

Table 1 Suppl. RG primers and amplicon characteristics for RT-qPCR in cauliflower.

Gene	Gene name	GenBank ID	Primer sequence (5'-3')	Amplicon size [bp]/ Tm [°C]	PCR efficiency [%]	(R <sup>2</sup> )
<i>ACT1</i>	actin-1-like	MN445988	F: CTGGAATGGTCAAGGCTGGTT R: AATGCCGTGCTCAATAGGGT	185/57.6 /55.4	114.86	0.9752
<i>ACT2</i>	actin-2-like	MN445987	F: GTTGGAATGGGACAGAAAGA R: TTCAGTAAGAAGCACAGGGT	189/53.4	110.81	0.9993
<i>ACT3</i>	actin-like protein	MN445986	F: AAGGCTATTAGGGAGCAACT R: CAACAGCATAAAGGGACAAC	100/53.4	114.75	0.9888
<i>ACT7</i>	actin 7	MN445989	F: GCTGACCGTATGAGCAAAGA R: GATCCTCCAATCCAGACACT	104/55.4	101.91	0.9858
<i>TUA2</i>	alpha-tubulin 2	MN445991	F: CAGCGTTGGAGAAGGATTAT R: TCAGACAGCAGGAGACAGAT	218/53.4 /55.4	102.10	0.9844
<i>TUB6</i>	tubulin beta-6	MN445992	F: TGAAGGAGCCGAGCTTATTG R: AGAGTTCCCATCCAGACCC	129/55.4 /57.4	106.27	0.9962
<i>EF1-α</i>	elongation factor 1-alpha	MN445990	F: GCCGAGCGTGAGCGTGGTAT R: CAGCAATCAGCCTGGGAG	145/53.4 /49.2	97.97	0.9917
<i>EF1-β</i>	elongation factor 1-beta	MN433710	F: AACTCTGACGCTGGATTGAA R: ACTGTGAGGTTGGTGGCTTA	127/53.4 /55.4	103.64	0.9799
<i>GAPDH</i>	glyceraldehyde-3-phosphate dehydrogenase	MN445982	F: AATGGTGGATACAGGAAAGG R: GTATTTGAGGAGATGCGTTG	177/53.4	103.84	0.9728
<i>HIS</i>	histone H1-like	MN445984	F: TTGGAAGACCCAGAAAGAAT R: GAGCCTGTATAGCTTGAACCAC	180/51.3 /57.7	117.90	0.9952
<i>KIN</i>	protein kinase	MN445981	F: CTTTCCCATAGCCATTCACG R: CTTCTTTCCAAGATCCACC	214/55.4	111.03	0.9856
<i>eIF4A-1</i>	eukaryotic initiation factor 4A-1	MN445980	F: GGGTATCTATGCCTATGGTTT R: ACTGGACAAGGGTGAAGTCG	167/53.7 /57.4	114.37	0.9981
<i>FBA5</i>	fructose-bisphosphate aldolase 5	MN445983	F: GGCTAACTCGGACGCTACAC R: CAAACCGTATCAAACGCAGA	124/59.5 /53.4	117.65	0.9999
<i>SAMDC</i>	S-adenosyl-L-methionine decarboxylase	MN433711	F: GTGACGCAATCTCCACTATCC R: GAGTCGTAGCCCTTCTGTGC	187/57.6 /59.5	101.47	0.9632
<i>UBQ</i>	polyubiquitin	MN445985	F: CAATCGACAATGTGAAGGCTAA R: ACGCAGCACAAGGTGAAGAGT	152/53.9 /57.6	106.73	0.9736

Table 2 Suppl. The cycle threshold (Ct) values of the 15 candidate genes. SD – standard deviation.

Rank	Gene	Ct	SD
1	<i>ACT7</i>	22.859	0.525
2	<i>TUA2</i>	23.360	0.877
3	<i>GAPDH</i>	23.644	1.544
4	<i>EF1-β</i>	24.654	0.749
5	<i>ACT2</i>	24.705	0.940
6	<i>TUB6</i>	24.845	0.794
7	<i>HIS</i>	24.939	0.856
8	<i>UBQ</i>	25.561	1.049
9	<i>FBA5</i>	25.729	2.236
10	<i>eIF4A-1</i>	26.030	0.906
11	<i>ACT1</i>	26.143	0.576
12	<i>EF1-α</i>	26.418	0.929
13	<i>KIN</i>	26.425	1.007
14	<i>SAMDC</i>	27.334	1.322
15	<i>ACT3</i>	28.855	0.592

Table 3 Suppl. Gene expression stability across sample sets calculated by *geNorm*. The numbers in brackets indicate the stability ordering of genes under different stresses.

