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: Segmented marine worm (*Thysanocardia nigra*)
Species Code: si-THNI
eDNA qPCR Tool: eTHNI3
eDNA qPCR Format: TaqMan
Gene Target: MT-ND1
Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.3 95% CI 0.2-0.6 Copies/Rxn LOQ 1.3 95% CI 0.9-2.3 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
si-THNI	Segmented marine worm (*Thysanocardia nigra*)	Yes	5	British Columbia
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-NOHE	Notomastus (*Notomastus hemipodus*)	No	2	British Columbia
an-PRJU	Prionospio (*Prionospio jubata*)	No	2	British Columbia
an-PRLI	Prionospio [*Prionospio (Minuspio) lighti]*	No	2	British Columbia
an-CACAW	Capitella (*Capitella capitata*)	No	2	British Columbia
an-OPAC	Ophelina (*Ophelina acuminata*)	No	2	British Columbia
an-MASA	Maldane (*Maldane sarsi*)	No	2	British Columbia
an-RIPI	Hydrothermal vent worm (*Ridgeia piscesae*)	No	2	British Columbia
an-PRPA	Praxillela (*Praxillella pacifica*)	No	1	British Columbia
ma-HOSA	Human (*Homo sapiens*)	No	1	Netherlands
ma-CAFA	Canine (*Canis lupus familiaris*)	No	1	British Columbia
ma-FECA	Cat (*Felis catus*)	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

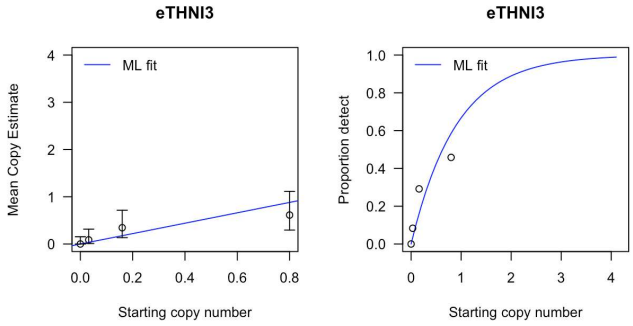
3. Langlois, VS, Allison, MJ, Bergman, LC, To, TA, and Helbing, CC (2020) The need for robust qPCR-based eDNA detection assays in environmental monitoring and risk assessments. Environmental DNA, 3: 519-527. doi: 10.1002/edn3.164

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

Helbing Lab
eDNA Inventory

eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

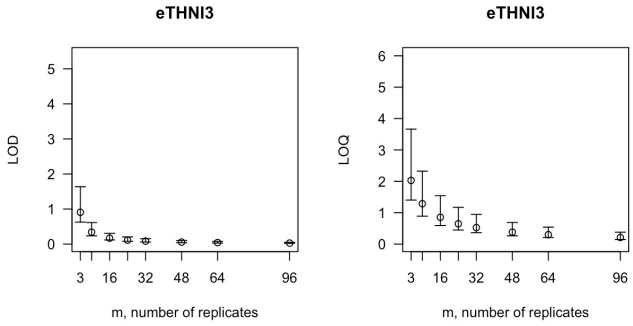
To calculate tables for different numbers of replicates, raw csv data files can be accessed here:
https://onlineacademiccommunity.uvic.ca/helbinglab/edna/



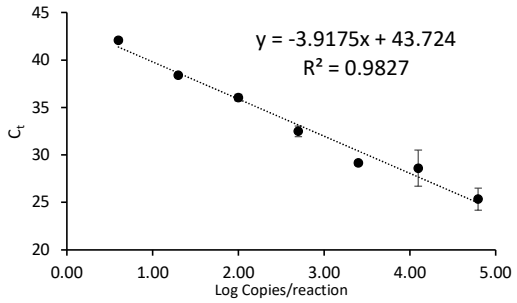
From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.12	0.12
2	0.26	0.2
3	0.43	0.27
4	0.63	0.35
5	0.89	0.46
6	1.26	0.63
7	1.89	0.95

Determined using eLowQuant R code[4].



Applied to reactions with 100% 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 92%

Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-ND1	Mitochondrial NADH dehydrogenase subunit 1 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