**Library preparation protocols**

In detail at LAB\_A the eDNA was used for Miseq (Illumina, San Diego, USA) library preparation following a two-step protocol: a first round of reverse transcriptase polymerase chain reaction (RT PCR) amplification was performed by using custom tagged primers. These were designed and validated using the EDITTAG software (Faircloth & Glenn, 2012) and consist of the Illumina overhang adapter, a seven base-pair tag and the fish specific MiFish-U primers (Miya *et al*., 2015, Tab S1). DNA amplifications were performed in triplicates in a final volume of 25ul. The amplification mixture contained 1X PCR Gold Buffer (Thermo Scientific, Waltham, USA), 2mM MgCl2 (Thermo Scientific), 0.4 μg/μL bovine serum albumin (BSA—Thermo Scientific), 0.4 μM of each of the tailed primers, 260nM dNTPs, 5X SYBR Green I Nucleic Acid Gel Stain and 1U AmpliTaq Gold DNA Polymerase (Thermo Scientific). PCR conditions consisted of an initial denaturation at 95° C for 5 min, followed by 35 cycles of 30 s at 95° C, 30 s at 65° C, and 60 s at 72° C, and a final elongation step at 72° C for 10 min. All PCRs were performed in the presence of both a negative and positive control (*i.e*., a mock community with a known composition of fish species). Amplification success at each step was determined by gel electrophoresis. All PCRs replicates per sample were pooled and purified using CleanNGS beads (CleanNA) to remove primer dimers.

For the second round of PCR amplification standard Illumina index primers are used to add the second set of tags and the Illumina sequencing adaptors to the PCR product from the first round of amplification (Tab S2). The reactions were performed according to the Illumina’s 16S Metagenomic Sequencing Library Preparation protocol.

The second PCR amplicons were sized using a TapeStation D1000 ScreenTape System (Agilent, Santa Clara, USA) and normalized to 4nM using the RT-qPCR LC480 with the Kapa Library quantification kit (Roche, Basel, Switzerland). The final pooled amplicon library was sequenced on an Illumina MiSeq with a V3 2 × 300 bp kit with a 15% PhiX control spike.

At LAB\_B all benches were decontaminated with ChemGene. Each step of the process had dedicated space, equipment, reagents and consumables. In the first step, purified DNA was amplified with MiFish-U primers (Miya *et al*. 2015). Tails were added at the 5′ end to be complementary with Illumina Nextera index primers. DNA amplifications were performed with 4 replicates in a final volume of 10 μL. The amplification mixture contained 1X Phusion Green Hot Start II High-Fidelity PCR Master Mix (Thermo Scientific), 0.4 μM of each of the tailed primers, 0.8 μg/μL bovine serum albumin (BSA—Thermo Scientific), 3% of Dimethyl Sulfoxide (DMSO) (Thermo Scientific), 1.5 mM of MgCl2 (Invitrogen, Waltham, USA), and topped up with PCR grade water (Thermo Scientific). PCR conditions consisted of an initial denaturation at 98° C for 3 min, followed by 45 cycles of 20 s at 98° C, 15 s at 69° C, and 15 s at 72° C, and a final elongation step at 72° C for 5 min.

A second PCR amplification with a final volume of 20 μL was used to index these purified amplicons. Triplicate index PCRs were performed per sample and indexes were added according to the Illumina’s 16S Metagenomic Sequencing Library Preparation protocol. PCR reactions contained 1X Phusion Green Hot Start II High-Fidelity PCR Master Mix (Thermo Scientific), 2 μL of Nextera XT i7 Index Primer (Illumina), 2 μL of Nextera XT i5 Index Primer (Illumina), 4 μL of PCR grade water (Thermo Scientific), and 2 μL of purified first-round PCR product. The second-round PCR products were purified using Mag-Bind TotalPure NGS (OMEGA BIOTEK) magnetic beads with a ratio 1:1 (beads:DNA). Purified index PCRs were quantified using a Qubit dsDNA HS Assay Kit, sized using a TapeStation D1000 ScreenTape System (Agilent) and normalized to 4 nM. The libraries were pooled in equimolar concentrations and sequenced on an Illumina MiSeq with a V2 2 × 250 bp kit, the final library was loaded at 12 pM with a 10% PhiX control spike.

**Table S1.** DNA extracted from fish fin clips and coming from different species were mixed together in order to create the mock communities with mix of 6, 9 and 14 species (M6, M9, M14 respectively). The numbers in the table correspond to the percentage of DNA in the mock for each species. In M14, a very low amount of DNA belonging to *Gobio gobio*, *Esox lucius* and *Lota lota* were included in order to simulate extremely rare specimens. These percentages were in good agreement (p*<*0.001) with the corresponding fraction of OTUs obtained with the HTS analyses.

|  |  |  |  |
| --- | --- | --- | --- |
| **Fish species DNA %** | **M6** | **M9** | **M14** |
| *Coregonus lavaretus* | 5.52840645 | 4.03780583 | 7.031004198 |
| *Salvelinus alpinus* | 16.9128286 | 12.3526949 | 21.50966469 |
| *Perca fluviatilis* | 22.1300063 | 16.1631872 | 28.14484940 |
| *Salmo trutta* | 32.9574927 | 24.0713047 | 22.35477404 |
| *Squalius cephalus* | 17.1885664 | 12.5540867 | 11.65885167 |
| *Abramis brama* | 5.2826995 | 3.85834779 | 3.583208065 |
| *Silurus glanis* | 0 | 5.72471137 | 1.329120201 |
| *Cyprinus carpio* | 0 | 13.9757931 | 3.244793970 |
| *Ameiurus melas* | 0 | 0 | 0.434475552 |
| *Tinca tinca* | 0 | 0 | 0.648704173 |
| *Rutilus rutilus* | 0 | 0 | 0.049014490 |
| *Gobio gobio* | 0 | 0 | 0.010541298 |
| *Esox lucius* | 0 | 7.26206855 | 0.000843026 |
| *Lota lota* | 0 | 0 | 0.000155226 |