Gene name	Heat stress	Cold stress	Drought stress	Salt stress	MeJA stress	Total
<i>ACT1</i>	0.543(7)	0.473(1)	0.210(3)	0.296(6)	0.299(3)	0.435(1)
<i>ACT2</i>	0.578(8)	0.731(8)	0.627(8)	0.821(14)	0.382(6)	0.852(8)
<i>ACT3</i>	0.273(1)	0.877(12)	0.536(7)	0.316(7)	0.228(1)	0.565(4)
<i>ACT7</i>	0.316(3)	0.681(7)	0.449(6)	0.170(1)	0.228(1)	0.435(1)
<i>TUA2</i>	0.424(5)	0.535(3)	0.379(5)	0.461(9)	0.527(11)	0.698(6)
<i>TUB6</i>	0.388(4)	0.473(1)	1.124(13)	0.261(5)	0.423(8)	0.898(9)
<i>EF1-α</i>	0.795(12)	0.838(11)	0.971(12)	0.529(10)	0.484(10)	0.982(11)
<i>EF1-β</i>	0.273(1)	0.612(5)	0.130(1)	0.236(4)	0.441(9)	0.498(3)
<i>GAPDH</i>	0.990(14)	0.550(4)	1.254(14)	0.742(13)	0.829(14)	1.147(14)
<i>HIS</i>	0.671(10)	0.797(10)	0.342(4)	0.170(1)	0.347(5)	0.638(5)
<i>KIN</i>	0.849(13)	0.764(9)	0.855(11)	0.598(11)	0.317(4)	0.791(7)
<i>eIF4A-1</i>	0.508(6)	0.645(6)	0.709(9)	0.396(8)	0.698(13)	0.941(10)
<i>FBA5</i>	1.126(15)	0.919(13)	1.427(15)	1.024(15)	1.036(15)	1.304(15)
<i>SAMDC</i>	0.623(9)	1.010(14)	0.789(10)	0.194(3)	0.600(12)	1.056(13)
<i>UBQ</i>	0.738(11)	1.105(15)	0.130(1)	0.672(12)	0.406(7)	1.014(12)

Table 4 Suppl. Pairwise variation (Vn/n+1) analysis of 15 candidate reference genes calculated by *geNorm*.

Vn/ Vn+1	Heat	Cold	Drought	Salt	MeJA	Total
V2/ V3	0.103	0.171	0.082	0.063	0.105	0.161
V3/ V4	0.106	0.119	0.115	0.066	0.072	0.142
V4/ V5	0.087	0.128	0.076	0.054	0.074	0.135
V5/ V6	0.103	0.102	0.089	0.055	0.067	0.119
V6/ V7	0.080	0.097	0.101	0.045	0.060	0.135
V7/ V8	0.072	0.101	0.106	0.076	0.048	0.113
V8/ V9	0.077	0.082	0.101	0.070	0.050	0.100
V9/ V10	0.077	0.082	0.101	0.073	0.061	0.094
V10/ V11	0.087	0.082	0.093	0.076	0.061	0.089
V11/ V12	0.082	0.079	0.120	0.081	0.076	0.081
V12/ V13	0.079	0.080	0.142	0.079	0.092	0.084
V13/ V14	0.126	0.103	0.134	0.086	0.110	0.110
V14/ V15	0.128	0.106	0.162	0.153	0.154	0.146

Table 5 Suppl. Gene expression stability of all sample sets by *NormFinder*.

