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DNA extraction and DNA quality control from fish tissue using the QIAwave DNA Blood & Tissue Kit (QIAGEN) or NucleoSpin Tissue Kit (MACHEREY-NAGEL)

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https://www.mn-net.com/media/pdf/5b/d0/d9/Instruction-NucleoSpin-Tissue.pdf

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We use this protocol and it's working

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Abstract

This protocol describes a column-based method for extracting genomic DNA from fish tissue (e.g., *Salvelinus alpinus*) using either the QIAwave DNA Blood & Tissue Kit (QIAGEN) or the NucleoSpin Tissue kit (MACHEREY-NAGEL), adapted from the manufacturers' recommendations. The protocol is also well-suited for DNA extraction from other fish species. A summary sheet of the DNA extraction procedure is included to support its practical implementation in the laboratory.

Attachments

PDF

Rogissart-Chardon-DN...

198KB

Image Attribution

Hervé Rogissart

Materials

- **Samples**

- Fish tissue
- Preserved in ethanol (final concentration > 90 % of ethanol) or at -20 °C
- Weight needed = 2-10 mg

- **Reagents**

To clean the workspace
- DNA/RNA-ExitusPlus
- Ethanol (70 %)

For prepare sample:
- DNA/RNA-ExitusPlus
- Ethanol 96 - 100 %
- Water

For DNA extraction
- DNA extraction kit: **Qiagen Kit** or **Macherey-Nagel Kit**
- Ethanol 96 - 100 %, molecular grade to prepare wash buffer
- Ultrapure water

- **Materials** (excluding solutions preparation)

- Bunsen burner
- Chisel and lab pincer
- Balance
- Specific DNA-work station (sterile area equipped with air filtration)
- Microcentrifuge for 1.5 to 2 mL tubes (relative centrifugal force needed: 6000 to 19000 x g)
- Horizontal vortexer with microtube holder
- Vortexer
- Stove or dry bath (to have 56 °C)
- Pipettes: 100-1000 µL; 10-100 µL
- 2 trash cans: 1 for liquid and 1 for solid

- **Consumables**

- Tips with filter:
> 1000 µL
> 100 µL
- 2 mL sterile microcentrifuge tube: 2 per sample
  1 to prepare and lyse tissue (steps 1-2-3) and 1 to elute DNA (step 6)
- Gloves

Protocol materials

Qubit™ dsDNA BR Assay Kit **Thermo Fisher Scientific** **Catalog #**Q32853

NucleoSpin Tissue **Macherey-Nagel** **Catalog #**740952.250

DNA/RNA-ExitusPlus **Applychem** **Catalog #**A7089

QIAwave DNA Blood & Tissue Kit **Qiagen** **Catalog #**69556

Safety warnings

Buffers used in these kits require safety precautions, mainly Buffer AL and Buffer AW1 or Buffer B3 and BW which contain guanidine salt.

Safety informations of all buffers are availabe at :
Qiawave DNA Blood & Tissue Kit (QIAGEN)
NucleoSpin Tissue (MACHEREY-NAGEL)

Before start

- Wear gloves througout the extraction process

1. Preheat dry bath or stove to 56 °C
2. Clean the workspace with DNA/RNA-ExitusPlus **Applychem Catalog #**A7089 followed by [M] 70 % volume **ethanol**
3. Unpack QIAwave DNA Blood & Tissue Kit **Qiagen Catalog #**69556 **(Qiagen Kit)**

or

NucleoSpin Tissue **Macherey-Nagel Catalog #**740952.250 **(Macherey-Nagel Kit)**

4. Check/Prepare buffers

5.1. If using **Qiagen Kit**

- **Buffer ATL** and **Buffer AL** may precipitate during storage

- Warm to 56 °C until fully dissolved

- Before first use, prepare **Buffer AW1, Buffer AW2** and **Buffer AE** as indicated on the manufacturer's protocol. The information below is for a 250 columns kit.

→ **Buffer AW1:** In a 250 mL sterile glass bottle, transfer the entire volume of **Buffer AW1/C** (98 mL) and add **ethanol** [M] 96-100 % volume (130 mL). Cap the glass bottle tightly and mix thoroughly by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.
The final volume of **Buffer AW1** is 228 mL . This solution is stable for 1 year when stored closed at room temperature.

