Supplemental Methods

Mini-Tn*7* Derivative Construction and Characterization. Mini-Tn*7* derivatives for constitutive expression of fluorescent proteins in *S. oneidensis* were constructed as follows. The *Eco*RI-*Xba*I mini-Tn*7*KmΩSm1 fragment from pBK-miniTn*7*-KmΩSm1 (**Koch et al. 2001**) was cloned into the *Eco*RI site of the small mobilizeable R6K-based plasmid pUX66 (D. Lies and G. Roberts, unpublished), after the digested plasmid and insert fragment were treated with Klenow DNA polymerase to generate blunt ends, to create plasmid pURR21. The *Not*I fragment from pBK-miniTn*7*-*gfp*3 (**Koch et al. 2001**) containing the *gfp*mut3* gene expressed from the *lac* $P_{A1/04/03}$ promoter derivative was cloned into the *Not*I site of pURR21 to generate the transposon mini-Tn*7*-KSGFP and the plasmid pURR25. Mini-Tn*7*-KSGFP contains the *Not*I fragment after the Ω cassette in the transposon while mini-Tn*7gfp*3 has the *Not*I fragment between the Ω cassette and the kanamycin resistance cassette. An ECFP expression cassette was constructed by cloning the pURR25 *Not*I fragment onto a version of pOK12 (**Vieira and Messing 1991**) lacking *Sph*I and *Hind*III sites and replacing the *gfp*mut3* gene from this fragment with an *ecfp* gene amplified by PCR from pMP4641 (**Bloemberg et al. 2000**) using ECFPN (5'-GACCGCATGCTGAGCAAGGGCGAGGAGCTG-3') and ECFPC (5'-GGTGAAGCTTACTTGTACAGCTCGTCCATGCC-3') primers. This ECFP expression cassette was cloned as a *Not*I fragment into pURR21 to generate the transposon mini-Tn*7*-KSCFP and the plasmid pURR27.

The plasmid pURR21 and its derivatives were constructed to provide a mini-Tn*7* and fluorescent protein gene delivery vehicle with a plasmid R6K-based origin of

replication. We examined the utility of these plasmids in *S. oneidensis* using donor strains containing pURR25 compared with the pMB1-based plasmid pBK-miniTn*7*-*gfp*2, which was previously used as a mini-Tn*7* donor to produce GFPmut3* expression in *S. oneidensis* MR-1 (**Thormann et al. 2004**). The *E. coli* donor strain WM3064 (**Saltikov and Newman 2003**) carrying either plasmid was mated with *S. oneidensis* MR-1 using a 1:5 donor:recipient ratio and incubated for 5h at 30ºC on LB agar plates containing 300 μM diaminopimelic acid, in the presence or absence of WM3064 containing the transposition helper plasmid pUX-BF13 (**Bao et al. 1991**). In the absence of pUX-BF13, matings between WM3064(pBK-miniTn*7*-*gfp*2) and MR-1 yielded *S. oneidensis* transconjugants resistant to 15 μg/ml gentamicin at frequencies of 10^{-5} per recipient. Plasmid extractions from 12 of these transconjugants all contained plasmid DNA of the same size as pBK-miniTn*7*-*gfp*2. Similar matings in the presence of pUX-BF13 yielded the same number of transconjugants and also contained additional plasmid DNA. Matings performed with WM3064(pURR25) and *S. oneidensis* MR-1 in the absence of pUX-BF13 yielded transconjugants resistant to 50 μg/ml kanamycin at frequencies of 10^{-9} per recipient. Matings between WM3064(pURR25), WM3064(pUX-BF13) and *S. oneidensis* MR-1 yielded kanamycin-resistant transconjugants at frequencies of 10^{-5} per recipient. No additional plasmid DNA was detected in plasmid extractions from two transconjugants from matings lacking pUX-BF13 or from five transconjugants from matings in the presence of pUX-BF13.

These results indicate that pBK-miniTn*7*-*gfp*2 replicates in *S. oneidensis* MR-1 and cannot easily be used for delivery of mini-Tn*7* derivatives at single copy into the genome of MR-1. We have confirmed this observation with seven other derivatives of

this group of mini-Tn*7* delivery plasmids reported by **Lambertsen et al. 2004** and have demonstrated that the plasmid pRK2013 is also maintained in *S. oneidensis* MR-1. Thus, contrary to a previous report (**Myers and Myers 1997**) but consistent with a more recent report (**Groh et al. 2005**), we find that pMB1- and ColE1-based plasmids replicate in MR-1. Plasmid R6K-based derivatives such as those constructed here and reported elsewhere (**Choi et al. 2005**) are more useful vehicles for delivery of mini-Tn*7* derivatives.

Tn*7* inserts in most Gram-negative bacteria in a site- and orientation-specific manner downstream of the *glmS* gene (**Craig 1989; Choi et al. 2005**). We analyzed 20 MR-1 transconjugants containing mini-Tn*7*-KSGFP by PCR using transposon-specific primers (Tn7aphAUp: GCCAGTTTAGTCTGACCATCTC and Tn7catEndDown: TGTCGGCAGAATGCTTAATGA) and primers targeting *glmS* and the downstream gene *menB* from MR-1 (MR1glmSDown: CGCCACTGATTTACACTATCCC and MR1menBUp: CGATCAAGACTTCTCAGCCTTC). Specific PCR products for all 20 transconjugants could be obtained with the primer pairs MR1glmSDown-Tn7catEndDown and Tn7aphAUp-MR1menBUp but not with other combinations of these primers. Only the MR1glmSDown-MR1menBUp primer pair yielded a specific PCR product from wild-type MR-1 DNA, which was absent in the transconjugants presumably because of the large size of the inserted transposon. These results indicate that Tn*7* inserts between *glmS* and *menB* in *S. oneidensis* MR-1, oriented with the Tn*7*R end near *glmS* and the Tn*7*L end near *menB*. We sequenced the specific upstream and downstream PCR products from five of the mini-Tn*7*-KSGFP transconjugants to determine the specific insertion site for the transposon in MR-1. In all five of the

transconjugants, mini-Tn*7*-KSGFP inserted following base pair (bp) 4951342 of the MR-1 genome sequence (NCBI accession number AE014299.1) and duplicated the six-bp sequence GCCAGT (bp 4951347-4951342 of the MR-1 genome). This sequence is part of an imperfect 42 bp inverted repeat presumably capable of forming a stem-and-loop structure and putatively serving as the *rho*-independent transcriptional terminator of *glmS*, which would be interrupted by insertion of the transposon.

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