Rank	Heat	Cold	Drought	Salt	MeJA	Total
1	<i>EF1-β</i> 0.095	<i>EF1-β</i> 0.023	<i>ACT1</i> 0.069	<i>HIS</i> 0.059	<i>ACT3</i> 0.066	<i>ACT1</i> 0.226
2	<i>ACT7</i> 0.097	<i>ACT1</i> 0.136	<i>EF1-β</i> 0.185	<i>SAMDC</i> 0.067	<i>ACT7</i> 0.149	<i>ACT7</i> 0.310
3	<i>ACT3</i> 0.115	<i>ACT7</i> 0.294	<i>UBQ</i> 0.256	<i>TUB6</i> 0.115	<i>ACT1</i> 0.169	<i>EF1-β</i> 0.326
4	<i>TUB6</i> 0.243	<i>eIF4A-1</i> 0.304	<i>TUA2</i> 0.410	<i>ACT1</i> 0.166	<i>KIN</i> 0.190	<i>ACT3</i> 0.407
5	<i>eIF4A-1</i> 0.325	<i>TUA2</i> 0.436	<i>ACT3</i> 0.432	<i>EF1-β</i> 0.173	<i>HIS</i> 0.191	<i>HIS</i> 0.411
6	<i>TUA2</i> 0.331	<i>TUB6</i> 0.448	<i>ACT2</i> 0.479	<i>ACT7</i> 0.199	<i>UBQ</i> 0.207	<i>TUA2</i> 0.561
7	<i>ACT1</i> 0.333	<i>KIN</i> 0.462	<i>ACT7</i> 0.479	<i>ACT3</i> 0.272	<i>TUB6</i> 0.215	<i>TUB6</i> 0.569
8	<i>SAMDC</i> 0.417	<i>HIS</i> 0.493	<i>HIS</i> 0.509	<i>eIF4A-1</i> 0.318	<i>ACT2</i> 0.227	<i>eIF4A-1</i> 0.613
9	<i>ACT2</i> 0.468	<i>ACT2</i> 0.520	<i>eIF4A-1</i> 0.673	<i>TUA2</i> 0.403	<i>EF1-β</i> 0.264	<i>KIN</i> 0.634
10	<i>HIS</i> 0.557	<i>GAPDH</i> 0.533	<i>EF1-α</i> 0.788	<i>EF1-α</i> 0.445	<i>EF1-α</i> 0.408	<i>ACT2</i> 0.635
11	<i>UBQ</i> 0.712	<i>EF1-α</i> 0.591	<i>KIN</i> 0.794	<i>KIN</i> 0.632	<i>TUA2</i> 0.540	<i>UBQ</i> 0.666
12	<i>EF1-α</i> 0.748	<i>ACT3</i> 0.674	<i>SAMDC</i> 0.943	<i>GAPDH</i> 0.646	<i>SAMDC</i> 0.702	<i>EF1-α</i> 0.688
13	<i>KIN</i> 0.790	<i>FBA5</i> 0.743	<i>TUB6</i> 1.165	<i>UBQ</i> 0.647	<i>eIF4A-1</i> 0.901	<i>SAMDC</i> 0.747
14	<i>GAPDH</i> 1.118	<i>SAMDC</i> 0.995	<i>GAPDH</i> 1.169	<i>ACT2</i> 0.929	<i>GAPDH</i> 0.972	<i>GAPDH</i> 0.960
15	<i>FBA5</i> 1.325	<i>UBQ</i> 1.094	<i>FBA5</i> 1.673	<i>FBA5</i> 1.590	<i>FBA5</i> 1.601	<i>FBA5</i> 1.515

Table 6 Suppl. Gene expression stability of all sample sets by *BestKeeper*.

