

Table S3. Comparison of unique primer sequences created by combination of primers with low number of degenerated sites Vs. one primer including all degenerations

Name	S/As	Sequence (5' to 3')	Length (nt)	Number of unique primer sequences
FW1	S	ACGCTTAACAAC M ARATCAAAGAA	24	4
FW2	S	ACGCTTAACAACAAAATC A DARAAG	25	6
FW3	S	ACGCTTAACGACAAAA H CAGARAAG	25	6
FW4	S	ACGCTTAACAGCTAAAA A CYAGAAG	25	2
FW5	S	ACGCTTAAC A RCAAAATCTTATAAG	25	2
Total number of unique primer sequences using 5 FW primers with low number of degenerations				20
Hypothetical FW primer using one highly degenerated primer	S	ACGCTTAAC R RCHARAH M HNADAA	24	5184
REV1*	As	C MGGGTAYTTRTAYTCATAYTGRTC	25	64
REV2	As	CTGGATATTTGTAYTCATAYTGATC	25	4
REV3	As	CAGGATATTTATATTCATACTGGTC	25	1
Total number of unique primer sequences using 3 REV primers with low number of degenerations				69
Hypothetical REV primer using one highly degenerated primer	As	C MGGRTAYTTRTAYTCATAYTGRTC	25	128
Probe1*	S	AACAC C YCTACAATGGA	17	2
Probe2*	S	AACACTACTACAATGGA	17	1
Total number of unique primer sequences using 2 probes with low number of degenerations				3
Hypothetical Probe using one highly degenerated probe	S	AACAC Y YCTACAATGGA	17	4

Oligonucleotides with * are identical to original protocol. S: sense primers, As: antisense primers. Number of unique primer sequences generated by “primer3” in Geneious Prime® 2022.1.1 (Biomatters, Auckland, New Zealand. Degenerated sites are shown **in bold**.