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ND5 sequencing of *Oncorhynchus masou masou*

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1 Works for me dx.doi.org/10.17504/protocols.io.bmf7k3rn

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MATERIALS TEXT

MATERIALS

Phusion High-Fidelity DNA Polymerase - 100 units **New England Biolabs Catalog #M0530S**

Agarose S **Nippon Gene Catalog #312-01193**

1 Amplify the *ND5* region (1597 bp) with the following PCR mixture and program.

Component	Amount
5× Phusion HF buffer (New England BioLabs)	2 µl
Phusion DNA Polymerase (New England BioLabs)	0.1 µl
10 mM dNTPs	0.2 µl
10 µM primer ND5-F1	0.5 µl
10 µM primer ND5-R	0.5 µl
Genomic DNA	10–50 ng
Nuclease-free water	Variable
Total volume	10 µl

PCR mixture

Step	Temperature	Time	Number of cycles
1	98°C	30 sec	1
2	98°C	10 sec	40
3	64°C	20 sec	
4	72°C	60 sec	
5	72°C	2 min	1
6	8 °C	Hold	N/A

PCR program

2 Electrophorese 1 µl PCR product through a 1% agarose–TAE gel.
Check for the presence of non-specific amplification products and/or primer-dimers, and calculate the approximate concentration of the specific amplified DNA fragment by comparing its band intensity to that of the marker.

3 Sequence the PCR product with the primers ND5-F1 or ND5-F2.

Note: The PCR product can be used directly as a sequence template without purification or clean-up reagents if there is no non-specific amplification, no primer-dimer contamination, and can be diluted more than 5-fold in the sequence reaction solution.

4 Assemble and align the ND5 sequences by using ATGC Ver. 4.3.5 software (GENETYX Co.).

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