Screening for *Wolbachia*

DNA Protocols for The Wolbachia Project

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Wolbachia Project

Introduction

The Wolbachia Project

The purpose of these lab protocols is to integrate a national research project into the high school classroom and laboratory. Each student involved becomes a research scientist helping to collect important information and data to answer unknown questions of the relationship of microorganisms and their host. The Wolbachia project focuses on a particular endocellular strain of bacterium, Wolbachia (explained in more detail below). These lab protocols are a collection of the necessary lab protocols to transform a high school lab bench into a research station for the purpose unlocking the mysteries of the greatest bacterial pandemic in the world.

The Wolbachia Project is an integrative approach to understanding the fundamental importance of micoorganism-host interaction. The prevalence of such symbiotic relationships is great in the natural world, and their influence on individual organisms and populations alike is immeasurable. This project hopes to shed some light on this phenomenon by closely studying Wolbachia-host interactions. It is massive undertaking that is aimed at ascertaining the genetic (genomic) diversity of Wolbachia, genetic changes that occur from host transfer, extent of gene transfer from Wolbachia to hosts, and the patterns of movement of Wolbachia globally and within communities. This project is also aimed at unraveling the evolutionary relationship between insects and Wolbachia. Moreover, the question of Wolbachia's influence on insect diversity world-wide can be explored.

What is Wolbachia

Wolbachia is an endocellular bacteria that is found within the gonads many different species arthropods (insects). Wolbachia may occur in up to 70% of arthropod species (Werren, Zhang, Guo. 1995). It is also known infect some species of nematodes. The effects of Wolbachia on its hosts are vast. Being a maternally transferred symbiont, Wolbachia strains have evolved a wide array of tactics to ensure the development of the female host. One effect is cytoplasmic incompatibility. It is known that in Mosquitoes, males that are infected with Wolbachia can only mate with females that are infected with the same strain. *Wolbachia* is also known to kill males during early development. Another more unworldly known effect is that of parthenogenesis. Parthenogenesis is a process by which females can reproduce without the fertilization by males. If the effects of Wolbachia are not weird enough, it is known to convert males to females. It has been documented that male woodlice are capable of male to female conversion when Wolbachia blocks male hormone release.

References:

Werren JH, Zhang W, Guo L: **Evolution and phylogeny of *Wolbachia:* reproductive parasites of arthropods.**
Proc R Soc London Ser B 1995, **251:**55-71.

Wolbachia Project

Insect Identification

Adapted from Bob Minckley

Background

Insects are among the most diverse and abundant animals. If the services insects provide everyday (for free) disappeared suddenly, humans would soon disappear. Insects keep us healthy and fed because they clean water, pollinate flowers that produce 1/3 of our food, breakdown waste and decompose plants and animals. Despite their importance, insects remain little appreciated and poorly understood primarily because of their small size. Most of the 1-20 million species (of which only 700,000 have names) are less than 1/3 inch long. This entire world of small creatures exists literally under your feet. The goals of the following activity are to introduce you to the incredible diversity of insects, to some basic methods of insect identification and classification, and to provide sorted and characterized insects to screen for *Wolbachia* and other insect bacterial symbionts by PCR (polymerase chain reaction), the next exercise in this series.

Procedure

INTRODUCTION

In this activity we assume that you have a batch of insects in ethanol that have been stored in the freezer, either you collected from a previous exercise or that were provided to you by a biological supply company or other source. You will sort the collections into "morphospecies" (individuals that have similar morphology and are probably members of the same species), identify the common morphospecies to taxonomic order, and prepare vouchers and specimens that will be analyzed for microorganisms. Vouchers are "copies" of the insects you will test for microorganisms and represent the only way the insect hosts can be linked to the bacteria that inhabit them.

