

**Comprehensive insights into the remarkable function and
regulatory mechanism of FluG during asexual development
in *Beauveria bassiana***

Fang Li¹, Haiying Zhong¹, Jianming Chen^{1*}, Juefeng Zhang^{1*}

¹ Institute of Plant Protection and Microbiology, Zhejiang Academy of Agricultural Sciences, Hangzhou, 310021, China

*** Correspondence:**

Jianming Chen, chenjm63@163.com;

Juefeng Zhang, zhangjf@zaas.ac.cn

Supplementary tables

Table S1 Paired primers used in manipulation of *fluG*, *brlA*, *abaA*, and *wetA* in *B. bassiana* and identification of their deletion/complement mutants.

Primers	Paired sequences (5'-3') *	Purposes
FluGup-F/R	<u>CCGGAATTC</u> GTAAAGGCACTTGTCATCCGC / <u>CGCGGATCC</u> GGTGGCAAGGGCAGTAAATC	Cloning <i>fluG</i> 5' (1599 bp) for deletion
FluGdn-F/R	<u>CCGCTCGAG</u> AGCATCCAGCGATTCCAGT / <u>GGAAGATCT</u> CTTTGGTGCGTGGTAGCCTC	Cloning <i>fluG</i> 3' (1639 bp) for deletion
BrlAup-F/R	<u>CCGGAATTC</u> GGGGGAGAGAGACTATGGAATC / <u>CGCGGATCC</u> AAGAAGGTAAAGGCTGGTGA	Cloning <i>brlA</i> 5' (1544 bp) for deletion
BrlAdn-F/R	<u>CTAGTCTAGACA</u> ATGTCAAGCCTGAACCTG / <u>GGAAGATCT</u> CCTCCTCGTAAAAGATAGCGT	Cloning <i>brlA</i> 3' (1689 bp) for deletion
AbaAup-F/R	<u>CGCGGATCC</u> GAAGAGATGGAATAGATGAGGCA / <u>CCCAAGCTT</u> GCGTGTATGTGGTGTATGTGTGT	Cloning <i>abaA</i> 5' (1695 bp) for deletion
AbaAdn-F/R	<u>CCGCTCGAG</u> ACAGACAGGCACACAAGGATAC / <u>GGAAGATCT</u> TGGAGAAGGAGTCAACAGGT	Cloning <i>abaA</i> 3' (1741 bp) for deletion
WetAup-F/R	<u>CGCGGATCC</u> AGACCTGGACAACGGAGAA / <u>CCCAAGCTT</u> GTTTGTGGTGACCCTATGAG	Cloning <i>wetA</i> 5' (1805 bp) for deletion
WetAdn-F/R	<u>CTAGTCTAGA</u> ATGTATTCAACCACGAGCGG / <u>GGAAGATCT</u> AAGGCAATAAAAGCCAGGG	Cloning <i>wetA</i> 3' (1846 bp) for deletion
FluGfl-F/R	<u>GGGGACAAGTTTGTACAAAAAAGCAGGCT</u> TGTCTGCTACCTAAACGAGTC / <u>GGGGACCACCTTTGTACAAGAAAGCTGGGT</u> CTCTCCACATTGCTAACG	Cloning full-length <i>fluG</i> (4590 bp) for complementation
BrlAfl-F/R	<u>GGGGACAAGTTTGTACAAAAAAGCAGGCT</u> AGTCTTGCCAGTCTCTTTTG / <u>GGGGACCACCTTTGTACAAGAAAGCTGGGT</u> TGTCATTATTGCGAGGTT	Cloning full-length <i>brlA</i> (4281 bp) for complementation
AbafI-F/R	<u>GGGGACAAGTTTGTACAAAAAAGCAGGCT</u> AGGACAGGGCAAAGAAGGTG / <u>GGGGACCACCTTTGTACAAGAAAGCTGGGT</u> CTCTCCTCCTCAACAACAGC	Cloning full-length <i>abaA</i> (5880 bp) for complementation
WetAfl-F/R	<u>GGGGACAAGTTTGTACAAAAAAGCAGGCT</u> ATTCTCATTTCCTCATTGTC / <u>GGGGACCACCTTTGTACAAGAAAGCTGGGT</u> GCTTGACCTGTCTGGATACC	Cloning full-length <i>wetA</i> (5234 bp) for complementation
idFluG-F/R	TCACAAAGAGGAAGGCGGTAT / CCTTGTTGACCTTCGGTATGC	PCR detecting of deleted /rescued <i>fluG</i>
idBrlA-F/R	TCAACTCTCTCTTGTTACCAG/TAGATGCGTGGCACAACCTAC	PCR detecting of deleted/rescued <i>brlA</i>
idAbaA-F/R	CGATACAGACGCTTCATACCACT/GCAAAGAGTAAAGATGTATTCGCT	PCR detecting of deleted/rescued <i>abaA</i>
idWetA-F/R	TGGTTGCCTGTTCTTCACTTTG/AGTGAGATGCCCTCCAGACG	PCR detecting of deleted/rescued <i>wetA</i>
RTidFluG-F/R	GGCAAGCGGTAGAGCAAC / GCGGAAACATTGTGGCTACC	RT-PCR detecting of deleted /rescued <i>fluG</i> (599 bp)
RTidBrlA-F/R	CTTTTCGTCTGCCTCTCCG / CGTAGGAAACAGAGCAATAGGG	RT-PCR detecting of deleted/rescued <i>brlA</i> (469 bp)
RTidAbaA-F/R	CACCAGCCAACACAAGACTATG / TTTCCGCCCATGGAAACTT	RT-PCR detecting of deleted/rescued <i>abaA</i> (482 bp)
RTidWetA-F/R	TGGCCTGACACCGACGTT / TGGGGTCAACATCATAGCCG	RT-PCR detecting of deleted/rescued <i>wetA</i> (280 bp)
18S-F/R	TGGTTTCTAGGACCGCCGTAA / CCTTGGCAAATGCTTTCCG	RT-PCR and qRT-PCR detecting
qFluG-F/R	CCATCAGGATACATCGTCTT / AGATTCATAGTCTCGCTCAA	qRT-PCR detecting of <i>fluG</i> transcript
qBrlA-F/R	GACCAGTTCAACAGACAAG / CAGTAATCTTCGTGCTTCTC	qRT-PCR detecting of <i>brlA</i> transcript
qAbaA-F/R	GCAAGTCTCCAGCCATAT / CTCCTCTTCGCATAGTAGTC	qRT-PCR detecting of <i>abaA</i> transcript
qWetA-F/R	CGCAGACGAATTTGACTT/ GCTGGTGGTTGAATACAT	qRT-PCR detecting of <i>wetA</i> transcript

