I used the QIAGEN DNeasy PowerSoil HTP Kit for 384 samples.

Remember:

- Use VIAFLO for all the steps
- The assembled S- Block and QIAmp 96 plate doesn't fit properly in the centrifuge. It will complain about the balance. However, you could push the plates already in the tilted position (do not be gentle) and start spinning (clockwise) the rotor. Close the lid and start the centrifuge. Stop the centrifuge before it starts jumping around: Just few seconds are enough to push the liquid through the filtering membrane.
- 4500 g = 4500 rpm, but our centrifuge arrives at 3220.

To prepare Solution C5-D before first use, add equal volume (120 ml) of 100% ethanol. Mix well.

1. Remove Square Well Mat from a PowerBead Plate. Add up to 0.25 g of soil sample. Note: Avoid cross contamination between sample wells. I loaded 100 uL of thawed microbial samples from frozen 96-well plates.

   This is an appropriate stopping point. You can store the PowerBead Plate at 2–8°C covered with the Square Well Mat.

2. Add 750 µl of PowerBead Solution to the wells of the PowerBead Plate. VIAFLO 2 x 300 + 150 uL. Note: This is the first part of the lysis procedure. The PowerBead Solution is a buffer that will disperse the soil particles. 1 box

3. Add 60 µl of Solution C1.VIAFLO. Secure the Square Well Mat tightly to the plate.
   Note: Solution C1 contains SDS, which is a detergent that aids in cell lysis. SDS breaks down fatty acids and lipids associated with the cell membrane of microorganisms. If it gets cold, SDS will precipitate. Heating at 60°C will dissolve the SDS. Solution C1 can be used while it is still warm. 1 box

4. Place PowerBead Plate with mat securely fastened between 2 Adapter Plates on a 96 well plate shaker or TissueLyser. Alm Lab. I kept the samples in a styrofoam box with ice while walking there and coming back. **(For our samples without real soil, we can just shake on our own shakers with 900 rpm)**

5. Shake at speed 20 Hz for 10 min. Re-orient plates so that the side that was closest to the machine body is now furthest from it and shake again at speed 20 Hz for 10 min. **(For our samples without real soil, we can just shake on our own shakers with 900 rpm)**
   Note: This protocol uses a combination of mechanical and chemical lysis. Mechanical lysis is introduced at this step. By randomly shaking the beads, they collide with one another and with microbial cells causing them to break open.

6. Centrifuge at room temperature for 6 min at 4500 x g.
   Note: Particulates, including cell debris, soil, beads and humic acids, will form a pellet at this point. DNA is in the supernatant.

7. Discard the Square Well Mat. Transfer the supernatant to a clean 1 ml Collection Plate.
   Note: The supernatant may still contain some soil particles. 4 box

8. Add 250 µl of Solution C2. VIAFLO.
   Note: Solution C2 contains a reagent to precipitate non-DNA organic and inorganic material, including humic substances, cell debris and proteins. It is important to remove contaminating organic and inorganic matter that may reduce DNA purity and inhibit downstream DNA applications. 4 box

9. Apply Sealing Tape to plate. Vortex for 5 s.

10. Incubate at 2–8°C for 10 min.
    Note: You can skip the 10 min incubation. However, if you have already validated DNeasy PowerSoil extractions with the incubation we recommend you retain the step.

11. Centrifuge the plate at room temperature for 6 min at 4500 x g. Discard Sealing Tape.

12. Avoiding the pellet, transfer entire volume of supernatant to a new 1 ml Collection Plate. VIAFLO.
    Apply Sealing Tape to plate. 4 box

13. Repeat steps 11-12 once. Then move on to step 14.

14. Add 200 µl of Solution C3. VIAFLO.

15. Repeat steps 10–12 once. Then apply Sealing Tape to the plate and centrifuge at room temperature for 6 min at 4500 x g.
    Note: Solution C3 is a second reagent to precipitate additional non-DNA organic and inorganic material, including humic substances, cell debris and proteins. It is important to remove contaminating organic and inorganic matter that may reduce DNA purity and inhibit downstream DNA applications.

16. Transfer no more than 650 µl of supernatant to a 2 ml Collection Plate. VIAFLO.

17. Add 1300 µl of Solution C4 to each well of the plate. VIAFLO 300 x 4 + 100 uL.
    Note: Solution C4 is a high-concentration salt solution. DNA binds tightly to silica at high salt concentrations, and this solution will adjust the salt concentration to allow binding of DNA, but not non-DNA organic and inorganic material that may still be present at low levels, to the spin plate.

    You can pause here and store the samples covered with Sealing Tape at 2–8°C.

18. Pipet samples up and down to mix. VIAFLO (aspire 300 - mix 300).

19. Place a spin plate onto an S-Block. Load approximately 650 µl into each well of the spin plate. VIAFLO (300 x 2 + 50 uL). Seal the plate with an AirPore Tape Sheet.

20. Centrifuge at room temperature for 3 min at 4500 x g. Discard the flow-through and place the spin plate back on the same S-Block. Discard the AirPore Tape Sheet.

21. Repeat steps 19 and 20 until all the supernatant has been processed. Discard the final flow-through.Place the spin plate back on the same S-Block.
    Note: In the high-salt solution, DNA is selectively bound to the silica membrane in the spin plate. Contaminants pass through the silica membrane, leaving only DNA bound to the membrane.

22. Add 500 µl of Solution C5-D to each well of the spin plate. VIAFLO (300 +200 uL). Seal the plate with an AirPore Tape Sheet.
    Note: Solution C5-D is an ethanol-based wash solution used to further clean the DNA bound to the silica membrane in the spin plate. This wash solution removes residues of salt, humic acid and other contaminants while allowing the DNA to stay bound to the silica membrane. The flow-through is waste containing ethanol wash solution and contaminants that did not bind to the silica membrane.
    Note: You can wash more than one time to further clean DNA if desired. In some cases where soils have very high humic acid content, it will be beneficial to repeat this wash step. There is 10% extra Solution C5-D in the bottle for this purpose. Solution C5-D is also sold separately (cat. no. 12955-4-5D).

23. Centrifuge at room temperature for 3 min at 4500 x g. Discard the flow-through and place the spin plate back on the same S-Block. Seal with an AirPore Tape Sheet.
    Note: This step removes residual Solution C5-D. It is critical to remove all traces of wash solution because it can interfere with downstream DNA applications.

24. Centrifuge again at room temperature for 5 min at 4500 x g. Discard flow-through.
    Note: Once again it is important to avoid any traces of the Solution C5-D.

25. Carefully place the spin plate onto Racked Elution Microtubes. Discard the AirPore Tape Sheet.

26. Allow to air dry for 10 min at room temperature.
    Note: This step removes residual Solution C5-D.

27. Add 100 µl of Solution C6 to the center of each well. VIAFLO. Seal plate with an AirPore Tape Sheet.
    Note: Placing Solution C6 (elution buffer) in the center of the membrane will make sure the entire membrane is wet. This will result in more efficient and complete release of the DNA from the silica spin plate membrane.

28. Centrifuge at room temperature for 3 min at 4500 x g. Discard the Spin Plate.
    Note: As Solution C6, which is a low salt solution, passes through the silica membrane, DNA that was bound in the presence of high salt is now selectively released.

29. Seal Elution Microtubes with the Caps provided. The DNA is now ready for downstream applications.

30. Store DNA frozen (–15 to –30°C or –65 to –90°C) as Solution C6 does not contain EDTA.

31. If you submit to BMC, transfer samples into pcr plates leaving at least one empty well per plate (they will add controls).