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Kappaphycus alvarezii DNA Extraction by CTAB (2X) and precipitation in ammonium acetate and isopropanol

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We use this protocol and it's working

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Abstract

Our optimized CTAB protocol offers a reliable solution for high-quality genomic DNA extraction from Kappaphycus alvarezii, a red seaweed, samples.

Materials

Reagentes:
- Tampão CTAB 2X: 15 ml
- 2-MERCAPTOETHANOL 99% (Acros Organics, Cat#125470010): 0,2% ul
- POLYVINYL PYRROLIDONE (PVP) 40 (Sigma, Cat#PVP40-100G): 2% g
- Clorofórmio, Molecular Biology Grade (MPBiomedicals, Cat#193814): 15 ml
- Isoamyl alcohol, (molecular biology) ≥98.5% (Sigma, Cat#I9392)
- Isopropanol, Molecular Biology Grade (Sigma Aldrich, Cat#I9516-500ML): 1 volume ml
- Acetato de amônio, 7,5 M (P/ BIOMOL): 0,08 volume ul
- Etanol, Molecular Biology Grade (Sigma Aldrich, Cat#E7023-500ML): 5 ml
- "TE high salt" (BIOMOL) feito no lab: 5 ml
- "TE minus NaCl" (BIOMOL) feito no lab: 250 ul
- Água Molecular Biology Grade (Sigma Aldrich, Cat#W4502-1L): 10 ml
- Água sanitária diluída (0.5%): 200 ml (NA necessário para 1 experimento)
- Álcool spray ou LGC Cleaner: 1 ml (NA necessário para 1 experimento)

Material plástico DNAse 26 RNAse Free dedicados para extração de DNA:
- Ponteria com filtro, 1000 µl: 5 Unidade
- Ponteria com filtro, 200 µl: 4 Unidade
- Ponteria com filtro, 10 µl: 1 Unidade
- Microtubos, 1.5 mL: 5 Unidade
- Falcon 15 ou 50 mL (para preparo de etanol 75%): 1 Unidade (NA necessário para 1 experimento)

Material para dissecção:
- Pinça
- Haste para estilete nº 11
- Lâmina para estilete nº 11 (descartável)
- Placa de Petri estéril
- Papel toalha
- Papel lenço em rolo (para forrar a bancada)
- LGC Cleaner

Equipamentos e espaços:
- Micropipetas dedicadas para extração de DNA (p1000, p200, p10)
- Microcentrífuga com refrigeração (para eppendorfs e com velocidade até 12000xg)
- Capela (em todas as etapas com Clorofórmio:alcool isoamílico)
- Bancada pré-PCR

Before start

Considerations for bench and solution prep:
Make sure the bench and materials have been thoroughly cleaned and sterilized. Use diluted chlorine (at 0.5%), followed by an ethanol 70% cleanup, and after it has dried, spray LGC Cleaner, let it soak for 5 minutes, and then cleanup with ethanol and a piece of paper.
Always prepare fresh solutions and aliquots, and use molecular biology-grade water for dilutions.

CTAB (2X)

25m

1 This buffer consists of Tris-HCl (at a final concentration of 0.1 M), NaCl (1 M), EDTA (0,2 M), and CTAB (at 2%), without PVP and b-mercaptoethanol (to be added immediatedly prior to use).

1.1 To prepare a 100 ml solution, add 10 ml of Tris-HCl (at 1 M, pH 8.0), 46,66 ml of NaCl solution (at 3 M) and 4 ml of EDTA (at 0.5 M). 5m

1.2 Add 2 g of CTAB, and adjust the volume up to 100 ml with distilled water.

1.3 Autoclave the solution for 20 minutes, at 1 ATM and 121ºC. 20m

1.4 Before use of the solution, for 15 ml (one sample) add 0.3 g of PVP (for a 2% final concentration) and 30 ul of β-mercaptoethanol (for 0.2%).

TE high salt

25m

2 This buffer consists of Tris-HCl (at a final concentration of 0.01 M), NaCl (1 M), and EDTA (1 mM).

2.1 To prepare a 50 ml solution, add 0.5 ml of Tris-HCl (at 1 M, pH 8.0), 16.66 ml of NaCl solution (at 3 M) and 100 ul of EDTA (at 0.5 M). Adjust the volume up to 50 ml with distilled water. 5m

2.2 Autoclave the solution for 20 minutes, at 1 ATM and 121ºC. 20m

TE high minus NaCl

3 This buffer consists of Tris-HCl (at a final concentration of 0.01 M), and EDTA (1 mM).

3.1 To prepare a 50 ml solution, add 0.5 ml of Tris-HCl (at 1 M, pH 8.0), 16.66 ml of NaCl solution (at 3 M) and 100 ul of EDTA (at 0.5 M). Adjust the volume up to 50 ml with distilled water.

