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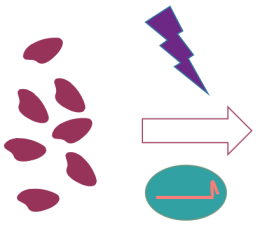
Protocol for introducing fluorescently labeled CRISPR/Cas9 RNP complex into heterotrophic dinoflagellates



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Protocol status: In development
We are still developing and optimizing this protocol

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Abstract

Introducing CRISPR/Cas9 machinery into the cell as RNP complexes reduces the number of steps necessary to edit the genome, does not require codon optimization or a strong promoter, can result in higher editing efficiencies with fewer off target effects, and has lower risks of cell toxicity (Woo et al., 2015; Liang et al., 2015; Liang et al., 2017; Farboud et al., 2018). This protocol utilizes IDTs fluorescently labeled tracrRNAs to visually check to see whether the CRISPR/Cas9 RNP molecule can make it into non photosynthetic dinoflagellates. This protocol serves as a good starting point to evaluate the CRISPR/Cas9 RNP method, the relative transformation efficiency, and can be used to help sort cells that contain the CRISPR/Cas9 RNP molecule from cells that do not.

Guidelines

The protocol utilizes Lonza's SG Cell Line 4D-Nucleofector™ X Kit S and IDTs Alt-R™CRISPR/Cas9 crRNA and tracrRNA – ATTO™ 550

Materials

MATERIALS

Lonza Nucleofector 4d **Lonza Catalog #**AAF-1002X

Before start

Make sure your dinoflagellate culture is healthy and narrow down an antibiotic cocktail that can be used to reduce the growth of bacteria in the culture.

Check out the **IDT application note** regarding the Alt-R™ CRISPR/Cas9 tracrRNA – ATTO™ 550

1. Prepare the RNP complex from IDT

1 Combine 1 uL Alt-R™ CRISPR/Cas9 tracrRNA – ATTO™ 550 (100 uM) + 1 L Alt-R™ CRISPR/Cas9 crRNA (100 uM) + 18 uL IDT Nuclease Free Buffer

1.1 Note: the concentrations can and should be adjusted to determine optimal [RNP] and Cas9:gRNA ratios

2 Incubate at 95˚C for 5 minutes, let cool to room temperature on bench

3 Meanwhile, combine 2.67 uL Alt-R Cas9 Enzyme (61 uM) + 37.33 uL IDT Nuclease Free Buffer

4 Form RNP complex by combining 1-part gRNA (part 1) and 1-part Cas9 (part 3) and let incubate at room temperature for 20 mins.

4.1 Note: RNP complexes can be kept at 4˚C for a month or 5 months at -80˚C

Collecting *O. marina*

5 Concentrate healthy *Oxyrrhis marina* cells using a flashlight. Make sure that under green light the cells have no background fluorescence (this may require not feeding them for some time)

6 Count cell concentration of these healthy cultures using a Sedgewick Rafter Counting Chamber. Goal is to use 200,000 cells/well for electroporation

7 Spin down the *O. marina* cells that are necessary for the experimental set-up (need to include the NPC (no pulse controls) and scrambled gRNA control). Use 2500g for 2.5 mins

8 Remove all but 2-5 mL of liquid, being very careful to not disturb the pellet. Transfer gently to 2 mL test tubes

9 Centrifuge at 900g for 1 minute and remove all remaining liquid

10 Resuspend in electroporation buffer

10.1 For each experimental well use Lonza's 16.4 uL SG Buffer, Lonza's 3.6 uL Supplement 1 Solution, and 2uL RNP complex (Step 4)

Electroporation Steps

11 Place 22 uL into each well of Lonza's Nucleocuvette™ 16-well strips very gently and electroporate using desired setting

12 Immediately add 80 uL of filtered and autoclaved seawater containing the antibiotic cocktail to limit bacteria growth

13 After 20-30 minutes transfer each well to a 24-well culture plate that already contains 1.4 mL of the same seawater described above

Observation Steps

14 After 1.5-2 hours try to observe the cells for red fluorescence to demonstrate RNP complex uptake under green light

15 Continue to observe cells for fluorescence for 3 days (many experiments have shown RNP complexes are mostly degraded after 72 hours)

16 Isolate gDNA after ~72 hours and perform T7E1 endonuclease assay to determine gene editing efficiency