

Supplement legends

Table S1. Cycle threshold mean (Ct), standard deviation (SD), and coefficient of variation (CV%) using qPCR with probe-based fluorescent. GOI refers to gene of interest, and plus (+) and minus (-) signs indicate positive (+) and negative (-) for GOI.

Table S2. Concentration (Conc, copies/uL), standard deviation (SD), and CV using ddPCR with fluorescent probe-based. GOI refers to gene of interest, and plus (+) and minus (-) signs indicate positive (+) and negative (-) for GOI.

Table S3. Oligonucleotide primer and probe sequences of the exogenous constructs and endogenous reference gene.

Fig. S1. Standard Curve assay for *LpCul4* (a), 1SST-6G-FFT (b), and *hph* (c), using qPCR with SYBR Green I fluorescence.

Fig. S2. Standard curve assay for 1SST-6G-FFT construct (FAM in blue) and *LpCul4* (HEX in green) (a), and *hph* (FAM in blue) and *LpCul4* (HEX in green) (b) using qPCR with fluorescent probes.

Fig. S3. 1D fluorescence amplitude plot of the ddPCR assay using a gradient of temperature (65-55°C) for 1SST-6G-FFT construct (FAM in blue) and *LpCul4* (HEX in green) (a), and *hph* (FAM in blue) and *LpCul4* (HEX in green) (b).

Fig. S4

Detection and quantification of *hph* construct, using qPCR with SYBR Green I fluorescence (a), and fluorescent probes (b). In the probe-base assay, the target construct (*hph*, FAM) is shown in blue and the reference gene (*LpCul4*, HEX) in green. The Y-axis shows relative fluorescence unit (RFU), and the X-axis denotes PCR cycle number.

Fig. S5

Detection and quantification of *hph* construct (FAM in blue) using ddPCR. **(a)** 1D fluorescence amplitude plot, where set threshold is shown with a pink line, blue plots indicate presence of the *hph* sequence in the droplet, and grey plots indicate absence of the sequence. **(b)** Ratio of *hph* construct and *LpCul4* (HEX). UT and T stand for untransformed and transformed, respectively. Error bars indicate the Poisson 95% confidence intervals for each measurement.

Fig. S6

Limit of detection and limit of quantification of 1SST-6G-FFT construct (FAM in blue) **(a)** and *hph* (FAM in blue) **(b)** using *LpCul4* (HEX in green) as the reference gene in a qPCR probe-based assay.

Fig. S7

Limit of detection and limit of quantification of *hph* construct (FAM in blue) and *LpCul4* (HEX in green) as reference gene, using droplet digital PCR. Blue and green plots indicate the concentration of positive droplets (counts/ μ L; Y-axis on the left side) for the *hph* and *LpCul4* sequences, respectively, and the average concentration is shown the left side of each plot. Orange plots show the copy number ratio of *hph* (Y-axis on the right side), which was calculated through the average concentration of FAM-positive droplets divided with that of HEX-positive droplets. Error bars indicate the Poisson 95% confidence intervals for each measurement.

Fig. S8

Melting curve assay and TapeStation of qPCR products for 1SST-6G-FFT (a and b respectively), and *hph* (c and d) and using SYBR Green I fluorescence.

Table S1

Tissue	GOI	Primers	Mean Ct	SD	CV (%)
Leave	-	1SST-6G-FFT	35.0	0.0	0.0
		LpCull4	24.0	0.4	1.7
Leave	+	1SST-6G-FFT	25.7	0.7	2.6
		LpCull4	24.2	0.6	2.5
Tiller	-	1SST-6G-FFT	34.9	0.3	0.8
		LpCull4	24.5	0.6	2.6
Tiller	+	1SST-6G-FFT	25.6	0.8	3.2
		LpCull4	24.2	0.7	2.9
Silage	-	1SST-6G-FFT	34.4	0.8	2.3
		LpCull4	27.7	1.6	5.7
Silage	+	1SST-6G-FFT	30.7	1.0	3.3
		LpCull4	28.2	0.8	2.8
Hay	-	1SST-6G-FFT	35.0	0.0	0.0
		LpCull4	23.1	0.6	2.8
Hay	+	1SST-6G-FFT	24.2	0.6	2.7
		LpCull4	23.1	0.6	2.6
Seed	-	1SST-6G-FFT	35.0	0.0	0.0
		LpCull4	24.9	0.2	1.0
Seed	+	1SST-6G-FFT	29.0	1.3	4.5
		LpCull4	25.0	0.4	1.6
Pollen	-	1SST-6G-FFT	35.0	0.0	0.0
		LpCull4	23.4	1.2	5.1
Pollen	+	1SST-6G-FFT	27.0	0.5	1.7
		LpCull4	24.6	1.6	6.5

