

Figure S1. Quantification of the radial growth (in diameter) of B05.10 at 3 days post inoculation (DPI) on PDA plates supplemented with different concentrations of G418. ND: Not detected. Data represent means \pm standard deviations (SD) from three independent experiments in which triplicate colonies were analyzed for each treatment. The letters above columns indicate significant differences analyzed with SPSS software at $p < 0.05$.

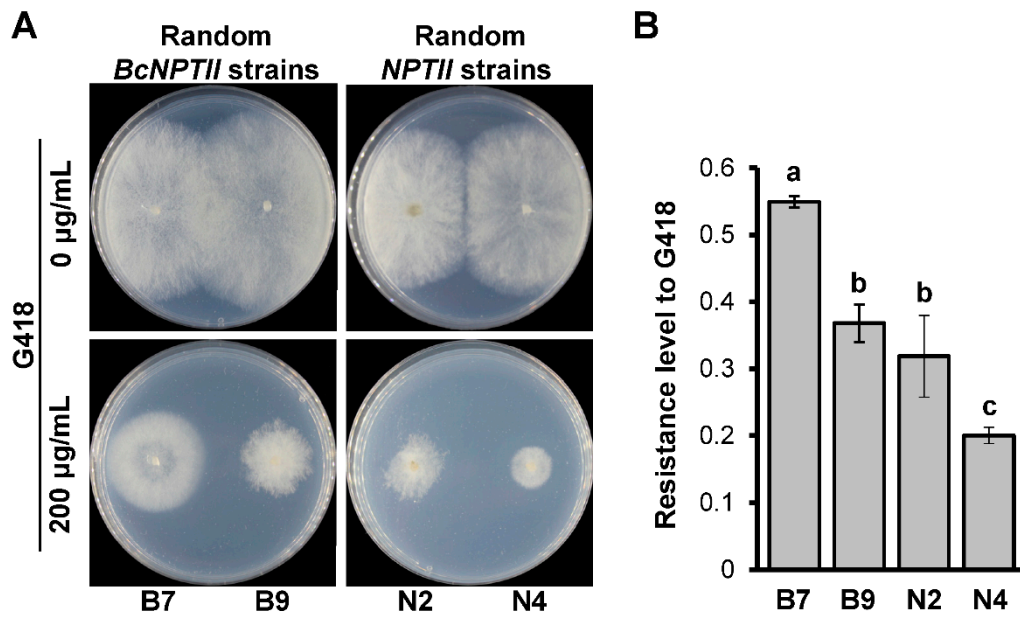


Figure S2. The transformants derived from the codon-optimized gene *BcNPTII* show varying degrees of increased resistance to geneticin. (A) Mycelial plugs of 4 randomly selected geneticin-resistant transformants were inoculated on PDA plates supplemented with or without G418. Photographs were taken at 2 DPI. (B) Quantification of the resistance levels of 4 randomly selected transformants derived from different resistance genes. The resistance level of a strain is reflected by calculating the percentage of the colony size with G418 compared to that without G418. The letters above columns indicate significant differences analyzed with SPSS software at $p < 0.05$. B7 and B9 were derived from *BcNPTII* transformation; N2 and N4 were derived from *NPTII* transformation.

Table S1. Codon usage of fungal genes and the two geneticin resistance genes

Parameter	Value for indicated organism or gene ^a							
	<i>M.</i> <i>oryzae</i>	<i>N.</i> <i>crassa</i>	<i>A.</i> <i>gossypii</i>	<i>F.</i> <i>oxysporum</i>	<i>S.</i> <i>sclerotiorum</i>	<i>B.</i> <i>cinerea</i>	<i>NPTII</i>	<i>BcNPTII</i>
Coding GC content (%)	56.3	56.1	52.8	51.2	47.6	46.6	59%	48%
Codon usage (%)								
Ala								
GCT	19.9	24.3	19.0	39.5	38.2	34.2	26(9)	40(14)
GCC	42.8	41.4	25.4	40.2	26	24.1	34(12)	29(10)
GCA	18.1	14.5	21.8	7.9	27.4	29.6	17(6)	31(11)
GCG	19.3	19.9	33.8	12.4	8.3	12	23(8)	0
Arg								
AGA	9.3	12.8	15.7	20.2	27.9	27.1	0	40(8)
AGG	18.5	19.1	14.2	6.0	10.8	10.2	15(3)	0
CGT	14.6	14.4	12.5	15.5	23.2	20.2	10(2)	25(5)
CGC	30.9	28.5	28.4	35.2	13.8	14.6	40(8)	0
CGA	12.8	11.4	6.5	23.2	19.8	21.1	5(1)	35(7)
CGG	13.9	13.8	22.7	0.0	4.4	6.8	30(6)	0
Asn								
AAT	23.8	27.7	39.1	17.2	43.4	51.9	100(3)	67(2)
AAC	76.2	72.4	60.9	82.8	56.6	48.1	0	33(1)
Asp								
GAT	35.6	42.4	42.1	44.1	69.2	66.5	48(12)	68(17)
GAC	64.4	57.6	57.9	55.9	30.8	33.5	52(13)	32(8)
Cys								
TGT	27	30.3	35.8	31.7	46.7	53.1	20(1)	60(3)
TGC	73	69.7	64.2	68.3	53.3	46.9	80(4)	40(2)
Glu								
GAA	29.8	34.5	36.9	17.0	53.1	54.7	56(10)	56(10)
GAG	70.2	65.5	63.1	83.0	46.9	45.3	44(8)	44(8)
Gln								
CAA	36	39.4	29.1	19.2	71.8	67.7	27(3)	73(8)
CAG	64	60.6	70.9	80.8	28.2	32.3	73(8)	27(3)
Gly								
GGT	23.4	25.5	21.9	35.4	43.7	35.7	24(5)	43(9)
GGC	45.8	40.4	40.1	44.8	16.1	20.9	48(10)	14(3)
GGA	18	18.9	15.0	17.5	34.3	33.4	14(3)	43(9)
GGG	12.7	15.2	23.1	2.3	6	10.1	14(3)	0
His								
CAT	31.8	39.0	41.7	48.4	50.2	58.2	57(4)	57(4)
CAC	68.2	61.0	58.3	51.6	49.8	41.8	43(3)	43(3)

