DNA EXTRACTION (A. Chistoserdov)

1. To the thawed and shredded filter membranes add:
   0.5 mL of 10% SDS
   250 μL of Proteinase K (10 mg/mL)
2. Incubate at 50 ^0C for 10'.
3. Freeze at –80 ^0C for 15'.
4. Repeat 2 and 3 twice → total of 3 times.

Phenol-Chloroform extraction

5. To the sample add 1 vol of water saturated phenol solution
6. Vortex until you have a milky-like consistency.
7. Centrifuge at 5000 rpm for 5' at 4 ^0C.
8. There should be two layers ⇒ carefully remove the upper layer by aspiration, transfer the supernatant to a fresh tube.
9. To the treated mix add 1 vol of chloroform. Vortex briefly.
10. Centrifuge at 5000 rpm for 15' at 4 ^0C.
11. Remove supernatant as in 8 and transfer to fresh tube.

PRECIPITATION

12. To the sample add
    1/10 vol of NaAc 3M
    2 vol of chilled 100% EtOH
13. Vortex VIGOROUSLY → 30" each tube.
14. Freeze at –80 ^0C for at least 2 h, or overnight.
15. Without melting, centrifuge at 10000 rpm for 45'.
16. Remove and STORE supernatant for possible recovery.
17. Spin again BRIEFLY and remove any remaining liquid.
18. Wash pellet with 2 mL of 70% EtOH → move liquid CAREFULLY around the walls. Spin BRIEFLY. Remove any remaining liquid.
19. Dry pellet at room temperature until there's no EtOH left → MAKE SURE the tubes don't smell like EtOH.
20. Dissolve the pellet in 100 μL of sterile HPLC grade water. If pellet doesn't dissolve add another 100 μL.