PCR (Polymerase Chain Reaction) with nematode extract template SOP

PPE: Nitrile gloves, lab coat, goggles or safety glasses, closed toe shoes

Job Hazard Analysis: See JHA No: 8 (Skantar 2020), JHA No: 9 (Skantar 2020)

Introduction to PCR: https://www.ncbi.nlm.nih.gov/probe/docs/techpcr/

Example optimization guide: https://www.neb.com/tools-and-resources/usage-guidelines/guidelines-for-pcr-optimization-with-taq-dna-polymerase

Notes: Read the instructions for the Taq you are working with for optimum cycle extension times and temperature range, necessary buffers/reagents, etc. Program the thermal cycler before assembling the reactions. Keep all reagents cold (on ice or in an approximately 4 degrees Celsius cooling block) while working with them, and return them to the freezer as soon as possible. Work as steadily and quickly as possible with no or minimal process interruption. If smaller amounts of a larger quantity of reagents are frequently used, make working aliquots of all reagents ahead of time in order to avoid air contact and freeze/thaw cycles which lower enzyme/component activity and shelf life. PCR reactions can be set up on the bench if you have no PCR hood, and with non-filtered pipet tips as long as they are pre-sterilized and DNAse/RNAse free, but contamination with airborne microorganisms may cause issues especially if you are using universal or non-specific primers. Gloves should be worn primarily to protect the materials from contamination with human DNA. When working with more than a few reactions, it is easiest to make a "master mix" of all reagents except template DNA, add that to each tube, then add the DNA.

If you are new to using micropipets, view an online introductory tutorial or request a demonstration from a colleague. Practice pipetting water into and between PCR/microcentrifuge tubes until you become familiar with the "first/soft" (precise volume) and "second/hard/blowout" plunger stops.

Materials:

PCR hood (we use Airclean model AC632LFUVC) with up-to-date HEPA and pre-filters
Thermal cycler (we use Biometra cyclers)
Sterile 1.5 mL, 0.5 mL, and 0.2 mL PCR/microcentrifuge tubes with attached lids
Molecular biology grade water (available in small bottles from several suppliers)
Chill rack for PCR tubes (we use one that holds 1.5 mL and 0.2 mL tubes)
Micropipetters (we use Rainin LTS with magnetic-assist plunger) in 2, 20 (or 10), 200, and 1000 microliter (uL) sizes
Compatible pre-sterilized, DNAse and RNAse-free pipet tips, preferably with filters
Reaction reagents (Taq, buffer, dNTPs, primers/oligos, other kit components as necessary)

Example reaction recipe for 25 microliter (uL) final volume:

---H20 (to 25 uL total reaction volume)
2.5 uL 10X DreamTaq buffer (includes MgCl2 at 2.0 mM final reaction concentration)
0.5 uL dNTPs mix (10 mM each dNTP, 0.2 mM final reaction concentration per dNTP)
0.75 uL primer 1 (0.3 uM final primer concentration)
0.75 uL primer 2 (0.3 uM final primer concentration)
0.125 uL DreamTaq (0.625 units)
2 to 3 uL template DNA (60-killed single-nematode extract in ~20 uL nematode extraction buffer)

Procedure:

1. Turn on the PCR hood, sterilize the working surface with 70% ethanol and kimwipe, and gather materials in the hood. Use sterile technique and use a new pipet tip for each step throughout the procedure. Pre-label the appropriate number of PCR and master-mix tubes and place/keep them in the chill rack as much as possible while working with them.
2. Add all ingredients in the order and amounts listed above to a 0.2 mL PCR tube, or a larger tube and minus the template DNA if preparing a master mix. Tip: When adding reagents subsequent to the water, place the end of the pipet tip just under the surface of the liquid already in the tube, gently pipetting up and down once or twice to get all of the tip contents into the tube. This is especially helpful with Taq, which is very viscous.
3. When using a master mix, mix it gently but thoroughly by pulsing on a vortexer (make sure the tube lid is closed) or stirring with a pipet tip. Add the proper amount (usually 22 or 23 microliters) to each tube. When working with more than a few PCR reaction tubes, close the tube lids after adding the master mix to prevent evaporation. After the master mix has been added to all the tubes, add the template DNA, gently and thoroughly stirring the contents of each tube with the pipet tip and then closing the lids as you go.
4. Flip the power switch on the back of the thermal cycler to the on position. When the boot-up sequencing is complete (check the screen display), open the lid and place the tubes into the thermal cycler block. Arrange them so that they are near the center but fairly evenly distributed throughout the block to avoid tube buckling. Empty tubes in the corners of the block help prevent tube buckling. Close the lid and seal it internally by turning the top dial clockwise until it clicks three times. Choose or create the appropriate program from the list using the button pad (or other user interface as applicable). Start the program. You may want to observe the screen display for a few minutes to ensure the program runs as expected.
5. When the run is finished and the samples are being held at 15 degrees Celsius (or lower depending on the brand/model), turn the dial on the top of the lid counter-clockwise until you feel the latch release, then open the lid. Remove the PCR tubes from the block and store at -20 degrees or 4 degrees Celsius if you will not be using them right away. Close the thermal cycler lid and flip the power switch to the off position.
6. See the Agarose Gel SOP and others for down-stream applications of the PCR reactions.

*Updated 7/31/2020 by Maria Hult