

Title: Supplementary Movie 1

Description: Transconjugant viability following acquisition of the *orf20* mutant plasmid under selective pressure. Donor and recipient cells were mixed for 4 h on a filter before being imaged every 10 min by time-lapse microscopy, with a continuous flow of fresh medium supplemented with ampicillin. Recipient cells, exhibiting red mCherry fluorescence, are sensitive to ampicillin. In the absence of complementation of the *orf20* mutation by the pOrf20 plasmid (R without pOrf20, right panel), some recipient cells appear elongated with an increased width, continuing to elongate over time before undergoing bulging and eventual cell lysis. Non-fluorescent donor cells and transconjugants (which exhibit both mCherry and sfGFP fluorescence) are resistant to ampicillin due to expression of the *blaOXA-48* gene encoded by pOXA-48. Donors *E. coli* *ilvA::PBiofab-tetR* / pOXA-48- Δ *orf20*-PtetR-sfGFP/ pOrf20 (LY4161); recipients left panel: *E. coli* *ilvA::Ptac-mcherry* / pOrf20 (LY4140), right panel *E. coli* *ilvA::Ptac-mcherry* (LY1593). Scale bar: 10 μ m

Title: Supplementary Data 1

Description: Tn-seq data analysis