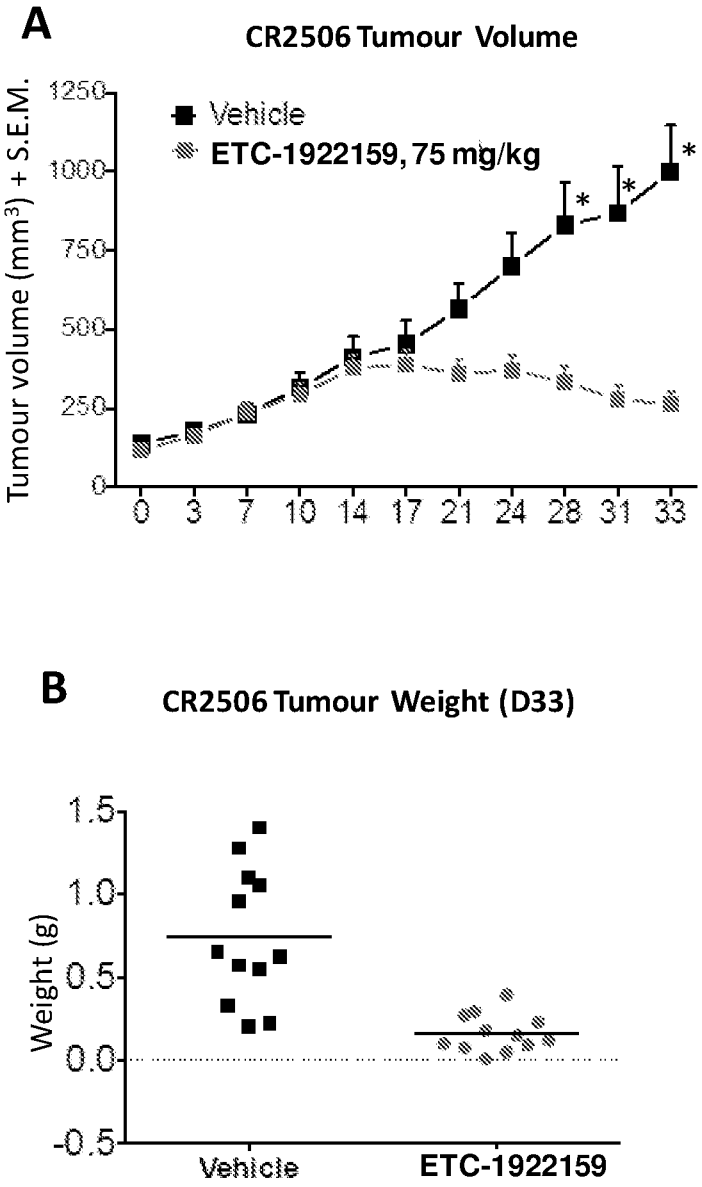
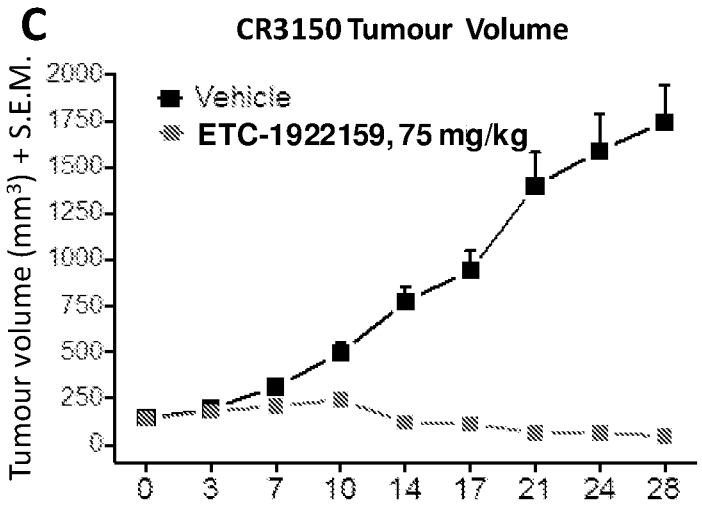

A
CR2506 Tumour Volume
Tumour volume (mm³) + S.E.M.
1250
1000
750
500
250
0
Vehicle
ETC-1922159, 75 mg/kg
*
*
*
0 3 7 10 14 17 21 24 28 31 33
B
CR2506 Tumour Weight (D33)
Weight (g)
1.5
1.0
0.5
0.0
-0.5
Vehicle
ETC-1922159

Figure 1



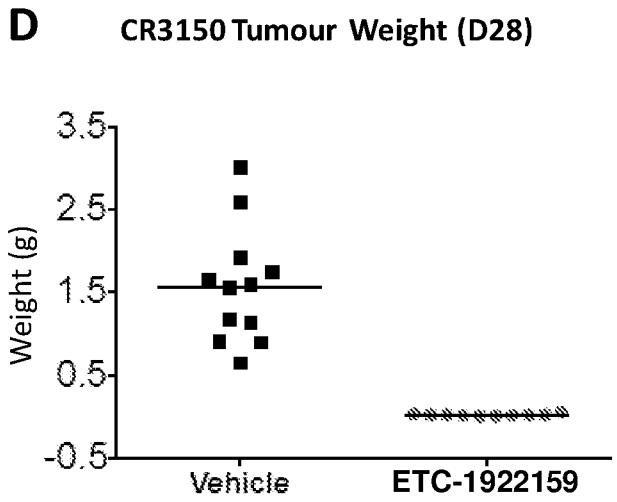


Figure 1 (Continued)

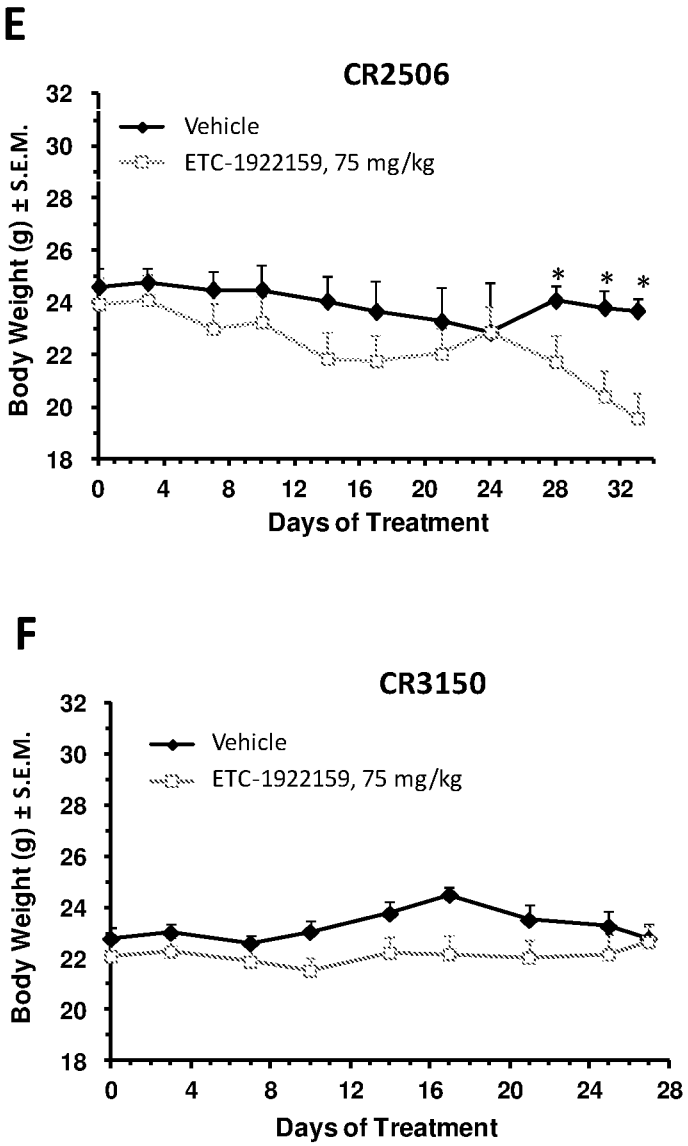
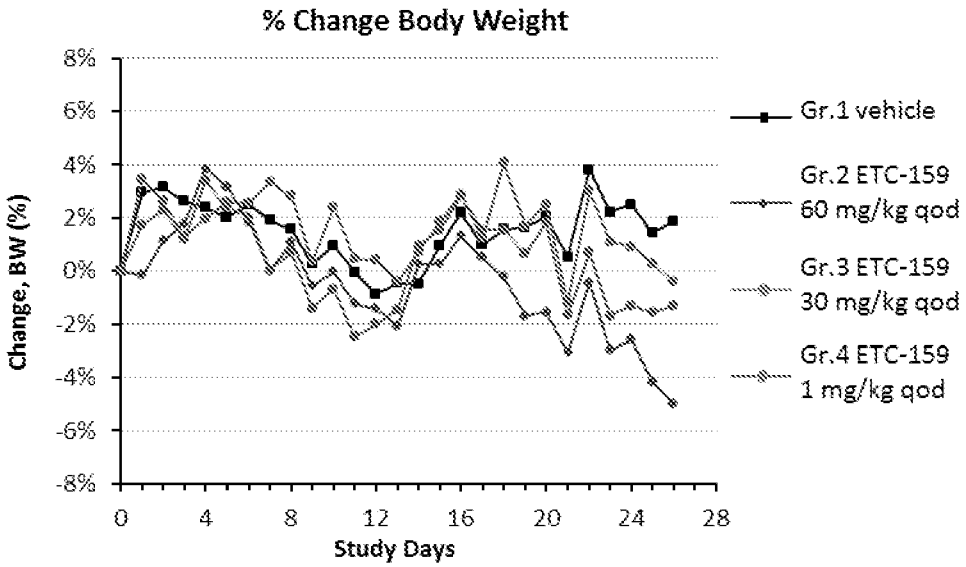


Figure 1 (Continued)

A


% Change Body Weight
Change, BW (%)
8%
6%
4%
2%
0%
-2%
-4%
-6%
-8%
0
4
8
12
16
20
24
28
Study Days
Gr.1 vehicle
Gr.2 ETC-159
60 mg/kg qod
Gr.3 ETC-159
30 mg/kg qod
Gr.4 ETC-159
1 mg/kg qod

B

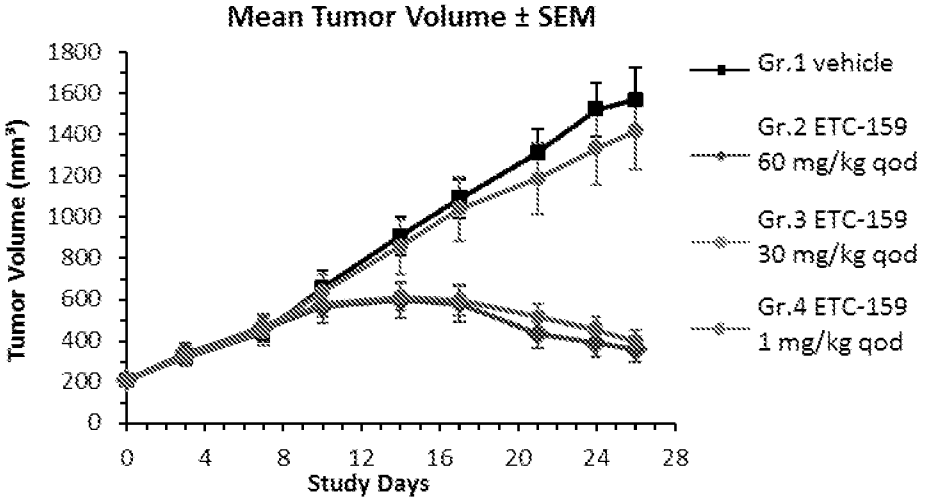

Mean Tumor Volume ± SEM
Tumor Volume (mm³)
1800
1600
1400
1200
1000
800
600
400
200
0
0
4
8
12
16
20
24
28
Study Days
Gr.1 vehicle
Gr.2 ETC-159
60 mg/kg qod
Gr.3 ETC-159
30 mg/kg qod
Gr.4 ETC-159
1 mg/kg qod

Figure 2

C

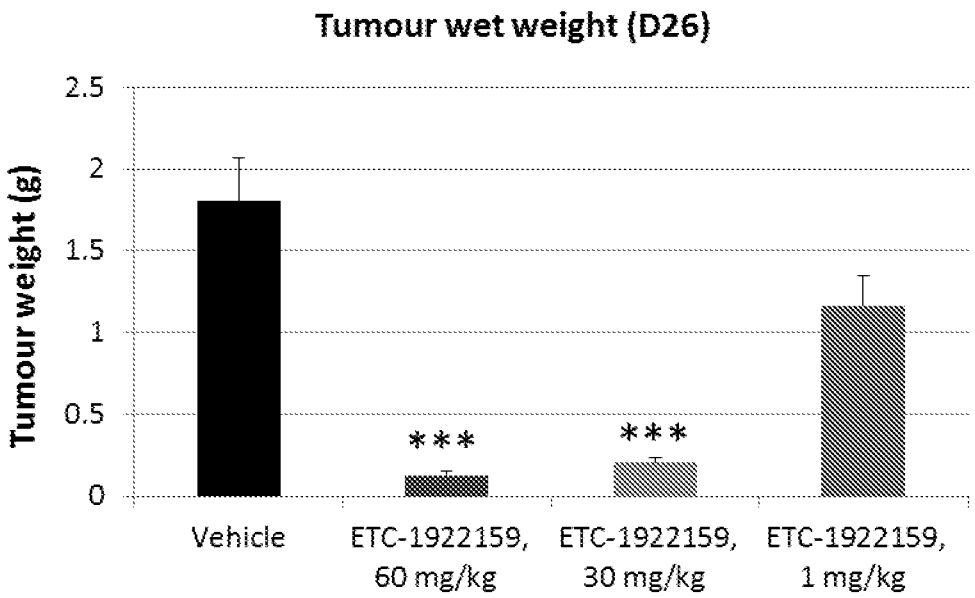


Figure 2 (Continued)

A

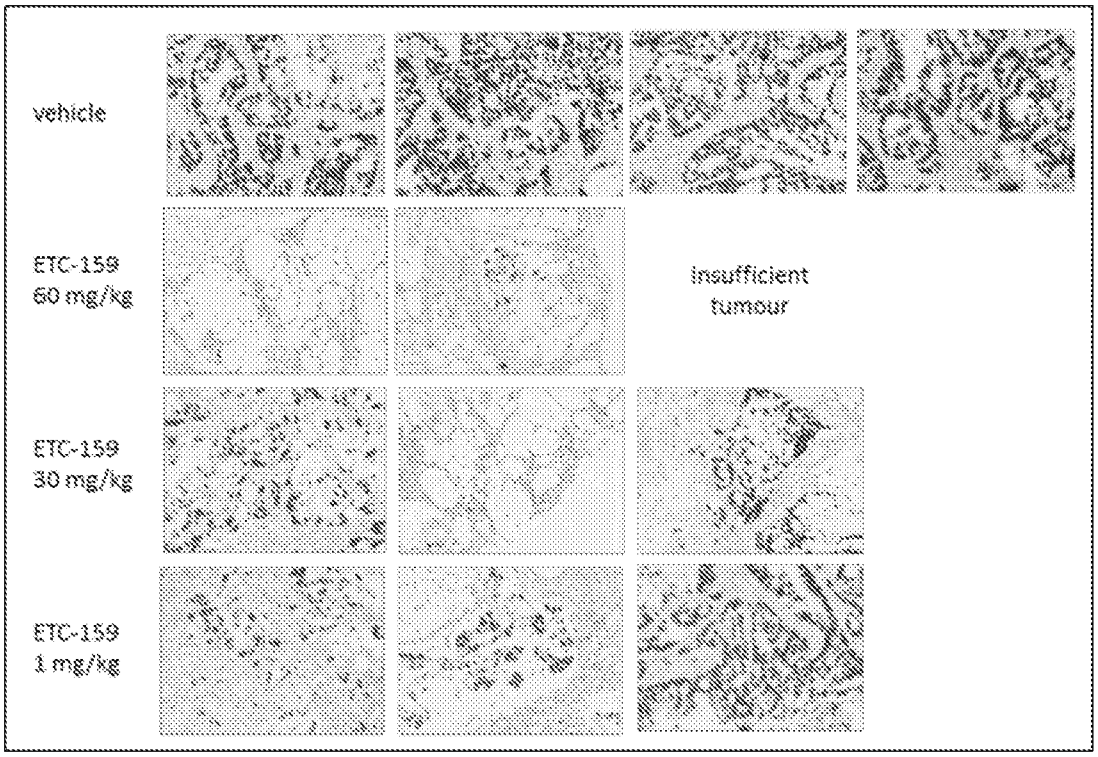
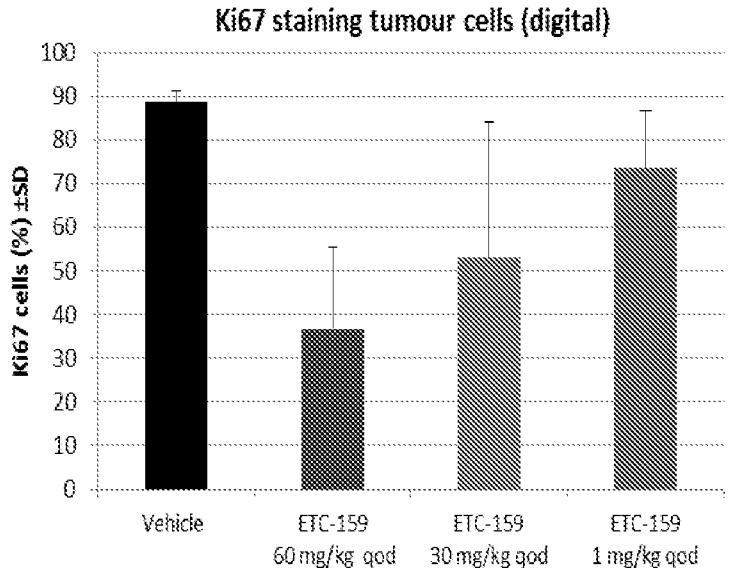


Figure 3

B



C

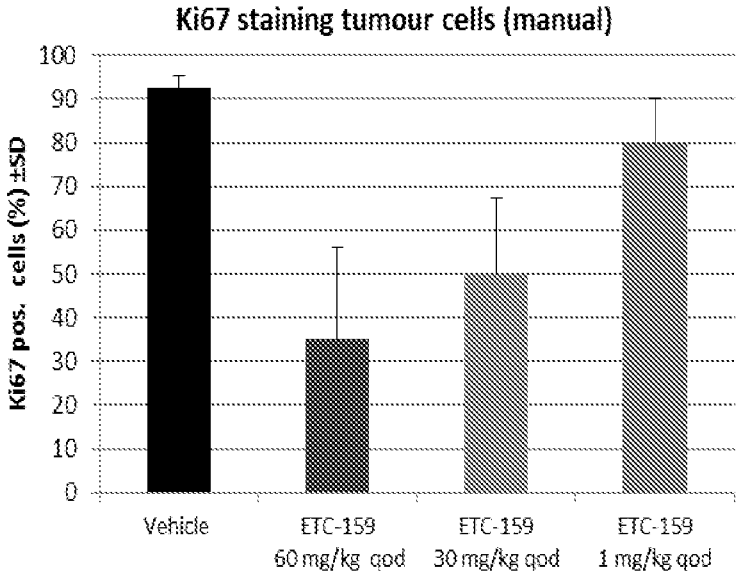
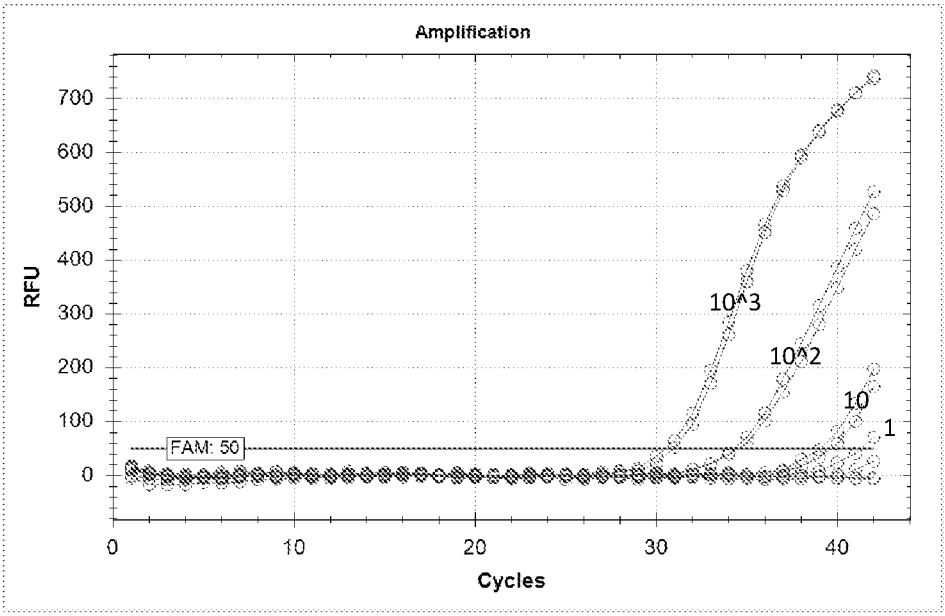


Figure 3 (Continued)

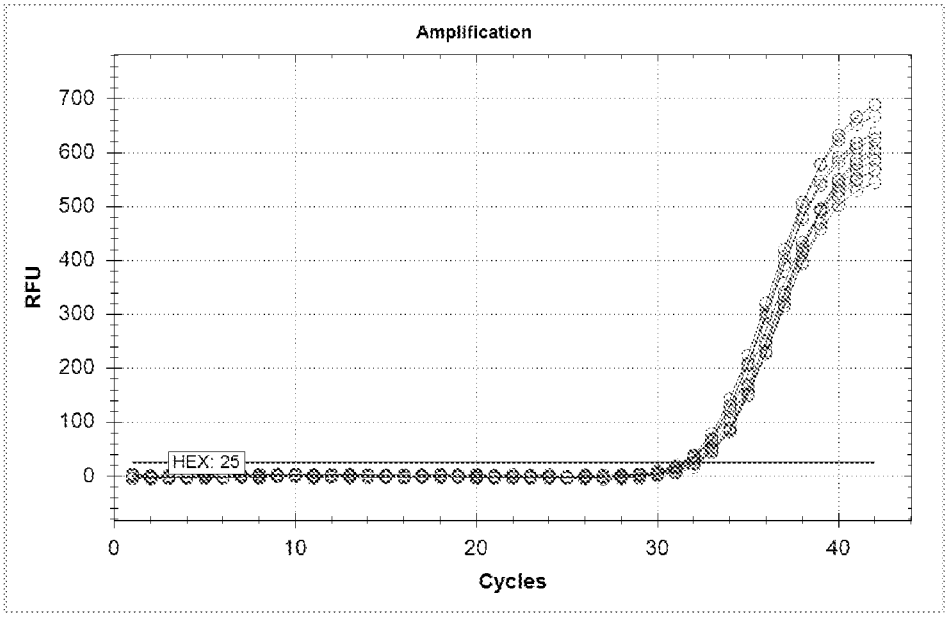
A



PTPRK-RSPO3 fusion: FAM			
PTPRK+RSPO3 plasmid (cp/ul)	*Ct value (Duplicates)*		*Average*
1000	30.49	30.93	30.71
100	34.27	34.51	34.39
10	39.05	39.62	39.34
1	No Ct	41.29	41.29
NTC	No Ct	No Ct	-

Figure 4

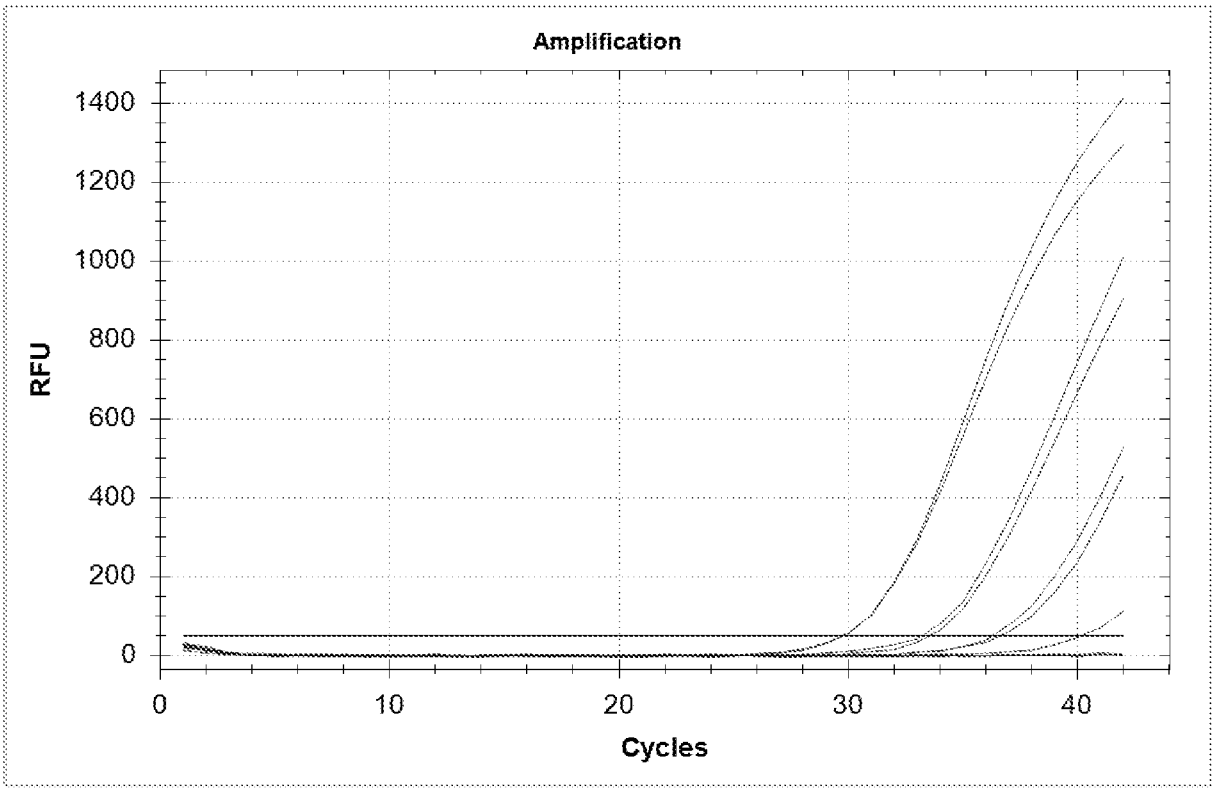
B



IC77: HEX			
PTPRK+RSPO3 plasmid (cp/ul)	*Ct value (Duplicates)*		*Average*
1000	32.10	32.10	32.10
100	31.57	32.00	31.79
10	32.29	31.26	31.78
1	31.89	31.46	31.68
NTC	31.38	31.38	31.38

Figure 4 (Continued)

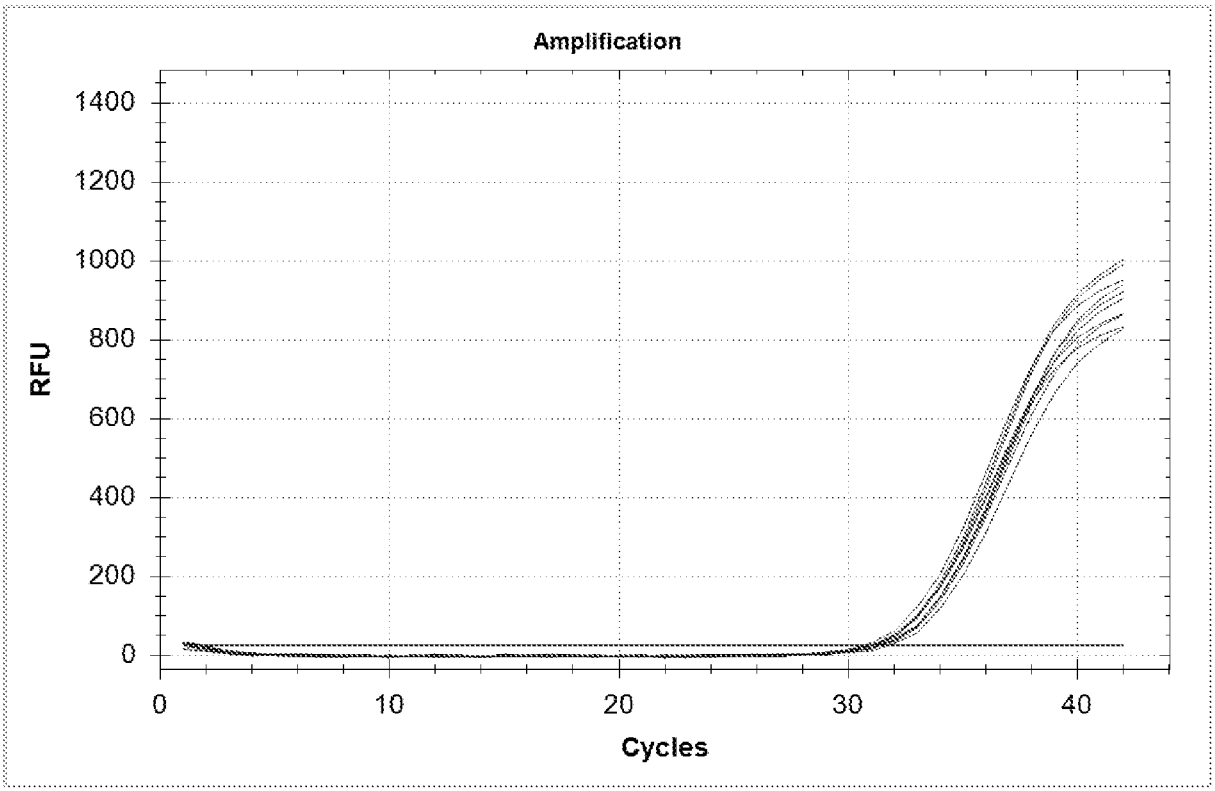
A



PTPRK: TxRd			
PTPRK plasmid (cp/µl)	*Ct value (Duplicates)*		*Average*
1000	29.76	29.75	29.76
100	33.53	33.20	33.37
10	36.28	36.62	36.45
1	No Ct	40.15	40.15
NTC	No Ct	No Ct	-

Figure 5

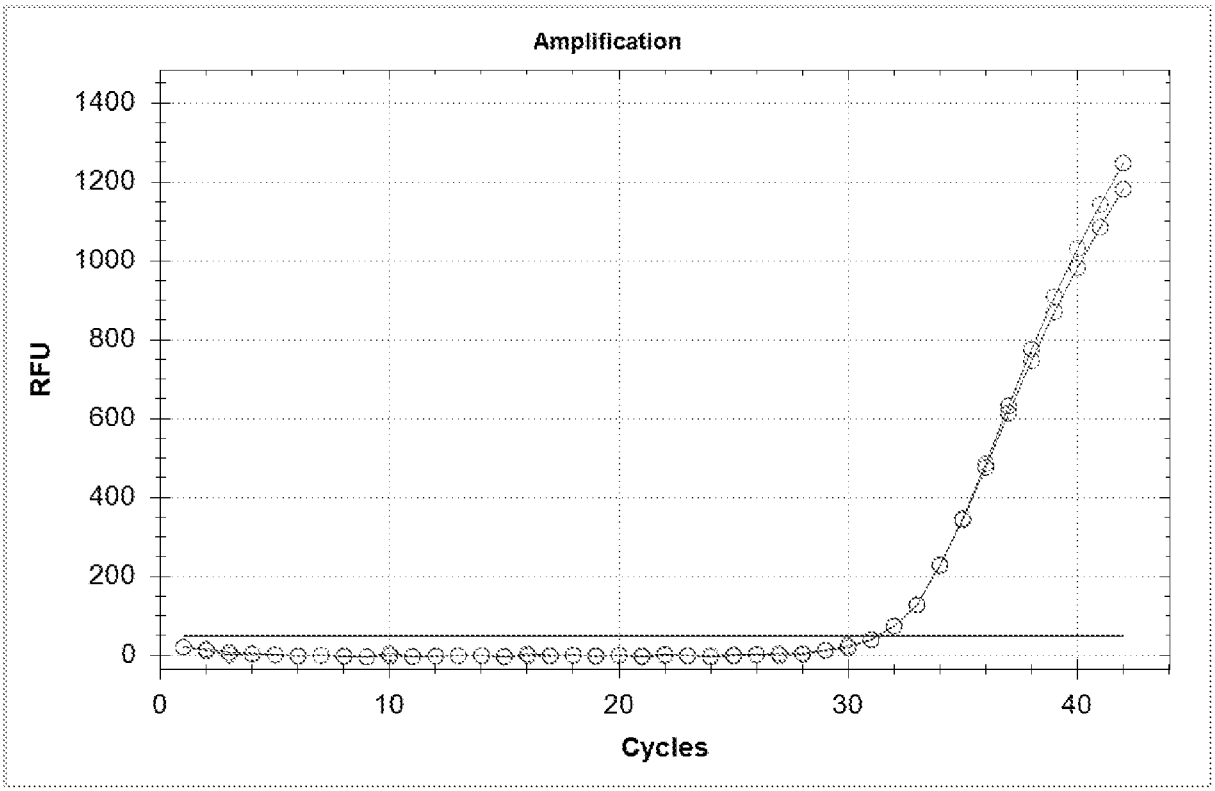
B



IC77: HEX			
PTPRK plasmid (cp/µl)	*Ct value (Duplicates)*		*Average*
1000	31.68	31.37	31.53
100	31.05	30.95	31.00
10	31.07	30.63	30.85
1	31.32	31.38	31.35
NTC	31.00	31.00	31.00

Figure 5 (Continued)