**Table S2.** Custom Tagged forward and reverse primers for the first round of polymerase chain reaction amplification. The Miseq sequencing primers are in grey, the tags are underlined and the MiFish-U primers targeting the mitochondrial 12S rRNA (Miya *et al*. 2015) are in italics.

|  |  |
| --- | --- |
| **Primer ID** | **Sequence (5’-3’)** |
| MiFish-U-F-T113 | **TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTACTAC*GTCGGTAAAACTCGTGCCAGC*** |
| MiFish-U-R-T113 | **GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTACTAC*CATAGTGGGGTATCTAATCCCAGTTTG*** |

Table S3. Illumina tagged primers used in the second round of polymerase chain reaction amplification. The underlined sequences represent the MiSeq flowcell adaptors, grey sequences are a portion of Miseq sequencing primers and [i5] and [i7] are Nextera XT Index Kit A to D.

|  |  |
| --- | --- |
| **Primer ID** | **Sequence (5’-3’)** |
| P5 indexed primer | **AATGATACGGCGACCACCGAGATCTACAC-[i5]-TCGTCGGCAGCGTC** |
| P7 indexed primer | **CAAGCAGAAGACGGCATACGAGAT-[i7]-GTCTCGTGGGCTCGG** |

Table S4. Blast search result of the sequences assigned at *Coregonus* genus.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Description** | **Scientific Name** | **Max Score** | **Total Score** | **Query Cover** | **E value** | **Pident** |
| *Coregonus clupeaformis* isolate NEFC F17-265 mitochondrion | *Coregonus clupeaformis* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus clupeaformis* isolate NEFC F17-264 mitochondrion | *Coregonus clupeaformis* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus ussuriensis* mitochondrion | *Coregonus ussuriensis* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus maraena* voucher CZ12 small subunit ribosomal RNA gene | *Coregonus maraena* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus clupeaformis* isolate A7 mitochondrion | *Coregonus clupeaformis* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus clupeaformis* isolate A6 mitochondrion | *Coregonus clupeaformis* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus clupeaformis* isolate A3 mitochondrion | *Coregonus clupeaformis* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus clupeaformis* isolate A2 mitochondrion | *Coregonus clupeaformis* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus clupeaformis* isolate A1 mitochondrion | *Coregonus clupeaformis* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus lavaretus* isolate N16 mitochondrion | *Coregonus lavaretus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus lavaretus* isolate N12 mitochondrion | *Coregonus lavaretus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus lavaretus* isolate N15 mitochondrion | *Coregonus lavaretus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus lavaretus* isolate N7 mitochondrion | *Coregonus lavaretus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus lavaretus* isolate N18 mitochondrion | *Coregonus lavaretus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus lavaretus* isolate N5 mitochondrion | *Coregonus lavaretus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus lavaretus* isolate N20 mitochondrion | *Coregonus lavaretus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus lavaretus* isolate N8 mitochondrion | *Coregonus lavaretus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus lavaretus* isolate R3 mitochondrion | *Coregonus lavaretus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus lavaretus* isolate R8 mitochondrion | *Coregonus lavaretus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus lavaretus* isolate R13 mitochondrion | *Coregonus lavaretus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus lavaretus* isolate R15 mitochondrion | *Coregonus lavaretus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus lavaretus* isolate R10 mitochondrion | *Coregonus lavaretus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus lavaretus* isolate R11 mitochondrion | *Coregonus lavaretus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus lavaretus* isolate R16 mitochondrion | *Coregonus lavaretus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus lavaretus* isolate R5 mitochondrion | *Coregonus lavaretus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus lavaretus* isolate R1 mitochondrion | *Coregonus lavaretus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus lavaretus* isolate R18 mitochondrion | *Coregonus lavaretus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus lavaretus* isolate R4 mitochondrion | *Coregonus lavaretus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus lavaretus* isolate F6 mitochondrion | *Coregonus lavaretus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus lavaretus* isolate F8 mitochondrion | *Coregonus lavaretus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus lavaretus* isolate F7 mitochondrion | *Coregonus lavaretus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus lavaretus* isolate F5 mitochondrion | *Coregonus lavaretus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus lavaretus* isolate T4 mitochondrion | *Coregonus lavaretus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus lavaretus* isolate T6 mitochondrion | *Coregonus lavaretus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus lavaretus* isolate T3 mitochondrion | *Coregonus lavaretus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus lavaretus* isolate T2 mitochondrion | *Coregonus lavaretus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus lavaretus* isolate T7 mitochondrion | *Coregonus lavaretus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus lavaretus* isolate T5 mitochondrion | *Coregonus lavaretus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus lavaretus* isolate K2 mitochondrion | *Coregonus lavaretus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus lavaretus* isolate K7 mitochondrion | *Coregonus lavaretus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus lavaretus* isolate K5 mitochondrion | *Coregonus lavaretus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus lavaretus* isolate K6 mitochondrion | *Coregonus lavaretus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus lavaretus* isolate K8 mitochondrion | *Coregonus lavaretus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus lavaretus* isolate K4 mitochondrion | *Coregonus lavaretus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus oxyrinchus* isolate V21 mitochondrion | *Coregonus oxyrinchus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus oxyrinchus* isolate V\_S1 mitochondrion | *Coregonus oxyrinchus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus oxyrinchus* isolate V12 mitochondrion | *Coregonus oxyrinchus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus oxyrinchus* isolate V\_S2 mitochondrion | *Coregonus oxyrinchus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus oxyrinchus* isolate V7 mitochondrion | *Coregonus oxyrinchus* | 315 | 315 | 100% | 7.00E-82 | 100 |
| *Coregonus oxyrinchus* isolate V1 mitochondrion | *Coregonus oxyrinchus* | 315 | 315 | 100% | 7.00E-82 | 100 |

