Genomic Diversity Laboratory
Department of Ecology and Evolutionary Biology
University of Michigan

HOW-TO MANUAL

Cleaning

How to clean your bench?
A good practice is to clean your bench before and after you work in the lab. Wipe the surface with 10% bleach and then with 70% ethanol. Keeping a clean environment will prevent contamination.

How to clean general glassware?
Use brush, detergent, and then finish up with a couple of rinses with regular tap water, followed by final rinse with distilled water (from the sink).

How to clean the centrifuges?
Use paper towel moisten with deionized water. Wipe down the centrifuge interior with the moist towel. If it is required, wipe the centrifuge and its parts with ethanol to disinfect. Do not use bleach for cleaning. The centrifuge should not be dipped into water or other liquid. If you spilled liquid inside the tube holders ask for help to the technician. Do not leave it dirty!

How to clean the photodocumentation system?
Wipe the glass surface with a lint free tissue and deionized water, making sure that no agarose gel residuals are left. Dispose the wipe in hazardous waste buckets.

How to clean the transilluminator (uv) box (in the dark room)?
After you use the transilluminator, wipe off the steel frame including the filter glass using deionized water. Do not use bleach for cleaning. In case of intense contamination it can be wiped with ethanol. Dispose all trash in hazardous waste buckets.

How to clean the EtBr area?
For decontamination, use gloves and do not allow any contact with your skin. 10% bleach will clean up EtBr spills (although it does not completely remove the EtBr). Clean contaminated area using paper towel from the outside of the spill to inside to avoid spreading the spill. Any materials used to clean up EtBr are considered hazardous waste and must be disposed of into hazardous waste buckets.
How to clean the gel electrophoresis systems?
After running a gel, the equipment should be immediate rinsed with deionized water. Do not use detergent or bleach for cleaning, and do not leave the systems with buffer.

How to clean the dry baths?
Use paper towel moisten with deionized water. Take off the blocks and wipe them. Wipe down the dry bath interior with the moist towel, and dry it with a new dry paper towel. Do not use bleach for cleaning. If you spilled liquid inside the tube holders ask for help to the technician. Make sure that liquid never stays inside the dry bath.

How to clean the thermocyclers?
Prior to using a thermocycler, check if no debris has settled into the wells. This will affect heat transfer from the block to the tube and may adversely affect your PCR. Only clean the external surface of the machine using a paper towel dipped in 10% bleach and then use ethanol. Do not remove or wet any area of the equipment. In case of spillage, please report it to the lab manager immediately, and do not use that machine.

How to clean the scales (balances)?
When powder has been spilled around the scale clean it up with a dry paper towel. If liquid is spilled soak the liquid with paper towels and then clean the surface with a paper towel dipped in deionized water. Let it dry out before you use it.

How to clean the microwave?
The microwave located in room 83 is exclusively for making agarose gels. If agarose is spilled on the dish or surrounding area, please do not wait until it solidifies because hardened agarose is hard to clean off. Use a paper towel to remove the agarose solution and wipe the inside with a wet paper towel. If you need to wash the glass dish, use a brush, detergent and running water. Dry the glass dish before placing it back in the microwave.

How to clean the pipettes?
Cleaning the pipettes regularly prevents cross-contamination. A good practice is to clean your pipettes before you start to work in the lab. Wipe your pipettes with ethanol. Let them dry out before using them. If you suck up liquid into the pipette please give it to the lab manager for her to clean it. If you notice that your pipettes need calibration, please also give them to the lab manager, who will replace that particular pipette.

How to clean the incubators?
In the case of spill, turn off the incubator and wipe the inside with a wet paper towel. Be aware that the incubator may be very hot. If that is the case wait until the temperature lowers and proceed to cleaning. Never leave the incubator dirty.
**Use of equipment**

How to use a pipette?
First, wipe your pipette with a lint-free tissue wet with ethanol. Then, set the volume within the range specified for the pipette (never use the maximum volume of a pipette or pass the allowed volume, instead use a larger volume pipette or pipette a smaller amount of your reagent more than once). Place a tip on the discharge end of the pipette (make sure the tip is tightly inserted). Do not allow the pipet tip to touch any object. Press the operating button to the first stop. Dip the tip into the solution, and slowly release the operating button. Wait 1-2 seconds and withdraw the tip from the liquid. Dispense the liquid into the receiving container by gently pressing the operating button to the first stop and then press the operating button to the second stop. This action will empty the tip. Dispose the tip. Avoid turning the pipette on its side when pipetting or when there is liquid in the tip, as it will cause inaccuracies in the loaded volume and because liquid might go to the interior of the pipette and contaminate it.

