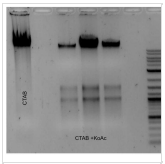


Jul 26, 2021

Slime away: a simple CTAB-based high molecular weight DNA and RNA extraction protocol for "difficult" invertebrates (rev20200518)

Sergio Vargas[1], Cüneyt Caglar[1], Gabi Büttner[1], Simone Schätzle[1], Fabian Deister[1], Gert Woerheide[1]

[1]Department of Earth and Environmental Sciences, Paleontology & Geobiology, Ludwig-Maximilians-Universität München, Richard-Wagner-Str. 10, 80333 München, Germany

dx.doi.org/10.17504/protocols.io.bwcwpaxe

Sergio Vargas
Department of Earth and Environmental Sciences, Paleontology...

DISCLAIMER

Before starting please check the **Guidelines & Warnings and the Materials sections**.

Be sure you understand all the steps and the risks (if any) associated with them.

This protocol is provided free of charge without warranty. Modifications might be necessary to optimize it for your group of interest.

ABSTRACT

Protocols for the extraction of nucleic acids of recalcitrant non-model invertebrates are lacking. Here we present an extraction protocol for the extraction of DNA and RNA from mucus-rich invertebrate species. We have successfully used the protocol for the extraction of high-molecular-weight DNA from cnidarians and poriferans and to recover high-quality RNA from samples impossible to extract with commercial methods.

DOI

dx.doi.org/10.17504/protocols.io.bwcwpaxe

PROTOCOL CITATION

Sergio Vargas, Cüneyt Caglar, Gabi Büttner, Simone Schätzle, Fabian Deister, Gert Woerheide 2021. Slime away: a simple CTAB-based high molecular weight DNA and RNA extraction protocol for "difficult" invertebrates (rev20200518). **protocols.io** https://dx.doi.org/10.17504/protocols.io.bwcwpaxe

KEYWORDS

CTAB, Invertebrates, Difficult samples, Non-model organisms, Mucus, sponges, corals, porifera, cnidaria

IMAGE ATTRIBUTION

DNA/RNA extraction of the octocoral *Xenia* sp. comparing the results of the classical CTAB and our modified protocol on a difficult, mucus-rich octocoral. Photo: Cüneyt Caglar

CREATED

Jul 06, 2021

LAST MODIFIED

Jul 26, 2021

PROTOCOL INTEGER ID

51318

GUIDELINES

Before starting with this protocol, please read the MSDS of the reagents used in it and be sure you fully understand the dangers associated with each of them.

Check with your lab. manager whether you are allowed to use this protocol and use the necessary reagents.

Wear gloves and safety goggles. Different gloves might be necessary for different chemicals! Be sure you know which type of gloves you need to wear to handle the different reagents used in this protocol. The regulations can change from country to country, **be sure you know what safety regulations apply in your locality**.

Citation: Sergio Vargas, CÃÂ¼neyt Caglar, Gabi BÃÂ¼ttner, Simone SchÃÂ¤tzle, Fabian Deister, Gert Woerheide (07/26/2021). Slime away: a simple CTAB-based high molecular weight DNA and RNA extraction protocol for "difficult" invertebrates (rev20200518). https://dx.doi.org/10.17504/protocols.io.bwcwpaxe

MATERIALS TEXT

1. Use 2ml microcentrifuge tubes.

2. Before starting the procedure, pre-heat the CTAB buffer in the oven to 60ºC. **ALL BUFFERS should be prepared with MilliQ water and autoclaved.**

Optional:
If RNA extraction will be done with the buffers, **all buffers BUT Tris-HCl must be treated with DEPC overnight and autoclaved**.

Table 1: Stock and working buffer concentration for DNA/RNA extraction:

A	B	C
Solution	**Stock Concentration**	**Volume for 4,9 ml**
PVP	10%	1ml
Tris-HCL pH 8.0	1M	500µl
EDTA pH 7.5	500mM	250µl
NaCl	5M	2ml
H2O	-	150µl
CTAB	10% (store at 60°C)	1ml

Other solutions needed for extraction:

- β-Mercaptoethanol
- Chloroform
- 80% Ethanol
- Isopropanol
- DNase-free Water

Optional solutions and enzymes:

- 5M Potassium acetate (KOAc)
- RNAse A

SAFETY WARNINGS

β-Mercaptoethanol is toxic use it only in the fume hood and be careful handling it.

Chloroform is highly volatile and flammable, use it only under the fume hood and be careful while handling it. Be sure the plastic wear you use is resistant to chloroform.

