MtDNA copy number

PLOS Genetics

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Cage Studies

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EXTERNAL LINK

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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 To determine mtDNA copy number, total DNA was extracted using DNeasy Blood and Tissue Kit (Qiagen #69582).

2 The relative fold change of mtDNA copy number was determined by quantitative PCR (qPCR) using a 72 well Rotorgene-3000 (Corbett Research, Cambridge, UK) with Sybr Green (Bio-Rad, CA, USA).

3 The mtDNA was quantified by amplifying ND4 and the nuclear gene *Act88* and corroborated independently by amplifying lrRNA and the nuclear gene *rp49Â* using the following primers: ND4 forward 5â€™-AACTGGAGCTTCAACATGAGC-3â€™, reverse 5â€™-AGCCAGAACGTTTACAAGCTG-3â€™; *Act88* forward 5â€™-ATCGAGCACGGCATCATCAC-3â€™, reverse 3â€™-CACGCGCAG CTCGTTGTA-5â€™; lrRNA forward 5â€™-TCGTCCAACCATTCATTCC-3â€™, reverse 3â€™-ATAAAGTCTAACCTGCCCACTGA-5â€™; *rp49* forward 5â€™-AGATCGTGAAGAAGCGCACC-3â€™, reverse 3â€™-CACCAGGAACTTCTTGAATC-5â€™.

4 The qPCR program included denaturing at 95Â° C for 5 min and amplification in 40 cycles of 95Â° C for 10 s followed by 60Â° C for 30 s.

5 Amplification was followed by a melting curve from 72Â° C to 95Â° C, rising by steps of 0.5Â° C, to verify that a single product was amplified.

6 The mean Ct values of the mitochondrial-encoded genes ranged from 10.3-13.2.

7 The mean Ct values of the housekeeping genes ranged from 16.2-19.4.

8 The mtDNA copy number was expressed as fold change of mean mtDNA copy number relative to Alstonville.

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