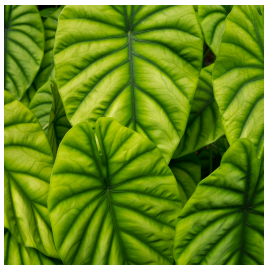
Jun 02, 2023 Version 1

Spin column TNA extraction from plants - GITC method V.1



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We use this protocol and it's working

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Abstract

This protocol is designed for extracting total nucleic acids (TNA) from plant material. In reality the drying step probably means that you won't isolate plant mRNA but viral RNA (and probably plant ribosomal RNA) are recovered. This protocol is based on the plant tissue TNA extraction method from **bomb.bio** but modified to use inexpensive 96 well spin column plates. The reasons for this are three-fold:

1. SPRI bead protocols are complicated to perform for multiple plates simultaneously. Using this protocol, up to four plates of extractions can be performed simultaneously in a deep well plate centrifuge such as the Heraeus Megafuge 40R.
2. SPRI beads require very clean supernatant after grinding to prevent plant particles binding within the bead pellet and causing downstream contamination whereas the spin columns and filter plates mechanically filter the lysate.
3. Spin columns allow a larger amount of input material than the bomb.bio protocol.

This protocol is significantly improved through the use of high volume multichannel electronic repeaters (e.g. a **Gilson PIPETMAN M P8X1200M**) or a 96 well pipette (e.g. the **Gilson Platemaster**) but can be performed using manual multichannel pipettes.

Attachments

PDF 1.5X_GITC_buffer-Ave... 186KB

PDF TNES_buffer-Avery_71... 182KB

Guidelines

Note on centrifugation in this protocol:
Maximum x g throughout is determined by both the centrifuge and the tolerances of the plastic labware you are using. If you find that not all of the liquid is moving through the spin column or that debris pellets are not pelleting, you can increase centrifugation times without any detriment to the process. Similarly, if you find plastic labware is breaking during centrifugation (this can happen with certain 0.5 ml deepwell plates), reduce the speed/g of the centrifuge and increase the centrifugation time to compensate.

Note on blocked spin columns:
Large samples and gelatinous samples can occasionally block the spin column. This can be dealt with by:

1. Increasing centrifuge times.
2. Decreasing input material.
3. Increasing the centrifugation time in the debris pelleting stage.

Materials

Buffers and reagents:

- Sodium chloride
- 1 M Tris-HCl
- 0.5 M EDTA
- Nuclease-free water
- SDS
- Sarkosyl
- Antifoam 204
- Guanidine HCl
- 100 % ethanol

For collection and initial lysis:

- 2 mL screw-cap tube for bead-beating
- 3mm hardened carbon steel ball bearings

For 96-well spin-column protocol:

- 2.2 mL deep well plates (or up to 1.5 mL) for initial protein denaturation
- 0.5 mL deep well plates for archiving
- 2.2 mL deep well plates for spin-column flow-through (these can be bleached and reused across sessions)
- Silica membrane 96-well spin-column plates
- 96-well filter plates
- 0.5 mL deep-well 96-well plates to collect eluted DNA
- Breathable plate seals
- Plate seals for long-term storage

Preparation of plant material

1w 0d 12h 30m

1 Source steel beads (ball bearings) for tissue grinding (Tungsten beads are not usually necessary). We use hardened **carbon steel** or **stainless steel bearings from simplybearings.co.uk**. This protocol requires three beads per sample tube. 1w

2 Beads are usually shipped coated in manufacturing oil (especially the carbon steel beads). To remove this, place beads in a borosilicate glass beaker or Duran bottle with the pouring lip and lid removed then bake for at least 12 hours at 250 ^{O}C. 12h

Figure 1: Depending on baking time, carbon steel beads will change colour, this is normal.

Note

Baked beads should be stored in a closed airtight DNA free container until needed, do not touch with bare hands to prevent contamination.

