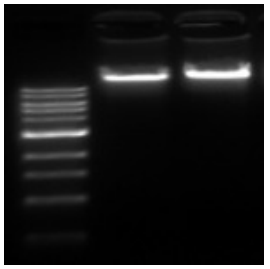
Feb 09, 2017 Version 1

Skeletonema DNA extraction by Plant DNAzol V.1



DOI
dx.doi.org/10.17504/protocols.io.g6bbzan

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OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.g6bbzan

Protocol Citation: Adrian Clarke: Skeletonema DNA extraction by Plant DNAzol. **protocols.io https://dx.doi.org/10.17504/protocols.io.g6bbzan**

Protocol status: Working

Created: February 09, 2017

Last Modified: March 15, 2018

Protocol Integer ID: 5027

Preparations

1 - Prepare **Plant DNAzol** by adding RNAse (100ug/mL, make 0,6mL/sample)
- Prepare **Wash solution,** 1 part **Plant DNAzol** to 0,75 parts 99% ethanol (2×0,6mL/sample)
- Prepare **75% ethanol** solution (5*0,6mL/sample)

Pellet Skeletonema culture (1500 x g, 5 min), remove growth me and dissolve in f/2 or growth media ~250uL.

2 Use Swing-out rotor

Put liquid N2 in mortar, drop Skeletonema suspension into N2 and grind once its evaporated.

3

Transfer ground cells to 2mL eppendorf tube and add 600uL prepared DNAzol (with RNAse).

4

Mix by inversion; incubate at 25°C (RT) for 5 min with a little shake.

5

Add 600uL Chloroform, mix by vortexing in short bursts, and incubate at 25°C (RT) for 5 min with a little shake.

6

Centrifuge 10 min at 12000g

7

Transfer the supernatant (aqueous phase) to a fresh eppendorf tube. (Around 800 uL)

8

Add 1200uL 99% ethanol to aqueous phase, mix by inversion and incubate for 5 min at RT.

9 Adjust volume to match 75% of supernatant if using other than 800uL

Pellet DNA by centrifugation, 4 min, 7000g. Remove the supernatant.

10

Add 600uL Wash solution to percipitate, mix by pipetting,

11

Incubate samples 5 min at RT.

12

Pellet DNA by centrifugation, 4 min, 7000g. Remove the supernatant by pipett

13

Repeat the Washing Procedure one more time (Step 11-

14

. Add 600uL 75% ethanol solution

15

Pellet DNA by centrifugation, 4 min, 7000g. Remove the supernatant as much as possible.

16

Repeat step 15+16 three-four additional times.

17

Evaporate the remaining ethanol in speed

18

. Dissolve the DNA in 80uL Water

19

Centrifuge 12000g for 5 min

20

Take the supernatant (75uL); add 0,75 ul 10xTE.

21 Makes for 0.1xTE for storage in 4C or -20