



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 sensitivity¹⁻³.

General eDNA Assay Information

Target Species: Glycera (*Glycera nana*) eDNA qPCR Tool: eGLNA3 Gene Target: MT-ND6
 Species Code: an-GLNA eDNA qPCR Format: TaqMan Published in: _____

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.2 95% CI 0.2-0.4 Copies/Rxn LOQ 0.9 95% CI 0.6-1.6 Copies/Rxn LOB 0 hits/8
 LOQ_{continuous} 4 Copies/Rxn

Binomial-Poisson model for 8 technical replicates determined using eLowQuant R code⁴. 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)

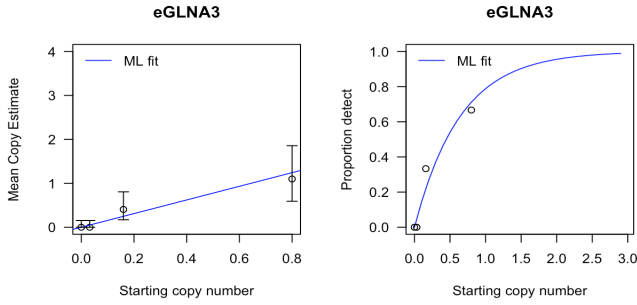
Species	Common Name (<i>Species</i>)	Detection	# Voucher		Sample Sources/Locations
			Specimens		
an-CACAW	Capitella (<i>Capitella capitata</i>)	No	2		Southwestern British Columbia
an-DEGR	Decamastus (<i>Decamastus gracilis</i>)	No	2		Southwestern British Columbia
an-GLNA	Glycera (<i>Glycera nana</i>)	Yes	5		Southwestern British Columbia
an-HEFI	Heteromastus (<i>Heteromastus filobranchus</i>)	No	2		Southwestern British Columbia
an-MASA	Maldane (<i>Maldane sarsi</i>)	No	2		Southwestern British Columbia
an-NOHE	Notomastus (<i>Notomastus hemipodus</i>)	No	2		Southwestern British Columbia
an-OPAC	Ophelina (<i>Ophelina acuminata</i>)	No	2		Southwestern British Columbia
an-PRJU	Prionospio (<i>Prionospio jubata</i>)	No	2		Southwestern British Columbia
an-PRLI	Prionospio [<i>Prionospio (Minuspio) lighti</i>]	No	2		Southwestern British Columbia
an-PRPA	Praxillela (<i>Praxillela pacifica</i>)	No	1		Southwestern British Columbia
an-RIPI	Hydrothermal vent worm (<i>Ridgeia piscesae</i>)	No	2		Southwestern British Columbia
ma-CAFA	Canine (<i>Canis lupus familiaris</i>)	No	1		Southwestern British Columbia
ma-FECA	Cat (<i>Felis catus</i>)	No	1		Southwestern British Columbia
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1		Netherlands
si-THNI	Segmented marine worm (<i>Thysanocardia nigra</i>)	No	2		Southwestern British Columbia

References

- 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>
- 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>
- 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
- 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

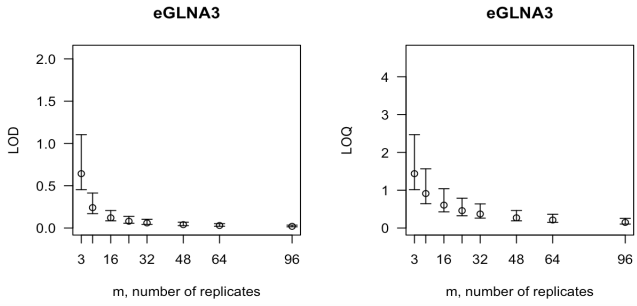
To calculate tables for different numbers of replicates, raw csv data files can be accessed here:
<https://onlineacademiccommunity.uvic.ca/helbinglab/edna/>



From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.086	0.088
2	0.19	0.14
3	0.3	0.19
4	0.45	0.25
5	0.63	0.32
6	0.89	0.44
7	1.34	0.67

Determined using eLowQuant R code⁴.

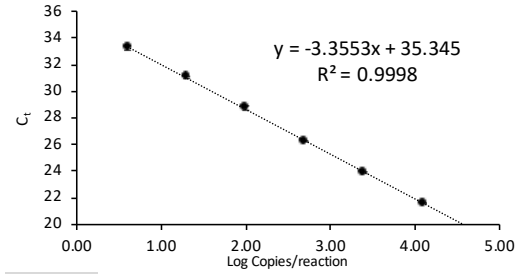


Binomial-Poisson model: No intercept

Determined using eLowQuant R code⁴.

Based on a 2 µL DNA input in a total 15 µL reaction

Applied to reactions with 100% positive hits



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-ND6	Mitochondrial NADH dehydrogenase subunit 6 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