Supplementary Material 2. qPCR optimization protocols per assay

UIBK: Sperm whale assay
Gradient qPCR to optimize melting temperature. Triplicate dilution series of sperm whale DNA (1:10 1 ng/µL, 6 points and one No Template Control (NTC) and nine Negative Controls were included on the plate.

Reagent	Volume per reaction
2x TaqMan Environmental MM	5 µL
Primer/probe SW	1 µL
Nuclease-free water	1 µL
DNA extract or +/- Control	3 µL

Step		Temp. (deg C)	Time (min:sec)	Ramping Rate (deg C/sec)
Enzyme Activation	Hold	95	10:00	4
Denaturation	40 cycles	95	00:15	1.6
Annealing/Extension		57.5 - 62	01:30	1.6

Target/nontarget testing
Nontarget species used to test for primer specificity in qPCR: Dwarf sperm whale (*Kogia sima*), Common dolphin (*Delphinus delphis*), Harbour porpoise (*Phocoena phocoena*), Fin whale (*Balaenoptera physalus*), Grey seal (*Halichoerus grypus*) at 1 ng/µL and 0.1 ng/µL.

INRAE: Porbeagle shark assay
Gradient qPCR to optimize melting temperature. Triplicate dilution series of porbeagle shark DNA (1:10 0.526 ng/µL, 6 points), one No Template Controls (NTC) and one Negative Control were included on the plate.

Reagent	Volume per reaction
1x TaqMan Environmental MM	10 µL
Primer LnND1-F209 and LnND1-R380 (0.5 µM)	1 µL
Probe LnND1-P242 (0.13 µM)	1 µL
Nuclease-free water	4 µL

Sample or +/- Control	3 µL

Step		Temp. (deg C)	Time (min:sec)	Ramping Rate (deg C/sec)
Enzyme Activation	Hold	95	10:00	Default
Denaturation	49 cycles	95	00:30	Default
Annealing/Extension		52-66	00:50	Default

Target/nontarget testing
Nontarget species used to test for primer specificity: Nursehound (*Scyliorhinus stellaris*) at 1 ng/µL and 0.1 ng/µL

UCC: Dolphin assay
Target/nontarget testing
Nontarget species used to test for primer specificity: Common dolphin and harbour porpoise at 5 ng/µL and 0.5 ng/µL.

IMR: Basking shark assay
Target/nontarget testing
Nontarget species used to test for primer specificity in qPCR: porbeagle shark (*lamna nasus*) at 1 ng/µL and 0.1 ng/µL.