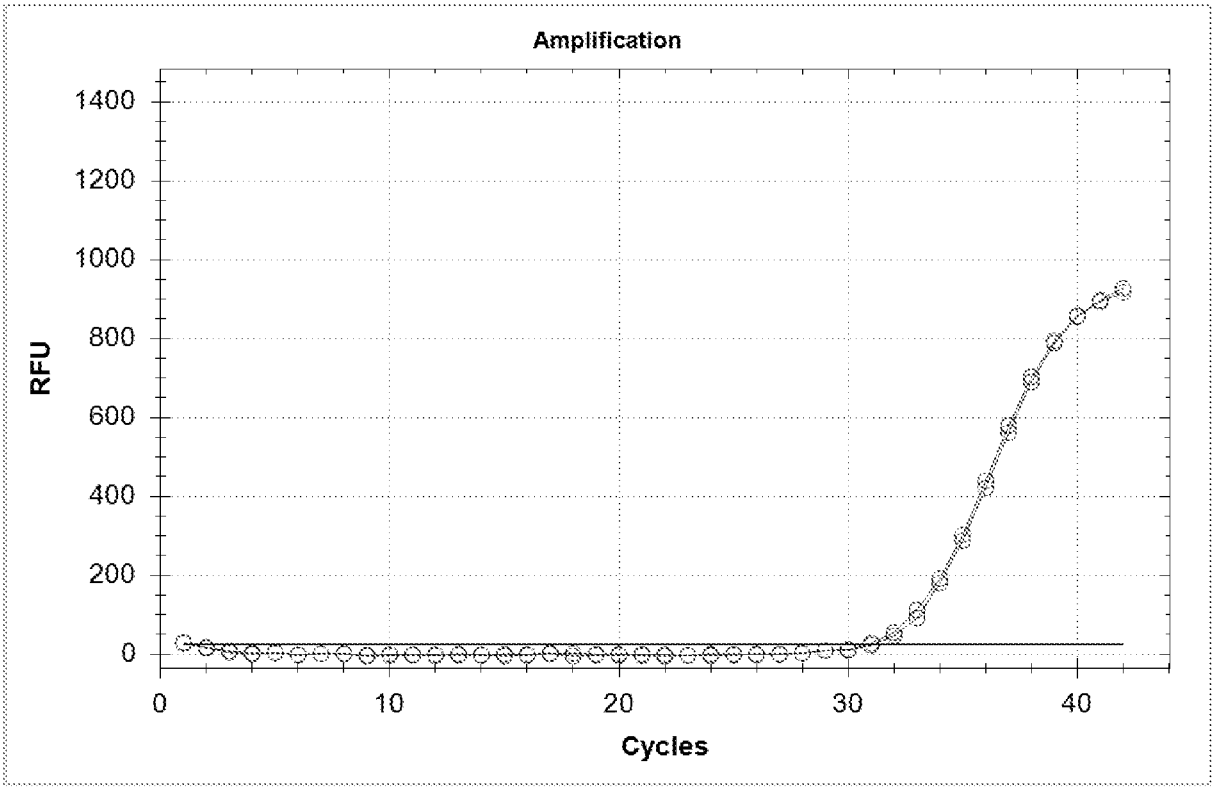
C



PTPRK: TxRd			
Human Total RNA (ng/µl)	*Ct value (Duplicates)*		*Average*
2	31.27	31.30	31.29

Figure 5 (Continued)

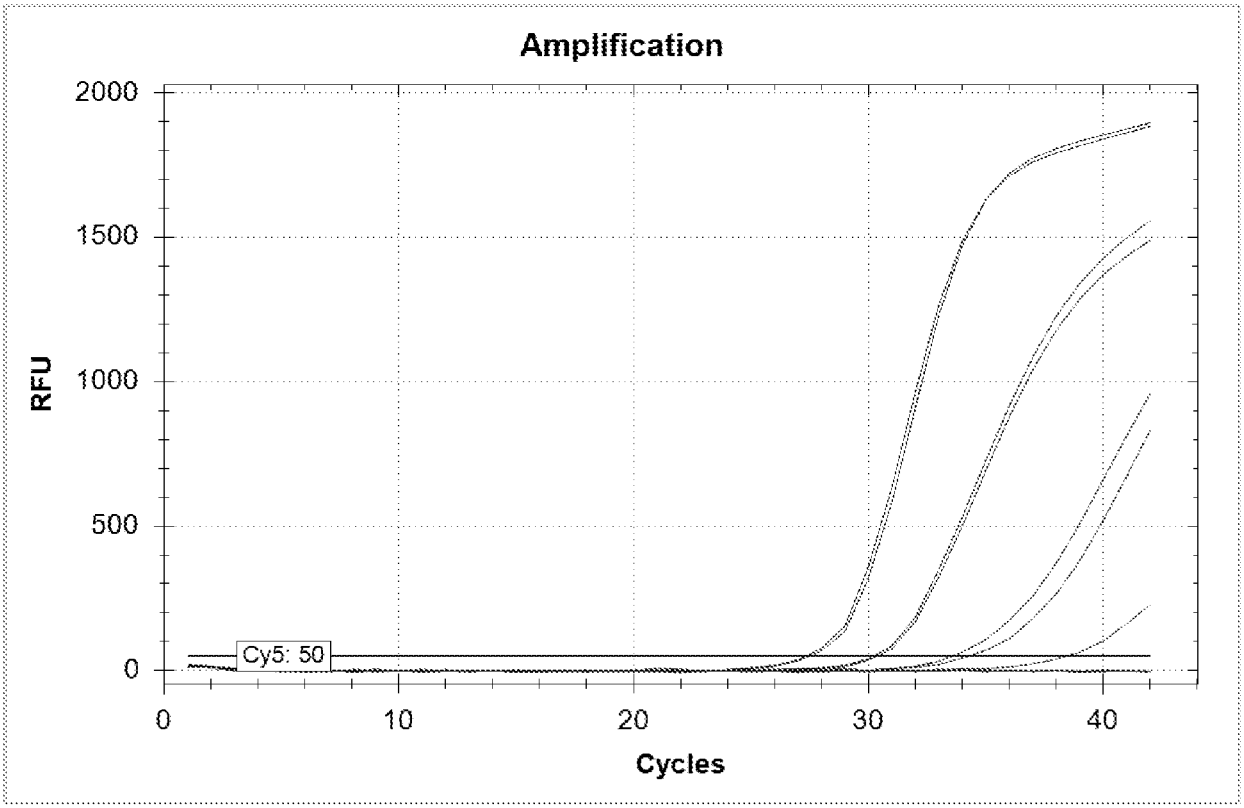
D



IC77: HEX			
Human Total RNA (ng/ul)	*Ct value (Duplicates)*		*Average*
2	30.82	31.08	30.95

Figure 5 (Continued)

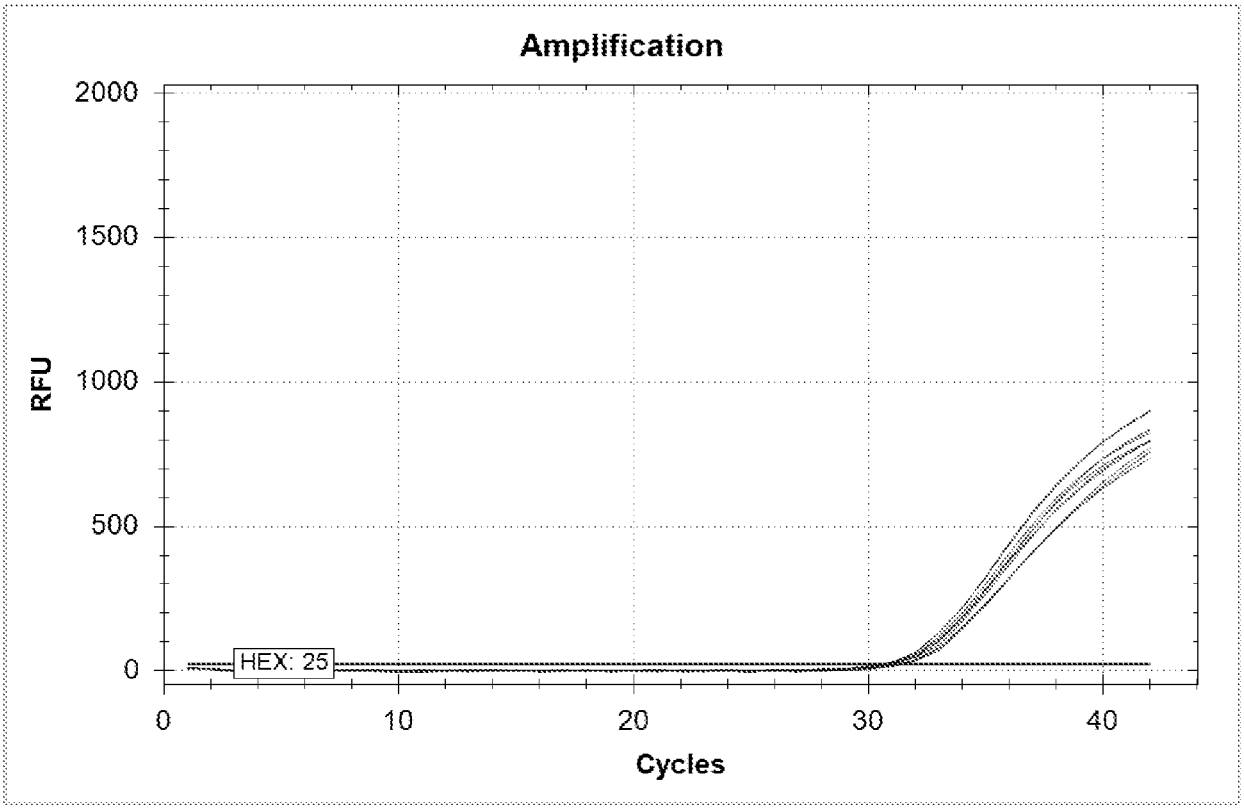
A



RSPO3: Cy5			
RSPO3 plasmid (cp/µl)	*Ct value (Duplicates)*		*Average*
1000	27.32	27.46	27.39
100	30.35	30.23	30.29
10	34.23	33.55	33.89
1	No Ct	38.43	38.43
NTC	No Ct	No Ct	-

Figure 6

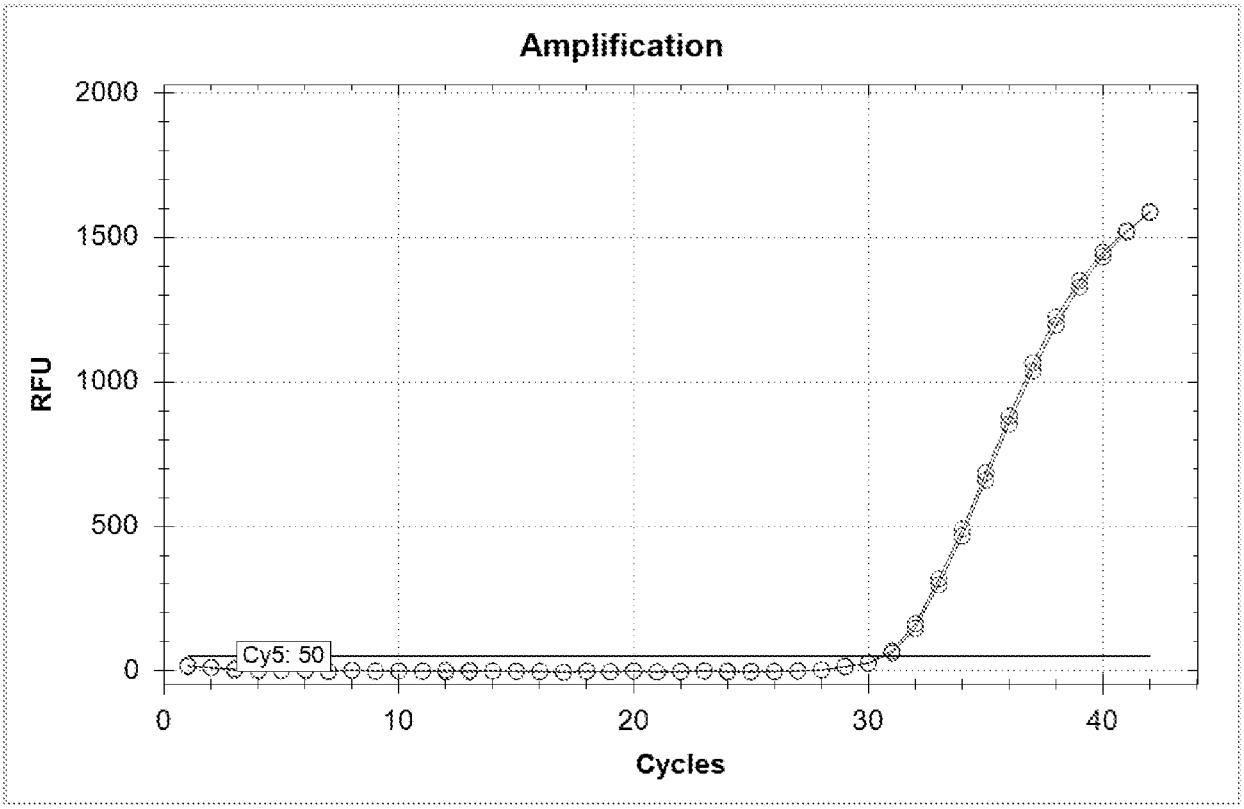
B



IC77: HEX			
RSPO3 plasmid (cp/µl)	*Ct value (Duplicates)*		*Average*
1000	31.31	31.51	31.41
100	31.37	30.94	31.16
10	30.99	31.02	31.01
1	30.75	31.10	30.93
NTC	30.59	30.74	30.67

Figure 6 (Continued)

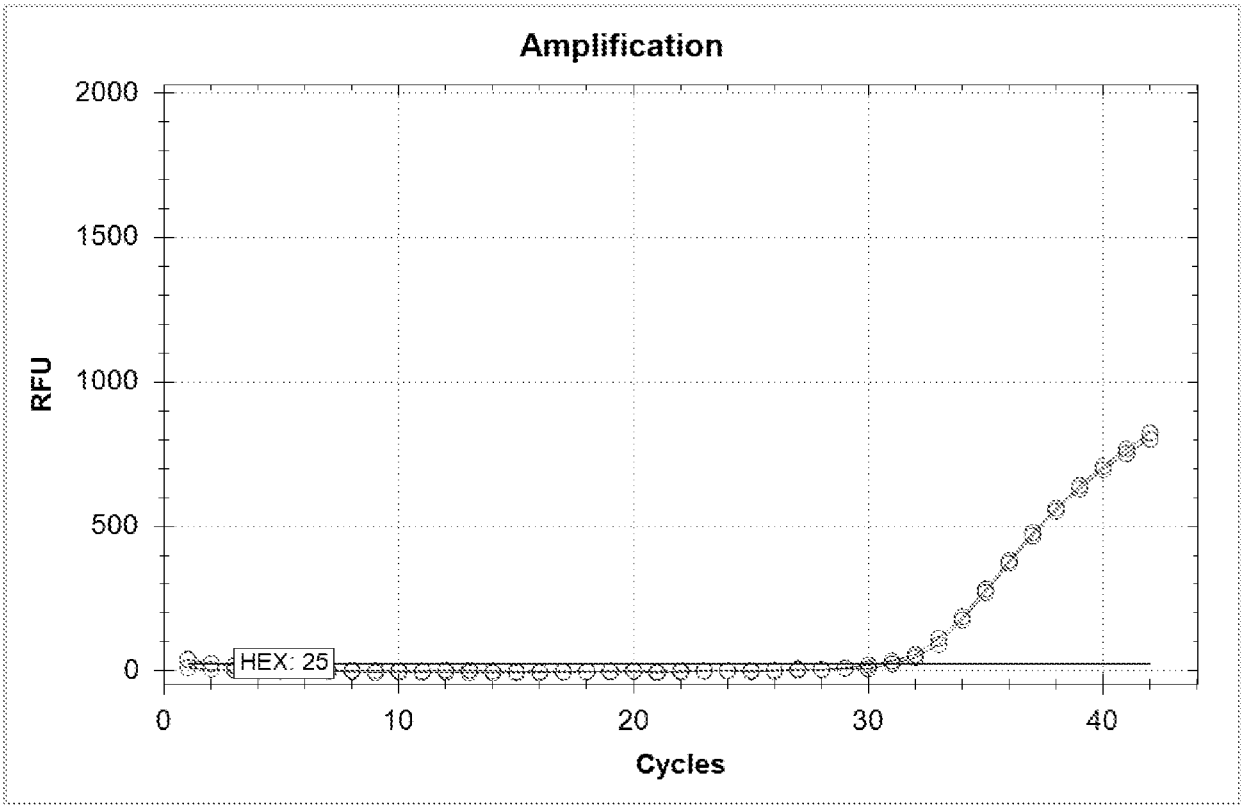
C



RSPO3: Cy5			
Human Total RNA (ng/µl)	*Ct value (Duplicates)*		*Average*
2	30.55	30.67	30.61

Figure 6 (Continued)

D



IC77: HEX			
Human Total RNA (ng/µl)	*Ct value (Duplicates)*		*Average*
2	31.05	30.33	30.69

Figure 6 (Continued)

A

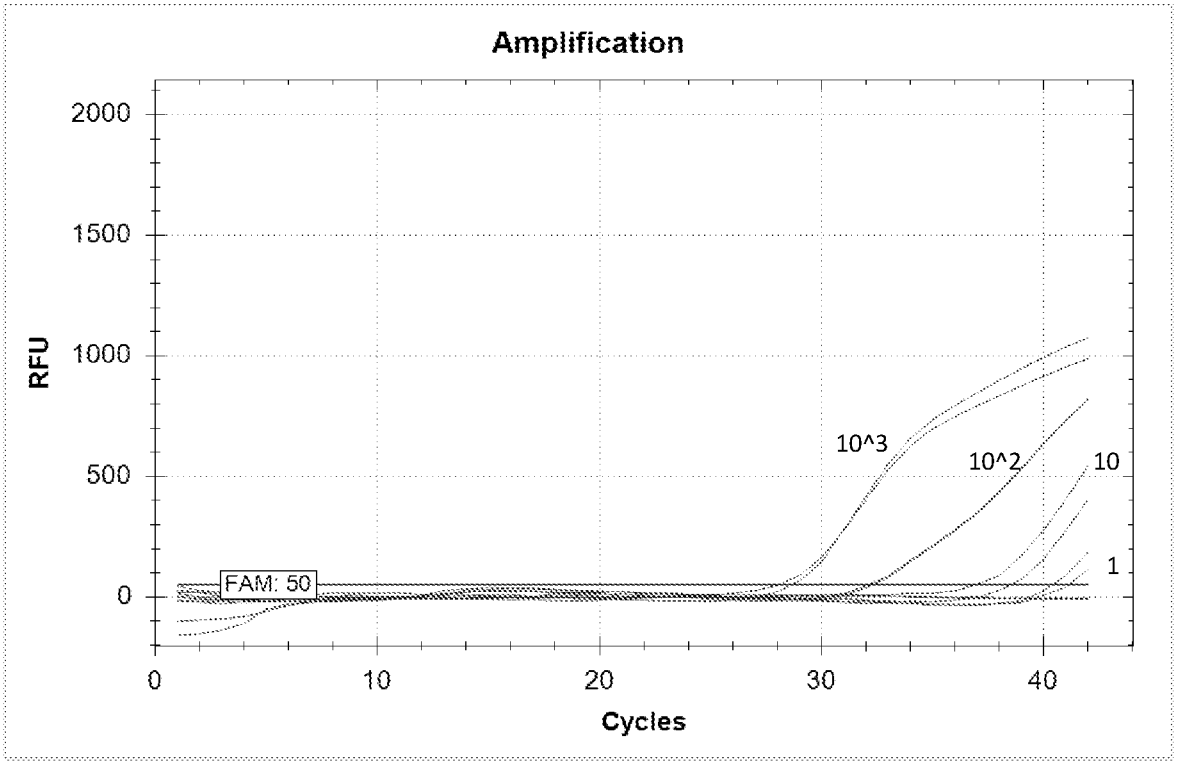
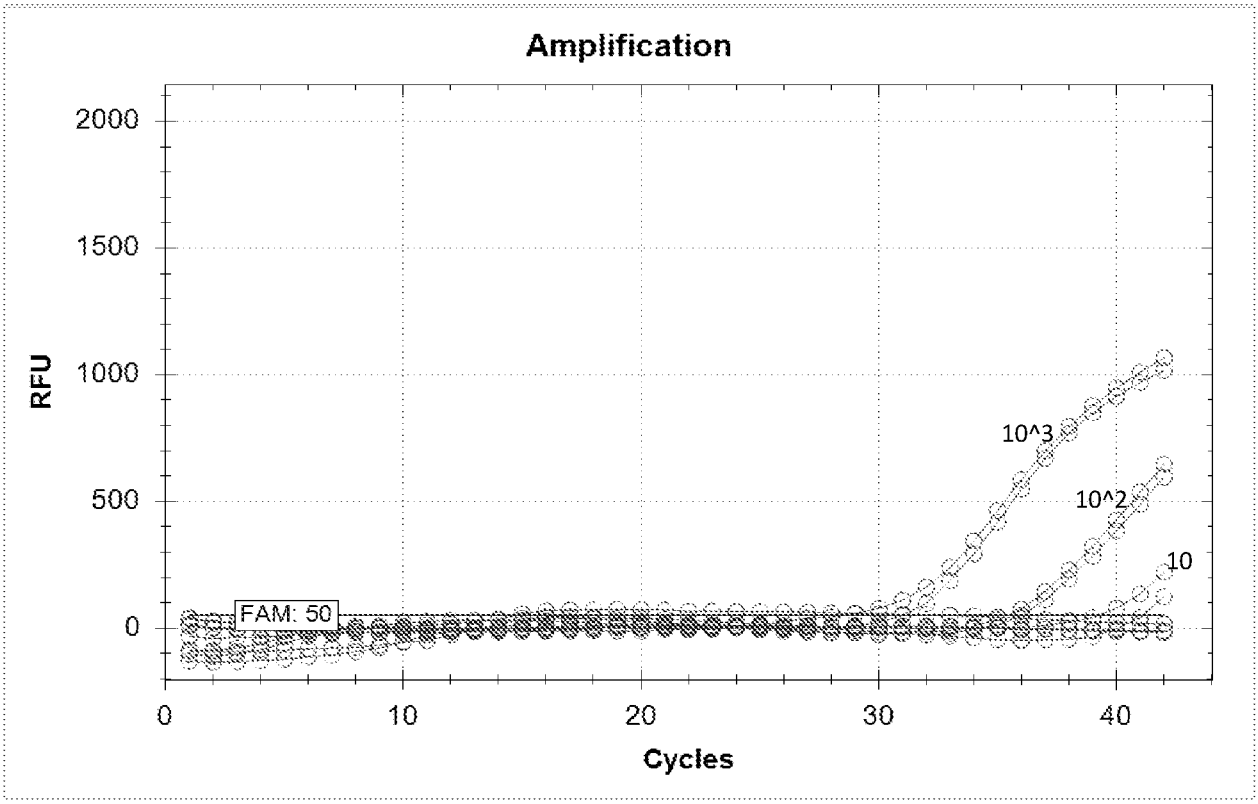

Amplification
RFU
2000
1500
1000
500
0
FAM: 50
10^3
10^2
10
1
0
10
20
30
40
Cycles

Figure 7

B



	Fusion A: FAM						
Fusion A plasmid (cp/ul)	3plex			1plex			ΔCt (3plex-1plex)
	Ct values (duplicates)		Average	Ct values (duplicates)		Average	
1000	28.02	28.64	28.33	30.94	28.40	29.67	-1.34
100	32.08	32.28	32.18	35.40	36.00	35.70	-3.52
10	36.90	38.50	37.70	39.22	41.10	40.16	-2.46
1	41.12	40.39	40.75	No Ct	No Ct	-	x
NTC	No Ct	No Ct	-	No Ct	error	-	-

Figure 7 (Continued)

C

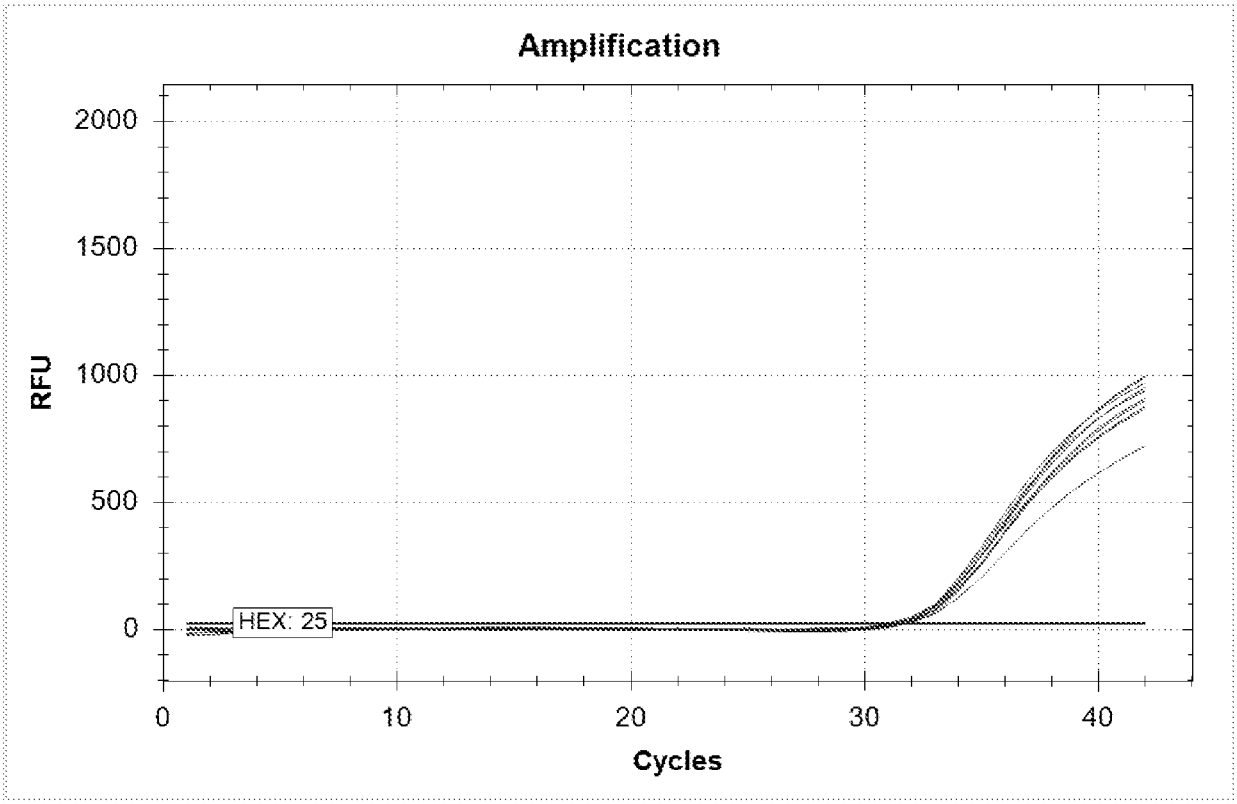
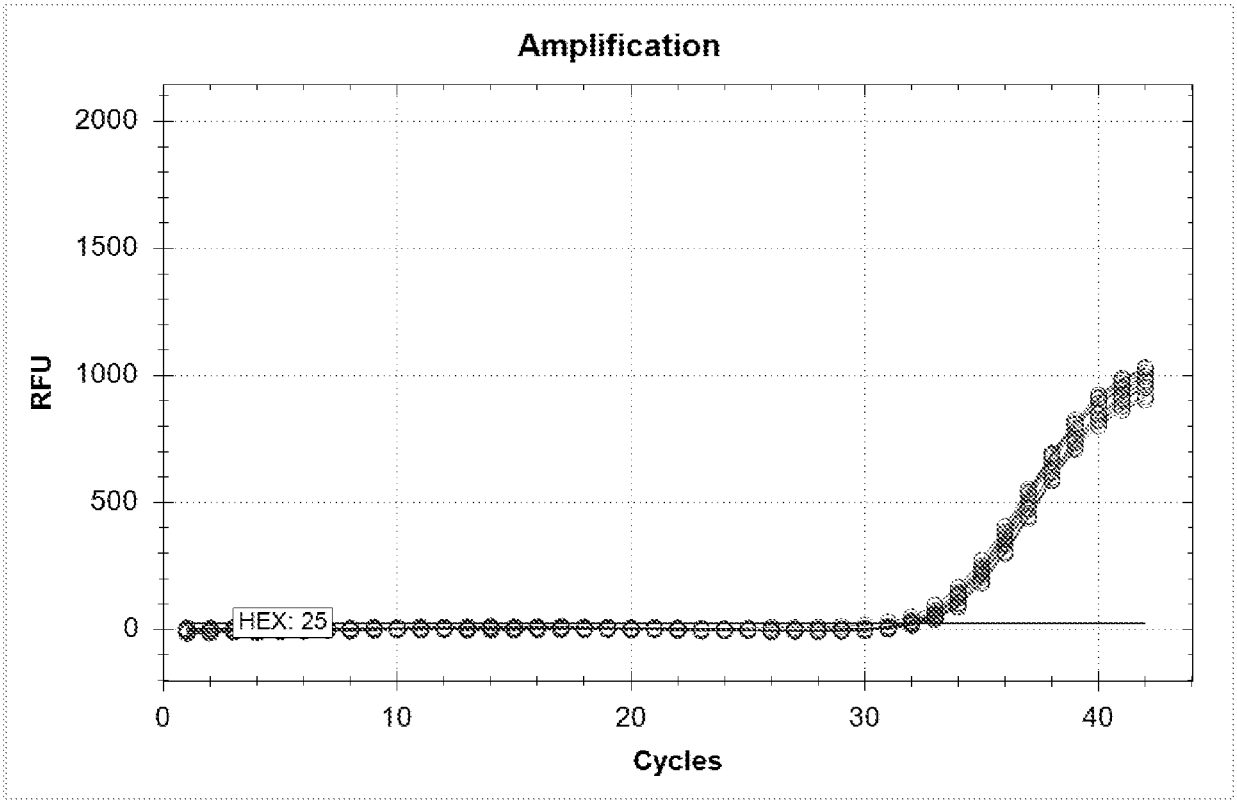


Figure 7 (Continued)

D



	IC77: HEX						
Fusion A plasmid (cp/ul)	3plex			1plex			*ΔCt* (3plex-1plex)
	Ct values (duplicates)		*Average*	*Ct values (duplicates)*		*Average*	
1000	31.75	31.25	31.50	32.29	30.57	31.43	0.06
100	31.65	31.82	31.74	32.18	32.42	32.30	-0.56
10	31.35	31.52	31.44	31.67	31.96	31.81	-0.38
1	31.34	31.75	31.55	31.57	31.94	31.76	-0.21
NTC	31.27	31.04	31.16	32.22	32.00	32.11	-0.95

Figure 7 (Continued)

A

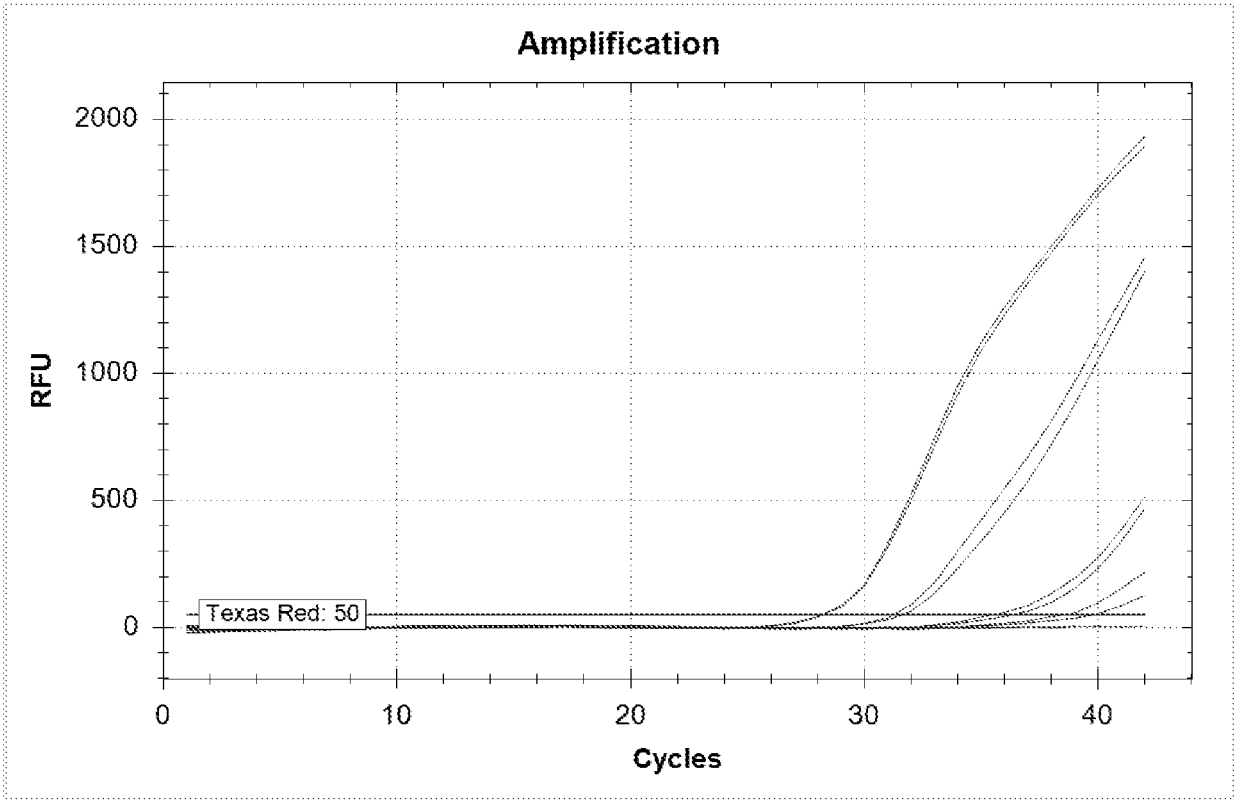
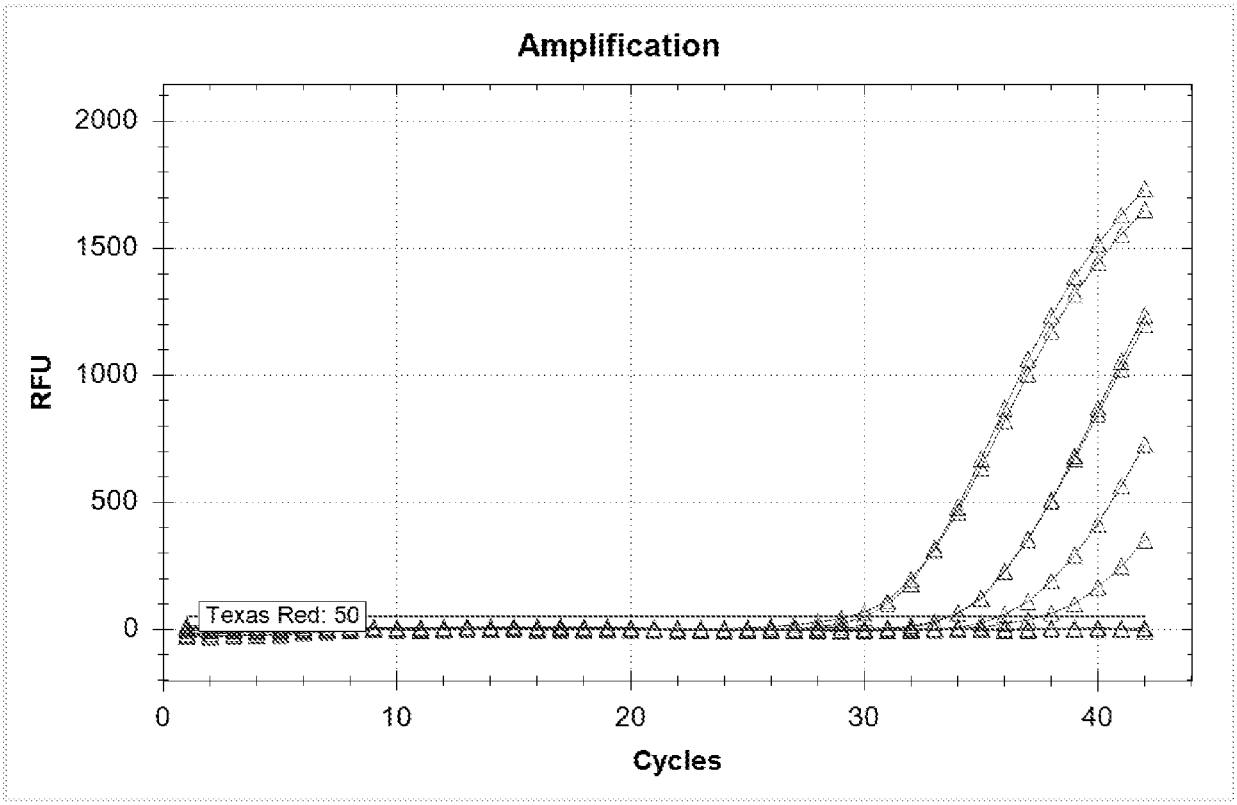

