MD_1 University of Wisconsin Madison     ESKAPE Test     Recorded Fridag November 15, 2019 by Ting Earthling  Add Test Results ESKAPE     Isolate 894     Isolate 894		Dashboard Public Database <ul> <li>Sol Stample 8942 / Culture 8943 / Indiate 8945</li> <li>Isolate 8945 / Indiate 8945</li> <li>Disolate 8945 / Indiate 8945</li> <li>Disolate 8945 / Indiate 8945</li> <li>ESKAPE Test <ul> <li>Bedidate Priday, November 15, 2019 by Ting, Earthing</li> </ul> <b>Add Test Results ESKAPE ESKAPE</b> </li></ul>



# New Isolate Record: 16S rRNA PCR Results

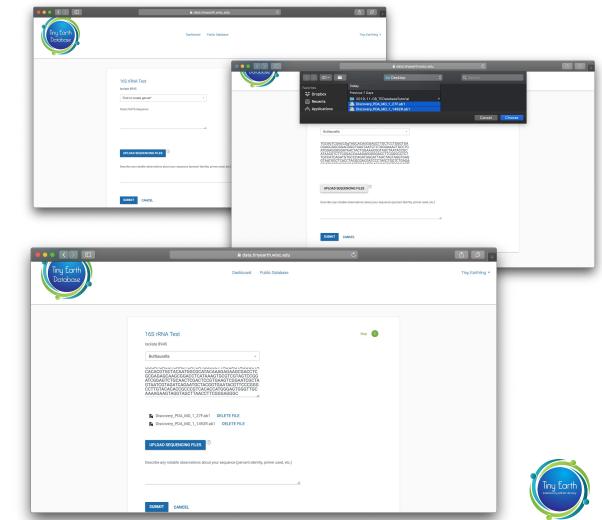
Clicking on "16S rRNA TEST" under "Add test results" will bring you to this page.

Enter the genus of your isolate by choosing from the dropdown menu or typing in a genus that is not present.

Copy and paste the 16S rRNA sequence

Click "UPLOAD SEQUENCING FILES" to submit the original files from 16S sequencing. For example, upload the actual sequence file, not a Word document with the sequence.

Click "SUBMIT"



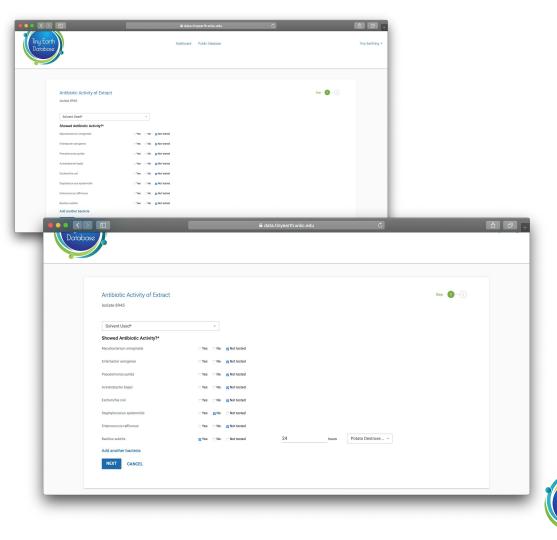
# New Isolate Record: Step 1: Antibiotic Activity of Extract

Back on the isolate homepage, you will see a link to "ANTIBIOTIC ACTIVITY OF EXTRACT" under "Add test results."

Here, select the solvent used for extraction, or enter a different one if yours does not appear on the dropdown menu.

Select appropriate boxes for screening with the chemical extract, following the same procedure as the "Isolate ESKAPE Screen"

Click "NEXT"



# New Isolate Record: Step 2: Antibiotic Activity of Extract

Describe your procedure for chemical extraction on Step 2 of "Antibiotic Activity of Extract."

Note if there were any deviations from the protocol described in the Tiny Earth Manual.

