

Running head: Functions of fungal Cdc14

Cytokinesis-required Cdc14 is a signaling hub of asexual development and multi-stress tolerance in *Beauveria bassiana*

Jie Wang, Jing Liu [†], Yue Hu [†], Sheng-Hua Ying and Ming-Guang Feng ^{*}

Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, 310058, People's Republic of China

[†] These authors contribute equally to the study.

* Corresponding author: M.-G. Feng, Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, 310058, People's Republic of China. Phone and fax: 86-571-88206178. E-mail: mgfeng@zju.edu.cn

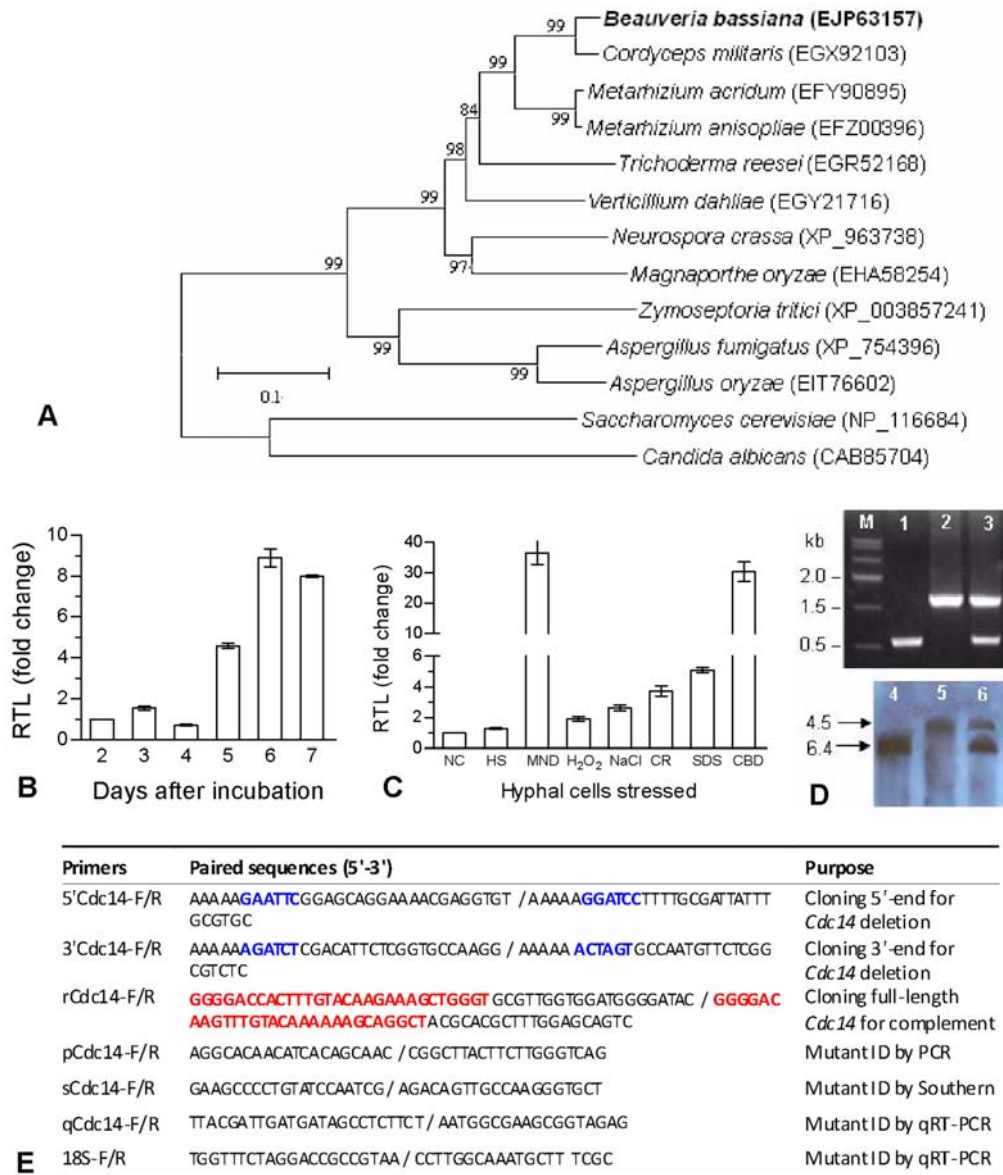


Fig. S1. Phylogenetic and transcriptional features of *B. bassiana* *Cdc14* and creation of its disruption and complemented mutants. (A) Phylogenetic tree for *Cdc14* orthologues in fungal and yeast species (NCBI accession codes in parentheses). Scale bar: branch length proportional to genetic distance assessed with neighbor-joining method. (B) Relative transcript levels (RTL) of *Cdc14* (assessed via qRT-PCR) in wild type during 7-day growth (versus day 2) on SDAY at 25°C. (C) RTL of *cdc14* in wild-type cultures grown for 3 days on 1/4 SDAY supplemented with menadione (MND, 0.2 mM), H₂O₂ (2 mM), NaCl (0.8 M), Congo red (CR, 0.5 mg/ml), SDS (0.2 mg/ml) and carbendazim (CBD, 0.5 µg/ml) at 25°C respectively or exposed for 1 h to heat shock (HS) at 40°C (NC: normal culture not stressed). Error bars: SD. (D) Identification of *Cdc14* disruption and complement mutants via PCR (lanes 1–3) and Southern blotting (lanes 4–6). Lanes 1 and 4: wild type. Lanes 2 and 5: $\Delta Cdc14$. Lanes 3 and 6: $\Delta Cdc14/Cdc14$. (E) Paired primers used for the manipulation of *B. bassiana* *Cdc14* and the identification of its mutants (Blue: the sites of restriction enzymes *EcoRI/Bam*HI and *Bgl*II/*Spe*I. Red: fragments for gateway exchange).

