Isolation Of Total DNA From NC64A Chlorella

David Dunigan and Irina Agarkova

Abstract

Citation: David Dunigan and Irina Agarkova Isolation Of Total DNA From NC64A Chlorella. **protocols.io** dx.doi.org/10.17504/protocols.io.ethbej6
Published: 13 Jun 2016

Guidelines

MATERIALS:

1) NC64A chlorella
2) Sterile 500 mL flasks
3) MBBM
4) 50 mM $NaHPO_4$, pH 7.4, 2.0 M NaCl
5) 20% SDS
6) Proteinase K, 25 mg/mL, autodigested at 37°C, 60 min prior to use
7) RNAse A, 10 mg/mL, heated at 80°C, 10 min prior to use
8) 5 M KOAc
9) 3 M NaOAc
10) 100% EtOH
11) 70% EtOH
12) Isopropanol
13) 50 mM Tris-HCl, pH 8.0, 10 mM EDTA
14) 10 mM Tris-HCl, pH 8.0, 1 mM EDTA (1X TE)
15) Sterile d-H_2O

Before start

Autodigest Proteinase K, 25 mg/mL, at 37°C 60 min prior to use
Heat RNAse A, 10 mg/mL, at 80°C 10 min prior to use

Protocol

Step 1.

Inoculate 500 mL flasks with NC64A *chlorella*, each flask to contain 360 mL of cells at 1.2 X 10^6 cells/mL in MBBM.

Step 2.

Incubate the flasks at 25°C for 72 hours, with continuous light and shaking.

DURATION

12:00:00

Step 3.
Count the cells.

NOTES

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Cells concentration should be 1.0-2.0 X 10^7 cells/mL.

Step 4.
Concentrate aliquots of NC64A *chlorella*, each aliquot to contain 6.0 X 10^9 cells.

Step 5.
Centrifuge the required volume of cells for each aliquot in the Sorvall GSA rotor at 5,000 rpm, 5 min, 4°C.

DURATION

00:05:00

Step 6.
Resuspend each aliquot with 160 mL of fresh MBBM.

NOTES

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The final concentration of cells is to be 4.0 X 10^7 cells/mL.

Step 7.
Incubate the flasks for 45-60 min at 25°C for the cells to acclimate to the new media.

DURATION

01:00:00

Step 8.
If the cells are to be infected with virus, infection should be at an moi (multiplicity of infection) of 3-5.

Step 9.
Incubate the flasks for the desired length of time at 25°C with continuous light and shaking.

Step 10.
Centrifuge the samples in the Sorvall GSA rotor at 5,000 rpm, 5 min, 4°C to harvest the cells.

DURATION

00:05:00

Step 11.
Discard the supernatants.

Step 12.
Wash the cells 1X with sterile d-H_2O in the Sorvall HB-4 rotor at 5,000 rpm, 5 min, 4°C.

DURATION

00:05:00

Step 13.
Quick freeze the cells pellets with liquid N_2 and store the frozen pellets at -80°C until ready for processing.

Step 14.
Resuspend each frozen sample with 5.0 mL of 50 mM $NaHPO_4$, pH 7.4, 2.0 M NaCl.

Step 15.
Break the cells in the MSK mechanical homogenizer with 5.0 gm of 0.3 mm glass beads for 60 sec, with CO_2 cooling.

DURATION
00:01:00

NOTES
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Take care not to freeze the samples.

Step 16.
Heat the samples at 65°C for 30 min (leave in the MSK flasks during the heating).

DURATION
00:30:00

Step 17.
Break the samples a second time in the MSK for 30 sec with CO_2 cooling.

DURATION
00:00:30

Step 18.
Recover the homogenates to clean tubes (SS34 plastic tubes).

Step 19.
Wash the glass beads with 50 mM $NaHPO_4$, pH 7.4, 2.0 M NaCl.

Step 20.
Add the washes to the homogenates.

NOTES
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The final volume is to be 10.3 mL.

Step 21.
Remove a 0.3 mL aliquot from each sample to microfuge tubes for determination of the original DNA concentration for each sample (use the fluorometric procedure).

Step 22.
Treat each sample with 500 µL of proteinase K for 60 min at 37°C (add 200 µL/sample).

DURATION
01:00:00

Step 23.
Heat the samples at 65°C for 5 min.

DURATION
00:05:00

Step 24.
Add 20% SDS to each sample to a final concentration of 1% (add 500 µL/sample).

Step 25.
Add 2.7 mL of 5 M KOAc to each sample, mix well (a final concentration of 1 M).

Step 26.
Incubate the samples in the cold room for 30 min.

DURATION
00:30:00

Step 27.
Centrifuge the sample in the Sorvall SS34 rotor at 14,000 rpm, 20 min, 4°C.

DURATION
00:20:00

Step 28.
Decant the supernatants to clean tubes.

Step 29.
Treat the samples with RNAse at 200 µg/mL for 2 hours at 37°C (add 200 µL/sample).

DURATION
02:00:00

Step 30.

Precipitate the DNAs in the samples by adding 2X volumes of 100% EtOH (approximately 30 mL/sample).

Step 31.
Store at -20°C for 2-3 hours.

DURATION
03:00:00

Step 32.
Centrifuge the tubes in the Sorvall HB-4 rotor at 10,000 rpm, 15 min, 4°C.

DURATION
00:15:00

Step 33.
Discard the supernatants.

Step 34.
Resuspend each DNA sample with 3.5 mL of 50 mM Tris-HCl, pH 8.0, 10 mM EDTA.

Step 35.
Dialyze the samples overnight at 4°C against several changes of 50 mM Tris-HCl, pH 8.0, 10 mM EDTA.

DURATION
18:00:00

Step 36.
Add 375 µL of 3 M NaOAc to each sample.

Step 37.
Precipitate the DNAs by adding an equal volume of isopropanol to each tube.

Step 38.
Mix well and hold at room temperature for 30 min.

DURATION
00:30:00

Step 39.
Centrifuge the samples in the Sorvall SS34 rotor at 10,000 rpm, 20 min, 4°C.

DURATION
00:20:00

Step 40.
Discard the supernatants.

Step 41.
Wash the DNA pellets 1X with 10 mL of 70% EtOH in the Sorvall SS34 rotor at 10,000 rpm, 5 min, 4°C.

DURATION
00:05:00

Step 42.
Dry the pellets briefly (10-15 min) in the vacuum desiccator to remove the EtOH.

DURATION
00:15:00

Step 43.
Resuspend the pellets with 2.0 mL of 1X TE buffer.

Step 44.

Determine the DNA concentration of the samples using the fluorometric procedure.

Step 45.
Run CsCl gradients.