Table S2

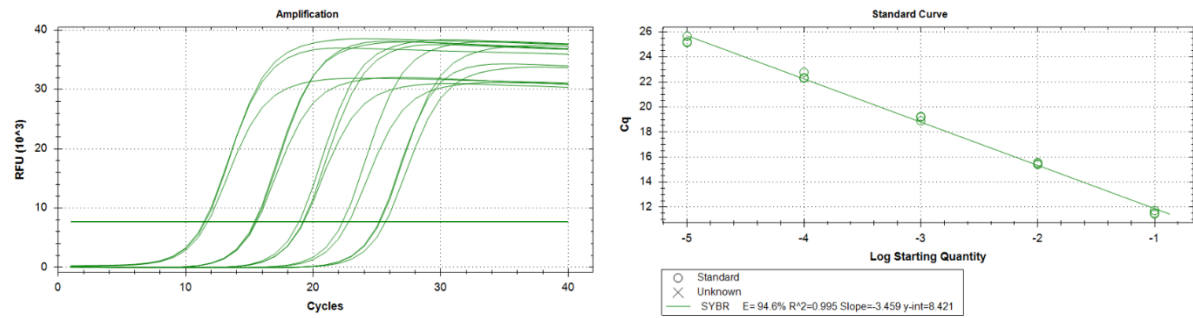
Tissue	GOI	Primers	Conc(copies/uL)	SD	CV (%)
Leave	-	1SST-6GFFT	3.8	0.1	1.8
		LpCull4	474.6	14.7	3.1
Leave	+	1SST-6GFFT	287.8	5.8	2.0
		LpCull4	463.4	22.9	4.9
Tiller	-	1SST-6GFFT	0.3	0.0	5.8
		LpCull4	495.8	8.9	1.8
Tiller	+	1SST-6GFFT	305.3	2.2	0.7
		LpCull4	547.6	8.0	1.5
Silage	-	1SST-6GFFT	3.8	0.2	4.0
		LpCull4	25.9	0.5	2.1
Silage	+	1SST-6GFFT	26.4	0.5	1.9
		LpCull4	22.0	1.0	4.5
Hay	-	1SST-6GFFT	0.4	0.0	8.5
		LpCull4	774.2	3.7	0.5
Hay	+	1SST-6GFFT	381.6	5.7	1.5
		LpCull4	754.2	25.8	3.4
Seed	-	1SST-6GFFT	2.6	0.2	7.1
		LpCull4	303.6	5.8	1.9
Seed	+	1SST-6GFFT	108.0	1.7	1.6
		LpCull4	316.3	5.8	1.8
Pollen	-	1SST-6GFFT	71.3	1.0	1.4
		LpCull4	162.5	4.0	2.5
Pollen	+	1SST-6GFFT	42.0	2.3	5.4
		LpCull4	57.6	3.0	5.2

