Supplementary Figures

Figure S1. Multiple sequence alignment of selenoprotein Grxs. Catalytic Sec (U) and Cys residues are highlighted in red. Resolving Cys residues in catalytic motif are highlighted in blue. Two variant amino acid sequences shown in the GSH binding region of selenoprotein Grxs are indicated by arrowheads. GenBank accession numbers of *Alkaliphilus metalliredigens* and *Moorella thermoacetica* complete genomes are NC_009633 and CP000232, respectively.

Figure S2. SDS-PAGE analysis of purified Grxs (A) and Western blot analysis for determination of selenoprotein cGrx1 concentration (B). (A) Purified Grxs were subjected to SDS-PAGE and the protein bands were visualized by Coomassie staining. (B) Purified cGrx1 was appropriately diluted and the diluted samples were subjected to Western blot analysis using anti-His antibodies. The cGrx1/U13C protein was used as an internal standard and the protein concentration was determined by quantifying the blot signals with ImageJ program. In the figure, 4 μl-loading sample's blot signal of 1:20 dilution was interpolated onto the calibration curve of standard protein concentration and the cGrx1 protein concentration was calculated to be 128 ng/μl.

Figure S3. Insertion ratio of Trp to Sec in selenoprotein cGrx1. The insertion ratio of Trp to Sec was calculated based on the number of spectra identified each peptide using UPLC-ESI-q-TOF tandem mass spectrometry. (A) Insertion ratio of Trp to Sec. (B) A tandem mass spectrum of Trp-containing peptide, ^{2}AKEVIVYTSNT**W**PHCFTVK^{20}. (C) A tandem mass spectrum of Sec-containing peptide, ^{4}EVIVYTSNT**U**PHCFTVK^{20}.

Figure S1

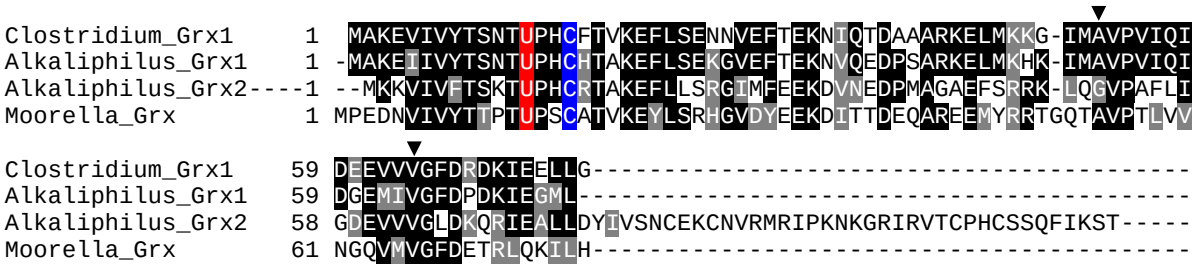
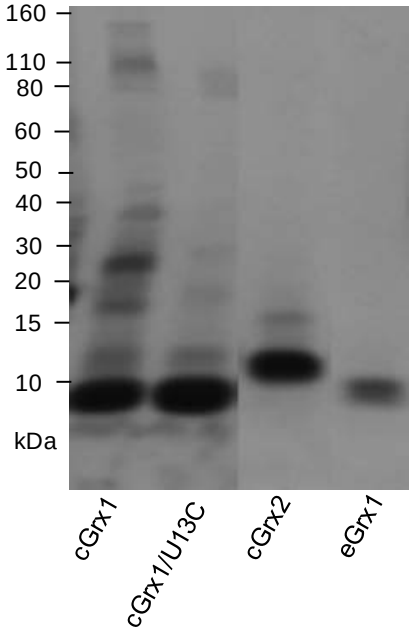
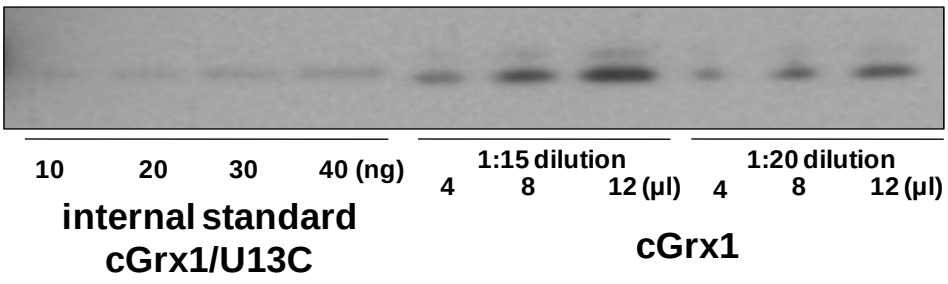