Rank	Heat	Cold	Drought	Salt	MeJA	Total
1	<i>ACT3</i> 0.62±0.18	<i>ACT1</i> 0.81±0.21	<i>TUA2</i> 1.49±0.36	<i>TUB6</i> 0.62±0.15	<i>ACT2</i> 0.91±0.23	<i>ACT7</i> 1.84±0.42
2	<i>EF-1β</i> 1.02±0.26	<i>TUB</i> 1.37±0.33	<i>ACT1</i> 1.63±0.43	<i>HIS</i> 0.69±0.17	<i>TUB</i> 0.94±0.23	<i>ACT3</i> 1.53±0.44
3	<i>ACT7</i> 1.36±0.31	<i>EF-1β</i> 1.40±0.33	<i>HIS</i> 1.80±0.45	<i>SAMDC</i> 0.74±0.21	<i>UBQ</i> 0.92±0.24	<i>ACT1</i> 1.86±0.49
4	<i>TUB6</i> 1.50±0.38	<i>GAPDH</i> 1.63±0.36	<i>EF-1β</i> 1.88±0.47	<i>ACT7</i> 1.11±0.25	<i>HIS</i> 1.08±0.27	<i>TUB</i> 2.04±0.51
5	<i>TUA2</i> 2.03±0.49	<i>ACT7</i> 1.63±0.37	<i>UBQ</i> 1.97±0.51	<i>ACT1</i> 1.06±0.27	<i>ACT3</i> 1.00±0.29	<i>HIS</i> 2.50±0.62
6	<i>SAMDC</i> 1.82±0.51	<i>ACT2</i> 1.91±0.46	<i>ACT7</i> 2.27±0.52	<i>EF-1β</i> 1.12±0.27	<i>ACT1</i> 1.47±0.39	<i>EF-1β</i> 2.55±0.63
7	<i>eIF4A-1</i> 2.05±0.55	<i>ACT3</i> 1.91±0.55	<i>eIF4A-1</i> 2.28±0.58	<i>eIF4A-1</i> 1.14±0.30	<i>KIN</i> 1.55±0.42	<i>ACT2</i> 2.70±0.67
8	<i>ACT1</i> 2.43±0.64	<i>TUA</i> 2.55±0.58	<i>EF-1α</i> 2.46±0.64	<i>ACT3</i> 1.31±0.38	<i>SAMDC</i> 1.55±0.43	<i>TUA2</i> 3.00±0.70
9	<i>ACT2</i> 2.88±0.70	<i>eIF4A-1</i> 2.37±0.60	<i>ACT2</i> 3.19±0.79	<i>EF-1α</i> 1.95±0.51	<i>ACT7</i> 2.02±0.46	<i>eIF4A-1</i> 2.75±0.72
10	<i>EF-1α</i> 2.87±0.75	<i>FBA5</i> 2.77±0.65	<i>ACT3</i> 2.83±0.81	<i>TUA</i> 2.41±0.56	<i>EF-1β</i> 2.16±0.53	<i>EF-1α</i> 2.89±0.76
11	<i>UBQ</i> 3.03±0.76	<i>KIN</i> 2.59±0.68	<i>SAMDC</i> 4.15±1.14	<i>UBQ</i> 2.57±0.66	<i>eIF4A-1</i> 2.56±0.67	<i>UBQ</i> 3.27±0.84
12	<i>HIS</i> 2.99±0.77	<i>HIS</i> 3.17±0.76	<i>KIN</i> 4.49±1.18	<i>GAPDH</i> 2.88±0.67	<i>EF-1α</i> 2.70±0.73	<i>KIN</i> 3.40±0.90
13	<i>GAPDH</i> 4.03±0.99	<i>EF-1α</i> 3.20±0.85	<i>TUB</i> 4.79±1.21	<i>KIN</i> 3.06±0.80	<i>TUA</i> 3.82±0.87	<i>SAMDC</i> 3.56±0.97
14	<i>KIN</i> 4.22±1.11	<i>SAMDC</i> 5.07±1.30	<i>GAPDH</i> 5.10±1.25	<i>ACT2</i> 4.40±1.11	<i>GAPDH</i> 5.84±1.43	<i>GAPDH</i> 5.73±1.35
15	<i>FBA5</i> 5.20±1.34	<i>UBQ</i> 5.24±1.30	<i>FBA5</i> 7.32±1.93	<i>FBA5</i> 7.16±1.83	<i>FBA5</i> 6.35±1.76	<i>FBA5</i> 7.96±2.05

Table 7 Suppl. Gene expression stability for all sample sets by the  $\Delta Ct$  method.

