



### 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: Mammalian species eDNA qPCR Tool: eMammal1 Gene Target: MT-RNR1  
Species Code: ma-Mammal eDNA qPCR Format: TaqMan Published in: \_\_\_\_\_

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

LOD 1.2 95% CI 0.9-2.1 Copies/Rxn LOQ 4.6 95% CI 3.2-8.1 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 (Species)                               | Detection | # Voucher |  | Sample Sources/Locations |
|---------|---|-----------|-----------|--|--------------------------|
|         |   |           | Specimens |  |                          |
| am-LICA | Bullfrog ( <i>Lithobates (Rana) catesbeiana</i> )   | No        | 1         |  | British Columbia         |
| ma-ALAL | Moose ( <i>Alces alces</i> )                        | Yes       | 1         |  | British Columbia         |
| ma-CEEL | Elk ( <i>Cervus elaphus</i> )                       | Yes       | 1         |  | British Columbia         |
| ma-FECA | Cat ( <i>Felis catus</i> )                          | Yes       | 1         |  | British Columbia         |
| ma-HOSA | Human ( <i>Homo Sapiens</i> )                       | Yes       | 1         |  | Netherlands              |
| ma-ODHE | Black Tailed Deer ( <i>Odocoileus hemionus</i> )    | Yes       | 1         |  | British Columbia         |
| ma-SOBE | Pacific Water/Marsh Shrew ( <i>Sorex bendirii</i> ) | Yes       | 1         |  | Washington               |
| ma-SOCI | Cinereus Shrew ( <i>Sorex cinereus</i> )            | Yes       | 1         |  | Washington               |
| ma-SOTR | Trowbridge's Shrew ( <i>Sorex trowbridgii</i> )     | Yes       | 1         |  | Washington               |
| ma-URAM | American Black Bear ( <i>Ursus americanus</i> )     | Yes       | 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, 3: 970-981. doi: 10.1002/edn3.220



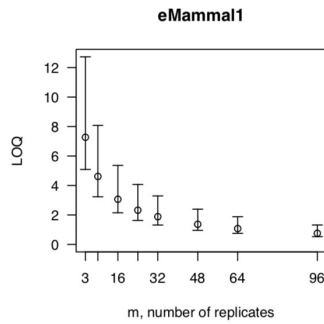
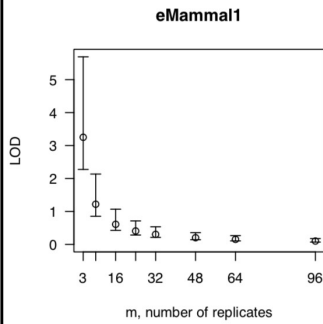
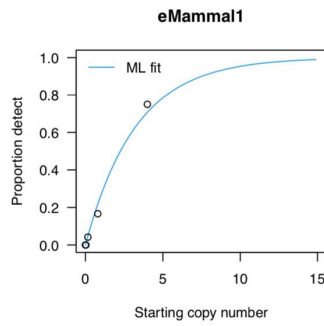
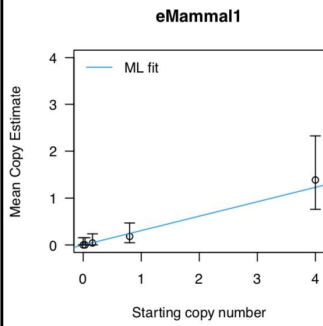
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

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

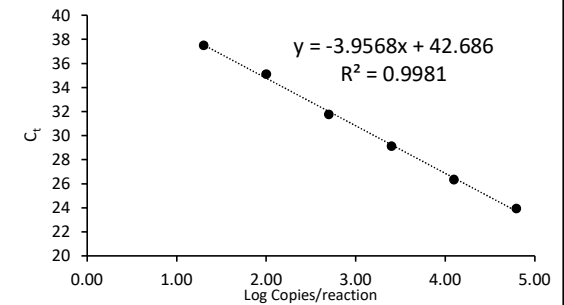
| # Detects | # Copies | SE   |
|-----------|----------|------|
| 0         | 0        | 0    |
| 1         | 0.43     | 0.45 |
| 2         | 0.94     | 0.7  |
| 3         | 1.53     | 0.95 |
| 4         | 2.26     | 1.25 |
| 5         | 3.2      | 1.64 |
| 6         | 4.51     | 2.23 |
| 7         | 6.77     | 3.39 |

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



Field Sample Validation

Known  
Sample Type Presence # Samples Detected Location

| Abbreviations |   |         |  |
|---------------|---|---------|--|
| 95% CI        | 95% Confidence interval                           | LOQ     | Limit of quantification                          |
| eDNA          | Environmental DNA                                 | MT-RNR1 | Mitochondrial 12S ribosomal RNA 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                                   |