DISCLAIMER:

Before starting please check the **Guidelines & Warnings and the Materials sections**.

Be sure you understand all the steps and the risks (if any) associated with them.

This protocol is provided free of charge without warranty. Modifications might be necessary to optimize it for your group of interest.

BEFORE STARTING

Prepare the following solutions:

Citation: Sergio Vargas, CÃÂ¼neyt Caglar, Gabi BÃÂ¼ttner, Simone SchÃÂ¤tzle, Fabian Deister, Gert Woerheide (07/26/2021). Slime away: a simple CTAB-based high molecular weight DNA and RNA extraction protocol for "difficult" invertebrates (rev20200518). https://dx.doi.org/10.17504/protocols.io.bwcwpaxe

A	B	C	D	E	F	G	H
Chemical name	Short name	Company	Cat-No.	Molar mass (g/mol)	Concentration (M)	Volume (l)	Amount (g)
Cethyltrimethylammoniumbromid	CTAB	Carl Roth	9161,1	-	10%	0.2	
Ethyldiamin-tetraacetic acid Disodium salt	EDTA	Carl Roth	8043,2	372.24	0.5	0.2	37.224
Sodium Chloride	NaCl	Carl Roth	P029.2	58.44	5	0.2	58.44
Polyvinylpyrrolidon K30	PVP	Carl Roth	4607,1	-	10%	0.2	
Potassium acetate	KoAC	Sigma Aldrich	P1190	98.14	5	0.2	98.14
TRIS (hydroxymethyl)aminoethan	Tris	Sigma Aldrich	15,456-3	121.14	1	0.2	24.228

Autoclave all solutions before using them for extraction.

**If the solutions will be used for RNA extraction or if co-extracting DNA and RNA from the same sample is necessary, treat all solutions (but Tris) with DEPC overnight before autoclaving.

Protocol 1h 11m

1 Mix the CTAB Buffer in a 15ml falcon tube and preheat it to **60 °C** before starting the extraction.

2 Add **100 µl** β-Mercaptoethanol to the preheated CTAB Buffer under the fume-hood directly before you start with the extraction.

3 Cut a small piece of tissue and place it in a 2ml tube. Try to smash the tissue to make cells accessible to the buffer.

(Optional)

- Transfer the frozen/preserved tissue to a mortar pre-cooled with liquid nitrogen. **Best practice:** let the tissue "swim" in the liquid nitrogen until the mortar is dry. The mortar should be kept cold, you can achieve this by placing the mortar in a small cool box with liquid nitrogen in it.

- Grind the tissue until you get a fine powder.

- Using a small spatula, transfer the powder to a 2mL nuclease-free microcentrifuge tube. **Best practice**: pre-cool the spatula and the tube by placing them in the cool box containing liquid nitrogen this helps to keep the powder dry and cold.

4 Add **550 µl** of pre-heated (**60 °C**) extraction buffer and **50 µl** Proteinase K (**20 mg/ml**) to the tube.

5 Mix thoroughly until ALL powder OR smashed tissue is in solution. If needed, vortex lightly for ca. **00:01:00** .

Incubate at least **00:15:00** to overnight at **56 °C** with moderate mixing (~ **400 rpm**). In general, if the tissue was ground OR smashed, after two hours the tissue is digested. It is a good practice to manually agitate the digestion approx. every 15 minutes to avoid the formation of clumps of tissue.

(Optional) If samples have a high polysaccharide content:

Citation: Sergio Vargas, CÃÂ¼neyt Caglar, Gabi BÃÂ¼ttner, Simone SchÃÂ¤tzle, Fabian Deister, Gert Woerheide (07/26/2021). Slime away: a simple CTAB-based high molecular weight DNA and RNA extraction protocol for "difficult" invertebrates (rev20200518). https://dx.doi.org/10.17504/protocols.io.bwcwpaxe

- Add **150 µl** KOAc (**[M]5 Molarity (M)**) to the tubes, mix by inversion and incubate **00:10:00** at **60 °C** with moderate mixing.
- Centrifuge at max. speed **00:15:00**
- Recover (**600 µl**) supernatant trying to avoid the transfer of any pellet or viscous precipitate.

(Optional) If you expect RNA contamination e.g. in fresh samples:

- Add **5 µl** RNase A, mix gently by inversion and incubate at **37 °C** for **00:30:00** .

