Introduction

In this protocol, you will be using chemically competent E coli that have been placed in a calcium chloride solution prior to freezing. The solution acts to neutralize the cells so that they don't repel each other. The bacteria and plasmid mixture will be chilled on an ice for 30 minutes. Placing the mixture in a 42 degrees Celsius water bath for 30 seconds will heat shock the mixture, which will cause the transformation. Once the LB is added and mixed with the transformed bacteria, it can be plated on the LB plate with an antibiotic. Since a lot of the bacteria will not be transformed after the heat shock, plating E. coli with an antibiotic will ensure only the transformed E. coli survive.

Before doing this lab, please watch this video: https://vimeo.com/25201947. Note that we do not have a control for our experiment, and we are not using a thermomixer.

Separate reagents from materials.

Safety precautions

This is a benign lab protocol, but don't forget that PPE is necessary at all times. The *E. coli* you will be working with will be non-pathogenic but it should still be handled properly, which means it should not come into contact with your skin or gloves. If you are removing materials from the -80°C freezer, do not use your bare hands or regular lab gloves. Use gloves designated specifically for the freezer.

Materials

Reagents

- Competent E. coli cells
- 950 µL of SOC
- LB plate supplemented with antibiotics

Equipment

- Set of micropipettes and pipette tips
- 5 x1.5 mL – microcentrifuge tube
- Ice bath
- Thermomixer
- Streaker

Procedure

1. Thaw competent E. coli **on ice**
    a. Competent cells will be in the -80 degrees Celsius freezer
    b. **DO NOT** thaw cells by hand, if they are warmed by hand they will no longer be competent
2. Mix the cells gently with a pipette tip (**DO NOT** pipette up and down)
3. Aliquot 50µL of bacteria into a pre-chilled 1.5mL-microcentrifuge tube.

4. Add 1-2 µL of the plasmid to the bacterial sample, and mix gently with the pipette tip (**DO NOT** pipette up and down)
5. Incubate the tube on ice for 30 minutes.
6. While the bacteria are on ice, ensure the water bath is set to 42°C
7. Put 950µL of SOC in a microcentrifuge tube and place it in the 37°C incubator to warm up during this time
8. After the 30 minute incubation period, place the tube in the 42°C water bath (mixing OFF) for exactly 30 **(45 seconds for E. Coli DH10 beta)** seconds
9. Place the tube on ice for two (2) minutes
10. Add 950 µL of pre-warmed SOC
11. Shake in 37℃ incubator, 300 rpm, for 1-2 hours
12. Spread 100 µL of the transformed bacteria on an LB plate supplemented with the appropriate antibiotic
13. Label plate with name, date, and what it is (i.e. iGEM 2016- Date- Transformed E.coli- Your name- Media Number)
14. Allow the plates to dry inverted, then place in the 37°C incubator O/N

Acknowledgements

Adapted from iGEM Toronto 2015.