Supplemental Figure on rarefaction writeup

**Testing the effects of rarefaction depth on estimates of relative alpha diversity between coral species**

The GCMP dataset was sequenced in such a way as to ensure a sequencing depth of at least 1000 sequences per sample for most samples. In this study, we retained this threshold. The choice of a higher rarefaction depth could influence our estimates in two ways: 1) improving them by allowing for more precise estimates of alpha diversity within each sample or 2) degrading them by causing many biological samples below the rarefaction depth to be removed from the analysis, thereby worsening biological replication.

Examination of sequencing depths across the GCMP suggested that using a substantially higher rarefaction depth would result in loss of many samples in key species. For example using a rarefaction depth of 10,000 sequences would reduce biological replication for Acropora hyacinthus to 2 (vs 10 at 1,000 sequences). However, such loss of biological replication might be worth it if it dramatically improved estimates of which species had the most vs. least divers microbiomes.

To test whether our use of a rarefaction depth of 1000 sequences per sample might bias microbiome results, we used samples with high sequencing depth (>100,000 sequences per sample) as an internal control to test the effect of sequencing depth on estimated relative alpha diversity. We emphasize relative rather than absolute alpha diversity because the focus of our analysis was correlating TIR containing gene repertoires with relative alpha diversity across coral species.

Coral skeletal samples were used for this analysis as skeleton is the compartment where TIR containing gene repertoires best correlated with microbiome diversity in our results. Samples were first rarefied to 100,000 reads and then those same samples were re-rarefied to 1,000 reads. If rarefaction depth biases our estimates of relative coral microbiome biodiversity, then the samples with the greatest apparent microbiome richness in the 100,000 read rarefaction should be only weakly correlated with apparent microbiome richness of the same samples at 1,000 reads. This was tested in both raw terms (correlation between observed features at 1k verses 100k reads) and by rank (correlation between rank observed features at 1k verses 100k reads).

Results from testing the validity of using 1000 sequences rather than something more stringent, such as 5,000 reads, shows that rarefaction depth has little effect on relative alpha diversity (Pearson R2 = 0.95, p = 3.14 x 10-25; Spearman R2 = 0.989, p = 3.51 x 10-37). These results indicate that the rank alpha diversity of individual GCMP samples is approximately 99% similar across a wide range of rarefaction depths.