



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: Dungeness crab (*Metacarcinus magister*) eDNA qPCR Tool: eMEMA2 Gene Target: MT-COIII
 Species Code: ar-MEMA eDNA qPCR Format: TaqMan Published in: _____

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 1 95% CI 0.8-1.6 Copies/Rxn LOQ 3.9 95% CI 2.9-6.2 Copies/Rxn LOB 0 hits/8
 LOQ_{continuous} 20 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)

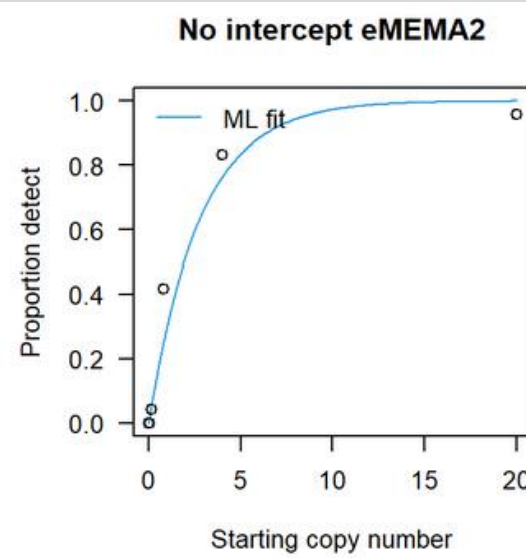
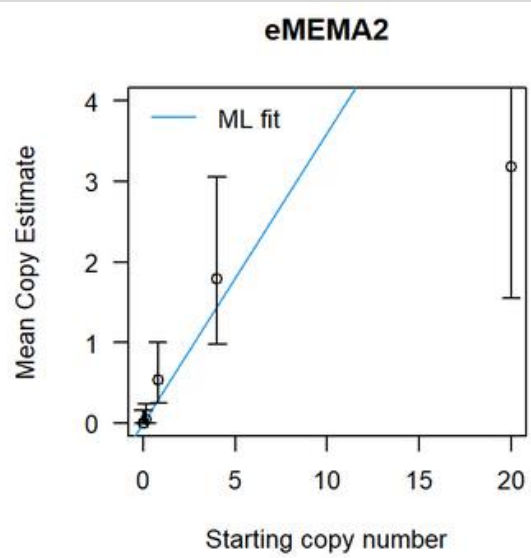
Species	Common Name (<i>Species</i>)	Detection	# Voucher	
			Specimens	Sample Sources/Locations
ar-CAMA	European green crab (<i>Carcinus maenas</i>)	No	1	British Columbia
ar-CAPR	Red rock crab (<i>Cancer productus</i>)	No	1	British Columbia
ar-ERSI	Chinese mitten crab (<i>Eriocheir sinensis</i>)	No	1	British Columbia
ar-EUOR	Oregon fairy shrimp (<i>Eubranchipus oregonus</i>)	No	1	British Columbia
ar-HEOR	Yellow shore crab (<i>Hemigrapsus oregonensis</i>)	No	1	British Columbia
ar-MEGR	Graceful rock crab (<i>Metacarcinus gracilis</i>)	No	1	British Columbia
ar-MEMA	Dungeness crab (<i>Metacarcinus magister</i>)	Yes	1	British Columbia
ar-ORRU	Red spotted crayfish (<i>Orconectes rusticus</i>)	No	1	British Columbia
ar-PACO	Snake River pilose crayfish (<i>Pacifastacus connectens</i>)	No	1	Idaho
ar-PAFO	Shasta crayfish (<i>Pacifastacus fortis</i>)	No	1	California
ar-PAGA	Pilose crayfish (<i>Pacifastacus gambelii</i>)	No	1	Wyoming
ar-PALE-kl	Signal crayfish (<i>Pacifastacus leniusculus klamathensis</i>)	No	1	British Columbia
ch-CACA	Great white shark (<i>Carcharodon carcharias</i>)	No	1	California
ma-CALUfa	Dog (<i>Canis lupus familiaris</i>)	No	1	British Columbia
ma-FECA	Domestic cat (<i>Felis catus</i>)	No	1	British Columbia
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1	Netherlands
mo-CRGI	Pacific oyster (<i>Crassostrea gigas</i>)	No	1	British Columbia
te-CLPA	Pacific herring (<i>Clupea pallasii</i>)	No	1	British Columbia
te-PRWI	Mountain whitefish (<i>Prosopium williamsoni</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 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



