Supplementary Materials

Effect of the Matrix and Target on the Accurate Quantification of Genomic and Plasmid DNA by Digital Polymerase Chain Reaction

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Table S1. Collected primer/probe sets targeting 11 commonly used exogenous elements.

Target	No.	Primer/Probe	Sequence	Amplicon size
NPTII	1	qNPTIIF63	CTATGACTGGGCACAACAGACA	101 bp
		qNPTIIR163	CGGACAGGTCGGTCTTGACA	
		qNPTIIP90	FAM-CTGCTCTGATGCCGCCGTGTTCCG-BHQ1	
	2	NPTII-F1	AGGATCTCGTCGTGACCCAT	183 bp
		NPTII-R1	GCACGAGGAAGCGGTCA	
		NPTII-P1	FAM-CACCCAGCCGGCCACAGTCGAT-BHQ1	
pFMV35S	3	pFMV35S-F	CAAAATAACGTGGAAAAGAGCT	78 bp
		pFMV35S -R	TCTTTTGTGGTCGTCACTGC	
		pFMV35S -P	FAM-CTGACAGCCCACTCACTAATGC-BHQ1	
	4	FMV35S-QF	AAGACATCCACCGAAGACTTA	210 bp
		FMV35S-QR	AGGACAGCTCTTTTCCACGTT	
		FMV35S-QP	FAM-TGGTCCCCACAAGCCAGCTGCTCGA-BHQ1	
	5	P-FMV-F	CGAAGACTTAAAGTTAGTGGGCATCT	79 bp
		P-FMV-R	TTTTGTCTGGTCCCCACAA	
		P-FMV-P	FAM-TGAAAGTAATCTTGTCAACATCGAGCAGCTGG-BHQ1	
Bar	6	RapB-F1	ACAAGCACGGTCAACTTCC	60 bp
		RapB-R1	GAGGTCGTCCGTCCACTC	
		RapB-S1	FAM-TACCGAGCCGCAGGAACC-BHQ1	
	7	Bar-F	ACAAGCACGGTCAACTTCC	175 bp
		Bar-R	ACTCGGCCGTCCAGTCGTA	
		Bar-P	FAM-CCGAGCCGCAGGAACCGCAGGAG-BHQ1	
T-NOS	8	180-F	CATGTAATGCATGACGTTATTTATG	84 bp

		180-R	TTGTTTTCTATCGCGTATTAAATGT	
		Tm-180	FAM-ATGGGTTTTTATGATTAGAGTCCCGCAA-BHQ1	
	9	NOS-QF	ATCGTTCAAACATTTGGCA	165 bp
		NOS-QR	ATTGCGGGACTCTAATCATA	
		NOS-QP	FAM-CATCGCAAGACCGGCAACAGG-BHQ1	
HPT	10	qHPTF286	CAGGGTGTCACGTTGCAAGA	110 bp
		qHPTR395	CCGCTCGTCTGGCTAAGATC	
		qHPTFP308	FAM-TGCCTGAAACCGAACTGCCCGCTG-BHQ1	
pCaMV35S	11	p35s-F	ATTGATGTGATATCTCCACTGACGT	101 bp
		p35s-R	CCTCTCCAAATGAAATGAACTTCCT	
		p35s-P	FAM--CCCACTATCCTTCGCAAGACCCTTCCT-BHQ1	
	12	35S-FTM	GCCTCTGCCGACAGTGGT	83 bp
		35S-RTM	AAGACGTGGTTGGAACGTCTTC	
		35S-TMP	FAM-CAAAGATGGACCCCCACCCACG-BHQ1	
	13	35S-QF	CGACAGTGGTCCCAAAGA	74 bp
		35S-QR	AAGACGTGGTTGGAACGTCTTC	
		35S-QP	FAM-TGGACCCCCACCCACGAGGAGCATC-BHQ1	
	14	sF	CGTCTTCAAAGCAAGTGGATTG	79 bp
		sR	TCTTGCGAAGGATAGTGGGATT	
		35S core	FAM-TCTCCACTGACGTAAGGGATGACGCA-BHQ1	
	15	35SEnh187F	CATCATTGCGATAAAGGAAAGGC	125 bp
		35SEnh311R	TGCTTTGAAGACGTGGTTGGA	
		35SEnh230P	FAM-CCGACAGTGGTCCCAAAGATGG-MGB	
PMI	16	PMIF240	ACTGCCTTTCCTGTTCAAAGTATTAT	96 bp
		PMIR335	TCTTTGGCAAAACCGATTTCAGAA	
		PMIP267	FAM-CGCAGCACAGCCACTCTCCATTCAGG-BHQ1	

	17	PMI-F	CCGGGTGAATCAGCGTTT	59 bp
		PMI-R	GCCGTGGCCTTTGACAGT	
		PMI-P	FAM-TGCCGCCAACGAATCACCGG-BHQ1	
TE9	18	TE9-F	TGAGAATGAACAAAAGGACCATATCA	87 bp
		TE9-R	TTTTTATTCGGTTTTCGCTATCG	
		TE9-P	FAM-TCATTAACTCTTCTCCATCCATTTCCATTTCACAGT-BHQ1	
Tg7	19	Tg7-F	ATGCAAGTTTAAATTCAGAAATATTTCAA	97 bp
		Tg7-R	ATGTATTACACATAATATCGCACTCAGTCT	
		Tg7-P	FAM-ACTGATTATATCAGCTGGTACATTGCCGTAGATGA-BHQ1	
T35S	20	T35SM-F	CCCTTAGTATGTATTTGTATTTGTAAAATACTTC	83 bp
		T35SM-R	GGATTTTAGTACTGGATTTTGGTTTTAG	
		T35S-P	FAM-TATCAATAAAATTTCTAATTC-MGB	
P-NOS	21	P-NOS-F	GTGACCTTAGGCGACTTTTGAAC	79 bp
		P-NOS-R	CGCGGGTTTCTGGAGTTTAA	
		P-NOS-P	FAM-CGCAATAATGGTTTCTGACGTATGTGCTTAGC-BHQ1	
	22	p-nos-F1	AAGCACATACGTCAGAAACCATTATT	94 bp
		p-nos-R	TCAGTGGAGCATTTTTGACAAGAA	
		p-nos-Tm	FAM-CGCGTTCAAAAGTCGCCTAAGGTCAC-BHQ1	
	23	P-NOS-QF	GACAGAACCGCAACGTTGAA	76 bp
		P-NOS-QR	TTCTGACGTATGTGCTTAGCTCATT	
		P-NOS-QP	FAM-AGCCACTCAGCCGCGGGTTTC-BHQ1	
CruA	24	qCruAF	GGCCAGGGCTTCCGTGAT	101 bp
		qCruAR	CCGTCGTTGTAGAACCATTGG	
		qCruFP	FAM-AGTCCTTATGTGCTCCACTTTCTGGTGCA-BHQ1	

