Supplementary Methods

IHMS DNA extraction protocol #1

Cell lysis

- Turn on heating block 70°C in advance.
- Take samples out of -80°C and before thawing of stool, add to samples:
    - 250µL Guanidine Thiocyanate *4M*
    - 40µL N-Lauroyl sarcosine 10%,
    - And homogenize with a sterile tooth pick (1 per sample).
- Add 500µL N-Lauroyl sarcosine 5%.
- Vortex vigorously.
- Incubate for 1h at 70°C on heating block.
- Weigh in cupules :
    - 15mg PVPP per sample
    - 200mg for 20 ml of TENP solution per batch of 12 samples.
- After incubation, add 750µL sterile glass beads (0,1mm) to each sample.
- Vortex vigorously.
- Apply bead-beating for 10 minutes in homogenizer set at 25r/s.

Removal of impurities

- Add 15mg of PVPP powder to each sample
- Vortex vigorously.
- Centrifuge 5 minutes at 12,700 RPM at 4°C.
- Transfer supernatant to sterile 2mL tube.
- Add to pellet 500µL of TENP solution (Homogenize TENP suspension before pipeting).
- Vortex vigorously until complete dissociation of the pellet. (Use sterile tooth pick if needed)
- Centrifuge 5 minutes at 12,700 RPM at 4°C.
- Collect surnageant and pool with the first one.
- Repeat operation of pellet wash with TENP two more times (3 washes total).
- Centrifuge pooled supernatants for 10 minutes at 12,700 RPM at 4°C.
- Divide supernatant in two equal volumes (≅ 850µl) in two 2mL tubes containing 1 mL isopropanol 2 (2 tubes per sample).

(Attention : take care not to draw the pellet).

Precipitation of nucleic acids and proteins

- Gently mix by turning the tubes a few times. (DNA floculation not always visible).
- Let settle for 10 minutes at room temperature.
- Centrifuge 15 to 30 minutes at 12,700 RPM at 4°C.

Attention : pellet not always visible.

- Discard supernatant by inversion of the tube into liquid biohazards bin.
- Let dry.

Attention : for fecal samples in stabilizing suspensions or liquid stool, supernatant must be discarded using a pipet and tips (not by tube inversion).

Removal of proteins

- Add 450/540µL phosphate buffer (PH8 0,1M) and 50/60µL potassium acetate (5M) in the first tube and flick pellet in suspension.

Attention : adjust volume depending on size of pellet, but use the same volume for all samples of a given project.

- Transfer all liquid of first tube containing resuspended pellet into second tube.
- Resuspend pellets by aspiration using P1000 automatic pipet (adjusted to 350µl).
- Complete resuspension by aspiration using P200 automatic pipet (adjusted to 150µl).
- Leave on ice in the fridge for 1h30 minutes minimum.

Precipitation of purified DNA

- Centrifuge 30 minutes at 12,700 RPM at 4°C.
- Turn on heating block 37°C in advance.
- After centrifugation, transfer cupernatant to sterile 1,5mL tube.
- Add 2µL Rnase (10mg/mL) to each DNA preparation (stored at -20°C)
- Incubate for 30 minutes à 37°C in heating block.
- Add 50µL sodium acetate 3M and 1mL absolute ethanol kept at -20°C.
- Mi Gently mix by turning the tubes a few times.
- Leave overnight at -20°C.
- Centrifuge 15 to 30 minutes at 12,700 RPM at 4°C (depending on the amonut of visible DNA flock).
- Discard supernatant in liquid biohazard bin.
- Eliminate droplets by tapping tubes upside down on tissue paper.
- Add 1mL 70% ethanol to wash the pellet.
- Centrifuge 5 minutes at 12,700 RPM at 4°C.
- Discard supernatant in liquid biohazard bin.
- Eliminate droplets by tapping tubes upside down on tissue paper.
- Repeat the wash in 70% ethanol a second time.
- Eliminate droplets of 70% ethanol with P200 automatic pipet.
- Set pellets to dry for 10 minutes in laminar flow hood.
- Resuspend DNA pellet using a pipet in a fixed volume of TE buffer (50 to 300µL depending on pellet size and sample origin).
- Discard all spoiled/exposed material in biohazard bins.

Storage

Store long term at -20°C or less.

Preparation of solutions

- Phosphate buffer pH 8, 1M
    - 9,32 mL Na2HPO4 : 14,2 g for 100mL H2O (dissolve on heating stirrer)
    - 0,68 mL NaH2PO4 (1M) : 12 g for 100mL H2O
    - 90 mL H2O
    - Check pH = 8 with pH paper

- EDTA, 2 H2O pH 8, 0.5M
    - 9,305g qs for 50 mL H2O (dissolve by heating)
    - Adjust to pH 8 with NaOH pellet (approximately one) using a pH meter
- Tris-HCL (pH 7.5, 1M or pH 8.0, 1M)
    - 6,05 g Trizma base qs for 50mL H2O.
    - Adjust to pH 7.5 or 8.0 with concentrated HCL using a pH meter
- TENP(50 mM Tris pH8, 20 mM EDTA pH8, 100 mM NaCl, 1% of PVPP)
    - 1,5 mL Tris-HCL pH 8, 1M
    - 1,2 mL EDTA pH 8, 0.5M
    - 0,6 mL NaCl, 5M
    - 0,3 g PVPP (attention, will not dissolve)
    - qs for 30ml H2O.
- Guanidine Thiocyanate 4M
    - 12,37g guanidine thiocyanate in a Falcon tube, manipulation under hood (careful with toxicity)
    - 13,5 mL H2O
    - 2,6 mL Tris-HCL 1M pH7.5
    - Shake overnight on a rocking agitator: in closed flacon, protected from light by aluminum foil
    - Completing to 26.1 mL of H2O
    - Heat in Dry bath or in an oven at 60-70 ° C for 10min (if not totally dissolved)
    - Filter through 0.2 microns Millipore filter
    - Store at 4°C protected from light
- NaCl 5M
    - 14.6g qs for 50mL H2O (in a Falcon tube)
- N-Lauroyl Sarcosine 10%
    - 2g for 20mL H2O (in a Falcon tube)
- N-Lauroyl Sarcosine 5%
    - 1g for 20mL phosphate buffer pH8, 0,1M (in a Falcon tube)
- TE pH 8 (10mM Tris.HCl pH8, 1mM EDTA pH8)
    - 200µl Tris HCL 1M pH8
    - 40µl EDTA 0,5M pH8
    - qs for 20mL H2O (in a Falcon tube)
- Potassium acetate (5M for Acetate , 3M for potassium)
    - 29,44g Potassium acetate
    - + 11,5 mL Glacial acetate
    - + 28,5 mL H2O
    - qs for 100 mL with H2O
- *Sodium acetate 3M*
    - 12,304g in 40mL d' H2O (in a Falcon tube)
    - Adjust pH at 5,2 with Glacial acetate
    - qs for 50 mL with H2O
- Rnase (Ribonuclease) 10mg/mL

IHMS DNA extraction protocol #2

Synopsis of the method

The protocol involves the following steps

- chemical and mechanical lysis of cells
- removal by precipitation and centrifugation of aromatic compounds, cellular debris and proteins
- enzymatic digestion of RNAs
- alcoholic precipitation of purified DNAs

Products

Na2HPO4	M : 142 g/mol	*Sigma*	
NaH2PO4	M : 120 g/mol	*Sigma*	*S3139*
EDTA	M : 372,2 g/mol	*Sigma*	*E5134*
Trizma base	M : 121,1 g/mol	*Sigma*	*T8524*
NaOH	M : 40,0 g/mol	*Prolabo*	*28.244.295*
HCl *concentrated*	M : 36,48 g/mol	*Prolabo*	*20.252.290*
PVPP(polyvinylpoly-pyrrolidone)		*Sigma*	*P6755*
Guanidine Thiocyanate	M : 118,2 g/mol	*Sigma*	*G6639*
NaCl	M : 58.44g/mol	*Prolabo*	*28.244.295*
N-Lauroyl Sarcosine	M : 293.38g/mol	*Sigma*	*L9150*
Acétate de Potassium	M :98.14g/mol	*Sigma*	*P3542*
Acétate de Sodium	M : 82.03g/mol	*Sigma*	*S7545*
Acide acétique glacial	M : 60.05g/mol :	*Prolabo*	*20.104.243*
Isopropanol = Propan-2-ol = 2-Propanol	M : 60.1 g/mol	*Merck*	*20842.298*
Ethanol 100% (Analyse)	M : 46.07 g/mol	*Merck*	*1.00983.1000*
Rnase (Ribonuclease)	10mg/mL	*Sigma* ou *Amersham*	*R6513* *E78020Y*

Materials

Filtres 0.22µm	Millipore	
Glass beads 0.1mm	Bioblock	B74471
Sterile screw-cap tubes 2mL, round bottom	ATGC	0214209510
Eppendorf Tubes 2 mL; 1,5 mL autoclaved		
Tooth picks autoclaved		
Bench-top Micro centrifuge		
Refrigerated Centrifuge (4°C)		
Heating blocks at 70°C & 37°C		
Bead-Beater™ (Biospec Products,USA)		

Solutions

Wear gloves at all times.

Use only MilliQ water (mq) sterile.

