



### Helbing Laboratory eDNA Technical Bulletin

All eDNA tools are validated through a rigorous multi-step evaluation protocol that includes tests of DNA target specificity and amplification sensitivity<sup>1-3</sup>.

#### General eDNA Assay Information

Target Species: Cutthroat Trout (*Oncorhynchus clarkii*)  
Species Code: te-ONCL  
eDNA qPCR Tool: eONCL4  
eDNA qPCR Format: TaqMan  
Gene Target: MT-ND1  
Published in:

#### eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.5 95% CI 0.3-1.1 Copies/Rxn LOQ 2 95% CI 1.3-4.3 Copies/Rxn LOB 0 hits/8  
LOQ<sub>continuous</sub> 4 Copies/Rxn

Binomial-Poisson model for 8 technical replicates determined using eLowQuant R code<sup>4</sup>. When the LOQ < LOD, use the LOD for the LOQ. Enzyme: Immolase

#### eDNA Assay Specificity Test Information

Each qPCR reaction in the specificity assay contained 10 picograms of voucher target gDNA (n=25 technical replicates)

| Species   | Common Name ( <i>Species</i> )                                   | Detection | # Voucher |  | Sample Sources/Locations     |
|-----------|--|-----------|-----------|--|------------------------------|
|           |  |           | Specimens |  |                              |
| ma-HOSA   | Human ( <i>Homo Sapiens</i> )                                    | No        | 1         |  | Netherlands                  |
| te-ONCLcl | Coastal Cutthroat Trout ( <i>Oncorhynchus clarkii clarkii</i> )  | Yes       | 5         |  | British Columbia             |
| te-ONCLle | Westslope Cutthroat Trout ( <i>Oncorhynchus clarkii lewisi</i> ) | Yes       | 9         |  | Alberta                      |
| te-ONGO   | Pink Salmon ( <i>Oncorhynchus gorbuscha</i> )                    | No        | 1         |  | British Columbia             |
| te-ONKE   | Chum Salmon ( <i>Oncorhynchus keta</i> )                         | No        | 1         |  | British Columbia             |
| te-ONKI   | Coho Salmon ( <i>Oncorhynchus kisutch</i> )                      | No        | 1         |  | British Columbia             |
| te-ONMY   | Rainbow Trout ( <i>Oncorhynchus mykiss</i> )                     | No        | 6         |  | Alberta and British Columbia |
| te-ONNE   | Sockeye Salmon ( <i>Oncorhynchus nerka</i> )                     | No        | 1         |  | British Columbia             |
| te-ONTS   | Chinook Salmon ( <i>Oncorhynchus tshawytscha</i> )               | No        | 1         |  | British Columbia             |
| te-SACO   | Bull Trout ( <i>Salvelinus confluentus</i> )                     | No        | 4         |  | Alberta                      |
| te-SAFO   | Brook Trout ( <i>Salvelinus fontinalis</i> )                     | No        | 4         |  | Alberta                      |
| te-SAMA   | Dolly Varden ( <i>Salvelinus malma</i> )                         | No        | 1         |  | Alberta                      |
| te-SASA   | Atlantic Salmon ( <i>Salmo salar</i> )                           | No        | 1         |  | Nova Scotia                  |

#### References

- Hobbs, J, Adams, IT, Round, JM, Goldberg, CS, Allison, MJ, Bergman, LC, Mirabzadeh, A, Allen, H, Helbing, CC (2020) Revising the range of Rocky Mountain tailed frog, *Ascaphus montanus*, in British Columbia, Canada, using environmental DNA methods. Environmental DNA. 2020; 2: 350-361. <https://doi.org/10.1002/edn3.82>
- Hobbs, J, Round, JM, Allison, MJ, Helbing, CC (2019) Expansion of the known distribution of the coastal tailed frog, *Ascaphus truei*, in British Columbia, Canada, using robust eDNA detection methods. PLOS ONE 14(3): e0213849. <https://doi.org/10.1371/journal.pone.0213849>
- Langlois, VS, Allison, MJ, Bergman, LC, To, TA, and Helbing, CC (2020) The need for robust qPCR-based eDNA detection assays in environmental monitoring and risk assessments. Environmental DNA, 3: 519-527. doi: 10.1002/edn3.164
- Lesperance, M, Allison, MJ, Bergman, LC, Hocking, MD, and Helbing, CC (2021) A statistical model for calibration and computation of detection and quantification limits for low copy number environmental DNA samples. Environmental DNA, 00: 1-12. doi: 10.1002/edn3.220



