Plant DNA Extraction Kit

For Research Use Only

Cat. No: EX6131 **Quantity: 50 Reactions**

Store kit at: RT **Store RNase A at: -20℃**

Kit Contents:

Lysis Buffer	25ml
B-mercaptoethanol	0.6ml
Precipitation Buffer *	2ml
Chloroform	2×25ml
Binding Buffer	20ml
Wash I Buffer	25ml
Wash II Buffer **	10ml
Elution Buffer	5ml
RNase A (20mg/ml)	250μl
Mini Column	50
Protocol Handbook	1

*** Please refer to Reconstitution of buffers**

**** Before first use, add absolute ethanol (ACS grade or better) into Wash II Buffer (Add 40ml absolute ethanol in Wash II Buffer before use)**

Product Disclaimer

SinaClon Genomic DNA Plant kit is for research use only. All due care and attention should be taken in every procedure in this handbook.

Storage and Stability

SinaClon Genomic DNA Plant kit is shipped at ambient condition. Basically all components are stable at room temperature (15 ~ 25°C). A precipitate can be formed in **Lysis Buffer** and **Binding Buffer** under cool ambient condition. In such a case, heat the bottle at 65°C until completely dissolving before use.

β-mercaptoethanol (β-ME) must be added to Lysis Buffer before use in each reaction.

Safety Information

Lysis Buffer, B-mercaptoethanol and **Chloroform** contain irritant which is **harmful** when in contact with skin or eyes, or when inhaled or swallowed. Care should be taken during handling. Always wear gloves and eye protector, and follow the standard safety precautions.

Immediately after usage, close all bottles especially Chloroform and Wash II Buffer.

Binding Buffer and **Wash I Buffer** contains chaotropic salt, which is irritant. Take appropriate laboratory safety measures and wear gloves when handling. It can form highly reactive compounds when combined with bleach. Do NOT add bleach or acidic solutions directly to the sample-preparation waste.

Add appropriate β-ME in Lysis Buffer. Dispense in a fume hood and wear appropriate protective clothing.

Product Specifications

Sample size	~100mg
Preparation time	<60mins
Maximum loading volume	700µl
Typical yield	2-15µg
Elution volume	30-50 µl

Introduction

Plant Kit provide a simple and easy method for the small, scale purification of total DNA from various plant tissues.The procedure can be done in just 60minutes,yielding a pure genomic DNA suitable for various downstream applications without further manipulation. Purified total DNA can be directly applicable in conventional PCR, realtimePCR, Southern blotting, SNP genotyping, RFLP, AFLP and RAPD.

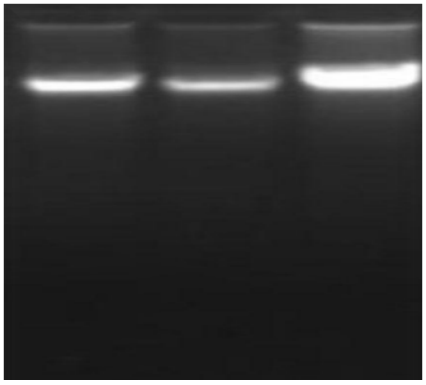

Fig1.Genomic DNA prepared from various plant tissues using SinaClon Plant DNA Extraction kit. Purified DNA was resolved on 0.7% agarose gel.

Sample weight	100mg
Elution vol.	50µl
Loaded vol.	5µl

General Considerations

Starting sample size

There is an optimized sample size for Plant Kit procedures. 100mg (wet weight) of starting sample material is optimized for the procedures. For dried or lyophilized tissue, it is 25mg. If the size of starting sample is larger than the optimized, tissue lysis cannot be performed efficiently, and this will bring about poor DNA recovery.

Sample preparation, disruption and lysis

In the purification of DNA from plants, sample preparations and disruptions are the most important steps for good result. Harvested plant sample or ground tissue powder should be stored at -80˚ C after frozen in liquid nitrogen for future use. Lyophilized tissue can be stored at room temperature. Fresh and young plant tissues will be best for

higher yield and good quality of DNA. Before lysis, tissue sample should be ground completely for efficient lysis, and this step must be performed at low temperature (below 0˚C) as quick as possible for optimized result. Mortar and pestle under liquid nitrogen is a typical and good method for grinding of sample. Lyophilized tissue can be ground at ambient condition.

