Apr 08, 2019

Working

Fecal DNA extraction by bead beating

Version 2

Chin Yee Tan[1]

[1]Duke University

dx.doi.org/10.17504/protocols.io.zvrf656

Chin Yee Tan

ABSTRACT

From Surana Lab protocols

This protocol is suitable for extracting DNA from either human or mouse feces. Best results will be obtained with 10-60 mg of starting material

1 **For each 2ml screw cap tube,**

- Add **400 µl beads**
- Add **550 µl Phenol/chloroform**
- Add **250 µl SDS**
- Add **500 µl PB buffer** .

2 Bead beat on Precellys, setting 2.

3 Spin down the tubes for **00:05:00** at 4000 RPM in microcentrifuge.

4 Proceed to PCR purification kit – for the purification of up to 10 ug PCR products.

5 Label & Place a QIAquick column in a 2ml collection tube (provided).

6 Apply the aqueous top layer of your sample onto the column and centrifuge for **00:01:00** , 17,900g (13,000 RPM), at room temperature.

7 After the spin, dump the contents of the collection tube.

8 Wash the column by adding **750 µl** of Buffer PE and repeat the spin at the same conditions for **00:01:00** .

9 Repeat steps 7 and 8.

10 After the second wash and spin, and after dumping the contents of the collection tube, Spin the column and collection tube one last time to remove residual wash buffer for **00:01:30** .

11 Place each QIAquick column in a clean **1.5 ml** microcentrifuge tube.

12 To elute DNA, add **50 µl** Buffer EB (provided in kit) OR water (pH of 7.0-8.5) to the center of the Qiaquick membrane and allow the tube to sit for **00:02:00** .

13 Centrifuge the column for **00:01:30** 17,900g (13,000 RPM), at room temperature.

14 Quantify with the Qubit dsDNA BR Assay kit. Alternatively, a nanodrop suffices.