* The underlined regions are the introduced cleavage sites of restriction enzymes for the deletion of *fluG* (*Eco*RI/*Bam*HI and *Xho*I/*Bgl*II), *brlA* (*Eco*RI/*Bam*HI and *Xba*I/*Bgl*II), *abaA* (*Bam*HI/*Hind*III and *Xho*I/*Bgl*II), and *wetA* (*Bam*HI/*Hind*III and *Xba*I/*Bgl*II). The underlined and italicized regions are the recognition fragments for the gateway exchange in each complementary plasmid.

Table S2 Paired primers used in *B. bassiana* for confirming data validation of transcriptome by qRT-PCR.

Gene name/id	Paired sequences (5'-3') *	Purposes
18S-F/R	TGGTTTCTAGGACCGCCGTAA / CCTTGGCAAATGCTTTCGC	qRT-PCR detecting
BBA_00001	TCTCAACTTCACAGTCTTC / CTGCCTGTCTGTATATCG	qRT-PCR detecting
BBA_00004	CCTCACCCAGAACTACTA / CCATCTCGTCAAGAATCTC	qRT-PCR detecting
BBA_00083	CAACTCCTACCGATTCTC / TTACCTATCGGCACATTG	qRT-PCR detecting
BBA_00193	CGACGGAGACAATAATACA / GCAGGACTGAAATACCAT	qRT-PCR detecting
BBA_00235	CAGGATTCCCTCCATCAC / AACTGGTCGTCGTAGTAG	qRT-PCR detecting
BBA_00319	GAGGTTGAGGAGATTGAG / GTGCTGTCTGTAGATGTAG	qRT-PCR detecting
BBA_01324	AAGTCTGCTATCGCTTTC / CCGCATTCTCTATCAGTAG	qRT-PCR detecting
BBA_01327	CTTCGTCGTCTGATTCTT / AGTGGCTAGTGTAGATGA	qRT-PCR detecting
BBA_01509	GTGCTTTGCCATCATTAC / GCTCAACTCTACACTATCC	qRT-PCR detecting
BBA_02892	TGAAAGCCAACACCATTG / CCCTTGATAATCCCAGAAT	qRT-PCR detecting
BBA_03099	AATACCTGGCATCCTTG / CTCTGGCTTAGTGTAGTC	qRT-PCR detecting
BBA_03642	GCCAATATCCTCATCGTT / GGTGATTCTCATCTTCCTT	qRT-PCR detecting

