



Transcriptomics

 PLOS Genetics

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Cage Studies

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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](https://doi.org/10.1371/journal.pgen.1007735)

PROTOCOL STATUS

Working

- 1 RNA was extracted from female third instar wandering larvae sourced from the side of the bottle that had developed on 1:2 and 1:16 P:C diets (4 replicates per mitotype-diet combination) using TruSeq kit (Illumina, CA, USA) with poly(A) pull-down
- 2 RNA quality was verified by Agilent Bioanalyser (Agilent Technologies, CA, USA) with all samples having an RNA Integrity Number greater than 8
- 3 Samples were sequenced on an Illumina HiSeq2000 sequencer at the Ramaciotti Center to produce 100 bp paired-end reads
- 4 At least 80 million read pairs were generated per sample. Reads were mapped to the NCBI *D. melanogaster* genome GCF_000001215.4_Release_6_plus_ISO1_MT using the Subread aligner [195] and assigned to genes using featureCounts
- 5 Genes were filtered as not expressed if they failed to achieve at least 0.25 counts per million in at least 4 samples. Trimmed means of M-values (TMM) scale normalisation was applied and read counts were transformed to log2-counts-per-million using the edgeR package
- 6 Statistical bioinformatics analysis used the limma package
- 7 To adjust for unwanted nuisance technical effects, extra surrogate variables were estimated by performing a singular value decomposition of the residuals, with emphasis on highly variable genes. Differential expression between groups was assessed using empirical Bayes t-statistics allowing for an abundance trend in the standard errors and for robust estimation of the Bayesian hyperparameters
- 8 Benjamini-Hochberg's method was used to control the false discovery rate (FDR).

9 KEGG pathway analyses were conducted using the `kegga` function in `limma`.

10 To produce heatmaps, log-count-per-million values were first batch corrected using `removeBatchEffect()`.



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