Buffers and Stocks

5X diluted Silica-coated Magnetic Beads

Component	For 50 ml
Silica-coated Magnetic Beads	10 ml
Add TE buffer to	50 ml

- Home-Made Magnetic Beads

TE buffer

Component	Final conc.	For 50 ml
1 M Tris-HCl, pH 8.0	10 mM	0.5 ml
0.5 M EDTA, pH 8.0	1 mM	0.1 ml
Add water to		50 ml

1.5X GITC Lysis Buffer

Component	Final conc.	For 50 ml
GITC (G54000)	6 M	35.46 g
1 M Tris HCl, pH 8.0	75 mM	3.75 ml
Sarkosyl	3%	1.5 g
EDTA	30 mM	3 ml of 0.5 M stock
Adjust pH with HCl to 7.6-8.0 and adjust the volume with water to 50 ml		

- Heat at 65˚C to dissolve.

TNES Buffer

Component	Final conc.	For 50 ml
1 M Tris HCl, pH 8.0	100 mM	5 ml
5 M NaCl	25 mM	0.25 ml
0.5 M EDTA	10 mM	1 ml
SDS	10% w/v	5 g

- Add 25 µl of RNase A (10 mg/ml) per 1 ml TNES Buffer before use.

Wash Buffer

Component	Final conc.	For 1 L
Ethanol	80%	800 ml
Add water to		1 L

Other materials

- Isopropanol

Procedures

1. Collect approximately 100 mg of plant tissue in two 2 ml collection tubes or 8 strip collection tube with two 4 mm beads. Freeze the samples at -80˚C in a refrigerator or liquid nitrogen.

   !!! info "For 8 strip collection tube, don't sample excessive tissue"

2. (**Highly recommend**) Lyophilize the samples overnight to enhance extraction, especially for root samples (Open the lid and cover with press ‘n seal wrap and poke a hole in it).

3. Homogenize the samples using a MiniG at 1500 rpm for 45 seconds. Re-freeze the samples and repeat the homogenization process until all the samples are ground into a powder.

   !!! info "Make sure the cap is completely sealed"

4. Add 200 µl of TNES Buffer with RNase A to the homogenized samples and mix by vortex.

   !!! info "When using 8 strip collection tube, tilt-tap the tube first to prevent powder aggregate at the bottom"

5. Incubate the samples at 37 °C for 15 minutes. A longer incubation time could degrade DNA.

   !!! info "When using 8 strip collection tube, briefly spin down the sample to prevent cross contamination"

6. Add 400 µl of 1.5X GITC Lysis Buffer, mix by vortex, and incubate for 5 min at RT.

   !!! info "When using 8 strip collection tube, briefly spin down the sample to prevent cross contamination"

7. Add 300 µl of Chloroform and mix by vortex.

8. Centrifuge at top speed for 5 min or 4000 g for 10 min (8 strip collection tube).

9. Prepare the following plates:

   - **Sample-plate** (96-deep well plate{target="_blank"}):
     - 444 µL isopropanol
     - 222 µL 5X Silica-coated diluted magnetic beads (Mix well!!!!!)
     - 333 µL DNA sample supernatant from step 8
   - **IPA-plate** (96-deep well plate): 1 ml Isopropanol

- **Wash-plate-1** (96-deep well plate): 1 ml Wash Buffer
- **Wash-plate-2** (96-deep well plate): 1 ml Wash Buffer
- **Wash-plate-3** (96-deep well plate): 1 ml Wash Buffer
- **Elution-plate** (Standard 96-well plate{target="_blank"}): 100 µL / 150 µl nuclease-free Water (after evaporation, only ~40 µl / 90 µl remain)
- **Tip-comb-plate:** KingFisher 96 Tip Comb for DW Magnets{target="_blank"} in a Standard 96-well plate.

10. Run the **DNA_Extraction_General** protocol (1 hour) located in KingFisher Flex machine.

Notes

- In this method, magnetic beads were diluted with TE buffer, therefore, you can use multichannel pipetteman to dispense.