

## Assessing eDNA sequences

You will use the BLAST database to determine what species are present in Trout Creek. This database includes a library of known eDNA sequences (genes) for many different wildlife species. It is a tool scientists use to determine which wildlife are living in an area.

**Link to BLAST database:** Open the link to the BLAST database: [https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE\\_TYPE=BlastSearch&LINK\\_LOC=blasthome](https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome).

### To use the BLAST database:

- 1 Copy one of the eDNA sequences from **page 3**, using Ctrl C or Command-C. (If you try to type it in, you're likely to introduce errors.) Include only the letters, not the title or sequence number.

The screenshot shows the NCBI BLAST Standard Nucleotide BLAST interface. The interface is divided into several sections: 'Enter Query Sequence', 'Choose Search Set', and 'Program Selection'. The 'Enter Query Sequence' section has a large text input field for the query sequence, a 'Clear' button, and a 'Query subrange' section with 'From' and 'To' input fields. Below this is an 'Or, upload file' section with a 'Choose File' button and a 'Job Title' input field. The 'Choose Search Set' section includes a 'Database' dropdown menu set to 'Nucleotide collection (nr/nt)', an 'Organism' input field, and several checkboxes for 'Exclude' and 'Limit to' options. The 'Program Selection' section has three radio buttons for 'Optimize for' options: 'Highly similar sequences (megablast)', 'More dissimilar sequences (discontiguous megablast)', and 'Somewhat similar sequences (blastn)'. At the bottom, there is a 'BLAST' button, a 'Search database' dropdown set to 'Nucleotide collection (nr/nt)', and a 'Show results in a new window' checkbox. A '+ Algorithm parameters' link is also visible at the bottom left.

**Standard Nucleotide BLAST**

**1** Paste the eDNA sequence here, using Ctrl V or Command-V.

**2** Make sure "Nucleotide collection (nr/nt)" is selected here. Leave the other search options blank.

**3** Make sure "Highly similar sequences (megablast)" is selected here.

**4** Push the BLAST button.

**5** Push the BLAST button.

BLAST® » blastn suite » RID-F3CYHTVM015

Home Recent Results

BLAST Results

Edit and Resubmit Save Search Strategies Formatting options Download

Job title: Nucleotide Sequence

YouTube How to read this page Blast report description

Click here to see the

RID F3CYHTVM015 (Expires on 06-02 07:08 am)

Query ID Icd|Query\_119629  
 Description None  
 Molecule type dna  
 Query Length 218

Database Name nr  
 Description Nucleotide collection (nt)  
 Program BLASTN 2.9.0+ Citation

Other reports: Search Summary Taxonomy reports Distance tree of

+ Graphic Summary

- Descriptions

6 After a few seconds, the program will list any matches from the database. Close matches or hits are shown at the top of the list. (If you see a bunch of red lines, click Graphic Summary above the results window to hide them.)

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max Score	Total Score	Query Cover	E value
<input type="checkbox"/> <a href="#">Cottus asper mitochondrial COI gene for cytochrome oxidase subunit I, partial cds, isolate: 051</a>	396	396	99%	2e-106
<input type="checkbox"/> <a href="#">Cottus asper voucher NXG2014142 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial</a>	396	396	99%	2e-106
<input type="checkbox"/> <a href="#">Cottus asper isolate PAD5 cytochrome oxidase 1 (COI) gene, partial cds; mitochondrial</a>	396	396	99%	2e-106
<input type="checkbox"/> <a href="#">Cottus asper isolate OL2 cytochrome oxidase 1 (COI) gene, partial cds; mitochondrial</a>	396	396	99%	2e-106
<input type="checkbox"/> <a href="#">Cottus asper isolate NIL12 cytochrome oxidase 1 (COI) gene, partial cds; mitochondrial</a>	396	396	99%	2e-106
<input type="checkbox"/> <a href="#">Cottus asper isolate MOL6 cytochrome oxidase 1 (COI) gene, partial cds; mitochondrial</a>	396	396	99%	2e-106
<input type="checkbox"/> <a href="#">Cottus asper isolate MOL1 cytochrome oxidase 1 (COI) gene, partial cds; mitochondrial</a>	396	396	99%	2e-106
<input type="checkbox"/> <a href="#">Cottus asper isolate ML8 cytochrome oxidase 1 (COI) gene, partial cds; mitochondrial</a>	396	396	99%	2e-106
<input type="checkbox"/> <a href="#">Cottus asper isolate ML1 cytochrome oxidase 1 (COI) gene, partial cds; mitochondrial</a>	396	396	99%	2e-106
<input type="checkbox"/> <a href="#">Cottus asper isolate MEL1 cytochrome oxidase 1 (COI) gene, partial cds; mitochondrial</a>	396	396	99%	2e-106
<input type="checkbox"/> <a href="#">Cottus asper isolate HL9 cytochrome oxidase 1 (COI) gene, partial cds; mitochondrial</a>	396	396	99%	2e-106
<input type="checkbox"/> <a href="#">Cottus asper isolate HL8 cytochrome oxidase 1 (COI) gene, partial cds; mitochondrial</a>	396	396	99%	2e-106
<input type="checkbox"/> <a href="#">Cottus asper isolate FC2 cytochrome oxidase 1 (COI) gene, partial cds; mitochondrial</a>	396	396	99%	2e-106

7 Look at the first two Latin words on the first line. These are the genus and species name of the animal with the best match. Check the "Monitoring a forest stream" student page to learn its common name. On the student page, record the eDNA sequence you BLASTed, writing the sequence number (1-9) in the row for the matching species.

