



### 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: Coastal Tailed Frog (*Ascaphus truei*) eDNA qPCR Tool: eASTR4 Gene Target: MT-CYB  
Species Code: am-ASTR eDNA qPCR Format: TaqMan Published in: 2

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

LOD 0.8 95% CI 0.6-1.3 Copies/Rxn LOQ 3.1 95% CI 2.2-5.1 Copies/Rxn LOB 0 hits/8  
LOQ<sub>continuous</sub> 20 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: Immolase

#### eDNA Assay Specificity Test Information

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

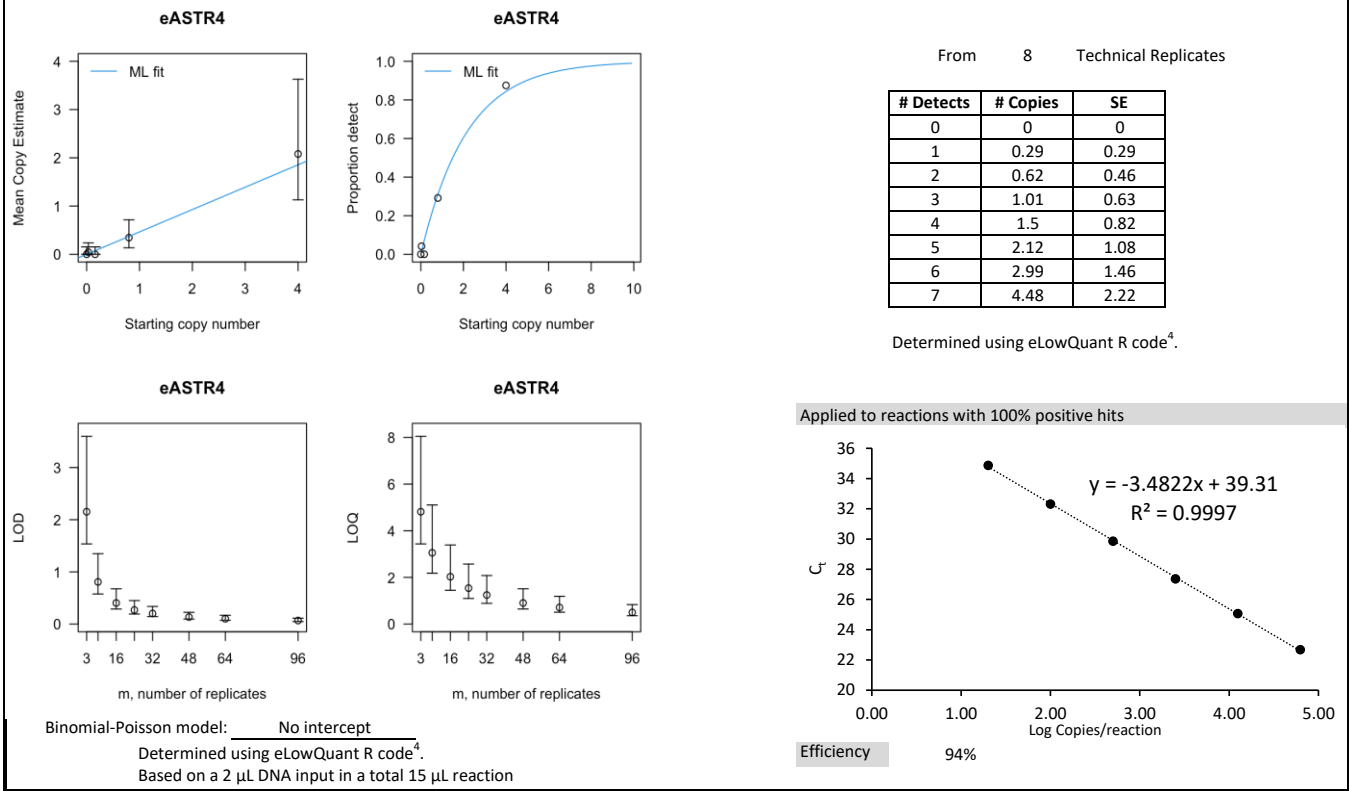
Species	Common Name ( <i>Species</i> )	Detection	# Voucher		Sample Sources/Locations
			Specimens		
am-ANBO-VI	Western Toad ( <i>Anaxyrus (Bufo) boreas</i> ) - Sourced from Vancouver	No	1		British Columbia
am-ASMO	Rocky Mountain Tailed Frog ( <i>Ascaphus montanus</i> )	No	1		British Columbia
am-ASTR	Coastal Tailed Frog ( <i>Ascaphus truei</i> )	Yes	5		British Columbia
am-LICA	Bullfrog ( <i>Lithobates (Rana) catesbeiana</i> )	No	1		British Columbia
am-PSRE	Pacific Chorus Frog ( <i>Pseudacris (Hyla) regilla</i> )	No	1		British Columbia
am-RAAU	Northern Red-legged Frog ( <i>Rana aurora</i> )	No	1		British Columbia
am-RALU	Columbia Spotted Frog ( <i>Rana luteiventris</i> )	No	1		British Columbia
am-TAGR	Rough-skinned Newt ( <i>Taricha granulosa</i> )	No	1		British Columbia
am-XELA	African Clawed Frog ( <i>Xenopus laevis</i> )	No	1		South Africa
ma-HOSA	Human ( <i>Homo sapiens</i> )	No	1		Netherlands

#### 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



Field Sample Validation

Sample Type	Known		Detected	Location	Reference
	Presence	# Samples			
Water	Y	55	Y	British Columbia	2

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-CYB	Mitochondrial cytochrome B 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