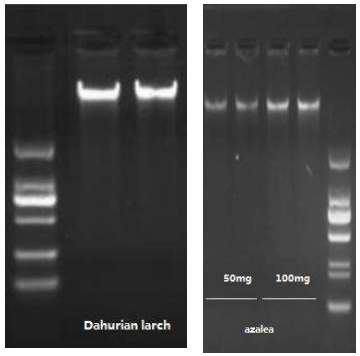
Example2: The extraction of plant tissues rich in polysaccharides and polyphenols



Company Information

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Biospin Omni Plant Genomic DNA Extraction Kit

Kit Components

Cat#	BSC13S1B	BSC13M1B
Components	**50Tests**	**100Tests**
LP Buffer	22.5 mL	45 mL
LP plus Buffer	22.5 mL	45 mL
DA Buffer	7.5 mL	15 mL
P Binding Buffer	14mL	28 mL
G Binding Buffer	25mL	50 mL
Wash Buffer	25.2mL	50.4 mL
Elution Buffer	10mL	20 mL
Spin column	50	100
Handbook	1 copy	1 copy

Storage

- The kit should be stored at 15-25℃.
- All reagents, when stored properly, are stable for 18 months.

Introduction

The kit provides a very simple, fast and economic way for the isolation of pure high–molecular-weight genomic DNA from plant tissues, adopting the Genomic DNA Buffer Set. The simple purification procedure, based on the remarkable selectivity of Biospin membrane, allows isolation of high yields of pure genomic DNA less than 1 hour. It not requires expensive equipment, involves only few steps, and completely avoids the use of toxic and hazardous reagents such as phenol and chloroform. In general, 1-30 μg genomic DNA can be acquired from up to 100 mg tissue by using this kit.

The pure DNA can be applied extensively in PCR/Real-time PCR, sequencing, Southern blot, mutant analysis, SNP and the others.

Principle

At first, the plant tissue is lysed in LP buffer, and then DNA in the sample is liberated. After adding DA Buffer and centrifuging, the impurity will be discarded. Released DNA is bound exclusively and specifically to the Biospin membrane in presence of a P Binding Buffer under appropriate salt iron and pH conditions. Denatured protein and other contaminants are removed by several washing procedures. The DNA is then eluted from the membrane with the Elution Buffer.

Apparatus and Materials to Be Supplied by the User

* Sterile 1.5ml microcentrifuge tubes
* 10μl/100μl/1000μl tips
* Microcentrifuge capable of 14,000g
* Absolute ethanol
* Vortex mixer

Important notes

1. Please add 37.8ml absolute ethanol (BSC13S1B) or 75.6ml absolute ethanol (BSC13M1B) to Wash Buffer and mix thoroughly before the first use.
2. Please add 28ml absolute ethanol (BSC13S1B) or 56ml absolute ethanol (BSC13M1B) to P Binding Buffer and mix thoroughly before the first use.
3. LP Buffer may form precipitates upon storage. In case of precipitate forming, please incubate the buffer at 37°C until the precipitate has fully dissolved.

Protocol

1. Grind the plant tissue into powder under the liquid nitrogen or ice bath.
2. Transfer up to 100mg tissue to a 1.5 or 2.0ml microcentrifuge tube. Note: the grinded degree of sample will be affect cell lysis.
3. Add 450μL LP Buffer（*Optional :add 4μL of 100mg/ml RNase A*）, Mix thoroughly.
   Note: If the plant tissues are rich in polysaccharides and polyphenols, please use the LP plus buffer.
4. Incubate at 65℃ for 15 minutes (you can vortex the tube 2~3 times during the incubation). And remove the tube from 65℃. If the sample is difficult to be lysised，please extend the incubation time。
5. Add 150μL DA Buffer and mix thoroughly，the incubate for 5 minutes on ice.
6. Apply the lysate to the Shredder Spin Column and centrifuge for 3 min at 14,000 x g. Or centrifuge the lysate at 14,000 x g for 3 minutes, transfer the supernatant to the Shredder Spin Column, then centrifuge at 12,000 x g for 1 minute.
7. Transfer the flow-through fraction to a new 1.5ml tube.
8. Add 750μL (or 1.5 volumes) of the P Binding Buffer. Mix thoroughly.
9. Transfer the mixture to the spin column. Centrifuge at 6,000 x g for 1 minute. Discard flow-through. Sample volume is more than 750μl simply load and spin again.
10. Add 500μL of the G Binding Buffer into the spin column. Centrifuge at 10,000 x g for 30 seconds. Discard flow-through.
11. Add 600μL Washing Buffer to the spin column. Centrifuge at 10,000 x *g* for 30 seconds. Discard flow-through.
12. Repeat step 11th.
13. Centrifuge for an additional 1 minute at 10,000 x g and transfer the spin column to a sterile 1.5ml microcentrifuge tube.
14. Add 100μL to 200μL Elution Buffer, Incubate at room temperature for 1 minute.
15. Centrifuge at 12,000 x g for 1 minute. The buffer in the microcentrifuge tube contains the DNA.
16. The purified DNA can be used directly for kinds of downstream molecular biological experiments. Store at -20℃ if not used immediately.

FAQ

Q1: We can extract DNA from which plant tissue using the kit?

A: We can extract DNA from leaves, flowers, caudexes (as the parts of bark which contain cells), roots (as the pileorhiza), fruits, and seeds (as soybean, corn and so on).

Q2: When should be centrifuged before filtration?

A: Some samples will be very dense after put into DA Buffer ice bath. We can transfer the above clear liquid into shredder Spin Column if centrifugate before that.

Q3: What can we do when DNA extraction yield is low?

A: DNA yield relates with lysis efficiency very much. We can improve lysis efficiency by extend incubate time (we can adopt full night lysis incubate to some samples), and then improve DNA yield.

Q4: How to wipe off the RNA included in the extracted DNA?

A: We can add RNase A to digest the RNA following with the method listed in Specification.

Q5: How long about the extracted genomic DNA fragments?

A: Length of the extracted genomic DNA fragments is about 30~50KB in general.

Analysis DNA

⊕Absorbance anlysis

Get some DNA，diluted in a advisable factor with elution buffer.

Survey the OD_{260} ， OD_{280} and OD_{320}.

expressions：concentration（μg/ml）＝$50\times OD_{260}\times$dilution fact

target： $2.0\geq OD_{260-320}/\ OD_{280-320}\geq 1.7$

Notice: $1.0\geq OD_{260}\geq 0.1$, the result of ratio is much reliable.

⊕Agarose Gel Analysis

0.8～1% Agarose gel

Example 1：Rice leaves