Bacterial DNA Concentration qPCR

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Prokaryotic DNA concentration changes across titrations (Fig. 1), indicating the proportion of bacterial DNA from the unmixed pre- and post-exposure samples in a titration is not consistent with the mixture design. A qPCR assay targeting the 16S rRNA gene was used to quantify the concentration of prokaryotic DNA in the titrations. An in-house standard curve with concentrations of 20 ng/ul, 2ng/ul, and 0.2 ng/ul was used. Standard curve efficiency 91.49, and R^2 0.999. If the proportion of prokaryotic DNA is the same between pre- and post-exposure samples the slope of the concentration estimates across the two-sample titration would be 0. For individuals where the proportion of prokaryotic DNA is higher in the pre-exposure samples the slope will be negative and positive when the proporition is higher for post-exposure samples. The slope estimates are significantly different from 1 for individuals all individuals excluding E01JH0011 (Fig. 1). These results indicate that the proportion of prokaryotic DNA is lower in the post-exposure when compared to the pre-exposure samples for E01JH0004 and E01JH0017 and higher for E01JH0016 and E01JH0038.

Across all titrations median prokaryotic DNA concentration varied by individual, with E01JH0011 having the highest concentration, 3.84 $ng/\mu l$, and E01JH0038 having the lowest concentration, 0.61 $ng/\mu l$. As the DNA concentration for the unmixed samples was normalized to 12.5 $ng/\mu l$ prior to generating the titration series, the proportion of DNA in the samples targeted by 16S sequencing method ranged from 0.31 to 0.05.

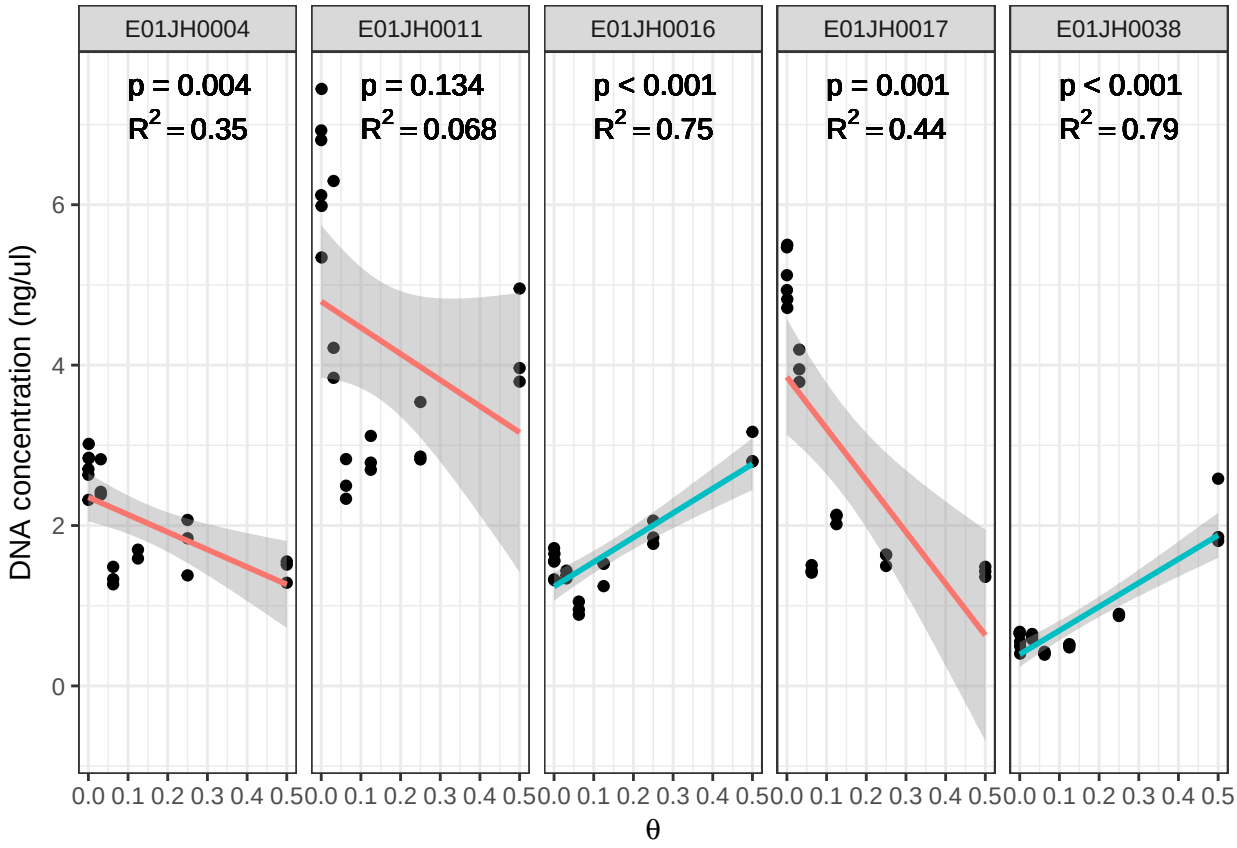

Figure 1: Prokaryotic DNA concentration (ng/ul) across titrations measured using a 16S rRNA qPCR assay. Separate linear models, [DNA]~$ heta$ were fit for each individual, R^2 and p-values were reported for each model. Red lines indicate negative slope estimates and blue lines positive slope estimates. p-value indicates signficant difference from the expected slopes of 0. Multiple test correction was performed using the Benjamini-Hochberg method. One of the E01JH0004 PCR replicates for titration 3 was identified as an outlier , with a concentration of 0.003, and was excluded from the linear model. The linear model slope was still significantly different from 0 when the outlier was included.