The
Wolbachia
Project

Discover the Microbes Within!

The Wolbachia Project Database

Guide

Table of Contents

The *Wolbachia* Project: Discover the Microbes Within! was developed by a collaboration of scientists, educators, and outreach specialists. It is directed by the Bordenstein Lab at Vanderbilt University.

https://www.vanderbilt.edu/wolbachiaproject

About The *Wolbachia* Project Database

The *Wolbachia* Project Database is an interactive data repository featuring the biodiversity, frequency, and distribution of the obligate intracellular endosymbiont *Wolbachia*. Each entry includes arthropod information, taxonomic classification, experimental methods, and results. An integrated BLAST feature allows users to search for related organisms based on DNA sequence identity and all data can be exported as a .csv file.

Students upload their data and methods to The *Wolbachia* Project Database, where they can BLAST their sequence, compare results, search by investigative group (school), or *Wolbachia* infection status.

Ideally, each student will create their own account and take responsibility for uploading their data to the database. By using an account, they can later visit their entry to edit.

Appropriate Use

The *Wolbachia* Project Database is designed for student and professional scientists. The *Wolbachia* Project expects all users to conduct themselves in a professional manner, and only upload original appropriate language and images. Plagiarism or inappropriate posts will not be tolerated. The *Wolbachia* Project reserves the right to remove and/or delete any post at any time.

Summary	The *Araneae* was found to be postive for *Wolbachia*.

⚠ Report Inappropriate Post

At the bottom of each published post, there is a "report inappropriate post" button. If anyone is found to be using inappropriate or stolen language or images, their post will be removed, and their teacher or supervisor will be immediately contacted.
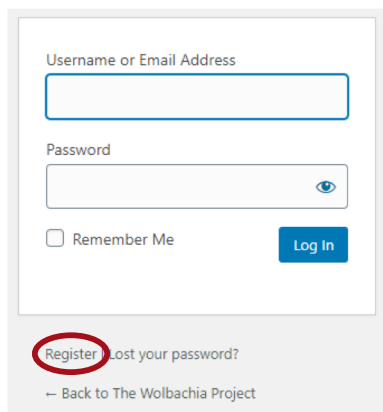
All users must first create an account in order to upload data into The *Wolbachia* Project Database. Each student or student group should create their own account, which will be linked to school or organization.
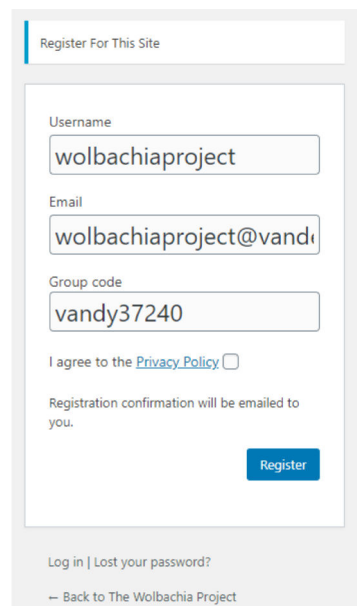
1. Visit https://wolbachiaprojectdb.org
2. Click the gold "Submit data!" button in the upper right-hand of the page.

Submit data!

3. Click "Register," under the login screen.

4. Register for the database.
    a. Choose a suitable username: this will be displayed to other users. The username should be school-appropriate without any identifying characteristics.
    b. Enter your e-mail. If you do not have an email address, your instructor will enter your data.
    c. Enter the group code that was sent to you by your instructor or coordinator. NOTE: This group code is unique to your school.
    d. Read and confirm agreement to the privacy policy.

5. The Database will send a welcome email containing a link to create your password. Once complete, you officially have your credentials to The *Wolbachia* Project Database, and are ready to submit data.
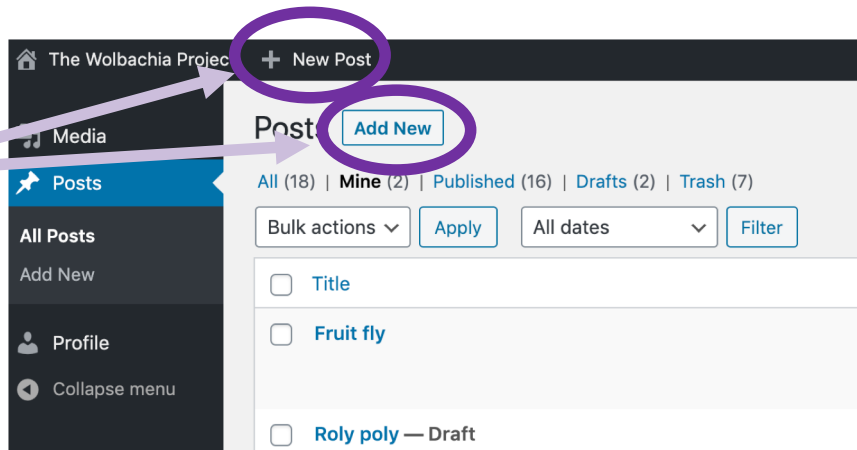
Once you log in to the database with your credentials, pressing the gold "Submit data!" button in the upper right-hand of the page will create a new post.

Submitting Data

The
Wolbachia
Project

Discover the Microbes Within!