Rank	Heat	Cold	Drought	Salt	MeJA	Total
1	<i>ACT7</i> 0.431	<i>GAPDH</i> 0.417	<i>ACT7</i> 0.771	<i>ACT7</i> 0.476	<i>TUA2</i> 0.92	<i>ACT7</i> 1.049
2	<i>TUA2</i> 1.186	<i>ACT7</i> 0.877	<i>TUA2</i> 1.421	<i>GAPDH</i> 0.827	<i>ACT7</i> 1.094	<i>TUA2</i> 1.404
3	<i>ACT2</i> 1.191	<i>TUA2</i> 0.964	<i>GAPDH</i> 1.831	<i>TUA2</i> 0.931	<i>GAPDH</i> 1.864	<i>GAPDH</i> 1.605
4	<i>GAPDH</i> 1.489	<i>FBA5</i> 1.43	<i>ACT2</i> 1.974	<i>EF1-<math>\beta</math></i> 1.579	<i>EF1-<math>\beta</math></i> 2.252	<i>EF1-<math>\beta</math></i> 2.319
5	<i>TUB6</i> 1.776	<i>EF1-<math>\beta</math></i> 1.808	<i>EF1-<math>\beta</math></i> 2.139	<i>TUB6</i> 1.722	<i>TUB6</i> 2.514	<i>ACT2</i> 2.355
6	<i>UBQ</i> 1.863	<i>HIS</i> 1.921	<i>HIS</i> 2.175	<i>HIS</i> 1.873	<i>ACT2</i> 2.634	<i>TUB6</i> 2.454
7	<i>EF1-<math>\beta</math></i> 2.218	<i>ACT2</i> 2.057	<i>TUB6</i> 2.491	<i>ACT2</i> 2.32	<i>HIS</i> 2.763	<i>HIS</i> 2.52
8	<i>HIS</i> 2.271	<i>TUB6</i> 2.168	<i>eIF4A-1</i> 2.64	<i>FBA5</i> 2.477	<i>eIF4A-1</i> 3.336	<i>UBQ</i> 2.96
9	<i>FBA5</i> 2.291	<i>UBQ</i> 2.392	<i>UBQ</i> 2.857	<i>UBQ</i> 2.622	<i>UBQ</i> 3.47	<i>FBA5</i> 3.079
10	<i>EF1-<math>\alpha</math></i> 2.518	<i>eIF4A-1</i> 2.649	<i>EF1-<math>\alpha</math></i> 2.992	<i>ACT1</i> 2.641	<i>ACT1</i> 3.557	<i>eIF4A-1</i> 3.292
11	<i>KIN</i> 2.621	<i>SAMDC</i> 3.017	<i>ACT1</i> 3.125	<i>KIN</i> 2.999	<i>KIN</i> 4.015	<i>ACT1</i> 3.371
12	<i>ACT1</i> 2.831	<i>ACT1</i> 3.105	<i>KIN</i> 3.128	<i>EF1-<math>\alpha</math></i> 3.059	<i>EF1-<math>\alpha</math></i> 4.026	<i>EF1-<math>\alpha</math></i> 3.566
13	<i>eIF4A-1</i> 2.962	<i>KIN</i> 3.494	<i>FBA5</i> 3.159	<i>eIF4A-1</i> 3.275	<i>FBA5</i> 4.44	<i>KIN</i> 3.571
14	<i>SAMDC</i> 3.892	<i>EF1-<math>\alpha</math></i> 3.637	<i>SAMDC</i> 3.98	<i>SAMDC</i> 4.001	<i>SAMDC</i> 4.581	<i>SAMDC</i> 4.214
15	<i>ACT3</i> 4.497	<i>ACT3</i> 5.175	<i>ACT3</i> 4.948	<i>ACT3</i> 4.776	<i>ACT3</i> 5.453	<i>ACT3</i> 5.289

Table 8 Suppl. The comprehensive ranking of the 15 RGs for gene expression.

