Supplemental materials and methods

Bacterial strains and plasmids. *Escherichia coli* strain DH5α (1) containing conjugative plasmid pJC01 was used as donor strain and streptomycin-resistant derivative *E. coli* BL21 (DE3) (2) was used as recipient strain expressing T7 RNA polymerase in HTC assay. *E. coli* DH5α (1) containing different conjugative and mobilizable plasmids (Table S1) and a rifampicin-resistant derivative of *E. coli* MDS52 (3) were used as donor and recipient strains respectively in plate-conjugation assays. *Salmonella enterica* Serovar *typhimurium* SV4522 was used as donor strain of a kanamycin-resistant derivative of pSLT plasmid (4). *Acinetobacter baumannii* ATCC 19606 (provided by Hospital de Valdecilla, Spain), *Vibrio cholera* N16961 (provided by Didier Mazel, Institut Pasteur, France), *Salmonella enterica* Serovar *typhimurium* SV4939 (provided by Dr. J. Casadesús, Universidad de Sevilla, Spain), *Agrobacterium tumefaciens* C58 (provided by Dr D. Pérez-Mendoza, Estación Experimental del Zaidín, Spain) and *Pseudomonas putida* KT2440 (provided by Dr. F. Rojo, Centro Nacional de Biotecnología, Spain) were used as donor strains of R388. Nalidixic and rifampicin-resistant derivatives of *E. coli* BW27783 (5) were used as donor (containing R1drd19 plasmid) and recipient strains respectively in liquid matings. *E. coli* DH5α (1) containing pHP161 (6) and pKM101ΔMOB (7) was used as donor strain to combine *oriT*-MOB of R388 and MPF apparatus of pKM101, respectively.

Reagents. When appropriate, antibiotics (Apollo) were added at the following concentrations: ampicillin sodium salt (Ap; 100 μg/ml), chloramphenicol (Cm; 25 μg/ml), nalidixic acid (Nx; 20 μg/ml), rifampicin (Rif; 50 μg/ml), streptomycin (Sm;

300 µg/ml), tetracycline (Tc; 10 µg/ml) and trimethoprim (Tp; 10 µg/ml). DMSO (Sigma-Aldrich) was used as solvent and IPTG (Sigma-Aldrich) as T7 RNA polymerase inductor. Bacterial cultures were set up in LB-broth and LB-agar (Pronadisa). M9 broth (Sigma-Aldrich) were used to resuspend bacteria after mating and perform serial dilutions.

Construction of pJC01. The *Xba*I fragment of plasmid pUA66 (8) encoding the GFPmut2 protein (9) was inserted into the *Xba*I site of plasmid pET3a (Novagen) to obtain pETGFP3, where the *gfpmut2* gene is under the control of the T7 φ10 promoter. Then, the chloramphenicol resistance gene (Cm^r) from pSB1C3 (http://parts.igem.org/Part:pSB1C3) was amplified by PCR using the primers Cm^r sense (CGTAAGATCTTCCAACTTTCACCATAATG) and Cm^r antisense (AGCTAGATCTCAAATTACGCCCCGCCCTG), which introduce *Bgl*II sites at both ends of the amplicon. The Cm^r fragment was digested with *Bgl*II and inserted in the same site of pETGFP3 (flanking the *gpfmut2* gene in a divergent orientation) to obtain the construct pETGFP-Cm6. This plasmid was used as template to amplify the region containing the adjacent (and divergent) genes *gfpmut2* and Cm^r by PCR using the primers Sense-EcoRI (CAGCGAATTCAGCTTCCTTTCG GGCTTTG) and Antisense-SacI (AGTGGCGAGCTCGATCTTCCCCATCGGTG). Subsequently, the amplified *gfpmut2-*Cm^r cassette was digested with *EcoRI* and *Sac*I enzymes and inserted into the conjugative plasmid R388 (10), previously digested with the same enzymes, to obtain the final construction pJC01.

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