Table S5. Blast search result of the sequences assigned at Coregoninae family.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Description** | **Scientific Name** | **Max Score** | **Total Score** | **Query Cover** | **E value** | **Per. ident** |
| *Coregonus clupeaformis* isolate NEFC F17-265 mitochondrion | *Coregonus clupeaformis* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus clupeaformis* isolate NEFC F17-264 mitochondrion | *Coregonus clupeaformis* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus ussuriensis* mitochondrion | *Coregonus ussuriensis* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus maraena* voucher CZ12 small subunit ribosomal RNA gene | *Coregonus maraena* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus clupeaformis* isolate A7 mitochondrion | *Coregonus clupeaformis* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus clupeaformis* isolate A6 mitochondrion | *Coregonus clupeaformis* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus clupeaformis* isolate A3 mitochondrion | *Coregonus clupeaformis* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus clupeaformis* isolate A2 mitochondrion | *Coregonus clupeaformis* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus clupeaformis* isolate A1 mitochondrion | *Coregonus clupeaformis* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate N16 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate N12 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate N15 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate N7 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate N18 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate N5 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate N20 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate N8 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate R3 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate R8 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate R13 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate R15 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate R10 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate R11 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate R16 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate R5 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate R1 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate R18 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate R4 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate F6 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate F8 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate F7 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate F5 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate T4 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate T6 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate T3 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate T2 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate T7 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate T5 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate K2 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate K7 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate K5 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate K6 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate K8 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate K4 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus oxyrinchus* isolate V21 mitochondrion | *Coregonus oxyrinchus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus oxyrinchus* isolate V\_S1 mitochondrion | *Coregonus oxyrinchus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus oxyrinchus* isolate V12 mitochondrion | *Coregonus oxyrinchus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus oxyrinchus* isolate V\_S2 mitochondrion | *Coregonus oxyrinchus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus oxyrinchus* isolate V7 mitochondrion | *Coregonus oxyrinchus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus oxyrinchus* isolate V1 mitochondrion | *Coregonus oxyrinchus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus oxyrinchus* isolate V2 mitochondrion | *Coregonus oxyrinchus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus oxyrinchus* isolate V4 mitochondrion | *Coregonus oxyrinchus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus oxyrinchus* isolate V16 mitochondrion | *Coregonus oxyrinchus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus oxyrinchus* isolate V19 mitochondrion | *Coregonus oxyrinchus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus oxyrinchus* isolate V15 mitochondrion | *Coregonus oxyrinchus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus oxyrinchus* isolate V6 mitochondrion | *Coregonus oxyrinchus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus oxyrinchus* isolate V13 mitochondrion | *Coregonus oxyrinchus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus oxyrinchus* isolate V14 mitochondrion | *Coregonus oxyrinchus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus oxyrinchus* isolate V17 mitochondrion | *Coregonus oxyrinchus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus oxyrinchus* isolate V5 mitochondrion | *Coregonus oxyrinchus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate Ro8 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate Ro4 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate Ro10 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate Ro3 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate Ro9 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate Ro13 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate Est4 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate Est7 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate Est22 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate Est8 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate Est10 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate Est5 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* isolate Est6 mitochondrion | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus lavaretus* mitochondrial DNA | *Coregonus lavaretus* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Coregonus migratorius* isolate Bar377 mitochondrion | *Coregonus migratorius* | 305 | 305 | 98% | 4.00E-79 | 99.4 |
| *Coregonus migratorius* isolate Bar376 mitochondrion | *Coregonus migratorius* | 305 | 305 | 98% | 4.00E-79 | 99.4 |
| *Coregonus baicalensis* isolate 14mm mitochondrion | *Coregonus baicalensis* | 305 | 305 | 98% | 4.00E-79 | 99.4 |
| *Coregonus baicalensis* isolate ss11 mitochondrion | *Coregonus baicalensis* | 305 | 305 | 98% | 4.00E-79 | 99.4 |
| *Coregonus baicalensis* isolate Chiv1 mitochondrion | *Coregonus baicalensis* | 305 | 305 | 98% | 4.00E-79 | 99.4 |
| ***Stenodus leucichthys* mitochondrion** | *Stenodus leucichthys* | 305 | 305 | 98% | 4.00E-79 | 99.4 |
| *Coregonus clupeaformis* mitochondrion | *Coregonus clupeaformis* | 303 | 303 | 100% | 2.00E-78 | 98.82 |
| *Coregonus clupeaformis* isolate A10 mitochondrion | *Coregonus clupeaformis* | 303 | 303 | 100% | 2.00E-78 | 98.82 |
| *Coregonus lavaretus* isolate R14 mitochondrion | *Coregonus lavaretus* | 303 | 303 | 100% | 2.00E-78 | 98.82 |
| *Coregonus migratorius* isolate Kul52 mitochondrion | *Coregonus migratorius* | 300 | 300 | 98% | 2.00E-77 | 98.81 |
| *Coregonus artedi* isolate NEFC F16-263 mitochondrion | *Coregonus artedi* | 300 | 300 | 98% | 2.00E-77 | 98.81 |
| *Coregonus zenithicus* isolate 4D3 12S ribosomal RNA gene | *Coregonus zenithicus* | 300 | 300 | 98% | 2.00E-77 | 98.81 |
| *Coregonus zenithicus* isolate 4B3 12S ribosomal RNA gene | *Coregonus zenithicus* | 300 | 300 | 98% | 2.00E-77 | 98.81 |
| *Coregonus sardinella* isolate 4B2 12S ribosomal RNA gene | *Coregonus sardinella* | 300 | 300 | 98% | 2.00E-77 | 98.81 |
| *Coregonus laurettae* isolate 4D1 12S ribosomal RNA gene | *Coregonus laurettae* | 300 | 300 | 98% | 2.00E-77 | 98.81 |
| *Coregonus laurettae* isolate 4B1 12S ribosomal RNA gene | *Coregonus laurettae* | 300 | 300 | 98% | 2.00E-77 | 98.81 |
| *Coregonus albula* isolate CA\_EVO1 small subunit ribosomal RNA gene | *Coregonus albula* | 300 | 300 | 98% | 2.00E-77 | 98.81 |
| *Coregonus artedi* strain NEFC\_F16-524 mitochondrion | *Coregonus artedi* | 300 | 300 | 98% | 2.00E-77 | 98.81 |
| *Coregonus artedi* voucher NEFC\_F16-375 mitochondrion | *Coregonus artedi* | 300 | 300 | 98% | 2.00E-77 | 98.81 |
| *Coregonus nasus* mitochondrion | *Coregonus nasus* | 300 | 300 | 97% | 2.00E-77 | 99.39 |
| *Coregonus maraena* voucher CZ13 small subunit ribosomal RNA gene | *Coregonus maraena* | 300 | 300 | 98% | 2.00E-77 | 98.81 |
| *Coregonus hoyi* voucher NEFC F18-197 mitochondrion | *Coregonus hoyi* | 300 | 300 | 98% | 2.00E-77 | 98.81 |
| *Coregonus maraena* voucher CZ14 small subunit ribosomal RNA gene | *Coregonus maraena* | 296 | 296 | 97% | 3.00E-76 | 98.8 |
| *Coregonus artedi* isolate NEFC F16-262 mitochondrion | *Coregonus artedi* | 294 | 294 | 98% | 1.00E-75 | 98.21 |
| *Coregonus sardinella* isolate 4C2 12S ribosomal RNA gene | *Coregonus sardinella* | 294 | 294 | 98% | 1.00E-75 | 98.21 |
| *Coregonus laurettae* isolate 4A1 12S ribosomal RNA gene | *Coregonus laurettae* | 294 | 294 | 98% | 1.00E-75 | 98.21 |

