Helbing Lab

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[1-3].

General eDNA Assay Information

Target Species: Pacific oyster *(Crassostrea gigas)*	eDNA qPCR Tool: eCRGI2	Gene Target: MT-TL1
Species Code: mo-CRGI	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	0.7	95% CI	0.5-1.1 Copies/Rxn	LOB	0 hits/8

$LOQ_{continuous}$ 4 Copies/Rxn

Binomial-Poisson model for 8 technical replicates determined using eLowQuant R code[4].   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*)	Detection	# Voucher Specimens	Sample Sources/Locations
mo-COFL	Asian clam (*Corbicula fluminea*)	No	1	British Columbia
mo-CRGI	Pacific oyster (*Crassostrea gigas*)	Yes	13	British Columbia
mo-DRBU	Quagga mussel (*Dreissena bugensis*)	No	1	Ontario
mo-DRPO	Zebra mussel (*Dreissena polymorpha*)	No	1	Ontario
mo-MAIN	Pointed macoma (*Macoma inquinita*)	No	1	British Columbia
mo-MYAR	Softshell clam (*Mya arenaria*)	No	1	British Columbia
mo-Myspp	Mytilus (*Mytilus sp*)	No	1	British Columbia
mo-OSLU	Olympia oyster (*Ostrea lurida*)	No	1	British Columbia
mo-VEPH	Manila clam (*Venerupis philippinarum*)	No	1	British Columbia
mo-OSED	European flat oyster (*Ostrea edulis*)	No	1	British Columbia
ma-FECA	Cat (*Felis catus*)	No	1	British Columbia
ma-HOSA	Human (*Homo sapiens*)	No	1	Netherlands
ma-CALUfa	Dog (*Canis lupus familiaris*)	No	1	British Columbia

References

1. 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

2. 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

3. 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

4. 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/

eCRGI2

eCRGI2
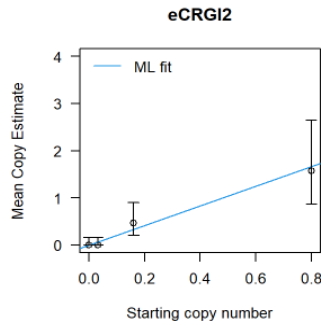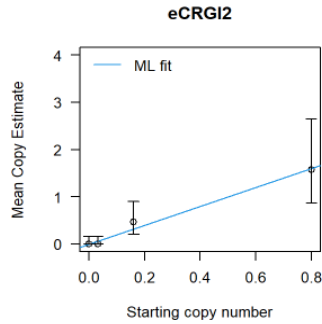
From ____8____ Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.067	0.096
2	0.142	0.115
3	0.245	0.153
4	0.352	0.193
5	0.493	0.254
6	0.688	0.346
7	1.04	0.545

Determined using eLowQuant R code[4].
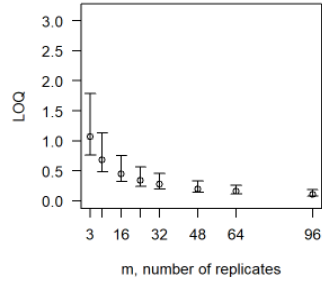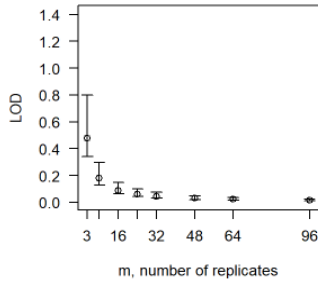
Applied to reactions with 100% positive hits

$$y = -3.3252x + 38.855$$
$$R^2 = 0.9901$$
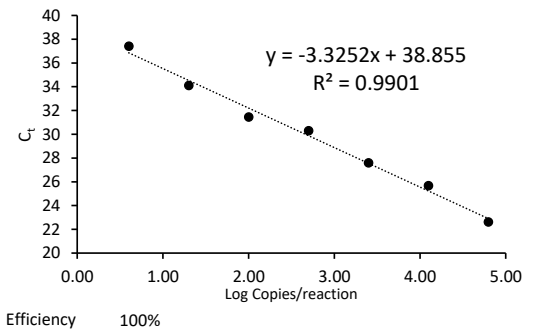
Binomial-Poisson model: ____No intercept____

Determined using eLowQuant R code[4].
Based on a 2 µL DNA input in a total 15 µL reaction

Efficiency   100%

Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-TL1	Mitochondrial transfer 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