- Phosphate buffer pH 8, 0.1M
    - 9,32 mL Na2HPO4 : 14,2 g for 100mL H2O (dissolve using heating stirrer)
    - 0,68 mL NaH2PO4 (1M) : 12 g for 100mL H2O
    - 90 mL H2O pH ≈ 8
- EDTA, 2 H2O pH 8, 0.5M
    - 9,305g and H2O to 50 mL (dissolve using heating stirrer)
    - Adjust pH at 8 avec NaOH in pellets (~one)
- Tris-HCL (pH 7,5 at 1M or pH 8 at 1M)
    - 6,05 g Trizma base and H2O to 50mL.
    - Adjust pH at 7,5 or 8.0 with concentrated HCL
- TENP(50 mM Tris pH8, 20 mM EDTA pH8, 100 mM NaCl, 1% de PVPP)
    - 20 mL H2O (Falcon tube)
    - 1,5 mL de Tris-HCL pH 8, 1M
    - 1,2 mL EDTA pH 8, 0.5M
    - 0,6 mL NaCl, 5M
    - 0,3 g de PVPP (attention, unsoluble : it will remain in suspension)
- Guanidine Thiocyanate 4M
    - 12,37 g guanidine thiocyanate under hood in Falcon tube (attention toxic)
    - 13,5 mL H2O
    - 2,6 mL Tris-HCL 1M pH7.5
    - Let closed vessel protected from light, overnight in rotating device.
    - Adjust with H2O to 26.1 mL
    - Warm up at 60-70°C for 10min
    - Filter with 0,2 µm Millipore filter
    - Store at 4°C protected from light
- NaCl 5M
    - 14.6g and H2O to 50mL (in Falcon tube)
- N-Lauroyl Sarcosine 10%
    - 2g in 20mL H2O (inFalcon tube)
- N-Lauroyl Sarcosine 5%
    - 1g in 20mL phosphate tampon pH8 , 0,1M (in Falcon tube)
- TE pH 8 (10mM Tris.Cl pH8, 1mM EDTA pH8)
    - 200µl Tris HCL 1M pH8
    - 40µl EDTA 0,5M pH8
    - H2O to 20mL (in Falcon tube)
- Potassium Acetate (5M Acetate , 3M de Potassium)
    - 29,44g potassium acetate
    - + 11,5 mL glacial acetic acid
    - + 28,5 mL H2O
    - H2O to 100 mL
- Sodium Acetate 3M
    - 12,304g in 40mL H2O (in Falcon tube)
    - Adjust pH at 5,2 with glacial acetic acid.
    - H2O to 50 mL
- Isopropanol = Propan-2-ol = 2-Propanol
- Ethanol 100% for Analyses stored at –20°C
- Ethanol 70%

    - 70mL ethanol 100% + 30mL sterile H20, stored at –20°C
- Rnase (Ribonuclease) 10mg/mL
    - 20 mg Rnase,
    - + 20 µl Tris.Cl 1M pH 7,5
    - + 6 µl NACL 5M
    - H2O to 2 ml
    - If necessary, bring to 100°C, 15 min, and let cool at room temperature
    - Aliquot in 50µl, store at -20°C

Aliquoting biological samples

Samples may be aliquotes in sterile Sarstedt screw cap tubes

- take frozen samples (-80°c) and work on a bed of dry-ice
- Use scalpel to cut pieces of sample on aluminium foil, to make 200mg
- Keep tubes on dry-ice or at -80°C

DNA extraction

- Turn on heating block set at 70°C
- in each tube containing 200mg stool aliquot, add 250µL Guanidine Thiocyanate
- Add 40µL N-Lauroyl sarcosine 10%
- Add 500µL N-Lauroyl sarcosine 5%
- Crush the stool aliquot with tooth pick (1 per sample)
- Vortex to homogeneity

It is possible to stop here and freeze samples overnight at –20°C

- Give a quick spin before incubation (centrifugation at 14000RPM for a few seconds)
- Incubate at 70°C in heating block for 1h (OK up to max 2h)

Note: the chemical treatment contributes to cell lysis and prevents degradation of nucleic acids. The Guanidine Thiocyanate inhibits nucleases and N-Lauroyl Sarcosine is a detergent lysing cells.

- During incubation, prepare Eppendorf tubes 2 ml containing ~750µL glass beads 0.1 mm. (1 per sample)
- weigh 15 mg PVPP on aluminium (1 per sample) + 300mg PVPP for the TENP solution
- at the end of incubation, add the beads to each sample.
- Shake in Bead-Beater™ for 5min (average speed)
- Let Bead-Beater™ still for 5min
- Shake again in Bead-Beater™ for 5min

Note: this ensures mechanical lysis of cells.

- Add 15mg PVPP per sample

Note: PVPP precipitates/adsorbs aromatic molecules

- Vortex : PVPP should not separate or float
- Centrifuge 3min at 14000RPM
- Collect supernatant in 2mL sterile tube with pipett

Note: possible to stop for up to 2 hours setting samples at 4°C

- Add 500µL TENP to the pellet (TENP homogenized before use)
- Vortex to fully resuspend the pellet
- Centrifuge 3min at 14000RPM
- Collect supernatant and pool with first one
- Repeat this TENP washing operation twice (3 washes total)

- Centrifuge the 2mL tube containing the pooled supernatants for 1min at 15000rpm

Note: this eliminates any particle in suspension.

- Dispense supernatant in two equal volumes in 2mL Eppendorf tubes (2 tubes per sample)
- Add 1mL isopropanol (propanol-2) in each tube

Note: isopropanol will precipitate nucleic acids

- mix gently by returning tubes a few times (DNA may be visible as a floculum)
- Leave for 10 min at room temperature (or overnight at 4°C)
- Centrifuge 5min at 14000RPM
- discard supernatant (using pipett)
- Dry tubes upside down on a kimwipe (making sure pellet is stable)
- Add 225µL phosphate buffer
- Add 25µL potassium acetate

Note : the latter precipitates proteins

- Vortex to resuspend the pellets
- Pool the 2 of the same sample in one of the 2 tubes
- Vortex and use a P200 pipett to dissolve
- leave 1h30 minimum on ice

Note: possible to stop for up to 2 hours at 4°C or overnight setting samples on ice

- Centrifuge 30min at 14000RPM at 4°C
- turn on heating blocks at 37°C
- collect supernatant containing DNA in 1.5mL Eppendorf tubes
- Add 2µL Rnase (10mg/mL)/vortex/spin
- Incubate 30 minutes at 37°C

Note : this ensures full digestion of RNA

- Add 50µL sodium acetate
- Add 1mL Ethanol 100% from –20°C
- mix gently by returning tubes a few times
- Leave 5 min at room temperature

Note: possible to stop overnight at 4°C or for longer periods at -20°C

Note: This step leads to DNA precipitation

- Prepare a 1.5mL tube with 500 µL cold ethanol 70% per sample
- If the DNA forms a floc, it is collected with the sterile tip of a Pasteur pipette and transferred to the tube containing 500µl ethanol 70%.
- Otherwise, collect the DN by centrifugation 3mns at 14000RPM remove ethanol and rinse with 500 µl Ethanol 70%, vortex
- Centrifuge 3 mns at 14000RPM and discard ethanol (wash 1)
- Do a second wash with 500 µl Ethanol 70%, vortex
- Centrifuge 3 mns at 14000RPM and discard ethanol (wash 2)
- Eliminate residual ethanol tapping tubes gently on a kimwipe
- Dry for 1h30 in laminar flow hood
- re suspend pellet in 200µL TE (more if necessary)
- leave at room temperature for 1 to 2h
- Vortex and re suspend completely using P200 pipette
- Store frozen at –20°C ; or at 4°C if to be used within a couple of days

IHMS DNA extraction protocol # 3

MoBio PowerSoil (HMP modification)

Step 1:

- Measure out 2.0 grams stool sample in a 15ml Falcon tube
- Add 5 ml of Bead Solution
- Vortex until the stool is homogenized with the stool sample
- Centrifuge for 5 mins @ 1500 g
- Split the supernantant into 5 bead tubes (750µl/tube)
- Incubate sample 10 min at 65°C, then 10 min at 95°C.
- Place @ -80°C overnight

Step 2:

If Solution C1 is precipitated, heat to 60°C until dissolved before use.

- Add 60µl of Solution C1 and vortex briefly.
- Secure PowerBead Tubes on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes.
- Spin tubes at 10,000 x g for 30 seconds at room temperature.
- Transfer the supernatant to a clean 2 ml Collection Tube. Expect ~400-500µl supernatant.
- Add 250µl of Solution C2 and vortex for 5 sec. Incubate at 4°C for 5 min.
- Spin the tubes at room temperature for 1 minute at 10,000 x g.
- Transfer no more than, 600µl of supernatant to a 2 ml Collection Tube.
- Add 200µl of Solution C3 and vortex briefly. Incubate at 4°C for 5 minutes.
- Spin tubes at 10,000 x g for 2 minutes at room temperature..
- Transfer no more than 750µl of supernatant to a 2 ml Collection Tube
- Add 1200µl of Solution C4 to the supernatant and vortex for 5 seconds.
- Load 675µl onto a Spin Filter and spin at 10,000 x g for 1 minute at room temperature. Discard the flow through. Repeat a total of three times to process all sample.
- Add 500µl of Solution C5 and spin for 30 seconds at 10,000 x g.
- Discard the flow through.
- Centrifuge again at room temperature for 1 minute at 10,000 x g.
- Place Spin Filter in a clean 1.5 ml tube avoiding splashing Solution C5 onto the Spin Filter.
- Add 100µl of sterile DNA-Free PCR Grade Water to the center of the white filter membrane.
- Spin for 30 seconds at 10,000 x g.
- Store frozen (-20° to -80°C).

IHMS DNA extraction protocol #4

- Turn on heating block to 75°C
- Label up screw-capped tubes, add 200 mg glass beads to each tube
- Take frozen fecal samples out of freezer to thaw
- Add 300 µL SLX buffer (from Omega Bio-Tek E.Z.N.A.® Stool DNA Kit) to each tube
- Add 10 µL proteinase K solution to each tube (20mg/mL proteinase K in 0.1mM $CaCl_2$)
- Invert tube 6 times to mix, add 200 mg of each fecal sample to prepared screw capped tubes
- Bead beat screw capped tubes 4 x 45 sec
- Incubate tubes at 70°C for 10 min (after 8 minutes has elapsed, turn heating block up to 95°C)
- Incubate for a further 5 min
- Incubate on ice for 2 min
- Add 100 µL Buffer P2 (from Omega Bio-Tek E.Z.N.A.® Stool DNA Kit), vortex for 30 sec
- Incubate on ice for 5 min
- Spin at 14500 x g for 5 min
- Remove supernatant to new 1.5 mL tube (discard pellet)
- Add 200 µL HTR reagent (from Omega Bio-Tek E.Z.N.A.® Stool DNA Kit) to each tube using a wide bore tip, vortex for 10 sec (mix HTR reagent bottle well before pipetting)
- Incubate at room temperature for 2 minutes (prepare Maxwell kit components (from Promega's Maxwell®16 DNA Purification Kit)while waiting)
- Spin at 14500 x g for 2 min
- Add supernatant to Maxwell cartridge (adding 300 µL Elution buffer to each blue collection tube), run through Maxwell 16 Instrument cycle.
- Transfer DNA from elution tube into labelled screw-cap 1.5 mL tube, store in -80°C freezer

IHMS DNA extraction protocol #5

Adapted from Zhongtang Yu and Mark Morrison, BioTechniques, 36:808-812.
This procedure is known as Repeated Bead-Beating (RBB) or the "double bead-beater procedure".

