AEM-AEM05257-11-s01.pdf. Central nitrogen metabolism in *E. coli* (Fig. S1); calculated cellular percentage of total 2OG amount by two theoretical subtraction strategies (Fig. S2); data fitting of external 2OG concentration with a model including leakage and active uptake (Fig. S3); alignment of *kgtP* promoter and coding regions (Fig. S4).

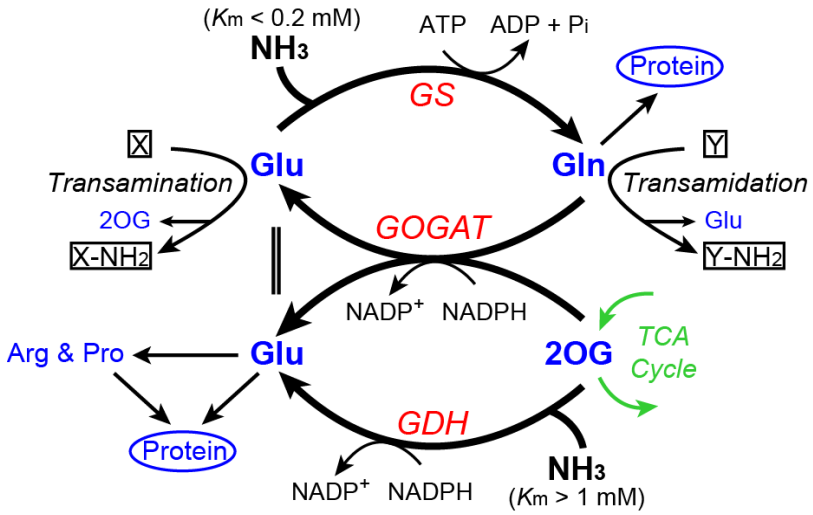

FIG. S1. Central nitrogen metabolism in *E. coli*. The circuit consists of three enzymes: GS, GOGAT, and GDH. Gln and Glu are the only two central nitrogen intermediates. 2OG comes from the TCA cycle and serves as the sole carbon skeletal substrate of the circuit. Boxed X and Y represent metabolic recipients of the amine from Glu and the amide from Gln, respectively. 2OG molecules are nitrogen carriers of the system. They are mostly recycled through transaminations. Some are incorporated into protein in residues of glutamine, glutamate, arginine, and proline.

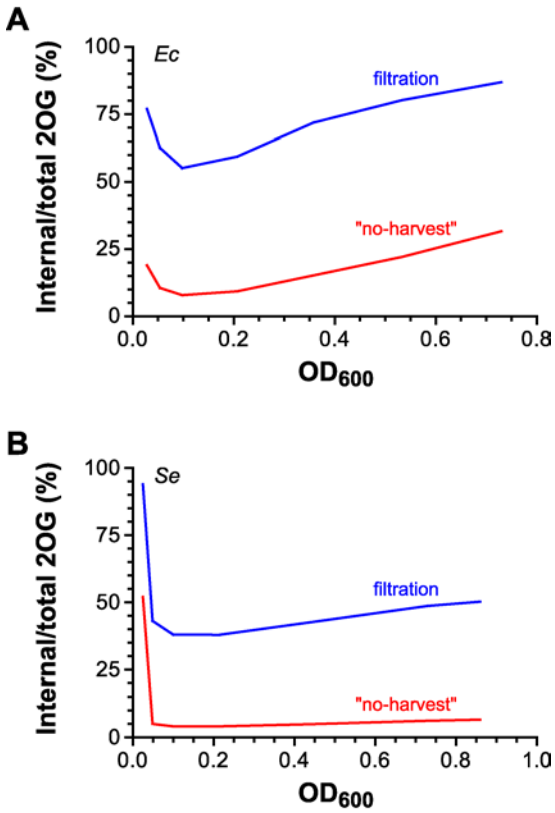

FIG. S2. Calculated cellular percentage of total 2OG amount by two theoretical subtraction strategies. (A) Estimate based on the external 2OG data of *E. coli* culture in Fig. 2A and an internal concentration of 0.5 mM. (B) Estimate based on the external 2OG data of *Salmonella* serovar Typhimurium culture in Fig. 6B and an internal concentration of 1.3 mM. External amount of 2OG is from real data and internal amount of 2OG is from the average of pool concentration. Total amount is the calculated sum of internal plus external amount. The "no-harvest" line illustrates percentage estimate from hypothetical "no-harvest" samples, which represent direct acid extraction of 100 µl whole culture containing both cells and medium. The filtration line illustrates percentage estimate from hypothetical filtration samples, which represent 1 ml of culture harvested by filtration without any wash, with 70 µl medium retained on the filter. Each line separates internal portion (below the line) and external portion (above the line). The lower the line locates, the larger error calculation may cause when one applies the subtraction strategy.