Amplification
RFU
2000
1500
1000
500
0
Texas Red: 50
0
10
20
30
40
Cycles

Figure 8

B



	PTPRK: TxRd						
PTPRK plasmid (cp/µl)	3plex			1plex			ΔCt (3plex-1plex)
	Ct values (duplicates)		Average	Ct values (duplicates)		Average	
1000	28.18	28.21	28.20	29.32	30.05	29.69	-1.49
100	31.61	31.24	31.43	33.62	33.62	33.62	-2.19
10	36.33	35.48	35.90	35.73	37.52	36.63	-0.73
1	39.75	38.52	39.14	No Ct	No Ct	-	x
NTC	No Ct	No Ct	-	No Ct	No Ct	-	-

Figure 8 (Continued)

C

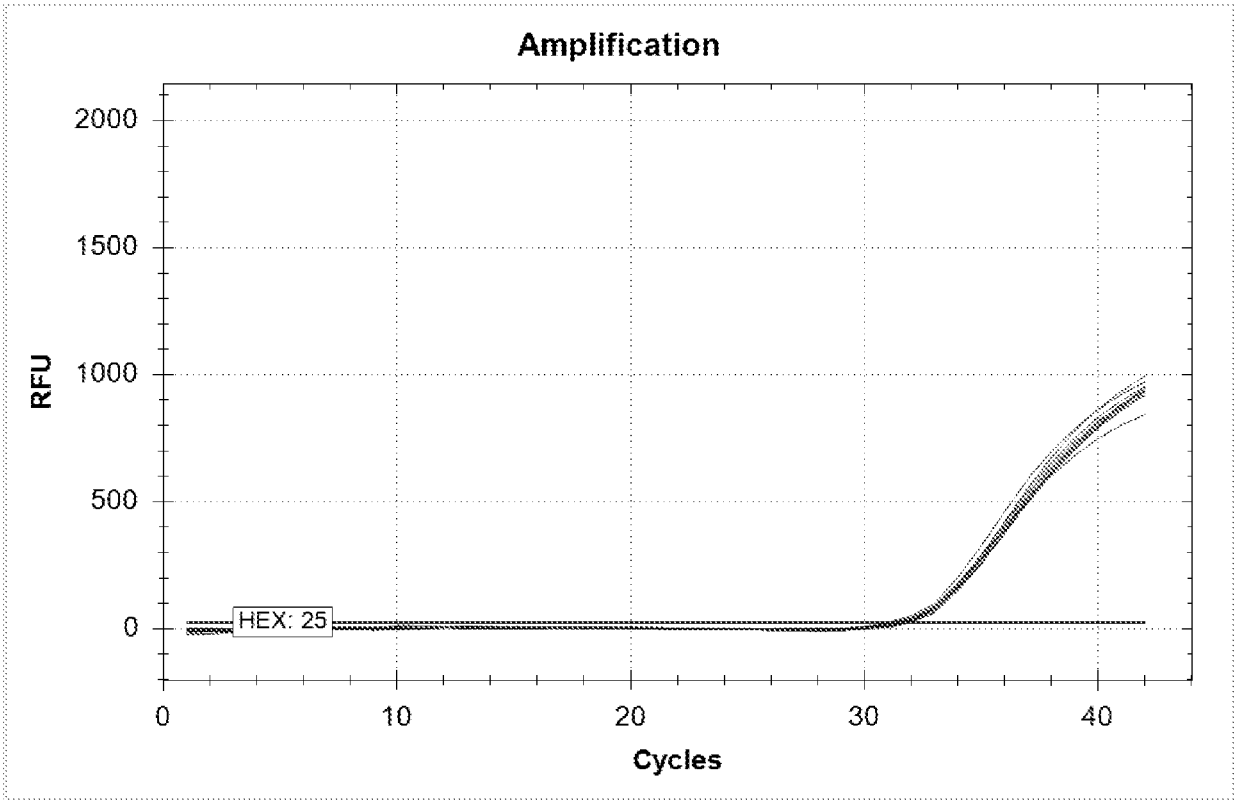
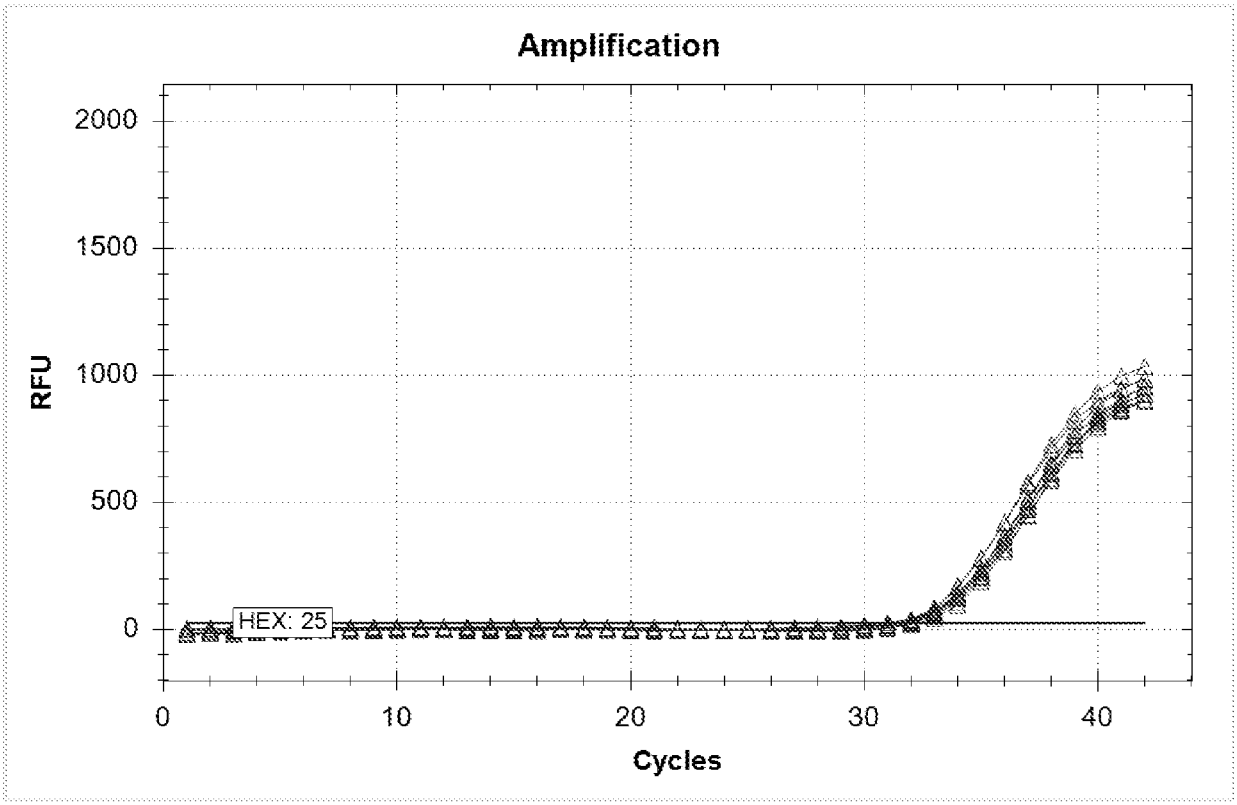


Figure 8 (Continued)

D



	IC77: HEX						
PTPRK plasmid (cp/ul)	3plex			1plex			ΔCt (3plex-1plex)
	Ct values (duplicates)		Average	Ct values (duplicates)		Average	
1000	31.63	31.90	31.77	30.82	32.10	31.46	0.31
100	31.98	31.71	31.85	32.03	32.26	32.15	-0.30
10	31.26	31.61	31.43	31.90	31.68	31.79	-0.36
1	31.48	31.74	31.61	31.32	31.48	31.40	0.21
NTC	31.27	31.04	31.16	31.38	31.39	31.38	-0.23

Figure 8 (Continued)

A

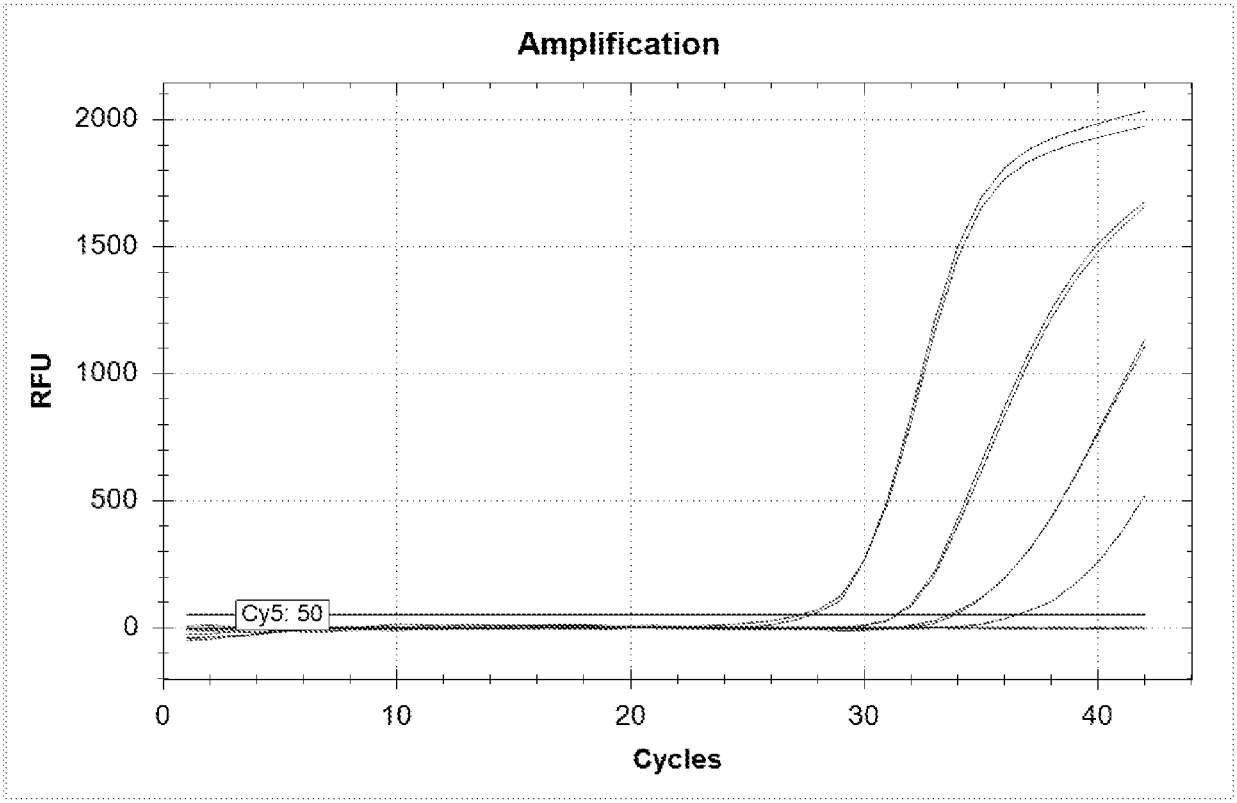
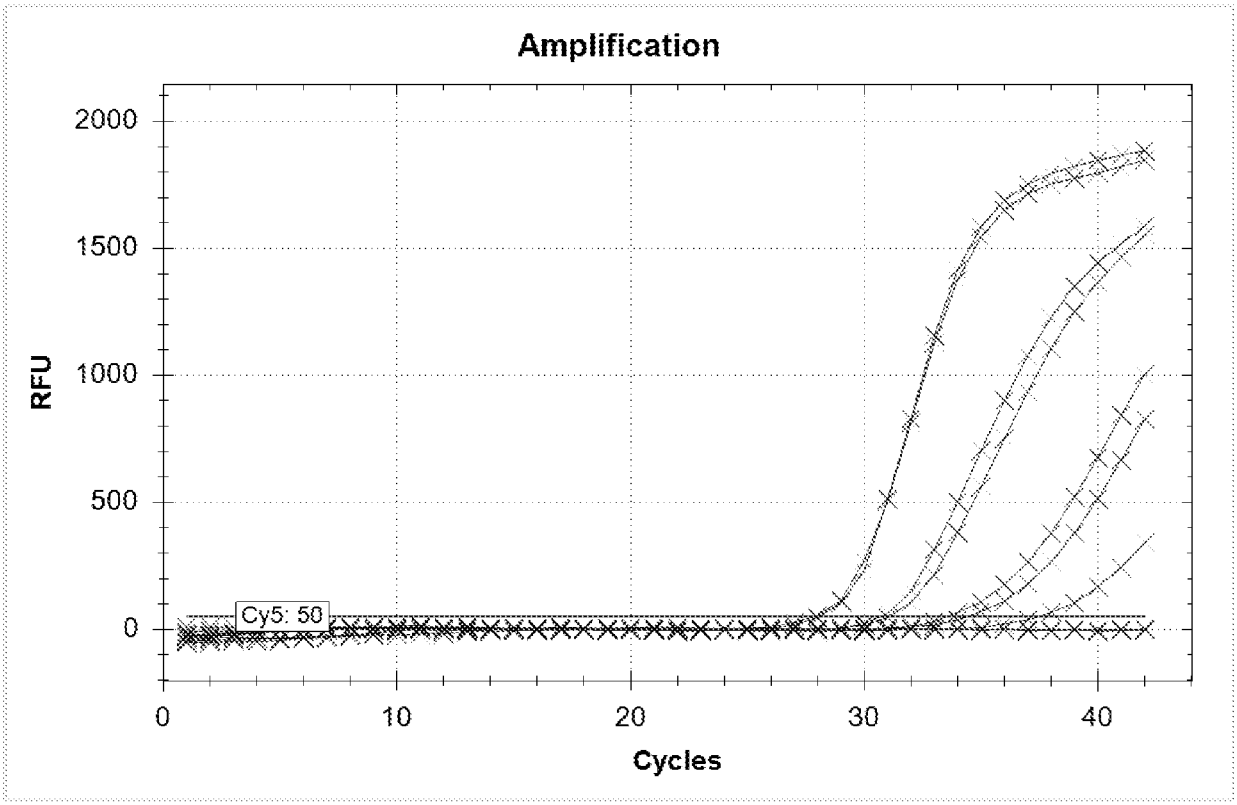

Amplification
RFU
2000
1500
1000
500
0
Cy5: 50
0
10
20
30
40
Cycles

Figure 9

B



	RSPO3: Cy5						
RSPO3 plasmid (cp/µl)	3plex			1plex			*ΔCt* (3plex-1plex)
	Ct values (duplicates)		*Average*	*Ct values (duplicates)*		*Average*	
1000	27.21	27.68	27.45	27.86	28.07	27.97	-0.52
100	31.35	31.36	31.36	31.07	30.68	30.88	0.48
10	33.83	33.56	33.70	34.39	33.53	33.96	-0.26
1	36.48	No Ct	36.48	No Ct	37.43	37.43	-0.95
NTC	No Ct	No Ct	-	No Ct	No Ct	-	-

Figure 9 (Continued)

C

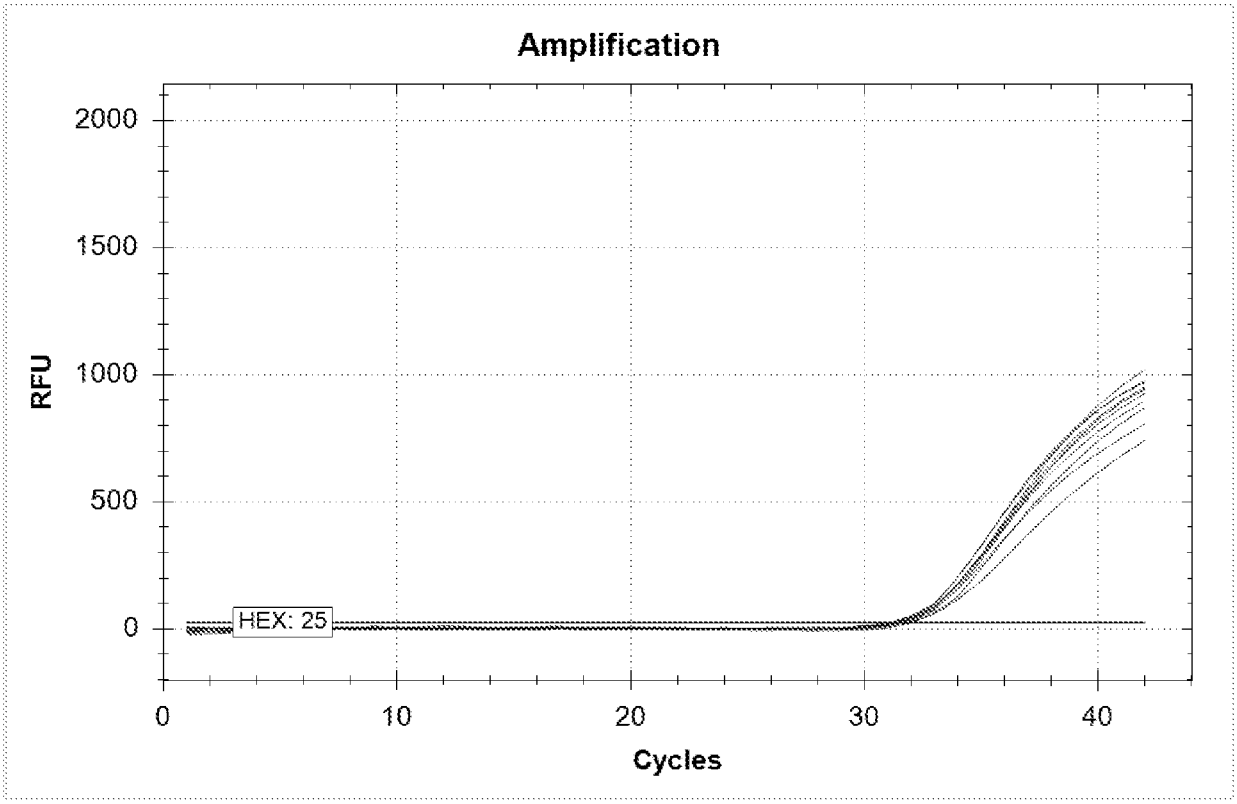
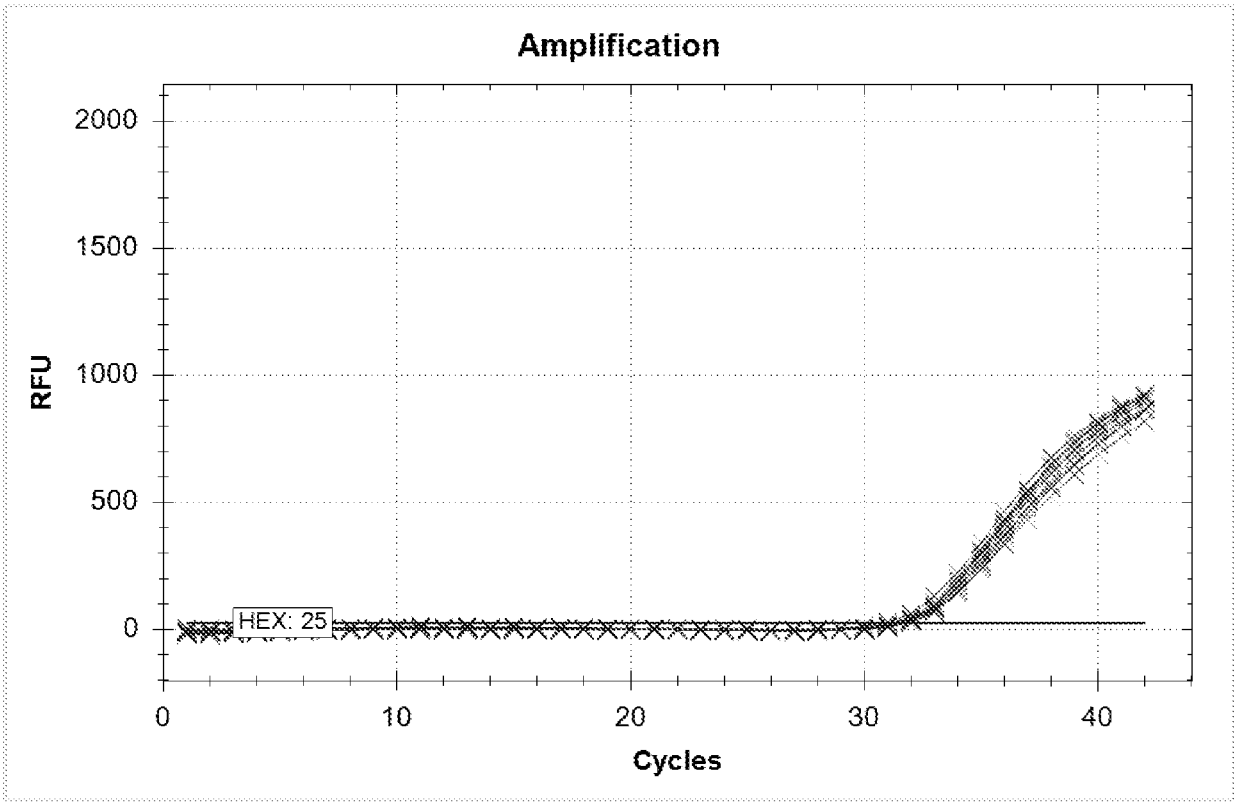


Figure 9 (Continued)

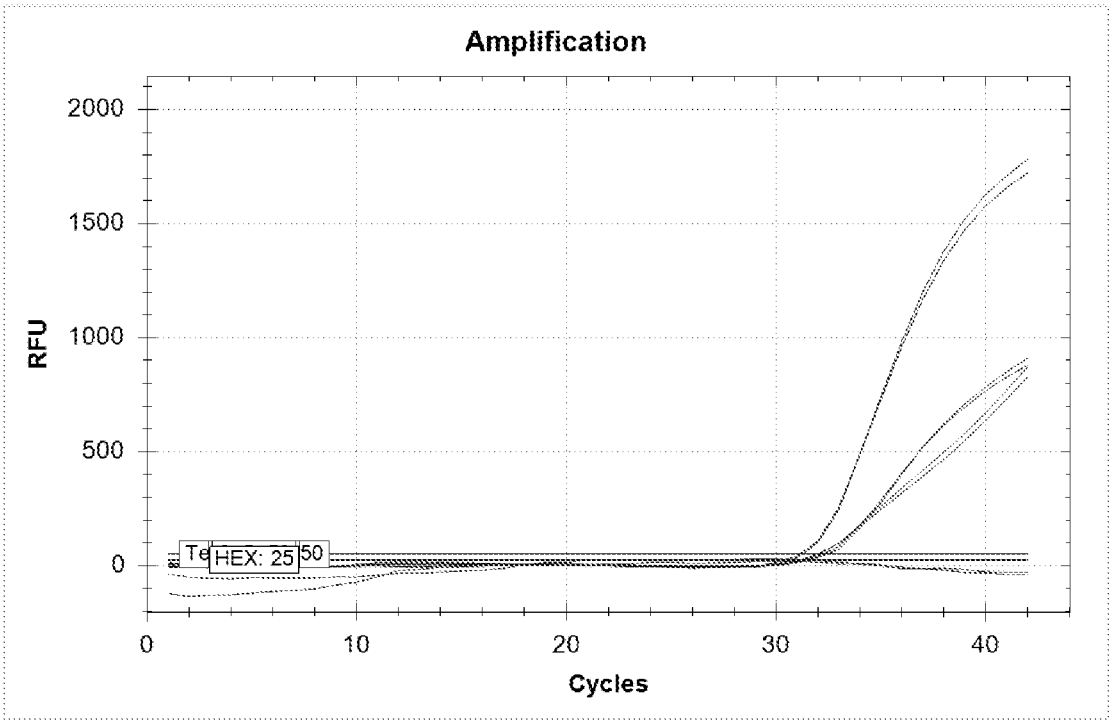
D



	IC77: HEX						
RSPO3 plasmid (cp/µl)	3plex			1plex			*ΔCt* (3plex-1plex)
	Ct values (duplicates)		*Average*	*Ct values (duplicates)*		*Average*	
1000	31.67	31.44	31.56	31.37	31.50	31.44	0.12
100	31.39	32.03	31.71	31.33	31.77	31.55	0.16
10	31.47	31.23	31.35	31.16	30.78	30.97	0.38
1	31.69	31.44	31.57	31.51	31.30	31.41	0.16
NTC	31.27	31.04	31.16	31.85	31.48	31.67	-0.51

Figure 9 (Continued)

A


Amplification
RFU
2000
1500
1000
500
0
HEX: 25
0
10
20
30
40
Cycles

B

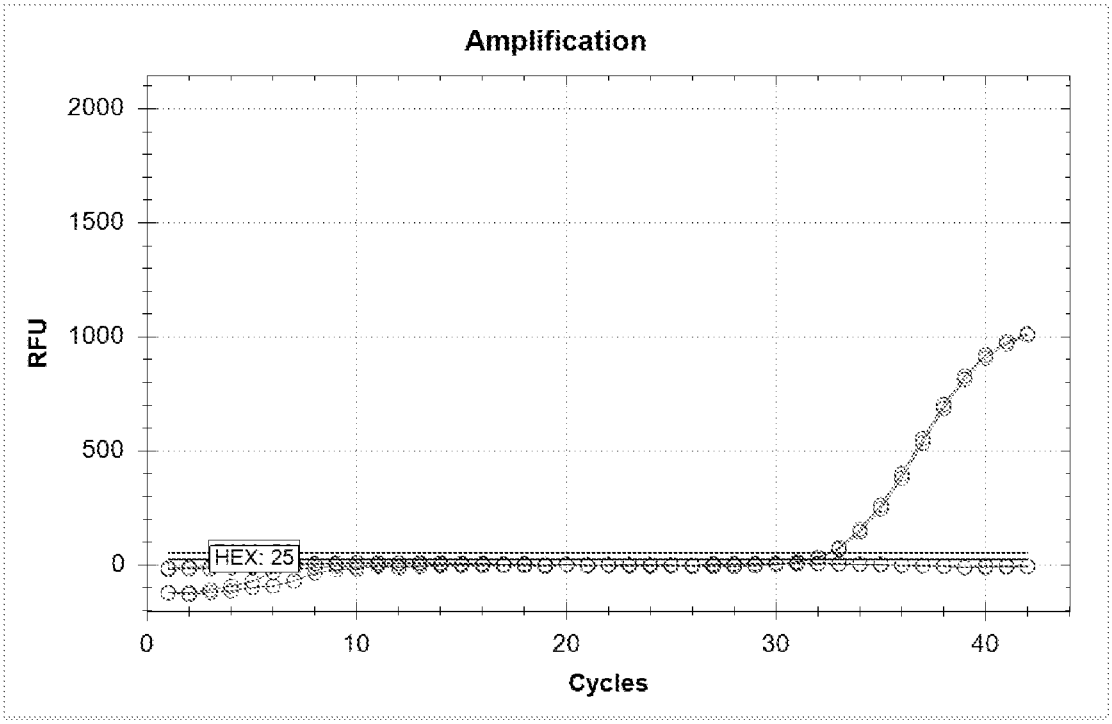

Amplification
RFU
2000
1500
1000
500
0
HEX: 25
0
10
20
30
40
Cycles

Figure 10

C

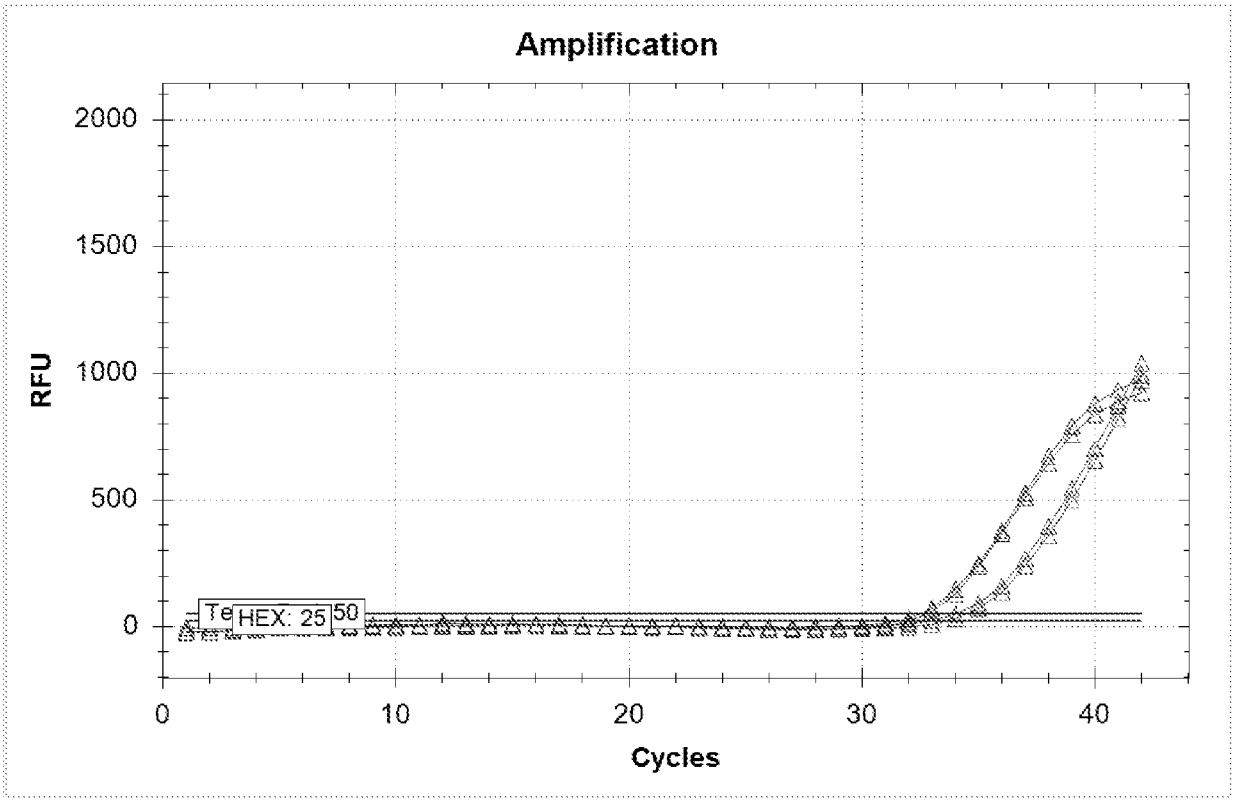
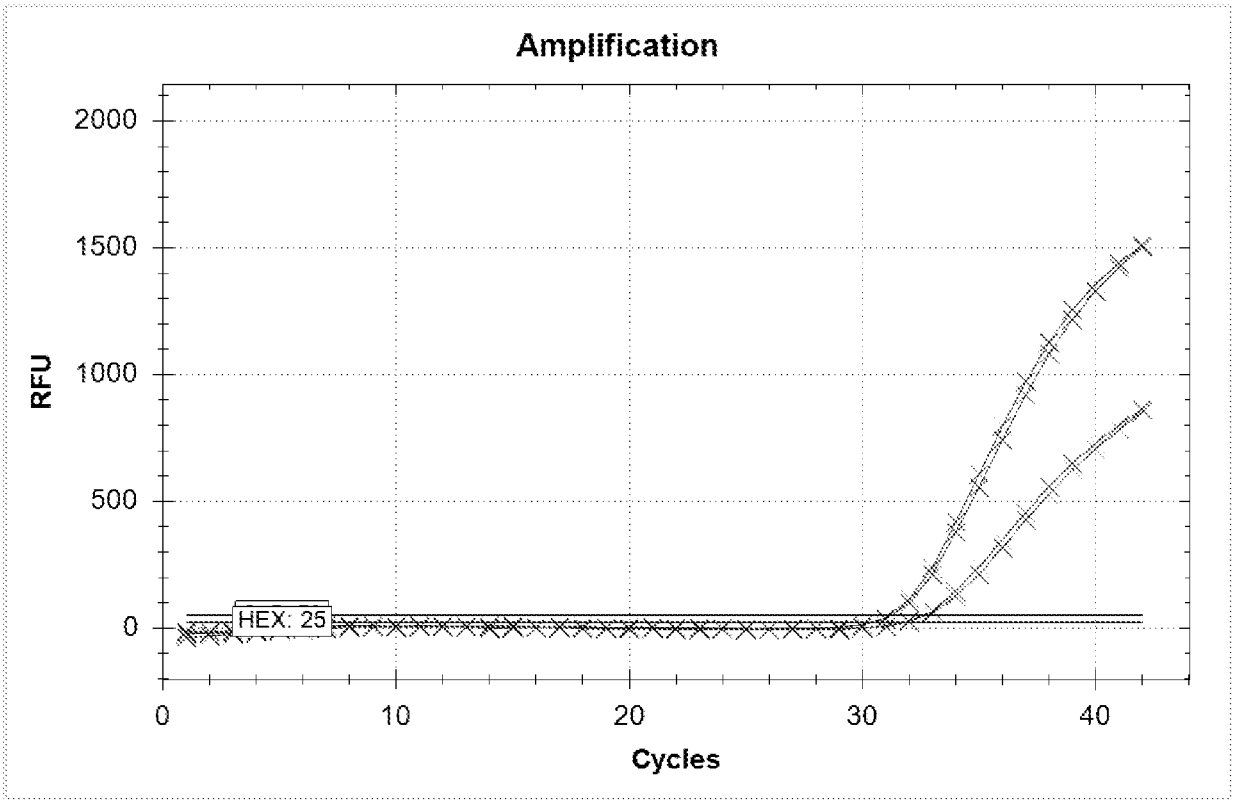