Alternatively, bead beater or vortex with glass or stainless steel beads can be adopted. Complete and quick pulverization of sample tissue will guarantee the optimized result, while incomplete grinding or thawing ground sample by delayed or poor handling will result in low yields and degraded DNA. After addition of Lysis Buffer, no clumps should be visible in sample mixture. Because clumped tissue will not lyse appropriately and will therefore result in a lower yield of DNA, complete resuspending by vortexing or pipetting must be carried out for good result. For typical preparations from leaf tissue, lysis at 65˚C for 30 minutes would be sufficient. Occasional mixing by shaking or inverting of sample tube will accelerate the disruption of cells. Incubation in shaking water bath or equivalents will be the best. Lysis time may be prolonged depending on the tissue type used, but it may be sufficient to incubate for 30-60 minutes in most case.

Elution

Purified DNA can be eluted in low salt buffer or deionized water depending on the downstream applications. Purified DNA can be eluted in low salt buffer or deionized water depending on the downstream applications. Elution Buffer contains 10mM TrisCl, pH 8.5. The volume of elution buffer can be adjusted, but it has to be over the minimum requirement. For higher concentration of DNA, decrease the volume of elution buffer to minimum. **For higher overall yield, increase the volume of elution buffer and repeat the elution step once again. Optimal results may be obtained by eluting twice.**

Plant Protocol for 100 mg of plant tissue

Additional equipment or materials to be supplied by user

- 1.5ml and 2ml Micro centrifuge tubes
- Water bath or heating block at 65°C
- Absolute ethanol
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Reconstitution of buffers

* Before first use, add absolute ethanol (ACS grade or better) to Wash II Buffer as indicated on the bottle. (Add 40ml absolute ethanol in Wash II Buffer before use) please after usage Immediately close the bottle.

* Lysis Buffer prewarm in 65°C water bath before starting protocol. Before use, add 10µl [2% (v/v) b-mercaptoethanol for each reaction and keep the buffer warm while grinding tissue.

*** Before each experiment and for each reaction** prepare 1ml **P solution** by adding 40µl Precipitation buffer to 960µl Chloroform. Chloroform is a volatile substance please after usage immediately close the bottle.

1. **Grind fresh or frozen plant tissue to a fine powder quickly and completely, using a mortar and pestle under liquid nitrogen. Place up to 100mg (wet) or 25mg(dried) of ground tissue into a 1.5ml or 2ml tube.**
   Quick and complete disruption of tissue is essential for good result in preparation. Lyophilized tissue can be ground at room temperature. Grinding under liquid nitrogen is the best method for good result.
2. **Add 500ul of Lysis Buffer and Vortex vigorously.**
   Any clumps should not be visible. Resuspend the mixture by pipetting or vortexing to remove any tissue clumps.

3. **Incubate for 30 mins at 65˚C. Mix 5~6 times during incubation time by inverting or vortexing.**
   Occasional mixing will accelerate the lysis.
4. **Centrifuge for 5 min at full speed. (>10,000xg or 14,000rpm).**
   After tissue lysis, the lysate may have some cell debris and salt precipitates, and these should be removed from lysate to avoid clogging of column at binding step.
5. **Transfer the supernatant into a new 1.5 ml tube.**
   Add 5µl of RNase A solution (20mg/mL, provided) and incubate for 15 mins at 37˚C. Mix 5~6 times during incubation time by inverting.
6. **Cool the tubes to room temperature and add equal volume of P solution to the lysate Shake and vortex gently until the two phases form an emulsion, then centrifuge at 14,000 rpm for 5 min in 4 ˚C.**
   Transfer the aqueous phase carefully without transferring or disrupting middle phase into a new tube and repeat step 6.
   If transfer middle phase with supernatant of aqueous phase it can cause protein contamination in gDNA isolation.
7. **Collect the aqueous phase carefully and add 400µl Binding Buffer, mix by pipetting and incubating 5 min in room temperature.**
8. **Transfer the mixture to spin column and centrifuge at 14,000 rpm for 2 min in room temperature.**
   If the mixture volume is larger than 700 µl, apply the mixture twice; apply 700 µl of the mixture, spin down, discard the pass-through, re-insert empty collection tube, and repeat the step again until all of the mixture has been applied to the mini column.
9. **Discard flow-through. (You can discard collection tube and place column in new 2ml tube (not included).**
10. **Wash the spin column with 500µl of Wash I buffer by centrifugation at 13.000 rpm for 3 min. Discard flow-through**
11. **Wash the spin column with 500µl of Wash II buffer by centrifugation at 13.000 rpm for 3 min. Discard flow-through**.

