Boudehen et al. An improved Xer-cise technology for the generation of multiple unmarked mutants in Mycobacteria.

**Supplemental material**

Supp Table S1. Oligonucleotides used to construct and verify a quadruple mutant in *M. smegmatis*

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| Oligonucleotides used to construct allelic exchange substrates a |
| SMEG1517-Up-Fw | GAGACGACAACACGTGACGG |
| SMEG1517-Up-Rv-Zeo | CAGTCGATCCACGTGGAGATGAGCAGCGTGGA**CAC**GGTGG |
| SMEG1517-Zeo-Dw-Fw | CCACTGAGCGTCAGACCCACGTGCTCGACCGCGCCGGCCGAG**TAG**CCG |
| SMEG1517-Dw-Rv | CGATCACCTTGGTGTACGGG |
| SMEG3155-Up-Fw | GCACTTGGACACCACATGATGG |
| SMEG3155-Up-Rv-Zeo | CAGTCGATCCACGTGGAGAGACAGCACCTTC**CAT**CGGTAAC |
| SMEG3155-Zeo-Dw-Fw | CCACTGAGCGTCAGACCCACGTGCTCGGAGCAG**TAA**GACACCGGCCG |
| SMEG3155-Dw-Rv | CGCTCACCGGAAATGTGAG |
| SMEG4576-Up-Fw | CATCGTGGTGCACCGCCTGG |
| SMEG4576-Up-Rv-Zeo | CAGTCGATCCACGTGGAGTAGAGCATGTC**CAT**GATTCGCCTC |
| SMEG4576-Zeo-Dw-Fw | CCACTGAGCGTCAGACCCACGTGCTCGCCGCGAGTTGTT**TAG**CGGCG |
| SMEG4576-Dw-Rv | CAGCCAGCGCCGCACCGATG |
| SMEG6059-Up-Fw | GCAAGCATGAACGTCAATGCG |
| SMEG6059-Up-Rv-Zeo | CAGTCGATCCACGTGGAGCCATGCCACACCATCGGCCAAGC |
| SMEG6058-Zeo-Dw-Fw | CCACTGAGCGTCAGACCCACGTGCTCCGACTAGGGCGTTCGGCGAACG |
| SMEG6058-Dw-Rv | GTGAGTGGTTTCACTCCG |
| Oligonucleotides used to verify by PCR a quadruple mutant in *M. smegmatis*  |
| SMEG1517-Up-Fw2 | GAAATCCACCCGCGTCTACATC |
| SMEG1517-Dw-Rv2 | TCATCTGCGGCATCCGGTAATC |
| SMEG1517-Int | GCCGCAGGTAAGAAGGAACT |
| SMEG3155-Up-Fw | GCACTTGGACACCACATGATGG |
| SMEG3155-Dw-Rv | CGCTCACCGGAAATGTGAG |
| SMEG3155-Int | CCAACAAGGTGTGGCTCGT |
| SMEG4576-Up-Fw2 | AGTCGCCAGTTGTGTGAACG |
| SMEG4576-Dw-Rv2 | TTGGTTGAGGCCGTCGAGAATG |
| SMEG4576-Int | ATCATCGGCAAGAGCAACCT |
| SMEG6059-Up-Fw | GCAAGCATGAACGTCAATGCG |
| SMEG6058-Dw-Rv | GTGAGTGGTTTCACTCCG |
| SMEG6059-Zeo-Dw-Fw | CCACTGAGCGTCAGACCCACGTGCTCCCACTGAGTGACCACTGAGTACC |

a Blue letters show nucleotides specific of the right or left end of the zeocin-resistance cassette. Nucleotides specific of the start or stop codon of the ORF of interest are in bold letters.

Supp Table S2. Oligonucleotides used to construct and verify a triple in-frame deletion mutant in *M. tuberculosis* a

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| 0968-Up-Fw | GTTGCTCATCGTCCACGAG |
| 0968-Up-Rv-Zeo | CAGTCGATCCACGTGGAGCCATGCCACAC**CAT**CGCGGATG |
| 0968-Zeo-Dw-Fw | CCACTGAGCGTCAGACCCACGTGCTCCCAC**TGA**CGTTCTTTCTGACACCG |
| 0968-Dw-Rv | GGTAAGCACCGAAGAACATTG |
| 0968-int | GGAGCCTTCACCACCATCT |
| 1993c-Up-Fw | CGTGAGTTGGCGGCTCTG |
| 1993c-Up-Rv-Zeo | CAGTCGATCCACGTGGAGGTAAC**CAC**GGTCAGTTCTCC |
| 1993c-Zeo-Dw-Fw | CCACTGAGCGTCAGACCCACGTGCTCCGAG**TGA**CGACTGTAGTTGACGCCGAG |
| 1993c-Dw-Rv | CAGCGGATAAGCCCATGCGG |
| 1993c-int | CAGGCCCCACTCCATCAC |
| 3269-Up-Fw | AGCGATACTCGACGATTC |
| 3269-Up-Rv-Zeo2 | CAGTCGATCCACGTGGAGGC**CAT**TGCCTGTACCTTTCTTCC |
| 3269-Zeo-Dw-Fw | CCACTGAGCGTCAGACCCACGTGCTCCCACTGAGCGCCTCGCCATGACC |
| 3269-Dw-Rv | TTCTCGCGCAGGATGAGGCTTG |
| 3269-int | CACGTCGGCCACCTTTAG |

a Blue letters show nucleotides specific of the right or left end of the zeocin-resistance cassette. Nucleotides specific of the start or stop codon of the ORF of interest are in bold letters.