Rank	Heat stress	Cold stress	Drought stress	Salt stress	MeJA stress	Total
1	<i>EF1-β</i>	<i>ACT1</i>	<i>TUA2</i>	<i>HIS</i>	<i>ACT7</i>	<i>ACT7</i>
2	<i>ACT7</i>	<i>EF1-β</i>	<i>EF1-β</i>	<i>ACT7</i>	<i>ACT3</i>	<i>EF1-β</i>
3	<i>ACT3</i>	<i>TUB6</i>	<i>ACT1</i>	<i>TUB6</i>	<i>ACT2</i>	<i>ACT1</i>
4	<i>TUA2</i>	<i>GAPDH</i>	<i>UBQ</i>	<i>SAMDC</i>	<i>ACT1</i>	<i>TUA2</i>
5	<i>TUB6</i>	<i>ACT7</i>	<i>ACT7</i>	<i>EF1-β</i>	<i>TUB6</i>	<i>TUB6</i>
6	<i>ACT2</i>	<i>TUA2</i>	<i>HIS</i>	<i>ACT1</i>	<i>HIS</i>	<i>HIS</i>
7	<i>eIF4A-1</i>	<i>eIF4A-1</i>	<i>ACT2</i>	<i>TUA2</i>	<i>UBQ</i>	<i>ACT3</i>
8	<i>ACT1</i>	<i>ACT2</i>	<i>eIF4A-1</i>	<i>GAPDH</i>	<i>KIN</i>	<i>ACT2</i>
9	<i>SAMDC</i>	<i>HIS</i>	<i>ACT3</i>	<i>eIF4A-1</i>	<i>TUA2</i>	<i>GAPDH</i>
10	<i>UBQ</i>	<i>FBA5</i>	<i>GAPDH</i>	<i>ACT3</i>	<i>EF1-β</i>	<i>UBQ</i>
11	<i>HIS</i>	<i>KIN</i>	<i>EF1-α</i>	<i>EF1-α</i>	<i>GAPDH</i>	<i>eIF4A-1</i>
12	<i>GAPDH</i>	<i>ACT3</i>	<i>TUB6</i>	<i>UBQ</i>	<i>EF1-α</i>	<i>SAMDC</i>
13	<i>EF1-α</i>	<i>EF1-α</i>	<i>KIN</i>	<i>KIN</i>	<i>eIF4A-1</i>	<i>KIN</i>
14	<i>KIN</i>	<i>SAMDC</i>	<i>SAMDC</i>	<i>ACT2</i>	<i>SAMDC</i>	<i>EF1-α</i>
15	<i>FBA5</i>	<i>UBQ</i>	<i>FBA5</i>	<i>FBA5</i>	<i>FBA5</i>	<i>FBA5</i>

