

SUPPLEMENTARY MATERIAL

TABLE. S1 Primers Designed for Q5 Site-Directed Mutagenesis Insertion of 6xHisTag into pDO6935. Primers were designed with NEBaseChanger with the forward and reverse primers having a length of 30 and 31 base pairs respectively. The calculated annealing temperature for both primers to conduct Q5 PCR was 62°C. The forward primer encodes two histidine residues and a HindIII cut site. The reverse primer encodes four histidine residues.

Primer Label	Primer Sequence	Annealing Temperature
Q5SDM_10/15/2023_F	5'-caccaca <u>agctt</u> GTGCTCCACCGAGATGTG-3'	T _m = 65°C
Q5SDM_10/15/2023_R	5'-gtgatgatgatgAAGAACCCGATGAGCAAGC-3'	T _m = 61°C

TABLE. S2 Primers Designed for DsbA Knockout Strain Confirmation. Primers were designed in Snapgene with internal and external primers within the kanamycin cassette and the downstream *yihF* gene. All expected PCR products were successfully amplified with 30 cycles of 95°C for 25 seconds, 61°C for 40 seconds, and 68°C for 55 seconds.

Primer	Primer Sequence	Annealing Temperature	GC Content
pUC19-193F	5'-GTGAAATACCGCACAGATGC-3'	T _m = 56.1°C	50%
pUC19-355R	5'-GGCGTTACCCAACTTAATCG-3'	T _m = 55.9°C	50%
F_ProposalKan	5'- AAACATCGCATCGAGCGAGCAC-3'	T _m = 62°C	55%
F_K1	5'- AGGCTATTCGGCTATGACTGGG-3'	T _m = 61°C	55%
R_new_yihF	5'-TTCGACACCAGGCTGAATTTGC- 3'	T _m = 60°C	50%

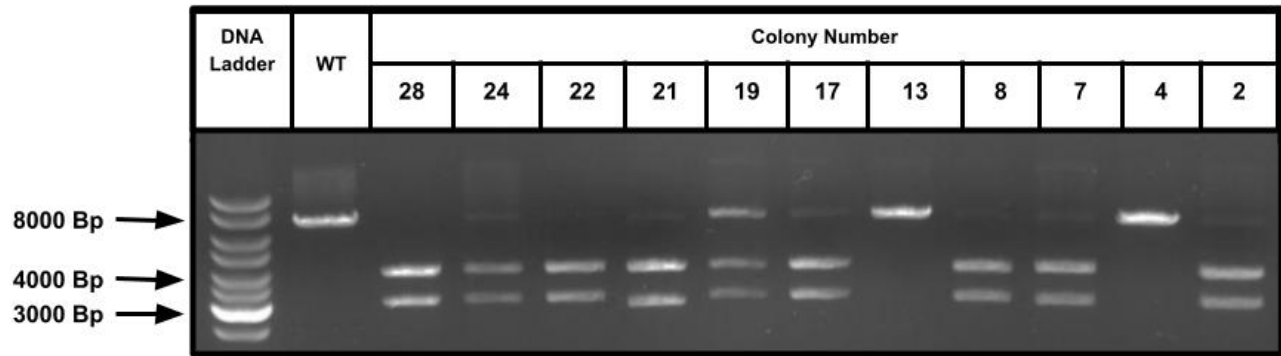


FIG. S1 Restriction Digest at HindIII of Potential pDO6935-6xHisTag Constructs. Expected constructs should have two HindIII restriction sites, one inserted by Q5 mutagenesis and one innately found within pDO6935. Expected digestion is seen in colonies 28, 24, 22, 21, 19, 17, 8, 7, and 2. Digestion products were found at the expected 3964 bp and 2987 bp fragment sizes.

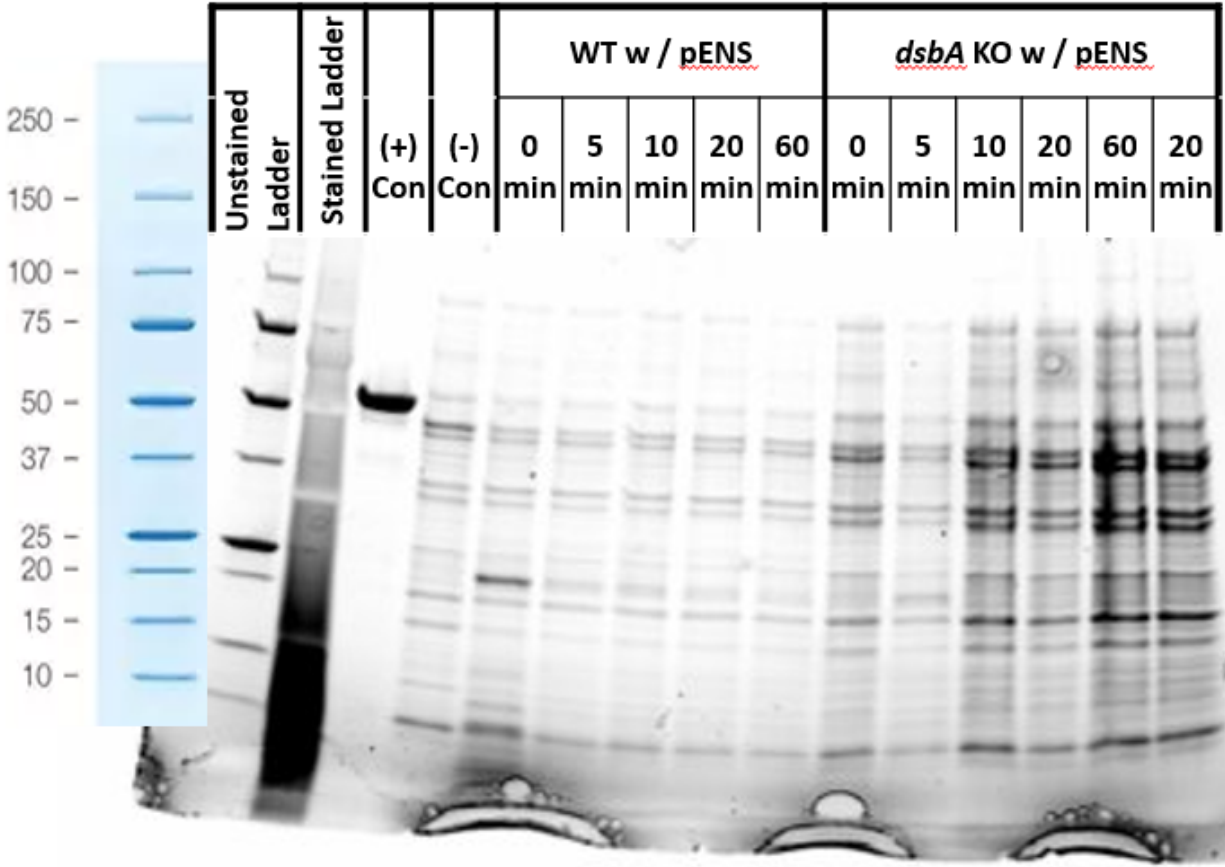


FIG. S2 Protein Concentrations in First Western Blot. WT-pENS and Δ dsbA-pENS cells were digested with 200 μ g/ml of bovine trypsin for 0, 5, 10, 20 and 60 minutes then resolved with SDS-PAGE and imaged with the ChemiDoc MP Imaging system to visualize the protein levels in the gel. Blue rectangle on the left of the figure is a clearer presentation of the expected unstained ladder, Precision Plus ProteinTM Unstained Protein Standards (BIO-RAD #1610363).