Materials

- Gloves
- 1.5 ml eppendorf tubes (B74085-BIOplastics)
- 2.0 ml eppendorf tubes (623 201 Greiner)
- 2.0 ml screw cap tubes (B91211-BIOplastics)
- screw caps (B91303-BIOplastics)
- Glass beads 3mm
- Silicium / Zirkonium beads 0.5 mm (11079101 BioSpec)
- RNase-free Filtertips 10 (B95012-BIOplastics)
- RNase-free Filtertips 200 (4810-Corning)
- RNase-free Filtertips 1000 (B95210-BIOplastics)
- Nuclease free water (Promega-P1193)
- RNase H (Promega- M428A)
- Ethanol, >99% (Merck-)
- Ammonium acetate (Merck-)
- 2-Propanol (Merck 1.01040)
- Ethanol, pure (Merck 1.00983)
- QIAamp DNA stool Minikit (Qiagen 51504)

Equipment

- Thermoblock (<100oC)
- waterbath (<100oC)
- eppendorf centrifuge
- eppendorf centrifuge with cooling (5417R)
- Nanodrop-ND-1000
- Beat Beater (Precellys 24, Bertan Technologies)

Solutions

- Lysis buffer
- 500 mM NaCl,50 mM Tris-HCl (pH 8), 50 mM EDTA, 4 % SDS.
- 10 M ammonium acetate
- Measure 192.7gr $C_2H_7NO_2$ and fill to 250ml with water.
- 70% ethanol
- 35ml pure ethanol, add 15 ml water.

Cell lysis

1. Add 0,5g of 0,1mm zirconia beads and 4 glass beads (3 mm) to a 2,0ml screw-cap tube, then sterilize.
2. Weigh 0,25 g of faeces into the tube and add 1,0 ml of Lysis buffer.
    a. If buffer is precipitated heat at +70°C

3. Treat sample in FastPrep at room temperature (RT) at 5,5 ms for 3x 1min (cool samples on ice in between).
4. Heat at 95°C for 15 min mix samples shaking by hand every 5 min.
5. Centrifuge at +4°C for 5 min at full speed (to pellet stool particles).
    a. Transfer the supernatant into new 2ml eppendorf tube.
6. Add 300 ul of fresh lysis buffer to the lysis tube and repeat steps 3-4, then pool the supernatants.

Precipitation of nucleic acids:

7. Add 260 ul of 10 M ammonium acetate to each lysate tube, mix well, and incubate on ice for 5 min
8. Centrifuge at 4°C for 10 min at full speed in a cooled centrifuge. Discard pellet.
9. Transfer the supernatant to two 1,5 ml eppendorf tubes, add one volume of isopropanol and mix well, and incubate on ice for 30 min
10. Centrifuge at RT for 15 min at full speed, remove the supernatant by decanting. Wash nucleic acids pellet with 500 µl 70 % EtOH for 2 min. and dry the pellet to air with cups turned up-side down.
11. Dissolve the nucleic acid pellet in 200 ul (100 ul each) of TE buffer, leave at 4°C overnight and pool the two aliquots.

Removal of RNA, protein and purification (QIAmp DNA Mini Kit):

12. Add 2 ul of DNase-free RNase (10 mg/ml) and incubate at 37°C for 15 min.
13. Add 15 ul of proteinase K and 200 ul of Buffer AL mix well and incubate at 70°C for 10 min.
    a. Do not mix proteinase K and Buffer AL in advance!
14. Add 200 ul of EtOH and mix well. Transfer to a QIAmp column and centrifuge for 1 min at 13.000 rcf.
15. Discard the flow through, add 500 ul of Buffer AW1 and centrifuge for 1 min at RT at 13.000 rcf.
16. Discard the flow through, add 500 ul of Buffer AW2 and centrifuge for 1 min at RT at 13.000 rcf.
17. Dry the column by centrifugation at RT for 1 min, leaving the cup open.
18. Add 100 ul of Buffer AE and incubate at room temperature for 1 min. Then centrifuge at 13.000 rcf for 1 min.
19. Re-use the elute with the DNA, incubate for 1 min. Then centrifuge at 13.000 rcf for 1 min.

IHMS DNA extraction protocol #6

Protocol of the Repeated Bead Beating Plus Column (RBB+C) Method

(Improved extraction of PCR-quality community DNA from digesta and fecal samples. Zhongtang Yu and Mark Morrison. Short Technical Reports. BioTechniques 36:808-812 (May 2004)

Cell lysis:

1. Transfer 0.25 g of sample into a fresh 2-mL screw-cap tube. Add 1 mL of lysis buffer [500 mM NaCl, 50 mM Tris-HCl, pH 8.0, 50 mM EDTA, and 4% sodium dodecyl sulfate (SDS)] and 0.4 g of sterile zirconia beads (0.3 g of 0.1 mm and 0.1 g of 0.5 mm).
2. Homogenize for 3 min at maximum speed on a Mini-Beadbeater™ (BioSpec Products, Bartlesville, OK, USA).
3. Incubate at 70°C for 15 min, with gentle shaking by hand every 5 min.
4. Centrifuge at 4°C for 5 min at 16,000× g. Transfer the supernatant to a fresh 2-mL Eppendorf® tube.
5. Add 300 µL of fresh lysis buffer to the lysis tube and repeat steps 2–4, and then pool the supernatant.

Precipitation of nucleic acids:

6. Add 260 µL of 10 M ammonium acetate to each lysate tube, mix well, and incubate on ice for 5 min.
7. Centrifuge at 4°C for 10 min at 16,000× g.
8. Transfer the supernatant to two 1.5-mL Eppendorf tubes, add one volume of isopropanol and mix well, and incubate on ice for 30 min.
9. Centrifuge at 4°C for 15 min at 16,000× g, remove the supernatant using aspiration, wash the nucleic acids pellet with 70% ethanol, and dry the pellet under vacuum for 3 min.
10. Dissolve the nucleic acid pellet in 100 µL of TE (Tris-EDTA) buffer and pool the two aliquots.

Removal of RNA, protein, and purification:

11. Add 2 µL of DNase-free RNase (10 mg/mL) and incubate at 37°C for 15 min.
12. Add 15 µL of proteinase K and 200 µL of Buffer AL (from the QIAamp DNA Stool Mini Kit), mix well, and incubate at 70°C for 10 min.
13. Add 200 µL of ethanol and mix well. Transfer to a QIAamp column and centrifuge at 16,000× g for 1 min.
14. Discard the flow through, add 500 µL of Buffer AW1 (Qiagen), and centrifuge for 1 min at room temperature.
15. Discard the flow through, add 500 µL of Buffer AW2 (Qiagen), and centrifuge for 1 min at room temperature.
16. Dry the column by centrifugation at room temperature for 1 min.
17. Add 200 µL of Buffer AE (Qiagen) and incubate at room temperature for 2 min.
18. Centrifuge at room temperature for 1 min to elute the DNA.
19. Aliquot the DNA solution into four tubes. Run 2 µL on a 0.8% gel to check the DNA quality.

20. Store the DNA solutions at -20°C.

IHMS DNA extraction protocol # 7

Initial samples of 200mg stool => n=8, split into several aliquots of approximately 20mg each (see below).

DNA Extraction Procedure

- Take out the sample tube (feces pellet aliquot) from freezer.
- Add X µL Tris-SDS solution, homogeneize and dispatch in Y tubes (with cutted tips).
- Add 0.3 g glass beads, Z µl tris-SDS and 500 µl TE-saturated phenol

Sample name	Weight (mg)	X µl Tris-SDS	Y tubes	Z µL tris-SDS
A1-033	123	600 µl	6	200 µl
A1-083	185	900 µl	9	200 µl
A1-133	152	700 µl	7	200 µl
A1-183	183	900 µl	9	200 µl
B1-033	182	900 µl	9	200 µl
B1-083	203	1 ml	10	200 µl
B1-133	196	1 ml	10	200 µl
B1-183	175	800 µl	8	200 µl
C1-010	bacterial pool	300 µl	1	0
C1-030	from 0,9 ml	300 µl	1	0

- Shake the tube vigorously using FastPrep at 5.0 power level for 30 seconds to disrupt the bacterial cells.
- Centrifuge the tube (15,000 rpm × 5 min, 4˚C).
- Transfer 400 µl of the supernatant into a new 2ml screw cap micro tube. Add 400 µl phenol/chloroform/isoamyl alcohol (25:24:1).
- Shake the tube vigorously using FastPrep FP120 at 4.0 power level for 45 seconds.
- Centrifuge the tube (15,000 rpm × 5 min, 4˚C).
- Transfer 250 µl of the supernatant into a new 1.5 ml screw cap micro tube.
- Add 25 µl 3 M sodium acetate (pH5.2).
- Add 300 µl isopropanol, and mix the solution by inversion.
- Centrifuge the tube (15,000 rpm × 5 min, 4˚C).
- Discard the supernatant.
- Add 500 µl 70% ethanol.
- Centrifuge the tube (15,000 rpm × 5 min, 4˚C).
- Discard the supernatant.
- Dry the pellet by heating the tube on a heat block incubator at 60˚C.
- Add 100 µl TE. Vortex the solution by pulses to dissolve the pellet.
- Let the tubes one night at 4°C
- Add 1µl of RNase (20 mg/ml), heat 30 minutes at 37°C
- store at -80°C

