**PCR Preparation**

Thaw all components required for PCR. Create entry in lab notebook with a table including the index for the tubes, genotype of the DNA in each tube, and the primer used. Number the PCR tubes according to the index in the notebook. Once thawed, pipette PCR components in the following order:

1. 25 μL GoTaq Green Master Mix 12.5 μL
2. 5 μL Forward Primer (5 μM dilution) 2.5 μL
3. 5 μL Reverse Primer (5 μM dilution) 2.5 μL
4. 10 μL Nuclease-free or Ultrapure water 5 μL
5. 5 μL DNA (flick DNA tube to mix before adding) 2.5 μL

Once DNA is added carefully pipette to mix. Start up the PCR and place the tubes in the machine. Input the pin (1019) and find the program. Start the program. Note: using half volumes to reduce supply use works.

*Primer Working Concentration Preparation*

Add the amount of nuclease-free or ultrapure water noted on the primer information sheet to the dry primer to make the stock solution. Vortex to mix. In a new tube, add 20 μL of the stock solution to 380 μL of nuclease-free or ultrapure water to attain the 5 μM working concentration.

*PCR Settings*

The PCR program begins with 3 minutes at 95° for initial denaturing. The program then cycles through the following stages 30 times:

1. Denaturing stage: 1:00 minute at 95°
2. Annealing stage: 1:30 minutes at 53° (may change depending on the primer used)
3. Extending stage: 1:30 minutes at 72°

After 30 cycles, the program ends with 5 minutes at 72° and drops to 4° for preservation until the product can be retrieved.

**Gel Preparation**

Determine the number of lanes required and select a gel rig. Place gel box in position to seal the edges. Place comb in desired position.

Determine the necessary volume. Mix TBE 1X with the agarose in an Erlenmeyer flask. Dissolve agarose in the TBE using the microwave at 30 second intervals, swirling the flask in between. Microwave until liquid is completely clear (solutes or bubbles). Allow liquid to cool and then add ethidium bromide using a micropipette. Swirl to mix and poor carefully onto gel rig. Allow to cool for 30 – 40 minutes. The component amounts are below:

|  |  |  |  |
| --- | --- | --- | --- |
| Size | TBE 1X | Agarose (2%) | Ethidium Bromide |
| Small: 35 mL | 35 mL | 0.7 g | 1.2 μL |
| Medium: 75 mL | 75 mL | 1.5 g | 2.6 μL |
| Large: 150 mL | 150 mL | 3.0 g | 5.1 μL |
| Large: 200 mL | 200 mL | 4.0 g | 6.8 μL |

\*Double Agarose for 4% gel to increase separation

*Preparing 1X TBE*

Add 200 mL 5X TBE Buffer to 1000 mL volumetric flask. Fill to 1000 mL with ultrapure water. Place in glass, screw top container, label, and store at room temperature.

*Preparing 5X TBE Buffer*

Add 800 mL of ultrapure water to a beaker. Place on a hotplate with a stir bar and stir while adding the following substances:

* 54 g Tris Base/Trizma
* 27.5 g Boric Acid
* 4.7 g EDTA

Once all substances are dissolved, fill to 1000 mL. Place in a glass, screw top bottle, label container, and store at room temperature.

**Running the Gel**

Remove comb from gel and position gel so the wells are close to the anode (black). The current will run toward the cathode (red), allowing the DNA with a partially negative charge to migrate toward the middle of the gel. Fill gel rig with 1X TBE buffer until the gel is covered completely.

Pipet 10 μL of PCR product into each well and write down the well order using the index numbers associated with each tube. When using a ladder, pipet 8 μL of loading dye on a piece of parafilm and mix 2 μL of the ladder. Set pipet to 10 μL and transfer bead to one well on the gel.

Cover the gel rig and plug into a power source. The power source output should be at the following settings:

* Volts = 100
* mAmps = 350

Run for 45 minutes. Check to make sure the tracking dye is migrating away from the wells.

\*If using a higher percentage gel (4%), decrease volts to 30, keep mAmps the same, and increase time to at least 4 hours.

**Imaging the Gel**

If camera is off, turn on machine by flipping switch on back. Use arrow buttons on front to navigate to the setting 2 for EtBr. Under transillumination, push the “UV” button until the green light turns on. Focus settings on the camera should be rotated to 2 on the top ring and 5.6 on the bottom ring. Open door, center gel with gel box on glass. Remove any obstructions that may remain from other users. Flip transilluminator to 302 nm in lower right corner with door open.

Log in to the computer (password: Science). Click the “FluorChem” shortcut on the desktop. Click “Acquire.” If a pop-up appears, click “continue anyway.” Once window opens check the following:

* All display boxes are NOT marked
* Settings at the bottom right match that of the machine (setting 2, “UV” on)
* Exposure is set to value close to 2 seconds

Use the zoom toward the top of the window to improve the gel display. Click the red “acquire” option. Once the picture is captured, use the gamma settings to improve display. Save the image in “Documents/Travers” using a descriptive file name including the data (mmddyyyy). Print the image and paste in notebook.