Table 2

Region	Name	Sequence (5'→3')	Tm (°C)	Amplicon (bp)
Large sequence (L)	Vac-ex_LF	CCATCGAGTCTTTGAACGCA	57.3	275
	Vac-ex_LR	CACTTCAGGGTCAAATGGGC	59.4	
Small sequence (S)	Vac-ex_sF	GCATTGCGTCACCCACTC	58.2	131
	Vac-ex_sR	ACTTGTCGTTGAATGTCCGTCA	57.3	
Large sequence 2 (L2)	Frw_Large 2	TTGCAGAATCCCGTGAACCA	57.3	230
	Rev_Small 2	TTTAGCAACCACCACTTGTCGT	58.4	
Small sequence 2 (S2)	Frw_Small 2	TGAAGGCACGTCTGCCTG	58.2	~~162~~170
	Rev_Small 2	TTTAGCAACCACCACTTGTCGT	58.4	

Table 3

Primer set	Sample	Cq	Cq Mean	Cq Std. Dev
L2	1072/10/12	25.61	25.37	0.212
		25.28		
		25.21		
S2	1072/10/12	26.17	25.89	0.247
		25.70		
		25.80		
L	1072/10/12	24.88	25.11	0.203
		25.20		
		25.25		

Table 5

Probe	Sample	Expected result	Result
M-FAM	*V. myrtillus*	Amplification of *V. myrtillus*	Figure 4
	E. nigrum		
	S. nigra		
	V. oxycoccos		
	V. corymbosum		
	V. macrocarpon		
M-FAM	*V. myrtillus* 100%	~~No~~ Amplification	Figure 5(a)
	E. nigrum 100%	No Amplification	
	V. myrtillus 95% *E. nigrum* 5%	Proportional amplification with the percentage of *V. myrtillus*	
	V. myrtillus 98% *E. nigrum* 2%		
	V. myrtillus 99% *E. nigrum* 1%		
E- HEX	*V. myrtillus* 100%	No Amplification	Figure 5(b)
	E. nigrum 100%	Amplification	
	V. myrtillus 95% *E. nigrum* 5%	Proportional amplification with the percentage of *E. nigrum*	
	V. myrtillus 98% *E. nigrum* 2%		
	V. myrtillus 99% *E. nigrum* 1%		

The amplification results were proportional to the content of the target species (**Figure 6**).

Example 2 – *Vaccinium myrtillus* dry-extract residual DNA identification

For each sample, two independent isolations of residual DNA were performed (biological replicates) and for each extracted DNA three technical replicates were tested, **Figure 7**.

The whole procedure was initially performed on four samples: 32549/H76, 32549/H80, 32549/H83, 32549/H84. After the residual DNA isolation and quantification (**Table 6**), these