IHMS DNA extraction protocol #8

Necessary Material

- Scale
- Water bath
- Heating blocks
- Homogenizer-Beater Retsch MM200
- Micro-Centrifuge
- Vortex
- Spatula
- Pipetts
- Sterile cones
- Sterile Microtubes 1,5 and 2 mL
- Lysozyme solution:
    - Lysozyme
    - Tris-HCl 1 M, pH 8
    - EDTA 0.5 M, pH 8
    - Triton X-100
    - H2O
- QIAamp DNA Stool Mini Kit (Qiagen)
- Zirconium beads 0,1 mm
- Absolute ethanol

Method

- Aliquot 300 mg zirconium beads per sample.
- Pre-heat heating block at 37°C take stool samples of the freezer.
- Prepare lysozyme :
- Weigh 100 mg lysozyme
- Add 100 µl Tris-HCL 1M pH8
    - 20 µl EDTA 0,5M pH8
    - 60 µl Triton X-100
    - 5 ml H_2O
- for 200 mg stool in a 2 mL screw-cap tube, give a short pulse of centrifuge to pellet stool material.
- Add 180 µl lysozyme solution to the 200 mg stool.
- Vortex to homogeneity of the suspension.
- Incubate 30 min at 37°C. ste the ASL buffer at 37°C at the same time.
- Add 1,220 ml of ASL buffer to each tube and vortex 15 seconds.
- Set the heating block at 95°C.
- Add 1 aliquot of zirconium beads (300 mg) to each tube and insert tubes in bead beater MM200 ; run during 3 min at 30 revolutions per second (maximum speed).
- Incubate 10 min at 95°C and vortex 15 sec.
- Centrifuge 1 min at 13000 rpm at room temperature.
- Transfer 1,2 ml of supernatant in an Eppendorf 2 mL tube.
- Add an InhibitEX pill to each tube, vortex untill complete dissolution (approximately 1 minute) and leave 1 min at room temperature.
- Set the heating block at 70°C.

- Centrifuge 6 min at 13200 rpm.
- Make sure that InhibitEX did pellet. If necessary, recentrifuge 6 min at 13200 rpm.
- Transfer supernatant to an Eppendorf 1,5 mL tube.
- Centrifuge 3 min at 13200 rpm.
- Dispense 15 µl of proteinase K in new Eppendorf 1,5 mL tubes.
- Transfer 200 µl of surnageant in tubes containing proteinase K; store remaining supernatant at -20°C.
- Add 200 µl of AL buffer and vortex to complete homogeneity.
- Incubate 10 min at 70°C and centrifuge a few seconds.
- Add 200 µL absolute ethanol and vortex. give a short pulse of centrifuge.

Tubes may be kept at 4°C for a maximum of 2 hours.

- Draw out mini-columns and label them.
- Set the content of the tubes on the columns (Volume is ~700µL).
- Centrifuge 2 min at 13200 rpm. Discard filtrate and reset in collection tube.
- Add 500 µL of AW1 buffer.
- Centrifuge 1 min at 13200 rpm. Discard filtrate and reset in collection tube.
- Add 500 µL of AW2 buffer.
- Centrifuge 1 min at 13200 rpm. Discard filtrate and reset in collection tube.
- Perform a second wash with 500µl of AW2 buffer.
- Centrifuge 3 min à 13200 rpm. Discard filtrate and transfer column to a 1,5 mL tube.
- Add 200 µL of AE buffer.
- Incubate 5 min at room temperature
- Centrifuge 1 min at 13200 rpm. Discard column.
- Store eluate at -20°C.

IHMS DNA extraction protocol #9

Fecal DNA extraction with Repated Beat Beating (RBB) method

Cell lysis

1. Add 0,25g of Ø 0,1mm zirconia/silica beads (BioSpec, Cat. No. 11079101z) and 3 glass beads (Ø 3 mm) into 2,0ml screw-cap tube (Sarstedt, 72.693).
2. Weigh 0,125g of faeces and add 0,5 ml of Lysis buffer (500 mM NaCl,50 mM Tris-HCl (pH 8), 50 mM EDTA, 4 % SDS) If buffer is precipitated heat at +70°C.
3. Treat sample in FastPrep®-24 Instrument (116004500) with CoolPrep Adapter (6002-528) (MP biomedicals) at 5,5 ms for 3x1 min (after every min wait for 5 min samples on ice to cool down the instrument). Use cool prep adapter with small amount of dry ice.
4. Incubate at 95°C for 15 min with gentle shaking by thermomixer.
5. Centrifuge at +4°C for 5 min at full speed (to pellet stool particles)
   Transfer the supernatant into new 2 ml eppendorf tube and store supernatant on ice.
6. Add 150 ul of fresh lysis buffer to the lysis tube and repeat steps 3-5, and then pool the supernatants.

Precipitation of nucleic acids

7. Add 130 ul of 10 M ammonium acetate to each lysate tube, mix well, and incubate on ice for 5 min.
8. Centrifuge at +4°C for 10 min at full speed.
9. Transfer the supernatant to 2 ml eppendorf tube, add 750 ul of isopropanol and mix well, and incubate on ice for 30 min.
10. Centrifuge at +4°C for 15 min at full speed, remove the supernatant using aspiration, wash nucleic acids pellet with 70 % EtOH (0,5 ml) and dry the pellet under vacuum for 3 min.
11. Dissolve the nucleic acid pellet in 200 ul of TE buffer .

Removal of RNA, protein and purification: (with QIAamp DNA Minikit (Qiagen, 51306)

12. Add 2 ul of DNase-free RNase (10 mg/ml) (Roche, 10109142001) and incubate at +37°C for 15 min.
13. Add 15 ul of proteinase K and 200 ul of Buffer AL mix well and incubate at +70°C for 10 min.
14. Add 200 ul of EtOH and mix well. Transfer to a QIAmp column and centrifuge for 1 min at full speed.
15. Discard the flow through, add 500 ul of Buffer AW1 and centrifuge for 1 min at RT.
16. Discard the flow through, add 500 ul of Buffer AW2 and centrifuge for 1 min at RT.
17. Dry the column by centrifugation at RT for 1 min.
18. Add 100 ul + 100 ul of Buffer AE and incubate at room temperature for 1 min.
19. Centrifuge at RT for 1 min to elute the DNA.

IHMS DNA extraction protocol #10

Lysis

- Resuspend fecal sample (100-200 mg) in 750 µl Lysis buffer and transfer to 2 ml screw-cap tube containing 300 mg of zirconium beads (0.1 mm, BioSpec Products).
- Incubate at 37˚C for 30 min.
- Add 85 µl 10 % SDS solution and 40 µl Proteinase K (15 mg/ml) and incubate 30 min at 60˚C.
- Add 500 µl of Phenol:Chloroform:Isoamyl alcohol (25:24:1).
- Disrupt cell that did not lyse with enzymatic lysis using a bead beater (BioSpec Products) set on high/homogenize for 2 min.
- Put on ice (2-5 min).
- Spin 13,000 rpm for 5 min, remove top layer and put into 1.5 ml eppendorf tube.
- Extract with Phenol:Chloroform:Isoamyl alcohol (25:24:1), vortex, spin at 13,000 rpm for 5 min and carefully remove upper phase.
- Extract once with Chloroform:Isoamylalcohol, vortex, spin, carefully remove top layer into new eppendorf tube.
- Precipitate DNA with 2.5 Vol ethanol and 1/10 vol 3 M sodium acetate (pH 5.2) and leave at -20 at least 1 hour (can be over night), or -80 for 30 min
- Spin at 13,000 rpm for 5 min to pellet DNA.
- Carefully discard Ethanol without disturbing DNA pallet (which may be brown) and air dry for 30 min.
- Resuspend DNA in 200 µl Tris Buffer (10 mM, pH 8) or H2O.
- *Comment: From here use the DNeasey Blood and Tissue kit and follow their instructions or instructions below.*
- Add 200 µl Buffer AL (without added ethanol). Mix thoroughly by vortexing.
- Ensure that ethanol has not been added to Buffer AL (see “Buffer AL”, page 18).
- Buffer AL can be purchased separately (see page 56 for ordering information).
- It is essential that the sample and Buffer AL are mixed immediately and thoroughly by vortexing or pipetting to yield a homogeneous solution.
- Add 200 µl ethanol (96–100%) to the sample, and mix thoroughly by vortexing. It is important that the sample and the ethanol are mixed thoroughly to yield a
- homogeneous solution.
- Pipet the mixture into the DNeasy Mini spin column placed in a 2 ml collection tube (provided). Centrifuge at _6000 x g (8000 rpm) for 1 min. Discard flow-through and collection tube.*
- Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 µl Buffer AW1, and centrifuge for 1 min at _6000 x g (8000 rpm). Discard flow-through and collection tube.*
- Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 µl Buffer AW2, and centrifuge for 3 min at 20,000 x g (14,000 rpm) to dry the DNeasy membrane. Discard flow-through and collection tube.
- It is important to dry the membrane of the DNeasy Mini spin column, since residual ethanol may interfere with subsequent reactions. This centrifugation step ensures that no residual ethanol will be carried over during the following elution. Following the centrifugation step, remove the DNeasy Mini spin column carefully so that the column does not come into contact with the flow-through, since this will result in carryover of

ethanol. If carryover of ethanol occurs, empty the collection tube, then reuse it in another centrifugation for 1 min at 20,000 x g (14,000 rpm).

- Place the DNeasy Mini spin column in a clean 1.5 ml or 2 ml microcentrifuge tube (not provided), and pipet 200 µl Buffer AE directly onto the DNeasy membrane. Incubate at room temperature for 1 min, and then centrifuge for 1 min at _6000 x g (8000 rpm) to elute.