Table S6. Blast search result of the sequences assigned at Cyprinidae family.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Description** | **Scientific Name** | **Max Score** | **Total Score** | **Query Cover** | **E value** | **Per. ident** |
| *Cyprinus carpio* CBM:ZF:18642 mitochondrial gene for 12S rRNA | *Cyprinus carpio* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio* CBM:ZF:17566 mitochondrial gene for 12S rRNA | *Cyprinus carpio* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio* CBM:ZF:17105 mitochondrial gene for 12S rRNA | *Cyprinus carpio* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio* 'Guilin' isolate Guilin mitochondrion | *Cyprinus carpio* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio* mitochondrion | *Cyprinus carpio* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio* CBM:ZF:19572 mitochondrial gene for 12S rRNA | *Cyprinus carpio* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio* CBM:ZF:19257 mitochondrial gene for 12S rRNA | *Cyprinus carpio* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio* mitochondrion | *Cyprinus carpio* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio* isolate CC\_EVO1 small subunit ribosomal RNA gene | *Cyprinus carpio* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio* KAUM:I:89444 mitochondrial gene for 12S rRNA | *Cyprinus carpio* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio* isolate CES005 tRNA-Phe gene | *Cyprinus carpio* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio* isolate NEFC F16-248 mitochondrion | *Cyprinus carpio* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio* isolate NEFC F16-117 mitochondrion | *Cyprinus carpio* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio* isolate NEFC F16-042 mitochondrion | *Cyprinus carpio* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio* 'Ying hybrid' mitochondrion | *Cyprinus carpio* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio* mitochondrial DNA | *Cyprinus carpio* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio* mitochondrial DNA | *Cyprinus carpio* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio* 'xingguonensis' mitochondrion | *Cyprinus carpio* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio carpio* mitochondrion | *Cyprinus carpio carpio* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus* sp. CBM ZF 11624 mitochondrial DNA | *Cyprinus sp.* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio nudus* mitochondrion | *Cyprinus carpio nudus* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio* mitochondrion | *Cyprinus carpio* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio* mitochondrion | *Cyprinus carpio* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio* mitochondrial gene for 12S rRNA | *Cyprinus carpio* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio* mitochondrial gene for 12S rRNA | *Cyprinus carpio* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio* mitochondrial gene for 12S rRNA | *Cyprinus carpio* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus acutidorsalis* mitochondrion | *Cyprinus acutidorsalis* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio carpio* mitochondrion | *Cyprinus carpio carpio* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio haematopterus* mitochondrion | *Cyprinus carpio haematopterus* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio* isolate Zujiang mitochondrion | *Cyprinus carpio* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio* isolate Oujiang mitochondrion | *Cyprinus carpio* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Procypris mera* mitochondrion | *Procypris mera* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio* mitochondrion | *Cyprinus carpio* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Pseudorasbora parva* mitochondrion | *Pseudorasbora parva* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio* mitochondrion | *Cyprinus carpio* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio* 'wananensis' mitochondrion | *Cyprinus carpio* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio color* mitochondrion | *Cyprinus carpio* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio wuyuanensis* mitochondrion | *Cyprinus carpi* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio haematopterus* mitochondrion | *Cyprinus carpio haematopterus* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio xingguonensis* mitochondrion | *Cyprinus carpio* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio carpio* mitochondrion | *Cyprinus carpio carpio* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio* var. baisenensis mitochondrion | *Cyprinus carpio* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Cyprinus carpio* complete mitochondrial genome | *Cyprinus carpio* | 315 | 315 | 100% | 8.00E-82 | 99.42 |
| *Carassius* sp. 'Ginbuna' CBM:ZF:19573 mitochondrial gene for 12S rRNA | *Carassius sp.* | 309 | 309 | 100% | 4.00E-80 | 98.84 |
| *Carassius* sp. 'Ginbuna' CBM:ZF:19352 mitochondrial gene for 12S rRNA | *Carassius sp.* | 309 | 309 | 100% | 4.00E-80 | 98.84 |

