2×CTAB Protocol for predominantly host DNA isolation from symbiotic Aiptasia

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Abstract

This protocol is kit-free and can be used to isolate high quality genomic DNA of predominantly Aiptasia from symbiotic anemones which can be used e.g. as PCR template for genotyping.

It is based on the method described in Coffroth et al., 1992.

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Guidelines

Make sure to use Phenol:Chloroform:Isoamyl alcohol (25:24:1) which is buffered with TE to a neutral to slightly basic pH (everthing starting from 6.7 should work) since DNA stability decreases at acidic pH (the opposite is true for RNA).

Be sure not to exceed the maximal ×g for which your microcentrifuge tubes are certified otherwise they might get damaged during centrifugation and release all the hazards substances (and the DNA of course) into the rotor. You don't want that!

If making your own stock solutions of RNase A from powder be aware that some products contain DNase impurities. If this should be the case you need to boil the stock solutions once for 15-20 min at 100°C and let cool down at room temperature to inactivate the DNases.

Before start

Recipes:
2×CTAB Buffer
(Coffroth et al. (1992) Marine Biology 114: 317-325)

End concentrations:		**For 100 ml buffer mix:**
1.4 M	NaCl	28 ml of a 5 M stock solution
20 mM	EDTA (pH 8)	4 ml of a 0.5 M stock solution
100 mM	Tris/HCl (pH 8)	10 ml of a 1 M stock solution
2% (w/v)	CTAB powder	2 g
Add after filter sterilization under a hood:		
0.2% (v/v)	β-mercaptoethanol (**TOXIC!!!**)	200 µl

- Add ddH_2O to just under 100 ml.
- Warm to 65°C under stirring to bring the CTAB into solution.

- Once dissolved, bring final volume to 100 ml using a graduated cylinder.
- Filter sterilize (0.2 µm) into sterile 50 ml Falcon tubes and store at −20°C.

Heat to 65°C before usage since freezing leads precipitation of the CTAB.

Materials

0.5 mm Zirconia/Silica Beads 11079105z by Bio Spec Products Inc.

Glass beads, acid-washed, 425-600 µm (30-40 U.S. sieve) G8772-100G by Sigma Aldrich

Ethyl alcohol, Pure 200 proof, for molecular biology E7023 by Sigma Aldrich

2-mercaptoethanol M-6250 by Sigma-aldrich

Hexadecyltrimethylammonium bromide (CTAB) H9151 by Sigma Aldrich

✓ Water bath at 65°C by Contributed by users

✓ Microcentrifuge by Contributed by users

✓ Microcentrifuge tubes (1.5 or 2 ml, screw-cap or safe-lock) by Contributed by users

✓ 0.5 M EDTA Stock Solution (adjusted to pH 8.0 with NaOH) by Contributed by users

✓ 1 M Tris/HCl Stock Solution (dissolved Tris base adjusted to pH 8.0 with HCl) by Contributed by users

✓ Vortexer/Multivortexer (<=2000 rpm) by

Contributed by users

✓ Proteinase K (20 mg/ml) by Contributed by users

Phenol/Chloroform/Isoamyl alcohol (25:24:1), stabilized, saturated with 100 mM Tris-EDTA to pH 8.0 AC327111000 by Fisher Scientific

Chloroform – isoamyl alcohol mixture 25666 by Sigma Aldrich

✓ 70% Ethanol by Contributed by users

✓ Nuclease-free water (e.g. MilliQ or HPLC grade water) by Contributed by users

✓ RNase A (10 mg/ml stock) by Contributed by users

Protocol

Sample Preparation

Step 1.

Homogenize anemone with a rotor-stator in 500 µl 2× CTAB buffer individually in screw-cap or safe-lock 1.5-2 ml microcentrifuge tubes.

This is important for the Phenol extraction; simple tubes might leak!Make sure your tubes tolerate the chemicals and centrifugation forces!

Make sure to clean the rotor thouroughly afterwards.

SAFETY INFORMATION

The 2-Mercaptoethanol in the CTAB buffer is toxic if inhaled, swallowed or at skin contact.

Sample Preparation

Step 2.

Do 5 rounds of sheering through a 25-gauge needle.
Important: *DNA in host-algae homogenate in SDS or water will degrade rapidly with every freeze-thaw cycle! If you want to perform DNA isolation from these samples later, freeze the samples after step 6.*

Sample Preparation

Step 3.
Pellet algae at 10,000 ×g for 5-10 min.

Sample Preparation

Step 4.
Transfer the supernatant into a new tube. Discard the tube with the algal pellet.

