Microbiome assay

PLOS Genetics

Wen Aw[1]

[1]z3314717@unsw.edu.au

dx.doi.org/10.17504/protocols.io.shjeb4n

Wen Aw

EXTERNAL LINK

https://doi.org/10.1371/journal.pgen.1007735

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Aw WC, Towarnicki SG, Melvin RG, Youngson NA, Garvin MR, Hu Y, Nielsen S, Thomas T, Pickford R, Bustamante S, Vila-Sanjurjo A, Smyth GK, Ballard JWO (2018) Genotype to phenotype: Diet-by-mitochondrial DNA haplotype interactions drive metabolic flexibility and organismal fitness. PLoS Genet 14(11): e1007735. doi: 10.1371/journal.pgen.1007735

PROTOCOL STATUS

Working

1 Extract total DNA from 6 sets of 4 guts of early third instar larvae using the NucleoSpinÂ® Tissue XS DNA extraction kit (Machery-Nagel, DÃ¼ren, Germany) following the manufacturerâ€™s instructions.

2 Amplify the V4 region of the 16S rRNA gene by PCR in duplicate for each sample, using the 515 forward 5â€™-GTGCCAGCMGCCGCGGTAA-3â€™ and 806 reverse 3â€™-GGACTACHVGGGTWTCTAAT-5â€™ primer.

3 Pool duplicate PCR products and sequence using a MiSeq platform with 2 x 250 bp chemistry

4 Merge paired-end sequences into contigs, and cluster into Operational Taxonomic Units (OTUs) at 97% sequence similiarty using Mothur (http://www.mothur.org) and its standard operating procedure for MiSeq data, but with minor changes (here, singleton contigs were removed after the pre-clustering step).

5 Taxonomically classify OTUs using the Ribosomal Database Project taxonomy (http://rdp.cme.msu.edu) with 60% confidence threshold, and compare communities at a genus level.

6 Rarefy each sample to the same total number of sequences (n=41,859) to account for differenti sequencing depth, then convert to relative abundance for analysis.