Lysis buffer:

- 200 mM NaCl
- 100 mM Tris (pH 8.0)
- 20 mM EDTA
- Prepare and autoclave
- Add Lysozyme to 20 mg/ml before use
- PBS (per liter):
- 8 g NaCl
- 0.2 g KCl
- 1.44 g Na_2HPO_4
- 0.24 g KH_2PO_4

IHMS DNA extraction protocol # 11

Overview of Steps and Material needed

- lysis of stool samples in Buffer ASL
- adsorption of impurities to InhibitEX matrix
- purification of DNA on QIAamp Mini spin columns (QIAamp DNA Stool Mini Kit)

Equipment and Reagents Needed – not in Kit

- Ethanol (96-100%)
- BSA (for downstream PCR)
- 1.5 and 2 ml microcentrifuge tubes (locking caps/screw caps)
- Pipet tips with filter and barrier
- Microcentrifuge
- Water bath for incubation or heat block at 70˚ C
- Spatulas
- Vortex
- Ice

Kit Contents

Number of preps 50

QIAamp Mini Spin Columns	50
Collection Tubes (2 ml)	200
InhibitEX® Tablets	50
Buffer ASL	140 ml
Buffer AL*	33 ml
Buffer AW1* (concentrate)	19 ml
Buffer AW2† (concentrate)	13 ml
Buffer AE	12 ml
Proteinase K	1.4 ml

- Buffer ASL (store at room temp)
    - mix buffer ASL by shaking
    - if precipitate has formed, incubate at 70˚ C
- Buffer AL (store at room temp)
    - mix buffer AL by shaking
    - if precipitate has formed, incubate at 70˚ C
    - DO NOT add proteinase K directly to Buffer AL
- Buffer AW2 (store at room temp)
    - Add 30 ml ethanol to Buffer AW2 concentrate (indicated on the bottle)

    - Mix thoroughly before use
- InhibitEX Tablets
    - Pop tablet directly out of packet into suitable 2 ml tube without touching
    - 2 ml tubes should be check to make sure mouth is wide enough to accomodate tablet
- QIAamp Mini Spin Columns - PRECAUTIONS
    - Carefully apply the sample or solution to the Mini spin column. Pipet into column without moistening the rim of the column.
    - Change pipet tips between all transfers (aerosol barrier pipet tips only)
    - Do not touch pipet tip to the Mini spin column membrane
    - After all vortexing steps, centrifuge tubes to remove drops from inside lid
    - Close Mini spin column before placing in microcentrifuge.
    - Remove both Mini spin column and collection tube from the centrifuge. Place Mini spin column into a new collection tube. Discard the filtrate and collection tube in Biohazard bin.
    - Open only one QIAamp Mini spin column at a time and avoid generating aerosols.
    - Set up a rack with multiple labeled collection tubes for transferring the Mini spin columns after centrifugation.
- Centrifugation
    - All centrifugation steps are carried out at room temperature.
    - Speed should be 20,000 x g (14,000 rpm). If centrifuge cannot reach 20,000 x g, increase time proportionately (e.g. as a proxy, at 10,000, centrifuge for double the time required at 20,000)

Set up

- Check that 2 ml tubes used in step 5 are wide enough to accommodate InhibitEX tablet.
- Prepare all buffers according to label instructions.
- Deal with any precipitate in buffer solutions by placing in 70°C water bath until precipitate dissipates.
- Prepare a 70°C water bath for buffers and heat block for other steps (3 and 12).

Procedure

Lyse cells in Buffer ASL

NOTE: Steps 1-8 involve material that is a biohazard. Use all precautions appropriate for handling biohazards.

1. Weigh 180–220 mg (best at 190-210 mg) stool in a 2 ml microcentrifuge tube, weigh the tube and place the tube on ice. If the sample is frozen, use a scalpel or spatula to scrape bits of stool into a 2 ml microcentrifuge tube on ice. Do not allow sample to thaw.
2. Add 1.4 ml Buffer ASL (700 ul and 700 ul) to each stool sample. Vortex continuously for 1 min or until the stool sample is thoroughly homogenized. Note: It is important to vortex the samples thoroughly and this step usually requires > 1 min. This helps ensure maximum DNA concentration in the final eluate.
3. Heat the suspension for 5 min at 70°C (up to 95°C for hard-to-lyse bacteria). Use heat block rather than water bath. This heating step increases total DNA yield 3- to 5-fold and helps to lyse bacteria and other parasites. The lysis temperature can be increased to 95°C for cells that are difficult to lyse (such as Gram-positive bacteria).

4. Vortex for 15 s and centrifuge sample at full speed for 1 min to pellet stool particles.
5. Pipet 1.2 ml (600 µl and 600 µl) of the supernatant into a new 2 ml microcentrifuge tube and discard the pellet. Note: The 2 ml tubes used should be wide enough to accommodate an InhibitEX Tablet. Transfer of small quantities of pelleted material will not affect the procedure.

Absorb inhibitors with InhibitEX

6. Add 1 InhibitEX Tablet to each sample and vortex immediately and continuously for 1 min or until the tablet is completely suspended. Incubate suspension for 1 min at room temperature to allow inhibitors to adsorb to the InhibitEX matrix.
7. Centrifuge sample at full speed for 3 min to pellet inhibitors bound to InhibitEX matrix.
8. Pipet all the supernatant into a new 1.5 ml microcentrifuge tube and discard the pellet. Centrifuge the sample at full speed for 3 min. Transfer of small quantities of pelleted material from step 7 will not affect the procedure.

Digest Proteins

9. Pipet 15 µl proteinase K into a new 1.5 ml microcentrifuge tube.
10. Pipet 200 µl supernatant from step 8 into the 1.5 ml microcentrifuge tube containing proteinase K.
11. Add 200 µl Buffer AL and vortex for 15 s. Note: Do not add proteinase K directly to Buffer AL. It is essential that the sample and Buffer AL are thoroughly mixed to form a homogeneous solution.
12. Incubate at 70°C for 10 min. Centrifuge briefly to remove drops from the inside of the tube lid (optional).
13. Add 200 µl of ethanol (96–100%) to the lysate, and mix by vortexing. Centrifuge briefly to remove drops from the inside of the tube lid (optional).

Bind DNA in Spin Column

14. Label the lid of a new QIAamp spin column placed in a 2 ml collection tube. Carefully apply the complete lysate from step 13 to the QIAamp spin column without moistening the rim. Close the cap and centrifuge at full speed for 1 min.
15. Place the QIAamp spin column in a new 2 ml collection tube, and discard the tube containing the filtrate. Close each spin column in order to avoid aerosol formation during centrifugation. If the lysate has not completely passed through the column after centrifugation, centrifuge again until the QIAamp spin column is empty.

Wash Spin Column

16. Carefully open the QIAamp spin column and add 500 µl Buffer AW1. Close the cap and centrifuge at full speed for 1 min. Place the QIAamp spin column in a new 2 ml collection tube, and discard the collection tube containing the filtrate.
17. Carefully open the QIAamp spin column and add 500 µl Buffer AW2. Close the cap and centrifuge at full speed for 3 min. Discard the collection tube containing the filtrate. Note: Residual Buffer AW2 in the eluate may cause problems in downstream applications. Some centrifuge rotors may vibrate upon deceleration, resulting in the flow-through, which contains Buffer AW2, contacting the QIAamp spin column. Removing the QIAamp spin column and collection tube from the rotor may also cause flow-through to come into contact with the QIAamp spin column.

18. Recommended (definitely do): Place the QIAamp spin column in a new 2 ml collection tube and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min. This step helps to eliminate the chance of possible Buffer AW2 carryover.

Elute DNA

19. Transfer the QIAamp spin column into a new, labeled 1.5 ml microcentrifuge tube. Carefully open the QIAamp spin column and pipet 200 µl Buffer AE directly onto the QIAamp membrane. Close the cap and incubate for 1 min at room temperature, then centrifuge at full speed for 1 min to elute DNA.

IHMS DNA extraction protocol # 12

- Starting material: 200 mg solid faeces or 200 µL faeces-water suspension contained in a 2 mL Eppendorf tube.
- 400 µL NucleoSENS Lysis Buffer (Biomerieux) is added and mixed by vortexing.
- Next, shaking for 5 min at maximum speed on a TissueLyser (Qiagen).
- Centrifugation for 2 min at 13.000 rpm.
- 100 µL supernatant is mixed with 2 mL NucleoSENS Lysis Buffer and incubated at room temperature for 10 min.
- 2.1 mL sample is transferred to sample vessel on the Easymag (Biomerieux) apparatus.
- Easymag protocol: “Specific A” with 140 µL magnetic silica and 110 µL output volume.

IHMS DNA extraction protocol # 13

Protocol for Isolation of DNA from Stool Sample
(Qiagen stool DNA extraction kit)

Homogenize a fresh fecal sample (kept cold for a maximum of 12 hours) by kneading in a strong plastic bag (preferably at 4 °C, keep samples cold throughout). DNA extraction can be performed on previously frozen stool (avoid multiple freeze/thaw cycles).