Once you log in to the database with your credentials, you will be taken to your home post screen.
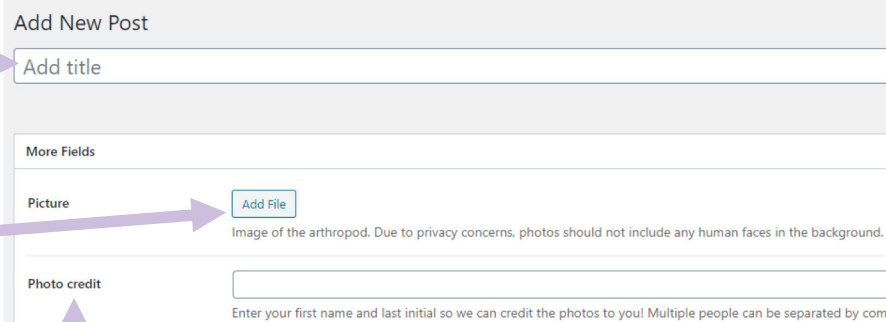
To create a new data entry, press "Add New" or "+ New Post" at the top of the screen.

🏠 The Wolbachia Project   + New Post

🎵 Media

📌 Posts

All Posts

Add New

👤 Profile

◀ Collapse menu

Post   Add New

All (18) | **Mine** (2) | Published (16) | Drafts (2) | Trash (7)

Bulk actions ⌄   Apply   All dates ⌄   Filter

☐ Title

☐ **Fruit fly**

☐ **Roly poly** — Draft

Alternatively, once you are signed into the database, pressing the gold "Submit data!" button in the upper right-hand of the page will take you to a new post.

To submit your data, first add a title. It can be named whatever you like- we recommend using the common name of your arthropod. This title can always be modified in the future.

Add a few pictures. If possible, try to capture an image of your arthropod in its natural habitat. We highly recommend submitting a detailed photo; this can be taken post-collection on a solid background. Do not submit images of arthropods in collection tubes or those containing identifiable people.

All images will be made available to the public under a creative commons license (see page 2). If you'd like credit, please enter your first name and last initial.

Add New Post

Add title

More Fields

Picture   Add File

Image of the arthropod. Due to privacy concerns, photos should not include any human faces in the background.

Photo credit

Enter your first name and last initial so we can credit the photos to you! Multiple people can be separated by comm

Submitting Data

Location

Lookup location (city, postal code, etc.)

Lookup Location
Go to my location

Approximately where was your arthropod collected? Enter a location, latitude/longitude, or by click and drag the map. Do not enter your home address! We recommend using your city zip code, school address, or local park.

Latitude

Longitude

Enter the date your arthropod was collected, and if it was cultivated (e.g., purchased in a store) or wild-caught.

Collection date

Captive / Cultivated?
○ Captive / Cultivated
○ Wild-caught

Observations

Add Media

Visual Text

Paragraph ▼ **B** *I* ☰ ☰ ❝ ☰ ☰ ☰ 🔗 ⬚ ⤢ ⌨

Enter observations about your arthropod collection. These could include an image of the environment, details about the season and weather, and description of the arthropod's physical habitat. Clear and concise descriptions will help scientists learn more about your organism.
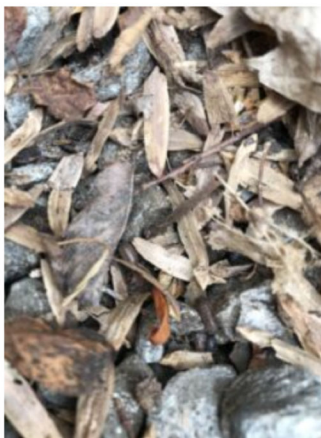
I found this roly poly under some leaves near school. I collected it in the afternoon, about 4pm, and it was overcast and about 70 degrees F outside. I attached a picture of the area that I collected it from. There were many roly polys under the leaves, but I only collected one.

Submitting Data

Now, enter your arthropod's taxonomic classification. We recommend using apps like iNaturalist to identify the arthropod to *at least* order. As with all data, this can be modified as you collect more information.

Click the little (+) to expand the classification You are only able to choose one entry; therefore, identify to the best of your ability. It is very possible you may only be able to identify to order or subphylum.

The list does not include all >1million arthropod species names. If you are unable to find your arthropod, choose the closest option and type in the collect identity here. Please make sure to spell it correctly as this will help us update the database.

Putative identification

Armadillidae x   Search   ▲

+ ◯ Arachnida
+ ◯ Diplopoda
+ ◯ Hexanauplia
+ ◯ Insecta
− ◯ Malacostraca
    ◯ Amphipoda
    ◯ Cumacea
    ◯ Decapoda
− ◯ Isopoda
        ◉ Armadillidae
+ ◯ Mysida
    ◯ Tanaidacea

What is it?

Arthropod not listed?

If your arthropod isn't listed above, select the closest option, and write in what it is here! We'll get it added.

Select your protocols. If your protocol is not listed here, email us at wolbachiaproject@ vanderbilt.edu to add it.

Upload an image of your gel. See page 10 for instructions on labeling the gel.

Extraction kit	DNeasy (Qiagen)
DNA extraction location	Abdomen
Gel image	arthropod gel
	Add File
	Image of gel
Gel electrophoresis system	MiniPCR
Buffer	TBE
DNA Stain	GelGreen

Submitting Data

Protocol notes

Include any additional notes about the methods. Was anything unexpected? Did you make an error during any of the steps? If you are unable to label your gel, identify each lane in this area.

Add Media | Visual | Text

Paragraph ▼ B I ☰ ☷ ❝ ☰ ☰ ☰ 🔗 ▦ ⤢ ⌨
ABE — A ▼ 📋 ⬧ Ω ⬦ ⬦ ↶ ↷ ❓

DNA extraction: I made sure to crush the arthropod really well. I dissected the abdomen out, and did not include the exoskeleton.
Gel electrophoresis lanes for CO1 arthropod PCR:
1- 1Kb ladder.
2- The second well is my negative DNA extraction control.
3- The third well is the positive DNA extraction control.
4- The fourth well is my positive PCR/DNA control.
5- The fifth well is my negative PCR control.
6- The sixth well is my sample.
Analysis: My controls worked, but my sample did not have a band at all. There could have been a problem with my DNA extraction.

