**Preparation of chromosomal DNA**

Materials, for two strains:
- 10 mL TNE buffer
- 2 mL TNEX
- 200 µL Lysozyme (5 mg/ml)
- 50 µL Proteinase K (20 mg/ml)
- 200 µL 5M NaCl
- 96% EtOH
- 100mL dH$_2$O

1. Harvest 500 µl culture: Spin down cells (5 min at 7000 g) and decant.
2. Wash with 1 ml TNE: Resuspend in 1 ml TNE; vortex; spin down cells (5 min at 7000 g); decant.
3. Resuspend pellet in 270 µl TNEX.
4. Add 30 µl of a freshly prepared lysozyme solution (5 mg/ml in H$_2$O).
5. Add 7.5 µl Proteinase K solution (20 mg/ml in H$_2$O)
6. Incubate for 90 min at 37°C and then at 65°C for another 90 min.
7. Add 15 µl 5 M NaCl and mix gently by inverting the tube.
8. Add gently 1 ml 96% EtOH. After 2-3 min mix gently and incubate 15 min at -20°C.
9. Spin at 15000 g for 10 min at 4°C.
10. Wash pellet with 1 ml ice cold 96% EtOH. Be careful! Don’t loose the DNA!
11. Spin at 15000 g for 10 min at 4°C.
12. Remove remaining EtOH by pipetting followed by incubation at 37°C for 3-5 min with the lid open (do not over dry pellet).
13. Add 500 µl dH$_2$O and mix gently by inverting the tube. Store at 20°C o.n. to dissolve the DNA; then at -20°C
16 S rDNA PCR amplification on chromosomal DNA from 2 isolates.

Materials
Sterile MilliQ water
10xPCR buffer
dNTP 10mM
9F primer, 5’-GAGTTTGATCTGGCTCAG-3’ (100µM)
1512R primer, 5’-ACGGCTACCTTGTACGACTT-3’ (100µM)
DMSO
Taq-polymerase

Important – Keep everything on ice until the PCR is running and work sterile!

The 16S rDNA from 2 isolates is amplified by the use of PCR with the primers 9f and 1512r

1. Prepare a PCR master mix with 4 x each of the components written below, except the DNA template and Taq-polymerase.
2. Pipette 48.5 µl of the master mix in 3 PCR tubes and add 1 µl chromosomal DNA solution to 2 of the tubes; one tube for each species! The third tube is a negative control without DNA.
3. The Taq-polymerase should be added just before the run. The master mix is made in excess to cover losses during pipetting.

The final content of each of the 3 samples should be:
34.5 µl sterile MilliQ water
5 µl PCR buffer (tag buffer with (NH₄)₂SO₄, no MgCl₂)
2 µl dNTP
1 µl 9F primer
1 µl 1512R primer
1 µl DMSO
4 µl MgCl₂
1 µl DNA template (or dH₂O in the case of the negative control!)
0.5 µl Taq-polymerase

The PCR is done with the following temperature profile: 95°C 5 min; 30 cycles of:
94°C 30 sec,
53°C 30 sec,
72°C 90 sec,
72 °C 5 min;
thereafter 4°C until storage at -20°C.

PCR products are sent for sequencing