

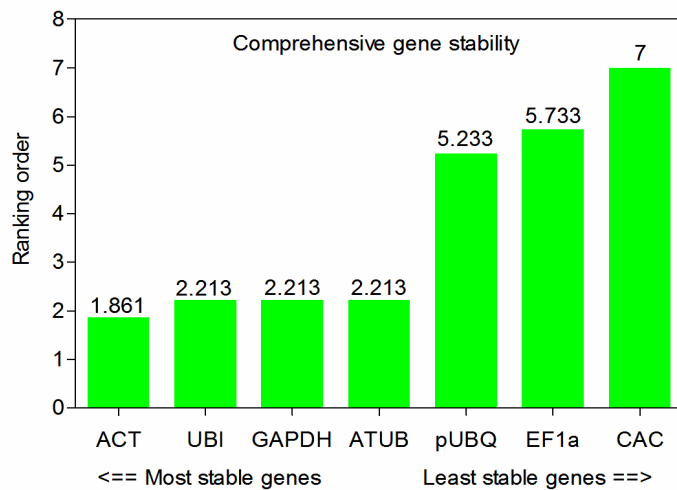
RT-qPCR assay details

Primer for candidate reference genes

Gene ID*	Gene symbol	Name	Oligo sequence (5' – 3')	Amplicon size (bp)	E	R ²	Reference
MA_10427661g0030	ACT	actin	F: TGAGCTCCCTGATGGGCAGGTGA R: TGGATACCAGCAGCTTCCATCCCAAT	104	0.9	0.998	Yakovlev et al. 2006
MA_93486g0010	ATUB	tubulin α -3 chain	F: GGCATACCGGCAGCTCTTC R: AAGTTGTTGGCGGCGTCTT	66	0.99	0.996	Yakovlev et al. 2006
N/A	CAC	clathrin adaptor complex subunit	F: ACGGAATAATGATGTGCTAA R: CCCTCTTGTTGATGTAAA	108	n.d.**	n.d.**	de Vega-Bartol et al. 2013
MA_434977g0010	EF1a	elongation factor 1- α	F: GGATTGCCACACTTGCCACA R: CTTGGGTTCTTCTCCAGTTCC	94	0.91	0.999	Yakovlev et al. 2014
MA_130835g0010	GAPDH	glyceraldehyde-3-phosphate dehydrogenase	F: TGCTGCAAAGGCAGTTGGAAGG R: GCGACATGTCAGATCCACGACTG	110	0.92	0.994	Yakovlev et al. 2006
MA_14427g0010	pUBQ	polyubiquitin	F: TGGTCGTACTCTGGCCGATTATA R: ACACCTAGCGGCACACAGTTAA	97	0.9	0.998	Yakovlev et al. 2006
MA_8884419g0010, MA_10435699g0020	UBI	ubiquitin	F: GTTGATTTTTGCTGGCAAGC R: CACCTCTCAGACGAAGTAC	101	0.91	0.997	Schmidt and Gershenzon, 2007

*ConGenIE database (<http://congenie.org/>); F and R: forward and reverse primer; E: amplification efficiency; R²: correlation coefficient of standard curve; **not determined (Cq values out of quantitative range or not detected)