P

Write up an analysis of your results, did you get a band on your gel? Multiple bands? What size(s)?

Wolbachia positive?

○ Unknown
○ Yes
● No

Based on your results, determine if your arthropod is *Wolbachia* positive.

Confidence level

○ Low
○ Medium
● High

How confident are you in the results?

How confident are you in your results?

Explain your confidence level

Add Media | Visual | Text

Paragraph ▼ B I ☰ ☷ ❝ ☰ ☰ ☰ 🔗 ▦ ⤢ ⌨

Describe your results in terms of experimental controls. Did all your controls work? Did your experiment go as planned?
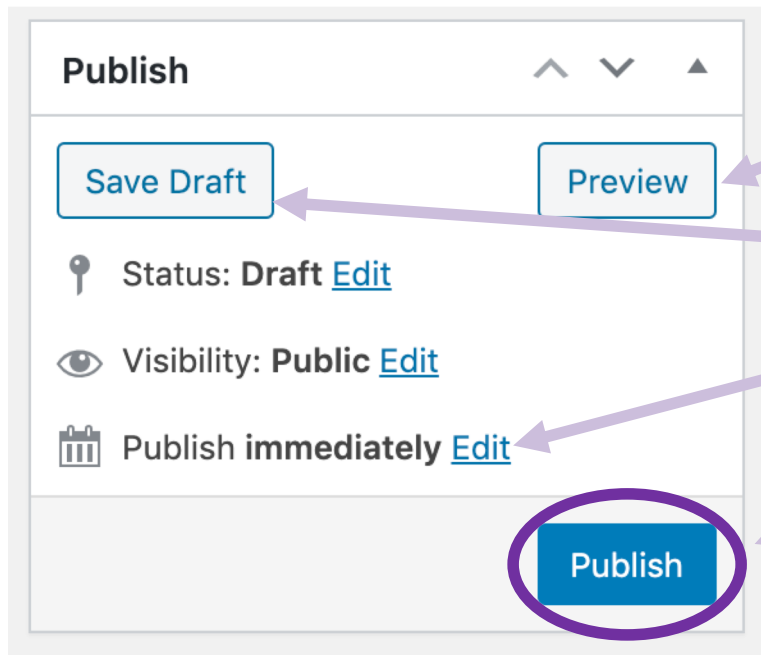
All of my controls worked as expected. I did not have any problems with the protocol, and I was not surprised by any bands on the gel. Therefore, I am confident my arthropod is not infected with *Wolbachia*.

P

Explain your confidence level: Did the controls work? How do you know you can trust your results?

Publishing Data

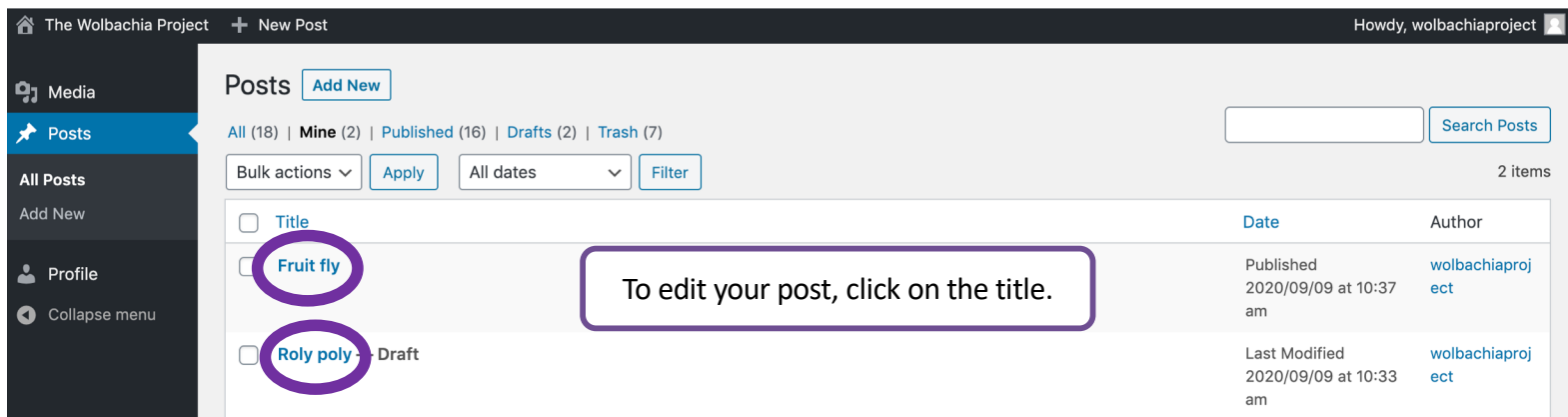To save your work, look at the floating menu on the right of your data entry.

- Preview will open your entry in a new browser tab for review.

- Save Draft will save your entry; it will not be publicly visible in the database.

- By selecting "Edit" after "Publishing immediately", you may designate a specific publication date and time.

- "Publish" will add your entry to the public database.

Editing Data Submission Posts

To edit your posts, press the gold "Submit data!" button in the upper right hand of the page. Log in with your previously established credentials and if you have already created a post, it will be visible as a draft or a published post.

To edit your post, click on the title.

Your post must be published to be visible to the community.

Annotating Gel Images: PowerPoint

Import your image into PowerPoint.