PLD	25	KVM-159	TGGTGAGCGTTTTGCAGTCT	64 bp
		KVM-160	CTGATCCACTAGCAGGAGGTCC	
		TM-013	FAM-TGTTGTGCTGCCAATGTGGCCTG-BHQ1	

Table S2. Sensitivity test of collected primer/probe sets

Template	Total reactions	Positive reactions											
		P-FMV35S			HPT	P-NOS			T-NOS		TE9	Tg7	T-35S
		pFMV35S-F/R/P	FMV35-QF/R/P	P-FMV-F/R/P	qHPTF286/R395/P308	P-nos-F1/R/Tm	P-NOS-Q/R/PF	P-NOS-F/R/P	180-F/180-R/Tm-180	NOS-QF/R/P	Te9-F/R/P	Tg7-F/R/P	T35SM-F/R/P
20 copies	8	8	8	8	8	8	8	8	8	7	8	8	8
10 copies	8	7	6	8	6	8	8	8	6	7	8	8	8
5 copies	8	7	4	6	7	5	8	8	0	5	7	6	5
1 copy	8	7	2	2	2	2	0	3	0	3	0	1	2
Blank	8	0	0	0	0	0	0	0	0	0	0	0	0

Continued

Template	Total reactions	Positive reactions										
		PMI		Bar		NPTII		P-35S				
		PMIF240/R335/P267	PMI-F/R/P	RapB-F1/R1/S1	Bar-F/R/P	qNPTIIF63/R163/P90	NPTII-F1/R1/P1	p-35s-F/R/P	35S-QF/R/P	35S-FTM/RTM/TMP	sF/sR/35Score	35SEnh187F/311R/230P
20 copies	8	8	7	8	8	8	8	8	8	8	8	8
10 copies	8	8	4	8	8	8	8	8	8	8	8	8
5 copies	8	8	7	7	4	8	8	8	6	7	8	8
1 copy	8	7	3	5	3	6	4	4	5	3	5	2
Blank	8	0	0	0	0	0	0	0	0	0	0	0

Table S3. Assessment of ddPCR assays using collected primer/probe sets.

Template type	Total reactions	Measured copy number ratio of screening element and reference gene by ddPCR (mean±SD)											
		P-FMV35S			HPT	P-NOS			T-NOS		TE9	Tg7	T-35S
		pFMV35S-F/R/P	FMV35-QF/R/P	P-FMV-F/R/P	qHPTF286/R395/P308	P-nos-F1/R/Tm	P-NOS-Q/R/PF	P-NOS-F/R/P	180-F/180-R/Tm-180	NOS-QF/R/P	Te9-F/R/P	Tg7-F/R/P	T35SM-F/R/P
Plasmid DNA (element/PLD)	6	0.98±0.02	0.97±0.11	0.97±0.04	0.95±0.05	0.98±0.04	1.00±0.08	0.96±0.06	0.97±0.03	0.94±0.06	0.99±0.04	0.96±0.05	1.00±0.03
Plasmid DNA (element/CruA)	6	0.98±0.03	0.97±0.10	0.97±0.05	0.95±0.06	0.98±0.05	1.00±0.10	0.96±0.08	0.97±0.02	0.94±0.05	0.99±0.04	0.96±0.04	1.00±0.04
SDrice gDNA (element/PLD)	6	0.94±0.05	0.79±0.01	1.03±0.03	0.94±0.03	0.84±0.01	1.06±0.01	0.97±0.06	0.98±0.03	0.83±0.05	1.02±0.05	0.93±0.03	1.04±0.01
SDrape gDNA (element/CruA)	6	0.48±0.05	0.38±0.02	0.49±0.05	0.48±0.03	0.45±0.01	0.55±0.03	0.50±0.02	0.50±0.02	0.44±0.02	0.53±0.02	0.45±0.07	0.51±0.02

Continued

Template type	Total reactions	Measured copy number ratio of screening element and reference gene by ddPCR (mean±SD)										
		PMI		Bar		NPTII		P-35S				
		PMIF240/R335/P267	PMI-F/R/P	RapB-F1/R1/S1	Bar-F/R/P	qNPTIIF63/R163/P90	NPTII-F1/R1/P1	p-35s-F/R/P	35S-QF/R/P	35S-FTM/RTM/TMP	sF/sR/35S	35SEnh187F/311R/230P
Plasmid DNA (element/PLD)	6	0.98±0.02	1.01±0.04	0.96±0.02	0.99±0.08	0.98±0.03	0.97±0.06	0.98±0.03	0.93±0.04	0.93±0.06	0.89±0.08	0.99±0.06
Plasmid DNA (element/CruA)	6	1.00±0.02	1.03±0.04	0.96±0.03	0.99±0.06	0.98±0.04	0.97±0.05	0.98±0.04	0.93±0.04	0.93±0.05	0.89±0.07	0.99±0.05
Sdrice gDNA (element/PLD)	6	0.98±0.03	-	1.03±0.02	0.97±0.06	1.07±0.10	0.89±0.05	1.00±0.03	0.97±0.03	0.95±0.01	0.94±0.02	0.92±0.06
Sdrape gDNA (element/CruA)	6	0.51±0.03	0.54±0.05	0.50±0.08	0.45±0.04	0.51±0.02	0.48±0.02	0.48±0.04	0.48±0.06	0.47±0.04	0.43±0.02	0.48±0.05

Note, the primer/probe set selected for subsequent analysis is shown in grey shadow, "-" indicates no amplification using the primer/probe set PMI-F/R/P because partial sequence of PMI gene was deleted during transformation of SDrice.

Table S4. Constructed standard curves and calculated amplification efficiencies using homogenous gDNA and plasmid as calibrants

Calibrant	Target	Standard curves	Amplification efficiency (%)
SDrice genomic DNA	PLD	Y=-3.55X+39.96	91.19
	T-NOS	Y=-3.47X+38.94	94.17
	P-CaMV35S	Y=-3.48X+39.29	93.80
SDrape genomic DNA	CruA	Y=-3.45X+39.61	94.92
	T-NOS	Y=-3.54X+38.62	91.64
	P-CaMV35S	Y=-3.18X+37.84	106.51
pBI121-Screening	PLD	Y=-3.21X+39.57	105.12
	CruA	Y=-3.16X+39.43	107.42
	T-NOS	Y=-3.21X+39.13	105.07
	P-CaMV35S	Y=-3.21X+39.28	105.90

Table S5. Quantitative results of test samples by qPCR targeting T-NOS using genomic and plasmid DNA as calibrants.

