



### 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<sup>1-3</sup>.

#### 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 0.2 95% CI 0.2-0.4 Copies/Rxn LOQ 0.9 95% CI 0.6-1.6 Copies/Rxn LOB 0 hits/8

Binomial-Poisson model for 8 technical replicates determined using eLowQuant R code<sup>4</sup>. When the LOQ < LOD, use the LOD for the LOQ. Enzyme: Immolase

#### eDNA Assay Specificity Test Information

Each qPCR reaction in the specificity assay contained 10 picograms of voucher target gDNA (n=25 technical replicates)

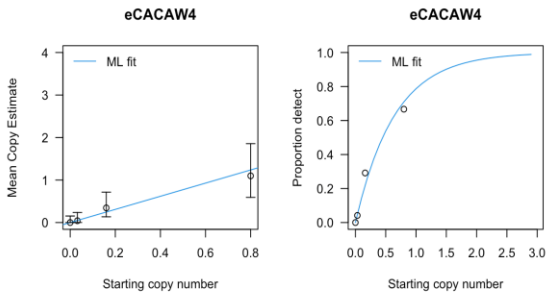
Species	Common Name ( <i>Species</i> )	Detection	# Voucher		Sample Sources/Locations
			Specimens		
an-CACAW	<i>Capitella capitata</i>	Y	3		British Columbia
an-HEFI	<i>Heteromastus filiobranchus</i>	N	6		British Columbia
an-GLNA	<i>Glycera nana</i>	N	2		British Columbia
an-DEGR	<i>Decamastus gracilis</i>	N	5		British Columbia
an-PRMU	<i>Prionospio multibranchiata</i>	N	1		British Columbia
an-PRLI	<i>Prionospio lightii</i>	N	6		British Columbia
an-PRJU	<i>Prionospio jubata</i>	N	6		British Columbia
an-NOHE	<i>Notomastus hemipodus</i>	N	2		British Columbia
an-RIPI	<i>Ridgea piscescae</i>	N	1		British Columbia
ma-HOSA	Human ( <i>Homo sapiens</i> )	N	1		Netherlands
ma-CAFA	Dog ( <i>Canis lupus familiaris</i> )	N	1		British Columbia
ma-FECA	Cat ( <i>Felis catus</i> )	N	1		British Columbia

#### 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. 2020; 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



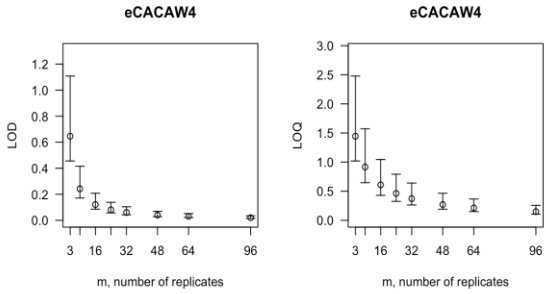
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA



From 8 Technical Replicates

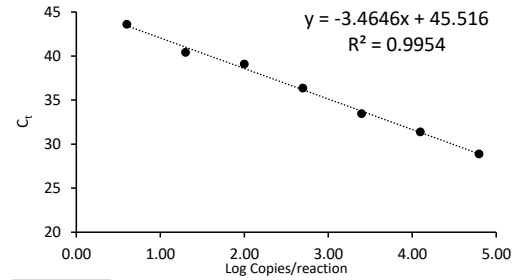
# Detects	# Copies	SE
0	0	0
1	0.09	0.09
2	0.19	0.14
3	0.3	0.19
4	0.45	0.25
5	0.63	0.33
6	0.9	0.44
7	1.35	0.67

Determined using eLowQuant R code<sup>4</sup>.



Binomial-Poisson model:                      No intercept  
 Determined using eLowQuant R code<sup>4</sup>.  
 Based on a 2 µL DNA input in a total 15 µL reaction

Applied to reactions with 100% positive hits



Efficiency 94%

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