If you crop the image (right-click > Crop), make sure the wells, lanes, and ladder remain visible.

Under the "Insert" tab, insert a text box, and input your first label.

Change your text color to be a contrasting color of your gel and increase the size of the text. Here, we used white 24pt font. Use the rotate handle on top of the text box to vertically rotate your text.
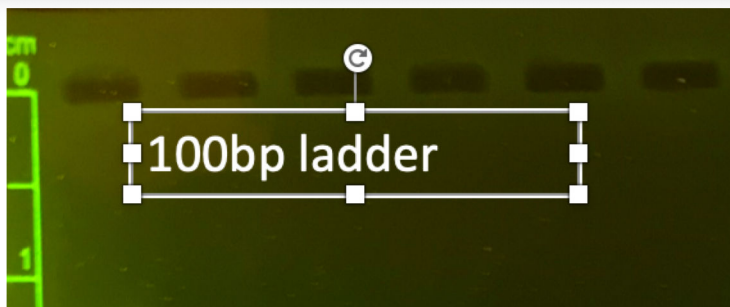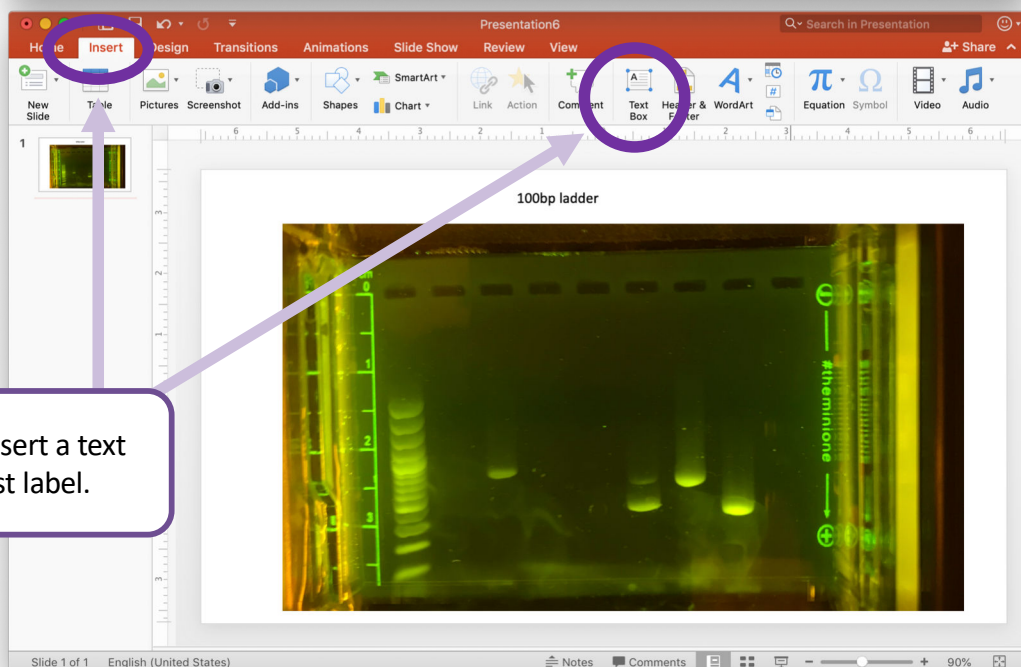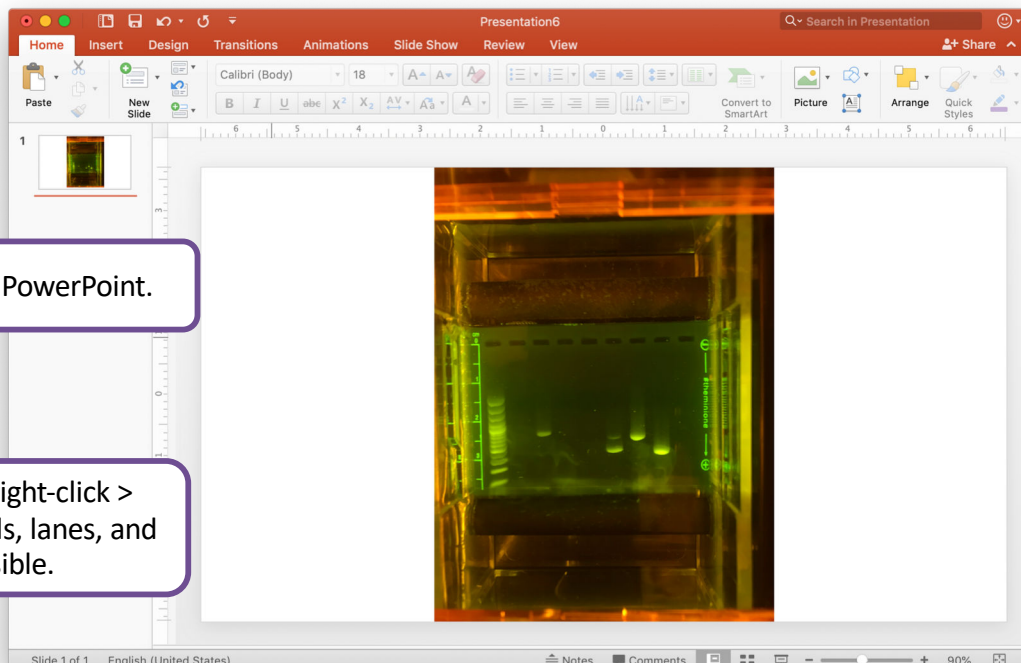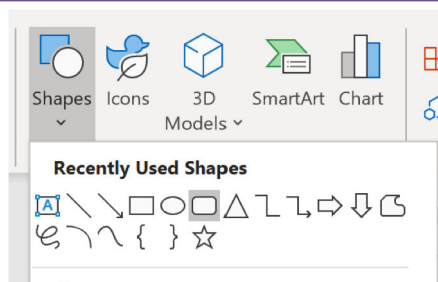
Annotating Gel Images: PowerPoint

