Transiom Plant gDNA kit

Cat No: TPLA-50

Contents

Introduction..... 2

Quality Control..... 2

Kit contents 3

Before Starting..... 3

Plant gDNA kit Protocol..... 4

Trouble shooting guide..... 7

Limited Use and Warranty..... 8

Introduction

The Plant DNA Kit provides a fast and simple method to isolating gDNA from Plant tissues. In the process, sample crushed with liquid nitrogen and lysed further with Lysis buffer (TPL). In the presence of binding buffer with chaotropic salt, the genomic DNA in the lysate binds to the silica column. The DNA is bound to the column while proteins and other impurities are removed by wash buffer.

Quality Control

Plant gDNA kit was tested by isolating of genomic DNA from 50 mg young and dry leaves. The purified DNA was quantified with Nanodrop 8000 and yield of genomic DNA was up to the 10 µg with the OD260/OD280 ratio of 1.8-2.0 .However, due to the tremendous variation in water and polysaccharide content of plants, sample size should be limited to ≤100 mg. The yield of DNA varies from plant to plant or depends on the nature of tissues. Best results are obtained with young leaves.

Materials supplied by user:

- **Sterile pestle & mortar**
- **Nuclease-free microfuge tubes**
- **Liquid nitrogen**
- **Water bath Equilibrated to 65ºC.**
- **Nuclease free ddH_2O**
- **Absolute (96%-100) ethanol**
- **β-mercaptoethanol.**
- **Chloroform/Isoamyl alcohol (24:1)**

Kit Contents

Product	TPLA-50	Storage
Buffer TPL	48 mL	RT
Buffer TPB	36 mL	RT
Buffer TWI	15 mL	RT
Spin Column	50	RT
Collection tube	50	RT
RNAse A	10 mg	-20°C
Elution Buffer	15 mL	RT
User Manual	1	

Before starting:

- **Buffer TWI:** Add 60 ml absolute (96%-100%) ethanol per bottle.
- **RNAse A**: Prepare an RNAse A stock solution with dissolving in 500 µl of elution buffer (Store stock solution at -20°C and thaw before use).

Plant gDNA Kit Protocol

Step 1: Plant tissue selection and crushing.

1. Take 50-100 mg of fresh or stored plant tissues and grind in liquid nitrogen to make fine powder using mortar and pestle.

2. Transfer it into a microfuge tube (Not provided)

Step 2: Lysis

3. Add 800 µl of Buffer TPL with 15 µl β-mercaptoethanol (Not provided) to the tube and mix it well. Add 10 µl of RNAse A into the lysate before incubation to remove the RNA.

Note: Dissolve RNAse A with elution buffer (refer to before starting note).

4. Incubate tissue lysate result from above step at 65 °C for 45 min. Mix sample during incubation by inverting tube.

5. Add 700 µl Chloroform/ Isoamyl alcohol (24:1) to the same microfuge tube and vortex to mix. Centrifuge at 10,000 rpm for 5-10 min.

6. Carefully aspirate supernatant resulting supernatant to a new 2 mL microfuge tube, make sure not to disturb the pellet or transfer any debris.

Step 3: Binding of DNA

7. Add 600 µl of Buffer TPB to the resultant supernatant from step 6 and mix well with inverting tubes 20-25 times.

8. Transfer 650 µl lysate to the DNA spin column, and centrifuge at 12,000 rpm for 1 min. Discard the flow through liquid.

9. Repeat above step, until the entire sample has been processed and retain column for further processing.

Step 4: DNA Washing

10. Place the column into same collection tube. Add 650 µl of Buffer TWI. Centrifuge at 10,000 rpm for 1 min. Discard the flow through.

11. Repeat the above "Step-10"

Note: Buffer TWI must be diluted with absolute (96%-100%) ethanol prior to use (refer to before starting note).

12. Place empty DNA spin column, with the lid open into the same collection tube and centrifuge at 10,000 rpm for 2 min.

Step 5: DNA Elution

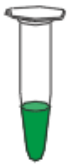
12. Place the column into a new sterile 1.5 ml eppendorf tube, add 30 µl preheated Elution buffer. Incubate at room temperature for 5 min. (perform this step twice 30 µl + 30 µl = 60 µl)

13. Centrifuge 10,000 rpm for 1 min to elute pure bacterial gDNA. The first elution normally yields 60-70 % of DNA bound. A second elution with another 30 µl buffer will yield another 20 % of the DNA.

Note: Elution volume may vary as per downstream process

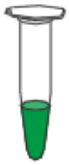
14. Discard the Column, and save elute. Do not reuse binding columns or collection tubes.

Flow Chart



Preparation of samples

- Take 50-100 mg plant sample and grind it with LN2



Lysis

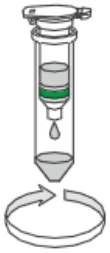
- Add 800 µl of Buffer TPL with 15 µl β-mercaptoethanol

Incubation & Phase Separation

- Incubate at 65^{0}C for 45 min
- Add Chloroform / Isoamyl alcohol

Binding to silica column

- Adjust binding of phase with Buffer TPB



Washing

- Washing twice with wash buffer

Elution of Pure DNA

Hand on time

Trouble Shooting Guide

Problems	Possible reason	Suggestions
Clogged column	Incomplete Lysis	Add the correct volume of Buffer TPL and incubate for specified time at 65°C. It may be necessary to extend incubation time by 10 min.
	Sample is too high	If using more Plant Tissue sample increase volume of β-mercaptoethanol, Buffer TPL, Buffer TPB. Pass aliquots of lysate through one column successively.
Low DNA Yield	Clogged Column	See above
	Poor elution	Repeat elution or increase elution volume. Incubation of column at 70°C for 5 min with elution buffer may increase yields.
	Improper washing	TWI buffer concentrate must be diluted with ethanol.
Low A260/280 Ratio	Extended centrifugation during elution	Resin from the column may be present in elute and effect the OD absorbance. Avoid centrifugation at speed higher than 12,000 rpm.
	Poor cell lysis due to incomplete mixing with buffer TBA	Repeat the procedure, this time making sure to vortex the sample with Buffer TPL immediately and completely.
No DNA Eluted	Poor Cell Lysis due to improper mixing with Buffer TBA	Mix thoroughly with buffer TPL.

Limited Use and Warranty

This product is intended for in vitro research use only. Transiom guarantees the performance of all products in the manner described in our product literature. We reserve the right to change, alter, or modify any product to enhance its performance and design. No other warranties of any kind express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Transiom, Transiom sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of Transiom, to replace the products, Transiom shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product. This product is warranted to perform as described in its labeling and in Transiom's literature when used in accordance with instructions. If a Transiom product does not meet your expectations, simply call Transiom Technical Support Team.

For technical support or for more product information, please visit our website at www.transiom.net