Table S3

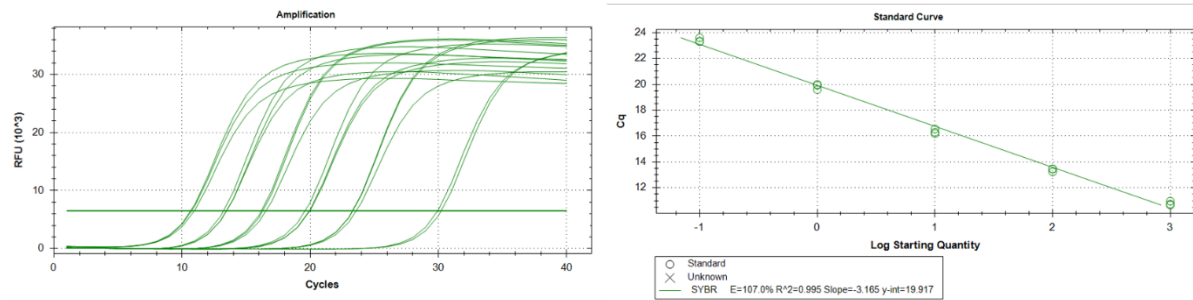
Target	Primer	Sequence (5'–3')	Product (bp)
Endogene	<i>LpCul4</i> -f	CCGATTAAGCCAGCGGATA	122
	<i>LpCul4</i> -r	CATCTCTTTCATGGCTGTCA	
	<i>LpCul4</i> -P (HEX)	ACCTGGAGAGAGACCGAAGTA	
Exogene	<i>Lp1SST-6G-FFT</i> -f	AGGCATAGCCCAGCTAGTTA	137
	<i>Lp1SST-6G-FFT</i> -r	CGCGTACGCATAAGGAAGCA	
	<i>Lp1SST-6G-FFT</i> -P(FAM)	CCCGCGGTGAATTCATGGAGTCCC	
Exogene	<i>Hph</i> -f	ATTTTCGGCTCCAACAATGTC	105
	<i>Hph</i> -r	AGATGTTGGCGACCTCGTAT	
	<i>Hph</i> -P (FAM)	TTGACTGGAGCGAGGCGATGTTC	

Figure S1

a



b



c

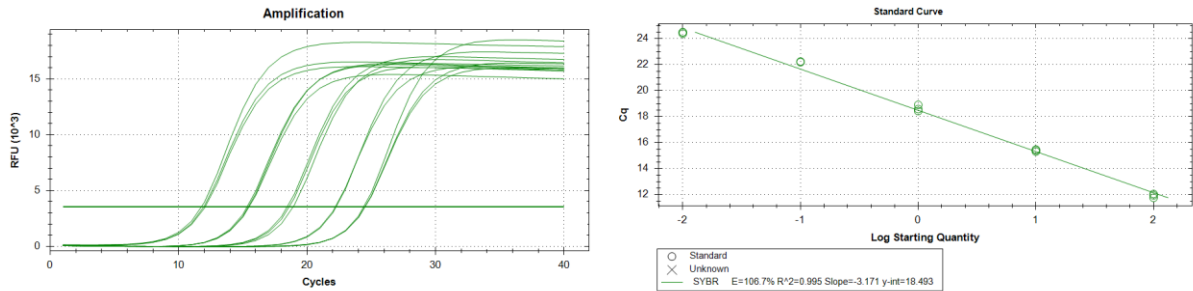
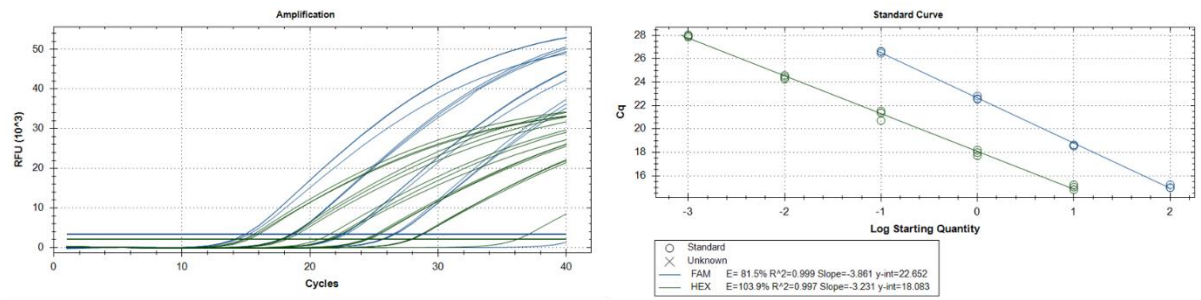


