Supporting Information

Voytek and Joyce 10.1073/pnas.0903397106

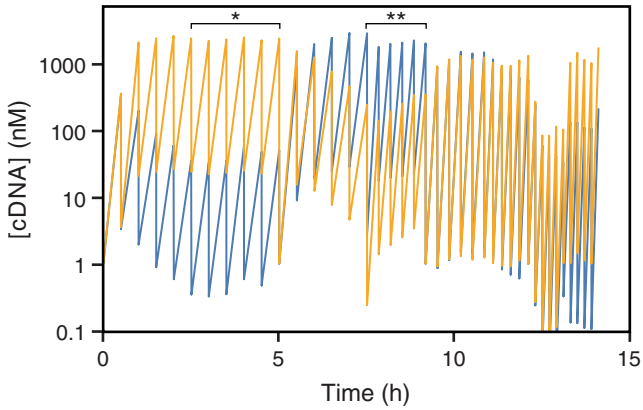

Fig. S1. Time course of 40 transfers of continuous coevolution using a single substrate. The concentration of the CL1 (orange) and DSL (blue) enzymes was determined (based on cDNA) before and after each transfer. Single and double asterisks denote periods when anti-CL1 and anti-DSL antisense oligos, respectively, were present in the reaction mixture.

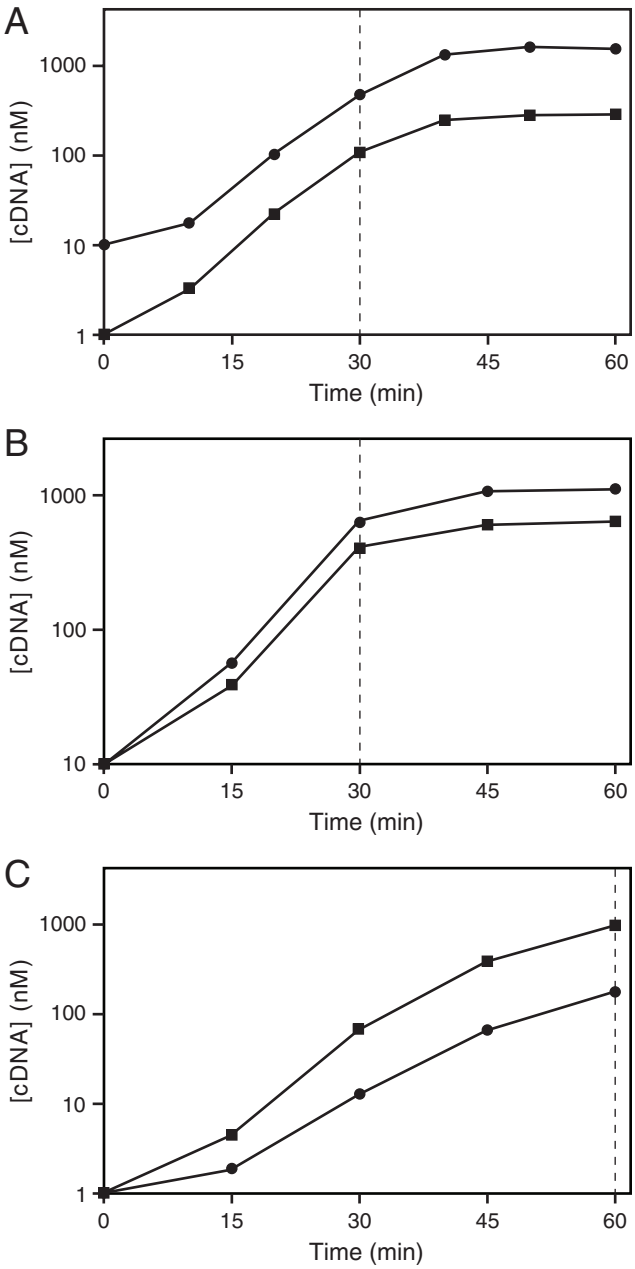

Fig. S2. Representative amplification profiles (based on cDNA) of the CL1 (circles) and DSL (squares) enzymes at the initiation of the coevolution experiment employing 5 substrates (*A*), following transfer 11 (*B*), and following transfer 25 (*C*). Dashed lines indicate the time at which dilution for the next transfer was performed.

