



### 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<sup>1-3</sup>.

#### General eDNA Assay Information

Target Species: Prionospio (*Prionospio (Minuspio) lighti*) eDNA qPCR Tool: ePRLI4 Gene Target: MT-CYB  
Species Code: an-PRLI eDNA qPCR Format: TaqMan Published in:

#### eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.3 95% CI 0.2-0.6 Copies LOQ 1.2 95% CI 0.8-2.2 Copies LOB 0 hits/8  
LOQ<sub>continuous</sub> 4 Copies/Rxn

Binomial-Poisson model for 8 technical replicates determined using eLowQuant R code<sup>4</sup>. 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> )	# Voucher		Sample Sources/Locations
		Detection	Specimens	
an-CACAW	Capitella ( <i>Capitella capitata</i> )	No	6	British Columbia
an-DEGR	Decamastus ( <i>Decamastus gracilis</i> )	No	5	British Columbia
an-DRLO	Drilonereis ( <i>Drilonereis longa</i> )	No	2	British Columbia
an-GLNA	Glycera ( <i>Glycera nana</i> )	No	6	British Columbia
an-HEFI	Heteromastus ( <i>Heteromastus filobranchus</i> )	No	6	British Columbia
an-MASA	Maldane ( <i>Maldane sarsi</i> )	No	6	British Columbia
an-NOHE	Notomastus ( <i>Notomastus hemipodus</i> )	No	5	British Columbia
an-OPAC	Ophelina ( <i>Ophelina acuminata</i> )	No	6	British Columbia
an-PRGR	Praxillela ( <i>Praxillela gracilis</i> )	No	5	British Columbia
an-PRJU	Prionospio ( <i>Prionospio (Prionospio) jubata</i> )	No	6	British Columbia
an-PRLI	Prionospio ( <i>Prionospio (Minuspio) lighti</i> )	Yes	6	British Columbia
an-PRMU	Prionospio ( <i>Prionospio (Minuspio) multibranchiata</i> )	No	1	British Columbia
an-PRPA	Praxillela ( <i>Praxillela pacifica</i> )	No	6	British Columbia
an-RIP1	Hydrothermal vent worm ( <i>Ridgeia picescae</i> )	No	4	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

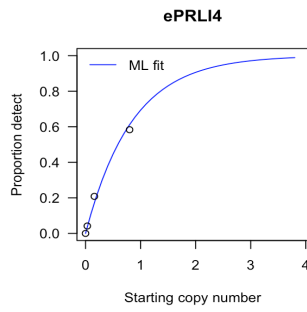
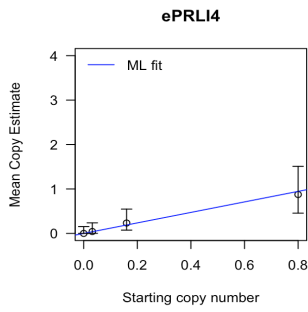
- Hobbs, J, Adams, IT, Round, JM, Goldberg, CS, Allison, MJ, Bergman, LC, Mirabzadeh, A, Allen, H, Helbing, CC (2020) Revising the range of Rocky Mountain tailed frog, *Ascaphus montanus*, in British Columbia, Canada, using environmental DNA methods. Environmental DNA. 2020; 2: 350-361. <https://doi.org/10.1002/edn3.82>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

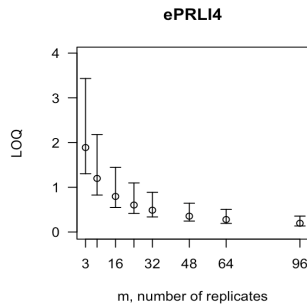
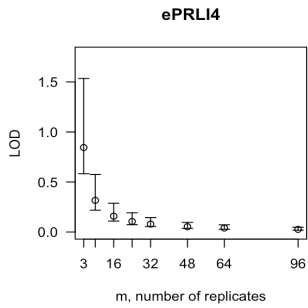
To calculate tables for different numbers of replicates, raw csv data files can be accessed here:  
<https://onlineacademiccommunity.uvic.ca/helbinglab/edna/>

From 8 Technical Replicates



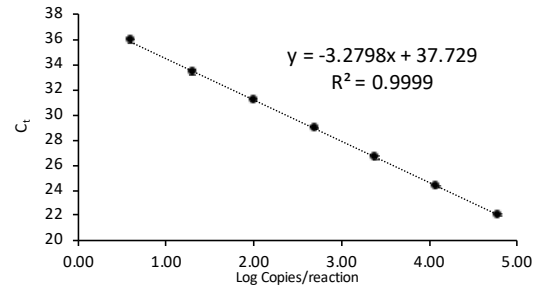
# Detects	# Copies	SE
0	0	0
1	0.11	0.11
2	0.24	0.18
3	0.4	0.25
4	0.59	0.33
5	0.83	0.43
6	1.17	0.58
7	1.76	0.89

Determined using eLowQuant R code<sup>4</sup>.



Binomial-Poisson model: No intercept  
Determined using eLowQuant R code<sup>4</sup>.  
Based on a 2 µL DNA input in a total 15 µL reaction

Applied to reactions with 100% positive hits



Efficiency 102%

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