Kelp DNA extractions - HMW

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ABSTRACT

DNA extraction protocol for Durvillaea antarctica with extremely high polysaccharide levels.

PROTOCOL CITATION

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https://protocols.io/view/kelp-dna-extractions-hmw-bwk7pczn

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1 Add 500mg of freeze dried kelp powder to 7mls of lysis buffer (4% CTAB, 2% PVP 40K, 80mM borax, 250mM EDTA, 20mM tris pH 8) and 20ul proteinase K.

This solution is extremely viscous, it couldn't pipette it - instead I poured it into a 15ml tube.

2 Mix together and lyse overnight at 65 degrees

3 Centrifuge for 10 minutes at 4,000rcf and transfer supernatant to new tube (easier to pour it into new tubes than to pipette it).

4 Cool to room temperature and add 7mls of chloroform:isoamyl alchol, mix thoroughly, centrifuge for 20 minutes at 4,000rcf

5 Transfer upper phase to new tube and add 0.5 volumes of 10% CTAB and 0.25 volumes of 500mM borax. Mix thoroughly.

6 Incubate at 65 degrees for 20 minutes

7 Cool to room temperature and add an equal volume of chloroform isoamyl alcohol. Mix thorougly.

8 Centrifuge for 20 minutes at 4,000 rcf

9 Transfer upper phase to new tube and repeat steps 7-8.

10 To the upperphase, add 1 volume of 100% isopropanol. You may notice that the isopropanol isn't fully miscible in the solution, add 1ml of H2O at a time till the solution is mostly miscible.

11 Incubate on ice for 10 minutes, then centrifuge at 4,000rcf for 5 minutes.

12 Pour off supernatant and add 5mls of 70% Ethanol and mix thoroughly through inversion (use a pipette tip full of 70% ethanol to disoldge the pellet). Centrifuge for 5 minutes at 4,000rcf

13 Repeat step 12

14 Remove supernatant, spin briefly, then remove any residual ethanol.

15 Dry pellet (I do this using a vacuum centrifuge set to dessicate mode)

16 Add 500ul to 1ml of H2O to 10mM Tris to resuspend