Protocol	#6.7
Title	**BOMB TNA extraction from environmental samples using GITC lysis**
Keywords	HT TNA isolation, carboxyl-beads, silica beads, GITC, environmental
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Online	https://bomb.bio/protocols/
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Summary

Isolation of total nucleic acid (TNA) is a basic wet lab technique and the starting point for many analysis pathways. This protocol describes a high-throughput magnetic bead-based protocol to purify total nucleic acid (TNA) from environmental samples like lake water. Material is collected by centrifugation (or other means) and then lysed in GITC buffer [1]. If RNase A is added at the beginning, only DNA will be isolated (see BOMB protocol #7.1). This protocol can also be coupled with an on-bead DNase I digest to extract only RNA (see BOMB protocol #8.2). It utilizes a sarkosyl and guanidinium-isothiocyanate (GITC) based lysis buffer and isopropropanol to drive precipitation of the nucleic acid to the paramagnetic beads. Variations of this protocol exist for isolation from mammalian cells and tissues, plants and yeast. Volumes can be adjusted, however, should remain at a consistent ratio of 2:3:4, beads:lysate:isopropanol.

Chemicals

Name	Provider	PN	MW [g/mol]	Safety codes	
Ethanol (C_2H_6O, 99.9%)	Honeywell/ Riedel-de Haën	34963	46.07	 Danger	H: 225-319 P: 210-240-305+351+338-403+233
Guanidine isothiocyanate (GITC, $C_2H_6N_4S$)	Roth Chemicals	2628.4	118.16	 Warning	H: 302+312+332-412-EUH032 P: 273-280-302+352-304+340-312
Tris(hydroxymethyl)-aminomethane (Tris, $C_4H_{11}NO_3$)	Roth Chemicals	AE15.3	121.14	 Warning	H 315-319-335 P: 280-302+352-305+351+338-312
N-Lauroylsarcosine sodium salt (Sarkosyl, $C_{15}H_{29}NO_3Na$)	Sigma (Merck)	L9150-50G	293.38	 Danger	H: 315-318-330 P: 260-280-284-305+351+338-310
Antifoam 204	Sigma (Merck)	A8311-50ML	n.a.	n.a.	n.a.
Ethylenediamine tetraacetic acid disodium salt dihydrate (EDTA ($C_{10}H_{14}N_2Na_2O_8 \cdot 2\ H_2O$)	Roth Chemicals	8043.1	372.24	 Danger	H: 332-373 P: 260-314
Isopropanol (C_3H_8O)	Acros Organic	184130025	60.01	 Danger	H: 225-319-336 P: 210-233-240-305+351+338-403+235

Please consult appropriate MSDS information before working with these chemicals! Use lab coat, gloves and eye protection at all times! The chemicals are available from other providers as well. No preference is given to the indicated vendors.

Buffers and solutions

Carboxyl-coated or **silica-coated magnetic beads** in **TE buffer** (160 µl per sample, 16 ml for a 96-well plate)

TE buffer (160 µl per sample, 16 ml per 96-well plate. Used for bead dilution, see above)

10 mM Tris pH 8.0
1 mM EDTA

Lysis buffer (stable for at least several weeks at RT. 240 µl per sample, 24 ml per 96-well plate)

Reagent	Concentrations	For 50 ml
GITC	4 M	23.64 g
Tris HCl pH 7.6-8.0	50 mM	2.5 ml of 1 M stock
Sarkosyl	2%	1 g
EDTA	20 mM	2 ml of 0.5 M stock
Antifoam (optional)	0.1 %	50 µl

adjust pH with HCl to 7.6-8.0 and adjust the volume with water to 50 ml

Isopropanol (720 µl per sample, 72 ml for a 96-well plate)

80% ethanol (600 µl per sample, 60 ml for a 96-well plate)

Equipment and setup

Fume hood

Microtiter plate orbital shaker (e.g. IKA MS 3 basic)

Magnetic stand for 96-well plate (e.g. BOMB microplate magnetic rack)

Heat block (e.g. Roth, Rotilabo®- block thermostat H250 – PN: Y264.1)

Multichannel Pipettes

30 – 300 µl (e.g. Eppendorf, Eppendorf Research® plus 8-channel – PN: 3125000052)