Look carefully at the samples. Notice there are many species that range in size from so small you can barely see them to very large. Are some males and females of the same species, or are they different species in groups? That these samples were taken in the same location increases the chance that similar-looking specimens are the same species. However, determining the scientific name of insects is very difficult because there are so many species. By keeping those parts of the animal you do not use to search for microorganisms, as well as an individual that looks similar to the specimen you destroy, we can eventually determine the name of the host later yet continue to look for interesting microorganisms now. **So, it is essential to maintain a voucher for each "morphospecies" that will be screened for *Wolbachia.***

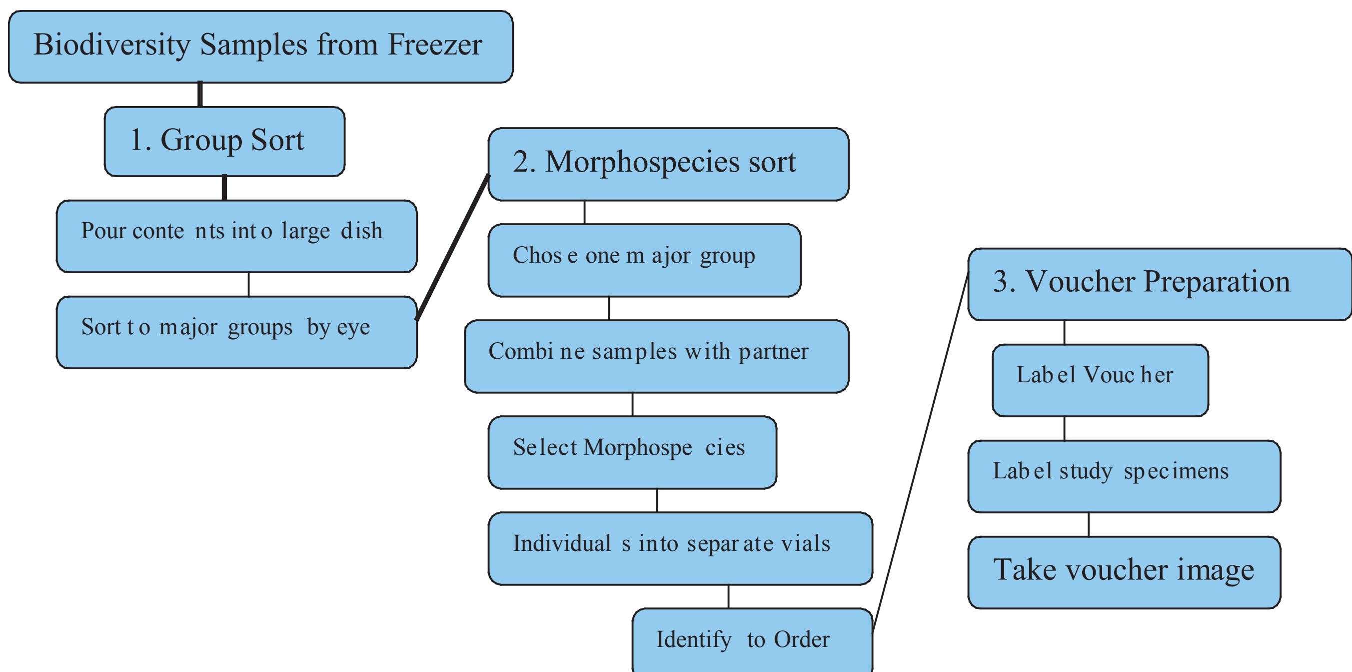
By the end of this exercise we will 1) discover the variety of insects one can collect in one location, 2) sort the collection into "morphospecies", 3) choose five species to test for microorganisms, and 4) prepare one sample that will be the specimen used to examine for microorganisms and another voucher of the specimens you believe are members of the same "morphospecies".

MATERIALS
Bent probes
Micron pens
24-32 lb. Bond paper
Forceps
Petri dishes (35 x 10 mm, 100 x 15 mm, 150 x 20 mm – 4 per workstation)
Dissecting scopes
Small squirt bottles for ethanol
Gloves
Plastic eye drops
95% ethanol
sharpie permanent ink pens

EQUPMENT
Dissecting scope
Illuminator
Digital Camera (optional but recommended).

IDENTIFYING INSECTS:
A. Basics: The basic procedure is that we will be sorting insects into morphospecies using a microscope, while keeping the insects in ethanol. The reason for the ethanol is that this preserves the DNA for future screening of the insects by PCR. Insects in ethanol should always be stored in the freezer when not being used in the lab. We will then take a "voucher" for a set of insects and identify them to Order (or better) using a set of on-line identification keys and books. We will then select 1 or more specimens from a set of morphospecies for screening by PCR.

