Protocol	#6.4
Title	**BOMB TNA extraction from plants using TNES/GITC lysis**
Keywords	TNA isolation, carboxyl-beads, silica beads, GITC, TNES, plants
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Online	https://bomb.bio/protocols/
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Summary

This protocol describes a magnetic bead-based protocol to purify total nucleic acid (TNA) from plant tissue homogenized and lysed in the low-salt and detergent TNES buffer, followed by addition of a high concentration GITC lysis buffer (1.5X) to denature DNA associated proteins and to maintain integrity of total RNA [1].

Where only DNA is required, plant tissue is first homogenized in a small volume TE buffer, followed by lysis in TNES buffer supplemented with RNaseA. This is followed by the addition of a GITC lysis buffer (1.5X) to denature DNA associated proteins.

Once tissue is homogenized and cells are lysed by either method, nucleic acid can be precipitated to either BOMB silica- or carboxyl-coated beads using isopropanol. Volumes can be adjusted, however, should remain at a consistent ratio of 2:3:4, beads:lysate:isopropanol. Below are sensible volumes for 1.5 ml microcentrifuge tubes or deep-well plates, but lower volumes with 0.2 ml PCR plates is 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	
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
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

Name	Provider	PN	MW [g/mol]	Safety codes	
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** (1:10 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

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

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

Micropestle (e.g. Eppendorf™ Autoclavable Safe-Lock Micropestle – PN: 10683001)

Note: can be attached to a hand held cordless drill to aid homogenisation

Fume hood

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

Magnetic stand for 1.5 ml microcentrifuge tubes (numerous vendors available)

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

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

Benchtop centrifuge

96-well Cell culture plate

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)

1.5 ml microcentrifuge tubes (numerous vendors available)

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)

<u>Optional</u>

Tissue lyser (e.g. Qiagen TissueLyser II – PN: 85300)

Collection microtubes (racked) (e.g. Qiagen collection microtubes – PN: 19560)

Collection microtube caps (e.g. Qiagen collection microtube caps – PN: 19566)

BOMB TNA extraction – low-medium throughput

Step	Sample collection and lysis – TNA	Time	☑
1	Collect up to 20mg of plant tissue in 1.5 ml microcentrifuge tubes and snap-freeze in liquid Nitrogen		☐
2	Add 60 µl **TNES buffer** to frozen tissue and grind using micropestle until tissue is homogenous. To help homogenise the tissue, the micropestle can be attached to a small, hand-held cordless drill. Alternatively, (if available) a tissue lyser can be used to homogenise the tissue (add x2 3mm glass beads to the tube prior to lysis)	10 min	☐
⚠	*Ensure tissue remains as cold as possible to maintain integrity of the total RNA*		
3	Once tissue is homogenized, snap-freeze the sample in liquid Nitrogen		
🧊	*At this point, the samples can be stored at -80 °C and processed later; or proceed at Step 4 when ready*		
Step	**TNA purification**		
4	Spin frozen samples at 13,000 rpm for 3 minutes until sample has thawed and cell debris has pelleted	3 min	☐
5	Add 120 µl of **1.5X GITC lysis buffer** to the cell lysate and mix by pipetting	1 min	☐
⚠	*The lysis buffer contains guanidine isothiocyanate, a chemical which can cause burns and sensitivity and produces hydrogen cyanide when mixed with bleach/acids*		
6	Add 120 µl TE-diluted **Carboxyl-coated** or **silica-coated magnetic beads** to the cell lysate and mix by pipetting	1 min	☐
5	Add 240 µl **isopropanol**, close lid and invert tube 5-10 times and leave for 5 min for beads to capture TNA	6 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**. Close lid and invert tube 5-10 times and leave 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	5 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)*		
11	Add 70 µl of **nuclease free water** and tap tube every now and then for at least 5 min to resuspend (centrifuge shortly if beads stick to the walls)	5 min	☐
⚠	*If the liquid is too viscous to pipette off and/or the beads don't settle, double the elution volume until it works*		
12	Settle the magnetic beads on a magnetic stand and transfer the supernatant to a collection tube	5 min	☐
End	Measure concentration	~45 min hands-on	
🧊	Store @ -80 °C		

