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Introduction

SQ Plant DNA Kit is designed for rapid and reliable isolation of total DNA from various plant samples.. The SQ Plant DNA Kit uses a proprietary buffer system to remove polysaccharides and proteins to isolate high molecular weight genomic DNA. There is no toxic substance such as phenol/chloroform or guandine salts involved in this system. The system can be easily scaled up or down, allowing for the purification from various amounts of starting materials.

Principle

If using the SQ Plant DNA Kit for the first time, please read this booklet to become familiar with the procedure and its various modifications. Samples are first lysed in a specially formulated buffer. The protein is precipitated by adding SQ2. After removal of the protein, the supernatant is mixed with 1 volume of isopropanol to precipitate the DNA. The DNA pellet is washed with 70% ethanol and dissolved with water or low ionic strength buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

Storage and Stability

All components of the SQ Plant DNA Kit are stable for at least 12 months from date of purchase when stored at Room Temperature except Rnase A which should be stored at 2-8°C. During shipment, or storage in cool ambient conditions, precipitates may form in the some of the buffers. Dissolve such deposits by warming the solution at 37°C.

Kit Contents

SQ Plant DNA Kit

SQ Plant DNA Kit			
Product No.	**D3095-00**	**D3095-01**	**D3095-02**
Amount of Tissue processed per kit	1 Gram	10 Grams	40 Grams
SQ1	35 ml	350 ml	2 x 700 ml
SQ2	12 ml	120 ml	500 ml
RNase A	110 µl	1.1 ml	4 x 1.1 ml
EB Buffer	3 ml	30 ml	120 ml
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Buffer EB = 10 mM Tris Hcl pH 8.5

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A. 10-25 mg SQ Plant DNA protocol

Material and Equipments supplied by User
Have the following reagents and supplies ready before starting procedure:

- Table top centrifuge capable at least 13,000 x g
- 1.5 or 2 ml Nuclease-Free centrifuge tubes
- 70% ethanol
- 100% Isopropanol
- waterbath or incubator

Before starting:

- Preheat a water bath or incubator to 65°C
- Warm up the SQ1 at 37°C water bath
- Under cool ambient conditions, crystals may form in some of the buffers. This is normal and the bottle should be warmed to re-dissolve the salt

Procedure

1. Add 750 µL of SQ 1 Buffer to a 1.5 mL Centrifuge Tube. Grind fresh plant tissue finely in liquid nitrogen with a porcelain mortar and pestle. Weigh 10-25 mg frozen ground tissue and transfer to the 1.5 ml centrifuge tube containing the 750µl of SQ1 Buffer.
2. Homogenize the sample by vortexing for 30 seconds
3. Add 250µl SQ2 Buffer to the cell lysate.
4. Invert the tube gently 10-15 times and incubate on ice for 5 minutes.
5. Centrifuge at ≥13,000 x g at room temperature for 5 minutes.
6. Carefully transfer the cleared supernatant into a new 1.5 ml microtube. Note: Do not disturb the pellet because it contains proteins
7. Add 2µL of Rnase A to the sample. Mix the sample by vortexing for 5 seconds.
8. Add an equal volume of isopropanol to the sample. Invert the tube 10-20 times.

9. Centrifuge at ≥13,000 x g at room temperature for 5 minutes. The DNA will form a small translucent pellet.

10. Carefully remove the supernatant.

11. Add 750µl 70% ethanol to the tube. Wash the DNA pellet by inverting the tube 5-10 times.

12. Centrifuge at ≥13,000 x g at room temperature for 5 minutes. Carefully remove the supernatant and drain tube on a clean absorbent paper. Air dry the DNA pellet for 10 minutes.

13. Add 100 µl of Buffer EB to the tube. Vortex the tube for 10 seconds. Allow the DNA to re-hydrate at 65°C for at least 30 minutes. Store the sample at -80°C

B. 100-200 mg SQ Plant DNA protocol

Material and Equipments supplied by User
Have the following reagents and supplies ready before starting procedure:

- Centrifuge capable at least 4,000 x g
- 70% ethanol
- 100% Isopropanol
- 15 mL Centrifuge Tube**s**
- waterbath or incubator

Before starting:
- Preheat a water bath to 65°C
- Warm up the SQ1 at 37°C water bath
- Under cool ambient conditions, crystals may form in some of the buffers. This is normal and the bottle should be warmed to re-dissolve the salt

Procedure

1. Add 6 mL of SQ 1 Buffer to a 15 mL Centrifuge Tube. Grind fresh plant tissue finely in liquid nitrogen with a porcelain mortar and pestle. Weigh 100-200 mg frozen ground tissue and transfer to the 15 ml microfuge tube containing the 6 mL of SQ1 Buffer.