Sample type	Target	Sample	Expected value (%)	Genomic DNA calibrants (%)							Plasmid DNA calibrants (%)						
				Rep 1	Rep 2	Rep 3	Mean	SD	RSD	Bias	Rep 1	Rep 2	Rep 3	Mean	SD	RSD	Bias
SDrice/rice	T-NOS	S1-G-rice	5	5.28	4.40	4.94	4.87	0.45	9.13	-2.53	5.79	6.92	6.46	6.39	0.57	8.89	27.80
		S2-G-rice	1	1.00	0.95	0.95	0.97	0.03	2.73	-3.45	1.24	1.18	1.19	1.20	0.03	2.57	20.17
		S3-G-rice	0.5	0.46	0.57	0.45	0.49	0.06	12.92	-1.56	0.61	0.73	0.59	0.64	0.08	11.76	28.67
		S4-G-rice	0.1	0.14	0.07	0.12	0.11	0.04	33.77	11.51	0.21	0.11	0.18	0.16	0.05	31.38	62.48
	P-*CaMV*35s	S1-G-rice	5	5.08	5.46	4.85	5.13	0.31	6.01	2.54	6.07	6.4	6.87	6.45	0.40	6.24	28.93
		S2-G-rice	1	1.11	0.95	0.95	1	0.09	9.24	0.33	1.28	1.15	1.16	1.20	0.07	6.05	19.67
		S3-G-rice	0.5	0.6	0.64	0.43	0.55	0.11	19.83	10.93	0.62	0.72	0.57	0.64	0.08	12.00	27.33
		S4-G-rice	0.1	0.12	0.08	0.09	0.1	0.02	22.53	-0.89	0.22	0.16	0.16	0.18	0.03	19.25	80.00
SDrape/rape	T-NOS	S1-G-rape	5	4.97	4.69	5.65	5.10	0.50	9.73	2.09	6.50	8.19	6.38	7.02	1.01	14.39	40.48
		S2-G-rape	1	0.87	0.89	1.02	0.93	0.08	9.16	-7.28	1.47	1.72	1.73	1.64	0.15	9.13	63.77
		S3-G-rape	0.5	0.66	0.62	0.44	0.57	0.12	20.71	14.40	1.02	0.64	0.85	0.84	0.19	22.64	67.16

		S4-G-rape	0.1	0.09	0.12	0.13	0.11	0.02	15.63	14.69	0.16	0.20	0.15	0.17	0.03	15.61	67.77
	P-*CaMV*35s	S1-G-rape	5	5.39	4.83	6.45	5.56	0.82	14.75	11.12	5.93	7.2	6.33	6.49	0.65	10.01	29.73
		S2-G-rape	1	0.92	0.88	1.06	0.95	0.09	9.72	-4.62	1.64	1.72	1.52	1.63	0.10	6.19	62.67
		S3-G-rape	0.5	0.46	0.43	0.49	0.46	0.03	5.75	-7.73	0.74	0.89	0.88	0.84	0.08	10.02	67.33
		S4-G-rape	0.1	0.11	0.12	0.14	0.12	0.02	12.39	23.33	0.19	0.15	0.18	0.17	0.02	12.01	73.33
Plasmid/rice	T-NOS	S1-P-rice	5	6.56	5.95	7.73	6.75	0.90	13.39	34.91	5.86	6.00	6.79	6.22	0.50	8.07	24.33
		S2-P-rice	1	1.29	1.25	1.48	1.34	0.12	9.04	33.86	1.26	1.46	1.47	1.40	0.12	8.34	39.55
		S3-P-rice	0.5	0.96	0.92	0.46	0.78	0.28	36.00	55.69	0.91	0.60	0.77	0.76	0.16	20.71	51.59
		S4-P-rice	0.1	0.17	0.21	0.21	0.20	0.02	11.74	96.67	0.17	0.20	0.16	0.18	0.02	11.75	79.68
	P-*CaMV*35s	S1-P-rice	5	6.56	5.95	7.73	6.75	0.9	13.39	34.91	4.66	6.75	4.94	5.45	1.13	20.82	9.01
		S2-P-rice	1	1.29	1.25	1.48	1.34	0.12	9.04	33.86	1.26	1.32	1.16	1.24	0.08	6.53	24.49
		S3-P-rice	0.5	0.69	0.65	0.72	0.69	0.04	5.42	37.59	1.01	0.84	0.74	0.86	0.14	15.81	72.67
		S4-P-rice	0.1	0.24	0.18	0.19	0.20	0.03	15.81	103.33	0.16	0.15	0.2	0.17	0.03	15.56	70.00
Plasmid/rap	T-NOS	S1-P-rape	5	6.63	5.72	7.15	6.50	0.72	11.15	29.96	9.13	7.75	9.91	8.93	1.09	12.23	78.58

e		S2-P-rape	1	1.46	1.74	1.47	1.55	0.16	10.22	55.46	1.72	2.09	1.73	1.84	0.21	11.30	84.45
		S3-P-rape	0.5	0.70	0.89	0.84	0.81	0.10	12.42	62.16	0.76	1.00	0.93	0.90	0.12	13.55	79.82
		S4-P-rape	0.1	0.18	0.25	0.18	0.20	0.04	19.88	103.33	0.18	0.26	0.18	0.21	0.05	22.35	106.67
	P-*CaMV*35s	S1-P-rape	5	5.8	5.97	9.15	6.98	1.89	27.05	39.5	7.48	7.76	11.97	9.07	2.51	27.72	81.39
		S2-P-rape	1	1.63	1.39	1.35	1.45	0.15	10.61	45.41	1.72	1.81	1.76	1.77	0.05	2.58	76.64
		S3-P-rape	0.5	0.52	0.78	0.66	0.65	0.13	19.77	30.82	0.69	1.04	0.87	0.87	0.18	20.67	73.51
		S4-P-rape	0.1	0.23	0.17	0.19	0.20	0.03	15.53	96.67	0.21	0.17	0.19	0.19	0.02	10.53	90.00

Table S6. Measurement data and statistical analysis of test samples by ddPCR.

Sample	Target	Measured copy number (copies/μL)			Measured copy number ratio (%)			Mean (%)	SD (%)	RSD (%)	Bias (%)
		C1	C2	C3	R1	R2	R3				
S1-G-rape	*CruA*	1662.67	1612.67	1657.67							
5%	*Bar*	90.00	94.10	90.40	5.41	5.84	5.45	5.57	0.23	4.18	11.34
	P-35S	99.00	97.00	95.00	5.97	5.85	5.73	5.85	0.12	2.06	17.01
	P-FMV35S	96.90	95.40	96.60	5.84	5.75	5.83	5.81	0.05	0.82	16.16
	T-NOS	92.20	93.10	92.50	5.56	5.62	5.58	5.59	0.03	0.49	11.70
	HPT	89.70	96.10	93.90	5.41	5.80	5.66	5.62	0.20	3.49	12.46
	NPTII	91.80	93.20	92.90	5.54	5.62	5.60	5.59	0.04	0.80	11.74
	P-NOS	97.00	98.00	98.00	5.85	5.91	5.91	5.89	0.03	0.59	17.81
	T-35S	98.00	97.30	97.00	5.91	5.87	5.85	5.88	0.03	0.53	17.53
	PMI	99.00	93.00	88.00	5.97	5.61	5.31	5.63	0.33	5.90	12.59
	T-E9	96.00	97.20	95.00	5.79	5.86	5.73	5.79	0.07	1.15	15.88
	T-g7	99.00	94.00	92.00	5.97	5.67	5.55	5.73	0.22	3.80	14.60
S2-G-rape	*CruA*	1775.00	1613.00	1667.67							
1%	*Bar*	19.00	18.40	19.00	1.07	1.14	1.14	1.12	0.04	3.60	11.68
	P-35S	20.90	18.10	20.60	1.24	1.07	1.22	1.18	0.09	7.74	17.57
	P-FMV35S	20.50	20.10	19.00	1.21	1.19	1.12	1.18	0.05	3.91	17.57
	T-NOS	20.50	18.60	18.90	1.21	1.10	1.12	1.14	0.06	5.28	14.42
	HPT	18.40	16.20	19.60	1.09	0.96	1.16	1.07	0.10	9.54	6.92
	NPTII	18.20	18.50	17.90	1.08	1.09	1.06	1.08	0.02	1.65	7.71
	P-NOS	19.90	17.00	19.60	1.18	1.01	1.16	1.11	0.09	8.47	11.46
	T-35S	19.50	21.50	19.10	1.15	1.27	1.13	1.19	0.08	6.42	18.56
	PMI	20.50	17.80	19.90	1.21	1.05	1.18	1.15	0.08	7.31	14.81

