DNA extraction using MoBio PowerSoil htp 96 well DNA Extraction Kit

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Objectives

Obtain total DNA from subsampled samples for downstream molecular analyses, including PCR and sequencing.

Materials

1. Multi-channel pipettemen
2. Autoclaved pipette tips and barrier pipette tips (various volumes)
3. Disposable media trays

Procedures (mostly folow the kit protocol with some modifications)

Experienced User Centrifugation Protocol

NOTE:

- Please wear gloves at all times
- BEFORE THE FIRST USE ONLY, Solution C5-D must be prepared. Add an equal amount of 100% Ethanol to Solution C5-D (for the 4 prep kit = 120 ml, or for the 12 prep kit = 360 ml). Mix well. Put a check mark in the "ethanol added" box on the bottle cap label.
- Use a new sterile disposable medium tray for each solution. If everything was sure kept sterile, pour ecess extraction solution back into the bottles.
- Centrifugation rotor: S5700.
- Also make sure the plate adapters sit flat in the rotor bucket before centrifugation.
- Minimal tips need per 96 extraction:
  - 3 boxes and 7 columns of Clip-tip 1250µl.
  - 5 boxes of 200µl VWR tips.

Detailed Steps (tip counts are for one 96 well plate):

- [ ] 1. Thaw your pre-filled PowerSoil-htp 96 well plate.
- [ ] 2. Lift the square well mat from pre-filled PowerSoil-htp 96 well plate. Save the mat and lay it upside down on a clean kim-wipe.
  - you can put a kim-wipe on top of the mat to keep dusts or other things to fall on it.
- [ ] 3. Add 750 µl of PowerSoil -htp Bead Solution to the wells of the PowerSoil -htp Bead Plate.

  - use E1 Clip-tip 1250µl, program `Htp-750`

  - hover tips over the wells, do not touch. use 8 tips.

- [ ] 4. Check Solution C1. If Solution C1 has precipitated, heat solution at 60°C until the precipitate has dissolved.
  - solution C1 contains SDS. If it gets cold, it will precipitate. Heating at 60°C will dissolve the SDS. Solution C1 can be used while it is still warm.

- [ ] 5. Add 60 µl of Solution C1. Secure the Square Well Mat tightly to the plate.
  - use E1 Clip-tip 1250µl, program `Htp-repeat60µl`

  - this program will dispense 12X 60µl repeatly with a pre-dispense before the first 60µl.

  - hover tips over the wells, do not touch. use 8 tips.

- [ ] 6. Place Bead Plate with mat securely fastened between 2 metal shaker adapter plates (MO BIO Catalog# 11990)
  and place them in the 96 well Plate Shaker (MO BIO Catalog# 11996).
  Reference the protocol provided with the adapter plates for proper placement.

- [ ] 7. Shake at speed 20 for 10 minutes
  - use program 2 on the Plate Shaker

- [ ] 8. Re-orient plates so that the side closest to the machine body is now furthest from the machine body and shake again at speed 20 for 10 minutes.
  - use program 2 on the Plate Shaker

- [ ] 9. Centrifuge at room temperature for 6 minutes at 4500 x g(RCF).

- [ ] 10. While waiting for the samples to come out of the centrifuge, take a clean 1ml Collection Plate, add 250 µl of Solution C2.
  - use E1 Clip-tip 1250µl, program `Htp-repeat250µl`

  - this program will repeatly pipette 4X 250µl with a pre-dispense step.

- [ ] 11. When step 9 is done, remove and discard the Square Well Mat.

- [ ] 12. Transfer the supernatant (~700µl) to the C2 filled 1 ml Collection Plate from step 10.
  - use 8 channel 300µl multichannel pipetteman.
  - Transfer 175µl at a time, total 4 times. Use the tip ledges as guide. The fourth transfer can be done like shown below
  - Pipette up and down to mix during the 4th transfer.

  - The supernatant may still contain some soil particles.

  - change pipette tips between columns. use 96 tips.

- [ ] 13. Apply **Sealing Tape**.

- [ ] 14. Incubate at 4°C for 10 minutes.

- [ ] 15. Centrifuge the plate at room temperature for 6 minutes at 4500 x g(RCF).

- [ ] 16. Remove and discard **Sealing Tape**.

- [ ] 17. Avoiding the pellet, transfer ~800µl of supernatant to a new 1 ml Collection Plate.
  - use 8 channel 300µl multichannel pipetteman.
  - Transfer 200µl at a time, total 4 times.
  - change tips between columns. use 96 tips.

- [ ] 18. Apply **Sealing Tape** to plate.

- [ ] 19. Centrifuge the plate again at room temperature for 6 minutes at 4500 x g(RCF).

- [ ] 20. While waiting for step 19 to complete, ta a new 1ml Collection Plate, and add 200 µl of Solution C3.
  - use E1 Clip-tip 1250µl, program `Htp-repeat200µl`
  - this program will repeatly dispense 6X 200µl with a pre-dispense step.