Clostridium_Grx1 1 MAKEVIVYTSNTUPHCFTVKEFLSENNVEFTEKNIQTDAAARKELMKKG-IMAVPVIQI
Alkaliphilus_Grx1 1 -MAKEIIVYTSNTUPHCHTAKEFLSEKGVEFTEKNVQEDPSARKELMKHK-IMAVPVIQI
Alkaliphilus_Grx2----1 --MKKVIVFTSKTUPHCRTAKEFLLSRGIMFEEKDVNEDPMAGAEFSRRK-LQGVPAFLI
Moorella_Grx 1 MPEDNVIVYTTPTUPSCATVKEYLSRHGVDYEEKDITTDEQAREEMYRRTGQTAVPTLVV
Clostridium_Grx1 59 DEEVVVGFDRDKIEELLG----------
Alkaliphilus_Grx1 59 DGEMIVGFDPDKIEGML----------
Alkaliphilus_Grx2 58 GDEVVVGLDKQRIEALLDYIVSNCEKCNVRMRIPKNKGRIRVTCPHCSSQFIKST-----
Moorella_Grx 61 NGQVMVGFDETRLQKILH----------

Figure S2

A. SDS-PAGE analysis of purified Grxs



B. Western blot analysis for determination of cGrx1 concentration



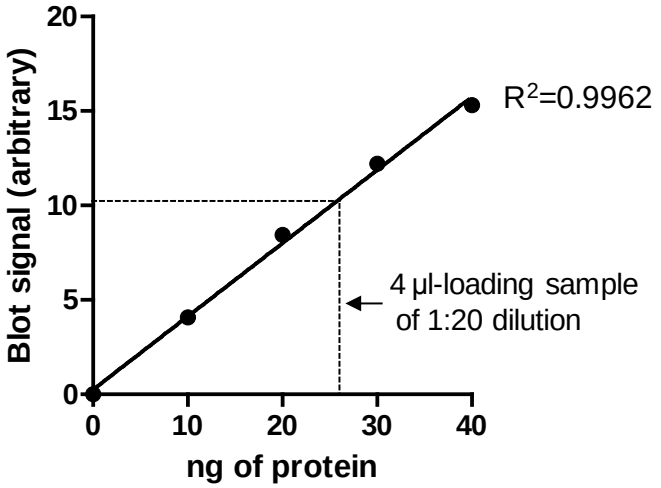
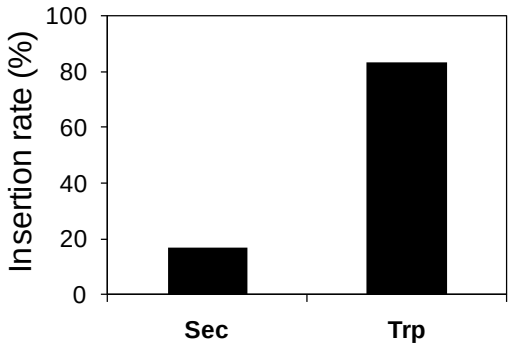


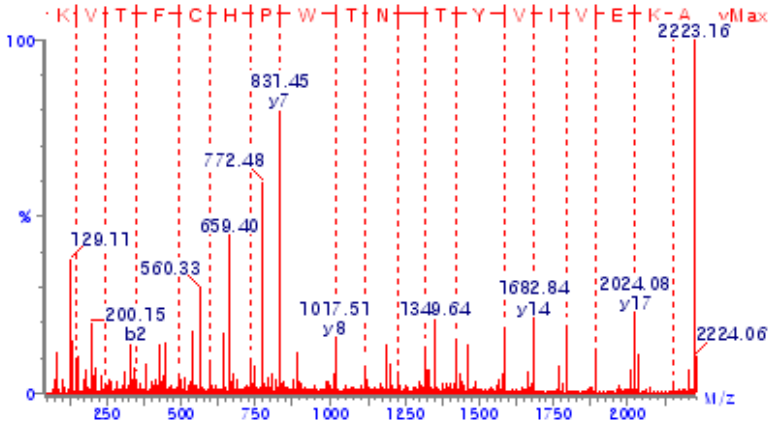
Figure S3

A



B

^{2}A K E V I V Y T S N T **W** P H C F T V K ^{20}



C

^{4}E V I V Y T S N T **U** P H C F T V K ^{20}