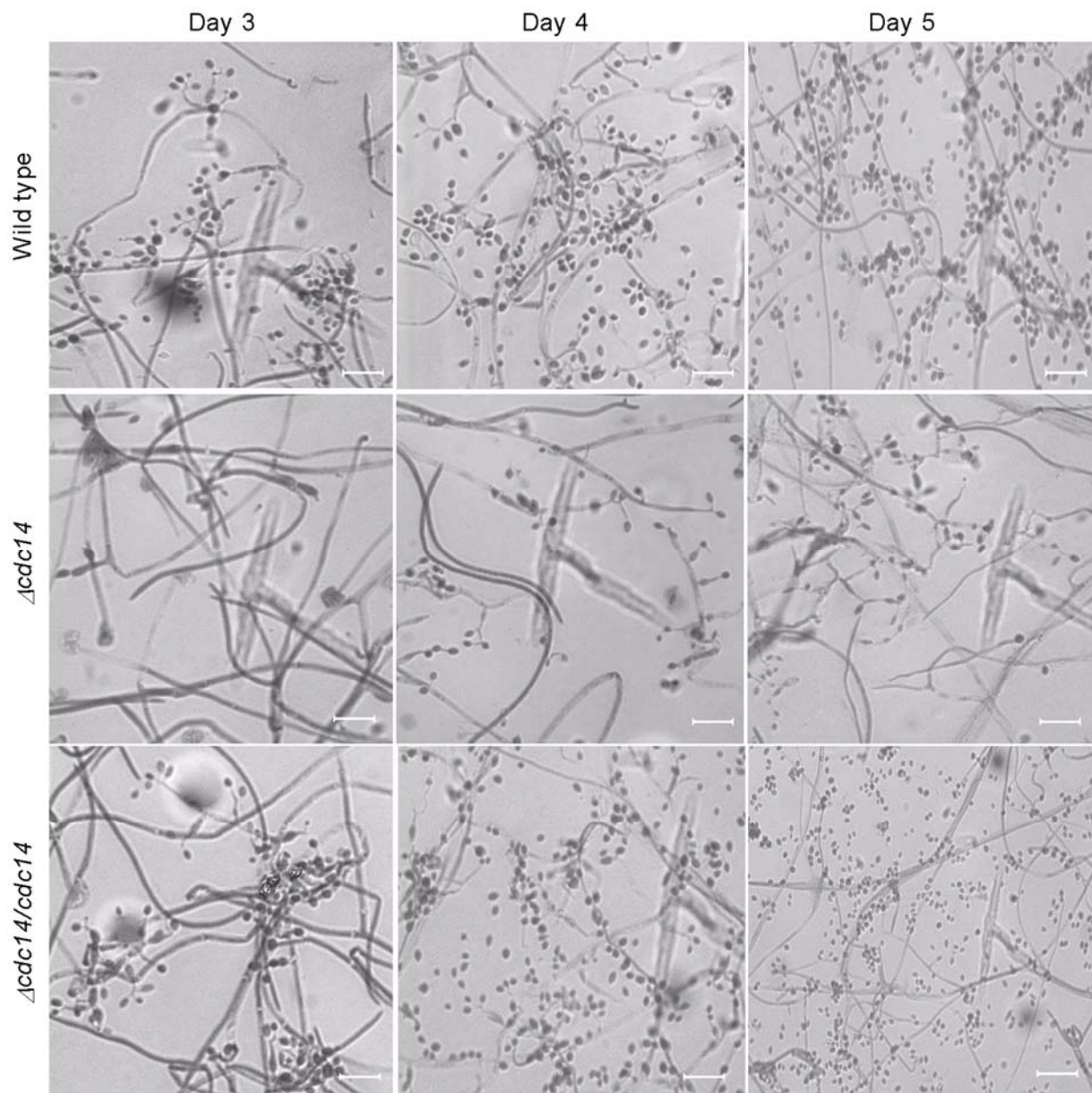


Fig. S2. Morphological comparison of colony samples taken during the conidiation of *B. bassiana* wild-type strain and *cdc14* mutants grown on SDAY plates at 25°C. Scale bars: 10 μ m.

Table S1. Paired primers used for assessing the transcript levels of 88 genes in the *Cdc14* disruption and complement mutants versus wild type of *B. bassiana* via qRT-PCR.