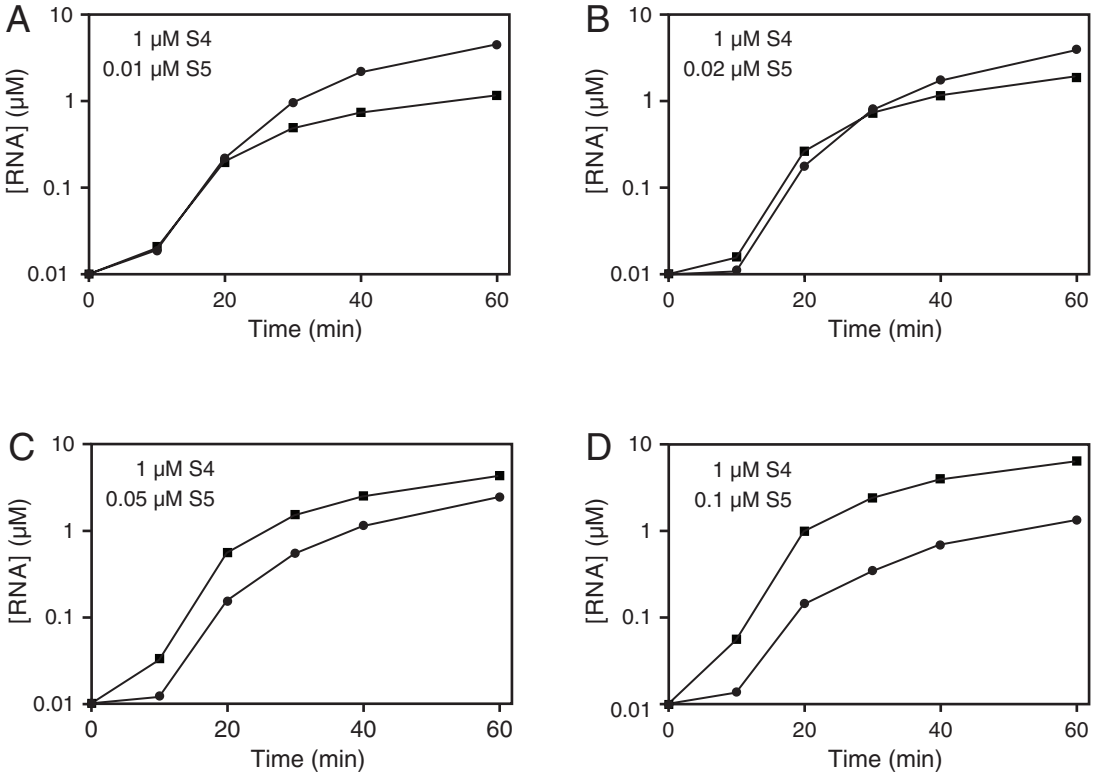

Fig. S3. Amplification profiles of the CL1 (circles) and DSL (squares) enzymes in a common reaction mixture containing 1 μM S4 and varying amounts of S5. The starting concentration of each enzyme was 0.01 μM. Concentrations were measured over the course of the reaction based on incorporation of [α-^{32}P]ATP into newly synthesized RNAs.

Table S1. Substrates and corresponding promoter strengths

Substrate	Promoter sequence	Transcription rate, nM min^{-1}	Relative efficiency
S0	TAATACGACTCACUAUU	3.8	1.00
S1	TAATACGACTC**G**CUAUU	0.41	0.11
S2	TAATACGACTCACU**G**UU	0.34	0.09
S3	TAATACGACTCACUA**C**U	0.75	0.20
S4	TAATACGACTCACUAU**C**	2.4	0.63
S5	TAATACGACTCACU**U**UU	0.89	0.23

Sequence of the 17-nucleotide T7 RNA polymerase promoter region of substrates used in continuous coevolution. The S0 promoter conforms to the ϕ2.5 consensus sequence (1); alternative promoter sequences were chosen based on Imburgio et al. (2). Nucleotides in bold indicate point mutations relative to S0. Transcription rate was measured in a reaction mixture that mimicked continuous evolution conditions, employing 10 nM RNA/cDNA heteroduplex template corresponding to the CL1 enzyme. Linear regression coefficients from 6 timepoints were in the range of 0.975–0.999.

1. Dunn JJ, Studier FW (1981) Nucleotide sequence from the genetic left end of bacteriophage T7 DNA to the beginning of gene 4. *J Mol Biol* 148:303–330.
2. Imburgio D, Rong M, Ma K, McAllister WT (2000) Studies of promoter recognition and start site selection by T7 RNA polymerase using a comprehensive collection of promoter variants. *Biochemistry* 39:10419–10430.