Supplemental Material

Gel shift assay

The Double-stranded artificial DNA binding sequence for the HDH-ADB1, HK-ADB2, and TS-ADB3 of the pSC-1 plasmid was produced by PCR amplification of pSC-1 plasmid template with oligonucleotides end-labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase (New England Biolabs, Beverly, MA, USA). PCR products were purified and incubated with 10 μg of each ADB-fused enzyme (HDH-ADB1, HK-ADB2, or TS-ADB3) in binding buffer [4 mM Tris-HCl (pH 7.5), 80 mM NaCl, 0.5 mM $ZnSO_4$, 1 mM EDTA, 0.5 mM DTT, 5% glycerol] at 25°C for 30 min. Samples were electrophoresed for 3 hours at 100 V on a nondenaturing polyacrylamide gel (6% [29:1] acryl-bisacrylamide, 0.25× TBE, 5% glycerol).

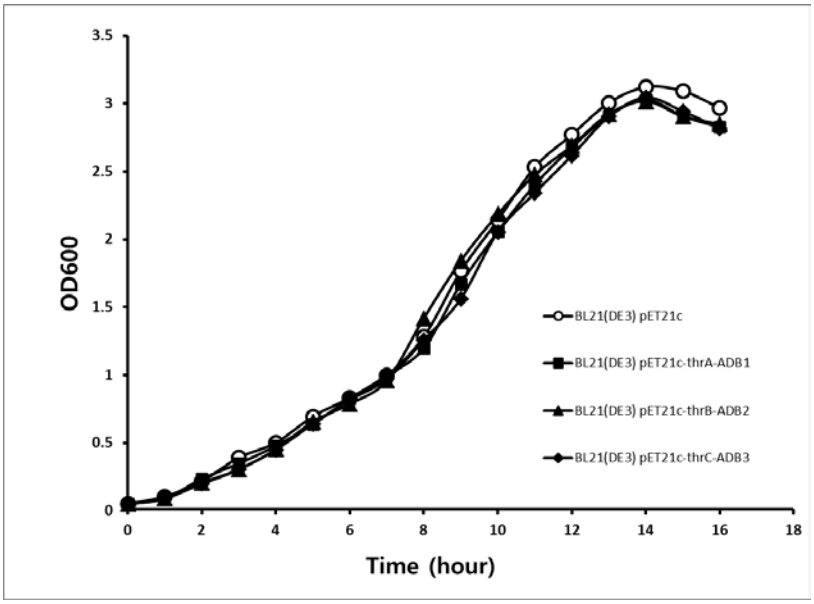

Figure S1. Effect of ADB-fused enzyme overexpression on the growth of *Escheichia coli*. Cells harboring each plasmid (pET21c-thrA-ADB1, pET21c-thrB-ADB2, or pET21c-thrC-ADB3) for the expression of ADB-fused enzymes (HDH-ADB1, HK-ADB2, or TS-ADB3, respectively) were cultivated in a 500 ml flask containing 100 ml of LB medium supplemented with 0.1 mM of IPTG. During the cultivation, the growth of cells was measured every hour by spectrophotometry.

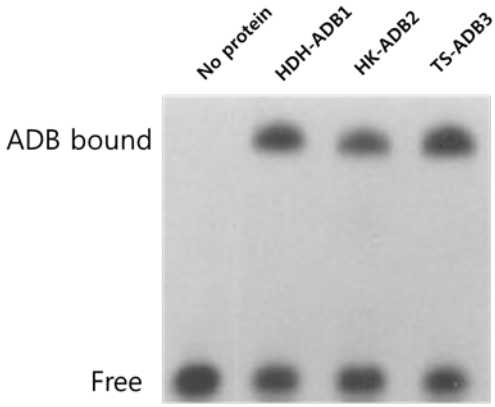

Figure S2. Binding of the ADB-fused enzymes (HDH-ADB1, HK-ADB2, and TS-ADB3) to their target DNA sequences. The binding of ADB-fused enzymes to their target sequences were confirmed by a gel shift assay using the 5' end-labeled target DNA sequence of the pSC-1 plasmid (refer to the gel shift assay in the Supplemental Material).

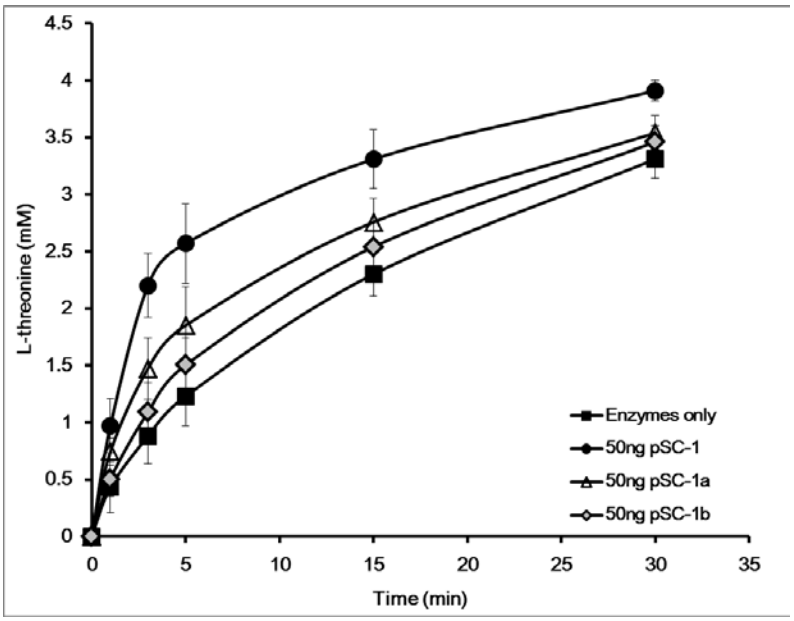

Figure S3. ***In vitro*** **L-threonine production assay.** Threonine production rates from the three different scaffold plasmids (pSC-1, pSC-1a, and pSC-1b) which have different distances (20-, 15-, 25-bp, respectively) between the binding sites of ADB-fused enzymes were measured by the *in vitro* enzymatic conversion of aspartate semialdehyde to threonine (refer to Materials and Methods). The data shown are the means for three independent experiments.