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GWAS Fecal Collection and DNA Extraction for Downstream Sequencing Applications

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1 *Works for me* This protocol is published without a DOI.

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MATERIALS

NAME	CATALOG #	VENDOR
Dry ice		
Denville Posi-click 1.7ml Eppendorf Tubes	C2172	
Quick-DNA Fecal/Soil Microbe Kits	D6010	

Feces Collection

1 Collect fresh feces – this must be directly from the animal.
Dried feces from the night before, or in the cage will not yield good DNA for sequencing.
Make sure the tube is labeled with an "A" for BSL, a "B" for Post-ShA, "C" for Post-LgA

2 Take approximately two small pieces of fresh feces by gently holding the rat by the tail with the front paws on a cart and bounce the animal so the hind legs come gently off the cart – this will make the animal defecate in 30sec-1min.

If the animal is not defecating, palpate the colon to determine if there are fecal pellets. If there is a fecal pellet, gently squeeze behind the pellet to release the feces from the colon.

3 Place the fresh feces into a marked tube and immediately put in powdered dry ice until it can be transferred to the -80 where it will be stored. Avoid freeze thaw cycles.

DNA Isolation for Sequencing

4 Add up to 150mg fecal sample to ZR Bashing Bead Lysis Tube

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5 Add 750ul Lysis Solution to the tube

6 Secure in a bead Beater fitted with 2ml holder assembly and process at max speed for 5 min.

7 Centrifuge for 10K for 1min

8 Transfer 400ul Supernatant to Zymo Spin IV Filter in a collection tube and centrifuge for 7K rpm

9 Add 1.2ml Fecal DNA Binding Buffer to the filtrate in the collection Tube

10 Transfer 800ul of mixture from step 6 to Zymo-Spin IIC column in a new collection tube and centrifuge at 10K for 1min.

11 Discard flow-though from step 7 and repeat.

12 Add 200ul DNA Pre-wash Buffer to the Zymo-Spin IIC column in a new collection tube and centrifuge at 10K for 1 min.

13 Add 500ul Fecal DNA Wash Buffer to the Zymo-spin IIC column and centrifuge at 10K for 1 min.

14 Transfer Zymo-Spin IIC column to a clean 1.7ml centrifuge tube (Denville Posi click) and add 100ul DNA Elution Buffer directly to column. Centrifuge at 10K for 30 seconds.

15 Transfer eluted DNA from step 10 to prepared Zymo-spin IV HRC filter - into a clean 1.7ml centrifuge tube and centrifuge exactly 8,000g for 1 min.

16 The filtered DNA is now suitable for PCR and downstream applications.

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