- [ ] 21. Once step 19 is done, transfer ~750µl of supernatant to the C3 filled 1 ml Collection Plate from step 20.
  - use 8 channel 300µl multichannel pipetteman.
  - Transfer 187.5µl at a time, total 4 times.
  - Pipette up and down to mix during the 4th transfer.
  - change tips between columns. use 96 tips.

- [ ] 22. Apply **Sealing Tape** to plate.

- [ ] 23. Incubate at 4^{o}C for 10 minutes.

- [ ] 24. Centrifuge at room temperature for 6 minutes at 4500 x g(RCF).

- [ ] 25. Remove and discard **Sealing Tape**.

- [ ] 26. Avoiding the pellet, transfer ~750µl of supernatant to a new 1 ml Collection Plate.
  - use 8 channel 300µl multichannel pipetteman.
  - Transfer i187.5µl at a time, total 4 times.
  - change tips between columns. use 96 tips.

- [ ] 27. Apply **Sealing Tape** to plate. Centrifuge the plate again at room temperature for 6 minutes at 4500 x g(RCF).

- [ ] 28. While waiting for step 27 to complete, take a 2ml Collection Plate. Add 2X 650 µl of Solution C4 to each well of the plate.
  - use E1 Clip-tip 1250µl, program `Htp-650µl`
  - use 8 tips.

- [ ] 29. Once step 27 is done, avoid the pellet and transfer no more than 650 µl of supernatant to the C4-filled 2 ml Collection Plate from step 28.

  - use 8 channel 300µl multichannel pipetteman.
  - Transfer 162.5µl at a time, total 4 times.
  - Pipette up and down to mix during the 4th transfer.
  - change tips between columns. use 96 tips.
  - It is safe to stop the protocol at this step and store the samples covered with a **Foil Sealing Tape** (not supplied in the kit) at 4°C.

- [ ] 30. Place Spin Plate onto a new 0.5 ml Collection Plate.
- [ ] 31. Pipet samples from step 29 "up and down" to mix. Load 650 µl into each well of the Spin Plate and apply **Centrifuge Tape**.
  - use E1 Clip-tip 1250µl, program `Htp-mix650µl`
  - this program will mix the solution by pipetting up and down 3 times. Then uptake 650µl.
  - do not dip the pipette tip all the way in. The wells will overflow.
  - change tips for each well. use a total of 3X 96 tips.
- [ ] 32. Centrifuge at room temperature for 3 minutes at 4500 x g(RCF).
- [ ] 33. Discard the flow through and place the Spin Plate back on the **same** 0.5 ml Collection Plate. Discard the **Centrifuge Tape**.
  - dump flow through directly down the sink or in a tray temporarily.
  - I usually tap the collection plate on kim-wipes/paper towels to get rid of axcess liquid before putting the Spin Plate back on.
- [ ] 34. Repeat steps 31-33 until all the supernatant has been processed. Discard the final flow through.
- [ ] 35. Place the Spin Plate back on the **same** 0.5 ml Collection Plate.
- [ ] 36. Confirm that ethanol has been added to Solution C5-D (see note).
- [ ] 37. Add 500 µl of Solution C5-D to each well of the Spin Plate.
  - use E1 Clip-tip 1250µl, program `Htp-repeat500µl`
  - this program will repeatly pipette 2X 500µl with a pre-dispension step.
  - hover over, do not touch the wells. use 8 tips.
- [ ] 38. Apply **Centrifuge Tape** to the Spin Plate.
- [ ] 39. Centrifuge at room temperature for 3 minutes at 4500 x g(RCF).
- [ ] 40. Discard the flow through and place the **same** 0.5 ml Collection Plate beneath the Spin Plate.
- [ ] 41. Centrifuge again at room temperature for 5 minutes at 4500 x g(RCF).
- [ ] 42. Carefully place the Spin Plate onto a Microplate.

- [ ] 43. Remove **Centrifuge Tape** and discard.

- [ ] 44. Allow to air dry for 10 minutes at room temperature.
  - lay a kim-wipe on top of the Spin Plate to prevent dusts from falling into wells.

- [ ] 45. Add 100 µl of Solution C6 to the center of each well of the Spin Plate. Apply **Centrifuge Tape**.
  - use E1 Clip-tip 1250µl, program `Htp-repeat100µl`
  - this program will repeatly pipette 12X 100µl with a pre-dispension step.
  - aim the tips straight above the wells, do not touch the wells. Use 8 tips.

- [ ] 46. Let C6 sit in the Spin Plate for 1 min.

- [ ] 47. Centrifuge at room temperature for 3 minutes at 4500 x g(RCF).

- [ ] 48. Remove Spin Plate and discard.

- [ ] 49. Cover the wells of Microplate with the **Elution Sealing Mat** provided. DNA is now ready for any
  downstream application.
  - store DNA at -80^{o}C.
  - solution C6 does not contain EDTA.

- [ ] 50. Clean up!
  - [ ] Discard used paper towels, kim-wipes, plates, etc.
  - [ ] Wipe down the bench top using 70% ethanol.
  - [ ] Open the centrifuge lid and turn it off.
  - [ ] Refill and autoclave the pipette tips for next person to use.