Fig. S1. Procedures for construction of *crgA* null mutants and complemented strains. (A) Disruption of *crgA* with split-marker strategy. In round 1, primer pairs 5f/5r and 3f/3r were used to amplify 5′ and 3′ flanks, and the selectable marker was amplified from plasmid pCSN44 with primer pair hF/hR. In round 2, 5′ and 3′ flanks were fused to 5′-hp and hp-3′ fragments by two separate overlap PCR using primer pairs 5f/hf and hr/3r, respectively. In round 3, the two deletion cassettes (5′-hp and hp-3′ fragments) were co-transformed into protoplasts, such that part of *crgA* is replaced by *hph* through homologous recombination. (B) Construction of complemented strains with split-marker strategy. In round 1, primer pairs Cu/Cd and Cf/Cr were used to amplify 5′ and 3′ regions of *crgA* gene by PCR and two fragments (*crg* and *rgA*) with partial overlap were obtained. In round 2, the two partially complemented fragments (*crg* and *rgA*) were co-transformed into protoplasts to replace *hph* with *crgA* through homologous recombination.

Fig. S2. Sequence alignment for *crgA* and its deduced protein from strain (-) of *B. trispora* with those from (+) strain. (A) Nucleotide sequence alignment for *crgA* from the opposite mating type strain of *B. trispora*. The nucleotides are numbered in 5′ to 3′ direction and the identical bases are shaded in gray. The boxed letters of GTG and TAA represent the starting codon and stop codon, respectively. The doubly and singly underlined sequences indicate the putative TATA box and exons of gene *crgA*, respectively. Sequence marked by dashed lines and wavy line denote the putative promoter and intron, respectively. (B) Sequence alignment of deduced protein (CrgA) from the opposite mating type strain of *B. trispora*. The identical amino acid

residues are marked with a grey background. The doubly underlined and boxed sequences indicate the RING-finger (RF1 and RF2) and the disrupted LON domains, respectively. Heptapeptide (HP) and isoprenylation (IP) sequences are marked by dash line and solid line, respectively.

Fig. S3. Isolation of *crgA* null mutants on the solid media and PCR analysis demonstrating the replacement of *crgA* gene by homologous recombination. (A) The transformants of *B. trispora* was grown on the selection media containing 200 μg/ml hygromycin B and 0.1% Trition×100, compared with no growth for the WT strain. (B) Four pairs of primers were designed to characterize the *crgA* locus from the wild-type and mutant strains. P1 and P2 were used for amplification of the middle sequence of *crgA* from WT strain. Primer pairs P3/P4, P5/P6 and hR/hF were used to confirm knockout of *crgA*. (C) Electrophoretic analysis of PCR amplicons from WT and three *crgA* null mutants using primers indicated in panel B. There are four lanes from left to right, corresponding to WT and three mutant strains, respectively. M = DNA ladder.

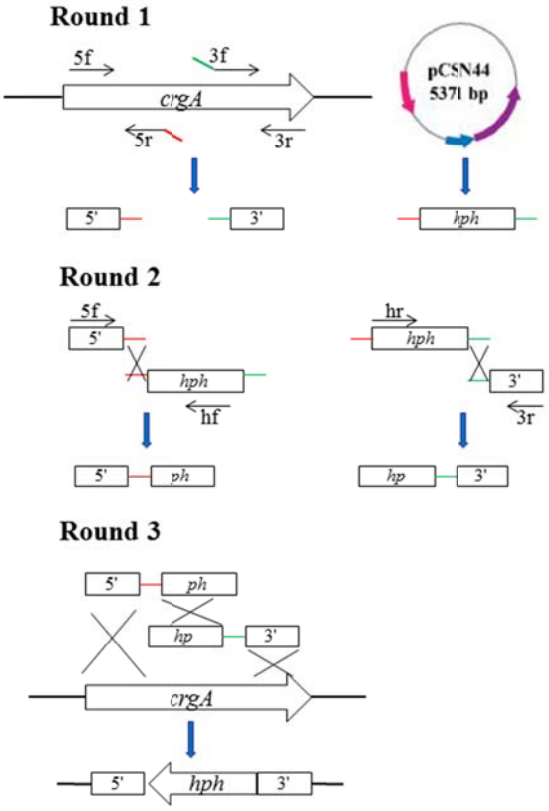
Round 1
5f
3f
crgA
5r
3r
pCSN44
5371 bp
5'
3'
hph
Round 2
5f
5'
hph
hf
5'
ph
hr
hph
3'
3r
hp
3'
Round 3
5'
ph
hp
3'
crgA
5'
hph
3'

