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: Brainworm (*Parelaphostrongylus tenuis*)
Species Code: ne-PATE
eDNA qPCR Tool: ePATE1
eDNA qPCR Format: TaqMan
Gene Target: MT-COI
Published in:

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD 0.4 95% CI 0.3-0.6 Copies
LOQ 1.6 95% CI 1.1-2.5 Copies
$LOQ_{continuous}$ 20 Copies/Rxn
LOB 0 hits/8

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

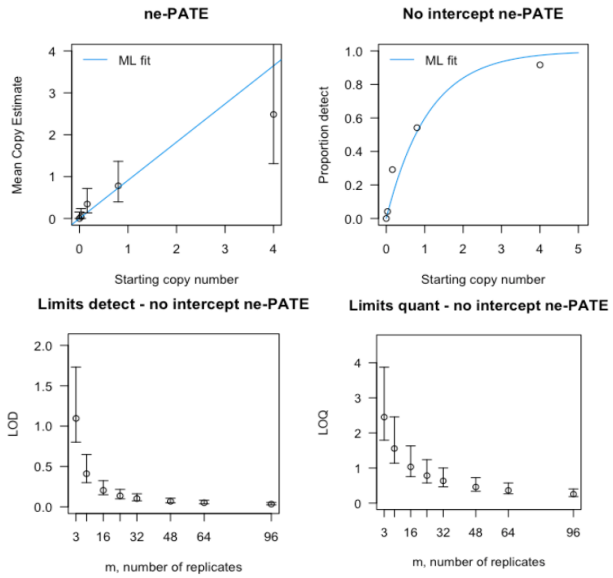
Each qPCR reaction in the specificity assay contained 10 picograms of voucher target gDNA (n=4 technical replicates)

Species	Common Name (*Species*)	Detection	# Voucher Specimens	Sample Sources/Locations
ne-PATE	Brainworm (*Parelaphostrongylus tenuis*)	Yes	4	Quebec
ma-ODVI	White-tailed deer (*Odocoileus virginianus*)	No	4	Quebec
ma-ALAM	American moose (*Alces americanus*)	No	4	Quebec
ma-HOSA	Human (*Homo sapiens*)	No	1	Cell line ATCC
ma-CALUfa	Domestic dog (*Canis lupus familiaris*)	No	1	Quebec
ma-FECA	Domestic cat (*Felis catus*)	No	1	Quebec

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. 2020; 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

eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA



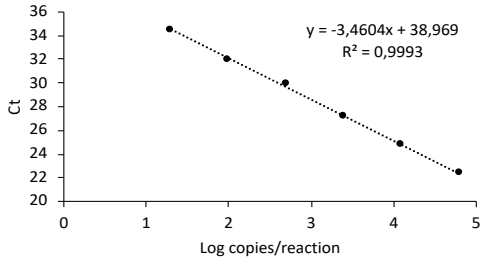
To calculate tables for different numbers of replicates, use raw csv data files.

From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0,146	0,149
2	0,315	0,231
3	0,516	0,316
4	0,76	0,413
5	1,076	0,54
6	1,521	0,73
7	2,281	1,111

Determined using eLowQuant R code[4].

Applied to reactions with ≥ 95% 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 95%

Field Sample Validation

Sample Type	Known Presence	# Samples	Detected	Location

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-COI	Mitochondrial cytochrome oxidase subunit 1 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