



### 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: Columbia Spotted Frog (*Rana luteiventris*) eDNA qPCR Tool: eRALU2 Gene Target: MT-ND1  
Species Code: am-RALU eDNA qPCR Format: TaqMan Published in:

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

LOD 0.9 95% CI 0.6-1.5 Copies/Rxn LOQ 3.4 95% CI 2.4-5.6 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-AMGR	Northwestern Salamander ( <i>Ambystoma gracile</i> )	36%	1		British Columbia
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-ENES	Ensatina ( <i>Ensatina eschscholtzii</i> )	16%	1		British Columbia
am-LICA	Bullfrog ( <i>Lithobates (Rana) catesbeiana</i> )	No	1		British Columbia
am-LICL	Green Frog ( <i>Lithobates (Rana) clamitans</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> )	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



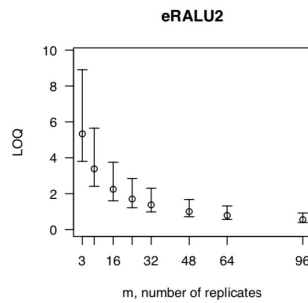
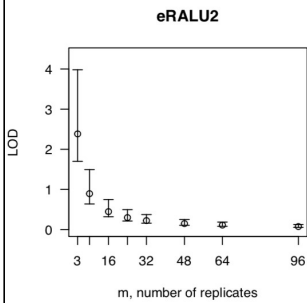
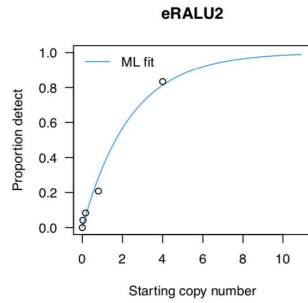
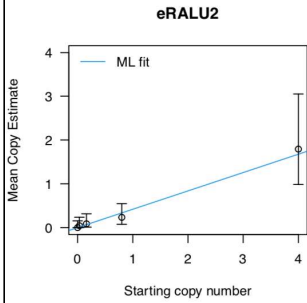
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

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

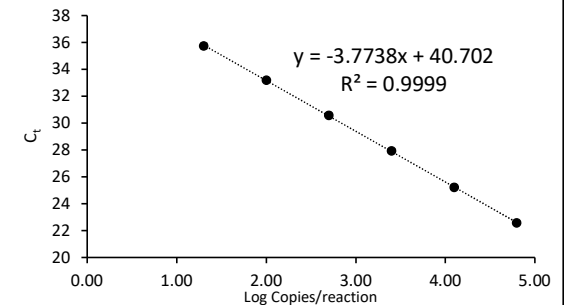
From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.32	0.32
2	0.69	0.51
3	1.12	0.69
4	1.65	0.01
5	2.34	1.19
6	3.31	1.61
7	4.96	2.54

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



Applied to reactions with  $\geq 95\%$  positive hits



Efficiency 84%

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

Field Sample Validation

Sample Type	Known		
	Presence	# Samples	Detected Location

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-ND1	Mitochondrial NADH dehydrogenase 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