Protocol	#6.3
Title	**BOMB TNA extraction from mammalian tissue using GITC lysis**
Keywords	HT TNA isolation, carboxyl-beads, silica beads, GITC, mammalian cells
Authors	Oberacker P*, Stepper P*, Bond DM*, Höhn S, Focken J, Meyer V, Schelle L, Sugrue VJ, Jeunen GJ, Moser T, Hore SR, von Meyenn F, Hipp K, Hore TA# and Jurkowski TP#
Citation	*Oberacker et al., Bio-On-Magnetic-Beads (BOMB): Open platform for high-throughput nucleic acid manipulation. Submitted*
Online	https://bomb.bio/protocols/
Revision	V1.0 (13th August 2018)

Summary

This protocol describes a magnetic bead-based protocol to purify total nucleic acid (TNA) from mammalian tissues lysed in GITC buffer[1]. If extracting from snap-frozen tissue, homogenisation in GITC is required. This can be done (for soft tissues) by passing through a pipette-tip prior to freezing, or tissue can be ground/shaved whilst still frozen. Addition of 2% β-mercaptoethanol may assist in cell lysis (e.g. sperm) or RNAse suppression (e.g. spleen).

Where only DNA is required, a high-throughput 2-step protocol may be simpler whereby tissue lysis and protein digestion are first undertaken in a low-salt/detergent buffer (TNES) with Proteinase K (ProtK) and RNaseA. Following this incubation, a higher concentration of GITC lysis buffer (1.5X) is added, denaturing DNA associated proteins.

Once cells are lysed by any method, nucleic acid can be precipitated to either BOMB silica- or carboxylate-coated beads using isopropanol. Volumes can be adjusted, however, should remain at a consistent ratio of 2:3:4, beads:lysate:isopropanol. Below is a sensible volume for deep-well plates, but lower volumes on 0.2 ml PCR plates are also possible (i.e. 40 µl of beads, 60 µl of lysate and 80 µl of isopropanol), and may further reduce costs.

Chemicals

Name	Provider	PN	MW [g/mol]	Safety codes	
β-mercaptoethanol	Sigma (Merck)	M6250-10ML	78.13	 Danger	H: 301+331-310-315-317-318-373-410 P: 261-280-301+310+330-302+352+310-305+351+338+310-403+233
Antifoam 204	Sigma (Merck)	A8311-50ML	n.a.	n.a.	n.a.
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
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

Name	Provider	PN	MW [g/mol]	Safety codes	
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
Isopropanol (C_3H_8O)	Acros Organic	184130025	60.01	 Danger	H: 225-319-336 P: 210-233-240-305+351+338-403+235
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
Proteinase K	Invitrogen (ThermoFisher)	AM2546	n.a.	 Danger	H: 316-334 P: 304+340+332+313+261+342+311+284
RNase A	Serva	34390.02	n.a.	 Danger	H: 334 P: 261-284-304+340-342+311
Sodium Chloride (NaCl)	Sigma (Merck)	S3014-500G	58.44	n.a.	n.a.
Sodium dodecyl sulfate (SDS, $NaC_{12}H_{25}SO_4$)	Sigma (Merck)	L3771-100G	288.38	 Danger	H: 228-302+332-315-318-335-412 P: 210-261-280-301+312+330-305+351+338+310-370+378
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

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 Speed beads (1:50 diluted in TE) or **silica-coated magnetic beads** (1:50 diluted from stock; 120 µl per sample, 12 ml for a 96-well plate)

TE buffer (stable for at least a year at RT)

10 mM Tris pH 8.0
1 mM EDTA

Lysis buffer (stable for at least several weeks at RT; 180 µl per sample, 18 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

1.5X GITC Lysis buffer (stable for at least several weeks at RT; 120 µl per sample, 16 ml per 96-well plate)

Reagent	Concentrations	For 50 ml
GITC	6 M	35.46 g
Tris HCl pH 7.6-8.0	75 mM	3.75 ml of 1 M stock
Sarkosyl	3%	1.5 g
EDTA	30 mM	3 ml of 0.5 M stock
Antifoam (optional)	0.15 %	75 µl

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

TNES buffer (stable for at least several weeks at RT; 60 µl per sample, 6 ml per 96-well plate)

Reagent	Concentrations	For 50 ml
Tris HCl pH 7.6-8.0	100 mM	5 ml of 1M stock
NaCl	25 mM	1.25 ml of 1 M stock
EDTA	10 mM	1 ml of 0.5 M stock
SDS	10 % w/v	5 g

Proteinase K (20 mg/ml, 2 µl per sample, 0.2 ml for a 96-well plate)

RNase A (DNase inactivated, 10 mg/ml, 3 µl per sample, 0.3 ml for a 96-well plate)

Isopropanol (240 µl per sample, 24 ml for a 96-well plate)