Table S7. Blast search result of the sequences assigned at *Esox* genus.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Description** | **Scientific Name** | **Max Score** | **Total Score** | **Query Cover** | **E value** | **Per. ident** |
| *Esox lucius* isolate EL\_EVO2 small subunit ribosomal RNA gene | *Esox lucius* | 305 | 305 | 100% | 5.00E-79 | 99.4 |
| *Esox lucius* isolate EL\_EVO1 small subunit ribosomal RNA gene | *Esox lucius* | 305 | 305 | 100% | 5.00E-79 | 99.4 |
| *Esox lucius* isolate smp216 mitochondrion | *Esox lucius* | 305 | 305 | 100% | 5.00E-79 | 99.4 |
| *Esox lucius* isolate smp148 mitochondrion | *Esox lucius* | 305 | 305 | 100% | 5.00E-79 | 99.4 |
| *Esox lucius* isolate smp1109 mitochondrion | *Esox lucius* | 305 | 305 | 100% | 5.00E-79 | 99.4 |
| *Esox lucius* voucher NEFC F18-125 mitochondrion | *Esox lucius* | 305 | 305 | 100% | 5.00E-79 | 99.4 |
| *Esox lucius* isolate smp349 mitochondrion | *Esox lucius* | 300 | 300 | 100% | 2.00E-77 | 98.81 |
| *Esox flaviae* isolate smp43 mitochondrion | *Esox flaviae* | 300 | 300 | 100% | 2.00E-77 | 98.81 |
| *Esox flaviae* isolate smp42 mitochondrion | *Esox flaviae* | 300 | 300 | 100% | 2.00E-77 | 98.81 |
| *Esox lucius* mitochondrial DNA | *Esox lucius* | 300 | 300 | 100% | 2.00E-77 | 98.81 |

