Helbing Lab
eDNA Inventory

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: Capitella capitata | eDNA qPCR Tool: eCACAW4 | Gene Target: MT-ND5
Species Code: an-CACAW | eDNA qPCR Format: TaqMan | Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.4 | 95% CI 0.3-0.6 Copies/Rxn | LOQ 1.5 | 95% CI 1.1-2.4 Copies/Rxn | LOB 0 hits/8

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
an-CACAW	*Capitella capitata*	Y	3	British Columbia
an-HEFI	*Heteromastus filiobracnhus*	N	6	British Columbia
an-GLNA	*Glycera nana*	N	2	British Columbia
an-DEGR	*Decamastus gracilis*	N	5	British Columbia
an-PRMU	*Prionospio multibranchiata*	N	1	British Columbia
an-PRLI	*Prionospio lightii*	N	6	British Columbia
an-PRJU	*Prionospio jubata*	N	6	British Columbia
an-NOHE	*Notomastus hemipodus*	N	2	British Columbia
an-RIPI	*Ridgea piscescae*	N	1	British Columbia
ma-HOSA	Human (*Homo sapiens*)	N	1	Netherlands
ma-CAFA	Dog (*Canis lupus familiaris*)	N	1	British Columbia
ma-FECA	Cat (*Felis catus*)	N	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*
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
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.
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

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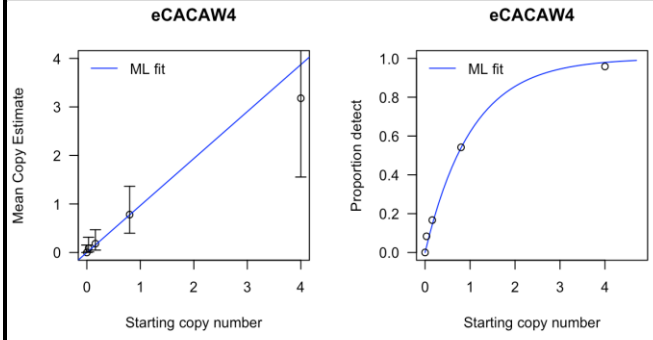
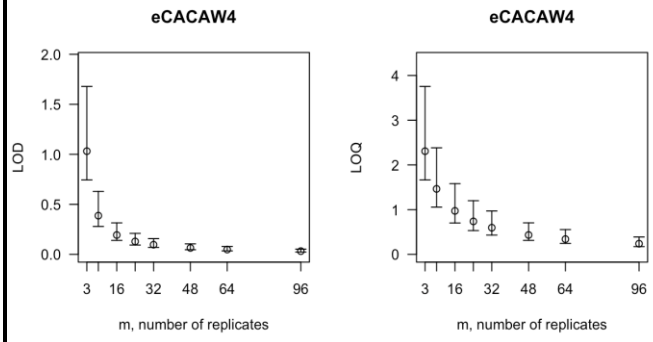
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA



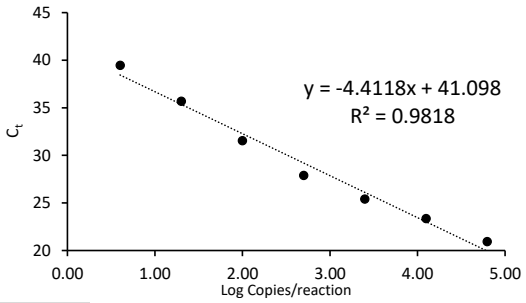
From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.14	0.14
2	0.3	0.22
3	0.49	0.3
4	0.72	0.39
5	1.01	0.51
6	1.43	0.69
7	2.15	1.06

Determined using eLowQuant R code[4].



Applied to reactions with 100% positive hits



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 69%

Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-ND5	Mitochondrial NADH dehydrogenase subunit 5 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