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

Equipment and setup

Vessel for harvesting tissue in liquid nitrogen

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 and lysis – snap frozen tissue	Time	☑
1	Collect up to 10mg of snap-frozen tissue that is prepared for homogenisation in lysis buffer (i.e. it has been finely sliced so it can be pipetted, or ground/shaved whilst frozen)		☐
2 ⚠	Add 180 µl **lysis buffer** and mix by pipetting or vortex until homogenous *The lysis buffer contains guanidine isothiocyanate, a chemical which can cause burns and sensitivity and produces hydrogen cyanide when mixed with bleach/acids*	5 min	☐
🧊	*At this point, the samples can be frozen at -20 °C and processed later; proceed at Step 4 when ready*		
Step	**Sample collection and lysis – non-snap frozen tissue, for DNA only**		
1	Collect up to 20 mg of tissue. Slicing tissue may help homogenisation		☐
2	Add 60 µl **TNES buffer** + 2 µl **Proteinase K** + 3 µl **RNAse A**, incubate at 55 °C for 3-4 h, or overnight, or until homogenous	variable	☐
3 ⚠	Add 120 µl **1.5X GITC lysis buffer** and mix by pipetting or vortex until homogenous *The lysis buffer contains guanidine isothiocyanate, a chemical which can cause burns and sensitivity and produces hydrogen cyanide when mixed with bleach/acids*	5 min	☐
🧊	*At this point, the samples can be frozen at -20 °C and processed later; proceed at Step 4 when ready*		
Step	**TNA purification**		
4	Add 240 µl **isopropanol**, seal and shake at RT at 1400 rpm for 5 min	10 min	☐
5	Add 120 µl TE-diluted **Carboxyl-coated** or **silica-coated magnetic beads** to the cell lysate	5 min	☐
6 ⚠	Settle the magnetic beads on a magnetic stand and discard the supernatant *Ensure that the beads are completely settled*	5 min	☐
7	Remove the plate from the magnetic stand and add 400 µl **isopropanol**. Shake at RT at 1400 rpm for 2 min	5 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	~45 min hands-on	
🧊	Store @ -80 °C		

Troubleshooting

Problem	Solution
Beads sticking to the sides of the tubes	• Sonicate briefly 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

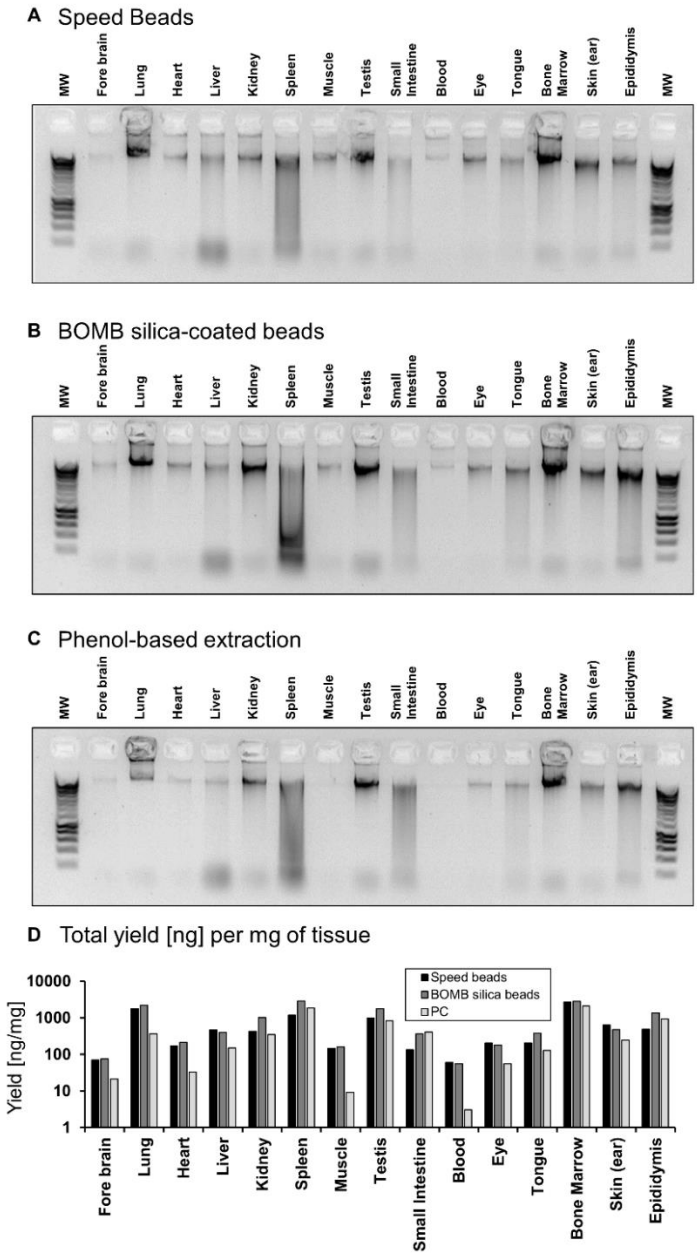

***Fig 1:* gDNA isolation from various rabbit tissues.** The protocol above was used with (A) Speed Beads and (B) BOMB silica beads. Comparison to (C) Phenol-chloroform based extraction is also shown. MW in all panels represents Hyperladder I (Bioline). Inevitably, some tissues (like bone marrow) produce far greater yields per mg of input compared to other tissues. However, the bead-based methods generally outperform phenol-chloroform extractions in our hands. Note, rabbit tissues were not preserved immediately after animal death, hence why tissues like spleen have experienced some DNA degradation. (D) yields per mg of input material, compared to other tissues. However, the bead-based methods generally outperform phenol-chloroform extractions in our hands. Note, rabbit tissues were not preserved immediately after animal death, hence why tissues like spleen have experienced some DNA degradation.

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