Figure 10 (Continued)

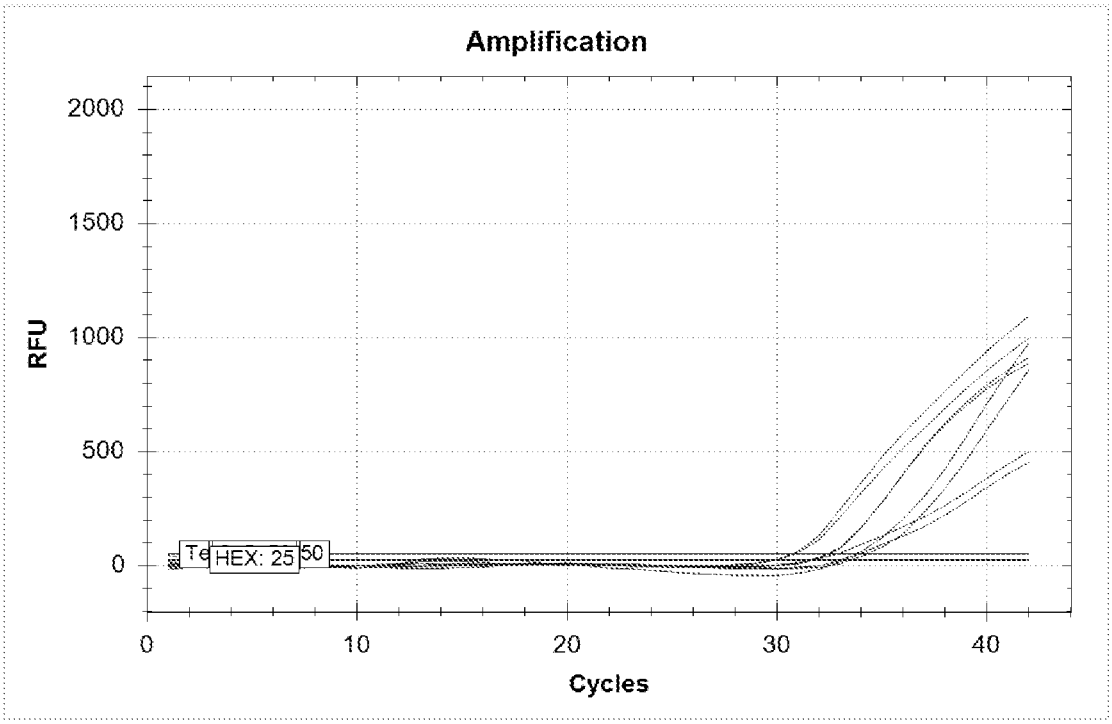
D



Assay	Fusion A: FAM			PTPRK: TxRD			RSPO3: Cy5			IC77: HEX		
	Ct values (duplicates)		*Average*	*Ct values (duplicates)*		*Average*	*Ct values (duplicates)*		*Average*	*Ct values (duplicates)*		*Average*
3plex	No Ct	No Ct	-	31.91	32.03	31.97	31.13	31.23	31.18	31.34	31.51	31.43
Fusion A/IC	No Ct	No Ct	-							31.59	31.67	31.63
PTPRK/IC				34.45	34.02	34.24				32.12	31.64	31.88
RSPO3/IC							31.20	31.12	31.16	31.78	31.85	31.82
ΔCt (3plex-1plex)						-2.27			0.02			

Figure 10 (Continued)

A


Amplification
2000
1500
1000
500
0
RFU
HEX: 25
0
10
20
30
40
Cycles

B

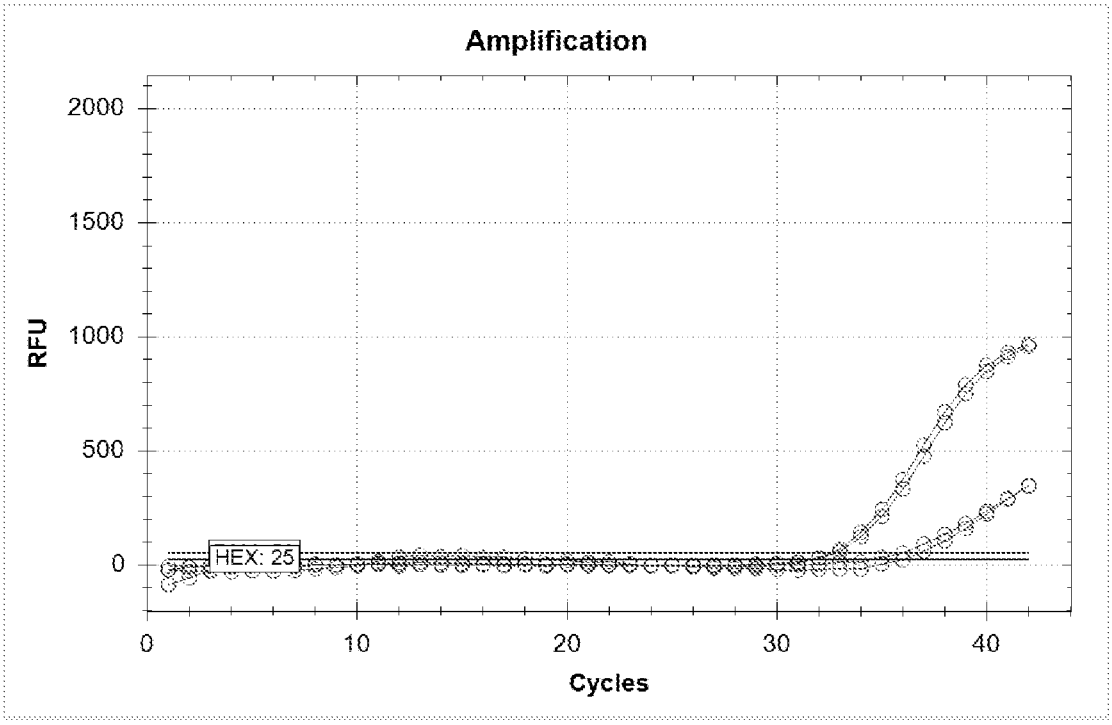

Amplification
2000
1500
1000
500
0
RFU
HEX: 25
0
10
20
30
40
Cycles

Figure 11

C

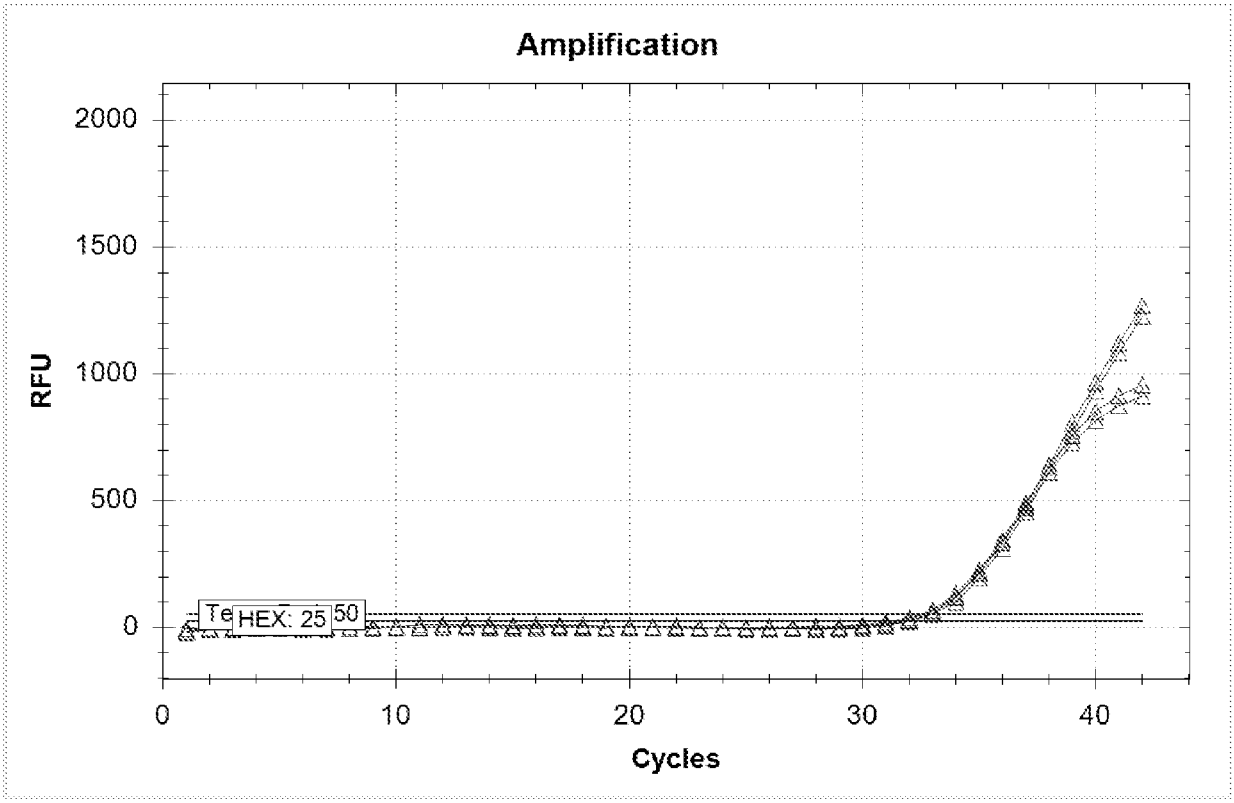
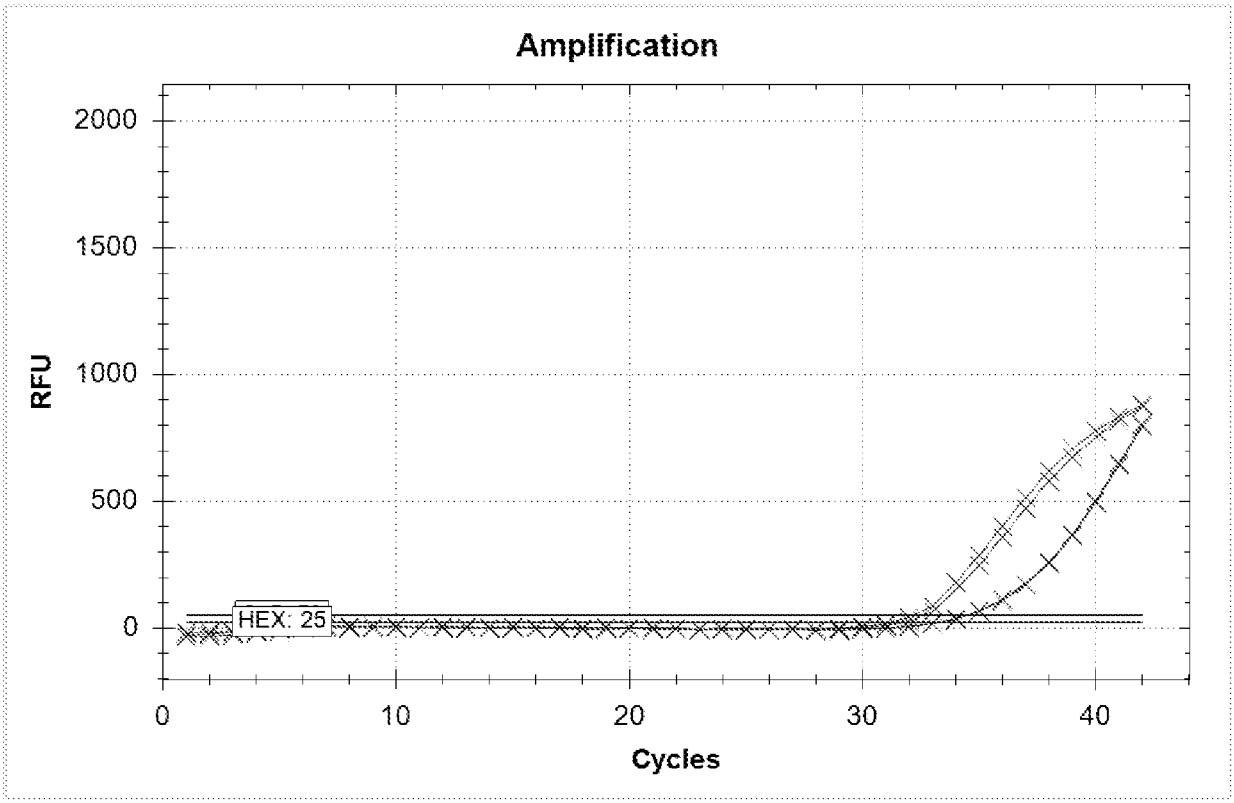


Figure 11 (Continued)

D



Assay	Fusion A: FAM			PTPRK: TxRD			RSPO3: Cy5			IC77: HEX		
	Ct values (duplicates)		*Average*	*Ct values (duplicates)*		*Average*	*Ct values (duplicates)*		*Average*	*Ct values (duplicates)*		*Average*
3plex	32.78	33.89	33.34	30.79	30.66	30.72	33.69	34.24	33.96	31.52	31.67	31.60
Fusion A/IC	35.88	36.72	36.30							31.70	31.75	31.72
PTPRK/IC				32.83	32.43	32.63				31.96	31.73	31.85
RSPO3/IC							34.47	34.36	34.42	31.31	31.83	31.57
ΔCt (3plex-1plex)			-2.96			-1.91			-0.46			

Figure 11 (Continued)

A

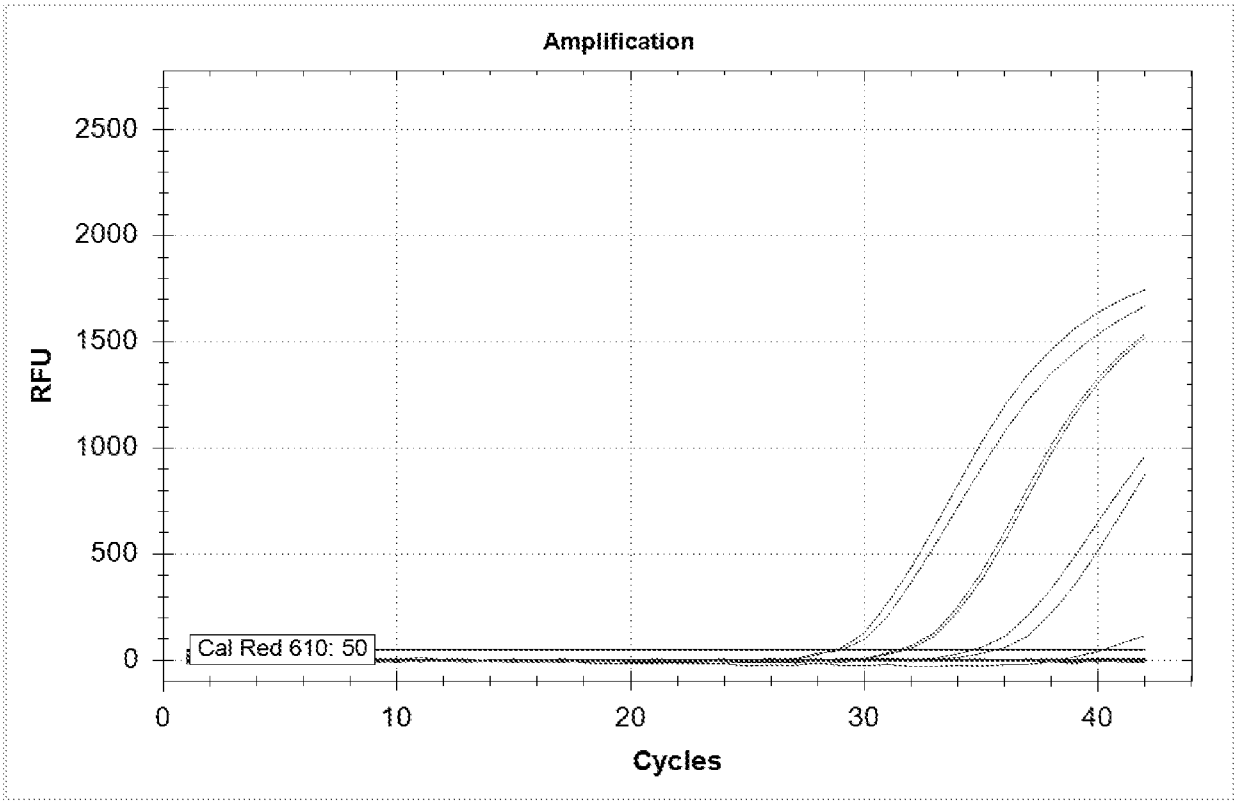
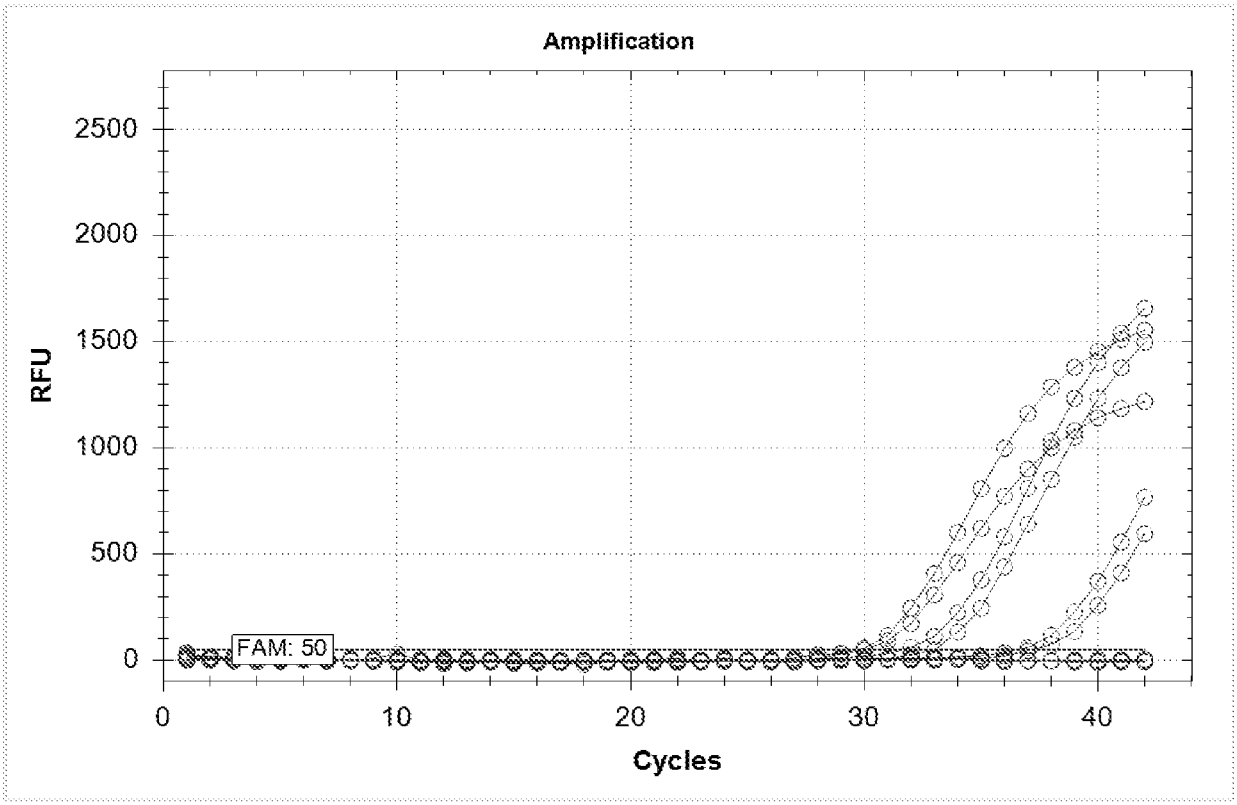

Amplification
2500
2000
1500
1000
500
0
RFU
Cal Red 610: 50
0
10
20
30
40
Cycles

Figure 12

B



	Fusion B: FAM						
Fusion B plasmid (cp/µl)	**3plex** (smooth)			**1plex** (circle)			***ΔCt*** (3plex-1plex)
	Ct values (duplicates)		*Average*	*Ct values (duplicates)*		*Average*	
1000	29.06	28.76	28.91	30.06	29.70	29.88	-0.97
100	31.78	31.45	31.62	33.02	31.64	32.33	-0.71
10	34.64	35.66	35.15	36.61	37.18	36.90	-1.75
1	No Ct	40.23	40.23	No Ct	No Ct	-	40.23
NTC	No Ct	No Ct	-	No Ct	No Ct	-	-

Figure 12 (Continued)

C

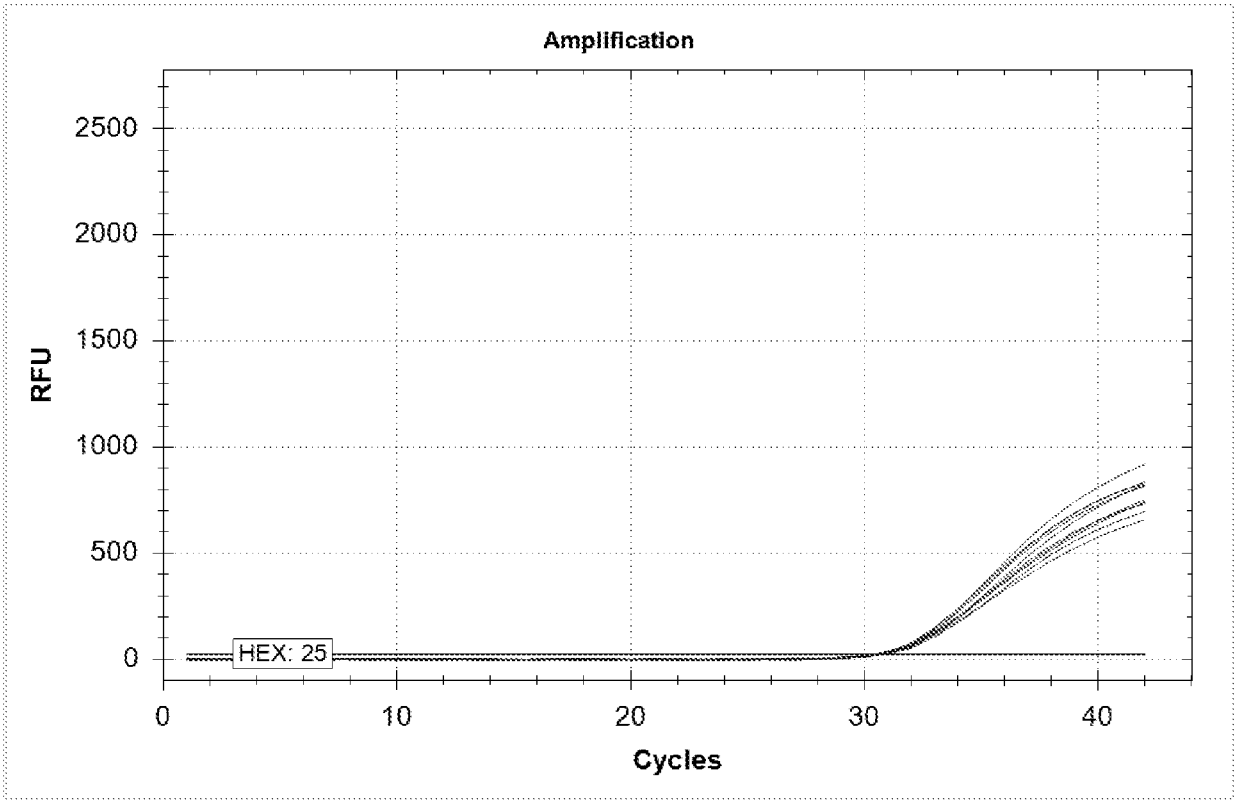
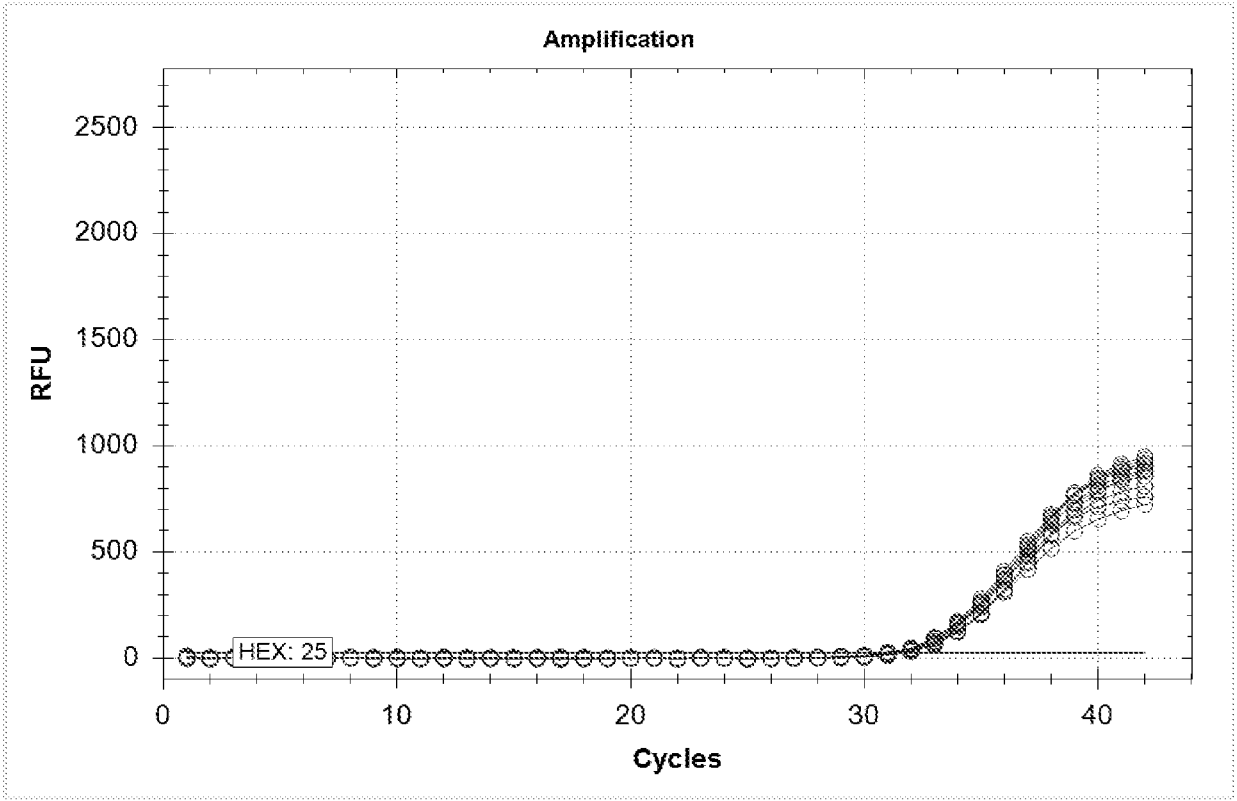


Figure 12 (Continued)

D



	IC77: HEX						
Fusion B plasmid (cp/µl)	**3plex** (smooth)			**1plex** (circle)			***ΔCt*** (3plex-1plex)
	Ct values (duplicates)		*Average*	*Ct values (duplicates)*		*Average*	
1000	30.61	30.71	30.66	31.51	30.77	31.14	-0.48
100	30.90	30.49	30.70	31.09	31.14	31.12	-0.42
10	30.45	30.42	30.44	30.84	31.02	30.93	-0.49
1	30.66	30.81	30.74	31.47	30.91	31.19	-0.45
NTC	30.41	30.50	30.46	31.32	31.65	31.49	-1.03

Figure 12 (Continued)

A

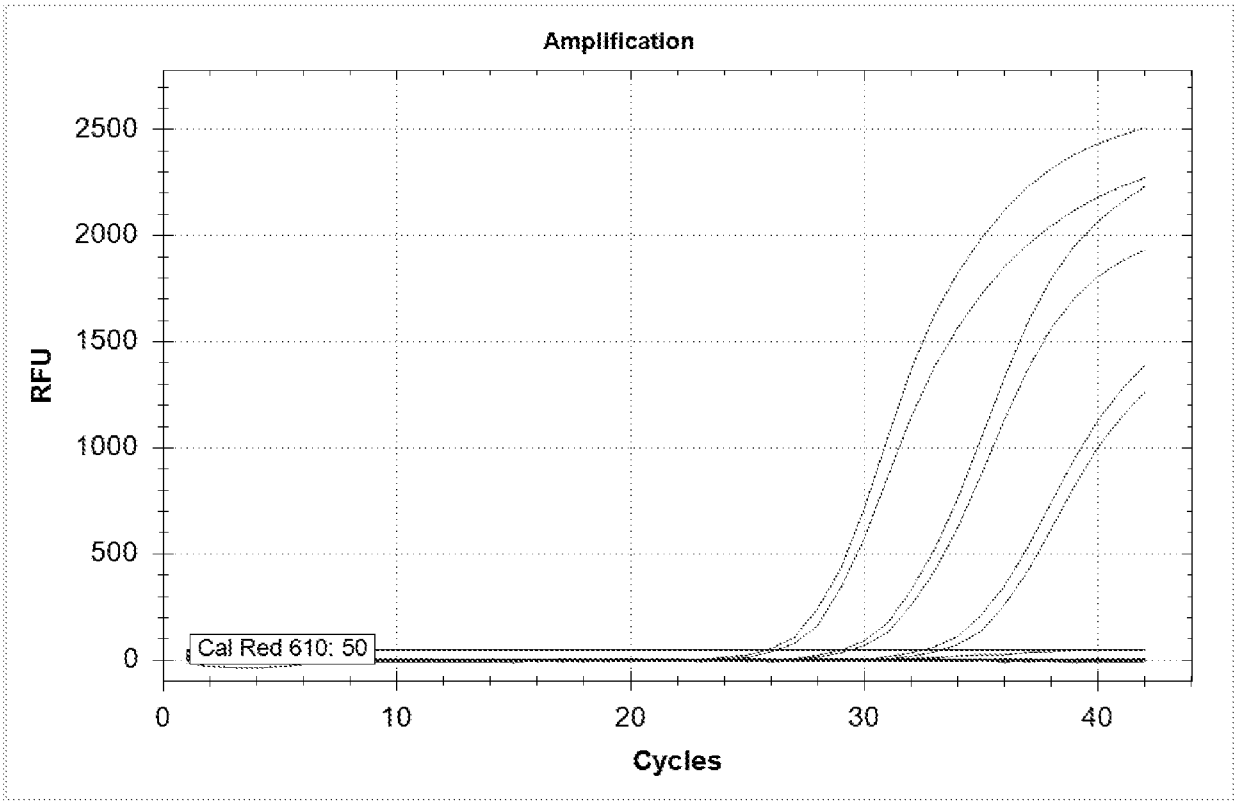
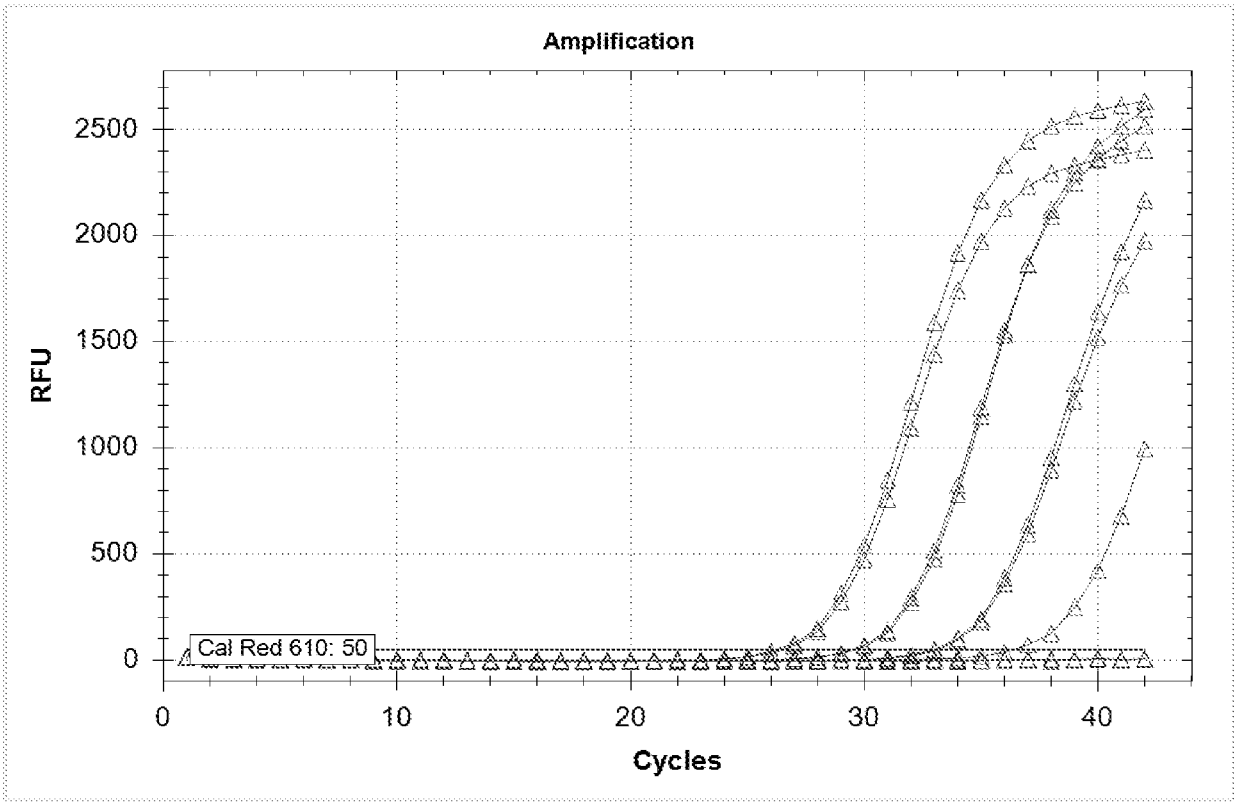

Amplification
2500
2000
1500
1000
500
0
RFU
Cal Red 610: 50
0
10
20
30
40
Cycles

Figure 13

B



	PTPRK: Cal Red 610						
PTPRK(e7,e8) plasmid (cp/µl)	**3plex** (smooth)			**1plex** (triangle)			***ΔCt*** (3plex-1plex)
	Ct values (duplicates)		*Average*	*Ct values (duplicates)*		*Average*	
1000	26.29	25.88	26.09	26.51	26.24	26.38	-0.29
100	29.10	29.46	29.28	29.50	29.60	29.55	-0.27
10	32.56	33.33	32.95	33.14	33.01	33.08	-0.13
1	No Ct	No Ct	-	36.36	No Ct	36.08	-36.08
NTC	No Ct	No Ct	-	No Ct	No Ct	-	-