Tag code*	Gene	Annotation	Sequences (5'-3') of paired primers
<i>Involved in cytokinesis</i>			
BBA_08543	<i>wee1</i>	WEE protein kinase	CTCGTCTACCAGCATCTT / CACCTGTTCAACCTTGTGTC
BBA_07180	<i>mid1</i>	calcium channel subunit	ATGAATTACACAGACAAG / ACATATCGGTGATAGTAG
BBA_01304	<i>ace2</i>	transcription factor	CAAGAAGAAGACATTATGC / ATCATCATTCTGGAACAT
BBA_03872	<i>eng1</i>	endo-1,3-beta-glucanase	AACACCTACTACAATGAT / TAGTCCTTGTCTTATCG
BBA_01202	<i>dse1</i>	WD domain-containing protein	ATCTCTACTGTTATTGTAAG / ATGATAATATCGGTGTCT
BBA_01489	<i>slil5</i>	inner centromere protein	CTCCAGATTGTTCAAGCAGT / GTTTCGTTCTCATCAATATAC
BBA_00440	<i>ask1</i>	subunit Ask1	CAAGCAGAAATATGAAATCG / TTTGTAATAGTTGGATGGT
BBA_05079	<i>ase1</i>	microtubule associated protein	AAGAAGGAAATGATTGAC / GGATAAGTTATTGTGATGT
BBA_03315	<i>cdc15</i>	CDC15 protein kinase	AATCTACCAACTGCCTCAAC / GTCATATACACGCCAACAAAG
BBA_03150	<i>mob1</i>	Mob1 family protein	ATGAAGGCTACTGACGAATT / AGGTCATCAACTGCTCAAT
BBA_08042	<i>plo1</i>	serine/threonine-protein kinase	CATCATCTCTTCAAGACAGT / ATCGTTGAGGATATAGCCAAG
BBA_03681	<i>spg1</i>	septum-promoting GTP-binding protein 1	ATCAAGGAGTGGTATAGAC / ATCTTGGATTGAAAAGTTG
BBA_02370	<i>sid2</i>	serine/threonine-protein kinase	CGACAGGAGTATCTATCTTG / AGAACATTTCCGCAATATAG
BBA_04438	<i>cbk1</i>	Serine/threonine-protein kinase	CTTCCAGGACACTTACTTT / AATGTAGAAGCGAGTTATGT
BBA_06124	<i>cts1</i>	CTP synthase	TATCTTGGCATTCTGCTCAG / CGGTCTTCCCTAAATAGTCTC
BBA_08017	<i>scw11</i>	cell wall glucanase	AACATTTGGCAGCAGACT / TTGAAGAAGCGTGGATG
BBA_10084	<i>hym1</i>	conidiophore development protein hymA	AAGACCTTGTCCACGATAT / TTAATAGACTGACGCTTGGT
BBA_04198	<i>tao3</i>	cell morphogenesis protein	CCTTGTCAAATATCTCATT / ATCTGTAATATGGATGTTG
<i>Involved in growth and asexual development</i>			
BBA_04942	<i>fluG</i>	developmental protein	CCTCCCTAGTTTGGTCGCTTCTC / CGTGTGCGGTAATCTGCTCCTC
BBA_02968	<i>flbA</i>	developmental regulator	CCAATCCACTCGCCGCTCTC / CGGAGGAAAGAGAATCGGTAGAGG
BBA_06988	<i>flbB</i>	bZIP transcription factor	GCACTGACACGCCACAAGAGC / CCGCCGCCGAAGCCTGTTG