Label your ladder and label your samples by aligning the text box with the well of the gel. Do not cover any DNA bands. Use labels that make sense to people reading your gel. You may provide additional details in the Protocol Notes box.
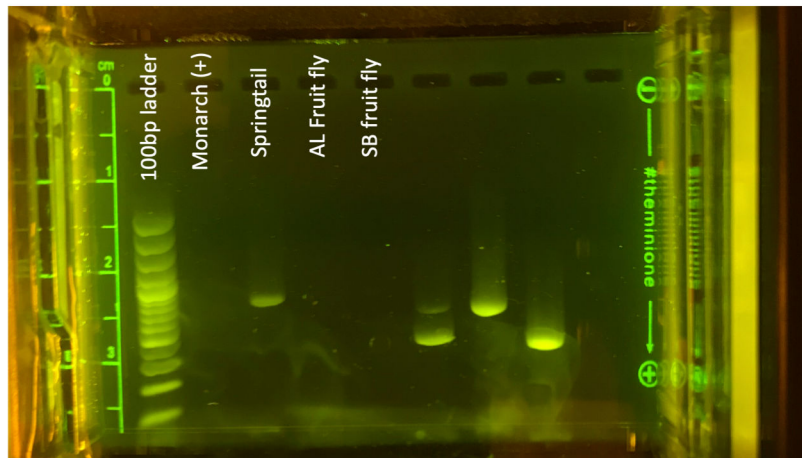
Label your controls.
(+) control – Arthropod positive for *Wolbachia*.
(-) control – Arthropod negative for *Wolbachia*.
***Wolbachia* DNA** – DNA extracted from an arthropod that was positive for *Wolbachia*.
Water/H2O control – PCR water control.
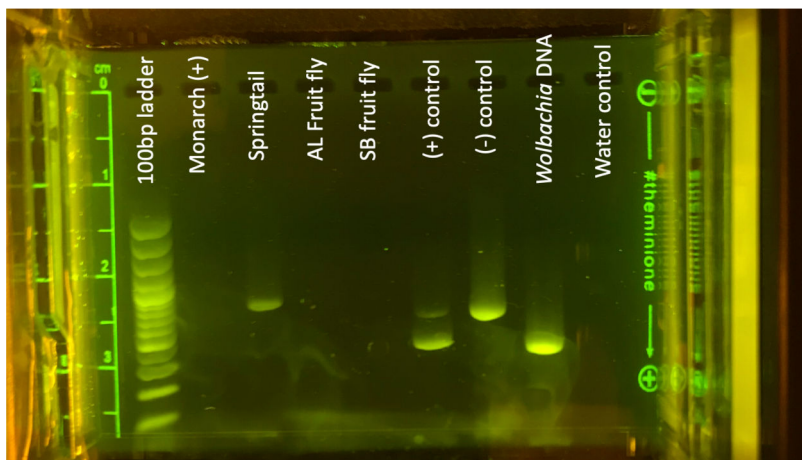
In order to distinguish the sample you are summitting to the Database, use Insert > Shapes and select a shape to insert.

