UvA UNIVERSITEIT VAN AMSTERDAM

eDNA isolation from PES 0.45 µm filters with enhanced CTAB buffer.

Based on: Improving eDNA yield and inhibitor reduction through increased water volumes and multi-filter isolation techniques

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Materials:

-Phenol/chloroform/isoamylalcohol (25:24:1) =**PCI**	(K169, VWR)
-3-bromochloropropane =**BCP**	(ACRO106860010, VWR)
-100 % ethanol (EtOH)	(1.00983.1000, VWR)
-70 % ethanol (EtOH)	
-3 M Sodium Acetate (NaAce) pH 5,2	(3957201, Brunschwig chemie bv)
-EchoCLEAN DNA Cleanup Kit	(Bio echo; 020-002-030-050)

-CTAB enhanced lysis buf. Per ml:

EDTA 0.5M	(E7889, Sigma Aldrich)	40 µl
Tris 1M (ph7,5)	(15567027, Thermofischer)	100 µl
NaCl 5M	(E529-500ml, VWR)	280 µl
CTAB	(H9151, Sigma Aldrich)	20 mg
PVP 40	(PVP40, Sigma Aldrich)	40 mg
B-mercaptoethanol	(M3148, Sigma Aldrich)	5 µl
RNase/DNase free H2O	(10977-035, Thermofischer)	up to 1 ml

Note: CTAB and PVP 40 dissolves slow in H2O, incubate at 55 °C for 10-20 min.

1. Pipet the lysis buffer from the filter jar to a 2 ml Eppendorf tube (ca. 750ul)
2. Use a clean tweezer to fold the filter 2 times in the jar and transfer it to the 2ml vial with lysis buffer.
3. Ad 4 µl β-mercaptoethanol. (lysis buffer can turn to purple, this is normal)
4. Vortex at room temperature for 10 min.
5. Option 1: Store the tube with filter overnight at room temperature and incubate the next day for 30 min at 55 ˚C.
6. Option 2: Incubate directly for 2 hours at 55 ˚C
7. Spin the tubes shortly.
8. Add 800 µl PCI and vortex for 10 min or until the PES filter is fully disintegrated.

9. Spin for 15 min at 15000 x g.
10. Transfer the upper phase carefully (≈800 µl) to a new 2 ml vial
11. Add 400 µl BCP and mix well.
12. Spin for 10 min at 15000 x g.
13. Transfer the upper aqueous phase to a new 2 ml tube.
14. Add 0.1-part 3M NaAce and mix.
15. Add 1 ml 100% EtOH and mix well
16. Precipitate 30 min at -20 ˚C
17. Spin for 15 min at 17000 x g at room temperature
18. Decant the supernatant, be careful not to disturb the pellet.
19. Wash the pellet by adding 750 µl 70 % EtOH
20. Spin 5 min at 17000 x g and carefully pipet of the supernatant.
21. Dry the pellet to the air. Alternatively, to speed things up, dry it in a vacuum centrifuge.
22. Dissolve the pellet in 90 µl H2O for the use in Echo CLEAN kit.
23. Vortex an Echo CLEAN column and place in a 2 ml waste tube, let it stand for 10 min.
24. Punch a hole in the cap with the Cap Puncher (delivered with Kit) and snap of bottom closure. Place column back in waste tube.
25. Spin for 1 min at 1000 x g to elute column buffer.
26. Discard waste tube and place column in a clean prelabeled 1,5 ml Eppendorf tube.
27. Pipet the 90 µl dissolved eDNA via the puncture hole slowly on the column.
28. Spin for 1 min at 1000 x g
29. The eluted eDNA should be colorless and is ready to use.
30. Use duplicates of 10 in a 30 µl qPCR reaction