University Hospital of RWTH Aachen, Inst. Med. Microbiology, Clavel lab
Collaborative Research Center 1382, Project Q02 "Integrative Microbiota Analysis", Dr. Nicole Treichel
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DNA extraction
Shortened Godon protocol with optional enzymatic lysis

Godon JJ, Zumstein E, Dabert P, Habouzit F, Moletta R. Molecular microbial diversity of an anaerobic digestor as determined by small-subunit rDNA sequence analysis. Appl Environ Microbiol. 1997 Jul;63(7):2802-13. doi: 10.1128/aem.63.7.2802-2813.1997. PMID: 9212428; PMCID: PMC168577.

Prepare:

- TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA)
- Bead-beater-tubes (with 500 mg+/- 10 mg Zirconia/Silicia beads, autoclaved)
- 4M Guanidinethiocyanate in 0,1M Tris pH 7,5 (consider appropriate disposal)
- 5 % N-laurolylsarcosine in PBS (Dulbecco's phosphate Buffered Saline) (consider appropriate disposal)

Sample preparation:

Consider first if you want to use the enzymatic lysis step!

- For extraction from bacterial culture: Take 2 ml of a well grown culture, centrifuge it at 12.000 x g for 10 min, and discard the supernatant
- For faecal samples stored premixed 1:6 **with** DNA Stabilizer (recommended for protocol used without enzymatic step): let thaw at room temperature.
- For faecal samples stored **without** DNA Stabilizer, add DNA stabilizer (1:6) to frozen sample, mix and thaw at room temperature
- If protocol is used **with enzymatic step do not** use DNA Stabilizer

DNA Isolation:

For enzymatic extraction

Prepare Lysozyme-Solution (TE Buffer with 15 µg/µl Lysozyme):

$m_{Lysozyme}$ = (#Samples(+1)) * 7.5 mg

$V_{TE\ buffer}$ = (#Samples(+1)) * 0.5 ml

Weigh lysozyme into TE buffer (leave lysozyme stock on ice!)

1. Resuspend bacterial pellet or faecal sample in 500 µl lysozyme-solution
2. Incubate for 30 min at 37 °C
3. Add 50 µl 10 % SDS
4. Add 15 µl ProteinaseK (20 mg/ml)
5. Incubate for 1-2 h at 50 °C (liquid should be totally clear)

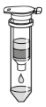
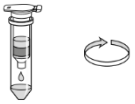
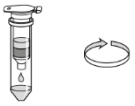
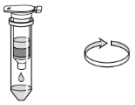
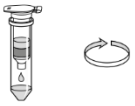
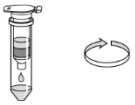
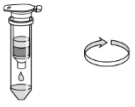
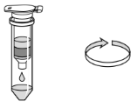
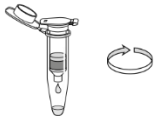
Continue with step 2 below

1. For extraction without the enzymatic step, resuspend the bacterial pellet in 600 µl DNA Stool Stabilizer or use 600µL of faecal sample in DNA stabilizer
2. Transfer sample into bead-beater-tubes (with 500 mg+/- 10 mg Zirconia/Silicia beads, previously autoclaved)
3. Add 250 µl 4M Guanidinethiocyanate
4. Add 500 µl 5 % N-laurolylsarcosine → vortex
5. Incubate at 70 °C while shaking (700 rpm) for 60 min; Set centrifuge to 4 °C
6. Bead-beat 3 x with the following program; re-fill the cooling adapter of the bead-beater with dry ice between each round:

   CY; 40 s; 6,6 m/s, cooled with dry ice

7. Add 15 mg PVPP [Poly(vinylpolypyrrolidone)]
   Vortex, then centrifuge at 15.000 x g, 4 °C, 3 min
8. Transfer clear supernatant into a new 2 ml tube
   Centrifuge at 15.000 x g, 4 °C, 3 min
9. Take 500 µl *clear supernatant* into a new 2 ml tube
10. Add 5 µl RNase (10 mg/ml), incubate at 37 °C while shaking (700 rpm) for 20-30 min (heat incubator afterwards to 70 °C, heat up DE)
11. Proceed with the NucleoSpin protocol below

NucleoSpin® gDNA Clean-up follow protocol:

		NucleoSpin® gDNA Clean-up	adjusted to larger volume
1	**Adjust DNA binding conditions**	 150 µL sample + 450 µL DB Vortex 5 s (For smaller sample volumes adjust to 150 µL with water, for larger sample volumes increase binding buffer proportionally.)	500 µL sample + 1500 µL binding buffer (DB); vortex 5 s
2	**Bind DNA**	 Load sample on NucleoSpin® gDNA Clean-up Column 11,000 x *g* 30 s	load 670 µL sample on NucleoSpin® gDNA Clean-up Column (max. volume of column is 700 µL) centrifuge at 11,000 x g for 30 s discard folw through repeat 3 times
3	**Wash silica membrane**	 **1st** + 700 µL DW Vortex 2 s 11,000 x *g* 30 s  **2nd** + 700 µL DW Vortex 2 s 11,000 x *g* 30 s	 **1st** + 700 µL DW Vortex 2 s 11,000 x *g* 30 s  **2nd** + 600 µL DW Vortex 2 s 11,000 x *g* 30 s  **3rd** + 600 µL DW Vortex 2 s 11,000 x *g* 30 s
4	**Dry silica membrane**	 11,000 x *g* 1 min	 11,000 x *g* 1 min
5	**Elute DNA**	 50 µL DE RT 1 min 11,000 x *g* 30 s *(Optional:* Repeat elution with first eluate *or another 50 µL of fresh Buffer DE. Heating elution buffer to 70°C might further promote elution.)*	Heat some elution buffer (DE) to 70°C; load 40 µL DE on silica membrane; incubate at RT for 1 min; centrifuge at 11,000 x g for 1 min (possible adjustment depending on expected amount of DNA: a) repeate step with euate (low); b) elute column a second time with 40 µL fresh DE buffer (high))

Material:

- ProteinaseK; Carl Roth (7528.1)
- NucleoSpin® gDNA Clean-up; Macherey-Nagel (740230.250)
- RNase; Sigma-Aldrich (R6513-50mg)
- Guanidinethiocyanate; Sigma-Aldrich (G9277-100G)
- Zirconia/Silicia beads; Biozym (55D1132-01TP)
- N-laurolylsarcosine; Sigma-Aldrich (61743-25G)
- TE buffer; Sigma-Aldrich (T9285-100ML)
- Poly(vinylpolypyrrolidone); Sigma-Aldrich (P5288-100G)
- 1.5 mL and 2 mL Eppendorf tubes