Table S8. Blast search result of the sequences assigned at *Salmo* genus.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Description** | **Scientific Name** | **Max Score** | **Total Score** | **Query Cover** | **E value** | **Per. ident** |
| *Salmo trutta* isolate ST\_EVO1 small subunit ribosomal RNA gene | *Salmo trutta* | 309 | 309 | 100% | 4.00E-80 | 99.41 |
| *Salmo trutta* mitochondrial genome | *Salmo trutta* | 309 | 309 | 100% | 4.00E-80 | 99.41 |
| *Salmo trutta* mitochondrial genome | *Salmo trutta* | 309 | 309 | 100% | 4.00E-80 | 99.41 |
| *Salmo trutta* mitochondrionial geneome | *Salmo trutta* | 309 | 309 | 100% | 4.00E-80 | 99.41 |
| *Salmo trutta* isolate CES069 tRNA-Phe gene | *Salmo trutta* | 309 | 309 | 100% | 4.00E-80 | 99.41 |
| *Salmo trutta* voucher NEFC\_F16-334 mitochondrion | *Salmo trutta* | 309 | 309 | 100% | 4.00E-80 | 99.41 |
| *Salmo trutta* voucher NEFC\_F16-333 mitochondrion | *Salmo trutta* | 309 | 309 | 100% | 4.00E-80 | 99.41 |
| *Salmo trutta* voucher NEFC\_F16-157 mitochondrion | *Salmo trutta* | 309 | 309 | 100% | 4.00E-80 | 99.41 |
| *Salmo trutta* strain NEFC\_F16-147 mitochondrion | *Salmo trutta* | 309 | 309 | 100% | 4.00E-80 | 99.41 |
| *Salmo trutta* mitochondrial gene for 12S rRNA | *Salmo trutta* | 309 | 309 | 100% | 4.00E-80 | 99.41 |
| *Salmo trutta fario* mitochondrion | *Salmo trutta fario* | 309 | 309 | 100% | 4.00E-80 | 99.41 |
| *Salmo trutta* mitochondrion | *Salmo trutta* | 309 | 309 | 100% | 4.00E-80 | 99.41 |
| *Salmo trutta* voucher St001 12S ribosomal RNA gene | *Salmo trutta* | 309 | 309 | 100% | 4.00E-80 | 99.41 |
| *Salmo trutta trutta* complete mitochondrial genome | *Salmo trutta trutta* | 309 | 309 | 100% | 4.00E-80 | 99.41 |
| *Salmo ischchan* isolate 30 mitochondrion | *Salmo ischchan* | 303 | 303 | 100% | 2.00E-78 | 98.82 |
| *Salmo ischchan* isolate 26 mitochondrion | *Salmo ischchan* | 303 | 303 | 100% | 2.00E-78 | 98.82 |
| *Salmo ischchan gegarkuni* isolate sevan4 mitochondrion | *Salmo ischchan gegarkuni* | 303 | 303 | 100% | 2.00E-78 | 98.82 |
| *Salmo ischchan* gegarkuni isolate issikkul mitochondrion | *Salmo ischchan gegarkuni* | 303 | 303 | 100% | 2.00E-78 | 98.82 |
| *Salmo ischchan* gegarkuni mitochondrion | *Salmo ischchan gegarkuni* | 303 | 303 | 100% | 2.00E-78 | 98.82 |
| *Salmo ischchan* isolate Li4 mitochondrion | *Salmo ischchan* | 303 | 303 | 100% | 2.00E-78 | 98.82 |
| *Salmo ischchan* isolate Li30 mitochondrion | *Salmo ischchan* | 303 | 303 | 100% | 2.00E-78 | 98.82 |
| *Salmo ischchan* isolate BO6 mitochondrion | *Salmo ischchan* | 303 | 303 | 100% | 2.00E-78 | 98.82 |
| *Salmo trutta* mitochondrial gene for 12S rRNA | *Salmo trutta* | 303 | 303 | 100% | 2.00E-78 | 98.82 |
| *Salmo trutta* fario mitochondrial DNA | *Salmo trutta fario* | 303 | 303 | 100% | 2.00E-78 | 98.82 |
| *Salmo trutta* caspius mitochondrial DNA | *Salmo caspius* | 303 | 303 | 100% | 2.00E-78 | 98.82 |
| *Salmo labrax* tRNA-Phe and 12S ribosomal RNA genes | *Salmo labrax* | 303 | 303 | 100% | 2.00E-78 | 98.82 |
| *Salmo trutta fario* tRNA-Phe and 12S ribosomal RNA genes | *Salmo trutta fario* | 303 | 303 | 100% | 2.00E-78 | 98.82 |
| *Salmo obtusirostris* voucher NMW\_67984 mitochondrion | *Salmo obtusirostris* | 298 | 298 | 100% | 8.00E-77 | 98.24 |
| *Salmo ischchan* isolate BO1 mitochondrion | *Salmo ischchan* | 298 | 298 | 100% | 8.00E-77 | 98.24 |
| *Salmo salar* mitochondrial gene for 12S rRNA | *Salmo salar* | 287 | 287 | 100% | 2.00E-73 | 97.06 |
| *Salmo salar* mitochondrial DNA | *Salmo salar* | 287 | 287 | 100% | 2.00E-73 | 97.06 |
| *Salmo salar* mitochondrion | *Salmo salar* | 287 | 287 | 100% | 2.00E-73 | 97.06 |
| *Salmo salar* mitochondrion | *Salmo salar* | 287 | 287 | 100% | 2.00E-73 | 97.06 |
| *Salmo salar* voucher Ss001 12S ribosomal RNA gene | *Salmo salar* | 287 | 287 | 100% | 2.00E-73 | 97.06 |
| *Salmo salar* haplotype Hap\_1 12S ribosomal RNA gene | *Salmo salar* | 287 | 287 | 100% | 2.00E-73 | 97.06 |
| *Salmo salar* mitochondrion | *Salmo salar* | 287 | 287 | 100% | 2.00E-73 | 97.06 |
| *Salmo salar* mitochondrion | *Salmo salar* | 287 | 287 | 100% | 2.00E-73 | 97.06 |

Table S9. Blast search result of the sequences assigned at *Silurus* genus.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Description** | **Scientific Name** | **Max Score** | **Total Score** | **Query Cover** | **E value** | **Per. ident** |
| *Silurus glanis* complete mitochondrial genome | *Silurus glanis* | 303 | 303 | 100% | 2.00E-78 | 98.82 |
| *Silurus grahami* mitochondrion | *Silurus grahami* | 298 | 298 | 100% | 8.00E-77 | 98.24 |
| *Silurus asotus* CBM:ZF:19363 mitochondrial gene for 12S rRNA | *Silurus asotus* | 292 | 292 | 100% | 4.00E-75 | 97.65 |
| *Silurus asotus* mitochondrial gene for 12S rRNA | *Silurus asotus* | 292 | 292 | 100% | 4.00E-75 | 97.65 |
| *Silurus asotus* mitochondrial gene for 12S rRNA | *Silurus asotus* | 292 | 292 | 100% | 4.00E-75 | 97.65 |
| *Silurus asotus* mitochondrial gene for 12S rRNA | *Silurus asotus* | 292 | 292 | 100% | 4.00E-75 | 97.65 |
| *Silurus asotus* isolate wujiang-8 12S ribosomal RNA gene | *Silurus asotus* | 292 | 292 | 100% | 4.00E-75 | 97.65 |
| *Silurus soldatovi* mitochondrion | *Silurus soldatovi* | 287 | 287 | 100% | 2.00E-73 | 97.06 |
| *Silurus asotus* mitochondrion | *Silurus asotus* | 287 | 287 | 100% | 2.00E-73 | 97.06 |
| *Silurus soldatovi* mitochondrial DNA | *Silurus soldatovi* | 287 | 287 | 100% | 2.00E-73 | 97.06 |

