DNA Isolation from Insect Tissue

(based on Bay Area Biotechnology Education Consortium protocol)

Solutions

Edward’s Buffer

2.5mls 0.5M EDTA

2.5mls 5 M NaCL

2.5mls 10% SDS

10 mls 1 M Tris, pH 8.0

32.5 mls distilled water for a total volume of 50mls

TE buffer

1ml 1 M Tris, pH 8.0

200ul 0.5 M EDTA

99 mls distilled water for a total volume of 100mls

1. Place insect on a Kim wipe to remove excess ETOH or isopropanol.
2. Transfer insect into a labeled 1.5ml microfuge tube.
3. Add 200ul Edward’s Buffer to each tube.
4. Remove the abdomen of the insect and cut off a ~2mm long and ~2mm wide piece. If insect is smaller than this, use the entire abdomen or entire insect.
5. Use a micropestle and grind each insect for 30 seconds to 1 minute.
6. Once the insect is macerated add 800ul more Edward’s Buffer.

When processing multiple insects it is recommended to macerate one

directly, then add the 800ul of Edward’s Buffer before moving to the

next insect.

1. Heat samples @ 95 degrees C for 5 minutes. Caps should be wrapped in parafilm to keep them for popping open.
2. Gently mix tubes by tilting back and forth.
3. Centrifuge at 13,000 rpm for 2 minutes to pellet debris.
4. Transfer 350ul of supernatant to a new labeled 1.5 ml microfuge tube.
5. Add 400ul cold isopropanol to each sample.

Store at -20 degrees C until ready for the PCR step.

1. Spin tubes at 13, 000 rpm for 5 minutes to pellet insect DNA.

Orient the hinge of the tube to point away from the middle of the centrifuge. Pellet may be seen at the bottom of the tube under the hinge.

1. Pour off supernatant and blot tube on a paper towel or kimwipe.
2. Spin again at 13,000 rpm for 1 minute. Remove supernatant with a pipette being carefully not to disturb the DNA pellet. Air dry for 10 minutes to ensure all Isopropanol is gone.
3. Resuspend with 100ul of TE buffer.
4. Proceed to PCR method of choice. Samples should be removed from thermal cycler immediately after the program is completed to avoid potential DNA degradation.