Figure S1

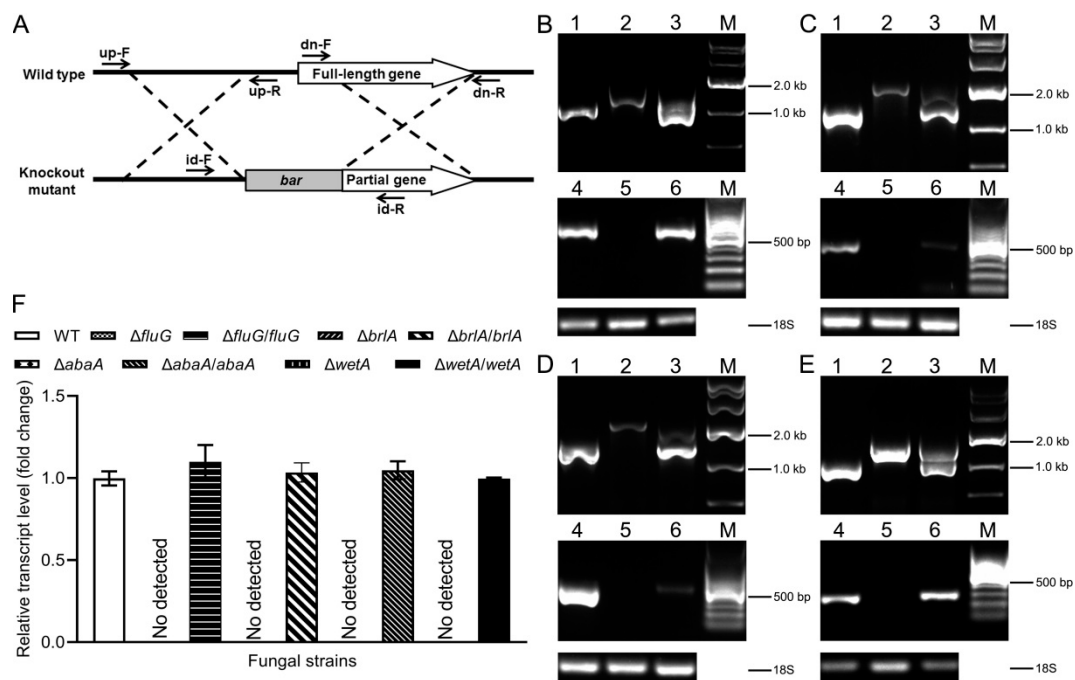


Figure S1. Constructing and identifying the single deletion of *fluG*, *brlA*, *abaA*, and *wetA* in *B. bassiana*. (A) Schematic diagram for the disruption of each gene. In knockout mutants, part of the coding sequence is replaced by the coding region of the *bar* gene with the promoter and terminator of *trpC*. (B, C) The mutants of *fluG* and *brlA* identified respectively via PCR (lanes 1–3) and RT-PCR (lanes 4–6). Lanes 1 and 4: wild type. Lanes 2 and 5: mutant. Lanes 3 and 6: complement. The bands of last row showed with internal reference 18S in different samples. (D, E) The mutants of *abaA* and *wetA* identified respectively via PCR (lanes 1–3) and Southern blotting (lanes 4–6). Lanes 1 and 4: wild type. Lanes 2 and 5: mutant. Lanes 3 and 6: complement. The bands of last row showed with internal reference 18S in different samples. (F) Relative transcript level of *fluG*, *brlA*, *abaA*, and *wetA* in the 4-day-old SDAY cultures of the deletion and complementation mutants versus the wild-type strain. Error bars: standard deviation of the mean from three cDNA samples of each strain detected in qRT-PCR experiments. See Table S1 for a list of paired primers used in PCR, RT-PCR and qRT-PCR.