Table S10. Blast search result of the sequences assigned at *Oncorhynchus* genus.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Description** | **Scientific Name** | **Max Score** | **Total Score** | **Query Cover** | **E value** | **Per. ident** |
| *Oncorhynchus mykis*s isolate CES077 tRNA-Phe gene | *Oncorhynchus mykiss* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Oncorhynchus mykiss* voucher NEFC\_F16-293 mitochondrion | *Oncorhynchus mykiss* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Oncorhynchus mykiss* voucher LodgeLab Omykiss\_1 mitochondrion | *Oncorhynchus mykiss* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Oncorhynchus mykiss* mitochondrial DNA | *Oncorhynchus mykiss* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Oncorhynchus gilae* apache isolate SNARRC19-001 mitochondrion | *Oncorhynchus gilae apache* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Oncorhynchus gilae* isolate SNARRC18-172 mitochondrion | *Oncorhynchus gilae* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Oncorhynchus gilae* isolate SNARRC18-171 mitochondrion | *Oncorhynchus gilae* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Oncorhynchus gilae* isolate SNARRC18-170 mitochondrion | *Oncorhynchus gilae* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Oncorhynchus mykiss* voucher NEFC F18-195 mitochondrion | *Oncorhynchus mykiss* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Oncorhynchus mykiss* mitochondrion | *Oncorhynchus mykiss* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Oncorhynchus mykiss* mitochondrion | *Oncorhynchus mykiss* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Oncorhynchus mykiss* mitochondrion | *Oncorhynchus mykiss* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Oncorhynchus mykiss* mitochondrion | *Oncorhynchus mykiss* | 309 | 309 | 100% | 3.00E-80 | 99.41 |
| *Oncorhynchus nerka* isolate FB1 mitochondrion | *Oncorhynchus nerka* | 303 | 303 | 100% | 2.00E-78 | 98.82 |
| *Oncorhynchus nerka* isolate FB7 mitochondrion | *Oncorhynchus nerka* | 303 | 303 | 100% | 2.00E-78 | 98.82 |
| *Oncorhynchus nerka* isolate FB2 mitochondrion | *Oncorhynchus nerka* | 303 | 303 | 100% | 2.00E-78 | 98.82 |
| *Oncorhynchus mykiss* mitochondrial gene for 12S rRNA | *Oncorhynchus mykiss* | 303 | 303 | 100% | 2.00E-78 | 98.82 |
| *Oncorhynchus mykiss* mitochondrion | *Oncorhynchus mykiss* | 303 | 303 | 100% | 2.00E-78 | 98.82 |
| *Oncorhynchus mykiss* voucher Om001 12S ribosomal RNA gene | *Oncorhynchus mykiss* | 303 | 303 | 100% | 2.00E-78 | 98.82 |
| *Oncorhynchus nerka* mitochondrion | *Oncorhynchus nerka* | 303 | 303 | 100% | 2.00E-78 | 98.82 |
| *Oncorhynchus mykiss* mitochondrion | *Oncorhynchus mykiss* | 303 | 303 | 100% | 2.00E-78 | 98.82 |
| *Oncorhynchus clarkii henshawi* mitochondrion | *Oncorhynchus clarkii henshawi* | 303 | 303 | 100% | 2.00E-78 | 98.82 |
| *Oncorhynchus mykiss albino* mutant 12S ribosomal RNA | *Oncorhynchus mykiss* | 302 | 302 | 100% | 6.00E-78 | 98.82 |
| *Oncorhynchus mykiss* 12S ribosomal RNA | *Oncorhynchus mykiss* | 302 | 302 | 100% | 6.00E-78 | 98.82 |
| *Oncorhynchus mykiss albino* mutant 12S ribosomal RNA gene | *Oncorhynchus mykiss* | 302 | 302 | 100% | 6.00E-78 | 98.82 |
| *Oncorhynchus mykiss* 12S ribosomal RNA gene | *Oncorhynchus mykiss* | 302 | 302 | 100% | 6.00E-78 | 98.82 |
| *Oncorhynchus clarkii lewisi* isolate FWI1 12S ribosomal RNA gene | *Oncorhynchus clarkii lewisi* | 298 | 298 | 100% | 8.00E-77 | 98.24 |
| *Oncorhynchus clarkii* mitochondrial gene for 12S rRNA | *Oncorhynchus clarkii* | 298 | 298 | 100% | 8.00E-77 | 98.24 |
| *Oncorhynchus clarkii* mitochondrial gene for 12S rRNA | *Oncorhynchus clarkii* | 298 | 298 | 100% | 8.00E-77 | 98.24 |
| *Oncorhynchus gorbuscha* mitochondrial gene for 12S rRNA | *Oncorhynchus gorbuscha* | 298 | 298 | 100% | 8.00E-77 | 98.24 |
| *Oncorhynchus gorbuscha* mitochondrial gene for 12S rRNA | *Oncorhynchus gorbuscha* | 298 | 298 | 100% | 8.00E-77 | 98.24 |
| *Oncorhynchus gorbuscha* mitochondrial gene for 12S rRNA | *Oncorhynchus gorbuscha* | 298 | 298 | 100% | 8.00E-77 | 98.24 |
| *Oncorhynchus gorbuscha* mitochondrion | *Oncorhynchus gorbuscha* | 298 | 298 | 100% | 8.00E-77 | 98.24 |
| *Oncorhynchus nerka* mitochondrial gene for 12S rRNA | *Oncorhynchus nerka* | 296 | 296 | 100% | 3.00E-76 | 98.24 |
| *Oncorhynchus clarkii stomias* voucher LodgeLab OCstomias\_2 mitochondrion | *Oncorhynchus clarkii stomias* | 292 | 292 | 100% | 3.00E-75 | 97.65 |
| *Oncorhynchus clarkii stomias* voucher LodgeLab OCstomias\_1 mitochondrion | *Oncorhynchus clarkii stomias* | 292 | 292 | 100% | 3.00E-75 | 97.65 |
| *Oncorhynchus mykiss* mitochondrion complete genome | *Oncorhynchus mykiss* | 292 | 292 | 100% | 3.00E-75 | 97.66 |
| *Oncorhynchus nerka* mitochondrial gene for 12S rRNA | *Oncorhynchus nerka* | 291 | 291 | 100% | 1.00E-74 | 97.65 |
| *Oncorhynchus keta* mitochondrial gene for 12S rRNA | *Oncorhynchus keta* | 291 | 291 | 100% | 1.00E-74 | 97.65 |
| *Oncorhynchus keta* mitochondrial gene for 12S rRNA | *Oncorhynchus keta* | 291 | 291 | 100% | 1.00E-74 | 97.65 |
| *Oncorhynchus keta* mitochondrial DNA | *Oncorhynchus keta* | 291 | 291 | 100% | 1.00E-74 | 97.65 |
| *Oncorhynchus kisutch* isolate FB4 mitochondrion | *Oncorhynchus kisutch* | 287 | 287 | 100% | 2.00E-73 | 97.06 |
| *Oncorhynchus kisutch* CBM:ZF:18912 mitochondrial gene for 12S rRNA | *Oncorhynchus kisutch* | 287 | 287 | 100% | 2.00E-73 | 97.06 |
| *Oncorhynchus kisutch* voucher NEFC\_F16-295 mitochondrion | *Oncorhynchus kisutch* | 287 | 287 | 100% | 2.00E-73 | 97.06 |
| *Oncorhynchus kisutch* voucher NEFC\_F16-322 mitochondrion | *Oncorhynchus kisutch* | 287 | 287 | 100% | 2.00E-73 | 97.06 |
| *Oncorhynchus tshawytscha* mitochondrial gene for 12S rRNA | *Oncorhynchus tshawytscha* | 287 | 287 | 100% | 2.00E-73 | 97.06 |
| *Oncorhynchus kisutch* mitochondrial gene for 12S rRNA | *Oncorhynchus kisutch* | 287 | 287 | 100% | 2.00E-73 | 97.06 |
| *Oncorhynchus kisutch* mitochondrial gene for 12S rRNA | *Oncorhynchus kisutch* | 287 | 287 | 100% | 2.00E-73 | 97.06 |
| *Oncorhynchus clarkii virginalis* isolate SNARRC19-023 mitochondrion | *Oncorhynchus clarkii virginalis* | 287 | 287 | 100% | 2.00E-73 | 97.06 |
| *Oncorhynchus clarkii virginalis* isolate SNARRC19-022 mitochondrion | *Oncorhynchus clarkii virginalis* | 287 | 287 | 100% | 2.00E-73 | 97.06 |
| *Oncorhynchus tshawytscha* voucher Ot001 12S ribosomal RNA gene | *Oncorhynchus tshawytscha* | 287 | 287 | 100% | 2.00E-73 | 97.06 |
| *Oncorhynchus kisutch* mitochondrion | *Oncorhynchus kisutch* | 287 | 287 | 100% | 2.00E-73 | 97.06 |
| *Oncorhynchus tshawytscha* mitochondrion | *Oncorhynchus tshawytscha* | 287 | 287 | 100% | 2.00E-73 | 97.06 |
| *Oncorhynchus masou* 'Biwa' mitochondrion | *Oncorhynchus masou 'Biwa'* | 285 | 285 | 100% | 6.00E-73 | 97.06 |

