



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*) eDNA qPCR Tool: eACFU1 Gene Target: MT-ND1
Species Code: te-ACFU eDNA qPCR Format: TaqMan Published in: _____

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.7 95% CI 0.5-1.1 Copies/Rxn LOQ 2.6 95% CI 1.9-4.1 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: QIAcuity

eDNA Assay Specificity Test Information

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

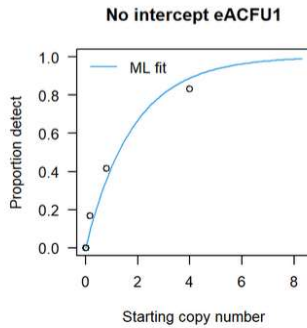
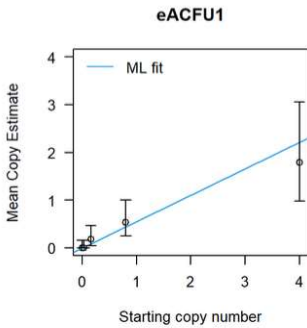
Species	Common Name (<i>Species</i>)	Detection	# Voucher		Sample Sources/Locations
			Specimens		
ma-CALUfa	Dog (<i>Canis lupus familiaris</i>)	No	2		British Columbia, Quebec
ma-FECA	Cat (<i>Felis catus</i>)	No	3		British Columbia, Quebec
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1		Netherlands
te-ACFU	Lake sturgeon (<i>Acipenser fulvescens</i>)	Yes	6		Quebec
te-ACME	Green Sturgeon (<i>Acipenser medirostris</i>)	No	2		California
te-ACTR	White sturgeon (<i>Acipenser transmontanus</i>)	No	2		British Columbia
te-CACAch	Salish sucker (<i>Catostomus catostomus chehalis</i>)	No	1		Alberta
te-CACO	White sucker (<i>Castostomus commersonii</i>)	No	1		Ontario
te-COAR	Cisco/Tullibee (<i>Coregonus artedi</i>)	No	1		British Columbia
te-COCL	Lake whitefish (<i>Coregonus clupeaformis</i>)	No	1		Quebec
te-ESLU	Northern pike (<i>Esox lucius</i>)	No	1		British Columbia (RIC-22-003)
te-LOLO	Burbot (<i>Lota lota</i>)	No	1		Yukon
te-ONCLle	Westslope cutthroat trout (<i>Oncorhynchus clarkii lewisi</i>)	No	1		Alberta
te-ONKE	Chum salmon (<i>Oncorhynchus keta</i>)	No	1		British Columbia
te-ONKI	Coho salmon (<i>Oncorhynchus kisutch</i>)	No	1		British Columbia
te-ONMY	Rainbow (steelhead) trout (<i>Oncorhynchus mykiss</i>)	No	1		Alberta
te-ONNE	Sockeye salmon (<i>Oncorhynchus nerka</i>)	No	1		British Columbia
te-ONTS	Chinook salmon (<i>Oncorhynchus tshawytscha</i>)	No	1		British Columbia
te-SACO	Bull trout (<i>Salvelinus confluentus</i>)	No	1		British Columbia
te-SAFO	Brook trout (<i>Salvelinus fontinalis</i>)	No	1		Alberta
te-SANA	Lake trout (<i>Salvelinus namaycush</i>)	No	1		Alberta
te-SASA	Atlantic salmon (<i>Salmo salar</i>)	No	1		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

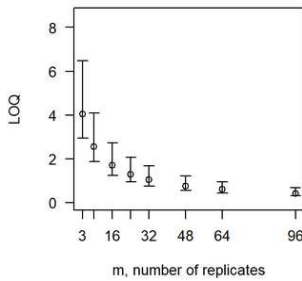


To generate tables for different numbers of replicates, use raw csv data files.

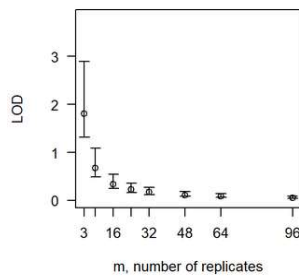
From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.242	0.246
2	0.521	0.383
3	0.851	0.522
4	1.254	0.683
5	1.775	0.893
6	2.509	1.208
7	3.763	1.839

Limits quant - no intercept eACFU1

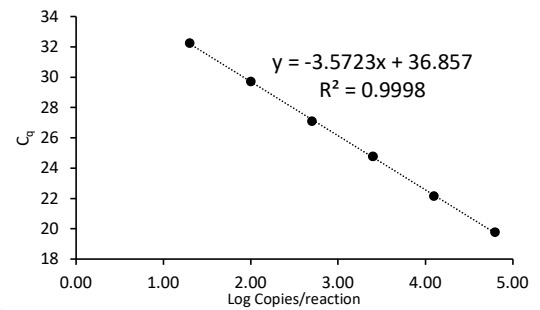


Limits detect - no intercept eACFU1



Determined using eLowQuant R code⁴.

Applied to reactions with $\geq 95\%$ positive hits



Binomial-Poisson model: No intercept

Determined using eLowQuant R code⁴.

Based on a 2 μ L DNA input in a total 15 μ L reaction

Efficiency 93%

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