# Optimized incorporation of a fluorescent unnatural amino acid into T7 DNA polymerase provides a signal to measure conformational dynamics governing high-fidelity DNA replication

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**Supplementary figures S1- S5**

**Other SI files:**

peptide_table.xlsx – list of matched peptides from MS/MS data for T7 DNA polymerase E514Cou variant

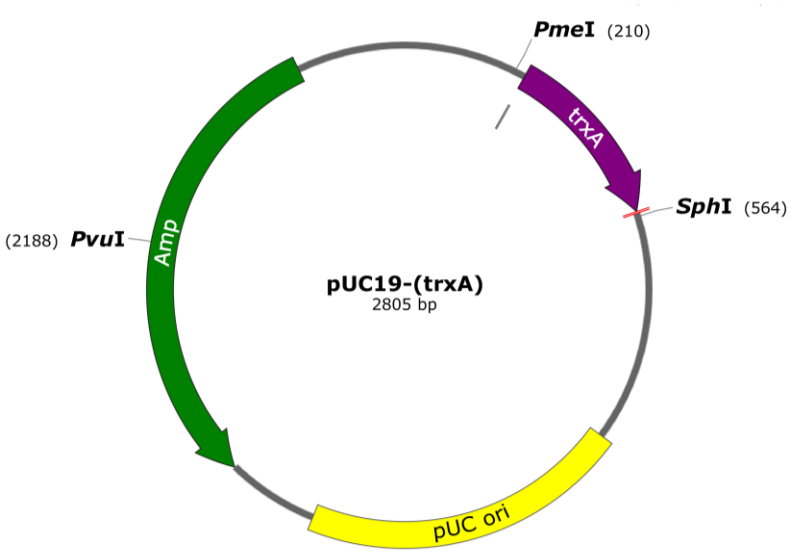


**Figure S1:** Plasmid map for pUC19-(trxA): This plasmid was the source for the trxA gene (purple) used to create the bicistronic expression plasmid pcI$^{ts}$-(T7 G5x-, trxA). Also contains the ampicillin resistance gene (Amp, green) and pUC origin of replication (pUC ori, yellow).

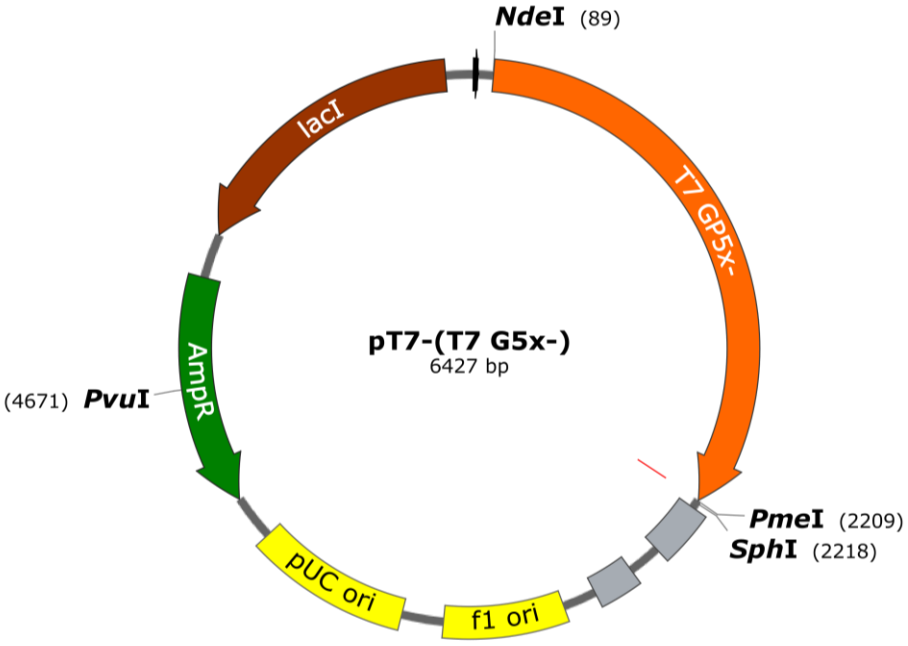


**Figure S2:** Plasmid map for pT7-(T7 G5x-): This plasmid was the source for the exonuclease deficient T7 gene 5 (T7 GP5x-, orange) used to create the bicistronic expression plasmid pcI$^{ts}$-(T7 G5x-, trxA). T7 gene 5 is under the T7 promoter (black). Also contains the ampicillin resistance gene (AmpR, green), lacI gene (brown) and pUC and f1 origins of replication (yellow).