DNA Isolation

Step 5.
Add 3.6 µl of Proteinase K (20 mg/ml). Mix by inverting several times.

DNA Isolation

Step 6.
Incubate at 65°C for 30-60 min. Invert occasionally while incubating.
The suspension should become green and less opaque when the cells lyse. 20 min should be enough for less dense samples.

DURATION

00:30:00 Additional info: 65°C incubation

DNA Isolation

Step 7.
Add 600 µl Phenol:Chloroform:Isoamyl alcohol (25:24:1, TE-buffered to pH ≥ 7, very TOXIC!!!), mix thoroughly by vortexing several seconds.
This should result in a milky emulsion with two phases starting to form.

SAFETY INFORMATION

Phenol is carcinogenic and causes chemical burns at skin contact. Chloroform is a carcinogen and an irritant.

DNA Isolation

Step 8.
Centrifuge for 10 min at 14,000 ×g to separate the phases.

DNA Isolation

Step 9.

Take 550 µl of the aqueous, upper phase without disturbing the interphase and transfer to a new tube.

DNA Isolation

Step 10.

Add 8 µl of RNase A (10 mg/ml stock concentration) to the sample, mix well and incubate at 37°C for 30 min.

During this step the RNA from the sample is degraded and removed during the next steps. If you started with a lot of sample material, prolong this step to 1 h.

DURATION

00:30:00 Additional info: RNase A treatment

DNA Isolation

Step 11.

Add 600 µl Phenol:Chloroform:Isoamyl alcohol (25:24:1, TE-buffered to pH ≥ 7, very TOXIC!!!), mix thoroughly by vortexing several seconds.

This should result in a milky emulsion with two phases starting to form.

SAFETY INFORMATION

Phenol is carcinogenic and causes chemical burns at skin contact. Chloroform is a carcinogen and an irritant.

DNA Isolation

Step 12.

Centrifuge for 10 min at 14,000 ×g to separate the phases.

DNA Isolation

Step 13.

Take 500 µl of the aqueous, upper phase without disturbing the interphase and transfer to a new tube.

DNA Isolation

Step 14.

Add 500 µl of Chloroform:Isoamyl alcohol (24:1), mix thoroughly by vortexing several seconds.

You can also use plain Chloroform.

SAFETY INFORMATION

Chloroform is a carcinogen and

an irritant.

DNA Isolation

Step 15.

Centrifuge for 10 min at 14,000 ×g.

DNA Isolation

Step 16.

Take 450 µl of the aqueous, upper phase and transfer to a new tube.

Do NOT disturb the interphase!

DNA Isolation

Step 17.

Add 1 ml of 100% ethanol (molecular biology grade) and mix well.

DNA Isolation

Step 18.

Incubate for ≥30 min at RT.

DURATION

00:30:00 Additional info: Incubation at room temperature

DNA Isolation

Step 19.

Centrifuge for ≥30 min at 14,000 ×g at RT.

Be sure to orient all tubes in the same direction to know on which side the pellet will form.

DNA Isolation

Step 20.

Decant supernatant.

You might not see any pellet at this step. Just be careful to not scratch off the DNA from the side where the pellet should be.

DNA Isolation

Step 21.

Add 500 µl 70% ethanol, don't mix, centrifuge for 5 min at 14,000 ×g.

DNA Isolation

Step 22.

Remove the supernatant carefully with a pipet, without disturbing the pellet.

It helps to use a 1000 µl pipette with a respective tip and add a 200 µl tip.

DNA Isolation

Step 23.

Air-dry for 10 min or until no Ethanol is visible.

DURATION

00:10:00 Additional info: Drying

DNA Isolation

Step 24.

Add 30 µl ddH_2O or 10 mM Tris/HCl pH 8.5 to the pellet.

You can also use TE (Tris-EDTA) buffer but be aware that the EDTA might interfere with downstream enzymatic reactions.

DNA Isolation

Step 25.

Store the samples at −20°C indefinitely or use them directly.

Warnings

Safety Remarks: Be sure to follow chemical safety procedures. β-Mercaptoethanol and Phenol are very toxic if inhaled, ingested or by skin contact. So, read the safety data sheets, work under a hood for at least the Phenol and Chloroform steps, wear nitrile gloves (don't re-use them), safety glasses and a lab coat. If you never worked with Phenol:Chloroform before, ask someone who did about handling of it!

Waste Disposal: Follow guidelines of your intitution for disposal. E.g. discard bottom organic layers in liquid Phenol-Chloroform waste container, the tubes and glass beads into solid Phenol-Chloroform waste container.