Figure 1. Flow chart of insect identification laboratory



B: Procedure in Steps:

1. Save all labels with locality and collection information in a safe place.
2. Slowly pour all or some of the contents of the collection bag or vial into the largest-sized petri dishes. The specimens are delicate so pour them out very carefully. Rinse small insects from the bag/vial with clean 95% ethanol using a squirt bottle. Use as many petri dishes as needed to hold the contents in the bag. Always cover the petri dishes when they are not in use to prevent the ethanol from evaporating away.
3. If needed, remove extra fluid in the dish with the transfer pipette (= eyedropper) taking care not to remove small insects at the same time. Look through the dissecting scope as you do this.
4. While maintaining the insects in ethanol in the Petri dish, sort specimens into the general groups, such as earwigs, butterflies, wasps, and flies. Using the forceps, place each of these groups into separate medium- or small-sized Petri dishes.
5. Now pair up with a partner <u>who has specimens from the same collection</u>. Do not combine specimens from different collections! Select a group you would like to sample for microorganisms and then combine your sample of that group with the sample of the same group your partner sorted out. Your partner will then combine samples from you and him or her of a completely different group of insects.

About Morphospecies:

Morphospecies are organisms that have been seperated based the differences of body appearance. Difference in morphology (body appearance) are a typical and classic method of seperating out species. Differences in morphology include body shape, size, textures, external/internal color, and other body structures.

6. Now, sort similar looking specimens into morphospecies. Choose **five** morphospecies for the microorganism study. In general, it is best to use smaller species because these are easier to dissect and they preserve better so are more likely to have bacterial endosymbionts (big insects rot more quickly which degrades the DNA).
7. Place **two** individuals of these morphospecies into glass vials filled halfway with clean ethanol. Use large vials for large specimens such as butterflies, and small vials for small specimens. Return the specimens you will not use to a large vial or the plastic bag they came in originally.
8. Make 2 labels for each vial and the original sample on bond paper with the micron pens. Record all the information on the original label on one of these labels. Record your name, the date, the Order you identified the species to, and the voucher number (see examples below).

USA: Arizona, Cochise Co. 30km east of Douglas 26 March 2001, A. Romero by Malaise traps	USA: Arizona, Cochise Co. 30km east of Douglas 26 March 2001, A. Romero by Malaise traps

9. Identify specimens to Order. With computers, identification no longer requires an expert. The web site we will use is at the following address: http://pick4.pick.uga.edu/mp/20q?guide=Insect_orders

Recall that insects have three main body regions, head, thorax and abdomen. On the head of most insects are conspicuous eyes, mouthparts, and antennae, on the thorax are the wings and legs, and on the abdomen are the reproductive organs (where many of the endosymbiotic bacteria live). Not surprising, however, it is common for there to be exceptions to the body plan. Many insects lack wings or legs, some are eyeless, etc. These features allow us to classify them as groups. Insect Orders are the large groups you are probably familiar with such as moths and butterflies (Order Lepidoptera), beetles (Order Coleoptera), bees, wasps and ants (Order Hymenoptera), and flies (Order Diptera). Notice that each of the Order names end the same way in –ptera. This word means wing and the wings are one of the most conspicuous ways Orders of insects differ. The key we are using allows you to look at the wings and several other features of the legs, mouths and abdomen. What is nice about these kinds of identification keys is that you can correctly identify your insect even if you cannot answer all of the questions. Answer those questions you understand. By clicking on the picture, an more detailed explanation appears that often helps those of us who are "insect challenged". Anytime in the process of answering questions you can click on the **identify** button. On the left will be the groups that have the characteristic or characteristics you have chosen. Click on the simplify button and this will eliminate unimportant characteristics from the list and show only the characteristics that might help you identify your organism from others.