Figure S2

a



b

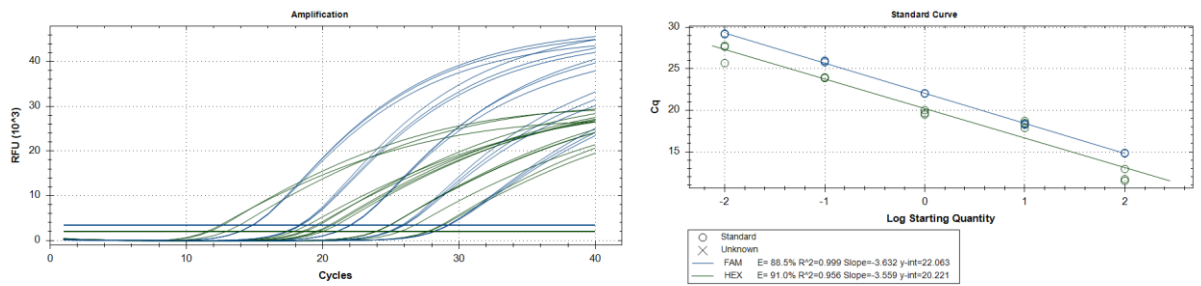
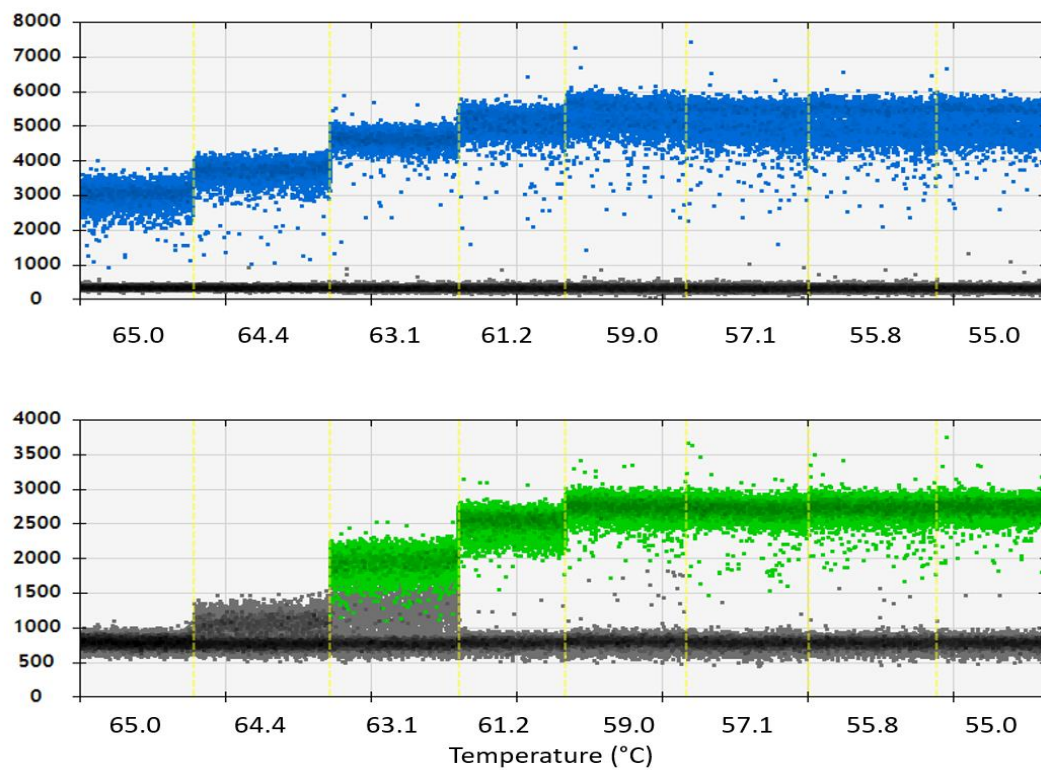


