

**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: Blanding's turtle (*Emydoidea blandingii*) eDNA qPCR Tool: eEMBL4 Gene Target: MT-ND5
 Species Code: re-EMBL eDNA qPCR Format: TaqMan Published in: _____

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 1 95% CI 0.7-1.6 Copies/Rxn LOQ 3.7 95% CI 2.6-6.1 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: Qiacity**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		
ma-CALUfa	Domestic dog (<i>Canis lupus familiaris</i>)	No	1		British Columbia
ma-FECA	Domestic cat (<i>Felis catus</i>)	No	1		British Columbia
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1		Netherlands
re-ASSE	Six-lined racerunner (<i>Aspidoscelis sexlineatus</i>)	No	1		Texas
re-CHBO	Rubber boa (<i>Charina bottae</i>)	No	1		British Columbia
re-CHPI	Painted turtle (<i>Chrysemys picta</i>)	No	1		Ontario
re-CHSE	Snapping turtle (<i>Chelydra serpentina</i>)	No	1		Ontario
re-CLGU	Spotted turtle (<i>Clemmys guttata</i>)	No	1		Ontario
re-COCO	Western yellow-bellied racer (<i>Coluber constrictor</i>)	No	1		British Columbia
re-COTE	Sharp-tailed snake (<i>Contia tenuis</i>)	No	1		British Columbia
re-ELCO	Alligator lizard (<i>Elgaria coerulea</i>)	No	1		British Columbia
re-EMBL	Blanding's turtle (<i>Emydoidea blandingii</i>)	Yes	3		Ontario
re-GLIN	Wood turtle (<i>Glyptemys insculpta</i>)	No	1		Nova Scotia
re-GRGE	Nothern map turtle (<i>Graptemys geographica</i>)	No	1		Ontario
re-HOLA	Plateau spot-tailed earless lizard (<i>Holbrookia lacerata</i>)	No	1		Texas
re-HOSU	Tamaulipin spot-tailed earless lizard (<i>Holbrookia subcaudalis</i>)	No	1		Texas
re-POMU	Common wall lizard (<i>Podarcis muralis</i>)	No	1		British Columbia
re-SCOL	Texas spiny lizard (<i>Sceloporus olivaceus</i>)	No	2		Texas
re-STOD	Eastern musk turtle (<i>Sternotherus odoratus</i>)	No	1		Ontario
te-CLPA	Pacific herring (<i>Clupea pallasii</i>)	No	1		British Columbia

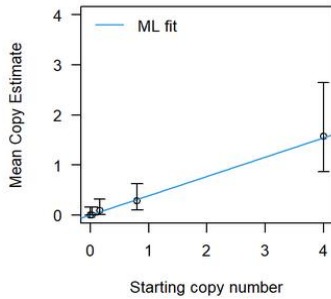
References

- 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

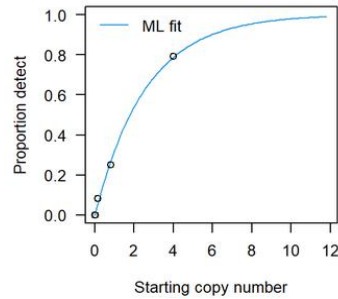
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

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

eEMBL4



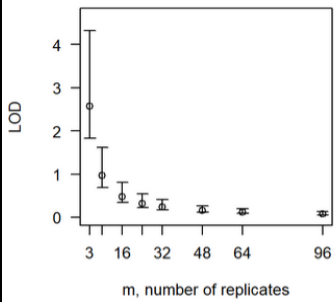
No intercept eEMBL4



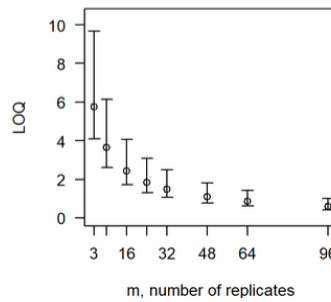
From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.345	0.352
2	0.743	0.549
3	1.213	0.749
4	1.789	0.984
5	2.531	1.288
6	3.578	1.744
7	5.364	2.654

Limits detect - no intercept eEMBL4

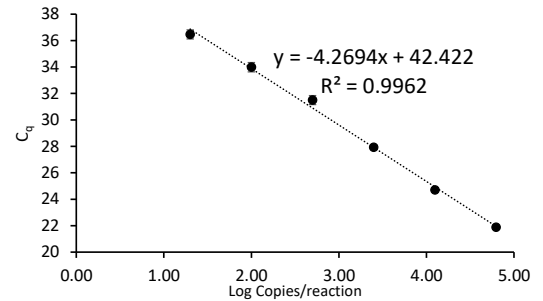


Limits quant - no intercept eEMBL4



Determined using eLowQuant R code⁴.

Applied to reactions with ≥ 95% positive hits



Efficiency 71%

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-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