Microbial DNA isolation (Adapted from the MOBIO PowerSoil® DNA isolation kit manual*)

1. Obtain 2 swabs per person for every member of your group from the TAs.
2. Wet the first swab by dipping it quickly in sterile water and then rub the swab in a twirling motion along the back of your right ear 25 times.
3. Place swab in a power bead tube and while twirling move the swab in and out of the beads for 15s. You may now discard the swab and recap the tube.
4. Remove the second swab from its packaging and place inside your mouth on the inside of your right check.
5. While twirling the swab gently rub the inside of you check in a circular motion 25 times.
6. Place swab in the second power bead tube and while twirling move the swab in and out of the beads for 15s. You may now discard the swab and recap the tube.
7. Label the tubes with group ID your initials and the body site (e.g., INS-CG-E for the instructor group, Christopher Gaulke, and ear sample tube).
8. Gently vortex both tubes to mix.
9. Check Solution C1. If Solution C1 is precipitated, heat solution to 60°C until dissolved before use.
10. Add 60 μl of Solution C1 and invert several times or vortex briefly.
11. Incubate at 65˚C for 10m.
12. Secure PowerBead Tubes horizontally using the MO BIO Vortex Adapter tube holder and vortex for 10m on the highest setting.
13. Make sure the PowerBead Tubes rotate freely in your centrifuge without rubbing. Centrifuge tubes at 10,000 x g for 30 seconds at room temperature. ***CAUTION: Be sure not to exceed 10,000 x g or tubes may break.***
14. Transfer the supernatant to a clean 2 ml Collection Tube (provided).
    Note: Expect between 400 to 500 μl. Supernatant may still contain some particles.
15. Add 250 μl of Solution C2 and vortex for 5 seconds. Incubate at 4°C for 5 minutes.
16. Centrifuge the tubes at room temperature for 1 minute at 10,000 x g.
17. Avoiding the pellet, transfer up to, but no more than, 600 μl of supernatant to a clean 2 ml Collection Tube (provided).
18. Add 200 μl of Solution C3 and vortex briefly. Incubate at 4°C for 5 minutes.
19. Centrifuge the tubes at room temperature for 1 minute at 10,000 x g.
20. Avoiding the pellet, transfer up to, but no more than, 750 μl of supernatant into a clean 2 ml Collection Tube (provided).
21. Shake to mix Solution C4 before use. Add 1200 μl of Solution C4 to the supernatant and vortex for 5 seconds.
22. Load approximately 675 μl onto a Spin Filter and centrifuge at 10,000 x g for 1 minute at room temperature. Discard the flow through and add an additional 675 μl of supernatant to the Spin Filter and centrifuge at 10,000 x g for 1 minute at room temperature. Load the remaining supernatant onto the Spin Filter and centrifuge at 10,000 x g for 1 minute at room temperature and decant the supernatant.
    Note: A total of three loads for each sample processed are required.
23. Add 500 μl of Solution C5 and centrifuge at room temperature for 30 seconds at 10,000 x g.
24. Discard the flow through.
25. Centrifuge again at room temperature for 1 minute at 10,000 x g.
26. Carefully place spin filter in a clean 2 ml Collection Tube (provided). Avoid splashing any Solution C5 onto the Spin Filter.
27. Add 50 μl of Solution C6 to the center of the white filter membrane.
28. Centrifuge at room temperature for 30 seconds at 10,000 x g.

29. Discard the Spin Filter. The DNA in the tube is now ready for any downstream application. No further steps are required. Store DNA at -20° to -80°C until use.

*Original protocol can be obtained at http://www.mobio.com (protocol 12888; Version: 11212013)