Protocol	#6.5
Title	**BOMB TNA extraction from yeast using GITC/lyticase lysis**
Keywords	HT TNA isolation, carboxyl beads, silica beads
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Online	https://bomb.bio/protocols/
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Summary

Isolation of total nucleic acids from yeast cells follows a two-step protocol. Freshly collected yeast cells are treated with lyticase which degrades the yeast cell wall [1], followed by a standard TNA extraction protocol with the GITC.

Chemicals

Name	Provider	PN	MW [g/mol]	Safety codes	
Antifoam 204	Sigma (Merck)	A8311-50ML	n.a.	n.a.	n.a.
1,4-Dithiothreitol (DTT)	Roth Chemicals	6908.1	154.2	 Warning	H: H302-H315-H319-H335
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
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
Glycerol (≥99,5 %, p.a., anhydrous)	Roth Chemicals	3783.3	92.09	n.a.	n.a.
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
Lyticase from *Arthrobacter luteus*	Sigma	L4025	n.a.	 Danger	H: H334 P: P261-P342 + P311
Ribonuclease A (RNase A)	Serva	34390.02	n.a.	 Danger	H: 334 P: 261-284-304+340-342+311
D-Sorbitol (≥98 %)	Roth Chemicals	6213.1	182.18	n.a.	n.a.

Name	Provider	PN	MW [g/mol]	Safety codes	
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 or **silica-coated magnetic beads** (30 µl per sample, 3 ml for a 96-well plate)

25x lyticase stock (stable for less than a year at -20 °C)

8500 U/ml lyticase
10 mM NaPi buffer pH 7.0
50% glycerol

Weigh few mg of the lyticase powder, calculate the required amount of buffer (dependent on the specific activity per mg written on the side of the purchased batch) and dissolve by pipetting.

Lyticase solution (prepare freshly, 250 µl per sample, 25 ml per 96-well plate)

Reagent	Concentrations	For 50 ml
Sorbitol	1.2 M	10.93 g
EDTA	50 mM	5 ml of 0.5 M stock
DTT	10 mM	0.5 ml of 1 M stock
Lyticase (add freshly)	340 U/ml	2 ml of 25x lyticase stock

adjust to 48 ml with ddH2O and add lyticase last (final 50 ml)

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

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

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 Yeast TNA extraction

Step	Sample collection	Time	☑
⚠	*It is not recommended to freeze the cell pellets before or during the procedure. Freezing causes cell disruption and release of the cell content during spheroplast isolation which complicates the procedure*		
1	Collect 2 ml of stationary phase yeast culture in a single well of a deepwell plate		☐
2	Pellet the cells via centrifugation at 2,500g for 1 min, discard the supernatant	5 min	☐
3	Re-suspend pellet with 1 ml of 50 mM EDTA solution and centrifuge at 2500g for 1 min, discard supernatant	5 min	☐
Step	**Spheroplast isolation**		
4	Re-suspend the cell pellet in 250 µl of the **lyticase buffer** with gentle pipetting or shaking at 300-400 rpm and incubate at 37 °C for 30 min	35 min	☐
5	Pellet the cells via centrifugation at 1,000g for 5 min and gently discard the supernatant	10 min	☐
Step	**Lysis and TNA purification**		
6	Add 480 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	☐
7	Add 640 µl of **isopropanol**, seal and shake at RT at 1400 rpm for 5 min	10 min	☐
8	Add 30 µl of **coated magnetic beads**, seal and shake at RT at 1400 rpm for 5 min	10 min	☐
9	Settle the magnetic beads on a magnetic stand and discard the supernatant	5 min	☐
⚠	*Ensure that the beads are completely settled*		
10	Remove the plate from the magnetic stand and add 400 µl **isopropanol**. Shake at RT at 1400 rpm for 2 min	10 min	☐
11	Settle the magnetic beads on a magnetic stand and discard the supernatant	5 min	☐
12	Wash twice with 300 µl of **80% ethanol** as above	10 min	☐☐
13	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	☐
14	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	☐
15	Settle the magnetic beads on a magnetic stand and transfer the supernatant to a collection plate/tube	5 min	☐
End	Measure concentration	**~2.5 h** (1.5 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
After lyticase treatment pellet is loose	• Freezing the yeast cells causes disruption of the cell membrane and release of the cell content. The cell wall prevents spillage of the cell content, yet when it is degraded by the lyticase the genomic DNA is released causing a loose, sticky pellet being formed after centrifugation. To avoid this, use freshly collected cells and pipette them gently throughout the procedure
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

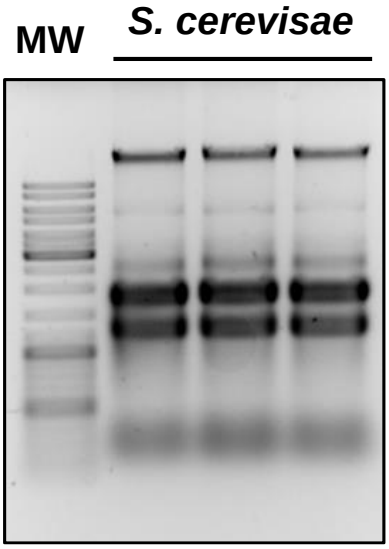

Fig 1: Quality control of BOMB yeast TNA extraction. *Agarose gel of TNA isolated from cultured yeast cells. MW: GeneRuler DNA Ladder Mix, Thermo Scientific*

References

1. https://www.babraham.ac.uk/files/download/832cfff60c09aea