Figure S2

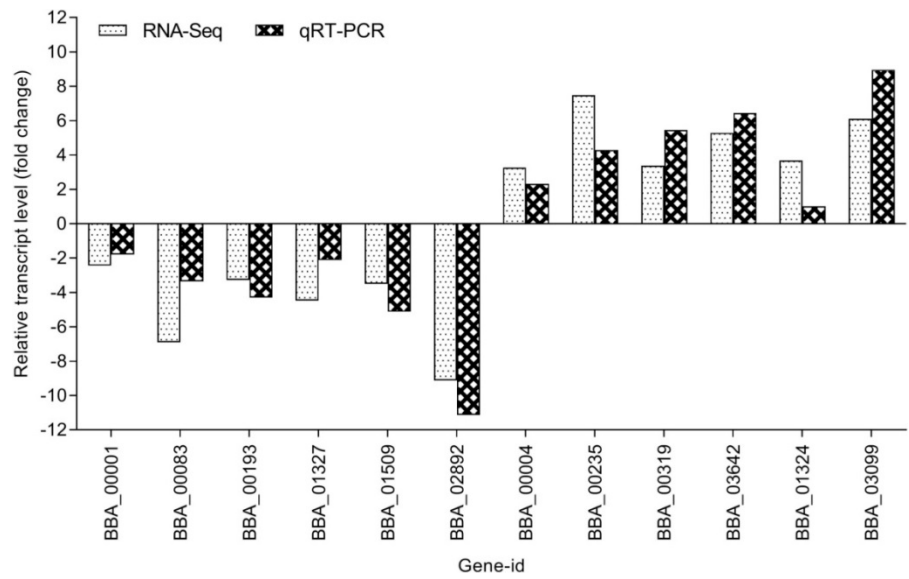


Figure S2. Comparison of gene expression patterns obtained by RNA-Seq and qRT-PCR. Changes in relative transcript levels of all 12 genes from the same sample were compared by transcriptome and qRT-PCR with paired primers in the Table S2.

Figure S3

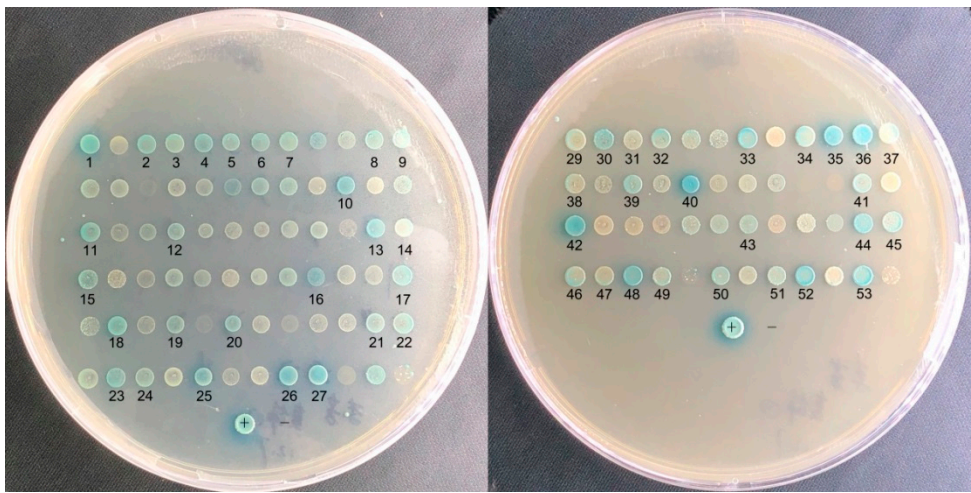


Figure S3. Positive clones on the re-screening plate. Pick the blue clones from the SD/-Leu/-Trp/-His/X- α -gal (TDO/X) screening plate and transfer them to SD/-Leu/-Trp/-His/-Ade/X- α -gal/AbA (QDO/X/A) plate for re-screening. 53 positive clones were successfully cloned and sequenced.