Sample	Target	Measured copy number (copies/μL)			Measured copy number ratio (%)			Mean (%)	SD (%)	RSD (%)	Bias (%)
		C1	C2	C3	R1	R2	R3				
	T-E9	19.00	18.90	21.20	1.12	1.12	1.25	1.17	0.08	6.60	16.59
	T-g7	18.40	20.20	21.30	1.09	1.20	1.26	1.18	0.09	7.33	18.16
S3-G-rape	*CruA*	1692.67	1690.00	1681.33							
0.50%	*Bar*	10.80	9.80	8.90	0.65	0.59	0.53	0.59	0.06	9.67	18.08
	P-35S	10.2	10.7	9.7	0.60	0.63	0.58	0.60	0.03	4.66	20.84
	P-FMV35S	10.10	11.0	10.10	0.61	0.65	0.61	0.62	0.03	6.04	23.22
	T-NOS	9.30	9.40	9.80	0.56	0.56	0.59	0.57	0.02	2.79	14.08
	HPT	9.80	10.30	9.00	0.59	0.62	0.54	0.58	0.04	6.76	16.48
	NPTII	10.50	10.60	10.10	0.63	0.64	0.61	0.62	0.02	2.54	24.89
	P-NOS	10.60	10.10	9.60	0.64	0.61	0.58	0.61	0.03	4.95	21.28
	T-35S	9.6	10.9	10.4	0.57	0.65	0.62	0.62	0.04	7.21	23.07
	PMI	10.10	9.60	10.90	0.61	0.58	0.65	0.61	0.04	6.43	22.49
	T-E9	9.90	10.00	9.70	0.59	0.60	0.58	0.59	0.01	1.55	18.48
	T-g7	10.8	10.4	10.2	0.64	0.62	0.61	0.62	0.01	2.25	24.39
S4-G-rape	*CruA*	1724.00	1665.00	1796.33							
0.10%	*Bar*	2.10	1.90	2.40	0.12	0.11	0.13	0.123	0.01	7.97	23.18
	P-35S	2.10	1.50	1.80	0.12	0.09	0.10	0.104	0.02	15.57	4.03
	P-FMV35S	2.00	2.30	1.70	0.12	0.14	0.09	0.116	0.02	18.71	16.26
	T-NOS	2.30	1.60	2.00	0.13	0.10	0.11	0.114	0.02	16.51	13.61
	HPT	2.10	2.30	2.00	0.12	0.14	0.11	0.124	0.01	10.91	23.76
	NPTII	1.50	1.80	2.00	0.09	0.11	0.11	0.102	0.01	12.94	2.15
	P-NOS	2.10	1.90	2.00	0.12	0.11	0.11	0.116	0.01	4.69	15.75

Sample	Target	Measured copy number (copies/μL)			Measured copy number ratio (%)			Mean (%)	SD (%)	RSD (%)	Bias (%)
		C1	C2	C3	R1	R2	R3				
	T-35S	2.47	1.97	1.70	0.14	0.12	0.09	0.119	0.02	20.51	18.80
	PMI	2.40	1.80	1.70	0.14	0.11	0.09	0.114	0.02	20.06	13.99
	T-E9	1.90	1.50	1.60	0.11	0.09	0.09	0.096	0.01	12.36	-3.54
	T-g7	1.60	1.50	1.70	0.09	0.09	0.09	0.093	0.00	2.47	-7.49
S1-P-rape	*CruA*	1694.67	1722.67	1666.67							
5%	*Bar*	85.50	82.60	84.00	5.05	4.88	4.97	4.97	0.09	1.73	-0.66
	P-35S	89.00	87.10	87.50	5.26	5.15	5.17	5.19	0.06	1.14	3.87
	P-FMV35S	75.24	88.40	88.00	4.45	5.23	5.20	4.96	0.44	8.93	-0.84
	T-NOS	80.10	85.00	79.70	4.73	5.02	4.71	4.82	0.17	3.62	-3.54
	HPT	79.40	84.26	80.40	4.69	4.98	4.75	4.81	0.15	3.15	-3.83
	NPTII	81.50	87.20	80.80	4.82	5.15	4.78	4.92	0.21	4.22	-1.68
	P-NOS	83.20	77.10	79.70	4.92	4.56	4.71	4.73	0.18	3.83	-5.43
	T-35S	76.80	87.36	77.50	4.54	5.16	4.58	4.76	0.35	7.33	-4.77
	PMI	78.80	78.60	79.20	4.66	4.65	4.68	4.66	0.02	0.39	-6.77
	T-E9	86.40	87.00	88.00	5.11	5.14	5.20	5.15	0.05	0.93	3.00
	T-g7	80.60	78.90	81.00	4.76	4.66	4.79	4.74	0.07	1.39	-5.23
S2-P-rape	*CruA*	1675.00	1757.00	1688.00							
1%	*Bar*	17.70	17.70	17.60	1.02	1.02	1.02	1.02	0.00	0.33	2.13
	P-35S	17.20	17.40	17.60	0.99	1.01	1.02	1.01	0.01	1.15	0.59
	P-FMV35S	16.90	16.00	15.30	0.98	0.92	0.88	0.93	0.05	4.99	-7.12
	T-NOS	14.50	16.00	17.93	0.84	0.92	1.04	0.93	0.10	10.65	-6.67
	HPT	17.40	17.20	16.00	1.01	0.99	0.92	0.98	0.04	4.49	-2.49

