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Non-destructive DNA extraction from DESS preservation solution

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We use this protocol and it's working

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Abstract

DESS is a widely used storage solution to preserve DNA in biological tissue samples. DESS consists of 20% dimethyl sulfoxide (DMSO), 250 mM ethylenediaminetetraacetic acid (EDTA), and saturated sodium chloride (NaCl), and its efficacy has been confirmed in a variety of taxa and tissues. We introduce non-destructive DNA extraction and DNA barcoding using a portion of the DESS supernatant of a nematode specimen stored at room temperature for 10 years in DESS. This technique can also be used for the preservation and non-destructive DNA extraction of specimens of various species collected in the field. By immersing samples in DESS in the field to prevent DNA degradation, and then immersing them in new DESS in the laboratory after separation and identification to extract DNA from the supernatant, non-destructive DNA barcoding can be performed. Here, we provide full protocols on how to extract DNA from DESS solutions, and how to use the extracted DNA for DNA barcoding.

Materials

1. **DESS**

1.1 **Requirements for making DESS solution (ref:** https://www.youtube.com/watch?v=ye_1FRIR8bY)

Reagents

- ♦ Saturated sodium chloride (NaCl)
- ♦ 250mM EDTA (pH 8.0)
- ♦ 20% DMSO
- ♦ Deionized / Milli-Q water

Equipment and disposables

- ♦ Measuring cylinder/volumetric flask
- ♦ Conical flask / Beaker
- ♦ Magnetic stirrer

1.2 **DESS preparation and recipe**

Equipment and disposables

To prepare 1000 mL of DESS

- ♦ 500 mM EDTA (pH 8.0, 500 mL)
- ♦ DMSO (200 mL)
- ♦ NaCl (288 g)

Fill up to 1000 mL with sterile water.

Sterilize solutions by autoclaving for 20 min at 15 psi (1.05 kg/cm^2) on a liquid cycle.

Store the DESS solution at room temperature (10~25˚C).

*Although NaCl may precipitate over time, this may not pose a problem; use the clear supernatant portion.

2. **DNA extraction**

1.2 **Requirements for DNA extraction**

Reagents

- ♦ Silica (FUJIFILM: wakosil [232-00841])
- ♦ 0.01 mol/L Hydrochloric acid
- ♦ AMpure (Beckman Coulter) or SeraPure (refer to the method described below for the preparation)
- ♦ Ethanol (99.5%)
- ♦ Ethanol (75%)
- ♦ 500 mM EDTA (pH 8.0)
- ♦ 1 M Tris-HCl (pH 8.0)
- ♦ Distilled water
- ♦ TE buffer (pH 8.0)
- ♦ 0.1× TE buffer (pH 8.0)
- ♦ Guanidine thiocyanate
- ♦ Triton X100
- ♦ Tween 20
- ♦ 500 mM Sodium acetate (pH 5.2)

Equipment and disposables

- ♦ Low DNA binding tube (e.g., Eppendorf LoBind tube, Cat.#0030108051)

2.2 **Preparation before DNA extraction**

Silica solution

To prepare 11 mL of silica solution

♦Wakosil (FUJIFILM [232-00841]) 2.5 g

♦0.01 N HCl 11 mL

*Before starting the DNA extraction, aliquot 10 µL of the solution into new low-bind tubes.

SeraPure (Rohland et al. 2012)

To prepare 50 mL of SeraPure

♦Sera-Mag SpeedBead Carboxylate-Modified [E3] Magnetic Particles 1 mL

♦PEG8000 9 g

♦NaCl 2.92 g

♦1 M Tris-HCl (pH 8.0) 500 µL

♦500 mM EDTA (pH 8.0) 100 µL

♦50-mL tube

♦1.5-mL tube

1. Take 1 mL of well-mixed Sera-Mag SpeedBeads in a 1.5-mL tube by inverting the tube.
2. Place the tube on a magnetic stand and wait for 5 min.
3. Remove the supernatant with a pipette tip and discard it.
4. Add 1 mL of TE buffer, remove the tube from the magnetic stand, and mix well by inverting.
5. Place the tube on the magnetic stand and wait for 5 min.
6. Remove the supernatant with a pipette tip and discard it.
7. Add 1 mL of TE buffer, remove the tube from the magnetic stand, and mix well by inverting.
8. Place the tube on the magnetic stand and wait for 5 minutes.
9. Remove the supernatant with a pipette tip and discard it.
10. Add 1 mL of TE buffer, remove the tube from the magnetic stand, and mix well by inverting.
11. In a 50-mL centrifuge tube, add 9 g of PEG8000 and 2.92 g of NaCl.
12. Add 500 µL of 1 M Tris-HCl (pH 8.0) and 100 µL of 500 mM EDTA (pH 8.0).
13. Fill up to 45 mL with sterile distilled water and completely dissolve the PEG.
14. Add 27.5 µL of Tween-20 and 1 mL of the solution from step 10.
15. Fill up to 50 mL with sterile distilled water.