Figure 13 (Continued)

C

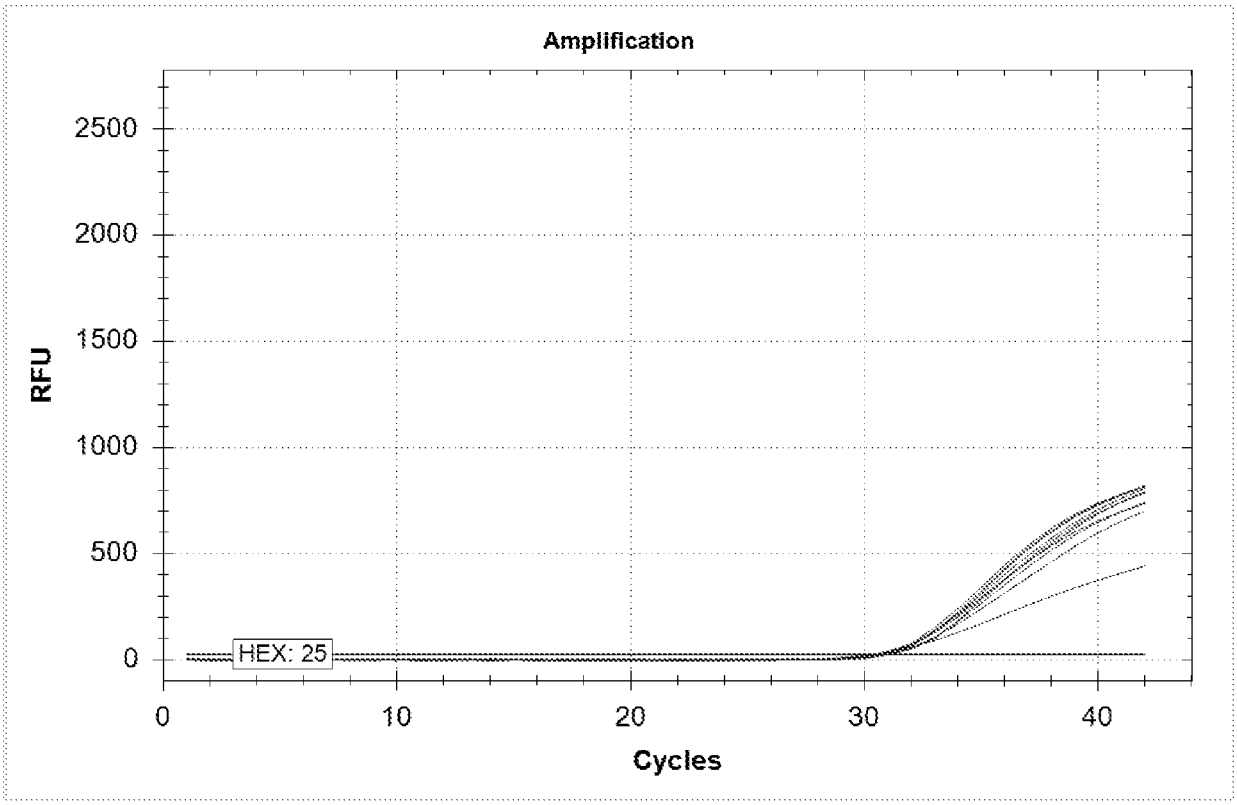
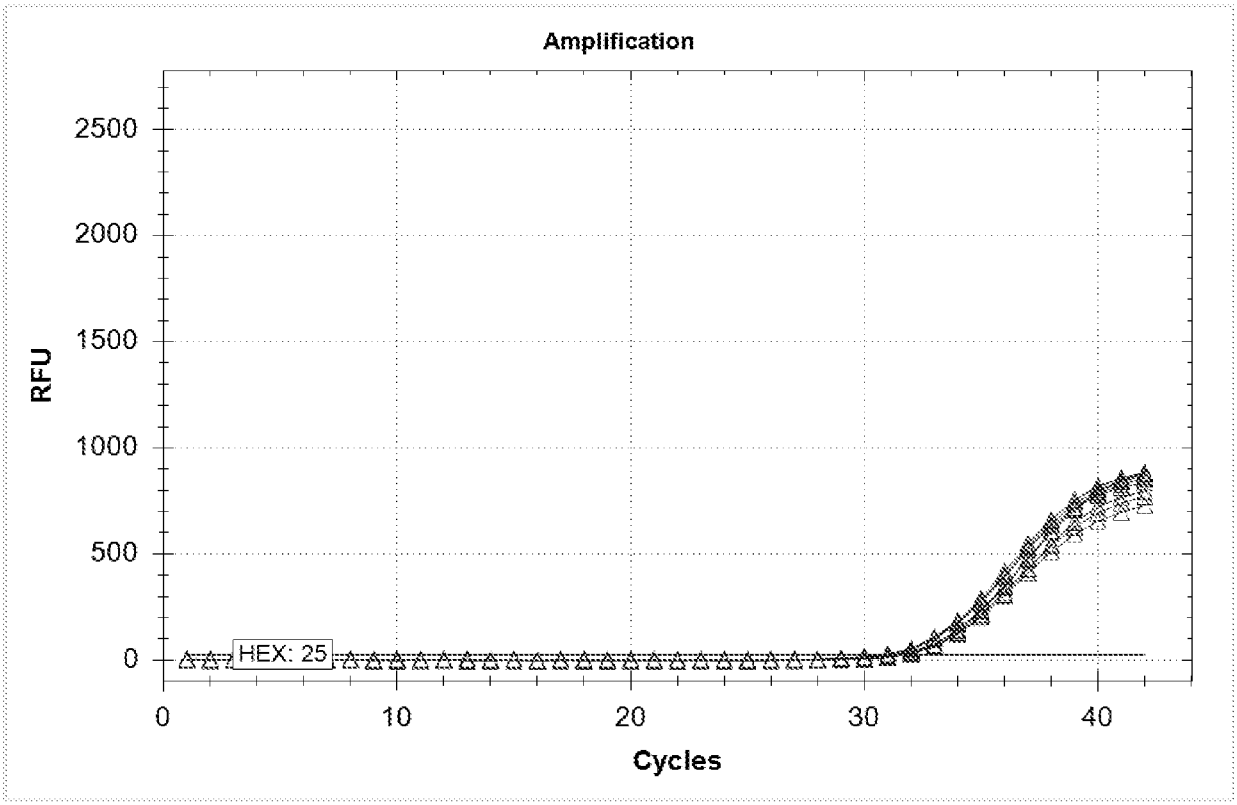


Figure 13 (Continued)

D



PTPRK(e7,e8) plasmid (cp/µl)	IC77: HEX						
	3plex (smooth)			**1plex** (triangle)			***ΔCt***
	Ct values (duplicates)		*Average*	*Ct values (duplicates)*		*Average*	(3plex-1plex)
1000	30.86	30.53	30.70	31.61	31.66	31.64	-0.94
100	30.41	31.07	30.74	30.82	30.83	30.83	-0.08
10	30.59	31.05	30.82	31.43	31.25	31.34	-0.52
1	30.41	30.63	30.52	30.76	31.62	31.19	-0.67
NTC	30.41	30.50	30.46	30.90	31.11	31.01	-0.55

Figure 13 (Continued)

A

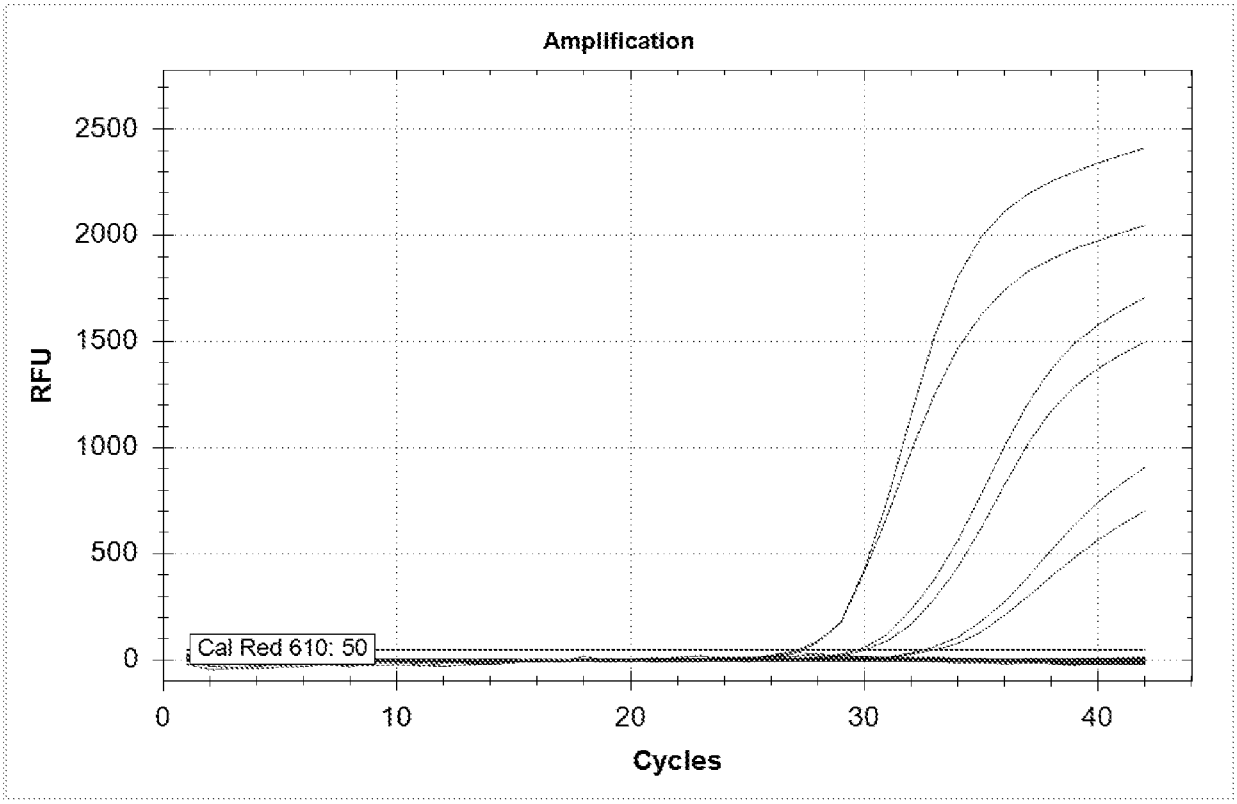
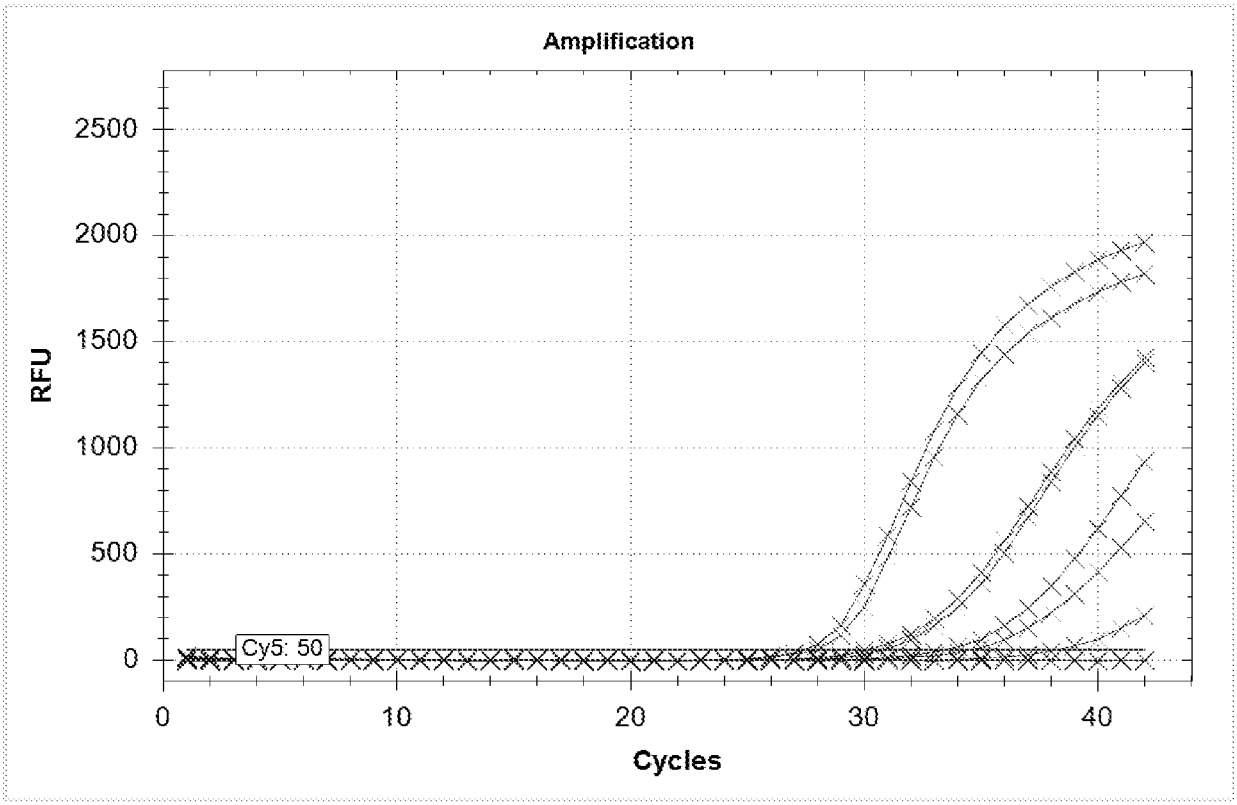

Amplification
RFU
2500
2000
1500
1000
500
0
Cal Red 610: 50
0
10
20
30
40
Cycles

Figure 14

B



RSPO3(e1,e2) plasmid (cp/µl)	RSPO3: Cy5						
	3plex (smooth)			**1plex** (cross)			***ΔCt***
	Ct values (duplicates)		*Average*	*Ct values (duplicates)*		*Average*	(3plex-1plex)
1000	27.39	27.21	27.30	28.06	27.42	27.74	-0.44
100	29.72	30.08	29.90	30.15	30.84	30.50	-0.59
10	33.10	32.60	32.85	34.61	33.43	34.02	-1.17
1	No Ct	No Ct	-	38.29	No Ct	38.29	-38.29
NTC	No Ct	No Ct	-	No Ct	No Ct	-	-

Figure 14 (Continued)

C

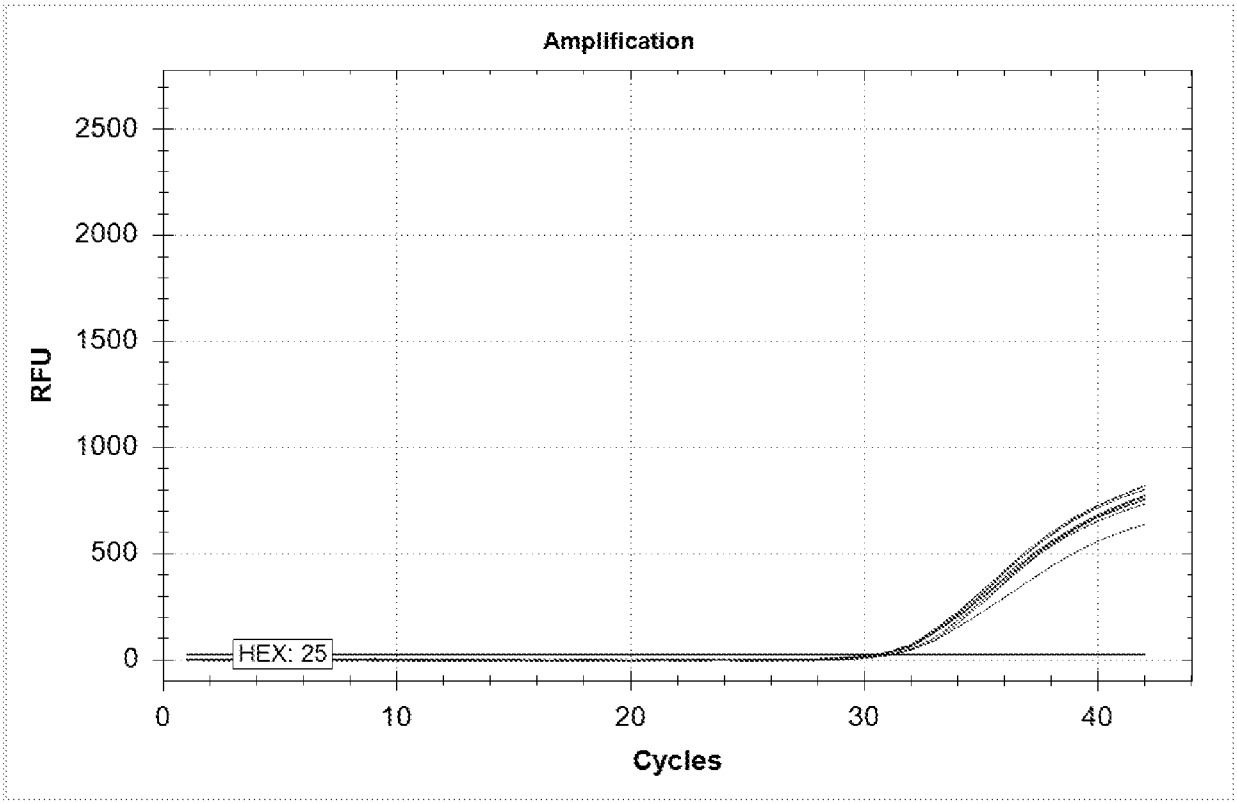
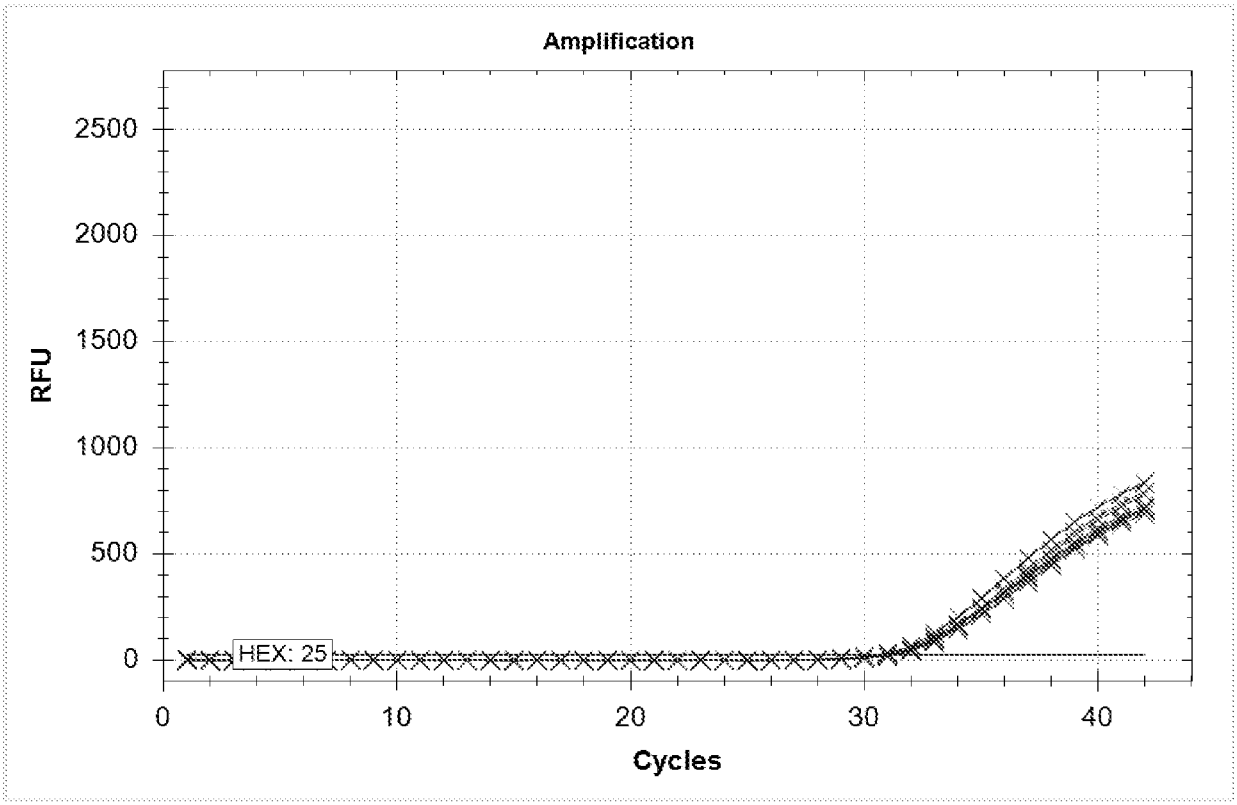


Figure 14 (Continued)

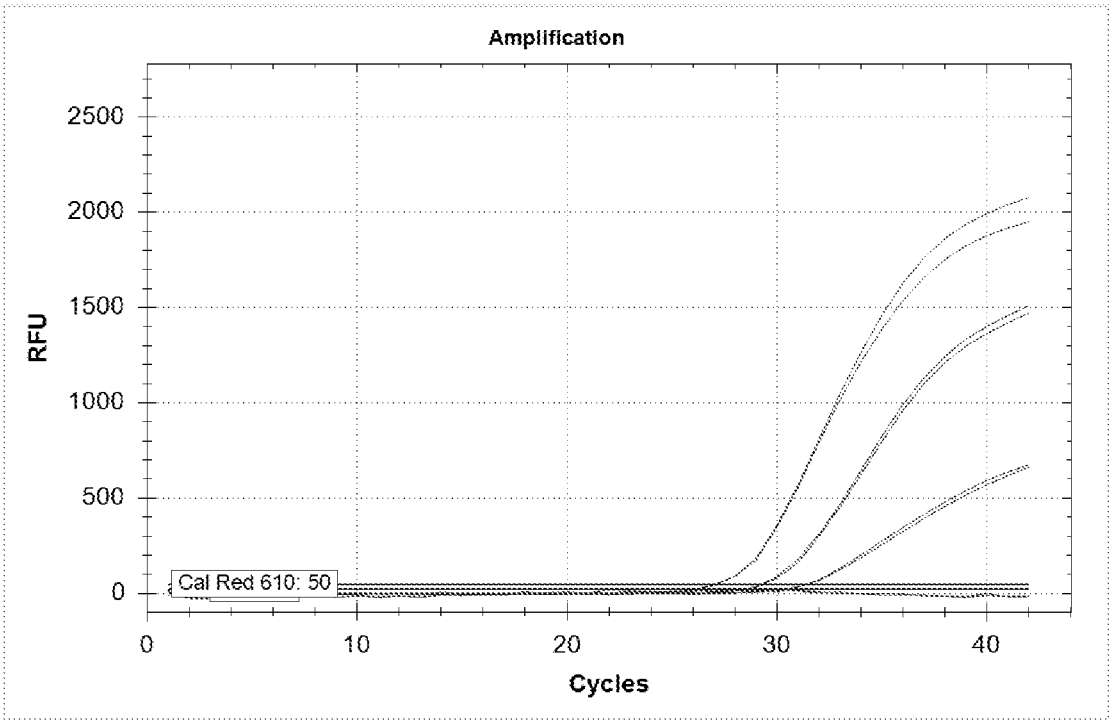
D



	IC77: HEX						
RSPO3(e1,e2) plasmid (cp/µl)	**3plex** (smooth)			**1plex** (cross)			***ΔCt*** (3plex-1plex)
	Ct values (duplicates)		*Average*	*Ct values (duplicates)*		*Average*	
1000	31.17	31.10	31.14	31.31	30.93	31.12	0.02
100	30.66	30.54	30.60	30.53	30.80	30.67	-0.06
10	30.46	31.10	30.78	30.85	31.07	30.96	-0.18
1	30.50	30.39	30.45	31.11	30.96	31.04	-0.59
NTC	30.41	30.50	30.46	30.47	30.58	30.53	-0.07

Figure 14 (Continued)

A


Amplification
2500
2000
1500
1000
500
0
RFU
Cal Red 610: 50
0
10
20
30
40
Cycles

B

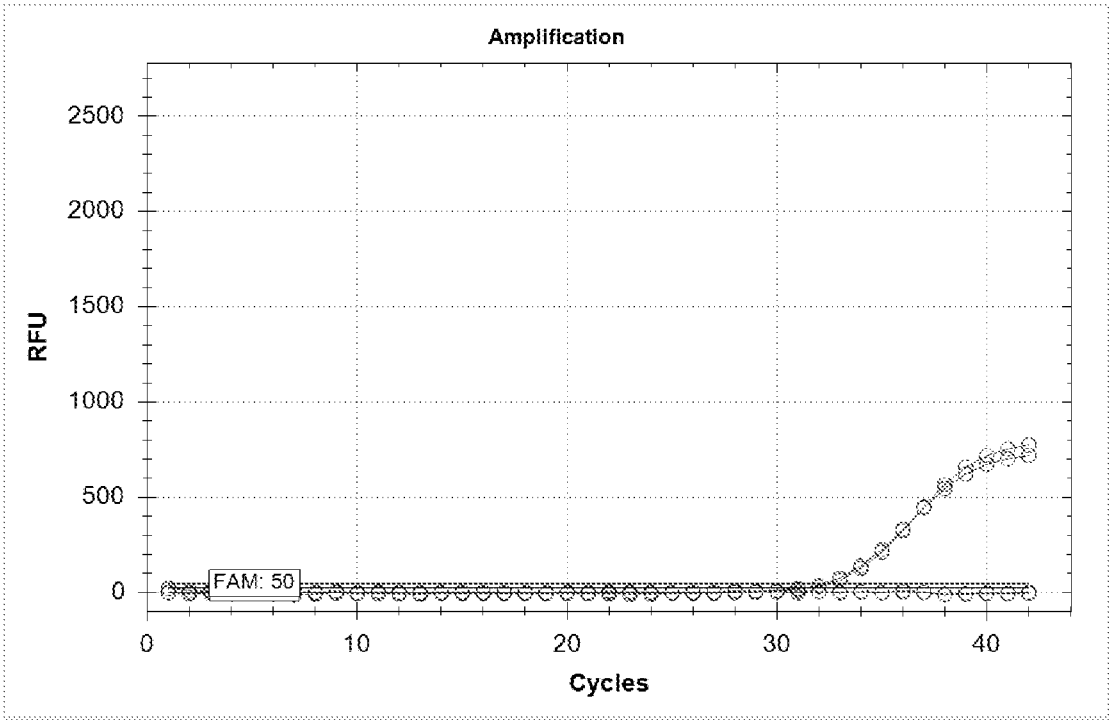

Amplification
2500
2000
1500
1000
500
0
RFU
FAM: 50
0
10
20
30
40
Cycles

Figure 15

C

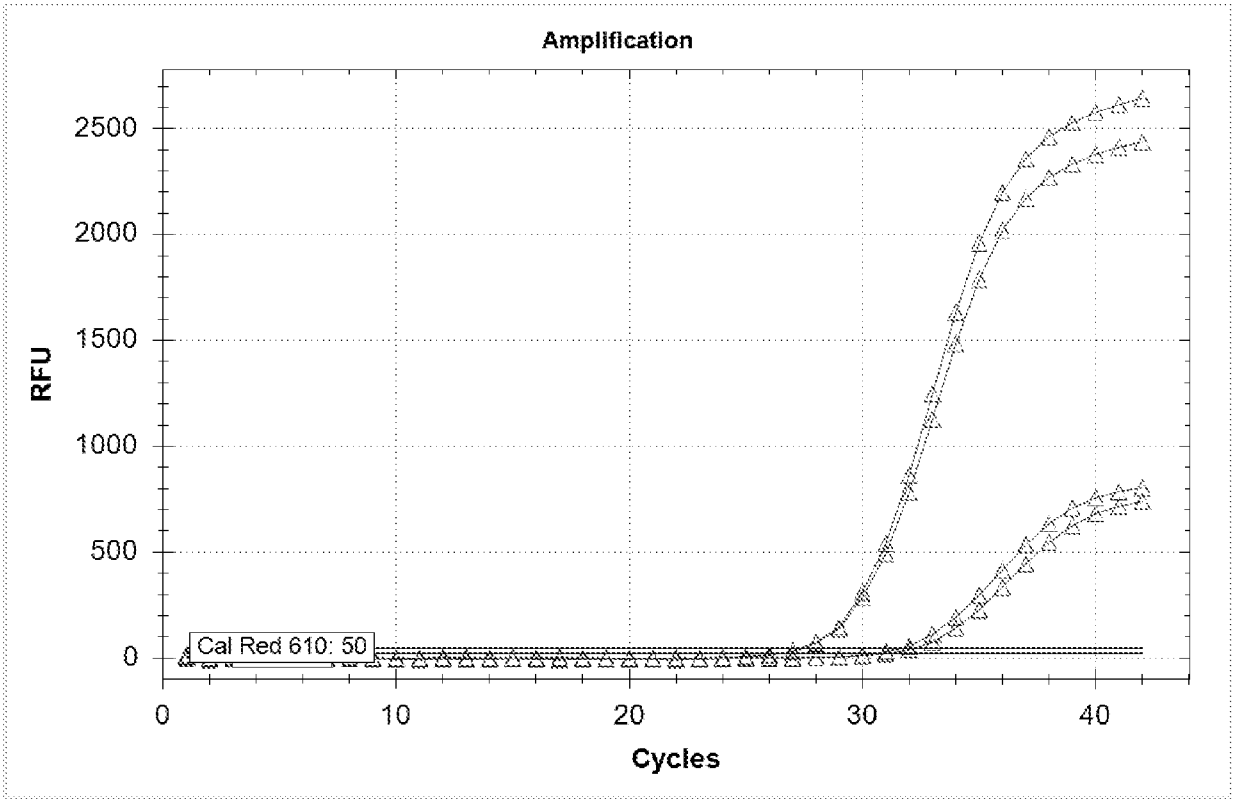
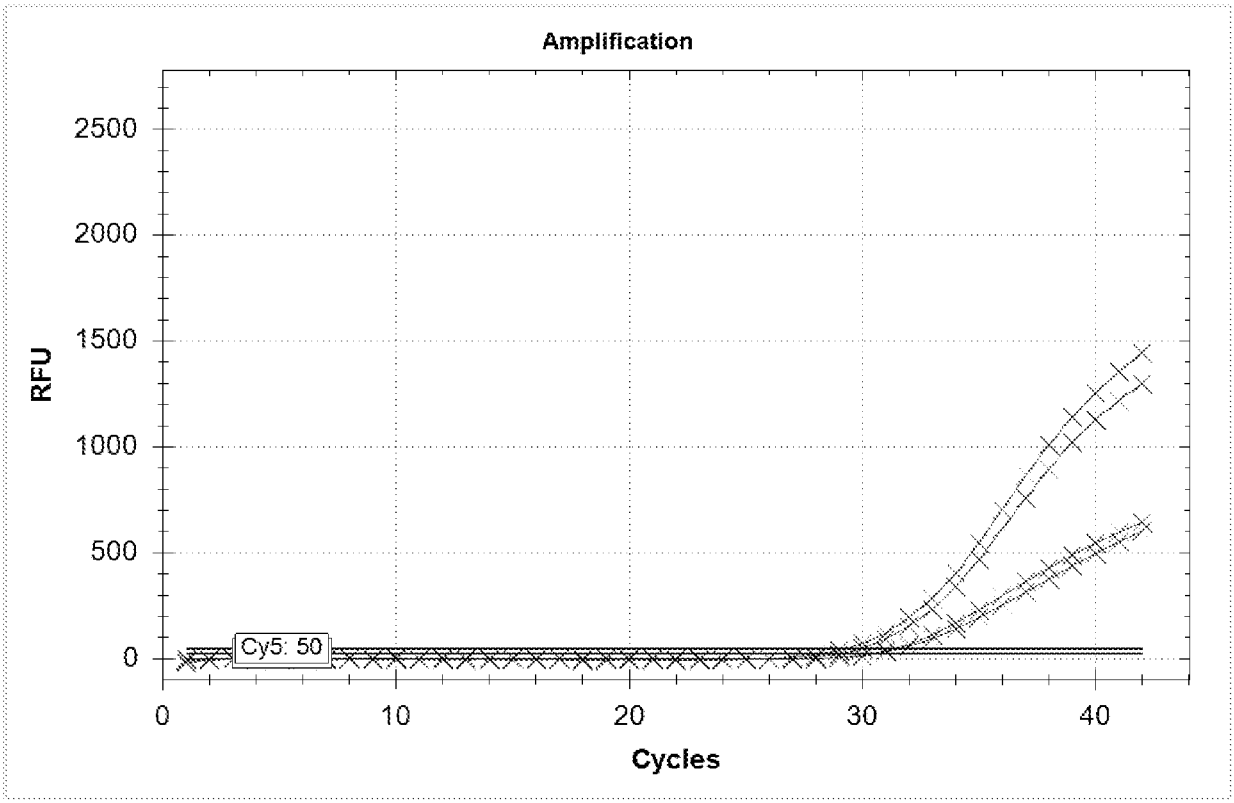


Figure 15 (Continued)