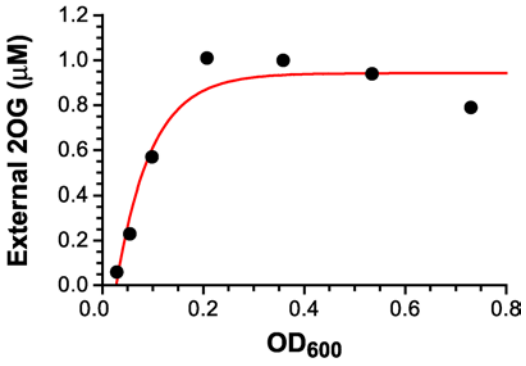

FIG. S3. Data fitting of external 2OG concentration with a model including leakage and active uptake. Data points are from Fig. 2A. The time-dependence of external 2OG concentration C_{ext} is given by $dC_{ext}/dt = v_L C_{in} - v_U C_{ext}$, where C_{in} is internal 2OG concentration, v_L is velocity of leakage, and v_U is velocity of uptake. One expects that leakage and uptake rates increase with cell density, i.e., $v_L \propto \mathrm{OD}$ and $v_U \propto \mathrm{OD}$. With x denoting $\mathrm{OD} = \mathrm{OD}_{t=0} \cdot e^{\lambda \cdot t}$ and λ being the growth rate, one has $v_L = a \cdot x$ and $v_U = b \cdot x$. Here, $a = P_{2\mathrm{OG}} \cdot A/V$ is related to the permeability coefficient of 2OG $P_{2\mathrm{OG}}$ (regardless of charge states) and the area to volume ratio A/V of a cell. $b = v_{max}/K_{2\mathrm{OG}}$ is set by the maximal uptake rate of KgtP v_{max} and the affinity of KgtP for 2OG $K_{2\mathrm{OG}}$. One then has $dC_{ext}/dt = a \cdot x \cdot C_{in} - b \cdot x \cdot C_{ext}$ with solution $C_{ext}(x) = \frac{a}{b} C_{in} + (C_{ext,t=0} - \frac{a}{b} C_{in}) \cdot e^{-b \cdot x/\lambda}$. With C_{in} = 0.5 mM and λ = 0.67 hr^{-1}, the fitting curve shows an initial accumulating stage at low cell densities and a later plateau at ~1 μM 2OG. The best fits of a = 0.01 hr^{-1} OD^{-1} and b = 6 hr^{-1} OD^{-1} result in a rate difference between leakage and uptake of ~600-fold, which is approximately the fold difference between internal and external 2OG concentrations at the plateau. From the values of fitting parameters, one can deduce $P_{2\mathrm{OG}} \approx 10^{-10}$ cm/sec (for an area to volume ratio $A/V \approx 3$ $\mu\mathrm{m}^{-1}$) and $v_{max}/K_{2\mathrm{OG}} \approx 2 \times 10^{-3}$ sec^{-1} OD^{-1}. The pKa of 2-oxoglutaric acid is 4.68 and cellular pH is ~3 units higher where 2OG is largely in its charged form. The permeability coefficient $P_{2\mathrm{OG}}$ obtained is for the anion form, which presumably is less permeable than its neutral form. From the partitioning of charged and uncharged forms of 2OG under the intracellular pH condition, one may estimate that the permeability coefficient of 2OG in its neutral form is on the order of 10^{-7} cm/sec.