→ **Buffer AW2:** In a 250 mL sterile glass bottle, transfer the entire volume of **Buffer AW2/C** (6 mL) and add **ultrapure water** (60 mL) + **ethanol** [M] 96-100 % volume (160 mL). Cap the glass bottle tightly and mix thoroughly by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.
The final volume of Buffer AW2 is 226 mL . This solution is stable for 1 year when stored closed at room temperature.

→ **Buffer AE:** In a 150mL sterile glass bottle, transfer the entire volume of buffer **AE/C** 10 mL (10 mL) and add **ultrapure water** 110 mL . Cap the glass bottle tightly and mix thoroughly by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.
The final volume of Buffer AE is 120 mL .

- *Prepare aliquots of each solution as needed*

5.2. If using **Macherey-Nagel Kit**

- **Buffer T1** and **Buffer B3** may precipitate during storage

- Warm between 50 °C and 70 °C until fully dissolved

- Before first use, prepare **Buffer B5** and **Proteinase K solution** as indicated on the manufacturer's protocol.

→ **Buffer B5**: add appropriate volume of **ethanol** [M] 96-100 % volume to **Buffer B5 Concentrate** as indicated on the bottle and write the date on the bottle. This solution is stable for 1 year when stored closed at Room temperature .

→ **Proteinase K solution**: add appropriate volume of Proteinase Buffer PB (provided) to lyophilized **Proteinase K**. Ensure all powder is dissolved and solution is homogeneous. Transfert **Proteinase K solution** in 1.5 or 2 mL sterile microtube. This solution is stable for 6 months at -20 °C .

- *Prepare aliquots of each solution as needed*

Prepare sample:

1 ▪ Before start
- For each sample, label a 2 mL sterile microcentrifuge tube (Eppendorf safe-lock) with an unique identifier
- Decontaminate cutting tools: clean with DNA ExitusPlus, rinse with water and cover with ethanol
- Sterilize cutting tools using bunsen burner

▪ Cut up to approximatively **2-10 mg de tissue**

▪ Place tissue in annotated tube

Note

- Decontaminate or replace cutting tools for each sample to prevent cross-contamination
- Cutting the tissue into small pieces to enable more efficient lysis
- Store at +4 °C for quick use (< 8h) or at -20 °C for further use (> 8h)

Lyse sample 1/2:

2 ▪ Prepare the lysis mixture containing the following per sample

	A	B
	Qiagen kit	**Macherey-Nagel kit**
	180 µL buffer ATL	180 µL buffer T1
	20 µL Proteinase K	25 µL Proteinase K

Lysis mixture composition

Note

*- Homogenize **Buffer** and **Proteinase K***
- When preparing the mix for multiple samples, allow a marge for 1-2 additional samples

▪ Add lysis mixture to each sample

	A	B
	Qiagen kit	**Macherey-Nagel kit**
	200 µL	205 µL

Lysis mixture volume / sample

- Vortex briefly 00:00:05 by pulse-vortexing
- Centrifuge , 00:00:05 in a microcentrifuge

Note

Check that tubes are tightly sealed to prevent evaporation during incubation, and ensure that tissue samples are fully immersed in the lysis buffer. If this was not the case, a brief centrifugation was performed to re-establish contact between the tissue and the solution.

- Incubate at 56 °C for at least 02:00:00 in a dry bath or in a stove

Note

- If using a thermomixer set to 300 rpm
- Make sure the incubator is preheated to 56°C otherwise the lysis time must be increased
Overnight *incubation is applied to* ensure that digestion will be complete *and/or to* facilitate the organization of extraction.

Lyse sample 2/2 & Adujst DNA binding conditions:

3

- To save time while waiting for the **lysis incubation**, per sample prepare and annotate:

- spin columns for DNA purification placed in waste tubes (provided)
- 2 mL microtubes for DNA elution

- Few minutes before the end of incubation, prepare **buffer and ethanol mixture** containing the following per sample

	A	B
	Qiagen kit	**Macherey-Nagel kit**
	200 µL AL	200 µL B3

Buffer and ethanol mixture composition

Note

*- Homogenize **Buffer** and **ethanol mixture***
- When preparing the mix for multiple samples, allow a marge for 1-2 additional samples

- Each sample must be vortexed briefly 00:00:15 and centrifuged briefly 00:00:05
- Add **buffer and ethanol mixture**

	A	B
	Qiagen kit	**Macherey-Nagel kit**
	400 µL	410 µL

Buffer and ethanol mixture volume / sample

- Immediately vortex for 00:00:15 to mix and centrifuge , 00:00:05 in a microcentrifuge

Note

*- It is important to immediately mix the samples by vortexing or pipetting after adding the **Buffer and ethanol mixture** to ensure a homogeneous solution. Failure to do so may affect DNA yield.*

Bind DNA:

4 - Transfer the mixture samples to **spin column** placed in a waste tube (provided and annotated)

- Centrifuge at Room temperature to **capture the DNA** on the column membranes. Follow the parameters indicated in the table below.