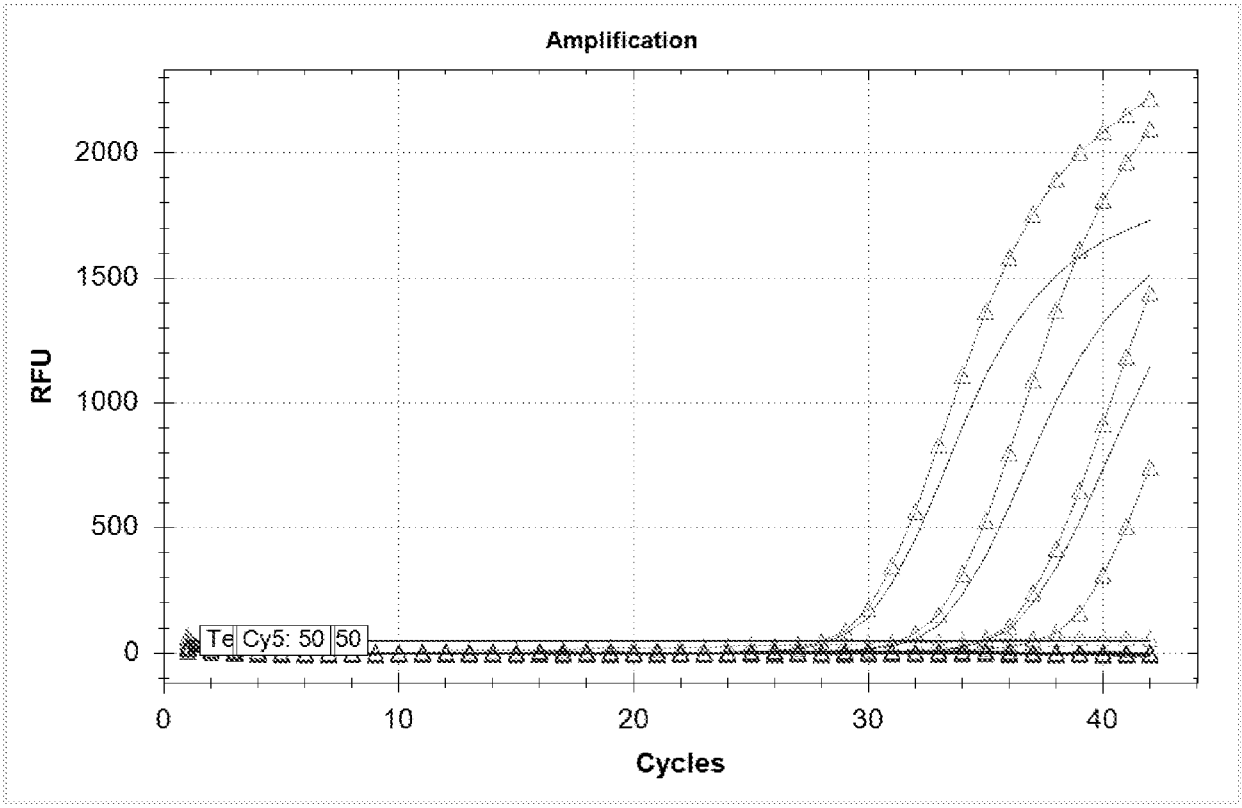
D



Assay	Fusion B: FAM			PTPRK: Cal Red 610			RSPO3: Cy5			IC77: HEX		
	Ct values (duplicates)		*Average*	*Ct values (duplicates)*		*Average*	*Ct values (duplicates)*		*Average*	*Ct values (duplicates)*		*Average*
3plex	No Ct	No Ct	-	27.17	27.11	27.14	29.38	29.29	29.34	30.47	30.51	30.49
Fusion B/IC	No Ct	No Ct	-							31.26	31.48	31.37
PTPRK/IC				27.48	27.38	27.43				31.16	30.62	30.89
RSPO3/IC							29.99	29.28	29.64	30.38	30.57	30.48
ΔCt (3plex-1plex)						-0.29			-0.30			

Figure 15 (Continued)

A

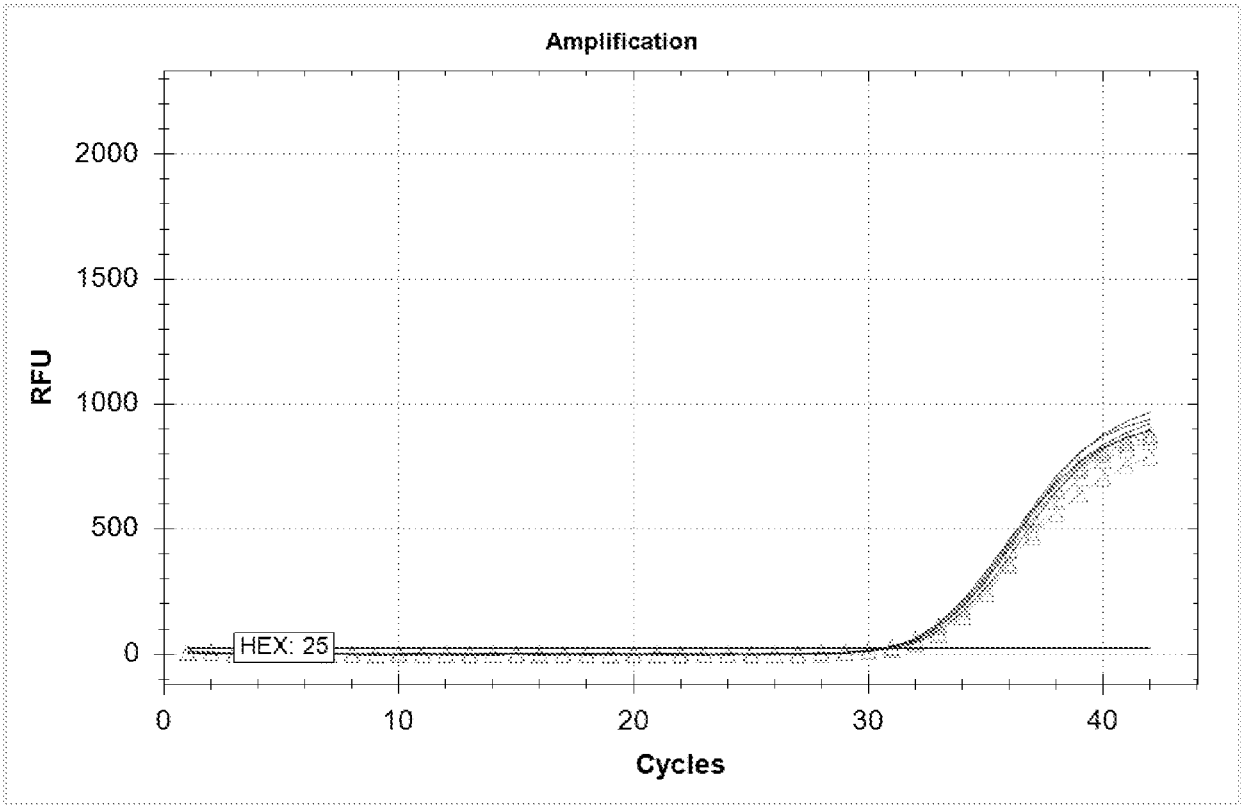


Fusion D: FAM			
Fusion D plasmid (cp/µl)	*Ct value* **Set 1** (smooth)	**Set 2** (triangle)	***ΔCt*** *(Set 1-Set 2)*
1000	28.36	28.10	0.26
100	31.82	31.32	0.50
10	34.99	34.72	0.27
1	No Ct	37.13	-37.13
NTC	No Ct	No Ct	-

Average ΔCt: 0.34
(excludes Ct for 1 cp/ul)

Figure 16

B

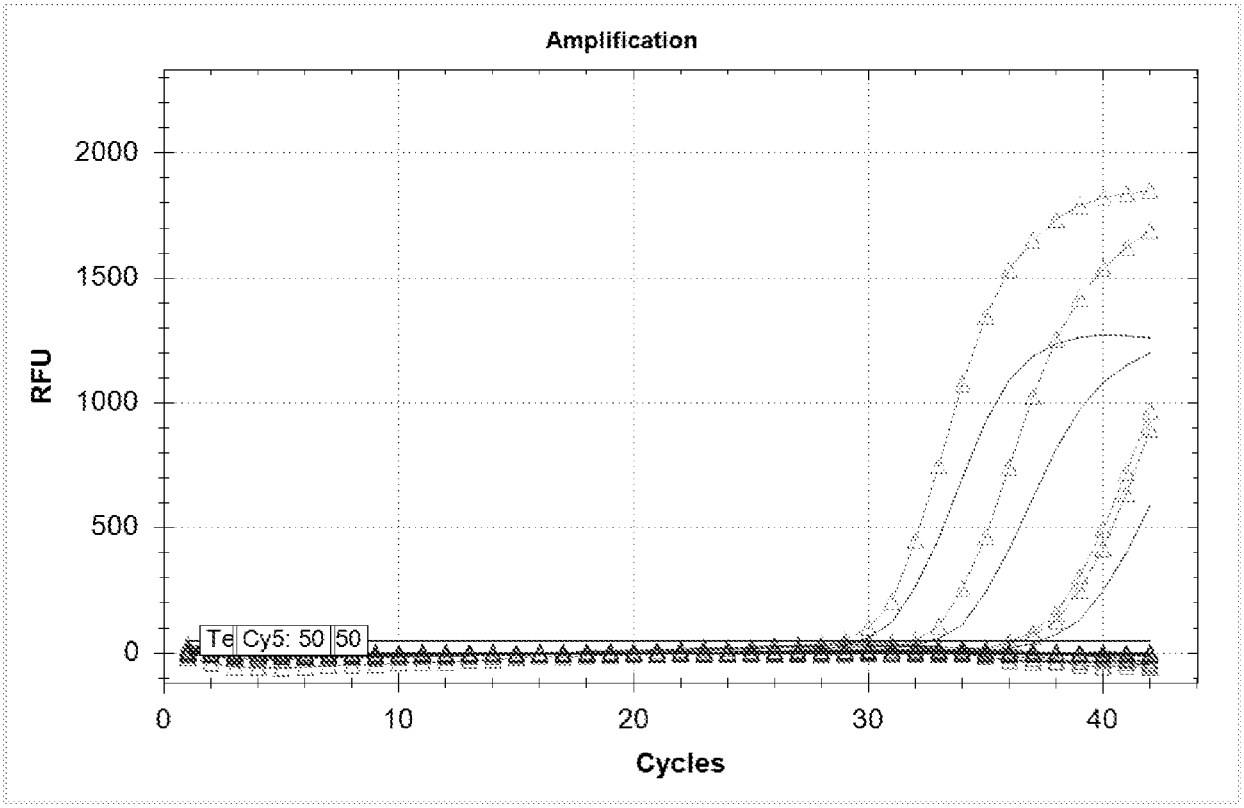


IC77: HEX			
Fusion D plasmid (cp/µl)	*Ct value* **Set 1** (smooth)	**Set 2** (triangle)	***ΔCt*** *(Set 1-Set 2)*
1000	30.75	31.15	-0.40
100	30.68	30.99	-0.31
10	30.63	31.21	-0.58
1	30.59	30.72	-0.13
NTC	30.98	30.82	0.16

Average ΔCt: -0.25

Figure 16 (Continued)

A

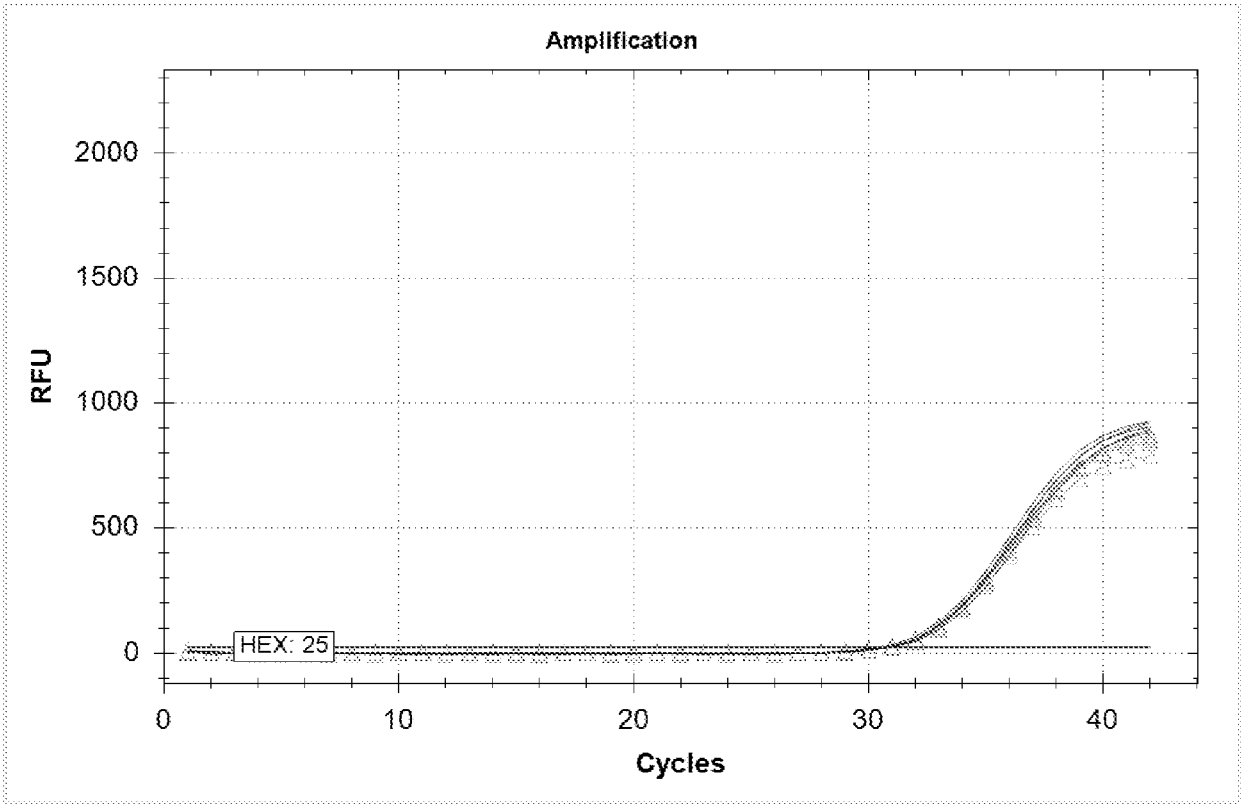


EIF3E: TxRd			
EIF3E(e1,e2) plasmid (cp/ul)	*Ct value*		***ΔCt***
	Set 1 (smooth)	**Set 2** (triangle)	*(Set 1-Set 2)*
1000	29.64	29.00	0.64
100	32.68	31.95	0.73
10	37.32	36.43	0.89
1	No Ct	36.13	-36.13
NTC	No Ct	No Ct	-

Average ΔCt: 0.75
(excludes Ct for 1 cp/ul)

Figure 17

B

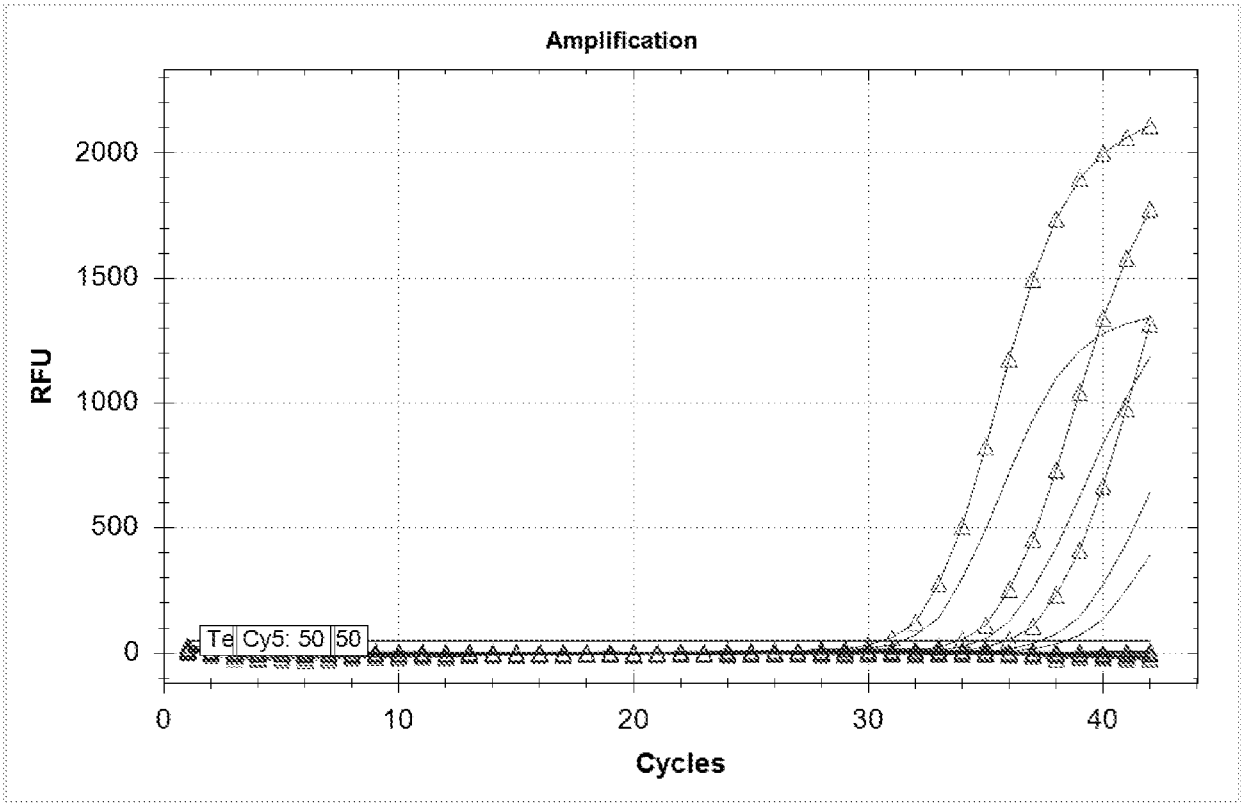


IC77: HEX			
EIF3E(e1,e2) plasmid (cp/ul)	*Ct value* **Set 1** (smooth)	**Set 2** (triangle)	***ΔCt*** *(Set 1-Set 2)*
1000	30.89	31.03	-0.14
100	30.43	30.82	-0.39
10	30.79	30.79	0.00
1	30.83	30.80	0.03
NTC	30.98	30.82	0.16

Average ΔCt: -0.07

Figure 17 (Continued)

A

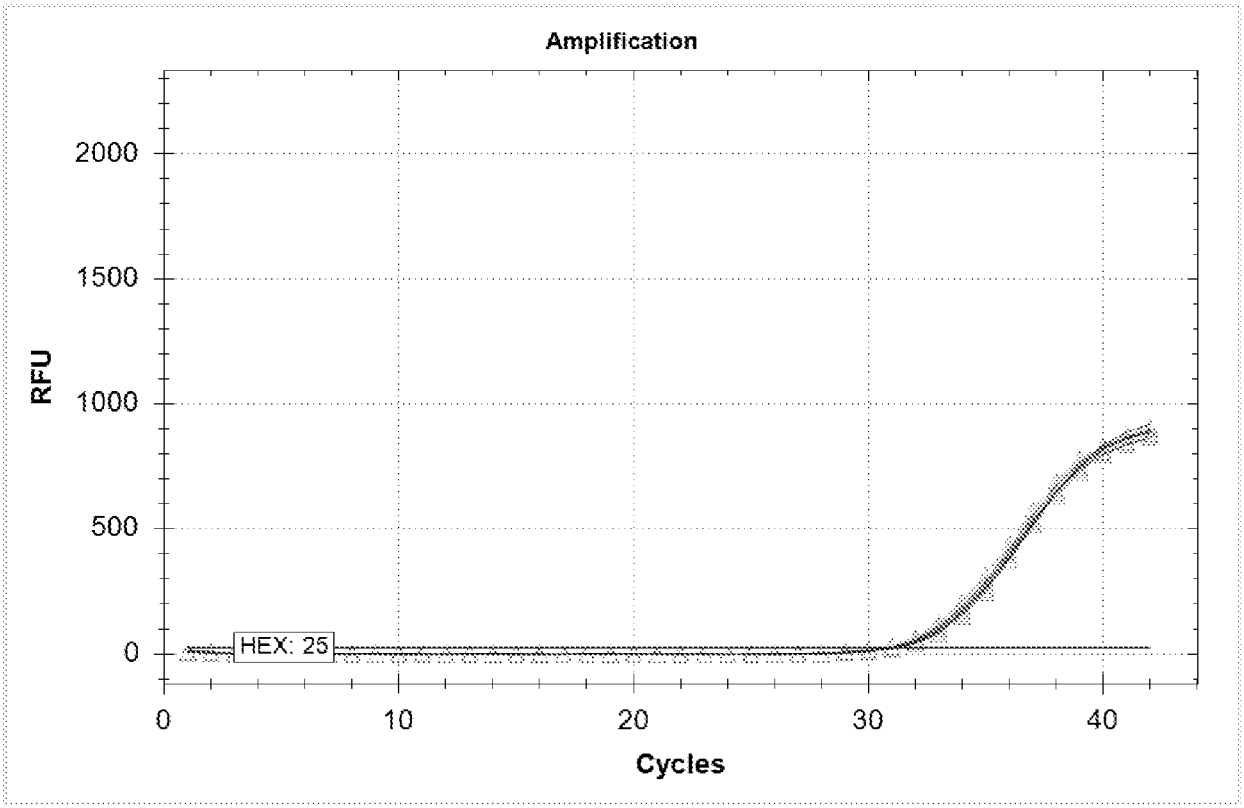


RSPO2: Cy5			
RSPO2(e1,e2) plasmid (cp/ul)	*Ct value*		***ΔCt***
	Set 1 (smooth)	**Set 2** (triangle)	*(Set 1-Set 2)*
1000	31.45	30.59	0.86
100	34.77	33.85	0.92
10	38.37	35.89	2.48
1	37.31	No Ct	37.31
NTC	No Ct	No Ct	-

Average ΔCt: 1.42
(excludes Ct for 1 cp/ul)

Figure 18

B



IC77: HEX			
RSPO2(e1,e2) plasmid (cp/ul)	*Ct value* **Set 1** (smooth)	**Set 2** (triangle)	***ΔCt*** *(Set 1-Set 2)*
1000	30.78	31.20	-0.42
100	30.87	30.98	-0.11
10	31.07	31.09	-0.02
1	31.18	30.49	0.69
NTC	30.98	30.82	0.16

Average ΔCt: 0.06

Figure 18 (Continued)

A

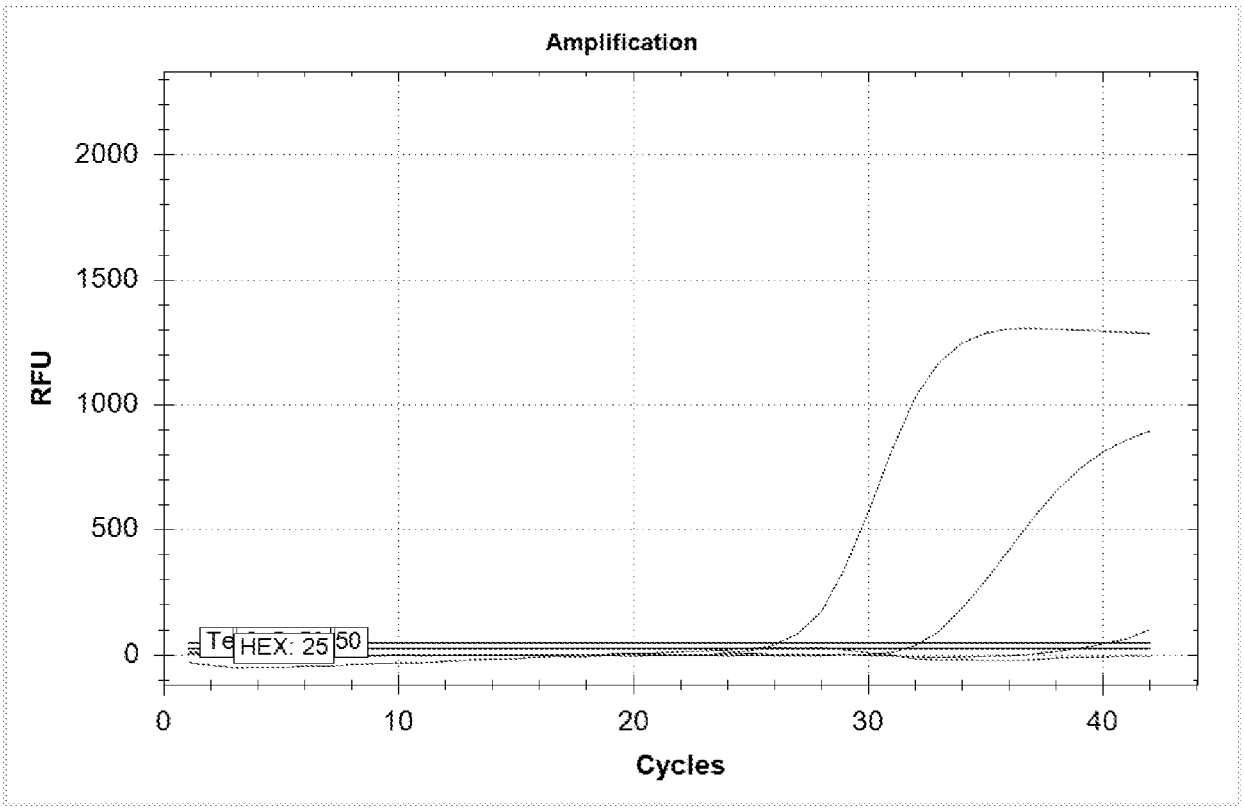
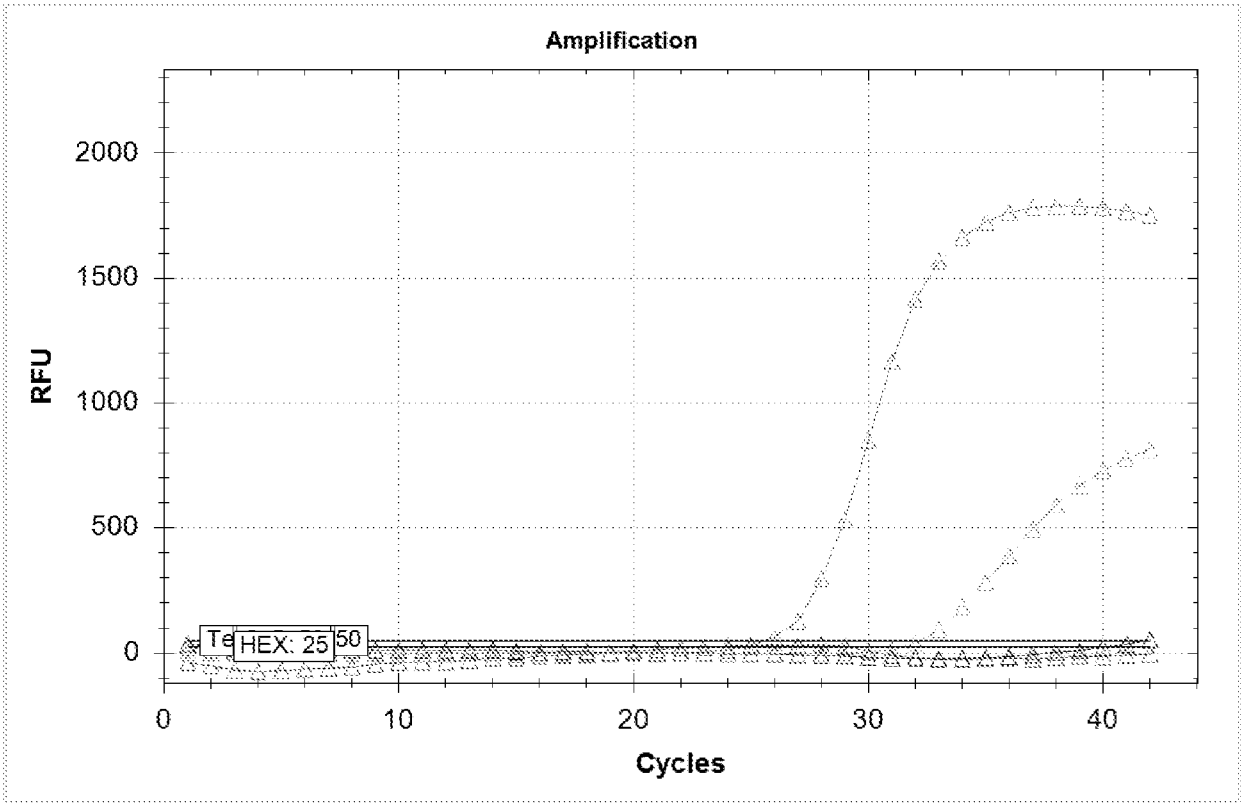

Set 1
Amplification
RFU
2000
1500
1000
500
0
HEX: 25
0
10
20
30
40
Cycles

Figure 19

B

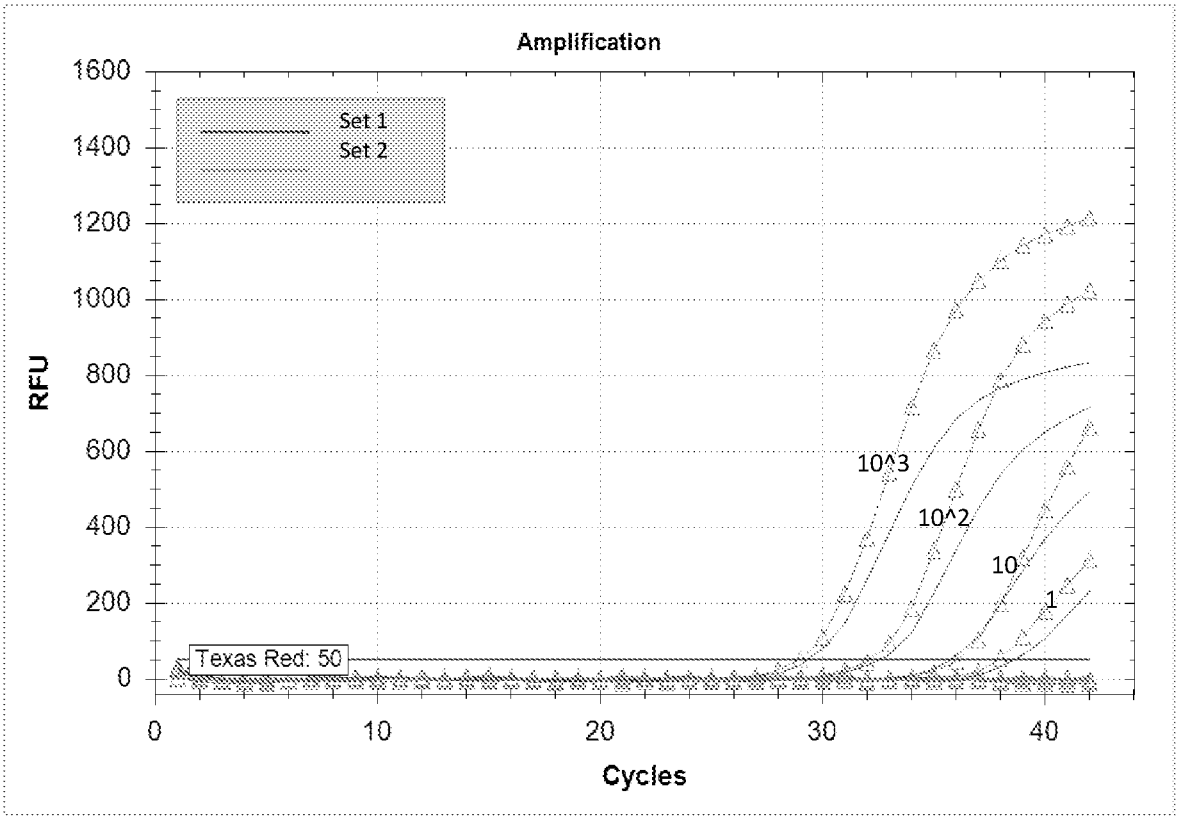
Set 2



Assay	*Ct value*			
	Fusion D: FAM	**EIF3E: TxRd**	**RSPO2: Cy5**	**IC77: HEX**
Set 1 (smooth)	No Ct	26.19	40.24	31.52
Set 2 (triangle)	No Ct	25.59	41.82	31.49
ΔCt (Set 1-Set 2)	-	0.60	-1.58	0.03

Figure 19 (Continued)

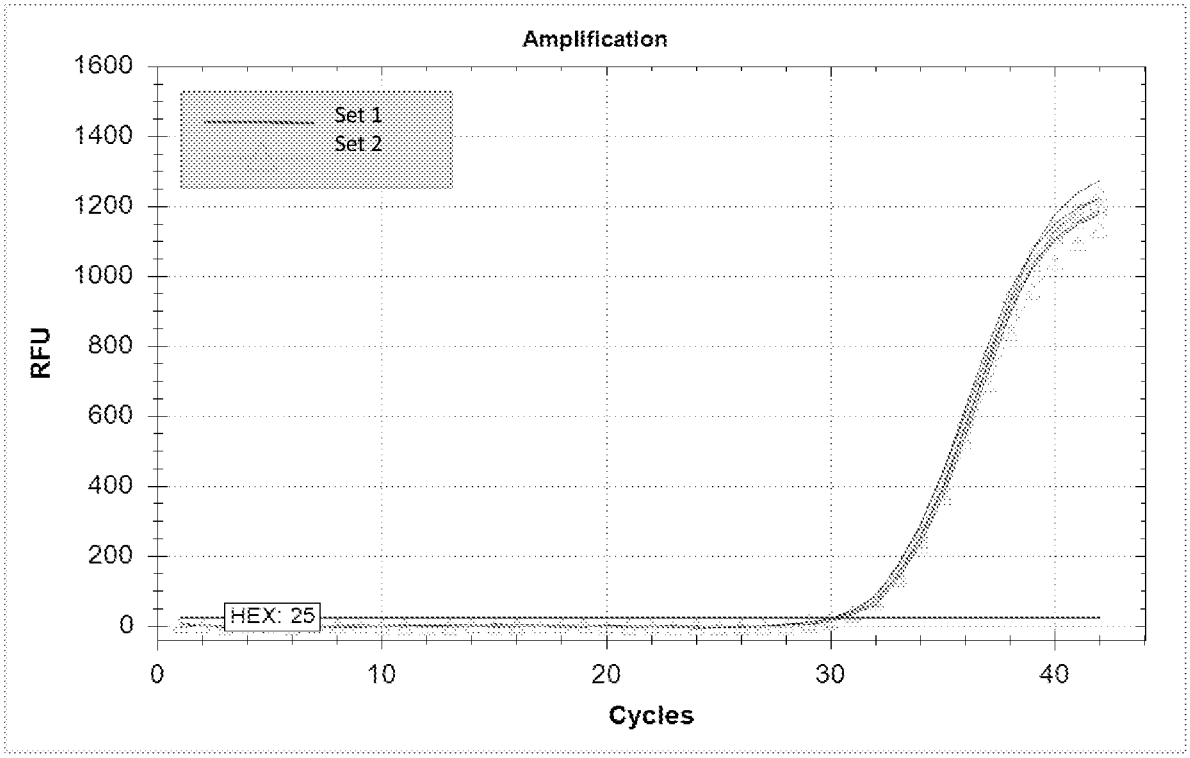
A



Fusion C: FAM			
Fusion C plasmid	*Ct value*		***ΔCt***
(cp/ul)	**Set 1** (smooth)	**Set 2** (triangle)	*(Set 1-Set 2)*
1000	29.27	28.91	0.36
100	32.48	32.10	0.38
10	35.69	36.06	-0.37
1	38.55	37.86	0.69
NTC	No Ct	No Ct	-

