



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: Zebra mussel (*Dreissena polymorpha*) eDNA qPCR Tool: eDRPO5 Gene Target: MT-TG (tRNA Gly)
 Species Code: mo-DRPO eDNA qPCR Format: TaqMan Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.3 95% CI 0.2-0.5 Copies/Rxn LOQ 1.1 95% CI 0.8-2 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>)	# 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>)	No	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-Myspp	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

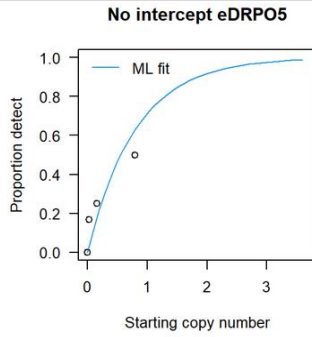
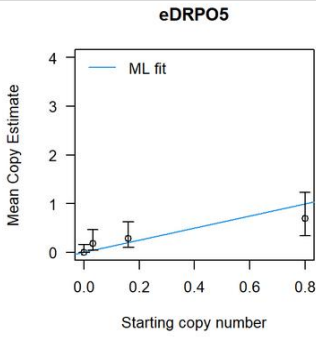


eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

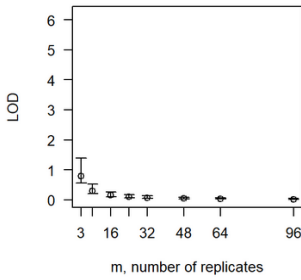
To calculate tables for different numbers of replicates, raw csv data files can be accessed here:
<https://onlineacademiccommunity.uvic.ca/helbinglab/edna/>

From 8 Technical Replicates

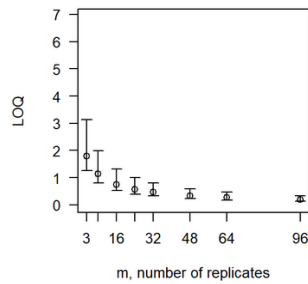
# Detects	# Copies	SE
0	0	0
1	0.107	0.11
2	0.231	0.171
3	0.377	0.235
4	0.557	0.309
5	0.787	0.405
6	1.113	0.548
7	1.67	0.835



Limits detect - no intercept eDRPO5

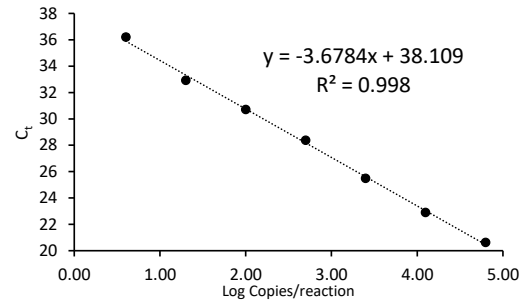


Limits quant - no intercept eDRPO5



Determined using eLowQuant R code⁴.

Applied to reactions with $\geq 95\%$ positive hits



Efficiency 87%

Binomial-Poisson model: No intercept
Determined using eLowQuant R code⁴.
Based on a 2 μ L DNA input in a total 15 μ L reaction

Field Sample Validation

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

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-TG	Mitochondrial tRNA glycine gene (tRNA Gly)
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