1. Weigh 200-300 mg (half pea sized) solid stool in a 2 ml microcentrifuge tube and place the tube on ice. Use 300-500 µl of loose stool (more for watery stools)
2. Put 4-5 glass beads along with 1 ml 0.05 M phosphate buffer, vortex until the stool sample is thoroughly homogenized.
3. Spin down at full speed (table centrifuge >10.000 rpm) and save pellet (pellet needs to be clearly visible). Add 1 ml 0.05 M phosphate buffer to wash, vortex and spin down again, save the pellet.
4. Add 1.4 ml buffer ASL to each tube. Vortex continuously for 1 min or until the sample is completely suspended.
5. Heat the suspension for 5 min at 70° C.
6. Add 0.3 g zirconia beads and fill with ASL buffer, beat for 3 min on “homogenize”.
7. Centrifuge the sample at full speed for 1 min to pellet the particles.
8. Pipet 1.2 ml of the supernatant into a new 2 ml microcentrifuge tube and discard the pellet.
9. Add 1 InhibitEX tablet to each sample and vortex immediately and continuously for 1 min or until the tablet is completely suspended. Incubate suspension for 1 min at room temperature to allow inhibitors to absorb to the inhibitEX matrix.
10. Centrifuge sample at full speed for 3 min to pellet inhibitors bound to InhibitEX.
11. Pipet the supernatant into a new 1.5 ml microcentrifuge tube and discard the pellet. Centrifuge the sample at full speed for 3 min.
12. Pipet 25 µl Proteinase K into a new 1.5 ml microcentrifuge tube.
13. Pipet 400 µl supernatant from step 8 into the 1.5 ml microcentrifuge tube containing Proteinase K.
14. Add 400 µl buffer AL and vortex for 15 s.
15. Incubate at 70° C for 10 min.
16. Add 400 µl ethanol of 200 proof to the lysate, and mix by vortexing.
17. Label the lid of TWO QIAamp spin columns placed in 2 ml collecting tubes. Carefully apply 610 ul of the complete lysate from step 16 to each of the two QIAamp spin column without moistening the rim. Close the cap and centrifuge at full speed for 1 min. Discard the filtrate. Place the QIAamp spin column in a new 2 ml collection tube, and discard the tube containing the filtrate.
18. Carefully open the QIAamp spin column and add 500 µl buffer AW1. Centrifuge at full speed for 1 min. Place the QIAamp spin column in a new 2 ml collection tube, and discard the collection tube containing the filtrate.
19. Carefully open the QIAamp spin columns and add 500 µl buffer AW2. Centrifuge at full speed for 3 min. Discard the collection tubes containing the filtrates.
20. Preheat the AE buffer at 65° C for 5-10 min.

21. Transfer the QIAamp spin columns into new, labeled 1.5 ml microcentrifuge tubes and pipet 100 µl preheated buffer AE directly onto the QIAamp membranes. Incubate for 5 min at room temperature, then centrifuge at full speed for 1 min to elute DNA.
22. Mix the two DNA samples together (200µl total) then split into on tube containing 175µl and the other containing 25µl. Ethanol precipitate the larger volume and dry the resulting pellet in a biosafety hood. Store the other sample (25µl) at -70°C.

IHMS DNA extraction protocol #14

gDNA extraction using FastDNA SPIN Kit for Soil (new instructions 2011)

- Remove samples for gDNA extraction from -80 °C freezer and place straight on ice, add 122 µl MT buffer and allow to thaw. Flick to get air out of beads, then add ~800 µl Sodium Phosphate Buffer into tube, do not add too much buffer or it will leak out of top of tube.
- Homogenize in the FastPrep® Instrument for 30 seconds at a speed setting of 6.5.
- Centrifuge at 14,000 x g for 5 minutes to pellet debris.
- Transfer supernatant to a clean 2.0 ml microcentrifuge tube. Add 250 µl PPS
- (Protein Precipitation Solution) and mix by shaking the tube by hand 10 times.
- Centrifuge at 14,000 x g for 5 minutes to pellet precipitate. Transfer supernatant to
- a clean 15 ml Corning tube.
- Resuspend Binding Matrix suspension and add 1.0 ml to supernatant in 15 ml tube.
- Place on rotator or invert by hand for 2 minutes to allow binding of DNA. Place tube
- in a rack for 3 minutes to allow settling of silica matrix.
- Remove and discard approx 1000 µl of supernatant being careful to avoid settled Binding
- Matrix.
- Resuspend Binding Matrix in the remaining amount of supernatant. Transfer approximately 600 µl of the mixture to a SPIN™ Filter and centrifuge at 14,000 x g for 1 minute. Empty the catch tube and add the remaining mixture to the SPIN™ Filter and centrifuge again at14,000 x g for 1 minute. Empty the catch tube again. Note – may take longer spins to get all liquid through.

- Add 500 µl prepared SEWS-M wash buffer using the force of the liquid from the pipette tip to gently resuspend the pellet.

NOTE: Ensure that ethanol has been added to the Concentrated SEWS-M.

- Centrifuge at 14,000 x g for 1 minute. Empty the catch tube and replace.
- Without any addition of liquid, centrifuge a second time at 14,000 x g for 2 minutes to “dry” the matrix of residual wash solution. Discard the catch tube and replace with a new, clean 1.5 ml eppendorf tube.
- Air dry the SPIN™ Filter for 5 minutes at room temperature.
- Gently resuspend Binding Matrix (above the SPIN filter) in 180 µl of DES (DNase/Pyrogen-Free Water). Incubate for 5 minutes at 55˚C in a heat block.
- Centrifuge at 14,000 x g for 1 minute to bring eluted DNA into the eppendorf tube. Discard the SPIN filter. DNA is now ready for PCR and other downstream applications.
- Store 50 µl aliquot at -20°C for backup and remainder at 4°C for use.

Do not store DNA in tubes supplied in the kit. Lids do not seal well and evaporation will occur.

IHMS DNA extraction protocol #15

Extraction of bacterial DNA from fecal samples using the QIAamp DNA stool kit (Qiagen, Hilden, Germany) with a modified protocol for cell lysis.

- Homogenization of 220 mg feces with 1.2 ml ASL lysis buffer of the kit by vortexing for 2 min in a 2 ml tube containing 0.75 g of sterile zirconia/silica beads (0.1 mm in diameter)
- Suspension is incubated for 15 min at 95°C with continuous shaking (1,400 min-1, Thermomixer 5436, Eppendorf)
- The sample is allowed to cool down on ice for 2 min
- Cells are mechanically lysed by running the Fastprep™ Instrument (Thermo Electron Corperation) for 8 min 15 sec
- After cooling down on ice for 2 min coarse particles cell debris and the zirconia/silica beads are spun down by centrifugation (20,000 x g, 4°C, for 1 min)
- Supernatant is transferred to a 2 ml tube
- The pellet is mixed with 350 µl ASL lysis buffer of the kit, vortexed for 1 min and incubated for 5 min at 95°C with continuous shaking as described above.
- After centrifugation at 20,000 x g and 4°C for 1 min the supernatants are combined
- InhibitEX tablet provided by the kit is added to the supernatant (DNA-damaging
- substances and PCR inhibitors adsorb to InhibitEX matrix, sample is vortexed immediately and continuously for 1 min, incubation of the suspension for 1 min at RT
    - The InhibitEX matrix is separated by centrifugation at 20,000 x g for 6 min at RT
    - The supernatant is collected and filled up to 1 ml with sterile phosphate-buffered
- saline (pH 7)
    - DNA was purified with the QIAcube machine (Qiagen) and eluted from the silica-based membrane with 200 µl ultra-pure water.
- QIAcube machine steps
- see Protocol QIAamp DNA Stool Handbook p. 16 from 9.-18.

IHMS DNA extraction protocol #16

DNA extraction with PSP® Spin Stool DNA Kit (Invitek)

1. Sample homogenization and prelysis

- Weigh 50 mg of stool sample (frozen) into a 2.0 ml Safe-Lock-Tube and add 1.2 ml Lysis Buffer P to each stool sample .Vortex vigorously for 1 min.
- Incubate the homogenized sample for 10 min at 95°C in a thermomixer under continuous shaking at 900 rpm.
- Incubate the sample on ice for 3 minutes
- Add 5 Zirconia Beads II to the homogenate.
- Put the sample back to the 95°C thermo block, incubate for further 3 min at 95°C under continuous shaking at 900 rpm.
- Vortex the sample for 2 min.
- Centrifuge the sample at 13.400 x g (12.000 rpm) for 1 min to pellet solid stool particles.

2. Removal of PCR Inhibitiors

- Transfer the supernatant into an InviAdsorb-Tube and vortex vigorously for 15 sec. Incubate

3. Second Sample Cleanup

- Transfer the supernatant completely into a new 1.5 ml Receiver Tube. Discard the pellet.
- Centrifuge the sample again at full speed for 3 min.

4. Proteinase K digestion

- Transfer 25 µl Proteinase K into a new 1.5 ml Receiver Tube and pipet 400 µl of the supernatant from step 3 to the 1.5 ml Receiver Tube containing Proteinase K,
- mix shortly by vortexing and incubate the sample for 10 min at 70°C in a thermomixer under continuous shaking at 900 rpm.

5. Binding of the DNA

- Add 200 µl of Binding Buffer P to the lysate and mix shortly by vortexing or by pipetting up and down several times.
- Transfer the mixture completely onto the membrane of the RTA Spin Filter. Incubate for 1 min at room temperature and centrifuge at 9.300 x g (10.000 rpm) for 2 min. Discard the filtrate and the RTA Receiver Tube.

6. Washing Steps

- Put the RTA Spin Filter in a new RTA Receiver Tube.
- Add 500 µl Wash Buffer I to the membrane of the RTA Spin Filter.
- Close the lid and centrifuge at 9.300 x g (10.000 rpm) for 1 min.
- Discard the filtrate and the RTA Receiver Tube.
- Put the RTA Spin Filter in a new RTA Receiver Tube.
- Add 700 µl Wash Buffer II to the membrane of the RTA Spin Filter.
- Close the lid and centrifuge at 9.300 x g (10.000 rpm) for 1 min.
- Discard the filtrate and put the RTA Spin Filter back to the same RTA Receiver Tube.

7. Ethanol Removal

- To eliminate any traces of ethanol, centrifuge again for 3 min at maximum speed, discard the RTA Receiver Tube

8. DNA Elution

- Place the RTA Spin Filter into a new 1.5 ml Receiver Tube
- Add 100 µl preheated (70°C) PCR-H2O.
- Incubate for 15 min. Centrifuge at 9.300 x g (10.000 rpm) for 1 min to elute the DNA.
- Finally discard the RTA Spin Filter.