Figure 20

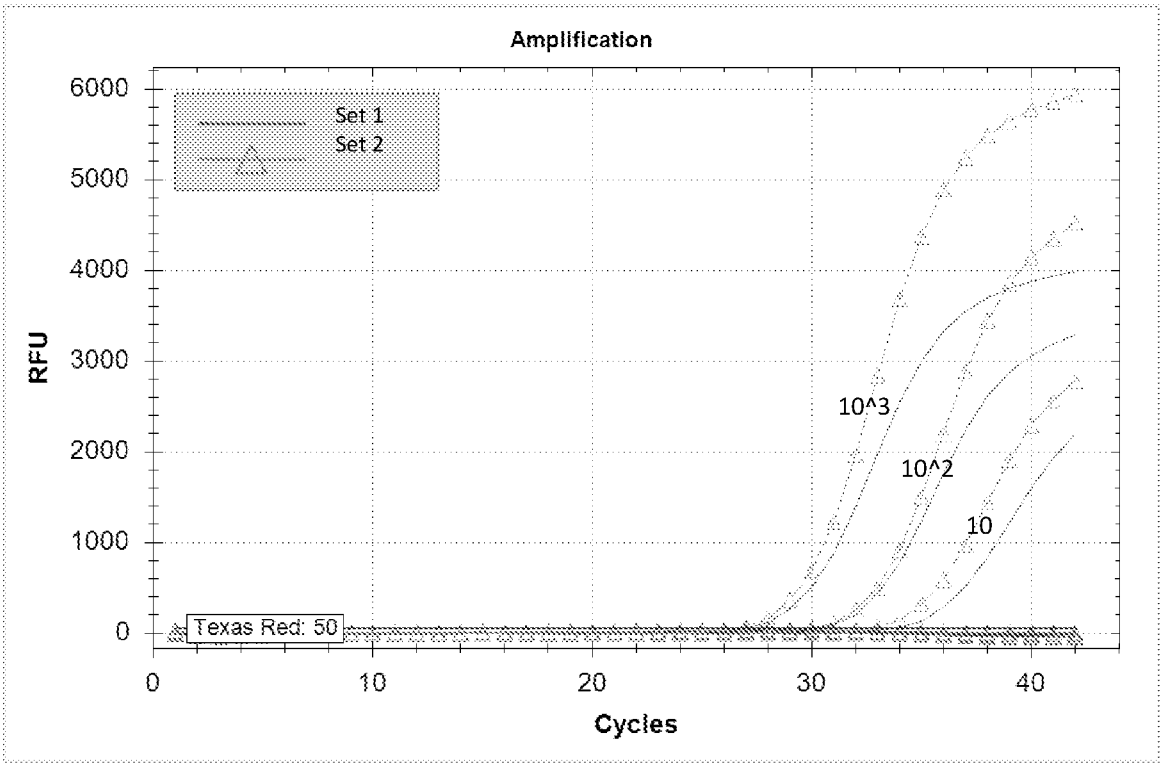
B



IC77: HEX			
Fusion C plasmid	*Ct value*		***ΔCt***
(cp/ul)	**Set 1** (smooth)	**Set 2** (triangle)	*(Set 1-Set 2)*
1000	30.10	30.56	-0.46
100	30.33	30.32	0.01
10	30.08	30.18	-0.10
1	30.52	30.34	0.18
NTC	30.26	30.40	-0.14

Figure 20 (Continued)

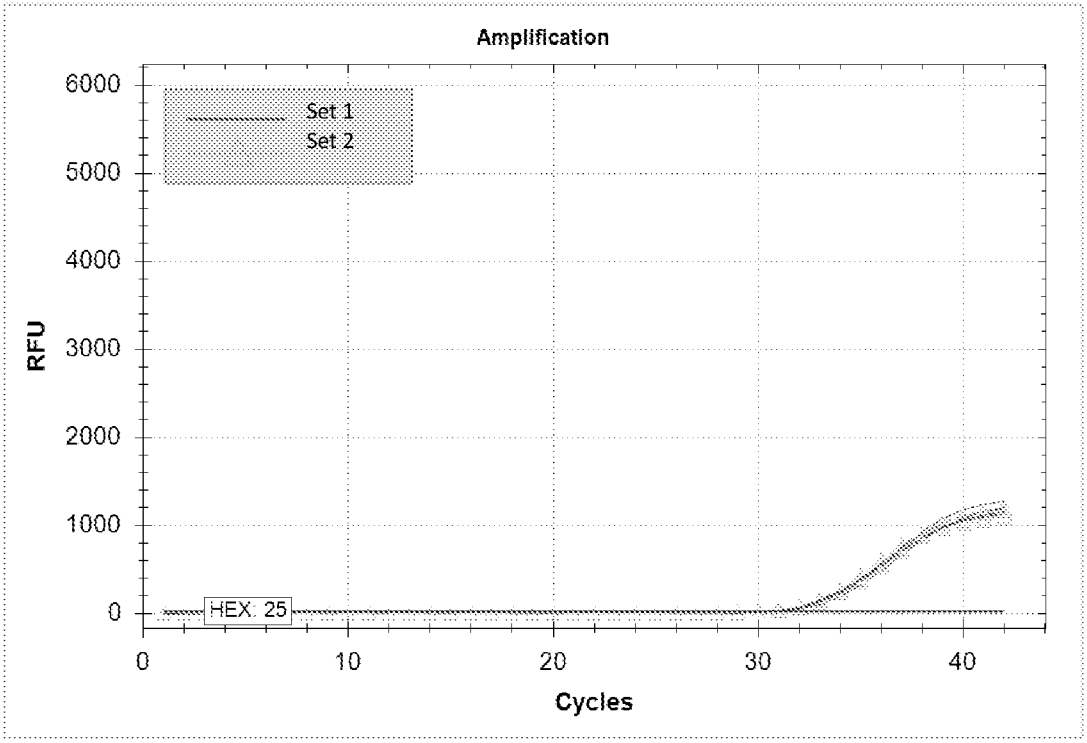
A



PTPRK: TxRd			
	Ct value		***ΔCt***
PTPRK(e12,e13,e14)plasmid (cp/ul)	**Set 1** (smooth)	**Set 2** (triangle)	*(Set 1-Set 2)*
1000	26.86	26.48	0.38
100	30.08	29.86	0.22
10	33.65	32.53	1.12
1	No Ct	No Ct	-
NTC	No Ct	No Ct	-

Figure 21

B



IC77: HEX			
	Ct value		***ΔCt***
PTPRK(e12,e13,e14)plasmid (cp/ul)	**Set 1** (smooth)	**Set 2** (triangle)	*(Set 1-Set 2)*
1000	31.11	30.40	0.71
100	30.59	30.46	0.13
10	30.40	30.73	-0.33
1	30.49	30.29	0.20
NTC	30.26	30.40	-0.14

Figure 21 (Continued)

A

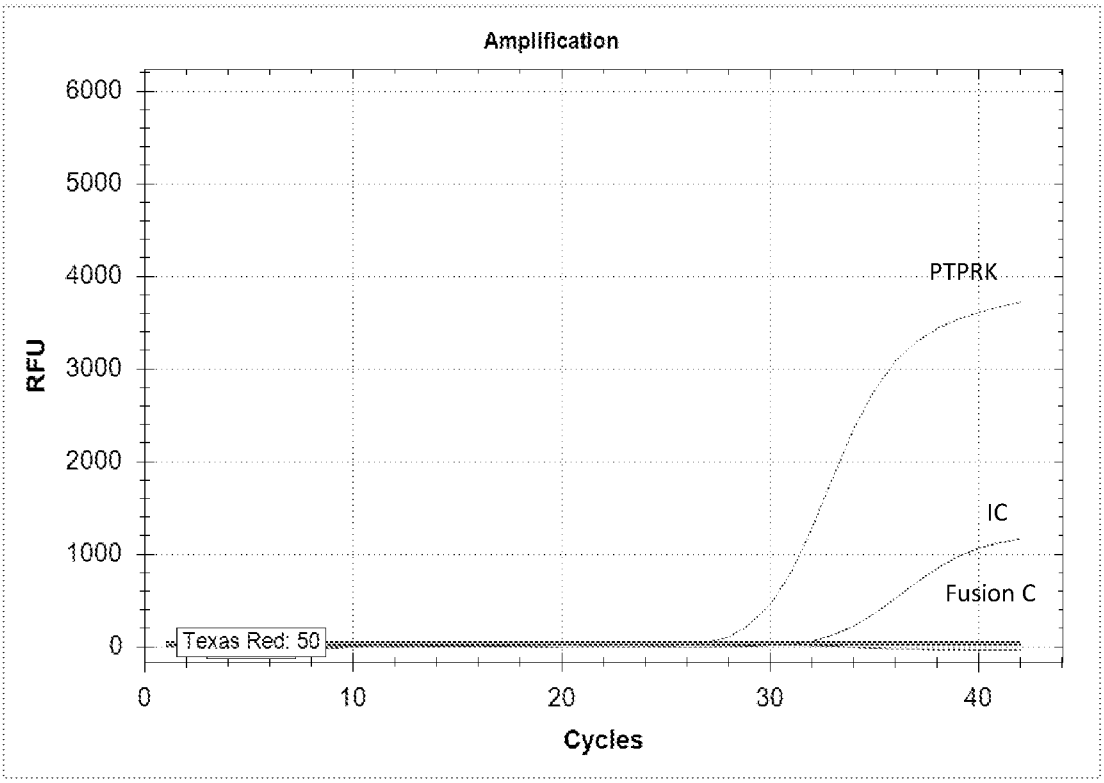
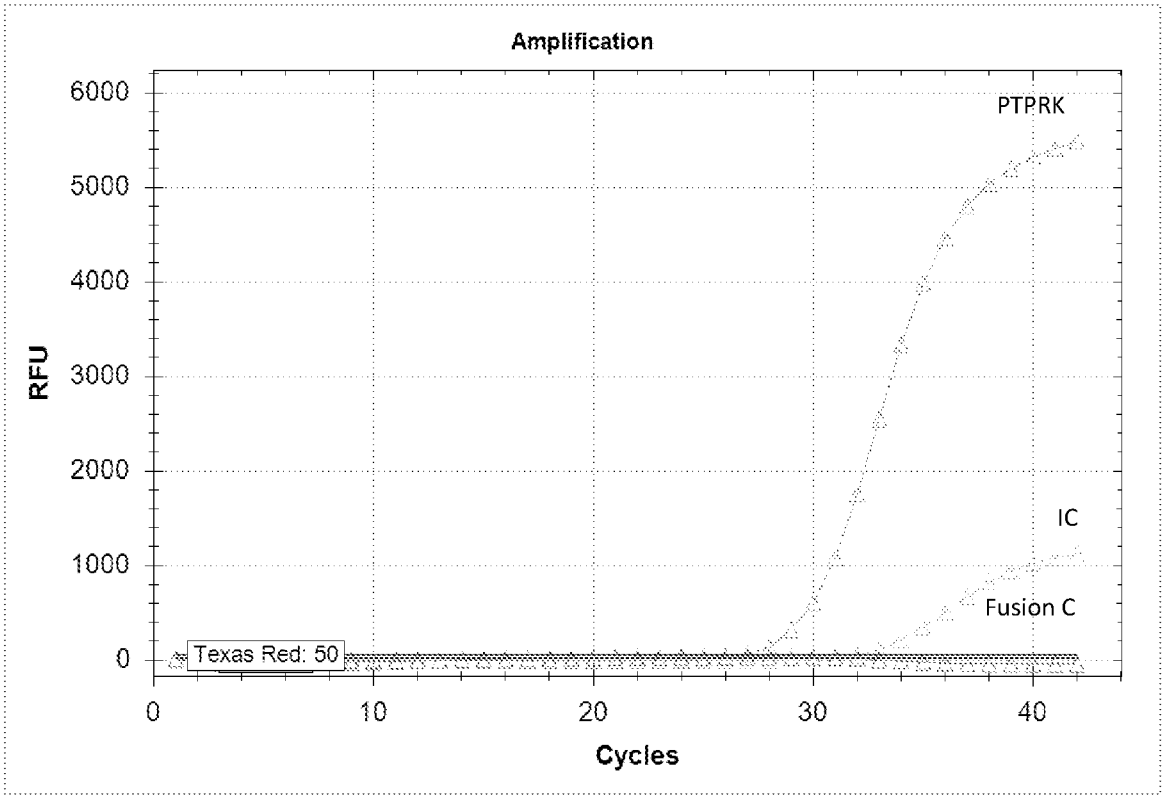

Amplification
RFU
6000
5000
4000
3000
2000
1000
0
0
10
20
30
40
Cycles
PTPRK
IC
Fusion C
Texas Red: 50

Figure 22

B



Assay	*Ct value*		
	Fusion C: FAM	**PTPRK: TxRd**	**IC77: HEX**
Set 1 (smooth)	No Ct	26.99	30.79
Set 2 (triangle)	No Ct	26.61	31.01
ΔCt (Set 1-Set 2)	-	0.38	-0.22

Figure 22 (Continued)

A

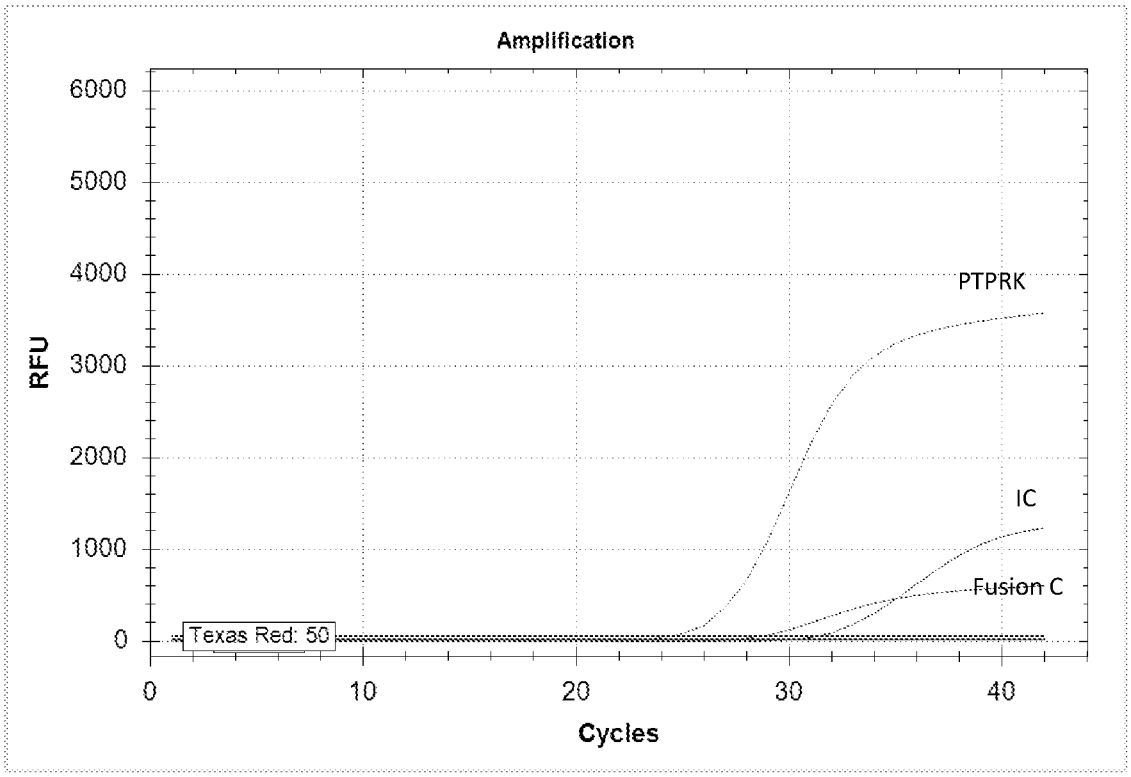
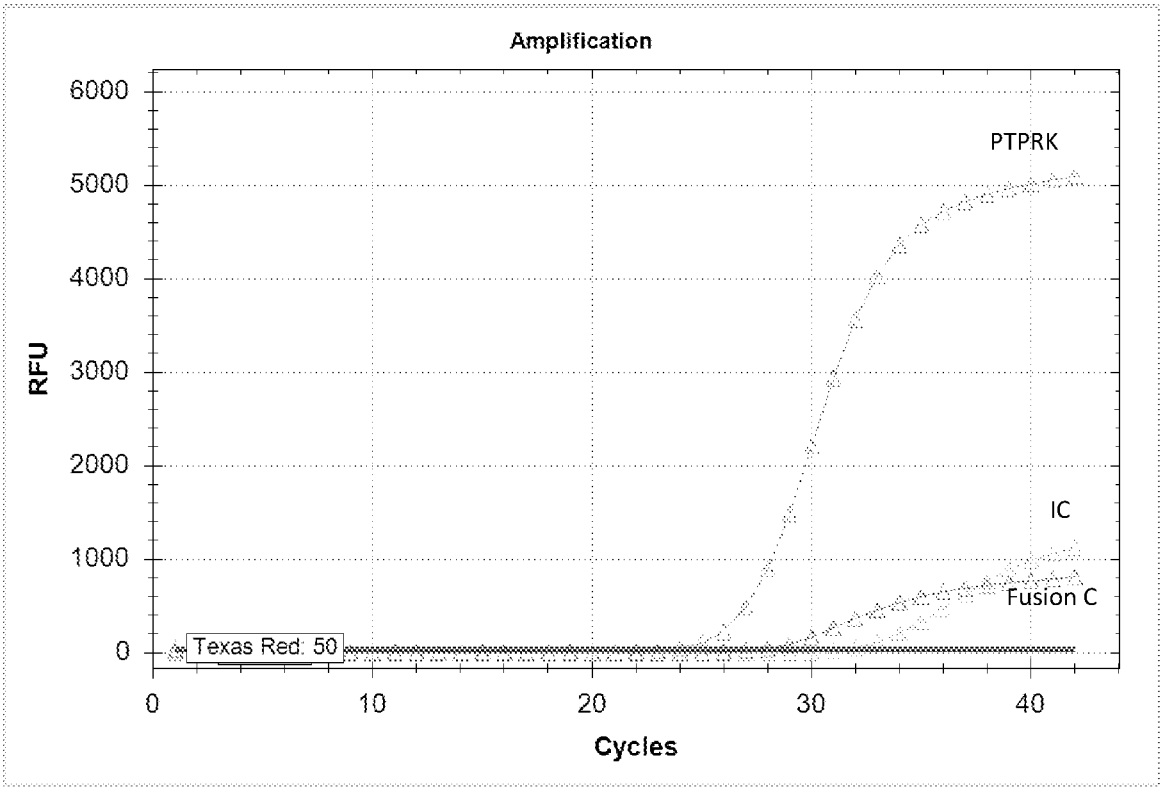

Amplification
RFU
6000
5000
4000
3000
2000
1000
0
0
10
20
30
40
Cycles
PTPRK
IC
Fusion C
Texas Red: 50

Figure 23

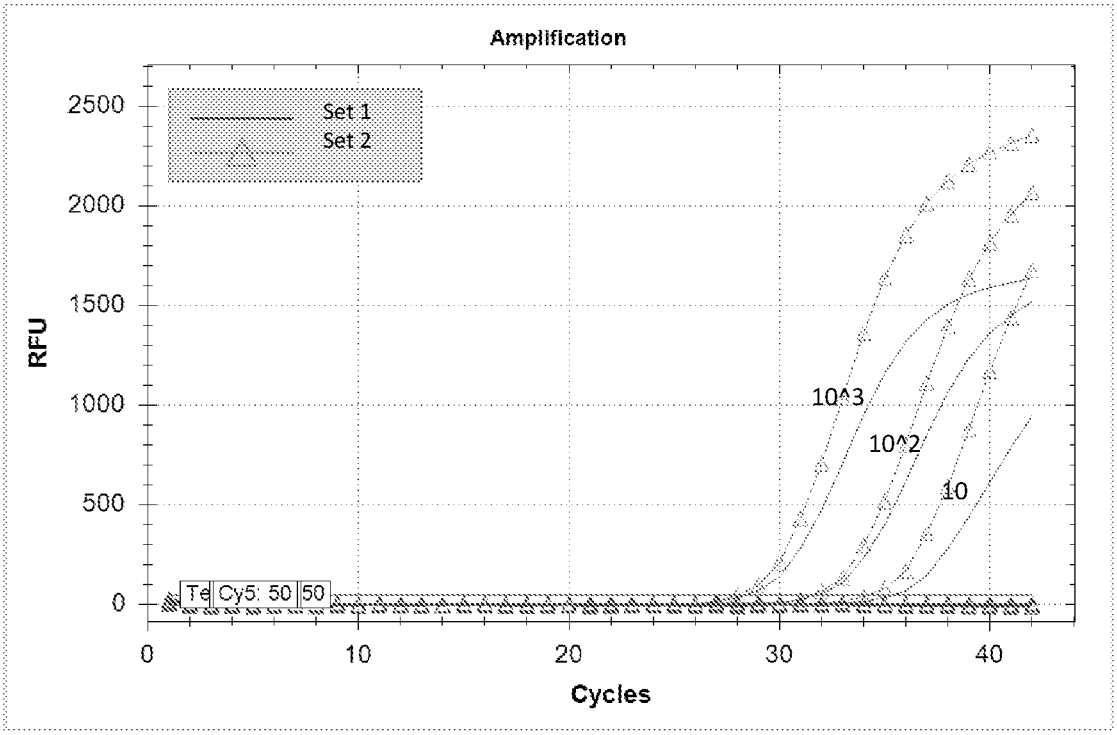
B



Assay	*Ct value*		
	Fusion C: FAM	**PTPRK: TxRd**	**IC77: HEX**
Set 1 (smooth)	28.53	24.25	30.37
Set 2 (triangle)	28.15	24.01	31.23
ΔCt (Set 1-Set 2)	0.38	0.24	-0.86

Figure 23 (Continued)

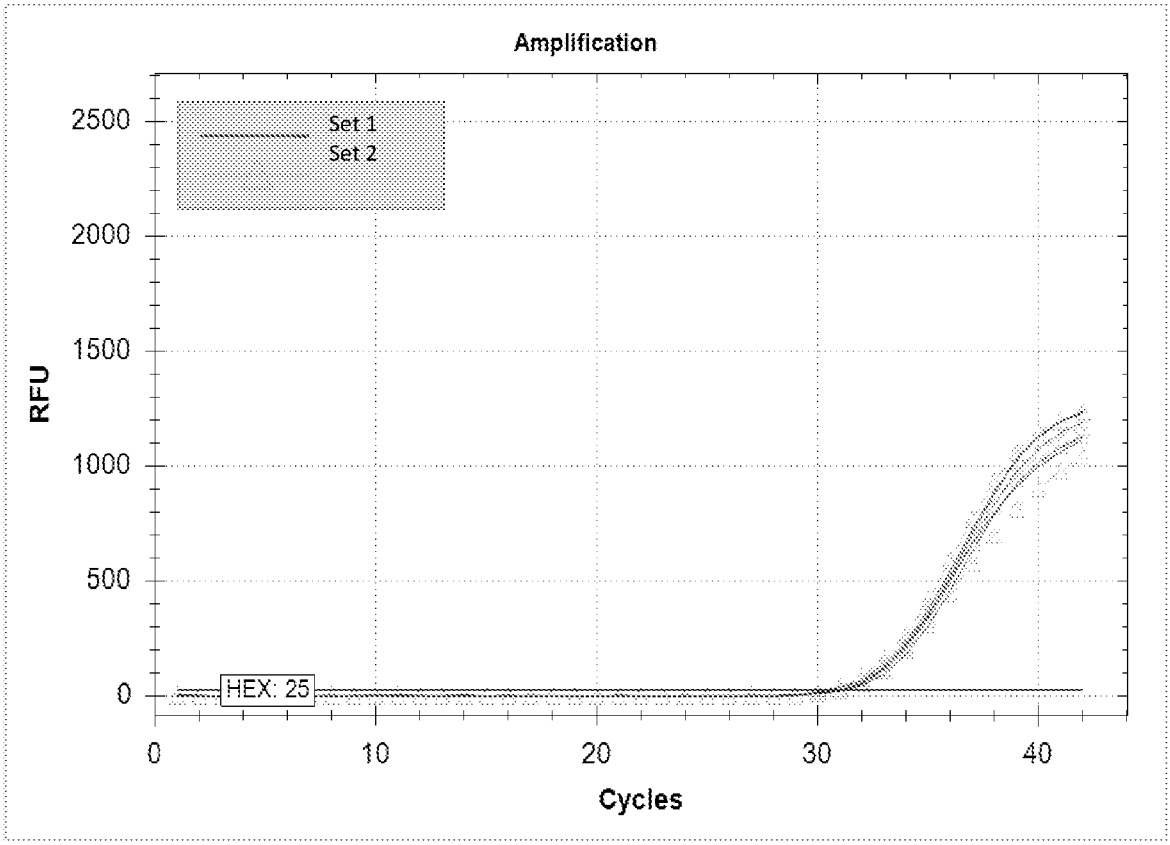
A



Fusion D: FAM			
Fusion D plasmid (cp/ul)	*Ct value* **Set 1** (smooth)	**Set 2** (triangle)	***ΔCt*** *(Set 1-Set 2)*
1000	28.43	28.06	0.37
100	31.88	31.60	0.28
10	35.43	34.25	1.18
1	No Ct	No Ct	-
NTC	No Ct	No Ct	-

Figure 24

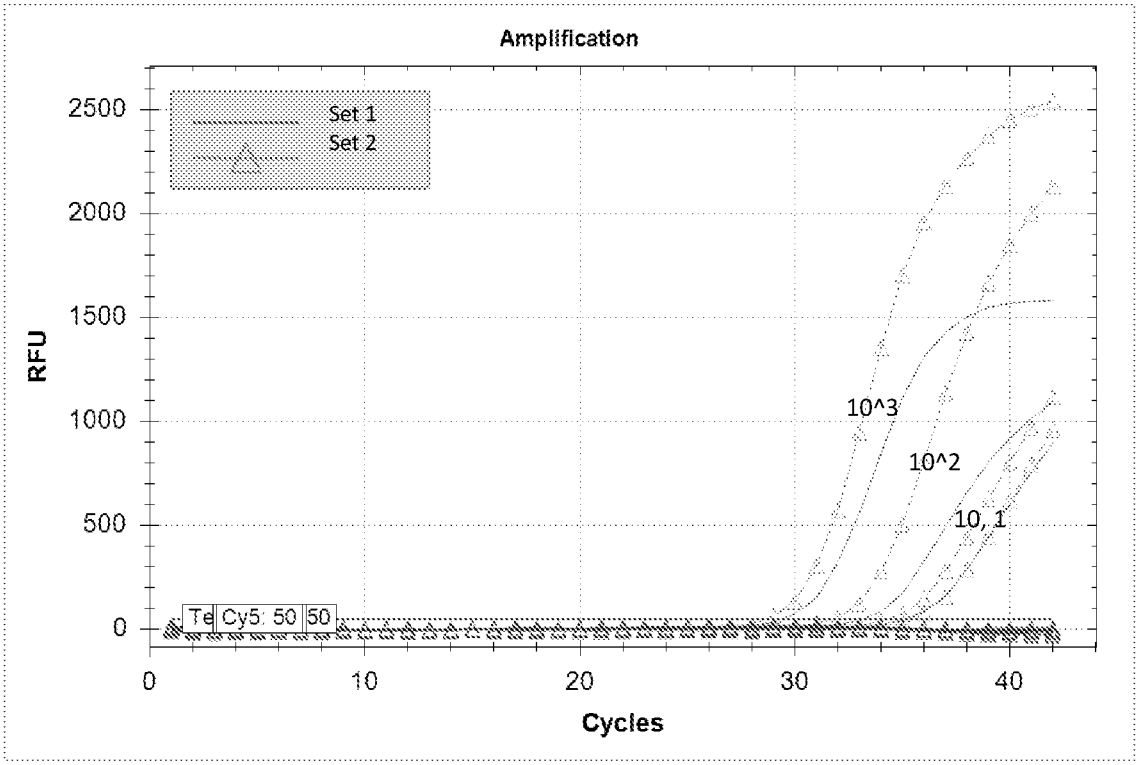
B



IC77: HEX			
Fusion D plasmid (cp/ul)	*Ct value* **Set 1** (smooth)	**Set 2** (triangle)	***ΔCt*** *(Set 1-Set 2)*
1000	31.07	30.98	0.09
100	31.16	30.57	0.59
10	30.91	30.12	0.79
1	30.41	30.24	0.17
NTC	30.97	30.46	0.51

Figure 24 (Continued)

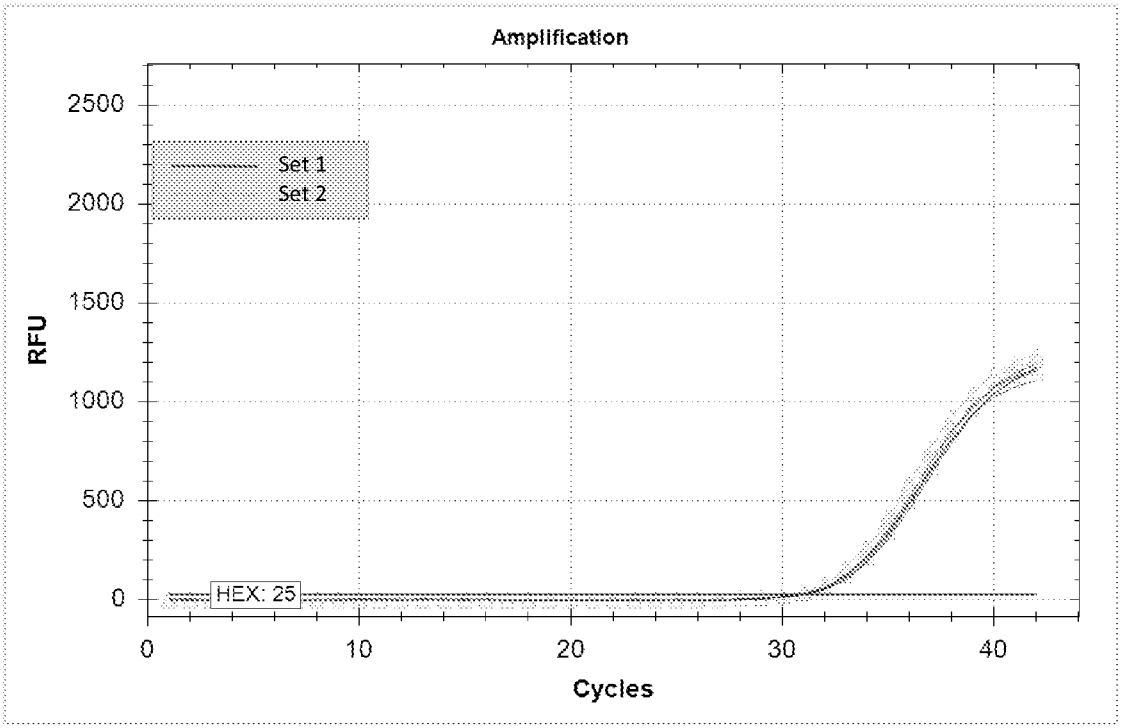
A



EIF3E: TxRd			
EIF3E(e1,e2) plasmid (cp/ul)	*Ct value*		***ΔCt***
	Set 1 (smooth)	**Set 2** (triangle)	*(Set 1-Set 2)*
1000	29.38	28.63	0.75
100	33.17	31.75	1.42
10	35.11	34.32	0.79
1	No Ct	35.26	-35.26
NTC	No Ct	No Ct	-

Figure 25

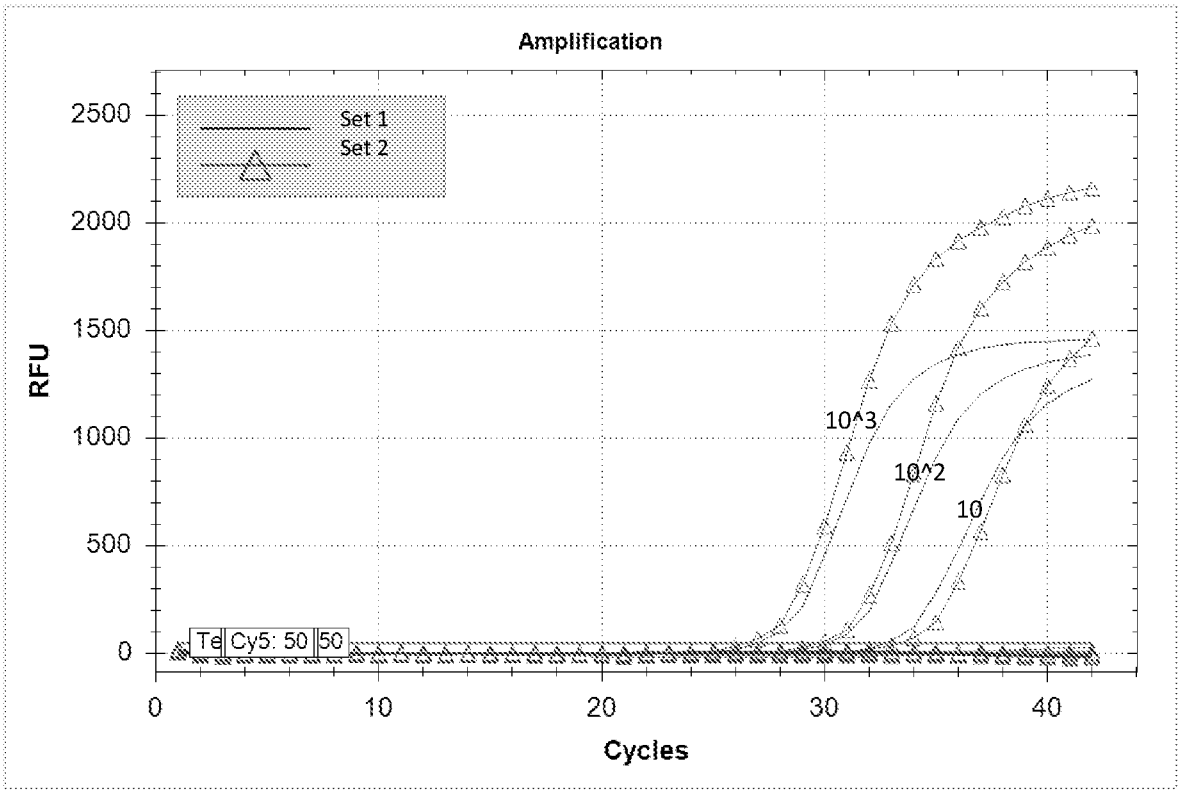
B



IC77: HEX			
EIF3E(e1,e2) plasmid (cp/ul)	*Ct value* **Set 1** (smooth)	**Set 2** (triangle)	***ΔCt*** *(Set 1-Set 2)*
1000	31.03	30.55	0.48
100	30.65	30.23	0.42
10	30.75	30.25	0.50
1	30.60	30.14	0.46
NTC	30.97	30.46	0.51

Figure 25 (Continued)