3 Prepare TNES buffer: 30m

	A	B	C	D
	1	Reagent	Required concentration in buffer	Amount per 100 ml
	2	Sodium Chloride	25 mM	0.701 g
	3	1M Tris-HCl	100 mM	5 ml
	4	0.5M EDTA	10 mM	4 ml
	5	SDS	10%	10g

Note

You will require 50 ml of TNES buffer per plate of extractions.

4 Prepare 5 ml screwcap collection tubes (**e.g. Eppendorf Cat. No. 0030122305**) containing three 3 mm hardened steel beads in batches of 92 tubes.

5 Using forceps (either disposable forceps or ethanol and flame steel forceps between samples), place ~ 50 - 100 mg of **wet plant material** into the pre-prepared tubes containing the hardened steel ball bearings.

6 Place plates in a -80 freezer for at least 30 min before grinding. Or alternatively snap freeze in liquid nitrogen.

7 **Everything from this point forward needs to be performed in pairs of plates so that your centrifuge is balanced.**

8 Place tubes while still cold in tissue homogeniser and grind the plant material in a tissue homogeniser until homogenised.

Note

We use a Geno/Grinder 2010 at full speed (1750 RPM) for 1-2 minutes in 1 min batches but different machines or even tube sizes and sample volumes will require separate optimised settings.

9 To the plant material, add 500 µl of freshly-prepared **TNES**, and place in a tube shaker for 5 minutes or tissue homogeniser (at 1750 RPM for one minute) to mix.

Note

Ensure that the powdered plant material is thoroughly mixed with the TNES so that the TNA can dissolve into the supernatant.

10

Note

Note on centrifugation times: *All centrifugation times and speeds below are based on a* ***Megafuge 40R****. This centrifuge cannot reach the same speeds as the* ***Qiagen Centrifuge 4-16****. If you have this faster centrifuge, then refer to the* ***Qiagen DNAeasy 96 protocol*** *for speeds and times.*

**The benefit of the Megafuge 40R over the Qiagen 4-16 is that despite being slightly slower, it can process four 96 well plates in a sessioncompared to two for the Qiagen 4-16.*

