Supplementary Materials and Methods

Details of the strains construction

To construct the *S. coelicolor* strain carrying the *tetO* array, first we constructed a Tn*5* derivative, Tn*5431*, containing 120 tandemly arrayed copies of *tetO*, an apramycin resistance gene and an *oriT* site to facilitate delivery of the transposon to *S. coelicolor* following *in vitro* transposition to *S. coelicolor* cosmids [1,2]. To construct Tn*5431*, a 4400 bp fragment containing 120 *tetO* cassettes was excised from pLAU44 [3], a kind gift from Prof. David Sherratt, with EcoRI/BglII and ligated into the EcoRI/BamHI sites of pUC18, creating pUC44. The *apra-oriT* cassette was excised from pIJ773 [4] using EcoRI/HindIII and ligated into the same sites of pUC44 to create p44FRT. This plasmid was digested with NheI/HindIII to excise the *tetO-apra-oriT* fragment that was ligated into pMOD<MCS> digested with XbaI/HindIII. This created p44FMOD that carried the transposon Tn*5431* that was excised from p44FMOD using PvuII and used for *in vitro* transposition [1]. Cosmid StH18 [2,5], encompassing the *oriC* region (position 4269844-4270777 of the *S. coelicolor* chromosome) was selected for tagging with Tn*5431* by *in vitro* transposition [1]. Transposed cosmids were recovered by transformation of *E. coli* JM109 (selecting for apramycin and kanamycin resistance). Tn*5431* insertion sites were determined by DNA sequencing of transposed cosmids using sequencing primer EZR1 [1]. One transposed cosmid, termed EJTH31A, carried a copy of Tn*5341* integrated ~29 kb from *oriC* region (position 4240808 of the *S. coelicolor* genome; this position refers to the first base of the duplicated CCGTAGAGC Tn*5* target site), in the intergenic region between SCO*3855* (possible membrane protein) and SCO*3856* (probable peptidyl-prolyl cis-trans isomerase). The insertion was 956 bp upstream start codon of SCO*3855* and 575 downstream stop codon of SCO*3856,* and was expected not to interfere with neighbouring genes. This cosmid was selected for construction of an *oriC* tagged strain of *S. coelicolor* by intergeneric conjugation [6]. Analysis of transconjugant colonies showed that about 10% had the apra^{R}, km^{S}, phenotype that was indicative integration of Tn*5431* into the M145 chromosome via a double recombination event (S1A Fig). One of the transconjugants, termed EJTH31, was used for the subsequent introduction of *tetR-mcherry*.

To construct the *tetR-mcherry* fusion the *mCherry* gene was amplified from pRSET*cherry* (a kind gift from Prof. Roger Tsien) with primers cherry_f and cherry_r (S2 Table) and cloned between the NdeI and NsiI sites of pMS83 containing the *tetR* gene under the control of constitutive promoter from phage II9 SF14 [7]. EJTH31 was used to integrate pMS83-mCherry plasmid (in $att_{\varphi BT1}$ site) to yield the strain DJ-NL102 (FROS strain).

The cosmid EJTH31A and integrating plasmid pMS83-*mCherry* were introduced into *S. coelicolor* J3310 (*parB-egfp*)[8]; *S. coelicolor* J3305 (Δ*parB*)[9], *S. coelicolor* J3306 (Δ*parA*)[10], *S. coelicolor* J3337a (*DnaN-egfp*) and *S. coelicolor* J3336a (*DnaN-egfpΔparA*) (see below). Apra^{R}, km^{S},

hyg^R exconjugates were selected indicating a double recombination of Tn*5431* and integration of pMS83-mCherry. The obtained strains were verified by PCR and Southern hybridisation. Additionally, SDS-PAGE analysis confirmed the production of the TetR-mCherry fusion protein (S1 Fig).

To introduce Tn*5431* to *S. coelicolor* expressing *dnaN-egfp* we modified J3337 [11] and J3336 (constructed by recombination of J3306 (Δ*parA*) strain with H18 *dnaN-egfp-apra* cosmid), to remove the apramycin resistance cassette, yielding strains J3337a and J3336a. The apramycin cassette was removed from the cosmid H18 *dnaN-egfp-apra* [11] by using FLP recombinase to yield StH18*dnaN-egfp*. Subsequently the *amp* gene in SuperCos of StH18*dnaN-egfp* was exchanged for *apra-oriT* cassette (amplified from pIJ773 with primers Blap1apraFW and Blap1apraRV). This construct was used for conjugation into *S. coelicolor* J3337 and J3336. Exconjugants were screened for the loss of both apra^R and Km^R, indicating a double cross-over allelic exchange of *dnaN* locus. The obtained strains J3337a and J3336a were verified by PCR using primers DnaNspr_fw and DnaNspr_rv (S2 Table). The presence of DnaN-EGFP in *S. coelicolor* cell extracts was examined by scanning SDS-PAGE gel with a scanner equipped with 488-nm laser.

In order to construct DJ598 strain (*parA$_{mut}$, parB-egfp*), *egfp–apra* cassette was amplified using primers ParBgfplink and Bdownrev, as described before (S2 Table) [8] and was used to target the cosmid H24*parA S249Y,E250V* [12] downstream of *parB* gene. The cosmid H24*parA S249Y,E250VparB-egfp-apra* was used for conjugation into *S. coelicolor* strain BD08 [12]. Apra^R exconjugants were screened for the loss of km^R, indicating the presence of a double cross-over allelic exchange, yielding DJ598 strain (S1 Table).

To obtain the strain overproducing ParA (DJ532) we used the construct pIJ6902*parA* in which *parA* was expressed from the thiostrepton-inducible *tipA* promoter [13]. The pIJ6902*parA(hyg)* plasmid was introduced into *S. coelicolor* J3310 (*parB-egfp*) [8], yielding DJ532 (S1 Table). The increased level of ParA (about 4 times exceeding wild type level) for cultures grown in presence of thiostrepton (5μg/ml) was confirmed by Western blot with antibodies against ParA.

Strain BD05 was obtained by recombination of K112 [14] with H24 *parB-egfp-apra* cosmid and apra^R exconjugants were screened for the loss of km^R, indicating the presence of a double cross-over allelic exchange.

AK101 strain was constructed by recombination of J3310 strain (*parB-egfp*) with H18 *dnaN-mcherry-apra* cosmid. First the *mcherry-apra* cassette amplified with primers DnaNRSwa and DnaNF and recombined with H18 using PCR targeting. H18 *dnaN-mcherry-apra* cosmid was introduced to J3310 strain and apra^R exconjugants were screened for the loss of km^R, indicating the presence of a double cross-over allelic exchange. The presence of DnaN-mCherry in *S. coelicolor* cell extracts was examined by scanning SDS-PAGE gel with a scanner equipped with 488-nm laser.

References

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