Click "SUBMIT"

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Tiny Earth Database	Dashboard Public Database	Tiny, Earthling 🛩
	Antibiotic Activity of Extract         Joans 8945         Describe your experiment design: (*)         Allowed instructions in Tiny Earth manual "Chemical Extraction of Antibiotics" opage 132:137.         SUMMT         Activity         Activity         Activity         Report Issues By Clicking Here	210 <b>0</b>



# New Isolate Record: Step 1: Eukaryotic Inhibition

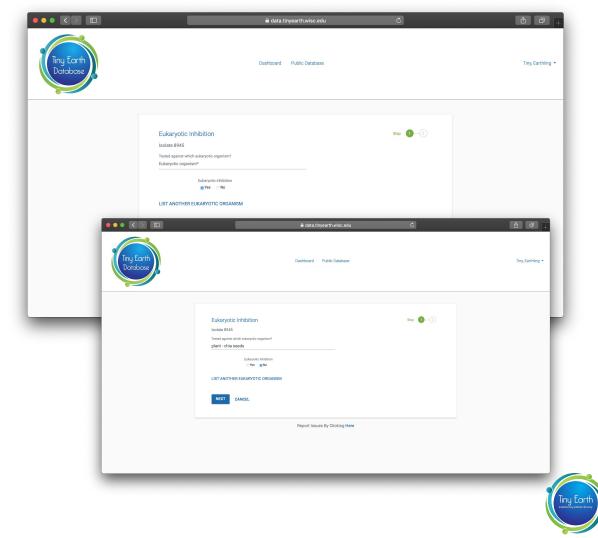
Back on the isolate homepage, you will see a link to "EUKARYOTIC INHIBITION" under "Add test results."

List the organism that your isolate or extract was tested against.

Indicate "yes" or "no" if there was inhibition.

List as many organisms as you tested.

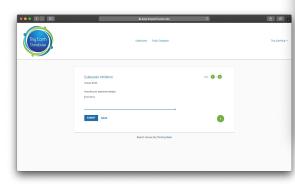
Click "NEXT"

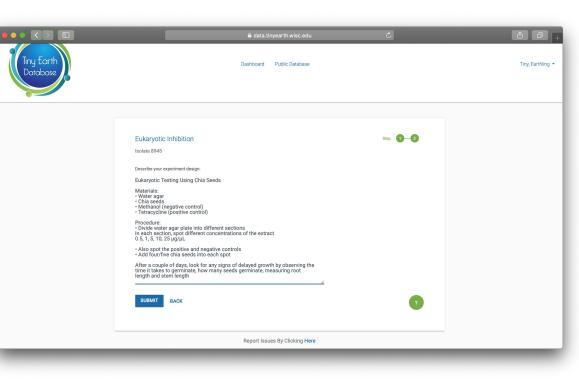


# New Isolate Record: Step 2: Eukaryotic Inhibition

Describe, in detail, your experimental design on Step 2 of "Eukaryotic Inhibition."

Click "SUBMIT"





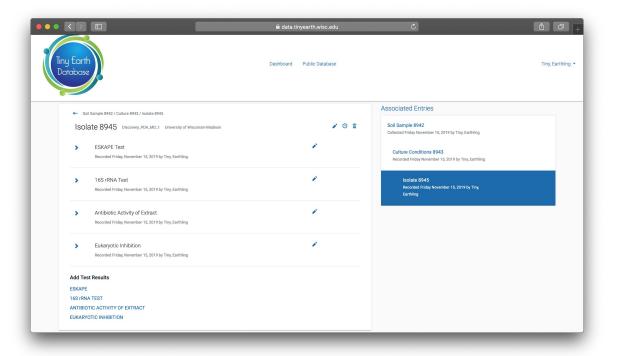


## Complete Isolate Record Page

All isolate test records will appear under the isolate's page.

Click on "Soil Samples" or "Culture Conditions" to add more sample data and information.

Click on "Dashboard" to see all samples you recorded in the database.

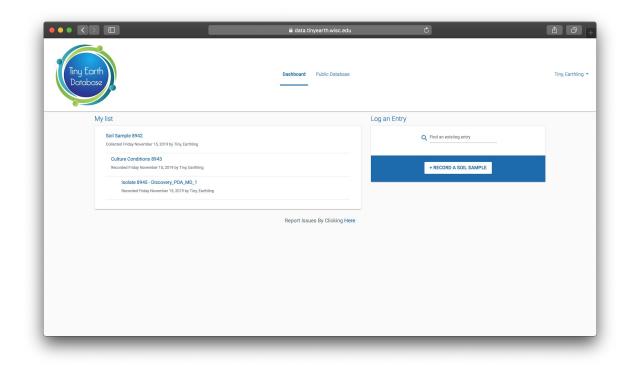




# The Finished Dashboard

Congrats! You have successfully entered soil data, culture conditions, and isolate test results on the Tiny Earth Database.

You are able to enter multiple Culture Conditions under one Soil Sample and multiple Isolates under each Culture Condition.



Tiny Earth website: tinyearth.wisc.edu Tiny Earth Database website: data.tinyearth.wisc.edu



Questions? Email tinyearth@wid.wisc.edu