11 Centrifuge at maximum x *g* for 10 minutes to pellet plant debris.

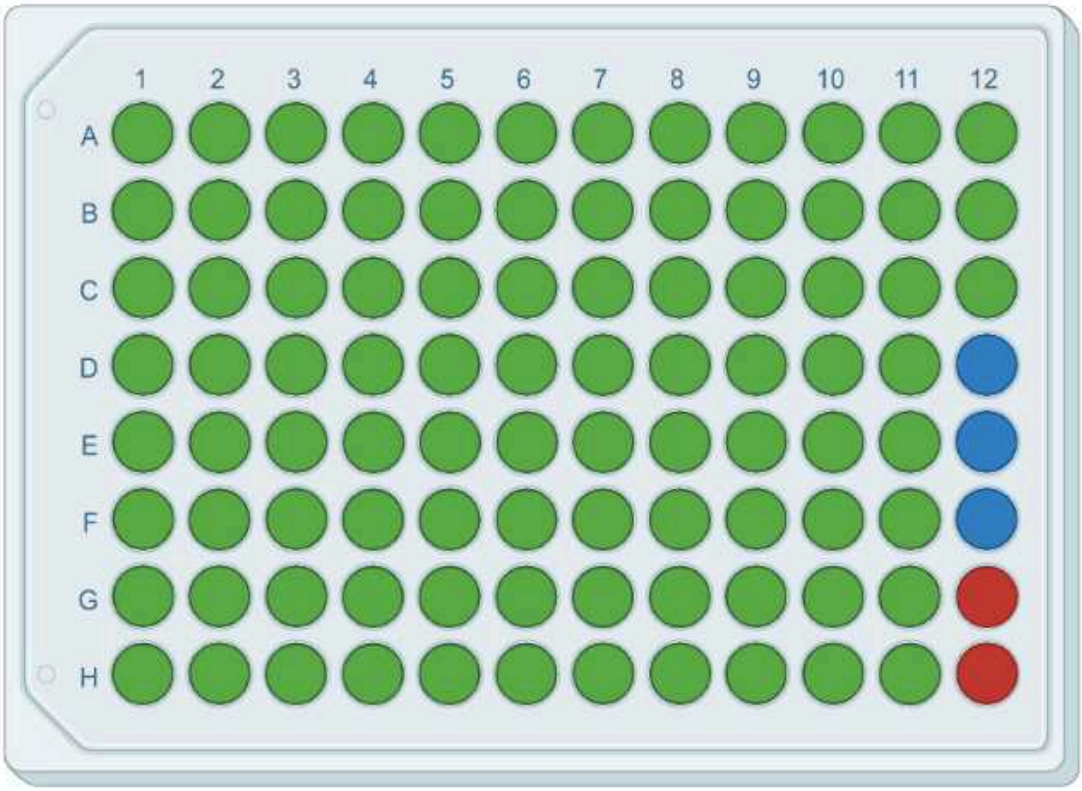
Note

For our centrifuge, a Megafuge 40R, max xg is 1500 xg. For centrifuges with lower speeds, you can increase centrifugation time.

12 Arrange 5 ml tubes in a 96-well format according to a predefined sample order (or randomly and record tube order). **Starlab 5ml tube racks are useful for this. 3D printed options exist as well.**

