Colony PCR

1. 8 colonies of each plasmid done in total.
2. Colonies 3mL and 2mL (DH10β & LZ) chosen for 2 spectiomycin plates.
3. Prepared 80ulL of PCR stock solution in 16 PCR tubes (2x sets of 8, for colnies 3ml and 2ml).
4. On each spec. petri dish, drew grid lines to mark 16 areas (in a 4x4 pattern).
5. For plate 1 of sample DH10β (2mL):
    a. Used thin end of sterile toothpicks to do a bacterial swab of 1 individual colony from the plate.
    b. Toothpick then dabbed onto area 1 on spec. Plate (determined by grid lines).
    c. Toothpick then gently shaken in 1 of the PCR tubes with PCR solution already present to introduce some colony bacteria into the tube.
    d. Repeated for steps a) - c) for 8 more samples from the gene plate onto the spec plate.
6. For plate 2 of sample DH10β (3mL):
    a. Repeated steps 5. a) - d).
7. **Table 1**: A master mix of PCR volumes/ concentrations was made using Lulu's template of:

Substance/ Concentration	Volume (uL)	Temperature (degrees Celsius)	Duration (seconds)
5x buffer	2	94	600 (=10 min)
25um DNTP's	0.08	98	30
10um Forward	0.25	98	10
10um Reverse	0.25	55	10
Taq enzyme	0.2	72	10
H20	6.22	72	60
Bacterial dilution	1	15	Keep until use

Note: The Forward, Reverse, and Taq were run at 35 cycles.

8. **Table 2**: To make the PCR master mix, the volumes of each of the substances were increased 18x from original volumes to generate:

Substance/ Concentration	Volume (uL)
5x buffer	36
25um DNTP's	1.44

10um Forward	4.5
10um Reverse	4.5
Taq enzyme	3.6
H20	111.96
Bacterial dilution	18

9. The substances, along with their respective volumes, were all pipetted into a PCR tube, starting with the water first (to prevent contamination).
10. The master mix was then centrifuged for 30sec to ensure all contents were mixed thoroughly.
11. 8 new, empty, PCR tubes were dedicated to sample DH10β (2mL), and 7 new, empty, PCR tubes for sample DH10β (3mL), creating 17 PCR tubes. The final PCR tube (#18) was used as a control with just water added.
12. 9mL of the PCR master mix was added to each new tube for both samples.
13. 1mL of the old PCR tubes (where the toothpick containing the bacteria was swished inside) was added to each new PCR tubes for both samples, with respect to the sample (i.e old PCR tubes containing the 2mL bacteria was placed in the new PCR tubes designated for the 2mL samples, etc).
14. Centrifuge both sample sets of the PCR tubes for 30sec to mix contents. Make sure no bubbles were introduced. If there are bubbles, centrifuge again.
15. Put both PCR tube sets in the incubator, following the temperature and duration instructions from **table 1**, taking note of the cycle number.