DNA isolation and amplification from Drosophila wings

PROTOCOL FOR:
Non-lethal PCR genotyping of single Drosophila

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LEGEND

ATTENTION
HINT
REST

REAGENTS
• Buffer A (10 mM Tris-Cl at pH 8.2, 1 mM EDTA and 25 mM NaCl)
• Protease K (MP Biomedicals, Solon, OH, USA) (400 μg/mL in Buffer A, prepared fresh from a 20 mg/mL frozen stock)
• iProof high-fidelity DNA polymerase and iProof HF buffer (Bio-Rad, Hercules, CA, USA)
• dNTPs
• Custom primers

PROCEDURE
1. Anesthetize flies to be genotyped and section both wings immediately distal to the base (exact location is not critical but using most of the wing will ensure optimal results) with a razor blade.
2. Place wing pair at the bottom of a 0.2-mL PCR tube and carefully cover with 10 μL protease K (400 μg/mL in buffer A).
   * Ensure that wings remain submerged in the solution. Due to their hydrophobicity, wings will float if added to the solution already in the tube. Transferring the wings to the dry tube and covering with the solution circumvents this problem.
3. Incubate at 37°C for 1 h.
4. Inactivate the protease K by incubating at 95°C for 2 min.
5. PCR-amplify gene of interest. Thermocycler conditions: 1 cycle of 98°C for 30 s; 35 cycles of 98°C for 10 s, Tm+3°C for 15 s, 72°C for 15s/kb; 1 cycle of 72°C for 10 min.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>final concentration</th>
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<tbody>
<tr>
<td>iProof HF buffer (Bio-rad)</td>
<td>1×</td>
</tr>
<tr>
<td>dNTPs</td>
<td>0.2 mM each</td>
</tr>
<tr>
<td>custom primers</td>
<td>0.5 μM</td>
</tr>
<tr>
<td>DNA template</td>
<td>3 μL of wing extract solution</td>
</tr>
<tr>
<td>iProof high-fidelity DNA polymerase</td>
<td>0.4 U</td>
</tr>
<tr>
<td>ddH2O</td>
<td>to a total volume of 20 μL</td>
</tr>
</tbody>
</table>

6. Run 5 μL of each reaction on an agarose gel.

**EQUIPMENT**
- Single-edged no. 9 industrial razor blades (Surgical Carbon Steel) (Cat. no. 55411-050; VWR, West Chester, PA, USA)
- 0.2-mL Ultra tubes for PCR (Cat. no. 1695; Sorenson Bioscience Inc. Salt Lake City, UT, USA)
- PTC-200 Peltier Thermo Cycler (MJ Research, St. Bruno, Quebec, Canada)