# Metallochaperones regulate intracellular Copper levels

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### ICP-MS Control Samples

|  |  |  |  |
| --- | --- | --- | --- |
| Description | Average  (uM) | Std.Dev  (uM) | Replicates |
| MilliQ water (sample tube wash) | 0.161 | 0.174 | 3 |
| MilliQ water, metal free | 0.003 | 0.004 | 2 |
| Basal Salts Solution (BSS), metal free | 0.346 | 0.071 | 3 |
| Complete Medium (CM) | 0.530 | NA | 1 |
| CM + Uracil | 0.689 | 0.045 | 2 |
| CM + Mevinolin + Uracil | 1.841 | 2.013 | 3 |

**Table S1**. Cu abundance in ICP-MS control samples

### Metallochaperone deletion phenotypes

We assayed metallochaperone deletion mutants for growth under varying concentrations of Cu. Unlike knockouts for *VNG1179C* or *yvgX*, metallochaperone knockout mutants exhibited no phenotypic defects (Figure S5).

### Expression vector construction

The promoter for VNG0700G (yvgX), cloned directly from the *H. salinarum* genome, is the intergenic space upstream of and including the predicted protein start codon (approx. 200bp). GFP (smRSGFP1044) was cloned from pJAM1044 [[1](#_ENREF_1)] . Since transcript termination signals have yet to be identified in *H. salinarum*, the pr0700>smRSGFP1044 fusion is divergent of ferrodoxin promoter (prFdx) fusions to avoid aberrant overexpression of GFP. The genes for VNG0702H and VNG2581H were each cloned from the *H. salinarum* genome according to predicted ORF boundaries. Overexpression was confirmed using qPCR (Figure S6).

### Quantitative PCR protocol

Total RNA was extracted with mirVana™ miRNA Isolation Kit (Ambion – AM1561) and treated with DNase (DNA-free kit-AM1561) to remove genomic DNA contamination. The integrity of the RNA samples was checked using an Agilent Bioanalyzer. Power SYBR® Green RNA-to-CT ™ 1-Step Kit (Applied Biosystems – 4389986) was used to prepare one-step RT-PCR reaction mix with SYBR® Green reagents. Reactions were cycled and measured on a 7900HT from Applied Biosystems in 384-well plate format. Absolute transcript levels were quantified using a standard curve and gene-specific primers for VNG0702H and VNG2581H.

### References

1. Reuter CJ, Maupin-Furlow JA (2004) Analysis of proteasome-dependent proteolysis in Haloferax volcanii cells, using short-lived green fluorescent proteins. Applied and environmental microbiology 70: 7530-7538.