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: *Salish Sucker (Catostomus sp. cf. catostomus)*	eDNA qPCR Tool: eCACA2 Gene Target: MT-CYB
Species Code: te-CACA	eDNA qPCR Format: TaqMan Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD   1   95% CI   0.7-1.7   Copies/Rxn   LOQ   3.8   95% CI   2.7-6.5   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
am-LICA	American Bullfrog (*Lithobates (Rana) catesbeiana*)	No	2	British Columbia
ma-HOSA	Human (*Homo sapiens*)	No	1	Netherlands
te-CAAU	Goldfish (*Carassius auratus*)	No	2	British Columbia
te-CACAch	Salish Sucker (*Catostomus sp. cf. catostomus*)	Yes	8	British Columbia
te-COCO	Slimy Sculpin (*Cottus cognatus*)	No	2	British Columbia
te-ESLU	Northern Pike (*Esox lucius*)	No	1	British Columbia
te-GAAC	Three Spine Stickleback (*Gasterosteus aculeatus*)	No	1	British Columbia
te-LEGI	Pumpkinseed Sunfish (*Lepomis gibbosus*)	No	2	British Columbia
te-ONCLcl	Coastal Cutthroat Trout (*Oncorhynchus clarkii clarkii*)	No	1	British Columbia
te-ONKI	Coho Salmon (*Oncorhynchus kisutch*)	No	1	British Columbia
te-ONMY	Rainbow Trout (*Oncorhynchus mykiss*)	No	1	British Columbia
te-SAMA	Dolly Varden (*Salvelinus malma*)	No	1	British Columbia

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

eCACA2

No intercept eCACA2

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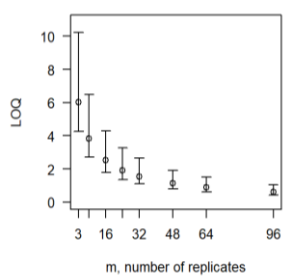https://onlineacademiccommunity.uvic.ca/helbinglab/edna/

From ___8___ Technical Replicates
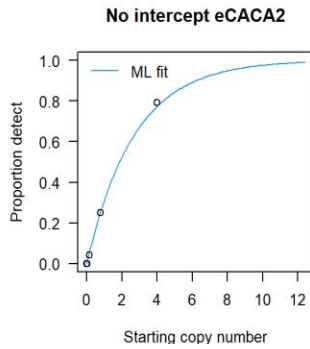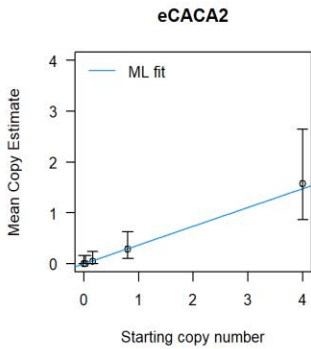
# Detects	# Copies	SE
0	0	0
1	0.36	0.37
2	0.78	0.57
3	1.27	0.79
4	1.87	1.03
5	2.65	1.35
6	3.74	1.83
7	5.61	2.78

Determined using eLowQuant R code[4].
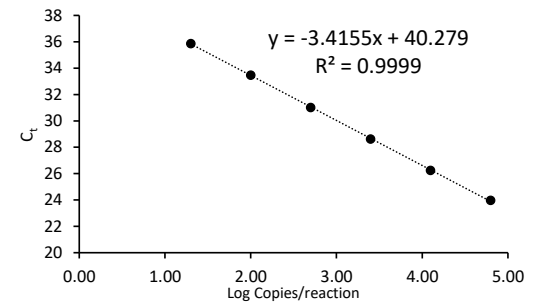
Limits detect - no intercept eCACA2
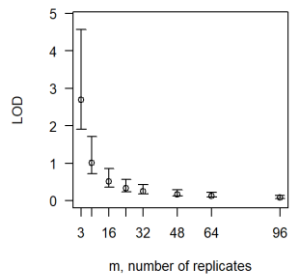
Limits quant - no intercept eCACA2

Applied to reactions with ≥ 95% positive hits

$y = -3.4155x + 40.279$
$R^2 = 0.9999$

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   96%

Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location
Water	Y	3	Y	Manual Pond, British Columbia
Water	Y	1	Y	Agassiz Slough, British Columbia

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-CYB	Mitochondrial cytochrome B 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