BBA_03181	<i>flbC</i>	C ₂ H ₂ conidiation transcription factor	TCCATCTCCAACCTGCTGGTCTC / GGCGCGTAGGCGGAAGG
BBA_07259	<i>flbD</i>	MYB conidiophore development protein	CGGCAAGCGATGGGCAGAGATTG / ACGAGCAAGGTGACGGTAGAGGTG
BBA_06599	<i>hyd1</i>	hydrophobin	TTCTCAGCGATCTTGATCTT / GCACTTGTGTGCGATTGG
BBA_03015	<i>hyd2</i>	class I hydrophobin	CATGGTGGAAGGATCTG / ATCTTGGTCGCTTCTCG
BBA_10084	<i>hymA</i>	conidiophore development protein	TCGCAAGATGGTTCAGTA / GGACAAGAAGTAAGAAGTT
<i>Involved in response to oxidative stress</i>			
BBA_02311	<i>sod1</i>	Cu/Zn superoxide dismutase	GCGGCTTCCACATCCACACCTTTG / GGTCCAGCGTTGCCAGTCTTGAG
BBA_09706	<i>sod2</i>	cytosol Mn-superoxide dismutase	CCAGTGTGGCATTGACATG / TCAGCCGCTTCCAGTTGATG
BBA_09382	<i>sod3</i>	mitochondrial Mn-superoxide dismutase	TCTCCGGCAAGATTATGGAGC / TTGGCGTCACTTCTGGCCT
BBA_04317	<i>sod4</i>	superoxide dismutase	CGAGATGGCTTACGGCTTCTCAG / GCTCCCAGGTGTTGAGGCATAG
BBA_01984	<i>sod5</i>	superoxide dismutase	CGGCGACCTCAGCGCAAGTAC / GCCAGCAACAACAGGGACCGTAGG
BBA_05603	<i>cat1</i>	catalase B	CCTCTGACGTTGGCGCCCTTTC / CCGTGTCCGTGCTGCCTCGTG
BBA_06186	<i>cat2</i>	catalase A	CCGTCTGGGCATCAACTGGGAAG / GCTGGGCGTGGTCTGGTATG
BBA_06567	<i>cat3</i>	Catalase	TCAAGTCGGTTCAGGAGATGGAG / TTGTTGCTCTTCAATCGGAGTG
BBA_09109	<i>cat4</i>	catalase C	GAGGAGCCCAGCAACGCACAAGAG / CTGAGGACGACAAGGCCCCATTC
BBA_09338	<i>cat5</i>	catalase P	GCTGGGCTGATCTGCTGGTCTTCTG / TCCTTGTCTGTAACGGTGGCTGTCTG
BBA_09760	<i>cat6</i>	catalase D	CGGCTCGGTGTCTTGTCCATAC / CCTTGTGCGGCTTCTGGCGAAG
BBA_08024	<i>pod1</i>	cytochrome c peroxidase	GCCGCCGCTTCTACTCGTCTG / CGTTGCTGCCGCCAGTACCG
BBA_00792	<i>pod2</i>	peroxidase	CCGCCAGAATGCCGCCGAGTG / GAATGATGGGTCCGCCGAGGAG
BBA_08023	<i>pod3</i>	NADH oxidase	CACACGCACCGACGACTACG / GTTCCGCCGCTGCTGAC
<i>Involved in cell wall integrity</i>			
BBA_09307	<i>chn1</i>	acidic chitinase endochitinase 1	TGACAACTCGCTTATCAGT / TTATCATACTTGGTCTCAGGAT
BBA_05353	<i>chn2</i>	class III chitinase	GCAATTTGACTACCTCTG / CGTATTGGCAATGTTCTT

