



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: Graceful rock crab (*Metacarcinus gracilis*)
Species Code: ar-MEGR

eDNA qPCR Tool: eMEGR3
eDNA qPCR Format: TaqMan

Gene Target: MT-CO1
Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.2 95% CI 0.2-0.4 Copies/Rxn LOQ 0.9 95% CI 0.7-1.6 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 (Species)	Detection	# Voucher Specimens	Sample Sources/Locations
ar-MEGR	Graceful Rock Crab (<i>Metacarcinus gracilis</i>)	Yes	5	British Columbia
ar-CAPR	Red Rock Crab (<i>Cancer productus</i>)	Inconsistent ^{a,b}	5	British Columbia
ar-EUOR	Oregon Fairy Shrimp (<i>Eubbranchipus oregonus</i>)	No	1	British Columbia
ar-MEMA	Dungeness Crab (<i>Metacarcinus magister</i>)	No	1	British Columbia
ar-HEOR	Yellow Shore Crab (<i>Hemigrapsus oregonensis</i>)	No	1	British Columbia
ar-ERSI	Chinese Mitten Crab (<i>Eriocheir sinensis</i>)	No	1	British Columbia
ar-ORRU	Red Spotted Crayfish (<i>Orconectes rusticus</i>)	No	1	British Columbia
ar-PALE-kl	Signal Crayfish (<i>Pacifastacus leniusculus klamathensis</i>)	Inconsistent ^{a,b}	1	Oregon
ar-PAFO	Shasta Crayfish (<i>Pacifastacus fortis</i>)	No	1	California
ar-PAGA	Pilose Crayfish (<i>Pacifastacus gambelii</i>)	No	1	Wyoming
av-CAGU	Hermit Thrush (<i>Catharus guttatus</i>)	No	1	British Columbia
am-LICA	North American Bullfrog (<i>Lithobates (Rana) catesbeiana</i>)	No	1	British Columbia
te-SASA	Atlantic Salmon (<i>Salmo salar</i>)	No	1	Nova Scotia
te-SEBA	Redbanded Rockfish (<i>Sebastes babcocki</i>)	No	1	British Columbia
mo-CRGI	Pacific Oyster (<i>Crassostrea gigas</i>)	No	1	British Columbia
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1	Netherlands
ma-FECA	Domestic Cat (<i>Felis catus</i>)	No	1	British Columbia
ma-CALUfa	Dog (<i>Canis lupus familiaris</i>)	No	1	British Columbia

^aIndicates possible survey confounders

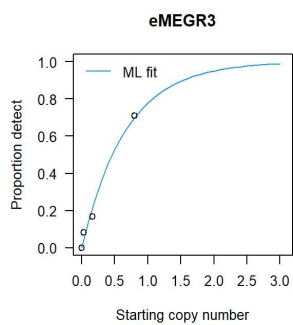
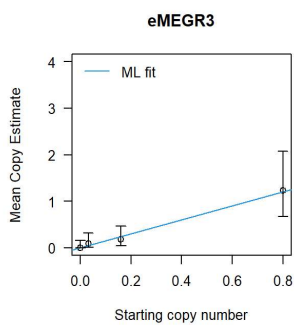
^bWeak signal. Maybe a survey confounder if in high numbers.

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

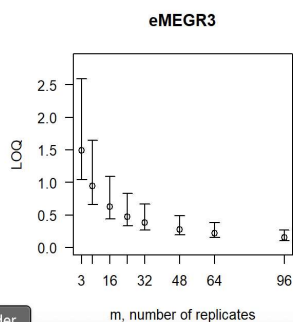
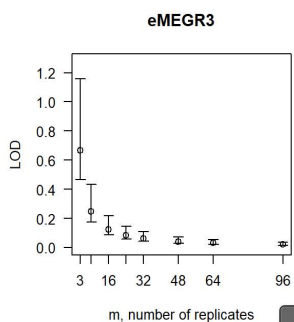


To calculate tables for different numbers of replicates, use raw csv data files.

From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.089	0.091
2	0.192	0.142
3	0.313	0.195
4	0.461	0.256
5	0.653	0.336
6	0.923	0.455
7	1.384	0.692

Determined using eLowQuant R code⁴.

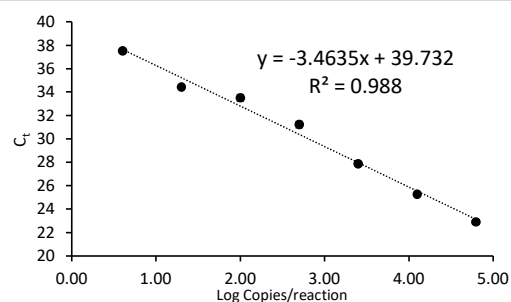


Binomial-Poisson model: No intercept

Determined using eLowQuant R code⁴.

Based on a 2 µL DNA input in a total 15 µL reaction

Applied to reactions with ≥ 95% positive hits



Efficiency 94%

Field Sample Validation

Sample Type	Known		Detected	Location
	Presence	# Samples		
Water	Yes	32	Yes	Sydney Spit
Sediment	Yes	32	Yes	Sydney Spit
Water	Yes	9	Yes	Sooke Basin
Sediment	Yes	9	Yes	Sooke Basin

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-CO1	Mitochondrial cytochrome oxidase 1
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