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Promega Wizard DNA extraction - Drosophila whole body protocol

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ABSTRACT

Edited version of the 'Promega Wizard Genomic DNA Purification' protocol optimised to work on pooled whole-body fruit fly samples.

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Protocol status: Working
We use this protocol and it's working

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Lysis & Incubation

1 Add **10 flies into a 1.5ml Eppendorf tube** and cover with 300 µL Nuclei Lysis Solution . 2m

2 Use a **micro-tube pestle to crush** the flies until they are fully ground. 5m

2.1 For the initial crushing, use your hands and the pestle only.

Once the flies are partially disintegrated an electronic homogeniser can be used to increase efficiency.

Take care not to froth the solution up too much.

3 Once the flies are throughly homogenised, add another 300 µL of Nuclei Lysis Solution . 2m

By this point, the solution should be opaque and red-brown in colour.

4 **Vortex** the homogenised solution then **incubate** at 65 °C for 20 minutes . 20m

5 Remove the samples from the heat and lower the set temperature to 55 °C .

6 Allow for the **samples to coo**l for a few moments, then add 17.5 µL Proteinase K . 2m

(This is not included in the Promega Wizard kit).

7 **Vortex the samples** throughly then **incubate** at 55 °C for 3 hours . 3h

Vortex the samples regularly throughout this time.

Protein Precipitation 4m

8 Remove the samples from the heat block and **allow to cool** to room temperature.

9 Add 200 µL Protein Precipitation Solution to each sample & **vortex** for **20 seconds** (until thoroughly mixed).

10 Leave the samples On ice for 5 minutes . 5m

11 **Centrifuge** the samples at high speed: 13200 rpm , for 4 minutes . 4m

12 **Carefully transfer the supernatant** (liquid) to a new (labelled) micro-centrifuge/Eppendorf tube. 2m

Take care not to disturb the pellet.

13 *If any tissue or protein precipitate (white mass) remains in the supernatant,* ***repeat steps 11 & 12.***

14 Once the supernatant is clear of tissue mass and protein precipitate, add 600 µL isopropanol (at room temperature). 2m

DNA elution and cleansing 31m

15 **Gently invert** the samples to mix the isopropanol and supernatant. 5m

White threads of DNA may or may-not form; if no threads are visible after ~5 minutes, continue on with the protocol.

16 **Centrifuge** the samples at 13200 rpm , for 1 minute . 1m

17 Taking care not to disturb the pellet (of DNA), **remove the supernatant**. 4m

If the supernatant does not contain the pellet, it can be discarded.

18 Add 600 µL 70% ethanol , then **gently invert** the tube several times (to wash the DNA). 3m

19 **Centrifuge** at 13200 rpm , for 1 minute . 1m

20 Making sure that the DNA pellet is not disturbed, **remove the ethanol** (this can be discarded). 2m

21 **Invert the (open) tube** onto clean absorbent paper; **leave for 10-15 minutes**. 15m

T*owards the end of this time, set the heating bloc to* 65 °C .

22 Add 100 µL DNA Rehydration Solution **(Nuclease Free Water)** and **incubate** at 65 °C for 1 hour .

Mix the solution by gently tapping and shaking the tubes.

23 Store the DNA at 2-8 °C .