DNA Extraction from cecal content

1. Take a very small amount of Caecal content (0.1g), dilute with 1 ml PBS. Centrifuge at 11,000 rpm for 2 mins. Discard the supernatant. Repeat 3 times.
2. Resuspend cell pellets thoroughly in 480µl of 50mM EDTA. Then add 50µl of 20mg/ml lysozyme.
3. Incubate the samples at 37°C for 45 minutes.
4. Centrifuge at 11,000xg (rcf) for 2 minutes then discard the supernatant.
5. Add 600µl of lysis buffer 32µl of 10mg/ml proteinase-K, pipette up and down. Incubate samples for 10 minutes at 80°C.
6. Add 5ul of RNase solution, mix by inverting and incubate at 37°C for 30 minutes.
7. Add 200ul 6M NaCl to the cell lysate and vortex.
8. Incubate on ice 5 minutes.
9. Centrifuge at 11,000xg (rcf) for 5 minutes and transfer the supernatant to a new 1.5ml microcentrifuge tube.
10. Add 600ul of Isopropanol and mix well by inverting the tubes several times.
11. Transfer DNA thread to filter column + isopropanol (700ul total volume).
12. Centrifuge at 11,000xg (rcf) for 2 minutes. Discard the supernatant.
13. Add 600µl 70% ethanol, then spin at 11,000xg (rcf) for 1 minute 30 seconds, repeat 2x
14. Spin one time without adding ethanol to remove the excess at 10,000 rpm for 3.5 mins.
15. Replace bottom tube with 1.5ml Eppendorf tube (place blue column inside tube).
16. Add 50ul of TE buffer, wait 2 minutes.
17. Spin at 14,500xg for 3.5 minutes, remove blue filter column.
18. Incubate Eppendorf tube at 65°C to 60 minutes.
19. Store DNA samples at 4°C after reaching room temp.