BBA_02017	<i>chn3</i>	chitinase 3	CGGCTCATCATACTACC / AATGTCGGGCTGCGTAAT
BBA_06297	<i>chn4</i>	class III chitinase, chitinase 4	TGAATGGCATAAATCCTG / CGTCTTGATGTCCTCTTC
BBA_09585	<i>chn5</i>	chitinase 5	AATCTGGTTACTGTGGTT / TTTGGAGGATCTGAGAAC
BBA_09259	<i>chn6</i>	symbiotic chitinase	CAGATGTGAATAATCCAATG / AAGAATATGAGCCAAGTTAG
BBA_02230	<i>chn7</i>	chitinase	GTATCAACTACCTCAAGT / TCATATAGACAAATGTAATCG
BBA_08982	<i>chn8</i>	class V chitinase	TAATGCTAATGCCACTTATG / AGAGATATTGATGCGTTTGT
BBA_00297	<i>chn9</i>	class V chitinase	TACATCTCCAATGCTGAAATC / CCACTGGTCTTGTCGTAT
BBA_03814	<i>chn10</i>	chitinase	AATGTATACTCTACCGTCAG / CTTGCTGGAAATGTAACCTT
BBA_06317	<i>chn11</i>	chitinase	GACCATTGTGCCGATTCT / GGTGGACAGGGTGTATTATG
BBA_04667	<i>chs1</i>	chitin synthase 1	ACCTATATCAACATTCTC / GACAATCTCATTCTTCTC
BBA_03236	<i>chs2</i>	chitin synthase 2	TGTGTCTTACTGATTGCT / TAATGATGGCGTAGATGAT
BBA_03590	<i>chs3</i>	chitin synthase 2	TCTTCAACACCGTCAACAT / TCCTACCGAGCAAGTCTT
BBA_08043	<i>chs4</i>	chitin synthase 4	CAGTCAGATTATCCTTATGTCAT / GATATACCAGTCACCTTCC
BBA_02360	<i>chs5</i>	chitin biosynthesis protein	ATGAGAGCGTGATTGAAG / CTGCTTTTCTTTTCTC
BBA_06858	<i>chs6</i>	chitin synthase 6	TGGAATTGTATCAAGAACT / GAGAATAATGGCAGAGAT
BBA_03793	<i>chs7</i>	chitin synthase chaperone-like protein	CGTAATGATGGTCTACAAG / CTCCTCAGGAAGTAACTC
BBA_06859	<i>chs8</i>	class V chitin synthase	GACTACAAGAACAACAAC / ACGAGTATCATTGGTATAG
BBA_08396	<i>chs9</i>	chitin synthase	CAACAAGAAGAAGCCAAG / ATAATGACGAGTCCACTAAT
BBA_07346	<i>chs10</i>	class VII chitin synthase	CCTCTTTATTCTTTTATCG / GGTTGTTGATGTAATGAC
BBA_06846	<i>chs11</i>	class VII chitin synthase	GGAGATTATGGAAGCGAT / TAGACAATAGTGCGATGAC
BBA_06144	<i>mhp1</i>	cell wall biogenesis protein	GGACCAACTCCATACCACAAGC / GCTCACCGATAGGACACCGTTCTC
BBA_06689	<i>ssd1</i>	cell wall biogenesis protein phosphatase	GCTCTCCCACCGTCGCAACCAATC / CACCTCCACCACCCCTTCCACCTC