Figure S3

a



b

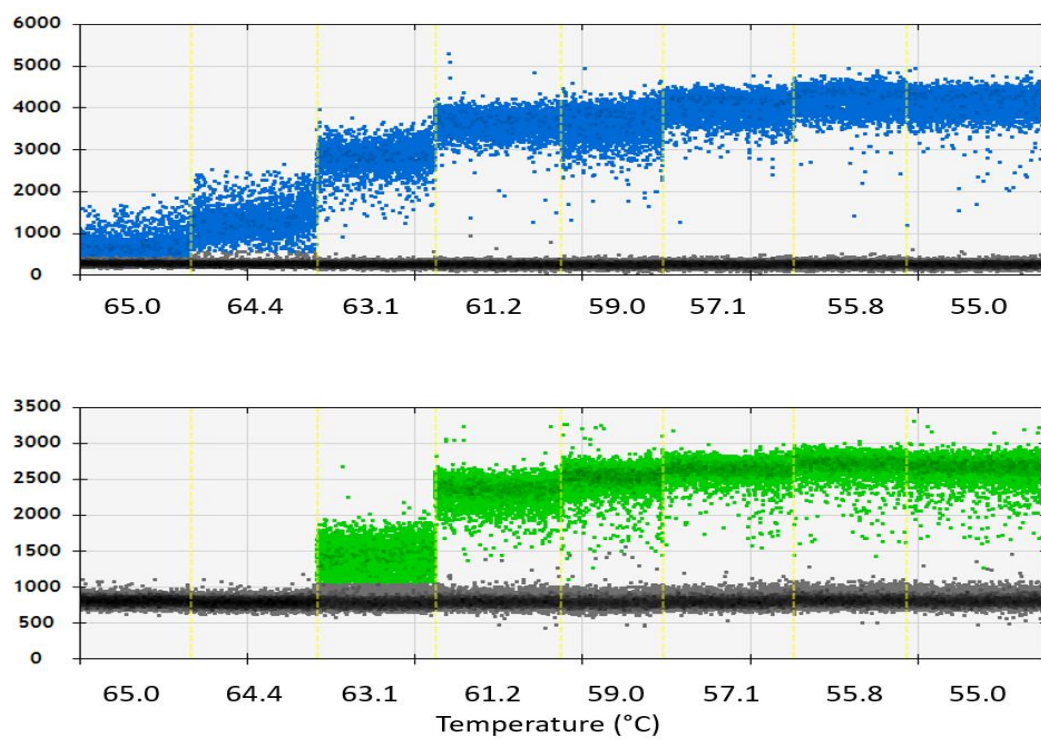
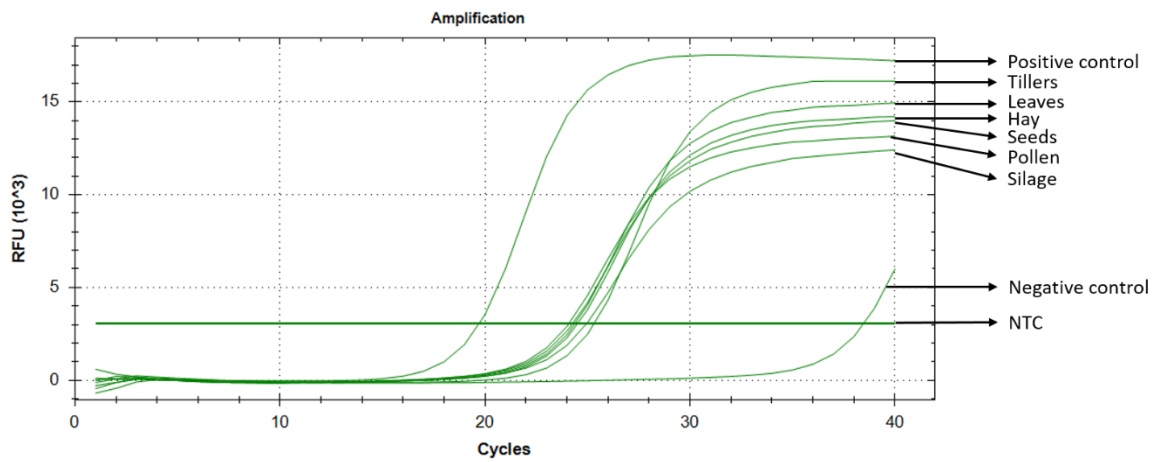