Sample	Target	Measured copy number (copies/μL)			Measured copy number ratio (%)			Mean (%)	SD (%)	RSD (%)	Bias (%)
		C1	C2	C3	R1	R2	R3				
	NPTII	16.30	17.50	17.20	0.94	1.01	0.99	0.98	0.04	3.67	-1.72
	P-NOS	19.30	17.80	15.60	1.12	1.03	0.90	1.02	0.11	10.59	1.56
	T-35S	18.30	18.40	17.50	1.06	1.06	1.01	1.04	0.03	2.73	4.45
	PMI	169.00	173.00	174.00	0.98	1.00	1.01	0.99	0.02	1.54	-0.56
	T-E9	17.90	19.30	18.80	1.03	1.12	1.09	1.08	0.04	3.80	7.92
	T-g7	16.80	16.20	17.90	0.97	0.94	1.03	0.98	0.05	5.08	-1.91
S3-P-rape	*CruA*	1608.00	1613.00	1735.00							
0.50%	*Bar*	8.20	10.00	8.60	0.50	0.61	0.53	0.55	0.06	10.58	9.70
	P-35S	8.80	8.20	8.40	0.54	0.50	0.52	0.52	0.02	3.61	3.97
	P-FMV35S	8.40	10.30	7.10	0.52	0.63	0.44	0.53	0.10	18.71	5.60
	T-NOS	7.90	8.40	10.30	0.49	0.52	0.63	0.54	0.08	14.28	8.88
	HPT	8.00	8.00	9.80	0.49	0.49	0.60	0.53	0.06	12.08	5.60
	NPTII	8.60	10.30	8.90	0.53	0.63	0.55	0.57	0.06	9.79	13.79
	P-NOS	9.20	8.90	7.40	0.56	0.55	0.45	0.52	0.06	11.35	4.37
	T-35S	8.50	9.50	9.00	0.52	0.58	0.55	0.55	0.03	5.56	10.51
	PMI	77.50	82.60	78.70	0.48	0.51	0.48	0.49	0.02	3.35	-2.26
	T-E9	9.30	7.30	9.20	0.57	0.45	0.56	0.53	0.07	13.10	5.60
	T-g7	10.60	8.50	8.50	0.65	0.52	0.52	0.56	0.07	13.18	12.97
S4-P-rape	*CruA*	1711.67	1672.67	1714.00							
0.10%	*Bar*	1.60	2.50	2.20	0.09	0.15	0.13	0.12	0.03	22.84	23.76
	P-35S	1.50	1.40	1.70	0.09	0.08	0.10	0.09	0.01	8.93	-9.83
	P-FMV35S	2.50	1.50	2.10	0.15	0.09	0.12	0.12	0.03	23.71	19.42

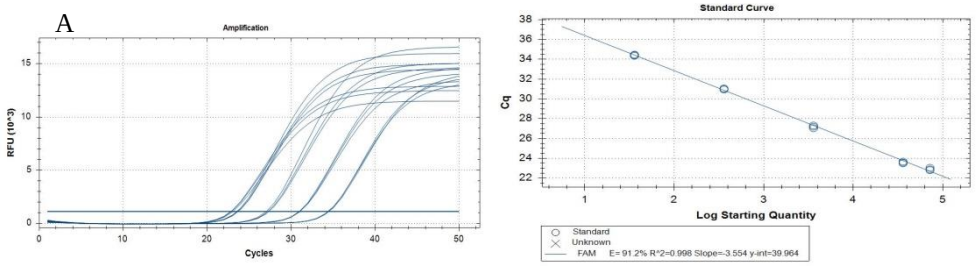
Sample	Target	Measured copy number (copies/μL)			Measured copy number ratio (%)			Mean (%)	SD (%)	RSD (%)	Bias (%)
		C1	C2	C3	R1	R2	R3				
	T-NOS	2.20	1.50	2.10	0.13	0.09	0.12	0.11	0.02	18.41	13.58
	HPT	2.10	2.30	1.50	0.12	0.14	0.09	0.12	0.03	22.15	15.90
	NPTII	1.90	2.30	1.80	0.11	0.14	0.11	0.12	0.02	14.67	17.84
	P-NOS	1.90	2.20	1.70	0.11	0.13	0.10	0.11	0.02	14.37	13.90
	T-35S	1.70	1.80	1.90	0.10	0.11	0.11	0.11	0.01	5.62	5.93
	PMI	1.51	1.30	1.72	0.09	0.08	0.10	0.09	0.01	12.76	-11.24
	T-E9	1.70	1.90	2.10	0.10	0.11	0.12	0.11	0.01	10.47	11.81
	T-g7	2.10	2.30	1.60	0.12	0.14	0.09	0.12	0.02	19.07	17.85
S1-G-rice	*PLD*	1827.00	1869.67	1687.67							
5%	*Bar*	84.25	95.80	105.00	4.66	5.29	5.80	5.25	0.57	10.94	5.01
	P-35S	91.00	91.00	95.00	5.03	5.03	5.25	5.10	0.13	2.50	2.04
	P-FMV35S	83.80	85.40	87.20	4.63	4.72	4.82	4.72	0.09	1.99	-5.54
	T-NOS	90.00	88.90	90.00	4.97	4.91	4.97	4.95	0.04	0.71	-0.94
	HPT	93.94	82.90	83.50	5.19	4.58	4.61	4.80	0.34	7.15	-4.09
	NPTII	102.00	99.00	93.80	5.64	5.47	5.18	5.43	0.23	4.22	8.60
	P-NOS	86.60	83.90	86.80	4.79	4.64	4.80	4.74	0.09	1.89	-5.21
	T-35S	93.90	92.20	92.60	5.19	5.09	5.12	5.13	0.05	0.96	2.67
	PMI	97.37	82.90	85.70	5.38	4.58	4.74	4.90	0.42	8.66	-2.02
	T-E9	89.90	91.60	93.70	4.97	5.06	5.18	5.07	0.11	2.08	1.38
	T-g7	88.10	86.70	91.50	4.87	4.79	5.06	4.91	0.14	2.78	-1.90
S2-G-rice	*PLD*	1824.00	1843.67	1847.33							
1%	*Bar*	20.10	19.00	18.30	1.09	1.03	0.99	1.04	0.05	4.74	3.84

Sample	Target	Measured copy number (copies/μL)			Measured copy number ratio (%)			Mean (%)	SD (%)	RSD (%)	Bias (%)
		C1	C2	C3	R1	R2	R3				
	P-35S	18.30	18.70	17.90	0.99	1.01	0.97	0.99	0.02	2.19	-0.68
	P-FMV35S	17.90	17.20	17.50	0.97	0.93	0.95	0.95	0.02	2.00	-4.84
	T-NOS	20.50	19.20	18.60	1.11	1.04	1.01	1.05	0.05	5.00	5.47
	HPT	17.20	17.60	18.30	0.93	0.96	0.99	0.96	0.03	3.15	-3.93
	NPTII	23.10	19.40	19.60	1.25	1.05	1.06	1.12	0.11	10.05	12.35
	P-NOS	20.10	17.20	16.80	1.09	0.93	0.91	0.98	0.10	9.97	-2.13
	T-35S	19.50	18.30	18.80	1.06	0.99	1.02	1.02	0.03	3.19	2.40
	PMI	17.60	18.00	17.90	0.96	0.98	0.97	0.97	0.01	1.17	-3.21
	T-E9	21.90	22.40	17.59	1.19	1.22	0.95	1.12	0.14	12.82	11.97
	T-g7	18.80	16.90	18.90	1.02	0.92	1.03	0.99	0.06	6.19	-1.22
S3-G-rice	*PLD*	1825.33	1884.00	1868.67							
0.50%	*Bar*	9.50	10.10	9.40	0.51	0.54	0.51	0.52	0.02	3.92	3.96
	P-35S	9.80	8.50	9.80	0.53	0.46	0.53	0.50	0.04	8.01	0.73
	P-FMV35S	9.40	8.70	10.20	0.51	0.47	0.55	0.51	0.04	7.96	1.45
	T-NOS	11.00	9.70	9.10	0.59	0.52	0.49	0.53	0.05	9.78	6.82
	HPT	10.60	8.90	10.60	0.57	0.48	0.57	0.54	0.05	9.78	7.90
	NPTII	11.60	10.40	10.80	0.62	0.56	0.58	0.59	0.03	5.59	17.58
	P-NOS	8.20	7.10	9.80	0.44	0.38	0.53	0.45	0.07	16.23	-10.02
	T-35S	10.70	9.60	10.10	0.58	0.52	0.54	0.54	0.03	5.44	8.98
	PMI	8.60	9.20	9.30	0.46	0.49	0.50	0.49	0.02	4.19	-2.85
	T-E9	8.40	8.30	10.80	0.45	0.45	0.58	0.49	0.08	15.44	-1.42
	T-g7	9.10	10.00	9.30	0.49	0.54	0.50	0.51	0.03	4.99	1.81

