Protocol Name: Transformation of the Gibson assembly product into pSB1C3
Category: Naringenin Operon Biosynthesis
Date: 10/10/18
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Source(s): NEBuilder HiFi DNA Assembly Transformation Protocol
Time Required: 3 hours

Additional Notes:
Materials

- SOC from SOB broth with H_2O that has had filter sterilised glucose added after it has been autoclaved.
- LB plates with chloramphenicol antibiotic.
- DH5α competent cells.
- NEBuilder HiFi DNA Assembly Master Mix.
- Naringenin operon.
- Positive control DNA.

Procedure

1. Thaw chemically competent cells on ice.
2. Add 2 µl of the chilled assembled product to the competent cells. Mix gently by pipetting up and down or flicking the tube 4 – 5 times. Do not vortex.
3. Place the mixture on ice for 30 minutes. Do not mix.
4. Heat Shock at 42°C for 30 seconds. Do not mix.
5. Transfer tubes to ice for 2 minutes.
6. Add 950 µl of room temperature SOC media to the tube.
7. Incubate the tube at 37°C for 60 minutes. Shake vigorously (250 rpm) or rotate.
8. Warm selection plates with ampicillin for the positive control and chloramphenicol for the assembled product to 37°C.
9. Have a negative control with DH5α cells without any plasmid.
10. Spread 100 µl of the cells onto the selection plates.
11. Incubate overnight at 37 °C.