Supplementary Data S2

The Supplementary Date S2 contains Figures S1, S2, S3 and S4. Data presented here will give a comparison of DNA binding properties of His-Fur and His-tag-removed Fur (Fur for short).

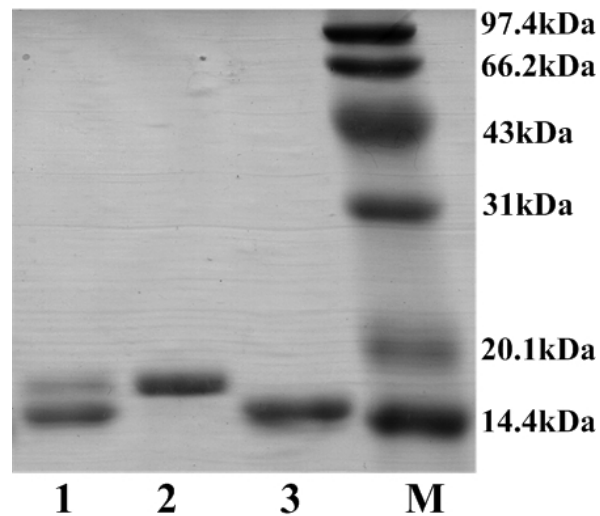
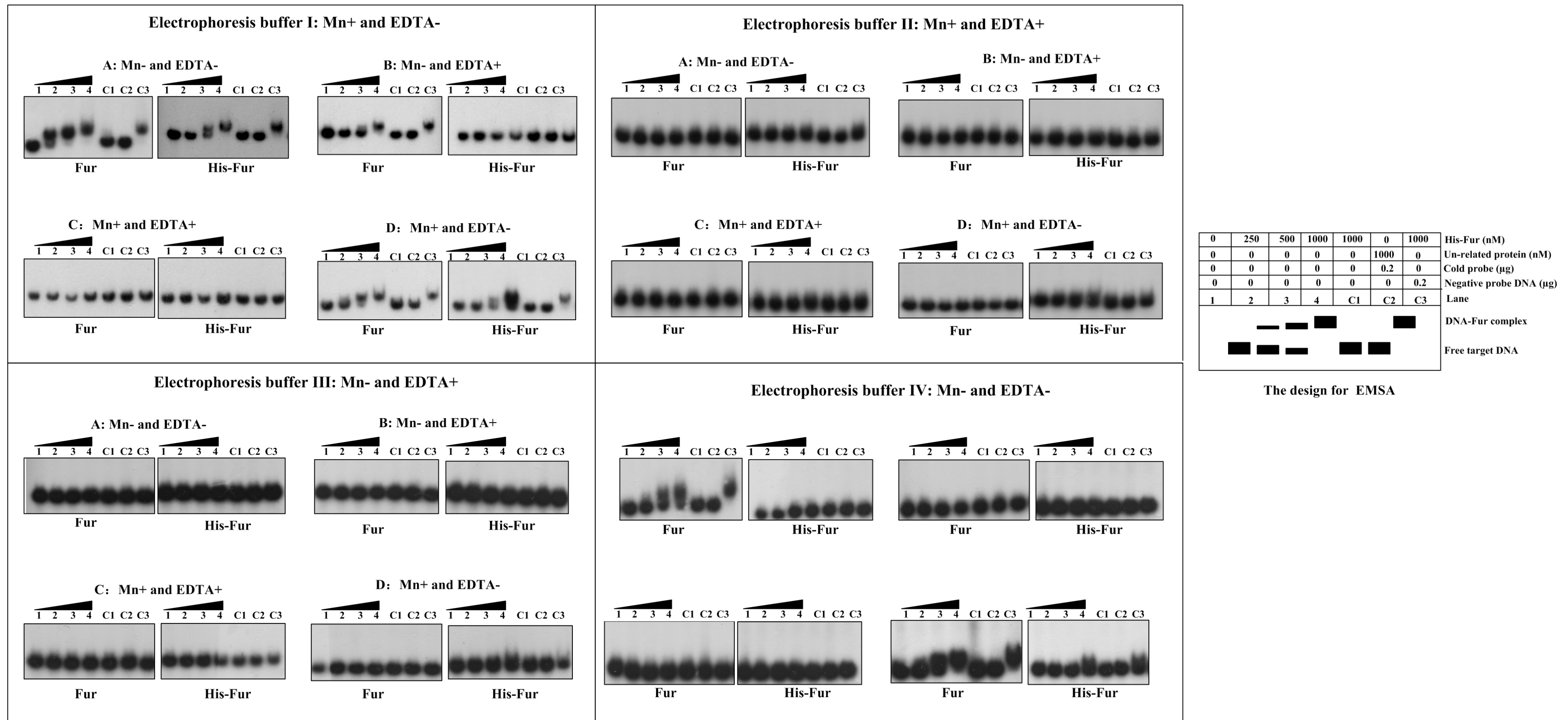


Figure S1. 15% SDS-PAGE analysis of His-Fur/Fur

2.5µg of each purified protein sample was loaded in each lane. Lane 1: His-Fur + Fur; Lane 2: His-Fur; Lane 3: Fur; Lane M: molecular markers.



