Ver.260501

ODP412

Seaweed Genomic DNA Kit

ISO 13485:2016 ISO 9001:2015

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Spin Column

(ODP412)

Kit Contents

Contents	50rxns
Buffer MT	100mL
Buffer MP	40mL
Buffer SA	5mL
Buffer AB	5mL
Buffer GD	13mL
Buffer PW	15mL
Buffer TE	10mL
Proteinase K	1mL
Spin Columns (CB3) & Collection Tubes	50 Nos

Storage

Seaweed Genomic DNA Kit can be stored dry at room temperature (15-25°C) for up to 12 months without showing any reduction in performance and quality. For longer storage, the kit can be stored at 2-8°C. Proteinase K has to be stored at -20°C for 1 year.

(Note: Check buffers for precipitate before use and dissolve at 37°C for 10 minutes if necessary.).

Introduction

The Seaweed Genomic DNA Kit provides a fast, simple, and cost- effective genomic DNA extraction method for routine molecular biology laboratory applications. The kit uses silica membrane technology to eliminate the cumbersome steps associated with loose resins or slurries. The kit is ready for use and can extract the genomic DNA from a wide variety of seaweed. Extracted DNA is suitable for PCR, restriction endonuclease digestion and Southern hybridization.

Chemicals required but not provided

1. Isopropanol
2. Chloroform
3. Isoamyl alcohol
4. β-Mercaptoethanol
5. Ethanol (96-100%)

Important Notes

1. Add appropriate amount of ethanol (96-100%) to Buffer GD and Buffer PW as indicated on the bottle before use.
2. Increasing the time of absorption and elution could improve recovery efficiency of DNA.
3. All centrifugation steps are carried out at 13,000 rpm in conventional tabletop microcentrifuge at room temperature.
4. The recovery efficiency is related to starting DNA quantity and elution volume. The less starting quantity or elution volume, less the recovery efficiency.

Protocol

Ensure that Buffer GD and Buffer PW have been prepared with appropriate volume of ethanol (96-100%) as indicated on the bottle and shake thoroughly.

Note: Prepare an aliquot of Buffer MP & TE and pre-warm at 65°C for 2 minutes before step 6 & 19.

1. Place 100mg fresh or frozen sample in a 1.5mL microcentrifuge tube. Add 200µL MT buffer to the tube

   Note: Samples may be frozen by dipping them in liquid nitrogen.
   Samples were ground using a micropestle.
2. Add 800µL of Buffer MT to the tube containing the sample.
3. Add 10µL β-Mercaptoethanol to the tube.
4. Vortex for 30 seconds and centrifuge at 5,000 rpm for 5 minutes.
5. Discard supernatant.

   Note: The Buffer MT wash may be repeated for challenging samples where the supernatant from the first wash is found to be viscous, densely turbid or dark brown in colour.
6. Add 800µL pre-warmed Buffer MP to the pellet.
7. Add 14µL β-Mercaptoethanol to tube.
8. Vortex for 30 seconds, make sure to disperse all clumps and then incubate for 1 hour at 65°C, mix by inverting the tube for several times during incubation.
9. Add 15µL Proteinase K and immediately invert the tubes a few times.
10. Incubate at RT for 5 minutes.
11. Add 600µL Chloroform:isoamyl alcohol (24:1), mix by vortexing the tube, centrifuge for 15 minutes at 12,000 rpm (~13,400 ×g).

    Note: Chloroform:isoamyl alcohol (24:1) has to be prepared freshly before extraction.
12. Carefully transfer the aqueous phase to a fresh tube.

    Note: For samples with high polysaccharides content, repeat the chloroform:isoamyl alcohol extraction for both the aqueous and organic phases, then combine the aqueous phases from both tubes into a new tube and continue the extraction.
13. Add 100µL Buffer SA followed by equal volume of ice-cold isopropanol (Aqueous phase: isopropanol = 1:1). Mix by gentle inversion.
14. Incubate at -20°C for 1 hour to enhance DNA precipitation.

    Note: The extraction can be paused at this stage and continued on the next day. The mixture needs to be stored at -20°C during this pause point.
    Before transferring the lysate to Spin column CB3, add 100µL Buffer AB to the centre of Spin column CB3 (in a 2mL collection tube), and incubate at room temperature for 5 minutes. Centrifuge at 12,000 rpm for 2 minutes and discard flow-through.
15. Pipette all the mixtures from step 14, including any precipitate that may have formed, into the spin column CB3 (place the spin column CB3 in the collection tube). Close the lid and incubate for 10 minutes at room temperature. Centrifuge at 12,000 rpm (~13,400 ×g) for 30 seconds. Discard the filtrate and replace the spin column CB3 in to the collection tube.

    Since the capacity of spin column CB3 is 700µL, the centrifugation step must be repeated for processing all the mixture from step 14.
16. Carefully open the spin column CB3 and add 500µL Buffer GD. Close the lid and centrifuge at 12,000 rpm (~13,400 ×g) for 30 seconds then discard the filtrate and replace the spin column CB3 into the collection tube.
17. Add 700µL Buffer PW to the spin column CB3 to wash the membrane, and centrifuge for 30 seconds at 12,000 rpm (~13,400 ×g), discard the flow-through, and replace the spin column CB3 in the collection tube.

18. Centrifuge for 2 minutes at 12,000 rpm (~13,400 ×g) to remove residual wash Buffer PW. Discard the collection tube and transfer the spin column CB3 to a clean 1.5mL or 2mL microcentrifuge tube. Open the lid of the spin column CB3 and incubate the assembly at room temperature (15-25°C) for 2 minutes to dry membrane completely.
    Note: Residual ethanol from Buffer PW may inhibit subsequent enzymatic reactions.
19. Pipette 50–100µL Buffer pre-warmed TE directly onto the membrane, incubate for 2-5 minutes at room temperature (15–25°C), and then centrifuge for 2 minutes at 12,000 rpm (~13,400 ×g) to elute.
    Note: The volume of Buffer TE must be at least 50µL, or it may affect recovery efficiency. The pH value of eluted buffer will have some influence in eluting, we suggest Buffer TE or distilled water (pH 7.0-8.5) to elute DNA. For long-term storage of DNA, eluting in Buffer TE and storing at –20°C is recommended, since DNA stored in water is subject to acid hydrolysis.
    To increase DNA yield, introduce the eluted buffer TE to the spin column CB3 and centrifuge for 2 minutes at 12,000 rpm.