SUPPLEMENTARY METHOD

Plasmid construction. The pHL1277 plasmid was constructed by inserting the galactose operon (from -75 to +4333) between the *Eco*RI and *Bam*HI sites of pCC1BAC (Epicenter Biotechnologies, USA). PCR primers used to amplify the galactose operon from genomic DNA are listed in Table S1. For site-directed mutagenesis, synthetic primers containing the desired mutations were designed. After obtaining the DNA fragments containing different mutants of the *gal* operon through PCR amplification of the pHL1277 plasmid, the DNA fragments were used as a “mega primer” for the next round of PCR (1). The resulting PCR fragments were digested with *EcoR*I and *Hind*III and then ligated into pHL1277 (2) to generate pHL1750 (*EHMM1*), pHL2185 (*E-Hairpin-restored*), pHL1930 (*DH1200*), pHL2088 (*galT-SD1/ DH1200*), pHL2089 (*galT-SD2/ DH1200*), pHL2090 (*galT-SD3/ DH1200*), pHL1657 (*galE* stop° mutant) and pHL1931 (*galE* stop°*/ DH1200*). For the construction of pausing mutants -1G, -1A, -1G -10A, and -1A -10A (Fig 7B), the DNA fragments containing different mutants of the *gal* operon through PCR amplification of the pHL1277 plasmid were obtained and ligated between *EcoRI* and *HindIII* of pHL1277, generating plasmids pHL1963, pHL1965, pHL2253, and pHL2252.

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