Fig. S1A

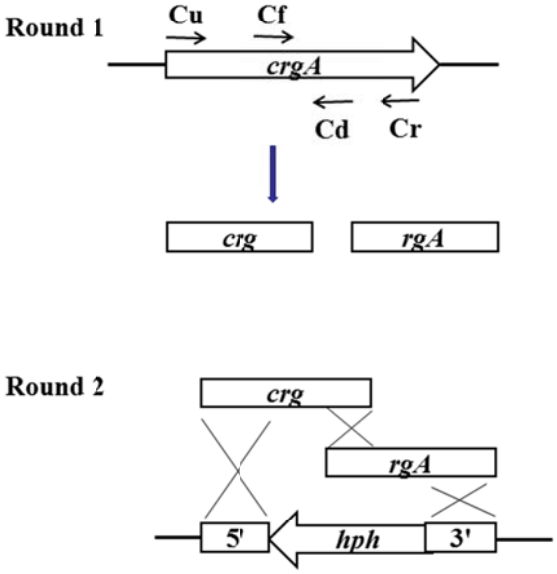

Fig.S1B

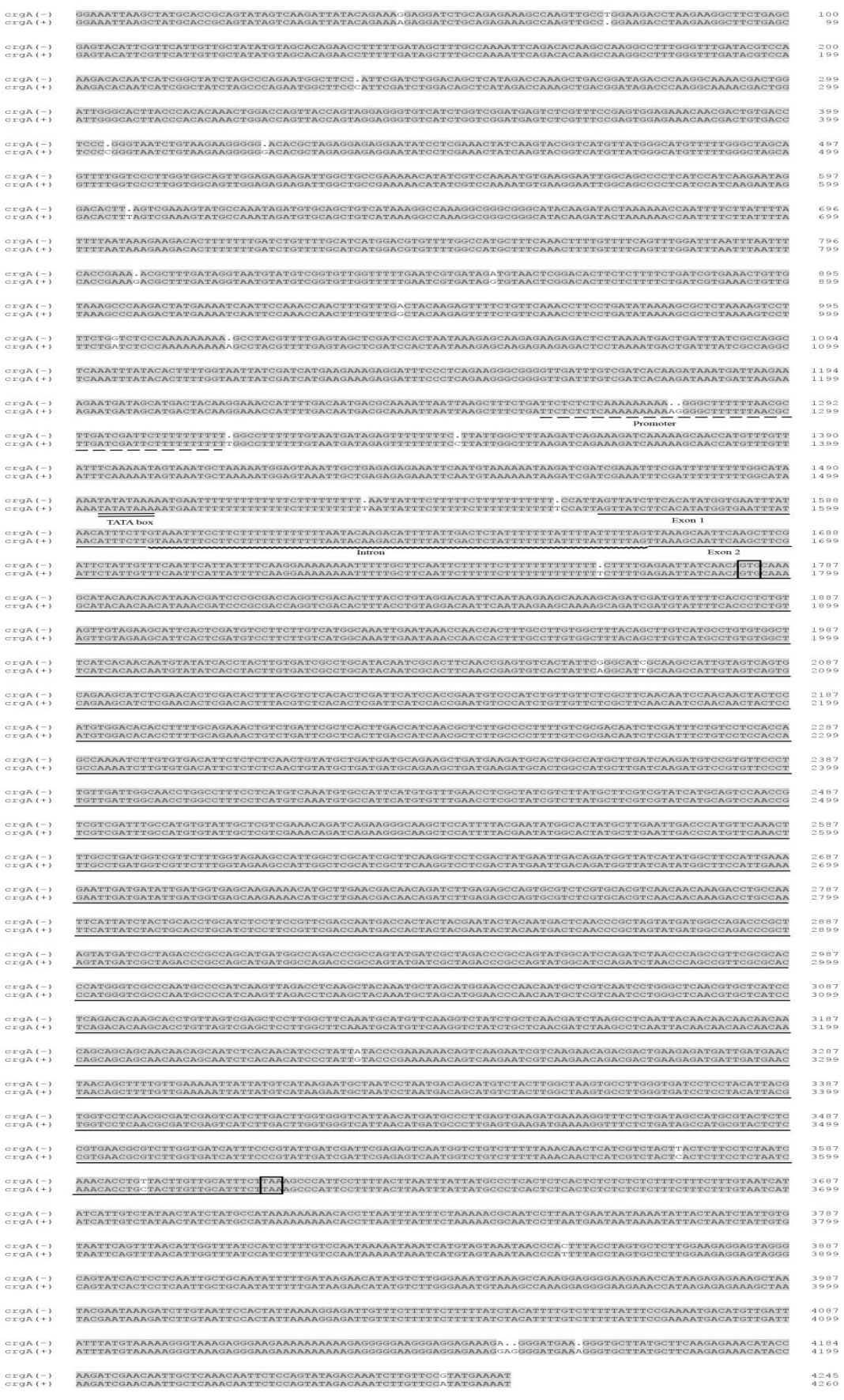

Fig.S2A

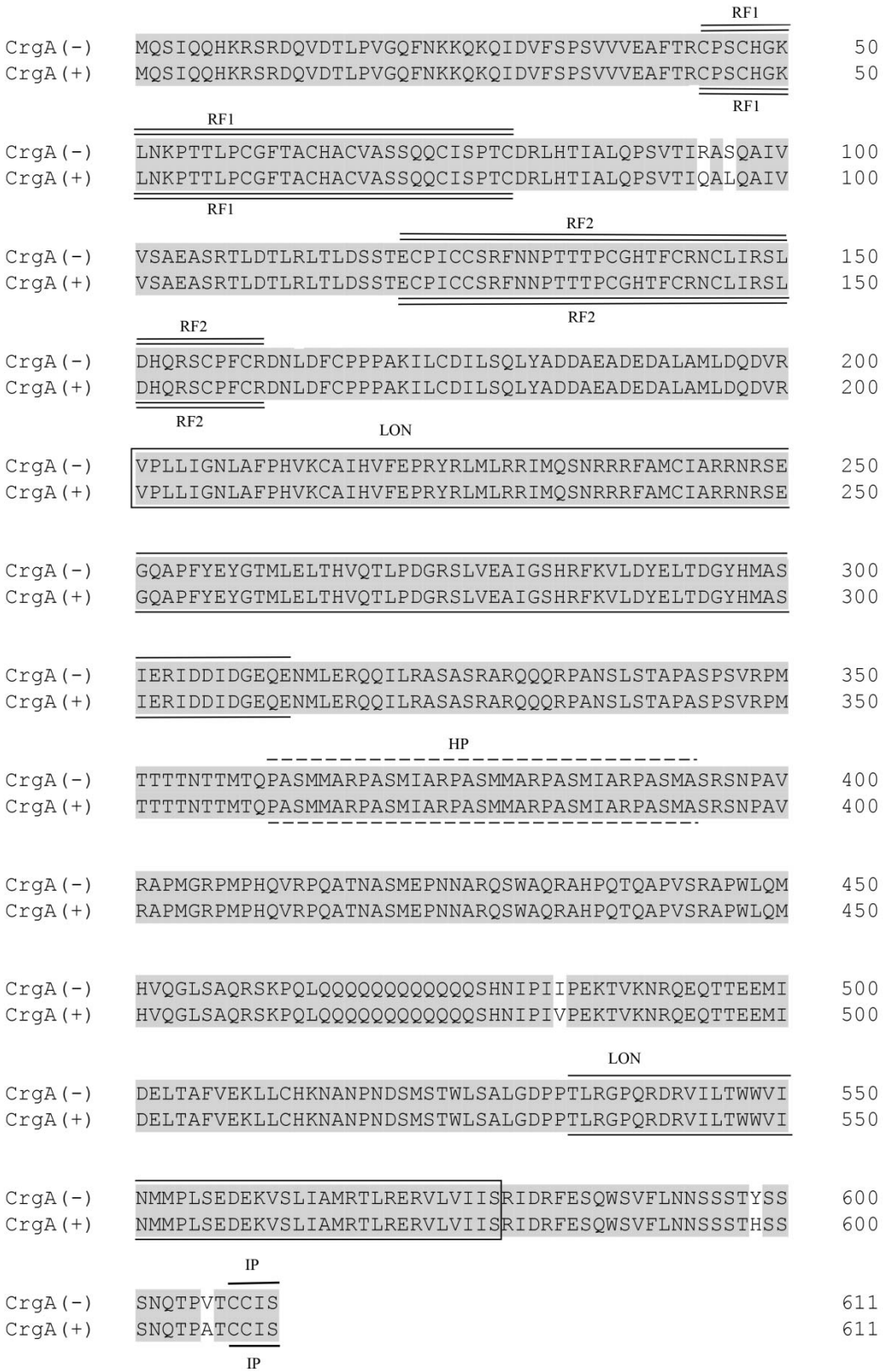
RF1
CrgA(-) MQSIQQHKRSRDQVDTLPVGQFNKKQKQIDVFSPSVVVEAFTRCPSCHGK 50
CrgA(+) MQSIQQHKRSRDQVDTLPVGQFNKKQKQIDVFSPSVVVEAFTRCPSCHGK 50
RF1
RF1
CrgA(-) LNKPTTLPCGFTACHACVASSQQCISPTCDRLHTIALQPSVTIRASQAIV 100
CrgA(+) LNKPTTLPCGFTACHACVASSQQCISPTCDRLHTIALQPSVTIQALQAIV 100
RF1
RF2
CrgA(-) VSAEASRTLDTLRLTLDSSTECPICCSRFNNPTTTPCGHTFCRNCLIRSL 150
CrgA(+) VSAEASRTLDTLRLTLDSSTECPICCSRFNNPTTTPCGHTFCRNCLIRSL 150
RF2
RF2
