Microbiome Scale Theta Estimate Results

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2017-07-27

Statement regarding the number of features per biological replicate/ pipeline Statement about MA plot

Consistent deviation across pipeline and titration from the expected θ values indicates our assumptions regarding the composition of the mixtures are not valid (Fig. 2). Our assumptions are that the DNA from the pre- and post-treatment samples were titrated following a log2 dilution series and that the pre- and post-treatment samples contained similar proportions of bacterial DNA. The qPCR spike-in results indicate that the unmixed pre- and post-treatment samples were mixed volumetrically according to our expectations. Based on the qPCR bacterial DNA concentration assays, the pre-treatment E01JH0038 sample had the lowest concentration of bacterial DNA between the five biological replicates and the bacterial DNA concentration was higher in the pre-treatment sample compared to the post-treatment sample for only E01JH0017 (Fig **qPCR Bacterial Concentration**).

Table 1: Number of features used to estimate theta by biological replicate and pipeline.

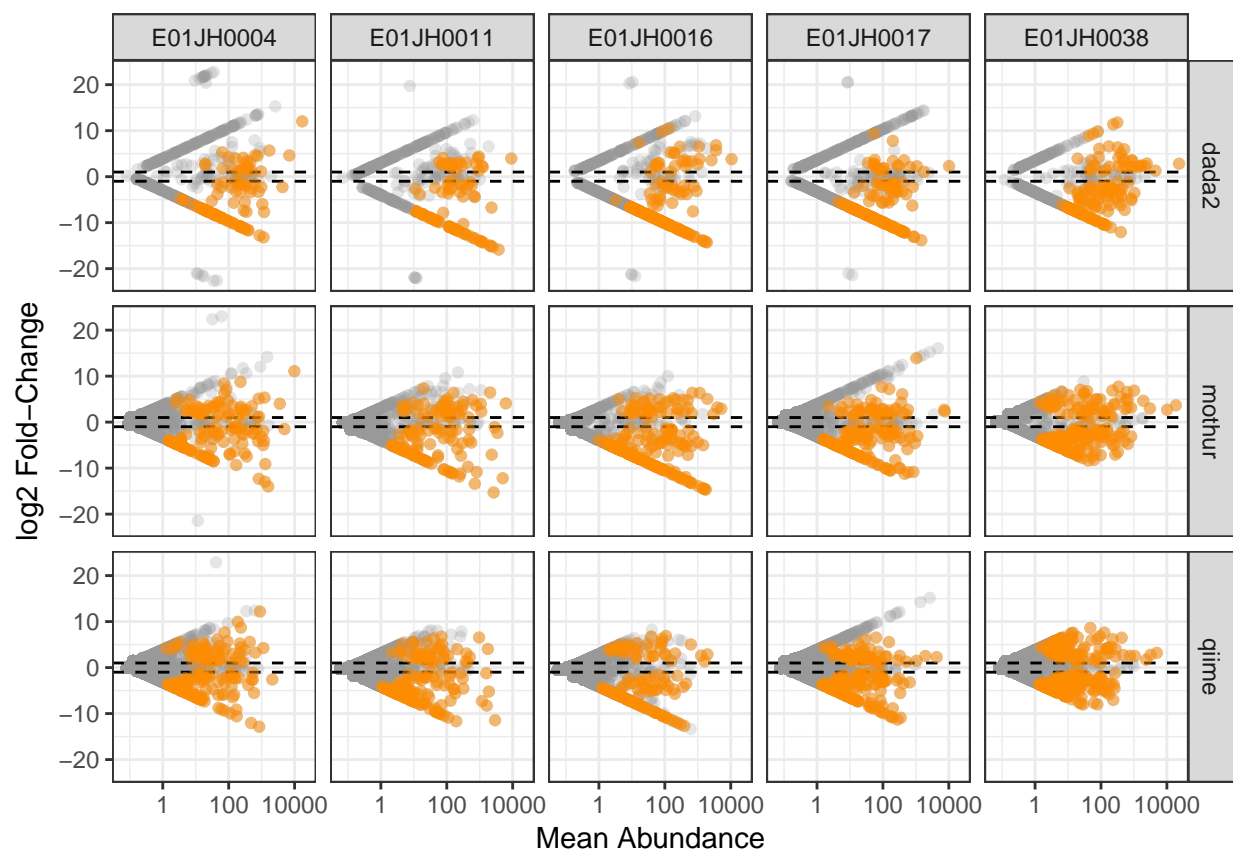| pipe	E01JH0004	E01JH0011	E01JH0016	E01JH0017	E01JH0038
dada2	102	91	151	142	136
mothur	131	123	207	164	193
qiime	165	189	118	183	231

