Primordial Genetics

Electroporation of PG10B Electroporation Competent Cells

1. Chill 1 mm cuvette on ice.
2. Chill sterile microfuge tube on ice
3. Thaw electrocomp cells on ice.
4. Pipet 25 ul of electrocomp cells into microfuge tube.
5. Add 1 ul of DNA.
6. Transfer the DNA-cell mixture to prechilled cuvette. Gently tap the cuvette to ensure the cells settle to bottom of cuvette.
7. Wipe moisture from cuvette and place into electroporator.
8. Electroporate. The recommended settings are 1.7 kV, 200 ohms resistance, and 25 uF capacitance.
9. Remove cuvette, add 975 ul recovery media. Pipet multiple times to ensure cells are mixed well.
10. Transfer cells to a 14 ml centrifuge tube, incubate in a 37^{o}C shaker (225 rpm) for 60-90 minutes.
11. Plate appropriate volume of cells. If entire contents of tube is to be plated on a single agar plate, transfer cells to microfuge tube, centrifuge for 2 min at 12,000 rpm, and resuspend pellet in TE at desired volume and plate immediately.