Transformation by Electroporation for *L.reuteri*

Buffer:
0.5M sucrose
10 % (v/v) glycerol

Plates:
MRS-broth
5 µg/ml erythromycin

DAY 1

Cultivation

1. Pipet 5ml MRS-broth in a 10-15ml tube.
2. Scrape up a small amount of glycerol stock of the *L.reuteri* and transfer them to the tube.
3. Incubate overnight (~16-24h) at 37° C. Take note that *L.reuteri* is more likely to lysate if not matured properly.

DAY 2

Follow the protocol *Measurement and control of Optic Density*

Competent cells

– From here on the cells must **ALWAYS** be kept on ice and **NEVER** be vortexed.

1. Centrifuge the culture **in the cold room** for 5 min with 5000 rpm. Keep them on ice afterwards.
2. Pour off the supernatant and resuspend the pellet carefully in 20 ml cold buffer.
3. When the pellet is in resuspention add additionally 20 ml of cold buffer.
4. Repeat steps 1 to 3 once. Finish by centrifuging one last time in the cold room, 5 min with 5000 rpm.
5. Pour off the supernatant and add buffer until the total volume is 1/5 of the initial volume, which is 800 µl (40/50ml=0.8ml). **Start with adding 600 µl and resuspend the pellet carefully. If there is less than 800 µl add buffer to make up the difference**

6. Add 100 to cooled and marked individual Eppendorf tubes. Keep them on ice.

Electroporation

1. Add 1 – 5 µl plasmid DNA to one Eppendorf tube with competent cell and add 5 µl water another tube with competent cells for control.
2. Carefully pipet the DNA mix to a precooled and labelled electroporation cuvette (2 mm). Place the pipet tip at a 45^{o} angle against the wall of the cuvette while pipetting.
3. Put the lid on and carefully tap the cuvette for an even distribution and make sure that there aren't any air bubbles. Keep the cuvettes on ice.
4. Start the electroporation on the settings: **2500 V**, **25 µF** and **400 Ω**. Keep the cuvettes on ice afterwards. The anticipated time constants are around 8 – 10 ms.
5. Add 1 ml room tempered MRS-broth when all transformations have been completed and mix by gently inverting the cuvette twice.
6. Incubate the cells for 2,5 h at 37^{o} C for recovery.

Plating the cells

1. Make sure you invert the cuvettes 2 – 3 times before pipetting.
2. Make sure that the plates (MRS + 5 µl/ml erythromyesin) are dry and that there are is no liquid left after spreading.
3. Spread 1, 10 and 100 µl on the plates.
4. The rest can be transferred to a tube with 10 ml MRS-broth with the same antibiotic concentration as the plates (to find transformants at low numbers ; after cultivation for 16 – 24 h, the culture has to be plated on agar plates if they are to be used).
5. Place the MRS-pates in an anaerobic jar with a gaspack or in an anaerobic chamber (if in an anaerobic chamber the plates have to be covered with parafilm). OBS! L.reuteri can grow in an aerobic environment although with lessened efficiency.