Figure 1: MA plot (x-axis mean abundance, y-axis log2 fold change between unmixed pre- and post-exposure samples. Orange points indicate features used to estimate theta. Dashed lines indicate the +/- 1 log2 fold change filter used when to pick features.
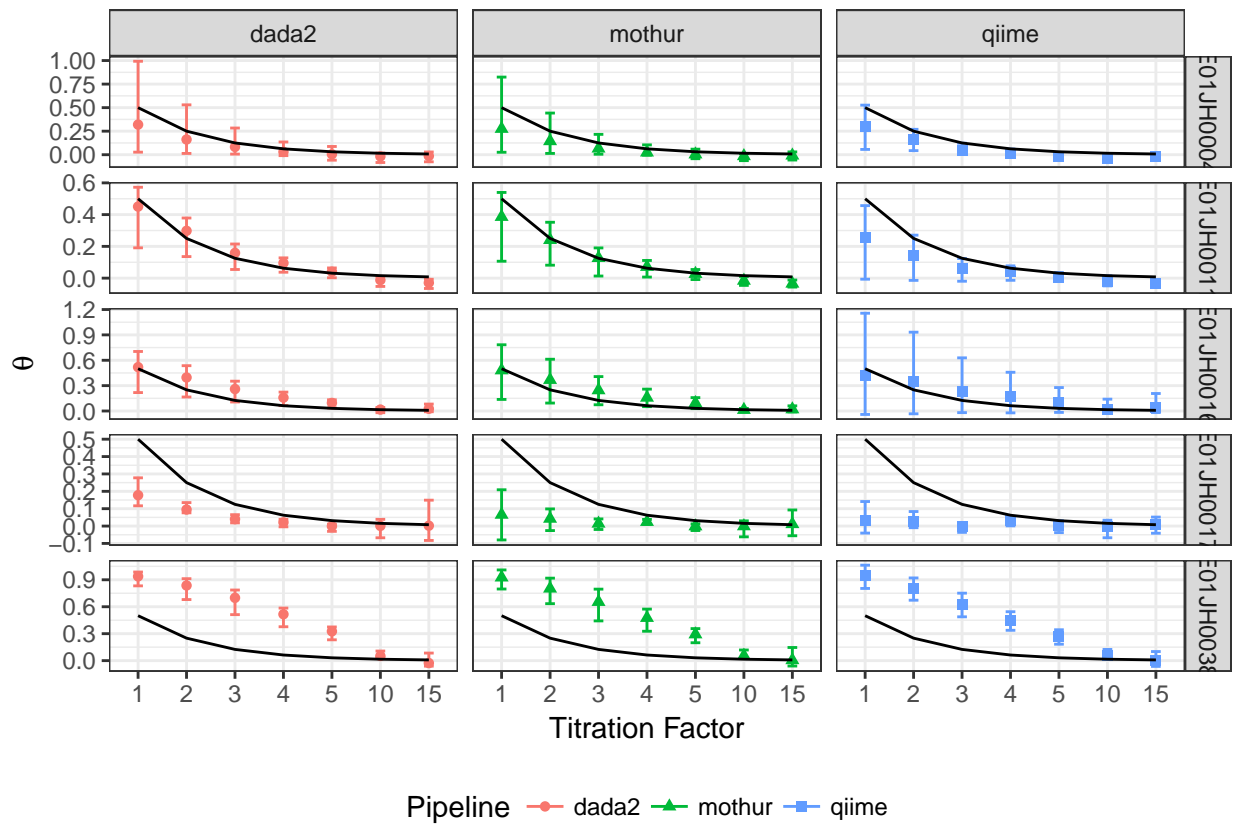
Figure 2: Theta estimates by titration, biological replicate, and bioinformatic pipeline. The points indicate mean estimate of 1000 bootstrap theta estimates and errorbars 95% confidence interval. The black line indicates the expected theta values. Theta estimates below the expected theta indicate that the titrations contains less than expected bacterial DNA from the post-treatment sample. Theta estimates greater than the expected theta indicate the titration contains more bacterial DNA from the pre-treatment sample than expected.