



### 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: Surf Smelt (*Hypomesus pretiosus*)  
Species Code: te-HYPR  
eDNA qPCR Tool: eHYPR4  
eDNA qPCR Format: TaqMan  
Gene Target: MT-COI  
Published in: 5

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

LOD 1.2 95% CI 0.8-2.2 Copies/Rxn LOQ 4.6 95% CI 3.2-8.2 Copies/Rxn LOB 0 hits/8  
LOQ<sub>continuous</sub> 20 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 |  |                          |
| am-LICA | Bullfrog ( <i>Lithobates (Rana) catesbeiana</i> )  | No        | 1         |  | British Columbia         |
| ma-HOSA | Human ( <i>Homo sapiens</i> )                      | No        | 1         |  | Netherlands              |
| te-AMPE | Pacific Sand Lance ( <i>Ammodytes personatus</i> ) | No        | 6         |  | British Columbia         |
| te-HYPR | Surf Smelt ( <i>Hypomesus pretiosus</i> )          | Yes       | 7         |  | British Columbia         |
| 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-ONNE | Sockeye Salmon ( <i>Oncorhynchus nerka</i> )       | No        | 1         |  | British Columbia         |
| te-ONTS | Chinook Salmon ( <i>Oncorhynchus tshawytscha</i> ) | No        | 1         |  | British Columbia         |
| te-THPA | Eulachon ( <i>Thaleichthys pacificus</i> )         | No        | 1         |  | British Columbia         |

#### References

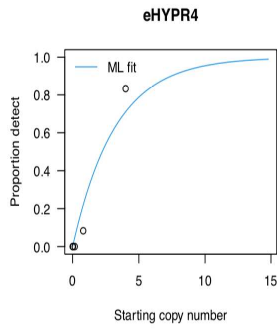
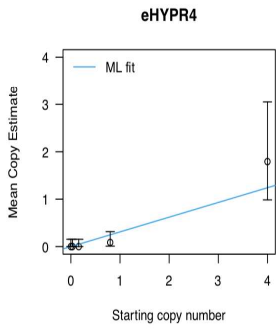
- Hobbs, J, Adams, IT, Round, JM, Goldberg, CS, Allison, MJ, Bergman, LC, Mirabzadeh, A, Allen, H, Helbing, CC (2020) Revising the range of Rocky Mountain tailed frog, *Ascaphus montanus*, in British Columbia, Canada, using environmental DNA methods. Environmental DNA. 2020; 2: 350-361. <https://doi.org/10.1002/edn3.82>
- 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
- Robinson, CLK, Bergman, LC, Allison, MJ, Huard, J, Sutherst, J, and Helbing, CC (2022) The utility of environmental DNA to detect intertidal habitat use by forage fish. Ecological Indicators, 142: 109306. doi: 10.1016/j.ecolind.2022.109306



**eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA**

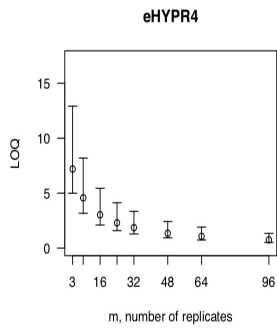
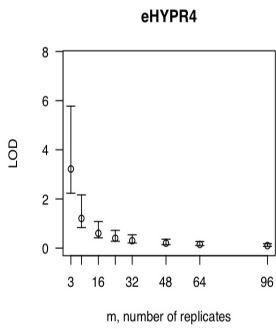
To calculate tables for different numbers of replicates, raw csv data files can be accessed here:  
<https://onlineacademiccommunity.uvic.ca/helbinglab/edna/>

From 8 Technical Replicates



| # Detects | # Copies | SE   |
|-----------|----------|------|
| 0         | 0        | 0    |
| 1         | 0.43     | 0.44 |
| 2         | 0.93     | 0.69 |
| 3         | 1.52     | 0.95 |
| 4         | 2.23     | 1.25 |
| 5         | 3.16     | 1.64 |
| 6         | 4.47     | 2.22 |
| 7         | 6.7      | 3.37 |

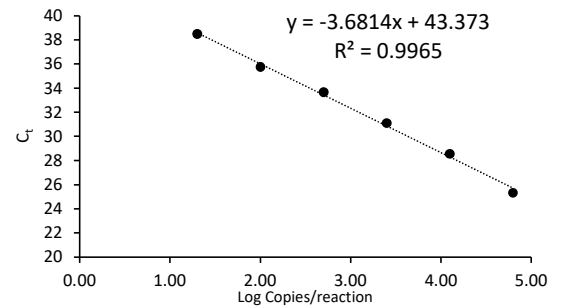
Determined using eLowQuant R code<sup>4</sup>.



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 87%

**Field Sample Validation**

Known  
Sample Type Presence # Samples Detected Location

| Abbreviations |   |        |  |
|---------------|---|--------|--|
| 95% CI        | 95% Confidence interval                           | LOQ    | Limit of quantification                          |
| eDNA          | Environmental DNA                                 | MT-COI | Mitochondrial cytochrome oxidase 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                                   |