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Western Blotting (Fly Heads)

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Daniel's workspace

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ABSTRACT

This protocol describes how to perform a west blotting technique using fly heads.

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1 Homogenize desired number of fly heads in 1 X Laemmli sample buffer.

2 Heat samples to **100 °C** for **00:10:00** , spin briefly before loading. 10m

3 Load premade gel into western blotting apparatus. Fill reservoir with Running Buffer:
Running Buffer:
6 g Tris-HCL
28.9 g glycine
Fill to **1 L** with distilled water
Add **5 mL** 20% SDS

4 Load samples on gel and attach electrodes.

5 Run gel at 120 V until dye front reaches the bottom of the gel, **01:00:00** .Run longer for greater separation. 1h

6 Remove gel and transfer using Trans-Blot Turbo.

7 Perform antigen retrieval by microwaving **00:09:00** in PBS. 9m

8 Block membrane in 1X PBS with 0.05% Tween-20 and 3% dry milk for **01:00:00** . 1h

9 Add primary antibody at correct dilution in PBSTween + milk and incubate with shaking **Overnight** at **4 °C** .

10 Wash blot 3x in PBSTween, **00:05:00** each, with shaking. 5m

11 Add secondary antibody at the correct dilution in PBSTween + milk, incubate with shaking at **Room temperature** for **03:00:00** . 3h

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12 Wash blot in PBSTween **00:30:00** with frequent wash changes. 30m

13 Develop with ECL substrate or image fluorescence, as appropriate.

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