Figure S4

a



b

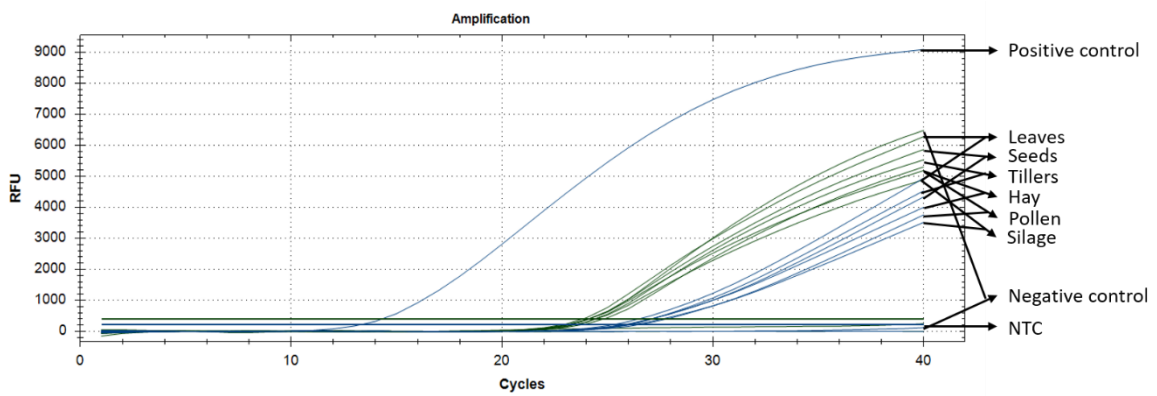
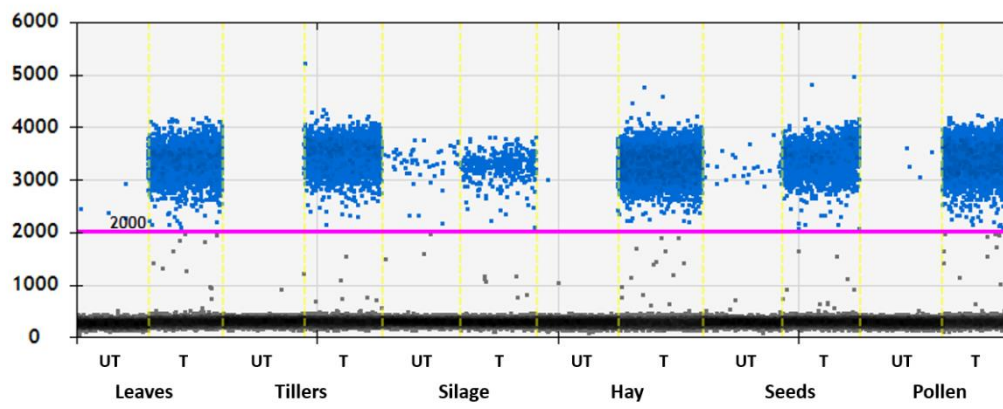


Figure S5

a



b

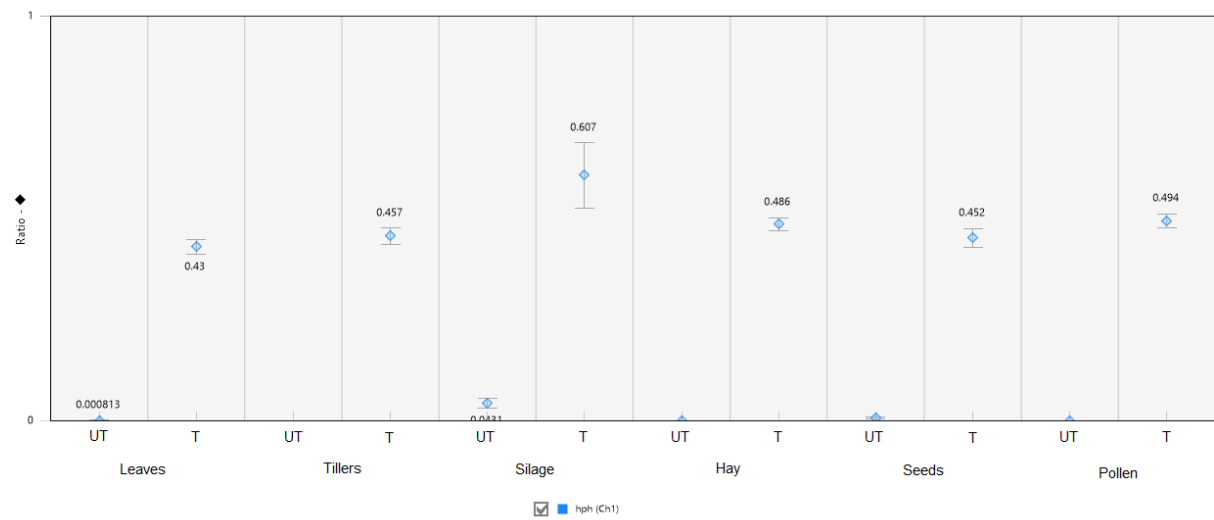
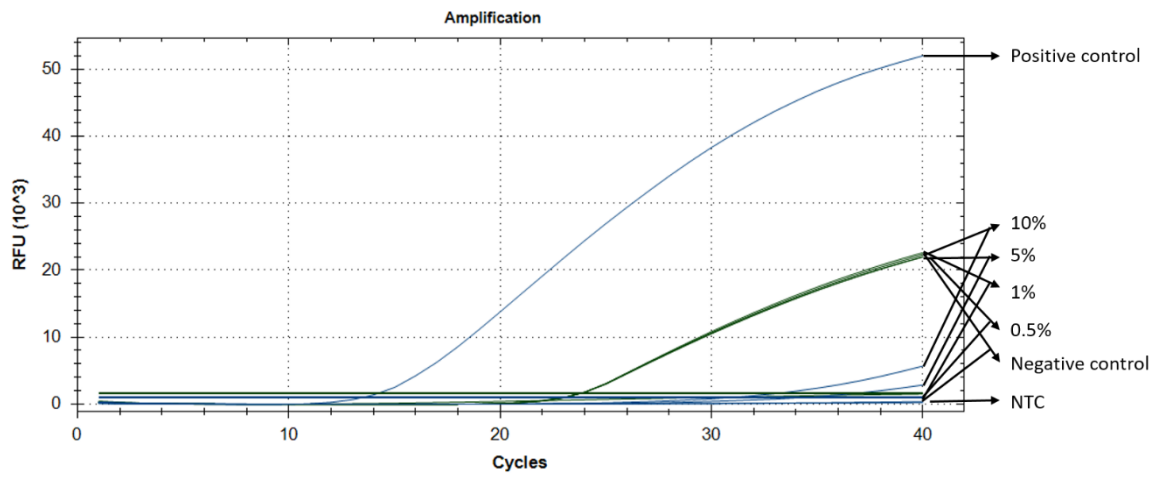


