Fluid-preserved specimen

It’s important to test beforehand the efficacy of this protocol on a few samples (not all of them at once), just in case you have to adjust your protocol (lysis time, elution volume,...).

1. DNA extraction

1.1. Genomic DNA from Tissue – NucleoSpin Tissue® – Machery-Nagel

| Consumables          | - 1.5 ml microcentrifuge tubes for sample lysis and DNA elution  
| Laboratory equipment | - Manual pipettors  
|                      | - Centrifuge for microcentrifuge tubes  
|                      | - Vortex mixer  
|                      | - Heating-block for incubation at 70°C  
|                      | - Equipment for sample disruption and homogenization  
|                      | - Personal protection equipment (lab coat, gloves)  
| Reagents             | - 96-100 % Ethanol  
|                      | - NucleoSpin® Tissue Kit :  
|                      | - Lysis Buffer T1  
|                      | - Buffer B1  
|                      | - Buffer B2  
|                      | - Wash Buffer (BW)  
|                      | - Wash Buffer B5 (concentrate)  
|                      | - Elution Buffer BE  
|                      | - Proteinase K (lyophilized)  
|                      | - Proteinase Buffer PB  

1.1.1. Storage conditions and preparation of working solutions

- Clean the bench top with alcohol before and after setting up extractions.
- Use the filter-plugged tips to avoid contamination of samples and reagents.
- All kit components can be stored at RT and are stable up to one year.
- If a white precipitate occurs in buffer T1, B1 or B3, it can be easily dissolved by incubating the bottle at 50-70°C before use.
- Before starting the extraction protocol, prepare the following:
  - Prepare the **Lysis Buffer B3** : transfer the total contents of Buffer B1 to Buffer B2 and mix well, it’s Buffer B3.
Fluid-preserved specimen DNA Extraction Procedure – December 2013

- Prepare the **Wash Buffer B5**: add the indicated volume of ethanol (96-100 %) to the Wash Buffer B5 concentrate.
- Prepare the **Proteinase K Solution**: add the indicated volume of Proteinase K Buffer PB to dissolve lyophilized Proteinase K. This solution is stable at -20°C for up to 6 months.

<table>
<thead>
<tr>
<th>Products</th>
<th>10 Preps</th>
<th>50 Preps</th>
<th>250 Preps</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysis Buffer B3</td>
<td>Add Buffer B1 (6 ml) to Buffer B2 (1.5 ml)</td>
<td>Add Buffer B1 (12 ml) to Buffer B2 (3 ml)</td>
<td>Add Buffer B1 (60 ml) to Buffer B2 (15 ml)</td>
</tr>
<tr>
<td>Wash Buffer B5</td>
<td>4 ml - Add 16 ml Ethanol</td>
<td>2 x 7 ml - Add 28 ml Ethanol to each bottle</td>
<td>2 x 40 ml - Add 160 ml Ethanol to each bottle</td>
</tr>
<tr>
<td>Proteinase K</td>
<td>6 mg – Add 260 µl Proteinase buffer</td>
<td>30 mg – Add 1.35 ml Proteinase buffer</td>
<td>2 x 75 mg – Add 3.35 ml Proteinase buffer to each vial</td>
</tr>
</tbody>
</table>

### 1.1.2. Preparation of the sample

- Set an incubator or water bath to **37°C** and **56°C**.
- Preheat **Elution Buffer BE** to **70°C**.
- If the specimens are not fresh and were stored in ethanol, the subsampled tissues need to be completely dried before the DNA extraction. Incubate at **37°C for about 15 min** with the cap slightly loosened, to evaporate the ethanol.

!! **If the specimens were stored in another DNA-friendly preservation fluid, it’s better to transfer it into ethanol 1 day prior to DNA extraction.**

- Place no more than **25 mg** of the sample cut into small pieces into a microcentrifuge tube.