6 Add one volume (**600 µl**) of Chloroform to the sample and shake vigorously until the solution turns milky.

7 Centrifuge (full speed) at room temperature for **00:15:00** .

8 Remove the tube carefully from the centrifuge, trying to leave the two phases undisturbed. If the phases mix, centrifuge again. Transfer 2x **200 µl** of the upper phase to a new 2ml tube. Be careful not to transfer the interphase or traces of Chloroform.

9 Add 1 volume (**400 µl**) of isopropanol to the solution containing the DNA. Mix gently by the hand and incubate at room temperature for **00:10:00** . You may see tiny bubbles and sometimes white flakes forming.

10 Centrifuge at **16 °C** for at least **00:30:00** at full speed.

11 Discard supernatant (by pipetting) without disturbing the pellet.

12 Add **1 mL** ethanol (**[M]80 % (v/v)**) to the DNA pellet and centrifuge at maximum speed for **00:15:00** at **16 °C** . The pellet should be visible by now.

13 Remove the supernatant carefully (watch for the pellet). If wanted, you can repeat the previous step one more time. If not proceed to 14.

14 Centrifuge shortly and remove any remaining EtOH with the 10 µl pipette.

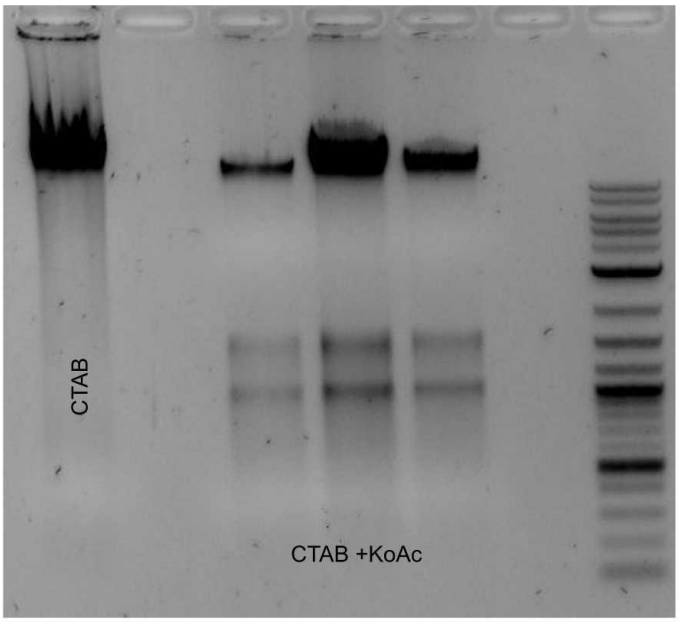
15 Leave the tube open for about **00:02:00** to dry the pellet. You can also check whether the pellet is transparent and proceed to the next step.

Citation: Sergio Vargas, CÃÂ¼neyt Caglar, Gabi BÃÂ¼ttner, Simone SchÃÂ¤tzle, Fabian Deister, Gert Woerheide (07/26/2021). Slime away: a simple CTAB-based high molecular weight DNA and RNA extraction protocol for "difficult" invertebrates (rev20200518). https://dx.doi.org/10.17504/protocols.io.bwcwpaxe

16 Resuspend the DNA in ~ **30 µl** water. (Optional) Incubate at **37 °C** in the Thermomixer for **00:20:00** .

17 Load **2 µl** of the extracted DNA on a 1% Agarose gel and let the gel run at 40V for **04:00:00** to **06:00:00** hours. If high molecular weight DNA is expected, use the lambda Hind-III marker to assess the quality of the extracts. Quantify the DNA concentration using the NanoDrop: record the concentration, the 260/230 and 260/280 ratios. A good extraction should have 260/230 and 260/280 ratios above 1.8

18 The protocol should yield high-quality DNA and RNA. Below is an example using the recalcitrant octocoral *Xenia* sp. Without KoAc, RNA cannot be recovered on a regular basis. Also, note the quality of the DNA obtained using the protocol. **Note:** you can use different commercial kits *in tandem* to isolate either DNA or RNA and purify the extracts further.



Nucleic acid extraction of the octocoral *Xenia* sp. using the Slime away protocol.

Citation: Sergio Vargas, CÃÂ¼neyt Caglar, Gabi BÃÂ¼ttner, Simone SchÃÂ¤tzle, Fabian Deister, Gert Woerheide (07/26/2021). Slime away: a simple CTAB-based high molecular weight DNA and RNA extraction protocol for "difficult" invertebrates (rev20200518). https://dx.doi.org/10.17504/protocols.io.bwcwpaxe