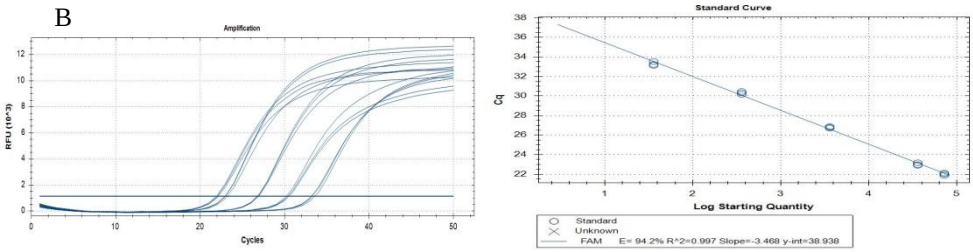
Sample	Target	Measured copy number (copies/μL)			Measured copy number ratio (%)			Mean (%)	SD (%)	RSD (%)	Bias (%)
		C1	C2	C3	R1	R2	R3				
S4-G-rice	*PLD*	1820.67	1874.00	1852.33							
0.10%	*Bar*	2.40	2.60	1.90	0.13	0.14	0.10	0.12	0.02	15.43	24.38
	P-35S	1.65	2.75	2.40	0.09	0.15	0.13	0.12	0.03	23.51	22.31
	P-FMV35S	2.00	2.40	2.00	0.11	0.13	0.11	0.12	0.01	9.63	15.30
	T-NOS	2.00	2.60	2.30	0.11	0.14	0.12	0.12	0.01	11.63	24.25
	HPT	1.80	1.80	1.80	0.10	0.10	0.10	0.10	0.00	1.45	-2.64
	NPTII	2.50	2.10	2.30	0.14	0.11	0.12	0.12	0.01	10.14	24.51
	P-NOS	2.10	2.10	2.70	0.12	0.11	0.15	0.12	0.02	14.94	24.39
	T-35S	2.77	1.73	2.40	0.15	0.09	0.13	0.12	0.03	24.23	24.67
	PMI	2.00	1.50	1.40	0.11	0.08	0.08	0.09	0.02	21.05	-11.51
	T-E9	2.20	2.00	1.80	0.12	0.11	0.10	0.11	0.01	11.00	8.24
	T-g7	2.40	2.00	1.50	0.13	0.11	0.08	0.11	0.03	23.87	6.51
S1-P-rice	*PLD*	1816.33	1841.67	1781.33							
5%	*Bar*	85.40	81.40	83.80	4.72	4.50	4.63	4.62	0.11	2.41	-7.61
	P-35S	74.00	80.90	82.60	4.09	4.47	4.57	4.38	0.25	5.75	-12.44
	P-FMV35S	84.92	72.78	77.49	4.70	4.03	4.29	4.34	0.34	7.81	-13.29
	T-NOS	80.00	82.60	78.90	4.42	4.57	4.36	4.45	0.11	2.36	-10.96
	HPT	78.90	80.60	78.10	4.36	4.46	4.32	4.38	0.07	1.61	-12.40
	NPTII	82.50	81.60	83.80	4.56	4.51	4.63	4.57	0.06	1.34	-8.60
	P-NOS	83.50	80.10	86.10	4.62	4.43	4.76	4.60	0.17	3.61	-7.94
	T-35S	79.50	85.10	79.90	4.40	4.71	4.42	4.51	0.17	3.83	-9.86
	PMI	84.59	82.54	83.29	4.68	4.56	4.61	4.62	0.06	1.24	-7.68

Sample	Target	Measured copy number (copies/μL)			Measured copy number ratio (%)			Mean (%)	SD (%)	RSD (%)	Bias (%)
		C1	C2	C3	R1	R2	R3				
	T-E9	85.50	82.80	86.10	4.73	4.58	4.76	4.69	0.10	2.07	-6.21
	T-g7	74.40	80.20	76.30	4.11	4.44	4.22	4.26	0.16	3.84	-14.87
S2-P-rice	*PLD*	1800.67	1819.67	1841.67							
1%	*Bar*	17.30	18.00	16.80	0.95	0.99	0.92	0.95	0.03	3.47	-4.61
	P-35S	19.20	17.30	17.40	1.05	0.95	0.96	0.99	0.06	5.95	-1.32
	P-FMV35S	17.50	15.70	16.40	0.96	0.86	0.90	0.91	0.05	5.49	-9.19
	T-NOS	17.40	17.80	15.70	0.96	0.98	0.86	0.93	0.06	6.57	-6.81
	HPT	15.50	17.10	16.80	0.85	0.94	0.92	0.90	0.05	5.16	-9.56
	NPTII	17.70	18.70	15.40	0.97	1.03	0.85	0.95	0.09	9.80	-5.16
	P-NOS	16.60	16.10	17.00	0.91	0.88	0.93	0.91	0.02	2.72	-9.01
	T-35S	16.30	14.50	16.40	0.90	0.80	0.90	0.86	0.06	6.80	-13.58
	PMI	143.00	152.00	159.00	0.86	0.90	0.88	0.88	0.02	2.45	-11.83
	T-E9	16.60	11.40	17.80	0.91	0.63	0.98	0.84	0.19	22.28	-16.15
	T-g7	16.10	17.00	15.30	0.88	0.93	0.84	0.89	0.05	5.27	-11.39
S3-P-rice	*PLD*	1821.00	1855.00	1909.67							
0.50%	*Bar*	7.70	9.20	8.30	0.41	0.49	0.44	0.45	0.04	8.99	-10.04
	P-35S	7.80	8.60	8.70	0.42	0.46	0.47	0.45	0.03	5.90	-10.40
	P-*FMV*35S	7.70	6.90	7.80	0.41	0.37	0.42	0.40	0.03	6.61	-20.04
	T-NOS	8.30	8.70	8.00	0.44	0.47	0.43	0.45	0.02	4.21	-10.75
	HPT	8.30	7.90	8.00	0.44	0.42	0.43	0.43	0.01	2.58	-13.61
	NPTII	9.10	7.60	8.90	0.49	0.41	0.48	0.46	0.04	9.54	-8.61
	P-NOS	8.30	8.50	8.50	0.44	0.46	0.46	0.45	0.01	1.37	-9.68

