

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: Heteromastus filobranchus eDNA qPCR Tool: eHEFI7 Gene Target: MT-CYB
 Species Code: an-HEFI eDNA qPCR Format: TaqMan Published in: _____

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.3 95% CI 0.2-0.5 Copies LOQ 1.1 95% CI 0.8-1.9 Copies 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: 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
an-CACA	<i>Capitella capitata</i>	No	3	British Columbia
an-DEGR	<i>Decamastus gracilis</i>	No	4	British Columbia
an-GLNA	<i>Glycera nana</i>	No	2	British Columbia
an-HEFI	<i>Heteromastus filobranchus</i>	Yes	4	British Columbia
an-NOHE	<i>Notomastus hemipodus</i>	No	2	British Columbia
an-PRJU	<i>Prionospio (Prionospio) jubata</i>	No	2	British Columbia
an-PRLI	<i>Prionospio (Minuspio) lighti</i>	No	4	British Columbia
an-PRMU	<i>Prionospio (Minuspio) multibranchiata</i>	No	1	British Columbia
an-RIPI	Hydrothermal vent worm (<i>Ridgeia piscesae</i>)	No	3	British Columbia
ma-CALUfa	Domestic dog (<i>Canis lupus familiaris</i>)	No	1	British Columbia
ma-FECA	Domestic cat (<i>Felis catus</i>)	No	1	British Columbia
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1	Netherlands

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. 2020; 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

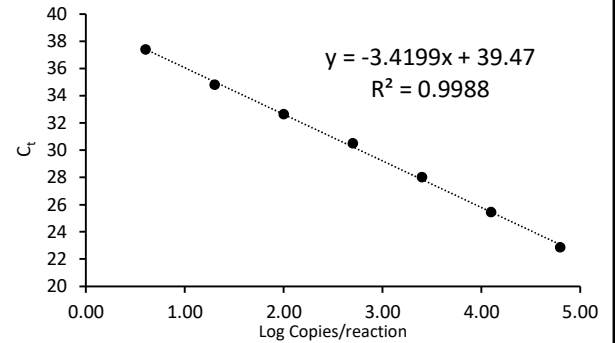
To calculate tables for different numbers of replicates, raw csv data files can be accessed here:
<https://onlineacademiccommunity.uvic.ca/helbinglab/edna/>

From 8 Technical Replicates

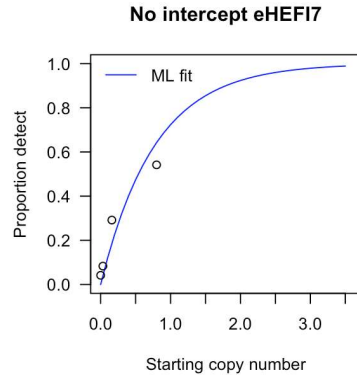
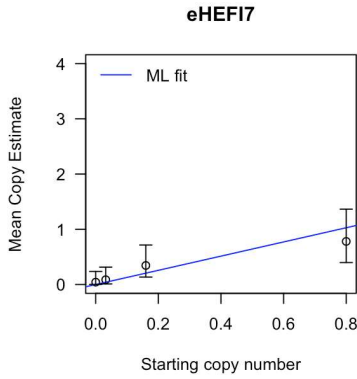
# Detects	# Copies	SE
0	0	0
1	0.1	0.11
2	0.22	0.17
3	0.37	0.23
4	0.54	0.3
5	0.76	0.39
6	1.08	0.53
7	1.62	0.81

Determined using eLowQuant R code⁴.

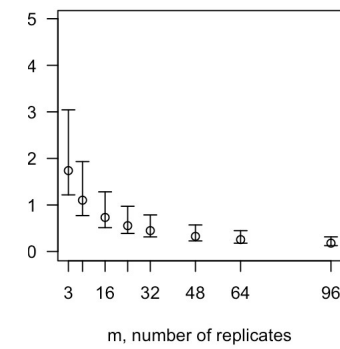
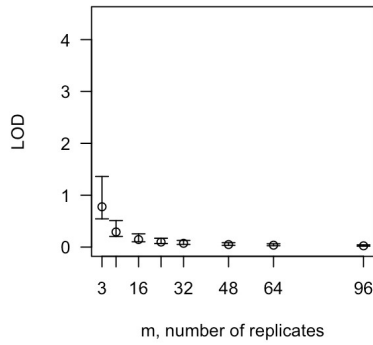
Applied to reactions with 100% positive hits



Efficiency 96%



Limits detect - no intercept eHEFI7 Limits quant - no intercept eHEFI7



Binomial-Poisson model: No-Intercept
 Determined using eLowQuant R code⁴.
 Based on a 2 μ L DNA input in a total 15 μ L reaction

Field Sample Validation

Known
 Sample Type Presence # Samples Detected Location

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-CYB	Mitochondrial cytochrome b 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