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: *Decamastus Gracilis* | eDNA qPCR Tool: eDEGR2 | Gene Target: MT-ND4

Species Code: an-DEGR | eDNA qPCR Format: TaqMan | Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 1.2 | 95% CI 0.9-2.1 Copies/Rxn | LOQ 4.6 | 95% CI 3.2-8.1 Copies/Rxn | LOB 0 hits/8

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-DEGR	Decamastus (*Decamastus gracilis*)	Yes	6	CRD/METVAN BC
an-HEFI	Heteromastus (*Heteromastus filobranchus*)	No	6	CRD/METVAN BC
an-GLNA	Glycera (*Glycera nana*)	No	2	CRD/METVAN BC
an-CACAW	Capitella (*Capitella capitata*)	No	4	CRD/METVAN BC
an-PRMU	Prionospio (*Prionospio (Minuspio) multibranchiata*)	No	1	CRD/METVAN BC
an-PRLI	Prionospio (*Prionospio (Minuspio) lighti*)	No	6	CRD/METVAN BC
an-PRJU	Prionospio (*Prionospio (Prionospio) jubata*)	No	6	CRD/METVAN BC
an-NOHE	Notomastus (*Notomastus hemipodus*)	No	2	CRD/METVAN BC
an-RIPI	Hydrothermal vent worm (*Ridgeia picescae*)	No	4	Endeavor Hydrothermal Vent
ma-HOSA	Human (*Homo sapiens*)	No	1	
ma-CAFA	Domestic dog (Canis lupus familiaris)	No	1	
ma-FECA	Cat (*Felis catus*)	No	1	

References

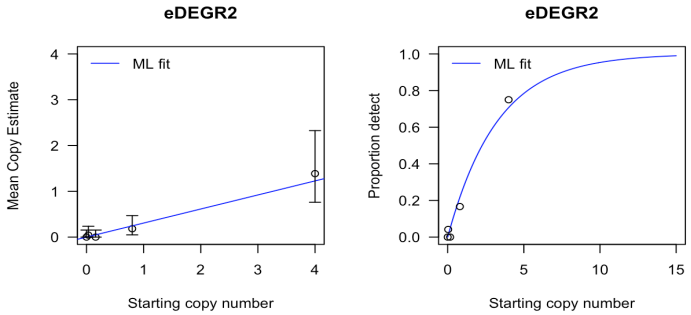
1. Hobbs, J, Adams, IT, Round, JM, Goldberg, CS, Allison, MJ, Bergman, LC, Mirabzadeh, A, Allen, H, Helbing, CC (2020) Revising the range of Rocky Mountain tailed frog, *Ascaphus montanus*, in British Columbia, Canada, using environmental DNA methods. Environmental DNA, 2: 350-361. https://doi.org/10.1002/edn3.82

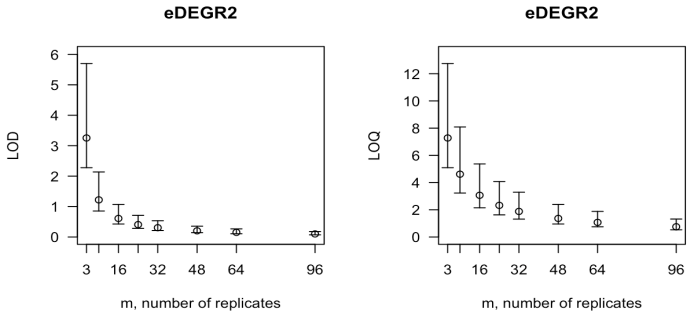
2. Hobbs, J, Round, JM, Allison, MJ, Helbing, CC (2019) Expansion of the known distribution of the coastal tailed frog, *Ascaphus truei*, in British Columbia, Canada, using robust eDNA detection methods. PLOS ONE 14(3): e0213849. https://doi.org/10.1371/journal.pone.0213849

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





Determined using eLowQuant R code[4].
Based on a 2 µL DNA input in a total 15 µL reaction

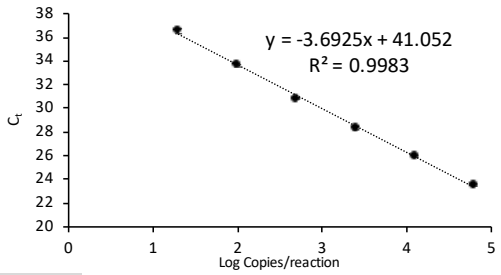
To calculate tables for different numbers of replicates, use raw csv data files.

From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.44	0.45
2	0.94	0.7
3	1.53	0.95
4	2.26	1.25
5	3.2	1.64
6	4.52	2.23
7	6.78	3.39

Determined using eLowQuant R code[4].

Applied to reactions with ≥ 95% positive hits



Efficiency 87%

Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location
Water	Y	280	Y	Vancouver, BC, Canada

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-ND4	Mitochondrial NADH Dehydrogenase subunit 4
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