

Supplementary materials:

Genome-Wide Screening and Stability Verification of the Robust Internal Control Genes for RT-qPCR in Filamentous Fungi

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Table S1. The 10 samples of *Flammulina filiformis* and 8 samples of *Neurospora crassa* involved in this study

NO.	Materials	Abbreviation	Notes
1	Mycelia	MY	Heterokaryon
2	Aerial hyphal knot	AHK	Heterokaryon
3	Primordia	PR	Fruiting body of strain
4	Bud	BUD	Fruiting body of strain
5	Pileus in young fruiting body	YFP	Fruiting body of strain
6	Stipe in young fruiting body	YFS	Fruiting body of strain
7	Pileus in elongation stage	ELP	Fruiting body of strain
8	Stipe in elongation stage	ELS	Fruiting body of strain
9	Pileus in maturation stage	MAP	Fruiting body of strain
10	Stipe in maturation stage	MAS	Fruiting body of strain

NO.	Abbreviation	Treatment condition
11	WT	The temperature of 25°C
12	ΔSpds	The temperature of 25°C
13	BT	The temperature of 25°C, the blue light treatment for 3 d, and the light intensity of 3 μmol·m ⁻² ·s ⁻¹
14	BS	The temperature of 25°C, the blue light stress for 3 h, and the light intensity of 3 μmol·m ⁻² ·s ⁻¹
15	CS	Cold stress 4 °C, cultured for 30 min
16	HS	Heat stress 42 °C, cultured for 30 min
17	JA	The concentration of 0.01 mmol/L
18	H ₂ O ₂	The concentration of 5 mmol/L

The two different combinations of sample sets:

A	<i>F. filiformis</i> samples	Samples of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10
B	<i>N. crassa</i> samples	Samples of 11, 12, 13, 14, 15, 16, 17 and 18

Table S2. The corresponding accession number of 12 ICGs *F. filiformis* and *N. crassa*

Gene symbol	Gene name	Accession number in Ff-L11	Accession number in Nc-OR74A
<i>ACTB</i>	β -actin	OP354401	NCU04173
<i>beta-TUB</i>	β -tubulin	OP354402	NCU04054
<i>GAPDH</i>	Glyceraldehyde 3-phosphate dehydrogenase	OP354403	NCU01528
<i>SPRYp</i>	SPRY-domain-containing protein	OP354404	NCU03678
<i>Ras</i>	Ras-2 protein	OP354405	NCU03616
<i>Vps26</i>	Vacuolar protein sorting protein 26	OP354406	NCU02743
<i>Cwf15</i>	Pre-mRNA-splicing factor cwc15	OP354407	NCU00335
<i>DnaJ</i>	ER associated DnaJ chaperone	OP354408	NCU03335
<i>HUL4</i>	E3 ubiquitin-protein ligase NEDD4	OP354409	NCU03947
<i>VAMP</i>	ATP-binding cassette, subfamily B (MDR/TAP), member 1	OP354410	NCU08956
<i>RNB</i>	Exosome complex exonucleaseDIS3/RRP44	OP354411	NCU01197
<i>V-ATP</i>	V-type H ⁺ -transporting ATPase subunit A	OP354412	NCU01207

Table S3. Descriptions of 12 ICGs candidates and the primers used in *F. filiformis*

