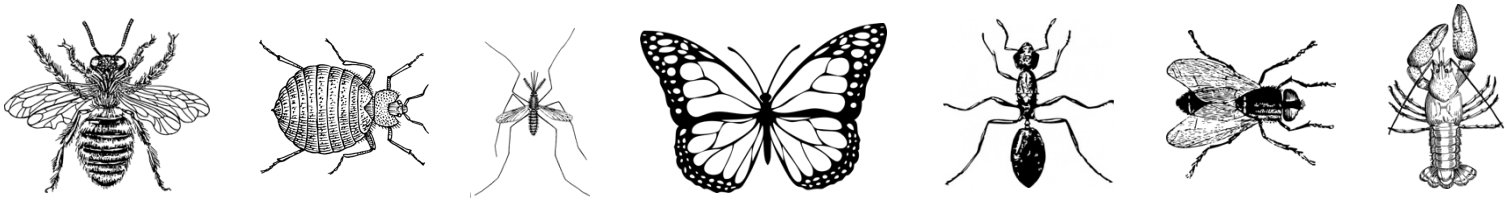
The Wolbachia Project

Discover the Microbes Within!

Getting Started

Teacher Resource Guide



Teacher Resource Guide

Are you interested in joining The *Wolbachia* Project, but are not sure which protocols and equipment are the best fit for your classroom? This guide provides recommendations based on the combined experience of our research lab and other *Wolbachia* educators. It is not intended to be a "Best of Guide" as we have not tested all available products and do not endorse one item/vendor over another. For each section, we highlight the distinction between education- and research-use. In the research setting, scientists generally purchase equipment that offers flexibility and greater sensitivity. This, of course, correlates with a higher price tag and may require technical expertise. In the classroom setting, however, we recommend products that are easy-to-use and deliver consistent results at an affordable price point. Major project needs are:

Equipment

- Computer with Internet
- Dissecting Microscopes
- Pipettes
- Vortex Mixer
- Mini Centrifuge
- Water/Dry Bath
- Thermal Cycler
- Electrophoresis System
- Transilluminator

Molecular Biology Reagents

- DNA Extraction Kit
- PCR Primers
- Insect Controls & DNA
- Ethanol
- DNase-free water
- Taq Polymerase
- Agarose Gel & Running Buffer
- DNA Stain
- DNA Ladder

Once you have selected your preferred equipment and reagents, proceed to The *Wolbachia* Project Shopping List to properly stock your classroom. The Shopping List includes recommended quantities and catalog numbers. It is broken into two sections: shared class resources and individual group supplies. Many items are non-specific; the exact brand doesn't matter.

DNA Extraction (Lab 2)

The path to *Wolbachia* Project success begins with a quality DNA extraction. If you have prior experience with a DNA Extraction method, we recommend staying within your comfort level. If you are looking for a new option, two methods are discussed below: Edward's DNA Extraction and Qiagen DNeasy Kit. Alternative protocols are also provided in our Resource Library, including Bio-Rad InstaGene Matrix, Promega Wizard SV kit, and Sigma-Aldrich Extract- and Amp-.

DNA Extraction Method

	Edward's DNA Extraction	Qiagen DNeasy Kit
Overview	SDS-based lysis buffer with alcohol precipitation step	Silica-based DNA purification; spin columns
Major equipment needs	▪ 95 °C water bath, heat block, or hot plate with beaker of boiling water ▪ Vortex mixer ▪ Mini-centrifuge (~10,000 x *g*) ▪ P200 pipette	▪ 56 °C water bath, heat block, or incubator ▪ Vortex mixer ▪ Mini-centrifuge (~20,000 x *g*) ▪ P200 pipette ▪ P1000 pipette
Time	1-2 class periods	1-2 class periods
Relative price point	$	$$$

Edward's DNA Extraction is cost-effective with fewer equipment needs. More room for student error.

Considerations:

- The DNA may not be as "pure" as a column-based kit with potential variability across students/arthropods.
- More hands-on steps lead to greater potential for error. Students must pipet supernatant away from cellular debris and they may lose the DNA pellet if not careful during final alcohol precipitation steps.
- The final DNA pellet may be very hard and require extra incubation/agitation prior to PCR lab.
- If you are on a tight budget, this is an excellent option and will yield plenty of DNA for PCR.

Qiagen DNeasy is a kit-based, long-time favorite of The *Wolbachia* Project.

Considerations:

- Columns may result in less yield (i.e., smaller amount of genomic DNA).
- Equipment needs are more sophisticated; reagents are more expensive. If you do not have access to a 20,000 *x g* centrifuge, allow extra time in the protocol for a potential ethanol evaporation step. While the protocol is optimized for 20,000 *x g*, we've had consistent success with a 10,000 *x g* mini-centrifuge.
- Molecular grade ethanol must be added to the buffers. Make sure your school has the proper permits to purchase ethanol.

In our Research Lab: We use multiple DNA Extraction protocols based on the specific needs of an experiment. For general extractions, we prefer Qiagen's Gentra Puregene Tissue Kit (# 158667). This protocol is not included in The *Wolbachia* Project because it requires a one-hour incubation.

