Genomic DNA extraction experiment using Exgene™ Plant SV mini kit from tiny amount of sea algae samples which are difficult to extract nucleic acid

Experimental Conditions

Materials Required

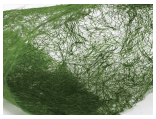
- Exgene™ Plant SV mini (100 preps: 117-101 / 250 preps: 117-152)
- Tissuelyser II (85300, supplier Q) or another bead beating device
- Liquid nitrogen (LN_2)
- Methanol (CH_3OH, CAS No.: 67-56-1, ≥99.8%)
- 1.5 ml or 2.0 ml microcentrifuge tube
- Vortex mixer
- Centrifuge (Max. speed 14,000 rpm)
- Pipette & sterile pipette tips
- Suitable protector (e.g., lab coat, disposable gloves, goggles, etc.)
- Ice

Sample Information

- Sample type:


Kelp
(*Saccharina japonaca*)


Sea lettuce
(*Ulva rigida*)


Green laver
(*Ulva prolifera*)

- Sampling: after collecting fresh algae samples, put them in a bag and seal it
- How to store: store in -70 ˚C deep freezer
- Sample preparation
  1. Rapidly freeze the frozen samples in LN_2.
  2. Prepare the two each of 5 mg sea algae samples.
  3. Homogenize the samples using Tissuelyser II (85300, supplier Q)
- Elution volume: 50 µl

Protocol

1. Prepare two each of three types of the sea algae samples measured 5 mg which are rapidly frozen. Homogenize the sea algae samples for 10 seconds at 30 Hz using TissueLyser II.
2. After homogenizing, first samples are treated with 1 ml methanol for removing salt, and second samples are not treated with 1 ml methanol.

- The methanol treatment method for removing salt and substances in the sea algae samples.

① Vortex after add the 1 ml of methanol in samples.
② Remove the supernatant solution after centrifugation for 1 min at ≥13,000 rpm.
③ Vortex the samples after add the nuclease-free water.
④ Remove the supernatant solution after centrifugation for 1 min at ≥13,000 rpm.

3. The next step for gDNA extraction is according to the protocol of Exgene™ Plant SV mini.

- Exgene™ Plant SV mini Protocol

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

① Add 400 µl Buffer PL and 4 µl of RNase A solution (100 mg/ml). vortex vigorously.
② Incubate for 10-15 min at 65 ˚C. Mix 2-3 times during incubation by vortexing. Occasional mixing will accelerate the lysis.
③ Add 140 µl of Buffer PD to the lysate. Vortex to mix, and incubate for 5 min on ice.
(Optional:) Centrifuge for 5 min at full speed (>10,000 x g or 14,000 rpm) if the lysate becomes very viscous or sticky after addition of Buffer PD.
④ Apply the lysate to EzSep™ Filter (blue) and centrifuge for 2 min at full speed.
⑤ Transfer the pass-through to a new 1.5 ml microcentrifuge tube by pipetting or decanting carefully not to disturb the cell debris pellet.
※ CAUTION: In step 4, small cell debris pellet can be formed in 2 ml collection tube after centrifugation. Please be careful not to disturb this pellet in next step.
⑥ Add 1.5 volumes of Buffer BD to the lysate and mix immediately by pipetting or inverting. Adjust of volume of Buffer BD on the basis of correct volume of lysate.
⑦ Apply 700 µl of the mixture to Column Type G (green) with collection tube. Centrifuge for 30 sec and discard the pass-through. Reuse the collection tube.
⑧ Apply the all of remaining lysate to Column Type G. Centrifuge for 30 sec and discard the pass-through. Reuse the collection tube.
⑨ Apply 700 µl Buffer CW to Column Type G. Centrifuge for 30 sec and discard the pass-through. Reinsert Column Type G to the collection tube.
⑩ Apply 300 µl Buffer CW to Column Type G. Centrifuge for 2 min. Transfer carefully Column Type G to a new 1.5 ml microcentrifuge tube. If carryover of Buffer CW occurs, centrifuge again for 1 min before proceeding to next step.
⑪ Apply 50 µl of Buffer AE directly onto the center of column membrane. Incubate for 5 min at room temperature, and centrifuge for 1 min.

Result

#	Sample Type	Conc. (ng/µl)		$A_{260/280}$		$A_{260/230}$		Yield (µg)	
		+	-	+	-	+	-	+	-
1	Kelp	8	9.2	1.61	1.99	0.97	1.01	**0.4**	**0.46**
2		7.7	10.5	1.93	1.77	1.17	1.04	**0.39**	**0.53**
3	Sea lettuce	0.4	0.7	3.51	2.04	-0.05	-0.08	**0.02**	**0.04**
4		0.4	0.6	5.01	9.46	-0.05	-0.06	**0.02**	**0.03**
5	Green laver	1.5	2.2	1.55	2.78	-0.28	-0.39	**0.08**	**0.11**
6		1.3	2.5	9.34	2.19	-0.18	-0.42	**0.07**	**0.13**

Table 1. The results of sea algae gDNA yield and purity measurement about condition of treatment in 1 ml methanol (+) and non-treatment in 1 ml methanol (-).
+: methanol 1 ml treatment
-: methanol 1 ml non-treatment
※ Absorbance measurement instrument: NanoDrop™ 2000/2000c (ND-2000, supplier T)

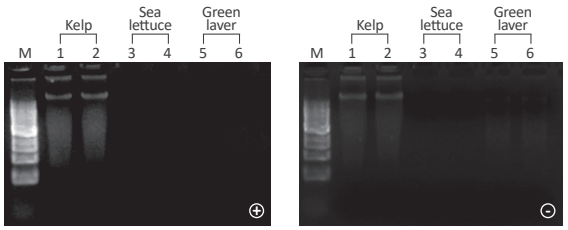


Figure 1. The result of electrophoresis about condition of treatment in 1 ml methanol (+) and non-treatment in 1 ml methanol (-). It is possible to see the DNA band of kelp samples.
M: GENESTA™ 1 kb DNA ladder with 5X loading dye (GA-100, GeneAll®, 1 µl loading)
※ Electrophoresis conditions: 1.0% agarose gel (150 V, 25 min, 10 µl loading)

Conclusion

- As a result of extracting genomic DNA from tiny amounts of sea algae samples difficult to extract nucleic acid using Exgene™ Plant SV mini kit, it is possible to confirm that a little amounts of DNA are extracted.
- Large amounts of salt contained in the sea algae samples conduct as an inhibitor interrupted to extract nucleic acid.
- For comparison of removing salt, we proceeded to treat 1 ml methanol in homogenized sea algae samples or not. But, it is not possible to confirm as a meaningful result of research.