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DNA extraction from fish tissue using the Wizard® SV 96 Genomic DNA Purification System (Promega)

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We use this protocol and it's working

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Abstract

This protocol describes a method for extracting genomic DNA from fish tissue (e.g., fin tissue) using the Wizard Genomic DNA Purification kit (Promega), adapted from the manufacturers' recommendations. A summary sheet of the DNA extraction procedure is included to support its practical implementation in the laboratory.

Attachments

PDF

Rogissart-et-al-DNA-...

204KB

Image Attribution

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Materials

- **Samples**

- Fish tissue preserved in ethanol (final concentration > 90 % of ethanol) or at -20 °C
- Weight required = 10-15 mg

- **Reagents**

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

For prepare sample:
- Ethanol 96 - 100 %
- Water

For DNA extraction
- DNA extraction kit: **Wizard Genomic DNA Purification kit**
- Ethanol 96 - 100 %, molecular grade to prepare wash buffer
- Nuclease-Free Water

- **Materials** (excluding solutions preparation)

- Vac-Man® 96 Vacuum Manifold
- Vacuum trap for waste collection
- Vacuum pump with tubing
- 96-well deep well plate
- Adhesive plate sealers
- Bunsen burner
- Chisel and lab pincer
- Balance
- Specific DNA-work station (sterile area equipped with air filtration)
- Horizontal vortexer with microtube holder
- Vortexer
- Stove (to have 55 °C)
- Pipettes: 100-1000 µL; 10-100 µL or Multichannel pipettors: 10-1,000 µL
- 2 trash cans: 1 for liquid and 1 for solid

- **Consumables**

- Tips with filter:
> 1000 µL
> 100 µL
- Gloves

Protocol materials

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

Wizard® SV 96 Genomic DNA Purification System **Promega** **Catalog #**A2370

Proteinase K 20 mg/ml SOLUTION (STABILISEE) **eurobio** **Catalog #**GEXPRK01-I5

Troubleshooting

Safety warnings

Safety informations of all buffers are availabe at: **Safety Data Sheets**

Before start

Wear gloves througout the extraction process.

1. Preheat dry bath or stove to 55 °C
2. Clean the workspace with DNA-ExitusPlus followed by [M] 70 % volume ethanol
3. Unpack Wizard® SV 96 Genomic DNA Purification System **Promega** **Catalog #**A2370
4. Check/Prepare solutions

→ Proteinase K Solution

- Ready-to-use solution: Proteinase K 20 mg/ml SOLUTION (STABILISEE) **eurobio** **Catalog #**GEXPRK01-I5
- Store at 4 °C

→ Column Wash Solution (CWA):

- Add [M] 95 % volume ethanol to the **CWA** bottle as directed on the bottle label
- Store at Room temperature

Prepare sample:

1 ▪ Before start
- Organize the samples based on the plate map
- Decontaminate cutting tools: clean with DNA ExitusPlus, rinse with water and cover with ethanol
- Sterilize cutting tools using bunsen burner

▪ Cut approximatively **10–15 mg of tissue**

▪ Place tissue in dedicated well of 96-Well Deep Well Plate

Note

- Decontaminate or replace cutting tools for each sample to prevent cross-contamination
- Cutting the tissue into small pieces may enable more efficient lysis
- Store at +4 °C for quick use (< 08:00:00) or at -20 °C for further use (> 08:00:00)

Lyse sample:

2 ▪ Prepare the lysis mixture containing the following per sample

	Digestion Solution Master Mix
	200 µL Nuclei Lysis Solution
	50 µL 0.5M EDTA (pH 8.0)
	20 µL proteinase K, 20 mg/ml
	5 µL RNase A Solution, 4 mg/ml

Lysis mixture composition

Note

- Homogenize Digestion Solution Master Mix
- When preparing the mix for multiple samples, include an extra volume equivalent to 1 to 1.5 additional samples per every 8 samples

▪ Add 275 mL lysis mixture to each sample in the 96-well deep well plate

- Incubate at 55 °C for at least 18:00:00 in a stove

Note

- Make sure the incubator is preheated to 55°C otherwise the lysis time must be increased
- Cover the plate with an adhesive plate sealer
- To prevent condensation or liquid accumulation on the sealer, place a sheet of aluminum foil on top
- Overnight incubation is applied to ensure that digestion will be complete and/or to facilitate the organization of extraction.

Purification of Genomic DNA:

3

- Following incubation, dispense 250 µL of the **Wizard SV Lysis Buffer** into each well of the deep-well plate containing lysate

Note

- Lysate must be warm during processing
- Mix each well's contents by pipetting up and down three times to ensure homogeneity

- Prepare the Vacuum Manifold (see figure on summary sheet)
- Place the Binding Plate in the Vacuum Manifold Base
- Orient the Binding Plate in the Vacuum Manifold with the numerical column headers toward the vacuum port
- Attach the vacuum line to the vacuum port on the Manifold Base
- Transfer the tissue lysates to the wells of the Binding Plate
- Apply vacuum until all of the lysate has passed through the Binding Plate

Note

*- **Caution**: Always place a liquid trap (e.g., vacuum flask) between the manifold and the vacuum pump to avoid damaging the pump with aspirated liquid*
- Vacuum pressure should be approximately 508 mBar (or 50.8 kPa) for efficient processing
- To maintain strong vacuum across the wells, cover the plate with adhesive plate sealer

Wash & Dry:

4

- Add 900 µL of **Column Wash Solution (CWA)** to each well of the Binding Plate
- Apply vacuum until the **CWA** passes through the Binding Plate
- Repeat two more times for a total of **3 washes** with the **CWA**
- After the wells have emptied, continue to apply vacuum for an additional 00:06:00 to allow the binding matrix to dry
- Turn off the vacuum
- Release the vacuum line from the Manifold Base, and snap it into the vacuum port in the Vacuum Manifold Collar
- Remove the Binding Plate from the Manifold Base
- Blot by gently tapping onto a clean paper towel to remove residual ethanol and repeat if necessary to remove all residual ethanol

Note

- A fourth wash can be performed if necessary

Elute:

5

- Place the **96-Well Deep Well Plate** in the Manifold Bed and position the Vacuum Manifold Collar on top
- Orient the plate with the numerical column headers toward the vacuum port
- Position the Binding Plate on top of the Manifold Collar
- Place the Manifold Collar containing the Binding Plate on top of the 96-Well Deep Well Plate sitting on the manifold bed
- The Binding Plate tips must be centered on the Deep Well Plate wells, and both plates must be in the same orientation
- Add 250 µL of Room temperature **Nuclease-Free Water** to each well of the Binding Plate and incubate for 00:02:00 at Room temperature
- Apply vacuum until the **Nuclease-Free Water** passes through the Binding Plate
- Release the vacuum and remove the Wizard SV 96 Binding Plate
- Optional: transfer the samples into 1.5 mL tubes

Safety information

- Gently detach the Manifold Collar ensuring the Deep Well Plate stays properly aligned in the Manifold Bed
- In case droplets are visible on the upper edge of the wells, gently tap the plate on the bench to allow them to settle at the bottom

DNA storage

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

- 4 °C for use a few days
- -20 °C for use within a few weeks
- -70 °C for long-term storage

Note

To store the plate, seal it tightly using a plate sealer

DNA quantification and DNA quality control:

7 ***NanoDrop measurement:***

- Use 2 µL of **elution buffer** (without DNA) as a blank to calibrate the NanoDrop and correct for background absorbance
- To validate the blank, add 2 µL of **elution buffer** onto the NanoDrop pedestal and measure

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 onto the NanoDrop pedestal and measure

- 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

8 ***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)