Highlight the gel lane of the arthropod you are submitting to the database. Make sure all bands are visible inside the shape.
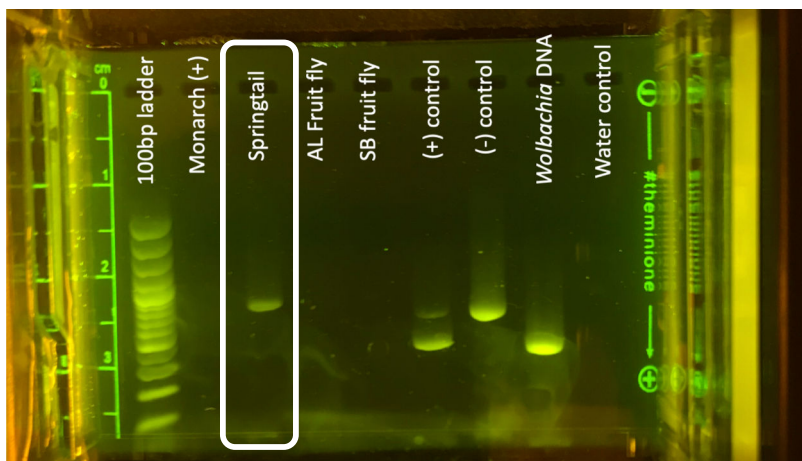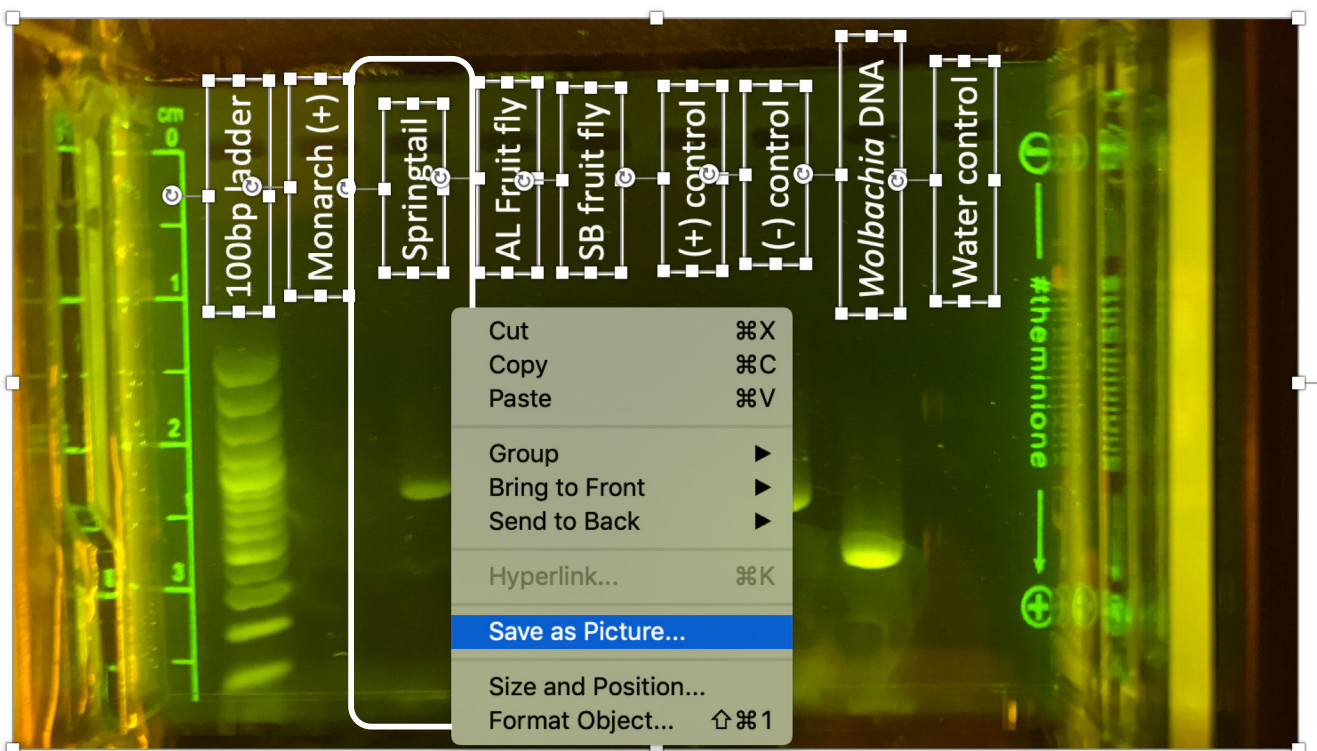
Make sure your shape has a contrasting color outline, and the fill of your shape is set to "no fill". You may need to increase the line thickness to make your shape clearly visible. These options can be found by right-clicking your shape.
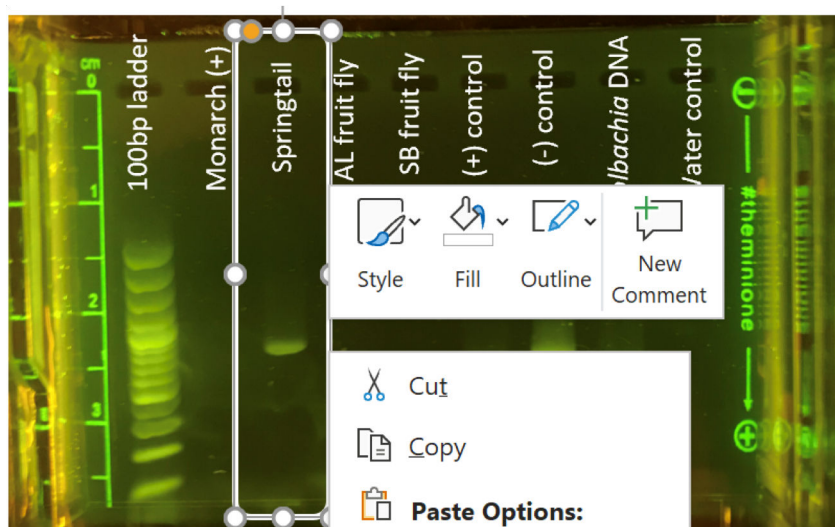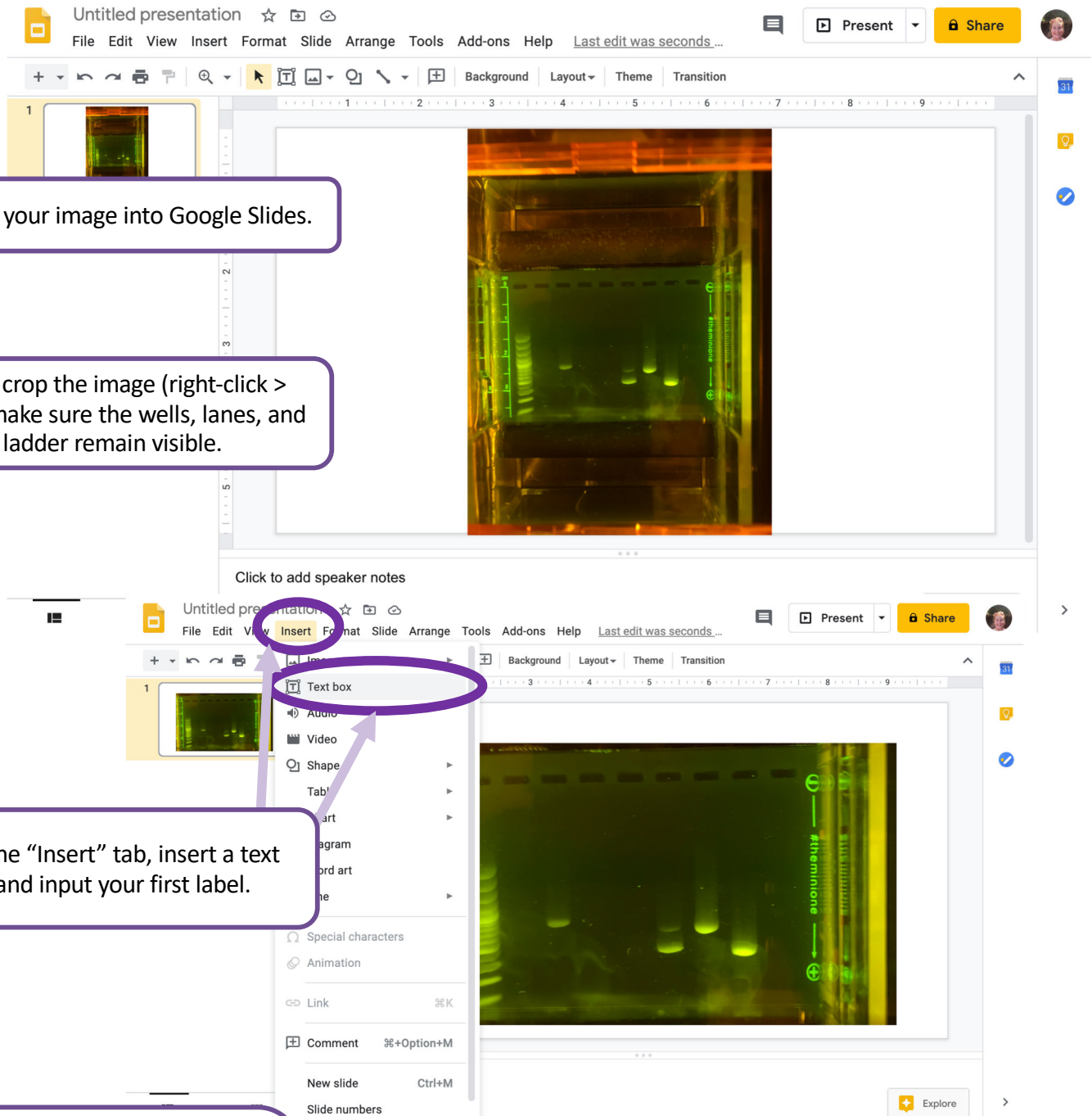
Select your gel and all of your labels. Right click and select "Save as Picture". Alternatively, take a screenshot of your labeled image.

