



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: Mountain whitefish (*Prosopium williamsoni*) eDNA qPCR Tool: ePRWI4 Gene Target: MT-ND1
Species Code: te-PRWI eDNA qPCR Format: TaqMan Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.1 95% CI 0.1-0.2 Copies/Rxn LOQ 0.4 95% CI 0.3-0.7 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		
ma-CALU	Wolf (<i>Canis lupus</i>)	No	1		British Columbia
ma-FECA	Cat (<i>Felis catus</i>)	No	1		Québec
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1		Netherlands
te-COAR	Cisco/Tullibee (<i>Coregonus artedi</i>)	No	1		Alberta
te-COCL	Lake whitefish (<i>Coregonus clupeaformis</i>)	No	2		Alberta
te-GAAC	Three-spined stickleback (<i>Gasterosteus aculeatus</i>)	No	1		British Columbia
te-MIDO	Smallmouth bass (<i>Micropterus dolomieu</i>)	No	1		British Columbia
te-MISA	Largemouth bass (<i>Micropterus salmoides</i>)	No	1		British Columbia
te-ONCLle	Westslope cutthroat trout (<i>Oncorhynchus clarkii lewisi</i>)	No	1		Alberta
te-ONGO	Pink salmon (<i>Oncorhynchus gorbuscha</i>)	No	1		British Columbia
te-ONNE	Sockeye salmon (<i>Oncorhynchus nerka</i>)	No	1		British Columbia
te-ONKE	Chum salmon (<i>Oncorhynchus keta</i>)	No	1		British Columbia
te-PRCY	Round whitefish (<i>Prosopium cylindraceum</i>)	No	1		Yukon
te-PRWI	Mountain whitefish (<i>Prosopium williamsoni</i>)	Yes	6		Alberta
te-RHUM	Umatilla dace (<i>Rhinichthys umatilla</i>)	No	1		British Columbia
te-SAFO	Brook trout (<i>Salvelinus fontinalis</i>)	No	1		Alberta
te-THAR	Arctic grayling (<i>Thymallus arcticus</i>)	No	1		Yukon

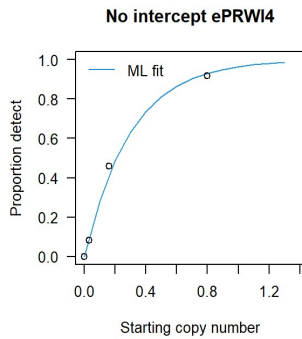
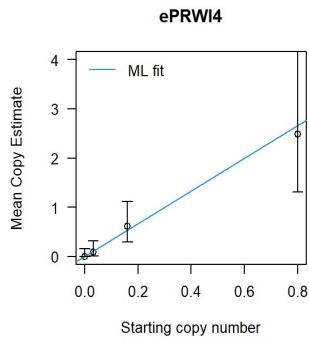
References

- 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/>

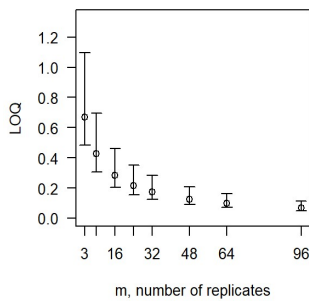
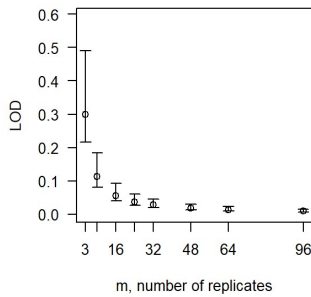
From 8 Technical Replicates



# Detects	# Copies	SE
0	0	N/A
1	0.040	0.041
2	0.086	0.064
3	0.14	0.087
4	0.21	0.11
5	0.29	0.15
6	0.42	0.20
7	0.62	0.31

Limits detect - no intercept ePRWI4

Limits quant - no intercept ePRWI4



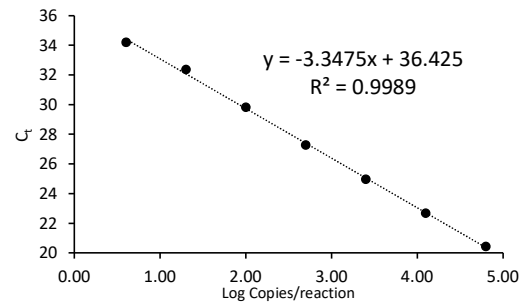
Binomial-Poisson model: No intercept

Determined using eLowQuant R code⁴.

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

Determined using eLowQuant R code⁴.

Applied to reactions with 100% positive hits



Efficiency 99%

Field Sample Validation

Sample Type	Known		Detected	Location
	Presence	# Samples		

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-ND1	Mitochondrial NADH dehydrogenase subunit 1 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