Colony PCR and Agarose Gel Electrophoresis Protocol

- **Colony PCR:**

1. Thaw, mix and spin down all solutions, keep on ice. The PCR setup can be performed at room temperature.

2. Prepare the reaction mix as follows.

Reagent	Volume
Phire Plant Master Mix	10 µl
Forward Primer	1 µl
Reverse Primer	1 µl
Sample	x (2 µl of culture or colony)
H_2O	Up to 20 µl
Total	20 µl

3. Place the tubes in the thermal cycler with the following program:

Cycles	Steps	Temperature	Duration of cycle
1x	Initial denaturation	98˚C	5 minutes
35x	Denaturation	98˚C	5 seconds
	Annealing	61˚C	5 seconds
	Extension	72˚C	20 seconds
1x	Final extension	72˚C	1 minute

- **Agarose Gel Electrophoresis**

1. Measure 0.5 g of agarose powder.

2. Mix agarose powder with 50 mL 1xTAE in a microwavable flask.

3. Microwave for 1-3 min until the agarose is completely dissolved. Gently swirl the flask halfway through to prevent the solution from boiling over.

4. Let agarose solution cool down to about 50 °C (about when you can comfortably keep your hand on the flask), about 5 mins.

5. Add 2 µl of SYBR™ Safe DNA Gel Stain to the agarose solution.

6. Pour the agarose into a gel tray with the well comb in place

7. Let sit at room temperature for 20-30 mins, until it has completely solidified.

8. Remove the comb carefully, then place the agarose gel into the gel box.

9. Fill gel box with 1xTAE until the gel is covered.

10. Carefully load a molecular weight ladder and your samples into the wells of the gel.

11. Run the gel at 120 V for 40 minutes.

12. Turn OFF power, disconnect the electrodes from the power source, and then carefully remove the gel from the gel box.

13. Visualize your DNA fragments using a gel imager.