Supplementary Text S6 Determination of tetracycline concentration by high-performance liquid chromatography

Standard tetracycline solutions (10, 20, 30, 40, and 50 mg/L) were prepared and sterilized by filtration through a 0.22 μm membrane. Two genetic evolution systems were established in PBS buffer: one with *E. coli* MG1655 carrying the RP4 plasmid, and the other with *E. coli* MG1655 harboring the pWH1266 plasmid, both of which were exposed to 20 mg/L tetracycline. Both systems were incubated at 30°C for 24 h. A defined volume of the culture was filtered through a 0.22 μm membrane to obtain the tetracycline-containing sample solution. Tetracycline concentrations were determined using high-performance liquid chromatography (HPLC) equipped with a C18 column, with a mobile phase of methanol and 0.02 mol/L KH_2PO_4 (12:88, v/v). Column temperature was maintained at 40°C, with a flow rate of 1.0 mL/min and an injection volume of 10 μL. A gradient elution program was applied, and UV detection was performed at 280 nm.