Protocol	#7.1
Title	**BOMB gDNA extraction using GITC lysis**
Keywords	HT DNA isolation, carboxyl beads, silica beads
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Online	https://bomb.bio/protocols/
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Summary

Isolation of genomic DNA from bacteria and eukaryotic cells is a basic wet lab technique and the starting point for many analysis pathways. This protocol describes a simple and rapid way for either total nucleic acid extraction or, if RNase A is added at the beginning, isolation of only DNA. It utilizes a sarkosyl and guanidinium-isothiocyanate (GITC) based lysis buffer [1] and isopropropanol to drive precipitation of the nucleic acid to the paramagnetic beads. This protocol follows an easy to remember ratio of 2:3:4, beads:lysis:isopropanol. Isolation of DNA was tested successfully with cultured mammalian cells, blood and bacteria.

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
Ribonuclease A (RNase A)	Serva	34390.02	n.a.	 Danger	H: 334 P: 261-284-304+340-342+311
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** (20 µl per sample, 2 ml for a 96-well plate)

TE buffer (with freshly added 3% RNase A, if only DNA is to be extracted. 140 µl per sample, 14 ml per 96-well plate)

10 mM Tris pH 8.0
1 mM EDTA
30 µg/ml RNase A, DNase inactivated (3%, from 10 mg/ml stock)

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)

Elution buffer (stable, can be stored for at least 1 year at RT. 70 µl per sample, 7 ml per 96-well plate)

5 mM Tris-HCl, pH 8.5

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 Genomic DNA extraction

Step	Sample collection	Time	☑
1	Collect up to 10^6 cultured mammalian cells or 2 ml of over-night cultured *E.coli* in a single well of a deep-well plate		☐
2	Pellet the cells via centrifugation at 500g and discard supernatant		☐
	At this point, the pellets can be frozen at -20 °C and processed later		
Step	**DNA purification**		
3	Add 140 µl of **TE buffer** (containing RNase A, DNA isolation only) to each cell pellet	5 min	☐
4	Seal the plate with a foil and shake for 5 min at 1400 rpm	5 min	☐
5	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	☐
6	Add 320 µl of **isopropanol**, seal and shake at RT at 1400 rpm for 5 min	10 min	☐
7	Add 20 µl of **coated magnetic beads**, seal and shake at RT at 1400 rpm for 5 min	10 min	☐
8	Settle the magnetic beads on a magnetic stand and discard the supernatant ⚠ *Ensure that the beads are completely settled*	5 min	☐
9	Remove the plate from the magnetic stand and add 400 µl **isopropanol**. Shake at RT at 1400 rpm for 2 min	10 min	☐
10	Settle the magnetic beads on a magnetic stand and discard the supernatant	5 min	☐
11	Wash twice with 300 µl of **80% ethanol** as above	10 min	☐☐
12	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	☐
13	Add 70 µl of **elution buffer** to the wells and shake for at least 5 min to resuspend (centrifuge shortly if beads stick to the walls) ⚠ *If the eluate/liquid is too viscous to pipette off and/or the beads don't settle, double the elution volume until it works*	5 min	☐
14	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** (1 h hands-on)	
	Store @ -20 °C or 4 °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

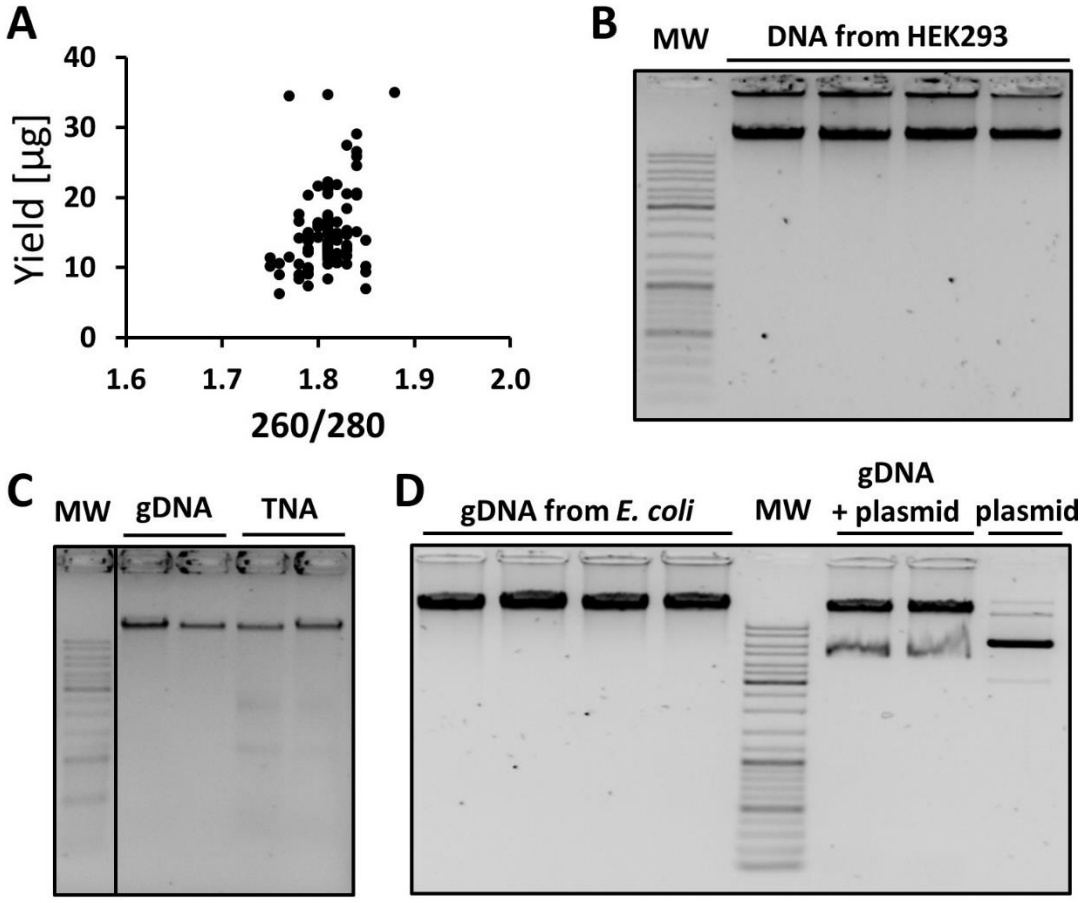

Fig 1: Quality control of BOMB DNA extraction. *(A) Total yield of extracted DNA from HEK293 cells, plotted over A260 nm/A280 nm ratio. (B) Representative agarose gel of isolated gDNA from HEK293 cells (200 ng each). (C) Total nucleic acids (TNA) can be extracted if RNase A is omitted. (D) Agarose gel of DNA isolated from TOP10* E. coli*. The first four lanes contain purified DNA from untransformed cells, whereas the three rightmost samples contain the DNA of two pellets of E. coli transformed with a pHAGE-EFS-insert plasmid and also the purified plasmid itself. MW: Gene Ruler 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