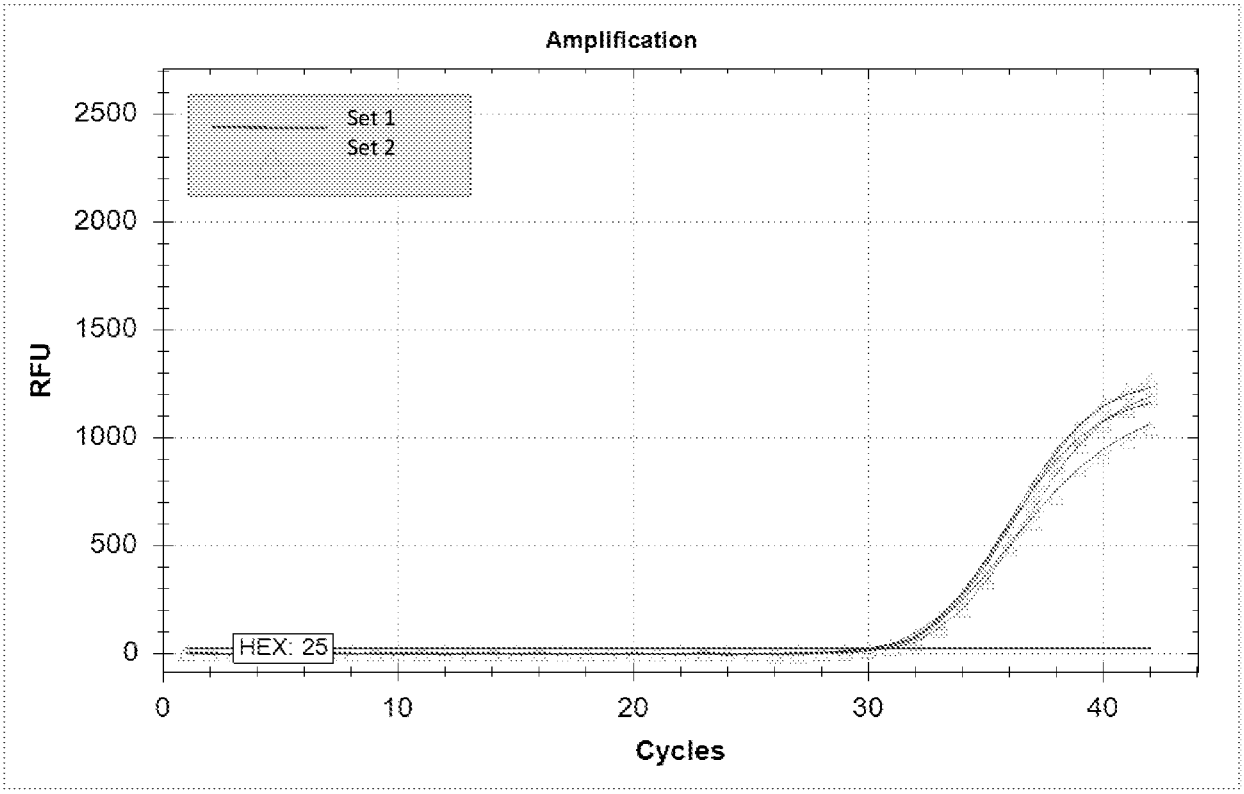
A



RSPO2-004: Cy5			
RSPO2-004(e1,e2) plasmid (cp/ul)	*Ct value*		***ΔCt***
	Set 1 (smooth)	**Set 2** (triangle)	*(Set 1-Set 2)*
1000	27.01	26.44	0.57
100	30.15	29.76	0.39
10	32.74	33.36	-0.62
1	No Ct	No Ct	-
NTC	No Ct	No Ct	-

Figure 26

B



IC77: HEX			
RSPO2-004(e1,e2) plasmid (cp/ul)	*Ct value*		***ΔCt***
	Set 1 (smooth)	**Set 2** (triangle)	*(Set 1-Set 2)*
1000	30.52	30.58	-0.06
100	30.09	30.53	-0.44
10	29.97	30.72	-0.75
1	30.13	30.31	-0.18
NTC	30.97	30.46	0.51

Figure 26 (Continued)

A

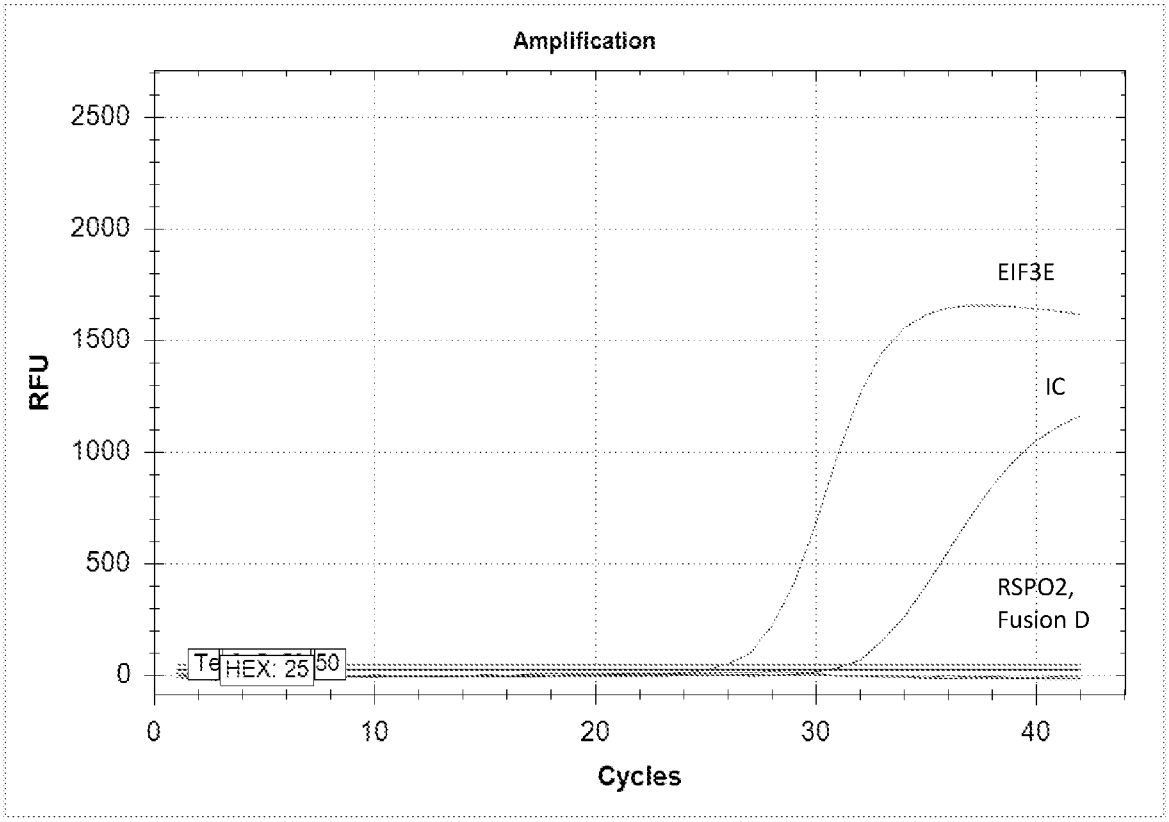
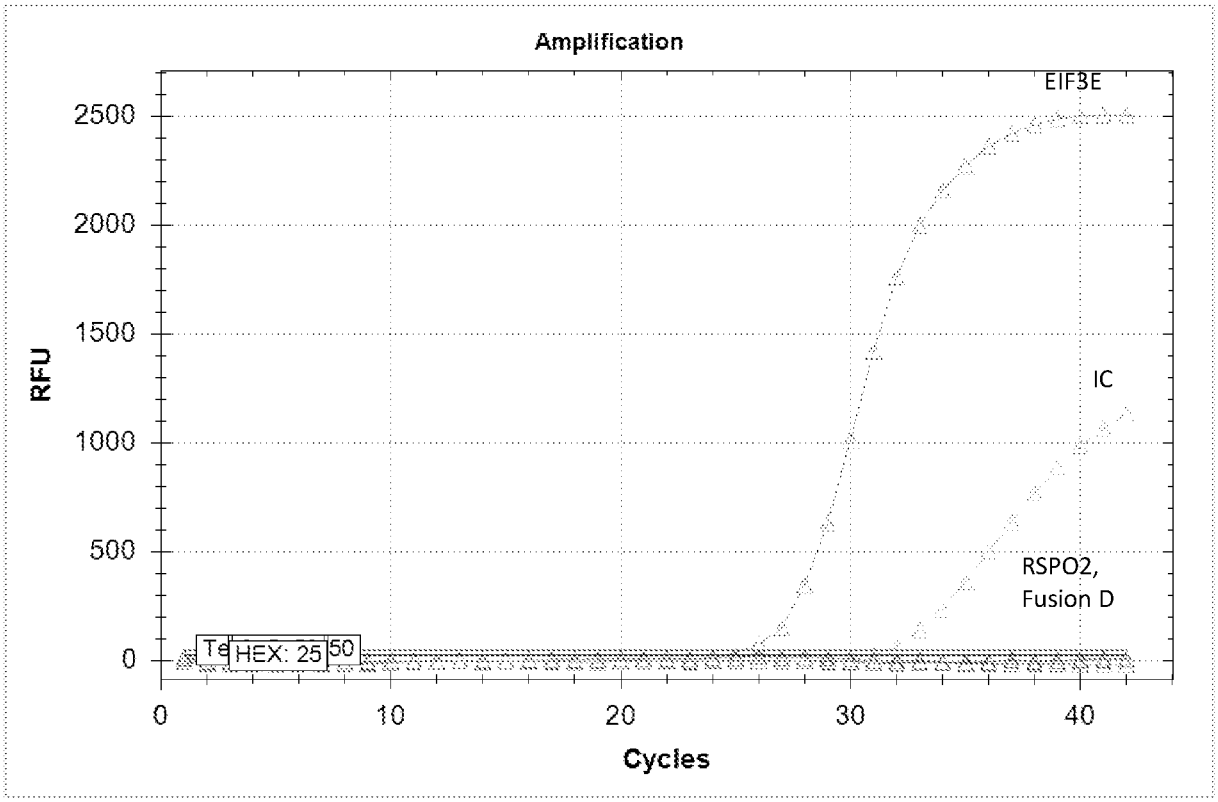

Amplification
2500
2000
1500
1000
500
0
RFU
EIF3E
IC
RSPO2,
Fusion D
HEX: 25
0
10
20
30
40
Cycles

Figure 27

B



Assay	*Ct value*			
	Fusion D: FAM	EIF3E: TxRd	RSPO2-004: Cy5	IC77: HEX
Set 1 (smooth)	No Ct	25.92	No Ct	30.42
Set 2 (triangle)	No Ct	25.32	No Ct	30.77
ΔCt (Set 1-Set 2)	-	0.60	-	-0.35

Figure 27 (Continued)

A

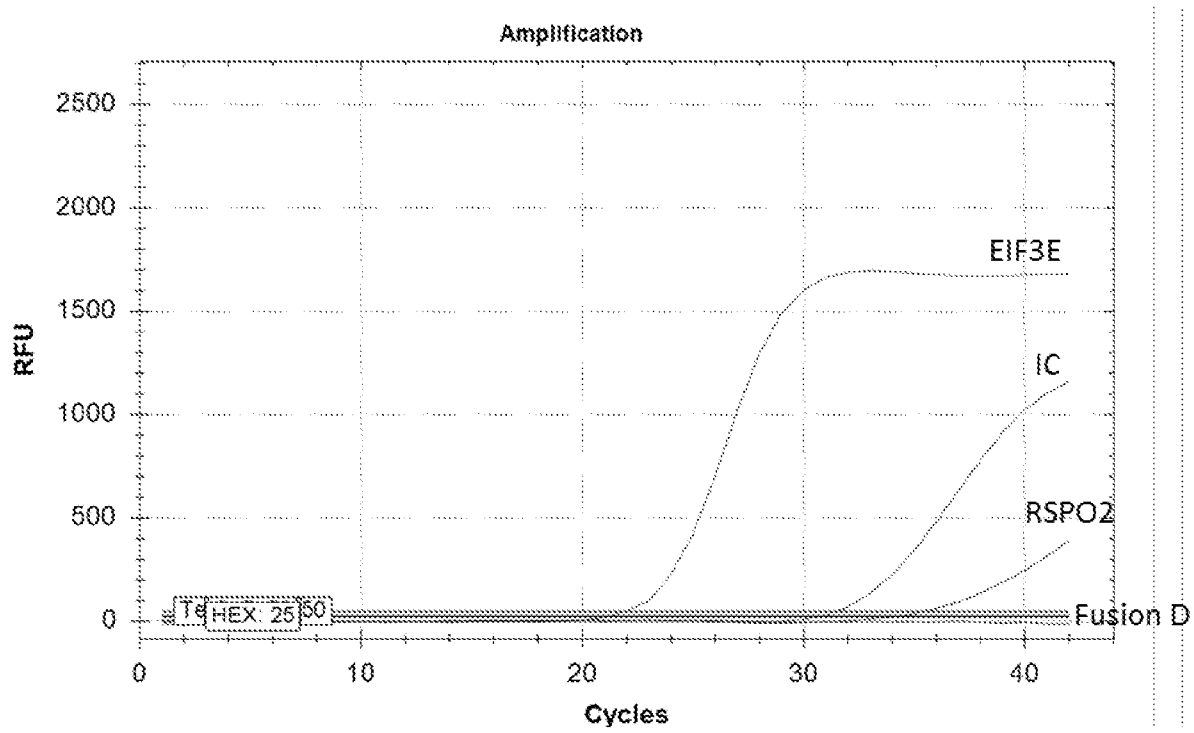
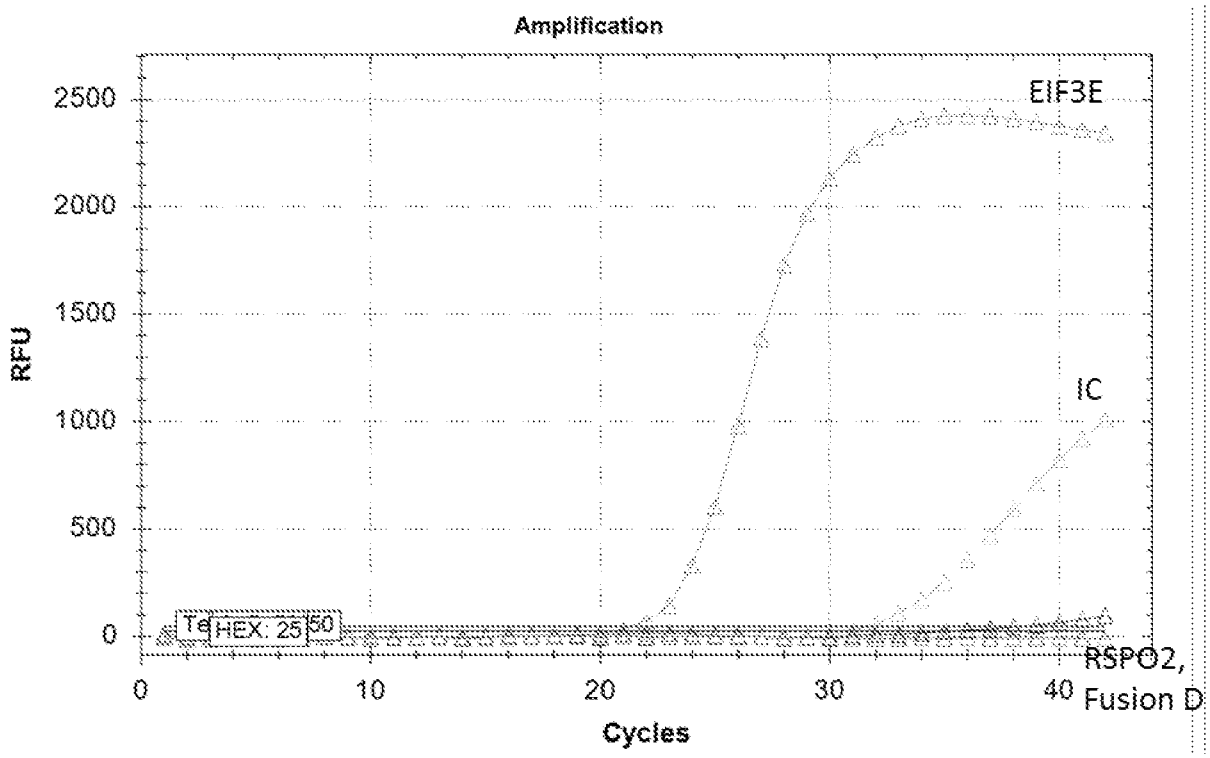

Amplification
2500
2000
1500
1000
500
0
RFU
EIF3E
IC
RSPO2
Fusion D
T
HEX 25
50
0
10
20
30
40
Cycles

Figure 28

B



Assay	*Ct value*			
	Fusion D: FAM	**EIF3E: TxRd**	**RSPO2-004: Cy5**	**IC77: HEX**
Set 1 (smooth)	No Ct	21.92	35.46	30.71
Set 2 (triangle)	No Ct	21.39	38.49	30.83
ΔCt (Set 1-Set 2)	-	0.53	-3.03	-0.12

Figure 28 (Continued)

A

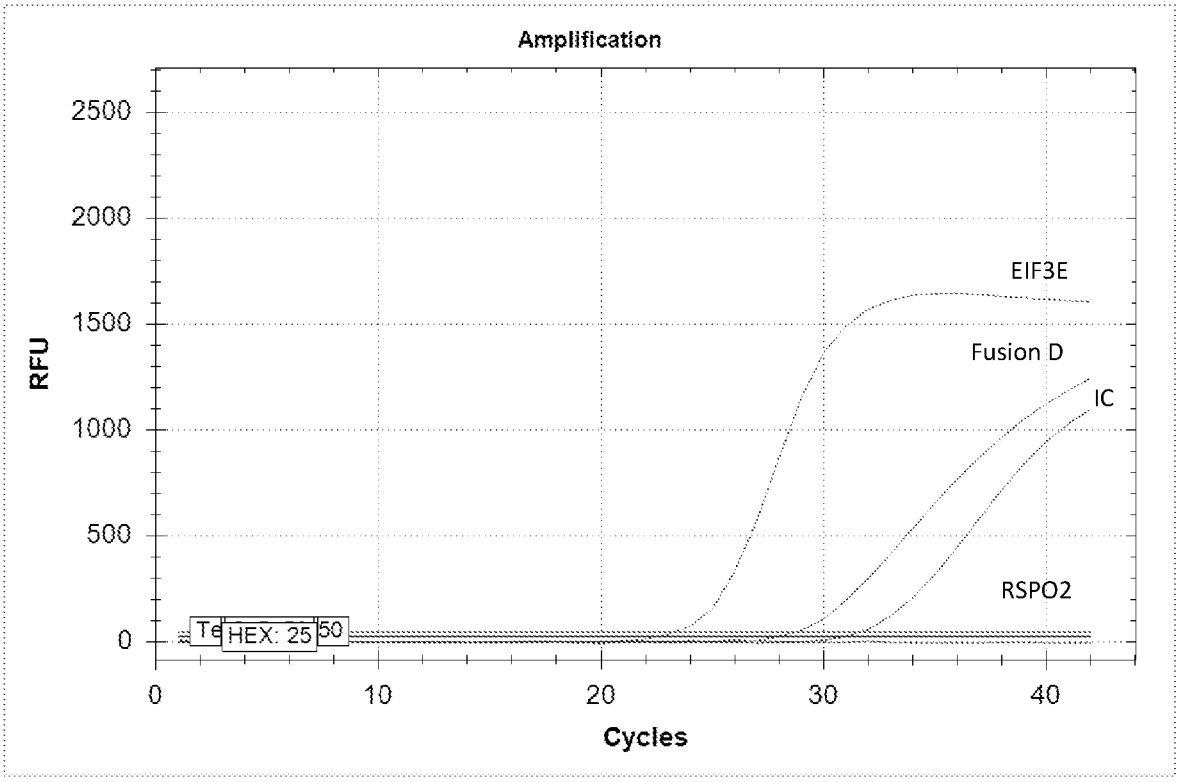
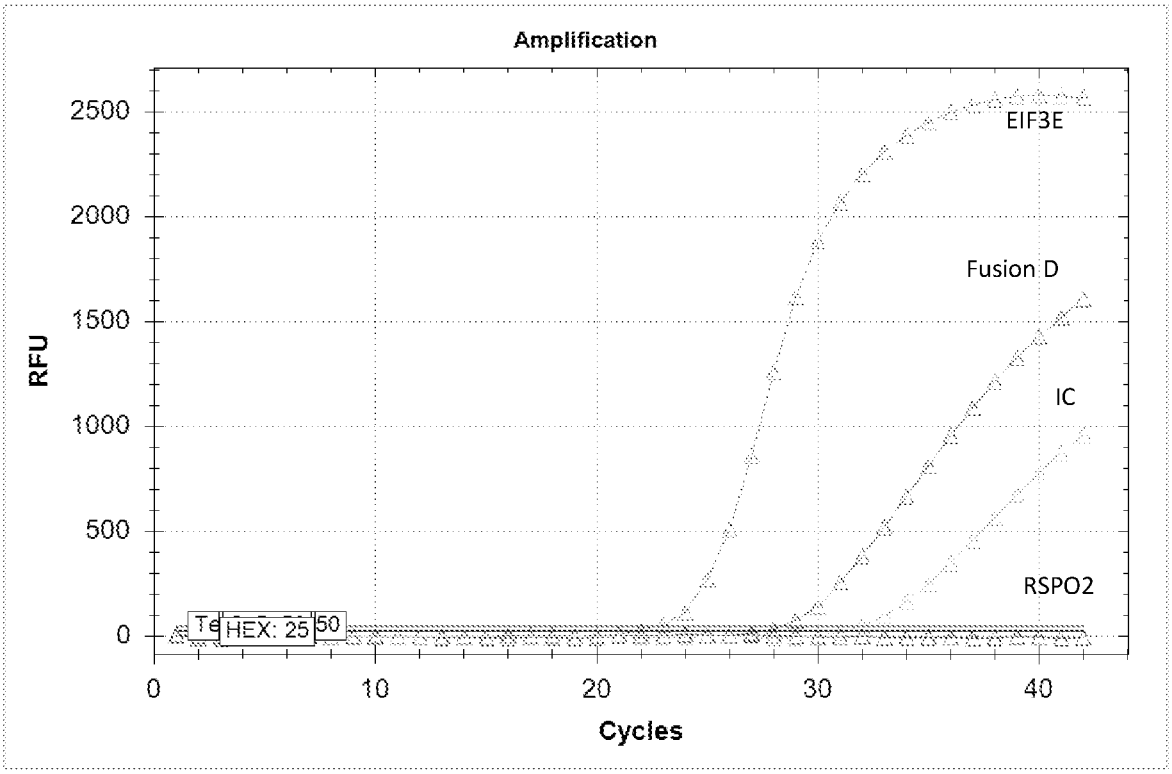

Amplification
RFU
2500
2000
1500
1000
500
0
0
10
20
30
40
Cycles
EIF3E
Fusion D
IC
RSPO2
HEX: 25

Figure 29

B



Assay	*Ct value*			
	Fusion D: FAM	**EIF3E: TxRd**	**RSPO2-004: Cy5**	**IC77: HEX**
Set 1 (smooth)	28.79	23.33	No Ct	30.87
Set 2 (triangle)	28.35	22.71	No Ct	31.17
ΔCt (Set 1-Set 2)	0.44	0.62	-	-0.30

Figure 29 (Continued)

A

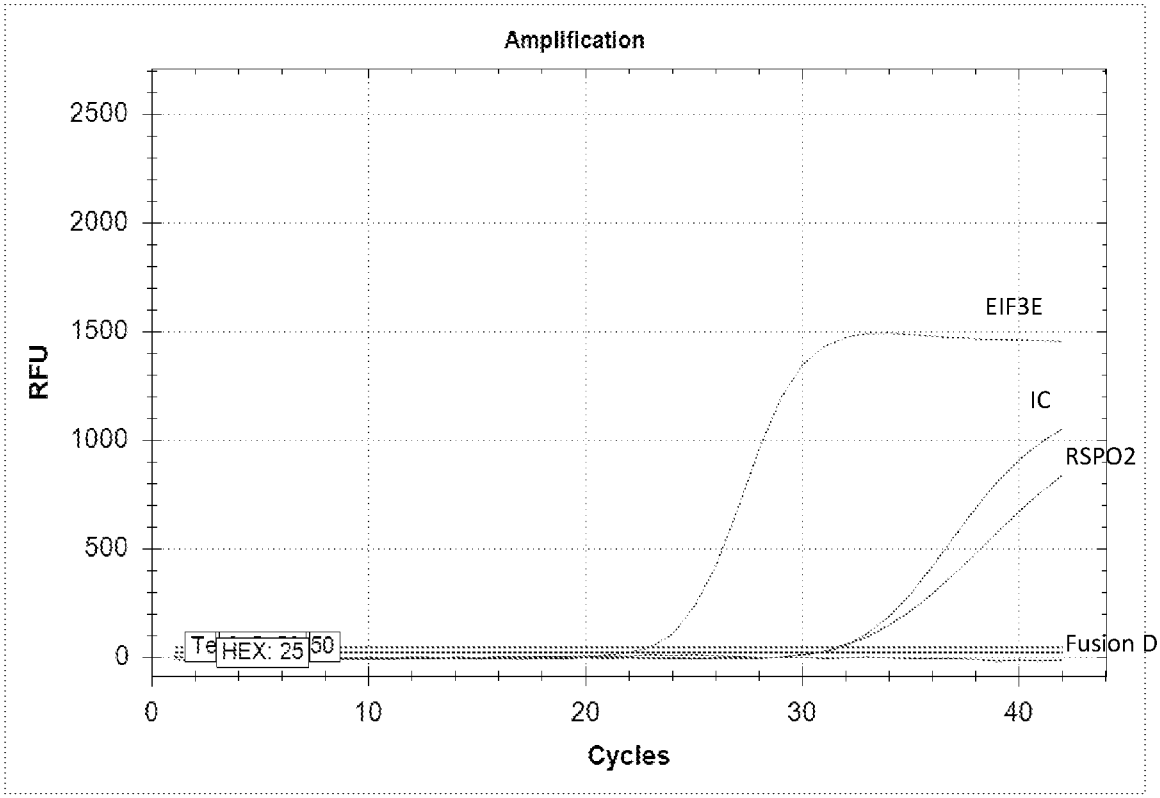
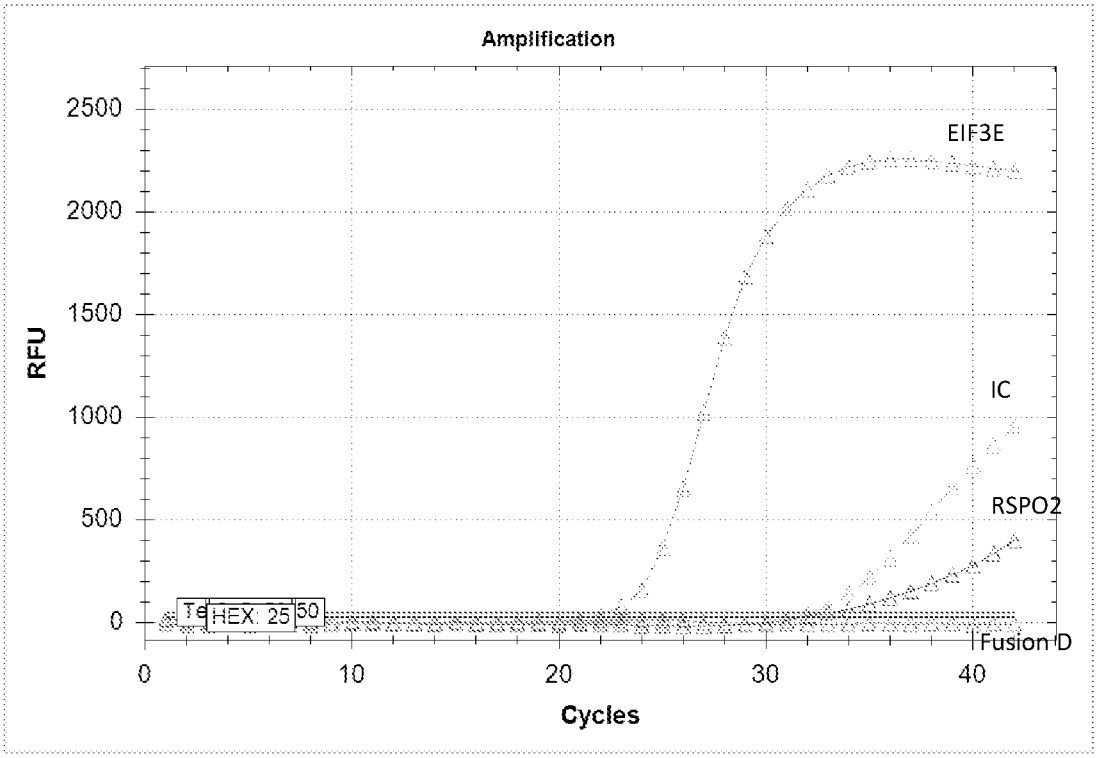

Amplification
2500
2000
1500
1000
500
0
RFU
EIF3E
IC
RSPO2
Fusion D
HEX: 25
0
10
20
30
40
Cycles

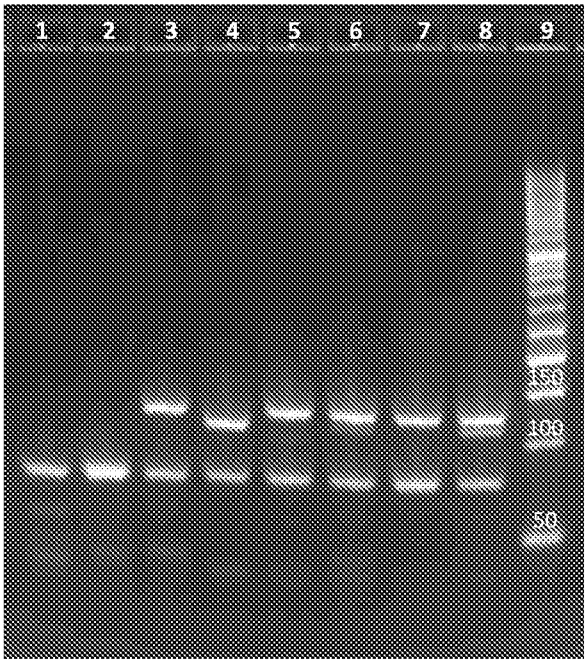
Figure 30

B



Assay	*Ct value*			
	Fusion D: FAM	**EIF3E: TxRd**	**RSPO2-004: Cy5**	**IC77: HEX**
Set 1 (smooth)	No Ct	22.97	31.62	30.86
Set 2 (triangle)	No Ct	22.26	33.02	31.22
ΔCt (Set 1-Set 2)	-	0.71	-1.40	-0.36

Figure 30 (Continued)



Target	Amplicon Size (bp)
EIF3E(e1)+RSPO2(e2)	80
EIF3E	123
RSPO2-004	114
Internal control	77

Figure 31

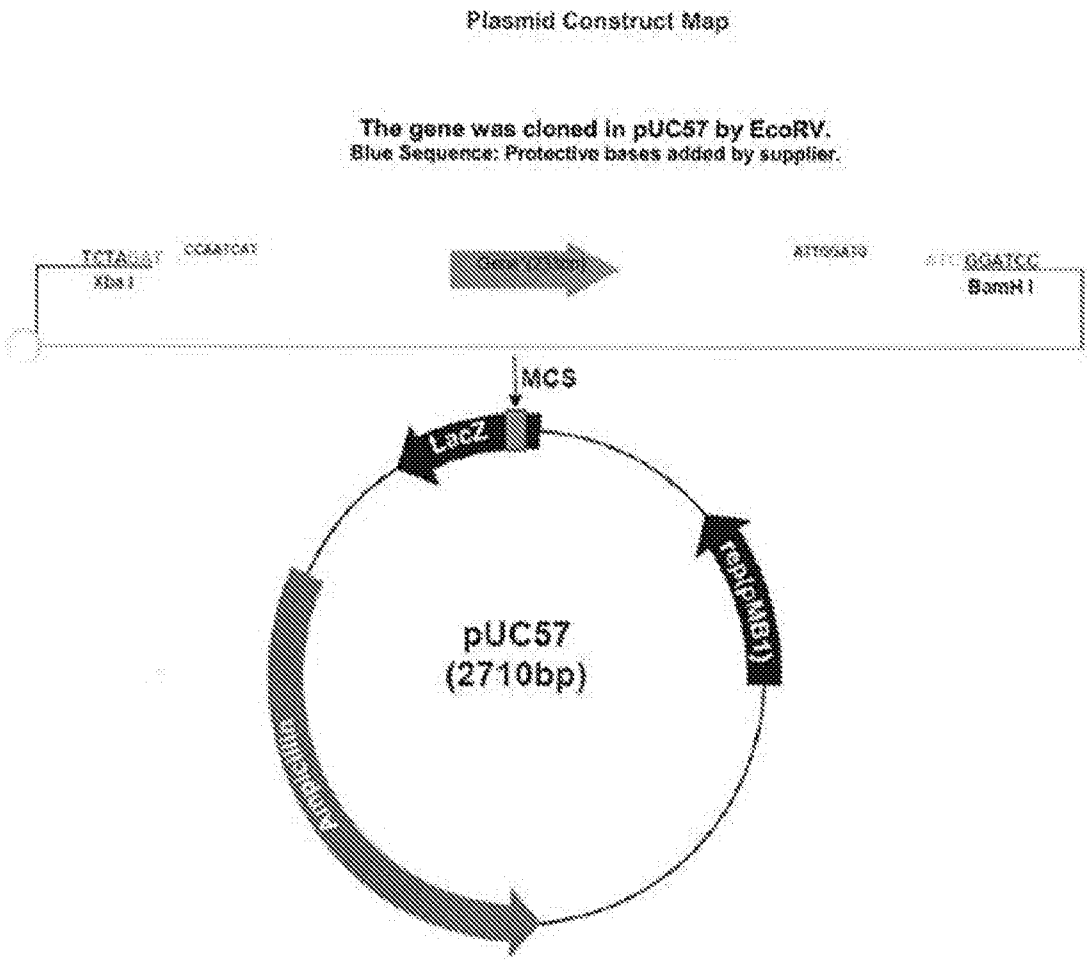

Plasmid Construct Map
The gene was cloned in pUC57 by EcoRV.
Blue Sequence: Protective bases added by supplier.
TCTAGA
Xba I
GGATCC
BamH I
MCS
LacZ
pUC57
(2710bp)

Figure 32