Genomic DNA extraction performance of customized protocol of Exgene™ Plant SV maxi from Halophyte (*Spergularia marina*)

Experimental Conditions

Materials

Product	Exgene™ Plant SV maxi	
Cat. No.	117-310	117-326
Number of preparation	10	26

Sample & Extraction Information

Sample	Halophyte (*Spergularia marina*)	
Sample amount	1 g	4 g
Target	Genomic DNA	
Elution volume	1 ml	

Protocol

Exgene™ Plant SV maxi (customized protocol for *Spergularia marina*)

*** For more details and methods, please refer to the manual of Exgene™ Plant SV maxi.**

1. The frozen sample using liquid nitrogen is ground finely using a mortal and pestle.
2. Transfer the 1 g (or 4 g) of fine powder to 50 ml conical tube. Add 5 ml of Buffer PL and 40 µl of RNase A (100 mg/ml) Solution. Vortex to mix completely.
3. Incubate for 20 min at 65 ˚C. Mix the sample by inverting 10 times every 5 min during incubation.
4. Add 1.8 ml of Buffer PD to the lysate and vortex to mix completely.
5. [Customized Step] Incubate for 10 min on ice.
6. Centrifuge at 4000 xg for 5 min at room temperature.
7. Carefully transfer the supernatant to EzSep™ Filter (maxi).
8. Centrifuge at 4000 xg for 5 min at room temperature.
9. Transfer the pass-through to a new 50 ml conical tube.
10. [Customized Step] Add 2 volume of absolute isopropanol (about 15 ml) to the lysate and mix 10 times by inverting.
11. Apply the sample mixture from previous step to Column Type G (maxi).
12. Centrifuge at 4000 xg for 2 min at room temperature and discard the pass-through and re-insert Column Type G (maxi) to the collection tube. If any residual mixture remains, repeat steps 11 and 12 to completely filter out any remaining mixture.
13. Apply 13 ml of Buffer CW to Column Type G (maxi). Centrifuge for 5 min at 4000 xg and discard the pass-through, and re-insert Column Type G (maxi) to the collection tube.
14. Apply 10 ml of Buffer CW to Column Type G (maxi). Centrifuge for 5 min at 4000 xg and discard the pass-through, and re-insert Column Type G (maxi) to the collection tube.
15. Centrifuge for 10 min at 4500 xg (not to apply the buffer) and place Column Type G (maxi) in a new 50 ml tube.
16. Add 1 ml of Buffer AE directly onto the center of Column Type G (maxi) membrane. Incubate for 5 min at room temperature and centrifuge for 5 min at 5000 xg.

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Results

Exgene™ Plant SV maxi	Original protocol				Customized protocol			
	Yield (μg)	CV	A_{260}/A_{280}	CV	Yield (μg)	CV	A_{260}/A_{280}	CV
Spergularia marina (1 g)	10.8	0.04	1.69	0.02	30.5	0.16	1.47	0.02
Spergularia marina (4 g)	24.8	0.06	1.80	0.01	109.2	0.07	1.28	0.02

Table 1. DNA yield and purity
Genomic DNA was extracted from *Spergularia marina* using Exgene™ Plant SV maxi (n=3). The yield (μg) and purity (A_{260}/A_{280}) of the extracted nucleic acids were measured using NanoDrop™ 2000. It was confirmed that a higher yield was obtained when genomic DNA was extracted using a custom protocol rather than the original protocol.

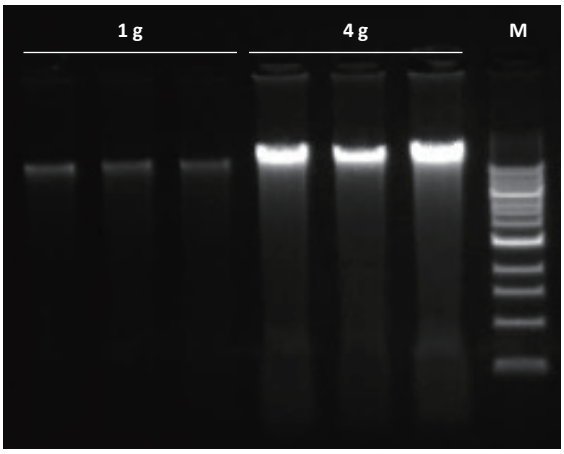

Figure 1. Agarose gel electrophoresis
The extracted each gDNA was running electrophoresis (1% agarose gel with 0.5 X TBE, 150 V, 18 min). The size of the separated DNA fragment was confirmed through GENESTA™ 1 Kb DNA ladder (GA-100), and the electrophoresis result was analyzed with SmartView Pro 1100 Imager System (UVCI-1100).

Conclusion

- More effective results were obtained when using customized protocol rather than standard protocol to increase nucleic acid yields from halophytes.
- Customized protocols (incubation on ice & isopropanol precipitation) for efficient salt removal improve the performance of nucleic acid extraction.
- The purity is low due to halophytes containing high salt compared to general samples, but the gel electrophoresis results show larger than 10 Kb of clear genomic DNA bands, so extracted DNA can be expected to apply most downstream applications.

Ordering Information

Cat. No.	Product	Size
117-101	Exgene™ Plant SV mini	100 prep
117-152	Exgene™ Plant SV mini	250 prep
117-226	Exgene™ Plant SV midi	26 prep
117-201	Exgene™ Plant SV midi	100 prep
117-310	Exgene™ Plant SV maxi	10 prep
117-326	Exgene™ Plant SV maxi	26 prep