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: 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

$LOQ_{continuous}$ __4__ Copies/Rxn

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-CACAW	Capitella (*Capitella capitata*)	No	6	British Columbia
an-DEGR	Decamastus (*Decamastus gracilis*)	No	5	British Columbia
an-DRLO	Drilonereis (*Drilonereis longa*)	No	2	British Columbia
an-GLNA	Glycera (*Glycera nana*)	No	6	British Columbia
an-HEFI	Heteromastus (*Heteromastus filobranchus*)	No	6	British Columbia
an-MASA	Maldane (*Maldane sarsi*)	No	6	British Columbia
an-NOHE	Notomastus (*Notomastus hemipodus*)	No	5	British Columbia
an-OPAC	Ophelina (*Ophelina acuminata*)	No	6	British Columbia
an-PRGR	Praxillela (*Praxillella gracilis*)	No	5	British Columbia
an-PRJU	Prionospio (*Prionospio (Prionospio) jubata*)	No	6	British Columbia
an-PRLI	Prionospio (*Prionospio (Minuspio) lighti*)	Yes	6	British Columbia
an-PRMU	Prionospio (*Prionospio (Minuspio) multibranchiata*)	No	1	British Columbia
an-PRPA	Praxillela (*Praxillella pacifica*)	No	6	British Columbia
an-RIPI	Hydrothermal vent worm (*Ridgeia picescae*)	No	4	British Columbia
ma-CALUfa	Domestic dog (Canis lupus familiaris)	No	1	British Columbia
ma-FECA	Domestic cat (*Felis catus*)	No	1	British Columbia
ma-HOSA	Human (*Homo sapiens*)	No	1	Netherlands

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. 2020; 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

ePRLI4

ePRLI4

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[4].
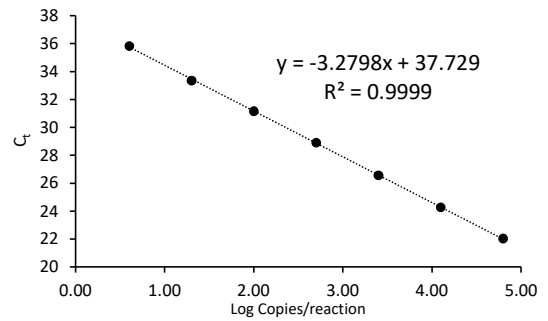
ePRLI4

ePRLI4
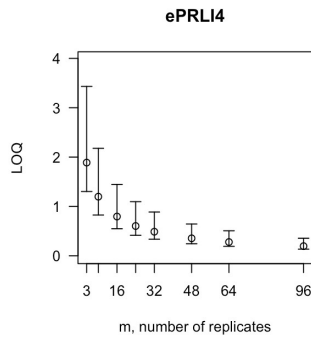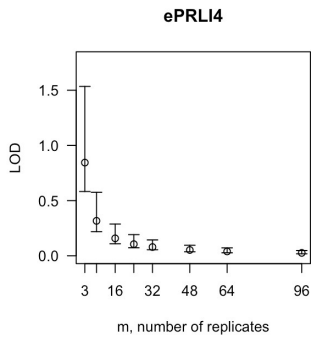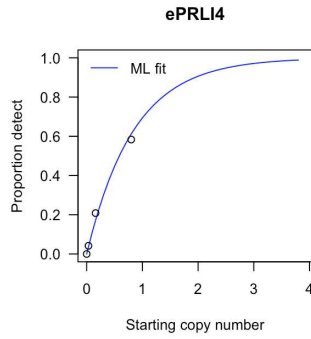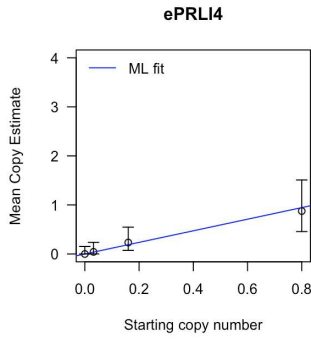
Binomial-Poisson model: ____No intercept____

Determined using eLowQuant R code[4].

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

Applied to reactions with 100% positive hits

$$y = -3.2798x + 37.729$$
$$R^2 = 0.9999$$

Efficiency   102%

Field Sample Validation

Sample Type	Known 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