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: Chloroplasts (Plant/Algae)	eDNA qPCR Tool: ePlant5	Gene Target: 23S
Species Code: IntegritE-DNA	eDNA qPCR Format: TaqMan	Published in: 1, 2, 5

eDNA Assay Sensitivity Test Summary using gBlocks™ Synthetic DNA

LOD N/A 95% CI N/A Copies LOQ N/A 95% CI N/A Copies LOB N/A 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: SensiFAST Probe

eDNA Assay Specificity Test Information

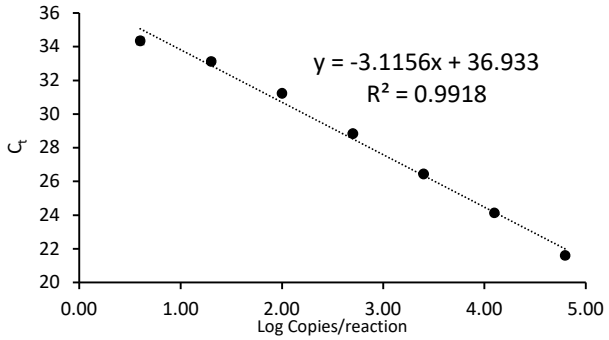
Each qPCR reaction in the specificity assay contained 10 picograms of voucher target gDNA (n=25 technical replicates)

Chloroplast DNA is used as an endogenous quality control for eDNA samples when testing for animal DNA.
A broad range of plants and algae from fresh water, marine water, sediments, air, and soil have been tested and pass.
Fresh, unopened bottled water typically does not have plant or algae DNA in it.
Typically the C_q cutoff is 27 for pass/fail for most applications.

"Fail" indicates poor quality sample with inhibition and/or degradation.
Refer fo references 1 and 2 for application examples.

The continuous standard curve is based upon n=8 technical replicates with very small standard error.
Based on a 2 µL DNA input in a total 15 µL reaction

Applied to reactions with 100% positive hits



Efficiency 100%

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

5. Veldhoen, N., Hobbs, J., Ikonomou, G., Hii, M., Lesperance, M., and Helbing, C.C. 2016. Implementation of novel design features for qPCR-based eDNA assessment. PLoS One, 11: e0164907. DOI: 10.1371/journal.pone.0164907

Abbreviations

23S	23S ribosomal RNA	NTC	qPCR no template control
eDNA	Environmental DNA	qPCR	Quantitative real-time polymerase chain reaction
gDNA	Total genomic DNA extracted from voucher specimen	SE	Standard error