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Custom Bead-Beating and Extraction Protocol

Source: Expired (2016) DNA extraction kits from MoBio called PowerSoil and PowerFecal.

Goal: Create soil DNA extractions for measurement of pH of solutions at early steps in lysating and "cleaning".

ALL TUBES

1. Remove all pre-loaded buffer solution from any bead tubes with such a solution. These have partially and differently evaporated, so add water to bring the final volume to the expected volume, which will bring the buffer to the correct concentration.

2. Add 750 microL of bead-beating solution.

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3. Add weighed soil (0.25 g) to bead-beating tubes and briefly vortext.

4. Add 60 microL of C1 solution.

5. Bead-beating specs: 45 seconds at 6.0 m/sec.

6. Centrifuge tubes at 10,000 x g for 30 seconds.

7. Transfer 500-600 microL of the supernatant to clean tubes.

8. Draw off 100 microL of this solution and add it to the pH multiplate labeled "after C1 solution".

9. Add 250 microL of C2 solution and incubate at 4 C for 5 minutes.

10. Centrifuge tubes at 10,000 x g for 1 minute.

11. Transfer 600-700 microL of the supernatant to clean tubes.

12. Draw off 100 microL of this solution and add it to the pH multiplate labeled "after C2 solution".

13. Freeze these tubes alongside the two pH multiplates.

14. Meaure pH of the multiplates.