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Bulk DNA Extraction (Ding Lab)

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Abstract

Bulk DNA Extraction

1 Cut tissue (~5 mg) and place in a 1.5 mL microcentrifuge tube. Add 180 µL Buffer ATL and 20 µL proteinase K, mix by vortexing and incubate at 56°C until completely lysed (1–3 h).

2 Add 2 µL RNase (100 mg/ml) and incubate for 30 min. at 37°C

3 Add 200 µL Buffer AL. Vortex for 15 sec.

4 Incubate at 70°C for 10 min. Briefly centrifuge the tube to remove drops from the lid.

5 Add 200 µL ethanol (96–100%). Vortex for 15 sec. Briefly centrifuge the tube to remove drops from the lid.

6 Pipet the mixture onto the QIAamp Mini spin column in a 2 mL collection tube. Centrifuge at 8,000 rpm for 1 min. at room temperature. Discard the flow-through and collection tube.

7 Place the QIAamp Mini spin column in a new 2 mL collection tube and add 500 µL Buffer AW1. Centrifuge at 8,000 rpm for 1 min. at room temperature. Discard the flow-through and collection tube.

8 Place the QIAamp Mini spin column in a new 2 mL collection tube and add 500 µL Buffer AW2. Centrifuge at 14,000 rpm for 3 min. at room temperature. Discard the flow-through and collection tube.

9 Place the QIAamp Mini spin column in a new 2 mL collection tube and centrifuge at full speed for 1 min. at room temperature.

10 Place the QIAamp Mini spin column in a new 1.5 mL microcentrifuge tube, add 50 µL DNase free water and incubate at room temperature for 1 min. Centrifuge 8,000 rpm for 1 min. at room temperature.

11 Repeat step 10 with a further 50 µL DNase free water.