Save your labeled gel to upload to the database.

Annotating Gel Images: Google Slides

Import your image into Google Slides.

If you crop the image (right-click > Crop), make sure the wells, lanes, and ladder remain visible.

Under the "Insert" tab, insert a text box, and input your first label.

Change your text color to be a contrasting color of your gel and increase the size of the text. Here, we used white 22pt font. Use the rotate handle on top of the text box to vertically rotate your text.

Label your ladder and label your samples by aligning the text box with the well of the gel. Do not cover any DNA bands. Use labels that make sense to people reading your gel. You may provide additional details in the Protocol Notes box.
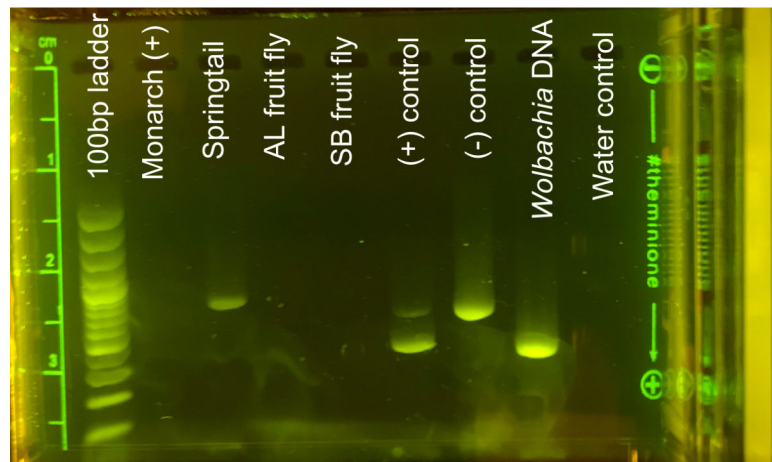
Label your controls.
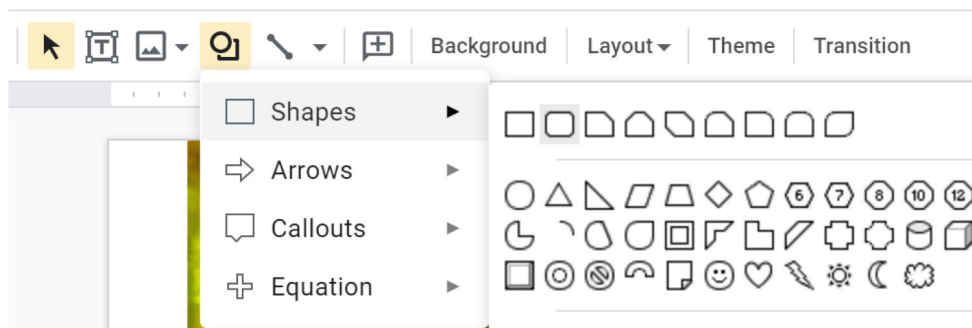**(+) control** – Arthropod positive for *Wolbachia*.
(-) control – Arthropod negative for *Wolbachia*.
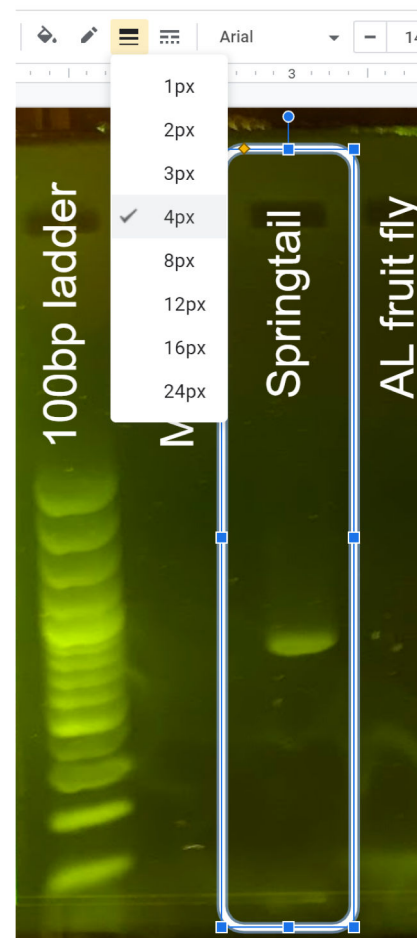***Wolbachia* DNA** – DNA extracted from an arthropod that was positive for *Wolbachia*.
Water/H2O control – PCR water control.

