**Sečoveljske soline protocol**

1. Weigh 0,5 g / 0,25 g of mud or petola into the tube.
2. Add 0,4 g glass beads and 500 μl lysis buffer (100 mM Tris-HCl, 100 mM NaEDTA, 1,5 M NaCl, 1 % CTAB, pH 8).
3. Homogenize with FastPrep for 45 s at 6,5 m/s.
4. Add 15 μl each of lysozyme (50 mg/ml) and 3 μl proteinase K (10 mg/ml) ~~and 0,75 μl RNase A (10 mg/ml) - use RNAse carefully, filter tips here!~~ For now, try without RNase.
5. Incubate for 30 min at 37 °C.
6. Add 100 μl SDS (20 %).
7. Incubate for 2 hours at 65 °C with vigorous shaking by hand every 30 min.
8. Vortex for 3 min at max speed.
9. Centrifuge for 10 min at 6000 g at RT.
10. Collect the supernatants in a 15 ml falcon.
11. Resuspend pellet with 250 μl lysis buffer.
12. Homogenize on vortex.
13. Incubate for 10 min at 65°C.
14. Centrifuge for 10 min at 6000 g at RT.
15. Transfer the supernatant to a collection falcon.
16. Repeat steps 11-15.
17. Add equal V of chloroform to the supernatants (in the fume hood! Parafilm on the falcon!).
18. Mix the solution and centrifuge for 15 min at 6000 g.
19. Transfer the water phase to new 15 ml falcon tubes or eppi (do not touch the interphase!).
20. Add 0,6 V isopropanol.
21. Store overnight at 4°C.
22. Centrifuge for 30 min at 10.000 g at 4°C and pipette out the supernatant (pipette carefully, don’t touch the pellet).
23. Purify the precipitate in 1 ml 70% EtOH (cold, 4°C).
24. Centrifuge for 15 min at 10.000 g at 4°C.
25. Remove the supernatant (pipette carefully) and air dry the pellet completely (cca 10 min).
26. Resuspend the pellet in 50 μl TE buffer (10 mM Tris-HCl, 1 mM NaEDTA, pH 8).