Figure 4

A

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
contains 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, TNGCGNTNCGC, was defined within the regulatory sequences of target genes detected by the bacterial one-hybrid system.