1. **TITLE:** General PCR
2. **PURPOSE**

For the preparation of master mix, running of PCR, detection of PCR product, gel electrophoresis and product visualization

1. **NEEDED MATERIALS**

Specific master mix Electrophoresis chamber

PCR tubes Gel-red or SYBR safe or equivalent

Thermocycler Electrophoresis agar

1x SB buffer UV transilluminator

1. **PROCEDURE**
2. Preparation of Amplification Reaction Mixture

Note: samples and reagents should be kept cold either on ice or in a frozen cryo-rack during all assembly procedures.

1. Get out required materials needed and start to UV the AHRB 272 Air Clean Hood.
2. While it is getting UV’d record appropriate data for each sample to be tested by PCR on a worksheet. The gel photo will be later affixed to this worksheet.
3. [Calculate](https://docs.google.com/spreadsheets/d/1TBocluPbry5tXUUesB-Mc5iEBqE3I4X_/edit?gid=432492739#gid=432492739) the amount of each reagent to go into the “Master Mix” (MM) according to the number of samples to be processed. Make a copy of the calculator and label into your own project folder.

**Note:** recommend printing out and having next to you while working in hood.

1. Once the hood is sterile, prepare MM in a 1.5 mL microcentrifuge tube by adding PCR reagents, except volume of DNA, in the order listed on the spreadsheet, adding water first and Taq polymerase last. Keep all reagents cold in frozen cryo-rack or on ice during mixing and return them to freezer immediately after use.

**Note:** The master mix spreadsheet makes enough MM for at least one more sample than being tested to compensate for retention of solution in pipette tips.

1. Once everything has been added, close cap and vortex for 3 seconds and spin down briefly.
2. Return to the hood and place specified volume of MM into each PCR tube or plate wells. Then load specified volume of each DNA to the appropriately labeled PCR tubes or plate wells. To avoid cross contamination always avoid touching the sides of the tube. Close caps tightly or seal PCR plate.
3. Running the PCR
4. Before loading into thermocycler, vortex the tubes or plate for 3 seconds to mix, then give them a “quick spin” to ensure that all reagents and samples are drawn down from sides of tube.
5. Load the samples tubes of PCR plate into the machine wells (follow manufacturer’s protocols).
6. If not already, program thermocycler for appropriate cycle conditions and run reaction.
7. After cycling, tubes may have a ring of condensation near rim of cap. Before opening tubes or unsealing plates, perform a “quick spin” to draw this fluid down into the reaction area of the tube and reduce the possibility of aerosol contamination upon opening tubes.
8. PCR products can be refrigerated for up to a month following amplification (or for longer storage they may be frozen at -20°C).
9. Detection of Product
10. Procedure for preparing the gel (refer to the [Glass Lab Gel Electrophoresis General Guidelines](https://docs.google.com/document/d/11N2sXqXQCQcnwp7jBMzDRco01JLdstMVU1ryjSzfJWs/edit?tab=t.0) for more in depth SOP):
11. Assemble the gel tray and position the well comb in tray according to the guidelines.
12. Prepare 1.5 to 2% agarose gel according to the volume recommended for specific gel rig.
    * Small rig: 30mL SB Buffer + 0.30g agarose
    * Medium rig: 80mL SB buffer + 0.8g agarose
    * Large rig: 125mL SB buffer + 1.25g agarose
13. Weigh appropriate amount of agarose and add to proper volume of 1X SB buffer in a conical flask.
14. Using rubber heat protectors, heat solution in microwave to near boiling until agarose is completely dissolved. Add appropriate volume of DNA stain such as Gel-Red or Sybr-Safe and swirl gently to thoroughly mix.
15. Allow solution to slightly cool, pour agarose solution into gel rig. Avoid the formation of bubbles.
16. Allow gel to cool completely for about 30 minutes and then carefully remove the comb.
17. Position the gel into the chamber with the sample end (wells) positioned closest to the negative (black) electrode.
18. Slowly fill the chamber with the remaining 1X SB buffer solution until the top of the gel surface is submerged or until the MAX fill line.
19. Load samples into wells as indicated for each assay.
20. For each tube of PCR product to be visualized, mix 1 uL of gel loading dye to every 5 uL of PCR product needed to fill each well formed in the gel. Mix the sample and the dye by repeated expulsion on wax paper or in separate tube prior to loading. (If loading dye is already included in your MM, then all you need to do is transfer 5 uL into the gel.)
21. When the sample and the dye are adequately mixed, carefully place the pipette tip containing the mixture over an individual well of the agarose gel, and load the well with the sample. Repeat for all the wells, being sure to include the DNA molecular weight standard (one with bands in the 100 – 1000 bp range) for base pair reference, and positive and negative controls.
22. Electrophoresis
23. Put the lid on the gel rig (black to black, red to red).
24. Turn on the electrophoresis machine and adjust the settings as needed.
25. Approximately 70-100 volts for 60 minutes or until tracking dye front approaches the edge of the gel (this is dependent of gel width, buffer, size of DNA fragments and goal of the visualization).
    1. Too high of voltage going too fast can cause smearing of bands. Too low of voltage going too slow can cause low band resolution.
26. Start the electrophoresis machine and check for bubbles forming on both ends of the rig, indicating electricity is running.
27. Visualize the DNA
28. Place gel on UV light source or inside a UV transilluminator and carefully record locations of bands on positive control samples in relation to the DNA molecular weight standard. Band locations of positive controls should be at the anticipated locations according to primers used and their (bp) size.

**Note:** use UV protective goggles or face shield if not using an enclosed transilluminator.

1. Note any unusual band occurrences. Negative controls should not have any bands. Contamination suspicions indicate the samples should be re-run from template DNA tube.
2. Photo document all PCR gels and attach to worksheet containing sample information.
3. Annotate worksheet accordingly and clean lab equipment.