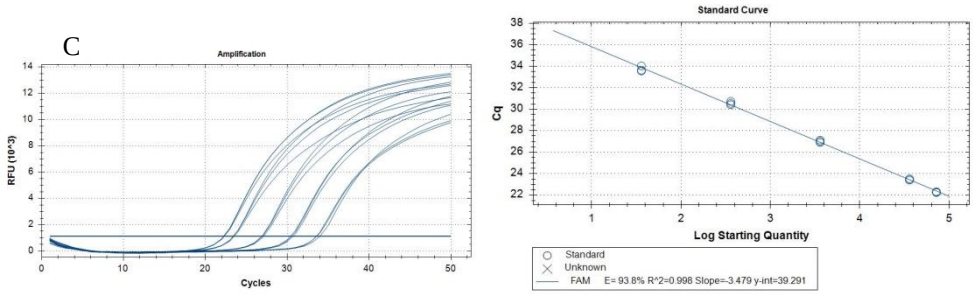
Sample	Target	Measured copy number (copies/μL)			Measured copy number ratio (%)			Mean (%)	SD (%)	RSD (%)	Bias (%)
		C1	C2	C3	R1	R2	R3				
	T-35S	9.70	8.60	8.30	0.52	0.46	0.44	0.47	0.04	8.31	-5.04
	PMI	7.47	7.98	8.59	0.41	0.43	0.45	0.43	0.02	4.65	-14.00
	T-E9	8.20	7.10	10.20	0.44	0.38	0.55	0.46	0.08	18.49	-8.97
	T-g7	7.50	7.80	7.90	0.40	0.42	0.42	0.41	0.01	2.69	-17.18
S4-P-rice	*PLD*	1805.67	1822.67	1765.67							
0.10%	*Bar*	1.80	1.70	2.20	0.10	0.09	0.12	0.11	0.01	13.93	5.61
	P-35S	2.20	2.20	1.80	0.12	0.12	0.10	0.11	0.01	11.17	14.88
	P-*FMV*35S	1.90	2.10	1.90	0.11	0.12	0.11	0.11	0.01	5.87	9.32
	T-NOS	1.90	1.80	1.60	0.11	0.10	0.09	0.10	0.01	8.65	-1.80
	HPT	2.10	1.60	1.80	0.12	0.09	0.10	0.10	0.01	13.73	1.91
	NPTII	1.90	1.90	2.20	0.11	0.11	0.12	0.11	0.01	8.66	11.17
	P-NOS	1.80	1.90	2.10	0.10	0.11	0.12	0.11	0.01	7.90	7.47
	T-35S	2.00	2.20	1.80	0.11	0.12	0.10	0.11	0.01	10.00	11.17
	PMI	13.90	13.90	15.70	0.10	0.09	0.10	0.10	0.00	3.14	-2.67
	T-E9	1.50	1.40	2.10	0.08	0.08	0.12	0.09	0.02	22.72	-7.36
	T-g7	1.80	2.30	1.80	0.10	0.13	0.10	0.11	0.02	14.68	9.32



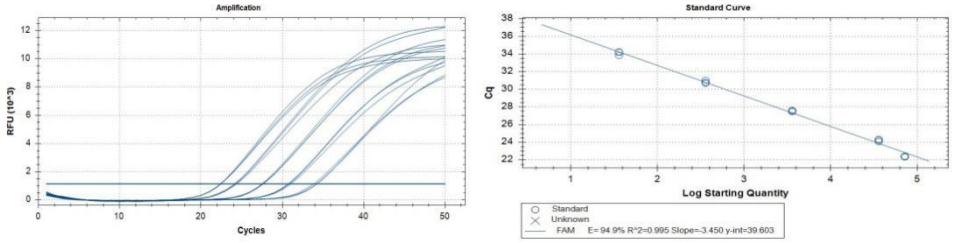
B


Amplification
RFU (10^3)
Cycles
Standard Curve
Cq
Log Starting Quantity
Standard
Unknown
FAM E= 94.2% R^2=0.997 Slope=-3.468 y-int=38.938

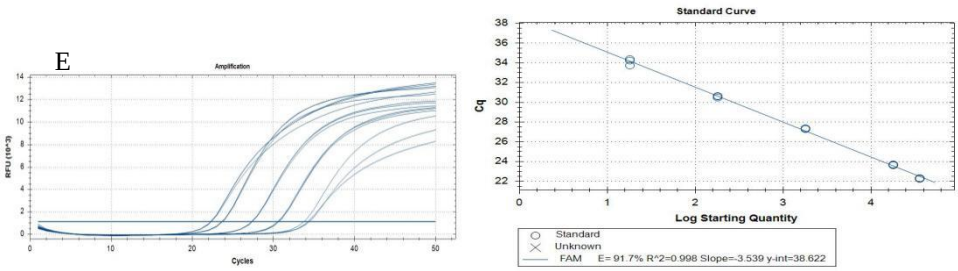
C


Amplification
RFU (10^3)
Cycles
Standard Curve
Cq
Log Starting Quantity
Standard
Unknown
FAM E= 93.8% R^2=0.998 Slope=-3.479 y-int=39.291

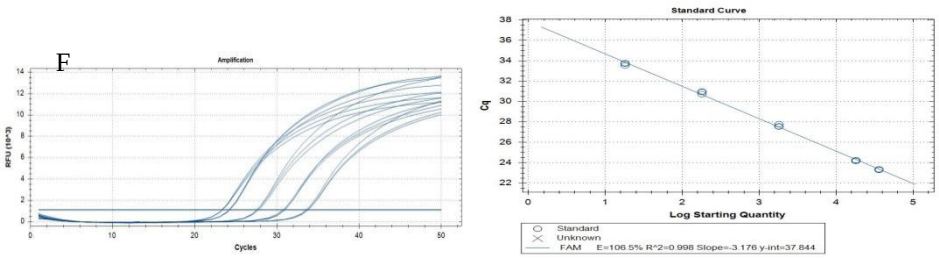
D


Amplification
RFU (10^3)
Cycles
Standard Curve
Cq
Log Starting Quantity
Standard
Unknown
FAM E= 94.9% R^2=0.995 Slope=-3.450 y-int=39.603

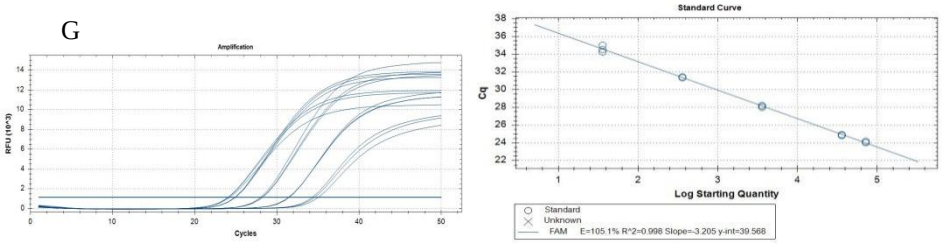
E


Amplification
RFU (10^3)
Cycles
Standard Curve
Cq
Log Starting Quantity
Standard
Unknown
FAM E= 91.7% R^2=0.998 Slope=-3.539 y-int=38.622

F


Amplification
RFU (10^3)
Cycles
Standard Curve
Cq
Log Starting Quantity
Standard
Unknown
FAM E=106.5% R^2=0.998 Slope=-3.176 y-int=37.844

G


Amplification
RFU (10^3)
Cycles
Standard Curve
Cq
Log Starting Quantity
Standard
Unknown
FAM E=105.1% R^2=0.998 Slope=-3.205 y-int=39.568

