Modified genomic DNA extraction method for Heterosigma akashiwo

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Abstract

This protocol was developed for isolating high quality genomic DNA from Heterosigma akashiwo for the purpose of Next generation sequencing technologies. We successfully repeated this protocol to obtain genomic DNA.

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Protocol

Step 1.

Starting material: Start with 2 x 100 ml axenic culture, about 2.5 x 10^5 cell/ml

Step 2.

Divide into 50 ml round bottom tubes and centrifuge in a swing rotor at 2,000 rpm for 2’

Step 3.

Pour off liquid and add 2 ml of DNA Extraction Buffer per tube

DNA Extraction Buffer

(final concentrations are shown)

CTAB (hexadecyltrimethylammonium bromide)	2% (w/v)
Tris Buffer pH 8.0	100 mM
EDTA (Ethylenediaminetetraacetic acid)	20 mM
NaCl	1.4 M
PVP (Polyvinylpyrrolidone, MW 40,000)	1% (w/v)

Prepared in DNase/RNase-free water

Add 2% (v/v) β-mercaptoethanol to the amount of extraction buffer needed each time just before starting

Note

Prepare all buffers with sterile solutions and bottles so you could skip autoclaving very viscous solutions. CTAB-containing buffers will need overnight stirring for CTAB to dissolve completely

Step 4.

Homogenize [PT3100 Polytron] at max speed 10”-15” to disrupt pellet

Step 5.

Combine homogenate in two 15 ml tubes

Step 6.

Add 8 ml DNA Extraction Buffer to each tube

For the following steps, use gentle inversion to mix. DO NOT vortex!

Step 7.

Incubate at 65°C for 1 h gently mixing every 10’

Step 8.

Add RNase A to a final concentration of 100 µg/ml, and incubate at 37°C for 30’

Step 9.

Centrifuge at 13,000 rpm, at room temperature for 10’

Step 10.

Transfer supernatant to a new tube, extract with same volume of phenol:chloroform:isoamyl alcohol (IAA) (25:24:1, pH6.6), and centrifuge at 13,000 rpm for 2’ at 4°C

Step 11.

Transfer the aqueous upper phase and add 1/10 volume of pre-warmed 65°C High Salt Buffer and mix well by inversion

High Salt Buffer

(final concentrations are shown)

CTAB	10% (w/v)
NaCl	0.7 M

Prepared in DNase/RNase-free water

Note

CTAB is added to the above solution in batches and allowed to dissolve overnight

Step 12.

Extract with same volume of phenol:chloroform:IAA as in step 10

Step 13.

Transfer the aqueous upper phase, and extract with same volume of chloroform:IAA (24:1); should have pretty clean aqueous/organic interface at this step

Step 14.

Transfer the aqueous upper phase into a new tube, add 0.8 volume of isopropanol and 40 µg of glycoblue

Step 15.

Precipitate genomic DNA at -80°C overnight

Step 16.

Centrifuge at 13,000 rpm, 4°C for 30’

Step 17.

Discard supernatant and wash pellet twice with 70% (v/v) ethanol

Step 18.

Air dry the DNA pellet and dissolve it in 200 µl Qiagen elution buffer

Expected yield 25-30 µg