His-Fur (nM)	0	250	500	1000	1000	0	1000
Un-related protein (nM)	0	0	0	0	0	1000	0
Cold probe (μg)	0	0	0	0	0	0.2	0
Negative probe DNA (μg)	0	0	0	0	0	0	0.2
Lane	1	2	3	4	C1	C2	C3

Figure S2. Effect of Mn^{2+} and EDTA on binding affinities of His-Fur and Fur

A primer pair was designed to amplify a 458bp DNA fragment upstream from the translational start site for the *ybtP* gene. The PCR-generated DNA sample was use as the DNA probe in EMSA. Various modified versions of the prototypic electrophorsis buffer or binding buffer indicated in Materials and Methods were employed. It was shown that the presence of Mn^{2+} (100μM) as well as the absence of EDTA in both the binding reaction and the electrophoresis buffer would give a strongest binding affinity of both His-Fur and Fur.

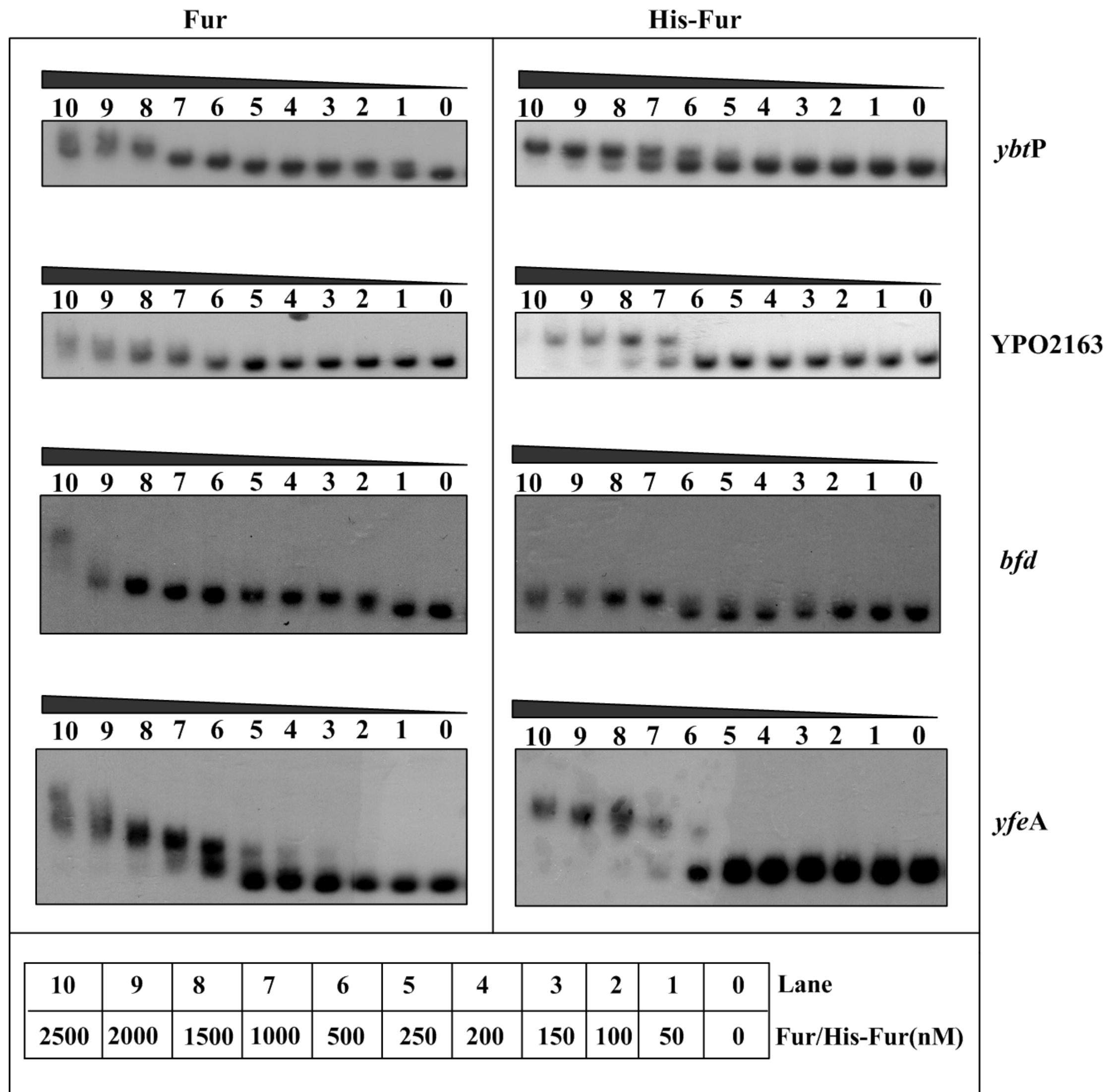


Figure S3. DNA binding affinities of His-Fur and Fur

A 400 to 500 promoter region of each of the four genes (*yptP*, YPO2163, *bfd*, and *yfeA*) was analyzed by EMSA to compare the DNA binding affinities of His-Fur/Fur. The band of free promoter DNA disappears with the increase of amounts of His-Fur or Fur protein, and a retarded DNA band with decreased mobility turns up, which presumably represents the protein-DNA complex. For a specific promoter DNA, the lower concentration of protein gave the retarded DNA band, the higher affinity of protein was. Accordingly, Fur had a DNA binding affinity slightly stronger than His-Fur.

