DNA Extraction from Symbiodinium Cultures

Tingting Xiang,Arthur Grossman

Abstract

Dinoflagellates are unicellular algae that can have photosynthetic or nonphotosynthetic lifestyles. Dinoflagellates in the genus *Symbiodinium* can enter endosymbiotic associations with corals, providing the metabolic basis for the highly productive and biologically diverse coral-reef ecosystems (Hoegh-Guldberg, 1999), as well as with other cnidarians, including sea anemones and jellyfish, and noncnidarian hosts (Trench, 1993; Lobban *et al.*, 2002; Mordret *et al.*, 2016).

Here I describe a protocol for isolating total DNA from *Symbiodinium* cells.

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Guidelines

Prepare the lysis buffer before you start.

Before start

Prepare 2X lysis buffer in this composition:
2% SDS,
400mM NaCl,
40 mM EDTA,
100mM Tris-HCl, pH 8.0

Keep the buffer at room temperature, SDS will precipitate in the coldness. Warm up the buffer in advance at 37ºC into clear liquid if it was precipiated.

Materials

- RNase A 19101 by Qiagen
- Sodium Chloride S271 by Fisher Scientific
- EDTA, disodium salt, dihydrate S312-500 by Fisher Scientific
- Sodium dodecyl sulfate 436143-25G by Sigma

Aldrich

UltraPure™ Phenol:Chloroform:Isoamyl Alcohol (25:24:1, v/v) 15593031 by Thermo Fisher Scientific

Chloroform:Isoamyl alcohol 24:1 C0549 by Sigma Aldrich

Tris hydrochloride 10812846001 by Sigma Aldrich

Ethanol 64-17-5 by Contributed by users

UltraPure™ Agarose 16500500 by Invitrogen - Thermo Fisher

Protocol

Grow Symbiodinium cells

Step 1.

Grow Symbiodinium cells in IMK or IMK+casein hydrolysate medium to mid log phase, the concentration is about 10^6 cells/mL.

Harvest Symbiodinium cells

Step 2.

Spin down the cells at 1000 *g* at room temperature for 5 minutes, and remove the supernatant.

Prepare the lysis buffer

Step 3.

Warm up the 2X lysis buffer if precipitated. Dilute the buffer using equal volume of water, and make it into 1X lysis buffer.

NOTES

Approximately 500 µl of lysis buffer is needed for 1 million cells. Prepare the appropriate amount of lysis buffer based on the numer of samples for processing.

Lyse Symbiodinium cells

Step 4.

Add the 500 µl 1X lysis buffer to the cell pellet;

Lyse Symbiodinium cells

Step 5.

Vortex the pellet to resuspend it well at room temperature.

Lyse Symbiodinium cells

Step 6.

Add 500 µl Phenol:Chloroform:Isoamyl alcohol (25:24:1) to the lysis buffer, and mix well by inverting for a few times.

Lyse Symbiodinium cells

Step 7.

Separate phases by centrifugation at 13,000 *g* for 5 minutes, and transfer the upper layer which contains the DNA to a new 1.5 mL eppendorf tube.

RNaseA treatment to get rid of RNA

Step 8.

Treat the supernatant with 4 µl Ribonuclease A (20mg/mL), and incubate the solution for 30 minutes at 37°C.

Purify the DNA - get rid of the proteins

Step 9.

Add 500 µl Phenol:Chloroform:Isoamyl alcohol (25:24:1) to the solution and mix well by inverting for a few times;

NOTES

Make sure get clean upper layer from this extraction, do not touch the middle layer. Extract withChloroform:Isoamyl alcohol (24:1) for additional times if needed to fully get rid of proteins.

Purify the DNA - get rid of the proteins

Step 10.

Separate phases by centrifugation at 13,000 *g* at room temperature for 5 min;

Purify the DNA - get rid of the proteins

Step 11.

Transfer the upper layer phase to a new 1.5 mL eppendorf tube;

Purify the DNA - get rid of the proteins

Step 12.

Add 500 µl Chloroform:Isoamyl alcohol (24:1) to the solution and mix well by inverting a few times;

Purify the DNA - get rid of the proteins

Step 13.

Separate phases by centrifugation at 13,000 *g* at room temperature for 5 min;

Purify the DNA - get rid of the proteins

Step 14.

Tranfer the upper layer phase to a new 1.5 mL eppendorf tube.

Precipitate the DNA

Step 15.

Add 2.5 volumes of absolute Ethanol, incubate on ice for 30 min or at -20 ºC overnight;

Precipitate the DNA

Step 16.

Centrifuge the solution at 13,000 *g* at room temperature for 20 minutes;

Precipitate the DNA

Step 17.

Wash the pellet once with 1mL of 70% Ethanol;

Precipitate the DNA

Step 18.

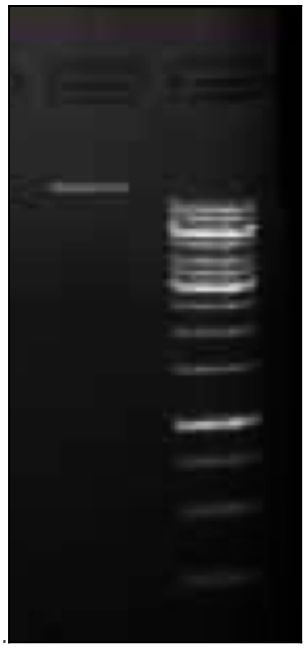
Dry the pellet and resupsend in 50 µl H_2O;

Precipitate the DNA

Step 19.

Quantify the DNA concentration by NanoDrop, and run a small amount (for example 1 µl) on 1% agrose gel.

NOTES



Agarose gel electrophoresis of Symbiodinium genomic DNA obtained using this protocol.