CrgA(-) DHQRSCPFCRDNLDFCPPPAKILCDILSQLYADDAEADEDALAMLDQDVR 200
CrgA(+) DHQRSCPFCRDNLDFCPPPAKILCDILSQLYADDAEADEDALAMLDQDVR 200
RF2
LON
CrgA(-) VPLLIGNLAFPHVKCAIHVFEPRYRLMLRRIMQSNRRRFAMCIARRNRSE 250
CrgA(+) VPLLIGNLAFPHVKCAIHVFEPRYRLMLRRIMQSNRRRFAMCIARRNRSE 250
CrgA(-) GQAPFYEYGTMLELTHVQTLPDGRSLVEAIGSHRFKVLDYELTDGYHMAS 300
CrgA(+) GQAPFYEYGTMLELTHVQTLPDGRSLVEAIGSHRFKVLDYELTDGYHMAS 300
CrgA(-) IERIDDIDGEQENMLERQQILRASASRARQQQRPANSLSTAPASPSVRPM 350
CrgA(+) IERIDDIDGEQENMLERQQILRASASRARQQQRPANSLSTAPASPSVRPM 350
HP
CrgA(-) TTTTNTTMTQPASMMARPASMIARPASMMARPASMIARPASMASRSNPAV 400
CrgA(+) TTTTNTTMTQPASMMARPASMIARPASMMARPASMIARPASMASRSNPAV 400
CrgA(-) RAPMGRPMPHQVRPQATNASMEPNNARQSWAQRAHPQTQAPVSRAPWLQM 450
CrgA(+) RAPMGRPMPHQVRPQATNASMEPNNARQSWAQRAHPQTQAPVSRAPWLQM 450
CrgA(-) HVQGLSAQRSKPQLQQQQQQQQQQQQSHNIPIIPEKTVKNRQEQTTEEMI 500
CrgA(+) HVQGLSAQRSKPQLQQQQQQQQQQQQSHNIPIVPEKTVKNRQEQTTEEMI 500
LON
CrgA(-) DELTAFVEKLLCHKNANPNDSMSTWLSALGDPPTLRGPQRDRVILTWWVI 550
CrgA(+) DELTAFVEKLLCHKNANPNDSMSTWLSALGDPPTLRGPQRDRVILTWWVI 550
CrgA(-) NMMPLSEDEKVSLIAMRTLRERVLVIISRIDRFESQWSVFLNNSSSTYSS 600
CrgA(+) NMMPLSEDEKVSLIAMRTLRERVLVIISRIDRFESQWSVFLNNSSSTHSS 600
IP
CrgA(-) SNQTPVTCCIS 611
CrgA(+) SNQTPATCCIS 611
IP

