



### 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: Burbot (*Lota lota*) eDNA qPCR Tool: eLOLO4 Gene Target: MT-ND4  
Species Code: te-LOLO eDNA qPCR Format: TaqMan Published in:

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

LOD 0.3 95% CI 0.3-0.6 Copies/Rxn LOQ 1.3 95% CI 0.9-2.1 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> )	# Voucher		Sample Sources/Locations
		Detection	Specimens	
ma-CALUfa	Dog ( <i>Canis lupus familiaris</i> )	No	1	British Columbia
ma-FECA	Cat (domestic) ( <i>Felis catus</i> )	No	1	British Columbia
ma-HOSA	Human ( <i>Homo sapiens</i> )	No	1	Netherlands
te-ANRO	American eel ( <i>Anguilla rostrata</i> )	No	1	Prince Edward Island
te-CACA	Longnose sucker ( <i>Catostomus catostomus</i> )	No	1	British Columbia
te-CACO	White sucker ( <i>Catostomus commersonii</i> )	No	1	Alberta
te-COCL	Lake whitefish ( <i>Coregonus clupeaformis</i> )	No	1	British Columbia
te-ESLU	Northern pike ( <i>Esox lucius</i> )	No	1	British Columbia
te-LOLO	Burbot ( <i>Lota lota</i> )	Yes	6	Yukon
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-ONCL	Cutthroat trout ( <i>Oncorhynchus clarkii</i> )	No	1	Alberta
te-ONMY	Rainbow trout ( <i>Oncorhynchus mykiss</i> )	No	1	Alberta
te-PRCY	Round whitefish ( <i>Prosopium cylindraceum</i> )	No	1	Yukon
te-SACO	Bull trout ( <i>Salvelinus confluentus</i> )	No	1	British Columbia
te-SAFO	Brook trout ( <i>Salvelinus fontinalis</i> )	No	1	Alberta
te-SANA	Lake trout ( <i>Salvelinus namaycush</i> )	No	1	Alberta
te-THAR	Arctic grayling ( <i>Thymallus arcticus</i> )	No	1	Alberta

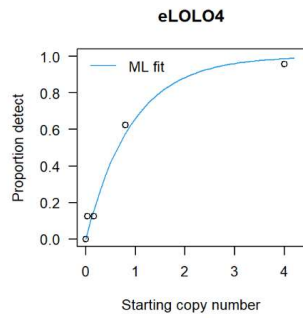
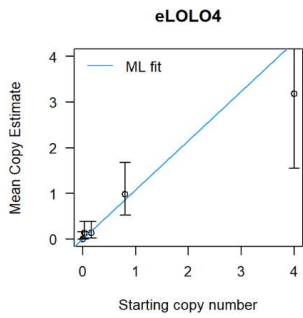
#### References

- 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*
- 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**

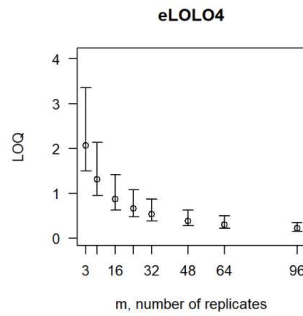
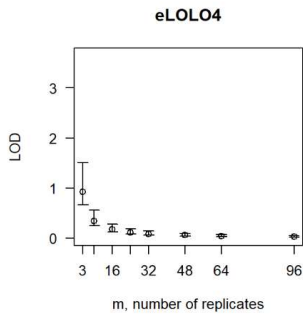
To calculate tables for different numbers of replicates, raw csv data files can be accessed here:  
<https://onlineacademiccommunity.uvic.ca/helbinglab/edna/>

From 8 Technical Replicates



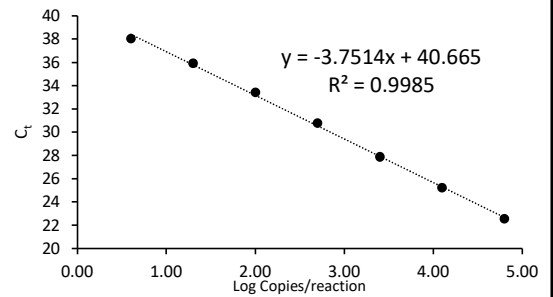
# Detects	# Copies	SE
0	0	0
1	0.12	0.13
2	0.27	0.2
3	0.44	0.27
4	0.64	0.35
5	0.91	0.46
6	1.28	0.62
7	1.92	0.94

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 85%

**Field Sample Validation**

Sample Type	Known		
	Presence	# Samples	Detected Location

**Abbreviations**

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-ND4	Mitochondrial NADH dehydrogenase subunit 4 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