(continued on page 4)

DNA Extraction (Lab 2)

Incubator

	Water bath	Dry bath / heat block	Hot plate with 1000 ml beaker
Temperature range	Adjustable	Adjustable	Some hot plates have adjustable temperature
Sample temperature	Longer preheat time, but samples heat faster and more evenly	Faster preheat time, but samples take longer to heat and may experience temperature fluctuations	Monitor closely to ensure stable temperature
Sterility	Routine maintenance required; prone to contamination	Autoclavable	Easy to clean
Container size	Most flexible	Fixed, based on block size	Limited; one round float rack will fit
Accessories	Float rack required; forceps recommended	Additional blocks may be purchased to accommodate different container sizes	Float rack required; forceps recommended
Relative price point	$$$	$$	$

Considerations:

- For protocols that require specific temperatures (i.e., 56 °C or 65 °C), we recommend adjustable water or dry baths. They offer the most flexibility.
- If you only have access to a hot plate and beaker, we recommend using the Edward's DNA Extraction method.

In our Research Lab: Our lab favorite is the Stovall Belly Dancer. It features a fast-heating water bath with adjustable shaker. While it is perfect for 1.5 ml tubes, it may not accommodate larger containers.

PCR (Lab 3)

Thermal Cycler

	Standard Thermal Cycler	MiniOne *
Overview	Flexibility for multiple uses	Designed to complete an entire PCR run within a class period
# wells	Variable, up to 384	16
Program interface	Built-in	Requires mobile device or computer
Relative price point	$$-$$$$	$

***** *miniPCR offers a similar product*

MiniOne offers an affordable thermal cycler that fits in the palm of your hand. It is lightweight, portable, and designed to complete a PCR reaction within one class period. Students can download the app and program the PCR protocol prior to class. Once the run begins, they are able to visualize PCR cycles in real-time. We recommend this type of system for educational use.

Standard Thermal Cyclers offer greater flexibility. Options include variable well capacity, temperature gradients, fast PCR, and more. They are heavier and require more bench space. We recommend this type of system for research use.

In our Research Lab: Our lab purchased Veriti thermal cyclers from Applied Biosystems over 10 years ago and they are still running strong. While we often use the VeriFlex temperature control technology when optimizing new PCR programs, these types of features are typically not necessary in the classroom.

Taq Polymerase

Selecting a Taq polymerase can be overwhelming due to the vast number of options on the market. For the purpose of The *Wolbachia* Project, most Taq chemistries will work.

Considerations:

- Carefully review the product information sheet provided with each Taq polymerase and note any protocol recommendations.
- We highly recommend master mixes. While they may be slightly more expensive than purchasing all items separately (Taq, buffer, $MgCl_2$, dNTPs, running dye), it is worth the investment in terms of prep time and error rate.
- Note the concentration of your Taq master mix (i.e., 2X vs. 5X) and use the correct amount.
- If using a MiniOne system, consider the MiniOne Taq master mix for faster PCRs.

In our Research Lab: We use multiple types of Taq polymerase depending on experimental needs. For general PCR, we prefer Promega GoTaq Green Master Mix. The mix contains two running dyes – a blue dye migrates at the same rate as 3–5kb DNA fragments while a yellow dye migrates at a rate faster than the primers. Therefore, *Wolbachia* Project bands will always be located between the blue and yellow dyes. Loading dyes are not necessary in the MiniOne system because students visualize bands in real-time with blue light as they migrate.

Gel Electrophoresis (Lab 4)

Electrophoresis System

	Standard System	MiniOne *
Overview	Standard systems are often comprised of a stand-alone gel rig, power supply, and transilluminator	All-in-one
Gel Size	Unit sizes vary. Larger gels can accommodate more samples and require more reagents	Fixed
Voltage	Flexible	Fixed
DNA Stain	Select a stain based on transilluminator (UV vs blue light)	Stain must be compatible with blue light
Prep Time	Requires more prep time to prepare running buffers and pour gels	Gels are ready within minutes; no post-staining required
Visualization	Post-run	During run
Relative price point	$$-$$$$	$

** miniPCR offers a similar product*

MiniOne offers an affordable all-in-one electrophoresis system that is easy to use and will save significant class time and resources. We recommend this type of system for educational use.

Considerations:

- Students are able to visualize DNA as it migrates through the gel
- Small gel size only accommodates 6-9 wells; however, run times are much shorter
- GreenGel cups reduce teacher prep time and eliminate student error
- Blue light is not compatible with GelRed or other UV-based stains

Standard electrophoresis systems provide more flexibility for a broader range of uses. We recommend this type of system for research use.

Considerations:

- Post-stain step substantially lengthens class time; no 'real-time' visualization
- Variable casting tray sizes allow for more wells and offer flexibility with well sizes; larger gels require more reagents and longer run times
- UV transilluminators are harmful and require special glasses and safety shields
- Higher voltage is a safety concern for students but may be required in the laboratory setting

In our Research Lab: Our lab uses the Owl EasyCast B2 Mini Gel System with a UV transilluminator and GelRed DNA stain. We prefer LB buffer (at 300V) for general use and TBE buffer (at 80V) for special applications.

DNA Stain

	GelRed	GelGreen	SYBR Safe	FastBlue
Transilluminator	UV	UV or blue light	UV or blue light	None
DNA Sensitivity	< 0.1 ng * Twice as sensitive as GelGreen	< 0.1 ng	0.5 ng	50 ng
Relative price point	$$$$	$$$	$$	$