Culturing

Making our own Chemical competent cells

Source: https://www.neb.com/protocols/2012/06/21/making-your-own-chemically-competent-cells (Retrieved 28-08-2017)

1. Culture desired strain O/N 0.5mL/50mL LB in a Greiner tube.
2. Grow to OD_{600} 0.6 in 0.1/10mL LB.
3. Centrifuge at 8000rpm for 5 min.
4. Resuspend in 5mL ice-cold $CaCl_2$.
5. Distribute 1.5mL over 3 Eppendorfs and spin for 30 seconds, 8000rpm.
6. Resuspend pellet in 0.5mL ice-cold $CaCl_2$, flick the tube gently.
7. Aliquot into 50µL Cryo-tubes and store at -80ºC until transformation.

Transformation using Heat-Shock

Source: https://www.neb.com/protocols/2012/05/21/transformation-protocol (Retrieved 28-08-2017)

1. Add plasmid DNA (1µL of miniprep/2µL of ligation mixture/...µL of cesium-purified DNA).
2. Incubate on ice for 30 min.
3. Heat shock at exactly 42ºC for exactly 30 seconds, then chill on ice again for 5 minutes.
4. Culture in 1mL SOC for 1 hour at 37ºC and pre-warm the agar plates in the meantime.
5. Spin the culture down at 3000rpm for 3 minutes, discard 900µL and resuspend the pellet in the remaining 100µL.
6. Plate accordingly on agar plates with the appropriate antibiotic, if applicable.

Making our own Electrocompetent cells

Source: https://www.neb.com/protocols/2012/06/21/making-your-own-electrocompetent-cells (Retrieved 28-08-2017)

1. Inoculate the strain to be made electrocompetent into 10mL SOB medium and incubate overnight at 37ºC, 200rpm.
2. Add two drops of this culture to 2x 250mL pre-warmed SOB in 1L flasks. Incubate in 37ºC until an OD_{600} of ~0.6 has been reached. Meanwhile, pre-chill the centrifuge to 4ºC.
3. Place cultures on ice for 15 minutes. Pour each culture into a cooled 500mL centrifuge bottle.
4. Centrifuge at 5000rpm for 10 minutes. Pour off the supernatant.
5. Resuspend the cell pellets in 250mL 10% glycerol and centrifuge again at 5000rpm for 10 minutes.
6. Pour off the supernatant again and resuspend in 250mL 10% glycerol.
7. Centrifuge one last time at 5000rpm for 10 minutes, pour off the supernatant and resuspend in the remaining liquid present in the bottle.
8. Cells are now ready for electroporation, or can be stored at -80ºC until further use.

Transformation using Electroporation

Source: https://www.neb.com/protocols/1/01/01/electroporation-protocol-c2986 (Retrieved 28-08-2017)

1. Pre-chill the electroporation cuvette on ice, thaw the electrocompetent cells on ice as well and mix them by flicking gently.
2. Transfer the amount of cells required for the electroporation cuvette in question (e.g. 50µL). Add 1µL of DNA solution.
3. Insert the correct specifications (Voltage, Resistance, etc.) for the used transformation strain into the pulse generator. Place the electroporation cuvette and apply the pulse.
4. Immediately add up the volume to 1mL with SOC medium (e.g. 950µL) and incubate the culture for 1 hour at 37ºC.
5. Spin the culture down at 3000rpm for 3 minutes, discard 900µL and resuspend the pellet in the remaining 100µL.
6. Plate accordingly on agar plates with the appropriate antibiotic, if applicable.
7. Incubate the plates overnight at 37ºC.

Glycerol stocks

1. Take 500µL of an overnight culture and add it to a sterile cryotube.
2. Add 500µL of a sterile 50% glycerol solution and invert 2-3 times.
3. Place the glycerol stock in -80ºC until needed again.