Figure S4

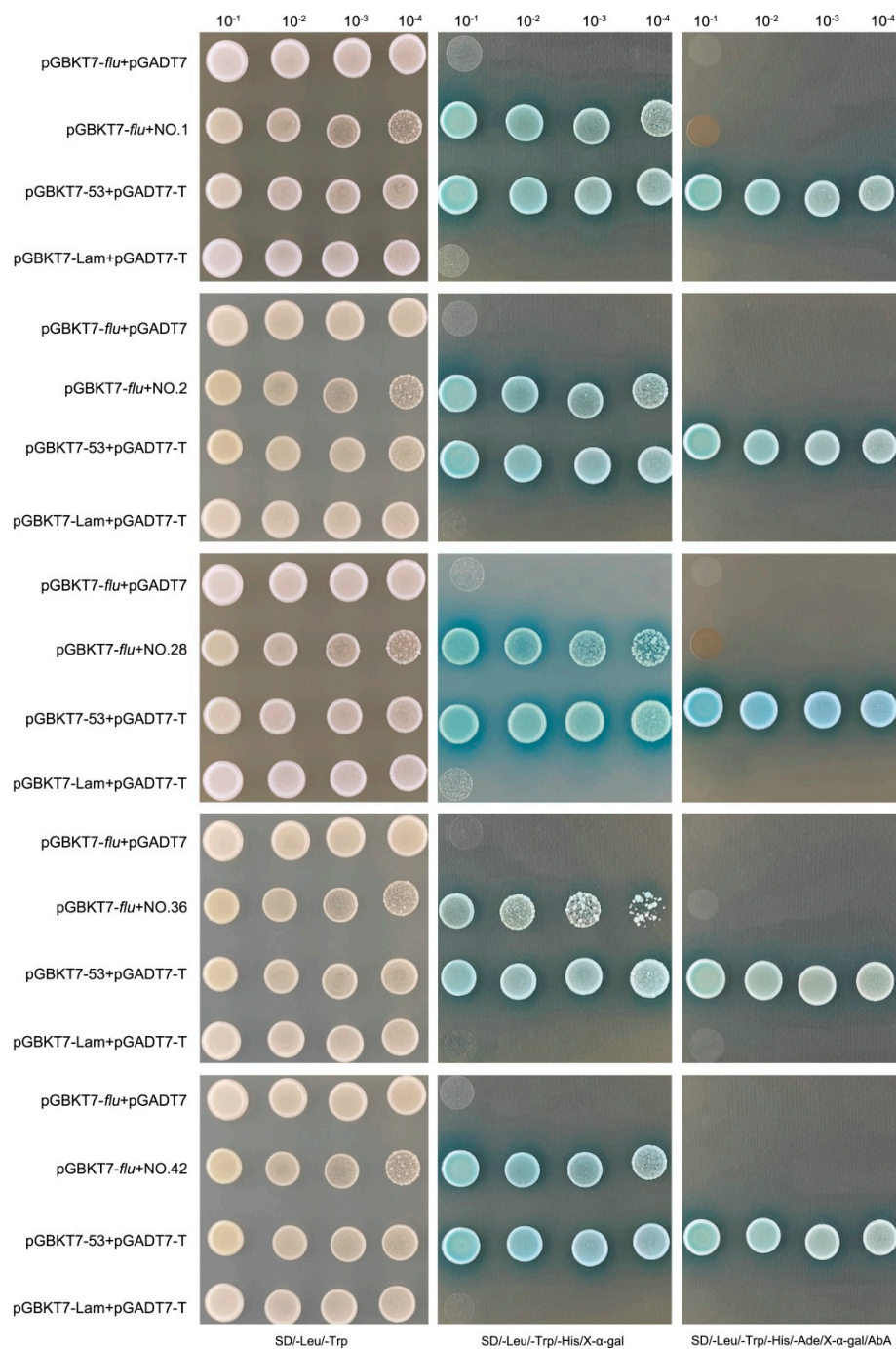


Figure S4. One - to - one yeast interaction validation. Five proteins were randomly selected to confirm the reliability of yeast interaction. The plasmids of pGBKT7-*fluG* and selected protein were used to confirm the interaction on different selection pressure plates.