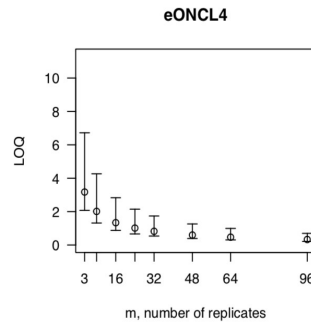
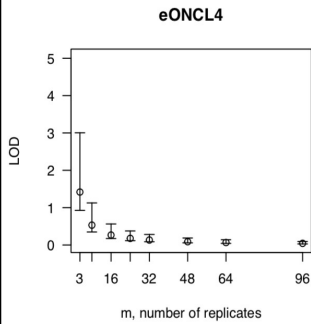
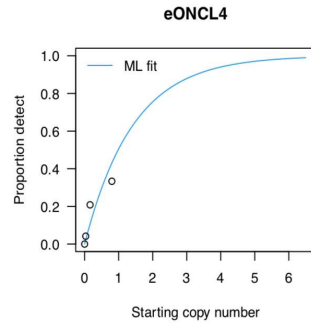
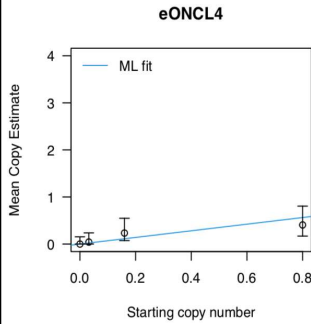
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

To calculate tables for different numbers of replicates, raw csv data files can be accessed here:  
<https://onlineacademiccommunity.uvic.ca/helbinglab/edna/>

From 8 Technical Replicates

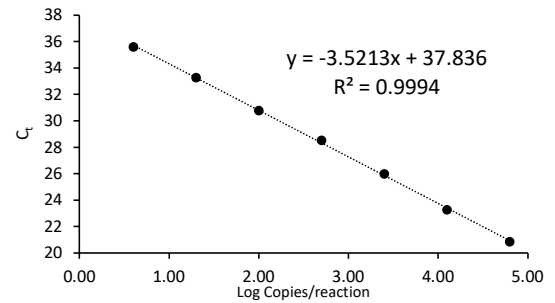
| # Detects | # Copies | SE   |
|-----------|----------|------|
| 0         | 0        | 0    |
| 1         | 0.19     | 0.2  |
| 2         | 0.41     | 0.31 |
| 3         | 0.67     | 0.43 |
| 4         | 0.98     | 0.57 |
| 5         | 1.39     | 0.75 |
| 6         | 1.97     | 1.02 |
| 7         | 2.95     | 1.55 |

Determined using eLowQuant R code<sup>4</sup>.



Binomial-Poisson model: No intercept  
Determined using eLowQuant R code<sup>4</sup>.  
Based on a 2 µL DNA input in a total 15 µL reaction

Applied to reactions with 100% positive hits



Efficiency 92%

Field Sample Validation

Known  
Sample Type Presence # Samples Detected Location

| Abbreviations |   |        |  |
|---------------|---|--------|--|
| 95% CI        | 95% Confidence interval                           | LOQ    | Limit of quantification                          |
| eDNA          | Environmental DNA                                 | MT-ND1 | Mitochondrial NADH subunit 1 gene                |
| gDNA          | Total genomic DNA extracted from voucher specimen | NTC    | qPCR no template control                         |
| LOB           | Limit of blank                                    | qPCR   | Quantitative real-time polymerase chain reaction |
| LOD           | Limit of detection                                | SE     | Standard error                                   |