IHMS DNA extraction protocol # 17

Fecal DNA extraction protocol with adapted G'NOME kit (BIO 101)

Equipment and materials used

- Centrifuge
- Speed Vacuum
- Screw-cap tubes & eppendorf tubes
- Glass beads 0.1 mm (Fisher Bioblock Scientific B74471)
- Water bath 55°C
- Bead-beater
- toothpicks

Reagents

- Cell Lysis/Denaturing solution (from kit)
- RNase Mix (from kit)
- Protease Mix (from kit)
- "Salt-Out" Mixture (from kit)
- PVPP (PolyVinylPolyPyrrolidone) (Sigma)
- TENP (50 mM Tris pH 8, 20 mM EDTA pH 8, 100 mM NaCl, 1% PVPP)
- Molecular water (Eurobio)
- Isopropanol
- Ethanol 100%
- Ethanol 70%
- TE buffer

Solutions preparation

- EDTA pH 8, 0.5M
    - MM: 372.2 g.mol-1
    - 9.305 g for 50 mL H2O sterile
    - Adjust pH with NaOH
- Tris-HCl pH 8 1M
    - MM: 121.1 g.mol-1
    - 6.05 g for 50 mL H20 sterile
    - Adjust pH with HCl
- NaCl 5M
    - MM: 58,44 g.mol-1
    - 14.61 g for 50 mL H2O sterile
- TENP (50 mM Tris pH 8, 20 mM EDTA pH 8, 100 mM NaCl, 1% PVPP)
    - 20 mL H2O sterile
    - 1.5 mL Tris-HCl pH 8 1M
    - 1.2 mL EDTA pH 8 0.5M
    - 0.6 mL NaCl 5M

- 0.3 g PVPP

Extraction protocol

From 200 mg of feces sample (conserved at -80°C). :

- Add 550 µL of Cell suspension Solution (buffer). Use a toothpick to homogenize.
- Add 50 µL of RNase Mix (4°C). Vortex vigorously.
- Add 100 µL of Cell Lysis/Denaturing Solution. Vortex.
- Incubate at 55°C for 30 minutes.
- Add 25 µL of protease mix and vortex.
- Incubate 55°C for 120 minutes.
- Add 750 µL of glass beads 0.1 mm in each sample.
- Put samples for 10 minutes on Bead beater.
- Add 15 mg of PVPP and vortex vigorously.
- Centrifuge 3 minutes at 20 000g
- Retrieve the supernatant in new tubes (Lysate)
- Add 400 µL of TENP to the remaining pellet and vortex (to fully resuspended the pellet)
- Centrifuge 3 minutes at 20 000g.
- Pool the supernatant with the first one for each sample.
- Repeat the washing operation twice.
- Centrifuge 3 minutes at 20 000g all the retrieved supernatant.
- Transfer 750 µL of the supernatant in a new tub (almost half of the whole supernatant).
- Add 1mL of cold Isopropanol (-20 °C) and mix slowly. Let 10 minutes at -20°C. Centrifuge 5 minutes at 20 000g and discard the supernatant. Dry the pellet with the speed vaccum. Take back the DNA pellet with 400 µL of molecular H20.
- Add 100 µL of "Salt Out" mixture. Mix slowly. Let 10 minutes 4°C. Centrifuge 10 minutes at 20 000g. Put the supernatant in a new sterile tub.
- Add 1.5 mL of Ethanol 100% (-20°C) and mix slowly. Let 5 minutes at ambient temperature and centrifuge 5 minutes at 20 000g. Discard the supernatant.
- Take back the pellet with 1 mL of Ethanol 70% (-20°C). Centrifuge 5 minutes at 20 000g.
- Discard the supernatant. Residual ethanol should be removed using Speed Vaccum for 5
- minutes.
- Take back the pellet with 150 µL of TE buffer (10 mM pH 7.5, 1 mM EDTA)
- Transfer in a screw tub.

IHMS DNA extraction protocol #18

Preparation of fecal sample suspension

Collected feces were immediately suspended in 20% glycerol (Wako) / phosphate buffer saline (PBS) solution (GIBCO), frozen in liquid nitrogen, and stored at -80 °C until use. In each experiment, 1.0 g of those of stool were used, respectively.

Recovery of bacteria from fecal samples

Frozen fecal sample (1.0 g of human feces) was thawed gradually on ice and suspended vigorously in a 50 mL tube (Falcon). The suspension of feces was filtered with a 100 µm-mesh nylon filter (Falcon) to separate bacterial cells from eukaryotic cells and other debris. The debris on the filter was washed using a glass or plastic bar with 10 ml of PBS buffer twice. The filtrate was centrifuged at 5,000 rpm for 10 min at 4 °C and the supernatant was then discarded. The bacterial pellet was rinsed with 35 ml of PBS buffer twice, and finally rinsed with 35 ml of TE10 (10mM Tris-HCl, 10mM EDTA, pH8.0) buffer. The bacterial pellet was used for microbial DNA isolation. Bacterial cells suspended in glycerol (20%)-PBS buffer were quickly frozen in liquid nitrogen and could be stored in freezer at -80 °C for at least half a year without degradation.

Microbial cell lysis and DNA isolation by enzymatic lysis method

The bacterial pellet was suspended in 10 mL of TE10 buffer. The suspension was incubated with lysozyme (SIGMA, final conc. 15 mg/mL of cell suspension) at 37 °C for 1 h with gentle mixing. Purified achromopeptidase (Wako, final conc. 2,000 units/mL of cell suspension) was then added and the sample was incubated at 37 °C for 0.5 h. The sample was treated with 10 % (wt/vol) sodium dodecyl sulfate (SDS; final conc. 1 %) and proteinase K (Merck, final conc. 1 mg/mL of cell suspension) and incubated at 55 °C for 1 h. The solution was mixed with equal volume of phenol/chloroform/isoamyl alcohol (Invitrogen) and centrifuged at 5,000 rpm for 10 min. DNA was precipitated by adding 1/10 volume of 3 M sodium acetate (pH 4.5, Wako) and 2 volume of ethanol (Wako). DNA was pelleted by centrifugation at 5,000 rpm at 4 °C for 15 min. DNA pellet was rinsed with 75 % ethanol, dried in vacuum and dissolved in TE buffer.

IHMS DNA extraction protocol #19

Magna Pure LC DNA III Isolation Kit (Bacteria, Fungi), Cat. No. 03 264 785 001

All preparations are performed sterile under the laminar flow hood

- prepare Lysozyme (100mg/ml): 100mg ad 1ml 5% Glycerol/PBS

Stool: Take a peanut-size stool-sample and suspend it in 500µl PBS (2 ml tube)
Do NOT centrifuge!
Take 100µl of the stool-suspension into a MagnaLyser tube
Immediately (!) proceed with step 1) into Magnalyser Bead tube

Procedure

- add 130µl Bacteria Lysis buffer (BLB) to 100µl sample into Magnalyser tubes, mix well
- homogenize at 6500rpm/20sec
- add 5,75µl lysozyme (100mg/ml) to 230µl BLB/sample mixture and mix well,
- Incubate at 37°C for 30min
- add 20µl Proteinase K (Roche ProteinaseK Magna Pure LC, dissolved in 1,2 ml Elution
- buffer)
- mix thoroughly, incubate for 10min at 65°C
- Incubate for a further 10min at 95°C, spin down, cool samples on ice (5min)
- centrifuge 2min at full speed (RT)
- transfer 100µl of the lysate supernatant into a MagnaPure sample tube

Magna Pure preparations

- Use buffers free from precipitates, use buffers at room temperature.
- dissolve Prot.K (1,2ml Elution buffer); mix completely; RT!
- Magnetic Glass Particles: vortex immediately before use!, add the MGPs to the container just before starting the run
- mark 1,5ml-Eppis for Eluates, fill Magnalyser
- select the protocol: ‘DNA Bacteria III’ (sample volume: 100µl, elution volume: 100µl) and follow the instructions of the software
- transfer samples from Sample cartridge to marked 1.5mL tubes and store at -20°C

IHMS DNA extraction protocol # 20

Protocol modified from the QIAamp DNA stool handbook (Stool pathogen detection pp15-18)(Cat#51504)

Before starting: Make sure 70°C water bath is pre-heated.

1. Record the weight of the Dry Bead tubes (MoBio Cat# 12811-100-DBT).
2. Place a pea size sample (for human stool) into each bead tube.

NB. Protocol will work for smaller amounts of 50mg and up to 250mg.

3. Record the weight of the tube again to identify the amount of stool sample used.
4. Add 1.4 mL Buffer ASL to each stool sample. Mechanically lyse cells using Fastprep/homogenizer for 1 minute (repeated twice) at a frequency of 5.5.

NB. Make sure that ASL buffer has not precipitated. If it has, put in 70°C water bath to dissolve.

5. Heat the suspension for 5 minutes in a 70°C water bath.
6. Vortex for 15 sec and centrifuge sample at 15000 rpm for 1 min to pellet stool particles.
7. Add 1 InhibitEX Tablet into new 2.0 mL centrifuge tubes.
8. Pipet 1.2 mL of the supernatant from Step 6 into the 2.0 mL centrifuge tubes containing InhibitEX Tablet and vortex immediately until the tablet is completely suspended. Incubate suspension for at least 1 min at room temperature.
9. Centrifuge sample at 15000 rpm for 3 min to pellet inhibitors bound to InhibitEX matrix.
10. Pipet all the supernatant into a new 1.5 mL centrifuge tube and discard the pellet. Centrifuge the sample at 15000 rpm for 3 min.
11. Pipet 15 µL proteinase K into a new 1.5 mL centrifube tube.
12. Pipet 200 µL supernatant from Step 10 into the 1.5 mL centrifuge tube containing proteinase K.
13. Add 200 µL Buffer AL and vortex for 15 sec.
14. Heat the tubes for 10 minutes in a 70°C water bath.
15. Add 200 µL of 100% ethanol to the lysate and mix by vortexing.
16. Centrifuge sample for 30 sec to remove drops from inside of the tube lid.
17. Pipet the complete lysate from Step 13 to the QIAamp spin column without moistening the rim. Centrifuge at 15000 rpm for 1 min. Place the QIAamp spin column into a new 2 mL collection tube and discard the tube containing the filtrate.
18. Add 500 µL Buffer AW1 to the spin column. Centrifuge at 15000 rpm for 1 min. Place the QIAamp spin column into a new 2 mL collection tube and discard the tube containing the filtrate.
19. Add 500 µL Buffer AW2. Centrifuge at 15000 rpm for 3 min. Place the QIAamp spin column into a new 2 mL collection tube and discard the tube containing the filtrate. Centrifuge again at 15000 rpm for 1 min to eliminate possible Buffer AW2 carryover. Transfer the QIAamp spin column into new 1.5 mL centrifuge tube.
20. Add 30 µL Buffer AE. Incubate for at least 5 min at room temperature. Centrifuge at 15000 rpm for 1 min to elute DNA.