Binding Buffer

To prepare 120 mL of binding buffer

♦Guanidine thiocyanate 70.9 g

♦Triton X100 4.8 g

♦Tween 20 12 %

♦500 mM Sodium acetate (pH 5.2) 240 µL

*If preparing this buffer is difficult, try using Buffer AL (QIAGEN, Cat. No.19075) as an alternative.

Wash Buffer

Stock wash buffer:ethanol = 1:4 ratio. (e.g., 20 mL stock wash buffer + 80 mL ethanol)

Toprepare 50 mL of Stock wash buffer

♦500 mM EDTA (pH 8.0) 50 µL

♦5 M NaCl	1 mL
♦1 M Tris-HCl (pH 8.0)	100 µL

Protocol materials

AL buffer **Qiagen Catalog #**19075 Step 4

1 **Sample storage using DESS solution (see Materials page)**

To transport or store the DNA as undamaged as possible, place the specimen or environmental sample in DESS solution.

*Cautions when the sample is an animal
Since the DESS solution has low toxicity, animals are not immediately killed when placed in the DESS.
It is advisable to kill it somehow before putting the animals into the DESS.
This treatment is not particularly necessary for microscopic specimens (e.g. meiofauna).

Samples in DESS should be stored in the dark at room temperature (10 ~ 35 ˚C)

2 Add 10 µL silica solution into new 1.5mL LoBind tube.

Note

Silica solution *mix well before aliquote
2.5g wakosil (FUJIFILM, Cat.#232-00841)
11mL 0.01N HCl

3 Transfer X µL (eg. 500 µL) of DESS supernatant with the sample to the tube.

4 Add 1 volume of Binding buffer (eg. 500 µL) to the tube.

Note

Binding Buffer
5M Guanidine Thiocyanate
4% Triton X100
10% Tween 20
10mM Sodium acetate

*If it is not possible to prepare the Binding Buffer, AL buffer **Qiagen Catalog #**19075 can also be used.

5 Add 1 volume of Ethanol (eg. 500 µL) to the tube.

6 Mix by turning upside-down for 00:05:00 at Room temperature . 5m

7 9100 x g, 20°C, 00:01:00 and discard the supernatant. 1m

8 Add 500 µL of Wash Buffer 1 and mix by vortexing.

Note

Wash Buffer
stock buffer : Ethanol = 1: 4 ratio

Stock buffer
10mM TrisHCl pH8.0
100 mM NaCl
0.5mM EDTA pH8.0

9 9100 x g, 20°C, 00:01:00 and discard the supernatant with a pipette (or carefully with an aspirator). 1m

10 Add 500 µL of Wash Buffer 2 and mix by vortexing.

11 9100 x g, 20°C, 00:01:00 and discard the supernatant with a pipette (or carefully with an aspirator). 1m

12 9100 x g, Room temperature, 00:01:00 and completely discard the supernatant with a pipette (or carefully with an aspirator). 1m

13 Air dry for 00:01:00 at Room temperature . 1m

*Be careful not to over-dry.

14 Add 103 µL TE and mix by vortexing.

15 Incubate at Room temperature for 00:10:00 . (DNA dissolves in TE.) 10m

*While incubating, prepare the following
1) Prepare a new 1.5mL LoBind tube.
2) Add 10 µL 3M sodium acetate to the tube.
3) Add 1 µL glycogen *optional

16 9100 x g, Room temperature, 00:01:00 1m

17 Transfer the 100 µL supernatant to a new 1.5 mL LoBind tube prepared in step 15.

18 Add 100 µL isopropanol to the tube and mix by vortexing.

19 -20 °C 00:05:00 ~ 5m

*Cool the centrifuge at this time.

20 20400 x g, 4°C 00:30:00 and discard the supernatant with a pipette (or carefully with an aspirator). 30m

*max speed

21 Add 1 mL 75% Ethanol to the tube .
*Don't mix

22 20400 x g, 4°C 00:01:00 and discard the supernatant with a pipette (or carefully with an aspirator). 1m

*max speed

23 9100 x g, 00:01:00 and completely discard the supernatant with a pipette (or carefully with an aspirator). 1m

*A small benchtop centrifuge can be used.

24 Air dry for 00:10:00 ~ 00:30:00 at Room temperature in the dark. 40m

*Be careful not to leave any ethanol residue and not to over-dry.

25 Add 10 µL 0.1 TE to the tube and mix by vortexing.

26 Incubate for 00:10:00 at Room temperature in the dark. 10m

27 PCR using 1 µL supernatant.

Protocol references

1) DESS solution: https://www.youtube.com/watch?v=ye_1FRIR8bY)