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# ssDNA Extraktion

PLOS One

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1 Works for me dx.doi.org/10.17504/protocols.io.8m9hu96

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ABSTRACT

ssDNA Extraction Protocol

This protocol is based on the Oligonucleotide extraction protocol of the NEB PCR & Clean-up Kit and the Qiagen DNeasy Protocol with a few modifications to maximize DNA output. First results of this protocol will be published soon, including first sequencing results of the so extracted DNA. Please understand that this protocol is so far only tested for tissue (legs and abdomen) of invertebrates (Lepidoptera, genus *Hyles*). I recommend testing this protocol with tissue of non valuable specimens first.

EXTERNAL LINK

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KEYWORDS

ssDNA, extraction, aDNA, museum

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GUIDELINES

Make sure to not touch the specimen/tissue at any given point, **always wear gloves** and **clean all used forecepts before and after usage** with DNAaway or similar.
Make sure to clean the sample if possible before you start the Lysis (by UV-light or chlor, not possible in insect legs or soft tissue).

Only use this Extraction protocol in a cleaned environment, idealy in an aDNA-laboratory.
Take full pre-cautions;

- clean your whole bench before DNA extraction
- always clean your gloves by using DNAaway after touching the tube of a sample
- take care of aerosols! do not touch/open any tube while your gloves are still wet
- of cause only use filter-tips

MATERIALS

| NAME | CATALOG # | VENDOR |
|---|---|---|
| Monarch® PCR & DNA Cleanup Kit (5 µg) | T1030 | New England Biolabs |
| Lysis Buffer FN | NAP41031 | LGC biosearch |
| Proteinase K | 845-CH-0010006 | Analytik Jena |

SAFETY WARNINGS

don´t drink the Ethanol ;)

BEFORE STARTING

Input amount of DNA to be purified should not exceed the binding capacity of the column (5 µg). A starting sample volume of 50 µl is recommended. For smaller samples, nuclease-free water can be used to adjust the volume to the recommended volume range. Centrifugation should be carried out at 16,000 x *g* in a standard laboratory microcentrifuge at room temperature.

Buffer Preparation:

Add ethanol to Monarch DNA Wash Buffer prior to use (4 volumes of ≥ 95% ethanol per volume of Monarch DNA Wash Buffer).

- For 50-prep kit add 20 ml of ethanol to 5 ml of Monarch DNA Wash Buffer
- For 250-prep kit add 100 ml of ethanol to 25 ml of Monarch DNA Wash Buffer

Always keep all buffer bottles tightly closed when not in use.

**All centrifugation steps should be carried out at 16,000 x *g*. (~13K RPM in a typical microcentrifuge).**
This ensures all traces of buffer are eluted at each step.

Elution

1 Place dry tissue in 1.5 ml microcentrifuge tube and grind it, using an oscillating mill (MM 400, Retsch) for **1 min** at a frequency of **25 Hz**, using two sterile stainless steel balls with a diameter of 3 mm

Lysis

2 Insert to the ground tissue

**45 µl Lysis buffer**

**5 µl Proteinase K**



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**50 µl total volume** mix well by handshaking, **don´t vortex!** and gently spin down the solution.

3 Put the tube in an

Mixing Block (MB-102, Thermocell)

**56 °C**

**08:00:00 at least, better overnight**

Mix gently, and spin again.

Extraction

4 Add

**100 µl DNA Cleanup Binding Buffer**

to the

**50 µl sample**

mix by pipetting up and down.

5 Add

**300 µl ethanol (≥ 95%)**

Mix well by pipetting up and down or flicking the tube. **Do not vortex.**

6 Insert column into collection tube,
load sample onto column and close the cap.

**00:01:00 Centrifuge**

then discard flow-through

7 Re-insert column into collection tube.

Add

**500 µl DNA Wash Buffer**

and

**00:01:00 Centrifuge**

Discard flow-through.

8 Repeat Step 7.
This step is recommended for removal of enzymes that may interfere with downstream applications (e.g., Proteinase K).

9 **Transfer column** to a clean 1.5 ml microfuge tube.
Use care to ensure that the tip of the column does not come into contact with the flow-through.
If in doubt, re-spin for 1 minute to ensure traces of salt and ethanol are not carried over to the next step.

Two-Step-Elution

10 Add

**17.5 µl Elution Buffer**



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to the center of the matrix
Wait for
**00:10:00 Incubation**
then spin for
**00:01:00 Centrifuge**
to elute the DNA.

11 Repeat Step 10 to extract all DNA



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