



### 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: Northern pikeminnow (*Ptychocheilus oregonensis*) eDNA qPCR Tool: ePTOR1 Gene Target: MT-ND1  
Species Code: te-PTOR eDNA qPCR Format: TaqMan Published in: \_\_\_\_\_

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

LOD 0.2 95% CI 0.1-0.3 Copies/Rxn LOQ 0.6 95% CI 0.4-1 Copies/Rxn 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> )	Detection	# Voucher		Sample Sources/Locations
			Specimens		
te-CACAch	Salish sucker ( <i>Catostomus catostomus (chehalis)</i> )	No	1		British Columbia
te-COCL	Lake whitefish ( <i>Coregonus clupeaformis</i> )	No	1		Alberta
te-COCO	Slimy sculpin ( <i>Cottus cognatus</i> )	No	1		British Columbia
te-ESLU	Northern pike ( <i>Esox lucius</i> )	No	1		British Columbia
te-MYCA	Peamouth chub ( <i>Mylocheilus caurinus</i> )	No	1		Ontario
te-ONCL	Some variety of cutthroat trout ( <i>Oncorhynchus clarkii</i> ?)	No	1		Alberta
te-ONGO	Pink salmon ( <i>Oncorhynchus gorbuscha</i> )	No	1		British Columbia
te-ONKI	Coho salmon ( <i>Oncorhynchus kisutch</i> )	No	1		British Columbia
te-ONMY	Rainbow (steelhead) trout ( <i>Oncorhynchus mykiss</i> )	No	1		Alberta
te-ONNE	Sockeye salmon ( <i>Oncorhynchus nerka</i> )	No	1		British Columbia
te-ONTS	Chinook salmon ( <i>Oncorhynchus tshawytscha</i> )	No	1		British Columbia
te-PLGR	Flathead chub ( <i>Platygobio gracilis</i> )	No	1		Ontario
te-PTOR	Northern pikeminnow ( <i>Ptychocheilus oregonensis</i> )	Yes	5		Ontario
te-RHCA	Longnose (nooksack) dace ( <i>Rhinichthys cataractae</i> )	No	1		Ontario
te-SAFO	Brook trout ( <i>Salvelinus fontinalis</i> )	No	1		Alberta
te-SANA	Lake trout ( <i>Salvelinus namaycush</i> )	No	1		British Columbia
te-SAVI	Walleye ( <i>Sander vitreus</i> )	No	1		Alberta
ma-CALUfa	Dog ( <i>Canis lupus familiaris</i> )	No	1		British Columbia
ma-FECA	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, 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

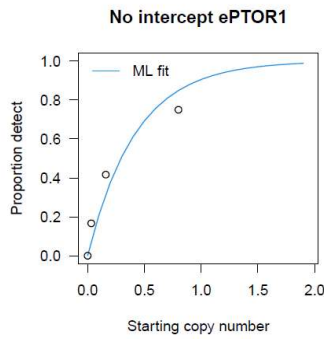
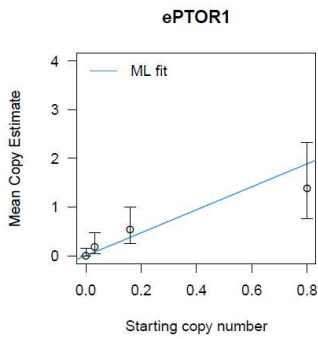
**eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA**

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

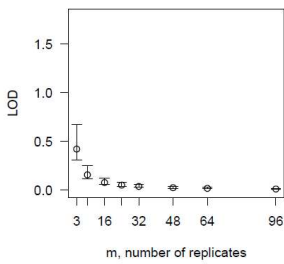
From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.048	0.065
2	0.119	0.097
3	0.203	0.133
4	0.305	0.175
5	0.437	0.231
6	0.624	0.315
7	0.943	0.483

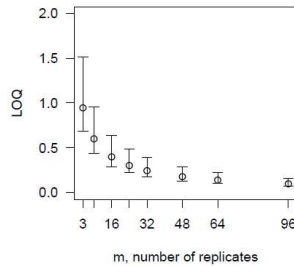
Determined using eLowQuant R code<sup>4</sup>.



**Limits detect - no intercept ePTOR1**



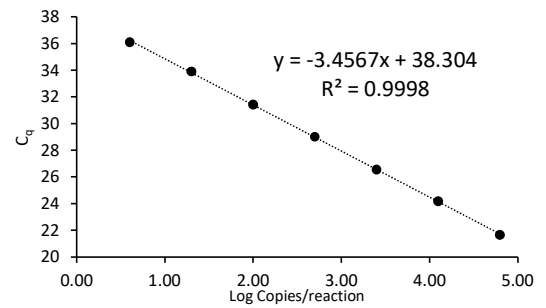
**Limits quant - no intercept ePTOR1**



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 ≥ 95% positive hits



Efficiency 95%

**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