If the purified DNA will be used for salt sensitive applications, let the mini column stand for 5 min after addition of Washing Buffer, making some amount of wash buffer flow through the column by gravity before centrifugation.

12. **Repeat the step 11.**

Avoid contaminating the column with ethanol. Ensure that the column is dry (YOU CAN USE heating block AT 65°C, 5-10 MIN TO ENSURE NO ETHANOL CONTAMINATION), and no ethanol contaminates the tip of the column. If you observe residues of ethanol, place the column in a new reaction tube.

13. **Centrifugation empty column at 13.000 rpm for 3 min.**
14. **Carefully transfer the column to a new 1.5ml tube (Not included). Place 30-50µl 65°C pre heated elution buffer in the center of the column, close lid and incubate for 5 - 10min at 65°C. Thereafter, centrifuge 13.000 rpm for 2 min to elute the DNA.**
15. **Repeat step 14.**

For higher overall yield, increase the volume of elution buffer and repeat the elution step once again. Optimal results may be obtained by eluting twice.

More 20~40% DNA can be acquired by repeat of eluting once again. A new 1.5ml tube can be used to prevent dilution of the first elute.

The elution volume depends on the sample: If high DNA amounts are expected, a higher elution volume may increase the DNA yield. Generally, 30-50µl elution volume gives satisfactory results.

Troubleshooting Guide

Facts	Possible Causes	Suggestions
Low or no recovery	Too much starting material	Too much starting material lead to Inefficient lysis and spin column clogging,Followed by poor DNA yields. Reduce theAmount of starting material.
	Too old or mis-stored Sample used	Refer " Sample preparation,disruption And lysis "
	Insufficient disruption	Refer " Sample preparation, disruption and lysis "
	Insufficient lysis	Too much starting material can lead to poor lysis, followed by significant decrease in recover yield.
	Improper elution	The conditions of optimal elution should be low salt concentration with weak alkaline pH (7<pH>9).When water or other buffer was used as eluent, ensure that condition. After eluent is applied on the center of spin column membrane, it is essential to incubate at least for 5 minutes at room temperature.
Low purity	Incomplete precipitation	Any cell debris or precipitates must be removed before addition of Binding Buffer.
	Insufficient lysis	Too much starting material can lead to poor lysis, followed by low purity of DNA.
	Transfer or disrupting middle phase in step 6	Refer to step 6 guideline.
Clogging spin Column	Incomplete removal of precipitate	Any cell debris or precipitates must be removed before addition of Binding Buffer, otherwise spin Column may be clogged by it.
	Lysate too viscous or sticky	Reduce the amount of starting sample, or increase the amount of Lysis Buffer.
DNA sheared	Too much starting materials	Too much starting material can make the lysate very viscous and lead to shearing of DNA. Reduce the amount of starting material.
	Too old or mis-stored sample used	Refer "Sample preparation, disruption and lysis"
	Too viscous lysate	In some plants, the lysate may become too viscous, so reduce the amount of starting sample, or increase the amount of Lysis Buffer.
Enzymatic reaction is not performed well with purified DNA	High salt concentration in eluate	Ensure that washing step was carried out just in accordance with the protocols. Repeat of washing step may help to remove high salt in eluate.
	Residual ethanol in eluate	Ensure that the wash step in protocols is performed properly. Spin Column membrane should be completely dried by additional centrifugation or air-drying before elution.