2. Homogenize the sample by vortexing or using 5-10 times strokes with a microfuge tube pestle.

3. Add 2 mL SQ2 Buffer to the cell lysate.

4. Invert the tube gently 10-15 times and incubate on ice for 5 minutes.

5. Centrifuge at ≥4,000 x g at room temperature for 15 minutes.

6. Carefully transfer the cleared supernatant into a new 15 ml microtube. Note: Do not disturb the pellet because it contains proteins

7. Add 20 µL of Rnase A to the sample. Mix the sample by vortexing for 5 seconds.

8. Add an equal volume of isopropanol to the sample. Invert the tube 10-20 times.

9. Centrifuge at 4,000 x g at room temperature for 15 minutes. The DNA will form a small translucent pellet.

10. Carefully remove the supernatant.

11. Add 6 mL 70% ethanol to the tube. Wash the DNA pellet by inverting the tube 5-10 times.

12. Centrifuge at 4,000 x g at room temperature for 15 minutes. Carefully remove the supernatant and drain tube on a clean absorbent paper. Air dry the DNA pellet for 10 minutes.

13. Add 500 µl of Buffer EB to the tube. Vortex the tube for 10 seconds. Allow the DNA to re-hydrate at 65°C for at least 30 minutes. Store the sample at -80°C

C. 500 mg SQ Plant DNA protocol

Material and Equipments supplied by User
Have the following reagents and supplies ready before starting procedure:

- Centrifuge capable at least 4,000 x g
- 70% ethanol
- 100% Isopropanol
- 50 mL Centrifuge Tube**s**
- waterbath or incubator

Before starting:
- Preheat a water bath or incubator to 65°C
- Warm up the SQ1 at 37°C water bath
- Under cool ambient conditions, crystals may form in some of the buffers. This is normal and the bottle should be warmed to re-dissolve the salt

Procedure

1. Add 15 mL of SQ 1 Buffer to a 50mL Centrifuge Tube. Grind fresh plant tissue finely in liquid nitrogen with a porcelain mortar and pestle. Weigh 500 mg frozen ground tissue and transfer to the 50 ml centrifuge tube containing the 15 mL of SQ1 Buffer.

2. Homogenize the sample by vortexing or using 5-10 times strokes with a microfuge tube pestle.

3. Add 5 mL SQ2 Buffer to the cell lysate.

4. Invert the tube gently 10-15 times and incubate on ice for 5 minutes.

5. Centrifuge at ≥4,000 x g at room temperature for 15 minutes.

6. Carefully transfer the cleared supernatant into a new 50 ml microtube. Note: Do not disturb the pellet because it contains proteins

7. Add 50 µL of Rnase A to the sample. Mix the sample by vortexing for 5 seconds.

8. Add an equal volume of isopropanol to the sample. Invert the tube 10-20 times.

9. Centrifuge at 4,000 x g at room temperature for 15 minutes. The DNA will form a small translucent pellet.

10. Carefully remove the supernatant.

11. Add 15 mL 70% ethanol to the tube. Wash the DNA pellet by inverting the tube 5-10 times.

12. Centrifuge at 4,000 x g at room temperature for 15 minutes. Carefully remove the supernatant and drain tube on a clean absorbent paper. Air dry the DNA pellet for 10 minutes.

13. Add 1.5 ml of Buffer EB to the tube. Vortex the tube for 10 seconds. Allow the DNA to re-hydrate at 65°C for at least 30 minutes. Store the sample at -80°C

Trouble Shooting

Problem	Likely Cause	Suggestions
Low nucleic acid yield	Incomplete disruption and homogenization of	See cell lysis and homogenization instruction. If the lysate is too viscous, a mechanic homogenizer may be needed.
	DNA degraded during sample storage	Make sure the sample is properly stored and make sure the samples are processed immediately after collection or removal from storage.
	Loss of DNA pellet during operation	Be careful not to lose the DNA pellet during the operation
	Ethanol carryover	Make sure the ethanol is completely removed before DNA rehydration