List of the common, conspicous orders:

Coleoptera (= beetles; 370,000 species) Fore wing a hard protective cover, hind wing membranous

Collembola (=spring tails; 6,000 species) Tiny wingless creatures with a "spring" on the end of the abdomen

Dermaptera (=earwigs; 1,200 species) First pair of wings very short, abdomen exposed, "forcep-like" appendages at tip of abdomen

Dictypotera (=cockroaches and mantids; 6,000 species) Two pair of wings, the forewings thickened and the hindwings membranous

Diptera (=flies, 120,000 species) Single pair of membranous wings

Ephemeroptera (=mayflies; 2,000 species) Two pair of membranous wings which they can fold over their back

Hemiptera (=true bugs; 50,000 species) First pair of wings thickened at the base, sucking/ piercing mouthparts

Homoptera (=aphids, scale, whiteflies; 32,000 species) Often wingless, but the forewing is uniform (all membrane or all thickened) when wings present

Hymenoptera (=bees, wasps, ants; 108,000 species) Both pair of wings membranous and hooked together

Isoptera (termites; 1,900 species) When winged, both pair membranous and similar in size

Lepidoptera (= butterflies, moths, skippers; 140,000 species) 2 pair of wings covered with small scales
Nueroptera (=dobsonflies, lacewings, antlion, owlflies; 4,700 species)
Odonata (=dragonflies, damselflies; 5,000 species) 2 pair of wings with many veins, aquatic larvae
Orthoptera (=grasshoppers, crickets, katydids; 17,000 species). Two pairs of wings, hindleg often enlarged
Siphonaptera (=fleas 2,300 species) Permanently wingless ectoparasites of vertebrates
Thysanura (=silverfish, firebrats; 350 species) Wingless with three long "tails" at the end of the abdomen
Trichoptera (caddisflies, 7,000 species) 2 pair of hairy wings

10. Optional: Take a voucher image with a digital camera

Insect Preparation

All insects collected should be stored in 95% ethanol until DNA isolation is ready to be conducted. Insects too large to be stored in alcohol can be frozen in a freezer. Smaller insects (i.e. flies, ants, small beetles) can be ground up whole. DNA isolation from larger insects (dragonflies, large beetles, etc.) should be done with only a small portion of the insect. In the latter case the posterior region of the abdomen should be taken and placed into the Eppendorf tube for maceration. Roughly the bottom 1/4 of the vial should be filled.

DNA Isolation

This protocol is spread over several days to allow students to see all aspects of the method, while allowing for minimum wait time.

Student Procedure: Day One

1) Transfer insects (or parts of insects) from 95% ethanol into a sterile Eppendorf tube. Make sure that the ethanol is allowed to evaporate completely so that it does not interfere with the isolation. This can be done by placing the specimen on a Kimwipe and allowing to thoroughly air dry.
2) Add 90ul of ATL buffer to 2ml Eppendorf tube containing insect sample.

Caution: Be sure to change pipette tips with the addition of ATL buffer to each new tube.

About ATL Buffer:
The ATL, or tissue lysis buffer, is designed to enhance and optimize the enzymatic activity of Proteinase K in the lysing of tissue (more about Proteinase K below). Lysis can be understood as the disintegration or breaking down of organic material (i.e. cellular membrane)

3) Sterilize micropestle with a Kim Wipe soaked in 100% ethanol.
4) Macerate insect sample for 1-2 minutes or until no recognizable parts are present.
5) Add another 90ul of ATL buffer to the Eppendorf tube containing the insect sample. Use buffer to rinse off the micropestle and sides of the tube.
6) Place tissue samples in refrigerator overnight.

Teacher Preparation: Day Two

Should be done 1-2 hours prior to lab in order to have samples thoroughly prepared for students

1) Bring samples to room temperature and add 20ul of Proteinase K. Mix tissue sample mixture thoroughly by vortexing.
2) Incubate insect tissue sample at 55 degrees Celsius for 1-1.5 hours.

About Proteinase K:
Proteinase K is an enzyme desinged to break down organisms' tissue in order to expose cells and cellular content for further degredation.

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DNA Isolation

Background

The following protocol is designed to yield high quality DNA from whole or partial insect specimens. DNA is found within the nucleus of cells, where it is protected from the environment. We will use both mechanical and chemical methods to break open cell to access and purify DNA. Once we have isolated clean DNA we can then use it for a variety of different techniques.

