Laboratory Operating Procedure

DNA EXTRACTION PROTOCOL FROM CHICKEN GUT

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LOP-Version: 1

Attachments: none

Introduction

Different sections of the chicken gut can be collected and analysed by using targeted or shotgun sequencing. This protocol can be adapted to different gut sections such as caeca, ilea and crops.

Sample Material

-Aseptically collect the targeted chicken gut section into a 2 ml criovial to be immidiatly frozen in liquid nitrogen before storage at -80°C.

Equipment and Reagents

Successful DNA extraction requires a nuclease-free environment and nuclease-free consumables.

Equipment and consumables

- Refrigerated centrifuge with adaptors for up to 2 ml tubes.
- Pipettes 10 µl, 100 µl, 1000 µl
- Sterile and DNA free pipet tips with filters
- Thermal mixer
- Water bath
- Vortex
- Biospectrometer
- Mini-Beadbeater
- Disposable gloves
- 1.5 and 2 ml sterile tubes
- 15 ml and 50 ml sterile tubes

Reagents

- QIAamp Fast DNA Stool Mini Kit (Qiagen, #51604)
- 10 M ammonium acetate
- Isopropanol
- Ethanol and 70% ethanol
- DNase-free RNase

General remarks

The QIAamp Fast DNA Stool Mini Kit enables rapid purification of high-quality genomic DNA from fresh or frozen stool samples. The novel InhibitEX Buffer replaces cumbersome inhibitor removal tablets to efficiently remove PCR inhibitors commonly present in stool samples.

Literature

- https://www.qiagen.com/it/shop/sample-technologies/dna/genomic-dna/qiaamp-fast-dna-stool-mini-kit/#resources
- Danzeisen et al., 2011. PloSOne 6:e27949
- https://figshare.com/articles/SOP_-_DNA_Isolation_QIAamp_Fast_DNA_Stool_Modified/3475406

Before getting started

- Switch on the thermos mixer at 70°C, the water bath at 70°C and the centrifuge at 4°C.
- Prepare the lysis buffer as it follows

Contents	Volume/Mass
NaCl (final 500mM)	7.3 g
Tris-HCl pH 8.0 (final 50mM)	25ml of 500mM stock
EDTA (final 50mM)	25ml of 500mM stock
4% SDS	10g
ddH2O	200ml
Total Volume	250ml

Procedure

1. Transfer 0.25g of fecal sample or about 250ul gut content into MagNa Lyser Green Beads tube
2. Add 1ml lysis buffer heated at 70°C per 5 min
3. Homogenize for 3 min at maximum speed on a Mini-Beadbeater
4. Incubate at 70°C for 15 min at 300 rpm
5. Centrifuge at 4°C for 5 min at 13000 rpm
6. Transfer the supernatant to a fresh 2ml microcentrifuge tube
7. Add 300ul of fresh lysis buffer to the lysis tube
8. Homogenize for 3 min at maximum speed on a Mini-Beadbeater
9. Incubate at 70°C for 15 min at 300 rpm
10. Centrifuge at 4°C for 5 min at 13000 rpm
11. Transfer the supernatant in the 2ml microcentrifuge tube of point 6
12. Add 260ul (or 1/5 volume of lysate) of 10 M ammonium acetate to each lysate tube and vortex to mix well
13. Incubate on ice for 5 min
14. Centrifuge at 4°C for 10 min at 13000 rpm
15. Transfer about 2 aliquots of 400 ul of supernatant each to 2 x 1.5 ml microcentrifuge tube
16. Add one volume (about 400 ul) of isopropanol and vortex to mix well
17. Incubate on ice for 30 min
18. Centrifuge at 4°C for 15 min at 13000 rpm
19. Remove the supernatant
20. Wash the pellet with 70% ethanol
21. Centrifuge at 4°C for 5 min at 13000 rpm
22. Remove the supernatant
23. Centrifuge at 4°C for 1 min at 13000 rpm
24. Remove the trace of ethanol by air drying the pellet for 3 min or under a vacuum for about 10 min
25. Dissolve the pellet in 100ul TE buffer or water
26. Add a volume (about 5 ul) of DNase-free RNase to a final concentration of 0.1ug/ul (Roche #11579681001)
27. Store at 4°C overnight

28. Join the two replicates of the same sample in a single 1.5 ml microcentrifuge tube
29. Add 1 ml InhibitEX Buffer pre-heated at 70°C for 5 min
30. Vortex continuously for 1 min or until the sample seems homogenous
31. Incubate at 95°C for 7 min at 300 rpm
32. Centrifuge for 5 min at 13000 rpm room T
33. Pipet 30 µl proteinase K into a new 2 ml microcentrifuge tube
34. Pipet 400 µl supernatant from step 32 into the 2 ml microcentrifuge tube containing the proteinase K
35. Add 400 µl Buffer AL pre-heated at 70°C for 5 min
36. Vortex for 15 sec
37. Incubate at 70°C for 10 min at 300 rpm
38. Add 400 µl of ethanol (96–100%) to the lysate, and mix by vortexing
39. Carefully apply 600 µl lysate from step 38 to the QIAamp spin column. Close the cap and centrifuge at 13000 rpm for 1 min T amb
40. Place the QIAamp spin column in a new 2 ml collection tube, and discard the tube containing the filtrate
41. Repeat step 39 until all of the lysate has been loaded on the column. Close the cap and centrifuge at 13000 rpm for 1 min T amb
42. Place the QIAamp spin column in a new 2 ml collection tube, and discard the tube containing the filtrate
43. Carefully open the QIAamp spin column and add 500 µl Buffer AW1
44. Centrifuge at 13000 rpm for 1 min room T
45. Place the QIAamp spin column in a new 2 ml collection tube, and discard the collection tube containing the filtrate
46. Carefully open the QIAamp spin column and add 500 µl Buffer AW2
47. Centrifuge at 13000 rpm for 3 min room T. Discard the collection tube containing the filtrate
48. Place the QIAamp spin column in a new 2 ml collection tube and discard the old collection tube with the filtrate
49. Centrifuge at 13000 rpm for 3 min at room T
50. Transfer the QIAamp spin column into a new 1.5 ml microcentrifuge tube and pipet 50 µl Buffer ATE or nuclease free water directly onto the QIAamp membrane
51. Incubate for 3 min at room temperature
52. Centrifuge at 13000 for 1 min at room T to elute DNA
53. Measure DNA quality and quantity by using a BioSpectrometer and keep the DNA at -20°C