



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: Boreal chorus frog (*Pseudacris maculata*) & Western Chorus Frog (*Pseudacris triseriata*) qPCR Tool: ePSpp2 Gene Target: MT-RNR2
Species Code: am-PSMA and am-PSTR eDNA qPCR Format: TaqMan Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.6 95% CI 0.4-1.2 Copies/Rxn LOQ 2.1 95% CI 1.4-4.7 Copies/Rxn LOB 0 hits/8
LOQ_{continuous} 4 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: QIAcuity

eDNA Assay Specificity Test Information

Each qPCR reaction in the specificity assay contained 10 picograms of voucher target gDNA (n=25 technical replicates)

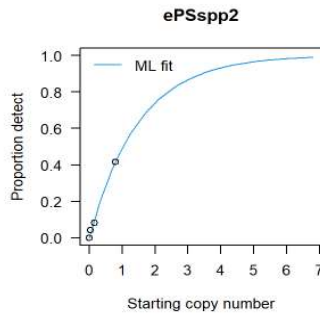
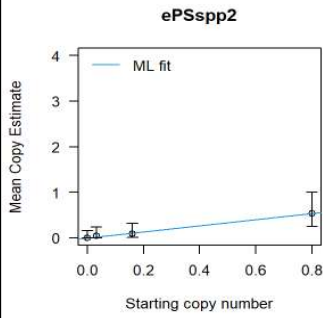
Species	Common Name (<i>Species</i>)	Detection	# Voucher		Sample Sources/Locations
			Specimens		
am-AMTI	Tiger salamander (<i>Ambystoma tigrinum</i>)	No	1		British Columbia
am-ANBO	Western Toad (<i>Anaxyrus (Bufo) boreas</i>)	No	2		British Columbia and Northwest Territories
am-LICA	Bullfrog (<i>Lithobates (Rana) catesbeiana</i>)	No	1		British Columbia
am-LICL	Green Frog (<i>Lithobates (Rana) clamitans</i>)	No	1		British Columbia
am-LIPI	Northern Leopard Frog (<i>Lithobates (Rana) pipiens</i>)	No	2		Alberta
am-LISY	Wood Frog (<i>Lithobates sylvaticus</i>)	No	2		British Columbia
am-PSMA	Boreal chorus frog (<i>Pseudacris maculata</i>)	Yes	7		Ontario
am-RAPR	Oregon Spotted Frog (<i>Rana pretiosa</i>)	No	1		British Columbia
am-SPIN	Great Basin Spadefoot (<i>Spea intermontana</i>)	No	1		British Columbia
ma-CALUfa	Canine (<i>Canis lupus familiaris</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 (2021) 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

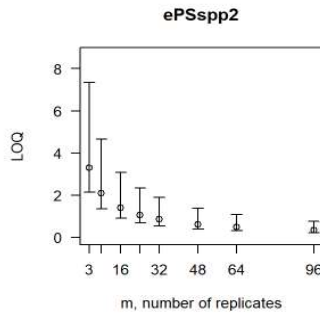
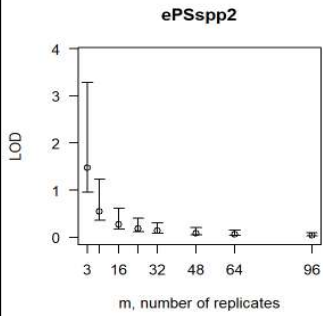


To calculate tables for different numbers of replicates, raw csv data files can be accessed here:
<https://onlineacademiccommunity.uvic.ca/helbinglab/edna/>

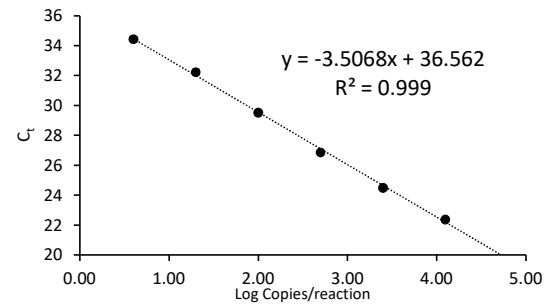
From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.2	0.21
2	0.43	0.33
3	0.69	0.45
4	1.02	0.61
5	1.45	0.81
6	2.05	1.11
7	3.09	1.71

Determined using eLowQuant R code⁴.



Applied to reactions with 100% positive hits



Efficiency 93%

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

Field Sample Validation

Sample Type	Known		Detected	Location
	Presence	# Samples		

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-RNR2	Mitochondrial 16S 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