Generation of competent *Escherichia coli* DH5α cells

Competent *Escherichia coli* DH5α cells were used for BioBrick cloning.

Chemically competent DH5α cells

1. Streak out frozen glycerol stock of bacterial cells (DH5α etc.) onto an LB plate (no antibiotics). Grow plate overnight at 37 °C.
2. Select a single colony from fresh LB plate and inoculate a 5 mL starter culture of LB. Grow culture at 37 °C in shaker overnight.
3. Inoculate 400 mL of LB media with 5 mL of starter culture and grow in 37 °C shaker. Measure the OD_{600} every hour, then every 15-20 minutes when the OD gets above 0.2.
4. When the OD_{600} reaches 0.35-0.4, immediately put the cells on ice. Chill the culture for 20-30 minutes, swirl occasionally to ensure even cooling.
5. Split the 400mL culture into ice cold 50 mL falcon tubes and harvest the cells by centrifugation at 3000x g for 15 minutes at 4 °C.
6. Decant the supernatant and gently resuspend each pellet in 20 mL of ice cold $MgCl_2$. Combine two suspensions into one 50 mL falcon tube.
7. Harvest the cells by centrifugation at 2000x g for 15 minutes at 4 °C.
8. Decant the supernatant and resuspend the pellet in 20 mL of ice cold $CaCl_2$. Combine two suspensions into one 50 mL falcon tube. Keep the suspension on ice for at least 20 minutes.
9. Harvest the cells by centrifugation at 2000x g for 15 minutes at 4 °C.
10. Decant the supernatant and resuspend the pellet in 10 mL ice cold 85 mM $CaCl_2$, 15 % glycerol. Combine two suspensions into one 50 mL falcon tube.
11. Harvest the cells by centrifugation at 1000x g for 15 minutes at 4 °C.
12. Decant the supernatant and resuspend the pellet in 2 mL of ice cold 85 mM $CaCl_2$, 15 % glycerol.
13. Aliquot 50 µL into sterile 1.5 mL microfuge tubes and snap freeze with liquid nitrogen.
14. Store frozen cells in the -80 °C freezer.

Electrocompetent DH5α cells

1. Streak out frozen glycerol stock of bacterial cells (DH5α etc.) onto an LB plate (no antibiotics). Grow plate overnight at 37 °C.
2. Select a single colony from fresh LB plate and inoculate a 5 mL starter culture of LB. Grow culture at 37 °C in shaker overnight.
3. Inoculate 250 mL of 2x YT media with 500 µL of starter culture and grow in 37 °C shaker. Measure the OD_{600} every hour, then every 15-20 minutes when the OD gets above 0.2.
4. When the OD_{600} reaches 0.5 split the 250 mL culture into sterile ice cold 50 mL falcon tubes and harvest the cells by centrifugation at 5000 rcf for 15 minutes at 4 °C.
5. Decant the supernatant and resuspend the pellet in 25 mL ice cold VE-H_2O. Combine the suspensions into two 50 mL falcon tubes.
6. Harvest the cells by centrifugation at 5000 rcf for 10 minutes at 4 °C.

7. Decant the supernatant and resuspend the pellet in 25 mL ice cold VE-H_2O.
8. Harvest the cells by centrifugation at 5000 rcf for 10 minutes at 4 °C.
9. Decant the supernatant and resuspend the pellet in 25 mL ice cold 10% glycerin.
10. Harvest the cells by centrifugation at 5000 rcf for 10 minutes at 4 °C.
11. Decant the supernatant, 1-2 mL of 10% glycerin remain in the 50 mL falcon tube. Resuspend the pellet in the remaining 10% glycerin.
12. Aliquot 50 µL into sterile 1.5 mL microfuge tubes and snap freeze with liquid nitrogen.
13. Store frozen cells in the -80 °C freezer.

Generation of competent *Vibrio natriegens* cells

Electrocompetent *Vibrio natriegens* cells were generated according to the Marburg 2018 protocol.

Electrocompetent *Vibrio natriegens* cells

1. Streak out frozen glycerol stock of bacterial cells (*Vibrio natriegens*) onto an LB plate containing v2 salts (no antibiotics). Grow plate overnight at 37 °C.
2. Select a single colony and inoculate a 5 mL starter culture of LB containing v2 salts. Grow culture at 37 °C in shaker overnight.
3. Inoculate 600 mL of the same growth medium with the starter culture with a final OD_{600} of 0.05.
4. Grow the culture in 37 °C shaker and measure OD_{600} every hour, then every 15-20 minutes when the OD gets above 0.2.
5. When the OD_{600} reaches 0.5 split the 600 mL culture into sterile ice cold 50 mL falcon tubes and chill the culture on ice for 15 minutes.
6. Harvest the cells by centrifugation at 3000x g for 20 minutes at 4 °C.
7. Decant the supernatant and resuspend the pellet in 10 mL ice cold electroporation buffer. Combine the suspensions in 50 mL falcon tubes.
8. Harvest the cells by centrifugation at 3000x g for 15 minutes at 4 °C.
9. Decant the supernatant and resuspend the pellet in 10 mL ice cold electroporation buffer. Fill up the 50 mL falcon tube with additional ice cold electroporation buffer (max. 50 mL in total).
10. Harvest the cells by centrifugation at 3000x g for 15 minutes at 4 °C.
11. Decant the supernatant and resuspend the pellet in 10 mL ice cold electroporation buffer. Fill up the 50 mL falcon tube with additional ice cold electroporation buffer (max. 50 mL in total).
12. Harvest the cells by centrifugation at 3000x g for 15 minutes at 4 °C.
13. Decant the supernatant, 1-2 mL of electroporation buffer remain in the 50 mL falcon tube. Resuspend the pellet in the remaining electroporation buffer.
14. Measure the OD_{600} in a 1:20 dilution against electroporation buffer. The volume is adjusted with additional electroporation buffer to reach the final OD_{600} of 16.
15. Aliquot 80 µL into sterile 1.5 mL microfuge tubes and snap freeze with liquid nitrogen.
16. Store frozen cells in the -80 °C freezer.