I

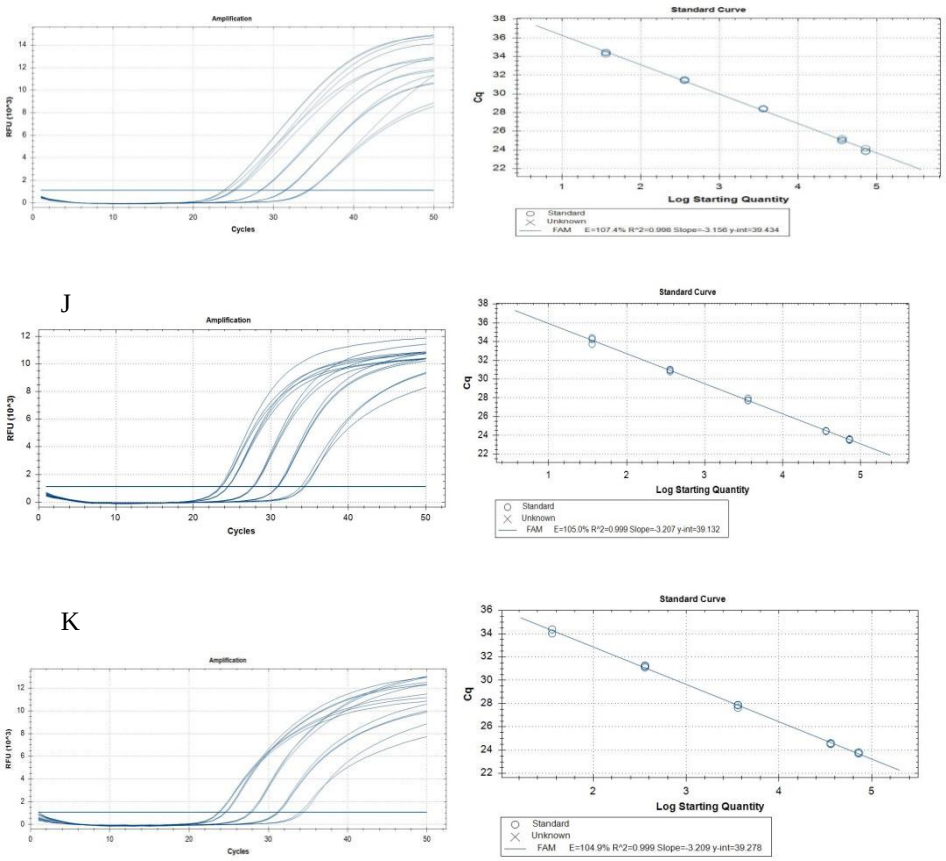


Figure S1. The amplification plots and standard curves of *PLD*, *CruA*, P-*CaMV*35S, T-NOS using gDNA or pDNA as calibrators. A-C, the amplification plots and standard curves of *PLD*, P-*CaMV*35S, T-NOS using SDrice gDNA as calibrators; D-F, that of *CruA*, P-*CaMV*35S, T-NOS using SDrapeseed gDNA as calibrators; G-K, that of *PLD*, *CruA*, P-*CaMV*35S, T-NOS using pBI121-Screening pDNA as calibrators.

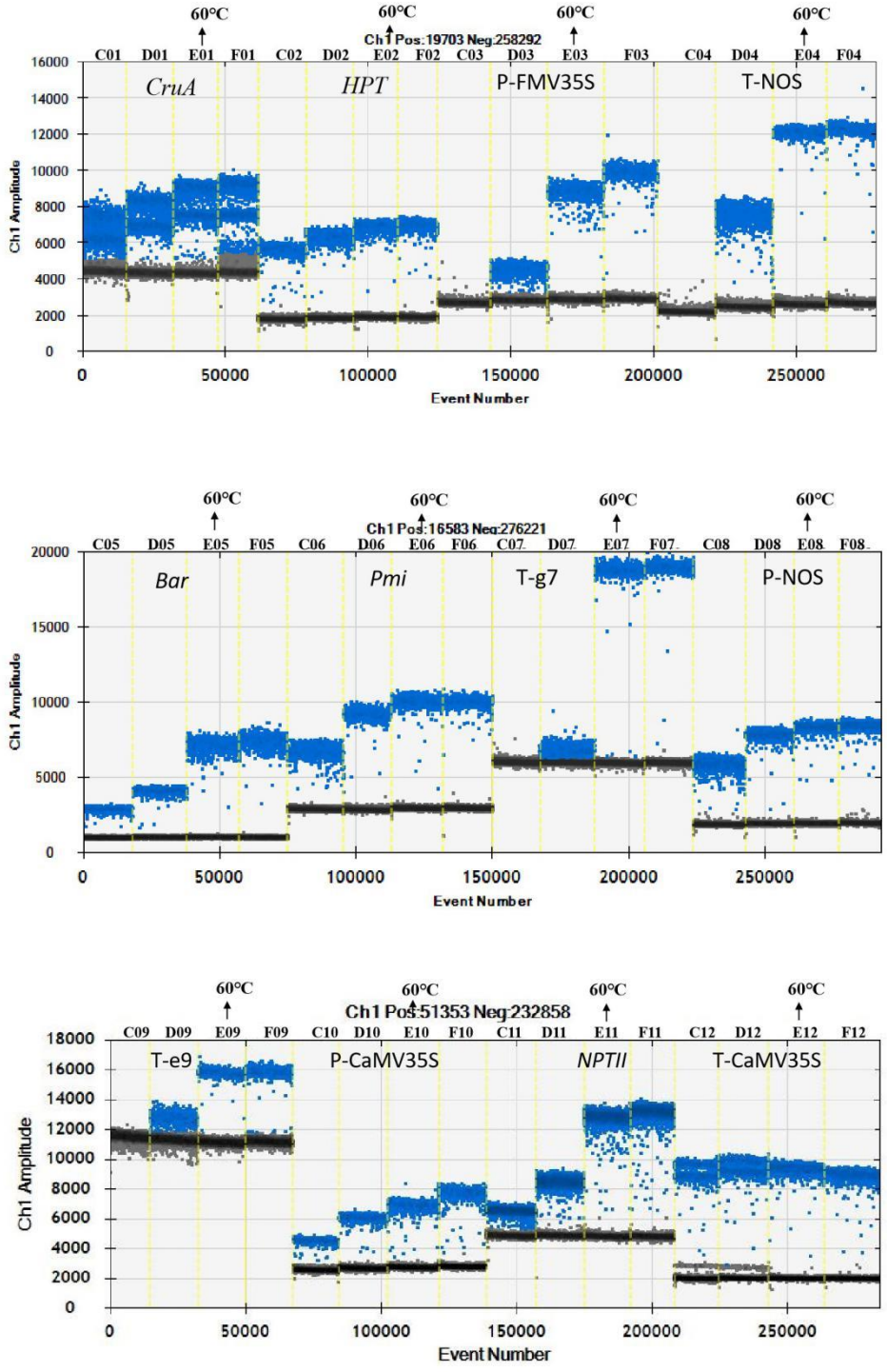


Figure S2. Testing of the optimum annealing elongation temperature for the 11 target dPCR assays.