



**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: Dreissena spp. eDNA qPCR Tool: eDRsp7 Gene Target: MT-RNR1 (12S)  
 Species Code: mo-DRsp eDNA qPCR Format: TaqMan Published in:

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

LOD 0.2 95% CI 0.2-0.4 Copies/Rxn LOQ 0.8 95% CI 0.6-1.4 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 (Species)	# 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
mo-COFL	Asian clam ( <i>Corbicula fluminea</i> )	No	1	British Columbia
mo-CRGI	Pacific oyster ( <i>Crassostrea gigas</i> )	No	1	British Columbia
mo-DRBU	Quagga mussel ( <i>Dreissena bugensis</i> )	Yes	4	Ontario
mo-DRPO	Zebra mussel ( <i>Dreissena polymorpha</i> )	Yes	4	Ontario
mo-MAIN	Pointed Macoma ( <i>Macoma inquinata</i> )	No	1	British Columbia
mo-MYAR	Softshell clam ( <i>Mya arenaria</i> )	No	1	British Columbia
mo-MYsp	Mussel ( <i>Mytilus spp.</i> )	No	1	British Columbia
mo-NUOB	Mahogany clam ( <i>Nuttallia obscurata</i> )	No	1	British Columbia
mo-OBOL	Freshwater mussel ( <i>Obovaria olivaria</i> )	No	1	Quebec
mo-OSLU	Olympia oyster ( <i>Ostrea lurida</i> )	No	1	British Columbia
mo-VEPH	Manila clam/Japanese littleneck ( <i>Venerupis philippinarum</i> )	No	1	British Columbia
te-PEOM	Trout-perch ( <i>Percopsis omiscomaycus</i> )	No	1	Ontario
mo-ANCA	California floater ( <i>Anodonta californiensis</i> )	No	1	British Columbia

**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

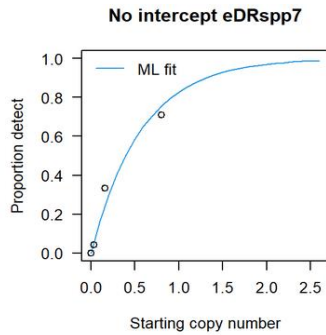
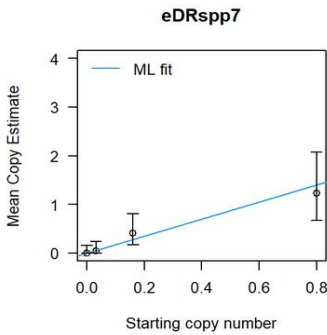


eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

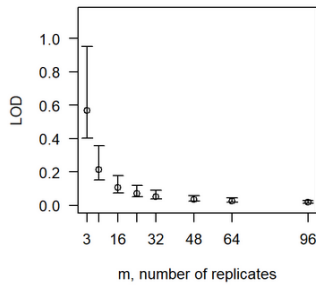
To calculate tables for different numbers of replicates, raw csv data files can be accessed here:  
<https://onlineacademiccommunity.uvic.ca/helbinglab/edna/>

From 8 Technical Replicates

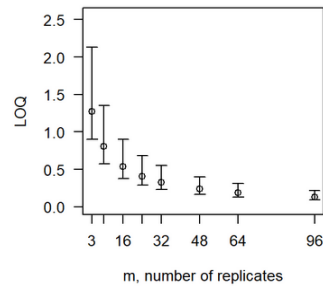
# Detects	# Copies	SE
0	0	0
1	0.076	0.078
2	0.164	0.121
3	0.267	0.165
4	0.394	0.217
5	0.557	0.284
6	0.788	0.384
7	1.182	0.585



Limits detect - no intercept eDRspp7

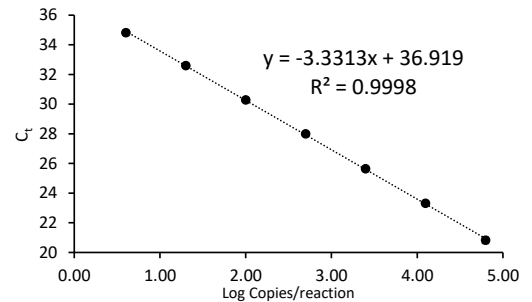


Limits quant - no intercept eDRspp7



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

Applied to reactions with ≥ 95% positive hits



Efficiency 100%

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

Field Sample Validation

Sample Type	Known			Location
	Presence	# Samples	Detected	
Water	Y	6	Y	Manitoba
Water	Y	12	Y	Manitoba

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-RNR1 (12S)	Mitochondrial 12S ribosomal RNA 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