**Supplemental File 2 : Comparison with Genome-Scale Essentiality Indices**

A previous group performed a genome-scale analysis of gene function of *Methanococcus maripaludis* via a saturated mutagenesis technique on rich and minimal media (1). Although this dataset does not contain the same quality of knockout data as actual knockout experiments, it provides a valuable “first pass” test set for gene essentiality of our model. For minimal medium in particular, their data included 2 whole genome libraries of mapped insertions, each of which contained growth data for 7 (T1) and 14 generations (T2). Reasoning that essential genes would likely be conserved across mutants, they correlated number of insertions at a particular gene location with gene essentiality by calculating an “essentiality index” (EI) for each location. Based upon a set of “known essential” genes, they set a cutoff of EI ≤ 3 for essential genes, effectively creating predictions of gene essentiality for all genes.

Considering the 4 sets of library:generation combinations—Lib.1:T1, Lib.1:T2, Lib.2:T1, Lib.2:T2—each gene could be predicted to be essential in 0-4 cases. Rather than globally classify gene essentiality based on all 4 cases, we created 4 separate sets of essential genes by setting different essentiality thresholds. For example, in “4 instances”, only genes that were predicted as essential in all 4 libraries were treated as essential genes and all other genes were considered non-essential; in “1 instance”, all genes that were predicted as essential in at least 1 library were treated as essential genes. The iMR540 reconstruction shared 538 genes with this dataset, thus we were able to compare gene essentiality predictions across nearly the entire model.

As shown by Figure S1, different thresholds had a great effect on the EI predictions; a lower threshold necessarily caused an increase in negative (no-growth) outcomes and a decrease in positive (yes-growth) outcomes. Our model experienced no change in its gene essentiality predictions in relation to threshold, hence a decrease in threshold resulted in improved performance on negative predictions and decreased performance on positive predictions. The threshold’s effect on overall performance, displayed in Figure S2, shows that our model’s predictive accuracy in the four cases ranged from 61.3-65.2% and was maximized in the “3 instances” dataset, whereas MCC ranged from 0.283-0.326 and was highest for “2 instances”. This small discrepancy reflects the difference in how these metrics are calculated, with MCC putting greater emphasis on our model’s improved ability to predict true negative outcomes.

Overall, this analysis revealed a slight positive correlation between EI predictions and gene essentiality predictions from out model. It is important to keep in mind that EI, like our reconstruction, is a model of gene essentiality and should not be confused for actual knockout data. Through different methods, both models provide hypotheses for gene functions outside known metabolism and could fuel future investigations to directly measure gene essentiality.

Figure S1: Comparison of model predictions with genome-scale essentiality indices (EI) on minimal media across 4 libraries. Instances indicate the threshold of libraries for qualifying a gene as lethal. Positive results indicate predicted non-lethal genes, negative results indicate predicted lethal-genes. TP: true positive, model and EI both predict non-lethality; TN: true negative, model and EI both predict lethality; FP: false positive, model predicts non-lethality, EI predicts lethality; FN: false negative, model predicts lethality, EI predicts non-lethality.

Figure S2: Matthews Correlation Coefficient (MCC; left y-axis) and predictive accuracy (ACC; right y-axis) comparing model predictions with genome-scale essentiality indices (EI) on minimal media across 4 libraries. Instances indicate the threshold of libraries for qualifying a gene as lethal.

1. **Sarmiento F**, **Mrázek J**, **Whitman WB**. 2013. Genome-scale analysis of gene function in the hydrogenotrophic methanogenic archaeon *Methanococcus maripaludis*. Proc Natl Acad Sci **110**:4726–4731.