Procedure

Introduction

Proper preparation and good organization can make the difference between a successful DNA isolation and an unsuccessful one (one that does not work or is contaminated with airborne bacteria and other organic material such as amino acids, lipids, and nucleic acids). It is important to make sure that all material used has been sterilized to reduce contamination from other insects, bacteria, and/or your own DNA. Use gloves to reduce the risk of contamination. To save time, be sure that all water baths and incubators are set to the proper temperatures well before starting the protocol.

Record keeping is extremely important. Make sure that all tubes are properly labeled to maintain consistency and to reduce the possibility of mixing up samples. List and label sample names and identification markers on containers in notebook in order to keep track of sample for future reference.

Isolate No.	Order	Family	Genus	Species	Common Name
0001	Odonata	Libelluidae	*Libellula*	*julia*	Dragonfly

Materials Required

All materials must be assembled and prepared (i.e. sterilized) prior to the DNA isolation procedure. Sterilization can be done using a UV stratalinker, autoclave, or baking oven. Improperly sterilized reagents and materials can lead to contamination of DNA samples.

- ☐ 3 - 2ml Eppendorf tubes for each sample you will be isolating DNA from (e.g., you will need 15 tubes for 5 extractions). One tube is for the homogenization step and the other two are for each elution steps.
- ☐ Sterilized micropestle to crush up the insect. This must be compatible with a 2ml Eppendorf tube.
- ☐ Water bath set to 55˚C. Make sure that the water bath is on and set to the proper temperature prior to starting the isolation protocol.
- ☐ Extraction Kit. This will contain the materials and any other necessary equipment for use (i.e. collecting tubes) that you will need for this experiment.
- ☐ Forceps to transfer insects.
- ☐ Laboratory notebook
- ☐ Gloves: for safety and contamination purposes. They should be worn at all times.

Student Procedure: Day Two

1) Vortex samples and add 200ul of AL buffer to sample and mix by vortexing.
2) Place tubes in incubating bath set at 70 degrees Celsius for 10 minutes (Make sure this is set prior to the beginning of lab to reduce waiting time).

About AL Buffer:
Incubation at 70 degrees Celsius of proteinase K treated tissues causes the actual cells to lyse. The AL buffer Helps stabilize the cellular components released from lysed cells - this is especially the case for released DNA. Without the AL buffer enzymes would break down cellular contents.

3) After incubation add 200ul of 100% ethanol to insect sample and mix by vortexing.

About 100% Ethanol:
100% ethanol causes the DNA released from the cells to precipitate out of solution. This step allows for the filtration of DNA from other cellular components. As the solution passes through the filter unit, the DNA sticks to it and the rest passes through and is discarded.

4) Prepare one filter unit and two collection tubes for each sample by placing them in a rack. Make sure they are properly labeled and that the tops are open. (Collection tubes differ from Eppendorf tubes in that they have no lid and are designed to receive the filter unit. They are intended to collecting waste and to be immediately discarded)
5) Pipette out the liquid portion of the insect sample (approx. 630ul) and place it in one collection tube.
6) Place collection tubes in microfuge set at 8000 rpm/minute for 1 minute.

Caution: It is important to evenly balance the microfuge to avoid damaging it.

7) Remove collection tube from microfuge and place on a rack. Take filter unit of the collection tube out and place it in the other 2ml collection tube. Discard the flow-through in the old collection tube.
8) Add 500ul of AW1 buffer to the filter unit and centrifuge set at 8000 rpm/minute for 1 minute.

About AW1 Buffer:
This buffer helps to wash undesirable cellular componenets through the filter unit. Cells contain many different kinds of proteins and fats (triglycerides) that must be washed through with special buffers in order to obtain pure DNA - the function of AW1 buffer.

9) Remove collection tube from microfuge and place on a rack. Take filter unit of the collection tube out and place in a new 2ml collection tube. Discard the flow-through in the old collection tube.
10) Add 500 micro liters of AW2 buffer to collecting tube

About AW2 Buffer:

This buffer helps to wash undesirable cellular componenets through the filter unit. Cells contain many different kinds of proteins and fats (triglycerides) that must be washed through with special buffers in order to obtain pure DNA - the function of AW2 buffer.