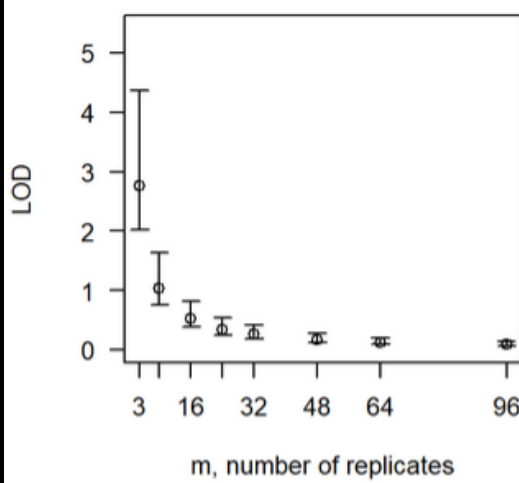
To calculate tables for different numbers of replicates, raw csv data files can be accessed here:
<https://onlineacademiccommunity.uvic.ca/helbinglab/edna/>

From 8 Technical Replicates

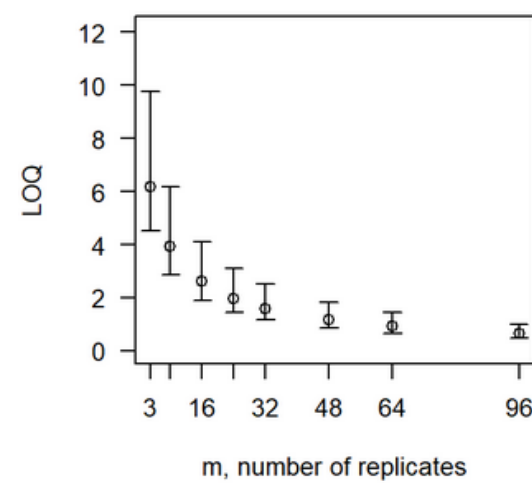
# Detects	# Copies	SE
0	0	0
1	0.37	0.377
2	0.797	0.585
3	1.302	0.797
4	1.921	1.043
5	2.718	1.363
6	3.841	1.842
7	5.757	2.803

Determined using eLowQuant R code⁴.

Limits detect - no intercept eMEMA2

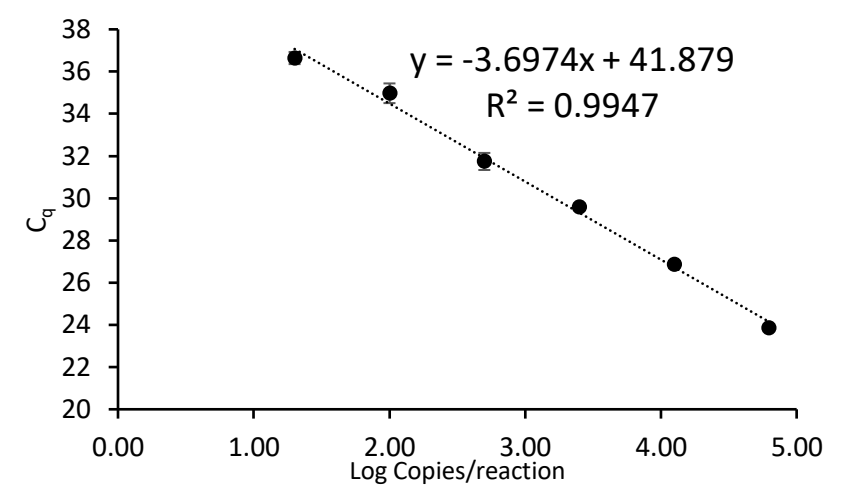


Limits quant - no intercept eMEMA2



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



Field Sample Validation

Sample Type	Known		Detected	Location
	Presence	# Samples		

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-COIII	Mitochondrial cytochrome oxidase subunit 3 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