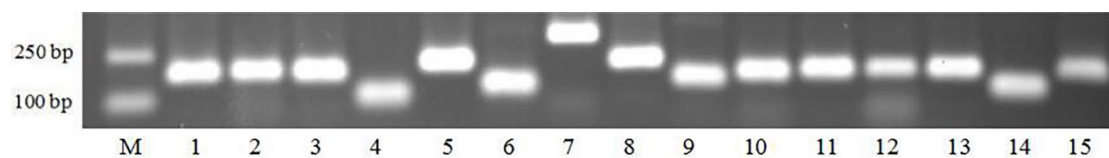
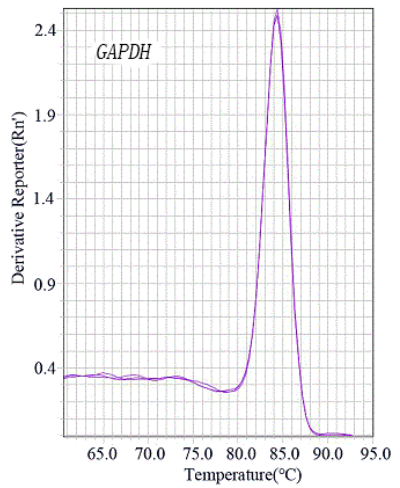
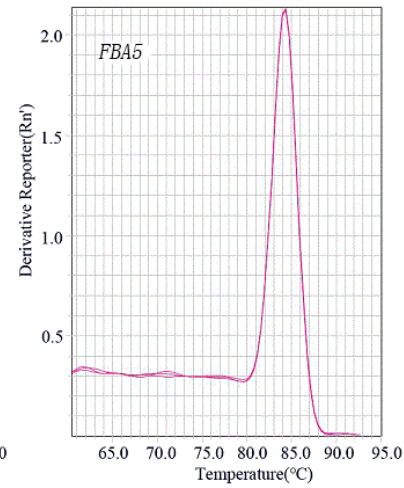
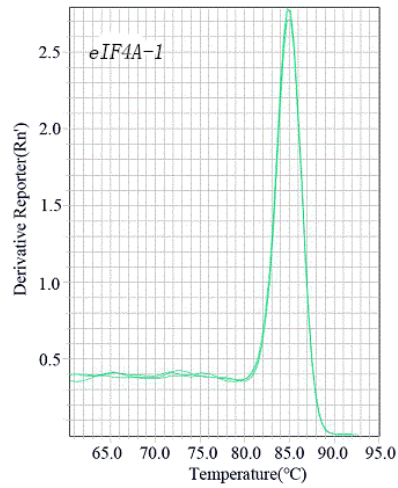
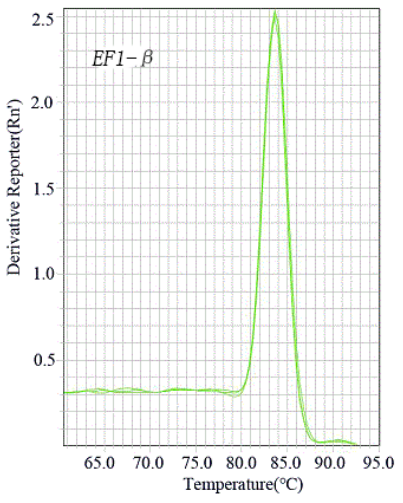
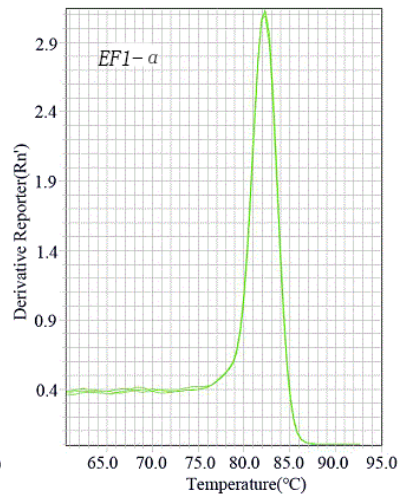
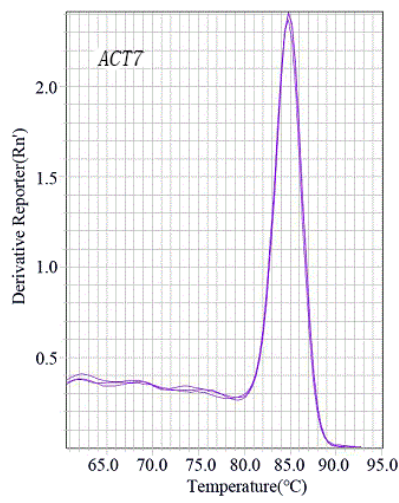
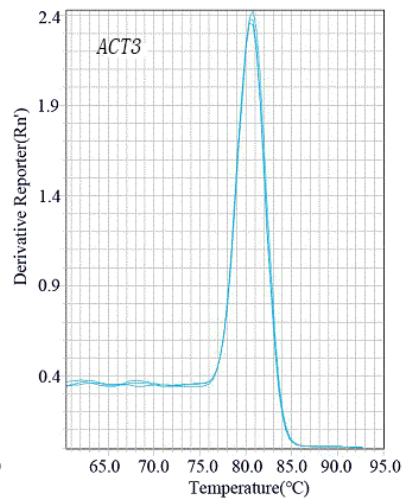
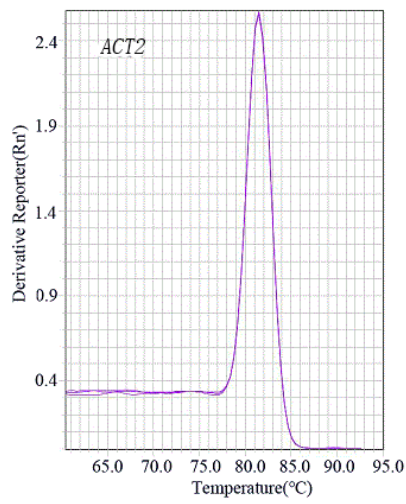
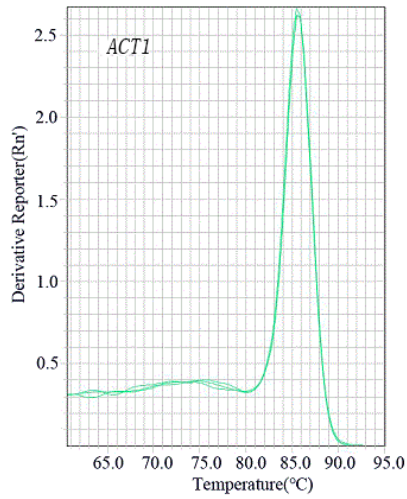


Fig. 1 Suppl. Agarose gel electrophoresis of 15 candidate reference genes for PCR specificity verification. M - DL2000 Marker; 1 - 15 were *ACT1*, *ACT2*, *ACT3*, *ACT7*, *TUA2*, *TUB6*, *EF1-α*, *EF1-β*, *GAPDH*, *HIS*, *KIN*, *eIF4A-1*, *FBA5*, *SAMDC* and *UBQ* PCR products.