11) Centrifuge set at max rpm/minute for 3 minutes
12) Prepare two new 2ml sterile Eppendorf tubes. These are not collecting tubes. These are for collecting DNA. The difference is that the Eppendorf tubes have caps and collecting tubes do not
13) Transfer filter unit of collection tubes into the newly prepared 2ml Eppendorf tubes. (Steps 14 -18 are the DNA removal steps from the filter)
14) Add 200 micro liters of AE buffer and let the sample sit for 1 minute at room temperature.

About AE Buffer:

AE buffer is an elution buffer that washes the isolated, precipitated DNA through the filter unit. Essentially, it resuspends the nucleic acids in solution.

15) Place centrifuge tube in centrifuge set at 8000 rpm/minute for 1 minute.
16) Remove filter and place in the second 2ml centrifuge tube.
17) Repeat steps 15 and 16 for new tube.
18) Remove filter and discard. DO NOT discard the 2-2ml Eppendorf tubes and their contents. This is the isolated DNA.
19) Combine the two tubes using a micropipetter set at 200 microliters
20) Place isolated DNA in freezer for storage

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Polymerase Chain Reaction (PCR)

Background

Polymerase Chain Reaction (PCR) is laboratory procedure that quickly amplifies specific regions of DNA. Using specially designed DNA replication primers, desired section of DNA can be amplified for further use. These primers are designed to match specific regions of an organism's genome, and guide the replication of it. Within a matter of hours billions of copies of the target DNA can be obtained from small amounts of DNA. It is superior in terms of speed and efficiency to other methods of DNA replication and amplification.

Procedure

Introduction:

Doing a PCR is not time consuming, but it is important to have prepared all materials prior to the laboratory procedure. Having done this, the procedure will run smoothly and efficiently. Begin preparing by reading over this procedure before attempting to do a PCR in class.

All frozen samples should be thawed before beginning a PCR reaction. In order to protect the integrity of the materials used, a container with ice should be ready at each lab workspace. DNA primers should be prepared and kept on ice throughout the experiment. Failure to keep on ice may cause them to degrade and they will become useless.

Good record keeping is extremely important when conducting PCR. It is important to label, in your notebook, the position of each of the samples in accordance with their PCR strip tubes. It is also important to write down the primers worked with and for which samples.

Materials:

- ☐ Ice bucket (with ice)
- ☐ 2 Eppendorf tubes. One for the PCR cocktail, and one for prepared primers
- ☐ PCR cocktail chemicals (found below in procedure)
- ☐ PCR Strip Tubes
- ☐ Prepared Primers (Forward and Reverse primers)
- ☐ Thawed DNA

PCR Procedure:

1) Acquire PCR strip tubes and place them on a rack (The number of tubes needed will vary depending on the number of samples).
2) Mix the PCR cocktail mix into an appropriately labeled 2ml Eppendorf tube. Each value should be multiplied by the number of insect specimens used:
   - 2.5ul 10x PCR buffer
   - 2.5ul dNTPS
   - 17.85ul of dH_2O
   - 0.15ull *Taq* Polymerase (To be added later!)

   Store cocktail on ice

3) Primer preparation:

- Mix 1ul of both the forward and reverse primers (multiply time n the number of insect samples being used) in a 2ml Eppendorf tube.
- Label the 2ml tube containing the primers appropriately.

About Primers:

Primers are short oglionucleotide chains that are designed to bind to a specific sequence of DNA. They initiate DNA replication at the sites where they bind to DNA template. They are introduced in pairs - forward and reverse - in order to a region of DNA that lies between them

4) Dot 2ul of the primer mix under the lip of the PCR tubes. (Caution: be sure to use a new tip for the addition of different primers to each of the PCR strip tubes).
5) Add 1ul of the isolated DNA template to the primer template dot. (Caution: be sure to use a new micropipette tube for each addition of the DNA template to the tubes).
6) Add the .15ul of *Taq* polymerase to the labeled PCR cocktail mix. Using a pipetter, mix the cocktail by pipetting the mix up and down.

