XpressDNA Plant Kit

Protocol for isolation of high quality total genomic DNA from various plant species and tissue types like leaf, seeds, stem, roots and other plant parts.

Process Flow

Kit Contents

Components	Storage Conditions	Shipping Conditions
Plant buffer A	RT	RT
Plant buffer B	RT	RT
Plant buffer C	RT	RT
Plant buffer D	RT	RT
RNase A	2 - 8 °C	RT
Plant MagNa Mix	RT	RT
Plant Wash Buffer 1	RT	RT
Plant Wash Buffer 2	RT	RT
Plant Elution Buffer	RT	RT
MagNa Stand (optional)	RT	RT

* RT denotes 15 - 25°C.

Materials not provided with the kit

1. 100% Ethanol to Wash Buffers as indicated on the bottle.
2. Water bath/heat block at 56°C.
3. Liquid Nitrogen
4. Mortar & Pestle

Important

Pay attention to standard lab practices and safety information before beginning the procedure. For more information, refer the appropriate Material Safety Data Sheet (MSDS) available from the product supplier or download from our website http://www.maggenome.com/

Technical Support

For any product related queries please write to us on info@maggenome.com, sales@maggenome.com, support@maggenome.com.

Protocol

Plant Lysate Preparation	1. Weigh **100 mg** fresh, young leaf tissue, dry seed or other plant part and transfer it to a frozen mortar and pestle. 2. Add liquid nitrogen to the mortar and grind the tissue thoroughly until it becomes a fine powder. Transfer the pulverized tissue to a 2 ml tube before the tissue gets thawed. *(Note: For dry seeds homogenization in liquid nitrogen is optional. Instead, they may be crushed thoroughly via mortar and pestle).* 3. Add **200 µl of Plant Buffer A** to the tube and vortex briefly and thoroughly. 4. Add **650 µl of Plant Buffer B** and vortex briefly and thoroughly. 5. For soft tissues like leaves, add **85 µl of Plant Buffer C.** For hard tissues like dry seeds, add **125 µl of Plant Buffer C** and vortex thoroughly for 1 minute. 6. Incubate at 56°C for 30 minutes with intermittent vortexing. 7. Add **20 µl of RNase A,** mix the components by vortexing and incubate at RT for 10 minutes. 8. Add **170 µl of Plant Buffer D** mix thoroughly by vortexing. Incubate in ice for 10 minutes. 9. Centrifuge at 14000 x g for 5 minutes. For samples like dry seeds centrifuge the tubes for 10 minutes at 14000 x g. 10. Transfer the supernatant to a fresh 1.5 ml tube.

DNA Binding	*(Note: Vortex the MagNa Mix thoroughly before the next step.)* 11. Add **400 µl of Plant MagNa Mix** to the supernatant and invert the tube 10 - 15 times to mix properly. Do not vortex. 12. Incubate the samples at RT for 5 minutes. 13. Place the tube on a magnetic stand until the solution becomes clear. 14. Carefully discard the supernatant without removing the tube from the MagNa Stand. Ensure the magnetic nanoparticles are not disturbed.

DNA Washing	15. To the magnetic nanoparticles, add **750 µl of Plant Wash Buffer 1** then remove the tube from the MagNa Stand and resuspend the magnetic nanoparticles by pipette mixing thoroughly to ensure complete dispersal of the particles. 16. Place the tube back on the MagNa Stand for 1 minute and discard the supernatant without removing the tube from the MagNa Stand. *(For samples with less pigment content including dry seeds can be directly proceeded to Step 18 while other samples like leaves and flowers to step 17.)* 17. Repeat steps **15 and 16.** 18. Add **750 µl of Plant Wash Buffer 2** and gently invert the stand 6 - 8 times without removing the tube from the MagNa Stand *(Surface washing).*

	19. Discard the supernatant without removing the tube from the MagNa Stand. 20. Repeat steps **18 and 19.** 21. Air dry the magnetic nanoparticles without removing the tube from the MagNa Stand for 10 - 15 minutes without over-drying them.

DNA Elution	22. After drying, remove the tubes from the MagNa Stand. 23. Add **50 µl of Plant Elution buffer (EB) or Nuclease free water** and resuspend the magnetic nanoparticles by pipette mixing thoroughly. 24. Incubate at 56°C for 5 minutes with intermittent tapping. 25. Place the tube back on the MagNa Stand for 10 minutes or until the solution becomes clear. 26. Discard the magnetic nanoparticles in the appropriate hazard container. *(Note: During elution, if it takes more than 10 minutes to clarify, centrifuge the tubes at 14,000 x g for 5 minutes and transfer the supernatant to a fresh microcentrifuge.)*

Note: To improve the purity profile and concentrate the eluted DNA, follow the below steps.

Rebinding and Washing (Optional)

1. To 50 µl eluted DNA, add **100 µl of Plant MagNa mix** and invert mix 10 – 12 times. Incubate at RT for 5 minutes.
2. Keep the tube on MagNa Stand for 5 minutes until the solution gets cleared.
3. Carefully discard the supernatant without removing the tube from the MagNa stand.
4. Add **300 µl of Plant Wash Buffer 2** and gently invert mix 6 - 8 times.
5. Discard the supernatant without removing the tube from the MagNa Stand.
6. Repeat steps **4 and 5.**
7. Air dry the pellet for 5 - 10 minutes at RT keeping the tube open on a MagNa Stand and proceed to the re-elution step.

Re-elution of DNA

8. After drying, remove the tube from the MagNa Stand.
9. Add **30 µl of Plant Elution buffer (EB)** or Nuclease free water and resuspend the magnetic nanoparticles until the solution becomes homogenous.
10. Incubate at 56°C for 3 minutes with intermittent tapping.
11. After incubation, keep the tube on a MagNa Stand for 10 minutes.
12. Transfer the supernatant containing the DNA to a fresh 1.5ml tube and discard the magnetic nanoparticles in the appropriate hazard container.

Note: During elution, if it takes more than 10 minutes to clarify, centrifuge the tubes at 14,000 x g for 5 minutes and transfer the supernatant to a fresh microcentrifuge

Troubleshooting Guide

Observation	Possible Reason	Recommended solution
Low DNA yield	Mature leaf tissue	Preferably fresh and young leaf tissue
	Poor homogenization	Tissue has to be pulverized thoroughly using a mortar and pestle in presence of liquid nitrogen
	Incomplete lysis	For hard tissues lysis time could be increased subjectively.
	Ethanol was not added in Wash Buffers	Add required volume of ethanol in both Wash Buffers
DNA incompatible for downstream applications	Contaminants like secondary metabolites	Proceed to Re-binding step
	Salt carry over	Ensure Wash steps were done correctly
	Ethanol carry over	Air dry the magnetic nanoparticles completely before proceeding to elution step
Sliminess in eluted DNA	Presence of water-soluble polysaccharides	Wash the pulverized tissue using TE buffer to wash away the water-soluble polysaccharides. Repeat the steps until the sliminess is completely removed and proceed to Plant Buffer A
Pigment carry over	High pigment content	Proceed to Re-binding step
Poor purity profile	Carryover of possible contaminants	Proceed to Re-binding step
RNA contamination	Absence of addition of RNase A	Add required volume of RNase A