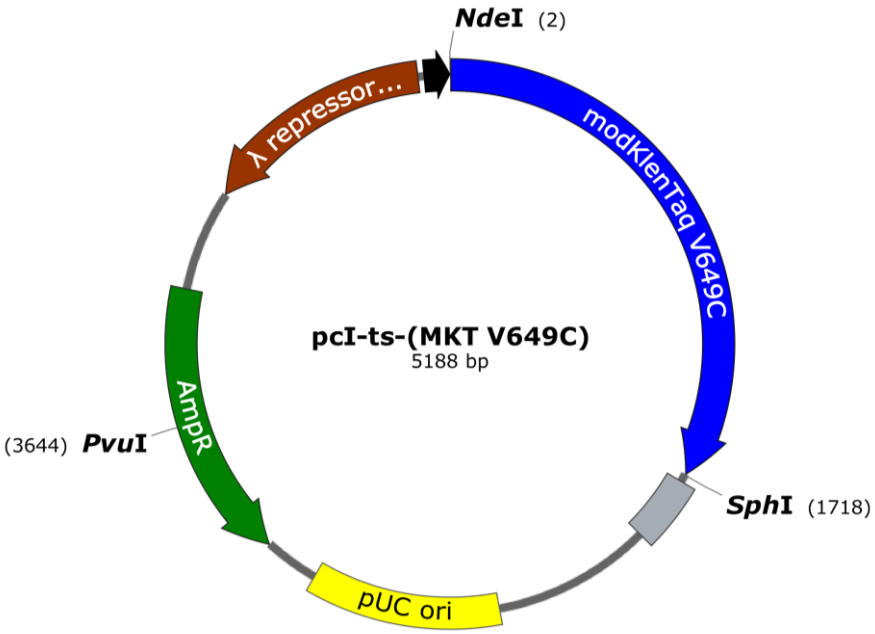


**Figure S3:** Plasmid map for pcI$^{ts}$-(MKT V648C): This plasmid was the source for the plasmid backbone containing the heat inducible λ promoter system used to create the bicistronic expression plasmid pcI$^{ts}$-(T7 G5x-, trxA). The λ promoter (black) drives expression of ModKlenTaq (blue), and the λ cI$^{ts}$ repressor (brown). Also contains the ampicillin resistance gene (AmpR, green), pUC origin of replication (yellow), and *E. coli* rrnBT1T2 transcription terminator (grey).