IHMS DNA extraction protocol #21

Standard Operating Procedure for isolation of genomic DNA from feces, in preparation for molecular analysis

PSP® Spin Stool DNA Kit (Invitek)

Equipment and reagents

- microcentrifuge
- thermomixer (for 70°C AND 95°C)
- bead beater (MagNA Lyser, Roche)
- measuring cylinder (250 ml)
- disposable gloves
- RNase-free Filtertips 10
- RNase-free Filtertips 200
- RNase-free Filtertips 1000
- reagents reservoirs for multichannel pipets
- Optional: RNase A (10 mg/ml)
- 96 - 100% ethanol
- Ultra pure water (Milli-Q)
- vortexer or other homogenizer
- Glass beads 3mm
- Silicium / Zirkonium beads 0.5 mm (BioSpec)
- 2.0 ml screw cap tubes
- screw caps
- PSP Spin Stool DNA Kit (Invitek)

Preparing tubes for bead beating

Add 0,5g of 0,1mm zirconia beads and 4 glass beads (3 mm) to a 2,0ml screw-cap tube, then sterilize.

Preparing reagents and buffers for the PSP® Spin Stool DNA Kit

1. adjust the thermomixer to 70°C.
2. dissolve Proteinase K in ddH2O
3. warm up the needed amount of Elution Buffer D to 70°C, (200 µl Elution Buffer D are needed per sample).
4. heat heating blocks (e.g. thermomixer) to 70°C and 95 °C
5. label the needed amount of 2.0 ml RTA Spin Filter Sets
6. label the needed amount of 1.5 ml Receiver Tubes (per sample: 1 Receiver Tube), add the needed amount of ethanol to the Wash Buffer I and II

3 or 10 total DNA extractions:
add 250 µl ddH2O to Proteinase K, mix thoroughly and store the tube at -20°C Wash Buffer I and II are ready to use
50 total DNA-extractions:
add 1.5 ml ddH2O to Proteinase K, mix thoroughly and store the tube at -20°C add 30 ml 96-100% ethanol to the bottle Wash Buffer I

add 42 ml 96-100% ethanol to each bottle Wash Buffer II mix thoroughly and always keep the bottle firmly closed
250 total DNA-extractions:
add 1.5 ml ddH2O to Proteinase K, mix thoroughly and store the tube at -20°C add 80 ml 96-100% ethanol to each bottle Wash Buffer I add 105 ml 96-100% ethanol to each bottle Wash Buffer II mix thoroughly and always keep the bottle firmly closed

Important Notes:

The centrifugation steps were made with the Centrifuge 5415 D from Eppendorf.

The indicated settings refer to this centrifuge.

Preheat the Elution Buffer D to 70°C (e.g. transfer the needed volume into a tube and place it at the appropriate temperature into a thermomixer)

1. Sample homogenization and prelysis

- Add 0,5g of 0,1mm zirconia beads and 4 glass beads (3 mm) to a 2,0ml screw-cap tube, then sterilize.
- Weigh 200 mg of stool sample (fresh or frozen) into the 2.0 ml screw-cap tube and add 1.2 ml Lysis Buffer P.

Important: If the sample is liquid, pipet 200 µl into the 2.0 ml screw-cap tube. Cut-off the end of the pipet tips to make pipetting easier (sterilize the tips after cutting!). If the sample is frozen, use a scalpel or spatula to scrape bits of stool into the provided 2.0 ml screw-cap tube on ice. Take care, that this samples do not thaw until Lysis Buffer P is added, otherwise the DNA in the sample may degrade. After addition of the buffer, the following steps can be performed at RT or like recommended.

- Treat sample in Magna Lyser at room temperature (RT) at 5,5 ms for 3x 1min (cool samples on ice in between).
- Incubate the sample for 10 min at 95°C in a thermomixer under continuously shaking at 900 rpm.
- Centrifuge the sample at 13.400 x g (12.000 rpm) for 1 min to pellet solid stool particles and beads.

2. Removal of PCR inhibitors

- Transfer the supernatant into an InviAdsorb-Tube and vortex vigorously for 15 sec.
- Incubate the suspension for 1 min at room temperature.
- Centrifuge the sample at full speed for 3 min.

3. Second sample cleanup

- Transfer the supernatant completely into a new 1.5 ml Receiver Tube.

- Discard the pellet.
- Centrifuge the sample again at full speed for 3 min.

4. Proteinase K digestion

- Transfer 25 µl Proteinase K into a new 1.5 ml Receiver Tube and pipet 400 µl of the supernatant from step 3 to the 1.5 ml Receiver Tube containing Proteinase K.
- Mix shortly by vortexing and incubate the sample for 10 min at 70°C in a thermomixer under continuous shaking at 900 rpm.

Optional: Removing traces of RNA

Invisorb® RTA Spin filter can also purify low amounts of RNA besides DNA. For the elimination of RNA (if necessary) add 20 µl RNase A (10 mg/ml) before adding the Binding Buffer P. Vortex briefly and incubate the sample at room temperature for 5 minutes. Then go on as described in the protocol.

5. Binding of the DNA

- Add 200 µl Binding Buffer P to the lysate and mix shortly by vortexing or by pipetting up and down several times.
- Transfer the mixture completely onto the membrane of the RTA Spin Filter. Incubate for 1 min at room temperature and centrifuge at 9.300 x g (10.000 rpm) for 2 min.
- Discard the filtrate and the RTA Receiver Tube.

6. Washing steps

- Put the RTA Spin Filter in a new RTA Receiver Tube.
- Add 500 µl Wash Buffer I to the membrane of the RTA Spin Filter.
- Close the lid and centrifuge at 9.300 x g (10.000 rpm) for 1 min.
- Discard the filtrate and the RTA Receiver Tube.
- Put the RTA Spin Filter in a new RTA Receiver Tube.
- Add 700 µl Wash Buffer II to the membrane of the RTA Spin Filter.
- Close the lid and centrifuge at 9.300 x g (10.000 rpm) for 1 min.
- Discard the filtrate and put the RTA Spin Filter back to the same RTA Receiver Tube.

7. Ethanol removal

- To eliminate any traces of ethanol, centrifuge again for 3 min at maximum speed, discard the RTA Receiver Tube.

8. DNA Elution

- Place the RTA Spin Filter into a new 1.5 ml Receiver Tube and add 100µl preheated (70°C) Elution Buffer D to the sample.
- Incubate for 3 min at RT.
- Centrifuge at 9.300 x g (10.000 rpm) for 1 min. to elute the DNA.
- Add another 100µl preheated (70°C) Elution Buffer D to the filter membrane
- Incubate for 3 min at RT.
- Centrifuge at 9.300 x g (10.000 rpm) for 1 min. to elute the DNA.
- Finally discard the RTA Spin Filter.

IHMS DNA extraction protocol Q

Fecal DNA extraction with the use of Qiagen QIAamp DNA stool kit

1. Homogenize the 150 to 200mg frozen feces with 1.0mL ASL lysis buffer of the kit by vortexing for 2min in a 2mL tube containing 0.3g of sterile zirconia beads Ø 0,1mm zirconia (BioSpec, Cat. No. 11079101z). [if buffer shows precipitate, heat at 70°C before use]
2. Incubate for 15min at 95°C.
3. Cells are mechanically lysed by running the Fastprep™ Instrument for 8min15sec (series of beating 1 min and resting 5 min are preferable).
4. Samples are allowed to cool down on ice for 2min.
5. Samples are centrifuged at 16000 x g, 4°C, for 5min.
6. Supernatant is transferred to a new 2mL tube.
7. The pellet is mixed with 300µL ASL lysis buffer of the kit, and steps 2-5 are repeated.
8. Supernatants are pooled in the new 2mL tube.

9. Add 260µl of 10M ammonium acetate to each lysate tube, mix well, and incubate on ice for 5 min.
10. Centrifuge at 16000 g, 4°C, for 10min.
11. Transfer the supernatant to two 1.5mL Eppendorf tubes, add one volume of isopropanol, mix well, and incubate on ice for 30 min.
12. Centrifuge at 16000 g, 4°C, 15min, remove the supernatant using aspiration, wash nucleic acids pellet with 70 % EtOH (0,5mL) and dry the pellet under vacuum for 3min.
13. Dissolve the nucleic acid pellet in 100µL of TE (Tris-EDTA) buffer and pool the two aliquots.
14. Add 2µL of DNase-free RNase (10mg/mL) and incubate at 37°C, 15 min.
15. Add 15µL proteinase K and 200µL AL buffer to the supernatant, vortex for 15sec and incubate at 70°C for 10 min.
16. Add 200µL of ethanol (96-100%) to the lysate, and mix by vortexing.
17. Transfer to a QIAamp spin column and centrifuge at 16000 g for 1min, at Room Temperature (RT).
18. Discard flow through, add 500µL buffer AW1 (Qiagen) and centrifuge at 16000 g for 1min, at RT.
19. Discard flow through, add 500µL buffer AW2 (Qiagen) and centrifuge at 16000 g for 1min, at RT
20. Dry the column by centrifugation at RT for 1min.
21. Add 200µL Buffer AE (Qiagen), incubate for 1min at RT
22. Centrifuge for 1min at 16000 g to elute DNA.

Quality control: use 1% agarose gel
Sample concentration: use Nanodrop or Qubit