



### 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: Cascades Frog (*Rana cascadae*) eDNA qPCR Tool: eRACA2 Gene Target: MT-CYB  
Species Code: am-RACA eDNA qPCR Format: TaqMan Published in: \_\_\_\_\_

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

LOD 0.6 95% CI 0.4-1 Copies/Rxn LOQ 2.2 95% CI 1.6-3.8 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: QIAcuity

#### 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 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> )	Yes	1	British Columbia
am-RALU	Columbia spotted frog ( <i>Rana luteiventris</i> )	No	1	British Columbia
am-RAPR	Oregon Spotted Frog ( <i>Rana pretiosa</i> )	No	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



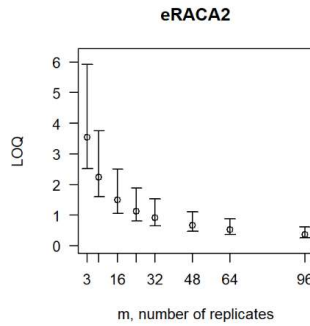
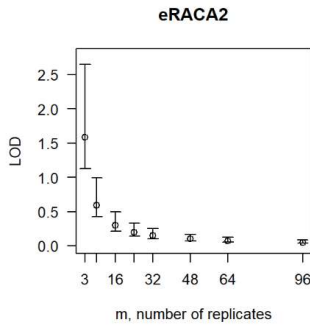
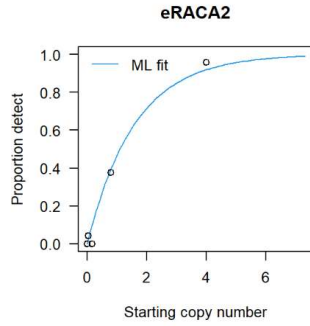
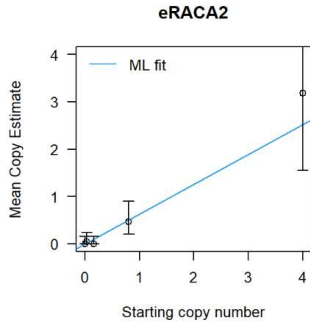
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

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

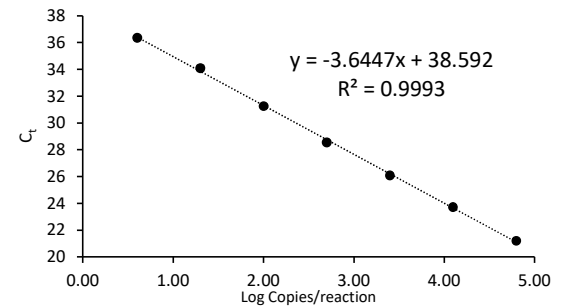
# Detects	# Copies	SE
0	0	0
1	0.21	0.22
2	0.46	0.34
3	0.75	0.46
4	1.1	0.6
5	1.56	0.79
6	2.2	1.07
7	3.3	1.63

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 ≥ 95% positive hits



Efficiency 88%

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