	A	B	C
		Qiagen kit	**Macherey-Nagel kit**
	Column name	DNeasy Mini Spin Column	NucleoSpin Tissue Column
	Centrifugation	6000 x g - 1 min	11000 x g - 1 min

- Discard the flow-through and reuse the **Waste Tube**

Wash & Dry silica membrane:

5

- To **clean DNA**, do 2 washes as described in the table below
- Between each centrifugation, discard the flow-through and reuse the **Waste Tube**
- To **dry silica membrane**, do an additional centrifugation described in table below

	A	B	C
		Qiagen	**MN**
	1st wash	500 µL AW1 6000 x g - 1 min	500 µL BW 11000 x g - 1 min
	2nd wash	500 µL AW2 19000 x g - 2 min	600 µL B5 11000 x g - 1 min
	Dry silica membrane	19000 x g - 1 min	11000 x g - 1 min

Elute:

6

- Place the **Spin Column** in a 2 mL annoted clean tube

- For each sample check the correspondence of Spin Column label with 2 mL tube label

- Add 100 µL of elution buffer (provided in the kit) to the membrane of each column

	A	B
	Qiagen kit	**Macherey-Nagel kit**
	Buffer AE	Buffer BE

- Incubate at Room temperature 00:01:00
- To elute DNA from the membrane,centrifuge by respecting these instructions

	A	B
	Qiagen kit	**Macherey-Nagel kit**
	6000 x g - 1 min	11000 x g - 1 min

Safety information

- Position the lid inward the center of the centrifuge
- Caution do not discard the eluate

- *Eluting with* 100 µL *increases the DNA concentration but reduces the overall DNA yield*

DNA storage:

7 **DNA is ready** to use immediately or store at:

- 4 °C for use a few days
- -20 °C for use within a few weeks
- -80 °C for long-term storage

Note

- Before using, allow the sample to equilibrate to Room temperature
- Perform pulse-vortexing for 00:00:05 *followed by microcentrifugation*

DNA quantification and DNA quality control:

8 ***NanoDrop measurement:***

- Use 2 µL of **used elution buffer** as blank as standard with the NanoDrop
- To validate blank, add 2 µL to **used elution buffer** into the surface of the NanoDrop and read

Equipment

NanoDrop™ One UV-Vis Spectrophotometer	NAME
spectrophotometer	TYPE
Thermo Scientific	BRAND
ND-ONE-W	SKU
https://www.thermofisher.com/order/catalog/product/ND-ONE-W	LINK
Sample Volume (Metric): Minimum 1µL; Spectral Bandwidth: ≤1.8 nm (FWHM at Hg 254 nm); System Requirements: Windows™ 8.1 and 10, 64 bit; Voltage: 12 V (DC); Wavelength Range: 190–850 nm	SPECIFICATIONS

- To measure DNA concentration of sample, add 2 µL of sample DNA into the surface of the NanoDrop and read

- Note down the A260/280, A260/230 and concentration (ng/µl)
- or export the data to a USB storage device

Note

- Clean the surface between each read

9 ***Qubit Measurement:***

- Ensure the Qubit is calibrated before starting

Equipment

Invitrogen™ Qubit™ 3 Fluorometer	NAME
Accurately measures DNA, RNA, and protein using the highly sensitive fluorescence-based Qubit quantitation assays	TYPE
Invitrogen™ Q33216	BRAND
Q33216	SKU
https://www.fishersci.co.uk/shop/products/qubit-3-0-fluorometer/15387293	LINK

- Use the
  Qubit™ dsDNA BR Assay Kit **Thermo Fisher Scientific** **Catalog #**Q32853
- Analyze 1 µL of **extracted DNA**
- Note the concentration (ng/µl)