How to use the photodocumentation system?
Make sure the transilluminator (inside the box) is turned on. Place the agarose gel on the transilluminator’ glass surface. Turn on the equipment and press live bottom. Set white light on. Place the agarose gel in the center of the transilluminator and manually adjust the zoom of the camera, so the gel image covers most of the screen. Close the door of the transilluminator and turn on the UV light (make sure the door is closed before you turn on the UV light). Make manual adjustments to the focus of the image. If needed, adjust the light using the up and down arrows. Press freeze and turn off the UV light. Press print to take your photo (make sure that the thermal printer is on). After using the photodocumentation system remove the agarose gel and dispose it into the hazardous waste buckets. Turn of the machine and wipe the glass surface with deionized water. Leave the door close.

How to use the centrifuges?
Always balance your tubes in the rotor, unbalanced tubes may permanently damage the centrifuge. Balance your tubes by mass not by volume. For balancing put your tubes in opposite directions. Keep the lid closed while the centrifuge is spinning.

How to use the SpeedVac centrifuges?
Place open tubes into the SpeedVac rotor in a balanced pattern. The open lids of the tubes should lie facing the center of the rotor. Close the lid of the SpeedVac, turn on the power of the SpeedVac, set the SpeedVac switches to high-medium or low heat (this will regulate the speed of the evaporation process, but be aware that if it is set to high it may damage the DNA). Check the progress of concentration at 10 minute intervals. When you have finished turn off the machine and remove your tubes. In case of spills wipe the inside of the SpeedVac with a dry wipe and then with ethanol.
How to use the dry bath incubator?
   Turn on the dry bath incubator. Set the thermometer in the machine and wait until it reaches the desire temperature. Place tubes into the wells. Remember to turn off the dry bath before using it; the blocks will remain hot for some time after use.

How to use the gel systems and power supplies?
   Position the tray that has your agarose gel into the electrophoresis chamber filled with TBE 0.5X. Make sure the buffer completely cover the surface of your gel and do not over pass the red reference line. Load your samples into the sample wells using the pipettes that are in this area (do not bring any of your bench pipettes over to the EtBr area!). Place the lid and power leads on the apparatus. Turn on the equipment and set the voltage and time (regularly 70-100V, and 10-40 min). Check that the current is flowing by observing bubbles coming off the electrodes. DNA will migrate towards the positive electrode. Make sure that you have placed your gel and connected the apparatus properly or your samples will go out of the gel and will be lost.

How to use the microwave in gel running area?
   After you mix agarose powder with buffer to the desired concentration, place the mix in the microwave and heat it until it is completely melted. Prepare your agarose mix using a container that fits your needs (regularly 150 -250 ml flasks work well). Put your agarose mix into the microwave. Push the power button and then set the time for 1 minute (enough for a 60 ml solution). The solution will boil. Make sure to turn the microwave off when the solution starts boiling or it may spill over. Wait a few seconds before taking the solution out of the microwave as it may splatter over and burn you. Wearing hot-resistant gloves remove the flask from the microwave. If the agarose has not been completely dissolved, gently mix the solution with circular movements and return the flask back into the microwave and add 40 seconds. When the mix is done, remove the flask from the microwave. If there is a spillage, clean it up immediately with a paper towel and dispose it in the hazardous waste bucket.

How to use the scale?
   Turn on the scale. Place the container (tubes, weigh paper, trays) that you will use to weigh the sample in the scale and set the measurement to zero. Then, add your sample. Use a container that fits your needs, the heavier the container the higher error. Keep the scale clean to avoid errors from spills or residuals powders, use deionized water for cleaning.

How to use the incubators?
   Turn on the incubator and set the desired temperature. Once the desired temperature is reached, place your samples and turn on shaker if need it. Make sure to keep a clean environment. Wipe down doors, handles and all external surfaces with ethanol. Avoid spills.
How to use the vortex?
Make sure your tubes are closed to avoid spills. Place the tube on the vibrating device and hold in it until the solution is mixed (for a 1.5 ml tube, ~10 seconds).

