Helbing Lab
eDNA Inventory

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[1-3].

General eDNA Assay Information

Target Species: Northern sea otter (*Enhydra lutris*) | eDNA qPCR Tool: eENLU6 | Gene Target: MT-CYB
Species Code: ma-ENLU | eDNA qPCR Format: TaqMan | Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.3 | 95% CI 0.2-0.6 Copies/Rxn | LOQ 1.3 | 95% CI 0.9-2.1 Copies/Rxn | LOB 0 hits/8
$LOQ_{continuous}$ 20 Copies/Rxn

Binomial-Poisson model for 8 technical replicates determined using eLowQuant R code[4]. 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 (*Species*)	Detection	# Voucher Specimens	Sample Sources/Locations
ENLU	Northern sea otter (*Enhydra lutris*)	Yes	5	British Columbia
NEVI	American mink (*Neovision vision*)	No	5	British Columbia
BAAC	Minke whale (*Balaenoptera acutorostrata*)	No	2	British Columbia
CAUR	Northern fur seal (*Callorhinus ursinus*)	No	1	British Columbia
ESRO	Gray whale (*Eschruchtius robustus*)	No	2	British Columbia
EUJU	Stellar sea lion (*Eumetopias jubatus*)	No	2	British Columbia
LAOB	Pacific white-sided dolphin (*Lagenorhynchus obliquidens*)	No	2	British Columbia
LOCA	River otter (*Lontra canadensis*)	No	2	British Columbia
MENO	Humpback whale (*Megaptera movaeangliae*)	No	2	British Columbia
PHDA	Dall's porpoise (*Phocoenoides dalli*)	No	2	British Columbia
PHLE	Southern elephant seal (*Phoca leonina*)	No	1	Antarctica
PHPH	Harbour porpoise (*Phocoena phocoena*)	No	2	British Columbia
PHVI	Harbour seal (*Phoca vitulina*)	No	2	British Columbia
ZACA	California sea lion (*Zalophus californianus*)	No	2	British Columbia
URAM	American black bear (*Ursus americanus*)	No	2	British Columbia
URAR	Grizzly bear (*Ursus arctos*)	No	2	British Columbia
HOSA	Human (*Homo sapiens*)	No	1	Netherlands
CAFA	Canine (*Canis lupus familiaris*)	No	1	British Columbia
FECA	Cat (*Felis catus*)	No	1	British Columbia

References

1. 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

2. 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

3. 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

4. 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

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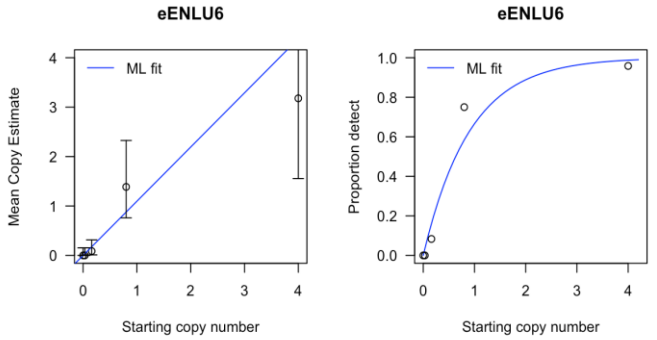
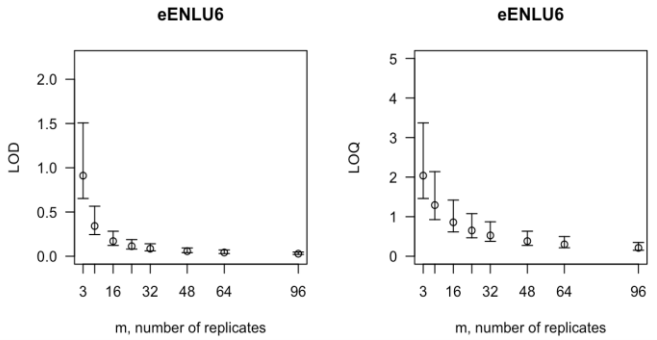
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA



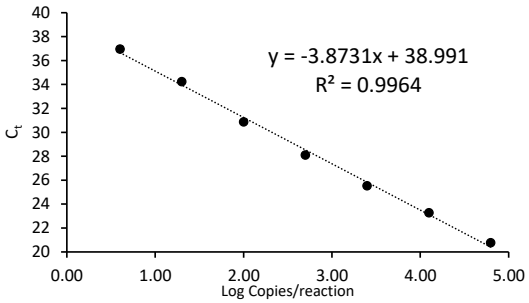
From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.12	0.12
2	0.26	0.19
3	0.43	0.26
4	0.63	0.35
5	0.9	0.45
6	1.26	0.61
7	1.9	0.94

Determined using eLowQuant R code[4].



Applied to reactions with 100% positive hits



Binomial-Poisson model: No intercept

Determined using eLowQuant R code[4].

Based on a 2 µL DNA input in a total 15 µL reaction

Efficiency 93%

Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location
Water	Y	2	Y	British Columbia
Water	Y	4	Y	British Columbia

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