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The application of a novel Cas12a nuclease aligned CRISPR system in Ulva prolifera

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We use this protocol and it's working

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Abstract

The protocol describes a novel CRISPR Cas12a-aligned gene-editing system in the green seaweed Ulva prolifera. It has 3 main steps:
1. Gamete induction and collection by phototaxis.
2. CRISPR genome editing RNP assembly and delivery by modified Polyethene Glycol (PEG) 4000 system.
3. Mutant screening by 2-fluoroadenine (2-FA).

Materials

Sorbitol (TCI S0065)
0.5M Tris-HCl pH 7.5
1M CaCl2
PEG 4000 (Wako)
PEG 8000 (MPbio)
2-Fluoroadenine (TCI F0647)
EnGen sgRNA Synthesis Kit (NEB #E3322S),
Monarch RNA Clean up Kit (NEB #T2040)
Cas9 Nuclease (NEB #M0646T)
crRNA (IDT)
ST8
AsCas12a (IDT 1081068)
Distilled water

Gamete collection

1 Prepare the Low Salinity Buffer:
Sorbitol 1.822 g
Distilled water 8.7 mL
1x PES medium 1 mL
0.5M Tris-HCl pH 7.5 200 µL
1M CaCl2 100 µL

2 The gametes are collected by phototactic migration after cells are obtained by centrifugation at 5,000 g for 5 min at 20°C. Finally, resuspend thoroughly in Low Salinity Buffer at a density of 1.0×10^^4 per µL.

RNP preparations

2.1 For CRISPR/Cas9 Genome Editing:
Mix 1 µL crRNA+1 µL tracrRNA and incubate at 95°C for 5 min as the synthetic sgRNA. After cooling down, Cas9 proteins are added to the synthetic sgRNA and incubated at 37°C for 5-10 min to make the CRISPR RNP complexes.

2.2 For CRISPR/AsCas12a and ST8 Genome Editing:
Mix 1 µL dissolved crRNA with AsCas12a/ST8 (3.34 µmol/µL), then incubate at room temperature for 10-20 min as the CRISPR RNP complexes.

Transformation

3 Prepare the Transformation Buffers:
40% PEG 4000:
PEG 4000 4 g
Distilled water 10 mL
40% PEG 4000 with CaCl2:
PEG 4000 4 g
Distilled water 9.7 mL
0.5M Tris-HCl pH 7.5 200 µL
1M CaCl2 100 µL
Final concentration: 10mM Tris-HCl pH 7.5, 10mM CaCl2, 40% PEG 4000.
60% PEG 4000:
PEG 4000 6 g
Distilled water 10 mL
40% PEG 8000:
PEG 8000 4 g
Distilled water 10 mL
60% PEG 8000:

PEG 8000 6 g
Distilled water 10 mL
Prepare the reaction as following:
Gametes 100 µL
60% PEG or UTB2 (Prefer 60% PEG) 100 µL
CRISPR RNP complex 2.5 µL
Gently mix the reagents and incubate at room temperature for 30 min.
Centrifuge at 5,000 × g for 5 min and remove the supernatant.
Wash the gametes with filtered seawater and centrifuge at 5,000 g for 5 min to obtain the pellet.
Resuspend the pellet in 1/2 PES and add to a 60 mm dish.
Incubate the dish at 20°C in the dark for 36-48 hours before 2-FA treatment.

2-FA selection

4 The 1/2 PES medium was removed, and the selection medium (10 uM 2-FA) was added to the dish and cultured for 2-3 weeks; change the selection medium every week.
Transfer the 2-FA-resistant thalli to 1/2 PES medium for the following experiments and change the medium every week.

Protocol references

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