Continued on the following page

Parameter		Value for indicated organism or gene ^a							
		M. <i>oryzae</i>	N. <i>crassa</i>	A. <i>gossypii</i>	F. <i>oxysporum</i>	S. <i>sclerotiorum</i>	B. <i>cinerea</i>	<i>NPTII</i>	<i>BcNPTII</i>
Ile	ATT	30.5	31.4	33.9	37.6	38.3	44.8	20(2)	60(6)
	ATC	56.4	59.4	43.1	61.4	51.3	39.6	70(7)	40(4)
	ATA	13	9.2	23.0	1.1	10.4	15.6	10(1)	0
Leu	TTA	3.7	3.3	8.2	3.1	11.4	11.4	0	0
	TTG	15.3	18	19.4	20.3	23.5	23.2	13(4)	28(9)
	CTT	15.2	17.2	12.6	22.3	25.4	24.4	25(8)	59(19)
	CTC	30.3	32.3	15.8	34.9	25.5	20.7	19(6)	13(4)
	CTA	6.4	7.2	13	2.3	6.5	9.5	9(3)	0
	CTG	28.9	22	31	17.2	7.7	10.7	34(11)	0
Lys	AAA	24.7	22.5	31.0	7.5	37.3	45.7	50(2)	50(2)
	AAG	75.3	77.6	69.0	92.5	62.7	54.3	50(2)	50(2)
Phe	TTT	38.4	34.8	42.2	21.9	30.8	41.8	18(2)	55(6)
	TTC	61.6	65.2	57.8	78.1	69.2	58.2	82(9)	45(5)
Pro	CCT	21	23.4	20.7	36.9	34.6	31.6	18(2)	45(5)
	CCC	31.8	34.8	21.9	39.0	14	17.9	18(2)	0
	CCA	21.1	19.2	25.8	19.4	42.3	39.1	18(2)	55(6)
	CCG	26.1	22.6	31.6	4.7	9.1	11.4	45(5)	0
Ser	AGT	8.3	10.6	10.1	10.3	13.7	16.3	0	20(2)
	AGC	24.9	21.3	20.8	23.2	12.8	12.9	0	0
	TCT	13.6	14.6	17.2	23.7	22.5	22.8	40(4)	40(4)
	TCC	18	24.4	18.4	25.1	26.4	16.6	20(2)	20(2)
	TCA	12.5	11.3	11.9	7.6	14.7	19.4	20(2)	20(2)
	TCG	22.7	17.7	21.6	10.0	9.9	12	20(2)	0
Thr	ACT	17.4	18.6	20.6	39.6	33.9	31.6	20(2)	40(4)
	ACC	38	41.1	26.8	40.5	37.5	27.1	40(4)	20(2)
	ACA	19.6	17.9	22.2	12.7	19.6	28.8	10(1)	40(4)
	ACG	25	22.5	30.3	7.2	9	12.4	30(3)	0
Tyr	TAT	27.6	32.7	35.6	38.2	45.7	49.3	75(3)	50(2)
	TAC	72.4	67.3	64.4	61.8	54.3	50.7	25(1)	50(2)
Val	GTT	21.8	23.2	21.3	37.6	38.4	36.7	19(3)	50(8)
	GTC	43.5	41.7	24.4	48.5	38.3	31.5	25(4)	44(7)
	GTA	8.9	9.1	13.4	1.1	12	16.2	6(1)	6(1)
	GTG	25.8	26.0	40.9	12.8	11.2	15.6	50(8)	0

^a Numbers in parentheses indicate codon numbers in geneticin resistance genes. Bold numbers indicate codons mainly used in *NPTII* but underrepresented in *B. cinerea* and *S. sclerotiorum*

compared to other fungi. GC content and codon frequencies of fungal coding sequences were taken from <http://www.kazusa.or.jp/codon/>. The fungi analyzed were as follows: *Magnaporthe oryzae* (*M. oryzae*), *Neurospora crassa* (*N. crassa*), *Ashbya gossypii* (*A. gossypii*), *Fusarium oxysporum* (*F. oxysporum*), *Sclerotinia sclerotiorum* (*S. sclerotiorum*), *Botrytis cinerea* (*B. cinerea*).

Note: A site for the commonly used restriction enzyme *Pst* I was eliminated from the final sequence of *BcNPTII*.