Fig.S2B

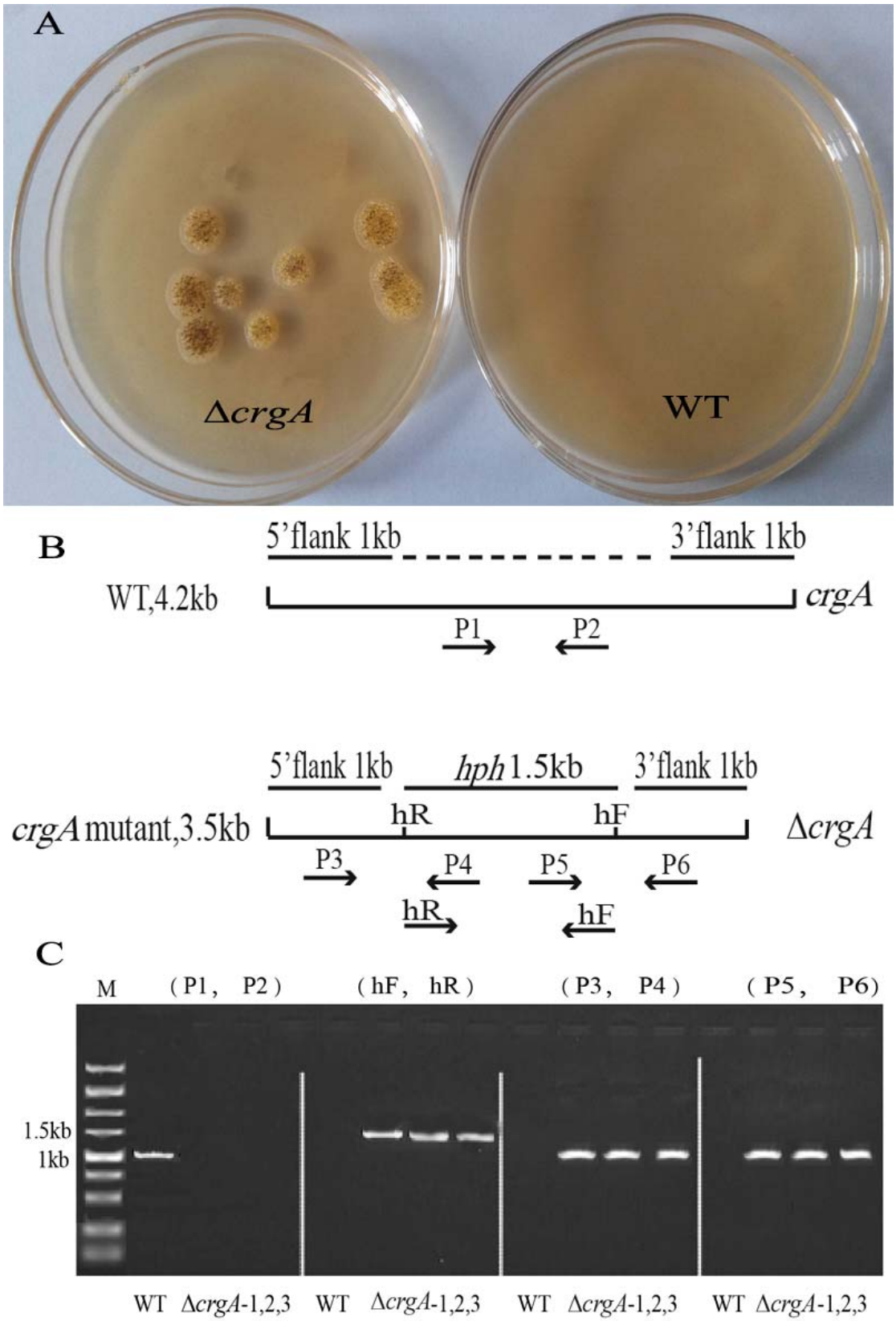
A
ΔcrgA
WT
B
5'flank 1kb
3'flank 1kb
WT,4.2kb
crgA
P1
P2
5'flank 1kb
hph 1.5kb
3'flank 1kb
crgA mutant,3.5kb
hR
hF
ΔcrgA
P3
P4
P5
P6
hR
hF
C
M
(P1, P2)
(hF, hR)
(P3, P4)
(P5, P6)
1.5kb
1kb
WT
ΔcrgA-1,2,3
WT
ΔcrgA-1,2,3
WT
ΔcrgA-1,2,3
WT
ΔcrgA-1,2,3

Fig.S3