Finally, draw a box around the gel lane you are submitting an entry for by drawing a box around it.
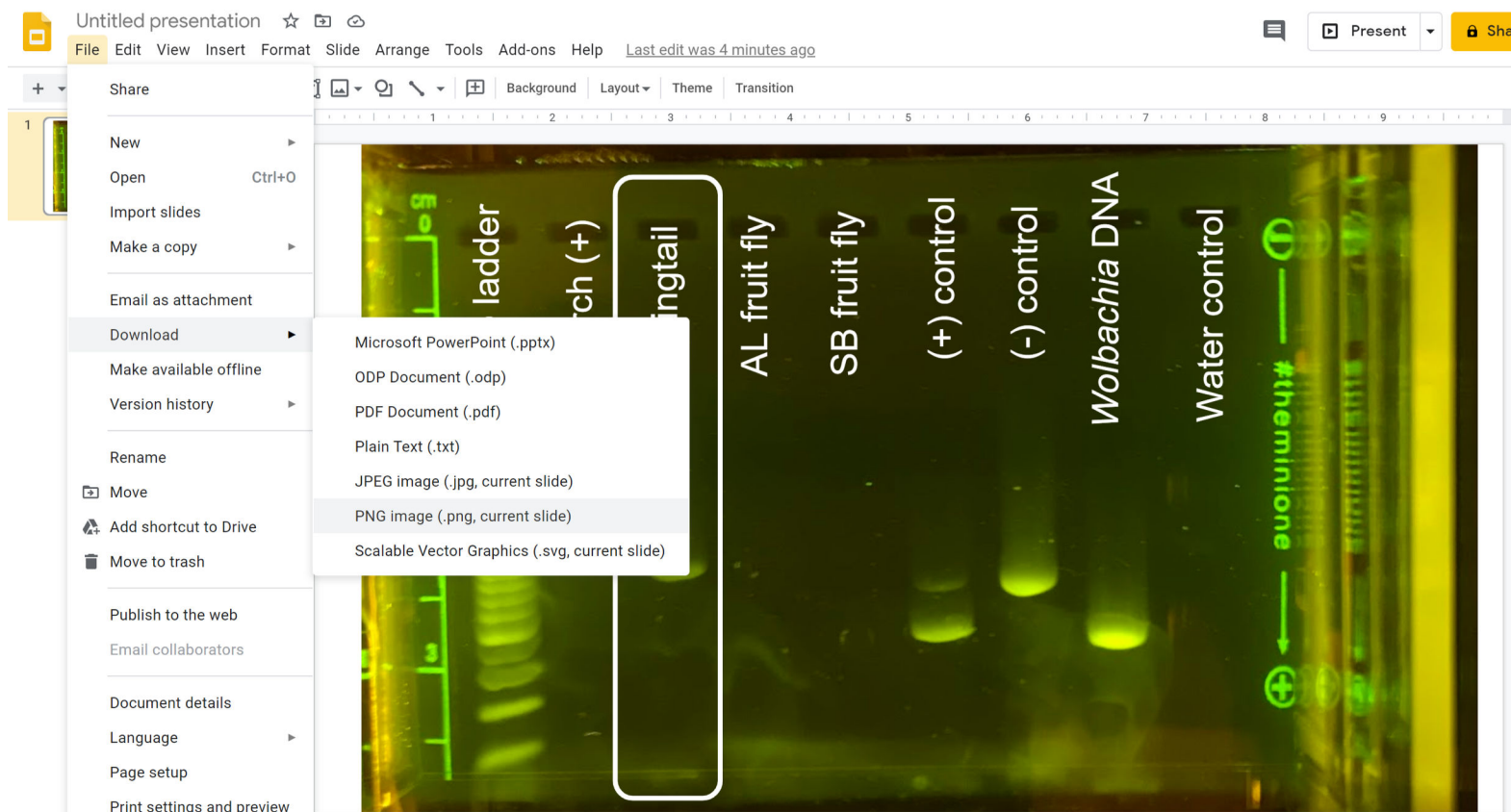

Make sure your shape has a contrasting color outline, and the fill of your shape is set to "transparent". You may need to increase the line thickness to make your shape clearly visible. These options are found on the toolbar above your slide.

Once you've labeled your gel, click "File", "Download", then save your image as a .png or .jpg. Alternatively, take a screenshot of your labeled image.

Save your labeled gel to upload to the database.