



### Helbing/Langlois 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: Cougar (*Puma concolor*) eDNA qPCR Tool: ma-ePUCO13 Gene Target: MT-ND1  
Species Code: ma-PUCO eDNA qPCR Format: TaqMan Published in: \_\_\_\_\_

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

LOD 0 95% CI 0-0 Copies/Rxn LOQ 0.1 95% CI 0.1-0.1 Copies/Rxn LOB 0 hits/8  
LOQ<sub>continuous</sub> 0.8 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)

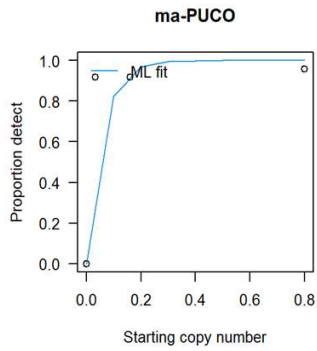
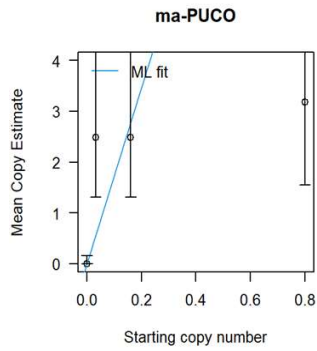
Species	Common Name ( <i>Species</i> )	Detection	# Voucher		Sample Sources/Locations
			Specimens		
ma-PUCO	Cougar ( <i>Puma concolor</i> )	Yes	6		MFFP
ma-CALUfa	Canine ( <i>Canis lupus familiaris</i> )	No	1		INRS
ma-FECA	Cat ( <i>Felis catus</i> )	No	1		INRS
ma-HOSA	Human ( <i>Homo sapiens</i> )	No	1		INRS
ma-LYCA	Canada Lynx ( <i>Lynx canadensis</i> )	No	6		MFFP
ma-LYRU	Bobcat ( <i>Lynx rufus</i> )	No	6		MFFP

#### 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

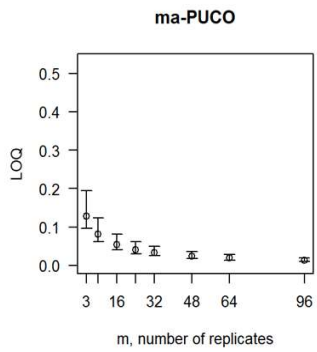
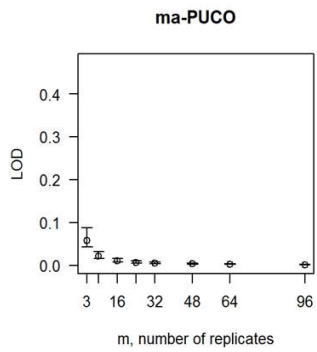


To generate tables for different numbers of replicates, use raw csv data files.

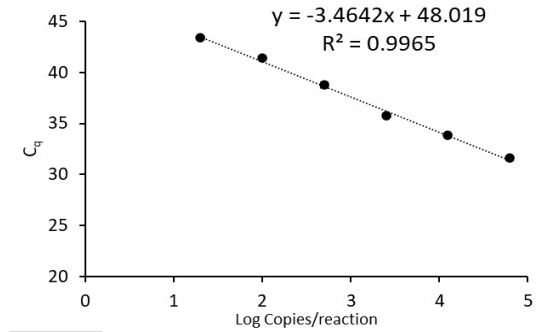
From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.008	0.008
2	0.017	0.012
3	0.027	0.016
4	0.04	0.022
5	0.057	0.028
6	0.08	0.038
7	0.12	0.058

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



Applied to reactions with  $\geq 95\%$  positive hits



Binomial-Poisson model: No intercept

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

Based on a 2  $\mu$ L DNA input in a total 15  $\mu$ L reaction

Efficiency 109%

Field Sample Validation

Sample Type	Known		Detected	Location
	Presence	# Samples		
Water	Y	1	Y	Zoo St-Felicien, Québec

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-ND1	Mitochondrial NADH dehydrogenase 1
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