SOP for Microbiota Sequencing

Material
- QIAmp FAST DNA stool Mini Kit (#51604 QIAGEN)
- 0.1 mm glass beads (#SI-BG01 Scientific Industries)
- Pellet pestle Motor (#Z359971-1EA Sigma-aldrich)
- TissueLyser
- Heatblock

Sample collection
1. Collect 5-6 fecal pellets per mouse for DNA extraction to a 2mL eppendorf tube.
2. Freeze dry pellet at -80°C ASAP or extract immediately after collection.

DNA-extraction protocol (QIAmp FAST DNA Stool Mini Kit (#51604) + mechanical extraction)
01.06.16

1. Add 0.5 ml of InhibitEX buffer to each eppendorf containing the frozen fecal samples and mix with the blender for about 30 s or until homogenized. Then add another 0.5mL of buffer ASL.
2. **Add 0.4-0.5 g of 0.1 mm autoclaved glass beads and vortex.**
3. **Lyse the cells in the tissue lyser, 2x 1 min 30 m/s.**
4. Heat suspension for 7 min at 95 ºC (heat block).
5. Vortex for 15 s and centrifuge at full speed (14000 rpm) for 1 min to pellet the fecal particles and the glass beads.
6. Pipet 15 µl proteinase K into a new 1.5 ml microcentrifuge tube (not provided).
7. Pipet 200 µl of supernatant from step 5 into the 1.5 ml tube containing proteinase K.
8. Add 200 µl of Buffer AL and vortex 15 s.
9. Incubate at 70ºC for 12 min.
10. Add 200 µl of ethanol (96-100%) to the lysate, and mix by vortexing.
11. Label the lid of a new QIAmp spin column placed in a 2 ml collection tube. Carefully apply the complete lysate from step 14 to the QIAmp spin column without moistening the rim. Close the cap and centrifuge at full speed for 1 min. Place the QIAmp spin column in a new 2 ml collection tube, and discard the tube containing the filtrate.
12. Carefully open the QIAmp spin column and add 500 µl of Buffer AW1. Close the cap and centrifuge at full speed for 1 min. Place the QIAmp spin column in a new 2 m collection tube, and discard the collection tube containing the filtrate.
13. Carefully open the QIAmp spin column and add 500 µl of Buffer AW2. Close the cap and centrifuge at full speed for 3 min.
14. Place the QIAmp spin column in a new 2 ml collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min.
15. Transfer the QIAmp spin column into a new, labelled, 1.5 ml microcentrifuge tube (not provided). Carefully open the QIAmp spin column and pipet 100-200 µl of Buffer ATE directly onto the QIAmp membrane. Close the cap and incubate for 1 min at room temperature, then centrifuge at full speed for 1 min to elute DNA.
16. Store DNA at -20ºC.
Sequencing

1. Request in agendo NGS – Metagenomics Experiment (16S V4 region).
2. Send 15-20 µl for sequencing.