A Novel Fluorescent Reporter Side-by-Side Assay for Detecting Plasmid-Mediated Gene Transfer in Bacteria

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This study describes the design of an engineered fluorescent reporter system to detect plasmid co-residence resulting from horizontal gene transfer (HGT) within individual bacterial cells. The system uses a split-GFP strategy in which two compatible plasmids encode complementary fragments of green fluorescent protein (GFP1–10 and GFP11). Fluorescence occurs only when both plasmids are present in the same cell, allowing the system to function as a biological “AND-gate” reporter of plasmid co-residence consistent with horizontal gene transfer. Plasmid constructs were designed and verified computationally to ensure sequence integrity, compatible replication origins, and independent antibiotic resistance markers. Following commercial synthesis, plasmid identity was confirmed through Sanger sequencing and restriction digest simulations. Individual plasmids were successfully introduced into Escherichia coli HB101 and maintained under selective conditions. Sequential transformation experiments demonstrated that dual-plasmid co-residence could be established, although recovery depended on plasmid introduction order. Growth analysis using OD600 measurements showed that cultures maintaining both plasmids reached less than half the final density of plasmid-free controls, indicating a measurable physiological burden. This reduced growth highlights a system constraint but does not prevent stable co-residence, the structural requirement for reporter function. Together, these results demonstrate that the system supports dual-plasmid maintenance in a single host cell, providing foundational validation of the reporter architecture and a basis for further optimization toward visualization of horizontal gene transfer events.

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