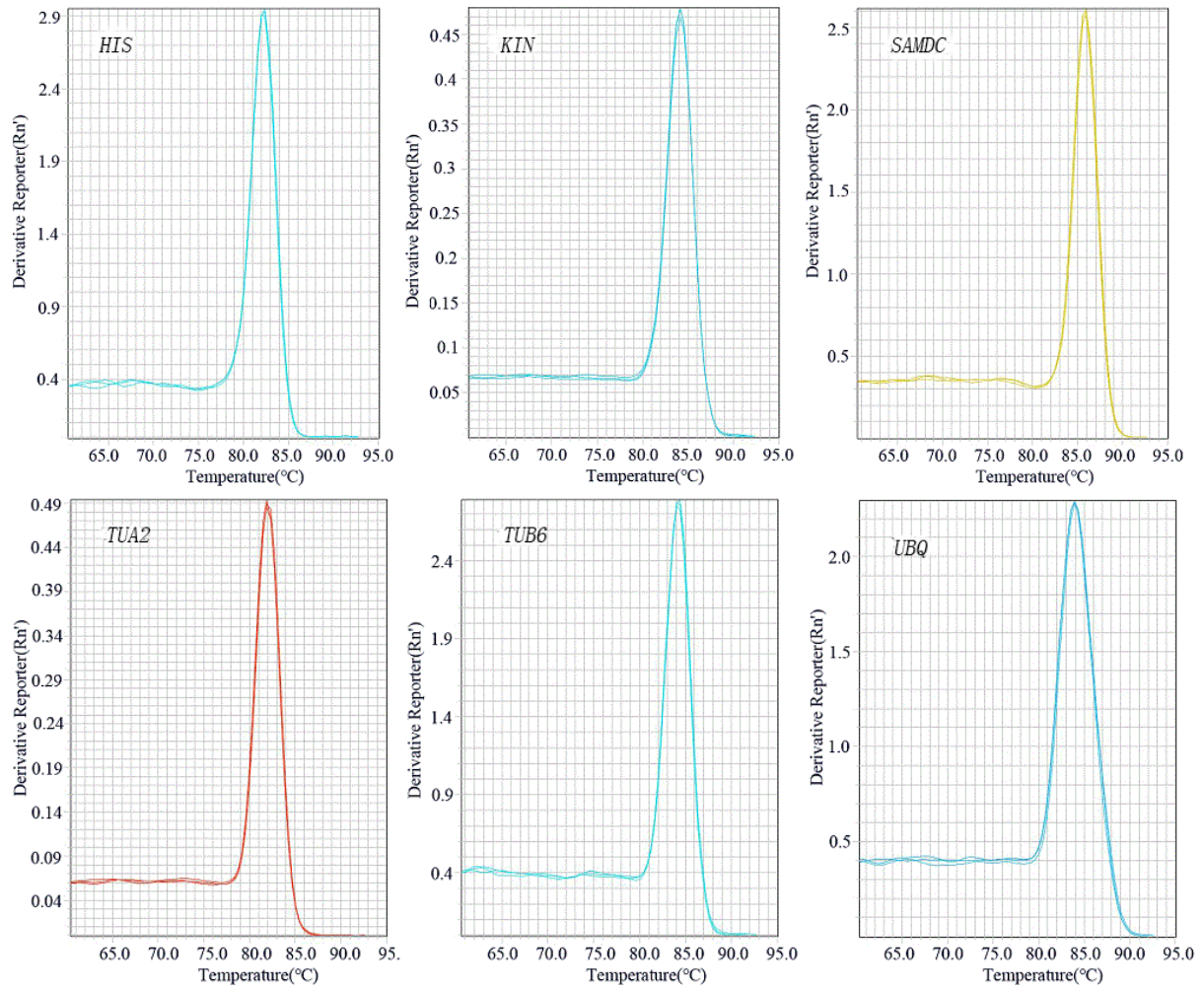


Fig. 2 Suppl. Melting curve of the chosen 15 reference genes.