**Table S11**. Species detected in 2018 in Lake Bourget with a traditional survey compared to those obtained with eDNA metabarcoding analysis performed in this study.

|  |  |  |  |
| --- | --- | --- | --- |
| **Name** | **Number of fish** | **Biomass (g)** | **Species identified with eDNA** |
| *Abramis brama* | 59 | 2781 | X |
| *Esox lucius* | 10 | 4808 | X |
| *Carassius carassius* | 3 | 61 |  |
| *Squalius cephalus* | 6 | 3620 | X |
| *Coregonus lavaretus* | 56 | 2872 | X |
| *Rutilus rutilus* | 335 | 19392 | X |
| *Gobio gobio* | 2 | 11 | X |
| *Gymnocephalus cernua* | 36 | 267 | X |
| *Lota lota* | 2 | 21 |  |
| *Perca fluviatilis* | 1151 | 15916 | X |
| *Lepomis gibbosus* | 19 | 618 | X |
| *Ameiurus melas* | 39 | 1890 | X |
| *Scardinius erythrophthalmus* | 38 | 10816 | X |
| *Silurus glanis* | 9 | 10600 | X |
| *Tinca tinca* | 7 | 0 | X |



**Figure S1.** Rarefaction curves built by using the eDNA metabarcoding reads obtained by the samples collected in the Lake Bourget (BAB = low Lake Bourget, BAM = medium Lake Bourget, BAH = high Lake Bourget)



**Figure S2.** Regression analysis built by using the eDNA metabarcoding reads obtained by the samples collected in the Lake Bourget by the two labs (Lab\_A and Lab\_B) and traditional survey data (number of fish and biomass) collected in 2018 (Table S11).