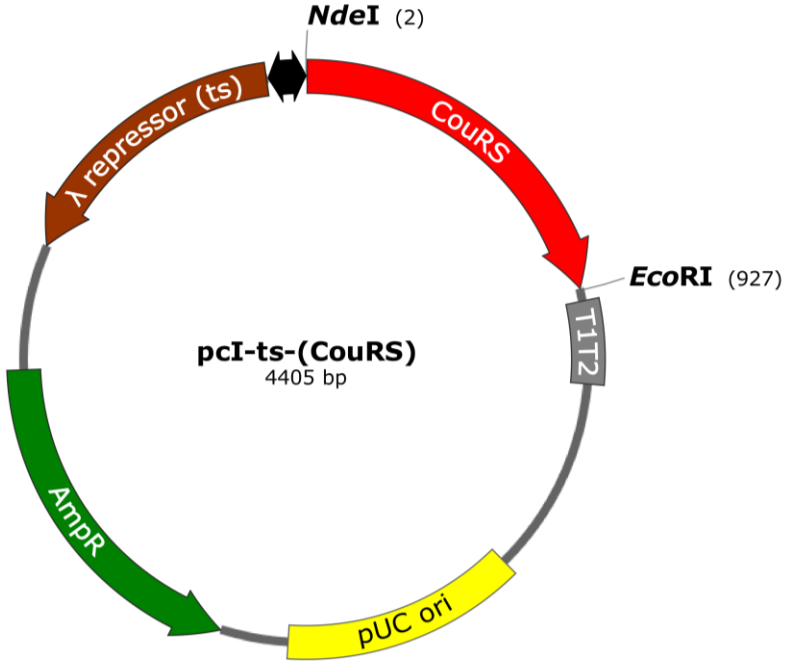


**Figure S4:** Plasmid map for pcI$^{ts}$-(CouRS): This plasmid has the CouRS gene (red) cloned into the pcI$^{ts}$ backbone which was used to add an inducible copy of CouRS into the amber suppression plasmids. Other features are the same as in Figure S3.

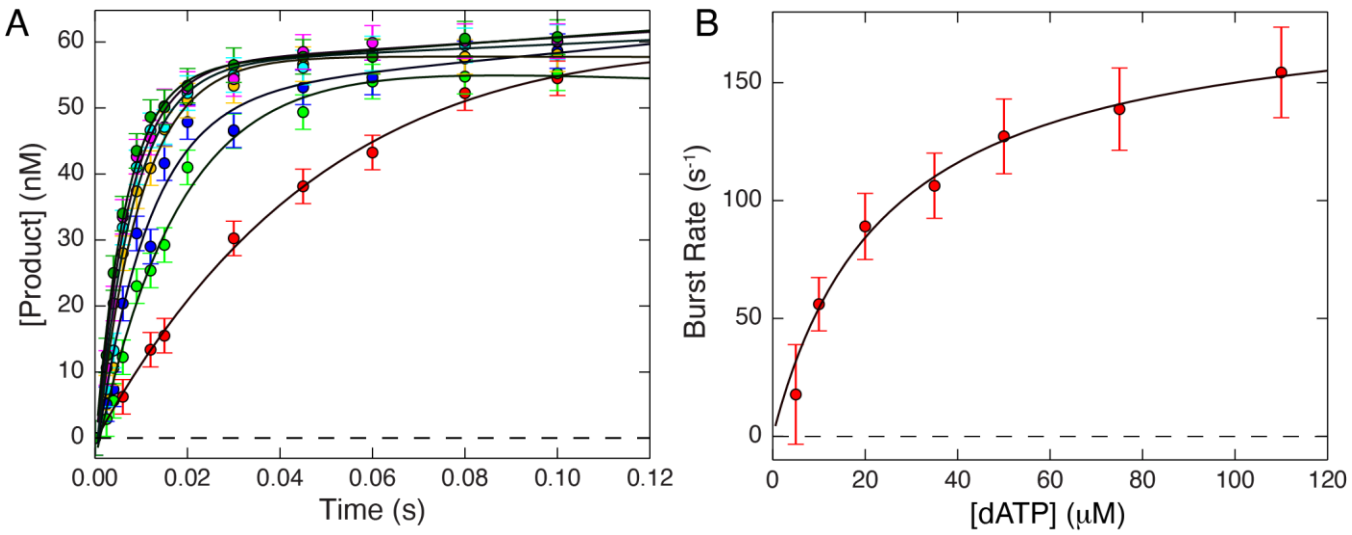


**Figure S5**. Conventional equation-based data fitting. (A) Data given in Figure 9A were fit using a burst equation: $Y = A_0 + A_1(1 - e^{-b_1 t}) + b_2 t$, illustrated by the black lines. Note the fluctuations of fitted amplitude and negative linear phase in some of the fitted curves compared to the global fitting in Figure 9A. (B) The dATP concentration dependence of the observed burst rate was fit to a hyperbolic function to get $k_{pol}$ = 186 ± 26 $s^{-1}$ and $K_{d,app}$ = 24 ± 9 μM, affording calculation of $k_{pol}/K_{d,app}$ = 8 ± 3 $\mu M^{-1}s^{-1}$.