



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 sensitivity¹⁻³.

General eDNA Assay Information

Target Species: Oregon Spotted Frog (*Rana pretiosa*)
Species Code: am-RAPR

eDNA qPCR Tool: eRAPR2
eDNA qPCR Format: TaqMan

Gene Target: MT-CYB
Published in: _____

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.5 95% CI 0.4-0.8 Copies/Rxn LOQ 2 95% CI 1.4-3.2 Copies/Rxn LOB 0 hits/8
LOQ_{continuous} 20 Copies/Rxn

Binomial-Poisson model for 8 technical replicates determined using eLowQuant R code⁴. 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 | |
|---------|--|-----------|-----------|--------------------------|
| | | | Specimens | Sample Sources/Locations |
| am-ANBO | Western Toad (<i>Anaxyrus (Bufo) boreas</i>) | No | 1 | Northwest Territories |
| am-ASMO | Rocky Mountain Tailed Frog (<i>Ascaphus montanus</i>) | No | 1 | British Columbia |
| am-LICA | Bullfrog (<i>Lithobates (Rana) catesbeiana</i>) | No | 1 | British Columbia |
| am-PSRE | Pacific Chorus Frog (<i>Pseudacris (Hyla) regilla</i>) | No | 1 | British Columbia |
| am-RAAU | Northern Red-legged Frog (<i>Rana aurora</i>) | No | 1 | British Columbia |
| am-RACA | Cascades Frog (<i>Rana cascadae</i>) | No | 1 | British Columbia |
| am-RALU | Columbia Spotted Frog (<i>Rana luteiventris</i>) | Yes* | 1 | British Columbia |
| am-RAPR | Oregon Spotted Frog (<i>Rana pretiosa</i>) | Yes | 1 | British Columbia |
| am-TAGR | Rough-skinned Newt (<i>Taricha granulosa</i>) | No | 1 | British Columbia |
| ma-HOSA | Human (<i>Homo sapiens</i>) | No | 1 | Netherlands |

References

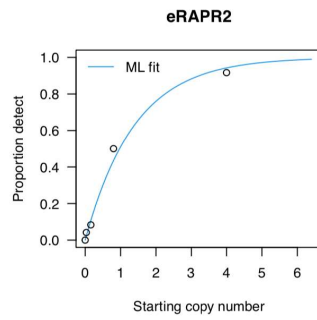
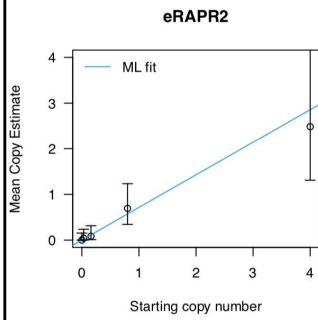
- Hobbs, J, Adams, IT, Round, JM, Goldberg, CS, Allison, MJ, Bergman, LC, Mirabzadeh, A, Allen, H, Helbing, CC (2020) Revising the range of Rocky Mountain tailed frog, *Ascaphus montanus*, in British Columbia, Canada, using environmental DNA methods. Environmental DNA, 2: 350-361. <https://doi.org/10.1002/edn3.82>
- 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

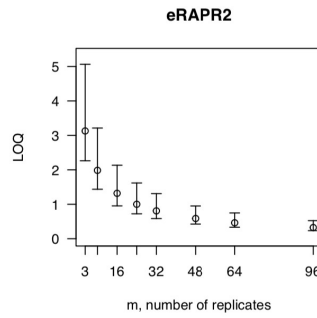
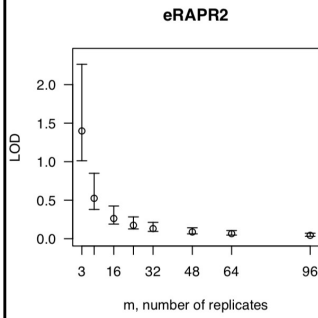
To calculate tables for different numbers of replicates, raw csv data files can be accessed here:
<https://onlineacademiccommunity.uvic.ca/helbinglab/edna/>

From 8 Technical Replicates



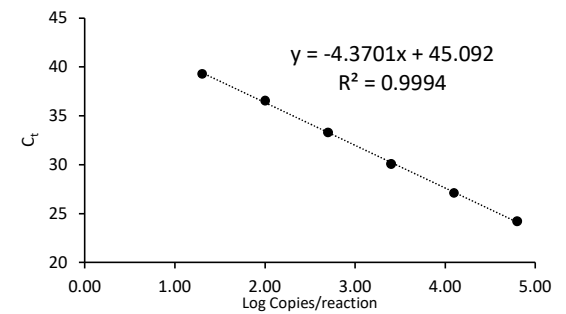
| # Detects | # Copies | SE |
|-----------|----------|------|
| 0 | 0 | 0 |
| 1 | 0.19 | 0.19 |
| 2 | 0.4 | 0.3 |
| 3 | 0.66 | 0.4 |
| 4 | 0.97 | 0.53 |
| 5 | 1.37 | 0.69 |
| 6 | 1.94 | 0.94 |
| 7 | 2.91 | 1.43 |

Determined using eLowQuant R code⁴.



Binomial-Poisson model: No intercept
Determined using eLowQuant R code⁴.
Based on a 2 µL DNA input in a total 15 µL reaction

Applied to reactions with ≥ 95% positive hits



Efficiency 69%

Field Sample Validation

Known
Sample Type Presence # Samples Detected Location

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