Gene symbol	Gene name	Primer	Sequences (5'-3')	Product size (bp)	Annealing temperature (°C)
<i>ACTB</i>	β -actin	F5510QF F5510QR	GATCGTATGCAGAAGGAGTTGACAC CCACTCTCGTCGTACTCTTGCTTG	211	62
<i>β-TUB</i>	β -tubulin	F346QF F346QR	CAAATGCAGAACGTCCAGAAC GTGAACTCCATCTCGTCCATAC	242	62
<i>GAPDH</i>	Glyceraldehyde 3-phosphate dehydrogenase	F5082QF F5082QR	GTTTCCGTTGTTGACCTTGTGTGCC CGAAGTTGCCGTTGAGCTGGATAC	118	62
<i>SPRYp</i>	SPRY-domain-containing protein	F5226QF F5226QR	CACAGTAATATCTCCAGACGCGATC TCCTACCACTCCTTCGCCTGTTG	237	62
<i>Ras</i>	Ras-2 protein	F5609QF F5609QR	TCAATGCGACGAGTAAAGAGAGG CATAGGTCCCACATCTACATTTCTG	233	62
<i>Vps26</i>	Vacuolar protein sorting protein 26	F898QF F898QR	CGAGGCGAAACTATCCCAATAC TATCGTCTGTTCTCCTCGTCTAC	134	62.5
<i>Cwf15</i>	Pre-mRNA-splicing factor cwc15	F7920QF F7920QR	CTGAAAGAGCTGGAGAAGATCAA CAGCGTCTCTTTACGGAGAAA	206	62
<i>DnaJ</i>	ER associated DnaJ chaperone	F2761QF F2761QR	CTCATCCTCTTTGCCCTTTCA CTGTCGGGTTGACGAAGTATT	157	62
<i>HUL4</i>	E3 ubiquitin-protein ligase NEDD4	F6860QF F6860QR	CCGAGGAGAACAAGAAGGAATAC ACTTGGTCCAATCGTCCATATC	198	62
<i>VAMP</i>	ATP-binding cassette, subfamily B (MDR/TAP), member 1	F7311QF F7311QR	GAGAAGATCAAAGAGGCTGAGG GAAGAGGTAGGTCTGTGATGAATAC	246	62
<i>RNB</i>	Exosome complex exonuclease DIS3/RRP44	F2240QF F2240QR	TTGTTCTCTGTGTTCTCATCTC GACATCCTTCGTGGACCAATAG	216	62
<i>V-ATP</i>	V-type H ⁺ -transporting ATPase subunit A	F5791QF F5791QR	GCAGAAATGGTATGAGCGAGTAT TCTGGGACAGCTTGAACATAAG	173	62

Table S4. Descriptions of 12 ICGs candidates and the primers used in *N. crassa*

Gene symbol	Gene name	Primer	Sequences (5'–3')	Product size (bp)	Annealing temperature (°C)
<i>ACTB</i>	β -actin	NCU04173QF NCU04173QR	CTCCATCATGAAGTGCGATGT TTCATGGAAGAAGGAGCAAGAG	138	56
β - <i>TUB</i>	β -tubulin	NCU04054QF NCU04054QR	GTCTCCATGAAGGAGGTTGAG GAAGGTGGAGGACATCTTGAG	141	58
<i>GAPDH</i>	Glyceraldehyde 3-phosphate dehydrogenase	NCU01528QF NCU01528QR	GGTTGTCTCTCCGACATGAA GCATCGACCTTGGAGATGTAG	168	57
<i>SPRYp</i>	SPRY-domain-containing protein	NCU03678QF NCU03678QR	TTCGAGCAAGGCAGTATTC TGATCGTGTGCGGTTATGAG	151	55
<i>Ras</i>	Ras-2 protein	NCU03616QF NCU03616QR	CCTGTGCCGATTATGCTAGTG TCAACATTTGTGCGTGTCTTTG	140	56
<i>Vps26</i>	Vacuolar protein sorting protein 26	NCU02743QF NCU02743QR	CCGCATCTACTTCTTGCTTGTA GGAATGGTCTCTCCTCTTGATG	162	57
<i>Cwf15</i>	Pre-mRNA-splicing factor cwc15	NCU00335QF NCU00335QR	AGACTCCTCCGATTCTGATTCC CTTCTCGCGCTGTTCTTCTTC	157	58
<i>DnaJ</i>	ER associated DnaJ chaperone	NCU03335QF NCU03335QR	CAAACGACGTCAGGCATTATTC GGCCAACCTTCTCTTGATCTGTA	170	56
<i>HUL4</i>	E3 ubiquitin-protein ligase NEDD4	NCU03947QF NCU03947QR	GGTAGTGATGGTCCTCGTAGA CATAGTCTCCTCCACAGCAATC	156	58
<i>VAMP</i>	ATP-binding cassette, subfamily B (MDR/TAP), member 1	NCU08956QF NCU08956QR	CCATCGCATCTCTCCAGAAC GAAGATATAGGCCAGCAGGAAG	203	58
<i>RNB</i>	Exosome complex exonuclease DIS3/RRP44	NCU01197QF NCU01197QR	CTCTGACATCAGAAAGGGTATGG GGGTACGTGAAATGAGTGTAGAG	141	58
<i>V-ATP</i>	V-type H ⁺ -transporting ATPase subunit A	NCU01207QF NCU01207QR	GATTTCTCTGATCCCGTCACTTC GGTACTCCCTCTCATACCACTT	163	58

