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Supplemental materials

Improving *Escherichia coli* FucO for Furfural Tolerance by Saturation Mutagenesis of Individual Amino Acid Positions

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FIG. S1 Growth-based screening by using the Bioscreen C, When OD ranged from 1.1 to 1.4, 15 µl seed cultures (AM1 medium, 5% xylose, 50 µg ml^{-1} Amp,) was inoculated into 0.39 ml screening culture (AM1medium, 5% xylose, 12.5 µg ml^{-1} Amp, 0.025 mM ITPG, 12.5 mM furfural). Plates were incubated at 37ºC and shaken for 10 seconds at 30 minutes intervals.

1. **FIG. S2** Prediction of RNA secondary structure and free energy calculation by the mfold web server (Zuker, M., Nucleic Acids Res. 31:3406-3415, 2003). A total of 45 nucleic acid bases (nt -7 to nt 37) were used for calculation. One RNA secondary structure was predicted for wild-type *fucO* gene (A). The three most stable structures for the *fucOL7F* mutant are included for comparison (B, C, D).