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: Ophelina (*Ophelina acuminata*)   eDNA qPCR Tool: eOPAC1   Gene Target: MT-ND2

Species Code: an-OPAC   eDNA qPCR Format: TaqMan   Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD   0.5   95% CI   0.4-0.9   Copies/Rxn   LOQ   2   95% CI   1.4-3.3   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
an-CACAW	Capitella (*Capitella capitata*)	No	2	Netherlands
an-DEGR	Decamastus (*Decamastus gracilis*)	No	2	British Columbia
an-GLNA	Glycera (*Glycera nana*)	No	2	British Columbia
an-HEFI	Heteromastus (*Heteromastus filobranchus*)	No	2	British Columbia
an-MASA	Maldane (*Maldane sarsi*)	No	2	British Columbia
an-NOHE	Notomastus (*Notomastus hemipodus*)	No	2	British Columbia
an-OPAC	Ophelina (*Ophelina acuminata*)	Yes	5	British Columbia
an-PRJU	Prionospio (*Prionospio jubata*)	No	2	British Columbia
an-PRLI	Prionospio [*Prionospio (Minuspio) lighti*]	No	2	British Columbia
an-PRPA	Praxillela (*Praxillella pacifica*)	No	1	British Columbia
an-RIPI	Hydrothermal vent worm (*Ridgeia piscesae*)	No	2	British Columbia
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
si-THNI	Segmented marine worm (*Thysanocardia nigra*)	No	2	Alberta

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
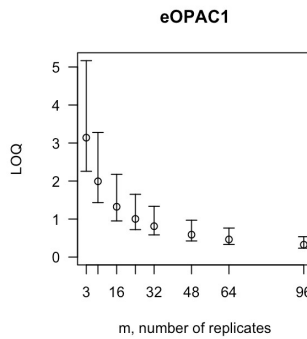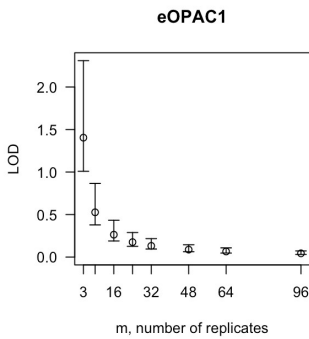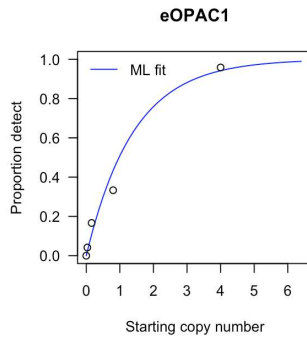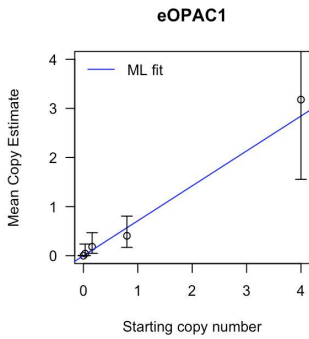
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

eOPAC1

eOPAC1

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	0.19	0.19
2	0.41	0.3
3	0.66	0.41
4	0.98	0.53
5	1.38	0.7
6	1.95	0.95
7	2.92	1.44

Determined using eLowQuant R code[4].

eOPAC1

eOPAC1

Applied to reactions with 100% positive hits

$y = -3.4653x + 37.707$
$R^2 = 0.9995$

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   94%
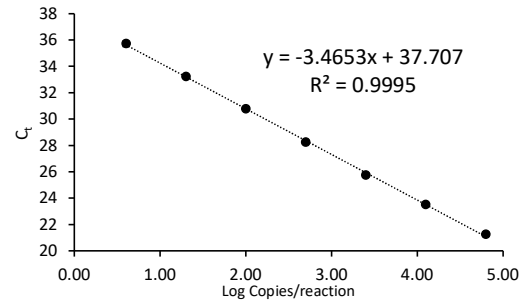
Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-ND2	Mitochondrial NADH dehydrogenase subunit 2 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