RefFinder (Xie et al. 2012) ranking of candidate reference genes



Primer for genes of interest

Gene ID*	Gene symbol	Name	Oligo sequence (5' – 3')	Amplicon size (bp)	E	R ²
MA_10204459g0010	CALM	calmodulin	F: GCGTTGAAGGAGGCATTTA R: CGGTGATGGAGGAGAAGAA	91	0.999	0.994
MA_10280648g0010	CALM	calmodulin	F: GCTGAAGGAGGCGTTTAAT R: CGATGAAGGAGGAGGAGAA	89	0.899	0.999
MA_52889g0010	CALM/CML	calmodulin / calcium-binding protein CML	F: AAATTGCGGAGTTCAGAGAG R: CCAGCTCCTTGGTTGTTATG	75	0.963	0.980
MA_10313114g0010	CHIB	basic endochitinase B	F: CCACGGACGAGGACCTATT R: CGGGTTGTTTATGAGATCGTATCC	88	0.971	0.990
MA_8921185g0010	CHIB	basic endochitinase B	F: CCGCATCGGGTTCTACAAA R: GGCCTCTGGTTGTACAGTC	81	1.07	0.992
MA_5735g0010	CHS	chalcone synthase	F: CTGCCCAGACAATTCTTCC R: GTAAGTCCGACCTCTCTCAA	67	0.999	0.990
MA_10431324g0020	CML	calcium-binding protein CML	F: TCATACTTGGGACCTCTCTC R: CAGTAACCCTTGTACGTCTTC	88	0.999	0.993
MA_115550g0010	CPK	calcium-dependent protein kinase	F: TCGTGGAGGAGAGACATTTA R: CATCAGCTTCAGGACCATAAT	92	0.848	0.994
MA_9458g0010	CPK	calcium-dependent protein kinase	F: GACTAACAGCTCAGCAAGTG R: CCCAAGAGGAACATTAGGAAC	74	1	0.994
MA_35694g0010	CTR1	serine/threonine-protein kinase CTR1	F: GGATTGGAGCAGGTTCTTT R: CTTCGTGGAAGTCTTGTTCTAT	96	0.985	0.993
MA_76780g0010	CYP75B1	flavonoid 3'-monooxygenase	F: GCAACCCACAGATGATGAA R: GACTCCTTTAGCGTCCTTTG	79	0.981	0.958
MA_10431201g0020	EDS1	enhanced disease susceptibility 1 protein	F: AAGAGGATGTAACAAACATGGA R: CGATCACGCACATAATGAAAC	87	0.918	0.994
MA_101621g0020	EDS1	enhanced disease susceptibility 1 protein	F: AAGTTGATACGGAATCCTCTTAC R: TTCAATTTCTCCTGCTACTTGA	101	0.999	0.997
MA_20516g0010	EIN3	ethylene-insensitive protein 3	F: CAAGAGCAAGCGAGAAGAA R: GAGCCTTACAAACCTCCATC	88	0.988	0.990
MA_10434030g0010	HSP90A	molecular chaperone HtpG	F: TAGCCTCATCATCAACACTTT R: TTTATCCGTTAGCCCTTCG	112	1	0.996
MA_12191g0010	HSP90A	molecular chaperone HtpG	F: GTCTGATCTCGTGAACAACCT R: CAAGTGCTTCCATAAACTCTTTG	68	1.05	0.999
MA_10431770g0010	HSP90B	heat shock protein 90kDa beta	F: CTTCGGGAGCTTGTTAGTAATG R: TCTCTAAGTCACCACCATCTC	100	1.06	0.998
MA_10431031g0010	HSP90B	heat shock protein 90kDa beta	F: CGACACCAAGCTCGAAAT R: CCACGATCTCGGATTGAAAG	69	1.05	0.998
MA_10194g0020	MKK9	mitogen-activated kinase kinase 9	F: CCTGATGGGTATGGTGGAAAG R: GCGCACACTCCAGTAAAGATA	79	1.05	0.999
MA_10437020g0010	MPK6	mitogen-activated protein kinase 6	F: TCCCTCCGCTATTTCTTATT R: GCAACTTGCTCATTTGGTTTC	82	1	0.988
MA_10435905g0020	MYC2	transcription factor MYC2	F: TGGCCAGAGGAACTTTGAG R: CAGAGTTCAGGGACTGGATTTTC	100	0.856	0.991
MA_53673g0010	PR1	pathogenesis-related protein 1	F: CGTGATGGCTTGGGTAAAC R: TCTCCACACCACTTGAGTATAG	100	0.999	0.986
MA_133206g0010	SGT1	suppressor of G2 allele of SKP1	F: CCTCGGCGAACATTAAACTC R: GGTATGCCTTTGCCAGATTAG	93	1.12	0.991

*ConGenIE database (<http://congenie.org/>); F and R: forward and reverse primer; E: amplification efficiency; R²: correlation coefficient of standard curve

References:

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- Yakovlev IA, Fossdal C-G, Johnsen Ø, Junttila O, Skrøppa T: Analysis of gene expression during bud burst initiation in Norway spruce via ESTs from subtracted cDNA libraries. *Tree Genetics and Genomes* 2006;2;39-52.
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