Involved in response to osmotic stress

BBA_08887	<i>sho1</i>	high osmolarity signaling protein	AGCGTCAACAACCTCGTCTAC / AGGCGTGGAGTTCTCAAAG
BBA_02191	<i>sln1</i>	sensor histidine kinase/response regulator	TCGGTGCCATCACTCCTTCCAACG / GCGGACGAGCAATGCCAACAGC
BBA_02621	<i>ssk1</i>	two-component response regulator	AGAACTTTAGCACCGAGCCCTTTC / AGAAGCAGCAGCAGACGATTGG
BBA_07057	<i>ypd1</i>	phosphotransmitter protein	TGACCAGGTCGAGGAGACGTTTGC / TCGGCGTCGGGTTCTGATGAGC
BBA_00971	<i>msn2</i>	stress response element binding protein	GCCCCCAGCCCATCTAC / ACCGAGGTCTCAACCGAGTCAAAC

Involved in signal transduction pathways

BBA_05916	<i>pkA</i>	cAMP-dependent protein kinase subunit	GCATCCGAGCAGCAGAAC / GCTTGACTGAACGAGGTGAAC
BBA_07006	<i>pkC</i>	protein kinase C	ATTGAAGAGATATGAAGAAC / AAGATTCCGAGTATTGAT
BBA_04261	<i>snf1</i>	Protein kinase	GCGAGAGGAACATATAGC / GGATTACGACTAGCATT
BBA_04387	<i>ras1</i>	Ras GTPase protein	GGCGGTGGCGTGGTAAATC / TGCGGGGAGGTGATGGAGTAG
BBA_04671	<i>ras2</i>	Ras GTPase protein	CCTCGGTGACGGTGGTGTAG / GACTCCTTGACTCGTGAATCTG
BBA_01318	<i>bck1</i>	MAP kinase kinase kinase (Bck1)	GACGCCTGATTACCTCTAACAC / CCTGACGCTGATTCTTGGAC
BBA_00937	<i>sskB</i>	MAP kinase kinase kinase (SskB)	GCAGAAGCACTCGGCACCATTTTC / GCGTTGCGGAAGAGGATCGGACTG
BBA_02280	<i>ste11</i>	MAP kinase kinase kinase (Ste11)	TATGCTTAACTCGTATGGT / TCGTGGAGATATGACAGA
BBA_01095	<i>mkk1</i>	MAP kinase kinase (Mkk1)	CCGCCGACCTCAGATTCC / CGGTTGACCACCTTGAGAGACAG
BBA_04254	<i>mkk2</i>	MAP kinase kinase (Mkk2)	GCCGAGAACC GCCCAGACTTTG / TGCTCACAGTGCCGCCGTTTC
BBA_02330	<i>pbs2</i>	MAP kinase kinase (Pbs2)	CGCTCAATGCCGATGTCTTG / GGCTGAGTTGATATGCTTGAC
BBA_01660	<i>mkk4</i>	MAP kinase kinase (Mkk4)	GAGGCGACTGAGGAGGACTGAG / CGGCAGAGGACGGACCAAGAG
BBA_05588	<i>Mkk5</i>	MAP kinase kinase (Mkk5)	TTCTCAGACAGCACCAAT / GGCAGACTCTCATAATGTAT
BBA_01244	<i>mpk1</i>	MAP kinase (Mpk1)	CTGCTCTCAACGCCAATG / GCTGGTGGTATAATCCTTGCC
BBA_03334	<i>slt2</i>	MAP kinase (Slit2)	ACCGACGCACACTTTCAATCCTTC / GCCCACAGACCACATCAATAGC
BBA_04298	<i>maf1</i>	MAP kinase (Maf1)	CGTTCTGTTGTGCCCGTCAATTC / CCGCCAATCTCATCGTCTCTCC
BBA_05209	<i>hog1</i>	MAP kinase (Hog1)	GGCTGGCGTCTTCATCGTGACC / AAGGGCTTCCGTCGAGCATCTC
BBA_09043	<i>sty1</i>	MAP kinase (Sty1)	ATCGCAGGCTCGAAATACG / GGCTGGCTGGCTGTTATCC

* Gene accession codes in the annotated genome of *B. bassiana* under the NCBI accession ADAH00000000.