BOMB DNA extraction – high throughput

Step	Sample collection and lysis – DNA	Time	☑
1	Collect ~5mg of plant tissue in to each well of a 96 well cell culture plate. Alternatively, if a tissue lyser is available, collect plant tissue into collection microtubes (racked) containing x2 3mm glass beads		☐
2	Add 20 µl **TE buffer** to tissue and grind using micropestle until tissue is homogenous. To help homogenise the tissue, the micropestle can be attached to a small, hand held cordless drill. Alternatively, if a tissue lyser is available, seal tubes with collection microtube caps and grind tissue following manufacturers' instructions	15 min	☐
3	Add 40 µl **TNES buffer** + 0.3 µl **RNAse A**, incubate at 37 °C for 15 mins. Transfer to a 1.2 ml 96-well deep well plate	18 min	☐
4 ⚠	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	☐
5	Add 120 µl TE-diluted **Carboxyl-coated** or **silica-coated magnetic beads** to the cell lysate	5 min	☐
6	Add 240 µl **isopropanol**, seal and shake at RT at 1400 rpm for 5 min	10 min	☐
7 ⚠	Settle the magnetic beads on a magnetic stand and discard the supernatant *Ensure that the beads are completely settled*	5 min	☐
8	Remove the plate from the magnetic stand and add 400 µl **isopropanol**. Shake at RT at 1400 rpm for 2 min	5 min	☐
9	Settle the magnetic beads on a magnetic stand and discard the supernatant	5 min	☐
10	Wash twice with 300 µl of **80% ethanol** as above	10 min	☐☐
11 ⚠	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	☐
12 ⚠	Add 70-100 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	☐
13	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 @ -20 °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

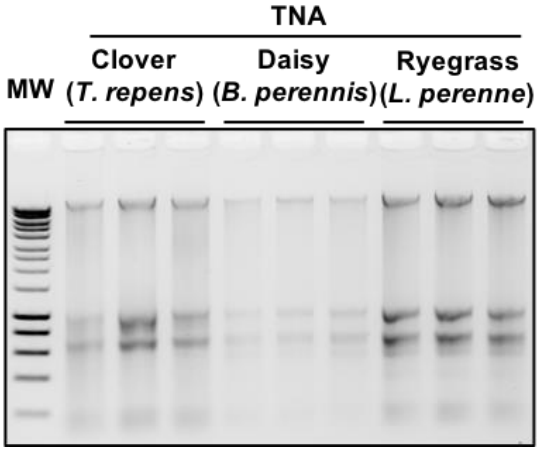

Fig 1: Example TNA isolation from plants. *TNA isolation from clover (*Trifolium repens*), daisy (*Bellis perennis*) and ryegrass (*Lolium perenne*) with carboxyl-coated magnetic beads. MW: Hyperladder I (Bioline).*

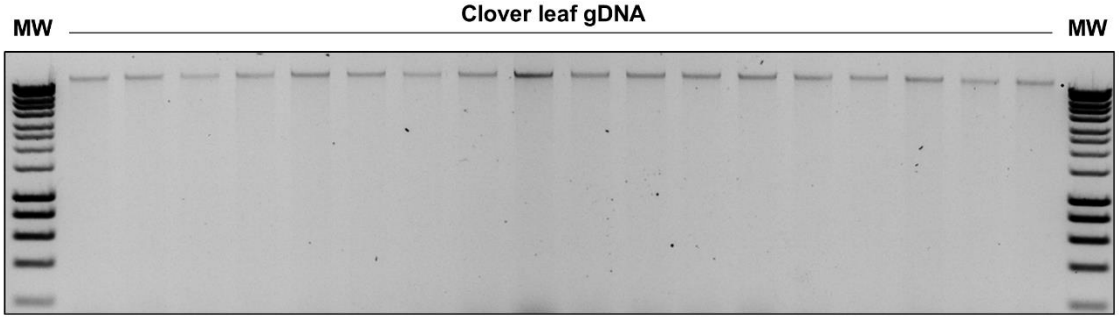

Fig 2: gDNA isolation from single clover leaves. *A subset of representative samples from 96 gDNA extractions (leaves harvested into a 96-well cell culture plate and grounded using a micropestle attached to a small, hand held cordless drill) with carboxyl-coated magnetic beads. MW: Hyperladder I (Bioline).*

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