Helbing Lab

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 sensitivity[1-3].

General eDNA Assay Information

Target Species: White sturgeon (*Acipenser transmontanus*) | eDNA qPCR Tool: eACTR4 | Gene Target: MT-Dloop
Species Code: te-ACTR | eDNA qPCR Format: TaqMan | Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.4 95% CI 0.3-0.8 Copies/Rxn LOQ 1.5 95% CI 1-2.8 Copies/Rxn LOB 0 hits/8
$LOQ_{continuous}$ 4 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
ma-CALUfa	Domestic dog (*Canis lupus familiaris*)	No	1	British Columbia
ma-FECA	Domestic cat (*Felis catus*)	No	1	British Columbia
ma-HOSA	Human (*Homo sapiens*)	No	1	Netherlands
te-ACFU	Lake sturgeon/yellow sturgeon (*Acipenser fulvescens*)	Yes*	2	Quebec
te-ACME	Green sturgeon (*Acipenser medirostris*)	No	3	California
te-ACTR	ı (*Acipenser transmontanus*)	Yes	15	British Columbia (Upper and Lower Fraser River, Nechako River, Columbia River, Kootenay Lake)
te-CAAU	Goldfish (*Carassius auratus*)	No	1	British Columbia
te-CACA	Longnose sucker (*Catostomus catostomus*)	No	1	British Columbia
te-CACAch	Salish sucker (*Catostomus catostomus (chehalis)*)	No	1	British Columbia
te-CACO	White sucker (*Catostomus commersonii*)	No	1	Ontario
te-CAMA	Largescale sucker (*Catostomus macrocheilus*)	No	1	Ontario
te-CLPA	Pacific herring (*Clupea pallasi*)	No	1	British Columbia
te-COAR	Cisco/Tubilee (*Coregonus artedi*)	No	1	Alberta
te-COCL	Lake whitefish (Coregonus clupeaformis)	No	1	Alberta
te-COCO	Slimy sculpin (*Cottus cognatus*)	No	1	British Columbia
te-ESLU	Northern pike (*Esox lucius*)	No	1	British Columbia
te-GAAC	Threespine stickleback (*Gasterosteus aculeatus*)	No	1	British Columbia
te-HYPR	Hypomesus pretiosus (*Hypomesus pretiosus*)	No	1	British Columbia
te-LEGI	Pumpkinseed (*Lepomis gibbosus*)	No	1	British Columbia
te-LOLO	Burbot (*Lota lota*)	No	1	Alberta
te-MIDO	Smallmouth bass (*Micropterus dolomieu*)	No	1	British Columbia
te-MISA	Peamouth chub (*Mylocheilus caurinus*)	No	1	British Columbia
te-ONCLle	Westslope cutthroat trout (*Oncorhynchus clarkii lewisi*)	No	1	Alberta
te-ONGO	Pink salmon (*Oncorhynchus gorbuscha*)	No	1	British Columbia
te-ONKE	Chum salmon (*Oncorhynchus keta*)	No	1	British Columbia
te-ONKI	Coho salmon (*Oncorhynchus kisutch*)	No	1	British Columbia
te-ONMY	Rainbow (steelhead) trout (*Oncorhynchus mykiss*)	No	1	Alberta
te-ONNE	Sockeye salmon (*Oncorhynchus nerka*)	No	1	British Columbia
te-ONTS	Chinhook salmon (*Oncorhynchus tschawytscha*)	No	1	British Columbia
te-PEFL	Yellow perch (*Perca flavescens*)	No	1	Quebec
te-PRWI	Mountain whitefish (*Prosopium williamsoni*)	No	1	Alberta
te-PTOR	Northern pikeminnow (*Ptychocheilus oregonensis*)	No	1	Ontario
te-RHCA	Longnose (nooksack) dace (*Rhinichthys cataractae*)	No	1	Ontario
te-SACO	Bull trout (*Salvelinus confluentus*)	No	1	Alberta
te-SAFO	Brook trout (*Salvelinus fontinalis)*	No	1	Alberta
te-SAMA	Dolly varden (*Salvelinus malma*)	No	1	British Columbia
te-SANA	Lake trout (*Salvelinus namaycush*)	No	1	Alberta
te-SPTH	Longfin smelt (*Spirinchus thaleichthys*)	No	1	Washington
te-THPA	Oolichan/Eulachon (*Thaleichthys pacificus*)	No	1	British Columbia