Figure S6

a



b

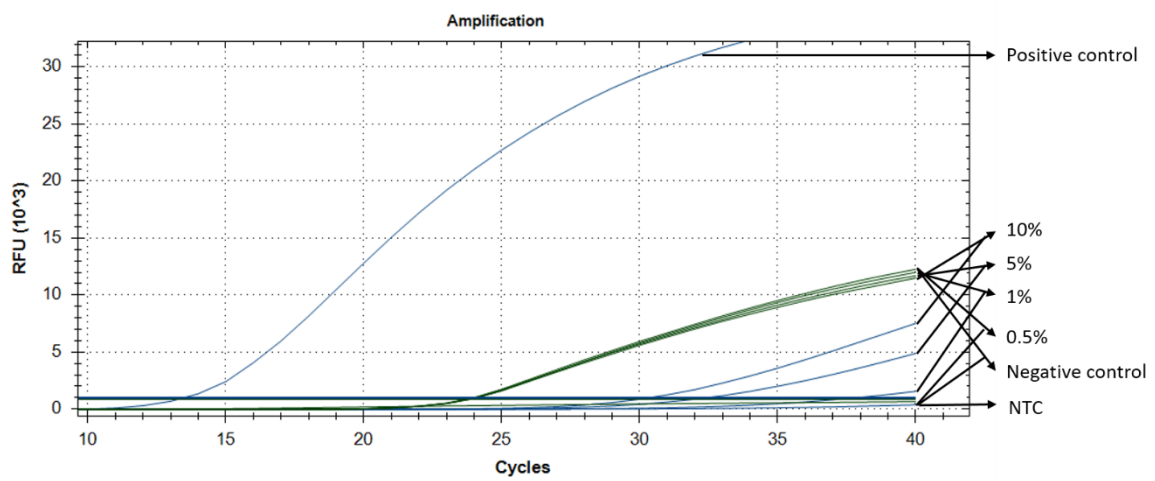


Figure S7

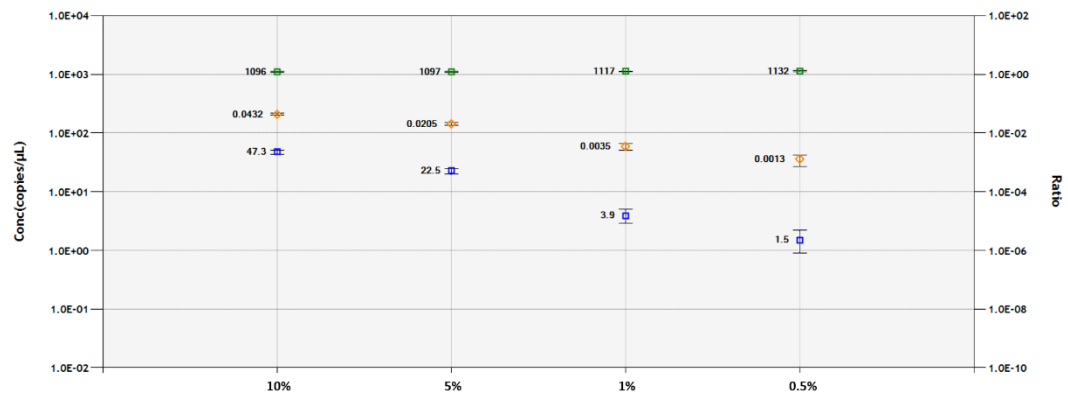
