Protocol 2: High Efficiency Transformation of NEB DH5-alpha Competent E. *coli* Cells

Purpose: The goal of this Protocol is to insert a plasmid into an E. *coli* cell.

Material:

- Gloves
- Sterilized solution: ethanol
- Sterilized petri dish
- 20 µl, 200 µl, and 1000 µl pipettes and pipette tips
- A frozen tube of 0.2 ml of NEB 5-alpha competent E. *coli* cells
- 25 ml of room temperature (37℃) SOC outgrowth medium
- Sample DNA: blotting paper of specific CAHS plasmid
- Sterilized exact-o-knife
- Transformation tube
- Ice
- Ice bucket
- Heat Block (42℃)
- Shaking Incubator (37℃, 250 rpm)
- 2 media plates containing Luria Broth (LB) and Kanamycin (Kan) (provided by lab or made before using a different protocol)

Methods:

1. Thaw tube of 0.2 NEB DH5-alpha competent E. *coli* cells on ice until ice crystals disappear. Carefully pipette 50 µl of cells into a transformation tube, then gently mix by flicking. Put on ice.
2. On a sterilized petri dish use a sterilized exact-o-knife to cut a 1 cm x 1 cm square of the specific CAHS plasmid blotting paper. Place square in transformation tube, then flick tube 4-5 times to mix cells and DNA. Do not vortex.
3. Place transformation tube on ice for 30 minutes. Do not mix.
4. Heat shock at 42℃ using the Heat Block for exactly 30 seconds. Do not mix.
5. Immediately place on ice for 5 minutes. Do not mix.
6. Use 1000 µl pipette to add 950 µl of room temperature SOC into transformation tube.
7. Place transformation tube in shaking Incubator at 37℃ and 250 rpm for 60 minutes.
8. Label the two media plates with initials, date, LB, Kan, E.*coli*, and one with 20 µl and another with 150 µl. Warm plates in Incubator at 37℃.
9. Remove transformation tube from Incubator and mix cells by flicking or inverting the tube.
10. Remove transformation tube from Incubator and mix cells by flicking or inverting the tube.
11. Store media plates in Incubator overnight at 37℃. Alternatively, incubate at 30℃ for 24-36 hours or at 25℃ for 48 hours.

Analysis: Check media plates the next day for results. If successful, the media plates will have white colonies throughout. The plate with 150 µl will have more colonies compared to the 20 µl plate. If unsuccessful, the media plates will not have any growth and the protocol must be repeated.

References:

New England Biolabs. Protocol guidelines from NEB 5-alpha competent E. *coli* (High Efficiency).