8 Repeat steps 1-7 for the other sequences.

## eDNA sequences from Trout Creek

Deoxyribonucleic acid, or DNA, is the genetic material that carries the instructions for an organism's growth, functioning and reproduction. The information in DNA is stored as a nucleotide sequence made up of four chemical bases: adenine (A), guanine (G), cytosine (C) and thymine (T).

You have isolated the following eDNA sequences from your water sample from Trout Creek. Use the BLAST database to determine what species each is likely from.

### eDNA SEQUENCE 1:

CGCAGGAGCCTCTGTTGACCTAACGACCT  
TCTCCCTTACCTAGCAGGTATCTTCTAT  
TCTTGAGCAATCAACTTTATCACAATAT  
CATTAAATATGAAACCCCTGCTATTTCTCA  
ATACCAGACCCCTATTCGTATGGTCTGTT  
CTTATTACTGCTGCTACTGCTTCTTTCCC  
TCCCCGTAATGCGCCGGCATCACAATG  
CTCCTAA

### eDNA SEQUENCE 2:

TTTTGCTAGTACCACCAACGCCTGACTAAT  
GGGAGAATGAGAAATTCACCAACTATCACA  
CCCCCTAGCAACTACAACAGTAATATTAGC  
CCTCGCTCTCAAGCTTGGACTAGCACCCGT  
TCACTTCTGACTCCCAGAAGTCTTCAAGG  
ACTTGAACCTACCACAGGATTAATCCTCTC  
AACCTAACAATAACTCGCACCTTCGCAC  
TTATGATTCAAGTAGCCCCAACCATCAACT  
CTTCCCTACTTGTGCAATTGGCCTTCTATC  
GACACTTGTGGGAGGTTGAGGT

### eDNA SEQUENCE 3:

TGTCACCCACCAACTATTATTCGAAAAA  
CTCACCCCTTCTATCACTAGGTAATAGCAT  
GCTAGTTGACATTCCTTCTCTGCTAAGATC  
TCCGCTGATGATGTTTTGGCTCACTTTTG  
AACCGATGTCGTATCTTACCAATTATTACAG  
GATTAATCTTTGATACATGCCTCCTATAA  
CACTGAACTAGCCTTTTCG

### eDNA SEQUENCE 4:

TTTTGCTAGCACCACCAACGCCTGACTTGT  
AGGAGAGTGAGAAATTCACCAGCCAACAC  
ACCCCTTAGCAACTACAACAGCAATATTGG  
CCCTTGCCCTCAAACCTTGGACTAGCCCCG  
TTCACCTTTGACTACCAGAAGTCTTCAAG  
GACTTGAACCTACAACAGGACTAATCCTGT  
CAACCTGACAAAACTAGCACCTTTTGCA  
CTTATAATTCAAGTAGCCCCAACCATTA  
CTTCTACTACTTGAATTGGCCTTCTATC  
AACACTTGTGCGGAGGCTGAGGG

### eDNA SEQUENCE 5:

AAGTCTGGTGCCAGCAGCCGCGTAATTC  
CAGCTCCAATAGCGTATACTACAGTTGTTG  
CAGTTAAAAAGCTCGTAGTTGGATTTGTGG  
CTGGTCACAGTGGCGTGCCCTATTGGGTT  
CGCTGTTTGTGTCTGCCATCTTTGGGTGGA  
ATCTAGGTGGCATTAGTTGTGCGCTGGGG  
GATGCCCATCGTTTACTGTGAAAAAATTAG  
AGTGTTCAAAGCAGGCTTATGCCGTTGAAT  
ATGTTAGCATGGAATAATGATATAGGACCTT  
GGTACTATTTGTTGGTTTGCACACTGAGG  
TAATGATTAATA

### eDNA SEQUENCE 6:

TTTTGCAAAAAAATGATTTCGGTTATTT  
AGGAATGGTTTATGCAATGTTATCTATAGGT  
TTATTAGGTTGATTGTATGGGCACATCATAT  
GTTTACTGTAGGTTAGATGTAGATGCTAGA  
GCTTATTTTTCAGCAGCTACTATGATTATTG  
CGGTACCTACGGGTATTAATCTTTAGTTG  
GTTAGCAACTTTAT

### eDNA SEQUENCE 7:

TGTGATGAAATTTTGGCTCACTCCTGGGCC  
TCTGCCTTATCACACAAATCCTAACAGGAT  
TATTTCTTGCAATACACTACACAGCTGACAT  
TTCAACAGCCTTCTCCTCTGTGCGCCACAT  
CTGCCGAGATGTGAACCTACGGATGACTAAT  
TCGAAACATTCATGCAAACGGGGCCTCTT  
TCTTCTTCAT

### eDNA SEQUENCE 8:

TCTTAAATTAATAATTTGAATTTAACTTTTA  
AGTGAAAAGGCTTAAATTATCTAGAGTGAC  
GATAAGACCCTATAAACTTTATATTTTAAA  
ATAATAGTTAGTTTTATTTAAGAGTTTTATT  
TGAAATATTTTATTGGGGTGATAAAGATATA  
AATTAATAACTGTCTTTTTTTTTTACAAT  
AATATTTGAATTAATGATCCTAAGAAAGGA  
GTAAAAGATCAAGTTACTTTA

### eDNA SEQUENCE 9:

AAAATACACCATCCTCGAGCAACAGAAGC  
AGCAACTAAATATTTTTTAATCCAAGCGTC  
TGCCTCAGCTTTAATCTTATTTTCAATAACA  
TTAAATCTTGATTAACAGGACATTGAATAA  
TTACAAATATAATTCACCATTTTCCCTCTATA  
GTTTTAACAATTGCACTTACTATAAAAAC  
GTGTCGCACCATTTTCATATATGACTG