How to use the autoclave?
The autoclave is on the 3rd floor and can be used for sterilizing equipment and solutions. Always attach a piece of autoclave tape to your autoclave items. When you sterilize liquids, bottles should be 3/4 or less full. In all cases lids should be slightly loosened. If no lids are used loosely cover the bottle with aluminum foil. Take your autoclave items placed on plastic trays to the autoclave room. Once there, using the pedal open the door of the autoclave. Then, load your items. Do not overload the autoclave and do not exceed the size and height of it, leave an appropriate space between trays. Close the door using the pedal. Select cycle 5 for solids and 3 for liquids. The process will run for 1 hour. Sign the spreadsheet and when the cycle has finished remove your samples.

Notify

How to report any accidents?
Always report any accident to the lab manager. Do not try to fix any equipment, this can increase the damage. And, if you experience a spill check the MSDS sheets to evaluate the level of hazard.

How to report non-working equipment?
Always avoid using equipment that is not working properly and report any problem ASAP to the lab manager. Leave a note on the equipment describing the problem so other people will not use it.

How to report when hazardous waste containers are full?
All hazardous waste containers (both solid and liquid) should be kept in room 83 under the bench area. If more buckets or bottles are needed there will be empty ones available in the same area. Please use the appropriate labels for hazardous waste (in the first drawer of file cabinet in room 86) and let the lab manager know that waste should be removed soon. The lab manager will call OSEH to schedule a waste pick up to 734-763-4568.
Primer design & PCR Optimization

How to design new primers?

In order to allow the primers to have adequate specificity and appropriate binding to the template at the annealing process, you should consider (1) the length of the primer, (2) base composition, (3) melting temperature, and (4) annealing temperature. An optimal length is 17-28 bp. A base composition should be ~60% G and C and not having a base repeated more than three times in a row. Also, it is recommended that primers should not have more than 2 G or C repeated at the end. A melting temperature (temperature at which the DNA will dissociate to become single stranded) in a range of 52-58 ºC will indicate more stability to the DNA, whereas temperature above 65 ºC will have a tendency for secondary annealing. Lastly, too high annealing temperature will produce insufficient primer-template and a too low may possibly lead to non-specific products caused by a high number of base pair mismatches.

How to order primers?

One option is to go to Marketsite, select Life Science Products and then type “oligo.” Select “Invitrogen Custom DNA Oligos.” Then, based on the number of primers that you want to order select “Basic Entry” (upload primer sequence at a time) or “Bulk Upload” (upload multiple sequences at a time). Your primers will be shipped dry unless you request special handling options to be shipped diluted in water (not recommended).

How to dilute your primers?

When you order primers they are shipped dry so you need to dilute them in water. To get a standard concentration of 100 µM stock solution, multiply the number of nmoles reported in the “Amount of Oligo” section by ten, then add this amount of ddH₂O to your tube. Then, you have to vortex the tube to resuspend the primer well and store it at –20 ºC.

To prepare working stock solution of your primer you need to dilute your stock solution in ddH₂O. This working stock will be used for running PCR reactions. A factor dilution of 1:9 will make your working concentration for PCR primers of 10 µM.

How to optimize your primers?

An optimal and specific amplification of a PCR product will be translated in a successful PCR. Remember that small fragments (~100-200 bp) are amplified with higher efficacy than longer fragments (do not amplify fragments <75 bp due to these can be confused with primer dimers). Also, design the primers based on primer specificity and properties (primer length, composition, and melting and annealing temperatures; see above).

A Touchdown PCR will allow you to try different annealing temperatures in a single reaction tube and generally results in specific amplification of desired PCR product without optimizing the PCR protocol. First, you need to determine the melting temperature of the primers. Set up a touchdown program with ±5 degrees respect with the primers melting temperature (starting with
5 degree higher than the melting temperature and reduce 1 degree per cycle until it reaches 5 degrees lower than the melting temperature). Then, continue amplification using the last annealing temperature.

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Alternatively, a Gradient PCR can be conducted where a range of temperatures are tested in different PCR tubes. Each column on the thermocycler will use different annealing temperatures. For instance if we tried a gradient between 65-55 °C, column 1 will be at 55 °C, column 2 at 55.8 °C, so on increasing 0.8 °C each column up to reach column 12 which will be at 65 °C. It is recommended to test a single template under different temperatures. To do not overuse the template, samples can be tested every three-four intervals and then conduct another PCR covering the range where better amplification occurred.

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