Helbing/Langlois Lab
eDNA Inventory

Helbing/Langlois 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[1-3].

General eDNA Assay Information

Target Species: Cutlip minnow (*Exoglossum maxillingua*)	eDNA qPCR Tool: eEXMA1	Gene Target: MT-ND1
Species Code: te-EXMA	eDNA qPCR Format: TaqMan	Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD __0.4__   95% CI __0.7-1.2__ Copies/Rxn   LOQ __1.7__   95% CI __1.2-2.7__ Copies/Rxn   LOB __0__ hits/8

$LOQ_{continuous}$ __20__ Copies/Rxn

Binomial-Poisson model for 8 technical replicates determined using eLowQuant R code[4].   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)

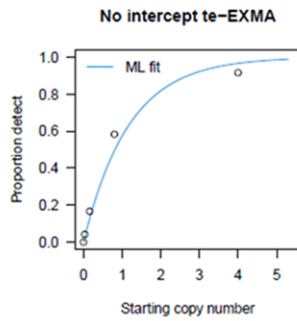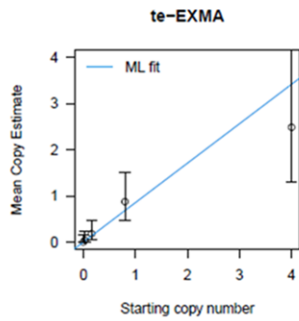| Species	Common Name (*Species*)	Detection	# Voucher Specimens	Sample Sources/Locations
te-EXMA	Cutlip minnow	Yes	4	Canada: Quebec/Ontario
te-PIPR	Fathead minnow	No	2	Canada: Quebec
te-LEGI	Sunfish/pond perch	No	1	Canada: Quebec
te-PEFL	Yellow perch	No	1	Canada: Quebec
te-LEPE	Northern sunfish	No	1	Canada: Quebec
te-LEMA	Bluegill	No	1	Canada: Quebec
ma-CALUfa	Canine (*Canis lupus familiaris*)	No	1	Canada: Quebec
ma-FECA	Cat (*Felis catus*)	No	2	Canada: Quebec
ma-HOSA	Human (*Homo sapiens*)	No	1	Canada: Quebec

References

1. 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

2. 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

3. 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

4. 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
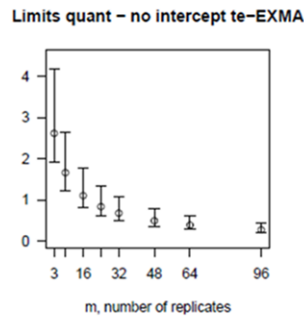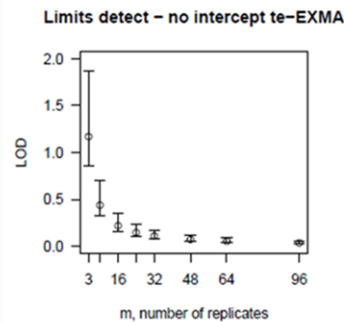
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

te-EXMA

No intercept te-EXMA

To generate tables for different numbers of replicates, use raw csv data files.

From ____8____ Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.155	0.159
2	0.337	0.248
3	0.55	0.337
4	0.821	0.45
5	1.156	0.586
6	1.646	0.803
7	2.52	1.264

Determined using eLowQuant R code[4].

Limits detect – no intercept te-EXMA

Limits quant – no intercept te-EXMA

Applied to reactions with ≥ 95% positive hits

$$y = -3.5781x + 38.984$$
$$R^2 = 0.9994$$

Binomial-Poisson model: ____No intercept____
Determined using eLowQuant R code[4].
Based on a 2 µL DNA input in a total 15 µL reaction

Efficiency	90%

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 1
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