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: European green crab (*carcinus maenas*) eDNA qPCR Tool: earCAMA1 Gene Target: MT-COII
Species Code: ar-CAMA 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	0.8-1.8	Copies/Rxn	LOB	0	hits/8
					$LOQ_{continuous}$	20			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)

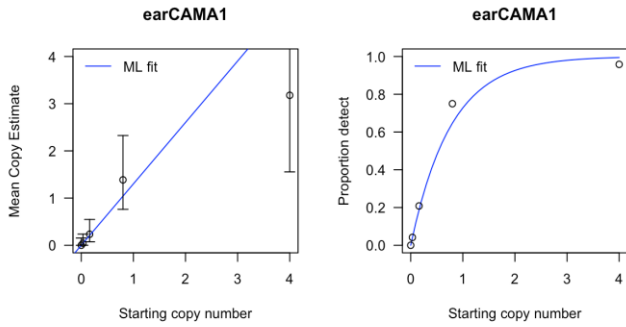| Species	Common Name (*Species*)	Detection	# Voucher Specimens	Sample Sources/Locations
ar-EUOR	Oregon fairy shrimp (*Eubranchipus oregonus*)	No	1	British Columbia
ar-MEGR	Graceful rock crab (*Metacarcinus gracilis*)	No	1	British Columbia
ar-MEMA	Dungeness crab (*Metacarcinus magister*)	No	1	British Columbia
ar-HEOR	Yellow shore crab (*Hemigrapsus oregonesis*)	No	1	British Columbia
ar-CAMA	European green crab (*Carcinus maenas*)	Yes	5	British Columbia
ar-ERSI	Chinese mitten crab (*Eriocheir sinensis*)	No	1	British Columbia
ar-ORRU	Red spotted crayfish (*Orconectes rusticus*)	No	1	British Columbia
ar-PALE-kl	Signal crayfish (*Pacifastacus leniusculus klamathensis*)	No	1	British Columbia
ar-PAFO	Shasta crayfish (*Pacifastacus fortis*)	No	1	British Columbia
ar-PAGA	Pilose crayfish (*Pacifastacus gambelii*)	No	1	British Columbia
ar-PACO	Snake River pilose crayfish (*Pacifastacus connectens*)	No	1	British Columbia
ma-HOSA	Human (*Homo sapiens*)	No	1	Netherlands
ma-FECA	Domestic cat (*Felis catus*)	No	1	British Columbia
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

earCAMA1

Mean Copy Estimate vs Starting copy number (ML fit)

earCAMA1

Proportion detect vs Starting copy number (ML fit)

earCAMA1

LOD vs m, number of replicates

earCAMA1

LOQ vs m, number of replicates

Binomial-Poisson model: ___No intercept___
Determined using eLowQuant R code[4].
Based on a 2 µL DNA input in a total 15 µL reaction

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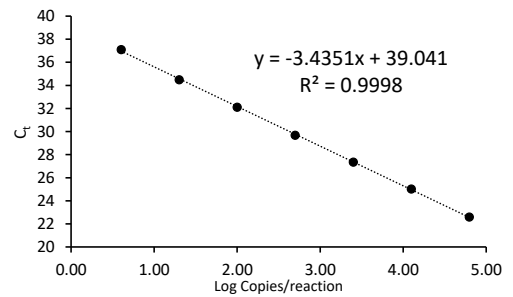https://onlineacademiccommunity.uvic.ca/helbinglab/edna/

From ___8___ Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.1	0.11
2	0.22	0.16
3	0.36	0.22
4	0.53	0.29
5	0.75	0.38
6	1.07	0.52
7	1.6	0.78

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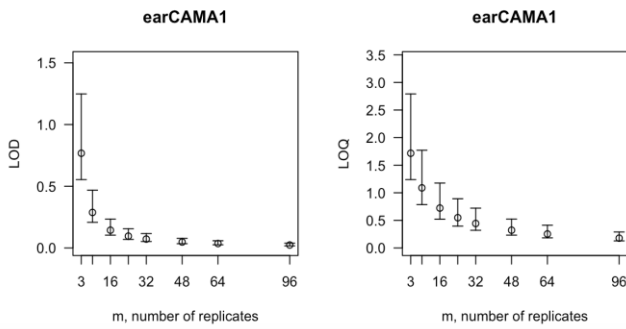
Applied to reactions with 100% positive hits

$y = -3.4351x + 39.041$
$R^2 = 0.9998$

C_t vs Log Copies/reaction

Efficiency	95%

Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-COII	Mitochondrial cytochrome oxidase subunit 2 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