



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 sensitivity¹⁻³.

General eDNA Assay Information

Target Species: Fathead minnow (*Pimephales promelas*) eDNA qPCR Tool: ePIPR2 Gene Target: MT-COX2
Species Code: te-PIPR eDNA qPCR Format: TaqMan Published in: _____

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.5 95% CI 0.1-0.3 Copies/Rxn LOQ 0.7 95% CI 0.5-1.2 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)

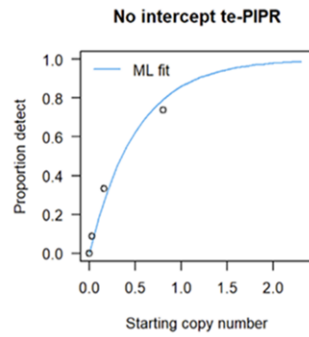
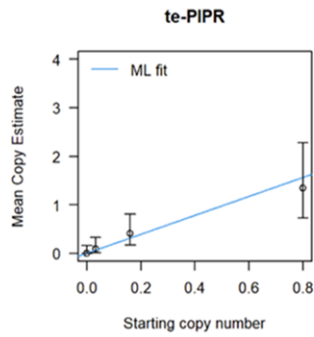
Species	Common Name (<i>Species</i>)	Detection	# Voucher	
			Specimens	Sample Sources/Locations
te-PIPR	Fathead minnow (<i>Pimephales promelas</i>)	Yes	3	Canada: Quebec: INRS Facilities
te-PEFL	Yellow perch (<i>Perca flavescens</i>)	No	2	Canada: Quebec
te-LEPE	Northern sunfish (<i>Lepomis peltastes</i>)	No	2	Canada: Quebec
te-LEGI	Pumpkinseed (<i>Lepomis gibbosus</i>)	No	2	Canada: Quebec
te-LEMA	Bluegill (<i>Lepomis macrochirus</i>)	No	2	Canada: Quebec
te-EXMA	Cutlip minnow (<i>Exoglossum maxillingua</i>)	No	2	Canada: Quebec
ma-CALUfa	Canine (<i>Canis lupus familiaris</i>)	No	2	Canada: Quebec
ma-FECA	Cat (<i>Felis catus</i>)	No	1	Canada: Quebec
ma-HOSA	Human (<i>Homo sapiens</i>)	No	2	Canada: Quebec

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



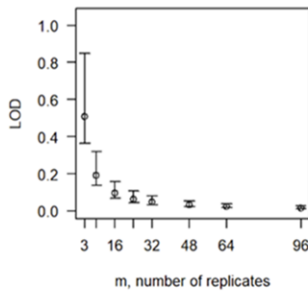
To generate tables for different numbers of replicates, use raw csv data files.

From 8 Technical Replicates

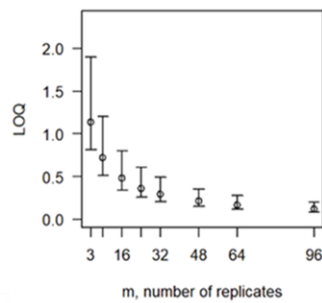
# Detects	# Copies	SE
0	0	0
1	0.077	0.08
2	0.147	0.108
3	0.287	0.181
4	0.363	0.199
5	0.525	0.269
6	0.767	0.382
7	1.167	0.6

Determined using eLowQuant R code⁴.

Limits detect - no intercept te-PIPR



Limits quant - no intercept te-PIPR

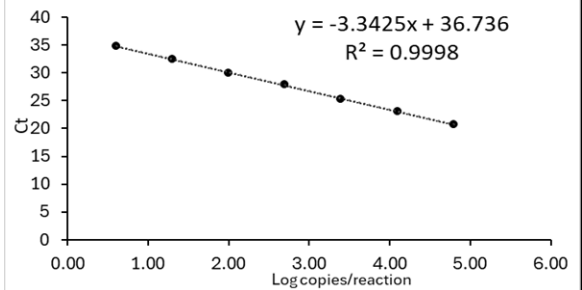


Binomial-Poisson model: No intercept

Determined using eLowQuant R code⁴.

Based on a 2 µL DNA input in a total 15 µL reaction

Applied to reactions with ≥ 95% positive hits



Efficiency 99%

Field Sample Validation

Known
Sample Type Presence # Samples Detected Location

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-COX2	Mitochondrial cyclooxygenase 2
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