3.2 Autoclave the solution for 20 minutes, at 1 ATM and 121ºC.

Tissue prep

10m

4 Use properly sterilized material, as described above.

5 If the samples are not being extracted immediately after sampling, place the algae pieces in aluminum foil, wrap and properly identify the sample. Immerse the sample in liquid nitrogen, and store it at -80 oC until processing.

6 Place small algae pieces in a crucible, and pour liquid nitrogen into it until the pieces are fully covered. Macerate the pieces into a fine powder, constantly replacing the liquid nitrogen, not letting it evaporate completely. 10m

Extração de DNA

6h 20m

7 Transfer 5 g of the algae powder into a 50 ml falcon, with 15 ml of CTAB (with 2% PVP and 0.2% b-mercaptoethanol). Add 15 ml of chloroform:isoamyl alcohol (24:1). Gently invert the tubes to homogenize the content. 5m

8 Centrifuge the falcons at maximum speed (5000 rpm) for 20 minutes, at room temperature. 20m



9 After centrifugation, two phases will form. Transfer the aqueous phase into a new falcon and add 1 V (about 15 ml) of isopropanol.

10 Keep samples at -20 oC overnight or at -80 oC for 1 hour. 1h



11 Centrifuge the falcons at maximum speed (5000 rpm) for 20 minutes, at room temperature. Discard the supernatant. 20m



12 Add 5 ml of ethanol 80% and 0.08 V (400 ul) of ammonium acetate 7.5 M.

13 Centrifuge the falcons at maximum speed (5000 rpm) for 20 minutes, at room temperature. Discard the supernatant. 20m



14 Add 5 ml of ethanol (100%). Incubate the tubes for 20 min at room temperature.



15 Centrifuge the falcons at maximum speed (5000 rpm) for 20 minutes, at room temperature. Discard the supernatant.

16 Resuspend the pellet in 5 ml in "TE high salt", incubate at 60 oC por 1-2 hours. 2h

17 Add 5 ml of CTAB 2X (without PVP and b-mercaptoethanol) and 5 ml of chloroform:isoamyl alcohol (24:1).

18 Centrifuge the falcons at maximum speed (5000 rpm) for 20 minutes, at room temperature. Discard the supernatant. 20m

19 After centrifugation, two phases will form. Transfer the aqueous phase into a new falcon and add 1 V (about 15 ml) of isopropanol.

20 Keep samples at -20 oC overnight or at -80 oC for 1 hour. 1h

21 Centrifuge the falcons at maximum speed (5000 rpm) for 20 minutes, at room temperature. Discard the supernatant.

22 Add 5 ml of ethanol 80% and 0.08 V (400 ul) of ammonium acetate 7.5 M.

23 Centrifuge the falcons at maximum speed (5000 rpm) for 20 minutes, at room temperature. Discard the supernatant. 20m

24 Add 5 ml of ethanol (100%). Centrifuge the falcons at maximum speed (5000 rpm) for 20 minutes, at room temperature. Discard the supernatant and let the pellets dry at room temperature. 20m

25 Resuspend the pellets in 250 ul of "TE minus NaCl".

26 For RNA removal, add 0.25 ul of RNase A and incubate at 37 oC for 15 min 15m

27 Assess sample quality and DNA concentration by NanoDrop and Qubit, and integrity by eletrophoresis by agarose gel (0.8%).

Acknowledgements

This procedure was originally created by *Milica Markovic_*.

Procedural Steps:
13. Centrifugar na velocidade máxima (para falcão de 50ml) por 20 minutos.
14. Após a centrifugação, duas fases se formarão: o fundo com sujeira e clorofórmio e o sobrenadante aquoso.
15. Transfira o sobrenadante para um novo falcon e adicione 1 volume de isopropanol;
16. Deixe a -20ºC durante a noite;
17. No dia seguinte, Centrifugue na velocidade máxima (para um falcon de 50 ml) por 20 minutos.
18. Descarte o sobrenadante;
19. Adicionar 5 ml de etanol 80% e 0,08v de acetato de amônio 7,5M.
20. Centrifugue por 20 minutos;
21. Descarte o sobrenadante e adicione 5ml de etanol 100%. Centrifugue por 20 minutos novamente em máxima velocidade.
22. Descarte o sobrenadante e deixe o pellet secar;
23. Ressuspenda o pellet em 250 ul de "TE minus NaCl"
24. Adicionar 0,25ul de RNase (Sigma-Aldrich R4642-10MG) incubar a 37ºC por 15´.
25. O DNA pode ser avaliado por NanoDrop e eletroforese em agarose (0,8%), e quantificado por fluorimetri (Qubit).