### 1.1.3. Lyse of the sample

- Add **180 µl Buffer T1** and **25 µl Proteinase K Solution**. Vortex to mix. The sample should be covered with the lysis solution. If you use an insect leg, pay attention that the leg is indeed in contact with the lysis solution and not stuck on the side of the microcentrifuge tube.
- Incubate at **56°C** until complete lysis (**1 to 3 hours**). Vortex occasionally or use a shaking incubator. Samples can be incubated overnight as well. If you want to remove residual bones or hair, centrifuge 5 min at high speed and transfer 200 µl of the supernatant in a new tube.
1.1.4. DNA extraction

- After digestion, centrifuge at **1500 g** for **15 seconds** to remove any condensate from the cap.

- Vortex the samples. Add **200 µl Buffer B3**, vortex vigorously and incubate at **70 °C for 10 min**. Vortex briefly. If insoluble particles are visible, centrifuge 5 min at maximum speed and transfer the supernatant into a new microcentrifuge tube.

- Add **210 µl ethanol** (96-100 %) to the sample and vortex vigorously. In case of precipitates, load the sample to the column regardless.

- For each sample, place one column into a collection tube. Apply the sample to the column. Centrifuge for **1 min at 11 000 g**. Discard the flow-through and place the column back into a new collection tube. Repeat this step if any lysis solution remains. Always position tubes in the centrifuge in the same orientation, i.e. cap facing outward, this will allow the pellet to remain glued to the same side of the tube during repeated centrifugations and minimize the loss of DNA pellets.

- 1st wash : Add **500 µl buffer BW**. Centrifuge for **1 min at 11 000 g**. Discard flow-through and place the column back into a new collection tube.

- 2st wash : Add **600 µl Buffer B5** to the column and centrifuge for **1 min at 11 000 g**. Discard flow-through and place the column back into a new collection tube.

- Dry silica membrane by centrifuging the column for **1 min at 11 000 g**.

- Elute DNA by placing the column into a new 1.5 ml microcentrifuge tube and add **100 µl pre-warmed buffer BE** (70 °C). Incubate at RT for **1 min**. Centrifuge **1 min at 11 000 g**. You can also performed two elution steps, i.e. 2 x 60 µl or 1 x 80 µl and 1 x 100 µl.

- Store at **-20 °C** (See 2. DNA storage for other options).

1.2. DNeasy Blood & Tissue Kit – QIAGEN

<table>
<thead>
<tr>
<th>Consumables</th>
<th>- 1.5 ml microcentrifuge tubes for sample lysis and DNA elution</th>
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<tbody>
<tr>
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<td>- Disposable tips</td>
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<td>- Equipment for sample disruption and homogenization</td>
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</tbody>
</table>
**1.2.1. Storage conditions and preparation of working solutions**

- Clean the bench top with alcohol before and after setting up extractions.
- Use the filter-plugged tips to avoid contamination of samples and reagents.
- All kit components can be stored at RT and are stable up to one year.
- If a white precipitate occurs in buffer AL and ATL, it can be easily dissolved by incubating the bottle at 56°C before use.
- **Buffer AW1 et AW2** are supplied as concentrates. Before using it for the first time, add the appropriate volume of ethanol (96-100%) as indicated on the bottle and shake thoroughly.

**1.2.2. Preparation of the sample**

- Set an incubator or water bath to 37°C and 56°C.
- If the specimens are not fresh and were stored in ethanol, the subsampled tissues need to be completely dried before the DNA extraction. Incubate at 37°C for about 15 min with the cap slightly loosened, to evaporate the ethanol.

**!! If the specimens were stored in another DNA-friendly preservation fluid, it’s better to transfer it into ethanol 1 day prior to DNA extraction.**

- Place no more than 25 mg of the sample cut into small pieces into a microcentrifuge tube.

**1.2.3. Lyse of the sample**

- Add 180 µl **Buffer ATL** and 20 µl **Proteinase K Solution**. Vortex to mix. The sample should be covered with the lysis solution. If you use an insect leg, pay attention that the leg is indeed in contact with the lysis solution and not stuck on the side of the microcentrifuge tube.
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- Incubate at 56°C until complete lysis (1 to 3 hours). Vortex occasionally or use a shaking incubator. Samples can be incubated overnight as well. If you want to remove residual bones or hair, centrifuge 5 min at high speed and transfer 200 µl of the supernatant in a new tube.