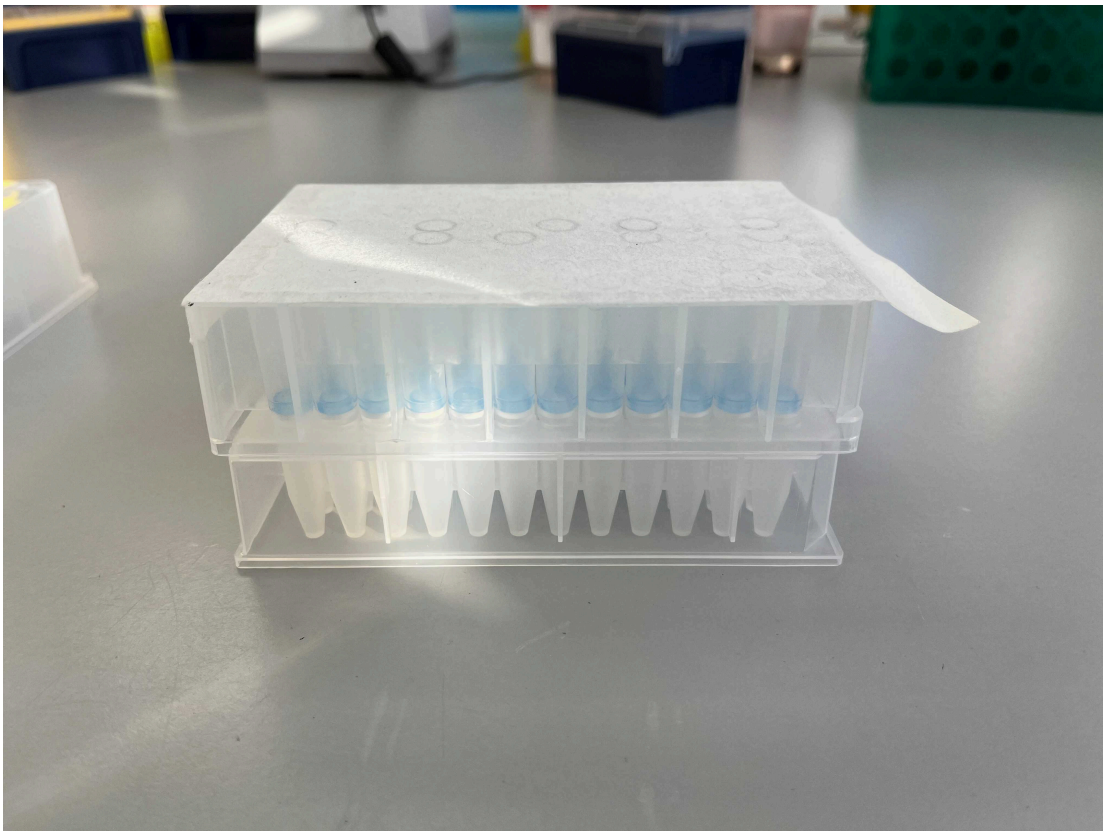
Note

Ensure you leave spaces for positive and negative controls on each plate.



Our plate layout with positive controls in blue and extraction and qPCR negatives in red.

13 Transfer the supernatant (approximately 400 µl) to a new 96 well filter plate (**NBS biologicals #SD5006**) placed on top of a 0.5 ml deep well plate (**Sarstedt #82.1969.002 or similar**). Seal the filter plate with a breathable plate seal (**Starlab #E2796-3005**). Make sure you record the location of each sample on the plate.



FIlter plate on 0.5 mL deep well plate sealed with breathable film.

14 Place both filter plate and deepwell plate in centrifuge at maximum x *g* for 5 minutes.

15 Transfer 60 µL of the supernatant to a clean 1.2 mL deep-well 96-well plate. Seal the 0.5ml plate with a non-breathable plate seal or caps.

Note

The remaining lysate in the 0.5ml plate can now be stored at -20 °C for backup/future work.

16 Add 120 µL **1.5X GITC buffer** (detailed in the sub-step below) and 240 µL of **Isopropanol** to each well of the 1.2 mL 96-well plate and mix by pipetting or seal and shake on a plate shaker.

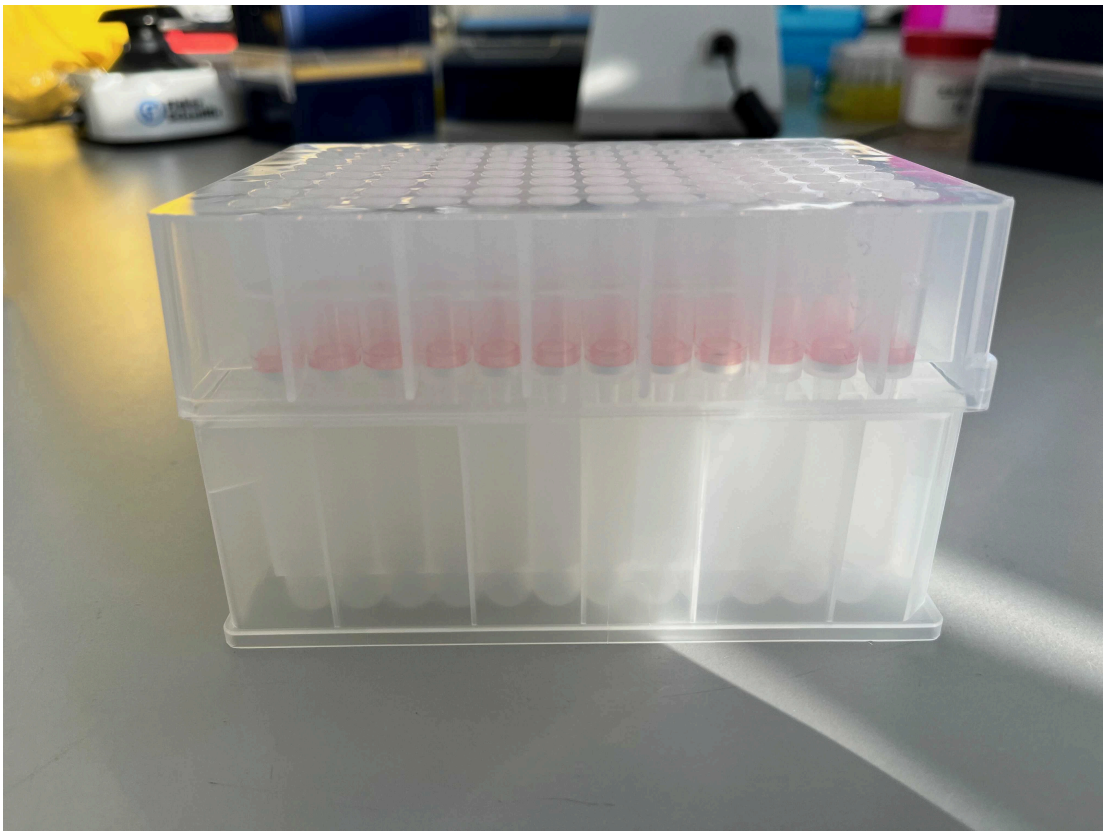
16.1 **1.5X GITC** should be comprised of the following reagents:

