Transformation of *E. coli*

Introduction

Transformation of *E. coli* with USER Cloning assembled product (using competent cells).

Materials

- Incubator at 37 °C
- Warm agar plates (media and supplements)
- USER reaction products
- 1.5 mL Eppendorf tubes

Procedure

1. Aliquot 50 µL of chemically competent cells into 1.5 mL Eppendorf tubes (one for every transformation plus 2 for the positive and negative controls) on ice.
2. Warm selection plates to 37ºC.
3. Add 10 µL of the chilled assembly product to the competent cells (or all the mixture). Mix gently by pipetting up and down or by flicking the tube 4-5 times. Do not vortex.
4. Place the mixture on ice for 30 minutes. Do not mix.
5. Heat shock at 42 ºC for 30 seconds. Do not mix.
6. Transfer tubes to ice for 2 minutes.
7. Spread all the mixture (around 60 µL) of cells onto the selection plates.
8. Remember to make positive and negative controls.
9. Incubate overnight at 37 ºC.