Stoeck Protocol

DNA-Extraction

1. cells were collected on 47-mm Durapore membranes (pore size: 0.65 µm)
2. immidiately after filtration, membranes were individually frozen (ca. –200 °C) in DNA extraction buffer* with proteinase K (final concentration, 100 µg ml^{-1})
3. for extraction: thaw frozen filter and transfer filter and storage buffer (DNA extraction buffer*) to 15 ml tube
4. add 5 ml DNA extraction-buffer*
5. add 25 µl proteinase K (20 mg/ml)
6. shake sample horizontally for 30 min at 37°C, 225 rpm
7. add 0.8 ml SDS (20%)
8. incubate for 2 h in 65°C waterbath, invert occasionally (every 15-20 minutes)
9. transfer "supernatant" to 50 ml centrifuge tube (PC or PPCO), filter remains in 15 ml tube
10. extract filter again, by adding 2.5 ml DNA extraction-buffer and 0.25 ml SDS (20%)
11. vortex 10 sec, incubate for 10 min in 65°C waterbath
12. combine supernatant with the supernatant already in 50 ml centrifuge tube (PC or PPCO
13. add 1 vol chloroform-isoamylalkohol (24:1)
14. mix
15. centrifuge at 6,000 g for 10 min at RT
16. transfer upper aqueous layer containing nucleic acids to clean 50 ml centrifuge tube (PPCO)
17. add 0.7 vol isopropanol (RT)
18. precipitate DNA for 1h at RT
19. centrifuge at 16,000 g for 20 min at RT
20. wash pellet with 3 ml EtOH (70%, RT) (light and short vortexing)
21. centrifuge at 16,000 g for 20 min at RT
22. dsicard EtOH
23. dry pellet
24. resuspend pellet in 500 to 1000 µl TE buffer (pH 8) by pipetting up and down, eventually resuspend over night at 4°C

*DNA extraction buffer:

100 mM TrisHCl pH8
100 mM Na2EDTA pH8
100 mM NaPhosphatebuffer pH8
1.5 M NaCl
1 % CTAB