

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 sensitivity¹⁻³.

General eDNA Assay Information

Target Species: Canada lynx (*Lynx canadensis*) eDNA qPCR Tool: eLYCA3 Gene Target: MT-ND5
Species Code: ma-LYCA eDNA qPCR Format: TaqMan Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.1 95% CI 0.1-0.2 Copies/Rxn LOQ 0.3 95% CI 0.2-0.7 Copies/Rxn LOB 0 hits/8

LOQ_{continuous} 0.8 Copies/Rxn

Binomial-Poisson model for 8 technical replicates determined using eLowQuant R code⁴. 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-LYCA	Canada lynx (<i>Lynx canadensis</i>)	Yes	3		British Columbia
ma-LYRU	Bobcat (<i>Lynx rufus</i>)	No	4		British Columbia
ma-PUCO	Cougar (<i>Puma concolor</i>)	No	1		British Columbia
ma-CALU	Wolf (<i>Canis lupus</i>)	No	1		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-CALA	Coyote (<i>Canis latrans</i>)	No	1		British Columbia
ma-CEEL	Red deer (<i>Cervus elaphus</i>)	No	1		British Columbia
ma-GUGU	Wolverine (<i>Gulo gulo</i>)	No	1		British Columbia
ma-LEAM	Snowshoe hare (<i>Lepus americanus</i>)	No	1		British Columbia
ma-MUMU	House mouse (<i>Mus musculus</i>)	No	1		British Columbia
ma-NEVI	American mink (<i>Neovision vision</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		British Columbia
ma-RATAca	Woodland (boreal) Caribou (<i>Rangifer tarandus caribou</i>)	No	1		British Columbia
ma-SOBE	Pacific water/marsh shrew (<i>Sorex bendirii</i>)	No	1		British Columbia
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-VUVU	Fox (<i>Vulpes vulpes</i>)	No	1		British Columbia
ma-CALUfa	Dog (<i>Canis lupus familiaris</i>)	No	1		British Columbia
ma-FECA	Cat (domestic) (<i>Felis catus</i>)	No	2		British Columbia
ma-HOSA	Human (<i>Homo sapiens</i>)	No	1		Netherlands

References

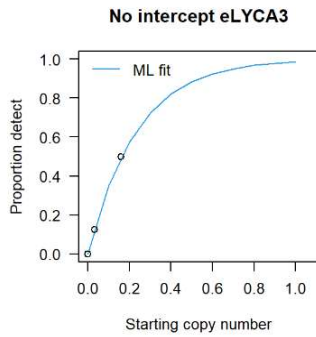
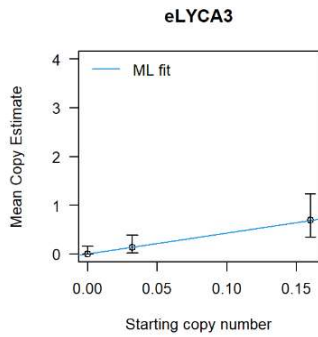
- Hobbs, J, Adams, IT, Round, JM, Goldberg, CS, Allison, MJ, Bergman, LC, Mirabzadeh, A, Allen, H, Helbing, CC (2020) Revising the range of Rocky Mountain tailed frog, *Ascaphus montanus*, in British Columbia, Canada, using environmental DNA methods. Environmental DNA, 2: 350-361. <https://doi.org/10.1002/edn3.82>
- 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/>

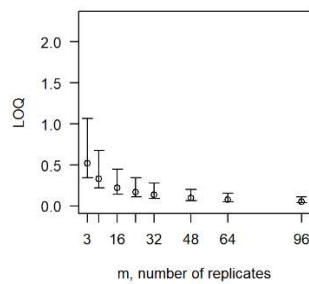
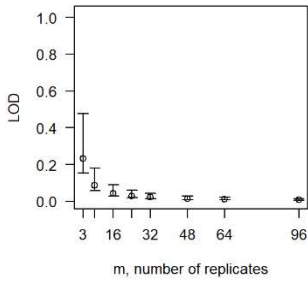
From 8 Technical Replicates



# Detects	# Copies	SE
0	0	0
1	0.031	0.032
2	0.067	0.051
3	0.109	0.07
4	0.161	0.092
5	0.228	0.122
6	0.323	0.166
7	0.484	0.252

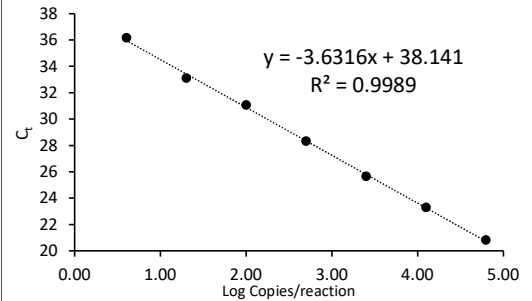
Limits detect - no intercept eLYCA3

Limits quant - no intercept eLYCA3



Determined using eLowQuant R code⁴.

Applied to reactions with 100% positive hits



Efficiency 89%

Binomial-Poisson model: No intercept

Determined using eLowQuant R code⁴.

Based on a 2 μ L DNA input in a total 15 μ L reaction

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 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