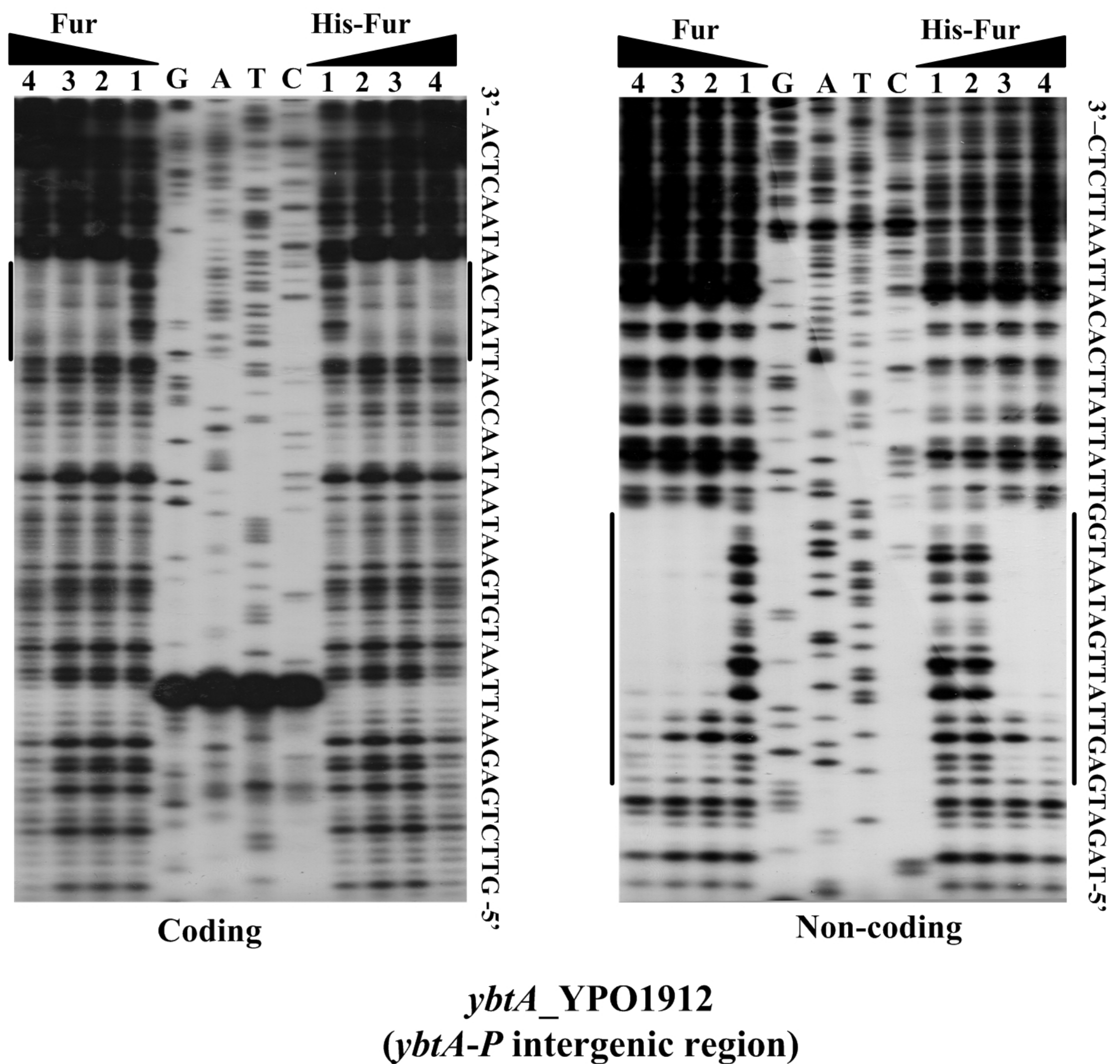


Figure S4. DNA binding sites of His-Fur and Fur

Coding and noncoding strands containing the predicted Fur box region were labeled with [γ-^{32}P] at the 5' end, incubated with increasing amounts of purified Fur or His-Fur (lanes 1, 2, 3 and 4 contained 0, 500, 1000 and 2000 nM, respectively), and subjected to DNase I footprinting assays. Lanes G, A, T and C represented the Maxam-Gilbert sequence reactions. Each protected region was indicated with a bold line. The DNA sequences of footprints were shown from the bottom (5') to the top (3'). It was shown that both His-Fur and Fur gave an identical footprint, i.e. the identical binding site. In addition, compared to His-Fur, a lower amount of Fur gave a complete protection against DNase I digestion, which was consistent with the notion that the DNA binding affinity of Fur was stronger than that of His-Fur, as seen in EMSA (Figure S3).