1.2.4. **DNA extraction**

- After digestion, centrifuge at 1500 g for 15 seconds to remove any condensate from the cap.
- Vortex the samples for 15 s. Add 200 µl Buffer Al, vortex vigorously. Then add 200 µl of ethanol (96-100%) and mix again thoroughly by vortexing.

- For each sample, place one column into a collection tube. Apply the sample to the column. Centrifuge for 1 min at 6000 g. Discard the flow-through and place the column back into a new collection tube. Repeat this step if any lysis solution remains. Always position tubes in the centrifuge in the same orientation, i.e. cap facing outward, this will allow the pellet to remain glued to the same side of the tube during repeated centrifugations and minimize the loss of DNA pellets.

- 1st wash : Add 500 µl buffer AW1. Centrifuge for 1 min at 6000 g. Discard flow-through and place the column back into a new collection tube.

- 2nd wash : Add 500 µl buffer AW2. Centrifuge for 3 min at 20000 g. Discard flow-through and place the column back into a new collection tube. Remove the DNeasy Mini spin column carefully so that the column does not come into contact with the flow-through, since this will result in carryover of ethanol.

- Elute DNA by placing the column into a new 1.5 ml microcentrifuge tube and add 100 µl buffer AE. Incubate at RT for 1 min. Centrifuge 1 min at 6000 g. You can also performed two elution steps, i.e. 2 x 60 µl or 1 x 80 µl and 1 x 100 µl.

- Store at -20 °C (See 2. DNA storage for other options).

2. **DNA Storage**

According to the DNA extraction protocols described above, exclusively use buffers to elute DNA. For storage, DNA can be stored :

- At -20°C as a working solution to be used for PCR amplification.
- At -80°C for long term storage in GenTegra™ DNA Tubes.
- At room temperature, in GenTegra™ DNA Tubes for long term storage : the transparent matrix applied to the bottom of this tubes allows storage of DNA at room temperature in a manner that
preserves DNA integrity, quality and purity. The DNA samples are protected from hydrolysis, oxidation and microbial growth.

2.1. DNA application in GenTegra™ DNA Tubes

- If the samples are frozen, incubate at 38°C for **1 minute**.
- Centrifuge at low speed for a few seconds.
- Apply **20-250 µl** of the samples in each GenTegra™ tube according to the manufacturer’s protocol and mix by pipetting up and down 6 times to solublize the GenTegra™ matrix.

2.2. Drying and storage of DNA in GenTegra™ DNA Tubes

- Put in the FastDryer. Ensure the tube holder is inserted in the FastDryer. Place rack of tubes with lids off or unsealed microplate on the top of the tube holder. Ensure that the power cord is lugged in. Close the fan lid.
- Press the red on/off switch to operate the FastDryer. Leave on for **16 hours** to dry the samples or overnight.
- When drying is complete, cap or seal the tubes/plates and store at room temperature (21-25°C)

2.3. DNA recovery from GenTegra™ DNA Tubes

- When you want to recover DNA, apply a volume of molecular biology-grade water according to the guidelines in the manufacturer’s protocol (usually **35-250 µl**).
- Incubate at room temperature (21-25°C) for **15 minutes**.
- Mix to solubilize the DNA. Cap the tubes and vortex **1 minute** or pipette up and down **10 times** for the microplate.
- Following the recovery, an aliquot of DNA may be removed for use and the sample dried again. This procedure may be repeated multiple times until a maximum of 75% of the original sample is removed.

For example: if a 200 µl sample is applied into a tube, the volume of the sample can’t drop below 50 µl (25% of the original volume). If it has to drop below 50 µl, you have to freeze it at -80°C.
3. Additional remarks

Silica membrane based kits are superior for routine extractions of most taxa regarding a lot of criteria, including efficiency, sample purity, fragment length, handling time and material cost.

Based on our experience and previous studies, we recommend either the use of these two kits: Genomic DNA from Tissue, NucleoSpin Tissue® (Machery-Nagel) or DNeasy Blood & Tissue Kit (QIAGEN).

In the literature, you can also find several studies using one of these two kits for extracting DNA from ethanol-fixed samples. Here is a non-extensive list:


