



# Microbiome DNA Isolation

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## 1 Abstract

The method describes efficiently isolating DNA from stool samples using a combination of mechanical and chemical lysis. The isolated DNA is suitable for NGS analysis such as 16S rDNA amplicon sequencing and shotgun metagenomics.

## 2 Materials and equipment

### 2.1 Reagents

- 0.1mm Zirconia/Silica Beads [Fisher Scientific, 11079101Z]
- Phenol:Chloroform:IAA, 25:24:1, pH 6.6 [Thermo Fisher, AM9732]
- SDS [Sigma-Aldrich, 75746-250G]
- QIAquick 96 PCR Purification Kit [Qiagen, 28181]
- Axygen 2.0mL Self-Standing Screw Cap Tubes [Fisher Scientific, 14-222-626]
- Quant-iT™ dsDNA Assay Kit, broad range [Thermo Fisher, Q33130]
- Corning™ 96-Well Solid Black Plates [Fisher Scientific, 07-200-590]
- Hard-Shell® 96-Well PCR Plates, low profile, thin wall, skirted, white/clear [Bio-Rad, HSP9601]
- Eppendorf Combitips advanced [Fisher Scientific, 13-683-724]
- ClipTip Filtered Pipette Tips [Fisher Scientific, 14-387-978]
- Tris (1 M), pH 8.0 [Thermo Fisher, AM9855G]
- EDTA (0.5 M), pH 8.0 [Thermo Fisher, AM9260G]
- NaCl (5M) [Thermo Fisher, AM9760G]
- ZymoBIOMICS Microbial Community [Zymo Research, D6300]
- 96-Well Deep Well Plates [VWR, 10755-250]
- Miscellaneous (tips etc.)

### 2.2 Equipment

- QIAVac 96 Vacuum manifold [Qiagen, 19504]
- BioSpec Mini-Beadbeater-96 [Biospec Products, 1001]
- Bead loader [Custom]
- Microcentrifuge (>3000g) [Eppendorf, 5424]
- Miscellaneous (Pipettes etc.)

## 3 Safety precautions

### 3.1 Human specimen handling

Universal precautions should be followed when handling human samples considered potentially infectious. Minimal protective clothing requirements are lab coat, gloves and safety glasses.

### 3.2 Waste

Dispose of all waste as biohazardous material.

### 3.3 Specific precautions

- Phenol:Chloroform:Isoamyl alcohol is toxic if swallowed, in contact with skin, or inhaled. It causes severe skin burns and eye damage. It is also suspected of causing cancer and may cause damage to organs through prolonged or repeated exposure. Impervious gloves such as Viton or Neoprene should be worn when handling Phenol:Chloroform:Isoamyl alcohol and all manipulations should be performed within a chemical fume hood.
- QIAquick Buffer PB and Buffer PM contain guanidine hydrochloride, which can form highly reactive compounds when combined with bleach. It also contains isopropanol and is considered highly flammable. Harmful if swallowed. Irritating to eyes and skin. Wear suitable protective clothing, gloves, and eye protection.

## 4 Method

### 4.1 Reagent preparation

#### 4.1.1 Phenol/Chloroform/Isoamylalcohol

1. Add Tris buffer to glass bottle to set pH to 7.9
2. Let stand for two hours at room temperature
3. Store bottle at 4C

#### 4.1.2 Zirconia/Silica beads

Bake at 180C for 2 hours to destroy DNA.

#### 4.1.3 50 mL DNA Buffer A (store at room temperature)

	<b>Reagent</b>	<b>Add</b>	<b>Final</b>
1 M	Tris, pH 8	1 mL	20 mM
500 mM	EDTA	0.2 mL	2 mM
5 M	NaCl	2 mL	200 mM
	H <sub>2</sub> O	46.8 mL	
	<b>Total</b>	50 mL	

Filter-sterilize through a 0.2um filter.

#### 4.1.4 50 mL 20% SDS (store at room temperature)

	<b>Reagent</b>	<b>Add</b>	<b>Final</b>
	SDS	10 g	20 %
	H <sub>2</sub> O	50 mL	
	<b>Total</b>	50 mL	

Initially dissolved in 35 mL, heat solution under to 37C to help solution of SDS, adjust volume to 50 mL and filter through a 0.22um filter.

#### 4.1.5 Extraction buffer

<b>1.3 mL Extraction buffer</b>				
	<b>Reagent</b>	<b>Per sample</b>	<b>Final</b>	<b>110 Samples</b>
	Phenol/Chloroform/IAA	0.55 mL	42 %	60.5 mL
20 %	SDS	0.2 mL	3.1 %	22.0 mL
	DNA Buffer A	0.282 mL	22 %	31.0 mL
	QIAquick PM buffer	0.268 mL	21 %	29.5 mL
	<b>Total</b>	<b>1.3 mL</b>		

Bring phenol/chloroform/IAA to to 20C and swirl thoroughly to form a single, clear, homogenous phase. Remove desired amount of material from the bottom layer, leaving the protective top argon layer in the bottle.

## 4.2 Procedure

### 4.2.1 Preprocessing for fluid samples (e.g. BAL).

1. Centrifuge for 10 min at 300g and collect supernatants as acellular fraction and pellet as cellular fraction.
2. Centrifuge acellular supernatant for 30 min at 30.000g to pellet bacteria, remove supernatant.
3. Continue standard extraction procedure with acellular and cellular pellets.