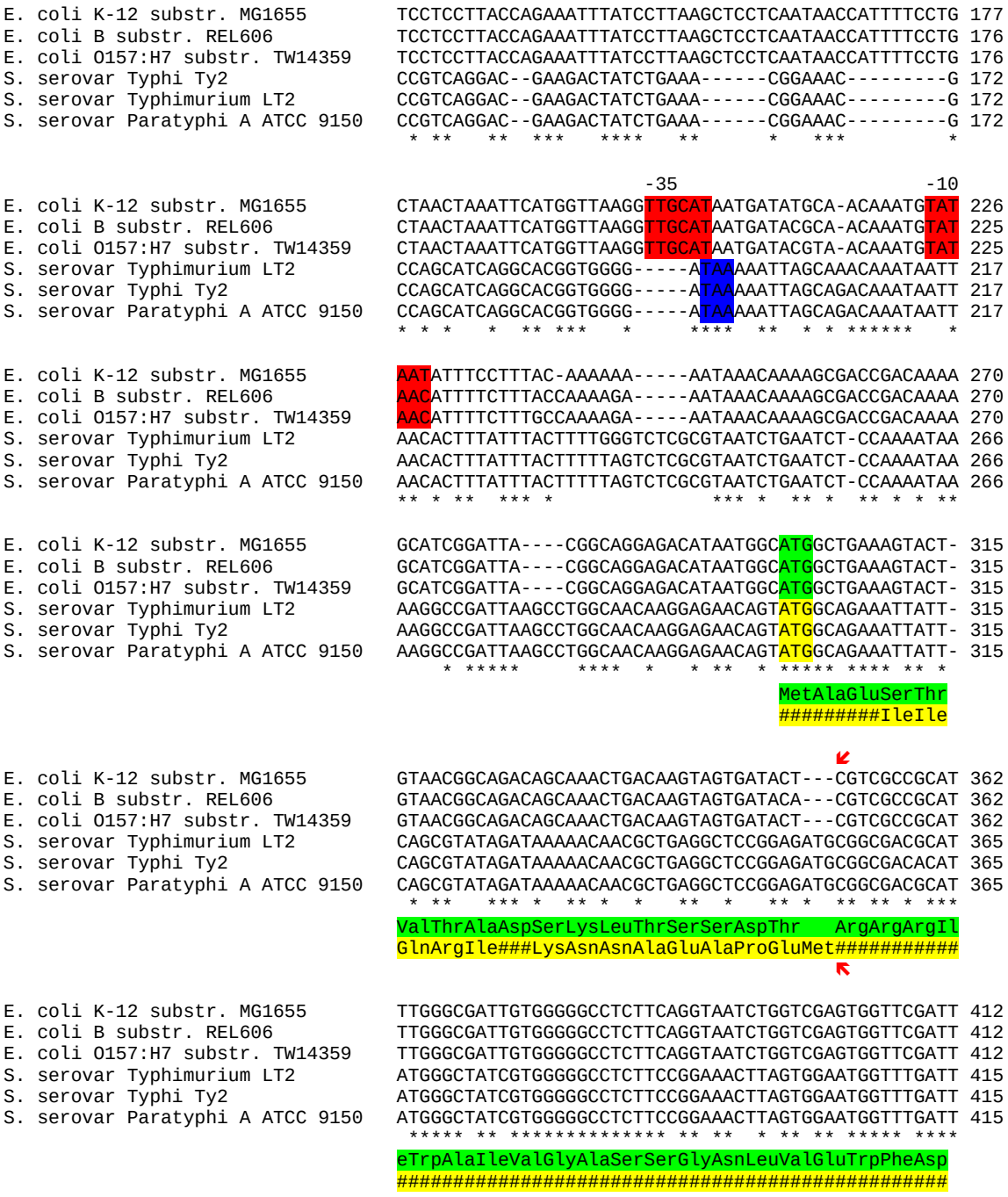

FIG. S4. Alignment of *kgtP* promoter and coding regions. Highlights: green, start codon and N-terminal amino acid residues of *E. coli kgtP*; yellow, start codon and N-terminal amino acid residues of *S. enterica kgtP*; red, -35 and -10 motifs of *E. coli kgtP* promoter; blue, stop codon of an inserted, putative cytoplasmic protein in *S. enterica*. *, identical nucleotide. ###, identical amino acid residue. Based on the nucleotide and protein sequences, the insertion appears to occur at ~50 bp into the coding region of *kgtP* in *S. enteric* (see the two red arrows). Although an in-frame start codon remains, *S. enterica kgtP* loses the self promoter possessed in *E. coli* due to the insertion.