***1/25 replicates obtained a positive hit**

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

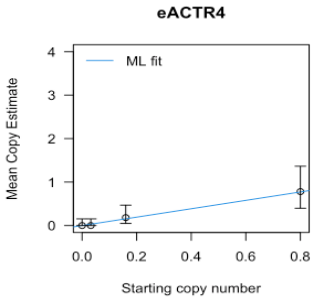
2. Hobbs, J, Round, JM, Allison, MJ, Helbing, CC (2019) Expansion of the known distribution of the coastal tailed frog, *Ascaphus truei*, in British Columbia, Canada, using robust eDNA detection methods. PLOS ONE 14(3): e0213849. https://doi.org/10.1371/journal.pone.0213849

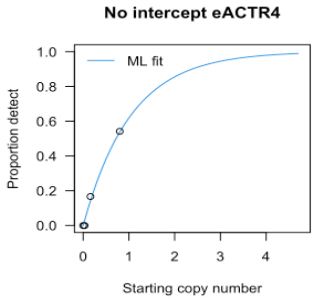
3. Langlois, VS, Allison, MJ, Bergman, LC, To, TA, and Helbing, CC (2020) The need for robust qPCR-based eDNA detection assays in environmental monitoring and risk assessments. Environmental DNA, 3: 519-527. doi: 10.1002/edn3.164

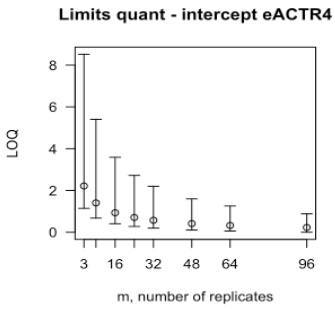
4. Lesperance, M, Allison, MJ, Bergman, LC, Hocking, MD, and Helbing, CC (2021) A statistical model for calibration and computation of detection and quantification limits for low copy number environmental DNA samples. Environmental DNA, 3: 970-981. doi: 10.1002/edn3.220

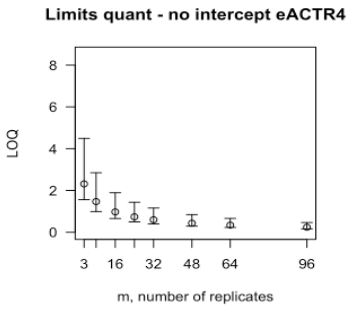
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

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







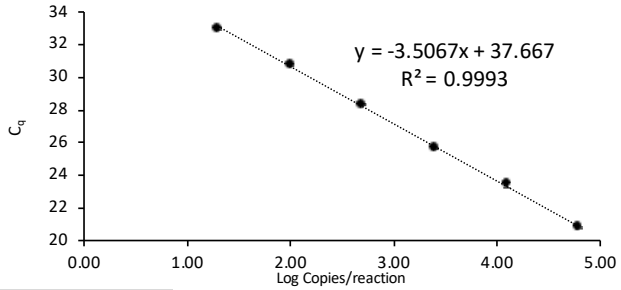


From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.138	0.143
2	0.298	0.224
3	0.487	0.308
4	0.718	0.407
5	1.016	0.536
6	1.437	0.727
7	2.155	1.106

Determined using eLowQuant R code[4].

Applied to reactions with ≥95% positive hits



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 93%

Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-Dloop	Mitochondrial displacement loop (D-loop) 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