



### 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: Great white shark (*Carcharodon carcharias*) eDNA qPCR Tool: echCACA2 Gene Target: MT-ND5  
Species Code: ch-CACA eDNA qPCR Format: TaqMan Published in: \_\_\_\_\_

#### eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.2 95% CI 0.1-0.3 Copies/Rxn LOQ 0.7 95% CI 0.3-1.2 Copies/Rxn LOB 0 hits/8  
LOQ<sub>continuous</sub> 4 Copies/Rxn

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: 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
ch-CACA	Great white shark ( <i>Carcharodon carcharias</i> )	Yes	6	California
ch-GAGA	School/southern shark ( <i>Galeorhinus galeus</i> )	No	1	Unknown
ch-ISOX	Shortfin mako shark ( <i>Isurus oxyrinchus</i> )	No	1	Unknown
ch-LADI	Salmon shark ( <i>Lamna ditropis</i> )	No	2	Unknown
ch-PRGL	Blue shark ( <i>Prionace glauca</i> )	No	4	Unknown
ot-HEGR	Bluntnose sixgill shark ( <i>Hexanchus griseus</i> )	No	2	Unknown
ma-CALUfa	Canine ( <i>Canis lupus familiaris</i> )	No	1	British Columbia
ma-FECA	Cat ( <i>Felis catus</i> )	No	1	British Columbia
ma-HOSA	Human ( <i>Homo sapiens</i> )	No	1	Netherlands
te-HIST	Pacific halibut ( <i>Hippoglossus stenolepis</i> )	No	1	British Columbia
te-CLPA	Pacific herring ( <i>Clupea pallasii</i> )	No	1	British Columbia
te-SERU	Yelloweye rockfish ( <i>Sebastes ruberrimus</i> )	No	1	British Columbia

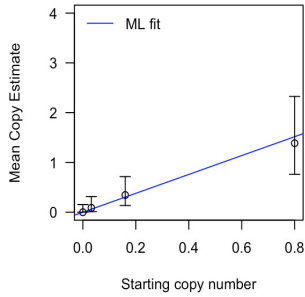
#### References

- 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, 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

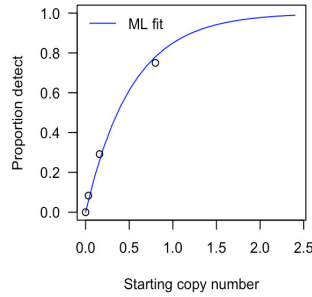


eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA

echCACAA2



echCACAA2

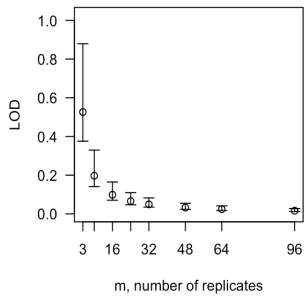


From 8 Technical Replicates

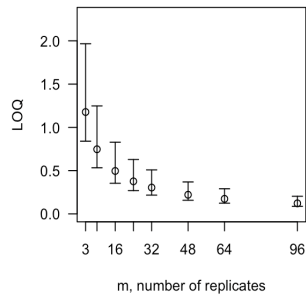
# Detects	# Copies	SE
0	0	0
1	0.07	0.07
2	0.15	0.11
3	0.25	0.15
4	0.37	0.2
5	0.52	0.26
6	0.73	0.37
7	1.1	0.54

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

echCACAA2



echCACAA2

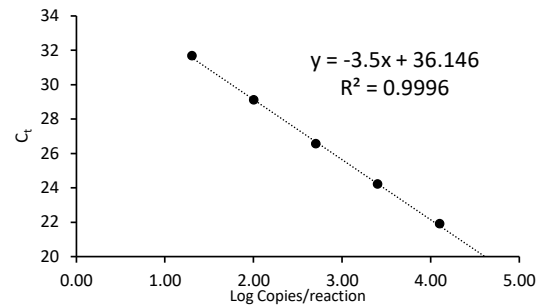


Binomial-Poisson model:            No intercept

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 92%

Field Sample Validation

Sample Type	Known		Detected	Location
	Presence	# Samples		

Abbreviations

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