SUPPLEMENT 1

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Variability in the relative quantity of human DNA resulted from metagenomic analysis of gut microbiota

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Method 1. Silico-zirconium beads (BioSpec Products, USA) with diameters of 0.1 mm (300 mg per sample) and 0.5 mm (100 mg per samples) were used. Freezed fecal samples (150 mg) were vortexed with beads and 1200 ul of warm lysis buffer (500mM NaCl, 50mM Tris-HCl, pH 8.0, 50mM EDTA, 4% SDS), and then homogenized by 3 min shaking in MiniBeadBeater (BioSpec Products, USA). Homogenized samples was incubated at 70°C during 15 min, then centrifuged 20 min at 14000 rpm on Mikro 22R (Hettich Zentrifugen). Supernatant was collected in new separate tubes and placed on ice (0°C). New aliquote of lysis buffer was added to pellets and homogenization by MiniBeadBeater was repeated. Supernatants from two homogenization steps were combined, then 2 volumes of 96% ethanol and 0.1 volume of 3M sodium acetate were added. Samples were incubated at -20°C not less then 1 hour, then were centrifuged at 14000 rpm during 20 min. Pellets were washed in 80% etanole twice, dried on air and diluted in deionized water.

Method 2. Total DNA was extracted from freezed fecal samples by Stool Mini Kit (Qiagen, Germany). 1.4 ml of ASL solution were added to freezed samples (150 mg), combined with 0.2 g of 0.1 mm silico-zirconium beads (BioSpec Products, USA). Samples were homogenized by MiniBeadBeater (BioSpec Products, USA) during 4 min. Suspension was centrifuged during 2 min at 14000 rpm, supernanant collected in clean tube and used for DNA extraction as described in manufacturers' manual with single modification: lysis was performed at 95 °C.

Method 3. Freezed fecal samples (125mg) were resuspended in 125 ul of 4M GTC solution (pH 7.4) and 18.75 ul of 10% N-lauroylsarcosine solution. Added 500 ul of 5% N-lauroylsarcosine solution in 0.1M phosphate buffer and incubated for 1 hour at 70°C. Then homogenized with 750 mg of 0.1 mm silico-zirconium beads (BioSpec Products, USA) by MiniBeadBeater (BioSpec Products, USA) during 3 min. Combined with 15 mg of dry polyvinylpyrrolidone (PVP) and vortexed.

Centrifuged during 3 min at 12000 rpm, supernatant collected in clean tubes and placed on ice. Pellets were washed three times with 500 ul of TENP (50mM Tris (pH 8,0); 20mM EDTA (pH 8.0); 100mM NaCl; 1% PVP) and centrifuged during 3 min at 12000 rpm. Supernatants from all washes were combined, same volume of isopropanol was added and samples were incubated at -20°C not less then 1 hour. Then samples were centrifuged at 20000 rpm for 15 min, pellets resuspended in 450 ul of phosphate buffer. 50 ul of 5M potassium acetate were added, samples were incubated on ice for 90 min and then centrifuged at 16000 rpm for 30 min. Supernatants were collected in clean tubes, combined with 4 ul of RNAse (5 mg/ml) and incubated at 37°C for 30 min. Then 1 ml of 96% ethanol and 50 ul of 3M sodium acetate were added. Samples were incubated at -20°C not less then 1 hour, then were centrifuged at 12000 rpm during 15 min. Pellets were washed in 70% etanole twice, dried on air and diluted in 400 ul of TE-buffer (10 mM Tris-HCl; 1 mM EDTA (pH 8,0))

Method 4. Fecal samples (500 mg) were resuspended in 500 ul of buffer (200 mM Tris, pH 8.0; 200 mM NaCl; 20 mM EDTA), 210 ul of 20% SDS, 500 ul of phenol-chlorophorm mixture (1:1, pH 7.9) and 500 ug of 0.1 mm silico-zirconium beads (BioSpec Products, USA). Lysis was performed by 2 min homogenization in BeadBeater (BioSpec Products, USA). After following 15min centrifugation at 20000 rpm supernatant was transferred to new tube and combined with equal volume of phenol-chlorophorm mixture (1:1, pH 7.9), thoroughly vortexed and centrifuged again at 20000 rpm during 10 min. Water phase was transferred into new tube, combined with equal volume of isopropanol and incubated at -20°C not less then 1 hour. Then sample was centrifuged 20 min at 20000 rpm, supernatant was discarded, and pellet was washed by 70% ethanol twice (with subsequent centrifugations at 20000 rpm during 10 min). After that pellet was dried on air and diluted in 200 ul of TE-buffer.

Method 5. Fecal samples (500 mg) were resuspended in 500 ul of phosphate buffer (8mM Na_2HPO_4; 137mM NaCl; 2,7mM KCl; 1,5mM KH_2PO_4) and centrifuged during 10 min at 500 rpm and 4°C. Pellet was resuspended in 5 ml of TE-buffer (10mM Tris-HCl; 1mM EDTA, pH 8,0), combined with 1.5 mg of lysozime and incubated 1 hour at 37°C on rocker mixer. Then 2 mg of proteinase K was added and sample was incubated 5 min at 55°C. After that 600 ul of 10% SDS was added and sample was incubated 1 hour at 55°C. Then sample was combined with equal volume of phenol-chlorophorm mixture (1:1, pH 7.9), thoroughly vortexed and centrifuged at 20000 rpm during 15 min. Water phase was transferred into new tube, combined with 1/10 of volume of 3M

sodium acetate and 2 volumes of 96% ethanol and incubated 1 hour at -20°C. Then sample was centrifuged 20 min at 20000 rpm, supernatant was discarded, and pellet was washed by 70% ethanol twice (with subsequent centrifugations at 20000 rpm during 10 min). After that pellet was dried on air and diluted in 200 ul of TE-buffer.

Method 6. Fecal samples (500 mg) were resuspended in 5 ml of phosphate buffer (20 mM sodium phosphate, 150 mM sodium chloride, pH 7,5) and vortexed during 5 min, with regular cooling in ice. Suspension was centrifuged 10 min at 20000 rpm at 4°C, supernatant was stored in ice and pellet was resuspended in 5 ml PBS. Such threatment was repeated 4 times. Supernatants were combined and centrifuged at 4°C and 13500 rpm during 20 min. Then pellets from all iterations were combined, resuspended in 1 ml of PBS with 0.1% Tween-20 and vortexed during 2 min with regular cooling in ice. Suspension was centrifuged 20 min at 13500 rpm at 4°C, supernatant was stored in ice and pellet was resuspended in 1 ml PBS with 0.1% Tween-20. Such treatment was repeated until supernatant became transparent. DNA was extracted from obtained pellet mass according to Method 1.

Method 7. Fecal samples (150 mg) were resuspended in 1.5 ml of TE-buffer (10 mM Tris pH 8,2; 100 mM EDTA), vortexed and centrifuged at 10°C and 500rpm during 10 min. Supernatant was transferred to clean tube and stored on ice. Such treatment was performed with pellet one more time. Supernatants then were centrifuged 15 min at 3000g and 10°C. DNA was extracted from pellets according to Method 1. Supernatants were transferred to clean tubes, combined with 1/10 of volume of 3M sodium acetate and 2 volumes of 96% ethanol and incubated 1 hour at -20°C. Then sample was centrifuged 20 min at 20000 rpm, supernatant was discarded, and pellet was washed by 70% ethanol twice (with subsequent centrifugations at 20000 rpm during 10 min). After that pellet was dried on air and diluted in deionized water.