

Protocol for DNA Isolation

1. Put 250 μ L extraction buffer in 1.5 mL Eppendorf tube.
2. Add wing punch.
3. Add 20 μ L Proteinase K (20 mg/mL). Mix well.
4. Incubate at 55°C overnight or until wing punch is dissolved.
5. Add 500 μ L phenol-chloroform-isoamyl alcohol (PCI).
6. Shake gently by hand for 10 minutes.
7. Centrifuge for 10 minutes at 7000 RPM.
8. Pull off top (aqueous) layer to a new tube. Discard lower layer.
9. Repeat steps 5-8.
10. Add 500 μ L chloroform.
11. Shake gently by hand for 10 minutes.
12. Centrifuge for 10 minutes at 7000 RPM.
13. Pull off top (aqueous) layer to a new tube. Discard lower layer.
14. Repeat steps 10-13.
15. Add 11.1 μ L of 4.5M NaCl. (Final concentration of salt = 0.2M).
16. Add 2 volumes of ice-cold 100% ethanol.
17. Incubate overnight at -20°C.
18. Centrifuge for 30 minutes at 14000 RPM at 4°C.
19. Carefully pour off ethanol, keeping track of the DNA pellet at the bottom of the tube.
20. Dry the pellet under vacuum or on benchtop, until thoroughly dry.
21. Resuspend the DNA in 15-20 μ L of 1/10 TE.
22. Incubate at room temperature for 24 hours to allow DNA to go back into solution.
23. Quantify DNA with a fluorometer.
24. Dilute to a working concentration of 10 ng/ μ L in 1/10 TE for PCR.
25. Run a checking gel to determine the size of recovered DNA.

Recipes:

DNA Extraction Buffer

- 100 mM NaCl (1.11 mL of 4.5M NaCl)
- 10 mM Tris-HCl, pH 8.0 (0.5 mL of 1M Tris-HCl, pH 8.0)
- 10 mM EDTA, pH 8.0 (0.5 mL of 1M EDTA, pH 8.0)
- 0.5% SDS (1.25 mL of 20% SDS)
- dH₂O to 50 mL final volume.
(Hint: do not combine concentrated salt directly with concentrated SDS, or SDS will come out of solution. First, add most of the water to dilute other ingredients.)

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