### 4.2.2 Zymo Mock Community Control

1. Thaw standard completely. Mix thoroughly by vortexing and aliquot 75 ul for future use.
2. Thaw one 75 ul of the standard for each DNA extraction (expected yield ~2ug).

### 4.2.3 Standard extraction procedure

1. Add 400 ul of beads with the bead loader (Pump twice for 400 ul of beads).
2. Mix extraction buffer by shaking for 20s and add 1.3 mL Extraction Buffer to each tube. Make sure to shake again before each addition due mix the layers.
3. Place tubes in plastic holders and bead-beat for 5 minutes.
4. Centrifuge for 5 minutes at 4000 RPM in bucket centrifuge.
5. Transfer 200 ul of the top aqueous layer to a deep well plate containing 650µL PM Buffer per well.
6. Mix by pipetting 3 times and transfer to a Qiagen 96 well PCR purification plate.

7. Assemble QIAvac 96 vacuum manifold as below with waste collection tray (#2).

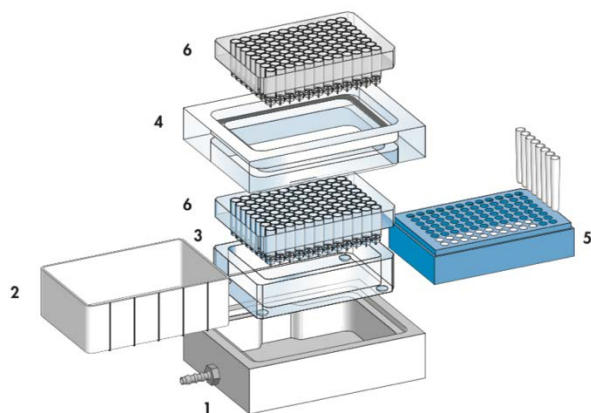
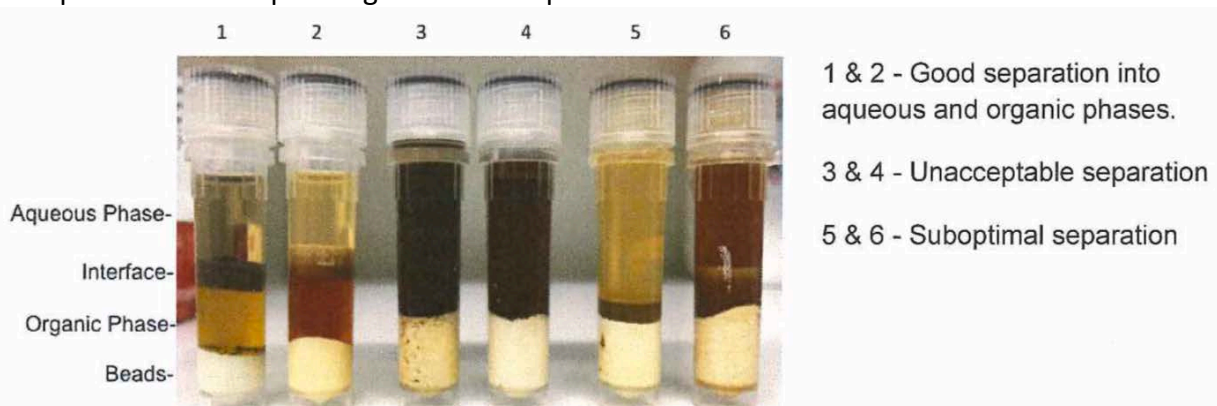


Figure 1. Components of the QIAvac 96 manifold. 1) QIAvac base, which holds a waste tray, plate holder, a plate or a microtube rack 2) Waste tray 3) Plate holder (shown with 96-well plate) 4) QIAvac 96 top plate with aperture for 96-well plate 5) Microtube rack. 6) 96-well plate\*. From QIAvac-handbook (<https://www.qiagen.com/us/resources/download.aspx?id=d6ac98ca-1403-407f-bf06-df65dd7c9d6f&lang=en>).

8. Process the 96-well plate on the QIAvac 96 vacuum manifold. Turn on the vacuum until all of lysate has run through each column. If the columns get clogged, spin the plate (resting it on top of a deep 96-well plate) for 5 min at 4000rpm.
9. Wash each column or well of the PCR purification plate twice with 900 $\mu$ L PE buffer.
10. Centrifuge the columns or plate for 5 minutes at 4000rpm at room temperature to remove all traces of ethanol or apply vacuum for 10 minutes.
11. Place Qiagen 96 well PCR purification plate into barcoded standard 96 well plate.
12. Add 60 $\mu$ L of EB buffer to each well, incubate for 1 min and centrifuge for 5 minutes at 4000rpm or place in the plate holder (#3) and apply vacuum.
13. Store samples at -80C.

#### 4.2.4 Trouble shooting & tips

Examples of how samples might look after phenol extraction:



If samples look like tubes 3-6 the following after centrifugation repeat bead-beating and centrifugation steps. Otherwise let them stand overnight and repeat bead-beating and centrifugation the next day. If this still fails repeat the procedure with less input material.