	A	B	C
	Reagent	**Required concentration in buffer**	**Amount per 100 mL**
	Guanidine Isothiocyanate	6 M	70.9 g
	1M Tris HCl pH 7.6 - 8.0	75 mM	7.5 mL
	Sarkosyl	3%	3 g
	0.5 M EDTA	30 mM	6 mL
	Antifoam 204	0.15 %	150 µL

17 Add all of the sample solution (~ 420 µL) to a well in a 96-well silica membrane spin column (**we use SD5007 from NBS Biologicals**) and cover with **a breathable seal**.

Note

*Ensure there is a suitable reservoir beneath into which the flow-through will go (e.g., **Sarstedt 2.2ml megablock - #82.1972.002**. These reservoirs can be bleached and reused across extraction sessions to save plastic waste.*



Spin column plate on 2.2 mL deep well waste plate

18 Leave for 5 min then centrifuge at maximum speed for 10 minutes.

Note

If the centrifuge cannot reach ~6000 x g, a longer centrifugation will work.

19 Add 400 µL **Isopropanol** to each spin column, cover with a breathable seal and leave for two minutes.

20 Centrifuge at maximum speed for 5 minutes and discard the flow-through.

21 Wash spin columns **three times** as in substeps below with **80% Ethanol solution**.

21.1 Add 300 µL of **80% Ethanol solution** to each well and centrifuge at maximum speed for 10 minutes.

22 Centrifuge spin columns for two further minutes at full speed to remove any last traces of ethanol from the silica membranes.

Note

Note on waste plate handling
At this point, your waste plate below the spin column will contain ~1720 µL of waste and will be close to the bottom of the spin column tips. Be extremely careful when handling plates to ensure that spin columns do *not come in contact with waste.*

This can be helped by having two waste plates for each spin column plate (i.e. you will need four 2.2ml plates if processing two spin column plates at a time and eight if processing four at a time). Simply change waste plates after the second wash buffer wash.

23 Carefully move the spin column plate to a new 0.5 mL DNA collection plate.

24 Add 100 µL **Elution Buffer or nuclease free water** directly to the silica membrane and leave it at room temperature for 5 minutes, covering with a breathable seal.

24.1 **Elution Buffer** should be pH ~7 and comprised of the following reagents:

	A	B	C	D
	Reagent	**Required concentration in buffer**	**Chemical molarity**	**Amount per 100 mL**
	1 M Tris-Hcl	10 mM	-	1 mL
	H2O	-	-	99 mL

25 Centrifuge at ≥ 6000 x *g* for 2 minutes. The DNA is now in the collection plate and can be taken forward to amplification.

Note

Steps 26 - 27 can be repeated for increased DNA yield but a lower overall concentration.

If the centrifuge cannot reach 6000 x g, a longer centrifugation (e.g., 5 minutes) will work, although should not be necessary for this step.