

Supporting Information

Ferric uptake regulator (Fur) binds a [2Fe-2S] cluster to regulate intracellular iron homeostasis in *Escherichia coli*

Chelsey R. Fontenot, and Huangen Ding

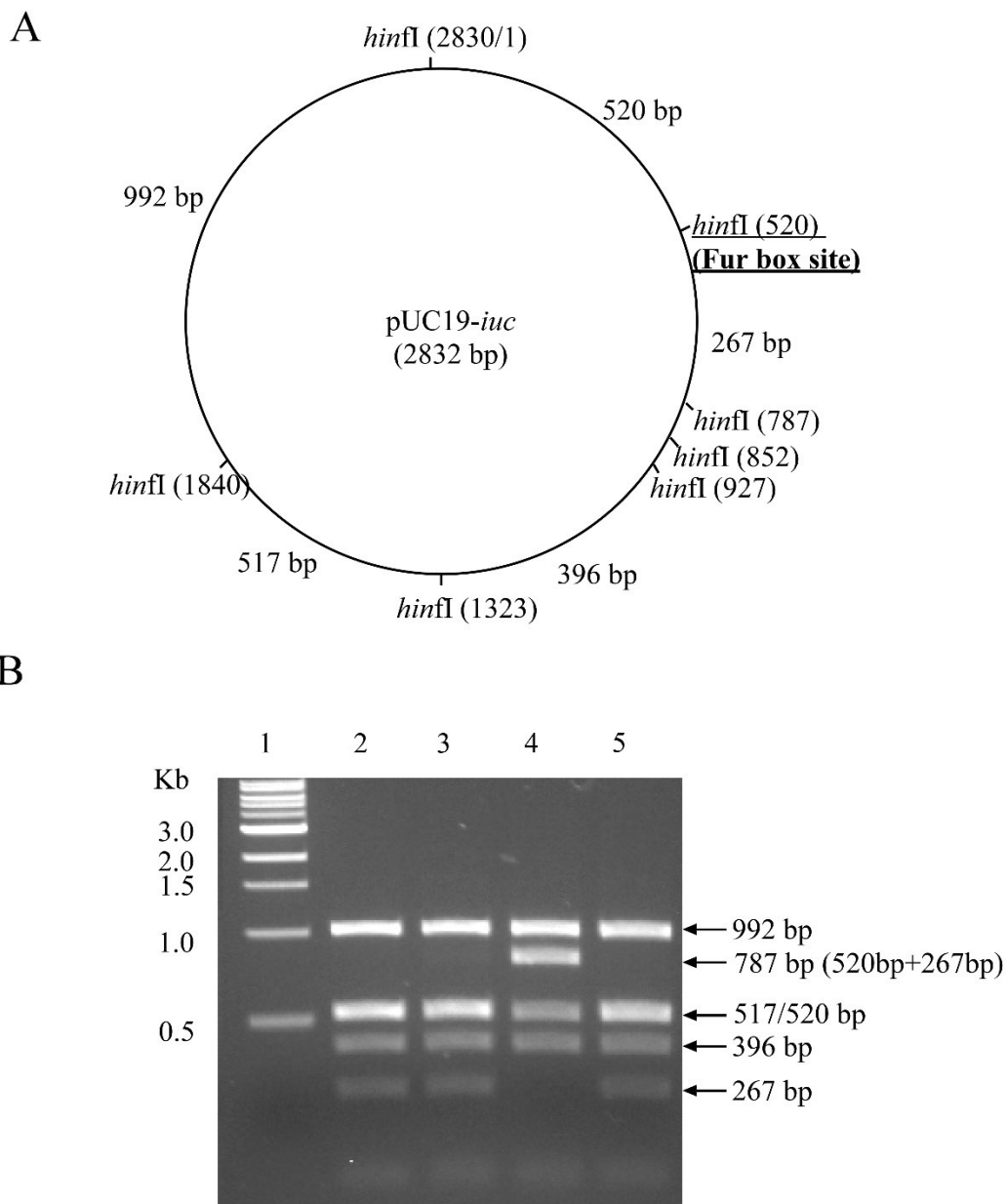
Supplemental Figure 1. Restriction map of pUC19-*iuc*

Supplemental Figure 2, Fur binds a [2Fe-2S] cluster in *E. coli* GC4468 cells grown in M9 medium supplemented with increasing concentrations of iron

Supplemental Figure 1. Restriction map of pUC19-*iuc*

(A) Restriction map of pUC19-*iuc*. The underlined *hin*fl site is within the Fur-box.

(B) pUC19-*iuc* (3.4 nM) was incubated with restriction enzyme *Hin*fl (1.0 unit) in 10 μ L reaction solution containing NaCl (150 mM), MgCl₂ (2 mM), BSA (0.1 mg/ml), and Tris (20 mM, pH 8.0) at 37°C for 10 min. The products were separated by 1.5% agarose electrophoresis gel. Lane 1, 1kb ladder. Lane 2, pUC19-*iuc* with no protein added. Lane 3, pUC19-*iuc* was pre-incubated with ferredoxin (2.0 μ M). Lane 4, pUC19-*iuc* was pre-incubated with Red-Fur (2.0 μ M). Lane 5, pUC19-*iuc* was first digested with *Hin*fl, followed by addition of Red-Fur (2.0 μ M).



Supplemental Figure 2. Fur binds a [2Fe-2S] cluster in *E. coli* GC4468 cells grown in M9 medium supplemented with increasing concentrations of iron

(A) VU-Visible absorption spectra of purified Fur. Spectra 1-5, Fur purified from *E. coli* GC4468 cells grown in M9 medium supplemented with 0.0 μM , 0.5 μM , 1.0 μM , 2.0 μM or 10.0 μM $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2$, respectively Purified Fur proteins (50 μM) were in buffer containing NaCl (500 mM) and Tris (20 mM, pH 8.0). **(B)** *E. coli* Fur binds the [2Fe-2S] cluster in wild type *E. coli* GC4468 cells in response to increasing concentrations of iron in M9 medium. The [2Fe-2S] cluster occupancy of Fur was calculated from the amplitude of the absorption peak at 410 nm (panel A) and plotted as a function of the iron concentrations in M9 medium.

