Name: Asma Khimani
Date: 8/29/19

Goal:

1. Transform original mcherry

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1. Transform original mcherry using NEB 5 alpha electrocompetent cells.

Protocol:

Electroporation

1. Placed SOC recovery medium in a 37°C water bath. Pre-warmed selective plates at 37°C for 1 hour.
2. Placed electroporation cuvettes (1 mm) and microcentrifuge tubes on ice.
3. As a **positive control** for transformation, used 1 ul of BBa_J04450 psB1C3 RFP Construct and 25 ul of competent cells.
4. Thawed NEB 5-alpha Electrocompetent cells on ice (about 10 min) and mixed cells by flicking gently. Transferred 25 µl of the cells (or the amount specified for the cuvettes) to a chilled microcentrifuge tube. Added 1 µl of the mCherry DNA solution.
5. Carefully transferred the cell/DNA mix into a chilled cuvette without introducing bubbles and made sure that the cells deposited across the bottom of the cuvette. Electroporated using the following conditions for BTX ECM 630 and Bio-Rad GenePulser electroporators: 1.7 kV, 200 Omega, and 25 µF. The typical time constant is 4.8 to 5.1 milliseconds.
6. Immediately added 975 µl of 37°C SOC to the cuvette, gently mixed up and down twice, then transferred to the 17 mm x 100 mm round-bottom culture tube.
7. Shook vigorously (250 rpm) or rotated at 37°C for 1 hour.
8. Diluted the cells as appropriate then spread 100-200 µl cells onto pre-warmed **Chlor plates**.
9. Incubated plates overnight at 37°C. 3 Original mCherry plates in incubator @ 4:40 08/29/2019 AK.