Heat shock transformation

1. Defrost 100µL of competent DH5α cells on ice for 5 minutes
2. Add 10 µL of the assembly mixture from Gibson assembly, mix carefully in the tube using a pipette tip. *NB! Remember that competent cells are stressed already!*
3. Chill on ice for 30 min
4. Heat shock: Transfer the tube with cells and assembly mixture to a heat block at 42 ºC. Keep the tube for 45 seconds in the heat block, then transfer to ice.
5. Chill on ice for 5 minutes
6. Add 1 mL LB medium
7. Incubate at 37°C shaker for 60 minutes at 200-300 rpm.
8. Plate out either 100 uL or concentrate the cells by centrifuging them at 8000 rpm for 3 minutes
9. Discard supernatant. Pour supernatant into the table trash bucket. There will be some medium (ca. 100 uL) left in the tube which you will use to resuspend your cells in.
10. Plate 100 µL on selective LA plates with amp100.
11. Incubate overnight at 37°C. Place the plates in the incubator cabinets shown below.
12. Prepare LB medium with ampicillin by pouring LB medium (50 mL) in a sterile tube (50 mL). Then add amp100 (50 µL). Mix by turning the tube several times.
13. Label two sterile tubes per sample. Pour LB amp (5 mL) into each tube (13 mL).
14. Per sample, two tubes should be prepared by adding one colony in each tube.