



### 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: North American Bullfrog (*Lithobates [Rana] catesbeiana*) eDNA qPCR Tool: eLICAS Gene Target: MT-ND5  
Species Code: am-LICA eDNA qPCR Format: TaqMan Published in:

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

LOD 0.6 95% CI 0.4-1.5 Copies LOQ 2.4 95% CI 1.5-5.5 Copies 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 (Species)	Detection	# Voucher		Sample Sources/Locations
			Specimens		
am-LICA	Bullfrog ( <i>Lithobates [Rana] catesbeiana</i> )	Yes	5		British Columbia
am-ANBO	Western Toad ( <i>Anaxyrus [Bufo] boreas</i> )	No	2		Northwest Territories
am-LICL	Green frog ( <i>Lithobates [Rana] clamitans</i> )	No	1		British Columbia
am-LIPI	Northern leopard frog ( <i>Lithobates [Rana] pipiens</i> )	No	2		Alberta
am-LISY	Wood frog ( <i>Lithobates [Rana] sylvaticus</i> )	No	1		British Columbia
am-PSRE	Pacific chorus frog ( <i>Pseudacris [Hyla] regilla</i> )	No	1		British Columbia
am-PSMA	Boreal chorus frog ( <i>Pseudacris maculata</i> )	No	1		Ontario
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> )	No	1		British Columbia
am-RAPR	Oregon spotted frog ( <i>Rana pretiosa</i> )	No	1		British Columbia
am-SPIN	Great basin spadefoot toad ( <i>Spea [Scaphiopus] intermontana</i> )	No	1		British Columbia
am-XELA	African clawed frog ( <i>Xenopus laevis</i> )	No	1		South Africa
am-AMGR	Northwestern salamander ( <i>Ambystoma gracile</i> )	No	1		British Columbia
am-AMMA	Long toed salamander ( <i>Ambystoma macrodactylum</i> )	No	1		British Columbia
am-AMMV	Eastern tiger salamander ( <i>Ambystoma mavortium</i> )	No	1		British Columbia
am-ANBO	Western toad ( <i>Anaxyrus [Bufo] boreas</i> )	No	1		British Columbia
am-ANAM	American toad ( <i>Anaxyrus americanus</i> )	No	1		British Columbia
ma-CALUfa	Canine ( <i>Canis lupus familiaris</i> )	No	1		British Columbia
ma-FECA	Cat ( <i>Felis catus</i> )	No	1		British Columbia
ma-HOSA	Human ( <i>Homo sapiens</i> )	No	1		Netherlands

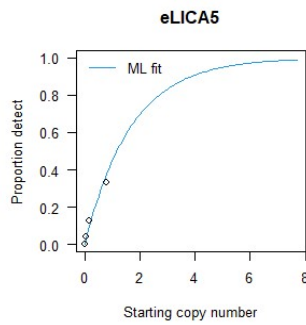
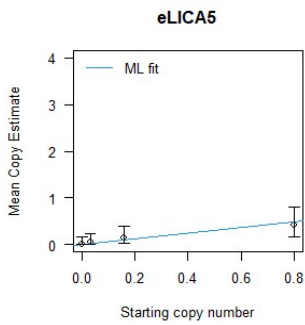
#### References

- 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**

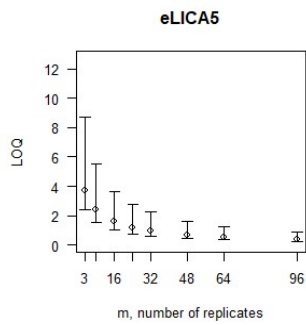
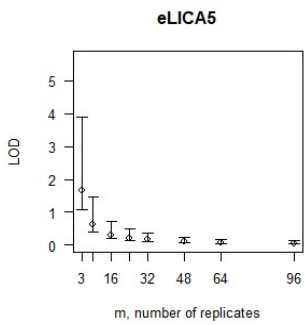
To calculate tables for different numbers of replicates, raw csv data files can be accessed here:  
<https://onlineacademiccommunity.uvic.ca/helbinglab/edna/>

From 8 Technical Replicates

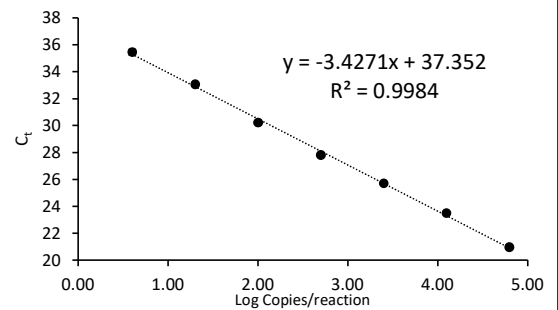


# Detects	# Copies	SE
0	0	0
1	0.22	0.24
2	0.49	0.39
3	0.81	0.55
4	1.20	0.74
5	1.71	0.99
6	2.42	1.36
7	3.63	2.07

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



Applied to reactions with 100% positive hits



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

Efficiency 96%

**Field Sample Validation**

Sample Type	Known Presence	# Samples	Detected	Location
Freshwater	Y	1	Y	Victoria, BC

**Abbreviations**

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-ND5	Mitochondrial NADH dehydrogenase subunit 5 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