

### 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: Fisher (*Pekania pennanti*) eDNA qPCR Tool: ePEPE2 Gene Target: MT-CYB  
Species Code: ma-PEPE eDNA qPCR Format: TaqMan Published in:

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

LOD 0.7 95% CI 0.5-1.2 Copies/Rxn LOQ 2.6 95% CI 1.9-4.4 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: 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		Sample Sources/Locations
			Specimens		
ma-PEPE	Fisher ( <i>Pekania pennanti</i> )	Yes	5		British Columbia
ma-ALAL	Moose ( <i>Alces alces</i> )	No	1		British Columbia
ma-ANPA	Pallid bat ( <i>Antrozous pallidus</i> )	No	1		British Columbia
ma-CALU	Wolf ( <i>Canis lupus</i> )	No	1		British Columbia
ma-CALUfa	Dog ( <i>Canis lupus familiaris</i> )	No	1		British Columbia
ma-CEEL	Red deer ( <i>Cervus elaphus</i> )	No	1		British Columbia
ma-ESRO	Gray whale ( <i>Eschrichtius robustus</i> )	No	1		British Columbia
ma-FECA	Cat (domestic) ( <i>Felis catus</i> )	No	1		British Columbia
ma-HOSA	Human ( <i>Homo sapiens</i> )	No	1		Netherlands
ma-LOCA	River otter ( <i>Lontra canadensis</i> )	No	1		British Columbia
ma-MUMU	House mouse ( <i>Mus musculus</i> )	No	1		British Columbia
ma-ODHE	Mule deer ( <i>Odocoileus hemionus</i> )	No	1		British Columbia
ma-ODVI	White-tailed deer ( <i>Odocoileus virginianus</i> )	No	1		Washington
ma-ORCU	European rabbit ( <i>Oryctolagus cuniculus</i> )	No	1		British Columbia
ma-PHPH	Harbour porpoise ( <i>Phocoena phocoena</i> )	No	1		British Columbia
ma-PHSO	Southern elephant seal ( <i>Phoca leonina</i> )	No	1		British Columbia
ma-PHVI	Harbour seal ( <i>Phoca vitulina</i> )	No	1		British Columbia
ma-SCCA	Eastern grey squirrel ( <i>Sciurus carolinensis</i> )	No	1		British Columbia
ma-SOBE	Pacific water/marsh shrew ( <i>Sorex bendirii</i> )	No	1		Washington
ma-SONA	Cardilleran water shrew ( <i>Sorex navigator</i> )	No	1		Washington
ma-URAM	American black bear ( <i>Ursus americanus</i> )	No	1		British Columbia
ma-USAR	Grizzly bear ( <i>Ursus arctos</i> )	No	1		British Columbia
ma-ZACA	California sea lion ( <i>Zalophus californianus</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**

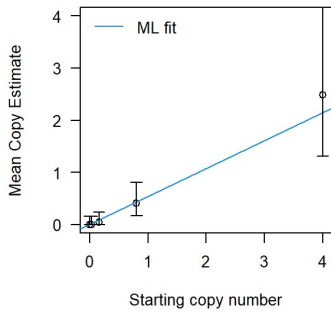
To calculate tables for different numbers of replicates, raw csv data files can be accessed here:  
<https://onlineacademiccommunity.uvic.ca/helbinglab/edna/>

From 8 Technical Replicates

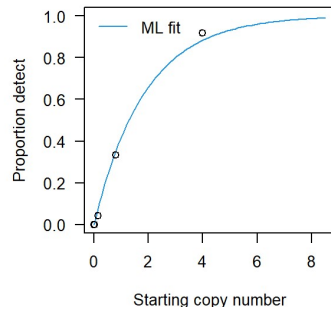
# Detects	# Copies	SE
0	0.00	0.00
1	0.25	0.25
2	0.54	0.40
3	0.87	0.54
4	1.29	0.71
5	1.82	0.93
6	2.58	1.25
7	3.87	1.91

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

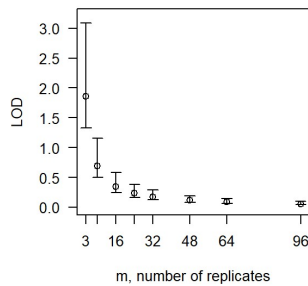
**ePEPE2**



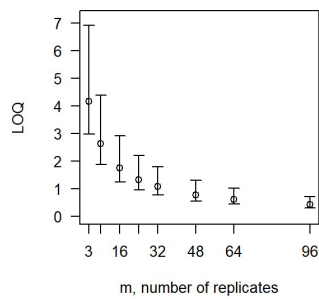
**No intercept ePEPE2**



**Limits detect - no intercept ePEPE2**



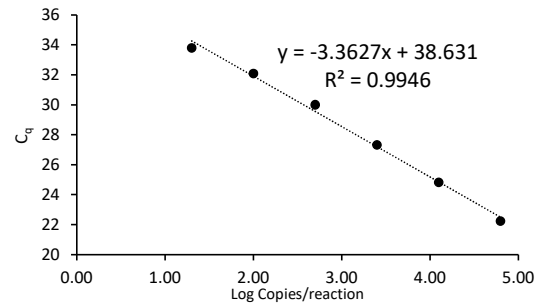
**Limits quant - no intercept ePEPE2**



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



**Field Sample Validation**

Sample Type	Known Presence	# Samples	Detected	Location

**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