



Microbiome assay [↗](#)

PLOS Genetics

Wen Aw¹

¹z3314717@unsw.edu.au

[dx.doi.org/10.17504/protocols.io.shjeb4n](https://doi.org/10.17504/protocols.io.shjeb4n)

Wen Aw

EXTERNAL LINK

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

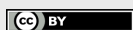
THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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 JW (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](https://doi.org/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 NucleoSpin[®] Tissue XS DNA extraction kit (Machery-Nagel, D[®]ren, Germany) following the manufacturer's instructions.
- 2 Amplify the V4 region of the 16S rRNA gene by PCR in duplicate for each sample, using the 515 forward 5'-GTGCCAGCMGCCGCGGTAA-3' and 806 reverse 3'-GGACTACHVGGGTWTCTAAT-5' 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 similarity 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 different sequencing depth, then convert to relative abundance for analysis.



This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited