Helbing Lab
eDNA Inventory

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: Asian Clam *(Corbicula fluminea)*
Species Code: mo-COFL
eDNA qPCR Tool: eCOFL13
eDNA qPCR Format: TaqMan
Gene Target: MT-RNR2
Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.3 95% CI 0.2-0.4 Copies/Rxn
LOQ 1 95% CI 0.7-1.6 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: Immolase

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	Canine *(Canis lupus familiaris)*	No	1	British Columbia
ma-FECA	Cat *(Felis catus)*	No	1	British Columbia
ma-HOSA	Human *(Homo Sapiens)*	No	1	Netherlands
mo-COFL	Asian Clam *(Corbicula fluminea)*	Yes	8	Ontario and British Columbia
mo-DRBU	Quagga Mussel *(Dreissena bugensis)*	No	6	Ontario
mo-DRPO	Zebra Mussel *(Dreissena polymorpha)*	No	6	Ontario
mo-CRGI	Pacific oyster (Crassostrea gigas)	No	3	British Columbia
mo-ANCA	California Floater (Anodonta californiensis)	No	3	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. 2020; 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 (2021) 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

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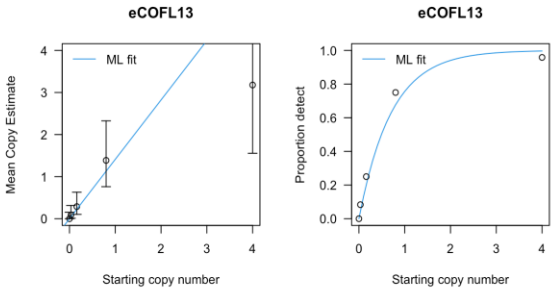
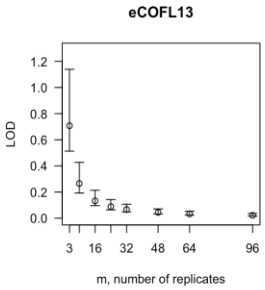
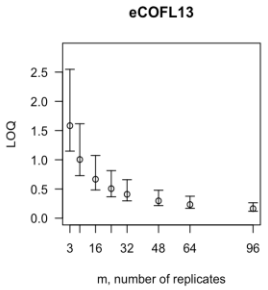
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA





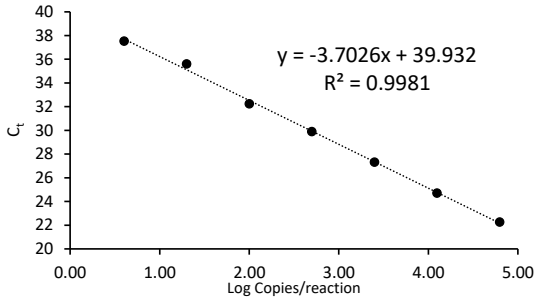


From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.095	0.096
2	0.2	0.15
3	0.33	0.2
4	0.49	0.27
5	0.7	0.35
6	0.98	0.47
7	1.47	0.72

Determined using eLowQuant R code[4].

Applied to reactions with 100% positive hits



Efficiency 86%

Binomial-Poisson model: No intercept
Determined using eLowQuant R code[4].
Based on a 2 µL DNA input in a total 15 µL reaction

Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location
Water	Yes	3	Yes	British Columbia
Water	Yes	1	Yes	British Columbia

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-RNR2	Mitochondrial 16S 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