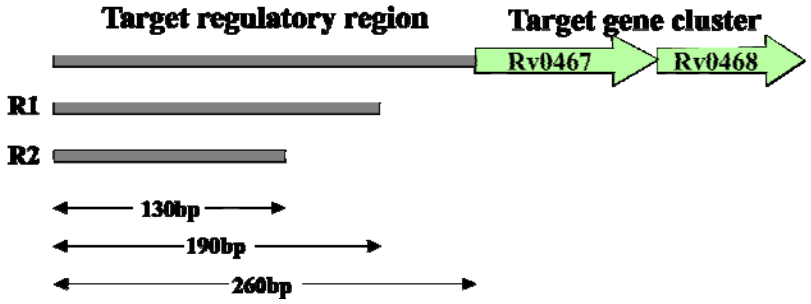
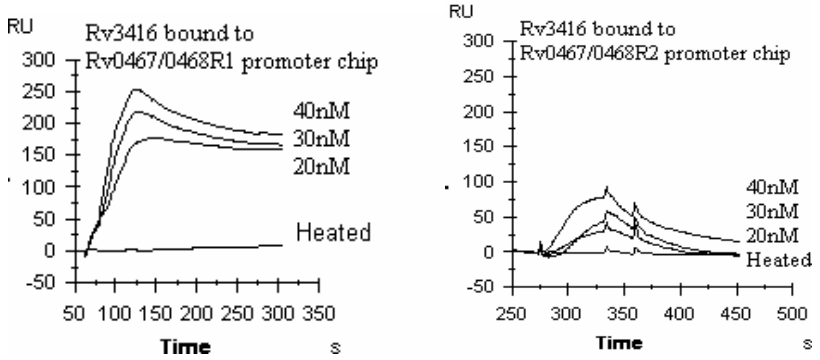
Figure 4

A



B



C

```
Rv0467-0468 GTGG-ACCAGCGTTGACACGCTGTG-CCATGGTCGA--GTTAGCACACCAGTGAA-GCTGCGCCG
Rv0053-0060 GTCGAGGGCTGTGGTGTAGCCTGG--GCGAGTTGCCGACGCAGGCGACCCTCCTG-CCA-CGGAT
Rv0244c     ATAGCGATCAAATGAAGAATATGCG--GAGT-CTAGGGCGGCAGCGCCTGGCAGCGTA-GATCAT
Rv0350-0351 GCGGGAACAAGACCCG-CACGACCAGCGTTAGC-ATGCTCAGTAAGTTGAGTGCATCAGGCTCA-
Rv0469      --GTCGAGGTTTCTACACGTACTGAAGTGTATGA-ACGCCCCCA--GGCTTG-ACGCAAGGCGA
Rv0551c     CCCTCGTAATCTCGAAGGATC----------A-CTACGCTTGGAGCCCATGGCCGATGC-AGACCT
Rv0757-0758 GTCGCAATACCCACGAGGGTTTGG--CCGAGGTTCATTCTGCGAGTGTATTCACG-GCG-CGCCG
Rv1094      CTGGCCAGGGCGTTTC-----CGCT-----CTACGACGGCTCGAGGAGTGGAGTCTGGTCGG-CC
Rv1460-1466 GCGC-GCCAGCGC-GGTGAGCTCGG-TAGTGGTCAGCGGTGCACCCACCGCCTGC-TCGTCGCCA
Rv2031      TCACCA-TGGTGTCCGGCATGATCAACCTCCGCTGTTCGATATCACCCCGATCTTTCTGAACGGC
Rv2735c-273 TTCG-GCTACTGTGGTGATCATTCGGAGCAGCCGACTTGT-CAGTGGCTGTCTCTAGTGTCACGG
Rv2875      CTTGAGGTGCGGCCCAGCAAGGGGCTACA-GGTTTTTTCCTTCACCTACGGATGAA-T--ATC-C
Rv2930-2939 CTGGCGCTGCG--CAGACATGCTGCGGAA-GCAGAACTGCGTAATCGTCAGGTGGC-TT-GGT-C
Rv3197A     GTCA-ATCACCGCGGCCGCCCTCCTCTCGTGTCGCG---CGCGGTTGCCAGCCCC-CCAATGCCA
Rv3417c-341 ACGGCGCGAGGGCGCG-GACGA---ATGCCGAA-TCACCTGGTAATTCGGACGGTTCCGGGGCAC
Rv3504-3505 TTGCCGTTGGCGTTGGTTGTCGACAGC--TAGTGGACGGCTGCTGACGGCCAGTGATAAAGAGGC
Rv3537-3538 GCCA--GTAGCGCCCGACCCGAGCAATTCTA-TAACGTGTTCTACA-TGACTGTGCAGGAGTTCG
Rv3543c-354 TCATCGTTGAAGCCACCCGC-AGC---CCCATCGGCAAACGCAACGGATGGC-TGTCGG-GG-CT
Rv3550-3552 ATCACGGTCGCGCCCTGTTCGGCGAATACCGAGCTGATGC-CCGCGCCTACCCCGCG-GACACCG
Rv3864-3871 TCACCA-AAAAATTCG-TGCACCAACCCCCCTCCGAGCGCTGCTAAGCTCAATGTGCAGTGCAAA
Rv3876-3877 CAGGCAATGGGTTCGACCGAAGGCAACGTCACTGGGATGTTGCATAGGGCAACGCCGAGTTCGC
```

Interaction between WhiB3 and the regulatory region of Rv0467/Rv0468 gene cluster. (**A**) Schematic diagrams of different deleted mutants of the regulatory region. Two mutant regulatory regions R1 and R2 were cloned into the reporter vector of pBTXcm-T, respectively. R2 is shorter than R1 which contains an additional 60-bp region. (**B**) SPR assays for the interactions between WhiB3 and the regulatory region of Rv0467/Rv0468 gene cluster. Two truncated DNA fragments, R1 and R2, were immobilized on the SA chip and SPR assays were conducted to further map the binding region of WhiB3 within the regulatory sequences. An about 60-bp region was shown to

contain potential target site due to a significantly reduced binding signal of WhiB3 detected on the shorter fragment of R2, which lost the 60-bp region. (**C**) A blast assay for searching the potential binding site of WhiB3. A non-conserved DNA-binding motif, TNGCGNTNNCGC, was defined within the regulatory sequences of targets genes detected by the bacterial one-hybrid system.