

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 0.7 95% CI 0.5-1.1 Copies LOQ 2.8 95% CI 2.1-4.2 Copies LOB 0 hits/8
LOQ_{continuous} 20 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 (Species)	Detection	# Voucher		Sample Sources/Locations
			Specimens		
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 (Minuspilio) lighti</i>	No	4		British Columbia
an-PRMU	<i>Prionospio (Minuspilio) multibranchiata</i>	No	1		British Columbia
an-RIP1	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

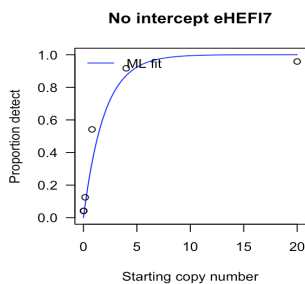
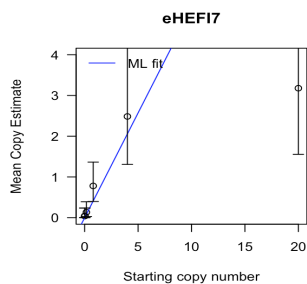
- Hobbs, J, Adams, IT, Round, JM, Goldberg, CS, Allison, MJ, Bergman, LC, Mirabzadeh, A, Allen, H, Helbing, CC (2020) Revising the range of Rocky Mountain tailed frog, *Ascaphus montanus*, in British Columbia, Canada, using environmental DNA methods. Environmental DNA. 2020; 2: 350-361. <https://doi.org/10.1002/edn3.82>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

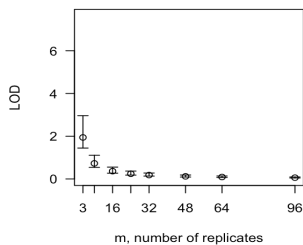
From 8 Technical Replicates



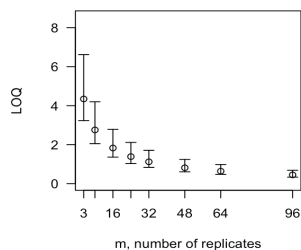
# Detects	# Copies	SE
0	0	0
1	0.26	0.26
2	0.56	0.41
3	0.91	0.56
4	1.35	0.73
5	1.91	0.95
6	2.7	1.28
7	4.05	1.95

Determined using eLowQuant R code⁴.

Limits detect - no intercept eHEFI7

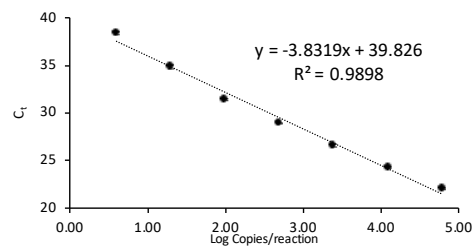


Limits quant - no intercept eHEFI7



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



Efficiency 82%

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-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