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Red and Brown Algal DNA Extraction

Purpose: To extract algal genomic DNA

Procedure:

1. Grind .5µL of sample in liquid nitrogen
2. Add 500µL of Red Algal Buffer for Red Algae or 500µL of Brown Algal Buffer for Brown Algae
3. Add 10µL of 10mg/mL ProK
4. Add 50µL 10% Tween
5. Incubate on rotator for an hour
6. Freeze at -20 °C for twenty minutes
7. Centrifuge at 13.2 KxG for ten minutes
8. Warm Elution Buffer (Buffer PE) to 65 °C
9. Pipette supernatant into filtration column
10. Centrifuge at 11 KxG for two minutes
11. Add 450µL of Buffer PC to flow-through and pipette up and down five times to mix
12. Pipette 700µL supernatant/Buffer PC solution into binding column
13. Centrifuge at 11 KxG for one minute and discard flow-through
14. Repeat previous two steps for remainder of solution
15. Apply 700µL Buffer PW2 Wash solution
16. Centrifuge at 11 KxG for one minute and discard flow-through
17. Apply additional 200µL of Buffer PW2 Wash solution
18. Centrifuge at 11 KxG for two minutes
19. Place column in fresh 2mL tube
20. Add 50µL of Elution Buffer (65 °C Buffer PE)
21. Incubate at 65 C for five minutes

22. Centrifuge at 1 KxG for one minute
23. Repeat previous three steps with another 50µL of Elution Buffer and elute into the same tube
24. Freeze at -20 °C

Reagents

Red Algal Buffer for 100 mL (from Saunders 1993 JPhyc 29:251-254)

Reagent	**Amount**	**[Final]**
1M Trizma	4.48 mL	0.448M*
1M Tris HCl	5.52 mL	0.552M*
0.5 M Na_2EDTA	10 mL	0.05M
NaCl	1.165 gm	0.2M
Potassium Acetate	24.54 gm	2.5M

* = 0.1M total Tris

Brown Algal Buffer for 100mL (from McDevit and Saunders 2009 JSP:132)

Reagent	**Amount**	**[Final]**
1M Tris base	4.48 mL	0.448M*
1M Tris HCl	5.52 mL	0.552M*
0.5 M Na_2EDTA	10 mL	0.05M
NaCl	1.169 gm	0.2M
$CaCl_2$	3.329 gm	0.3M

* = 0.1M total Tris

Adjust to pH 8.0 andtop off to 100ml

Other Buffers

A Machery-Nagel NucleoSpin Plant II kit for DNA, RNA, and protein provided the other buffers.

ProK and Tween diluted from stock