Western Blot

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EXTERNAL LINK

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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 Groups of 10 female third instar wandering larvae were collected, frozen in liquid nitrogen and were homogenised in 2Ã— lysis buffer (50 mM TRIS, pH 8.0; 300 mM NaCl; 2 mM EDTA; 1% SDS; 2% Triton X-100) with protease inhibitor cocktail (Roche 11873580001).

2 Homogenates were spun down at 13,000 RPM for 30 seconds, and supernatant was collected

3 Laemmli buffer was added to supernatant and the resulting solution was boiled at 96Â° C for 7 minutes

4 Samples were run on SDS/PAGE gel, and transferred to nitrocellulose using semi-dry blotting

5 Blots were labelled with monoclonal antibodies to the Î²-subunit of ATP synthase (Abcam AB14730) diluted 1/1500, actin (Abcam AB8224) diluted 1/25,000, the NDUFS3 subunit of Complex I (Abcam AB14711) diluted 1/800