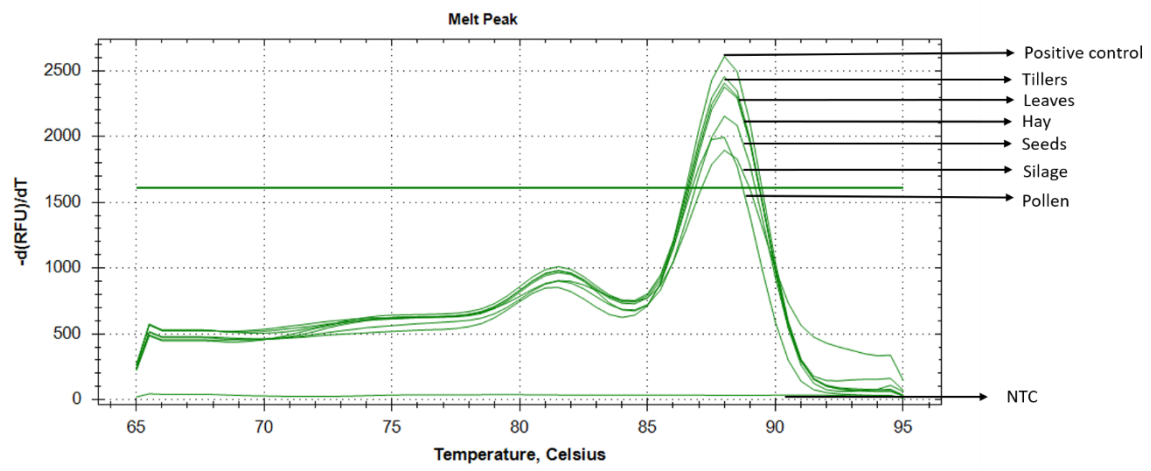


Figure 8

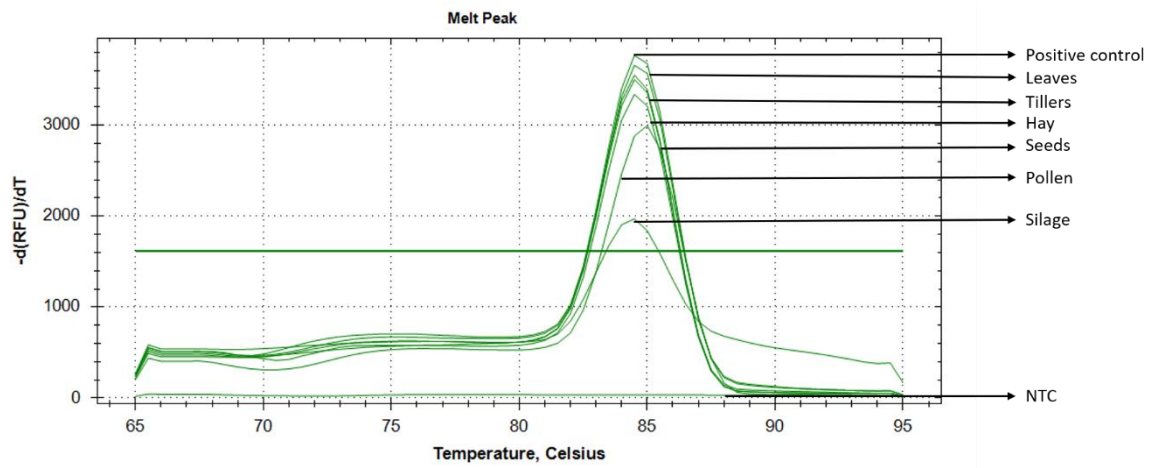
a



b



c



d

