



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: Lake sturgeon (*Acipenser fulvescens*)
Species Code: te-ACFU

eDNA qPCR Tool: eACFU1
eDNA qPCR Format: TaqMan

Gene Target: MT-ND1
Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.7 95% CI 0.5-1.2 Copies/Rxn LOQ 2.8 95% CI 2-4.5 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: 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		
te-ACOX	Atlantic sturgeon (<i>Acipenser oxyrinchus</i>)	No	6		Quebec
ma-CALufa	Dog (<i>Canis lupus familiaris</i>)	No	1		Quebec
ma-FECA	Cat (<i>Felis catus</i>)	No	2		Quebec
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1		Cell line ATCC
te-ACFU	Lake sturgeon (<i>Acipenser fulvescens</i>)	Yes	6		Quebec

References

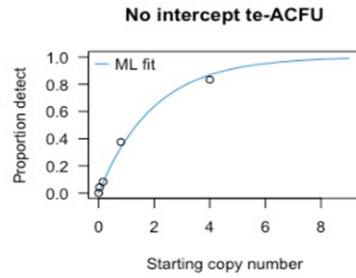
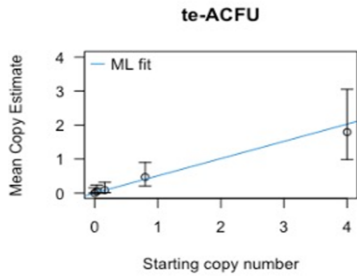
- Hobbs, J, Adams, IT, Round, JM, Goldberg, CS, Allison, MJ, Bergman, LC, Mirabzadeh, A, Allen, H, Helbing, CC (2020) Revising the range of Rocky Mountain tailed frog, *Ascaphus montanus*, in British Columbia, Canada, using environmental DNA methods. *Environmental DNA*, 2: 350-361. <https://doi.org/10.1002/edn3.82>
- 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

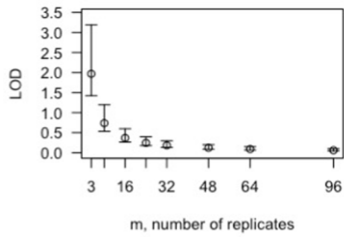
To generate tables for different numbers of replicates, use raw csv data files.

From 8 Technical Replicates

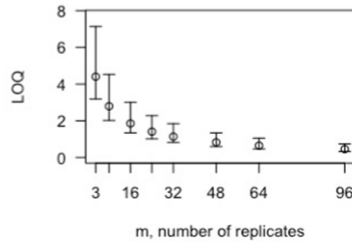


# Detects	# Copies	SE
0	0	0
1	0.263	0.269
2	0.568	0.418
3	0.927	0.57
4	1.367	0.747
5	1.934	0.976
6	2.734	1.32
7	4.101	2.011

Limits detect - no intercept te-ACFU

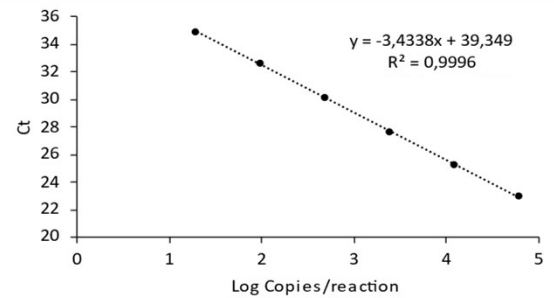


Limits quant - no intercept te-ACFU



Determined using eLowQuant R code⁴.

Applied to reactions with ≥ 95% positive hits



Efficiency 96%

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		
Water	Y	50	Y	Rivière Chaudière, Quebec
Water	Y	2	Y	Rivière Maicasagi, Quebec

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