50 – 1200 µl (e.g. VWR, Multi channel pipette, 8-channel – PN: 613-5422)

96-well PCR plate (e.g. Sarstedt, 96 PCR-Plate half skirt flat – PN: 170134)

1.2 ml 96-well deep well plates (e.g. Sarstedt, MegaBlock® 96 Well – PN: 82.1971.002**)**

Reservoirs (e.g. Roth, Rotilabo®-liquid reservoirs – PN: E830.2)

Seals (e.g. Bio-Rad, Microseal® 'B' Adhesive Seals – PN: MSB1001)

BOMB TNA extraction

Step	Sample collection	Time	☑
⚠	*The key point of this step is to collect biomaterial containing nucleic acids that could be extracted. Here we used centrifugation, yet any other method to collect the desired biomaterial can be used instead, including water filtration systems (especially when larger volumes have to be processed), affinity capture or others.*		
1	Collect up to 50 ml of lake water or other environmental samples in a centrifuge tube (or a deepwell plate, if using low volumes)		☐
2	Pellet the samples via centrifugation at 5000g and discard supernatant		☐
🧊	*At this point, the pellets can be frozen at -20 °C and processed later*		
Step	**TNA purification**		
3	Add 240 µl of **lysis buffer**, seal and shake at RT at 1400 rpm for 5 min		
⚠	*The lysis buffer contains guanidine isothiocyanate, a chemical which can cause burns and sensitivity and produces hydrogen cyanide when mixed with bleach/acids*	10 min	☐
4	Add 320 µl of **isopropanol**, seal and shake at RT at 1400 rpm for 5 min	10 min	☐
5	Add 160 µl of **coated magnetic beads** (diluted 1:50 in **TE** from stocks), seal and shake at RT at 1400 rpm for 5 min	10 min	☐
6	Settle the magnetic beads on a magnetic stand and discard the supernatant	5 min	☐
⚠	*Ensure that the beads are completely settled*		
7	Remove the plate from the magnetic stand and add 400 µl **isopropanol**. Shake at RT at 1400 rpm for 2 min	10 min	☐
8	Settle the magnetic beads on a magnetic stand and discard the supernatant	5 min	☐
9	Wash twice with 300 µl of **80% ethanol** as above	10 min	☐☐
10	Remove the supernatant completely and dry the beads for approximately 5-10 min		
⚠	*Make sure to remove all remaining ethanol. Silica-coated beads should be completely dried (at 50 °C), whereas carboxyl-coated ones should be only dried briefly (at RT)*	5 min	☐
11	Add 70 µl of **nuclease free water** to the wells and shake for at least 5 min to resuspend (centrifuge shortly if beads stick to the walls)		
⚠	*If the liquid is too viscous to pipette off and/or the beads don't settle, double the elution volume until it works*	5 min	☐
12	Settle the magnetic beads on a magnetic stand and transfer the supernatant to a collection plate/tube	5 min	☐
End	Measure concentration	**~1.5 h** (45 min hands-on)	
🧊	Store @ -80 °C		

Troubleshooting

Problem	Solution
Beads sticking to the sides	• Sonicate shortly in the sonic bath and/or push the beads down with a pipette tip
Beads stay at the bottom of the well when mixing	• Use a good foil to cover the plate well and invert a few times
Elution very viscous	• We observe this problem when too many cells were used for extraction. Add more elution buffer (concentration should still be very high), vortex strongly or pipette up and down. Heat up to 65 °C

Exemplary Results

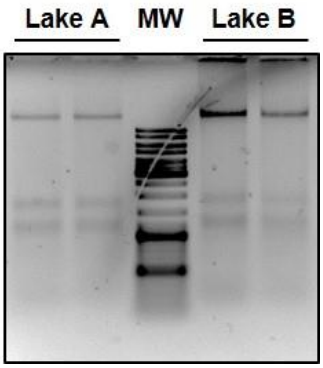

Fig 1: **Quality control of BOMB TNA extraction from environmental samples.** Total nucleic acid (TNA) was extracted from 50 ml of water from two different lakes. MW: GeneRuler DNA Ladder Mix, Thermo Scientific.

References

1. Chirgwin JM, Przybyla AE, MacDonald RJ, Rutter WJ. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry. 1979;18: 5294–5299. doi:10.1021/bi00591a005