Table S5. Raw Ct values of *ACTB* and *β -TUB* genes of *N. crassa* in WT strains on LC96 and CFX96 instruments

Gene	<i>ACTB</i>		<i>β-TUB</i>	
Instrument	LC96	CFX96	LC96	CFX96
Raw Ct value	27.25	27.18	22.51	24.55
	27.13	27.93	22.13	23.77
	27.06	27.12	21.94	24.05
	28.59	28.60	23.86	22.99
	27.94	28.69	23.66	23.32
	27.87	28.59	23.18	22.79
	28.01	28.98	21.98	22.42
	28.16	29.05	21.76	23.15
	28.22	29.04	22.44	22.68
Mean Ct	27.80333333	28.35333333	22.60666667	23.30222222
Standard deviation	0.505261209	0.719320667	0.732378166	0.656908209
P value	0.0958		0.0628	

Notice: LC96: LightCycler® 96 Instrument; CFX96: Bio–Rad CFX96 Real-Time PCR Detection System. *P* values were derived from unpaired t tests.

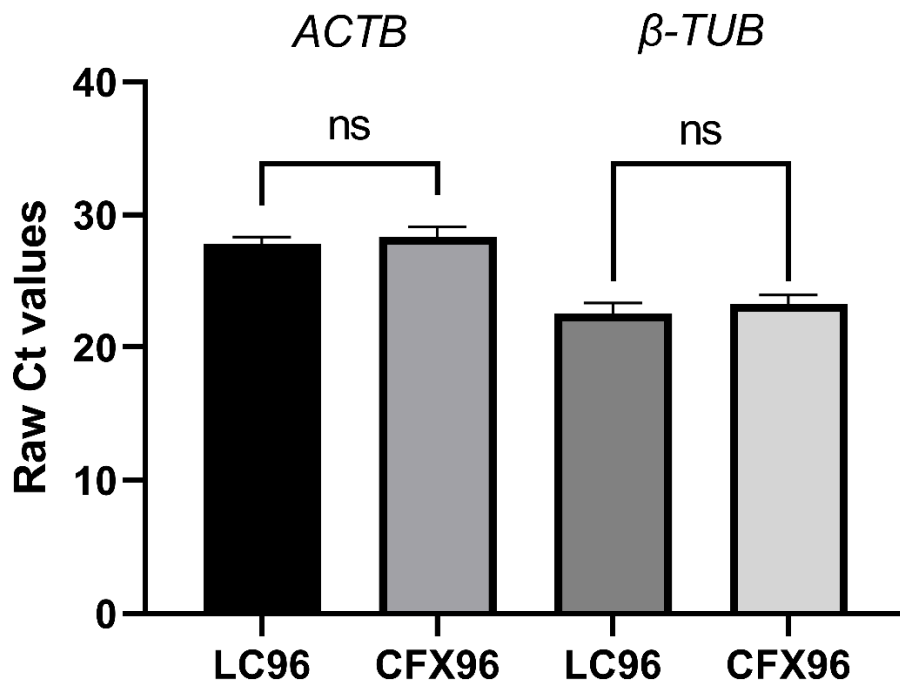


Figure S1. Raw Ct values bar graph of *ACTB* and *β-TUB* genes of *N. crassa* in WT strains on LC96 and CFX96 instruments, respectively. Average Ct value of three biological replicates and three technical replicates were shown on the Y-axis. The two different instruments LC96 (LightCycler® 96 Instrument) and CFX96 (Bio-Rad CFX96 Real-Time PCR Detection System) were shown on the X-axis. The values are the means \pm SD of three independent experiments. (Student's t test: ns: no significant).