About *Taq* polymerase:

Taq polymerase is a protein that replicates DNA, and operates optimally at a higher temperature than most other DNA polymerases. Because of its high optimal temperature it is the only polymerase that can replicate DNA during PCR. Taq is used in PCR as the primary polymerase of these qualities

7) Add 23ul liters of the PCR cocktail mix to each of the PCR tubes containing DNA templates and primers.
8) Microfuge the PCR tubes (Caution: Be sure to balance microfuge equipment. Balancing a microfuge is as simple as making sure to place approximately the same weight on the opposite side of the microfuge). Spin the samples to briefly, up to 1500rpms and then stop. This should drop the liquids to the bottom of the PCR tubes.

About Positive and Negative Controls:

P

9) Place mixture in thermocycler.
Temperature cycles:

1	95 °C – for 5 min
2	94 °C – for 15 sec *
3	56 °C – for 30 sec *
4	72 °C – 30 sec *
5	72 °C – 7 min
6	10 °C – holds

The starred temperature cycles 2-5 are repeated 35 times during the thermocycling process.

About thermocyclying (PCR):

The higher end temperatures (94-95 C) during PCR causes what is known as denaturation. This causes the DNA double helix to split apart and exposes the DNA bases on each strand. The lowest temperature phase (~56 C) causes what is known as annealing. It is during this phase that the DNA primers mixed into solution bind to their corresponding DNA segments. The final mid-grad temperature set (~72 C) allows for extension. During extension Taq polymerase binds to the annealed primers and synthesizes the desired DNA segments for PCR. This cycle is repeated approximately 35 times to obtain thousands of copies of the desired DNA segments.

10) Once PCR process has been completed store sample on ice until gel electrophoresis can be done. If a long term storage is necessary store sample DNA PCR product in freezer

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Gel Electrophoresis

Background

Important in the process of extracting and amplifying DNA is analyzing what has been obtained throughout this lab. Analytical DNA procedures allow for the separation and visualization of amplified DNA. Gel electrophoresis is one of the most efficient simplest methods on analyzing DNA. This method separates DNA fragments (and other macromolecules) by size, electrical charge, and other physical properties. An electrical charge pulls these DNA molecules through a thick medium whereby separating it out.

Procedure

Introduction:

It is important to adequately prepare materials used prior to beginning loading and running a gel. In order to save time, a gel should be poured in advance and while it is polymerizing PCR and dye should be made ready. An important precaution that must be kept in mind when working with a gel is safety. Be sure to wear gloves throughout this procedure. You will be working with hazardous materials when staining a gel, be sure to wear gloves at all times when working with laboratory materials.

As it should be with all lab work it is important to keep and maintain good records. Keep a log in a notebook of the gel loading order. Make sure it can be recalled from a notebook what was loaded into each well on the gel.

EX:

Well 1	**Well 2**	**Well 3**	**Well 4**	**Well 5**
Isolate No. 1	Isolate No. 2	Ladder	Isolate No. 3	Isolate No. 4

Materials Required:

- ☐ Electrophoresis rig (Gel box, well comb, cords, and charge box)
- ☐ Prepared loading dye
- ☐ PCR samples
- ☐ Staining container (insulated from light)
- ☐ Agarose
- ☐ 1XTBE buffer
- ☐ SYBR Gold (Stain for Gel)
- ☐ Gel viewing equipment

Agarose preparation procedure:

1) Prepare 0.8% agarose solution by adding 4 g of agarose in 500ml of 1XTBE buffer. If using an already prepared stock solution heat until it has completely liquefied.

2) Put well forming comb into place and pour liquefied agarose solution into gel box filling the box about half way up the combs.
3) Allow gel to polymerize fully (harden) before attempting to load PCR products.

Gel stain preparation:

1) Prepare fresh SYBR stain (if necessary). *Refer to procedure below*

SYBR Gold staining protocol:

Safety: SYBR Gold is stored in a 10,000X concentration in DMSO. DMSO is a chemical that enhances the ability of SYBR Gold stain to enter into your cells. It is absolutely imperative that you wear gloves at all times in working with this stain.

