Reverse transcription and qRT-PCR

PLOS Genetics

Wen Aw[1]

[1]z3314717@unsw.edu.au

dx.doi.org/10.17504/protocols.io.rtcd6iw

Cage Studies

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 For RNA extraction, 6 female third instar wandering larvae were homogenised in TRI reagent (Sigma) in a Precellys 24 homogeniser (Bertin Technologies, Ã®le-de-France, France).

2 RNA was extracted with the standard TRI reagent protocol.

3 1.5 Âµg of total RNA was treated with DNase I Amplification Grade (Sigma).

4 cDNA was prepared from 1.5 Âµg RNA template in 20 Âµl reaction mixture using a ProtoScript cDNA synthesis kit (New England Biolabs, MA, USA).

5 The comparative cycle threshold (Ct) method was used to analyse the qPCR studies.

6 The expression of mtDNA genes was quantified by the following primers: ATP6 forward 5â€™-AGAATAGCGGGTGTTCCTTGA-3â€™, reverse 3â€™-CCATCAGGTCATAATGGATCT-5â€™; ND4 forward 5â€™-AACTGGAGCTTCAACATGAGC-3â€™, reverse 3â€™-AGCCAGAACGTTTACAAGCTG-5â€™; ND6 forward 5â€™-AATTCATCCATTAGCTTTAGG-3â€™, reverse 3â€™-AGAGGCTAAAGATGTTACGTA-5â€™; 12srRNA forward 5â€™-TGGCGGTATTTTAGTCTATCT-3â€™, reverse 3â€™-AAGCTACACCTTGATCTGATA-5â€™; and lrRNA forward 5â€™-AGTCTAACCTGCCCACTGAAA-3â€™, reverse 3â€™-AGGGTCTTCTCGTCTTTTAAA-5â€™.

7 The expression of nuclear genes was quantified by the following primers: *GSTE1* 5â€™- TCTTCTTCGATGCCAGTGTAATC-3â€™, reverse 3â€™-CACTGGCATCGAAGAAGAGAC-5â€™
GstE5 5â€™- GGTAACTACATTTGGGACTCGC-3â€™, reverse 3â€™- ATCTCTGGGATACAGGGCATC-5â€™; *Notch* forward 5â€™-GTCGGCGACTACTGTGAACAC-3â€™, reverse 3â€™-GTTGCGAAAGGTCACCTGACA-5â€™;
CrebB forward 5â€™-ATGGACAACAGCATCGTCGA-3â€™, reverse 3â€™-ACGACATCGACCACGTCATT-5â€™; *eloF* forward 5â€™-

GCACATTGATTGGCTATCTGCT-3â€™, reverse 3â€™- GATTTGGTAGGCTTTCAGGACA-5â€™; *bmm* forward 5â€™-AAGTATGCACCGCATCTGTTG-3â€™, reverse 3â€™-CAAATCGCAGAGGAGACAGC-5â€™; *llp2* forward 5â€™-ATGAGCAAGCCTTTGTCCTTC-3â€™, reverse 3â€™-ACCTCGTTGAGCTTTTCACTG-5â€™; *ERR* forward 5â€™-GACCTCTCTATCCTGCGATTTG-3â€™, reverse 3â€™-CCACTTGTACCACTTCCTTTCAG-5â€™; *Zw* forward 5â€™-TTTGACGGCAAGATTCCGCA-3â€™, reverse 3â€™-CACCAGAGCGTGGGGTAGA-5â€™; *Inr* forward 5â€™-AAGCGTGGGAAAATTAAGATGGA-3â€™, reverse 3â€™-GGCTGTCAACTGCTTCTACTG-5â€™

8 The qRT-PCR 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.

9 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.

10 The mean Ct values of the mitochondrial-encoded genes and nuclear-encoded genes ranged from 7.9-11.5 and 20.8-27.8, respectively

11 The mean Ct values of the housekeeping genes ranged from 8.3-16.5.

12 The mRNA levels were expressed as the relative fold change against the normalisedÂ *rp49*Â and *Actin88* mRNA.

13 t-tests were used to determine significance. Benjamini-Hochbergâ€™s correction was used to control the FDR.