Protocol	#6.6
Title	**BOMB TNA extraction from *E. coli* using TRI reagent**
Keywords	HT TNA isolation, Silica-beads, TRI reagent, E. coli
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Summary

Piotr Chomczyński and Nicoletta Sacchi developed the rapid single-step TRI protocol for RNA isolation in 1987 [1]. This protocol has since been used extensively to purify high quality RNA [2]. However, it can also be used to isolate the total nucleic acid from a sample. It employs acid guanidinium-thiocyanate and phenol to lyse cells and inactivate proteins including RNases. Here, we combine it with capturing the TNA on silica-coated magnetic beads instead of centrifugation and phase separation. This protocol can be combined with an on-bead DNase I treatment and RNA clean-up to isolate only RNA (see BOMB protocol #8.1).

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
Guanidinium chloride (GuHCl, $CH_5N_3 \cdot HCl$)	Roth Chemicals	0037.1	95.53	 Attention	H: 302+332-315-319 P: 261-280-301+312-330-304+340+312-305+351+338-337+313
TRI reagent	Refer to #B BOMB TRI reagent		n.a.	 Danger	H: 301+311+331-314-335-341-373-412 P: 201-261-264-280-273-301+310-302+352-303+361+353-304+340-305+351+338

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

TRI reagent or **TRIzol** (200 µl per sample, 20 ml per 96-well plate)

90% ethanol (1.6 ml per sample, 160 ml per 96-well plate)

RNA binding buffer (0.8 ml per sample, 80 ml for 96-well plate, adjust pH to ~6)

Reagent	Concentrations	For 100 ml
Gu-HCl	1 M	9.55 g
Tween 20	0.05%	0.5 ml of 10% stock
Ethanol	~ absolute	100 ml of 99.9% stock

Equipment and setup

Fume hood

Temperature controlled incubation shaker or incubator (e.g. Infors HT Multitron Pro)

Plate centrifuge with swing-out rotor (e.g. Eppendorf Centrifuge 5804R)

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

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

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

Multichannel Pipettes

- 10 - 100 µl (e.g. Eppendorf, Eppendorf Research® plus 8-Channel – PN: 3122000035)
- 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)

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

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

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

BOMB TNA extraction

Step	Sample preparation	Time	☑
1	Collect up to 1 ml of an *E. coli* overnight culture in a single well of a deep-well plate		☐
2	Pellet the cells via centrifugation at 500g and discard the supernatant	5 min	☐
	Opt *At this point, the pellets can be frozen at -80 °C and processed later*		
3	Resuspend and lyse the cells in 200 µl **TRI reagent** (TRIzol or similar) at 1300 rpm for 15 min (or until dissolved)	30 min	☐
⚠	*Phenol (in TRIzol) is highly toxic; perform all manipulations with personal protective equipment. Always use a fume hood. Dispose phenol-containing trash following local requirements!*		
	Opt *At this point, the plates can be sealed and kept at 4 °C overnight or frozen at -20 °C and processed later*		
Step	**TNA purification**		
4	Add 200 µl of **RNA binding buffer**	5 min	☐
5	Add 40 µl **silica-coated magnetic beads** (BOMB protocol #2.1, 1:10 diluted from stock) and shake for 5 min at 1100 rpm	10 min	☐
6	Settle the magnetic beads on a magnetic stand, remove and discard the cleared supernatant. Aspirate the solution slowly to avoid losing the beads	5 min	☐
7	Add 200 µl **TRI reagent** and 200 µl **RNA binding buffer** and mix for 5 min to remove any remaining RNases	10 min	
8	Settle the magnetic beads on a magnetic stand, remove and discard the cleared supernatant	5 min	
9	Remove the plate from the magnetic stand and add 400 µl **90% ethanol** and mix well	5 min	☐☐ ☐☐
10	Settle the magnetic beads on a magnetic stand and discard the supernatant	5 min	☐☐ ☐☐
11	**Repeat** steps **9-10 three** more times for a total of **four washes**	15 min	☐
12	Dry the beads on a heat block at 50 °C for ~30 min	45 min	☐
👁	*The beads are dry when they turn brownish. Do not elute earlier!*		
13	Add 40 µl of **nuclease-free water** to elute TNA, mix at 1300 rpm for 5 min	10 min	☐
14	Pellet the magnetic beads on a magnetic stand and transfer the eluted TNA to a fresh 96-well collection plate	10 min	☐
End	Measure concentration and purity	**~3.5 h** (1.5 h 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 seal the plate well and invert a few times
Incomplete lysis	• Resuspend the pellets by pipetting if they did not dissolve by shaking
Degraded RNA	• Process samples immediately after collection • Make sure to perform the additional wash step with TRI reagent as recommended for sources with high content of RNases like bacteria
TRI reagent spillage	• Have a bottle of PEG 300 or 400 nearby and use this to wipe off immediately and repeatedly
Elution very viscous	• We observe this problem when too many cells were used for extraction. Add more water (concentration should still be very high), vortex strongly or pipette up and down

Exemplary results

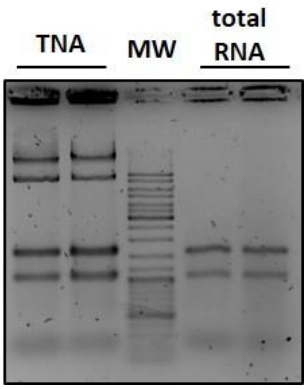

Fig 1: Quality control of BOMB TNA extraction using TRI-reagent from *E. coli*. TNA isolation from 1 ml of an overnight culture of TOP10 *E. coli* (left two lanes) followed by DNase I digest to isolate only RNA (right two lanes). M: Gene Ruler DNA Ladder Mix, Thermo Scientific

References

1. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium extraction by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem. 1987;162: 156–159. doi:10.1016/0003-2697(87)90021-2

2. Chomczynski P, Sacchi N. The single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction: Twenty-something years on. Nat Protoc. 2006;1: 581–585. doi:10.1038/nprot.2006.83