1) Retrieve SYBR Gold from the freezer and allow to fully thaw. (The stain should be stored at least at –20 °C).
2) Fill gel-staining container with 100ml of 1XTBE buffer.
3) Adjust the pH of the staining solution by adding HCL. XTBE buffer is generally at a pH of about 8.5. Add about 8-10 drops of HCL to drop the pH to about 7.7.
4) Add 20ul of the SYBR Gold stain to the XTBE buffer.
5) Swirl container to evenly distribute the stain in solution.
6) Return stain to freezer immediately.

Safety:

Be careful when working with SYBR Gold stain. It is extremely hazardous avoid direct contact with chemical.

Not a preparation students will generally be doing

Gel Loading and Running Procedure:

1) Prepare ladder by adding 1ul of loading dye, 1ul water, and 4ul of ladder stock solution to a 2 ml Eppendorf tube and have ready.
2) Have ready a gel tray and mix 4ul of PCR reaction product with 1ul of loading dye.
3) Place loading dye back in fridge and place PCR samples back in freezer.
4) Once agarose gel has polymerized place gel box in loading rig. Be sure to place the wells of the gel at the negative end of the gel rig and the end of the gel at the positive end of the gel rig

About Agarose Gel:

Agarose gel, after is has polymerized forms a microscopic matrix. This matric is the medium through which DNA fragments are pulled by an electrical charge. The fragments travel from the negative end of the gel box to the positive end. Smaller DNA fragments travel through the matrix faster than the largeer pieces, creating different bands. These bands can be visualized to determine the DNA products.

5) Fill loading rig with 1XTBE buffer up to line that completely submerses the gel. Gently press down on gel box to remove any air bubbles that may have formed beneath it.
6) Set a micropipette to 5.5ul and take up PCR sample. Make sure there is no bubble in tip. This may cause your sample to splash out of the well when loading
7) Load PCR and dye products onto gel by inserting the micropipette tip into the well and gently releasing the products into the well. Be very careful when filling wells. This technique takes a steady hand and good eye to make sure you have placed the micropipette tip into the well
8) Load a ladder onto gel. Place the ladder in between, to the left, or to the right of the DNA samples.

About Ladders:

A ladder is a multipurpose tool when running a gel electrophoresis procedure. Teya re typically used to determine the size of DNA fragments that have been isolated and amplified. Ladders can also be used to determine if a particular DNA fragments was achieved. At the very least, they function as a positive control to determine if the gel and staining procedure were successful.

9) Plug gel rigs positive and negative wires into the charge box. The red wire corresponds to red input and it is positive. The black wire corresponds to black input and is negative.

About DNA Charge:

A gel is always run from the positve to the negative direction. The charge box emits a negative charge that runs from one side of the box to the other creather a negatively chaged pole (where the wells should be) and a positively chaged pole. DNA, which has a slight negative charge, runs towards the positively charged end of the gel (opposites attract!).

10) Turn on charge boxes and run gel at 80V ~ 100V (+/- depending on samples, gel size, and time constraints) until purple bands have migrated half the distance between upper and lower wells (explain DNA movement, charge, etc…).
11) Let gel electrophoresis run for approximately 1 hour
12) Turn off boxes and remove excess gel with razor. Discard excess gel into a designated solid waste container.
13) Place and fully submerge gel in staining container and cover. Make sure that the staining box is fully insulated from light as the SYBR Gold stain is photo-reactive and can be broken down by UV lights. (**Safety:** Be careful when working with SYBE Gold stain. It is extremely hazardous avoid direct contact with chemical)

About SYBR Gold:

SYBR Gold stain, like most DNA stains, binds to the DNA that is within the gel. By soaking the gel in the stain, it is able to diffuse through it and attach to the DNA. DNA stains bind directly to the DNA macromolecule usually by insertion between the base-pair rungs

14) Allow the gel to stain for 1 hour.
    (Teacher can remove, store, and image gels).

15) Take picture of with FotoAnalyst (if available). Most gels can be viewed using a UV light when photo equipment is unavailable.
16) When through make sure to dispose of gel in hazardous solid waste bucket. Clean up workspace and all laboratory materials used.