UvA UNIVERSITEIT VAN AMSTERDAM

eDNA isolation by precipitation.

Vs 4.2

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Materials:

-3M Sodium Acetate (NaAce) pH 5,2	(3957201, Brunschwig chemie bv)
-isopropanol	(190764-1L, Sigma-Aldrich)
-Phosphate Buffered Saline (PBS)	(15414499, Fischer Scientific)
-Purelink gDNA isolation kit	(10053293, Fischer Scientific)

1. Add 0.1 volume of 3 M Sodium Acetate to 1 volume of water sample (in a 15 or 50 ml tube).
2. Add 1 volume of isopropanol to the water sample.
3. Store overnight in 0-4 °C.
4. Centrifuge 1 h at 4600 x *g* in a centrifuge with swing-out rotor*****.
5. Decant and discard the supernatant.
6. Centrifuge 5 min at 4600 x *g*.
7. Carefully pipet the supernatant and discard it.
8. Air-dry the pellet for 30 min at room temperature.
9. Resuspend the pellet in 200 µL 1x PBS.
10. Short spin down
11. Transfer the 200 µl to a 1,5 ml Eppendorf tube.
12. Store at - 20°C or use directly in the Purelink gDNA isolation kit (step 13)

Use the following protocol to prepare lysate from the dissolved pellet. (Protocol extracted from manual Purelink gDNA isolation kit)

13. Set a water bath or heat block at 55°C.
14. Add 20 µL Proteinase K (supplied with the kit) to the sample.
15. Add 20 µL RNase A (supplied with the kit) to the sample, mix well by brief vortexing and incubate at room temperature for 2 minutes.
16. Add 200 µL PureLink Genomic Lysis/Binding Buffer and mix well by vortexing to obtain a homogenous solution.
17. Incubate at 55°C for 10 minutes to promote protein digestion.
18. Spin for 5 min at 13000 x *g* and transfer the supernatant to a clean 1,5 ml Eppendorf tube.
19. Add 200 µL 96–100% ethanol to the lysate. Mix well by vortexing for 5 seconds to yield a homogenous solution.
20. Add the lysate (~640 µL) prepared with PureLink Genomic Lysis/Binding Buffer and ethanol to the PureLinkR Spin Column.
21. Centrifuge the column at 10,000 x *g* for 30 sec. at room temperature.
22. Discard the collection tube and place the spin column into a clean PureLink Collection Tube supplied with the kit.

23. Add 500 µL Wash Buffer 1 prepared with ethanol to the column.
24. Centrifuge column at room temperature at 10,000 × *g* for 30 sec.
25. Discard the flow-through and place the spin column back into the PureLink collection tube.
26. Add 500 µL Wash Buffer 2 prepared with ethanol to the column.
27. Centrifuge the column at 13000 x *g* for 2 minutes at room temperature. Discard collection tube.
28. Place the spin column into a new and clean PureLink collection tube.
29. Centrifuge the column at 13000 x *g* for 5 minutes at room temperature. Discard collection tube.
30. Place the PureLink Spin Column in an Eppendorf lowbind 1,5 ml ep.
31. Elute the DNA by adding 25 µl elution buffer (provided in kit) and incubate for 1 minute at room temperature.
32. Centrifuge for 1 minute at 13000 x *g*.
33. The ≈23 µl eluate, containing the eDNA, is ready to use in an qPCR assay.
34. For qPCR of point samples use duplicates of 5 µL purified sample

*****do not spin above 4600 x *g.* The 15- or 50-ml tubes will break, although they are rated for 20 k x *g* this is not the case for a swing-out rotor where all the force concentrates in the tip of the tube!

Fixed angle rotor vs swingout rotor: